THE INFLUENCE OF ENDOMYCORRHIZAL INFECTION AND SOIL TEMPERATURE ON THE GROWTH AND SURVIVAL OF BARLEY ROOTS

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Karl M. Volkmar

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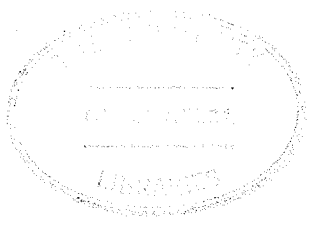

ABSTRACT

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Barley was grown at soil temperatures of 12, 16 and 20 C (air temperature 22/18 C day/night) in mycorrhizal and nonmycorrhizal soil. The root systems were segmented and examined for living biomass (amino acid and carbohydrate content and conductivity) and for lignification.

The optimum soil temperature for root and shoot growth of nonmycorrhizal plants was 16 C. Mycorrhizal infection appeared to shift the optimum to 12 C.

Mycorrhizal plants had a higher shoot/root ratio (dry weight) and greater root proliferation than nonmycorrhizal plants. Infection was associated with finer roots. Warm soil temperatures promoted optimum shoot growth of mycorrhizal plants and the greatest demands for soil water. Soil water deficits were attributed to heightened root proliferation of mycorrhizal plants grown at 16 and 20 C.

Enhanced phosphorus levels of mycorrhizal roots were associated with higher levels of root conductivity and amino acids. Root carbohydrate levels did not differ between mycorrhizal and nonmycorrhizal plants. Root amino acid and phosphorus contents increased with increasing soil temperature. Warm soil temperatures reduced the carbohydrate and conductivity levels of the roots of mycorrhizal and nonmycorrhizal plants.

Root lignification, a possible indicator of root age, was highest in root of mycorrhizal plants at the warmest soil temperature.

The improved nutrient and water uptake efficiency of the mycorrhizal roots may have been due to a larger surface area of the roots as well as to a delay in cortical cell degeneration resulting from localized mycorrhizal enhancement of root nutrient status.

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INTRODUCTION

Many soils are deficient in phosphorus in a form that is available to plants (Mengel and Kirkby, 1979). Two important factors that have been shown to influence the ability of plant roots to take up phosphorus are the age of the root and the microbial population in the vicinity of the root. (Clarkson and Robards, 1975; Martin, 1977).

Several studies have shown conclusively that mature regions of the root extending from near the root apex to the root base are capable of active uptake of phosphorus, and may therefore contribute significantly to the total phosphate supply of the plant. (Clarkson et al., 1968; Ferguson and Clarkson, 1975). However uptake of phosphorus in the mature regions of the root may be limited due to the rapidity of degeneration and collapse of epidermal and cortical cells a few centimeters behind the root apex (Martin, 1977). Under field conditions the rate of root deterioration may be hastened in the presence of indigenous non-pathogenic rhizosphere microflora. (Barber, 1973; Martin, 1977).

Some rhizosphere microorganisms have a beneficial effect on plant growth. Vesicular-arbuscular mycorrhizae are a form of plant-fungus symbiotic association that are widespread in natural soils and occur on most agronomic crops (Gerdemann, 1968; Mosse, 1973). Vesicular-arbuscular mycorrhizae can greatly improve phosphate supply of the host plant in phosphate deficient soils by making use of the absorbing capacity of the network of external hyphae associated with the infected root (Mosse, 1973; Gerdemann, 1975; Tinker, 1975). Improvement of supply of slow diffusing micronutrient ions such as zinc

and molybdenum has also been shown. (Gilmore, 1971; Mosse, 1973). In addition it has been demonstrated that mycorrhizal plants have reduced resistance to water transport as a consequence of the improved nutrient status of the plant. (Safir et al., 1972).

Very little information is available on the effects of mycorrhizae on general root anatomy and morphology. The improved localized nutrient status in the vicinity of the infection sites may contribute to longevity of function of the root cortex and may therefore be in part responsible for the improved phosphorus status of mycorrhizal plants. Ultrastructural studies of the infected cell have indicated increased cellular metabolism. (Kinden and Brown, 1975 a, b.). However, because the energy supply of the fungus is derived from host carbohydrate, it could be argued that the sink stress imposed on the host by the fungus may promote cell death.

Nutrient enhancement in localized regions of the root may influence root morphology. Several studies have shown that isolated root regions exposed to higher levels of available nitrate and phosphorus have stimulated root proliferation (Drew and Saker, 1975, 1978). The improved phosphate status of mycorrhizal roots may result in greater rates of root proliferation in the infected zones thereby furthering the nutrient uptake ability of the plant root.

Root temperature may be an important consideration since mycorrhizal infection is most influential on the plants' nutrient status during the plants vegetative stage, (Cox et al., 1975; Cox and Tinker, 1976), a time when soil temperature may be considerably lower than that of the air. Low soil temperatures (5 - 12 C) have been shown to reduce the rate of plant growth prolonging both vegetative and reproductive stages, but not final grain yield (Power et al, 1970). Cool root temperatures may therefore delay cortical cell deterioration as well as permit more extensive development of

mycorrhizal infection. In addition cool soil temperatures are known to induce root thickening and shortening (Neilson and Cunningham, 1964; Garwood, 1968). Cool soil temperatures may therefore restrict mycorrhizally enhanced root proliferation, if it occurs.

In order to clarify the role of mycorrhizae in improving the nutrient status of plants, a study was undertaken to investigate the influence of mycorrhizae on cortical cell deterioration and root proliferation.

LITERATURE REVIEW

Vesicular-Arbuscular Mycorrhizae

History and Taxonomy

Mycorrhizae are highly evolved symbiotic fungus plant root associations that occur among a diversity of fungal and plant species. The term mycorrhiza has been commonly used in reference to the fungus involved in this symbiotic association though strictly speaking this terminology is incorrect. Depending upon the context in which it is applied, both usages will be found in this paper.

Endotrophic mycorrhizae are distinguished from ectotrophic mycorrhizae by the presence of an extensive inter- and intracellular mycelial network in the host root cortex and the absence of a mycelial sheath or mantle around the root surface. Vesicular-arbuscular mycorrhizae (VAM) are endotrophic mycorrhizae recognizable from two other types of endotrophic mycorrhizae, the ericacean and the orchidacaous, by having aseptate mycelium, an extremely broad host range and a characteristic infection structure. Extensive surveys of Jones (1924), Butler (1939), Winter and Birgel (1953) and others have indicated that potentially the roots of most angiosperms are capable of acting as host to this group of fungi which have almost universal distribution in the soil.

Very few of the many plant families examined do not have VAM infection. Only the Ericales, Orchidaceae and certain ectomycorrhizal families such as Pinaceae and Betulaeceae are believed to definitely lack VAM, although it may be rare or absent from species in such families as Cruciferae, Chenopodiaceae, Cyperaceae and Polygonaceae (Gerdemann, 1968).

Rayner (1927), Butler (1938) and Mosse (1963) have reviewed the early mycorrhizal literature, while more recent research on mycorrhizae has been

reviewed by Gerdemann (1968), Harley (1968), Khan (1972), Mosse (1973), Gerdemann (1975) and Hayman (1978).

VAM have been assigned to the genus Endogone, family Endogonaceae, order Mucorales. This order is currently placed in the Zygomycotina. The genus Endogone includes zygosporic, chlamydosporic and sporangial species, though none of the sporangial species of Endogone are known to be mycorrhizal. (Gerdemann, 1968). Various species of Endogone are distinguished chiefly by the morphology of their large resting spores. These spores are generally round or oval and, when viable, contain oil globules. Spores range in diameter from 50 microns to nearly one millimeter, though most are between 80 to 250 microns in diameter (Mosse and Bowen, 1968; Gerdemann and Trappe, 1974).

Attempts to isolate Endogone from spores in pure culture have generally failed. Mosse (1959) obtained vigorous growth from Endogone chlamydospores but hyphae stopped growing before the nutrients in the spores was exhausted. Hyphae could not be subcultured. Attempts at obtaining two membered cultures of Endogone species and host plant under aseptic conditions had mixed success, (Mosse, 1962, 1963; Barrett, 1961; Gerdemann, 1969), until Mosse and Phillips (1971) successfully inoculated surface sterilized Endogone spores onto aseptically grown seedlings. Although vesicular-arbuscular mycorrhizae behave as obligate symbionts, they appear to have a surprising lack of specificity in the range of host plants that they can infect; the same species producing VAM on such diverse crops as corn (Zea mays), clover (Trifolium spp.), soybean (Glycine max.), onion (Allium cepa) and strawberry (Fragaria spp.) (Gerdemann, 1961).

Morphology and Ontogeny

Gross morphology and ontogeny of mycorrhizal infection have been described in several reviews (Gerdemann, 1968; Mosse, 1973; Hayman, 1978).

Upon germination of the resting spore, in the presence of a suitable host, the germ tube contacts the host root surface, and an appressorium is formed at the junction between two epidermal cells. Hyphae spread intercellularly in the middle and inner regions of the root cortex, growing parallel to the root axis. Hyphae are extremely variable in size and irregular in shape. Arbuscules develop within a host cell by repeated dichotomous branching of hyphal tips to form what appears as complex tree-like structures that may nearly fill the lumen of the cell. Vesicles develop intra- and extracellularly to form ovate to spherical swellings on or at the tips of distributive hyphae. External mycelium forms a loose network in the soil around the root. The main network of mycelium is formed from coarse hyphae 20 to 30 microns in diameter which are thick walled and knobbly (Nicolson, 1967). Fine hyphae 2 to 7 microns in diameter, which are thin walled and ephemeral, arise from the coarse hyphae. Large resting spores originate from the coarse external hyphae.

Bevege et al. (1975) estimated that external mycelial hyphae of Endogone mossae infected roots of clover represented about 1 percent of the total dry weight of the plant. However, from chitin assays, Hepper (1977) concluded that VAM may account for as much as 9 and 17 percent, respectively, of the dry weight of infected onion and clover roots. The internal mycelial network therefore comprises a large proportion of the fungal mass.

A three phase pattern of development involving sequentially, a lag phase, a phase of extensive mycorrhizal development and a constant phase in relation to the proportion of mycorrhizal to nonmycorrhizal roots was found to be characteristic of many plant species (Furlan and Fortin, 1973; Sutton, 1973; Sanders, 1975; Saif, 1977). The duration of the lag phase was related to the time required for spore germination, germ tube growth and host

penetration. Formation of external mycelium was observed to spread in the fungus through the root cortex and to initiate an abundance of new colonies in the roots. The third phase coincided with fruit development of the host, and continued until host senescence. In general, arbuscular development was prominent in the vegetative stage of the host corresponding to the exponential phase of mycorrhizal infection. In contrast, the reproductive stage of the host was characterized by a reduction in arbuscular infection and a greater number of vesicles. VAM spore number was shown to be related to the amount of root infection (Sutton, 1973). Maximum spore production was observed to occur after the period of maximum host root production (Saif, 1977).

Effects on Plant Growth

It is well established that VAM can increase plant growth, particularly on soils of low fertility, (Mosse, 1973; Gerdemann, 1968). Assai (1956; cited in Gerdemann, 1967) published the first good evidence for mycorrhizal related growth improvement. He compared the growth of plant species susceptible to mycorrhizal infection to plants resistant to infection and found that mycorrhizal species grew best when infected with mycorrhizal inoculum, whereas nonmycorrhizal species were uninfluenced, whether inoculum was present or not.

Mosse (1957) used sporocarps of E.Mosseae to inoculate apple (Malus spp) seedlings grown in autoclaved soil, and found that the mean size of mycorrhizal plants was greater than that of the controls. Baylis (1959) inoculated Grisselinia littoralis by growing seedlings in a soil containing VAM, then transferring the seedlings to sterile soil. He found that the infected plants grew normally whereas growth was stunted or ceased entirely in plants that failed to become infected.

The improved growth of mycorrhizal plants is considered to be largely due to an improved phosphorus status. Gerdemann (1964) inoculated maize grown in infertile steamed soil with sporocarps of E. mossae and observed that mycorrhizal plants had almost four times greater dry weight and had removed more soil phosphorus than controls. Bowen and Mosse (1965, cited in Gerdemann, 1967) utilizing ^{32}P as a tracer with two membered cultures and with pot-grown onion and clover plants found that mycorrhizal plants absorbed nearly two times as much phosphate as uninfected plants. Gray and Gerdemann (1969) found significantly greater radioactivity in the roots and shoots of mycorrhizal than nonmycorrhizal onion plants grown for 64 days, then exposed for 90 hours with H_3PO_4. They also noted that there was twenty-five times greater radioactivity in mycorrhizal root segments than in uninfected root segments of mycorrhizal plants, and five hundred times more radioactivity than that in nonmycorrhizal plants.

Mechanisms of Plant Growth Enhancement

Early studies attributed the mycorrhizal enhancement of phosphate nutrition to the ability of mycorrhizae to hydrolyze the insoluble fraction of soil phosphorus, thereby shifting the soil phosphorous equilibrium toward increased phosphate in solution. Daft and Nicolson (1966) found that addition of bonemeal to mycorrhizal tomato plants (Lycopersicon esculentum) improved growth compared to nonmycorrhizal plants. Murdoch et al. (1967) found that mycorrhizal corn plants grown in soil that received highly soluble sources of phosphate, viz. monocalcium and super phosphate, did not have a higher phosphate content than nonmycorrhizal plants while mycorrhizal plants grown in soil that received slightly soluble phosphate sources, viz. rock or tricalcium phosphate had a phosphate content much higher than nonmycorrhizal plants. Hayman and Mosse (1972 a) reported improved growth of mycorrhizal

onion plants in soils amended with rock phosphate compared to nonmycorrhizal plants.

Sanders and Tinker (1971), Hayman and Mosse (1972) and Mosse et al. (1973) investigated the source of the extra phosphate taken up by mycorrhizal and nonmycorrhizal onions. Where the pool of labile soil phosphorus had been labelled with ^{32}P, they found that mycorrhizal and nonmycorrhizal plants took up P with the same specific activity in all soils tested, indicating that both used the same or a similarly labelled fraction of soil phosphorus.

Sanders and Tinker (1971, 1973) calculated the inflow or the uptake per unit length of root per unit time of phosphorus to mycorrhizal and nonmycorrhizal onion plants using radioactive phosphorus in order to determine whether differences in uptake of phosphorus were due to an increased flux per unit surface area or an increased root area. Their results showed that mycorrhizal plants had a lower root to shoot ratio in terms of dry weight and in terms of root length than nonmycorrhizal plants. In addition phosphorus inflow into mycorrhizal roots was on average four times larger than that into nonmycorrhizal roots. The measured inflow of phosphorus to nonmycorrhizal roots was found to be very close to the maximum inflow of phosphorus to a root operating as a zero concentration sink. They concluded that the greater inflow of phosphate to roots of mycorrhizal plants was due to the ability of external hyphae to take up soluble phosphorus by extending beyond the phosphate depletion zone and translocating and releasing it to the host root cortex.

Macroautoradiographic studies using mycorrhizal onion plants have shown that external hyphae may extend from 1 to 7 cm beyond the root surface (Sanders and Tinker, 1973; Hattingh et al., 1973; Pearson and Tinker, 1975; Rhodes and Gerdemann, 1975). Tinker (1975) pointed out that root hairs are

less effective than mycorrhizal hyphae as absorbing organs because they are short lived, and because inter hair competition results in overlap of depletion zones which reduces their effective absorbing surface area. Baldwin et al.(1972) proposed that cylindrical zero sink absorbing organs compete seriously when the distance between them is less than the square root of the diffusion coefficient of that ion multiplied by the time of diffusion. This corresponds to 0.29 mm for phosphate ions. They suggested that root hairs which are typically 0.7 mm long (Lewis and Quirk, 1967) and present up to 50 hairs per mm (Drew and Nye, 1970) would compete seriously with one another up to a distance of 0.67 mm from the root surface, and in effect would behave like hairless root with a diameter of approximately 1.5 mm.

Baldwin et al.(1972) compared the efficiency of root hairs to mycorrhizal hyphae in taking up soil phosphorus. He estimated that external hyphae 4 cm long that penetrate the root surface 1 to 20 times per mm of root and have a diameter of approximately 25 microns (Mosse, 1959, Kessler, 1956; Bielesky, 1973), would have hyphae about 0.5 mm apart at the base and diffusion shells would overlap far less than for root hairs. The result would be an increase in uptake of phosphorus up to 60 times that of root hairs if diffusion was the limiting factor. Bowen et al (1975) calculated that 1 mg. of hyphae would have the same length as 1600 mg of root 0.4 mm in diameter or 1 to 4 mg of root hairs.

Owusu et al.(1979) used autoradiography to compare the depletion zones of ^{32}P labelled phosphate around mycorrhizal and nonmycorrhizal onion roots. They found that the effect of the mycorrhizae was to increase the radius of the depletion zone from 0.1 to 0.2 cm. The positive response of mycorrhizal plants to insoluble forms of phosphate that have been alluded to were explained by Tinker (1975) as being the consequence of randomly distributed hyphae reducing the mean distance between rock phosphate grains and the nearest

absorbing surface. Tinker (1975) did not completely exclude the possibility that hyphae may in addition have some special property of dissolving rock phosphate, for example, through local alteration in the pH, or cation concentration at the mineral surface.

The increase in surface area provided by mycorrhizal plants would be expected to influence uptake of ions other than phosphate that are in short supply. Gilmore (1971) demonstrated increased Zn uptake and plant growth by mycorrhizal peach (Prunus persica) seedlings in Zn deficient soil supplied with N, P, K, Ca Mg, B and Fe EDTA. Gray and Gerdemann (1973) demonstrated greater uptake of sulfur in mycorrhizal maize and clover plants by labelling sand with ^{35}S. Jackson et al (1973) found much greater ^{90}Sr uptake of mycorrhizal soybeans from ^{90}Sr amended soil.

Studies investigating the influence of mycorrhizae on uptake of N, K, Ca, Mg, Fe, Cu, Ma, Zn, Na, and B have given inconsistant results (Mosse, 1973). Gerdemann (1975) suggested that uptake of any element of limiting availability in the soil would likely be increased in mycorrhizal plants.

Few studies have investigated the influence of mycorrhizaë on water uptake. Safir et al.(1970) compared resistance to water transport in whole soybean plants infected with mycorrhizae to that of nonmycorrhizal control plants and found that resistances were similar for the first 21 days of growth but by 28 days resistance to water movement in nonmycorrhizal plants had increased by 40% over mycorrhizal plants. They also found that addition of nutrients to non-mycorrhizal plants eliminated differences in root resistance between the two treatments (Safir et al.,1972). The authors concluded that since root volumes and root dry weights were not significantly different between the infected and uninfected plants root growth was not involved, nor was increased hyphal surface area since addition of a fungal inhibitor PCNB did not affect resistances. They proposed that decreased water resistance observed in mycorrhizal plants is

related to an enhanced nutrient status of plants grown in low nutrient soils.

Transfer of host plant assimilate to the VA fungus has been reported. Ho and Trappe (1973) demonstrated that photosynthetic ^{14}C carbon travels from host to spores. The well documented accumulation of lipid droplets in external and internal hyphae, vesicles and spores of VAM (Mosse, 1973) was verified by Cox et al.(1975) who found that photosynthetically labelled ^{14}C carbon was transferred from host to mycelium where it was detected in lipids. Bevege et al.(1975) investigated the distribution of ^{14}C labelled photosynthate in infected and uninfected roots of mycorrhizal hoop pine (Araucaria cunninghamii) and onion and found that a high proportion of the ^{14}C lipid fraction occurred in the fungal hyphae external to the root. This was further confirmed by qualitative and quantitative studies of the lipid content of mycorrhizal and nonmycorrhizal onion roots (Cooper and Loesel, 1978). Lewis and Harley (1965) and Smith et al.(1969) showed that transfer of carbohydrates from host to fungus in fungal parasites and ecto mycorrhizas involves the conversion by the fungus of host derived glucose into other sugars such as trehalose or polyols such as mannitol which could not be metabolized by the host plant. Because trehalose or mannitol were not observed in the case of VAM, (Hepper and Mosse, 1972; Hayman, 1974; Bevege et al,,1975), Cox et al.(1975) suggested that the synthesis of lipid by the fungus could provide an alternative sink for host derived photosynthate. From the results of their studies Bevege et al (1975) proposed that sucrose and glucose were the principal host sugars transferred and that conversion of these sugars into glycogen and eventual incorporation into lipids, proteins and organic acids by the fungus would maintain a sufficient sink for further passive or facilitative sugar transfer.

Loesel and Cooper (1979) studied the utilization of exogenous carbon by mycorrhizal and nonmycorrhizal roots compared with that of photosynthate translocated from the leaves in order to assess the relative importance of

lipid and other types of metabolism in mycorrhizal roots. They found that exogenous carbon sources were incorporated into the same range of cell components as that derived from photosynthate, and that the rate and amount of incorporation of exogenous and endogenous carbon sources was greater in mycorrhizal plants. The authors reported no increase in incorporation of ^{14}C labelled photosynthate into lipids in mycorrhizal roots. They suggested that this may have been due to insufficient time allowed for synthesis of ^{14}C labelled lipid by the fungus. They did find, however, that exogenously applied acetate and glycerol were incorporated into lipids at an accelerated rate in mycorrhizal compared to nonmycorrhizal roots.

Lösel (1979) also observed that sucrose fed mycorrhizal roots had twice as much ^{14}C labelled soluble carbohydrate as sucrose fed nonmycorrhizal roots.

In agreement with a study by Jasper et al.(1979) Lösel found that there was approximately an 80% increase in alcohol soluble compounds derived by ^{14}C sucrose roots in mycorrhizal plants over that of controls.

Bevege et al.(1975) pointed out that extramatrical Endogone hyphae was only 1% of the total weight of the plant, reflecting a relatively small diversion of photosynthate to the fungus, yet this diversion increased the plant fresh weight by 150%.

Nutrient Exchange Mechanisms

Early observations of VAM (Demeter, 1923 cited from Cox and Tinker, 1976; Kelley, 1950) described the rupture of arbuscular tips and loss of fungal cytoplasm to the host cell. This led to the theory of nutrient exchange in mycorrhizal roots prevalent during the first half of this century (Rayner, 1927; Kelley, 1950);that the fungus was a parasite initially extracting nutrients from the host. The host however then made use of the fungus by

digesting its arbuscules, thereby obtaining mineral nutrients derived from the soil. More recently Harley (1969, 1975) urged workers to consider transfer across the membranes of living cells. He pointed out that nutrient transfer occurred in ectomycorrhizae without breakdown in fungal structures, and that the fine branching characteristics of arbuscules provided a structure well adapted for transfer and absorption of nutrients. Moreover, he stated that since transfer across a living interface could take place over an extended period, more nutrients than the fungus obtained at any one time could be transported to the host. Electron microscopical studies by workers in the mid-seventies have tended to support Harley's hypothesis.

Kinden and Brown (1975 a, b) in their ultrastructural study of mycorrhizal infection of poplar (Populus spp.) detected evidence of increased metabolic activity of the host cells in the presence of hyphae and arbuscular development as indicated by enlargement of host nuclei, decline in the level of starch, and an increase in host cytoplasmic volume. They also noted, as did Cox et al. (1975) a large number of deteriorated arbuscules, indicating an apparent rapid turnover of arbuscules during fungal infection.

Cox and Tinker (1976) used tracings of electron micrographs of mycorrhizal roots of onions using an image analyzer to derive estimates of the volume occupied by the fungal arbuscules, the increase in volume of the host cytoplasm after infection, and the area of interface between fungus and host. From these calculations, they found that the inflow of phosphorus from the fungus to the host solely from arbuscular digestion was less than 1% of the phosphorus flow as calculated by Sanders and Tinker (1973). Findings such as these have led to the conclusion that most of the nutrient transfer between fungus and host occurs prior to arbuscular collapse.

Callow et al. (1978) extracted polyphosphate from onion roots infected with

mycorrhizal, but not from nonmycorrhizal onions. They calculated that on a dry weight basis as much as 40% of the total phosphate in the fungus may be polyphosphate. Similarly, Cox et al. (1975) found polyphosphate granules in small vacuoles in VA fungal cytoplasm. They suggested that packets of polyphosphate could provide a means of translocation of P in the fungus. Their ultrastructural studies indicated that vacuoles lost their polyphosphate after they reached fine hyphae tips. Cox et al. (1975) hypothesized that this was the site of polyphosphate hydrolysis and transfer of phosphorous to the host.

Gianinazzi et al (1979) detected acid phosphatase activity in young developing arbuscules in onion roots which declined at arbuscular maturity, while alkaline phosphatase was prominent in the later stages of development in the vacuoles of mature arbuscules. This finding prompted them to conclude that vacuolar alkaline phosphate may be involved in the active mechanism of phosphate transport to the host.

Factors Influencing Mycorrhizal Infection

Important factors affecting the intensity of VA infection include fertilizers and plant nutrition, light intensity, air and soil temperature, soil moisture, pH, inoculum density, and plant susceptibility.

The extent to which a root system becomes mycorrhizal has been shown to be related to the availability of phosphorus in the medium. Baylis (1967) and Daft and Nicolson (1966) applied different amounts of phosphorus to soil and sand onion cultures, and found that the beneficial effects of VAM on the host were inversely related to the amount of available phosphorus. Hayman and Mosse (1971) examined the response of onion infected with mycorrhizae to growth in a wide range of soils varying in phosphorus availability and found that soils deficient in phosphorus induced greater responses to mycorrhizae than did less deficient soils. They found that mycorrhizal infection was

extensive in all soils, including those containing high amounts of phosphorus.

Khan (1972) noted a reduction in root infection of maize with increased soil phosphorus.

Endomycorrhizal fungi were found difficult to establish in agar media when the soluble phosphate level rose above 30 ppm (Mosse and Phillips, 1971).

Mosse (1973 b) found that onion plants infected with mycorrhizae were more susceptible to stunting of growth in the presence of added phosphorus than nonmycorrhizal plants. She attributed this to a build up of phosphorus in the plants to a toxic level. She suggested that added phosphorus caused the fungal growth habit in the plant to change. Fungal hyphae branched profusely, and penetration points were far more numerous, but hyphae were unable to spread into adjacent cells. She also found that in sandy soils lower phosphate concentrations were required to induce the same response as in clayey soils.

In confirmation of Mosse's (1973) findings, Mosse et al. (1976) found that where phosphate concentrations in mycorrhizal onion plants as well as in several legume species was around 0.17% inoculation always improved growth, but where the concentration was near 0.2%, growth increases were generally negligible.

Several studies have shown enhanced infection development in response to fertilizer addition. Bevege (1971, cited in Hayman, 1971), found a trend toward increased mycorrhizal infection with increased rates of applied nitrogen in field plots of hoop pine, in particular at intermediate levels of phosphorus (37 - 74 kg/ha) and nitrogen at 448 to 4032 kg/ha as urea. Hayman (1975) speculated that increasing the nitrogen level in the plants slowed down the decline in infection which would normally occur upon addition of phosphate by encouraging vegetative growth, thereby decreasing the concentration of phosphorus in the plant.

Other anomalies have been reported. Hayman (1975) could find no correlation between the amount of mycorrhizae at a particular field site and the fertility level of the soil, mycorrhizae being abundant in both poor and rich soils. Differences in the initial fertility levels of the soil were suggested as at least partly responsible for the lack of correlation. Sites may also have differed in initial inoculum densities and species, moisture levels, pH, and soil structure which all influence mycorrhizal response. For example, Kruckelman (1975) reported that spore numbers increased progressively in sandy soil plots manured for 50 years with N, P and K, or compost or both, while silty clay loam soils showed decreased spore number as a result of long term fertilizer treatment.

A study by Owusu-Bennoah and Mosse (1979) reported that mycorrhizal onion and alfalfa (Medicago sativa) showed increased response with increased phosphorus up to 14 ppm P., but that mycorrhizal barley (Hordeum vulgare) only responded to inoculum at lower phosphorus levels (8 - 11 ppm P.)

Several experiments have since been conducted that confirm Mosse's (1973 b) proposal that the phosphorus concentration of the plant determines the amount of VAM infection. Sanders (1975) found that phosphorus injected into leaves of mycorrhizal onion plants reduced the spread of mycorrhizae, the weight of external mycelium per cm of root, and the supply of phosphorous to the host via the fungus. Menge (1978), employing a split root technique found that phosphorus fertilization of half of the root system of sudan grass (Sorghum vulgare pers.) could reduce the spore number of the VA fungus in the unfertilized half of the root system. In a second experiment, sudan grass roots grown in pots containing either 6 or 600 ppm P were allowed to grow randomly into open topped screen vials containing soil with phosphate concentrations of either 6 or 600 ppm P. Pots were inoculated with Glomus fasciculatus. They found that the amount of mycorrhizal infection in roots

inside the vials was inversely correlated with the concentration of phosphorus in the roots outside the vials. Thus, vials containing soil with 600 ppm P but surrounded with soil containing 6 ppm P had evidence of infection which was similar to that present in vials with 6 ppm P. The results therefore supported those of Sanders (1975) and also illustrated that localized high concentrations of soil phosphorus did not inhibit infection if the root phosphorus concentration was low.

In an experiment designed to study the effect of phosphate placement on mycorrhizal development, Jasper et al. (1979) working with subterranean clover found that the proportion of root volume infected decreased with high rates of phosphorus. However the different concentrations of phosphorus in a particular root zone did not affect the extent of mycorrhizal infection in that zone. In another experiment they studied the effect of previous and current phosphorus supply on mycorrhizal infection and found that the amount of mycorrhizal infection was correlated with the concentration of phosphate in the plant at the initial stages of infection, but not with the phosphate concentration at the time of assessment. The alcohol soluble carbohydrate level in roots 10 days after transplantation was inversely proportional to phosphate level in the shoots and roots, and directly proportional to the level of infection 21 days later.

A number of recent papers have reported that plants infected with VAM contain generally higher internal phosphate concentrations than do uninfected plants of the same size. For example, Abbott and Robson (1977, 1978), Pairunan et al (1980), and Sparling and Tinker et al (1980) have observed this effect on subterranean clover (Trifolium subterraneum) and white clover (Trifolium repens), while similar results were reported for leek plants, (Allium porrum) (Stribley et al. 1980a). Stribley et al. (1980 b) interpreted these findings in terms of an inability of mycorrhizal plants to fully

exploit the improved phosphorous status because of fungal drain on host photosynthate. They pointed to studies by Cox and Tinker (1976) who reported increased root respiration and carbon accumulation in cortical cells infected with VAM. A study by Pang and Paul (1980) using ^{14}C labelling showed a 74% increase in CO_2 loss from mycorrhizal soybean roots. Stribley et al. (1980 a) suggested that reports of yield losses on mycorrhizal plants given phosphate fertilizer (Crush, 1975; Hall et al., 1977) may be attributed to plant carbohydrate losses to the fungus.

Furlan and Fortin (1973) examined the influence of three different air temperature regimes (11/16 C, 16/21 C, 21/26 C; Night/Day) on the chronology of development and reproduction of mycorrhizal infection on onion plants. They found that cool temperatures had a depressing and lengthening effect on each component of the infection development curve. Increased temperatures reduced the length of the lag phase, increased the intensity and the rate of infection and enhanced spore production. They also noted that the dry weight of the low temperature mycorrhizal plants did not differ significantly from that of nonmycorrhizal plants, whereas infected plants at the higher temperatures showed significant growth improvement.

Peyronel (1940, cited in Hayman, 1974) observed more VAM infection in cereals grown in sunny than in shady sites. Baylis (1967) found that shading to about one-third full daylight did not reduce the level of VAM infection of the shade dwelling plant Coprosma roual. Hayman (1974) reported that high light density, up to 25,000 lux increased the soluble carbohydrate content, plant growth and the mycorrhizal effect on onion plants. Low air temperatures (14 C) appeared to have the same effect as low light (13,000 lux). Air temperatures of 14^{o} C at 13,000 lux caused no growth improvement over uninoculated controls. Mycorrhizal plants kept at 13,000 lux for 6 hours at

18° C were over three times heavier than nonmycorrhizal controls. The effect of infection increased in longer day lengths and higher light intensities to 14.2 times the weight of controls with 18 hours at 25,000 lux.

Fürlan and Fortin (1977) contrary to observations by Hayman (1974) found that rates of maximum percent infection of mycorrhizal onion plants were lower at higher light intensities (15,000 to 20,000 lux) than at lower ones (5,000 to 10,000 lux). Growth enhancement was reported to be more significant and occurred over a longer time period under low light intensities. High light intensity stimulated spore production toward the later stages of growth.

Daft and El-Giahmi (1978) showed that partial or complete defoliation or exposure to low light intensity (6.7 and 19.7 Wm^{-2}) or short day lengths (6 to 12 hours) depressed the development of mycorrhizal infection in tomato, maize, alfalfa and a lawn grass mixture. They hypothesized that the supply of photosynthate to the fungus affects mycorrhizal development.

Apparent contradictions cited above regarding the effects of light on VAM infection may have been due to differences in response of different species of endophytes to different plant species and their interaction with the environment. It has been well documented that many soils harbor inefficient mycorrhizal species, and that inoculation of unsterilized field soils with strains of efficient fungi can increase plant growth and phosphorous uptake in the greenhouse (Mosse and Hayman, 1971; Mosse et al.,1976; Mosse, 1977; Powell and Daniel, 1978) and in the field (Khan, 1972, 1975; Black and Tinker, 1977; Powell, 1977; Hayman and Mosse, 1979; Owusu-Bennoah and Mosse, 1979). Powell and Daniel (1978) inoculated three successive crops of rye grass (Lolium perenne) grown in sterilized soil receiving soluble or rock phosphate, with several endophyte species and found that plants infected with an efficient fungus, e.g. Glomus tenuis were much more efficient than other introduced and indigenous mycorrhizal species at stimulating phosphorus uptake. Similar

findings were obtained by Owusu-Bennoah and Mosse (1979) when onions, alfalfa, and barley were inoculated with one or two endophytes placed below the seed in the field containing fairly high levels of indigenous mycorrhizal fungi, but low in phosphorus (8 - 14 ppm available P). They found that the extent of infection was similar for both endophytes, but that one species improved growth by 4 - 6 fold compared to a second species that increased growth only 80% over that of controls in alfalfa and onion. There is a shortage of information on environmental effects relating to mycorrhizal species.

Few studies have investigated mycorrhizal related nutrient uptake under water stress conditions. Work by Mexal and Reid (1978) and Theodorou (1978) on ectotrophic mycorrhiza has shown that mycorrhizal fungi can tolerate and grow in laboratory media with low water potentials. Reid and Bowen (1978) examined the uptake of ^{32}P by excised ecto-mycorrhizal roots of Pinus radiata in solution, adjusted with polyethylene glycol to water potentials ranging from 0 to -1.2 MPa. They found that uptake of phosphorus based on surface area of the roots was little affected by water potential in either infected or non-infected plants. They concluded that low moisture is more apt to have major effects on the transfer of ions to roots as a result of reduced hydraulic conductivity. The increased penetration of the soil by hyphal strands, they suggested would have large advantages under conditions of moisture stress.

A study by Rabatin (1979) measured the seasonal differences in the percent length of roots of Gramineae spp infected with an efficient mycorrhizal species, Glomus tenuis. They observed that the degree of infection declined in the warm summer months in soil cores taken where the percent soil moisture was low. In addition they suggested that increased soil moisture in the spring led to a rise in the level of soil microflora that could solubilize organic

soil phosphorous. This would result in roots shedding inefficient endophytes in favor of those that could better compete for soil phosphorus.

Little mention is made in the literature of pH effects on mycorrhizal infection. Mosse (1972) noted that Endogone strains did not grow well in very acid soils. In the same experiment in which she tested the effect of four introduced strains of Endogone on the growth of Paspalum notatum in Brazilian soil containing indigenous mycorrhizal species, she reported that the performance of the fungal strains was influenced considerably by liming and soil types; a pH of 5.8 enhancing the performance of one strain in one soil but not in the other, while another spore type was best at pH 4.8 in both soils. Mosse (1972) suggested that the differential effects may have been due to direct pH effects on fungal growth, phosphorus availability, or to changes in calcium concentration in the soil.

Mosse et al. (1976) examined the interaction between VAM, utilization of rock phosphate and nodulation on three legumes and onions grown in soils low in available phosphate and ranging in pH between 5.3 to 8.1. They found that for most soils rock phosphate utilization by mycorrhizal plants was not significantly improved over that of controls in neutral and alkaline soils, but that in acid soils adding rock phosphate increased uptake of phosphate.

Root Functioning

Symplast and Apoplast

Munch (1930) distinguished two pathways for water and ion movement through the root cortex. These have been designated the apoplast and the symplast. The apoplast is situated outside the plasmalemma and solute and solvent movement in the apoplast proceeds via the intermicellar and interfibrillar spaces of the cell wall as well as through intercellular air spaces. The symplastic component is delineated by the plasmalemma which constitutes the external surface of living protoplasm. Interconnection of protoplasts of different cells by plasmodesmata forms a symplastic system.

Crafts and Broyer (1938) are credited with one of the more commonly accepted hypotheses explaining ion transport in the root. They suggested that an ion pump located in the plasmalemma of the root epidermis, root hair and cortical cells actively transports ions into the symplast. In this hypothesis the apoplast of the root cortex can be penetrated by water and ions from the external medium. The plasmalemma of all cortical cells therefore provides a greatly expanded surface for active ion uptake into the symplast. The endodermis has long been recognized as a block to continuous apoplasmic movement into the stele (Priestly, 1926; Van Fleet, 1961). Clarkson and Robards (1975) described three prominent stages of endodermal development: Casparian bands composed of incrusted lignin and suberin laid down within a 5 to 7 millimeters of the root tip on the transverse and longitudinal radial walls of the proendodermis constitute the primary state (State I) endodermis; deposition of a thin continuous suberin lamella over the inside of the endodermal wall followed by production of a thick cellulosic wall-layer around the inner tangential and radial wall signifies formation of the State II and State III endodermis. Robards et al.,(1973) suggested that the latter

developmental states prevent direct exchange of solutes between the symplast and apoplast at the endodermis. Clarkson and Robards (1975) proposed three important functions of the endodermis: it provides a barrier to free diffusion of ions out of the root via the apoplast from the xylem; it allows development of root pressure in the xylem; and it ensures selectivity in ion transport toward the xylem, as all ions must traverse at least one set of membranes. Lauchli (1976), Spanswick (1976), and Luttge and Higginbotham (1979) have reviewed the current literature relating to apoplastic and symplastic transport.

Despite the evidence for considerable endodermal thickening behind the root apex, the rate of uptake and translocation of phosphorus and potassium has been observed to remain fairly constant along the length of the root. Russell and Sanderson (1967) found that translocation of phosphorus occurred to an approximately constant extent over the apical 6 cm of cereal roots.

Clarkson et al.,(1968) found that 2 - 3 week old barley plants grown in culture solution took up and translocated up to four times more radioactive phosphorus from the basal segments than from the tip. In contrast, very little ^{85}Sr, which is used as a tracer for calcium, was translocated from basal segments. In a subsequent experiment they demonstrated that phosphate is transferred to the stele via the symplast. They found that desiccating the basal root axis by lowering the culture solution level and forcing dry air over the exposed roots reduced the rate of phosphorus uptake to less than one twentieth of the former rate. Autoradiography of the desiccated site showed that most of the radioactive phosphorus was distributed in the cell debris outside the cortex. They suggested that the differential uptake of phosphorus and strontium was due to the restriction of passage of strontium through plasmodesma whereas phosphorus is freely able to traverse plasmodesma. Similar evidence was reported for corn by Ferguson and Clarkson (1975). In

this experiment, they also noted that DNP, CCCP and low temperature (4 C) treatments which would limit ion uptake which depends upon metabolic activity, reduced the amount of phosphate entering the stele by more than 90%.[1] Sanderson (1975) found that uptake was comparable for rapidly growing barley roots in the field and those grown in solution culture.

The importance of plasmodesmata in symplastic transport has not been disputed. (Gunning and Robards, 1976). However, details of their fine structure have been a matter of contention among electronmicroscopists. Gunning and Robards (1976) provided a detailed review on interpretation of structure of plasmodesmata as it relates to their function.

Root Decomposition

It may be concluded from the studies on phosphorus and strontium uptake presented above that not only the root tip, but also the mature regions of the root contribute considerably to the nutrient status of the plant. It is therefore relevant that a number of studies have indicated that behind the root apex, cortical cells undergo progressive degeneration with increasing age of the plant root.

Holden (1975) used nuclear staining to assess the rates of cell death in cortices of seminal roots of 3 to 4 week old wheat plants and observed that more than 70% of the cortical cells were dead. Rovira (1973) examined ultrathin sections of the root-soil interface of wheat plants and found that in the older regions of the plant root, bacteria lyse the walls of cortical cells which is followed by dense bacterial colonization. Similar observations were reported by Darbeyshire and Greaves (1973). Electron microscopical studies by Foster and Rovira (1976) also showed extensive invasion and degradation of epidermal and cortical tissue of roots grown in natural conditions.

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[1] DNP: 2, 4 - Dinitrophenol; CCCP: Carbonyl Cyanide-m-Chlorophenylhydrazone.

Three stages of root decomposition of wheat (Triticum aestivum) growing in non-sterilized soil were listed by Martin (1977) based upon his studies of the release of carbon compounds from wheat roots: a continuing release of low molecular weight compounds from degenerating epidermal and cortical cells occurs as a normal process during the stages leading up to flowering. Soil microflora accelerate this process, according to Martin (1977). Invasion of deteriorating cells by soil microflora marks the second stage causing extensive breakdown of cell walls. The decomposition of the endodermal tissue and plant death signifies the final stage.

Rhizosphere

Hiltner (1902) assigned the term rhizosphere to that zone of soil influenced by the root and found greater numbers of microorganisms in this region than in soil beyond the rhizosphere. Attempts to better define the zones of influence have led to such terms as outer and inner rhizosphere, and rhizoplane. (Rovira, 1965). Louw and Webley (1958) defined the rhizosphere as that soil which is removed from roots when the roots are shaken firmly in sterile water. Rouatt and Katznelson (1961) distinguished between the rhizosphere and rhizoplane microflora by shaking roots and soil to provide the rhizosphere sample and then after several washings, macerating and suspending the roots to provide the rhizoplane sample.

It has been demonstrated that soil microflora can strongly influence the rate of cortical cell lysis (Martin, 1977). It is important therefore to examine what factors influence microbial activity in the rhizosphere, and how the rhizosphere microflora influences nutrient uptake and plant growth.

Plant Factors Influencing the Rhizosphere Response

"The magnitude of the rhizosphere response in terms of microbial numbers is markedly influenced by physical, chemical and biological factors. Soil

moisture, temperature, aeration, reaction and fertility have been shown to affect the rhizosphere population directly or indirectly through their effect on the plant" (Darbeyshire and Greaves, 1977). The primary cause of the rhizosphere effect is the presence of soluble and insoluble compounds released from living and dead cells (Darbeyshire and Greaves, 1979).

Martin (1977) distinguished between root exudates defined by Rovira (1969) as substances released from healthy intact plant roots, and root lysates, which included compounds resulting from autolysis of root hairs, sloughed root cap cells and epidermal and cortical cells. The mucilaginous layer described by Jenny and Grossenbacher (1963) as resulting from an active secretory process involving specialized cells, was considered by Martin (1977) as a separate class of organic materials. He described the primary rhizosphere effect as a composite of the interaction of soil microflora with root exudates, root lysates, mucigel, wall residues and intact plant cells. The estimated amount of various lysates and exudates released from plant root has been recorded as ranging from 2% of the total root dry matter (Rovira and Davey, 1974) to 5% to 9% (Barber and Gunn, 1974) for plants grown in solution culture. Plants grown in sterile soil were estimated to exude between 7 - 13% of their total plant dry matter within 3 weeks, while under non-sterile conditions losses increased to 18 - 25%. (Barber and Martin, 1976).

Both the nature and amounts of substances exuded from plant roots has been found to vary with plant age, species and environmental conditions. Rovira (1959) compared exudation rates of tomato and sweet clover grown at low (25/21 C; day/night) and high (31/21 C; day/night) air temperatures for four weeks. He found that the amount of exudate per unit weight of root increased with increasing temperatures for all species, particularly the amount of glutamic acid. He also noted that high internal free amino acid concentrations did not correlate with increased amino acid exudation.

Rouatt et al. (1963) demonstrated specific changes in the rhizosphere microbial composition of wheat and soybeans grown at atmospheric temperatures of 16, 18 or 25 C for 21 to 27 days, and attributed this to changes in the exudate quantity and composition. He equated bacterial numbers in the rhizosphere of plants grown at different temperatures with the optimal growing conditions of the plant. Thus, for wheat the microbial population was largest at 16 C while for soybeans it was largest at 25 C.

Vancura (1967) grew corn and cucumber (Cucumis sativa) seedlings under favorable conditions (19 C), then exposed them to 5 C for 3 days. He observed a marked rise in exudation and detected three new oligosaccharides in the cold treated plants. Martin (1977) measured carbon loss from wheat plants grown in atmospheres containing $^{14}CO_2$, at soil temperatures of 10 C and 18 C for periods of 3 to 8 weeks. He reported that although 10 C plants had 70% less dry weight than 18 C plants, there was not a corresponding reduction in ^{14}C present in the soil. Introducing microorganisms to the soil at either temperature more than doubled the ^{14}C content of the soil. He also noted that the nature of the ^{14}C labelled material was quite similar for both sterile and non sterile soils.

Moisture stress has been demonstrated to affect the amount of root exudates. It has been shown that alternate drying and wetting of the rooting medium can stimulate increased exudation (Vancura, 1967).

Increasing light intensity from 300 to 1000 foot candles was found to double the total number of rhizosphere bacteria (Rovira, 1959). The proportion of amino acid requiring bacteria also increased from 30 to 65% at the higher light intensity.

Newman et al (1978) presented information on the influence of shoot nitrogen status on the amount of exudation of roots of four common grassland plants. They found that there was a high correlation between shoot nitrogen concentration and combined volume of bacteria and fungi per unit root surface area. They related this to increased exudation. This agreed with findings of Bowen (1967) in which plants supplied with extra mineral nitrogen exuded more amino acids. Reports have indicated that amino acids may be one of the major nutrients of rhizosphere microflora, (Rovira, 1965).

Soil structure is an important factor affecting exudation. Barber and Gunn (1974) found that exudation of amino acids and sugars from barley growing in dilute solutions in the presence of glass ballotini 1 mm in diameter was dramatically enhanced compared to plants grown in the absence of ballotine. The authors stated that this simulated pressure was small compared to pressures exerted in the soil.

Mechanical injury can alter exudation patterns of roots. Boulter et al. (1966) found seven times more amino acids in the root exudates of peas grown in quartz sand compared with plants grown in nutrient solution.

Kepert et al.(1978) used media of increasing abrasiveness (solution culture, sand, carborundum) to vary the amount of material lost from roots of onion plants in order to examine the effect of organic products lost from the root on dissolution of hydroxy apatite. They found that plants grown in carborundum and given high levels of hydroxy apatite exceeded the growth of sand cultured plants and approached that of plants grown in solution culture. Improved growth was presumed to be due to solubilization of hydroxy apatite by exudates.

Age and stage of development are factors that affect the quantity and kind of root exudate. Several investigators have shown that substantially smaller quantities of sugars, organic acids, and amino acids were released by plant roots during fruiting than in the earlier vegetative stages (Rovira, 1969). An investigation of the amino acids exuded from alfalfa plants demonstrated that age significantly affected exudation; amino-N per plant plant per week increased from 0.24 to 0.61 mg from 10 day to 51 day old plants (Richter et al,,1968). An analysis of the amino acid and sugar compositon of exudates of sorghum and tomato at 15 day intervals after seeding showed that the number of amino acids increased over 45 days, but relative amounts declined. The quantity of sugars also fell over the 45 day period.

Changes in the rhizosphere environment also occur as a consequence of the nature of the root as an absorbing organ (Dommergues, 1978). Nutrient and moisture depletion zones will affect the size and content of the microbial population in these zones.

Plant roots have been reported to alter the pH of the rhizosphere. The rhizosphere pH is regulated by hydration of CO_2 to carbonic acid (Brady, 1974), release of cellular organic acids (Moore, 1974) and by imbalance of cation and anion uptake and the associated release of protons or hydroxy ions, or bicarbonate moieties (Moore, 1974).

Microbial Influences on Plant Growth

Large numbers of bacteria and fungi have been reported in the soil (Clarke, 1967). Bacterial numbers in the rhizosphere in most soils was reported to be in the range of 2.0×10^9 cells per gram of soil. This is equivalent to approximately 0.2% of the soil weight, or about 2200 kg live weight of bacteria per hectare to a depth of 0.15 meters. Live weight of

fungi was reported to be more than double this (Clark, 1967).

Activity of microflora in the rhizosphere has been closely correlated with release of substances from the root. Warembourg and Billes (1978) observed two phases of heightened respiration in the rhizosphere of wheat seedlings grown in non-sterile solution culture, compared to a single phase for axenically grown plants. The second phase was attributed to heightened microbial activity in the presence of soluble compounds of plant origin. Studies by Campbell and Rovira (1973) and Foster and Rovira (1976) confirmed the progressive disappearance of diffusible and non-diffusible compounds along the root and away from the root tip.

Soil microflora have been shown to strongly influence plant growth. Mention was already made of their direct influence on cortical cell degeneration (Martin, 1977). The effect of microorganisms on the uptake and translocation of phosphorus has been extensively investigated. Gerretsen (1948) showed that uptake of phosphorus by plants growing in unsterilized sand containing insoluble phosphorus compounds increased upon addition of 1% by weight of unsterilized soil.

Bowen and Rovira (1966) found that both the accumulation in the roots and transfer of P to the shoots of tomato and clover seedlings were increased in the presence of microorganisms. Barber and Loughman (1967) examined the uptake of P over 24 hours by three week old barley plants in solution cultures labelled with ^{32}P varying in phosphate concentrations between .001 and 10 ppm P. They found that except at the lowest concentration examined, microorganisms had little effect on the total amount of phosphorus taken up. However their work as well as later studies by Barber and Frankenburg (1971) and Benians and Barber (1974) demonstrated that a larger quantity of absorbed P was incorporated into nuclear acids, phospholipids and phosphoproteins in non-sterile cultures and less into easily soluble phosphate fractions. Barber (1974) reasoned

that microorganisms absorbed phosphorus at the expense of the root and incorporated the phosphorus into complex organic compounds. He concluded that the greatest competitive effect by the root microflora was exerted in conditions of low or limiting nutrient availability.

Barber (1967) noted that low pH conditions (pH 4) reduced the competitive ability of microflora.

In view of the claims that certain bacteria solubilize unavailable forms of phosphorus, these effects have been much studied. Webley and Duff (1965) confirmed that bacteria that produce 2-ketogluconic acid can dissolve phosphate minerals in culture. Bacteria capable of hydrolyzing soil organic phosphorus have also been reported (Greaves and Webley, 1967). However, the quantities of phosphorus released by solubilizing bacteria was not felt to be of any significance to plants primarily because of the large quantities of phosphorus that would be required to shift the equilibrium between unavailable phosphorus and phosphorus in solution. (Tinker and Sanders, 1975).

There is some evidence that root inhabiting microorganisms produce substances that form complexes with trace elements which are more readily available to plants than EDTA chelates or free ions. For example, Barber and Lee (1974) identified a microbial product which stimulated absorption of manganese in non-sterile culture. The authors also noted increases in absorption of iron and zinc in the presence of microorganisms. Barber (1973) cited reports of improved uptake of iron, zinc and manganese chelates and sulfates, as well as KNO_3 and NH_4Cl. Caution should be taken in invoking direct microbial activity as the cause. The lysing activity of soil microflora on roots (Darbyshire and Greaves, 1973) may cause a shift in the rhizosphere pH through release of organic acids. Marchener and Barber (1975) have shown that concomitant with a reduction in solution pH, iron deficient sunflower plants (Helianthus annuus) exuded increased quantities of riboflavin

and substances capable of reducing the less available Fe^{+3} to Fe^{+2}, thereby overcoming the deficiency symptoms. Similarly, since the availability of phosphorus is pH regulated (Lindsay and Moreno, 1960) microbial activity may have altered uptake of phosphorus in non-sterile soils.

Rhizosphere microorganisms may influence plant growth through their influence on root morphology. Bowen and Rovira (1961) reported that microbes reduced root growth of several plant species by 25% to 52%, lateral root production by 20% to 53%, and root hair length and number. They suggested that this could lead to a 54 to 64% reduction in phosphorus uptake. Mosse (1963, cited in Rovira and Bowen, 1970) showed that mycorrhizal clover roots were longer than uninfected roots (21.6 mm vs 13.1 mm) and produced lateral roots more frequently (16.3% of roots with laterals vs. 1.3%).

The influence of soil microorganisms on root growth may be related to microbially derived plant growth regulating substances. Brown (1972) isolated microorganisms that produced substances with properties of gibberellins and IAA. Shoot growth was enhanced while root number, length and weight were reduced in the presence of these compounds. Smith and Russell (1969) showed that under anaerobic conditions ethylene could accumulate in the soil atmosphere at concentrations sufficient to inhibit extension of cereal roots in the laboratory. The topic of microbially produced compound that affect plant growth is thoroughly reviewed by Lynch (1976).

Soil Temperature Effects on Plant Growth

Barber (1979) commented that when plants are grown under conditions where a nutrient or an environmental condition is less than optimal, the plant responds by altering its root growth and demand for nutrients at the root in such a way as to provide more nutrients for the plant". The response of plants to various soil temperatures has been related mainly to changes in the metabolic

activity of the root (Wardlaw, 1968; Neilsen, 1974). Nutrient absorption requires respiration energy supplied by photosynthate transported from the shoot to the root. Reduction in soil temperatures reduces the metabolic rate of root along with its requirement for shoot assimilates. Slower metabolic activity due to reductions in root temperature from 27 C to 13 C have been shown to reduce the maximum rate of potassium uptake by nearly a factor of 8 (Ching, 1977, cited in Barber, 1979). Consequently suboptimal temperatures have been reported to reduce shoot growth as well as root growth. (Brouwer, 1962). Neilsen (1974) observed that increasing root temperature from suboptimal levels reduced the root-shoot ratio thereby altering the size of the absorption surface per unit of nutrient demand.

Several reports have indicated that the yields of barley grown at various temperatures have been best at about 10 C, but that optimums may vary depending on nutrient availability and plant age. (Power et al. 1963, 1970; Mack, 1965).

Power et al. (1963) found that there was a greater range of temperature optimums for barley when high levels of phosphorus were available. Plants grown at low soil temperatures (8 C) were less able to take up soluble phosphorus than those at warmer temperatures. At optimum temperatures, less phosphorus was required to produce a unit of plant material.

Mack (1965) observed that barley plants grown in 27 C soil were unable to use applied N, P and K fertilizers, while those at 9 C and 18 C were nearly equally capable of fertilizer use. Low moisture impaired utilization of fertilizer at all temperatures tested.

Power et al. (1970) demonstrated that as the barley plant matures its temperature optimum shifted from higher to lower temperatures. Rapid vegetative growth was observed at 22 C soil temperature, but continued warm temperature throughout heading reduced grain yield. Labananauska et al. (1974)

reported similar findings. They showed soil temperatures of 25 C during grain filling substantially lowered the grain yield of wheat compared to plants exposed to soil temperatures of 5 or 15 C during grain filling. In addition they noted that plants exposed to cool soil at heading took up more nutrients than warm temperature plants.

Cool soil temperatures have been shown to improve yields of barley. Power et al. (1970) demonstrated that low soil temperatures (9 C) affect plant growth primarily by reducing growth rate. Barley plants grown at low soil temperature yielded as well or better than those at higher temperatures. Power et al. (1970) concluded that uptake of nutrients was not restricting root growth at 9 C but that translocation of nutrients from roots to shoots was impeded. These results concurred with findings by Nielsen et al (1960) and Read and Ashford (1968) where it was reported that there was a marked accumulation of nutrients in roots at 5C to 10 C.

Soil temperatures can greatly influence the pattern of nutrient uptake. Rovira and Bowen (1973) noted that wheat plants grown at 20 C took up a large amount of phosphorus in the apical 3 cm, and very little behind this region up to the lateral root zone where uptake was high again. In contrast at 10 C uptake was low at the apex, and higher along the remainder of the root. They attributed this reduced uptake at the apex at low temperatures to a reduced rate of translocation of carbon compounds to the root tip, as indicated by a lower level of ^{14}C labelled assimilate located in this region. They speculated that higher levels of carbon assimilates in the older roots at lower temperatures may have given rise to an increased level of exudation, and therefore induced greater microbial activity.

Soil temperature influences root morphology. At cooler temperatures roots have been found to be usually whiter, thicker in diameter and less branched than at warmer temperatures. (Brouwer and Hoogland, 1964; Nielsen and Cunningham, 1964; Garwood, 1968). Brouwer and Hoogland (1964) observed that

for barley, root temperatures of 10 C and 35 C caused the formation of suberin closer to the apex than roots grown at 15 C and 20 C. Rovira and Bowen (1973) have suggested that the increased thickness of roots at cooler temperatures may have been the result of slower movement of assimilate along the root, which may have offered a greater opportunity for radial diffusion of assimilate out of the phloem and into the root cortex.

Root Proliferation

Hackett (1972) referred as far back as Nobbe (1862) in stating that root systems have been observed to proliferate in and around regions of the soil where mineral nutrients are concentrated. Hackett (1965) showed that when barley roots were grown for four weeks in phosphorus deficient solution culture, the total volume of the main axes and the primary lateral nodal roots was considerably reduced while the total surface area of the axial and primary lateral seminal roots remained unchanged compared to the P sufficient control plants. The volume of the secondary lateral roots was four times larger than that of the controls.

Studies by Drew and Saker (1975, 1978) have shown that a localized supply of phosphorus or nitrate promoted lateral root growth in the zone of nutrient application in solution and sand culture. They found that the increased lateral growth resulted in an enhanced rate of nutrient uptake per unit weight of root which compensated for a deficient nutrient supply in other zones.

Strong and Soper (1974) showed that enhanced recovery of applied phosphorus by flax (linum spp.) and wheat after funnelling the root systems through a simulated phosphorus reaction zone could be explained on the basis of increased root proliferation in that zone.

MATERIALS AND METHODS

Preparation of Materials

Soil

Soil used in this study was obtained from the former University of Manitoba Weed Research Station near Carman, Manitoba in mid August, 1979. This soil is an Almasippi very fine sandy loam. The soil was taken from plots that were seeded four years earlier to barley (Hordeum vulgare), but had been fallow since that time. No chemicals had been applied during the four year interval. Only soil from the surface 16 cm was used. This was stored in polyethylene bags which were loosely covered, and stored in a cool dark environment (5 C) until required. Several days prior to planting the soil was seived through a 2 mm mesh screen to remove most of the extraneous organic material. The seived soil was mixed with sand (2/1 soil:sand mixture), and was then either returned directly to the polyethylene bags or was given one cycle of pasteurization using a Lansa soil pasteurizer (Johnsen Machine Co. Ltd.). The pasteurized soil mixture was then transferred to clean polyethylene bags. The pasteurized soil was left uncovered at room temperature (23 C) and occasionally mixed in order to permit reestablishment of non mycorrhizal microbial activity in the soil. The field capacity of the pasteurized and unpasteurized soil/sand mixtures were determined according to the method of Shaykewich (1980). The soil mixtures were adjusted to 80% field capacity 3 - 4 hours prior to planting.

Available soil P was determined on the pasteurized and unpasteurized soil mixtures by extraction with sodium bicarbonate for 30 min. followed by a colorimetric determination based on reduction of phosphomolybdic acid

with stannous chloride (Olsen and Dean, 1965). Results of the soil phosphorous determination are shown in the Appendix, Table 15.

Mycorrhizal Inoculum

The source of inoculum consisted of the mixture of Endogone species indigenous to the Carman Research Station soil. Mycorrhizal spores were extracted from the soil using a flotation and bubbling method devised by Furlan and Fortin (1975). No attempt was made to quantify the numbers and species of VAM fungi present in the soil based on the endophytic spore population. However an estimate of the total spore density per gram of soil was obtained, and several VAM species present in the Carman soil were tentatively identified.

To facilitate counting of spores a filter paper was marked with a grid pattern to form 0.5 cm squares. A spore suspension was poured over the gridline filter paper fitted into a glass funnel, and was allowed to drain. The filter paper was placed on a 9 cm petri plate bottom and the spores were counted under a stereoscopic dissecting microscope (X50) with strong direct lighting. Using this method Carman soil was found to have an average of 384 mycorrhizal spores per gram dry weight of soil based on eight replicates. Mycorrhizal genera observed included what appeared to correspond to the descriptions provided by Gerdemann and Trappe (1974) of E. glomus, E. gigaspora, and E. acaulospora.

Planting Procedure

The plant material used for all experiments was barley, Hordeum vulgare, cv. bonanza. Four surface sterilized barley seeds were planted per pot, and seedlings were subsequently thinned to 2 plants per pot.

To minimize root crowding during the later stages of development plant containers were improvised from standard plastic sewer pipe, 11.0 cm inside diameter, and cut to a length of 38.0 cm. The tubes, about 3 litres in volume,

were then lined with 3 mil 48.0 x 18.0 cm polyethylene bags, three on the inside and two on the outside of the tube, to prevent water entry. An equal weight and volume of soil was then placed inside the plant containers so that the soil surface was approximately 2.0 cm from the lip of the container.

Method of Inoculation

Three mycorrhizal treatments were involved.

1. Pasteurized controls (PC) - Plants were grown in pots completely filled with nonmycorrhizal pasteurized soil. Pots received filtered unpasteurized soil water extract to ensure that control plants received the same microflora other than mycorrhizal fungi.
2. Pasteurized inoculated (PI) - Pots were filled to within 9.0 cm of the top with pasteurized soil. 290 g of unmixed unpasteurized seived soil was placed approximately 2 cm deep on the pasteurized soil. A third 5 cm deep layer of pasteurized soil was added to the second layer.
3. Unpasteurized (UP) - Plants were grown in entirely unpasteurized soil containing indigenous mycorrhizal fungi.

Growth Conditions

Plants were grown at three soil temperatures, 12, 16 and 20 C, under controlled growth chamber conditions. The 12 and 16 C soil temperatures were maintained using circulating water baths thermostatically controlled by Aquachiller refrigeration units (manufactured by Jewel Industries Inc.). The 20 C soil temperature was an average of the day/night ambient air temperature inside the growth chamber (22C/18C). The plants were exposed to 14 hours daylight, and a constant relative humidity of 50%. The photon flux density throughout the experiments was maintained at 380 microeinsteins $M^{-2}SEC^{-1}$.

The moisture content of the soil in the plant containers was maintained at approximately 80% field capacity. Throughout the growth period by addition

of distilled water to maintain a constant pot weight. 100 ml half strength Hoaglands solution minus P was applied to the soil 10 and 30 days after seedling emergence. The increase in contribution to pot weight due to plant growth was monitored every 10 days using a mycorrhizal and nonmycorrhizal control plants. The weight gain due to plant growth was taken into account when watering.

Plants were harvested at the same stage of development: at the initial stage of heading (10.1 on Feekes' scale).

Experimental Design

The arrangement of the water and air baths in the growth chamber is shown in Figure 1. Mycorrhizal treatments were randomized within temperature regimes in order to minimize variability due to location within the growth chamber.

Planting date was not expected to be a critical factor since experimentation was carried out in the growth chamber. Therefore to permit ample time for processing of the plant material, planting was spread out over approximately a two month time interval, with 4 days between each planting date. Randomization of planting dates within each temperature regime prevented bias of location. At each planting date, a control pot, a mycorrhizal-pasteurized and a mycorrhizal-unpasteurized pot was planted at 12, 16 and 20 C, making a total of 9 pots per planting date.

Sampling of Plant Material

Shoots

Plant shoots were separated from their root systems and shoot fresh weights were immediately determined. Shoot dry weights were obtained following oven drying for 48 hours at 80 C.

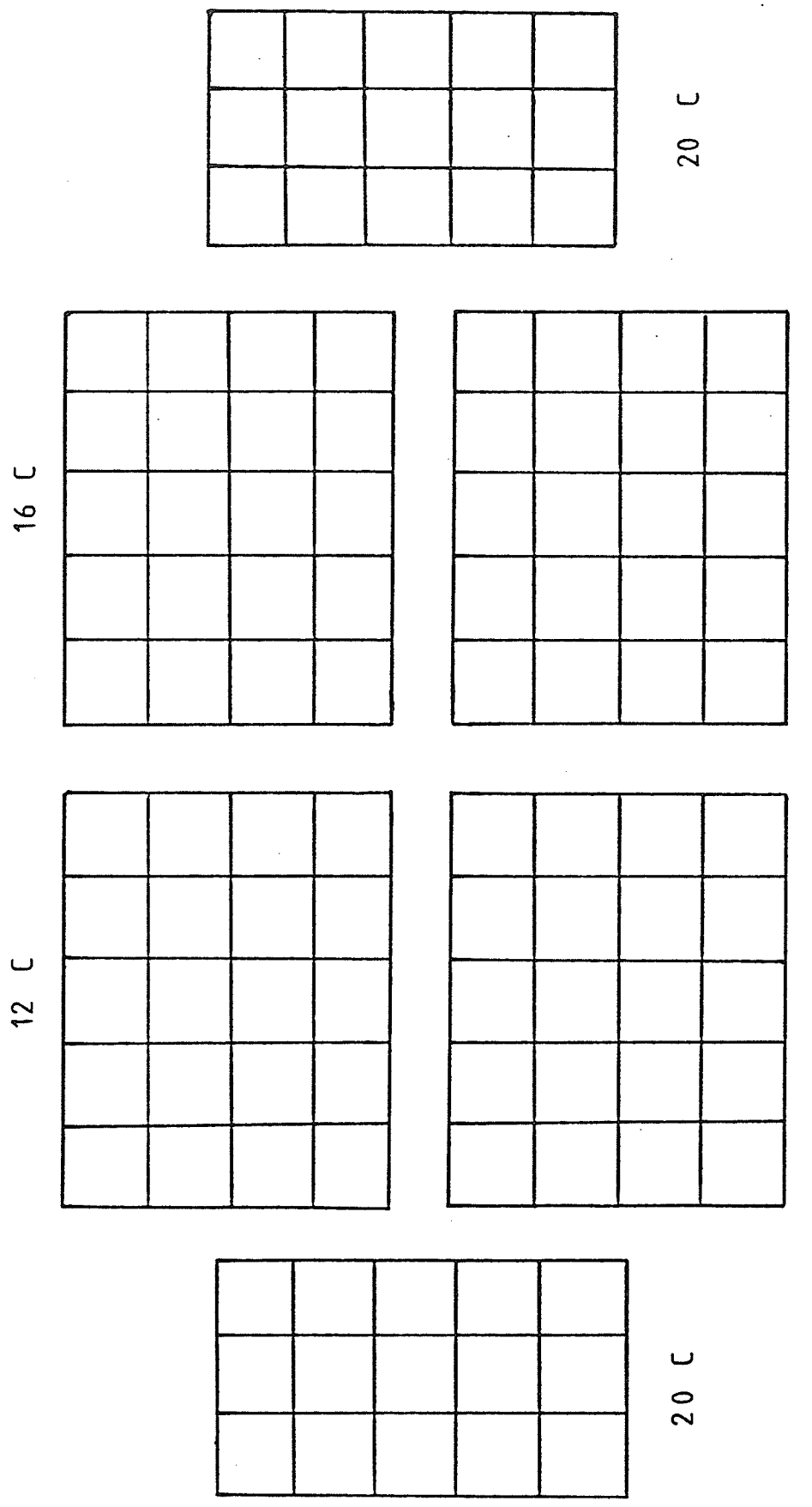


FIGURE 1. Arrangement of water (12, 16 C) and air (20 C) baths in the growth chamber showing individual spaces for plant containers.

Roots

After removal of the top growth, the columns of soil were pulled from the pots and soaked in water for 10 minutes to loosen the soil around the roots. The columns were then gently massaged and agitated in the water until most of the soil was removed. The root systems were rinsed four more times in fresh changes of water, the last two using distilled water. Effective rinsing was achieved by repeatedly dipping and raising the roots into and out of a tall water column.

The washed root systems were laid full length into a basin containing just enough water to submerse them. The root systems were then sectioned using sharp scissors into 9.0 cm segments starting from the root base. The length of the fourth segment varied from plant to plant depending on the total length of the individual root system.

The fresh weight of the segments was obtained after removing free water, after which they were wrapped in 14.0 x 24.0 cm sheets of absorbent paper, and rapidly frozen in liquid nitrogen. The frozen segments were dried on insulated racks in a freeze drying chamber. Dry weights of the segments were immediately obtained after freeze drying.

The dried root segments were sectioned into four 2.25 cm root subsegments. The root tip segment was sectioned into 2.25 cm subsegments starting at the region furthest from the root tip. The remainder after four subsegments had been obtained was discarded. This was because beyond the fourth subsegment considerable root disfiguration was observed as a consequence of the root systems contacting the bottoms of the pots. It was felt that it would have been invalid to compare disfigured roots with roots of normal appearance.

The 2.25 cm subsegments were further divided into approximately 0.5 cm pieces which were subsequently stored in 15.0 x 5.2 cm glassine pollinating bags until required for analysis. A schematic diagram of the root segmenting

process is shown in Figure 2.

Alternate root subsegments were examined starting from the root base down for total root length, presence and extent of mycorrhizal infection, and cortical cell wall and endodermal suberization and/or lignification. The amount of carbohydrate, free amino acids and water soluble phosphorus in aqueous root diffusates obtained from these subsegments was also measured. Conductivity measurements were also performed on the root diffusates.

In addition, measurements of root length, mycorrhizal infection and root diameter were obtained from subsections not examined in the above analysis.

Root Diffusate

Twenty-five ml deionized-distilled water was added to 30 ml glass vials containing a 0.1 cm sample of roots obtained from root subsegments. The vials were transferred to a vacuum desiccator for 30 minutes, with an intervening 1.0 minutes stirring period after the first 15 minutes. The samples were filtered through 9 cm No. 1 filter paper. Roots trapped on the filter paper were subsequently stained and analyzed for mycorrhizal infection.

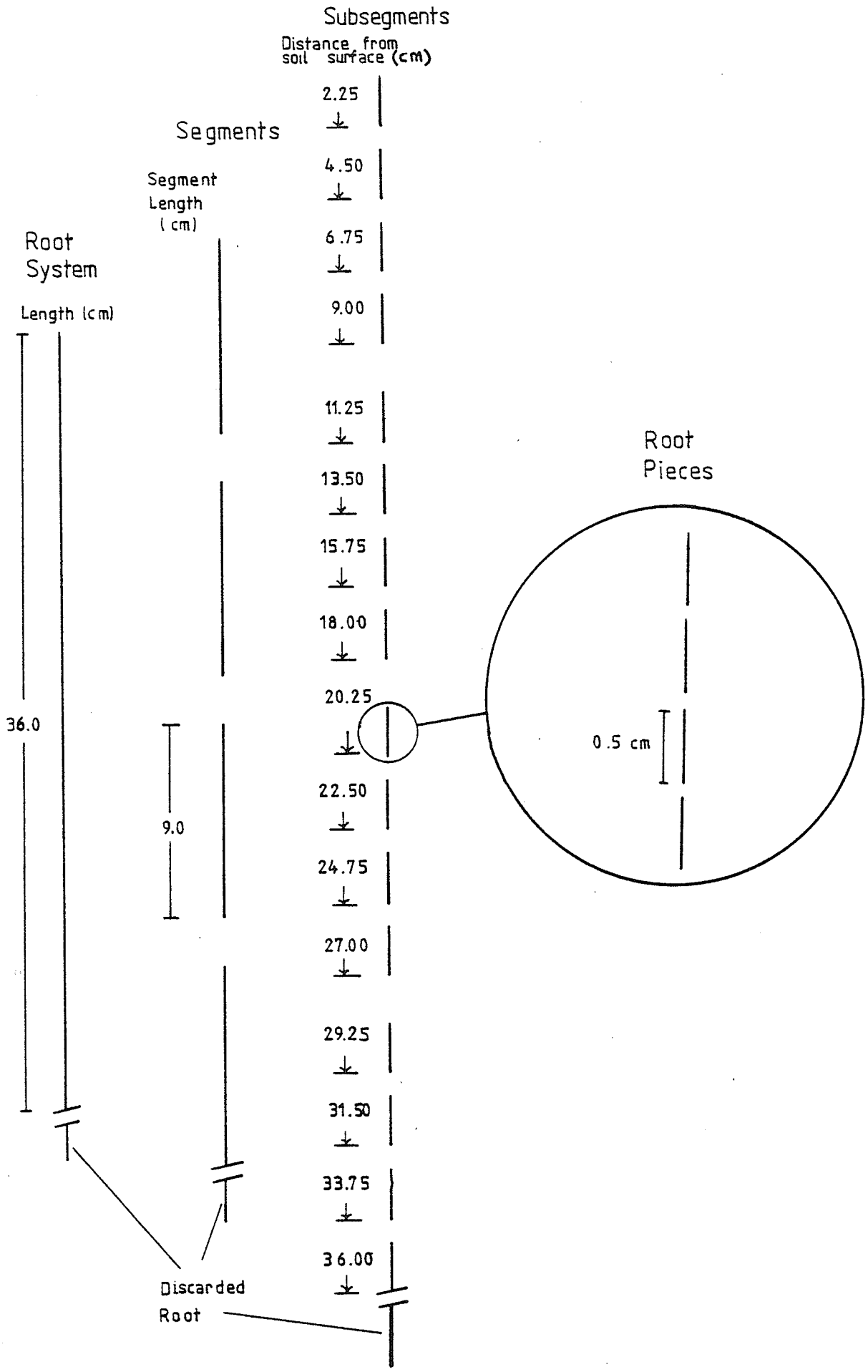
The diffusate was collected into centrifuge tubes and centrifuged for 10 minutes at 10.2 G in order to settle out cell wall debris. Chemical analysis was conducted on the supernatant.

One sample of diffusate was obtained from each root subsection. Chemical analysis was performed twice on each sample. Treatment means are based on values obtained from eight pots, two plants per pot.

Experimental Measurements

Soil Moisture

Moisture content of the soil in the plant containers at 6.0, 20.0 and 34.0 cm below the soil surface in pots in which were grown plants exposed to each of the three mycorrhizal treatments at each soil temperature was determined at 20


Subsegments
Distance from soil surface (cm)
2.25
4.50
6.75
9.00
11.25
13.50
15.75
18.00
20.25
22.50
24.75
27.00
29.25
31.50
33.75
36.00
Segments
Segment Length (cm)
9.0
Root System
Length (cm)
36.0
Discarded Root
Root Pieces
0.5 cm

and 45 days after seedling emergence. Values obtained were based on the average of two replications.

Indicators of Metabolic Activity

Introduction

Carbohydrates, amino acids and phosphorus; are principal constituents of most metabolic processes in plants and animals. Because the analytical methods for their quantitative determination is straight forward, measurement of the concentration of these components in plant cells would provide a reliable and simple method of evaluating the level of metabolic activity in localized regions of the plant root.

In this experiment chemical measurements were performed on the compounds that diffused through the disrupted cell walls and membranes into the suspending aqueous medium.

Total Soluble Carbohydrates

Total soluble carbohydrate content was determined colorimetrically using the phenol-sulfuric acid reaction described by Montgomery (1961) using glucose standards.

Free Amino Acids

Free amino acid content was determined colorimetrically by the procedure of Moore and Stein (1948) using the commercially prepared ninhydrin reagent, ninsol (Pierce Chemicals). Glutamate standards were used for calibration.

Phosphorous

Water soluble phosphorus content was determined according to the method described by Fiske and Surbarow (1925).

All colorimetric determinations were measured using a Bausch and Laumb Spectrometer-20 spectrophotometer.

Conductivity

The regulation of ion exchange across membranes in plant cells is an active process involving an expenditure of metabolic energy. Electrolyte leakage as a consequence of loss of control of ion movement due to cell death would be reflected in a reduction in the ion concentration in the cell. In this experiment where measurements were made on the root diffusate the conductivity of the diffusate would be a measure of the electrolytic concentration of the cell prior to root sampling.

Conductivity measurements were performed on a Model 31 conductivity bridge (Yellow Springs Instrument Co., Ohio).

Mycorrhizal Infection

Root samples collected on filter paper during preparation of the diffusate were cleaned in hot 10% KOH and stained in 0.1% trypan blue according to the procedure of Phillips and Hayman (1970). 1.0% Sudan III was added to the trypan blue staining solution to detect lipids in the mycorrhizal vesicles.

A modification of the method of Ambler and Young (1977) and Mosse (1980) was used to determine the extent of mycorrhizal infection. After staining of the root sample, excess stain was removed by rinsing the roots on a 0.1 mm nylon screen with an aerated stream of water. The roots were then placed into 30.0 ml glass vials containing 15.0 ml 50% glycerol. A 4.0 ml aliquot of stained roots in glycerol was transferred to a previously weighed 9.0 cm plastic petri plate bottom using a 5.0 ml plastic syringe with the tip bored to an 8.0 mm diameter. The inside of the petri plate had been earlier brushed lightly with a fine sandpaper to prevent water beading occurring when samples were placed in the plate. The petri plate with the root suspension was weighed and the fraction of the total suspension put on the plate was calculated.

Roots on the plate were dispersed using a dissecting needle, so that they were randomly distributed over the surface of the plate. The petri plate was then inserted into a specially prepared counting platform on which a grid line pattern had been drawn to form 1.0 cm squares. The plate was scanned along the grid lines under a Zeiss Dissecting Microscope at X40 magnification with high intensity direct illumination. Roots that intrasected the grid lines were counted. A record of the total number of roots with evidence of mycorrhizal infection intersecting the grid lines was kept on a styrofoam mounted hand-held type counter. After the first count, the plate was rotated 90 degrees and a second count was taken.

VAM infection was detected on the basis of recognition of characteristic, and readily identifiable vesicles and arbuscules. The chitinous fungal cell walls stain a dark blue with trypan blue. Sudan III stained lipid droplets in the mycelium and vesicles orange.

Total Root Length

Total root length was estimated on the basis of the total number of mycorrhizal and nonmycorrhizal root pieces intersecting the grid lines, using the method outlined for determination of mycorrhizal infection.

Root Diameter

Based on general observation on the distribution of various root sizes among samples, root diameters appeared to fall into three size categories, namely, 0.2, 0.4, and 0.6 mm.. Using this somewhat arbitrary size classification, the contribution to the total root length within each root sample by roots in each size category was estimated. A record of the total number of roots belonging to each size classification was maintained using the method outlined for determination of mycorrhizal infection. An eye piece graticule aided in root size differentiation.

Lignification

Endodermal cell walls have been observed to become heavily thickened with age as a consequence of deposition of lignin and suberin (Clarkson, 1974). Similar thickening was found in hypodermal cells in the older regions of the roots of some plant species (Ferguson and Clarkson, 1975). Since it is believed that these encrustations significantly increase the resistance to nutrient and water flow to the xylem, (Clarkson, 1974), any influence on their occurence as a result of mycorrhizal infection could significantly affect rates of nutrient and water uptake in these regions.

Because suberin is highly soluble in hot 10% KOH, (Johansen, 1940), it was not possible to detect its presence. However, lignin is insoluble in hot 10% KOH (Johansen, 1940), and is stainable with trypan blue (Ambler and Young, 1977).

A record of the total number of lignified roots was kept using the method outlined for determination of mycorrhizal infection.

Root number estimates obtained from measurement of mycorrhizal infection total root length, root diameter and lignification were converted to root length values. Details on the method of calculation of root length from line intersect counts is shown in Page 168 in the Appendix.

Final values obtained from the above measurements were means of two counts per sample, one sample per root subsegment, eight pots, and two plants per pot. The values are representative of one sample within a root subsegment.

Statistical Analysis

The experimental design for all experiments was a complete factorial of three mycorrhizal treatments, three soil temperatures and four, seven or eight soil depths. There were eight replicates per treatment, except for the soil moisture determinations which were replicated twice.

Prior to statistical analysis, Arcsine transformation was applied to all percentage measurements to ensure normal distribution of these variates. Analysis of variance was conducted with the aid of an IBM computer using statistical analysis system (SAS) programs. Significant differences were detected using the LSD test at the 5% level.

RESULTS AND DISCUSSION

SECTION I

Effects of Planting Dates on Root and Shoot Measurements

Date of planting had a significant influence on many of the root and shoot parameters that were measured in this study. This was contrary to what was expected, since experiments were conducted entirely in the growth chamber, and therefore should have been unaffected by such mutable factors as photoperiod, light intensity, soil moisture, etc.

Differences due to planting date in this study were interpreted as reflecting variability of the plant material. Pairs of plants in each pot represented one planting date. There was therefore no way to separate the effects due to variability in planting dates from that due to plant material. It was therefore not meaningful to attempt to determine trends in the root parameters measured due to effects of planting date.

It was felt that this did not detract from the meaningfulness of the influences of the other factors, since in all cases the effects of the other independent variables, viz- soil temperatures, mycorrhizal treatment and soil depth, considerably outweighed that of planting date in terms of significance. For this reason the effects of planting date were not discussed in the text, nor were they tabulated in the summary tables showing analysis of variance in the various dependent variables. However detailed analysis of variance tables inserted in the Appendix include the effects of planting date.

Length of Mycorrhizal Roots

The total length of barley roots infected with mycorrhiza was strongly dependent on whether infection was achieved using mycorrhizal inoculum in

pasteurized soil or using unpasteurized soil. In addition soil depth and soil temperatures influenced infection. A summary of the analysis of variance in Table 1 shows that not only the main effects were significant, but also that there were significant two and three way interactions between mycorrhizal treatment, soil temperature and soil depth. A detailed analysis of variance in total mycorrhizal infection is shown in Table 16 in the Appendix.

Table 1. Summary of Analysis of Variance in Total Mycorrhizal Infection in Root Segments of Mycorrhizal Barley Roots Grown at Three Soil Temperatures

Source of Variation	F Value
Soil Temperature (T)	19.17**
Soil Depth (SD)	46.17**
Mycorrhizal Treatment (MT)	269.42**
T X SD	3.13*
T X MT	28.14**
SD X MT	9.51**
T X SD X MT	3.69**

* Significant at P = .05

** Significant at P = .01

Figure 3 graphically shows the influence of mycorrhizal treatment, soil temperature and soil depth on the total length of mycorrhizal roots. At all soil depths and soil temperatures barley plants grown in unpasteurized soil, (designated "UP" barley plants), had a greater length of mycorrhizal roots than did plants grown in pasteurized inoculated soil, (designated "PI" plants). The notable exception was for the PI plants grown at 16 C where mycorrhizal infection was relatively heavy at the zone of inoculum placement.

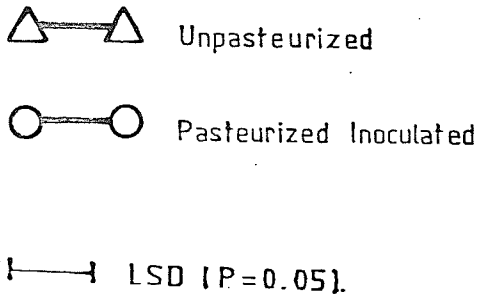
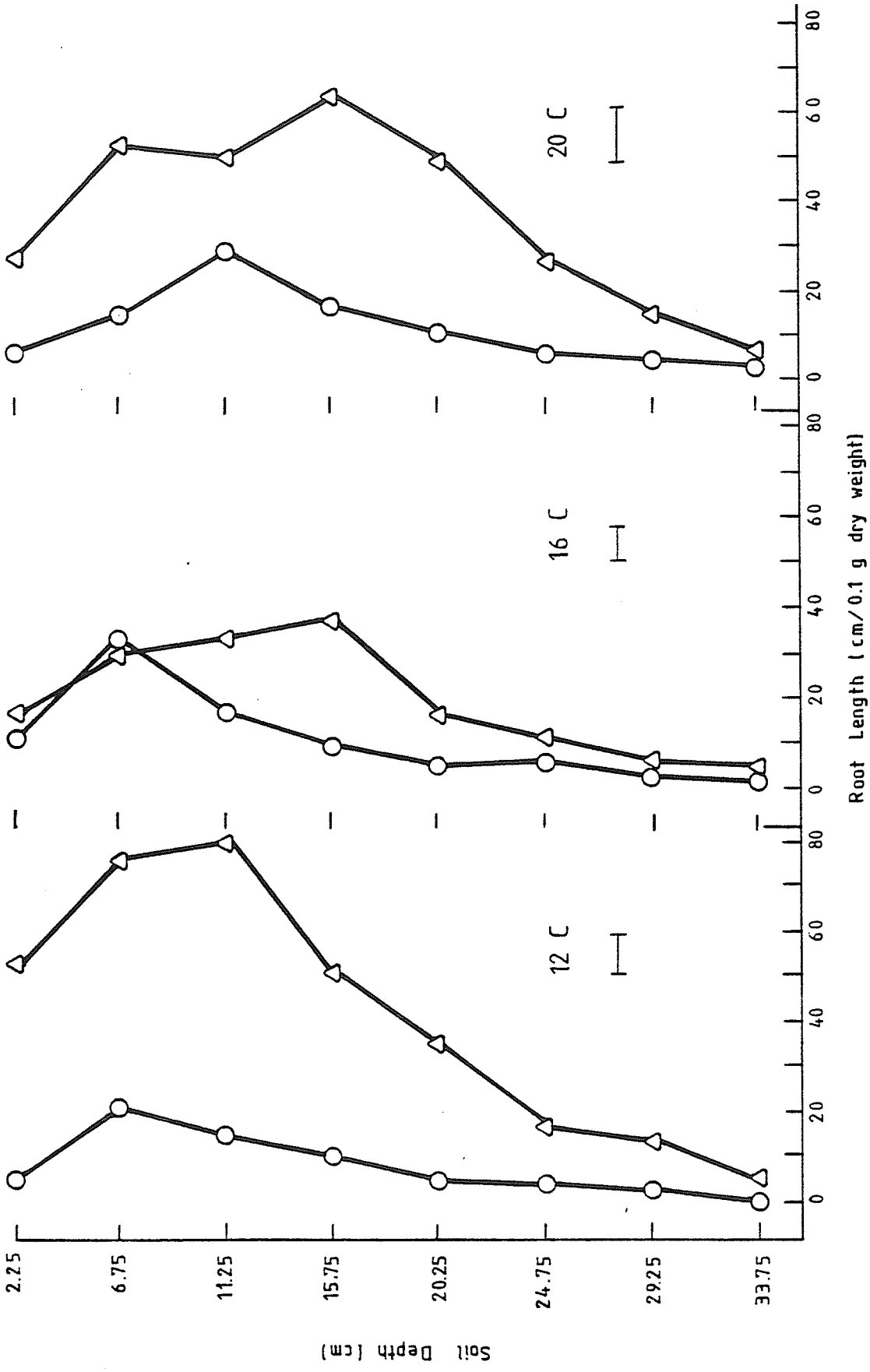


FIGURE 3. The Effect of Mycorrhizal Treatment on the Length of Mycorrhizal Roots at Eight Soil Depths and Three Soil Temperatures.


Soil Depth (cm)
2.25
6.75
11.25
15.75
20.25
24.75
29.25
33.75
0
20
40
60
80
12 C
16 C
20 C
Root Length (cm/0.1 g dry weight)

The only notable infection in roots of PI plants occurred at or a few centimeters below the site of inoculation. Slightly greater mycorrhizal infection was found at the warmer soil temperatures for these plants.

Mycorrhizal infection in roots of UP plants was predominantly in the upper half of the plant containers. Soil temperatures of 20 C favored a greater length of roots infected in the middle region of the pots while the coldest temperature resulted in the maximum root length infected occurring nearer the soil surface. The 16 C UP plants behaved similarly to those at 20 C., but the total length of infected roots for each root segment was less than at 20 C.

Root Length of Mycorrhizal and Nonmycorrhizal Plants

The differences in length of mycorrhizal roots at the various mycorrhizal treatments and soil temperatures may have been due to differences in total root length of each root segment. The results obtained from the calculations of total root length showed that the length of roots in each segment varied with respect to soil temperature, soil depth, and the mycorrhizal treatment. This is indicated in Table 2 which presents a summary of the analysis of variance in total root length for the variables listed above. Each of the independent variables had a strong influence on total root length. Two way interaction between soil temperature, mycorrhizal treatment and soil depth were also significant. A detailed analysis of variance in total root length is shown in Table 17 in the Appendix.

These results are depicted graphically in Figures 4, 5, and 6 showing only the significant two way interactions. Root lengths of individual segments increased from the tip of the root system to the root base, with a plateau region approximately 12 to 18 cm below the soil surface (Figures 5 and 6). The influence of soil temperature was reflected mainly in the root

length of segments near the bottom of the pots (Figure 5). Higher soil temperatures increased the root length of mycorrhizal and nonmycorrhizal plants in this region although the influence of soil temperature was slight for the roots of the UP plants (Figure 6). Roots of the PI plants were strongly and similarly influenced by soil temperature (Figure 6). The differences in variation in root length with soil depth of the pasteurized and unpasteurized soil plants was very apparent, both PC and PI roots having shorter root lengths than that of the UP plants (Figures 4 and 6).

Table 2. Summary of Analysis of Variance in Total Root Length of Roots of Mycorrhizal and Nonmycorrhizal Barley Plants Grown at Three Soil Temperatures

Source of Variation	F Value
Soil Temperature (T)	70.34**
Soil Depth (SD)	133.67**
Mycorrhizal Treatment (MT)	103.98**
T X SD	3.00*
T X MT	7.72**
SD X MT	2.29*
T X SD X MT	0.82^{NS}

* Significant at P = .05
** Significant at P = .01
NS Not Significant

The differences in total infected root length noted earlier could have been due to differences in total root length between the various treatments. However, the differential influence of soil treatment (pasteurization vs non-pasteurization) on root length indicated that mycorrhizal infection may not only have increased proportionally with increasing root length, but that it

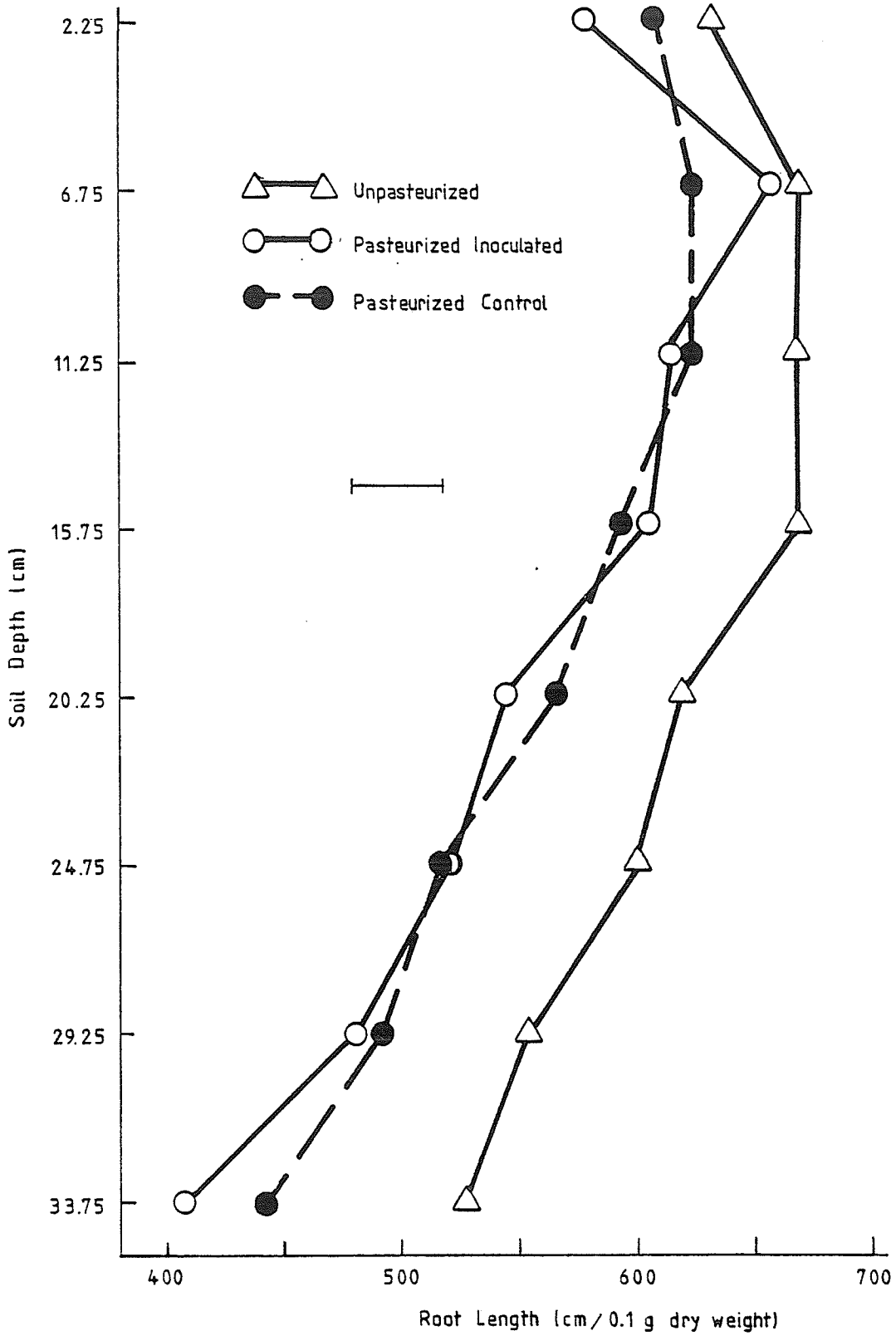


FIGURE 4. The effect of mycorrhizal treatment on total root length at eight soil depths.

⊢⊣ LSD (P=0.05).

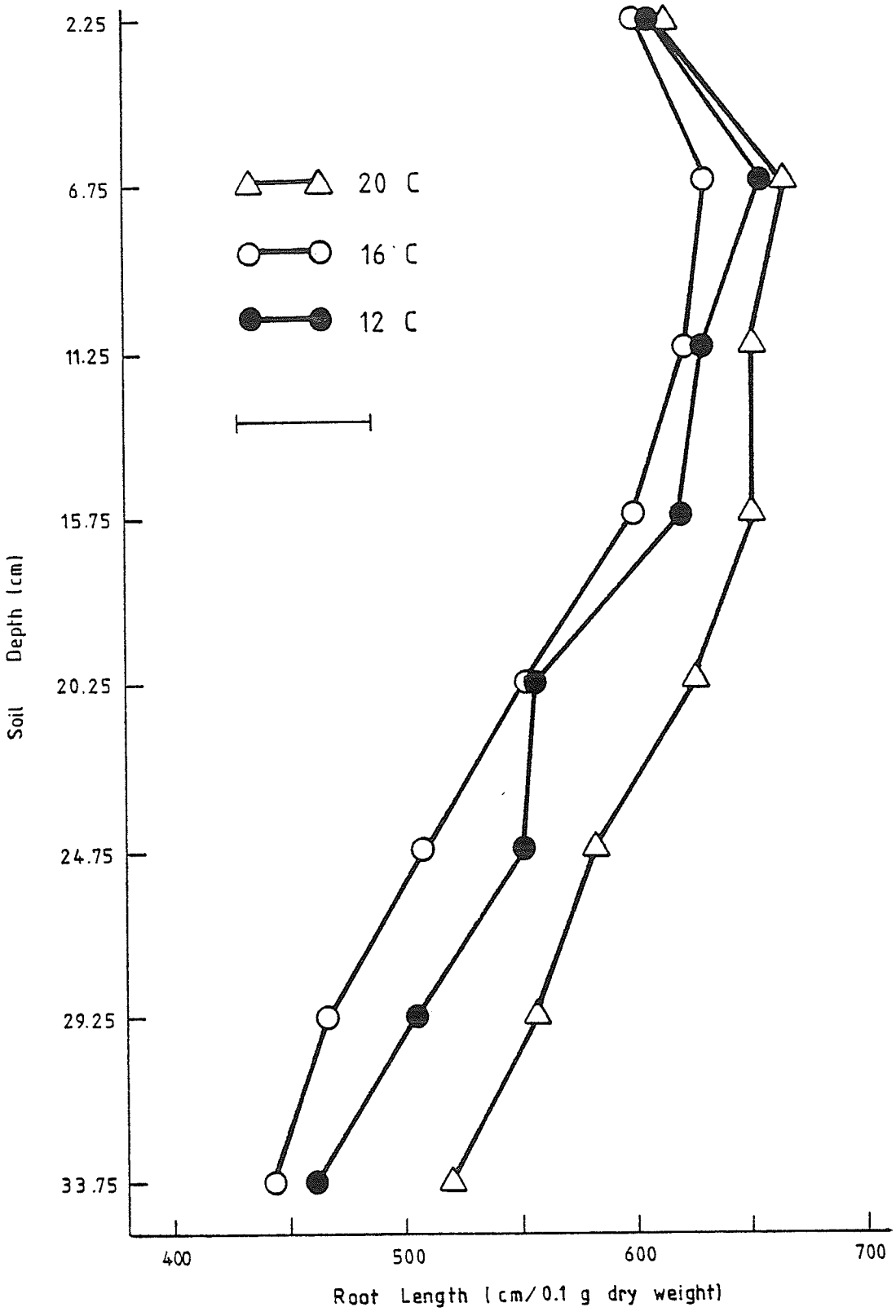


FIGURE 5. The effect of soil temperature on total root length at eight soil depths.

⊢⊣ LSD (P=0.05).

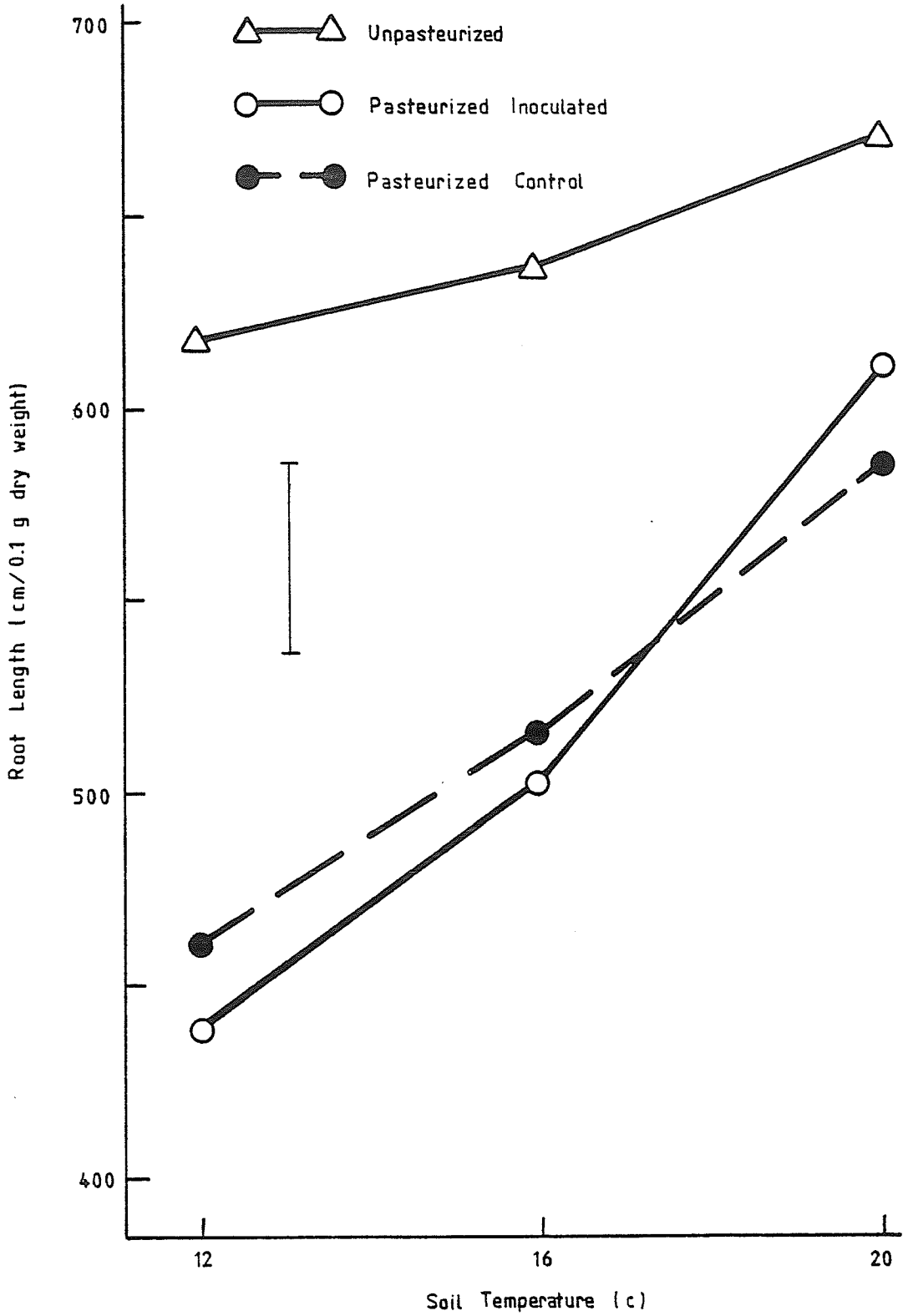


FIGURE 6. The effects of soil temperature and mycorrhizal treatment on total root length.

⊢—⊣ LSD (P=0.05).

may have actually stimulated an increase in total root length in regions where infection was more extensive. An inspection of the infection data on the basis of the percentage of the total root infected was made to obtain a clearer picture of the relationships indicated above.

Percentage of Total Root Length Mycorrhizally Infected

Table 3 shows a summary of the analysis of variance in the percentage of the total root length infected with mycorrhize in root segments of mycorrhizae barley roots grown at three soil temperatures.

Table 3. Summary of Analysis of Variance in the Percentage of the Total Root Length Infected with Mycorrhizae on Mycorrhizal Barley Plants Grown at Three Soil Temperatures

Source of Variation	F Value
Soil Temperature (T)	5.17*
Soil Depth (SD)	3.41*
Mycorrhizal Treatment (MT)	42.58**
T X SD	2.13^{NS}
T X MT	17.21**
SD X MT	7.42**
T X SD X MT	4.90**

* Significant at P = 0.05
** Significant at P = 0.01
NS Not Significant

The main effects of soil temperature and soil depth only slightly influenced the percentage of infection. However the temperature x mycorrhizal treatment and soil depth x mycorrhizal treatment interactions were highly significant. The three way interaction between these independent variables was also significant. A detailed analysis of variance is shown in Table 18 in the Appendix.

A comparison of Figures 3 and 7 which show the total length of mycorrhizal root and the percentage of the total root length infected, respectively, reveals the two graphs to be very nearly identical. The increase in the percentage of infected roots near the soil surface on the IP plants was expected. The isolated mycorrhizal infection reflected the localization of the inoculum, and indicated clearly that the rate of root development was considerably more rapid than that of infection. The only significant difference between these plants at the three temperatures was that with increasing soil temperature infection tended to spread to a lower soil depth. Hayman (1974) reported that cool soil temperatures delayed the onset of mycorrhizal infection, and retarded the rate of infection spread. In the present experiment the warmer soil temperatures may have promoted a more rapid descent of the infection.

Differences in the percentage of infected roots for a given mycorrhizal treatment between soil temperatures was also expected. Because soil temperature has been shown to influence both root (Power et al, 1970) and mycorrhizal infection development (Hayman, 1974), it is possible that temperature also influenced the association between the host and fungus.

More surprising than the influence of soil temperature on percent infection for each mycorrhizal treatment was the effect of soil depth on percent infection. The results in Figure 7 indicated that instead of a stable infection density along the root system in roots of the UP plants, the percent length of infected roots increased, reaching a peak in the upper half of the root system.

It was anticipated in this study that at a given soil temperature, the level of mycorrhizal infection would be directly proportional to the root length, and that the percentage of mycorrhizal root would be fairly stable

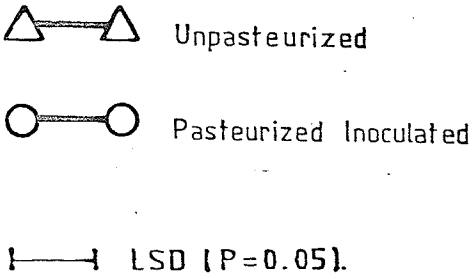
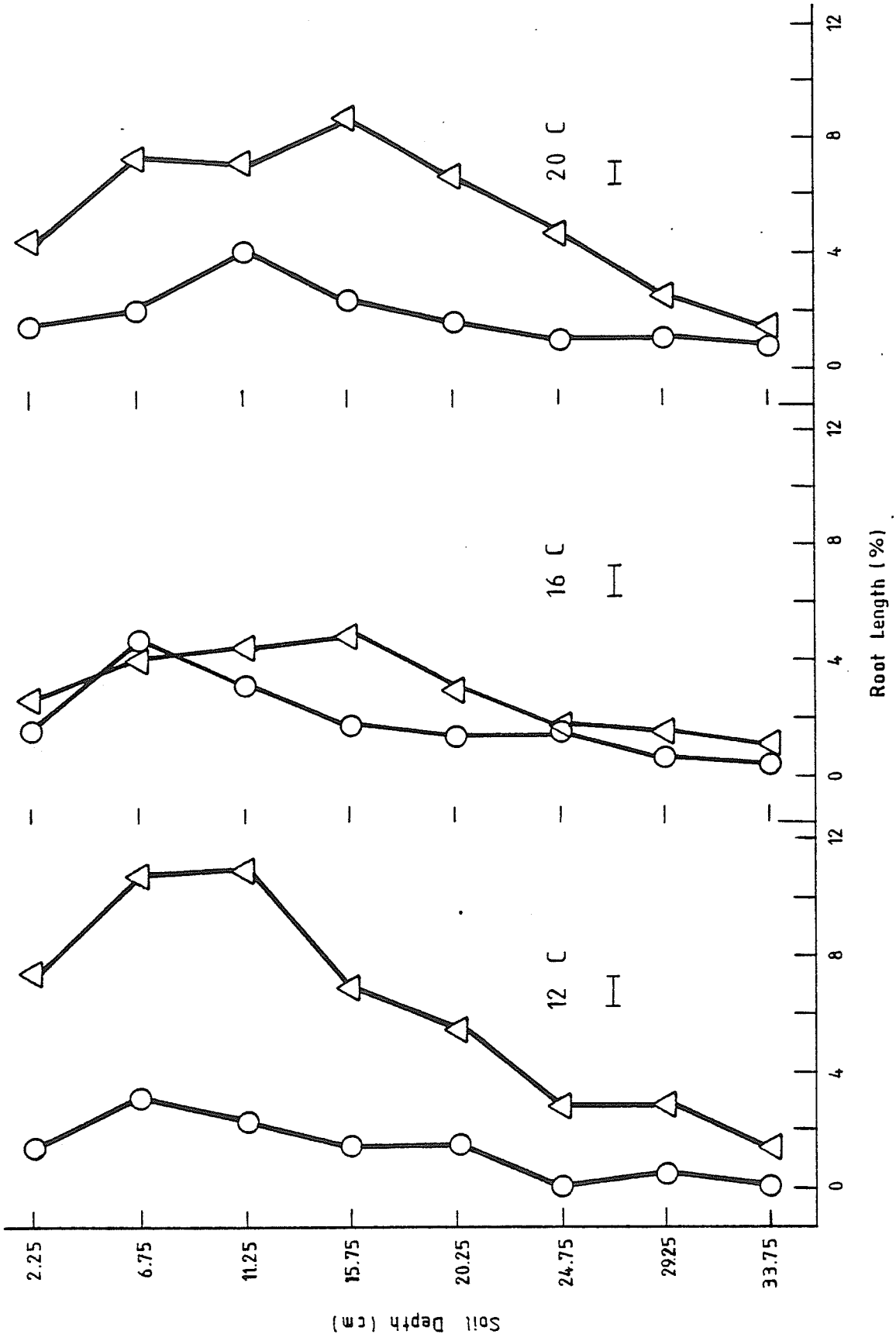


FIGURE 7. The Effect of Mycorrhizal Treatment on the Percentage of the Total Root Length Infected with Mycorrhizae at Eight Soil Depths and Three Soil Temperatures.


Soil Depth (cm)
2.25
6.75
11.25
15.75
20.25
24.75
29.25
33.75
0
4
8
12
12 C
16 C
20 C
Root Length (%)

over all soil depths in plants grown in unpasteurized soil. This was assuming that adequate time had been allowed to permit an equilibrium to be established between the rate of root development and mycorrhizal infection. It is possible that infection could have lagged behind rapid root growth, with the result that in regions where root growth was rapid, the percentage of mycorrhizal infection would have been lower than in areas of slower root development. It was felt that in this study ample time had been given for all plants to reach a stage of development at which an equilibrium between mycorrhizal infection and root growth could be established. Studies have indicated that by the heading stage, the percentage of mycorrhizal roots reaches a plateau, and that this percentage drops off during the grain filling stage (Furlin and Fortin, 1973; Hayman, 1974). Since plants in the present experiment were harvested at the same stage of development which corresponded to the plateau stage in mycorrhizal development, the differences in percent infection at various soil depths due to differences in stage of mycorrhizal development would have been minimal.

The decline in the percentage of mycorrhizal roots at the greater soil depths may have reflected the inability of mycorrhizal infection to keep pace with the rapid growth of the root near the apex of the root system. In the older regions of the root system mycorrhizal infection may have had more time to become established resulting in more widespread infection.

Although this explanation has some credence it may not adequately explain the increase in percentage of mycorrhizal roots nearer the soil surface. The larger total root length of samples taken near the soil surface (Figure 4) indicated the maintenance of a high level of root proliferation in this region. The percentage of mycorrhizal roots near the soil surface should therefore have not exceeded that at the greater soil depths on the basis of the above argument.

A second possible explanation that may account for the disproportionate increase in mycorrhizal roots in the region near to the soil surface is that there may have been a larger source of potentially infectable roots in this region compared to the roots further from the soil surface. It was strongly suspected that root size was in some way related to the percentage of infected roots that occurred at a given soil depth. The basis for this reasoning was that since all root samples had a dry weight of 0.1 g, assuming that the amount of extraneous debris on the roots was fairly constant throughout all samples examined, and that the density of a given root varied little over the root length, then for the length of roots per sample to be higher in one sample compared to another, the mean root diameter within the sample with the greater root length must have been smaller. Since there was an increased rate of infection with an increase in root length it was logical to suppose that the increase in mycorrhizal infection occurred in the roots with smaller root diameters. It remained then to test the validity of this supposition.

Root Diameter and Mycorrhizal Infection

A study was conducted to determine the relationship between root diameter and the incidence of mycorrhizal infection at different soil depths and temperatures.

Percentage of Mycorrhizal Roots 0.2 and 0.4 mm in Diameter

Table 4 shows a summary of the analysis of variance in the percentage of mycorrhizal roots 0.2 and 0.4 mm in diameter. A detailed analysis of variance is shown in Tables 19 and 20 in the Appendix.

Highly significant two way interactions occurred between soil temperature and mycorrhizal treatment, as well as between soil depth and mycorrhizal treatment for roots 0.2 mm in diameter. There was also a significant three way interaction between these variables at the 95% level of significance.

Table 4. Summary of Analysis of Variance in Percentage of Mycorrhizal Roots 0.2 and 0.4 mm in Diameter on Mycorrhizal Barley Plants Grown at Three Soil Temperatures+

Source of Variation	F Value	
	Root Diameter (mm)	
	0.20	0.40
Soil Temperature (T)	5.15**	2.15^{NS}
Soil Depth (SD)	3.65*	26.98**
Mycorrhizal Treatment (MT)	47.76**	23.68**
T X SD	2.01^{NS}	1.48^{NS}
T X MT	12.54**	0.26^{NS}
SD X MT	9.88**	7.06**
T X SD X MT	2.28^{NS}	0.74^{NS}

\+ Analysis of Variance conducted on Transformed Data.
* Significant at .05 level.
** Significant at .01 level.
NS Not Significant.

The three way interaction for the percentage of the total length of mycorrhizal roots 0.2 mm in diameter is shown graphically in Figure 8. Between 70 to over 90% total mycorrhizal root length of the UP plants were 0.2 mm in diameter over all three soil temperatures. This was essentially true also for roots of the PI plants, considering that the length of mycorrhizal roots was negligible below a soil depth of 25 cm. (Figure 3) on these plants.

The analysis of variance in the percentage of mycorrhizal roots 0.4 mm in diameter is shown in Table 4. Soil depth and soil temperature interacted significantly for roots of this size.

Figure 9 illustrates how soil temperature and mycorrhizal treatment influenced the extent of infection of roots 0.4 mm in diameter. Between 18.0 to 24.0% of the mycorrhizal roots of the UP plants were 0.4 mm in diameter in

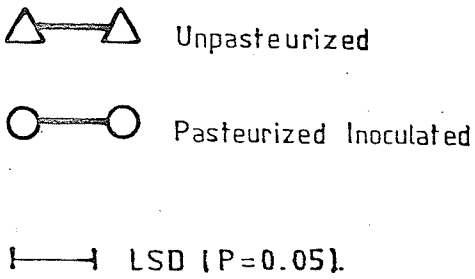
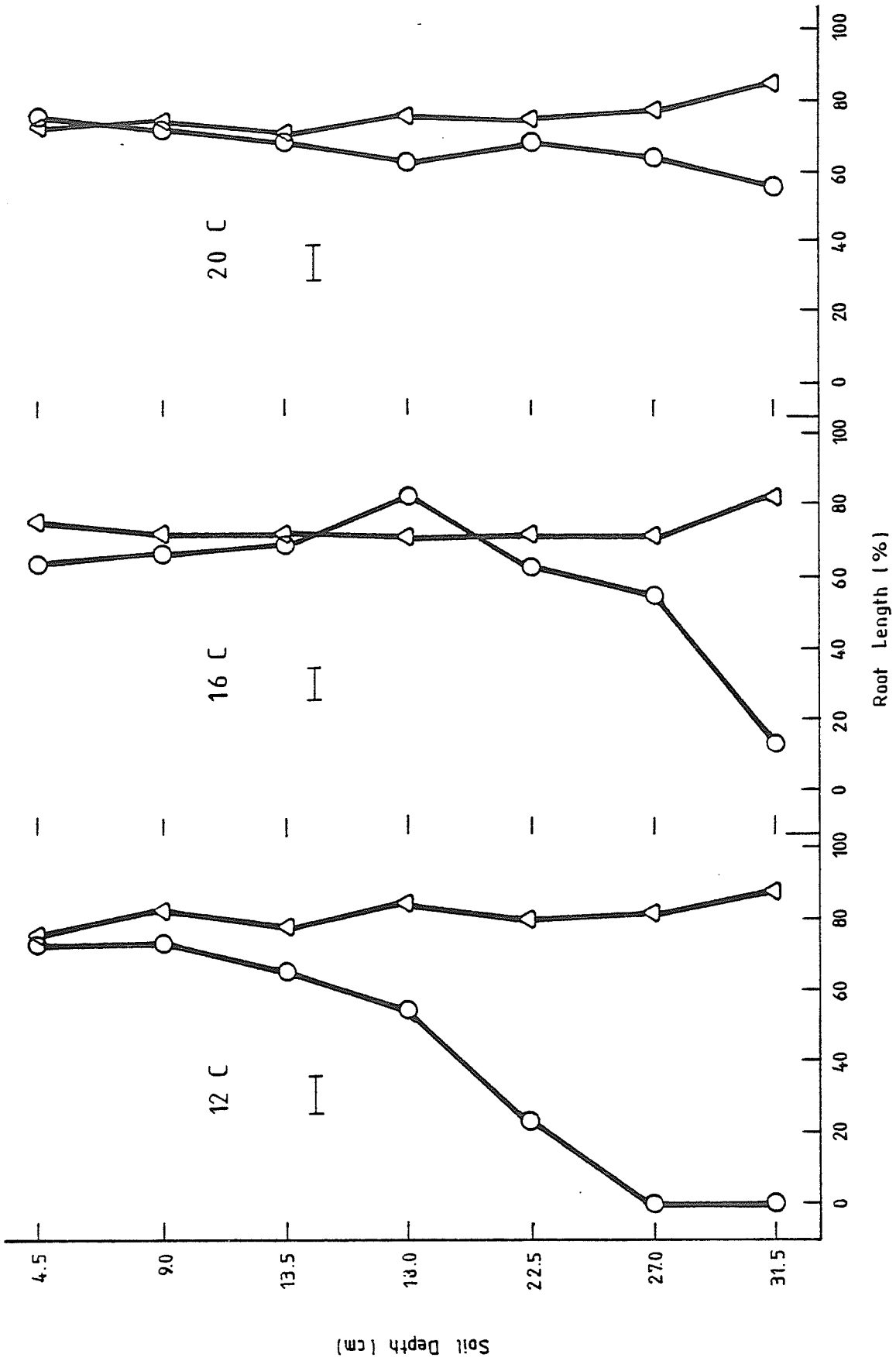


FIGURE 8. The Effect of Mycorrhizal Treatment on the Percentage of the Length of Mycorrhizal Roots 0.2 mm in Diameter at Eight Soil Depths and Three Soil Temperatures.


12 C
16 C
20 C
4.5
9.0
13.5
18.0
22.5
27.0
31.5
Soil Depth (cm)
0
20
40
60
80
100
Root Length (%)

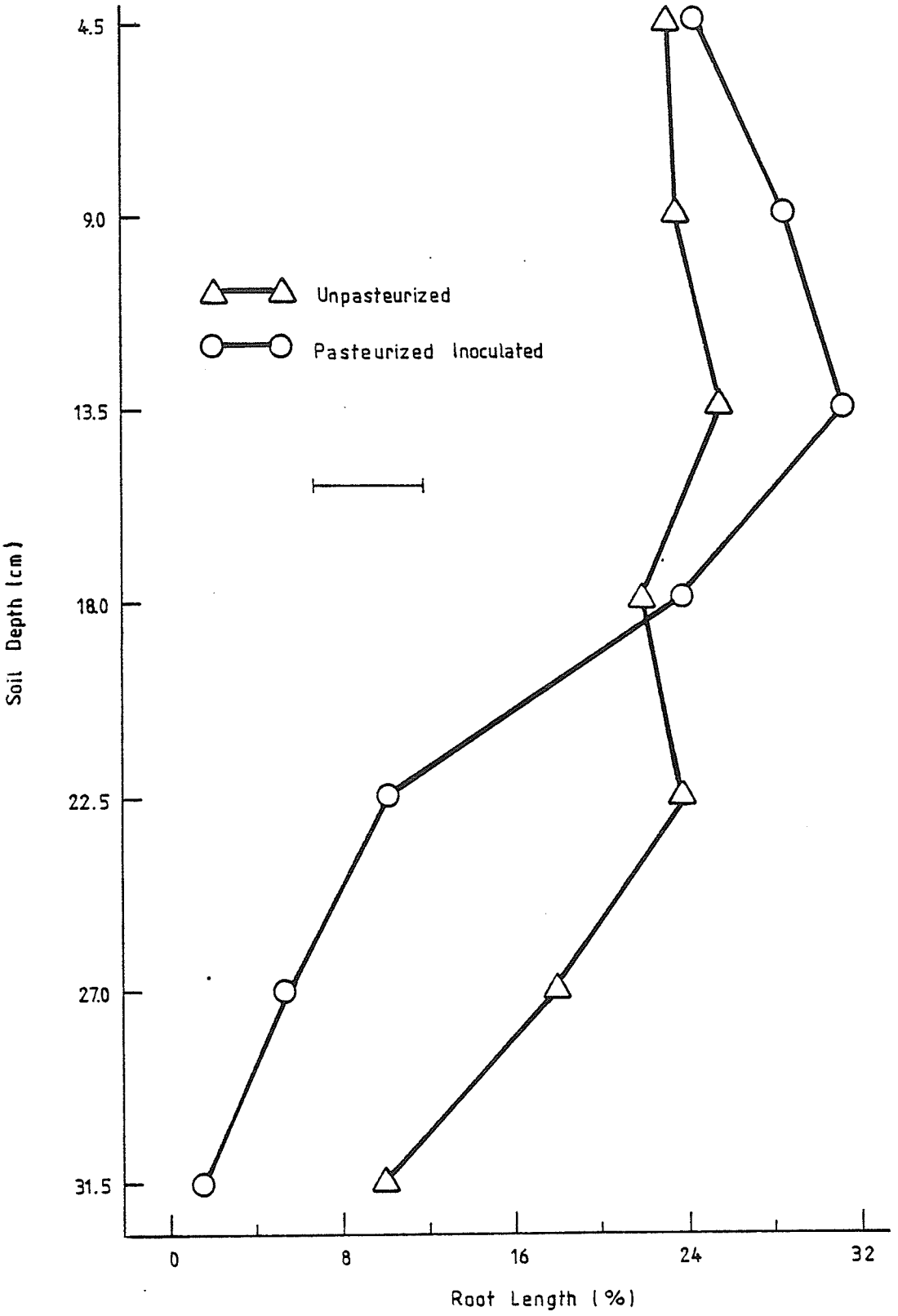


FIGURE 9. The effect of mycorrhizal treatment on the percentage of the length of mycorrhizal roots 0.4 mm. in diameter.

⊢⊣ LSD (P=0.05).

the case of all three soil temperatures, with the exception of the roots at the bottom of the pots, where no more than 4.0% of the total root length was mycorrhizal (Figure 7). Between approximately 24.0 to 30.0% of the mycorrhizal roots of the PI plants were 0.4 mm in diameter over the temperature range examined. Again this is considering the very small number mycorrhizal roots below a soil depth of 25 cm.

Mycorrhizal infection in roots 0.6 mm in diameter occurred very infrequently (no more often than once per root sample) and therefore was not considered to contribute significantly to the total length of infected root.

Mycorrhizal infection appeared to be predominantly confined to roots less than 0.4 mm in diameter. This may have been a reflection of the tendency of mycorrhizal infection to occur principally on newly developing roots, assuming that root diameter gave a rough approximation of root age. This was a valid assumption in that during root development at each successive stage of root development a thicker lateral gives rise to a lateral root finer than the one that preceded it (Weaver, 1926). Therefore, a measure of root diameter may also be an approximate measure of root age.

The delayed development of nodal roots which occurs approximately 15 - 20 days after that of seminal roots (Robards et al, 1973), introduced a weakness into the experiment. Nodal roots tend to be thicker than seminal roots. Their delayed onset would result in an overestimation of the length of older roots throughout the root system. However, since all plants were harvested at the same developmental stage, and since the length of this time period was sufficient for growth of the nodal roots to reach the bottom of the pot, it was felt that the presence of the nodal roots would not influence any one treatment or any single root segment more than another.

Percentage of Total Root Length 0.2, 0.4 and 0.6 mm in Diameter

In this section the results showing the extent of the inequality in distribution of coarse and fine roots at the various soil depths is presented.

A summary of the analysis of variance in the percentage of the total root length 0.2, 0.4 and 0.6 mm in diameter is shown in Table 5. A detailed analysis of variance for each of the root diameters is shown in Tables 21 - 23 (Appendix). For each of the root diameters examined, their number and distribution were found to be highly dependent on soil temperature, mycorrhizal treatment and soil depth. Two and three way interactions between soil temperature, soil depth and mycorrhizal treatment were also significant.

Table 5. Summary of Analysis of Variance in Percentage of the Total Length of Roots 0.2, 0.4 and 0.6 mm in Diameter on Mycorrhizal and Nonmycorrhizal Barley Plants Grown at Three Soil Temperatures+

Source of Variation	F Value		
	Root Diameter (mm)		
	0.20	0.40	0.60
Soil Temperature (T)	545.76**	387.76**	161.06**
Soil Depth (SD)	100.99**	64.55**	33.92**
Mycorrhizal Treatment (MT)	569.07**	375.85**	216.33**
T X SD	25.69**	15.65**	9.98**
T X MT	23.47**	19.14**	4.40**
SD X MT	14.02**	10.05**	5.41**
T X SD X MT	4.69**	3.33**	2.11**

\+ Analysis of variance conducted on transformed data.
* Significant at P = .05
** Significant at P = .01

Roots 0.2 mm in diameter comprised between 65 - 95% of the total root length while roots of 0.4 and 0.6 mm diameter made up approximately 5.0 - 25%

and 1.0 - 7.0% of the total root length, respectively, (Figures 10, 11 and 12). Figures 10, 11 and 12 show that both the mycorrhizal and nonmycorrhizal plants grown in pasteurized soil behaved very similarly with respect to the pattern of distribution of the three root diameters, and differently to the roots of the UP plants. Plants grown in pasteurized soil showed a gradual increase in the percentage of finer roots nearer the soil surface at lower soil temperatures coupled with a gradual decrease in the percentage of coarser roots. At the warmest temperature this difference in relation to soil depth was not apparent, the percentage contribution of each root size to the total root length, remaining fairly constant at all soil depths. The percentage of fine roots (0.2 mm) at 20 C in the bottom third of the pots of the pasteurized plants was greater than that at 16 and 12 C. The percentage of coarse roots in the lower half of the plant pots decreased with increasing temperatures.

The interaction between soil depth and soil temperature described for plants grown in the pasteurized soil was not evident for those grown in unpasteurized soil. At all soil temperatures and at all soil depths the percentage of roots in each size range fluctuated only slightly. A greater percentage of the roots were finer at all treatments compared to the plants grown in pasteurized soil, and conversely there was a lower percentage of coarser roots on the UP plants

The findings in this study were in agreement with other studies which have reported that plant roots grown in cool soil (5 - 10C) tended to be thicker than those exposed to warm soil conditions (Brouwer and Hoogland, 1964). It is not clear why this temperature effect was only noticeable in roots growing in the bottom third of the pots. Irradiation energy from the fluorescent lights may have been responsible for a slight increase in temperature at the soil surface causing roots in this region to be somewhat finer. However temperature measurements taken using thermistors at four

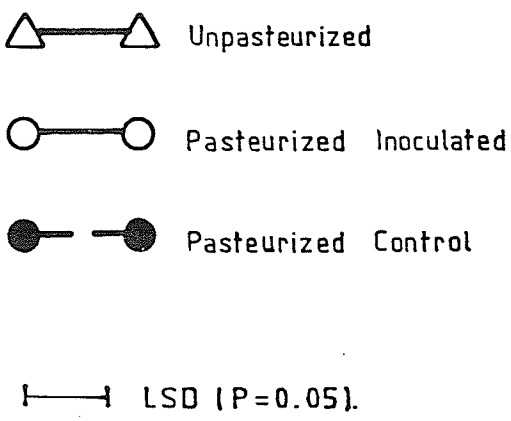
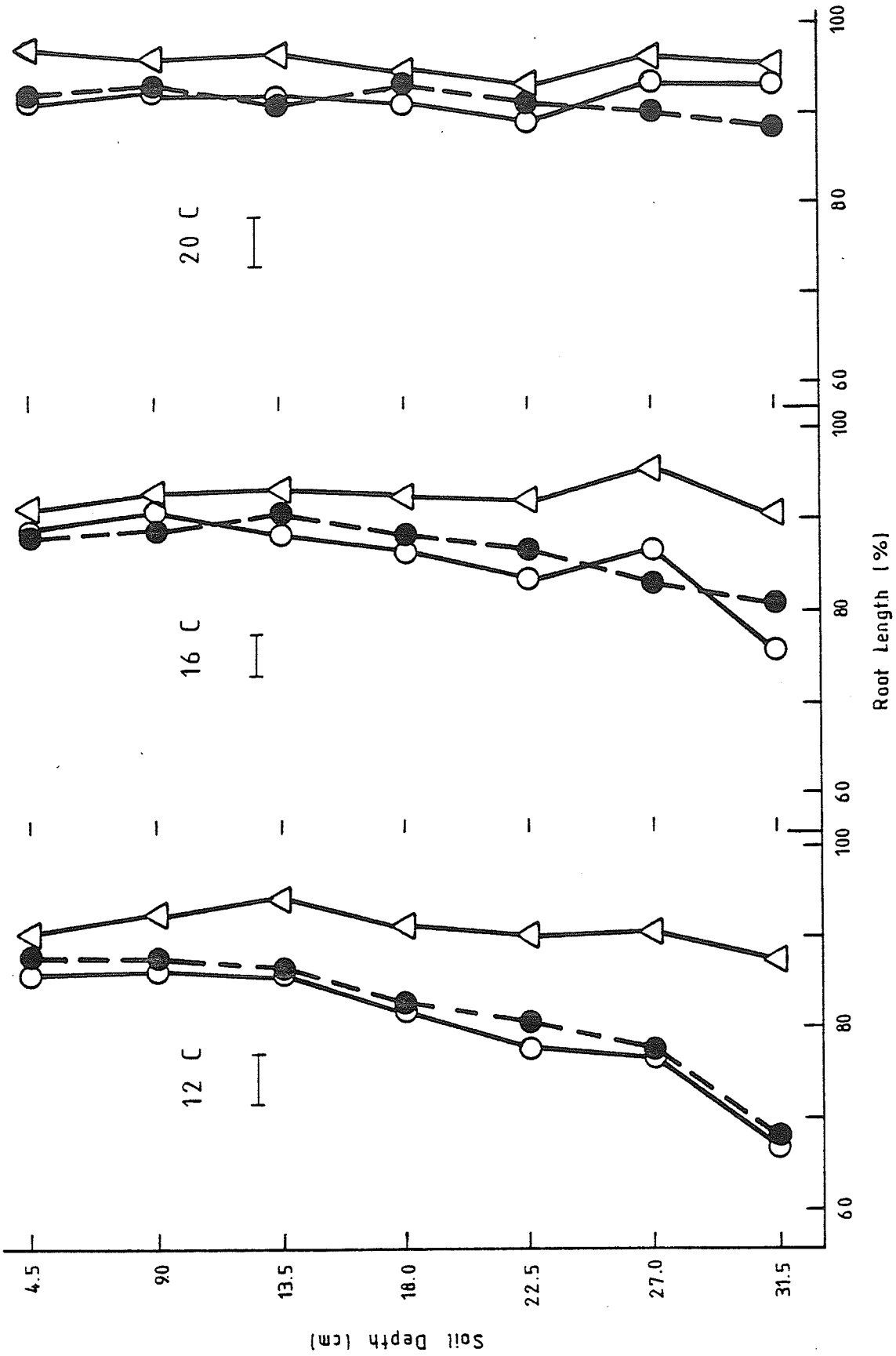


FIGURE 10. The Effect of Mycorrhizal Treatment on the Percentage of Total Root Length 0.2 mm in Diameter at Eight Soil Depths and Three Soil Temperatures.


Soil Depth (cm)
4.5
9.0
13.5
18.0
22.5
27.0
31.5
12 C
16 C
20 C
60
80
100
Root Length (%)

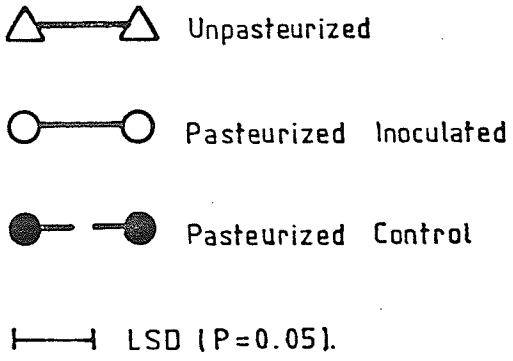
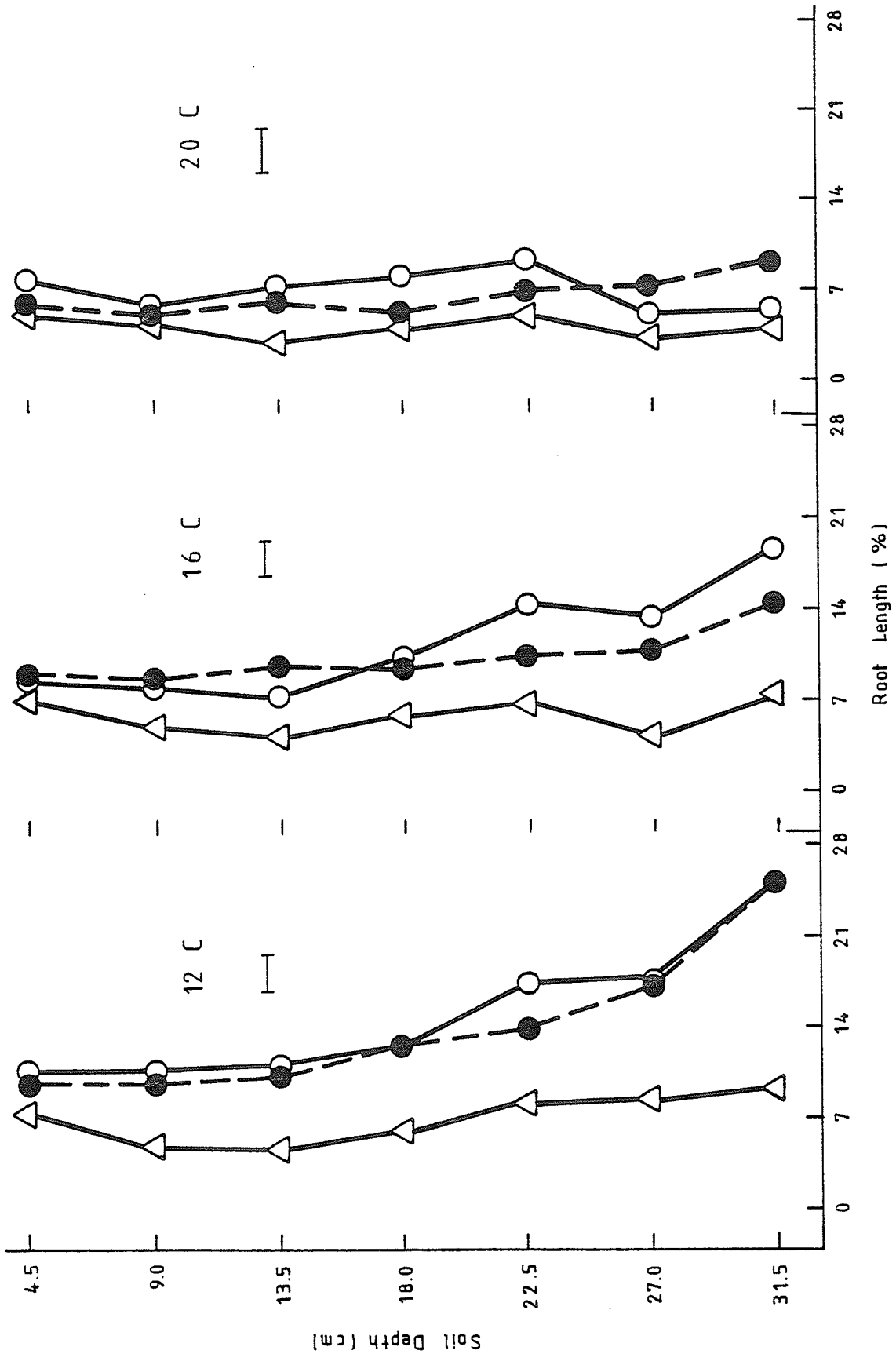


FIGURE 11. The Effect of Mycorrhizal Treatment on the Percentage of the Total Root Length 0.4 mm in Diameter at Eight Soil Depths and Three Soil Temperatures.


12 C
16 C
20 C
Soil Depth (cm)
4.5
9.0
13.5
18.0
22.5
27.0
31.5
0
7
14
21
28
Root Length (%)

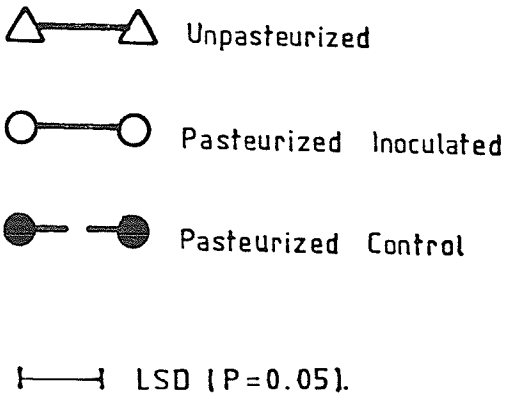
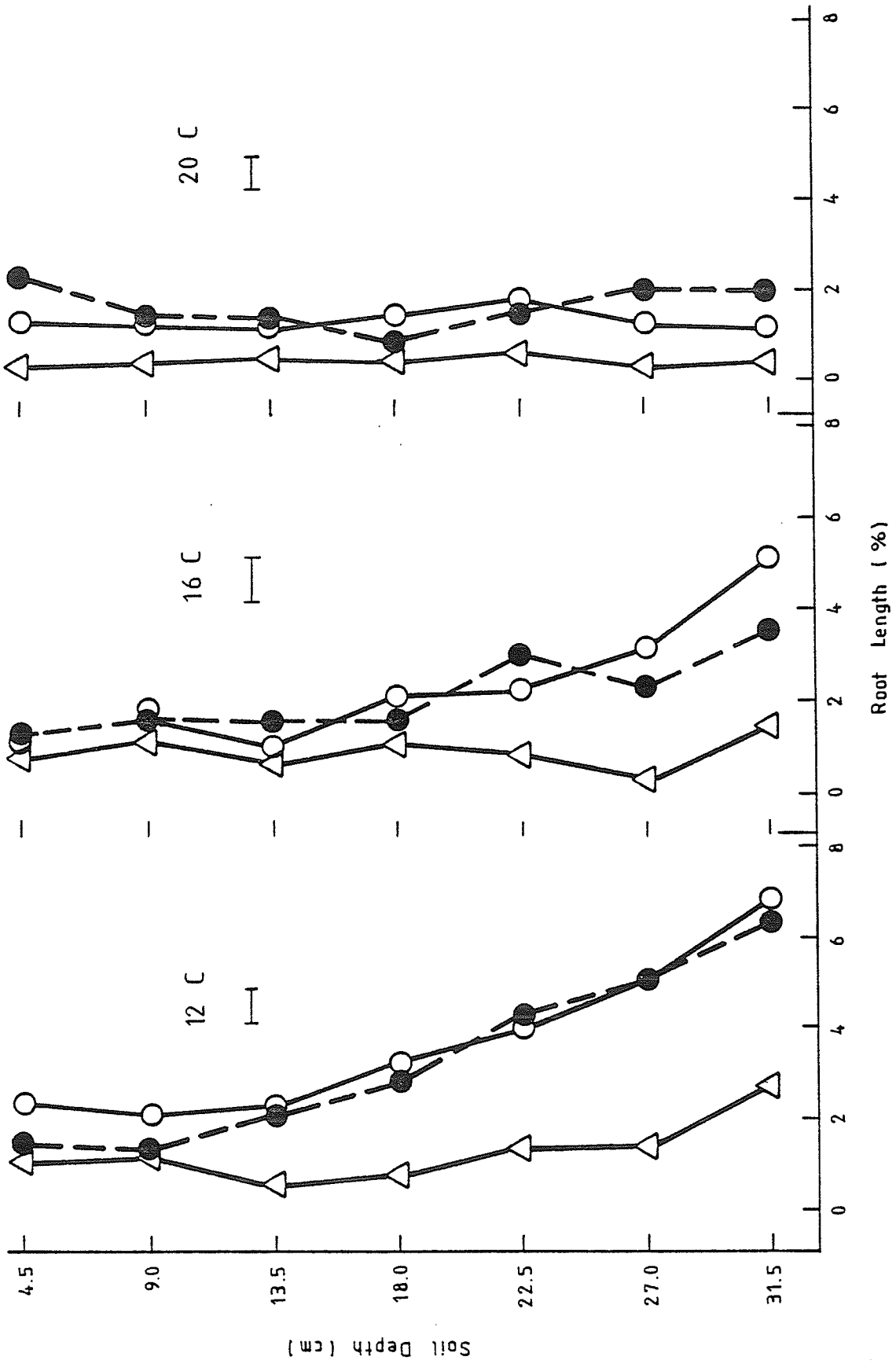


FIGURE 12. The Effect of Mycorrhizal Treatment on the Percentage of the Total Root Length 0.6 mm in Diameter at Eight Soil Depths and Three Soil Temperatures.


12 C
16 C
20 C
Soil Depth (cm)
4.5
9.0
13.5
18.0
22.5
27.0
31.5
0
2
4
6
8
Root Length (%)

soil depths indicated that soil temperature did not fluctuate more than one degree celsius throughout the pots at each of the temperature regimes.

Mechanical impedance imposed on barley roots by directing them through glass ballotini has been shown to cause an increase in thickness of the cortex over the root length affected (Wilson et al, 1975). In addition, root length was found to decrease by one-half or more. Similar findings have been reported for roots growing under natural conditions (Barley et al, 1965). Generally it has been found that when the extension of roots is retarded, differentiation is slowed less than extension (Drew, 1979). It is possible therefore that the coarser character of the bottom third of the root system was due to the resistance detected by the developing root when it contacted the bottom of the pot. The failure to observe the same degree of root thickening on plants grown in 20 C soil, as well as for those grown in unpasteurized soil compared to their counterparts removes some of this theory's credability.

The interaction between soil temperature, soil moisture and nutrient availability may have influenced the character of the rooting pattern in this study.

Soil Moisture

An uneven soil moisture distribution along the lengths of the plant containers was observed to have developed with time for both mycorrhizal and nonmycorrhizal plants, particularly at 16 and 20 C. This is shown in Table 6, which presents the results of determinations of soil water content at soil depths of 6.0, 20.0 and 34.0 cm and 20 and 45 days after seedling emergence. Within 20 days after emergence there was a significant decline in soil moisture with increasing soil depth for all three soil temperatures and mycorrhizal treatments. This is indicated in Table 7 which shows a summary of an analysis of variance in moisture content for the various

Table 6. Soil Moisture Content expressed as Percent Field Capacity of a very fine sandy loam/ sand soil mixture at three soil depths in pots containing mycorrhizal and nonmycorrhizal barley grown at three soil temperatures and sampled at 20 and 45 days after seedling emergence

		SAMPLING DATE+					
		20			45		
	Soil Depth	Soil Temperature (c)			Soil Temperature (c)		
	(cm)	12	16	20	12	16	20
Nonmycorrhizal Control	6	82.41	82.12	87.40	84.09	96.41	91.47
	20	76.36	83.67	78.89	74.61	69.18	72.37
	34	75.80	74.52	71.10	69.28	53.46	55.28
	LSD (P=0.05)	7.68	7.21	6.83	6.40	5.92	5.88
Mycorrhizal Pasteurized	6	79.28	89.43	85.62	86.45	94.60	98.49
	20	78.31	74.26	75.40	72.06	62.53	69.97
	34	74.47	68.64	73.35	60.33	49.84	52.33
	LSD (P=0.05)	7.96	7.04	6.69	6.68	6.11	5.50
Mycorrhizal Unpasteurized	6	86.52	91.41	89.54	96.64	98.59	102.48
	20	72.66	70.20	74.00	62.31	65.52	60.99
	34	67.35	62.68	61.31	51.50	48.46	38.31
	LSD (P=0.05)	7.63	6.98	6.43	6.24	5.43	5.61

* Values represent mean of 4 pots - 2 plants/pot

\+ Days Following Emergence

Table 7. Summary of Analysis of Variance in Soil Moisture Content at Three Soil Depths in pots containing Mycorrhizal and Nonmycorrhizal Barley Plants grown at three soil temperatures and sampled 20 and 45 days after seedling emergence.

Source of Variation	F Value
Temperature (T)	8.75**
Soil Depth (SD)	10.46**
Mycorrhizal Treatment (MT)	5.21**
Date of Sampling (D)	5.52**
T X SD	3.35*
T X MT	2.61*
T X DS	4.70*
SD X MT	2.72*
SD X DS	1.39^{NS}
MT X DS	2.84*
T X SD X MT	1.09^{NS}
T X SD X DS	3.21*
T X MT X DS	3.05*
SD X MT X DS	3.53**

* Significant at P = .05

** Significant at P = .01

NS Not Significant

treatments. A detailed analysis of variance is presented in the Appendix (Table 24). Table 6 shows that all of the main effects were significant as were most of the two and three-way interactions between the four independent variables. Although this made it difficult to sort out specific environmental effects on soil moisture, there were apparent trends that could be commented on.

The decrease in soil moisture with soil depth already noted was least evident after 20 days for the 12 C plants while the 16 and 20 C plants had increasingly larger differences in soil moisture with soil depth. This trend also occurred at day 45. However, the 20 C soil was significantly more moisture deficient than the 12 and 16 C soils at 20 and 36 cm below the soil surface. For all soil temperatures, the soil moisture content at the two deeper soil positions decreased from day 20 to day 45, with the largest reduction occurring at 20 C, for all mycorrhizal treatments. In treatments where soil moisture fell off rapidly with soil depth, moisture content was generally higher than 80% field capacity near the soil surface. The trends in moisture contents of the soils of the PC and PI plants were similar, and in most cases values of these two mycorrhizal treatments did not differ significantly from each other at any single treatment. The decline in the moisture content with soil depth of the UP soil treatments was significantly more rapid than that of the pasteurized treatments, at all soil treatments, particularly at day 45. The largest reduction in moisture content was in the unpasteurized soil at 20 C.

These results signified that soil moisture depletion became an increasingly serious problem as the plant aged. The plants that experienced this water deficit most acutely were those that were most heavily infected with mycorrhizae. Within this group, warmer soil temperatures accentuated the soil moisture deficiency. Plants that were uninfected had apparently the least problems with soil moisture deficiencies, although increasing the soil

temperature resulted in more severe soil water deficits. Presumably a moisture imbalance within the pots was a consequence of rapid water absorption by the roots of the plants. Differences in the severity of the water imbalance between treatments were likely related to the effect that each treatment had on the rate of growth of the plant. Extensive mycorrhizal infection and warm soil temperatures may have promoted an increased growth rate, along with a greater demand for soil water and nutrients. Warm soil temperatures have been reported to induce a shortening of the length of time for barley plants to attain maturity (Power et al., 1970). An increased growth rate would presumably have imposed greater damands on the moisture and nutrient supply around the roots. Daily replenishment of the water lost the previous day was meant to ensure that in the present experiment a serious water deficit did not develop in the soil used in this study. The very sandy texture of the soil should have permitted rapid percolation of the added water down to the bottom of the pots. It is possible that the decreased soil moisture with depth observed in this study occurred as a result of the low hydraulic conductivity across a wet/dry soil interface (Brady, 1974. As water flowed through the damp freshly moistened soil and contacted the relatively drier soil having a moisture content of 80% or less, its movement may have been impeded. This restriction in water flow may have been avoided if the pots had been irrigated to field capacity.

Water that remaind near the soil surface was most rapidly absorbed by heavily mycorrhizal plants as well as those grown at warm soil temperatures. At the cool soil temperature the rate of growth was likely reduced, hence moisture deficits did not occur to the same extent as for plants grown at 16 and 20 C. It is interesting to note the response of the three mycorrhizal treatments to different soil temperature. Most notable is that the PC and PI plants apparently responded more favorably to the 16 C soil temperatures than to the 12 and 20 C temperatures assuming that the rate of water absorption was

a rough indicator of the rate of shoot development. On the other hand there was very little increase in the soil water deficit of the 16 C plants when exposed to extensive mycorrhizal infection, whereas there was a considerable increase in the water deficit imposed on the 12 and especially the 20 C plants when grown in unpasteurized soil.

In common with the water deficit in the bottom third of the plant containers, there was an increase in the length of fine roots in this region as is shown in Figure 10. There was therefore strong reason to suspect that the primary cause of the increased root proliferation at the greater soil depths at the warmer soil temperatures and/or to extensive mycorrhizal infection was a reduction in the moisture content in these regions.

Root Fresh and Dry Weight

Differences in the effects of soil temperature and mycorrhizal treatment on root proliferation noted previously were also reflected in differences in fresh and dry root weight between the treatments. Table 8 shows a summary of the analysis of variance for fresh and dry root weight as well as for fresh/dry weight ratios. Soil temperature, soil depth and mycorrhizal treatment significantly influenced fresh and dry weight and the fresh and dry weight ratios with the exception that soil temperature had no significant influence on root dry weight. There were significant interactions between soil temperature and mycorrhizal treatment, and soil depth and mycorrhizal treatment for the fresh weight and fresh weight/dry weight ratio values. The only significant two way interaction that affected dry weight was between soil depth and mycorrhizal treatment. Only the fresh/dry weight ratio was influenced by the interaction between soil temperature and soil depth. A detailed analysis of variance is shown in the Appendix (Tables 25 - 27).

Table 8. Summary of Analysis in Variance in Fresh and Dry Root Weight and Fresh/Dry Root Weight Ratio of Mycorrhizal and Nonmycorrhizal Barley Plants grown at three soil temperatures and measured at four soil depths.

Source of Variation	F Value		
	Fresh Weight	Dry Weight	Fresh/dry Ratio
Temperature (T)	113.78**	2.96^{NS}	47.26**
Soil Depth (SD)	5.34**	6.62**	21.67**
Mycorrhizal Treatment (MT)	361.83**	119.85**	6.21**
T X SD	2.15^{NS}	1.18^{NS}	3.51**
T X MT	7.74**	0.76^{NS}	3.69**
SD X MT	7.17**	4.31**	3.60**
T X SD X MT	1.01^{NS}	1.01^{NS}	1.16^{NS}

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant

Two way interactions are shown numerically in Tables 9 a, b, and c. UP plant roots had considerably lower fresh and dry weights than the other two mycorrhizal treatments at all soil temperatures (Table 9 a). The PI plant roots had only slightly lower fresh and dry weights than the roots of the PC plants. Increased soil temperatures resulted in a steady decline in the fresh and dry weights of the roots of the mycorrhizal plants. The PC roots showed a slight increase in fresh and dry weight from 12 C to 16 C which declined sharply at 20 C; particularly the fresh weight. The fresh weight/dry weight ratio of the PI and UP roots tended to be higher than that of the controls at 12 and 20 C but were less than that of the control plants at 16 C. At soil temperatures of 12 and 20 C, the root fresh/dry weight ratios increased with an increase in the extent of mycorrhizal infection (Figure 3, Table 9). At 16 C there was a

decrease in the ratios as mycorrhizal infection increased (Figure 3, Table 9). The values of the ratios generally declined in the PI and UP roots with increasing soil temperature.

Table 9. Fresh and Dry Root Weight, and Fresh/Dry Root Weight Ratio of Mycorrhizal and Nonmycorrhizal Barley Plants grown at three soil temperatures. Table 9.a. Soil Temperature x Mycorrhizal Treatment

Mycorrhizal Treatment	Soil Temperature (C)	Fresh Weight (g)	Dry Weight (g)	Fresh/Dry Ratio (g)
Pasteurized Control	12	+13.47	2.14	6.47
	16	14.11	2.21	6.92
	20	9.53	2.13	5.11
	LSD (P=0.05)	1.01	0.32	0.60
Pasteurized Inoculated	12	12.70	1.89	6.90
	16	11.16	1.78	6.71
	20	8.14	1.55	5.58
	LSD (P=0.05)	0.77	0.30	0.50
Unpasteurized	12	7.14	0.97	7.61
	16	5.11	0.79	6.36
	20	3.83	0.68	5.96
	LSD (P=0.05)	0.43	0.13	0.38

+ Values represent mean of 8 pots, 2 plants per pot.

Table 9 b. shows the interaction between soil temperature and soil depth. Only the fresh/dry weight ratio showed significant responses (Table 8). At the lower soil temperatures, there was a pronounced reduction in the values of the fresh/dry ratio in the segments nearest the soil surface. This was not as evident for the plants grown at 20 C. Table 9.b. also shows the clear trend in reduction in fresh and dry total root weight with increasing soil temperature. A similar trend was observed in the fresh/dry ratio values.

Table 9 b. Temperature x Soil Depth

Soil Temp (C)	Soil Depth cm		Fresh Weight (g)	Dry Weight (g)	Fresh/Dry Ratio (g)
12	0.0 - 9.0		11.36	2.06	5.78
	9.0 - 18.0		11.23	1.51	7.84
	18.0 - 27.0		11.47	1.50	7.84
	27.0 - 36.0		10.36	1.60	6.83
		LSD (P=0.05) 1.45	0.28	0.46	
		TOTAL	44.1 ± 5.80	6.68 ± 1.10	6.64 ± 1.83
16	0.0 - 9.0		9.67	1.76	5.79
	9.0 - 18.0		10.41	1.48	7.28
	18.0 - 27.0		10.71	1.67	7.00
	27.0 - 36.0		9.79	1.46	6.97
		LSD (P=0.05) 1.84	0.35	0.62	
		TOTAL	40.52 ± 5.33	6.36 ± 1.41	6.37 ± 2.49
20	0.0 - 9.0		8.38	1.67	5.26
	9.0 - 18.0		7.04	1.45	5.73
	18.0 - 27.0		7.20	1.56	5.75
	27.0 - 36.0		6.05	1.15	5.46
		LSD (P=0.05) 1.30	0.41	0.55	
		TOTAL	28.67 ± 5.18	5.82 ± 1.62	4.93 ± 2.18

Values Represent mean of 8 pots, 2 plant/pot.
± Confidence interval (P = 0.05).

The soil depth x mycorrhizal treatment interactions are shown in Table 9 c. This interaction was highly significant for fresh and dry weight and for the fresh/dry weight ratios (Table 8). While the PC roots had the highest fresh and dry weights between 18.0 - 27.0 cm from the soil surface, both the PI and UP roots tended to have increasing fresh and dry weight with decreasing soil depth. The UP roots deviated slightly from this pattern in that the segments furthest from the soil surface had higher fresh and dry weights than the adjacent root segments.

The fresh/dry weight ratio appeared to reflect the level of mycorrhizal infection in the roots at the various soil depths. The values of the fresh/dry ratio varied only slightly with soil depth in the PC roots (Table 9 c), with the exception of a decline in the ratios in the segments nearest the soil surface. The PI roots followed a similar pattern except that there was a marked increase in the ratio of fresh/dry weights in the segments taken 9 - 18 cm from the soil surface, which corresponded to the region of denser mycorrhizal infection (Figures 3, 7). This was also the trend in the UP roots; greatest fresh/dry ratios occurred in the root regions characterized by heightened mycorrhizal activity.

The total root weights given in Table 9 c. indicated that the fresh and dry weights of roots declined in proportion to the intensity of mycorrhizal infection, with the UP roots having by far the lowest fresh and dry root weights. The totals of the fresh/dry weight ratios varied only slightly between mycorrhizal treatments although there was a trend toward increasing values with increasing mycorrhizal infection.

Fresh/dry weight ratio measurement is useful as an indicator of the water content per unit weight of plant material, and may roughly depict the relative amount of healthy root in a given sample. The higher fresh/dry root weight ratios of the most heavily infected plants indicated that although they were considerably finer in structure, a greater proportion of these roots were actively involved in water and nutrient uptake compared to the control plants.

There appeared to be an inverse relationship between root length and root weight. Plants grown in cool soil (12 C) in the absence of mycorrhizae had the largest wet and dry root weights. However as Figure 4 shows, their roots were shorter than those of plants grown at 16 and 20 C. Mycorrhizal infection had an influence similar to that of raising the soil temperature. However it

Table 9 c. Mycorrhizal Treatment X Soil Depth

Mycorrhizal Treatment	Soil Depth (cm)	Fresh Weight (g)	Dry Weight (g)	Fresh/Dry Ratio (g)
Pasteurized	0.0 - 9.0	11.66 +	2.25	5.22
	9.0 - 18.0	12.25	2.07	6.32
	18.0 - 27.0	14.18	2.54	6.38
	27.0 - 36.0	11.40	1.78	6.74
	LSD (P = 0.05)	1.38	0.35	0.72
	TOTAL	49.49 ± 5.53	8.64 ± 1.41	24.66 ± 2.89
Pasteurized Inoculated	0.0 - 9.0	11.85	2.26	5.39
	9.0 - 18.0	11.18	1.64	7.21
	18.0 - 27.0	10.27	1.52	6.96
	27.0 - 36.0	9.37	1.54	6.15
	LSD (P = 0.05)	1.14	0.23	0.54
	TOTAL	42.67 ± 4.54	6.96 ± 0.91	25.71 ± 2.18
Unpasteurized	0.0 - 9.0	5.90	0.98	6.21
	9.0 - 18.0	5.24	0.73	7.32
	18.0 - 27.0	4.92	0.67	7.24
	27.0 - 36.0	5.38	0.88	6.17
	LSD (P = 0.05)	0.74	0.11	0.48
	TOTAL	21.44 ± 2.96	2.96 ± 0.42	26.94 ± 1.92

+ Values represent mean of 8 pots, 2 plants/pot.
± Confidence Interval (P = 0.05).

appeared that mycorrhizal infection and soil temperature had opposite effects on the values of the fresh/dry weight ratios.

Increased soil temperature apparently reduced the fresh weight/dry weight root ratios within mycorrhizal treatments. A close look at the results in Table 9 reveals that the effect of soil temperature was most prominent for the plants most heavily infected, and was least for the PC roots. In fact, an increase in soil temperature from 12 to 16 C resulted in an increase in the fresh/dry weight ratio for the control plants, and only a slight decrease in the ratios of the PI roots. In addition, plants grown at 16 C responded differently to the presence of mycorrhizal infection than those at 12 and 20 C. Thus, the ratio values increased with increasing infection at 12 and 20 C, but decreased at 16 C. At 16 C the PC plants had ratios greater than those of the mycorrhizal plants. It would appear then that at least on the basis of the values of the fresh/dry weight ratios, plants responded favorably to mycorrhizal infection at 12 and 20 C whereas at 16 C infection was detrimental. A negative response to infection at 16 C may have indicated that 16 C was the optimal soil temperature for root development. In the presence of mycorrhizae, the temperature optimum may have shifted to 12 C as indicated by an increase in the fresh/dry root weight ratio with these mycorrhizal treatments. At 20 C, although the total length of roots increased (Figure 4) the moisture content per unit root weight decreased. Although this may have indicated a reduction in the length of functional roots, it may in fact have reflected only the relative decrease in cell size that likely was characteristic of roots of smaller diameter, which would manifest itself in a decrease in the fresh/dry weight ratio. Yet, in spite of the increase in root length induced by the presence of mycorrhizal infection, the fresh/dry weight root ratio increased at 12 and 20 C. This would seem to indicate a real increase in the amount of

functional root at these temperatures. This argument is strengthened by the finding that the increased fresh/dry ratios corresponded to an increased incidence of mycorrhizal infection in individual pots (Figure 7) as well as an increase in total root length (Figure 4).

Shoot Fresh and Dry Weight

To determine whether the alteration in root development by the presence of mycorrhizal organisms was translated into improved shoot growth, fresh and dry weights of shoots were obtained. Table 10 shows a summary of the analysis of variance in fresh and dry shoot weight, fresh/dry shoot weight ratio, and the shoot/root dry weight ratio of mycorrhizal and nonmycorrhizal barley plants grown at three soil temperatures. There were significant main effects of soil temperature and mycorrhizal treatment, as well as significant interactions between these independent variables on fresh and dry shoot weights. Temperature and mycorrhizal treatment had no effect on the shoot fresh/dry ratios. A detailed analysis of variance for this data is shown in Tables 28, 29 and 30 in the Appendix.

Table 10. Summary of Analysis of Variance in Fresh and Dry Shoot Weight Fresh/Dry Shoot Weight Ratio and Shoot/Root Dry Weight Ratio of Mycorrhizal and Nonmycorrhizal Plants grown at three soil temperatures.

Source of Variation	F Values			
	Fresh Shoot Wt.(g)	Dry Shoot Wt.(g)	Fresh/Dry Ratio	Shoot/Root Ratio
Soil Temperature (T)	4.88**	3.68*	1.19^{NS}	0.29^{NS}
Mycorrhizal Treatment (MT)	17.76**	20.38**	1.07^{NS}	19.42**
T X MT	7.73**	2.93*	0.96^{NS}	1.14^{NS}

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant

Mycorrhizal plants at all soil temperatures had significantly higher fresh and dry shoot weights than their nonmycorrhizal counterparts (Table 11). PC and PI plants had higher wet shoot weights at 16 C than those at 12 C. Shoot fresh weights of the PC plants was higher at 12 C than at 20 C. The fresh shoot weight of the PC and PI plants increased with increasing soil temperatures from 12 to 16 C. At 20 C, the fresh shoot weights of the PC and UP plants were generally lower than those at 12 and 16 C, while the PI plants had lowest values at 12 and 20 C. Shoot dry weights of all three mycorrhizal treatments did not differ between the three soil temperatures with the exception that at 16 C the dry shoot weight of the PC plant was higher than the 20 PC plants.

With respect to the shoot fresh/dry weight ratios soil temperature and mycorrhizal treatment had no influence on these values.

Apart from the obvious influence of mycorrhizal enhancement of fresh and dry shoot weight, the interactions between soil temperature and mycorrhizal treatment on shoot growth presented a somewhat complex pattern. Moderate to cool soil temperatures favored maximum shoot growth of nonmycorrhizal plants. The reduction in shoot fresh and dry weight of the PC plants at 20 C may have been in part related to the moisture deficiencies that developed at increasing soil depth with time (Table 6). The rapid growth rate characteristic of plants grown at warm soil temperatures (Power et al, 1970) may have served to deplete the readily available moisture supply at the greater soil depths.

It is possible that the reduced shoot growth at 20 C was due to an impaired nutrient uptake ability of the plant roots in direct response to higher soil temperatures as Mack (1965) observed for barley plants grown in 27 C soil compared to 9 and 18 C. However it seems doubtful that this was the main influence of soil temperature in this case inasmuch as 20 C is not as far removed from the 18 C theoretical soil temperature optimum for barley growth as is 27 C.

Table 11. Fresh and Dry Shoot Weight, Fresh/Dry Shoot Weight Ratio, Dry Root Weight and Shoot/Root Dry Weight Ratio of Mycorrhizal and Nonmycorrhizal Barley Plants Grown at Three Soil Temperatures

		SHOOTS			ROOTS	SHOOT/ROOT
Mycorrhizal Treatment	Soil Temp. (C)	Fresh Weight (g)	Dry Weight (g)	Fresh/Dry Ratio	Dry Weight (g)	Ratio
Pasteurized	12	+45.08	11.52	3.93	8.45	1.36
	16	48.62	11.94	4.07	8.83	1.35
	20	41.56	10.97	3.78	8.54	1.28
	LSD (P = 0.05)	2.97	0.79	0.37	0.82	.20
Pasteurized Inoculated	12	48.22	12.21	3.94	7.58	1.61
	16	52.25	13.01	4.02	7.12	1.82
	20	49.41	12.16	4.06	6.21	1.95
	LSD (P = 0.05)	3.15	0.92	0.26	0.63	0.24
Unpasteurized	12	60.78	14.36	4.23	3.88	3.70
	16	57.85	14.29	4.04	3.15	4.78
	20	54.62	13.64	4.00	2.73	4.99
	LSD (P = 0.05)	4.07	0.96	0.29	0.33	0.43

+ Values represent mean of 8 pots, 2 plants/pot.

The absence of a reduction in shoot fresh and dry weights of the 12 C mycorrhizal and nonmycorrhizal plants confirmed the findings of Power et al (1970) who found that reduced soil temperatures did not in themselves reduce final plant yield. The shift in the temperature optimum towards cooler soil temperatures during the later stages of growth reported by Brouwer and Hoogland (1968) may have been responsible for the weaker shoot growth of the 12 C plants during the initial developmental stages. Sampling prior to plant maturity may have given the warmer soil temperature plants an advantage over the 12 C plants, although as already noted, that was not the case for the 20 C plants.

Greatest improvement in fresh shoot weight in response to mycorrhizal infection occurred in the 12 C (increase of 15.7 g) and the 20 C (increase of 13.0 g) UP plants, as compared to an increase of approximately 9.2 g for the 16 C plants given the same mycorrhizal treatment (Table 12). This trend was closely approximated by the PI plants, although growth improvement of shoots was less than that for the heavily mycorrhizal plants. Also, the 16 C plants still had a slightly higher shoot fresh weight compared to those grown at 12 and 20 C. The improvement in growth of the mycorrhizal plants was directly related to the total percent length of roots that were mycorrhizal (Table 12). Extensive mycorrhizal infection resulted in a larger increase in shoot growth over the PI plants, but soil temperature strongly influenced the magnitude of this increase.

The same trend remarked on in the discussion on fresh and dry root weight, viz. a reduced response of the 16 C plants to mycorrhizal infection, was observed with respect to shoot weight. This trend indicated that plants grown at 16 C may have responded less to the presence of intensive infection because their growth was already proceeding at a rate faster than that of plants grown at 12 C and 20 C. The introduction of mycorrhizal inoculum to the soil containers

at 12 and 20 C increased the rate of growth of these plants so that they were comparable to that of the 16 C plants (Table 12). The substantial increase in the percentage of mycorrhizal infection of the 12 C plants in unpasteurized soil (Table 12) indicated that cool soil temperatures favored the beneficial balance between increased root growth apparently induced by the infection process, and reduced root growth stimulated by the cool soil temperatures.

Table 12. Total Length of Mycorrhizal Roots, Total Root Length, Total Percentage of Mycorrhizal Root, and Fresh and Dry Root Weight Increase of Mycorrhizal and Nonmycorrhizal Barley Plants grown at Three Soil Temperatures.

		Root Length*			Shoot Weight Increase+	
Mycorrhizal Treatment	Soil Temp (C)	Infected (cm)	Total (cm)	% Infected of Total	Fresh (g)	Dry (g)
Pasteurized Control	12		×3310.51			
	16		3904.62			
	20		4266.28			
	LSD (P = 0.05)		243.16			
Pasteurized	12	66.97	3154.27	2.10	3.14	0.69
	16	90.16	3655.75	2.48	3.63	1.07
	20	177.23	4347.19	4.08	7.85	1.23
	LSD (P = 0.05)	5.08	240.23	0.08		
Unpasteurized	12	354.60	4295.14	8.25	15.70	2.84
	16	176.45	4502.63	3.92	9.23	2.35
	20	271.45	4819.86	5.65	13.06	2.67
	LSD (P = 0.05)	7.15	258.89	0.30		

* Total Root Length obtained from seven root subsegments.
+ Comparisons made in relation to pasteurized controls.
× Values represent means of eight pots, 2 plants/pot.

Shoot/Root dry weight ratios shown in Table 11 provided perhaps the best evidence of influence of mycorrhizal infection on barley development. A summary of the analysis of variance in shoot/root dry weight is shown in Table 10. Mycorrhizal treatment significantly influenced the shoot/root dry weight ratio. No other factors were important. A detailed analysis of

variance in shoot/root dry weight ratio is shown in the Appendix, Table 31.

The shoot/root dry weight ratio of the UP plants was over three times larger than that of the pasteurized controls and 2.5 times larger than that of the PI plants, when averaged over three soil temperatures (Table 11). Shoot/root ratio values of the PI plants were larger than those of the PC plants at all three soil temperatures but differences were far less dramatic than for the UP plants. The shoot/root ratios of the nonmycorrhizal control plants did not vary over the three soil temperatures. Increasing the soil temperature from 16 to 20 C for the mycorrhizal plants resulted in no change in the shoot/root ratio values. However, the 12 C mycorrhizal plants had lower shoot/root ratios than their 16 and 20 C counterparts.

The trends of the shoot/root ratios exemplified the improved efficiency in growth of the mycorrhizal plants over that of the nonmycorrhizal control plants at all three soil temperatures. The improved growth efficiency of the warm temperature plants over the 12 C plants may have reflected higher metabolic activity at the warm temperatures. No doubt the greater surface area of the roots grown at 16 and 20 C improved their ability to extract slowly diffusing ions from the soil (Nye, 1966; Olsen and Kemper, 1968). The improved access of mycorrhizal plants to soil phosphorous by way of the extended hyphal surface area likely contributed to an enhanced efficiency of the roots (Sanders and Tinker, 1971, 1973). In addition the finer textured roots characteristic of the mycorrhizal plants in this study may have resulted in a superior nutrient and water uptake efficiency.

SECTION II

Root Viability

Introduction

The original intent of this study was to determine whether the enhancement effect of mycorrhizal infection on the level of phosphorus in the root delayed the onset of cortical cell deterioration that has been reported to occur in older regions of plant roots (Clarkson, 1974; Martin, 1977). It was assumed that the level of carbohydrates, amino acids, and conductivity, which in this study were designated as metabolic indicators, would provide a rough measure of the extent of cortical cell decay.

The finding in the previous section that the total root length within a root sample generally increased with increasing soil depth (Figure 4), placed uncertainty in the validity of the above assumption. An increase in the level of a given metabolic indicator may have been either due to an increase in the proportion of intact roots or simply to a greater length of root in the sample. Because both soil temperature and mycorrhizal infection significantly influenced root length it was difficult to distinguish whether these factors affected root viability or root length.

An alternative to examining the data directly was to convert the results so that values could be compared on the basis of a standard root length per sample. This would have eliminated the problem of distinguishing the effect of mycorrhizal treatment and soil temperature on root length and cell viability. There were two difficulties that made this solution undesirable. Firstly, differences in the values of the metabolic indicators at the various soil temperatures, soil depths, and mycorrhizal treatments were generally much less

than the differences in the root length values for the various levels of the independent variables. Standardizing the indicator values to a single root length therefore resulted in what might be considered as over correction. A consequence of this over correction was that when the corrected data were plotted the curves that resulted closely resembled mirror images of the original curves for total root length. Correction on the basis of root length therefore did not improve interpretability of the data. A second difficulty was that correcting for root length differences between samples did not accurately reflect the differences in the level of the metabolic indicators in samples having different root lengths. In the previous section in which the distribution of the various root diameters throughout the root system was analysed, it was observed that although the majority of the root system had root diameters of approximately 0.2 mm, over 30% of the roots in some of the root samples had root diameters greater than 0.2 mm, depending on the soil depth, temperature and mycorrhizal treatment (Figures 10, 12). Using root length as the correction factor without at the same time taking into account the differences in diameters of the roots that constituted the total root length presupposed the assumption that fine and thick roots contributed equally to the total amount of the metabolic indicator detected in a given sample. It seemed reasonable to assume otherwise; that in fact a given length of thick root contributed a larger proportion of the total amount of metabolic indicator than an equal length of fine roots.

To accommodate these two criticisms, and at the same time obtain a better representation of the data that would account for the uneven distribution in root length and root diameter, the original data was corrected to a standard root volume within each root sample taken over the length of the root system.

Root volumes were determined from a knowledge of the total length of roots of each diameter. The data for total root volume is shown numerically in Table

32 in the Appendix. Because the trends in root volume generally correspond to those for total root length a detailed discussion of the effects of soil temperature and mycorrhizal treatment on total root volume was not included in the discussion.

Root volume data were obtained from different soil depths than were the data on phosphorous content and the root viability parameters. Interpolation of volume data was necessary to obtain values that corresponded to the correct soil depth. Although this introduced further uncertainty into the data, it was felt that this would be minimal insofar as the trends in the volume data followed a smooth predictable curve when averaged over the eight planting dates (Table 32).

Results

The results obtained from performing chemical analysis and conductivity measurements on the root diffusates are shown in the Appendix (Tables 33 to 36).

The results of the chemical analysis subsequently corrected for a standard root volume are shown in Figures 13 to 18. The corrected data showed that soil temperature as well as mycorrhizal treatment strongly influenced the level of the indicators of metabolic activity at the various soil depths. Table 13 shows a summary of the analysis of variance for the corrected data. A detailed analysis of variance is shown in Tables 37 to 40 in the Appendix. The analysis of variance showed that along with the main effects of soil temperature, soil depth and mycorrhizal treatment, most of the two and three way interactions between these independent variables were also highly significant for the metabolic indicators. The exceptions included the main effect of soil temperature on the level of water soluble phosphorus, the two way interaction between temperature and soil depth for phosphorus, the two way interaction

Table 13. Summary of Analysis of Variance in Indicators of Metabolic Activity Corrected For Root Volume in Mycorrhizal and Nonmycorrhizal Barley Roots grown at three Soil Temperatures

Source of Variation	F Value			
	Carbohydrates	Free Amino Acids	Water Soluble Phosphorus	Conductivity
Soil Temperature (T)	208.31**	39.50**	1.32^{NS}	160.40**
Soil Depth (SD)	197.10**	38.45**	53.59**	112.30**
Mycorrhizal Treatment (MT)	17.78**	4.52**	125.82**	6.59**
T X SD	6.23**	3.84**	1.48^{NS}	7.86**
T X MT	3.71**	9.74**	52.62**	6.38**
SD X MT	0.84^{NS}	2.51**	7.52**	3.71**
T X SD X MT	0.74^{NS}	2.09**	3.86**	4.77**

* Significant at P = 0.05.

** Significant at P = 0.01.

NS Not Significant

between soil depth and mycorrhizal treatment on the level of soluble carbohydrates, and the three way interaction between soil temperature, soil depth and mycorrhizal treatment on the level of carbohydrates; all of which had effects that were not significant.

Carbohydrates

In general, warm soil temperatures resulted in lower levels of carbohydrate (Figure 13). Roots of PC plants grown at 12 C had higher levels of carbohydrate than those of the PC plants grown at 16 and 20 C; the latter two soil temperatures showing no difference in their influence on carbohydrate values (Figure 13). This trend continued for the PI plants as well as for the UP plants (Figure 13).

Carbohydrate values generally increased with increasing soil depth (Figure 14). Soil depth had very similar influences on the warmer temperature plants (Figure 14). Carbohydrate values for the 12 C plants declined with soil depth, then abruptly increased approximately 25 cm. from the root base (Figure 14).

Nonmycorrhizal plants grown at 20 C soil temperatures had higher carbohydrate values than the corresponding mycorrhizal plants (Figure 13). At 12 and 16 C mycorrhizal treatment had no effect on the level carbohydrate in the plant roots (Figure 13). Carbohydrate levels did not differ at 16 and 20 C within each of the mycorrhizal treatments (Figure 13). Carbohydrate values did not differ greatly between mycorrhizal treatments when averaged over the three soil temperatures (Figure 15).

Conductivity

The effects of soil temperature and mycorrhizal treatment on the level of conductivity in barley roots is shown graphically in Figure 16. The following discussion will refer to this set of graphs.

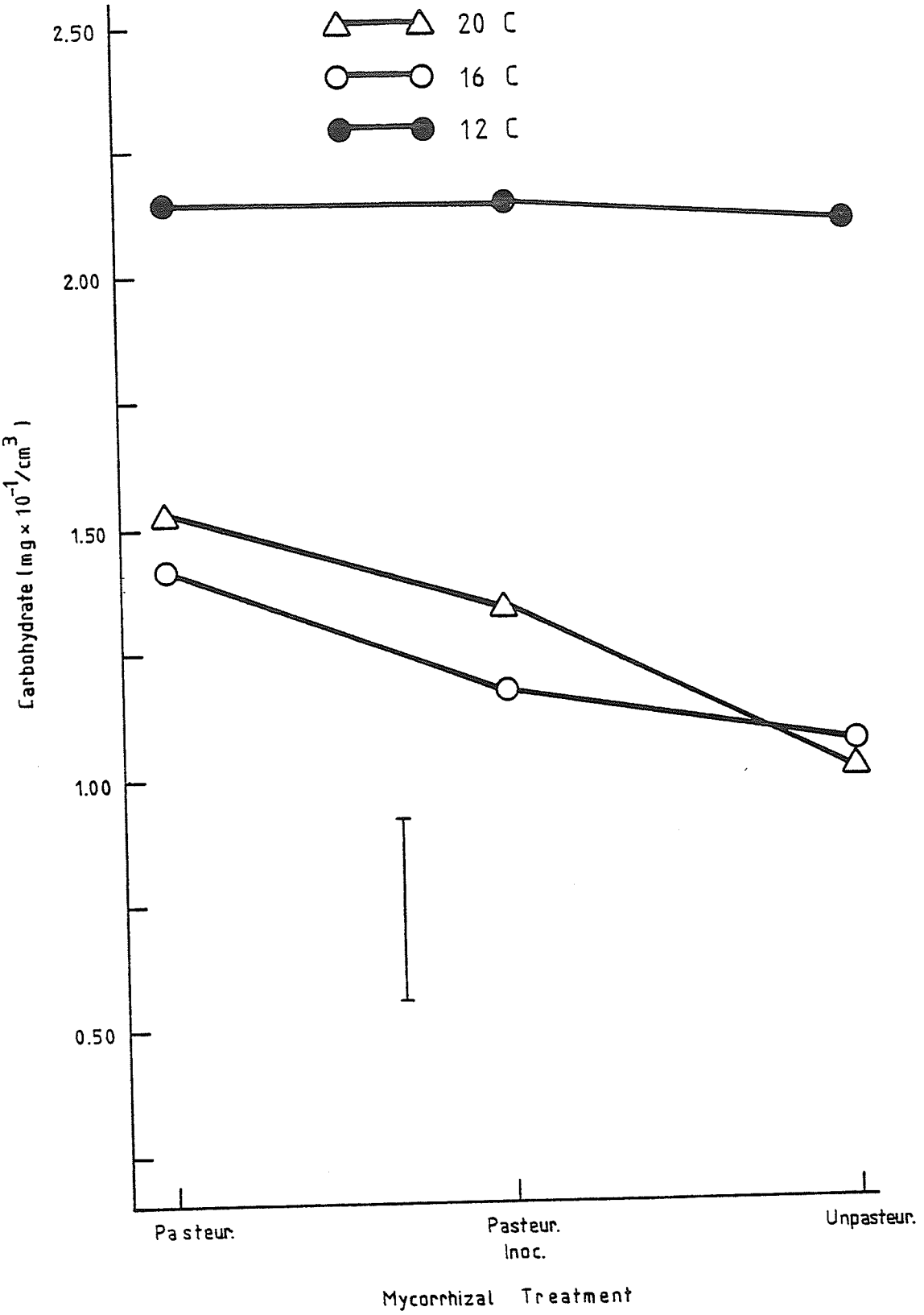


FIGURE 13. The effects of soil temperature and mycorrhizal treatment on root carbohydrate level.
⊢—⊣ LSD (P=0.05).

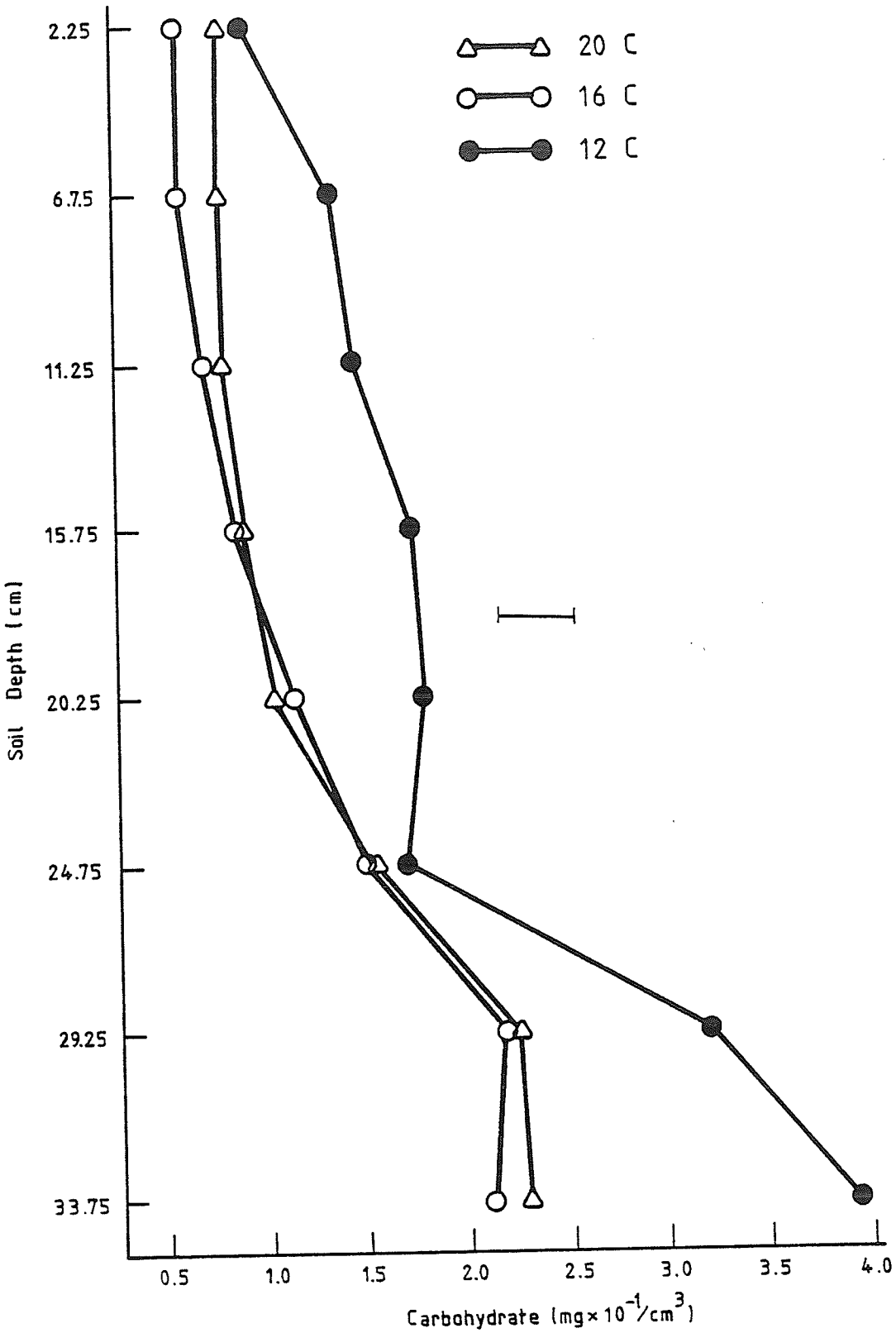


FIGURE 14. The effect of soil temperature on root carbohydrate level at eight soil depths.

⊢—⊣ LSD (P=0.05).

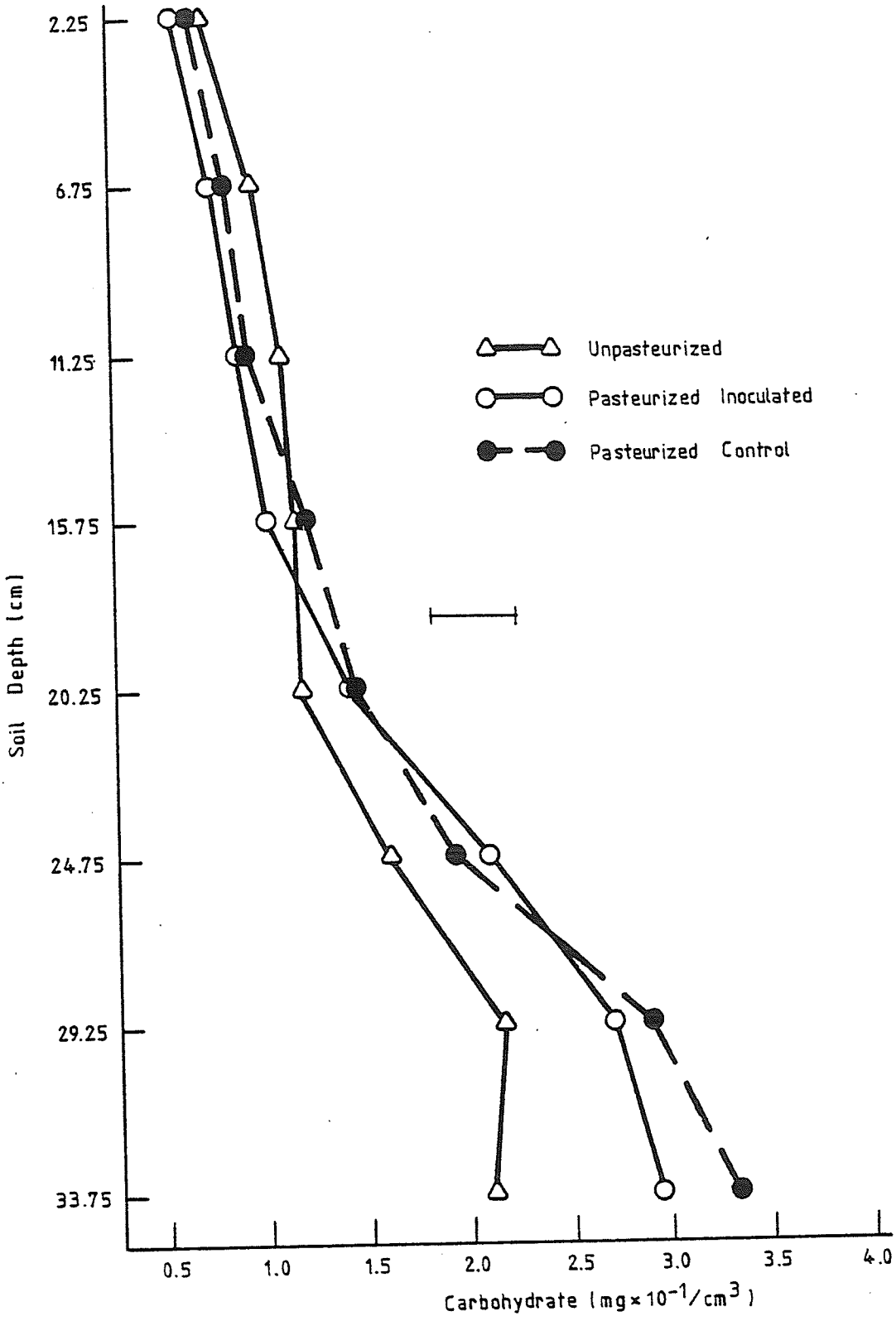


FIGURE 15. The effect of mycorrhizal treatment on root carbohydrate level at eight soil depths.

⊢—⊣ LSD (P=0.05).

For all three mycorrhizal treatments the level of conductivity in the root samples was generally the highest at 12 C. Sixteen and 20 C soil temperatures did not differ in their influence on the conductivity levels in the roots of the PC plants. The 12 C roots of the PC plants had higher conductivity values than the 16 and 20 C PC plant roots. Differences between the 12 and 16 C soil temperatures with respect to conductivity values were more noticeable in the mycorrhizal plants than in the controls. The 16 C PI plant roots had higher conductivity values than the roots of the PI plants at 20 C. The conductivity values of the UP roots grown at 20 C were similar to or lower than those of the UP roots grown at 16 C.

Conductivity values generally increased with increasing soil depth at all three soil temperatures and mycorrhizal treatments. The 16 C roots of the PI plants had conductivity values very similar to those of the 12 C PI roots in the upper half of the root system, but became increasingly similar to the 20 C PI roots in the lower half of the root system. A very prominent increase in conductivity was observed in the PI roots at 16 C approximately 7 cm below the soil surface. This corresponded to a region of high mycorrhizal activity (Figure 7). No such abrupt conductivity increases were noted in the 12 or 20 C PI roots or in the 12, 16 or 20 C UP plant roots. However, small increases in conductivity were noted in the 12 and 20 C UP roots which corresponded to regions having the highest percentage of mycorrhizal infection (Figure 7).

At 12 C the UP roots had higher conductivity values than PI plant roots grown in pasteurized soil, which in turn, were greater than those of the PC roots. At 16 C the mycorrhizal plants differed very little from each other and generally had higher conductivity values than the nonmycorrhizal plants. At 20 C conductivity values of all three mycorrhizal treatments differed only slightly from each other. The pasteurized soil treatments were quite similar

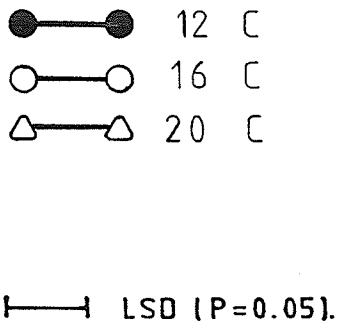
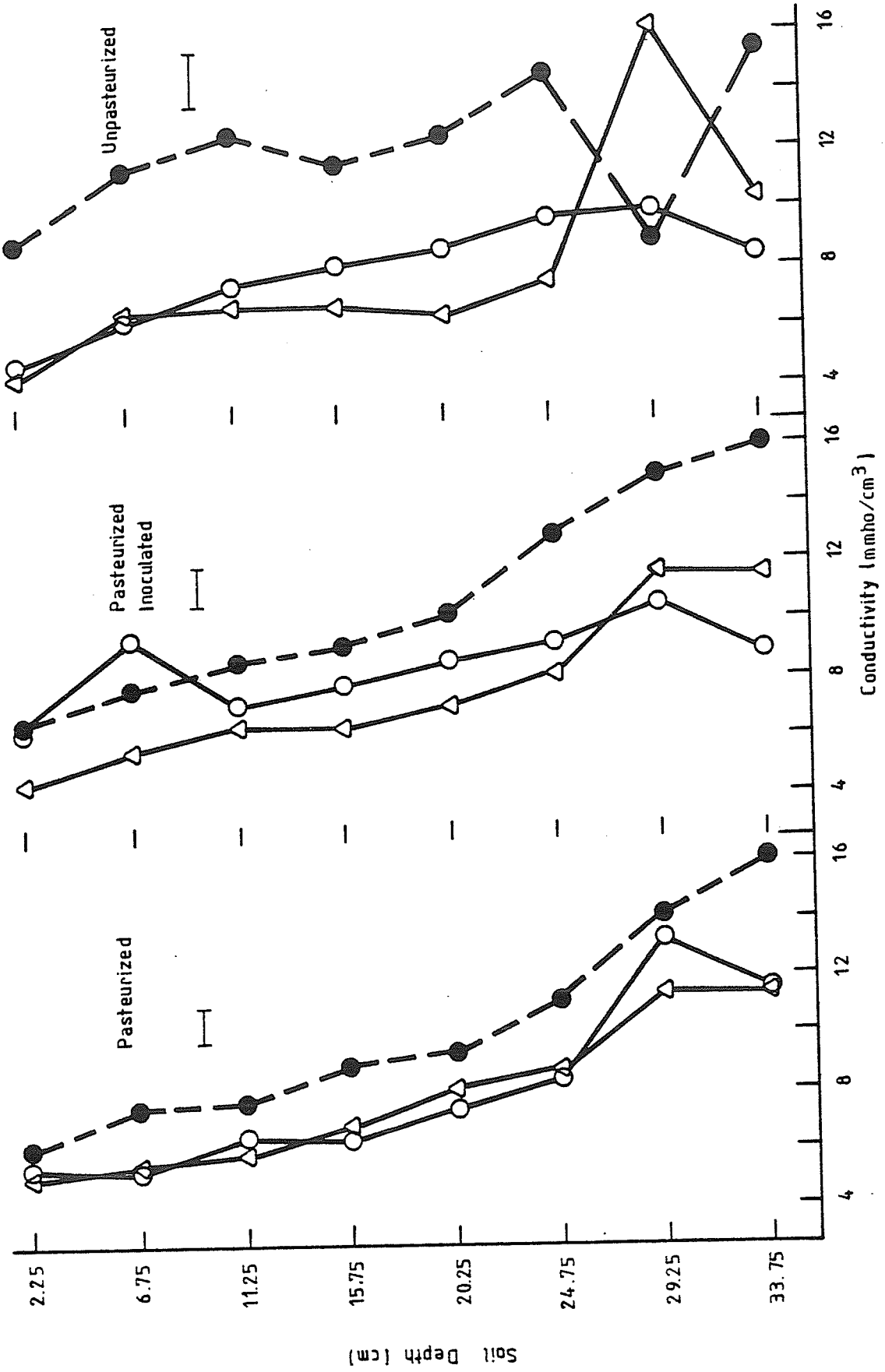


FIGURE 16. The Effect of Soil Temperature on the Level of Conductivity in Mycorrhizal and Nonmycorrhizal Barley Roots at Eight Soil Depths.


Unpasteurized
Pasteurized
Inoculated
Pasteurized
Conductivity (mmho/cm³)
Soil Depth (cm)
2.25
6.75
11.25
15.75
20.25
24.75
29.25
33.75
4
8
12
16

while in root zones where intense infection was observed the UP roots had slightly higher conductivity values than those grown in pasteurized soil. There were sharp differences in conductivity values in the 12 and 20 C UP roots near the tip of the root system.

Amino Acids

The following discussion on the levels of free amino acids in mycorrhizal and nonmycorrhizal barley at three soil temperatures is based on the three graphs shown in Figure 17.

Amino acid values of the PC plant roots grown at 12 C were usually higher than those at 16 and 20 C. The 20 C PC roots had higher values than the 16 C PC roots. This trend was not as distinct in the case of the PI plant roots. Less difference in the values of amino acids was observed over the three soil temperatures for these roots, although again the 12 C plants most often had higher values than the 20 C roots. A reversal in this temperature relationship was observed for the UP plant roots. The amino acid content of these roots was highest at 20 C and lowest at 12 C at most regions along the root.

Amino acid levels at 12 C were least in the UP plants, while the plants raised in pasteurized soil differed very little from each other with respect to amino acid values. At 16 and 20 C mycorrhizal treatments had very little effect on the level of amino acids. At 20 C UP plant roots often had higher amino acid levels than the PI roots.

Amino acid values increased with increasing soil depth. However, larger fluctuations were noted in these values over the length of the root. These fluctuations appeared to vary with soil temperature and mycorrhizal treatment. At 16 C the level of amino acids declined significantly just below the soil surface in the PI roots corresponding to an increase in the level of mycorrhizal infection in this region. A slight increase in the amino acid level was observed in the same region at 16 C in the PC roots. At 20 C the amino acid

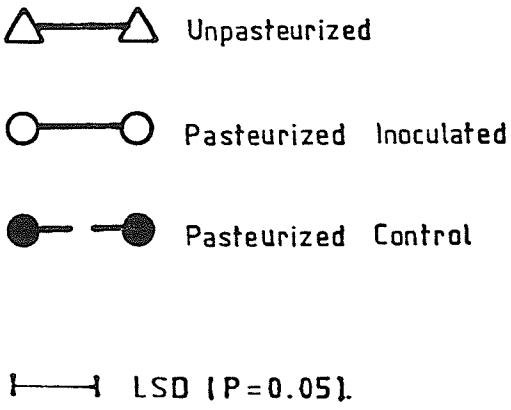
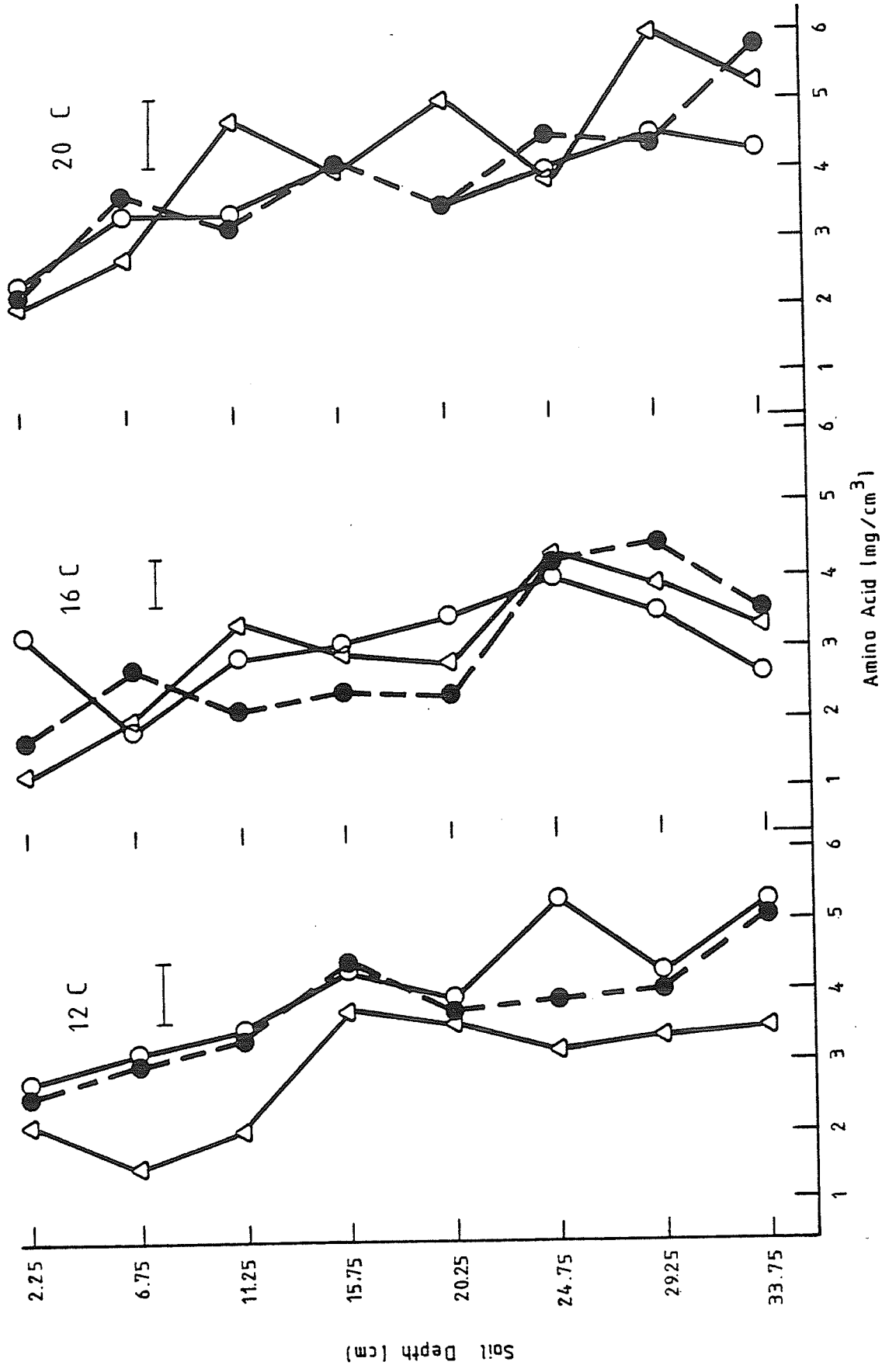


FIGURE 17. The Effect of Mycorrhizal Treatment on the Amino Acid Level in Barley Roots at Eight Soil Depths and Three Soil Temperatures.

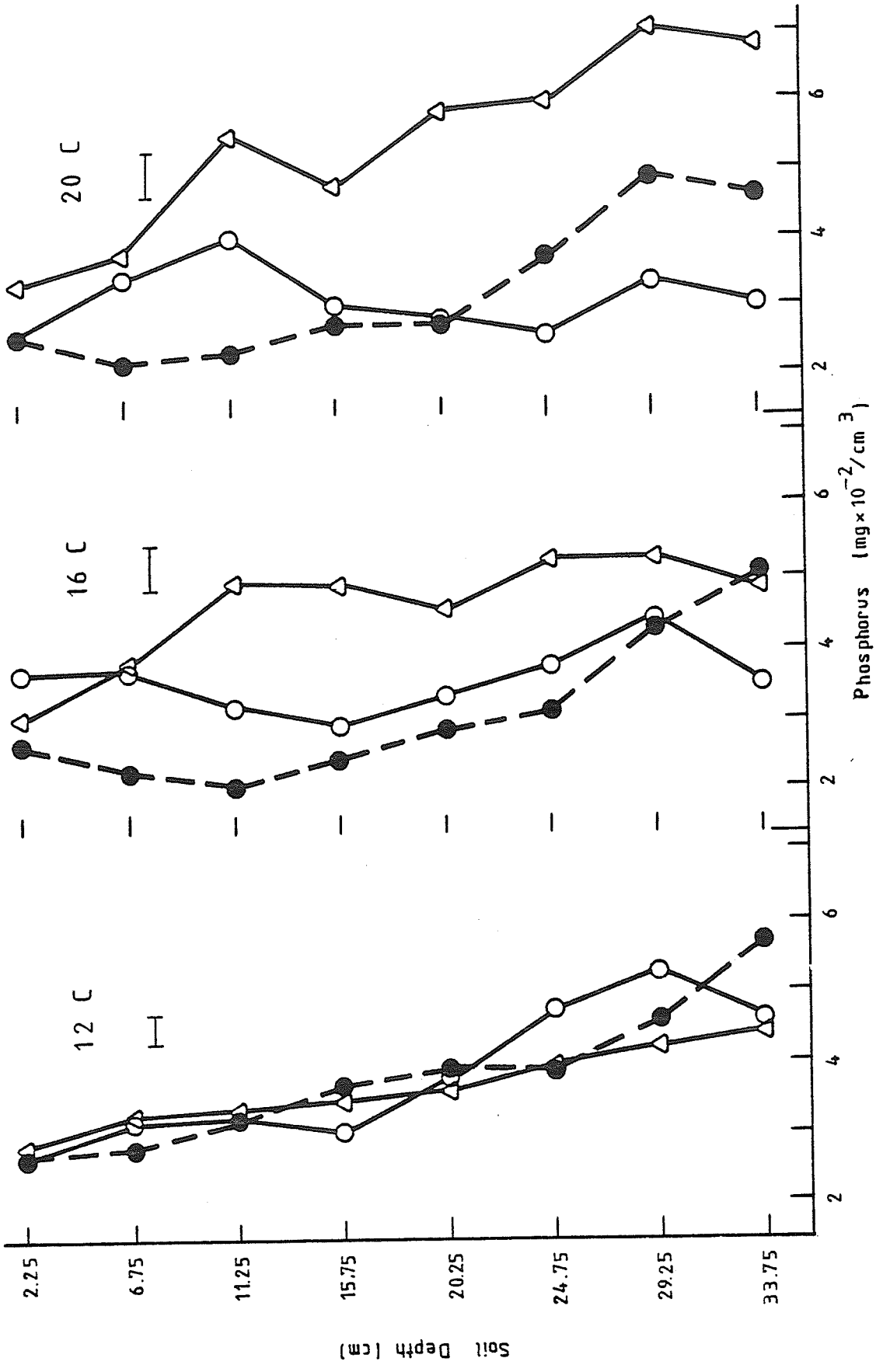

12 C
16 C
20 C
Amino Acid (mg/cm3)
Soil Depth (cm)
2.25
6.75
11.25
15.75
20.25
24.75
29.25
33.75

content of the UP roots increased in the region of the root where mycorrhizal infection was highest. At 12 C, the level of amino acids in the UP plant roots declined in the root region where mycorrhizal infection was greatest and increased at 16 and 20 C corresponding to a higher level of mycorrhizal infection. Such striking temperature effects on the level of amino acid were not noted for the control plants, although large fluctuations apparently unrelated to mycorrhizal infection were observed for those plants at the various soil depths.

Phosphorus

Figure 18 shows the effects of soil temperature and mycorrhizal treatment on the level of phosphorus in barley roots at different soil depths. At 16 and 20 C PC roots differed little from each other in phosphorus content and had lower levels of phosphorus than the roots with same mycorrhizal treatment grown at 12 C. Mycorrhizal plants had large differences in their root phosphorus contents at the three soil temperatures. These differences varied with soil depth, and mycorrhizal treatment. In the lower half of the root system the 16 and 20 C PI roots had the lowest phosphorus values. The level of phosphorus in the same plants was highest at 16 and 20 C in the upper half of the root system. This increase corresponded to an increase in the intensity of mycorrhizal infection in this region (Figure 7). Roots of UP plants contained more phosphorus at the higher soil temperatures. This was most noticeable at the greater soil depths.

Phosphorus levels at the 12 C soil temperature were not influenced by mycorrhizal treatment. This observation was in common with that for carbohydrate levels at 12 C. Soil temperatures of 16 and 20 C resulted in large differences in the phosphorus content between the three mycorrhizal treatments. The roots of UP plants had higher phosphorus levels at both 16 and 20 C than roots grown in pasteurized soil. At 16 C phosphorus values of the PC roots were significantly


12 C
16 C
20 C
Soil Depth (cm)
2.25
6.75
11.25
15.75
20.25
24.75
29.25
33.75
2
4
6
Phosphorus (mg × 10⁻²/cm³)

lower than those of the PI and UP roots at all but the bottom root segment. PI roots occupied a position between the other two mycorrhizal treatments with respect to phosphorus levels at 20 C. In the region where mycorrhizal infection occurred, the PI plant roots had higher phosphorus contents than the nonmycorrhizal plants, but this relationship was reversed in regions of the root where infection did not occur.

Discussion

The two methods of mycorrhizal inoculation that were used in this study permitted a comparison to be made of the effects of localized and widespread infection on the level of the metabolic indicators at the various soil depths. The widely reported improved phosphorous status of mycorrhizal plants may act as a metabolic stimulus that maintains a normal level of metabolism, thereby delaying the onset of cortical cell deterioration. If this was the case in the present study, an enhanced level of the metabolic indicators should have been observed in the regions of the root where mycorrhizal infection occurred.

Results in this study showed that significant increases in the level of phosphorus occurred in the region of the root where localized inoculation was performed (Figure 18). In addition, for plants grown in soil in which mycorrhizal inoculum was widespread, levels of phosphorus were highest where the intensity of mycorrhizal infection was highest (Figure 7). Soil temperature influenced this trend. At 12 C, the presence of mycorrhizal infection had no influence on the level of phosphorus in the roots, whereas at 16 and 20 C, the effect of infection was as described above. This may have been because the level of activity of the mycorrhizal fungus increased in response to warmer soil temperatures. This argument conflicted with the finding that the percentage length of root infected by mycorrhizae at 12 C in the root zone approximately 0 to 12 cm below the soil surface was greater than that at 20 C for the PI plant roots (Figure 7). This indicated that the low soil temperature did not

limit the rate of spread of the fungus. An important point to consider however is that a longer time was required for the 12 C plants to attain the same stage of maturity as the 20 C plants. An average of 66 days was required for the 12 C plants to reach the same stage that the 20 C plants attained after an average of 57 days (Untabulated data).

The longer growth period of the 12 C plants may have enabled the fungus to ramify further through the root system. Yet, the slower growth rate of the fungus at cool soil temperatures reported by Furlan and Fortin (1973) made it unlikely that the level of infection would have been comparable to that of the 20 C plants.

Although the cool temperature plants had a comparable infection density to the 20 C plants, their slower metabolic rate at the reduced soil temperature may have resulted in a slower influx of phosphorus per unit of mycelial surface area compared to that of the 20 C plants. Consequently, a greater amount of phosphorous may have accummulated in the 20 C plant roots.

Apart from its effects on the rate of development, soil temperature may have also influenced the level of phosphorus, as well as the levels of the metabolic indicators through its effect on the metabolic activity of the indigenous nonmycorrhizal rhizosphere microflora, the rate of cell metabolite translocation, and the amount and composition of root exudates.

Although no measurements were taken to determine differences in the size of the rhizosphere microbial population in the soils given the various mycorrhizal and temperature treatments there may have been significant differences in the number and composition of microorganisms between treatments. Because the inoculated pasteurized soil was exposed to contamination by airborne microorganisms before and after planting, this soil was not devoid of microbial activity. A large number of soil inhabiting microorganisms would have been expected in the pasteurized soil that had been locally inoculated

with unpasteurized soil. The largest and probably most diverse population of microbial soil colonizers was likely in the unpasteurized soil.

The destructive influence of the rhizosphere microflora on root cortical cells reported by Martin (1977) may have been the greatest in this study on the UP plant roots. The stimulative effect of warmer soil temperatures on the level of metabolic activity of most soil inhabiting microorganisms (Garrett, 1960) may have caused an even greater rate of root degradation in this study.

Warm soil temperatures have been shown to increase the quantity of exudate released per unit weight of root (Rovira, 1959). This would be expected to increase the microbial population as well as possibly alter its composition.

On the basis of these findings, in terms of the microbial influence of cortical cell decay, the least effects would likely have been observed on the roots of PC plants grown at 12 C.

Several investigators have reported a significant accummulation of nutrients in plant roots at soil temperatures between 5 and 12 C. (Nielsen et al.,1960; Read and Ashford, 1968). Power et al.(1970) concluded from their work that the uptake of nutrients at 9 C did not restrict shoot growth but that translocation from the roots to the tops was impeded. Rovira and Bowen (1973) noting a similar increase in the level of assimilates in the roots of plants grown at low soil temperatures, suggested that the increase was due to a reduced rate of translocation of metabolites to the root tip, resulting in an accumulation of assimilate in the older region of the root. This may have been an important factor influencing the level of metabolites at the cool soil temperatures in this study.

Soil moisture content may have had an influence on the level of activity of soil microorganisms, thereby influencing the rate of cortical cell decay in the roots. As discussed in the first section, warm soil conditions as well as mycorrhizal infection had a very notable depressing effect on the level of

soil moisture in the plant containers in this study. Vancura (1967) showed that alternate drying and wetting of the soil increased the rate of root exudation. It is possible that a similar drying and wetting cycle occurred in this study, particularly in the warm soil in which mycorrhizal roots were grown. This would have resulted in an improved nutrient supply for the soil microflora, with possible harmful consequences to the root.

The reduced soil moisture conditions may have reduced the mobility of slowly diffusible ions in this soil solution (Nye, 1966), resulting in not only a moisture deficit but also a nutrient deficit. Barber (1974), from the results of his study, claimed that the greatest competitive effect of the root microflora was exerted in conditions of limiting nutrient availability. Therefore at the warmer soil conditions, and also in soil containing mycorrhizal roots, the low soil moisture conditions may have made the plant roots more nutrient deficient both as a consequence of reduced ion mobility, and increased microbial competition for plant nutrients in the soil. The poorer nutrient status in these roots may have caused a reduction in the level of plant metabolites as well as a greater susceptibility to attack by degradative soil microorganisms.

The interactions of soil temperature and soil moisture with plant and microbial metabolism listed above may have accounted for the differences that were observed in the levels of phosphorus; as well as in the metabolic indicators in the plant roots studied. The higher phosphorus; content of the PC roots grown at 12 C compared to those grown at 16 and 20 C may have been due to a reduction in destructive microbial influence at 12 C, as well as to a reduction in the rate of export of phosphorus; from the root at the lower soil temperature. As already mentioned the increase in metabolic activity of the mycorrhizal fungus at the warm soil temperatures may have been the cause of the higher phosphorus; levels in the mycorrhizal plants.

Because the highest phosphorus levels were observed in mycorrhizal plants at the warmest soil temperatures a corresponding increase in the level of the other metabolic indicators was expected in these plants at the warmest soil temperature if the enhanced phosphorus status delayed the onset of cortical cell deterioration. The amino acid content of the UP plant roots tended to increase with soil temperature, while conductivity and carbohydrate values in the roots of these plants declined with increasing soil temperature, indicating that factors besides mycorrhizal infection influenced the level of these metabolic indicators. However, at any given soil temperature the mycorrhizal plants tended to have higher conductivity values than that of the nonmycorrhizal plants. The reduction in conductivity values with increasing soil temperature indicated that microbial activity at the warmer soil temperatures may have led to an increase in the rate of cortical cell decay. The low level of microbial activity in the mycorrhizal plants at the lower soil temperature as well as the improved soil moisture status and nutrient availability may have reduced the rate of cortical cell degradation. Since the level of microbial activity at any given soil temperature would likely have been similar, differences in conductivities at any one soil temperature would have been related to the level of mycorrhizal infection in the pots, and to the size and makeup of the microbial population characteristic to that mycorrhizal treatment. The fact that mycorrhizal plant roots generally had higher conductivity values at each soil temperature compared to the nonmycorrhizal plant roots even at the higher soil temperatures, indicated that mycorrhizal infection may have enhanced the level of conductivity in the roots.

The lower carbohydrate values of the mycorrhizal plants at each of the soil temperatures indicated that the improved phosphorus status of the mycorrhizal plants had no enhancing effect on carbohydrate levels. One possible explanation is that the mycorrhizal fungus used the plant derived carbon compounds as an

energy source and as structural components. In this way, the carbohydrates may have been subsequently released as CO_2 through respiration or converted into carbon containing structures not detectable by the analytical methods used in this study.

Alternatively nonmycorrhizal plants may have had higher carbohydrate values than mycorrhizal plants because of the lower level of microbial activity in the soil in which the nonmycorrhizal plants grew, resulting in less physical damage to their roots. The improved water status of these plants as well as the reduced microbial completion for nutrients may have also been a factor.

Higher carbohydrate values at the lower soil temperatures may have been due to low soil temperatures favoring the accumulation of carbohydrates. Alternatively the reduced soil temperature may have reduced the activity of the mycorrhizal fungus, lessening the sink effect created by the fungus through its requirement for carbohydrates.

Since an increase in phosphorus values in the upper half of the root generally corresponded to an increase in mycorrhizal infection intensity, a similar increase in the level of the metabolic indicators was expected in these regions. Amino acid and carbohydrate values of the mycorrhizal plants did not show any consistent positive response to the presence of mycorrhizal infection in this region of the root system. For example the PI plant roots grown at 16 C showed a decrease in the amino acid content in the same region of the root system where mycorrhizal infection was highest and in the same root zone where the level of amino acids increased in the nonmycorrhizal plant roots.

The increased level of conductivity in the PI plant roots grown at 16 C approximately 7 cm below the soil surface corresponded to an increased level of mycorrhizal infection in this region (Figure 16). However, it would be incorrect to ascribe this increased conductivity to an increase in cortical

cell viability due to the presence of mycorrhizal infection since, for the same mycorrhizal treatment, plants grown at 20 C showed no change in conductivity in this region of the root system, despite the fact that in the same root zone mycorrhizal infection was highest. The warmer soil temperature may have influenced the rate of microbially induced cortical decay. No change was observed in the level of conductivity of the UP plant roots grown at 16 and 20 C in the region where mycorrhizal infection was intense and where phosphorus levels were highest. At 12 C, conductivity values rose significantly in the same region where phosphorus levels showed the least change. There was therefore very little evidence to suggest from these results that a mycorrhizally induced enhancement of the phosphorus content in the root resulted in an increased root cortical cell viability.

In both PC and PI plant roots the level of carbohydrates, amino acids and conductivity was highest at the coolest soil temperature except where mycorrhizal infection was observed. In the latter case, the conductivity and amino acid values were highest at 16 and 20 C respectively in the region of the root where infection occurred. The higher levels of the metabolic indicators at the lower temperatures may have been because of a reduced translocation rate of plant metabolites out of the root at the lower soil temperatures, and because of the lower level of microbial activity at 12 C, which may have resulted in less cortical cell destruction.

An alternative explanation for the temperature effects on the level of the metabolic indicators relates to the influence of soil temperatures on root diameter that was reported in the first section. In that section it was noted that the root diamter of plants grown in cool soil was always greater than that of plants grown at 16 and 20 C. A consequence of this may have been that the larger diameter roots may have been composed of cells that on average exceeded in size those from smaller diameter roots. If this was the case, the larger

roots would have had a larger cytoplasmic volume to cell wall surface area ratio than that of the smaller roots. A consequence of this would have been that for a root sample having the same calculated total cylindrical volume, the sample with a higher proportion of large diameter roots would have contained a greater amount of cytoplasm. This would have been reflected in higher levels of each of the metabolic indicators.

If this explanation was correct, warm soil treatments would have resulted in plant roots with lower metabolic indicator values. This conformed to the results in this study. Since the previous section also showed that mycorrhizal roots had significantly finer roots than nonmycorrhizal plants (Figure 8), plant roots grown in mycorrhizal soil should have had lower levels of the metabolic indicators at each of the soil temperatures than nonmycorrhizal roots. The results showed that this was in fact the case for carbohydrates at the 16 and 20 C soil temperatures, and for amino acids at 12 C. The fact that the level of the other metabolic indicators was as high or higher in the mycorrhizal plant roots compared to the nonmycorrhizal roots indicated that real increases in the levels of the indicators did occur in roots infected with mycorrhizae. When the results are examined in this perspective, the absence of differences in amino acids and values between the mycorrhizal and nonmycorrhizal roots may be significant. In addition the higher conductivity values of the mycorrhizal plants especially at 12 and 16 C become quite important.

The general rise in the level of the metabolic indicators with increasing soil depth noted in this study in the mycorrhizal and nonmycorrhizal roots at all three soil temperatures indicated a higher level of metabolic activity in the roots in the region near the top of the root system. Previous findings in this study pointed to a greater length of finer and presumably younger roots near the soil surface (Figure 10). On the basis of these findings, there should

have been a higher level of root metabolites in the upper half of the root system. The apparent contradiction may be explained again on the basis of the larger cytoplasmic volume to cell wall surface area ratio for the roots with larger diameters. That this explanation suitably fits the results can be shown by comparing the level of each of the metabolic indicators, including phosphorus, near the tip of the root system. Roots grown in warm soil as well as those that were mycorrhizal had lower indicator values in the root tip region than the cool soil temperature and nonmycorrhizal plants, despite what influences mycorrhizal infection had on these values in the upper region of the root. The only notable exception was for the phosphorus levels in the UP plant roots grown at 20 C (Figure 18). This may have been the result of an overall improvement in the phosphorus level of these plants.

The high degree of variability in the conductivity values in the roots of the UP plants at 12 and 20 C was apparently unrelated to mycorrhizal effects. Relatively large changes in the level of animo acids also observed along the root system indicated that more than the presence or absence of mycorrhizal infection influenced the level of this metabolic indicator. The fact that root properties unrelated to the influence mycorrhizal infection would induce such significant changes in the level of these metabolic indicators placed some doubt on the validity of ascribing the differences in the level of the metabolic indicators to the effects of mycorrhizal infection.

SECTION III

Root Lignification

Results

Table 14 shows a summary of an analysis of variance in the percentage of the total root length that was lignified. The main effects, and the two and three way interactions between soil temperature, mycorrhizal treatment and soil depth showed significance. Mycorrhizal treatment as well as the interaction between soil temperature and mycorrhizal treatment had very high F values. A detailed analysis of variance in root lignification is shown in Table 41, in the Appendix.

Table 14. Summary of Analysis of Variance in Percentage of Length Lignified on Mycorrhizal and Nonmycorrhizal Barley Plants Grown at Three Soil Temperatures †

Source of Variation	F Value
Soil Temperature	32.04**
Soil Depth	6.64**
Mycorrhizal Treatment	329.59*
T X SD	2.67*
T X MT	84.04*
SD X MT	3.38**
T X SD X MT	3.94**

† Analysis of Variance was conducted on transformed data.
* Significant at P = 0.05.
** Significant at P = 0.01.

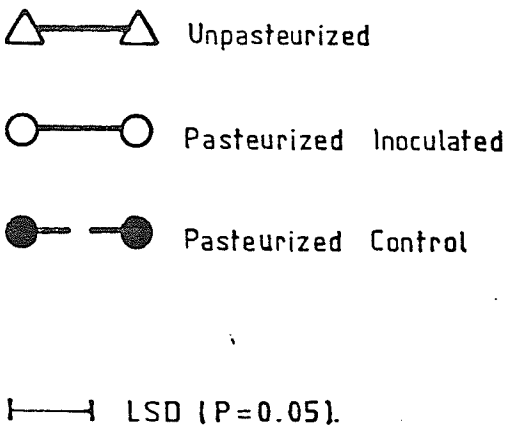
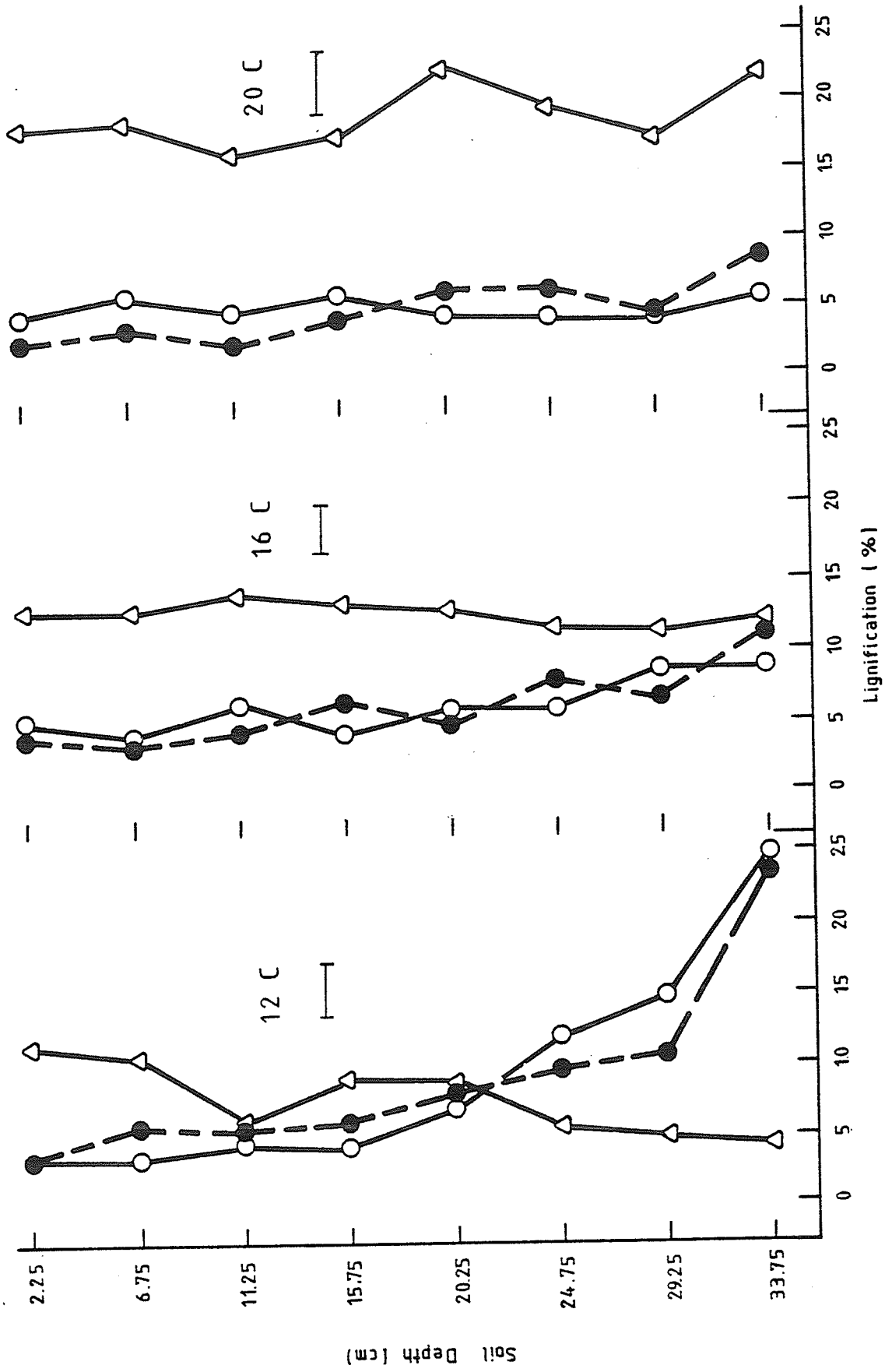


FIGURE 19. The Effect of Mycorrhizal Treatment on the Percent Lignification on Barley Roots at Eight Soil Depths and Three Soil Temperatures.


Soil Depth (cm)
2.25
6.75
11.25
15.75
20.25
24.75
29.25
33.75
0
5
10
15
20
25
12 C
16 C
20 C
Lignification (%)

Discussion will focus primarily on Figure 19 which shows the influence of soil temperature and mycorrhizal treatment on the percentage of barley roots having extensively lignified cortical cell walls. Table 42 in the Appendix shows the total length of lignified mycorrhizal and nonmycorrhizal barley roots grown at three soil temperatures.

The results show that no more than approximately 20% of the total root length had visually detectable lignification. UP plant roots had the largest percentage of lignified roots. Lignification was most extensive in these plants at 20 C and declined with decreasing soil temperature, so that at 12 C the percentage of root length that was lignified was only slightly greater than in the roots of the PC and PI plants. The extent of lignification was very similar between the PC and PI roots. Increasing the soil temperature had a small effect on the percentage of the total root length that was lignified in the roots of the PC and PI plants. This was only noticeable at the greater soil depths in the 12 C plants which showed increasing lignification near the tip of the root system. This did not occur in the PC and PI roots at the warmer soil temperatures. The UP plants grown at 12 C tended to have decreasing lignification with increasing soil depth. At 16 and 20 C the PC, PI and UP roots changed very little in the percentage of their total root lengths that were lignified, with soil depth. Although variation with soil depth in the percentage of lignification that occurred in the UP roots was significant, no interpretable trends were evident.

Discussion

The amount of lignification that occurred in the roots seemed to be less related to the presence of mycorrhizal infection than to the level of activity of the microbial population. There was no trend toward an increase in root lignification in the root zones where mycorrhizal infection was most dense.

A clear differentiation was evident between the level of lignification in the roots grown in pasteurized and unpasteurized soil since mycorrhizal infection seemed to have had no influence on the extent of lignification, the only other factor distinguishing the two sets of soils was the difference in the level of microbial colonization. Presumably the larger and more diverse rhizosphere microbial population present in the unpasteurized soil stimulated the plant roots to become more lignified. The warm soil temperatures may have increased the level of metabolic activity of the microorganisms inhabiting the rhizosphere, thereby further stimulating the microbially induced increase in root lignification.

The increased level of lignification observed in the UP roots may have been in response to the drier soil conditions that were noted for plants grown in this soil. Warmer soil temperatures were also found to decrease the moisture status of these soils. However there was evidence that seemed to argue against the theory that reduced soil moisture levels induced enhanced levels of lignification. Firstly, there was very little variability in the percentage of the total root length that was lignified in the UP roots grown at 16 and 20 C, even though there was a considerable reduction in the moisture level with increased soil depth in these soils (Table 10). Secondly, although there was generally an increasing water deficit in the soil with increasing soil temperature, there was very little difference in the extent of lignification between the two pasteurized soil treatments.

The dramatic rise in the percentage of lignification that occurred in the 12 C PC roots could be explained by neither an increase in microbial activity nor a decrease in the soil moisture content. An explanation that may account for this anomolous behaviour relates to the relative root lengths of the three root diameters that were measured in this study. Table 43 in the Appendix shows this data. The length of the 0.2 mm diameter roots decreased rapidly

from 540.9 cm near the root base down to 156.2 cm near the root tip, whereas the length of 0.4 and 0.6 mm diameter roots remained nearly constant or increased, respectively, with increasing soil depth. In the case of the 12 C nonmycorrhizal roots, even though the total length of lignified root remained virtually the same (Table 40, Appendix), the large reduction in total root length resulted in the percentage length of lignified root increasing. Because the lack of appreciable change in the extent of lignification corresponded to a similar lack of change in the root length of the thicker diameter roots, it was tempting to suggest that the bulk of the lignification observed in this experiment occurred in the thicker roots. Although this seemed to be the case at 12 C, the results obtained for the 16 and 20 C UP roots conflicted with this hypothesis. Even though the length of lignified roots was largest for these plants (Table 40, Appendix), the total length of thick roots decreased, and the total length of fine roots on these plants increased relative to the 12 C plant roots. This indicated that a large number of the fine roots had become lignified.

GENERAL DISCUSSION

The higher percentage of root length infected by mycorrhiza at the shallow soil depths may have been due to a larger source of potentially infectable roots in this region compared to the regions further from the soil surface. Results showed that mycorrhizal infection was confined predominantly to roots less than 0.4 mm in diameter (Figure 8). Measurements of the distribution of each of the root diameters selected in this study showed that mycorrhizal and nonmycorrhizal plants grown in pasteurized soil had a higher percentage of fine roots in the upper half of the root system compared to the lower half at the cooler soil temperatures. As soil temperatures increased the percentage of roots in the lower half of the plant containers increased. The percentage of fine roots in the UP plants did not fluctuate over the length of the root system. The UP roots generally had a greater percentage of fine roots at all soil depths and at all three soil temperatures than the roots of the PC and PI plants.

The differences in the effects of soil temperature and mycorrhizal treatment on the extent of the soil water deficits noted in the pots may have reflected the differences in growth rate of the plants in response to the treatments. Through their depressing effect on the soil moisture content at the greater soil depths, warm soil temperatures as well as mycorrhizal infection may have caused an increase in the proliferation of fine roots in the deeper regions of the soil. Bowen and Rovira (1970) found that although the length of the main axis of the primary seminal roots of barley was reduced at a lower soil water content (10%) the number and total length of lateral roots increased resulting in a greater total root length per plant.

Apart from this direct influence of soil moisture on root development the apparent increase in the rate of root proliferation may have been also caused by a reduction in nutrient availability. The importance of an adequate soil moisture supply in relation to the transmission of ions by mass flow and diffusion to the plant root has been discussed in detail by a number of investigators (Lewis and Quirk, 1967; Olsen and Kemper, 1968; Barber, 1970). The absorption of water by plant roots causes a flow of water toward the root which results in the transport of nutrients by mass flow. Mathematical relations describing mass flow to the root as a cylindrical sink have been developed by several workers (Passioùirra, 1963; Nye, 1966). Diffusion of ions to the root occurs when ions at the root surface are absorbed faster than they are carried to the root surface. The resultant lowering in the ion concentration at the root surface creates a concentration gradient between the root surface and the soil, bringing about a diffusion process. The rate of diffusion is expressed by the equation

$$F = -D\ A\ (dc/dx) \qquad \text{(Jacobs, 1967)}$$

where F, the rate of diffusion depends upon D, the diffusion coefficient for a particular ion, and the area of diffusion, and dc/dx, the concentration gradient. In practice diffusion is much more important than mass flow in terms of the relative amount of ions contributed by each process to the total nutrient supply of the plant (Domergues, 1978). It has been observed that as soil moisture content is reduced the diffusion coefficient of an ion decreases due to an increase in tortuosity of the diffusion path of the ion to the root and to a decrease in the cross sectional area of the pathway for diffusion (Rowell et al.,1967). For ions such as potassium and phosphate for which their supply to the plant root primarily depends on diffusion, reduced soil moisture conditions have been found to impair their availability to the plant (Olsen and Watanabe, 1968).

The results obtained in the present study indicated that water depletion may have occurred in the case of mycorrhizal plants and for plants grown at warmer soil temperatures, particularly in the lower regions of the plant containers. However the increase in the proportion of finer roots in this region was an apparent contradiction to what would have been expected if moisture depletion had had a significant effect on nutrient availability.

A number of studies have shown that a localized supply of nutrients promoted lateral root growth in the zone of nutrient application in solution, sand, and soil cultures (Drew et al., 1975, 1978; Strong and Soper, 1974). The evidence of these findings carries with it the implication that in zones of nutrient depletion, root proliferation would be reduced.

The observed increase in root proliferation in the lower region of the plant containers, in contrast to what might have been expected had the reduction in moisture content in this region significantly affected the supply of nutrients to the plant, indicated nutrient limitation was not the predominant factor influencing root development. The results therefore lent strong support to the hypothesis that the increase in the proportion of finer roots was in direct response to a shortage of soil moisture. The increased root surface area exposed to the soil would have improved the ability of the root to extract the limited amount of water still remaining in the soil.

Nye (1966) showed using mathematical models, that the rate of ion absorption per unit surface area of root increases as root radius decreases. It has been demonstrated that lateral roots contribute considerably to the total nutrient uptake of plants. Bowen and Rovira (1970) labelled wheat roots with radioactive phosphate and sulfate, and found that the laterals of seminal roots of wheat grown in soil for 14 days accounted for

over 70% of the uptake of sulfate and phosphorus. It is likely therefore that increased lateral root production witnessed in the present study not only improved the plants water status, but may have also partly compensated for the reduction in nutrient absorption due to decreased ion mobility in the soil.

Both increased soil temperature and extensive mycorrhizal infection promoted an increase in the rate of water uptake by barley plants in this study. It was suggested earlier in the discussion that mycorrhizal infection was more extensive in root zones where new roots, characterized by a narrow root diameter, were found. In addition the warm soil temperature was observed to increase the total root length at a given root position, likely by stimulating production of finer roots. The evidence therefore indicated that the rise in the rate of water absorption noted for the warm temperature and the extensively infected mycorrhizal plants was the result of production of a finer root system by these plants.

Disentangling cause and effect in this study was difficult. For example, it may be argued that mycorrhizal infection stimulated the production of finer roots, thereby improving the plants' nutrient and water uptake capabilities. It was already mentioned that root proliferation has been shown to occur in regions where there are localized improvements in nutrient availability (Drew and Saker, 1975, 1978). In their studies Drew and Saker (1975,1978) found that the enhanced rates of nutrient uptake due to increased root proliferation occurred when the disparity between the enriched and unenriched roots zones in nutrient supply was very large. In the present study the reduced nutrient availability as a consequence of the conditions of moisture stress at the greater soil depths coupled with the improved nutrient status of the mycorrhizal roots may have created the conditions conducive for increased root proliferation

in the regions of the root infected with mycorrhizae. Mycorrhizal infection then may have rapidly spread intercellularly as well as at the root surface keeping pace with the increased rate of root production. The enlarged surface area resulting from the localized increase in lateral root production would have thereby led to an increase in nutrient and water absorption.

An alternative explanation is that the increased surface area provided by the network of mycorrhizal mycelium external to the root may have permitted the mycorrhizal roots to extract more water from the soil than nonmycorrhizal roots. As argued earlier the reduction in soil moisture may have induced an increased rate of production of lateral roots. Safir et al. (1973) indicated in their paper that the decrease in resistance to water absorption by mycorrhizal plants compared to that of the nonmycorrhizal controls that they observed in their study was attributable to the improved nutrient status of the mycorrhizal plants. The characteristically larger mycorrhizal plants apparently had a greater demand for water, and consequently resistance to radial water flow across the root was reduced. They detected no significant differences in root dry weights and volumes that could account for the variability in resistance to water flow. The present experiment indicated that real differences in root size existed between mycorrhizal and nonmycorrhizal plants that could have possibly accounted for the differences in soil moisture levels of the various treatments.

The results of the study on fresh and dry root weights clearly indicated that contrary to the findings of Safir et al. (1973), who worked with soybeans, dry weights of mycorrhizal barley plant roots differed significantly from that of nonmycorrhizal plant roots at all of the temperatures studies (Table 9). However, contrary to what was expected, namely an increase in root dry weight with increasing root length, root dry weight declined as root length in the

segments increased. This decline seemed to have been inversely related to the length of finer roots in the sample. This was logical in terms of the relation between the fresh/dry root weight ratio and the incidence of mycorrhizal infection since ratio values reflected the level of infection, and infection occurred mainly on fine roots.

The increase in the fresh/dry root weight ratio with increasing mycorrhizal infection that was observed indicated that not only did the mycorrhizal plant roots have a greater absorbing surface exposed to the soil, but that a greater proportion of these roots were functional compared to the nonmycorrhizal plant roots. The greater length of functional root may have been due to a larger proportion of new roots in the regions where mycorrhizal infection occurred or to a reduction in the rate of epidermal and cortical cell delay, or to a combination of both. It was not possible to differentiate the causes in this study.

The increased water demand of the mycorrhizal plants was reflected in higher fresh and dry shoot weights compared to the nonmycorrhizal plants (Table 11). The poorer shoot growth of the 20 C plants for all three mycorrhizal treatments seemed to implicate a lower level of efficiency in assimilate production and/or accumulation at 20 C. However, the finding that the largest shoot/root ratios were at the warmest soil temperature (Table 11), argued against a reduction in efficiency at warmer soil temperatures. The most plausible explanation for the reduction in shoot growth at 20 C for all three mycorrhizal treatments is that the greater water deficit at the warmer soil temperatures retarded the growth potential of the plants. Since root proliferation was generally least for the nonmycorrhizal plants their reduced ability to extract water and nutrients at 20 C was most pronounced under the conditions of low soil water availability. Similarly the improved shoot growth of the

12 and 16 C plants may have been indicative of more favorable soil water conditions.

The ability of the mycorrhizal plants to not only tolerate the stressful water deficit situation but to produce larger shoots than the nonmycorrhizal plants illustrated the benefits derived from mycorrhizal infection. An interesting point is that despite the increased root proliferation observed in the 20 C nonmycorrhizal plants, the 12 and 16 C PI plants which had substantially less root growth than the 20 C PC plants (Table 12), had larger fresh and dry shoot weights than the 20 C PC plants (Table 11). In addition the roots of the plants grown in pasteurized, inoculated soil at 16 C were capable of extracting more soil water than the nonmycorrhizal plant roots grown at 20 C despite the greater root length of the nonmycorrhizal plants (Table 6). The improved growth of both mycorrhizal and nonmycorrhizal plants at 16 C may partly explain this inconsistency. Barley plants may be physiologically adapted to 16 C soil temperature and consequently may have a lower Km with respect to water and nutrient uptake at that soil temperature compared to that at the lower and higher temperatures. This may also explain why mycorrhizal infection tended to be reduced in roots at this soil temperature. It has been established that the extent of mycorrhizal infection is regulated mainly by the phosphorous status of the plant (Sanders, 1975; Menge et al, 1978). The improved nutrient status of the 16 C plants may have retarded the rate of spread of the fungus in these plants.

However the improved shoot growth of the 12 C PI plants over that of the 20 C PC plants (Table 11) indicated that the beneficial effect of the mycorrhizal association may have been not only on an increased rate of root proliferation, but also on an improved nutrient uptake efficiency. This may have been due to the greater proportion of functional roots as indicated by

the higher fresh/dry root weight ratio of the mycorrhizal roots (Table 9.C.). The heightened microbial activity in the rhizosphere at the warmer soil temperatures may have resulted in a greater rate of cortical cell collapse (Martin, 1977). This would have seriously impaired the nutrient uptake ability of the plant. The absence of any improvement in the uptake efficiency of the nonmycorrhizal plant roots with increasing soil temperature, even though the proportion of fine roots increased on these plants at the warmer soil temperature, may have reflected an increase in the rate of cortical cell collapse at the warmer soil temperature. It may have also indicated that even with the greater proportion of fine roots at the warmer soil temperatures, the nonmycorrhizal roots were unable to exploit the less available soil water. This would imply that in the absence of mycorrhizal infection, plant roots were capable of extracting a limited amount of soil water even with an increase in root surface area. The much greater efficiency of the UP plant roots may have been attributable to the greater surface area provided by the external mycelial network on the host plant. The increase in the shoot/root ratio of the mycorrhizal plants at the warmer soil temperatures (Table 11) may have been due to an increase in root surface area, a greater extension of mycorrhizal mycelium along the root, a delay in the decay of mature roots due to mycorrhizal nutrient enhancement, or to a combination of the above. The higher percentage total root length infected with mycorrhizae at 12 C compared to the 16 and 20 C plants seemed to indicate that, if anything, there may have been more external mycorrhizal mycelium per unit surface area at the lower soil temperatures than at 16 and 20 C.

The very large surface area of the mycorrhizal roots no doubt contributed to the improved root efficiency, yet the destructive activity of the indigenous rhizosphere microflora should have reduced the efficiency of these roots as was apparently the case for the nonmycorrhizal plants. There was some reason

to believe therefore that the improved mycorrhizally induced nutrient and water status of the mycorrhizal plants may have limited the extent of cortical deterioration.

The results of Section II on the level of metabolic indicators in mycorrhizal and nonmycorrhizal roots were inconclusive. An improved phosphorus status of mycorrhizal roots over that of nonmycorrhizal roots observed in this study (Figure 18) was not reflected in higher carbohydrate levels (Figure 13). Nor were there any clear improvements in the amino acid concentrations in the mycorrhizal roots compared to the nonmycorrhizal roots (Figure 17). On the other hand, mycorrhizal plant roots generally had higher conductivity values at each of soil temperatures than the nonmycorrhizal plants (Figure 16). Possible explanations of the results of this section have been discussed in some detail already and will not be restated here except to say that the effects of soil temperature and moisture on microbial and plant metabolism may have overshadowed the influence of mycorrhizal infection.

Incrustation of epidermal and cortical cells by compounds such as lignin and suberin is known to restrict the uptake of certain plant nutrients that are incapable of traversing the symplasmic pathway across the endodermis to the xylem. (Luttge and Higginbotham, 1979). The fact that the plants with roots most heavily lignified (Figure 19) had shoots with the highest fresh and dry weights (Table 12) apparently contradicted the supposed detrimental effects of cell wall thickening. The greater surface area of the roots on these plants may have compensated for any decline in the nutrient uptake ability that might have been incurred as a consequence of lignification.

It was not unusual that the roots exposed to unpasteurized soil had higher levels of lignification. Akai and Fukutomi (1980) reported several instances

in which lignification acted as an effective physical barrier to the penetration of exposed plant tissue by plant pathogenic organisms. A pathogen induced stimulation of lignification is known to occur for a number of host-pathogen associations (Beckman, 1980). It is possible that the increased lignification may have been in response to the presence of the fungal endophyte which may have been most effective at inducing this response at the warm soil temperatures.

In as much as older roots are characterized by more advanced lignification than young roots (Clarkson and Robards, 1975) the generally higher incidence of lignification detected in the root zones having dense mycorrhizal infection seemed to indicate that mycorrhizal infection was not restricted to younger roots as had been earlier surmised. However, this deduction was highly speculative in view of the fact that microbial activity seemed to stimulate the advanced occurence of lignification. Also, since no record was kept of whether mycorrhizal roots corresponded to those that were lignified, it was not impossible that mycorrhizal infection only occurred in the absence of lignification. In addition, even if evidence of mycorrhizal infection was detected on heavily lignified root there was no way of knowing the age or functionality of the infection structure. Although mycorrhizal arbuscules are normally degraded within a few days of their formation (Kinden and Brown, 1976), mycorrhizal vesicles may function as reproductive structures after the root has died (Kinden and Brown, 1975 a). Therefore lignification may have occurred any time before, during or after mycorrhizally induced nutrient enhancement had occurred.

Nevertheless it might be argued that if mycorrhizal infection structures were formed in older lignified cortical cells, it would have neutralized the negative influence of lignification on nutrient uptake. The weakness of this

argument is that phosphorus, which is the major plant nutrient transferred to the plant via the mycorrhizal association, is readily taken up by regions of the root that have undergone secondary thickening (Clarkson et al., 1968). This is not to say that the mycorrhizal infection would not have increased the availability of phosphorus in more mature regions of the root, but that infection may have had no more beneficial effect in this region than in the younger root regions.

The improved water uptake efficiency characteristic of the mycorrhizal plants in this study may be linked to the occurence of infection in older roots. Secondary thickening of cell walls may reduce the water permeability of the root. The presence of mycorrhizal infection in the regions of the root that have undergone secondary thickening would have maintained an unhindered flow of water to the xylem.

The improvement in mycorrhizal root water and nutrient uptake efficiency noted in this study was attributed both to an increase in surface area available for absorption and to a reduction in the degradation of mature root cortices possibly due to an improved nutrient status of mycorrhizal roots. While an enhanced phosphate status may have retarded cortical cell deterioration it is also possible that the presence of the mycorrhizal fungus in the root may have rendered the plant root resistant to invasion by root pathogens. A number of reports have indicated that both ecto and endomycorrhizal plant roots exhibited substantially improved shoot and root growth (dry weights) (Davis et al., 1978; Schenck, 1981), even though, in the case of vesicular-arbuscular mycorrhizae plants had a higher incidence and severity of disease, (Davis et al., 1979, 1980) compared to nonmycorrhizal plants exposed to a root pathogen. The improved growth of root pathogen exposed mycorrhizal plants has been linked to the unique physical and chemical soil environment surrounding the mycorrhizal

root which in turn is characterized by a distinct "Mycorrhizosphere" microflora (Marx, 1972, 1973). The microbial and chemical composition of this environment is believed to be important in restricting the establishment and development of root pathogens (Marx, 1973).

While the results of the above studies indicated that resistance to root pathogen invasion was conferred upon roots infected with mycorrhizae, it cannot be inferred from this that all saprophytic microbial activity would be repressed at the root surface and rhizosphere as well. The steady release of root exudates, mucigel and sloughed off epidermal and cortical cells would encourage a large saprophytic micorbial rhizosphere population. However, whatever mycorrhizae conferred resistance factors are involved in repressing pathogen invasion may also have been responsible for neutralizing the alleged microbially induced enhancement of cortical cell deterioration reported by Martin (1977).

No attempt was made in this study to evaluate the influence of nonmycorrhizal rhizosphere inhabiting microorganisms on barley root development. This may have been a serious omission since microhizal infection was achieved by growing the plants in unpasteurized soil and differences in plant structure and composition which appeared to have been mycorrhizally related may have been just as likely due to the action of nonmycorrhizal microorganisms. The soil microflora in proximity to the rhizosphere may have improved plant growth through the release of plant growth promoting substances (Libbert et al., 1968). Plant growth regulating substances derived from all soil microorganisms have been shown to influence root morphology (Lynch, 1976). Thimann (1972) reviewed the biological activity of auxin. Auxin inhibits root elongation and stimulates root thickening and lateral root growth, with an overall reduction in root dry weight. At low concentrations, auxin stimulates root growth.

The effects of different soil temperature and soil moisture conditions on the level of micorbially derived plant growth regulating substances has not been well studied. It is very possible that the warm temperature induced increases in root proliferation may have been related to higher auxin concentrations in the soil at 20 C, while the increased root weight and root diameters observed on the cool soil temperature plants may have been due to low stimulatory auxin concentrations.

Phytotoxic compounds released from decomposing plant residues are also known to seriously inhibit plant growth. (Tousson, et al., 1968; Patrick, 1971). These substances, including aliphatic acids such as acetic, malonic, and malic acids, and phenolic acids such as p-coumaric, vanillic and ferulic acid, may be breakdown products of both plants and microbes. The influence of soil temperature and moisture on their accumulation in this study may have been significant but without actual quantitation, difficult to predict. It might be expected however, that a more rapid decomposition of organic matter, particularly sloughed off epidermal and cortical cells at the warm soil temperatures resulted in higher levels of toxic metabolites which may have caused a reduction in root development. This may have accounted for the lower fresh and dry root weights of the 20 C roots in this study. The effects of both growth regulating as well as phytotoxic substances may have been accentuated in this study because of the absence of drainage holes at the bottom of the plant containers, which would have otherwise permitted leaching out of these substances.

Oxygen concentration is a critical factor regulating the amoung of microbial metabolites in the soil. As Lynch (1976) pointed out "whereas many microbially derived compounds are only produced anaerobically, their subsequent metabolism to carbon dioxide and other products is favored under aerobic conditions." In

the present study it is possible that near anaerobic conditions developed at the greater soil depths due to the reduced oxygen diffusion rates through the very moist soil near the soil surface. The reduced oxygen concentrations near the bottoms of the pots may have reduced the rate of metabolism of microbially derived substances, and thereby extended the time period of exposure to the roots.

Anaerobic conditions which may have occurred at the greater soil depths may have also favored the buildup of hydrogen sulfide, a product of anaerobic sulfate-reducing bacteria (Le Gall and Postgate, 1973). Normally oxidized or converted to the nontoxic sulfhydryl ion, it does not build up to toxic levels. However under acidic conditions, or under water logged, or poorly structured soils, hydrogen sulfide may be damaging to plants. Since these soil conditions did not prevail in the present study, it was unlikely that sulfide toxility occurred.

Beneficial microbial effects may have occurred through solubilization of less available minerals such as phosphorus (Webley and Duff, 1965). Deleterious effects of soil sterilization on plant performance due to the removal of beneficial microbial activity in or near the rhizosphere have been reported (Bowen and Rovira, 1966).

PROBLEMS AND IMPROVEMENTS

Several inherent weaknesses in this study made the data difficult to interpret, and to draw conclusions from. The method by which root viability was assessed created the greatest problems.

The inability to discriminate between the effects of mycorrhizal infection on the root sample from those on individual cells made the method used to determine root viability less than satisfactory. Differences in the level of the metabolic indicators may have been due not only to the relative proportion of viable cells in the root sample but also to localized differences in the level of plant metabolites in completely viable cells. The same environmental factors that influenced cortical cell degeneration may have also influenced the relative concentration of these metabolites in the root. Thus, warm soil temperatures are known to favor an increase in the rate of translocation of nutrients from the root to the shoot, (Nielsen, 1974) as well as enhance the rate of decay of the root (Martin, 1977). Similarly an increase in phosphorus content in a particular root zone may either have reflected a greater number of healthy roots in the sample from that zone or a small number of healthy roots that had a high level of cellular phosphorous and other plant metabolites.

In addition the values of the metabolic indicators obtained were the product of between 400 to over 15,000 individual 0.5 cm root subsegments whose origin with respect to the seminal roots and primary lateral roots was unknown. Nor was it known to what extent each root subsegment contributed to the final indicator value. It was assumed in evaluating the data that the contribution to the total value of the indicator was proportional to root

volume. It was possible that this was an inappropriate conversion factor, the correct factor perhaps being total surface area.

The selection of carbohydrate and amino acid levels as indicators of cell metabolism was appropriate in view of the diversity of metabolic pathways in which these compounds are involved in viable cells. However the mechanisms that regulate their level in the cell themselves respond to the total amino acid, protein and carbohydrate compliment in the whole plant which in turn are influenced by the same environmental factors that affect the metabolism at the cellular level. Because of the many variables that may have influenced the level of these metabolites, including variability in the plant population itself, this method of assessing plant root viability has its limitations.

A more reliable approach would have been to scan a single root along its entire length and, using an appropriate method, correlate the presence or absence of infection with cell viability. There are a number of possible microscopical techniques by which a quantitative estimate of the viability of a localized region of the root could be obtained. Vital stains, that is, stains that can be used on fresh plant material without interfering with cell metabolism, are available that can differentiate living and dead cells on the basis of their ability to be absorbed into the cell (Stadelmann and Kinzel, 1972). Evans blue is an example of this (Gaff and Okong'ogola, 1971). Some vital stains are capable of traversing the tonoplast into the cell vacuole only if the cell is metabolically active. Acridine orange is an example of such a stain (Stadelmann and Kinzel, 1972).

Several fluorescence methods for the detection of living cells have been tested, but mainly on microbial cells. For example the differential staining method developed by Anderson and Slinger (1975) to enumerate soil propagules

and comprising two components: europium chelate which stains nucleic acid containing living cells, and fluorescent brightener, which stains dead cells and organic matter has been adapted for use in rhizosphere microbiology. Another promising staining combination involves fluorescein diacetate and phenosafranine, which stains live and dead cells respectively (Widholm, 1972).

The method used in assessing mycorrhizal infection and root length and diameter was very effective. A study has shown that this method or a variant of it, is superior to other methods previously used to assess mycorrhizal infection (Mosse, 1980).

A more efficient mycorrhizal inoculum source may have invoked even more dramatic differences in the growth parameters examined in this study between mycorrhizal and nonmycorrhizal plants. Most studies have indicated that indigenous mycorrhizal fungi are less effective in improving phosphate nutrition than introduced mycorrhizal species (Mosse and Bowen, 1971; Mosse, 1977). The inability to obtain reliable mycorrhizal infection from inoculation of barley roots using isolated spores in preliminary studies may be partly attributable to the low infectivity of the indigenous mycorrhizal fungi. Future studies directed toward determination of the influence of mycorrhizal infection cortical cell viability should employ isolated mycorrhizal spores as the inoculum source instead of a mixture of contaminating soil and spores. This would largely eliminate the difficulty of differentiating the effects of nonmycorrhizal rhizosphere microflora on root growth from those of the mycorrhizal fungi.

The improvised plant containers used in this study were beneficial in permitting examination of roots largely unaffected by the restrictions on root growth common in conventional clay pots. Conditions which impair the normal extension of the developing root at the apex of the root system may also

influence root growth nearer the soil surface. To eliminate possible interference due to physical restrictions, longer pots, possibly up to 1.5 meters in length, should be used. This may create problems in maintaining an adequate moisture level at the greater soil depths since in the present study soil water deficits were noted in the pots having the relatively short length of 38.0 cm. However, as has already been mentioned a soil water deficit with increasing soil depth observed in this study may have been avoided by irrigating the pots to field capacity instead of to 80% field capacity. Devising an irrigation method for elongated pots may be one of the more troublesome obstacles in further studies of this nature.

SUMMARY AND CONCLUSIONS

Barley plants grown in unpasteurized soil (UP) had a greater length of mycorrhizal roots than that of plants grown in inoculated pasteurized soil (PI) based on measurements taken from 0.1 g (dry weight) root samples along the length of the root system. Mycorrhizal infection in roots of UP plants was mainly in the upper half of the root system. Mycorrhizal infection in PI roots was isolated to the region of innoculation. Roots of UP plants grown at 12 and 20 C had greater lengths of mycorrhizal roots than those grown at 16 C. PI plants had the greatest lengths of infected roots at 16 and 20 C.

Total root lengths of 0.1 gm (dry weight) samples declined with increasing soil depth. Roots of UP and PI plants grown at 12 and 16 C had greater total root lengths than nonmycorrhizal plants grown in pasteurized soil (PC) at 16 and 20 C. PC and PI plants had shorter root lengths than UP plants at all soil temperatures.

The percent length of mycorrhizal roots on the UP plants increased with decreasing soil depth, reaching a peak in the upper half of the root system.

Between approximately 70 to 90% of the total length of mycorrhizal roots of both PI and UP plants were 0.2 mm in diameter while approximately 10 to 30% of the mycorrhizal roots in these treatments were 0.4 mm in diameter.

Roots 0.2 mm in diameter comprised between 65 to 95% of the total root length while roots that were 0.4 and 0.6 mm in diameter made up approximately 5 to 25% and 1 to 7% of the total root length, respectively, averaged over all the treatments. The percentage of fine roots on the PC and PI plants increased at the greater soil depths with increasing soil temperature. No

such temperature effect was observed for the UP plants. The roots of the UP plants had a greater percentage of fine roots at all soil depths and at all three soil temperatures than the PC and PI plants.

There was a significant decline in soil moisture with increasing soil depth for all three soil temperatures and mycorrhizal treatments. The severity of the soil moisture deficit increased with time. Soil in which heavily mycorrhizal plants were grown had greater soil moisture deficits than soils in which nonmycorrhizal plants had grown. Soil moisture content decreased with increasing soil temperature. Differences in the level of soil moisture between treatments was related to differences in the treatment effects on plant growth. Extensive mycorrhizal infections and warm soil temperatures promoted an increased growth rate which was reflected in a greater demand for soil water. The slow rate of water descent in the pots possibly due to the failure to irrigate the soil to field capacity coupled with the greater demand for soil water by the mycorrhizal and warm temperature plants resulted in a soil moisture deficit developing at the greater soil depths.

The depressed soil moisture levels near the bottom of the plant containers exposed to warm soil temperatures and widespread mycorrhizal infection corresponded to an increase in proliferation of fine roots under the same conditions. The greater rate of root proliferation may have been in direct response to a reduced soil moisture content. The increased surface area provided by the network of mycorrhizal mycelium may have permitted mycorrhizal roots to extract more water from the soil than nonmycorrhizal roots. In addition, the mycorrhizal association may have provided localized regions of enhanced nutrient supply which may have stimulated root proliferation. In either case the increased root surface area exposed to the soil that resulted from an increase in root proliferation may have compensated for the reduced soil moisture and nutrient availability.

Total fresh and dry root weights declined in proportion to the extent of mycorrhizal infection. Roots of UP plants had by far the lowest fresh and dry root weights. Fresh and dry weights of mycorrhizal and nonmycorrhizal roots generally declined with increasing soil temperatures. Fresh and dry weights of mycorrhizal roots generaly increased with decreasing soil depth, while corresponding weights of nonmycorrhizal roots peaked in the mid regions of the pots.

Fresh/dry root weight ratios were highest in the nonmycorrhizal roots grown at 16 C and lowest at 20 C. Mycorrhizal roots had the lowest ratio values at 20 C and the highest at 12 C. Increased mycorrhizal infection resulted in lower fresh/dry ratios at 16 C but increased ratio values at 12 and 20 C. The fresh/dry weight ratio of the roots of mycorrhizal plants increased in segments which were infected with mycorrhizal fungi. No such increase was observed for the roots of nonmycorrhizal plants.

Mycorrhizal plants had higher fresh and dry shoot weights than those of the nonmycorrhizal plants. Fresh and dry shoot weights of nonmycorrhizal plants were highest at 16 C and lowest at 20 C. Shoot weights of the UP roots were lowest at 20 C, and did not differ at 12 and 16 C. Greatest improvement in fresh shoot weight in response to mycorrhizal infection occurred in the 12 and 20 C UP roots. Shoot growth improvement was direcly related to the total percent length of roots that were infected.

The shoot/root dry weight ratio of the UP plants was over 3 times larger than that of the PC plants and 2.5 times larger than that of the PI plants. The 12 C PI and UP plants had lower shoot/root ratios than that of the 16 and 20 C PI and UP plants.

The improvement of the efficiency in dry matter production of the mycorrhizal plants over the nonmycorrhizal plants was attributed to an increase in root surface area contributed both from fungal hyphal elements extending beyond

the root surface, as well as from an enhanced rate of lateral root proliferation in the zone of mycorrhizal infection. Mycorrhizal infection enhanced the level of phosphorus in barley root compared to control plants. Roots of the PC plants grown at 16 and 20 C differed little from each other in the level of phosphorus, and had lower levels of phosphorus than roots given the same mycorrhizal treatment at 12 C. Phosphorus levels at 12 C were not significantly influenced by mycorrhizal treatment.

Cool temperatures and the absence of mycorrhizal infection favored the highest carbohydrate levels along the roots. Cool temperatures also resulted in higher conductivity levels. Mycorrhizal and nonmycorrhizal plant roots grown at 12 and 16 C generally differed very little in conductivity values. Increases in conductivity were noted in localized regions where mycorrhizal infection occurred, and in general plant roots having the most extensive mycorrhizal infection had the highest conductivity levels. Amino acid values were generally highest at 12 C in the PC and PI roots, but were lowest in the roots of plants grown in unpasteurized soil at this temperature. At 12 C UP roots had lower amino acid contents than the PC and PI roots. PC and PI roots had lower amino acid values than UP roots at 20 C.

The higher levels of conductivity and amino acids of the mycorrhizal roots at the warmer soil temperatures indicated that the improved phosphorus status of the mycorrhizal plants may have reduced the extent of cortical cell deterioration of mycorrhizal roots. The interactions of soil temperature and soil moisture with plant and microbial metabolism may have been responsible for many of the differences in the levels of phosphorus and the metabolic indicators that were observed in this study. Higher levels of lignification were observed in roots grown in unpasteurized soil at warmer soil temperatures. This was attributed primarily to the microbially induced stimulation of

lignification in this soil. There was some possibility that the increased lignification in these roots was in response to mycorrhizal infection. In addition, it may have been an indication of the ability of mycorrhizal infection to occur in mature roots that have undergone secondary thickening.

In conclusion, the improvement in soil water and nutrient uptake efficiency observed in the mycorrhizal roots particularly at the warm soil temperature was attributed to an increased in root surface area resulting from heightened root proliferation and possibly to a reduction in the rate of mature root cortical cell decay.

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APPENDIX

Calculation of Total Root Length

Root length from line and root piece intersections was calculated using the formula:

$$R = \frac{\pi N A}{2 H}$$

where R is the estimated root length on the plate, H is the length of the scanning line along which intersections are counted, N is the number of intersections between the lines and the 0.5 cm root pieces, and A is the area over which root pieces were spread (Newman, 1966).

For the present study, the petri plate area was 58.09 cm^2, and H = 37.7 cm.

After calculating the total length of root on the petri plate, root length of the original sample was calculated from the known proportion of roots placed on the petri plate.

Table 15. Total Available Soil Phosphorous Extracted from Pasteurized and Unpasteurized Soil, Sand Mixture (2/1 soil:sand)

Soil Treatment	Phosphorous (ppm)
Unpasteurized	7.21 ± 0.58
Pasteurized	7.08 ± 0.55

Values are the means of 3 replications on 3 soil samples.
± Standard error P = 0.05.

Table 16. Analysis of Variance in Total Mycorrhizal Infection in Root of Mycorrhizal Barley Roots grown at Three Soil Temperatures

Source of Variation	Degrees of Freedom	Sum of Squares	F Value
Temperature (T)	2	2882.3594	19.17**
Soil Depth (SD)	7	24296.6432	46.17**
Mycorrhizal Treatment (MT)	1	20256.5651	269.42**
Date of Planting (D)	7	1808.5183	3.44*
T x SD	14	3293.7240	3.13*
T x MT	2	4231.6302	28.14*
T x D	14	1826.0990	1.73^{NS}
SD x MT	7	5003.3724	9.51**
SD x D	49	2559.2109	0.69^{NS}
MT x D	7	1070.9140	2.03^{NS}
T x SD x MT	14	3879.6198	3.69**
T x SD x D	98	7106.4844	0.96^{NS}
T x MT x D	14	4109.3281	3.90**
SD x MT x D	49	2822.9818	0.77^{NS}
Error	90	7368.0885	
Total	383		

* Significant at P = 0.05
** Significant at P = 0.01
NS Not Significant

Table 17. Analysis of Variance in Total Root Length of Mycorrhizal and Nonmycorrhizal barley plants grown at three soil Temperatures

Source of Variation	Degrees of Freedom	Sum of Squares	F Value
Soil Temperature (T)	2	486834.5035	70.34**
Soil Depth (SD)	7	2327961.0278	133.67**
Mycorrhizal Treatment (MT)	2	719620.2222	103.98**
Date of Planting (D)	7	80586.9444	3.33*
T x SD	14	145188.1910	3.00*
T x MT	4	106800.0174	7.72**
T x D	14	93466.6910	1.93^{NS}
SD x MT	14	110716.8889	2.29**
SD x D	49	189704.1389	1.12^{NS}
MT x D	14	118232.9722	2.44**
T x SD x MT	28	79308.4549	0.82^{NS}
T x SD x D	98	286610.3924	0.85^{NS}
T x MT x D	28	159225.2049	
SD x MT x D	98	352485.0278	1.04^{NS}
Error	196	678245.8785	
TOTAL	575		

* Significant at P = 0.05.
** Significant at P = 0.01
NS Not Significant

Table 18. Analysis of Variance in the Percentage of the Total Root Length Infected With Mycorrhizae on Mycorrhizal and Nonmycorrhizal Barley Plants Grown at Three Soil Temperatures

Source of Variation	Degrees of Freedom	Sum of Squares	F Value
Soil Temperature (T)	2	1.8299	5.17*
Mycorrhizal Treatment (MT)	1	7.5358	42.58**
Soil Depth (SD)	6	3.6210	3.40*
Planting Date (D)	7	0.4221	0.50^{NS}
T x MT	2	6.0917	17.21**
T x SD	12	4.5236	2.13^{NS}
T x D	14	1.0132	0.71^{NS}
MT x SD	6	7.8791	7.42**
MT x D	7	1.1913	0.96^{NS}
SD x D	42	2.8784	0.38^{NS}
T x MT x SD	12	10.4064	4.90**
T x MT x D	14	0.9408	0.38^{NS}
T x SD x D	84	2.9217	0.20^{NS}
MT x SD x D	42	3.1729	0.43^{NS}
Error	84	6.0512	
TOTAL	335		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant

Table 19. Analysis of Variance in Percentage of Mycorrhizal Roots 0.2 mm in Diameter on Mycorrhizal Barley Plants grown at Three Soil Temperatures †

Source of Variation	Degrees of Freedom	Sum of Squares	F Value
Soil Temperature (T)	2	1.3555	5.15*
Soil Depth (SD)	6	2.8780	3.65*
Mycorrhizal Treatment (MT)	1	6.2834	47.76**
Date of Planting (DT)	7	0.4641	0.50^{NS}
T x SD	12	3.1762	2.01^{NS}
T x MT	2	3.2989	12.54**
T x D	14	1.2998	0.71^{NS}
SD x MT	6	7.7972	9.88**
SD x D	42	4.9654	0.90^{NS}
MT x D	7	1.1913	1.29^{NS}
T x SD x MT	12	3.6028	2.28^{NS}
T x SD x D	84	10.4069	0.94^{NS}
T x MT x D	14	0.9408	0.51^{NS}
SD x MT x D	42	4.1217	0.75^{NS}
Error	84	11.0512	
Total	335		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant
† Analysis of Variable Performed on Transformed Data.

Table 20. Analysis of Variance in Percentage of Mycorrhizal Roots 0.4 mm in Diameter on Pot Grown Mycorrhizal and Nonmycorrhizal Barley Plants grown at Three Soil Temperatures.[+]

Source of Variation	Degrees of Freedom	Sum of Squares	F Value
Soil Temperature (T)	2	0.1815	2.15^{NS}
Soil Depth (SD)	6	6.8396	26.98**
Mycorrhizal Treatment (MT)	1	0.9972	23.63**
Date of Planting (DT)	7	0.1997	0.68^{NS}
T x SD	12	0.7498	1.48^{NS}
T x MT	2	0.0218	0.26^{NS}
T x D	14	0.5077	0.86^{NS}
SD x MT	6	1.7883	7.06**
SD x D	42	1.8689	1.05^{NS}
MT x D	7	0.1448	0.49^{NS}
T x SD x MT	12	0.7483	1.48^{NS}
T x SD x D	84	2.8932	0.82^{NS}
T x MT x D	14	0.4384	0.74^{NS}
SD x MT x D	42	2.2413	1.26^{NS}
Error	84	3.5443	
TOTAL	335		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant
+ Analysis of Variance was Conducted on Transformed Data.

Table 21. Analysis of Variance in Percentage of the Length of Root 0.2 mm in Diameter on Mycorrhizal and Nonmycorrhizal Barley Plants Grown at Three Soil Temperatures +

Source of Variation	Degrees of Freedom	Sum of Squares	F Value
Soil Temperature (T)	2	1.2053	545.76**
Soil Depth (SD)	6	0.6691	100.99**
Mycorrhizal Treatment (MT)	2	1.2569	569.07**
Date of Planting (D)	7	0.0063	0.81^{NS}
T x SD	12	0.3405	25.69**
T x MT	4	0.1037	23.47**
T x D	12	0.0920	5.95**
SD x MT	12	0.1858	14.02**
SD x D	42	0.0410	0.38^{NS}
MT x D	14	0.1487	9.62**
T x SD x MT	24	0.1243	4.69**
T x SD x D	84	0.0698	0.78^{NS}
T x MT x D	28	0.2691	8.70**
SD x MT x D	28	0.1612	1.74**
Error	168		
TOTAL	503		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant
\+ Analysis of Variance was conducted on Transformed Data.

Table 22. Analysis of Variance in Percentage of the Total Length of Root 0.4 mm in Diameter on Mycorrhizal and Nonmycorrhizal Barley Plants grown at Three Soil Temperatures †

Source of Variation	Degrees of Freedom	Sum of Squares	F Value
Soil Temperature (T)	2	0.8924	387.76**
Soil Depth (SD)	6	0.4456	64.55**
Mycorrhizal Treatment (MT)	2	0.8649	375.85**
Date of Planting (D)	7	0.0019	0.24^{NS}
T x SD	12	0.2161	15.65**
T x MT	4	0.0881	19.14**
T x D	12	0.0743	4.61**
SD x MT	12	0.1387	10.05**
SD x D	42	0.0534	1.10^{NS}
MT x D	14	0.1366	8.48**
T x SD x MT	24	0.0919	3.33**
T x SD x D	84	0.0820	0.85^{NS}
T x MT x D	28	0.3087	9.58**
SD x MT x D	28	0.1723	1.78**
Error	168		
TOTAL	503		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant
† Analysis of Variance Conducted on Transformed Data.

Table 23. Analysis of Variance in Percentage of the Total Length of Root 0.6 mm in Diameter on Mycorrhizal and Nonmycorrhizal Barley Plants grown at Three Soil Temperatures.†

Source of Variation	Degrees of Freedom	Sum of Squares	F Value
Temperature (T)	2	0.2899	161.06**
Soil Depth (SD)	6	0.1831	33.92**
Mycorrhizal Treatment (MT)	2	0.3893	216.33**
Date of Planting (D)	7	0.0188	2.98*
T x SD	12	0.1068	9.89**
T x MT	4	0.0158	4.40**
T x D	12	0.0570	4.53**
SD x MT	12	0.0584	5.41**
SD x D	42	0.0343	0.91^{NS}
MT x D	14	0.0454	3.60**
T x SD x MT	24	0.0455	2.11**
T x SD x D	84	0.0564	0.75^{NS}
T x MT x D	28	0.0816	3.24**
SD x MT x D	28	0.0634	0.84^{NS}
Error	168		
TOTAL	503		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant
† Analysis of Variance was conducted on Transformed Data.

Table 24. Analysis of Variance in Soil Moisture Content at Three Soil Depths in Pots containing Mycorrhizal and Nonmycorrhizal Barley Plants grown at three soil temperatures, and samples 20 and 45 days after seedling emergence.

Source of Variation	Degrees of Freedom	Sums of Squares	F Value
Temperature (T)	2	1162.2127	5.52**
Soil Depth (SD)	2	2204.0619	10.46**
Mycorrhizal Treatment (MT)	2	1098.2135	5.21**
Date of Sampling (DS)	1	921.5764	8.75**
Date of Planting (D)	1	214.7271	2.03^{NS}
T x SD	4	1412.8946	3.35*
T x MT	4	1101.2419	2.61*
T x DS	2	990.8614	4.70*
T x D	2	463.7208	2.20^{NS}
SD x MT	4	1147.3162	2.72*
SD x DS	2	1084.9395	5.14**
SD x D	2	387.4265	1.83^{NS}
MT x DS	2	1104.0304	5.24**
MT x D	2	296.3871	1.41^{NS}
DS x D	1	274.6439	2.61^{NS}
T x SD x MT	8	2407.4236	2.85**
T x SD x DS	4	1356.2892	3.21*
T x SD x D	4	418.9617	0.99^{NS}
T x MT x DS	4	1285.4403	3.05*
T x MT x D	4	321.0509	0.76^{NS}
T x DS x D	2	204.8926	0.97^{NS}
SD x MT x DS	4	1487.6419	3.53**
SD x DS x D	2	137.4206	0.65^{NS}
SD x MT x D	4	264.3892	0.63^{NS}
MT x DS x D	2	102.4637	0.49^{NS}
T x SD x MT x DS	8	759.2146	0.90^{NS}
T x MT x DS x D	4	392.5763	0.93^{NS}
T x SD x MT x D	8	637.5201	0.75^{NS}
SD x MT x DS x D	4	271.4135	0.64^{NS}
Error	8	842.6834	
TOTAL	107		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant

Table 25. Analysis of Variance in Fresh Weight of Mycorrhizal and Nonmycorrhizal Roots grown at Three Soil Temperatures and Measured at Four Soil Depths.

Source of Variation	Degrees of Freedom	Sums of Squares	F Value
Temperature (T)	2	807.0891	113.78**
Soil Depth (SD)	3	56.8166	5.34**
Mycorrhizal Treatment (MT)	2	2566.5030	361.83**
Date of Planting (D)	7	78.4035	3.16*
T x SD	6	45.6538	2.15^{NS}
T x MT	4	109.7631	7.74**
T x D	14	68.5954	1.38^{NS}
SD x MT	6	152.5104	7.17**
SD x D	21	89.9835	1.21^{NS}
MT x D	14	77.2353	1.56^{NS}
T x SD x MT	12	43.1167	1.01^{NS}
T x SD x D	42	79.8068	0.54^{NS}
T x MT x D	28	221.6975	2.23*
SD x MT x D	42	116.7468	0.78^{NS}
Error	84	297.9136	
TOTAL	287		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant.

Table 26. Analysis of Variance in Dry Weight Mycorrhizal and Nonmycorrhizal Roots grown at Three Soil Temperatures and measured at Four Soil Depths.

Source of Variation	Degrees of Freedom	Sum of Squares	F Value
Temperature (T)	2	2.2569	2.96^{NS}
Soil Depth (SD)	3	7.5546	6.62**
Mycorrhizal Treatment (MT)	2	91.2404	119.85**
Date of Planting (D)	7	8.7475	3.28**
T x SD	6	2.6914	1.18^{NS}
T x MT	4	1.1502	0.76^{NS}
T x D	14	3.9677	0.74^{NS}
SD x MT	6	9.8351	4.31**
SD x D	21	8.8134	1.10^{NS}
MT x D	14	6.9988	1.31^{NS}
T x SD x MT	12	4.6268	1.01^{NS}
T x SD x D	42	18.1252	1.13^{NS}
T x MT x D	28	13.9104	1.31^{NS}
SD x MD x D	42	10.7678	0.67^{NS}
Error	84		
TOTAL	287		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant

Table 27. Analysis of Variance in the Fresh/Dry Root Weight Ratio of Mycorrhizal and Nonmycorrhizal Plants grown at Three Soil Temperatures and Measured at Four Soil Depths.

Source of Variation	Degrees of Freedom	Sums of Squares	F Value
Temperature (T)	2	118.2695	47.26**
Soil Depth (SD)	3	81.3509	21.67**
Mycorrhizal Treatment (MT)	2	15.5356	6.21**
Date of Planting (D)	7	35.6319	4.07**
T x SD	6	26.3338	3.51**
T x MT	4	18.4452	3.69**
T x D	14	29.8395	1.70^{NS}
SD x MT	6	27.0401	3.60**
SD x D	21	32.2509	1.23^{NS}
MT x D	14	25.8607	1.48^{NS}
T x SD x MT	12	17.4734	1.16^{NS}
T x SD x D	42	73.7609	1.40^{NS}
T x MT x D	28	56.3339	1.61^{NS}
SD x MT x D	42	57.7658	1.10^{NS}
Error	84	105.0959	
TOTAL	287		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant.

TABLE 28. Analysis of Variance in Fresh Weight of Shoots of Mycorrhizal and Nonmycorrhizal Plants Grown at Three Soil Temperatures.

Source of Variation	Degrees of Freedom	Sums of Squares	F Values
Temperature (T)	2	302.8761	4.98**
Mycorrhizal Treatment (MT)	2	1101.3245	17.76**
Date of Planting (D)	7	56.0614	0.26NS
T x MT	4	958.1346	7.73**
T x D	14	39.2012	1.26NS
MT x D	14	69.3896	0.09NS
Error	28	889.7209	
TOTAL	71		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant

Table 29. Analysis of Variance in Dry Weight of Shoots of Mycorrhizal and Nonmycorrhizal Plants Grown at Three Soil Temperatures

Source of Variation	Degrees of Freedom	Sums of Squares	F Values
Temperature (T)	2	24.3561	3.68*
Mycorrhizal Treatment (MT)	2	134.9418	20.38**
Date of Planting (D)	7	56.3294	2.43*
T X MT	4	38.7472	2.93*
T x D	14	118.8238	2.56*
MT x D	14	104.6620	1.59NS
Error	28		
TOTAL	71		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant

Table 30. Analysis of Variance in the Fresh/Dry Shoot Weight Ratio of Mycorrhizal and Nonmycorrhizal Plants Grown at Three Soil Temperatures.

Source of Variation	Degrees of Freedom	Sums of Squares	F Values
Temperature (T)	2	2.9250	1.19NS
Mycorrhizal Treatment (MT)	2	2.5038	1.07NS
Date of Planting (D)	7	2.8420	1.99NS
T x MT	4	1.1654	0.96NS
T x D	14	4.0320	1.42NS
MT x D	14	2.4009	0.84NS
Error	28	5.6932	
TOTAL	71		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant.

Table 31. Analysis of Variance in the Shoot/Root Dry Weight Ratio of Mycorrhizal and Nonmycorrhizal Barley Plants at Three Soil Temperatures.

Source of Variation	Degrees of Freedom	Sums of Squares	F Value
Temperature (T)	2	0.7762	0.29NS
Mycorrhizal Treatment (MT)	2	50.5129	19.42**
Date of Planting (D)	7	13.1963	1.40NS
T x MT	4	6.1385	1.14NS
T x D	14	20.4291	1.08NS
MT x D	14	29.2735	1.55NS
Error	28	37.7694	
TOTAL	71		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant.

Table 32. Total Root Volume of Mycorrhizal and Nonmycorrhizal Barley Plants at Eight Soil Depths and Three Soil Temperatures.+

Soil Temp (c)	Soil Depth (cm)	Root Volume (cm^3) Mycorrhizal Treatment: Pasteurized	Pasteurized Inoc.	Unpasteurized
12	2.25	0.272*	0.270	0.288
	6.75	0.280	0.283	0.290
	11.25	0.280	0.286	0.277
	15.75	0.262	0.267	0.251
	20.25	0.242	0.230	0.242
	24.75	0.220	0.193	0.234
	29.25	0.180	0.174	0.220
	33.75	0.140	0.174	0.208
16	2.25	0.273	0.267	0.298
	6.75	0.283	0.273	0.290
	11.25	0.284	0.281	0.290
	15.75	0.273	0.273	0.280
	20.25	0.263	0.240	0.250
	24.75	0.240	0.210	0.223
	29.25	0.204	0.204	0.204
	33.75	0.173	0.204	0.204
20	2.25	0.280	0.290	0.280
	6.75	0.298	0.285	0.284
	11.25	0.280	0.287	0.280
	15.75	0.270	0.283	0.280
	20.25	0.250	0.272	0.262
	24.75	0.226	0.237	0.226
	29.25	0.202	0.200	0.200
	33.75	0.188	0.185	0.178

+ Volume Data Estimated in 0.1 gm (Dry Weight) Root Samples.

* Values are means of eight replicates, two plants per pot.

Table 33. Carbohydrate Level in Mycorrhizal and Nonmycorrhizal Barley Roots at Eight Soil Depths and Three Soil Temperatures +

Soil Temp (C)	Soil Depth (cm)	Carbohydrate (mg x10^{-3}) Mycorrhizal Treatment		
		Pasteurized	Pasteurized Inoc.	Unpasteurized
12	2.25	23.46*	20.14	31.45
	6.75	30.55	33.20	48.09
	11.25	36.66	39.58	47.54
	15.75	45.04	37.48	44.60
	20.25	43.89	40.85	40.98
	24.75	54.99	51.41	57.75
	29.25	59.25	53.10	64.99
	33.75	53.87	65.84	64.44
16	2.25	18.51	16.48	14.73
	6.75	18.68	17.45	18.51
	11.25	20.74	17.60	25.48
	15.75	25.35	21.54	30.46
	20.25	33.28	33.31	23.50
	24.75	40.93	32.28	29.11
	29.25	55.0	33.10	36.81
	33.75	45.95	42.69	29.35
20	2.25	16.78	14.14	17.69
	6.75	18.73	20.49	17.61
	11.25	23.9	21.48	28.53
	15.75	31.21	24.99	21.48
	20.25	33.25	27.85	23.19
	24.75	36.96	44.99	25.65
	29.25	50.68	51.95	33.96
	33.75	56.41	45.16	25.21

+ Carbohydrate Values Estimated in 0.1 gm (Dry Weight) Root Samples.

* Values are means of eight replicates, two plants per pot.

Table 34. Amino Acid Levels in Mycorrhizal and Nonmycorrhizal Barley Roots at Eight Soil Depths and Three Soil Temperatures +

Soil Temp (C)	Soil Depth (cm)	Amino Acid (mg x10^{-1}) Mycorrhizal Treatment		
		Pasteurized	Pasteurized Inoc.	Unpasteurized
12	2.25	0.702*	0.756	0.657
	6.75	0.848	0.934	0.458
	11.25	0.952	1.015	0.596
	15.75	1.166	1.161	0.941
	20.25	0.908	0.909	0.852
	24.75	0.869	1.023	0.761
	29.25	0.720	0.748	0.744
	33.75	0.714	0.913	0.742
16	2.25	0.491	0.878	0.387
	6.75	0.792	0.552	0.609
	11.25	0.639	0.829	0.986
	15.75	0.669	0.871	0.840
	20.25	0.623	0.840	0.713
	24.75	1.02	0.840	0.970
	29.25	0.912	0.716	0.806
	33.75	0.606	0.532	0.673
20	2.25	0.616	0.687	0.560
	6.75	1.073	0.988	0.781
	11.25	0.902	0.976	1.316
	15.75	1.107	1.160	1.12
	20.25	0.875	0.952	1.31
	24.75	1.004	0.948	0.848
	29.25	0.873	0.910	1.196
	33.75	1.989	0.788	0.935

+ Amino Acid Values Estimated in 0.1 gm (Dry Weight) Samples.

* Values are means of eight replicates, two plants per pot.

Table 35. Phosphorous Level in Mycorrhizal and Nonmycorrhizal Barley Roots at Eight Soil Depths and Three Soil Temperatures +

Soil Temp. (C)	Soil Depth (cm)	Phosphorus (mg x10^{-3}) Mycorrhizal Treatment: Pasteurized	Pasteurized Inoc	Unpasteurized
12	2.25	7.48*	7.43	8.06
	6.75	7.81	9.19	9.57
	11.25	9.24	9.43	9.42
	15.75	9.70	8.01	8.66
	20.25	9.56	8.69	8.46
	24.75	8.58	9.17	9.36
	29.25	8.28	9.61	9.35
	33.75	7.98	8.00	9.15
16	2.25	7.37	9.88	9.09
	6.75	6.23	10.10	11.02
	11.25	8.52	9.00	14.36
	15.75	6.55	7.92	13.86
	20.25	7.63	7.92	11.25
	24.75	7.44	7.88	11.60
	29.25	8.67	9.08	10.61
	33.75	8.82	7.14	10.00
20	2.25	7.00	7.25	9.24
	6.75	6.32	9.88	10.56
	11.25	6.30	11.48	15.12
	15.75	7.16	8.43	13.30
	20.25	6.75	7.48	15.06
	24.75	8.48	5.92	13.45
	29.25	9.90	6.50	13.80
	33.75	8.57	5.55	12.02

+ Phosphorous Value estimated in 0.1 gm (Dry Weight) Root Samples.

* Values are means of eight replicates, two plants per pot.

TABLE 36. Conductivity Levels in Mycorrhizal and Nonmycorrhizal Barley Roots at eight soil depths and three soil temperatures.†

Soil Temp (C)	Soil Depth (cm)	Conductivity (mmhos x10^{-3}) Mycorrhizal Treatment: Pasteurized	Pasteurized Inoc	Unpasteurized
12	2.25	2070.0*	2515.0	3028.8
	6.75	2406.3	2391.3	1987.5
	11.25	2405.6	2360.6	3441.3
	15.75	2260.0	2273.8	3028.8
	20.25	2345	2358.1	3181.3
	24.75	2113.8	2463.8	3448.3
	29.25	2082.5	2592.5	3883.8
	33.75	1725.0	1733.0	2547.5
16	2.25	1575.0	1734.4	1480.0
	6.75	1600.0	2131.3	1877.8
	11.25	1903.1	1996.3	2203.3
	15.75	1755.0	2132.5	2275.0
	20.25	2026.9	1998.8	2165.0
	24.75	2015.6	1892.5	2165.0
	29.25	2730.0	2052.5	2012.5
	33.75	2023.1	1787.5	1743.8
20	2.25	1471.3	1238.8	1326.3
	6.75	1642.5	1731.3	1781.3
	11.25	1746.3	1837.5	1860.0
	15.75	1865.0	1793.8	1875.0
	20.25	1996.9	1922.5	1921.3
	24.75	2015.0	1876.3	1860.5
	29.25	2326.8	2515.0	3257.6
	33.75	2118.8	2102.5	1776.3

† Conductivity values estimated in 0.1 gm (Dry Weight) Root Samples.

* Values are means of eight replicates two plants per pot.

Table 37. Analysis of Variance in Carbohydrates Corrected for Root Volume in Mycorrhizal and Nonmycorrhizal Barley Roots Grown at Three Soil Temperatures

Source of Variation	Degrees of Freedom	Sum of Squares	F Value
Soil Temperature (T)	2	9364.3321	208.31**
Soil Depth (SD)	7	31014.6348	197.10**
Mycorrhizal Treatment (MT)	2	772.4293	17.18**
Date of Planting (D)	7	47.9349	0.30^{NS}
T x SD	14	1959.1336	6.23**
T x MT	4	333.3054	3.71**
T x D	14	2237.2736	7.11**
SD x MT	14	2444.0122	7.77**
SD x D	49	930.2124	0.84^{NS}
MT x D	14	1836.2001	5.83**
T x SD x MT	28	468.6785	0.74^{NS}
T x SD x D	98	2856.3047	1.30^{NS}
T x MT x D	28	2969.5499	4.72**
SD x MT x D	98	2939.4119	1.30^{NS}
Error	196	4406.0303	
TOTAL	575		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Signficant.

Table 38. Analysis of Variance in Free Amino Acids Corrected for Root Volume in Mycorrhizal and Nonmycorrhizal Barley Roots Grown at Three Soil Temperatures

Source of Variation	Degrees of Freedom	Sum of Squares	F Value
Soil Temperature (T)	2	0.8847	39.50**
Soil Depth (SD)	7	3.0139	38.45**
Mycorrhizal Treatment (MT)	2	0.1012	4.52*
Date of Planting (D)	7	0.2501	3.19*
T x SD	14	0.6014	3.84**
T x MT	4	0.4362	9.74**
T x D	14	0.7390	4.71**
SD x MT	14	0.3939	2.51**
SD x D	49	0.5791	1.06^{NS}
MT x D	14	0.7308	4.66**
T x SD x MT	28	0.6539	2.09**
T x SD x D	98	1.0560	0.96^{NS}
T x MT x D	28	1.0996	3.51**
SD x MT x D	98	1.0149	0.92^{NS}
Error	196	2.1951	
TOTAL	575		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant.

Table 39. Analysis of Variance in Phosphorous corrected for Root Volume in Mycorrhizal and Nonmycorrhizal Barley Roots grown at Three Soil Temperatures.

Source of Variation	Degrees of Freedom	Sum of Squares	F Value
Soil Temperature (T)	2	0.0732	1.32^{NS}
Soil Depth (SD)	7	10.4420	53.59**
Mycorrhizal Treatment (MT)	2	7.0051	125.82**
Date of Planting (D)	7	0.4575	2.35*
T x SD	14	0.5761	1.48^{NS}
T x MT	4	5.8590	52.62**
T x D	14	1.8346	4.71**
SD x MT	14	2.9322	7.52**
SD x D	49	1.4235	1.04^{NS}
MT x D	14	2.3575	6.05**
T x SD x MT	28	3.0104	3.86**
T x SD x D	98	3.0591	1.12^{NS}
T x MT x D	28	3.6004	4.62**
SD x MT x D	98	3.1397	1.15^{NS}
Error	196	5.4561	
TOTAL	575		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant

Table 40. Analysis of Variance in Conductivity of Root Diffusate Corrected for Root Volume in Mycorrhizal and Nonmycorrhizal Barley Roots grown at Three Soil Temperatures.

Source of Variation	Degrees of Freedom	Sum of Squares	F Value
Soil Temperature (T)	2	11290547.3013	160.40**
Soil Depth (SD)	7	27660319.1352	112.30**
Mycorrhizal Treatment (MT)	2	463769.7761	6.59**
Date of Planting (D)	7	726497.7067	2.95**
T x SD	14	3871616.5616	7.86**
T x MT	4	897980.2357	6.38**
T x D	14	3312278.6675	6.72**
SD x MT	14	1827823.3358	3.71**
SD x D	49	1740652.0209	1.01^{NS}
MT x D	14	2292610.9851	4.65**
T x SD x MT	28	4894288.3795	4.97**
T x SD x D	98	3533381.0670	1.02^{NS}
T x MT x D	28	5055697.3107	5.13**
SD x MT x D	98	2630106.0766	0.76^{NS}
Error	196	6696448.8745	
TOTAL	575		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant.

Table 41. Analysis of Variance in Percentage of Total Root Length Lignified on Mycorrhizal and Nonmycorrhizal Barley Plants Grown at Three Soil Temperatures

Source of Variation	Degrees of Freedom	Sum of Squares	F Value
Soil Temperature (T)	2	921.0869	32.04**
Soil Depth (SD)	7	668.4322	6.64**
Mycorrhizal Treatment (MT)	2	9473.8846	329.59**
Date of Planting (D)	7	196.5977	1.96^{NS}
T x SD	14	696.3279	2.67*
T x MT	4	4832.0531	84.04**
T x D	14	216.2575	1.07^{NS}
SD x MT	14	679.5905	3.38**
SD x D	49	577.3331	0.82^{NS}
MT x D	14	318.2758	1.58^{NS}
T x SD x MT	28	1584.7370	3.94**
T x SD x D	98	1515.9278	1.08^{NS}
T x MT x D	28	802.3047	1.99*
SD x MT x D	98	1424.0051	1.01^{NS}
Error	196	2817.3143	
TOTAL	575		

* Significant at P = 0.05.
** Significant at P = 0.01.
NS Not Significant

Table 42. Total Length of Lignified Roots on Mycorrhizal and Non-mycorrhizal Barley Plants Grown at Three Soil Temperatures

Mycorrhizal Treatment	Soil Depth (cm)	Length of Lignified Root (cm) Soil Temperature (C) 12	16	20
Pasteurized Control	2.25	21.53	23.31	14.20
	6.75	30.82	25.29	23.31
	11.25	25.88	24.30	16.79
	15.75	30.42	35.76	27.66
	20.25	32.99	23.71	33.78
	24.75	31.02	33.78	27.46
	29.25	29.24	27.66	21.34
	33.75	48.60	26.27	31.02
Pasteurized Inoculated	2.25	16.99	29.24	23.71
	6.75	21.73	29.63	40.10
	11.25	32.20	39.12	29.63
	15.75	28.25	25.48	38.92
	20.25	32.20	24.89	27.06
	24.75	39.51	24.10	23.51
	29.25	37.73	24.50	22.92
	33.75	53.14	23.31	24.10
Unpasteurized	2.25	83.76	88.31	113.79
	6.75	74.28	90.28	130.19
	11.25	46.03	106.68	121.29
	15.75	65.39	83.96	130.98
	20.25	52.35	76.65	157.05
	24.75	28.25	73.29	115.37
	29.25	24.69	54.52	96.79
	33.75	19.36	50.57	102.92

Table 43. Total Length of Mycorrhizal and Nonmycorrhizal Barley Roots with Diameters of 0.2, 0.4, or 0.6 mm, Grown at Three Soil Temperatures.

Soil Temp (C)	Soil Depth (cm)	TOTAL ROOT LENGTH (cm) ROOT DIAMETER (mm) 0.2 Pasteur.	0.2 Pasteur. Inoc.	0.2 Unpast.	0.4 Pasteur.	0.4 Pasteur. Inoc.	0.4 Unpast.	0.6 Pasteur.	0.6 Pasteur. Inoc.	0.6 Unpast.
12	4.5	* 540.9	505.3	638.9	60.5	62.4	53.5	10.7	14.6	7.51
	9.0	556.5	557.1	677.4	61.4	64.9	40.5	11.5	12.8	9.1
	13.0	509.1	506.7	661.0	64.4	68.2	34.2	12.6	13.8	5.5
	18.0	411.7	394.7	562.4	66.6	66.2	39.9	14.6	15.4	5.9
	22.5	346.5	280.1	511.5	59.5	63.4	46.8	18.4	14.6	8.3
	27.0	263.5	229.4	476.5	57.1	52.9	41.9	17.2	15.0	8.1
	31.5	156.2	144.6	399.8	57.5	42.3	60.1	14.2	14.0	12.2
16	4.5	566.6	552.0	651.5	59.1	57.1	55.3	9.9	9.1	6.9
	9.0	592.7	589.3	687.3	55.5	52.2	38.1	10.7	8.7	8.1
	13.0	575.9	625.4	732.5	58.1	50.6	36.7	11.5	7.1	5.5
	18.0	543.1	500.2	608.3	56.9	58.7	41.3	10.3	12.6	6.7
	22.5	460.7	354.2	560.5	56.9	61.4	41.3	12.2	9.5	5.7
	27.0	404.6	316.7	524.7	49.6	52.5	24.9	11.1	12.4	2.2
	31.5	293.2	250.3	439.2	49.8	60.8	35.8	12.6	16.8	7.1
20	4.5	629.0	630.0	703.5	45.8	52.9	42.9	17.0	8.9	3.6
	9.0	680.8	659.2	717.3	40.9	44.8	32.4	11.5	9.3	3.6
	13.0	625.4	629.6	774.8	43.1	49.4	25.5	9.7	8.1	4.0
	18.0	610.0	608.1	685.2	39.5	54.3	35.0	5.7	10.1	3.6
	22.5	533.6	534.4	642.6	40.3	54.7	35.4	9.1	10.5	5.9
	27.0	443.7	476.5	566.4	35.0	29.8	20.5	10.1	6.5	2.6
	31.5	388.8	449.4	486.7	38.7	28.6	22.7	8.8	5.1	2.2

* Values are means of eight replicates, two plants per pot.