THE ECOLOGY OF *RHODODENDRON PONTICUM* L. WITH SPECIAL REFERENCE TO ITS COMPETITIVE AND INVASIVE CAPABILITIES.

by

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PLATE 1 : Rhododendron ponticum in flower .

PLATE 2 : Invasive R.ponticum at Cordwell near Sheffield .

DEDICATION

To my parents and my brothers, Neil and Stewart.

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SUMMARY

A survey of the current status of Rhododendron ponticum in the Peak District and Sheffield area was carried out. It was found to be widespread over much of the area, particularly on free-draining, nutrient-poor, acidic soils, in sheltered, moist situations. It was largely absent from the Carboniferous and Magnesian Limestone Series, from the high altitude Kinder/Bleaklow massif and from areas subject to intensive agricultural or industrial development. Disturbance of habitats through forestry, grazing or recreational activity seems to encourage invasion of suitable areas.

The role of mycorrhizal infection in R. ponticum was investigated. Mycorrhizal plants had lower Root/Shoot Ratios, higher Relative Growth Rates and showed increased yield compared to non-mycorrhizal plants. The benefits of infection were strongest on nutrient-poor soils without added nutrients. Infection of roots was visible after around six weeks and the effects of mycorrhizas were increasingly apparent during the following six weeks. The source of fungal inoculum was investigated and considered.

The 'interference' phenomenon described by earlier workers was investigated. A mixture of competitive and allelopathic influences upon test seedlings in bioassays was demonstrated. The toxicity found, was closely related to the presence of R. ponticum roots in the soil. Both living or dead roots produced the effects. These were not removed by nutrient addition. Interference was not dependent on mycorrhizal infection of the R. ponticum roots.

To help an understanding of the interference demonstrated, a survey was undertaken of the 'free' phenolic compounds occurring in R. ponticum tissues, associated soil and litter, and in canopy throughfall. These compounds have been implicated in allelopathic interactions involving other members of the Ericaceae. R. ponticum tissues were found to have very high concentrations of 'free' phenolic compounds compared to other plant species examined. Considerable variation in form and amount was found with tissue type and age. Phenolic compounds were detected in associated soil and litter, as well as in canopy throughfall from R. ponticum.

It was shown that the interference cannot be fully explained by competition for water and/or nutrients. In some situations a toxic influence perhaps due to aromatic and aliphatic acids released from the roots, has a major effect on the interaction between R. ponticum and associated vegetation. Competition for nutrients and/or water clearly occurs in some field situations, particularly when R. ponticum bushes are encroaching on established vegetation. However, with bare-zones (either in the field or under artificial conditions) competition factors may be almost totally eliminated by the toxicity which inhibits root formation. Since the seedlings have very restricted root development, they are barely able to compete for nutrients or moisture, and the toxic effects dominate the interaction. The natural situation in the field is complicated by the acidification of soils associated with R. ponticum, the physical and chemical effects of its litter, shading and the overall influence of the plant on soils and nutrient cycling.

The large quantity of 'free' phenolic compounds in R. ponticum tissues (especially new leaves and new stems) probably have anti-herbivore and/or anti-pathogen functions. This would explain the observed lack of damage to the plant by invertebrate herbivores, diseases or parasites. These compounds would thereby enhance the growth and competitive ability of R. ponticum.

The ecological success and invasive nature of R. ponticum may be attributed to a mixture of factors. It has a high fecundity with effective dispersal. The high phenolic content of its tissues gives resistance to attack from invertebrate (and possibly vertebrate) herbivores or diseases. Mycorrhizal infection produces more effective growth under the conditions in which R. ponticum is usually found in the field. The highly competitive ability in favourable habitats may be aided in at least some situations by an allelopathic influence on competing plants. It is a remarkable attribute of R. ponticum that (as demonstrated) it can actively modify its environment to make it more suitable. Through processes such as the acidification of associated soil, the edaphic range exploited by R. ponticum (and indeed other ericaceous plants) is expanded.

These factors are discussed, and their importance in terms of the ecological success and invasive nature of R. ponticum as an alien in the British Isles, is assessed.

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CHAPTER 1

INTRODUCTION

The status of Rhododendron ponticum in the British Isles and north-west Europe is that of an alien. Now widely naturalized, its invasive nature in both managed and semi-natural habitats, gives it ecological and financial interest. It is an extremely important weed of commercial forest plantations and a threat to some semi-natural oakwoods.

The genus Rhododendron is a large group of shrubs in the family Ericaceae. This rather extensive family includes other well-known genera such as Erica, Calluna, Vaccinium and Gaultheria. The family is of almost world-wide distribution. Its largest genus is Rhododendron, with around 1200 species. The bulk of these are found in the Far East where China, Tibet, Burma and Assam all meet. A much smaller number are native to southern Europe and the Middle East. It is this latter group which includes R. ponticum.

R. ponticum was divided into two sub-species: R. ponticum ponticum, native to the Mediterranean region around Turkey, Asia Minor and Lebanon, and R. ponticum baeticum occurring in a small area of south-west Spain, central and southern Portugal. C.F. Chamberlain (pers comm.) does not consider the division into sub-species to be valid.

In addition to its natural distribution R. ponticum is now naturalized in Belgium, France and the British Isles (Cross, 1975). It was introduced to Britain in 1763 (Elton, 1958). According to N. Burston (pers comm.) 1763 was probably the year in which seeds reached Kew Gardens. The commercial introduction of plants to estates was probably somewhat later (1770-1780) by the nurseryman Conrad Loddiges. The late H.G. Hurrell (pers comm.) believed the original site of introduction to be Lyndhurst in the New Forest.

R. ponticum was widely planted on estates throughout the British Isles from the late 1770's to around 1930. Planting was for ornamental purposes, as cover for game and/or wildlife and to form windbreaks. Seedlings of R. ponticum were also raised for commercial grafting of

hybrid rhododendrons which have been developed over the last two hundred years as a result of the great interest in cultivation of both natural species and hybrids. Hundreds of species were introduced into gardens and collections from all over the world. Particularly important were the introductions by Joseph Hooker during the mid-late 1800's and by Frank Kingdom-Ward in the 1920's and 1930's.

Despite the large number of species introduced, only two, R. ponticum and R. luteum, have become naturalized in the British Isles. (R. luteum is only naturalized at few sites especially in the West Highlands (D.M. Henderson, pers comm.)). A number of other species will produce self-sown seedlings in the mild, humid conditions of some of the estates in western Scotland (D.F. Chamberlain, pers comm.; A.C. Leslie, pers comm.). Some of the American species (e.g. R. maximum and R. catawbiense) are very similar to R. ponticum, grow well in Britain and are more hardy. Even so, they have failed to establish themselves in the wild.

R. ponticum is now thoroughly naturalized in suitable habitats throughout the British Isles. This is especially the case in areas where it was planted on a vast scale in the shrubberies and avenues of country estates. Where conditions are neither too dry, nor too exposed, with soil which is acid to neutral (especially on sandy, podsolic soils), it thrives. It is now a major forest weed. (Brown, 1953a, 1953b; Cross, 1975).

R. ponticum produces great quantities of fertile seed and in addition spreads freely by natural layering. Cross (1975) estimated seed production by a bush 2m high with a 10m circumference, to be over one million, with about 90% viability during the following season. The seeds are very light, being about 2×10^{-5}g dry weight each (Brown, 1953a, 1953b). They are therefore easily windblown over considerable distances.

A number of studies have been devoted to the spread of R. ponticum at sites in England (Fuller and Boorman, 1977; Brown, 1953a, 1953b) and in Ireland (Cross, 1973, 1981; Robinson, 1971, 1980). Robinson (1980) cites a number of examples of the rapid spread of R. ponticum in Irish forests. Following introduction around 1921 or later in the sites named below the plant has now spread to occupy the areas given in brackets:-

Ballyporeen (198 ha), Bansha (136 ha), Cahir (155 ha), Galtee (80 ha), Glengarra (288 ha) and Glen of Aherlow (33 ha).

According to Robinson (1971) 445 ha or 14% of the total area under forest at Clogheen, has been invaded by R. ponticum during a fifty year period. At Killarney, 3200 ha has been invaded since the mid-1800's (Cross, 1979 in Robinson, 1980).

The character and form of the plant varies with its situation. It is taller and spreading in woodland, and smaller and more compact in open sites. According to Cross (1975) the British plants more closely resemble the ssp. ponticum. Cox and Hutchinson (1963) believed them to be hybrids of R. ponticum and R. catawbiense. As already noted, the division into sub-species is no longer acceptable. The variation seen in naturalized populations is now believed to be due to environmental factors, variability within the species not associated with hybridization, and in some populations, to introgressed characters, indicating a considerable degree of hybridization (A.C. Leslie, pers comm.; D.M. Henderson, pers comm.).

An evergreen shrub 2-8m high, R. ponticum is monopodial at first then sympodial and when mature, has several major axes arising from a large, irregular base. Due to poor mechanical strength, the plant tends to spread laterally rather than vertically, except when it is supported by trees or other shrubs. The mauve flowers are borne freely in late May and June, in compact racemes.

Under favourable conditions R. ponticum grows into a rank and impenetrable shrub layer up to c. 8m high in woods and c. 5m high in the open. This growth crowds, shades and competes with other vegetation. Large mature bushes may transmit only about 2% of total daylight (Cross, 1975). The most extreme effect of this growth habit is the elimination of herbaceous vegetation and the choking of shrubs and small trees (such as Betula sp. and Ilex aquifolium). Regeneration of trees and shrubs is prevented and ultimately woodland in mild, humid climates might be totally replaced by a monospecific blanket of R. ponticum. According to

Brown (1953a) there is no available information on the longevity of R. ponticum in Britain. Sites occupied for two hundred years or more, as yet show few signs of degeneration of the mature plants.

Once established in a suitable area, R. ponticum can be extremely difficult to eradicate. Simple cutting results in a proliferation of shoots and the development of very dense stands. Clearance by hand is laborious and costly in terms of manpower. Heavy machinery such as bulldozers and rotovators are expensive to operate, destructive to the habitat and their use is not always feasible in rough or inaccessible terrain. Unless combined with the use of a suitable arboricidal spray, these methods are of little permanent value. R. ponticum regenerates rapidly from cut stumps and roots, and also recolonizes such disturbed open areas by seed. (The seed often being spread liberally over the site during clearance of the bushes!). Hand-cutting and subsequent maintenance may be useful as a last resort for small areas of high conservation value.

Brown (1953a) noted that whatever method of control was chosen, it would be very costly. R. ponticum is very resistant to most herbicides. Ammonium sulphamate, 2,4,5-T, glyphosate and triclopyr have been used successfully to kill R. ponticum. Such applications need to be followed by mechanical clearance and subsequent periodic spraying to prevent regeneration of R. ponticum (Aldous and Hendrie, 1966; Robinson, 1980). Practical problems with the use of ammonium sulphamate and 2,4,5-T led to work by Robinson (1980) which suggests glyphosate or triclopyr as potential commercial control agents.

Robinson (1980) concludes that R. ponticum in commercial plantations is a serious threat to the profitability of a potential crop. The major costs arise through competition with the crop, hindrance to accessibility and forestry operations or through the application of control measures. For complete eradication Robinson states that a fully integrated weed control programme is necessary. It seems unlikely that such comprehensive control could be implemented throughout the large areas of commercial plantations infested in England and Wales, and particularly in west Scotland and Ireland.

A degree of biological control may be achieved by planting tree crops which cast a heavy shade, to either eliminate R. ponticum, or at least to retard its spread. Suitable species are Tsuga heterophylla, Picea sitchensis and Pseudotsuga menziesii (Robinson, 1980).

The extent of spread of R. ponticum, together with the problems of control, pose a threat to many areas of native, semi-natural woodlands in the west of the British Isles.

If R. ponticum is to be controlled, then the reasons for its success need to be understood. Cross (1975) notes the poor competitive ability of the seedling phase. R. ponticum at this stage is readily smothered by other vegetation or litter. However, the seedlings are able to survive for long periods if not smothered or desiccated making virtually no growth until conditions become more suitable.

Once established, the mature plants are highly competitive in suitable habitats. R. ponticum grows rapidly, casting a dense shade and presumably exerting strong root competition on neighbouring plants. The shallow root system however, leaves the plant particularly vulnerable to drought. Observations in North Wales after the 1976 drought showed extensive areas of mature R. ponticum which had complete 'die-back' of all aerial parts.

Elton (1958) suggests that R. ponticum may be a successful invader due to it having a poor herbivore fauna associated with it. There are a number of examples such as the introduction of Opuntia stricta to Australia, in which an alien plan has become invasive, only to be eventually controlled by a herbivore. Elton's idea is that R. ponticum has become isolated from its normal food-chain and that native British animals are not adapted to feed on it. However, it may be that restricted herbivory is a natural feature of R. ponticum, perhaps associated with aspects of its biochemistry rather than its country of origin. R. ponticum and other species of Rhododendron seem to be as successful and dominating in their native regions as they are as aliens (H.G. Hurrell, pers comm.; Whittaker, 1962; H. Gurtan, pers comm. via J.R. Cross; Cross, 1973). In the case of R. ponticum, Cross (1973) notes it as being very plentiful in Turkey at 2000m in clearings among Picea orientalis and forming a thick

ground cover under forests, especially if not too shaded. He states that it is clearly a commercially important weed for forestry in Turkey, one of its countries of origin. It is the case that R. ponticum appears to be relatively free of herbivory (both vertebrate and invertebrate) and also of serious diseases (Cross, 1973, 1975).

Herbivory by either vertebrates or invertebrates can be of great importance to the ecology of individual plant species and to vegetation community dynamics. Removal of grazing pressure by large herbivores such as mammals can drastically alter a community and the outcome of interspecific competition within a community. Changes in grassland communities following the decline of the rabbit in Britain after myxomatosis during the 1950's are an example. Susceptibility or resistance to herbivory may have considerable influence on the outcome of competition between plant species. This may be a subtle effect, not necessarily discernable without controlled experiments (e.g. Bentley and Whittaker, 1979; Bentley, Whittaker and Malloch, 1980).

Successful biological control has been employed in areas where a plant had been introduced to a suitable environment which lacked the plant's associated herbivores. Under these circumstances the plant initially becomes highly invasive throughout suitable habitats due to the absence of herbivore pressure. When suitable herbivores are successfully introduced from the plant's country of origin, a considerable measure of control can be achieved, the plant's invasiveness being curtailed. Examples of this are the spread of Opuntia stricta in Australia and its subsequent control by Cactoblastis cactorum, and that of Hypericum perforatum in California and its control by Chrysolina beetles (Krebs, 1972).

Herbivores and pathogens damage plants in a variety of ways. Damage may have obvious effects through seed or seedling predation, or catastrophic damage to mature plants (Chew and Rodman, 1979; Waloff and Richards, 1977). Generally, however, heterotroph damage may not have clearly observable effects on a plant's performance. Mineral loss via herbivory damage to a leaf for example, may be a more important factor than the more obvious loss of photosynthetis capacity.

The developmental stage of the plant is important in terms of the damage inflicted by a herbivore and also in terms of the degree of protection the plant may have through either physical or chemical means.

Palatability of the plant tissues and their nutritive value to a herbivore may vary considerably with age. Similarly the investment of plant resources in tissues will also vary, along with the ability of the plant to recover from damage.

The effects of herbivory may therefore be very difficult to predict. It may have a negative effect on growth and reproduction of the plant, or little overall effect, or even a stimulatory effect. This depends largely on the timing of damage, the intensity and also the interaction of the plant with its competitors (Chew and Rodman, 1979).

The invertebrate fauna recorded as feeding on R. ponticum in the British Isles is very poor (Cross, 1975). It does seem likely that the existing fauna could be expanded with further research. Bushes under canopies of Quercus or other deciduous trees may sometimes be severely affected by phytophagous insects. These are presumably 'raining' down from the tree canopy above and hence not especially adapted to, or dependent on R. ponticum. Indeed they may suffer from toxic effects of the foliage, but damage to the plant would be effected by a fresh fall of insects from above. The amount of invertebrate herbivory in its natural habitat is not known.

Grazing or browsing by wild mammals or domestic livestock is usually very limited. R. ponticum contains a compound known as 'andromedo toxin'. This is highly toxic if ingested (Forsyth, 1954). Animals (usually juveniles) will sometimes nibble young leaves and shoots. This rarely causes substantial damage and animals probably learn to avoid R. ponticum. The high phenolic content of its leaves may also render it unpalatable and possibly toxic.

Cross (1975) gives a list of fungi and algae which occur on R. ponticum either pathogenically or as epiphytes. None of these seems to have generally severe effects on vigorous plants. One fungal disease of possible importance is 'Rhododendron bud-blast'. This is caused by the fungus Pycnostysanus azalaea, probably spread by the leafhopper Graphocephala coccinea (Baillie and Jepson, 1951). Bud-blast may kill-

off up to 50% of R. ponticum flower buds (Cross, 1975). However, with the prolific seed production by R. ponticum, this is unlikely to be of ecological significance.

Cross (1973, 1975) concluded that slightly higher relative growth rate at low light levels, than competing species such as Ilex and Quercus, may give it a competitive edge. This same work suggested that its capacity to photosynthesise in winter may also be important.

The studies of Cross (1973, 1975) and other workers such as Brown (1953), largely overlook the potential importance of mycorrhizal infection. Cross (1973) notes work suggesting beneficial effects of infection on the ericaceous host in nutrient-deficient soils, but is also strongly influenced by work suggesting the relationship to be relatively unimportant to the host plant and possibly a case of controlled parasitism (Leach, 1962, Singh, 1964).

There is now a considerable body of evidence showing the beneficial effects of ericaceous mycorrhizas to their host plants. This is especially the case on free-draining, nutrient-poor soils. The benefit is largely in terms of increased dry matter production and enhanced nitrogen and phosphorus uptake (Pearson and Read, 1973, 1975; Stribley and Read, 1974, 1976, 1980; Read and Stribley, 1975; Cooke, 1977). It seems likely that these benefits to the host should apply to R. ponticum and could confer a substantial competitive advantage. Cross (1973, 1975) notes the poor performance of R. ponticum on waterlogged sites. This might well be through inhibition of mycorrhizas which would occur under such conditions.

Another possible effect of R. ponticum on competing plants is that of an allelopathic influence. Cross (1973) suggest this might be an explanation for the deleterious effects on Ilex in woodland and for decreased cover of vascular plants around bushes at Winterton National Nature Reserve in Norfolk. Roff (1964) examined this latter phenomenon with regard to 'bare-zones' around Calluna vulgaris bushes at Winterton and in the Norfolk Brecklands. He concluded in favour of some form of toxicity associated with soil long-occupied by Calluna roots. The fact that a number of supposed examples of allelopathy do involve ericaceous

plants is also of interest (Chou and Muller, 1972; Robinson, 1971, 1972; Ballester, Albo and Vieitez, 1977; Carballeira, 1980; Carballeira and Cuervo, 1980; Read and Jalal, 1980; Jalal and Read, 1983 I&II).

Chou and Muller (1972) state that pure stands of any long-lived plant species are very suggestive of chemical dominance. Dense thickets formed by many of the Ericaceae (such as R. ponticum) might be examples of this.

Allelopathic effects of R. ponticum could manifest themselves through decreased growth of competitors around the perimeter of the bushes or of either emergent trees and shrubs within stands of R. ponticum or potential colonizers within such stands. Although the dense shade cast by R. ponticum may well suppress germination and growth of seedlings, areas within stands where the canopy has fallen away for up to several metres square (thus eliminating the shading effect) still show no signs of regeneration of any vegetation. (Examples of this may be seen at Strawberry Lee Plantation, South Yorkshire.) This might be explained by intense root competition, drought, toxicity, a physical effect of the Rhododendron litter, or a combination of these.

Success attributable to either low herbivore pressure or allelopathic effects on competing plants, requires a mechanism by which to operate. Studies of secondary plant metabolites and of their role in interactions between plants have increased markedly in recent years (Rosenthal and Janzen (ed.), 1979; Harborne (ed.), 1972; Harborne, 1977). It is clearly of interest that the Ericaceae have been shown to be rich in such secondary metabolites, notably the phenolics (Cross, 1975; Read and Jalal, 1980). Cases of suspected allelopathy involving the Ericaceae have also implied that phenolic compounds are agents of toxic effects (Chou and Muller, 1972; Ballester, Albo and Vieitez, 1977; Carballeira, 1980; Carballeira and Cuervo, 1980; Read and Jalal, 1980; Jalal and Read, 1983 I&II).

Current evidence and opinions of the possible role of the flavonoid compounds and of tannins (including proanthocyanidins) in the interaction of plants and herbivores are discussed by Harborne (1979) and Swain (1979). Flavonoids have an established function in plants as floral pigments and hence as attractants for insect pollinators. The functions

of the widespread 'colourless' flavones and flavonols often present in leaves, are rather obscure. According to Harborne (1979) however, there is evidence to suggest that they may be of considerable significance as feeding deterrents. Some have been shown to be insecticidal and others have hormonal effects on grazing mammals and birds. Control of insect attach is only one of a number of possible explanations for the complexity of flavonoids and associated glycosides found in plant tissues (Harborne, 1979).

Tannins are known to be extremely important in defence of higher plants against attack by either herbivores or by fungi. Tannins reduce both the nutritional availability of soluble plant proteins and polysaccharides and the activity of the digestive enzymes and symbiotic micro-organisms of the herbivore's own gut. The importance of these compounds is stressed by Swain (1979). The proanthocyanidins are the most widely distributed tannins in higher plants. They are formed by condensation of the flavan-3-ols, catechin, epicatechin or gallocatechin. A number of proanthocyanidins and their catechin and epicatechin preccusors are important constituents of the spectrum of simple phenolics in R. ponticum tissues. These simple phenolics might therefore be important through anti-herbivore functions and hence contribute directly to the plant's ecological success.

An aspect of Rhododendron biochemistry which is widely accepted as a contributor to its success, is the presence of 'andromedo toxin' as already mentioned (Forsyth, 1954; Wood, Stromberg, Keresztesy and Horning, 1954; Cross, 1973, 1975; Jaynes, 1975). This compound is extremely toxic to grazing mammals and undoubtedly confers a considerable advantage in this respect.

Another way in which R. ponticum may influence the growth of its competitors is via changes in the nature of its associated soil. In common with other ericaceous plants, R. ponticum has a tendency on suitable soils to cause acidification and podsolization. This is discussed further in a later chapter. Cross (1975) suggests it may have a deleterious effect on soils by mobilizing cations, directly or indirectly by the production of polyphenols. Doekson (1964) found that ground rhododendron leaves caused a reduction in the number of

earthworms, Lumbricus rubellus in peaty soil. Many earthworms are very sensitive to soil pH and avoid acid soils. Elimination or a decline in earthworms at a site would decrease soil mixing, slow the breakdown of organic litter and favour increased podsolization.

Soils with dominant R. ponticum tend to develop a layer of very coarse, unfragmented litter, lying over a layer of decomposing litter, permeated by fine hair roots. This layer usually covers an acid, peaty humus, again with hair roots. These layers are of variable thickness and lie usually over a peat or a sandy soil that was originally present. The accumulation of a thick layer of undercomposed litter, together with a large amount of above-ground biomass, suggests that R. ponticum could have a drastic effect on nutrient cycles at such a site. On nutrient-poor soils R. ponticum may compete effectively with other plants for a limited supply of nutrients. Having been absorbed, these minerals are removed from the system and only recycled to a relatively minor degree. Acidification and podsolization of the soil will also encourage nutrient loss via leaching. As R. ponticum is only shallow-rooting, once nutrients are leached to the lower soil horizons they will be effectively unavailable to it. In a community dominated by R. ponticum these nutrients would be removed from the vegetation as a whole. The outcome of such an effect would be to encourage low-nutrient, acidic soils, precisely the conditions in which R. ponticum is likely to be a successful competitor.

The work undertaken and presented here took the form of an investigation of salient aspects of the ecology of R. ponticum as an invasive alien.

Firstly, the extent of invasion of a large area of rather diverse geology, topography and land-use was assessed, together with detailed surveys at three sites within this area. The region chosen for this study was the Peak District and Sheffield area.

Secondly, it was considered essential to assess the neglected question of the importance of mycorrhizal infection to the plant's success. It is likely that mycorrhizas could enhance nutrient uptake and hence

competitive ability in suitable situations. Experimental work was carried out to test whether R. ponticum benefits from infection and how this is affected by soil nutrient status.

Thirdly, it was necessary to test whether some of the effects of R. ponticum on competing vegetation could be due to allelopathy. The interference phenomenon was investigated experimentally. Conditions were designed to eliminate effects such as competition for moisture or nutrients, or canopy effects such as throughfall drip or dense shading.

Fourthly, to assist in the interpretation of the information obtained from the above studies, aspects of the biochemistry of R. ponticum were investigated. This was with particular reference to secondary compounds suspected in some cases of being allelopathic agents, namely the phenolics. A qualitative and quantitative survey of simple 'free' phenolics in R. ponticum tissues, associated soils and canopy throughfall was undertaken.

NOTES ON THE STATISTICAL ANALYSIS

The Student's t-test was normally applied where appropriate. In some instances data were subjected to an analysis of variance or 'anovar'. For almost all cases, the anovar analysis of the experimental data revealed the same pattern of significance as that obtained using the less appropriate Student's t-test. Where the test routinely refers to 'significance' this does refer to the results of Student's t-test. The analysis by Student's t-test (supplemented in some cases by comparison of confidence limits) is either unnecessary in some instances, or is insufficient in others. In the first case, the effects observed in some of the toxicity experiments (e.g. 4.4.2.2 Expt. 4, 4.4.2.3. Expt. 5 and 4.4.2.4. Expt. 6) are as clear cut, that statistical analysis is not required. The drastic effects in some cases, giving very poor seedling growth or fatalities, have skewed the data away from a normal distribution. This invalidates the Student's t-test on untransformed data. Secondly, there are cases where analysis by Student's t-test alone is invalid. The complexity of some of the experiments makes these tests effectively 'multiple t-tests'. The chances of statistically significant effects occurring is increased unacceptably by this. In these cases, the data need to be subjected to an analysis of variance. The details of the anovar used, the results obtained and the overall critique of the original statistical analysis are presented along with notes on all the statistical methods used, in Appendix 7.

CHAPTER 2

2.1 THE INTRODUCTION, SPREAD AND CURRENT DISTRIBUTION OF *RHODODENDRON PONTICUM* IN THE PEAK DISTRICT AND SHEFFIELD AREA

2.1.1 INTRODUCTION

As an invasive alien the status and spread of R. ponticum has been studied at a number of individual sites within the British Isles (Cross, 1973, 1981; Robinson, 1971, 1980; Fuller and Boorman, 1977). Its spread over the country as a whole has been documented by Brown (1953a, 1953b), Elton (1958) and Cross (1975).

The introduction, spread and current distribution of the plant within the Peak District and surrounding areas has received little attention. R. ponticum was not mentioned in the floras of Lees (1888), Linton (1903) or Moss (1913). The first reference in local or regional floras is in the 'Flora of Derbyshire' (Clapham (ed.), 1969). This states that R. ponticum is an introduced species often planted in woods and elsewhere, spreading freely on suitably moist, acid soils, both under shade and in the open. It is described as being locally abundant. A number of sites are recorded on soil derived from both the Millstone Grit and Coal Measures Series (Upper Derwent Dale (SK19); Taxal (SK0080); Blacka Moor (SK2880); Buxton (SK07); Grindleford (SK2778); Rowsley (SK2865); Beauchief (SK3381); Cordwell (SK3076); Ogston (SK3759)). There is also one record on the Carboniferous Limestone at Fenny Bentley (SK1750). (Some of these records, for example that at Grindleford (SK2778), may be inaccurate.) Further records in the 'Supplement to Flora of Derbyshire, 1969' (Hollick and Patrick, 1980) include one for Lathkill Dale (SK16) also on the Carboniferous Limestone.

According to Anderson and Shimwell (1981), R. ponticum was planted in some early coniferous plantations that were established as coverts. They note that it is locally dominant in the Chunal Plantation south of Glossop, in plantings at the southern end of Beeley Moor and around Park Hall in Little Hayfield. At this latter site it is invading the adjacent heather moorland. It is also noted as a prominent component of some

mixed and deciduous plantations, as in Lyme Park, Disley. They describe the Errwood Hall woodlands as being the home of Rhododendron in the Peak District, with some 40,000 specimens being planted there in the 1850's.

A major difficulty in tracing the introduction and spread of R. ponticum is that because of its alien status, botanists have tended to neglect it, even though considerably rarer exotics may be recorded. It is also frequently absent from records of gardens and estates since it was the 'common rhododendron' and perhaps not worthy of note, despite being planted on a massive scale.

Piecing together the picture of the introduction and spread of R. ponticum in the Peak District and Sheffield area must, therefore, rely on currently extractable information.

2.1.2 METHOD

Firstly, a survey was carried out to establish the present distribution on a 1Km. square basis. Areas were visited and numerous appeals for information were made to local naturalists, landowners and the general public. The response was good and many squares with naturalized R. ponticum were found. Aerial photographs of the area were also examined.

Secondly, sites of known or suspected introduction were identified. Where possible, information was obtained from landowners, local library archives or other data sources, concerning dates and reasons for introduction.

Thirdly, a general survey of relevant local natural history publications and other literature supplemented the above.

2.1.3 RESULTS

Known sites and dates of introduction of R. ponticum are presented in Table 2.1.1. The earliest records of introduction in this area are from around 1830 on the major estates such as Chatsworth (in the east of the Peak District) and Errwood (in the west). These were as part of large-scale landscaping schemes for gardens and estates and presumably cover for game. Alderwasley Hall (in the east) and Lyme Park (in the west) may have followed relatively close behind, sometime between 1850 and 1890.

The first known introduction in Sheffield was by the Wilsons at Beauchief, between 1850 and 1870. The same family was responsible for introductions to Ecclesall Woods (c.1870), Cordwell (c.1870-1890) and Upper Derwent Dale (c.1900). The Wilsons were also responsible via friends or relatives, for the introduction of R. ponticum to numerous sites throughout the area east of the Peak and west or north of Sheffield (e.g. Broomhead Hall, Fairthorn Lodge, Sugworth Hall, Sydnope Hall and possibly Ogston Hall, all c.1900). All these were primarily for wildlife cover and ornament, although it seems likely that in exposed sites such as Broomhead, they may also have served as wind-breaks.

The period 1890-1900 also saw introductions taking place to the south-east of Sheffield at Renishaw and to the north-west of Sheffield at Strawberry Lee Plantation and Longshaw. Again these were primarily for ornament and at Strawberry Lee, probably also as a shelter-belt.

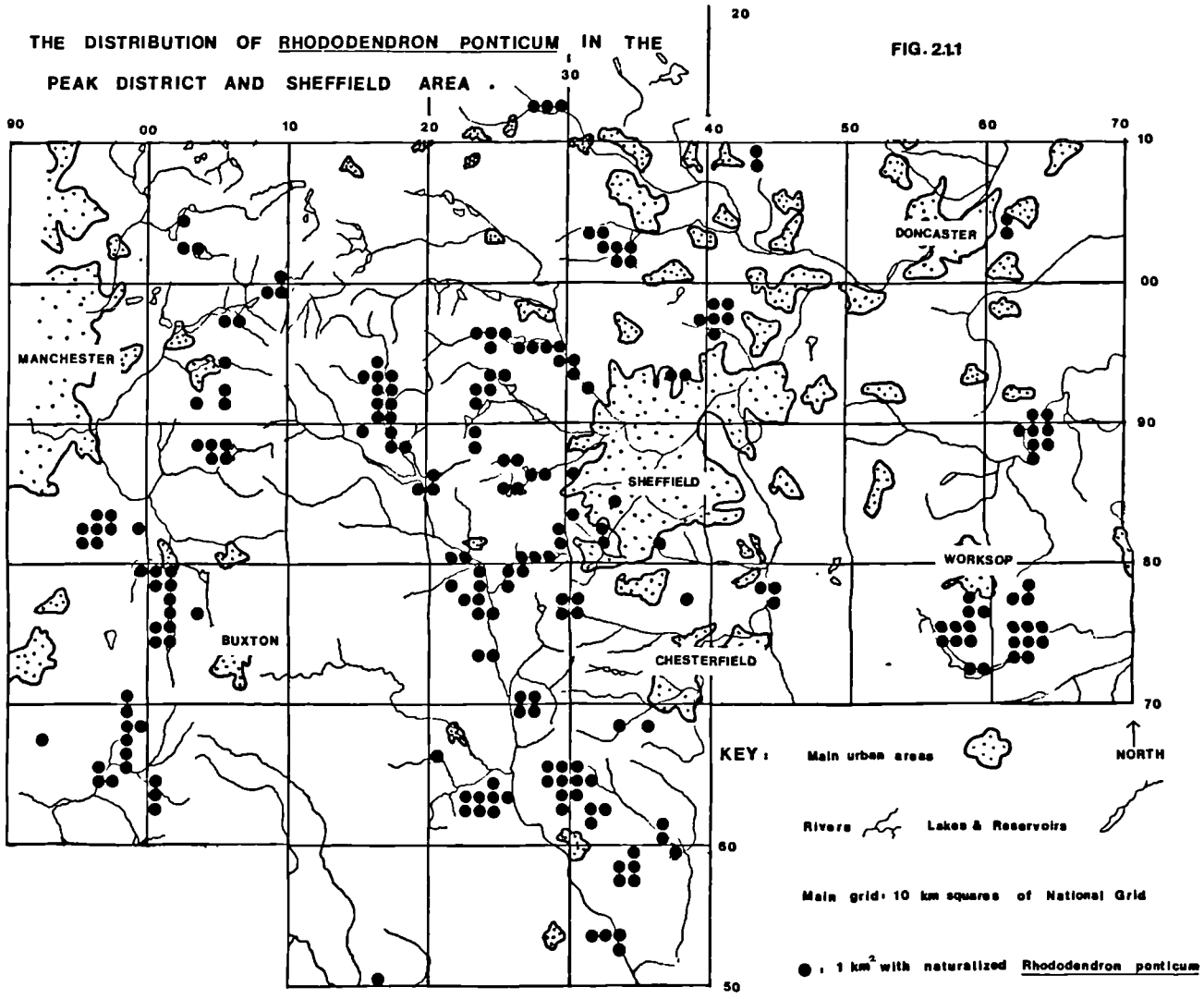
By the turn of the century, R. ponticum had already been introduced to many of the sites from which it has since spread. The main introductions since then have been on estates to the east of Sheffield, during the period 1920-1930. These were primarily for game cover. Figure 1 shows the current distribution of R. ponticum in the Peak District and Sheffield area.

2.1.4 DISCUSSION

The present distribution reflects the pattern of introduction, the suitability of habitats and the degree of management employed. A major factor limiting the spread of R. ponticum is the availability of suitable sites for seedling germination and survival. Relatively open moss-covered ground and humid conditions are essential (Cross, 1973).

THE DISTRIBUTION OF RHODODENDRON PONTICUM IN THE PEAK DISTRICT AND SHEFFIELD AREA.

FIG. 2.1.1



TABLE

2.1.1

1. INTRODUCTION OF *R. PONTICUM*

Site	Grid Ref	Estate	Date	Reason	Information Source
Alderwasley Hall	SK.3253	Hurt	c.1850?	?	Mrs B W Brook, Alderwasley Hall
Beauchief Hall	SK.3281	Wilson	1850-1870	Wildlife Cover/Ornament	Miss E WIlson
Broomhead Hall	SK.2496	(Wilson)	c.1900	Wildlife Cover/Ornament	Miss E Wilson
Bradfield/Strines	SK.2492	Fitzwilliam	c.1920	Game Cover	Mr G J R Broadhead, Fitzwilliam Estates
Chatsworth	SK.2670	Devonshire	1830-1840	Game Cover/Ornament	Mr M Pearman, Chatsworth Estate
Clumber	SK.6375	Newcastle	1920-1930	Game/Wildlife Cover	National Trust
Cordwell	SK.3077	Wilson	1870-1890	Wildlife Cover/Ornament	Miss E Wilson
Ecclesall Woods	SK.3282	Wilson/ Fitzwilliam	c.1870	Wildlife Cover/Ornament	Miss E Wilson
Errwood Hall	SK.0074	Grimshawes	c.1830-1850	?	Mr J B Kingsmill, Forestry Commission
Fairthorn Lodge	SK.2585	(Wilson)	c.1900	Wildlife Cover/Ornament	Miss E Wilson
Kinder Reservoir	SK.0588	?	Late 1800's?	Landscaping around reservoir	Mr B P Annikin, N.W. Water Authority
Longdendale	SK.0899	Tollemache	?	Game Cover	N.W. Water Authority
Longshaw	SK.2679	Rutland	c.1890	Ornament	National Trust
Lyme Park	SK.9682	Newton	Late 1800's	Rhododendron Collection	Mrs K M Atkinson, Lyme Park
Ogston Hall	SK.3759	Turbot	c.1900 ?	Wildlife Cover/Ornament	Miss E Wilson
Renishaw Hall	SK.4378	Sitwell	1890-1900	Ornament	Mr P Hollingworth, Sitwell Estates
Rivelin Lodge	SK.2786	?	c.1900	Ornament	
Strawberry Lee Plantation	SK.2780	Rutland	c.1890-1900	Ornament	Sheffield City Recreation Dept.
Sugworth Hall	SK.2389	(Wilson)	c.1900	Wildlife Cover/Ornament	Miss E Wilson
Sydnope Hall	SK.2964	(Wilson)	c.1900	Wildlife Cover/Ornament	Miss E Wilson
Upper Derwent Dale	SK.1789	Wilson	c.1900	Wildlife Cover/Ornament	Miss E Wilson
Wentworth	SK.3997	Fitzwilliam	c.1920	Game Cover	Mr G J R Broadhead, Fitzwilliam Estates

(Wilson) : Estates owned by friends or relatives of the Wilsons.

Disturbance of vegetation and soil by forestry management, grazing animals, or other events such as moorland fires, appear to considerably increase the availability of such sites.

At sites where such disturbance occurs, R. ponticum actively invades surrounding vegetation such as woodland (e.g. Chatsworth), moorland (e.g. Hallam Moor (SK2686), Blacka Moor (SK2880), Broomhead Moor, Park Hall) or rough grassland (e.g. Ewden Valley below Broomhead Hall, Cordwell, Matlock Forest near Sydnope Hall).

At sites which are less disturbed, spread is by vegetative means with apparently restricted regeneration from seed. Examples of this are Ladies Spring Wood near Beauchief Hall and Strawberry Lee Plantation. In the latter case, no seedlings or young bushes were found in the central more open area of the wood. The only spread apparently being vegetative from the original planting around the perimeter. Spread by seed is occurring on the adjacent heather moor, as shown by two small R. ponticum bushes, presumably originating from wind-blown seed.

Spread may occur over distances up to at least 1Km from the original site. This is probably as a result of dispersal of the very small seeds which are produced in profusion and can be carried over considerable distances by strong winds which characterize the Peak District uplands.

Some control and eradication work is now being carried out either by the Forestry Commission or with the aid of conservation volunteers at sites owned by the National Trust or private estates like Chatsworth. Such attempts, however, are expensive and labour intensive. They are also of restricted success or application (for reasons discussed elsewhere). At a number of sites such as Stand Wood, Chatsworth or Upper Derwent Dale, R. ponticum is cleared from within woodlands and maintained as an 'amenity screen' around woodland edges and roadsides. The obvious drawback with such a policy is the constant source of abundant seed, adjacent to managed woodland which provides ideal regeneration habitats.

The picture which emerges is that R. ponticum has been introduced to what are often the ideal situations for it:- acid soils, sheltered moist woods and valleys with abundant sites for regeneration by seed. In addition to this, the exposure of some sites to periodic very strong winds provides an ideal means of dispersal.

Within the Peak, R. ponticum is generally absent from the Carboniferous Limestone. Where it has been introduced (presumably on the more acid soils) its spread is clearly restricted by lack of suitable soils. Being shallow rooted, it is able to grow in relatively thin layers of acid soil overlying calcareous soil or rock.

Around the perimeter of the White Peak, R. ponticum is abundant and widespread in the horseshoe shaped regions of Millstone Grit and associated geology to the west, east and north. It is most successful on the wooded slopes below the Gritstone edges of river valleys to the east (e.g. Chatsworth) and the west (e.g. Errwood). The occurrence and spread in the northern Gritstone area is probably restricted by the bleak, open, high altitude topography of the Kinder/Bleaklow massif. The river valleys along either side of this central area of the Dark Peak and further south the White Peak have abundant R. ponticum.

The maximum altitude at which R. ponticum occurs in the Peak District is between 300m and 400m. High altitude sites include Broomhead Moor (320m), Kinder Reservoir (305m), Wood's Cabin on Kinder (SK0592)(380m), Strawberry Lee Plantation (380m), Fairthorn (380m), Chatsworth (305m) and Errwood (300-400m).

In the regions east of the Peak District, R. ponticum is less abundant though still widespread. It occurs most frequently on sites that either are or were parts of estates (large or small) during the late 1800's and early 1900's. Bushes in gardens at Nether Edge, Sheffield for example, pre-date the present houses (c.1930). The bushes originate from the grounds of the local hall, which have since been absorbed into the suburban development. Throughout the Coal Measures regions around Sheffield the occurrence of R. ponticum is restricted by extensive housing and industrial development.

Further east R. ponticum is generally restricted to the large estates, particularly on the Bunter Sandstone of the Dukeries around Worksop. It appears to be absent from the Magnesian Limestone for much the same reasons as from most of the Carboniferous Limestone. It is probably further restricted to the east by increasingly intensive land-use for agriculture.

The presence of R. ponticum in the area clearly provides a serious problem for management of commercial forests. Its effects on wildlife depend on the habitat being invaded. Of the major suitable habitats, heather moorland is unlikely to be seriously affected, R. ponticum probably becoming just another member of a largely ericaceous plant community. In very moist sites such as moorland bogs, invasion is severely restricted due to waterlogging, so the problem is minimal (e.g. Reddicar Bog SK2687). *Acidic grasslands which suffer some grazing* pressure seem to be vulnerable to invasion as at Cordwell. At all sites where grazing livestock are present, there is the potential problem posed by the toxicity of R. ponticum foliage.

Undoubtedly, woodland habitats are the most threatened by invasion. Relatively unmanaged or undisturbed woods seem less suitable for regeneration from seed. Managed amenity/commercial woods such as Stand Wood, Chatsworth, may therefore pose the major problems. Semi-natural oakwood relics such as at Padley Gorge (SK2579) are also being invaded. At Padley this is from the introduction at Longshaw. This wood is both grazed and suffers severe human disturbance. The abundant apparently suitable regeneration sites may encourage further invasion, but the situation at present appears to be stable.

The impact of R. ponticum on the local environment is thus made up of a mixture of harmful and beneficial effects. Whilst creating problems for woodland management and swamping existing vegetation, it adds diversity to some areas. The dense scrub which it forms provides ideal nesting sites for many birds, including regionally rare species such as the nightingale at Clumber. Many important winter roosts of finches and thrushes are in extensive R. ponticum beds. The dense cover also provides shelter for mammals such as badgers which may have their setts within large patches of R. ponticum.

In addition, R. ponticum has considerable amenity value, being very popular for its spectacular displays of flowers in June (such as at Cordwell and at Errwood). It is very useful in providing cover, screening and impenetrable protection for areas subject to intense visitor pressure, such as Chatsworth and Clumber.

It is unlikely and also of questionable desirability that R. ponticum will be fully controlled or eliminated from the area under study. Spread of the species to new sites within the region should be relatively easy to control. Control within large areas already infested may prove impossible except where large amounts of manpower or finance are available. Key areas to be monitored for signs of further encroachment are the semi-natural oakwoods and possibly some moorland areas. With greater understanding of the ecological background to the problem, management may be better placed to discourage further spread. One obvious area in which careful monitoring and control may be useful is the inadvertent creation of regeneration sites. Intensive management for forestry, grazing or amenity may well create suitable sites and thus increase the likelihood of further spread by seed.

2.2 THE SPREAD OF *R. PONTICUM* AT THREE SITES IN THE SHEFFIELD AREA

2.2.1 INTRODUCTION

In addition to the main survey, the current status of R. ponticum at three sites was mapped in detail. The area covered by R. ponticum at each site was estimated.

2.2.2 METHOD

The distribution of R. ponticum was mapped onto 1 : 2500 scale ordnance survey maps. This was done using aerial photographs and by site visits.

Having mapped the site coverage, the area was estimated by means of a tracing transferred to graph paper and cut out. The cut graph paper was then weighed and compared to the weight of a known area of graph paper. The area was then adjusted to the scale of 1 : 2500.

A difficulty with assessing the spread of R. ponticum rather than simply its current status, is in deciding the extent of coverage at known times in the past. As detailed records of R. ponticum have not been kept at these sites, this can only be done by considering the initial plantings, supplemented by comments from individuals who have known the sites over a long period, photographs of the sites from different dates and other sources such as comments in local newspapers. It is assumed that the bushes were planted within fairly restricted areas (which are known approximately), at intervals of about 2m between adjacent bushes. From this beginning, the extent and pattern of spread over the period from the dates of introduction to the present day, can be reasonably assessed.

2.2.3 RESULTS

Extensive vegetative spread has occurred at all three sites. (See Table 2.2.3.1.) The original pattern of planting is reflected in the current distribution at Strawberry Lee (around the perimeter of the wood) and at Beauchief (along the woodland edge). At Strawberry Lee the population appears to be all R. ponticum. At Beauchief one or two other species or

TABLE

2.2.3.1 ENCROACHMENT BY *R. PONTICUM* AT THREE SITES IN THE SHEFFIELD AREA

Dates of Introduction

1.	Beauchief/Ladies Spring Wood	:	1850 - 1870
2.	Cordwell	:	1870 - 1890
3.	Strawberry Lee Plantation	:	c.1890 - 1900

Approximate Current Area of

1.	Beauchief/Ladies Spring Wood	:	20,400 sq.m
2.	Cordwell (a) West of Road	:	27,200 sq.m
	(b) East of Road	:	21,300 sq.m
	Total	:	48,500 sq.m
3.	Strawberry Lee Plantation	:	21,100 sq.m

KEY:

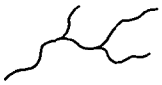
Rhododendron ponticum 

Woodland  

Footpath 

Track 

Road 

Stream 

Scale :-1 : 2500

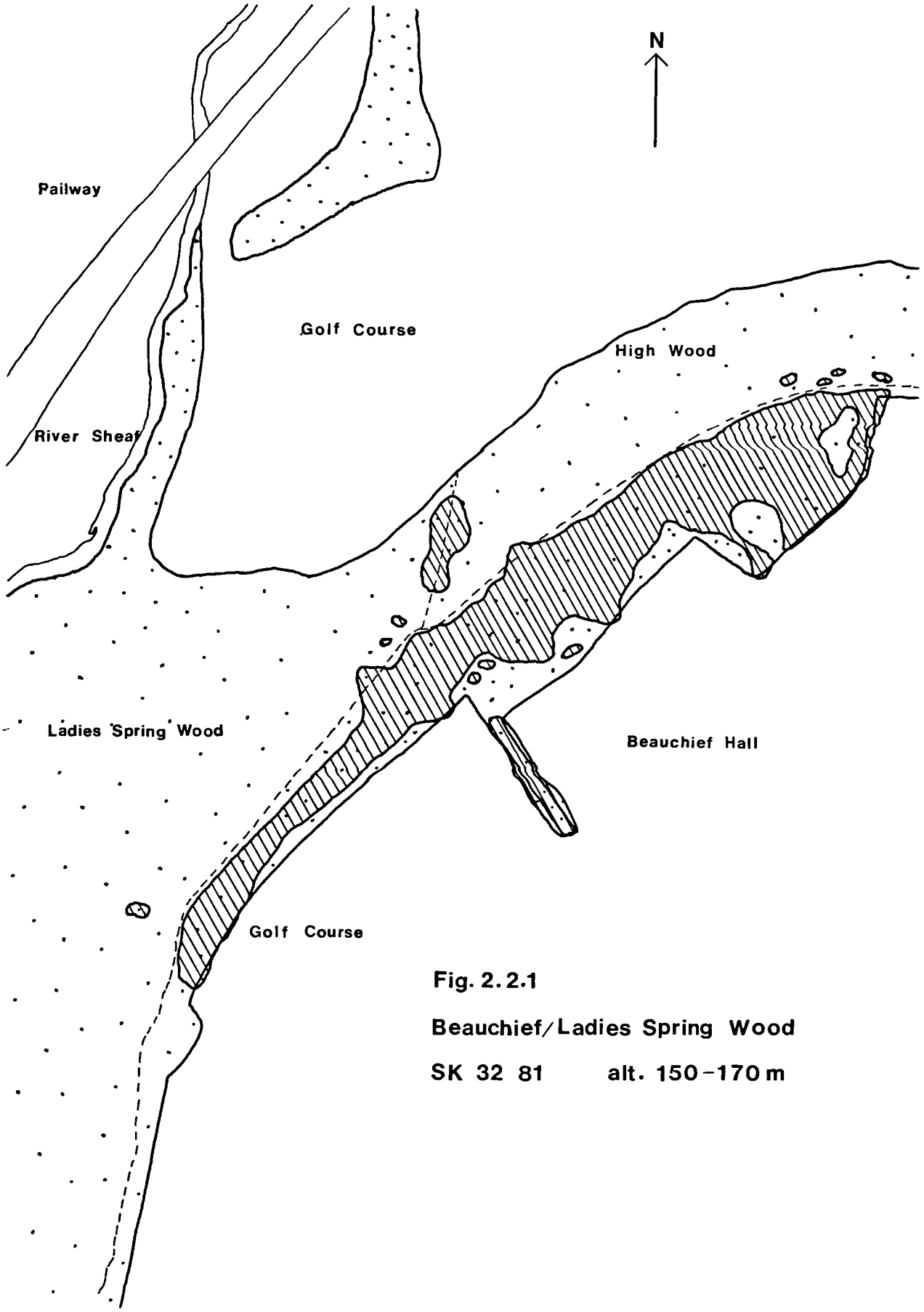


Fig. 2.2.1

Beauchief/Ladies Spring Wood

SK 32 81 alt. 150-170 m

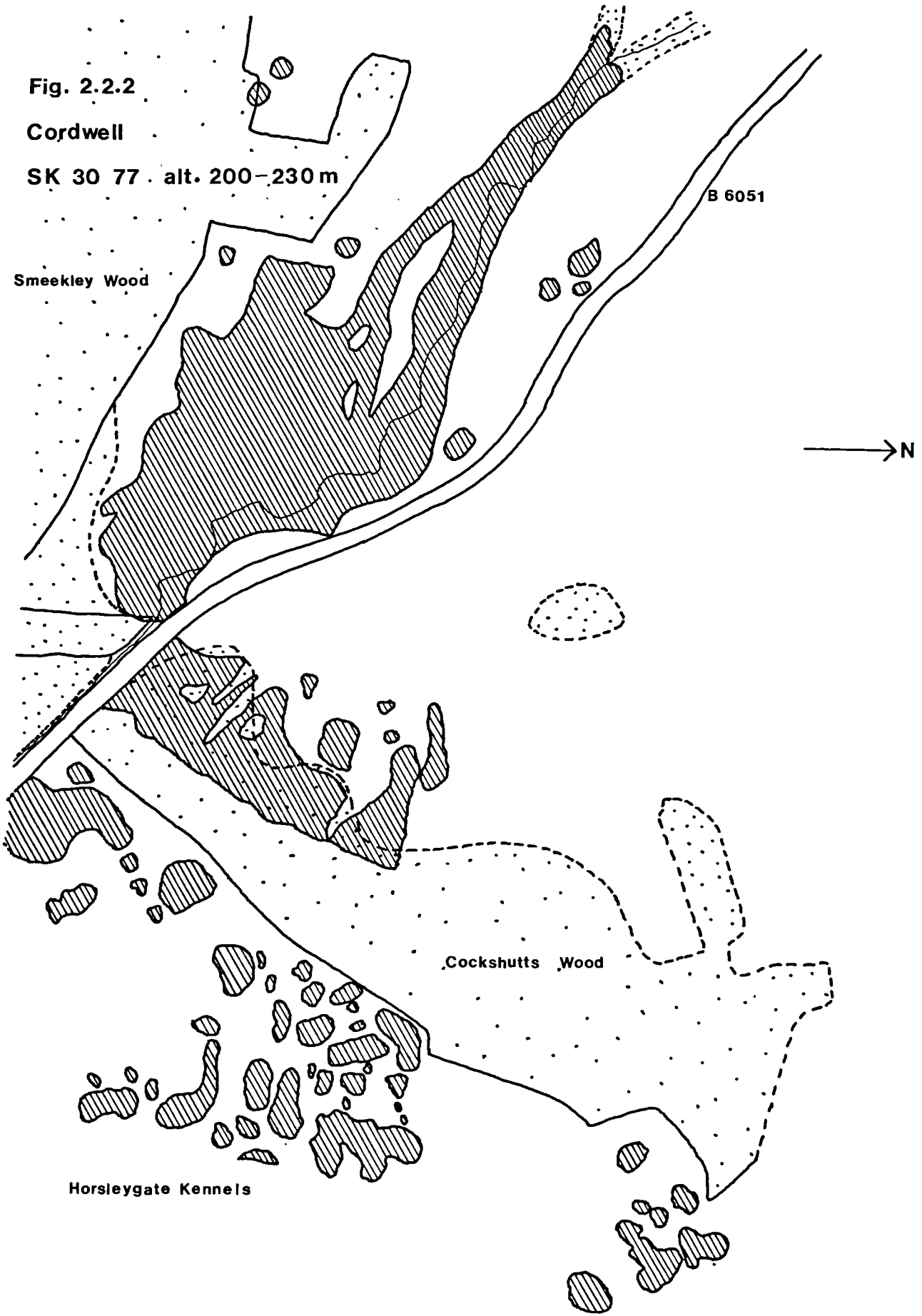
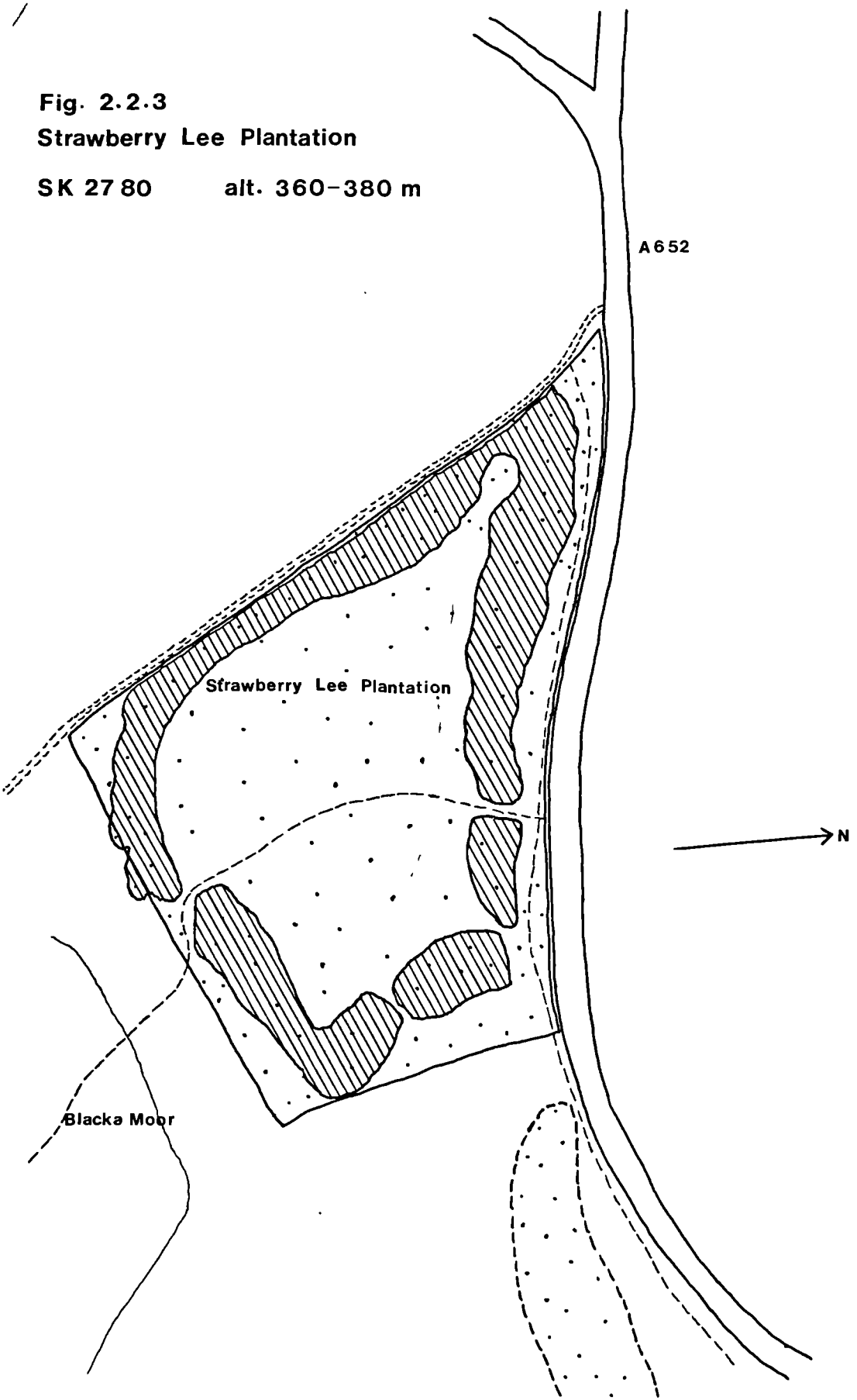


Fig. 2.2.2
Cordwell
SK 30 77 . alt. 200–230 m
B 6051
Smeekley Wood
N
Cockshutts Wood
Horsleygate Kennels

Fig. 2.2.3
Strawberry Lee Plantation

SK 27 80 alt. 360–380 m



hybrids are present in the edge nearest the Hall (south of the wood), presumably the area originally planted. At Strawberry Lee there are no obvious examples of spread from seed within the wood. There are two smallish bushes on the moor to the south. At Beauchief there are some young bushes clearly showing spread from seed. Again, however, this is rather restricted.

At Cordwell it is convenient to consider the area as two separate sites. Firstly, to the south of the road adjacent to Smeekley Wood. Secondly, to the north of the road around Horsleygate Kennels. This second site was only mapped as far as the kennels, but it does extend east to Horsleygate Hall itself.

The planting at this site was apparently more scattered than at the previous two. In the case of the first sub-site (south of the road) the main area planted was around a small reservoir in the heart of the current R. ponticum bed. Since the planting around 1870-1890, the whole of the lower valley has been almost totally engulfed.

Some invasion has also occurred in Smeekley Wood (a conifer plantation). Much of the area is not intensively managed, the main disturbance being from people on foot or on horseback. R. ponticum seedlings were abundant in some of these disturbed areas. There may also be occasional sheep grazing. The surrounding vegetation is acid grass/heath with extensive coverage by Pteridium aquilinum.

The vegetation invaded at the second sub-site (north of the road) is a mixture of woodland and acid grassland with some scrub. The area is grazed. Spread from the original plantings at this sub-site is far more patchy, indicating colonization by seed. It may also be that coalescence of bushes by vegetative expansion (as has occurred south of the road) has been restricted by grazing.

Within all the sites mapped, the blanketing Rhododendron was effectively eliminating all ground flora and regeneration of trees and shrubs. Emergent trees and shrubs were still present.

2.2.4 DISCUSSION

Invasion of the surrounding vegetation at these three sites has occurred over a period of between 80 and 130 years. Around 20,000 sq.m has been covered by R. ponticum at each end of Strawberry Lee Plantation and Beauchief and over 50,000 sq.m at the total Cordwell site (not all mapped).

Regeneration by seed seems to be very restricted in relatively unmanaged, undisturbed and ungrazed areas. This is probably due to lack of suitable regeneration sites. In these cases spread is primarily by vegetative means.

Within the dense R. ponticum beds, ground flora and regenerating trees and shrubs are totally eliminated.

CHAPTER 3

MYCORRHIZAL INFECTION IN *RHODODENDRON PONTICUM*

3.1 INTRODUCTION

The major general of the Ericaceae (Calluna, Erica, Vaccinium and Rhododendron) all have dense, matted root systems which terminate in fine, absorbing, mycorrhizal rootlets or 'hair roots' (Beijerinck, 1940). These hair roots have one to three layers of cortical cells surrounding a central stele. Root hairs are absent. When mycorrhizal infection occurs, it is only the cortical cells that are invaded. There is no infection of the stele and little infection of the older, suberized portions of root, the apical meristem or the zone of elongation.

The endomycorrhizas formed by the Ericaceae are known as 'ericaceous mycorrhizas' or 'ericoid endomycorrhizas'. They are also found in the closely related Epacridaceae (Harley, 1969).

The endomycorrhizal fungus or 'endophyte' develops a weft of sparcely septate hyphae over the root surface. Fine lateral hyphae then penetrate the cortical cells. They normally enter the large epidermal cells directly through their outer walls. Closely interwoven coils of slender hyphae are formed within the host cell. These characteristic hyphal masses or 'intracellular coils' may almost totally fill the infected cell (Gordon, 1937).

There were many attempts by early workers to isolate and culture the endophyte of ericaceous mycorrhizas. Supposedly successful isolation of the fungus and identification of it as Phoma radicis (Ternetz, 1907; Rayner, 1915; Rayner and Smith, 1929; Rayner and Levisoln, 1940) was supported by other workers such as Addoms and Mounce (1931, 1932). This early work suggested obligate symbiosis with systemic infection of the host plant by fungal hypae, infection of the seed coat whilst in the ovary and dispersal of the inoculum with the seed. 'Cyclic' infection then occurred when the emerging radicle was inoculated by fungus on the seed coat following germination. Other work did not support this hypothesis

(Christoph, 1921; Doak, 1928; Knudson, 1929; Friesleben, 1933,1934; Bain, 1937; Gordon, 1937) and it has since been discredited. Boerema (1967) showed a fungus identical to that isolated by Rayner, to be a contaminant of air, woodpulp and soil (Pearson and Read, 1973). This fungus is assumed to be either a widespread contaminant of aerial plant organs or an artifact of the preparation.

Typical mycorrhizas were successfully synthesized using isolates of slow-growing, dark, sterile mycelia obtained from roots of ericaceous plants (Doak, 1928; Friesleben, 1933,1934,1936; Bain, 1937; Burgeff, 1961; McNabb, 1961). Pearson and Read (1973) confirmed the ericaceous endophyte to be a slow-growing, normally sterile, dark fungus with little host specificity. This fungus has a specialized capacity to form mycorrhizas with ericaceous plants. The perfect form of the fungus was first observed by growing inoculated plants of Calluna vulgaris on soil partially sterilized by gamma irradiation (Read, 1974). Apothecia are often freely produced following this treatment, although the time before their production is variable. Isolates of the fungus (Pezizella ericae Read) have been shown to form mycorrhizas with a range of ericaceous plants. Pearson and Read (1973) consider that all isolates from ericaceous mycorrhizas will ultimately be recognised as Ascomycetes of the same or a closely related genus. It is unlikely that the endophyte isolated from roots of R. ponticum in Britain is genetically the same as that originally associated with it. As the plant was introduced to the country as seed, it must have become infected by indigenous ericaceous endophytes from the native vegetation.

There is considerable evidence for the mycorrhizal relationship being a truly mutualistic one. Firstly, the host/fungus pathway has been demonstrated to provide for translocation of nutrients and metabolites. Pearson and Read (1973) showed that glucose and orthophosphate could be translocated by the endophyte in pure culture. Orthophosphate was absorbed by the fungus and passed to the host plant. Photosynthates from the host were translocated to the endophyte. Stribley and Read (1974) demonstrated the presence of carbohydrates associated with fungal metabolism in mycorrhizal roots, but not in non-mycorrhizal roots. This work suggested that the endophyte may derive a supply of carbohydrate from

the host, but the importance of considering net flow of carbon was stressed. The ability of the endophyte to translocate nutrients in the direction of a nutrient sink has been shown by Read and Stribley (1975).

Secondly, mycorrhizal infection may increase both the levels of nitrogen and phosphorus of tissues and the total yield of the host. Read and Stribley (1973) showed an increased proportion of nitrogen and phosphorus in C. vulgaris and V. macrocarpon, in mycorrhizal compared to non-mycorrhizal. Total nitrogen content on a percentage dry weight basis for the whole plant, was more than doubled in mycorrhizal V. macrocarpon compared to non-mycorrhizal six months post-inoculation. The mycorrhizal plants were also larger and healthier in appearance than their non-mycorrhizal equivalents. Stribley and Read (1974) again found an increase in nitrogen content of mycorrhizal seedlings. They also found that additional nitrogen (possibly from organic sources) was being taken up by mycorrhizal plants. This was in addition to the usual inorganic sources and has important implications for mineral nutrition on nutrient-poor soils. Read and Jalal (1980) found increased yield and percentage nitrogen content of mycorrhizal vs. non-mycorrhizal C. vulgaris after 3 months and 6 months following inoculation. In V. macrocarpon also, mycorrhizal infection increased yield and Relative Growth Rate and decreased Root/Shoot Ratio in seedlings, but when soil treatment caused high nitrogen mineralization, the mycorrhizas had no significant effect (Stribley, Read and Hunt, 1975). The effects of mycorrhizas were again shown to be insignificant at high levels of ammonium addition (Stribley and Read, 1976). They also showed enhanced uptake of ammonium by mycorrhizal V. macrocarpon seedlings at intermediate levels of ammonium, but not at the lowest levels. (The endophyte possibly proving a drain on the host at the very low levels, the cost outweighing the benefits.) Stribley and Read (1980) showed that mycorrhizal seedlings of V. macrocarpon were able to utilize amino acids as a source of nitrogen. This was totally dependent upon mycorrhizas, the infected plants using the amino acids as readily as they would ammonium.

The ability of the endophyte to use inisotol hexaphosphates as a source of phosphorus may be of benefit to the host on soils low in free, inorganic phosphates. The commonest sources of phosphorus on nutrient-poor soils are inisotol phosphates (Pearson and Read, 1975).

Thirdly, the mycorrhizal fungus seems to have a reduced capacity for free-living, saprophytic existence. It is apparently limited in its ability to independently utilize some complex carbohydrates. Pearson (1971) noted this restricted saprophytic ability. Although able to produce pectinase and carboxymethylcellulase (Nieuwdorp, 1969), it may be unable to produce the enzymes capable of degrading native cellulose or other insoluble polymers in the soil. The use of both simple and complex carbohydrates by the endophyte was demonstrated by Pearson and Read (1975) but again the use of cellulose was limited. Organic sources of phosphorus and nitrogen were readily utilized and considerable acid-phosphatase activity was shown.

In summary, the fungus has been shown to use simple organic sources of nitrogen, phosphorus and carbon, but is restricted in its capacity to use carbohydrates (particularly cellulose). It is able to translocate nitrogen and phosphorus from fungus to host and to receive photosynthates in the reverse direction. The balance of such effects and the degree with which infection occurs seems strongly affected by nutrient levels in the medium. In particular, relatively high levels of 'available' nitrogen decrease infection and the consequent benefits to the host may be lost. Infection at very low nutrient levels may also be of little benefit to the host.

In freely drained, low nutrient, acid soils, mycorrhizal infection appears to enhance uptake of nitrogen and phosphorus, and leads to an increase in yield by the host. Simple organic sources of nitrogen and phosphorus, unavailable to non-mycorrhizal plants, become available when infection occurs. These soil conditions are typical of many areas vegetated by ericaceous plants including R. ponticum.

The details of the nutrient/metabolite balance between host and fungus are complex and variable. As well as transfer and release both ways, there is the antagonistic effect of host cells reacting to infection by the eventual lysis of the intracellular hyphal coils. This must retrieve some of the host photosynthate which the endophyte has accumulated. The importance of this aspect of the relationship is not known. Without more

information on the saprophytic abilities of the fungus, the possibility of a net flow of carbohydrate to the host under certain conditions is also speculative. The degree of dependence of the endophyte on the host plant is also uncertain.

Until relatively recently, most detailed ecological studies of ericaceous plants have largely ignored the possible importance of mycorrhizal infection. The effects of mycorrhizas on R. ponticum are only discussed very briefly by Cross (1973, 1975). Clearly, the possession of mycorrhizal roots is potentially of prime importance to the plant's competitive ability and its invasive nature.

In order to assess the importance of mycorrhiza formation, the occurrence and timing of infection during the development of Rhododendron need to be studied, along with its effects on host plant growth. To do this it is necessary to obtain mycorrhizal and non-mycorrhizal plants. Plants of each category have to be grown under controlled conditions and this growth compared. Attempts were therefore made to isolate the mycorrhizal endophyte and to re-inoculate it into aseptically grown seedlings. The performance of inoculated plants was then compared with that of uninoculated controls under a range of conditions.

The occurrence of viable endophyte in field soils was also investigated as this has important implications regarding the mycorrhizal nature of invasive R. ponticum.

3.2 METHOD

3.2.1 ISOLATION OF THE ENDOPHYTE AND ITS ASEPTIC CULTURE

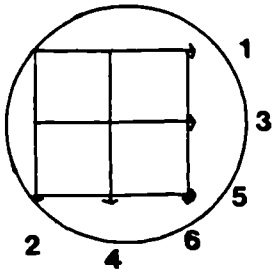
The technique adopted was based on that of Pearson and Read (1973). Young, healthy roots were collected from mature R. ponticum in the field. The roots were separated from leaf litter and soil debris. The fine hair roots were carefully selected and cut away from the other root material.

The hair roots were then washed in tap water to remove any remaining coarse debris. They were placed in a muslin bag and suspended beneath a running tap for twelve hours.

Having been removed from the muslin bag, the roots were placed in sterile water in a glass vial. This was then shaken vigorously on a mechanical shaker for five minutes. This process was repeated for forty serial washings. Washing under the tap, followed by serial washings was designed to remove as far as possible, surface-contaminating bacteria and fungi from the roots.

The roots were then removed from the vial and macerated in a sterile, ground-glass macerator. The macerate was sub-sampled and plated onto 0.5% sterile water agar in petri dishes. The samples were incubated at 20°C.

After development of the culture, the plates were examined microscopically for the presence of endophyte. The periodically coiled hyphae of the endophyte are easily recognised. Other fungi twisting their hyphae at the agar/dish interface may produce superficially similar coils but never with the regularity or form of the endophyte. Suspected endophyte was sampled with a sterile needle and transferred to plates of weak malt agar, plated as grid of crossing lines.



1-6 represent successive strokes of the sampling needle across the plate.

The cultures were again incubated at 20°C and carefully examined daily for the development of recognisable endophyte. As the endophyte is slow-growing it was found that speedy recognition and isolation of the fungus was vital to avoid contamination by more vigorous fungi or bacteria.

The endophyte was recognised as slow-growing, dark, sterile cultures. These were subsampled and transferred by sterile needle to plates of 5% malt agar.

At least one further subsample onto 5% malt agar was usually necessary to ensure there was no contamination. Pure cultures on 5% malt agar were sealed in dishes by plastic tape and then stored in sealed poly bags at 20°C.

All subsampling and transferrence of materials beyond the initial washing under the tap, was carried out in sterile conditions in a 'Microflow' cabinet.

The endophyte was also successfully cultured in Norkran's Solution. This was found to be suitable for maintaining viable endophyte in sterile cultures over long periods of time.

3.2.2 CULTURING OF NON-MYCORRHIZAL SEEDLINGS OF *R. PONTICUM*

To eliminate the contamination of sterile media for growing non-mycorrhizal seedlings, it is necessary to 'surface sterilize' the seeds before sowing. The method used was that employed by Pearson and Read (1973).

10g of calcium hypochlorite was dissolved as far as possible in 140ml of distilled water. The solution/suspension was filtered on Whatman No. 1 paper in a glass funnel. The filtrate was collected for use as a sterilant.

A small quantity of R. ponticum seed was placed in 20ml of sterilant and shaken for two minutes. The seeds and sterilant were decanted into another filter paper in a glass funnel. Sterile water was poured over the seeds to wash off excess sterilant and prevent further sterilization and the death of the seeds.

The seeds were transferred to 0.5% sterile water agar using a sterile needle. The petri dishes of agar plus seeds were taken in sealed poly bags to the growth-room. They were then incubated at 20°C in good light conditions. Germination usually occurred after around two weeks.

Again, treatment and transfer of materials were carried out, as far as possible, in sterile conditions in a 'Microflow' cabinet.

It was found that where aseptic material was not required, non-mycorrhizal seedlings could be readily obtained by sowing seed onto partially sterilized (gamma irradiated) soil. Healthy, vigorous R. ponticum seedlings were more successfully germinated and grown in this way.

3.2.3 INOCULATION OF SEEDLINGS

Non-mycorrhizal seedlings of R. ponticum were transferred onto either acid-washed sand, 'steam-sterilized' soil or gamma-irradiated soil.

The endophyte was then taken from culture on agar or applied directly from liquid culture (sampled with a sterile needle and loop). When taken from agar, the fungus was cut from a known area of culture, macerated and suspended in a known volume of sterile water. The suspension could then be applied by pipette, a known volume having been used. The inoculum was simply applied to the soil (or other medium) around the seedling roots.

Mycorrhizal infection can be easily obtained under non-sterile conditions by growing seedlings on field soil from under R. ponticum or other ericaceous plants.

3.2.4 STAINING OF ROOTS TO SHOW MYCORRHIZAL INFECTION

Young, healthy roots were separated from the main root mass (in the case of older plants) or whole seedling root systems were used. They were carefully washed in water to remove macroscopic debris.

The roots were touched dry on a tissue paper and then immersed in trypan blue solution (0.5% in lactophenol) for three minutes. Following removal from the stain, the roots were washed of excess trypan blue with glycerol.

They were then placed on a slide with a drop of glycerol. With a cover slip placed carefully over the root, the preparation was ready for microscopic examination. Slides may be kept this way in a closed container for up to several weeks.

Fungal hyphae within the mycorrhizal roots show under microscopic examination as being stained dull, turquoise-blue. The intracellular coils are usually discernable. Non-mycorrhizal roots or uninfected areas of roots are usually a clear royal blue, with only host cytoplasm and cell inclusions visible.

PLATE 3 : Mycorrhizal root of R ponticum showing internal and external hyphae × c. 500

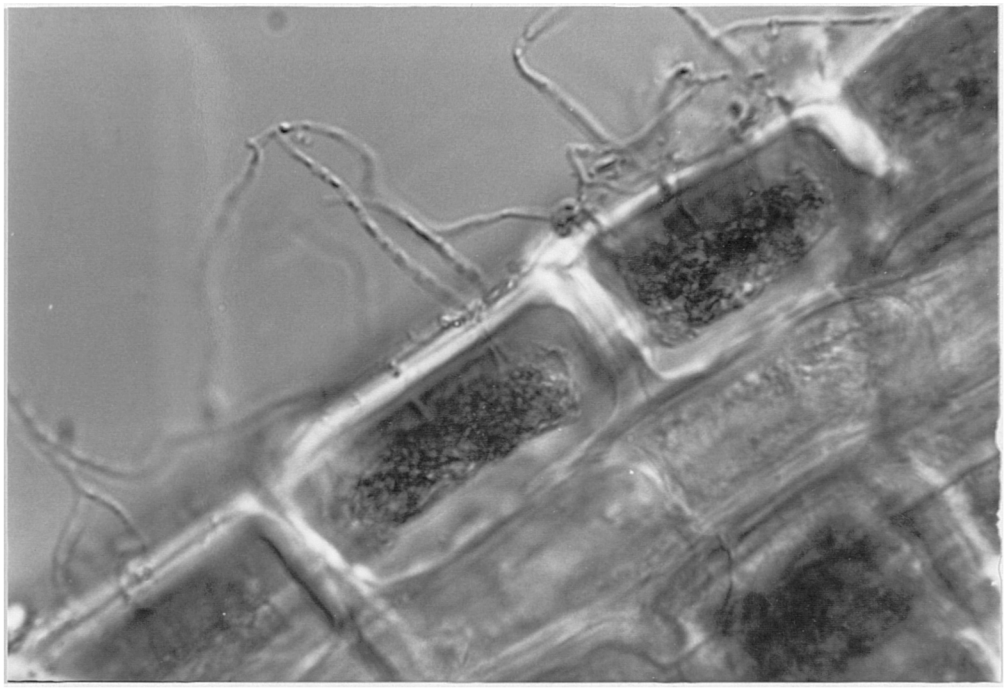


PLATE 4 : As Pl. 3 , × c. 150

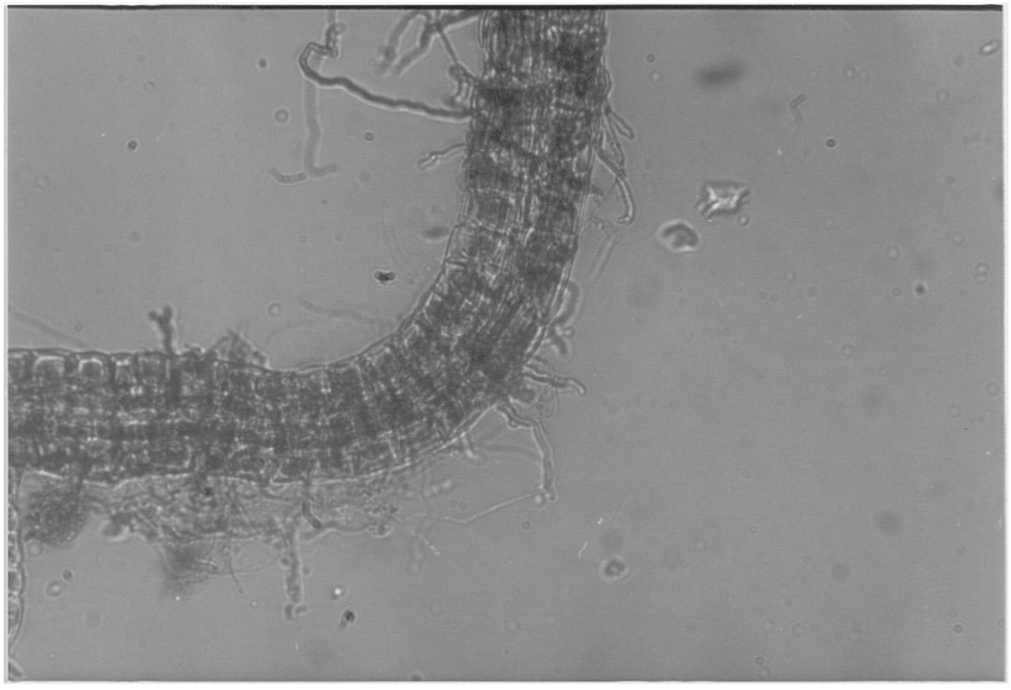
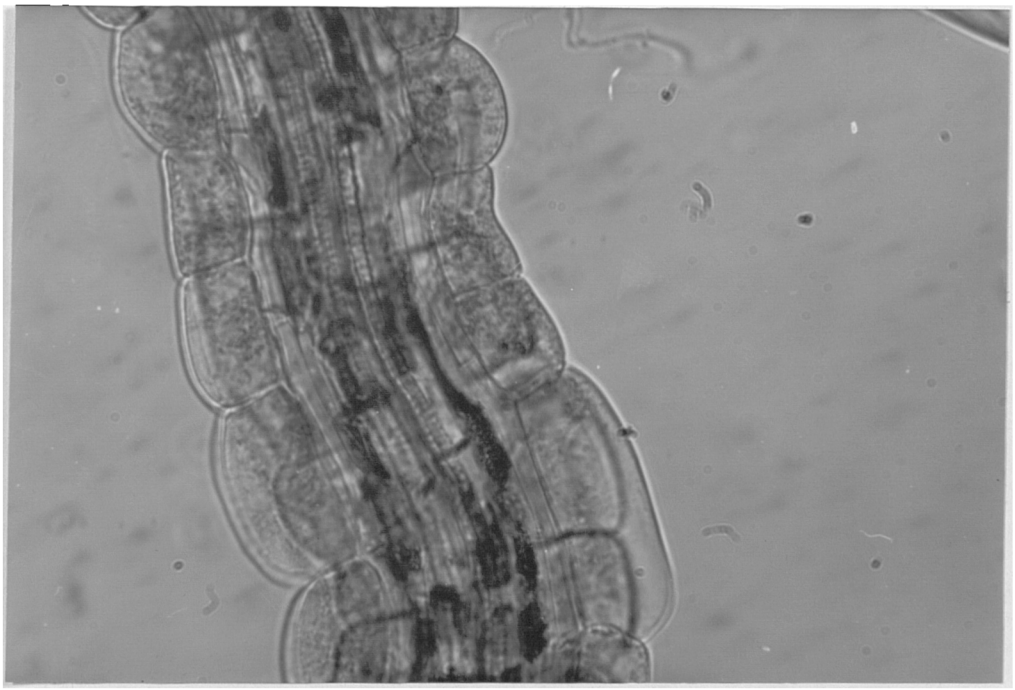


PLATE 5: Mycorrhizal root tip of R.ponticum. × c. 150

PLATE 6: As Pl. 3, × c. 300



3.3 EXPERIMENTAL WORK AND RESULTS

3.3.1 ISOLATION OF THE ENDOPHYTE

Isolates were obtained from plants at a number of sites. Five isolates were maintained as stock cultures on malt agar (Table 3.3.1.1).

Table 3.3.1.1

	Source Plant	Site	Date
a.	R. ponticum	Longshaw, North Derbyshire SK 7926	10.78
b.	"	"	"
c.	"	Winterton Dunes, Norfolk TG 4821	11.78
d.	"	"	"
e.	R. maximum	Smoky Mountains, U.S.A.	1980

The first culture was used exclusively for the experimental work with synthesized mycorrhizas.

The isolate from R. maxiumum is of interest as it is the naturally occurring endophyte of a rhododendron. All the other endophytes must originally have been derived from native ericaceous vegetation, not including any rhododendron.

The isolates from each site seem to be consistent with regard to colour, growth rate and growth form. There was considerable variation between different sites. The isolate from R. maximum was much faster growing than the other cultures.

Culture growth-rates were estimated by measuring the increase in area of cultures grown on plates of 5% malt agar (Table 3.3.1.2). 0.5cm diameter cores of each endophyte culture were transferred to plates of 5% malt agar. The cultures were then kept at 20°C for 66 days. Culture diameter was then measured ten times for each plate, with five plates for each different endophyte culture.

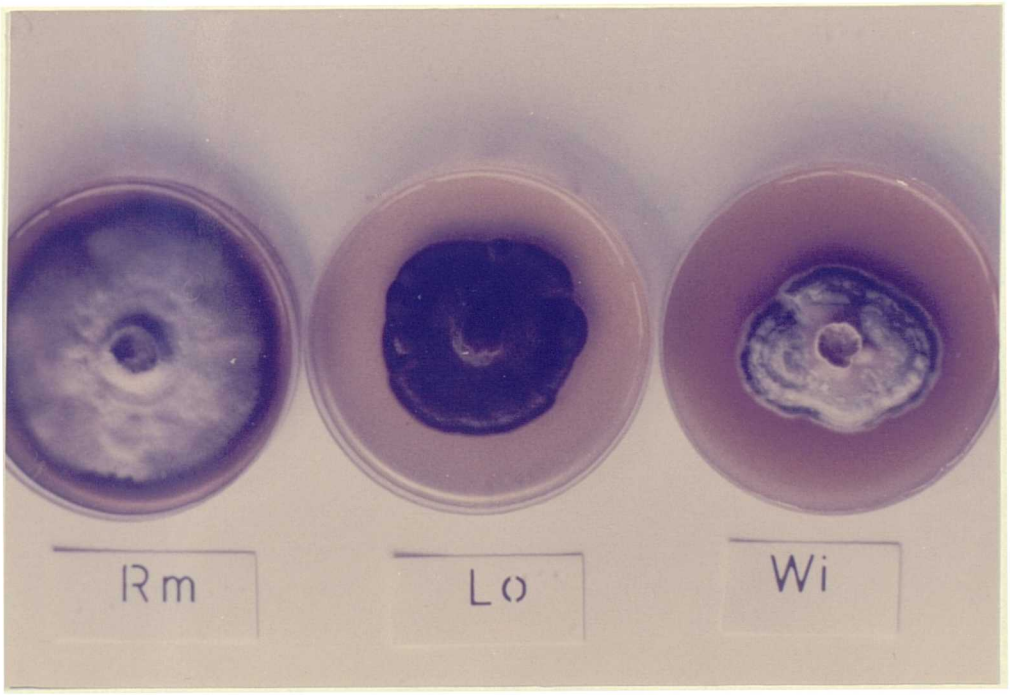
Culture colour was described using 'A Mycological Colour Chart' (Rayner, R.W., 1970).

Table 3.3.1.2

Culture	Colour	Increase in culture diameter over the experimental period (cm per week)	Increase in culture area over the experimental (sq.cm per week)
Longshaw (R. ponticum)	Pale greyish sepia - dark sepia	0.53	2.4
Winterton (R. ponticum)	Greyish vinaceous buff - greyish sepia	0.47	1.9
Smoky Mountains (R. maximum)	Pale greyish sepia - greyish dark sepia	0.70	4.1

PLATE 7 : Cultured endophyte on malt agar plates :-

Rm : R. maximum endophyte .
Lo : R.ponticum endophyte from Longshaw.
Wi : R.ponticum endophyte from Winterton.



3.3.2 THE DEVELOPMENT OF MYCORRHIZAS IN YOUNG *R. PONTICUM* SEEDLINGS GROWN ON FIELD SOIL

3.3.2.1 Introduction

The time taken for mycorrhizas to develop in seedling roots is very important with respect to the age of the seedling at which infection has an effect. In this experiment the development of microscopically visible infection was followed for seedlings grown on field soil.

3.3.2.2 Method

Freshly collected soil from a Rhododendron back-dune site at Winterton, Norfolk, was placed in 2½ inch diameter plastic pots. The pots were then sown with R. ponticum seed (from Clumber, North Nottinghamshire (N.Notts.)). They were then watered with distilled water and placed in a growth-room with a 16 hour/20°C day and 8 hour/15°C night.

The seeds were sown on the 4th May and germination was visible by the 16th. Following germination, ten seedlings were sampled at weekly intervals. Their roots were washed and stained. They were then examined meticulously for the presence of mycorrhizas.

3.3.2.3 Results

The first signs of mycorrhizal infection were after 65 days from the emergence of the radicle or 77 days after sowing (Table 3.3.2.1). Infection was very light and there was little visible, external fungus around the root. Intracellular infection of root tissue was clearly observed.

Examination of roots the following week (72 days after germination) showed increased infection with both more intracellular development and considerably more hyphal development around the root.

After 65 days (post-germination) a maceration of isolated R. ponticum endophyte was added to some pots. Examination of the seedlings from these pots at the last harvest showed their mycorrhizal development to be more advanced than those from the uninoculated pots. This suggests that limited availability of endophyte in the soil may be restricting the formation of mycorrhizas.

Further tests with pre-germinated, non-mycorrhizal seedlings of R. ponticum showed that mycorrhizas were visible under a light microscope around 6 weeks after inoculation with macerated endophyte. This is in agreement with observations by Duddridge and Read (1982) using electron microscopy.

Table 3.3.2.1

Number of days after sowing	Number of days after germination	Observation
21	9	Non-mycorrhizal
28	16	"
35	23	"
42	30	"
49	37	"
56	44	"
63	51	"
70	58	"
77	65	Slight mycorrhizal infection
84	72	Mycorrhizal

3.3.2.4 Discussion

Mycorrhizal infection of seedlings may have been restricted by the availability of endophyte, the availability of suitable root for infection and then by the time taken for mycorrhizas to establish when these were available. The results suggest infection of established seedlings would take around six weeks if the fungus is readily available. For seedlings germinating in situ, around nine weeks may be taken for infection to become visible. Further time will of course be necessary for the mycorrhizas to become sufficiently well established to be of physiological importance.

3.3.3 EXPERIMENT I : EFFECTS OF MYCORRHIZAL INFECTION ON THE GROWTH OF *RHODODENDRON PONTICUM*

3.3.3.1 Introduction

It was necessary to compare the growth of mycorrhizal with non-mycorrhizal seedlings. This was done by growing non-mycorrhizal seedlings on a range of soils and inoculating half of them with endophyte. The dry weights of harvested seedlings were then compared.

3.3.3.2 Method

Soils were collected from three Rhododendron sites at Winterton (East Norfolk, TG 4821), Clumber (N.Notts. SK 6375) and Cropton (North Yorkshire (N.Yorks.) SE 7696). They were coarse sieved and then subjected to gamma irradiation at a dose rate equivalent to 1.8 mega-rads. This is sufficient to kill any Rhododendron endophyte present in the soil.

The soils were then placed in 2½ inch diameter, sterilized, plastic pots with clear plastic tops. Sixteen pots were set up for each soil and sown with R. ponticum seed (from Clumber, N.Notts.). After germination the seedlings were thinned to give five seedlings per pot. Half the pots were then inoculated with R. ponticum endophyte (from Longshaw, North Derbyshire (N.Derbys.)).

Four harvests of seedlings were taken. Firstly, immediately prior to inoculation, and then at six-weekly intervals. Ten seedlings were harvested each time for each treatment. Seedlings harvested were carefully extracted from the soil, washed and then oven-dried at 80°C for 24 hours. Control pots were also set up to check mycorrhizal infection at each harvest.

The experiment was conducted in a growth-room with a 16 hour/15°C day and an 8 hour/10°C night. All the pots were watered with distilled water.

The soils used in the experiment were analysed for 'available' nitrogen (as ammonium and as nitrate), 'available' phosphorus, organic content and pH.

3.3.3.3 Results

The results varied somewhat with the different soils used (Tables 3.3.3.1, 3.3.3.2, 3.3.3.3 and 3.3.3.4).

Winterton Back-Dune Soil (Figure 3.1)

There was little apparent difference in growth between mycorrhizal and non-mycorrhizal seedlings after 6 and 12 weeks. At the third harvest however, both categories of seedlings were smaller than at the previous one. By the final harvest, growth had improved with mycorrhizal plants having smaller roots but larger shoots than the non-mycorrhizal ones. None of the differences were significant.

Clumber Soil (Figure 3.2)

Very slightly greater growth of mycorrhizal seedlings was apparent after 6 weeks. After 12 weeks the situation was reversed with non-mycorrhizal seedlings being larger in both root and shoot. By the final harvest, at 18 weeks, the mycorrhizal seedlings were significantly larger than non-mycorrhizal, in root, shoot and total dry weights (significant at $p = 0.05$).

Cropton Soil (Figure 3.3)

While mycorrhizal seedlings had greater root, shoot and total dry weight after both 12 weeks and 18 weeks (post-inoculation), the differences between mycorrhizal and non-mycorrhizal seedlings were not significant.

The changes with time of the Relative Growth Rate (R') (Figure 3.4) are difficult to interpret, the trends differing with the different soils. On Clumber and Cropton soils, R' either increased or decreased slightly from H1/H2 to H2/H3 and then decreased to H3/H4. In all cases R' was greater for mycorrhizal compared with non-mycorrhizal seedlings during the final period.

In almost all cases, the Root/Shoot Ratio (R/S) decreased during the experiment (Figure 3.5). The exceptions were very slight increases for non-mycorrhizal seedlings on Winterton and Cropton soils at the final harvest. There was little obvious difference between the values or trends for mycorrhizal and non-mycorrhizal plants.

Table 3.3.3.1 Dry weight of *R. ponticum* seedlings

Key: * Difference significant at p = 0.05 or less
1,2,3,4 : Harvests
Wint. : Winterton soil
Clum. : Clumber soil
Cropt. : Cropton soil
Myc : Inoculated seedlings
NMyc : Uninoculated (non-mycorrhizal) seedlings

Treatment Harvest No.	Mean dry weight (mg) of ten harvested seedlings. Standard deviation in ( ) Root	Shoot	Total
1. Wint. NMyc	0.24(0.10)	0.29(0.13)	0.53(0.15)
2. Wint. NMyc	0.84(0.35)	2.28(1.13)	3.12(1.25)
2. Wint. Myc	1.22(0.77)	1.89(0.66)	3.11(0.94)
3. Wint. NMyc	0.63(0.21)	2.09(0.99)	2.72(1.08)
3. Wint. Myc	0.65(0.38)	2.06(1.57)	2.71(1.87)
4. Wint. NMyc	3.30(0.97)	9.35(2.35)	12.65(2.87)
4. Wint. Myc	2.84(2.10)	11.70(7.61)	14.54(9.58)
1. Clum. NMyc	0.15(0.05)	0.22(0.08)	0.37(0.12)
2. Clum. NMyc	0.43(0.10)	0.69(0.10)	1.12(0.23)
2. Clum. Myc	0.56(0.15)*	0.77(0.30)	1.33(0.39)
3. Clum. NMyc	2.50(0.90)	5.82(2.74)	8.32(3.23)
3. Clum. Myc	2.11(0.91)	5.67(3.67)	7.78(4.51)
4. Clum. NMyc	1.18(0.33)	9.54(3.36)	10.72(3.31)
4. Clum. Myc	2.88(1.40)*	17.71(10.28)*	20.59(11.25)*
1. Cropt. NMyc	0.17(0.07)	0.22(0.08)	0.39(0.11)
2. Cropt. NMyc	0.38(0.19)	2.24(0.85)	2.62(0.97)
2. Cropt. Myc	0.46(0.18)	2.23(0.87)	2.69(0.95)
3. Cropt. NMyc	1.85(0.97)	11.57(7.25)	13.42(7.59)
3. Cropt. Myc	2.60(0.98)	13.34(8.36)	15.94(8.83)
4. Cropt. NMyc	6.83(3.26)	32.53(17.63)	39.16(20.48)
4. Cropt. Myc	7.78(3.18)	44.99(25.08)	52.77(27.58)

Table 3.3.3.2 Dry weight of seedlings with 95% confidence limits

Treatment	Mean dry weight (mg) of ten harvested seedlings + or - limits		
	Root	Shoot	Total
1. Wint. NMyc	0.24 ± 0.08	0.29 ± 0.10	0.53 ± 0.11
2. Wint. NMyc	0.84 ± 0.27	2.28 ± 0.85	3.12 ± 0.94
2. Wint. Myc	1.22 ± 0.58	1.89 ± 0.50	3.11 ± 0.71
3. Wint. NMyc	0.63 ± 0.16	2.09 ± 0.75	2.72 ± 0.81
3. Wint. Myc	0.65 ± 0.29	2.06 ± 1.18	2.71 ± 1.41
4. Wint. NMyc	3.30 ± 0.73	9.35 ± 1.77	12.65 ± 2.16
4. Wint. Myc	2.84 ± 1.58	11.70 ± 5.74	14.54 ± 7.22
1. Clum. NMyc	0.15 ± 0.04	0.22 ± 0.06	0.37 ± 0.09
2. Clum. NMyc	0.43 ± 0.08	0.69 ± 0.08	1.12 ± 0.17
2. Clum. Myc	0.56 ± 0.11*	0.77 ± 0.23	1.33 ± 0.29
3. Clum. NMyc	2.50 ± 0.68	5.82 ± 2.07	8.32 ± 2.44
3. Clum. Myc	2.11 ± 0.69	5.67 ± 2.77	7.78 ± 3.40
4. Clum. NMyc	1.18 ± 0.25	9.54 ± 2.53	10.72 ± 2.50
4. Clum. Myc	2.88 ± 1.06*	17.71 ± 7.75*	20.59 ± 8.48*
1. Cropt. NMyc	0.17 ± 0.05	0.22 ± 0.06	0.39 ± 0.08
2. Cropt. NMyc	0.38 ± 0.14	2.24 ± 0.64	2.62 ± 0.73
2. Cropt. Myc	0.46 ± 0.14	2.23 ± 0.66	2.69 ± 0.72
3. Cropt. NMyc	1.85 ± 0.73	11.57 ± 5.47	13.42 ± 5.72
3. Cropt. Myc	2.60 ± 0.74	13.34 ± 6.30	15.94 ± 6.66
4. Cropt. NMyc	6.83 ± 2.46	32.33 ± 13.29	39.16 ± 15.44
4. Cropt. Myc	7.78 ± 2.40	44.99 ± 18.91	52.77 ± 20.80

Table 3.3.3.3 Relative Growth Rates for seedlings

Time period considered	Treatment	$\bar{W}$ (mg.)	dW (mg.)	dT (weeks)	R'(per week)
Harvest 1-2	Wint. NMyc	1.83	2.59	6	0.24
	Wint. Myc	1.82	2.58	6	0.24
Harvest 2-3	Wint. NMyc	2.92	-0.40	6	-0.02
	Wint. Myc	2.91	-0.40	6	-0.02
Harvest 3-4	Wint. NMyc	7.69	9.93	6	0.22
	Wint. Myc	8.63	11.83	6	0.23
Harvest 1-2	Clum. NMyc	0.75	0.75	6	0.17
	Clum. Myc	0.85	0.96	6	0.19
Harvest 2-3	Clum. NMyc	4.72	7.20	6	0.25
	Clum. Myc	4.56	6.45	6	0.24
Harvest 3-4	Clum. NMyc	9.52	2.40	6	0.04
	Clum. Myc	14.19	12.81	6	0.15
Harvest 1-2	Cropt. NMyc	1.51	2.23	6	0.25
	Cropt. Myc	1.54	2.30	6	0.25
Harvest 2-3	Cropt. NMyc	8.02	10.80	6	0.22
	Cropt. Myc	9.32	13.25	6	0.24
Harvest 3-4	Cropt. NMyc	26.29	25.74	6	0.16
	Cropt. Myc	34.36	36.83	6	0.18

Calculation of Relative Growth Rate (R'):-

(Ref. Hunt, R., 1978)

$$R' = \frac{\text{Change in whole plant dry weight}}{\text{Time between harvests}} \times \frac{1}{\text{Mean dry weight}}$$

or

$$R' = \frac{dW}{dT} \times \frac{1}{\bar{W}}, \quad \bar{W} \text{ taken as } \frac{W(\text{Harv.1}) + W(\text{Harv.2})}{2}$$

Table 3.3.3.4 Root/Shoot Ratio of harvested seedlings

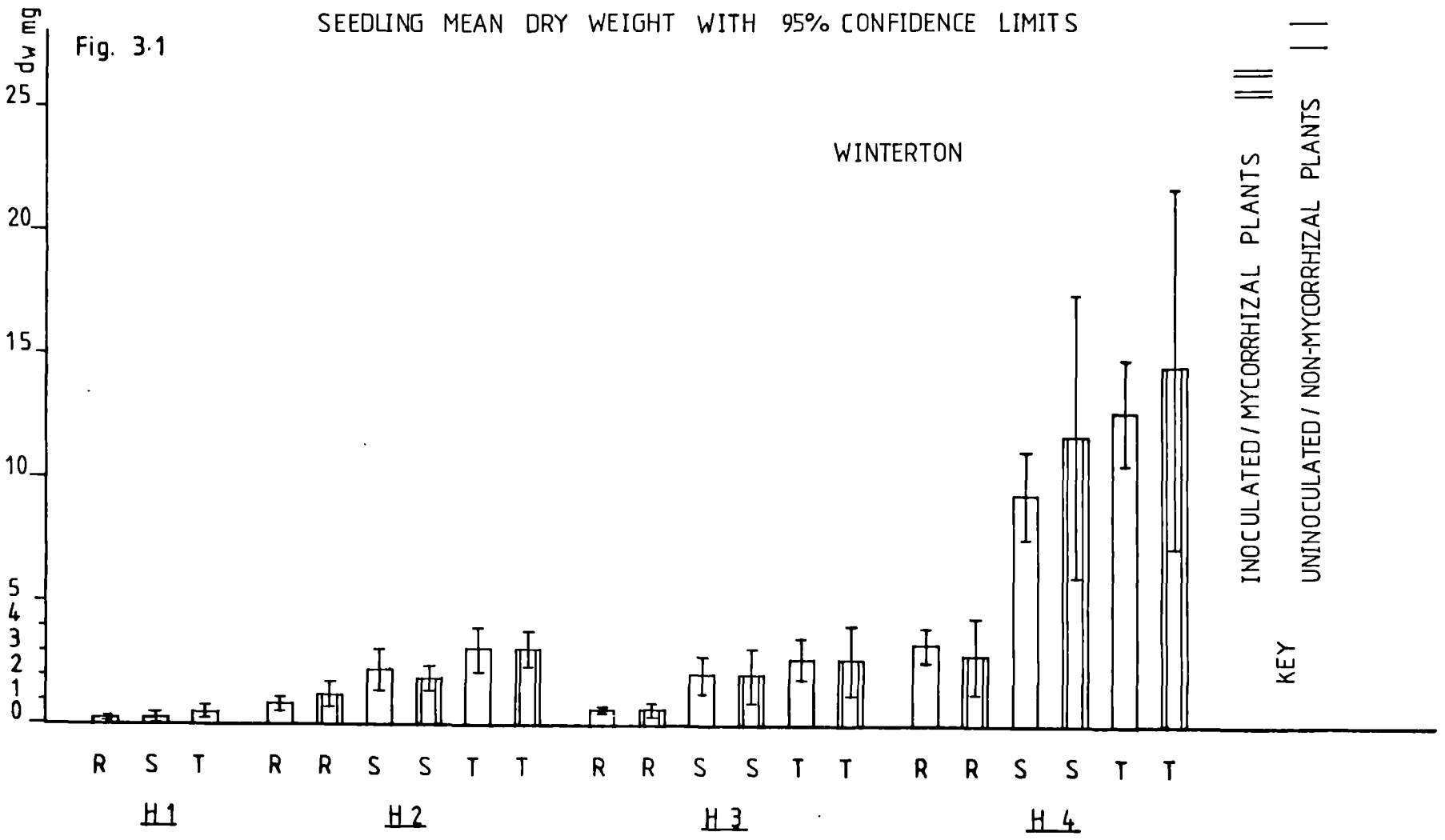
Harvest	R/S	Harvest	R/S	Harvest	R/S
1. Wint. NMyc	0.83	1. Clum. NMyc	0.68	1. Cropt. NMyc	0.77
2. Wint. NMyc	0.37	2. Clum. NMyc	0.62	2. Cropt. NMyc	0.17
2. Wint. Myc	0.65	2. Clum. Myc	0.73	2. Cropt. Myc	0.21
3. Wint. NMyc	0.30	3. Clum. NMyc	0.43	3. Cropt. NMyc	0.16
3. Wint. Myc	0.32	3. Clum. Myc	0.37	3. Cropt. Myc	0.20
4. Wint. NMyc	0.35	4. Clum. NMyc	0.12	4. Cropt. NMyc	0.21
4. Wint. Myc	0.24	4. Clum. Myc	0.16	4. Cropt. Myc	0.17

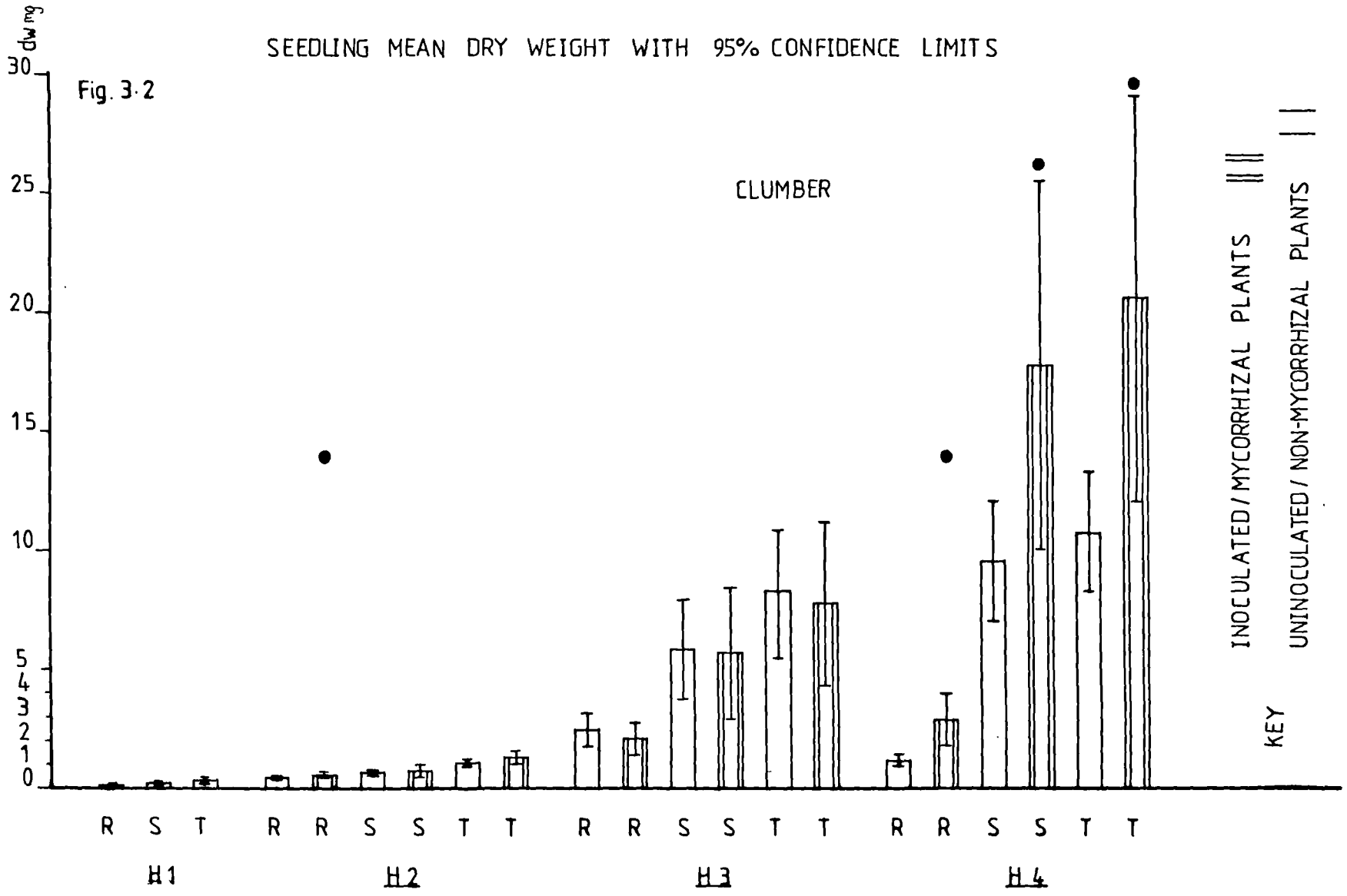
Table 3.3.3.5 Examination of seedlings for mycorrhizal infection

Treatment	Comments on infection of roots
2. Wint. Myc	Very slight infection
3. Wint. Myc	Very slight infection
4. Wint. Myc	Roots quite heavily infected
2. Clum. Myc	Slight infection
3. Clum. Myc	Slight infection
4. Clum. Myc	Roots heavily infected
2. Cropt. Myc	Roots lightly infected
3. Cropt. Myc	Roots heavily infected
4. Cropt. Myc	Roots heavily infected

KEY :- Results tested with Student's t-test differences from Controls significant at $p = 0{\cdot}05$ or less. ●

Fig. 3.1


SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS
mg dw
25
20
15
10
5
4
3
2
1
0
WINTERTON
INOCULATED / MYCORRHIZAL PLANTS
UNINOCULATED / NON-MYCORRHIZAL PLANTS
KEY
R S T R R S S T T R R S S T T R R S S T T
H1
H2
H3
H4


SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS
Fig. 3·2
dw mg
30
25
20
15
10
5
4
3
2
1
0
CLUMBER
KEY
INOCULATED/MYCORRHIZAL PLANTS
UNINOCULATED/NON-MYCORRHIZAL PLANTS
R S T R R S S T T R R S S T T R R S S T T
H1
H2
H3
H4

SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Fig. 3.3

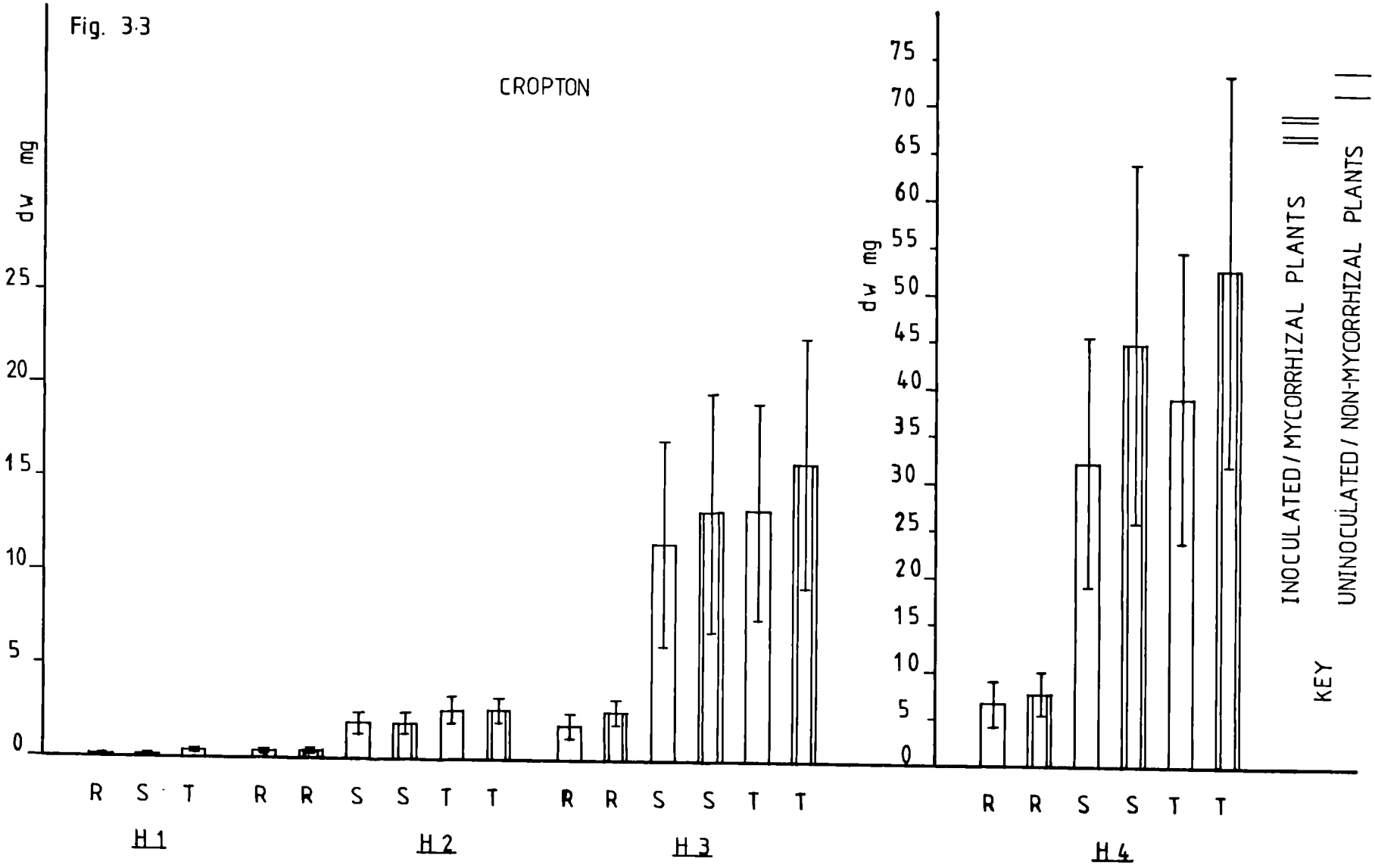
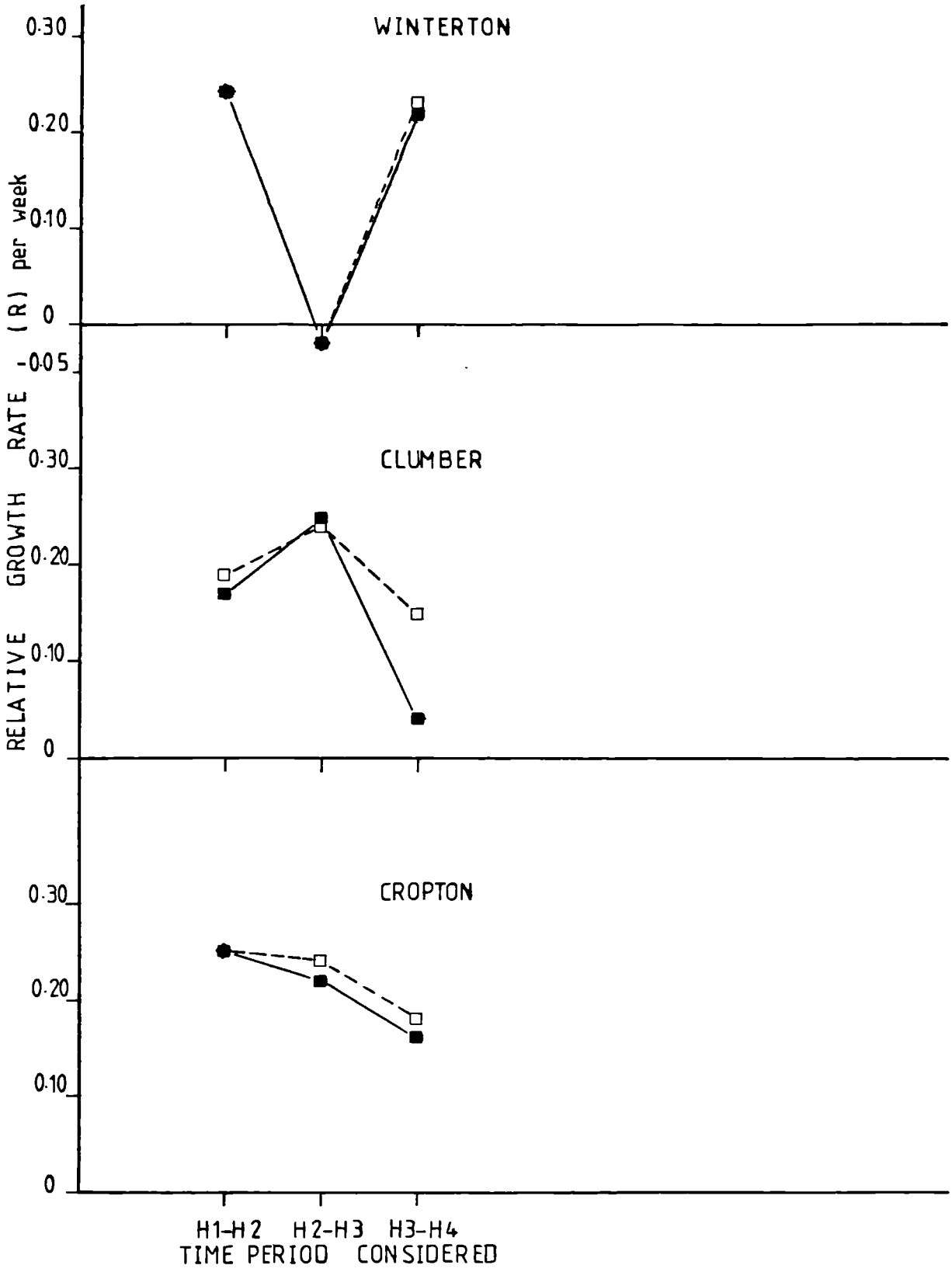


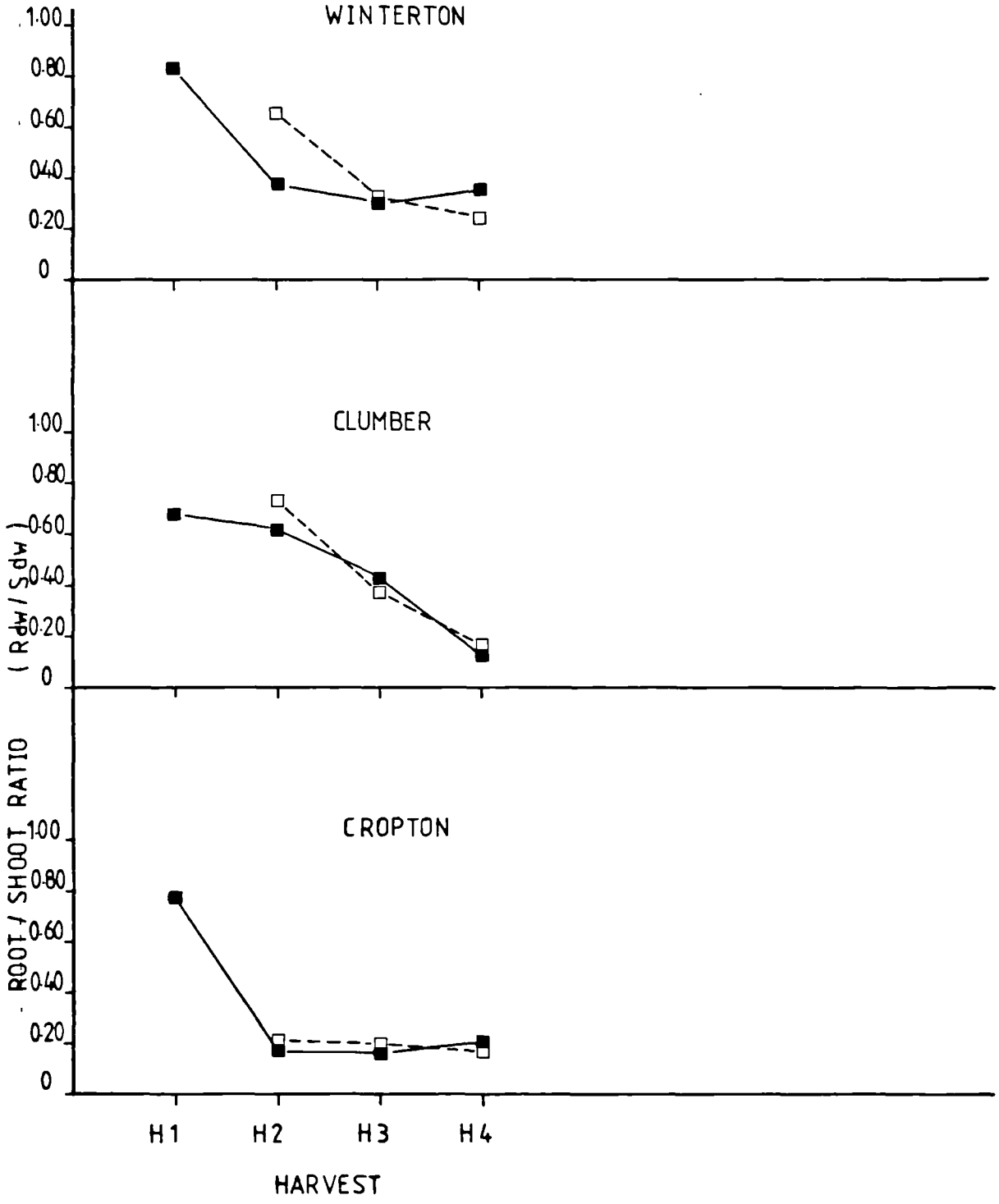
Fig. 3.4 RELATIVE GROWTH RATES OF INOCULATED AND UNINOCULATED SEEDLINGS



KEY :- INOCULATED/MYCORRHIZAL PLANTS : □

UNINOCULATED/NON-MYCORRHIZAL PLANTS: ■

Fig. 3.5 ROOT / SHOOT RATIO OF INOCULATED AND UNINOCULATED SEEDLINGS



KEY :- INOCULATED / MYCORRHIZAL PLANTS : □

UNINOCULATED / NON-MYCORRHIZAL PLANTS: ■

Table 3.3.3.6 Soil analysis

Soil	Org.Co.%.	Available nitrogen (as ammonium and as nitrate) and phosphorus in ppm. Av.P.	Av.N(Amm.).	Av.N(Nit.).	Av.N Total
Cropt. Irr.		7.2	343.4	14.2	357.5
Cropt. Unt.	62.2	3.6	155.1	9.1	164.2
Clum. Irr.		3.1	12.8	0.3	13.1
Clum. Unt.	2.1	-	0.6	0.9	1.5
Wint. Irr.		3.3	29.7	3.8	33.5
Wint. Unt.	3.2	-	9.8	3.5	13.3

(Note: When considering the above values relative to each other, it should be noted that the volume of the Cropton soil was considerably larger than that of the other soils. The highly organic Cropton soil was perhaps 5-10 X less dense, although this is very variable due to its compressible nature. The values of available N and P on a volume for volume basis would still be highest in the Cropton soil, but by a far smaller margin.)

Table 3.3.3.7 Soil pH in distilled water after 24 hours

Soil	Coarse sieved	Fine sieved
Cropt. Irr.	3.65	3.65
Cropt. Unt.	3.80	3.80
Clum. Irr.	3.85	3.85
Clum. Unt.	3.85	3.95
Wint. Irr.	-	4.30
Wint. Unt.	-	4.15

3.3.4 EXPERIMENT II : EFFECTS OF MYCORRHIZAL INFECTION ON THE GROWTH OF *RHODODENDRON PONTICUM* WITH AND WITHOUT NUTRIENT ADDITION

3.3.4.1 Introduction

To develop further the findings of the previous experiment (3.3.3), a comparison of mycorrhizal with non-mycorrhizal growth was again undertaken. The growing conditions were modified to increase temperature, decrease moisture loss (and hence decrease the watering required) and to eliminate possible contamination of uninoculated seedlings with the mycorrhizal fungus. The number of seedlings harvested for each condition was increased to make the data more reliable.

In order to compare the relative effects of inoculation with the endophyte and of addition of nutrients, half the pots were watered with a 10X concentration of full-strength Robbins' solution. As a relatively low strength, well-balanced nutrient addition, Robbins' solution has been used consistently throughout this work. In this particular experiment a reasonably high level of nutrient addition was desired, so the 10X concentration was used.

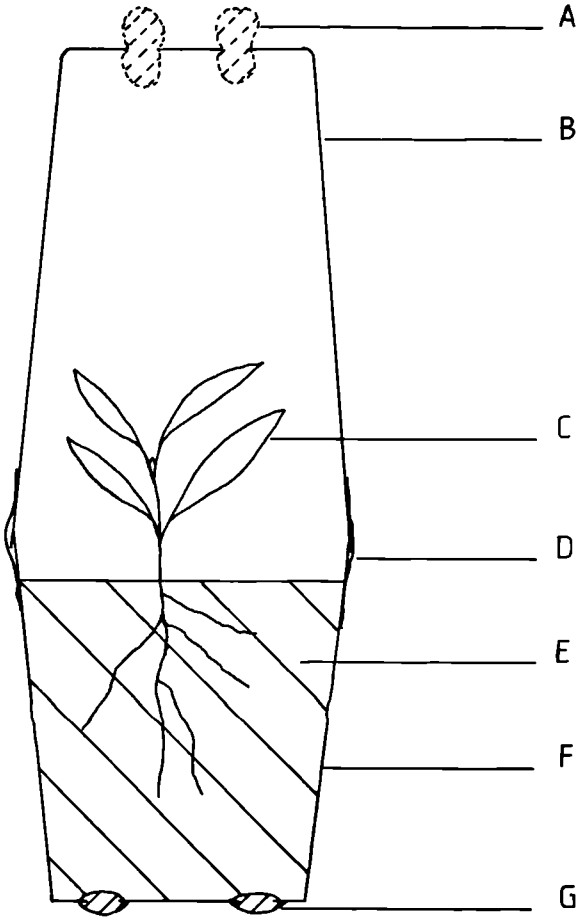
3.3.4.2 Method

Soil from Clumber (N.Notts.) was collected from under established R. ponticum bushes. The soil was then partially sterilized by gamma irradiation at a dose rate equivalent to 1.8 mega-rads.

The soil was then placed in 2½ inch diameter, sterilized, plastic pots with clear plastic propagator tops. The pots were planted with non-mycorrhizal seedlings of R. ponticum and set up, semi-sealed as shown in the diagram (Figure 3.10).

Five seedlings were planted in each pot. Half the pots were then inoculated with R. ponticum endophyte (from Longshaw, N.Derbys.). The pots were watered at weekly intervals, half with 25 mls. of distilled water and half with 25 mls. of 10X full-strength Robbins' solution (ION). All additions of water, nutrient solution or endophyte were by sterile

Fig. 3·10 POT FOR GROWING INOCULATED AND UNINOCULATED SEEDLINGS OF RHODODENDRON PONTICUM



KEY :-

A : Cotton wool bung for air-hole in propagator top. Water or nutrient solution injected through the bung with a sterile syringe.

B : Clear plastic propagator lid.

C : Seedling

D : Sticky plastic tape sealing the lid to the pot.

E : Soil

F : Plastic pot (2·5 inch diameter)

G : Drain-holes plugged with silicone rubber sealant.

syringe injected through the cotton wool bung in the plastic top. The pots were placed in a growth-room with a 16 hour/20°C day and an 8 hour/15°C night.

Four harvests of seedlings were taken. The first harvest was of untreated seedlings at the time of planting, followed by three more harvests at six-weekly intervals. Four pots (20 seedlings) were taken at each harvest for each treatment. Pots were selected at random for each harvest. The pots were numbered and correspondingly numbered cards were drawn blindly to select the harvested pot for each treatment. Additional pots were set up for the second harvest. The seedlings in these were harvested and their roots examined to verify their mycorrhizal status. Seedlings were carefully extracted from the soil, washed, dried at 80°C for 24 hours and weighed.

The soil used for the experiment was analysed for available nitrogen and phosphorus, organic content and pH.

3.3.4.3 Results

The results of Experiment II showed clear trends.

After 6 weeks there was little difference between mycorrhizal and non-mycorrhizal plants (Figure 3.6). There were obvious increases in root, shoot and total dry weights for all those with added nutrients. These differences were significant, but less so for Myc.+N vs. Myc.-N, than for NMyc.+N vs. NMyc.-N.

By 12 weeks the effects of mycorrhizal infection were showing as increased root, shoot and total dry weight of mycorrhizal compared to non-mycorrhizal plants (Figure 3.7). Seedlings with added nutrients were considerably larger than those without. The mycorrhizal seedlings without nutrients, although slightly smaller, were of a comparable size to the non-mycorrhizal seedlings with nutrients added. The increased shoot and total dry weights of mycorrhizal vs. non-mycorrhizal (both without added nutrients) and with nutrients vs. without, were significant at $p = 0.05$ or

better. Mycorrhizal seedlings with added nutrients had larger roots, shoots and total dry weights than non-mycorrhizal with nutrients, but they were significant only at p = 0.10 (root and shoot) and p = 0.05 (total).

At the final harvest (Figure 3.7) there was little difference between the mycorrhizal and non-mycorrhizal with added nutrients (the mycorrhizal plants being slightly larger, with smaller roots and larger shoots, but not significant at p = 0.10). All the seedlings without added nutrients were relatively reduced in growth. This was significant at p = 0.001 except for Myc.+N vs. Myc.-N roots, significant at p = 0.01. The increased growth of mycorrhizal compared to non-mycorrhizal seedlings, with no nutrient addition was significant at p = 0.02 (shoots and total) and p = 0.10 (roots).

Relative Growth Rate (R') showed consistent trends for Myc.+N, Myc.-N and NMyc.-N, with values decreasing in that order (Figure 3.8). R'increased from H1/H2 to H2/H3 and then decreased to H3/H4. For NMyc.+N, R' began relatively high, remained level from H1/H2 to H2/H3, and then increased to H3/H4, being the highest value of all for the final period.

Root/Shoot Ratio (R/S) (Figure 3.9) decreased slightly at first for both non-mycorrhizal treatments. With nutrient addition the value carried on falling steadily. With no addition of nutrients, the value rose sharply from H2 to H3, and then fell to H4, but was still by far the highest value at the final harvest. For mycorrhizal plants, the value of R/S was already quite high by H2, having risen considerably relative to the non-mycorrhizal seedlings that were planted (H1). The value for Myc.+N then fell very sharply to H3 and continued falling to H4, to be the lowest value at the final harvest. For Myc.-N the trend was similar but R/S neither rose so sharply nor fell so low. By the final harvest the R/S values were in the following order:- NMyc.-N (highest), Myc.-N and NMyc.+N (equal), Myc.+N (lowest).

Table 3.3.4.1 Dry weights of the *Rhododendron* seedlings

Treatment	Mean dry weight (mg) of twenty harvested seedlings. Standard deviation in ( )		
	Root	Shoot	Total
Harvest 1			
Transplanted seedlings	0.10(0)	0.31(0.11)	0.40(0.12)
Harvest 2			
Myc. + N	0.51(0.57)	0.74(0.51)	1.25(1.06)
Myc. - N	0.22(0.15)	0.52(0.30)	0.74(0.42)
NMyc. + N	0.38(0.30)	0.76(0.37)	1.14(0.61)
NMyc. - N	0.15(0.10)	0.50(0.18)	0.65(0.24)
Harvest 3			
Myc. + N	1.10(0.92)	4.74(4.43)	5.84(5.21)
Myc. - N	0.75(0.44)	1.98(1.31)	2.72(1.53)
NMyc. + N	0.66(0.30)	2.66(1.68)	3.22(1.69)
NMyc. - N	0.65(0.51)	0.98(0.50)	1.63(0.96)
Harvest 4			
Myc. + N	3.07(2.33)	19.41(11.42)	22.48(13.23)
Myc. - N	1.34(1.03)	5.99(6.90)	7.33(7.74)
NMyc. + N	3.85(2.94)	17.38(14.34)	21.23(17.08)
NMyc. - N	0.86(0.49)	1.72(1.40)	2.58(1.72)

Key:- Myc. : mycorrhizal; NMyc. : non-mycorrhizal
+ N : with 10X Robbins' solution; -N : with distilled water

Table 3.3.4.2 Dry weight of seedlings with 95% confidence limits

Treatment	Mean dry weight (mg) of twenty harvested seedlings + or - limits		
	Root	Shoot	Total
Harvest 1			
Transplanted seedlings	0.10 ± 0	0.31 ± 0.05	0.40 ± 0.06
Harvest 2			
Myc. + N	0.51 ± 0.27	0.74 ± 0.24	1.25 ± 0.50
Myc. - N	0.22 ± 0.07	0.52 ± 0.14	0.74 ± 0.20
NMyc. + N	0.38 ± 0.14	0.76 ± 0.17	1.14 ± 0.29
NMyc. - N	0.15 ± 0.15	*0.50 ± 0.08*	*0.65 ± 0.11*
Harvest 3			
Myc. + N	1.10 ± 0.43	4.74 ± 2.07	5.84 ± 2.44
Myc. - N	0.75 ± 0.21	1.98 ± 0.61	2.72 ± 0.72
NMyc. + N	0.66 ± 0.14	2.66 ± 0.79	3.22 ± 0.79
NMyc. - N	0.65 ± 0.24	0.98 ± 0.23	1.63 ± 0.45
Harvest 4			
Myc. + N	3.07 ± 1.09	19.41 ± 5.35	22.48 ± 6.19
Myc. - N	1.34 ± 0.48	5.99 ± 3.23	7.33 ± 6.19
NMyc. + N	3.85 ± 1.38	17.38 ± 6.71	21.23 ± 7.99
NMyc. - N	0.86 ± 0.23	1.72 ± 0.67	2.58 ± 0.81

Table 3.3.4.3 Statistical significance of the treatments

The seedling dry weights were tested with 'Student's' t-test to show the significance or otherwise of the addition of nutrient solution and the infection of the roots by mycorrhizas on seedling growth.

Key:-

NS	:	Not significant at p = 0.05 or 5% level
*	:	Significant
		Degree of significance given as percentage level
Myc.	:	Inoculated seedlings
NMyc.	:	Uninoculated seedlings
+ N	:	Added nutrients
- N	:	No added nutrients

Treatments	Harvest 2			Harvest 3			Harvest 4		
Compared	R	S	T	R	S	T	R	S	T
Myc. + N	5%				2%	2%	1%	0.1%	0.1%
vs. Myc. - N	*	NS	NS	NS	*	*	*	*	*
NMyc. + N	1%	1%	1%		0.1%	0.1%	0.1%	0.1%	0.1%
vs. NMyc. - N	*	*	*	NS	*	*	*	*	*
Myc. + N						5%			
vs. NMyc. + N	NS	NS	NS	NS	NS	*	NS	NS	NS
Myc. - N					1%	2%		2%	2%
vs. NMyc. - N	NS	NS	NS	NS	*	*	NS	*	*

The data obtained from this experiment were subjected to an anovar, the results of which are presented in Appendix 7.

Table 3.3.4.4 Relative Growth Rates for seedlings

Time period considered	Treatment	$\bar{W}$ (mg.)	dW (mg.)	dT (weeks)	R'(per week)
Harvest 1-2	Myc. + N	0.83	0.85	6	0.17
	Myc. - N	0.57	0.34	6	0.10
	NMyc. + N	0.77	0.74	6	0.16
	NMyc. - N	0.53	0.25	6	0.08
Harvest 2-3	Myc. + N	3.55	4.59	6	0.22
	Myc. - N	1.73	1.98	6	0.19
	NMyc. + N	2.18	2.08	6	0.16
	NMyc. - N	1.14	0.98	6	0.14
Harvest 3-4	Myc. + N	14.16	16.64	6	0.20
	Myc. - N	5.03	4.61	6	0.15
	NMyc. + N	12.23	18.01	6	0.25
	NMyc. - N	2.11	0.95	6	0.08

Table 3.3.4.5 Root/Shoot Ratio of harvested seedlings

Treatment	Mean Root/Shoot Ratio for each harvest			
	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Myc. + N	-	0.69	0.23	0.16
Myc. - N	-	0.42	0.38	0.22
NMyc. + N	-	0.28	0.25	0.22
NMyc. - N	0.32	0.30	0.66	0.50

(transplanted seedlings)

Table 3.3.4.6 Soil analysis

1. pH in distilled water after 24 hours	:	3.80
2. Available Nitrogen a. as ammonium	:	10.0 ppm.
b. as nitrate	:	1.2 ppm.
c. Total	:	11.2 ppm.
3. Available Phosphorus	:	3.5 ppm.
4. Organic content	:	1.5%

Nitrogen and Phosphorus Addition in Nutrient Solution

25 mls of 10 N Robbins' solution added where required, once per week. This contains approximately 96 ppm. of nitrogent (as nitrate) and 23 ppm. of phosphorus (as phosphate). Expressed as weight added, this is approximately 2.4 mg N. and 0.58 mg P.

Mycorrhizal Status of Seedlings

Seedlings examined for mycorrhizal infection at the second harvest were mycorrhizal for both treatments in which endophyte was added and non-mycorrhizal for both in which no endophyte was added.

Fig. 3.6

SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

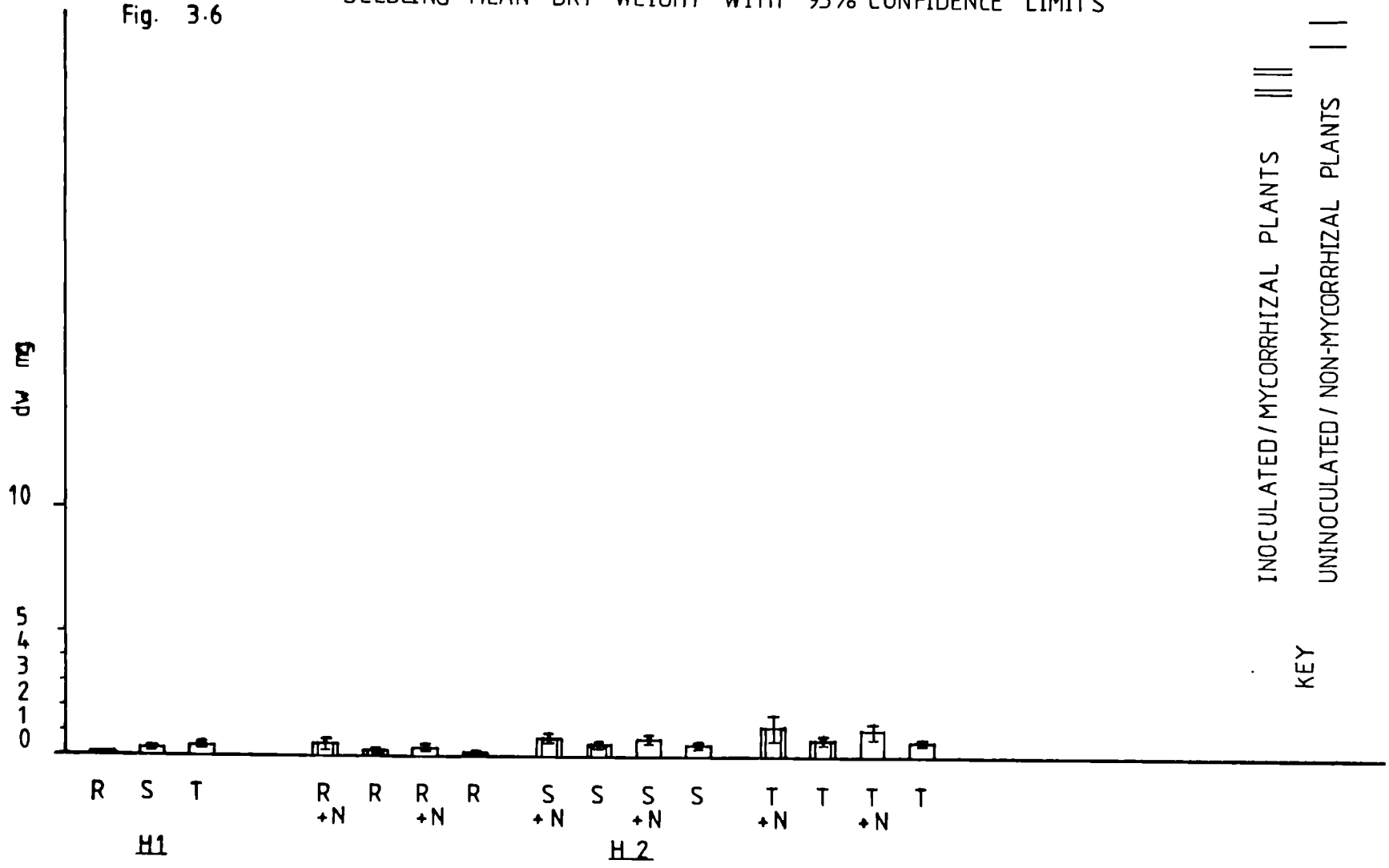


Fig. 3·7 SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

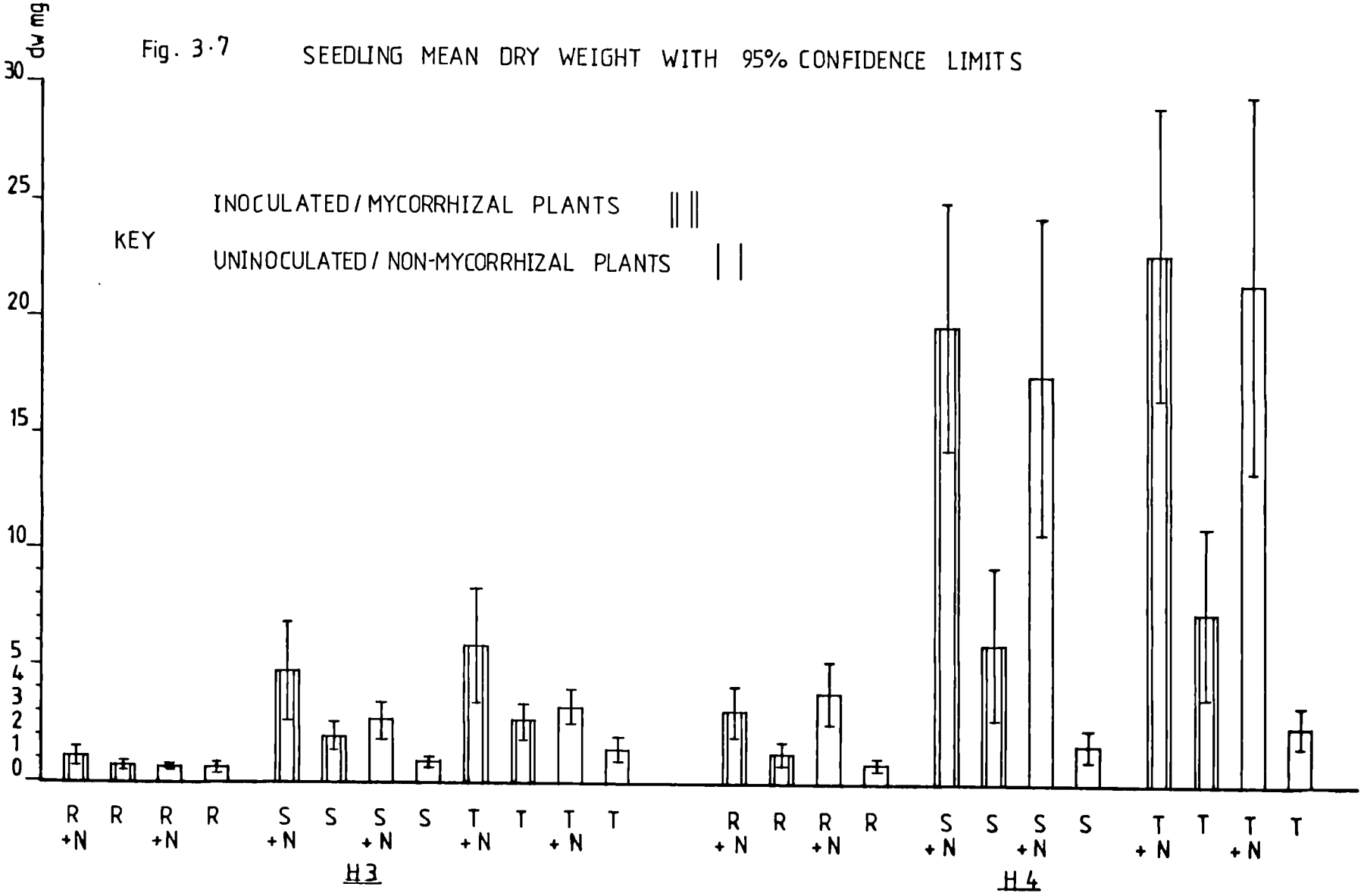


Fig. 3.8 RELATIVE GROWTH RATES OF INOCULATED AND UNINOCULATED SEEDLINGS

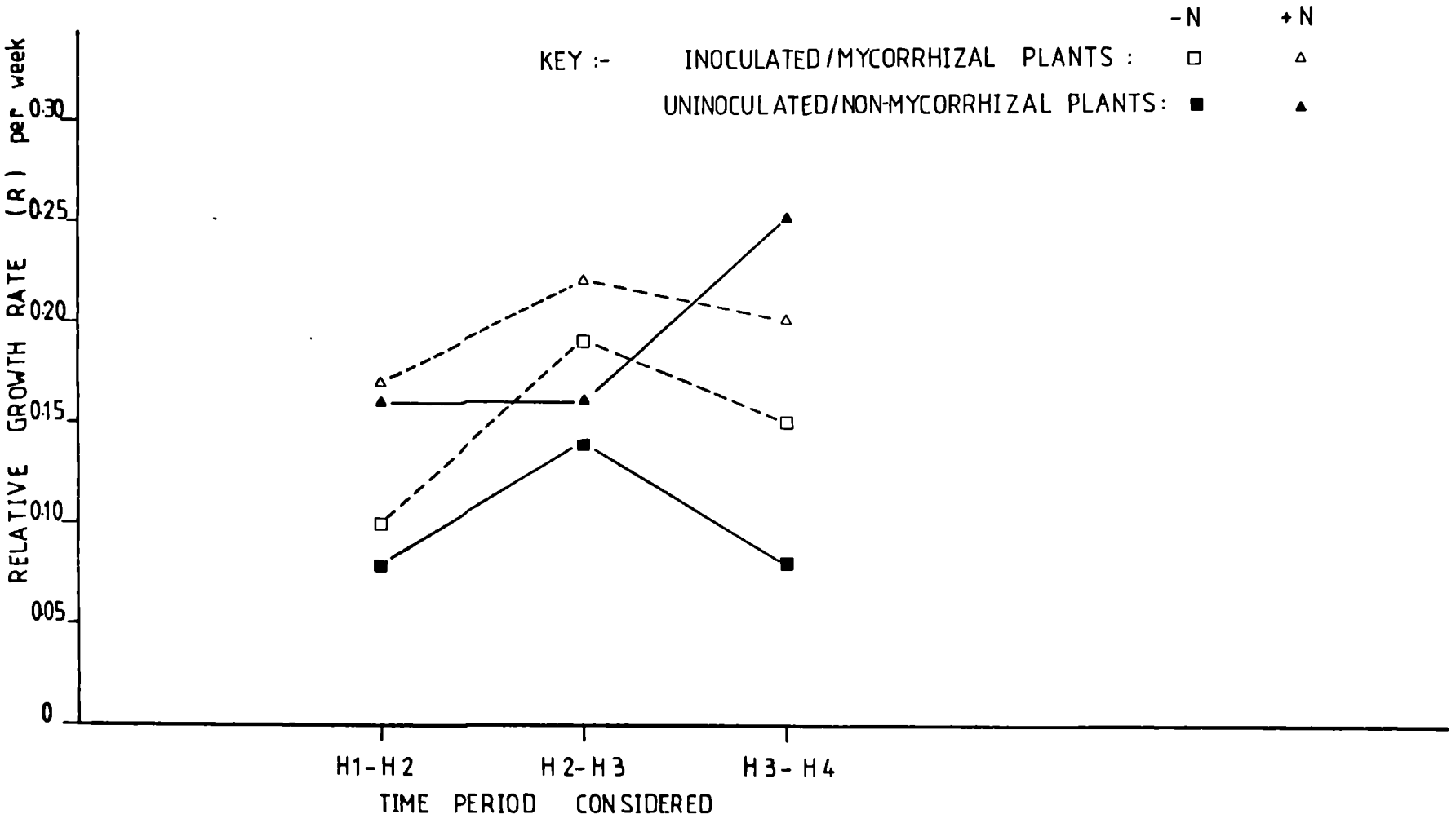
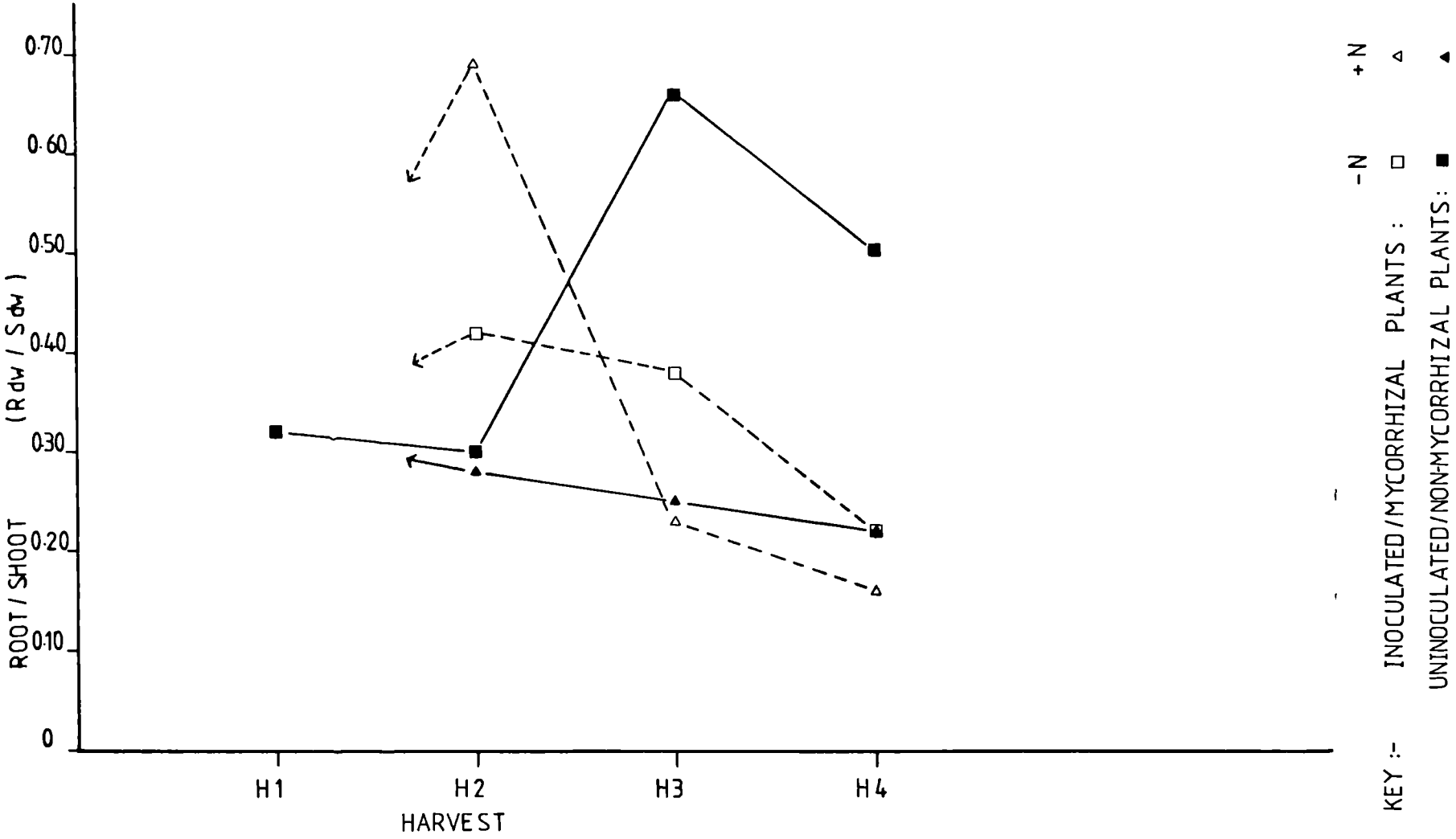


Fig. 3·9 ROOT / SHOOT RATIO OF INOCULATED AND UNINOCULATED SEEDLINGS



3.3.5 THE OCCURRENCE OF *RHODODENDRON* ENDOPHYTE IN A RANGE OF FIELD SOILS

3.3.5.1 Introduction

With the rejection of Rayner's ideas of 'cyclic' infection of ericaceous mycorrhizas, the question of the source of viable endophyte for infection becomes of particular interest. Clearly when invasive R. ponticum spreads by seed into a new area, any potential benefit from mycorrhizal infection will be totally dependent on a source of viable inoculum.

It was therefore decided to examine a range of field soils for viable endophyte.

3.3.5.2 Method

Field soils were collected from a range of sites and placed in sterilized plastic pots. All collecting equipment was sterilized with ethanol on site, immediately prior to collecting a sample.

The soils were then sown with R. ponticum seed collected from Clumber, N.Notts.. Three pots were set up for each sample used. After germination and development of the seedlings, they were harvested at intervals and the roots were examined for mycorrhizas.

All pots were watered with distilled water. The acidity of the soil samples was measured.

Table 3.3.5.1 Description of sites

Non-ericaceous/non-rhododendron sites

1. Moss Valley, N.Derbys. SK 378 814. Non-ericaceous. Acid leached soil with sparce grass cver beneath Quercus canopy.

2. Graves Park, S.Yorks. SK 357 826. Non-ericaceous. Acid soil with dense grass sward beneath Acer pseudoplatanus canopy.

3. Ecclesall Woods, S.Yorks. SK 326 828. Non-ericaceous. Acid soil with dense grass and forb sward in mature, mixed woodland.

4/5 Same site as 8.

6/7 Soil and sand from the University Experimental Garden at Tapton, Sheffield.

Rhododendron sites

8. Chatsworth, N.Derbys. SK 276 700. R. ponticum below Pinus canopy.
9. Clumber, N.Notts. SK 618 747. R. ponticum, pure stand in open.
10. Winterton, Norfolk TG 485 215. R. ponticum, pure stand in open.
11. Strawberry Lee Plantation, S.Yorks. SK 279 805. R. ponticum, pure stand within mixed woodland.

The Moss Valley site was well away from any ericaceous vegetation (probably well over 1 km.). The Graves Park site has planted rhododendrons at about 300m distance. The Ecclesall Woods site has R. ponticum within about 50m.

3.3.5.3 Results

As shown in Table 3.3.5.2 field soils which have not supported ericaceous plants did not produce mycorrhizal infection of R. ponticum seedlings. Field soils from sites with ericaceous vegetation produced mycorrhizal infection of R. ponticum seedlings within two months. Pinus soil and litter from a site very near to R. ponticum bushes gave infection after between two and a half months and six months.

Table 3.3.5.2 Examination of roots. 10 seedlings per sample (seed sown : 13.7.79)

Vegetation	Site	6.9.79	25.9.79	20.12.79
Non-ericaceous/ Non-Rhododendron	Moss Valley	-	-	-
	Graves Park	-	-	-
	Ecclesall Woods	-	-	-
	Tapton Gardens sand	-	-	-
	Tapton Gardens soil	-	-	-
Ericaceous/ Rhododendron	Chatsworth	+	+	+
	Clumber	+	+	+
	Winterton Back-Dune	+	+	+
	Strawberry Lee Plant.	+	+	+
Non-ericaceous/ Non/Rhododendron but near Rhododendron	Chatsworth Pinus litter	-	-	+ (2 pots)
	Chatsworth Pinus soil	-	-	+

Key: + : mycorrhizas present in seedlings from all pots unless stated otherwise.
- : no mycorrhizas found.

In the Tapton Gardens soil the Rhododendron roots were very stunted and by the final harvest all the seedlings had died.

Sample pH's are presented in Table 3.3.5.3. All samples except those from Tapton Gardens were very acidic (pH c. 3.40 - 3.80). Seedling growth on the Tapton soil (pH 7.20) was very poor.

Table 3.3.5.3 Acidity of soil samples

Soil	pH measured in distilled water after 24 hours
1. Moss Valley	3.65
2. Graves Park	3.55
3. Ecclesall Woods	3.55
4.. Chatsworth	3.40
5. Clumber	3.40
6. Winterton Back-Dune	3.40
7. Strawberry Lee Plantation	3.40
8. Chatsworth *Pinus* litter	3.80
9. Chatsworth *Pinus* soil	3.80
10. Tapton Gardens sand	6.15
11. Tapton Gardens soil	7.20

3.3.6 THE OCCURRENCE OF *RHODODENDRON* ENDOPHYTE IN FIELD SOILS FROM WINTERTON DUNES, NORFOLK

3.3.6.1 Introduction

The results of the previous experiment suggest the absence of viable Rhododendron endophyte from field soils with non-ericaceous vegetation. The situation was now examined further in a fairly self-contained area of vegegation with clearly defined, different vegetation types, including a large proportion of ericaceous heath and invasive R. ponticum. The site was at Winterton Dunes in Norfolk (TG 4821).

3.3.6.2 Method

Field soils were collected in the same manner as was employed for the previous experiment. In this case however, samples were taken from different habitats within the same vegetation system.

The soils were placed in sterilized 2½ inch plastic pots, sown with R. ponticum seed (from Clumber, N.Notts.) and watered with distilled water. Half the pots were inoculated with macerated Rhododendron endophyte (from Longshaw, N.Derbys.). Four pots were set up for each sample, two with added endophyte and two without.

The seeds were sown 15.2.80, endophyte added where required 4.3.80 and the seedlings were harvested and their roots examined for mycorrhizas, 13.5.80.

The pH of each soil sample was measured.

3.3.6.3 Results

Field soils from the 'back-dune' areas all produced heavy mycorrhizal infection in R. ponticum seedlings (Table 3.3.6.1). The other field soils produced little or no infection. Root development on the 'fore-dune' soils was poor (Table 3.3.6.1) and any infection would have been difficult to detect. On the 'main-dune' soils roots developed well but infection was either absent or only light.

Table 3.3.6.1

	No added endophyte		Added endophyte	
Pot:	1	2	1	2
Soil				
1. Fore-Dune	-	-	-!	-!
	Roots stunted			
2. Fore-Dune	-	-	-!	-!
(Top of Ridge)	Roots stunted			
	but less than 1.			
3. Main-Dune (Crest)	-	(+)	(+)	(+)
4. Main-Dune	(+)	(+)	+	(+)
(Base, Landward)	Level of infection variable. Some roots stunted but			
	others of quite healthy appearance.			
5. Main-Dune/Slack	-	-	++	+
6. Fore-Dune, South	-!	-!	-!	-!
7. Main-Dune, South	-	-	+	+
8. Back-Dune (Grass)	++	++	++	++
9. Back-Dune	++	++	++	++
(Calluna)				
10. Back-Dune	++	++	++	++
(Rhododendron)				

Key: - : no visible infection

! : heavy infection of root by non-mycorrhizal fungi and bacteria. Roots often more or less stunted and distorted.

(+) : light mycorrhizal infection

\+ : moderate amount of mycorrhizal infection

++ : heavy mycorrhizal infection

The pH of each of the samples is presented in Table 3.3.6.2. The high values for all the fore-dune samples was probably responsible for the poor root development of the seedlings. All the other soils were acidic, values ranging from 5.50 (Main-dune base, Landward) to 3.40 (Back-Dune, Rhododendron).

On the more acidic, 'main-dune' soils which did not produce mycorrhizal infection (Table 3.3.6.1), the addition of endophyte did result in *R. ponticum* seedlings developing mycorrhizas.

Table 3.3.6.2 Acidity of soil samples

Soil	pH measured in distilled water after 24 hours
1. Fore-Dune	7.10
2. Fore-Dune, Top of Ridge	7.00
3. Main-Dune, Crest	5.30
4. Main-Dune, Base, Landward	5.50
5. Main-Dune, Slack	4.50
6. Fore-Dune, South	6.60
7. Main-Dune, South	3.85
8. Back-Dune, Grass	3.60
9. Back-Dune, *Calluna*	3.50
10. Back-Dune, *Rhododendron*	3.40

3.3.7 OBSERVATIONS ON ASCOCARP FORMATION BY *RHODODENDRON* ENDOPHYTE

3.3.7.1 Introduction

The formation of ascocarps by the endophyte under experimental conditions, is of interest with regard to the potential importance of ascospores in the dispersal of the fungus to new sites in the field.

3.3.7.2 Method

A detailed study of this phenomenon was not undertaken. However, during the course of a number of experiments into either the growth of R. ponticum or other aspects of its competitive ability, ascocarp formation by the mycorrhizal fungus was observed. These observations were collected and are presented below.

3.3.7.3 Results

Ascocarp formation was observed on potted soils containing R. ponticum seedlings (Table 3.3.7.1). This usually followed inoculation with previously isolated and cultured endophyte onto soil partially sterilized by gamma irradiation or 'steam sterilization'. It has also been observed on potted field soil containing R. ponticum seedlings watered with Robbins' solution or with distilled water.

Ascocarp formation occurred from 2-12 months after inoculation on irradiated soil. On untreated soil they formed after about 13 months when watered with Robbins' solution and 14 months with distilled water. On 'steam sterilized' soil they formed after 2½ months (Table 3.3.7.1).

Once fruiting had begun, it was observed to continue freely for at least 3 years. This was the case on both acid, low-nutrient sand (Clumber and Winterton soils) and acidic, organic soil (Cropton soil).

Table 3.3.7.1 Miscellaneous observations of ascocarp formation by *R. ponticum* endophyte

1. R. ponticum seed sown on Winterton Back-Dune soil : 1.6.79
   Addition of Robbins' solution to some pots begun : 15.5.79
   Watering otherwise with distilled water only
   Ascocarps appeared in pots with Robbins' solution : 15.7.80 (c. 13 months)
   Ascocarps appeared in pots with distilled water : 12.8.80 (c. 14 months).

2. Pots of Irradiated Clumber soil planted with non-mycorrhizal R. ponticum : 19.2.80
   (Seed from Clumber, N.Notts. sown on irradiated Cropton soil 20.11.79)
   Inoculated with macerated endophyte : 2.6.80
   Ascocarps appeared on inoculated soils : 4.8.80 (c. 2 months).

3. Pots with Winterton Back-Dune soil and with Clumber soil sown with R. ponticum seed (Clumber, N.Notts.) : 10.4.79.
   Ascocarps developed : 16.1.80 (c. 9 months).

4. Winterton Back-Dune soil was 'Steam Sterilized' at 60°C, 65°C and 70°C and then placed in crystallizing dishes
   Soil treated : 23.1.79
   Seed sown (Clumber, N.Notts.) : 25.1.79
   Germinating well by : 6.2.79
   Ascocarps developing : 21.4.79 (c. 2½ months)
   Present for all soils including the control, exposed only to room-temperature. The number of ascocarps was however lower for the untreated soil and increased with increasing temperature used.

5. Ascocarps took 6-12 months to form following inoculation of R. ponticum on irradiated Cropton soil (Duddridge, J. pers. comm.).

PLATE 8: Ascocarps of *P. ericae*.
(Each c. 1mm diameter).

PLATE 9 : As Pl. 8.

3.3.9 DISCUSSION

Observed mycorrhizal development after 5-6 weeks, agrees with other workers (Gordon, 1937; Duddridge and Read, 1982). Inoculation of seedlings with endophyte culture may speed up infection, depending on the amount applied and the method of application. Presumably four main factors affect formation:-

1. Availability of viable endophyte in the soil.
2. Availability of suitable roots and their proximity to available endophyte.
3. The time taken for mycorrhizas to actually form following infection.
4. Suitability of soil conditions, particularly nutrient and moisture levels.

Physiological effects of mycorrhizas on plant growth are unlikely to be significant during the first 5-6 weeks following exposure of non-mycorrhizal plants to the endophyte. Experimental observations were in agreement with this.

In the preliminary investigation into mycorrhizas and growth, (Experiment I (3.3.3)), it proved difficult to obtain vigorous mycorrhiza formation and only slight or very slight infection occurred in roots on Clumber and Winterton soils after 12 weeks. This was probably due to the presence of excess moisture in the soil. In order to avoid possible drought effects (to which R. ponticum is very susceptible) the plants were well watered. The room temperature however, was rather low (15°C day/10°C night). Moisture loss through evapotranspiration was therefore low. The effects of the cool, moist conditions may have inhibited both plant growth and mycorrhiza formation. Watering was decreased from Harvest 3 and the plastic lids on the pots were removed. Subsequently, increased growth, mycorrhizal establishment and increased relative growth of mycorrhizal plants were observed. The conditions for the mycorrhizal growth experiment II (3.3.4) were modified accordingly.

By Harvest 3 on Clumber soil, there was a decrease in growth of mycorrhizal vs. non-mycorrhizal plants. This could have been due to a drain on the resources of seedlings imposed by establishing mycorrhizas in a low nutrient soil. Such decreases have been observed previously by Stribley and Read, 1976. The trend was reversed at the final harvest.

In Experiment II (3.3.4), mycorrhizas increased yield and Relative Growth Rate (R'), but decreased Root/Shoot Ratio. The exception was the high R' of NMyc. + N at the final harvest. The effects of mycorrhizal infection were modified considerably by the addition of nutrients to the soil. These general observations are in accordance with previous work (Read and Stribley, 1973; Stribley, Read and Hunt, 1975; Read and Jalal, 1980).

R' increased both with nutrient addition and with mycorrhizal infection. Following an increase during early growth and seedling establishment, R' declined over the final period. Comparison of R' values for mycorrhizal and non-mycorrhizal seedlings (both with added nutrients), shows R' to be higher at first for mycorrhizal ones, but this reverses during the last period. This may be an ontogenetic change, the timing of which is affected by infection. The peak value of R' for NMyc. + N plants may be delayed relative to that for Myc. + N plants, due to differences in time taken for an effective root system to establish. In this case the value for NMyc. + N would be expected to fall at subsequent harvests. Whether it would be above or below that of the Myc. + N plants is uncertain. The actual increase in biomass during the final period was higher for NMyc.+ N than for Myc. + N. However, this included a relatively greater root growth by the non-mycorrhizal plants. Shoot growth was virtually the same for both sets of seedlings. At the higher nutrient levels it is possible that mycorrhizal infection aided the rapid development of an effective root system, but later growth was slightly retarded due to a drain imposed by the fungus (nutrients being readily available to both mycorrhizal and non-mycorrhizal plants). The degree of infection was not obviously affected by nutrient addition.

The greater mean dry weight of the mycorrhizal seedlings during the final period would in fact contribute to a decrease in R' relative to the non-mycorrhizal seedlings, even if there was no difference in Absolute Growth Rate.

The changes in Root/Shoot Ratio indicate early development of mycorrhizal roots, followed by rapid shoot growth in mycorrhizal plants. For NMyc.+ N the steady decline in R/S may be due to root establishment taking longer than for the equivalent mycorrhizal plants. The 'take-off' of growth is correspondingly delayed. This agrees with the observed changes in R'. NMyc. - N seedlings showed a rapid increase in R/S from H2 to H3. Roots were establishing slowly and shoot development was poor. By the final harvest, non-mycorrhizal seedlings without nutrient addition, had a relatively high R/S and a low R'. They were considerably smaller than either mycorrhizal seedlings (with or without added nutrients) or non-mycorrhizal seedlings with added nutrients. At the final harvest, mycorrhizal plants had smaller roots and larger shoots than their non-mycorrhizal equivalents.

Pearson and Read (1973) found ericaceous endophyte in soils from sites with no ericaceous vegetation. This is tentatively put forward as part of the explanation for the apparent universal occurrence of mycorrhizas in ericaceous plants in the field, in the absence of the 'cyclic' inoculation of seedlings suggested by Rayner. The suggestion being that ericaceous endophyte is widespread (possibly almost universal in suitable climatic regions) both in soils which support ericaceous vegetation and those which do not. The fungus either persisting in a resistent, dormant form or occurring as a weak saprophyte.

Both the 'non-ericaceous' sites tested by Pearson and Read were associated with moorlands. Presumably ericaceous plants were present relatively close by and may have been present at the sites themselves in times past. Potential means of spread of the fungus are spores and hyphal fragments or cysts. These might be dispersed by wind or animals, either on soil particles or independently. The possible importance of these depends on the ability of the fungus to sporulate in the field, the saprophytic ability of the free-living fungus and the longevity of hyphal fragments or spores. None of these is yet known for certain.

Cooke (1977) cites Gordon (1937) as having found endophyte in non-ericaceous soil. However, the soil referred to was 'potting soil' and no details of its actual source of history were given. In order to be certain that no possible contamination of samples by endophyte from other

sources (trowels, clothing, pots, etc.) has occurred, strict precautions need to be taken in sampling technique. When this has been the case (3.3.5 and 3.3.6) a somewhat different picture emerges. Endophyte does not appear to be ubiquitous, but does occur in soils from all ericaceous sites and nearby non-ericaceous ones. Even at a potentially very mobile system such as Winterton Dunes, endophyte was not found in all apparently suitable soils. Wind-blown soil particles would be expected to disperse the fungus throughout the dune system. The presence of viable endophyte might therefore have been predicted from all the soil samples in which healthy root development occurred. This was not the case.

Experimental work on heat-treated or 'steam-sterilized' soil and endophyte in liquid culture (see Appendix 1) suggests the occurrence of a resistent form of the fungus in the soil. Heating to 80-90°C for 60 minutes was required to eliminate viable endophyte from soil samples. In liquid culture, endophyte was destroyed by 50°C for 5 minutes or more, or 70°C for less than 1 minute. Such a resistent form of endophyte in the soil could be the agent of dispersal to new sites, or of maintenance at existing sites currently without ericaceous vegetation.

The possible importance of sporulation as a means of dispersal is difficult to assess. Fruiting usually occurs following inoculation of non-mycorrhizal seedlings in partially sterilized soil, with previously isolated and cultured endophyte. Once sporulation occurs, infection of any soils in the vicinity is usual. Such infected, mycorrhizal plants also produce ascocarps quite readily. All cases of fruiting on non-treated soils must be interpreted cautiously due to the possibility of contamination by spores from the aforementioned source. The initiation of fruiting may be stimulated either by the isolation/culturing procedure for the fungus, or by conditions in the medium. The latter seems most likely. (Formation of ascocarps by endophyte cultured on Melin's agar in the absence of any host plant, or other influences, has been reported by Vegh, I., Fabre, E. and Gianinazzi-Pearson, V., 1979.) If this is the case, then there seems little reason to suppose that this phenomenon does not occur in the field. The difficulty is perhaps in forecasting the conditions required and then finding the ascocarps. A quite extensive search was carried out, but none were found. The ascocarps are very small (c. 0.1-0.5mm diameter) and therefore inconspicuous. The formation of

ascocarps in the field would solve the dilemma of the apparently universal infection by mycorrhizas of even isolated ericaceous plants in 'natural' vegetation.

The importance of mycorrhizal infection to R. ponticum invading free-draining, nutrient-poor, acid soils is clearly indicated by the experimental results. Infection led to increased Relative Growth Rate, decreased Root/Shoot Ratio and an overall increase in dry matter production sustained over the whole experimental period. Non-mycorrhizal plants without added nutrients grow badly with a low Relative Growth Rate and a High Root/Shoot ratio. Inoculation with endophyte appeared to encourage rapid development of an effective root system, subsequently reflected by increased shoot and total growth. The effects of mycorrhizal infection were apparent at the second harvest (six weeks post-inoculation) as increased root biomass. Shoot growth at this stage was little altered. By the third harvest (twelve weeks post-inoculation) shoot dry weight of mycorrhizal plants was higher than that of non-mycorrhizal ones, significantly so when there was no nutrient addition. R. ponticum seedlings invading an area of vegetation may benefit considerably from mycorrhizal infection within the first 6-12 weeks of growth. This is providing that edaphic conditions are suitable and endophyte is available for inoculation.

The presence of viable endophyte in the soil of a site being invaded is extremely important if the seedlings are to benefit from mycorrhizas. Without exception, all R. ponticum plants collected in the field and later examined for the presence of mycorrhizas were infected. How the fungus dispersed to new areas is not clear, although some form of resistent propagule or possibly an ascospore could be the agent.

The possession of mycorrhizas will considerably enhace the competitive ability of R. ponticum. This will firstly aid the establishment of young plants in competition with herbaceous vegetation and possibly with trees or with other shrubs. Established plants under suitable conditions grow vigorously to form a dense and often dominating shrub layer. The increase in growth, perhaps through more effective uptake of nitrogen and phosphorus, is undoubtedly a major factor in enabling the domination and elimination of competitors to arise and to be maintained.

CHAPTER 4

THE POSSIBLE IMPORTANCE OF ALLELOPATHY IN THE ECOLOGY OF *RHODODENDRON PONTICUM*

4.1.1 INTRODUCTION

The capacity of ericaceous plants to dominate heathland vegetation often to the complete exclusion of other species has received considerable attention (Watt, 1955; Gimingham, 1960, 1972). Competition for mineral nutrients and light has usually been cited as the key reason for the success of the dominant plant. Recent work by Read et al (various), has demonstrated the importance of mycorrhizal infection in the successful competition for nitrogen and phosphorus in heathland soils poor in mineral nutrients. However, some observations suggest that even together with competition for other environmental factors (such as light, water and space) these attributes may not provide a complete explanation for the dominance in all situations. Investigations into the stunting of tree growth in areas of vigorous Calluna (known as 'Calluna-check') have suggested the involvement of phytotoxins and fungitoxins. These might be produced by raw humus from Calluna (Handley, 1963; McVean, 1963) or by living Calluna roots (Robinson, 1971, 1972). Roff (1964) examined Calluna - heathland in the East Anglian Brecklands with particular reference to 'bare zones' or 'interference zones' around mature heather bushes. According to Roff, the lack of a complete cover of vascular plants on the Breckland grass and Calluna heaths and in particular, the presence of bare ground vegetated only by lichens and bryophytes around bushes of Calluna and Rhododendron, is exceptional in Britain. Such paucity of cover may be expected in situations of extreme environmental pressures or disturbed ground, but is not usual in relatively stable systems. 'Interference zones' around Calluna bushes were observed by Roff in the Brecklands, at Winterton National Nature Reserve in Norfolk and at one site in each of Sussex and Devon.

Roff uses 'interference' to describe the influence of Calluna on other plants, manifesting itself as a considerable decrease in their cover within a certain distance of the bushes. This is a useful concept since it avoids the problems and assumptions associated with describing the

phenomenon as 'allelopathy'. Muller (1969) suggested the use of the term 'interference' to refer to the overall influence of one plant on another, thus encompassing both allelopathy and competition.

The 'interference zone' around Calluna or Rhododendron bushes shows a lower cover and density of angiosperms over a belt of variable width, up to about 0.50 m, from the bush perimeter. Very small bushes show no such zone around them. Although there is no cover of the interference zone by the bush canopy, Calluna and Rhododendron roots penetrate this band as a dense mat, well beyond the lateral projection of the crown. The interference zone is poor in terms of vascular plant cover, but is usually carpeted by lichens and bryophytes.

Between two or more Calluna or Rhododendron bushes, the interference zones may coalesce to form continuous areas more or less devoid of competing vascular plants. In the area of invasive Rhododendron at Winterton, patches of such bare ground exist totally enclosed by vigorously growing Rhododendron. These areas show no signs of activity by rabbits which might provide alternative explanations for the paucity of vascular plant cover.

Roff concluded that the suppression of growth of competing plants in the close vicinity of vigorously growing heather bushes, was not due to competition for nutrients or water. He suggested the lower yield of competing species such as Festuca ovina was caused by an inhibitory property of soils long occupied by Calluna roots. The roots or mycorrhizas of Calluna perhaps producing a substance which inhibits the growth of Festuca roots or mycorrhizas.

Nearby Calluna - heathland at Winterton has numerous examples of similar bare-zones around bushes of Calluna (Roff, 1964) and Rhododendron (Cross, 1973). The reasons suggested by Cross were either competition for water by Rhododendron causing reduced growth of competitors, the release of phytotoxins by Rhododendron, or possibly increased grazing by rabbits around the bushes. Roff (1964) showed that competition for water was not the controlling factor in the case of Calluna bare-zones. If, as seems likely, the phenomenon is the same in the case of both Calluna and Rhododendron, then competition for water may be ruled out for Rhododendron

also. Roff commented on the possibility that bare-zones are a result of rabbit activity. The interference phenomenon was not observed in the Brecklands until the disappearance of rabbits due to myxomatosis in the 1950's. Strong grazing by rabbits had previously restricted the size of Calluna bushes. With the departure of the rabbits, the bushes increased rapidly in size and the interference zones appeared. The main spread of invasive Rhododendron at Winterton has been linked to the same decline of rabbits during the 1950's (Fuller and Boorman, 1977). It seems likely that the interference zones may also have appeared for the same reasons. Areas of bare ground caused by rabbits in these vegetation systems are very distinct, with scratching and destruction of the lichen carpet and are quite different from the changes associated with interference zones.

Cross (1973) also suggests that Rhododendron may have a deleterious effect on Ilex through competition for nutrients, for water, or by the production of phytotoxins. Removal of Rhododendron from around Ilex trees apparently suffering such adverse effects, resulted in the sprouting of new shoots from the trunks. He believed this to be due to either increased light or the removal of growth inhibitors released from living Rhododendron leaves.

When conditions are suitable, Rhododendron forms dense, vigorous stands virtually devoid of herbaceous plants or tree/shrub saplings. Authors such as Cross (1973) have attributed this to competition for nutrients or water, shading or the release of phytotoxins. Chou and Muller (1972) state that pure stands of any long-lived species are highly suggestive of chemical dominance. The dense thickets formed by many species of the Ericaceae (such as R. ponticum) perhaps share with Arctostaphylos glandulosa var. zacaensis, a chemical basis to their strong dominance. A considerable amount of work in recent years has implied 'allelopathic' interactions between ericaceous and other plants (Handley, 1963; McVean, 1963; Chou & Muller, 1972; Robinson, 1970, 1972; Ballester, Albo & Vieitez, 1977; Carballeira, 1980; Carballeira & Cuervo, 1980; Read and Jalal, 1980; Jalal and Read, 1983 I & II).

The term 'allelopathy' was used by Molisch in 1937, referring to biochemical interactions between all types of plants including microorganisms. This included both detrimental and beneficial interactions. Detrimental, supposedly allelopathic effects have been

observed since the time of Pliny (A.D. 23-79), in the case of the black walnut (Juglans nigra). In Japan around 300 years ago Banzan Kumazawa recorded the detrimental effects of rain or dew washing off the leaves of red pine (Pinus densiflora), on crops growing below. In 1832, De Candolle suggested that some plants excrete chemicals from their roots that are harmful to other plants. Since then, there has been increasing interest with research and reviews such as Pickering (1917, 1919), Moblisch (1937), Bonner (1950), Grummer (1955), Borner (1960), Evenari (1961), Whittaker (1969), Tukey (1969), Rice (1974, 1979) and Stowe (1979). During the late 1970's there was an almost exponential increase in the number of papers dealing with allelopathy (Rice, 1979).

Allelopathy is still a rather controversial subject. Many reported instances of allelopathic interactions may be open to alternative interpretations. There are considerable problems of methodology and the interpretation of the results of a variety of bioassay techniques in relation to real field situations (Stowe, 1979).

Muller & Chou (1972) place allelopathy as one of several basic ecological processes acting as major factors in the environmental complex. The chemical influence of one plant on its neighbours acts alongside the traditionally recognized environmental components such as light, temperature, moisture and inorganic nutrients, and associated competitive interaction for these. Despite the problems of interpretation of bioassay results and of demonstrating allelopathic interactions satisfactorily in the field, a large amount of evidence in its favour has been amassed (McPherson, Chou & Muller, 1971; Chou & Muller, 1972; Newman & Rovira, 1975; Newman & Miller, 1977; Harborne, 1982; Jalal & Read, 1983 I & II).

Many examples of suspected allelopathic interactions have involved phenolic compounds acting as phytotoxins (DeBell, 1969; McPherson, Chou & Muller, 1971; Chou & Muller, 1972; Ballester, Albo & Vieitez, 1977; Carballeira, 1980; Carballeira & Cuervo, 1980; Jalal & Read, 1983 I & II). Usually the phenolics have been at least partly identified and quantified. They are not necessarily considered to be the total or even the major cause of toxicity (McPherson, Chou & Muller, 1971; Jalal & Read, 1983 I & II).

Ericaceous plants such as Calluna and Rhododendron are rich in phenolic compounds (Cross, 1975; Read & Jalal, 1980). Examples of suspected allelopathy by members of the Ericaceae (as already noted) have generally implicated the involvement of phenolics as phytotoxins.

To examine the possible importance of allelopathy or interference in the ecology of R. ponticum, a number of investigations were carried out:-

1. Examination of the 'interference' phenomenon
   a) In the field
   b) In controlled pot experiments under greenhouse or growth-room conditions.

2. Qualitative and quantitative examination of the simple phenolics found in R. ponticum tissues, soil and canopy throughfall.

3. Investigations into various other aspects of the apparent toxicity, such as regeneration at sites totally or partly cleared of dominant R. ponticum.

The work described in this chapter relates mainly to No. 1.

4.1.2 SUMMARY OF THE FIELD OBSERVATIONS AND EXPERIMENTAL WORK (4.2, 4.3, 4.4 & 4.5) PRESENTED IN CHAPTER 4

4.2 A qualitative comparison of vegetation in Wintergon grassland and Winterton *Rhododendron* interference zone

Vegetation of the stabilized 'back-dune' grassland was described and is presented with a comparable description for interference zone vegetation.

4.3 Investigation into residual toxicity of *Rhododendron* soil and litter

Two investigations (Experiments 1 and 2) are presented concerning the growth of test seedlings on field soil or litter from under R. ponticum or nearby vegetation.

4.4 Investigation into interference caused by *Rhododendron* under artificial conditions

This section consists of four studies involving artificially created 'interference zones' (Experiments 3, 4, 5 & 6). The effects of live R. ponticum, dead roots and mycorrhizal status on the growth of test seedlings were investigated.

4.5 Investigation of the interference phenomenon in the field

Aspects of interference investigated in 4.3 and 4.4 were studied under field conditions and the results are presented.

Note: Basic data and statistical analysis from this chapter are presented in Appendix 7.

As in Chapter 3, Experiment 2, primary analysis of data was by Student's t-test, but anovar was also carried out on all relevant occasions. These results are presented in Appendix 7.

4.2 A QUALITATIVE COMPARISON OF VEGETATION IN WINTERTON GRASSLAND AND WINTERTON *RHODODENDRON* INTERFERENCE ZONE

The grassland vegetation was growing on stabilized 'back-dune' sand, approximately 100 m inland from the main-dune system. The sand produces a nutrient poor, acidic soil (pH 3.60). The vegetation is a species-poor, acid heath-grassland with the following being the main plant species present:-

Galium saxatile
Rumex acetosa
Rumex acetosella
Luzula campestris
Deschampsia flexuosa
Festuca ovina
Agrostis canina montana
Carex arenaria
Rhododendron ponticum
Calluna vulgaris
Betula sp.
Dicranum scoparium
Polytrichum juniperum
Hypnum cupressiforme var. ericetorum

The vegetation within 0.5 m of a large vigorously growing R. ponticum bush was described. There was no obvious sign of the activity of rabbits directly affecting this vegetation. The only higher plant species found was Carex arenaria present as occasional individuals. The characteristic vegetation was a lichen/bryophyte carpet with the following species:-

Cladonia squamosa
Cladonia chlorophaea
Cladonia fimbriata
Cladonia impexa
Cladonia furcata
Cladonia macilenta
Dicranum scoparium
Polytrichum piliferum

PLATE 10 : Typical enclosed 'bare-zone' at Winterton, Norfolk.

4.3 INVESTIGATION INTO RESIDUAL TOXICITY OF *RHODODENDRON* SOIL AND LITTER EXPERIMENTS 1a, 1b, 2a, 2b, 2c, 2d AND 2e

4.3.1 INTRODUCTION

Two investigations (Experiments 1 and 2) were carried out with field soil/litter from under vigorously growing Rhododendron and from adjacent grassland. Some samples were sieved to remove root material (-R), others were unsieved (+R). Bioassays were done using seedlings of Festuca ovina, Rumex acetosa and Rhododendron ponticum. Some were watered with distilled water, others with nutrient solution (+N).

The study was to see whether the growth of test species on Rhododendron soil, litter or interference zone soil (referred to as bare-zone soil, Rh.bz), was reduced in comparison to that on soil or litter from adjacent grassland. The effects of removing Rhododendron roots from the soil, or adding nutrients were also investigated.

Relatively unfragmented R. ponticum litter without roots was taken from the upper litter layer at Strawberry Lee Plantation for Experiment 1. Litter used for Experiment 2 was from the same site but lower down the soil profile. This was well fragmented and permeated by mycorrhizal Rhododendron roots.

4.3.2 METHOD

Soils and associated litter were collected from under R. ponticum and adjacent grassland at the following sites:- Stand Wood (SW.), Chatsworth, North Derbyshire; Strawberry Lee Plantation (S.L.P.), South Yorkshire; Cordwell (Co.), North Derbyshire; Clumber (Cl.), North Nottinghamshire and Winterton (W.), Norfolk. The geological series from which these soils are derived, are:- Millstone Grit (S.W. and S.L.P.), Coal Measures (Co.), Bunter Sandstone (CL.) and a rather acidic, stabilized dune-sand (W.).

Soils were either sieved (-R) or left unsieved (+R) and placed in 2½ inch diameter plastic pots with clear plastic lids. The pots were watered with distilled water or full-strength Robbins' solution (+N). They were either sown with seed of the test species (Experiment 1) or planted with freshly

germinated seedlings (Experiment 2). In Experiment 1 two additional pots were set up for each soil and sown with R. ponticum seed. At the end of the experiment two seedlings were taken from each pot and examined for mycorrhizas.

The pots were placed in a growth-room with a 20°C/16 hour day and a 15°C/ 8 hour night.

The test species seedlings were harvested after 70 days (Experiment 1) and 49 days (Experiment 2).

Table 4.3.2.1 Sites and sample types

Experiment 1a	S.L.P.	Rhododendron soil	(Rh.S)
	"	" coarse litter	(Rh.L)
	"	grass soil	(GR)
1b	S.W.	Rhododendron soil	(Rh.S)
	"	grass soil	(GR)
2a	W.	Rhododendron soil	(Rh.S)
	"	" litter	(Rh.L)
	"	" bare-zone soil	(Rh.bz)
	"	grassland soil	(GR)
	"	Calluna soil	(Ca.S)
	"	" bare-zone soil	(Ca.bz)
2b	S.L.P.	Rhododendron soil	(Rh.S)
	"	" lower litter	(Rh.L)
	"	grass soil	(GR)
2c	S.W.	Rhododendron soil	(Rh.S)
	"	" litter	(Rh.L)
	"	grass coarse soil	(GR1)
	"	" " lower soil	(GR1 1)
	"	" fine soil	(GR2)
	"	" " lower soil	(GR2 1)
2d	Cl.	Rhododendron soil	(Rh.S)
	"	" lower soil	(Rh.S1)
	"	grassland soil	(GR)
	"	" lower soil	(GR1)

Experiment 2e	Co.	Rhododendron	(1)	soil	(Rh.S1)
	"	"	(1)	lower soil	(Rh.S1 l)
	"	"	(1)	litter	(Rh.L1)
	"	"	(2)	soil	(Rh.S2)
	"	"	(2)	lower soil	(Rh.S2 l)
	"	"	(2)	litter	(Rh.L2)
	"	grassland soil			(GR)
	"	"	lower soil		(GRl)
	"	Pteridium soil			(Pt.S)
	"	"	litter		(Pt.L)

Table 4.3.2.2 Key to abbreviations used

s	:	soil sieved to remove root material
l	:	lower horizon of soil or litter
Rh.	:	Rhododendron ponticum
Ca.	:	*Calluna vulgaris*
Pt.	:	Pteridium aquilinum
GR	:	grass/grassland
bz	:	bare-zone (or interference zone)
+R	:	unsieved soil
-R	:	sieved soil (roots removed)
+N	:	watered with Robbins' solution to add nutrients
R	:	root)
S	:	shoot) yield of test seedings as dry
T	:	total (root + shoot)) weight

4.3.3 RESULTS

4.3.3.1 Experiments 1a and 1b

4.3.3.1.1 Experiment 1a (Strawberry Lee Plantation)

There was a highly significant reduction in growth of both F. ovina and R. acetosa on Rhododendron soil compared to that on grass soil (Figures 4.1 and 4.2). Coarse Rhododendron litter significantly increased yield of F. ovina over that on the grass control.

R. ponticum root growth was significantly reduced on both Rhododendron soil and litter below that on the grass control (Figure 4.3). Shoot growth of R. ponticum on Rhododendron litter was significantly increased.

4.3.3.1.2 Experiment 1b (Stand Wood)

There were no significant differences in yield between test seedlings on Rhododendron and grass soils (Figure 4.4).

All the soils and litter were low in available nitrogen (Table 4.3.3.1), maximum levels being in S.L.P. GR. and S.L.P. Rh.L. Ammonium was the main form of available nitrogen. No detectable amounts of available phosphorus were found in any of the soils.

As Experiment 1a included 3 variables, the data have also been subjected to anovar. The results are given in Appendix 7.

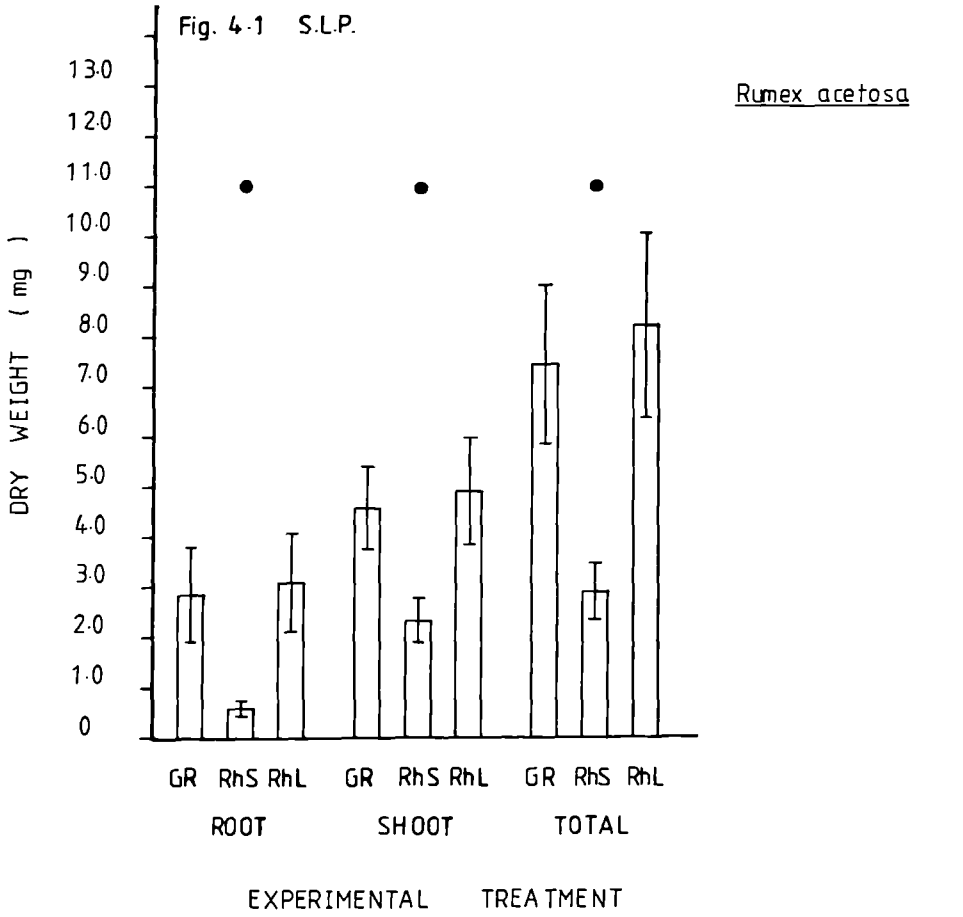
KEY :-

Results tested with Student's t-test, differences significant at p = 5 % or less. ●

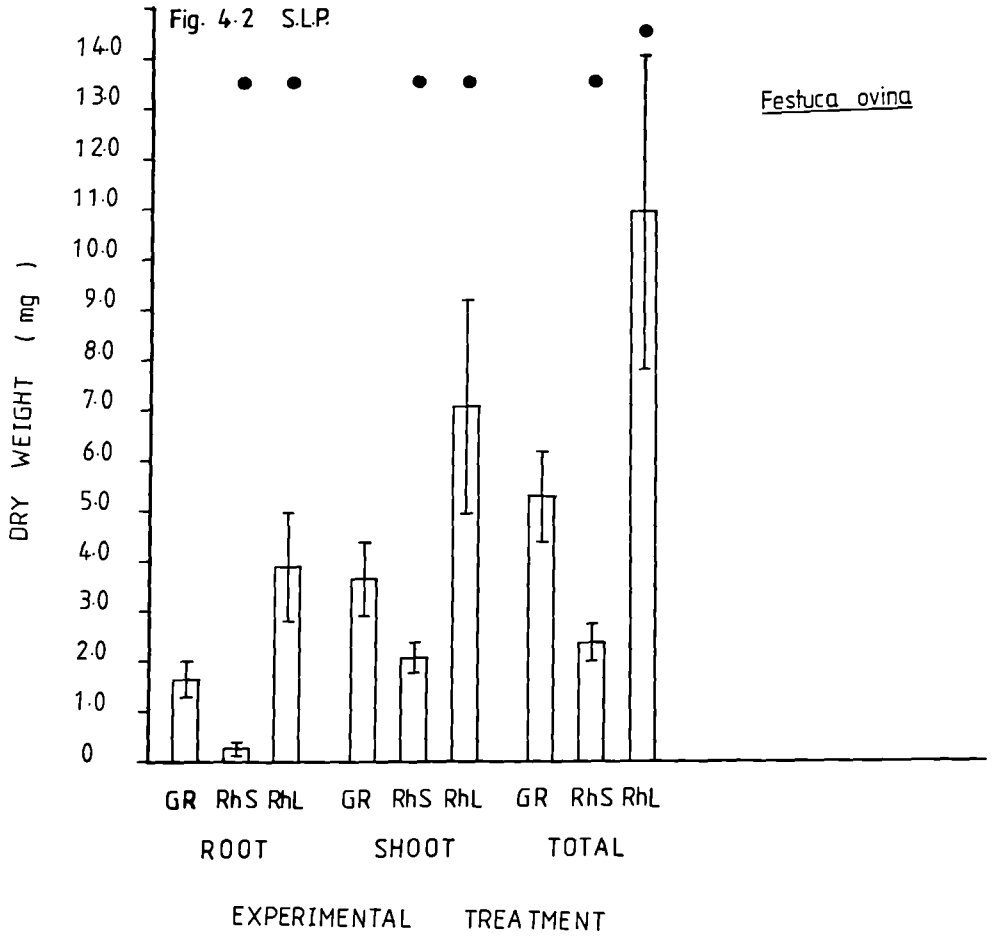
Controls taken as grass soil without added nutrients.

SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Fig. 4.1 S.L.P.



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

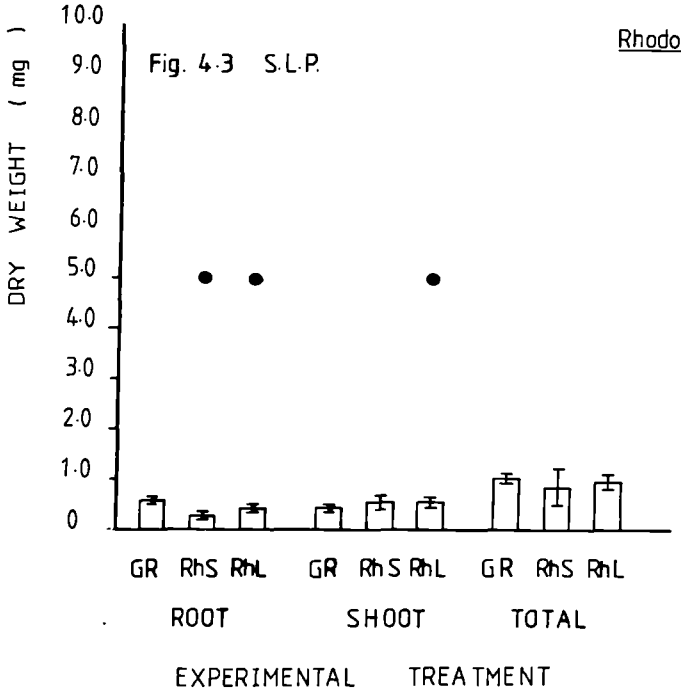


Fig. 4·3 S.L.P.

Rhododendron ponticum

SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

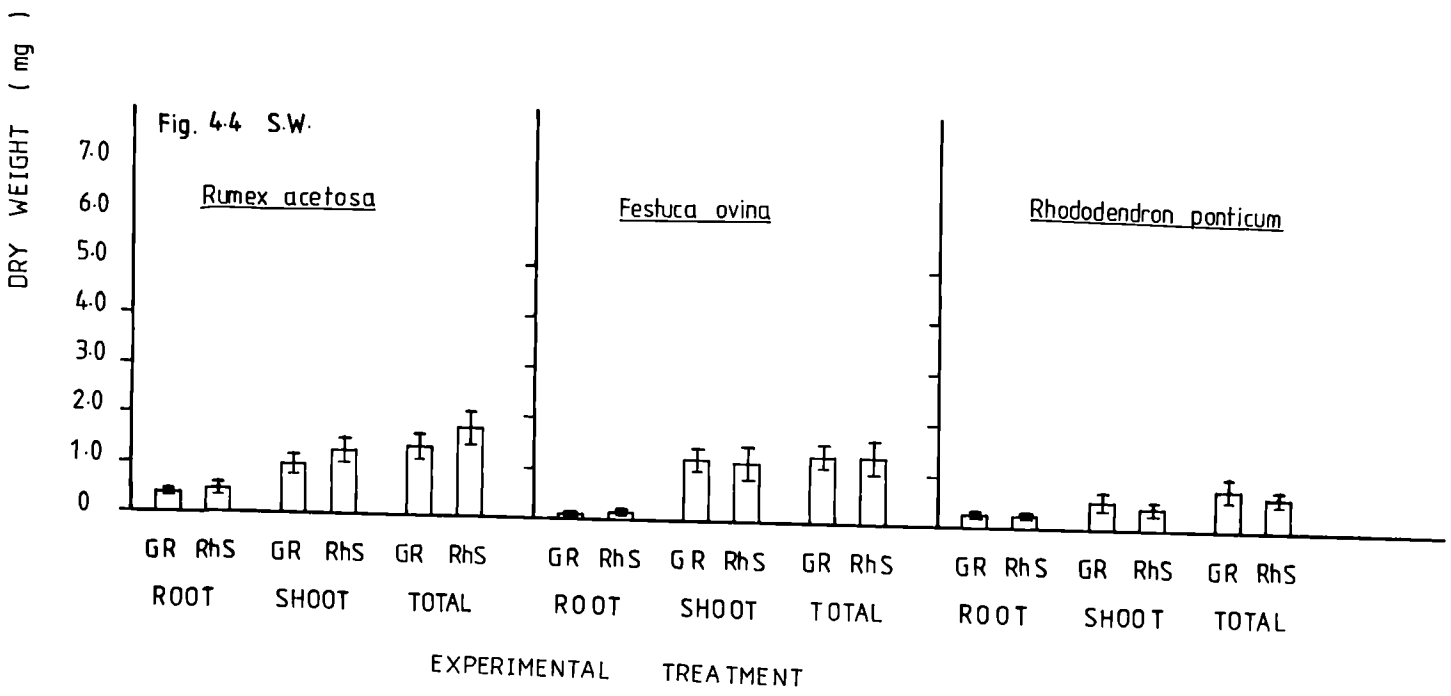


Fig. 4.4 S.W.

Table 4.3.3.1 Available nitrogen and phosphorus content of soils

(all values in ppm.)

Soil	Av.P.	Av. Ammonium-N	Av. Nitrate-N	Total Av.N
S.L.P. GR.	-	11.5	2.5	14.0
S.L.P. Rh.S.	-	5.0	2.3	7.3
S.L.P. Rh.L.	-	13.1	3.0	16.1
S.W. GR.	-	4.0	-	4.0
S.W. Rh.S.	-	4.1	-	4.1

Table 4.3.3.2 Mycorrhizal status of *R. ponticum* seedlings

S.L.P. GR : All four seedlings lightly infected.

S.L.P. Rh.S : All four seedlings heavily infected.

S.L.P. Rh.L : Three seedlings heavily infected, one lightly infected.

S.W. GR : Two seedlings heavily infected, two lightly.

S.W. Rh.S : All four seedlings heavily infected.

All of the soils produced heavily mycorrhizal roots in at least two of the four R. ponticum seedlings examined.

4.3.3.2 Experiments 2a, 2b, 2c, 2d and 2e

Those differences between dry weight yields of the controls on grass soils and test seedlings in other treatments which are significant at the 95% level are shown on Figures 4.5 - 4.26. As experiments 2a, 2b, 2c and 2d included more than 2 variables each, the data have also been subjected to anovar. The results are presented in Appendix 7.

4.3.3.2.1 Effects on *Festuca ovina* (Figurès 4.5 - 4.15)

4.3.3.2.1.1 Experiment 2a (Winterton) (Figures 4.5 and 4.6)

Rhododendron litter produced a significant increase in R and T. With added nutrients the increase was significant for R, S and T. Rhododendron soil significantly decreased R. Sieving of Rhododendron soil resulted in a significant increase in R and T. Addition of nutrients to sieved Rhododendron soil produced a significant increase in S and T. Calluna soil with added nutrients caused a significant increase in S and T.

4.3.3.2.1.2 Experiment 2b (Strawberry Lee Plantation) (Figures 4.7 and 4.8)

Nutrient addition significantly increased seedling dry weight over that of the controls. All Rhododendron soil and litter treatments led to decreased R and T. With the exception of sieved Rhododendron litter, S was also reduced. Significant decreases in R were produced by all Rhododendron litter treatments, Rhododendron soil with added nutrients and sieved Rhododendron soil. Sieved and unsieved Rhododendron woil both gave significantly reduced S. Rhododendron soil with added nutrients, sieved Rhododendron litter with added nutrients and Rhododendron soil all significantly reduced T.

4.3.3.2.1.3 Experiment 2c (Clumber) (Figures 4.9 and 4.10)

All Rhododendron soils produced significant decreases in R and T compared to the controls. All produced decreased S, significant except for Rhododendron lower soil. Nutrient addition did not eliminate the effect and in fact led to a significant decrease of R on the grass lower soil compared to the same without addition.

4.3.3.2.1.4 Experiment 2d (Stand Wood) (Figures 4.11 and 4.12)

All Rhododendron soils and litter, with all treatments, produced highly significant decreases in R, S and T. These effects were not removed by nutrient addition.

4.3.3.2.1.5 Experiment 2e (Cordwell) (Figures 4.13, 4.14 and 4.15)

Pteridium soil and litter caused significant reductions in R. The effect was removed by addition of nutrients for sieved litter, but not for soil. Rhododendron soil and litter samples showed no obvious trends. All Rhododendron (1) soil samples (all treatments, with or without nutrient addition) produced significant reductions in R.

4.3.3.2.2 Effects on *Rumex acetosa* (Figures 4.16 - 4.26)

4.3.3.2.2.1 Experiment 2a (Winterton) (Figures 4.16 and 4.17)

Again, Rhododendron litter resulted in increased R, S and T. These increases were highly significant when nutrients were added. In contrast, the Rhododendron soil caused decreased yield which was highly significant when no nutrients were added. With addition of nutrients the effect was diminished, but the reduction in R was still significant. Sieved Rhododendron soil produced significantly reduced R and T, though with nutrient addition this was no longer significant.

Soil from Rhododendron bare-zones gave significant decreases in R, S and T. These reductions were still significant with nutrient addition.

Calluna soil with added nutrients or when sieved, resulted in significantly decreased R. Sieved Calluna soil also caused a significant reduction in S and T.

4.3.3.2.2.2 Experiment 2b (Strawberry Lee Plantation) (Figures 4.18 & 4.19)

Nutrient addition significantly increased yield on grass soil above that of the controls. All Rhododendron soil and litter significantly reduced R, S and T and sieving and/or nutrient addition did not eliminate the effects.

4.3.3.2.2.3 Experiment 2c (Clumber) (Figures 4.20 and 4.21)

All Rhododendron soils caused highly significant reductions in seedling growth (R, S and T) compared to the controls. The effect was not removed by nutrient addition.

4.3.3.2.2.4 Experiment 2d (Stand Wood) (Figures 4.22 and 4.23)

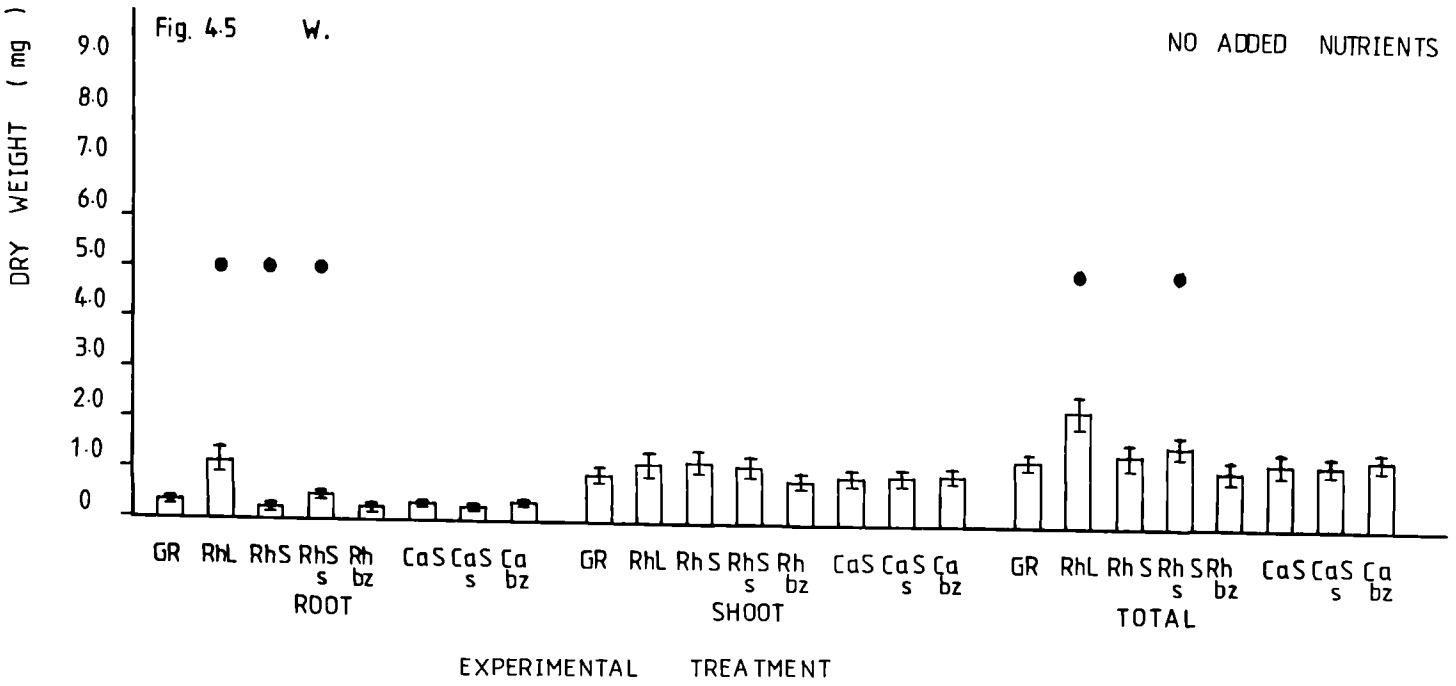
Adding nutrients significantly increased R and T on the control soil. Again, all Rhododendron soils and litter gave highly significant reductions in R, S and T. This was not eliminated by nutrient addition.

4.3.3.2.2.5 Experiment 2e (Cordwell) (Figures 4.24, 4.25 and 4.26)

Pteridium soil and litter, Rhododendron (1) soil and Rhododendron (2) litter produced highly significant decreases in R, S and T. These effects were not removed by sieving or by nutrient addition. Rhododendron (1) litter reduced seedling growth (significant for S and T) but this effect was eliminated by adding nutrients. Rhododendron (2) soil led to a reduction in R, S and T but this was only significant for R and T. The addition of nutrients did not remove the effect.

SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

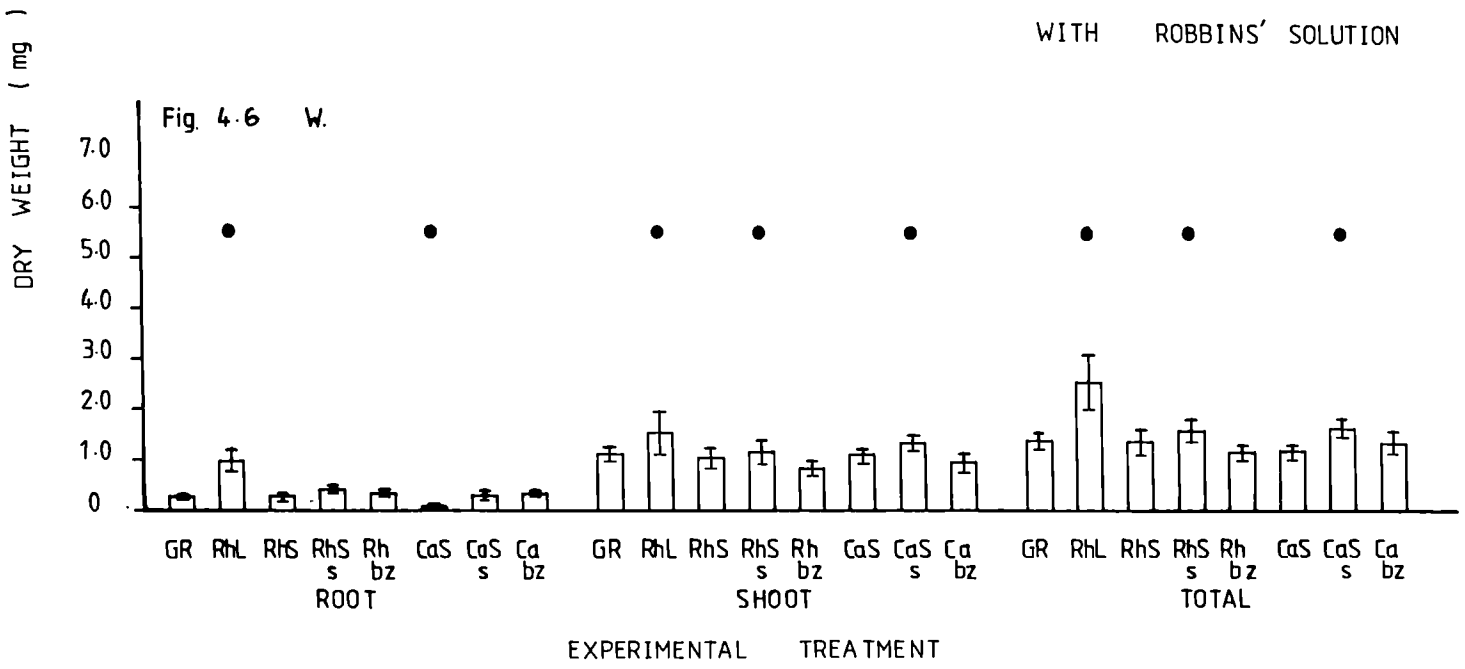
Festuca ovina



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Festuca ovina

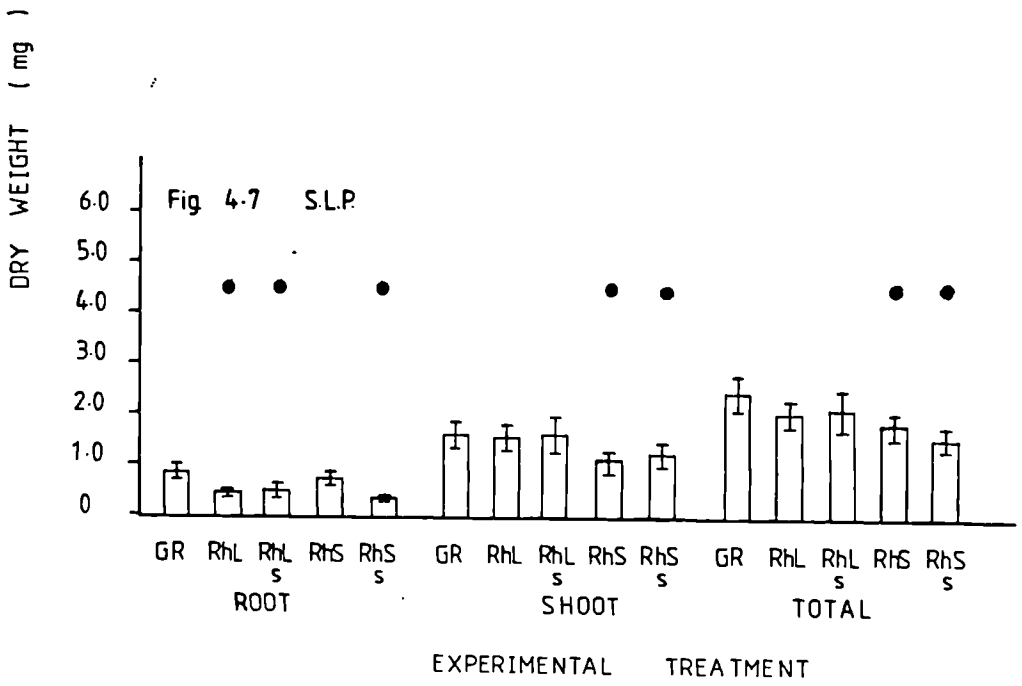
WITH ROBBINS' SOLUTION



SEEDLING MEAN DRY WEIGHT . WITH 95% CONFIDENCE LIMITS

Festuca ovina

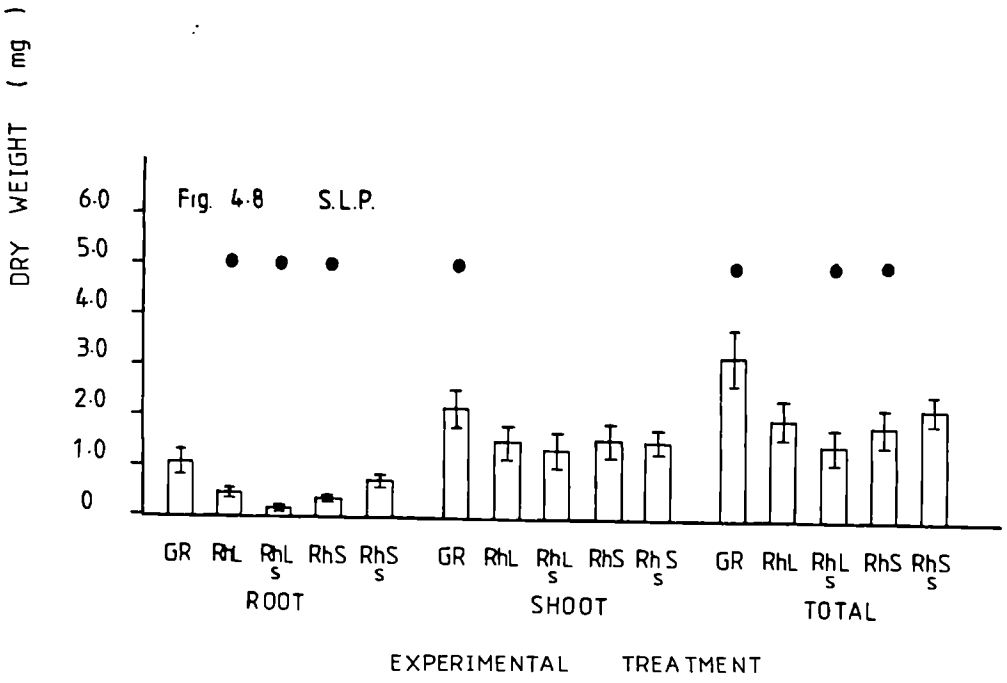
NO ADDED NUTRIENTS



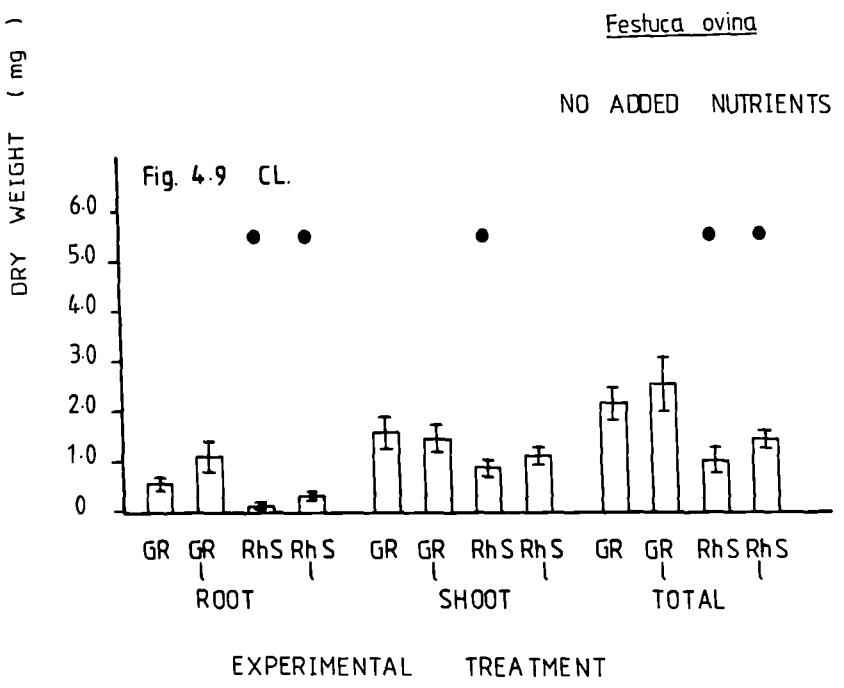
Festuca ovina

SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

WITH ROBBINS' SOLUTION



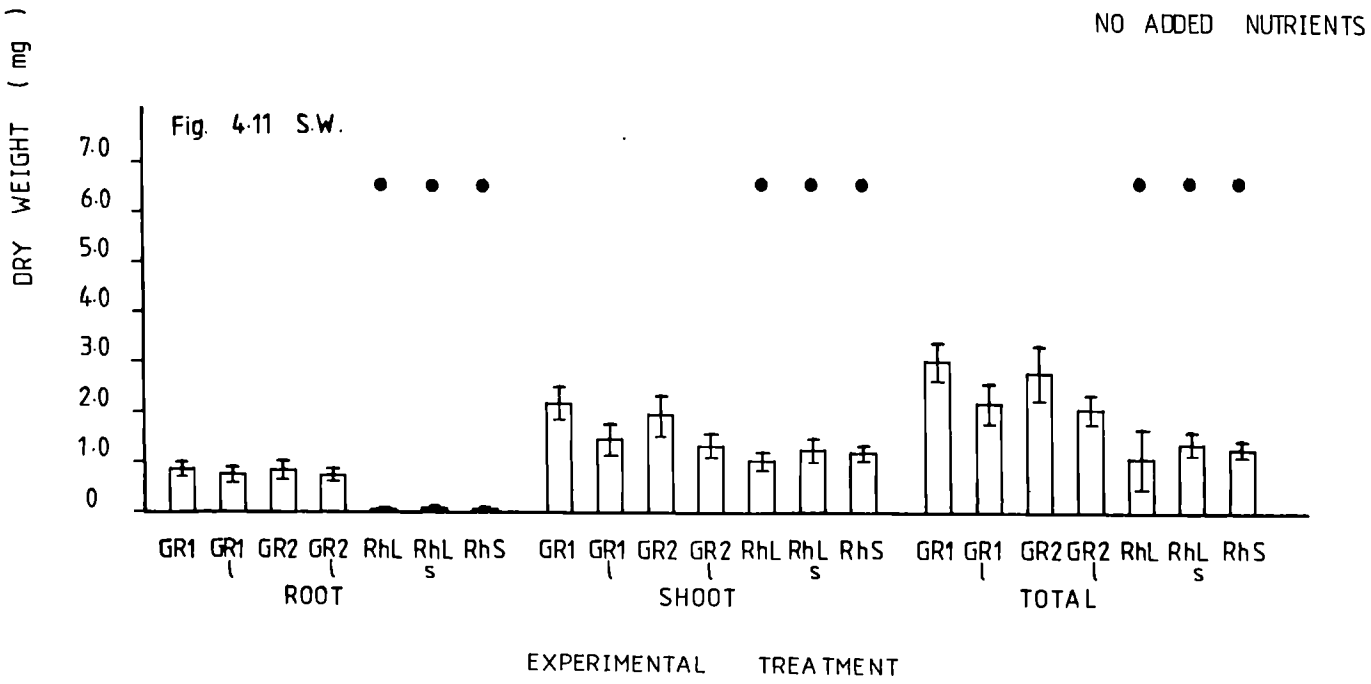
SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Festuca ovina

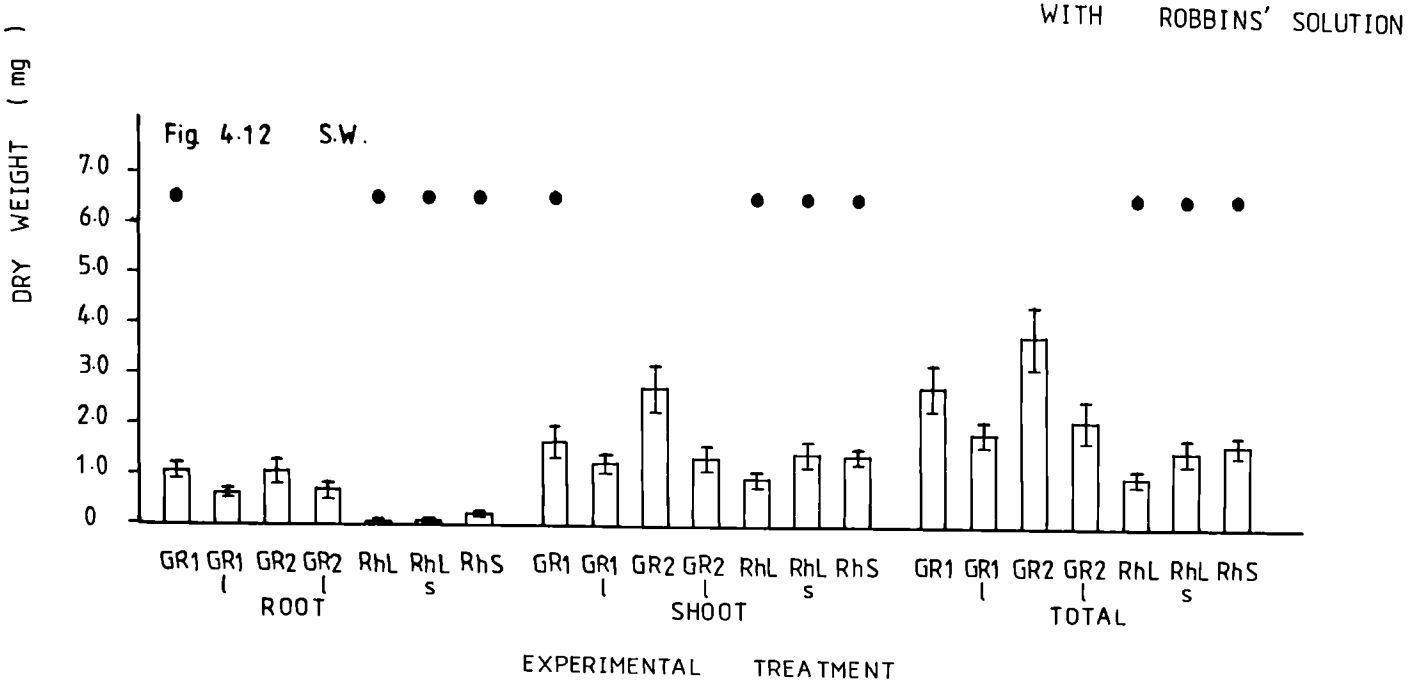
NO ADDED NUTRIENTS



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Festuca ovina

WITH ROBBINS' SOLUTION



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Festuca ovina

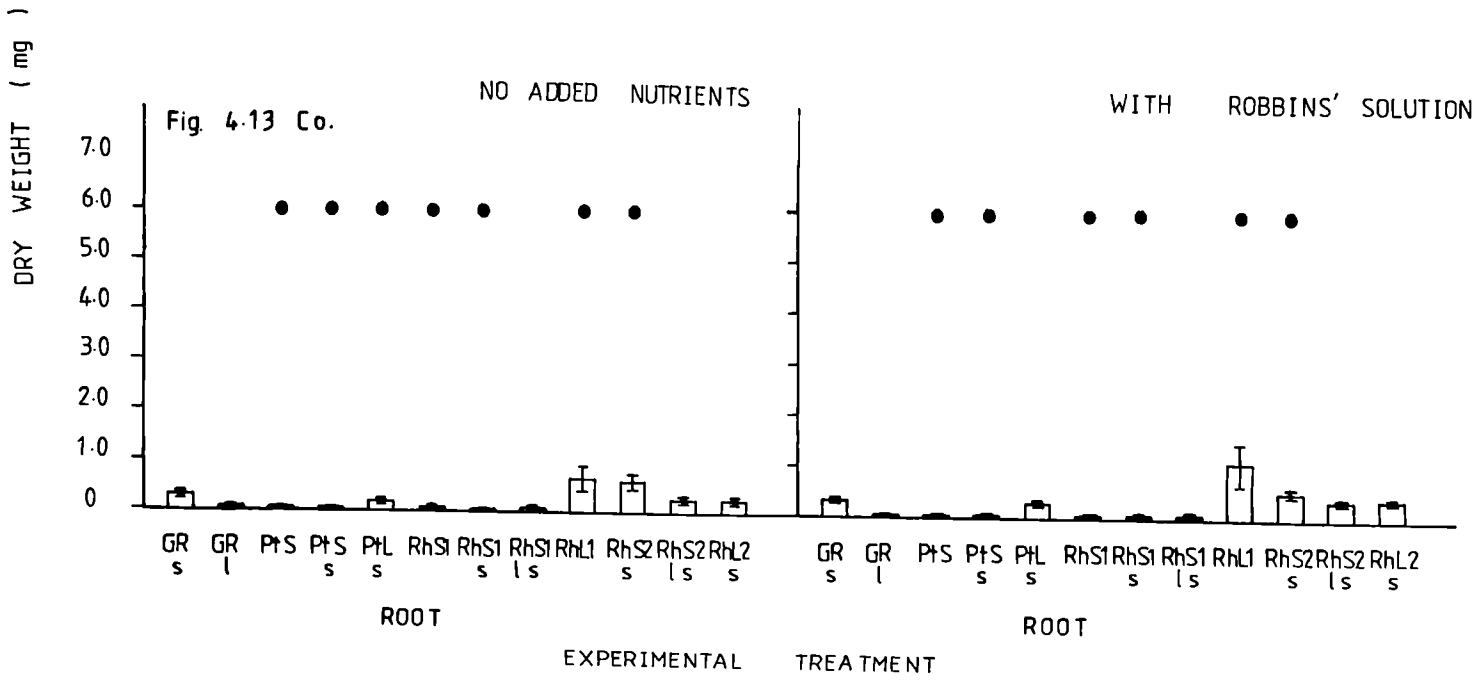


Fig. 4.13 Co.

SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Festuca ovina

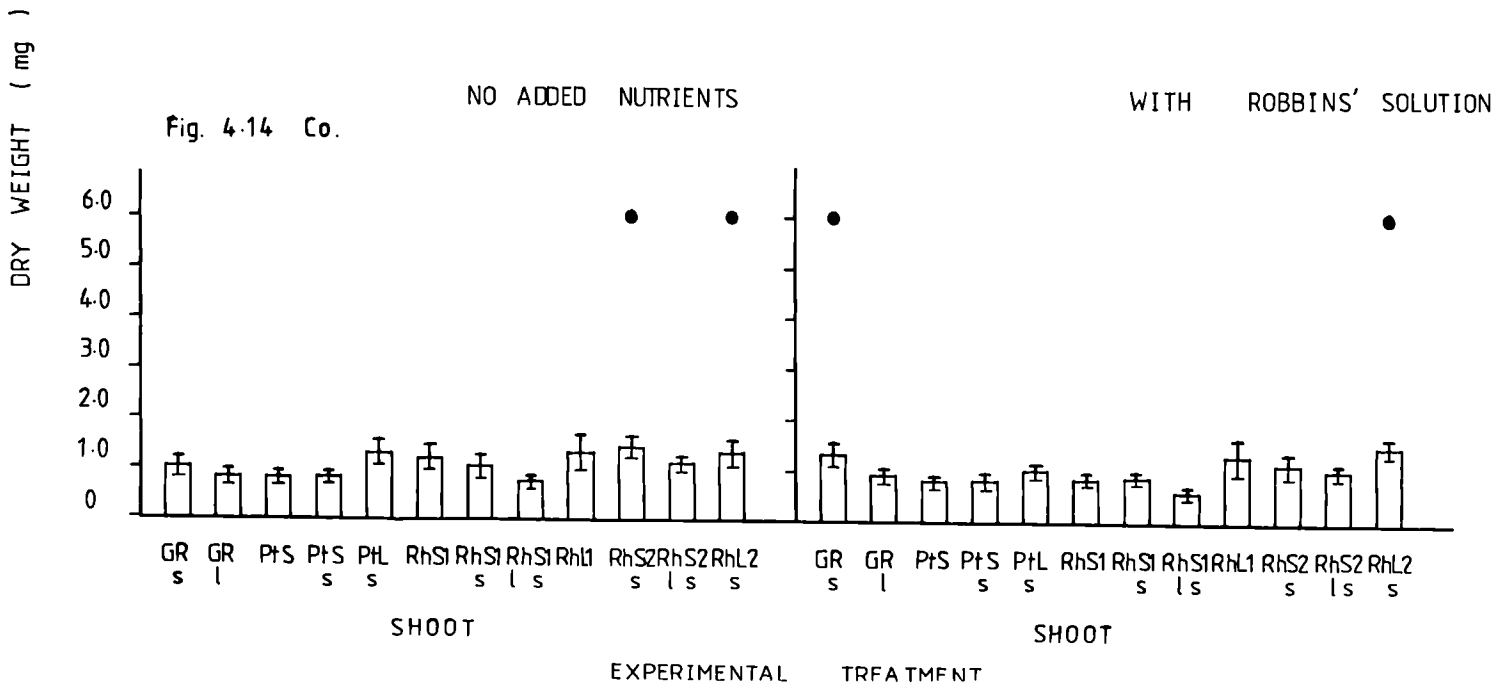
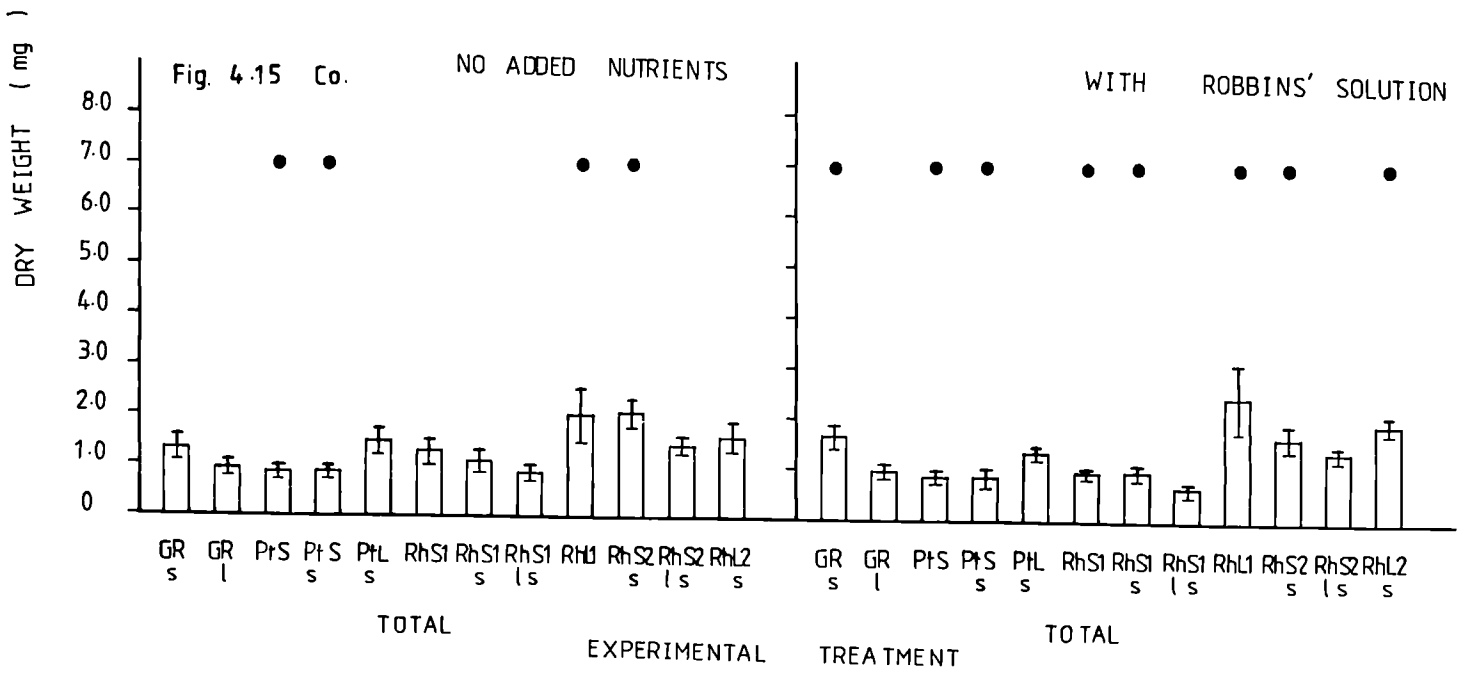


Fig. 4·14 Co.

SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Festuca ovina

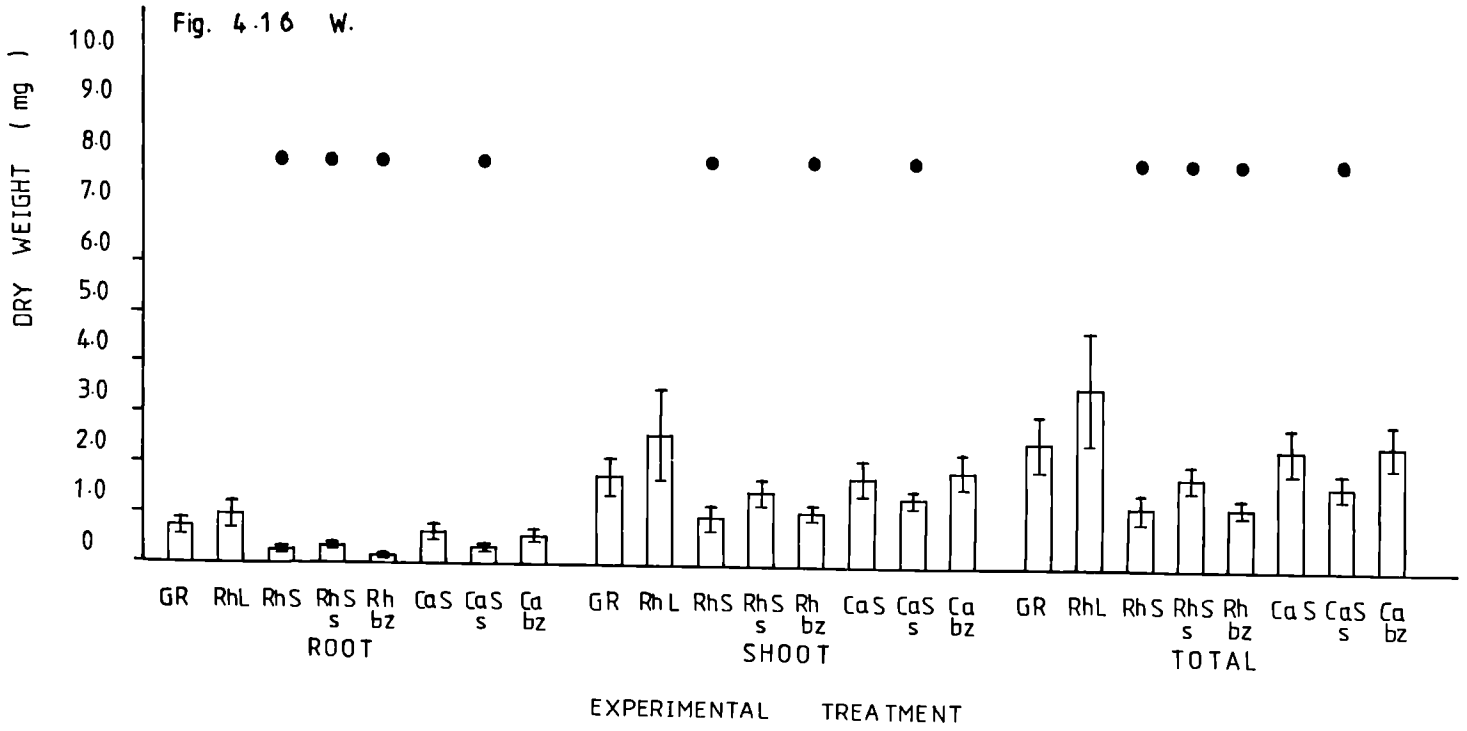


SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Rumex acetosa

NO ADDED NUTRIENTS

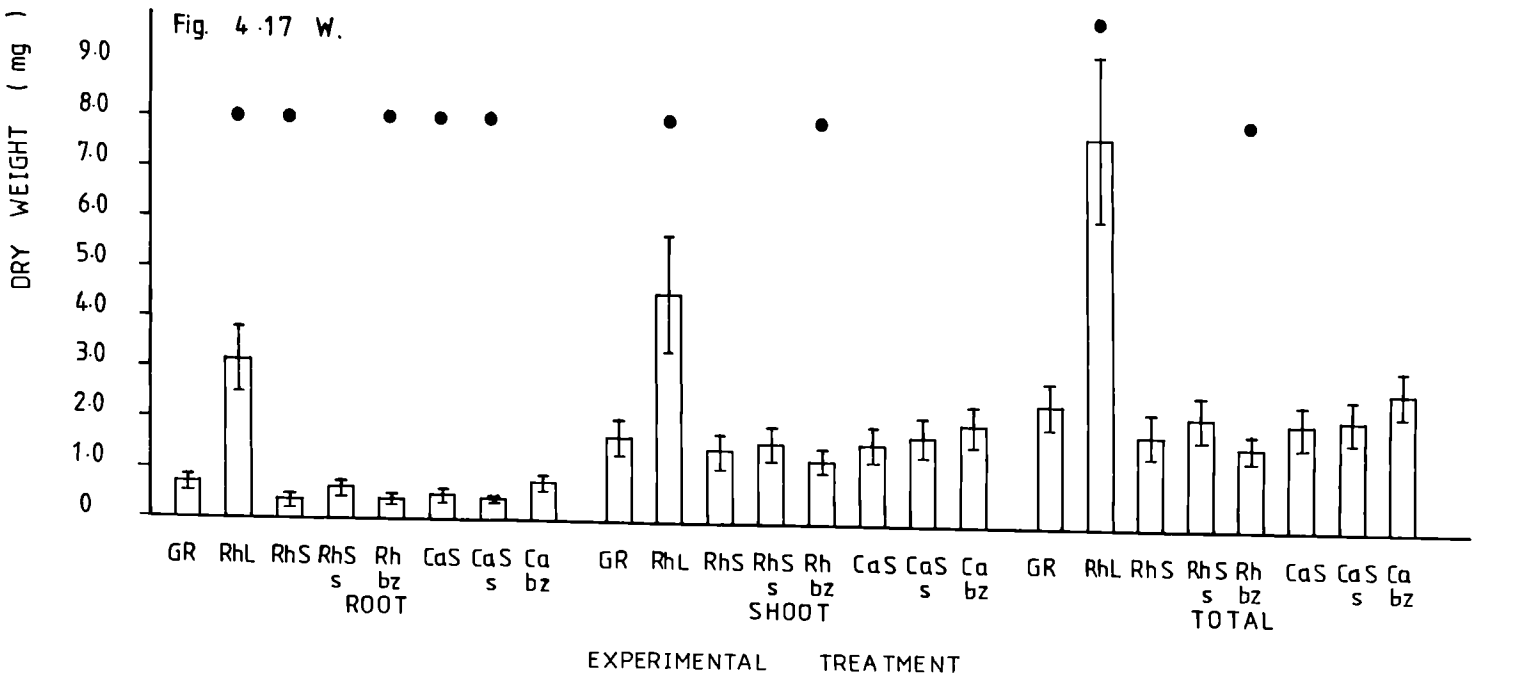
Fig. 4.16 W.

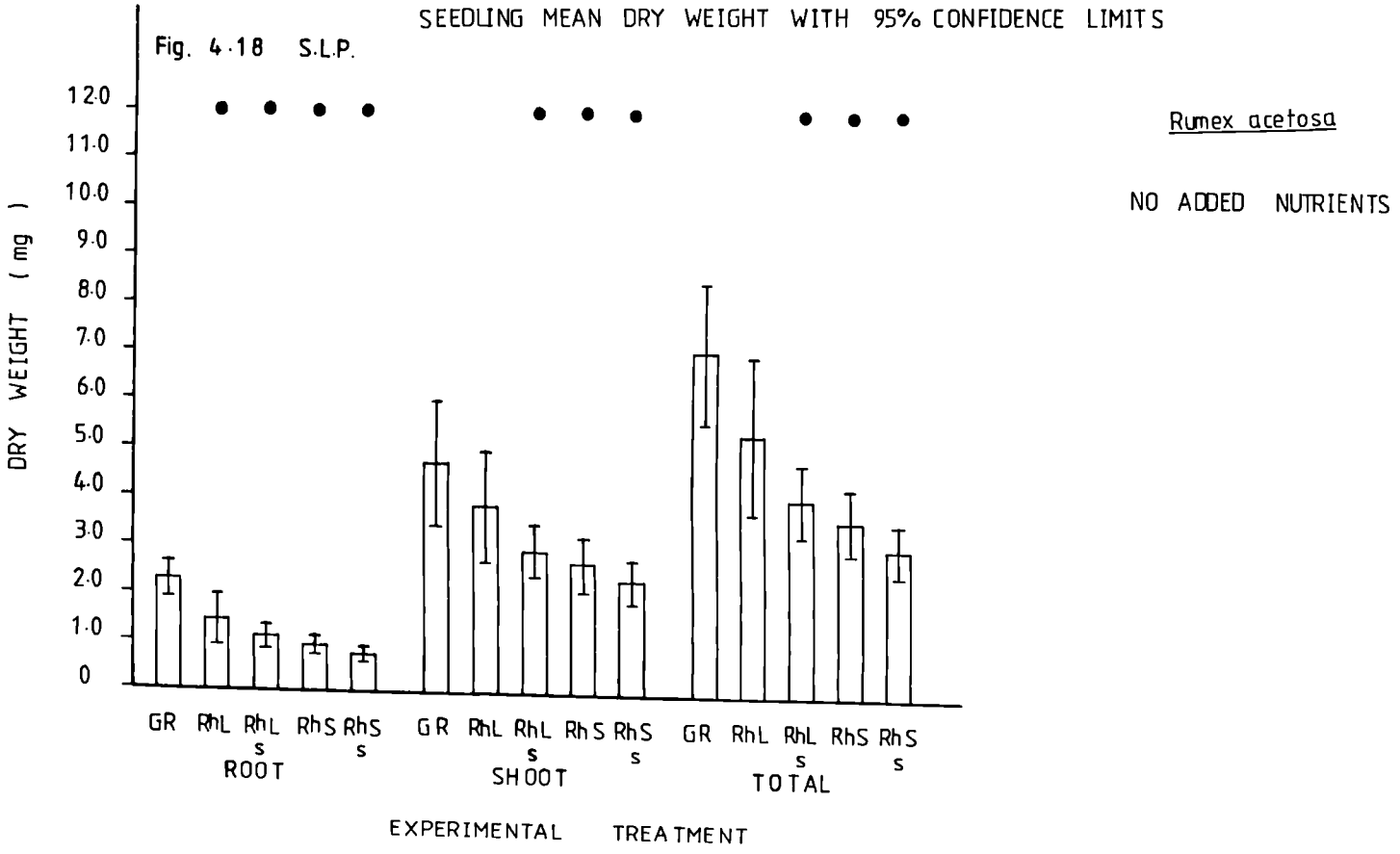


SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

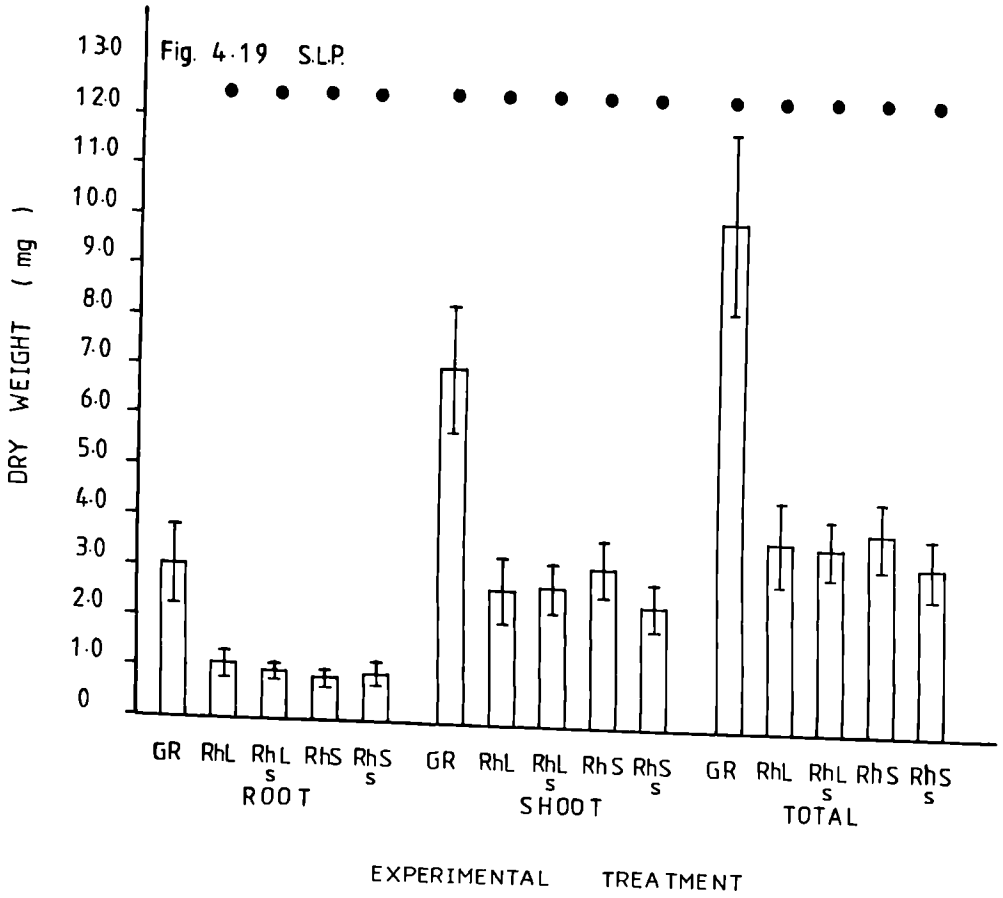
Rumex acetosa

WITH ROBBINS' SOLUTION




SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS
Fig. 4.18 S.L.P.
DRY WEIGHT (mg)
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
GR RhL RhLs RhS RhSs GR RhL RhLs RhS RhSs GR RhL RhLs RhS RhSs
ROOT
SHOOT
TOTAL
EXPERIMENTAL TREATMENT
Rumex acetosa
NO ADDED NUTRIENTS

SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS



Rumex acetosa

WITH ROBBINS' SOLUTION

SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Rumex acetosa

NO ADDED NUTRIENTS

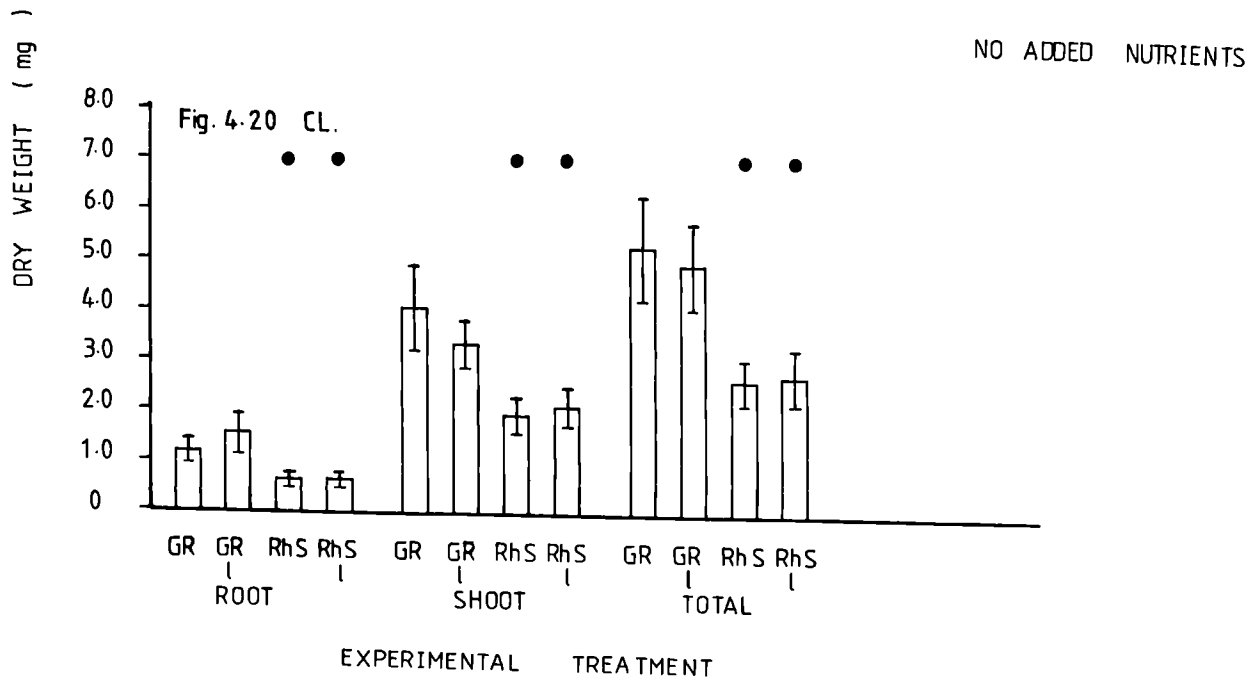


Fig. 4·20 CL.

SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Rumex acetosa

WITH ROBBINS' SOLUTION

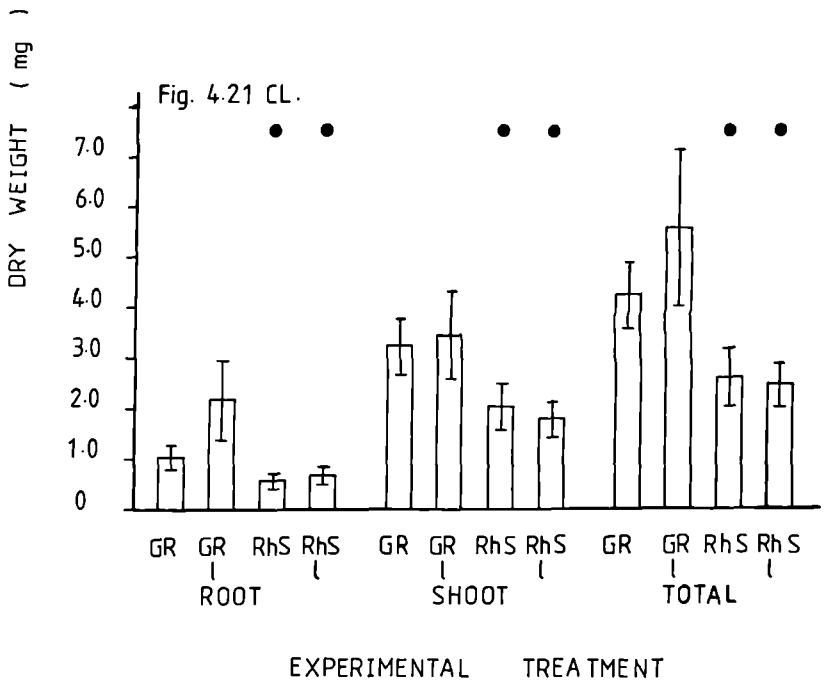


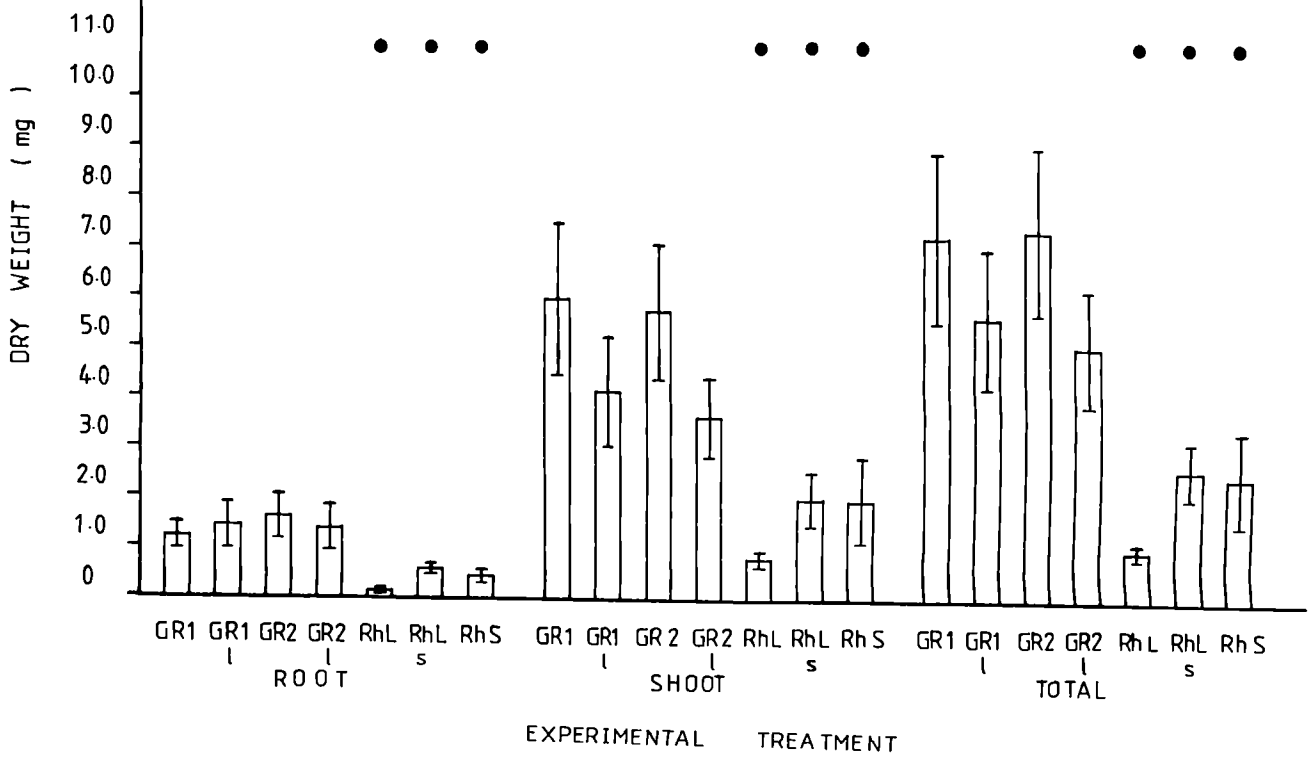
Fig. 4.21 CL.

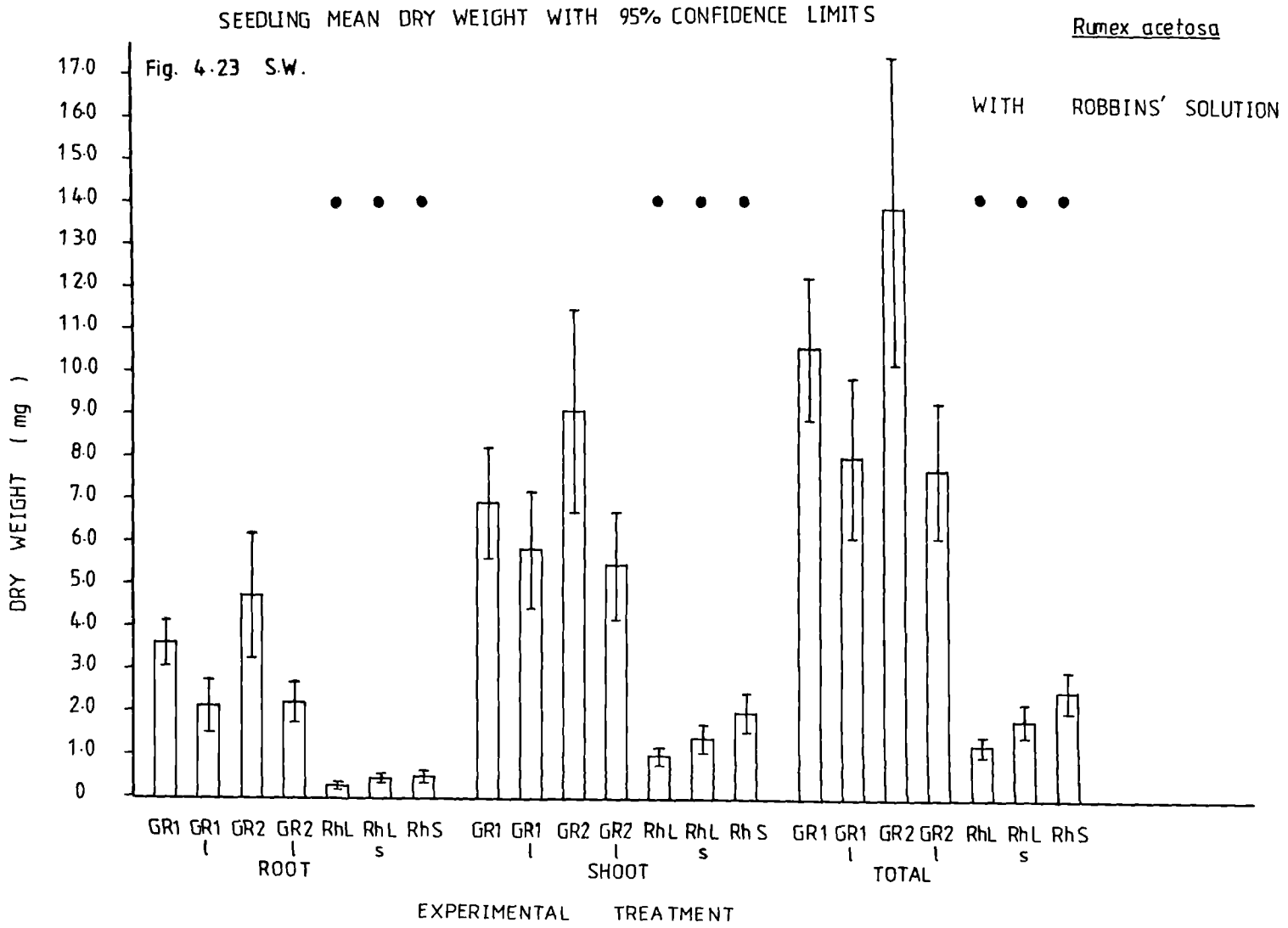
SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Rumex acetosa

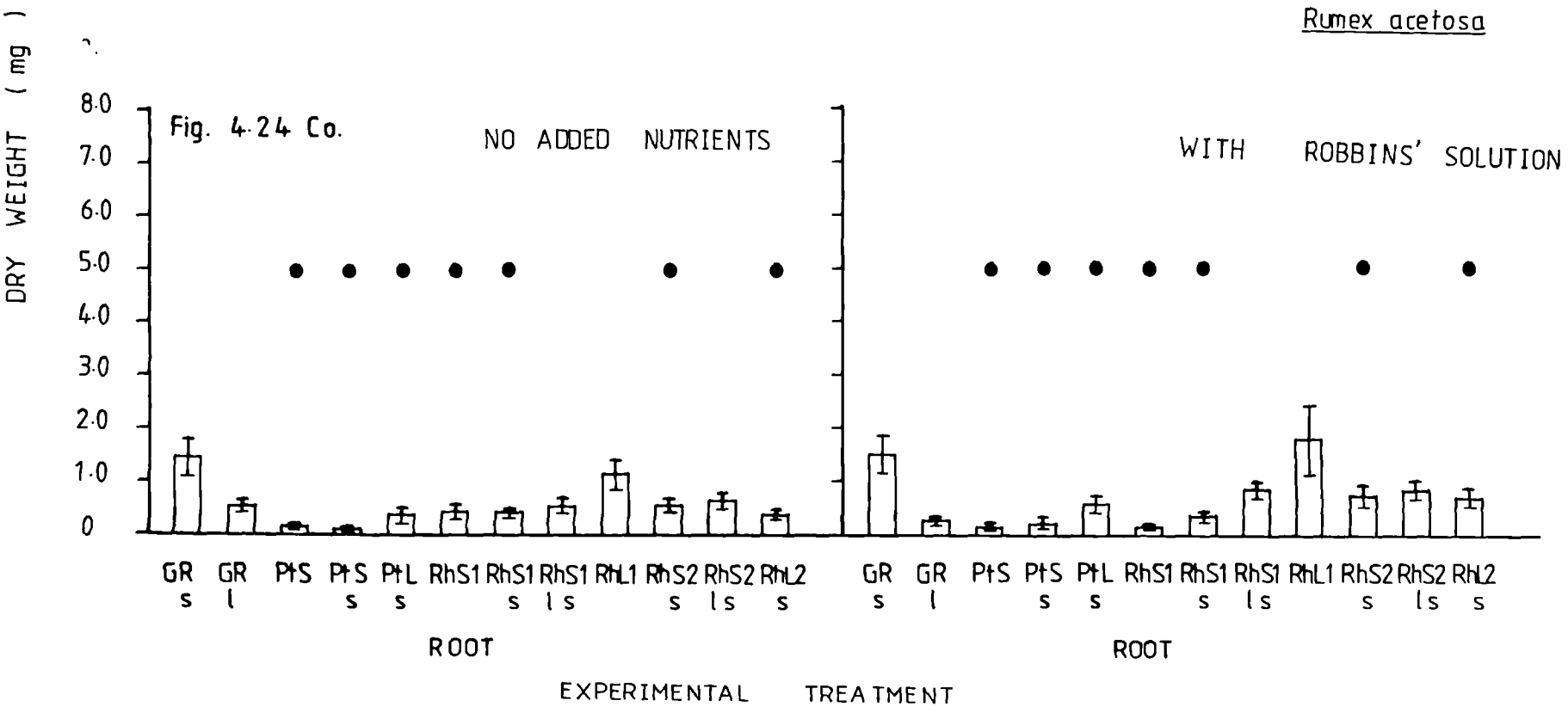
Fig. 4.22 S.W.

NO ADDED NUTRIENTS

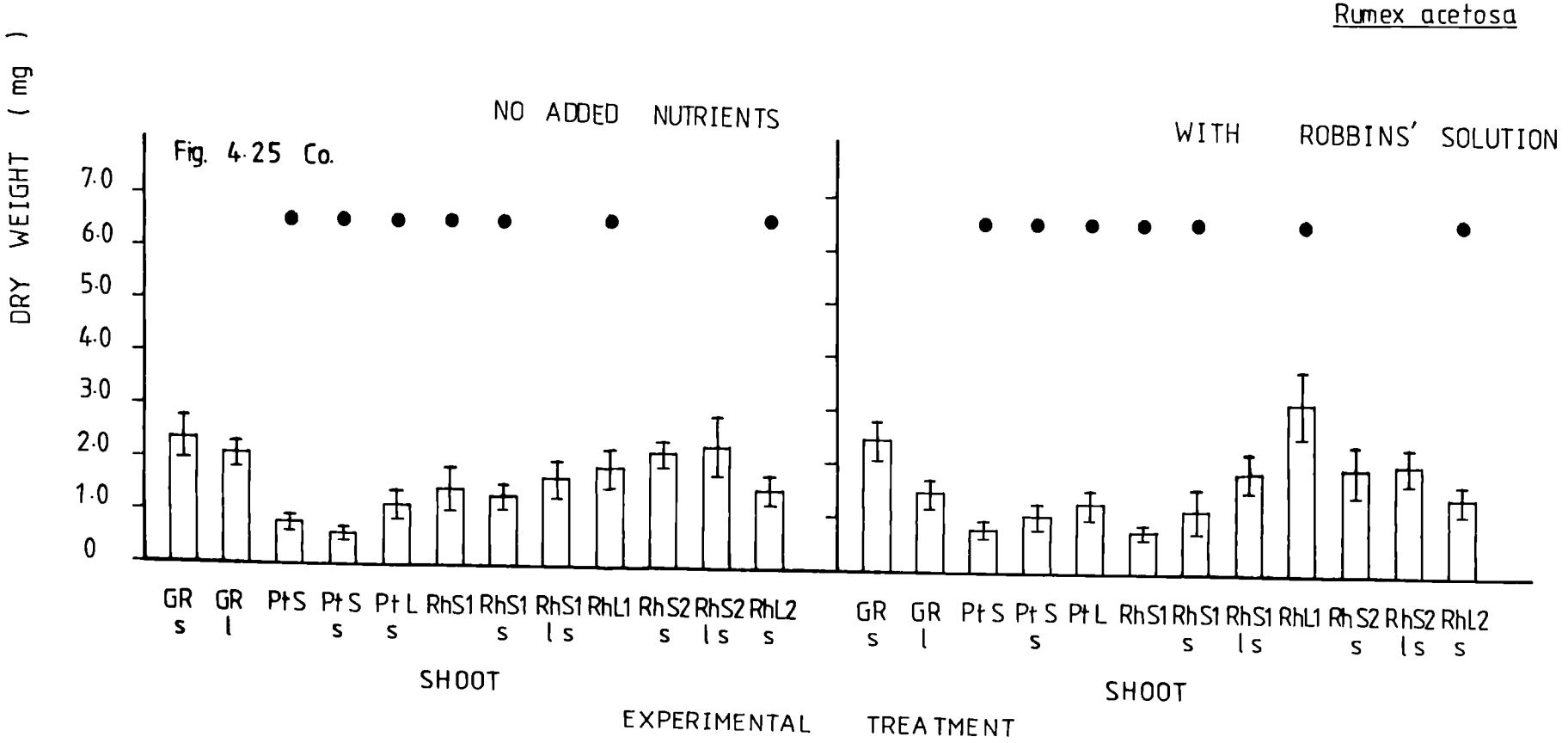



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS
Rumex acetosa
Fig. 4.23 S.W.
WITH ROBBINS' SOLUTION
DRY WEIGHT (mg)
17·0
16·0
15·0
14·0
13·0
12·0
11·0
10·0
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
GR1
GR1 l
GR2
GR2 l
RhL
RhL s
RhS
ROOT
SHOOT
TOTAL
EXPERIMENTAL TREATMENT

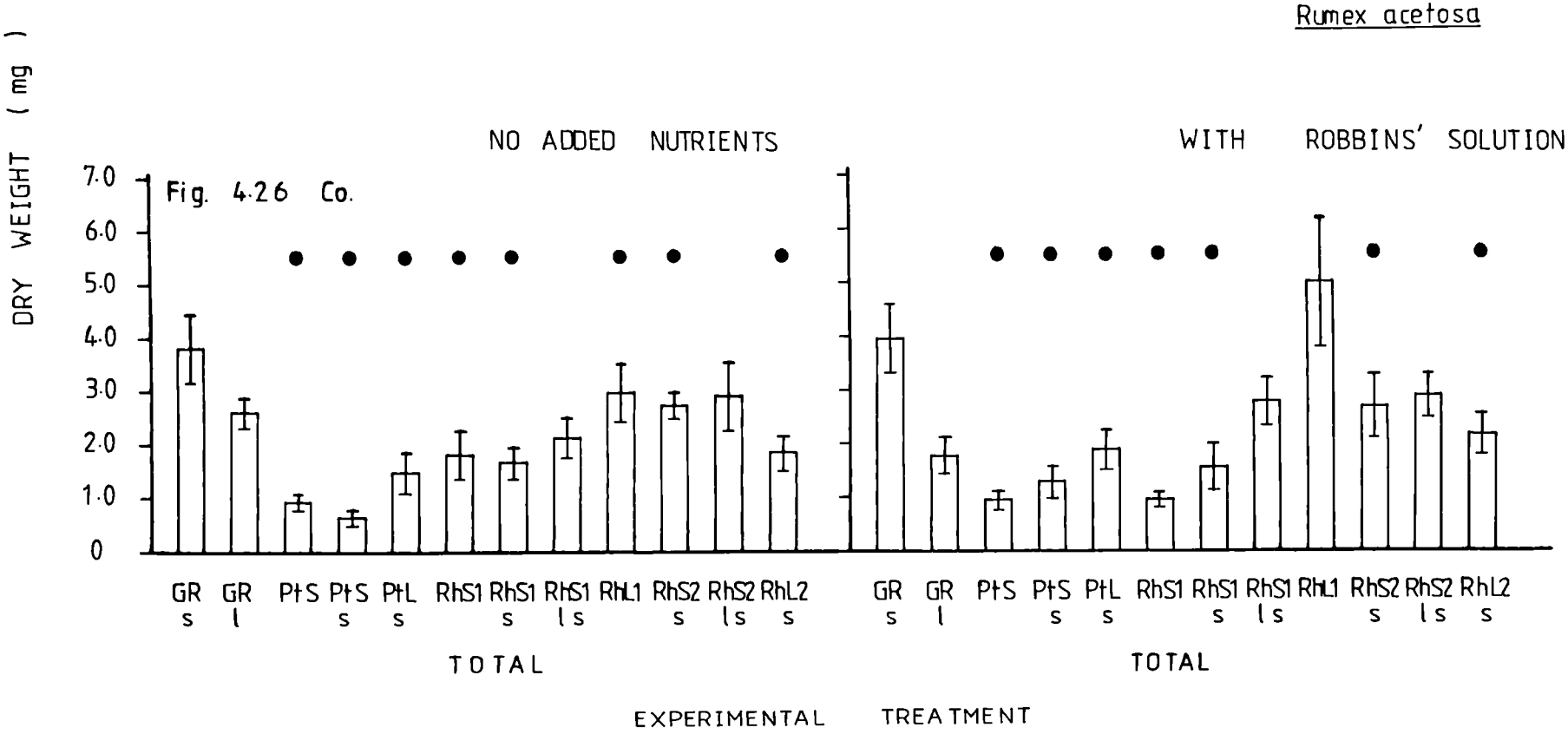
SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS



4.3.3.2.3 Soil and litter 'available' nitrogen and phosphorus, and pH

All the soils and litter used were fairly poor in 'available' nitrogen and phosphorus (Table 4.3.3.3). Rhododendron soil generally had slightly lower values that soil from adjacent grassland. Rhododendron litter had relatively high levels of 'available' nitrogen and phosphorus compared to the equivalent Rhododendron soil and in some cases (e.g. Winterton) these were also higher than those from grassland soil.

The pH of Rhododendron soil was consistently slightly lower than that of soil from adjacent grassland (Table 4.3.3.4). Rhododendron bare-zone soil from Winterton was also more acid than the grassland soil. Rhododendron litter was consistently less acid than Rhododendron soil, but usually more acid than grassland soil.

Table 4.3.3.3 Soil sample available nitrogen and phosphorus

Sample	Av.N (Ammonium)	Av.N (Nitrate)	Total	Av. P
W.GR	4.1	1.5	5.6	-
W.Rh.L	10.3	3.0	13.3	2.1
W.Rh.S	3.2	2.1	5.3	-
W.Rh.bz	4.0	1.1	5.1	-
S.L.P.GR	14.1	4.3	18.4	4.1
S.L.P.Rh.L	6.2	4.5	10.7	2.0
S.L.P.Rh.S	5.2	5.0	10.2	-
Cl.GR	3.7	1.1	4.8	-
Cl.GR 1	1.0	-	1.0	-
Cl.Rh.S	2.4	1.8	4.2	-
Cl.Rh.S 1	1.3	0.5	1.8	-
S.W.GR1	15.7	2.0	17.7	3.1
S.W.Rh.L	8.3	-	8.3	-
S.W.Rh.S	5.7	0.9	6.6	-
Co.GR	2.3	-	2.3	-
Co.Rh.S1	3.1	-	3.1	-
Co.Rh.L1	6.2	1.4	7.6	0.9
Co.Rh.S2	1.5	-	1.5	-
Co.Rh.L2	2.3	0.7	3.0	1.1

Table 4.3.3.4 Soil and litter acidity

All samples coarse sieved, mixed with distilled water and pH measured after 24 hours.

Sample	pH
W.GR	3.60
W.Rh.L	3.85
W.Rh.S	3.40
W.Rh.bz	3.40
W.Ca.S	3.50
W.Ca.bz	3.50
S.L.P.GR	3.65
S.L.P.Rh.L	3.55
S.L.P.Rh.S	3.40
CL.GR	3.60
CL.GR 1	3.80
CL.Rh.S	3.40
CL.Rh.S 1	3.40
S.W.GR1	3.90
S.W.GR1 1	3.80
S.W.GR2	7.10
S.W.GR2 1	7.00
S.W.Rh.L	3.40
S.W.Rh.S	3.35
Co.GR	5.20
Co.GR 1	5.00
Co.Pt.S	3.20
Co.Pt.L	3.70
Co.Rh.S1	3.45
Co.Rh.S1 1	4.20
Co.Rh.L1	4.35
Co.Rh.S2	3.55
Co.Rh.S2 1	3.65
Co.Rh.L2	3.55

4.3.4 DISCUSSION

Experiment 1a showed highly significant decreases in R, S and T of F.ovina and R. acetosa on Rhododendron soil compared to the control. Coarse Rhododendron litter was not toxic and produced significantly increased growth of F. ovina over the control.

Further investigation of this phenomenon (Experiment 2) revealed similar effects with soil and litter from a number of sites. Soils from under R. ponticum caused reduced growth of test seedlings, often with highly significant decreases in R. Results of sieving to remove roots were variable. Sieving did ameliorate the effect in some cases (e.g. W.Rh.S.s. for F. ovina) and sieving combined with nutrient addition, did so in other cases (e.g. W.Rh.S.s.+N for R. acetosa). Nutrient addition did not remove the generally observed reductions in growth.

Rhododendron bare-zone soil from Winterton, Norfolk, decreased seedling growth, though this was only significant for R. acetosa. The effect was not removed by adding nutrients.

Rhododendron litter gave reduced seedling yield in some cases (e.g. S.L.P.Rh.L for F. ovina and R. acetosa), but increased yield in others (e.g. W.Rh.L for F. ovina and R. acetosa). Coarse litter which lacked fine, mycorrhizal Rhododendron hair roots gave increased yields. More fragmented and decomposed litter, well permeated by Rhododendron roots, decreased yield of test seedlings.

Soil and litter from under Pteridium at Cordwell significantly reduced seedling yield. (For F. ovina this only applied to R). The effect on R, S and T of R. acetosa seedlings was not removed by nutrient addition.

4.4 INVESTIGATION INTO INTERFERENCE CAUSED BY *RHODODENDRON* UNDER ARTIFICIAL CONDITIONS EXPERIMENTS 3, 4, 5, 6a, 6b AND 6c

4.4.1 INTRODUCTION

Four investigations (Experiments 3, 4, 5 and 6) were carried out to look at various aspects of the interference phenomenon. The aim was to produce artificial 'interference zones' in either glass dishes or plastic pots containing soil. Various factors such as the presence of live or dead Rhododendron roots and the presence or absence of additional nutrients, were examined in bioassays with test species. This was in order to see whether interference could be artificially produced, and if so, whether its effects could be influenced by the experimental treatments.

4.4.2 METHOD

Experiments 3, 4 and 5 were carried out in a growth-room with a 20°C/16 hour day and a 15°C/8 hour night. Experiment 6 was done in the greenhouse at the University *Experimental Gardens* at Tapton, Sheffield. This was with daylight and supplementary lighting to give a 16 hour day.

Glass crystallizing dishes with glass tops (Experiments 3 and 4) or plastic pots (2½ inch diameter for Experiment 5; 5 inch diameter for Experiment 6) were set up with field soil. Freshly collected back-dune soil from Winterton, Norfolk was used for Experiments 3 and 5. Back-dune soil from the same site, but partially sterilized by gamma irradiation (equivalent to 1.8 mega-rads), was used for Experiment 4. Similarly treated soil from Clumber, North Nottinghamshire, was used for Experiment 6. Available nitrogen, available phosphorus and pH were measured for the experimental soils.

Experimental treatments included the presence or absence of live, mycorrhizal or non-mycorrhizal Rhododendron roots, of dead Rhododendron roots and various amounts of added nutrients. The pots or dishes were sown or planted with F. ovina, A. tenuis, R. acetosa or Trifolium repens as test species.

After harvesting, the test seedlings were oven-dried at 80°C for 24 hours and then weighed.

Some of the data are presented and compared in two forms. Firstly, as the mean dry weight and confidence limits for the seedlings still alive at the end of the experiment. Secondly, the same expressed in terms of the total number of seeds sown or seedlings planted for each treatment. The two types of assessment of the data are both important. The first shows the state of the surviving seedlings in different treatments. The second demonstrates the effects of treatments on the overall yield of the test seedlings. The two sets of data are only very different when seedling survival was poor.

4.4.2.1 Experiment 3 Effects of *Rhododendron* on root and shoot development in pre-germinated and in situ germinated test seedlings

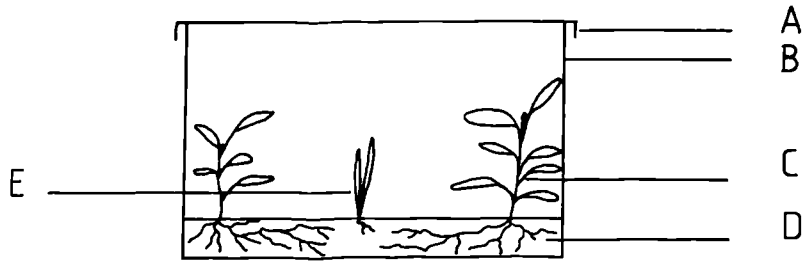
Crystallizing dishes containing Winterton back-dune soil were sown with R. ponticum seed to give 10 seedlings per dish. These were then grown for 4 months. Test species (F. ovina (Fe) and A. tenuis (Ag)) were then sown directly as seed or planted as 16-day old, pre-germinated seedlings, to give 10 per dish.

The test seedlings were harvested after 6 weeks when planted as seedlings, or 6 weeks + 16 days for those sown as seed. They were cleaned, their root and shoot lengths measured and were then dried and weighed.

4.4.2.2 Experiment 4 Effects of presence or absence of mycorrhizal or non-mycorrhizal *Rhododendron*, *Rhododendron* soil with the *Rhododendron* removed and the addition of nutrient solution, on *Festuca ovina* germinated in situ

Crystallizing dishes with irradiated Winterton back-dune soil were planted with non-mycorrhizal R. ponticum seedlings (Figure 4.27). After one month half of these were inoculated with R. ponticum endophyte. Control dishes were left without R. ponticum seedlings. All the dishes were watered with distilled water.

Fig. 4.27 DISH FOR LABORATORY INVESTIGATION OF INTERFERENCE USING ARTIFICIAL 'BARE-ZONES'



KEY:-

A: Glass lid
B: Glass crystallizing dish
C: Rhododendron seedling
D: Soil
E: Festuca seedling

After two months, during which time mycorrhizas had established in inoculated plants, the seedlings were harvested to give either 4 seedlings per dish or dishes with no seedlings. The harvested R. ponticum seedlings were examined to confirm their mycorrhizal or non-mycorrhizal status. The dishes were now sown with 10 F. ovina seeds per dish. Half the dishes were watered with distilled water and half with full-strength Robbins' solution. The F. ovina seedlings were harvested after six weeks.

4.4.2.3 Experiment 5 Effects of presence of live, mycorrhizal *Rhododendron* roots, dead roots, *Rhododendron* soil with *Rhododendron* removed and the addition of nutrients, on test seedlings

R. ponticum seed was sown in pots of Winterton back-dune soil, to give 5 seedlings per pot. After eleven months the seedlings were treated in the following ways:-

1. R. ponticum seedlings were fastened back to prevent shading of the pot (Rh.) (Figure 4.28).

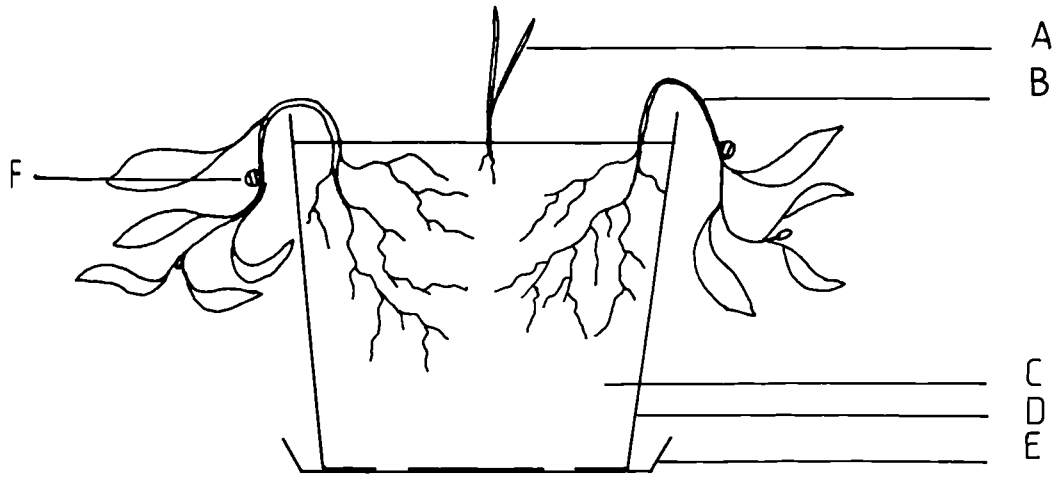
2. R. ponticum seedlings harvested; roots left (Rh.H+R).

3. R. ponticum seedlings harvested; roots carefully removed from the soil and the soil passed through a 2 mm mesh sieve (Rh.H-R).

4. Fresh Winterton back-dune soil was collected and placed in pots as a control (NRh.).

8 pots were set up for each of the treatments (1-4). 4 were watered with distilled water and 4 with full-strength Robbins' solution. Each pot was then planted with 5 8-day old, pre-germinated F. ovina seedlings. The F. ovina seedlings were harvested after 24 weeks.

Fig. 4.28 POT FOR LABORATORY INVESTIGATION OF INTERFERENCE USING ARTIFICIAL 'BARE-ZONES'



KEY:-

A: Festuca seedling
B: Rhododendron seedling tied back by rubber band (F).
C: Soil
D: Plastic pot
E: Plastic saucer

4.4.2.4 Experiment 6 Effects of mycorrhizal status, of presence or absence of *Rhododendron* roots and of different levels of nutrient addition on test seedlings

4.4.2.4.1 Experiment 6a

Seven month old R. ponticum seedlings were planted (1 per pot) in pots of irradiated Clumber soil (Rh.) (Figure 4.29). Some pots were left without R. ponticum (NRh.). To half the Rhododendron pots, a macerate of R. ponticum endophyte was added. The pots were then watered with distilled water.

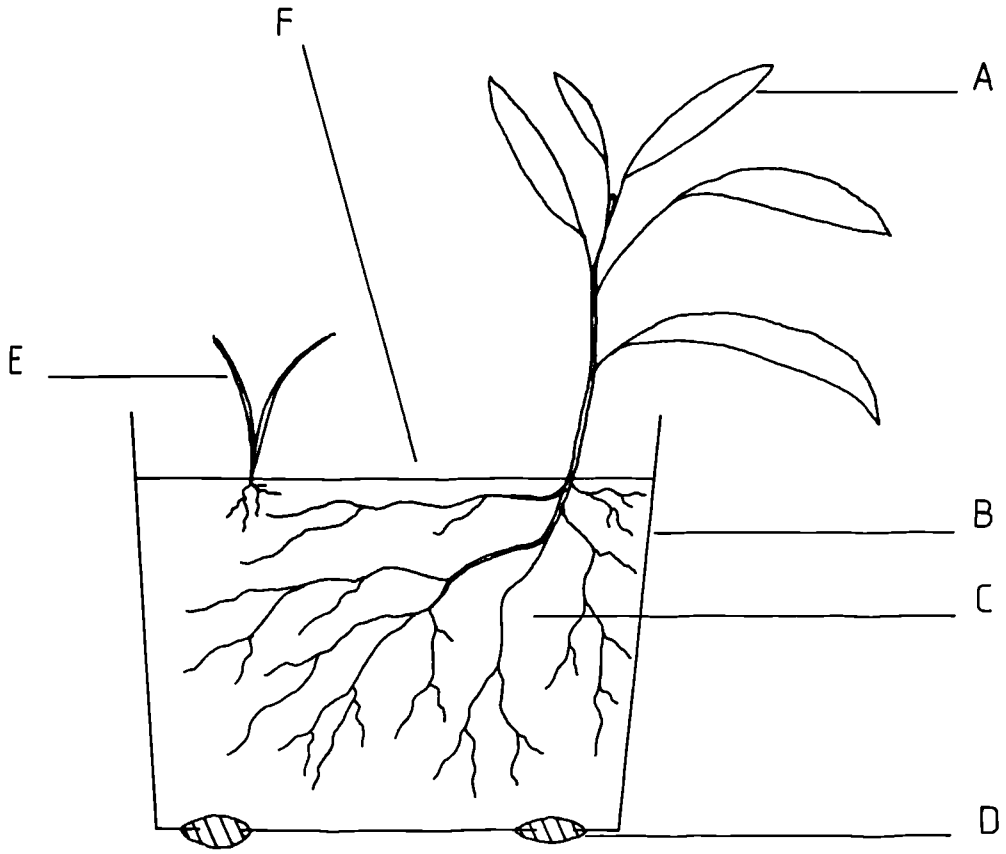
After approximately sixteen months, the pots were planted with pre-germinated seedlings of three test species : Festuca ovina, Rumex acetosa and Trifolium repens. Ten seedlings were planted in each pot, with two pots per treatment for each test species. The pots were watered with distilled water, full-strength Robbins' solution (1N) or double-strength Robbins' solution (2N). The test seedlings were harvested after six weeks.

4.4.2.4.2 Experiment 6b

The pots were then re-used to give non-Rhododendron controls (NRh.) and live Rhododendron controls (Rh.) (both as in Experiment 6a), together with Rhododendron harvested with dead roots left (Rh.H+R) and Rhododendron harvested with the dead roots sieved from the soil (Rh.H-R).

The pots were planted with 10 pre-germinated seedlings of F. ovina or R. acetosa, one pot per treatment. The pots were watered with distilled water, full-strength Robbins' solution, double-strength Robbins' solution or 10X-strength Robbins' solution (10N). The test seedlings were harvested after six weeks.

Fig. 4-29 POT FOR LABORATORY INVESTIGATION OF INTERFERENCE USING ARTIFICIAL 'BARE-ZONES'



KEY:-

A : Rhododendron seedling
B : Plastic pot (5 inch diameter)
C : Soil thoroughly permeated by Rhododendron roots
D : Drainage hole plugged with silicone rubber
E : Test seedling
F : 'Bare-zone'

4.4.2.4.3 Experiment 6c

The treatments in Experiment 6b were maintained and the pots were replanted with further test seedlings. These were again harvested after six weeks.

Soil pH was measured for each pot after each of the three harvests. 'Available' nitrogen and 'available' phosphorus were measured after the first harvest.

Table 4.4.2.1 Key to abbreviations used

Rh.	:	Rhododendron ponticum
Fe	:	Festuca ovina
Ag	:	Agrostis tenuis
Ru	:	Rumex acetosa
Tr	:	Trifolium repens
NRh.	:	soil without R. ponticum
Rh.NMyc	:	non-mycorrhizal R. ponticum
Rh.Myc	:	mycorrhizal R. ponticum
Rh.H	:	soil from which R. ponticum has been harvested
+R	:	roots left in soil
-R	:	roots removed from soil by sieving
-N	:	watering with distilled water only
+1N	:	watering with 1X Robbins' solution
+2N	:	watering with 2X Robbins' solution
+10N	:	watering with 10X Robbins' solution
R	:	root)
S	:	shoot) yield of test seedlings as dry weight
T	:	total (R+S))
+E	:	added endophyte (usually referring to addition to pots without R. ponticum).

4.4.3 RESULTS

4.4.3.1 Experiment 3

Table 4.4.3.1 Germination of seeds in situ

F. ovina : 10 seeds sown per dish.
Dish (1) : 9 germinated.
Dish (2) : 6 germinated.
Mean germination = 75%.

Control germination of F. ovina on filter paper, watered with distilled water:

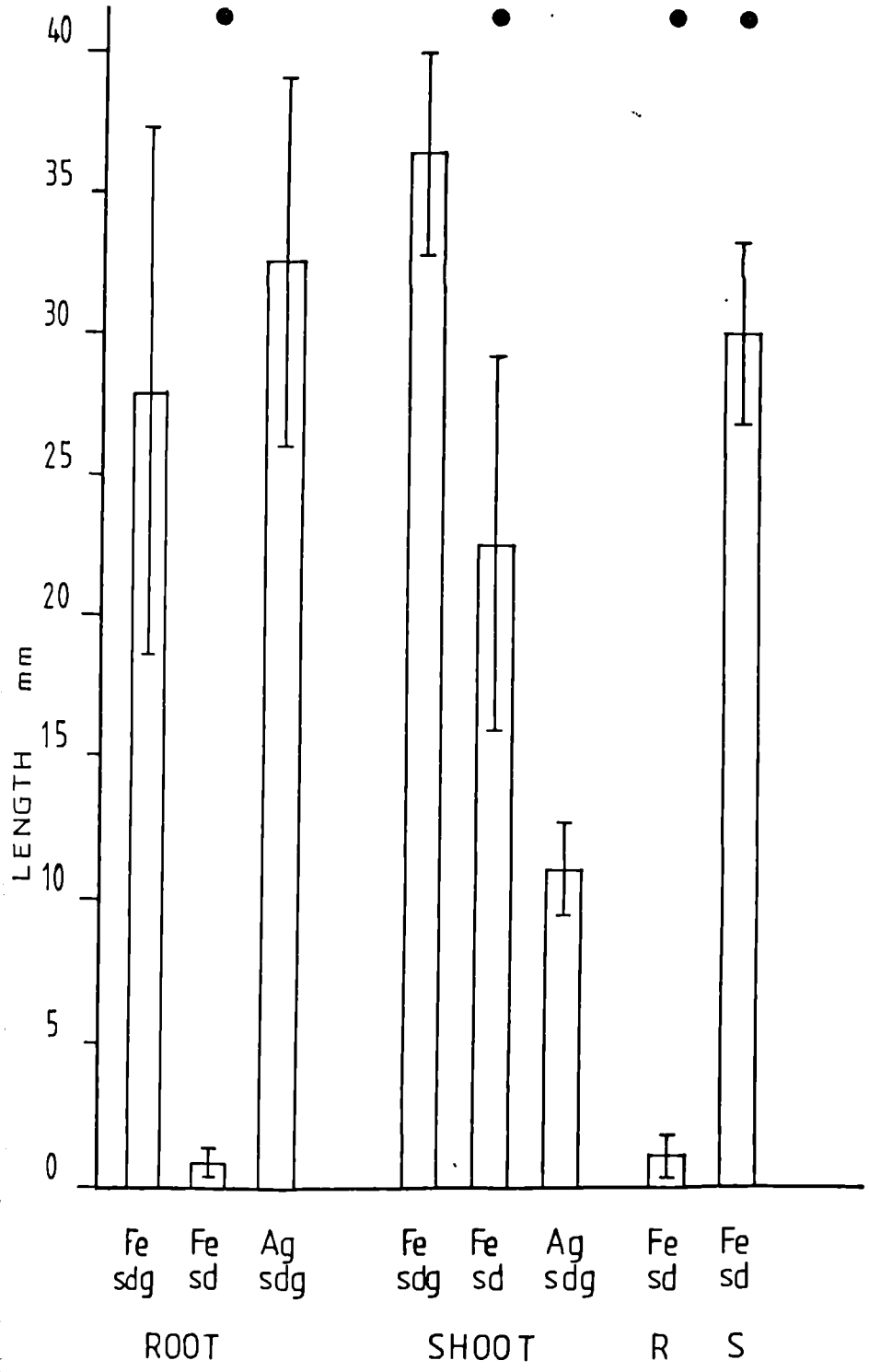
Dish (1) : 8 germinated.
Dish (2) : 7 germinated.
Mean germination = 75%.

Pre-germinated seedlings of F. ovina and A. tenuis grew well, producing healthy extensive root systems. F. ovina seedlings germinated in situ with live R. ponticum produced virtually no roots. Those produced were very stunted and pale brown in colour (contrasting with the clean, white, healthy roots of the pre-germinated seedlings). Root and shoot lengths of seedlings germinated in situ were significantly less than those of the pre-germinated seedlings (Figure 4.30). Root dry weight was also significantly reduced. Shoot dry weight was significantly increased for the surviving seedlings germinated in situ, but not when all twenty seeds sown were considered (Figure 4.31). The total dry weight yield was not significantly different from that of the pre-germinated seedlings.

Germination success of seeds in situ with live R. ponticum was the same as for seeds on filter paper (Table 4.4.3.1).

Fig. 4.30

SEEDLING ROOT AND SHOOT LENGTHS; MEANS WITH 95% CONFIDENCE LIMITS



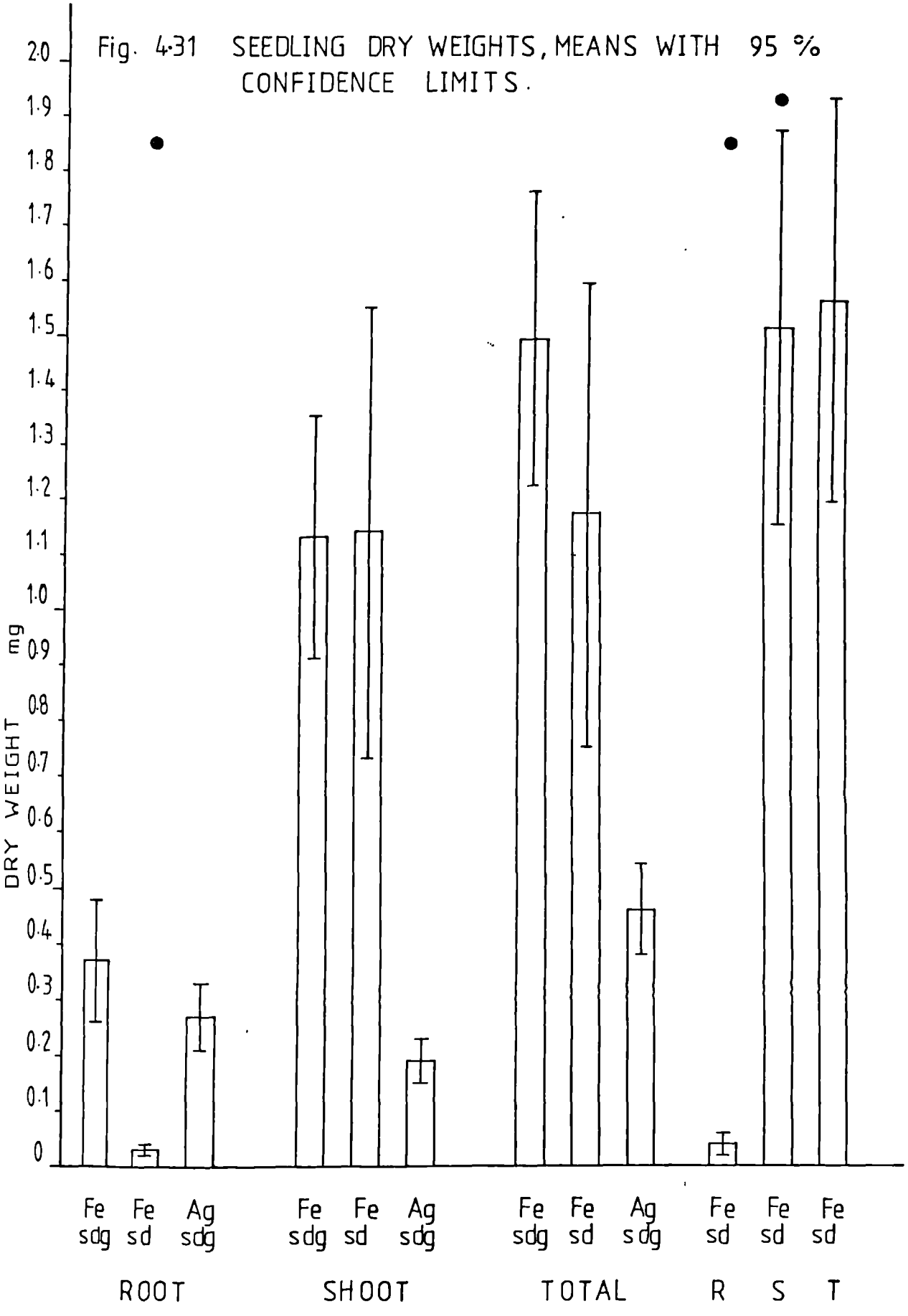
Means of 20 seedlings planted or 20 seeds sown.

Means of surviving seedlings.

sd = sown as seed.

sdg = planted as pre-germinated seedling.

Fig. 4·31 SEEDLING DRY WEIGHTS, MEANS WITH 95 % CONFIDENCE LIMITS.



Means of 20 seedlings planted or 20 seeds sown.

Means of surviving seedlings.

sd = sown as seed.

sdg = planted as pre-germinated seedling.

4.4.3.2 Experiment 4

Germination and subsequent survival of Festuca seedlings (Table 4.4.3.2, Figures 4.32 and 4.33) was reduced from 87.5% in dishes without Rhododendron, to between 5% and 10% for dishes with Rhododendron harvested and 5% for dishes with mycorrhizal Rhododendron. This effect was not removed by nutrient addition.

Mycorrhizal Rhododendron caused slightly poorer germination and survival than non-mycorrhizal. This might be due to the increased growth of Rhododendron with mycorrhizal infection *(see Chapter 3)*. *Nutrient* addition generally led to a slight increase in germination and survival.

All dishes with Rhododendron, either live or harvested, mycorrhizal or non-mycorrhizal, produced highly significant reductions in R, S and T of Festuca seedlings (Figures 4.34, 4.35, 4.36 and 4.37). Adding nutrients did not eliminate these effects.

The presence of Rhododendron in the soil was associated with a fall in pH from around 3.50 - 3.60 *(irradiated field soil and maintained in NRh.* dishes) to around 3.10 (Table 4.4.3.4). This decrease was maintained whether or not nutrients were added. Harvesting the Rhododendron seedlings lessened the effect, but did not eliminate it. This decrease in soil pH occurred with both mycorrhizal and non-mycorrhizal seedlings.

The soil used was low in 'available' nitrogen and phosphorus. The predominant form of available nitrogen was as ammonium (Table 4.4.3.5).

Fig. 4.32 PERCENTAGE OF SEEDS SOWN PRODUCING SEEDLINGS STILL ALIVE AT THE HARVEST

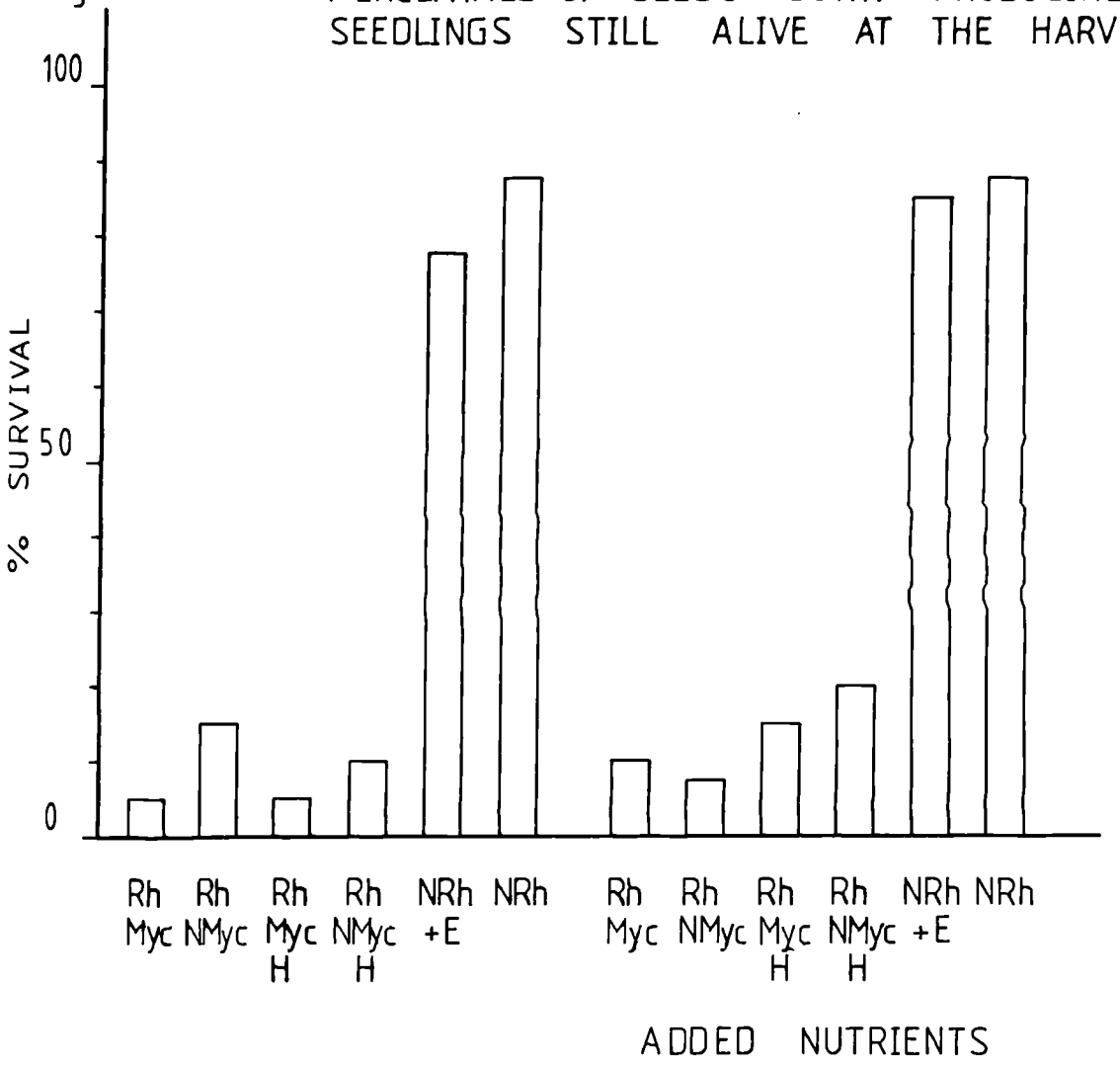
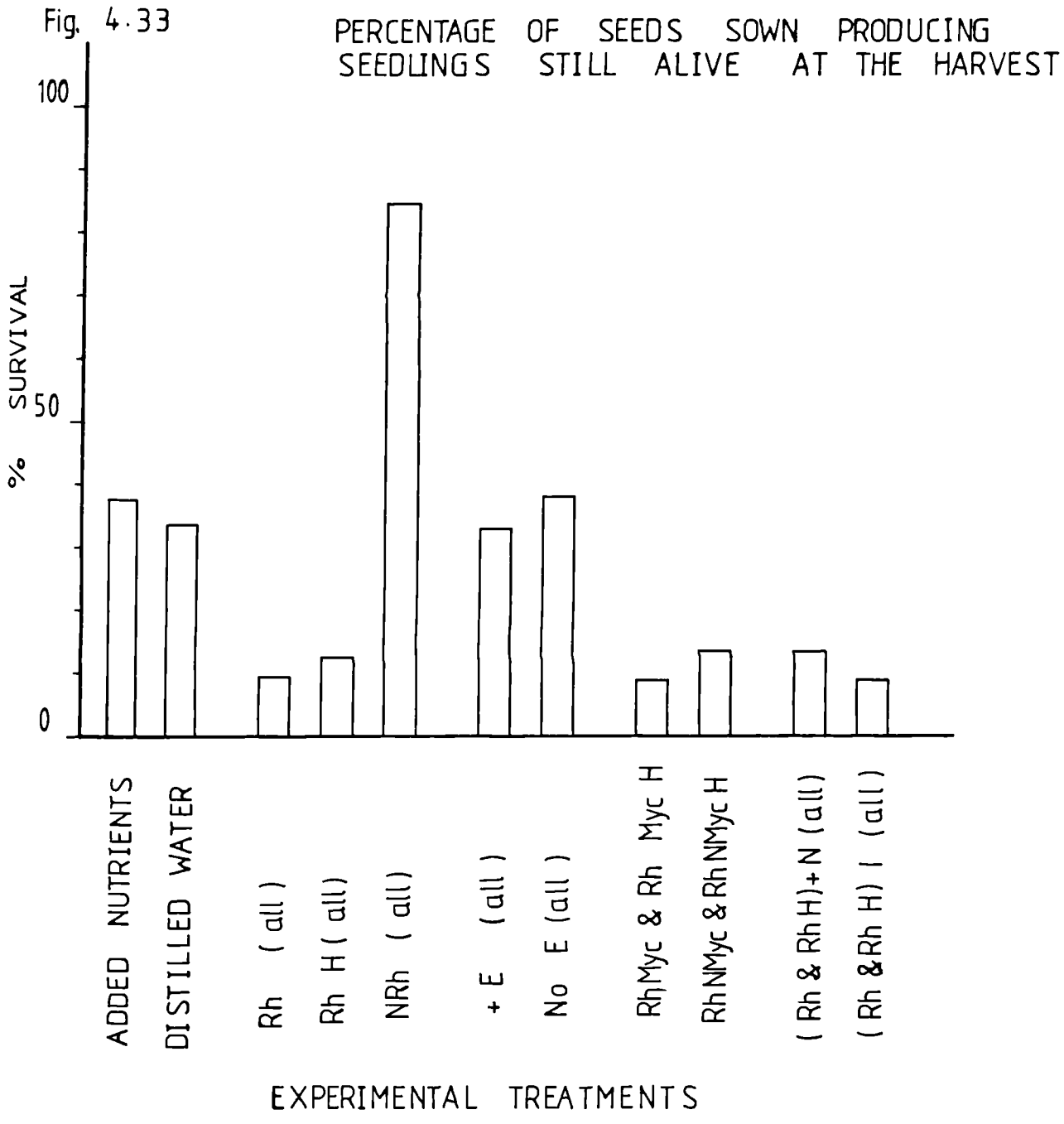


Fig. 4.33


PERCENTAGE OF SEEDS SOWN PRODUCING SEEDLINGS STILL ALIVE AT THE HARVEST
100
50
0
% SURVIVAL
ADDED NUTRIENTS
DISTILLED WATER
Rh (all)
Rh H (all)
NRh (all)
+E (all)
No E (all)
RhMyc & Rh Myc H
RhNMyc & RhNMyc H
(Rh & RhH)+N (all)
(Rh & RhH) I (all)
EXPERIMENTAL TREATMENTS

SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Fig. 4.34

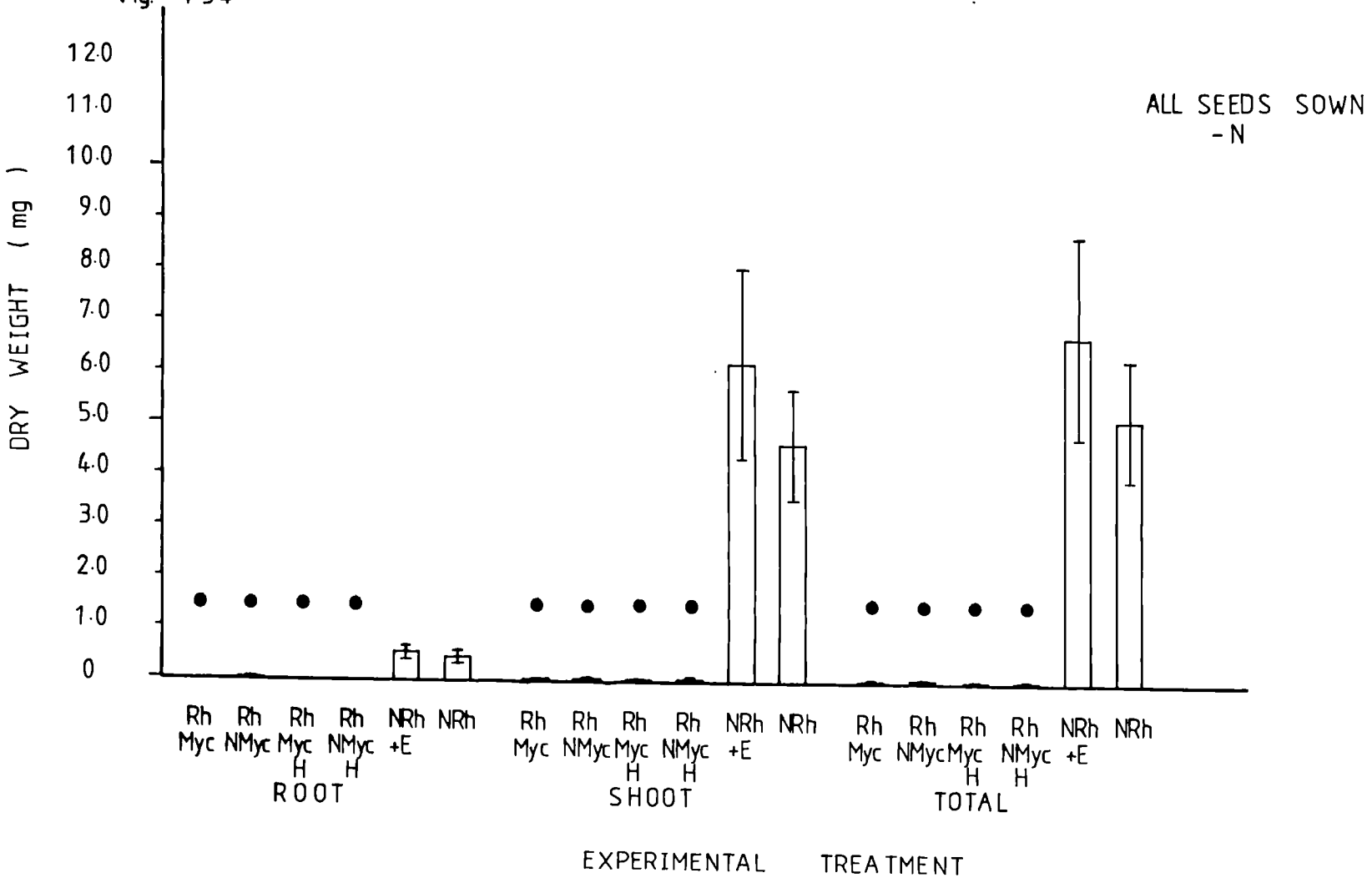


Fig. 4.35 SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

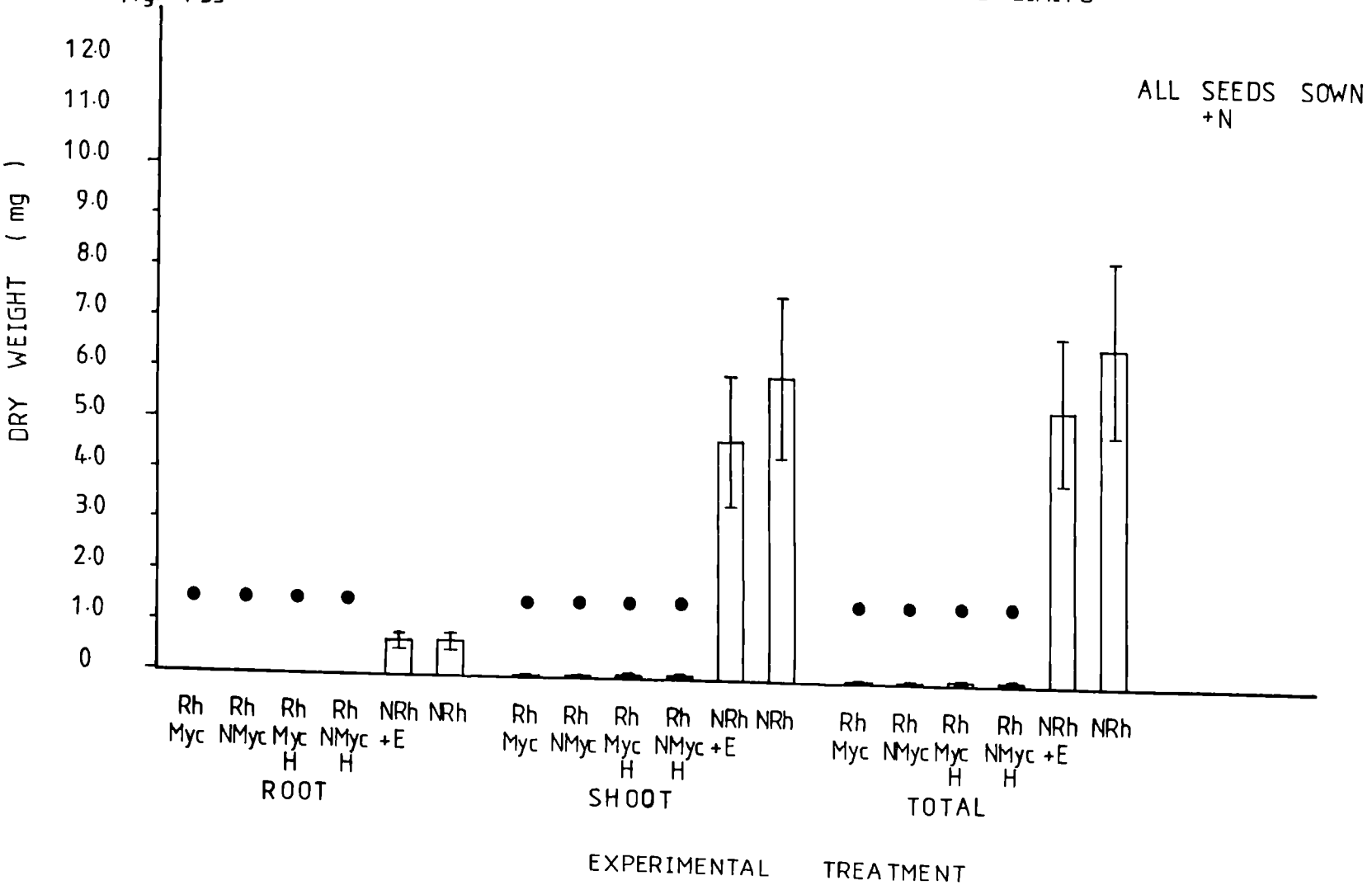


Fig. 4.36 SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

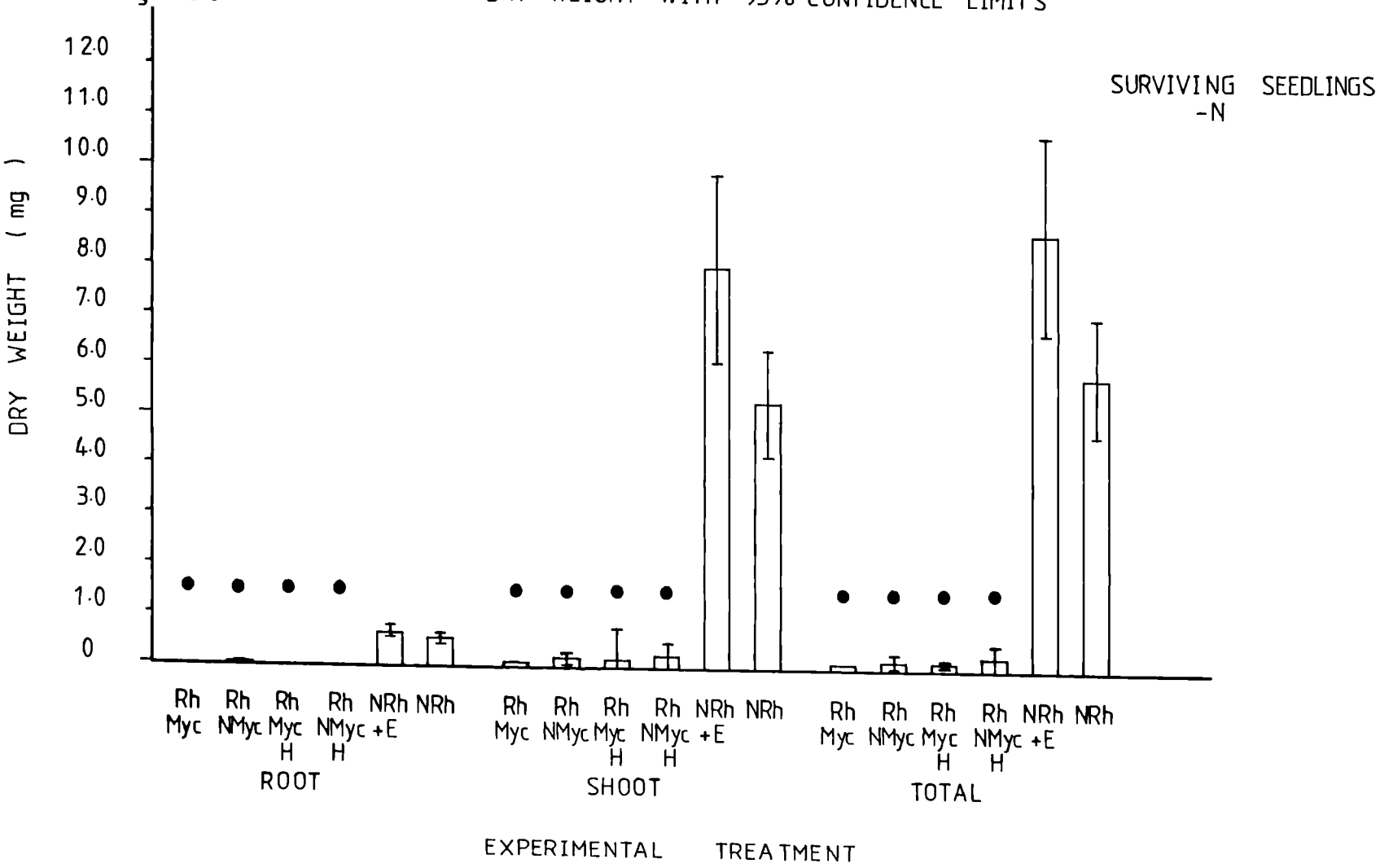


Table 4.4.3.2 Successful germination and subsequent survival of *F. ovina*

Treatment	No. seedlings per dish						
Dish	1	2	3	4	Total	Mean	%
1. +N							
Rh.Myc	2	1	0	1	4	1.00	10.0
Rh.NMyc	0	2	0	1	3	0.75	7.5
Rh.MycH	3	2	1	0	6	1.50	15.0
Rh.NMycH	2	3	2	1	8	2.00	20.0
NRh.+E	8	9	10	7	34	8.50	85.0
NRh.	8	8	10	9	35	8.75	87.5
2. -N							
Rh.Myc	0	1	1	0	2	0.50	5.0
Rh.NMyc	2	0	2	2	6	1.50	15.0
Rh.MycH	1	0	1	0	2	0.50	5.0
Rh.NMycH	1	1	1	1	4	1.00	10.0
NRh.+E	8	9	7	7	31	7.75	77.5
NRh.	8	9	8	10	35	8.75	87.5

Table 4.4.3.3 Summary of the above for different variables

Variable	Mean no. seedlings per dish	
Added nutrient solution	3.75	
Distilled water only	3.33	
Rh.	0.94	
Rh.H	1.25	
NRh.	8.44	
+E	3.29	
-E	3.79	
Rh.Myc and Rh.MycH	0.88	
Rh.NMyc and Rh.NMycH	1.31	
Rh. and Rh.H+N	1.31	) Both include Myc and
Rh. and Rh.H-N	0.88	) NMyc

Table 4.4.3.4 Changes in soil acidity with different experimental treatments

Original pH of the Winterton back-dune soil : 3.55 (Distilled water, 24 hours)
3.25 (Calcium chloride solution, 24 hours)

Treatment	pH in dist.water,24 hrs		pH in Calcium chloride solution, 24 hrs	
Dish	1	2	3	4
1. +N				
Rh.Myc	3.10	3.10	2.95	3.00
Rh.NMyc	3.10	3.10	2.90	2.95
Rh.MycH	3.20	3.20	3.00	2.95
Rh.NMycH	3.10	3.15	3.00	3.00
NRh.+E	3.55	3.40	3.15	3.20
NRh.	3.45	3.50	3.15	3.20
2. -N				
Rh.Myc	3.10	3.00	2.90	2.90
Rh.NMyc	3.10	3.10	2.85	2.95
Rh.MycH	3.10	3.10	2.95	2.90
Rh.NMycH	3.10	3.10	2.90	2.95
NRh.+E	3.60	3.35	3.10	3.10
NRh.	3.50	3.40	3.15	3.20

Table 4.4.3.5 Soil nutrient analysis

The Winterton back-dune soil used for the experiment was analysed for available nitrogen content (as ammonium and as nitrate), available phosphorus, and organic content.

	Untreated field soil	Irradiated soil
Av.nitrogen (Ammonium) :	2.7 ppm.	9.3 ppm.
(Nitrate) :	2.5 ppm.	3.2 ppm.
(Total) :	5.2 ppm.	12.5 ppm.
Av. phosphorus :	-	-
Soil organic content :	14700 ppm. or 1.47%	

4.4.3.3 Experiment 5

The survival of pre-germinated Festuca seedlings was reduced from 90% (NRh.) and 95% (Rh.H-R) to 55% for Rh.H+R and 25% for Rh. (Figure 4.38).

WIth added nutrients, all treatments produced survival of 50 or 55% (Figure 4.38). Survival was therefore increased in pots with live Rhododendron, unchanged in pots with Rhododendron roots and reduced in the other two treatments. (This reduction was almost certainly due to increased growth of Festuca seedlings resulting in increased intra-specific competition.)

Due to difficulties experienced with the non-Rhododendron control soil (NRh.) collected from the field (i.e. poor growth of test seedlings), RhH-R was taken as the control for statistical analysis.

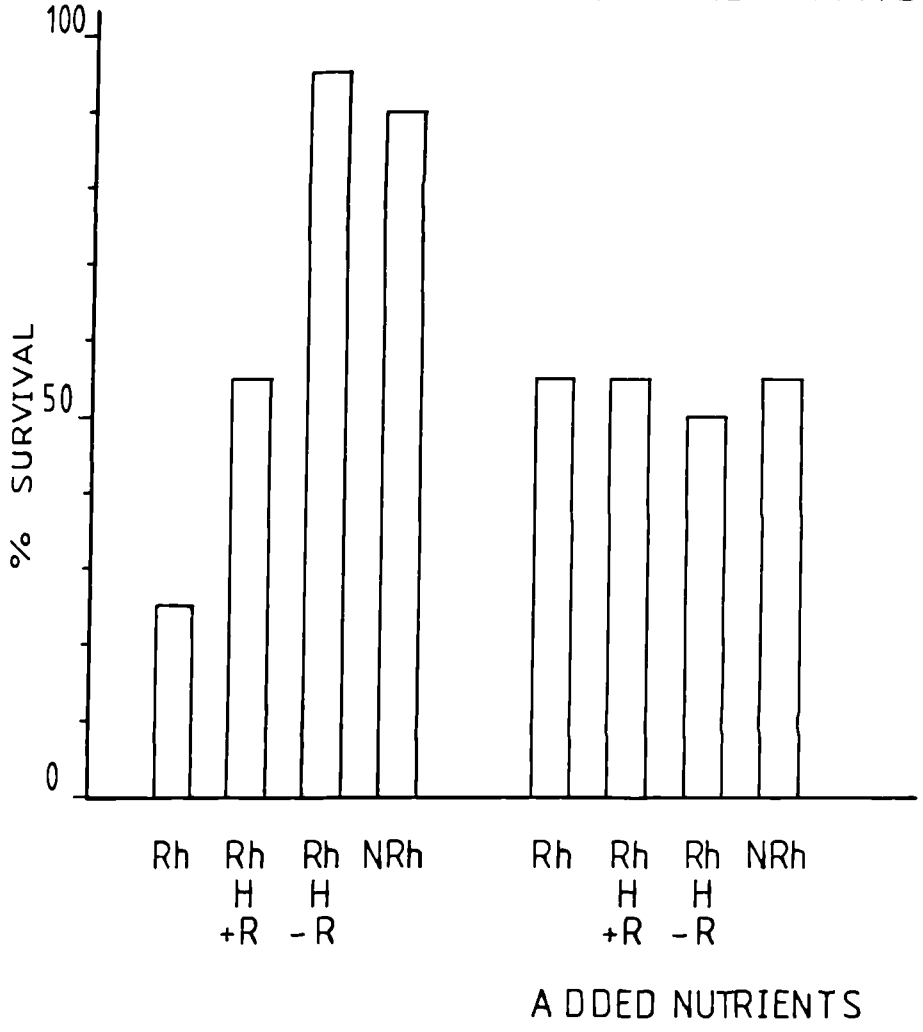
Presence of live Rhododendron or dead Rhododendron roots produced highly significant reductions of seedling R, S and T (Figures 4.39 and 4.41). The reduction was less significant when both control and other conditions had added nutrients (Figures 4.40 and 4.42).

Addition of nutrients resulted in increased production under all conditions (Figures 4.39 and 4.40, 4.41 and 4.42). This was only significant for pots with live Rhododendron and with non-Rhododendron soil. Increases in the pots with Rh.H+R and Rh.H-R were not significant. This was probably due to the intra-specific competition of Festuca seedlings producing some very large and some very small seedlings and hence very high standard deviations.

Soils analysed from each experimental treatment had very low levels of 'available' nitrogen and phosphorus (Table 4.4.3.6).

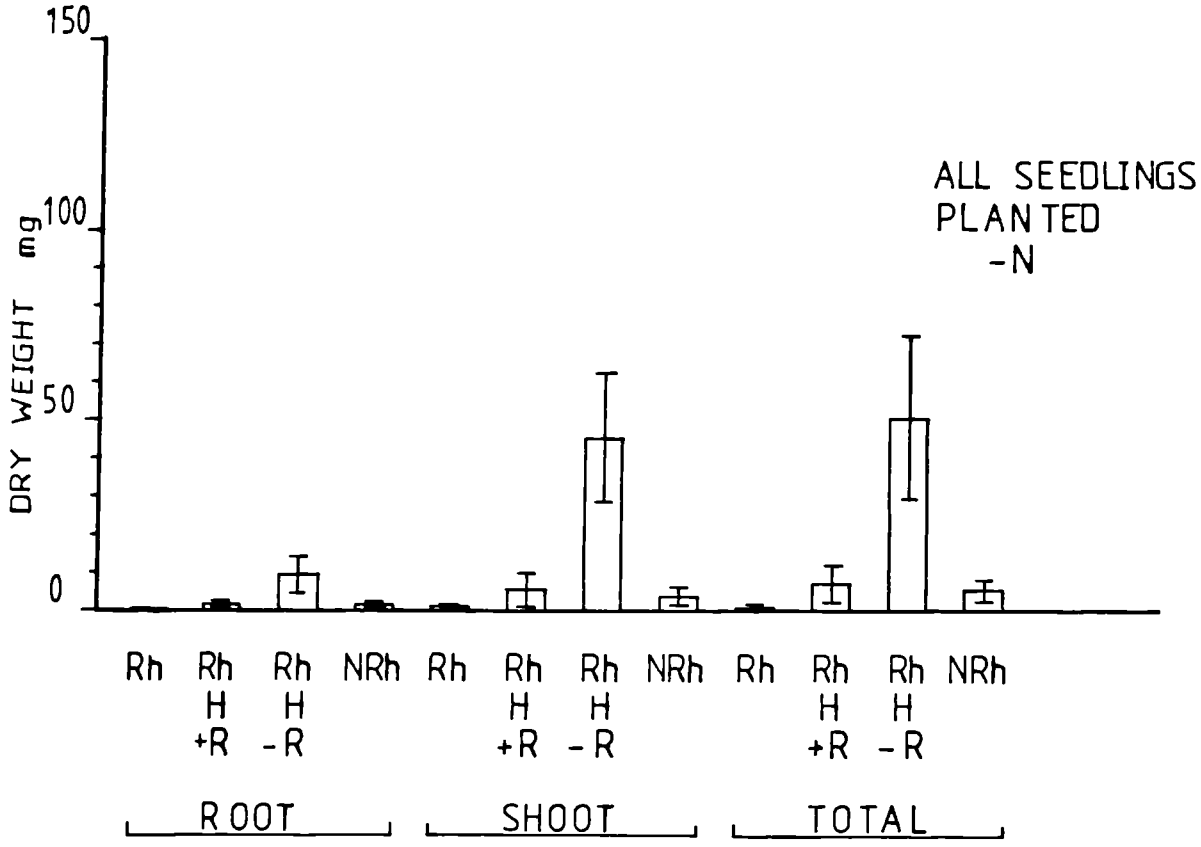
As in Experiment 4 (4.4.3.2), the presence of Rhododendron in the soil led to a decrease in pH (in this case from 3.70 to 3.40) (Table 4.4.3.7). Harvesting the Rhododendron seedlings (+R or -R) gave an increase in soil pH above that in the Rhododendron pots. Addition of nutrients produced a rise in pH, but the NRh. soil was still the least acid.

Fig. 4.38 PERCENTAGE OF SEEDLINGS PLANTED STILL ALIVE AT THE HARVEST



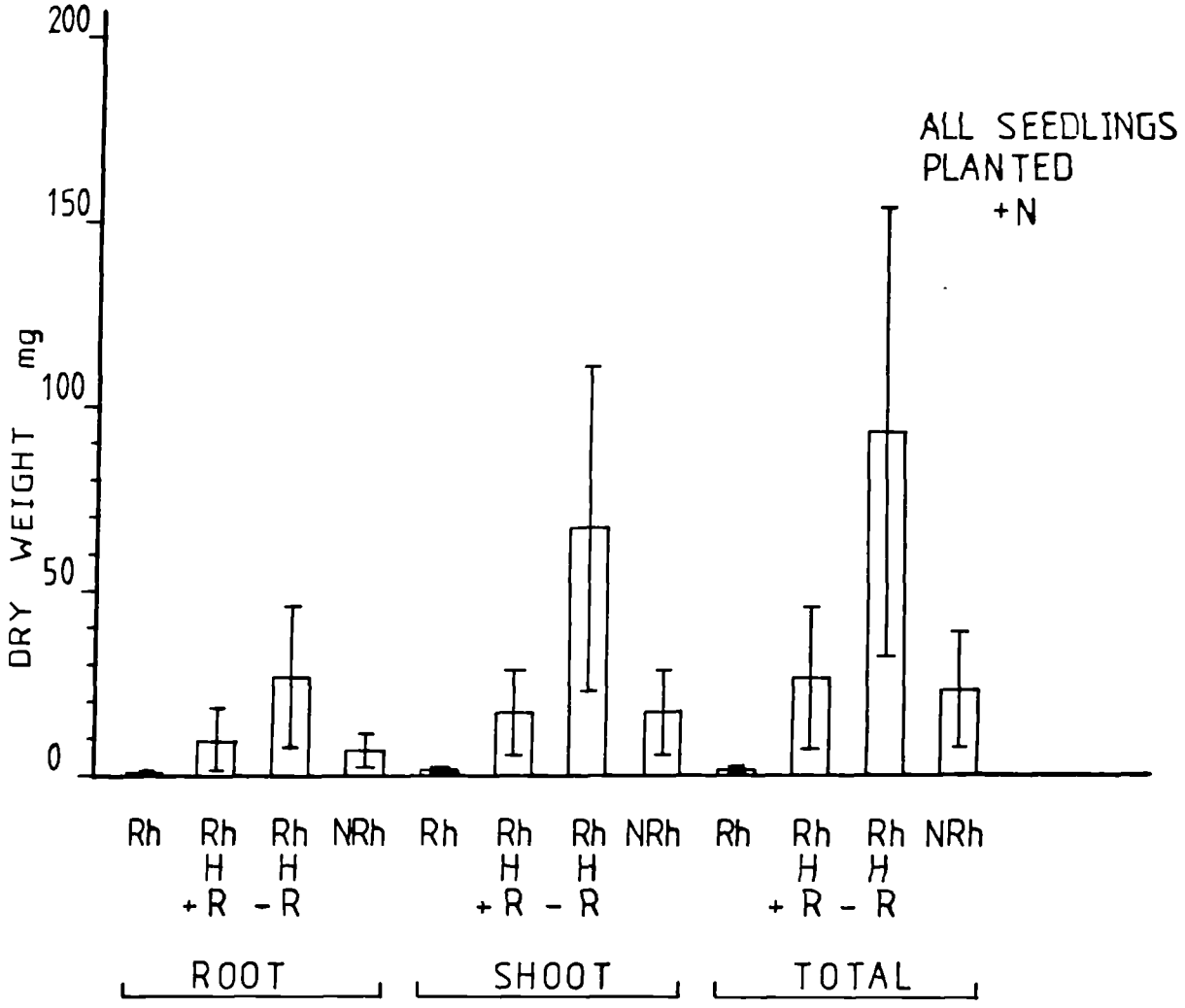
SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Fig. 4.39



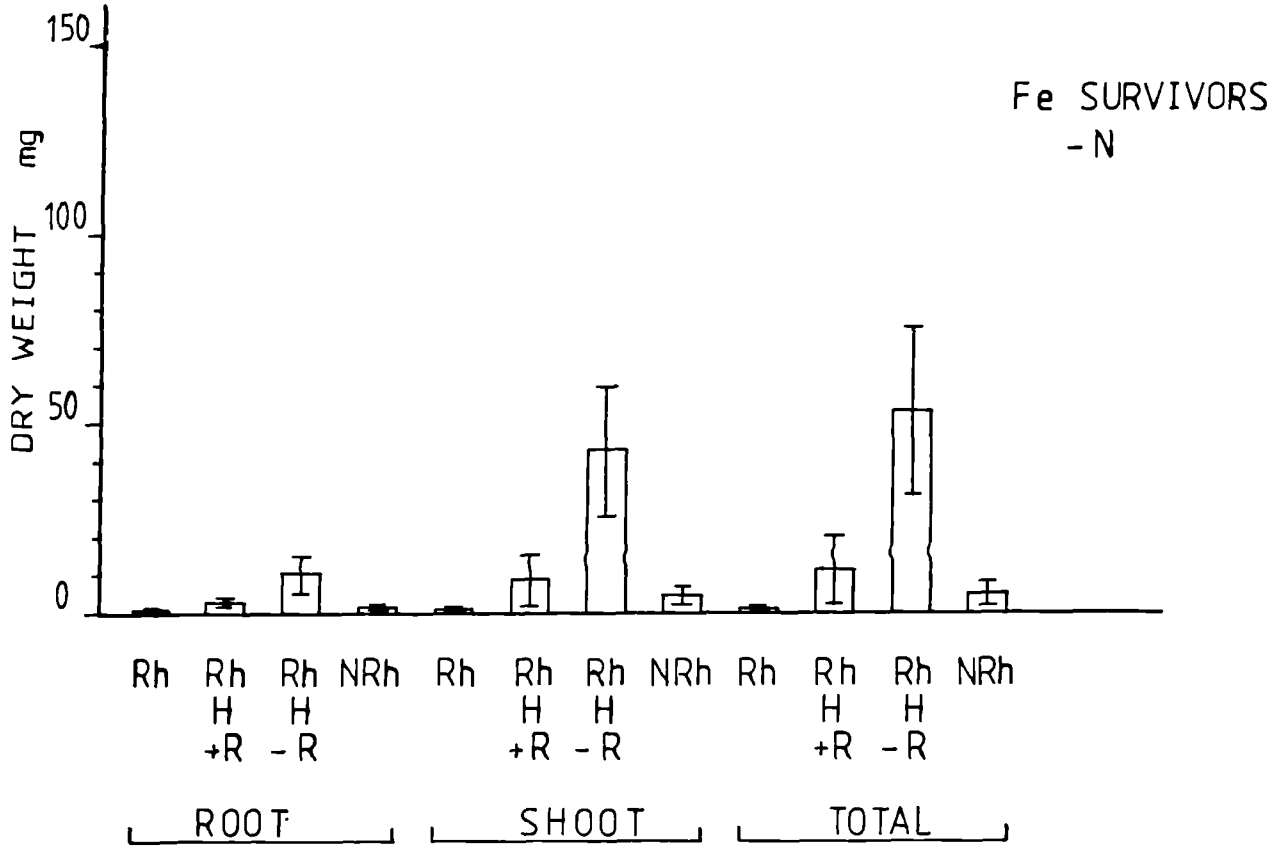
SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Fig. 4.40



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Fig. 4.41



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Fig. 4.42

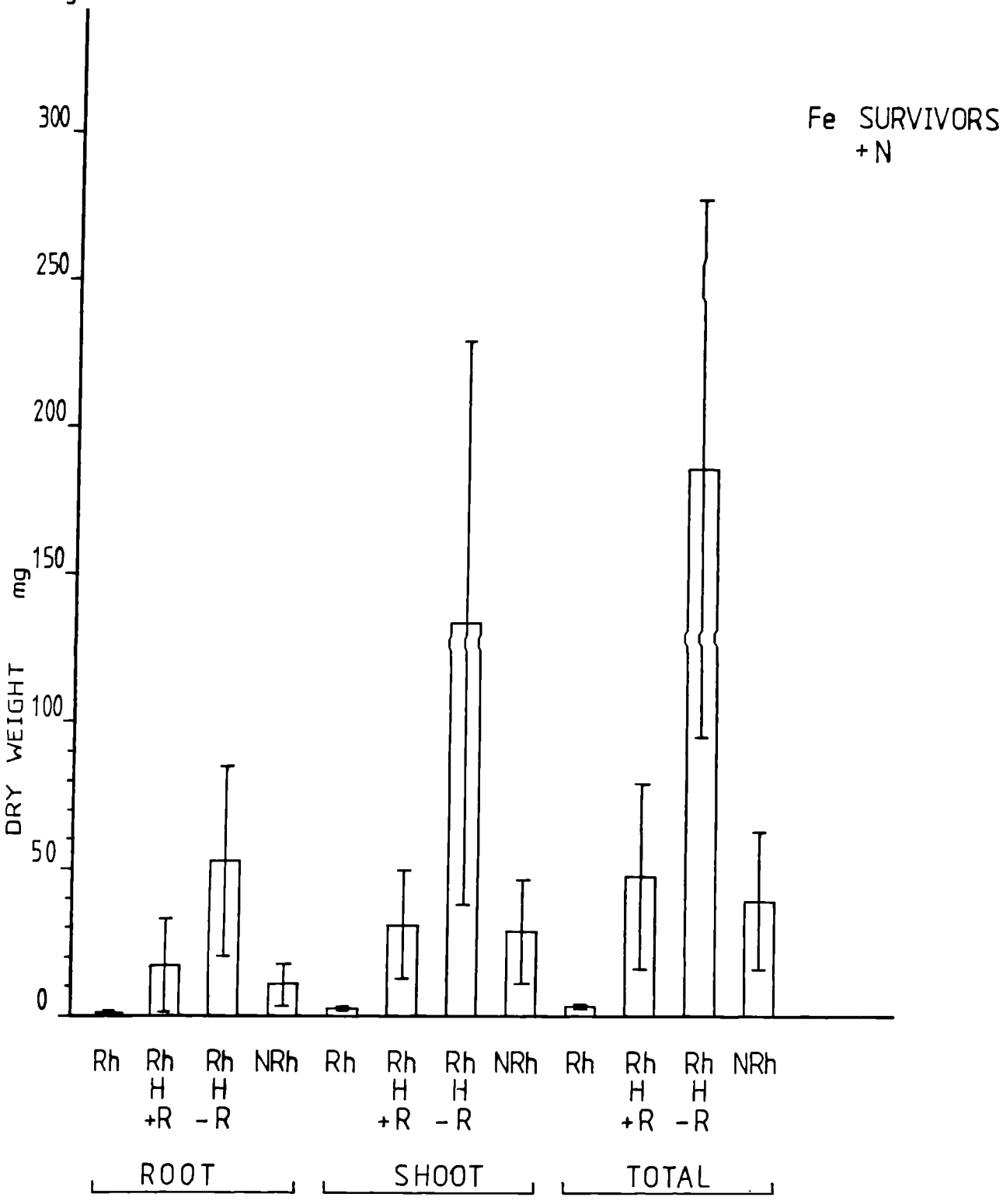


Table 4.4.3.6 Soil analysis

(All values in ppm.)

Treatment	Av.P.	Av.Ammonium-N	Av.Nitrate-N	Total Av.N
Rh.	-	0.7	0.2	0.9
Rh.H+R	1.0	0.3	-	0.3
Rh.H-R	0.7	1.0	0.3	1.3
NRh.	0.3	0.8	0.1	1.0
Rh.+N	0.4	-	1.9	1.9
Rh.H+R+N	0.5	-	0.4	0.4
Rh.H-R+N	0.7	1.1	0.7	1.8
NRh.+N	0.6	1.2	1.6	2.8

('N' in the 'Treatment' column refers to Robbins' solution, in the other headings to nitrogen.)

Table 4.4.3.7 Soil acidity for the different treatments

Treatment	pH in distilled water after 24 hours	pH in calcium chloride (aq.) after 24 hours
Rh.	3.40	3.30
Rh.H+R	3.65	3.40
Rh.H-R	3.65	3.40
NRh.	3.80	3.50
Rh.+N	3.70	3.40
Rh.H+R+N	3.80	3.50
Rh.H-R+N	3.65	3.45
NRh.+N	4.15	3.70
Original pH of soil	3.70	3.50

4.4.3.4 Experiment 6

4.4.3.4.1 Effects on *Rumex acetosa*

4.4.3.4.1.1 Experiment 6a (Figures 4.43 and 4.44)

Nutrient addition significantly increased R, S and T of Rumex for all NRh. pots. Increases for Rh.NMyc and Rh.Myc pots were either not significant or were less significant than for the NRh. pots.

Without added nutrients, R, S and T in Rh.Myc pots and R and T in Rh.NMyc pots were significantly reduced compared to the NRh. controls. When nutrients were added, these reductions in R, S and T dry weights were highly significant for both Rh.Myc and Rh.NMyc.

Mycorrhizal infection of the Rhododendron significantly reduced test seedling yield below that with non-mycorrhizal Rhododendron, in only one case. This was a decrease in S and T for 2N addition.

4.4.3.4.1.2 Experiment 6b (Figures 4.45, 4.46 and 4.47)

Only nutrient addition at the 10N level produced a significant increase in yield of the controls. For Rh.H-R and Rh.H+R pots the results were variable, with some significant reductions in growth with 1N and 2N addition, but both showing significant increases with 10N.

All Rhododendron treatments without added nutrients gave reduced R, S and T compared to the NRh. control. These were not significant for Rh.H-R, but were increasingly significant for Rh.H+R and Rh. With added nutrients these reductions were highly significant for both Rh.H-R and Rh.H+R.

Harvesting of Rhododendron resulted in increased R, S and T over the Rh. control, though this was not significant. Removal of roots by sieving, following harvesting of Rhododendron led to a highly significant increase in R, S and T of Rumex seedlings. The increases in R, S and T from pots with Rh.H-R compared to those with Rh.H+R were significant when no nutrients were added.

4.4.3.4.1.3 Experiment 6c (Figures 4.48, 4.49, 4.50, 4.51, 4.52 and 4.53)

Adding nutrients to the NRh. control pots produced variable results for 1N and 2N, but highly significant increases at 10N. The results of nutrient addiditon to Rh.H-R pots were variable and insignificant, but to Rh.H+R pots they were generally significant increases in yield.

All Rhododendron treatments without added nutrients led to reduced R, S and T compared to the NRh. controls. This was significant for Rh.H+R and Rh.

Rh.H-R and Rh.H+R both gave significant reductions in seedling growth compared to the NRh. controls, for 1N and most significantly for 10N. Growth increased, but not significantly for 2N.

Sieving of Rhododendron soil significantly increased the yield of surviving Rumex seedlings compared to the Rh. control. Growth on unsieved Rhododendron soil was not significantly different from the Rh. control.

In pots with Rhododendron harvested, sieving led to significantly increased yield when there was no nutrient addition. The effects of sieving when nutrients were added, were not significant.

4.4.3.4.2 Effects on *Festuca ovina*

4.4.3.4.2.1 Experiment 6a (Figures 4.54 and 4.55)

There was no significant increase in yield when nutrients were added to the NRh. control. Seedling growth was increased significantly by nutrient addition to Rh.Myc pots. S and T were significantly increased by 2N addition to Rh.NMyc.

Rhododendron soils (Rh.Myc and Rh.NMyc), with or without added nutrients, all produced significant reductions in R, S and T compared to the NRh. controls.

Mycorrhizal infection of Rhododendron plants did not produce any significant difference in test seedling yield compared to non-mycorrhizal Rhododendron.

4.4.3.4.2.2 Experiment 6b (Figures 4.56, 4.57, 4.58, 4.59, 4.60 and 4.61)

Addition of nutrients to the NRh. controls increased Festuca dry weight, but only significantly at 10N levels. The same applied to Rh.H-R pots. The increase in test seedling dry weight on Rh.H+R soils was significant at 2N and more so at 10N.

Pots with Rh.-N and Rh.H+R-N significantly reduced yield below that of the NRh.-N control.

Differences in yield between seedlings in Rh.H-R pots and the NRh. controls, both with added nutrients, were generally significant. Rh.H+R soils with added nutrients significantly reduced S and T of the test seedlings below the controls.

Without added nutrients, Rh.H+R resulted in increased yield compared to the Rh. control, but this was not significant. When the roots were removed (Rh.H-R), the increase was significant.

With Rhododendron harvested, sieving of roots significantly increased yield above that of pots with dead roots remaining, for -N, 1N and 2N (S only).

4.4.3.4.2.3 Experiment 6c (Figures 4.62, 4.63, 4.64, 4.65, 4.66 and 4.67)

Significant increases in yield with nutrient addition to the NRh. controls occurred only at 10N levels. At 1N there was a significant decrease in seedling growth. Nutrients added to Rh.H-R led to significantly increased R, S and T. For Rh.H+R a significant increase occurred only with 10N addition.

All Rhododendron soils (Rh., Rh.H+R and Rh.H-R) without added nutrients, gave significant decreases in yield compared to the NRh. control. This was least significant for Rh.H-R. With nutrient addition the differences were variable and generally not significant. At 10N levels, Rh.H-R significantly reduced seedling dry weight.

Harvesting the Rh.H+R did not significantly increase yield compared to that on soil with Rh. Rh.H-R did give a significant increase.

The increases in seedling dry weights in pots with Rh.H-R, compared to those with the Rh.H+R, were significant for S and T at -N, 1N and 2N levels.

4.4.3.4.3 Effects on *Trifolium repens*

4.4.3.4.3.1 Experiment 6a (Figures 4.68 and 4.69)

Yield of Trifolium was significantly increased by adding nutrients to the NRh. controls. Nutrient addition had no significant effect on yield in Rh.NMyc pots but increased it for Rh.Myc at 2N.

With added nutrients, both Rh.Myc and Rh.NMyc gave significant decreases in R, S and T compared to the NRh. controls.

Mycorrhizal infection of the Rhododendron plants did not significantly affect test seedling yield.

All the soil samples analysed for 'available' nitrogen and phosphorus following the first harvest gave very low results (Table 4.4.3.8). Soil acidity was also measured after this harvest (Table 4.4.3.9) and after the second and third harvests (Table 4.4.3.10). As in the earlier experiments (4.4.3.2 and 4.4.3.3), the presence of Rhododendron was associated with a fall in soil pH. This occurred with both mycorrhizal and non-mycorrhizal plants, with and without added nutrients. Harvesting and removal of roots may have alleviated the effect to some extent (Table 4.4.3.10).

The effects of experimental treatments on the survival of test seedlings showed few obvious trends (Tables 4.3.3.11 and 4.3.3.12). In experiment 6a, survival of Trifolium was clearly increased with nutrient addition.

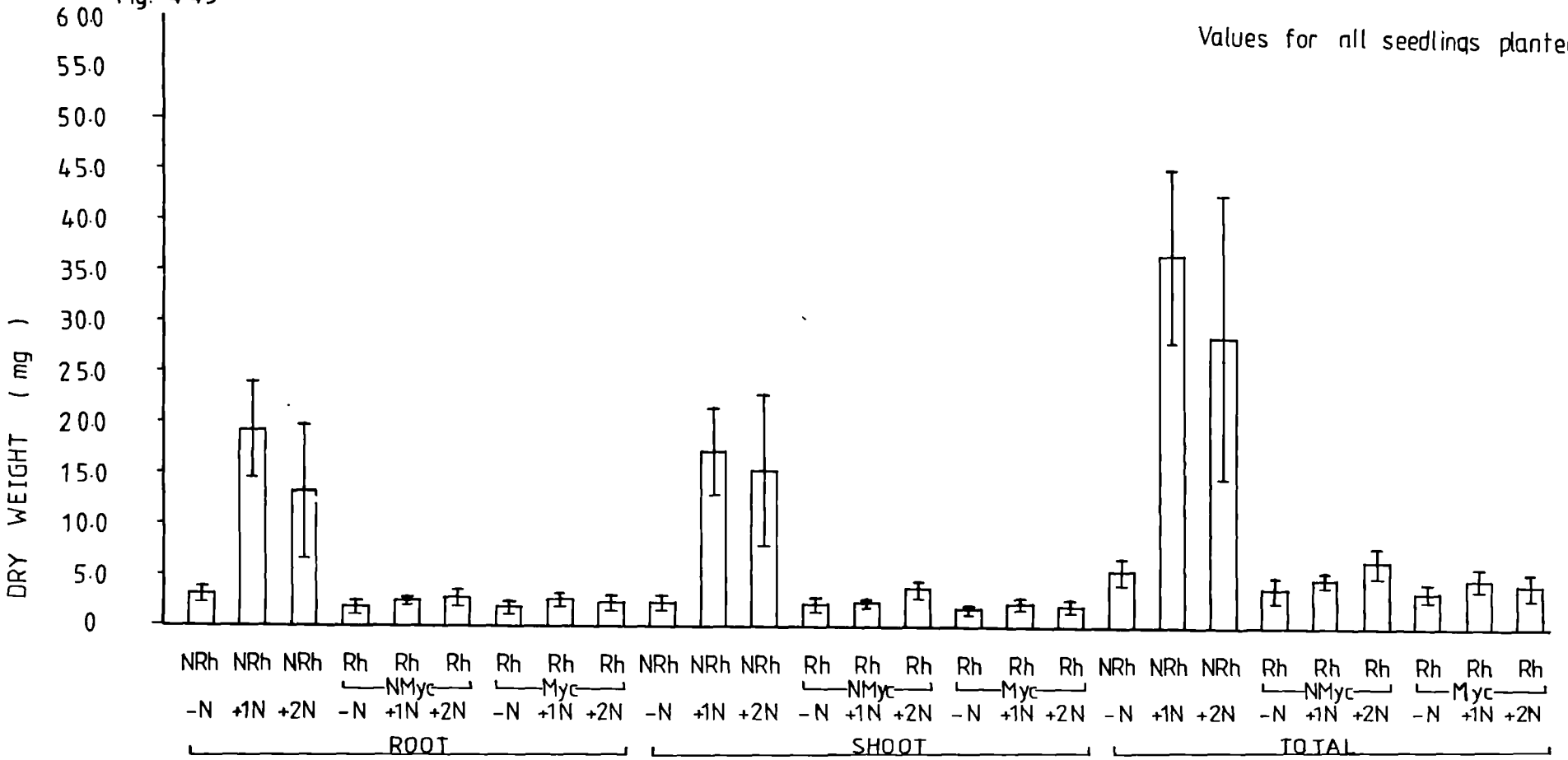
SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

6 A

Rumex acetosa

Fig. 4.43

Values for all seedlings planted

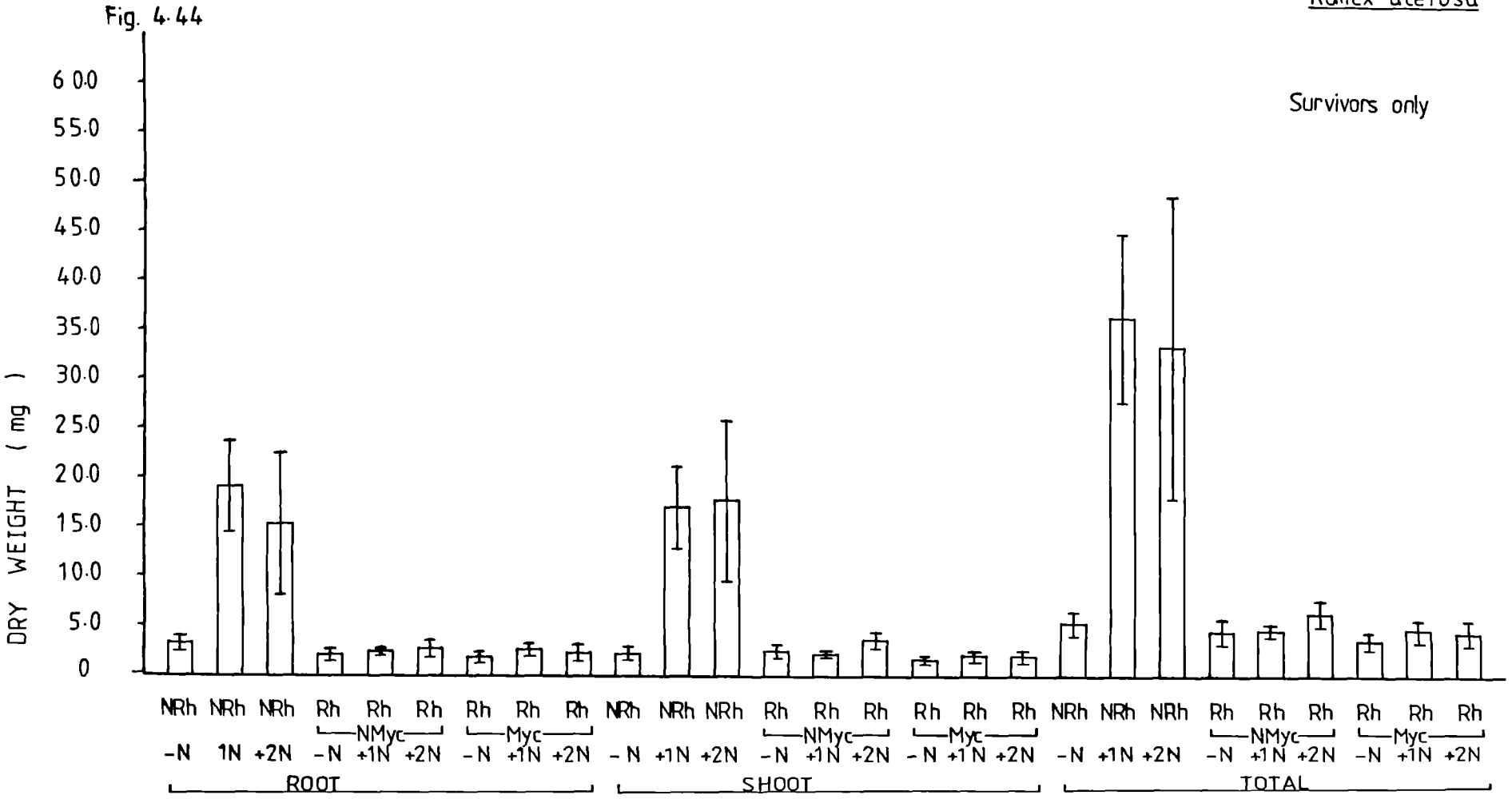


SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

6 A

Rumex acetosa

Fig. 4.44



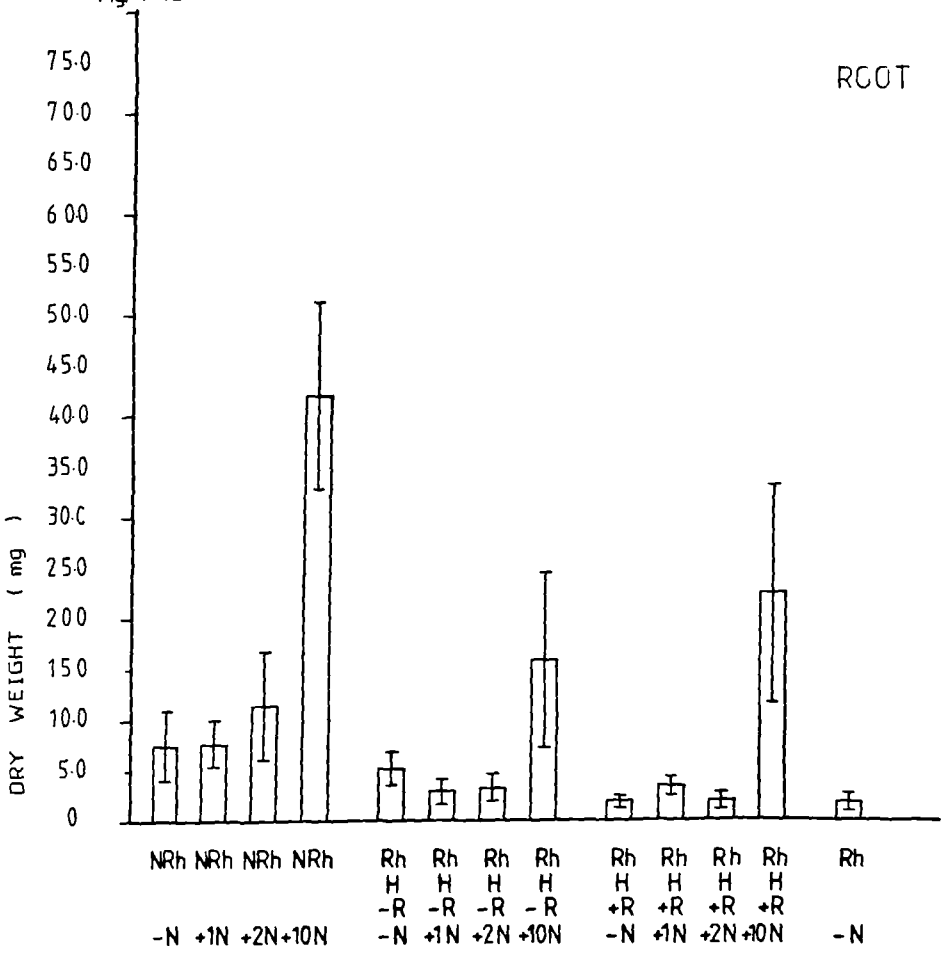
SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

6 B

Rumex acetosa

Values for all seedlings planted

Fig. 4.45



SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

6 B

Rumex acetosa

Values for all seedlings planted

SHOOT

Fig. 4.46

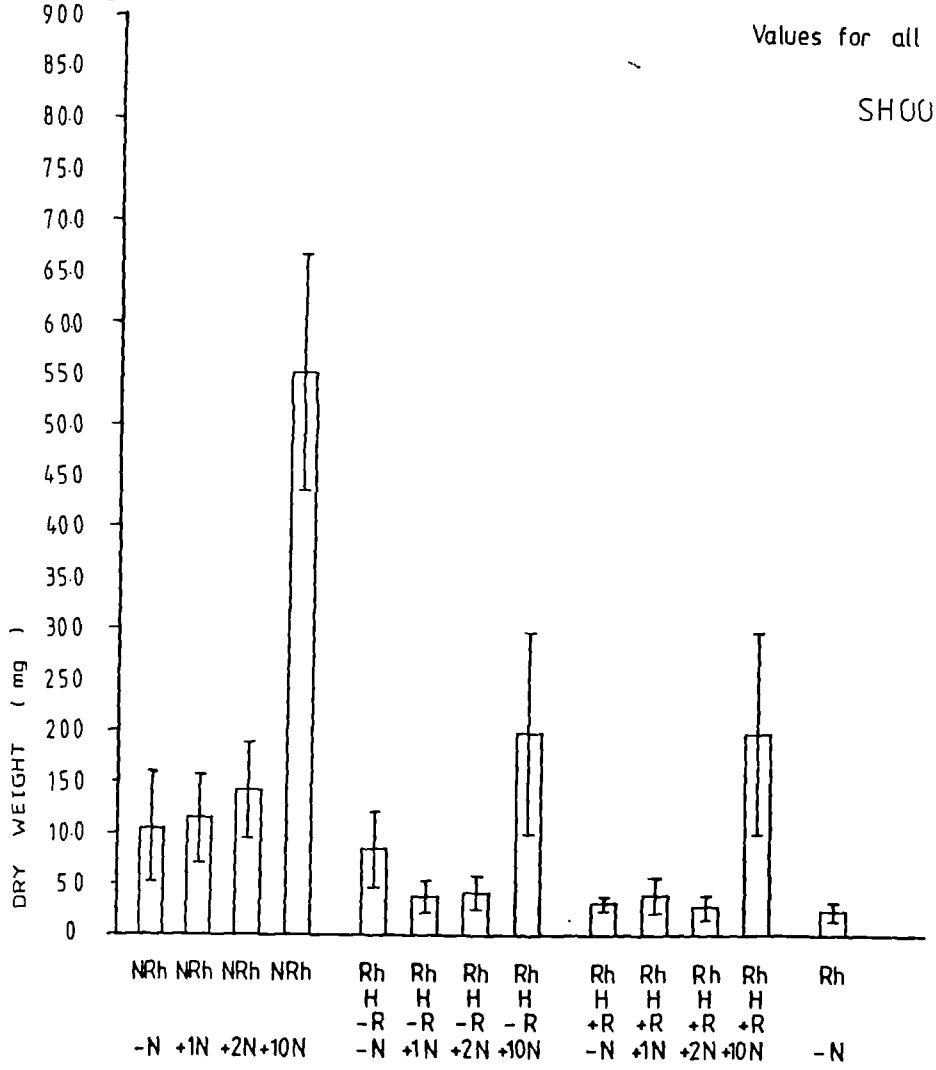


Fig 4.47

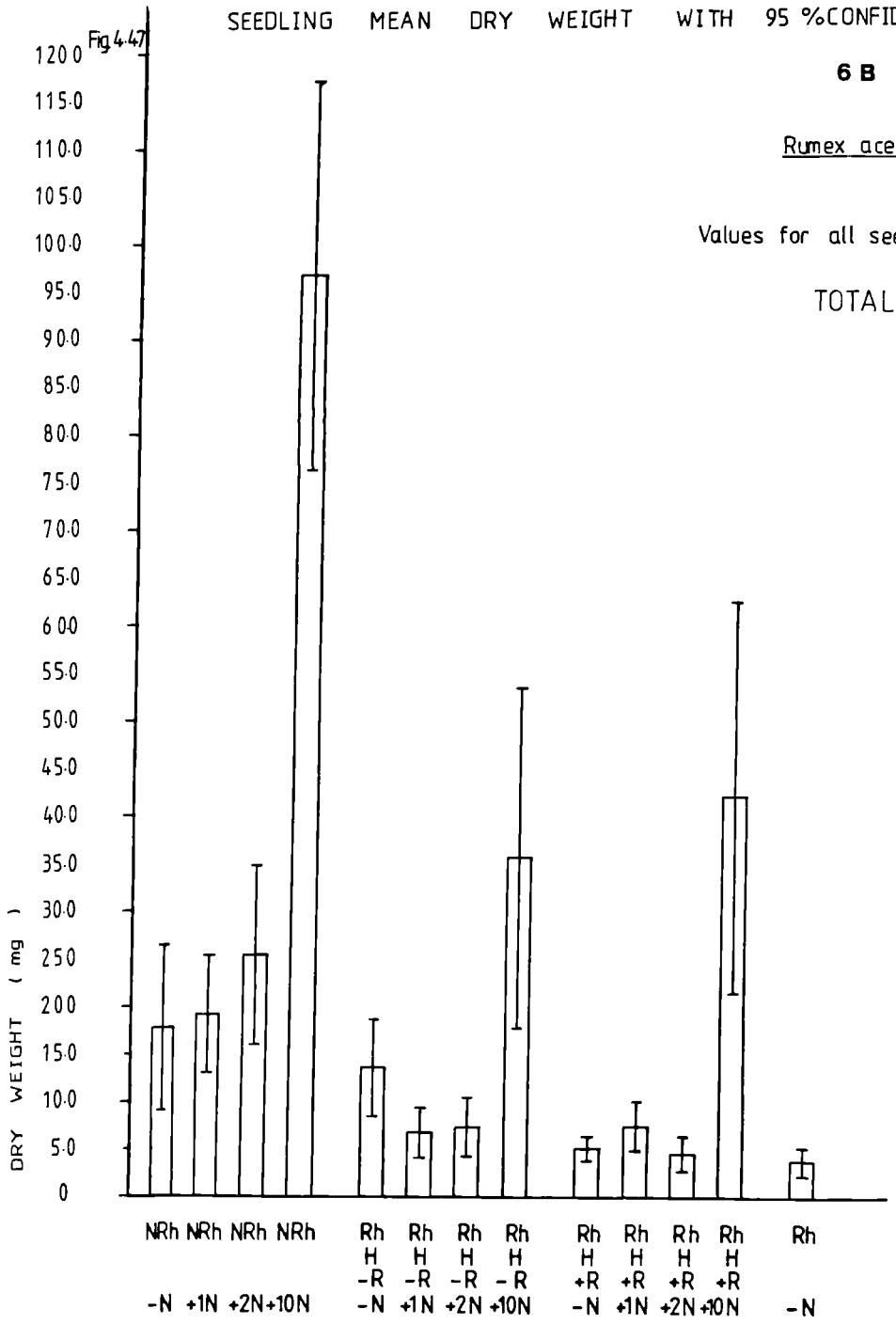
SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

6 B

Rumex acetosa

Values for all seedlings planted

TOTAL



SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

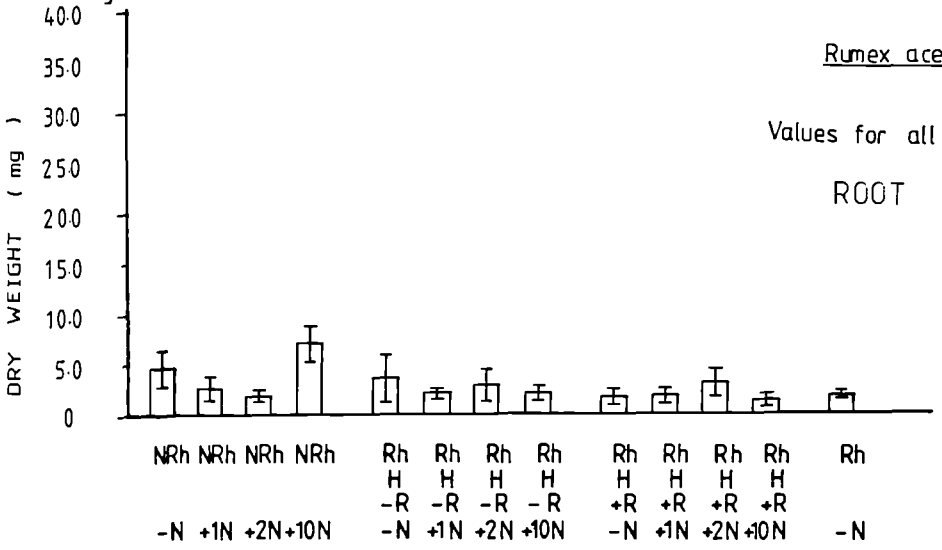
Fig. 448

6 C

Rumex acetosa

Values for all seedlings planted

ROOT



SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

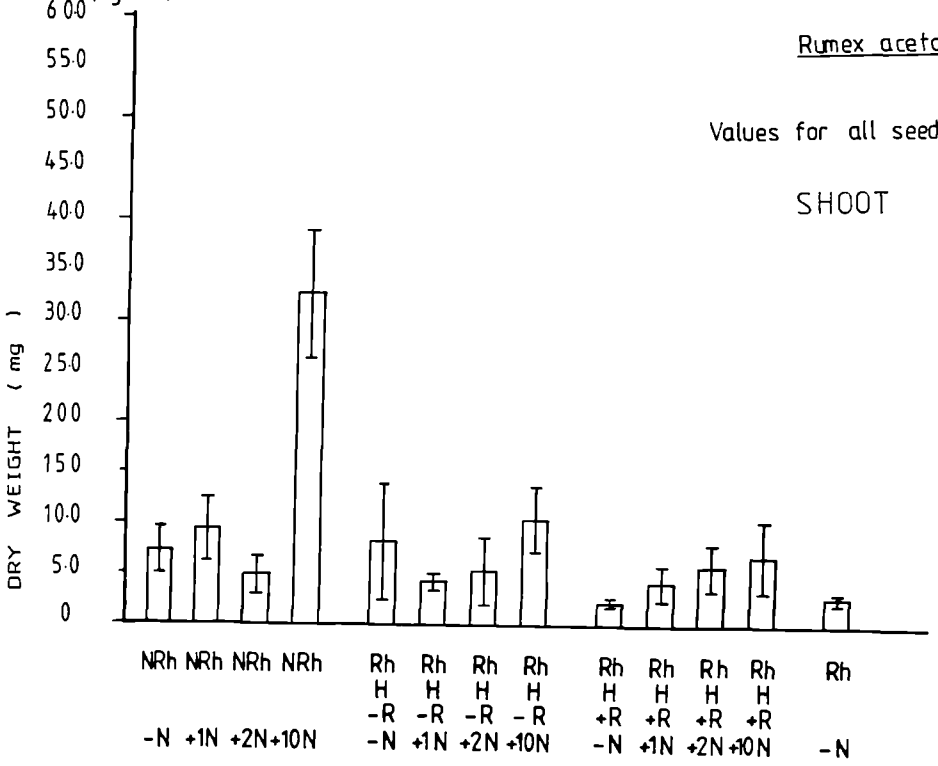
Fig. 449

6 C

Rumex acetosa

Values for all seedlings planted

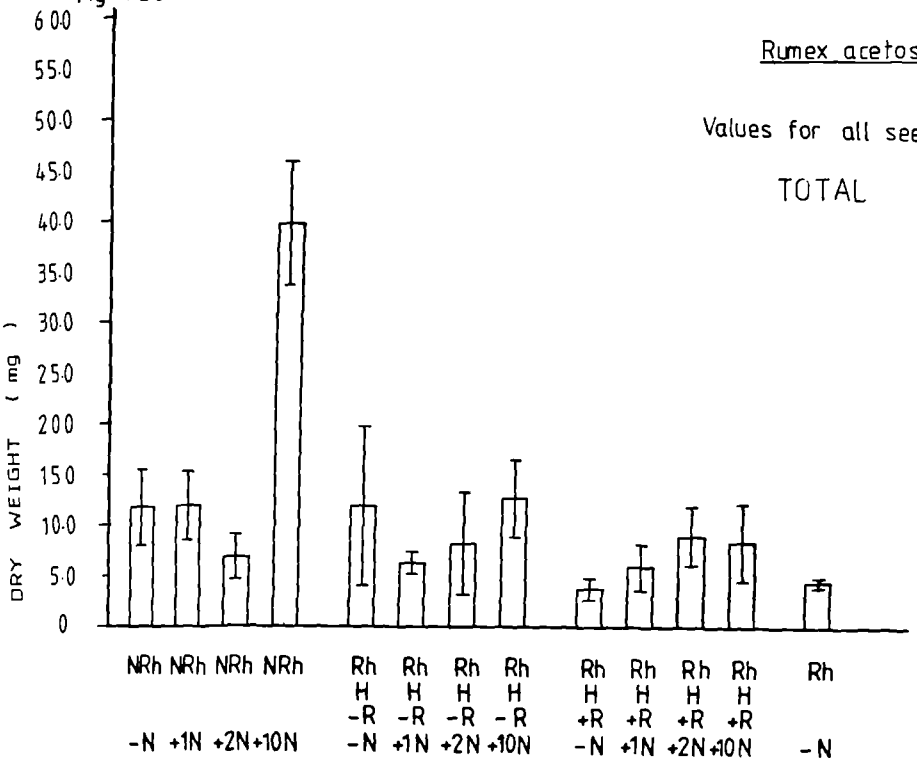
SHOOT



SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

6 C

Fig. 4.50



<u>Rumex acetosa</u>

Values for all seedlings planted

TOTAL

SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

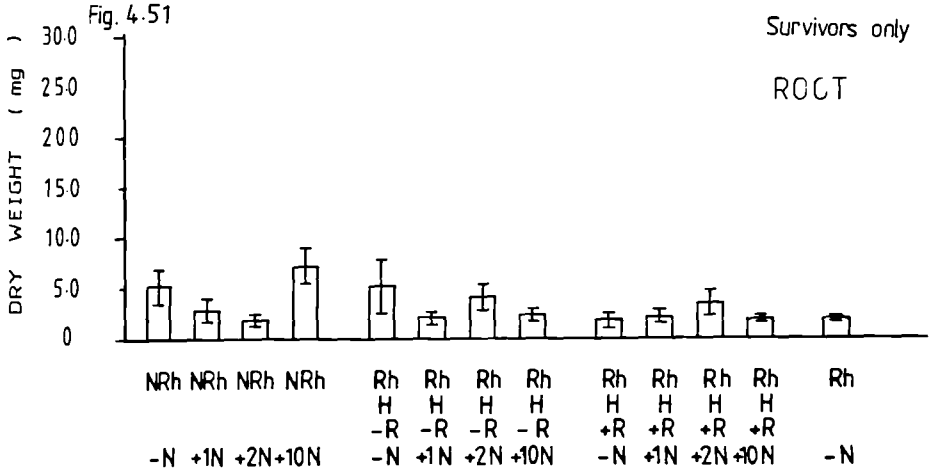
6 C

Rumex acetosa

Fig. 4.51

Survivors only

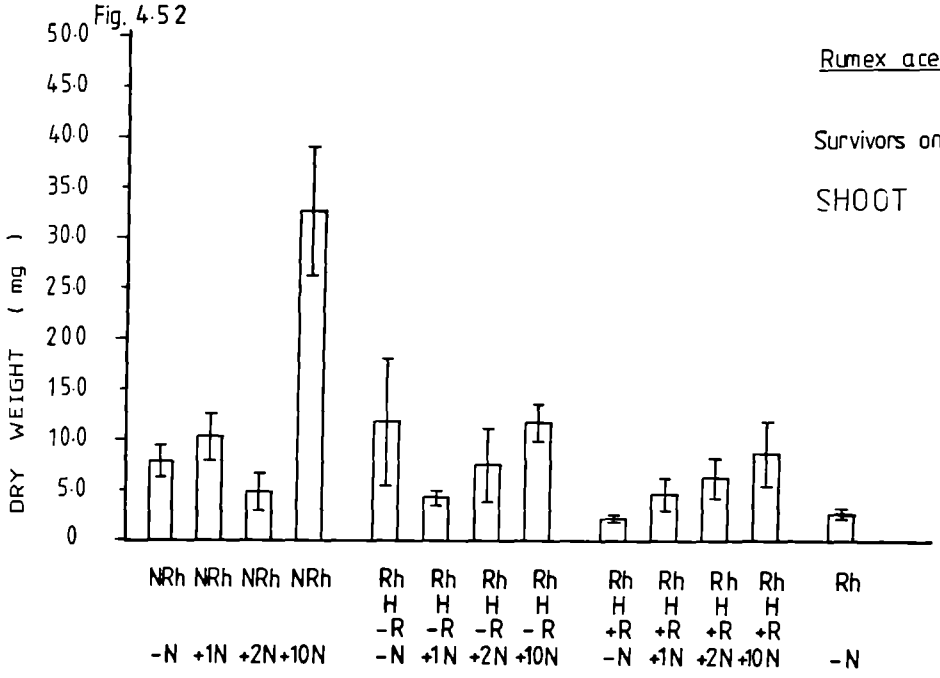
ROOT



SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

6 C

Fig. 4.52



SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

6 C

Fig. 4.53

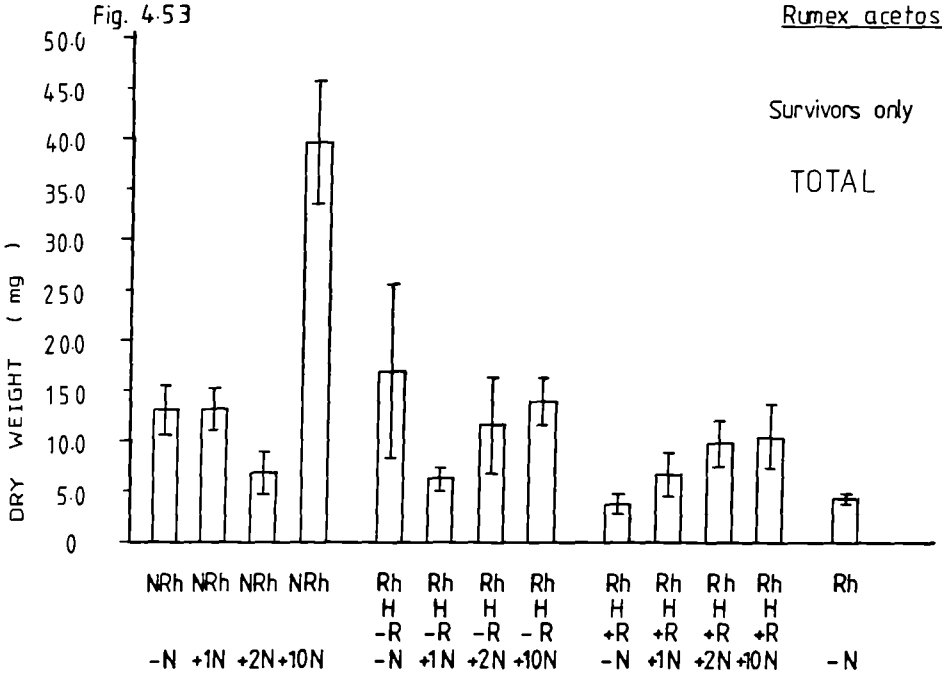


Fig. 4.54 SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

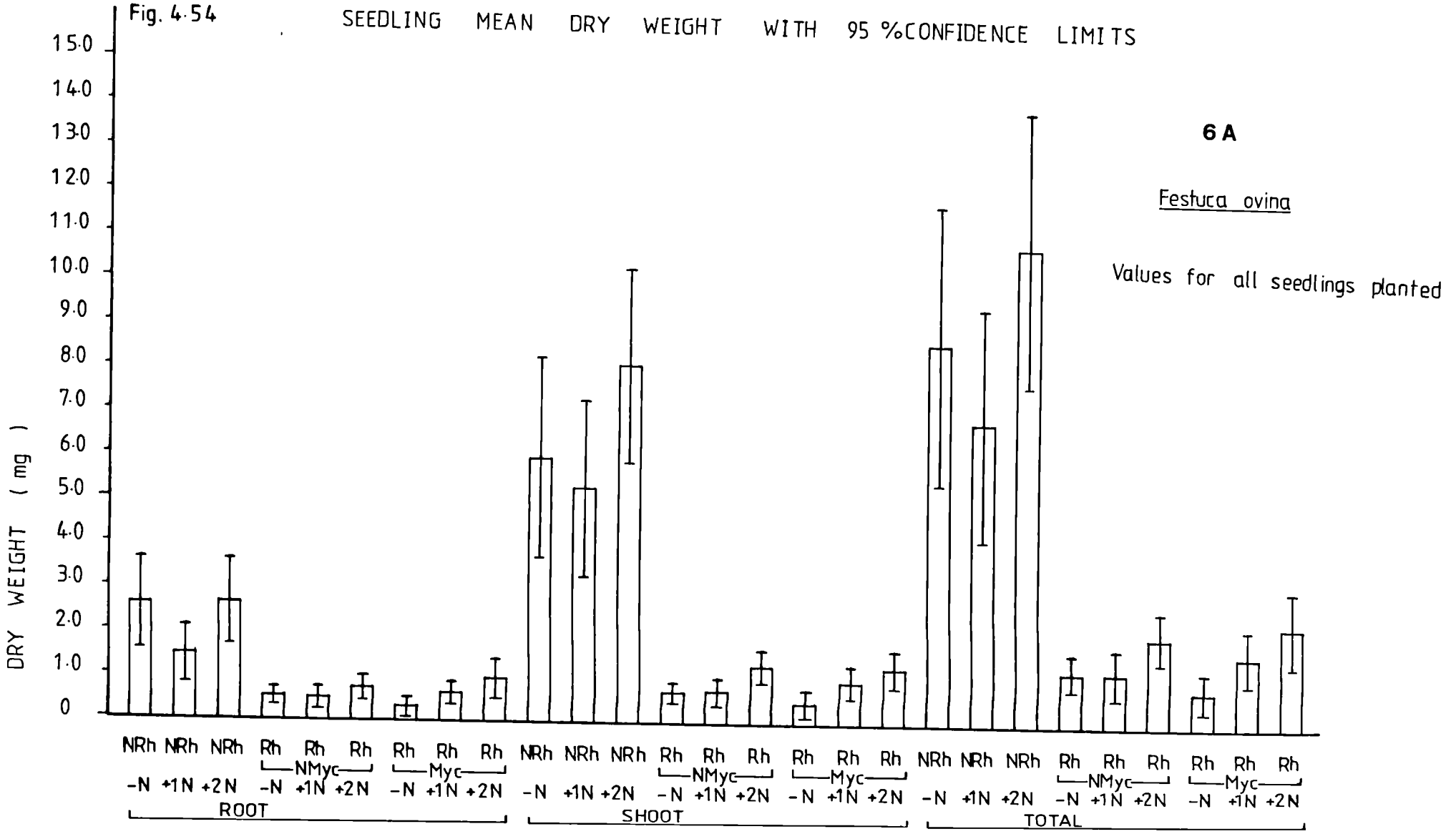
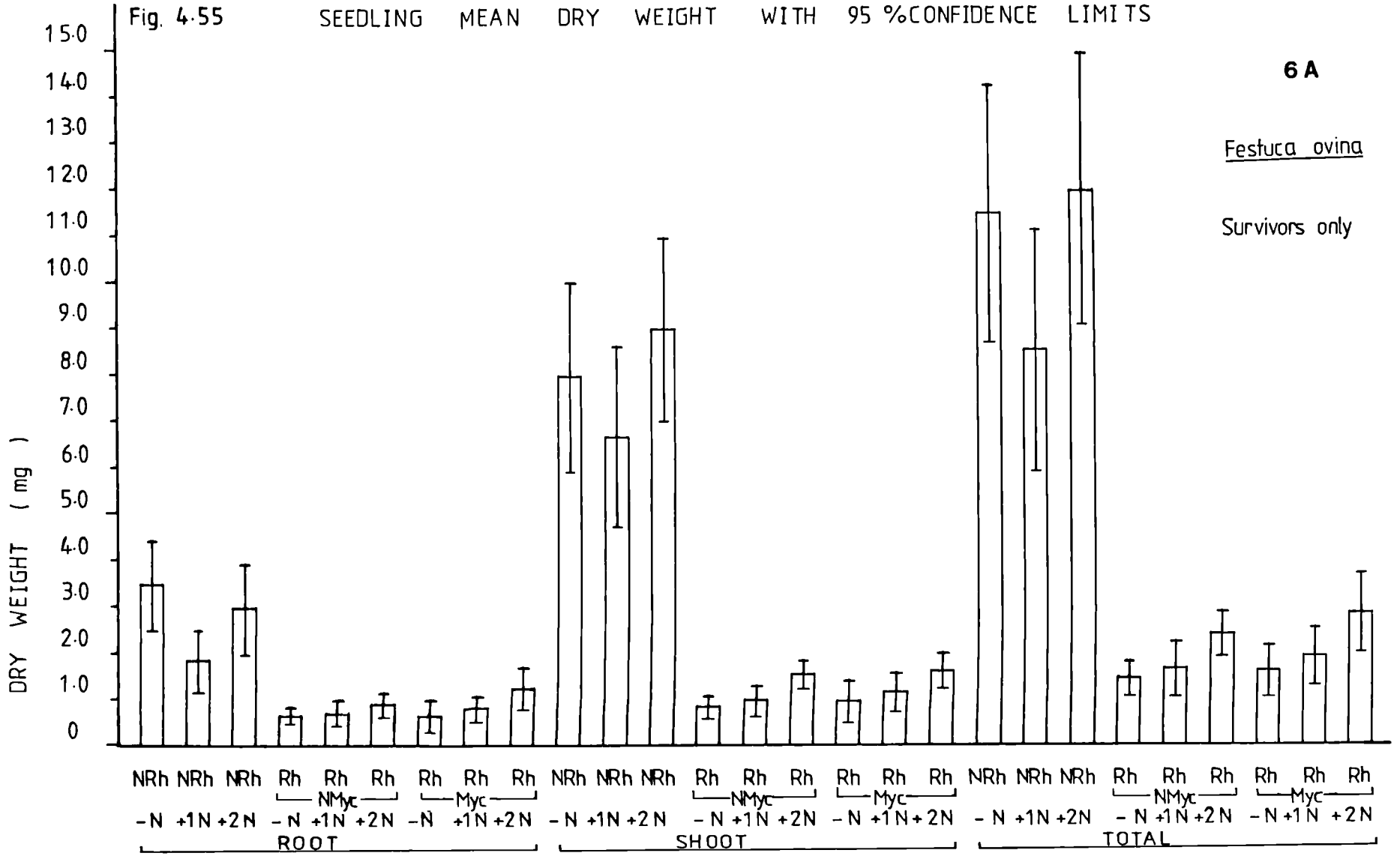


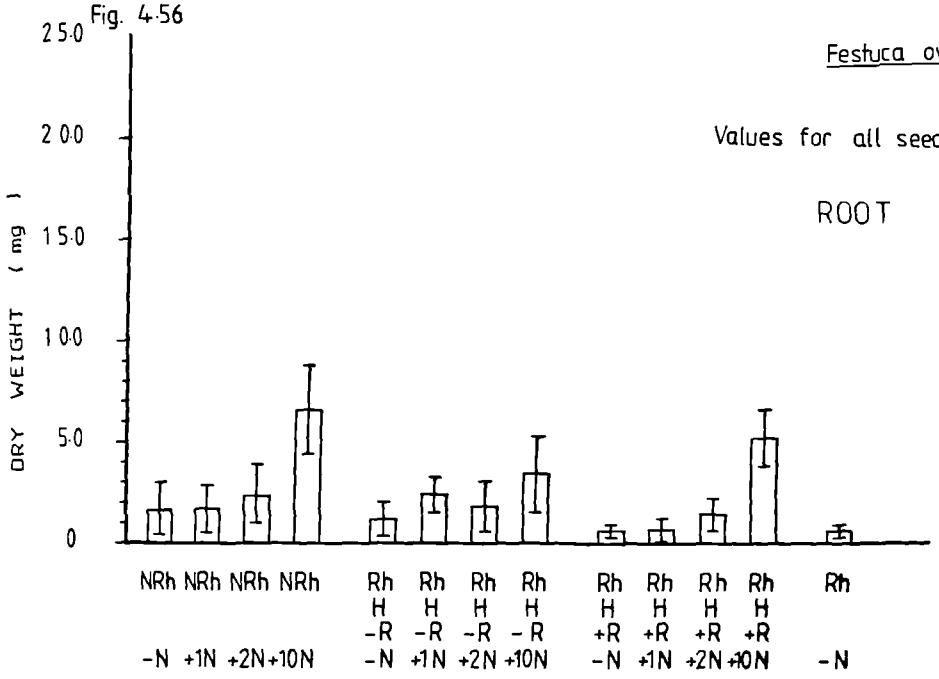
Fig. 4.55 SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS



SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

6 B

Fig. 4·56



Festuca ovina

Values for all seedlings planted

ROOT

SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

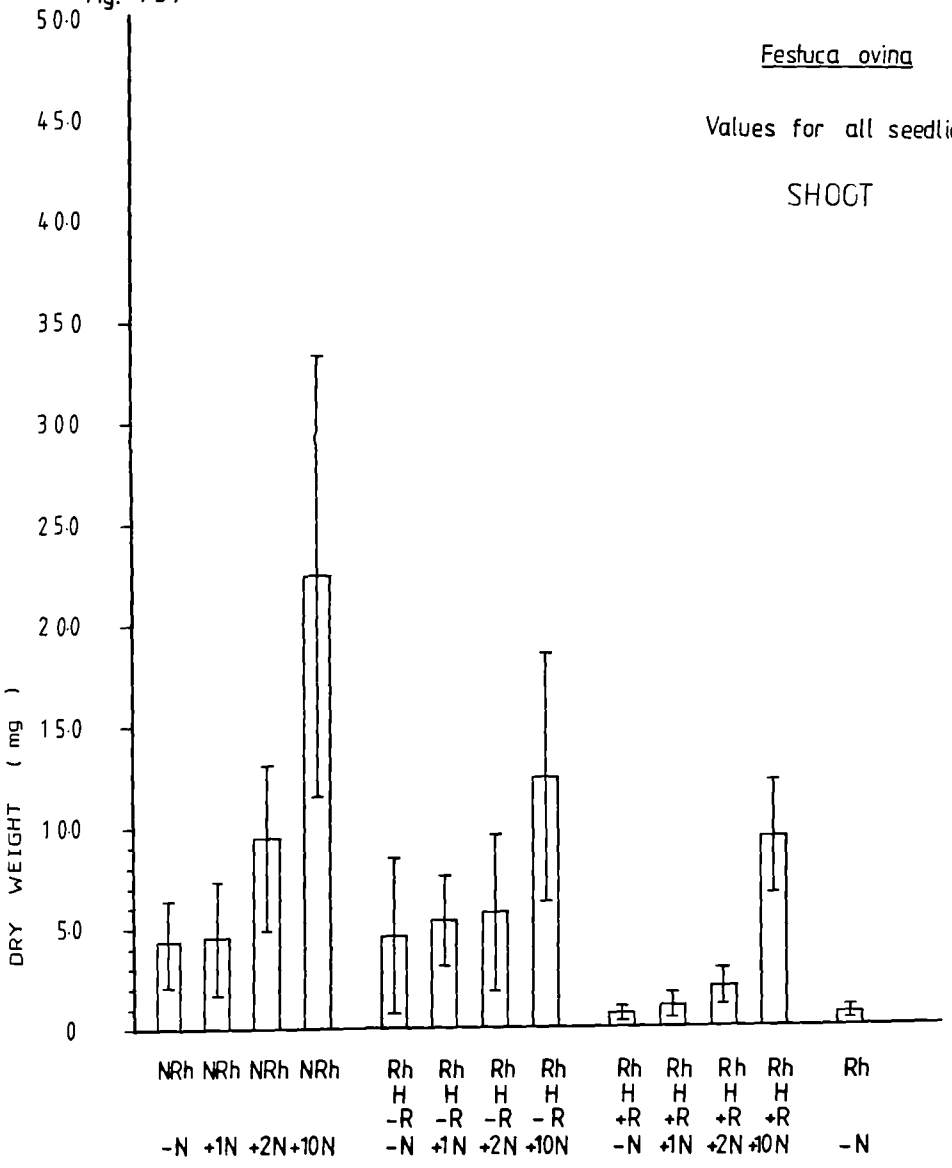
Fig. 4·57

6 B

Festuca ovina

Values for all seedlings planted

SHOOT



SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

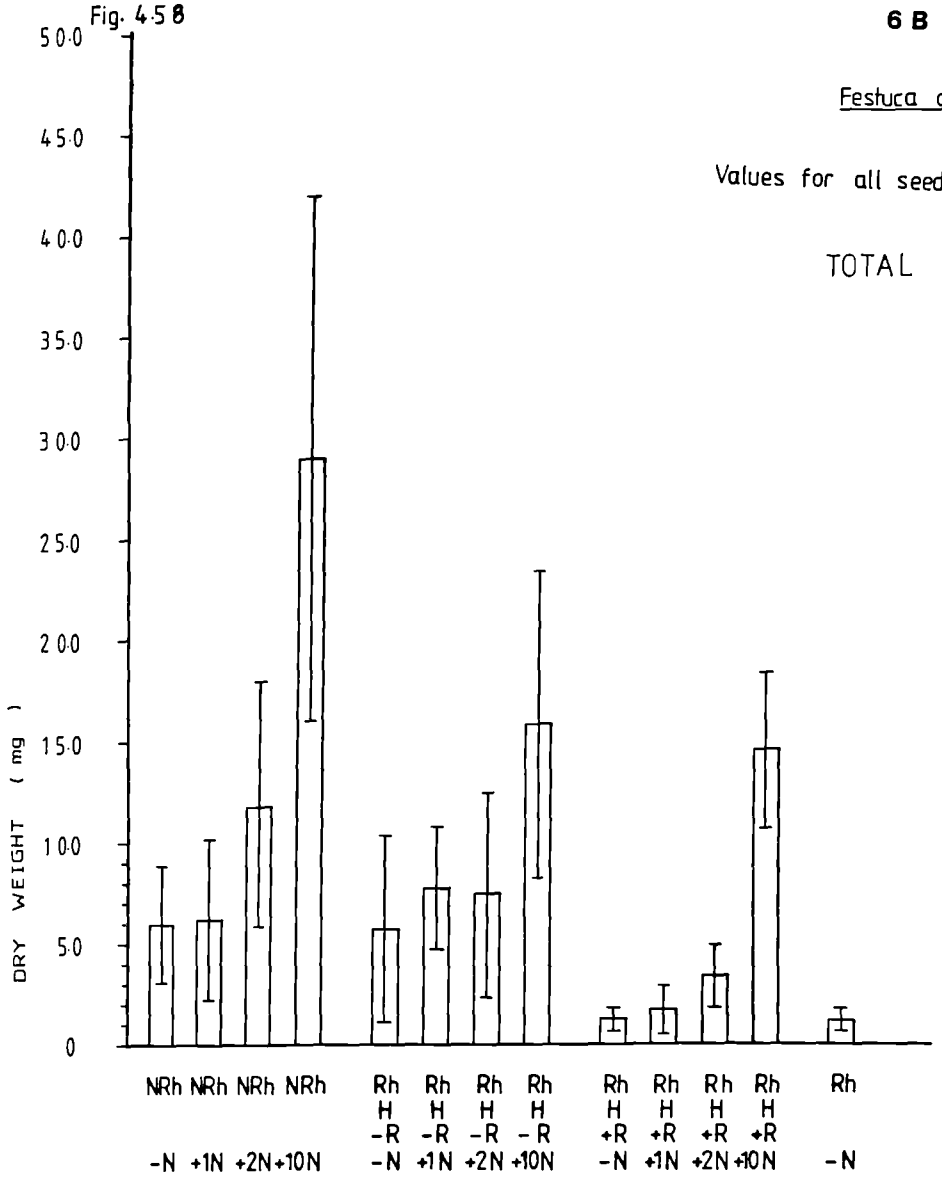
Fig. 4.5 B

6 B

Festuca ovina

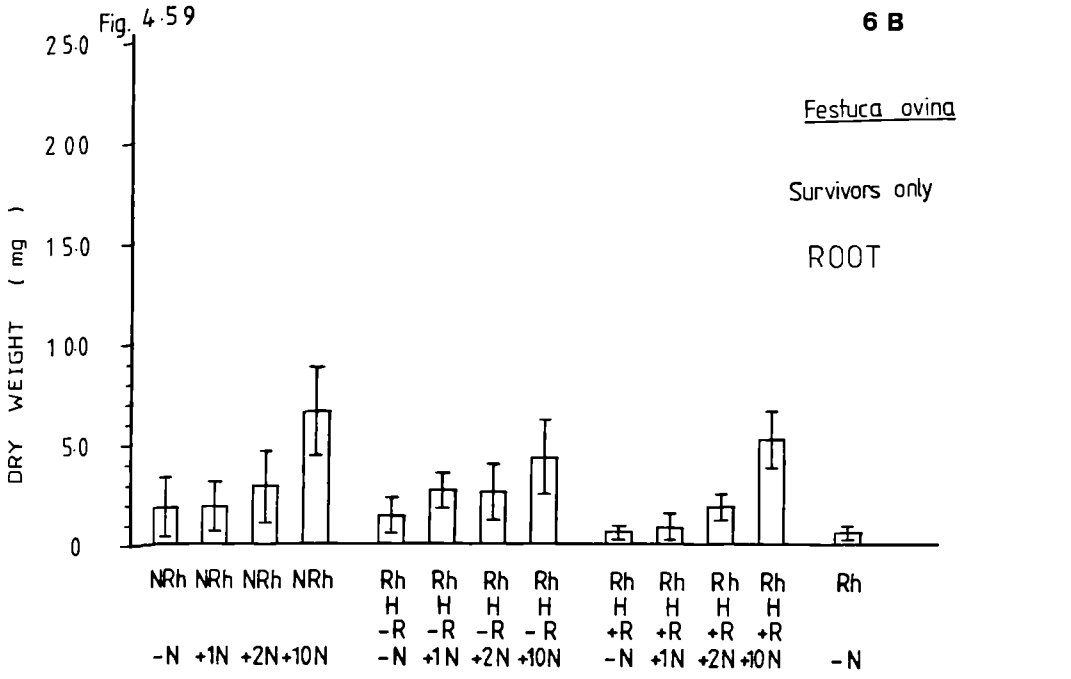
Values for all seedlings planted

TOTAL



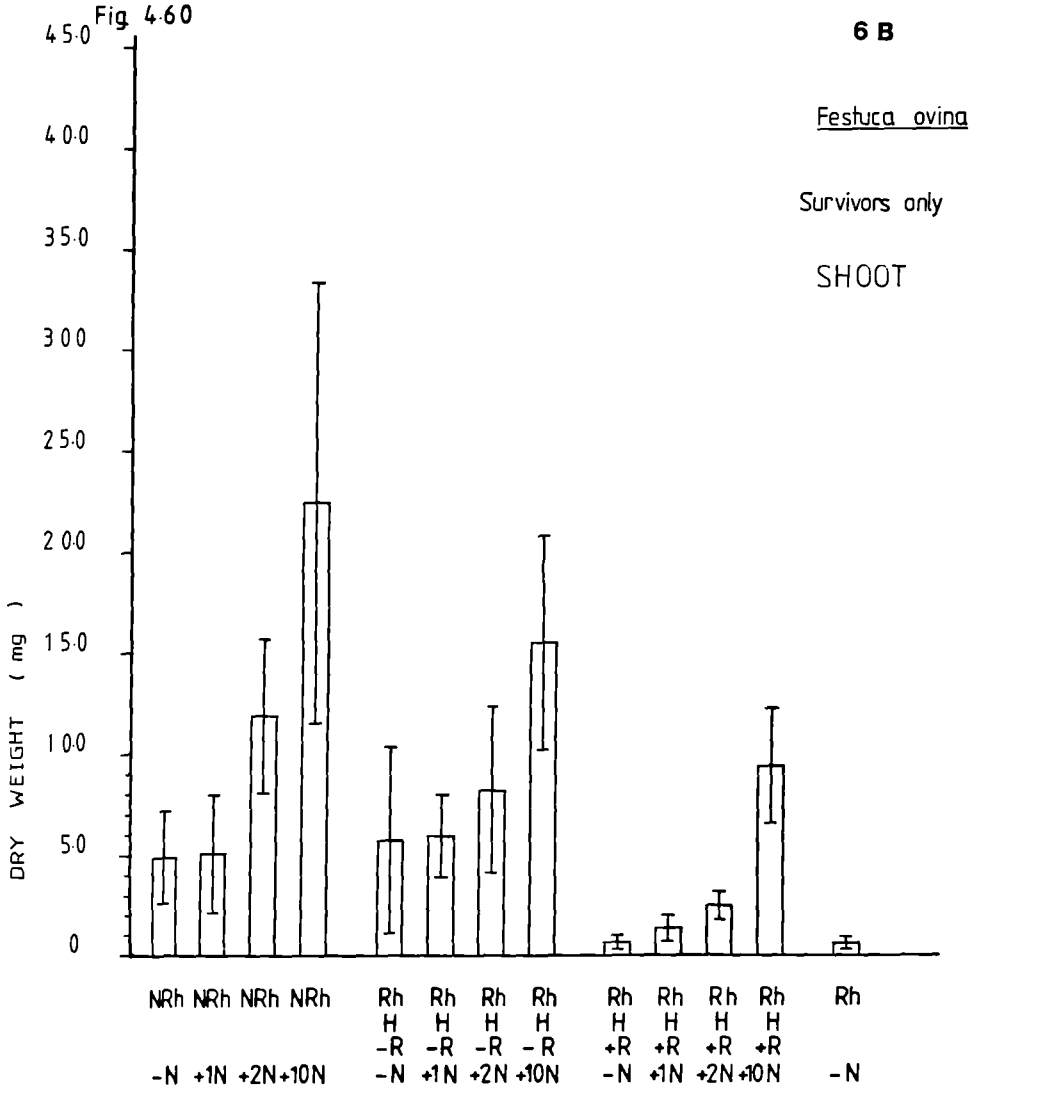
SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

Fig. 4·59



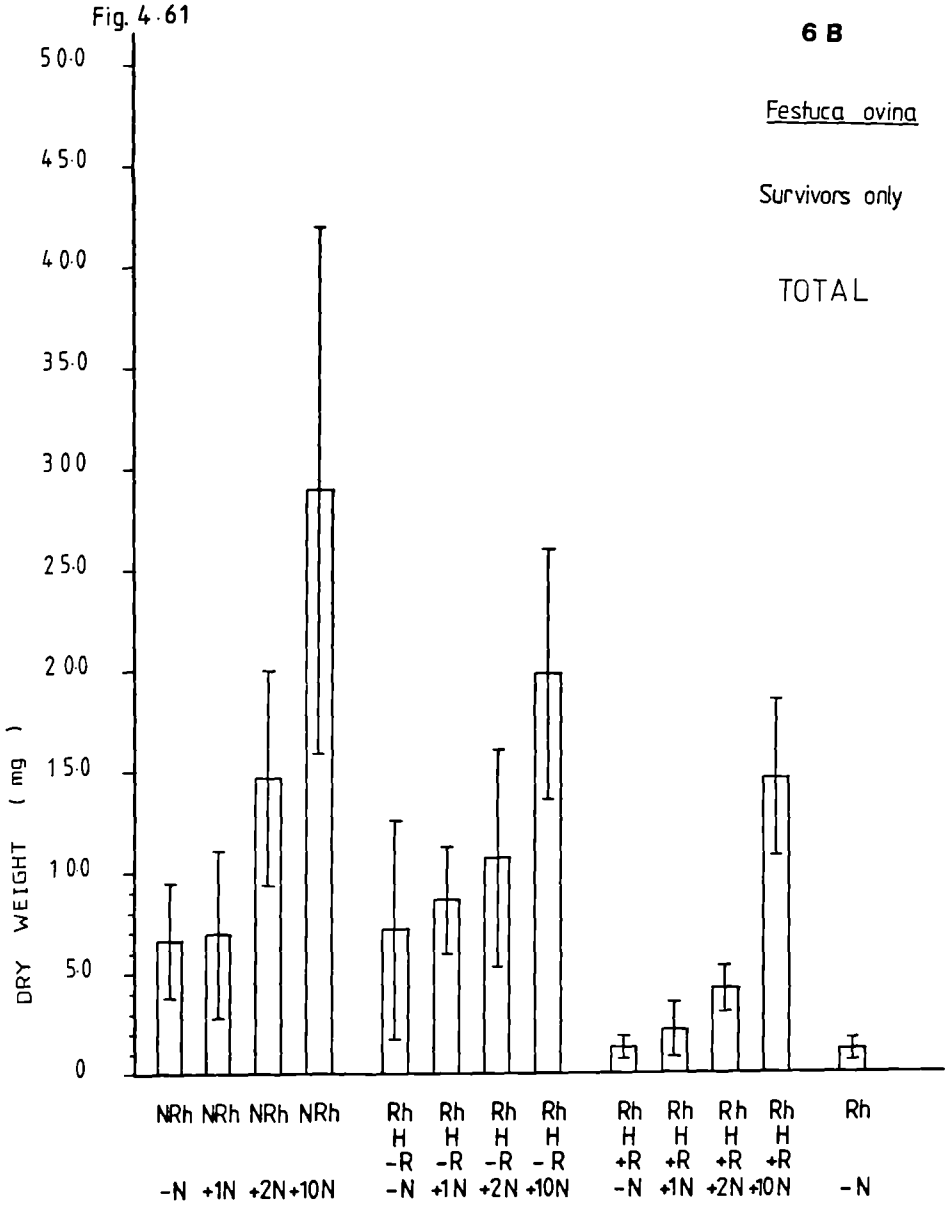
SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

Fig. 4.60



SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

Fig. 4.61



SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

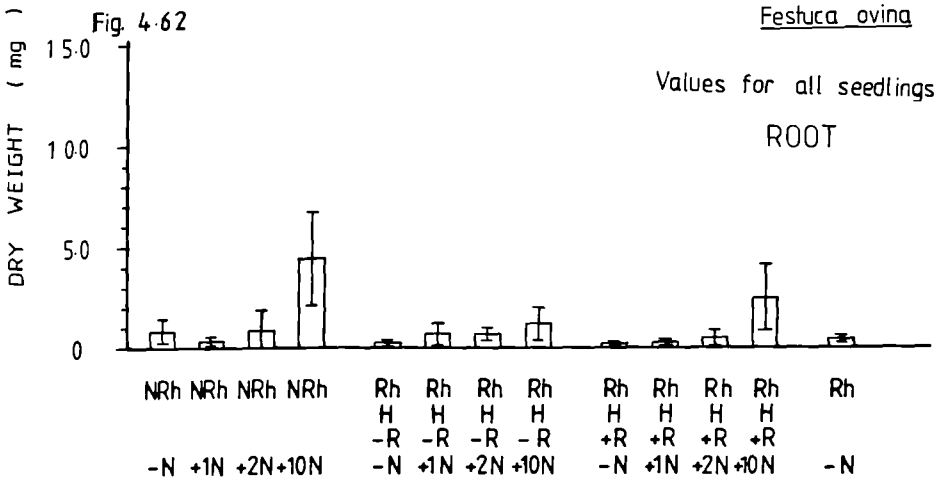
6 C

Fig. 4.62

Festuca ovina

Values for all seedlings planted

ROOT



SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

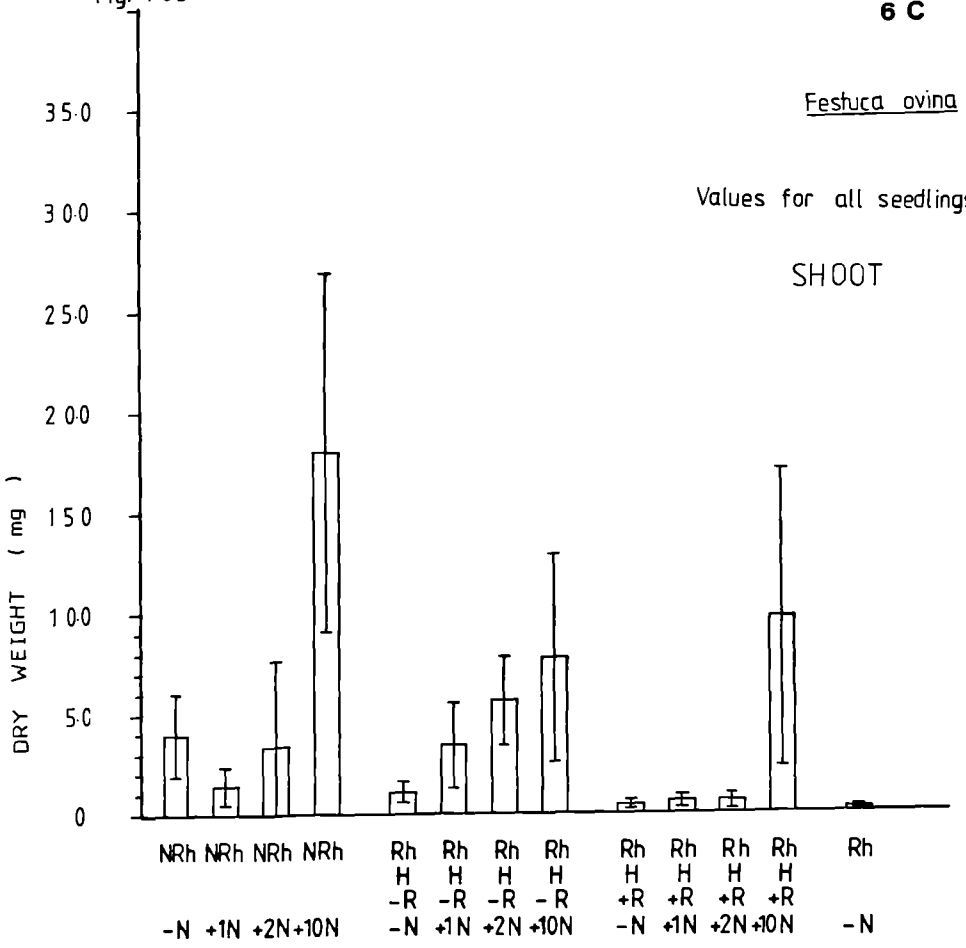
Fig. 4.63

6 C

Festuca ovina

Values for all seedlings planted

SHOOT



SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

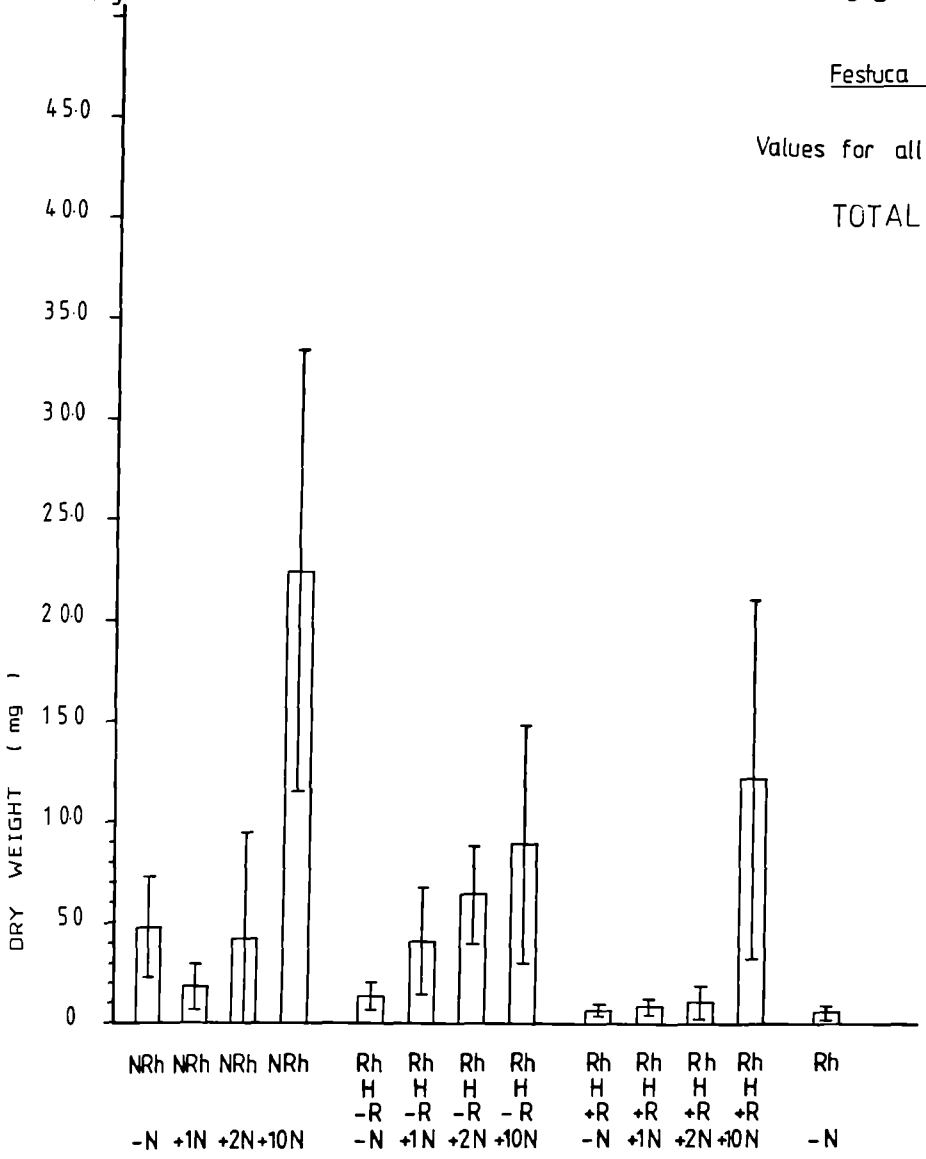
Fig. 4.64

6 C

Festuca ovina

Values for all seedlings planted

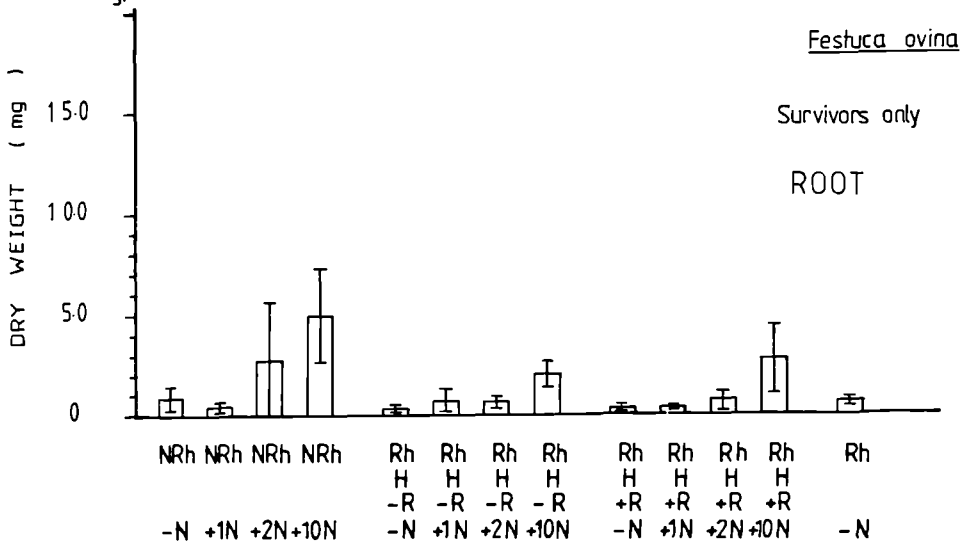
TOTAL



SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

6 C

Fig. 4·65



SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

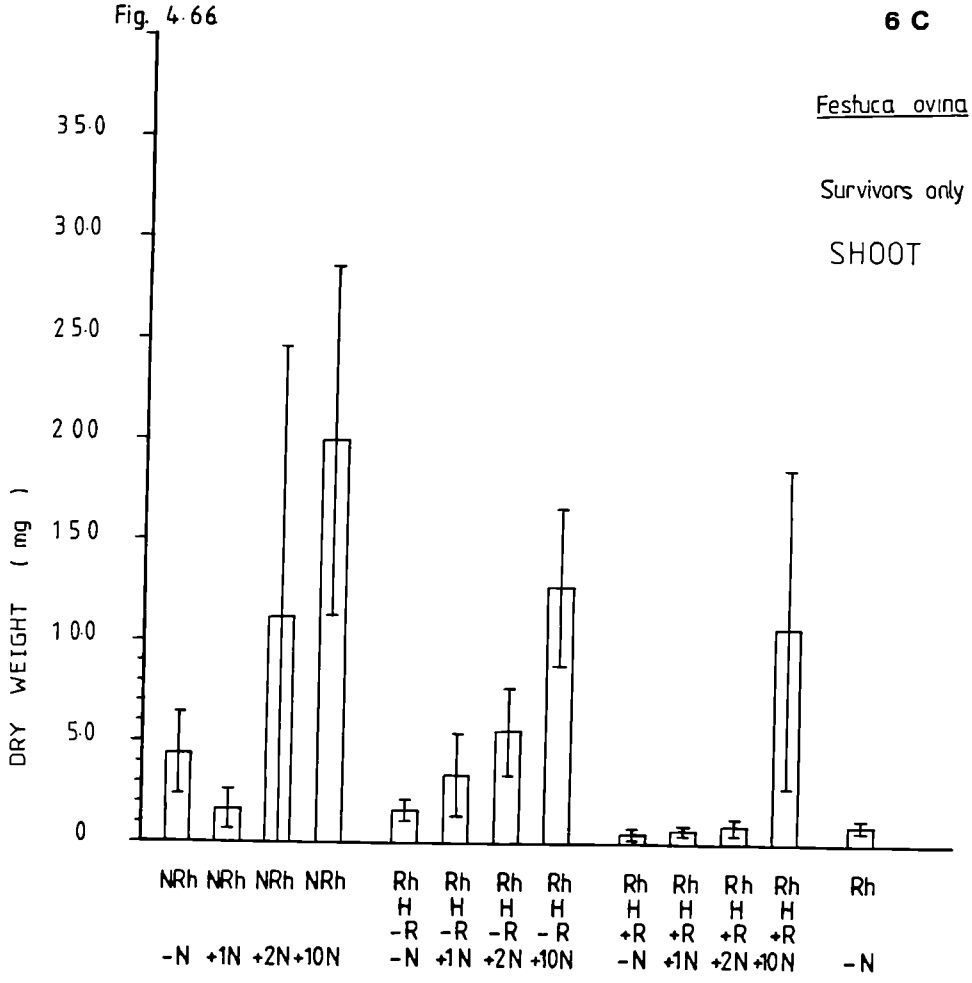
Fig. 4.66

6 C

Festuca ovina

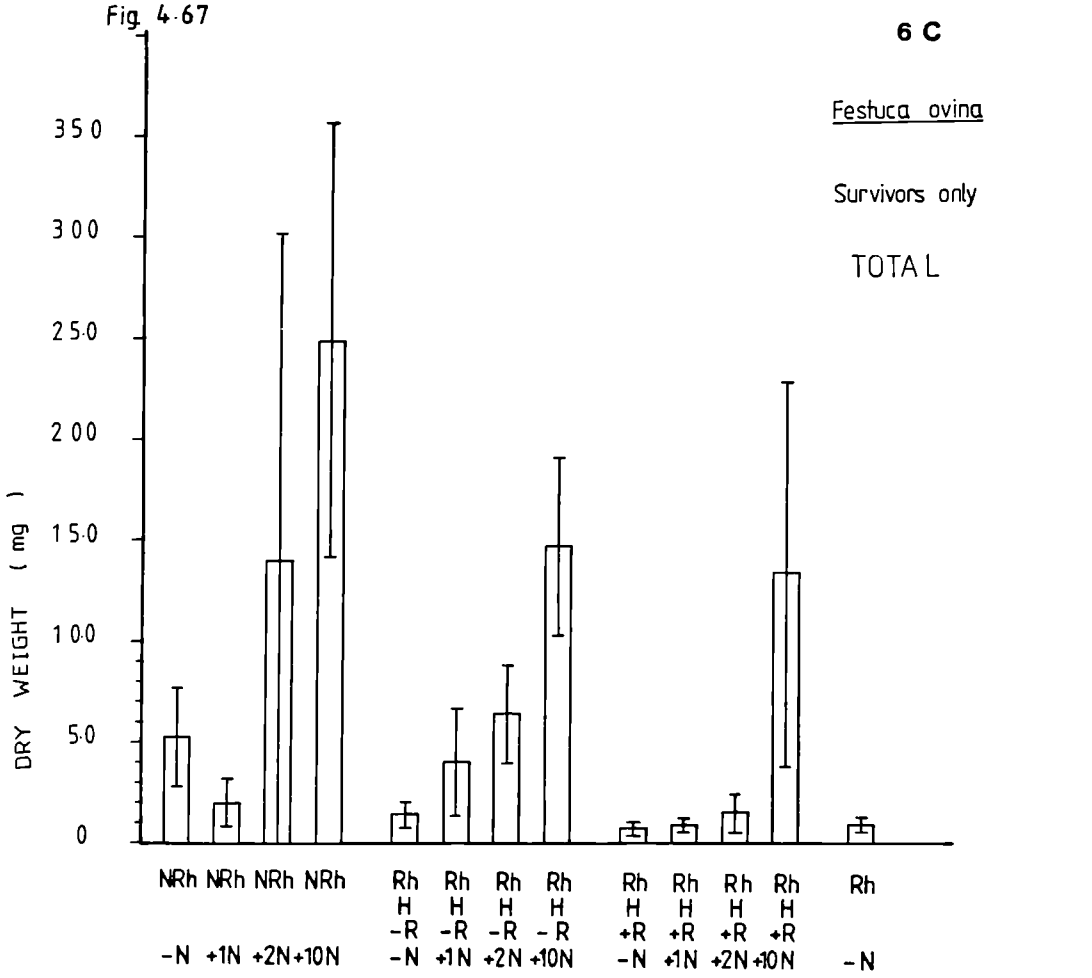
Survivors only

SHOOT



SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

Fig. 4.67



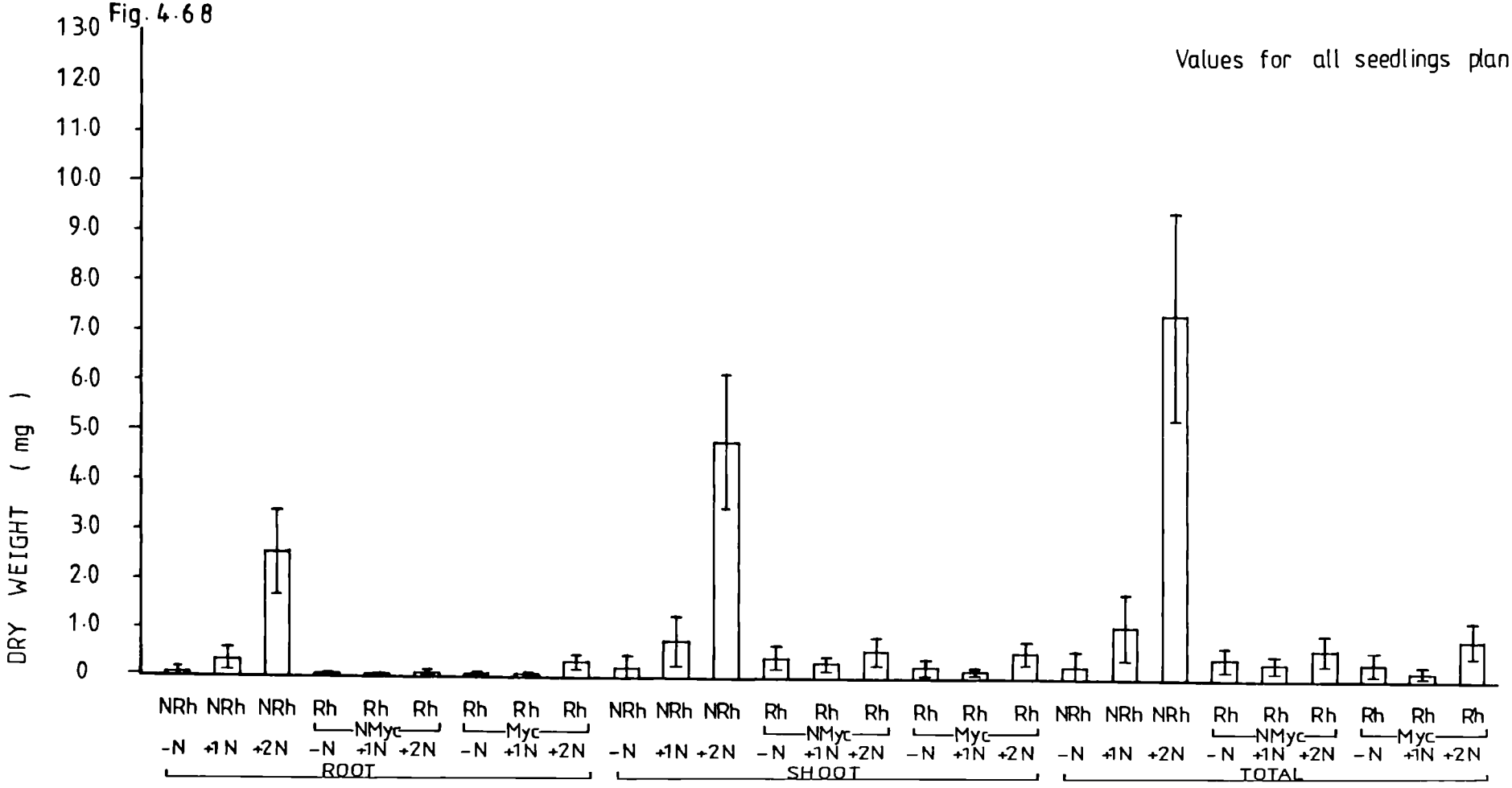
SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

6 A

Trifolium repens

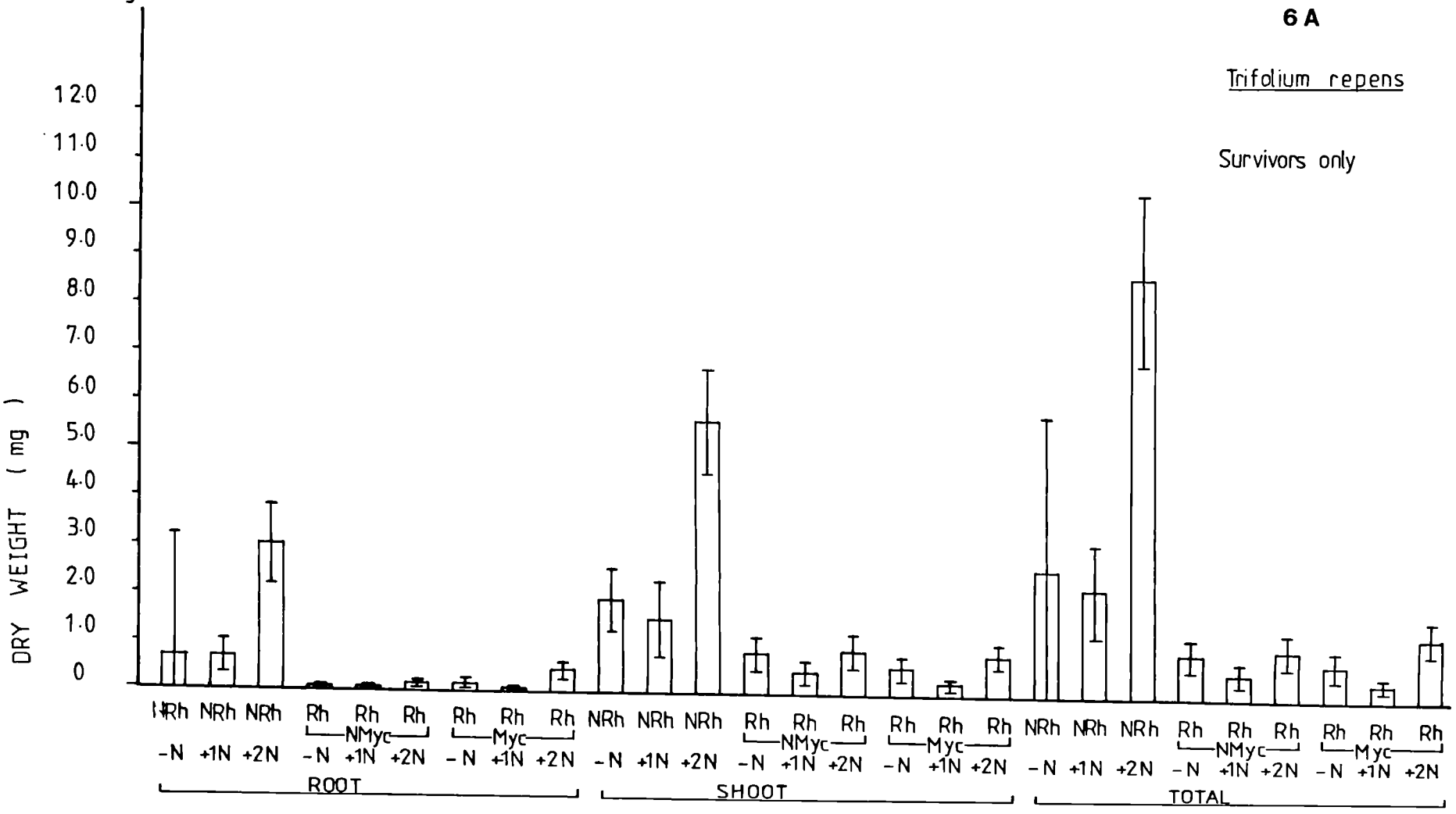
Fig. 4.68

Values for all seedlings planted



SEEDLING MEAN DRY WEIGHT WITH 95 %CONFIDENCE LIMITS

Fig. 4.69



4.4.3.4.4 Soil analysis

4.4.3.4.4.1 Available nitrogen and phosphorus, sampled after first harvest

Table 4.4.3.8

Treatment	Av.Ammonium N (ppm)	Av.Nitrate N (ppm)	Total Av.N (ppm)	Av.P (ppm)
NRh.-N	0.79	1.08	1.87	-
NRh.+1N	0.46	0.98	1.44	0.29
NRh.+2N	-	-	-	-
Rh.NMyc-N	-	-	-	-
Rh.NMyc+N	-	-	-	-
Rh.NMyc+2N	1.11	0.66	1.77	-
Rh.Myc-N	-	-	-	0.62
Rh.Myc+1N	-	-	-	1.28
Rh.Myc+2N	-	-	-	0.48

4.4.3.4.4.2 Soil pH

All measured in distilled water after 24 hours.

Original pH of irradiated soil : 3.75

Table 4.4.3.9

Treatment	Pot	Test species in pot		
		Fe	Ru	Tr
Experiment 6a				
NRh.-N	1	3.70	3.90	3.80
NRh.+1N	1	3.70	3.65	3.80
NRh.+2N	1	3.80	3.70	4.15
Rh.NMyc-N	1	3.50	3.40	3.40
	2	3.45	3.40	3.50
Rh.NMyc+1N	1	3.35	3.50	3.35
	2	3.55	3.55	3.35
Rh.NMyc+2N	1	3.50	3.55	3.45
	2	3.40	3.50	3.40
Rh.Myc-N	1	3.60	3.50	3.60
	2	3.60	3.55	3.55
Rh.Myc+1N	1	3.55	3.60	3.60
	2	3.50	3.50	3.45
Rh.Myc+2N	1	3.65	3.30	3.50
	2	3.55	3.50	3.60

All samples taken from pots and measured, immediately following the harvest.

Table 4.4.3.10

Treatment	Pot	Test species in pot			
		Experiment 6b		Experiment 6c	
		Fe	Ru	Fe	Ru
NRh.-N	1	3.70	3.70	3.80	3.70
NRh.+1N	1	3.65	3.75	3.80	3.90
NRh.+2N	1	3.90	3.85	4.05	3.75
NRh.+10N	1	3.80	3.80	3.75	3.75
Rh.H-R-N	1	3.80	3.95	3.90	4.30
Rh.H-R+1N	1	3.60	3.70	3.60	3.50
Rh.H-R+2N	1	3.95	3.55	3.90	3.60
Rh.H-R+10N	1	3.95	3.75	3.85	3.80
Rh.H+R-N	1	3.50	3.55	3.65	3.70
Rh.H+R+1N	1	3.50	3.60	3.50	3.70
Rh.H+R+2N	1	3.60	3.60	3.50	3.60
Rh.H+R+10N	1	3.85	3.65	3.80	3.75
Rh.-N	1	3.50	3.50	3.50	3.45

4.4.3.4.4.3 Seedling survival

Table 4.3.3.11

Treatment	Pot	Test species					
		Fe		Ru		Tr	
Experiment 6a							
NRh.-N	1	15		20		2	
NRh.+1N	1	16		20		10	
NRh.+2N	1	18		17		17	
Rh.NMyc	1	10	18	9	19	6	10
-N	2	8		10		4	
Rh.NMyc	1	6	14	10	20	5	13
+1N	2	8		10		8	
Rh.NMyc	1	10	18	10	20	8	13
+2N	2	8		10		5	
Rh.Myc	1	4	10	10	19	2	9
-N	2	6		9		7	
Rh.Myc	1	10	17	10	20	0	10
+1N	2	7		10		10	
Rh.Myc	1	6	15	9	19	6	15
+2N	2	9		10		9	

Table 4.3.3.12

Treatment	Pot	Experiment 6b		Experiment 6c	
		Fe	Ru	Fe	Ru
NRh.-N	1	9	10	9	9
NRh.+1N	1	9	10	9	9
NRh.+2N	1	8	10	3	10
NRh.+10N	1	10	10	9	10
Rh.H-R-N	1	8	10	9	7
Rh.H-R+1N	1	9	10	10	10
Rh.H-R+2N	1	7	10	10	7
Rh.H-R+10N	1	8	10	6	9
Rh.H+R-N	1	10	10	8	10
Rh.H+R+1N	1	8	10	9	9
Rh.H+R+2N	1	8	10	7	9
Rh.H+R+10N	1	10	9	9	8
Rh.-N	1	10	9	6	10

4.4.4 DISCUSSION

4.4.4.1 Experiment 3

The presence of live Rhododendron roots did not affect the germination of Festuca ovina, but it did significantly decrease root growth. Roots formed by seedlings germinated in situ with Rhododendron were brown in colour and very stunted. Root growth in pre-germinated seedlings (again planted with live Rhododendron) was much greater and the roots were white in colour and healthy in appearance.

Shoot length was significantly less for Festuca plants sown as seed with Rhododendron, than for those planted as seedlings. Considered as dry weights, overall shoot yield was significantly reduced. The mean shoot dry weight of the surviving seedlings produced from seeds germinated in situ, was actually significantly increased. This might be explained by the channelling of all the growth of these seedlings into shoot production, due to the inhibition of root development. In a field situation however, these seedlings would quickly die due to the lack of an effective root system.

4.4.4.2 Experiment 4

Live Rhododendron or soil which had been occupied by Rhododendron, but from which it had been removed, significantly reduced the growth of Festuca seedlings. Their germination and subsequent survival was also drastically reduced. These effects were not eliminated by nutrient addition and were not dependent upon mycorrhizal infection of the Rhododendron.

Once again, root formation by seedlings exposed to the various Rhododendron treatments was severely stunted. In field conditions these seedlings would not have survived.

4.4.4.3 Experiment 5

The survival rate of pre-germinated seedlings was reduced when dead Rhododendron roots were present in the soil and reduced further by the presence of live Rhododendron. With nutrient addition, the survival rate with live Rhododendron increased, but only to 55%, compared to 90% on the non-Rhododendron soil without added nutrients.

Live Rhododendron or dead Rhododendron roots led to highly significant decreases in yield by Festuca. Removal of the Rhododendron roots by sieving resulted in a significant improvement in the growth of test seedlings. The inhibition was still there when additional nutrients were present. Nutrient addition did increase yield, but only significantly for pots with live Rhododendron and those which had non-Rhododendron soil. This suggests that live Rhododendron was influencing the growth of test seedlings through competition (alleviated at least in part by nutrient addition) and some other form of interference, not removed by adding nutrients. The latter phenomenon is also associated with the presence of dead Rhododendron roots in soil or with Rhododendron soil from which the roots have been removed. In both cases, nutrient addition did not significantly increase test seedling yield.

4.4.4.4 Experiment 6

4.4.4.4.1 6a

Mycorrhizal infection of the R. ponticum did not strongly influence the level of interference observed in test seedlings.

The presence of Rhododendron significantly reduced the yield of Festuca and of Rumex. The survival of Trifolium without nutrient addition was very poor on all soils including the control. Most of the surviving seedlings of Trifolium on the control soil were considerably larger than those on the Rhododendron soil. The differences were not significant, because of the large standard deviations on the control soil.

When nutrients were added the effect was not removed. The reduction of growth in Rhododendron pots compared to the non-Rhododendron controls was of similar significance to that without nutrients, for Festuca and of increased significance for Rumex and Trifolium.

4.4.4.4.2 6b/6c

The interference effect of Rhododendron upon Festuca and Rumex was again clearly shown. The inhibition of test seedling growth was associated with both live Rhododendron and soil with either dead Rhododendron roots or Rhododendron soil from which the roots had been removed by sieving. Addition of nutrients did not eliminate the effect and did not always result in increased yield. At the higher levels of nutrient addition, despite increased growth on all soils, the inhibition was in some cases more significant. Removal of dead roots by sieving, either with or without adding nutrients, did significantly reduce the effect.

4.4.4.4.3 The overall interference phenomenon declined in the following order:-

Live Rhododendron (mycorrhizal or non-mycorrhizal)	Rhododendron harvested, dead roots left.	Rhododendron harvested, roots removed.	non-Rhododendron soil.

The effect of dead roots or soil from which roots had been removed was still present during the third experiment (6c), 6-12 weeks after harvesting of Rhododendron and the removal of root material. The causatic agent of this phenomenon therefore appears to be quite persistent within the soil.

The major findings of experiments 3, 4, 5 and 6 are summarized in tabular form in the following tables (p. 114a et seq.).

SUMMARY OF TOXICITY EVIDENCE FROM POT AND DISH EXPERIMENTS

Experiment 3

Live Rhododendron roots stunted root growth of in-situ germinated test seedlings.

Experiment 4

Live Rhododendron roots or soil in which Rhododendron had previously grown significantly reduced both growth and germination and subsequent survival of test seedlings. Nutrient addition did not remove the effect.

The above were not dependent on mycorrhizal infection of the Rhododendron.

Test seedling roots stunted by Rhododendron treatments.

Experiment 5

Test seedling growth reduced by live Rhododendron or dead Rhododendron roots compared to the same woil with the roots removed. Nutrient addition did not eliminate the interference effect.

Survival rate of test seedlings was also reduced.

Experiment 6

Interference occurred regardless of mycorrhizal status of Rhododendron roots. The effects of nutrient addition were variable, but did not consistently remove interference.

Interference generally decreased from live roots to dead roots to roots removed.

The effect was still apparent 6-12 weeks after harvesting of Rhododendron and, where relevant, removal of root material.

SUMMARY OF TOXICITY EFFECTS

EFFECT ON TEST SEEDLINGS		EXPERIMENT 3	4	5	6
Stunted roots		+	+	+	+
Decreased survival or germination and survival			+	+	+
Interference (reduced growth)	*Rhododendron* (live)		+	+	+
	Rhododendron (soil + dead roots)			+	+
	Rhododendron (soil - dead roots)		+		+
Effects not dependent on *Rhododendron* being mycorrhizal			+		+
Effects not removed by nutrient addition			+	+	+
Effects persistent in soil			(+)	(+)	+

SUMMARY OF TOXICITY DATA (TOTAL WEIGHTS ONLY)

Total dw. ± 95% confidence limits (mg). (Expts. 4, 5, 6 only)

	Rh.Myc.	Rh.NMyc.	Rh.Myc.H	Rh.NMyc.H	NRh.+E	NRh.
Expt. 4						
+N	0.09±0.05	0.12±0.20	0.21±0.15	0.26±0.11	6.28±1.43	7.53±1.71
n	4	3	6	8	34	35
-N	0.10	0.18±0.13	0.15±0.63	0.24±0.24	8.60±1.94	5.78±1.14
n	2	6	2	4	31	35

	Rh.	Rh.H + R	Rh.H - R
Expt. 5			
+N	3.24±2.99	47.46±31.39	185.55±91.74
n	11	11	10
-N	0.97±0.55	11.26±8.73	52.68±22.15
n	5	11	19

Seedling survival				NRh.
+N	55%	55%	50%	55%
-N	25%	55%	95%	90%
	Probable interspecific nutrient competition + interference		Probable intraspecific competition causing reduced survival with +N.	

Expt. 6a	NRh. -N	NRh. +1N	NRh. +2N	Rh. NMyc. -N	Rh. NMyc. +1N	Rh. NMyc. +2N	Rh. Myc. -N	Rh. Myc. +1N	Rh. Myc. +2N
Festuca	11.46 ± 2.76	8.51 ±2.58	11.96 ±2.85	1.50 ±0.38	1.70 ±0.59	2.46 ±0.49	1.65 ±0.54	1.98 ±0.62	2.90 ±0.81
Rumex	5.56 ±1.25	36.20 ±8.45	33.31 ±15.01	4.68 ±1.23	4.84 ±0.69	6.58 ±1.47	3.78 ±0.89	4.06 ±1.06	4.60 ±1.22

Expt. 6b	-N	+N	+ 2N	+ 10N
Festuca				
NRh.	6.62±2.84	6.92±4.17	14.71±5.34	28.93±13.01
Rh.-R	7.14±5.48	8.57±2.65	10.59±5.37	19.75±6.23
Rh.+R	1.26±0.57	2.13±1.35	4.23±1.16	14.57±3.83
Rh.	1.18±0.54			
Rumex				
NRh.	17.92±8.71	19.11±6.09	25.46±9.45	96.97±20.30
Rh.-R	13.61±5.10	6.78±2.60	7.41±3.08	35.76±17.98
Rh.+R	5.05±1.32	7.47±2.51	4.53±1.79	46.81±20.03
Rh.	4.22±1.32			

SUMMARY OF TOXICITY DATA FROM EXPT. 6c

Rumex	dw.(mg).	R	S	T
n = 10	Rh.-N	1.81±0.29	2.70±0.50	4.41±0.54
n = 10	Rh.+R-N	1.70±0.76	2.16±0.44	3.76±1.01
n = 7	Rh.-R-N	5.10±2.55	11.74±6.36	16.84±8.56
n = 9	NRh.-N	5.11±1.67	7.93±1.61	13.04±2.48
n = 8	Rh.+R+10N	1.80±0.31	8.66±3.14	10.46±3.18
n = 9	Rh.-R+10N	2.26±0.53	11.66±1.91	13.91±2.31
n = 10	NRh.+10N	7.04±1.78	32.51±6.45	39.55±6.10
Festuca				
n = 6	Rh.-N	0.55±0.21	0.30±0.18	0.85±0.32
n = 8	Rh.+R-N	0.24±0.11	0.46±0.28	0.70±0.33
n = 9	Rh.-R-N	0.27±0.22	1.16±0.53	1.43±0.72
n = 9	NRh.-N	0.85±0.54	4.39±2.01	5.24±2.48
n = 9	Rh.+R+10N	2.68±1.74	10.74±7.90	13.42±9.50
n = 6	Rh.-R+10N	2.00±0.69	12.72±3.93	14.72±4.44
n = 9	NRh.+10N	4.94±2.28	19.86±8.68	24.80±10.75

4.5 INVESTIGATION INTO THE INTERFERENCE PHENOMENON IN THE FIELD

(The competitive and possible allelopathic effects of *Rhododendron* on seedlings of test species in field trials at Stand Wood, North Derbyshire)

4.5.1 INTRODUCTION

The aim of this experiment was to indicate some of the possible factors involved in competitive and possibly allelopathic interactions of Rhododendron in a field situation.

Experimental factors involved the addition of nutrients, of lime, the removal of Rhododendron canopy and the presence of live Rhododendron roots (Rh.), dead Rhododendron roots (Rh.US), or no roots at all (Rh.S).

4.5.2 METHOD

Eight experimental plots were set up in parts of Stand Wood (Chatsworth, N. Derbyshire) dominated by R. ponticum as an almost complete shrub-layer. Four plots were in artificially created gaps (3M x 3M) within dense R. ponticum. The other four, were under dense canopy of R. ponticum adjacent to the gaps. The plots were therefore in pairs (shaded and unshaded) with very similar conditions of aspect, pedology and topography. Two pairs of plots were on the west-facing slope of Stand Wood and two pairs were on the flat upper part of the wood. The wood is a mature mixed plantation of both native and exotic species with an understorey of mainly R. ponticum with some Ilex and Taxus. Approximate altitude of the plots on the slope was 213m and on the flat was 230m.

The following experimental conditions were set up:-

Non-Rhododendron soil unsieved	(NRh.US)	
" " sieved	(NRh.S)	
Rhododendron soil unsieved	(Rh.US)	All in 2½ inch
" " sieved	(Rh.S)	diameter plastic
" " unsieved + fertilizer	(Rh.US+F)	pots.
" " sieved + fertilizer	(Rh.S+F)	
" " unsieved + lime	(Rh.US+L)	
" " sieved + lime	(Rh.S+L)	

Rhododendron soil, coarse litter removed (Rh.)		)
" " " " " + fertilizer	(Rh.+F)	) soil
" " " " " + lime	(Rh.+L)	) undisturbed

A = unshaded plots (Rhododendron canopy removed)
B = shaded plots (Rhododendron canopy intact)

For all conditions except those involving undisturbed soil, four pots were set up to be planted with F. ovina and four with R. acetosa. Five seedlings were planted per pot and an equivalent number directly into the undisturbed soil.

All soils used were those taken from the site of the particular plot except for the Non-Rhododendron soil. This was all collected from one grass-dominated site on the west-facing slope. As far as is known, this has not had any R. ponticum growing on it.

Sieving of the soil was with a 0.5 cm. mesh to remove as much root material as possible. A finer mesh could not be used as this would also have removed considerable amounts of coarse organic and mineral fractions of the soils to an unacceptable degree.

The application rate of both fertilizer and lime was equivalent to 50g per sq m. The plots were treated two weeks before planting.

No measurements of light input to the plots were made. This might have been useful with respect to the shaded and unshaded plots, but in the circumstances would have been difficult to achieve to a satisfactory degree of reliability. The nature of the terrain, with rough topography and in the case of two pairs of plots, the steep slope, together with a broken upper canopy of very large trees produced extremely variable light regimes at ground level. Instantaneous measurements of irradiance, or perhaps cumulative measurements over known periods of time, could not realistically have been extrapolated to produce comparable daily light regimes for the different plots. Apparatus was not available to record total daily irradiance for the plots. Cross (1973), found dense R. ponticum canopies to reduce total daylight by up to 98%. The reduction of total daylight in the Stand Wood plots with the R. ponticum canopy intact was probably of a similar magnitude.

The plots were set up with fertilizer and lime added where required (28.8.80). The test species were planted (14.9.80); sown (7.9.80) and the seedlings were harvested (12.10.80).

(Note: It was originally intended to harvest twice, after 3 weeks, and after 6 weeks. The setting up of the plots was however delayed by bad weather. The cool, wet conditions continued throughout the experimental period. It was therefore necessary to shorten the span of the experiment and harvest once, with a compromised time of 4 weeks.)

The fertilizer was a commercially available NPK fertilizer with the following:- Total nitrogen (5.1%); Total phosphorus (2.9%); Water soluble phosphorus (2.5%); Total potassium (6.7%). The lime was a commercially available fine ground calcium carbonate.

4.5.3 RESULTS

4.5.3.1 The results of the field trial show no obvious trends consistent between different plots. The original intention was to combine results from different plots (either all four, or as pairs from similar situations), to increase data reliability. However, the divergent results invalidate this approach. The data are presented as histograms (Figures 4.70 - 4.92).

Survival and growth was poorer under dense Rhododendron canopy than in the open plots. However, due to the mild, wet conditions prevailing throughout the experiment many seedlings is a very poor state survived. Under warmer, drier conditions these would have perished and increased the differences between some of the treatments.

Growth was generally quite good on the non-Rhododendron soil and poor when seedlings were planted directly into the ground with live Rhododendron roots. However, this and the effects of other variables such as sieving, presence of dead roots only, addition of fertilizer or of lime were all very variable. Again the high rainfall affected seedling survival and also presumably levels of nutrients or any potential toxins in the soil subject to leaching.

Variability between plots may have been produced by the uneven topography and differences in micro-climate.

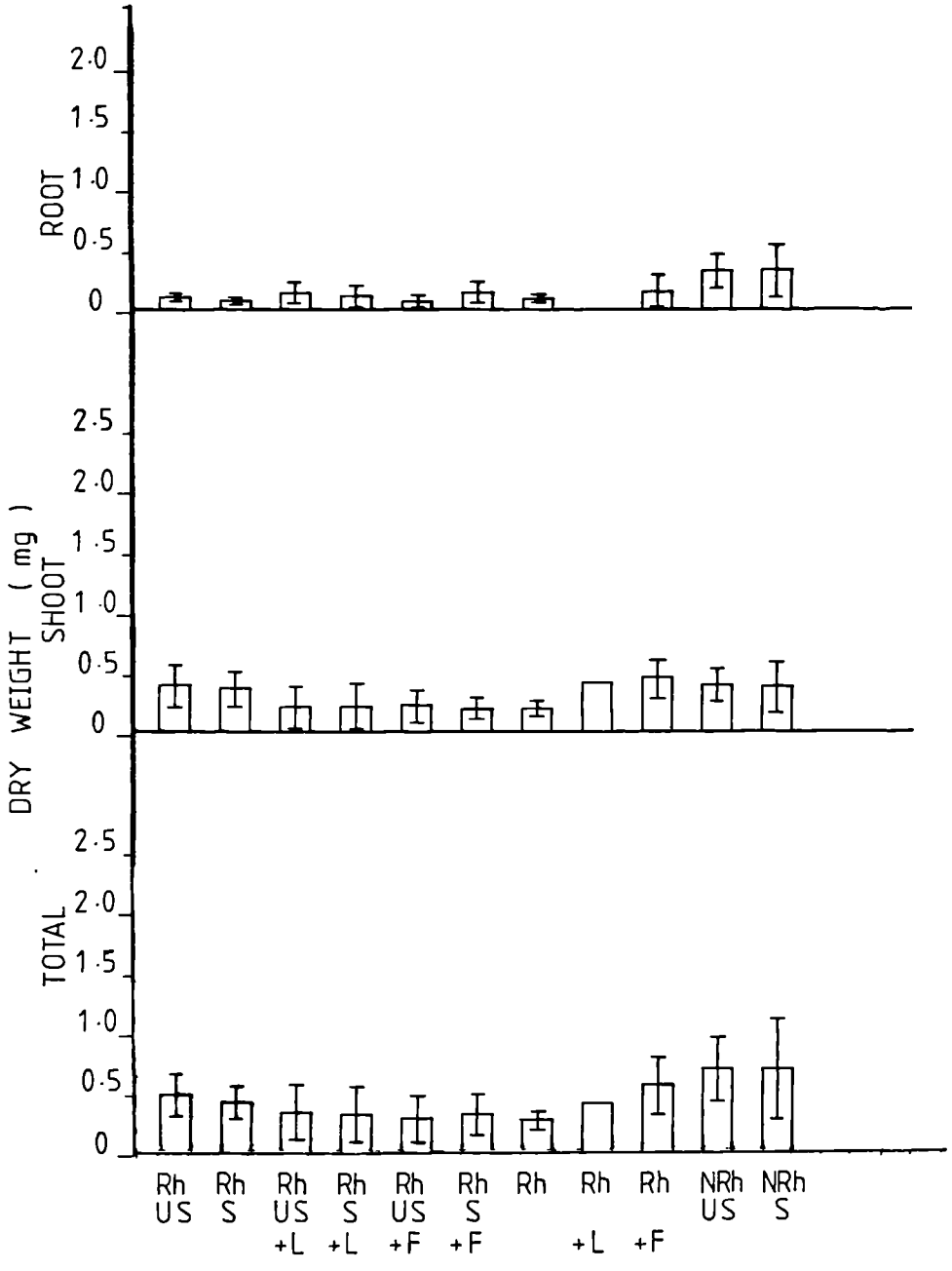
Soil analysis showed the Rh. and NRh.S/NRh.US soils to be relatively low in 'available' nitrogen and phosphorus. Levels in the potted Rhododendron soils were much higher. Nitrogen was mainly available as ammonium. These values were high even in soils without addition of F or L. The Rhododendron soils were rather organic and presumably these 'available' nutrients were released by breakdown of organic matter. The Rhododendron soils in plots 2, 3 and 4, were all more acidic than the NRh. controls. As would be expected, addition of lime raised soil pH. The pH value of all Rhododendron soils from plot 1 were surprisingly high (c. 5.50-6.70). This might be explained by surface run-off from the metalled road running across the slope just above this plot.

SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

1A Fe

Survivors only

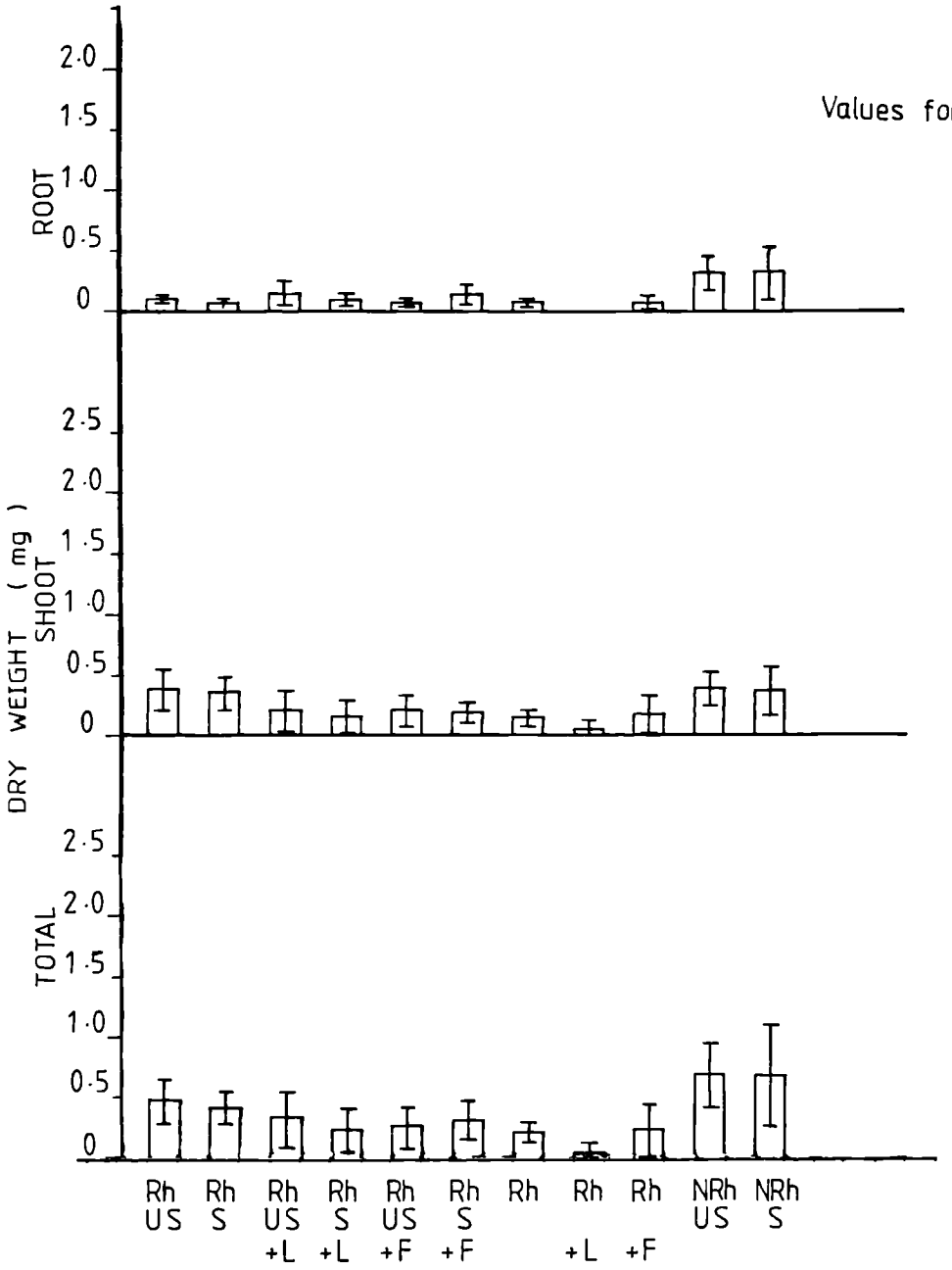
Fig. 4.70



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

1A Fe

Fig. 4.71

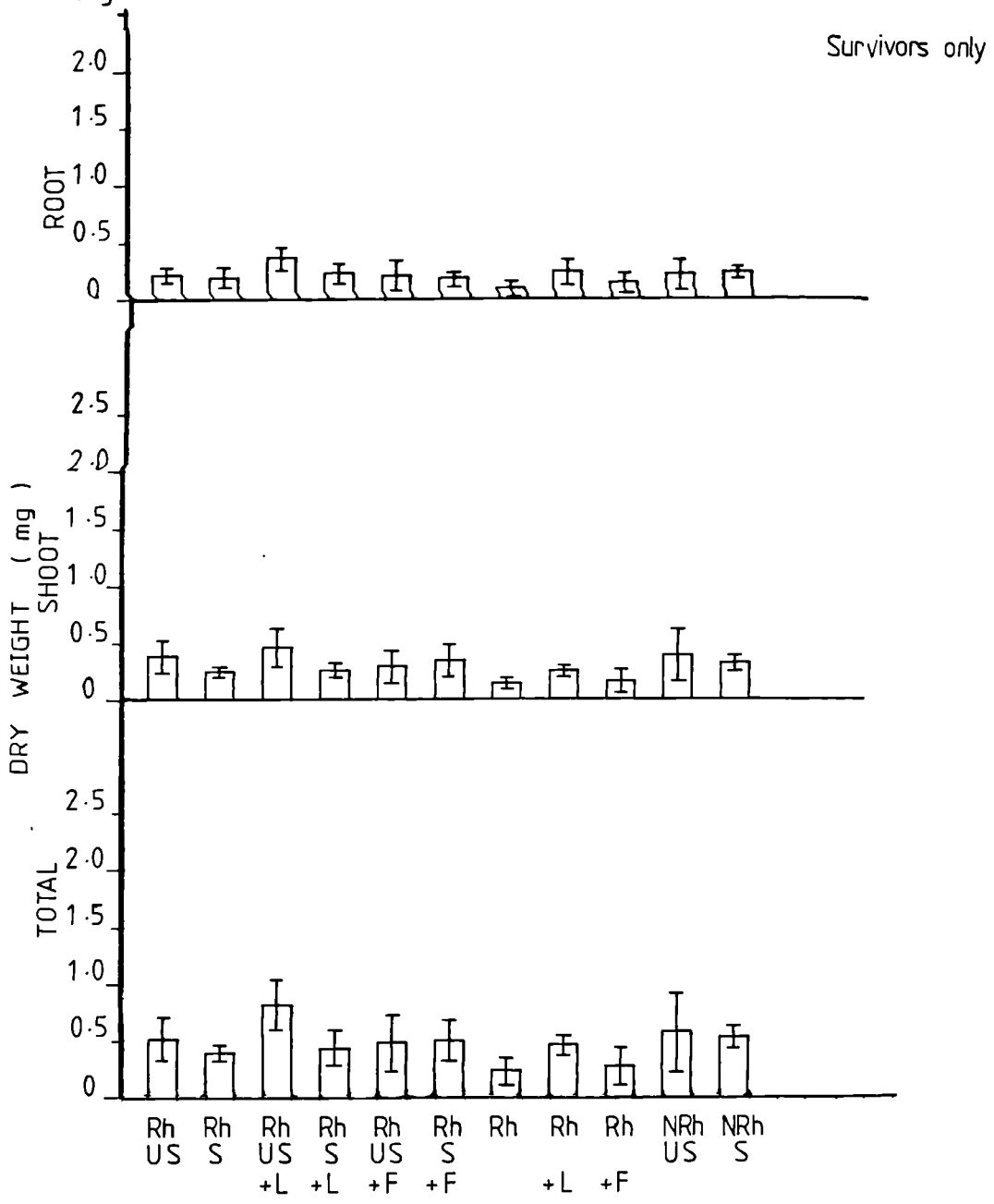


Values for all seedlings planted

SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

2A Fe

Fig. 4.72

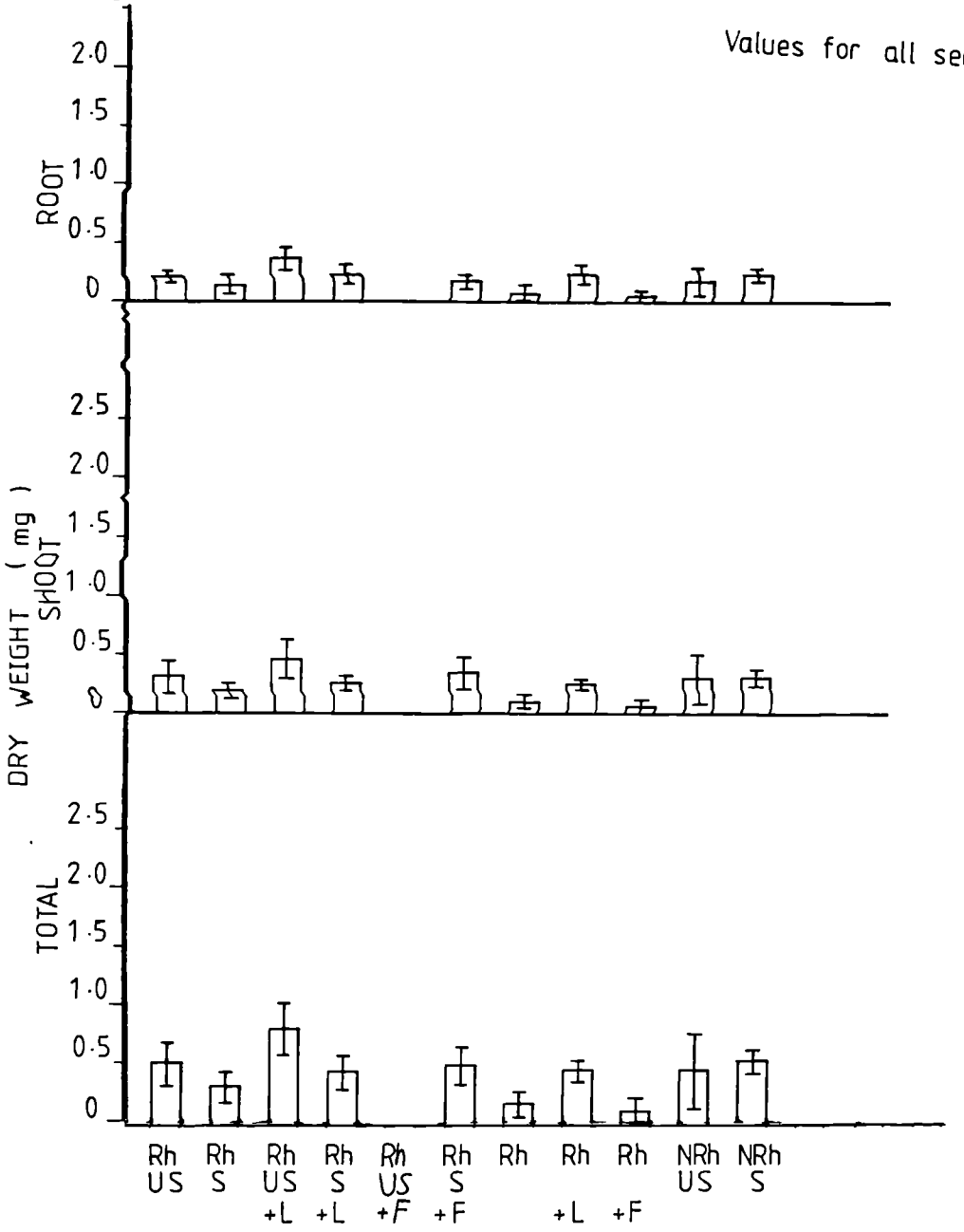


SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

2A Fe

Fig. 4·73

Values for all seedlings planted



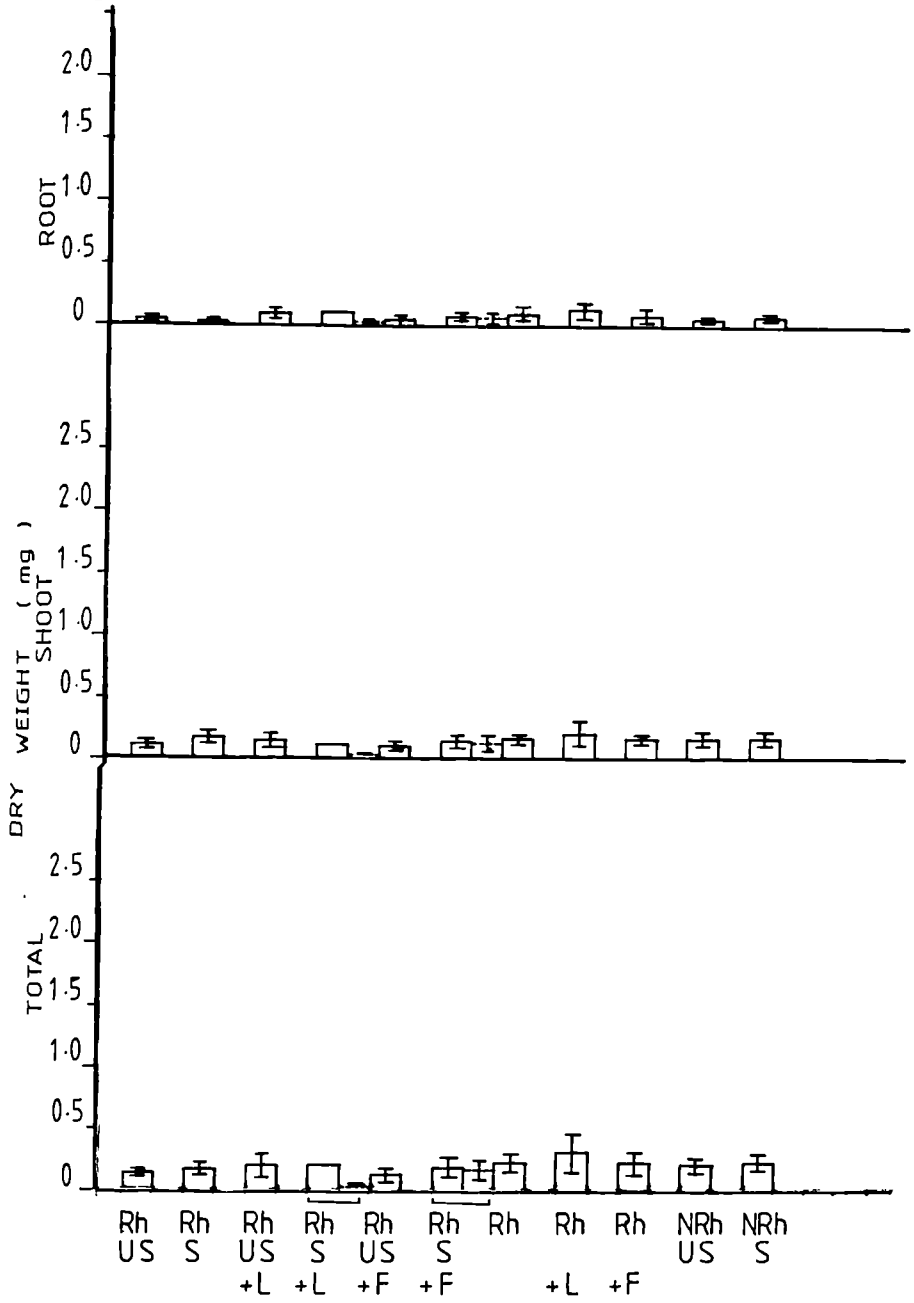
SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Survivors only ——

Values for all seedlings planted ---

3A Fe

Fig. 4.74



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

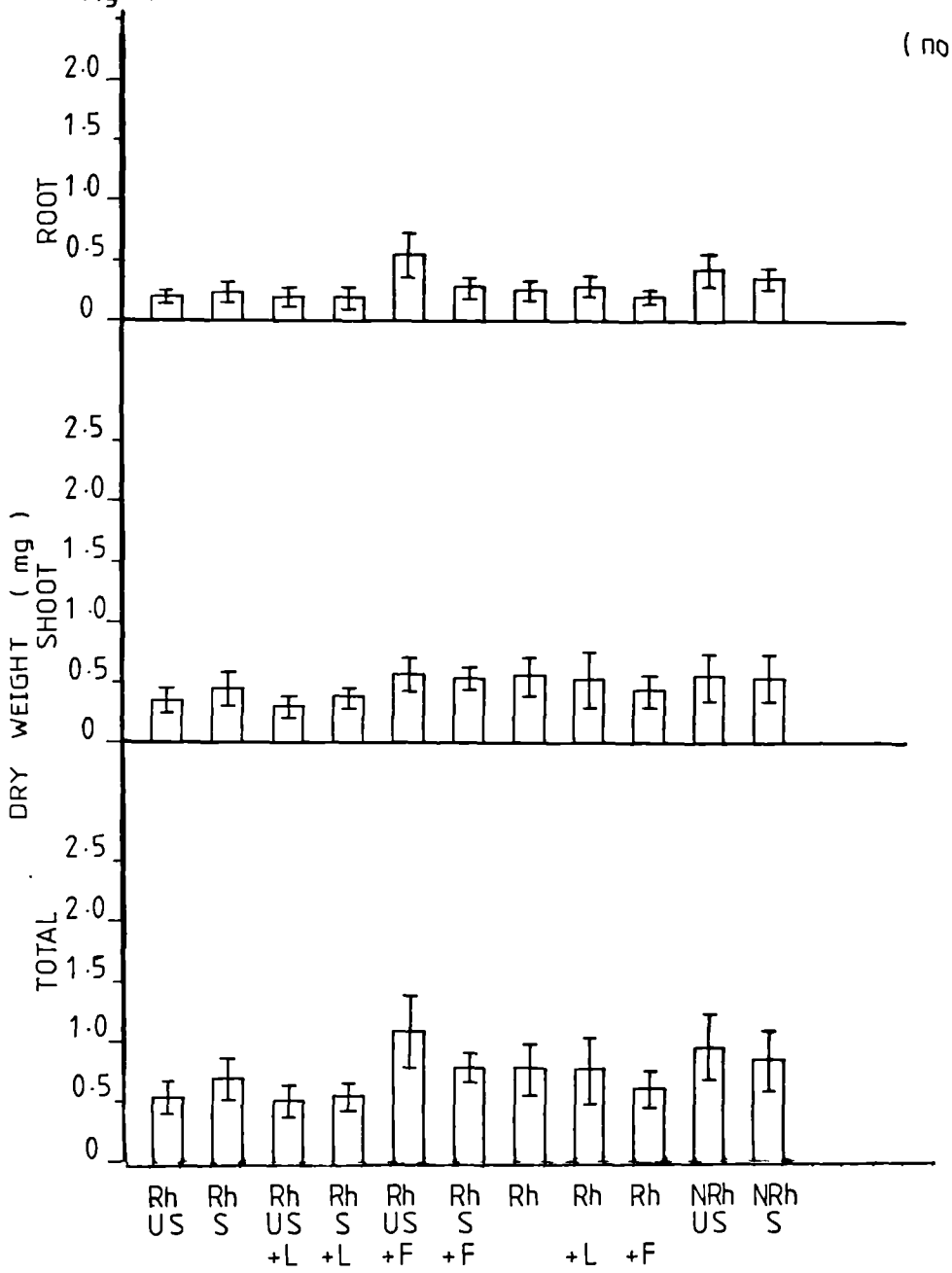
Survivors only

4A Fe

Values for all seedlings planted

(no change)

Fig. 4.75

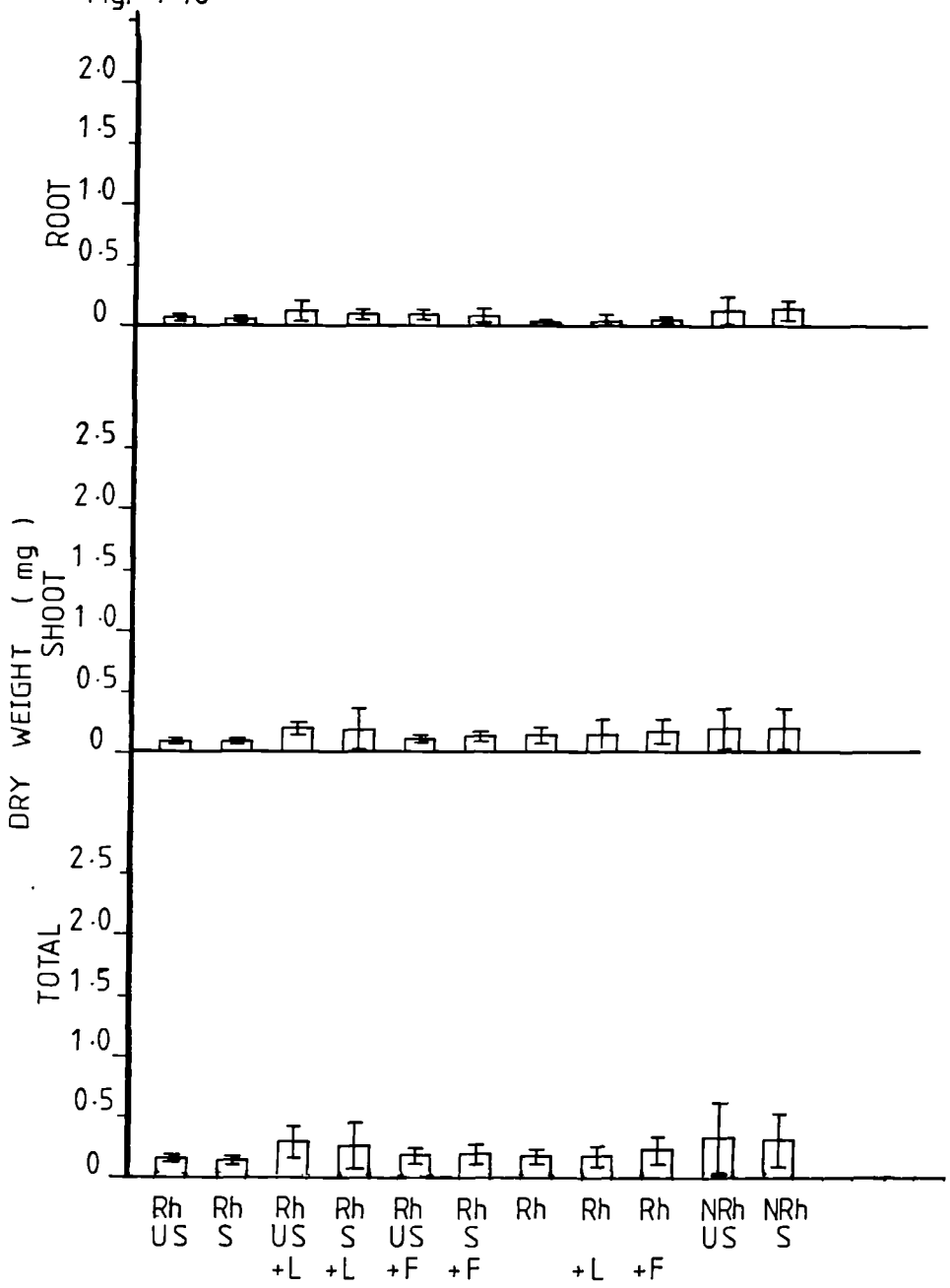


SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

1 B Fe

Survivors only

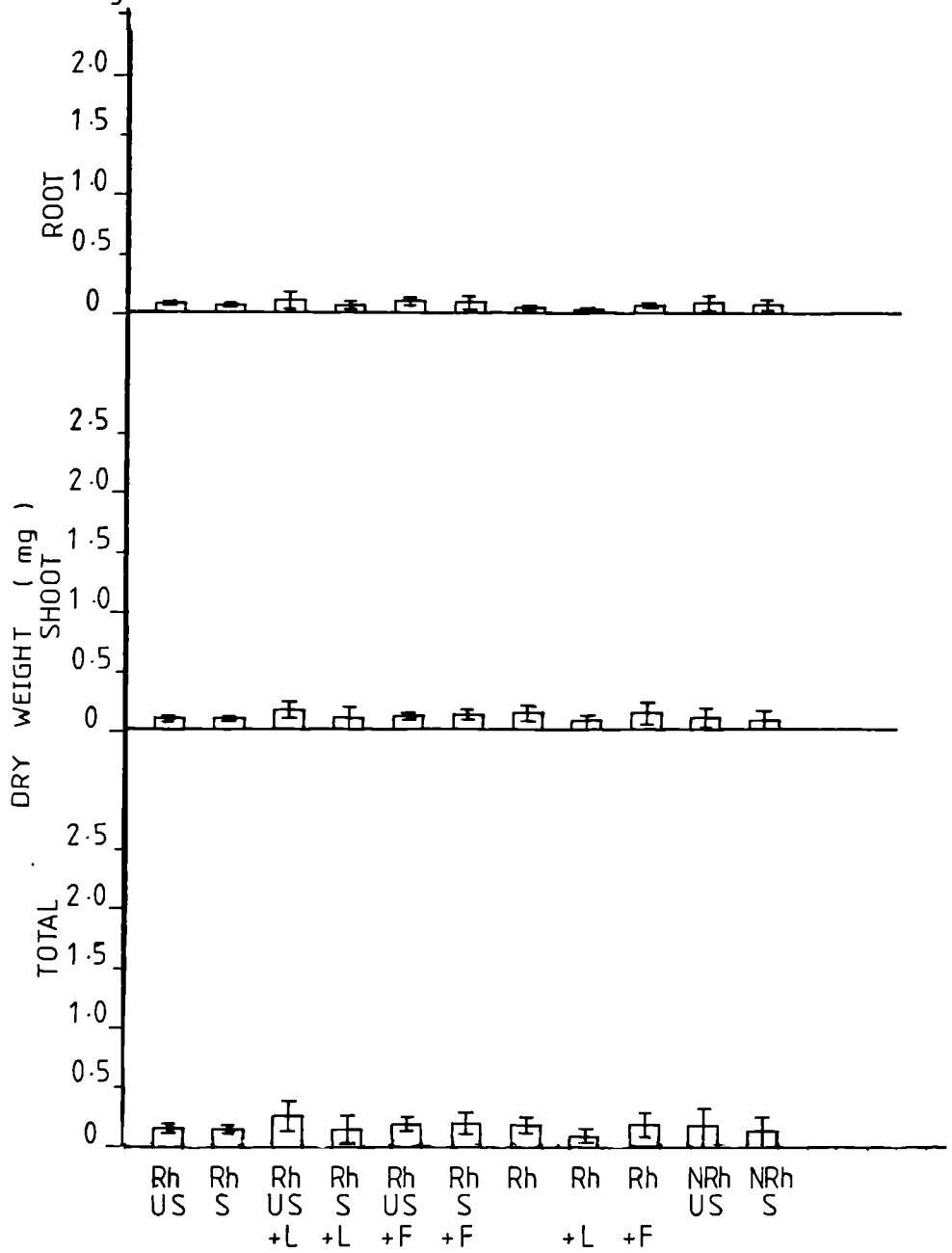
Fig. 4·76



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

1 B Fe

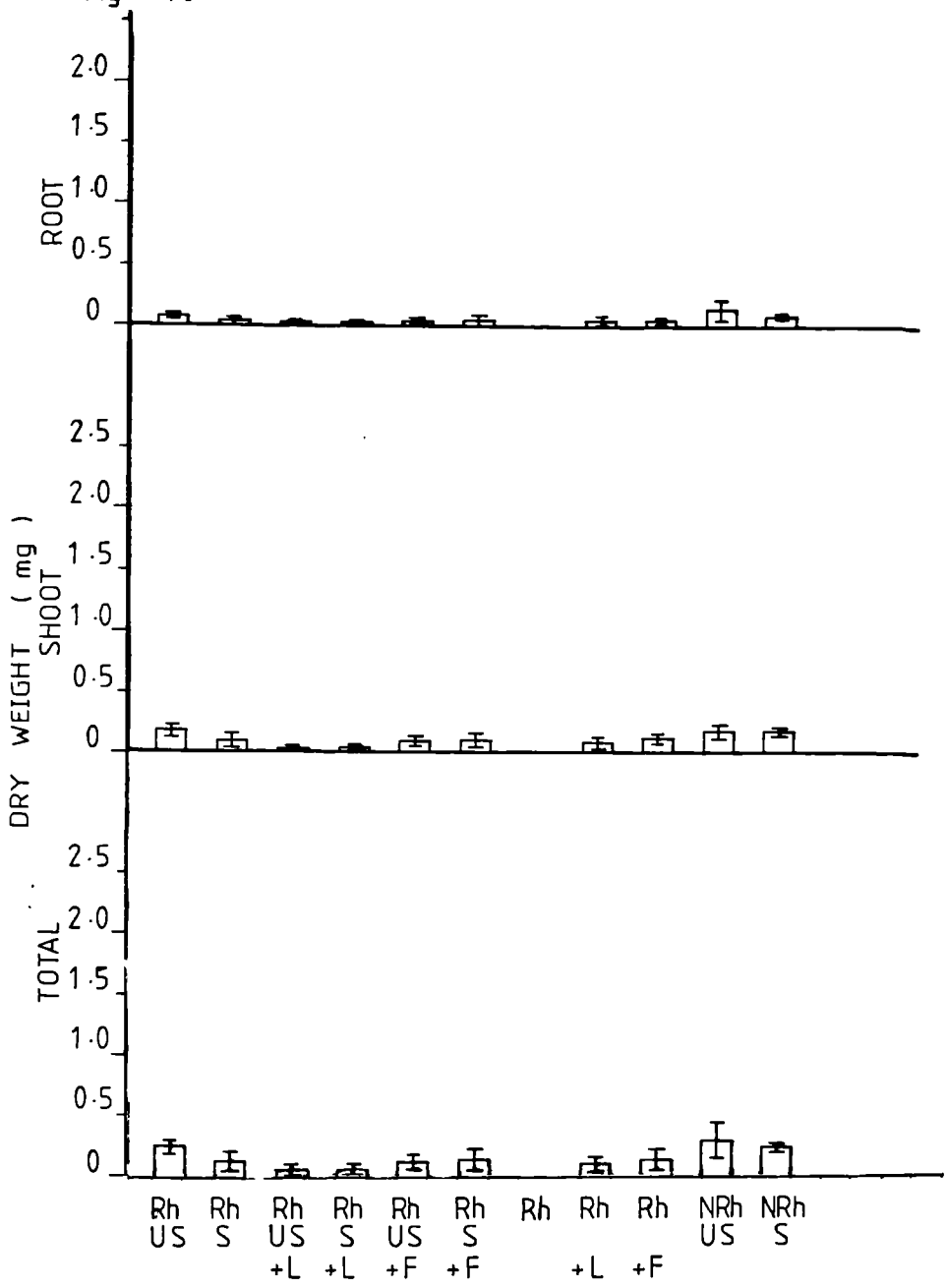
Fig. 4·77 Values for all seedlings planted



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

2 B Fe Values for all seedlings planted

Fig. 4.78

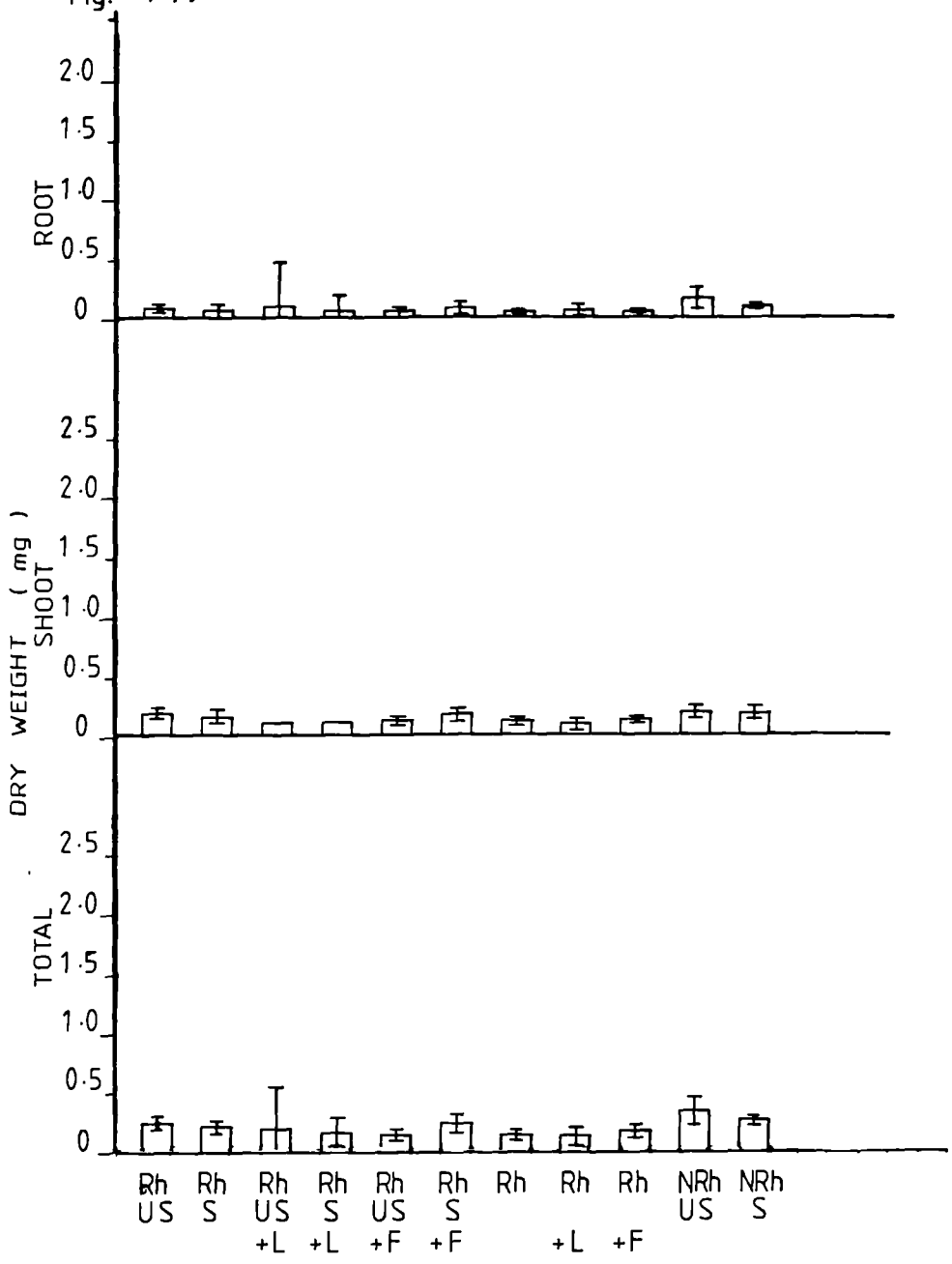


SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Survivors only

2B Fe

Fig. 4.79



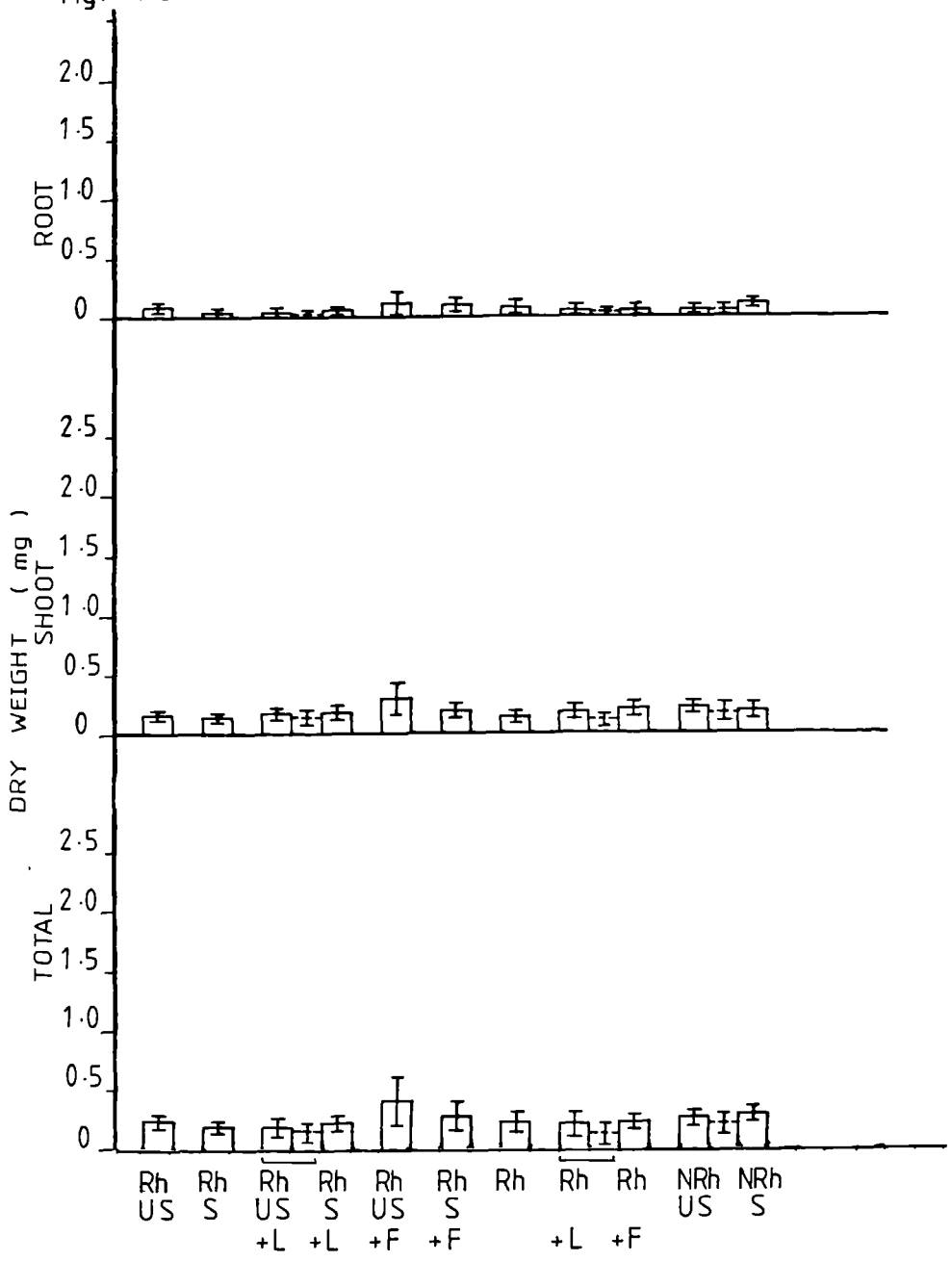
SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Survivors only ——

Values for all seedlings planted – –

3B Fe

Fig. 4·80

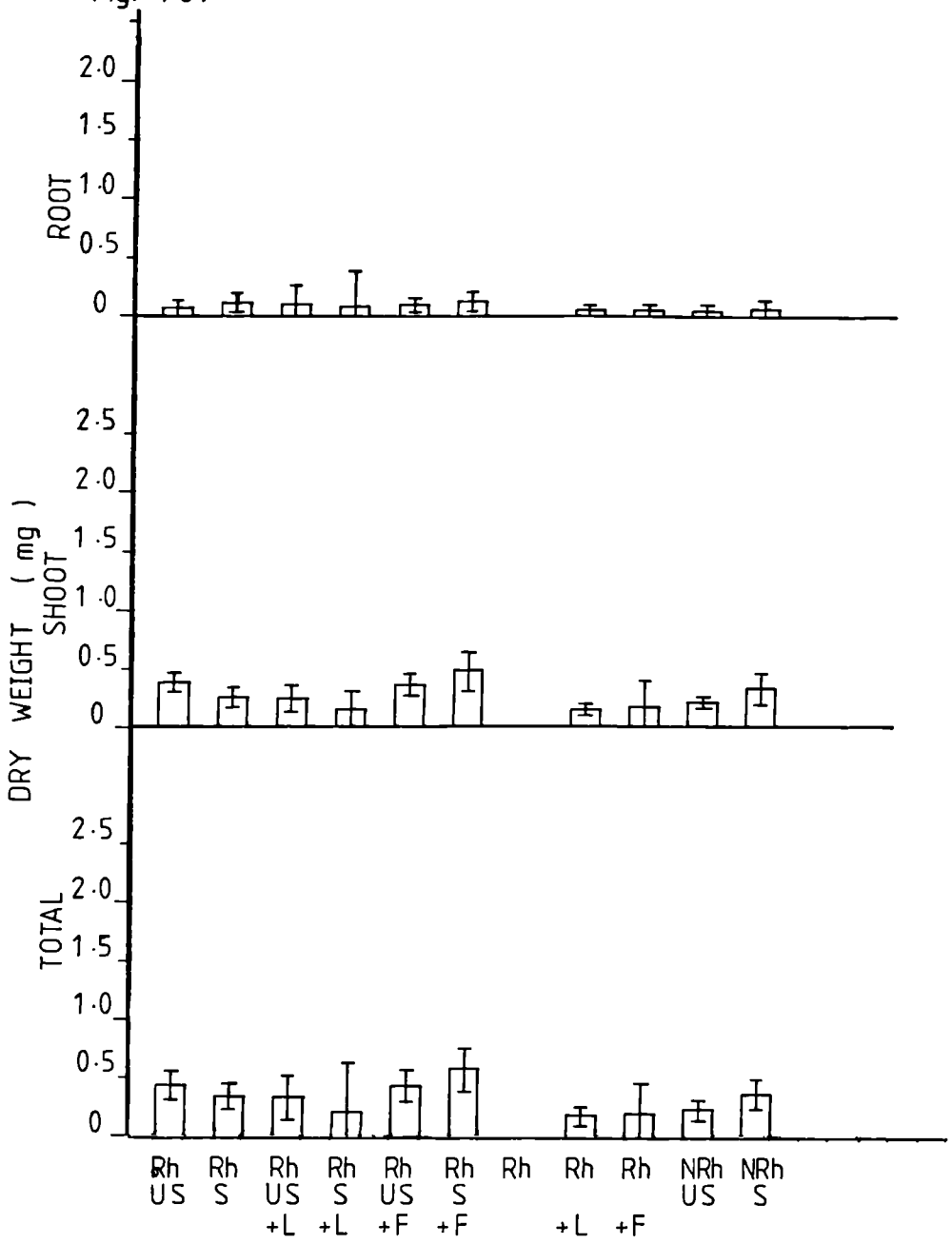


SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Survivors only

4B Fe

Fig. 4.81

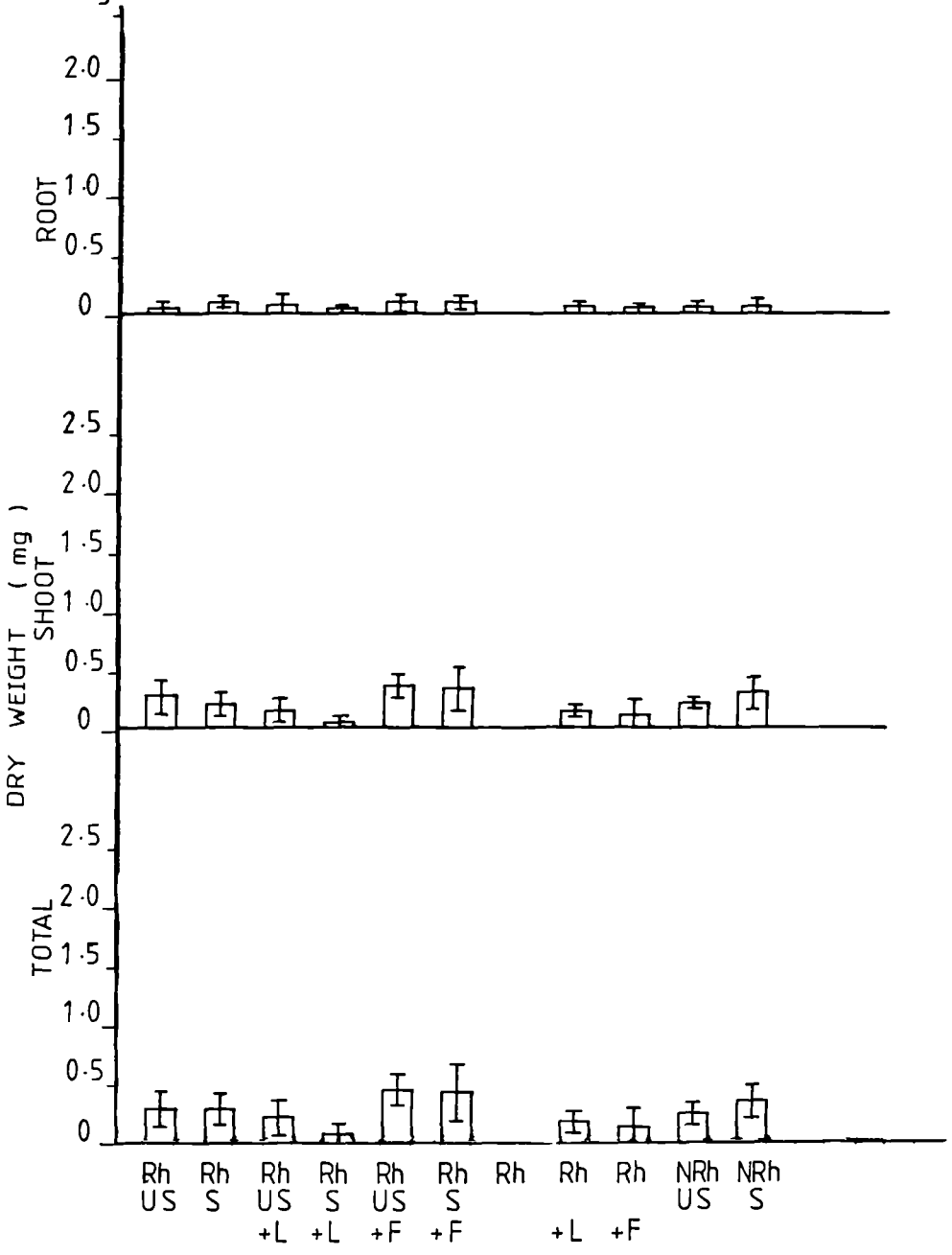


SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Values for all seedlings planted

4 B Fe

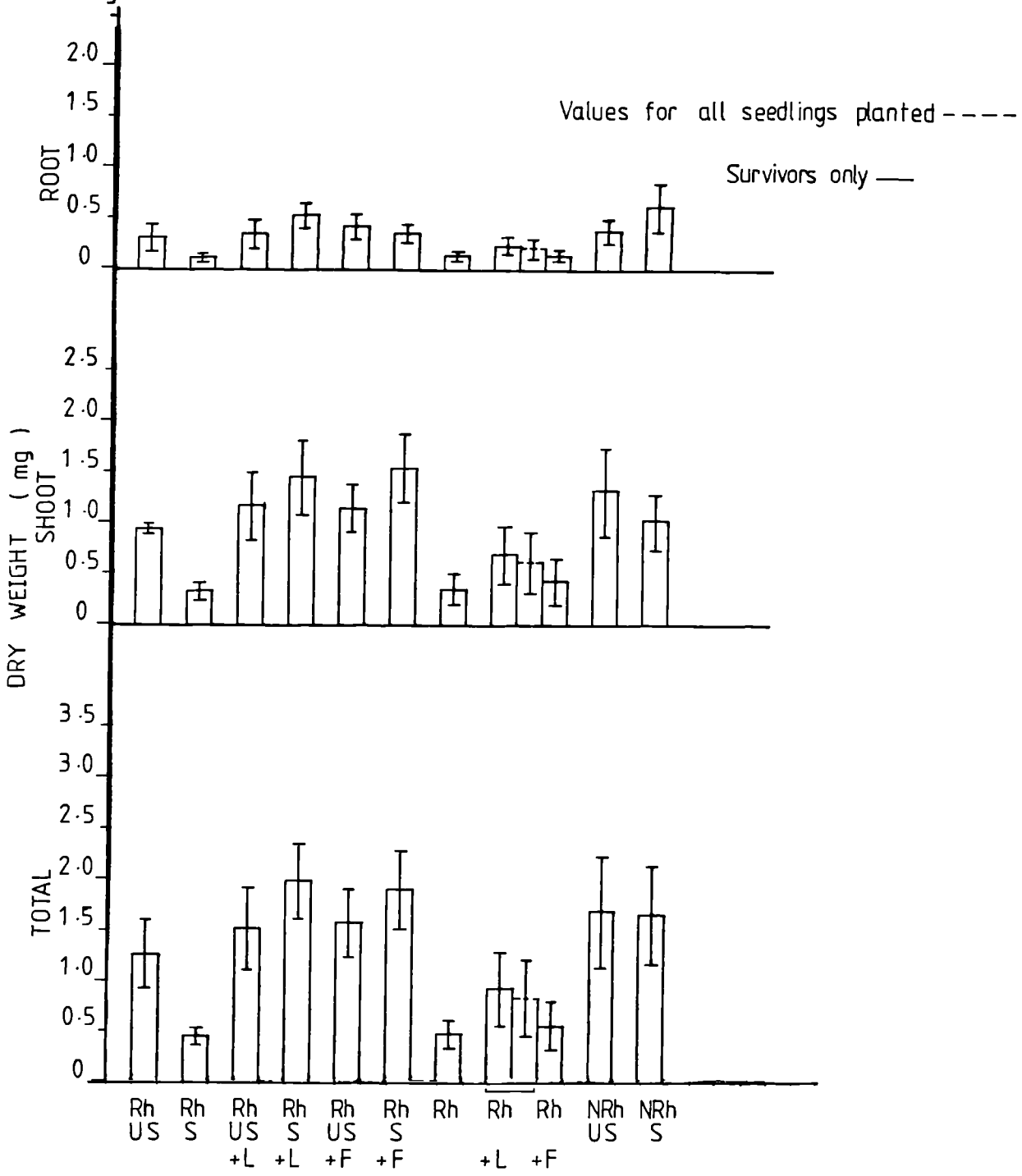
Fig. 4·8 2



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

1A Ru

Fig. 4·83



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

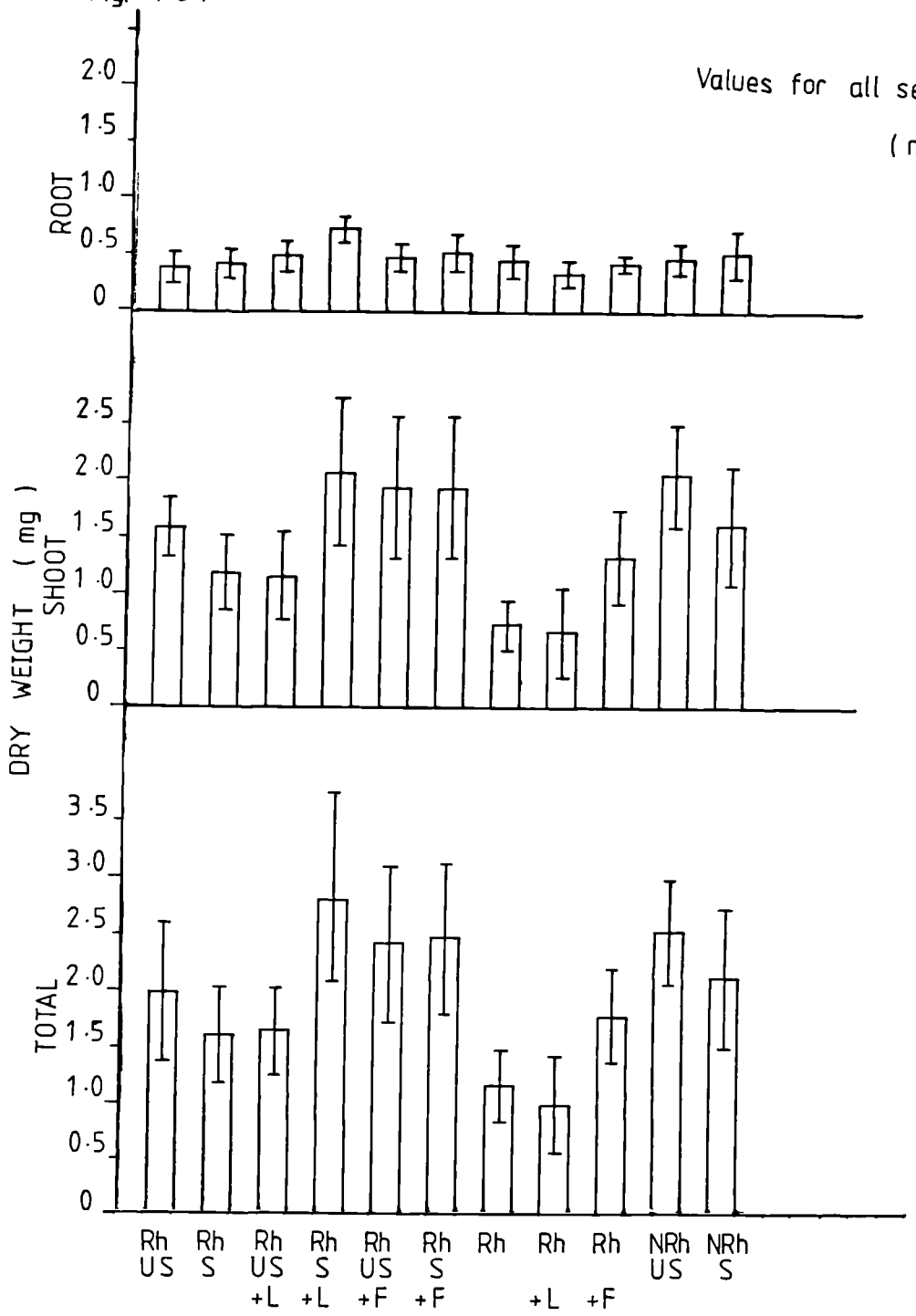
Fig. 4.84

2 A Ru

Survivors only

Values for all seedlings planted

(no change)

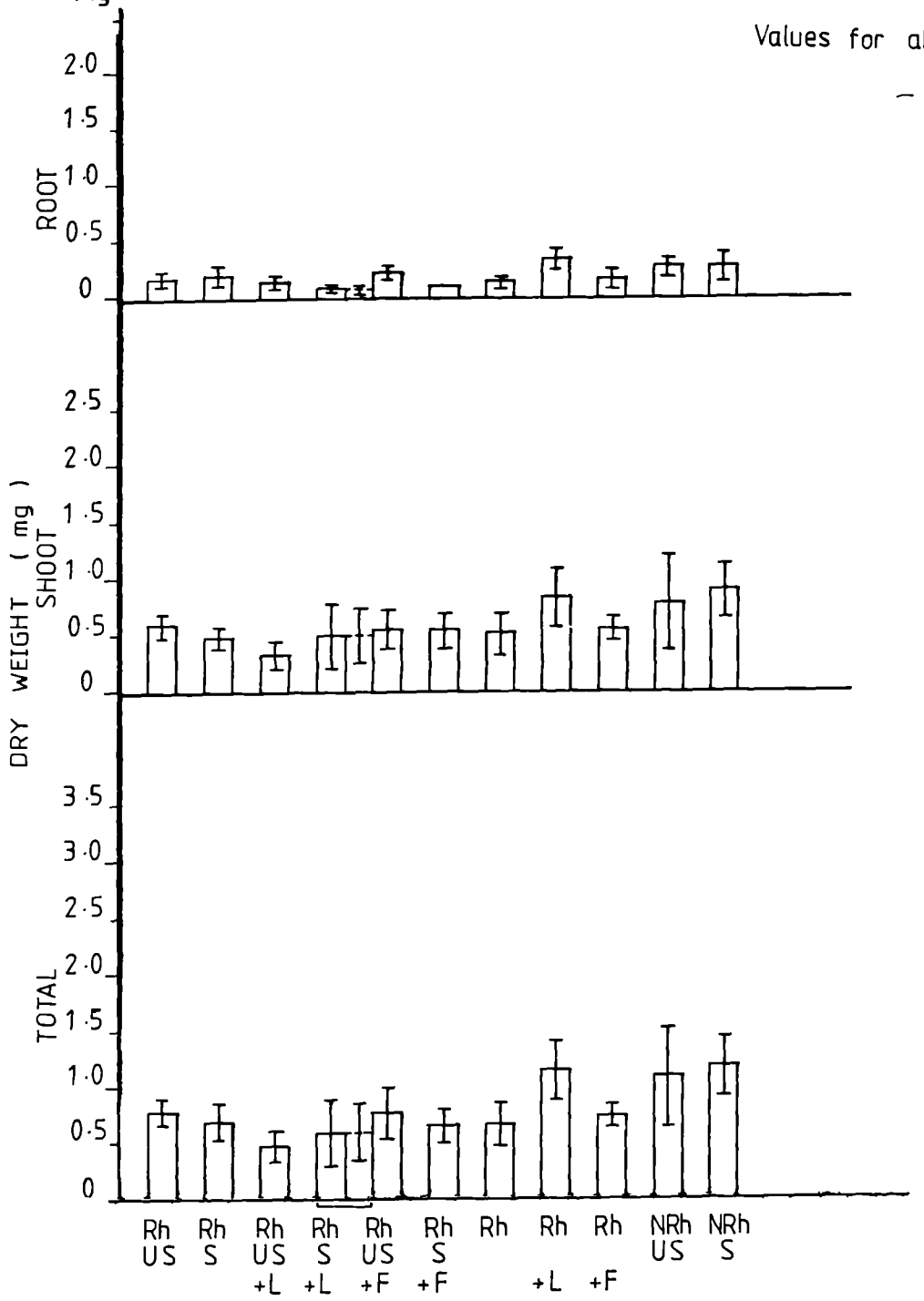


SEEDLING MEAN DRY WEIGHT WITH 95%CONFIDENCE LIMITS

3A Ru Survivors only ——

Fig. 4.85

Values for all seedlings planted – – –



SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

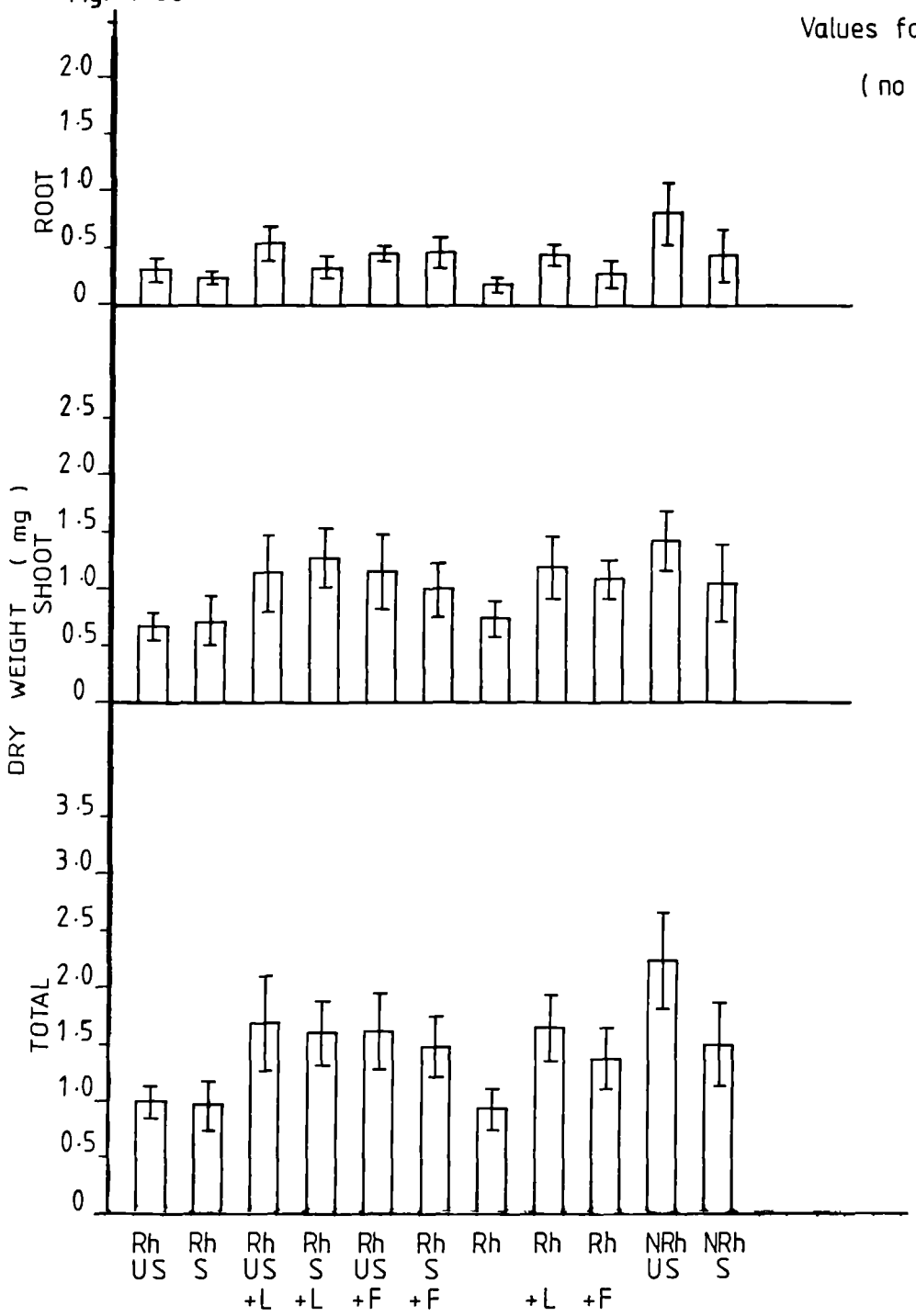
Survivors only

4A Ru

Fig. 4·86

Values for all seedlings planted

(no change)

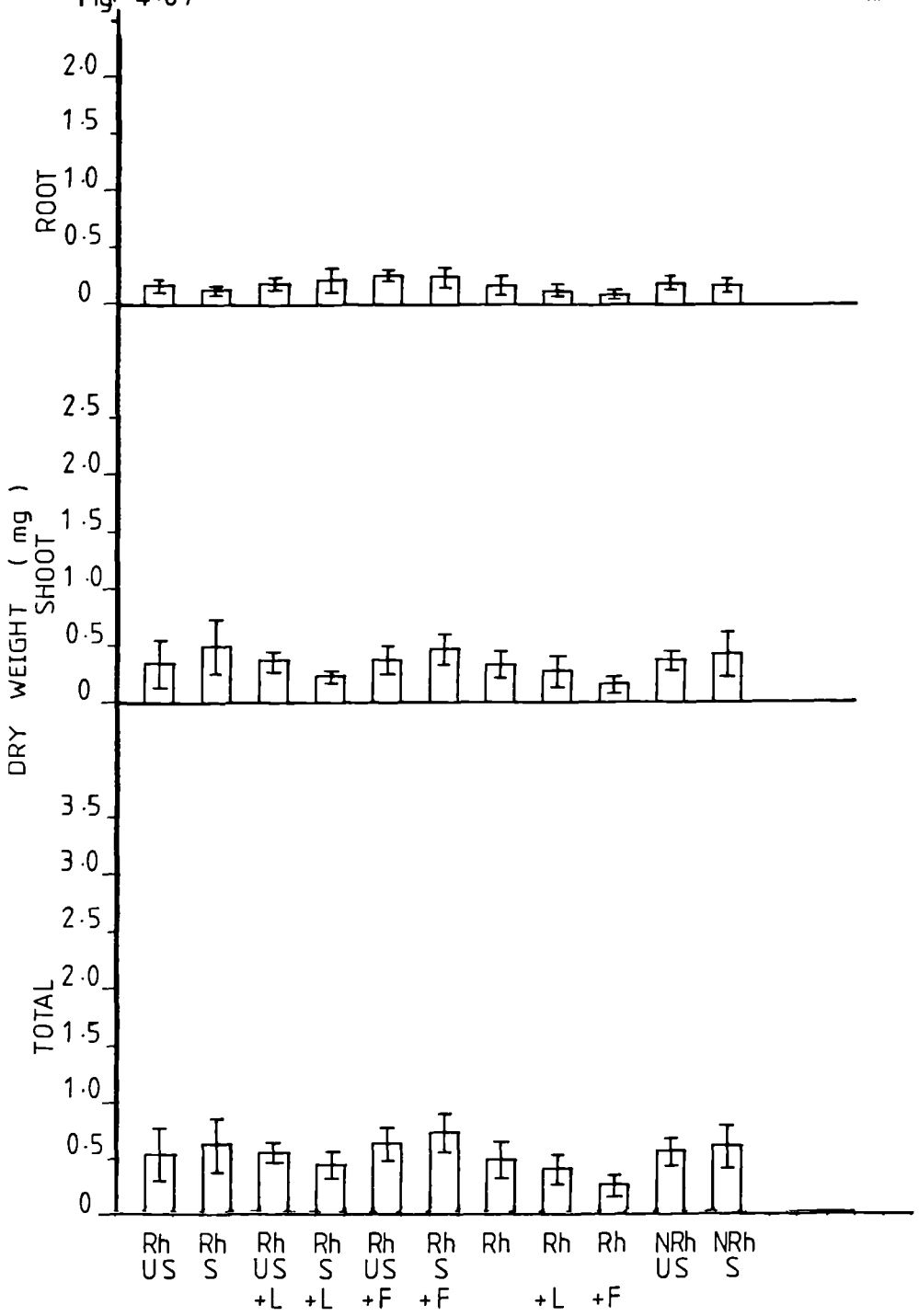


SEEDLING MEAN DRY WEIGHT WITH 95%CONFIDENCE LIMITS

1 B Ru

Fig. 4.87

Survivors only

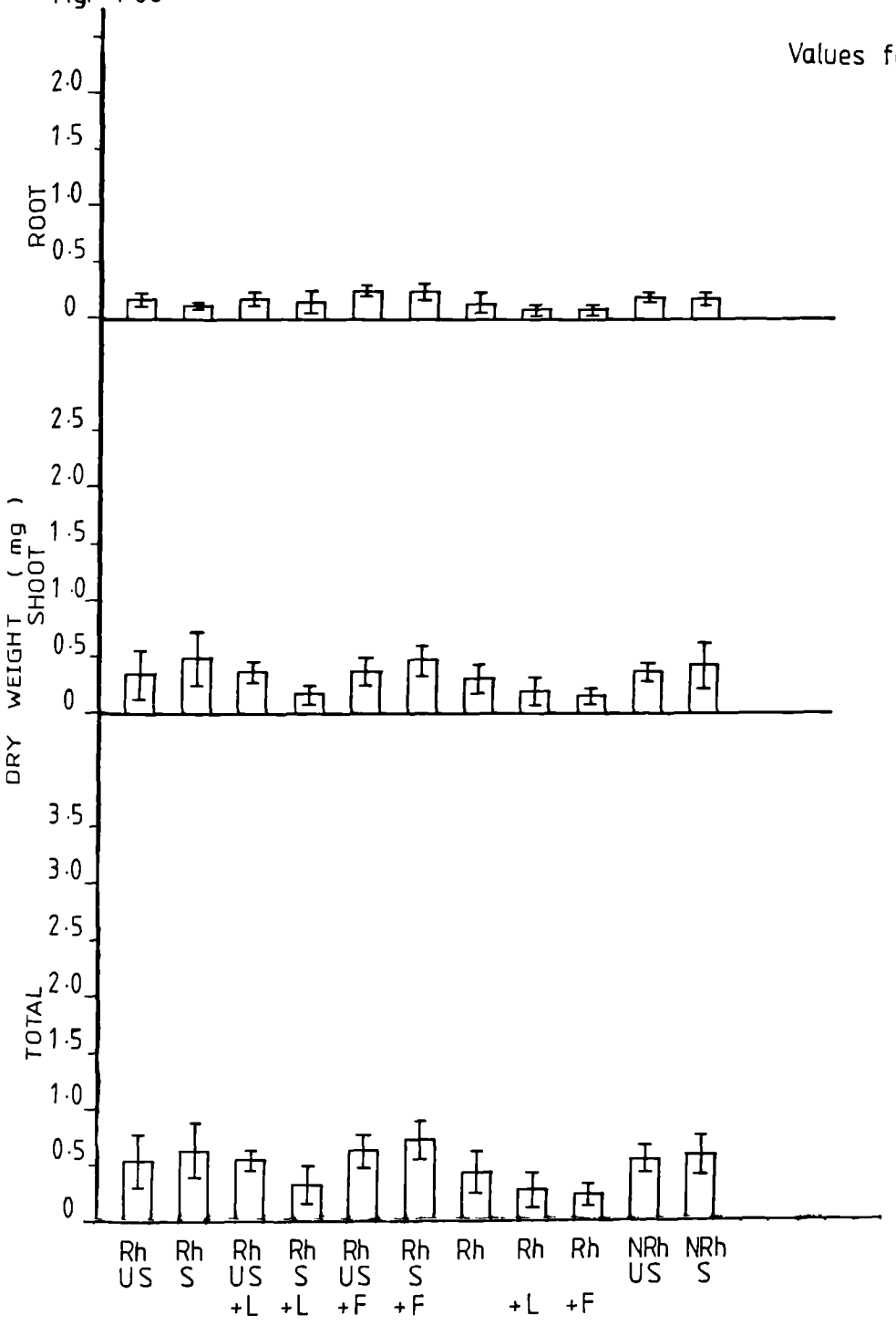


SEEDLING MEAN DRY WEIGHT WITH 95%CONFIDENCE LIMITS

1 B Ru

Fig. 4.88

Values for all seedlings planted

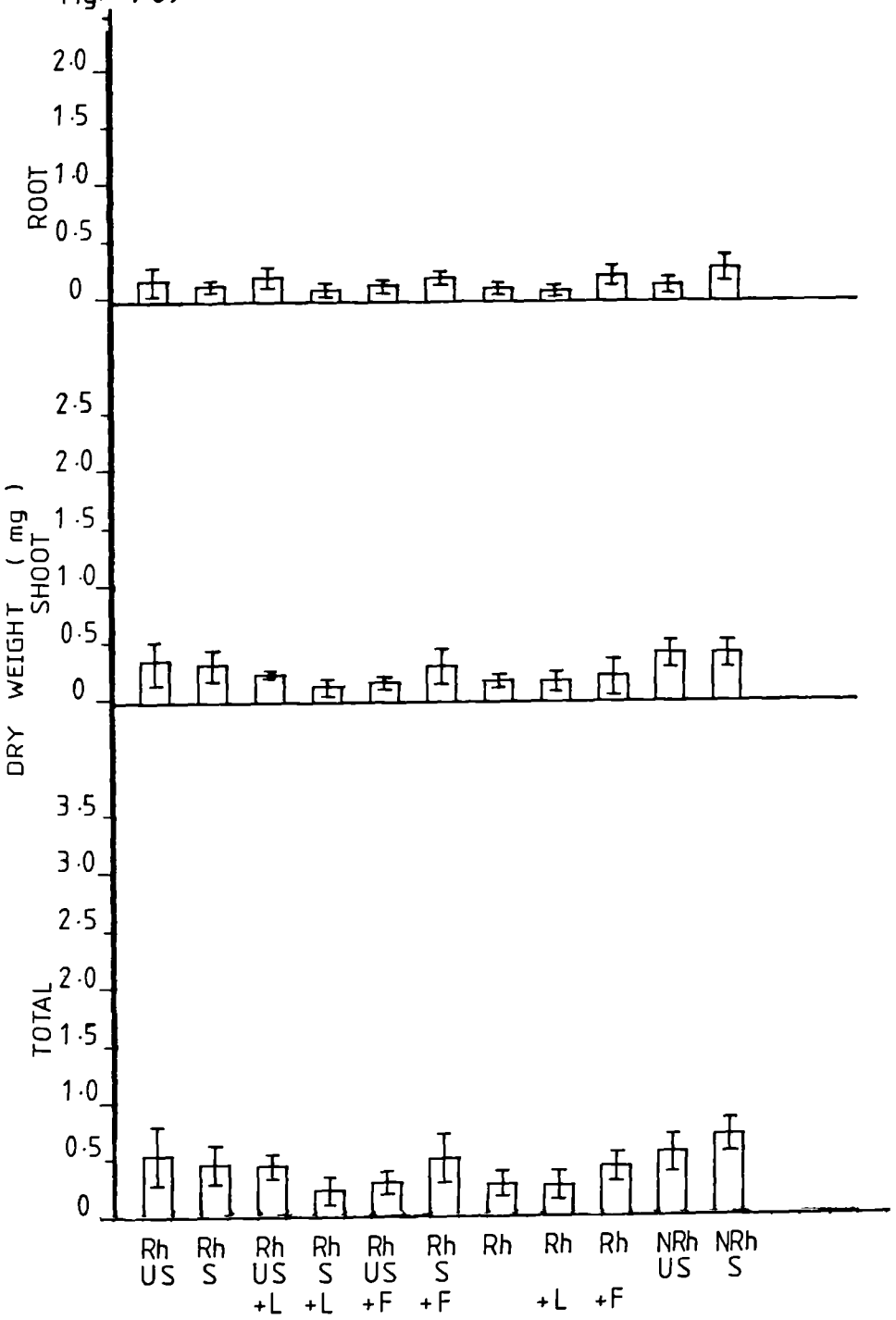


SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

Survivors only

2 B Ru

Fig. 4.89

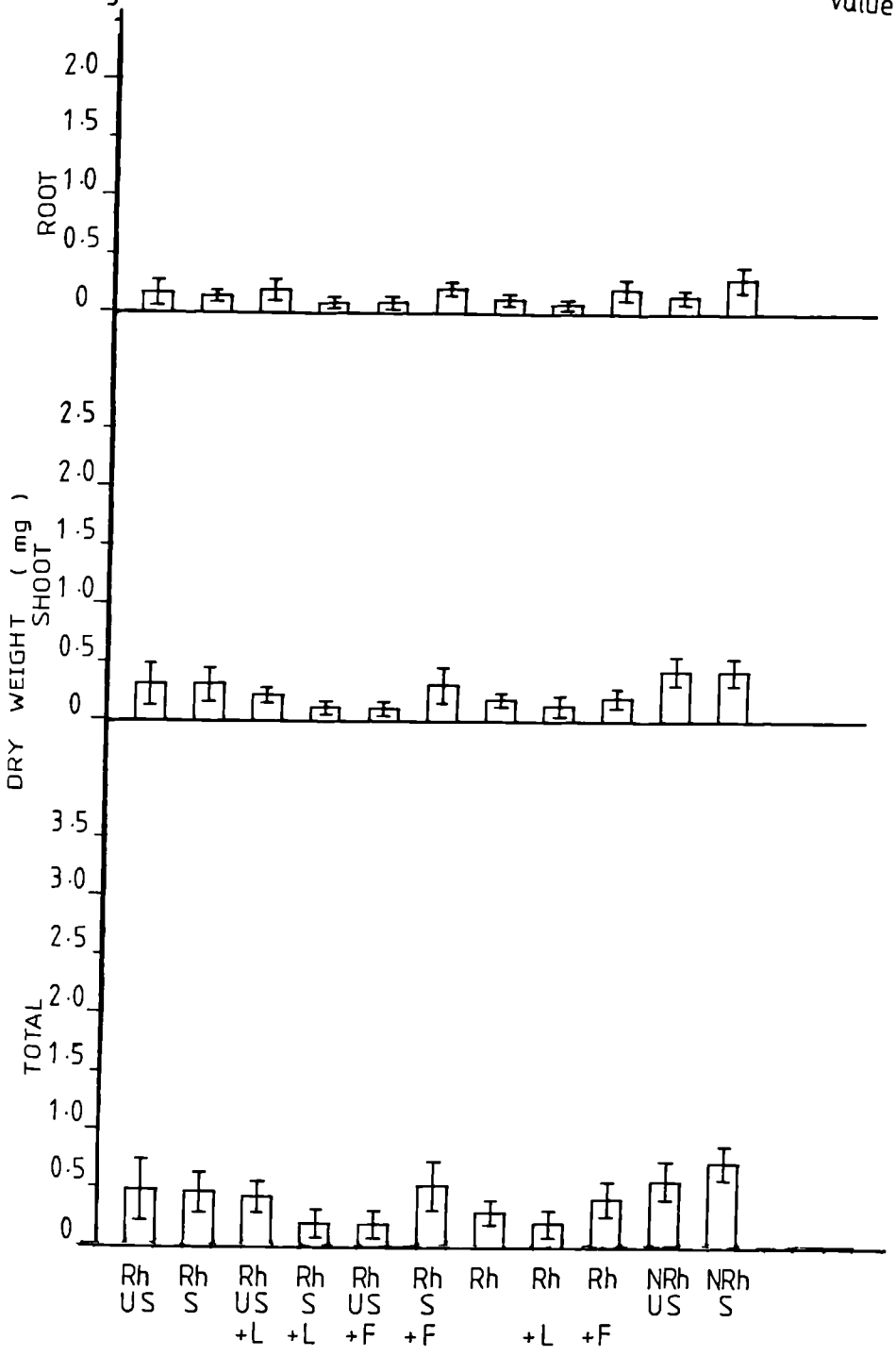


SEEDLING MEAN DRY WEIGHT WITH 95%CONFIDENCE LIMITS

2B Ru

Fig. 4.90

Values for all seedlings planted



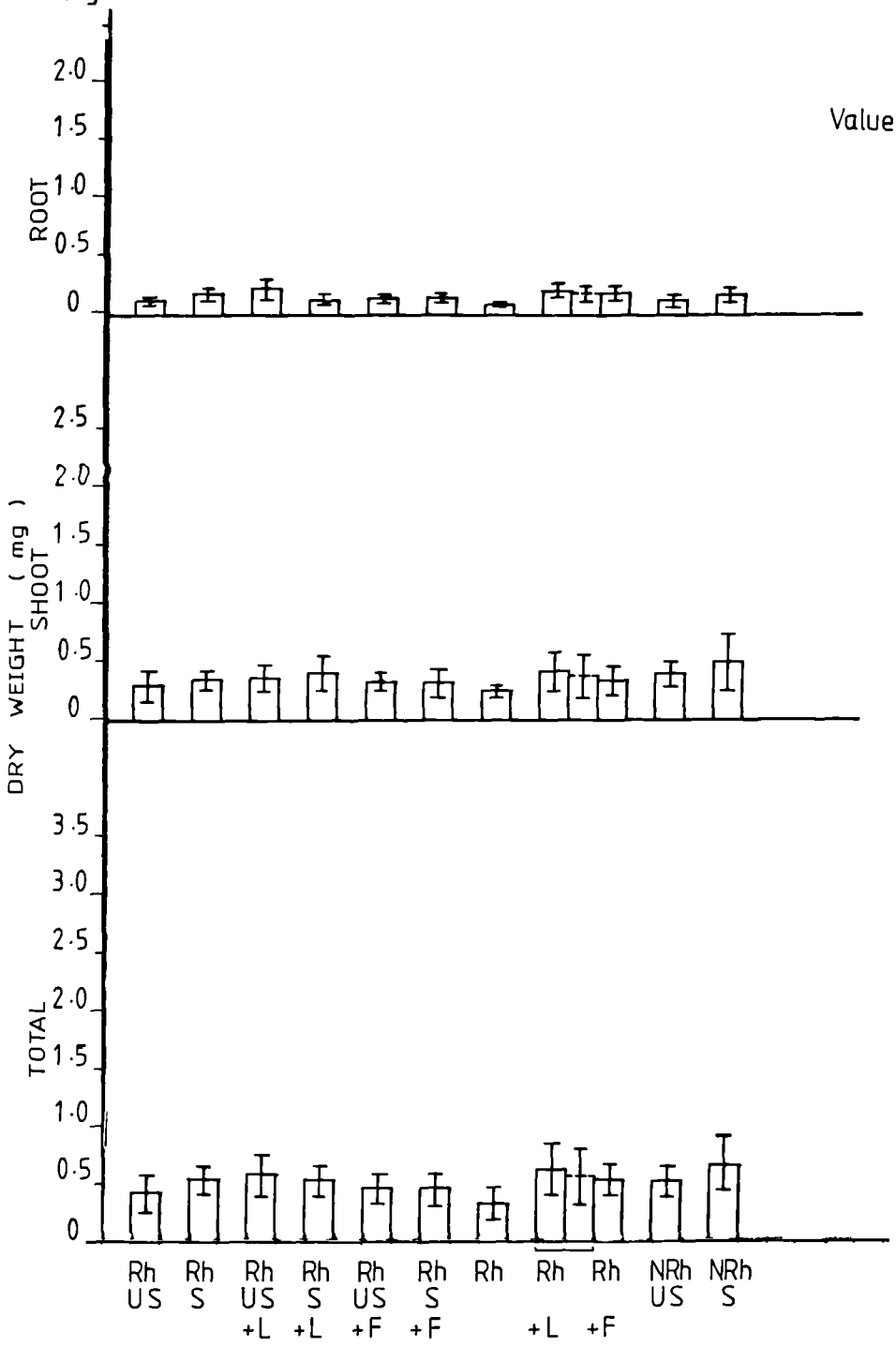
SEEDLING MEAN DRY WEIGHT WITH 95% CONFIDENCE LIMITS

3B Ru

Survivors only ——

Values for all seedlings planted – – –

Fig. 4.91



SEEDLING MEAN DRY WEIGHT WITH 95%CONFIDENCE LIMITS

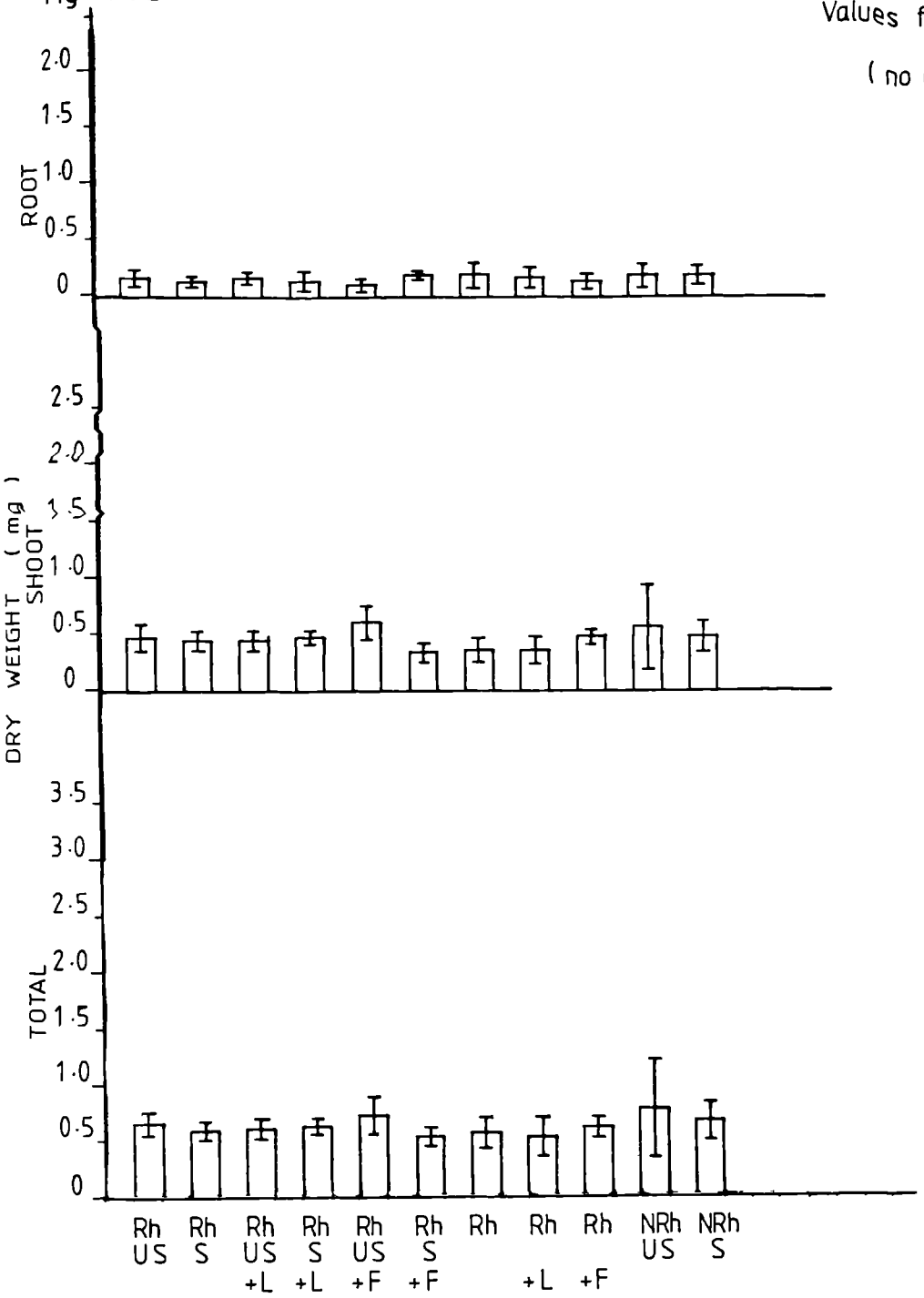
Survivors only

4 B Ru

Fig. 4.92

Values for all seedlings planted

(no change)



4.5.3.2 Seedling Survival

Table 4.5.3.1

	% seedlings surviving in each plot								
Treatment	1A	2A	3A	4A	1B	2B	3B	4B	(F.ovina)
Rh.US	100	100	100	100	90	100	100	70	
Rh.S	100	80	100	100	100	60	100	80	
Rh.US+L	100	100	100	100	80	20	80	60	
Rh.S+L	70	100	10	100	50	30	100	30	
Rh.US+F	90	100	100	100	100	80	100	100	
Rh.S+F	100	100	80	100	100	60	100	70	
Rh.	80	70	100	100	100	100	100	0	
Rh.+L	10	100	100	100	40	80	60	90	
Rh.+F	40	40	100	100	80	90	100	60	
NRh.US	100	80	100	100	50	90	80	100	
NRh.S	100	100	100	100	40	100	100	90	
Mean value :	80.9	88.2	90.0	100	75.5	73.6	92.7	68.2	
		89.8				77.5			

Table 4.5.3.2

	Mean % seedlings surviving for each treatment	
	A (Rhodo. canopy removed)	B (canopy intact)
Rh.US	100	90.0
Rh.S	95.0	85.0
Rh.US+L	100	60.0
Rh.S+L	70.0	52.5
Rh.US+F	97.5	95.0
Rh.S+F	95.0	82.5
Rh.	87.5	75.0
Rh.+L	77.5	67.5
Rh.+F	70.0	82.5
NRh.US	95.0	80.0
NRh.S	100	82.5

Table 4.5.3.3

	% seedlings surviving in each plot								
Treatment	1A	2A	3A	4A	1B	2B	3B	4B	(R. acetosa)
Rh.US	100	100	100	100	100	90	100	100	
Rh.S	100	100	100	100	100	100	100	100	
Rh.US+L	100	100	100	100	100	90	100	100	
Rh.S+L	100	100	80	100	70	80	100	100	
Rh.US+F	100	100	100	100	100	60	100	100	
Rh.S+F	100	100	100	100	100	100	100	100	
Rh.	100	100	100	100	90	100	100	100	
Rh.+L	90	100	100	100	70	70	90	100	
Rh.+F	100	100	100	100	90	90	100	100	
NRh.US	100	100	100	100	100	100	100	100	
NRh.S	100	100	100	100	100	100	100	100	
Mean value :	99.1	100	98.2	100	92.7	89.1	99.1	100	
		99.3				95.2			

Table 4.5.3.4

	Mean % seedlings surviving for each treatment	
	A (Rhodo. canopy removed)	B (canopy intact)
Rh.US	100	97.5
Rh.S	100	100
Rh.US+L	100	97.5
Rh.S+L	95.0	87.5
Rh.US+F	100	90.0
Rh.S+F	100	100
Rh.	100	97.5
Rh.+L	97.5	82.5
Rh.+F	100	95.0
NRh.US	100	100
NRh.S	100	100

4.5.3.3 Soil acidity

For each of the four pairs of plots, the soil pH for the different treatments was measured in both distilled water an in calcium chloride solution. Measurements taken after 2 hours and 24 hours. Values for 24 hours given below.

Table 4.5.3.5

Treatment	pH in distilled water				pH in calcium chloride			
Plot:	1	2	3	4	1	2	3	4
Rh.US	6.70	3.80	3.75	3.60	6.50	3.15	3.55	3.20
Rh.S	6.40	3.75	3.70	3.55	6.25	2.95	3.40	3.15
Rh.US+L	6.70	4.80	4.70	4.80	6.70	4.30	4.30	4.30
Rh.S+L	6.55	4.80	4.65	4.75	6.70	4.30	4.35	4.25
Rh.US+F	6.30	3.80	3.70	3.60	6.10	3.10	3.30	3.20
Rh.S+F	5.90	3.70	3.70	3.55	5.75	3.10	3.10	3.20
Rh.	6.00	3.70	3.60	3.55	5.70	3.50	3.30	3.10
Rh.+L	6.50	4.40	4.55	4.30	6.35	4.20	4.15	3.95
Rh.+F	5.90	3.80	3.60	3.60	5.85	3.60	3.40	3.20
NRh.US	4.35	4.05	4.25	4.30	4.00	3.65	3.95	4.00
NRh.S	4.05	4.00	4.10	4.15	3.50	3.30	3.90	3.85

4.5.3.4 Soil analysis : moisture content and description

Table 4.5.3.6 % moisture in fresh field samples

Treatment	Plot: 1	2	3	4
Rh.US	61%, org.	81%, v.org.	76%, v.org.	83%, v.org.
Rh.S	60%, org.	82%, v.org.	77%, v.org.	82%, v.org.
Rh.US+L	63%, org.	83%, v.org.	75%, v.org.	83%, v.org.
Rh.S+L	63%, org.	82%, v.org.	76%, v.org.	80%, v.org.
Rh.US+F	60%, org.	81%, v.org.	77%, v.org.	81%, v.org.
Rh.S+F	56%, org.	82%, v.org.	74%, v.org.	84%, v.org.
Rh.	60%, org.	83%, v.org.	77%, v.org.	83%, v.org.
Rh.+L	62%, org.	80%, v.org.	75%, v.org.	83%, v.org.
Rh.+F	59%, org.	84%, v.org.	76%, v.org.	83%, v.org.
NRh.US	41%, v.min.	40%, v.min.	39%, v.min.	42%, v.min.
NRh.S	42%, v.min.	37%, v.min.	40%, v.min.	39%, v.min.

4.5.3.5 Soil analysis : available nitrogen and phosphorus

Av. P. (ppm.)

Av. N- Ammonium, Av.-N Nitrate, Av.-N Total, (ppm.)

Table 4.5.3.7

Treatment	Plot: 1	2	3	4
Rh.US	3.5	22.1	15.2	17.1
	85.1,6.9,92.0,	166.9,-,166.9,	62.3,3.9,66.2,	60.1,-,60.1
Rh.S	3.5	15.8	13.7	20.2
	63.2,6.1,69.3,	111.3,10.8,122.1,	59.2,-,59.2,	42.0,3.4,45.4
Rh.US+L	1.0	39.1	25.3	9.1
	104.1,11.1,115.2,	215.9,-,215.9,	92.1,4.7,96.8,	57.5,-,57.5
Rh.US+F	19.1	19.6	18.3	22.9
	62.2,-,62.2	134.6,-,134.6,	75.9,-,75.9,	68.2,2.1,70.3
Rh.S+F	38.1	20.9	25.4	16.5
	82.1,5.2,87.3,	164.7,-,164.7,	70.1,3.2,73.4,	68.0,-,68.0,
Rh.	-	2.0	1.4	3.2
	8.2,1.0,9.2,	10.4,-,10.4,	13.8,1.1,14.9,	7.1,-,7.1,
Rh.+L	1.5	3.2	6.8	4.9
	9.7,-,9.7,	10.0,2.1,12.1,	15.9,-,15.9,	17.2,2.5,19.7
Rh.+F	10.0	11.3	10.3	12.3
	11.5,2.4,13.9,	14.4,-,14.4,	19.7,4.0,23.7,	18.5,3.2,21.7
NRh.US	0.1	-	0.5	-
	2.0,3.7,5.7,	-	-	3.2,3.5,6.7
NRh.S	-	-	1.0	1.1
	-	-	-	2.1,3.9,6.0

4.5.4 DISCUSSION

Due to the variability in the results achieved, it is difficult to draw meaningful conclusions from this particular experiment. Consideration of problems of uniformity of conditions and hence of data replication and also the implications of laboratory experiments on interference and on extractions of potential toxins from soils, suggests the following. A field experiment of this nature could be effectively carried out at another site such as Clumber (N.Notts) or Winterton (Norfolk). Uniformly level topography with a low-nutrient, acidic, sandy soil and vigorous *R. ponticum* are probably essential prerequisites of such a site. With hindsight and the information gathered during this research, it is clear that the site at Chatsworth was unsuitable for this particular work. The variable topography, rather variable (generally quite organic) soils and high rainfall are conditions least conducive to the clarification of the interference phenomenon.

CHAPTER 5

THE OCCURRENCE OF PHENOLIC COMPOUNDS IN *R. PONTICUM* AND IN ASSOCIATED SOIL, LITTER AND CANOPY THROUGHFALL

5.1 INTRODUCTION

Phenolic compounds have been widely considered to be implicated in allelopathic reactions between plants (McPherson, Chou and Muller, 1971; Chou and Muller, 1972; Ballester, Albo and Vieitez, 1977; Carballeira, 1980; Carballeira and Cuervo, 1980; Read and Jalal, 1980; Jalal and Read, 1983 I and II). They are a complex group of related compounds of widespread occurrence whose roles in both plant physiology and ecology are often not fully understood. For this reason, some analysis of their structural and biochemical characteristics, together with their occurrence in plants, is neccessary.

Ericaceous plants are known to be both qualitatively and quantitatively rich in phenolic ompounds (Davies, Coulson and Lewis, IV, 1964; Hegnauer, 1966; Harborne and Williams, 1971,1973; Thompson, Jacques, Haslam and Tanner, 1972; Read and Jalal, 1980; Jalal, Read and Haslam, 1981). Handley (1957) found large quantities of protein precipitating materials (suspected polyphenols) in the leaves of R. ponticum. Published data are now available concerning the flavonoids and simple phenols of the genus Rhododendron in particular (Harborne and Williams, 1971) and the family Ericaceae in general (Harborne and Williams, 1973); the catechins and procyanidins of R. ponticum (Thompson et al, 1972); the phenolic compounds of C. vulgaris (Read and Jalal, 1980; Jalal et al, 1981); as well as a variety of less comprehensive reports on phenolics in the Ericaceae, including numerous compounds cited for various species of Rhododendron.

Phenolic compounds are aromatic, organic chemicals with one or more hydroxyl groups. Other substitutions occur within the basic structure, together with polymerizations and combinations, to produce a vast array of naturally occurring phenols. Harborne (1964) defines them as 'all naturally occurring substances with a free or masked phenolic function'.

Biosynthetic studies suggest that phenols are formed by similar pathways in a wide variety of plants. The diversity of naturally occurring phenols are believed to be inter-related biosynthetically.

Harborne and Simmonds (1964) classified plant phenolics in fifteen major groupings. The flavonoids form the largest naturally occurring group. Simple monocyclic phenols, phyenylpropanoids and phenolic quinones are also numerous. Important polymeric compounds in plants, such as lignins, melanins and tannins, are polyphenolic. Proteins, alkaloids and terpenoids also occasionally possess phenolic units. The main groups relevant to this research are:-

1. Free phenols, phenolic acids and phenylpropanoids (cinnamic acids and coumarins). These are C_6, C_6-C_1 and C_6-C_3 structures.
2. Flavonoid compounds. These are C_6-C_3-C_6 based structures.

Free phenols are comparatively rare in nature. Hydroquinone is the most widely distributed, with catechol, orcinol and pyrogallol occasionally reported.

Some phenolic acids are of universal occurrence. Phenolic acids associated with lignin as ester groups, are found in the alcohol insoluble fraction of the leaf, or bound as glycosides in the alcohol soluble fraction. Protocatechuic, p-hydroxybenzoic, vanillic and syringic acids are universal in angiosperms. Gentistic acid is widespread and both salicylic and o-protocatechuic acids are characteristic of the Ericaceae. These phenolic acids are usually released from plant tissues by acid hydrolysis.

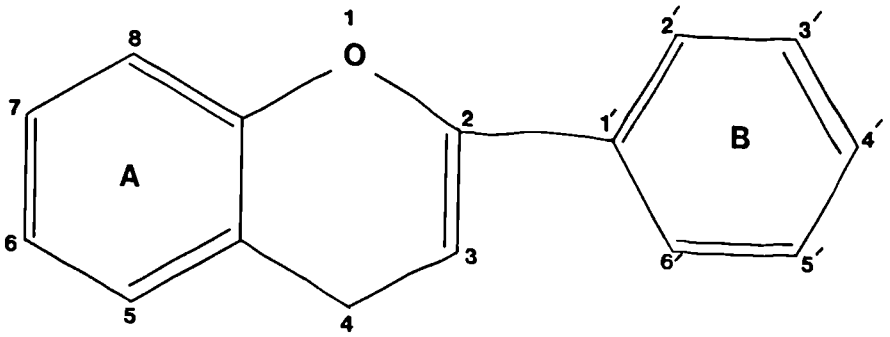
The most common phenylpropanoids are the hydroxycinnamic acids (important as the precursors of lignin, as well as for growth regulation and disease resistance). Ferulic, sinapic, caffeic and p-coumaric acids are almost ubiquitous in plants. They usually occur as esters and are extracted from tissues by mild, alkaline hydrolysis. Caffeic acid is often found as the quinic acid ester, chlorogenic acid. Hydroxy coumarins are found in a wide range of plants. Coumarin itself is very widespread and notable for its aromatic properties. Other phenylpropanoids do occur, but are of less importance.

Flavonoid compounds are present in all vascular plants. Anthocyanins are important, widespread pigments in petals, leaves and other tissues. Flavones and flavonols are universal, mainly colourless and occur as co-pigments to anthocyanins in petals, as well as being widespread in leaves. The commonest flavonols are kaempferol, quercetin and myricetin. Flavanones are of restricted and sporadic occurrence in a few higher plant families. Proanthocyanins, catechins and leucoanthocyanidins occur widely in 'woody' plants. They may have tanning abilities are are associated with tannin formation. They are mostly colourless and are found particularly in heartwood and leaves.

Flavonoids are derived structurally from flavone (which occurs as the white, mealy farina on Primula sp.). They are polyphenolic and weakly acidic. Harborne (1979) divides them into twelve groups, of which the following are relevant:-

1. Flavones
2. Flavanones
3. Flavonols
4. Anthocyanins
5. Catchins
6. Proanthocyanins
7. Leucoanthocyanidins

The Flavonoid Nucleus



Flavones are based on the above with oxygen at (4). Flavanones lack the double bond in the 2-3 position. Flavonols have a 3-hydroxyl subtituent. Anthocyanidins lack the carbonyl group at position (4). Proanthocyanidins are flavan-3-ol dimers or higher oligomers (and therefore polymeric in nature). Catechins are flavan-3-ols. Leucoanthocyanidins are flavan-3,4-diols.

Most flavonoids are water soluble, but this varies considerably, some being highly ether soluble and occurring in leaf waxes or bud exudates. They usually occur in the living cell as glycosides, mainly in the cell vacuole. The combination with a sugar molecule to form a glycoside is important in conferring sap-solubility, protection from enzymic oxidation and stability to light. Particularly in leaves, glycosylation may also be a means of storing toxic phenolic substances in a relatively inactive form (free flavonoids probably being more active as enzyme inhibitors, etc. than bound ones) (Harborne, 1979).

Any one flavonoid aglycone may be found in the same plant in a number of glycosidic combinations. The sugars most frequently found are the common plant sugars and in monosaccharide attachment. The linkage is usually β in the case of D-sugars, α in the case of L-sugars, with the exception of arabinose, which may be either α or β. The most frequent disaccharides are rutinose and sophorose. In some glycosides the situation may be further complicated by an acyl group being attached to one or more of the sugar hydroxyl groups. The acyl groups are often aromatic acids based on one of the four common hydroxycinnamic acids. Other linkages of the sugar to the flavonoid group may occur.

Flavones and flavonols may also occur in water soluble form in plants, covalently bound to inorganic sulphate. Many of the known examples of this have sugar attachments as well.

Bate-Smith (1962) highlighted the differing patterns of phenolic compounds occurring in 'woody' and in 'non-woody' plants. He suggested a distinction between 'woody' and 'non-woody' plants on the basis of the typically 'woody' pattern of phenolic constituents in the leaves of 'woody' dicotyledonous plants. These 'woody' phenolics are also common in

monocotyledonous plants, gymnosperms and pteridophytes. Bryophytes, algae, fungi and lichens have quite different patterns of phenolic compounds.

The occurrence of these 'woody' phenolics is not complete throughout the various genera. Systematic variations on a taxonomic basis have been shown. A basic set of eight commonly occurring 'woody' phenolics were listed:-

Leucoanthocyanins	(2) :	leucodelphinidin, leucocyanidin
Flavonols	(3) :	myricetin, quercetin, kaempferol
Hydroxy Acids	(3) :	ellagic, caffeic and p-coumaric acids.

There are also other fairly common constituents and an immense variety of less common phenolics. Almost all are structurally derivable from the basic eight.

Thompson et al (1972) divided the leucoanthocyanins (associated with tannin formation and 'woodiness' by Bate-Smith) into leucoanthocyanidins and procyanidins. They found procyanidins and catechins to be widespread in woody plants, but not so the monomer leucoanthocyanidins. The procyanidins and catechins were also found 'free' rather than bound as glycosides. On the basis of this work, they proposed the synthesis of procyanidins from catechins.

In addition to variation between major plant groupings, differences in quantity and quality of phenolics have been found to depend on the tissue sampled and environmental factors such as soil-type. Coulson, Davies and Lewis (1960 I), working on beech, showed an increase in quantity and diversity of phenolic substances from a mor humus site compared to a mull humus site, when examining fresh leaves. The quantity decreased in the following order:-

fresh green leaves > senescent leaves > dead leaves > fresh fallen leaves > decayed leaves, humus or stored, dry leaves.

An aqueous extract of fresh beech leaves was twice as rich in phenolics when from the mor site than from the mull site. An ethyl acetate extract was six times stronger from the mor than the mull.

Pot culture of seedlings suggested that high polyphenol content was associated with lack of soil nitrogen or phosphorus (Davies, Coulson and Lewis, 1964 IV). A link was suggested between nutrient deficient soil and increased organic acid synthesis linked to greater biosynthesis of carbohydrates. The outcome proposed was that the litter produced at a mor site would tend to be more acid. Soil conditions at a mor site would be likely to lead to relatively high tannin levels in the leaves of acid-tolerant plants associated with such sites. Plants capable of synthesizing leucoanthocyanins are common on such sites and are associated with mor formation. The influence of polyphenols from such plants on the mobility of iron compounds etc. in the soil, and on associated mor formation was demonstrated by Coulson et al (1960 II) and Davies et al (1964 III). Handley (1954) proposed the interaction between phenolics from Calluna and humic colloids to be responsible for mor humus formation.

Davies et al (1964 IV) presented the relative levels of anthocyanidins found in a range of species from both mull and mor sites:-

1. Mor Calluna > birch > beech > oak > sycamore > Douglas fir > scots pine
2. Mull Calluna > beech > birch > Douglas fir > oak/sycamore > scots pine.

The same workers (Coulson et al, 1960 I) showed that oxidized and polymerized phenolics were present in partially humified litter from both mull and mor sites. Relatively little polyphenol material was obtained from litter or humus using ethyl acetate as the extractant. More was extracted from mor litter and humus using a 'tannin-stripping' solvent (methanol/water).

The involvement of phenolic substances from ericaceous plants in competitive interactions has been strongly indicated by a number of studies such as Chou and Muller (1972), Carballeira and Cuervo (1980); Carballeira (1980); Ballester, Alba and Vieitz (1977) and Read and Jalal

(1980). It is the possible importance of plant phenolics to such interactions, as well as their potential anti-herbivore effects and their influence on soil formation, that are of interest to this investigation. Flower pigmentation and other possible functions, whilst of potential ecological significance, are therefore not considered.

As part of the investigation into the possible role of allelopathic interaction in the ecology of R. ponticum, qualitative and quantitative analyses of the 'free' phenolic and polyphenolic compounds in various R. ponticum tissues were carried out. To assess the potential importance of allelopathy, the occurrence of possible allelopathic agents in soil and in canopy throughfall needs to be examined. In addition to the surveys of phenolic compounds in R. ponticum tissues, analyses were also undertaken for these compounds in Rhododendron soil and canopy throughfall.

5.2 METHOD

5.2.1 EXTRACTION OF 'FREE' SIMPLE PHENOLIC AND POLYPHENOLIC COMPOUNDS FROM PLANT TISSUE

Seedlings were harvested or samples were collected from the field for immediate extraction. Coarse debris was carefully removed by hand and root and shoot material were separated. The samples were washed gently but quickly in cold water to remove superficial contamination. They were then carefully dried on a paper towel to eliminate excess water.

Sub-samples were then taken (usually 5g fresh weight for shoot material and 0.5 - 1.0g fresh weight for root material) and macerated by hand with a mortar and pestle using 100% methanol as the extractant. The macerate was centrifuged at 10000 r.p.m. to separate the extracted supernatant from the solid residue. At least four macerations and separations were usually required. The volume of extract was measured and a sub-sample (c. 5ml) taken for the estimation of total phenols.

The extract was dried to a solid residue using a rotary vacuum evaporator on a water bath at 40°C. Its dry weight was measured. The solid residue from the maceration and extraction was oven-dried at 80°C for 24 hours and weighed.

The extract was re-dissolved in water. In the case of chlorophyllous tissues the extract was now re-extracted in a separating funnel with chloroform to remove the pigments. This was repeated until the green pigment was no longer visible in the aqueous fraction. The aqueous fraction was re-extracted with ethyl acetate in a separating funnel, repeated 3 - 4 times (for both root and shoot samples). The ethyl acetate fraction was passed through anhydrous sodium sulphate in a funnel to remove water. The 'dry' extract was then evaporated to a solid on a rotary vacuum evaporator at 40°C. It was stored in a desiccator in a fridge for a short time until analysis. For further analysis the dried sample was taken up in a small volume of 100% methanol immediately prior to use.

The method was adapted from Coulson, Davies and Lewis (1960), Forrest and Bendall (1969), Thompson, Jacques, Haslam and Tanner (1972) and Harborne (1973) by Dr. M.A.F. Jalal (pers. comm.).

5.2.2 COLORIMETRIC ESTIMATION OF TOTAL PHENOLS

The method uses potassium titanium oxalate or P.T.O. ($K_2Ti\ O(C_2O_4)_2$) and hydroxymethyl methylamine or TRIS. ($NH_2C\ (CH_2O\ H)_3$). It gives an estimate of the amount of 'free' simple phenolics in an extract and is based on the method of Bendall and Hill (Forrest and Bendall, 1969) adapted by Dr. M.A. F. Jalal (pers. comm.). It is thought to be nearly specific for ortho-substituted dihydroxyphenols.

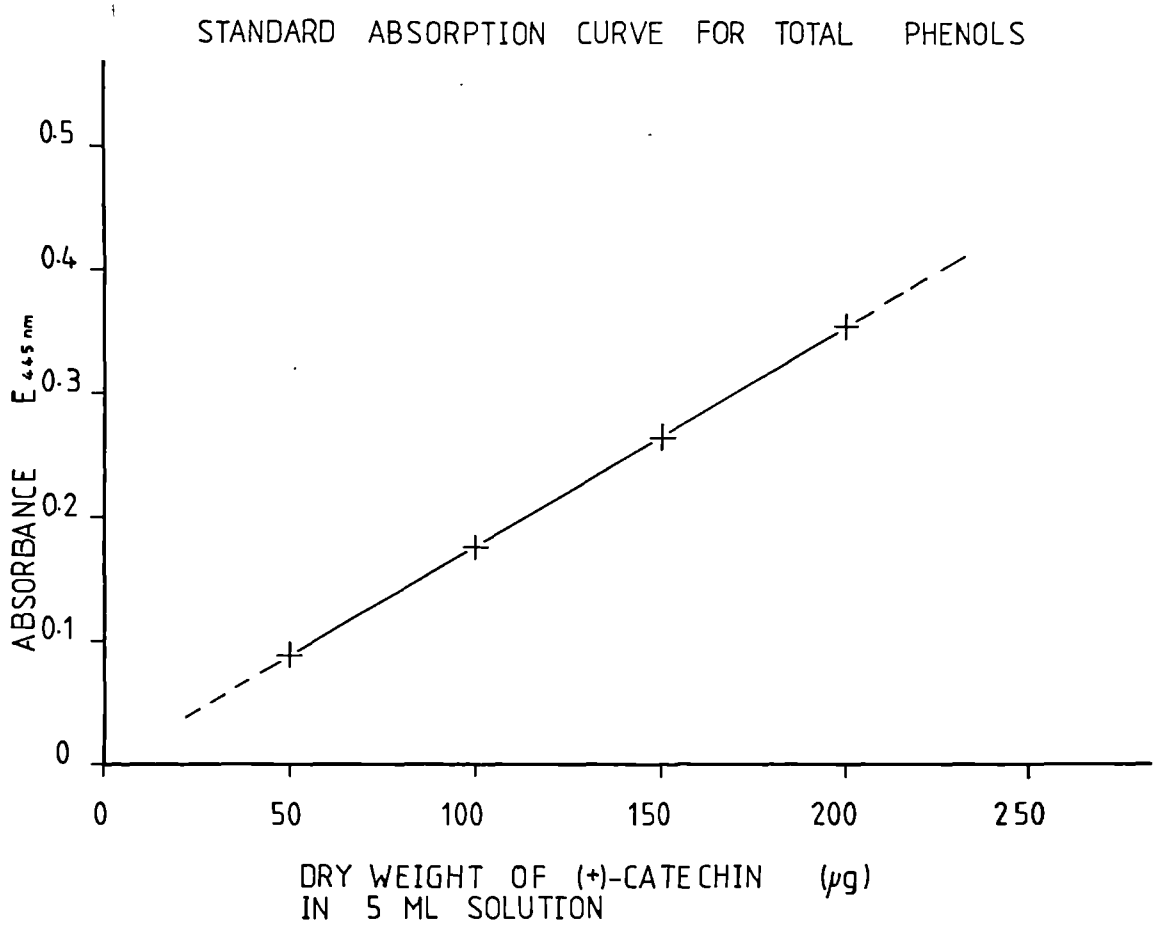
a. The sample is macerated and extracted as already described.
b. The solid residue is removed, dried and weighed.
c. The volume of the methanol-soluble fraction is measured.
d. A sub-sample of known volume is taken.
e. The remainder of the methanol-soluble fraction is taken and dried at 40°C on a rotary vacuum evaporator.
f. 0.1ml of the sub-sample is taken and treated as below:-
   0.1ml extract + 1.5ml mixed indicator solution + 3.4ml distilled water.

The mixed indicator solution is made up of P.T.O. and TRIS. mixed in the ratio 2:1. The pH of P.T.O. is 3.5 and of TRIS is 9.0. The mixed indicator is adjusted to a pH of 6.7.

The 5ml of sample, indicator and distilled water was then left for 5-10 minutes to allow the colour to develop. The colour is stable for around 12 hours.

The absorbance of the solution was read on the spectrophotometer at 445nm. All readings were made within 1 hour of the mixed solution being made up. Readings were compared to a standard calibration curve using known amounts of (+)-catechin (Figure 5.1a). The conversion used was that an absorbance of 0.175 was the equivalent of 100 micro-grammes of (+)-catechin.

Fig. 5·1· a


STANDARD ABSORPTION CURVE FOR TOTAL PHENOLS
ABSORBANCE E 445 nm
0·5
0·4
0·3
0·2
0·1
0
0
50
100
150
200
250
DRY WEIGHT OF (+)-CATECHIN (μg)
IN 5 ML SOLUTION

5.2.3 QUICK TEST FOR THE PRESENCE OF SIMPLE PHENOLIC ACIDS OR PROCYANIDINS

An indication of the possible phenolic content of an extract was gained by the following test. This is based on the known variety of colour reactions given by phenolic compounds following treatment with Gibb's Reagent (2,6-dibromobenzoquinone 4-chloroimide or 2,6-dichlorobenzoquinone 4-chloroimide).

a. The plant extract, soil extract or canopy throughfall extract was evaporated to a solid residue, and then taken up in methanol.
b. Concentrated sample in methanol was 'spotted' on a silica gell plate or a paper chromatogram and run one-way in 6% aqueous acetic acid.
c. The chromatogram was allowed to air-dry and then sprayed:-
   i) With 1% Gibb's Reagent in ethanol.
   ii) Saturated, aqueous sodium hydrogen carbonate.
d. The colours developed and colour changes with time were noted.

A variety of colours are given by different phenols and the method may be used to distinguish between similar compounds or isomers (Harborne, 1973). Phenolic acids not blocked in the para-position give blue colours (Smith, 1969). Ling-Lee, Chilvers and Ashford (1977) state that the formation of the blue salt of indophenol indicates the presence of phenols or other aromatic ring compounds.

As a general guide, the formation of blue colour indicates the presence of simple phenolic compounds. A mauve-purple colour indicates procyanidin compounds (Thompson et al, 1972).

5.2.4 TWO-WAY PAPER CHROMATOGRAPHY FOR SEPARATION AND EXAMINATION OF PHENOLIC COMPOUNDS

A bi-directional chromatographic separation method based on that of Thompson et al (1972) was used. A concentrated sample of extract in methanol was 'spotted' on to Whatman No. 1 paper and run first in 6%v/v aqueous acetic acid. The paper was then removed, air-dried in a fume cupboard and then re-run at right angles to the first run, this time in

butan-2-ol/acetic acid/water (14: 1:5, v/v). The paper was again removed and dried in the fume cupboard before further treatment. Once thoroughly air-dried, the chromatogram was examined and developed as below:-

a. Fluorescence/absorbance noted under ultra-violet light, before and after fuming with ammonia solution.

b. Half the papers were then sprayed with 1:1 1% potassium ferricyanide/1% ferric chloride solution containing a trace of potassium permanganate. The papers were immediately washed in water with concentrated hydrochloric acid added, and then air-dried in a fume cupboard.
In the presence of reducing compounds such as the readily oxidizable phenols, excess ferric ions are reduced to the ferrous state. This produces the blue ferro-cyanide (or Turnbull's Blue) (Ling-Lee et al, 1977). Phenolic compounds are thereby revealed on the chromatogram as prussian blue spots on a white background.

c. Half the papers were sprayed with 1% Gibb's Reagent in ethanol (the reagent used in this case was 2,6-dibromobenzoquinone 4-chloroimide). The papers were then air-dried in a fume cupboard and sprayed with saturated aqueous sodium hydrogen carbonate. Colours and colour changes were noted for the spots which appeared.

Information from a - c above, together with the r.f. values of the spots and their distributional pattern on the paper, was used to identify or to describe the compounds found. Comparisons were made with known compounds identified after purification by paper chromatography and column separation, and pure compounds supplied commercially, with the guidance of Dr. Jalal. Comparison of chromatograms was also made with published information (Smith, 1969; Thompson et al, 1972; Harborne, 1973; Ballester et al, 1977; Read and Jalal, 1980; Jalal et al, 1981) and various similar extracts from C. vulgaris with help from Dr. Jalal.

5.3 QUANTITATIVE ANALYSIS OF THE 'FREE' PHENOLIC CONTENT OF *RHODODENDRON PONTICUM* AND OF A RANGE OF ASSOCIATED HIGHER PLANT SPECIES

5.3.1 INTRODUCTION

Assessing the role of 'free' phenolics in the ecology of R. ponticum requires a knowledge of the total amounts extractable from the different tissues and of seasonal variation in this. Whether or not these amounts are unusual in comparison to those in other competing plants in the same community is important. If the success of R. ponticum is in some way related to the 'free' phenolics and their function, the amounts present might be expected to be unusually high.

The concentration of 'free' phenolics in different aged leaves will have considerably bearing on their potential as either anti-herbivore devices or as allelopathic agents. The latter would presumably be released either via canopy throughfall or decomposing leaf litter. Similarly, the quantities found in root tissues may be important in terms of allelopathic agents released directly from the roots.

5.3.2 METHOD

Samples of plant leaves, roots and stems were collected from Strawberry Lee Plantation, South Yorkshire. A number of part-samples were taken from the same plant for each tissue required. These were then taken back to the laboratory for immediate analysis.

The samples were carefully cleaned and separated into the required portions. The part-samples were then well mixed and sub-sampled for extraction and analysis as described previously. All the leaves were carefully dissected to remove the main veins and supporting tissue. The leaf tissue extracted was therefore primarily interfascicular.

The following R. ponticum tissues were sampled:-

a. New Leaf/New Stem : just emerged, freshly developed tissues.
b. Young Leaf/Young Stem : tissues c. 4-6 weeks old.
c. Mature Leaf/Mature Stem : fully formed, healthy leaves and stem c. 1 year old.
d. Senescent Leaf : yellowing, clearly senescent leaves, but not yet dead.
e. Fourth Year Stem : older stem with developing adventitious roots.
f. Mycorrhizal Hair Roots.
g. Non-mycorrhizal Hair Roots/Adventitious Roots.
h. Mature Main Root : mature, main root, from which the hair roots are borne.

Also for comparison, mycorrhizal and non-mycorrhizal seedlings were examined for total 'free' phenol content. R. ponticum seed was sown onto irradiated and untreated Cropton soil (from North Yorkshire) and the seedlings were harvested after 5 months.

5.3.3 RESULTS

5.3.3.1 Total 'free' phenol content of *R. ponticum* tissues and associated plant species

Particularly high levels of 'free' phenolics were found in the New Leaves of Rhododendron (Table 5.3.3.1). The order of decreasing levels on a dry weight basis in Rhododendron leaves was:-

New Leaf > Young Leaf > Mature Leaf > Senescent Leaf.

In fresh weight terms the Young Leaf was the lowest. The greater dry weight, presumably of supportive tissue, in Mature and Senescent Leaf resulted in their having lower values on a dry weight basis.

The trend for stem samples as dry weights was similar (Table 5.3.3.1):-

New Stem > Young Stem > Mature Stem > 2nd Yr. Stem > 4th Yr. Stem

Again the fresh weight value of the Young Stem phenolics was lower than that of Mature Stem and again this reverses in terms of dry weight.

For roots (Table 5.3.3.1) the order was:-

(Dry Weight) Non-myc. Hair > Myc. Hair > Mature Main Root
(Fresh Weight) Non-myc. Hair > Mature Main Root > Myc. Hair

Two samples were analysed for the Non-mycorrhizal Adventitious Hair Roots. Although the mean of the two samples was richer in phenolics than for Mycorrhizal Hair Roots, one sample was above and one was below.

Levels of 'free' phenolics were generally highest in the leaves. Roots, New Stem and Young Stem were also relatively rich (the latter only on a dry weight basis). Other Stem samples were poor in phenolics, particularly in terms of fresh weight.

Some work was done early on in the analysis, to compare levels of 'free' phenolics in mycorrhizal and non-mycorrhizal seedlings of R. ponticum. Little difference was found for either leaf or root levels in five month old seedlings.

Myc. Leaf 6.2% fw.	Non-myc. Leaf 5.9% fw.
Myc. Hair Root 3.1% fw.	Non-myc. Hair Root 3.2% fw.

Table 5.3.3.1 Total 'free' phenol content of a range of *Rhododendron* tissues

Sample	% total 'free' phenol content as mg (+)-catechin/g tissue	
	% fresh weight	% dry weight
New Leaf	13.7	57.0
Young Leaf	6.3	28.3
Mature Leaf	7.6	22.7
Senescent Leaf	7.1	20.8
New Stem	2.6	22.0
Young Stem	1.8	15.0
Mature Stem	2.0	8.7
Second Year Stem	1.5	4.1
Fourth Year Stem (with developing roots)	1.1	2.8
Mycorrhizal 'Hair' Roots	3.2	14.0
Non-mycorrhizal Adventitious Roots (1)	6.4	22.6
Non-mycorrhizal Adventitious Roots (2)	3.0	12.7
Adventitious Roots (1) & (2) mean	4.7	17.7
Mature Main Root	3.5	9.4

(Samples collected September 1981)

Phenol data p139 et seq

Key for abbreviations used:-

NL	New Leaf	NS	New stem
YL	Young Leaf	YS	Young stem
ML	Mature Leaf	MS	Mature stem
SL	Senescent Leaf	SYS	Second year stem
		FYS	Fourth year stem

MHR	Mycorrhizal hair root
NHR	Non-mycorrhizal hair root
MaR	Mature root

L	leaf	R	root

Be	Betula pubescens	Dr	Dryopteris dilatata
Qu	Quercus petraea	Pt	Pteridium aquifinum
Pi	Pinus Sylvestus	Ca	Calluna vulgaris
Fr	Fraximus excelsia	Ag	Agnostus tenius
Ac	Acer pseudoplatanus	Fe	Festuca ovina
Al	Alnus glutinosa	Mo	Molinia caernlea
Il	Ilex aquifolium		
Ru 1	Rumex acetosella		
Ru 2	Rumex acetosa		
Ho	Holcus mollis		

(See text p.137 et seq. for exact definitions)

Fig. 5.1 TOTAL 'FREE' PHENOLIC CONTENT OF TISSUES FROM RHODODENDRON AND FROM ASSOCIATED SPECIES (1)

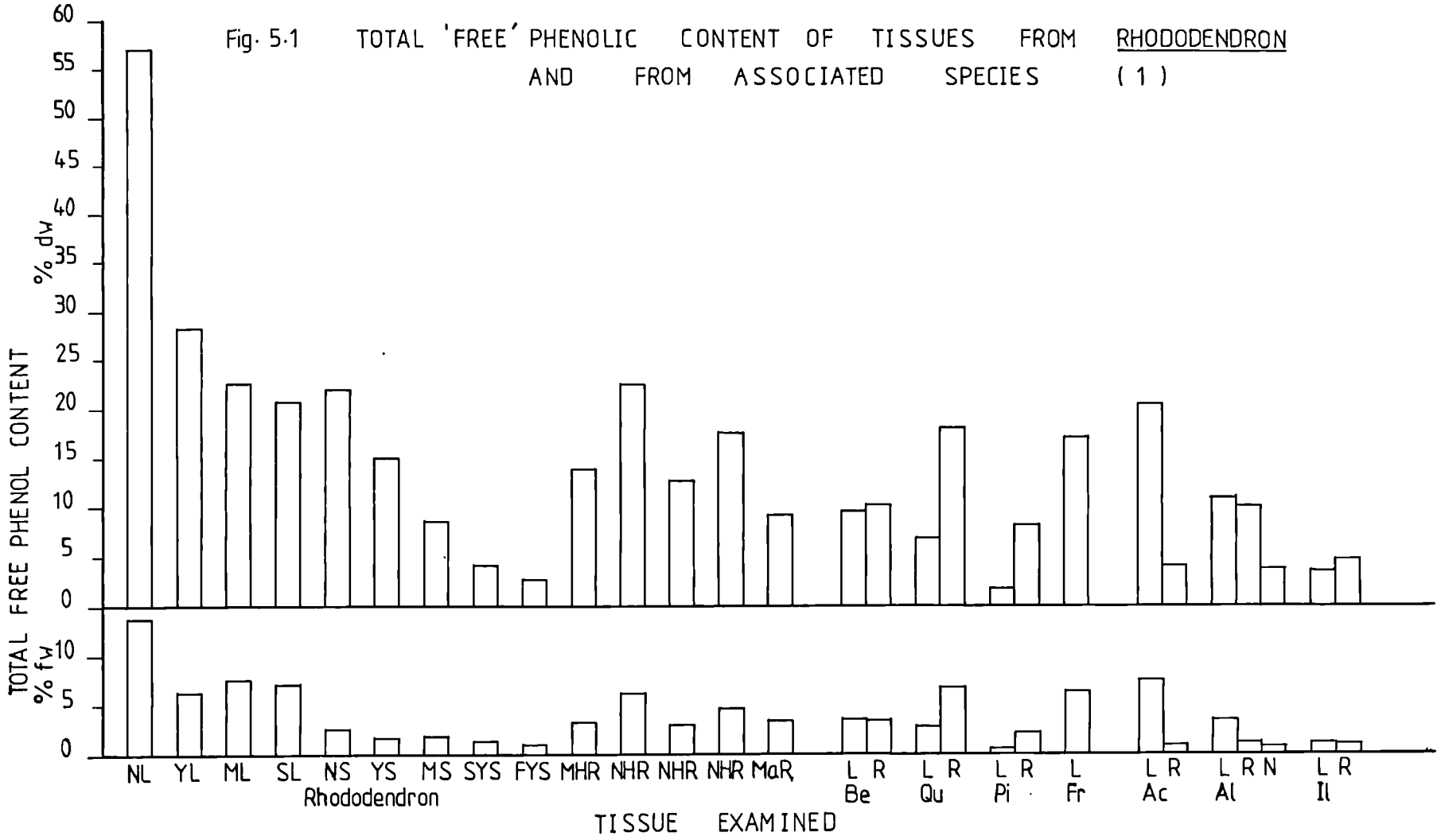
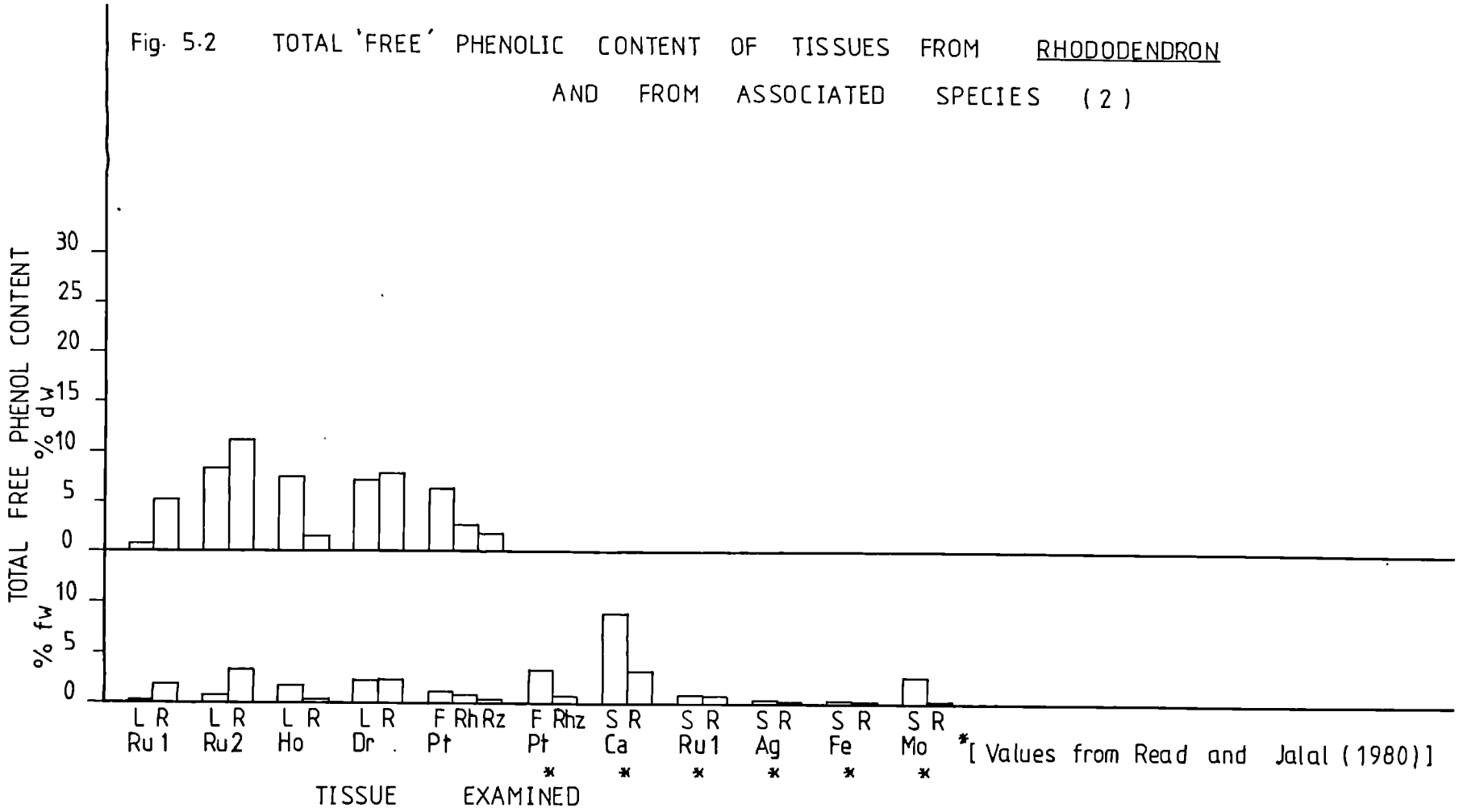


Fig. 5.2 TOTAL 'FREE' PHENOLIC CONTENT OF TISSUES FROM RHODODENDRON AND FROM ASSOCIATED SPECIES (2)



*[Values from Read and Jalal (1980)]

The amounts of 'free' phenolics were higher in Rhododendron tissues than in equivalent tissues from other plants sampled, except for Quercus root (Tables 5.3.3.1, 5.3.3.2 and 5.3.3.3; Figures 5.1 and 5.2).

The varying amounts of supportive tissue and the differing spectra of compounds involved, mean that some caution must be used in the detailed comparison of differences between species. There do appear to be general trends in the relative values. The tree and shrub species have relatively high values, particularly in their leaves. The ericaceous species (Calluna and Rhododendron) are the highest of all. The exceptions to the general trend are Pinus and Ilex, both of which had very small amounts of 'free' phenolics. The values for root samples show no obvious trends of this sort. The relative values are distributed differently to those in the leaf samples and trees, shrubs and herbaceous plants occur throughout the order. Both ericaceous species are in the upper half of the table and Rhododendron is next to the top for its mycorrhizal roots in dry weight terms.

The values given for Calluna 'shoot' include interfascicular leaf, vascular and supportive tissue and stem. The values for pure interfascicular leaf would probably differ considerably, perhaps being somewhat higher.

Table 5.3.3.2 Total 'free' phenol content of roots and leaves of a range of plant species occurring in the same community as *Rhododendron*

Sample	% total 'free' phenol as mg (+)-catechin/g tissue	
	% fresh weight	% dry weight
Betula pubescens		
Mature Leaf	3.6	9.7
Fine Root	3.5	10.4
Quercus petraea		
Mature Leaf	2.9	7.0
Fine Root	6.9	18.4
Pinus sylvestris		
Mature Leaf	0.6	1.9
Fine Root	2.3	8.4
Fraxinus excelsior		
Mature Leaf	6.5	17.3
Fine Root	(Sample not obtained)	
Acer pseudoplatanus		
Mature Leaf	7.6	20.7
Fine Root	1.0	4.2
Alnus glutinosa		
Mature Leaf	3.5	11.1
Fine Root	1.3	10.1
Root Nodule	0.9	3.9

Sample	% total 'free' phenol as mg (+)-catechin/g tissue	
	% fresh weight	% dry weight
Ilex aquifolium		
Mature Leaf	1.1	3.7
Fine Root	1.0	4.8
Rumex acetosella		
Mature Leaf	0.1	0.8
Fine Root	1.9	5.1
Rumex acetosa		
Mature Leaf	0.8	8.4
Fine Root	3.3	11.2
Holcus mollis		
Mature Leaf	1.7	7.5
Fine Root	0.3	1.5
Dryopteris dilatata		
Mature Frond	2.2	7.2
Fine Rhizoid	2.3	7.9
Pteridium aquilinum		
Mature Frond	1.2	6.4
Fine Rhizoid	0.8	2.8
Mature Rhizome	0.4	1.9
Pteridium aquilinum (*)		
Mature Frond	3.2	-
Mixed Rhizome & Rhizoid	0.7	-

Sample	% total 'free' phenol as mg (+)-catechin/g tissue	
	% fresh weight	% dry weight
Calluna vulgaris (*)		
Mature Shoot	9.0	-
Fine Root	3.1	-
Rumex acetosella (*)		
Mature Leaf	0.8	-
Fine Root	0.7	-
Agrostis tenuis (*)		
Shoot	0.4	-
Root (less than)	0.1	-
Festuca ovina (*)		
Shoot	0.3	-
Root	0.1	-
Molinia caerulea (*)		
Shoot	2.6	-
Root	0.1	-

All samples collected from Strawberry Lee Plantation (S.Yorks.) on the same day in September 1981, except where marked (*). (*) samples are adapted from Read and Jalal (1980) for comparative purposes. All data given to the nearest 0.1%.

Data from Read and Jalal are based on three determinations of an extract from each sample. All other data are based on two determinations for each sample. Method as described.

The tables below give the values found, in order of decreasing amounts.

Table 5.3.3.3 Mature leaf or frond

Fresh weight	Dry weight
Calluna (*)	Rhododendron
Rhododendron/Acer	Acer
Fraxinus	Fraxinus
Betula	Alnus
Alnus	Betula
Pteridium (*)	Quercus
Quercus	Rumex acetosa
Molinia (*)	Holcus
Dryopteris	Dryopteris
Holcus	Pteridium
Pteridium	Ilex
Ilex	Pinus
Rumex acetosella (*)/Rumex acetosa	Rumex acetosella
Pinus	
Agrostis (*)	
Festuca (*)	
Rumex acetosella	

Table 5.3.3.4 Fine root or rhizoid

Fresh weight	Dry weight
Quercus	Quercus
Rhodo. Non-myc.	Rhodo. Non-myc.
Betula	Rhodo. Myc.
Rumex acetosa	Rumex acetosa
Rhodo. Myc.	Alnus
Calluna (*)	Betula
Pinus/Dryopteris	Pinus
Rumex acetosella	Dryopteris
Alnus	Rumex acetosella
Acer/Ilex	Ilex
Pteridium	Acer
Pteridium (*)/Rumex acetosella (*)	Pteridium
Holcus	Holcus
Festuca (*)	
Molinia (*)	
Agrostis (*)	

(N.B. Data marked (*) are from Read and Jalal (1980). No dry weight data are available for these.)

5.3.3.2 Seasonal variation

5.3.3.2.1 Roots

Total 'free' phenolics (Table 5.3.3.5; Figure 5.3) varied from 2.2% fresh weight (June 1980) to 5.0% fresh weight (February 1981) and 10.0% dry weight (August 1980) to 33.9% dry weight (April 1981). This seems to be a reflection of the dry weight/fresh weight ratio, which is lowest in April. At this time there is presumably active growth of new roots, relatively rich in 'free' phenolics and with relatively little fibrous material.

5.3.3.2.2 Leaves

Levels of total 'free' phenolics (Table 5.3.3.5; Figure 5.3) ranged from 4.1% fresh weight (June 1980) to 15.5% fresh weight (December 1980) and 24.5% dry weight (June 1980) to 31.6% dry weight (December 1980). The dry weight/fresh weight ratio was lowest for June 1980 and relatively stable at c. 40% for the other samples. Apart from a low in terms of fresh weight in June 1980, 'free' phenol levels appear to be fairly stable throughout the year in both fresh and dry weight values.

Table 5.3.3.4 Seasonal variation in total 'free' phenol content of *Rhododendron* leaf and root tissue

Sample date	Tissue	Total 'free' phenol mg/g fw.	% fw.	Total 'free' phenol mg/g dw.	% dw.
Feb. 1980	Leaf	45.31	4.5	-	-
	Root	39.37	3.9	-	-
Apr. 1980	Leaf	78.28	7.8	-	-
	Root	17.86	1.8	-	-
Jun. 1980	Leaf	40.99	4.1	245.10	24.5
	Root	21.63	2.2	119.77	12.0
Aug. 1980	Leaf	111.66	11.2	284.83	28.5
	Root	36.80	3.7	100.27	10.0
Oct. 1980	Leaf	112.18	11.2	256.85	25.7
	Root	42.33	4.2	201.52	20.2
Dec. 1980	Leaf	155.14	15.5	316.04	31.6
	Root	27.68	2.8	126.19	12.6
Feb. 1981	Leaf	112.48	11.3	270.10	27.0
	Root	49.51	5.0	162.29	16.2
Apr. 1981	Leaf	144.86	14.5	302.65	30.3
	Root	39.52	4.0	338.65	33.9

Additional data for comparison

Sample date	Tissue	Total 'free' phenol mg/g fw.	% fw.	Total 'free' phenol mg/g dw.	% dw.
Sep. 1981	Leaf	76.34	7.6	226.71	22.7
	Root	31.54	3.2	139.81	14.0
Calluna:					
Feb. 1981	Leaf	102.86	10.3	-	-
	Root	11.05	1.1	-	-
Apr. 1980	Leaf	102.58	10.3	-	-
	Root	11.84	1.2	-	-

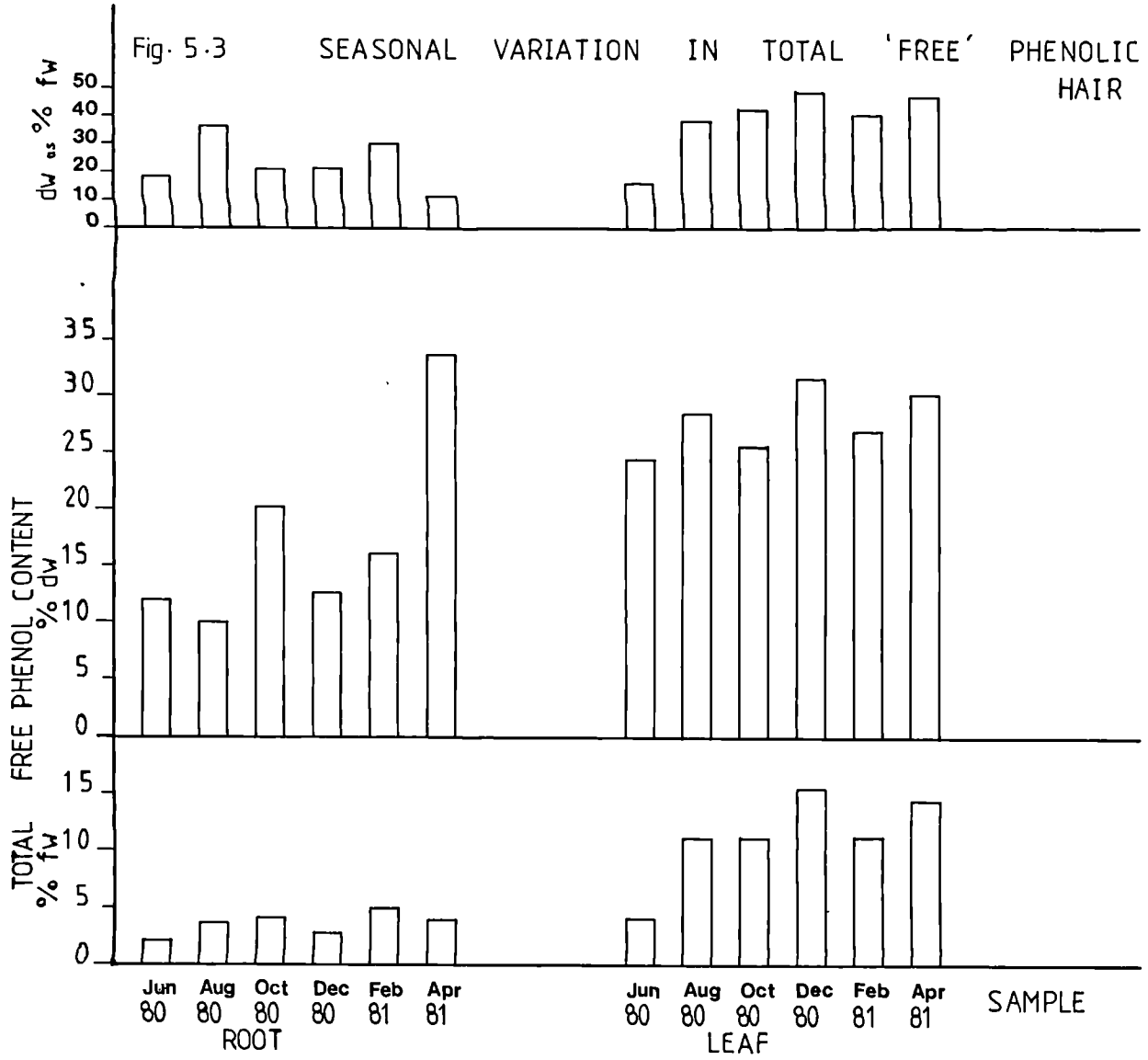
Table 5.3.3.5 Summary of data from June 1980 to April 1981 inclusive

	Total 'free' phenol content			
	% fw.	% dw.	% of methanol extractable fraction	dw. as % fw.
Root				
Jun. 1980	2.2	12.0	75.7	18.1
Aug. 1980	3.7	10.0	65.3	36.7
Oct. 1980	4.2	20.2	63.0	21.0
Dec. 1980	2.8	12.6	62.4	21.9
Feb. 1981	5.0	16.2	69.1	30.5
Apr. 1981	4.0	33.9	69.5	11.7
Leaf				
Jun. 1980	4.1	24.5	61.3	16.7
Aug. 1980	11.2	28.5	57.0	39.2
Oct. 1980	11.2	25.7	52.0	43.7
Dec. 1980	15.5	31.6	55.2	49.1
Feb. 1981	11.3	27.0	58.1	41.6
Apr. 1981	14.5	30.3	60.1	47.9

All values for total phenol are expressed in terms of mg (+)-catechin per g of tissue (either fresh weight, fw., or dry weight, dw.) or of a particular fraction of the extract.

All root samples were of very fine, mycorrhizal 'hair' roots. All leaf samples were of healthy, mature leaves, taking only the leaf blade and removing the major veins.

Fig. 5.3 SEASONAL VARIATION IN TOTAL 'FREE' PHENOLIC CONTENT OF RHODODENDRON HAIR ROOTS AND MATURE LEAVES



5.3.4 DISCUSSION

According to Harborne (1979) there is relatively little information available on the quantitative aspects of flavonoids in plants. Anthocyanin flower pigments may generally be from 0.3% to 4% of tissue dry weight. In deeply pigmented fruits or flowers they may be up to 30%. He suggests that concentrations in leaves usually range from 1% to 10% of dry weight, with occasionally higher concentrations. The exception cited is Camellia sp. leaves which have a total catechin content of 20% dry weight or more. Jalal, Read and Haslam (1982) recorded up to 28.4% dry weight, as total 'free' phenol content in Calluna shoot and up to 9.5% in Calluna root. These estimates were by the same technique as in the present study and are therefore directly comparable. Results of other workers such as Forrest and Bendall may not always be directly comparable since their data are usually expressed in terms of a different phenolic standard and often given only as a percentage of the fresh weight of the tissue.

The concentration of 'free' phenolics in R. ponticum leaves was generally high (20% - 30% dw.) and was comparable to that found by Jalal, Read and Haslam (1982) for Calluna shoot. These values are high in comparison to the other plants examined. Concentrations in mature Camellia leaves are probably of a similar magnitude (Forrest and Bendall, 1969; Harborne, 1979). The value of 57% dw. found in new leaves of R. ponticum was by far the highest value recorded and highlights the need to carefully consider levels in a range of tissue types and ages. Levels declined with age on a dry weight basis. The decline in levels from mature to older leaves agrees with the findings of Forrest and Bendall (1969) for Camellia, although their data were only for fresh weights. Concentrations found in the leaves of some of the tree species were also quite high (e.g. Acer : 20.7% dw.; Fraxinus : 17.3% dw.). In the herbaceous plants, the amounts of 'free' phenolics in leaves were relatively low.

'Free' phenol concentrations in R. ponticum stem samples were generally lower than in leaf samples. New stem had 22% dw. as 'free' phenolic compounds. This is much lower than the equivalent leaf sample, but is still a high concentration. Levels again declined with age. Forrest and Bendall (1969) found total phenol content to be relatively low in stem samples of Camellia.

In R. ponticum roots the non-mycorrhizal, adventitious root samples had the highest concentration of 'free' phenols and the mature, main root the lowest. The high fibre content of the mature, main roots has an obvious effect in giving low values, particularly on a percentage dry weight basis. Again, the amounts of 'free' phenolic compounds found in R. ponticum roots are high compared to most other plants sampled. There were no clear trends for herbaceous or 'woody' plants. Some herbaceous species had quite high levels (e.g. Holcus with 11.2% dw.). The values for R. ponticum mycorrhizal hair root were higher than those found by Jalal, Read and Haslam (1982) for Calluna. Read and Jalal (1980) give a percentage fresh weight value for mycorrhizal hair roots of Calluna of 3.1% 'free' phenolics. This is about the same as found for R. ponticum.

Seasonal variation in the concentration of 'free' phenols in R. ponticum hair roots was relatively greater than in mature leaf samples. Both roots and leaves had lowest concentrations (% fw.) in June. On a dry weight basis, levels in roots were lowest in August. The peak in concentration of 'free' phenols was 33.9% (dw.) for roots in April and 31.6% (dw.) in December and 30.3% (dw.) in April for leaves. The concentration of 'free' phenols perhaps reflects metabolic activity, the development of fresh tissues and related to this, the ratio of dry weight/fresh weight of these tissues. These factors will vary with the seasons and from tissue to tissue. Forrest and Bendall (1969) found many polyphenols to be restricted to the sites of active cell growth and maturation (except root tips). Concentrations seemed to be highest at either sites of active metabolism (young shoots and vascular cambial regions) for monomeric flavonols and wood or bark tissues for leucoanthocyanins and their polymers.

Clearly, seasonal variation has implications for any proposed ecological function of these compounds. Such seasonal fluctuations have been related to anti-herbivore functions of tannins (including catechins) in Q. robur (Feeny, 1968, 1969, 1970). Values are affected by changes related to the stage of growth, the physiological status of the plant and also to environmental conditions (Harborne, 1979). Almost all of the concentrations found in tissues of R. ponticum were high and remained high, even with seasonal variations. Harborne (1979) states that biologically active flavanoids are effective in vitro in solution, at

concentrations of 0.1% - 1%. The levels found in R. ponticum suggest that they could be effective in terms of anti-herbivore functions. Forrest and Bendall (1969) propose structural functions for the catechins in tannin polymers and also in the control of polymerization of tannins. Similar functions could also occur in R. ponticum.

5.4 QUALITATIVE ANALYSIS OF THE 'FREE' PHENOLIC COMPOUNDS OF *RHODODENDRON PONTICUM*

5.4.1 INTRODUCTION

A number of workers have found polyphenols or suspected polyphenols in leaf and stem tissues of rhododendrons (Rangaswami and Verkateswarku, 1966; Raudnitz, 1957; Love and Brown, 1959; Harborne and Williams, 1971). Some have also been reported from R. ponticum (Handley, 1957; Hegnauer, 1966; Harborne and Williams, 1971; Thompson et al, 1972; Pigott pers. comm. in Cross, 1975).

The range of 'free' phenolic compounds in R. ponticum and its variation from tissue to tissue may reflect their functions. This variation is therefore of interest in a study of the possible roles of 'free' phenolic compounds as allelopathic agents or as anti-herbivore devices.

The aim of this survey was to identify as many as possible of the compounds found and to examine their occurrence in different tissues, together with seasonal variations. The total number of compounds (both identified and unidentified) was also estimated for the same.

5.4.2 METHOD

Tissues from mycorrhizal and non-mycorrhizal seedlings and mature R. ponticum were sampled as in the previous experiment (5.3). The sample extracts were analysed by paper chromatography as already described (5.2).

5.4.3 RESULTS

5.4.3.1 Different *R. ponticum* tissues

5.4.3.1.1 Leaves

Leaf extracts (Tables 5.4.3.4.1 and 5.4.3.4.2) generally had between 20 and 30 'free' phenolic compounds. The minimum was 18 (Mycorrhizal Seedling) and the maximum was 28 (Mature Plant). Leaves from the same plant, but of differing ages had between 21 and 25 compounds. The minimum was 21 (Young Leaves) and the maximum was 25 (Senescent Leaves).

(+)-catechin was consistently present as a major compound. (-)-epicatechin was present in all samples, but in relatively small amounts except for the Mature Plant and Young Leaf samples. U1 was found in large quantities in both non-mycorrhizal and mycorrhizal seedlings and in moderate amounts in Senescent Leaf. U3 was present in large amounts in New Leaf and Mature Leaf and in moderate amounts in Senescent Leaf. Both U1 and U3 were found in all other leaf samples, either in small amounts or as traces. D1 was absent from New and Young Leaf samples, but present in samall amounts and moderate amounts in Mature Leaf and Senescent Leaf respectively. It was also present in both seedling samples, in small amounts, or in the case of Mycorrhizal Seedlings, moderate amounts. C1 was absent except for traces in both seedling samples.

The flavonoid glycosides (U4-U7) were present in most samples, but absent except for traces of U7 in both seedling samples. The flavonoid aglycones (U8-U10) were only found as traces, except for U10 in small amounts for both seedlings, New Leaf and Young Leaf, and U8 and U9 present in small and moderate amounts respectively in New Leaf.

Table 5.4.3.1 Compounds found in leaves of all stages and ages

(+)-catechin, (-)-epicatechin
Procyanidin : B1, B2, B3, B4, B5, B8, C1, C2
Flavonoid Glycosides : (4) : U4-U7
Flavonoid Aglycones : (3) : U8-U10
Totally Unidentified : U1, U2, U3, U11, U12, U13, U14, U15, U16, U17, U18, U19, U28, U29 + several others in very small quantities.

5.4.3.1.2 Stems

Stem samples (Table 5.4.3.4.5) had between 15 and 20 'free' phenolic compounds. The minimum was 15 in New Stem and the maximum was 20 in Second Year Stem.

(+)-catechin was present in large amounts in all samples except for Fourth Year Stem which had only moderate amounts. (-)-epicatechin was present in all samples but only small amounts or traces in New, Young and Mature Stem. In Second and Fourth Year Stem, (-)-epicatechin was present in large amounts and was the dominant 'free' phenol.

Procyanidin B1 was found in moderate amounts in New, Young and Mature Stem, but absent in Second and Fourth Year Stem. B3 was present as a trace in New Stem, increased to large amounts in Young and Mature Stem and then declined to small amounts in Second and Fourth Year Stem. D1 was a trace in New and Young Stem, small amounts in Mature and Second Year Stem and large amounts in Fourth Year Stem.

U1 was absent in New Stem, present as a trace in Young Stem and in small or moderate amounts in older stems. U3 was present only in New Stem, in which it was in large quantities.

Flavonoid glycosides were absent from all stem samples, but the aglycones were found as traces in most.

Table 5.4.3.2 Compounds found in stem samples of all ages and stages

(+)-catechin, (-)-epicatechin
Procyanidin : B1, B3, B5, C2, A2, D1
Flavonoid Aglycones : (3) : U8-U10
Totally Unidentified : U1, U3, U11, U12, U20, U21, U22, U24, U25 + several others in very small amounts.

5.4.3.1.3 Roots

Root samples (Tables 5.4.3.4.3 and 5.4.3.4.4) contained between 7 and 13 'free' phenolic compounds. the minimum was 7 in Mycorrhizal Hair Roots (mature plant). The maximum was 13 in Non-mycorrhizal Hair Roots of seedlings.

(+)-catechin and (-)-epicatechin were ubiquitous, the former being dominant in all samples except Mature Main Root. (-)-epicatechin was present in large amounts in all the non-mycorrhizal root samples (i.e. Non-mycorrhizal Hair Root of seedlings, Non-mycorrhizal Adventitious Root and Mature Main Root of mature plants). It was found in small amounts in Mycorrhizal Hair Root of mature plants and moderate amounts in Mycorrhizal Hair Root of seedlings.

Procyanidin B1 was absent from all roots except for very small amounts in both seedling samples. B3 was present in large amounts in all samples except Mature Main Root (absent) and Non-mycorrhizal Adventitious Root (moderate amounts). C2 was found in all hair roots, but not in Mature Main Root.

Flavonoid glycosides and aglycones were absent from all root samples except for possible traces.

Table 5.4.3.3 Compounds found in roots of all ages and stages

(+)-catechin, (-)-epicatechin
Procyanidin : B1, B3, B4, C2, A2, D1
Totally Unidentified : U27, U29 + several others in very small quantities.

5.4.3.1.3 Roots

Root samples (Tables 5.4.3.4.3 and 5.4.3.4.4) contained between 7 and 13 'free' phenolic compounds. the minimum was 7 in Mycorrhizal Hair Roots (mature plant). The maximum was 13 in Non-mycorrhizal Hair Roots of seedlings.

(+)-catechin and (-)-epicatechin were ubiquitous, the former being dominant in all samples except Mature Main Root. (-)-epicatechin was present in large amounts in all the non-mycorrhizal root samples (i.e. Non-mycorrhizal Hair Root of seedlings, Non-mycorrhizal Adventitious Root and Mature Main Root of mature plants). It was found in small amounts in Mycorrhizal Hair Root of mature plants and moderate amounts in Mycorrhizal Hair Root of seedlings.

Procyanidin B1 was absent from all roots except for very small amounts in both seedling samples. B3 was present in large amounts in all samples except Mature Main Root (absent) and Non-mycorrhizal Adventitious Root (moderate amounts). C2 was found in all hair roots, but not in Mature Main Root.

Flavonoid glycosides and aglycones were absent from all root samples except for possible traces.

Table 5.4.3.3 Compounds found in roots of all ages and stages

(+)-catechin, (-)-epicatechin
Procyanidin : B1, B3, B4, C2, A2, D1
Totally Unidentified : U27, U29 + several others in very small quantities.

KEY:- (For tables 5.4.3.4.1.-5. and 5.4.3.5.-6.)

-	:	Absent
+	:	Present
++++	)	Relative intensity of spot on chromatogram taken as an
+++	)	approximate indication of the relative amount of each
++	)	compound in a particular sample.
+	)	Range from ++++ (large amount, very intense spot) to
(+)	)	((+)) (very small trace, spot barely detectable).
((+))	)	
?	:	Identification as a particular compound not certain.
U	:	Unidentified.
BAW	:	Butanol/Acetic acid/Water.

Tables 5.4.3.4.1-5 Spectrum of phenolic compounds found in *Rhododendron ponticum*

Table 5.4.3.4.1 Leaves of mycorrhizal and non-mycorrhizal plants

Compound	Leaf Non-mycorrhizal Seedling	Leaf Mycorrhizal Seedling	Leaf Mature Plant
(+)-Catechin	+++	++++	++++
(-)-Epicatechin	+	+	+++
Procyanidin B1	+	(+)	+
B2	+	-	(+)
B3	++	+	+
B4	+	+	(+)
B5	+	+	++
B6	-	-	-
B7	-	-	-
B8	-	+	(+)
C1	(+)	(+)	-
C2	+	+	+
A1	-	-	-
A2	?	?	?
D1	+	++	+
D2	-	-	-
E	-	-	+?
Polymeric Procyanidins	++	++	++
BAW. Basal Streak	-	(+)	-
Mixed Non-polar Compounds	-	-	-
Unidentified 1	++++	+++	(+)
2	-	-	-
3	+	+	+
4)	-	-	+
5) Flavonoid	-	-	+
6) Glycosides	-	-	+
7)	(+)	(+)	+
8) Flavonoid	((+))	((+))	((+))
9) Aglycones	((+))	((+))	(+)

Compound		Leaf Non-mycorrhizal Seedling	Leaf Mycorrhizal Seedling	Leaf Mature Plant
10)		+	+	(+)
11		-	-	-
12		((+))	-	-
13		((+))	-	-
14	Procyanidin G?	-	-	-
15)	Possibly	+	+	(+)
16)	simple	-	-	-
17)	phenolic	(+)	+	(+)
18)	acids	-	-	-
19)		-	-	-
20		-	-	-
21		-	-	-
22		-	-	-
23		-	-	-
24		-	-	-
25		-	-	-
26		-	-	-
27)	Possibly	-	-	-
28)	simple	-	-	-
29)	phenolic acids	-	-	-
Others		((+))	-	(+)
				((+))
				((+))
				((+))
				((+))
				((+))
Total Number of Compounds		21	18	28

Table 5.4.3.4.2 Leaves of different ages

Compound		New	Young	Mature	Senescent
(+)-Catechin		+++	++++	++++	++++
(-)-Epicatechin		+	+++	+	+
Procyanidin B1		-	-	-	(+)?
B2		+	-	(+)	+
B3		+	+	+	+
B4		-	-	-	(+)?
B5		+	+	+	+++
B6		-	-	-	-
B7		-	-	-	-
B8		-	-	-	-
C1		-	-	-	-
C2		+	+	+	+
A1		-	-	-	-
A2		?	?	?	?
D1		-	-	+	++
D2		-	-	-	-
E		-	-	-	-
Polymeric Procyanidins		+	+	+	+
BAW. Basal Streak		-	+	+	-
Mixed Non-polar compounds		-	-	-	-
Unidentified 1		+	(+)	((+))	++
2		+	++	+	-
3		++++	+	+++	++
4	Flavonoid Glycosides	+	+	+	+
5	Flavonoid Glycosides	+	(+)	+	+
6	Flavonoid Glycosides	++	-	++	++
7	Flavonoid Glycosides	+	+	+	+
8	Flavanoid Aglycones	+	((+))	((+))	(+)
9	Flavanoid Aglycones	++	((+))	((+))	((+))?
10	Flavanoid Aglycones	+	+	-	-
11		((+))	((+))	((+))	((+))
12		((+))	((+))	((+))	((+))
13		((+))	((+))	((+))	-

Compound		New	Young	Mature	Senescent
14	Procyanidin G?	-	-	-	- (+)
15	Possibly	+	+	(+)	+
16	simple	+	-	-	-
17	phenolic	(+)	-	-	-
18	acids	(+)	+?	(+)?	-
19		((+))	-	-	-
20		-	-	-	-
21		-	-	-	-
22		-	-	-	-
23		-	-	-	-
24		-	-	-	-
25		-	-	-	-
26		-	-	-	-
27	Possibly	-	-	-	-
28	simple	-	-	-	((+))
29	phenolic acids	-	-	-	((+))
Others		-	((+))	((+))	(+)
			((+))	((+))	((+))
				((+))	
Total number of compounds		24	21	24	25

Table 5.4.3.4.3 Roots of different types

Compound		Mycorrhizal Hair Root	Non-mycorrhizal Adventitious Hair Root	Non-mycorrhizal Mature Main Root
(+)-Catechin		++++	++++	++
(-)-Epicatechin		+	+++	+++
Procyanidin B1		-	-	-
B2		-	-	-
B3		+++	++	-
B4		+	++	(+)
B5		-	-	-
B6		-	-	-
B7		-	-	-
B8		-	-	-
C1		-	-	-
C2		+	+	-
A1		-	-	-
A2		(+)	+	++++
D1		+	(+)	+
D2		-	-	-
E		-	-	-
Polymeric Procyanidins		-	+	-
BAW. Basal Streak		-	-	+
Mixed Non-polar Compounds		(+)	+	++
Unidentified 1		-	-	-
2		-	-	-
3		-	-	-
4	Flavonoid Glycosides	-	-	-
5		-	-	-
6		-	-	-
7		-	-	-
8	Flavonoid Aglycones	-	-	-
9		-	-	((+))?
10		-	-	-
11		-	-	-
12		-	-	-

Compound		Mycorrhizal Hair Root	Non-mycorrhizal Adventitious Hair Root	Non-mycorrhizal Mature Main Root
13		-	-	-
14	Procyanidin G?	-	((+))?	-
15)	Possibly	-	-	-
16)	simple	-	-	-
17)	phenolic	-	-	-
18)	acids	-	-	-
19)		-	-	-
20		-	-	-
21		-	-	-
22		-	-	-
23		-	-	-
24		-	-	-
25		-	-	-
26		-	-	-
27)	Possibly	-	-	-
28)	simple	-	-	-
29)	phenolic acids	-	-	(+)
Others				((+))
				((+))
				((+))
Total Number of Compounds		7	8	10

Table 5.4.3.4.4 Roots from different plants

Compound		Mycorrhizal Hair Root: Mature Plant	Mycorrhizal Hair Root: Seedling	Non-mycorrhizal Hair Root: Seedling
(+)-Catechin		++++	++++	++++
(-)-Epicatechin		+	++	+++
Procyanidin B1		-	(+)	(+)
B2		-	-	-
B3		+++	+++	+++
B4		++	+	+
B5		-	-	-
B6		-	-	-
B7		-	-	-
B8		-	-	-
C1		-	(+)	(+)
C2		+	+	+
A1		-	-	-
A2		+	(+)	((+))
D1		+	+	+
D2		-	-	-
E		-	-	-
Polymeric Procyanidins		++	+	+
BAW. Basal Streak		(+)	++	++
Mixed Non-polar Compounds		((+))	(+)	(+)
Unidentified 1		-	-	-
2		-	-	-
3		-	-	-
4		-	-	-
5	Flavonoid	-	-	-
6	Glycosides	-	-	-
7		-	-	-
8	Flavonoid	-	-	-
9	Aglycones	-	((+))?	((+))?
10		-	((+))?	((+))?
11		-	-	-
12		-	-	-

Compound		Mycorrhizal Hair Root: Mature Plant	Mycorrhizal Hair Root: Seedling	Non-mycorrhizal Hair Root Seedling
13		-	-	-
14	Procyanidin G?	-	-	-
15	Possibly simple phenolic acids	-	-	-
16		-	-	-
17		-	-	-
18		-	-	-
19		-	-	-
20		-	-	-
21		-	-	-
22		-	-	-
23		-	-	-
24		-	-	-
25		-	-	-
26		-	-	-
27	Possibly simple phenolic acids	((+))	-	-
28		-	-	-
29		((+))	-	-
Others			((+))	((+)) ((+))
Total Number of Compounds		9	12	13

Table 5.4.3.4.5 Stem of different ages

Compound		New	Young	Mature	2nd Yr.	4th Yr. (rooting)
(+)-Catechin		++++	++++	++++	+++	++
(-)-Epicatechin		+	(+)	+	++++	++++
Procyanidin B1		++	++	++	~	-
B2		-	-	-	~	-
B3		(+)	+++	+++	+	+
B4		-	-	-	~	-
B5		-	(+)	(+)	+	(+)
B6		-	-	-	~	-
B7		-	-	-	~	-
B8		-	-	-	~	-
C1		-	-	-	~	-
C2		(+)	+	+	(+)	(+)
A1		-	-	-	~	-
A2		-	-	-	+	+
D1		(+)	(+)	+	+	+++
D2		-	-	-	~	-
E		-	-	-	~	(+)?
Polymeric Procyanidins		-	+	-	+	+
BAW. Basal Streak		((+))	-	((+))	~	-
Mixed Non-polar compounds		(+)	-	-	(+)	(+)
Unidentified 1		-	(+)	+	++	+
2		-	-	-	~	-
3		+++	-	-	~	-
4	)	-	-	-	~	-
5	) Flavonoid	-	-	-	~	-
6	) Glycosides	-	-	-	~	-
7	)	-	-	-	~	-
8	) Flavonoid	((+))	-	-	((+))	((+))
9	) Aglycones	((+))	(+)	((+))	((+))	((+))
10	)	((+))	(+)	((+))	-	((+))
11		-	-	((+))	((+))	-
12		((+))	((+))	((+))	((+))	((+))
13		-	-	-	-	-

Compound		New	Young	Mature	2nd Yr.	4th Yr. (rooting)
14	Procyanidin G?	-	-	(+)?	-	-
15	Possibly simple phenolic acids	-	-	-	-	-
16		-	-	-	-	-
17		-	-	-	-	-
18		-	-	-	-	-
19		-	-	-	-	-
20		(+)	(+)	-	(+)	-
21		(+)	(+)	(+)	(+)	-
22		-	-	-	(+)	(+)
23		-	-	-	(+)	((+))
24		-	((+))	((+))	((+))	((+))
25		(+)	((+))	((+))	((+))	-
26		-	-	-	-	-
27	Possibly simple phenolic acids	-	-	-	-	-
28		-	-	-	-	-
29		-	-	-	-	-
Others		((+))	((+))	-	(+) ((+))	((+))
Total number of compounds		15	16	16	20	17

5.4.3.2 Seasonal variation

5.4.3.2.1 Leaf samples (Table 5.4.3.5)

(+)-catechin was consistently the dominant 'free' phenol except for December, when it was exceeded by one of the flavonoid glycosides, U6. (-)-epicatechin was present in small amounts throughout the year, excepting October when it was absent.

Procyanidin D1 was generally present in large or moderate amounts, as was the flavonoid glycoside U6.

The total number of compounds present was around 18 or 19 from June to December. This increased to 20 in February and 23 in April. The main fluctuations in the spectrum and total number of compounds found were in the traces.

5.4.3.2.2 Root samples (Table 5.4.3.6)

Between 9 and 13 compounds were found. . The number remained at 9 from June to October, increased to 10 in December, 12 in February and 13 in April. The spectrum of compounds remained fairly stable except for the occasional appearance of traces.

The amounts of some of the major compounds did vary. (+)-catechin was consistently dominant. (-)-epicatechin was co-dominant in June, present only in small quantities in August, large amounts in October and moderate amounts for all other samples.

Procyanidins B3 and B2 varied considerably, but with no obvious pattern. Both were always present. A2 was found as a trace or in small amounts for all samples except December and February, when it was present in large amounts.

Table 5.4.3.5 Seasonal changes in the spectrum of phenolic compounds in mature leaves of *Rhododendron ponticum*

		Jun. 1980	Aug. 1980	Oct. 1980	Dec. 1980	Feb. 1981	Apr. 1981
(+)-Catechin		++++	++++	++++	+++	++++	++++
(-)-Epicatechin		+	+	-	+	+	+
Procyanidin B1		-	-	-	-	(+)	(+)?
B2		+	+	+	+	+	+
B3		++	+	+	+	++	+
B4		-	-	-	-	-	-
B5		+	+	+	++	+	+
B6		-	-	-	-	-	-
B7		-	-	-	-	-	-
B8		-	-	-	-	(+)	-
C1		-	-	-	-	-	-
C2		+	+	+	+	+	+
A1		-	-	-	-	+	-
A2		?	?	?	?	?	?
D1		+++	+	+++	++	+	+++
D2		-	-	-	-	-	-
E		-	-	-	-	-	-
Polymeric Procyanidins		+	+	+	+	+	+
BAW. Basal Streak		+	+	+	+	-	+
Mixed Non-polar compounds		+	+	+	+	-	(+)
Unidentified 1		++	+	+	+	++	+++
2		-	-	-	-	-	-
3		(+)	+	+	(+)	+	+
4		+	+	+	+	(+)	+
5	Flavonoid	+	+	+	+	+	+
6	Glycosides	++	++	++	++++	+	+++
7		+	+	+	+	(+)	+
8	Flavonoid	-	-	+	+	-	-
9	Aglycones	((+))	(+)	+	+	((+))	+
10		-	-	+	+	((+))	+
11		-	-	-	-	-	-
12		-	-	-	-	-	-

		Jun. 1980	Aug. 1980	Oct. 1980	Dec. 1980	Feb. 1981	Apr. 1981
13		-	-	-	-	-	-
14	Procyanidin G?	(+)	(+)	(+)	(+)	(+)	+
15	Possibly	(+)	-	-	-	-	+
16	simple	+	+	+	-	(+)?	-
17	phenolic	-	-	-	-	-	-
18	acids	((+))	+	+	-	-	-
19		((+))	((+))	((+))	-	-	(+)
20		-	-	-	-	-	-
21		-	-	-	-	-	-
22		-	-	-	-	-	-
23		-	-	-	-	-	-
24		-	-	-	-	-	-
25		-	-	-	-	-	-
26		-	-	-	-	-	-
27	Possibly	-	-	-	-	-	-
28	simple	-	-	-	-	-	-
29	phenolic acids	-	-	-	-	-	-
Others		-	-	-	(+)	-	(+) + ((+)) (+)
Total number of compounds		19	18	19	18	20	23

Table 5.4.3.6 Seasonal changes in the spectrum of phenolic compounds in mycorrhizal hair roots of *Rhododendron ponticum*

Compound		Jun. 1980	Aug. 1980	Oct. 1980	Dec. 1980	Feb. 1981	Apr. 1981
(+)-Catechin		++++	++++	++++	++++	++++	++++
(-)-Epicatechin		++++	+	+++	++	++	++
Procyanidin B1		-	-	-	-	-	-
B2		-	-	-	-	-	-
B3		+	+++	+	(+)	+	+++
B4		+	+	++	+	+	+
B5		-	-	-	-	-	-
B6		-	-	-	-	-	-
B7		-	-	-	-	-	-
B8		-	-	-	-	-	-
C1		-	-	-	-	-	-
C2		+	+	+	+	(+)	(+)
A1		-	-	-	-	-	-
A2		+	(+)	+	+++	+++	+
D1		+	+	(+)	+	+	(+)
D2		-	-	-	-	-	-
E		-	-	-	-	-	-
Polymeric Procyanidins		+	+	+	(+)	+	+
BAW. Basal Streak		-	-	-	-	(+)	-
Mixed Non-polar compounds		++	+	+	+	++	+
Unidentified 1		-	-	-	-	-	-
2		-	-	-	-	-	-
3		-	-	-	-	-	-
4	)	-	-	-	-	-	-
5	) Flavonoid	-	-	-	-	-	-
6	) Glycosides	-	-	-	-	-	-
7	)	-	-	-	-	-	-
8	) Flavonoid	-	-	-	-	-	((+))?
9	) Aglycones	-	-	-	-	((+))?	((+))?
10	)	-	-	-	-	-	((+))?
11		-	-	-	-	-	-
12		-	-	-	-	-	-

Compound			Jun. 1980	Aug. 1980	Oct. 1980	Dec. 1980	Feb. 1981	Apr. 1981
	13		-	-	-	-	-	-
	14	Procyanidin G?	-	-	-	-	-	-
	15	Possibly simple phenolic acids	-	-	-	-	-	-
	16		-	-	-	-	-	-
	17		-	-	-	-	-	-
	18		-	-	-	-	-	-
	19		-	-	-	-	-	-
	20		-	-	-	-	-	-
	21		-	-	-	-	-	-
	22		-	-	-	-	-	-
	23		-	-	-	-	-	-
	24		-	-	-	-	-	-
	25		-	-	-	-	-	-
	26		-	-	-	-	++	(+)
	27	Possibly simple phenolic acids	((+))	-	-	((+))	((+))	-
	28		-	((+))	(+)	(+)	-	-
	29		((+))	((+))	(+)	(+)	-	-
Others							(+)	(+)
							(+)	(+)
Total number of compounds			9	9	9	10	12	13

5.4.4 DISCUSSION

In a survey of phenols in the genus Rhododendron, Harborne and Williams (1971) found myricetin and quercetin to occur as simple glycosides. These were usually as the 3-galactoside, but also as the 3-rhamnoside, 3-arabinoside and 3-glucoside. They took R. ciliatum as a representative member of the genus for detailed testing. The glycosides mentioned are widespread in the angiosperms, with the exception of the 3-arabinoside, which was previously found only in Vaccinium macrocarpon and Lysmachia punctata. Azaleatin was found in leaves and flowers, mostly as the 3-rhamnoside, azalein. They considered the yellow flavonol, gossypetin to be a characteristic constituent of the genus, found in leaves and corollas. The genus is particularly large and considerable interspecific variation in phenolic constituents may occur.

Handley (1957) found large amounts of protein precipitating materials in the leaves of R. ponticum. These were readily soluble in water at room temperature and my well have been phenolic. Cross (1975) cites Pigott (pers. comm.) as having found large quantities of the anthocyanidin, cyanidin and traces of delphinidin and pelargonidin in R. ponticum after acid-hydrolysis. Hegnauer (1966) notes the following in R. ponticum:-

> (+)-catechin (a lot isolated), (-)-catechin (a lot demonstrated), gallocatechin (a lot demonstrated), caffeic acid (demonstrated) and chlorogenic acid (demonstrated).

Harborne and Williams (1971) found a number of phenolic compounds following acid-hydrolysis of R. ponticum leaves:- quercetin, gossypetin, myricetin, azaleatin and 5-methylkaempferol or 5-methylmyricetin (all flavonoid aglycones), and p-hydroxybenzoic acid, protocatechuic acid, vanillic acid and syringic acid (all simple phenolic acids). These findings are of considerable interest in terms of the phenolic compounds occurring in Rhododendron in general and R. ponticum in particular. However, they do rely on drastic treatment of tissues for extraction (usually acid-hydrolysis). This releases a range of phenolic constituents normally closely bound to large, organic polymers.

Of more relevance to this study are the findings of Thompson et al (1972). After extraction from R. ponticum leaves using methanol, they found the following phenolic compounds in 'free' form:-

> (+)-catechin (major component), procyanidin B3, procyanidin B6 (major component), procyanidin B8, procyanidin C2, procyanidin D1 (major component) and procyanidin G.

They also found traces of other procyanidins in all samples examined in more concentrated form.

These more freely available phenolic compounds are of interest since they are more likely to be biologically active in any allelopathic or anti-herbivore roles. The method of extraction used in this present study was similar to that of Thompson et al.

The spectrum of compounds extracted was similar to that found by Thompson et al, but considerably more extensive. (-)-epicatechin was found, sometimes in large amounts, along with several procyanidins additional to those of Thompson et al. Procyanidins B6 and G were not found. Four flavonoid glycosides and three flavonoid aglycones were extracted but not identified. A large number of totally unidentified phenolic compounds was also found. These were mostly present as traces, although some (such as U1 and U3) were in large quantities in some samples.

The largest number of compounds was found in leaves, with between 18 and 28. Leaves from seedlings and young leaves from mature plants had fewer compounds, whilst mature leaves and senescent leaves from mature plants had the most. (+)-catechin was always present as a major component. Flavonoid glycosides and aglycones were present in large amounts. A range of procyanidins was present, mostly in fairly small quantities. B5 was in large amounts in senescent leaves, whilst D1 was absent from new leaves and young leaves, but present in increasing amounts in mature and senescent leaves.

Stem samples had fewer compounds (15-20). (+)-catechin was again present in most samples as a major compound. (-)-epicatechin was the major constituent in second and fourth year stems. Flavonoid glycosides were

absent, but traces of aglycones were found. Forrest and Bendall (1969) similarly found stem samples of Camellia to have a rather restricted range of 'free' phenolic compounds.

Roots of R. ponticum had considerably fewer compounds (7-13) than either stem or leaf samples. (+)-catechin was the dominant 'free' phenolic compound in most samples. (-)-epicatechin was present in large amounts in non-mycorrhizal root samples. The range of compounds was very restricted with far less unidentified compounds, no flavonoid glycosides or aglycones (except possible traces) and a small number of procyanidins which occurred consistently throughout the samples. Interpretation of the results from root samples is complicated by the effects of mycorrhizal infection. The fungus may utilize or modify the phenolic compounds produced by the plant cells. Jalal, Read and Haslam (1982) related some differences between root samples and some seasonal changes to the presence of mycorrhizas or the balance between the production of phenols (probably affected by the amount of active growth) in host root cells and their utilization by the fungus. In Camellia *the 'free' phenolics in roots were very restricted,* with only (-)-epicatechin and leucoanthocyanins being found (Forrest and Bendall, 1969).

The range of phenolic compounds in detectable quantities in R. ponticum tissues, together with their relative amounts, clearly varies from one major tissue type to another (i.e. leaf, stem or root). They also vary within a tissue type, with the developmental stage (or age) and between mycorrhizal and non-mycorrhizal plants. Mycorrhizal and non-mycorrhizal roots on the same plant also differ. Jalal, Read and Haslam (1982) found similar differences between their shoot and root samples from Calluna, with 9-22 compounds identified in shoot and 4-13 in root samples. (+)-catechin, (-)-epicatechin, procyanidins, flavonoid glycosides, flavonoid aglycones and simple phenolic acids were the 'free' phenols found in Calluna. Again, the flavonoid glycosides were in large amounts in shoot material, but only small quantities and much less diverse in roots.

The pattern of 'free' phenolic compounds present in the mature leaf samples from mature R. ponticum was rather stable during the year. (+)-catechin was dominant except when it was exceeded by a flavonoid glycoside

in December. (-)-epicatechin was consistently present but in small quantities. The number of compounds detected increased slightly from 18 or 19 to 23 in April.

A similarly stable pattern of 'free' phenolic compounds during the year was found in mycorrhizal hair roots from mature R. ponticum in the field. (+)-catechin was again dominant, although (-)-epicatechin was present in quite large amounts. The number of compounds again increased in February and April (from 9 to 13). This increase was due to the presence of trace compounds, apparently flavonoid aglycones.

The traces of additional 'free' phenolic compounds in the samples from early spring may be linked to increased metabolic activity prior to the new growing season. Jalal, Read and Haslam (1982) found a similar increase in the number of 'free' phenolic compounds in early summer, with less compounds during the winter. Feeny (1968) found considerable changes in levels of tannins in leaves of Q. robur (including catechins and other flavonoids) from the spring to late summer. The changes in both quality and quantity are believed to be of considerable ecological importance, through anti-herbivore function (Feeny, 1970). Large seasonal changes in levels of phenols in leaves of Quercus might be predicted due to its deciduous nature. A more constant pattern in terms of both quality and quantity of compounds would be expected in R. ponticum leaves since they are evergreen. This is apparently the case.

5.5 EXTRACTIONS FROM FIELD AND ARTIFICIAL SOILS

5.5.1 INTRODUCTION

A range of potentially phytotoxic substances are present in Calluna heathland soils. These are not normally removed by aqueous leaching, but can be displaced by a mild, non-hydrolytic, alkaline extraction. This suggests that these compounds are loosely attached, probably by hydrogen bonding to active sites on the humic and fulvic acid polymers (Jalal and Read, 1983 I and II).

Chou and Muller (1972) showed that some phenolic acids could be obtained by aqueous extraction from soil of low (5%) organic content. With increasing organic matter, it became more difficult to extract them. At an organic matter content of 29%, no water soluble phenolics were released. The binding capacity of Calluna heathland soil (85% organic content in the case of Jalal and Read) or some Rhododendron soils must be very high.

Whitehead (1964) isolated p-hydroxybenzoic, vanillic, p-coumaric and ferulic acids from soils of low organic content (less than 14%). This was by aqueous extraction. The same compounds plus syringic acid were also found in agricultural soils, using an alkaline extraction technique (Wang, Cheng and Tung, 1967). Chou and Muller (1972) extracted the same compounds from soil under Arctostaphylos. Carballeira (1980) obtained them from methanolic extracts of Erica soil, together with protocatechuic acid. Jalal and Read (1983 I and II) also found o-hydroxybenzoic (salicylic), p-methoxybenzoic and benzoic acids in 'free' form in Calluna soil. These are especially interesting as they have been shown to be particularly phytotoxic in seedling bioassays (Prill, Barton and Solt, 1949; Lynch, 1980). Phenolic acids have been shown to cause inhibition of ion uptake by roots (Glass, 1973).

In addition to the phenolic acids, Jalal and Read (1983 I and II) detected a range of hydroxyalkanoic acids in Calluna soil. These were present in amounts either equal to, or greater than the aromatic acids. They

consider that such compounds may also occur in other soils dominated by Ericaceous plants but have been overlooked by earlier workers due to the analytical techniques used.

A number of workers have shown aliphatic acids, such as octanoic and decanoic, to have considerable phytotoxic properties (Priss et al, 1949; Van Overbeek and Blondeau, 1954; Takijima, 1964; Jackson and Taylor, 1970; Lee, 1977).

Nonanoic acid has also been shown to have fungitoxic properties (Robinson, Park and Garrett, 1968; Hobot and Gull, 1980; Garrett and Robinson, 1969). Similar properties have been shown for octanoic acid (Pedersen, 1970). Fungitoxic effects have been suggested to be due to direct action on the structure and function of the plasma membrance, by an interaction involving fatty acids and the lipophilic parts of the membrane. Such an effect has been demonstrated in fungi by Lode and Pedersen (1970) and in higher plants by Lee (1977).

The main aqueous-extractable materials are expected to be those associated with the large amounts of fulvic acid (molecular weight less than c.10,000) and humic acid (molecular weight above c.5,000 up to 1,000,000's) in heathland soils. Jalal and Read (1983 I & II) found large amounts of fulvic acid in Calluna heathland soil; up to 600 mg/100g soil in the Ah horizon in July.

The presence of oxidized and polymerized phenolics was shown in both mull and mor, partially humified beech litter by Coulson et al (1960 I). They obtained little polyphenol material from litter or humus using ethyl acetate, but more were extracted from mor humus and litter with a 'tannin-stripping' solvent (i.e. methanol/water). The work of Coulson et al linked polyphenols from vegetation with cation-mobilization and podsolization. Cross (1975) suggests that R. ponticum in common with other ericaceous plants, has a deleterious effect on soil, mobilizing cations, either directly or indirectly by the production of polyphenols.

The effects of ericaceous plants upon associated soils have been noted by numerous researchers (Handley, 1954 (with regard to podsolization); Chou and Muller, 1972; Jalal and Read, 1983 I & II; Grubb, Green and

Merrifield, 1969). The influence of plants (including ericaceous ones) that are rich in polyphenols on the formation of 'mor' humus and podsols have been demonstrated (Coulson, Davies and Lewis, 1960 I & II; Davies, Coulson and Lewis, 1964 III & IV; Bruckert and Jacquin, 1969).

The consequences of the effects of ericaceous plants on soils and vegetation have been considered by various workers. A number have looked at apparent 'toxicity' or 'interference' phenomena affecting competing plants.

Roff (1964) considered the phenomenon of 'bare-zones' around Calluna bushes and possible interference effects. Similar effects of Rhododendron bushes were observed by Cross (1973). Chou and Muller (1972) examined the formation of bare-zones and allelopathic effects associated with Arctostaphylos. Possible allelopathic interactions have been investigated by Ballester, Albo and Vieitez (1977) with Erica scoparia; Carballeira (1980) with Erica australis and Jalal and Read (1983 I and II) with Calluna. Various other workers have examined the interaction between Calluna and tree species known as 'Calluna-check' (Weatherell, 1953; Handley, 1963; McVean, 1963; Robinson, 1972).

These effects on soils and vegetation seem to be spread throughout a wide range of ericaceous species. In suitable habitats, it might be expected that R. ponticum would exert similarly important influences on the biochemistry and physical structure of soils and upon associated vegetation.

If phenolic compounds from Rhododendron are to be implicated in such effects as discussed, it is necessary to demonstrate their presence in suitable quantities and degrees of availability in the soil system with which the plant is associated. The experiments and extractions which follow attempt to see whether the phenolics, as already described, are present in soil associated with R. ponticum. They were done in two main parts.

Firstly, extractions from field soils and secondly, extractions from field soils or acid-washed sand in pots with vigorous R. ponticum.

5.5.2 AQUEOUS LEACHATE AND ALKALINE ETHANOLIC EXTRACTION OF FIELD SOILS

5.5.2.1 Introduction

Soil and litter samples were collected from under mature R. ponticum bushes at Strawberry Lee Plantation, South Yorkshire. Samples were taken at bimonthly intervals and removed to the laboratory for immediate analysis. The amount of extract from the various samples was measured and the presence of phenolics was tested for. In some samples where phenolics were found, the amounts were also measured.

5.5.2.2 Method

5.5.2.2.1 Aqueous leachate

100-200g (fresh weight) of soil was placed on a Whatman No. 1 filter paper in a large, glass funnel. 1000 mls of distilled, deionized water was slowly poured into the sample and collected below in a large, glass flask. The leachate was recycled periodically during each day and each day's extract was evaporated to a solid at 40°C on a rotary vacuum evaporator. The leaching was carried out for five days in a cold-room at 5°C.

The soil sample fresh weights were measured and sub-samples were taken. The sub-samples were fresh weighed, dried at 80°C in the oven and dry weighed. The moisture content of the samples was then calculated.

The combined extracts, evaporated to a solid as noted, were then stored in a desiccator in the fridge to ensure that they were thoroughly dried. They were then weighed. Sub-samples were taken and tested for the presence of phenolics using the methods described earlier (5.2).

5.5.2.2.2 Alkaline ethanolic extraction

Soil and litter samples were extracted using an alkaline ethanolic solution. This technique is sufficiently strong to release adsorbed organic acid anions, but too weak to cause hydrolysis of most organic compounds. The method was suggested by Dr M.A.F. Jalal (pers. comm.) and is shown schematically over:-

100g soil or litter (fresh weight) in a 400 ml glass flask

↓

Suspended in 200 ml ethanol with a few drops of concentrated sodium hydroxide solution, giving an extraction pH of 11.4

↓

Suspension thoroughly shaken and left to stand for 15 hours

↓

Filtered on Whatman No. 1 paper and the residue washed with fresh extractant

↓

Solid residue discarded

Filtrate evaporated on rotary vacuum evaporator at 40°C to a small volume, then made up to 100 ml with distilled water.

↓

Acidified to pH 2.0 using conc. HCl

↓

Extracted four times in ethyl acetate

↓

Dried by passing over anhydrous sodium sulphate

↓

Extracted in 5% aqueous sodium hydrogen carbonate

↓

Acidified to pH 2.0 using conc. HCl

↓

Extracted in 15% ethyl acetate in petroleum ether *(repeated several times as necessary)*

↓

Extract evaporated to dryness at 40°C on the rotary vacuum evaporator

↓

Sample stored in desiccator in fridge to ensure complete dryness

(Note: all the serial extractions were carried out using a separating funnel.)

5.5.2.2.3 Soil acidity

Soil and litter samples were also taken for the measurement of pH in distilled water. Seasonal patterns were noted.

5.5.2.2.4 Phenolic content

Aqueous leachates and alkaline ethanolic extracts were tested for the presence of phenolics using one-way paper chromatography as already described (5.2). Alkaline ethanolic extracts for two of the bimonthly samples were also examined by two-way paper chromatography and their total phenolic content was measured.

5.5.2.3 Results

Extraction of field soil (either sieved of root material or unsieved) by aqueous leaching, did not release detectable phenolic compounds (Table 5.5.2.3.4). A considerable amount of brown organic material was extracted. This may well have been fulvic acid polymers such as found by Jalal and Read (1983 I and II). The amount of aqueous extract peaked in October (and to a lesser degree) in April (Figure 5.4). The sieving of soil had little obvious effect, except that the peak in October was far higher from unsieved soil than from soil with Rhododendron roots sieved out.

Alkaline ethanolic extraction released considerably more material from litter than from soil (Figure 5.5). This was especially on a dry weight basis. Both soil and litter released maximum amounts of material to alkaline ethanolic extraction during the summer. A slight increase in the amount of material released in February was followed by a decrease in April.

Analysis of the alkaline exthanolic extract showed detectable phenolic compounds in all but two samples (Table 5.5.2.3.4). The phenolic content of extracts from two of the bimonthly samples was determined (Table 5.5.2.3.5). This was 0.16% and 0.40% of the extract dry weight from sieved soil and 0.16% and 0.20% of the extract dry weight from litter. As a percentage of either soil or litter dry weight (Table 5.5.2.3.6), these were 0.0074% and 0.0068% from sieved soil and 0.0044% and 0.0147% from litter. Two phenolic compounds were detected by two-way paper chromatography (Table 5.5.2.3.7). The same two compounds were present in extracts of both soil and litter.

Table 5.5.2.3.1 Seasonal variation in aqueous and alkaline ethanolic extractable fractions of *Rhododendron* soil and litter. (I) Aqueous leachate

	Aqueous leachate			
Sample and date	mg/g soil (fw)	%(fw)	mg/g soil(dw)	%(dw)
Soil Sieved, Feb. 1980	0.24	0.024	0.79	0.079
Soil Unsieved, Feb. 1980	-	-	-	-
Litter Sieved, Feb. 1980	-	-	-	-
Soil Sieved, Apr. 1980	0.40	0.040	1.00	0.100
Soil Unsieved, Apr. 1980	-	-	-	-
Litter Sieved, Apr. 1980	-	-	-	-
Soil Sieved, Jun. 1980	0.32	0.032	0.79	0.079
Soil Unsieved, Jun. 1980	0.28	0.028	0.69	0.069
Litter Sieved, Jun. 1980	-	-	-	-
Soil Sieved, Aug. 1980	0.42	0.042	0.94	0.094
Soil Unsieved, Aug. 1980	0.45	0.045	1.02	0.102
Litter Sieved, Aug. 1980	-	-	-	-
Soil Sieved, Oct. 1980	0.38	0.038	1.02	0.102
Soil Unsieved, Oct. 1980	0.85	0.085	2.27	0.227
Litter Sieved, Oct. 1980	-	-	-	-
Soil Sieved, Dec. 1980	0.31	0.031	0.88	0.088
Soil Unsieved, Dec. 1980	0.43	0.043	1.24	0.124
Litter Sieved, Dec. 1980	-	-	-	-
Soil Sieved, Feb. 1981	0.37	0.037	0.90	0.090
Soil Unsieved, Feb. 1981	0.48	0.048	1.17	0.117
Litter Sieved, Feb. 1981	-	-	-	-
Soil Sieved, Apr. 1981	0.63	0.063	1.77	0.177
Soil Unsieved, Apr. 1981	0.57	0.057	1.61	0.161
Litter Sieved, Apr. 1981	-	-	-	-

Table 5.5.2.3.2 Seasonal variation in aqueous and alkaline ethanolic extractable fractions of *Rhododendron* soil and litter. (II) Alkaline ethanolic extract

	Alkaline ethanolic extractable sub-fraction			
Sample and date	mg/g soil (fw)	%(fw)	mg/g soil(dw)	%(dw)
Soil Sieved, Feb. 1980	-	-	-	-
Soil Unsieved, Feb. 1980	-	-	-	-
Litter Sieved, Feb. 1980	-	-	-	-
Soil Sieved, Apr. 1980	-	-	-	-
Soil Unsieved, Apr. 1980	-	-	-	-
Litter Sieved, Apr. 1980	-	-	-	-
Soil Sieved, Jun. 1980	-	-	-	-
Soil Unsieved, Jun. 1980	-	-	-	-
Litter Sieved, Jun. 1980	-	-	-	-
Soil Sieved, Aug. 1980	0.15	0.015	0.34	0.034
Soil Unsieved, Aug. 1980	-	-	-	-
Litter Sieved, Aug. 1980	0.84	0.084	2.84	0.284
Soil Sieved, Oct. 1980	0.02	0.002	0.04	0.004
Soil Unsieved, Oct. 1980	-	-	-	-
Litter Sieved, Oct. 1980	0.15	0.015	0.43	0.043
Soil Sieved, Dec. 1980	0.10	0.010	0.29	0.029
Soil Unsieved, Dec. 1980	-	-	-	-
Litter Sieved, Dec. 1980	0.11	0.011	0.46	0.046
Soil Sieved, Feb. 1981	0.19	0.019	0.46	0.046
Soil Unsieved, Feb. 1981	-	-	-	-
Litter Sieved, Feb. 1981	0.23	0.023	0.92	0.092
Soil Sieved, Apr. 1981	0.06	0.006	0.17	0.017
Soil Unsieved, Apr. 1981	-	-	-	-
Litter Sieved, Apr. 1981	0.06	0.006	0.22	0.022

Table 5.5.2.3.3 Seasonal variation in soil and litter acidity

Sample and date	pH (after 24 hrs. in distilled water)
Rh. Soil Sieved, Feb. 1980	3.30
Rh. Soil Unsieved, Feb. 1980	3.30
Rh. Litter Sieved, Feb. 1980	3.55
Grass Soil Sieved, Feb. 1980	3.60
Rh. Soil Sieved, Apr. 1980	3.40
Rh. Soil Unsieved, Apr. 1980	3.40
Rh. Litter Sieved, Apr. 1980	3.60
Grass Soil Sieved, Apr. 1980	3.65
Rh. Soil Sieved, Jun. 1980	3.45
Rh. Soil Unsieved, Jun. 1980	3.45
Rh. Litter Sieved, Jun. 1980	3.65
Grass Soil Sieved, Jun. 1980	3.70
Rh. Soil Sieved, Aug. 1980	3.30
Rh. Soil Unsieved, Aug. 1980	3.30
Rh. Litter Sieved, Aug. 1980	3.45
Grass Soil Sieved, Aug. 1980	3.60
Rh. Soil Sieved, Oct. 1980	3.50
Rh. Soil Unsieved, Oct. 1980	3.50
Rh. Litter Sieved, Oct. 1980	3.60
Grass Soil Sieved, Oct. 1980	3.65
Rh. Soil Sieved, Dec. 1980	3.35
Rh. Soil Unsieved, Dec. 1980	3.35
Rh. Litter Sieved, Dec. 1980	3.50
Grass Soil Sieved, Dec. 1980	3.60

Seasonal variation in soil and litter acidity

Sample and date	pH (after 24 hrs. in distilled water)
Rh. Soil Sieved, Feb. 1981	3.65
Rh. Soil Unsieved, Feb. 1981	3.65
Rh. Litter Sieved, Feb. 1981	3.80
Grass Soil Sieved, Feb. 1981	3.90
Rh. Soil Sieved, Apr. 1981	3.40
Rh. Soil Unsieved, Apr. 1981	3.40
Rh. Litter Sieved, Apr. 1981	3.50
Grass Soil Sieved, Apr. 1981	3.65

Table 5.5.2.3.4 The presence of phenols in *Rhododendron* soil and litter

Sample and date		Test for simple phenolics using Gibb's Reagent Spots on 1-way paper chromatograph	+ve for phenolics
1. Aqueous leachate			
Soil Sieved,	Aug. 1980	1 Pink, 1 Grey-Green	NO
	Oct. 1980	" "	NO
	Dec. 1980	" "	NO
	Feb. 1981	" "	NO
	Apr. 1981	" "	NO
Soil Unsieved,	Aug. 1980	1 Brown, 1 Grey-Green	NO
	Oct. 1980	1 Pink, 1 Grey-Green	NO
	Dec. 1980	" "	NO
	Feb. 1981	" "	NO
	Apr. 1981	" "	NO
2. Alkaline ethanol extract			
Soil Sieved,	Aug. 1980	1 Pink	NO
	Oct. 1980	1 Pink, 2 Pale Blue	YES
	Dec. 1980	1 Pink, 1 Pale Blue	YES
	Feb. 1981	1 Pink, 1 Blue, 1 Yellow	YES
	Apr. 1981	1 Pink, 1 Blue, 1 Pale Blue	YES
Litter Sieved,	Aug. 1980	1 Pink, 2 Pale Blue, 1 Grey-Green	YES
	Oct. 1980	Nothing	NO
	Dec. 1980	1 Pink, 2 Blue	YES
	Feb. 1981	1 Pink, 1 Blue, 1 Yellow	YES
	Apr. 1981	1 Pink, 2 Blue, 1 Grey-Green	YES

Table 5.5.2.3.5 Extractable simple phenolics in *Rhododendron* soil and litter

Sample and date	% phenol as mg (+)-catechin/g extract
Alkaline Ethanolic Extract	
Soil Sieved, Feb. 1981	0.16
Litter Sieved, Feb. 1981	0.16
Soil Sieved, Apr. 1981	0.40
Litter Sieved, Apr. 1981	0.20

Using the amount of extract obtained from each soil/litter sample, the amount of simple phenolic material per unit weight of soil/litter was obtained.

Table 5.5.2.3.6

Sample and date	% phenol as mg (+)-catechin/g soil	
	Soil/Litter fw.	Soil/Litter dw.
Soil Sieved, Feb. 1981	0.0030	0.0074
Litter Sieved, Feb. 1981	0.0037	0.0147
Soil Sieved, Apr. 1981	0.0024	0.0068
Litter Sieved, Apr. 1981	0.0012	0.0044

Table 5.5.2.3.7 Two-way paper chromatography of alkaline ethanolic extracts of *Rhododendron* soil and litter

Extracts from samples collected in February 1981 were run two ways as already described. Three spots were produced for each sample corresponding with the spots already obtained by one-way paper chromatography.

The papers were tested both with Gibb's Reagent and Ferricyanide etc. Each sample was run twice, firstly a test run, followed by a more concentrated sample.

Soil, Feb. 1981 3 spots

1. Intense yellow fluorescence with UV. light, blue-yellow with Ferricyanide, pale purple with Gibb's tending to green for the more concentrated sample plus a basal streak behind the BAW. front.
   Mixed Non-polar compounds.

2. Pale blue fluorescence with UV. light to blue with ammonia. Level with BAW. front. Corresponds to No. 2 in the 'Litter'.
   Simple phenolic.

3. Pale blue fluorescence, pink with Gibb's (only showed for the higher concentration).
   Unknown.

Litter, Feb. 1981 3 spots

1. As for soil.

2. As for soil, but also gave blue with Gibb's for the more concentrated sample.

3. As for soil.

KEY:-

% dry weight ————————

% fresh weight - - - - - - - - -

Litter ●

Soil ○

Soil unsieved ◉

Soil sieved to remove root material ◎

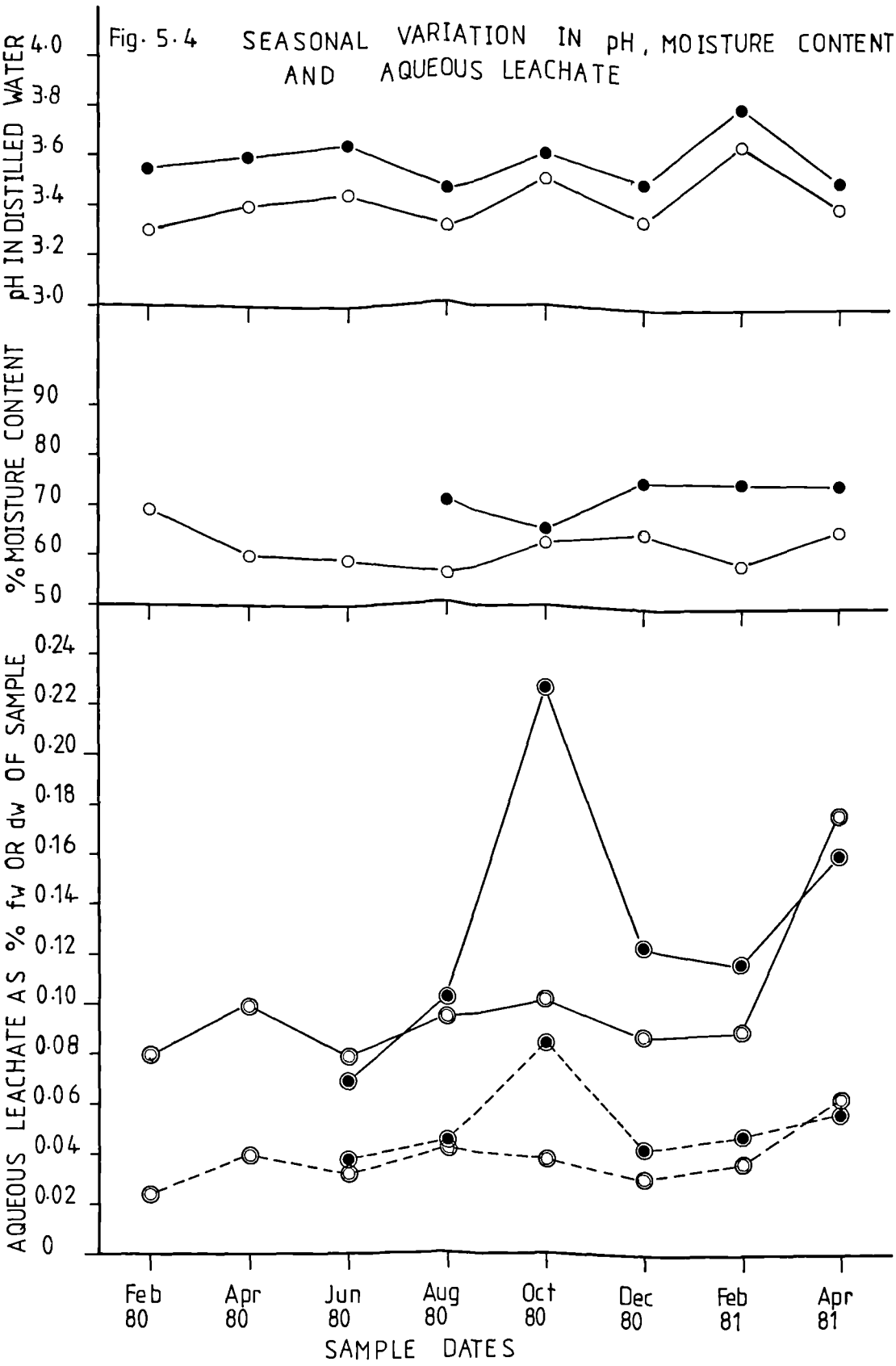
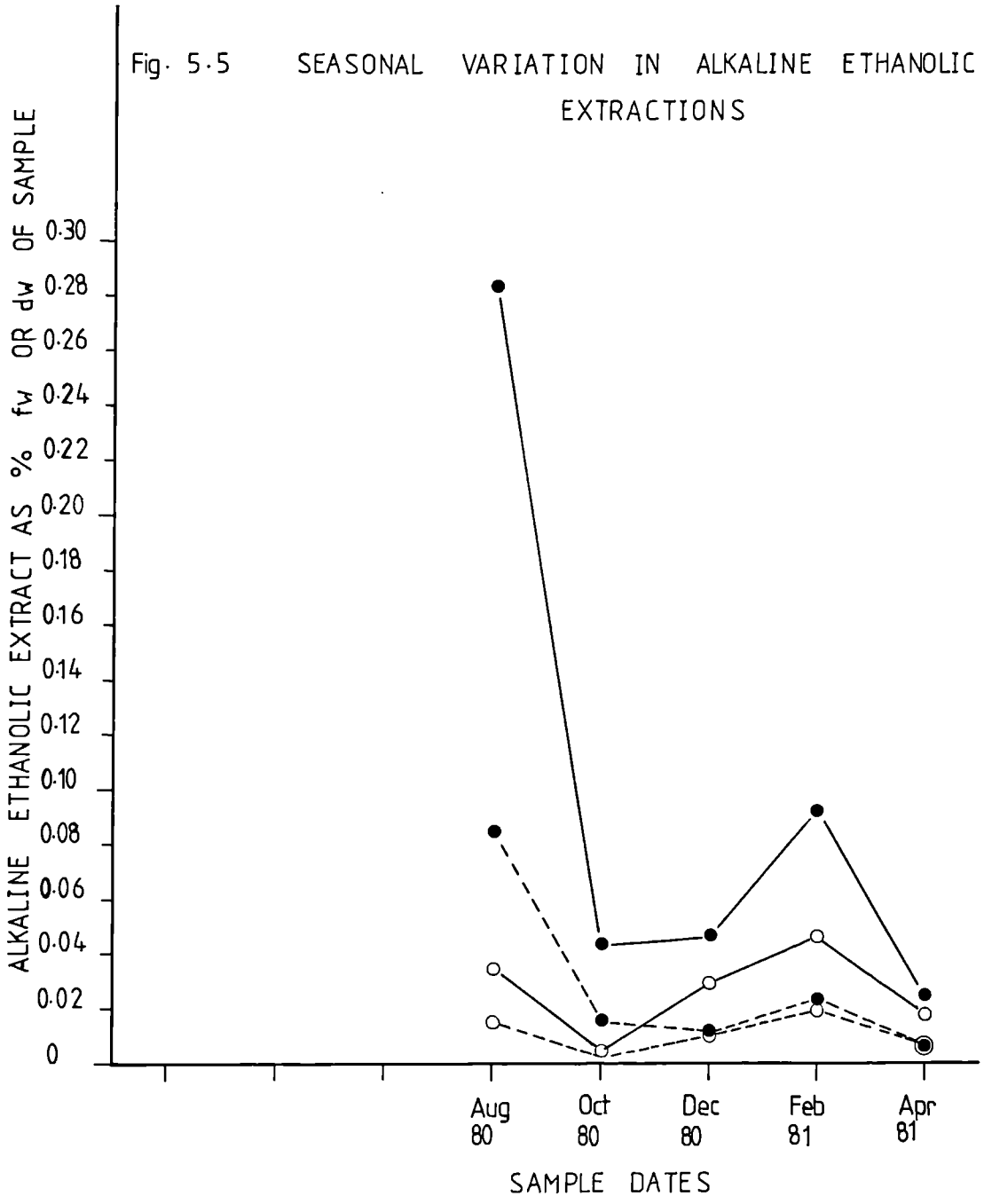


Fig. 5·4 SEASONAL VARIATION IN pH, MOISTURE CONTENT AND AQUEOUS LEACHATE

Fig. 5·5 SEASONAL VARIATION IN ALKALINE ETHANOLIC EXTRACTIONS



5.5.2.4 Discussion

Aqueous leaching of Rhododendron soil released organic material (probably fulvic acid polymers), but no detectable 'free' phenolic compounds. A more drastic extraction procedure, using alkaline ethanolic extraction did release 'free' phenols. This method was designed to release phenolic compounds weakly bonded to organic polymers in soil or litter. Two unidentified phenolic compounds were obtained from both soil and litter collected from under R. ponticum. The total amount of phenolic material was similar to that obtained from Calluna soil by Jalal and Read (1983 II).

5.5.3 EXTRACTIONS FROM SANDY SOIL AND ACID-WASHED SAND IN POTS, WITH AND WITHOUT *RHODODENDRON* SEEDLINGS

5.5.3.1 Aqueous leaching of Clumber soil in pots, with and without *Rhododendron* seedlings

5.5.3.1.1 Introduction

Chou and Muller (1972) have shown that the ease of extraction of phenolic acids from soils, declines with increasing organic content. The soil selected for this extraction was very sandy Clumber soil with little organic matter. Extraction was with distilled water so that only loosely bound 'free' compounds would be released.

5.5.3.1.2 Method

R. ponticum seedlings were grown on irradiated Clumber soil. They were 18 months old at the time of the leaching and had been grown in the pots of irradiated Clumber soil (1 per pot) for 15 months. The seedlings were grown in the greenhouse lit by normal daylight, together with supplementary lighting during dull periods. Daytime temperatures ranged from 20°C to 30°C.

The seedlings and treatments were as below:-

Pot with or without seedling	Watering/nutrient addition
1. R. ponticum, non-mycorrhizal	Robbins' solution (X1)
2. " "	" " (X2)
3. " "	Distilled water
4. " , mycorrhizal	Robbins' solution (X1)
5. " "	" " (X2)
6. " , "	Distilled water
7. No seedling	Robbins' solution (X1)
8. "	" " (X2)
9. "	Distilled water.

The pots were then removed to the laboratory for leaching. 1000 ml distilled deionized water was added in 250 ml. aliquots by a slow drip (see Figure 5.6). Leaching occurred over a period of 24 hours. The leachate was collected in an ice-cooled, glass beaker and then filtered Whatman No. 1 paper and concentrated at 40°C on a rotary vacuum evaporator. It was then taken into ethyl acetate and dried with anhydrous sodium sulphate. The sample was evaporated to dryness on a rotary vacuum evaporator at 40°C. The solid residue was collected and stored in a desiccator in a fridge.

The samples were then examined for the presence of possible phenolic compounds by gas liquid chromatography (G.L.C.). Samples of p-hydroxybenzoic, vanillic, protocatechuic, p-coumaric and ferulic acid were used as standards.

5.5.3.1.3 Results

None of the G.L.C. traces produced peaks indicative of phenolic compounds though a series of peaks was obtained. These were produced by all the samples and seem to be either similar, related compounds or 1-2 main compounds with associated breakdown products. No clearly characteristic peaks were obtained from the Rhododendron soils alone. All these major peaks had retention times that were too long for simple phenolic compounds. There was also a considerable amount of heterogeneous background contribution to most of the traces.

5.5.3.1.4 Discussion

There was no positive evidence for the existence of freely available phenolic compounds in these soils. If such 'free' phenolics exist, it may be that they are bound to the soil in some way (perhaps by hydrogen bonds to the soil organic matter or clay minerals). They would then not be freely available to aqueous leaching. There are two ways of overcoming this problem. Firstly, to grow the seedlings on a simpler medium such as acid-washed sand. In a simple and relatively pure medium such as this, the capacity for 'fixing' or degrading phenolics released from roots,

should be reduced or eliminated. The other possibility would be to use a more drastic extraction technique. The following experiments attempt both these.

Such compounds as were obtained may have been from the vegetation/ microflora of the original field soil (perhaps modified by the gamma irradiation treatment) or from microbial activity in the potted soils.

5.5.3.2 Aqueous leaching of acid-washed sand in pots, with and without *Rhododendron* seedlings

5.5.3.2.1 Introduction

In an attempt to test whether or not 'free' phenolics can be obtained from soil systems in which R. ponticum is growing, a simplified 'soil' system was devised. Using acid-washed sand as the growing medium in pots should have two major advantages over field soil. Firstly, any phenolic material found in the sand will have come from the sand/root system and not from other sources as is possible in the field soil. Secondly, any phenolic compounds released into the sand from the roots should be fairly easy to remove with a mild extractant.

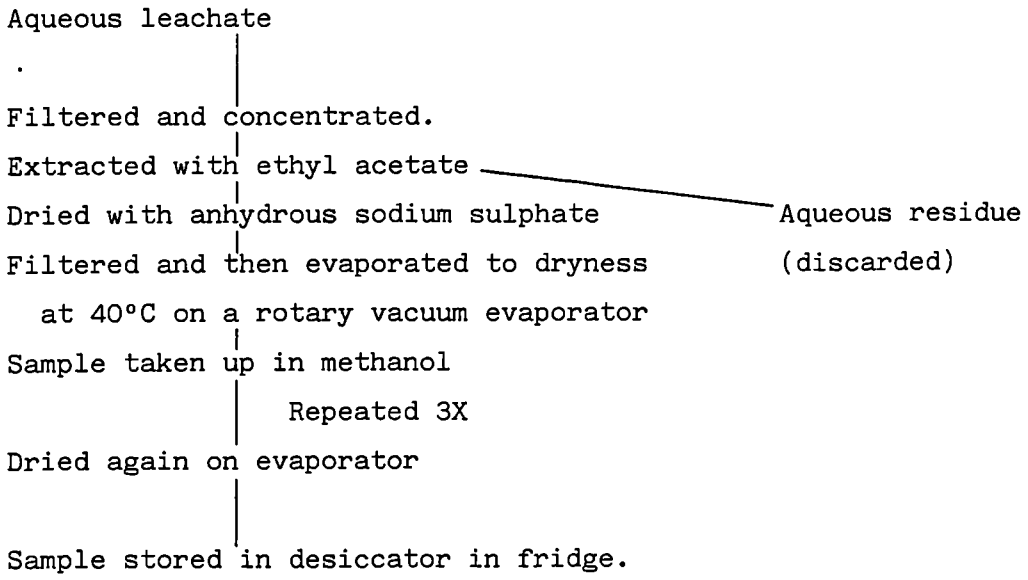
5.5.3.2.2 Method

Two year old mycorrhizal R. ponticum seedlings were taken from irradiated Cropton soil. Their roots were carefully cleaned of all visible macro-debris, washed in distilled water and transferred to acid-washed sand with a pH of 6.00.

The seedlings were then grown in the pots of acid-washed sand for 3½ months in the greenhouse. Lighting was by natural daylight which was supplemented during dull periods. Daytime temperatures were between 20°C and 30°C. The pots were watered with full-strength Robbins' solution. The seedlings were grown under these conditions from 25.6.81 - 2.10.81. The surface of the sand was covered by acid-washed, black, alcathene beads.

The pots were then removed to the laboratory for leaching. 1000 ml. distilled, deionized water was added in 250 ml. aliquots by a slow drip, using the system shown in the diagram (Figure 5.6). Leaching occurred over a period of 24 hours. The leachate was collected in an ice-cooled, glass beaker. It was then filtered on Whatman No. 1 paper, concentrated at 40°C on a rotary vacuum evaporator, taken into ethyl acetate and then dried with anhydrous sodium sulphate. The sample was then evaporated to

dryness on a rotary vacuum evaporator at 40°C. The solid residue was collected and stored in a desiccator in a fridge. The system is shown schematically below:-



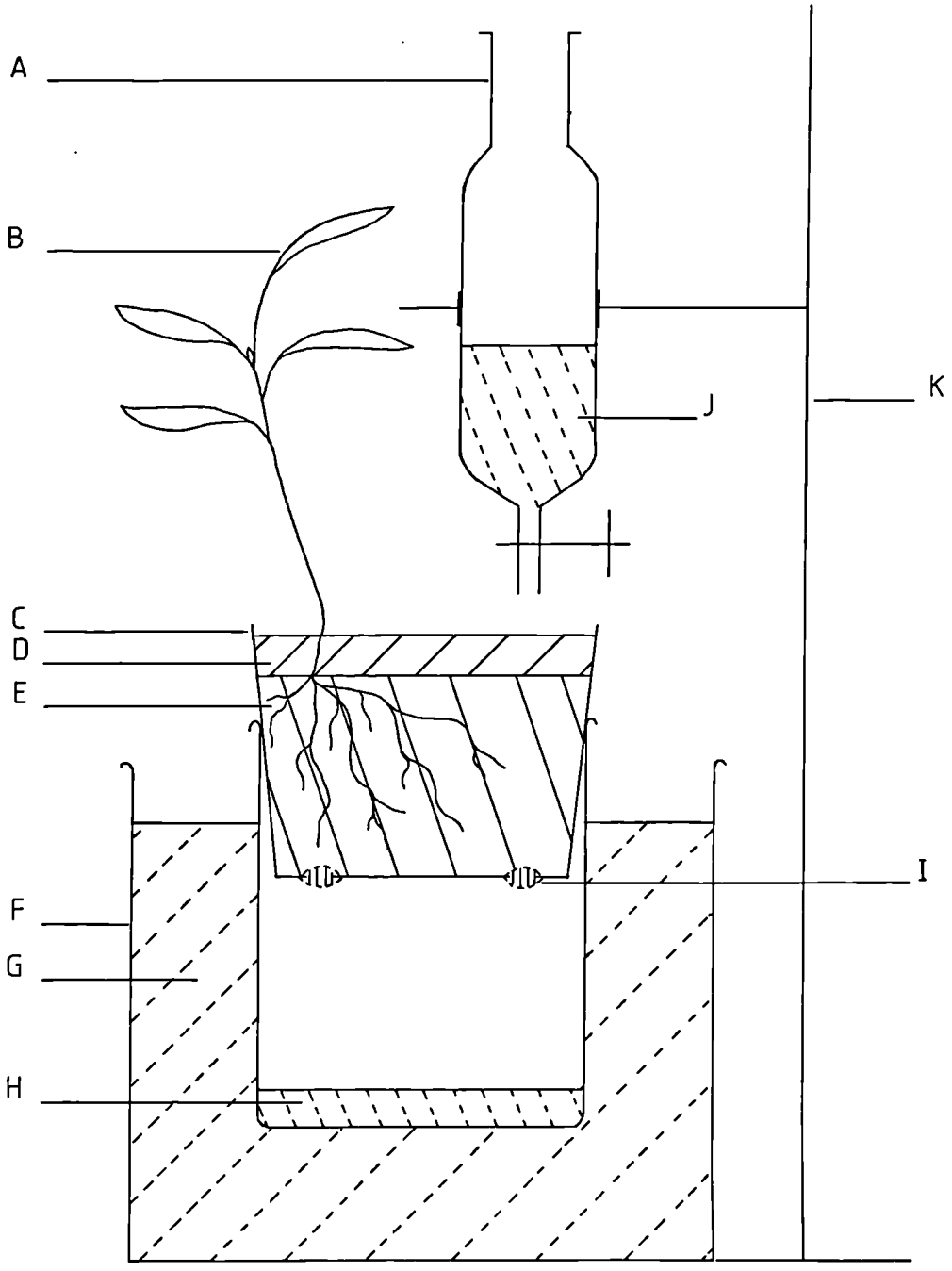
The samples were then examined for the presence of possible phenolic compounds by gas liquid chromatography. The following phenolic acids were used as standards:-

p-hydroxybenzoic acid
vanillic acid
protocatechuic acid
p-coumaric acid
ferulic acid.

KEY :-

A : Glass separating funnel
B : Rhododendron seedling
C : Plastic pot (5 inch diameter)
D : Black alcathene beads
E : Acid-washed sand
F : Large glass beaker
G : Ice
H : Leachate
I : Silicone rubber bungs ---- perforated to allow leaching
J : Leaching liquid
K : Retort stand and clamp

Fig. 5·6 APPARATUS FOR LEACHING SOIL OR ACID-WASHED SAND



5.5.3.2.3 Results

Pot extracted	Result of G.L.C. analysis
Sand + R. ponticum (1)	Negative for phenolics
Sand + R. ponticum (2)	" " "
Sand without R. ponticum with no added nutrients	" " "
Sand without R. ponticum but with Robbins' solution.	" " "

No G.L.C. peaks indicative of phenolic compounds were found in any of the extracts. All the peaks present had much longer retention times than the simple phenolic acids used as standards.

Several clearly defined peaks were present to a greater or lesser extent in all the samples (both with and without R. ponticum). The main G.L.C. peaks varied from sample to sample, but there was always a corresponding minor peak in the other samples. This could be due to closely related compounds perhaps differing slightly from sample to sample, or one major compound with various breakdown products of the basic structure.

The peaks were considerably higher for the non-Rhododendron than the Rhododendron samples. This is not necessarily significant since the extractions were not strictly quantitative. The amount of background interference and the minor peaks also increased in the non-Rhododendron samples.

5.5.3.2.4 Discussion

The compounds extracted could be of microbial origin, from activity within the acid-washed sand system. The amount of material obtained might therefore be greater from the pots without Rhododendron, due to the greater bulk of sand being extracted. The background interference might

also increase for the same reason. The quality and quantity of such material may vary from pot to pot with the addition of nutrient solution or distilled water and with the presence or absence of mycorrhizal roots.

Microbial activity occurring around the sand particles and within the soil solution may have produced a capacity to 'fix' organic compounds released by the roots. This could be by hydrogen bonding to organic debris around sand particles, or by the active metabolic action of the microflora/fauna. Release of such bonded organic compounds would then require a more drastic extractant.

5.5.3.3 Extraction from sand and from root washings with sodium bicarbonate in aqueous solution

5.5.3.3.1 Introduction

The previous two experiments indicated possible difficulties in obtaining 'free' phenolic compounds even from relatively simple soil systems with little organic matter. The extraction used in this experiment was an attempt to detect any such compounds occurring in the soil or on the root surface, using a slightly more drastic treatment.

5.5.3.3.2 Method

Two year old R. ponticum seedlings were transferred from irradiated Cropton soil to acid-washed sand in pots. The roots were carefully cleaned of macro-debris and then washed in distilled water prior to replanting. The seedlings were mycorrhizal, having been inoculated with cultured endophyte. The seedlings were then removed to the greenhouse with the same conditions as described for the previous experiments (5.5.3.1 and 5.5.3.2). They were grown in the pots of acid-washed sand (pH 6.0) for 7 months (25.6.81 - 21.1.82) and watered with Robbins' solution. Control pots were set up without seedlings and watered with distilled water alone or with Robbins' solution.

For the extraction, the root system and sand were carefully separated. The roots (which were very fine and pale cream in colour) were in a tight mass, completely filling the pot. They were present to the sand surface and just above (between the lower alcathene beads and the top of the sand). Microscopic examination of a sample of fine roots, after extraction from the pot, separation from the sand and washing in sodium bicarbonate, showed heavy mycorrhizal infection. There was no sign of breakdown of gross cellular structures or any other damage.

Not much sand came off the roots during washing, suggesting that the separation of root from the sand was effective. Very little fine root material was present in the sand washing. This was removed after filteration, together with a small amount of fine, brown, organic matter,

the combined fresh weight being less than 0.50 g. This again suggests that the separation was effective and that root damage (and hence contamination of the sand by the contents of lysed cells) was minimal.

Procedure:-

1. Roots gently removed from the pot.
2. Excess sand carefully shaken off.
3. Root and sand fractions separated and treated as 5.5.3.3.2.1 (sand) or 5.5.3.3.2.2 (roots).

5.5.3.3.2.1 Extraction from sand

The sand was removed from the pot and washed in 5% sodium bicarbonate (aq.) in an end-over-end shaker. 1000 ml. was used in two washings:-

i) 500 ml. for 10 minutes.

ii) 500 ml. for 20 minutes.

Following washing, the pH was checked to make sure it was above 7.0.

The extract was filtered, the sand washed with a small amount of distilled water following filtration and the combined extracts centrifuged.

Supernatant acidified to pH 2.0 with conc. HCl.

Concentrated to c. 250 ml. on the rotary vacuum evaporator at 40°C.

Extract in ethyl acetate in separating funnel. (Repeated 3X).

Aqueous fraction remaining after third extraction in ethyl acetate.

Ethyl acetate fraction evaporated to dryness on rotary vacuum evaporator at 40°C.

Hydrolyse by addition of conc. HCl to make 1-2 N solution and boil on steam bath for 30 minutes.

Cool and extract in ethyl acetate as before.

Add a few drops of methanol (Repeat 3X)

Re-evaporate as before

Store in desiccator.

Sand fresh weight and dry weight also measured for each pot.

5.5.3.3.2.2 Extraction from roots

Root fraction

Measure fresh weight of total sample, fresh and dry weight of sub-sample.

Shake gently for 10 mins. in 250 ml. 5% sodium bicarbonate.

Centrifuge extract.

Acidify to pH 2.0 with conc. HCl

Concentrate to c. 25 ml. on rotary vacuum evaporator at 40°C.

Extract in same way as for sand extract (5.5.3.3.2.1).

5.5.3.3.2.3 Subsequent analysis of extracts

The ethyl acetate fractions were dried and then weighed. Sub-samples were then taken for analysis to determine the presence or absence of phenolic compounds. The techniques used were for the measurement of total phenolic content and two-way paper chromatography as described previously (5.2).

Key to abbreviations

Rh. 1	:	Rhododendron pot number 1
Rh. 2	:	Rhododendron pot number 2
NRh. + N	:	Pot without Rhododendron but with nutrient solution added
NRh. - N	:	Pot without Rhododendron but without added nutrient solution
Root	:	Sample from root washing
Sand	:	Sample from sand washing
Hydr.	:	Extract from hydrolysed remnant of sample, following the first extraction into ethyl acetate.

5.5.3.3.3 Results

The total dry weight of extract from the sand washing of each pot (Table 5.5.3.3.3.2, Figure 5.7) decreased as:-

Rh. 1 sand > NRh. + N sand > NRh. - N sand > Rh. 2 sand.
and Rh. 1 sand hydr. > NRh. + N sand hydr. > NRh. - N sand hydr. > Rh.2 sand hydr.

The total phenolic acid content of the extracts (Table 5.5.3.3.3.4, Figure 5.8) decreased as:-

Rh. 1 sand > NRh. + N sand > NRh. - N sand > Rh. 2 sand
and Rh. 1 sand hydr. > NRh. + N sand hydr. > Rh. 2 sand hydr > NRh. - N sand hydr.

Considered as dry weight of extract per unit dry weight of sand (Table 5.5.3.3.3.2, Figure 5.9), values were:-

Rh. 1 sand > NRh. + N sand > Rh. 2 sand > NRh. - N sand
Rh. 1 sand hydr. > Rh. 1 sand hydr. > NRh. + N sand hydr. > NRh. - N sand hydr.

The phenolic acid content of extracts as dry weight per unit dry weight of sand (Table 5.5.3.3.3.4, Figure 5.9) values were in the following order:-

Rh. 1 sand > NRh. + N sand > NRh. - N sand > Rh. 2 sand
Rh. 1 sand hydr. > Rh. 2 sand hydr. > NRh. + N sand hydr. > NRh. - N sand hydr.

Combining the extracts from root and sand (Table 5.5.3.3.3.3) gave the following trends for total extract dry weight:-

Rh. 1 + Rh. 1 sand > NRh. + N sand > Rh. 2 + Rh. 2 sand > NRh. - N sand (and the same for hydrolysed samples).

The combined extracts considered as dry weight per unit dry weight of sand (Table 5.5.3.3.3.3) showed the following pattern:-

Rh. 1 + Rh. 1 sand > NRh. + N sand > Rh. 2 + Rh. 2 sand > NRh. - N sand and (Rh. 1 + Rh. 1 sand) hydr. > (Rh. 2 + Rh. 2 sand) hydr.> NRh. + N sand hydr. > NRh. - N sand hydr.

Combined root and sand extracts (Table 5.5.3.3.3.5, Figure 5.10) gave total phenolic acid contents in the following order:-

Rh. 1 + Rh. 1 sand > NRh. + N sand > Rh. 2 + Rh. 2 sand > NRh. - N sand and (Rh. 1 + Rh. 1 sand) hydr. > (Rh. 2 + Rh. 2 sand) hydr.> (NRh. + N sand) hydr. > (NRh. - N sand) hydr.

Phenolic acid contents per unit dry weight of sand (Table 5.5.3.3.3.5, Figure 5.10) for combined extracts were in order of:-

Rh. 1 + Rh. 1 sand > Rh. 2 + Rh. 2 sand > NRh. + N sand> NRh. - N sand. and (Rh. 2 + Rh. 2 sand) hydr. > (Rh. 1 + Rh. 1 sand) hydr.> (NRh. + N sand) hydr. > (NRh. - N sand) hydr.

The mean values of Rhododendron and non-Rhododendron pots (Table 5.5.3.3.3.2) gave the following for total extracts:-

Rh. hydr. > NRh. hydr.> Rh. > NRh.
Rh. + Rh. hydr. > NRh. + NRh. hydr.

Per unit dry weight of sand extracted (Table 5.5.3.3.3.2), this was:-

Rh. hydr. > Rh. >NRh. hydr. > NRh.
Rh. + Rh. hydr. > NRh. + NRh. hydr.

The mean values for total phenolic acid content (Table 5.5.3.3.3.5, Figure 5.11) were:-

Rh. > Rh. hydr.> NRh. hydr.> NRh.
Rh. + Rh. hydr. > NRh. + NRh. hydr.

Compared by unit dry weight of sand extracted (Table 5.5.3.3.3.5, Figure 5.11) this was:-

Rh. hydr. Rh. NRh. hydr. NRh.
Rh. + Rh. hydr. NRh. + NRh. hydr.

The amount of phenolic compounds in the extracts ranged from 0.10% to 0.95% (Table 5.5.3.3.3.4). Values were generally higher for the extract before hydrolysis of the sample than in the extract of the hydrolysed remnant of the first extraction. This was the case in all root samples and all sand washings except Rh. 2 sand.

The highest percentages of phenolics in the total extracts from any samples were 0.95% in the Rh. 2 root washing and 0.68% in the Rh. 1 sand washing. Levels varied within the different fractions of each sample. However, the combined pre-hydrolysed and hydrolysed sample extracts for non-Rhododendron pots with and without added nutrients had the same values (0.23%). This was slightly lower than the overall combined fraction values for the Rhododendron pots (Rh. 1 = 0.28%; Rh. 2 = 0.30%).

The presence of a range of phenolic compounds was demonstrated by two-way paper chromatography of extracts from both Rhododendron root and Rhododendron sand washings (Table 5.5.3.3.3.6). The amounts were very small, but some of the same compounds were present in both the Rhododendron root washings. The range of compounds in all other Rhododendron samples was much reduced. Some of the phenolic compounds indicated in the root washings (notably spots number 3, 5 and 10 in Rh. 1) recur in the sand washing (Rh. 1 sand).

All the hydrolysed samples and all the non-Rhododendron sand washings produced only a few spots on the chromatograms (Table 5.5.3.3.3.6). These were mainly spots number 6, 11 and 12. Spot number 6 represents mixed non-polar compounds expected from any extract of soil or similar substances (M.A.F. Jalal, pers. comm.). 11 and 12 are apparently low molecular weight, polar compounds.

Some of the compounds observed from root or sand washings of Rhododendron, were present in the same region of the chromatogram as compounds extracted from tissues of R. ponticum. None of the other samples produced any spots

of this nature. According to M.A.F. Jalal (pers. comm.), two spots (numbers 5 and 10) might represent the simple phenolic acids, ferulic acid (number 5) and caffeic acid (number 10).

Fig. 5.7 TOTAL EXTRACT DRY WEIGHT FOR EACH SAMPLE

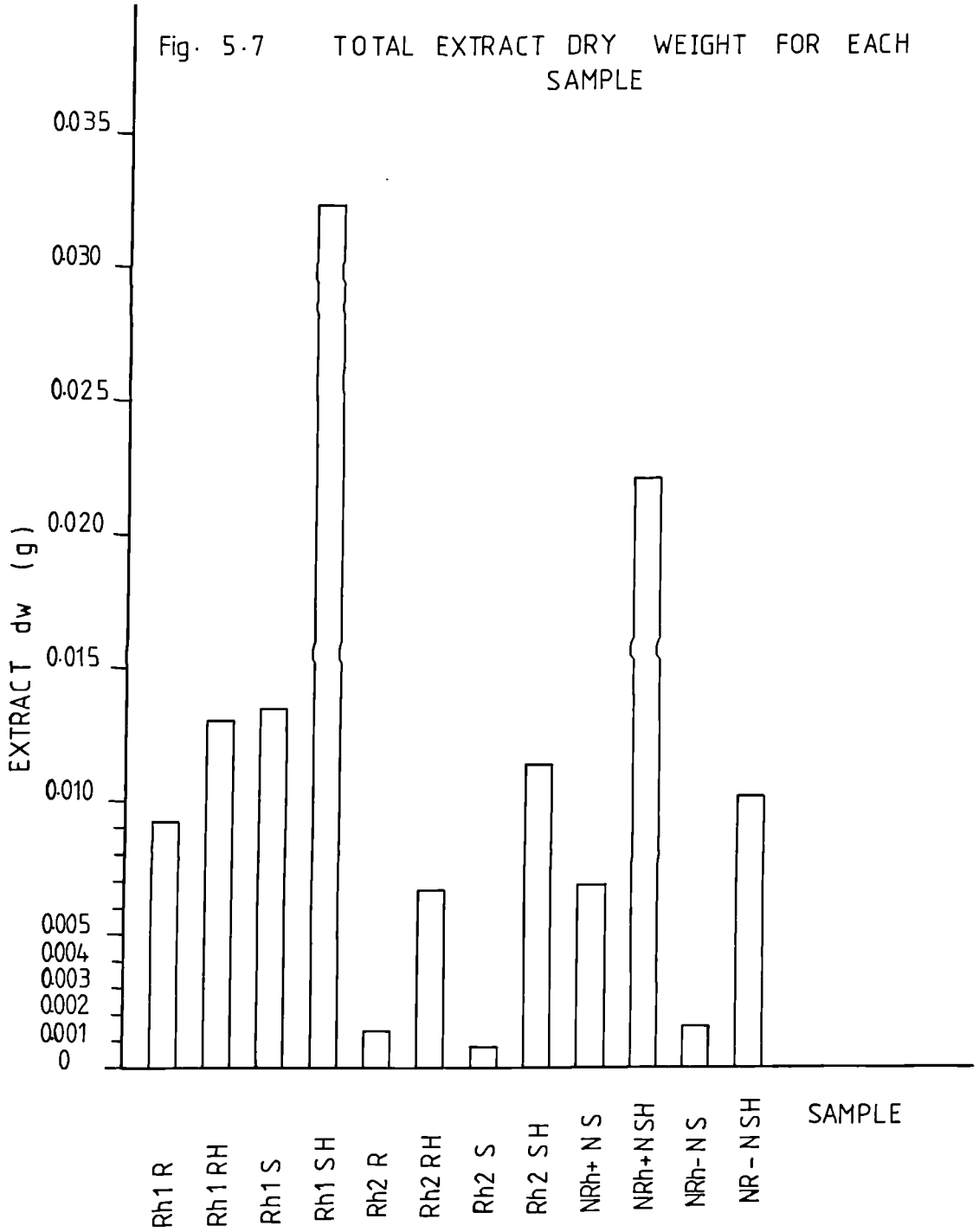


Fig. 5.8 TOTAL PHENOLIC CONTENT OF SAMPLE EXTRACTS

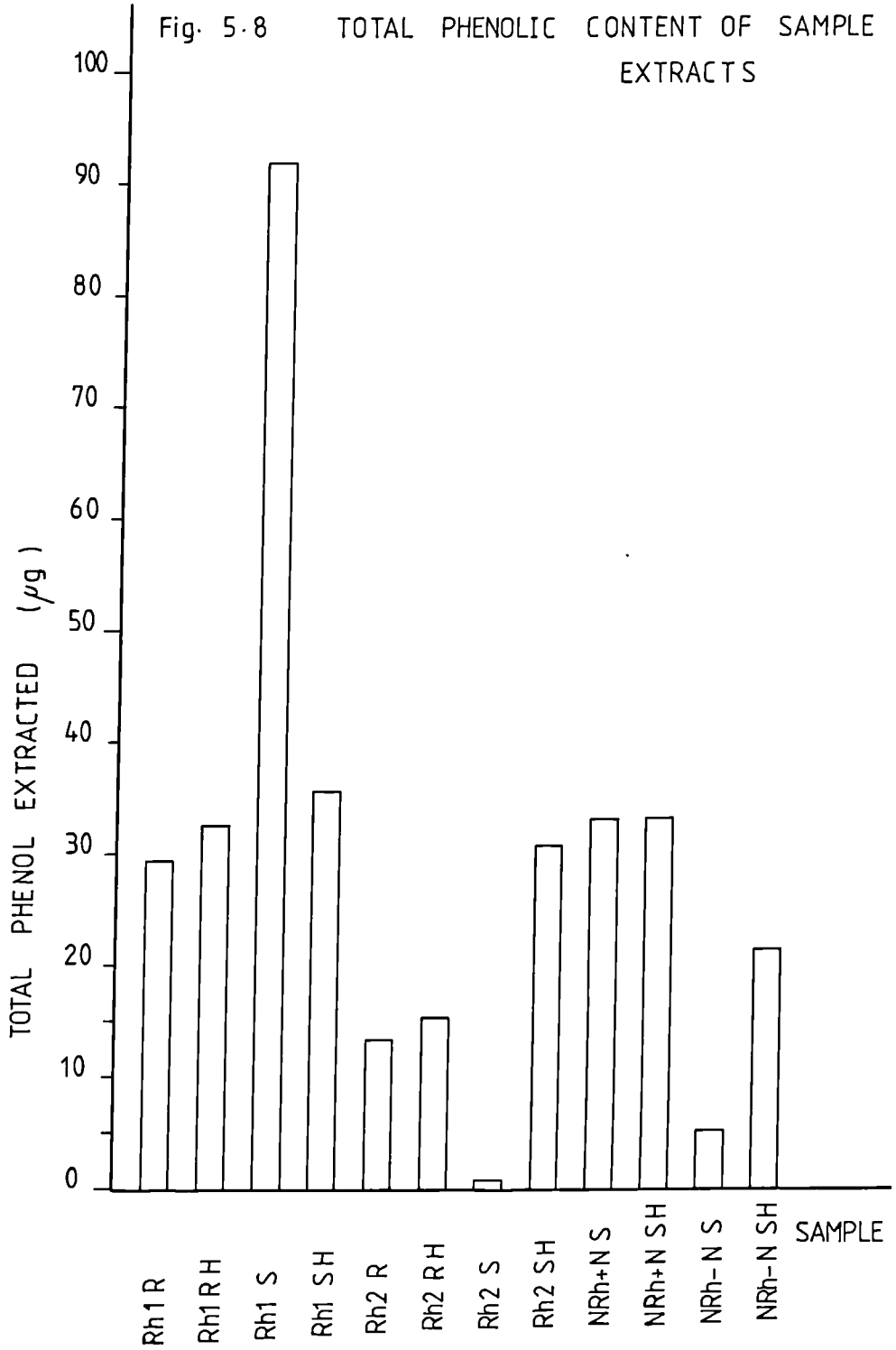


Fig. 5.9 DRY WEIGHT OF EXTRACT AND OF PHENOLICS PER UNIT DRY WEIGHT OF SAND EXTRACTED

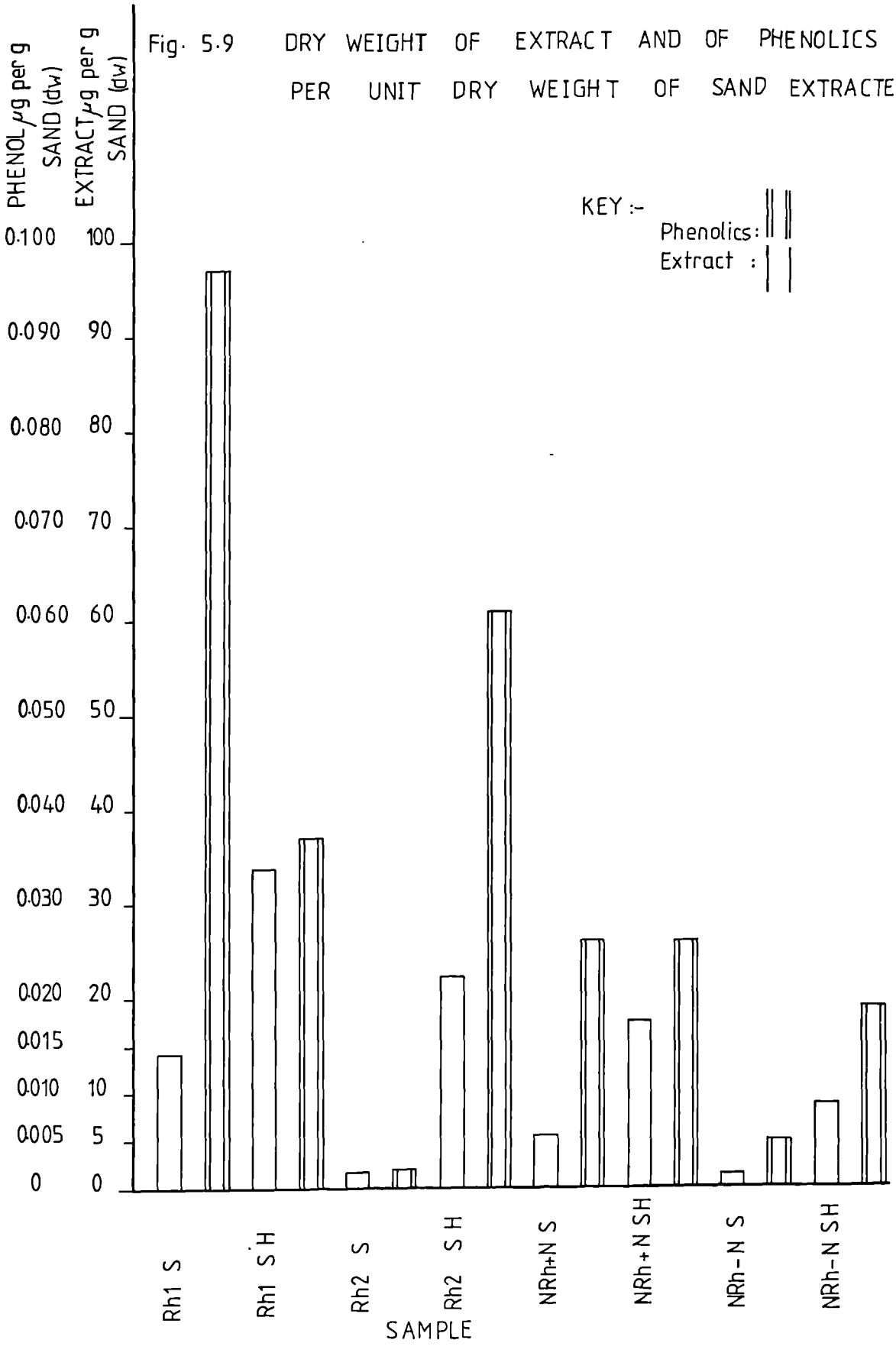
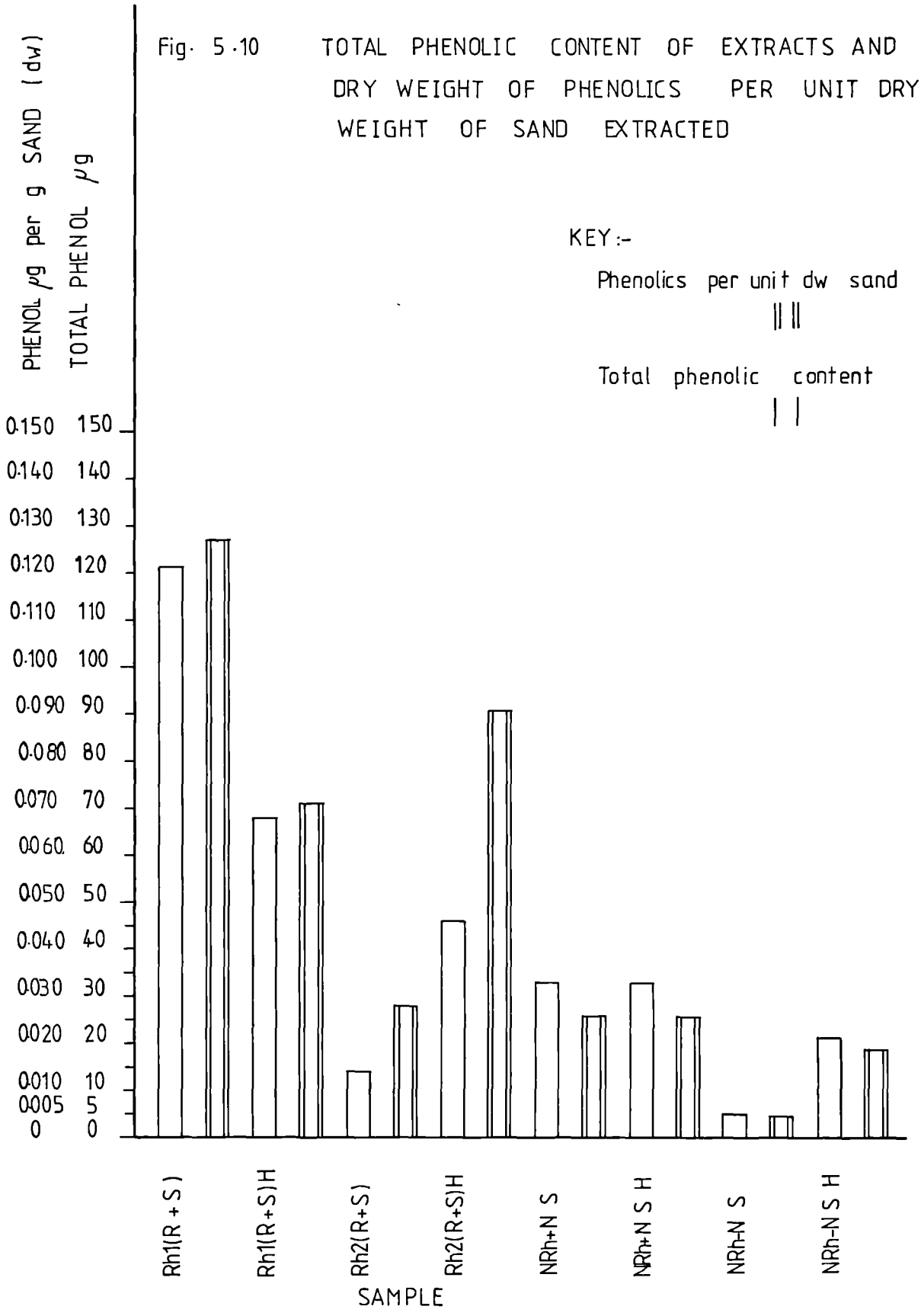


Fig. 5.10 TOTAL PHENOLIC CONTENT OF EXTRACTS AND DRY WEIGHT OF PHENOLICS PER UNIT DRY WEIGHT OF SAND EXTRACTED



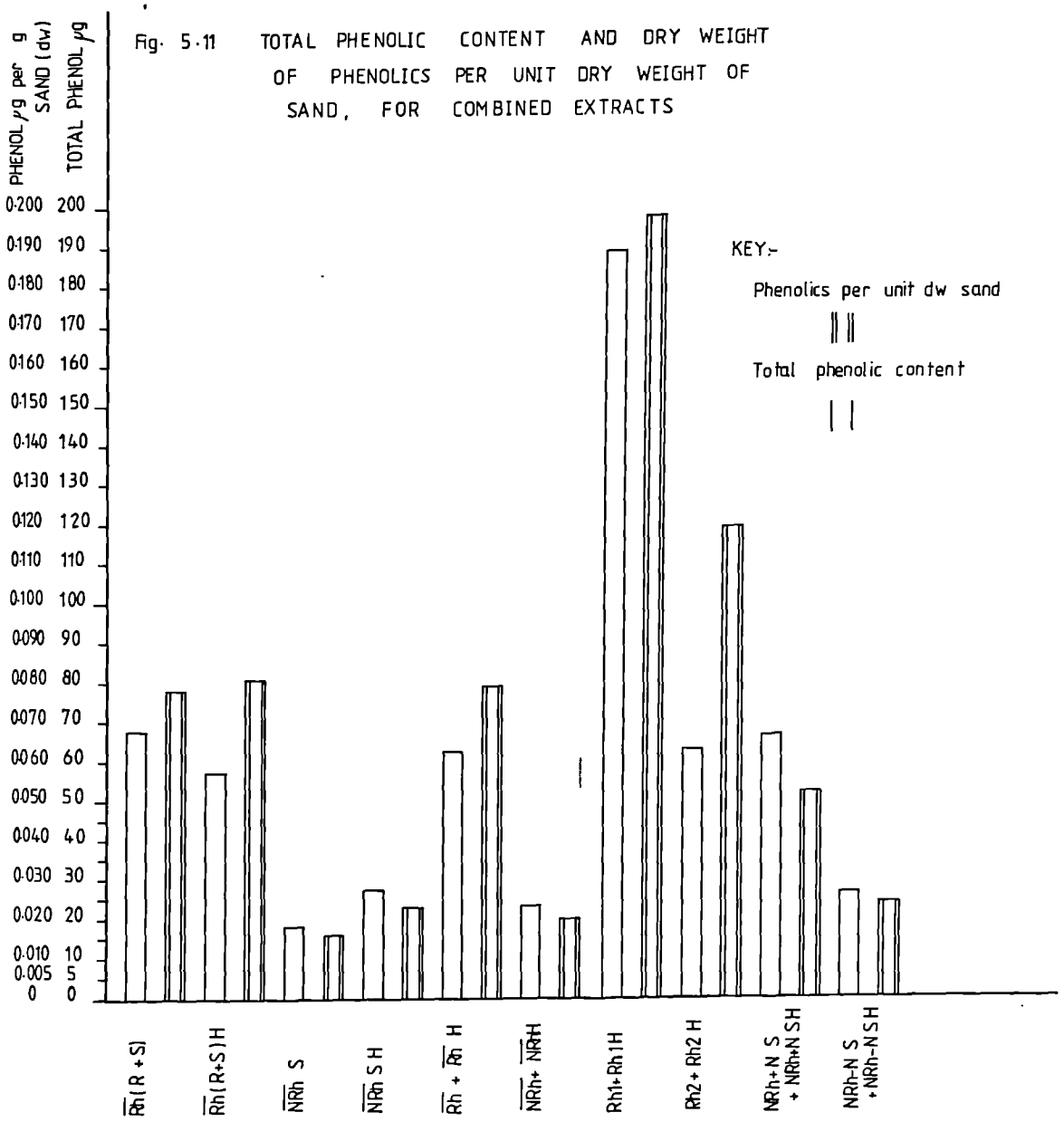


Fig. 5·11 TOTAL PHENOLIC CONTENT AND DRY WEIGHT OF PHENOLICS PER UNIT DRY WEIGHT OF SAND, FOR COMBINED EXTRACTS

Table 5.5.3.3.3.1 Sample data and descriptions

Rhododendron (1)

Sand fresh weight : 1002.0 g; dry weight : 954.4 g.
Sand pH in distilled water : 4.90
Root fresh weight : 22.3 g; dry weight : 12.4 g.
Shoot fresh weight : 21.1 g; dry weight : 9.4 g.

Root washing : dark, reddish-brown liquid.
Sand washing : pale, " " "

Rhododendron (2)

Sand fresh weight : 528.5 g; dry weight : 508.4 g.
Sand pH in distilled water : 5.30
Root fresh weight : 10.7 g; dry weight : 6.0 g.
Shoot fresh weight : 10.6 g; dry weight : 4.1 g.

Root washing : pale, reddish-brown liquid.
Sand washing : pale, brown liquid.

Sand with Robbins' solution

Sand fresh weight : 1333.7 g ; dry weight : 1253.7 g.
Sand pH in distilled water : 6.30
Sand washing : pale, straw coloured liquid.

Sand with distilled water

Sand fresh weight : 1233.9 g; dry weight : 1153.7 g.
Sand pH in distilled water : 6.30
Sand washing : pale, straw coloured liquid.

Table 5.5.3.3.3.2

Extract	Sand dw.(g)	Extract dw.(g)		Extract dw. per g dw. sand (µg)	
Rh. 1 root		0.0092			
Rh. 1 root hydr.		0.0130			
Rh. 1 sand	954.4	0.0135)	0.0458	14.2)	48.0
Rh. 1 sand hydr.		0.0323)		33.8)	
Rh. 2 root		0.0014			
Rh. 2 root hydr.		0.0067			
Rh. 2 sand	508.4	0.0008)	0.0122	1.6)	24.0
Rh. 2 sand hydr.		0.0114)		22.4)	
NRh. + N sand	1253.7	0.0069)	0.0290	5.5)	23.1
NRh. + N sand hydr.		0.0221)		17.6	
NRh. - N sand	1153.7	0.0016)	0.0118	1.4)	10.2
NRh. - N sand hydr.		0.0102)		8.8)	
Rh. Mean (root + sand)		0.0130		17.2	
Rh. Mean hydr. (root + sand)		0.0317		43.3	
NRh. Mean (sand)		0.0043		1.8	
NRh. Mean hydr. (sand)		0.0162		13.4	

Table 5.5.3.3.3.3

	Total extract dw.(g)	Extract dw.(µg), g^{-1} (sand dw.)
Rh. 1 + Rh. 1 sand	0.0227	23.8
Rh. 2 + Rh. 2 sand	0.0022	4.3
NRh. + N	0.0069	5.5
NRh. - N	0.0016	1.4
(Rh. 1 + Rh. 1 sand) hydr.	0.0453	47.5
(Rh. 2 + Rh. 2 sand) hydr.	0.0181	35.6
NRh. + N hydr.	0.0221	17.6
NRh. - N hydr.	0.0102	8.8

Table 5.5.3.3.3.4

Extract	Total phenol (µg)		µg phenol/ g dw. sand		% phenol in extract
Rh. 1 root	29.4				0.32
Rh. 1 root hydr.	32.5				0.25
Rh. 1 sand	91.8)	127.3	0.097)	0.134	0.68
Rh. 1 sand hydr.	35.5)		0.037)		0.11
Rh. 2 root	13.3				0.95
Rh. 2 root hydr.	15.4				0.23
Rh. 2 sand	0.8)	31.6	0.002)	0.063	0.10
Rh. 2 sand hydr.	30.8)		0.061)		0.27
NRh. + N sand	33.1)	66.3	0.026)	0.052	0.48
NRh. + N sand hydr.	33.2)		0.026)		0.15
NRh. - N sand	5.1)	26.5	0.005)	0.024	0.32
NRh. - N sand hydr.	21.4)		0.019)		0.21

Table 5.5.3.3.3.5

Extract	Total phenol (µg)	µg phenol/g dw. sand
Rh. 1 root + sand	121.2	0.127
Rh. 1 (root + sand) hydr.	68.0	0.071
Rh. 2 root + sand	14.1	0.028
Rh. 2 (root + sand) hydr.	46.2	0.091
NRh. + N sand	33.1	0.026
NRh. + N sand hydr.	33.2	0.026
NRh. - N sand	5.1	0.005
NRh. - sand hydr.	21.4	0.019
Rh. Mean (root + sand)	67.7	0.078
Rh. Mean hydr. (root + sand)	57.1	0.081
NRh. Mean (sand)	19.1	0.016
NRh. Mean hydr. (sand)	27.3	0.023
Rh. 1 + Rh. 1 hydr.	189.2	0.198
Rh. 2 + Rh. 2 hydr.	60.3	0.119
(NRh. + N) + (NRh. + N hydr.)	66.3	0.052
(NRh. - N) + (NRh. - N hydr.)	26.5	0.024
Rh. Mean + Rh. Mean hydr.	62.4	0.079
NRh. Mean + NRh. Mean hydr.	23.2	0.020

(Note: All values for phenolic content in µg (+)-catechin equivalent.)

Table 5.5.3.3.3.6 Two-way paper chromatography of sample extracts

(The papers were treated with Gibb's Reagent, potassium ferricyanide/ ferric chloride and ultraviolet light (with and without fuming ammonia solution). Twelve distinct compounds were located.)

Rh. 1 root

10 spots.

1-4 : blue with ferricyanide; no fluorescence.

5 : " " " ; blue fluorescence/intense blue with ammonia; blue with Gibb's.

6 : blue with ferricyanide; intense yellow fluorescence; blue with Gibb's.

7 : violet fluorescence.

8,9 : pale blue fluorescence.

10 : pale blue fluorescence/intense blue with ammonia.

Rh. 1 root hydr.

4 spots.

6.

7.

11 : blue with ferricyanide; pale blue fluorescence.

Plus one other : blue with ferricyanide; violet fluorescence.

Rh. 1 sand

8 spots.

3.

5.

6.

10.

11.

Plus three others : 3 pale blue fluorescence.

Rh. 1 sand hydr.

3 spots.
6.
11.
12 : blue with ferricyanide; similar position to 7 but lacking fluorescence.

Rh. 2 root

10 spots.
2? : blue with ferricyanide.
4? : " " " ; pale blue with Gibb's.
5.
6.
Plus six others : 1 blue with ferricyanide; 1 yellow fluorescence; 2 pale yellow fluorescence; 2 pale blue fluorescence.

Rh. 2 root hydr.

3 spots.
6.
11.
12.

Rh. 2 sand

1 spot.
6.

Rh. 2 sand hydr.

4 spots.
6.
7.
11.
Plus one other : blue with ferricyanide; pale blue fluorescence.

NRh. + N sand

1 spot.
6.

NRh. + N sand hydr.

2 spots.
11.
12.

NRh. - N sand

1 spot.
6.

NRh. - N sand hydr.

3 spots.
6.
11.
12.

5.5.3.3.4 Discussion

The amount of extract (including phenolic compounds) obtained from Rhododendron pot 2 was clearly much lower than for Rhododendron pot 1. This was probably due to the plant in pot 2 being considerably smaller and less vigorous than that in pot 1. The amount of sand in pot 2 was also only approximately half that in the other pots. When the amounts of extract were calculated on the basis of a unit dry weight of sand washed, the values for Rhododendron pot 2 were increased relative to the others.

Consideration of Rhododendron pot 1 or the mean of both Rhododendron pots, shows the amount of extract and of phenolic compounds obtained, to be much greater than from the non-Rhododendron pots. This applied to extracts both before and after hydrolysis. A larger amount of total extract was obtained from samples after hydrolysis of the remnant from the first extraction. The hydrolysis releases less polar compounds (including organic compounds such as phenolics and phenolic glycosides) which are bound to more polar compounds in the aqueous phase during separation and extraction. The phenolic compounds were more readily taken with the first extraction, relatively less being obtained in most cases following hydrolysis. Most phenolic compounds, except those in some way bound to polar compounds (perhaps as salts with inorganic molecules), would be expected to be readily taken into the first ethyl acetate extraction. Any phenolics so obtained represent those more freely available and hence more potentially active biologically within the soil system.

Up to 0.95% of the total extract of Rhododendron root washing and up to 0.68% of the total extract of Rhododendron sand washing was phenolic. Some of the phenolic compounds obtained from R. ponticum root washing extract appeared to be present in the equivalent sand washing extract. The quantities were too small to facilitate identification.

Some 'background' phenolic compounds may have been present in the extract of sand washings from pots without R. ponticum. This background of both total extract and phenolic compounds increased in the pots with added nutrients. This might be a result of microbial activity within the sand.

5.5.4 DISCUSSION

The presence in the soil of 'bound' phenolic compounds with varying degrees of availability has been demonstrated by a number of workers (Coulson et al, 1960 I; Chou and Muller, 1972; Carballeira, 1980; Jalal and Read, 1983 I & II). These are often bound to the soil matrix by hydrogen bonding to organic polymers (Chou and Muller, 1972; Jalal and Read, 1983 I & II). The demonstration of 'free' phenolic compounds in soils can therefore be very difficult. Chou and Muller (1972) found that an organic content of 29% or more, prevented the release of water-soluble phenolics. The lack of 'free' phenolics in the aqueous leachate of field soil might therefore have been predicted. A considerable amount of brown organic material was obtained in these leachates. This was probably mostly fulvic acid polymers of the type found in Calluna soil by Jalal and Read (1983 I & II).

A more drastic treatment of the soil was by alkaline ethanolic extraction, as used by Jalal and Read (1983 I). This will free adsorbed organic acid anions but is too weak to cause hydrolysis of most organic compounds. Most of the extracts of Rhododendron soil and litter gave a positive response to tests for phenolic acids. The amount of phenolic acid released was between 0.16% and 0.40% of the soil extract and between 0.16% and 0.20% of the litter extract. This was 0.0068% - 0.0074% of the soil dry weight and 0.0044% - 0.0147% of the litter dry weight. These levels are similar to those found by Jalal and Read (1983 II) for Calluna soil. Paper chromatography revealed two phenolic compounds in the alkaline ethanolic extracts of both soil and litter.

The amount of material released by aqueous leaching was high in October and again in April. The soil with Rhododendron root sieved out released less material during the October peak, although differences were slight at other times. Aqueous leachates of Calluna soil produced a similar pattern of a major peak and at least one minor peak during the year (Jalal and Read, 1983 II). The seasonal distribution of these peaks differed from that found for Rhododendron soil. This might be accounted for by varying phenologies of the two dominant species at the sites and by different climates (due to differences in geographic location, topography, etc.).

Using an alkaline ethanolic extraction, the amounts of material released from litter and from soil were highest during the summer with a minor peak in February. Litter consistently released more material, especially on a dry weight basis. Similar variations were found for Calluna soil and litter by Jalal and Read (1983 II), although the seasonal trends were again somewhat different.

The pH of Rhododendron soil and litter fluctuated little over the year. That of litter was consistently slightly higher than that of soil. The pH of soil from adjacent grassland was a little above that of the Rhododendron litter. These results are very similar to those of Jalal and Read (1983 II) for Calluna soil and litter.

Extractions from soils with R. ponticum seedlings growing in pots highlighted the problems of methodology already noted. The first extraction was by aqueous leaching of a very sandy field soil from Clumber, North Nottinghamshire. No phenolic compounds were found and the major compounds, located by gas liquid chromatography, were present in various forms in all the soils, with or without R. ponticum.

Substituting a *simpler soil system (acid-washed sand) for the field soil* and repeating the extraction and analysis, again failed to detect phenolic compounds. The final extraction utilized the simplified soil system as before, but with a 5% aqueous solution of sodium bicarbonate as the extractant. This was a more drastic treatment designed to release organic acids weakly bound to organic and/or inorganic materials within the soil matrix. Extraction with 5% sodium bicarbonate solution successfully released detectable phenolic compounds. Rhododendron root and sand washings appeared to release at least some phenolic compounds in common. Again, there was considerable background material extracted even from pots of sand without R. ponticum. Quantitative measures of phenolic content indicated some in all samples. It is suggested that some organic compounds (including phenolics) were present in the acid-washed sand due to microbial activity. That nutrient addition increased the levels of background material, supports this idea.

5.6 THE COLLECTION AND ANALYSIS OF CANOPY THROUGHFALL

5.6.1 INTRODUCTION

A number of investigations have shown or implied effects of the interaction of rainfall and vegetation canopies, on associated soils and competing plant species. Polyphenols washed out of foliar shoots by rainfall have been shown to be important in podsolization. (See the previous section on soils). If such compounds are derived directly from living shoots rather than from decomposing litter, they should be detectable in canopy throughfall.

The same and similar compounds have been suggested to act as phytotoxins. McPherson and Muller (1969) obtained effective toxins in rain drip and fog drip collected from Adenostoma fasciculatum. Both field samples and artificially produced drip collections in the laboratory were used. They found the compounds responsible to be deposited on the leaf surface during normal metabolism. They seemed to accumulate during periods of atmospheric drought and were rapidly depleted by as little as 5-10mm of rain. Nine identifiable phenolic compounds (glycosides and phenolic acids) were found:-

arbutin, ferulic acid, p-hydroxybenzoic acid and syringic acid, together with unidentified compounds.

All showed toxicity to seedling growth and most inhibited germination in bioassay tests.

Work by Hook and Stubbs (1967) and De Bell (1969) (both in Muller & Chou, 1972) showed a toxic aqueous leachate of Quercus falcata var pagodaefolia to contain salicylic acid and another less abundant compound.

The leaves of many Eucalyptus species are rich in phenolics (Ashton and Willis, 1982). Del Moral and Muller (1969) found the natural foliar fog drip of Eucalyptus globulus, grown as an exotic in California, inhibited the germination and growth of understorey herbs. Other similar effects of eucalypts have been demonstrated (Al-Mousawi and Al-Naib, 1975, 1976; Al-Naib and Al-Mousawi, 1976; Del Moral, Willis and Ashton, 1978).

Aqueous foliar leachate of the ericaceous shrub Arctostaphylos glandulosa contained eight identified and two unknown phenolic compounds (Chou and Muller, 1972). The compounds were:-

arbutin, hydroquinone, gallic acid, unknown A, chlorogenic acid, protocatechuic acid, ferulic acid, unknown B, p-hydroxybenzoic acid, vanillic acid, syringic acid, o-coumaric acid and p-coumaric acid (in approximate order of decreasing abundance).

The rain drip was shown to be toxic.

Read and Jalal (1980) found both shoots and roots of Calluna vulgaris to lose simple phenolic compounds through aqueous leaching. The spectrum of compounds found was slightly different from that in the methanolic extract. This was ascribed to hydrolysis having occurred during leaching. Caffeic acid was therefore probably derived from chlorogenic acid, hydroquinone from arbutin and free orcinol from orcinol-β-D-glucoside. The phenolic compounds found were:-

Shoot aqueous leachate	:	caffeic acid, hydroquinone, orcinol-β-D-glucoside, orcinol
Root aqueous leachate	:	ferulic acid, vanillic acid.

A well-known example of allelopathy is the suppression of competing plants around the North American Black Walnut, Juglans nigra. Part of the effect is now attributed to a toxin, bound as a non-toxic glucoside, leached from leaves, stems and branches. This is hydrolysed and oxidized to release the toxin which kills off annual plants beneath the Juglans canopy. The toxin is a phenol, 5-hydroxynapthoquinone or 'juglone' (Harborne, 1982).

Dead plant material has also been implicated in allelopathic interactions involving throughfall. Phytotoxins (probably caffeic and ferulic acids) were leached from dead, standing bracken fronds and thought to cause the suppression of herbs (Gliessman and Muller, 1978).

Coulson, Davies and Lewis (1960 l.) suggested the quantity of simple polyphenol reaching the ground to be at its peak in spring and early summer. Rain showers will constantly remove polyphenols by dialysis from growing leaves or from leaf-surface exudates. This was considered to be more important than the contribution from senescent leaves during fall.

Ingham (1950) commented on the washing off of adsorbed materials from leaf surfaces and Stenlid (1958) noted the leaching of substances from plant tissues by rain. Interception of rainfall by the plant canopy may affect the chemistry of throughfall in a variety of ways. Materials within the leaves, exuded onto the leaf surface or impacted (wet or dry) from the atmosphere, may all be involved (Carlisle, Brown and White, 1966 b).

Firstly, the rainfall contains chemicals which may be deposited during a particular storm and held in the canopy. These then appear in the throughfall, not in that period of rain but after a subsequent storm.

Secondly, chemicals may be leached from the leaf into the throughfall. These then pass to the ground or may be intercepted by other leaves and possible re-deposited as water evaporates on the leaf surface.

Thirdly, there is usually a net increase in concentration of materials in throughfall compared to the original rainfall. This may be accounted for, at least in part, by evaporation. Some chemicals may be absorbed from the water by leaves, or taken up by the epiphytic microflora (Eaton, Likens and Bormann, 1973).

The more mobile bases (Na and K) are readily leached, especially from senescent or dead leaves. Less mobile bases (Ca and Mg) are also leached but to a lesser degree. The acidity of rainfall (affected by the formation of carbonic acid and especially sulphurous/sulphuric acids) may have a strong effect on this leaching.

Carlisle, Brown and White (1966 b) found the following effect of a Quercus petraea canopy on throughfall:-

Elements	Throughfall	Rainfall	Throughfall & Litter
N	8.82	9.54	49.88
P	1.31	0.43	3.50
K	28.14	2.96	38.65
Ca	17.18	7.30	41.01
Mg	9.36	4.63	13.23
Na	55.35	35.34 !	57.22

(All values in kg $ha^{-1}yr^{-1}$)

(! : high value due to oceanic influence at West Coast, U.K. site)

They found 67.80 kg $ha^{-1}yr^{-1}$ of carbohydrate in throughfall in August. This was mainly melezitose (a trisaccharide found in honeydew) plus glucose and fructose. There was a complex exchange system between the canopy and the rainfall. Inorganic nitrogen and phosphorus were removed from precipitation as it passed through the canopy. As would be expected for a deciduous plant, most organic matter was washed from the canopy when the trees were in leaf, particularly from June to August.

Carlisle, Brown and White (1967) estimated the polyphenols derived from a Q. petraea canopy:-

Canopy Throughfall	:	11.68 kg $ha^{-1}yr^{-1}$
Stemflow	:	0.65 " "
Total	:	12.53 " "

They also estimated the following:-

	Polyphenols (ppm)		Total Organic Matter		Soluble Carbohydrate	
	Max.	Min.	Max.	Min.	Max.	Min.
Canopy Throughfall :	2.16	0.42	34.0	9.2	8.5	3.1
Stemflow :	9.00	2.00	142.0	31.0	14.1	1.1
Incident Rainfall :	-	-	7.48	2.58	-	-

They found throughfall compared to rainfall, decreased inorganic and total nitrogen, but increased organic nitrogen reaching the soil. Phosphorus, potassium and calcium increased. Magnesium was about the same and sodium was decreased. Organic matter increased from 76.56 kg $ha^{-1}yr^{-1}$ in rainfall to 292.47 kg $ha^{-1}yr^{-1}$ in total throughfall, with 277.86 kg $ha^{-1}yr^{-1}$ in canopy throughfall and 14.61 kg $ha^{-1}yr^{-1}$ in stemflow. Soluble carbohydrate was increased from zero in rainfall to 68.42 kg ha yr in canopy throughfall and 1.12 kg $ha^{-1}yr^{-1}$ in stemflow (total 69.54 kg ha^{-1} yr^{-1}).

Malcolm and McCracken (1968) examined simulated canopy drip collected from Quercus falcata var pagodaefolia, Q. virginiana and Pinus palustris. An estimated 20 kg $ha^{-1}yr^{-1}$ of organic matter was added to the soil from this source. They found polyphenols, reducing sugars and organic acids. These were active components responsible for the mobilization of iron and aluminium within the soil profile. Canopy drip was shown to be an important source of mobile soil organic matter for podsolization and other pedogenic processes. L-catechin (or (-)-epicatechin) was identified in canopy drip of Q. falcata, along with numerous unidentified phenolic compounds. The amount of phenolic material was not measured. Infra red spectra suggested that organic acids in addition to the phenolics were also present, perhaps with potential for podsolization or other effects. They suggested that organic exudates onto the leaf surface would probably be associated with bases similarly exuded.

Nihlgard (1970) found the effect of the vegetation canopy on the pH of impacted rainfall to vary from species to species. Beech canopy generally increased pH and spruce decreased it. (Values given were:- rainfall: 5.2; beech: 5.7; spruce: 4.5). He suggested that acidification was by the leaching of acid organic compounds. Increasing pH was possibly effected by a shift in the carbonic acid equilibrium to the right, through uptake of carbon dioxide from solution in throughfall by leaves:-

$$HCO_3^- + H^+ \rightleftharpoons H_2O + CO_2$$

Alcock and Morton (1981) examined throughfall from under canopies of Betula pendula and Pinus sylvestris. They looked at the possible importance of sulphur deposition on leaves on throughfall pH. Sulphur concentration was generally higher in throughfall than rainfall, but the

total amount reaching the ground was about the same. They found the pH of throughfall to be reduced beneath both canopies, but no evidence of it being due to the washing off of adsorbed sulphate. The effect on pH was variable.

The same workers also found evidence suggesting that when rainfall is quite acid, H^+ ions may be absorbed by the foliage. This results in leaching by ion exchange, giving a less acid throughfall richer in other cations.

Malcolm and McCracken (1969) found the pH of simulated canopy drip to vary from species to species.

Carlisle, Brown and White (1967) showed the pH of Q. petraea throughfall varied considerably over the sampling period. Stemflow was consistently more acid (pH 3.5-3.9) than incident rainfall (pH 4.1-4.6), canopy throughfall (pH 4.1-4.6) or Pteridium aquilinum throughfall (pH 4.5-4.6). They found that pH decreased as base concentration (for Ca, Mg and Na, but not K) increased. This suggested an indirect relationship between the adsorption of bases, the leaching of organic acids and the leaching of bases. They also cite data from Pozdnyakor (1956) showing the variation of stemflow pH from species to species and with time:-

Pinus sylvestris	:	pH 3.6-3.7
Betula sp.	:	pH 4.7-4.8
Larix sp.	:	pH 3.8-5.3

The extent of interception of rainfall by a canopy will vary considerably from species to species and with topographic, meteorological and plant community factors. The size and form of the canopy, together with the intensity and duration of rainfall, are clearly very important. Seasonal variation in leaf cover will also be important.

According to Eaton, Likens and Bormann (1973), after a dry period approximately 1-3mm of rain are required to wet a forest canopy before significant penetration of water to the forest floor. Once the canopy has become relatively saturated, rainfall penetrates mainly as throughfall or stemflow.

The amount leached per unit quantity of rain has been shown to be greater during a low intensity rain than a heavy storm (Mecklenburg and Tukey, 1964; Attiwill, 1966). Maximum leaching from within the leaf as well as washing off of surface deposits, is during the early part of a storm. The leaching of some materials however, does increase during prolonged rainfall. The physical and physiological condition of the leaves and other aerial parts will clearly have an important influence on the nature of throughfall.

Tukey, Mecklenburg and Morgan (1965) suggested the leaching of cations by a process of exchange and diffusion at the leaf surface. They state that nutrients in young, growing tissues are usually quickly metabolized and therefore difficult to leach. In older tissues nutrients are in exchangeable forms and hence more easily leached. Again this is a feature which will vary both *seasonally and with different species*. The 'wetting' properties of leaves are also important.

The geographic location of a site has a significant influence on the solute and particulate content of both rainfall and atmospheric fallout. Precipitation varies in chemistry depending on the origin of the air masses involved. Oceanic influence leads to high sodium content and a relatively low Ca/Mg ratio. Continental influences lead to a relatively low sodium content and relatively high Ca/Mg ratio. Sources of atmospheric pollutants are also particularly important in affecting rainfall quality. Acidity effects of pollutants such as sulphur dioxide clearly influence rainfall pH and hence throughfall quality. Topographic and geographic factors leading to exposure to strong and prevalent winds might also influence the rainfall/canopy interaction. All these factors may affect the leaching, exchange and evapo-concentration effects and hence the chemical nature of throughfall.

In order to gauge the potential importance of canopy throughfall from R. ponticum on the interaction between the plant, competing species and associated soils, natural and artificial throughfall was collected and analysed. Some of the samples were also used in bioassay experiments on seedling germination and growth (see appendix 3).

5.6.2 LABORATORY COLLECTIONS

5.6.2.1 Laboratory shoot washings in methanol and in distilled water

5.6.2.1.1 Introduction

To help an understanding of the availability of phenolic compounds for leaching from the R. ponticum canopy, extractions less drastic than the maceration in methanol, were carried out. These were of whole shoot material in methanol and in water.

5.6.2.1.2 Method

Fresh, young R. ponticum shoots were collected from Strawberry Lee Plantation, South Yorkshire and immediately taken back to the laboratory for extraction.

Extraction was carried out by washing the shoots in a flask as shown in the diagram (Figure 5.12), in either distilled water or 70% methanol. The washings were filtered on Whatman No. 1 Paper. The methanol washing was evaporated to a solid residue on a rotary vacuum evaporator at 40°C and the aqueous washing was evaporated to a solid residue using a freeze drier.

Extract dry weight and phenolic content were measured, using the method described earlier (5.2). Samples were also examined for phenolic constituents by 2-way paper chromatography.

5.6.2.1.3 Results

5.6.2.1.3.1 Methanol washing

15g fresh weight of shoots, washed in 100ml of 70% methanol shaken for 30 minutes, gave 0.1925g dry weight of exudate. This was 7.39% phenolic (as (+)-catechin equivalent), or 14.2mg of phenolic material.

Paper chromatography revealed 15 spots excluding a basal streak. Compounds U8, U9 and U10 (flavonoid aglycones), (+)-catechin,

KEY:-

A , C : Metal foil cap
B , D : Rubber band
E : Rhododendron shoots
F : Glass flask
G : Methanol or distilled

KEY:-

A : Plastic chamber (c. 1m × 2m × 1m)
B : Metal foil cap
C : Rhododendron shoots
D : Humidifier
E : Plastic collecting tray
F : Leachate
G : Retort stand and clamp
H : Rubber band

Fig. 5·12 APPARATUS FOR SHOOT WASHING

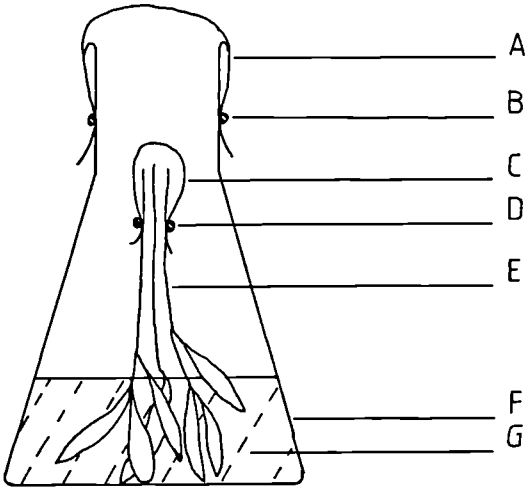
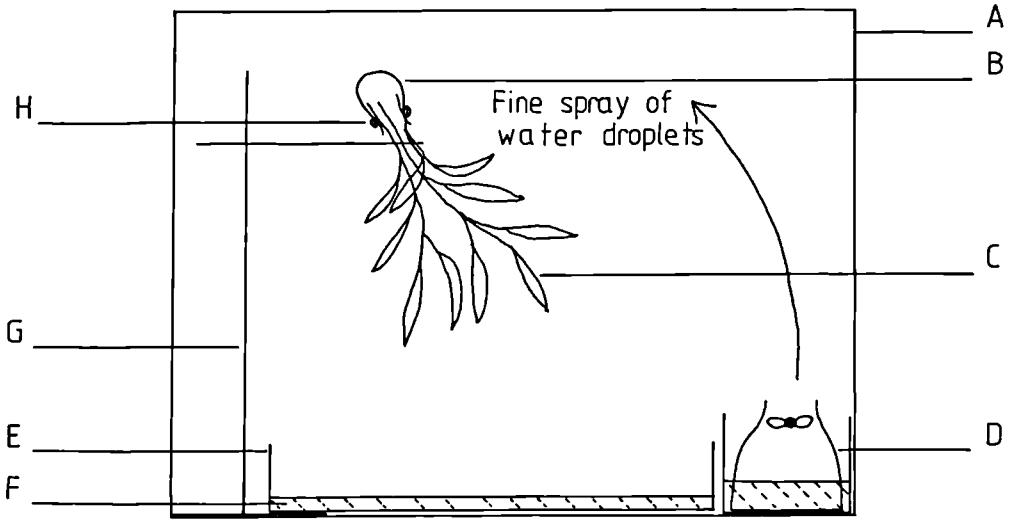


Fig. 5·13 HUMIDIFIER FOR SHOOT WASHING



(-)-catechin, procyanidins B3, B5 and possible C2 and A2 were shown. Other spots seemed to correspond with compounds U5, U12, U13 and U2.

(+)-catechin was the most intense, followed by U9 and U8.

5.6.2.1.3.2 Aqueous washing

15g fresh weight of shoots, washed in 100ml of distilled water shaken for 30 minutes, gave 0.0115g dry weight of exudate. This was 5.17% phenolic (as (+)-catechin equivalent), or 0.6mg of phenolic material.

Paper chromatography revealed 13 spots, excluding a basal streak. Compounds identified were:- U8, U9 and U10 (flavonoid aglycones), (+)-catechin and (-)-epicatechin. Some procyanidins may have been present, but the spots were too faint to be sure.

5.6.2.2 Laboratory shoot washing with distilled water in a humidifier

5.6.2.2.1 Introduction

Experiment 1 was a relatively drastic treatment, not representative of field conditions. Leaching using a fine spray, more nearly approaches the field situation.

5.6.2.2.2 Method

Fresh, mature R. ponticum shoots were collected from Strawberry Lee Plantation, South Yorkshire. They were brought back to the laboratory and immediately set up in a humidifier chamber as shown in the diagram (Figure 5.13). The spray produced fine water droplets, simulating fine rain or fog drip. This was deposited on the shoots and ran off to be collected in the plastic tray below.

The shoots were exposed to the spray for 18 hours and 1145 ml of drip were collected. After filtering on Whatman No. 1 Paper, the washings were evaporated to dryness on a rotary vacuum evaporator at 40°C.

Extract dry weight and phenolic content were measured. Samples were examined for phenolic constituents by 2-way paper chromatography.

5.6.2.2.3 Results

0.2213g dry weight of exudate and surface deposits were collected. This was 0.11% phenolic (as (+)-catechin equivalent), or 0.2mg of phenolic material.

Using 2-way paper chromatography a rather complex pattern of spots was found. Their positions suggested low molecular weight compounds with a range of polarities. Many of the compounds were clearly not phenolic. A number of phenolic compounds were shown, but they did not correspond to any of the earlier extracts.

5.6.2.2.4 Discussion

Washings of Rhododendron shoot material with methanol and with distilled water both released a range of phenolic compounds. Some were identified as compounds previously found in extracts from macerated leaves. As would be expected, considerably more material was released by the methanol extraction than by distilled water.

The results of aqueous leaching with a humidifier producing a fine spray were more complex. The material collected had some phenolic compounds, but they did not correspond to those previously identified. It may be that the method of collection allowed the compounds to degrade, complex or be hydrolysed (as suggested under similar circumstances by Read and Jalal, 1980).

5.6.3 COLLECTION AND ANALYSIS OF CANOPY THROUGHFALL

5.6.3.1 Collections made during 1979 and 1980

5.6.3.1.1 Introduction

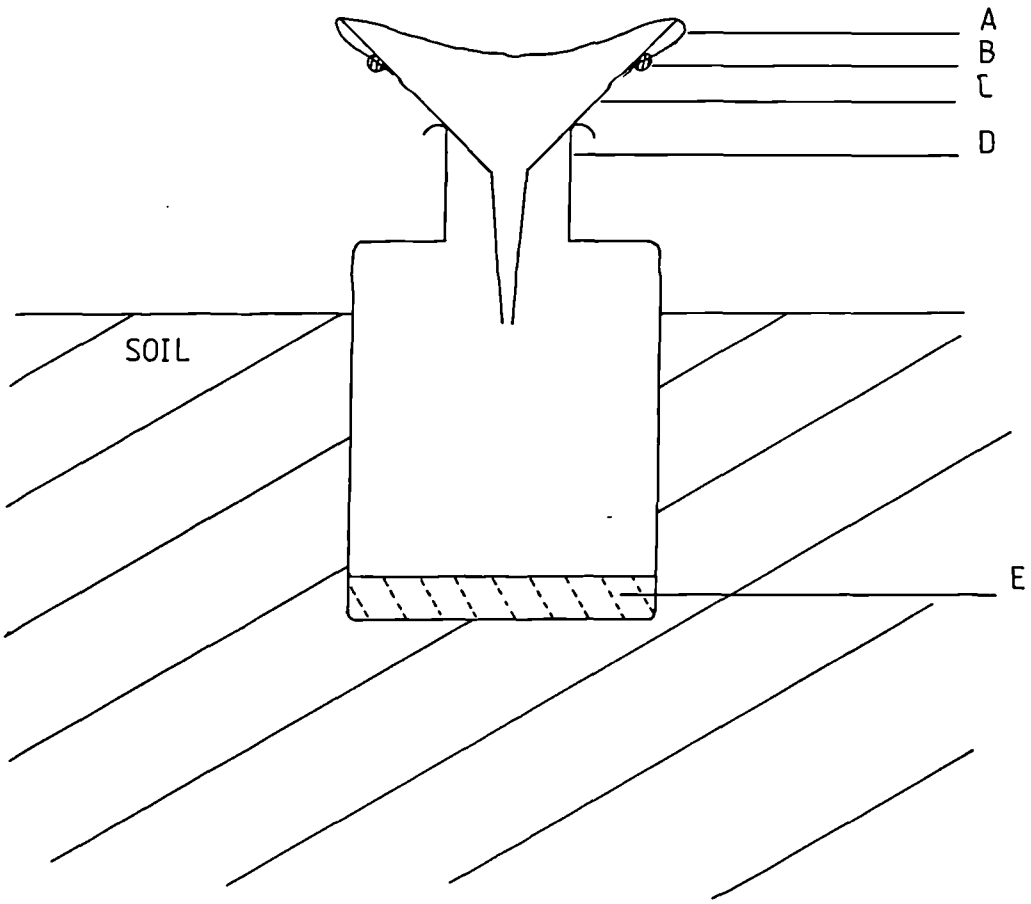
Since large quantities of 'free' phenolic compounds have been found in R. ponticum leaves, their possible occurrence in throughfall is of great interest. Samples were collected from under a R. ponticum canopy and for comparison, fron under canopies of other species. These samples were examined for total solute contents, presence of phenolic compounds and in some cases, for their effects on seedling growth in bioassays.

5.6.3.1.2 Method

Collecting vessels were set up as shown in the diagram (Figure 5.14), at field sites at Cordwell (North Derbyshire) and Strawberry Lee Plantation (South Yorkshire). The vessels were either in the open or under vegetation canopies. Throughfall was collected as soon as possible after rainfall and returned to the laboratory for analysis.

The samples were filtered on Whatman No. 1 Paper on a Buchner funnel. The filtrate was then measured for volume and (where required) evaporated to a solid residue on a rotary vacuum evaporator at 40°C. Some samples were used for bioassay tests of toxicity. Dried samples were weighed and tested for presence or absence of phenolic compounds using Gibb's Reagent. One sample of R. ponticum throughfall was examined by 2-way paper chromatography and its phenolic content measured.

Fig. 5.14 COLLECTING FUNNEL FOR THROUGHFALL OR RAINFALL



KEY :-
A: Terylene netting (1 mm mesh)
B: Silicone rubber
C: Plastic funnel (15 cm diameter)
D: Polythene bottle
E: Sample

Table 5.6.3.1.1 Sites and collections

Sample	Date	Collection period	Site	Comments
1.	22.5.79	15.5-22.5	Strawberry Lee	Large vol., heavy rain
2.	1.6.79	23.5- 1.6	" "	" " " "
3.	26.6.79	22.6-26.6	" "	Small vol, after hot, dry spell
4.	22.7.79	15.7-22.7	" "	Dry, cool period, rain on 21.7 & 22.7
5.	31.7.79	28.7-31.7	" "	Warm dry spell, rain (heavy) from 28.7-31.7
6.	27.7.80	24.7-27.7	Cordwell	Dry, warm weather with very heavy continual rain on 26.7. Samples collected 12 a.m. 27.7.

5.6.3.1.3 Results

The solute dry weight of Rhododendron and Calluna throughfall was usually higher than that of the other species (Table 5.6.3.1.3.1). The solute content per unit volume of throughfall was quite high for some samples from Molinia and Eriophorum. These were not consistent however, and were relatively low when considered in terms of the collecting area. Solute concentration was higher in the Rhododendron throughfall than in the corresponding rainfall (Figure 5.15). The difference was greater on a volume of sample basis than in terms of solute weight per unit of collecting area. With low rainfall, the amount of solute and the relative volumes of rainfall and throughfall were variable. The relative volume of Rhododendron throughfall to rainfall increased slightly with increasing rainfall (Figure 5.15).

Phenolic compounds were demonstrated in throughfall collected under M. caerulea (all samples), E. vaginatum (one of four samples), C. vulgaris (all samples) and R. ponticum (three of four samples) (Table 5.6.3.1.3.2).

Tests on rainfall were all negative for phenolic compounds. The intensity of the chromatogram spots indicated relatively large amounts in Calluna and Rhododendron throughfall.

A sample of R. ponticum throughfall was analysed for phenolic acid content. This was 2.36% phenolic acid as (+)-catechin equivalent. Compounds located by two-way paper chromatography were not identified and did not correspond to known compounds from R. ponticum tissues.

The results of bioassays using throughfall, rainfall and distilled water were variable and difficult to interpret (see Appendix 3).

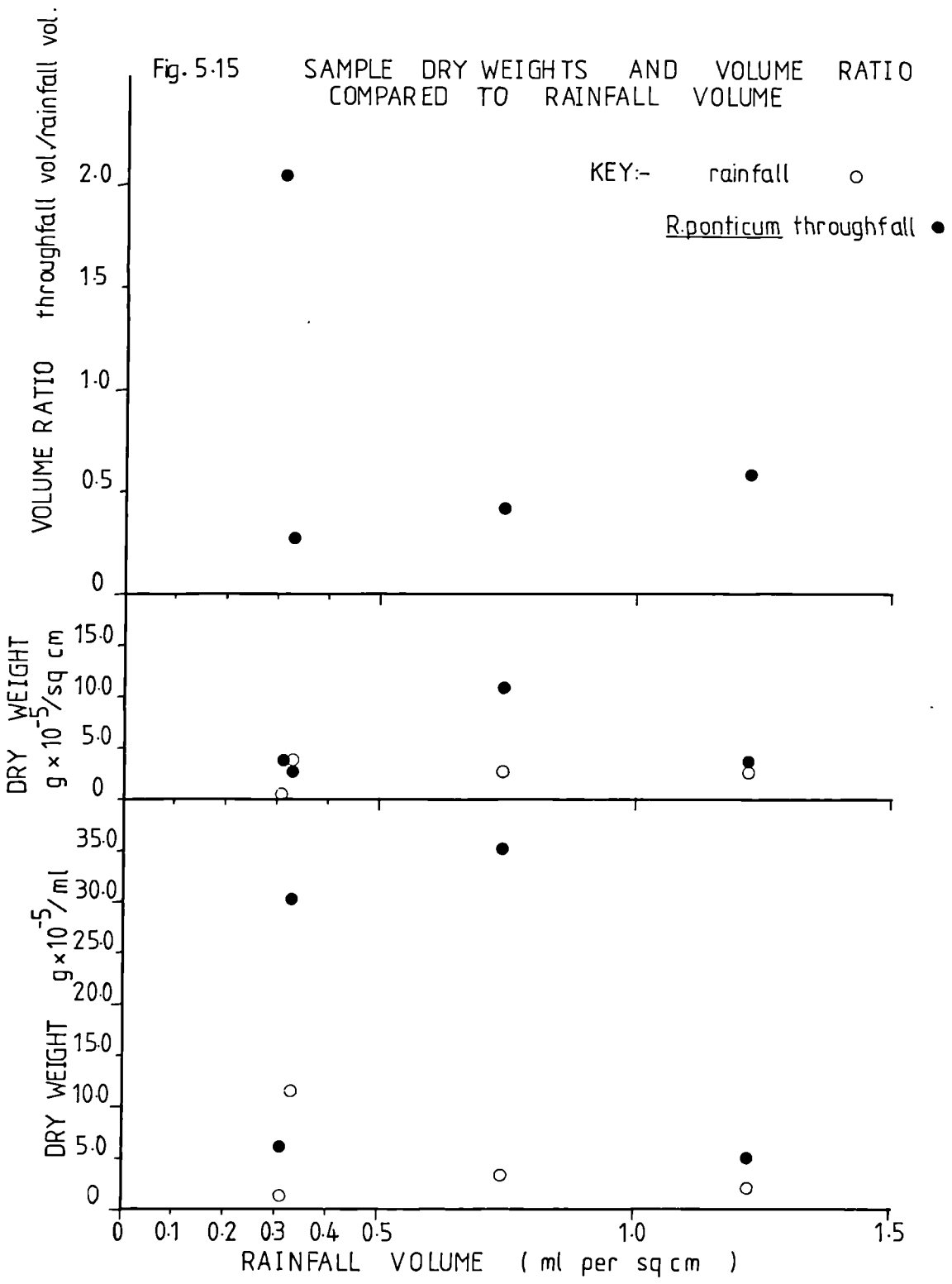


Fig. 5.15 SAMPLE DRY WEIGHTS AND VOLUME RATIO COMPARED TO RAINFALL VOLUME
KEY:- rainfall ○
R.ponticum throughfall ●
VOLUME RATIO throughfall vol/rainfall vol.
2.0
1.5
1.0
0.5
0
DRY WEIGHT g × 10⁻⁵/sq cm
15.0
10.0
5.0
0
DRY WEIGHT g×10⁻⁵/ml
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0
0 0.1 0.2 0.3 0.4 0.5 1.0 1.5
RAINFALL VOLUME (ml per sq cm)

Table 5.6.3.1.3.1 Collected throughfall samples

Sample	Canopy	No. of vessels	Vol.(ml)	Dry weights in ($gx10^{-5}$) Total dw	dw/cm	dw/ml
1	Molinia caerulea	1	86	330	1.88	3.84
	Eriophorum vaginatum	1	60	400	2.28	6.67
	R. ponticum	3 (area = 531 sq.cm)	332(0.63m/ sq.cm)	2080	3.92	6.27
	C. vulgaris	2	113	1370	3.88	12.13
	Open	2 (area = 354 sq.cm)	110(0.31m/ sq.cm)	150	0.44	1.36
2	M. caerulea	1	103	890	5.03	8.64
	E. vaginatum	1	123	450	2.56	3.66
	R. ponticum	3	375(0.71m/ sq.cm)	1890	3.56	5.04
	C. vulgaris	2	528	3270	9.23	6.19
	Open	2	431(1.22m/ sq.cm)	910	2.56	2.11
3	Very small volume collected, not recorded.					
4	M. caerulea	1	very small volume not recorded.			
	E. vaginatum	1	5	60	0.04	12.00
	R. ponticum	3	47(0.09m/ sq.cm)	1430	2.68	30.43
	C. vulgaris	2	very small volume not recorded.			
	Open	2	118(0.33m/ sq.cm)	1360	3.84	11.53

Sample	Canopy	No. of vessels	Vol.(ml)	Dry weights in ($gx10^{-5}$) Total dw	dw/cm	dw/ml
5	M. caerulea	1	36	640	3.62	17.78
	E. vaginatum	1	19	630	3.70	33.16
	R. ponticum	3	165(0.31m/sq.cm)	5790	10.90	35.09
	C. vulgaris	2	62	1750	4.94	28.23
	Open	2	262(0.74m/sq.cm)	900	2.54	3.44
6	R. ponticum	9	290	16110	10.11	55.55

(N.B.: each collecting vessel was served by a funnel with a collecting area of 177 square cm.)

Table 5.6.3.1.3.2 Samples tested for phenolics

Plant sp.	Sample	Spots on chromatogram	Whether +ve for phenolics
M. caerulea	1	1 pale blue	YES
	2	1 pale blue	YES
	5	1 pale blue	YES
E. vaginatum	1	None	NO
	2	None	NO
	4	None	NO
	5	1 pink, 1 blue	YES
C. vulgaris	1	1 pink, 2 blue	YES
	2	1 pink, 2 blue	YES
	5	1 pink, 1 blue	YES
R. ponticum	1	1 pink, 2 blue, 1 grey-green, 1 yellow-green	YES
	2	1 pink, 1 pale blue	YES
	4	1 pink, 1 grey-green	NO
	6	2 blue, 1 grey-green, 1 yellow-green	YES
Open Site, No canopy	1	None	NO
	2	None	NO
	4	None	NO
	5	None	NO

Note:- R. ponticum sample 6 gave 3.80 mg dry weight of phenolic material (as (+)-catechin equivalent), or 2.36% phenolic acid on a sample dry weight basis.

The extract from the same sample was examined by 2-way paper chromatography. No compounds were found which were obviously related to those extracted from R. ponticum leaves. The spots which seemed to be phenolic were rather low molecular weight, polar compounds.

5.6.3.1.4 Discussion

The relationship between rainfall and throughfall is obviously complex in terms of both relative volumes and solute contents. The variability leads to similarly variable patterns of inhibition and stimulation of seedling growth in bioassays (see Appendix 3). The passage of rainfall through a plant canopy frequently results in the addition of phenolic compounds in detectable quantities. Relatively large amounts of these compounds appeared in throughfall from Calluna and from Rhododendron. Throughfall is usually concentrated as it passes through the canopy.

5.6.3.2 Collections made during 1982

5.6.3.2.1 Introduction

The earlier collections of throughfall highlighted their variability in quality, in quantity and in their effects in bioassays. They also showed phenolic compounds to be regular constituents of throughfall. A more detailed analysis of a series of collections of rainfall and of Rhododendron throughfall was therefore undertaken. This was to examine phenol content, solute content (including different broad categories of solute material), rainfall and throughfall acidity and the relative volumes of rainfall and throughfall.

5.6.3.2.2 Method

The collection technique was similar to the previous section (5.6.3.1). The collecting vessel was modified by means of a poly-bag inserted to hold the throughfall (as shown in the diagram Figure 5.16). The sample was thus easier to collect with less risk of contamination by debris. After collection of a sample, the poly-bag was simply replaced with a fresh, clean one. Following collection, the samples were removed to the laboratory and treated as shown below:-

Sample collected

|

Filtered on Whatman No. 1 Paper

(Volume and pH measured)

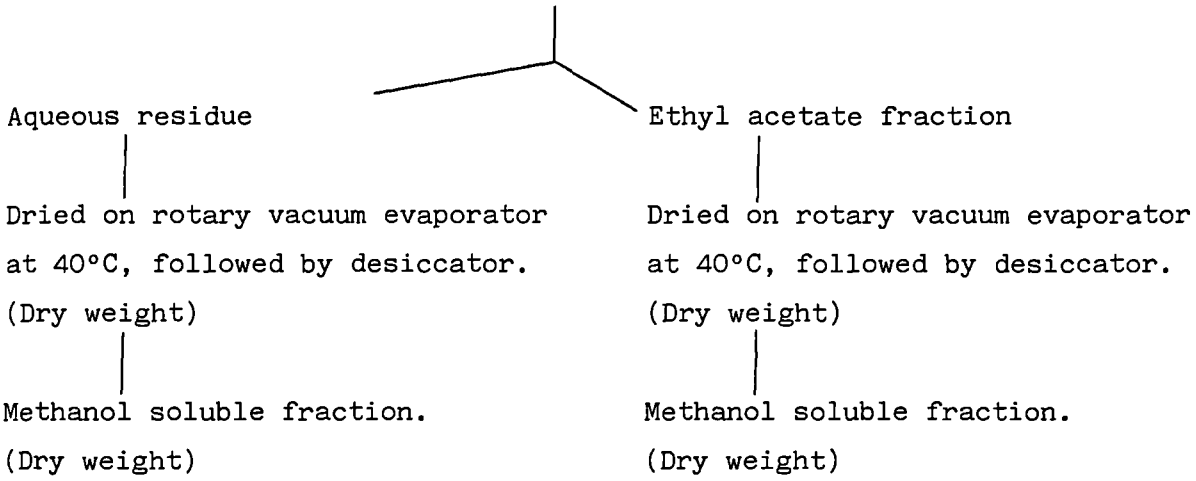
|

Concentrated on rotary vacuum evaporator at 40°C.

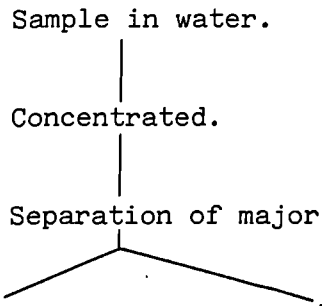
|

Extraction in ethyl acetate in separating funnel.

|


Aqueous residue
Dried on rotary vacuum evaporator
at 40°C, followed by desiccator.
(Dry weight)
Methanol soluble fraction.
(Dry weight)
Ethyl acetate fraction
Dried on rotary vacuum evaporator
at 40°C, followed by desiccator.
(Dry weight)
Methanol soluble fraction.
(Dry weight)

Rationale for the extractions



Aqueous fraction

More polar compounds, including salts of acids (inc. simple organic), sugars, amino acids, possibly some glycosides where there is a lot of sugar and not much phenol.

Dried

Take up in methanol. Most of the organic fraction.

Residue, insoluble in methanol. Mostly inorganic material with organic substances having inorganic components.

Ethyl acetate fraction

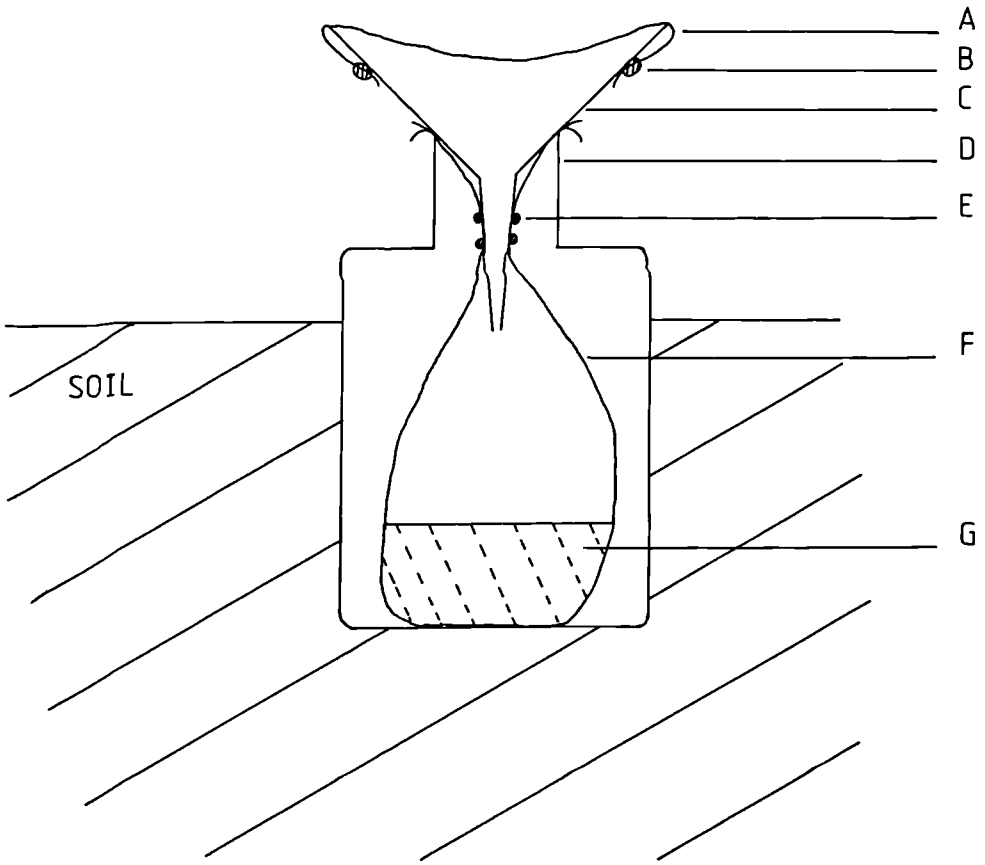
Less polar compounds, especially those with long organic components Includes all the phenolics and most phenolic glycosides.

Dried

Take up in methanol. Probably includes most of the Ethyl acetate fraction.

Dried.

Fig. 5.16 MODIFIED COLLECTING FUNNEL FOR THROUGHFALL OR RAINFALL



KEY:-

A : Terylene netting (1 mm mesh)
B : Silicone rubber
C : Plastic funnel (15 cm diameter)
D : Polythene bottle
E : Rubber band
F : Polythene bag
G : Sample

5.6.3.2.3 Results

The ratio of throughfall volume to rainfall volume rose from approximately 0.30 with low rainfall, to approximately 0.60 with increasing rain (Figure 5.19). It then fell to about 0.30 at higher levels of rainfall. This difference in volumes collected is probably a result of evaporative loss from water on the aerial plant organs. The effect is a concentration of the liquid collected. This is reflected in the relatively high solute content in throughfall as dry weight per ml (Figures 5.17 & 5.18). As dry weight per unit collecting area, the differences between throughfall and rainfall largely disappear or may even be slightly reversed (Figures 5.17 & 5.18).

The relative levels of the major extract fractions were consistent from sample to sample for both throughfall and rainfall and between the two. The bulk of the solutes dissolved in water were not extractable into ethyl acetate. Most of the ethyl acetate fraction was usually soluble in methanol. Of the aqueous residue following separation and extraction into ethyl acetate, about a third was then soluble in methanol.

The concentration of the main solute fractions declined with increasing rainfall in both throughfall and rainfall (Figures 5.19 & 5.20). Levels were consistently higher for throughfall. The decline of total solute content and the aqueous residue (after ethyl acetate extraction) was less in throughfall than in rainfall.

As dry weight of solute per unit collecting area, the amounts seem quite similar for corresponding samples of throughfall and rainfall (Figures 5.19 & 5.20). The pattern with increasing rainfall however, does seem rather different. Solute content and the levels of all major fractions increase for throughfall with increasing rainfall and then reach a plateau with little further change. Solute content in rainfall was rather more variable.

The major solute fractions as percentages of the total solute content were fairly constant for rainfall (Figure 5.20). For throughfall (Figure 5.19) the levels of both the ethyl acetate fraction and the methanol soluble aqueous residue fall with increasing rainfall. The aqueous residue correspondingly increases as a percentage of the total.

The acidity of both throughfall and rainfall was rather variable (Figure 5.20). Their changes with increasing rainfall and their relationship with each other do not seem simple. Throughfall may be more or less acidic than the corresponding rainfall. The fluctuations in rainfall pH appearing somewhat buffered in throughfall. The change in hydrogen ion concentration from rainfall to throughfall (Figure 5.21) closely reflected the rainfall pH. When pH was above approximately 5.0, there was an increase in hydrogen ion concentration. Below this approximate value, there was a decrease in hydrogen ion concentration from rainfall to throughfall.

The phenolic acid content of throughfall declined as a proportion of the total solute content with increasing rainfall (Figure 5.21). The total phenolic acid content showed no clear changes, possibly remaining relatively constant after an initial increase with increasing rainfall.

KEY :-

Total Sample : **TS**
Aqueous Residue : **AR**
Aqueous Residue Methanol Fraction : **AR MF**
Ethyl Acetate Fraction : **EAF**
Ethyl Acetate Fraction, Methanol Fraction : **EAF MF**

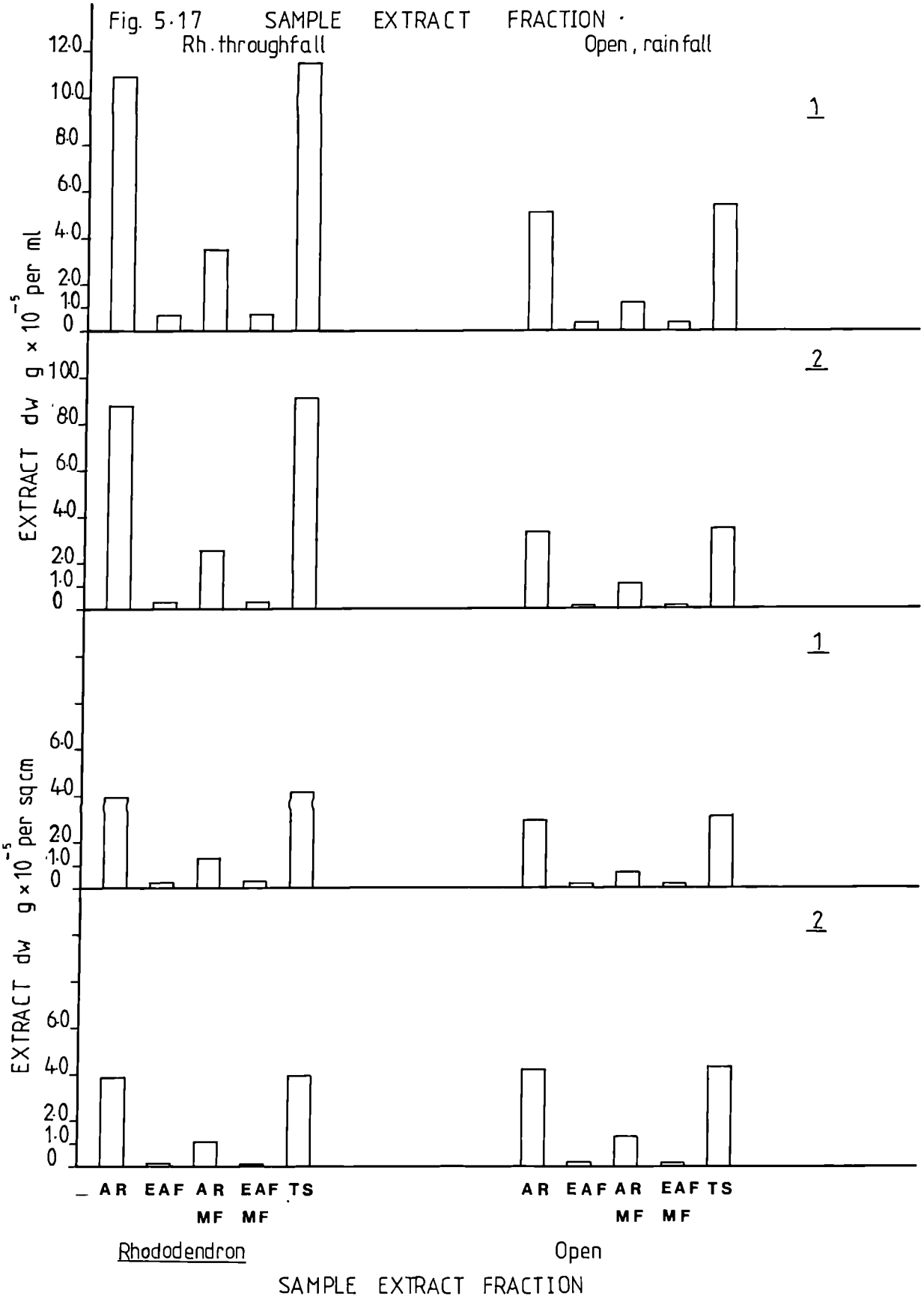


Fig. 5·17 SAMPLE EXTRACT FRACTION ·
Rh. throughfall
Open, rain fall
EXTRACT dw g x 10⁻⁵ per ml
EXTRACT dw g x 10⁻⁵ per sq cm
1
2
12·0
10·0
8·0
6·0
4·0
2·0
1·0
0
AR EAF AR EAF TS
MF MF
Rhododendron
Open
SAMPLE EXTRACT FRACTION

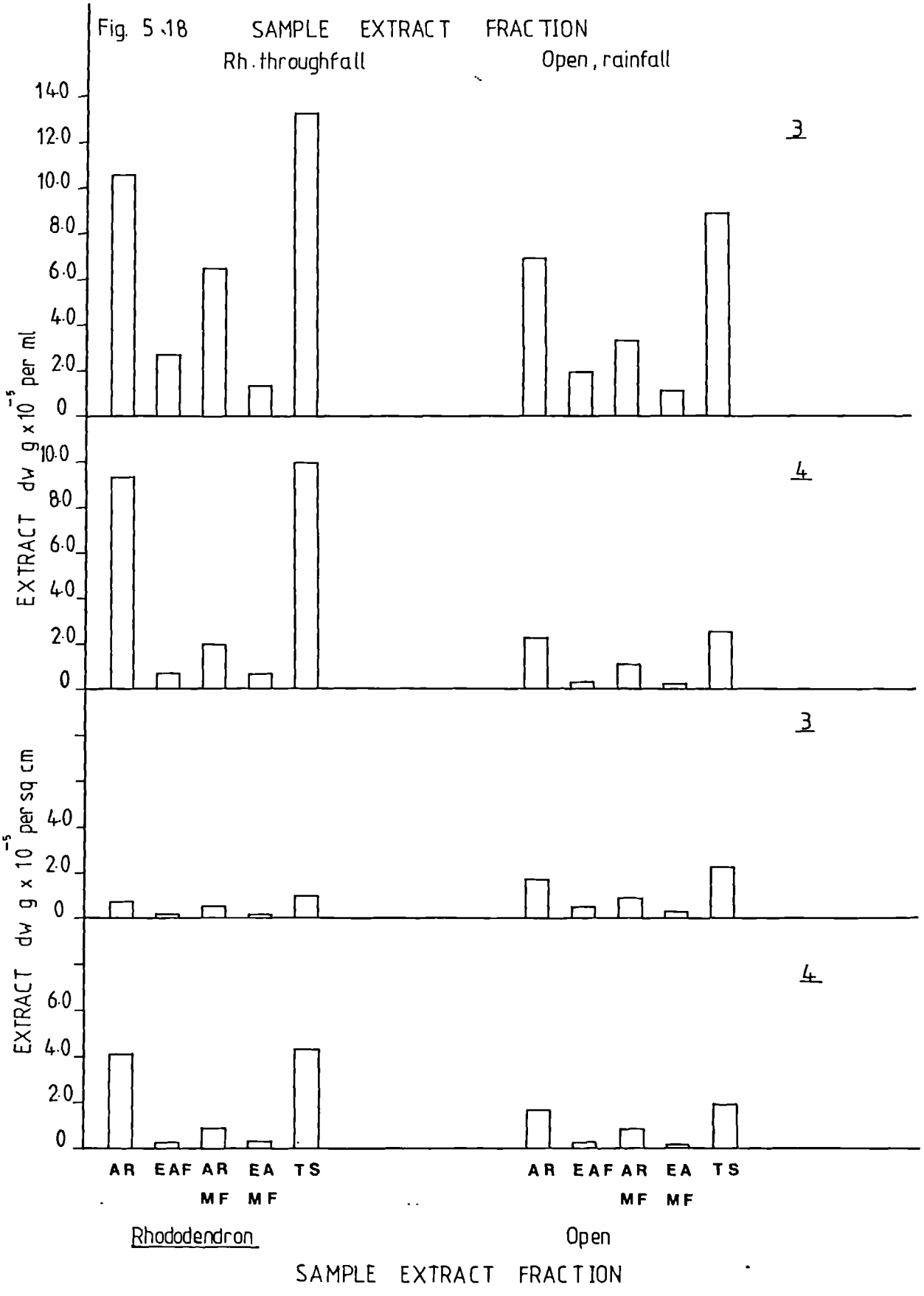


Fig. 5.18 SAMPLE EXTRACT FRACTION
Rh. throughfall
Open, rainfall
EXTRACT dw g x 10⁻⁵ per ml
EXTRACT dw g x 10⁻⁵ per sq cm
14·0
12·0
10·0
8·0
6·0
4·0
2·0
0
3
4
AR EAF AR MF EA MF TS
Rhododendron
Open
SAMPLE EXTRACT FRACTION

KEY:-

pH values : throughfall : ●
rainfall : ○

Extract Fractions :

Total Sample : ○
Aqueous Residue : ●
Aqueous Residue Methanol Fraction: ▲
Ethyl Acetate Fraction: □
Ethyl Acetate Fraction, Methanol Fraction: ■

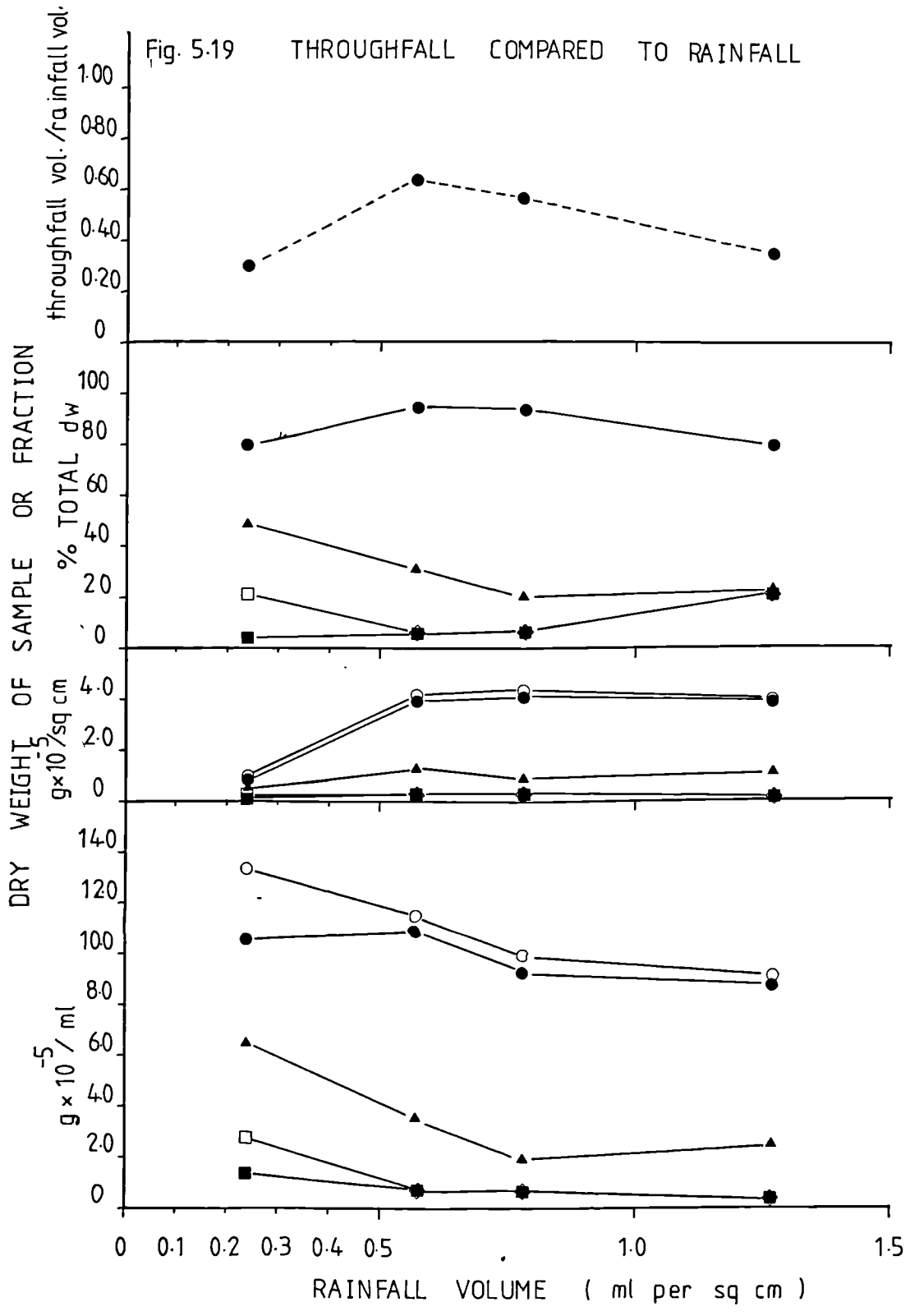


Fig. 5.19 THROUGHFALL COMPARED TO RAINFALL
throughfall vol./rainfall vol.
1.00
0.80
0.60
0.40
0.20
0
DRY WEIGHT OF SAMPLE OR FRACTION
% TOTAL dw
100
80
60
40
20
0
g×10⁻⁵/sq cm
4.0
2.0
0
g×10⁻⁵/ml
140
120
100
80
60
40
20
0
0 0.1 0.2 0.3 0.4 0.5 1.0 1.5
RAINFALL VOLUME (ml per sq cm)

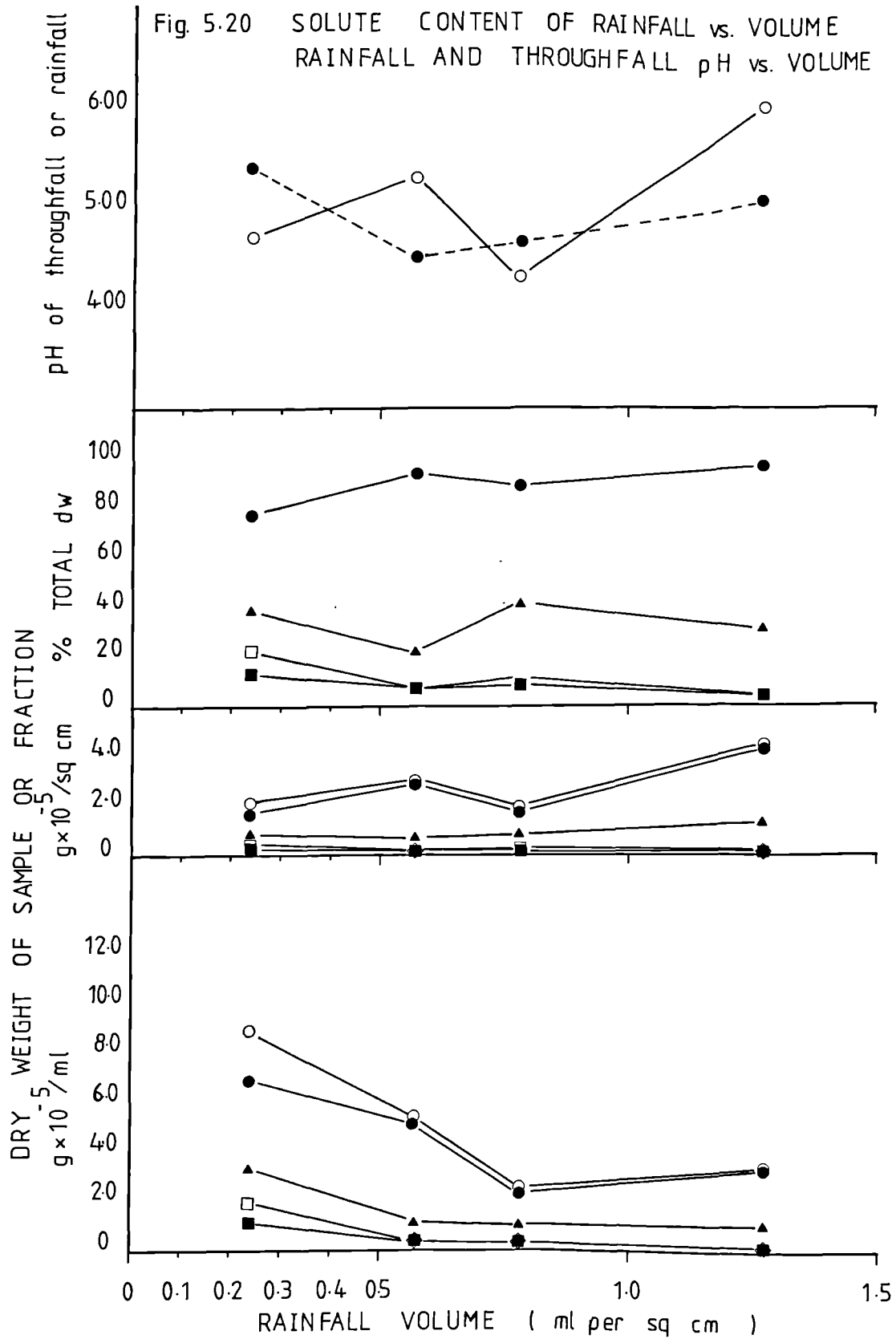
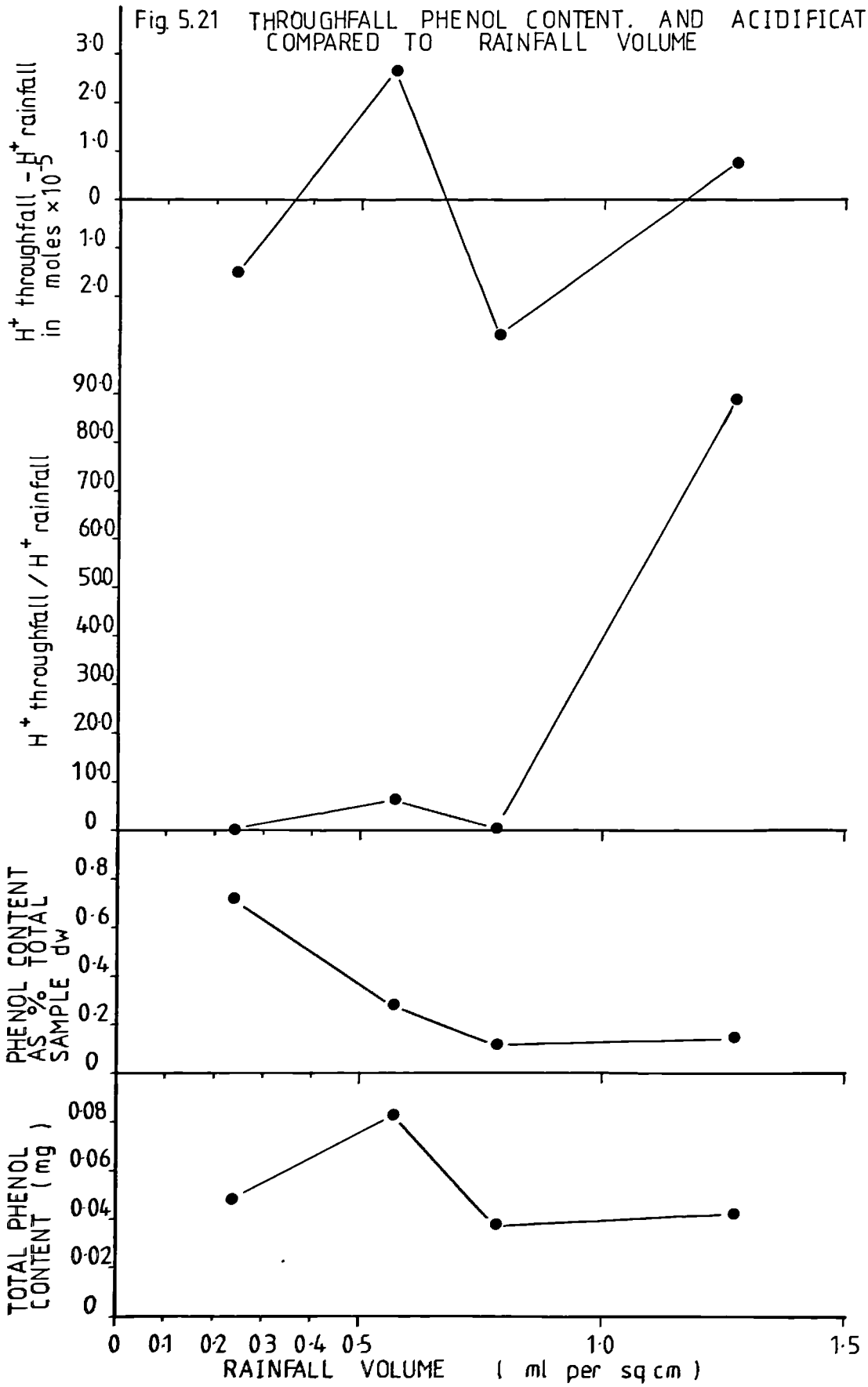


Fig. 5.20 SOLUTE CONTENT OF RAINFALL vs. VOLUME
RAINFALL AND THROUGHFALL pH vs. VOLUME
pH of throughfall or rainfall
6·00
5·00
4·00
DRY WEIGHT OF SAMPLE OR FRACTION
% TOTAL dw
100
80
60
40
20
0
g×10⁻⁵/sq cm
4·0
2·0
0
g×10⁻⁵/ml
12·0
10·0
8·0
6·0
4·0
2·0
0
0 0·1 0·2 0·3 0·4 05 1·0 1·5
RAINFALL VOLUME (ml per sq cm)

Fig. 5.21 THROUGHFALL PHENOL CONTENT. AND ACIDIFICATION COMPARED TO RAINFALL VOLUME



All samples were collected from under dense, mature R. ponticum or in adjacent open areas at Cordwell, North Derbyshire.

Table 5.6.3.2.3.1

Sample	pH	No. collecting funnels	Volume collected (ml)	Volume (ml/cm^2)	Total sample dw. mg.
R. ponticum 1.	4.50	4	252	0.36	29.0
Open 1.	5.30	3	300	0.57	16.2
R. ponticum 2.	5.10	4	312	0.44	28.2
Open 2.	6.05	3	676	1.27	23.2
R. ponticum 3.	5.40	4	51	0.07	6.8
Open 3.	4.70	3	128	0.24	11.3
R. ponticum 4.	4.65	4	312	0.44	31.0
Open 4.	4.30	3	416	0.78	10.4

Total collecting area : 708 sq. cm. (Rhododendron)
531 sq. cm. (Open)

Table 5.6.3.2.3.2

Sample 1 : 30.4.82 Light rain followed 3 weeks of warm, dry weather.

Sample 2 : 4.5.82 Quite heavy rain followed rather cool period.

Sample 3 : 7.4.82 Generally rather cold, dry spell with night frost. Sample collected after short, heavy shower.

Sample 4 : 16.6.82 Cool, dry weather for a week, followed by heavy rain in 2 falls over a 30 hour period. R. ponticum with flowers and emergent young shoots.

Table 5.6.3.2.3.3 Dry weights of different fractions

Sample:	1.		2.		3.		4.	
Fraction	Rh.	O.	Rh.	O.	Rh.	O.	Rh.	O.
1. Aqueous Residue	10.87	5.07	8.74	3.30	10.59	6.88	9.30	2.21
2. Ethyl Acetate Fraction	0.64	0.33	0.29	0.13	2.75	1.95	0.64	0.29
3. Aqueous Residue, Methanol Fraction	3.53	1.17	2.50	1.04	6.47	3.36	1.99	1.03
4. Ethyl Acetate Fraction, Methanol Fraction	0.64	0.33	0.29	0.13	1.37	1.17	0.64	0.22
5. Total sample	11.51	5.40	9.04	3.43	13.33	8.83	9.94	2.50
	(values in $g(x10^{-5})/ml$)							
1. Aqueous Residue	3.87	2.86	3.86	4.20	0.76	1.66	4.10	1.73
2. Ethyl Acetate Fraction	0.23	0.19	0.13	0.17	0.29	0.47	0.28	0.23
3. Aqueous Residue, Methanol Fraction	1.26	0.66	1.10	1.30	0.47	0.81	0.88	0.81
4. Ethyl Acetate Fraction, Methanol Fraction	0.23	0.19	0.13	0.17	0.10	0.28	0.28	0.17
5. Total sample	4.10	3.05	3.98	4.37	0.96	2.13	4.38	1.96
	(values in $g(x\ 10^{-5})/sq.cm.$)							
1. Aqueous Residue	94.5	93.8	79.6	96.1	79.4	77.9	93.6	88.5
2. Ethyl Acetate Fraction	5.5	6.2	20.4	3.9	20.6	22.1	6.4	11.5
3. Aqueous Residue, Methanol Fraction	30.7	21.6	22.7	30.2	48.5	38.1	20.0	41.4
4. Ethyl Acetate Fraction, Methanol Fraction	5.5	6.2	20.4	3.9	10.3	13.3	6.4	8.7
5. Total sample	100	100	100	100	100	100	100	100
	(fractions as % of total sample dry weight)							

Key:- Rh. = Rhododendron throughfall

O. = Rainfall collected in open.

Table 5.6.3.2.3.4

Sample	Volume ratio	Phenolic content of R. ponticum sample: Dry weight	Phenolic content of R. ponticum sample: % total sample	Change in H^+ ion concentration (Moles)
1.	0.63	0.0829 mg	0.28	$+ 2.66 \times 10^{-5}$M or x 6.31
2.	0.35	0.0429	0.15	$+ 0.79 \times 10^{-5}$M or x 89.25
3.	0.29	0.0486	0.72	$- 1.50 \times 10^{-5}$M or x 0.20
4.	0.56	0.0372	0.12	$- 2.77 \times 10^{-5}$M or x 0.44

Explanation of terms in the above table:-

Volume Ratio : Volume collected under R. ponticum / volume collected in open (both expressed on a volume per unit area basis).

Change in hydrogen ion concentration : This is expressed in two ways. Firstly, the pH of throughfall from under R. ponticum minus that of rainfall from the open, both expressed as hydrogen ion concentration. Secondly, the ratio of these two values.

5.6.3.2.4 Discussion

The relative amounts of the different solute fractions were modified by the passage of rainfall through a Rhododendron canopy. The overall amount of solute materials in terms of dry weight per unit collecting area, was not very different from rainfall to throughfall. Qualitative changes did occur however and the dilution effect of increasing rainfall seemed slightly buffered in throughfall. The amount of material per unit area was more constant in throughfall than in rainfall, with increasing levels of rainfall.

Phenolic compounds were present in all the throughfall samples. The proportion of phenols in the total solute content declined with increasing rainfall. Possibly most of the phenols were removed by a relatively small amount of rain.

The pH of rainfall strongly affected the pattern of pH change from rainfall to throughfall. It may also have an important influence on leaching and exchange processes occurring in throughfall.

5.6.4 DISCUSSION

The interactions between incident rainfall and a vegetation canopy are very complex. The age, structure, seasonal status (and hence physiological condition) and the topographic situation of the plant will strongly influence throughfall quality and quantity. Similarly, the intensity and duration of rainfall, as well as the geographic location of the precipitating air mass will affect the physical and chemical processes involved. Rainfall solute content and pH vary from storm to storm. This not only affects the overall chemical properties of both rainfall and derived throughfall, but also its interaction with the plant canopy.

This complex exchange system seems to produce very variable quality and quantity of throughfall. Rainfall and throughfall pH fluctuated quite widely as was reported by earlier workers (Carlisle, Brown and White, 1967; Alcock and Morton, 1981). It is probable that a process of exchange and diffusion of cations occurs at the leaf surface, along with leaching of organic compounds and dissolution of compounds exuded onto the leaf surface. Throughfall pH, the leaching of organic acids and of bases and the adsorption of bases, are probably interrelated in a rather complicated and variable system. The whole process is further modified by impacted solid fall-out from the atmosphere and throughfall solutes re-deposited within the canopy due to evaporation before throughfall reaches the ground. (Tukey, Mecklenburg and Morgan, 1965; Carlisle, Brown and White, 1967.)

Most of the rainfall solute content is inorganic salts with a small amount of organic matter. (Carlisle, Brown and White, 1967, estimated between 7.48 and 2.58 ppm. total organic matter in rainfall.) This is altered in various and variable ways by passage through a vegetation canopy. As well as concentration, the total and relative amounts of inorganic bases may be increased or decreased and there may be a considerable increase in the organic content. (Carlisle, Brown and White, 1967, estimated the total organic matter in throughfall collected under a Q. petraea canopy to be between 34.0 ppm. and 9.2 ppm.)

Carlisle, Brown and White (1967) also calculated the polyphenol content of Q. petraea throughfall to range between 2.16 ppm. and 0.42 ppm. This compares with estimates from the present work of 13.10 ppm. to 0.12 ppm. in R. ponticum throughfall.

Following extraction of the ethyl acetate soluble fraction, approximately 30% of the aqueous residue was then soluble in methanol. This would probably include sugars, amino acids and simple organic acids. Malcolm and McCracken (1968) suggested that in addition to phenolic acids, aliphatic acids were present in simulated canopy throughfall for Q. falcata.

Both increases and decreases in throughfall pH compared to rainfall, have been noted by a number of workers. The changes in pH vary from species to species, from one sampling period to another and also between canopy throughfall and stemflow (Carlisle, Brown and White, 1967; Malcolm and McCracken, 1968; Nihlgard, 1970). Alcock and Morton (1981) suggested that when rainfall is quite acid, hydrogen ions may be absorbed by the foliage to give leaching by ion exchange. The result is a throughfall which is less acid and is richer in other cations. This could help explain the increase in pH of R. ponticum throughfall observed when rainfall was quite acid.

The rather complicated situation which emerges regarding both quality and quantity of canopy throughfall makes an assessment of its possible ecological significance very difficult. The presence of phenolic compounds and possibly of other organic acids as reported by earlier workers for other plants, might have implications for allelopathic interactions. Some of the major phenolic compounds found in 'free' form in R. ponticum tissues have been reported in throughfall from vegetation (e.g. (-)-epicatechin reported in simulated canopy throughfall from Q. falcata by Malcolm and McCracken, 1968). Phenolic compounds from canopy throughfall have been implicated in allelopathic or pedogenic interactions by a number of workers (Coulson et al, 1960 I & II; Davies et al, 1964 III & IV; McPherson and Muller, 1968, Chou and Muller, 1972) and might produce similar effects associated with R. ponticum.

The rather variable results of the bioassays could be explained by the variability in quality and quantity of both throughfall and rainfall collected. Without far more detailed investigations of these phenomena it is impossible to evaluate the ecological importance of Rhododendron canopy throughfall.

CHAPTER 6

GENERAL DISCUSSION

As a widespread and increasingly invasive alien in the British Isles, R. ponticum is a plant of considerable economic as well as ecological interest. Under suitable conditions its invasive behaviour causes severe problems in both commercial forests and semi-natural woodlands. Various features of its ecology and physiology have been suggested as reasons for its invasive and dominating nature. These include low herbivore pressure due to its alien status (Elton, 1958); high Relative Growth Rate compared to competitors at low light levels, and a capacity for winter photosynthesis (Cross, 1973, 1975); high fecundity with easily dispersed seeds (Brown, 1953 a and b) and a lack of serious diseases or parasites (Cross, 1975). It seems likely that a combination of these factors, together with other features not fully considered by earlier workers, is responsible for the invasive behaviour and ecological success of this species.

An important conclusion of the surveys undertaken (Chapter 2), was that the invasive behaviour of R. ponticum in woodlands was closely related to habitat disturbance by forestry management, grazing animals or other human influences. Cross (1981) has come to very similar conclusions following extensive studies in the Killarney woodlands of south-west Ireland:-

'Given time, it seems highly probable that R. ponticum would certainly displace Ilex aquifolium, but it is clear that the woods are far more disturbed than Tansley realized, and that Rhododendron therefore behaves as a characteristic alien species. In an ungrazed or lightly grazed wood unaltered by man it would probably be largely restricted to naturally unstable areas, for example around cliffs, on poorly consolidated soil on steep valley sides, and on soil exposed by wind-thrown trees. It would therefore be expected to occur as clumps or isolated bushes, while the natural understorey shrub, Ilex aquifolium would persist in areas with a well-developed ground flora, where soil conditions exclude Rhododendron ponticum or where litter accumulates. The success of R. ponticum in the Killarney Woods and elsewhere must therefore be considered largely a reflection of habitat disturbance.'

Recent work by Read et al has demonstrated the importance of mycorrhizas in the ecological success of ericaceous plants. On free-draining, low-nutrient soils, ericaceous mycorrhizas result in increased growth of the host plant, probably through enhanced uptake of nitrogen and phosphorus. The potential importance of such mycorrhizas was largely ignored by earlier research into the ecology of R. ponticum.

A priority for study was to establish the role of mycorrhizas in the ecology of R. ponticum. Firstly, to see whether the benefits of mycorrhizal infection shown for other ericaceous species also applied to R. ponticum. Secondly, it was of interest to know whether the interference phenomenon was itself dependent on mycorrhizal infection.

Experimental work comparing the growth of mycorrhizal and non-mycorrhizal R. ponticum showed the plant to benefit significantly in terms of yield at low levels of soil nutrients. With increased nutrient addition the benefits of infection declined, though in the early stages of growth, the mycorrhizal plants did establish more rapidly than the non-mycorrhizal ones. Infection resulted in a lower Root/Shoot Ratio (R/S). The mycorrhizal plant was therefore able to commit more resources to photosynthetically productive aerial organs and less into roots. This, together with the more rapid establishment of an effective root system (involving relatively less investment of energy and materials than the non-mycorrhizal equivalent) gave an increased Relative Growth Rate (R'). With very small seeds and a correspondingly limited supply of stored nutrients for the germinating seedlings, the benefits of rapid establishment of an effective root system, are likely to be particularly important to R. ponticum. Enhanced uptake of nitrogen and phosphorus, and access to forms unavailable to the non-mycorrhizal plant, are benefits of infection also expected since they have been demonstrated for other ericaceous species by Read et al.

Observations confirmed that infection of roots could be expected after around six weeks from germination, with physiological effects and benefits being experienced increasingly during the following six weeks.

The source of fungal inoculum for seedlings of invasive R. ponticum in the field is uncertain when the existing vegetation is not predominantly ericaceous. A resistent propagule in the soil and/or ascospores are possible explanations.

A considerable amount of work in recent years has suggested the involvement of ericaceous plants in allelopathic interactions with their competitors.

Cross (1973) noted the effect of vigorous R. ponticum on Ilex aquifolium in the Irish woodlands, and upon adjacent vegetation at Winterton Dunes, Norfolk. Some form of toxicity was suggested as one of the possible explanations for these effects. The latter interaction manifested itself as a zone of interference around the bush, in which the growth of competing plants was suppressed and the cover by higher plants was very incomplete. Similar interference zones or 'bare-zones' around Calluna bushes were studied in detail by Roff (1964). He concluded that the suppressed growth was due to a toxic effect associated with soil long-occupied by Calluna roots.

Investigation into the interference phenomenon revealed intense suppression of test seedling growth under experimental conditions. This occurred with both soil collected from under R. ponticum in the field or from R. ponticum bare-zones, as well as with artificially created bare-zones in pots or dishes.

Germination of test species seeds was not inhibited, but in the presence of live R. ponticum or soil associated with R. ponticum (with or without dead roots) the development of roots was very stunted. Successful germination and survival of seedlings was much reduced by R. ponticum (e.g. 4.4. Experiment 3, 90% survival with NRh. reduced to 25% with Rh.).

Interference was shown to involve competition for nutrients and also some form of inhibition that was not alleviated by nutrient addition (4.4 Experiment 3). In some cases the addition of nutrients to non-Rhododendron controls and to Rhododendron treatments, actually increased the statistical significance of the interference (4.4 Experiment 4). The suppression of test seedling growth was induced by the presence of live R.

ponticum roots, by dead R. ponticum roots and to a lesser extent by soil from which R. ponticum roots had been removed. In some cases, the inhibition produced by soil with dead R. ponticum roots was as strong as that with live R. ponticum. Removal of the roots from the soil did decrease the effect, sometimes with and sometimes without the addition of nutrients. The interference effect was not dependent upon mycorrhizal infection of the roots.

R. ponticum tissues were found to be very rich in 'free' phenolic compounds when compared to a range of other plant species. Considerable qualitative and quantitative differences between tissue types and ages were shown. The compounds found included those such as the catechins, associated with tanning ability, anti-herbivore functions and defence against fungal attack (Feeny, 1968, 1969; Harborne, 1979; Swain, 1979). The concentrations present (c. 20-55% dw. of interfascicular leaf tissue) suggest that they should be biologically active in these respects. The very high levels in new leaves (57% dw.) and new stems (22% dw.) could be especially important in protecting these organs from herbivore attack. Being softer and more delicate, with less fibrous material and hence a presumably higher nutritive value than older tissues, they are particularly vulnerable to attack. Damage to young, developing organs would be a serious loss to the plant.

High levels (c. 30% dw.) of 'free' phenols were maintained throughout the year in mature leaves, perhaps associated with the evergreen nature of R. ponticum. Deciduous species such as Quercus which have similar compounds performing an anti-herbivore function, show strong seasonal changes in concentrations (Feeny, 1968, 1969). In the light of other work (Feeny, 1968, 1969; Harborne, 1979, 1982; Swain, 1979), these results suggest that the relatively small amount of damage to the plant by invertebrate herbivores, pathogens or parasites (Elton, 1958; Cross, 1973, 1975), may be due to the biochemistry of R. ponticum rather than its alien status in Britain. If this is the case, then the high content of 'free' phenolic compounds is a major factor contributing to the success of R. ponticum. There is no evidence that more herbivore or disease damage occurs in its natural habitats. If the phenolic compounds do have an anti-herbivore or

anti-pathogen function, then there would be no reason for any major difference in such damage to the plant occurring as a native or as an alien.

Another possible function of these 'free' phenolic compounds is that of allelopathic agents. For an allelopathic interaction, a number of possible sources of toxins may be proposed. In the case of R. ponticum these are:-

1. Toxins leached from aerial organs into the canopy throughfall.

2. Toxins leached or released by decomposition from fallen litter.

3. Toxins exuded directly from the roots.

Canopy throughfall was shown to contain 'free' phenolic compounds derived from the aerial organs of R. ponticum. However, the effects of throughfall on test seedlings in bioassays were very variable. It is possible that throughfall could be toxic in some situations, but it is unlikely to be a consistently important feature of the ecology of R. ponticum. Bare-zones around bushes and within bushes have been found with no canopy overhead and hence no throughfall. Similarly, the interference effect has been successfully demonstrated under laboratory conditions in the absence of either canopy throughfall or shading.

The growth of test seedlings on R. ponticum litter was variable. Coarse, relatively undecomposed litter without R. ponticum roots had a stimulatory effect on test seedlings (4.3 Experiments 1 and 2). Fine, well-decomposed litter from lower down the soil profile and well-permeated by fine hair roots of R. ponticum was toxic. In the field it may be that the coarser, upper litter does inhibit colonization of R. ponticum clumps by physical effects (through desiccation) rather than by a chemical influence. (Field experiments at Strawberry Lee Plantation indicated such an effect.) Again, interference was successfully produced under controlled laboratory conditions without litter. Bare-zones at Winterton are also influenced only by live R. ponticum roots and not by litter, canopy throughfall or shading.

The interference phenomenon is therefore associated with the presence of the roots of R. ponticum in soil. This supports the earlier findings of Roff (1964). This influence of roots may be three-fold. Firstly, competition for water. Secondly, competition for nutrients, and thirdly, the toxicity factor within the overall interference effect. To account for this toxic effect, a causative agent needs to be found and demonstrated, in both the plant and the soil. R. ponticum hair roots contained between c.10% and c.30% dw. 'free' phenolic compounds. These compounds could be released into the soil and they or their derivatives be responsible directly for the inhibition observed.

To help assess the possible importance of this, field soils from under R. ponticum and artificial soils with R. ponticum grown in pots, were extracted and the extracts examined for phenolic materials. There are considerable problems in extracting 'free' phenols from soils, especially with increasing organic matter content (Muller and Chou, 1972). A simplified soil system (acid-washed sand) washed with 5% sodium bicarbonate solution did release phenolic compounds. These were obtained from both the washings of sand and of the whole R. ponticum root system (extracted from the pot and separated from the sand). The quantities obtained were very small and it is difficult to assess their ecological significance. Clearly though, this is a potential source of an allelopathic agent. According to Rovira (1969), the exudation of compounds with specific biological activity (stimulatory or inhibitory) is often at such low concentrations that they are barely detectable by chemical or chromatographic techniques. Exudation may also be affected in several ways by micro-organisms. This may be through an effect upon the permeability of root cells, an effect on root cell metabolism or the absorption of certain compounds in root exudates by micro-organisms and the excretion of other compounds (Rovira, 1969). The mycorrhizal status of the roots extracted may have had considerable bearing on the compounds released. Infection could increase the amount of exudation (perhaps by increased 'leakiness' of the roots) or decrease it due to absorption or breakdown of exudate by the fungus. The interaction between mycorrhizal roots and the rhizosphere micro-organisms may also be very important. (As already noted however, the interference effect is independent of mycorrhizal infection.)

Aqueous extractions from field soil and from pot soil failed to release detectable quantities of phenolic compounds. Alkaline ethanolic extraction did release phenolic material from both R. ponticum soil and litter. (This was equivalent to c. 0.0070% dw. for soil and 0.0044%-0.0147% dw. for litter.) These compounds could be bound (perhaps by weak hydrogen bonding) to large organic polymers within the soil complex. Such phenolic compounds might still be biologically active, even though they are too strongly held to be released by simple aqueous leaching.

The toxic effects may result from a mixture of organic compounds, perhaps including both simple aromatic and aliphatic acids. Other workers investigating allelopathy and phenolic compounds have suggested that the phenols were not the only agents, or even necessarily the main ones (Muller and Chou, 1972). Rovira (1969), suggested the balance of commonly exuded compounds and/or the presence of compounds peculiar to a particular plant species will be important in ecological interactions. Jalal and Read (1983 I and II) extracted simple aliphatic acids from Calluna soil. These compounds are potentially highly toxic. Their origin was suggested to be litter, but if similar compounds are implicated in the Rhododendron interference a direct source from the roots must be proposed. This present work demonstrated phenolic compounds released in this way and it seems likely that aliphatic acids could be similarly exuded. Rovira (1969) notes a wide range of compounds released from intact roots, including sugars, amino acids, peptides, enzymes, vitamins, organic acids, nucleotides, fungal stimulators, inhibitors and attractants.

The dense mass of fine adventitious roots and hair roots of R. ponticum have abundant potential sites for the release of compounds into the soil. The presence of a dense root mass in a shallow band from the soil surface to around 15-30 cm depth, provides an easily visualized means of dispensation to the target plants. Exudation from intact roots functioning normally was estimated to be around 0.1% - 0.4% of the carbon photosynthesized by the plant (Rovira, 1969). The zone of root around the tip and older regions, especially where adventitious roots emerge were considered to be major sources of exudates. Compounds may also be released from the root-hair zone and from root-hairs themselves (Rovira,

1969). The interference effect and the detection of released phenolic compounds in soil were closely linked to the presence of a dense mass of fine Rhododendron hair roots.

A major complication with experimentation into the interference exhibited by R. ponticum or C. vulgaris, is the lowering of soil pH associated with these species. Separation of direct toxicity from acidification effects may be difficult. Comparison of soil from under R. ponticum and the equivalent soil horizon from adjacent grassland usually shows a fall in pH of around 0.20 - 0.30 pH units (Table 4.3.3.4). In pot and dish experiments when R. ponticum was grown in soil, similar acidification was observed (0.30 -0.40 pH units, Tables 4.4.3.4, 4.4.3.7 and 4.4.3.9). The range of soil pH involved was from c. 3.0 to c. 4.5. This is the range over which toxic effects of aluminium and manganese (Rorison, 1960), organic acids (Lee, 1977) and direct toxic effects of acidity (Arnon & Johnson, 1942) might be expected. *In many acid soils the presence of* aluminium is associated with the stunted growth of susceptible species (Rorison, 1960). The roots of these plants fail to elongate and produce only stunted laterals. Leaves of such plants turn red, indicating phosphorus deficiency. These symptoms are similar to those observed in some cases of interference. The toxicity caused by aluminium is removed above pH 5.0, by the metal being precipitated. Wright (1943) found that at low pH's aluminium caused the precipitation of phosphorus in roots and hence created an actual deficiency in the various meristematic regions of the plant. According to Clarkson (1966, in Russell, 1973), aluminium also has an inhibitory effect on sugar phosphorylation in the living cell of a susceptible plant. Arnon and Johnson (1942) examined pH associated soil toxicity within a range from 3.0 - 9.0. They found that direct toxic effects of pH were important at pH 3.0 and pH 9.0. From pH 4.0 - 8.0 the effects were largely indirect via other soil factors. They did not rule out the possibility of effects on nutrient uptake being directly due to primary injury to the absorbing root cells. At pH 3.0 their test seedlings suffered complete failure of root development.

At first sight the acidification could be an explanation for the poor performance of test seedlings on R. ponticum soil. The effects on root development are very similar to those described for pH-linked aluminium toxicity by Rorison (1960) and Wright (1943). However, the interference

effect has been produced artificially at a range of pH's. These values overlap with toxic effects in some experiments occurring at pH's which in other experiments produced no such effects (Tables 4.4.3.4, 4.4.3.7, 4.4.3.9 and 4.4.3.10). Indeed Rh.+R and Rh.-R soils (4.4 Experiment 5), both had pH's of 3.65. Rh.+R however, showed a significant interference effect compared to Rh.-R, and this was not removed by nutrient addition.

The experimental evidence indicates that the prime interference effect with the species tested, did not rely on acidification. When R. ponticum causes soil pH to fall into the range 3.00 - 3.40, it seems likely that an interference effect would be increased by direct pH toxicity. The test plants used in bioassays were acid-tolerant species or ecotypes and therefore presumably able to tolerate adverse effects of aluminium and manganese in the pH range 3.50 - 4.50. Seedlings used by Rorison (1960) which proved susceptible to such toxicity were calcicoles.

Another possible influence of soil pH would be by altering the availability of the organic acids suspected of direct phytotoxic activity.

As already noted, R. ponticum is clearly able to lower soil pH in the field and under laboratory conditions. This could happen in a variety of ways:-

1. The uptake of bases (as found for Calluna and Ulex by Grubb and Suter, 1971). This would probably occur very effectively by means of the dense mat of mycorrhizal hair roots of R. ponticum.

2. The release of acidic materials from litter. This would be expected with the high phenolic content of R. ponticum leaves demonstrated in the assays (Chapter 5.2).

3. The release of acidic materials into the canopy throughfall, as found in samples collected from the field and from laboratory collections (Chapter 5.6).

4. Exudation of acidic compounds from the roots as was found to occur in controlled pot experiments and was indicated as a possible source by analysis of field soils (Chapter 5.5).

The effect of acidic compounds released will be determined by their strength and quantity. The soil involved may be very acid (c. 3.5-4.0) even before acidification by R. ponticum. These compounds might act indirectly by encouraging the leaching of cations from the upper soil horizons and movement down the profile. Mechanisms of this type have already been clearly established in relation to podsolization. Grubb and Suter (1971) found c. 0.5 m equiv./100 g soil of low molecular weight organic acids in soil from under Ulex and Calluna. They suggested that these were unlikely to contribute directly to the soil acidification (from pH c. 5-6 to pH c.3.5 - 4.5 under Calluna). Organic acids (aliphatic and aromatic) were suggested to be involved in complexing and mobilizing iron and aluminium, together with calcium, magnesium and potassium. This would cause their removal by leaching from the upper soil horizons, a process likely to occur with R. ponticum also. The organic acids might inhibit bacterial decomposition of litter. This would have implications for nutrient cycling, podsolization and acidification. These effects probably occur in the field situation, but were eliminated from the artificial interference zones studied in the present work. It is of interest that like the bare-zones at Winterton and in the Brecklands, the acidification phenomenon studied by Grubb et al followed the loss of rabbits through myxomatosis in 1954. Decreased grazing resulted in increased growth of Calluna and Ulex, and the associated decrease in soil pH.

Acidification of field soil by R. ponticum may therefore occur by the uptake and immobilization of bases in plant tissues and derived litter. The litter is broken down only slowly and builds up into a thick layer at the top of the soil profile. Organic acids may be leached into throughfall from aerial organs as demonstrated, released from litter or exuded by roots (as shown in Chapter 5). Leached bases will be easily removed from the rooting zone (which for R. ponticum is very shallow). This again will limit nutrient recycling and encourage acidification.

In the laboratory experiments, acidification of soil by R. ponticum involved neither litter nor throughfall. It was therefore associated solely with the presence of R. ponticum roots. Probably a combination of very effective uptake of bases by the dense root mass, together with

organic acids exuded from the roots was responsible. In the pot experiments leaching might also have some effect, but this was not possible in the dish experiments.

The processes producing acidification and those yielding the toxicity associated with interference may occur together and are probably complementary, but the toxic effects are not entirely dependent on the acidification of the soil.

The ecological success of R. ponticum, both as an invasive alien in suitable habitats in the British Isles, and also in its natural habitats is attained by a mixture of key factors. One of these is the prolific production of very small, fertile seeds which allows dispersal over considerable distances. Research by Cross (1973, 1975 and 1981) highlighted the vulnerability of the seedling phase and the restricted occurrence of suitable regeneration sites. The high fecundity helps ensure that available regeneration sites are effectively exploited.

Once dispersed to a site suitable for regeneration, mycorrhizal infection together with biochemical protection from herbivory and pathogens, must be important in favouring successful growth and survival. A high phenolic content of the leaves probably decreases invertebrate herbivory and/or fungal attack. 'Andromedo toxin' perhaps aided by phenolic compounds, discourages feeding by vertebrates.

The benefits of mycorrhizal infection are dependent upon the presence of fungal inoculum in the soil or its introduction by means of effective dispersal mechanisms into soil in the vicinity of the seedling. Assuming that inoculum is available, then increased Relative Growth Rate, decreased Root/Shoot Ratio and enhanced uptake of nitrogen and phosphorus should occur within 2-3 months after germination. Maximum benefit from infection will occur on free-draining, low-nutrient soils.

During the seedling phase, the ability of R. ponticum to survive making virtually no growth when conditions are unsuitable, may be another factor in its success. This feature has also been noted by Cross (1981) and may again be related partly to the high phenolic content of the leaves, perhaps protecting such seedlings from herbivore or fungal attack.

When established in a favourable habitat R. ponticum forms a dense, blanketing shrub-layer. This effectively eliminates all competing vegetation, with the exception of emergent trees and shrubs, and occasionally individuals of Pteridium aquilinum which may survive within small clumps of R. ponticum or in peripheral areas. The ability to take-over and dominate areas of vegetation must be greatly helped by enhanced growth through mycorrhizal infection and the benefits of low herbivory as already discussed. Comparatively higher Relative Growth Rate than its competitors at low light intensities, together with the capacity to photosynthesise in winter may also be important in this respect (Cross, 1973, 1975).

Having formed such dense monospecific blankets, R. ponticum is well placed to exert strong root competition (for water and nutrients) and heavy shading, upon its competitors. Interference effects consisting of a mixture of competition and in some situations allelopathic influences, may explain how R. ponticum becomes so dominant. Colonization of these areas of established R. ponticum is restricted by shading. When the canopy is not intact and the ground is relatively unshaded, the physical nature of coarse R. ponticum litter may prevent colonization. If the upper litter layer is removed, the lower litter layer or soil (both with mycorrhizal hair roots), may have a toxic effect on potential colonizers.

This analysis of interference may explain the deleterious influence of R. ponticum on emergent Ilex bushes (Cross, 1973). However, the clearest examples of the overall interference effect including toxicity, are the bare-zones around R. ponticum bushes at Winterton in Norfolk. This present work has supported the earlier conclusions of Roff (1964) regarding interference by Calluna. This is not simply a case of severe competition for nutrients and/or water. The suppression of the growth of competing higher plants appears to be brought about partly by competition but with a strong allelopathic influence as well. The outcome is a zone c. 0.5m wide around vigorous R. ponticum bushes, relatively unvegetated except for lichens and bryophytes. The roots of R. ponticum penetrate beneath this band and provide a ready source for the release of potential phytotoxins. By inhibiting the growth of competitors in this zone, R. ponticum presumably benefits by increased availability of water and nutrients, and perhaps by decreased shading from competitors.

Experimental observations suggest that although the germination of test seedings is itself not inhibited, root growth of germinating seedlings is. The interference effect is exerted strongly on pre-germinated seedlings, but much more strongly on those germinating in situ. The early seedling phase was observed because the plants would be most sensitive to adverse conditions at this stage. Rorison (1960) considered this to be particularly important in such investigations. The effects on competitors from the time of germination onwards need to be considered and proved very important in this case. The response of a plant to adverse conditions may be affected by the amount and type of seed reserve, or by the stage of development at which it exposed to such conditions. The use of established transplants for bioassays could strongly influence the outcome (Rorison, 1960). Another important feature of experiments of this type, carried out under controlled conditions, is that seedling survival is increased above that which would occur under field conditions. Many seedlings with poor root development would have died during even a short period of drought, and the effect of interference on survival would have been even more marked. Rorison (1960) found similarly increased survival under greenhouse conditions.

If the vegetation at a site like Winterton was dominated largely by annual herbs, then the interference phenomenon might be even more striking. A number of examples of strong allelopathic effects have involved relatively arid sites with annual herb species (Muller and Chou, 1972; Rice, 1979).

The mechanism of the allelopathic part of interference is not clear. It is closely associated with the presence of R. ponticum roots in the soil. It may be due to organic acids released in some way from the roots. The mechanism or agent is capable of remaining active in potted soils for up to at least 12 weeks without replenishment. The effect is not dependent upon mycorrhizal infection of R. ponticum roots.

The interactions of R. ponticum with its competitors and with its environment are obviously complex. The work undertaken has highlighted some important features in the ecology of this species, but there is considerable potential for further research.

Firstly, further elucidation of the details of interference, and particularly of the effects on root development of suppressed plants at the cellular level need consideration. Attempts should be made to isolate suspected agents of the effects described and to induce the toxicity with controlled applications of these compounds. The possible role of aliphatic acids such as described for Calluna soil by Jalal and Read (1983, I and II) also needs to be assessed. The complex interaction of toxicity and acidification should perhaps be considered.

The second suggestion concerns interference in the field situation. An understanding of this phenomenon may ultimately depend on carefully controlled field trials at a suitable site such as either Winterton or Clumber.

The final recommendation relates to the important question of herbivore pressure. Now that the high phenol content of R. ponticum tissues has been confirmed, further experiments regarding the effects of these compounds on herbivores would be interesting. This is particularly so, in view of work along similar lines for Quercus by Feeny (1968, 1969) and the suggestions by Harborne (1979) and Swain (1979) concerning the possible roles of phenolic compounds.

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