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MYCORRHIZAL ASSOCIATIONS AND CALLUNA HEATHLAND AFFORESTATION

By

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OXFORD

LONDON: HER MAJESTY'S STATIONERY OFFICE
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FOREWORD

Ever since its establishment in 1919, the Forestry Commission has taken an active interest in the afforestation of heathlands dominated by the common heather, *Calluna vulgaris*. These heaths hold a large reserve of plantable land, but are difficult areas for the good growth of most timber trees. Much research work has therefore been carried out on their problems, from several angles of approach.

This present bulletin, by Dr. W. R. C. Handley of the Commonwealth Forestry Institute, Oxford University, presents the results of enquiries into those heathland mycorrhizal associations—that is the inter-relationships between trees, plants, and fungi, which appear to be important to the practising Forester. It summarises work done at intervals over the past sixteen years, mainly at Oxford but also at several of the Commission's heathland forests, notably Allerston in Yorkshire and Wareham in Dorset. Throughout this period the Commission's research staff have co-operated with Dr. Handley in field studies.

It is believed that this publication will throw valuable light on a field of study that is important to both foresters and other scientists concerned with soils and plant life.

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INTRODUCTION

It has now been established beyond reasonable doubt (Dimbleby 1952 a) that the marked podzolization occurring on the heather moorlands of north-east Yorkshire has largely arisen since Bronze Age times.

Pollen analyses of the former mineral soil surface, which has remained buried since Bronze Age times, indicate that before this period, where there is now moorland, there was deciduous forest containing species of *Betula*, *Corylus*, *Alnus*, *Quercus*, *Tilia*, *Ulmus*, with *Acer*, *Salix* and *Pinus* as minor constituents. It seems most probable that at that time these species were associated with a brown forest soil.

Similar vegetation changes appear to have occurred, although perhaps not always during exactly the same period of time, in other regions of Atlantic Europe where extensive, dense Callunetum associated with marked podzolization of the soil is to be found now or at any rate until quite recently. These vegetation changes are almost certainly the result of the activities of man commencing with the destruction of the deciduous forests. Although it is probable that a number of changes in the type of vegetation took place before *Calluna vulgaris* became dominant, it is likely that these areas have supported a vegetation consisting largely of *Calluna vulgaris* for centuries at least. The Callunetum has been maintained by burning and grazing.

It is by no means impossible, however, for the long continued dominance of *Calluna vulgaris* to be broken and for this species to be partly or wholly replaced by other species. For example, Borggreve (1873) mentions that pine, willow, aspen, birch, juniper and oak will colonize *Calluna* heathland in such numbers that the latter disappears.

The colonization of strongly podzolized heather covered soils by self sown tree seedlings has been studied in some detail by Dimbleby (1953). As might be expected the distribution of self sown pine and birch on the heathland seems to be closely dependent on the proximity of seed trees and wind direction. However, the occurrence of very scattered seedlings, often confined to local disturbances of the moor surface, and the considerable numbers of seedlings occurring where there has been considerable mechanical disturbance of the Calluna vegetation and the heath soil on parts of the moor most remote from seed trees is interpreted as indicating that establishment of tree seedlings only takes place where the dominance of the heather is broken. Dimbleby is doubtful as to whether birch can invade dense Callunetum and the instances of establishment of birch seedlings which were studied in detail had occurred soon after destruction of the *Calluna* by fire, which often occurs for one reason or another before the *Calluna* becomes senescent. Dimbleby records that the most important colonizers of the north-east Yorkshire *Calluna* moors are Scots pine and birch. Oak, rowan and willows are the only other woody species occurring but he considers that they are restricted to certain habitats and cannot be regarded as colonizers of the open moor.

According to Galoux (1953) birch, oak and pine recolonize the land spontaneously at Gare de As after burning of the Callunetum.

The status of the once extensive heathlands of Halland in S.W. Sweden has been discussed by Malmström (1937). On old maps the older heathlands were shown as being treeless in 1652. Other heathlands seem to be of more recent origin and to be about 150–200 years old. Burning of heathland has ceased at various times in various districts since about 1850. In 1890 the heaths were recorded as being either without trees, or almost so, where burning had ceased most recently. Where burning had ceased earlier, scattered pine and birch occurred along with numerous juniper bushes. *Sorbus aucuparia* and *Salix repens* are also noted as species occurring on the heathlands. Malmström's studies indicate that colonization of the heathlands by birch and pine occurs soon after burning, and evidence supporting this is provided in the even-aged stands of self-sown trees which have developed on former heathland. He also reported the presence in the area of Norway spruce trees up to one hundred years old and individuals or quite small groups of this species which seem to have come into being without planting by man. Since 1872 Norway spruce has been introduced into this area to a very large extent and stands of this species occupy about half the area. Observations made by Malmström in the years around 1930

indicate that Norway spruce occurred in some, though not all, of the birch and pine stands from which *Calluna* was absent or to be found only as isolated or scattered plants. Vigorous groups of self-sown Norway spruce occurred where beech forest had been clearfelled and in alder-birch swamp forest, *Calluna* being absent from both sites. It is perhaps significant that, in contrast, Norway spruce is not recorded as occurring in the nearby heath areas where *Calluna* is recorded as being abundant and where woody species such as *Betula pubescens*, *B. verrucosa*, *Sorbus aucuparia*, *Juniperus communis*, *Quercus* sp., *Salix repens* and *Pinus silvestris* are to be found, even though it is recorded that good stands of Norway spruce have been obtained by planting on this *Calluna* heathland although the treatment of the heath site before planting is not clear.

The heaths of Norholm in the south-west of Jutland were studied over a number of years (1921–37) by Bornebusch (1943) who considers that the original heath vegetation contained *Juniperus communis*, *Sorbus aucuparia*, *Rhamnus frangula*, *Salix cinerea*, *Betula* (largely *pubescens*), *Salix repens* and *Populus tremula*. Most of these species were present on the heathland plots examined and in addition acorns are carried on to the heath by various animals, but although they germinate readily and the seedlings survive for some years most of them eventually disappear. As usual on heathlands the vegetation has been subjected to a number of hazards including fire, grazing and exposure to wind, which between them bring about considerable variations in the plant population from time to time. Although the extent of the vegetation changes in the years immediately preceding the period of observation is not indicated, it is of interest to examine Bornebusch's results for the variations in the plant populations of the various plots especially with regard to Norway spruce and *Calluna*. The results demonstrate, if nothing else, the influence of the prevailing wind on seed dispersal from the small number of localized seed trees. Those plots (Nos. 6, 12 and 13) which are near the spruce seed trees and contain appreciable numbers of spruce plants also show increasing numbers of spruce and birch plants as the area covered by *Calluna* diminishes although this trend was not maintained by birch in the most recent period possibly because of the activities of deer as suggested by Bornebusch. The spruce plants were still quite young and there had been no opportunity to determine the capacity of the Norway spruce to maintain itself on the heath although Bornebusch states that the lower branches of the spruce remain green and spread over the *Calluna* and smother it, which is perhaps an indication that he considered that there is generally a struggle between *Calluna* and the young trees. On the other hand Bornebusch considers that climatic conditions are the reason for Scots pine and Norway spruce being unable to maintain themselves on the heathland at the present time. In only two of the plots for which vegetation descriptions are given (Nos. 14 and 19) does *Calluna* attain anything like complete dominance during the period of observation; in the case of plot 14 both mountain pine and spruce increase in numbers as the area covered by *Calluna* diminishes whilst in the case of plot 19 the increase in the number of birch plants ceases, possibly due to deer damage as mentioned earlier, when the *Calluna* covers most of the plot; spruce is virtually absent at all times although it is not certain that much spruce seed would fall on this plot.

Semb and Nedkvitne (1957) have described the vegetation of deciduous woodland, associated with brown forest soils at Jaeren in Norway, and that of the surrounding heathland where *Calluna* covers most of the surface of the soil. The woodlands contain hazel, birch, rowan, ash, *Salix caprea*, *Sorbus aria*, *Populus tremula*, *Sambucus racemosa* and oak, and presumably seeds of some or all of these species are carried on to the *Calluna* heathland; but for one reason or another the species noted as occurring there are *Betula* spp., *Juniperus communis*, *Salix aurita*, *Salix repens*, *Salix* spp., and *Sorbus aucuparia*.

Calluna-dominated heathlands of southern Sweden have been studied recently by Damman (1957).

Although there are certain differences in the plant species associated with *Calluna* in the Scandinavian heaths as compared with the heathlands of Holland and N.W. Germany there is invasion by trees and shrubs as there is elsewhere. Damman concludes that the most important species in this respect are *Juniperus communis*, *Picea abies* and *Betula* spp., although his own tables giving the composition of the vegetation of the various *Calluna*-dominated heaths would seem to indicate that the tree and shrub species found there were *Pinus sylvestris*, *Betula pubescens*, *Sorbus aucuparia*, *Juniperus communis* and *Genista pilosa*.

The observations of various investigators regarding the *Calluna* heathlands of Scandinavia, the Netherlands and N.W. Germany have been collected together and summarized by Beijerinck (1940). Thus Nordhagen recorded small numbers of *Betula nana* and *Juniperus nana* occurring on *Calluna* heathland in Central Scandinavia at an altitude of 850 m. Raunkiaer observed *Salix repens* in one of the types of *Calluna* heath in Jutland. Observations on the vegetation of 10 *Calluna* heaths in the Netherlands and N.W. Germany, by various investigators, indicate the presence of small numbers of birch trees on 8 of the 10 heaths, *Pinus sylvestris* on 5, *Juniperus communis* on 4, *Quercus robur* on 6, *Sorbus aucuparia* on 4, *Salix repens* on 2, and *Salix aurita* on 3. Although *Calluna* covers more than three quarters

of the area in some parts of all these heaths, by no means all the heath is covered to this extent in all cases.

These various observations suggest that throughout the *Calluna* heath region of western Europe there is a relatively small number of tree and non-Ericaceous shrub species able to colonize the heathland whilst the dominance of *Calluna* is temporarily broken. These species are *Betula pubescens*, *B. verrucosa*, *Pinus sylvestris*, *Juniperus communis*, *Sorbus aucuparia*, *Quercus* spp., *Populus tremula*, *Salix aurita*, *Salix repens*, *Salix* spp., and *Genista* spp. They are not characterized by a particular type of seed, e.g. wind distributed. Although there are no doubt a number of factors concerned in determining whether a species can colonize *Calluna* heathland it is perhaps important that even though other species may grow on adjacent areas and produce seed, some of which probably reaches the heathland, they do not seem to colonize the heathland even though they may be able to grow on the heathland under certain conditions.

Experiences in the afforestation of *Calluna* heathland show that whilst some tree species such as Scots pine and larch can be successfully established on these areas other species such as Norway and Sitka spruce, Lawson Cypress and Silver fir have been found to be more difficult to establish with certainty under the same conditions and using the same methods.

P. E. Müller (1897) was perhaps the first to observe and describe the checking of the growth of Norway spruce and silver fir on *Calluna* heathlands in western Jutland. He commented that although cultivation of the heathland soil, ploughing three times and harrowing several times in the course of three years, resulted in satisfactory growth of the spruce, this was only maintained so long as heather did not re-invade the site. Prevention of re-invasion by heather allowed the spruce to go on growing but once the heather covered the site again stagnation of the growth of spruce ensued and this effect was more pronounced where there had been more rapid growth of the spruce following cultivation of the soil.

Duchaufour (1950) records observations by M. Vazeilles in "la Corrèze" on the "Plateau de Millevaches" that spruce planted on *Calluna* land, which was very little leached but having up to 10 cms of raw humus, either died soon after planting or went into check; the needles are then yellowish in colour and growth is practically nil. On the same sites Scots pine grows satisfactorily. Duchaufour also records similar observations made by M. Oudin in the Puy de Dôme in 1937. Here again spruce planted on *Calluna* land went into check and the needles became yellow whilst Scots pine grew well.

Similar findings have been recorded by Braathe (1950) for the heaths of Denmark and North Germany and to some extent in South Sweden and in Vestland in Norway. He states that when planted on land where there is much *Calluna vulgaris*, Norway spruce, Sitka spruce, silver fir and some other species have a shorter or longer period of little growth which may last for a few years up to 20–25 years or more, whereas Scots pine and mountain pine do not undergo the same growth inhibition when planted under the same conditions. In the case of the *Calluna* heathlands of north-east Yorkshire, Weatherell (1953) has observed that the growth of Norway spruce, Sitka spruce and Lawson's cypress is checked by the *Calluna* whilst Scots pine and Japanese larch are not similarly affected. The checked growth of spruce in the presence of heather has also been observed in northern Bohemia by Nemec (1954).

The poor growth of Norway spruce and some other species in the presence of *Calluna* on *Calluna* heathland may therefore be regarded as a widespread phenomenon which seems to differ from the temporary check to growth frequently observed when young spruce plants which have been grown in nurseries established on former agricultural soils are planted in the forest.

It has, however, been observed that the effect of *Calluna* is apparently overcome under some conditions. Müller (1897 and 1903) observed that Norway spruce planted alone on the *Calluna* heathlands of Jutland grew a little during the first year but soon ceased growth and eventually died whereas in the mixed plantations of spruce and mountain pine of E. M. Dalgas the growth of spruce was much improved. He also noted that even in areas of *Calluna* heathland where stagnation of the growth of spruce occurs there are small areas, in which the soil and nature of the site are not visibly different from the rest, where the spruce continued to grow in the absence of mountain pine and, having reached a height of 10 feet, had almost closed canopy: further, that checking of the growth of spruce by *Calluna* is not found in some of the heaths of east Jutland nor in Zealand or on the island of Bornholm. Even in west Jutland the effect is not observed, even though the ground is completely covered by heather, if the spruce is growing on former farmland.

In 1938 Weatherell (1953 and 1957) observed that spruce grown for about 11 years, on shallow ploughed *Calluna* heathland, in mixture with Scots pine or adjacent to Japanese larch, had begun to emerge from check.

Spruce planted on ploughed *Calluna* heathland, where the *Calluna* has been destroyed at least partially for a time, has, according to the observations of M. Oudin in the Puy de Dôme (Duchaufour (1950)), grown very much better than spruce planted on an untreated *Calluna* site.

Rayner and Neilson-Jones (1944) observed that when an area of *Calluna* heathland was tractor ploughed there was a marked improvement in the subsequent growth of pines and of such members of the natural vegetation as reappeared spontaneously although many of the failures and inconsistencies of tree growth persisted locally. They remarked on the improved soil drainage and aeration resulting from the ploughing. These observations are reminiscent of those indicating that trees frequently only appear to be able to invade *Calluna* heathland when the dominance of the *Calluna* is broken by local disturbance or fire and it may well be that, in addition to the effects on drainage and aeration, an important effect of the tractor ploughing is due to the dominance of the *Calluna* being broken.

Weatherell (1953) has found that Norway spruce, Sitka spruce and Lawson's cypress, whose growth was severely checked in the presence of dense *Calluna*, became a healthier colour and grew more rapidly when the *Calluna* had been killed by the application of a heavy mulch of *Calluna* shoots or by rotary hoeing. It was subsequently observed that needle colour and height growth of checked Sitka spruce are improved by the presence of *Sarothamnus scoparius*, as previously observed by Müller (1903) for Norway spruce, or addition of nitrogenous fertilizers, although phosphatic fertilizers had no detectable effect, even though *Calluna* might still be present.

Checking of the growth of spruce in the presence of *Calluna* has also been found to be diminished by the application of a mulch of heather by Nemec (1954) who also observed that application of powdered basalt resulted in a much smaller improvement in the condition of the spruce.

Thus the domination of other plant species by *Calluna* only occurs under certain conditions and can be modified by the application of nitrogenous fertilizers and overcome by measures which kill the *Calluna*, e.g. heavy mulching, or prevent the return to dominance of the *Calluna* following ploughing of the heathland, e.g. by the growth of species such as pine and larch, which are apparently less sensitive to the influence of *Calluna*, and dominate the returning *Calluna* by shading.

The reasons for the checking of the growth of trees and especially of species such as spruce, aptly described as "heather sensitive" by Weatherell (1953), by *Calluna* are still uncertain. Müller (1897) was convinced that the effect on the spruce is exclusively due to the heather itself and could not be due to the influence of the wind, the raw humus, the leached sand or the hardpan since soil working modified all the soil factors yet when the heather returned the spruce became sickly and stopped growing. He wondered whether it is a bacteriological or soil physiological problem of local occurrence and whether it could be solved by inoculation with appropriate soil. Later Müller (1903) suggested that the effect of mountain pine in overcoming the checking of the growth of spruce by *Calluna* is due to the ability of its mycorrhizal associations to assimilate atmospheric nitrogen which is passed on to the spruce on the death of these associations. Duchaufour (1950) put forward the observations of M. Oudin in the Puy de Dôme in 1937 as demonstrating that lack of moisture is not the reason for the failure of spruce planted on *Calluna* heathlands, which is in agreement with the observations of Levisohn (1952), and that the checked growth of spruce in this region must, as on the Plateau de Millevaches, be attributed to nitrogen deficiency. The differing behaviour of pine and spruce in this respect he considers to be due partly to the tap root developed by the pine in the A_2C horizon and partly to the numbers and various types of mycorrhizal associations developed by the pine, and supplying its nitrogen requirements, compared with the absence or insufficiency of mycorrhizal associations formed by spruce under these conditions. The previously mentioned observations by Weatherell (1953) on the effect of *Sarothamnus scoparius* and the application of nitrogenous fertilizers on the growth of spruce in the presence of reinvading *Calluna* were interpreted as suggesting a nitrogen deficiency as being the cause of the growth of the spruce being checked. Arising out of some experiments on the establishment of various broadleaf species on *Calluna* heathland in north-east Yorkshire, Dimbleby (1958) concludes that competition from *Calluna* has seriously affected the growth of most species and he considers that the effect is mainly concerned with the nitrogen supply. The presence of broom was again found to influence the growth of some species favourably.

In a recent most useful and comprehensive account of experiments concerning the afforestation of upland heaths by the Forestry Commission, Zehetmayr (1960) concludes that manuring has proved of little importance compared with cultivation in the establishment of trees on *Calluna* heathland. He suggests that the reasons for the checking of the growth of trees by *Calluna* is due to competition with the *Calluna* for water and nutrients, that the effect of cultivation of the *Calluna* heathland on the growth of trees is due to improved drainage and aeration of the soil, that the "nursing" effect by trees less sensitive to *Calluna* is due more to the provision of improved conditions for the tree roots than to any sheltering effect and that conditions favouring the establishment of "heather sensitive" tree species on *Calluna* heathland are elimination of heather competition by ploughing, suppression of heather regrowth by a "nurse" species and stimula-

tion of growth by phosphatic manuring or legumes.

Levisohn (1953a) found that, when the growth of trees was released from check by mulching, soil analyses did not indicate any change in the phosphate status of the soil as a result of mulching. In addition, although the application of a bracken mulch resulted in appreciable amounts of potassium being added to the soil, the treatment of checked Lawson Cypress plants with potassium sulphate produced no apparent change in their condition.

Unfavourable physical soil conditions, excessive acidity of the soil, deficiency of trace elements in the soil, deficiency of mycorrhizal fungi and severe competition from *Calluna* are listed by Braathe (1950) as causes which have been suggested as being responsible for the inhibition of the growth of spruce on *Calluna* heathland. He considers, however, that whilst one or more such factors might possibly be operative on the first occasion on which spruce is planted on *Calluna* heathlands which have been without trees for many centuries, they cannot explain the many examples of inhibition of growth in the second generation of spruce on an area from which the first generation, which had grown satisfactorily, was removed by clear felling before replanting and the area then subsequently invaded by *Calluna* in a few years. Braathe finds it impossible to believe that the soil can change so markedly in two or three years that it results in the growth of the spruce plants coming to a standstill or that the *Calluna* at the time of invasion should monopolize all the nutrients so completely that there is nothing left for the spruce. He therefore concluded that *Calluna* has a biological effect on spruce and suggested that *Calluna* produces a substance which in some way inhibits the growth of spruce. Experiments by Braathe to test this hypothesis did not give conclusive results but this point will be considered again later. Laing (1932) found that decaying *Calluna* leaves had an adverse effect on seedlings of *Picea excelsa* growing in water culture whereas *Molinia* leaves had no such effect under the same conditions.

Although he does not give any reasons for doing so, Björkman (1949) discounts the probability that *Calluna* litter contains, or that *Calluna* produces, substances having a toxic effect on spruce and prefers to ascribe the effect to nutritional conditions especially regarding nitrogen and phosphorus.

When the growth of spruce on *Calluna* heathland is being favourably influenced by the presence of pine or larch the spruce roots develop very largely beneath the "nurse" species where they branch freely, travel below the layer of larch litter, and only occasionally do side branches descend into the ploughed soils (Weatherell (1953 and 1957)). On the other hand, spruce roots growing in the area occupied by the spruce plant, and where *Calluna* was present, were shorter and thinner and had much smaller tips. In the case of spruce plants adjacent to larch but remaining in check it was found that their roots were confined to the *Calluna* dominated area. The distribution of the roots of various tree species planted on *Calluna* heathland has also been investigated by Yeatman (1955) and it seems clear that when spruce roots spread into an area occupied by pine roots they will be in the zone of free fibrous rooting of the pines. It may therefore be expected that under these conditions there will be severe competition for moisture and nutrients yet the spruce grows much better than when its roots are restricted to soil also occupied by *Calluna* roots.

Similarly Duchaufour (1950) has recorded that spruce grows well on the slightly leached soils of the Plateau de Millevaches when the *Calluna* vegetation has been replaced by *Genista pilosa* or bracken as a result of the abandonment of sheep grazing whereas on pedologically similar sites (the C/N ratio of the soil organic matter is somewhat higher), in the midst of a good spruce area with *Genista pilosa*, but carrying a vegetation of pure *Calluna*, the growth of spruce is checked. Although the similarity of the rooting zones of spruce, *Genista pilosa* and bracken is not so certain as in the case of spruce, *Calluna* and most species such as pine and larch, spruce is able to grow on these soils in the presence of *Genista pilosa* and bracken but not in the presence of *Calluna*. Again, it has been observed that when grazing is abandoned the old pastures situated in Swedish coniferous forests become thickets of spruce (Romell 1957); in this case also the spruce seems to be able to overcome any competition from the pasture vegetation for nutrients and moisture. Similarly spruce readily establishes itself in the dense herbaceous vegetation of alpine meadows. It is clear that under a variety of conditions spruce is not without appreciable capacity to compete with other plant species for water and nutrients even though it appears to be unable to compete with *Calluna*.

In this connection the findings of Ingestad (1957, 1959 and 1960), who grew birch, spruce and pine seedlings over a range of nutrient conditions in water culture, are interesting. He found that birch and pine seem to require a larger supply of mineral nutrients, and especially of nitrogen, than spruce to produce the same amount of dry matter. This could mean that in the colonization of *Calluna* heathlands the nutrient requirements of birch and pine would not seem to confer any advantages on these species or account for their apparent superiority over spruce as a colonizing species; if anything, the reverse would seem to be indicated. This seems to be supported by the results from some of the experiments reported by Zehetmayr (1960) showing that the "heather sensitive" Sitka spruce is growing faster than its "nurses"

under the nutrient conditions of the *Calluna* heathland and showing greater resistance to exposure.

Although it seems clear that the struggle between *Calluna* and tree species on heathlands ultimately represents competition for mineral nutrients it would seem to have unusual features. Usually a species more successful in the competition for water and nutrients grows faster than the less successful which is overtopped and suppressed; *Calluna*, however, does not overtop the trees whose growth it checks. Some tree species are able to establish themselves sufficiently quickly on heathland, from which *Calluna* has been eradicated, that they can prevent the return of the *Calluna* by the shade of their branches, their roots being able to obtain sufficient nutrients and water from the soil from which the *Calluna* is now excluded. Other tree species, such as the "heather sensitive" spruce, although unable to become sufficiently established during the time the *Calluna* is absent from the heathland that they can effectively exclude the returning *Calluna* from a sufficient area of heathland, can none the less, on the same sites, compete with tree species which are able to withstand the returning *Calluna*. Therefore in the absence of *Calluna* the root of the "heather sensitive" spruce seems to be an equally effective organ in the competition for water and nutrients as the roots of the species, pine and larch, which are less sensitive to the effect of *Calluna*. It seems evident therefore that the basis of the "checking" effect of the *Calluna* must be sought in an effect on the functioning of the root system of the tree, probably by interference with the mechanism whereby mineral nutrients are taken into the root.

Whatever factor or factors are concerned it would seem that they must have a differential effect on various tree species, although it is possible for all species to be affected, and must be associated with the presence of living *Calluna*, for in the absence of living *Calluna* the differential effect on different tree species disappears. It is perhaps important to remember here that Løfting (1951) has described how continental races of Scots pine of slender branching habit, when planted on podzolized heathland, often maintain their perfectly straight stems and short fine lateral branches so that they are incapable of suppressing the *Calluna* and are consequently liable to be unable to flourish. On the other hand isolated pines which have recently colonized heathlands have widely spreading lower branches which prevent the growth of *Calluna* beneath them; the latter is also true for birch.

Since the effect of *Calluna* seems to be brought about through its influence on the tree roots it is desirable to try and ascertain whether there is any evidence that tree roots display any unusual characteristics when growing in the presence of *Calluna*.

Changes in the root systems of checked Sitka spruce when the *Calluna* surrounding them is killed by the application of a heavy *Calluna* mulch have been described by Yeatman (1955). The growth of Sitka spruce planted into prepared patches on the *Calluna* heathland is checked in the second year and the number of survivors gradually diminishes. The root systems of survivors consist of adventitious roots extending over the surface of the *Calluna* raw humus. The original root system developed in the nursery usually fails to develop further after planting and is surrounded by fine *Calluna* roots. When the living *Calluna* is suppressed by a heather mulch, a mass of fibrous spruce rootlets develops in the *Calluna* debris lying on the surface of the raw humus; but new roots growing in the raw humus are not produced at least within a period of three years after suppression of the *Calluna*, during which time the needles have changed from a sickly yellow to dark green and there is a pronounced growth of leading shoots. To maintain this improved condition the recolonization by *Calluna* must be prevented.

The seeds of various species of pine were sown on *Calluna* heathland by Rayner and Neilson Jones (1944) and they subsequently recorded that a large proportion of the seedlings died outright or passed into a condition of more or less complete check, although it was still possible after 14–16 years to find surviving seedlings a few inches high whose roots are characterized by marked deficiency of short roots and poverty of mycorrhizal associations. No actual root disease or parasitic attack by any soil organism was ever observed nor was there any sign of pseudomycorrhizal associations. The same authors also observed that when Norway spruce is sown or planted in *Calluna* heath soil, either in field plots or pots, there is arrest of root growth and absence of mycorrhizal associations, along with complete check to shoot growth and severe chlorosis of the foliage. In these cases also there was no evidence that failure to grow was associated with root attack by any soil fungus. The fact that checked trees can remain alive for many years in the presence of *Calluna* and respond at any time to suppression of the *Calluna* or application of fertilizers, also suggests that the checked trees are not being subjected to attack by pathogenic microorganisms.

Although actual numbers are not given, Duchaufour (1950) found that checked spruce on *Calluna* heathlands in France had an absence or insufficiency of mycorrhizal associations, whereas pine had fair numbers of various types of such associations which are considered to ensure the nitrogen supply of the pines. Similarly Rayner and Neilson Jones (1944) reported that on the untreated *Calluna* heathland of Wareham Forest root growth is arrested and the formation of mycorrhizal associations is com-

pletely inhibited in Norway spruce; they concluded that the absence of mycorrhizal associations was directly related to arrested growth and chlorosis of the spruce plants. Subsequently Levisohn (1952) observed in the same area that whilst the roots of checked spruce show arrested development and either absence or remarkable scarcity of mycorrhizal associations, the roots of previously checked plants, which had been mulched and whose colour had improved, had well developed monopodially branched root systems characteristic of healthy Norway spruce; the short roots were fully mycorrhizal and pseudomycorrhizal associations were absent. Changes in the roots were observed before improvement in shoot development was noticeable "externally" (Levisohn (1953a)).

Vigorous young birch trees colonizing the *Calluna* heathlands of north-east Yorkshire were found by Dimbleby (1953) to have developed mycorrhizal associations especially in old rotten roots and tree stumps and occasionally in the humus layer which is sometimes strongly developed just above the pan. None of the pine seedlings growing in the same area appeared to have formed mycorrhizal associations.

In the case of the pine heaths of Sweden, where *Calluna* is the predominant constituent of the ground vegetation, Melin (1948) has pointed out that it has long been known that in such plant communities mycorrhizal associations are formed with difficulty by trees as is also the case in old, slow growing spruce woods with inactive raw humus where the ground flora contains a considerable amount of *Vaccinium myrtillus*. In 1924 Hesselman set up trenched plot experiments in pine heath forests near Vindeln. Some of these plots were hoed; this resulted in the death of the *Calluna* and as reported by Romell and Malmström (1945) the seedlings showed better development of roots and mycorrhizal associations.

There seems to be evidence therefore that when tree seedlings grow poorly in the presence of living *Calluna* they are usually characterized by lack of development of characteristic ectotrophic mycorrhizal associations.

In the colonization of many *Calluna* heathlands by trees, the conflict between *Calluna* and tree seedlings seems to be characterized by the following features. Usually few, if any, tree species can invade dense and undisturbed Callunetum, but if there is local disturbance and the dominance of the *Calluna* is broken then a number of tree species will be able to grow and of these some will be able to compete successfully with the re-invading *Calluna* whilst others such as spruce will not be able to do so. Those trees which successfully colonize the disturbed heathland will be found to have halted the return of the *Calluna* by shade produced by the extending branches. Where the trees are sufficiently close together this process continues until canopy is closed. The check to the growth of tree species such as spruce can be overcome by measures such as mulching which kill the heather, or by the application of nitrogenous fertilizers; and it is not observed in spruce growing in the presence of *Calluna* on heathland which has been subjected to agricultural practices or in spruce whose roots are growing beneath the canopy of another species which has completely shaded out the *Calluna*. The check to the growth of trees is likely to recur if conditions arise which permit recolonization by vigorous heather.

Individuals of both "sensitive" and "less sensitive" tree species which have been unsuccessful on the *Calluna* heathland seem to be characterized by lack of development of characteristic ectotrophic mycorrhizal associations.

It must be remembered that the factor or factors responsible for the effect of *Calluna* on spruce and other plant species seem likely to be markedly modified by a change in circumstances.

The situation regarding the problem of the stagnation of the growth of spruce and other species in the presence of *Calluna* would still seem to be well summed up by Müller's (1897) verdict: "Vi haar her lige overfor et x, en ubekendt, om en fysisk, en kemisk eller en jordbundsfysiologisk Faktor vilde det vore af største Vigtighed normere at undersøge*."

However, no matter what the function of characteristic ectotrophic mycorrhizal associations may be with regard to tree growth; the formation of such associations appears to be associated with more satisfactory tree growth and recovery of trees from the influence of *Calluna* on *Calluna* heathlands. Therefore if the basis of this lack of development of mycorrhizal associations can be ascertained it may be of assistance in understanding the failure of spruce and other species to grow in the presence of living *Calluna* and the ability of other tree species to compete successfully with returning *Calluna*.

Such information should help towards a better understanding of the ecological problems associated with the colonization of *Calluna* heathlands by trees and may be of assistance in forestry practice in such areas.

An attempt has therefore been made to inquire into the reasons for the lack of development of characteristic ectotrophic mycorrhizal associations on the roots of trees whose growth has been checked by the presence of *Calluna*.

*"Here we come up against an *x*, an unknown. It would be of the greatest importance to examine more closely whether this is a physical, a chemical, or a soil physiological factor."

Chapter 1

FACTORS AFFECTING THE FORMATION OF ECTOTROPHIC MYCORRHIZAL ASSOCIATIONS

Ectotrophic mycorrhizal associations are close associations between very different kinds of organisms. If such associations are to be formed on a particular site the higher plant must be able to remain alive on the site and must be in an appropriate condition to be infected by the fungus and in a condition to promote the formation of an association with the fungus. The fungus must be able to persist on the site sufficiently long for it to be able to reach or promote conditions enabling it to form an association with the higher plant. Although details of the processes leading to the formation of such associations are not known it is probable that such an intimate relationship between two different kinds of organisms will be influenced by a variety of factors acting on the activities of either or both the organisms concerned; the different factors varying in importance according to circumstances.

In order to approach the more specific problem of the factors controlling the formation of ectotrophic mycorrhizal associations in trees growing on *Calluna* heathland sites, information concerning the factors influencing the formation of ectotrophic mycorrhizal associations generally will be considered as well.

The problem of trying to ascertain the factors influencing the formation of ectotrophic mycorrhizal associations has been approached in a number of ways.

A broad approach to the problem has been made by various investigators by noting the variations in the abundance of mycorrhizal associations developed by trees growing in different kinds of soil.

This quite naturally led to the consideration of the possible influence of mineral nutrients in the soil on the formation of mycorrhizal associations; for example, the influence of availability of mineral nutrients in the soil on internal conditions in the host and on the production of short roots and root exudate materials by the host.

Much effort has been expended in ascertaining the nutritional characteristics and metabolic activities of mycorrhizal fungi and the influence of various factors of the soil environment on their nutrition and metabolic activities.

In recent times there has been increasing awareness of the possible interactions between various microorganisms of the soil environment and the mycorrhizal fungi, and between mycorrhizal fungi and plant roots.

(i) Variations in the abundance of mycorrhizal associations in different soils

Lack of ectotrophic mycorrhizal associations is not only to be found in trees moribund or growing poorly on *Calluna* heathland soils. Indeed, soon after the nature of the association had been established, variations in the occurrence of such associations in the same tree species when grown on different kinds of soil were apparently the cause of conflicting reports on their occurrence in various species.

Early observations indicated that a tree species forming abundant ectotrophic mycorrhizal associations under forest conditions was frequently devoid of such associations when grown in nurseries or botanic gardens and on agricultural soils. Subsequently more detailed observations on the differential distribution of ectotrophic mycorrhizal associations were made and the general trend of such observations is well illustrated by the findings of Melin (1923a, 1925) that mycorrhizal associations are poorly developed on weakly acid or neutral soils such as the hardwood meadows of Central Sweden and the coniferous forests rich in herbs growing on soil containing much lime and having a reaction of pH 6–7. Atypical mycorrhizal associations occur, but only sporadically, on the roots of pine and spruce in such forests. It may be important from the present point of view that in such soils groups of ectotrophic mycorrhizal associations often develop on roots growing in pieces of decayed wood or bark. On the other hand Tansley (1939) described how the slender ramifying rootlets of beech densely fill the shallow limestone soils, which have a reaction of pH 77·2–8·4 and contain free Ca CO_3, and form abundant mycorrhizal associations, although it may be that these associations are formed by a rather different type of fungus compared with those concerned in the formation of Melin's A and B type associations in pine and spruce.

Although Melin (1925) asserts that ectotrophic

mycorrhizal associations are abundant on the roots of pine and spruce in non-calcareous mull soils, Björkman (1949) apparently considers that these are not characteristic associations but merely the hyphae of mycorrhizal fungi enveloping short roots without forming characteristic associations, even though Björkman (1942) himself describes young spruce plants growing on non-calcareous beech mull and having up to 96 per cent of their short roots as A and B type mycorrhizal associations with Hartig nets; also in the case of experimental pine and spruce plants growing on apparently non-calcareous mull soils, Björkman (1940) obtained high percentages of short roots forming A and B type mycorrhizal associations.

Melin (1925) states that ectotrophic mycorrhizal associations reach optimum development in coniferous forests with so-called "good" or "active" raw humus. In these forests the forest floor is rich in moss and samples of the raw humus show considerable nitrogen mobilization on incubation. In the case of pine heaths and forests of slow-growing old spruces with "inactive" raw humus, i.e. raw humus which shows little, if any, tendency for nitrogen mobilization when treated with lime or in samples removed and incubated, mycorrhizal associations are only formed sparingly and as previously mentioned the ground vegetation of the pine heaths is predominantly *Calluna vulgaris*.

Björkman (1942) divided ectotrophic mycorrhizal associations into a number of morphological types which he designated A, B, C, D and pseudomycorrhizal types. As far as is known at present the A, B and C types of mycorrhizal association are formed mainly by association between tree roots and certain Hymenomycetes and Gasteromycetes such as species of *Boletus*, *Tricholoma*, *Rhizopogon*, *Amanita*, etc. Type D are black mycorrhizal associations and the pseudomycorrhizal type are associations formed with *M.r. atrovirens*. He examined the completely undamaged root systems of 20–100 small pine and spruce plants and parts of the root systems of older trees from a number of different kinds of forest stand, and determined the number of short roots which had formed one or other of the various types of mycorrhizal association. One or more of the various types of mycorrhizal association may be formed by different short roots of one and the same plant.

Although the more general observations such as those of Melin (1925) and the more detailed observations of Björkman (1942) indicate that the formation of mycorrhizal associations varies under different conditions, it is not possible to ascertain from these observations how the formation of mycorrhizal associations is controlled in different types of forest stand; one must agree with Björkman's conclusion that the factors governing the formation of mycorrhizal associations cannot be established by observations in nature alone. It is probable that a number of factors are involved and that their relative importance varies under different conditions. Observations on the occurrence of mycorrhizal associations in the field do however suggest that there are some factors which have an overriding influence, e.g. calcareous mull soils and *Calluna* growing on base-deficient soils; but even in these cases the unfavourable factor or factors can be overcome locally by the presence of fairly substantial pieces of decaying woody tissue. Similarly if there is any significance in the differential distribution of A, B and C type mycorrhizal associations on the one hand, and D and pseudomycorrhizal associations on the other, representatives of the two groups occurring on one and the same plant, it would seem to be necessary that some factor or factors having a discontinuous and rather localized distribution in either the soil or the root must be exerting a controlling influence.

It is not surprising therefore that attempts have been made to carry out experiments to elucidate the factors controlling the formation of ectotrophic mycorrhizal associations.

(ii) **Experimental investigations into the effect of level of mineral nutrients on the formation of mycorrhizal associations**

Early observations on the occurrence of ectotrophic mycorrhizal associations led to the opinion that they were only formed in soils having a high organic matter content. Wider experience has shown that this is not the case and that associations are to be found in sandy soils containing very small amounts of organic matter. In addition, as mentioned previously, they may be absent from the roots of trees growing in the highly organic raw humus layers of *Calluna* heath.

As the observations on the occurrence of ectotrophic mycorrhizal associations in different kinds of soil increased in number, the idea developed that the level of available nutrients in a soil was the factor determining the formation of these associations. In 1937 Hatch put forward a schematic diagram to illustrate the relationship between the development of ectotrophic mycorrhizal associations in various habitats and the nutrient availability in the soils of those habitats (see Fig. 1 overleaf). This might be taken to imply that the absence of ectotrophic mycorrhizal associations in trees growing on heathlands is due to inadequate supplies of nutrients and especially mineralized nitrogen.

The view that the level of available nutrients in the soil controls the formation of ectotrophic mycorrhizal associations is now widely held. However, since ectotrophic mycorrhizal associations do not seem to be developed by trees whose growth is checked in

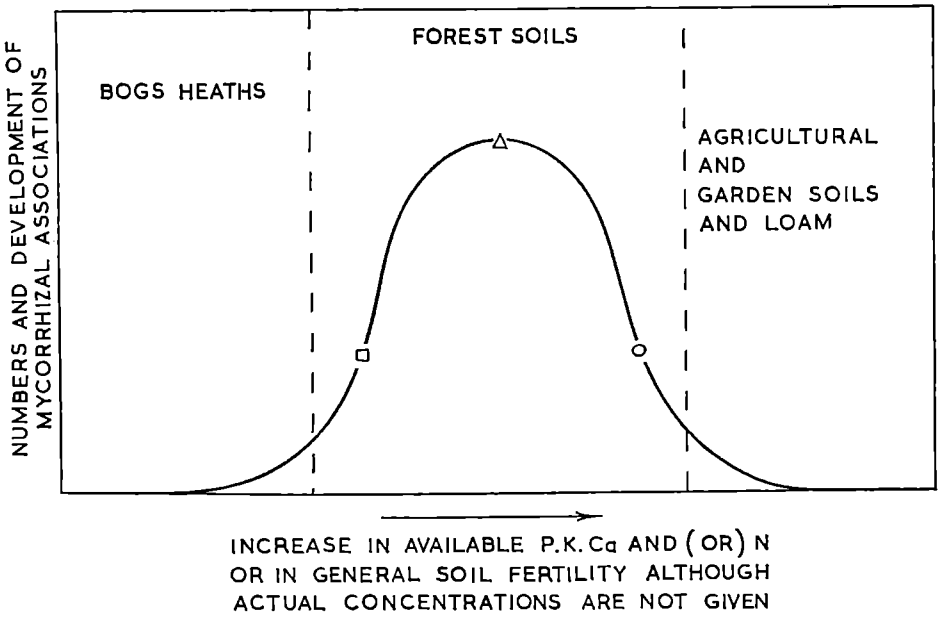

Fig. 1. Schematic Diagram to illustrate the Development of Ectotrophic Mycorrhizal Associations in Various Habitats and Soils. (After Hatch 1937).

the presence of living *Calluna*, whilst such associations are well developed when the trees are released from check by suppression of the heather, even though they are growing in competition with individuals of the same or another tree species, suggests that the evidence for this view should be more closely scrutinized.

A number of attempts have been made to investigate experimentally the influence of mineral nutrients on the formation of mycorrhizal associations although the determination of available soil nutrients presents great difficulties.

Hesselman (1927) and Melin (1927) grew pine seedlings in mixtures of sand and raw humus from four different forest sites having the following characteristics,

I. Open pine heath difficult to regenerate,
II. Regeneration group beneath older pines on pine heath,
III. Old, slow-growing and lichen-covered spruce forest,
IV. From site of old slow-growing and lichen-covered spruce forest clear felled 14 years previously.

The plots were watered either with distilled water or freshly prepared aqueous extract of fresh raw humus. After three growing seasons the pine plants were examined for development of mycorrhizal associations and nitrogen mobilization in the soils was determined by chemical analyses of samples, which had been incubated for three months, for NH_4 and NO_3 content. Analyses of similarly treated samples were made in the autumn of the first and second growing seasons; the three values for each type of raw humus are taken as a measure of nitrogen mobilization in the experimental sand-raw humus mixtures over the three year experimental period. The results for the nitrogen analyses and the development of mycorrhizal associations are given in Table I which has been prepared from the results of Hesselman and Melin.

The raw humus from site IV yielded comparatively large amount of NH_4 and NO_3 nitrogen even at the end of the third growing season, whilst in the case of the raw humus from the other sites the amounts of mobilized nitrogen diminished each year and were very small in the third year. Similar trends were shown whether watering was carried out with distilled water or with raw humus extract. The nitrogen contents of the pine plants were apparently not determined so it is not possible to ascertain whether the nitrogen absorbed by the plants was related to the assessments of nitrogen mobilization in the soils. It should be remembered, however, that the assessments of nitrogen mobilization were made each year after the pine seedlings had absorbed previously mobilized nitrogen for their growth, and that the amount of nitrogen mobilized under the experimental conditions is likely to be greater than in the field because of the effects of the unavoidable disturbance

TABLE I

Soil ammonia and nitrate nitrogen and the formation of ectotrophic mycorrhizal associations from the results of Hesselman (1927) and Melin (1927).

Site from which humus was collected	*Whether watered with humus extract or distilled water*	*Growing season*	*NH_4 and NO_3 nitrogen mobilized in incubation expts. mg/kg.*	*Seedling number*	*Mycorrhizal associations present at the end of the experimental period*									
					Type A		*Type B*		*Type C*		*Type D*		*Pseudomycorrhiza*	
					Number	*as percentage of short roots*	*Number*	*as percentage of short roots*	*Number*	*as percentage of short roots*	*Number*	*as percentage of short roots*	*Number*	*as percentage of short roots*
I	water	1922	10·9	1	15	5	41	15	2	1	0	0	212	79
		1923	9·7	2	0	0	10	4	12	5	0	0	227	91
		1924	0·4											
	extract	1922	19·7	1	3	1	31	8	1	<1	6	2	336	89
		1924	1·5	2	8	3·5	32	13·5	12	5	3	1	184	79
				3	82	31	13	5	2	1	1	<1	165	63
II	water	1922	35·8	1	1	<1	29	9	3	1	94	29	200	61
		1923	15·7	2	0	0	8	5	1	1	36	21	123	73
		1924	0·2											
	extract	1922	45·1	1	21	10*	32	16	1	<1	33	16	114	57
		1924	1·0	2	8	0	26	15	3	2	47	27	88	51
III	water	1922	31·7	1	1	1	9	6·5	5	3·5	24	17	99	72
		1923	28·3	2	22	18	38	32	1	1	6	5	53	44
		1924	8·8											
	extract	1922	38·8	1	4	3	13	9	7	5	53	37	65	46
		1924	0·8	2	22	14	18	11·5	4	3	21	13·5	90	58
				3	59	20	3	1	0	0	68	23	162	56
IV	water	1922	51·3	1	294	96	0	0	0	0	4	1	10	3
		1923	30·1	2	419	87	0	0	0	0	31	6	33	7
		1924	46·4	3	601	96	0	0	0	0	13	2	12	2
	extract	1922	73·4	1	388	96	0	0	0	0	15	4	0	0
		1923	48·5	2	556	97	0	0	0	0	12	2	5	1
		1924	47·2											

*This would appear to be an error but it is as it appears in Melin's paper.

which takes place when the samples are obtained and which will result in the death of severed roots and other changes. The total mobilization of nitrogen over three years, as represented by the measurements made, was greater in the case of humus IV than for humus I, II and III, but this is largely because the amount of nitrogen mobilized in humus IV remains more or less constant whilst in the case of humus I, II and III there is diminution with time and especially in the third year. In the first year the level of nitrogen mobilization in II and III was of the same order as for all the years for humus IV. Melin examined the roots of the pine plants at the end of the experiment and assessed the numbers of various types (A, B, C, D, E, F and pseudomycorrhizal (characterized by intracellular fungal infection)) of mycorrhizal associations which had developed. Although the significance of the formation of pseudomycorrhizal associations as compared with the formation of types A–F is not thoroughly understood, it was found that predominantly pseudomycorrhizal associations had been formed at the end of three years, when the mobilized nitrogen (as measured) was very low, in the case of humus samples I, II and III, whereas in humus sample IV mycorrhizal associations were formed in similar numbers but predominantly of the A type. This could be taken to indicate the influence of nitrogen mobilization on the proportion of short roots which become type A mycorrhizal associations. If, as seems likely, however, mycorrhizal associations are largely formed anew each year (Melin 1923 and Robertson 1954) it would have been more indicative that other factors were not involved if it had been possible to examine plants at the end of the first and second years, when presumably there might have been a marked difference in the type of mycorrhizal association formed, compared with the third year, if as the findings indicate the amount of mobilized nitrogen decreased in the case of humus samples I, II and III.

In 1930 Gast (1937) carried out a similar experiment but in this case the seedlings were grown under a range of light intensities and their nitrogen contents were determined. *Pinus sylvestris* seedlings were grown in sand mixed with samples of raw humus from different sources and showing varying levels of nitrogen mobilization after incubation for three months. The sources from which the raw humus samples were obtained were:

1. An undisturbed overmature slow growing spruce forest of the *Hylocomium*-rich *Vaccinium myrtillus* type.
2. A similar site adjacent to (1) but which had been clear-cut and burned in 1927—F layer burned but H layer unaffected.
3. A rapidly growing mixed stand of spruce, pine and birch developing after burning.
4. An area on which Scots pine had been clear felled five years previously and at the time of sampling had a herbaceous flora and the soil was said to be well on the way to becoming a mull.

The assessment of the numbers of mycorrhizal associations which had developed at the end of the experiment gives no indication as to whether different types of mycorrhizal associations were developed or not. The results obtained are shown in Table 2 derived from Gast (1937) and Hatch (1937).

Although the precise mathematical relationships may not be clear certain trends seem to emerge from these results. It is necessary to assume that nitrogen mobilization and availability will be similar in the same sand-raw humus mixtures when exposed to different intensities of radiation. As the radiation intensity increases there is a tendency for the seedling dry weight, the amount of nitrogen absorbed by the seedlings, the number of short roots per seedling and the number of short roots per seedling which develop into mycorrhizal associations, to increase. Bearing in mind that there is no differentiation of the various types of mycorrhizal association there is no marked trend in the proportion of short roots which form mycorrhizal associations as radiation intensity increases. It may be deduced from these results that for a given light intensity one may expect the number of short roots forming mycorrhizal associations to tend to increase as the nitrogen concentration in the seedlings increases and also as the mobilized nitrogen in the soil, as measured in this experiment, increases.

In 1932 Hatch (1937) grew seedlings of *Pinus strobus* on mixtures of sand and mineral soil from the upper mineral horizons of four soils, suspected to be of differing fertility for tree growth, supporting different types of hardwood stand. The soil samples were kept moist until placed in the pots except in the case of one (Mid-slope (poor)) which became thoroughly air dried. The nitrogen status of the four soils was determined by direct chemical analysis (Mitchell 1934) and found to be:

NH_4+NO_3 as mg. nitrogen per kg. of soil on an air-dry basis:

Cove soil	Mid slope (good) soil	Mid slope (poor) soil	Ridge soil
484	365	362	308

Gast (1937) considers that nitrogen mobilization values, as determined in storage tests, do not provide a measure of available nitrogen in humus-sand cultures comparable with the concentration of free ions in nutrient solution sand cultures and Mitchell (1934) also states that there is little reason to suppose that there is necessarily any relation between the amounts of nutrients extracted from soils by chemical reagents in the laboratory and the amounts which can be taken up by plants, since such reagents do not

TABLE II

The effect of variation in light intensity on the development of mycorrhizal associations by Pine seedlings grown in raw humus from various sources. After Gast (1937) and Hatch (1937)

Humus sample	*NH_4 and NO_3 nitrogen mobilized in the humus-sand mixtures in incubation tests. mg./kg.*	*Dry weight of seedlings in mg.*	*Nitrogen content of seedlings as a percentage of dry weight*	*Nitrogen content of seedlings in mg.*	*Number of short roots*	*Number of mycorrhizal short roots per seedling*	*Mycorrhizal short roots as a percentage of total short roots per seedling*
				11% radiation			
1	1·3	13·7	1·57	0·215	83	29	35
2	46·2	16·1	2·01	0·324	102	79	77
3	49·6	19·0	2·36	0·448	70	38	55
4	75·4	18·4	2·68	0·493	73	47	64
				22% radiation			
1	1·3	21·4	1·12	0–240	195	68	35
2	46·2	29·6	2·16	0·639	195	165	84
3	49·6	48·2	2·52	1·315	293	258	88
4	75·4	36·4	2·89	1·052	196	163	83
				27% radiation (infra red removed)			
1	1·3	26·6	0·97	0·258	233	64	27
2	46·2	36·2	2·28	0·825	264	221	84
3	49·6	48·5	2·54	1·232	323	293	91
4	75·4	50·7	3·28	1·663	377	305	81
				50% radiation			
1	1·3	29·9	1·12	0·335	290	110	38
2	46·2	42·4	2·47	1·047	377	326	86
3	49·6	54·8	1·96	1·074	584	513	88
4	75·4	85·6	2·93	2·508	406	256	63

necessarily have extractive powers corresponding to those of plant roots. From sand culture experiments Mitchell deduced that the optimum nitrogen concentration for White pine seedlings is of the order of 300 p.p.m. and that dry weight production and nitrogen contents of the seedlings are proportional to the nitrogen concentration of the nutrient solution. From the dry weights and nitrogen contents of seedlings grown on the four soils, in the presence of added nutrients other than nitrogen, it was accordingly deduced that the available nitrogen contents of the soils in mg. per kg. were:

Cove	*Mid slope (good)*	*Mid slope (poor)*	*Ridge*
72·37	40·54	44·87	29·71

Mitchell considers that it is doubtful if any natural soil could supply the optimum nitrogen concentration of 300 mg. per kg. indicated by sand cultures.

In some cases nutrient solutions were added to the soils which resulted in additional amounts of 96·8 and 193·6 mg. of nitrogen, in the form of $NH_4 NO_3$, being added per litre of nutrient solution used. This means, according to Hatch, that 276 and 553 mg. of nitrogen were added per pot but it does not seem possible to deduce the concentration per litre or per kilogram of soil. The duration of the growing period is not clear and the mycorrhizal associations which developed were not separated into different types although it is stated that in the case of the Mid slope (poor) soil they were almost entirely of the *M.r. nigrostrigosum* type. The illustrations of the mycorrhizal associations formed in the other soils indicate that they may well have been characteristic ectotrophic types. This lack of information regarding the type(s) of mycorrhizal associations formed increases the difficulty of interpreting Hatch's results. It would seem however that addition of nitrogen to the Ridge soil, which would appear to contain relatively small amounts of mineralizable nitrogen compared with the other soils used, does not change the proportion of short roots forming mycorrhizal associations, presumably of the *M.r. nigrostrigosum* type, and there is perhaps a very small decrease in the number of mycorrhizal points and an increase in nitrogen concentration in the seedlings. In the case of the Cove soil, which apparently contains the largest amounts of mobilizable nitrogen, addition of nitrogen causes a decrease in the proportion of short roots becoming mycorrhizal associations, and in the numbers of mycorrhizal points, whilst at the same time the nitrogen concentration in the seedlings increases. As Hatch points out this disagrees with his own previous findings for the influence of external nitrogen concentration on the formation of mycorrhizal associations and also with the findings of Melin (1927), but it would seem that in this experiment the concentrations of ammonia and nitrate in the soils where the formation of mycorrhizal associations is decreased are likely to be very considerably greater than in his own previous experiment or that of Hesselman (1927) and Melin (1927).

From a single pot Hatch selected fifteen seedlings which differed widely in their development of mycorrhizal associations and determined their nitrogen and percentage nitrogen contents on a dry weight basis. He obtained a direct relationship between the percentage nitrogen content of the seedlings and the number of mycorrhizal associations developed which could be expressed as Nitrogen concentration (as percentage of dry weight) $= 0{\cdot}00164 \times$ Number of mycorrhizal points $+ 1{\cdot}31$. That the data are too few to be conclusive was pointed out by Hatch as was also the fact that his results for development of mycorrhizal associations and nitrogen concentration in similar seedlings grown in the various soils under various conditions cannot be considered to be in agreement with the results obtained for the fifteen seedlings from a single pot.

Despite the lack of agreement between the results of his various experiments Hatch concludes from his results as a whole that the abundance of mycorrhizal associations on the roots of pine seedlings is determined in normal forest soils by the availability of mineral salts and that mycorrhizal associations are produced in abundance under conditions of low availability of any one or more of the four elements —nitrogen, phosphorus, potassium and calcium or when there is lack of balance in the availability of these nutrients. Further, that in relatively poor soils, as used by himself (Hatch 1937, Gast 1937), Melin (1927) and other European investigators, the percentage of short roots which become mycorrhizal associations increases with increase in available nitrogen, whilst in soils richer in nutrients development of mycorrhizal associations decreases with increasing individual availability of P, K, Ca and N.

The effect of watering pine and spruce seedlings, growing in various soils, with ammonium nitrate solutions, whose concentrations formed a geometric series, on the formation of mycorrhizal associations was investigated by Björkman (1940). With one exception the soils were of the mull type and had reactions varying between pH 4·5 and pH 7·5. The ammonia nitrogen and nitrate nitrogen contents of the soils were determined at the start of the experiment when they were found to vary from 0·5 to 7 mg. per litre of soil for ammonia nitrogen and from 0 to 21 mg. per litre of soil for nitrate nitrogen. The nitrogen mobilized as nitrate in the various soils during storage for three months varied from 6 to 86 mg. per litre of soil and the amounts of nitrogen added as ammonium nitrate varied from 82 to 2579 mg. per litre of soil. The ammonia and nitrate

nitrogen remaining in the soils at the time of harvesting was determined and although in many cases these values showed decreases compared with the nitrogen present at the start of the experiment, the concentrations remaining are still very high in many cases. It seems probable that the higher concentrations of added nitrogen are likely to give rise to concentrations of available nitrogen which may well be a number of times greater than those likely to occur naturally even in the richest soils. There was little, if any, effect on the numbers and percentages of the roots forming A and B type mycorrhizal associations when up to 250–300 mg. of nitrogen as ammonium nitrate, even more in the case of spruce mull, were added per litre of soil; this applies in the case of all the soils and it is noteworthy that the wide variation in reaction of the soils, as indicated by pH measurements, does not appear to influence the formation of mycorrhizal associations. There was some depression in the numbers of short roots and in the percentage forming A and B type mycorrhizal associations at the higher rates of addition of ammonium nitrate, but even in these cases appreciable numbers of A and B type mycorrhizal associations were formed. The results of these experiments indicate that the highest concentrations of ammonia and nitrate nitrogen likely to occur naturally in soils are unlikely by themselves to be the cause of lack of development of A and B type mycorrhizal associations. There was no indication of any significant influence of changes in ammonia and nitrate nitrogen concentration on the formation of A and B type mycorrhizal associations over the lower part of the range. The results have been collected together in Table III overleaf.

Subsequently Björkman (1942), in experiments specifically designed to test Hatch's conclusions, grew pine seedlings for one growing season in mixtures of sand and two kinds of raw-humus and one kind of mull, the ammonia and nitrate nitrogen contents of the mixtures being determined initially and after storage for three months, to which nutrient solutions were added at intervals during the growing period. Nitrogen where added was at the rate of 180 mg. of nitrogen, as NH_4NO_3, to each litre of soil during the growing period. The easily soluble ammonia and nitrate nitrogen remaining in the sand mixtures at the end of the experiment was determined and the percentage of short roots which had become A and B type mycorrhizal associations was ascertained. The results are collected together in Table IV on page 17.

(*Text resumes on page* 18)

TABLE III

The influence of added nitrogen on the formation of mycorrhizal associations by pine and spruce growing in various soils (from Björkman 1940)

	Oakwood mull pH 4·9		*Spruce wood mull pH 4·5*			*Alder wood mull pH 4·8*			*Beech wood mull pH 6·0*			*Garden soil pH 7·3–7·5*			*Rawhumus + sand pH 4·9*				
	N_0	N_3	N_0	N_3	N_9	N_0	N_3	N_{27}	N_0	N_3	N_9*	N_0	N_1	N_3	N_0	N_1	N_3	N_9	N_{27}
Inherent ammonia nitrogen in soil at beginning of expt. in mgm/litre	5	5	7	7	7	2	2	2	0·5	0·5	0·5	1	1	1	2	2	2	2	2
Inherent nitrate nitrogen in soil at beginning expt. in mgm/litre	20	20	15	15	15	21	21	21	6	6	6	7	7	7	0	0	0	0	0
Nitrate nitrogen in soil after 3 months storage mgm/litre	86	86	84	84	84	69	69	69	30	30	30	20	20	20	6	6	6	6	6
Nitrogen added as ammonium nitrate mgm/litre of soil	—	221	—	240	721	—	287	2579	—	255	—	—	82	247	—	92	277	831	2492
Ammonia nitrogen soil at end of expt. mgm/litre	5	22	5	41	218	4	14	493	4	4	—	4	2	1	4	10	21	44	325
Nitrate nitrogen in soil at end of expt. mgm/litre	29	127	27	119	292	3	153	554	10	66	—	92	25	54	0·3	23	59	98	260
Ammonia + nitrate nitrogen in soil at end of expt. mgm/l	34	149	32	160	510	7	167	1047	14	70	—	94	27	55	4·3	33	80	142	585
Percentage of short roots which have become A and B type mycorrhizal association (Pine)	88	71	85	91	83	67	62	25	85	74	37	68	73	84	78	79	62	44	22
Percentage of short roots which have become pseudomycorrhizal assoc.	10	27	15	9	17	33	38	75	13	16	53	32	27	16	15	15	34	54	76
Percentage of short roots which have become A and B type myc. assoc. (Spruce)	78	68	90	92	85	70	57	37	68	59	53	57	68	79	65	68	74	60	26
Percentage of short roots which have become pseudomycorrhizal assoc.	21	26	10	8	15	30	43	63	20	23	36	43	32	21	28	25	20	35	72

*Note** = 2 year seedlings

TABLE IV

The effect of added mineral nutrients on the formation of ectotrophic mycorrhizal associations, from Björkman (1942)

	Raw humus (D) from mixed coniferous forest of pure 'Hylocomium' type				*Raw humus (K) from moss rich spruce forest of 'Vaccinium' type*				*Mull (M) from herb-rich beech forest*			
Ammonia nitrogen inherent in soil mgm/litre	6				3·5				10			
Nitrate nitrogen inherent in soil mgm/litre	2·5				3·0				2·5			
Ammonia nitrogen formed during 3 months storage mgm/litre	64				3·5				10			
Nitrate nitrogen formed during 3 months storage mgm/litre	6				3·0				28			
Ammonia nitrate and ammonia + nitrate nitrogen remaining in soil (mgm/litre) at end of experiment and percentage of short roots forming A and B type mycorrhizal associations when soil was watered with:	*Nitrate nitrogen*	*Ammonia nitrogen*	*Nitrate + ammonia nitrogen*	*Percentage A + B mycorrhizal assoc.*	*Nitrate nitrogen*	*Ammonia nitrogen*	*Nitrate + ammonia nitrogen*	*Percentage A + B mycorrhizal assoc.*	*Nitrate nitrogen*	*Ammonia nitrogen*	*Nitrate + ammonia nitrogen*	*Percentage A + B mycorrhizal Assoc.*
Distilled water	6	18	24	35	2	18	20	50	30	8	38	20
Nutrient solution B	111	157	268	1	110	150	260	6	237	105	342	1
,, ,, –N	6	18	24	28	2	18	20	76	10	10	20	25
,, ,, –P	110	145	255	17	125	150	275	12	242	120	362	1
,, ,, –N–P	8	20	28	40	3	17	20	75	16	6	22	30
,, ,, –K	112	180	292	1	165	217	382	2	210	135	345	1
,, ,, –Ca	110	210	320	1	125	175	300	2	233	105	338	1

Unlike the previous experiment, it would appear that in this case addition of nitrogen in the form of ammonium nitrate has stimulated the mineralization of soil nitrogen so that at the end of the experiment there has been a gain in mineralized nitrogen instead of a loss. Wherever ammonium nitrate was added, and especially when it was added along with phosphorus, the percentage of short roots forming A and B type mycorrhizal associations was severely diminished. By comparison with the previous experiment the pronounced reduction in the percentage of short roots forming A and B type mycorrhizal associations when ammonium nitrate is added seems to occur at lower concentrations of added ammonium nitrate when phosphorus is also added. It is perhaps also significant that in those cases where nitrogen and phosphorus were not added, and also where only distilled water was used for watering, the levels of nitrate and ammonia in the soil, as determined, were very similar to those in the untreated mull soils of the previous experiment yet the percentages of short roots forming A and B type mycorrhizal associations did not reach anything like the percentages attained in the previous experiment except in the case of raw humus K.

Björkman (1942) extended this line of enquiry in 1941 when he grew pine seedlings on raw humus from two different sources. (Raw Humus K from a moss-rich spruce forest of *Vaccinium* type and Torf H, a peat from a drained bog on which *Andromeda polifolia* was growing) to which were added varying combinations of various amounts of NH_4NO_3 and H_3PO_4. He determined the mineralized nitrogen present in the unamended soils initially and after storage for three months; at the end of the experiment the residual mineralized nitrogen in the soil of the experimental pots was determined. The NH_4 and NO_3 nitrogen in the soils was determined by the method of Olsen. Easily soluble phosphate remaining in the soils at the end of the experiment was determined by the method of Egner. The numbers of the various types of mycorrhizal association were ascertained and the dry weights and the nitrogen and phosphorus contents of roots and shoots were determined separately. Some of these results and the results from some calculations made using Björkman's data are given in Tables V and VI, pp. 19–20.

There is insufficient information available to ascertain whether there are any relationships between the percentage of short roots forming A and B type mycorrhizal associations and the concentrations of ammonia and nitrate nitrogen and easily soluble phosphorus in the soil. In terms of the amounts of nitrogen and phosphorus *added to the soils*, however, there is a tendency for the percentage of A and B type mycorrhizal associations formed to diminish, especially when nitrogen and phosphorus are added together, although there seems to be little correlation between the amounts of nitrogen and phosphorus added and the amounts recovered from the soil at the end of the experiment. This could be due to a number of reasons but, if the mineralized nitrogen and easily soluble phosphate, as determined, remaining in the soils at the end of the experiment are related to the formation of A and B type mycorrhizal relationships, it is not easy to see *how*, either in terms of actual levels of nitrogen and phosphorus or ratios of nitrogen and phosphorus.

The difficulty of relating the concentrations of mineralized nitrogen found in a soil by various methods to the concentration which influences the activities of roots and microorganisms growing in the soil is well known. In view of this it may be useful to compare the results of the above experiments in which soils were used with those obtained by Björkman (1942) in pure culture experiments. In 1939 he grew pine seedlings under aseptic conditions in culture solutions containing varying known amounts of nitrogen and phosphorus and other mineral nutrients and at two concentrations of glucose. Under these conditions the initial physiologically effective concentrations of N and P are presumably known more certainly. Some of the flasks were inoculated with *Rhizopogon roseolus*, which forms mycorrhizal associations with *Pinus sylvestris*, whilst similar flasks remained uninoculated. At the end of the experiment the percentage of short roots which had formed A and B type mycorrhizal associations was ascertained. The results obtained by Björkman will be found in Table VII. Here again in the presence of high concentrations of mineral nitrogen and phosphorus the percentage of short roots forming A and B type mycorrhizal associations is markedly reduced. In the lowest range of the concentrations used there is also a reduction in the percentage of short roots forming A and B type mycorrhizal associations although some are still formed. The lowest concentration of nitrogen used was 2·4 mgms/litre although there were only 40 ml. of culture fluid per flask and therefore the total amount of nitrogen available would be very small and considerable changes in concentration must have taken place by the end of the experiment.

If it is assumed that levels of mineral nutrients of this order are influencing the formation of A and B type mycorrhizal associations the problem is how to relate the various determinations of NH_4 and NO_3 in soils and the corresponding percentage of A and B type mycorrhizal associations formed to these findings. For example if the concentrations of NH_4 and NO_3 found by Björkman to be present in the soils at the end of the experiment, when considerable absorption of NH_4 and NO_3 may have taken place, be used, then in the case of P_1, P_2 and P_4 of the

TABLE V

Nutrients added to raw humus "K", ammonia and nitrate nitrogen and easily soluble phosphorus extractable from raw humus "K" at beginning and end of the experiment, nitrogen and phosphorus contents of plants, and percentages of short roots forming A and B mycorrhizal associations and pseudemycorrhizal associations. From Björkman's (1942) results

Ammonia nitrogen extractable from soil initially 5 mgm/litre of soil
Nitrate nitrogen extractable from soil initially 2 mgm/litre of soil
Ammonia nitrogen extractable from soil after 3 months storage 10 mgm/litre of soil
Nitrate nitrogen extractable from soil after 3 months storage 2 mgm/litre of soil

	Nitrogen in mgm. added as NH_4NO_3 to each 2 litre pot containing 25 plants	*Phosphorus in mgm. added as H_3PO_4 to each 2 litre pot containing 25 plants*	*Extractable from soil at end of experiment*			*Total nitrogen content of plant in mgm.*	*Total phosphorus content of plant in mgm.*	*Total nitrogen content / Total phosphorus content*	*Nitrogen content of root material in mgm.*	*Phosphorus content of root material in mgm.*	*Concentration of nitrogen in root material as a percentage of dry weight*	*Concentration of phosphorus in root material as a percentage of dry weight*	*Concentration of nitrogen in root material / Concentration of phosphorus in root material*	*Percentage of short roots forming A and B type mycorrhizal associations*	*Percentage of short roots forming pseudomycorrhizal associations*	*Concentration of easily soluble reducing substance in roots as percentage of glucose*
			Nitrate nitrogen mgm./litre of soil	*Ammonia nitrogen mgm./litre of soil*	*Easily soluble phosphorus as P_2O_5 mgm./kg. air dried soil*											
Distilled water	—	—	7	2	7·7	1·16	0·185	6·3	0·46	0·088	1·78	0·34	5·2	53	37	5·6
N_1	250	—	14	12	7·1	4·34	0·375	11·6	0·99	0·096	2·05	0·20	10·3	48	46	4·8
N_2	500	—	27	43	5·8	6·04	0·475	12·7	1·34	0·126	2·23	0·21	10·6	37	55	3·7
N_4	1,000	—	38	102	6	7·01	0·385	18·2	1·53	0·089	2·57	0·15	17·1	31	65	3·0
P_1	—	432	7	0	12	1·15	0·297	3·9	0·45	0·141	1·68	0·53	3·2	62	23	5·3
P_2	—	864	6	0	13	1·03	0·311	3·3	0·38	0·139	1·52	0·56	2·7	64	20	5·3
P_4	—	1,728	5	0	26	0·91	0·385	2·4	0·35	0·173	1·37	0·68	2·0	66	20	5·8
N_1P_1	250	432	10	2	10	4·45	0·789	5·6	1·11	0·254	2·23	0·51	4·4	40	50	3·7
N_1P_2	250	864	14	5	14	4·50	0·858	5·2	1·06	0·264	2·28	0·57	4·0	35	59	3·6
N_1P_4	250	1,728	14	6	30	4·12	0·941	4·4	0·92	0·279	2·18	0·66	3·3	28	68	3·7
N_2P_1	500	432	14	3	10	4·67	0·637	7·3	1·23	0·232	2·38	0·45	5·3	28	67	3·1
N_2P_2	500	864	33	2	23	4·61	0·696	6·6	1·19	0·243	2·34	0·48	4·9	21	74	3·4
N_2P_4	500	1,728	32	18	26	4·28	0·754	5·7	1·12	0·271	2·24	0·54	4·1	13	85	3·2
N_4P_1	1,000	432	75	70	11	4·78	0·468	10·2	1·16	0·154	2·78	0·37	7·5	16	84	2·8
N_4P_2	1,000	864	34	59	18	3·78	0·469	8·1	0·99	0·171	2·66	0·46	5·8	10	90	2·6
N_4P_4	1,000	1,728	75	45	41	3·36	0·523	6·4	0·91	0·170	2·57	0·48	5·4	5	95	2·3

TABLE VI

Nutrients added to torf H, ammonia and nitrate nitrogen and easily soluble phosphorus extractable from torf H, at beginning and end of the experiment, nitrogen and phosphorus content of plants and percentages of short roots forming A and B mycorrhizal associations and pseudomycorrhizal associations. From Björkman's (1942) results

Ammonia nitrogen extracted from soil initially 25 mgm/litre of soil
Nitrate nitrogen extracted from soil initially 2·5 mgm/litre of soil
Ammonia nitrogen extractable from soil after 3 months storage 50 mgm/litre of soil
Nitrate nitrogen extractable from soil after 3 months storage 5·0 mgm/litre of soil

	Nitrogen in mgm. added as NH_4NO_3 to each 2 litre pot containing 25 plants	*Phosphorus in mgm. added as H_3PO_4 to each 2 litre pot containing 25 plants*	*Extractable from soil at end of experiment*			*Total nitrogen content of plant in mgm.*	*Total phosphorus content of plant in mgm.*	*Total nitrogen content / Total phosphorus content*	*Nitrogen content of root material in mgm.*	*Phosphorus content of root material in mgm.*	*Concentration of nitrogen in root material as a percentage of dry weight*	*Concentration of phosphorus in root material as a percentage of dry weight*	*Concentration of nitrogen in root material / Concentration of phosphorus in root material*	*Percentage of short roots forming A and B type mycorrhizal associations*	*Percentage of short roots forming pseudomycorrhizal associations*	*Concentration of easily soluble reducing substance in roots as percentage of glucose*
			Nitrate nitrogen mgm./litre of soil	*Ammonia nitrogen mgm./litre of soil*	*Easily soluble phosphorus as P_2O_5 mgm./Kg. air dried soil*											
Distilled water	—	—	2	18	3·5	1·80	0·172	10·5	0·62	0·071	1·92	0·22	8·7	78	19	5·3
N_1	250	—	2	30	2·9	1·94	0·156	12·4	0·69	0·067	2·04	0·20	10·2	76	24	5·1
N_2	500	—	3	97	2·8	2·26	0·155	14·6	0·68	0·058	2·34	0·20	11·7	74	26	4·6
N_4	1,000	—	4	181	2·4	2·43	0·143	17·0	0·66	0·045	2·51	0·17	14·8	73	27	4·2
P_1	—	432	1	0	20	3·95	0·972	4·0	1·14	0·378	1·23	0·41	3·0	68	30	5·0
P_2	—	864	1	0	30	3·90	1·246	3·1	1·13	0·472	1·20	0·50	2·4	58	40	4·6
P_4	—	1,728	2	0	41	3·92	1·470	2·7	1·14	0·529	1·19	0·55	2·2	51	42	4·2
N_1P_1	250	432	3	29	17	7·71	1·269	6·1	1·57	0·381	1·81	0·44	4·1	18	80	3·1
N_1P_2	250	864	4	22	23	7·90	1·557	5·1	1·70	0·509	1·74	0·52	3·3	9	90	3·0
N_1P_4	250	1,728	5	22	36	8·49	1·789	4·7	1·67	0·552	1·76	0·58	3·0	5	91	2·7
N_2P_1	500	432	6	109	16	11·20	1·459	7·7	2·31	0·401	2·13	0·37	5·8	8	92	2·6
N_2P_2	500	864	8	67	19	11·24	1·604	7·0	2·37	0·495	2·15	0·45	4·8	3	97	2·3
N_2P_4	500	1,728	11	97	30	11·16	1·841	6·1	2·30	0·614	2·10	0·56	3·8	2	98	2·4
N_4P_1	1,000	432	8	192	16	12·81	1·275	10·0	2·87	0·341	2·52	0·30	8·4	3	97	2·3
N_4P_2	1,000	864	11	204	20	13·10	1·413	9·3	2·97	0·366	2·60	0·32	8·1	0	100	2·0
N_4P_4	1,000	1,728	15	135	30	12·71	1·858	6·8	2·95	0·511	2·48	0·43	5·8	0	100	2·1

TABLE VII

Björkman's (1942) pure culture experiment with Pinus sylvestris and Rhizöpogon roseolus. Nitrogen, phosphorus and glucose concentrations in culture solutions. Nitrogen and phosphorus contents of plants and root material and percentages of short roots forming A and B type mycorrhizal associations

	Nitrogen present in culture solution in mgm/litre	Phosphorus present in culture solution in mgm/litre	Nitrogen in culture solution / Phosphorus in culture solution	0·5% glucose									5·0% glucose							
				Total nitrogen content of plant in mgm	Total phosphorus content of plant in mgm	Nitrogen content of plant / Phosphorus content of plant	Nitrogen content of root material in mgm	Phosphorus content of root material in mgm	Concentration of nitrogen in root material	Concentration of phosphorus in root material	Concentration of nitrogen in root material / Concentration of phosphorus in root material	Percentage of short roots forming A and B type mycorrhizal associations	Total nitrogen content of plant in mgm.	Total phosphorus content of plant in mgm	Nitrogen content of plant / Phosphorus content of plant	Nitrogen content of root material in mgm	Phosphorus content of root material in mgm	Concentration of nitrogen in root material	Concentration of phosphorus in root material	Concentration of nitrogen in root material / Concentration of phosphorus in root material	Percentage of short roots forming A and B type mycorrhizal associations
N_0P_0	2·4	8·0	0·30	0·229	0·046	4·98	0·071	0·016	0·86	0·201	4·28	4	0·262	0·049	5·35	0·080	0·017	0·98	0·207	4·73	0
N_0P_1	2·4	173	0·13	0·293	0·070	4·19	0·079	0·031	1·35	0·171	2·59	8	0·272	0·069	3·94	0·081	0·028	1·34	0·195	2·92	4
N_0P_2	2·4	346	0·007	0·277	0·084	3·30	0·087	0·041	2·21	0·188	2·13	7	0·281	0·078	3·60	0·087	0·037	2·39	0·194	2·39	6
N_0P_4	2·4	692	0·003	0·312	0·109	2·86	0·094	0·062	2·87	0·200	1·52	7	0·318	0·126	2·52	0·094	0·059	3·10	0·204	1·61	8
N_1P_0	53	8·0	6·63	0·717	0·067	10·70	0·161	0·020	0·87	0·336	7·90	28	0·689	0·070	9·84	0·153	0·022	1·01	0·346	6·87	14
N_1P_1	53	173	0·31	0·778	0·108	7·20	0·190	0·042	1·58	0·346	4·57	32	0·801	0·126	6·36	0·206	0·050	1·47	0·358	4·11	30
N_1P_2	53	346	0·15	0·802	0·149	5·38	0·197	0·062	2·46	0·357	3·17	24	0·780	0·156	5·00	0·173	0·063	2·33	0·387	2·76	32
N_1P_4	53	692	0·08	0·858	0·196	4·38	0·207	0·098	2·97	0·359	2·11	22	0·706	0·220	3·21	0·193	0·092	3·25	0·388	2·10	33
N_3P_0	159	8·0	19·88	1·373	0·096	14·30	0·316	0·027	0·93	0·436	11·76	12	1·440	0·102	14·12	0·344	0·028	1·04	0·436	12·32	10
N_3P_1	159	173	0·92	1·714	0·186	9·22	0·325	0·047	1·43	0·451	6·89	14	1·895	0·223	8·50	0·415	0·069	1·26	0·457	6·02	40
N_3P_2	159	346	0·46	1·877	0·264	7·11	0·351	0·065	2·49	0·459	5·43	8	1·883	0·265	7·11	0·432	0·084	2·51	0·488	5·14	42
N_3P_4	159	692	0·23	1·785	0·335	5·33	0·317	0·089	2·92	0·481	3·59	0	1·730	0·318	5·44	0·385	0·110	3·18	0·475	3·50	35
N_5P_0	265	8·0	33·13	2·064	0·125	16·51	0·491	0·034	1·02	0·670	14·35	0	2·130	0·130	16·39	0·546	0·036	1·07	0·665	15·20	0
N_5P_1	265	173	1·53	2·375	0·270	8·80	0·466	0·056	1·48	0·702	8·27	5	2·760	0·262	10·53	0·614	0·073	1·38	0·657	8·38	18
N_5P_2	265	346	0·77	2·409	0·311	7·75	0·420	0·069	2·56	0·714	6·07	0	2·678	0·295	9·08	0·576	0·086	2·42	0·691	6·70	10
N_5P_4	265	692	0·38	2·165	0·401	5·40	0·453	0·106	3·08	0·723	4·26	0	2·319	0·431	5·38	0·484	0·104	3·34	0·715	4·67	0

Torf H series (Table VI) the order of concentrations is the same as that of the lowest concentration in the pure culture series yet very considerably higher percentages of A and B type mycorrhizal associations are formed in the Torf H series. The concentrations of NH_4 and NO_3 remaining at the end of the experiment in the other cases (pots watered with distilled water) where nitrogen was not added and the concentrations of NH_4 and NO_3 initially present in the soils, as determined and in the absence of storage, are all several times greater than the lowest concentration initially present in the pure culture experiment. Perhaps the most suitable concentration value available for comparative purposes is that of the NH_4 and NO_3 initially present in the soil, determined without storage, and it would appear that, in the case of both raw humus K and Torf H, the level of this concentration is considerably higher than that at which the development of types A and B mycorrhizal associations appears to be limited in pure culture experiments.

(iii) "Ecological" concentrations of NH_4 and NO_3 in soils

There have been a number of attempts to ascertain the concentration of NH_4 and NO_3 in various soils and these will be considered in relation to the above mentioned experimental findings of Björkman. The determinations have been made on aqueous extracts, or extracts obtained by the use of aqueous solutions of mineral acids and salts, of freshly collected samples of soil; but in some cases the results of determinations on stored samples are included for comparison. The soil moisture generally occupies only a fraction of the soil volume and therefore the concentrations of mineral nitrogen in the soil solution are likely to be considerably greater than those expressed on the basis of a litre of soil.

Investigations on old woodland soils, which had developed on different kinds of mineral material (base-rich and base-poor) and on which stands of various tree species (both conifer and hardwood) were growing, were carried out by Clarke (1924). Since the investigation was primarily intended to ascertain the influence of soil acidity on nitrate and ammonia production, the soils were divided into two groups according to reaction, i.e. pH 3·5–5 and pH 5–7. The samples were collected from the upper 3 inches of the mineral soil at intervals of a fortnight or three weeks from January to November and the amounts of NH_4 and NO_3, which could be extracted by water, determined within a few hours of collection. Bearing in mind the difficulties of the methods then available for the determination of ammonia and nitrate, Clarke's results indicate that in soils having a reaction of pH 3·5–5 the water soluble ammonia varied between 1 and 35 p.p.m. of soil heated for 24 hours at 100°C, whilst water soluble nitrate varied from 0–7 p.p.m.; the corresponding values for the soils of reaction pH 5–7 are 0–9 and 0–10 p.p.m. of dry soil respectively. The results also indicate considerable variation in the concentration of water-extractable ammonia in the case of soils with reaction pH 3·5–5 and in the concentration of nitrates, especially in the soils within the reaction range pH 5–7, with season, being low between about the middle of June and the middle of October. Even though expressed as mgms/kg of dry soil it is clear that these ammonia and nitrate concentrations are frequently considerably greater than those at which Björkman observed the formation of only small percentages of types A and B mycorrhizal associations in pure cultures.

A little later Aaltonen (1926) carried out an extensive investigation of the ammonia and nitrate contents of the soils of a range of forest types classified according to the method of Cajander. Samples from the surface organic layer and from the mineral soil beneath (0–5 cm depth) were taken at each site. Ammonia nitrogen (extractable with successive quantities of N/2 KCl) and nitrate nitrogen were determined immediately after sampling and also after storage of the samples for two months at a temperature of about 25°C. Samples were dried to constant weight at room temperature (about 20°C) and the moisture loss determined. Aaltonen's results for ammonia and nitrate extractable at the time of sampling and after incubation for two months are given in Tables VIII and IX. Although it is not known how the concentrations of mineralized nitrogen thus obtained are related to the corresponding physiologically active concentrations, and although considerable variations are encountered within a single forest type, it is perhaps of significance that even in those stands (CT) where *Calluna* is dominant in the ground flora and spruce is not recorded as being present, the concentrations of mineralized nitrogen extractable from the soils at the time of sampling are not characteristically different from those of the soils from other types of forest stand. If they are at all comparable with the concentrations in Björkman's pure cultures then they considerably exceed those at which a depression was observed in the percentage of A and B type mycorrhizal associations formed.

Similar conclusions may be drawn from the extensive observations of Glømme (1932). Although the soils having a preponderance of *Calluna* in the ground vegetation are in the lowest part of the range of levels of mobilized nitrogen content and may contain little or no nitrate and show little or no production of nitrate on incubation, they are not unique in this respect nor in the level of ammonia extractable initially or after incubation. As pointed out by Glømme, nitrogen mobilization seems to be more

TABLE VIII

Ammonia nitrogen extracted from soil samples from various kinds of forest stand during June to October, from Aaltonen (1926)

	Ammonia nitrogen extracted from humus layer				*Ammonia nitrogen extracted from mineral soil layer*					*Ammonia nitrogen extracted from humus layer after incubation*					*Ammonia nitrogen extracted from mineral soil layer after incubation*				
pH4·2	4·6	4·8	5·2	5·0	4·2	4·6	4·8	5·2	5·0	4·2	4·6	4·8	5·2	5·0	4·2	4·6	4·8	5·2	5·0
CT	VT	MT	OMT	OMAT	CT	VT	MT	OMT	OMAT	CT	VT	MT	OMT	OMAT	CT	VT	MT	OMT	OMAT
1	25	27	49	23	13	16	14	14	17	23	128	76	231	64	11	13	26	22	29
June																			
2	31	16	29	22	14	15	13	10	16	30	65	81	113	48	12	13	30	19	48
14	42	52	24	25	15	16	16	13	11	65	86	104	170	133	8	23	30	14	18
9	32	65	25	23	14	15	15	8	17	32	68	204	249	147	9	12	15	19	39
6	56	30	115	66	8	12	6	16	11	10	73	198	119	230	4	6	26	25	57
13	58	42	53	86	10	14	8	17	27	7	35	208	52	271	4	8	32	29	75
4	39	38	46	77	10	13	12	18	27	9	58	224	263	243	5	3	40	29	55
—	24	78	64	55	—	12	15	16	16	13	21	224	239	284	7	7	84	7	21
20	71	42	36	32	14	14	12	17	16	65	110	184	75	197	8	4	27	7	74
21	30	74	37	34	13	14	15	16	21	55	73	120	128	168	6	14	19	38	77
40	41	82	55	81	—	11	16	20	22	60	67	120	146	365	4	11	19	33	66
26	21	41	61	40	13	14	13	25	18	27	49	40	98	492	4	6	15	44	81
48	23	21	45	25	15	8	27	18	21	232	64	48	89	26	4	37	4	56	19
62	27	21	22	23	14	7	16	16	15	166	73	39	77	35	8	29	6	53	20
37	35	23	26	26	11	7	21	14	12	163	73	48	100	78	11	22	8	64	28
53	42	41	29	54	12	9	15	14	14	163	83	44	176	58	5	35	3	53	19
25	39	74	14	20	10	6	15	4	16	232	59	107	199	98	6	27	21	91	63
40	30	110	15	20	12	12	14	5	21	230	73	67	869	104	7	27	23	52	86
40	28	24	36	16	10	9	14	4	16	407	86	91	574	70	6	59	22	53	70
13	27	19	29	21	12	11	15	4	16	83	73	81	464	114	7	47	13	73	45
5	24	16	19	62	—	14	4	2	15	272	159	122	658	132	—	26	58	58	73
12	18	28	4	30	—	12	4	2	9	241	75	191	382	—	—	28	45	96	61
18	46	27	20	23	—	10	2	7	15	202	191	280	560	143	—	30	33	96	73
7	37	37	45	35	—	11	3	10	14	329	200	416	825	232	—	39	70	108	112
37	37	20	33	14	4	4	4	10	16	580	113	293	427	146	67	40	56	112	93
35	58	36	30	28	6	3	3	10	15	296	385	424	293	146	58	50	56	86	87
24	48	14	42	17	4	—	2	8	7	218	184	293	398	117	66	53	48	56	60
35	14	35	37	59	4	4	2	8	14	732	356	486	527	272	60	74	45	67	68
17	57	33	14	29	3	—	—	7	26	325	263	237	226	153	49	—	—	107	137
69	58	43	44	20	3	—	—	8	16	249	621	385	236	128	58	—	—	67	107
46	85	51	61	18	4	—	—	7	27	184	512	183	486	197	49	—	—	108	96
36	74	46	57	16	4	—	—	15	28	150	325	293	395	114	52	—	—	99	117
	42	97				—	4				293	258				—	70		
	57	70				—	9				234	365				—	74		
October	74	114				—	3				294	296				—	58		
	77	86				—	2				241	263				—	63		

NH_4 nitrogen in mgm/kg. of soil dried at 20°C

CT = Cajander's *Calluna* forest type
VT = Cajander's *Vaccinium* forest type
MT = Cajander's *Myrtillus* forest type
OMT = Cajander's *Oxalis/Myrtillus* forest type
OMAT = Cajander's *Oxalis/Majanthemum* forest type

TABLE IX

Nitrate nitrogen extracted from soil samples taken from various kinds of forest stand during June to October, from Aaltonen (1926)

Nitrate nitrogen extracted from humus layer					*Nitrate nitrogen extracted from mineral soil layer*					*Nitrate nitrogen extracted from humus layer after incubation*					*Nitrate nitrogen extracted from mineral soil layer after incubation*				
pH4·2 CT	4·6 VT	4·8 MT	5·2 OMT	5·0 OMAT	4·2 CT	4·6 VT	4·8 MT	5·2 OMT	5·0 OMAT	4·2 CT	4·6 VT	4·8 MT	5·2 OMT	5·0 OMAT	4·2 CT	4·6 VT	4·8 MT	5·2 OMT	5·0 OMAT
0·6	1·2	1·0	0·5	0·7	0·0	0·0	0·0	0·0	0·5	0·6	1·0	0·8	5	64·0	0·0	0·0	0·0	0·0	40·0
June																			
0·8	1·6	0·8	0·5	0·5	,,	,,	,,	,,	0·5	0·8	1·1	0·7	5	21·4	,,	,,	,,	,,	40·0
0·9	1·1	1·4	0·5	0·5	,,	,,	,,	,,	0·5	0·8	1·0	0·7	5	5·0	,,	,,	,,	,,	36·0
0·5	1·0	1·4	0·5	0·5	,,	,,	,,	,,	0·7	0·6	0·7	1·0	5	8·4	,,	,,	,,	,,	36·0
0·6	1·5	0·5	0·7	—	,,	,,	,,	0·5	0·6	0·5	2·0	0·5	1·0	7·5	,,	0·5	,,	35·2	12·8
0·8	1·6	0·6	0·7	1·0	,,	,,	,,	0·0	0·5	0·5	1·5	0·5	4·8	29·0	,,	0·0	,,	0·0	2·2
0·7	1·8	0·8	0·7	4·8	,,	,,	,,	,,	0·5	0·8	1·2	0·5	2·6	119·9	,,	,,	,,	15·2	0·5
—	—	0·6	0·7	8·9	,,	,,	,,	—	2·3	0·5	0·0	0·5	1·1	130·0	,,	,,	,,	22·0	18·0
0·5	0·7	0·6	0·5	0·5	..	,,	,,	0·0	0·5	0·5	1·1	0·0	(140)	74·0	,,	,,	,,	80·0	5·8
0·5	0·0	0·7	0·5	0·5	,,	,,	,,	,,	0·0	0·5	0·8	1·6	2·1	256·0	,,	0·5	,,	4·0	62·0
0·5	0·6	0·6	0·5	1·0	,,	,,	,,	,,	0·0	0·6	0·8	1·8	0·9	64·0	,,	0·0	,,	2·0	14·0
0·5	0·6	1·1	0·5	0·5	..	,,	,,	,,	0·5	0·5	0·7	0·0	2·4	1·0	,,	,,	,,	36·0	1·4
0·8	0·5	0·5	0·0	0·5	,,	,,	,,	,,	0·0	2·0	0·6	0·5	1·7	0·0	,,	,,	,,	12·7	28·6
0·9	0·6	0·5	0·0	0·5	,,	,,	,,	,,	0·0	1·7	0·8	0·5	1·9	4·6	,,	,,	,,	2·0	4·0
0·6	0·5	0·5	0·0	0·5	,,	,,	,,	,,	0·0	1·4	0·8	0·5	2·3	66·9	,,	,,	,,	0·0	6·2
0·5	0·0	—	0·0	0·5	,,	,,	,,	,,	0·0	1·2	0·7	0·5	2·5	6·3	,,	,,	,,	30·8	1·7
0·5	0·5	0·5	0·5	19·4	,,	,,	,,	,,	1·2	1·1	0·5	0·6	0·0	422·0	,,	,,	,,	0·0	6·9
0·5	0·5	0·5	0·6	21·8	,,	,,	,,	,,	8·0	1·3	0·5	0·6	1·3	476·0	,,	,,	,,	1·7	24·6
0·7	0·5	0·7	0·5	13·2	,,	—	,,	,,	1·6	0·7	0·5	0·9	1·8	396·0	,,	,,	,,	0·0	13·8
0·5	—	—	0·5	4·9	,,	—	,,	,,	1·3	0·5	0·5	0·5	1·5	422·0	,,	,,	0·5	,,	1·7
0·7	0·0	0·9	0·5	0·6	,,	0·0	,,	,,	0·5	1·1	0·0	0·9	1·0	337·0	,,	,,	0·0	,,	0·6
1·0	0·0	0·6	0·5	0·5	,,	,,	,,	,,	0·5	1·0	0·0	1·1	1·0	—	,,	,,	,,	,,	1·2
0·6	0·0	2·3	0·0	0·6	,,	,,	,,	,,	0·5	1·3	0·0	1·8	1·1	355·0	,,	,,	,,	,,	1·3
0·7	0·0	1·1	0·0	0·7	,,	,,	,,	,,	0·5	1·5	0·0	2·4	2·7	370·0	,,	,,	,,	3·0	1·3
0·6	0·9	0·6	1·2	0·5	,,	,,	,,	,,	0·5	0·0	0·7	1·1	8·0	1·8	,,	,,	,,	0·0	1·3
0·7	1·0	1·8	1·1	0·6	,,	,,	,,	,,	0·5	1·1	1·9	1·2	1·7	3·9	,,	,,	,,	,,	0·6
0·7	0·6	0·5	1·3	0·5	,,	,,	,,	,,	0·5	0·7	9	1·1	1·9	0·7	,,	,,	,,	,,	0·0
0·7	0·5	4·8	1·4	0·7	,,	,,	,,	,,	0·5	0·7	1·1	1·5	2·0	7·9	,,	,,	,,	,,	9·5
0·0	1·2	0·5	0·6	1·0	,,	,,	,,	,,	0·7	0·9	1·5	1·3	1·3	400·0	,,	,,	0·5	0·5	9·2
0·0	1·3	2·1	0·6	0·8	,,	,,	,,	,,	1·3	1·2	1·9	1·6	0·8	317·0	,,	,,	0·5	0·0	10·6
0·0	3·7	2·6	0·7	0·8	,,	,,	0·5	,,	0·0	1·1	2·5	1·8	4·6	63·4	,,	,,	0·5	,,	1·1
0·0	1·8	0·6	0·7	0·9	,,	,,	0·0	,,	0·0	0·9	1·2	1·4	0·9	370·0	,,	,,	0·5	,,	3·7
	2·0	0·7				,,	,,				1·1	1·5				,,	0·0		
	1·7	0·5				,,	,,				1·0	1·7				,,	0·0		
October																			
	2·2	1·1				,,	,,				1·3	2·5				,,	0·0		
	2·5	1·3				,,	,,				1·1	1·9				,,	0·0		

Nitrate nitrogen in mgms. per kilogram of soil dried at 20°C

CT = Cajander's *Calluna* forest type
VT = Cajander's *Vaccinium* forest type
MT = Cajander's *Myrtillus* forest type
OMT = Cajander's *Oxalis/Myrtillus* forest type
OMAT = Cajander's *Oxalis/Majanthemum* forest type

active, in some cases extraordinarily so, in Norwegian soils than in those of other parts of Scandinavia and Finland. Soils associated with *Calluna* are no exception in this respect the values being considerably greater than those obtained by Aaltonen (1926) for soils associated with *Calluna*. There is also great variation in the amounts of ammonia and nitrate nitrogen produced by the various soils after 3 months' incubation. This picture is supported by the recent investigation of Låg and Mork (1959) using *Calluna* raw humus.

Recently Schönar (1955) has estimated the ammonia and nitrate extractable, with N/10 HCl and 1 per cent potash alum respectively, from a number of forest soils. The forest stands were oak/beech on acid soils, first generation spruce on soils which were previously similar to those of the oak/beech stand and oak/beech on weakly acid soils. The reactions of the various soils, as indicated by pH measurements, covered a very similar range to those investigated by Aaltonen. Samples were taken from 0–3, 3–10 and 10–20 cm. depths in June, August, November and December. Extractable ammonia and nitrate were determined directly after sampling and also after storage for 7 and 28 days in the case of the June and August samples. In the freshly-collected samples from 3–20 cm depths, at no time was the presence of ammonia and nitrate demonstrable in any of the soils. In the freshly-collected samples from 0–3 cm depths, ammonia and nitrate were not detectable in the August samples and even at the other times the extractable ammonia and nitrate was frequently zero; in only one instance did either reach a value of 10 mgms nitrogen per litre of dry soil; 13 mgms of ammonia plus nitrate nitrogen was the maximum observed.

The samples from the 0–3 cms surface layer generally yielded higher concentrations of ammonia + nitrate nitrogen after storage than samples from 3–20 cms depth. In June the range of extractable mineralized nitrogen (NO_3 and NH_4) varied from 4 to 24 mgms/litre of dry soil after 7 days storage and from 18 to 78 mgms after 28 days storage. Corresponding figures for the August samples are 5 to 24 mgms and 22 to 77 mgms respectively. Although there is no mention of the matter there seems no reason to suppose that characteristic ectotrophic mycorrhizal associations would not be formed by the trees, including spruce, growing on these sites.

It has been recorded (Pearsall 1938 and Romell 1932) that even in soils in which nitrification can take place readily it is frequently not possible to detect the presence of nitrates at those times of the year when plants are actively growing on them.

Bearing in mind the difficulties of relating the concentrations of ammonia and nitrate nitrogen in various soils, as determined by various methods, to the physiologically active concentrations experienced by plant roots and microorganisms, it would appear that a wide range of concentrations is likely to be met with even in the same soil over a period of time and such evidence as is available does not suggest that some soils, including those having a ground vegetation which is predominantly *Calluna*, are characterised by concentrations, low or high, which would be associated with absence of development of A and B type mycorrhizal associations; indeed in the case of soils where such associations would be expected to occur, mineralized nitrogen seems at times, including those when mycorrhizal associations may be expected to be forming, to be undetectable and Björkman's (1940) own experiments indicate that A and B type mycorrhizal associations are formed equally well in soils apparently containing a wide range of ammonia and nitrate nitrogen concentrations.

(iv) **The influence of internal conditions in the higher plant on the formation of ectotrophic mycorrhizal associations**

Although, as Romell (1935) has pointed out, certain arte-facts or "sampling effects" are liable to arise in measurements of nitrogen mineralization in stored samples of soil, such measurements indicate that the amount of ammonia and nitrate produced over a period of time may vary considerably for different soils. Therefore, although at certain times the concentration of mineralized nitrogen to which plant roots and mycorrhizal fungi are exposed may be low or nil, due in all probability to absorption by plant roots, in a variety of soils it may well be that the rate of production of mineralized nitrogen varies considerably in different soils so that the amounts available for absorption by plants in one soil may, over a period time, be very different from those in another soil. Such differences may lead to differing growth rates and physiological conditions inside the plant, which in turn may influence the formation of ectotrophic mycorrhizal associations.

Hatch (1937) transplanted month-old seedlings of *Pinus strobus* into boxes of infertile soil taken from a *Pinus rigida* stand. Similar seedlings growing in fibre cartridges containing added mineral nutrients, equivalent to those found to be optimum for *Pinus strobus* by Mitchell (1934) in sand culture experiments, were planted in soil of the same kind. After three months the roots had grown out of the cartridge into the surrounding soil but very few of the roots of these plants had formed mycorrhizal associations whereas many mycorrhizal associations had been formed on the roots of plants which had grown in the absence of the mineral nutrient-containing fibre cartridge. Hatch contends that this observation supports the

belief that the concentration of nutrients inside the roots controls susceptibility to the formation of ectotrophic mycorrhizal associations. It would also seem to imply a rather uniform systemic condition throughout the root system, which does not seem to be at all certain since mycorrhizal, and apparently equivalent non-mycorrhizal, short roots can occur side by side, but it does not seem to be certain that mineral nutrients did not diffuse from the cartridge into the surrounding soil.

The possible role of conditions within the root on the formation of ectotrophic mycorrhizal associations was studied more closely by Björkman (1940 and 1942). He considered that the amounts of available mineral nutrients in the soil influence the amounts of these nutrients inside the roots which in turn influence the amounts of soluble carbohydrates in the roots and only when the concentration of soluble carbohydrate reaches a sufficient level can the formation of ectotrophic mycorrhizal associations take place. Björkman (1949) envisages that variations in the amount of phosphorus available to the plant will influence the production of carbohydrates by photosynthesis, whilst the amounts and proportions of nitrogen and phosphorus available to the plant will influence the utilization of soluble carbohydrate for tissue synthesis. Either abundant supplies of nitrogen or a very great deficiency, how these are to be characterized is not stated, will result in low concentrations of soluble carbohydrate in the plant. It is also considered that a large deficiency of phosphorus will reduce the sugar concentration in the plant by reducing CO_2 assimilation, whilst a moderate deficiency will allow of normal CO_2 assimilation but the synthesis of protein will be reduced and at times there will be a surplus of soluble carbohydrate. Under these circumstances characteristic ectotrophic mycorrhizal associations will be formed. Björkman (1940 and 1942) considers that this hypothesis is supported by experiments in which varying quantities of nitrogen- and phosphorus-containing mineral salts were added to soils in which pine seedlings were growing under different light intensities. As the amount of nitrogen or phosphorus *added to the soil* increased, and especially when both were added, the percentage of short roots forming A and B type mycorrhizal associations diminished; this was also the case as the light intensity in which the plants were growing decreased. The latter is in agreement with observations that the percentage of A and B type mycorrhizal associations tends to be small when trees are growing in shade in the forest (e.g. Björkman 1942). The roots and shoots of the plants grown under various conditions by Björkman were dried at 80°C, and after being powdered the reducing substances which could be extracted by distilled water were determined. The findings indicate that the percentage of short roots forming A and B type mycorrhizal associations increased as the concentration of reducing substances in the powdered root material increased. Essentially similar findings were obtained by Harley and Waid (1955) for beech seedlings grown under different light intensities. The greater the percentage of short roots which became A and B type mycorrhizal associations, the stronger was the reaction for starch in the roots. Björkman also points out that whilst such mycorrhizal associations are numerous in the autumn they are seldom to be found in the summer months; he concludes from these various observations that ectotrophic mycorrhizal fungi do not form associations with roots unless the latter contain more than a certain amount of soluble carbohydrate and if for any reason, e.g. because of unsuitable amounts of mineral nutrients or inadequate light intensity, the photosynthetic processes are unable to maintain an adequate level of soluble carbohydrates in the roots, then the formation of mycorrhizal associations will be interfered with.

It would seem, however, that the amounts of nitrogen and phosphorus contained by the plants are better indications of the nitrogen and phosphorus available in the soil for the plants, and also of the nitrogen and phosphorus relations inside the plant, than are the amounts of nitrogen and phosphorus added to the soil or found in the soil by analyses of one kind or another. If the values for the nitrogen and phosphorus contained by the plants and present in the roots of Björkman's experimental plants are calculated (see Tables V, VI and VII) it will be seen that there is no direct relationship between these values or the various nitrogen/phosphorus ratios and the percentages of short roots forming A and B type mycorrhizal associations, or the concentration of easily soluble reducing substance in the roots.

There is perhaps a slight indication from the results of Björkman's (1942) experiments with *Pinus sylvestris* and *Rhizopogon roseolus* in pure culture that at the lowest levels of nitrogen, 0·094 mgms of nitrogen as $(NH_4)_2 HPO_4$ per flask containing a single seedling, this nutrient is present in inadequate amounts and is perhaps restricting both the growth of the plants and the formation of A and B type mycorrhizal associations, in spite of the presence of adequate amounts of phosphorus: it is the only experiment in which there is an increase in the percentage of short roots forming A and B type mycorrhizal associations with increase in the amount of nitrogen supplied to the plant and in the amount of nitrogen absorbed by the plant; the lowest levels of nitrogen in all the other experiments were apparently considerably higher than the lowest levels in this experiment. Both the percentage of nitrogen in the dry matter of the root and shoot, and the total amounts of nitrogen absorbed, were larger, the latter

several times larger even in the case of soils watered with distilled water, than those for the lowest nitrogen levels in the pure culture experiment where at least half of the nitrogen of the seedling probably came from the seed itself. All the indications are, therefore, that the amount of nitrogen available, as distinct from the concentration, in those flasks where it appeared that this might be limiting the formation of mycorrhizal associations, is considerably less than the amount of mineralized nitrogen likely to be available during the course of a growing season even in poor forest soils.

Björkman's (1942) results for the concentration of reducing substances as a percentage of root material dry weight indicate a direct relationship between this and the percentage of short roots forming A and B type mycorrhizal associations. Björkman (1944) also found a direct relationship between the concentrations of reducing substance in one and two year old root material from strangled and untreated pine plants, and the number of newly formed mycorrhizal associations other than pseudomycorrhizal associations. In this experiment, however, the highest level of reducing substance, at which 70 per cent of the short roots formed mycorrhizal associations, in the root material is the same (2·1–2·3 per cent of the dry weight) as that at which few or no A and B type mycorrhizal associations were found in the previous study (Björkman 1942). Björkman ascribes this to seasonal differences and to the fact that whereas the root material analysed in the previous experiment was only one year old in the present case, 2 year old roots were included which would contain a higher relative weight of cell wall material.

In both the pure culture and other experiments Björkman expresses the number of short roots forming A and B type mycorrhizal associations as a percentage of the total number of short roots and, although he has pointed out (Björkman, 1940) that it is not always possible to distinguish from external appearances alone whether a lateral root is a true short root or a short root having the nature and potentialities of a long root, he must, therefore, consider at least some of those short roots which do not form A and B type mycorrhizal associations as being potentially similar to those which do. He postulates that the concentration of soluble carbohydrate must be of an adequate level in roots before A and B type mycorrhizal associations can be formed. As a result the percentage of short roots forming A and B type mycorrhizal associations shows a relationship to the concentration of easily soluble reducing substance in the root material as a whole. On the basis of these points it would seem that in order to explain the lack of formation of A and B type mycorrhizal associations in a proportion of the short roots, where there was otherwise apparently every opportunity for them to do so, of the pine plants growing in pure culture with *Rhizopogon roseolus*, the level of easily soluble reducing substance in these roots must have been too low for such associations to be formed and lower than the concentrations in those short roots on the same plants which formed A and B type mycorrhizal associations. Similarly, in the case of plants growing under conditions of reduced light intensity, it is presumably considered that there will be a smaller supply of photosynthetic material to the roots resulting in a reduced proportion of short roots forming type A and B mycorrhizal associations whilst the other short roots form pseudomycorrhizal associations, this presumably indicates that the latter are in fact short roots and not potential long roots, whose formation is presumably not affected in the same way by the level of the concentration of easily soluble reducing material; in such circumstances it would seem that even adjacent short roots would have to contain very different concentrations of easily soluble reducing material.

The formation of A and B type mycorrhizal associations by only a proportion of the short roots in Björkman's pure culture and other experiments would seem to indicate that any factor inside the root system which has a controlling influence on the formation of such associations has a discontinuous distribution within the root system, provided that the non-mycorrhizal short roots are otherwise equivalent to those which are mycorrhizal.

It would have been easier to accept Björkman's hypothesis if data concerning the concentration of reducing substances in root material from plants which had been grown under various appropriate conditions, but with the absence of formation of mycorrhizal associations, had been available. The fungal mantle and the Hartig net may form a considerable proportion of the material of a mycorrhizal association. Melin and Nilsson (1958) have determined the dry weights of the fungal sheath and the core for a number of mycorrhizal roots; in some cases the weight of the sheath, which does not of course include the fungal material of the Hartig net, is greater than the weight of the core. It has been shown that the mycelium of some species of fungi when grown under some conditions may contain very considerable quantities of readily hydrolysable carbohydrate material which usually seems to give glucose on hydrolysis although other hexoses have occasionally been obtained (Irani, R. J. and Ganapathi, K. (1959), Shu, P. and Thorn, J. A. (1952), Norman, A. G., Petersen, W. H., Houtz, R. C. (1932)). The conditions used for extraction and hydrolysis of the intracellular carbohydrates, although gelatinous material surrounding the hyphae may have been included in some cases, are generally somewhat more severe than those used by Björkman

in his determinations of reducing substances in root material. In view of the fact that values for reducing substances up to 60 per cent of the dry weight of the mycelium have been obtained, it would seem desirable that the possibility of there being quite considerable concentrations of reducing substances in the hyphal mantles of mycorrhizal associations should be considered. This possibility seems to be supported by the recent work of Melin and Nilsson (1957) who found that pine seedlings, which had formed mycorrhizal associations in pure culture, produced photosynthetic material which was rapidly transported to the hyphal mantles when the plants were exposed to $C^{14}O_2$ for a short period of $\frac{1}{2}$–1 hour; in addition the radioactivity values of the fungal mantles were somewhat higher than those of uninfected roots but this is not necessarily due to carbohydrate. The presence of even moderately increased concentrations of reducing substances in the hyphal mantles may be sufficient to account for the increases in concentration of reducing substances in the root material, as the percentage of short roots forming A and B mycorrhizal associations increases, without any appreciable difference in the concentration of reducing substances in the root tissue. It may well be significant in this connection that Björkman's values show far less variation in the concentration of reducing substances in shoot material grown under various conditions, than is the case for the corresponding root material. This would mean that the increased concentrations of reducing substance observed in the root material are the result and not the cause of the formation of A and B type mycorrhizal associations and would require that the mycelium of pseudomycorrhizal associations accumulate little easily soluble reducing substance. This aspect of the problem has recently been investigated experimentally (Handley and Sanders, 1962) and the results obtained do not suggest that there are any variations, such as would seem to be required by Björkman's hypothesis, in the concentration of easily soluble reducing substances in the roots of pine plants grown under the same mineral nutrient conditions but in different light intensities.

Indeed it is difficult to understand how a concentration of glucose or reducing substance within a root can influence a fungus outside the root unless it is reflected in, or associated with, some external root characteristic to which the fungus is able to react.

It is also possible that under some circumstances conditions arise inside the root which prevent the formation of mycorrhizal associations by interfering with the growth or metabolism of mycorrhizal fungi. For such a factor to be responsible for the lack of formation of mycorrhizal associations in spruce whose growth has been checked by living *Calluna*, it is necessary that it ceases to be effective when the living *Calluna* is killed, e.g. by mulching.

It therefore seems doubtful whether internal conditions in the plant, as represented by concentration of easily soluble reducing substance and the N/P ratio of the plant tissue, are directly related to the formation of A and B type mycorrhizal associations.

Chapter 2

THE INFLUENCE OF FACTORS OUTSIDE THE ROOT ON THE FORMATION OF ECTOTROPHIC MYCORRHIZAL ASSOCIATIONS

Instances of marked localized variation in the proportion of short roots forming ectotrophic mycorrhizal associations have been recorded.

Melin (1925) found that in calcareous mull soils, where ectotrophic mycorrhizal associations are not usually formed on the roots of coniferous species, excellent groups of mycorrhizal roots may occur locally in decayed wood or pieces of bark.

In beech woodland, Harley (1952) noted that roots colonizing decayed wood deep in the soil below the humus layer often develop mycorrhizal laterals in quantity which is in striking contrast to the development in the surrounding mineral soil.

The localised development of mycorrhizal associations by birch growing in old rotten roots and tree stumps (Dimbleby 1953) has already been mentioned.

These marked localized variations have been noticed on different parts of the root system of one and the same tree and draw attention to possible effects which variations in the soil environment may have on either partner of the mycorrhizal association. Melin (1927) concluded that the nature of the humus form had a direct or indirect significance for the formation of mycorrhizal associations by influencing either the virulence of the fungus or the activity of the root cells or both. It is conceivable that the different roots of the same tree growing in different soil environments do not have complete systemic metabolic and physiological continuity and as a result internal conditions, which influence the formation of mycorrhizal associations, may differ in roots occupying the two differing environments. That the external root environment may modify conditions inside the root is suggested by the results obtained by Dimbleby (1952 (b)) from analyses of the sap exuded from the cut roots of young birch trees growing on *Calluna* heathland.

The mycorrhizal fungi which have so far been found to form A and B type associations are relatively sensitive and nutritionally exacting organisms. It would not be surprising, therefore, if the localized differential development of ectotrophic mycorrhizal associations is an indication that in some soils there may be conditions which are inimical to the life of these fungi or to one or more of their activities on which formation of the association depends. This could be at least as important as the effect of environmental conditions on the root. The localized absence of mycorrhizal associations may well be due to different causes in the different environments. Discontinuous distribution of such inimical conditions, perhaps varying with time and very local differences or changes in vegetation, might well be the basis of Björkman's (1942) conclusion that no indication could be found from field studies for the very variable development of mycorrhizal associations within apparently uniform forest communities. The possible importance of factors outside the root which are in some way inimical to the life or activities of mycorrhizal fungi is perhaps emphasized by the previously mentioned lack of correlation between 'ecological' levels of mineral nutrients in soils or the mineral nutrient content of seedlings and the percentage of their short roots forming A and B type mycorrhizal associations and also, in the absence of some discontinuously distributed factor inside the root system, by the indications from Robertson's (1954) work that in young seedlings coming into contact with the mycorrhizal fungus at an early stage of growth the fungus would be expected to spread to all short roots from a single focus in the absence of factors affecting the spread of the mycelium adversely.

From the point of view of the formation of ectotrophic mycorrhizal associations by trees growing on *Calluna* heathland it is therefore important to know first of all whether trees growing in this environment are able to produce short roots and whether the mycorrhizal fungi are able to live there.

(a) Effect of soil conditions on the formation of short roots

The formation of mycorrhizal associations is dependent on the presence of suitable roots. Whilst it is sometimes contended that when a lateral root is infected by a mycorrhizal fungus its growth is retarded and it becomes a short root because of this, there are others (e.g. Aldrich Blake 1930, Hatch 1937) who consider that a short root is short because of the inherent physiological state or lack of vigour

in such roots. Observations indicate that the quantities of available nutrients in soils or culture solutions have a strong influence on the production of short roots. Where relatively large supplies of nutrients are available, root growth as a whole diminishes and short roots may be almost, if not completely, absent. At lower concentrations increasing numbers of short roots are produced and even at very low concentrations of nutrients short roots are by no means absent (Hatch 1937) and short roots, similar to those which form characteristic ectotrophic mycorrhizal associations, have been observed to be produced by pine plants growing in aseptic culture. Short roots which do not become infected by mycorrhizal fungi do not seem to persist for long.

Rayner and Neilson Jones (1944) examined the roots of various coniferous species growing on *Calluna* heathland. In the case of young pine plants there was a marked deficiency in the number of short roots and a corresponding poverty of mycorrhizal associations, although there was no evidence of root disease, attack by parasitic microorganisms or the formation of pseudomycorrhizal associations. Norway spruce grew very poorly on the same soil and showed arrest of root growth and absence of development of mycorrhizal associations; here again there was no evidence that failure of the plants to grow was associated with the roots being attacked by any soil fungus. On the other hand, although Sitka spruce grows very poorly on the same soil and has root systems which are very dark in colour and deficient in sublaterals, the majority of the short roots in this case form mycorrhizal associations which are imperfect in structure and apparently due to association with *M.r. atrovirens* or *Rhizoctonia sylvestris*.

Later Levisohn (1952) also examined the roots of plants of various conifer species growing on *Calluna* heathland and showing well developed symptoms of checked growth; she compared them with the roots of similar plants emerging from growth check as a result of being mulched with *Calluna* shoots. Plants of Norway spruce whose growth has been checked have considerably reduced root systems having a pronounced deficiency of short roots. In some areas these short roots are uninfected whereas in other areas they have formed pseudomycorrhizal associations with *M.r. atrovirens*. The mulched plants on the other hand had well developed monopodially branched root systems which were characteristic for healthy Norway spruce; the short roots were fully mycorrhizal and pseudomycorrhizal associations were absent. This is a rather different result from what might have been anticipated from the previously mentioned experiences of Laing (1932) in which decaying *Calluna* leaves had an adverse effect on seedlings of *Picea excelsa* growing in water culture. Whilst the roots of Sitka spruce plants showing checked growth were non-mycorrhizal, the roots of mulched plants, which were previously showing checked growth, had fully developed mycorrhizal associations. Similarly in the case of birch and the endotrophically mycorrhizal Lawson cypress, mulching greatly stimulated the formation of mycorrhizal associations.

The absence of characteristic A and B type ectotrophic mycorrhizal associations from the roots of trees whose growth is checked on the *Calluna* heathland is apparently not due to absence of short roots, assuming that the short roots which are present are of a type potentially capable of forming mycorrhizal associations, even though they may be considerably fewer in number than when the trees are growing normally: it may well be that if the formation of mycorrhizal associations once began the production of more short roots would be stimulated and subsequently more mycorrhizal associations would be formed, although as is well known the mere presence of short roots does not necessarily mean that all of them will form mycorrhizal associations. This suggests the probability that under *Calluna* heathland conditions some factor is preventing the growth or activities of ectotrophic mycorrhizal fungi on which the formation of type A and B mycorrhizal associations is dependent.

(b) The occurrence of ectotrophic mycorrhizal fungi in *Calluna* heathland soil

For the formation of ectotrophic mycorrhizal associations on the roots of trees growing on *Calluna* heathland the ability of the appropriate fungi to live and carry out any activities essential for the formation of such associations in that environment is at least equally as important as the ability of the tree to form short roots under the same conditions: it may be even more important in view of the possible effects of some of these fungi on root growth, both in pure culture (Slankis 1948) and in soils (Rayner and Neilson Jones 1944 and Levisohn 1953b and 1956), before mycorrhizal associations are formed.

Comparatively little is known with certainty about the ectotrophic mycorrhizal fungi as free living organisms in soils. From studies with pure cultures of these organisms, generally isolated from sporophores, it has been established that under these conditions they are nutritionally exacting and relatively sensitive to environmental conditions although the group of organisms as a whole shows a range of requirements. These organisms have been shown, especially from the extensive work of Melin (1953) and his collaborators, to require the provision of a number of substances, whose identity is known, for their growth in pure culture. These include simple carbohydrates, amino acids, thiamine or its consti-

tuents pyrimidine and thiazole, pantothenic acid, nicotinic acid, biotin and inositol. It has also been found that a number of unidentified substances stimulate the growth of these organisms in pure culture, they include yeast extract, materials extractable from freshly fallen leaf litter at a suitable dilution, and substances emanating from the roots of many different plants including those with which the fungi are not known to form associations. The temperature for optimum growth of ectotrophic mycorrhizal fungi of temperate climates may vary, under pure culture conditions, for different species and even for different strains of the same species (Moser 1959) but in many cases it seems to be in the region of 20°C. These fungi seem to survive longer in pure culture in the vegetative phase at considerably lower temperatures. Melin (1925) has pointed out that mycorrhizal associations develop well on trees growing in subalpine and sub-arctic regions. It seems probable that these fungi are capable of forming mycorrhizal associations over a considerable temperature range, and the *Calluna* heathland would not seem to be exceptional in this respect as is indicated by the fact that trees growing on such heathlands develop A and B type mycorrhizal associations in the absence of *Calluna*. For optimum growth rates in pure culture these fungi require low concentrations of salts and nutrients and a suitable reaction (pH). In pure culture optimum growth of the ectotrophic mycorrhizal fungi is obtained over the reaction range pH 3·5–6·5 and few of these organisms appear to be able to grow at all under these conditions at reactions above pH 7·0 (Modess 1941) although strains of some of them, at least, are able to grow, even though at a reduced rate, at reactions of pH 2·5 and some at pH 2·1. Therefore, although there may be some doubt concerning the suitability for the growth of mycorrhizal fungi of soils whose reaction is on the alkaline side of neutrality, there seems no reason why the reaction of *Calluna* heathland soil should be unsuitable.

It is perhaps to be expected that the presence of the vegetative mycelium of organisms having such characteristics would be difficult to detect in samples of soil by the use of culture media. This has proved to be the case, any mycelium of these organisms which may be present in samples of soil plated on culture media being rapidly overgrown by less exacting fungi. Even if isolated in this way their identification is a matter of great difficulty. These considerations have resulted in the widely accepted view that the mycorrhizal fungi are at a disadvantage as free living organisms in the soil compared with many other soil fungi. This view also seems to be supported by the small success which has attended attempts (e.g. Robertson 1954), to use mycorrhizal root material as a source of infection for uninfected plants growing in soil although Levisohn (1953b) claims it to be a satisfactory method.

It may well be however, that the characteristics of these organisms as observed in pure culture may not represent completely the capabilities of these organisms in the soil. In pure culture few ectotrophic mycorrhizal fungi have shown ability to utilize more than simple sugars, yet it would seem that when in association with roots they may be able to modify the materials of the middle lamellae between the root cortical cells and under some circumstances some of them, at least, penetrate the walls of the cortical cells. Whatever their capabilities in soil away from roots, when an association is formed with a root they seem to possess characteristics enabling them to dominate the environment immediately surrounding the root. In this respect the ectotrophic mycorrhizal fungi show similarities to other Hymenomycetes which appear to live mainly on litter and which are also rarely obtained in plate cultures of soils. These litter-destroying Hymenomycetes have been shown by Warcup (1951) to occupy definite zones, from which very few species of microfungi were isolated, in soils; this was especially true for material collected from beneath groups of sporophores. More recent work by Warcup (1959) indicates that Basidiomycetes may be isolated from soils if appropriate methods are used and that these organisms are particularly likely to be obtained from root material and the larger fragments of plant debris.

At present, knowledge of the existence of ectotrophic mycorrhizal fungi in particular soils is largely dependent on the formation of characteristic associations with roots and the production of sporophores, although the absence of either of these manifestations from a site cannot be taken to indicate that ectotrophic Hymenomycetes are not present as free living mycelium or spores. In many cases trees have not grown on *Calluna* heathlands for very considerable periods of time and the frequently observed lack of development of mycorrhizal associations by young trees in this environment might well be due to the absence of the appropriate fungi from these heathland soils. In this connection Warcup (1959) records the occurrence of sporophores of *Boletus granulatus* on pastures and the isolation of the mycelium of the same organism from pastures but he does not state whether or not there are trees in the vicinity of the pasture. Although for reasons already mentioned it is unlikely to be any easier to attempt to demonstrate the presence or absence of the mycelium of ectotrophic mycorrhizal fungi in *Calluna* heathland soils than in the case of any other soil, by the use of the customary dilution method and plate cultures, it might be possible to investigate the problem by the methods used by Warcup.

Rayner and Levisohn (1941) recorded the isola-

tion, from rhizomorphs occurring in heathland soil, of a mycelium which in pure culture had characteristics similar to those of pure cultures of *Boletus bovinus*. In 1955 Levisohn again isolated mycelia from rhizomorphs occurring in heathland soil and states that the fungi isolated were *Boletus bovinus*, *Boletus scaber* and *M.r. nigrostrigosum;* the hyphal strands from which the fungi were isolated were apparently similar to each other. In neither case does it seem probable that the organisms were isolated from soil in the vicinity of *Calluna* plants growing on undisturbed treeless heathland.

As a result of the work of Robertson (1954) and of mulching experiments by Levisohn (1952) it would appear that the problem should be looked at from a rather different angle. Experiments indicate that where the living heather around trees, whose growth is checked, is suppressed, for example, by mulching with materials such as heather or bracken, the vegetative mycelium of ectotrophic mycorrhizal fungi very soon either arrives or becomes active, whereas previously it was absent or inactive. In view of Robertson's findings there seems every possibility that the spores of these fungi will reach the surface of the *Calluna* heathland in sufficient numbers to ensure the presence of these organisms in the vegetative phase when conditions are suitable; this makes the problem of demonstrating the presence or absence of the mycelium of ectotrophic mycorrhizal fungi in the *Calluna* heathland soil, at any particular time, of diminished importance from the present point of view.

From the point of view of the nutrition of ectotrophic mycorrhizal fungi it would seem that the *Calluna* heathland soil is equally as suitable as other soils. Abundant development of ectotrophic mycorrhizal associations has been recorded for roots growing in coniferous raw humus, and *Calluna* heathland is usually characterised by the development of a raw humus layer which seems to arise in a similar manner to that of coniferous forests. Woody tissues are decomposed under *Calluna* heathland conditions, in all probability by wood-destroying Hymenomycetes which have a similar heterotrophy for vitamins as the mycorrhizal Hymenomycetes. It may reasonably be expected that *Calluna* litter will, like the leaf litter of other plant species, provide growth stimulating factors for the mycorrhizal Hymenomycetes and also that the roots of trees, and perhaps the roots of *Calluna* itself in this environment, would provide the Melin 'M' factor. In addition any mobilized nitrogen is likely to be in the more favourable ammonia form, rather than as nitrate, in *Calluna* heathland soils. The spores of mycorrhizal Hymenomycetes germinate with difficulty or not at all on culture media in the laboratory. Fries (1943) found that they would germinate in the presence of certain other soil microorganisms and it seems likely that organisms capable of bringing about the germination of spores of mycorrhizal Hymenomycetes will be present in the soils of *Calluna* heathlands.

There seems, therefore, to be little if anything to suggest that the *Calluna* heathland soil is inferior to other soils as a nutritional and physical environment for ectotrophic mycorrhizal fungi. The nutritional suitability of the *Calluna* heathland for mycorrhizal Hymenomycetes also seems to be indicated by the fact that as soon as the living *Calluna* is suppressed e.g. by mulching with bracken or heather, ectotrophic mycorrhizal associations are readily formed. There seems little reason to suppose that mulching significantly changes the nutritional or other environmental factors except perhaps moisture relations at the surface of the raw humus.

This seems to suggest that in some way the mulch removes or nullifies some factor(s) hindering the formation of mycorrhizal associations. Such a factor may be influencing the germination of the spores, or the growth and activities of the mycelium of the mycorrhizal Hymenomycetes, in the unmodified *Calluna* heathland soil supporting living *Calluna*.

Chapter 3

THE *CALLUNA* HEATHLAND SOIL AS AN ENVIRONMENT FOR THE GROWTH AND ACTIVITIES OF ECTOTROPHIC MYCORRHIZAL FUNGI

Since the problem of lack of formation of mycorrhizal associations by trees growing on *Calluna* heathland does not seem to be concerned with the actual presence or absence in the *Calluna* heathland soil of suitable propagules of the mycorrhizal Hymenomycetes, nor with nutritional and physical characteristics of the *Calluna* heathland soil as an environment for the mycorrhizal Hymenomycetes, but with some factor which has a deleterious influence on the germination of spores or the growth and activities of the mycelium of the mycorrhizal Hymenomycetes, it is desirable to consider the overall suitability of the *Calluna* heathland soil as an environment for ectotrophic mycorrhizal fungi; for it is evident that in those soils in which numerous ectotrophic mycorrhizal associations are formed by tree roots, overall conditions are suitable for these fungi or at least not inhibitory towards them.

There have been a number of attempts to grow ectotrophic mycorrhizal fungi on soils, raw humus, litter and extracts of these various materials.

In an investigation of the relationship between *Boletus elegans* and larch, Hammarlund (1923) reported that he obtained good growth from basidiospores of *Boletus elegans* placed on soil (apparently a garden soil) which had been treated with formaldehyde. Wishing to study the development of mycorrhizal fungi on various natural humus forms, and realizing the difficulty of sterilizing humus without at the same time producing chemical changes in it, Melin (1925) treated a 'good' raw humus with formaldehyde according to Hammarlund's method. Inocula from vigorous colonies of *Boletus elegans*, *B. variegatus* and *M.r. sylvestris* β and γ* failed to make further growth on this material and he considered that humus treated in this way has probably undergone chemical change making comparison with natural raw humus impossible. Subsequently Melin heated raw humus in a moist atmosphere at 50°C for three days. *Boletus variegatus* and *Boletus luteus* grew strongly on this material but after 3 or 4 days they were overgrown by vigorous mycelium arising from spores originally present in the soil and which had not been killed by the heat treatment. Melin sterilized raw humus by heating it to 100°C and found that mycorrhizal fungi only grew on it, and even then rather weakly, when the humus had been washed with water following sterilization; he considered the feeble growth to be due to incomplete removal of toxic substances produced during heating.

Recently, Moser (1958) has reported that he obtained good growth of a number of species of ectotrophic mycorrhizal fungi, including species of *Boletus*, *Amanita* and *Lactarius*, on peat (Niedermoortorf), with or without admixture of larch or spruce needles, which had been sterilized by autoclaving and to which glucose, phosphorus and nitrogen were added. Addition of calcium appeared to favour the growth of some species of mycorrhizal fungi and the growth of some species was favoured by addition of biotin and aneurin. There does not seem to be any suggestion, however, that autoclaving resulted in the production of substances toxic to the mycorrhizal fungi. The plant community whose remains gave rise to the peat is not indicated. With a similar aim in view—the promotion of the formation of mycorrhizal associations by forest trees in the field by inoculation with pure cultures of mycorrhizal fungi—Boker (1958) claims to have obtained pure cultures of various ectotrophic mycorrhizal fungi from basidiospores placed on nutrient agar and to have grown the resulting mycelia on a medium of loose fibrous peat, but insufficient details are given for a comparison to be made with the observations mentioned previously.

Melin (1925) considered that results obtained with unheated humus extracts sterilized by filtration were of greater interest. On such an extract prepared from 'good' raw humus, presumably a raw humus showing appreciable nitrogen mobilization on incubation, the ectotrophic mycorrhizal fungi *M. r. sylvestris a*, β and γ and *M.r. abietis* were able to grow without addition of amendments but only small colourless colonies were produced. Growth similar to that on a good nutrient medium was obtained when glucose was added to such a humus extract. Extracts prepared in a similar manner from beech and oak forest humus supported only poor or even scarcely perceptible growth of these organisms. The preparation of extracts in such a manner would almost cer-

*Note: The initials *M.r.* stand for *Mycelium radicis*.

tainly involve dilution, as compared with their concentration in undisturbed soil, of any factors unfavourable to the growth of ectotrophic mycorrhizal fungi even if they were not removed during sterilization by filtration.

Later Melin (1946) extracted the freshly fallen leaf litter of various plant species (*Acer platanoides* L., *Betula verrucosa* Ehrh., *Fagus sylvatica* L., *Pinus sylvestris* L., *Populus tremula* L., *Quercus robur* L., and *Glyceria maxima* (Hartm. Holmb.) with distilled water for 24 hours at 5°C. These extracts were sterilized either by autoclaving or Seitz-filtration and were then found to stimulate the growth of ectotrophic mycorrhizal fungi at low concentrations. At higher concentrations such extracts, with the exception of the extract of *Glyceria maxima* which had no such effect, exerted an inhibitory effect on the growth of these fungi. The magnitude of the inhibitory effect varied considerably, depending on the plant species from which the litter had been obtained, in the case of the Seitz-filtered extract whose growth-inhibitory effect Melin considers may be the same as that of the untreated extract. The variation in the inhibitory effect was smaller for similar extracts which had been autoclaved, a process which seems to have increased the inhibitory effect. Whilst the growth of the mycorrhizal Hymenomycetes was inhibited by the litter extracts at the higher concentrations, the growth of the litter-decomposing Hymenomycetes was not inhibited except by autoclaved extracts of the litter of *Acer platanoides* L. Subsequently Melin (1953) put forward the view that these inhibitory substances in litter may be the cause of the lack of formation of ectotrophic mycorrhizal associations in the litter layers but that, as these substances will in time disappear due to leaching or decomposition, any antibiotic substances present in forest humus are likely to be produced by micro-organisms.

Rayner and Neilson-Jones (1944) concluded that the superficial organic layer of the *Calluna* heathland soil contains substances actively injurious to root growth and that conditions in this layer are inimical to the formation of mycorrhizal associations. From observations on the behaviour of young trees in the field, and fungal cultures on the soil in the laboratory, it was concluded that the degree of toxicity in the *Calluna* heathland soil fluctuated with the season of the year, being at a maximum in late winter and decreasing during the summer. This suggested that excessive amounts of water in the soil increased the degree of toxicity and laboratory experiments showed that the toxicity of the soil towards fungi was maintained or even increased when the samples were kept under waterlogged conditions. Air drying and steaming for not more than 20 mins. rendered the soil non-toxic, and it remained so indefinitely if kept only moderately moist but gradually regained its toxicity when waterlogged. Steaming for one hour, autoclaving or treatment with alcohol removed the toxicity; the soil did not regain its toxicity even under waterlogged conditions until inoculated with untreated soil. This suggested that the toxicity is of biological origin and produced by anaerobic microorganisms; it was further suggested that the toxicity is due to hydrogen sulphide produced by anaerobic microorganisms although this could not be demonstrated. Neilson-Jones does point out that there is no direct evidence that the toxicity shown to be responsible for inhibition of fungal growth can act directly on the growth of vascular plants, and also that the production of deleterious substances or toxic conditions by causes other than waterlogging is not excluded.

Subsequently Brian, Hemming and McGowan (1945) isolated saprophytic fungi (e.g. *Penicillium* spp.) from the same *Calluna* heathland soil. In *pure culture* these organisms produced a substance, apparently gliotoxin, which inhibited the growth of various fungi when these were inoculated on to malt extract agar containing this substance (see Table X).

TABLE X

Gliotoxin µgm/ml.	*Boletus bovinus*	*Boletus elegans*	*M.r. nigrostrigosum*	*M.r. atrovirens*	*Rhizoctonia sp.*	*Phoma radicis callunae*
0	⊹ ⊹	⊹ +	⊹ ⊹	⊹ ⊹	⊹ ⊹	⊹ ⊹
5	—	⊹	⊹	⊹	⊹	⊹ ⊹
10	—	⊹	—	—	—	+ +
20	—	—	—	—	—	+
40	—	—	—	—	—	—

+ + = normal growth
⊹ = reduced growth
— = no growth

It still remained to be demonstrated whether or not gliotoxin is in fact produced in *Calluna* heathland soil. This problem was taken a step further by Wright (1956) in an investigation of the production of antibiotic substances in the soil itself from the same *Calluna* heathland. The presence of antibiotic, and its possible identity with gliotoxin, was investigated by extraction of the soil or other material with ether, followed by chromatography, of the extract alongside an authentic sample of gliotoxin; the antibiotics were assayed by placing the chromatograms on plates seeded with *B. subtilis*. The soil samples were not heated and contained the natural microflora. Based on the hypothesis that if antibiotics are produced in soil they will probably occur in localized zones where the supply of organic nutrients is favourable, straw was incorporated in the soil and in some cases the soil was inoculated with suspensions of spores of a strain of *Trichoderma viride* known to produce gliotoxin in pure culture. Although the straw in the soil not inoculated with *T. viride* supported a good growth of fungi *in no case* could antibiotic be detected in extracts of the straw or the soil surrounding the straw. In the soil inoculated with *T. viride* however, a substance apparently identical with gliotoxin was produced but only in the vicinity of the straw. When the reaction of the soil was adjusted to pH 6·5 or 7·3 by addition of $Ca(OH)_2$ the production of antibiotic was reduced almost to zero. On the other hand antibiotic yields were increased by addition of 1 per cent KNO_3, 1 per cent Ca superphosphate or 1 per cent $(NH_4)_2SO_4$ to soils inoculated with *T. viride* and to which straw had been added. $(NH_4)_2SO_4$ was the most effective in increasing antibiotic production. The absence of detectable antibiotic in the ether extract of acid *Calluna* heathland soil in the absence of inoculation with *T. viride* seems to be in agreement with the observation of Jackson (1958) that an acid humus soil having a reaction of pH 2·8 and supporting a vegetation of *Pteridium* and *Aira* only inhibited the germination of the spores of the acid-sensitive fungus *Acrostalagmus cinnabarinus* out of the eight test soil fungi used whereas arable or pasture soils having a circumneutral reaction showed thermolabile inhibitory effects on the germination of the spores of the majority of the same fungi. Although the inhibitory effect at the less acid reactions does not seem to be due to the reaction as such, it was found to be decreased when the acidity of the soils was increased.

It is difficult to see how the Rayner and Neilson-Jones fungistatic factor, developing in *Calluna* heathland soils under conditions of waterlogging, can be equated to gliotoxin which is produced by fungi, isolated from *Calluna* heathland soil, growing in pure culture under aerobic conditions. The observations of Wright that the presence of gliotoxin or similar antibiotic could not be detected in unheated *Calluna* heathland soil, in the absence of added straw and inoculation with spores of *Trichoderma viride*, make it improbable that any inhibition of the growth of mycorrhizal Hymenomycetes in these soils is due to gliotoxin or similar antibiotics. This is reinforced by Wright's finding that the antibiotic is produced in greater amounts in the presence of plant residues, since one would expect from this that ploughing of *Calluna* heathland, which results in the breaking up, burial and death of *Calluna* roots and shoots, and the application of nitrogenous fertilizers, would result in increased production of antibiotic, whereas in fact these procedures seem to markedly reduce the check to growth of trees and increase the formation of mycorrhizal associations.

Although aeration reduces the Rayner and Neilson-Jones fungistatic factor in previously waterlogged *Calluna* heathland soil, and ploughing alleviates the check to tree growth on *Calluna* heathland, when living *Calluna* surrounding checked spruce plants is killed by a mulch of *Calluna* shoots, conditions in the raw humus become considerably moister (e.g. Nemec 1954), yet the spruce and their mycorrhizal associations show improvement. As previously mentioned, Duchaufour (1950) found that spruce growing well and spruce showing checked growth and scarcity of mycorrhizal associations were growing on pedologically similar sites whilst Braathe (1950) records that even on a site from which a crop of spruce has been removed by clear felling, the growth of recently planted spruce will be checked if the site is colonized by *Calluna*.

There seems reason to doubt therefore whether an inhibitory factor having the characteristics described by Rayner and Neilson-Jones, or a substance having the characteristic of gliotoxin, can be the reasons for the checked growth and lack of development of mycorrhizal associations in spruce growing on typical *Calluna* heathland in the presence of living *Calluna*. This doubt seems to be strengthened by Levisohn's (1952) observations on *Calluna* heathland soils from the same area as used in the original investigations of Rayner and Neilson-Jones. A high degree of toxicity was expected in view of previous experiences because of the extremely wet conditions during the winter and spring of 1950–51, but in fact there was no indication of toxicity in any of the soil samples from various depths. It is also recorded that pine and spruce seedlings grown in this soil grew better and with more normal mycorrhizal associations than was the case in earlier years. This indicates that the factor responsible for fungal inhibition and depression of seedling growth cannot be perpetuated indefinitely merely by intermittent renewal of high water content in the soil and it is perhaps signi-

ficant that it seems likely that it is some time since this soil supported a vegetation of *Calluna*. The somewhat contradictory findings concerning the influence of raw humus on the growth of fungi and on the growth of mycorrhizal Hymenomycetes in particular also suggest that the effect of *Calluna* heathland soil, and especially of such a soil which is carrying a vegetation of living *Calluna*, on the growth of mycorrhizal Hymenomycetes, should be re-examined.

Chapter 4

AN EXPERIMENTAL RE-EXAMINATION OF *CALLUNA* RAW HUMUS AS AN ENVIRONMENT FOR THE GROWTH OF MYCORRHIZAL HYMENOMYCETES AND OTHER FUNGI

In various previously mentioned investigations into the effect of raw humus on the growth of fungi, including mycorrhizal Hymenomycetes, the solid material was used and it is clearly desirable to use this material if possible. However, the mycorrhizal Hymenomycetes are relatively slow-growing fungi which makes it desirable to use sterile substrates, but it is difficult to sterilize raw humus with certainty other than by autoclaving, a process which is clearly undesirable. In addition, for comparative purposes it is desirable to use if possible a more certainly homogeneous material than solid raw humus.

It seemed probable that any substances inhibitory to the mycorrhizal Hymenomycetes and present in the *Calluna* raw humus as a result of the activities of microorganisms or the roots of *Calluna* plants would be present in the soil water and extractable with water. Therefore, even though it was appreciated that extraction of raw humus with water would result in dilution of any inhibitory factor, it was decided to investigate the effects of aqueous extracts of *Calluna* raw humus on the growth of mycorrhizal Hymenomycetes and other fungi: a very brief mention of some of the findings has already been made (Harley, 1952).

(a) **Preparation of Extracts**

Calluna raw humus supporting a vigorous and well established but in no way senescent, monoculture of *Calluna vulgaris* was obtained from a characteristic podzol which has long been associated with this plant species in north-east Yorkshire. The moist, dark brown, almost black organic material was crumbled away from the *Calluna* roots by hand although many fragments of fine roots remained in the material. Two hundred gms. of this material were mixed with 500 ml. of distilled water and 20 ml. of chloroform. This mixture stood in a stoppered jar for four weeks at room temperature when a clear golden brown fluid having a reaction of approximately pH 3·7 was removed and kept in the presence of chloroform until required.

(b) **Method of assay of extracts for inhibition of fungal growth**

Incorporation of the extracts in agar slopes without further considerable dilution of the extract would have involved considerable heating of the extracts which it was desirable to avoid; there is also the risk of absorption on to the agar of any inhibitor which may be present. Attempts were therefore made to grow mycorrhizal Hymenomycetes in fluid cultures. Although growth of inocula of mycorrhizal Hymenomycetes was frequently unsatisfactory even in shallow layers of a suitable liquid medium, it was found that this could be remedied by supporting the inoculum, at or just above the surface of the liquid, on quartz sand or grit which was free from calcareous material and iron. The method of culture ultimately adopted was to sterilize, by autoclaving, 50 ml. Pyrex conical flasks plugged with cotton wool and containing 10 gms of the quartz sand or grit; subsequently 5 ml. of culture fluid and 0·05 ml of chloroform were added to each flask which was placed along with others in a large desiccator containing a shallow layer of chloroform. The flasks were kept in the desiccators for 4–6 weeks at room temperature. On removing the flasks from the desiccators they were heated in a waterbath for 20 minutes at a temperature of 62°C, to drive off any residual chloroform, and then allowed to stand overnight at room temperature before inoculation.

(c) **Effect of aqueous extracts of *Calluna* raw humus on the growth of fungi**

In the first experiments strains of the following fungi isolated from sporophores were used. *B. bovinus*, *B. variegatus*, *B. elegans*, *B. scaber*, *Rhizopogon luteolus* and a species of *Penicillium* isolated from *Calluna* raw humus.

It is difficult to assess the growth of fungi in this type of culture other than subjectively and growth is therefore indicated in the following terms. ++ indicates good vigorous growth, + a small amount of growth, and — no growth.

The extract of *Calluna* raw humus used had a reaction of pH 3·61 and one month after inoculation growth results shown in Table XI were recorded.

TABLE XI

Boletus bovinus	*B. variegatus*	*B. elegans*	*B. scaber*	*Rhizopogon luteolus*	*Penicillium spp.*
—	—	—	+	—	+

It seemed possible that shortage or absence of mineral or organic nutrients may be the cause of lack of growth of these fungi in the presence of *Calluna* raw humus extract. Therefore the following mineral and organic nutrients were added to each 100 ml. of *Calluna* raw humus extract before sterilization by chloroform:

0·5 ml 10 per cent NH_4Cl
0·5 ml 10 per cent $MgSO_4\ 7H_2O$
1·0 ml 10 per cent KH_2PO_4
1·0 gm glucose
2·0 ml 10 per cent solution of malt extract

Whenever mineral nutrients or malt extract have been used in these investigations, small volumes of concentrated solutions were added, glucose was added as a solid, to avoid unnecessary dilution of the experimental extracts, and equal quantities of water were added when comparisons with unamended extracts were being made.

The reaction of the *Calluna* raw humus extract after the e additions was pH 3·67. One month after inocula' on the following growth results were recorde((see Table XII).

TABLE XII

Boletus bovinus	*B. variegatus*	*B. elegans*	*B. scaber*	*Rhizopogon luteolus*	*Penicillium spp.*
—	—	—	++	—	++

Although the growth of *Boletus scaber* and the *Penicillium* species is greater than in the absence of added nutrients the other strains of fungi still failed to grow.

The reaction of the *Calluna* raw humus extract is on the acid side of the reaction at which optimum growth has been observed for many of the mycorrhizal Hymenomycetes in pure culture. The reaction of the *Calluna* raw humus extract was adjusted to pH 5·02 by addition of $^N/_{10}$ NaOH and the growth of the same fungi on this substrate under the same conditions as before was ascertained (see Table XIII).

TABLE XIII

Boletus bovinus	*B. variegatus*	*B. elegans*	*B. scaber*	*Rhizopogon luteolus*	*Penicillium spp.*
+	+	+	+	+	+

When mineral and organic nutrients were added to the *Calluna* raw humus whose reaction had been adjusted to pH 5·02 the reaction became pH 4·92 and the growth of the fungi on this substrate was as shown in Table XIV.

TABLE XIV

Boletus bovinus	*B. variegatus*	*B. elegans*	*B. scaber*	*Rhizopogon luteolus*	*Penicillium spp.*
++	++	++	++	++	++

To clarify the role of reaction in the growth of the fungi under these conditions the reaction of a medium containing only the mineral and organic nutrients was adjusted to pH 3·52 (i.e. approximately that of the *Calluna* raw humus extract) and the growth of the fungi on this medium, under the same conditions as before, compared with their growth on the *Calluna* raw humus extract and on the mineral and organic nutrient medium whose reaction (pH 4·85) had not been adjusted (see Table XV).

TABLE XV

	Boletus bovinus	*B. variegatus*	*B. elegans*	*B. scaber*	*Rhizopogon luteolus*	*Penicillium spp.*
Mineral and organic nutrient medium pH 3·52	++	++	++	++	++	++
Mineral and organic nutrient medium pH 4·85	++	++	++	++	++	++

From these results it would seem that the inability of some of the mycorrhizal Hymenomycetes to grow on the *Calluna* raw humus extracts is not due to an unfavourable reaction or to lack of nutrients but apparently to some inhibitory factor which is more active at more acid reactions. The strain of *B. scaber* used seems to be able to tolerate the inhibitory factor better than the strains of the other mycorrhizal Hymenomycetes tested.

In the case of a few of the extracts of *Calluna* raw humus which have been prepared, some of the strains of mycorrhizal Hymenomycetes other than *B. scaber* have shown some growth. This may be due to differences in the initial concentration of inhibitor in the various samples of *Calluna* raw humus used and which undergoes considerable dilution during the preparation of the extracts. It may well be that the concentration of inhibitor initially present in the raw humus is even sufficient to prevent the growth of this strain of *B. scaber*.

(d) **Removal of the factor present in extracts of *Calluna* raw humus which is inhibitory to the growth of mycorrhizal Hymenomycetes, by the growth of saprophytic microorganisms**

Ploughing of *Calluna* heathland, which results in increased aeration of the soil and the death of most of the *Calluna* plants, can result in better growth, for a time at least, of trees, including spruce, subsequently planted there. Both increased aeration and the residues from the killed *Calluna* plants may be expected to result in increased microbiological activity in the heathland soil.

Since ploughing is associated with improved tree growth, if there is a relationship between the factor in raw humus extracts which is inhibitory to the growth of mycorrhizal Hymenomycetes and the checked growth of spruce, then it may well be that removal of such an inhibitory factor is promoted by one or more of the effects of ploughing.

The experiences of Rayner and Neilson-Jones (1944) in nurseries established on former *Calluna* heathland, and the results of pot experiments with soils from such sites, indicate that the effect of any substance which may be directly or indirectly inhibitory to the growth of tree seedlings on these soils disappears only slowly in the absence of added materials such as composts of one kind or another, when the tree seedlings then develop an abundance of short roots and mycorrhizal associations. In addition Yeatman (1955) observed that when heather around Sitka spruce trees, whose growth had been checked, was killed by a *Calluna* mulch, the spruce developed new roots in the mulch but not in the undisturbed *Calluna* raw humus, for at least a few years after the mulch was applied. This again indicates that the inhibitory factor seems to take some time to disappear from *Calluna* raw humus. On the other hand, it has been observed that sporophores of *Boletus scaber* appear in the shade of young birch trees only a few years old which have become established on a *Calluna* heathland podzol where the *Calluna* plants have been killed by hoeing but the site not otherwise cultivated. In this case constituents of the birch litter may have promoted the removal of inhibitory substances from the *Calluna* raw humus.

It therefore seemed likely that removal of the factor present in extracts of *Calluna* raw humus which is inhibitory to the growth of mycorrhizal Hymenomycetes might be expedited by addition of material or microorganisms, from a compost found to be effective in the field, to such an extract when this was placed under conditions suitable for the growth and activity of saprophytic microorganisms.

An aqueous extract of *Calluna* raw humus prepared in the usual way and inhibitory to the growth of mycorrhizal Hymenomycetes was therefore subjected to various treatments in an attempt to remove the inhibitory factor. The treatments included addi-

tion of a small quantity of an aqueous extract of a compost (C_5) found by Rayner and Neilson-Jones (1944) to be effective in promoting the growth of tree seedlings in *Calluna* heathland nurseries. The extract was prepared by adding 500 ml. of distilled water and 50 ml. of chloroform to 200 gms of fresh, moist compost and allowing the mixture to stand with occasional shaking for four days. 300 ml. of a golden yellow fluid were obtained and this was stored in the presence of chloroform until required.

Separate 100 ml. quantities of the *Calluna* raw humus extract were subjected to the following treatments at room temperature.

1. Standing unaerated in the presence of chloroform.
2. As 1, but with 2 ml. of the compost extract added.
3. Air bubbled through until all chloroform had been removed, when very small particles of *Calluna* raw humus and C_5 compost were added and the aeration continued.
4. As (3) but with 2 ml. of extract of C_5 compost added.
5. Sterile air bubbled through the extract previously sterilized by chloroform.
6. As 5 but with 2 ml. of extract of C_5 compost (sterilized by chloroform) added.

After 4 weeks aeration was stopped. Growth of microorganisms was not detectable in flasks 1, 2, 5 and 6, but there was a heavy growth of microorganisms in flasks 3 and 4. The colour of the fluids in flasks 3 and 4, and especially in 4, had become much paler as a result of the treatment, whereas the colour of the extracts in the other flasks remained unchanged.

Where necessary the volume of the extract in each flask was made up to 102 ml. with distilled water; this was after removal of the growth of microorganisms by centrifuging in the case of flasks 3 and 4. The reaction (glass electrode) of the six fluids was then ascertained.

Treatment	*pH*
1	3·57
2	3·59
3	4·24
4	4·44
5	3·61
6	3·63

To ensure an adequate supply of mineral and organic nutrients for the growth of the mycorrhizal Hymenomycetes, 0·5 ml. of 10 per cent $MgSO_4$ $7H_2O$, 0·5 ml. 10 per cent NH_4Cl, and 1·0 ml. 10 per cent KH_2PO_4, 1·0 gm. glucose and 2 ml. of a 10 per cent solution of malt extract, were added to each of the six fluids. Fluid media (numbers 7 and 8) containing these mineral and organic nutrients added to distilled water, instead of *Calluna* raw humus extract, and with reactions adjusted to two different levels, were also prepared.

As the growth of microorganisms in fluids 3 and 4 was accompanied by considerable change in acidity, the reaction of half the volume of fluid from each of these flasks was adjusted to approximately that of the fluids 1, 2, 5 and 6.

The final reactions of the various test fluids before distribution in 5 ml. quantities among sterile 50 ml. conical flasks, containing 10 gms sand, and sterilization by chloroform were:

Fluid	pH (glass electrode)
1	3·66
2	3·70
3a	4·34
3b	3·65
4a	4·49
4b	3·65
5	3·73
6	3·72
7	4·89
8	3·68

After sterilization by chloroform in the usual way the flasks were inoculated with strains of various mycorrhizal Hymenomycetes. The growth results in Table XVI were recorded after the inoculated flasks had stood at room temperature for 3 weeks.

The general trend of these results indicates that the inhibition to the growth of mycorrhizal Hymenomycetes diminishes when the fluids are aerated in the presence of saprophytic microorganisms whereas aeration alone does not bring about disappearance of the inhibitory factor i.e. it is not readily volatile. The diminution of the inhibitory factor does not seem to be due to the change to a reaction more favourable for the growth of the mycorrhizal Hymenomycetes. There is nothing to suggest that the extract of C_5 compost is necessary for the diminution of the inhibitory factor but on the other hand there is no reason to suppose that C_5 compost and probably birch litter and dead *Calluna* plant material would hinder the removal of the inhibitory factor in the field, and they may assist in its removal.

The finding that the growth of microorganisms in aerated extract of *Calluna* raw humus results in removal of the inhibitory factor suggests that ploughing of *Calluna* heathland may assist the removal of such an inhibitory factor in the field and that the activities of saprophytic microorganisms may not be the source of the inhibitory factor.

(e) Investigation of *Calluna* litter as the source of the factor inhibitory to the growth of mycorrhizal Hymenomycetes in *Calluna* raw humus extracts

Although the vigorous development of spruce roots and mycorrhizal associations in the *Calluna* mulch applied around spruce trees, whose growth on

TABLE XVI

Fluid Number	*Amanita muscaria*	*Boletus elegans*	*Rhizopogon luteolus*	*Boletus scaber*	*Boletus variegatus*	*Boletus granulatus*	*Boletus luteus*	*Boletus bovinus*	*Penicillium spp.*
1	—	—	—	++	—	—	—	—	++
2	—	—	—	++	—	—	—	+	++
3a	++	++	+	++	++	+	++	++	++
3b	+	++	—	++	++	++	++	++	++
4a	—	+	+	++	—	+	++	—	++
4b	++	++	—	++	++	++	++	++	++
5	—	—	—	+	—	—	—	—	++
6	—	—	+	++	—	—	—	—	++
7	+	++	++	++	+	++	++	++	++
8	+	++	++	++	+	++	++	++	++

Calluna heathland has previously been checked in the presence of living *Calluna*, would not lead one to expect that inhibitors to the growth of mycorrhizal Hymenomycetes are added to the system by the mulching process, it must be remembered that much of the mulching material is moribund *Calluna* plant material and not dead *Calluna* litter. Melin (1946), however, found that the freshly fallen litter of maple, birch, beech, oak, aspen and pine contained water soluble material which inhibited the growth of many soil Hymenomycetes, whereas extracts of the litter of *Glyceria maxima* had no inhibitory effect on the fungi tested. In addition, very low concentrations of the inhibitory extracts stimulated the growth of the mycorrhizal and litter decomposing Hymenomycetes very considerably, and of course mycorrhizal associations are well developed in the raw humus layers developing from the litter of some of these tree species.

In order to ascertain whether or not *Calluna* litter contains a factor inhibitory to the growth of mycorrhizal Hymenomycetes, aqueous extracts of *Calluna* litter were prepared. The *Calluna* litter was collected from *Calluna* plants growing on a *Calluna* heathland podzol, in the same area as that from which the *Calluna* raw humus was collected, by shaking the plants over sheets of paper. The dead leaf material obtained in this way was air dried at room temperature and 50 gms of it were extracted with 500 ml. of distilled water and 20 ml. of chloroform. After standing for 5 days at room temperature, with occasional shaking, 410 ml. of pale yellow fluid were obtained. This fluid was clarified by centrifuging and then tested, in its original state and after amendment in various ways, for inhibitory activity towards mycorrhizal Hymenomycetes and other fungi, under the same conditions as the extracts of *Calluna* raw humus were tested:

The various fluids and their reactions were:

	pH
1. *Calluna* litter extract in original state	3·84
2. *Calluna* litter extract + mineral and organic nutrients	3·94
3. *Calluna* litter extract with reaction modified by addition of NaOH	4·92
4. *Calluna* litter extract + mineral and organic nutrients and reaction modified by addition of NaOH	4·92
5. Mineral and organic nutrients in distilled water, reaction adjusted by addition of HCl	3·78
6. Mineral and organic nutrients in distilled water	4·89

The mineral and organic nutrients added were 0·5 ml. 10 per cent $MgSO_4\,7H_2O$, 0·5 ml. 10 per cent NH_4Cl, 1·0 ml. KH_2PO, 1·0 gm. glucose, and 2 ml. of 10 per cent malt extract solution to 100 ml. of litter extract or distilled water. The following results for the growth of various fungi were recorded after the inoculated flasks had been standing at 21·5°C for 4 weeks. At present it is difficult to explain the failure of many of the fungi to grow in the extract of *Calluna* litter when the reaction had been adjusted to pH 4·92, unless it is due to some unknown experimental error. The remaining results do not point to *Calluna* litter as being the source of the inhibitory

TABLE XVII

The growth of strains of ectotrophic mycorrhizal fungi on aqueous extract of *Calluna* litter

Number of fluid	*Boletus luteus*	*Boletus elegans*	*Boletus scaber*	*Boletus bovinus*	*Boletus variegatus*	*Boletus granulatus*	*M.r. silv. α*	*Amanita muscaria*	*Rhizopogon luteolus*	*Rhizoctonia silvestris*	*M.r. atrovirens*	*Penicillium spp.*
1	+ +	+ +	+ +	+	+	+	|	|	+	+	+	+
2	+ +	+ +	+ +	+ +	+ +	+ +	|	+ +	+ +	+ +	+ +	+ +
3	|	|	+	|	|	|	+	|	|	|	+	+ +
4	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	|	+ +	+ +	+ +
5	+ +	+ +	+ +	+ +	+ +	+	+ +	+ +	+ +	+ +	+ +	+ +
6	+ +	+ +	+ +	+ +	+	+	+ +	+	+ +	+ +	+ +	+ +

factor observed in extracts of *Calluna* raw humus; if anything they point to the litter extract being nutritionally inadequate to support the growth of a few of the species of fungi.

The apparent absence of inhibitory factor in the leafy *Calluna* material may be the basis of the inconclusive results obtained by Braathe (1950) when spruce plants were watered with water which had stood in the presence of *Calluna* shoots and some root material for ½–1 hour, although the duration of the extraction time was extremely short.

(f) Concentration by vacuum distillation of the factor which can be extracted from *Calluna* raw humus and which is inhibitory to the growth of mycorrhizal Hymenomycetes

Raw humus was collected from a *Calluna* podzol in Berkshire in late autumn in 1958 after a wet and dull summer and autumn. The site was a very small opening in a pine stand; the *Calluna* growing there was subjected to considerable shading and was leggy with only sparse flowers. Occasional pine roots were found in the F and H layers of the *Calluna* raw humus. These roots were approximately 3 mm. in diameter and almost unbranched. A few short forked roots with very swollen, globular, almost white tips were found in the debris beneath a patch of moss. The much branched pine roots beneath the pine canopy, where *Calluna* was completely absent, had forked short roots with reddish brown or tan coloured mycorrhizal associations.

The raw humus from beneath the *Calluna* plants was very moist when collected. An aqueous extract was prepared by extracting the raw humus with distilled water in the presence of chloroform as before. When expressed and centrifuged the extract was clear golden brown in colour and had a reaction of pH 3·5. This extract was tested for its effect on the growth of mycorrhizal Hymenomycetes by the method previously described. In those cases where mineral nutrients were added to the extract a more complex salt mixture than previously, that of medium III of Norkrans (1950), was used and 0·15 ml. of the modified Hoagland's A–Z mixture (Robbins and Kavanagh 1938) were also added to each 150 ml. of extract. Since additional strains of *Boletus scaber* had become available these were included in the tests along with a number of non-mycorrhizal fungi. The growth results are given in Table XVIII.

These results suggest that the extract from *Calluna* raw humus associated with shaded and leggy *Calluna* is somewhat less inhibitory towards mycorrhizal Hymenomycetes, although it is active against some of the strains of *Boletus scaber*, than the extract of *Calluna* raw humus from north-east Yorkshire. The growth results with extracts to which mineral

TABLE XVIII

The effect of concentration of an extract of *Calluna* raw humus by vacuum distillation on the growth of mycorrhizal Hymenomycetes and other fungi

	Boletus scaber (heathland)	*Boletus scaber (deciduous woodland)*	*Boletus scaber (heathland)*	*Boletus scaber (moorland)*	*Boletus scaber (deciduous woodland)*	*Boletus carpini*	*Boletus elegans*	*Boletus bovinus*	*Boletus granulatus*	*Boletus luteus*	*Boletus variegatus*	*M.r. silvestris* ∝	*Rhizopogon luteolus*	*Amanita muscaria*	*M.r. nigrostrigosum*	*M.r. atrovirens* α	*Rhizoctonia silvestris*	*Marasmius peronatus*	*Mycena pura*	*Clitocybe nebularis*	*Polysticus sanguineus*	*Polyporus fragilis*	*Lenziites betulina*	*Hypholoma fasciculare*	*Penicillium* spp. *isolated from Calluna raw humus*
Unconcentrated extract Reaction pH 3·5	++	—	++	—	±	—	—	+	—	—	++	±	+	±	+	++	++	++	+	+	++	+	++	++	++
Unconcentrated extract with mineral nutrients, malt and glucose added. Reaction pH 3·5	++	—	++	—	—	—	++	—	—	—	++	++	++	—	++	++	++	++	+	++	++	++	++	++	++
Extract concentrated by vacuum distillation Reaction pH 3·5	++	—	++	—	—	—	—	—	—	—	—	—	—	—	—	++	++								
Extract concentrated by vacuum distillation with malt and glucose added. Reaction pH 3·5	++	—	++	—	—	—	—	—	—	—	++	—	—	—	—	++	++								
Culture medium containing mineral nutrients, malt and glucose. Reaction pH 3·5	++	+	++	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+	++	++	++

++ = growth extending over most or all of surface of grit in flask
+ = growth extending some way on to surface of grit around inoculum
± = further growth only on unoccupied surface of inoculum
— = no growth.

and organic nutrients have been added do not suggest that inadequate nutrition is influencing the growth of the mycorrhizal Hymenomycetes. The growth of the strains of mycorrhizal Hymenomycetes on a culture medium containing only the mineral salts and organic nutrients, and having the same reaction (pH 3·5) as the extract of *Calluna* raw humus, indicates that the reaction is not the cause of the decreased growth of some of the fungi in the *Calluna* raw humus extract. These results and those obtained for the extract of *Calluna* raw humus from north-east Yorkshire suggest that there may be a range of sensitivity among the various mycorrhizal Hymenomycetes to the inhibitory factor in *Calluna* raw humus.

In view of the apparently lower inhibitory activity towards mycorrhizal Hymenomycetes of the Berkshire sample of *Calluna* raw humus, compared with that of the north-east Yorkshire sample, part of the extract from the Berkshire *Calluna* raw humus was concentrated, by distillation under reduced pressure at a temperature of 40°C, to approximately 1/6th of its initial volume. The distillate was clear and colourless and had a reaction of pH 3·7. The concentrated residue was clarified by centrifuging, had a reaction of pH 3·25, and was tested, at a reaction of pH 3·5, for inhibitory activity towards various mycorrhizal Hymenomycetes and other fungi by the method previously described. Although malt extract and glucose were added to the concentrated extract in one case, additional mineral nutrients were not added in view of the increase in concentration of those already present as a result of the vacuum distillation. The results of these tests (Table XVIII) indicate that as a result of concentration the extract has become more inhibitory towards mycorrhizal Hymenomycetes and *M.r. nigrostrigosum*. Two strains of *Boletus scaber*, isolated from sporophores from *Calluna* heathland areas, were able to grow satisfactorily in the concentrated extract as is also the strain of *Boletus variegatus* when mineral and organic nutrients have been added.

These findings suggest that, perhaps as a result of the *Calluna* at present growing on the site being subjected to shading or to its having reached the leggy and possibly senescent stage, the level of inhibitor to mycorrhizal Hymenomycetes is now lower in this *Calluna* raw humus than in that from the open heathland of north-east Yorkshire, and that the inhibitor can be concentrated by distillation of the extract under reduced pressure.

(g) The effect of aqueous extracts of various soils and soil organic layers on the growth of mycorrhizal Hymenomycetes

The effect of aqueous extracts of two brown forest soils and the surface organic layers of a pine stand on the growth of mycorrhizal Hymenomycetes has been investigated.

Two mull soils were used, one supporting an even-aged stand of sessile oak, about 50 years old, growing on an acid sandy loam with a very sparse ground flora, the other supporting a similar stand of Wych elm growing on a less acid sandy soil having a ground flora of *Urtica dioica*, *Mercurialis perennis* and mosses.

The small amount of loose plant debris on the surface of the soil was removed before samples of the mull (0–10 cms.) were collected in early March. Stones, roots and other obvious plant material were removed from the samples before they were extracted with distilled water; equal weights of water and fresh soil, on an oven dry basis, being used. These mixtures stood at room temperature in the presence of chloroform for nine days when as much fluid as possible was squeezed out in a press. The extracts were clarified by centrifuging and their reactions were pH 4·24 for the oak soil and pH 5·34 for the Wych elm soil. Half of each extract was supplemented by addition of Norkrans salt mixture, Hoaglands A–Z mixture, glucose and malt and the reaction of both halves then adjusted to pH 3·6 by the addition of sulphuric acid. The capacity of the extracts to allow the growth of mycorrhizal Hymenomycetes was then tested by the method previously described. The growth results are given in Table XIX.

It is clear from these results that more strains of mycorrhizal Hymenomycetes were able to grow in the presence of extracts of oak and Wych elm soil, especially in the presence of added nutrients, than grew in the presence of extracts of *Calluna* raw humus.

As has been indicated previously ectotrophic mycorrhizal associations are developed abundantly by trees growing on *Calluna* heathlands from which living *Calluna* has been completely suppressed or removed. Therefore the surface organic layers of the soil beneath a 30–40 year old stand of maritime pine, devoid of ground flora and growing on a little-disturbed, characteristic *Calluna* podzol in Berkshire were examined for their capacity to inhibit the growth of mycorrhizal Hymenomycetes and other fungi. Samples of the following horizons were collected in mid-October.

(a) After removal of the L layer the 5 cm. thick F layer was sampled. It was slightly moist, consisted of easily recognizable brittle fragments of pine needles and was traversed by a number of pine roots having "Gabelmykorrhiza".

(b) Beneath the F layer of pine debris and sharply demarcated from it were the slightly damp surface organic layers of the soil associated with the former *Calluna* vegetation. Recognizable *Calluna* litter and hollow *Calluna* stems were still present on the surface of the old *Calluna* mor. The latter was about

TABLE XIX

The growth of mycorrhizal Hymenomycetes and other fungi on aqueous extracts of mull soils

	Boletus scaber	*Boletus elegans*	*Boletus bovinus*	*Boletus granulatus*	*Boletus luteus*	*Rhizopogon luteolus*	*M.r. nigrostrigosum*	*M.r. atrovirens*	*Control (uninoculated)*	*Control (uninoculated)*
Oak soil extract. Reaction pH 3·6	++	+	++	+	—	—	+	++	—	—
Oak soil extract and mineral nutrients, malt and glucose. Reaction pH 3·6	++	++	++	—	++	++	++	++	—	—
Wych elm soil extract. Reaction pH 3·6	—	—	+	+	+	—	+	++	—	—
Wych elm soil extract and mineral nutrients, malt and glucose. Reaction pH 3·6	++	++	++	—	—	++	++	++	—	—

++ = good vigorous growth over surface of grit
+ = some growth on surface of grit
— = no growth.

1 cm. in thickness and although dense and compact contained numerous much branched pine roots with reddish brown or yellow brown 'Gabelmykorrhiza' similar to those found in the F layer of the pine debris.

These materials were extracted for seven weeks at room temperature with approximately their own weights of deionized water in the presence of chloroform; sufficient water being added to the material, packed into a glass jar, that it was just submerged. The fluids were extracted by means of a press and centrifuged; their reactions were then found to be pH 3·30 for the pine F layer material and pH 2·95 for the old *Calluna* mor. The extracts were then assayed, by the method previously described, for their capacity to support or inhibit the growth of various strains of mycorrhizal Hymenomycetes and other fungi in the presence and absence of added mineral nutrients, malt and glucose. The results of these experiments are given in Table XX. Although some of the strains of mycorrhizal Hymenomycetes fail to grow in extracts of the pine F layer material they are all able to grow when glucose and malt are added although the growth of *Boletus carpini* was rather feeble. There is little evidence of the presence in the extract of a factor having an adverse influence on the mycorrhizal Hymenomycetes although it is not easy to understand why some strains failed to grow when the mineral salt mixture was added to the extract as well as glucose and malt. The mycorrhizal Hymenomycetes were able to grow, even though some strains grew rather feebly, in the presence of unamended extracts of the old *Calluna* raw humus; when nutrients were added to the extract the growth of some strains was improved whilst a few failed to grow. This extract of old *Calluna* raw humus is much less inhibitory to the growth of mycorrhizal Hymenomycetes than the extracts of *Calluna* raw humus bearing vigorous unshaded *Calluna* and any extractable inhibitory activity it may have had has been almost, if not completely, lost, as might be expected from the presence now in the old *Calluna* raw humus of pine roots with characteristic ectotrophic mycorrhizal associations. All the strains of fungi used in the test grew vigorously on a culture medium of Norkrans (1950) mineral salt mixture with glucose and malt added and having a reaction (pH 3·0) similar to that of the extracts. The increased growth of strains of fungi other than the mycorrhizal Hymenomycetes when organic nutrients were added to the extracts indicates that the already satisfactory growth of these organisms on the unamended extracts was only limited by inadequate supplies of nutrients.

The main points of the results arising from the experiments with extracts of *Calluna* raw humus and soils, or horizons of soils, not supporting a vegetation of *Calluna* may be summarized:

1. The growth of mycorrhizal Hymenomycetes is inhibited by extracts of *Calluna* raw humus supporting vigorous *Calluna* growing in full light. The inhibitory effect seems less marked with extracts of *Calluna* raw humus supporting *Calluna* growing in reduced light intensity.

2. The failure of mycorrhizal Hymenomycetes to grow on extracts of *Calluna* raw humus does not appear to be due to insufficiency of nutrients or meta-

TABLE XX

The effect of aqueous extracts of various soil organic horizons on the growth of mycorrhizal Hymenomycetes and other fungi

	Boletus scaber (heathland)	*Boletus scaber (Sweden—deciduous woodland)*	*Boletus scaber (heathland)*	*Boletus scaber (moorland)*	*Boletus scaber (woodland)*	*Boletus carpini*	*Boletus elegans*	*Boletus bovinus*	*Boletus granulatus*	*Boletus luteus*	*Boletus variegatus*	*M.r. silvestris* α	*Rhizopogon luteolus*	*Amanita muscaria*	*M.r. nigrostrigosum*	*M.r. atrovirens*	*Rhizoctonia silvestris*	*Phoma radici callunae*	*Polystictus sanguineus*	*Hypholoma fasciculare*	*Penicillium spp.*	*Uninoculated control*	*Uninoculated control*	*Uninoculated control*
Extract of Maritime pine F layer material. Reaction pH 3·3	++	+	++	±	+	±	—	++	+	±	++	++	±	++	++	++	++	++	++	++	++	—	—	—
Extract of Maritime pine F layer material with mineral nutrients added. Reaction pH 3·3	++	+	++	+	+	—	—	++	+	+	++	++	+	++	++	+++	++	++	++	++	+++	—	—	—
Extract of Maritime pine F layer material with mineral nutrients, malt and glucose added. Reaction pH 3·3	+++	++	+++	—	+	—	—	++	—	—	+++	+++	+++	—	+++	+++	++	+++	+++	+++	+++	—	—	—
Extract of Maritime pine F layer material with malt and glucose added. Reaction pH 3·3	++	+	++	+	++	±	++	++	++	++	++	++	++	++	++	+++	++	++	+++	++	+++	—	—	—
Extract of old Calluna mor from Maritime pine stand. Reaction pH 3·0	++	+	++	+	++	+	++	++	±	±	++	++	±	++	++	++	++	++	++	+++	++	—	—	—
Extract of old Calluna mor from Maritime pine stand with mineral nutrients added. Reaction pH 3·0	++	++	++	+	+	—	++	++	++	+	++	—	+	++	++	+++	++	++	++	+++	+++	—	—	—
Extract of old Calluna mor from Maritime pine stand with mineral nutrients, malt and glucose added. Reaction pH 3·0	+++	++	+++	++	—	—	+++	++	++	++	++	—	++	±	++	+++	++	+++	+++	+++	+++	—	—	—
Extract of old Calluna mor from Maritime pine stand with malt and glucose added. Reaction pH 3·0	++	+	++	+	+	+	++	—	+	—	++	++	+	++	++	+++	++	+++	++	+++	+++	—	—	—
Culture medium containing mineral nutrients, malt and glucose. Reaction pH 3·0	+++	++	+++	++	++	++	+++	++	++	++	++	++	+++	++	+++	+++	++	+++	+++	+++	+++	—	—	—

+++ vigorous dense growth over surface of grit.
++ growth extending over most or all of surface of grit.
+ growth extending some way on to surface of grit around inoculum.
± further growth only on unoccupied surface of inoculum.
— no growth.

bolites or to an unsuitable reaction i.e. the rather acid reaction.

3. The activity of the inhibitory factor seems to be less pronounced at less acid reactions.

4. The activity of the inhibitory factor can be removed by saprophytic soil microorganisms.

5. Whilst extracts of *Calluna* raw humus associated with unshaded, vigorous *Calluna* inhibit the growth of mycorrhizal Hymenomycetes, with the exception of a strain of *Boletus scaber*, similar extracts from soils or soil horizons not currently associated with a vegetation of *Calluna* allow the growth of most of the strains of mycorrhizal Hymenomycetes tested, especially when the extracts are amended by the addition of mineral and organic nutrients.

6. The factor inhibitory to the growth of mycorrhizal Hymenomycetes does not appear to be present in *Calluna* litter.

7. Some strains of *Boletus scaber*, originating from sporophores from *Calluna* heathland soils, seem to have considerable resistance to the inhibitory factor present in the extracts of *Calluna* raw humus although it must be borne in mind that the inhibitory factor must undergo considerable dilution during the process of extraction and is probably to a greater or lesser extent incompletely extracted so that in the *Calluna* raw humus in its natural state the concentration of inhibitory factor may well be sufficient to prevent the growth of these relatively resistant strains of *B. scaber*.

8. There is an indication that there may be a range of sensitivity among mycorrhizal Hymenomycetes to the inhibitory factor in *Calluna* raw humus.

Chapter 5

IMPLICATIONS OF THE EFFECT OF EXTRACTS OF *CALLUNA* RAW HUMUS ON THE GROWTH OF MYCORRHIZAL HYMENOMYCETES, AND ESPECIALLY THE DIFFERENTIAL EFFECTS ON STRAINS OF *BOLETUS SCABER*, FOR THE GROWTH OF TREES ON *CALLUNA* HEATHLAND

The method of extraction of the factor inhibitory to the growth of mycorrhizal Hymenomycetes from *Calluna* raw humus, and the fact that extracts prepared in a similar way from soils supporting other plant species do not exhibit a comparable inhibitory effect, makes it unlikely that the inhibitory effect is an artefact resulting from the method of preparation. The method of preparation inevitably results in dilution of the inhibitory factor and since it appears to be possible to concentrate the factor by vacuum distillation it seems probable that this factor could be present in some samples of *Calluna* raw humus at sufficient strength to inhibit the growth of all mycorrhizal Hymenomycetes. It may therefore be expected that this factor would prevent the formation of ectotrophic mycorrhizal associations on the roots of trees in contact with vigorous unshaded *Calluna* on heathlands of long standing.

Observations have been cited earlier which indicate that trees whose growth has been checked by the presence of *Calluna* are almost if not quite devoid of ectotrophic mycorrhizal associations and their roots may show no indications of attack or parasitism by microorganisms. The observations that trees may remain alive but in check for 30 years or so in the presence of living *Calluna* and then recover spontaneously and also respond quickly at any time to the application of nitrogenous fertilizers are also indications that an organism pathogenic in the usual sense of attacking plant tissue is not likely to be involved. In addition the roots of trees whose growth, on previously similar sites, is unchecked or has been released from check as a result of such treatments as mulching or the roots reaching the soil beneath trees which have formed canopy, are characterized by the presence of ectotrophic mycorrhizal associations. Such observations, along with those of Melin and Nilsson (1958) and Melin, Nilsson and Hacskaylo (1958) indicating that the mycorrhizal association promotes the absorption of mineral nutrients, suggest that the growth of trees under *Calluna* heathland and other conditions may be dependent on the formation of mycorrhizal associations for the absorption of mineral nutrients in competition with the roots of other plants especially if the supply of mineral nutrients is small. Therefore it seems feasible that a factor in *Calluna* raw humus inhibitory to the growth of ectotrophic mycorrhizal fungi is a very likely cause of the checked growth of trees on *Calluna* heathland, this being brought about by its effect on the formation of ectotrophic mycorrhizal associations by the tree which are the potential mineral nutrient absorbing system of the tree.

To take this hypothesis further, the apparently greater resistance of certain strains of *Boletus scaber* to the inhibitory factor of *Calluna* raw humus as compared with the resistance of the strains of other mycorrhizal fungi tested would seem to be of significance in the establishment of trees on *Calluna* heathland. Levisohn (1957a) has reported differing reactions by *B. scaber* compared with the reactions of other ectotrophic mycorrhizal fungi when in mixed cultures with strains of *Alternaria tenuis* and towards culture filtrates of *Alternaria tenuis*. In both cases the growth of *B. scaber* was much less inhibited than was that of the other fungi. Similar results appear to have been obtained with gibberellic acid (Levisohn 1960a). A comparative study of the cultural characteristics of a number of strains of *B. scaber* isolated from different sporophores, believed to be identifiable as *B. scaber* on an orthodox taxonomic basis, obtained from different types of plant habitat has also been made by Levisohn (1959). There was a sharp distinction between the cultural characteristics of the two strains from sporophores from *Calluna* heathland sites (they grew more readily and were much more active in relation to various substrates) and those of strains from sporophores from moorland and woodland sites.

The ability of mycorrhizal Hymenomycetes to grow in the presence of extracts of *Calluna* raw humus which has not supported living *Calluna* for some time, and the destruction of the inhibitory factor by microorganisms, could mean that when the living *Calluna* of the *Calluna* heathland is interfered with and virtually excluded for a time by ploughing or burning, the level of inhibitory factor will progressively decrease either by being leached out or being destroyed by the activity of microorganisms. As the concentration of inhibitory factor decreases a

level will presumably be reached at which strains of *Boletus scaber* resistant to this factor will be able to grow or their spores to germinate before other mycorrhizal Hymenomycetes are able to grow, although the possibility that there are strains of other species of mycorrhizal Hymenomycetes which are resistant to the inhibitory factor cannot be ruled out. It may be expected that any tree species which is able to form mycorrhizal associations with resistant strains of *Boletus scaber* will be able to establish itself more quickly before the site is massively reinvaded by *Calluna* (say about 4 or 5 years) and production of inhibitory factor is renewed. Once established and equipped with mycorrhizal associations and spreading lower branches such trees will gradually shade out more and more of the reinvading *Calluna* until canopy is formed.

Present knowledge, however, indicates a certain degree of specificity between various tree species and the species of mycorrhizal fungi although the picture is doubtless by no means complete. The formation of ectotrophic mycorrhizal associations between a particular higher plant species and a particular species of fungus may be indicated from two kinds of observations. The first depends on the fact that most if not all of the fungi considered to be able to form A and B type ectotrophic mycorrhizal associations are only known to produce sporophores in the presence of an appropriate higher plant and therefore the production of sporophores by one of these fungi in a pure stand of a particular higher plant species may be regarded as strong evidence of the formation of an association between the fungus and the higher plant. On the other hand the absence of sporophores of a particular mycorrhizal fungus does not necessarily mean that it cannot form associations with that particular higher plant. The second type of observation depends on culture experiments in which pure cultures obtained from sporophores of known Basidiomycetes and seedlings of the higher plant are grown together under aseptic conditions. The formation of mycorrhizal associations under such conditions may be regarded as strong evidence that the two organisms are likely to form associations but the absence of formation of mycorrhizal associations under such conditions does not necessarily mean that the two organisms are unable to form associations. The two types of observation should be used in conjunction with each other in assessing whether or not a particular fungus is likely to form associations with a particular higher plant in the field.

If the information concerning *Boletus scaber* is examined from this point of view it will be found, as is to be expected, that there are relatively few pure culture synthesis experiments and rather more records of the parallel occurrence of *Boletus scaber* sporophores and various tree species.

Melin (1923b) reported that *Boletus scaber* was one of the most widely distributed Hymenomycetes in birch and aspen woods and in woods of other broadleaved species but was only found in coniferous woods in exceptional cases. Like Thesleff he has observed *Boletus scaber* in association with larch, oak and alder; he also quotes Peyronel and Thesleff as stating that *B. scaber* may be found associated with oak and larch in Finland and with *Fagus*, *Corylus*, *Castanea* and *Sorbus aucuparia* in Northern Italy. Melin also sites a number of other references supporting the very frequent occurrence of *Boletus scaber* in birch woods.

It would seem to be significant that sporophores of *Boletus scaber* are to be found beneath isolated young birch trees of bushy habit and about one metre in height which have become established on *Calluna* heathland after disturbance of the previously vigorous heather.

Modess (1941) states that he has found *Boletus scaber* in pure coniferous woods on repeated occasions but he does not indicate whether they were of pine or spruce or both.

The occurrence of sporophores of *Boletus scaber*, along with those of other species of mycorrhizal fungi, in a pure pine stand 85–100 year old, has been reported by Björkman (1942).

In a survey of the Hymenomycetes to be found in various kinds of woods Malmström (1937) records that *Boletus scaber* occurs in beech and birch woods (containing silver fir in a few places) but he does not record it from the spruce woods examined. On the other hand Warcup (1951) records *Boletus scaber* from a pine stand although it does not seem certain whether other tree species were present or not. The only definite statement regarding the parallel occurrence of *Boletus scaber* and *Picea abies* seems to be that of Levisohn (1960b) who merely states that *Boletus scaber* has been observed to occur in the neighbourhood of *Picea abies* but whether in a pure stand or not is not stated. Her comment with regard to pines is that sporophores of *Boletus scaber* are not frequently met in pine stands and the fungus has not been acclaimed as a common mycorrhiza former for the genus *Pinus*.

In pure culture experiments Melin (1923b) observed that *Boletus scaber* formed mycorrhizal associations with birch and aspen seedlings. Subsequently, he recorded (Melin 1925) the results of pure culture experiments with *Boletus scaber* and pine and spruce seedlings. He found that although the hyphae of *B. scaber* surrounded the roots of *Pinus sylvestris* seedlings after 16 months, in only one case did they penetrate the roots and this occurred only in scattered cells of the tap root and mycorrhizal associations were not formed. In the case of Norway spruce

seedlings, although the fungus developed rapidly the seedlings were very weak and the hyphae penetrated the roots profusely, especially the cortical cells of the tap root, and the fungus was considered to be highly virulent towards the seedlings. In his assessment of these findings in relation to field conditions Melin is of the opinion that *Boletus scaber* may be a mycorrhizal associate of a secondary order for pine. He is uncertain how the species of *Boletus* (*B. scaber*, *B. variegatus* and *B. badius*) found to be highly virulent towards spruce in pure culture would behave towards this species in nature, since in general these fungi do not belong to the spruce forest, but suggests that they may act as mycorrhizal associates of a secondary order in cases where the fungus is less virulent and competition with other mycorrhizal fungi is not so strong.

It is unfortunate that in his extensive pure culture experiments with pine and spruce Modess (1941) did not use a strain of *Boletus scaber*. Both he and Melin found a number of other fungi which formed mycorrhizal associations with spruce under these conditions.

Young (1940) found that typical mycorrhizal associations were formed when seedlings of *Pinus taeda* and *Pinus caribaea* were grown under sterile conditions and inoculated with *Boletus scaber*.

Recently Levisohn (1960b) has found that, in pot cultures using sterilized sand, *Pinus sylvestris* seedlings developed abundant mycorrhizal associations when inoculated with *Boletus scaber*. The growth of the Pine seedlings was stimulated by comparison with the growth of control seedlings and of seedlings grown in the presence of *Boletus bovinus* when only a few mycorrhizal associations were formed.

Although the evidence is perhaps not so complete as one could wish, it does suggest that *Boletus scaber* is able to form mycorrhizal associations with birch, aspen, larch, oak, alder, beech, hazel, sweet chestnut, rowan and various species of pine. These tree species include a great proportion of those which have been recorded as being able to colonize *Calluna* heathland or are more readily established thereon in forestry practice. On the other hand it seems doubtful whether *Boletus scaber* forms mycorrhizal associations with Norway spruce nor does there appear to be any suggestion at present that associations are formed with other 'heather sensitive' species such as Lawson cypress, Sitka spruce and silver fir. It is therefore tempting to consider whether those tree species which have been recorded as being able to colonize disturbed *Calluna* heathland are those which in all probability seem to be able to form mycorrhizal associations with *Boletus scaber* whilst on the other hand the 'heather sensitive' species may not be able to form associations with *Boletus scaber*. There seems to be little if any evidence which is contradictory to this suggestion.

In terms of such a hypothesis the following sequence of events is envisaged for the growth of young trees on *Calluna* heathland following interference by burning or ploughing to such an extent that vigorous *Calluna* is virtually absent for several years. It is assumed that if the *Calluna* were not disturbed the growth of young trees on the heathland would be severely checked if not entirely prevented. Following the virtual obliteration of living *Calluna* the level of the factor in the soil inhibitory to the growth of mycorrhizal Hymenomycetes would be expected to decrease and observations suggest that it will be leached out and inactivated by microorganisms. Assuming that there is a threshold level of concentration of the inhibitory factor above which even the resistant forms of *Boletus scaber* may be unable to grow and form associations with tree roots then, after the concentration of the inhibitory factor has fallen below this level it may be expected that naturally occurring seedlings or young planted individuals of tree species, e.g. birch, pine and larch, which are able to form associations with *Boletus scaber* and are otherwise adapted to the *Calluna* heathland environment, will be able to establish themselves by the aid of such mycorrhizal associations. Indeed *Boletus scarber* sporophores are to be found beneath quite young birch trees which have invaded disturbed heathland. Such trees will have the maximum time in which to grow before the *Calluna* begins to return and production of inhibitory factor is renewed. If growth of the young trees has proceeded sufficiently before this occurs then, if the supply of mineral nutrients is adequate, one may expect the *Calluna* to be increasingly shaded out, as is especially the case with the multistemmed form of *P. montana* planted on the *Calluna* heathlands of Jutland, and its area of occupation reduced until the trees form a complete canopy and the *Calluna* is completely suppressed as in the case of pine and larch. It would be expected that plants of tree species which are unable to form associations with *Boletus scaber* would have to wait longer before the concentration of the inhibitory factor falls, after obliteration of the living *Calluna*, below the threshold level for the fungi which are more susceptible to the *Calluna* inhibitory factor and with which they are able to form associations. Thus they would have less time in which to grow before the reinvasion by *Calluna* and would find it more difficult to become established unless or until their roots can reach the soil beneath the canopy of a species able to form associations with *Boletus scaber*. In the soil beneath the canopy the concentration of inhibitory factor will be quite low enough for the roots of the 'heather sensitive' tree species to form associations with appropriate fungi which are more sensitive to the *Calluna* inhibitory factor. Such individuals of a 'heather sensitive' tree species will then

be able to grow and themselves shade out *Calluna*, and in time form complete canopy having been 'nursed' by the species able to form associations with *Boletus scaber*. In the case of trees planted on *Calluna* heathland the speed and manner in which this occurs will depend on the distribution pattern of the 'sensitive' and 'non-sensitive' tree species.

It is of course possible that there may be resistant strains of other species of mycorrhizal fungi but there seems to be no evidence of this so far from laboratory or field observations. If there are resistant strains of fungi able to form mycorrhizal associations with spruce it would be expected that spruce would be equal to birch and pine, etc. as a colonizer of *Calluna* heathland.

Chapter 6

THE POSSIBLE SOURCE OF THE FACTOR IN *CALLUNA* RAW HUMUS INHIBITORY TO THE GROWTH OF MYCORRHIZAL HYMENOMYCETES

Experiments with aqueous extracts of *Calluna* raw humus indicate that the factor inhibitory to the growth of mycorrhizal Hymenomycetes is inactivated or destroyed by the activities of soil organisms. On long established *Calluna* heathland tree seedlings or young planted trees surrounded by vigorous *Calluna* do not seem to develop ectotrophic mycorrhizal associations and their growth is checked. If the *Calluna* is shaded or killed the growth of the 'checked' trees will improve and ectotrophic mycorrhizal associations will develop on their roots. Although the 'heather sensitive' tree species are unable to compete with vigorous unshaded *Calluna* they are well able, when they have developed mycorrhizal associations, to compete with other tree species, less sensitive to the effect of *Calluna*, which have become established and formed canopy on the *Calluna* heathland; in fact there is on the contrary the well known 'nursing' effect of the sensitive species by the less sensitive species and it seems unlikely that the mineral nutrients required by the 'nurse' trees will be less than those needed by *Calluna*.

Such observations strongly suggest that the inhibitory effect is in some way associated with living *Calluna*. Since very heavy mulches of *Calluna* shoots result in the recovery of 'sensitive' tree species from 'check' and the factor inhibitory to the growth of mycorrhizal Hymenomycetes could not be detected in *Calluna* litter the problem seems to be narrowed still further to the living roots of the *Calluna* plant. As the inhibitory effect seems to disappear when the living *Calluna* is removed or killed this suggests that the inhibitory factor must be continuously produced to maintain the inhibitory level.

The inhibitory factor could arise in one or more of several ways from the *Calluna* root system or its immediate vicinity:

(a) from the roots themselves either with or without the influence of the associated endophyte,
(b) from the associated endophyte,
(c) from rhizosphere organisms,

The root tissues of a number of plant species have been shown to contain substances actually or potentially deleterious to plant roots or microorganisms. It is well known that juglone and the glucosides amygdalin, phloridzin and scopolin occur in the roots of various plants and that these substances or their hydrolytic products have a deleterious effect on plant roots or microorganisms.

Experiments with the roots of plants growing under aseptic conditions have shown that it may be expected that a variety of organic substances such as amino acids, sugars, vitamins, etc., will be released from root tissues into the medium surrounding the root but it seems very likely that, except perhaps in abnormal circumstances, in the case of intact plants most if not all of these substances arise from sloughed off root cells or are the products of their autolysis (e.g. Rovira 1956). On the other hand there has been much interest in the problem of the deleterious effect of flax on subsequent crops of the same plant and there have been a number of attempts to determine the nature and source of the effect. Becquerel and Rousseau (1941) found that when flax seedlings were grown under aseptic conditions in distilled water they apparently excreted materials which hindered the growth, based on comparisons of root elongation, of other flax seedlings, but not of the seedlings of other plant species, subsequently placed in contact with the water. They also found that the effect was much enhanced if the roots of the seedlings were killed by heating and suggested that when the flax crop is harvested the inhibitory factor passes into the soil when the root residues die. However, Börner, Martin, Clauss and Rademacher (1959) stated that aqueous extracts of flax root residues produced no appreciable effect on the growth of flax seedlings nor was there any inhibitory effect on the growth of flax seedlings of root excretions from flax seedlings grown under sterile conditions, although when in non-sterile culture solutions the growth of flax plants had a deleterious effect on the subsequent growth (stem length, dry weight of shoot and number of flowers but not on root growth) of other flax plants in the same culture solution even though the absorbed nutrients had been replaced. Hot water extracts of the roots of flax seedlings and alcohol extracts of the roots of flax plants just beginning to flower both contained materials which

hindered the germination and root growth of flax seedlings. There seems to be no clear evidence suggesting that, in an environment having physical and chemical characteristics corresponding to those of soil and in the absence of microorganisms, the roots of intact plants excrete organic material, such as the above mentioned toxic or potentially toxic substances, in the sense of excretion by the kidney for example whereby a considerable variety of organic substances may be excreted from the animal body. In the case of roots any excretory processes of this kind would have to be sustained and on a considerable scale for root constituents, such as those previously mentioned, to reach a concentration having an appreciable sphere of influence on the roots of other plants and microorganisms since the inhibitory substances concerned, or their toxic moieties, are liable to degradation and consequent inactivation by microorganisms. It must be borne in mind however that some of the aerial organs of some plants appear to be capable of excreting organic materials, e.g. during guttation from leaves and from nectaries.

Therefore there does not appear at present to be reason to suppose that in the absence of microorganisms the roots of *Calluna* might be able to excrete substances into the soil which would inhibit the growth of other plant cells. Even though some plants contain toxic materials which might be liberated into the soil in appreciable quantities from root residues under certain conditions, e.g. after harvesting a crop, thereby adversely affecting future crops, it seems clear that such a mechanism is not the basis of the effect of *Calluna* on mycorrhizal fungi since the effect produced by *Calluna* occurs in the presence of the whole plant growing vigorously and any treatment which results in the death of *Calluna* roots is generally followed by the formation of ectotrophic mycorrhizal associations and improvement of growth in the checked trees.

In spite of the above, what appear to be well substantiated examples of one plant species exerting an unfavourable influence on another under conditions where effects due to competition for water, light and mineral nutrients can be ruled out have been quoted by Börner (1960), Miller (1938) and Woods (1960). Such effects have only been reported for plants not growing under aseptic conditions. In none of these cases does the nature of the effect appear to have been determined in the sense of isolation or identification of a toxic factor. The possibility that microorganisms are involved in these effects, either by inducing root tissues to excrete toxic materials and at the same time stimulating increased production of toxic material by the root or producing toxic substances themselves should be considered. Findings such as those of Hughes and Swain (1960) and Barnes and Williams (1960) suggest that infection of plant tissues by pathogenic microorganisms may result in a marked increase in the content of fungistatic or other substances which are normal constituents of healthy plant tissue. In addition the observations of Martin (1958) indicate that culture filtrates from *Fusarium moniliforme* when added to the culture solution in which oats were growing under aseptic conditions resulted in a 3½ fold increase in the release of scopoletin (6-methoxy–7-oxycoumarin) from the roots without root growth being influenced, although exposure of the roots to the culture filtrate was of relatively short duration and it remains to be demonstrated whether the organism would produce the same effect under soil conditions. In some instances it seems that infection of higher plant tissue with microorganisms results in the production of substances not previously detectable in the tissues. Thus Gäumann and Kern (1959) and Gäumann, Nüesch and Rimpau (1960) have found that substances, either orcinol or substances of unknown composition, inhibitory to the growth of fungi are only produced in the tubers of 24 species of European orchids when infected by fungi and especially orchid mycorrhizal fungi or by 3 species of soil bacteria which often occur in the outer cells of the tubers, whilst Condon and Kuc (1959 and 1960) observed that a fungitoxic substance is only to be found in carrot tissue after inoculation with *Ceratocystis fimbriata*. Such substances may be produced either by the higher plant tissue in response to the action of microorganisms or by the microorganisms in the nutritional environment of the higher plant tissue.

Although the considerably greater populations of microorganisms of the rhizosphere suggest that nutritional conditions may be more favourable there than in the surrounding soil it seems unlikely, from the indications mentioned previously, that even in the rhizosphere will there be nutrients in sufficient quantity for the continued production, by rhizosphere microorganisms, of toxic substances in effective concentration. It is well known that the production of antibiotics by microorganisms requires very favourable nutritional conditions but adequate supplies of nutrients for vigorous growth is not necessarily the only requirement and supplies of specific materials may also be required before antibiotics or toxic substances are produced, even though growth is vigorous. As is therefore to be expected, active production of antibiotics by microorganisms in natural environments occurs either when they are growing in the presence of the living tissues of animals and higher plants or in the presence of considerable supplies of organic materials, e.g. plant and animal residues. Under such conditions the microorganisms producing the antibiotic frequently dominate the microbial population for a time at least. The

interior of the root may well provide a nutritional environment favourable for the production of antibiotic materials by microorganisms and the endophyte within the root cells in endotrophic mycorrhizal associations would appear to be particularly well placed from this point of view and continuous production of antibiotic over considerable periods would seem to be feasible.

It now seems quite clear that whilst on the one hand mineral nutrients can be absorbed from the soil by the hyphae of the ectotrophic mycorrhizal association, these are then conveyed inside the root and subsequently given up to the tissues of the higher plant, on the other hand the fungus takes up photosynthetic products from the higher plant and, in view of the ability of fungal mycelium to excrete elaborated materials such as enzymes, antibiotics etc., it seems feasible that the endophyte of the endotrophic mycorrhizal association could excrete elaborated antibiotics into the soil. Such antibiotics may well have little or no effect on higher plant cells compared with their effects on microorganisms.

The endophytes of endotrophic mycorrhizal associations have some connections with the soil and possibly considerably more ramifications in the soil than is commonly currently supposed (e.g. Mosse 1959). Therefore in view of the ability of fungal hyphae to transport and excrete various materials any elaborated antibiotics could be excreted directly into the soil from the mycelium external to the root. This would result in restriction of the species of microorganisms able to inhabit the rhizosphere and perhaps the soil environment in general for an appreciable distance in the vicinity of the root.

Such a system could also be the basis of the local dominance of ectotrophic mycorrhizal fungi in the rhizosphere of the roots with which they are associated even though the competitive saprophytic ability of these fungi may be low and the factors initially enabling them to form associations with roots still remain to be elucidated.

When the roots of other plants enter the sphere of influence of such an antibiotic producing system, and as described by Yeatman (1955) the roots of trees whose growth is checked in the presence of *Calluna* are closely invested by *Calluna* roots, they would not be able to develop mycorrhizal associations if the fungi normally forming such associations are susceptible to the influence of the antibiotic; hence under conditions where supplies of mineral nutrients are small such higher plants may not be able to obtain adequate supplies of mineral nutrients and be unable to compete with the other species.

In view of such possibilities it is of interest that, as has been mentioned previously, *Calluna* does not invariably have an untoward effect on other plant species. The effect of *Calluna* on other plants is reduced or removed if the *Calluna* is shaded. If the *Calluna* escapes being burned for between 20 and 30 years it will become senescent and the growth of trees which have remained in check during this time will begin to improve. The other circumstances, apart from shading and waterlogging, in which *Calluna* appears to lose its dominating influence usually seem to be associated with increased amounts of plant nutrients in the soil; this applies especially to nitrogen for other nutrients seem to have little influence although modification of the reaction of the soil by addition of Ca or Mg may, in some circumstances, have an effect similar to that of increased supplies of nitrogen.

According to Steinmann (1947) there is a number of general statements that *Calluna* only thrives on soils poor in nutrients and that it yields to the competition of other plants in soils richer in nutrients and on increasingly calcareous soils. The latter effect may be associated with increasing amounts of calcium, less acid reactions or increased supplies of plant nutrients especially mineral nitrogen in the soil. The factor inhibitory to the growth of ectotrophic mycorrhizal fungi appeared to be less active at less acid reactions and it may well be that the loss of dominance by *Calluna* on richer soils is in some cases in part at least associated with decreased activity of the inhibitory factor on these soils. Such effects recall P. E. Müller's (1897) observation that spruce was not affected by *Calluna*, even if it occupied the ground between the spruces completely, on old farmland. It is perhaps of importance in the same connection that Gimingham (1960) states that much animal manuring affects *Calluna* adversely. Beijerinck (1940) also seems to consider that animal manure is one of the factors which brings about a change from heath vegetation to sand grasses and herbs, whilst Nicholson and Robertson (1958) comment that the grazing habits of cattle and the effects of large quantities of dung and urine depress the growth of *Calluna* to a greater degree than the smaller and more selective grazing animals. It seems possible that the influence of rabbits in converting *Calluna* heathland to grass heath is in part due to a manurial effect from the faecal pellets of the rabbits. The dominance of *Nardus stricta* (commented on by King (1960)) on sheep paths across Calluneta may originate in a similar manner for otherwise *Nardus* only seems to be able to achieve dominance when the competitive power of *Calluna* is reduced, for example by senility of the *Calluna*. What appear to be analogous changes in vegetation have been recorded (Gillham 1960) for the sclerophyllous heath and scrub vegetation of coastal areas of Australia when colonized by sea-birds, the guano causing a change to annual, biennial and succulent species including

a considerable number of alien species where there are introducing agencies.

Rayner (1911) was impressed by the differential occurrence of *Calluna vulgaris* on the Berkshire and Wiltshire Downs, the plant growing where the layer of Clay with Flints is deepest and being absent where the chalk approached more closely to the surface. Her experimental investigations (Rayner 1913) into the causes of this differential distribution of *Calluna* seem to point to the importance of mineral nutrients in influencing its growth. Calluna seedlings grown in soil from where the heather grew on the Clay-with-Flints loam (i.e. "heather" soil) were bright green in colour and had a vigorous, much branched root system having an abundant characteristic endotrophic mycorrhizal association whereas the growth of the *Calluna* seedlings on the "chalk" soil, from which *Calluna* was absent in the field, was practically completely inhibited and root growth was almost completely checked; although many lateral roots began to develop, the tips were often recurved and had a brownish discoloration, similar ineffective efforts to develop a root system may be made repeatedly. In such seedlings fungal mycelium was present only in scanty amounts in the roots whereas numerous bacteria were present and even penetrated the root cortical parenchyma. When transplanted to the Clay-with-Flints "heather" loam such seedlings quickly recover and grow normally. *Calluna* seedlings growing normally in the "heather" soil were watered with aqueous extracts of the chalk soil and only after 6–7 months did the seedlings begin to show signs of injury; growth became stunted, the foliage yellowish and leaves on some of the older shoots had died whilst in addition root growth had been checked the tips having marked curvatures and often had associated dense mantles of bacteria, and the mycorrhizal association was poorly developed. The response of similarly treated *Calluna* seedlings growing in larger pots of "heather" soil, although similar, appeared much more slowly. *Calluna* seedlings growing in "chalk" soil were watered (a) with distilled water and (b) with an aqueous extract of "heather" soil. The seedlings watered with distilled water were dead after six months whilst those watered with extract of "heather" soil had made some progress the stem having four to six leaves and the root growth is said to have been more normal although the state of the mycorrhizal association is not indicated. Since the growth of the *Calluna* seedlings was influenced by filtered, unheated extracts of the soils and the injurious effects developed more slowly in plants growing in larger pots it was concluded that the unfavourable factor was chemical and not physical or biological in nature. Subsequently Rayner (1915 and 1921) grew *Calluna* seedlings in aqueous extracts of the "heather" and "chalk" soils and in mineral nutrient solution in the presence and absence of a fungus isolated from *Calluna* root material. The seedlings growing in "heather" soil extract grew normally, the cortical cells of the root being filled with colourless hyphae, whilst both root and shoot growth of seedlings in the "chalk" soil extract were strongly arrested and most of the seedlings were dead or moribund two months after planting, having the appearance of being severely parasitized by the fungus with which the cultures were inoculated. When *Calluna* seedlings free from microorganisms were grown in sterilized mineral nutrient solution they grew very feebly and produced virtually no roots whereas under similar conditions but in the presence of a fungus isolated from *Calluna* ovary tissue the *Calluna* seedlings produced considerable shoot growth and many roots were produced. The presence or otherwise of fungal associations in the roots does not seem to have been investigated although there was much growth of the fungus, in the vicinity of the roots, in the agar with which the nutrient solution had been solidified. Rayner (1921 and 1924) states that using a nutrient solution having the composition KNO_3, 1·0 gm, $MgSO_4$ 0·4 gm, $CaSO_4$ 0·5 gm, $CaH_4P_2O_8$ 0·5 gm, NaCl 0·5 gm, $FeCl_3$ trace, dist. water to 1000 ml, as a basis she found that optimum growth of *Calluna* seedlings was obtained when the total salt concentration was 0·1 or 0·05 per cent, growth being adversely affected at higher concentrations, and Rayner appears to have considered this to confirm previous suggestions that the *Calluna* plant is unable to thrive in any but weak nutrient solutions. It must be remembered however that the nutrient solution used by Rayner contained quantities of available mineral nutrients which very probably represent comparatively high "ecological" concentrations and that *Calluna* seedlings only grew in this solution in the presence of fungi which may well have reduced significantly the concentration of one or more nutrients, particularly nitrogen, during their growth.

In a study of the possibility of nitrogen fixation in the Ericaceae, Rayner (1922) commented on the association of *Calluna* and other ericaceous species with soils generally considered to be deficient in nitrates and posed the question as to whether *Calluna* could be cultivated in relatively alkaline soils if the nitrate and other salt content were low as is considered to be the case in heath soils. This seems to imply that Rayner was inclined to consider that the effect of the "chemical" factor of the "chalk" soil on the growth of *Calluna* seedlings may be due to its content of nitrate or other mineral nutrients although the hydrogen ion concentration may well have been a complicating factor. In the same paper Rayner records that, in the presence of a fungus isolated from *Calluna*, the growth of *Calluna* seedlings

was as vigorous and the seedlings a brighter green and healthier on mineral nutrient solutions solidified with agar to which combined nitrogen had not been added as on similar solutions containing 0·5 gm KNO_3 per litre.

Subsequently Rayner (1924–5) commented that "no satisfactory evidence was known to the writer that plants of ling ever occur under natural conditions or under cultivation entirely free from specific fungal infection and that it is impossible to cultivate the ling plant in the absence of its associate."

The following points significant in the present context seem to emerge from Rayner's work on *Calluna*.

1. *Calluna* forms typical mycorrhizal associations in soils generally considered to be deficient in nitrates—these associations may be deficient in the presence of other forms of combined nitrogen than nitrate.

2. *Calluna* seedlings grow vigorously under experimental conditions, in the presence of a fungus isolated from living *Calluna* plant material, where the supply of combined nitrogen in the medium would appear to be extremely small.

3. The young roots of *Calluna* seedlings growing in soils or extracts of soils likely to contain appreciable amounts of nitrate and/or other mineral nutrients show a remarkable investment with and penetration by bacteria and only scanty fungal mycelium in the roots.

It is of considerable interest that Rayner appears to consider that her experimental observations suggest that *Calluna* does not thrive in the presence of increased supplies of mineral nitrogen since this is in agreement with the field observations that *Calluna* loses its power to become dominant on richer soils and in the presence of applications of animal manure both of which may be expected to be associated with increased supplies of mineral nutrients and especially nitrogen. It is also of interest that increased supplies of mineral nitrogen appear to result in diminished incidence of mycorrhizal associations in *Calluna*. These findings appear to receive support from investigations into the effect of addition of fertilizers to soils on the growth and development of mycorrhizal associations in other plant species and especially members of the Ericaceae.

Recently Mosse (1962) reported that soluble nitrogen in various forms (KNO_3, $(NH_4)_2SO_4$, urea and asparagine) at a concentration of 0·05 per cent strongly inhibited the formation of associations between *Endogone* sp. and *Trifolium parviflorum*. The application of urea to the leaves is said to have increased plant size but not the number of associations. In addition, Cartwright and Snow (1962) found that spraying of urea on to the leaves adversely affected nodulation in seven species of legumes without impairing the growth of the plants.

The effect of added nutrients on the growth of cranberry and the formation of mycorrhizal associations in its roots has been investigated by Addams and Mounce (1931) and Bain (1937). The results obtained suggest that the development of mycorrhizal associations is less pronounced in the presence of added nutrients than when nutrients are not added but that the effect is by no means so marked as that obtained in the case of *Calluna* by Rayner.

More recently Brook (1952) and Morrison (1957) have investigated the effect of various levels of added mineral nutrients on the development of mycorrhizal associations and growth in *Pernettya macrostigma*. Brook used different levels of added nutrients: 22, 110 or 550 p.p.m. in the case of nitrogen, and two applications at these levels were made with an interval of five weeks between them. He observed that at the lowest rate of nutrient additions all the plants remained healthy, at the intermediate levels K and Ca added together depressed the growth of seedlings markedly, whilst some of the plants receiving Ca and some receiving nitrogen showed signs of injury. At the highest level of application of nutrients all plants receiving nitrogen and some receiving Ca had died at the cotyledon stage the plants of other treatments remaining vigorous. In spite of the wide range of growth response to the treatments most plants had copious mycorrhizal associations and the less intense infections were not clearly correlated with any element or combination of elements. The only positive result was that plants receiving phosphorus showed a somewhat reduced incidence of mycorrhizal associations although the dry weight of the stems of the plants increased with increasing doses of phosphorus. In the present context it is important that one of Brook's main conclusions was that the plant was adversely affected by dressings of nitrogen of the order normally used for crop plants. Morrison also carried out experiments on the effect of added nutrients (N, P, K) on *Pernettya macrostigma* growing in soil from the plant's natural habitat. In terms of the method of ascertaining the incidence of mycorrhizal associations, i.e. numbers of infected and uninfected epidermal cells in 10 fields under the microscope at random, all additions of nutrients brought about a reduced incidence of infection compared with the controls, but addition of NH_4NO_3 was especially associated with reduction (50 per cent) in infection and in the absence of added phosphate, the soil was considered to be deficient in available phosphate, in reduced growth of the plants; at the highest levels of addition (ca. 550 p.p.m. nitrogen) all the plants were dead.

Burgeff (1961) observed that the addition of 0·025 per cent NH_4NO_3 to the medium in which young seedlings of *Vaccinium oxycoccus* were grow-

ing resulted in the plants having dark green leaves and being about twice as long, and the leaves twice as large, as those of plants grown in the absence of added NH_4NO_3; the latter plants had light green leaves and their roots systems were much larger in volume with very slender and much branched roots. In addition there was much greater development of associations between fungi and the roots, both internally and externally, than was the case with the plants grown in the presence of added NH_4NO_3.

The investigations of Stalder and Schütz (1957) into a disease of nursery-grown *Erica gracilis* would seem to be of considerable significance. The disease, which is characterized by symptoms of nutrient deficiency and desiccation, is associated with death and browning of roots, the death within a short time of newly developed roots and a heavy infection of the roots of the diseased plants with a species of *Olpidium*. It is of interest that Levisohn (1957b) found that in the case of some species, such as Ash, although very deficient in mycorrhizal associations when growing in soils of high nutrient status they were characterized by a remarkably rapid growth rate and were presumably not affected by pathogenic organisms. The disease is never found in wild plants of *Erica carnea*, *Erica vagans* and *Calluna vulgaris* and *Olpidium* was only found in isolated outer cells of the roots even in plants of *E. carnea* planted close to but outside the area where the diseased plants were. It is striking that the young roots of these unaffected plants contained, almost without exception, well developed mycorrhizal associations. Stalder and Schütz raise the question as to whether wild plants of *Erica* are not attacked by *Olpidium* because the mycorrhizal associations of the *Erica* roots are antagonistic to *Olpidium*. It was also observed that even if cultivated *Erica gracilis* initially had profuse mycorrhizal associations these were lost completely in the second year when the flowering time of the plants was being controlled by the application of nitrogenous fertilizers during the second year.

In experimental investigations into this problem, which were intended to show up the relative importance of the various possible factors concerned, Stalder and Schütz observed the effect of addition of various amounts of N, P, K on the growth of non-mycorrhizal rooted cuttings of *Erica gracilis*. The greater the amount of nitrogen added the greater the shoot/root ratio became; this was associated with inability to absorb adequate supplies of water and increased deleterious effects from attacks by *Olpidium* and *Rhizophidium*. In a further experiment the effect of different levels of added nitrogen on the development of mycorrhizal associations by rooted cuttings of *Erica gracilis* was observed after 1 month's treatment with nutrients. There was little difference between the control plants, which did not receive additional nutrients, and those receiving

$$\frac{P\,N\,K}{100}\left(\frac{N}{100} = 1{\cdot}2 \text{ mgm } (NH_4)_2SO_4 \text{ per litre}\right)$$

whereas in the case of plants receiving P 3N K (3N = 353·2 mgm $(NH_4)_2\,SO_4$ per litre) the development of mycorrhizal associations was reduced to ¼. It is deduced that the addition of nutrients is hindering the formation of mycorrhizal associations and further, that the lack of difference between the control and the $\frac{P\,N\,K}{100}$ plants in this respect indicates that it is the added nitrogen which is affecting the formation of mycorrhizal associations. From the results of another experiment, in which mineral nutrients were not added, it is concluded that growth promotion following addition of nitrogenous nutrients differs from growth promotion following the formation of mycorrhizal associations in that whilst nitrogenous nutrients favour shoot growth but hinder root growth, mycorrhizal associations encourage both root and shoot growth, the roots of mycorrhizal plants being up to five times better developed than those of plants with few or no mycorrhizal associations.

The results of these various investigations suggest that the development of mycorrhizal associations and the growth of *Erica* spp. and *Calluna* are more adversely affected by additions of nitrogenous fertilizers than is the case with cranberry and *Pernettya*. Both Morrison and Stalder and Schütz draw attention to the apparently very small effect of nitrogenous fertilizers on the development of mycorrhizal associations and growth in spruce and pine. Thus Björkman (1940) found that some ectotrophic mycorrhizal associations were developed in tree seedlings growing in full light even when nitrogen was applied at the rate of 2500 mgm/kgm of soil although none were formed when 9000 mgm N were added per kgm soil. There is a tendency in this case for addition of nitrogen to be associated with the formation of fewer mycorrhizal associations the lower the light intensity but, as Björkman comments, it is remarkable that development of mycorrhizal associations took place at all at these very high levels of nitrogen. The development of the tree seedlings remained surprisingly steady in the presence of added nitrogen, unlike the effects produced on ericaceous plants.

It therefore seems possible to put forward a series in which addition of combined nitrogen is associated with decreasingly adverse effects on plant growth and the formation of mycorrhizal associations: *Erica* and *Calluna* $\geqslant$ cranberry and *Pernettya* $\geqslant$ Scots Pine and Spruce although recent results obtained by Richards (1961) from pot cultures of *Pinus taeda* and *Pinus caribaea* suggest that when NH_4NO_3

was added to soils having reactions approaching neutrality and resulting in levels of 71·4 and 115·3 p.p.m. of NO_3, the already rather low percentage of short roots forming A and B type ectotrophic mycorrhizal associations was greatly reduced. Whether it is the amount of nitrogen available over a period or the concentration of available mineral nitrogen in the soil which is important, remains to be elucidated.

Such a state of affairs could well account for the apparently decreased ability of *Calluna* to dominate the vegetation on richer soils and when nitrogenous fertilizers are used for, in addition to any assistance such improved nutritional conditions may afford to previously dominated species, the increased amounts of mineral nutrients, especially nitrogen, seems likely to have a deleterious effect on the growth and survival of *Calluna*. Conversely, when forests are cleared and the succeeding vegetation is repeatedly subjected to burning and crop removal the mineralizable soil nitrogen will be progressively depleted which may be expected to encourage the growth of *Calluna*. The ability of *Calluna* to thrive on small supplies of mineral nutrients does not alone seem to offer an explanation of the potentiality of *Calluna* to dominate the vegetation of sites apparently poor in available nitrogen since tree species which cannot compete with *Calluna* on heathland are able to compete with what would appear to be the equal or greater nutritional demands of other tree species on the same heathlands in the absence of added nutrients provided they have developed characteristic ectotrophic mycorrhizal associations. It is here that the suggestion of Stalder and Schütz that the mycorrhizal associate of *Erica* exerts an antagonistic effect on *Olpidium* becomes of considerable interest. This seems to be paralleled by the observations of Levisohn (1953c) that the intracellular haustorial infection found in the roots of pines is readily induced in seedlings lacking ectotrophic mycorrhizal associations whereas plants well furnished with ectotrophic mycorrhizal associations appear to be immune to infection. Also in the invasion of lily bulb scales by a pathogenic bacterium both antagonistic and synergistic effects due to different species of fungi have been reported by Bald and Solberg (1960).

An antagonistic action arising from the mycorrhizal roots of *Calluna*, whether from the root tissue itself under the influence of the endophyte or from the endophyte itself when in association with the root, on ectotrophic mycorrhizal Hymenomycetes, would fit well the observed presence of an inhibitor to the growth of mycorrhizal Hymenomycetes in aqueous extracts of *Calluna* raw humus associated with *Calluna* growing vigorously in full light on soils which are generally supposed to be poor in available nitrogen. Both these are in accord with observations that the lack of formation of ectotrophic mycorrhizal associations by checked spruce on *Calluna* heathlands is associated with the presence of living *Calluna* roots, closely investing the tree roots (Yeatman 1955), likely to have numerous mycorrhizal associations.

Although the identity of the endophyte of *Calluna* seems to be uncertain the organism would appear to be ubiquitous in *Calluna* heathland soils at least. If it is able to grow in the absence of *Calluna* there is no indication that it has an effect on tree growth such as is observed in the presence of vigorous living *Calluna* with abundant and vigorous development of mycorrhizal associations. Since there seems to be no precedent at present for the excretion of toxic materials by the *Calluna* roots *per se* it would seem to be profitable to enquire whether the endophyte induces the production of antibiotic by the root tissue or whether the endophyte produces an antibiotic or toxic factor when in association with *Calluna* roots and especially under the nutritional conditions of the *Calluna* heathland. It may be of significance in this connection that, as mentioned earlier, in the absence of the endophyte, apparently induced by increased supplies of mineral nitrogen, *Calluna* roots have been observed to have a considerable surface population of bacteria and these may even penetrate the root cortical cells. Any capacity of the *Calluna* endophyte to produce or induce production of antibiotic substances may be reduced or have disappeared some time before increased supplies of nitrogen have resulted in the disappearance of the endophyte from the *Calluna* roots.

It has already been suggested that mycorrhizal fungi may achieve dominance in and on the root as a result of antibiotic production with consequent restriction of the composition of the rhizosphere microflora. This could result in the exclusion of potential root pathogens and at the same time achieve a more effective and extensive system for the absorption of mineral nutrients from the soil. Any antibiotic conflict between systems of this kind in different plant species would place the plant with the dominated system in a very unfavourable position, even though its root tissue is undamaged, for the absorption of mineral nutrients when limited amounts of these are available and its growth may be expected to be curtailed.

Such a system would be a very effective form of competition between plant species, especially where the roots of individuals of the two species are in close contact as in the case of the roots of *Calluna* and trees, and seems to fit most if not all the observations regarding the behaviour of tree species in the presence of *Calluna:*

(a) The absence of check to the growth of young trees on *Calluna* heathland where the *Calluna* has been killed by ploughing or burning when one might

expect increased production of a toxic factor arising from saprophytic soil organisms whereas in fact everything suggests that any toxic factor disappears.

(b) Resumption of growth by trees, whose growth has been checked in the presence of living *Calluna*, following application of nitrogenous fertilizer. Such treatment in addition to suppressing the endophyte of the *Calluna* root system, thereby diminishing any production of antibiotics and increasing the possibility of development of ectotrophic mycorrhizal associations on tree roots, might be expected to allow the non-mycorrhizal, and apparently undamaged, roots of the checked trees to absorb increased supplies of mineral nutrients. The improved growth of the trees can only be expected to be maintained after the exhaustion of the added nitrogen if growth of the trees in the meantime has been sufficient to shade out an appreciable area of *Calluna*, and ectotrophic mycorrhizal associations have been formed; if this has not occurred the tree may then be in an even more vulnerable position, as observed by P. E. Müller (1897), when the *Calluna* is again growing at lower levels of available nitrogen.

(c) It is to be expected that the richer soils, where *Calluna* apparently lacks the capacity to dominate the vegetation, will be characterized by increased supplies of mineral nutrients including nitrogen and in many cases with a reaction less acid than that of the *Calluna* heathland. The greater supplies of nitrogen may be expected to reduce the incidence of mycorrhizal infection in *Calluna* and the less acid reaction to decrease the activity of a factor, similar to that observed in extracts of *Calluna* raw humus, inhibitory to ectotrophic mycorrhizal fungi. Either or both of these factors may be expected to reduce the capacity of *Calluna* to dominate other species.

(d) *Calluna* is intolerant of shading e.g. by the lower branches of trees, and this may be expected to interfere with the incidence and/or activity of endophytes in the *Calluna* roots which could result in the tree roots developing ectotrophic mycorrhizal associations and thereby being better fitted to compete for supplies of mineral nutrients. Artificial shading and mulching of the *Calluna* would therefore be expected to have the observed similar effect on the growth of trees checked by the presence of *Calluna*.

(e) During the senescent phase of *Calluna* stands the soil will become increasingly less occupied by active *Calluna* roots and therefore the root systems of other plants, e.g. trees in check, on the same site will be increasingly less influenced by any effects due to the *Calluna* roots until eventually it should be possible for the tree roots to form ectotrophic mycorrhizal associations and the trees may then be expected to begin to grow. This may well be the basis of the observation that trees whose growth is checked by *Calluna* may begin to grow after 20–30 years.

(f) The "nursing" effect of certain tree species towards others when growing on *Calluna* heathland, the greater "heather sensitivity" of some tree species and the characteristics of the tree species able to colonize *Calluna* heathland when the dominance of *Calluna* is broken, may all represent expressions of the same phenomenon, namely that those tree species best able to invade the *Calluna* heathland all appear to be able to form associations with *Boletus scaber*. Certain strains of this organism appear to be especially resistant to the inhibitory factor extractable from *Calluna* raw humus. If this factor is produced by the mycorrhizal associations of *Calluna* then any treatment which removes the *Calluna* or reduces the incidence of its mycorrhizal associations may be expected to tend to allow trees to form ectotrophic mycorrhizal associations and grow. Trees forming associations with *B. scaber* may be expected to form associations earlier after removal or suppression of *Calluna* than trees which do not and will therefore have a longer time in which to become established before the *Calluna* begins to return; this will increase the possibility for progressive shading out of the returning *Calluna* at the same time providing a root environment in which the concentration of the factor inhibitory to ectotrophic mycorrhizal Hymenomycetes is low and in which ectotrophic mycorrhizal associations may therefore be formed by those species e.g. spruce, which do not appear to form associations with *Boletus scaber* but only with fungi more susceptible to the inhibitory factor; the development of roots in the litter layer arising from the trees already established, and from which the inhibitory factor will be absent, will also assist the growth of the "heather sensitive" species. It may also be expected that the level of inhibitor in the soil may show wide variation depending on such factors as age of the *Calluna* and incidence of the endophyte in *Calluna* roots according to environmental conditions such as the mineral nutrients available in the soil and shading. It is also possible that there could be wide local variations in the level of inhibitor over short distances depending on the distribution of *Calluna* roots at any particular time.

Chapter 7

THE IMPLICATIONS FOR FORESTRY PRACTICE OF THE SUPPRESSION OF THE FORMATION OF A & B TYPE ECTOTROPHIC MYCORRHIZAL ASSOCIATIONS IN FOREST TREES BY *CALLUNA VULGARIS*

Whether a plant species succeeds on a particular site is related to inherent, genetically determined characteristics concerning its requirements for light, water, mineral nutrients etc. which determine whether the physical factors of the site are suitable, whether the supply of mineral nutrients is suitable, whether it is able to compete successfully with other plant species for which the site also offers a suitable environment and whether it is able to tolerate interference by man and grazing animals. When a number of plant species are potentially able to grow on a site in the absence of competition from other species dominance by a particular species in a mixed culture of such species may be achieved by various methods. For example one plant species may make more rapid growth, or grow to a greater height, than another with the result that the more slowly growing plant, unless shade tolerant, is eventually killed. Some plant species may for some reason be able to tolerate drought better than others and therefore survive to the exclusion of other species when water supplies become scarce. In yet other cases dominance may be achieved by the production of new plants by means of runners which are nourished by the parent plant, in the midst of plants of another species, until they have grown sufficiently to become established and suppress plants of other species by shading. Such methods do not seem to operate in the case of the competition between *Calluna vulgaris* and many other plants including tree species on base-poor heathlands, whereby the growth of the trees is checked for many years even though they are well able to grow on the heathlands in the absence of *Calluna*, where the *Calluna* may kill or suppress the growth of other plant species even though it does not overtop them.

The domination of tree species by *Calluna* seems to be associated with the lack of development of ectotrophic mycorrhizal associations by the trees which in turn seems to be associated with some factor produced by the living *Calluna* roots. The effect of *Calluna* is more marked in the case of "heather sensitive" tree species than with other tree species which appear to be characterized by their ability to form mycorrhizal associations with *Boletus scaber*.

When the *Calluna* heathland is subjected to burning or mechanical disturbance, resulting in the elimination of living *Calluna* for a time, colonization by various tree species such as birch or pine will occur if there are sources of seed. The effect of *Calluna* on the growth of planted trees can be counteracted by elimination of the *Calluna* by cultivation, by shading of the *Calluna*, by application of nitrogenous fertilizers and perhaps to some extent by making the reaction of the soil less acid. These considerations will have considerable importance for the establishment and subsequent growth of trees on *Calluna* heathland.

A heathland soil on which *Calluna* has grown for a long time usually supplies only small quantities of available nitrogen and, although additional amounts may be mobilized from the root and shoot residues following killing of the *Calluna* by cultivation or shading, it may be expected, from the nature of the *Calluna* effect as described above, that under these conditions the roots of pure or almost pure stands of *Calluna* will be associated with maximum intensity of checking of trees planted thereon.

Many years ago Danish experience indicated that cultivation alone was not the answer to the problem of the difficulties of tree growth on *Calluna* heathlands, the important factor being suppression of *Calluna*.

Initially the British Forestry Commission (as reported by Zehetmayr 1960) attempted to establish trees on *Calluna* heathland by hand planting methods involving a minimum of cultivation or preparation but the results were disappointing. Subsequently increasing intensities of cultivation were undertaken with a view to ameliorating what were considered to be unsuitable conditions of drainage and aeration of the soil. Experience suggests that the original belief that furrows were required to remove water from the heath is far from being the case once the pan is fractured or the soil disturbed to a sufficient depth, on the contrary water conservation may be needed in many areas, e.g. those having a rainfall below 35 in. In various places in his report Zehetmayr lays emphasis on different factors and it would appear that he feels uncertain as to the crux of the problem of the checking of tree growth on *Calluna* heathland. At

one point he concludes that every increase in soil disturbance, by increased coverage, depth and repetition, increases growth, breaking of the pan being of importance where this is near the surface, and that disturbance of the surface peat to promote its aeration and breakdown is the vital factor in this cultivation. Elsewhere, however, he points out that the success of furrow planting, well removed from decaying vegetation, makes the conclusion that mobilization of nutrients by decay of buried vegetation is largely responsible for the excellent early growth of young trees on ploughed heathland open to question. In addition, since the raw humus first began to accumulate on a well-aerated mull surface it would seem very unlikely that mechanical disturbance alone is likely to lead to its breakdown. Further, the results of the experiments in which various types and intensities of cultivation were used along with broom and/or Scots pine "nurses" could more realistically be interpreted as indicating that any effect of increased cultivation is due to suppression of an increased proportion of *Calluna* since deep ploughing over the whole area produced no better growth of Sitka spruce between the 6th to the 13th year than single furrow shallow ploughing, the trees being nursed by broom in both cases. These trees produced ten times as much height growth, in the same period, as similar trees growing on adjacent unploughed heathland. This interpretation may well be envisaged in Zehetmayer's definition of the favourable conditions for establishment of Sitka spruce as being elimination of heather competition by ploughing, suppression of heather regrowth by a nurse crop and stimulation of the spruce by phosphatic manuring or legumes. This is in essence the old Danish recipe the importance of which is further emphasized by the concept of the checking of tree growth on *Calluna* heathland being associated with the suppression of the formation of mycorrhizal associations by tree roots due to the activities of the *Calluna* root system.

The following measures (a, b, c and d) may be expected to assist in minimizing the effect of the *Calluna* roots and promote the development of either stands of heather sensitive species or of mixtures of "nurses" and heather sensitive species which will be able to form mycorrhizal associations without delay, start growth at the same time and have a better chance of making equal progress:

(a) Planting in the furrow of shallow single furrow ploughing so that the tree roots are in a medium which is, and has been, relatively free from *Calluna* roots. This has already been found to be beneficial in practice.

(b) Delay of planting after suppression of the *Calluna* by cultivation or other means to allow sufficient time for the death of *Calluna* roots and for the *Calluna* root factor to be leached out or destroyed. Experiments on the optimum time for planting after cultivation have so far resulted in the conclusion (Zehetmayr 1960) that weathering for one winter after cultivation is advantageous and two winters may elapse between cultivation and planting but that not more than one growing season should intervene because if a longer time is allowed to elapse the *Calluna* begins to return and severe checking of tree growth develops. It could well be that the initial growth of heather sensitive tree species would be assisted if planting could be delayed still further, the regrowth of *Calluna* being suppressed by selective herbicides, in order that the level of the *Calluna* root factor could be still further reduced. This seems to be supported by Dimbleby's (1953) findings that colonization of *Calluna* heath by pine is poor immediately following a fire but that it gradually builds up until a maximum is reached 3 to 5 years afterwards thereafter decreasing as the heather returns.

(c) It seems probable that cultivation, if only to provide a medium relatively free from *Calluna* roots in which the tree roots can develop, may well be unnecessary even for "heather sensitive" tree species planted without "nurses" if the *Calluna* could be eradicated, a few years before the trees are planted, by the use of herbicides which could be used subsequently to prevent recolonization by *Calluna* until the trees were in no danger from the *Calluna*. Where "heather sensitive" species are planted with "nurses" or other species, eradication of the *Calluna* for some time before planting and prevention of reinvasion by *Calluna* by the use of herbicides should ensure more equal growth of the heather-sensitive and other species and avoid the need to remove "nurses" which are in danger of suppressing the spruce but which if removed, may allow of resurgence of *Calluna* although of course different heaths may provide different environmental and nutritional conditions even when cleared of *Calluna* so that the reaction of individual species and provenances of species and the interactions between various species may be expected to differ to some extent on different heaths.

(d) The application of fertilizers and especially nitrogenous fertilizers, although the latter are usually short-lived in the soil, may be expected to assist the growth of trees and reduce the checking activity of the *Calluna* root system. The use of calcareous materials in sufficient quantities to reduce substantially the acidity of the heathland soil may also be expected to reduce the inhibitory effect due to *Calluna* root activity.

The sensitivity of a tree species to heather is no guide to its growth on a site in the absence of *Calluna* and therefore the nutrient-supplying status of a site as reflected by the growth responses of the trees following the addition of fertilizers is likely to be

masked or modified by the presence of *Calluna*. When canopy has formed the development of a ground flora is likely to be precluded for some time especially in stands containing only evergreen coniferous species and at this stage the growth responses of the trees to the addition of fertilizers should indicate which nutrients are particularly inadequate in available form on a particular site. Under such conditions the trees should be in a favourable position to utilize added mineral nutrients, especially nitrogen, assuming of course that the trees are suited to the physical factors of the site.

When the stand has grown sufficiently to need thinning, or openings are made for the introduction of other species, the danger of resurgence of the *Calluna* is considerable especially if the canopy is opened too rapidly and sufficient light reaches the forest floor to allow the growth and activity of *Calluna*. This danger may be expected to increase in even-aged heathland stands as they become more open towards the end of the rotation especially if seeding fellings, or clear fellings prior to replanting, are made. These dangers are underlined by observations such as those of Braathe (1950) on the checking of the second generation of Norway spruce following invasion of the site by *Calluna* after clear felling of the first crop, also by the failure of pine seedlings to prosper in the *Calluna*-dominated openings of the Swedish pine heaths, which had previously carried a fine pine stand, as described by Hesselman (1910) and by the cases mentioned by Zehetmayr (1960) of 25 year old pines which are double the height of their 100 year old predecessors which had battled against *Calluna* and of old pines on heaths which have developed new leaders after soil working nearby which suggests that as the tree has grown older the *Calluna* has crept in towards the stem and although the tree has survived it has grown more slowly than it might have done in the absence of *Calluna*.

In a private communication, Jägmästare Gustaf Kolmodin described how in the first experiments, about 1890, to try to obtain regeneration of Scots pine, on old *Calluna* ground, the soil was ploughed with a Finnplough; the latter is an implement the use of which probably did not result in the *Calluna* being suppressed to any considerable extent. Although there were only 2–12 old Scots pine trees per hectare, many seedlings were obtained during the course of many years but none survived beyond the early stages. The same results were obtained when the ploughing was combined with ditching. In 1938, powdered sodium chlorate was applied at the rate of 100 litres per hectare and the few old Scots pine trees subsequently gave rise to 6,500–15,000 seedlings per hectare where heather was decaying, the pine plants being 20 cms high when 4 years old, the last extension shoot being 10 cms long.

It may be possible in some cases to minimize the potential effect of reinvading *Calluna* by careful regulation of thinning so that the amount of light reaching the forest floor is always a minimum, or by the use of selective herbicides especially when groups of young trees are being introduced into openings so that there may be a steady succession of younger trees to replace the older trees when they are removed. It may be desirable in some cases at least to encourage non-ericaceous shrubs and herbs which will prevent light reaching the soil to the extent that *Calluna* is unable to grow; although such plants will use some of the available nutrients for growth their litter may catalyse the decomposition of coniferous litter and the liberation of mineral nutrients therefrom especially if calcareous material can be applied. Since as far as is known at present all the coniferous species are likely to give rise to raw humus on the base-poor *Calluna* heathlands the supply of mineral nitrogen is likely to be low and mineral nitrogen supplied as fertilizer is likely to become unavailable when it has been absorbed by the tree and returns to the soil in the litter. If however tree species whose litter does not give rise to raw humus on the base-poor *Calluna* heathlands (and it should be remembered that species able to form mycorrhizal associations with *B. scaber* are more likely to be able to compete with the returning *Calluna* provided they are otherwise suited to the environment) can be mixed with the conifers then more nitrogen may be expected to become available from the *Calluna* raw humus and the conifer litter than when non-ericaceous shrubs and herbs form the ground flora. Any such increases in supplies of available nitrogen should tend to reduce the development and intensity of the *Calluna* checking effect and at the same time promote the growth of the trees.

There are soils which are low or relatively poor in bases which have so far escaped the activities of raw humus-forming plant species. Such soils are usually in what may be regarded as a delicately poised condition from the point of view of soil development and especially raw humus formation. If pure stands of coniferous species are planted on them the reserves of mobilizable nitrogen will tend to be used up for the growth of the conifers and, especially in the case of such soils having a sandy texture, they will readily acquire a raw humus layer the nitrogen of which is likely to be difficult to mobilize. Supplies of mineral nitrogen will become meagre so that at a later stage if adequate light is allowed to reach the forest floor conditions will be suitable for invasion by *Calluna* and a deleterious effect on tree growth and tree regeneration may be expected to follow.

The effect of *Calluna* on the growth of trees and especially young trees is dramatic and the problem has therefore attracted much attention. If the effect is

associated, as there seems reason to believe, with the activity of the mycorrhizal roots of *Calluna* it may well be that other phenomena have a similar basis, although they are perhaps not quite so dramatic and have been ascribed to other causes. For example Petrini (1932–34 and 1935) concludes that in various spruce stands the vegetation type was more important in determining spruce regeneration than soil type and that of the vegetation types, that dominated by *Vaccinium* was the most unfavourable. Sodium chlorate has been used in Sweden to suppress *Vaccinium* so that spruce may regenerate more readily; this is in the same region where, as previously mentioned, spruce readily invades old pastures when grazing is abandoned. A similar effect associated with *Vaccinium* seems to have been observed in recent times in the spruce forests of the Alps (Trepp 1961) which is also in a region where, as mentioned previously, spruce is able to invade pastures. In the old Swedish spruce stands where the growth of the trees has become stagnant, though not necessarily permanently so, there is generally a ground vegetation of *Vaccinium* and as pointed out by Melin (1948) the trees form mycorrhizal associations with difficulty under such conditions. Tirén (1951) has concluded that under these conditions even the presence of sufficient and sufficiently high grade seed trees is valueless if care is not taken to see that screefing and burning are efficiently carried out. It could well be that *Vaccinium* has a similar, though perhaps not quite so dramatic, effect on tree growth as *Calluna* on soils with raw humus layers and it would be interesting to know whether other Ericaceous species and indeed plants from other genera, families or groups have similar properties.

Richards (1962) has studied the suppression of *Araucaria cunninghamii*, planted on the lateritic, podzolic soils of the coastal lowlands of South Queensland, by sclerophyll forest dominated by *Eucalyptus* sp. The growth of *Araucaria* on these sites is improved if cultivation is carried out before planting, or if adequate supplies of nitrogenous fertilizer are added. *Araucaria* will grow successfully on these sites in association with perennial legumes or *Pinus taeda* or *Pinus elliottii* in circumstances which suggest a very close parallel between the effect of the sclerophyll vegetation on *Araucaria cunninghamii* and the effect of *Calluna* on Norway spruce; it seems possible that similar mechanisms are involved in both cases.

For a considerable period of time it has been the opinion of those concerned with the production of certain crops that there are marked interactions between certain plant species which do not seem to be attributable to factors normally associated with plant competition, e.g. shade, nutrients and water. Many of these opinions have been concerned with the genus *Juglans* and the effect seems to be more pronounced the less productive the soil (Brooks 1951 and Schneiderhan 1927). Only some species of the genus (*J. nigra* and *J. cinerea*) appear to display activity which has lethal effects on some other plants e.g. apple, tomato, potato and ericaceous plants. The same species of the genus *Juglans* may have no effects on other plant species and perhaps even beneficial effects on yet other plant species. It is perhaps significant that in the cases where deleterious effects are observed there is apparently close root contact between the walnut and the affected plant. It is suspected that a toxic factor is produced only in close proximity to the walnut root, but the manner of excretion of whatever factor is involved appears to be unknown. It seems possible that a mechanism analogous to that suggested for the action of *Calluna* on other plant species may be involved.

Ivy, *Hedera helix*, although generally found growing on the more fertile soils, also appears to have an effect on many herbaceous plants which bears resemblances to the effect of *Calluna* on other plant species. Ivy may occupy extensive areas of the forest floor in broad-leaved woods to the exclusion of most, if not all, other plants. This appears to be achieved by creeping shoots which grow between the herbaceous plants and, without shading them, apparently cause the herbaceous plants to gradually fade away. It is of course known from the work of Boullard (1953 and 1954) that *Hedera* develops abundant mycorrhizal associations and it could be that these associations have an unfavourable effect on the roots of other plant species.

When two or more tree species are planted in intimate mixture it sometimes happens that one species fails to thrive and is ultimately suppressed and dies out. Such failure is often attributed to unsuitability of the soil or some other characteristic of the site for the particular species and some such factor is no doubt responsible in some cases. Instances of unfavourable action of one plant species on another have been recorded over a considerable period of time and instances are given by various authors such as Börner (1960) and Woods (1960). It is suggested that such instances should be examined from the point of view of interaction between plant species through the metabolic activities of the microorganisms associated with their roots whereby substances are produced which in some way, such as interference with the processes of absorption of nutrients by the roots e.g. by inhibiting the formation or functioning of mycorrhizal associations, have a deleterious effect on other plant species; such interactions being perhaps modified by the kind of soil in which the plants are growing.

There would also appear to be the same problem in reverse as it were. The specific peach, apple and ci-

trus decline and replant problems which occur in some regions but not in others are well known and have been investigated from a number of different angles without satisfactory conclusions concerning the causes being reached. There are also suggestions from time to time that the growth of some forest tree species tends to stagnate when they are growing in pure stands. In some of these cases at least inadequate supplies of mineral nutrients do not seem to be the cause and there seems to be little indication that pathogenic organisms have invaded the root tissue. The fact that for some years growth is satisfactory seems to indicate that the plants are not altogether unsuited for the environments where the replant and similar problems occur. These are undoubtedly difficult and probably complex problems but it is suggested that they bear a strong resemblance to the well known 'staling' effects observed in cultures of microorganisms. It should therefore be considered whether or not in some soils one or more of the microorganisms, e.g. mycorrhizal fungi, closely associated with the roots of plants of this kind and which in some way affect the growth of these plants, produce metabolites which are either relatively stable and over a period of time accumulate in the soil or gradually induce other microorganisms in the soil to produce unfavourable conditions so that the growth or activity of the mycorrhizal fungi is interfered with. After some time the growth of the plants already there and of plants of the same species planted on the same site is adversely affected. If such self-inhibitory plants are grown in intimate mixture with plants of other species the microflora of the roots of the latter plant species may inactivate or destroy inhibitory metabolites influencing the self-inhibitory plant species. It is not easy to visualize the quantitative and spatial relationships of such systems and one may ask why one or more of the many kinds of soil microorganisms are not active in such a process of inactivation or destruction in the absence of plant roots of another species, but it is interesting that there seems to be a rather similar specific system operating in the case of certain aquatic animals. For example, the well known fish *Lebistes reticulatus* appears to produce rather specific water borne substances which limit the population of the species (Rose 1959). Thus when scarcity of food is not a limiting factor both production and survival of the young fish decrease as the number of adults increases, the aquarium size being kept constant. The production of fish can be increased by replacing the water in the aquaria with water from other aquaria containing fish of other species. Also when *Lebistes reticulatus* is living under crowded conditions, so that production is limited even though there is no shortage of food, the survival of young fish is markedly increased when another species of fish (*Tanichthys albonubes*) is introduced into the aquarium even though food and oxygen demand is thereby still further increased. Under uncrowded conditions the introduction of *Tanichthys albonubes* produced no effect. On the other hand *Tanichthys albonubes* produces factors which very effectively limit its own populations. It is suggested that the self-inhibitory system produced by *Lebistes reticulatus* is inactivated or destroyed by the metabolic activities of *Tanichthys albonubes* or possibly by its associated microorganisms; if the latter is the case it would be of interest to know why the microorganisms of the water of the aquaria do not apparently inactivate or destroy the inhibitory material. Should certain plant species possess analogous characteristics then it may be expected that in some instances members of a species will grow better in mixture with other species than with their own kind.

Attempts should be made to isolate, identify and ascertain the properties of specific active substances from these contrasting interspecific and intraspecific inhibitory systems which could be of considerable importance in plant ecology and plant production.

SUMMARY

Information from widely scattered *Calluna* heathlands in Europe indicates that the woody species colonizing burned or mechanically-disturbed heathland are restricted to *Betula* spp., *Pinus sylvestris*, *Juniperus communis*, *Sorbus aucuparia*, *Quercus* spp., *Populus tremula*, *Salix* spp. and *Genista* spp. even though the seed of other species may be available.

The growth of trees planted on ancient *Calluna* heathland is usually checked if the dominance of the heather is not broken by measures such as cultivation. After the dominance of the heather has been temporarily broken by cultivation of the heathland the growth of trees planted thereon may be checked by the returning *Calluna*. Some tree species are more sensitive to the presence of *Calluna* than others. The check to growth can be alleviated to some extent by nitrogenous fertilizers and by anything which suppresses the returning *Calluna*, e.g. mulching, and the presence of species less sensitive to the returning *Calluna* provided they have grown to a sufficient size.

The suppression of tree growth by *Calluna* has been attributed to various factors but none seems to provide a satisfactory explanation.

"Heather sensitive" species such as spruce are well able to compete, under a variety of conditions, with other types of vegetation than *Calluna* for nutrients and water. The mineral nutrient requirements of spruce do not seem to be unusual and may even be less than those of species such as pine and birch which are less sensitive to the influence of *Calluna*. Although unable to resist the returning *Calluna* the "heather sensitive" species can compete successfully on heathland, in the absence of *Calluna*, with other tree species which are able to resist the returning *Calluna*.

The nature of the competition between tree species and *Calluna* on heathland is unusual in that the successful species, *Calluna*, does not outgrow and suppress the trees by overtopping.

It is concluded that the basis of the checking effect on the growth of trees by living *Calluna* must be sought in an effect on the functioning of the root system of the tree, probably by interference with the mechanism whereby mineral nutrients are taken into the root.

Trees whose growth is checked by the presence of living *Calluna* seem to be characterized by lack of development of ectotrophic mycorrhizal associations. If the basis of this lack of development of mycorrhizal associations can be ascertained it should be of assistance in understanding the failure of spruce and other species to grow in the presence of living *Calluna*.

Observations on the differential occurrence of ectotrophic mycorrhizal associations on tree roots in the field led to the idea that the level of available nutrients in the soil was the factor determining the formation of these associations. The results of experimental investigation by various workers into the influence of the level of mineral nutrients, especially nitrogen, in the soil, on the formation of ectotrophic mycorrhizal associations, do not seem to give any conclusive results. Bearing in mind the difficulties of relating the concentrations of ammonia and nitrate nitrogen in various soils, as determined by various methods, to the physiologically active concentrations experienced by plant roots and, microorganisms, it would appear that a considerable range of concentrations is likely to be met with even in the same soil over a period of time; but there is no indication as yet that soils in which development of A and B type ectotrophic mycorrhizal associations is scanty or lacking, can be characterized by their concentrations of ammonia and nitrate nitrogen.

It is concluded that it is doubtful whether internal conditions in the plant, as represented by concentration of easily soluble reducing substance and the N/P ratio of the plant tissue, are directly related to the formation of A and B type mycorrhizal associations by tree roots.

Examples of marked localized variation in the development of mycorrhizal associations on the same root system suggest that local variations in the external root environment may influence the formation of mycorrhizal associations by modifying conditions locally inside the root and/or by affecting the growth and activities of the ectotrophic mycorrhizal fungi.

The absence of characteristic A and B type ectotrophic mycorrhizal associations from the roots of trees whose growth is checked in the presence of living *Calluna* on heathland does not appear to be due to absence of short roots even though these may be considerably fewer in number than when trees are growing normally.

There seems to be little if anything to suggest that the *Calluna* heathland soil is inferior to other soils as a nutritional and physical environment for ectotrophic mycorrhizal fungi.

The prior presence in the *Calluna* heathland soil of the mycorrhizal Hymenomycetes in one form or another appears to be of little importance in the problem of lack of formation of mycorrhizal associations by trees growing on *Calluna* heathlands.

It looks as though under *Calluna* heathland conditions some factor associated with living *Calluna* is preventing the growth of activities of mycorrhizal Hymenomycetes on which the formation of ecto-

trophic mycorrhizal associations is dependent.

Available information concerning the presence and source of factors in soils and raw humus which are inhibitory to the growth of mycorrhizal Hymenomycetes is somewhat contradictory; a re-examination of the soil of *Calluna* heathland from this point of view seemed desirable.

Aqueous extracts of *Calluna* raw humus have been prepared which inhibit the growth of a number of mycorrhizal Hymenomycetes. The inability of these fungi to grow on such extracts appears to be due not to an unfavourable reaction or to lack of nutrients but to some positive inhibitory factor which is more active at more acid reactions. Some strains of *Boletus scaber* seem to show resistance to this inhibitory factor.

When saprophytic soil microorganisms were grown in an aerated extract of *Calluna* raw humus inhibitory to mycorrhizal Hymenomycetes, the inhibitory effect disappeared.

Experimental investigation does not point to *Calluna* litter as being the source of the inhibitory factor observed in extracts of *Calluna* raw humus.

Calluna raw humus associated with somewhat shaded, leggy and perhaps senescent *Calluna* yielded extracts containing a lower level of inhibitor to mycorrhizal Hymenomycetes than extracts of raw humus from open *Calluna* heathland. The inhibitor could be concentrated by distillation of the extract under reduced pressure. Several strains of *Boletus scaber* were used as test organisms and the two resistant to the concentrated extract were both from sporophores from heathland.

More strains of mycorrhizal Hymenomycetes were able to grow in the presence of aqueous extracts prepared from two mull soils, especially in the presence of added nutrients, than grew in the presence of extracts of *Calluna* raw humus.

Aqueous extracts of the F layer from pine debris and of old *Calluna* raw humus from a Maritime pine stand, both of which contained characteristic ectotrophic mycorrhizal pine roots, allowed the growth of almost all strains of mycorrhizal Hymenomycetes tested especially when malt and glucose were added. The extract of old *Calluna* raw humus is much less inhibitory to the growth of mycorrhizal Hymenomycetes than extracts of *Calluna* raw humus supporting vigorous, unshaded *Calluna*, and any extractable inhibitory activity it may have had, has been almost if not completely lost.

Any inhibitory factor will be diluted during preparation of extracts compared with its concentration in the *Calluna* raw humus, where even the resistant strains of *Boletus scaber* may be inhibited.

It is suggested that the factor in *Calluna* raw humus inhibitory to the growth of mycorrhizal Hymenomycetes is a very likely cause of the checked growth of trees on *Calluna* heathland due to the possibility of its effect on the formation of ectotrophic mycorrhizal associations by trees, since these are the potential mineral nutrient-absorbing system of the tree especially when the supply of mineral nutrients may be small.

The greater resistance of certain strains of *Boletus scaber* to the factor in *Calluna* raw humus inhibitory to mycorrhizal Hymenomycetes, and the apparent specificity between tree species and mycorrhizal Hymenomycete species, could well explain why some tree species are able to establish themselves more readily on the disturbed *Calluna* heathland than the "heather sensitive" species.

The effects of "nurse" trees and mulches on the growth of heather sensitive trees, whose growth is checked by *Calluna*, indicate that the effect of *Calluna* is associated with living *Calluna*. If the check to tree growth and the factor inhibitory to the growth of mycorrhizal Hymenomycetes have the same basis, then this would seem to be associated with living *Calluna* roots since mulches of *Calluna* shoots, which kill the *Calluna*, release the growth of trees from check, and extracts of *Calluna* litter do not inhibit the growth of mycorrhizal Hymenomycetes.

The disappearance of the factor inhibitory to mycorrhizal Hymenomycetes when *Calluna* is suppressed suggests that it must be continuously produced to maintain the inhibitory level.

In considering the possible source of the factor inhibitory to the mycorrhizal Hymenomycetes, which appears to be associated with living *Calluna* roots, there seems no precedent for the *Calluna* root tissue as such being the source and it is suggested that the endophyte of *Calluna* is in a nutritionally favourable environment for the production of antibiotic substances which could be excreted into the soil in the vicinity of the root. Such a system would explain the local domination by mycorrhizal fungi of the zone close to the root.

Calluna loses its power to dominate the vegetation on richer soils and in the presence of animal excreta, both of which may be expected to be associated with increased supplies of mineral nitrogen. Increased supplies of mineral nitrogen also appear to be associated with diminished incidence of mycorrhizal associations in the roots of ericaceous species and have a greater effect in this direction on the mycorrhizal associations and growth of *Calluna* than on the mycorrhizal associations and growth of species such as pine. Stalder and Schütz (1957) found that, in the case of *Erica gracilis*, in addition to having a deleterious effect on the balance of root and shoot growth and survival, the application of nitrogenous fertilizers also causes the disappearance of mycorrhizal associations and is associated with attack by pathogenic fungi; these authors suggest an antagonistic effect of

the endophyte of *Erica gracilis* on the pathogenic *Olpidium*.

An antagonistic action on mycorrhizal Hymenomycetes arising from the mycorrhizal roots of *Calluna* would fit well the observed presence of an inhibitor to the growth of these fungi in aqueous extracts of *Calluna* raw humus associated with *Calluna* growing vigorously in full light on soils which are generally supposed to be poor in available nitrogen.

Antibiotic conflict between the mycorrhizal systems of different plant species would place the dominated system in a very unfavourable position, even though the root tissue is undamaged, for the absorption of mineral nutrients when only limited amounts of these are available and the growth of the whole plant may be expected to be curtailed. Such a system would be a very effective form of competition between plant species, especially where the roots of individuals of the two species are in close contact as in the case of the roots of *Calluna* and trees, and seems to fit most if not all the observations regarding the behaviour of tree species in the presence of *Calluna*.

The suppression of the growth of trees by *Calluna* on *Calluna* heathlands seems to represent an unusual form of competition between plant species in that the dominant *Calluna* does not usually overtop its victims. This has important consequences for forestry practice on *Calluna* heathlands.

In the establishment of young trees on *Calluna* heathland the chief function of cultivation is the suppression of *Calluna*. Suppression can be increased and maintained by the planting of broom and trees less sensitive to the effect of *Calluna*. Single furrow ploughing, besides suppressing a certain amount of *Calluna*, probably helps the initial establishment of young trees by enabling them to be planted in a material which has been less intensively occupied by *Calluna* roots.

Delay of planting after cultivation whilst allowing more time for the disappearance of the factor inhibitory to the formation of mycorrhizal associations also decreases the time between planting of the trees and reinvasion by *Calluna* and it is suggested that attempts should be made to overcome the latter disadvantage by the use of herbicides. The use of these substances at the outset may even obviate the necessity for cultivation and allow of more equal growth of mixtures of the "heather sensitive" and other species when they are eventually planted.

The application of fertilizers, especially nitrogenous fertilizers, may be expected to assist the growth of trees and reduce the checking effect of *Calluna* although the effect of the nitrogen fertilizers will be shortlived. Maximum effect of fertilizers on tree growth is to be expected in the absence of *Calluna*, i.e. when the trees have formed canopy and possess mycorrhizal associations.

As the stands become older and require thinning, the danger of resurgence of *Calluna*, with its accompanying unfavourable effect on tree growth, will increase. Attempts should be made to counter such effects by keeping the amount of light reaching the forest floor to a minimum, possibly by using selective herbicides to suppress the resurgent *Calluna*, and by encouraging non-ericaceous shrubs and herbs which, besides preventing light reaching the soil surface and the redevelopment of *Calluna*, will produce litter which may catalyse the decomposition of coniferous litter and the liberation of mineral nutrients, especially if calcareous material can also be applied. The mixing of tree species whose litter does not give rise to raw humus with coniferous species, may also be expected to assist in the mobilization of mineral nutrients.

It is suggested that other species, e.g. *Vaccinium myrtillus*, may have a similar, although perhaps not such a dramatic, effect on tree growth as *Calluna* and also that interactions of a similar nature between tree species should not be overlooked.

The possibility that cases where individuals of one plant species seem to have a deleterious effect on each other, i.e. intraspecific interaction, should be regarded as the converse of interspecific action, is suggested and it seems possible that in such cases the introduction of other species may alleviate the condition.

Attempts should be made to isolate, identify and study the properties of specific substances from such systems which could be of considerable importance in plant ecology and plant production.

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