DISSERTATION

INTERACTION BETWEEN LIGHT, NITROGEN AND MYCORRHIZAL FUNGI ON PHOTOSYNTHESIS OF ECTOMYCORRHIZAL PINE

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY SEBASTIAN AKUMEFULA EKWEBELAM

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ABSTRACT OF DISSERTATION

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The importance of mycorrhizae to the physiological functions of plants is relatively well documented. Despite the obvious benefits of mycorrhizae for the enhancement of seedling growth, study of the relationship between applied cultural practices, such as shading, fertilization and mycorrhizae formation, and growth and photosynthesis of containerized nursery stock has been limited. The long-term objective of the present study, therefore, was to gain a more complete understanding of how the aggregate factors of light, nitrogen fertilization and mycorrhizae formation influence growth and photosynthesis of containerized seedlings, aimed at optimizing seedling production in a nursery environment.

Seedlings of lodgepole pine (Pinus contorta Dougl.) were grown for 16 weeks without ectomycorrhizae in the greenhouse at 3 levels of irradiance (high, medium and low) by use of shadecloth, and ammonium nitrate (3, 62 and 248 ppm N). Measurements at 5, 10 and 16 weeks of age indicated that biomass increased significantly with increasing levels of irradiance and nitrogen over the ranges studied. Although root/shoot ratios increased from low to high irradiance at each harvest, nitrogen application resulted in increased ratios from 3 to 62 ppm N, but decreased ratios at 248 ppm N. Nitrogen and phosphorus concentration generally decreased with increase in irradiance, but total N

content and photosynthesis per unit leaf area generally increased from low to high levels of both irradiance and nitrogen.

In a mycorrhizal fungi inoculation study, lodgepole pine seedlings were grown for 10 weeks without ectomycorrhizae at the aforementioned 3 levels of irradiance and nitrogen. At 10 weeks, mycorrhizal treatments, inoculation with either Pisolithus tinctorius or Suillus granulatus, were superimposed on the light and nitrogen treatments, and the seedlings were grown for an additional 6 weeks. Mycorrhizae formation increased with increase in irradiance, while fertilization with 62 ppm N resulted in greater mycorrhizae formation than either 3 or 248 ppm N. Further, inoculated plants had significantly greater biomass and nutrient contents than nonmycorrhizal seedlings. Inoculation with P. tinctorius and S. granulatus resulted in photosynthetic rates, 1.87 and 1.85 mg $CO_2 dm^{-2} h^{-1}$, respectively, significantly greater than nonmycorrhizal plants (1.41 mg $CO_2 dm^{-2} h^{-1}$). Although the increase in growth of the mycorrhizal seedlings was associated with increased photosynthesis, the magnitude of this response depended on specific combinations of irradiance and nitrogen fertilization. These results emphasize the importance of the interactions among irradiance, nitrogen fertilization, and mycorrhizae development in the growth of containerized seedlings.

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CHAPTER I

GENERAL INTRODUCTION

Forest tree roots and specific non-pathogenic fungi form a symbiotic relationship first recognized by Frank (1885) as a regular association of roots and fungal mycelia and termed 'mycorrhiza.' He later proposed a classification of mycorrhizae into 'ectotrophic' and 'endotrophic' types (Frank, 1887), and included observations that the mycorrhizal habit was beneficial to the growth of the host tree. He considered the fungal symbiont to function as an absorbing organ for the plant and to provide to the host nutrients and soluble organic matter from the humus. Frank's views on the functions and significance of the mycorrhizal association were not well received by contemporary leading forest pathologists (Hartig, 1888), who were at that time convinced that the fungus was parasitic. Thus, a controversy began which persisted for nearly half a century.

Mycorrhizae of importance in forest regeneration are the ectomycorrhizae (i.e., ectotrophic) which are common among conifer species. The beneficial effects of ectomycorrhizae on pines and their significance for successful plantation establishment have been demonstrated in many parts of the world where attempts to afforest virgin areas with exotic pines failed because of the lack of suitable mycorrhizal fungi in the soil (Harley, 1969; Hacskaylo and Vozzo, 1971). The introduction of inoculum containing appropriate mycorrhizal fungi often overcame these problems. Inoculation of ectomycorrhizal plants is also important

when afforestation of naturally treeless areas is envisaged (Mikola, 1970, 1973; Shemakhanova, 1962), and when establishing new conifer nurseries (Briscoe, 1959; Mikola, 1970).

In many parts of the world there is great demand for large quantities of wood for building and industrial use. In the tropical and subtropical regions, much of the land now available for plantation establishment, particularly in Africa, is savanna with restricted rainfall, high temperature, low soil fertility and low productivity. The afforestation of these areas has presented the forester with considerable problems. When reforestation with indigenous species usually proved impracticable, the introduction of high yielding exotic tree species to supplement supplies of native timber became imperative. In these areas, the genus Pinus has, in recent years, offered promise for plantation establishment. Several species of Pinus are known which can grow rapidly on poor sites under adverse climatic conditions that hinder the growth of other species (Ekwebelam, 1975), and produce timber suitable for pulp for paper, and general construction. Consequently, several countries in these regions, including Nigeria, have shown great interest in the establishment of pines. Several exploratory studies suggest that even though satisfactory growth of pines has been obtained, factors such as the mycorrhizal association still appear to be critical. Fungi which form mycorrhizae with pines are absent from the soils of the relevant areas, and results with nursery stock have been very unsatisfactory.

In the temperate regions, on the other hand, increase in wood demand and the capital investment involved in the production of bare-root and containerized conifer seedlings have led to interest in the

search for techniques which would result in increased growth, greater survival and substantial savings to forest operations. In the state of Colorado, for example, lodgepole pine (P. contorta Dougl.) represents one of the major species of conifers established for timber and poles, and accounted for 85 percent of the 1975 production of wood (Betters et al., 1977). Currently, over two million seedlings are produced annually, representing an investment of about one million dollars (M. Strachan, personal communication). Fertilization and shading to improve containerized seedling vigor have gradually become accepted procedure in nursery management. However, relatively little appears to be known of the implications of these cultural practices for mycorrhizae formation and seedling growth in containers. Also, the need to incorporate photosynthetic analyses in mycorrhizal studies has been stressed (Slankis, 1973), but little or no attempt has been made to quantify the effects of photosynthetic production on mycorrhizae formation and vice versa.

The present work, therefore, describes interactions between light, nitrogen fertilization, mycorrhizae formation, and growth and photosynthesis of containerized lodgepole pine (P. contorta Dougl.) seedlings. The study seeks to quantify a growth response curve and photosynthesis of lodgepole pine as affected by varied levels of light, nitrogen fertilization and mycorrhizae formation, and to correlate ectomycorrhizae formation with photoassimilation. Quantifying seedling growth parameters as affected by each component above will be useful in development of a cultural regime optimizing seedling production in a nursery environment.

CHAPTER II

REVIEW OF LITERATURE

Ectomycorrhizae and Nutrient Uptake

It is well known that the formation of ectomycorrhizae influences seedling growth and increases uptake of nutrients by their host, particularly N and P (Bowen, 1973; Harley, 1969; Hatch, 1937; Lamb and Richards, 1971; Young, 1940). Some authors (Marx, 1969, 1973; Marx and Davey, 1969; Zak, 1964) also suggest that ectomycorrhizae offer the host protection against attack by pathogens. Further, differences in seedling vigor and nutrient uptake may be brought about by inoculating with different ectomycorrhizal fungi (Bowen, 1973; Cline and Reid, 1982; Ekwebelam, 1975, 1980; Harley, 1969). Differences in uptake of nutrients, particularly P, by ectomycorrhizae have been attributed to specific differences of the fungal mantles (Bowen, 1973; Harley, 1969), differences in the capacity of the fungi to utilize soil volume tapped by the mycorrhizae through the production of mycelial strands (Bowen, 1973; Harley, 1969; Hatch, 1937) and differences in respiratory activity of ectomycorrhizal fungi (Kramer, 1951; McComb and Griffith, 1946). Such differences may be affected by external factors, such as temperature (Mejstrik, 1970), oxygen supply (Brierley, 1955), nutrient concentration in the soil (Bowen, 1968, 1973; Bowen and Rovira, 1969; Harley, 1969; Marx, 1980; Smith, 1972) and internal factors, such as carbohydrate availability (Bjorkman, 1942, 1970; Hacskaylo, 1973; Harley, 1969; Lister et al., 1968).

Most studies on nutrient uptake by ectomycorrhizae have dealt with major nutrients, such as N, P and K. However, the best documented advantage of improved nutrition to the host by the mycorrhizae is the increased absorption and accumulation of P, although absorption of other nutrients is also facilitated (Bowen, 1973; Harley, 1969). This is probably because P is the element usually in low concentration in the soil, and because it is relatively immobile (Harley, 1969; Lindsay, 1979; Mengel and Kirby, 1979; Tisdale and Nelson, 1975). Mycorrhizae accumulate more P from the soil solution than nonmycorrhizal roots because the hyphae emanating from mycorrhizae extend beyond the zone of P depletion that develops adjacent to the root epidermis (Bowen, 1973; Gerdemann, 1975; Harley, 1969). In examining the mechanism of P absorption by ectomycorrhizae, several authors (Alexander and Hardy, 1981; Ashford et al., 1975; Harley, 1969; Ling Lee et al., 1975) reported that during P uptake, inorganic phosphates passed into the host core and accumulated in the fungal sheath. The work of Bartlett and Lewis (1973) indicates that mycorrhizal roots may possess surface phosphatases which could allow them to exploit organic soil phosphates more efficiently than nonmycorrhizal ones. Research has also suggested that nutrients, such as phosphates, may be released from iron and aluminum complexes in soil by hydroxyacids which are sometimes products of fungal metabolism, thereby making phosphates more available for absorption by the plants (Alexander and Hardy, 1981; Graustein et al., 1977; Harley, 1969).

In common with other basidiomycetes, ectomycorrhizal fungi appear to grow better on ammonium and simple organic nitrogen compounds than on nitrates (Bowen, 1973; Carrodus, 1966, 1967; France, 1980; Harley, 1969;

Lundeberg, 1970; McFee and Stone, 1968; Smith, 1972), and some appear to lack the enzyme nitrate reductase (Bowen, 1973; Harley, 1969, 1978; Richards, 1974; Trappe, 1967). The preference for ammonium by ectomycorrhizae appears to be dependent on carbohydrate supply to roots (Harley, 1969, 1978; Smith, 1972). Ammonium has also been reported to promote P uptake by mycorrhizae (McFee and Stone, 1968; Riley and Barber, 1971; Taber and McFee, 1972; Thien and McFee, 1970), and there is evidence that amino acids and more complex organic nitrogen compounds may exert a stimulating effect on the growth of ectomycorrhizal fungi. For instance, Hatch (1937) reported that ectomycorrhizal fungi associated with P. strobus could use nucleic acids and peptone as the sole source of nitrogen. Melin and Nilsson (1953a) observed that the hyphae of mycorrhizae of P. sylvestris absorbed and transported ^{15}N-glutamic acid to the host plant, and Carrodus (1966, 1967) demonstrated the uptake of glutamic acid, aspartic acid, glutamine and asparagine by excised beech mycorrhizae. However, unlike P uptake, there are relatively few reports on the mechanism of N absorption. Nevertheless, the few reports in this regard indicate that like P, the main site of assimilation of nitrogen compounds is the fungal sheath (Bowen, 1973), and that the main product of assimilation is glutamine or glutamic acid which is transferred to the host. This suggests that these two amino acids are forms of nitrogen transported in ectomycorrhizal associations (Bowen, 1973; France, 1980; Harley, 1969; Lewis, 1975).

In addition to a requirement for simple energy substrates, some ectomycorrhizal fungi have a need for accessory growth factors. Pure culture studies have revealed that most ectomycorrhizal fungi are partially or wholly dependent on thiamine or one of its constituent

moieties for normal growth (Harley, 1969; Marx, 1969, 1973; Melin and Norkrans, 1948). Certain fungal species are deficient in other vitamins as well, and unidentified growth-promoting substances have been found in root exudates (Slankis, 1973). Fried and Shapiro (1961) found a very rapid movement of sulfates through the mantle of beech mycorrhizae in culture with a sulfate concentration lower than normally found in soils. However, Morrison (1962b) demonstrated no differences in uptake of sulfates between mycorrhizal and nonmycorrhizal P. radiata seedlings of high, medium and low sulfur status. Increased uptake of zinc and other trace elements by mycorrhizae has also been reported (Bowen, 1973; Wilde and Iyer, 1962).

Mycorrhizae may influence processes other than ion uptake. The hyphae of mycorrhizal roots may behave like root hairs in water uptake. Under conditions of water stress, roots normally shrink, creating air gaps between roots and soil, and the hyphae from mycorrhizal roots, like root hairs, provide continuity between roots and soil. They may also bind soil to roots, thereby lessening shrinkage gap discontinuity, and excrete substances which may increase soil-root contact (Slankis, 1973). Thus, plants associated with ectomycorrhizal fungi may benefit from the symbiotic relationship, particularly in arid and semiarid areas (Cromer, 1935; Goss, 1960; Lobanow, 1960; Safir et al., 1971; Worley and Hacskaylo, 1959).

Unlike nonmycorrhizal roots, the uptake characteristics of mycorrhizal associations are governed by the absorbing characteristics of both symbionts. The fungal sheath around the mycorrhizae forms a compartment external to the root. This presupposes that an ion will encounter the fungus first and then move to the cortical cells and

stele. Detailed mechanisms of ion uptake by ectomycorrhizae have been reviewed by Bowen (1973) and Harley (1969).

In forest ecosystems, mycorrhizae help to conserve and cycle nutrients (Ruehle and Marx, 1979). The fungal hyphae readily penetrate litter and decomposing organic matter, and may spatially compete with other soil microorganisms for organic and inorganic nutrients far more efficiently than nonmycorrhizal roots.

Carbohydrate Allocation in Ectomycorrhizae

Establishment of mycorrhizae between higher plants and fungi depends to a large extent on the physiology of the host plant. Hacskaylo (1973) suggested that if sugars exuded from the host plant support metabolic activities of the mycorrhizal fungi, then the internal carbohydrate status of short roots could be directly related to a complex of factors regulating ectomycorrhizae formation. Factors, such as photosynthetic activity, translocation of assimilates, light intensity and availability of nutrients affect the concentration and composition of carbon compounds, and may therefore have a direct influence upon establishment and maintenance of mycorrhizae (Harley, 1969). Although mycorrhizal fungi utilize carbohydrates provided by the host, there has not been agreement on whether or not a cause and effect relationship exists between the amount of soluble carbohydrates in the roots and the degree of mycorrhizae formation.

Among many efforts to define the mechanisms regulating ectomycorrhizae formation, perhaps the most prominent were those of Bjorkman (1942). Experimenting with various levels of soil fertility and light intensity, Bjorkman observed that optimal mycorrhizal development occurred on seedlings subjected to nitrogen deficiency and high light

intensity, conditions which did not elicit maximum plant growth. Analysis of root carbohydrates showed that sugar contents were lower in shaded plants and in plants grown at high nitrogen levels. He concluded that high nitrogen reduced sugar concentration by favoring a rapid conversion of carbohydrates into amino acids. Presumably low light intensities suppressed photosynthesis and carbohydrate production. From these observations and later work, Bjorkman (1949, 1970) proposed that mycorrhizal formation was dependent on a surplus of soluble sugars in the roots, and the levels of these sugars were in turn determined by mineral availability, especially N and P, and light intensity. Lister et al. (1968) reached a similar conclusion.

Hacskaylo and Snow (1959) reported that at given levels of available nitrogen and phosphorus, mycorrhizae developed best on Pinus virginiana, P. taeda and P. strobus at moderate levels of nutrients in full sunlight, but high levels of N and P and low light intensity suppressed the formation of mycorrhizae, thus supporting Bjorkman's hypothesis.

Other investigators (Handley and Sanders, 1962; Meyer, 1962; Schweers and Meyer, 1970) however, disagreed with Bjorkman's carbohydrate theory. Handley and Sanders (1962) grew seedlings of Pinus sylvestris in nutrient solutions similar to those of Bjorkman. Analysis of root carbohydrates showed that the concentrations of soluble reducing substances increased as a result of mycorrhizal infection. They therefore concluded that mycorrhizal infection caused, rather than resulted from, the different carbohydrate concentrations found by Bjorkman.

Meyer (1962) grew seedlings of Fagus sylvatica in soils of varying N and P fertility. In soils of high nitrogen and phosphorus fertilities, mycorrhizae formation was not reduced but increased, and the greatest amounts of reducing substances were found in these roots. He attributed the increased sugar content of mycorrhizal roots to the activity of mycorrhizal fungi and other rhizoplane microorganisms, and suggested that auxins produced by the fungi may have promoted the hydrolysis of carbohydrates in the tissues and prevented the accumulation of starch in the stele. He therefore concluded that fungi absorbed carbohydrates from the tissues thereby increasing the flow of soluble carbohydrates from stems to roots.

Schweers and Meyer (1970) exposed seedlings of Pinus sylvestris to labeled carbon dioxide. The amount of $^{14}CO_2$ respired by the root systems was measured at the end of the assimilation period. Forty percent of the assimilated $^{14}CO_2$ was detected in the mycorrhizal roots and less than 10 percent in the uninfected roots. When other plant parts were measured, the assimilative activity was found to have increased with increase in mycorrhizal frequency, though the shoot/root ratios of activity decreased. They therefore concluded that the rising levels of sugars in the roots were the result of mycorrhizal infection and not the cause. Other workers (Richards and Wilson, 1963; Slankis, 1961) have also disagreed with Bjorkman's carbohydrate theory.

Harley (1969) criticised the techniques of carbohydrate analyses employed by Bjorkman and other workers partly because easily soluble reducing substances contain substances other than sugar, and partly because preparatory treatments of materials could have resulted in the formation of new and different reducing substances. He contended that

Bjorkman's failure to estimate the presence of non-reducing disaccharides in mycorrhizae could have confounded his results. In response to his critics, Bjorkman (1970) re-examined his carbohydrate theory using more sophisticated techniques for carbohydrate analysis. He inoculated seedlings of Pinus sylvestris with Boletus bovinus and B. subtomentosus in soils provided with different levels of nitrogen, phosphorus and potassium under varying light intensities. Roots were later examined for mycorrhizae, and needles and roots were analyzed for carbohydrate contents the same day using the technique of Meyer (1962). He again obtained results similar to his earlier experiment, and thus disagreed with the findings of his critics as outlined above. He therefore concluded that high light intensity and low soil fertility increased the level of sugars in the roots and enhanced mycorrhizae development.

Irrespective of specific analytical techniques employed in carbohydrate analysis, previous work has been hampered because mycorrhiza formation in itself changes the carbohydrate composition of short roots. Analysis of roots after infection may not be a reliable indication of sugars present before infection (Lewis and Harley, 1969a, b). This led Marx et al. (1977) to state that the greatest deficiency in assessing the effect of mineral nutrition on mycorrhizal infection has been the failure to analyze roots prior to infection. They therefore subjected seedlings of loblolly pine (P. taeda L.) to varying fertilization regimes for 10 weeks without ectomycorrhizae, analyzed the roots for carbohydrate contents, then inoculated the seedlings with Pisolithus tinctorius and observed infection of all seedlings after 21 to 28 days under constant conditions, followed by carbohydrate analysis of roots. Their results showed that more ectomycorrhizae were formed where

nutrients were limiting, and that sucrose level in the roots was positively correlated with percent mycorrhizal infection ($r^2 = 0.85$), thus supporting Bjorkman's hypothesis.

In discussing the influence of auxins and other metabolites on mycorrhizae formation, Slankis (1961) argued that soluble carbohydrates were not the sole factor in ectomycorrhizae formation. He contended that mycorrhizal roots may revert to nonmycorrhizal roots in the presence of a high concentration of nitrogen and phosphorus in the nutrient solution, although the reversion process does not occur when there is a decrease or deficiency in soluble sugars in the roots. Field studies have shown, however, that excessive fertilization does not always inhibit mycorrhizae formation (Shemakhanova, 1962). Thus, a discrepancy exists in comparing axenic and field systems in terms of high mineral concentration on mycorrhizae development. Also, one readily notes inconsistencies and pitfalls in previous axenic investigations. Most of the carbohydrate analyses included tissues from large woody laterals as well as short roots. Since mycorrhizal fungi only infect primary cortical tissues of short roots (Harley, 1969; Lamb and Richards, 1974; Zak, 1973), the dilution of primary tissues with a large volume of secondary tissues reduces the reliability of results. In addition, many roots were assayed only for reducing substances which may have precluded detection of other metabolically important carbohydrates (Harley, 1969).

Ectomycorrhizal fungi possess many properties in common with other rhizosphere and rhizoplane microorganisms, including preference for simple carbon sources. Although early reports (Melin, 1925) indicated that these fungi utilize only simple sugars, more recent research

(Linkins and Antibus, 1982; Lundeberg, 1948; Norkrans, 1950; Palmer and Hacskaylo, 1970; Young, 1947) has shown that certain species also appear to have some limited ability to use complex carbohydrates, such as cellulose and pectin, by promoting the formation of 'adaptive' enzymes (Cochrane, 1958) and production of cellulase (see Hacskaylo, 1973).

Source-Sink Relations

The mycotrophic relationship of mycorrhizal plants and the associations of other heterotrophic organisms with roots in a symbiotic relationship appears to be one in which the heterotrophs are entirely dependent on their host plants for carbon which they absorb as simple carbohydrates or immobilized carbon stores (Christie et al., 1978; Hacskaylo, 1973; Harley, 1969; Williams et al., 1982). Powered by this carbon source, the symbionts in turn benefit their hosts by increasing the availability of nutrients, particularly nitrogen and phosphorus, water and other metabolites (Bowen, 1973; Harley, 1969; Kucey and Paul, 1982; Paul and Kucey, 1981; Slankis, 1958, 1973; Williams et al., 1982).

The heterotrophic symbionts, in most cases, do not appear to satisfy their carbon requirements from the humus of the forest soils which contain relatively small amounts of soluble carbohydrates, and are incapable of breaking down cellulose (Bowen, 1973; Harley, 1969). Considerable evidence suggests that these organisms therefore depend on current photosynthates produced by their hosts to satisfy their demands for carbon. In other words, carbon flows from the photosynthetic organs of the host plants acting as a source of carbohydrates to the roots and associated symbionts acting as a sink. Such a flow was

clearly demonstrated in ectomycorrhizae by Melin and Nilsson (1957). These authors exposed Pinus sylvestris seedlings inoculated with mycorrhizae to $^{14}CO_2$ and traced the movement of ^{14}C through the plants. The ^{14}C was subsequently detected in the external hyphae of the mycorrhizae. This type of carbon transfer has also been suggested to occur in ectomycorrhizae of some Pinus species by Lister et al. (1968), Nelson (1964), Schweers and Meyer (1970) and Shiroya et al. (1962). Harley (1971) cites a study claiming that two-thirds of the photosynthates of Pinus cembra may be translocated to ectomycorrhizae.

Experimenting with Fagus sylvatica, Lewis and Harley (1965a, b, c) elegantly demonstrated the source-sink relationship in beech mycorrhizae. They showed that the transfer of sucrose from the host to the fungus depends on the latter functioning as a metabolic sink which receives and stores carbohydrates principally as fungal carbohydrates, trehalose and mannitol, and ultimately the storage polysaccharide glycogen, none of which can be reabsorbed by the host, thus maintaining a concentration gradient for further movement of sucrose from source (i.e., host) to sink (i.e., fungus).

Reports of movement of photosynthate-carbon from host to fungus in endomycorrhizal plants and other associated symbioses have also been published. For instance, Losel and Cooper (1979) demonstrated that vesicular-arbuscular (VA) mycorrhizal infection of onion roots with Glomus mosseae increased their ability to incorporate carbon from external sources, such as sucrose, acetate and glycerol as well as from photosynthetic assimilates.

Paul and Kucey (1981) and Kucey and Paul (1982) assessed the carbon flows to VA mycorrhizal fungi and rhizobial symbionts of 4- to

5-week-old Vicia faba by measuring the distribution of $^{14}CO_2$-C fixed by above-ground plant parts. Mycorrhizal fungi of both nodulated and non-nodulated hosts utilized about 4 percent of the carbon fixed by their hosts. Nodules utilized 6 percent of the carbon fixed by non-mycorrhizal hosts and 12 percent of the carbon fixed by mycorrhizal hosts. Measured rates of CO_2 fixation for symbiotic beans were higher than non-symbiotic beans. Besides, nodulated root systems of mycorrhizal beans fixed more $^{15}N_2$ than nodulated root systems of the nonmycorrhizal plants. An increase in biomass for plants infected with both rhizobia and mycorrhizal fungi was concluded to be the major factor increasing nitrogen fixation rates.

Bevege et al. (1975), Cox and Tinker (1976), Ho and Trappe (1973), Levy and Krikun (1980), Patrick (1972b) and Pang and Paul (1980) also established that carbon does move from hosts to fungal symbionts in endomycorrhizal plants. Pate et al. (1979) found that host tissues subtending nodules had higher rates of CO_2 evolution than host tissues not associated with nodules.

Pearson and Read (1973) and Stribley and Read (1974a) demonstrated movement of ^{14}C from the host plant to the external mycelium of ericaceous mycorrhizae.

The mechanisms of carbon movement from source (i.e., host) to sink (i.e., symbionts) in symbiotic relationships are not clear. The reports above seem to indicate that while a considerable degree of common behavior involving similar compounds and related processes is evident, the heterotrophic symbionts may produce a 'sink' for translocated carbohydrates from their hosts in essentially three ways: (i) utilization of carbohydrates for biomass, probably for increased structural

components, (ii) conversion of the carbohydrates received from their hosts into forms which cannot be utilized by their hosts, for example the storage compounds, such as trehalose, mannitol and glycogen, and (iii) rapid utilization of carbohydrates into maintenance of the structure, for example the energy required for membrane integrity, and used by carrier systems for active ion uptake. It is not known which of the three alternatives above could be the more important sink. However, the work of Barnard and Jorgensen (1977), Routien and Dawson (1943), Schweers and Meyer (1970) and more recently Reid et al. (1982) seem to suggest that alternative three, by way of increased respiration, is the more important of the alternatives.

Although the utilization of carbohydrates by the symbionts in these associations may establish a 'source-sink' relationship that stimulates photosynthesis, and subsequent translocation of assimilates to roots, other possibilities for stimulation may also exist. For instance, Johnson et al. (cited by Kucey and Paul, 1982) found that CO_2 fixation rates for mycorrhizal Citrus jambhiri seedlings were elevated over noninfected controls and attributed the increased CO_2 fixation of the mycorrhizal plants to increased phosphorus levels in the leaf tissues. Levy and Krikun (1980) found that mycorrhizal lemon seedlings recovered from water stress more quickly than nonmycorrhizal plants, and therefore had higher photosynthetic rates. This they assumed was due to mycorrhizal effects on water balance of the plant.

In a plant system as a whole, the various growth and storage centers of the plant compete for assimilates, and each center has a certain competitive and mobilizing ability whereby it can pull or attract assimilates against the effects of other centers. In short,

although allocation of materials for export from a source leaf is determined partly by carbohydrate metabolism in the source, sink tissues play a significant role in determining photosynthetic rates through their ability to mobilize assimilates. The mobilizing ability of a sink is a measure of its ability to import assimilates and is given by the absolute growth rate of the whole plant or plant parts, and varies depending upon the rate of supply of assimilates, reaching its maximum potential value under conditions of non-limiting supply (Wareing and Patrick, 1975).

However, although increased photosynthesis may result from increased translocation of assimilates to roots and other sink regions, some researchers have doubted the plausibility of the explanation of increase in photosynthesis as a result of source-sink effects. For instance, Geiger (1976) has suggested that increases and decreases in CO_2 fixation rates are probably due to indirect mechanisms, possibly involving hormonal control rather than direct mechanisms of feedback control by product inhibition.

Photosynthesis in Conifers

Plants show considerable variations in their ability to fix CO_2 by photosynthesis. Most higher plants, including conifer species, fix CO_2 by the C_3 pathway. Of the C_3 species, only one, Camissonia claviformis has so far been reported to achieve unusually high rates of photosynthesis, up to 94 mg $CO_2 dm^{-2} h^{-1}$ (Mooney et al., 1976). Most C_3 plants photosynthesize at no more than half this rate, and studies suggest that rates for conifers are generally lower than those for deciduous species (see Larcher, 1969, 1980; Salisbury and Ross, 1978).

Jarvis and Jarvis (1964) found that the maximum net CO_2 uptake for Pinus sylvestris and Picea abies seedlings in a growth room was in the range of 5 to 10 mg $CO_2 dm^{-2} h^{-1}$. Larcher (1969, 1980) reported photosynthetic rates of 5 to 18 mg $CO_2 dm^{-2} h^{-1}$ and 4 to 18 mg $CO_2 g^{-1} h^{-1}$ for Pinus sylvestris seedlings, and 10 to 40 mg $CO_2 g^{-1} h^{-1}$ for Picea abies seedlings. However, Zelawski et al. (1974) reported net photosynthetic rates as high as 33 mg $CO_2 g^{-1} h^{-1}$ for P. sylvestris seedlings.

Krueger and Ferrell (1965) found the maximum net photosynthesis for young Pseudotsuga menziesii seedlings grown in controlled environments to be near 18 mg $CO_2 dm^{-2} h^{-1}$, and Brix (1967) reported a rate of 12 mg $CO_2 dm^{-2} h^{-1}$ for seedlings of the same species. Krueger and Ruth (1969) measured rates near 15 mg $CO_2 dm^{-2} h^{-1}$ for Pseudotsuga menziesii and Picea sitchensis seedlings grown in the understory of spruce-hemlock forest stands.

Net photosynthetic rates of 18 mg $CO_2 g^{-1} h^{-1}$ and 20 mg $CO_2 g^{-1} h^{-1}$ were reported for Pinus radiata seedlings by van den Driessche and Wareing (1966) and van den Driessche (1972), respectively.

Kramer and Decker (1944) reported 3.4 mg $CO_2 dm^{-2} h^{-1}$ for individual needles of Pinus taeda, and a net photosynthesis rate of about 15 mg $CO_2 dm^{-2} h^{-1}$ was calculated for individual needles of the same species from data in Kramer and Clark (1947).

It can be seen from the above that there are large variations in the photosynthetic rates reported by different workers even within the same genus. Differences in rates have been attributed to specific differences in: measurements of surface area of leaves (Krueger and Ruth, 1969; Zelawski and Walker, 1976); such morphological factors as shape, age and orientation of the needles and branches; the amount of

quanta absorbed by the plants per unit of time in a particular light environment (Brunes et al., 1980; Zelawski and Walker, 1976); assimilation chambers used for photosynthetic measurements (Benecke, 1980; Jarvis et al., 1971); mutual shading among the needles (Kramer and Clark, 1947; Kramer and Kozlowski, 1979; Larcher, 1969, 1980; Zelawski and Walker, 1976); growing conditions of the plants (Botkin et al., 1970; Helm, 1970, 1976; Hodges and Scott, 1968; Woodman, 1971) and cultural treatments of plant materials (Brix and Ebell, 1969; Poskuta, 1968). Because of the complexity of these factors, it is often difficult to make meaningful quantitative comparisons of photosynthetic efficiency between species as reported by different workers (Kramer and Kozlowski, 1979; Sestak et al., 1971; Zelawski and Walker, 1976).

Although measurements of rates of photosynthesis among conifer species have been estimated in terms of the amount of CO_2 absorbed per unit of surface area of seedling needles (i.e., mg $CO_2 dm^{-2} h^{-1}$), such estimates of surface area are often difficult. However, there are a variety of photoplanimetric and other techniques available for this purpose (Kvet and Marshall, 1971), including the glass bead technique of Thompson and Leyton (1971). Baumgartner (1969) and Denmead (1969) have applied the meteorological technique to measure photosynthesis in conifer stands. More work is involved in the measurement and estimation of surface area of needles, but the results are more meaningful than expression of CO_2 fixation on a dry weight basis.

Some workers have expressed their results of net photosynthesis on the basis of dry weight (i.e., mg $CO_2 g^{-1} h^{-1}$) of seedling material, partly because of the difficulties involved in measuring and calculating surface area of conifer needles, and partly because dry weight is related to biomass values. Such a basis of expression has been

severely criticized because it does not take into account the shape or content and distribution of photosynthesizing tissues which are likely to influence leaf resistance to CO_2 uptake (Kramer and Kozlowski, 1979; Sestak et al., 1971; Zelawski and Walker, 1976). Also, dry weight has little or no direct relationship with radiation absorption, CO_2 uptake and enzyme activity (Sestak et al., 1971).

Several studies (Govindjee, 1975; Gregory, 1977; Kramer and Kozlowski, 1979; Larcher, 1969, 1980; Salisbury and Ross, 1978; Zelitch, 1971) have listed a number of variables which are likely to influence the rates of photosynthesis in plants. These include plant and environmental factors, such as age and leaf morphology, stomatal responses, light, temperature, CO_2 concentration, relative humidity and soil nutrient status. Because of the special relevance to the present study, light and soil nutrient status with particular reference to nitrogen fertilization will be reviewed.

Influence of Light on Photosynthesis

Photosynthesis is influenced by a complex of environmental and internal factors which are often interacting. Of the external factors, light appears to be the one that affects the photosynthetic process directly. Light-catalyzed reactions provide not only the ATP and nicotinamide di-hydrogen phosphate ($NADPH_2$) needed for CO_2 fixation and reduction to carbohydrates, but also regulates the activity of several chloroplast enzymes involved in photosynthesis (Salisbury and Ross, 1978). Studies of the kinetics of photosynthesis undertaken with a variety of plants have served to establish the relationship between the rates of photosynthesis and the incident light energy. The rate of photosynthesis has been found to be proportional to light intensity at

relatively low light intensities, but to become increasingly independent of light intensity at higher intensities (Kozlowski, 1949; Kramer and Decker, 1944; Ronco, 1970).

Kozlowski (1949) studied the effects of light intensities varying from 300 to 10000 foot-candles (ft-c) on photosynthesis of Acer rubra, Cornus florida and Pinus taeda, and found that P. taeda showed a consistent increase in photosynthetic rate with increase in light up to 10000 ft-c. A. rubra and C. florida reached maximum or near maximum photosynthetic efficiency at relatively low light intensities.

Kramer and Decker (1944) compared the net photosynthesis of Quercus borealis maxima, Q. alba, C. florida and P. taeda seedlings at light intensities varying from 300 to 10000 ft-c. All four species showed rapid increases in photosynthesis with increase in light intensity at the lower intensities. Net photosynthesis of P. taeda increased with increase in light intensity up to the equivalent of full sun, but the three broadleaved species achieved maximum net photosynthesis at one-third of full light, and any further increase in light intensity produced no further increase in the rate of photosynthesis.

Ronco (1970) noted that net photosynthesis of P. contorta seedlings did not become light saturated even at 12000 ft-c (i.e., 129000 lux), whereas light saturation for Picea engelmannii occurred at 5023 ft-c (i.e., 54000 lux).

The above reports seem to indicate that light saturation for photosynthesis not only varies with species, but also deciduous tree seedlings appear to saturate at much lower light intensities than conifer species. Data from several species also indicate that photosynthesis on a surface area basis is higher for sun than shade leaves

(Lewandowska and Jarvis, 1977). The greater photosynthetic efficiency of sun leaves compared to shade leaves has been attributed to their greater volume and more chlorophyll per unit of leaf area (Boardmann, 1977; Kramer and Kozlowski, 1979), lower stomatal and mesophyll resistances to CO_2 uptake (Holmgren, 1968; Zelitch, 1971), and more carboxylating enzymes per unit of leaf area (Alberte et al., 1976; Natr, 1972, 1975; Osman and Milthorpe, 1971).

The response of photosynthesis to light among species varies with such plant factors as leaf morphology and orientation, and age, largely as a result of the amount of foliage and shading. For instance, Kramer and Kozlowski (1979) noted that the radial arrangement of the primary needles of juvenile conifer seedlings gives better exposure and less mutual shading than the clusters of needles found on older seedlings. Kramer and Decker (1944) attributed the lower efficiency of P. taeda at low light intensity to mutual shading of the needles by each other, based on the observation of Uhl (1937) that the rate of photosynthesis of two-needled pines is greater per unit of surface area than that of five-needled species because the needles in the larger clusters shade each other more. Woodman (1971) pointed out that in a 38-year-old Douglas-fir with 18 whorls of branches, maximum photosynthesis occurred in the current year needles of the branches of whorl 7, a whorl intermediate between full light and full shade. These observed differences in rates of photosynthesis among leaves of different ages and from different locations on the plant often emphasize the necessity for extensive sampling, especially in the field, for reliable estimates of net photosynthesis of an entire tree.

Response of Photosynthesis to Nitrogen Fertilization

The level of mineral nutrition may have a profound influence on the rate of photosynthesis. Recent reviews (Foth and Turk, 1972; Gutschick, 1981) indicate that perhaps no element has received as much attention as has nitrogen in studies related to plant nutrition. Nitrogen is found in greater quantities in young growing parts of plants than in older tissues, and is especially abundant in the leaves and seeds (Salisbury and Ross, 1978). Nitrogen is a constituent of every living cell, and hence its contribution to life is evident (Baath et al., 1978; Dangerfield and Ebell, 1979). It is a constituent of the chlorophyll molecule and proteins which serve as enzymes, and affects photosynthesis by affecting chlorophyll and protein syntheses (Foth and Turk, 1972), leaf size (Brix, 1971; Longstreth and Nobel, 1980) and stomatal responses (Ishihara et al., 1979; Longstreth and Nobel, 1980).

In agricultural as well as forest crops, nitrogen is clearly one of the most important mineral nutrients regulating photosynthesis (Natr, 1975; Zelitch, 1971). A positive correlation has been established between net photosynthesis and foliar N for many agricultural crops (see Natr, 1975) and tree species (Brix, 1971, 1981). Since chlorophyll content is more or less proportional to nitrogen supply over a wide range, a deficiency of nitrogen inhibits photosynthesis (Keller and Koch, 1962; Keller and Wehrmann, 1963). Brix (1971) reported that the effect of increased nitrogen on net photosynthesis of Douglas-fir was partly produced by increasing the leaf area and only partly by increasing the rate of photosynthesis per unit of leaf area. Since a large proportion of the leaf protein is found in the chloroplasts, the effect of

increased nitrogen is undoubtedly reflected in improved structure and enzymatic activity in these organelles (Natr, 1975; Osman and Milthorpe, 1971).

The photosynthetic response of nitrogen fertilization varies with the form in which nitrogen is applied. Forest tree seedlings supplied with fertilizers containing ammonium-N have been reported to make greater growth and have higher photosynthetic rates than plants treated with fertilizers containing nitrate-N (Brix, 1971, 1981; Ingestad and Molin, 1960; van den Driessche, 1971, 1972), partly because ammonium promotes better uptake of nutrients, such as phosphorus (McFee and Stone, 1968), and partly because of changes in soil pH associated with the two nitrogen sources (van den Driessche, 1972). The photosynthetic rates of young Pinus sylvestris seedlings were increased more by nitrogen fertilizer supplied as ammonium chloride than by ammonium nitrate (Lotocki and Zelawski, 1973; Zajaczkowska, 1973, 1974). Recently, Brix (1981) compared the effects of ammonium nitrate and urea on foliar N concentration, photosynthetic rates and growth of Douglas-fir, and found that ammonium nitrate provided for a higher foliar N concentration and better growth the first year than urea, probably as a result of increase in soil pH following urea hydrolysis. A significant correlation was obtained between foliar N concentration and photosynthetic rates with an optimum at 1.74 percent foliar N, without an effect of nitrogen source. However, studies indicate that prolonged nutrition with ammonium-N may result in accumulation of ammonia in leaves which unfavorably affects chloroplast structure, with a consequent decrease in net photosynthesis (Natr, 1972, 1975).

Plant nutrition can be considered in terms of both intensity and balance (Shear et al., 1946), and any change in the concentration of any one element in the plant is usually accompanied by a change in the concentration of the other elements. Increases in nitrogen supply level generally decrease foliar P and K concentrations (Cain, 1959). Hence, the response to nitrogen fertilization of net photosynthesis may depend on the relative levels of these elements, through their influences on certain plant processes connected with photosynthesis. For instance, phosphorus is a component of nucleotides, and the coenzyme adenosine triphosphate (ATP), which acts as an intermediate energy transfer compound in such cell functions as photosynthesis, respiration, biosynthesis, stomatal opening and transfer of organic solutes across membranes. Phosphorus deficiency therefore may impede photosynthesis by disturbing the energy transfer by ATP, although the depressing effect appears to be relatively weak (Pirson, 1958; Zelitch, 1971). Similarly, potassium is involved in the electron transport system of the thylakoids, and potassium ion flux is also related to stomatal responses (Longstreth and Nobel, 1980). The photosynthetic capacity of leaves may also be influenced by potassium nutrition since stomatal opening and closing are slow in potassium-deficient trees (Davies and Kozlowski, 1974). Consequently, shortage of potassium, like phosphorus, may impede photosynthetic energy transfer and increase respiration (Pirson, 1958), thus lowering the rate of photosynthesis.

CHAPTER III

NITROGEN FERTILIZATION AND LIGHT EFFECTS ON GROWTH AND PHOTOSYNTHESIS OF LODGEPOLE PINE SEEDLINGS

Introduction

The production of containerized seedlings for afforestation is expanding rapidly. The growth of such seedlings, when not limited by water, temperature and disease problems, may be influenced by mineral nutrition, such as nitrogen, and by light intensity.

The utility of shading and fertilizing nursery stock to optimize seedling vigor has been demonstrated (Tinus and McDonald, 1979), and the implications of such applied cultural practices in photosynthesis and yield of containerized conifer seedlings have received little but incidental attention. For instance, nitrogen is involved in many processes, in addition to photosynthesis (Gutschick, 1981), and nitrogen nutrition can be altered by changing its composition in the nutrient solution. Similarly, light fluctuates widely and its influence on seedling growth and photosynthesis may vary depending on its intensity, and the growth stage and mineral nutrition of the seedlings. Manipulations of both factors for optimum seedling growth as currently practiced in most conifer nurseries call for a better understanding of how these factors affect seedling growth and photosynthesis.

Studies have reported effects of nitrogen nutrition (Brix, 1971, 1981; van den Driessche, 1972) and light intensity (see Kramer and Kozlowski, 1979; Larcher, 1969, 1980) on growth and photosynthesis of

conifer seedlings. Although the relationships between seedling growth and photosynthesis as affected by nitrogen nutrition and light intensity have been studied for some conifer species, no previous comparative work concerning the interaction of varied levels of light and nitrogen fertilization on growth and photosynthesis of lodgepole pine (P. contorta Dougl.) seedlings is apparent.

The present work, therefore, examines light and nitrogen effects on growth and photosynthesis of lodgepole pine seedlings.

Materials and Methods

Establishment of Plant Material

Seed of lodgepole pine (P. contorta Dougl.) were surface-sterilized in 1.1 percent sodium hypochlorite for 20 min and sown directly into a sterile potting mix consisting of coarse vermiculite and finely-sieved peat (5:2 v/v) wetted with distilled water. One hundred and thirty-five presterilized "Ray-Leach" containers (150 cm^3 capacity) were filled with the sterile potting mixture. Four seeds were sown into each container and misted daily until germination. Four days after germination, seedlings were thinned to one per container.

All seedlings were grown in an electrostatic HEPA-filtered fiberglass chamber within a standard greenhouse, designed specifically to minimize air-borne contamination by fungal spores. Day/night temperatures were 30/26 ±2°C, and a 16-h photoperiod was maintained by supplemental lighting with fluorescent cool beam lamps.

Experimental Design

Seedlings were grown at three relative levels of irradiance established by use of commercial shadecloth netting (nominal rating of

55 percent shade). Placing two, one or no layer(s) of shadecloth above the seedlings gave about 100, 210 and 470 μEinstein (E)$m^{-2}sec^{-1}$ quantum flux density, respectively, as measured with a LI-170 Quantum Sensor (Lambda Instrument Co., Lincoln, Nebraska, USA) at midday during the part of the growing season with the lowest ambient sunlight. Maximum values reached without shadecloth were near 610 $\mu Em^{-2}sec^{-1}$. These three ambient levels of sunlight will hereinafter be referred to as low, medium, and high levels, respectively. Seedlings were fertilized with Hocking's (1971) nutrient solution with the following modifications: nitrogen was added as ammonium nitrate to give levels of 3, 62, and 248 ppm of N, calcium chloride was substituted for calcium nitrate, sulfur was changed from 150 to 64 ppm, and sequestrene 330 Fe was substituted for ferric chloride (see Appendix 1).

After thinning, seedlings were arranged into 9 groups of 15 seedlings each and grown without ectomycorrhizae for 16 weeks. Plants which were treated with ectomycorrhizae are reported in Chapter IV. The groups were randomly assigned to the light and nitrogen treatments, and randomized on the greenhouse bench weekly to minimize position effects. Fertilization commenced one day after thinning and daily thereafter. To maintain container concentrations of nitrogen near the applied levels, the nutrient solution was added daily in excess to allow flushing of the potting mix (R.W. Tinus, private communication). No other irrigation was necessary.

At 5, 10 and 16 weeks growth, a random sample of 5 seedlings was selected from each treatment combination for determination of net photosynthesis (P_n) and respiration (R_s) rates, growth parameters (i.e., dry weights of needles and shoots, roots and root/shoot ratios) and

foliar N and P. At 16 weeks, prior to photosynthetic measurements, stomatal conductance was determined on all seedlings.

Photosynthesis and Respiration Measurements

Net photosynthesis (P_n) and dark respiration (R_s) rates of seedlings were determined by CO_2 analysis using a Beckman model 315A (Beckman Instrument, Pasadena, California, USA) infrared gas analyzer (IRGA) in an open system (Fig. 1) designed to detect small changes in CO_2 concentrations. The principal components of the system included a 700 cm^3-capacity plexiglas assimilation chamber or cuvette (Fig. 2), a differential infrared gas analyzer, three drying tubes holding a mixture of anhydrous calcium sulfate and magnesium perchlorate (1:2 v/v) and flow meters. Ambient air was pumped through a 210-l mixing reservoir dehumidified with a mixture of calcium sulfate and magnesium perchlorate (1:2 v/v) and divided into sample and reference lines. The length and volume of the pathway was identical for both sample and reference lines. Flow rates (ca 1.5 l min^{-1}) were adjusted to maintain CO_2 concentrations in the sample cuvette within ±10 percent of ambient. The CO_2 concentration differential between the reference and sample lines was measured following calibration of the IRGA for a full-scale deflection of 50 ppm using standard gases. To provide vigorous mixing within the assimilation chamber and minimize boundary air layer resistances, a small fan was incorporated into the assimilation chamber, driven by a 4.5-volt motor (see Fig. 2). The fan caused very slight leaf flutter and eliminated variations in rate determinations.

Incandescent light was provided by seven 300-W narrow-spot General Electric cool beam reflector lamps positioned about 1 m above the assimilation chamber, and was filtered through a continuous flow

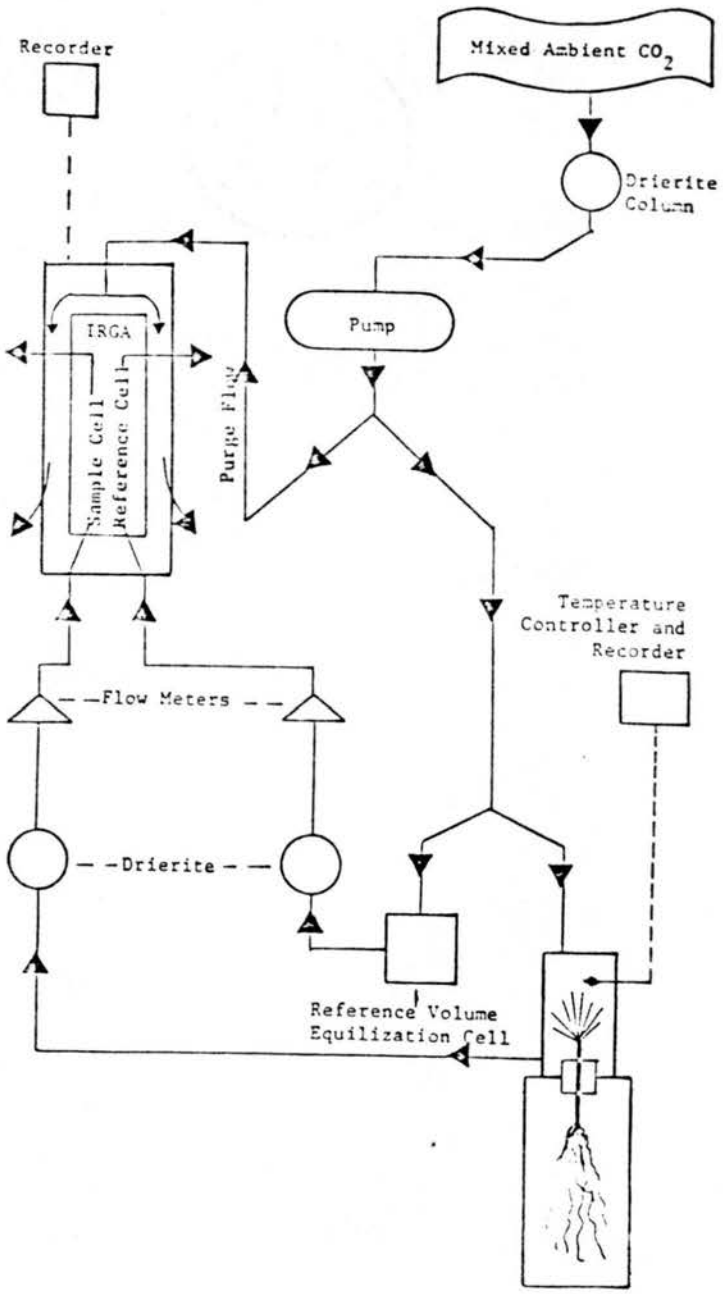


Figure 1. Schematic drawing of the open system of infrared gas analysis (IRGA) used to measure CO_2 exchange of pine seedlings.

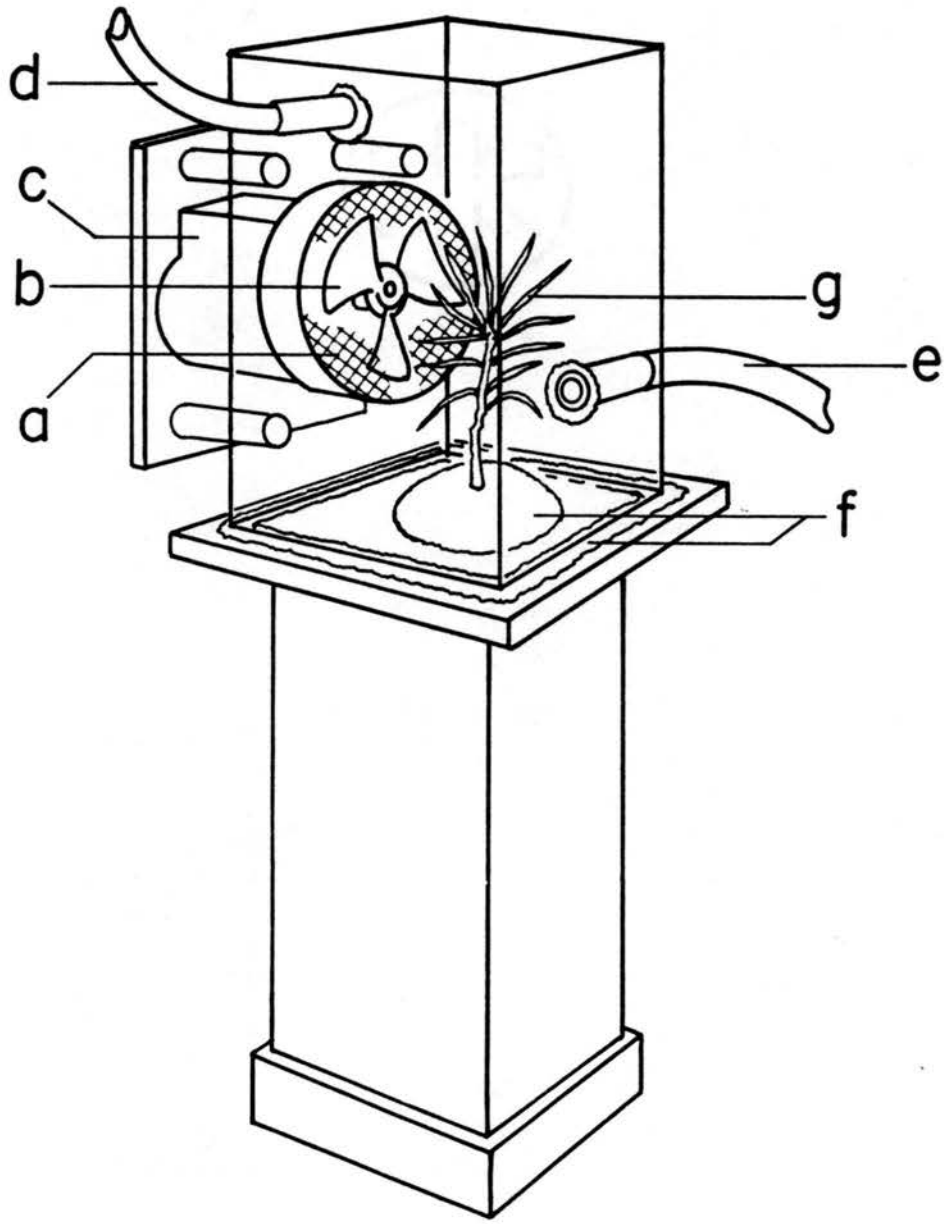


Figure 2. Diagrammatic representation of the assimilation chamber used to measure CO_2 exchange in an open system of infrared gas analysis (IRGA). (a) Nylon mesh. (b) Fan. (c) Magnetic motor. (d) Inlet tygon tube. (e) Outlet tygon tube. (f) Terostat sealant. (g) Pine seedling.

water bath 15 cm deep to reduce much of the infrared component. At 10- and 16-week harvests, P_n and R_s rates were determined at 100, 210 and 470 $\mu Em^{-2}sec^{-1}$ by use of shadecloth for seedlings grown at low, medium and high levels of irradiance, respectively; at 5 weeks all seedlings were measured at 470 $\mu Em^{-2}sec^{-1}$.

The sequence of selecting seedlings for photosynthetic measurements from each treatment combination was completely random. Seedlings remained in the vermiculite-peat mix during measurements and were well-watered prior to each measurement. Contribution of CO_2 to the system from the belowground portions of the seedlings was excluded by isolating the shoot cuvette from the root system. Terostat sealant (Terosan GmbH Heidelberg) was used to seal around the stem at the root collar. Prior to each experimental run, the seedlings were acclimatized for 60 min at the appropriate irradiance level. After this period, the assimilation cuvette was completely sealed at the base with Terostat and measurements of P_n taken for 20 min. Respiration rates also taken for 20 min were determined immediately after P_n by placing the cuvette system in a dark chamber maintained at the same temperature as under the lights. A 15-min adjustment period of the IRGA was allowed at the completion of each P_n and R_s measurement before a new run was started. Air temperature within the cuvette in all determinations of P_n and R_s rates were maintained at 31 ±2°C, and were continuously monitored with a thermistor probe model 8502-20 (Cole Palmer Instrument Co., Chicago, Illinois, USA). All measurements were recorded on a single strip chart recorder (Hewlett Packard, Pasadena, California, USA).

At the end of each measurement, the individual seedling needles were clipped and hand-sorted into green and nongreen portions. The

needle lengths and widths were measured for calculation of surface area. Since the majority of the needles were primary needles and the shape approximated a half cylinder, the surface area of the needles was calculated using the expression:

$$\text{Area } (dm^2) = \pi RT$$

where R and T represent average radius and total lengths of needles, respectively. The seedling components of shoots and roots were then placed in plastic bags, sealed and stored at -20°C until the end of the day when they were taken to the laboratory for determination of biomass and foliar N and P.

P_n rates were calculated on the basis of both surface area (SA) and dry weight (DWT) of aboveground green biomass. Calculation of R_s rates on the other hand, was based on the entire aboveground SA and DWT of entire seedling top. All computations expressed on mg CO_2 per h were computed by the expression:

$$P_n \text{ or } R_s = [(\text{ppm } CO_2 \cdot F/h \cdot 60 \cdot 10^{-6} \cdot 10^3)(MT_1P/LTP_1)/SA \text{ or DWT}]$$

P_n = net photosynthesis based on either surface area (mg $CO_2 dm^{-2} h^{-1}$) or dry weight (mg $CO_2 g^{-1} h^{-1}$) of aboveground green biomass

R_s = aboveground dark respiration (mg $CO_2 dm^{-2} h^{-1}$) or (mg $C0_2 g^{-1} h^{-1}$)

F = flow rates of 1.5 l per min

ppm CO_2 = CO_2 exchange rate in parts per million converted to volume fraction of CO_2 by multiplying by 10^{-6}, and then to mg CO_2 per h my multiplying by 10^3 and 60 min

M = volume weight of CO_2 (44.010 mg)

L = mole volume of CO_2 (22.414 l)

T_1 = 273°K

T = assimilation chamber air temperature in °K (304°K)

P = average barometric pressure (647 mm Hg)

P_1 = standard barometric pressure (760 mm Hg)

SA = surface area in dm^2 of green, including nongreen aboveground biomass for determination of R_s rates

DWT = dry weight in g of total green, including nongreen aboveground biomass for determination of R_s rates

Determination of Stomatal Conductance

Stomatal conductance was determined at 16 weeks using a null-balance porometer, LI-COR model 1600 (LI-COR, Inc., Lincoln, Nebraska, USA) with a conifer needle chamber cuvette. Seedlings remained in the vermiculite-peat mix and were well-watered prior to measurement. Stomatal conductance was determined at 100, 210 and 470 $\mu Em^{-2}sec^{-1}$ by use of shadecloth for seedlings grown at low, medium and high levels of irradiance, respectively.

The sequence of selecting seedlings for stomatal conductance measurements from each treatment combination was completely random, and all measurements were made at 31 ±2°C, corresponding to the temperature for photosynthetic measurements. Prior to each measurement, seedlings were acclimatized for 60 min under the appropriate light treatment.

Stomatal conductance expressed in cm sec^{-1} was calculated as the reciprocal of resistance, and obtained as previously described (Anon, 1980) using the expression:

$$R = (Rd + 0.15) \cdot \frac{A_t}{A}$$

where R, Rd, A and A_t represent actual diffusive resistance, diffusive resistance read on the instrument, pre-set area (10 cm^2) entered into the instrument microprocessor and actual area of needles, respectively. Needle area was obtained as previously described above.

Estimation of Biomass and Foliar Analysis

Root and shoot dry weights of seedlings were determined after oven-drying at 68°C for 48 h. Dry needle tissues (i.e., green and non-green) were digested by the method of Thomas et al. (1967). Nitrogen was determined by the salicylate isocyanurate method (Bigelow et al., 1982) and P by the molybdate blue method (Murphy and Riley, 1962).

Data Synthesis

Measured parameters (i.e., growth, photosynthetic rates, foliar N and P, stomatal conductance and ratio of surface area to dry weight of needles) were examined by factorial analysis of variance (ANOVA) (Steel and Torrie, 1980). When significant differences were found between or among treatments, treatment means were compared at the 5 percent probability level by Tukey's test for Honestly Significant Difference (HSD) (Steel and Torrie, 1980). To simplify data presentation and interpretation, in some cases treatments were pooled if no significant interaction occurred in the ANOVA.

Results

Dry Matter Production

Results of mean values for dry matter (i.e., shoot and root dry weights) of seedlings at 5, 10 and 16 weeks of harvest are shown in Fig. 3. Analysis of variance revealed that there were significant ($p = 0.05$) interactions between light and nitrogen levels on dry weights of

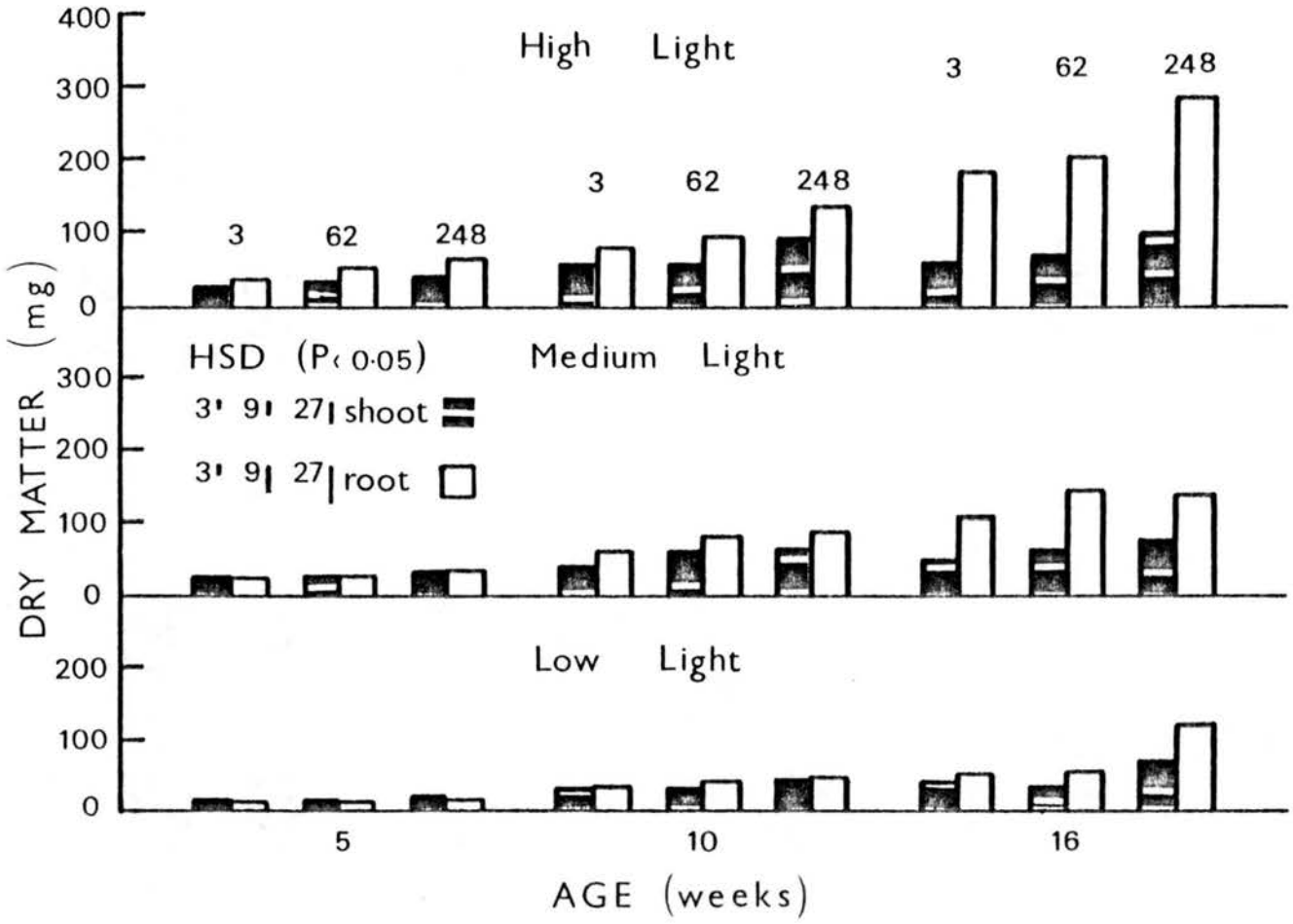


Figure 3. Dry matter production of P. contorta seedlings at 5, 10 and 16 weeks of age in response to 3 levels of irradiance (high, medium and low) and nitrogen (3, 62 and 248 ppm). Vertical bars labeled 3, 9 and 27 represent HSD ($p = 0.05$) for comparisons of 3, 9 and 27 means within any one, two and all treatments, respectively. All figures are means of 5 seedlings.

shoots and roots relative to the age of the seedlings. On each of the three harvest dates, increases in shoot and root dry weights were proportional to increases in light and nitrogen levels over the ranges studied. At the three nitrogen levels, high light resulted in a significant ($p = 0.05$) increase in production of shoot and root dry weights, compared to low light. Similarly, under the three light levels, maximum and lowest dry weights of shoots and roots were attained at the high (i.e., 248 ppm) and low (i.e., 3 ppm) nitrogen levels, respectively. At 5 and 10 weeks, the effect of nitrogen treatment on root biomass was greater as light intensity increased, and at 16 weeks root biomass doubled from 3 to 248 ppm N at low light. For example, at 5, 10 and 16 weeks of age, root dry weights averaged, respectively, 66.2, 137.2 and 284.2 mg for high nitrogen as compared to 42.2, 80.6 and 182.0 mg for low nitrogen levels at high light. At low light, root dry weights averaged 17.4, 46.6 and 92.8 mg for high nitrogen, compared to 12.4, 32.6 and 47.2 mg for low nitrogen at 5, 10 and 16 weeks of harvest, respectively.

Consistent with increase in dry matter, root/shoot ratios also increased significantly ($p = 0.05$) from low to high light at each period of harvest (Fig. 4). However, root/shoot ratios generally appeared to increase from 3 to 62 (i.e., intermediate) ppm N, and decreased from 62 to 248 ppm N during the three harvests (Fig. 4). For example, at 5, 10 and 16 weeks of age, and at high light, root/shoot ratios averaged, respectively, 1.30, 1.47 and 3.00 for low nitrogen, 1.38, 1.61 and 3.10 for intermediate nitrogen and 1.45, 1.48 and 2.88 for high nitrogen. At low light, root/shoot ratios averaged 0.66, 1.08 and 1.41 for low nitrogen, 0.74, 1.24 and 1.57 for intermediate nitrogen, and

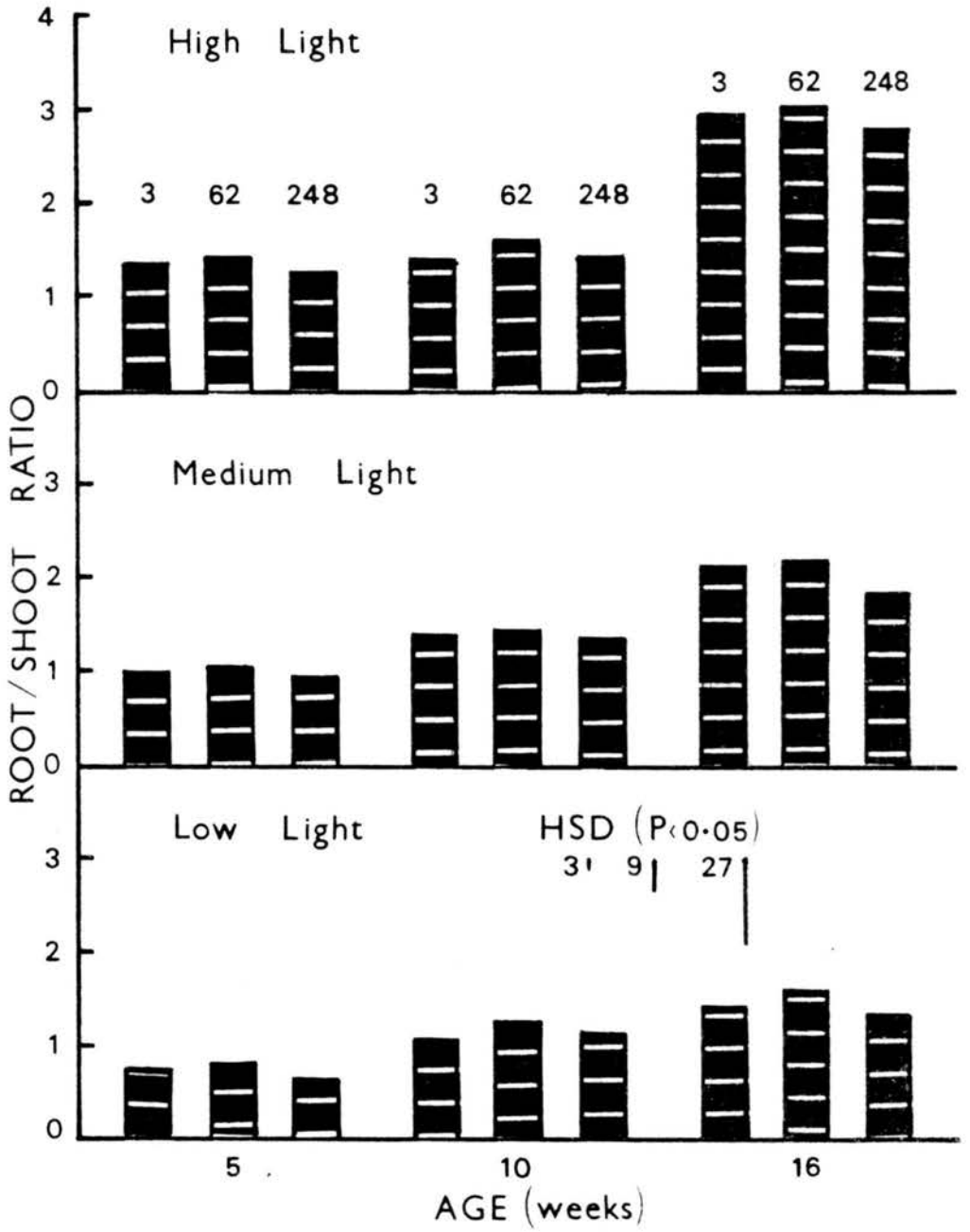


Figure 4. Root/shoot ratio of P. contorta seedlings at 5, 10 and 16 weeks of age in response to 3 levels of irradiance (high, medium and low) and nitrogen (3, 62 and 248 ppm). Vertical bars labeled 3, 9 and 27 represent HSD (p = 0.05) for comparisons of 3, 9 and 27 means within any one, two and all treatments, respectively. All figures are means of 5 seedlings.

0.80, 1.16 and 1.35 for high nitrogen at 5, 10 and 16 weeks of harvests, respectively.

Nutrient Concentration and Content

Analysis of the effects of light and nitrogen on N and P contents of foliage indicated significant ($p = 0.05$) interactions between light and nitrogen treatments at 5, 10 and 16 weeks of age. Therefore data on N and P concentrations and total contents are presented for each treatment combination during the harvests (Figs. 5 and 6). On each of the three harvest periods, N and P concentrations generally decreased significantly ($p = 0.05$) with increasing levels of light (Fig. 5). Higher concentrations of nutrients, especially N at low light probably indicate that light was limiting growth and indirectly limiting the use of N, such that N could not be fully used in biomass. However, at 5 and 10 weeks of age, N concentrations increased from low to high nitrogen levels at both high and low light levels (Fig. 5). At 16 weeks, N concentrations increased when soil N was increased from 3 to 248 ppm N at low light, but decreased from 3 to 248 ppm N at high light. At the intermediate light levels, percent N at 5 weeks increased from low to high nitrogen, but at 10 and 16 weeks, N concentrations decreased from low to high nitrogen levels.

Despite the low nutrient concentrations at high light as shown in Fig. 5, the general trend during the harvests was an increase in total N and P contents with increasing levels of both light and nitrogen (Fig. 6), reflecting increased dry matter (Fig. 3).

Figure 5. Foliar N and P concentrations of P. contorta seedlings at 5, 10 and 16 weeks of age in response to 3 levels of irradiance (high, medium and low) and nitrogen (3, 62 and 248 ppm). Vertical bars labeled 3, 9 and 27 represent HSD ($p = 0.05$) for comparisons of 3, 9 and 27 means within any one, two and all treatments, respectively. All figures are means of 5 seedlings.

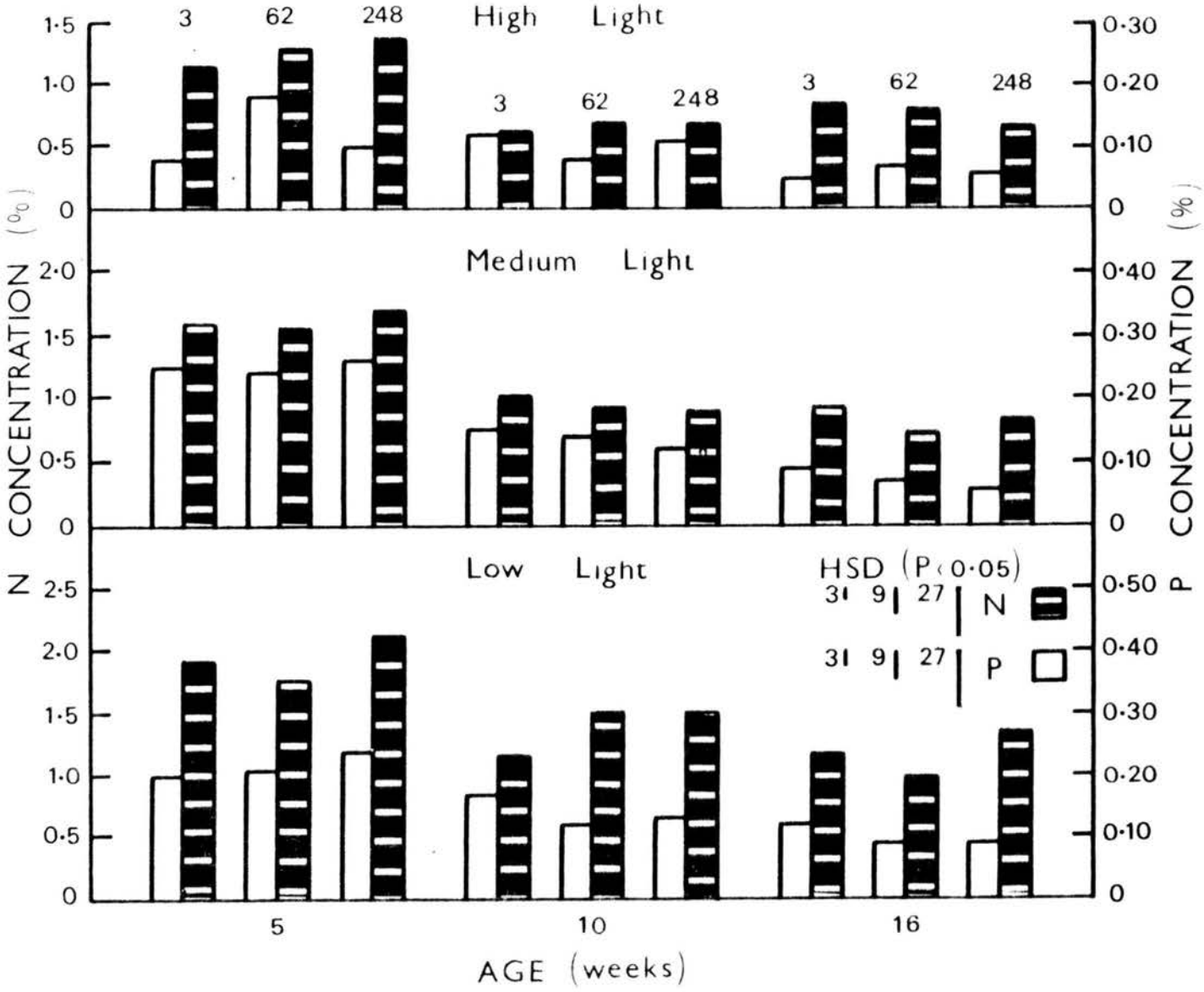
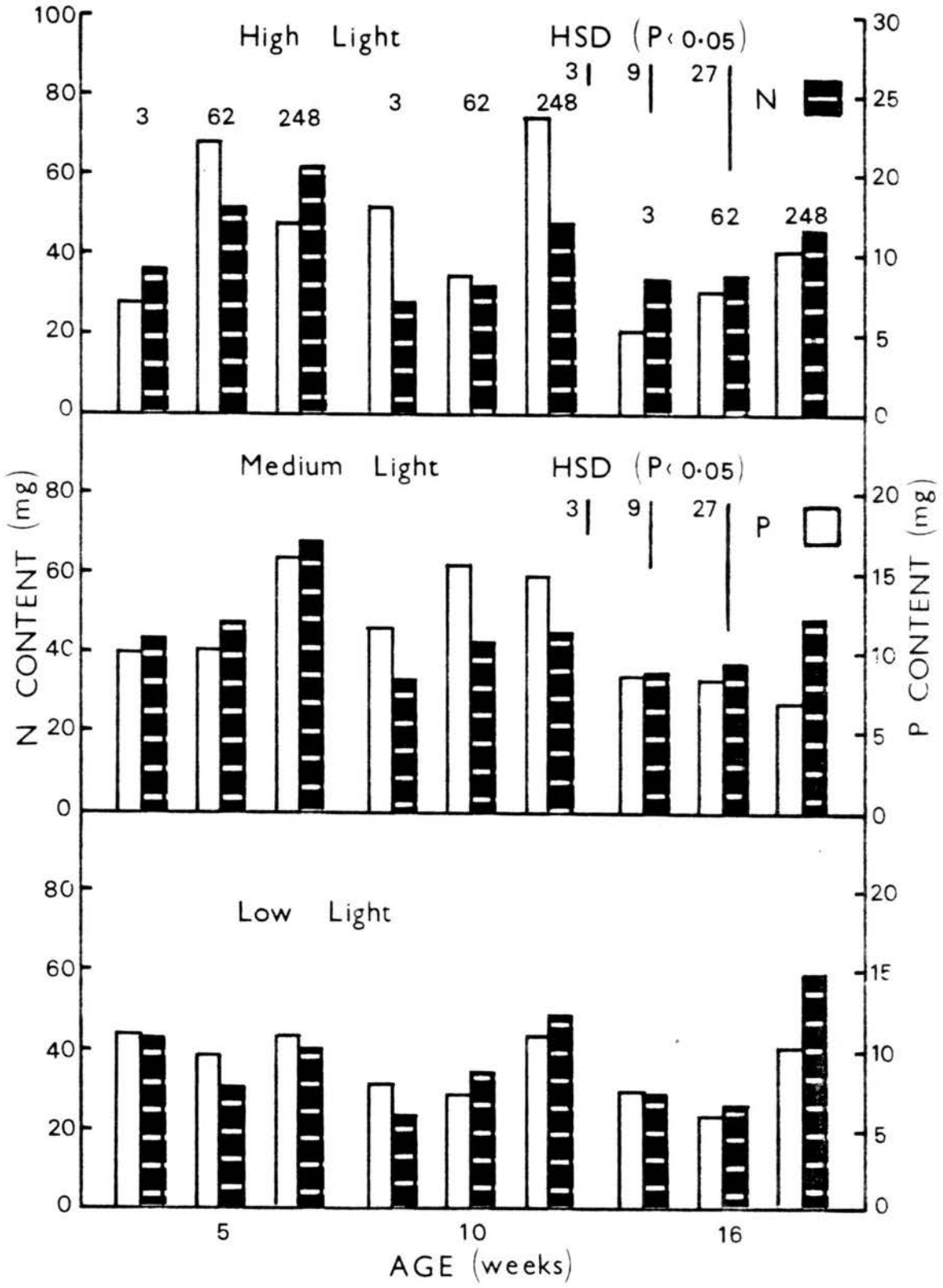

3
62
248
High Light
3
62
248
3
62
248
Medium Light
Low Light
HSD (P<0·05)
31 9 27 N
31 9 27 P
N CONCENTRATION (%)
P CONCENTRATION (%)
1·5
1·0
0·5
0
2·0
2·5
0·30
0·20
0·10
0·40
0·50
5
10
16
AGE (weeks)

Figure 6. Total foliar N and P contents of *P. contorta* seedlings at 5, 10 and 16 weeks of age in response to 3 levels of irradiance (high, medium and low) and nitrogen (3, 62 and 248 ppm). Vertical bars labeled 3, 9 and 27 represent HSD ($p = 0.05$) for comparisons of 3, 9 and 27 means within any one, two and all treatments, respectively. All figures are means of 5 seedlings.


High Light
HSD (P< 0·05)
3
9
27
N
3
62
248
3
62
248
3
62
248
Medium Light
HSD (P< 0·05)
3
9
27
P
Low Light
N CONTENT (mg)
P CONTENT (mg)
AGE (weeks)
5
10
16

Net Photosynthesis

Since analysis of variance of the effects of light and nitrogen indicated that there were no significant interactions between light and nitrogen treatments on P_n rates either in terms of surface area (mg $CO_2 dm^{-2} h^{-1}$) or dry weight (mg $CO_2 g^{-1} h^{-1}$) during the harvests, data on responses to light were pooled within nitrogen treatments and vice versa. In terms of P_n expressed on the basis of surface area (Table 1), high levels of light and nitrogen generally resulted in higher P_n rates. At 5 and 10 weeks, P_n rates for high light were significantly ($p = 0.05$) higher than at both the intermediate and low light levels, which also differed from each other; the low level of light produced the lowest P_n rates. Similarly, at 5 and 10 weeks, high nitrogen produced significantly ($p = 0.05$) higher P_n rates than low nitrogen levels (Table 1). At 16 weeks, high light and high nitrogen also appeared to produce higher P_n rates, compared to low levels of each component, although differences between them were not significant at the 5 percent level (HSD).

In terms of P_n on a dry weight basis (Table 2), except at 5 weeks when high nitrogen appeared to result in higher P_n rates relative to low nitrogen, high light and high nitrogen generally appeared to decrease P_n rates, although treatment mean comparisons with low levels of each component were never significant at the 5 percent level (HSD).

Respiration

Analysis of variance revealed that there were no significant interactions between light and nitrogen levels on R_s rates on the basis of either surface area (Table 3) or dry weight (Table 4) during the harvests. Therefore, light observations were pooled within nitrogen

Table 1. Net photosynthesis (mg $CO_2 dm^{-2} h^{-1}$) of P. contorta seedlings at 5, 10 and 16 weeks of age in response to irradiance and nitrogen levels. All values are means of 15 seedlings.[1]

Age (weeks)	Irradiance[2] Low	Medium	High	Nitrogen[2] (ppm) 3	62	248
5	2.41c[3]	3.02b	3.96a	2.25c	3.26b	3.88a
10	1.33b	1.82ab	2.27a	1.57b	1.74ab	2.13a
16	1.14a	1.46a	1.64a	1.23a	1.41a	1.60a

[1]Photosynthesis was measured under 100, 210 and 470 $\mu Em^{-2} sec^{-1}$ for seedlings grown at low, medium and high levels if irradiance, respectively.

[2]Means were compared between levels within each treatment (light or nitrogen) at each age.

[3]Means followed by similar letters are not significantly different ($p = 0.05$) by HSD.

Table 2. Effect of irradiance and nitrogen levels on net photosynthesis (mg $CO_2 g^{-1} h^{-1}$) of P. contorta seedlings at 5, 10 and 16 weeks of age. All values are means of 15 seedlings.[1]

Age (weeks)	Irradiance[2] Low	Medium	High	Nitrogen[2] (ppm) 3	62	248
5	10.27a[3]	10.05a	9.03a	9.34a	10.09a	9.93a
10	6.23a	6.49a	4.81a	6.47a	6.37a	4.68a
16	4.51a	3.48a	3.16a	3.85a	3.96a	3.34a

[1]Photosynthesis was measured under 100, 210 and 470 $\mu Em^{-2} sec^{-1}$ for seedlings grown at low, medium and high levels of irradiance, respectively.

[2]Means were compared between levels within each treatment (light or nitrogen) at each age.

[3]Means followed by similar letters are not significantly different ($p = 0.05$) by HSD.

Table 3. Respiration (mg $CO_2 dm^{-2} h^{-1}$) of P. contorta seedlings at 5, 10 and 16 weeks of age as affected by irradiance and nitrogen levels. All values are means of 15 seedlings.[1]

Age (weeks)	Irradiance[2] Low	Medium	High	Nitrogen[2] (ppm) 3	62	248
5	2.30a[3]	1.70b	2.22a	1.91b	1.83b	2.48a
10	0.92a	0.75a	0.96a	0.76a	0.86a	1.00a
16	0.65b	0.75ab	1.08a	0.84a	0.78a	0.86a

[1]Respiration was measured in the dark.

[2]Means were compared between levels within each treatment (light or nitrogen) at each age.

[3]Means followed by similar letters are not significantly different (p = 0.05) by HSD.

Table 4. Effect of irradiance and nitrogen levels on respiration (mg $CO_2 g^{-1} h^{-1}$) of P. contorta seedlings at 5, 10 and 16 weeks of age. All values are means of 15 seedlings.[1]

Age (weeks)	Irradiance[2] Low	Medium	High	Nitrogen[2] (ppm) 3	62	248
5	7.61a[2]	4.91b	4.46b	5.57a	5.07a	6.33a
10	1.28a	0.87a	0.63a	1.09a	0.88a	0.81a
16	1.67a	1.25a	1.38a	1.69a	1.37a	1.24a

[1]Respiration was measured in the dark.

[2]Means were compared between levels within each treatment (light or nitrogen) at each age.

[3]Means followed by similar letters are not significantly different (p = 0.05) by HSD.

treatments. In terms of surface area, R_s rates increased significantly ($p = 0.05$) at low and high irradiance levels compared to medium light at 5 weeks of age. Although R_s rates appeared to be little affected by light levels at 10 weeks, R_s rates increased significantly ($p = 0.05$) from low to high light at 16 weeks of age. Similarly, nitrogen application resulted in significantly ($p = 0.05$) higher R_s rates at 5 weeks, but R_s rates appeared to be little affected by nitrogen fertilization levels during the 10- and 16-week harvests (Table 3).

On the basis of dry weight (Table 4), seedlings grown under high light had decreased R_s rates compared to those grown under low light. At 5 weeks, R_s rates for seedlings grown at high light were significantly ($p = 0.05$) lower than for low light seedlings. Although high nitrogen also appeared to decrease R_s rates during the harvests, except at 5 weeks, differences in R_s rates between the high and low nitrogen levels were not significant at the 5 percent level (HSD).

Stomatal Conductance

Since analysis of variance of the effects of light and nitrogen levels indicated that there were no significant interactions between light and nitrogen on stomatal conductance at 16 weeks, data on responses to light were pooled within nitrogen treatments. Stomatal conductance was significantly ($p = 0.05$) lower at the lowest irradiance level (Table 5). Although the highest level of nitrogen appeared to result in higher stomatal conductance (Table 5), treatment mean comparisons with the low nitrogen levels were not significant at the 5 percent level (HSD).

Table 5. Stomatal conductance (cm sec^{-1}) of 16-week-old P. contorta seedlings in response to irradiance and nitrogen levels. All values are means of 15 seedlings.[1]

Irradiance[2]			Nitrogen[2] (ppm)		
Low	Medium	High	3	62	248
0.11b[3]	0.15a	0.15a	0.13a	0.13a	0.15a

[1]Stomatal conductance was measured under 100, 210 and 470 $\mu Em^{-2}sec^{-1}$ for seedlings grown at low, medium and high irradiance levels, respectively.

[2]Means were compared between levels within each treatment (light or nitrogen).

[3]Means followed by similar letters are not significantly different ($p = 0.05$) by HSD.

Ratio of Surface Area to Dry Weight of Needles

Because of the difference in P_n rates when expressed on the basis of surface area and dry weight during the harvests, it was thought that this might be related to the relationship between surface area (SA) and dry weight (DWT) of needles. Consequently, the ratios of SA/DWT of needles were calculated for each period of harvest. Analysis of variance of the effects of light and nitrogen indicated that there were no significant interactions between light and nitrogen on SA/DWT ratios during the harvests. Therefore, light observations were pooled within nitrogen treatments. On each of the harvest dates, SA/DWT ratios were inversely proportional to increases in the levels of both light and nitrogen (Table 6). The low levels of each component resulted in significantly ($p = 0.05$) higher SA/DWT ratios than the high levels.

Discussion

The results of the present study clearly demonstrate that variations in light and nitrogen levels affect growth, foliar nutrient

Table 6. Ratio of surface area (dm^2) to dry weight (g) of needles of P. contorta seedlings as affected by irradiance and nitrogen levels. All values are means of 15 seedlings.

Age (weeks)	Irradiance[1] Low	Medium	High	Nitrogen[1] (ppm) 3	62	248
5	4.39a[2]	3.40b	2.42c	3.96a	3.27b	2.99b
10	3.36a	2.99b	2.34c	3.38a	2.94b	2.38c
16	3.20a	2.47b	1.99c	2.83a	2.66a	2.17b

[1]Means were compared between levels within each treatment (light or nitrogen) at each age.

[2]Means followed by similar letters are not significantly different ($p = 0.05$) by HSD.

contents and photosynthetic rates of containerized conifer seedlings, the magnitude of the effects varying between the two components. Increases in both light and nitrogen within the ranges studied resulted in a significant increase in seedling biomass, nutrient uptake and photosynthesis on surface area basis, but decreased photosynthesis on dry weight basis.

Seedlings grown at high light which had the highest total dry matter (Fig. 3) also generally produced the greatest root size and the highest root/shoot ratio (Fig. 4). This suggests that increase in light levels within the ranges studied had a greater stimulating effect on root than shoot development of containerized conifer seedlings. Since root/shoot ratio is an indication of potential water and nutrient absorption (Brouwer and Dewit, 1968; Kriebel, 1963), the large root size of the high light seedlings in the present study would be of particular advantage to these seedlings when transplanted to the field, as they would enable the plants to absorb sufficient nutrients and water. The increased uptake of nutrients will, in turn, stimulate increased

biomass, and increased productivity generally. This response is clearly demonstrated in the results of total dry matter production (Fig. 3) and foliar nutrient content (Fig. 6) which were significantly greater for high light compared to low light seedlings.

However, in contrast to the increase in root size and root/shoot ratio with increasing light levels, root/shoot ratios generally appeared to increase from 3 to 62 ppm N, and then decrease from 62 to 248 ppm N. This relationship might be explained on the basis of the influence of nitrogen on root growth and physiology. It is possible that in the ranges of 3 to 62 ppm N, most of the nitrogen absorbed by the roots is rapidly incorporated into organic compounds and utilized in new root formation, thereby minimizing the amount of nitrogen reaching the shoots, and thus maintaining the shoot growth at a low level. But at 248 ppm N, more nitrogen is absorbed and translocated to the shoot as reflected in Figure 6, and carbohydrates are preferentially utilized in shoot growth, causing proportionally less carbohydrate translocation to the roots, thereby reducing root growth. It is concluded from the above results that high nitrogen and low light intensity stimulate shoot growth more than root growth, whilst low nitrogen and high light intensity have an opposite effect.

Figure 5 indicates that percent N and P decreased with increasing light levels. The higher concentrations of nutrients, especially N at low light might indicate that light was limiting growth and therefore indirectly limiting the use of N for construction of biomass. In short, the plants at low light were growing slowly as shown in Figure 3 and since growth is very dependent on light through photosynthesis, the foliage of the low light plants could be expected to show higher

nutrient concentrations than otherwise comparable high light foliage, as nutrient concentration was not diluted by carbohydrate synthesis and subsequent biomass production.

It is evident from Table 1 that net photosynthesis on surface area basis increased with increasing levels of both light and nitrogen. This suggests that the inhibition of growth (biomass) at low levels of each component is accompanied to a considerable extent by decreased photosynthesis, probably because of the inability of the plants at low levels of light and nitrogen to synthesize sufficient carbohydrates for construction of shoots as a result of the limiting effects of light and nitrogen. Studies have indicated that the effects of light and nitrogen deficiencies on conifer growth are manifested through several mechanisms. Lower photosynthetic rates of conifer seedlings grown at low light occur in low light (Kozlowski, 1949; Kramer and Clark, 1947) and decreased activity of carboxylating enzymes (Alberte et al., 1976; Natr, 1972, 1975). Although the results of Table 5 indicate that stomatal conductance was significantly lower at the lowest light level, it is not clear whether stomatal conductance has any effects on the rate of net photosynthesis (see Jarvis, 1981). Similarly, increases in photosynthesis following increases in nitrogen can result from such factors as increased chlorophyll concentration (Brix, 1981), decreased stomatal and mesophyll resistances to CO_2 diffusion (Osman et al., 1977), increased activity of carboxylating enzymes (Natr, 1975; Osman and Milthorpe, 1971), increased leaf size (Brix, 1971) and increased utilization of assimilates by sinks (Wareing and Patrick, 1975). In the results above (Table 1), stomatal resistance to CO_2 diffusion appeared to have played a negligible role in bringing about the increased photosynthesis

following increase in nitrogen levels because there were no significant differences in stomatal conductance of seedlings between the low and high nitrogen levels at 16 weeks of age (Table 5).

The maximum photosynthetic rates measured for the light and nitrogen treated seedlings during the three harvest periods were in the ranges of 1 to 4 mg $CO_2 dm^{-2} h^{-1}$. Although these values are lower than the 15 to 30 mg $CO_2 dm^{-2} h^{-1}$ reported for P. sylvestris (Larcher, 1969), 18 mg $CO_2 dm^{-2} h^{-1}$ for Douglas-fir (Krueger and Ferrell, 1965) and 10 mg $CO_2 dm^{-2} h^{-1}$ for P. elliottii (van den Driessche, 1972), they are similar to the values of 3.4 mg $CO_2 dm^{-2} h^{-1}$ for P. taeda (Kramer and Decker, 1944), 7 mg $CO_2 dm^{-2} h^{-1}$ for P. halopensis (Whiteman and Koller, 1964) and 5 to 10 mg $CO_2 dm^{-2} h^{-1}$ for P. sylvestris and Picea abies (Jarvis and Jarvis, 1964) in greenhouse and nursery studies.

The results of Table 2 indicate that photosynthetic rates expressed on dry weight basis proved to be inconsistent with the data based on surface area (Table 1). As shown in Table 1, P_n rates on surface area were higher for high light and high nitrogen levels, but lower for both components on dry weight basis (Table 2). This suggests that the smaller light- and nitrogen-deficient seedlings with less biomass had more surface area per unit of dry weight, and appeared photosynthetically more active than those at high light and high nitrogen when P_n was based on dry weight. The reasons for the observed difference were found in the ratio of surface area to dry weight of needles (Table 6) which indicated that at each harvest date, the ratios were significantly higher for low light and low nitrogen as compared to the high levels of both components, thus rendering P_n rates in terms of dry weight very unreliable. These results indicate that P_n rates on their own,

expressed on unit foliar dry weight of plant material, can be very misleading. Therefore, it is suggested that the area of the leaf intercepting the radiation provides the more suitable basis for calculating the photosynthetic rate, especially if irradiance is limiting. At low light, P_n rate data expressed on leaf dry weight may seem illogical, since according to Sestak et al. (1971) dry weight has little or no direct relationship with radiation absorption, CO_2 uptake or enzyme activity. These results therefore, emphasize the complications that may arise from selection of units for the expression of photosynthetic rates.

Considering the biomass, nutrient status and photosynthetic rates of seedlings at the three harvest periods, it can be seen from the results above that although biomass increased with age (Fig. 3), nutrient concentration (Fig. 5) and photosynthetic rates (Tables 1 and 2) declined with increasing age of the seedlings or needles. The increase in foliar nutrient concentration at 5 weeks relative to 10 and 16 weeks may be partially associated with changes in the proportion of structural materials to cytoplasm, for example, cuticle thickness and secondary thickening. Since growth of a plant is conditioned by both the external and internal factors operating during development, it seems possible that at 5 weeks, nutrient concentrations were high because demands by the developing seedlings were low. But at 10 and 16 weeks of age, increased size of the seedlings and later developmental stages resulted in increased demand and utilization of nutrients for tissue construction with a consequent drop in foliar concentrations. Magwick (1970) reported that the nutrient concentration of P. virginiana decreased from 1.2 percent in the first year to 1.0 percent in the third

year, and associated the decrease with increased structural development of the older foliage.

The photosynthetic rates of the 5-week-old seedlings were also found to be higher than at 10- and 16-week-old. This suggests that the age of needles is a significant factor in the photosynthesis of containerized conifer seedlings. The lower photosynthetic rates of the older needles result partly from their lower ratios of surface area to dry weight (see Table 6), and partly from possible changes in the photosynthetic activity of the older needles, such as cuticle and secondary thickening which may have produced increased resistances, both stomatal and mesophyll to CO_2 diffusion as the leaves aged. Ludlow and Jarvis (1971a) observed a sharp decline in photosynthetic capacity of P. sitchensis with increasing age of foliage, and correlated this with increasing leaf resistance, supporting observations of El-Sharkawy et al. (1968) that old leaves assimilated CO_2 more slowly than recently expanded ones.

CHAPTER IV

EFFECT OF LIGHT, NITROGEN FERTILIZATION AND MYCORRHIZAE FORMATION ON GROWTH AND PHOTOSYNTHESIS OF LODGEPOLE PINE SEEDLINGS

Introduction

"Tailoring" seedlings with mycorrhizae is slowly coming to be viewed as a valuable tool in achieving increased productivity in forestation. The importance of mycorrhizae to the physiological functions of conifer species is relatively well documented (Bowen, 1973). Mycorrhizal fungi affect their hosts' growth via several mechanisms, particularly by improving growth and nutrient relations of the hosts (Bowen, 1973), and there is evidence that differences in seedling vigor may be brought about by inoculating with different mycorrhizal fungi (Ekwebelam, 1979; Lamb and Richards, 1971). It is also well established that nutrient uptake by mycorrhizal plants is greater than that of non-mycorrhizal ones (Ekwebelam, 1979; Lamb and Richards, 1971) in infertile soils. Studies also indirectly have implicated that ectomycorrhizae of conifers (Lister et al., 1968; Nelson, 1964; Schweers and Meyer, 1970; Shiroya et al., 1962) and endomycorrhizae of nonwoody plants (Allen et al., 1981; Losel and Cooper, 1979; Kucey and Paul, 1982) cause increase in photosynthesis and translocation of photosynthates to roots of host plants. The opportunity to improve seedling quality and thereby seedling performance in nurseries and greenhouses through inoculation therefore becomes apparent.

Over the years, forestry practices have increasingly incorporated techniques for encouraging and manipulating microbial systems (Hacskaylo, 1972). The production of containerized conifer seedlings sufficiently large and vigorous to survive and grow in the field requires the maintenance of a balanced level of mineral nutrition, particularly N and P, and adequate levels of light intensity for plant development. There are reports that an inverse relationship exists between nutrient availability in the soil and mycorrhizal frequency (Bjorkman, 1942, 1970; Marx et al., 1977). High fertility in the rooting medium, especially N and P, inhibit mycorrhizal development, and a number of papers have implied that mycorrhizae formation is correlated with variations in light intensities (Bjorkman, 1942, 1970; Mitchell et al., 1937). However, reports of the relationship between the levels of individual mineral nutrients, light intensity and ectomycorrhizae formation on growth and photosynthesis of conifer species are lacking. Also, the need to incorporate photosynthetic analysis into ectomycorrhizal studies has been stressed (Slankis, 1973); information in this respect has remained meager and fragmentary. These facts have provided the rationale for the present study.

The present work, therefore, describes the interaction between light, nitrogen fertilization and mycorrhizae formation on growth and photosynthesis of lodgepole pine (P. contorta Dougl.) seedlings. The study seeks to quantify growth and photosynthesis of containerized lodgepole pine seedlings as affected by varied levels of light and nitrogen fertilization, and ectomycorrhize formation, and to correlate ectomycorrhizae formation with photoassimilation. Such information

will aid in the development of a cultural regime to optimize seedling production in a nursery environment.

Material and Methods

Inoculum Preparation

Inocula of two fungal species (Pisolithus tinctorius (Pers.) Coker and Couch and Suillus granulatus (L. ex Fr.) O. Kuntze were prepared in bulk by the technique of Marx and Bryan (1975). Inoculum was grown in 1.9-l mason glass jars with screw caps containing a sterile mixture of coarse vermiculite-peat (15:1 v/v) moistened with liquid modified Melin-Norkrans (MMN) nutrient solution (Marx, 1969). Initially, mycelia of each fungus were grown aseptically in 125-ml Erlenmeyer flasks containing 20 ml of sterile MMN liquid solution. After 5 weeks incubation, the cultures were slightly broken up in a sterile Waring blender, and 40 ml of slurry were aseptically transferred to each 1.9-l glass jar. After 4 months of incubation at room temperature, the inoculum was removed from the jars and leached with cold running tap water to remove excess nutrients. Inoculum was then placed in plastic bags and stored in a cold room at 4°C for about 5 h before use.

Establishment of Seedlings

Seed of lodgepole pine (P. contorta Dougl.) were surface-sterilized in 1.1 percent solution of sodium hypochlorite, and sown directly into an autoclaved mixture of vermiculite-peat (5:2 v/v) wetted with distilled water. One hundred and thirty-five sterile "Ray-Leach" containers (150 cm^3 capacity) were filled with the sterile potting mixture. Four seeds were sown into each container and misted daily until

germination. Four days after germination, seedlings were thinned to one per container.

All seedlings were grown in an electrostatic HEPA-filtered fiberglass chamber contained within a standard greenhouse, designed specifically to minimize air-borne contamination by fungal spores. Day/night temperatures were 30/26 ±2°C, and a 16-h photoperiod was maintained by supplemental lighting with fluorescent cool beam lamps.

Nitrogen Fertilization and Light Regimes

Seedlings were grown at three relative levels of irradiance established by use of commercial shadecloth netting (nominal rating of 55 percent shade). Placing two, one or no layer(s) of shadecloth above the seedlings gave about 100, 210 and 470 μEinstein(E)$m^{-2}sec^{-1}$ quantum flux density, respectively, as measured with LI-170 Quantum Sensor (Lambda Instrument Co., Lincoln, Nebraska, USA) at midday during the part of the growing season with the lowest ambient sunlight. Maximum values reached without shadecloth were near 610 $\mu Em^{-2}sec^{-1}$. The three ambient sunlight levels will hereinafter be referred to as low, medium and high light levels, respectively. Seedlings were fertilized with Hocking's (1971) nutrient solution with the following modifications: nitrogen was added as ammonium nitrate to give levels of 3, 62 and 248 ppm of N, calcium chloride was substituted for calcium nitrate to give 80 ppm of Ca, sulfur was changed from 150 to 64 ppm, and ferric chloride was changed to sequestrene 330 Fe (see Appendix 1).

After thinning, seedlings were randomly arranged into 9 groups of 15 seedlings each. The groups were randomly assigned to light and nitrogen treatments, and grown without ectomycorrhizae for 10 weeks. To minimize position effects, the groups were rerandomized on the

greenhouse bench weekly. Fertilization commenced one day after thinning and daily thereafter. To maintain container concentrations of nitrogen near the applied levels, nutrient solution was added daily in excess to allow flushing of the potting mix (R.W. Tinus, private communication). No other irrigation was necessary.

Mycorrhizal Fungi Inoculation

At 10 weeks, mycorrhizal treatments were superimposed on the light and nitrogen treatments in a 3^3 factorial combination design. Mycorrhizal treatments were noninoculated controls, inoculated with P. tinctorius, and inoculated with S. granulatus. The seedlings in each light and nitrogen combination were removed from their containers and their roots were rinsed with filtered tap water. Seedlings were then replanted into a fresh mix of autoclaved vermiculite-peat and fungal inoculum (3:1 v/v), 5 seedlings for each fungal species and 5 non-inoculated controls. To avoid cross infection of mycorrhizal fungi, the control seedlings were replanted first. One fungus was dealt with at a time, and as a further precautionary measure, hands and tools were thoroughly washed between treatments. The replanted seedlings were then placed at random on the greenhouse bench in the same light and nitrogen treatment groups, and rerandomized weekly on the bench.

After 6 weeks, a time selected to allow adequate time for ectomycorrhizae formation on seedling roots, but short enough to minimize excessive changes in short root physiology, the experiment was terminated and the seedlings used for determination of net photosynthesis and respiration rates, mycorrhizae development on seedling roots, growth parameters (dry weights of shoots, needles and roots, and root/shoot

ratios) and foliar N and P. Prior to photosynthetic measurements, stomatal conductance was determined on each seedling.

Net Photosynthesis and Respiration Measurements

Net photosynthesis (P_n) and dark respiration (R_s) rates were measured continuously with a Beckman model 315A (Beckman Instrument, Pasadena, California, USA) infrared gas analyzer (IRGA) in an open system (Fig. 1), calibrated daily for a full-scale deflection of 50 ppm using standard gases. Ambient air was pumped through a 210-l mixing reservoir dehumidified with a mixture of anhydrous calcium sulfate and magnesium perchlorate (1:2 v/v) and divided into sample and reference lines. The length and volume of the pathway was identical for both sample and reference lines. Flow rates (ca 1.5 l min^{-1}) were adjusted to maintain CO_2 concentrations in the sample cuvette within ±10 percent of ambient. Seedling shoots were sealed in a 700 cm^3 volume plexiglas assimilation chamber, isolated from the root system by Terostat sealant (Terosan GmbH Heidelberg). To provide vigorous air mixing within the assimilation chamber and minimize boundary air layer resistances, a small fan was incorporated into the assimilation chamber (Fig. 2), driven by a 4.5-volt D.C. motor.

The sequence of selecting seedlings for photosynthetic measurements was completely random. Seedlings remained in the vermiculite-peat mix and were well-watered prior to measurements. Seedlings were placed under a bank of seven 300-W incandescent flood lamps with a continuous flow water bath filter positioned about 1 m above the assimilation chamber. Net photosynthesis was determined at 100, 210 and 470 $\mu Em^{-2}sec^{-1}$ by use of shadecloth for seedlings grown at low, medium and high irradiance levels, respectively, and temperature within the

assimilation chamber was maintained at 31 ±2°C. Prior to each P_n measurement, a 60 min acclimation period was allowed for each seedling under each appropriate irradiance level, followed by a 20 min measurement of P_n rate. Dark R_s was determined by placing the seedling immediately in a dark growth chamber and measuring CO_2 exchange under similar temperature conditions as above. A 15-min adjustment period of the IRGA was allowed at the completion of each P_n and R_s rate before a new run was started.

Following the P_n and R_s measurements, individual seedling needles were clipped and hand-sorted into green and nongreen portions. The needle lengths and widths were measured for calculation of surface area as previously described in Chapter III. The seedling components of shoots and roots were then placed in plastic bags, sealed and stored at -20°C until the end of the day when they were taken to the laboratory for assessment of mycorrhizal infection on seedling roots and determination of biomass, and foliar N and P.

The CO_2 exchange rates for P_n were calculated on the basis of surface area (mg $CO_2 dm^{-2} h^{-1}$) and dry weight (mg $CO_2 g^{-1} h^{-1}$) of green needles. Calculation of R_s rates was based on the entire aboveground surface area and dry weight of entire seedling top.

Assessment of Mycorrhizal Infection on Seedling Roots

Mycorrhizae development was expressed as 'mycorrhizae percent,' the total number of mycorrhizal root tips expressed as a percentage of the total number of short roots and mycorrhizal root tips of individual seedling roots. Forking or dichotomy of the short roots was used as an indication of mycorrhizal infection. Since dichotomy in short roots of Pinus is not always associated with mycorrhizal infection (Levisohn,

1954; Slankis, 1958), representative samples of roots classified as mycorrhizal and nonmycorrhizal were verified by fixing in formalin-acetic-alcohol (FAA), sectioning and staining, and examining microscopically for evidence of Hartig net and fungal mantle.

Estimation of Biomass and Foliar Analysis

Seedling biomass (shoot and root dry weights) were determined after oven-drying at 68°C for 48 h. Dry needle tissues (i.e., both green and nongreen) were digested as previously described (Thomas et al., 1967). Nitrogen was determined by the salicylate isocyanurate method (Bigelow et al., 1982) and P by the molybdate blue method (Murphy and Riley, 1962).

Measurement of Stomatal Conductance

Stomatal conductance of seedlings was determined using a null-balance porometer, LI-COR model 1600 (LI-COR, Inc., Lincoln, Nebraska, USA) with a conifer needle chamber cuvette. Seedlings remained in the vermiculite-peat mix and were well-watered prior to measurement. Stomatal conductance was determined at 100, 210 and 470 $\mu Em^{-2}sec^{-1}$ irradiance levels established by use of shadecloth for seedlings grown at low, medium and high irradiance levels, respectively. The sequence of selecting seedlings for stomatal conductance measurements from each treatment combination was completely random, and all measurements were made at 31 ±2°C. Prior to each measurement, seedlings were acclimatized for 60 min under the appropriate light treatment.

Stomatal conductance expressed in cm sec^{-1} and calculated as the reciprocal of resistance was obtained as previously described (Anon, 1980) using the expression:

$$R = (Rd + 0.15) \cdot \frac{A_t}{A}$$

where R, Rd, A_t and A represent actual diffusive resistance, diffusive resistance read on the instrument, actual area of needles and pre-set area (10 cm^2) entered into the instrument microprocessor, respectively. Needle area was obtained as described above (see Chapter III).

Statistical Analysis of Data

Measured parameters (i.e., shoot and root dry weights, root/shoot ratios, foliar N and P, photosynthetic and respiration rates, number of short roots and mycorrhizae development, and stomatal conductance) were examined by factorial analysis of variance (ANOVA) (Steel and Torrie, 1980). When significant differences were found between or among treatments, treatment means were compared at the 5 percent probability of error level by Tukey's test for Honestly Significant Difference (HSD) (Steel and Torrie, 1980). To simplify data presentation and interpretation, in some cases treatments were pooled if no significant interaction occurred in the ANOVA. The relationship between net photosynthesis and foliar N and P, and stomatal conductance of all seedlings (i.e., both inoculated and noninoculated), and also the relationship between mycorrhizae percent and growth parameters (i.e., total dry matter, dry weights of shoots and roots), foliar N and P, and net photosynthesis of inoculated seedlings were examined by simple linear regression analyses (Steel and Torrie, 1980).

Results

Dry Matter Production

Since analysis of variance indicated no significant interactions between light, nitrogen fertilization and inoculation treatments on dry

matter of either shoots or roots at 16 weeks (i.e., 6 weeks following inoculation) (Fig. 7, Table 7), data on responses to inoculation were pooled within light and nitrogen treatments. High light (Fig. 7a) and high nitrogen (Fig. 7b) levels significantly ($p = 0.05$) increased shoot and root dry weights compared to low levels of each component. The average values for shoot and root dry weights were, respectively, 83.6 and 242.2 mg for high light and 87.0 and 186.4 mg for high nitrogen levels compared to 50.7 and 74.7 mg for low light and 54.4 and 123.5 mg for low nitrogen levels. The root weights of the high light seedlings were more than treble those of the low light plants. Although root/shoot ratios also increased significantly ($p = 0.05$) with increasing light levels (Fig. 7a), the ratios appeared to be little affected by nitrogen levels (Fig. 7b). The average root/shoot ratios were 2.99 for high light, 1.47 for low light, 2.14 for high nitrogen, and 2.27 for low nitrogen.

Despite the low average mycorrhizae percent of inoculated seedlings, 4.4 (range of 0.8 to 10.5) for P. tinctorius and 4.8 (range of 1.2 to 12.9) for S. granulatus, inoculation with both mycorrhizal fungi significantly ($p = 0.05$) improved both shoot and root dry weights over the noninoculated controls (Table 7). The average shoot and root dry weights were, respectively, 70.1 and 163.8 mg for P. tinctorius, 71.6 and 155.2 mg for S. granulatus, and 62.2 and 139.4 mg for the noninoculated controls. Regression analyses indicated that mycorrhizae percent was not correlated with either total dry matter ($r^2 = 0.02$), shoot ($r^2 = 0.001$) or root ($r^2 = 0.03$) dry weights.

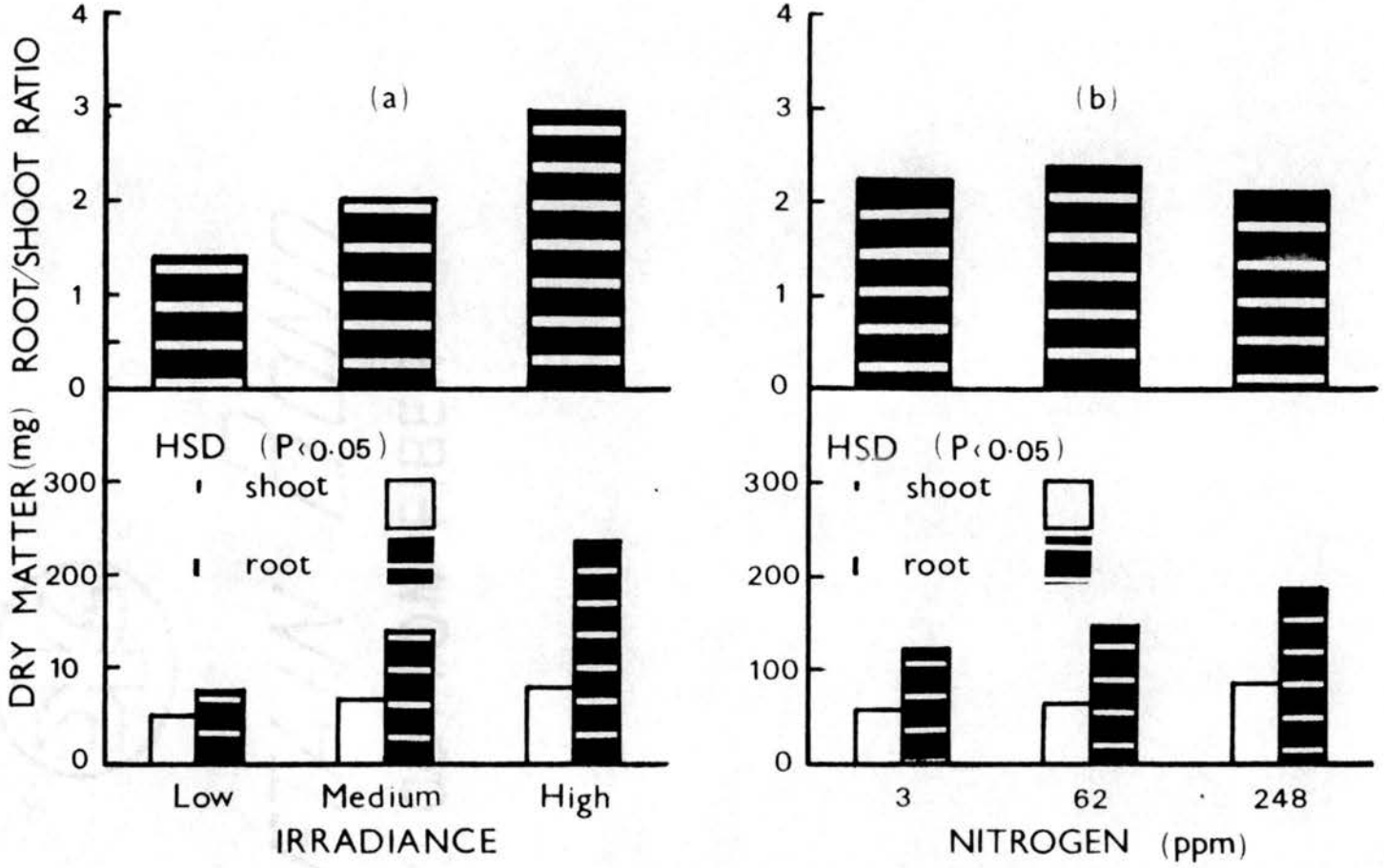


Figure 7. Effect of irradiance (a) and nitrogen (b) levels on dry matter and root/shoot ratio of P. contorta seedlings at 16 weeks, 6 weeks following inoculation with mycorrhizal fungi. Vertical bars represent HSD (p = 0.05). All figures are means of 45 seedlings.

Table 7. Effect of mycorrhizal fungi inoculation on dry matter, root/shoot ratio, number of short roots, mycorrhizae percent and dark respiration rates of 16-week-old P. contorta seedlings, 6 weeks following inoculation. All values are means of 45 seedlings.

Inoculation Treatment	Dry Matter (mg) Shoot	Root	Root/shoot Ratio	Number of Short Roots	Mycorrhiza (%)[1]	Respiration (mg $CO_2 dm^{-2} h^{-1}$)[2]	Respiration (mg $CO_2 g^{-1} h^{-1}$)[3]
Control (noninoculated)	62.2b[4]	139.4b	2.24a	530.0b	0.0b	0.83a	1.43a
P. tinctorius	70.1a	163.8a	2.34a	704.0a	4.4a	0.86a	1.26a
S. granulatus	71.6a	155.2ab	2.17a	775.0a	4.8a	0.88a	1.33a

[1]Proportion of the total number of short roots converted to mycorrhizae per seedling.

[2]Respiration on the basis of surface area (mg $CO_2 dm^{-2} h^{-1}$).

[3]Respiration on the basis of dry weight (mg $CO_2 g^{-1} h^{-1}$).

[4]Means in each column followed by similar letters are not significantly different at the 5 percent level (HSD).

Number of Short Roots

When number of short roots was examined at 16 weeks, analysis of variance indicated that there were no significant interactions between light, nitrogen fertilization and inoculation treatments with short roots, therefore inoculation treatment observations were pooled within light and nitrogen treatments. Number of short roots was significantly ($p = 0.05$) greater on seedlings grown at the high light (Fig. 8a) and high nitrogen (Fig. 8b) levels than both low and intermediate levels of each component; the low levels of each component were consistently poorest in the number of short roots. The average numbers of short roots were 928, 727 and 352 for high, intermediate and low light levels, respectively, and 820, 688 and 500 for high, intermediate and low nitrogen levels, respectively.

Inoculation with mycorrhizal fungi caused a significant ($p = 0.05$) increase in the number of short roots of seedlings over that of the noninoculated controls (Table 7). The average numbers of short roots were 704 for P. tinctorius, 775 for S. granulatus and 530 for the noninoculated controls.

Mycorrhizal Status

Six weeks following inoculation, all inoculated plants were mycorrhizal and noninoculated controls remained free of mycorrhizae. Analysis of variance indicated that there were significant main effects of light and nitrogen treatments on the proportion of the number of short roots converted to mycorrhizae (i.e., mycorrhizae percent) at the 5 percent probability of error (HSD). However, since there were no significant light, nitrogen and inoculation treatment interactions,

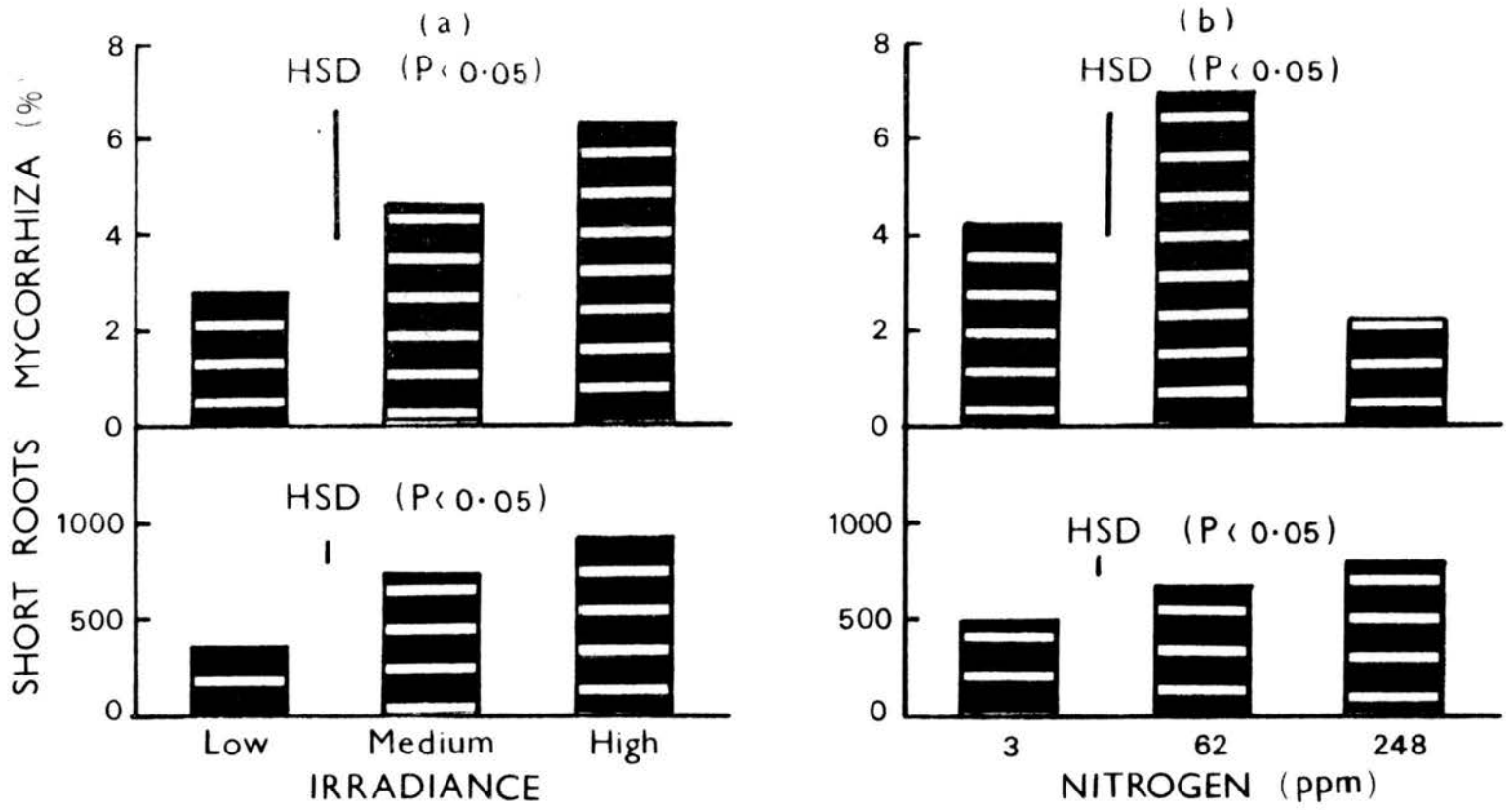


Figure 8. Number of short roots and mycorrhizae percent of 16-week-old P. contorta seedlings as affected by irradiance (a) and nitrogen (b) levels, 6 weeks following inoculation with mycorrhizal fungi at 10 weeks. Vertical bars represent HSD (p = 0.05). All figures are means of 45 seedlings.

inoculation treatment observations were pooled within light and nitrogen treatments.

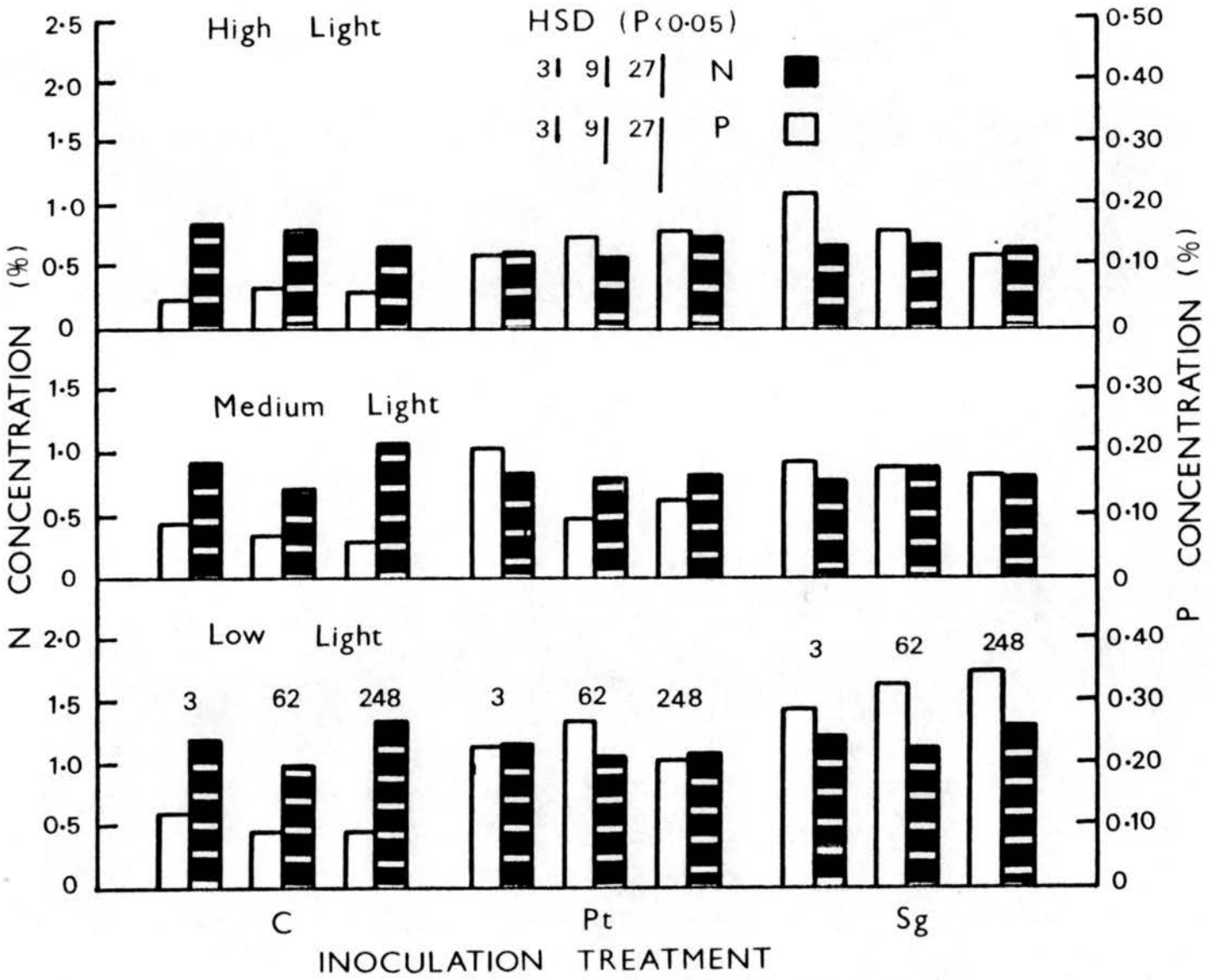
Mycorrhizae percent increased significantly ($p = 0.05$) with increasing light levels (Fig. 8a), and was more than double at the highest light treatment (i.e., 6.40 percent) as compared to low light (i.e., 2.74 percent). Also, nitrogen fertilization levels significantly ($p = 0.05$) influenced mycorrhizae formation. Although the highest nitrogen level (248 ppm) significantly ($p = 0.05$) decreased mycorrhizae percent, the intermediate level of 62 ppm gave significantly ($p = 0.05$) higher mycorrhizae percent (7.04) than either the lowest (3 ppm) or highest (248 ppm) nitrogen levels. There appeared to be little difference between the two fungi in mycorrhizae formation (Table 7).

Nutrient Concentration and Content

Analysis of the effects of light, nitrogen fertilization and inoculation treatments on N and P contents of foliage indicated significant ($p = 0.05$) interactions between treatments. Therefore, the data on N and P concentrations and total contents are presented for each treatment combination (Figs. 9 and 10). There appeared to be no clear-cut trend in N concentrations as affected by any single component (Fig. 9). However, P concentrations generally decreased with increasing levels of both light and nitrogen, partially as a result of dilution caused by increased dry matter production at those combinations of each component (Fig. 9).

Total N generally increased with increasing nitrogen levels (Fig. 10), and is probably associated with increased uptake and biomass production. The P contents of specific combinations of inoculation and nitrogen treatments within the high light and low light were rarely

Figure 9. Foliar N and P concentrations in 16-week-old *P. contorta* seedlings in response to mycorrhizal fungi inoculation at 10 weeks of age, 3 levels of irradiance (high, medium and low) and nitrogen (3, 62 and 248 ppm). C = noninoculated controls, Pt = inoculated with *Pisolithus tinctorius*, and Sg = inoculated with *Suillus granulatus*. Vertical bars labeled 3, 9 and 27 represent HSD ($p = 0.05$) for comparisons of 3, 9 and 27 means within any one, two and all treatments, respectively. All figures are means of 5 seedlings.


High Light
HSD (P<0·05)
3 9 27 N
3 9 27 P
Medium Light
Low Light
3 62 248 3 62 248 3 62 248
N CONCENTRATION (%)
P CONCENTRATION (%)
2·5
2·0
1·5
1·0
0·5
0
0·50
0·40
0·30
0·20
0·10
C
Pt
Sg
INOCULATION TREATMENT

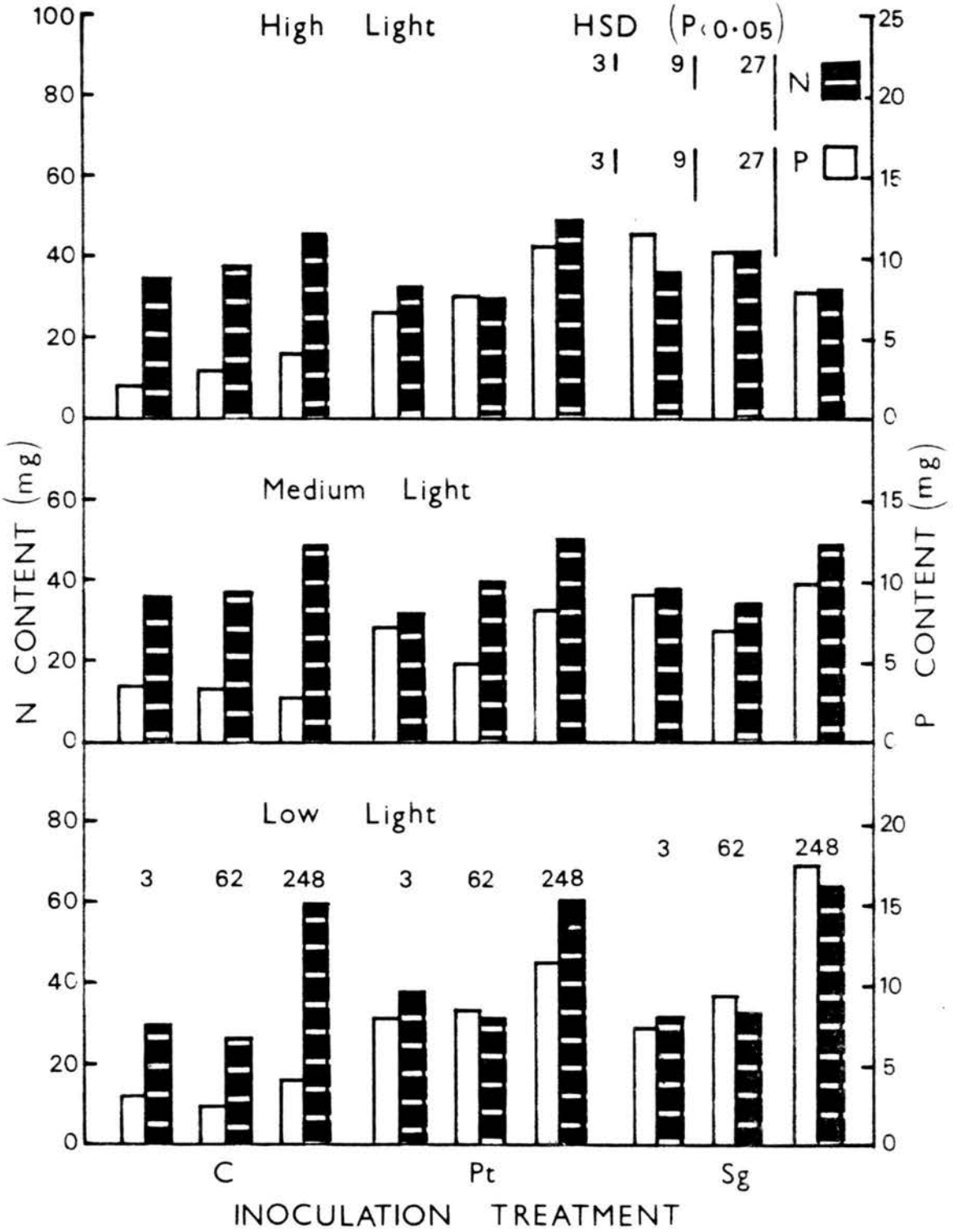


Figure 10. Total foliar N and P contents of 16-week-old P. contorta seedlings in response to mycorrhizal fungi inoculation at 10 weeks of age, 3 levels of irradiance (high, medium and low) and nitrogen (3, 62 and 248 ppm). Vertical bars labeled 3, 9 and 27 represent HSD (p = 0.05) for comparisons of 3, 9 and 27 means within any one, two and all treatments, respectively. All figures are means of 5 seedlings.

significant at the 5 percent level (HSD). However, total P generally increased significantly ($p = 0.05$) with increasing nitrogen levels (Fig. 10), except for the S. granulatus treatment at high light.

N concentration (Fig. 9) and content (Fig. 10) were little affected by inoculation treatments, thus suggesting that P, and not N, might be responsible for the marked responses in growth and yield to mycorrhizal fungi inoculation. At all three light and nitrogen levels, inoculation with either P. tinctorius or S. granulatus significantly ($p = 0.05$) increased total P contents (Fig. 10) over the noninoculated controls. Even P concentration was greater in the inoculated plants in spite of their greater shoot and root dry weights which might indicate a dilution effect on P. Thus, inoculation with mycorrhizal fungi seemed to primarily increase P uptake. Simple linear regression analyses indicated that mycorrhizae percent was not significantly correlated with either percent N ($r^2 = 0.28$), percent P ($r^2 = 0.13$), total N ($r^2 = 0.18$) or total P ($r^2 = 0.15$).

Net Photosynthesis

Since analysis of variance of the effects of light, nitrogen fertilization and inoculation treatments indicated that there were no significant interactions between light, nitrogen and inoculation treatments on P_n rates in terms of either surface area (mg $CO_2 dm^{-2} h^{-1}$) or dry weight (mg $CO_2 g^{-1} h^{-1}$), data on responses to inoculation were pooled within light and nitrogen treatments. In terms of surface area, high light P_n rates (2.05 mg $CO_2 dm^{-2} h^{-1}$) and high nitrogen P_n rates (1.99) were significantly ($p = 0.05$) greater than at low levels of each component, 1.45 and 1.52, respectively (Figs. 11a, b). Despite the low average mycorrhizae percent of inoculated seedlings, inoculation with

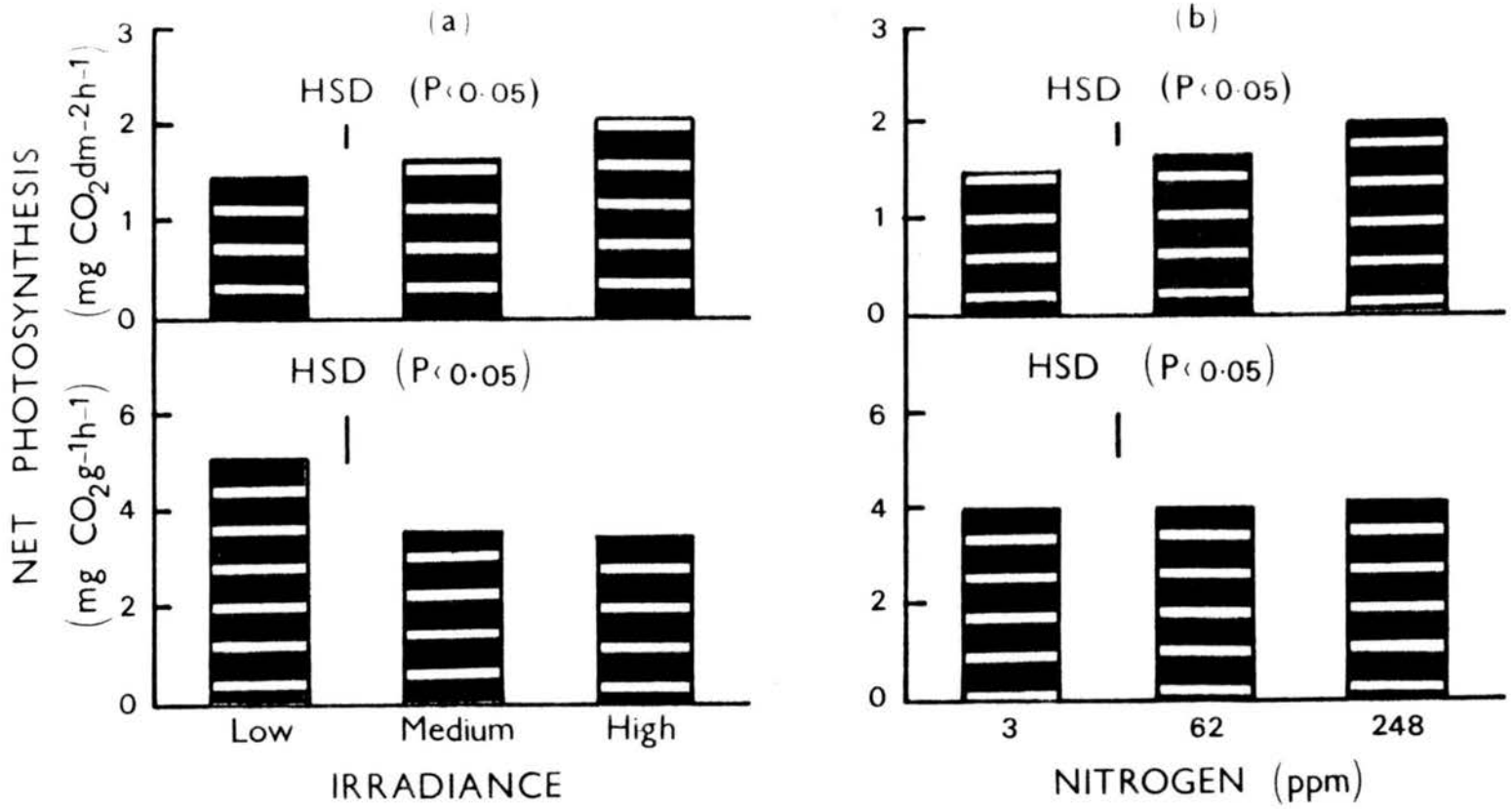


Figure 11. Effect of irradiance (a) and nitrogen (b) levels on net photosynthesis on the basis of surface area (mg $CO_2dm^{-2}h^{-1}$) and dry weight (mg $CO_2g^{-1}h^{-1}$) of 16-week-old P. contorta seedlings in response to mycorrhizal fungi inoculation at 10 weeks of age. Vertical bars represent HSD (p = 0.05). All figures are means of 45 seedlings.

either P. tinctorius or S. granulatus significantly ($p = 0.05$) increased P_n rates (Fig. 12) over the noninoculated controls, consistent with dry matter production and nutrient contents. The average P_n rates based on surface area were 1.87, 1.85 and 1.41 for P. tinctorius, S. granulatus and noninoculated controls, respectively. Simple linear regression analyses indicated that P_n rates were not significantly correlated with percent N ($r^2 = 0.05$), percent P ($r^2 = 0.0$), total N ($r^2 = 0.0$) or total P ($r^2 = 0.005$). Nor was mycorrhizae percent strongly correlated with P_n rates ($r^2 = 0.02$).

On the basis of dry weight, high light significantly ($p = 0.05$) decreased P_n rates compared to low light (Fig. 11a). There were no discernible effects of nitrogen levels on P_n rates (Fig. 11b). Although inoculated plants appeared to have improved P_n rates compared to noninoculated controls, differences between them were not significant at the 5 percent level (HSD) (Fig. 12).

Respiration

Analysis of variance of the effects of light, nitrogen ferilization and inoculation treatments on R_s rates indicated that there were no significant light, nitrogen and inoculation treatment interactions on R_s rates either in terms of surface area or dry weight. Therefore, data on responses to inoculation were pooled within light and nitrogen treatments. Plants grown under high light had significantly ($p = 0.05$) greater R_s in terms of surface area, compared to low light (Table 8), but decreased R_s on dry weight basis, probably for the same reason that P_n rates on dry weight basis decreased with increasing light levels. The average R_s rates were 1.06 and 1.22 for high light and

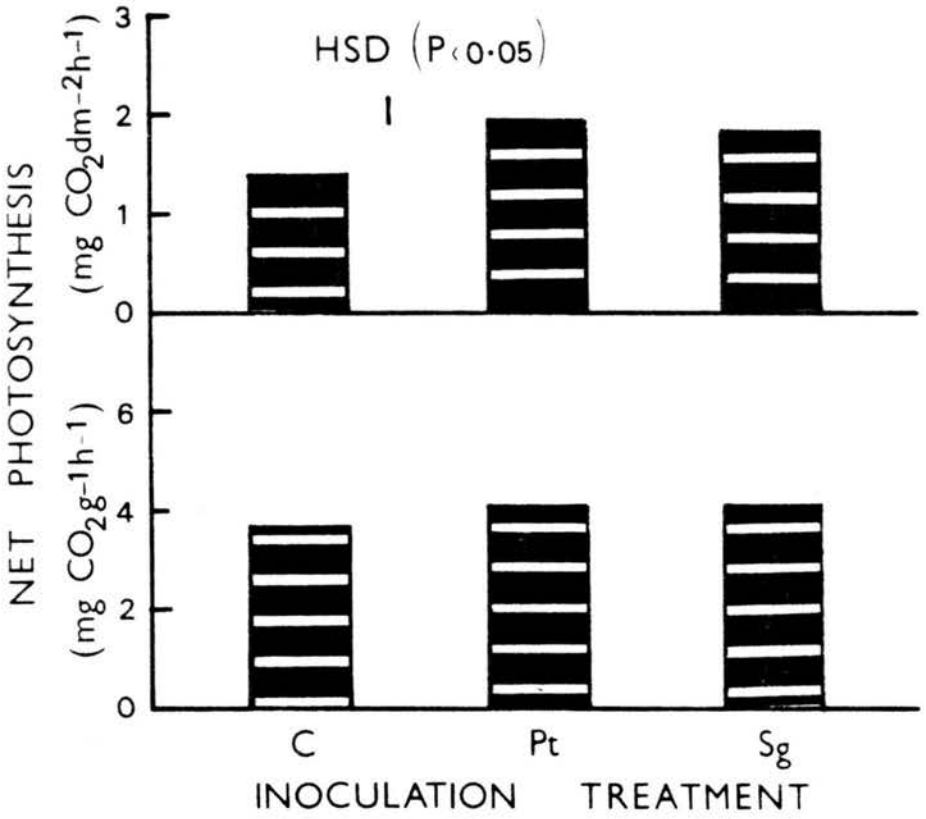


Figure 12. Net photosynthesis on the basis of surface area (mg $CO_2dm^{-2}h^{-1}$) and dry weight (mg $CO_2g^{-1}h^{-1}$) of 16-week-old P. contorta seedlings in response to mycorrhizal fungi inoculation at 10 weeks of age. C = noninoculated controls, Pt = inoculated with Pisolithus tinctorius, and Sg = inoculated with Suillus granulatus. Vertical bars represent HSD (p = 0.05). All figures are means of 45 seedlings.

Table 8. Respiration rates of 16-week-old P. contorta seedlings as affected by levels of irradiance and nitrogen following inoculation with mycorrhizal fungi at 10 weeks of age. All values are means of 45 seedlings.[1]

Parameter	Irradiance[2]			Nigrogen[2] (ppm)		
	Low	Medium	High	3	62	248
Respiration[3]	0.67b[5]	0.83ab	1.06a	0.83a	0.85a	0.88a
Respiration[4]	1.50a	1.31a	1.22a	1.45a	1.36a	1.22a

[1]Respiration was measured in the dark.

[2]Means were compared between levels within each treatment (light or nitrogen).

[3]Respiration rates on surface area basis (mg $CO_2 dm^{-2} h^{-1}$).

[4]Respiration rates in terms of dry weight (mg $CO_2 g^{-1} h^{-1}$).

[5]Means in each column followed by similar letters are not significantly different at the 5 percent probability of error (HSD).

0.67 and 1.50 for low light levels in terms of surface area and dry weight basis, respectively. There were no discernible effects of nitrogen levels (Table 8) and inoculation treatments (Table 7) on R_s rates either on surface area or dry weight basis.

Stomatal Conductance

Analysis of variance of the effects of light, nitrogen fertilization and inoculation treatments on stomatal conductance revealed significant interactions between light and nitrogen at the 5 percent level (HSD), but none between light, nitrogen fertilization and inoculation treatment. In some cases, stomatal conductance was significantly ($p = 0.05$) greater at high light levels compared to low light (Fig. 13). At high light, high nitrogen significantly ($p = 0.05$) increased conductance in the inoculated plants relative to the noninoculated controls. At the intermediate light levels, the intermediate and

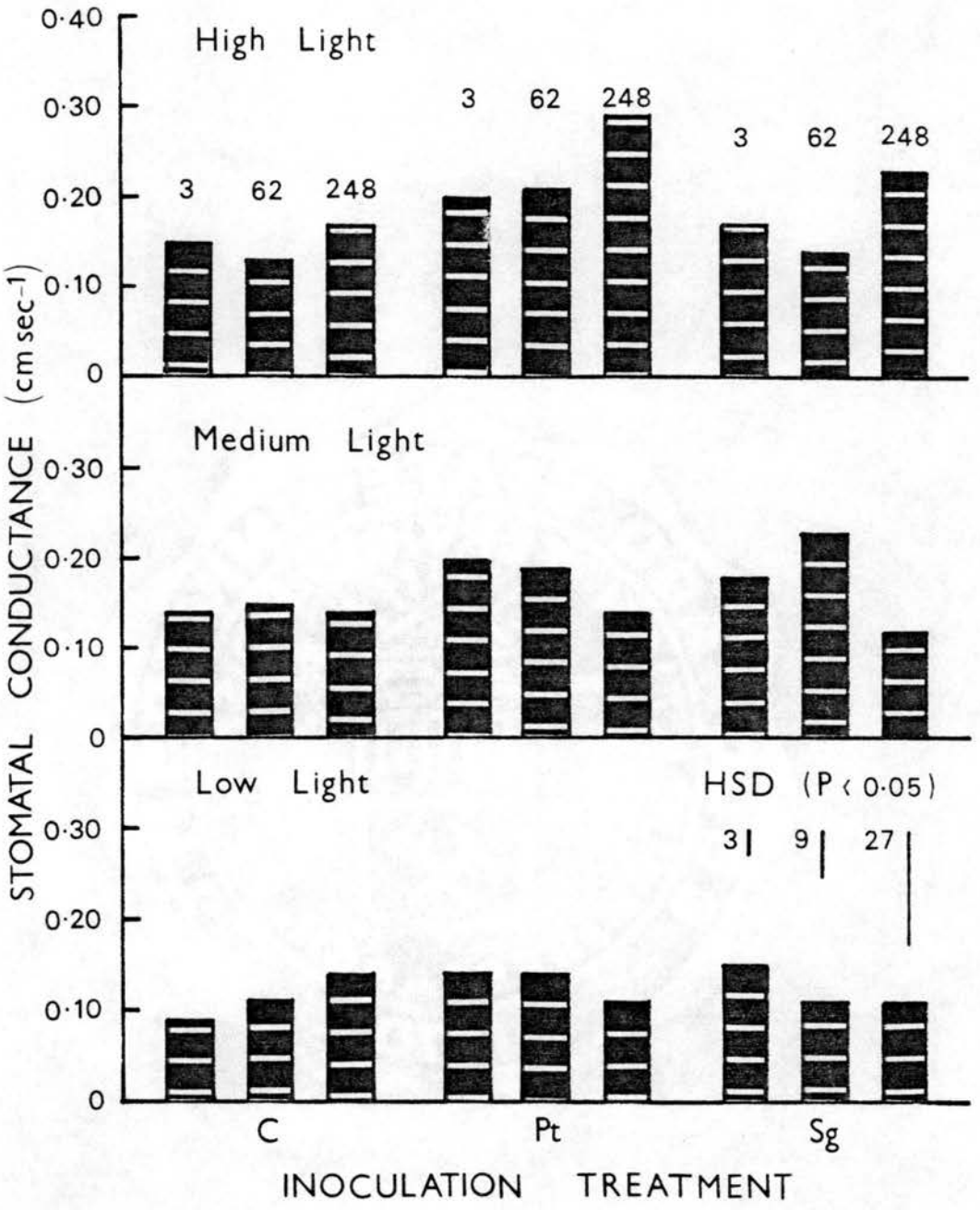


Figure 13. Stomatal conductance (cm sec^{-1}) of 16-week-old P. contorta seedlings in response to mycorrhizal fungi inoculation at 10 weeks of age, 3 levels of irradiance (high, medium and low) and nitrogen (3, 62 and 248 ppm). C = noninoculated controls, Pt = inoculated with Pisolithus tinctorius, and Sg = inoculated with Suillus granulatus. Vertical bars labeled 3, 9 and 27 represent HSD (p = 0.05) for comparisons of 3, 9 and 27 means within any one, two and all treatments, respectively. All figures are means of 5 seedlings.

low nitrogen levels significantly ($p = 0.05$) increased conductance in both P. tinctorius and S. granulatus seedlings. At low light levels, low nitrogen resulted in greater conductance in P. tinctorius and S. granulatus treated plants. Simple linear regression analyses indicated that stomatal conductance was not strongly correlated with P_n rates either on surface area ($r^2 = 0.25$) or dry weight ($r^2 = 0.002$) basis.

Discussion

Despite the obvious benefits of mycorrhizae, realizing their full potential to enhance seedling growth is contingent on a better understanding of how formation is affected by standard nursery practices, such as shading and fertilization. The results of the present study clearly demonstrate that inoculation of containerized conifer seedlings with ectomycorrhizal fungi can lead to some stimulation of plant growth, increased nutrient content and increased photosynthesis, even 6 weeks following inoculation. The magnitude of this improvement depended on specific combinations of light and nitrogen fertilization.

It is evident from the results that variations in light affect growth, yield and photosynthetic rates of containerized conifer seedlings. Increases in both shoot and root dry weights and root/shoot ratios were proportional to increases in light levels over the ranges studied (Fig. 7a). Plants grown at the highest light level were not only significantly heavier in total dry matter, especially root weight (Fig. 7a), but also had a higher root/shoot ratio than those grown at low light levels. These results indicate that increases in irradiance have a greater stimulating effect on root than shoot development. The advantages of high light were also supported by the observed increase in photosynthesis per unit area of leaf (Fig. 11a), increased number of

short roots, and increased mycorrhizae formation on seedling roots (Fig. 8a) as compared to low light. These results emphasize the importance of light levels in conifer growth and mycorrhizae formation on seedling roots.

Two trends emerged regarding the influence of light levels on P_n rates. It is seen from Figure 11a that P_n rates on surface area basis were significantly higher for high light than low light, but when expressed on dry weight basis, showed the opposite trend, thus suggesting that the smaller, low-light plants with less dry matter had more surface area per unit of foliar dry weight and appeared photosynthetically more active than those grown at high light. This is consistent with the results obtained earlier (see Chapter III).

Under the varied light levels, the differential effects of nitrogen fertilization levels were also evident in both shoot and root dry weights, root/shoot ratios, nutrient contents, number of short roots and mycorrhizae formation on seedling roots, and photosynthetic rates. Although maximum dry matter (Fig. 7b), number of short roots (Fig. 8b), nutrient contents (Figs. 9 and 10) and P_n rates (Fig. 11b) were attained at the highest nitrogen (248 ppm) levels, the root/shoot ratios as previously reported (see Chapter III) increased from 3 to 62 ppm N, and decreased from 62 to 248 ppm N (Fig. 7b), indicating that nitrogen levels at 248 ppm had a greater stimulating effect on shoot than root development, contrary to the results obtained with increases in light levels. This is in accord with the results obtained in Chapter III. In spite of the increased number of short roots produced on seedling roots at the highest nitrogen level (Fig. 8b), mycorrhizae formation on seedling roots was few at the highest nitrogen, more frequent at the

lowest nitrogen (3 ppm), but most abundant at the intermediate nitrogen (62 ppm) levels (Fig. 8b). It is inferred from these results that increase in the number of short roots is not necessarily an explanation for increased mycorrhizae formation on seedling roots. Also it is evident from the results that under favorable conditions of light, the frequency of occurrence and the degree of mycorrhizae formation on roots of containerized conifer seedlings are most predominant when seedlings are grown in a nutrient medium containing from 3 to 62 ppm N, and decrease from 62 to 248 ppm N. While these results emphasize the complications that may arise from nitrogen fertilization of conifer seedlings, they also point to the importance of a proper balance between the essential elements, principally N and P for conifer growth and mycorrhizae formation. In the results above, the best mycorrhizae formation at 62 ppm N represents an N/P ratio of 2:1, and the N/P ratios at 3 and 248 ppm N which gave the intermediate and poorest mycorrhizae formation were 1:10 and 8:1, respectively.

Considering growth, yield and P_n rates of seedlings following inoculation, it is unequivocally clear that inoculation with mycorrhizal fungi significantly increased dry matter, nutrient contents, number of short roots and P_n rates of seedlings over the noninoculated control plants, and there appeared to be little difference between the two fungi in their effects on growth, yield and P_n rates of host plants. It is perhaps significant that the marked responses in growth, yield and P_n rates following inoculation occurred in spite of the low average mycorrhizae percent of the inoculated seedlings, and only after 6 weeks from inoculation. Although there were some significant effects of inoculation on stomatal conductance (Fig. 13), it is not clear whether

the magnitude of this response will affect P_n rates (see Jarvis, 1981). At all three light and nitrogen levels where inoculation had no significant effects on stomatal conductance, P_n rates were still greater in inoculated than noninoculated control seedlings. It seems possible, therefore, that the increase in growth of the mycorrhizal seedlings is associated with increased photosynthesis stimulated by the mycorrhizal fungi. However, studies (Lister et al., 1968; Nelson, 1964; Schweers and Meyer, 1970; Shiroya et al., 1962) have suggested that ectomycorrhizal fungi may increase the photosynthesis of their hosts by establishing a physiological sink through utilization of photosynthates for fungal biomass and conversion of assimilates to either storage products or into energy for maintenance of metabolic processes, though some workers (Bidwell and Turner, 1966; Neales and Incoll, 1968; Sweet and Wareing, 1966; Geiger, 1976) have doubted the plausibility of the explanation of increased photosynthesis as a result of source-sink effects. Nevertheless, regardless of what causes increased photosynthesis in ectomycorrhizal plants, the results of the present study clearly indicate that the levels of light and nitrogen fertilization may be important in stimulation of photosynthesis by ectomycorrhizal fungi.

CHAPTER V

SUMMARY AND CONCLUSIONS

Studies were conducted in the greenhouse to examine the relationships between light, nitrogen fertilization, and mycorrhizal fungi inoculation on growth and photosynthesis of containerized lodgepole pine (Pinus contorta Dougl.) seedlings. The first study involved growing lodgepole pine seedlings for 16 weeks without ectomycorrhizae at 3 levels each of irradiance (high, medium and low) and ammonium nitrate (3, 62 and 248 ppm N) added to a basic nutrient solution as previously described by Hocking (1971). Growth parameters (dry weights of shoots, roots and root/shoot ratios), foliar N and P, and photosynthetic rates were determined at 5, 10 and 16 weeks of age. Stomatal conductance was measured at 16 weeks. Net photosynthesis was measured with an infrared gas analyzer in an open system.

The second study involved growing lodgepole pine seedlings for 10 weeks without ectomycorrhizae at the 3 levels of light and nitrogen fertilization described above and at 10 weeks, inoculating with the ectomycorrhizal fungi Pisolithus tinctorius and Suillus granulatus. The mycorrhizal treatments were superimposed on the light and nitrogen treatments, and the seedlings were grown for an additional 6 weeks. At 16 weeks, growth parameters, foliar N and P, and photosynthetic rates were determined as outlined above, as well as stomatal conductance and mycorrhizae formation on seedling roots.

At 5 weeks of age, nitrogen and light treatments resulted in differences in the amounts of dry matter, nutrient contents and photosynthetic rates of seedlings; and at 16 weeks, the growth and yield parameters showed a large response to treatments. Plants receiving the highest light showed a more vigorous development than those at low light. The former had more total dry matter, especially root biomass, and a higher root/shoot ratio than the latter. Thus, high light produced stocky plants with well-developed root systems. Since root/shoot ratio is a measure of potential absorption and transpiration (Brouwer and Dewit, 1968; Kriebel, 1963), such plants with well-developed root systems are preferred, particularly in semiarid afforestation, since they would enable the plants to extract more water and nutrients from the soil. It was also found that net photosynthesis per unit of leaf area and mycorrhizae formation on seedling roots were higher under high light treatments than under low light. These characteristics would be an additional advantage to seedling establishment in the field. These results emphasize the importance of light levels in conifer growth and mycorrhizae formation.

The results also showed that at each light level, fertilization with the high nitrogen level (248 ppm) increased total dry matter, especially shoot biomass, and photosynthesis of seedlings compared to low nitrogen level (3 ppm), but root/shoot ratios increased from 3 to 62 (i.e., intermediate nitrogen level) ppm N, and decreased from 62 to 248 ppm N. Although the number of short roots also increased with increasing levels of nitrogen, mycorrhizae were few on seedlings grown at 248 ppm N, fewer at 3 ppm N, but most abundant at 62 ppm N. These results emphasize the complications that may arise from nitrogen

fertilization of conifers. In nursery practices, high nitrogen fertilization might be undesirable.

Over the years, forestry practices have increasingly incorporated techniques for encouraging and manipulating microbial systems. To economize on space, minimize cost, and reduce weight during transportation of planting stock, small containers in which reserves of nutrients and water are much less than in a nursery seedbed or larger containers are used to grow seedlings. Fertilization of nursery stock is gradually becoming an accepted procedure in forest management practices, and all indications are that fertilization of container plants has presented many problems not encountered in conventional nursery practices (Brix and van den Driessche, 1974). The results above point to one of these problems. Undoubtedly, high nitrogen fertilization may result in increased dry matter production by favoring shoot development, nutrient content and photosynthesis of conifer seedlings; however, as evident from these results, such seedlings are not necessarily those best suited for plantation establishment if they lack sufficient mycorrhizae to carry over to the field, and root/shoot ratios are low. In contrast, although intermediate levels of nitrogen may decrease the above parameters, the overall effect is to favor root development and subsequent mycorrhizae formation on seedling roots. Since good ectomycorrhizae formation on the roots of conifer seedlings is essential for successful establishment of conifer plantations (Harley, 1969; Jorganson and Shoulders, 1967; Rosendahl and Wilde, 1942), such plants are preferred to those fertilized with excessive high nitrogen levels. In these studies, nitrogen fertilization at 62 ppm appeared to be the best level for mycorrhizae formation. Perhaps it is significant to note that

although these results are obtained for lodgepole pine seedlings, the fundamental principles are believed to be the same, and may therefore be extrapolated to other conifers, regardless of differences in the absorptive capacity between various conifer species. It is therefore concluded that in nursery fertilization of conifers, the impact of fertilizer prescriptions, either in containers or seedbeds, on mycorrhizae should be considered.

It was also found that inoculation of containerized pine seedlings with mycorrhizal fungi can lead to a stimulation of plant growth, nutrient content and photosynthesis of seedlings, some 6 weeks following inoculation. The magnitude of this response depended on the levels of light and nitrogen fertilization, but was independent of mycorrhizal development at these low percentages. Inoculated plants produced significantly more dry matter, took up more nutrients and were photosynthetically more active than the noninoculated seedlings. The potential for improved growth and increased productivity by the inoculation of nursery stock with mycorrhizal fungi prior to planting in the field seems apparent from these results. Bjorkman (1970) pointed out that if a practical means could be devised for the introduction of mycorrhizal fungi in forestation, such might constitute a suitable alternative to chemical fertilization, since nutrients, particularly N, P and K could be more readily utilized.

It is hoped that the results reported herein will be applicable to other situations, particularly in the lowland tropics where in recent years the genus Pinus has proved to be one of the most promising species for plantation forestry.

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APPENDIX 1

FORMULATION OF DAILY NUTRIENT LEVELS IN FERTILIZER APPLIED TO SEEDLINGS

Macronutrients		g/l		ppm
$MgSO_4 \cdot 7H_2O$		0.492	Mg	48
			S	64
K_2CO_3		0.276	K	156
$CaCl_2 \cdot 2H_2O$		0.294	Ca	80
H_3PO_4 (85%)		0.115 ml/l	P	31
NH_4NO_3	i	0.009	N	3
	ii	0.177	N	62
	iii	0.709	N	248

Micronutrients	g/l		ppm
H_3BO_3	0.022	B	0.04
M_oO_3	0.00005	M_o	0.03
M_nCl_2	0.0003	M_n	0.20
Z_nCl_2	0.0001	Z_n	0.05
$CuCl_2 \cdot 2H_2O$	0.00005	Cu	0.02
Sequestrene 330 Fe 0.24 g/l, then	0.21 ml/l	Fe	0.67