Why Forest Microbiology

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The efficient practice of forestry rests fundamentally on its biological side; upon a knowledge of the constitution of the associations of plants and animals which form the forests and of the interrelationships between the various members of them. Thus microbiology which is essentially a laboratory subject is studied as a means to an end, and not as an end in itself. Now, any laboratory requires extensive, expensive equipment if it is to deal effectively with the problems which arise and provide a useful service. Microbiology is only an exception insofar as parts of the equipment necessaary are specific to a microbiology laboratory. A very conservative estimate of the cost of equipping such a laboratory would be in the range of £1000. and this would only provide the absolute essentials for primitive research involving plate counting and nutritional studies. The question then must naturally arise: what is the position of microbiology in forestry and is it sufficiently important to warrant this expenditure on equipment and personnel?

On the other hand forest management continues to become more intensive as a growing demand for timber necessitates both an increase in forest production per unit area and the afforestation of areas which at one time were regarded as unsuitable for commercial forestry. Clearly, forestry is becoming more agricultural in approach and with more intensive management, there must be a greater awareness of the need for precise information on the mineral requirements of forest crops, the possibilities of improving growth and the prevention of soil impoverishment by applying mineral fertilizers.

Although trees are basically similar to other plants in their nutritional requirements, greater consideration must be given to the natural nutrient cycle for woodlands than for agricultural land, because of certain distinctive features of forest communities. The most striking feature of a woodland community from the point of view of nutrient circulation is the regular annual return of nutrients to the soil in the litter falling from the vegetation cover. The amount and type of litter fall, varies not only with tree species but also with age and has been calculated experimentally to be of the order shown in Table 1.

TABLE 1

Species	*Age (yrs.)*	*Annual litter—lbs./acre dry wt.*
Beech	30-60	3028
Norway Spruce	(30-60 (60-90 (140	(3032 (2582 (1319
Scots Pine	(25-50 (50-100	(2629 (1479

Thus it has been estimated that the build up of organic matter after 55 years of afforestation with Scots pine on open heath in England results in the accumulation of:—

	In litter (lbs/acre)	In Wood (lbs/acre)	Cwts/acre (approx.)
Calcium	574	129	6
Nitrogen	948	289	11
Potassium	272	59	3
Phosphorus	77	17	1
Magnesium	127	29	1½
Sodium	46	10	½

Here then is a vast store of nutrients effectively locked-up from the tree crop and depending upon the ability of the microflora to decompose cellulose and lignin for its release. How then does the crop obtain its supply of essential mineral elements such as nitrogen? Is the soil being constantly drained of its supply of nitrogen while this extensive source builds up and lies useless on the soil surface. Certainly, soil fungi and bacteria degradate some of this organic matter with the resultant release of ammonia, carbon dioxide, amino acids, soluble carbohydrates and fats. Certainly plants can utilise such compounds to some degree, but it is equally certain, that some, such as ammonia, must be converted by micro-organisms into more available forms before they can be utilised by many plants. Are these organisms present in our forest soils? More important still, are they active in our forest soils? The high water holding capacity of many of our forest soils, which suggests at best microaerophilic conditions, at least for parts of the year, plus the acidic nature of humus deposits under coniferous plantations, indicate a great reduction in the activity of these organisms.

But still the question remains, of how the tree gets its supply of nitrogen. Is its supply inadequate? and if so, how inadequate? Recently many investigators have drawn attention to the fact that soil nitrogen balance sheets do not in fact balance. A large number of major reports on this subject show an unexplained increment in soils of about 30-50 pounds of nitrogen per acre, per year. So that not alone are the trees assimilating at least some nitrogen, but in some mysterious way nitrogen is being built up about them. Considerable justification exists therefore, for the entertainment of the idea of nitrogen fixation by non-nodulated plants. This is supplemented by the fact that the inevitable association between plant surfaces and bacteria is not always a random arrangement, but definite associations have been noticed particularly on seed surfaces and on leaves. Data have been produced showing that teeming microbial populations occupy the leaf surfaces of plants. Moreover these populations include various nitrogen fixing bacteria as prominent common components of the population. It is not difficult then to envisage, on the one hand, cuticular excretion of salts and metabolites, providing nutrients for epiphyllic nitrogen fixing micro-organisms, and on the other, uptake of fixed nitrogen by the forest plants, either by the leaf surfaces or via foliar drip, to the soil and then to the roots. However, despite this build-up of total nitrogen about forest trees it is more than likely that many of our forest crops, especially those on the poorer type, wet and heavy soils do suffer from deficiencies of this element.

As the number of flowering plant species is of the order of 250,000 and of the Eubacteriales about 1000, it does not belittle the literature on plant-bacterium symbiosis to say that not much is known about the subject. Even though the Romans in the time of Julius Caesar were aware of the fact that the presence of clover plants in a grass sward improved the fertility level, it is true to say that plant-bacterium symbiosis is largely an unexplored field. The results of plant influence in the rhizosphere are a differently composed, numerically more dense, metabolically more active microflora, compared to that of the surrounding soil. Many investigators claim instances of non-legume response to inoculation with rhizosphere organisms. This response is not ascribed to nitrogen fixation but to the beneficial effects of rhizosphere organisms on the availability of nutrients already present in the soil. Responses of about 10% due to such inoculations have been reported for agricultural crops. Again this is quite creditable for it is well-known that prominent rhizosphere organisms can release nutrients in much the same way as *Bacillus megaterium var phosphaticum* can release available phosphorus.

CH_2 . OR
1 1
CH . OR
1 OH
CH_2 . OPO (CH) + $3H_2O$
O $(CH_2)_2$ 3 + Decithinase
OH

Lecithin (A phospholipid)

CH_2OH
1
CH OH
1 OH 1 (CH)
CH_2 OPO + ROH + R OH + $(CH_2)_2$ OH : N
OH OH

Glycerophosphoric Acid Fatty Acids Choline

Microorganisms often live together because certain bacteria and fungi produce substances that render the existence of other organisms possible. A similar connection exists between the higher autotrophic plants of the forests and microorganisms of forest soils. The microorganisms are dependent to a large extent on organic substances of the litter produced by the autotrophic plants and they in turn promote the autotrophic plants by making the mineral substances of the litter available to them. This however, is not regarded as true symbiosis, nor is the case of epiphytes, where organisms live on or in close proximity to the roots; the concept symbiosis is restricted to the living together of two organisms in immediate contact. There are however, innumerable cases in which the plants and organisms not only come into contact, but in which at least one of the symbionts lives partly on the tissues of the other; e.g. nodule bacteria, mycorhizal fungi or actinomycetes, which live in the roots of their host plants. This is a specialized form of living together in which the contact is close and there is a reciprocal influence on metabolism. It is possible and in fact probable, that the nodulation of *Myrica* and *Alnus* fall into this category, as do root nodules which are known to occur on some conifers and are considered to contain a microsymbiont. One is unable however, to conclude the identity of the latter organism since opinion is divided between fungi and bacteria.

In some cases such close mutual dependence has developed, that under natural conditions, neither of the partners is entirely capable of independent existence; e.g. several mycorhizal fungi form their fruiting bodies only in symbiosis with forest trees and the failure to afforest certain areas of prairie and peatland has frequently been attributed to the lack of mycorrhizal fungi. To what extent then should we, as foresters, worry about these mycorhizal fungi?; after all Ireland has always been a land of forests. How do these fungi get onto our trees? or to what extent are these, all too frequently generalised about mycorhizae present on our trees?

Dealing with these questions in rotation, it is not difficult to find reasons why we should be concerned about these fungi for it has been shown experimentally that pines with mycorhizae can absorb 234% more phosphorus, 86% more nitrogen and 75% more potassium than pines devoid of mycorhizae. Because of the absence of root hairs in the mycorhiza and because the fungal sheath covers those parts of the roots capable of absorption of nutrient salts and water the latter have no other course to the plants than through the fungal associate. Beyond this, the hyphae spreading into the surroundings from the fungal sheath contribute in a high degree to the enlargement of the absorbing surface. The mycorhizae actually represent absorptive organs of the trees and hyphae going forth from the fungal sheath into the substrate act in an analogous manner to root hairs in respect of salt absorption. Moreover pine and spruce grown in pure culture do not assimilate complex organic nitrogen compounds to any great degree, but mycorhizae can be shown to facilitate the absorption of ammonia and organically combined nitrogen and to be of special importance in those acid and poor soils in which nitrogen is combined in organic humus substances. They also make the plants more competitive for nitrogen with other fungi and this fact is borne out by the sparce development of mycorhizae in richer soil types. It has also been shown repeatedly under normal conditions that tree species devoid of mycorhizae on difficult sites become stunted and die. This is reported frequently on freshly drained peat bogs, on which pine and spruce seedlings are at first stunted. The mycorhizal fungi which spread rapidly after drainage then stimulate their growth. Furthermore, it has long been known that orchid seeds depend on mycorhizal fungi for successful germination and the same may very well hold true for the germination of many other seeds. For instance, acorns inoculated with an appropriate fungus, yield about 34% more plants than acorns without mycorhizae, while addition of mycorhizal soil increases the height of uninoculated seedlings by about 52%. Other functions frequently attributed to mycorhizae include the protection against pathogenic fungi, while the fixation of atmospheric nitrogen by mycorhizae has been mentioned over and over again. Because of these important considerations therefore, not only should foresters be conscious of these organisms but because of their great practical significance forestry should provide the requisite impetus for their intensive study.

As to the method of formation of these mycorhizae, there is every indication that primary infection is of the endotrophic nature. This primary infection seems to mobilise the defence forces of the host plant, which digest the intracellular mycelia and which push the fungus into an intercellular position, in which case an ectotrophic association is formed. If however, the fungus is able to resist digestion by the enzymes of the cortical cells it may remain in an intracellular position. The mycorhizal fungus, therefore, attacks the host primarily in the same way as a parasite. The volume of the infected cells often increases or the cells may be stretched in a radial direction. On the other hand the growth in length of the roots is often arrested. In this way the stocky form of the root arises, which is characteristic of many mycorhizae. Plants thus allow mycorhizae to penetrate only to a certain distance. On penetrating a certain distance hyphae of the fungus may be digested by the plant and on the basis of the degree of digestion different types of mycorhizae are now recognised. Thus a gradation from ectotrophic to endotrophic is now generally recognised.

In the absence of experimental evidence to prove otherwise, the only logical explanation for the relative success of many of our Irish forest crops on extremely difficult sites, is the presence on the roots of mycorhizae. Much of our blanket peat is otherwise, too acidic in nature, too high in moisture content to allow vigorous microbial activity and too impoverished in mineral nutrients to support tree growth. It seems to be also that the requisite fungi are not present in these bogs themselves but are carried on transplants at planting time.

Whatever section of forestry activities one takes the importance of microbiology is equally striking but none more so than the field of plant pathology. For instance, in a recent report on the pathology of forest trees in New Zealand, 93 fungal, 3 bacterial and 1 algae pathogen are recorded, together with one mycorhizal deficiency disease. However, apart from the undetermined damage caused by heart rots, few diseases have caused serious loss in either native or exotic plantations in Ireland. Two important reasons for this are, our geographical isolation from sources of infection and the wide generic differences between our exotics and our native trees, which has meant that few native pests or diseases have attacked our exotics. Nevertheless the danger cannot be overemphasised, for almost the whole of our forest industry is based upon a few exotic species. In such a situation an economic catastrophe may result from the introduction of a single primary pest or disease. It is becoming increasingly obvious that more fundamental information on the biology of the many forest organisms, both beneficial and pathogenic, is needed before field experiments or any control measures can be satisfactorily attempted. Recent developments in the biological control of *Fomes annosus* have emphasised this point for now the value of creosote as a protectant can be looked upon as a 'passive' measure and seriously questioned. The inoculation of the fresh stumps either naturally or artificially by fast cellulose decomposers such as *Trichoderma viride* or *Peniophora gigantea* and the alteration of stump tissues by chemical means have now been proven to be preferable methods. More recently still, at least two actinomycetes have been discovered producing antibiotics which inhibit the growth of Fomes. It is not stretching the imagination to extremes, to foresee the synthesising of these antibiotics and the injection of valuable trees or stands of trees with them, to prevent further damage to the timber. Investigations have also shown that fumigation of the soil with carbon disulphide may also lead to biological control of *Armillaria mellea*. After fumigation *Trichoderma viride*, a harmless fungus grows much faster than other fungi because of its tolerance to carbon disulphide and produces two antifungal antibiotics, Gliotoxin and Viridin which are antagonistic to Armillaria. Furthermore the living bark of European Larch may be expected to carry a microflora which contains both fungi and bacteria and frequently *Dasyscypha*. The necrotic areas which constitute a canker can be shown to carry a similar microflora, but *Dasyscypha* is not necessarily the most abundant or dominant organism nor is its presence necessary for the development of canker. What then is the importance of the fungus in these areas? or is it a harmless saprophyte. Again the microbiological examination of 250 Sitka Spruce seeds which had failed to germinate showed that 80% of them were infected with microorganisms.

69	carried	Bacteria
55	,,	Psychrophilic seed fungus.
43	,,	*Penicillia*
7	,,	*Cylindrocarbon*
5	,,	*Rhizoctania solani*
4	,,	*Verticillium*
3	,,	*Ceratobasidium*
2	,,	*Mucor*
1	,,	*Gliocladium roseum*
13	,,	Unidentified fungi

There are moreover numerous reports of up to 50% failure of Sitka Spruce seeds to germinate even though laboratory viability tests seldom show this failure rate. This discrepancy has frequently been attributed to the infection of weak and damaged seeds by microorganisms, some of which cause decay of radicles. However, no microbial infection of seeds can be found on cones direct from the tree; only slight seed infection on cones opening on the tree and 50-100% infection on seeds when the cones are stored in bins or storehouses. Furthermore fungal growth is noted on seeds stored at temperatures below freezing point and Table II will provide further evidence of the influence of the seedcoat microflora upon seed germination.

Effect of storing at 10°C. on germination at 20°C.

Days at 10°C.	*% Germination*	*% Ungerminated seeds yielding Bacteria*	*Penicillia*	*P.S. Fungus +*	*Other Fungi*
0	82	30	50	5	5
3	72	15	35	20	30
5	68	20	25	20	40
7	65	0	60	20	35
17	63	15	5	45	10
32	40	0	10	50	15

+ Psychrophilic seed fungus

Thus far I have confined myself to pointing out some of the microbiological problems of forestry, but with more intensive management of our forests, how are we to harness these useful micro-organisms and control the harmful ones? For instance is it possible to control the build up of organic material on the forest floor and gradually return to useful circulation this vast store of nutrients locked-up from the trees? Is it possible to utilise more efficiently those sites heretofore regarded as unfit for commercial forestry or will the tourists of future years looking upon our poor stunted effort to re-afforest our peatlands, re-echo the words of an eminent visitor, looking upon these same barren peatlands in the 17th century and say, (quote), 'that it is due to the wretchedness of the Irish, who took no care to tend or cultivate them'. (unquote). Well may this comment be true, for all of the available evidence suggests that high yielding Sitka Spruce can be grown even on the most difficult peats provided, (1) that additional nitrogen is provided: (2) that the pH is rectified to some degree, and (3) that some drainage is carried out. Under these circumstances, if Sitka spruce were given a few nitrogen top-dressings, sufficient nitrogen would be injected into the nutrient cycle to keep trees growing especially if the trees could by this time utilise the nitrogen reserves of the peat through increased microbiological action in breaking down the organic matter.

Again a comparison of the total annual litter fall under different thinning grades and the weight of the forest floor, illustrates the important influence of this silvicultural operation on the rate of decomposition of organic material, through its effect upon the micro-climate at the soil surface.

Monthly litter fall under different thinning grades (N. Spruce)
Dry Wt. lbs./acre.

Thinning grade	B	C	D	L.C.
Oct.	480	412	295	393
Nov.	478	379	334	318
Dec.	278	211	231	180
Jan.	355	304	281	263
Feb.	489	296	304	249
Mar.	683	420	342	557
Totals	2763	2022	1877	1960

Wt. of forest floor under different thinning grades.
Dry Wt. lbs./acre

Thinning grade	B	C	D	L.C.
	44,000	23,500	14,900	26,300

The greater litter fall and unfavourable microclimate under the heavy shade of the B grade thinning results in twice the weight of organic material accumulating compared with C and L.C. grades, and three times that under D grade. The large difference in the weights of the forest floor between C and D grades whose annual litter fall is similar, confirms that this is due to differences in the rate of decomposition and not merely to differences in litter fall. Perhaps this is a factor which ought to be borne in mind in laying-out future thinning experiments or perhaps we might take a lead from the agriculturalists, who have for long treated build-up of organic material in permanent pasture by disc-harrowing and rotovation, which practise is not foreign to many European foresters in dealing with the same problem. The true value of microbiology in the field of pathology has, despite recent advances, probably yet to be realised, in the biological control of the diseases which threaten our forests.

Agriculturalists do not have to contend, to the same extent in any case, with the build-up of organic matter; they are not so concerned with those fungi which play such an important role in feeding our forest crops; they do not, in Ireland in any case, sow pure crops in blocks of hundreds of acres, as the forester does. Therefore, microbiology is not as great a concern for them as it must be for the forester. Insofar as it is of concern they have not been slow to recognise its significance; e.g. the nitrification and nitrogen fixing processes in soil are probably the most researched aspects of the whole subject. Because of its great practical significance in forestry, then, the intensive study of microbial-microbial and microbial-plant symbiosis must be carried out by foresters, for forestry. I at least, have no doubt, but that forest microbiology is a vital link in the chain that is slowly but surely changing this Emerald Isle of bogs, (to quote another eminent visitor), into the Emerald Isle of woodlands, it undoubtedly once was.

GENERAL DISCUSSION

1. The question of the nitrogen supply to plants in the afforestation of peatlands continues to be of concern. It is generally conceded that the nitrogen supply from the peat itself is inadequate to meet the demands of a growing crop. The question automatically arises as to which form of fertilizer is most efficient in supplying the necessary nitrogen?

The general opinion at the moment seems to be that plants growing on peat can utilise all three forms of nitrogen. Most plants can readily utilise nitrate as a nitrogen source but the advisability of applying nitrate nitrogen to peat is questionable, since the nitrate ion is very mobile and is readily leached. Furthermore acid peats in general show a very strong denitrifying power. Application of nitrate therefore, would of necessity, have to be in the form of frequent light dressings, which over large areas would for economic reasons be unfeasible. Ammonia has been shown to be absorbed very rapidly by mycorhizae but addition of ammonium salts and nitrates diminish the intensity of root infection with symbiotic fungi. The ready availability of ammonia to plants, therefore, depends upon the presence of mycorhizae, and it is known that the microflora necessary for the conversion of ammonia to the nitrate form is either completely lacking or completely inactive in virgin peats. Organic nitrogen compounds, such as, glumatic and asportic acids, amino acids, urea, nucleic acids and protein hydrolysates are therefore more likely to be better sources of nitrogen than the ammonium or nitrate forms, but again the presence of mycorhizae must be assumed.

A question of practical significance now arises, for plants which are normally mycorhizal can be raised successfully as uninfected seedlings and can make very good growth in a short time under heavily manured conditions. But the relative poverty of available mineral salts determines the prevalence of mycorhizae on seedlings. Is it more important to the forester to grow large nursery stock although it is uninfected or to ensure that the seedlings become mycorhizal in the nursery at the expense of the degree of growth. Where exotics which are not habitually grown are being raised, it seems to be good policy to raise infected seedlings by suitable inoculation and treatment to ensure a source of inoculum in forest conditions. This is borne out by the fact that self-seeded pine and spruce seedlings on recently drained peat-bogs develop poorly and show signs of nutrient deficiency, such as are associated with nitrogen starvation.

The specificity of mycorhizal fungi and the possibility of the presence of these organisms in peat was queried. It was rather discouragingly admitted that it was unlikely that these fungi were present in peat. In fact, examination of the surface layers of Irish peats by some of our leading mycologists has shown that many fungi are present on or near the surface but that these are mainly Penicilli, Aspergilli, and Mucor sp. Although some unidentified fungi are recorded in these reports there is not even one mention of a mycorhizal fungus. As far as specificity is concerned most host species seem capable of forming mycorhizae with several fungi, and on any given root system, more than one type of mycorhiza may be observed, i.e. a single host may associate with members of several fungal species at one time. The most unspecific of all the mycorhizal fungi seems to be *Cenococcum grandiforme*, which has been found on on the root systems of species of *Pinus*, *Picea*, *Abies*, *Pseudotsuga*, *Tsuga*, *Tilia*, *Larix*, *Quercus*, *Fagus*, *Betula*, *Corylus*, *Corya;* *Populus*, *Salix* and *Alnus*. The greatest degree of specificity is shown by *Boletus elegans*, which is only known to associate with *Larix* sp. Between these two extremes there are many fungi from amongst the Phycomycetes, Ascomycetes and Basidiomycetes of intermediate host range.

The question of the role of phosphorus in plant nutrition was raised and it was observed that phosphorus is mainly present as a structural component of the nucleic acids, ribosenucleic acid (RNA) and deoxyribosenucleic acid (DNA). It is also present in the phospholipids, which are believed to play an essential role in the structure of the membrane. A deficiency of phosphorus is thus very serious for the cell, preventing the formation of new genetic material in nucleus and cytoplasm. Phosphorus is critically involved also in all energy transfer steps in the cell, since compounds such as adenosine diphosphate (ADP) and adenosine triphosphate (ATP) contain two and three phosphates.

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