MICROBIAL AND NUTRIENT CHANGES ASSOCIATED WITH THE 'ASHBED EFFECT'

by

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March, 1968

Thesis submitted for the degree of Master of Science in Botany, at the Australian National University

Aerial view of 15 year old P. radiata exhibiting 'ashbed effect' at Dunn's Pinch, A.C.T.

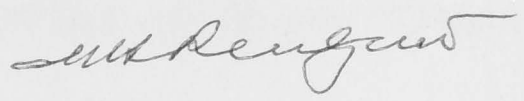
ACKNOWLEDGEMENTS

I would like to thank my supervisor, Professor L.D. Pryor, Professor of Botany, S.G.S., A.N.U., for the assistance he has given me during the preparation of this work. I am particularly indebted to my co-supervisor, Mr G.A. Chilvers, Lecturer in Botany, for his dedicated effort and guidance during the last three years.

Thanks are also due to Mr Bill Roberts of the Forestry Research Institute, Canberra, A.C.T. for recording soil temperatures during the burn, to Mr W. Twine, C.S.I.R.O., Canberra, A.C.T., for the total nitrogen and phosphorus analyses, to A.A.E.C., Lucas Heights, for irradiating soil samples, and to A.P.M. Pty Ltd through Mr R.N. Cromer for the loan of Cavette flasks.

For helpful advice and stimulating discussion I am grateful to Dr J.R. Freney and his associates, Division of Plant Industry, C.S.I.R.O., Canberra, A.C.T., Mr R.N. Cromer A.P.M. Forests Pty Ltd, Traralgon, Victoria, Mr F.R. Humphreys, Division of Wood Technology, Forestry Commission of N.S.W., and to the staff and research students in the Department of Botany, S.G.S., A.N.U.

In accordance with the regulations of the Australian National University, I wish to state that, with the exception of the work acknowledged, the experiments described in this thesis were carried out by myself under the supervision of Mr G.A. Chilvers.



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SUMMARY

When low grade native woodland is cleared in favour of pine plantations the original trees are bulldozed into windrows and burnt. Pine seedlings planted in the residual ashbeds frequently exhibit a dramatic growth response by comparison with the rest of the plantation. This 'ashbed effect' has excited considerable interest among foresters and a number of workers have studied tree growth and soil chemistry in relation to this phenomenon.

This study commences with the hypothesis that the most significant treatment in ashbedding is to heat-sterilize the soil, and it seeks to implicate micro-organisms in the subsequent stimulation of plant growth. A series of observations and experiments establish the following important points:

(i) soil may indeed be sterilized to a considerable depth;

(ii) micro-organisms commence to recolonise the ashbed as soon as adequate moisture is available;

(iii) the new population is very different from the original;

(iv) this new population is gradually replaced by species of the original population, taking about two years to return to normal;

(v) seedlings respond immediately when transplanted to an ashbed or heated soil, growth stimulation relative to control soil being maximal at the start and declining to zero within two to three years.

There is, therefore a very suggestive coincidence between changes in the stimulatory capacity of the ashbed soil and the microbial population within it. Attempts to establish a causal relationship between these are inconclusive. Changes in the level and form of nitrogen and phosphorus in the actual soil solution provide no obvious explanation for the growth stimulation and their duration is inconsistent with the duration of plant growth stimulation and microbial differences. On the other hand, there is clear evidence of microbe-induced changes in the nutrient status of heated soil.

The original hypothesis is modified by adding that the heat-treatment, in addition to killing the original micro-organisms, modifies the soil chemistry in a way which favours the development of a qualitatively different population which, directly or indirectly, gives rise to a superior soil environment, resulting in stimulation of plant growth.

INTRODUCTION

For almost 30 years it has been the practice in Australian forest plantation establishment to clear the low-yielding native forest by removing any merchantable wood and pushing over and bulldozing the remaining trees into windrows which are then burned. It has been clearly established that both Pinus spp. and Eucalyptus spp., when planted in the burned windrows, grow markedly better than in the adjacent unburned areas. Since the burned sites could be recognised by the presence of ash or charred remains of wood, the phenomenon of growth response came to be called the 'ashbed effect'. Although this effect has been observed in many parts of the world, the use of the term 'ashbed effect' has been restricted to Australia.

The response in growth on ashbeds is sufficiently marked to be clearly visible to the eye, especially during the early years of growth. Excellent examples of the effect are to be found in many parts of eastern Australia, both in coastal and tableland regions, as well as in Western Australia. The W.A. Forest Department (1957) report that the height of one year old Karri seedlings on ashbeds was 5-10 times that on adjacent unburned areas. The growth of loblolly pine seedlings in Livingston Parish, Louisiana, was twice as much on burned windrows at the end of the second and third years (Applequist, 1960). Kessell and Stoate (1938) found that after three years the growth rate of Pinus pinaster in Western Australia was 1.5 times better on ashbeds. In Sunny Corner, N.S.W.,

Humphreys and Lambert (1965) found the height of Pinus radiata to be 1.2 to 1.4 times greater after nine years.

Marked increases in the survival of seedlings both after direct sowing and planting have also been reported (W.A. Forest Department, 1957; Applequist, 1960).

The potential gain in wood production is very great. Humphreys and Lambert (1965) found that, on particular sites, there is two to three times the total volume of wood produced on ashbeds after nine years, whereas Cromer, Raupach and Clarke (1968) have shown that there is 50 per cent more merchantable volume after 17 years on ashbed sites in the A.C.T., and 50 per cent greater pulpwood volume at 12 years in Gippsland. As Cromer points out, a site, having a ten per cent ashbed cover capable of producing 50 per cent more volume, would show an increase in pulpwood volume of five per cent at the age of 12 years. If the basic principles underlying the growth response on ashbeds were understood, it may well lead to an economic means of reproducing the effect over whole sites.

It has been suggested that factors other than burning could have bearing on the apparent stimulation of growth on ashbeds. Pryor (1963) points out that the difference in growth between ashbed and non-ashbed sites need not necessarily represent a total gain on ashbeds, but simply a relative gain on ashbeds and a loss to the rest of the plantation. This is supported by the fact that during clearing not only the top soil, but also the aerial parts of the original stand are mechanically transported to the windrows, leading to a gross imbalance of potential nutrients over the site. The effect of soil transport during windrow heaping has been recently investigated by

Cromer (personal communication). In a pot trial using soil collected from windrows (A), disturbed areas between windrows (B), and undisturbed areas (C) at Flynn Creek, Gippsland, he found that the difference in growth of Pinus radiata was of a whole order more significant between (A) and (B) than between (A) and (C). He concluded that soil transport was a significant factor. The transport of vegetation to the windrows must also be regarded as a significant factor since potential nutrients are concentrated in these areas at the expense of the remainder of the site.

The effect of burning on the chemical properties of the soil has received considerable attention. Significant increases in soil nutrients have been reported for various types of forest fires, and since most forest fires do not raise the soil temperature to a significant degree (Heyward, 1938; Beadle, 1940; Bentley and Fenner, 1958; Hatch, 1959) it is probable that any changes in the nutrient levels of the burnt soils would be due to the presence of ash. Increases in several nutrients have been reported: phosphorus (Firsova, 1962; Austin and Baisinger, 1955), potassium (Edwards, 1938; Burns, 1952; Austin and Baisinger, 1955; Miller, 1962; Firsova, 1962), calcium (Heyward and Barnette, 1934; Burns, 1952; Austin and Baisinger, 1955), and magnesium (Austin and Baisinger, 1955). All workers found that the soil pH was increased after burning.

The chemistry of ashbed soil has only recently been investigated, and as yet there is little information available. Hatch (1960) investigated the chemical properties of ashbeds on Karri and Wandoo forest sites in Western Australia and found increases in soil pH, total

soluble salts, nutrients extracted by 2.5 per cent acetic acid, and calcium. These changes were most pronounced in the top inch of the soil and decreased rapidly with depth. Humphreys and Lambert (1965) examined several nine year old ashbed sites at Sunny Corner, N.S.W. and found that the only significant difference between soils on and off ashbeds was the higher content of aluminium-, calcium- and iron-bound phosphorus in ashbed soils. No significant difference in soil pH was observed. They concluded that the increase in available phosphorus was responsible for the ashbed effect. Working in Louisiana, Applequist (1960) reports marked increases in available P, K, Ca and Mg, as well as soil pH, in the top six inches of soil. However, the differences in available nutrients and soil pH were found to decrease with time. For instance, on one year old sites there was 30, 25, and 6 times more available P, Ca, and Mg respectively, but by the end of three years, there was only 3, 5, and 3 times more available P, Ca, and Mg respectively. The gain in available K remained constant over the three year period. The soil pH decreased by 1/10th each year and was still 1.3 times higher in ashbed soil at the end of three years.

There is very little data on the nitrogen status of ashbed soils. Hatch (1960) reports a loss in total nitrogen in the top inch of ashbed soil, and this is supported by McIntyre (personal communication). Humphreys and Lambert (1965) found no difference in total nitrogen in the top three inches of nine year old ashbed soil. However there was apparently less ammonia in ashbed soils from four of the six sites examined.

While changes in soil nutrients after forest fires can be attributed to the presence of ash, in the ashbed situation the direct effect of heat on the soil must also be considered. Soil temperature recordings during windrow burns have shown that the soil is heated to relatively high temperatures down to the depth of several inches (Humphreys and Lambert, 1965; Cromer and Vines, 1966). The work of Vlamis, Schultz and Biswell (1955) and that of Jamwal (1939) suggests that the degree to which the soil is heated during the burn is significant in determining the magnitude of growth stimulation. One of the earliest attempts to separate out the effect of nutrients from ash and the effect of heat treatment on the observed growth response was made by Griffith (1946). As a result of a carefully laid out field trial he was able to show that there was little or no difference in growth response between ash plus heat treatment and the heat treatment alone. Ash only was better than no treatment, but definitely inferior to either of the heat treatments. More recently, Pryor (1960) has reported that the addition of ash produced a 25 per cent increase in the growth of eucalypts, but that baking the soil at 150°C for several hours produced an increase of 100 per cent over the controls. The addition of ash to the baked soil did not produce any further increase. There is substantial evidence then, that ash plays only a minor role in the production of the 'ashbed effect', and that the essential factor is heat. The term 'ashbed effect' is consequently somewhat misleading.

The effect of heat on soil and subsequent plant growth aroused considerable interest around the turn of the century, when soil steaming became established as a

popular nursery practice. It was during this period also that growth stimulation in soils which had been heated in the absence of moisture, as opposed to steaming, was first reported.

Pickering (1908) found that apple trees grown in soil which had been heated in an oven at 200°C for two hours showed an increase of 63 per cent in new wood production and 44 per cent increase in dry matter of leaves. Several workers have since reported highly significant growth stimulation subsequent to heating soils over a wide range of temperatures above 100°C. Seaver and Clark (1912) observed increases in the growth of oats and lupins after heating at 120°C. More recently Pryor (personal communication) found that the growth of E. grandis seedlings was significantly better in soils heated at 100°C, 200°C, 300°C, 500°C and 900°C. Growth stimulation over a similar range has also been observed by Cromer (personal communication) for P. radiata seedlings. Florence and Crocker (1962) reported almost four times as much growth in E. pilularis in soil heated at 160°C for 48 hours.

In direct contrast to the results of Pickering, Pryor, Cromer, and Florence and Crocker, several workers have found that soils heated in the range of 135-300°C are toxic to plant growth (Seaver and Clark, 1912; G.W. Wilson, 1914; Johnson, 1916; Attiwill, 1962). It is important to note that in all these cases planting occurred immediately after heating, or the soil was kept in the dry condition until the time of planting. The significance of this becomes apparent after a closer examination of Pickering's work. Not all the apple trees planted in the soil

straight after heating survived. Those that did survive initially showed much poorer growth than those in the unheated soil, but subsequently became extremely vigorous and by far exceeded the growth in the unheated soil. It would appear then, that for a while after heating the soil is indeed toxic to plant growth, but, provided that the plants are able to withstand this toxicity, enhanced growth subsequently takes place. Pickering further showed that if planting was done after the heated soil had been watered constantly or kept under moist warm conditions, the plants showed an immediate increase in growth. Pryor achieved the same result by flooding the soil for a period of one week. Toxicity in heated soils, then, is only temporary provided that the conditions are favourable for the process of detoxification.

Evidence indicates that the toxic factor developed on heating soil is organic in nature. Pickering (1910) found that over a range of temperatures from 150^{o}C to low red heat, the degree of toxicity correlated closely with the amount of soluble organic material in the soil. Attiwill (1962) found that when extracts from both dry heated organic matter and soil at 300^{o}C were added to culture solutions, the growth of barley was inhibited, again suggesting that the toxins are organic compounds and further, that they are derived from the organic matter in the soil. Rovira and Bowen (personal communication) have demonstrated that the phytotoxins are readily leached and that their effects can be transmitted to sterile sand cultures. The case for the organic nature of the toxins is further strengthened by the fact that toxicity is only developed in the approximate range from 135^{o}C to 300^{o}C. Below this range organic material in the soil would not be expected to be

appreciably altered, whereas above it destruction of the organic matter would take place. Cromer (personal communication) found that after heating soil at 200°C, 400°C, 600°C and 800°C, there was a reduction in the organic carbon content of 20 per cent, 70 per cent, 90 per cent and 94 per cent respectively compared to unheated soil. Loss of total nitrogen from soil after heating at the same temperatures was found to be of the same order. Humphreys and Lambert (personal communication) have also demonstrated a marked reduction in total nitrogen after heating above 200°C. Attiwill (1962), who kept the heated soil in the dry state before planting, found that soil heated at 300°C was toxic to plant growth, whereas soil heated at 500°C showed no toxic effect on growth.

Allowing for the initial toxicity developed after heating between 135°C and 300°C, there is little doubt that heated soils are capable of supporting superior plant growth. Heat then must effect some change in the soil which results in higher productivity. The obvious solution would be that heating makes available extra plant nutrients, and thus it is perhaps not surprising that most of the attempts to explain the growth stimulation in heated soils have been made to date in the field of soil chemistry.

Increases in water soluble material after heating up to 200°C have been reported by Pickering (1910), Seaver and Clark (1912) and Kelly and McGeorge (1913). Whereas Pickering found that this increase was due entirely to the organic fraction, the other workers found that the soluble inorganic nutrients also increased with increase in temperature, though not to the same extent. Seaver and Clark (1912) report an increase in total soluble nitrogen

over the same range of temperatures. Significantly larger amounts of ammonia-nitrogen were found in a range of heated Hawaiian soils analysed by Kelly and McGeorge (1913). The values were abnormally large after heating at 200°C. Similar results have recently been obtained by Humphreys and Lambert (personal communication). In 1965, the same workers reported increases in Al-P, Ca-P and Fe-P after heating soil at 350°C for four hours. Increases in available phosphorus have also been reported by Grange and Fox (1955), Arbuckle (1955) and Fields et al. (1955). However heated soils have been found to contain less exchangeable calcium and aluminium (Humphreys and Lambert, 1965) and nitrate-nitrogen (Kelly and McGeorge, 1913). Nitrate-nitrogen was almost completely removed after heating at 200°C and above.

The increases in soluble inorganic nutrients and available phosphorus are certainly suggestive. Both Attiwill and Humphreys maintain that the growth response in heated and ashbed soils is explained in terms of the increased phosphorus availability. However, in view of the magnitude of plant response, these increases seem relatively small. Furthermore, Pryor has shown that the type of growth response obtained on ashbeds cannot be duplicated by phosphorus additions to unheated soil. Large doses of phosphorus only produce small increases in growth of eucalypts. The large amounts of ammonia-nitrogen found by Kelly and McGeorge in heated soils led them to believe that the superior growth in these soils was a response to ammonia. Yet evidence from plant nutrition studies indicate that, in general, plants require the nitrate form of nitrogen for healthy growth and that large amounts of

ammonia-nitrogen are seldom tolerated. Since the increase in ammonia-nitrogen is accompanied by a decrease in nitrate-nitrogen, it seems unlikely that growth enhancement can be explained purely in terms of the nitrogen status of the heated soil. Pryor has shown that luxury doses of nitrogen and phosphorus together may duplicate the effect of heating soil at 150°C but the levels required to achieve this were greatly in excess of those actually detected in ashbed soils. Soluble organic materials released by heating may contain special nutrient factors also, but as discussed earlier, the net effect of this fraction is to cause growth depression.

While it seems likely that changes in the availability of nutrients to plants growing in an ashbed is involved, no satisfactory explanation of the growth response has so far been provided in terms of changes in the nutrient levels in the soil.

Growth stimulation is not peculiar to heat pre-treatment of soil. Similar increases in growth have been reported as a result of steaming, fumigation and gamma irradiation. Increased yields after steaming have been reported by Frank (1888), Schmoeger (1893), Pfeiffer and Franke (1896), Liebscher (1893), Hassenbäumer, Coppenrath and König (1906), Pickering (1908), A. Wilson (1915), Peyronel (1926) and Tam and Clark (1943), to mention only a few. A variety of soil fumigants have been found to increase the productivity of the soil. The most commonly employed fumigant among the earlier workers was carbon bisulphide, which has been shown to enhance the growth of a wide range of species, including Helianthus, Cucurbita, oats, wheat, potato, beet, mulberry, Acacia and other tree

species (Dumas, 1875; Oberlin, 1895; Mach, 1896; Henry, 1901; Egorow, 1908; Sirker, 1909; Fred, 1916; Gainey, 1912; Miège, 1914; Peyronel, 1926). Other fumigants which have been shown to have similar effects on plant growth are ether (Clemens, 1847; Johannsen, 1896; Noble and Richter, 1904; Fred, 1916; Koch, 1912; Peyronel, 1926), chloroform (Johannsen, 1896), toluol (Gainey, 1912), formaldehyde (Peyronel, 1926; Tam and Clark, 1943), chloropicrin (Tam and Clark, 1943) and trizone, a gaseous or liquid mixture of methylene bromide, propargyl bromide and chloropicrin (Wright, 1964). Growth stimulation as a result of gamma irradiation has been reported by Florence and Crocker (1962) for Blackbutt seedlings and by Bowen and Cawse (1964) for barley and lettuce. Cromer (personal communication) observed enhanced growth of P. radiata seedlings in gamma irradiated clay soil.

While dry heat, steam and fumigants produce changes in the mineral fraction of the soil to varying degrees, gamma irradiation has been shown to have no significant effect on this fraction (McLaren, Lūse and Skujiņš, 1962). Thus all these treatments have only two effects in common, namely soil sterilization and subsequent stimulation of plant growth, suggesting that there may be a causal relationship between these two phenomena.

It is interesting to note in passing that Kopeloff and Coleman in 1917, while commenting on the difficulty of separating the effects of soil sterilization and the effects of chemical changes on plant growth, lamented the fact that there was no means of sterilizing soil without affecting the chemistry of the soil. It was not until the

early 1960s, when sources of gamma irradiation became available for commercial use, that such a method was realised.

The living component of the soil is seldom considered in direct relationship to plant growth, except when some organism or other becomes a pest and its presence can no longer be ignored. The plant nutritionist tends to regard the soil merely as a sink for plant nutrients, unconscious of the fact that the availability of nutrients for plant growth depends very much upon the activity of the soil micro-organisms, particularly in the case of nitrogen. But whatever role the soil population may play under normal soil conditions, it can hardly be denied that the destruction of this dynamic complex in heated soils could have profound implications with regard to plant growth in these soils.

The aim of this present work is to investigate the hypothesis that the changes brought about by heat in the microbial population of the soil are either directly or indirectly responsible for the so-called 'ashbed effect'. Three areas of study immediately suggest themselves:

First it is necessary to investigate the extent and nature of the changes in the microbial population, not only on heating but also subsequent to heating. Some specific questions that need to be answered in relation to ashbed soil are:

(i) Does effective sterilization take place?

Recordings obtained by Cromer and Vines (1966)

and Humphreys and Lambert (1965) for a number of windrow burns indicate that the temperatures, in general, are high enough to bring about at least partial sterilization to the depth of several inches. However, there is no microbial evidence for either the degree of sterilization or the extent of sterilization with depth.

(ii) How long is it before recolonisation commences?

This would to a large extent depend on the availability of a source of inoculum and the presence of sufficient moisture in the soil for microbiological activity. However, even if these conditions are met there could still be a delay if the environment of the ashbed soil was unfavourable for spore germination and subsequent microbial growth. This leads to the possibility that even whole groups of micro-organisms may be prevented from colonising the soil.

(iii) What is the nature of the microbial population that colonises the soil after sterilization?

Wright and Tarrant (1957) report that after logging and slash burning, the number of prokaryotic organisms is greater in the surface soil and that the ratio of actinomycetes to bacteria is lower than in the unburnt soil. There is no other information available about the microbial population of either ashbed or heat sterilized soil.

(iv) How long does it take for the ashbed population to return to normal or to reach a new equilibrium?

(v) Is the root surface flora on plants growing in ashbed soil different from that on plants growing in untreated soil?

Because these micro-organisms are so intimately associated with the plant root system, differences between treatments could well be directly related to the difference in plant response.

Secondly, more information about the nature of the plant response is required before a correlation between any differences that may be found in the ashbed microbial population and plant growth can be attempted. For example:

(i) Does all the soil heated during ashbedding have the same effect on plant growth?

Temperature recordings taken during windrow burns (Cromer and Vines, 1966; Humphreys and Lambert, 1965) indicate that there is quite a steep temperature gradient down the soil profile. Comparison of plant growth in ashbed soil from different depth could lead to useful information about the conditions required for optimum plant response, provided that the temperature of the soil and the degree of sterilization obtained at the different depths was known.

(ii) What is the time relationship between soil sterilization, planting and the onset of growth stimulation?

In order to avoid drawing invalid conclusions, it is important to have this information before attempting to attribute the enhanced growth to any particular difference that may be observed in the sterilized soil. In general, the temperatures employed in studies of heated soils fall in the range in which initial toxicity is known to develop, so that it is necessary to delay planting until

detoxification has taken place. The accepted procedure is to flood the soil for a period of approximately one week prior to planting. In forestry practice, planting takes place some considerable time after burning, so that in case of ashbeds there is even a longer time interval between sterilization and planting. Consequently, even if differences are observed in the sterilized soil during the interval before planting, they could hardly have direct bearing on growth. Strictly speaking, comparisons between plant growth and such differences as there may be in the nature of the sterilized soil, are only valid once growth stimulation becomes evident. It is necessary, then, to establish with some degree of accuracy, the time interval between planting and the onset of enhanced plant growth.

(iii) What is the duration of the period of growth stimulation?

Knowledge of the duration of the growth response would be of particular use in determining the relative value of any long term or short term changes that may be induced by sterilization. Humphreys and Lambert (1965) seem to be of the opinion that as long as height differences are in evidence, growth stimulation is still in operation. Curves obtained for the heights of P. radiata on and off ashbed sites showed that there was a significant height difference even after 11 years. However, Chilvers (personal communication) points out that if the curves obtained by Humphreys and Lambert are compared in terms of the extra time taken for the control trees to reach the same height as the ashbed trees, it appears that the special stimulatory factors of the ashbed are in operation for only three years and that half of the total ashbed effect is

generated in the amazingly short period of eight months (Figure 19). An intensive comparison of the growth of ashbed versus control trees over the first few months should therefore prove interesting. Most studies to date have made measurements only about once a year.

Thirdly, there is a need to obtain a better idea of the week to week changes in the levels of plant nutrients in the soil and to correlate them with the behaviour of the microbes and plants in an attempt to illuminate the interactions between all three. Most of the earlier studies of nutrient changes can be objected to on the grounds that they do not apply to soils which are known to be actively producing a growth response. For example, in most of the studies recently heated soils, which had not been detoxified by wetting or standing for a while before planting, were used. It seems certain that quite profound changes, possibly involving plant nutrient elements, are brought about by microbes during this detoxification period (page 54). Studies like those of Humphreys and Lambert on 11 year old stands are irrelevant if, as suggested by their own growth curves, the ashbed effect is completely generated within three years.

In summary, the following is an attempt to rationalise current studies on the ashbed effect by:

(i) investigating changes in the soil microflora, an area of study which has been largely neglected up to date;

(ii) evaluating microbial, nutrient and plant growth

changes in one and the same experimental soil (as far as possible simultaneously) so that reliable correlations may be sought which could provide clues to the important interactions involved;

(iii) concentrating studies on the early period of growth following treatment, since there is reason to believe that the ashbed factors are most active over the first few months of growth.

I. EFFECT OF ASHBEDDING ON SOIL MICRO-ORGANISMS

1. SOIL TEMPERATURES AND DEGREE OF STERILIZATION

(a) Materials and Methods

Location and site preparation

The location of the experimental blocks is shown in Figure 1. This area is particularly stoney and since the stones could have impeded the transferrence of heat to the soil, as well as made sampling of the soil difficult, they were carefully removed. To further ensure uniformity of treatment, the soil surface was raked even. The area was divided into two blocks, approximately 10' x 12', the more southerly of which was to be used for the ashbed.

Soil temperature recordings

Mr Bill Roberts of the Forest Research Institute, Canberra, A.C.T., kindly offered to record the temperatures of the soil at different depths during the burn. Thermocouples were placed at the surface and 1", 2", $2\frac{1}{2}$", $3\frac{3}{4}$", $6\frac{1}{2}$" and $8\frac{1}{2}$" below the surface of the soil in the centre of the intended ashbed block. These were connected to a metering unit, allowing a continuous series of temperature measurements to be recorded.

Degree of sterilization with depth

This was determined by comparing microbial counts before and after the burn. Since the block was to be used for further study, it was impossible to use the standard method of digging a soil face and then sampling at the depths required an inch or so in from the face, because

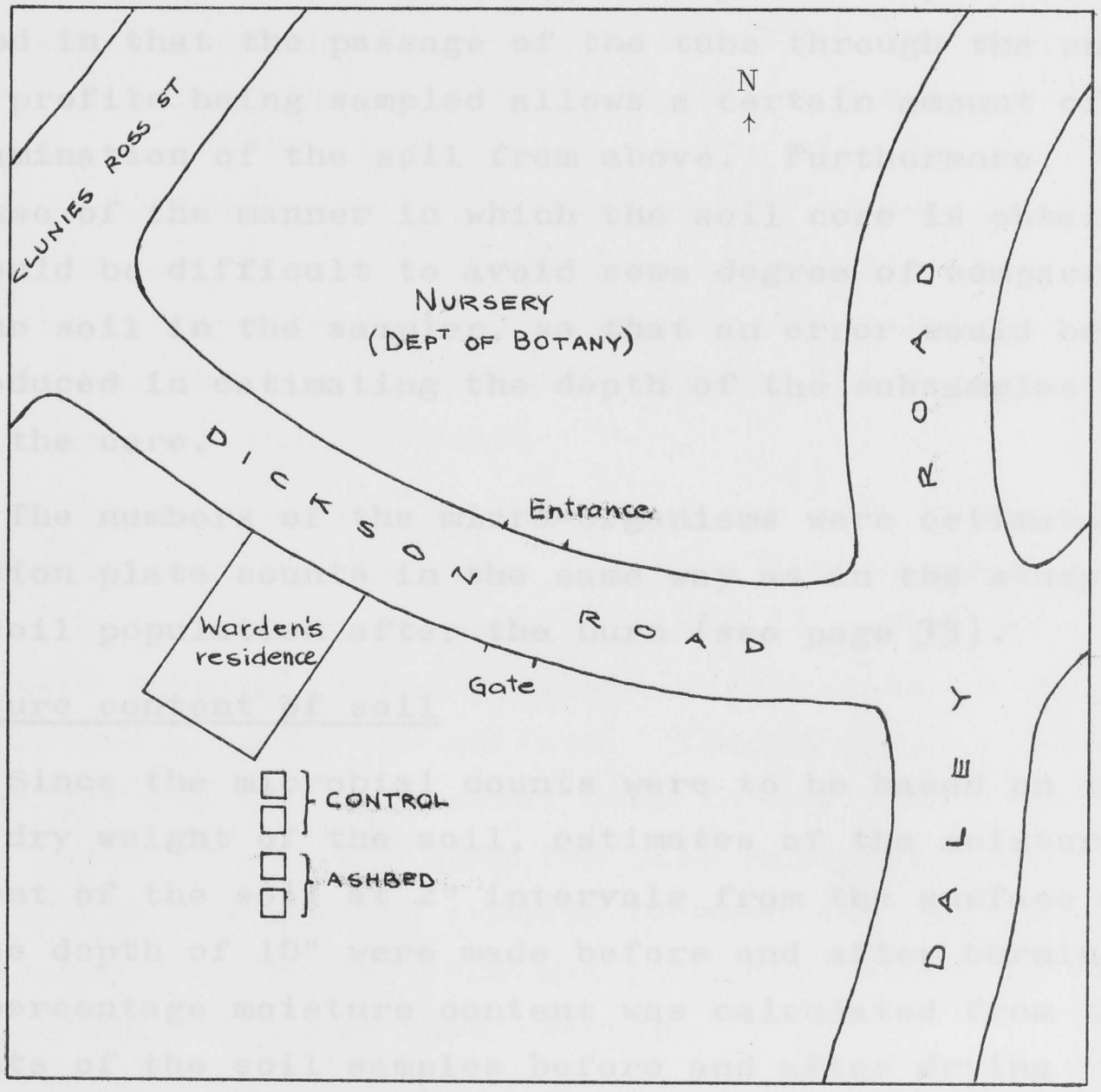


FIGURE 1 Diagram showing the location of the experimental plots in the grounds of the Australian National University

this would have destroyed the block. As an alternative, a metal tube was driven into the ground with a mallet and samples of the soil were taken from different depths of the soil column through holes drilled at 2" intervals in the wall of the tube. This is a comparatively crude method in that the passage of the tube through the entire soil profile being sampled allows a certain amount of contamination of the soil from above. Furthermore, because of the manner in which the soil core is obtained, it would be difficult to avoid some degree of compaction of the soil in the sampler, so that an error would be introduced in estimating the depth of the subsamples taken from the core.

The numbers of the micro-organisms were estimated by dilution plate counts in the same way as in the study of the soil population after the burn (see page 33).

Moisture content of soil

Since the microbial counts were to be based on the oven dry weight of the soil, estimates of the moisture content of the soil at 2" intervals from the surface down to the depth of 10" were made before and after burning. The percentage moisture content was calculated from the weights of the soil samples before and after drying in an oven at 105°C for 48 hours.

Preparation of the ashbed

Logs were carefully stacked on the intended ashbed block allowing for an overlap of approximately one foot on all sides, so that as even as possible heat treatment could be obtained over the whole block. The control block was covered to protect it from radiated heat during the burn (Plate 1).

(b) Results

Soil temperatures

Soil temperature curves obtained for the different depths (Figure 2) show that there was a considerable temperature gradient down the soil profile for some time after the burn. At the soil surface the maximum temperature reached was 660°C, at the depth of 1", 2", 2½", and 3¾" the maxima were 545°C, 365°C, 300°C and 210°C respectively, whereas below 6½" the temperatures only just reached 100°C. The soil at the lower depths took longer to reach a peak than nearer the surface. At all depths the soil took longer to cool than to heat.

It is interesting to note that the soil temperatures continued to rise long after the fire had subsided. Three minutes after the fire was lit (Plate 2), although the logs were burning fiercely, there was no rise in the soil temperature even at the surface. Thirty minutes later (Plate 3) heat had penetrated down to two inches and three hours later (Plate 4), when the pile had been reduced to a heap of ash and charred remains of wood and heat had penetrated down to 8½", the maximum temperature at the surface had still not been reached.

Lawrence (1956) states that most soil micro-organisms are killed if the soil is heated to approximately 70°C for ten minutes, and that all life in the soil is killed at 127°C. Thus, the temperatures to which the soil was heated as a result of the burn suggest that complete sterilization of the soil would have been achieved down to about four inches and a substantial kill down to 8½" or more.

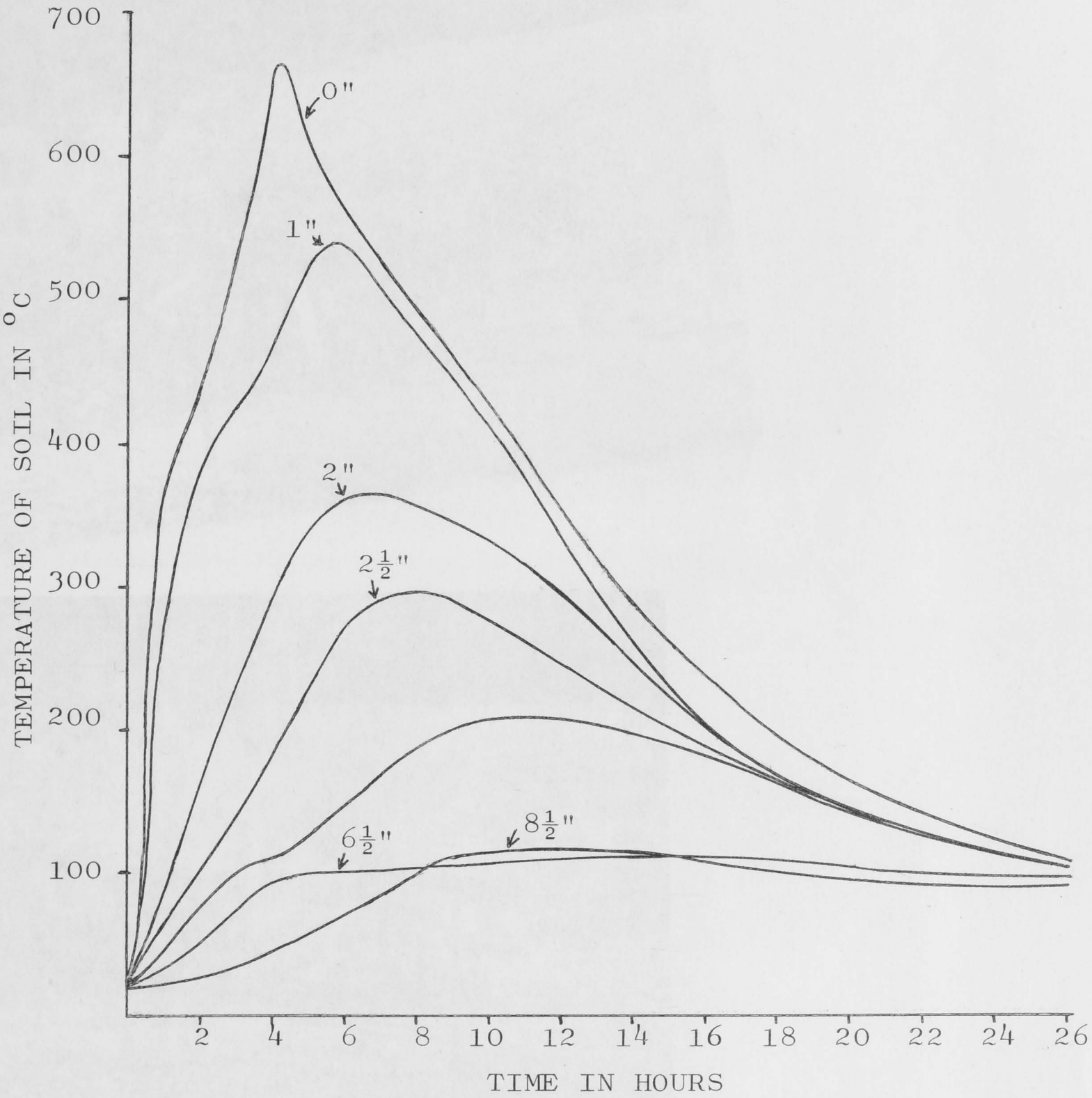


FIGURE 2 Temperature curves obtained at different depths during course of fire

Plate 1 Wood pile before fire

Plate 2 Three minutes after lighting of fire

Plate 3 Thirty minutes after lighting of fire

Plate 4 Three hours after lighting of fire

Microbial numbers

Table 1 shows that there was a complete kill of the mycelial organisms, namely the actinomycetes and fungi, down to a depth of ten inches and possibly down to 14". Some bacteria appear to have survived the treatment, but the numbers after heating represent only about 0.1 per cent of the total population before the burn. The numbers of apparent survivors at the depths of 4", 6", 8" and 10" are suspiciously constant, and although the temperatures below $6\frac{1}{2}$" were not high enough to allow complete sterilization, at 4" the temperature was far in excess of that required for a complete kill, suggesting that these are probably contaminants. No estimates of the microbial numbers are available from the depth of 14" prior to the burn, since it was not expected that the temperatures would be sufficiently raised below the top few inches of the soil. However, judging from the rate of decline of bacterial numbers with depth, it is unlikely that the count obtained after the burn reflects any appreciable kill of bacteria at this depth.

Soil moisture content

Table 2 shows the values obtained for the soil moisture content at the different depths before and after the burn. The date of the burn coincided with the tail-end of a severe drought period in the Canberra area, so that the moisture content of the surface soil was found to be much lower than normally encountered prior to windrow burns. In fact, windrows are usually only burnt after there has been some rain. More recent work of Roberts (personal communication) suggests that it was the low moisture

	BACTERIA		ACTINOMYCETES		FUNGI		TOTAL	
Depth	Before	After	Before	After	Before	After	Before	After
2"	1,900,000	-	667,000	-	153,000	-	2,720,000	-
4"	1,400,000	2,000	1,095,000	0	130,000	0	2,605,000	2,000
6"	950,000	3,000	1,162,000	0	48,000	0	2,160,000	3,000
8"	465,000	2,000	1,471,000	0	15,000	0	1,951,000	2,000
10"	70,000	2,000	800,000	0	12,000	0	882,000	2,000
14"	-	48,000	-	0	-	0	-	48,000

TABLE 1 Numbers of micro-organisms per gram of oven dry soil, at various depths before and after the burn.

DEPTH OF SOIL SAMPLE	PERCENTAGE MOISTURE CONTENT	
	PRE-BURN	POST-BURN
0-2"	2.1	0.1
2-4"	3.7	0.4
4-6"	5.3	0.6
6-8"	5.7	2.5
8-10"	6.1	6.1

TABLE 2 Percentage moisture content of soil at different depths before and after the burn.

content at the time of the burn that enabled such high temperatures to be attained in the soil.

I. EFFECT OF ASHBEDDING ON SOIL MICRO-ORGANISMS

2. QUANTITATIVE CHANGES IN MICROBIAL POPULATION

(a) Materials and Methods

Experimental plots

Both the ashbed and control blocks were subdivided into two plots, 5 feet by 5 feet, one of which was to be subsequently planted. Drainage channels were dug beside and between the plots to avoid the removal or transfer of the top soil during rainfall.

Soil samples

Because of the heterogeneous nature of soil with respect to both number and species of microbes, soil sampling must be regarded as one of the largest sources of error. The method usually recommended involves taking several large samples which are subdivided and then bulked. The bulked samples are again subdivided and replicate subsamples of these are finally used for analysis.

For the purpose of this study this method was impractical because it would have necessitated the progressive destruction of the plots, making a continuous study impossible, and because the minimum of eight subsamples per treatment that results from this method of sampling could not have been handled in a single day. Instead a large number of small samples were taken, aliquots of which were bulked and used for the analysis, so that only one sample per treatment had to be analysed.

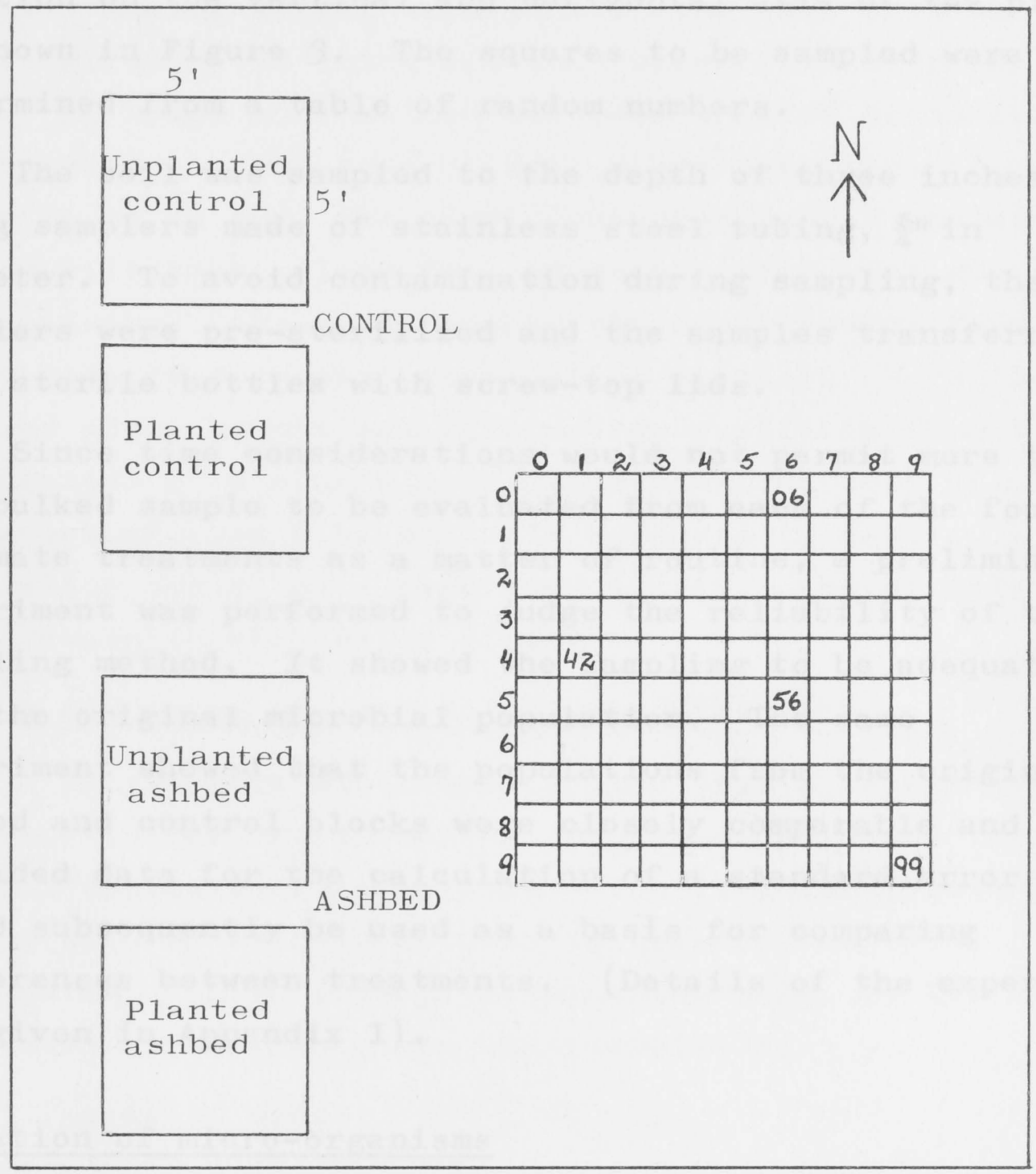


FIGURE 3 Diagram showing the layout of the experimental plots and the sampling grid.

In order to ensure a random set of samples, each of the four plots was subdivided into one hundred 6" x 6" squares by placing a wire grid over the surface of the plots. Each square was given a number according to its position on the vertical and horizontal axis of the plot as shown in Figure 3. The squares to be sampled were determined from a table of random numbers.

The soil was sampled to the depth of three inches using samplers made of stainless steel tubing, $\frac{3}{4}$" in diameter. To avoid contamination during sampling, the samplers were pre-sterilized and the samples transferred into sterile bottles with screw-top lids.

Since time considerations would not permit more than one bulked sample to be evaluated from each of the four ultimate treatments as a matter of routine, a preliminary experiment was performed to judge the reliability of the sampling method. It showed the sampling to be adequate for the original microbial population. The same experiment showed that the populations from the original ashbed and control blocks were closely comparable and it provided data for the calculation of a standard error which could subsequently be used as a basis for comparing differences between treatments. (Details of the experiment are given in Appendix I).

Isolation of micro-organisms

(i) Method

There are two widely used methods suitable for estimating microbial numbers in the soil. One is by direct examination of the soil, and the other the soil dilution plate method. The latter was chosen because

direct examination of the soil does not permit identification of the micro-organisms or their isolation for subsequent study.

The soil dilution plate method (Waksman, 1927) involves making serial dilutions of the soil with sterile water, aliquots of which are then mixed with nutrient agar. Although this method has been applied extensively to the general isolation of soil micro-organisms it has its limitations in that the conditions under which isolation customarily takes place fail to account for such groups as the anaerobes, the phototrophs, the slow-growing fungi or those requiring special germination stimuli. Nevertheless, it does give the maximum information available from the application of a single method, so that the soil dilution plate method should be entirely adequate to detect population trends of a magnitude likely to be of interest in relation to the ashbed effect.

(ii) Preparation of soil dilution series

A preliminary experiment showed that a considerable error is introduced in making the serial dilution from the initial concentrated soil suspension (1/10:soil/water) if a single 10 ml aliquot is used to prepare the 1/100 suspension. In an attempt to minimise this, 10 1ml aliquots were used. (Details given in Appendix II).

(iii) Isolation media

The isolation media chosen were aimed at isolating as large a proportion of the soil organisms as possible, and since the prokaryotic organisms (bacteria and actinomycetes) and fungi in general differ with regard to their basic growth requirements, two different media were used to reduce interference between the two groups. Both the media

contained a common comprehensive inorganic salt base, but differed in that the Bacterial Isolation Medium contained low levels of both Neopeptone and glucose and the monohydrogen salt of potassium phosphate to provide a pH around neutrality, whereas the Fungal Isolation Medium contained high levels of Neopeptone and glucose and had a lower pH due to the presence of the dihydrogen salt of potassium phosphate. The Fungal Isolation Medium also contained Streptomycin and Rose Bengal to suppress bacterial growth. (Appendix IIIa gives the detailed composition and preparation of the isolation media.)

The Bacterial Isolation Medium is a modification of Thornton's Isolation Medium in which glucose was used instead of mannitol and Neopeptone instead of asparagine. The modified medium was shown to give four times as many colonies as Thornton's Isolation Medium. (Details in Appendix IIIb.)

(iv) Plate replicates

In a preliminary trial it was found that 5 plates were sufficient to give representative counts in case of the bacteria and 10 plates in case of the fungi. (Details in Appendix IV.) The plates were incubated at 25°C and counted after four days growths. Counts were made of the plates at the dilution which gave 10 to 30 fungal colonies per plate and 30 to 100 bacterial and actinomycete colonies per plate (Warcup, 1960). The number of organisms was expressed per gram of oven dry soil.

The whole method is summarised in Figure 4.

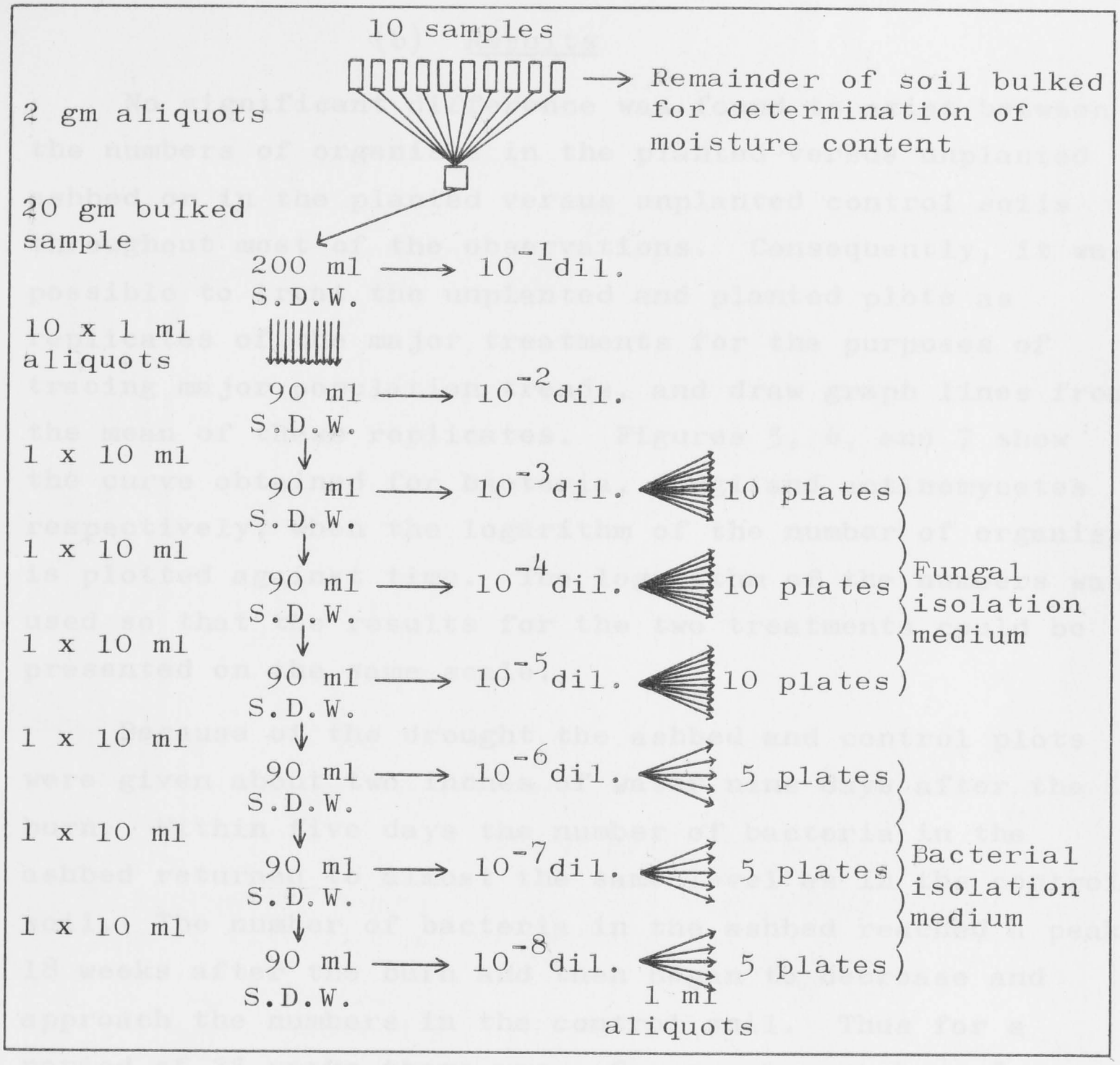


FIGURE 4 Summary of the sampling and isolation procedure used in estimating the microbial numbers in the ashbed and control soils.

(The dilution selected for plating depended on the anticipated number of organisms in the soil, being chosen such that the middle dilution gave a suitable number of colonies for counting.)

S.D.W. = sterile distilled water.

(b) Results

No significant difference was found to exist between the numbers of organisms in the planted versus unplanted ashbed or in the planted versus unplanted control soils throughout most of the observations. Consequently, it was possible to treat the unplanted and planted plots as replicates of the major treatments for the purposes of tracing major population trends, and draw graph lines from the mean of these replicates. Figures 5, 6, and 7 show the curve obtained for bacteria, fungi and actinomycetes respectively, when the logarithm of the number of organisms is plotted against time. The logarithm of the numbers was used so that the results for the two treatments could be presented on the same scale.

Because of the drought the ashbed and control plots were given about two inches of water nine days after the burn. Within five days the number of bacteria in the ashbed returned to almost the same level as in the control soil. The number of bacteria in the ashbed reached a peak 18 weeks after the burn and then began to decrease and approach the numbers in the control soil. Thus for a period of 35 weeks there was a far greater number of bacteria in the ashbed. By the end of 48 weeks after the burn the number of bacteria in the ashbed had returned to normal.

Fungal recolonisation of the ashbed was far more gradual and for a period of about 35 weeks the numbers were only about 1/100th of those in the untreated soil. At the end of 66 weeks the numbers were still only half the numbers in the control.

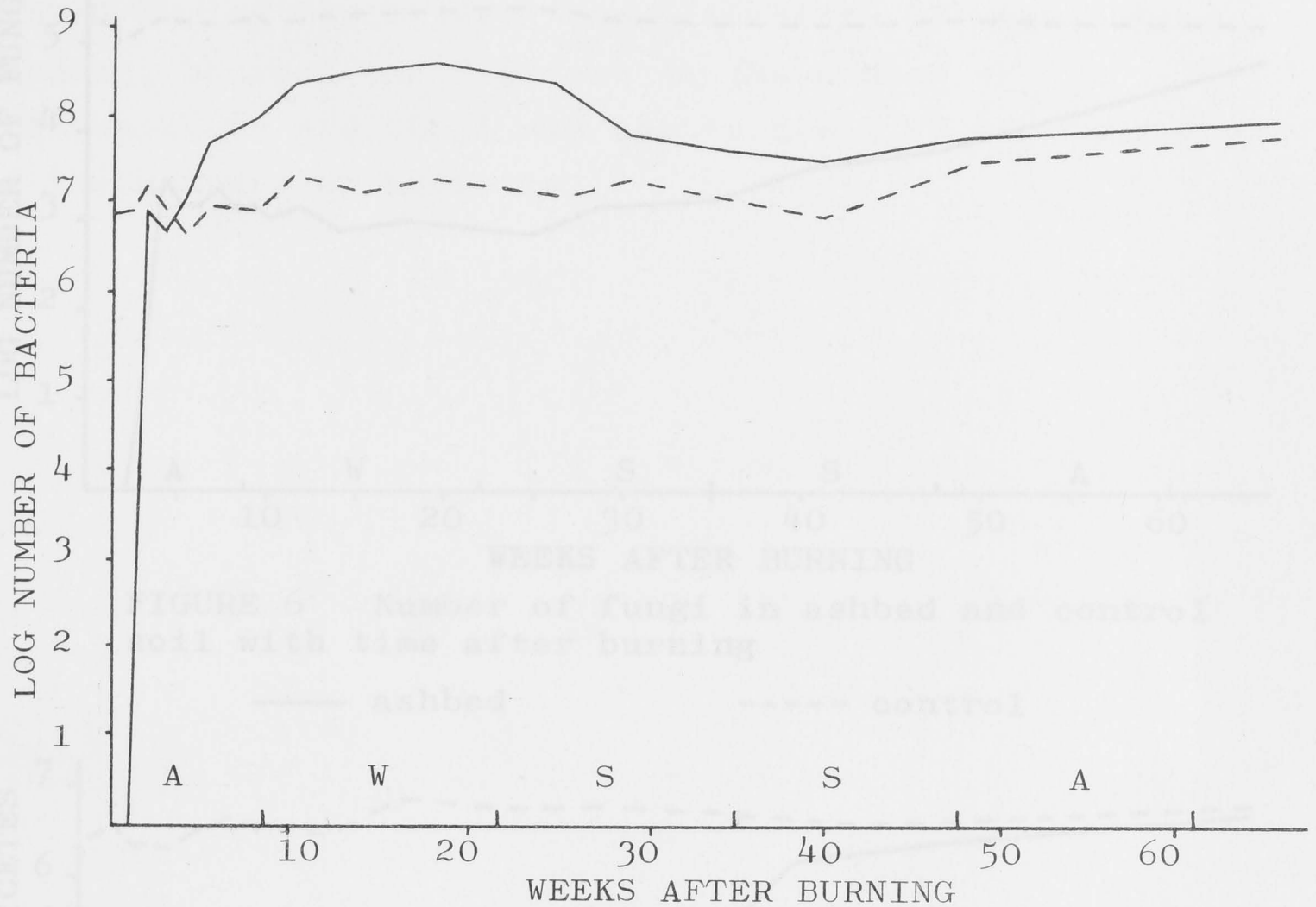


FIGURE 5 Number of bacteria in ashbed and control soil with time after burning

——— ashbed
- - - - control

The most marked differences between the ashbed and control soils was in the number of actinomycetes. The actinomycetes did not appear until 29 weeks after burning, yet by the end of 48 weeks the numbers in the control and ashbed soil were not significantly different. It must be pointed out that the actinomycetes could have been present in the ashbed soil in small numbers before they were first detected due to the very high dilutions of soil necessary to estimate the bacterial numbers: the chance of detecting them on the plates would have been very small until they approached at least 1/500th of the number of bacteria.

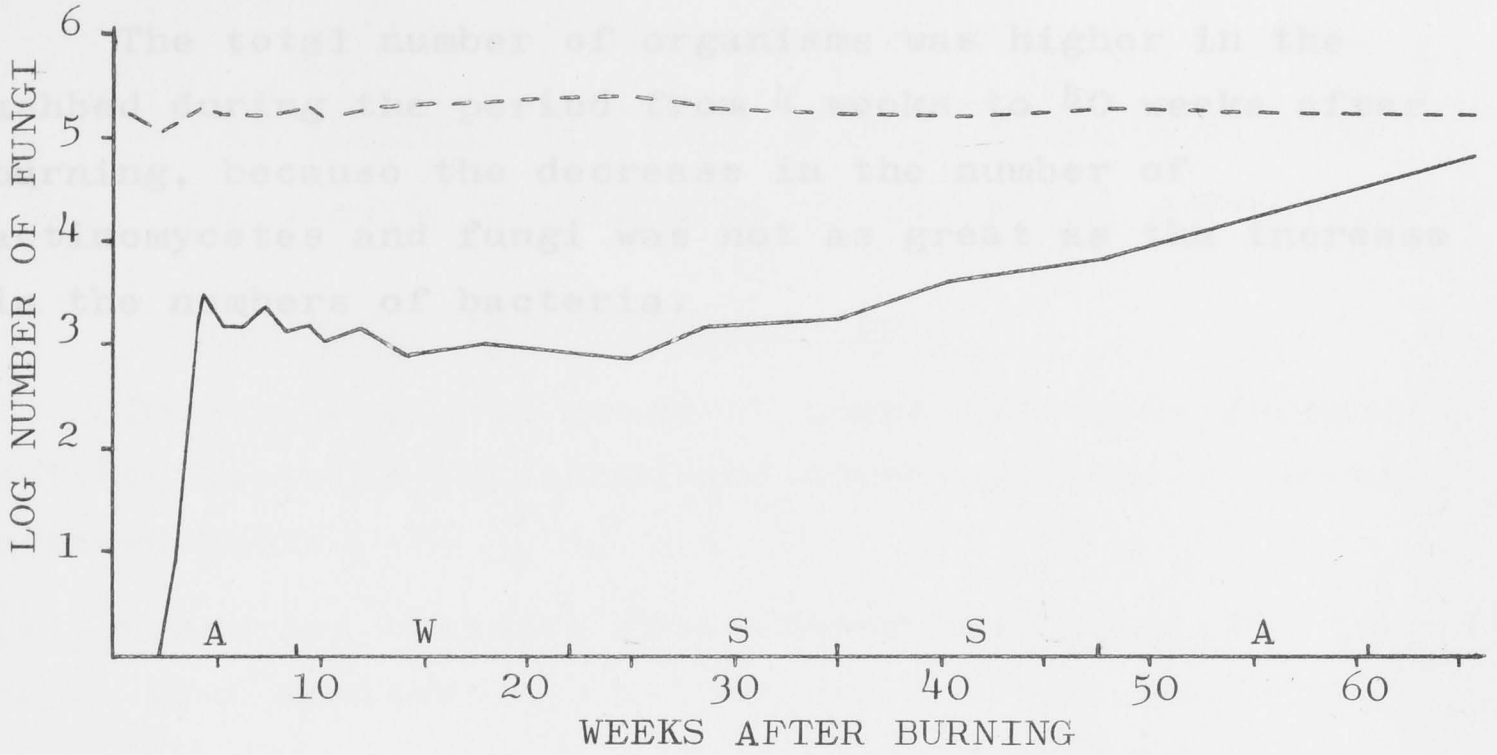


FIGURE 6 Number of fungi in ashbed and control soil with time after burning

—— ashbed ----- control

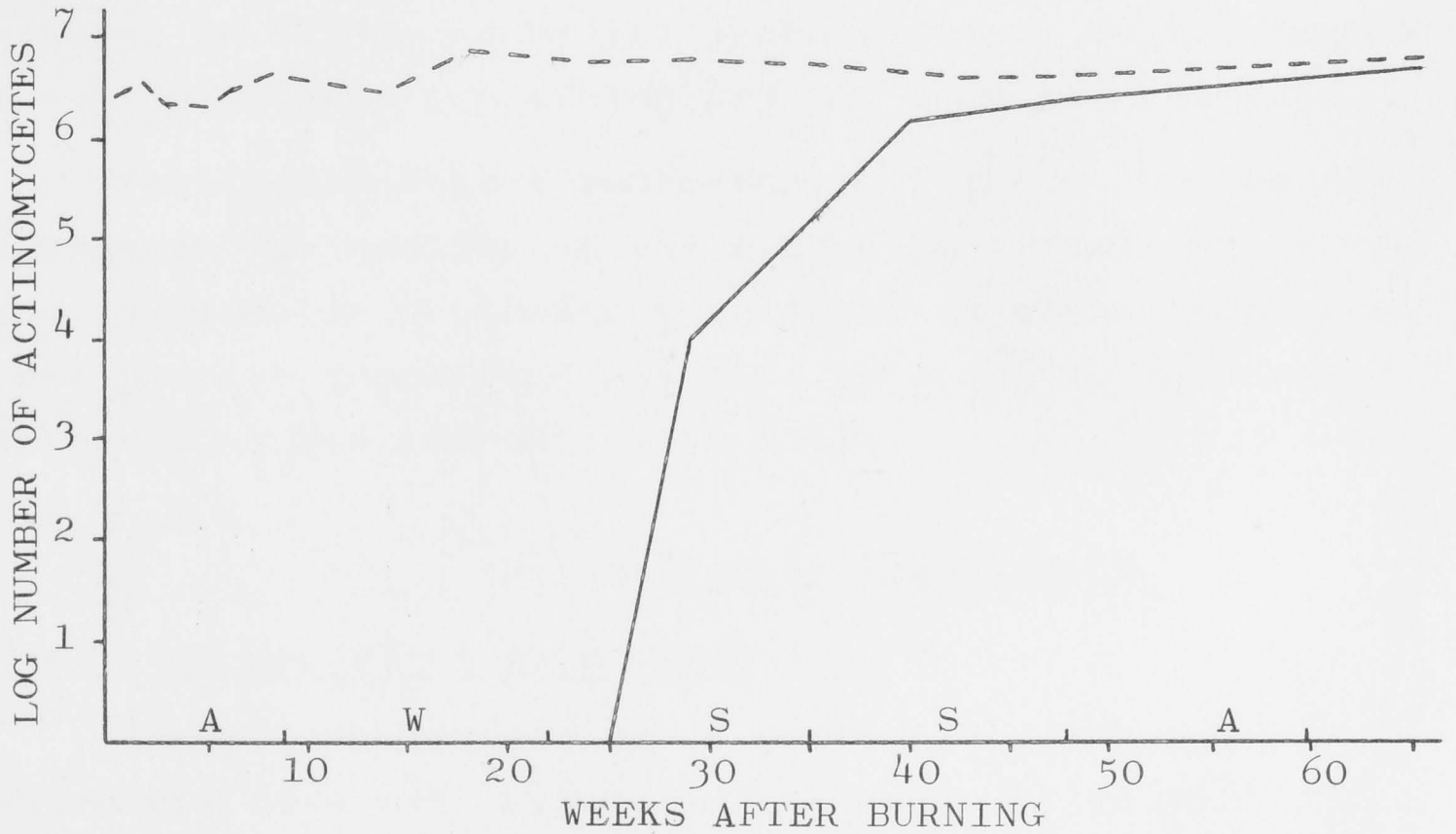


FIGURE 7 Number of actinomycetes in ashbed and control soil with time after burning

—— ashbed -----control

The total number of organisms was higher in the ashbed during the period from 4 weeks to 40 weeks after burning, because the decrease in the number of actinomycetes and fungi was not as great as the increase in the numbers of bacteria.

I. EFFECT OF ASHBEDDING ON SOIL MICRO-ORGANISMS

3. QUALITATIVE DIFFERENCES IN BACTERIAL POPULATION

(a) Introduction

In course of the study of quantitative differences between bacteria in ashbed and non-ashbed soils, it was noticed that:

(i) bacterial colonies from ashbed soil generally tended to be much smaller;

(ii) Bacillus spp., normally abundant, were absent from the ashbed soil;

(iii) a streptomycin resistant bacterium was present in the ashbed, as evidenced by its profuse growth on the fungal isolation medium containing 0.3 per cent streptomycin.

These differences indicated that there is also some change in the quality of the ashbed bacterial population and in order to determine the extent of these changes, a more precise comparison was made between the bacterial types in ashbed and untreated soils.

(b) Materials and Methods

Basis for bacterial comparison

Formal identification of bacterial species is extremely involved, based largely on a series of physiological reactions, so that comparison of the bacterial population in terms of species and genus would be a major study in its own right. For the purpose of this work, breakdown of the bacterial population into a

number of similar 'types' was based on their differential reaction to a standard set of antibiotics because:

(i) bacteria are fairly constant in their reaction to antibiotics;

(ii) a bacterium can be tested against a number of antibiotics in a single operation, either by placing individual antibiotic tablets on a single bacterial plate, or, as in this case, a paper disc impregnated with eight antibiotics commonly used in medical laboratories;

(iii) if 'typing' is based on the reaction over a spectrum of antibiotics, that is, according to resistance or susceptibility to all the antibiotics tested, a large number of subdivisions are possible depending on the number of antibiotics used. Over the range of eight antibiotics there is provision for over 250 different 'types'. It was thought that this degree of distinction should provide adequate information about the similarity or otherwise of the treated versus untreated bacterial populations.

Preparation of bacterial plates

One plate containing approximately 50 bacterial colonies was selected from the soil dilution plates obtained ten weeks after the burn (eight weeks after planting) for planted and unplanted control soil. Each bacterium on each of the plates selected was subcultured onto slopes of the bacterial isolation medium. The cultures were incubated at 27°C for four weeks.

Antibiotic reaction

A suspension of each bacterium, made in sterile distilled water under aseptic conditions, was poured onto

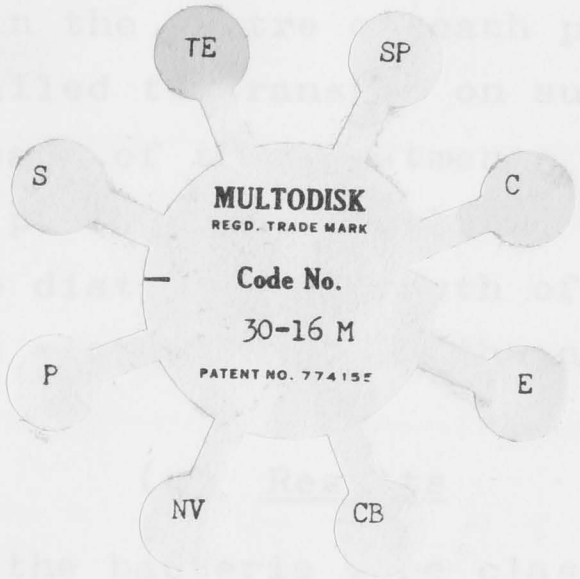


FIGURE 8 Antibiotic disc (by Oxoid Ltd)

P	-	Penicillin	1.5 units
S	-	Streptomycin	10 mcg
TE	-	Tetracyline	10 mcg
SP	-	Spiramycin	25 mcg
C	-	Chloramphenicol	10 mcg
E	-	Erythromycin	10 mcg
CB	-	Methicillin	10 mcg
NV	-	Novobiocin	5 mcg

a plate of solidified bacterial isolation medium and allowed to stand for a few minutes to permit the bacterial cells to settle onto the surface of the agar. The plates were then carefully drained and an antibiotic disc (Figure 8) was placed in the centre of each plate. Since some of the bacteria failed to transfer on subculturing, 30 cultures from each of the treatments were used for 'typing'. The plates were incubated at 27°C for four days after which the distance of growth of each bacterium from each antibiotic was measured to the nearest millimetre.

(c) Results

Initially the bacteria were classified as susceptible if there was a clear area between the edge of the colony and the disc, and resistant if there was growth right up to the disc. On this criterion of susceptibility or resistance to the eight antibiotics, 16 different types were distinguished (Table 3), of which only two were represented in both the ashbed and control populations. A greater variety of types were found in the ashbed soil which contained more bacteria resistant to the different antibiotics, as is shown in Table 4.

Just under 50 per cent of both the ashbed and control bacteria were found to be susceptible to all of the antibiotics. However, if these bacteria were compared on the basis of the pattern of reaction in terms of the actual distance between the colony margin and the edge of the disc (inhibition zone), a further 27 types were distinguished. Table 5 shows that of these 27 only seven were common to both the ashbed and control soils.

BACTERIAL TYPES								ASHBED			CONTROL		
P	S	TE	SP	C	E	CB	NV	Unplanted	Planted	Total	Unplanted	Planted	Total
-	-	-	-	-	-	-	-	14	15	29	16	13	29
x	-	-	-	-	-	-	-		1	1	12	15	27
x	-	-	-	-	-	x	-				2	2	4
x	-	-	-	-	-	-	x	1	1	2			
x	x	-	-	-	-	-	-	1	1	2			
x	-	-	-	-	-	x	x	1		1			
-	x	-	-	-	-	-	-	1		1			
-	x	-	x	-	-	-	-	1		1			
-	x	x	-	-	-	-	-		1	1			
-	-	-	x	-	-	-	-	1		1			
-	-	-	-	-	-	x	-	1		1			
x	x	-	x	x	-	x	-	1	1	2			
x	-	x	x	x	x	x	x	3	3	6			
x	-	-	x	x	x	x	x	1	1	2			
x	x	-	x	x	x	x	x	1	2	3			
x	x	x	x	x	x	x	x	3	4	7			

TABLE 3 Distribution of bacterial types, based on resistance over the whole range of antibiotics, in ashbed and control soil.

- susceptible to antibiotic
x resistant to antibiotic

RESISTANT TO	ASHBED	CONTROL
PENICILLIN (P)	43	52
STREPTOMYCIN (S)	27	-
TETRACYCLINE (TE)	23	-
SPIRAMYCIN (SP)	37	-
CHLORAMPHENICOL (C)	33	-
ERYTHROMYCIN (E)	30	-
METHICILLIN (CB)	37	7
NOVOBIOCIN (NV)	25	-

TABLE 4 Percentage of ashbed and control bacteria resistant to the different antibiotics. (Many of the bacteria were resistant to several antibiotics.)

On the same basis, the penicillin-resistant bacteria, abundant in the control population, could be broken down into 13 different types, none of which corresponded to the single representative in the ashbed population. (Details of the different types are given in Appendix Vb.)

Thus only seven of the distinguishable types were found both in the ashbed and control soil. Qualitative comparison of the planted and unplanted soil is difficult because in many cases only a single representative of a particular type was found. Even so, 58 per cent of the types were found both in the planted and unplanted ashbed and 38 per cent in both the planted and unplanted control populations. Compared with the 12 per cent coincidence of types in the ashbed and control, the differences between the planted and unplanted treatments are relatively small.

		ASHBED			CONTROL		
	TYPE	Unplanted	Planted	Total	Unplanted	Planted	Total
Types found in both populations	C	1		1	3	2	5
	D		2	2	1		1
	E		1	1		1	1
	I	1	1	2	1	1	2
	J	1		1	1	1	2
	N		1	1	1		1
	O	1		1		1	1
Types found only in control populations	B				2	2	4
	H				1	1	2
	L				1	1	2
	M				1	1	2
	Q					1	1
	X				1		1
	Y				1		1
	Z				1		1
	ZZ				1	1	2
Types found only in ashbed population	A	2	3	5			
	F	1	1	2			
	G	2	1	3			
	K	1	1	2			
	P	1	1	2			
	R	1		1			
	S		1	1			
	T		1	1			
	U	1		1			
	V	1		1			
	W		1	1			

TABLE 5. Distribution of the bacterial types showing some susceptibility to all of the antibiotics when reclassified in terms of the width of the inhibition zone.

(Appendix V(a) gives details of the criteria used to distinguish bacterial 'types' above.)

I. EFFECT OF ASHBEDDING ON SOIL MICRO-ORGANISMS

4. QUALITATIVE DIFFERENCES IN FUNGAL POPULATION

(a) Materials and Methods

Throughout the period of the ashbed study, a record was kept of the different species appearing on the soil dilution plates from the planted and unplanted ashbed and planted and unplanted control. The fungi were identified either from the colonies on the dilution plates or from subcultures on 'Difco' Czapek agar in case of the Penicillium spp. and 'Difco' potato dextrose agar in case of the other fungi.

Qualitative comparison between the ashbed and control fungal populations was made in terms of the number and variety of species. Time did not permit many of the fungi to be formally identified to species level, but all were continuously compared with type cultures maintained throughout the study.

(b) Results

Fungal population of control soil

No significant variation was found between the fungal species in the control soil either with respect to time or presence or absence of plants. All of the 33 species isolated were present both in the planted and unplanted soil over the whole period of the study.

Fungal population of the ashbed soil

For a considerable time after the burn there were far fewer species of fungi in the ashbed than there were in

the control soil. In addition, a number of species not found in the untreated soil, were predominant in the early stages of recolonisation.

The pattern of recolonisation with respect to kind and number of species is presented in a two-dimensional graph in Figure 9 in the case of the unplanted ashbed, and in Figure 10 in the case of the planted ashbed. Line (a) was obtained by plotting the total number of species that had appeared and line (c) by plotting the total number of species that had disappeared, against time since the burn. Thus the distance between these two lines represents the number of species present at any time during recolonisation. Line (b) was obtained by plotting the progressive total of distinctive 'ashbed species' (i.e. those not found at all in the control soil), so that the distance between lines (a) and (b) represents the number of species in the ashbed that were the same as in the control, and that between lines (b) and (c) the number of surviving species which were peculiar to the ashbed.

Comparison of the graphs shows that the recolonisation of the planted and unplanted ashbed followed the same pattern:

(i) the number of species isolated steadily increased with time until at the end of the 66 week period both the planted and unplanted ashbed yielded 31 species;

(ii) initially there was a predominance of species peculiar to the ashbed, but these gradually disappeared from the population;

(iii) the return of the species normally present in the soil was at first comparatively slow and only about half of the species had appeared by the end of 40 weeks. However, once

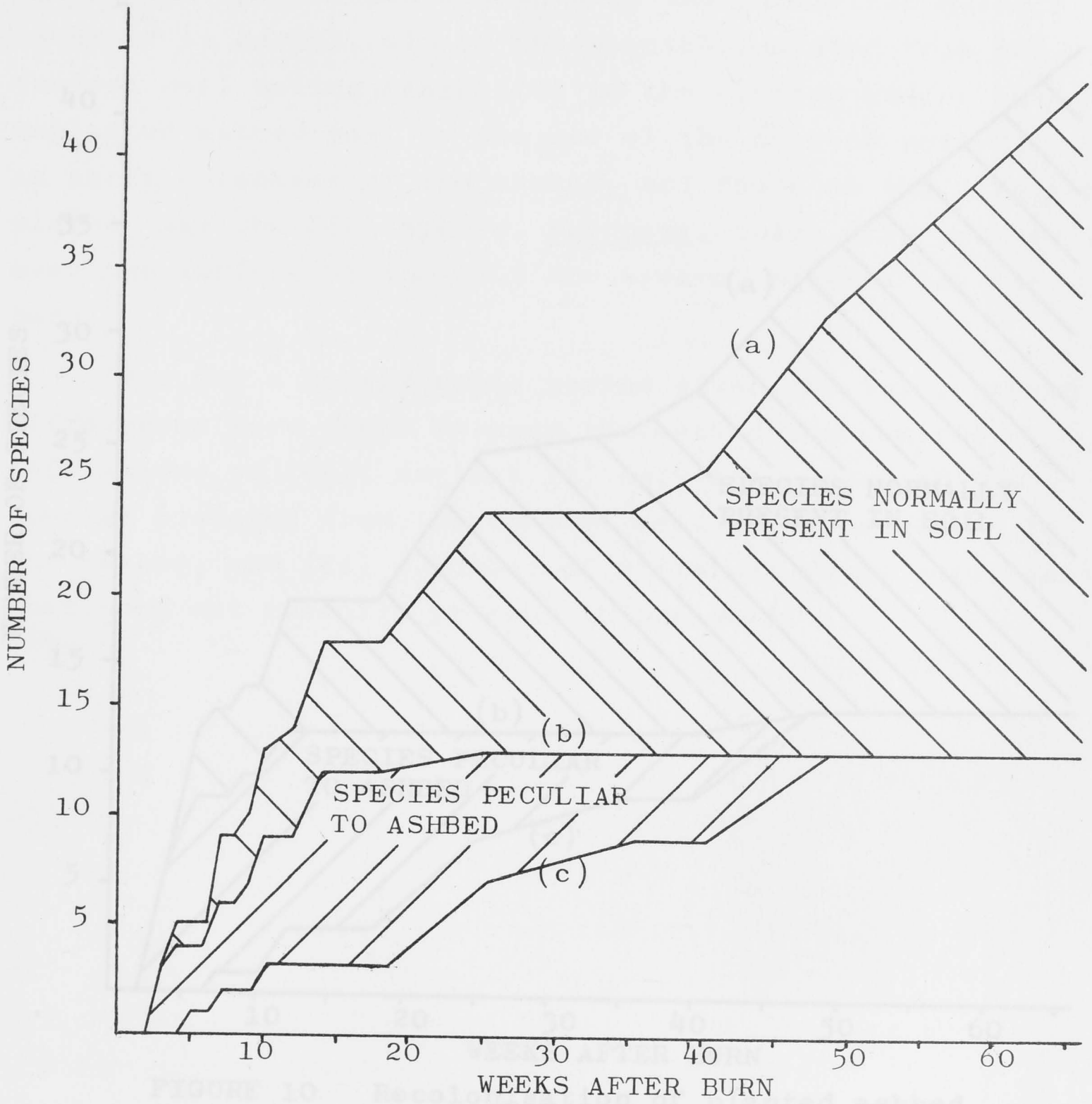


FIGURE 9 Recolonisation of unplanted ashbed

(a) Total number of all species appeared to date

(b) Total number of species peculiar to the ashbed appeared to date

(c) Total number of all species disappeared to date

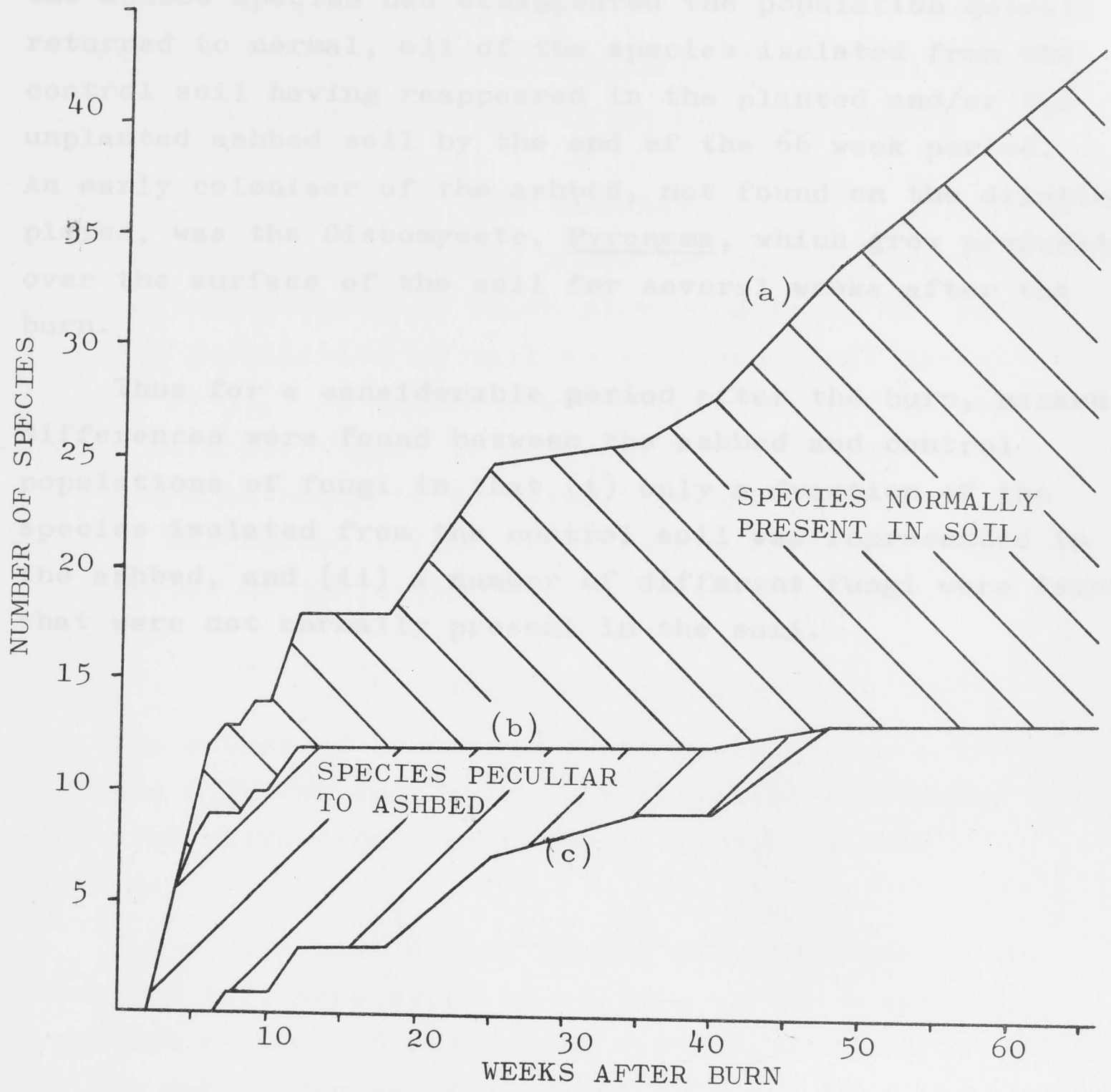


FIGURE 10 Recolonisation of planted ashbed

(a) Total number of all species appeared to date

(b) Total number of species peculiar to the ashbed appeared to date

(c) Total number of all species disappeared to date

the ashbed species had disappeared the population quickly returned to normal, all of the species isolated from the control soil having reappeared in the planted and/or the unplanted ashbed soil by the end of the 66 week period. An early coloniser of the ashbed, not found on the dilution plates, was the Discomycete, Pyronema, which grew profusely over the surface of the soil for several weeks after the burn.

Thus for a considerable period after the burn, marked differences were found between the ashbed and control populations of fungi in that (i) only a fraction of the species isolated from the control soil was represented in the ashbed, and (ii) a number of different fungi were found that were not normally present in the soil.

I. EFFECT OF ASHBEDDING ON SOIL MICRO-ORGANISMS

5. EFFECT OF ACTINOMYCETES ON PLANT GROWTH

(a) Introduction

Actinomycetes are well known for the production of antibiotics, several of which have been found to inhibit seedling germination as well as root and shoot growth (Brian, 1957). No actinomycetes were detected in the ashbed soil for almost 30 weeks after the burn, so that if they could be shown to be toxic to plant growth, at least part of the ashbed effect could be explained in terms of the removal of inhibition due to these organisms.

(b) Materials and Methods

The method of study was essentially the same as that employed by Bowen and Rovira (1961) in their study of the effect of micro-organisms on plant growth in sand cultures.

Six of the most common of the actinomycetes in the untreated soil were grown in culture on the bacterial isolation medium. A suspension containing approximately 750,000 spores per millilitre was prepared from each of the six actinomycetes. A 'paste' of spores was made with sterile distilled water in the culture tube. A loopful of this was transferred to a tube containing 4 ml of sterile distilled water and the tube shaken vigorously to separate spore clusters. The spores were washed three times by centrifuging at speed 7 for ten minutes, using fresh 4 ml lots of sterile water. In addition a mixed suspension of

spores of all six actinomycetes was prepared by mixing 1.0 ml aliquots of each suspension.

The test plant used was E. bicostata. Young, agar germinated seedlings were transplanted individually to 8" x 1" tubes containing 2" of sterile sand moistened to field capacity with half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1938). Twelve of these tubes were each inoculated with 0.2 ml of a spore suspension. In addition to the seven inoculated sets there was a set of sterile controls.

The plants were harvested after six weeks and the dry weights of the shoots obtained. Counts of the number of root tips were made, and the length of the tap root measured.

(c) Results

The oven dry weights of the shoots for the different treatments are shown in Table 6. Although the mean dry weights were generally lower for the actinomycete inoculated plants none of them were significantly different from the control at the 0.5 per cent level.

Comparison of the mean root length, or the mean number of root tips also failed to show up any differences between treatments.

Repl.	A	B	C	D	E	F	X	Control
1	14.1	30.8	33.0	28.4	36.5	28.8	31.3	33.6
2	11.7	26.7	31.9	25.1	33.9	32.6	-	27.8
3	30.5	11.7	19.2	30.6	28.7	28.9	36.4	22.9
4	30.5	32.5	28.1	27.8	33.9	19.0	24.1	33.7
5	30.4	28.5	33.4	33.8	32.4	31.7	30.2	30.9
6	21.7	28.4	27.1	24.1	28.7	22.2	32.0	-
7	22.5	33.0	28.8	27.5	30.8	12.2	25.0	28.6
8	34.5	16.0	30.7	28.9	29.8	26.1	32.0	31.9
9	32.4	32.6	27.4	40.0	8.3	27.8	33.8	35.0
10	13.4	34.2	-	22.0	36.1	22.5	22.2	34.4
11	30.6	23.2	23.3	25.6	30.5	25.5	28.5	27.2
12	-	25.8	30.1	9.3	35.4	31.3	34.6	24.7
MEAN	24.8	26.9	28.5	26.9	30.4	25.7	30.0	30.1

TABLE 6 Oven dry weights of shoots of E. bicostata seedlings grown for six weeks in sand culture.

A - F : six different actinomycetes

X : mixture of the six actinomycetes

I. EFFECT OF ASHBEDDING ON SOIL MICRO-ORGANISMS

6. DISCUSSION

The foregoing results provide more or less explicit answers to some of the questions posed in the introduction.

Firstly, there is no doubt that substantial sterilization of the soil was achieved. Microbial counts before and after the burn revealed that the depth to which the soil was sterilized was greater than would be expected according to the soil temperature, and that a substantial kill of the microbial population occurs at temperatures somewhat below 100°C. It seems likely then that Lawrence's figure of 70°C, rather than that of 127°C is sufficient to bring about effective sterilization of the soil. In light of this evidence the temperature recordings obtained for other burns (Beadle, 1940; Miller, Stout and Lee, 1955; Humphreys and Lambert, 1965; Cromer and Vines, 1966) show that in six out of the ten burns a substantial kill of the soil population would have occurred down to the depth of six inches or more, in three down to two or three inches, and only in one was there no effective sterilization. Thus, although the depth to which the soil is sterilized may vary from one burn to another, in general, sterilization is a common feature. Unfortunately it is not known whether these ashbeds were all capable of producing the ashbed effect.

Secondly, recolonisation commenced immediately the sterilized ashbed soil was watered. The plots were watered for two reasons: (i) to alleviate the drought

conditions prevalent at the time and (ii) to ensure detoxification of the soil prior to planting. Only bacteria were detected in the first flush of colonists, but the numbers attained within five days of watering were quite startling. Neither the wind-blown dust, which undoubtedly would have supplied a miscellany of organisms of the type normally found in the soil to the surface of the ashbed, nor the chlorinated water applied, which in spite of the treatment also contained a variety of organisms, could have singly or together accounted for the numbers of bacteria found in the soil, indicating that there was considerable reproductive activity during these first five days.

Sufficient water (about two inches) was supplied to simulate a short intensive rainfall, so that the moisture requirements for detoxification could be met. As was mentioned in the introduction, heated soil is readily detoxified if watered for about one week. In course of a later experiment in this work, however, it was found that if insufficient water is supplied to the heated soil, detoxification is delayed for some time. It is quite likely that under field conditions, detoxification of the ashbed soil could take even a month or more depending on the amount of rainfall after burning.

Since the initiation of detoxification was accompanied by a spectacular flush in the number of bacteria in the ashbed soil, it seems very likely that bacteria are in some way involved in removing the toxins from the soil. The biological nature of detoxification was proposed as early as 1908 by Pickering, when he noticed that the time taken for the growth response to appear varied from one

apple tree to another, arguing that 'chance reinoculation of the soil was very possible, whereas chance alteration of its chemical character was hardly so'. More recently Rovira and Bowen (personal communication) have shown that detoxification is in fact brought about by specific organisms: 40 per cent of the fungal isolates and 10 per cent of the bacterial isolates tested proved to be effective in removing the toxic factors from heated soil. Although their work suggests that fungi may be more effective agents, their absence in the ashbed soil during the period of detoxification leaves no doubt that bacteria alone are quite adequate. The phytotoxins are believed to be organic compounds (page 7) and apparently certain groups of organisms are able to metabolise them and thereby remove them from the soil.

The very removal of the toxins would undoubtedly alter the chemistry of the heated soil during detoxification, but what may be of greater significance in relation to the ashbed effect is the possibility that, as a result of the metabolic activity of these organisms, by-products in the form of plant nutrients may be formed. An attempt will be made in a later section of this work to follow the chemical changes taking place in the heated soil both during and after the period of detoxification.

Thirdly, the population of early colonisers is completely different, both quantitatively and qualitatively, from that in the soil before sterilization took place. In the early stages, for instance, no actinomycetes and only very small numbers of fungi (about 1/100th of the numbers before burning) were detected, and the bacterial population

was shown to contain markedly different types. The early fungal population too was shown to consist mainly of forms absent from the untreated soil or present in such small numbers that they could not be detected. As found previously by Wright and Tarrant (1957), the number of prokaryotic organisms (bacteria and actinomycetes) as well as the bacteria/actinomycete ratio was much higher in the ashbed soil than in the untreated soil.

As far as can be determined from the miscellany of data available, other sterilization treatments result in changes in the microbial population similar to those observed in the ashbed. After initial marked decreases or complete kill, subsequent large increases in bacterial numbers have been reported following fumigation (Buddin, 1914; Waksman and Starkey, 1923) and after steam sterilization (Osmun, 1906; Lodge and Smith, 1912; Russell and Hutchinson, 1909; Waksman and Starkey, 1923; Ludwig and Henry, 1943). Russell and Hutchinson also suggested that the bacterial population consisted of different types, but they failed to support this with experimental evidence. The fungal population after steam sterilization or fumigation has been shown to be quite different from that prior to treatment by many workers, among them Ludwig and Henry (1943), Warcup (1951a) and Martin (1950). The latter also found that the nature of the new fungal population depended on the degree of sterilization. In general, fungal recolonisation was found to be very slow, although Ludwig and Henry (1943) showed that if the sterilized soil was deliberately reinoculated a large increase in the number of fungi was observed after only four days incubation. Very little information is available about the microbial

population subsequent to irradiation, but Rambelli (1963) reports that in Co^{60} irradiated soils the return to normal conditions with respect to microbes is very slow.

The magnitude of the changes induced in the microbial population by ashbedding makes it entirely plausible that this could play a prominent role in the plant growth response. Establishing a causal relationship between changes in the microbial population and stimulation of plant growth is quite another matter.

The one attempt to establish such a relationship, namely between the absence of actinomycetes in the ashbed and plant growth, proved inconclusive. It could well be that other actinomycetes or even certain fungi do inhibit plant growth, so that until further experimental evidence is produced, the removal of a net inhibition cannot as yet be excluded as a possible factor in the ashbed effect.

Other possible factors involving microbes are:

(i) Plant pathogens are removed from the soil on sterilization. The practice of soil sterilization in nurseries has been carried on since before the turn of the century for this very purpose. Soil that has been used over and over again for growing the same crop tends to become saturated with pathogens so that subsequent crops are very much reduced, and it was found that if such soils are sterilized, the productivity of the soil is restored. One of the earliest accounts of this phenomenon was published in 1895 by Behrens, who found that it 'onion-sick ground' is treated with carbon bisulphide the crop was doubled. It is quite possible that the growth stimulation

reported by the earlier workers who used nursery soils, was in fact largely due to the removal of pathogens. However, this would not explain why Pinus spp. grow so well in ashbeds, because soil which had previously supported the growth of native Eucalyptus spp. would not be expected to contain a large population of microbial species pathogenic to Pinus.

(ii) Another popular contention is that on heating the soil microbes are killed, removing competition for plant nutrients so that plant growth is enhanced. For example, increased root development in subterranean clover when grown under sterile conditions has been reported by Rovira and Bowen (1960). This argument is not supported by the microbial data obtained for the ashbed. The soil does not remain sterile: recolonisation begins as soon as water is added to the soil. Furthermore, at the time of planting two weeks after the burn, the total number of microbes in the ashbed was actually ten times as great as that in the untreated soil. In fact, heat sterilized soils can only be planted after detoxification of the soil has taken place.

(iii) For a considerable time after the burn there was not only a qualitative difference between the ashbed and control populations, but also a marked imbalance of the relative proportions of the three major groups of microbes normally found in the soil. It seems very probable that due to these differences in the ashbed population the nutrient status of the ashbed soil could be considerably altered. Since all the nitrogen transformations in the soil are carried out by micro-organisms, it was thought that an intensive study of the levels of nitrogen after soil

sterilization might be profitable. This study will be treated in a later section.

(iv) Yet another possibility is that soil subsequent to sterilization contains a predominance of microbes, or even substances released from the microbial cells on their death, that are capable of directly stimulating plant growth. Panosyan, Arutyunyan and Avetisyan (1962) have shown that certain bacteria excrete metabolic substances capable of stimulating the growth of Azotobacter and nitrogen fixation by it. These substances were found to increase the stimulatory effect of Azotobacter on crop yields, while some of them were found to increase yields even in the absence of Azotobacter.

Finally, it was seen that the microbial population gradually returned to 'normal' by the gradual reappearance of the usual forms and the disappearance of the distinctive early colonisers, but even after 66 weeks some differences between the populations still existed, but these appeared to be negligible to those noted over the first few months of recolonisation. This time period is particularly interesting because it is of the same order as was suggested by the Pinus radiata growth curves of Humphreys and Lambert for the operation of 'ashbed factors'. The next section attempts to get more information about the duration of the ashbed factors in the ashbed in question.

If the time scale of events do coincide, then there is a strong possibility that the differences in the microbial population are important causal factors in the ashbed effect. It must be noted, though, that both the microbial population and the plants could, of course, be simultaneously altered by some yet unknown factor.

II. PLANT GROWTH IN ASHBED AND HEATED SOIL

1. TOTAL ASHBED EFFECT

(a) Materials and Methods

Two weeks after burning, nine E. bicostata seedlings were planted in the southern ashbed and control plots. Heights attained by the trees were recorded in centimetres at intervals after planting.

(b) Results

The mean growth curves on and off ashbed are shown in Figure 11. The control plants did not gain much height until the end of winter whereas the ashbed ones showed an immediate increase, and after only six weeks growth their mean height was already significantly greater (5 per cent) than that of the control trees. At the final measurement, a little over $2\frac{1}{2}$ years after planting, the mean height of E. bicostata in the ashbed was 1.9 times that in the control.

It is interesting to note that in spite of the large height difference between E. bicostata on and off the ashbed, mature leaves were formed at the same time after about two years growth.

Plates 5 and 6 illustrate the magnitude of the ashbed effect after one years growth.

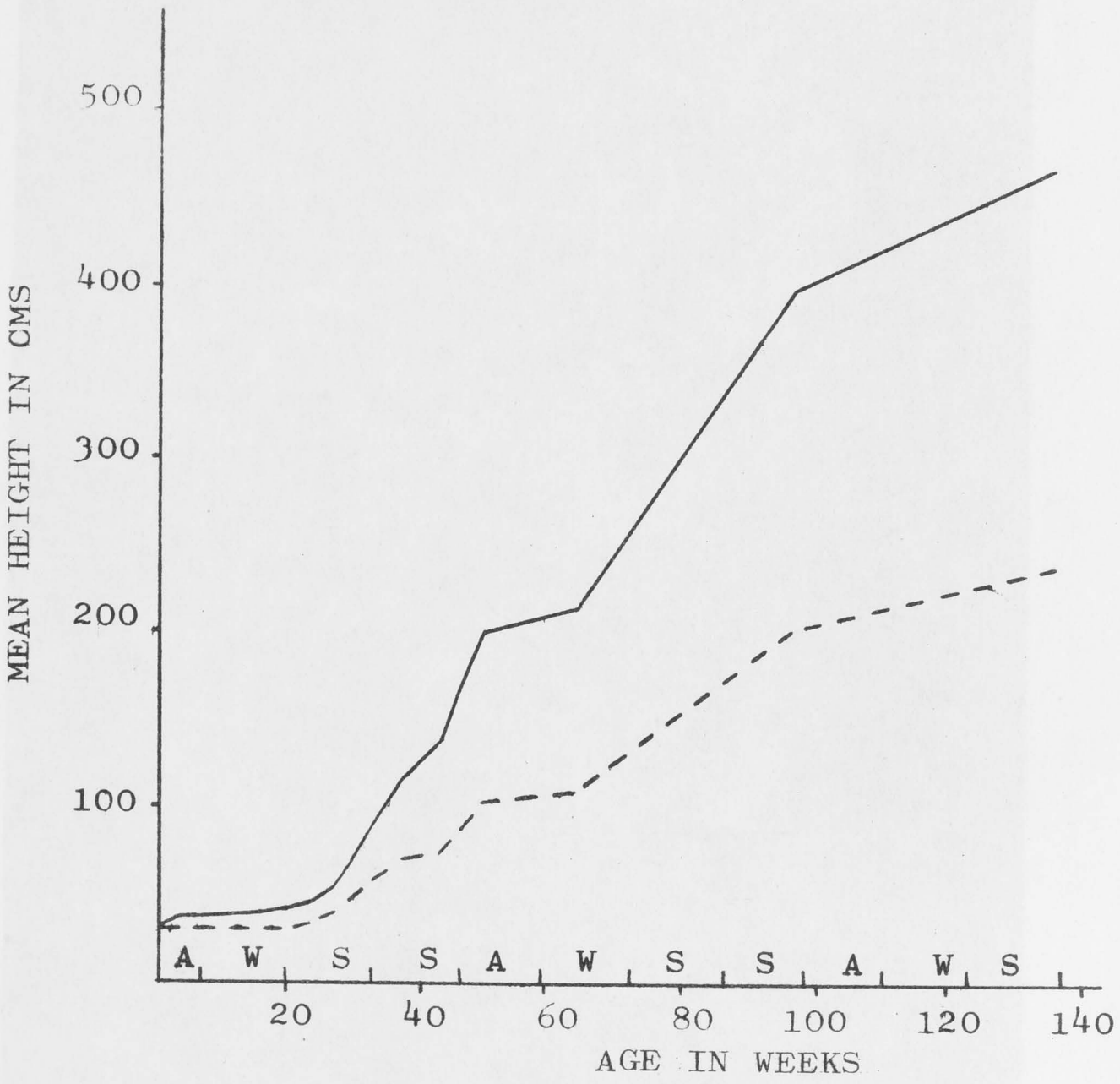


FIGURE 11 Growth curves of E. bicostata on and off ashbed

——— ashbed

- - - - control

PLATE 5 One year old E. bicostata in ashbed plot

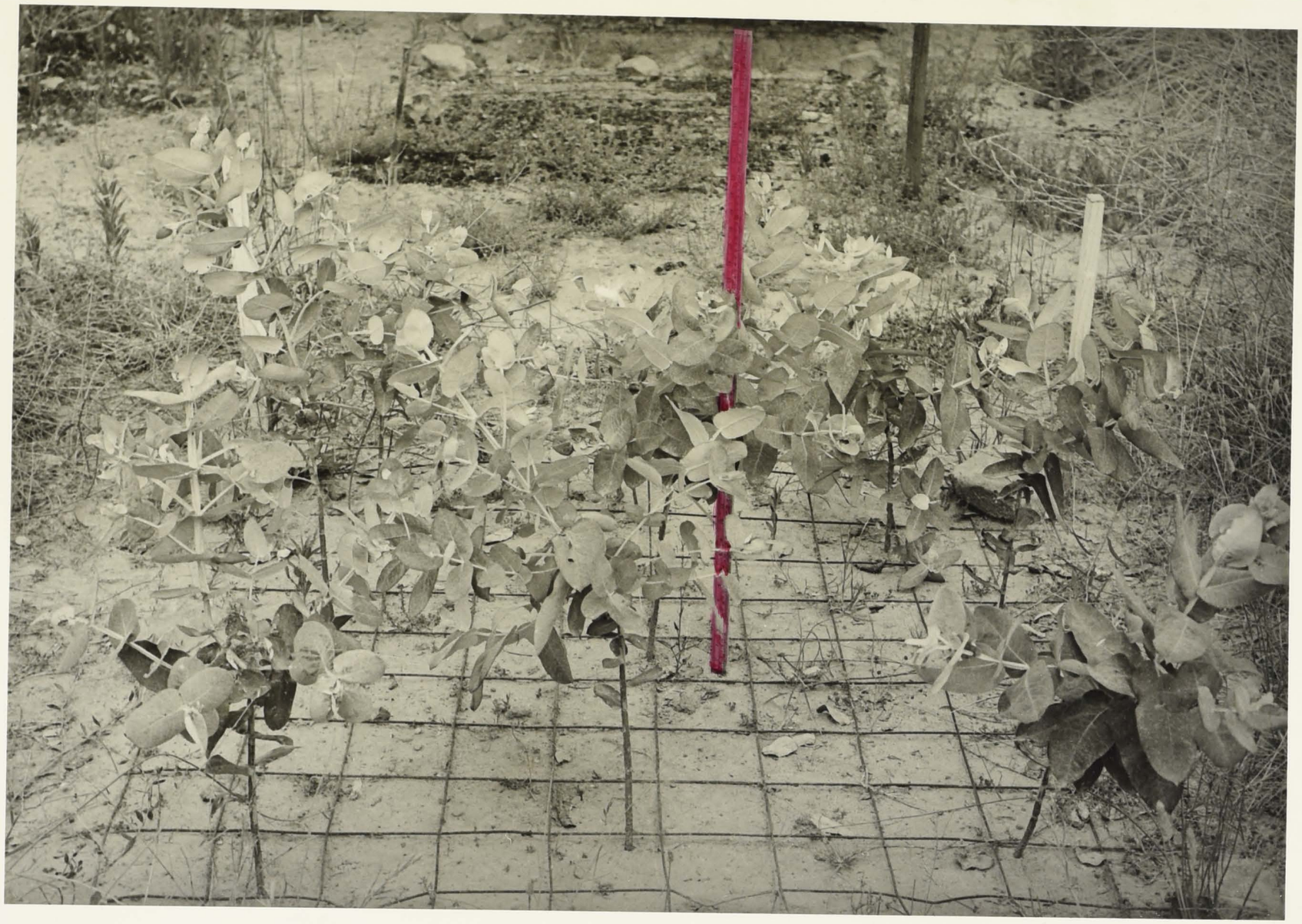


PLATE 6 One year old E. bicostata in control plot

II. PLANT GROWTH IN ASHBED AND HEATED SOILS

2. ASHBED EFFECT AT DIFFERENT DEPTHS IN SOIL PROFILE

(a) Materials and Methods

After burning, soil was collected from the area between the two ashbed and two control plots in conjunction with preparation of drains, samples representing soil depths of 0-2", 2-4", 4-6", 6-8" and 8-10". Soil from each depth and from both treatments was used to prepare eight pots which were planted with *E. grandis* seedlings on 12/4/65. The plants were harvested on 5/8/65 and the oven dry weights of the shoots and roots determined.

(b) Results

Figure 12 shows that the total dry weights of the plants in ashbed soil were significantly greater than those in the control soil at all depths except 0-2", in which case the dry weights were greater in the control, indicating that the top two inches of the ashbed soil were actually detrimental to growth.

In general there was a decline in growth response with increasing depth of soil reflecting the decline in soil fertility down the profile.

So that the ashbed effect with depth of soil could be assessed, the response due to differences in soil fertility down the profile was eliminated by expressing the ashbed response at a given depth as a percentage of the response in control soil at the same depth (Figure 13).

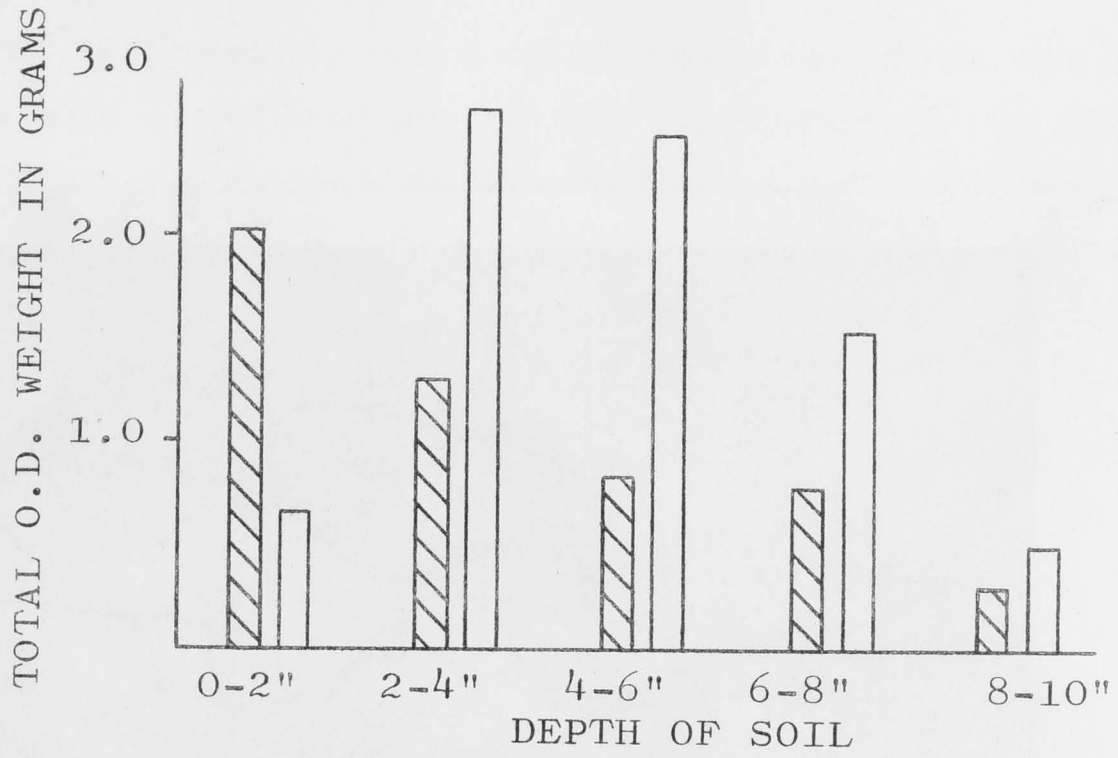


FIGURE 12 Total oven dry weights of E. grandis in pots of ashbed and control soil taken from different depths

control ashbed

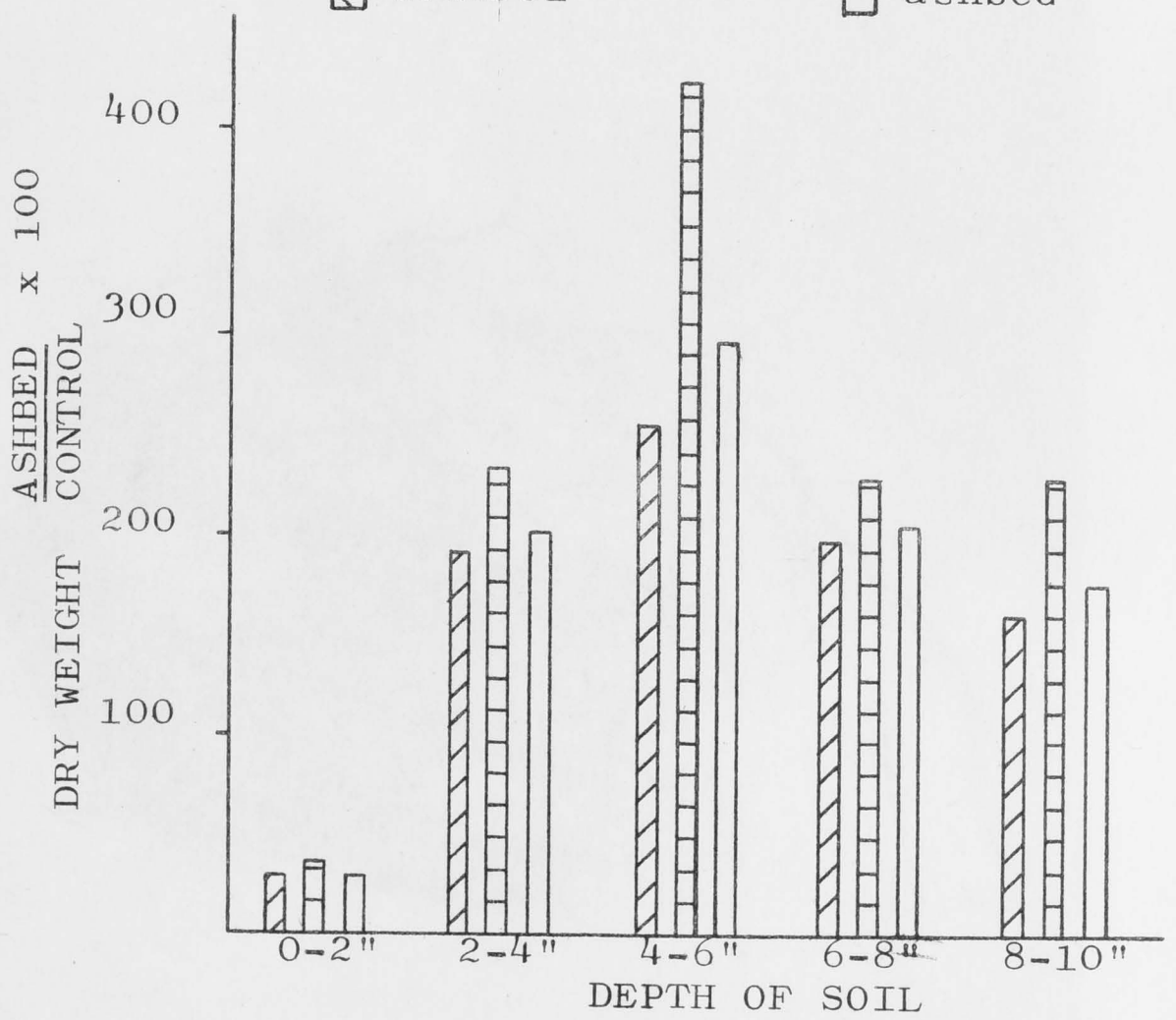


FIGURE 13 Response of E. grandis to different depths of ashbed soil expressed as percentage of control growth at same depth

shoots roots total

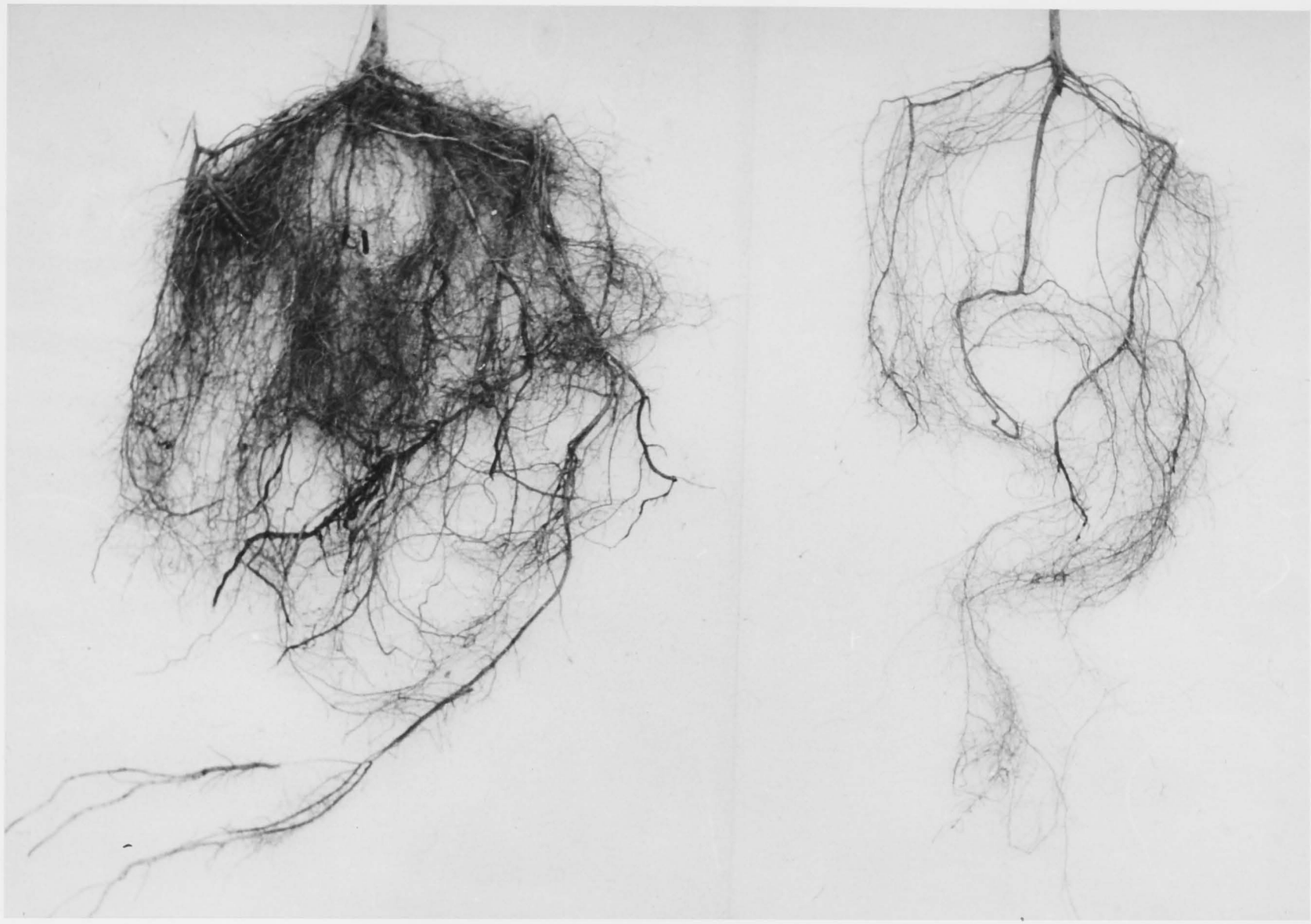


PLATE 5 Representative root system of E. grandis in ashbed soil on the left, and in control soil on the right (4-6" depth)

The differences in ashbed response down the soil profile are a reflection of the differences in the temperature treatment during ashbedding. In Figure 14 the ashbed response was plotted against the mean maximum temperature to which the soil was heated at the different depths. The maximum response was in the soil heated to approximately 150°C. The rate of decline in response with increase in temperature above 150°C suggests that 450°C is the maximum temperature to which the soil can be heated before the soil becomes detrimental to growth.

At all depths there was greater stimulation of the roots than of the shoots, which is reflected in the appearance of the ashbed and control root systems (e.g. Plate 7). The root systems of the plants in the ashbed soil were much larger and had more abundant coarse and fine roots. Figure 15 shows the positive correlation between the number of lateral roots greater than 1.0 mm in diameter in the ashbed and control plants and the total dry weights obtained.

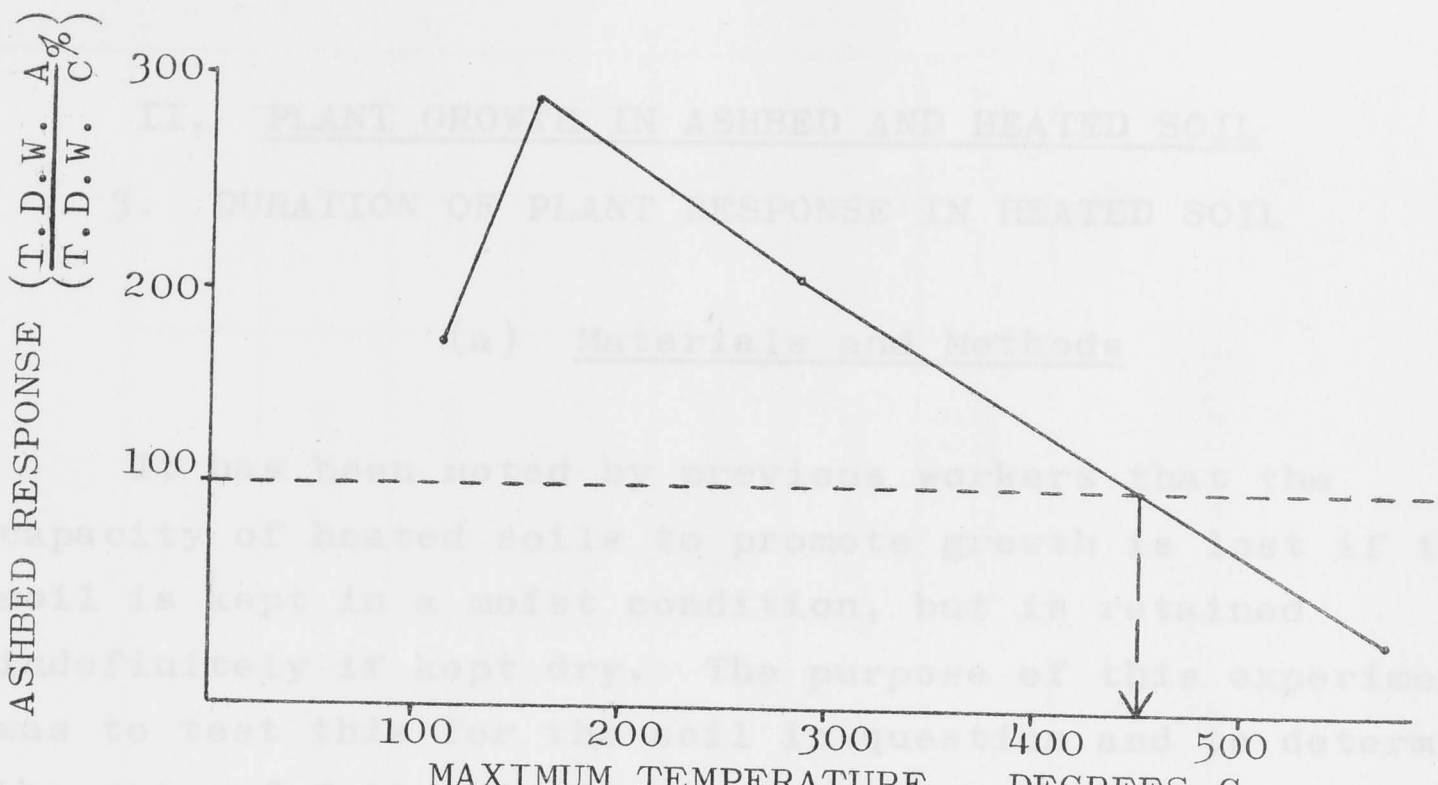


FIGURE 14 Relationship between the ashbed response and temperature attained during ashbedding. The dotted line indicates the limit between stimulation and inhibition of growth.

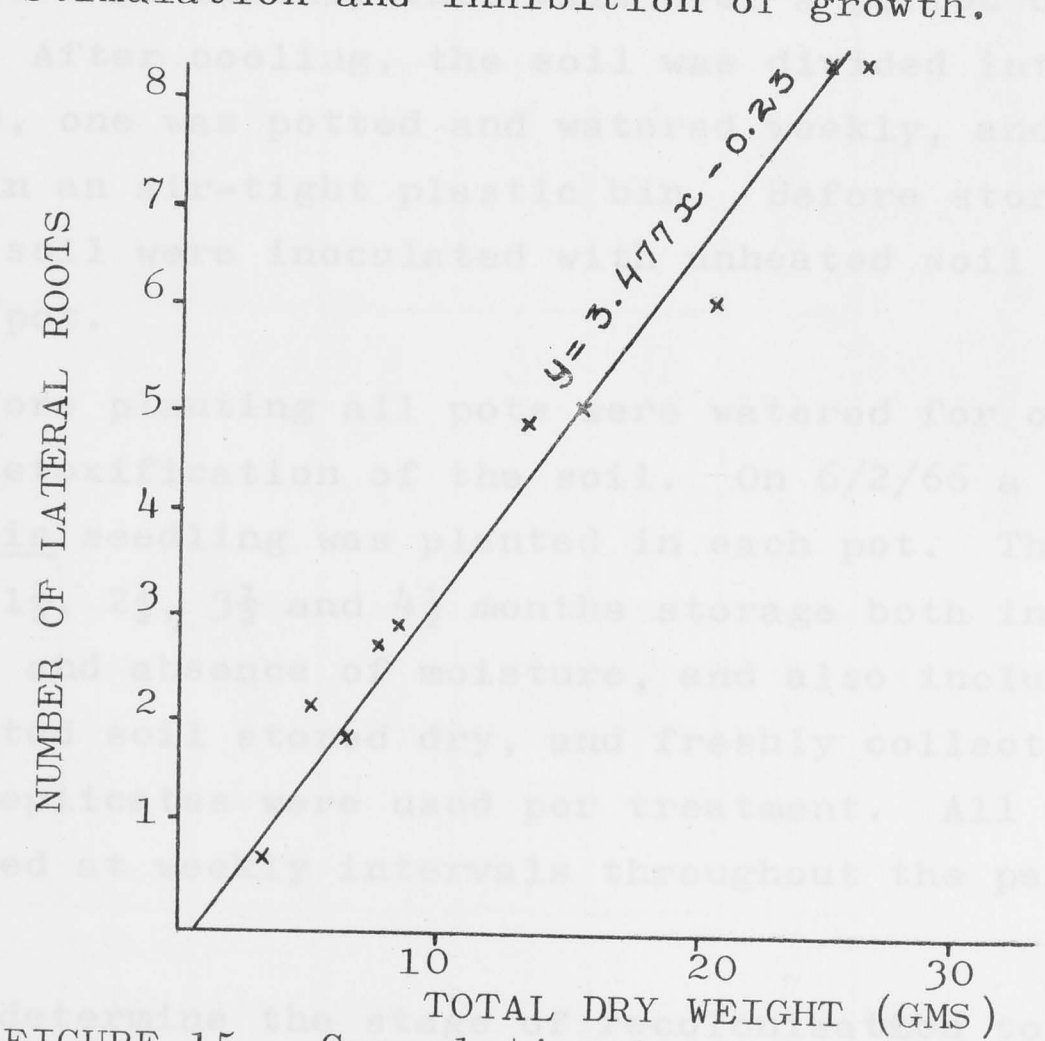


FIGURE 15 Correlation between total dry weight and number of lateral roots > 1.0 diameter (r = 0.9)

II. PLANT GROWTH IN ASHBED AND HEATED SOIL

3. DURATION OF PLANT RESPONSE IN HEATED SOIL

(a) Materials and Methods

It has been noted by previous workers that the capacity of heated soils to promote growth is lost if the soil is kept in a moist condition, but is retained indefinitely if kept dry. The purpose of this experiment was to test this for the soil in question and to determine the rate of fall-off of the response.

Air dried soil was heated in an oven at 150°C for three hours at monthly intervals over a period of five months. After cooling, the soil was divided into two portions, one was potted and watered weekly, and the other stored in an air-tight plastic bin. Before storage, both lots of soil were inoculated with unheated soil at the rate of 5 gm/pot.

Before planting all pots were watered for one week to ensure detoxification of the soil. On 6/2/66 a single *E. grandis* seedling was planted in each pot. The treatments were $\frac{1}{2}$, $1\frac{1}{2}$, $2\frac{1}{2}$, $3\frac{1}{2}$ and $4\frac{1}{2}$ months storage both in the presence and absence of moisture, and also included samples of unheated soil stored dry, and freshly collected soil. Twelve replicates were used per treatment. All pots were randomised at weekly intervals throughout the period of growth.

To determine the stage of recolonisation to which the soils had reached during storage, microbial numbers were

LENGTH OF STORAGE (MONTHS)	DRY			WET		
	BACT.	ACT.	FUNGI	BACT.	ACT.	FUNGI
0.5	539	120	40	8,000,000	40,000	500
1.5	503	98	28	32,000,000	120,000	2,000
2.5	400	109	34	22,000,000	1,050,000	35,000
3.5	570	89	54	67,000,000	840,000	24,500
4.5	440	125	38	34,000,000	2,280,000	45,800
Control	4,600,000	2,830,000	137,000	5,500,000	2,050,000	210,000

TABLE 7 Microbial numbers per gram O.D. soil before planting

estimated for all treatments just before planting. The method used was the same as for the ashbed study.

The plants were harvested after nine weeks growth and the oven dry weights determined.

(b) Results

Microbial counts

Results of the microbial counts are summarised in Table 7. The heated soils which had been stored in the absence of moisture show no evidence of recolonisation, indicating the need for moisture. The constant low numbers detected represent the numbers introduced in the inoculum before storage.

The rapid return of the actinomycetes in heated soil under moist conditions is surprising in view of the ashbed results. Bacterial and fungal recolonisation, however, compares favourably with that in the ashbed.

It is interesting to note that Pyronema which grew profusely over the ashbed for some time after the burn, also appeared on the surface of the heated soil in all pots within a few weeks of watering.

Growth response

The mean oven dry weights for the different treatments and the level of significant differences between wet and dry treatments are given in Table 8.

The magnitude of the growth response in the heated soils was expressed as the mean O.D. weight in heated soil/ mean O.D. weight in unheated soil. The mean of 0.66 was used for the unheated soil. The values obtained were

LENGTH OF STORAGE (MONTHS)	DRY / WET	MEAN O.D. WEIGHT (GMS)	t	LEVEL OF SIGNIFICANT DIFFERENCE
0.5	D	2.20	0.45	-
	W	2.10		
1.5	D	2.14	1.06	-
	W	1.94		
2.5	D	2.23	2.27	5%
	W	1.77		
3.5	D	2.20	2.90	1%
	W	1.58		
4.5	D	2.17	3.84	0.1%
	W	1.49		
Control	Stored air dry	0.65	0.19	-
	Fresh	0.67		

TABLE 8 Summary of growth response of E. grandis in heated soil stored in the presence and absence of moisture.

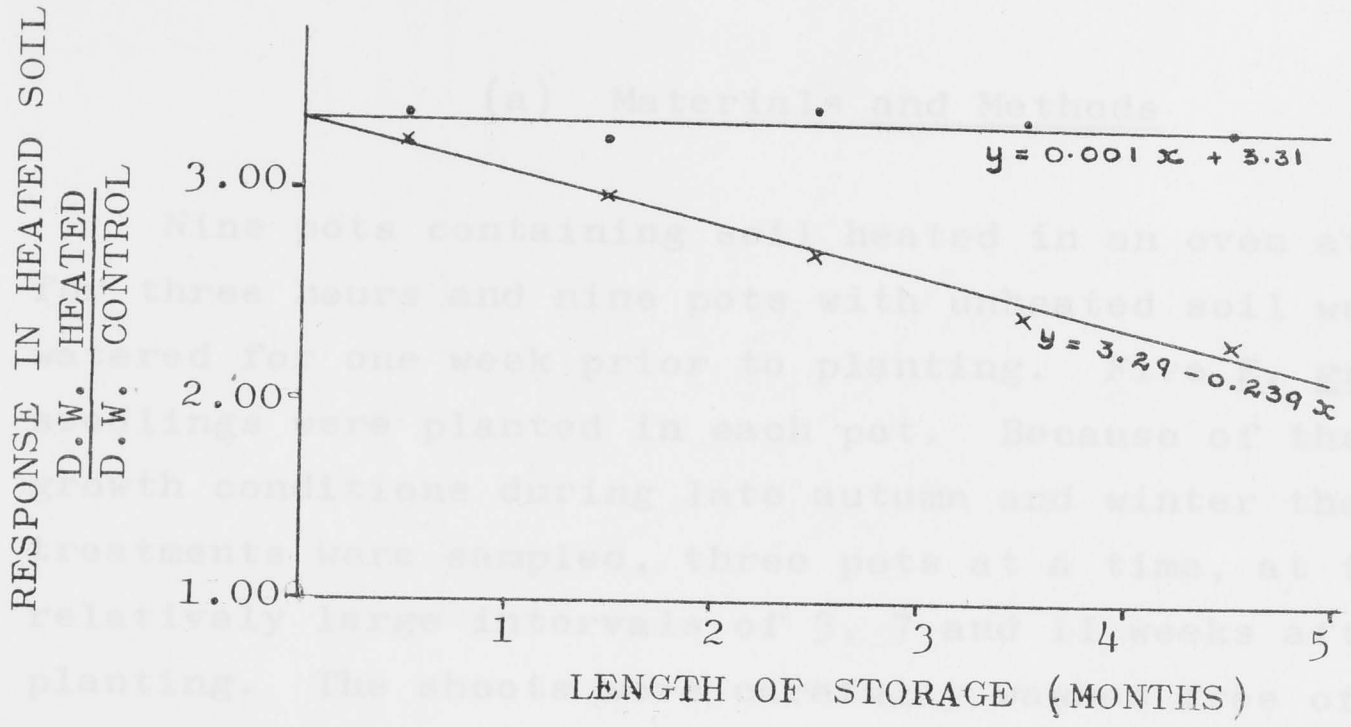


FIGURE 16 Growth response of E. grandis in soils stored in the presence or absence of moisture for varying lengths of time

• Dry storage

× Wet storage

plotted against length of storage (Figure 16) and the line of best fit calculated. There was no decline in the growth response with respect to length of storage under dry conditions, whereas there was a distinct decline in response in soils stored in the presence of moisture. Assuming that the growth response continues to decline linearly, it can be calculated from the line of best fit that heated soil, in the presence of moisture, will lose its capacity to stimulate plant growth in just over $9\frac{1}{2}$ months.

II. PLANT GROWTH IN ASHBED AND HEATED SOIL

4. ROOT DEVELOPMENT AND GROWTH IN HEATED SOIL

(a) Materials and Methods

Nine pots containing soil heated in an oven at 150°C for three hours and nine pots with unheated soil were watered for one week prior to planting. Five E. grandis seedlings were planted in each pot. Because of the poor growth conditions during late autumn and winter the treatments were sampled, three pots at a time, at the relatively large intervals of 3, 7 and 11 weeks after planting. The shoots were carefully washed free of soil, cleared in lactophenol containing 0.25 parts per thousand cotton blue (Smith, 1960), mounted in clear lactophenol and the number of root tips counted under a stereomicroscope.

(b) Results

The dry weights of the shoots and the number of root tips obtained for the two treatments are presented graphically in Figures 18 and 19 respectively. Both the number of root tips and the oven dry weights of the shoots were much greater for the seedlings in heated soil than those in the control soil. The rate of increase in shoot weight with time was very similar to the rate of increase in the number of root tips in both the heated and unheated soils.

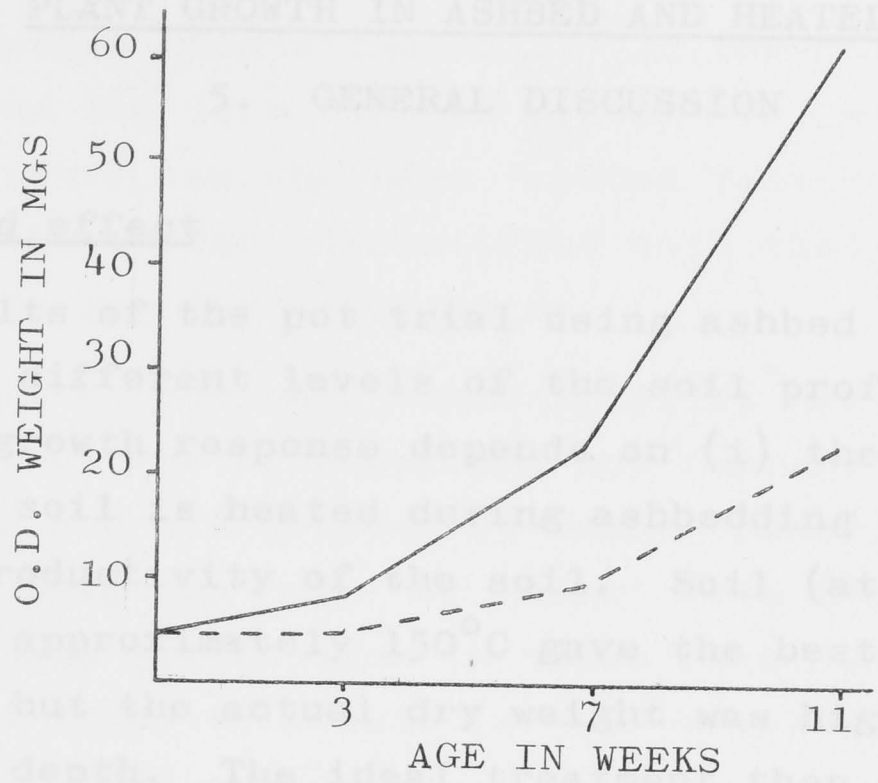


FIGURE 18 Oven dry weights of E. grandis seedlings grown in heated and unheated soils.

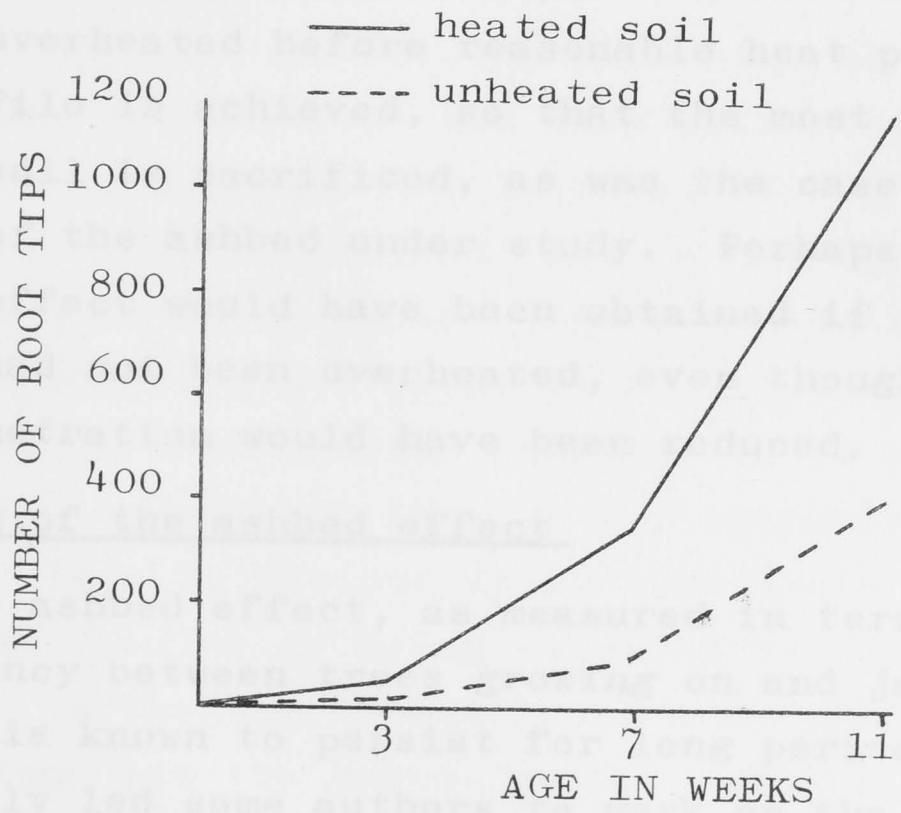


FIGURE 19 Number of root tips of E. grandis seedlings grown in heated and unheated soils.

——— heated soil

----- unheated soil

II. PLANT GROWTH IN ASHBED AND HEATED SOIL

5. GENERAL DISCUSSION

The ashbed effect

Results of the pot trial using ashbed and non-ashbed soil from different levels of the soil profile indicate that the growth response depends on (i) the temperature to which the soil is heated during ashbedding and (ii) the initial productivity of the soil. Soil (at 4-6" depth) heated to approximately 150°C gave the best relative response, but the actual dry weight was higher in the soil from 2-4" depth. The ideal treatment then appears to be to heat the soil to about 150°C down the whole profile. In practice, however, the top few inches of the soil tend to get overheated before reasonable heat penetration down the profile is achieved, so that the most productive part of the soil is sacrificed, as was the case of the top two inches of the ashbed under study. Perhaps a greater overall ashbed effect would have been obtained if the top two inches had not been overheated, even though the depth of heat penetration would have been reduced.

Duration of the ashbed effect

The ashbed effect, as measured in terms of a height discrepancy between trees growing on and just beside an ashbed, is known to persist for long periods. This has apparently led some authors to work on the assumption that the soil changes directly responsible for the stimulation are also long lasting. This does not necessarily follow of course. The assumption appears to arise out of semantic

confusion resulting from the use of the term 'ashbed effect' to describe both the plant stimulation and the causal changes within the soil. In the following discussions the term 'ashbed effect' is reserved for the plant response and the term 'ashbed factors' is used to describe the so far unidentified soil changes which are responsible for the stimulation.

Chilvers (personal communication) has shown that if the growth curves of Humphreys and Lambert for Pinus radiata on and off ashbeds at Sunny Corner, N.S.W. are compared along the 'age' or 'time' axis (Figure 20) to determine the discrepancy in times required by ashbed and non-ashbed trees to attain various heights, then 3 year old, 4 feet high ashbed trees are found to have acquired a maximum 'time lead' of $15\frac{1}{2}$ months over non-ashbed trees. This lead is maintained through the rest of the growth curves. In other words, the form of both curves beyond this point is identical and they differ only in being separated by a horizontal interval of $15\frac{1}{2}$ months. It seems that this 'time lead' may be the best way to measure the magnitude of an ashbed effect especially when comparing the phenomenon on different sites. But more than this, it enables a more meaningful estimate to be made of the period over which the ashbed factors are operating to produce the effect. This is because it is reasonable to assume that no extra stimulus is being received by the ashbed trees relative to the non-ashbed trees once the maximum time lead is attained and the growth curves take on an identical form. Thus in the case of the trees at Sunny Corner, the maximum time over which the ashbed factors could have operated was three years. More startling than this, one half of the

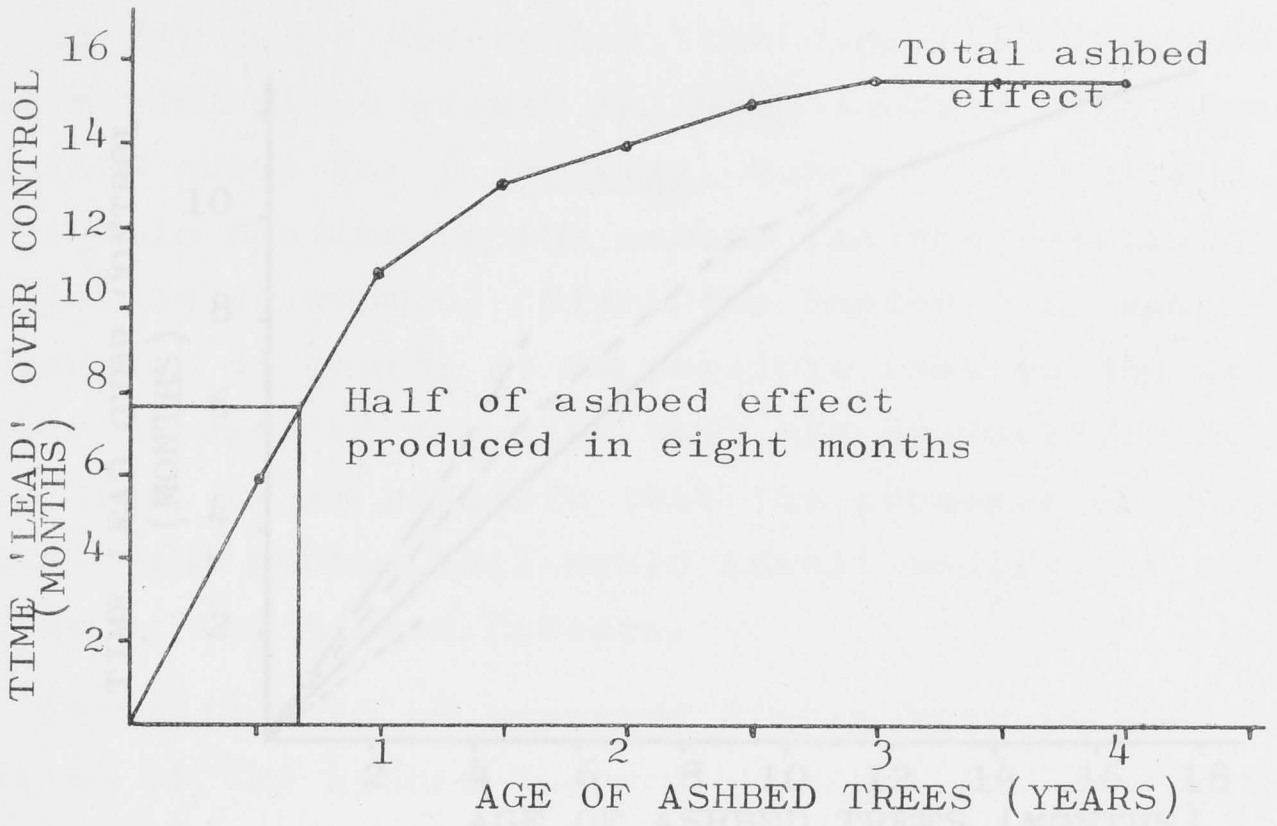


FIGURE 20 Graph showing progressive time lead of ashbed trees over control trees redrawn from P. radiata growth curves at Sunny Corner, N.S.W. (Humphreys and Lambert, 1965).

By courtesy of G.A. Chilvers, Department of Botany, A.N.U.

total ashbed effect, measured as a time lead, was apparently generated in only 8 months.

Unfortunately the growth of E. bicostata in the A.N.U. ashbed experiment was not sufficiently advanced to determine the point at which the time lead of the ashbed trees reached a maximum, but the portion of the curve that is available corresponds well with that obtained for Pinus radiata (Figure 21). Two sets of growth curves obtained by McIntyre (1966) for E. grandis at Coff's Harbour, N.S.W. were also used to construct time lead graphs during the first few months of growth. Although the time lead curves available

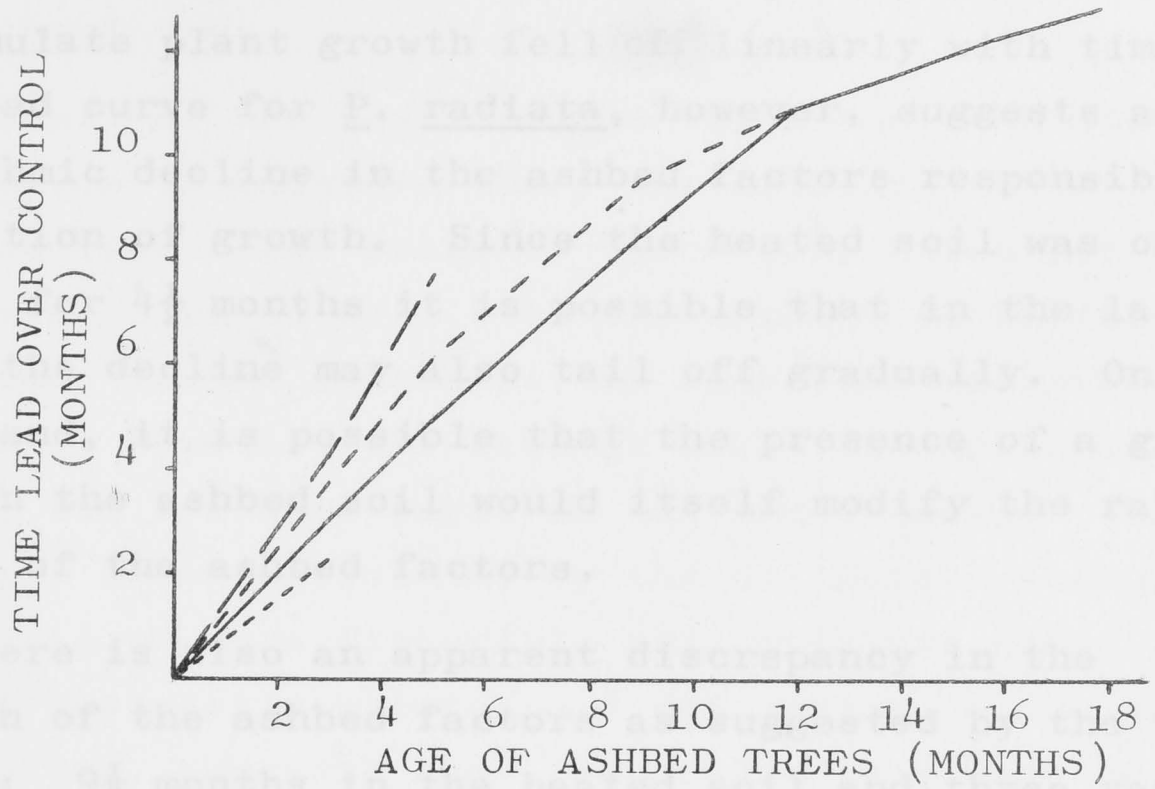


FIGURE 21 Comparison of 'time lead' curves for ashbed trees at four different sites.

——— P. radiata, Sunny Corner, N.S.W.

— — E. grandis, Coff's Harbour, N.S.W. (Site A)

------ E. grandis, Coff's Harbour, N.S.W. (Site B)

---- E. bicostata, A.N.U., A.C.T.

to date do not allow any firm conclusions to be drawn about the length of the period over which the ashbed factors are in operation, it is perhaps most significant that the early parts of the time lead curves, in spite of the variation in tree species and the widely separated sites, should in all cases be so similar.

Period of growth stimulation in heated soil

As far as it was traced, the capacity of heated soil to stimulate plant growth fell off linearly with time. The time lead curve for P. radiata, however, suggests a logarithmic decline in the ashbed factors responsible for stimulation of growth. Since the heated soil was only studied for $4\frac{1}{2}$ months it is possible that in the later stages the decline may also tail off gradually. On the other hand, it is possible that the presence of a growing plant in the ashbed soil would itself modify the rate of decline of the ashbed factors.

There is also an apparent discrepancy in the duration of the ashbed factors as suggested by the two sets of data: $9\frac{1}{2}$ months in the heated soil and three years in the ashbed. This discrepancy may be because the heated soil was watered regularly whereas the ashbed in question depended on intermittent rainfall. The heated soil experiment shows clearly how dependent on water the decay process is. The evidence on both counts is nevertheless consistent in pointing to a very short survival time of the ashbed factors.

Microbial numbers in heated soil

The counts obtained for the different treatments prior to planting not only lend further support to some of the observations made in the ashbed study, but also suggest that the microbes are quite intimately involved with the stimulatory factors in the sterilized soil.

(i) There was no evidence of recolonisation in the soils which had been stored in the absence of moisture showing that the mere application of inoculum is insufficient to initiate recolonisation if moisture is unavailable.

(ii) The factors responsible for growth stimulation can evidently be preserved indefinitely, provided the heated soil is kept dry.

(iii) In all of the heated soils which had been stored in the presence of moisture there was evidence of recolonisation. The stage to which recolonisation had progressed compared well with the numbers of bacteria and fungi found in the ashbed at the same time intervals after treatment. However, the rate at which the actinomycetes returned was far more rapid in the heated soil. This could have been facilitated by the deliberate inoculation of the soil or more favourable soil moisture conditions. The fact that actinomycetes did not appear in the ashbed until spring, suggests that temperature may also be of some importance in determining the rate of return of this group. Ludwig and Henry (1943) have shown that if sterilized soil is reinoculated and incubated, very large increases in actinomycete numbers are obtained within eight days of treatment. In view of this evidence the differences in the actinomycete numbers in the ashbed are possibly far less significant than was suggested in the previous section.

(iv) The decline in plant response was greatest in the soil which had been stored for the longest period of time and was consequently far more advanced in recolonisation. Thus, loss of the ability to stimulate plant growth appears to go hand in hand with the advance in recolonisation, or in other words, the closer the population in the sterilized soil comes to resemble that in the untreated soil, the lower the capacity of the sterilized soil to stimulate plant growth.

Root development and growth in heated soil

Since this experiment was carried out in mid-winter instead of summer the growth of E. grandis was much poorer than in the pot trial on duration of plant response in heated soil. The magnitude of the growth response in the heated soil was also very much reduced.

Two points of interest were observed in relation to seedling transplants:

(i) Apparently when the seedling root system was transplanted to soil most of it did not grow, and eventually died away. The old root system was readily distinguished from the newly formed roots because it failed to take up the lactophenol cotton blue stain.

(ii) The establishment of seedlings was dependent on the production of new roots. Obviously the faster a seedling can grow new roots, the sooner it will be able to establish itself and produce new foliage.

At the first sampling, three weeks after planting, the seedlings in the sterilized soil showed themselves to be well in advance of those in the untreated soil:

(i) Three times as many new roots were produced by the seedlings in the heated soil.

(ii) Already there was a statistically significant increase in the oven dry weights of the shoots. The heights of the seedlings in the two treatments, however, were identical at this stage.

There is little doubt, then, that the heated soil contains factors capable of stimulating growth and that they come into operation as soon as seedlings are planted.

In summary, the evidence presented in this section indicates not only that the factors responsible for growth stimulation may be in operation for only a relatively short period, but also that they commence to operate immediately after planting. Furthermore, the fall-off in growth response appeared to be inversely proportional to the stage of recolonisation in sterilized soil. It seems then that the factors responsible for growth stimulation in sterilized soil can best be sought in terms of changes observed during the period of recolonisation.

III. ROOT SURFACE FLORA IN ASHBED AND HEATED SOIL

1. DIFFERENCES WITH DEPTH OF ASHBED SOIL

(a) Materials and Methods

A subsample was taken from the root systems of the E. grandis seedlings grown in ashbed and control soils from different depths of the soil profile (page 64).

The roots were cleared in lactophenol containing 0.25 parts per thousand cotton blue (Smith 1960) and mounted on slides in clear lactophenol. The percentage of infected and uninfected root tips was determined microscopically.

(b) Results

All root tips that had mycorrhiza, superficial mycorrhiza or a black pseudomycorrhiza were scored as infected. Table 9 shows that the percentage of infected roots was far less in the ashbed soil than in the control soil, except at 0-2" depth, in which the growth response in the ashbed soil was actually less than in the control.

	ASHBED		CONTROL	
Depth of soil	Infected	Uninfected	Infected	Uninfected
0-2"	81.7	18.3	87.3	12.7
2-4"	45.7	54.3	84.3	15.7
4-6"	30.7	69.3	93.9	6.1

TABLE 9 Percentage of infected and uninfected root tips in E. grandis

III. ROOT SURFACE FLORA IN ASHBED AND HEATED SOIL

2. DEVELOPMENT OF ROOT SURFACE FLORA IN HEATED SOIL

(a) Materials and Methods

The number of mycorrhizal, superficially mycorrhizal, pseudomycorrhizal, uninfected and actively growing root tips in E. grandis seedlings were determined microscopically 3, 7, and 11 weeks after planting (page 69).

(b) Results

Figures 22 and 23 show the proportion of different types of root tips in heated and unheated soil respectively. Although the actual number of infected root tips was comparable in the two treatments, in the heated soil they represented only a comparatively small portion of the total number of root tips. Mycorrhizal development was only slightly retarded in the heated soil.

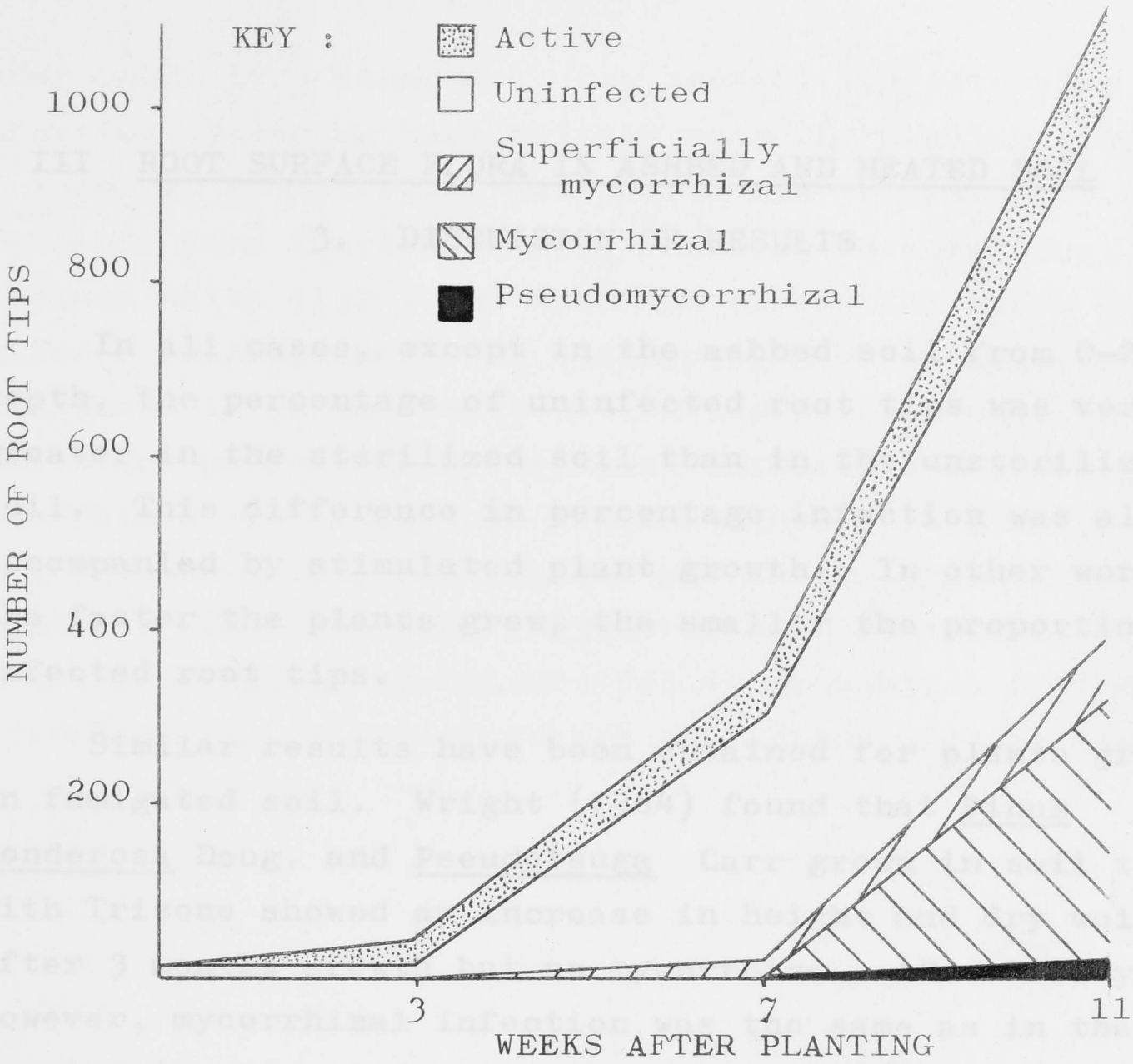


FIGURE 22 Development of various kinds of root tips of E. grandis in heated soil

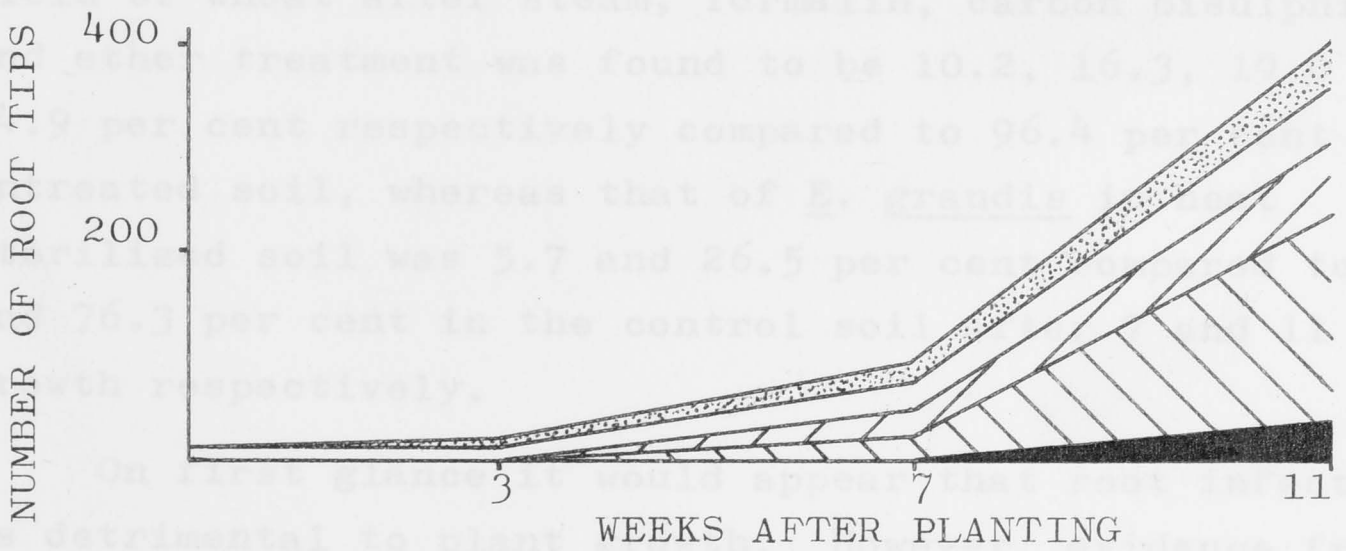


FIGURE 23 Development of various kinds of root tips of E. grandis in unheated soil

III ROOT SURFACE FLORA IN ASHBED AND HEATED SOIL

3. DISCUSSION OF RESULTS

In all cases, except in the ashbed soil from 0-2" depth, the percentage of uninfected root tips was very much greater in the sterilized soil than in the unsterilized soil. This difference in percentage infection was always accompanied by stimulated plant growth. In other words, the faster the plants grew, the smaller the proportion of infected root tips.

Similar results have been obtained for plants growing in fumigated soil. Wright (1964) found that Pinus ponderosa Doug. and Pseudotsuga Carr grown in soil treated with Trizone showed an increase in height and dry weight after 3 months growth but no mycorrhiza. After two years, however, mycorrhizal infection was the same as in the plants growing in untreated soil. Results of Peyronel (1926) showed that root infection was in inverse ratio to the yield of wheat after steam, formalin, carbon bisulphide and ether treatment was found to be 10.2, 16.3, 19.0 and 14.9 per cent respectively compared to 96.4 per cent in the untreated soil, whereas that of E. grandis in heat sterilized soil was 5.7 and 26.5 per cent compared to 57.9 and 76.3 per cent in the control soil after 7 and 11 weeks growth respectively.

On first glance it would appear that root infection is detrimental to plant growth. However, evidence from mycorrhizal studies indicates that this is not so. In general ectotrophic mycorrhizas are known to be developed

under conditions suboptimal for growth, the intensity of infection depending particularly upon the availability of supplies of nitrogen, phosphorus and potassium in an available form (Harley, 1959). Several workers, for instance Hatch (1934) and Björkman (1940) for pines and Chilvers (unpublished) for eucalypts, have established that there is an inverse relationship between nutrient levels and percentage mycorrhizal infections. These mycorrhizas are thought to be of advantage to the trees by their enhanced uptake of scarce and intermittent nutrient supplies. Thus the observed decrease in root infection in plants growing in sterilized soil is probably a further indication that superior growth conditions are prevalent.

IV. EFFECT OF STERILIZATION ON NUTRIENT STATUS OF SOIL

1. NITROGEN

(a) Materials and Methods

Three hundred grams each of untreated, heat sterilized (150°C for 3 hours) and gamma-irradiated soil were placed in plastic pots and brought to field capacity with distilled water. All the pots were inoculated with 5 gm of unheated soil, and incubated at 25°C in a humid incubator (to minimise evaporation). The moisture content was kept constant by watering when required.

The analyses were made on the soil solution at field capacity. Bowen and Cawse (1964) had found that analysis of the soil solution was a more sensitive indicator of changes in soil subjected to gamma-irradiation than the standard methods of soil analysis. The soil solution was extracted by centrifuging the soil at 2000 r.p.m. for 15 minutes. A diagram of the container used for collecting the solution is shown in Figure 24. Two bulked samples of the soil solution derived from four pots were analysed for each treatment.

Analytical methods

(i) Ammonia-nitrogen: A modification of the microdiffusion method described by Bremner and Shaw (1955) was used. One ml of soil solution was placed in the outer chamber of a Conway unit and 3 ml of 12 per cent magnesium oxide added. The ammonia was collected in 2 per cent (w/v) boric acid

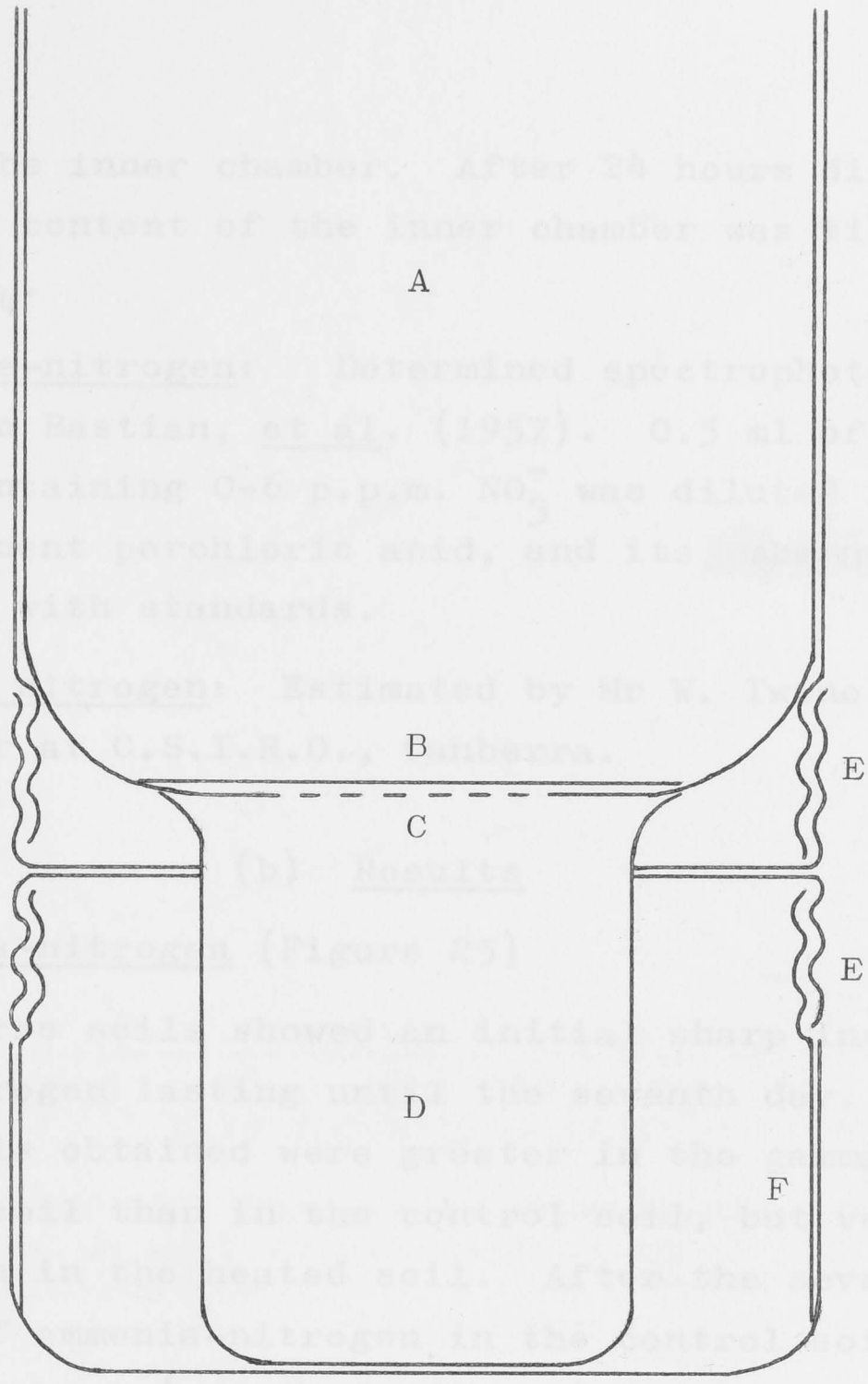


FIGURE 24 Diagram of Apparatus for extracting soil solution by centrifugation (x 1)

A - container for soil

B - filter paper (Whatman No.42)

C - perforated base

D - polyethylene beaker for collecting soil solution

E - screw-top lid

F - supporting base

The apparatus was constructed from two polyethylene bottles with screw top-lids (14.5 cms high and 8.5 cms in diameter).

placed in the inner chamber. After 24 hours distillation at 25°C the content of the inner chamber was titrated with 0.005N H_2SO_4.

(ii) Nitrate-nitrogen: Determined spectrophotometrically according to Bastian, et al. (1957). 0.5 ml of soil solution containing 0-6 p.p.m. NO_3^- was diluted to 10 ml with 5 per cent perchloric acid, and its absorption at 210 mμ compared with standards.

(iii) Total nitrogen: Estimated by Mr W. Twine in the autoanalyser at C.S.I.R.O., Canberra.

(b) Results

(i) Ammonia-nitrogen (Figure 25)

All three soils showed an initial sharp increase in ammonia-nitrogen lasting until the seventh day. The actual levels obtained were greater in the gamma-irradiated soil than in the control soil, but very much larger again in the heated soil. After the seventh day the level of ammonia-nitrogen in the control soil declined rapidly to normal (regained after 12 days). The level in the gamma-irradiated soil remained considerably higher than that in the control and actually rose again slowly to a fresh peak at 42 days, finally returning to normal on the seventieth day. The ammonia concentration in the heated soil did not fall at all, but continued to rise at a slow rate throughout the experiment, becoming approximately 100 times the level of that in the control and irradiated soils by the seventieth day.

(ii) Nitrate-nitrogen (Figure 26)

Nitrification in the heat-sterilized soil was

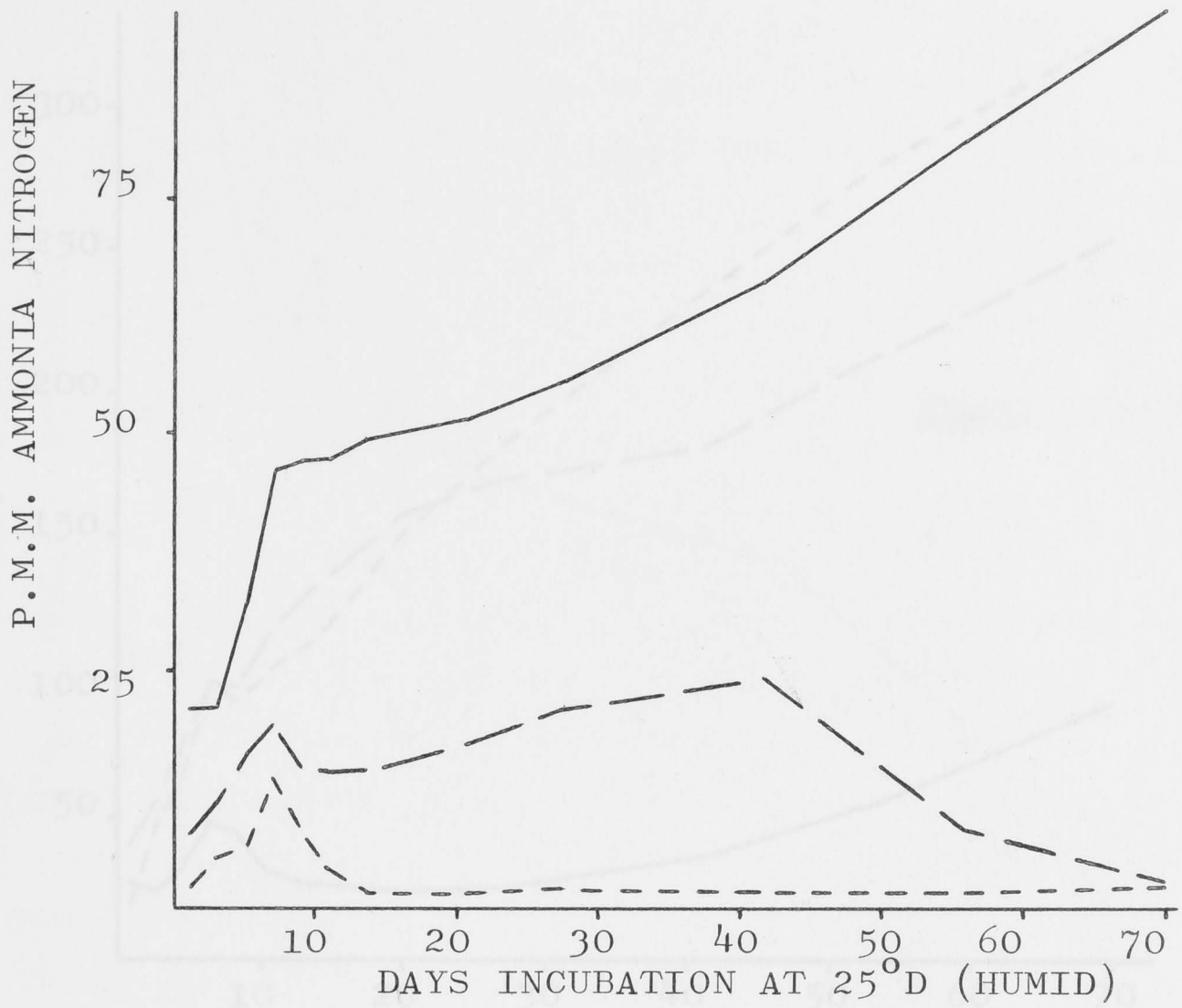


FIGURE 25 Changes in ammonia-nitrogen with time in soil solutions from three different soil treatments

—— heated

— — gamma-irradiated

---- untreated

inhibited for 28 days compared with the control after which the level of nitrate-nitrogen began to increase slowly. After 56 days the rate of nitrification was the same as in the other two soils. The amount of NO_3-N was only a small fraction of the amount in the untreated soil throughout the period of $2\frac{1}{2}$ months. The rate of nitrification in the gamma-sterilized soil was the same as

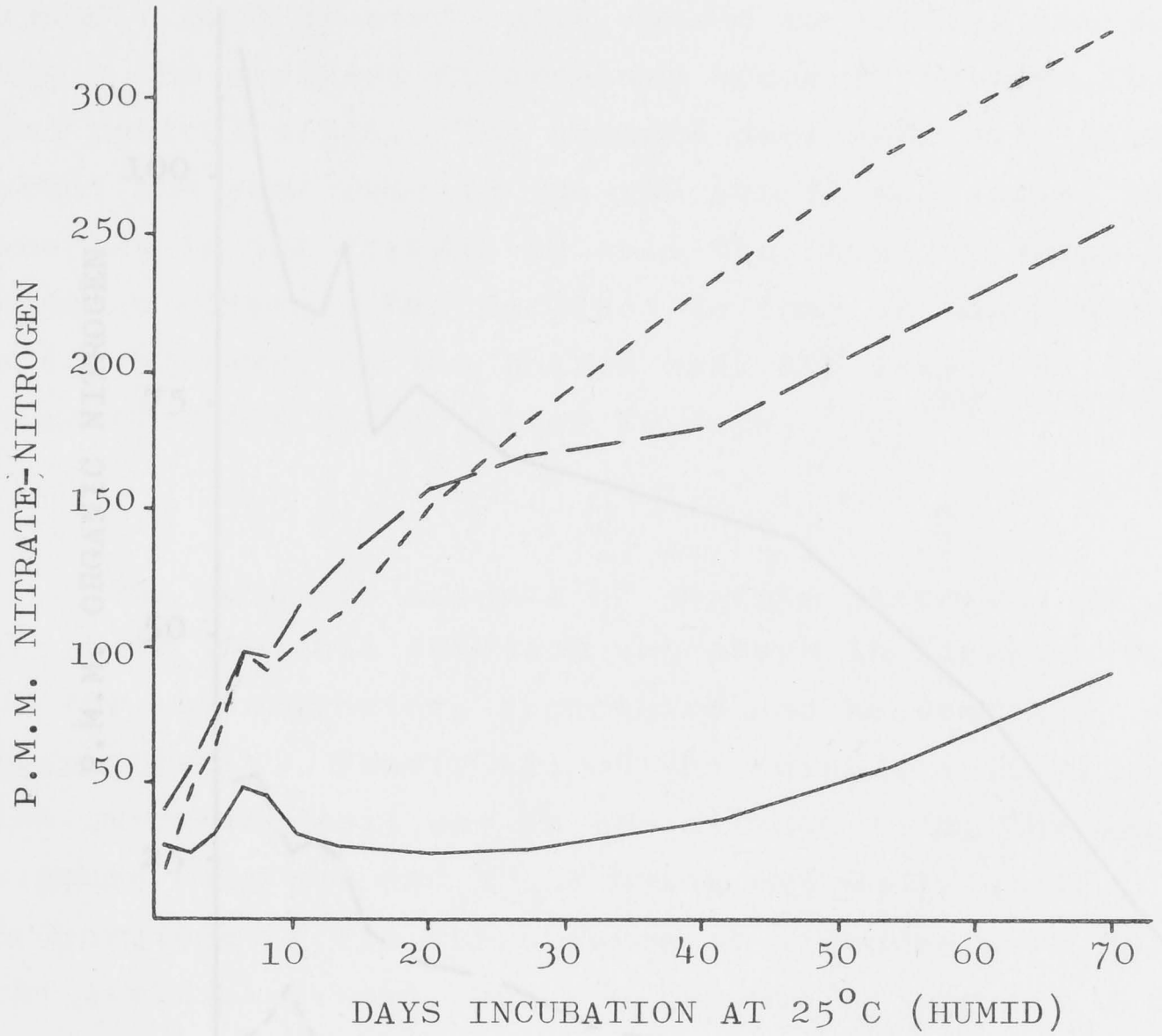


FIGURE 26 Changes in nitrate-nitrogen with time in soil solutions from three different soil treatments

——— heated
— — gamma-irradiated
----- untreated

in the untreated soil except between the thirtieth and fortieth day after treatment, when the rate was considerably lower in the irradiated soil.

(iii) Organic nitrogen (Figure 27)

Marked differences between the amounts of organic nitrogen in the soil solutions of the three soils were also

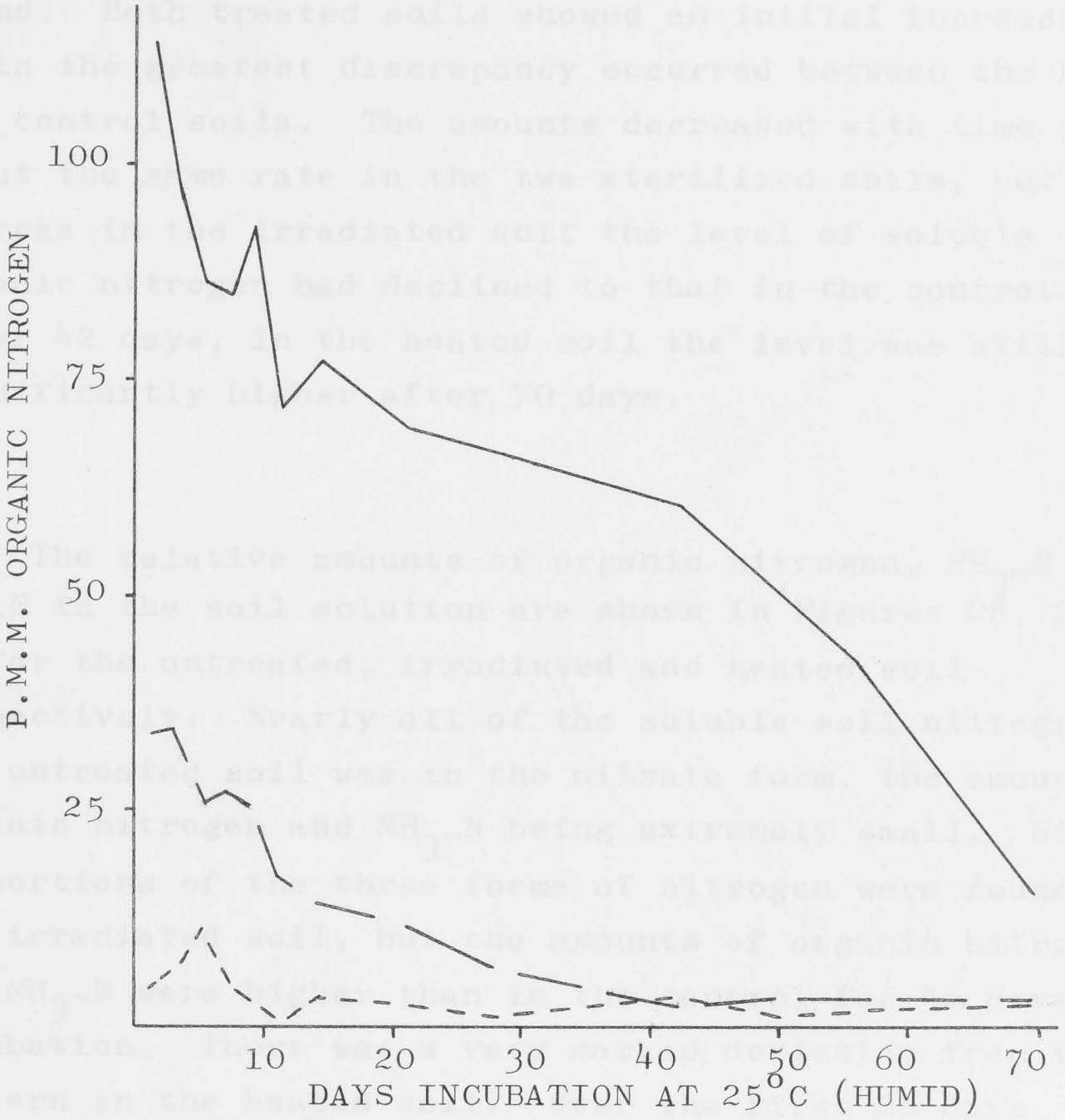


FIGURE 27 Changes in organic nitrogen with time in soil solutions from three different soil treatments.

——— heated

— — gamma-irradiated

----- untreated

found. Both treated soils showed an initial increase, but again the greatest discrepancy occurred between the heated and control soils. The amounts decreased with time at about the same rate in the two sterilized soils, but whereas in the irradiated soil the level of soluble organic nitrogen had declined to that in the control soil after 42 days, in the heated soil the level was still significantly higher after 70 days.

The relative amounts of organic nitrogen, NH_3-N and NO_3-N in the soil solution are shown in Figures 28, 29 and 30 for the untreated, irradiated and heated soil respectively. Nearly all of the soluble soil nitrogen in the untreated soil was in the nitrate form, the amounts of organic nitrogen and NH_3-N being extremely small. Similar proportions of the three forms of nitrogen were found in the irradiated soil, but the amounts of organic nitrogen and NH_3-N were higher than in the control for 56 days after incubation. There was a very marked deviation from this pattern in the heated soil. Over the first 28 days soluble organic nitrogen made up the larger portion of the soluble soil nitrogen. Subsequently, as the organic nitrogen decreased, ammonium-nitrogen became the most abundant form of nitrogen. For 42 days nitrate-nitrogen formed the smallest portion of the soil nitrogen but as the rate of nitrification increased it exceeded the amount of soluble organic nitrogen.

It is interesting to note that in the heated soil the amount of total soluble nitrogen beyond 21 days incubation was significantly less than in the untreated soil although initially it was much higher. In the irradiated soil the

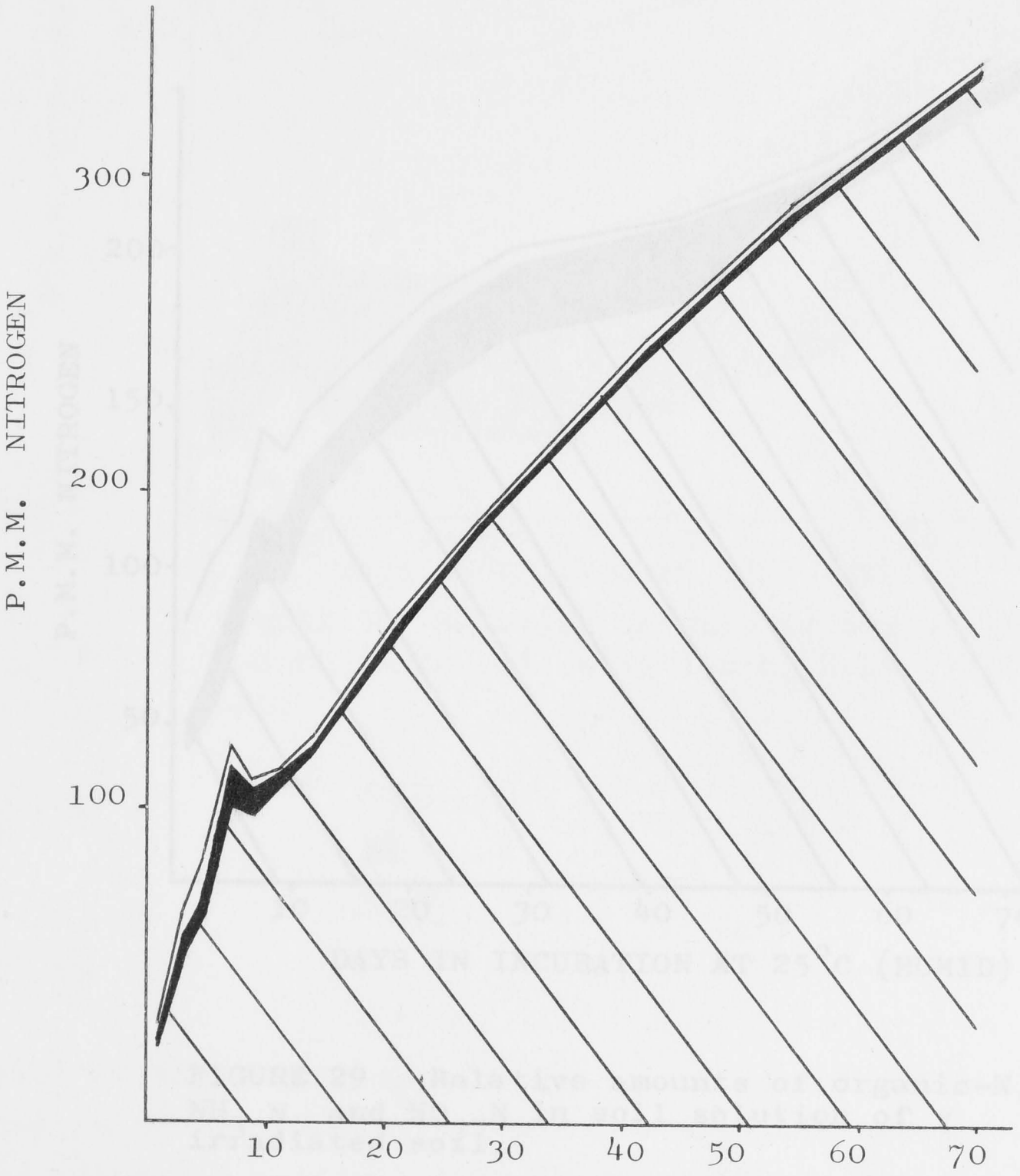


FIGURE 28 Relative amounts of organic-N NH_3-N and NO_3-N in soil solution of untreated soil

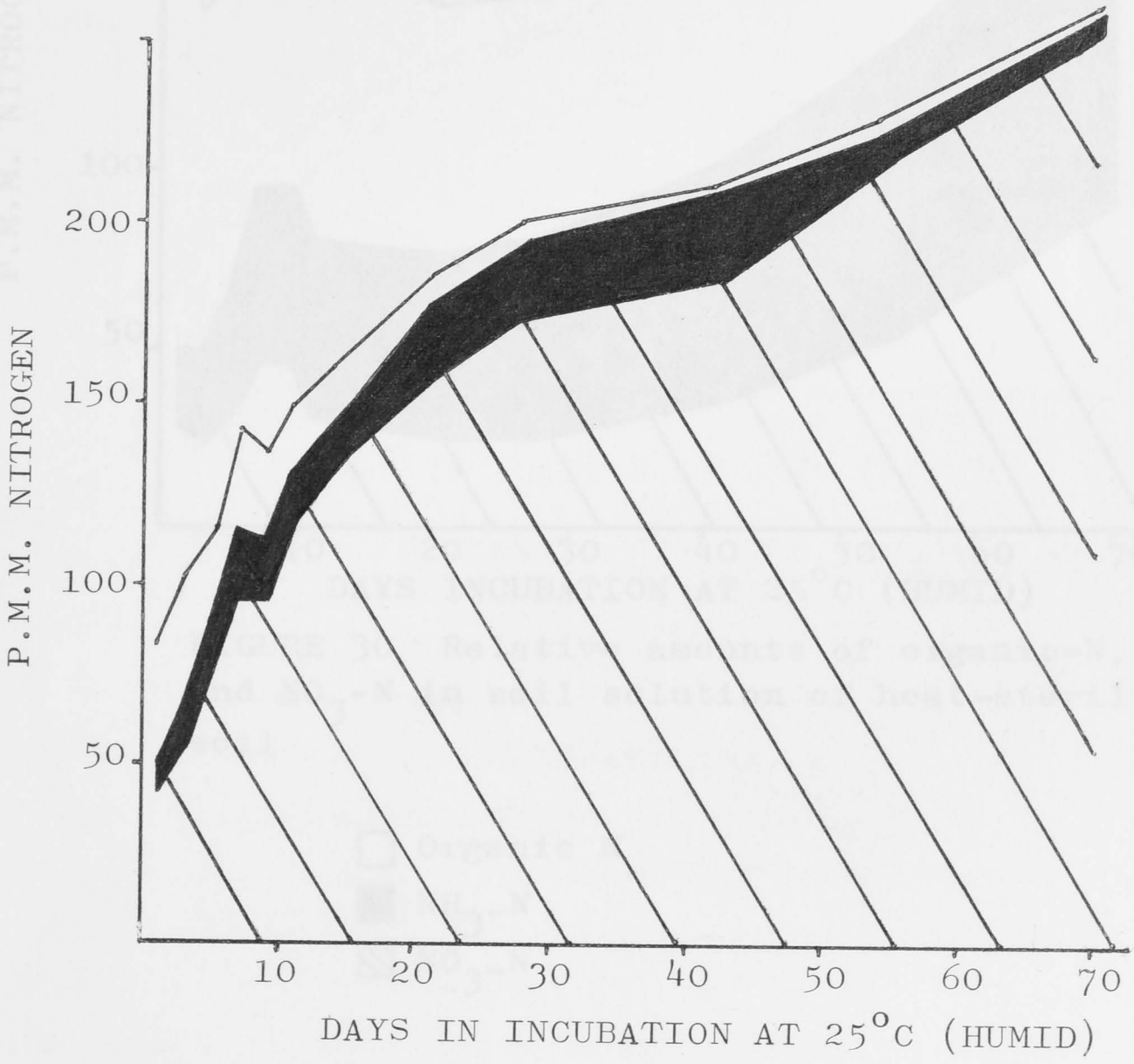


FIGURE 29 Relative amounts of organic-N, NH_3-N and NO_3-N in soil solution of γ irradiated soil.

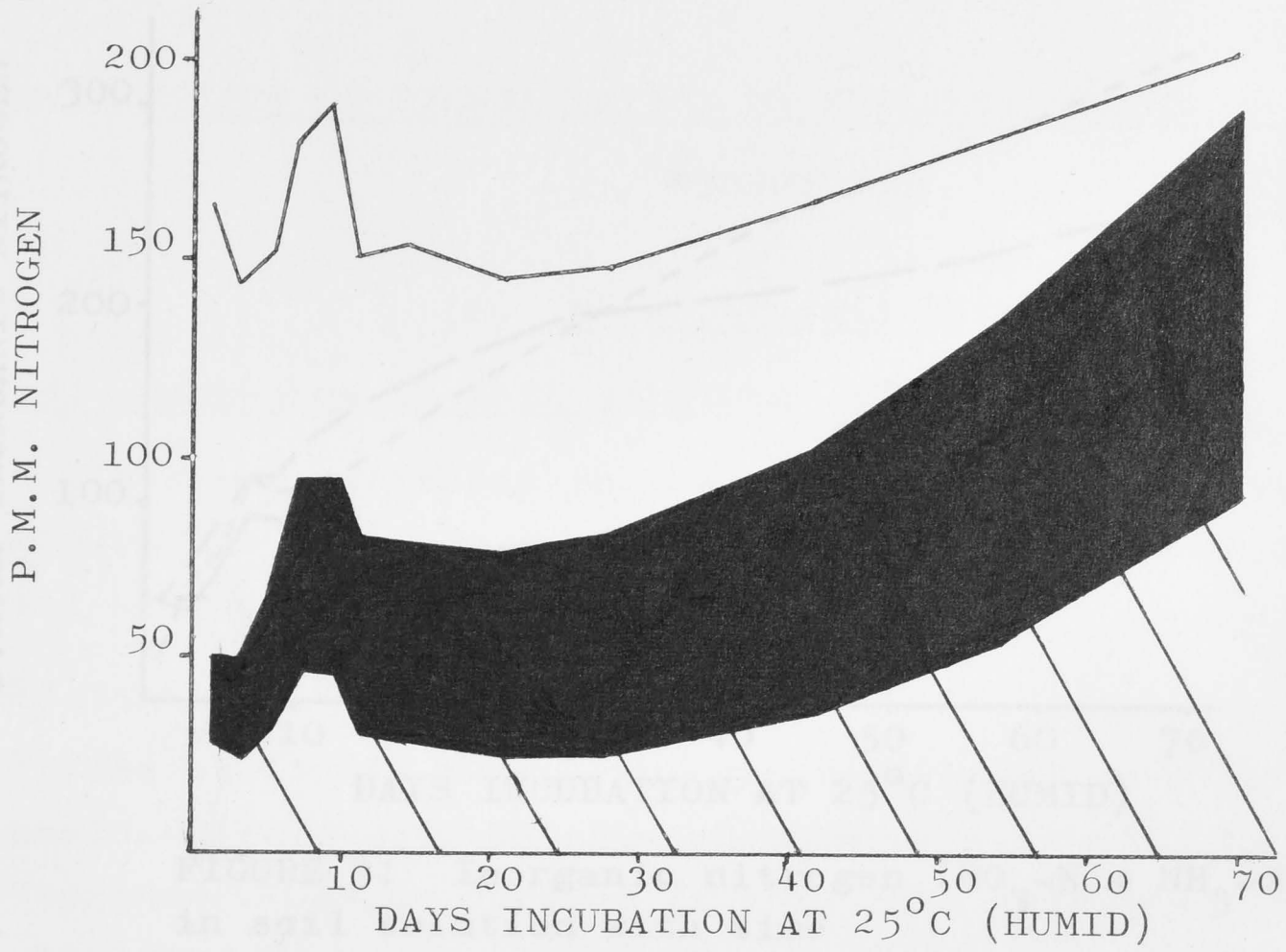


FIGURE 30 Relative amounts of organic-N, NH_3-N and NO_3-N in soil solution of heat-sterilized soil

level of total soluble nitrogen was also initially higher than in the untreated soil but did not fall below that in the untreated soil until the fortieth day. The discrepancy between the amount of soluble nitrogen in the gamma-irradiated soil and that in the control soil was much less than in the case of the heated soil.

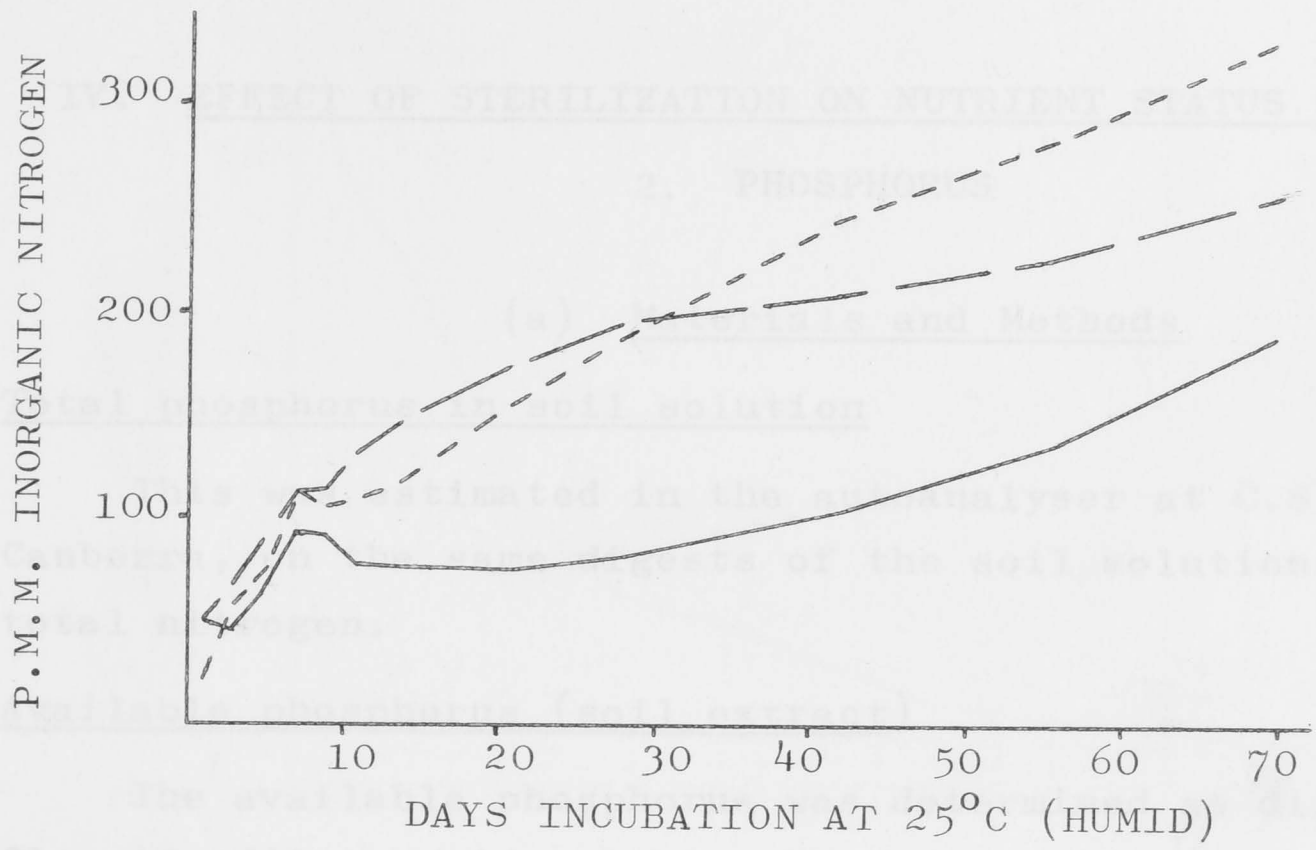


FIGURE 31 Inorganic nitrogen (NO_3-N + NH_3-N) in soil solution with time

——— heated

— — gamma-irradiated

----- untreated

Although the ammonification rate in the heated soil was very much higher in the heated soil than in the untreated soil, the total available nitrogen NO_3-N + NH_3-N) was significantly lower in the heated soil. The amount of available nitrogen in the soil solutions of the three soils is plotted against time in Figure 31.

IV. EFFECT OF STERILIZATION ON NUTRIENT STATUS OF SOIL

2. PHOSPHORUS

(a) Materials and Methods

Total phosphorus in soil solution

This was estimated in the autoanalyser at C.S.I.R.O., Canberra, on the same digests of the soil solutions as the total nitrogen.

Available phosphorus (soil extract)

The available phosphorus was determined as dilute fluoride-dilute acid soluble phosphorus according to the method described by Jackson (1958). The analyses were made on the soil after the soil solution had been extracted.

(b) Results

The soluble phosphorus was entirely organic in nature because no inorganic phosphorus, using the method of Truog and Meyer (1929), could be detected in any of the soil solutions. The relative amounts of soluble organic phosphorus in heated, irradiated and untreated soils are shown in Figure 32. Initially the soil solutions of both of the sterilized soils contained more organic phosphorus than that of the untreated soil. The amount of organic phosphorus brought into solution after heating, however, was very much larger than after gamma irradiation. Within 56 days the level of organic phosphorus in the sterilized soils had fallen to approximately the same level as in the control soil.

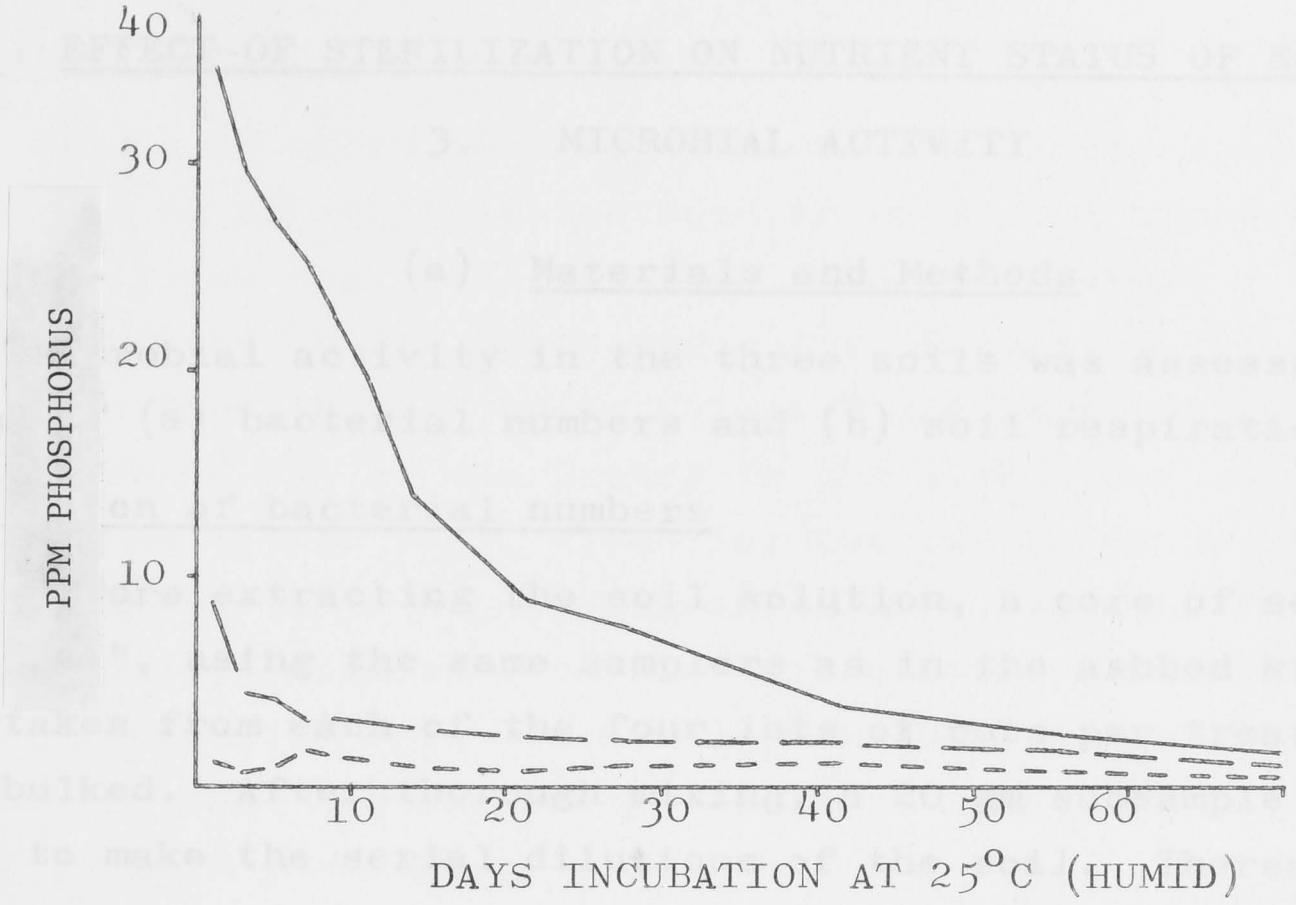


FIGURE 32 Changes in total phosphorus (organic) with time in soil solutions from three different soil treatments

——— heated
— — gamma-irradiated
----- untreated

The available soil phosphorus was determined 11 days after treatment to correspond with the time of planting in the pot trial (page 107). The heated, irradiated and untreated soil was found to contain 21.2, 7.8 and 8.0 p.p.m. available phosphorus/gm oven dry soil respectively.

IV. EFFECT OF STERILIZATION ON NUTRIENT STATUS OF SOIL

3. MICROBIAL ACTIVITY

(a) Materials and Methods

Microbial activity in the three soils was assessed in terms of (a) bacterial numbers and (b) soil respiration.

Estimation of bacterial numbers

Before extracting the soil solution, a core of soil, 3" x 3/4", using the same samplers as in the ashbed study, was taken from each of the four lots of pots per treatment and bulked. After thorough mixing, a 20 gm subsample was used to make the serial dilutions of the soil. Thereafter the same procedure was followed as in the estimation of the ashbed bacteria.

Soil respiration

At the same time that the pots were sampled for estimation of bacterial numbers, a similar core from each pot was transferred to the bottom of a Cavette flask giving two lots of four replicates per treatment. Exactly 1.0 ml of approximately 0.1N $Ba(OH)_2$ (0.2N in case of the sterilized soils after 5 and 9 days incubation) was placed in the well at the base of the stopper and the flasks sealed by means of metal springs. Four blanks were set up so that the amount of CO_2 in the air in the flask could be determined. After 12 hours incubation at 25°C the contents of the well in each flask was transferred quantitatively to a small beaker using 7 ml of distilled water and titrated with 0.02N HCl. The soil respiration was calculated as milligrams CO_2 evolved per 100 gm of soil.

(b) Results

Microbial numbers

Figure 33 shows that in both the sterilized soils there was an appreciable increase in bacterial numbers after only one day, while after 5 days the numbers exceeded those in the untreated soil. Bacterial recolonisation in both soils followed the same pattern as in the ashbed but it was faster in the irradiated soil than in the heated soil. Thus for the first few weeks more than ten times as many bacteria were isolated from the irradiated soil as from the heated soil. The bacteria in the irradiated soil reached a peak three weeks ahead of the heated soil and thereafter declined gradually until after 70 days the numbers were about the same as in the control soil. In the heated soil at this time there were still more than ten times as many bacteria as in the control soil.

Visual comparison of the bacterial plates from the three soils indicated that the population in the irradiated soil was indistinguishable from that in the untreated soil, whereas the population in the heated soil was quite different resembling that found in the ashbed (page 38). Unfortunately, because of the size of this experiment, there was no time to persue this further.

Yeasts, not detected in either the irradiated or untreated soil, were present in considerable numbers in the heated soil after three days, actually exceeding the number of bacteria. The number of yeasts gradually decreased until after 28 days no more could be detected. This does not necessarily mean that there were no more

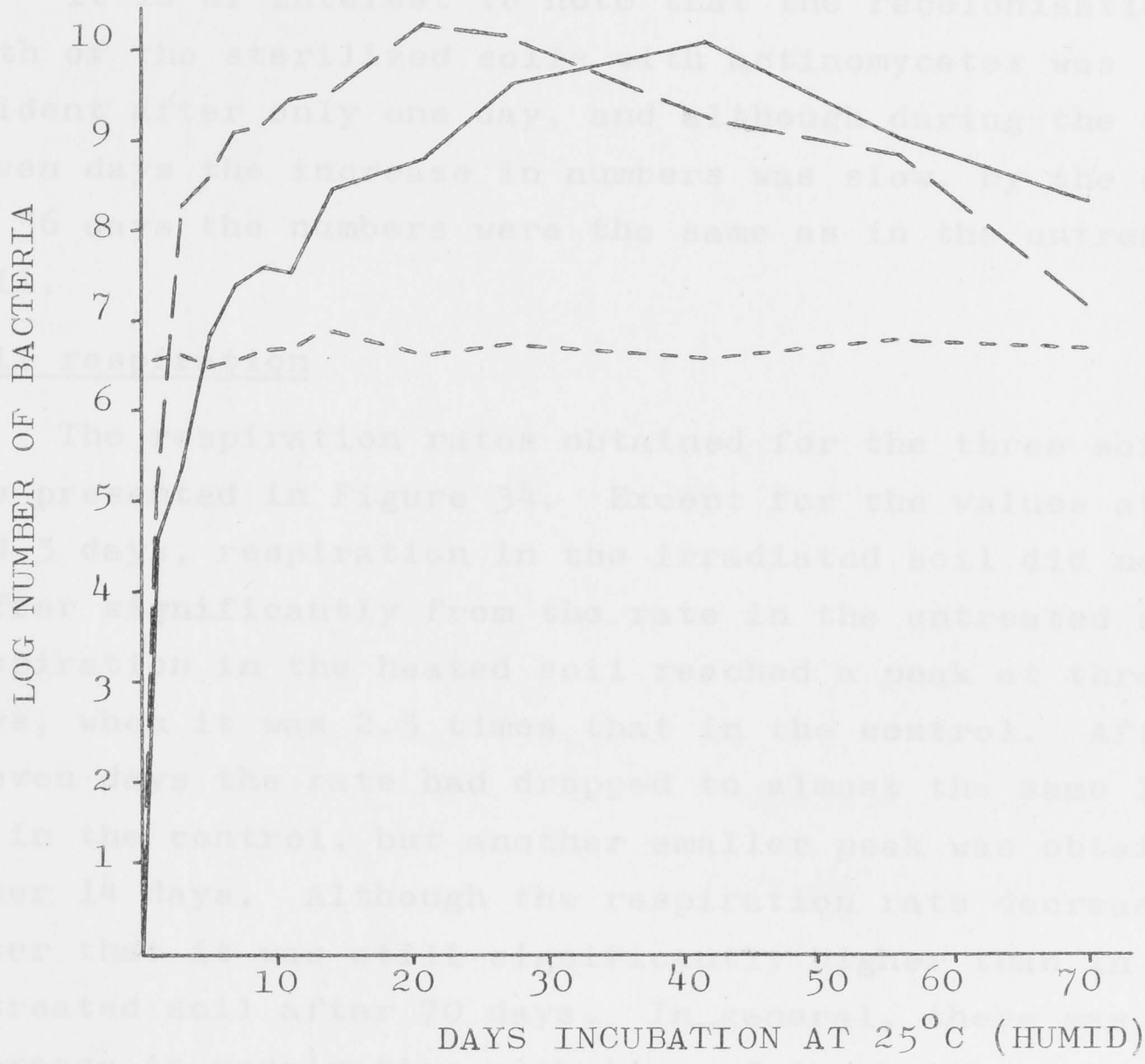


FIGURE 33 Changes in bacterial numbers with time after three different soil treatments

——— heated

— — gamma-irradiated

----- untreated

yeasts present: the high dilutions of soil necessary to obtain reliable counts of bacteria would have made their detection impossible.

It is of interest to note that the recolonisation of both of the sterilized soils with actinomycetes was evident after only one day, and although during the first seven days the increase in numbers was slow, by the end of 56 days the numbers were the same as in the untreated soil.

Soil respiration

The respiration rates obtained for the three soils are presented in Figure 34. Except for the values at 3 and 5 days, respiration in the irradiated soil did not differ significantly from the rate in the untreated soil. Respiration in the heated soil reached a peak at three days, when it was 2.5 times that in the control. After eleven days the rate had dropped to almost the same level as in the control, but another smaller peak was obtained after 14 days. Although the respiration rate decreased after that it was still significantly higher than in the untreated soil after 70 days. In general, there was a decrease in respiration with time of incubation.

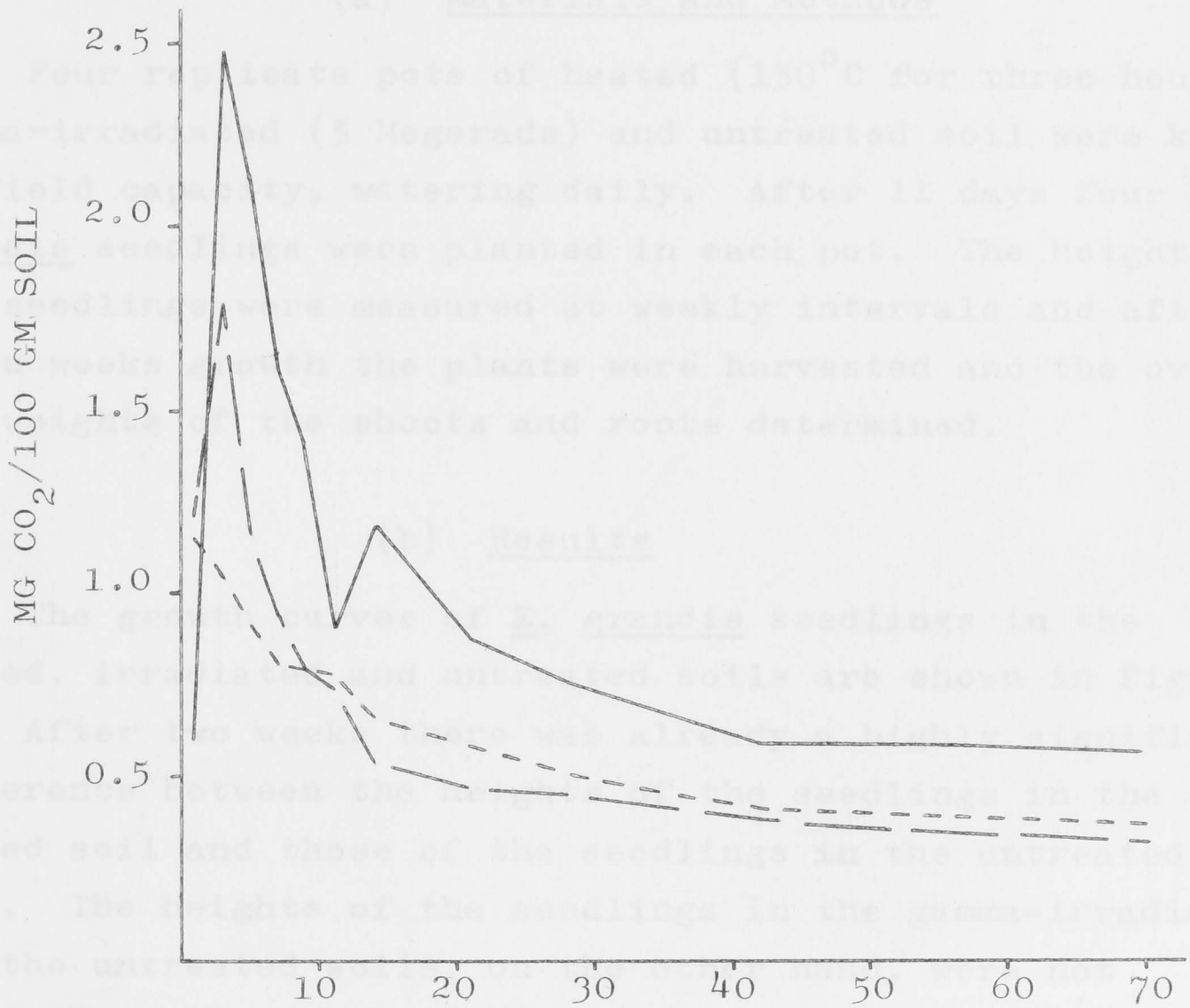


FIGURE 34 Soil respiration rates in three different soil treatments

IV. EFFECT OF STERILIZATION ON NUTRIENT STATUS OF SOIL

4. PLANT RESPONSE

(a) Materials and Methods

Four replicate pots of heated (150°C for three hours), gamma-irradiated (5 Megarads) and untreated soil were kept at field capacity, watering daily. After 11 days four E. grandis seedlings were planted in each pot. The heights of the seedlings were measured at weekly intervals and after seven weeks growth the plants were harvested and the oven dry weights of the shoots and roots determined.

(b) Results

The growth curves of E. grandis seedlings in the heated, irradiated and untreated soils are shown in Figure 35. After two weeks there was already a highly significant difference between the heights of the seedlings in the heated soil and those of the seedlings in the untreated soil. The heights of the seedlings in the gamma-irradiated and the untreated soils, on the other hand, were not statistically different at any time.

Comparison of the oven dry weights of the shoots and roots also failed to show up any differences between the growth in the gamma-irradiated and that in the untreated soil. In case of the heated soil, the difference between the oven dry weights was greater than the difference between the heights of the seedlings compared with the control. Whereas the seedlings in the heated soil were only 1.7 times

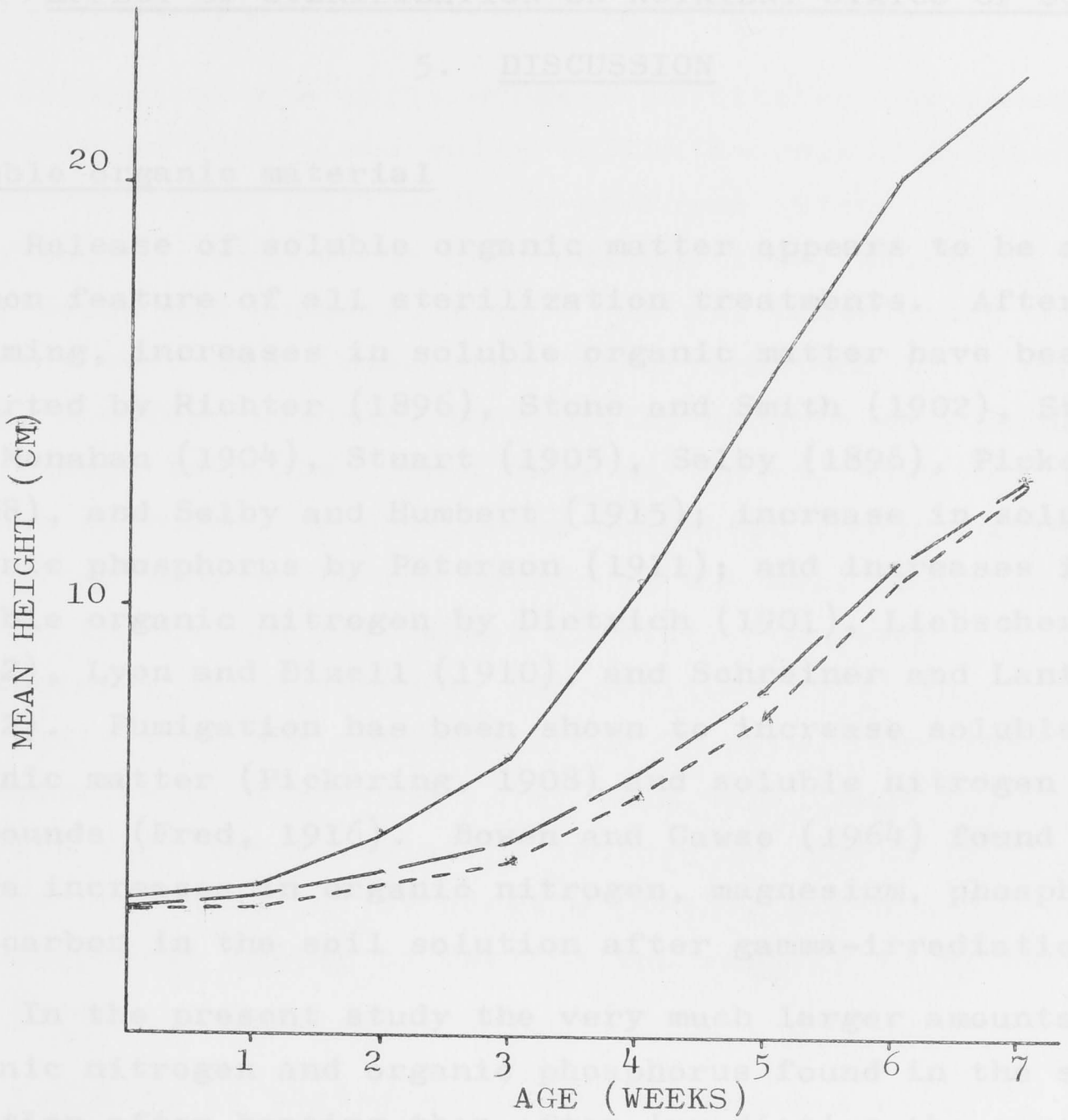


FIGURE 35 Growth of E. grandis in three different soil treatments

——— heated

— — gamma-irradiated

----- untreated

higher than those in the untreated soil, they were 2.8 times heavier.

IV. EFFECT OF STERILIZATION ON NUTRIENT STATUS OF SOIL

5. DISCUSSION

Soluble organic material

Release of soluble organic matter appears to be a common feature of all sterilization treatments. After steaming, increases in soluble organic matter have been reported by Richter (1896), Stone and Smith (1902), Stone and Monahan (1904), Stuart (1905), Selby (1896), Pickering (1908), and Selby and Humbert (1915); increase in soluble organic phosphorus by Peterson (1911); and increases in soluble organic nitrogen by Dietrich (1901), Liebscher (1892), Lyon and Bizell (1910), and Schreiner and Lanthrop (1912). Fumigation has been shown to increase soluble organic matter (Pickering, 1908) and soluble nitrogen compounds (Fred, 1916). Bowen and Cawse (1964) found large increases in organic nitrogen, magnesium, phosphorus and carbon in the soil solution after gamma-irradiation.

In the present study the very much larger amounts of organic nitrogen and organic phosphorus found in the soil solution after heating than after irradiating the same soil indicate that there is a significant difference between the two soil treatments. There is little doubt that the amounts released on irradiation come from the lysis of microbial cells since irradiation has been shown not to alter the organic matter or humus in the soil (Bowen and Cawse, 1964). Heating, however, is known to alter the organic fraction of the soil, so that much of the organic nitrogen and phosphorus in the soil solution of the heated

soil would appear to have come from the organic fraction of the soil, and only a small proportion from the lysis of microbial cells.

Several of the early workers attributed the increase in growth in sterilized soils to the increase in soluble organic material, particularly nitrogen (Stone and Smith, 1902; Selby, 1896; Stone and Monahan, 1906; Stoklasa, 1911; Selby and Humbert, 1915). In all these cases analyses were made immediately after treatment. In this study, however, although the initial amounts were indeed high enough to suggest that this may be so, both the organic nitrogen and phosphorus decreased rapidly with time after treatment; the levels in the soil solution, even in the heated soil which initially contained particularly large amounts, had declined until they were comparable with the controls after 70 days. Considering the evidence from section II of this work (page 73) which suggests that the stimulatory factors in heated soil operate over a period of about 9 months, organic nitrogen and phosphorus appear to decline too rapidly to be equated with them.

Toxicity in heated soils, as in steamed soils (Dietrich, 1901; Schulze, 1907; Schreiner and Lanthrop, 1907; Gedroitz, 1909), is believed to be caused by organic comp unds formed by the action of heat on the organic matter in the soil. The results of several workers (e.g. Pickering, 1908, 1910) suggests that the toxicity in due to the presence of soluble nitrogen-containing organic

compounds. In experiment 4 of this section, the heated soil was planted 11 days after treatment when it was no longer toxic to growth. Yet 62 per cent of the organic nitrogen and 56 per cent of the organic phosphorus initially released on heating was still present in the soil solution at this time. Apparently, then, the mere presence of these compounds does not necessarily render the soil toxic. It seems more likely that the heated soil will become toxic to growth when some critical level of soluble organic compounds is exceeded, or else the toxicity is due to some specific compounds which are readily metabolised by the early colonisers.

Nitrification

Nitrification in the heated soil was suppressed for a period of seven weeks. Coleman (1909) obtained almost identical results for a number of soils containing various levels of nitrate-nitrogen after treatment with measured sterilizing doses of carbon bisulphide. Smith (1938), Heinze (1906), and Kirkwood (1962) also report inhibition of nitrification after soil fumigation. Steaming (Deherain and De Moussey, 1896; Russell and Hutchinson, 1909) and irradiation from ^{60}Co in the field (Rambelli, 1963) have also been shown to inhibit nitrification for some time after treatment.

In contrast to Rambelli's results, nitrification in the gamma-irradiated soil used in this laboratory experiment continued unchecked in the period immediately following treatment. However, after 28 days, nitrification showed a marked inhibition which persisted until the 42nd day of the experiment. It is interesting to note that this

coincided exactly with a period during which ammonia levels reached a peak. Probably, then, the high ammonia levels are responsible for the inhibition, since in all the cases cited where nitrification was found to be inhibited, large amounts of ammonia-nitrogen were present. Coleman in fact found that once the ammonia level in the fumigated soil had dropped appreciably, nitrification proceeded at an even higher rate than in the untreated soil.

Ammonification

Increased ammonification has been reported following all forms of sterilization, namely steam (Richter, 1896; Koch and Luken, 1907; Deherain and De Moussey, 1896; Russell and Hutchinson, 1909; Lyon and Bizell, 1910; Schreiner and Lanthrop, 1912; Tam and Clark, 1943), fumigation (Smith, 1938; Tam and Clark, 1943; Kirkwood, 1962) and irradiation from ^{60}Co (Rambelli, 1963). It is difficult to say with certainty whether the increase in ammonia-nitrogen found by Bowen and Cawse (1964) in the soil solution after gamma-irradiation was due to the increase in ammonification or to the release of ammonia from the lysis of microbial cells, because the analyses were done after incubating the soil for two days. In the present study it was found that the rate of ammonification in the gamma-irradiated soil was the same as that in the untreated soil for a period of nine days, so that the increase in ammonia-nitrogen observed by Bowen and Cawse could well be the product of cell destruction as they themselves suggested.

The increase in ammonia-nitrogen in the soil solution of the gamma-irradiated soil over that in the untreated

soil after one day, appears at first glance to represent the amount of ammonia released from the microbial cells on death. However, bacterial and actinomycete numbers at this stage had already increased appreciably, so that part of this could have come from the metabolic activity of these early colonisers.

The soil solution from the heated soil after one day contained about three times as much ammonia-nitrogen as the irradiated soil. Since the number of microbes that had returned to the heated soil was comparable to that in the irradiated soil, the excess ammonia must have been derived from sources other than the ones already mentioned; for example, from the organic matter in the soil, or perhaps the clay minerals since heat is known to release ammonia from both of these. For three days ammonification in the heated soil was negligible, but between three days and nine days it was extremely rapid. It is probable that this may coincide with the act of detoxification.

In general, in both the sterilized soils, increases in ammonia-nitrogen were accompanied by decreases in soluble organic nitrogen, suggesting that the ammonia is produced from the organic nitrogen as a result of microbial metabolism.

In all respects the nitrogen status of the gamma-irradiated soil was very similar to that of the untreated soil, whereas that of the heated soil was markedly different. This would suggest nitrogen involvement in the production of the plant response. However, although the ammonia levels in the heated soil were very much higher,

the total available nitrogen was found to be considerably lower than in the other two soils.

Microbial activity

Bacterial recolonisation of the sterilized soils, in general followed the same pattern as in the ashbed, but occurred at a much faster rate. Perhaps this is not surprising, because under the experimental conditions of this study fairly optimal growth conditions were provided; namely constant temperature, constant water supply and a deliberate inoculation. The return of the actinomycetes was again found to be much faster than in the ashbed, confirming the results obtained in section II of this work. It may be concluded then that actinomycete recolonisation is very dependent upon the water supply and that their absence from the ashbed for a prolonged period is not causally related to the plant growth response.

The presence of yeasts in the heated soil indicates that sugars had been formed on heating; supporting the work of Seaver and Clark (1912) who showed that the water soluble organic matter extracted from heated soil displayed the properties of sugars and organic acids.

There seems to be very little if any correlation between the plate counts obtained and the rate of carbon dioxide production. Soil respiration in the gamma-irradiated treatment was equal to that in the untreated soil after only one day, whereas in the heated soil it was still very much smaller. Since the microbial numbers at this time were comparable in the two sterilized soils, it suggests that the difference in respiration rate is due to qualitative differences in the population. In fact, the

respiration rates in the gamma-irradiated and control soils were comparable throughout the experiment, while the respiration of the heated soil was considerably higher than either. As far as it was assessed, the microbial population to recolonise the gamma-irradiated soil consisted of the normal soil forms, whereas that recolonising the heated soil was quite different, suggesting that differences in respiration rates are due to differences in microbial population.

GENERAL DISCUSSION

While the results of the experiments carried out in this study do not provide final answers to the problem of growth response in ashbeds, a number of important points have been established which clarify it to some extent.

For instance, it is clear that ashbedding results in soil sterilization, so that the phenomenon can be equated in varying degrees to other sterilizing treatments such as dry heating, autoclaving, fumigation and irradiation. Moreover, although recolonisation commences immediately the soil is moistened, the new microbial population is very distinct from the original one at first, being replaced by the original forms slowly over a period of some months.

Earlier proposals that the heat treatment of the soil was the most important component of ashbedding are endorsed by the evidence from these studies. For instance, changes in the microbial populations of ashbed and heat treated soils followed a similar pattern. Gamma-irradiation however was an essentially different treatment in that no growth response was obtained and further that the microbes normally present in the soil recolonised the soil immediately after sterilization. Apparently, sterilization in itself has no direct effect on the subsequent plant growth, pointing to the dependence of the type of plant response obtained on the events following sterilization.

The rapid return of normal soil forms to the gamma-irradiated soil suggests that the conditions for microbial

growth must have been very similar, particularly with regard to the type of nutrients available since these are known to have a very important influence in determining the types of microbes which will be active. On the other hand, the distinctly different microbes recolonising the heated and ashbed soils point to the presence of an entirely different soil environment with regard to microbial growth. The development of toxicity in heated and ashbed soils is a clear indication of a profound chemical alteration in the soil and it may be presumed that the substances responsible for the toxicity also play a large part in determining the microbial population.

It is very likely also that chemical alteration of the soil leads to the growth stimulation, either directly through the production of growth promoting substances or available nutrients, or indirectly through alteration of the microbial population. The presence of larger amounts of available phosphorus in the heated soil cannot be disputed. However phosphorus additions to the soil alone fail to produce a growth response, and higher levels of phosphorus can still be detected in ashbed soils many years after treatment when there is no longer any evidence for the operation of ashbed factors. The indications are that growth stimulation is related to the distinctive microbial population. For instance, the capacity of heated soil to stimulate growth declined with the progressive return of the microbial population to normal, and the duration of the ashbed factors indicated by the 'time lead' growth curves was of the same order as the period during which the distinctive microbial population persisted.

Since all nitrogen transformations in the soil are brought about by microbial activity it is not surprising that the different microbial population produced marked changes in the nitrogen status of the heated soil. The increase in ammonia-nitrogen would appear at first sight to provide a ready answer to the growth stimulation. Yet, in view of the generality of response shown by various plant species, it is difficult to reconcile this with the fact that ammonia-nitrogen actually inhibits the growth of some species. Notable among these is the apple (Grasmanis and Leeper, 1967), a species that has in fact been shown to grow much better in heated soil.

Apart from the evidence provided by growth rates and the suppression of mycorrhizal development, direct evidence from plant analyses (Hassenbäumer, Coppenrath and König, 1906; Pfeiffer and Franke, 1896; Pickering, 1908; Tam and Clark, 1943) shows that plants take up more nitrogen in sterilized soils, suggesting that there is more available nitrogen in the soil. But it was found in this study that the total amount of available nitrogen (nitrate- and ammonia-nitrogen) in the soil was actually lower. This appears to create a paradox. However, it must be appreciated that to speak of 'available' nitrogen in the soil chemistry sense is merely to describe the state of the nitrogen; in other words that it is present in the form of certain inorganic ions which plants are known to utilise. But not all this 'available' nitrogen will necessarily be assimilated by plants. In the case of nitrate, for example, as well as being assimilated by plants, it may be leached out of the soil, assimilated by microbes or used to provide oxidising power with resulting

reduction to nitrite or ammonia. Moreover, at any given time, the total quantity of 'available' nitrogen represents an equilibrium situation between the rate of production and the rate of loss of these ions, so that the level of 'available' nitrogen is not necessarily a good guide to the amount which plants will obtain from the system because it gives no information about the rate of turnover. Thus a soil with a higher level of available nitrogen but with a low rate of turnover may supply much less nitrogen to a plant over a period of time than a soil with a lower level but a high rate of turnover. It is possible that such a discrepancy exists between heat-sterilized and control soils with their great differences in microbial populations. Certainly the heated soil is extremely active at this stage compared with the controls (viz. population growth rates and soil respiration). In any future work it would be interesting therefore to obtain information on the dynamics of these nutrient transformations using isotopic tracer techniques.

In addition to the rate at which available nitrogen may become accessible to the plant root, there remains the possibility that the different rhizosphere floras of plants growing in heated soil compared with those in untreated soil may affect the rate of nutrient uptake more directly. Bowen and Rovira (1966) and Barber and Loughman (1967) found that far more phosphorus was taken up by roots with a rhizosphere flora than those without. The profound modifications of the rhizosphere flora induced in the ashbed and heated soils may also create conditions at the root surface which are more favourable to nutrient uptake. In this regard it could again be useful to apply isotopic

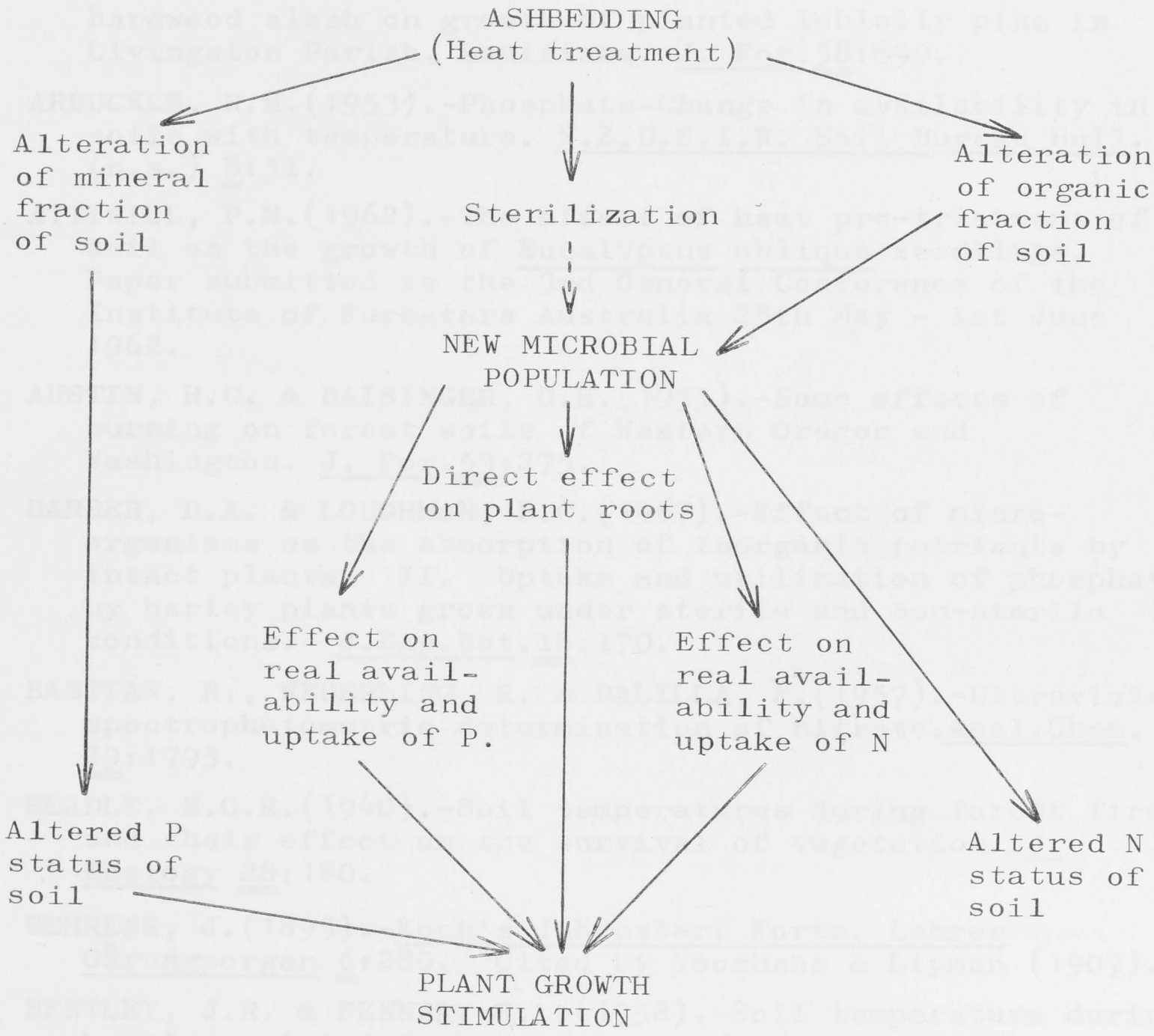
techniques to compare, say, the rates of uptake by ashbed plants with that by control plants of comparable size or perhaps by using excised roots of both.

Perhaps the most likely explanation of the growth response may be in terms of an N/P interaction. The work of McIntyre (1967) shows that eucalypts will not respond to applications of nitrogen if phosphorus is limiting, and this seems to be the situation in most Australian soils. Heat-sterilized soil certainly contains sufficient phosphorus to enable the plants to respond to any increases in the real availability of nitrogen. Any further work, therefore, must certainly seek to clarify this problem of nitrogen availability.

Finally, early root stimulation is obviously a very significant feature of the response, regardless of whether it is directly stimulated by increased nutrient availability or due to alleviation of a growth inhibition normally present in soils (for example, by destruction of microbes producing growth inhibitory substances). It means that plants in the heat sterilized soil rapidly gain access to a much larger volume of soil than those in untreated soil and subsequently will have more nutrients available to them on these grounds alone.

> Seaver and Clark pointed out in 1912 that -
>
> ... the effect of heating soil is a very complex one in which the experimental interprepretation of results depends upon his training and point of view; whether it be bacteriological, chemical or phytopathological. It is very likely that the truth of the matter lies somewhere on the borderline of the three sciences indicated.

From the results gained here and other pertinent studies since then, it is now possible to visualise the sequence of events following heat treatment and the more important inter-relations between these events as summarised below:



In other words, it is postulated that the new microbial population occupies a central position in the events subsequent to heat treatment and that the ultimate stimulation of plant growth is due to a composite of different factors operating together.

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APPENDIX I

Soil sampling error (ashbed study).

(a) Two sets of 10 soil samples from each block. Fungal counts (10^{-4} dil.)

	Control		Future Ashbed	
Plate	Set(a)	Set(b)	Set(a)	Set(b)
1	12	12	14	10
2	15	16	15	13
3	10	10	18	16
4	11	15	9	9
5	15	15	12	18
6	17	14	14	15
7	16	12	12	13
8	16	17	16	14
9	8	20	16	10
10	14	13	12	9
Mean	13.4	14.4	13.8	12.7

No significant difference between the control block intended ashbed block with respect to fungal counts.

(b) Bacterial/Actinomycete Counts (10^{-5} dil.)

	Control		Future Ashbed	
Plate	Set(a)	Set(b)	Set(a)	Set(b)
1	63	68	81	78
2	64	71	67	82
3	93	90	66	76
4	104	82	55	90
5	88	71	80	65
Mean	82.4	76.4	69.8	78.2

No significant difference between the control block and intended ashbed block with respect to bacterial/ actinomycete counts.

APPENDIX II

Differences in plate counts from different serial dilutions from the same soil suspension using 10ml aliquot/90mls.

Fungal counts/plate at dilution 10^{-4}.

Plate	1	2	3	4
1	12	16	24	11
2	8	15	26	3
3	11	17	19	7
4	9	14	16	9
5	14	12	12	12
6	15	19	28	14
7	18	13	32	9
8	9	12	18	10
9	12	10	18	8
10	10	9	20	12
Mean	11.8	13.7	21.3	9.5

Significant difference between series 3 and 4, and between series 3 and 1 and 2.

APPENDIX III

(a) Composition and preparation of media

1. Fungal isolation medium (per litre)

20gm	agar
1.0gm	KH_2PO_4
10.0gm	glucose
5.0gm	neopeptone
10ml	0.3% Rose bengal and Streptomycin
990ml	basal salt solution

2. Bacterial isolation medium (per litre)

20gm	agar
1.0gm	K_2HPO_4
1.0gm	glucose
0.5gm	neopeptone
1000ml	basal salt solution

3. Basal salt solution (per litre)

0.2gm	NH_4NO_3
0.1gm	$NaCl$
0.1gm	$CaCl_2$
0.5gm	$MgSO_4$
1 crystal	$FeCl_3$
4ml	trace element solution

Media were prepared without addition of agar, at room temperature. Deeps were prepared by placing 10ml aliquots of the medium in individual tubes containing 0.2gm agar. Tubes were capped and autoclaved and kept until use. Media were prepared in deeps to ensure that when plates were poured that each contained the same quantity of nutrient agar.

APPENDIX III

(b) Isolation of bacteria/actinomycetes on Thornton's vs modified Thornton's medium.

Counts of 10^{-5} dilution plates

	Thornton's		Modified Thornton's	
Plate	A	B	A	B
1	25	28	110	96
2	28	32	103	89
3	35	24	121	120
4	42	21	128	117
5	31	38	97	132
Mean	32.2	28.6	111.8	110.8

APPENDIX IV

Plate replicates

(a) Fungi.

Counts made at 10^{-4} dilution of soil.

Plate No.	SERIES I			SERIES II		
	No. of Colonies	No. of diff. species	Undeter-minable	No. of Colonies	No. of diff. species	Undeter-minable
1	7	4	1	4	4	0
2	4	4	0	4	3	0
3	4	4	0	5	3	0
4	8	3	1	6	3	1
5	4	3	0	2	2	0
6	4	4	0	5	4	1
7	5	3	1	5	4	1
8	5	3	2	0	0	0
9	7	4	0	5	4	1
10	7	3	2	9	5	3
11	3	3	0	2	2	0
12	2	1	0	5	3	2
13	6	5	0	7	4	1
14	6	5	1	5	5	0
15	4	3	0	3	2	0
16	9	5	2	2	2	0
17	5	3	2	8	6	1
18	4	2	1	2	2	0
19	8	4	3	-	-	-
20	0	0	0	-	-	-

10 plates sufficient to give representative count.

APPENDIX IV

(b) Bacteria/Actinomycetes.
Counts at 10^{-5} dilution of soil.

	SERIES I	SERIES II
Plate	Bacteria/ Actinomycetes	Bacteria/ Actinomycetes
1	50	42
2	49	102
3	86	78
4	60	86
5	44	92
6	48	78
7	83	81
8	98	110
9	40	96
10	60	50
11	90	48
12	66	86
13	100	74
14	50	93
15	43	69
16	72	78
17	94	84
18	89	98
19	77	101
20	56	52

5 plates sufficient to give representative counts.

APPENDIX V

(a) Reclassification of penicillin-resistant bacteria according to the width of the inhibition zone (m.m.) between the other seven antibiotics.

"Type"	Code No.	P	S	TE	SP	C	E	CB	NV
A	1	-	6.0	7.5	5.0	9.0	9.0	9.0	9.5
	3	-	5.5	6.5	5.0	10.0	9.0	8.5	10.0
	92	-	5.0	6.5	4.5	9.0	8.5	8.0	10.0
	18	-	5.5	7.0	5.0	9.0	9.0	9.0	10.5
	2(a)	-	5.0	6.0	4.5	9.0	8.5	8.0	9.5
B	44	-	6.0	6.0	4.0	6.0	9.0	7.5	9.0
	57	-	5.5	5.5	4.5	6.0	8.0	8.0	10.0
	58	-	5.5	6.0	4.5	6.0	9.0	8.0	9.0
C	6	-	7.0	11.0	8.0	10.5	10.5	10.5	12.0
	42	-	7.0	10.0	8.0	10.0	10.5	9.5	11.5
	173b	-	8.0	11.0	7.5	10.0	10.5	10.5	12.0
D	10	-	8.0	11.0	8.5	4.5	12.0	12.0	12.0
	56	-	8.0	10.0	8.0	4.0	12.5	12.0	11.5
E	43	-	6.5	12.5	5.0	2.0	10.5	12.0	13.5
	53	-	6.0	13.5	4.0	2.5	11.0	12.5	12.5
F	41	-	5.0	11.0	6.0	4.0	11.0	12.0	8.0
	96	-	5.0	11.0	6.0	5.0	11.0	11.0	9.0
	60	-	4.5	10.0	6.0	4.0	11.5	11.5	8.0
	91	-	5.0	10.0	5.5	4.5	10.5	11.0	8.0
G	5a	-	5.5	9.5	4.0	10.0	7.0	5.0	11.5
	172	-	6.0	8.5	5.0	11.0	8.0	5.0	12.0
H	133	-	6.0	9.0	6.5	7.0	9.0	12.0	9.0
I	97	-	6.0	9.0	6.0	6.5	14.0	14.0	14.5
J	13	-	5.0	7.5	5.5	2.0	10.0	9.5	7.0
K*	Ab2	-	8.0	3.0	6.5	6.0	12.0	3.0	3.0
L	90	-	7.0	10.0	7.5	12.0	11.0	12.0	8.5
M	171	-	6.0	7.0	2.0	2.0	9.0	9.0	9.0
N	81	-	10.0	13.0	9.5	11.0	12.0	11.0	11.0

* Ashbed type.

APPENDIX V

(b) Reclassification of bacteria, showing some susceptibility to all antibiotics, in terms of the width of the inhibition zone (m.m.).

"Type"	Code No.	P	S	TE	SP	C	E	CB	NV
A	108	7.5	4.0	8.0	5.0	12.0	12.0	12.0	11.0
	143	8.5	5.0	7.5	6.0	12.0	13.0	13.0	12.0
	Ab1	7.5	5.0	8.5	5.0	13.0	12.0	12.5	11.5
	37	8.0	5.0	7.5	5.0	12.5	12.0	11.5	11.0
	40	8.5	4.0	8.0	6.0	12.0	12.5	12.0	12.0
B	7	13.5	7.0	11.0	6.0	8.0	10.5	10.5	13.0
	180	14.0	7.0	12.0	6.5	9.0	11.5	12.0	14.0
	51	14.5	7.0	12.5	7.0	8.5	12.0	10.5	13.5
	139	14.0	7.0	11.5	6.5	8.5	11.5	11.0	13.5
C	130b	3.5	3.5	8.0	3.0	9.0	8.0	7.0	12.0
	5	4.5	3.5	8.0	3.5	10.0	8.5	7.5	12.5
	167	3.5	4.5	9.0	4.5	10.5	9.5	8.5	13.0
	55	4.0	4.5	9.5	4.0	9.5	9.0	8.5	13.5
	54(b)	4.5	4.0	8.5	3.5	9.5	8.5	7.5	13.5
		4.5	4.5	8.5	4.5	10.5	8.0	7.5	12.0
D	46(b)	3.0	6.5	9.0	5.0	7.0	7.5	6.0	12.5
	75	3.5	5.0	8.0	5.5	8.0	8.0	7.0	12.5
	116	4.0	5.5	8.5	4.0	8.5	7.0	6.5	13.0
E	131	5.0	6.0	7.0	8.0	9.5	10.5	8.5	8.0
	38	5.0	7.0	7.5	9.0	10.0	11.5	9.0	8.5
F	190	5.0	5.5	7.0	5.0	10.0	9.5	7.0	8.0
	78	6.0	6.0	7.0	6.0	11.5	10.0	8.5	9.0
G	62b	7.5	3.5	7.5	4.0	12.5	10.5	11.5	13.0
	68	8.5	4.0	8.5	4.5	13.5	11.5	12.0	14.0
	71	8.0	4.0	9.0	4.5	12.0	11.0	12.5	13.0
H	48b	5.0	3.5	8.0	5.0	10.0	9.0	7.0	14.0
	161b	5.0	4.5	9.5	5.0	10.5	9.0	8.0	13.5
I	122	5.0	3.5	12.0	5.0	10.0	10.0	7.0	4.5
	114	5.5	4.5	11.0	5.5	11.5	9.0	8.0	4.5
	190b	6.0	3.5	12.5	5.5	12.5	10.5	8.5	5.0
	86	5.0	5.0	12.0	5.0	11.5	10.0	7.5	5.5
J	47b	3.0	7.5	12.0	10.5	13.0	13.0	7.0	12.0
	136	4.0	7.5	14.0	11.0	12.0	14.0	7.0	13.5
	109	3.0	8.0	13.5	11.0	13.0	14.5	8.0	13.0

"Type"	Code No.	P	S	TE	SP	C	E	CB	NV
K	63b	11.5	3.5	7.5	5.0	9.0	8.0	10.0	12.0
	119	12.0	5.0	8.5	6.0	9.5	9.0	11.0	12.0
L	179	4.0	5.5	5.5	4.0	7.0	7.5	7.5	7.5
	177	4.5	6.0	6.5	4.0	7.0	8.5	8.0	8.0
M	85	12.0	6.0	7.0	9.5	13.0	12.0	10.5	12.5
	123	13.0	7.0	8.5	10.0	14.0	12.5	11.5	13.0
N	2	8.0	7.0	11.0	7.0	11.0	8.5	10.0	11.5
	152	9.0	8.0	11.5	7.5	11.0	9.5	10.5	12.0
O	106	5.0	4.0	6.0	5.0	8.0	12.0	8.0	12.0
	134	6.0	5.5	7.5	5.0	8.0	13.0	9.0	12.0
P	69	4.0	3.0	11.0	6.0	8.0	11.5	6.0	13.5
	111	5.0	3.0	12.0	5.5	9.0	12.0	6.5	14.0
Q	59b	5.0	3.0	7.0	4.0	5.5	6.5	14.0	14.0
R	189	3.0	3.0	7.0	4.0	13.5	11.5	10.5	13.5
S	199b	4.5	5.5	13.5	6.5	12.0	9.0	10.0	14.0
T	117	5.0	4.0	9.0	5.5	14.0	9.0	4.5	14.0
U	27	4.0	2.0	7.0	2.0	6.0	11.0	8.0	13.0
V	110	10.0	13.0	10.0	5.0	10.0	9.0	13.0	13.5
W	113	14.0	8.0	10.0	13.5	6.5	8.5	8.5	13.5
X	165	9.5	6.0	6.5	6.0	12.0	11.5	9.0	11.0
Y	Ca4	7.5	7.0	12.0	7.0	11.0	13.5	5.5	10.5
Z	50	9.0	11.0	11.0	9.5	10.0	14.0	14.0	14.5
ZZ	138	5.0	6.0	6.0	5.0	4.0	12.0	6.0	8.5
	132	5.0	6.5	6.5	5.0	4.5	12.0	6.5	8.5

Code nos 1-10;41-50;81-90;121-130;161-170 - Unplanted control
11-20;51-60;91-100;131-140;171-180 - Planted control
21-30;61-70;101-110;141-150;181-190 - Unplanted ashbed
31-40;71-80;111-120;151-160;191-200 - Planted ashbed

Code nos with prefix C - control
A - ashbed