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Title: The Influence of Ectomycorrhizae on Drought Tolerance Characteristics of Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) Seedlings

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Douglas-fir seedlings were inoculated with different species of ectomycorrhizae-forming fungi in order to test the concept that ectomycorrhizae enhance the drought tolerance of seedlings and to investigate the mechanisms responsible for this effect.

Seedlings were transplanted at age 6 to 8 weeks into pots containing pasteurized loam soil and inoculated with either Rhizopogon vinicolor (Rv), Laccaria laccata (Ll), or Hebeloma crustuliniforme (Hc), or left uninoculated. Rv and Hc colonization produced abundant hyphal growth, while Ll produced much less hyphae. After 4 months under well-watered greenhouse conditions, neither Rv- or Ll-colonized seedlings had significantly different dry mass and leaf N, P, K, and Ca concentrations compared to nonmycorrhizal controls. Higher nutrient concentrations of Hc-colonized seedlings resulted from suppressed growth, since total amounts of these nutrients were equal to or less than for nonmycorrhizal controls.

Seedlings were transferred to a growth room where photosynthesis, stomatal conductance, and plant water potential components were measured under well-watered and soil water-limiting conditions. Drought tolerance, as evaluated by net photosynthesis rate over the soil water potential range of -0.05 to -0.6 MPa, was clearly enhanced by Rv, somewhat enhanced by Hc, and decreased by Ll compared to nonmycorrhizal controls. Stomatal conductances closely followed net

photosynthesis rates. Compared to control seedlings, leaf water potentials of mycorrhizal seedlings were lower (Rv by 0.2 to 0.3 MPa) or similar (Ll and Hc) over the entire range of soil water potential. Significantly reduced root lengths (Rv 65% of control; Hc 70% of control; Ll 90% of control) may have counteracted a mycorrhizal benefit of efficient water absorption.

It is hypothesized that higher net photosynthesis rate and stomatal conductance despite lower leaf water potential, as observed for Rv-colonized seedlings, can arise from an ectomycorrhizae-altered carbon economy of the plants. According to this hypothesis, net photosynthesis rate and stomatal conductance are correlated with photosynthate sink demand, which here would be increased by export to the mycorrhizal fungus. Strong mycorrhizal demand, which occurs at some cost to plant growth, stimulates photosynthesis, to which the stomata respond by opening in spite of water stress. The degree to which this effect was observed in this study correlated with the visual abundance of hyphal growth which each fungal species developed.

The Influence of Ectomycorrhizae on Drought Tolerance Characteristics of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) Seedlings

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INTRODUCTION

A leading cause of failure of reforestation in the western United States is drought. Drought refers to low soil moisture, and results from hot, nearly rainless summers. Drought increases plant water stress resulting in reduced growth and survival. In order to survive and grow under limited soil moisture availability, seedlings must avoid excessive plant water stress by controlling water loss while maintaining water absorption, and/or adjust cellular conditions to tolerate greater degrees of stress. Logically, one way of improving reforestation success in areas of limited soil moisture would be to enhance the ability of seedlings to perform one or more of these functions.

It has long been known that certain fungi colonize plant roots and form mycorrhizae, a symbiotic association which improves plant growth. The necessity of mycorrhizae for the survival and growth of trees has been repeatedly demonstrated in afforestation efforts in many parts of the world (Molina and Trappe, 1982). Observations suggest that mycorrhizae afford plants with improved ability to survive and grow under limited soil moisture (Reid, 1979). Some recent studies infer that this improved performance is related to a modification of the plant's water uptake characteristics by the presence and activity of the mycorrhizae (Safir and Nelson, 1985). Others have suggested that stomatal control (e.g. Levi and Krikun, 1980) and osmotic adjustment (e.g. Allen and Boosalis, 1983) are enhanced. Thus, management of mycorrhizal associations might be a useful tool for improving reforestation success.

Past studies suggest that different fungus/host associations induce different plant growth and drought tolerance characteristics (Harley and Smith, 1983). Most research on this matter has been done

on vegetable and fruit crop plants and grasses that form vesicular-arbuscular (VA) mycorrhizae. Little-studied, but uniquely important to forestry, are the structurally dissimilar ectomycorrhizae common to conifers, including regionally important Douglas-fir. Ectomycorrhizae are formed by fungi which are taxonomically unrelated to those which form VA mycorrhizae.

Douglas-fir may form ectomycorrhizal associations with as many as 2000 different species of fungi (Trappe, 1977), most of which can be isolated, cultured and inoculated to Douglas-fir seedlings. There is evidence that some fungal species influence Douglas-fir growth and water relations more than others (Parke et al., 1983), and that some fungi provide marginal benefit or may even hinder Douglas-fir plantation performance (Alvarez and Trappe, 1983). In order to use ectomycorrhizae and maximize their potential benefit to Douglas-fir crops, it is important to select the best fungal symbionts from among the many possible choices.

Selection of the 'best' fungal symbiont will be enhanced by understanding the mechanisms by which these fungi impart a degree of drought tolerance to seedlings. It is not enough to only know the effect of mycorrhizal colonization on drought tolerance under one set of environmental conditions because the effect of a fungal symbiont on a plants' behavior may vary under different environmental conditions (Trappe, 1977). However, if we understand the fundamental mechanisms underlying the mycorrhizal effect, we should be able to predict mycorrhizal effects under different conditions. Combined with this understanding, then, comparative experiments under one set of conditions should allow us to identify those fungal symbionts which are most likely to improve seedling growth and survival in the field. Toward this broader goal, the objectives of this study were to:

1) determine if colonization by ectomycorrhizae-forming fungi influences the drought tolerance of Douglas-fir seedlings,
2) investigate mechanisms involved.

LITERATURE REVIEW

Drought Tolerance Characteristics of Plants

For the purpose of this study, drought tolerance refers to the ability of an individual plant to survive and grow under conditions where supply of soil water is limited (Kramer, 1980). In perennial plants, drought tolerance can be ascribed to regulatory capabilities which:

1) optimize and stabilize plant water potential and water content,
2) contribute to tolerance of suboptimal plant water potential and water content.

Processes and characteristics which enhance drought tolerance of plants can be grouped according to those which give the plant greater ability to:

1) extract soil water,
2) respond to limited water uptake by restricting water loss through leaves,
3) minimize detrimental effects of increased plant water stress.

If mycorrhizae influence the tolerance of plants to drought, we would expect a change in one or more of these characteristics.

It is generally accepted that under normal conditions, plant growth increases when root systems are colonized by mycorrhizae-forming fungi (Harley and Smith, 1983). Better growth has been attributed to improved nutrition, protection from root pathogens, improved tolerance of toxins and extremes of soil pH and temperature, and altered plant growth regulator balances conferred on the plant by this colonization (Zak, 1964; Marks and Kozlowski, 1973; Sanders et

al., 1975; Harley and Smith, 1983). Better growth is also commonly observed for mycorrhizal plants compared to nonmycorrhizal plants under limited soil moisture (Hardie and Leyton, 1981; Nelson and Safir, 1982b; Levy et al., 1983; Sweatt and Davies, 1984). Improved plant survivability of extreme drought conditions has also been suggested (Goss, 1960; Reid, 1979), and rate of recovery from moisture stress demonstrated (Safir et al., 1971, 1972; Levy and Krikun, 1980; Hardie and Leyton, 1981; Nelson and Safir, 1982b; Parke et al., 1983). Plant growth responses under moisture stress have been related to improved ability of the plant to take up soil water and tolerate water stress in tissues. Thus, the presence of mycorrhizae has been correlated to drought tolerance characteristics of host plants.

Types of Mycorrhizae

Mycorrhizae are distinctly different organs than non-colonized plant roots. They are structurally different because of the intimate association of fungal hyphae with root cells. Mycorrhizae are also functionally different than roots partly due to the structural differences, and partly resulting from plant and fungal physiological interactions with each other and with their environment (Harley and Smith, 1983). The physiological basis of mycorrhizal associations is that the fungus derives most, if not all, of its carbon from plant photosynthate while the plant receives any of several possible benefits from the fungus, including enhanced water and nutrient uptake capability.

Mycorrhizae are commonly classified according to gross morphological features (Harley and Smith, 1983). Two major types of mycorrhizae, namely vesicular-arbuscular (VA) mycorrhizae and ectomycorrhizae, exemplify the range of mycorrhizal morphological characteristics. VA mycorrhizae, are characterized by fungi with aseptate hyphae which flourish around the root surface and penetrate both between root epidermal and cortical cells and within these cells.

They typically form arbuscules within host cells and swollen vesicles inside and outside of host cells. Although the epidermal and cortical cell walls show some deformation at contact with fungal hyphae, little gross change in morphology of root tissue occurs and only microscopic investigation of roots can reveal if they are indeed VA mycorrhizal.

Ectomycorrhizae, at the other extreme, develop a mantel or sheath of hyphae which encloses the colonized root (Harley and Smith, 1983). Where the sheath occurs, root hair development is suppressed. From the sheath, hyphae and/or rhizomorphs grow outward into adjacent soil. Hyphae typically penetrate between, and rarely within, host cells to form a dense intercellular network of hyphae called a Hartig net. Some ectomycorrhizal fungi, however, characteristically develop only a rudimentary Hartig net. Where the Hartig net occurs, root cells are greatly enlarged in width, but not length, and intercellular spaces filled with hyphae become large (Harley and Smith,1983), but intercellular plasmodesmata connections remain undamaged (Nylund, 1980). Outwardly, the ectomycorrhizae appear as greatly thickened roots, some types with thick sheaths, and commonly more highly branched than roots (Gerdemann, 1971).

In both VA and ectomycorrhizae, colonization is limited to primary roots and the inward limit to hyphal penetration is the cortex adjacent to the endodermis (Marks and Foster, 1973). The vascular tissue is not penetrated. Colonization lasts until secondary differentiation of the root stele occurs, at which time, the fungal tissues are sloughed off along with the cortex (Harley and Smith, 1983). There are a few other types of mycorrhizae, but they generally exhibit different combinations or degrees of sheathing, host cell penetration and hyphal structures in the cortex.

These general groupings of mycorrhizal types are convenient because mycorrhizal morphologies are characteristic of closely related fungal taxa. VA mycorrhizae are formed by Zygomycetes of the family Endogonaceae, while ectomycorrhizae are formed primarily by higher Basidiomycetes and Ascomycetes (Trappe, 1977). Also to a significant degree, fungi which develop certain mycorrhizal types tend to associate with certain taxa of plants. In general, most crop plants

and grasses form VA mycorrhizae, while ectomycorrhizae are common to forest trees such as Douglas-fir.

Based upon structural differences between these two broad types of mycorrhizae, we might expect each of them to influence plants differently. Since the fungal and host taxa are also different between these two types of mycorrhizae, we might also expect physiological dissimilarities resulting in different influences on the plants. The specific nature of the functional difference between VA and ectomycorrhizae, however, has not been clarified. Of particular interest in this study are the ectomycorrhizal associations common to conifers of the family Pinaceae. However, most studies on changes in plant water relations responses due to mycorrhizal colonization have dealt with VA mycorrhizal associations.

Mycorrhizal Influence on Water Uptake by Plants

Mycorrhizal colonization appears to change the amount of roots of plants in several ways. First, where overall plant growth is stimulated by colonization, the total amount of roots is also generally increased. Second, while mycorrhizal colonization generally correlates with decreases in the proportion of plant biomass in root systems, it increases the life span of fine roots. In Pinus, these fine roots senesce within a few months after development unless they become mycorrhizal, in which case their life span increases to a year or more (Harley and Smith, 1983). This delay in maturation and senescence of primary root tissues clearly could increase the proportion of these fine absorbing structures.

Colonization could also greatly influence the efficiency of roots for water uptake. Common to nearly all types of mycorrhizae is a proliferation of fungal hyphae through the intercellular spaces of the colonized host root epidermis and cortical cells, and, to varying degrees, extending outward into the adjacent soil. It is easy to visualize these external fungal hyphae as extensions of absorbing

roots in a manner similar to root hairs, but much longer. In fact, it appears that these fungal hyphae are capable of functioning similar to roots for water absorption. Duddridge et al. (1980) and Brownlee et al. (1983) have shown quite conclusively that aggregated hyphal strands of ectomycorrhizae, rhizomorphs, do absorb and conduct physiologically significant quantities of water over long distances to the mycorrhiza cortex, where it is then transferred to by the plant. Rhizomorphs are highly differentiated, with large diameter central hyphae, generally lacking cytoplasmic contents, surrounded by dense, cytoplasmic, thick-walled hyphae. Duddridge et al. (1980) suggested that the large central hyphae provide a low resistance conduit for water flow, and that rhizomorphs assist seedlings in exploiting moist soil at long distances from the root surface.

The ability to produce rhizomorphs appears restricted to only some ectomycorrhizal fungi and only under certain environmental conditions (Skinner and Bowen, 1974). Further, different fungal species exhibit characteristic degrees of differentiation (Townsend, 1954). The rhizomorphs described by Duddridge et al. (1980) are comparable to the highest degree of differentiation found by Townsend (1954). Thus, not all mycorrhizae produce rhizomorphs, nor can those which do, be expected to conduct water to the same degree.

Closer to the plant root surface, individual external hyphae may also absorb water from soil in the vicinity of the mycorrhizae and translocate it toward the vascular tissue. Although these hyphae contain cytoplasm, movement of water through such tissues is known to occur at significant rates in response to osmotic pressure gradients (Jennings et al., 1974). Thus, external hyphae and rhizomorphs may be viewed in a general way to function like an increased fine root system with regard to water uptake and transport.

Although, mycorrhizal colonization theoretically could increase the water absorbing capability of plants, the actual ability, however, could depend upon soil water conditions. This idea has received little recognition and has been inadequately explored in the literature. Continuous hyphal conduits extending from the cortex to long distances out into the soil represent a modification of the water uptake flow

pathway from soil toward the leaves of plants. The water transmission characteristics of soil and root tissue are very different, so we can expect that hyphae would contribute differently to water flow through these two portions of the pathway. Since the water transmission capability of the soil changes radically as it dries, we can also expect that fungal hyphae would contribute differently to soil water flow depending upon how dry the soil is. Thus, depending upon mycorrhizal form and function, and soil water status, the effect of colonization on one of these portions of the water uptake pathway might dominate over others.

From Wet Soil

Closer examination of the influence of mycorrhizal colonization on the efficiency of water uptake by plant roots is facilitated by describing the water uptake flow as a continuum, where water flows along a gradient of decreasing water potential from soil through roots to the leaves, at a rate proportional to the magnitude of the gradient. The rate is limited by the transmission resistance of the pathway (Philip, 1957). Lower plant water potential in leaves is initiated and maintained by transpiration. The water uptake pathway can be conveniently divided into soil, root and xylem segments since the transmission characteristics of each of these segments differ greatly and have relatively well-defined boundaries.

For plants rooted in wet soil, the resistance to water flow through the plant is much larger than through the soil to the root surface (Jarvis, 1975). Most of this resistance occurs in the roots (Jensen et al., 1961; Tinklin and Weatherly, 1966), probably radially across the root to the xylem (Boyer, 1971), and perhaps dominantly across the endodermis, where flow must pass through at least two membranes to reach the xylem (Nobel, 1983). Water flow resistance through xylem probably is never large relative to the total resistance (Boyer, 1971; Herkelrath et al., 1977). Thus, water transport through

the soil to the root surface by hyphae and rhizomorphs would contribute little to water uptake by plants since the dominant limitation to water uptake flow under wet soil conditions lies within the roots and not in the wet soil. Under these conditions, hyphal transport of water could effect water uptake flow between the mycorrhizal surface and the endodermis.

Mycorrhizal colonization modifies the structure of absorbing roots in ways which might affect their permeability to water. In his review, Jarvis (1975) concluded that water moves across the root cortex primarily through cell wall pores and intercellular spaces (apoplast). Fungal hyphae occupy at least some of this intercellular space, especially in ectomycorrhizae with an extensive Hartig net. However, the amount of intercellular space may be increased to accommodate the added hyphae. Cortical cell wall structure is modified where contacted by fungal hyphae in some associations (Harley and Smith, 1983). Lack of information on the specific nature of changes in cell wall structure and free space volume make it impossible to speculate about the net result of these effects on apoplastic water flow.

Water may also flow through hyphae, and at significant velocities (Jennings et al., 1974), toward the endodermis. Where intercellular penetration is extensive, this pathway might short circuit root cortical resistance to flow. However, for this water to be transferred to the host, it must pass through the fungal plasma-limiting membrane, perhaps greatly decreasing the efficiency of this mechanism.

The permeability of root tissue to water is variable (Parsons and Kramer, 1974) and might respond to physiological conditions in the plant which could be influenced by mycorrhizae. Such physiological conditions include mineral nutrition and plant hormone balance.

It is well-established that mycorrhizae generally improve nutrient uptake, especially relatively immobile phosphate ions. Nelson and Safir (1982b) have found phosphorus concentrations in mycorrhizal onion plants to be much greater than even phosphorus fertilized uncolonized plants, when subjected to cyclical drought. They suggest that phosphorus levels may regulate, to some degree, membrane

permeability to water (Safir and Nelson, 1985). Radin and Eidenboch (1984), studying phosphorus deficient cotton, found reduced root hydraulic conductance, corroborating the findings of Nelson and Safir (1982b). Phosphate is an important component of membrane-forming lipids, but how low total tissue phosphorus levels translate into a limitation for water movement through membranes has not been examined.

If phosphorus nutrition does indeed influence membrane permeability, as Safir and Nelson (1985) hypothesize, then water movement through the cortex and endodermis will be affected. A major pathway for water to by-pass the casparian strip, is to pass through a membrane into the root symplast and diffuse toward the stele. The symplast may be entered at any cell from the epidermis to the outer edge of the endodermis. To enter the root xylem, water then passes out of the symplast through another membrane near the interior of the endodermis. Since membranes exhibit low water permeabilities compared to free space, and diffusion through cellular contents may be relatively slow, the symplast pathway is often considered to be a highly limiting pathway for water flow (Jarvis, 1975). Increased membrane permeability, then, will reduce a significant resistance in this pathway. Further, if better overall plant nutrition is the cause, then the entire rooting system, roots and mycorrhizae, become more efficient absorbing organs.

Safir and Nelson (1985) argue that in most reported cases where water relations of plants are altered by mycorrhizal colonization, these changes are due indirectly to improved plant nutrition as it effects root hydraulic resistance. They base their hypothesis on studies with soybeans (Safir et al., 1972) and onions (Nelson and Safir, 1982a), where water uptake resistance was reduced by fertilization of nonmycorrhizal plants to levels nearly equal to that of VA mycorrhizal plants. The study of Nelson and Safir (1982a) strongly implicates phosphorus as the key nutrient. Supportive evidence comes from good correlation between higher concentration of tissue phosphorus and lower uptake resistance in mycorrhizal clover (Hardie and Leyton, 1981; Allen et al., 1981), lower water uptake resistance in mycorrhizal plants than for nonmycorrhizal plants in

studies where no fertilizer was applied (Allen, 1982), and nearly equal resistances in studies where available soil phosphorus was very high (Levy and Krikun, 1980).

Plant hormones and growth regulators are known to alter the permeability of roots, but it is not known if mycorrhizae induce such changes. Parsons and Kramer (1974) found that hydraulic resistance of roots cycles diurnally and their data suggest that this is controlled by a growth regulator originating in the shoots. Markhart et al. (1979) demonstrated that exogenous application of abscisic acid lowers root hydraulic resistance of roots in soybeans, and suggested that it acts at the membrane level. Collins and Kerrigan (1974) confirmed Markhart's results for abscisic acid, and also found that kinetin application reduced water permeability of roots. Allen et al. (1980) found greatly increased cytokinin levels in roots and leaves of VA mycorrhizal grasses compared to uncolonized controls, but this did not translate into reduced water uptake permeability (Allen et al., 1981) as Collins and Kerrigan's data would suggest. Allen et al. (1982) also found greatly reduced abscisic acid levels in leaves of mycorrhizal grasses, but not in roots. Ectomycorrhizae and pure cultures of ectomycorrhizal fungi have been found to produce plant hormones (Crafts and Miller, 1974; Graham and Linderman, 1980; Ek et al., 1983; DeVries et al., 1987). When some of these substances were exogenously applied to roots, root growth was stimulated and some qualities of their structure resembled those which are unique to mycorrhizae (Slankis, 1973; Graham and Linderman, 1981; DeVries et al., 1987). However, there is no consistent evidence which correlates altered hormone or growth regulator levels in mycorrhizal plants with increased water uptake resulting from altered root permeability.

The relative importance of mycorrhizal changes to total amount and efficiency of roots on water uptake by plants from wet soil has been investigated in several studies. These studies evaluate the net effect of all contributing factors and provide clues to the dominant mechanisms of the effect. Safir et al. (1971) found that mycorrhizal soybean plants had a lower water uptake resistance from wet soil. They explained that mycorrhizae might do this in four possible ways. First,

hyphae in the soil absorb and translocate water to the root cortex, much like root hairs. Second, hyphal penetration of the root cortex provides a lower resistance pathway across it. Third, better nutrition could decrease root hydraulic resistance. Fourth, mycorrhizal root systems were larger, resulting in lower overall hydraulic resistance of the root systems. In their experiment, they found that root system sizes were not different. In view of this discussion, soil hyphae were probably not important since the soil was wet. However, lower cortical resistance is possible, and nutritional effects have since been substantiated (Safir et al., 1972).

Allen (1982) found lower resistance to water uptake in well-watered mycorrhizal grasses and hypothesized direct hyphal uptake and transport to the roots as the cause. Hardie and Leyton (1981) found lower root resistance in well-watered mycorrhizal clover, acknowledged the possibility of lower cortical resistance, but finally hypothesized better transport to the root as the dominant effect. These interpretations, however, lack substance since the soil portion of the pathway provides negligible resistance to water uptake flow when the soil is wet. It is apparent in these studies that, for wet soil conditions, too much interpretive value has been placed on the absorption of water by soil hyphae and transport to the surface of mycorrhizae.

Less resistance to water uptake from wet soil by mycorrhizal plants has not always been found. No effect was detected by Levy and Krikun (1980) for fertilized citrus, Nelson and Safir (1982a) in nutritionally similar onion plants, nor by Sands and Theodoreau (1978) in ectomycorrhizal pine seedlings. Two studies, one with Pinus (Sands and Theodoreau, 1978) and one with citrus (Levy et al., 1983), point out that mycorrhizal enhancement of efficiency with which plant roots take up soil water may not override the lack of a large, well-distributed plant rooting system.

From Dry Soil

As the soil dries, the permeability of roots to water becomes a relatively smaller factor in water uptake by plants compared to the ability of water to flow through the soil to the roots. Numerous studies have shown that for plants rooted in soil, the total resistance to water uptake from the soil to leaves increases as the soil dries (Sands and Theodoreau, 1978; Taylor and Klepper, 1975; among others). This implies that the decrease in water uptake (and transpiration) rate that is normally observed as the soil dries, is greater than can be simply explained by a change in the water potentials of soil and leaves. As soil dries, its ability to transmit water, as measured by unsaturated hydraulic conductivity, to the roots diminishes rapidly (Gardner, 1960). Because of this, the location of the dominant resistance to water uptake by plants shifts from the root, under wet soil conditions, toward the soil portion of the pathway as the soil dries.

Recent mathematical modelling of this segment of the water uptake pathway shows that the soil limitation to water uptake by plants becomes significant in soil as moist as -0.10 MPa water potential (McCoy et al., 1984). This water potential can be higher in very coarse textured soils and for plants with fewer absorbing roots and greater water uptake rates, and it can be lower in finer textured soils and for plants with more absorbing roots and lower water uptake rates (Gardner, 1964; McCoy et al., 1984).

Limitations to water flow develop very close to the root surfaces in dry soil. The water potential of the root decreases in response to added soil resistance in order to develop a stronger gradient for maintaining the same flow rate to roots. Lower water potential at the root surface is translated into lower soil water potential, and thus dryer soil, adjacent to the root surface which, in turn, increases the resistance even more. Because of this increase in soil hydraulic resistance, the location of the dominant limitation to water uptake shifts quickly from root to soil as the bulk soil dries and occurs no

farther from the root surface than a couple of millimeters (McCoy et al., 1984). The coarser the soil texture and the greater the water uptake rate per unit of absorbing root length, the more drastically the shift occurs. In this way, severe plant water stress can occur when bulk soil water potential is much higher than water potential in the plant. It is also important to note at this point that stomatal responses which change transpiration rate influence water uptake resistance in dry soil by changing the water uptake flux per unit root length. This point will be discussed later in the section on mycorrhizal influences on stomatal behavior.

It has also been hypothesized that a different water flow limiting resistance can develop at the soil-root interface as the soil dries. This limitation might develop because large pores at the soil-root interface drain under higher water potentials, causing an air gap between soil water and the root surface (Herkelrath et al., 1977). Such an air gap would effectively limit water uptake rate because of a decrease in cross section available for liquid flow. This air gap could be increased by root diameter shrinkage in response to increasing plant water stress (Huck et al., 1970). However, primary roots of many plants exude mucilagenous materials which may minimize any soil-root surface contact problem (Hale and Moore, 1979; Mengel and Kirkby, 1982).

As soil dries, soil and perhaps soil-root contact resistance become more limiting to water uptake by roots. Under these conditions, water-conducting soil hyphae, fungal sheaths and rhizomorphs of mycorrhizae can more greatly influence water uptake. Uptake and transport of water by these fungal structures from soil a few millimeters from the mycorrhizal surface to the sheath or cortex would provide lower resistance than that of the soil. In effect, hyphae and rhizomorphs provide a short bridge for water flow to roots in dry soil. A second benefit would be that the thin zone of high soil hydraulic resistance around the mycorrhizae would not develop as drastically as for an uncolonized root, since the water uptake flow through the soil to the mycorrhizal surface is effectively reduced. In effect, the mycorrhizal surface can remain a more active absorption

zone when the bulk soil is drier than that for a comparable root. Soil hyphae, rhizomorphs and the mycorrhizal sheath would have the same effect on any developing air gap. These benefits of conductive fungal hyphae would offset detriments of colonization such as reduced mucilage and suppression of root hair development (Harley and Smith, 1983).

Under extreme drought conditions, mycorrhizal colonization might protect roots from structural damage. Observations by Cromer (1935), and later by Goss (1960), on Pinus show that under severe drought stress, ectomycorrhizae sustain considerably less cortical cell collapse and less diameter shrinkage than uncolonized roots. During drought, this can help maintain soil-root contact to allow sustainable water quantities to flow into the plant. After drought, more undamaged root tissue could act to speed water and nutrient uptake by the plant, assisting in rapid recovery from severe plant water stress. Indeed, Goss (1960) found that a much higher proportion of mycorrhizal seedlings survived 17 days without rewatering and that growth resumed much sooner than for nonmycorrhizal seedlings. More rapid metabolic and water stress recovery of mycorrhizal plants than nonmycorrhizal plants has been observed (Levy and Krikun, 1980; Hardie and Leyton, 1981; Parke et al., 1983; Sweatt and Davies, 1984), but not for all fungal associations (Parke et al., 1983).

Long distance transport of water through soil to roots by rhizomorphs could also improve plant water uptake. As Brownlee et al. (1983) showed, sustainable quantities of water can be taken up by ectomycorrhizal rhizomorphs from distant water sources to plants rooted in very dry soil. In the field, distant water sources could be decaying logs and deeper horizons of wet soil as summer drought conditions prevail.

In theory, it appears that conductive hyphae, rhizomorphs and fungal sheaths make mycorrhizae more efficient absorbers of water from dry soil. The net effect is that the plant water potential need not go as low to develop sufficient gradient to maintain water uptake. As a result, the mycorrhizal plant sustains less water stress than uncolonized plants. In practice, however, there have been few studies

on whole intact plants which verify this result. For VA mycorrhizal grass rooted in dry soil, Allen et al. (1981) calculated lower water uptake resistance, but the data of Sands and Theodoreau (1978) clearly show higher resistance for ectomycorrhizal pine seedlings which lead to lower plant water potential. Further study is required to elucidate the causes of these contrasting results.

In summary, the literature is replete with hypotheses explaining why mycorrhizal colonization should improve the efficiency of water uptake from soil, and lead to higher plant water potential. Some of them demonstrate lack of fundamental knowledge about the limiting conditions to water uptake by plants. Furthermore, a significant proportion of relevant studies have not confirmed a net benefit. This could result from a lack of quantitative information regarding each of these mechanisms. This could also be because other mycorrhizal factors which could influence water uptake have not been considered. One such factor which has not been recognized, is that the mycorrhizal effect on both water uptake resistance and plant water potential is dependent upon how mycorrhizae influence water loss through stomata.

Mycorrhizal Influence on Stomatal Response to Plant Water Stress

The preceding discussion suggests that mycorrhizae could help minimize plant water stress in wet soil and during drought periods by enhancing water uptake from soil. This can occur through improved root growth and permeability when the soil is wet, and through hyphal uptake and transport through the soil as the soil becomes drier. Nelson and Safir (1982a) confirmed higher plant water potentials in mycorrhizal plants. However, most studies show mycorrhizal plants to have lower (Sands and Theodoreau, 1978; Sweatt and Davies, 1984) or similar plant water potential (Levy and Krikun, 1980; Allen et al., 1981; Allen, 1982; Allen and Boosalis, 1983; Huang et al., 1984; Stahl and Smith, 1984; Koide, 1985; Auge et al., 1986) compared to nonmycorrhizal plants under a wide range of soil water conditions.

More importantly, while mycorrhizae did not alleviate plant water stress in these studies, colonization consistently led to greater stomatal conductances at all but the extreme plant water stress conditions. These two observations imply that regardless of whether water uptake is enhanced or not, stomatal aperture tends to be greater in mycorrhizal plants for a given level of plant water stress. Mycorrhizal colonization appears to alter the relationship between stomatal aperture and plant water stress.

All other influences being equal, stomata become more closed as plant water potential decreases. This response is a regulatory action which limits water loss as water uptake becomes more limited in order to prevent excessive water stress in tissues. When the relationship between stomatal aperture and plant water stress changes, some physiological factor which influences stomata must be responsible.

Safir and Nelson (1985) have hypothesized that stomatal behavior is modified by mycorrhizal colonization through enhanced plant nutrition, particularly phosphorus. In general, extreme P deficiencies correlate with reduced stomatal aperture (Atkinson and Davison, 1972; Hak and Natr, 1984; Radin, 1984; Ackerson, 1985). However, Auge et al. (1986) tested Safir and Nelson's hypothesis and found that greater stomatal conductance of VA mycorrhizal rose plants could not be explained by enhanced P nutrition. Deficiencies of other nutrients, such as nitrogen and potassium, also correlate with reduced stomatal aperture (Bradbury and Malcolm, 1977; Radin and Parker, 1979a, 1979b; Radin and Ackerson, 1981; Hak and Natr, 1984), but these have not been rigorously tested in mycorrhizal systems.

Stomatal behavior is influenced by hormone balances. Cytokinins induce stomatal opening (Incoll and Jewer, 1987), which can be reversed by abscisic acid (Raschke, 1987). In grasses, VA mycorrhizal colonization has been correlated with higher leaf cytokinin and gibberellin concentrations (Allen et al., 1980) and lower leaf abscisic acid (Allen et al., 1982). This is consistent with observations of greater stomatal conductance in mycorrhizal plants, but there is no evidence to indicate whether these changes are due directly to the activity of the mycorrhizae, are a response to other

altered conditions in mycorrhizal plants, such as nutrition or water stress, or even that the degree of change in hormone concentrations which Allen et al. (1980,1982) found is capable of inducing a stomatal response. In spite of these uncertainties, and commonly without assessing hormone levels in their studies, several researchers have used this plant hormone hypothesis to explain modified stomatal behavior of mycorrhizal plants (Levy and Krikun, 1980; Allen and Boosalis, 1983; Sweatt and Davies, 1984; Koide, 1985).

Stomatal conductance could be influenced by altered photosynthate sink strength. Greater sink activity can be transduced, in several ways, to higher photosynthesis rate (Herold, 1980), which in turn can induce a stomatal opening response (Farquhar and Sharkey, 1982). Mycorrhizal root systems are known to be greater photosynthate sinks than nonmycorrhizal root systems (Harley and Smith,1983; Koch and Johnson, 1984), and higher net photosynthesis rates have been measured in mycorrhizal plants (Reid et al., 1983; Nylund and Unestam, 1987). Reid et al. (1983) attempted to test the hypothesis that enhanced photosynthate demand by mycorrhizal root systems could contribute to increased net photosynthesis rate, but their results were inconclusive due to greatly elevated N and P concentrations in their mycorrhizal plants, which themselves could lead to greater net photosynthesis rate (Natr, 1972). The hypothesis that mycorrhizal enhancement of root system sink strength could lead to greater stomatal conductance through stimulated photosynthesis is reasonable but has not yet been experimentally examined.

In several studies, greater stomatal conductance in mycorrhizal plants under drought stress has been attributed to enhanced osmotic adjustment (Levy and Krikun, 1980; Hardie and Leyton, 1981; Allen and Boosalis, 1983; Stahl and Smith, 1984), although only Allen and Boosalis (1983) actually measured osmoticum concentrations. Osmotic adjustment, which is an accumulation of solutes in leaf mesophyll cells, maintains leaf turgor as plant water potential declines. Presumably, this also applies to guard cells. However, there are some problems with this hypothesis for explaining greater stomatal conductance in mycorrhizal plants. First, osmotic adjustment generally

occurs after multiple cycles of severe drought (Turner and Jones, 1980) which does not explain observations of greater stomatal conductance in either wet soil or during initial drought stress cycles. Second, not all plants show an ability to osmotically adjust in response to increasing water stress. In their review of research on this behavior, Turner and Jones (1980) found evidence for significant osmotic adjustment in sorghum, sunflower and some grasses. However, they also cite several studies showing osmotic adjustment in some cultivars of tomato, wheat and soybean, but not in others. In conifers, osmotic adjustment has been demonstrated in Sitka spruce (Beadle et al., 1978), and only slightly, if at all, in stressed Douglas-fir seedlings (Joly, 1984). There is reason to believe that Joly's seedlings were mycorrhizal, since he potted his seedlings in unpasteurized soil collected from Douglas-fir forest lands, but mycorrhizal colonization was not investigated. It appears that osmotic adjustment alone cannot explain the general observation of greater stomatal conductance in mycorrhizal plants for given levels of water stress. Although osmotic adjustment might be a contributor under some circumstances, other mechanisms may dominate under other conditions.

Mycorrhizal Influence on Tolerance of Plants to Plant Water Stress

Mycorrhizal colonization might lead to physiological changes in the plant which lead to greater tolerance of water stress in tissues. Osmotic adjustment can be considered as a mechanism which minimizes detrimental affects of moderate water stress in plants (Turner and Jones, 1980). Osmotic adjustment has been associated with less stomatal closure in response to increasing water stress, which would maintain greater CO_2 uptake for growth under drought conditions. It also maintains tissue water content and turgor pressure for cell elongation and photosynthate translocation as plant water potential declines. Biochemical processes in plants are not significantly affected by moderate fluctuations in plant hydration or water

potential (Hanson and Hitz, 1982). Thus, under moderate water stress, enhanced osmotic adjustment in mycorrhizal plants could result in greater plant growth in spite of lower plant water potential. However, the possibility that, and the degree to which, osmotic adjustment might be enhanced by mycorrhizal colonization has not been adequately investigated.

As plant water stress increases, tissue dehydration begins to cause serious structural and biochemical problems for plants. At extreme levels of water stress, beyond full stomatal closure, most growth processes cease, so tolerance of plant water stress amounts to the prevention of cellular damage caused by dehydration (Hsaio, 1973). If damage is minimized, rehydration could lead to quicker resumption of growth. Under cyclical extreme drought, this can translate into greater growth. Presence of mycorrhizae has been correlated with more rapid relief from extreme stress (Goss, 1960) and better growth under cyclical drought (Nelson and Safir, 1982b; Hetrick et al., 1987).

The most immediate effect of tissue dehydration is turgor loss, which can cause rupture of plasma membranes (Gaff, 1980; Hsaio, 1973) and massive cellular disruption which leads to cell death. Further water loss causes significantly lower activity of water, solutes, and solute-sensitive enzymes, membrane degradation by metabolic waste products, and impaired respiration. At extreme dehydration, membranes rupture and proteins denature (Bewley, 1979; Gaff, 1980). Osmotic adjustment is a turgor-maintaining process (Turner and Jones, 1980). Yancey et al. (1982) suggest that cells undergoing dehydration produce organic osmolytes such as polyhydric alcohols (eg. glycerol and mannitol) and amino acids (eg. proline and betaine) which help to preserve enzyme conformation and membrane integrity. It is not known, however, if that is the primary function of these substances, or if they are merely a consequence of altered biochemical processes resulting from changes in water, solute and enzyme activities under severe dehydration (Stuart and Hanson, 1980). There have been no investigations into mycorrhizal effects on these biochemical mechanisms of stress tolerance.

Summary and Conclusions

Characteristics of drought tolerance in plants can be grouped according to those which give the plant greater ability to:

1) extract soil water,
2) respond to limited water uptake by restricting water loss from leaves, and
3) minimize detrimental effects of increased plant water stress.

The literature contains many examples of mycorrhizal effect on each of these three groups. Mycorrhizae can influence water uptake in many ways, most importantly by altering root growth, and also through root permeability when the soil is wet, and increasingly by hyphal uptake and transport of water through the soil as the soil becomes drier. Stomatal response to plant water stress is also altered by mycorrhizal colonization. Mycorrhizal effects on plant water stress tolerance have received little research attention beyond observations of rapid plant growth response after periods of extreme drought.

Several mechanisms for these effects have been suggested in the literature, some almost routinely, to account for these effects. They include hyphal water transport, enhanced mineral nutrition, altered plant hormone balance and enhanced osmotic adjustment. Many studies have failed to confirm each of these mechanisms of effect for any of several possible reasons. For example:

1) they were not acting in the experimental system,
2) not all of the parameters required to adequately evaluate them were measured,
3) they interacted in indistinguishable or unrecognizable ways,
4) other mechanisms which were not considered were dominating the plant response.

One mechanism which has not yet been investigated, but which could have broad implications for many drought tolerance characteristics, is

the influence of added photosynthate sink-strength due to mycorrhizal colonization.

Since the outward plant response to mycorrhizal colonization is a net effect of several possible mechanisms, quantitative information on each possibility is necessary to evaluate their relative importance. To do so, requires that many parameters be evaluated simultaneously on intact plants under controlled and well-defined environmental conditions.

The mycorrhizae formed by different fungal species could have different effects on a host plant. Based upon structural differences, such as the quantity of hyphal growth, mycorrhizae formed by different fungi could be quite different in their ability to affect nutrient or water uptake or photosynthate sink-strength. The physiology of these mycorrhizae may be just as variable. As a result, we can expect differences between types of mycorrhizae in their effect on drought tolerance characteristics of plants.

Mycorrhizal effects on drought tolerance characteristics of plants, and their causal mechanisms, have been investigated predominantly with VA mycorrhizal fungi on crop plants. In these systems, enhanced P nutrition appears to be a dominant mechanism of effect since many plant responses to mycorrhizal colonization appear similar to responses to P fertilization. Little research, however, has been done with ectomycorrhizae in this regard. Since ectomycorrhizae are structurally, and perhaps physiologically, very different from VA mycorrhizae, we can expect that other mechanisms might dominate the mycorrhizal effect and produce different plant responses. At this time, the influence of ectomycorrhizae on the response of Douglas-fir seedlings to drought, and their causal mechanisms remain speculative.

METHODS

Experimental Approach

Evaluation of Drought Tolerance

The first objective of this study was to determine if ectomycorrhizal colonization influences the drought tolerance of Douglas-fir seedlings. In this study, drought tolerance was defined as the ability of the plant to maintain greater growth-process activity under drought conditions. Net photosynthesis rate was used as the measure of growth-process activity and soil water potential was used as the measure of level of drought. Higher net photosynthesis rate at similar soil water potential indicates greater drought tolerance.

Evaluation of Drought Tolerance Characteristics

Review of the literature suggests that mycorrhizae can enhance drought tolerance of plants by affecting certain characteristics which are associated with drought tolerance. They can be grouped according to those which give the plant greater ability to:

1) extract water from soil,
2) respond to limited water uptake by limiting water loss from leaves,
3) minimize detrimental effects of increased plant water stress.

Drought tolerance characteristics were evaluated in this study from measurements of net photosynthesis rate, stomatal conductance and transpiration rate, and plant water potential of Douglas-fir seedlings and soil water potential under wet soil and dry soil conditions. Ability of seedlings to extract water from soil was evaluated by

calculating water uptake conductance, which is the transpiration rate divided by the water potential difference between bulk soil and the leaves. The ability of seedlings to restrict water loss was evaluated from stomatal conductance. Plant water stress was measured by plant water potential and was used to evaluate the overall balance between water uptake conductance and stomatal conductance of the seedlings. The response of growth activity to plant water stress was evaluated from the relationship between net photosynthesis rate and plant water potential. Higher net photosynthesis rate for similar levels of plant water potential indicate greater ability to minimize detrimental effects of plant water stress.

The effect of ectomycorrhizal colonization on drought tolerance characteristics was judged by comparing colonized seedlings with uncolonized seedlings. Since the literature suggests that different species of ectomycorrhizal fungi could have different effects on plant drought tolerance, three fungal species were examined in this study, in order to test this hypothesis.

Evaluation of Mechanisms of Ectomycorrhizal Effect

Several mechanisms are possible which might account for mycorrhizae-induced changes in these drought tolerance characteristics:

1) hyphal water uptake and transport,
2) enhance mineral nutrition,
3) altered plant hormone balance,
4) enhanced osmotic adjustment,
5) altered photosynthate sink-source relations.

The literature suggests that enhanced mineral nutrition, particularly P, is commonly a dominant mechanism of mycorrhizal effect with VA mycorrhizal plants. From theoretical considerations, all of the above, except perhaps for osmotic adjustment, could be important factors with ectomycorrhizal Douglas-fir.

Some of these mechanisms were tested directly. The relative contribution of hyphae to water uptake was evaluated from water uptake conductance under soil water-limiting conditions, and osmotic adjustment was evaluated from measurements of leaf sap osmotic potential under wet and dry soil conditions. Other mechanisms were evaluated indirectly by looking for correlations between mycorrhizal effects and what is known about how each mechanism should affect the seedlings. Based upon the literature, we expected ectomycorrhizal colonization to enhance P nutrition of the seedlings. However, there is little information in the literature on its consequences for drought tolerance characteristics. Therefore, a parallel experiment was included in this study to examine the effect of increasing P nutrition on drought tolerance characteristics of nonmycorrhizal seedlings. Mycorrhizal effects which could not be explained by P nutrition, hyphal water uptake, or osmotic adjustment, were considered as evidence of other non-nutritional effects. These effects might include photosynthate sink-strength and plant hormone balance.

Experiments were conducted under controlled environmental conditions in order to reduce sample variation and enhance the ability to discern small, but potentially meaningful, effects in the results. The details of experimental methods are explained in the following sections.

Treatments

Preparation of Fungal Inoculum

Three species of ectomycorrhizae-forming fungi were used in this study namely Rhizopogon vinicolor (Rv), Laccaria laccata (Ll), and Hebeloma crustuliniforme (Hc). These species are naturally occurring symbionts with Douglas-fir in western Oregon. The sources of these fungi are listed in Table 1. Seedlings were inoculated with Ll and Hc

Table 1. Sources of fungal isolates used in this study.

Laccaria laccata (Ll)
Source: Oregon St. Univ. isolate S238-A
Original Isolation: 1978, Benton County, Oregon
Elev.: 100m
Host: Western larch

Hebeloma crustuliniforme (Hc)
Source: Mark Coleman, Univ. of Washington, isolate HeCr2
Screened as water stress tolerant in PEG pure culture
Original Isolation: Oct. 1983, Bald Mtn., Oregon
Elev.: 1900 m
Host: White fir
Lodgepole pine

Rhizopogon vinicolor (Rv)
Source: Michael Castellano, USDA-Forest Service
isolate FSL 788-5
Original collection: May 1984, near Roseburg, Oregon
Elev.: 450 m

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by mixing vegetative inoculum with pasteurized potting soil, and with Rv, by mixing spores with the potting soil.

Vegetative inoculum of Ll and Hc was prepared according to recognized procedures (Marx and Kenny, 1982). Initial pure culture stocks were maintained on Modified Melin-Norkrans (MMN) agar media (Table 2) at 2°C. Mycelial biomass was increased by transferring subsamples of stock cultures to MMN-agar in petri plates, and incubating for three weeks at 22°C, in darkness. Petri plate mycelium was subsequently increased by transfer to MMN-vermiculite medium consisting of a 30:1:12 mixture (by unmixed volume) of horticultural grade no. 2 vermiculite, finely crushed sphagnum peat, and MMN-solution. The mixture was contained in 3-liter polypropylene bags or 1-liter canning jars and autoclaved twice at 0.1 MPa, 121°C for 1 hour, 24 hours apart, to ensure sterility. The agar cultures were transferred to this medium by blending cultures from 3 agar plates with 50 ml of sterile distilled water for a few seconds, then pouring the mixture into each MMN-vermiculite media container. At the same time, other containers were left uninoculated for use as nonfungal control "inoculum". All cultures were incubated for 3 months at about 22°C.

Prior to use as inoculum, an aliquot of each vermiculite culture was plated on MMN-agar to establish viability and purity for seedling inoculation. Additional nonfungal MMN-vermiculite was needed at the time of soil mixing for the nonmycorrhizal control treatments associated with Ll and Hc inoculum treatments. It was prepared in this same manner except that it was used 24 hours after autoclaving.

Rv spore inoculum was prepared from sporocarps collected from Douglas-fir forested lands in southwest Oregon (Table 1). The sporocarps were stored in water at 2°C for 2 years. For use as inoculum, 3 or 4 sporocarps were blended with 50 ml of sterile, distilled water just prior to inoculating seedlings. Spore concentration was roughly 6×10^5 spores per ml with viability estimated by a dye method to be about 25%.

Table 2. Modified Melin-Norkrans (MMN) nutrient solution used for pure fungal culture. MMN-agar is made using this solution by adding agar to it at 15 g l^{-1} of solution.

Ingredient	Quantity
	(g l^{-1} of solution)
$CaCl_2$ H_2O	0.05
NaCl	0.025
KH_2PO_4	0.5
$(NH_4)_2HPO_4$	0.25
$MgSO_4$ $7H_2O$	0.15
Sequestrene (Geigy)	0.02
Thiamine HCl	0.0001
Malt extract	3.0
Dextrose	10.0

Germination of Seedlings

Douglas-fir seed was acquired from a southwest Oregon source (Table 3). To overcome seed dormancy and induce uniform germination, a long period of cold, moist conditions (stratification) is required. About 8000 seeds were soaked in 4 liters of distilled water for 16 hours at room temperature, then spread out into a monolayer on filter paper over wet vermiculite in shallow pans. A dilute Captan solution (1/2 tsp., 10% Captan powder per liter distilled water) was used to wet the vermiculite and suppress mold growth. The trays were covered to prevent drying and placed in a cold room (2°C) for 9 weeks.

The stratified seeds were planted into 10 cm deep pots (1000 cm^3) filled with a commercial potting mix, consisting of a 1:1 mixture of sphagnum peat and vermiculite with a small amount of dolomite and 5-10-5 fertilizer added. Prior to planting, the potting mix was air-steam pasteurized to kill any possible mycorrhizal fungi propagules (65°C, 30 min.), then placed into pots and saturated with water and 20 ml of dilute Captan solution to suppress growth of Pythium and subsequent damping-off of emerging seedlings. The seeds were sewn on the surface of the potting mix, 16 seeds per pot, and then covered with 1 cm of potting mix.

All pots were maintained in a greenhouse and watered periodically to maintain moist soil conditions. Beginning 1 week after germination, pots were fertilized at every other watering (approximately weekly) with 50 ml of a modified Long-Ashton complete nutrient solution (Hewitt, 1966) described in Table 4. Three weeks after germination, supplemental high pressure sodium vapor lamps were used in the greenhouse to ensure a 15 hour daylength.

About 75% of the seeds germinated during the third week after planting. Over the following 6 weeks, this seedling population was reduced by eliminating seedlings whose shoots were obviously larger or smaller than the apparent average. About 40% of the germinants were discarded under these guidelines. About 10% of the remaining

Table 3. Identification of the source of Douglas-fir seed used in this study.

Species:	Douglas-fir (*Pseudotsuga menziesii* var. *menziezii* (Mirb.) Franco)
Location:	near Roseburg, Oregon
Processor:	Brown Seed Co., Vancouver, Washington
Identification:	seed lot DF 270S-20-78 processing lots 492/494/495 certification SIB 18265

Table 4. Modified Long-Ashton nutrient solution used for fertilizing seedlings in this study. (A) shows the ingredients for preparing the solution. (B) shows the nutrient element concentrations in the final solution.

(A)

Ingredient	mg/liter of solution
KNO_3	259
$MgSO_4$ $7H_2O$	368
$Ca(NO_3)_2$ $4H_2O$	594
NaH_2PO_4 H_2O	0, 89, or 222
$(NH_4)_2SO_4$	66
$MnCl_2$ $4H_2O$	1.98
$CuSO_4$ $5H_2O$	0.25
$ZnSO_4$ $7H_2O$	0.29
H_3BO_3	0.21
$(NH_4)_6Mo_7O_{24}$ $4H_2O$	0.04
$FeC_6H_5O_7$ $9H_2O$ (Fe citrate) (assay >16% Fe)	2.0
Citric acid	2.0

(B)

Nutrient element	for germinants ($mg\ kg^{-1}$)	for transplants ($mg\ kg^{-1}$)
NO_3-N	106	106
NH_4-N	14	14
P	50	0, 20, or 50
K	100	100
Ca	100	100
Mg	36	36
S	64	64
Fe	4.6	4.6
Mn	0.55	0.55
Zn	0.065	0.065
Cu	0.064	0.064
Mo	0.019	0.019
Cl	0.71	0.71
B	0.37	0.37
Na	37	0, 15, or 37

population was further discarded during transplanting because of abnormally large or small root systems.

Inoculation and Fertilization of Seedlings

Six weeks from emergence, 1000 seedlings were transplanted into 250 cm^3 (15 cm deep) tubes containing pasteurized mineral soil mixed with fungus-free vermiculite media. The soil was a fine sandy loam with very low organic matter and low available P (Table 5). This soil was considered to be fine textured enough to have desirable water release characteristics without compromising aeration, and with low enough P availability to allow some control over plant P nutrition through fertilization.

Prior to mixing with fungus-free media, the soil was steam pasteurized (65°C, 1 hour) to kill any native ectomycorrhizal fungi propagules in it. The MMN-vermiculite control medium was wrapped in cheesecloth in 3 liter batches and rinsed 3 times, each time in 9 liters of fresh, cool tap water to remove residual MMN-solution, then placed on a water extraction table overnight under 3.5 kPa tension. The rinsed medium was mixed immediately with the mineral soil or stored in a cold room at 2°C for up to 48 hours until mixing. The mineral soil and rinsed vermiculite medium were thoroughly mixed in a chlorine bleach-sterilized cement mixer at an unsettled volume ratio of 3:1.

Each seedling was transplanted with the soil mix, watered, then lightly shaken to settle the soil to 1.5 cm below the lip of the tube resulting in a soil volume of about 225 cm^3. All tubes were maintained at approximately this level over the next 4 months by adding more soil to tubes in which there was some further settling. Bulk density was measured on soil from 19 pots, 4 months after transplanting, and averaged 1.06 g cm^{-3} (std. dev. = .04 g cm^{-3}).

One week after transplanting, the seedlings were randomly assigned into 5 groups of 190 seedlings each. To 2 of the groups, 5 ml

Table 5. Soil and pot characteristics.

Soil Characteristics	
particle size analysis (<2mm fraction)	sand 53% silt 32% clay 15%
soil mass > 2mm	7.5%
organic matter	0.76%
CEC	18.9 cmol kg^{-1}
available P (Bray)	10 mg kg^{-1}
pH	6.5
Pot Characteristics	
pot depth	15 cm
soil volume (V)	225 cm^3 (std. dev.= 1 cm^3)
bulk density (BD)	1.06 g cm^{-3} (std. dev.= 0.04 g cm^{-3})
oven dry soil mass	238.5 g

of Rv spore suspension was injected into each tube using a manostat syringe. Injection started deep in the pot and continued as the needle was withdrawn toward the surface of the soil. This procedure was intended to ensure complete soil permeation by spores for contact with growing root tips. The other 3 groups were left uninoculated.

Eight weeks from emergence, about 600 more seedlings were transplanted into tubes using the same techniques as those for transplanting of 6-week-old seedlings. Two hundred seedlings were transplanted into soil mixed with fungus-free vermiculite medium, and 200 each into soil mixed with Ll and Hc inoculum.

All seedlings were maintained in the greenhouse under 15 hour daylength. This was ensured by use of supplemental high pressure sodium vapor lamps for the first and last 4 hour periods of each day during all months except June and July when seedlings were 3 to 5 months old. The seedlings were watered as needed to maintain moist soil conditions and fertilized every fifth watering (about every 2 weeks) with 15 ml of modified Long-Ashton complete nutrient solution (Table 4) according to the levels in Table 6. This fertilizer schedule was intended to produce low, but not deficient, nutritional status for all mineral nutrients except for P. The three different P fertilizer concentrations of 0, 20 and 50 mg kg^{-1} were intended to produce deficiency, adequate and luxury P levels in the plants.

The treatments produced 8 groups of seedlings. They are summarized in Table 6.

The seedlings were grouped by treatment along the greenhouse bench. They were rearranged every 3 weeks, both among and within treatment groups, to minimize any effect of environmental variation across the greenhouse bench. Rearrangement was systematic so that no treatment repeated in the same bench position.

Some transplant shock occurred which caused temporary growth cessation in nearly all seedlings. Most of these seedlings began growing again within 2 weeks after transplanting. Transplant mortality ranged from 1 to 5% depending upon the treatment. Twenty plants from each treatment with the largest shoots and 12 plants with the smallest shoots were discarded prior to experimentation to minimize size

Table 6. Experimental plan for fungal inoculation and P fertilization of seedlings at the indicated seedling age when transplanted (in weeks) and P fertilizer concentration (in mg kg^{-1}). Reference numbers are used for convenience to identify treatments.

Treatment reference no.	Age transplanted	Fungal treatment	Fertilizer P conc.
(weeks)		(mg kg^{-1})	
1	6	nonmycorrhizal	0
2	6	nonmycorrhizal	20
3	6	nonmycorrhizal	50
4	6	Rhizopogon vinicolor	0
5	6	Rhizopogon vinicolor	20
6	8	nonmycorrhizal	20
7	8	Laccaria laccata	20
8	8	Hebeloma crustuliniforme	20

variation within treatments, apparently caused by differences in when growth resumed after transplanting. From these final treatment populations, seedlings were randomly selected for growth room experiments.

Experimental Procedures

Introduction

Seedlings were transferred to a walk-in growth chamber where drought tolerance characteristics of seedlings were evaluated from measurements of stomatal conductance and net photosynthesis rate, leaf water potential and leaf sap osmotic potential, and predawn soil water potential. These data were collected according to two designs. One design was for plants in wet soil, a non water-limiting soil condition, and a second design was for drier, more water-limiting soil conditions. Seedling growth and nutrition measurements, and estimates of mycorrhizal colonization were made on seedlings used in these experiments. Experiments were conducted on all treatments in the same manner.

Experiments were begun when seedlings were 5.5 months old, which was adequate time (4 months) for mycorrhizal formation and for some growth differences between treatments to be apparent. Final budset occurred at about 5 months old, after which there was no regrowth. The wet soil experiments were conducted when the seedlings were 5.5 to 6.5 months old. The drought stress experiment was conducted when the seedlings were 6.5 to 9 months old. On the average, these two experiments were conducted about 2 months apart.

The experiments were designed to allow for measurements to be made on a maximum number of seedlings in order to distinguish differences between treatment populations in which wide variation in

seedling size and physiological behavior, and degree of fungal colonization were expected.

All plants were maintained in the greenhouse until used in growth room experiments. Seedlings were brought into the growth room at least 3 days before use in experiments in order to acclimatize them to growth room environmental conditions. In order to minimize the environmental change from greenhouse to growth room, the light period schedule for the growth room was set to begin and end at the same times of day as the greenhouse lights. Day and night temperatures were also selected to be similar to those in the greenhouse. Growth room conditions were:

1) 15 hour daylength from a bank of fluorescent and incandescent lamps,
2) light intensity about 350 umol m^{-2} s^{-1} in the 400 to 700 nm waveband during 13 hours per day, with stepwise on and off periods according to Figure 1,
3) 26°C daytime, 22°C night.
4) about 50% relative humidity daytime, 80% at night,
5) about .15 m s^{-1} windspeed,
6) ambient CO_2 concentration between about 13 and 15 mmol m^{-3}.

Wet Soil Experiment

In the first part of this experiment, with the soil well-watered, stomatal conductance and net photosynthesis rates were measured periodically through the course of one day. Gas exchange rates were measured repeatedly on entire shoots of plants using the LI-6000 Portable Photosynthesis Meter at 6:45 (predawn), 7:15, 7:45, 8:45, 14:15, 20:45, 21:45, and just after dark at 22:15 hours. These plants were harvested the following midday for analysis of leaf tissue concentration of N, P, K, and Ca. Nitrogen and phosphorus concentrations were determined colorimetrically from Kjeldahl digested

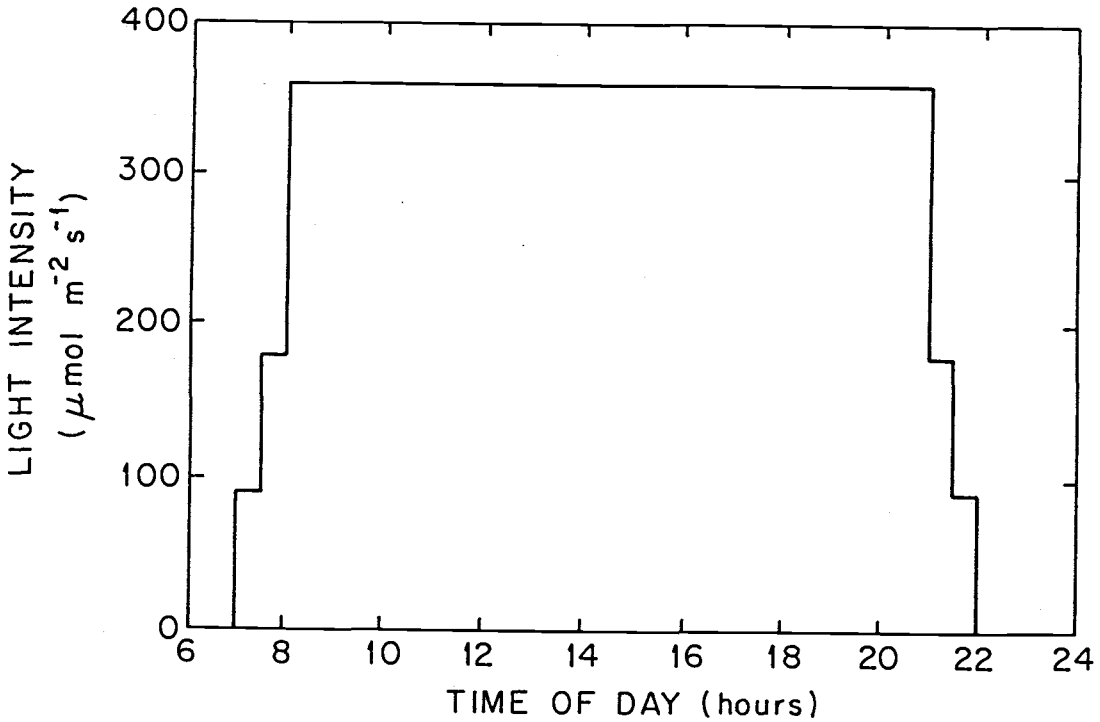


Figure 1. Schedule for the day/night light intensity cycle used in growth chamber experiments. Light intensity was measured by quantum sensor in the 400 to 700 nm waveband.

leaf tissue, while K and Ca were determined using an atomic absorption spectrophotometer on perchloric acid-digested samples. Measurements were made during 12 days with 1 seedling from each treatment being investigated on each day, for a total of 12 plants per treatment. Table 7 shows the order in which measurements were made in this gas exchange experiment.

The LI-6000 measurement system is a closed system, transient technique where the rate of CO_2 decrease and humidity increase of the cuvette atmosphere are directly dependent upon the enclosed plant net photosynthesis rate, transpiration rate, and cuvette volume. A 1981 cm^3 cylindrical cuvette was used to enclose whole shoots of Douglas-fir seedlings without touching the leaves (Figure 2).

During measurement periods, sensor readings were begun 30 seconds after enclosing the shoot in the cuvette, and observations were taken every 10 seconds for the following 90 seconds. This time period allowed an average CO_2 drawdown of about 25 ppm during the daytime. From each set of 10 observations, LI-6000 software performed a linear regression to arrive at the corrected value for the first observation. Thus, measurement results reported in this study represent the condition of the plants 30 seconds after enclosing them in the cuvette.

LI-6000 sensors measure temperature, relative humidity, and CO_2 concentration of the cuvette atmosphere. The LI-6000 software computes transpiration rate, stomatal conductance, net photosynthesis rate, and leaf internal CO_2 concentration from these measurements and input values for constants listed in Table 8. All LI-6000 computations were made using a constant leaf area of 100 cm^2. After the plants were harvested, actual leaf area was estimated for each plant, and an adjustment was made to these computed values. Actual leaf area was estimated from oven dry mass of needles and the conversion function

$$\text{Leaf Area} = -2.28 + 120.90\ (\text{Mass}) - 34.97\ (\text{Mass}^2) \qquad (1)$$

where area is in cm^2 and mass in g. To obtain this function, leaf area and dry mass were determined on 4 to 6 plants per treatment, selected

Table 7. Order in which samples were prepared and measured on each seedling used in the gas exchange experiment for plants growing in wet soil.

Day	Activity
1	Transfer seedling to growth chamber
3	Seedling well watered
4	LI-6000 measurement periodically over the day
5	Harvest plant; separate plant parts leaves: dry mass nutrient concentration stem: dry mass roots: degree of colonization root length dry mass

Figure 2. Photograph of the cuvette used in combination with the LI-6000 in these experiments.

Table 8. Values for constants used in LI-6000 computations.

Cuvette volume	1981 cm^3
Atmospheric pressure	1000 mb
Leaf temperature	same as air temperature (est. from Campbell, 1977)
Leaf area	100 cm^2
Diffusion resistance of the leaf boundary layer to water vapor	0.15 s cm^{-1} (est. from Nobel, 1983)
Ratio of stomatal diffusion resistance of upper leaf surface to stomatal diffusion resistance of the lower leaf surface	20

to represent the normal range of shoot sizes, for a total of 40 plants. For each plant, all leaves were removed from the shoot and placed side-by-side on 1 mm grid paper. The paper was covered with double-sided adhesive transparent tape to hold the leaves in place. Leaves were arranged to minimize overlap and vacant area. Thus, area of one side of the leaves was estimated for each plant, then the leaves were carefully removed from the tape and oven dried (65°C, 48 hrs,) for measurement of dry mass.

A scatterplot of these data revealed the absence of treatment differences, therefore data were pooled and fit to both linear and second order linear regression models. The best fit was obtained from the second order model, probably because larger plants had larger leaves, with lower leaf area to mass ratio, than for smaller plants. The scatterplot and the fit obtained with equation (1) are shown in Figure 3.

The values for transpiration, stomatal conductance, net photosynthesis, and leaf internal CO_2 concentration which were computed by the LI-6000 software were adjusted to actual values by multiplying (or dividing where appropriate) the computed value by the ratio of actual seedling leaf area (A) to the input leaf area of 100 cm^2. This simple adjustment method is exactly correct for transpiration and net photosynthesis rate, but slightly underestimates the actual values for stomatal conductance and leaf internal CO_2 concentration based upon LI-6000 computational formulas (for details, see LI-6000 Instruction Manual, Revision 2, Software 3.00, LI-COR, Inc., Lincoln, Neb.). The simple adjustment equation for stomatal conductance (SC) is,

$$\text{simply adjusted SC} = \text{unadjusted SC}\ (100/A) \quad .$$

The more complex, but more accurate adjustment equation is,

$$\text{accurately adjusted SC} = 1/[\{[(1/\text{unadjusted SC})-0.14]\ A/100\} - 0.14]).$$

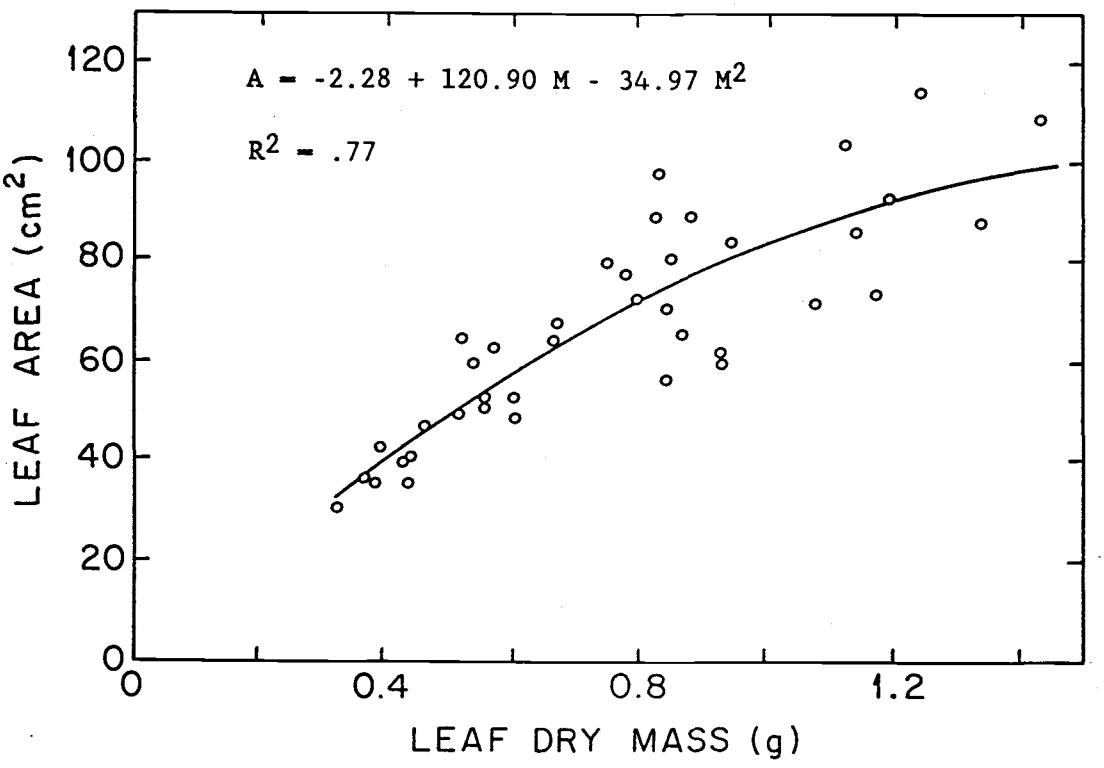


Figure 3. Relationship between leaf area and leaf dry mass obtained by using complete shoots of 8-month-old Douglas-fir seedlings. Each point represents an independent observation on a single seedling , and the solid line represents the second order regression function.

The absolute error of the simple adjustment method is largest (9%) for very rapidly transpiring plants (SC > 0.25 cm s^{-1}) where the leaf area adjustment is large (A = 65 cm^{-2}), and becomes negligible where stomata are nearly closed (Table 9). Since we are interested in relative differences between treatments, maximum error will appear when comparing a treatment with high transpiration rate and large leaf area adjustment (such as treatment A in Table 10) with a treatment which exhibits a lower transpiration rate and leaf area very close to 100 cm^2 (such as treatment B). Using worst case values from these experiments for this example, treatment A is underestimated by 9%, and treatment B by only 4% (Table 10). Thus, the difference in stomatal conductance between these two treatments is actually 5% greater than that which results from the simplified adjustment. This adjustment error is only slightly larger for leaf internal CO_2 concentration. Since these errors are small (even in our worst case example), the simple adjustment for actual leaf area will only negligibly affect comparisons based on LI-6000 computations.

In the second part of this wet soil experiment, measurements of concurrent leaf water potential and leaf sap osmotic potential were made periodically through the course of one day. These measurements were taken near 6:45 (predawn), 7:45, 8:30, 11:00, 20:30, and just after dark at 20:45 hours. Leaf water potential was measured on whole shoots with a pressure chamber, and then, a sample of leaves was immediately removed, frozen in sealed capsules on dry ice, and then thawed for osmotic potential measurement using a vapor pressure osmometer on expressed leaf sap. Osmometer values were adjusted to estimate average symplast water potential by assuming that apoplastic water is a constant 20% of the total leaf water content (estimated from Joly, 1984). The second part of this wet soil experiment was performed during 8 days with 1 seedling from each treatment at each measurement time for a total of 48 plants per treatment.

Leaves, stems, and roots were separated from all plants used in both parts of this experiment. Root systems were thoroughly washed, and microscopically examined to identify the colonizing mycorrhizae, and to visually estimate the percentage of all root tips on each root

Table 9. The magnitude of underestimation of stomatal conductance (SC) by using the simple leaf area adjustment method compared to that calculated by the accurate adjustment method, expressed as percent of the accurately adjusted value, for different measured leaf areas and unadjusted stomatal conductances, where a leaf area of 100 cm^2 was initially assumed.

Measured Leaf Area	Unadjusted SC	Underestimation
(cm^{-2})	(cm s^{-1})	(percent)
90	.025	1
90	.25	7
65	.025	1
65	.25	9

Table 10. The magnitude of underestimation of stomatal conductance (SC) by using the simple leaf area adjustment method compared to the accurate adjustment method, expressed as a percent of the accurately adjusted method, for the worst case analysis of treatment data collected in this study.

Treatment	Leaf Area	Unadjusted SC	SC underestimation
	(cm^{-2})	($cm\ s^{-1}$)	(percent)
A	65	.25	9
B	90	.15	4

system which had formed well-developed mycorrhizae. Root lengths were measured manually by the line-intersect method (Newman, 1966) only on the plants used for LI-6000 measurements. Roots, stems and leaves (including recovered pulp from osmotic potential samples) of all plants were oven dried (65°C, 48 hrs.) and weighed. Table 11 shows the order in which measurements were made in this plant water potential experiment.

Drought Stress Experiment

In this experiment, measurements of stomatal conductance, net photosynthesis rate, leaf water potential, and leaf sap osmotic potential were made over a wide range of soil water potentials. In order to do this, the soil was allowed to dry through normal transpiration for a few days until soil water-limiting conditions were approached. Measurements were taken daily thereafter. Preliminary data suggested that soil limitations to water uptake could become significant when the soil water potential decreased to about -0.15 MPa. Aluminum foil was loosely secured over the soil surface to reduce evaporation and encourage even soil drydown through root extraction only. Then, measurements were made on several plants from each treatment near the end of each day at 19:30 hours. Stomatal conductance and net photosynthesis rates were measured first, then the shoots of these plants were removed for immediate leaf water potential measurement, and finally, leaf samples were obtained for determination of leaf sap osmotic potential as described previously. Soil water content was measured after sealing the pots, with the shoots removed, in plastic bags for 12 hours.

Water content of the soil was measured gravimetrically and also obtained from psychrometric water potential measurements (SC-10A, Decagon Devices, Inc., Pullman, WA), converted to water content by the soil water release function (Figure 4). Water content values estimated from psychrometer measurements were used when water potentials were

Table 11. Order in which samples were prepared and measured on each seedling used in the water potential experiments for plants growing in wet soil.

Day	Activity
1	Transfer seedling to growth chamber
3	Seedling well watered
4	Harvest plant: plant water potential Separate leaf sample and freeze on dry ice
5	Measure osmotic potential of leaf sample and then combine pulp with the rest of the leaves Separate plant parts leaves: dry mass stem: dry mass roots: degree of colonization dry mass

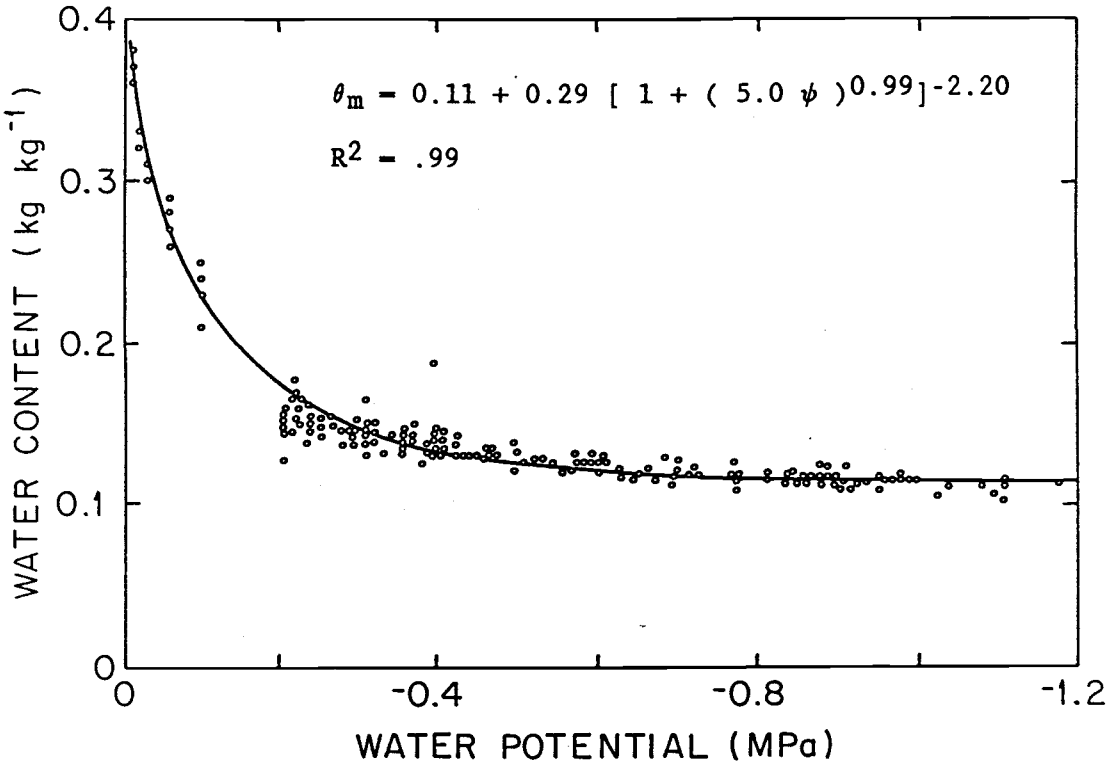


Figure 4. Soil water release function.

-0.2 MPa or lower, since this technique is more accurate in this range than gravimetric measurements. For soil wetter than that corresponding to -0.2 MPa, gravimetric water content measurements were used. These values for soil water content were adjusted to estimate water contents at predawn by adding the change in water content due to water loss during the day of measurement. For this correction, pots were weighed in the morning and again just after LI-6000 measurements to calculate the soil water loss during the day (equal to pot weight loss). Oven dry soil mass in each tube was assumed to be constant at 238.5 g (Table 5). Finally, predawn water contents were converted to water potentials through the soil water release function. Figure 5 is a flow diagram showing the procedure for estimating water potential of the soil at predawn.

Data for the soil water release function were collected from two sources. Soil water content was measured gravimetrically on soil mix samples on a pressure plate at -0.01, -0.02, -0.03, -0.06, and -0.10 MPa water potential. Nine samples were measured at each of these water potentials. Data pairs for soil drier than -0.2 MPa were taken from measurements of gravimetric water content and corresponding psychrometric water potential of pots in the drought stress experiment. One hundred and sixty-nine data pairs were used over a range of -0.2 to -1.3 MPa. A non-linear regression on an exponential model (Van Genuchten and Nielson, 1985) of the form

$$\theta_m = e + d\,[\,1 + (\,a\,\psi\,)^b]^c$$

produced an equation with $R^2 = .9875$, where

θ_m = water content in kg kg^{-1}
ψ = water potential in MPa
e = 0.10853613
a = 5.02093965
b = 0.98622474
c = -2.20443445
d = 0.28591877 .

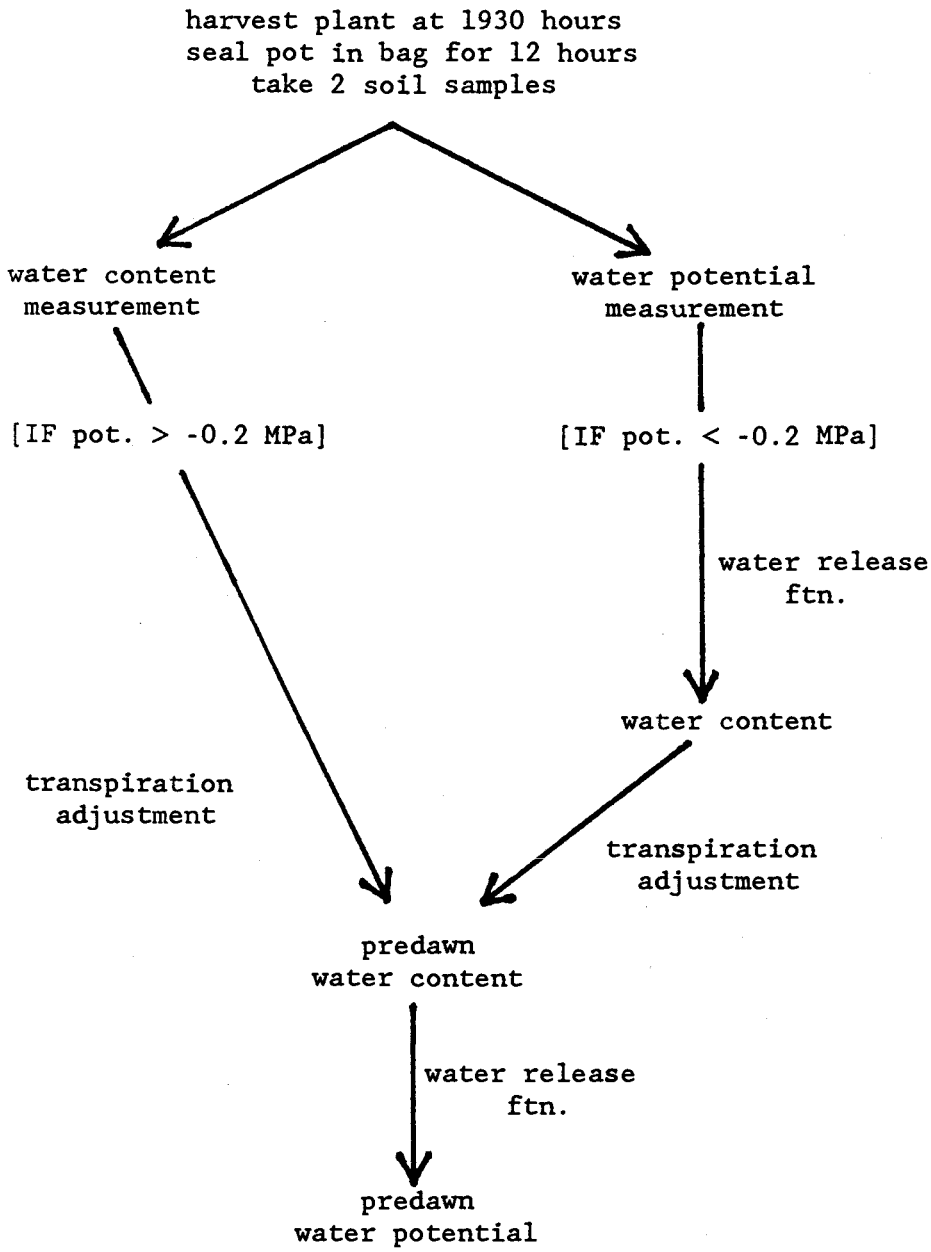


Figure 5. Flow diagram of the procedure used for estimating predawn soil water potential.

The data are plotted with this function in Figure 4.

This drought stress experiment was conducted on 70 seedlings from each treatment, in 7 sets. For all seedlings in each set, a target predawn pot weight was assigned, to which each seedling pot in the set would dry down before measurements would be taken. This was done to ensure that data from plants of all sizes and conditions would be represented evenly over a range of soil water potentials from about -0.05 to -1.0 MPa. Each morning, pots were weighed, and all plants which had dried to the target weight were sampled the following evening. This normally took 4 to 12 days depending upon plant transpiration rate and target pot weight.

After making soil water content and water potential measurements, root systems of the seedlings were thoroughly washed to identify the colonizing mycorrhizae, and to visually estimate the percentage of all root tips on each root system which had become well-developed mycorrhizae. Root lengths were measured manually by the line-intersect method (Newman, 1966). Roots and leaves (including leaf pulp recovered from osmotic potential measurement) were oven-dried (65°C, 48 hrs.) and weighed. Table 12 shows the order in which measurements were made in this drought stress experiment.

Statistical Design and Data Analysis

The treatments produced 8 groups of seedlings from which 6 comparisons were made:

The effect of seedling age when transplanted:

1) Treatments 2 and 6 (from Table 6) were compared for effects of seedling age when transplanted.

The effect of ectomycorrhizal colonization:

Table 12. Order in which samples were prepared and measured on each seedling used in the drought stress experiments.

Day	Activity
1	Transfer seedling to growth chamber
3	Seedling well watered
4-15	Weigh pot before lights come on Li-6000 measurement Weigh pot Harvest plant: plant water potential Separate leaf sample and freeze on dry ice Enclose pot in plastic bag
5-16	Measure osmotic potential on leaf sample then combine with the rest of the leaves Take soil sample: water potential Take soil sample: water content Separate plant parts leaves: dry mass roots: degree of colonization root length dry mass

2) Treatments 6, 7, and 8 were compared for effects of Ll and Hc colonization on seedlings at moderate soil P level.
3) Treatments 2 and 5 were compared for effects of Rv colonization on seedlings at moderate soil P level.
4) Treatments 1 and 4 were compared for effects of Rv colonization on seedlings at low soil P level.

The effect of level of P nutrition:

5) Treatments 1, 2, and 3 were compared for effects of level of P nutrition on nonmycorrhizal seedlings.
6) Treatments 4 and 5 were compared for effects of level of P nutrition on Rv-colonized seedlings.

These comparisons were made and are discussed in following chapters.

A completely randomized design was used in these experiments. For each treatment comparison outlined above, treatment means and their standard errors were calculated for all growth and nutrition data, and for all net photosynthesis, stomatal conductance, plant water potential, and leaf sap osmotic potential data from the wet soil experiment at each measurement time of day. Similarity of these treatment means were statistically tested by analysis of variance F-ratio for comparisons of 2 treatments, and by a blanket F-ratio test combined with the Least-Significant-Difference method (Fischer's protected LSD) for comparisons of three treatments. For x,y graph presentation of net photosynthesis, stomatal conductance, plant water potential, and leaf sap osmotic potential results, treatment mean values were plotted versus time of day and connected by straight lines to signify that no observations were made between these times of day.

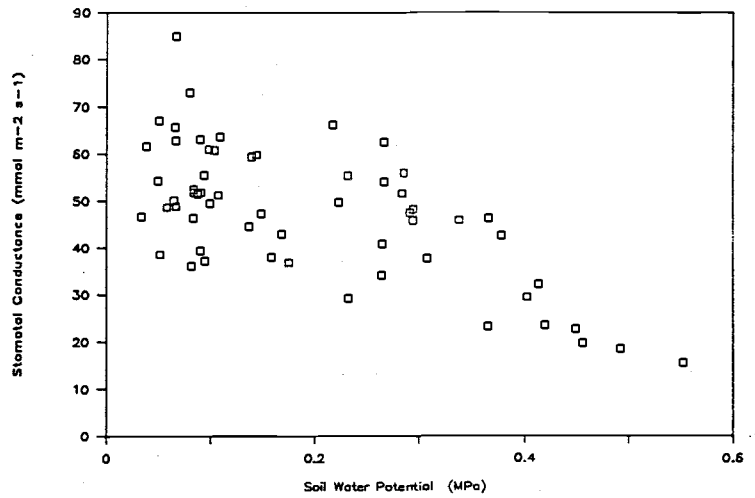
For the drought stress experiment, treatment means and their standard errors for net photosynthesis, stomatal conductance, and water potentials were calculated from all observations lying within discrete 0.1 MPa ranges of soil water potential. In all but a few cases, these treatment means included from 5 to 25 independent observations. For x,y graphs, continuous functions for each

relationship were hand fit using the treatment means and their standard errors, with the added assistance of a scatterplot containing smoothed or "running average" data points.

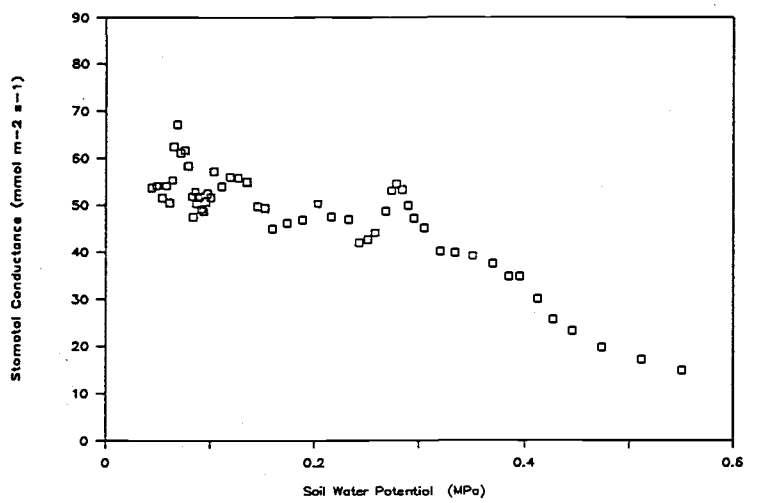
Running average points were generated in the following way: All x,y data pairs for the x,y graph relationship were ordered from lowest x to the highest, then the mean x,mean y point was calculated from the first five data pairs, then dropping the data pair with the lowest x value from that set of 5, and replacing it with the data pair having the next highest x value, the next mean x,mean y point was calculated from the new set of 5. This pattern was continued until their were no more higher x-valued data pairs to add to make up another set of 5. In this way, new sets of points were generated representing a sequence of mean values (running average) with much reduced variation between these points. Using a combined plot containing these running average points, and discrete x-range treatment means with their standard errors and sample sizes noted, a curve was hand fit to describe the continuous function relationship between the two variables. Figure 6 shows an example of the major steps used in this curve fitting technique. This technique produces realistic continuous functions and allows for some quantitative tests of treatment similarity through treatment means and standard errors. This technique does not introduce artifacts associated with regression which can be caused by requiring that functions fit specific mathematical models.

Prior to analysis of the data collected in the experiments just described, each plant was inspected for unusual features which could bias or otherwise lead to erroneous conclusions from the results. Inspection of the root systems of all 656 seedlings used in these experiments revealed that some had girdling-like damage at the root collar and others were colonized by unintended fungal species. On 13 plants, half of which were colonized by Rv, girdling damage appeared significant, with more than 1/4 of the root collar circumference damaged. This observation correlated with abnormally low xylem potential and stomatal conductance values. Sixteen other plants were found to be colonized with the unintended fungal species, or if

Scatterplot



Running Average Plot



Combined Plot

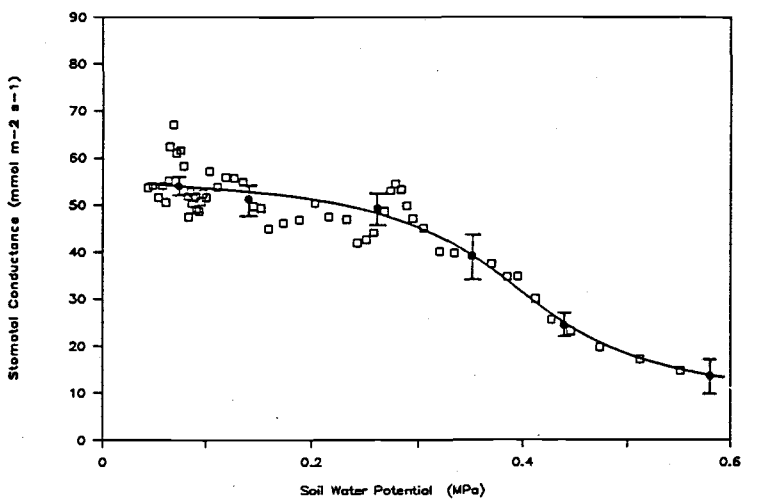


Figure 6. Diagrams showing the stepwise method used for deriving the continuous function for the relationship between stomatal conductance and predawn soil water potential.

originally uninoculated, were found to be mycorrhizal. All data collected on these seedlings were eliminated from the data analysis.

In the drought stress experiment, where plants were given up to 12 days to dry down to the target pot weight, a few plants did not reach the target. These plants were measured on day 12, but not included in the analyses, since their inclusion would bias the results by including a greater proportion of slowly transpiring plants under wetter soil conditions. This amounted to 1 or 2 plants, out of a total of 70 plants, from each treatment, except in treatment group 2, where this behavior was found in 8 plants. These plants were analyzed separately to investigate the reason for low transpiration rate.

The statistical methods outlined above were selected upon consultation with a statistician. They were judged to be appropriate for satisfying the study objectives within the constraints set by the experimental procedures.

RESULTS

Colonization of Seedlings by Ectomycorrhizal Fungi

Expected Results

The general morphological features of ectomycorrhizae have been summarized by Harley and Smith (1983) and Marks and Foster (1973). Roots are considered to be colonized when they have a well-developed ectomycorrhizal mantel. Hyphal mantels are the most prominent evidence of ectomycorrhizal colonization and generally appear at or near apices of slow growing young roots which have not yet sloughed off the cortex tissue through secondary differentiation. Thus, ectomycorrhizae generally appear as short branches in root systems.

It was expected that Rhizopogon, Laccaria, and Hebeloma mycorrhizae would have distinctly different structures on Douglas-fir seedlings. Different fungi tend to produce ectomycorrhizae that have a morphology that is characteristic of the type of fungus. General morphological descriptions of ectomycorrhizae formed by Rhizopogon, Laccaria, and Hebeloma species on conifers are found in Zak (1971), Molina (1980, 1982) and Danielson et al. (1984). Rhizopogon mycorrhizae have thick brown, rough-surface mantels with a moderate amount of hyphal growth into the soil, including well-developed rope-like rhizomorphs which are intertwined strands of hyphae. They commonly develop highly branched clusters of many short mycorrhizae. Laccaria typically form smooth, thin-manteled mycorrhizae, but the abundance of hyphal growth into the soil appears to be variable, as is the development of highly-branched clusters of mycorrhizae. Hebeloma mycorrhizae are distinguishable by smooth mantels and extremely abundant growth of white hyphae throughout the soil. Hebeloma mycorrhizae clusters have not been reported. It was expected that

these fungal genera would produce mycorrhizae of the same general description on Douglas-fir seedlings in this experiment.

It was also expected that mycorrhizae would not develop on all root tips, and that the number that did form would differ between the fungal types used in this study. The degree of colonization of root systems by different species of fungi is loosely considered a characteristic of the fungus, but can be greatly influenced by environmental and plant factors (Harley and Smith, 1983). Both Hebeloma and Laccaria are reported to be aggressive colonizers of conifer root systems, colonizing root systems quickly and almost completely (Molina 1980, 1982; Danielson et al., 1984). Rhizopogon colonization from spores tends to be less complete over similar time spans, but this is influenced by soil nutrient availability (Castellano et al., 1985). In general, severe soil nutrient deficiency and extremely high nutrient levels, particularly of P and N, appear detrimental to mycorrhizal colonization, while moderate deficiency promotes ectomycorrhizal colonization (Harley and Smith, 1983; Heilman and Ekuan, 1980). Based upon these studies, it was expected that Hebeloma and Laccaria would colonize nearly all slow growing root tips, while Rhizopogon would only partially colonize these roots in this experiment. Withholding P fertilization was expected to reduce Rhizopogon colonization.

Results

As expected, the general development of mycorrhizae on root systems was morphologically similar to published descriptions (Zak, 1971; Molina, 1980, 1981; Danielson et al., 1984). A few mycorrhizae formed by each fungus were examined in cross-section to verify the presence of a Hartig net as an indication that they were indeed mycorrhizae. Each of the three fungi used produced morphologically distinct mycorrhizae. Rhizopogon vinicolor mycorrhizae developed very thick dark brown and white mantels from which hyphae and thick brown

rhizomorphs (strands of intertwined hyphae) grew outward into the soil. Hyphae in the soil were not easily seen due to their brownish color, but their presence was evident by the moderate difficulty of washing these root systems free of soil compared to uncolonized root systems. Rhizopogon mycorrhizae were only occasionally branched and highly branched clusters of 6 to 12 mycorrhizae were uncommon, forming on about 10% of the seedlings. Interestingly, there were many blackened portions of long and short roots in regions of root systems where Rhizopogon mycorrhizae had developed, but there was no evidence of hyphal colonization of these roots. On the other hand, Laccaria laccata formed much less distinctive, smooth grayish mycorrhizae with little evidence of hyphal growth into the surrounding soil. Laccaria mycorrhizae were commonly twice as long as the typical uncolonized short root and branched in 1 or 2 places. Hebeloma crustuliniforme mycorrhizae were similar to Laccaria in that they were smooth, light colored and longer than typical uncolonized short roots. On the other hand, they were unbranched and developed a profusion of white hyphae and intertwined hyphal strands which grew along all root axes and permeated the entire soil volume, apparently to a much greater degree than Rhizopogon. Intertwined strands of Hebeloma hyphae were much finer and less well-developed than the ropy Rhizopogon rhizomorphs. These descriptions generally agree with those in the published literature, confirming that colonization of seedlings in this experiment did occur with the intended fungal species.

As expected, the degree of mycorrhizal colonization differed among fungal species. Table 13 shows results from visual estimates of the percentage of root tips on seedlings which had become well-developed mycorrhizae by about 4 months after inoculation. Data are from seedlings used in the wet soil experiment. The results are consistent with the literature which shows that Laccaria and Hebeloma are more complete colonizers of root systems than Rhizopogon. The degree of mycorrhizal colonization was difficult to assess accurately for Laccaria because of the relatively less distinctive mycorrhizal morphology, and for Hebeloma because of the profusion of hyphae external to mantels which sometimes concealed the structure of other

Table 13. Degree of mycorrhizal colonization of well-watered Douglas-fir seedlings at about 4 months and 6 months after inoculation for the indicated mycorrhizal fungus species and fertilizer P concentration. Degree of mycorrhizal colonization is expressed as the percentage of all root tips on a seedling which had become well-developed mycorrhizae.

Treatment		Colonization	
Fungal species	Fertilizer P conc.	4 months	6 months
Rhizopogon vinicolor	0	37 ± 3[a]	39 ± 2
	20	36 ± 2	43 ± 2
Laccaria laccata	20	73 ± 3	46 ± 3
Hebeloma crustuliniforme	20	93 ± 2	85 ± 2

[a] mean ± one standard error

root tips. Standard deviations of these estimates for degree of colonization were 11 to 19% which reflects this difficulty as well as large variation in actual colonization degree among seedlings.

In this experiment, withholding P fertilizer had no effect on the degree of Rhizopogon colonization of seedlings (Table 13). Apparently, neither P fertilizer schedule produced sufficiently extreme soil P availability, high or low, to influence Rhizopogon colonization.

Estimates of the degree of mycorrhizal colonization for seedlings at about 4 months and at about 6 months after inoculation are compared in Table 13. Both Rhizopogon and Hebeloma showed no apparent change in the degree of colonization, but Laccaria appeared to decrease greatly over the two months between experiments. It is not clear why the percentage of roots colonized by Rhizopogon did not increase over the intervening 2 months. It can only be concluded that initiation of new roots proceeded at the same pace as the ability of Rhizopogon to colonize them. This was also true of Hebeloma. However, for Laccaria, the apparent decrease in colonization could have been due to root initiation at a pace faster than Laccaria could colonize them, or simply reflect a gradual shift in the visual definition of these relatively less distinctive mycorrhizae over 4 months of microscopic evaluations. Since ectomycorrhizal longevity has been estimated at 9 months to several years (Harley and Smith, 1983) it is unlikely that this was due to an absolute decrease in numbers of Laccaria mycorrhizae.

Nutrition of Seedlings

Expected Results

It is well-established that increasing the availability of P to plant roots through fertilization increases plant uptake of P. Ectomycorrhizal colonization is also expected to increase plant uptake

of mineral nutrients, ostensibly through greater efficiency of ectomycorrhizae as nutrient absorbing organs (Marks and Koslowski, 1973; Harley and Smith, 1983). This usually occurs for soil nutrients which are limited in availability relative to the needs of the plant as is common for phosphorus and nitrogen (Hatch, 1937; Harley and Smith, 1983). Phosphorus uptake by conifers appears to be improved to a greater degree than other nutrients by ectomycorrhizal colonization (McComb, 1938; McComb and Griffith, 1946; Stone, 1949). As a result, greater quantities of mineral nutrients, especially P, were expected to be found in ectomycorrhizal seedlings than in uncolonized seedlings.

Each fungal species used in this study was expected to differ in its effect on nutrient uptake. Such differences have been demonstrated (Mejstrik, 1970; Mejstrik and Krause, 1973), and may be due to dissimilarities in structure and physiology of the mycorrhizae that different fungi produce (Harley and Smith, 1983). Not all changes in nutrient content of the plants can be attributed directly to mycorrhizal uptake _per se_. In an investigation of nutrient interactions on _Pinus contorta_, Rousseau and Reid (1985) found that P fertilization of uncolonized seedlings and mycorrhizal colonization both increased leaf P concentration, but decreased leaf N concentration. They suggested that mycorrhizae improved P uptake, but the plant responded by inhibiting N uptake. On the basis of Rousseau and Reid's (1985) conclusions, it was expected that mycorrhizae- or fertilizer-enhanced P uptake would negatively affect leaf N concentration.

Both leaf tissue concentration and total amount of each nutrient in leaves, leaf content, were evaluated in this experiment. Higher leaf tissue concentration often reflects improved nutrient uptake by plants, so long as plants are not severely stunted. But, if some other major limitation to plant growth is relieved, greater leaf growth can cause concentrations to decrease (Jarrell and Beverly, 1981). Under these conditions, leaf content is a better indicator of changes in nutrient uptake by the plant.

Results

Leaf tissue concentrations of 6 month old Douglas-fir seedlings, about 4 months after fertilizer and mycorrhizal treatments were begun, are shown in Table 14. In general, N and P levels were deficient compared to recognized foliar levels for optimum growth of nursery-grown Douglas-fir seedlings, and K and Ca were both at the lower end of their optimum ranges (Kreuger, 1967). However, under the conditions of this study, these concentrations may not be growth-limiting. Nutrient concentrations in conifer leaves are highly variable over a growing season, generally decreasing rapidly as new leaves expand (Mitchell, 1936). In this experiment, new flushes of leaf growth generally ceased one month before measurements were made, a time when concentrations would be at their lowest levels. There is also evidence that growing plants under low light intensity can reduce P uptake (Bhat, 1982; Son and Smith, 1988), a factor which might also contribute to low nutrient concentrations in leaves of greenhouse-grown seedlings. Thus, the relatively low leaf N and P concentrations measured in this experiment may be normal for the conditions of this study and not indicate growth limiting deficiency.

Proper nutrient balance can also be important for optimum growth of Douglas-fir seedlings. Investigations of Mohren et al. (1986) indicate that optimum growth of Douglas-fir can occur when the leaf concentration ratio of N:P is within the broad range of 4 to 30. Brix and van den Driessche (1974) suggested a range of 4 to 10. Outside this range some growth inhibition occurs. In this experiment, N:P ratios were within a range of about 5 to 14, indicating that a proper nutrient balance between N and P was maintained in spite of their low concentrations.

The response of seedling nutrition to changes in fertilizer P concentration was the same for mycorrhizal and nonmycorrhizal seedlings. Increasing fertilizer P concentration to nonmycorrhizal seedlings from none to 50 mg kg^{-1} increased leaf P concentration almost two fold ($p=.01$), tended to increase K and decrease N, and Ca

Table 14. Concentration of mineral nutrients in leaves of 6-month-old Douglas-fir seedlings grown under well-watered greenhouse conditions for the indicated mycorrhizal fungus treatment, fertilizer P concentration, and seedling age when transplanted. Leaf nutrient concentrations are expressed as percent of leaf dry mass.

Treatment			Concentration of nutrients in leaves			
Age transplanted	Fungal treatment	Fertilizer P conc.	N	P	K	Ca
(weeks)		(mg kg^{-1})	(percent)	(percent)	(percent)	(percent)
6	NM[a]	0	.68 ±.03[b]	.05 ±.01	.59 ±.03	.34 ±.02
		20	.64 ±.03	.06 ±.01	.66 ±.04	.33 ±.02
		50	.62 ±.02	.12 ±.01	.72 ±.05	.32 ±.02
	Rv	0	.77 ±.04	.06 ±.01	.70 ±.05	.31 ±.02
		20	.71 ±.03	.06 ±.01	.71 ±.05	.33 ±.02
8	NM	20	.63 ±.03	.08 ±.01	.76 ±.08	.31 ±.02
	Ll	20	.57 ±.02	.09 ±.01	.69 ±.04	.31 ±.02
	Hc	20	.74 ±.04	.11 ±.01	.82 ±.04	.41 ±.02

[a] fungal treatment abbreviations: NM, nonmycorrhizal; Rv, Rhizopogon vinicolor; Ll, Laccaria laccata; Hc, Hebeloma crustuliniforme

[b] mean ± one standard error

did not change (Table 14). For both nonmycorrhizal and Rhizopogon-colonized seedlings increasing fertilizer P from none to 20 mg P kg^{-1} resulted in the same trends, but changes were less pronounced and none were statistically significant (p=.05). Phosphorus fertilization stimulated both P and K uptake, but tended to reduce N uptake and had no effect on Ca. Reduced foliar N in response to P fertilization was expected for both uncolonized and ectomycorrhizal seedlings based upon the study by Rousseau and Reid (1985).

Mycorrhizal colonization improved leaf concentrations of nutrients to a greater degree when soil P was less available, as expected. Rhizopogon colonization tended to increase N, P, and K compared to nonmycorrhizal seedlings when no P fertilizer was applied, but only N and K tended to be higher, and to a lesser degree, when seedlings were fertilized with supplemental P (Table 14). These results were due mainly to the large response of nonmycorrhizal controls to P fertilization since nutrient concentrations in both Rhizopogon treatments were similar.

At the same fertilizer P concentration (20 mg kg^{-1}), neither Rhizopogon- nor Laccaria-colonized seedlings showed any clear differences in leaf concentration of nutrients compared to nonmycorrhizal controls, but Hebeloma-colonized seedlings had significantly greater N, P and Ca content (p=.01), and tended to have higher K content than either nonmycorrhizal or Laccaria-colonized seedlings (Table 14). Hebeloma-colonized seedlings were significantly smaller than either nonmycorrhizal or Laccaria-colonized seedlings (see section on Growth of Seedlings) which translated into lower amounts of N (p=.01) and K, and similar amounts of P and Ca in the leaves of these seedlings (Table 15). Except for low N in Hebeloma-colonized seedlings and high P in nonmycorrhizal seedlings fertilized at the highest fertilizer P level, there were no statistically significant differences (p=.05) in total leaf content of N, P, K, or Ca resulting from different mycorrhizal fungal species or P fertilizer levels.

Table 15. Quantity of mineral nutrients in leaves of 6-month-old Douglas-fir seedlings grown under well-watered greenhouse conditions for the indicated mycorrhizal fungus treatment, fertilizer P concentration, and seedling age when transplanted.

Treatment			Quantity of nutrients in leaves			
Age transplanted	Fungal treatment	Fertilizer P conc.	N	P	K	Ca
(weeks)		(mg kg^{-1})	(mg)	(mg)	(mg)	(mg)
6	NM[a]	0	5.3 ±.5[b]	.35 ±.03	4.5 ±.4	2.5 ±.2
		20	5.4 ±.4	.51 ±.05	5.9 ±.8	2.8 ±.3
		50	5.3 ±.2	1.00 ±.10	6.2 ±.5	2.6 ±.1
	Rv	0	5.4 ±.3	.42 ±.04	4.9 ±.4	2.2 ±.1
		20	4.7 ±.4	.41 ±.04	4.9 ±.7	2.2 ±.2
8	NM	20	5.4 ±.2	.68 ±.04	6.9 ±1.0	2.7 ±.2
	L1	20	5.0 ±.2	.82 ±.06	6.1 ±.4	2.7 ±.2
	Hc	20	4.3 ±.2	.68 ±.07	5.0 ±.5	2.5 ±.2

[a] fungal treatment abbreviations: NM, nonmycorrhizal; Rv, Rhizopogon vinicolor; L1, Laccaria laccata; Hc, Hebeloma crustuliniforme

[b] mean ± one standard error

Overall, mycorrhizal colonization did not greatly influence nutrient uptake by Douglas-fir seedlings, although some P uptake benefit tended to occur when P fertilizer was withheld. There were only small differences between mycorrhizal species in their effect on seedling nutrient uptake. P fertilization increased leaf P concentrations without any significant effect on concentrations of other nutrients, but there was a tendency for N to be reduced.

Growth of Seedlings

Expected Results

In general, ectomycorrhizal colonization increases production of plant dry matter (Harley and Smith, 1983). This is common where moderate soil nutrient deficiencies occur, especially P, and can be eliminated by enhancing nonmycorrhizal plant growth through fertilization (Stone, 1949; Harley and Smith, 1983; Rousseau and Reid, 1985). Such observations suggest that ectomycorrhizae increase plant growth by improving plant nutrition (Bowen, 1973), but there remain examples of better growth of ectomycorrhizal plants even when mineral nutrients are not limiting (McComb and Griffith, 1946; Vozzo and Hacskylo, 1971). This latter observation has led some researchers to suggest that plant growth regulators secreted by the fungus might also contribute to greater total growth of ectomycorrhizal plants (McComb and Griffith, 1946; Slankis, 1973).

There are also examples of less plant dry matter production in response to ectomycorrhizal colonization (Bowen, 1973; Sands and Theodoreau, 1978; Marx, 1979; Reid, et al., 1983), generally occurring under extremely low soil nutrient availability (Bowen, 1973; Harley and Smith, 1983) or under conditions where nutrients are not limiting (Nylund and Unestam, 1987). These observations suggest that ectomycorrhizae retard plant dry matter production under conditions

where mycorrhizae cannot relieve limitations to plant growth to a degree which makes up for photosynthate diversion to the fungus (Harley and Smith, 1983). Following this logic, low light levels, for example, could cause less plant growth in mycorrhizal plants, a hypothesis confirmed for VA mycorrhizal onion plants by Son and Smith (1988). Less plant growth could also be due to a lag time between photosynthate diversion to the developing mycorrhizal fungus and the return of significant benefits to the plant (Vozzo and Hacskaylo, 1971). This is probably more significant for small seedlings during initial colonization (Harley and Smith, 1983). For the same host, different fungal species or even strains can differ in their effect on plant growth (Bowen, 1973; Trappe, 1977; Marx, 1979). In view of this discussion, this could be the result of differences between fungi in their strength of photosynthate diversion, ability to benefit the plant, and timing of photosynthate diversion relative to derived benefits by the plant.

Ectomycorrhizal colonization is expected to alter the distribution of dry matter within seedlings in this experiment. Results of many studies show that colonization tends to decrease the proportion of plant dry matter in root systems (Harley, 1969; Alexander, 1981; Cline and Reid, 1982; Black, 1984), but not in every case (Trappe, 1977). Alexander's (1981) data shows that mycorrhizal root systems produce greater numbers of short roots per unit root system dry mass than nonmycorrhizal root systems. This has been interpreted to mean that root dry matter is distributed proportionally more into fine roots in response to colonization (Alexander, 1981; Harley and Smith, 1983), although there is no data which show this unequivocally. Thus, ectomycorrhizal colonization is expected to reduce the proportion of total plant dry matter in root systems, but appears to increase the proportion of root dry matter in finer roots.

The contribution of fungal biomass to measurements of root system biomass can be significant. Fungal hyphae in sheaths and Hartig nets are not easily separable from root tissue. It has been estimated that fungal sheaths can represent as much as 40% of the biomass of some types of ectomycorrhizae and 20% of the biomass of a whole root system

that is completely colonized (Harley and McCready, 1952; Harley and Smith, 1983). Since the fungal biomass can be significant, there probably is significantly less dry matter in root tissue of mycorrhizal plants than results of most studies indicate.

Biomass of hyphae which grow outward into the soil from the sheaths is considered to be substantial and certainly highly variable between species (Harley and Smith, 1983), but there are no published estimates from which to derive some perspective. Such variability could be a major contributor to fungal differences in effect on host plant growth since fungal hyphae represent both a photosynthate sink and a source for nutrient and water uptake.

Results

Total plant growth in response to fungal and fertilizer treatments is shown in Table 16. Mycorrhizal colonization influenced dry mass of whole seedlings. While Laccaria-colonized seedlings were similar in dry mass to nonmycorrhizal controls, Rhizopogon-colonized seedlings tended to be smaller by about 8% and Hebeloma-colonized seedlings were significantly smaller than nonmycorrhizal controls by 18% ($p=.01$). Different P fertilization levels had no effect on the dry mass of nonmycorrhizal seedlings or Rhizopogon-colonized seedlings. Seedlings transplanted at 8 weeks old became significantly larger ($p=.01$) than those transplanted at 6 weeks old, so that the total growth of seedlings colonized by Rhizopogon cannot be directly compared to those of Laccaria and Hebeloma.

The relative distribution of total dry mass of seedlings among leaves, stems, and roots is shown in Table 17. There was no effect of different P fertilization levels, transplant age, or fungal treatment, except for Hebeloma colonization, on the relative partitioning of dry mass in these Douglas-fir seedlings. For all treatments except Hebeloma, about 37% of the dry matter was in leaves, 23% in stems, and 40% in the root systems. For Hebeloma-colonized seedlings, however,

Table 16. Total dry mass of 6-month-old Douglas-fir seedlings grown under well-watered greenhouse conditions for the indicated mycorrhizal fungus treatment, fertilizer P concentration, and seedling age when transplanted.

Treatment			
Age transplanted	Fungal treatment	Fertilizer P conc.	Total dry mass
(weeks)		(mg kg^{-1})	(g)
6	NM[a]	0	2.13 ± .07[b]
		20	2.08 ± .06
		50	2.15 ± .06
	Rv	0	1.94 ± .06
		20	1.92 ± .06
8	NM	20	2.44 ± .06
	Ll	20	2.53 ± .05
	Hc	20	2.01 ± .06

[a] fungal treatment abbreviations: NM, nonmycorrhizal; Rv, Rhizopogon vinicolor; Ll, Laccaria laccata; Hc, Hebeloma crustuliniforme

[b] mean ± one standard error

Table 17. Distribution of total dry mass among leaves, stem, and roots of 6-month-old seedlings grown under well-watered greenhouse conditions for the indicated mycorrhizal fungus treatment, fertilizer P concentration, and seedling age when transplanted. Dry mass of each plant part is expressed as percent of the total seedling dry mass.

Treatment			Plant part		
Age transplanted	Fungal treatment	Fertilizer P conc.	Leaves	Stem	Roots
(weeks)		(mg kg^{-1})	(percent)	(percent)	(percent)
6	NM[a]	0	36[b]	24	40
		20	37	22	41
		50	37	23	40
	Rv	0	35	23	42
		20	37	22	41
8	NM	20	37	23	40
	Ll	20	38	22	40
	Hc	20	31	19	49

[a] fungal treatment abbreviations: NM, nonmycorrhizal; Rv, Rhizopogon vinicolor; Ll, Laccaria laccata; Hc, Hebeloma crustuliniforme

[b] standard errors for all values given in this table equal 1%

there was a shift in dry matter from leaves (31%) toward the roots (49%), while the stem remained relatively unchanged (19%).

The amount of leaf surface area of seedlings (Table 18) followed the same trend as total plant dry weight. Laccaria and Rhizopogon-colonized plants had similar or slightly less leaf area, respectively, than nonmycorrhizal controls, and Hebeloma-colonization reduced leaf area by 24% due to both overall reduction in plant dry mass and the shift of dry matter away from leaves to the roots. Phosphorus fertilization level had no effect on leaf area of nonmycorrhizal or Rhizopogon-colonized seedlings.

Root dry mass was not significantly altered by mycorrhizal colonization or P fertilization (Table 19). For Hebeloma-colonized seedlings, this resulted from a relative shift of biomass to roots which counteracted the overall decrease in seedling dry mass. In spite of the similarities in dry mass of roots, Table 19 also shows that there were some large differences in length of roots. Hebeloma-colonized seedlings tended to have less root length, by 20%, and Rhizopogon-colonized seedlings had greatly reduced root length, by 31 to 44%, than nonmycorrhizal controls. This important effect was not detectable in the root dry mass comparisons, and translates to less root length per unit root dry mass for Hebeloma and particularly for Rhizopogon-colonized seedlings. This result appears counter to the data of Alexander (1981) and McComb (1943) which suggests that colonization should lead to a greater proportion of root growth into fine, short roots which would increase the amount of root length per unit dry mass. In these experiments, less root length per unit root system dry mass indicates greater root density, larger diameter roots and/or inclusion of significant amounts of fungal material in the root dry mass. Probably all three are contributors.

Table 20 shows that seedling growth occurred between the ages of 6 and 8 months. Wet soil experiments were conducted when seedlings were about 6 months old and the drought stress experiments were conducted when the seedlings were about 8 months old. Between 6 and 8 months old, all treatments increased their leaf dry mass by 5 to 21%. Since there were no new flushes of shoot growth during the experiments

Table 18. Leaf area of 6-month-old Douglas-fir seedlings grown under well-watered greenhouse conditions for the indicated mycorrhizal fungus treatment, fertilizer P concentration, and seedling age when transplanted.

Treatment			
Age transplanted	Fungal treatment	Fertilizer P conc.	Leaf Area
(weeks)		(mg kg^{-1})	(cm^2)
6	NM[a]	0	68.8 ± 1.7[b]
		20	68.4 ± 1.8
		50	70.6 ± 1.6
	Rv	0	63.4 ± 1.6
		20	64.2 ± 1.7
8	NM	20	77.8 ± 1.4
	Ll	20	80.4 ± 1.1
	Hc	20	58.9 ± 1.5

[a] fungal treatment abbreviations: NM, nonmycorrhizal; Rv, Rhizopogon vinicolor; Ll, Laccaria laccata; Hc, Hebeloma crustuliniforme

[b] mean ± one standard error

Table 19. Root dry mass, root length, and root length per unit root dry mass of 6-month-old Douglas-fir seedlings grown under well-watered greenhouse conditions for the indicated mycorrhizal fungus treatment, fertilizer P concentration, and seedling age when transplanted.

Treatment					
Age transplanted	Fungal treatment	Fertilizer P conc.	Root dry mass	Root length	Root length per unit dry mass
(weeks)		(mg kg^{-1})	(g)	(m)	(m g^{-1})
6	NM[a]	0	.86 ± .03[b]	14.4 ± 1.2	16.8
		20	.85 ± .03	16.4 ± 1.6	19.3
		50	.86 ± .03	15.0 ± 1.1	17.4
	Rv	0	.81 ± .03	9.9 ± 0.5	12.2
		20	.80 ± .03	9.2 ± 0.7	11.6
8	NM	20	.97 ± .03	15.5 ± 1.1	16.0
	Ll	20	1.01 ± .03	15.5 ± 1.5	15.3
	Hc	20	.99 ± .03	12.4 ± 1.1	12.5

[a] fungal treatment abbreviations: NM, nonmycorrhizal; Rv, Rhizopogon vinicolor; Ll, Laccaria laccata; Hc, Hebeloma crustuliniforme

[b] mean ± one standard error

Table 20. Change in leaf dry mass, root dry mass, and root length of Douglas-fir seedlings from 6 months old to 8 months old grown under well-water greenhouse conditions for the indicated mycorrhizal fungus treatment, fertilizer P concentration, and seedling age when transplanted. Change is expressed as percent of the value for 6-month-old seedlings.

Treatment					
Age transplanted	Fungal treatment	Fertilizer P conc.	Leaf dry mass	Root dry mass	Root length
(weeks)		(mg kg^{-1})	(percent)	(percent)	(percent)
6	NM[a]	0	+ 8	+ 60	+ 56
		20	+ 7	+ 56	+ 27
		50	+ 16	+ 56	+ 47
	Rv	0	+ 5	+ 24	+ 35
		20	+ 6	+ 29	+ 47
8	NM	20	+ 13	+ 53	+ 63
	Ll	20	+ 21	+ 46	+ 47
	Hc	20	+ 11	+ 32	+ 49

[a] fungal treatment abbreviations: NM, nonmycorrhizal; Rv, Rhizopogon vinicolor; Ll, Laccaria laccata; Hc, Hebeloma crustuliniforme

this was probably due mainly to leaf expansion after final budset. Table 20 also shows that while leaf growth was small, roots grew rapidly over the time experiments were conducted. Root dry mass increased by 24 to 63% depending upon the treatment. Lower values, of 24 to 32%, were found for Rhizopogon and Hebeloma-colonized seedlings and larger increases of 46 to 69% were found for nonmycorrhizal and Laccaria-colonized seedlings. This indicates relatively slower root system growth in Rhizopogon- and Hebeloma-colonized seedlings during these experiments. Root growth also occurred as large increases in root length for all treatments, but root length did not show the clear mycorrhizal effect seen in root dry mass. Phosphorus fertilizer levels had no effect on either aspect of root growth. Root growth after a period of shoot growth is a normal growth pattern for Douglas-fir seedlings (Cleary et al., 1978).

In summary, mycorrhizal colonization tended to decrease plant dry mass under well-watered greenhouse conditions; Laccaria had the least effect, Rhizopogon was intermediate, and Hebeloma had the greatest effect in this regard. Soil P availability must not have been limiting seedling growth, since fertilizing at different levels of P had no effect on any aspect of growth of nonmycorrhizal or Rhizopogon-colonized seedlings. Hebeloma colonization caused a shift of dry matter from leaves to root systems. The other fungal species had no effect on dry matter distribution between leaves, stem and root system. Mycorrhizal colonization lead to less root length growth without much effect on root system dry mass, suggesting that these mycorrhizal root systems were not more finely divided as the literature had suggested. These seedlings were still growing during the period of experiments. Only a small amount of leaf growth occurred, but root system growth was large in all treatments.

Net Photosynthesis Rate

Expected Results

In the experiments where measurements were made periodically over the course of one day, net photosynthesis rate was expected to increase rapidly to a maximum when the lights came on. Photosynthesis and photorespiration rates require light energy and, therefore, closely follow the intensity of light, so that the net photosynthesis rate responds similarly. However, net photosynthesis rate achieves a maximum or light saturated level at light intensities usually much less than that of full sunlight. For Douglas-fir the light saturation level is at about 400 to 500 umol m^{-2} s^{-1} or about 1/4 of full sunlight (Brix, 1967,1971; Brix and Ebell, 1969), or even lower for plants grown at lower light intensities (Brix, 1967; Boardman, 1977), or where mineral nutrition is a limitation (Brix, 1971). These studies also show that as light intensity increases, net photosynthesis rate increases rapidly at first, and then more slowly as light saturation is approached (Brix, 1967, 1971; Brix and Ebell, 1969). Conversely, net photosynthesis rate was expected to decrease as light intensity decreased, and become negative in darkness because of continued metabolic respiration.

Net photosynthesis rate of conifers decreases when soil water becomes limiting (Clark, 1961; Brix, 1962; Babalola et al., 1968), mainly through its effect on plant water status. Lower plant water potential and turgor can cause stomatal closing which restricts CO_2 supply (Kramer, 1969; Boyer, 1976). Lower plant turgor can immediately reduce growth rate (Hsaio, 1973), leading to reduced demand for photosynthates and reduced phytohormone production which could feed back to chloroplasts and suppress photosynthesis (Sweet and Wareing, 1967; Herold, 1980). At more extreme plant dehydration, reduced biochemical capacity for photosynthesis and structural injuries will also contribute to reduce the photosynthesis rate (Hsaio, 1973). There

is also some evidence of a stomatal closure response to soil dryness which is independent of plant water status, but the mechanism is not known (Schulze, 1986).

There have been only a few studies on the effect of ectomycorrhizae on photosynthesis of plants. Under unlimited soil water availability, Reid et al. (1983), Ekwebelem and Reid (1983), and Nylund and Unestam (1987) measured higher net photosynthesis rate per unit leaf area in ectomycorrhizal pines than in uncolonized controls. Reid et al. (1983) suggested that this result was consistent with the concept that a high mycorrhizal demand for photosynthates from the plant could stimulate photosynthesis rate, but in their study, leaf N and P concentrations were also much higher in the mycorrhizal seedlings. Parke et al. (1983) measured higher, similar, and lower net photosynthesis rates, depending upon the fungal species, for ectomycorrhizal Douglas-fir compared to nonmycorrhizal seedlings under well-watered conditions. However, Rhizopogon vinicolor and Laccaria laccata were two species which did not alter seedling net photosynthesis rate. Seedling nutrition was not evaluated in their experiments. Parke et al. (1983) also measured net photosynthesis rates of ectomycorrhizal seedlings under extreme water stress which produced negative rates. There are no published studies on the influence of ectomycorrhizal colonization on net photosynthesis of plants at moderate levels of drought stress. However, if plant water potential is improved by mycorrhizae under moderate drought conditions, as expected, then net photosynthesis rate should be higher.

In general, fertilization leads to higher net photosynthesis rate through increased nutrient uptake when those nutrients are otherwise limiting the CO_2 exchange and fixation process (Natr, 1972; Jarvis and Sanford, 1986). Except at very high leaf concentrations, increasing leaf N, P, and K concentrations correlates with higher net photosynthesis rates (Keller, 1972; Natr, 1972; Longstreth and Nobel, 1980). The change in net photosynthesis rate becomes smaller for incremental increases in leaf N, P and K concentration, but there can be considerable differences in sensitivity depending upon the nutrient

in question. Data of Longstreth and Nobel (1980) suggest that increasing P and K concentrations increases photosynthesis rate, but only at levels of severe deficiency, while photosynthesis response to increasing N concentration is more gradual. This might account, in part, for the lack of strong correlations between net photosynthesis and leaf P and K concentrations and rather good correlations with N observed by Natr (1972) in his review of this subject. However, low light levels shift the entire photosynthesis response range to much lower leaf concentrations of N (Keller, 1972) and this might apply to P and K as well. Conifers appear no different from other plants in the relationship between photosynthesis and leaf nutrient concentration (Keller, 1972; Brix, 1981). Calcium does not directly participate in any major way in the photosynthesis process (Mengel and Kirkby, 1978) which might account for the lack of research in this regard.

Fertilization might not lead to greater net photosynthesis as nutrient uptake interactions and nutrient concentration imbalances might occur, and as fertilization can suppress beneficial microflora populations, such as mycorrhizae (Lister et al., 1968; Ekwebelem and Reid, 1983), which might influence plant photosynthesis in non-nutritional ways. Such mechanisms may be responsible for decreased photosynthesis and growth commonly observed for plants at very high fertilization rates (Lister, 1968; Keller, 1972; Mengel and Kirkby, 1978).

Based upon the studies reviewed here, it was expected that net photosynthesis rate of well-watered Douglas-fir seedlings would increase quickly as the lights came on, to a steady level during the day, and decrease quickly as the lights went off. Net photosynthesis rate would decrease in response to decreasing soil water potential, but more rapidly after water uptake becomes limited. Ectomycorrhizal colonization was expected to influence net photosynthesis rate in an unpredictable way under wet soil conditions. However, it would generally help the seedlings maintain net photosynthesis rates as the soil water potential declined, by helping to maintain high plant water potential. Phosphorus fertilization was expected to increase net

photosynthesis rate, but only if an extreme P deficiency developed when P fertilizer was withheld.

Results

Treatments were compared in 6 different combinations in order to determine the nature of the effect of ectomycorrhizae, P fertilizer level, and transplant age on the seedlings. These comparisons were outlined in the "METHODS" section covering "Statistical Design and Data Analysis", and are briefly reviewed here:

The effect of seedling age when transplanted:

1) The nonmycorrhizal treatments, 2 and 6 (see Table 6 for reference numbers), were used to exemplify "typical" seedling behavior, and to test for effect of transplant age on seedlings.

The effect of ectomycorrhizal colonization:

2) Hc and Ll (treatments 7 and 8) were compared with their nonmycorrhizal control, treatment 6, for moderate soil P levels.
3) Rv treatment 5 was compared with its nonmycorrhizal control, treatment 2, for moderate soil P levels.
4) Rv treatment 4 was compared to its nonmycorrhizal control, treatment 1, for seedlings where fertilizer P withheld.

The effect of P fertilization level:

5) For nonmycorrhizal seedlings, high P level (treatment 3) and the low P level (where fertilizer P was withheld, treatment 1) were compared with moderate P level (treatment 2).

6) For Rv, low P level (where fertilizer P was withheld, treatment 4) was compared with moderate P level (treatment 5).

In this section, comparisons are made in the above order. Furthermore, for each comparison made, 2 graphs are presented. The diurnal trend of net photosynthesis rate for 6-month-old seedlings growing in wet soil is described first, followed immediately by the relationship between net photosynthesis rate and predawn soil water potential found for 8-month-old seedlings.

The typical diurnal trend of net photosynthesis rate in this experiment is shown in Figure 7. Net photosynthesis increased quickly in response to increasing light, remained steady or decreased slightly over the course of 13 hours of daylight, then decreased rapidly as lights were turned off. Net photosynthesis rate of about 4 umol m^{-2} s^{-1} during the day in these experiments are similar to published values for Douglas-fir after adjusting for differences in light levels (Brix, 1971, 1981; Parke et al., 1983). As light intensity increased stepwise in the morning, net photosynthesis rate increased proportionally less with each step. The last step in light intensity from 180 to 360 umol m^{-2} s^{-1} resulted in only a 15% increase in net photosynthesis rate, indicating that these seedlings were approaching their light saturation level at 360 umol m^{-2} s^{-1} , which was expected from published values for Douglas-fir (Brix and Ebell, 1969; Brix 1969, 1971). Small negative values of net photosynthesis rate in darkness represent dark respiration rates and agree in magnitude to published dark respiration rates for Douglas-fir of about 10% to 20% of the maximum net photosynthesis rate (Brix, 1971). Interestingly, net photosynthesis rates under the same light intensity are slightly higher in the morning than in the evening. This occurs under light intensities of 90 and 360 umol m^{-2} s^{-1} and suggests that some small limitation to net photosynthesis rate developed over the course of the day.

The typical trend of net photosynthesis rate in relation to soil water potential in this experiment is shown in Figure 8. Soil water

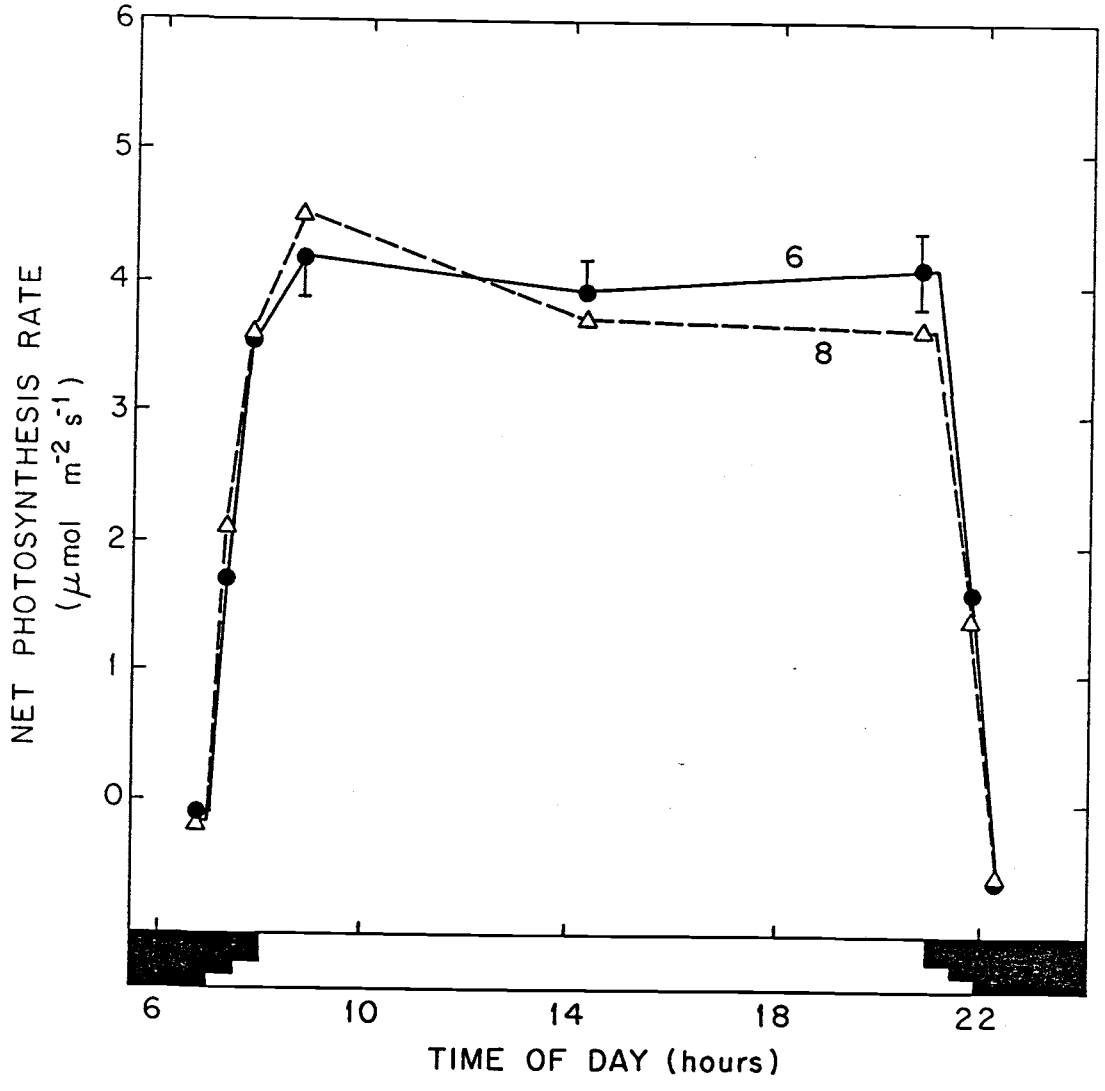


Figure 7. Net photosynthesis rate measured periodically over the course of one day under well-watered soil conditions for 6-month-old nonmycorrhizal Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: transplanted at 6 weeks old (●); transplanted at 8 weeks old (△).

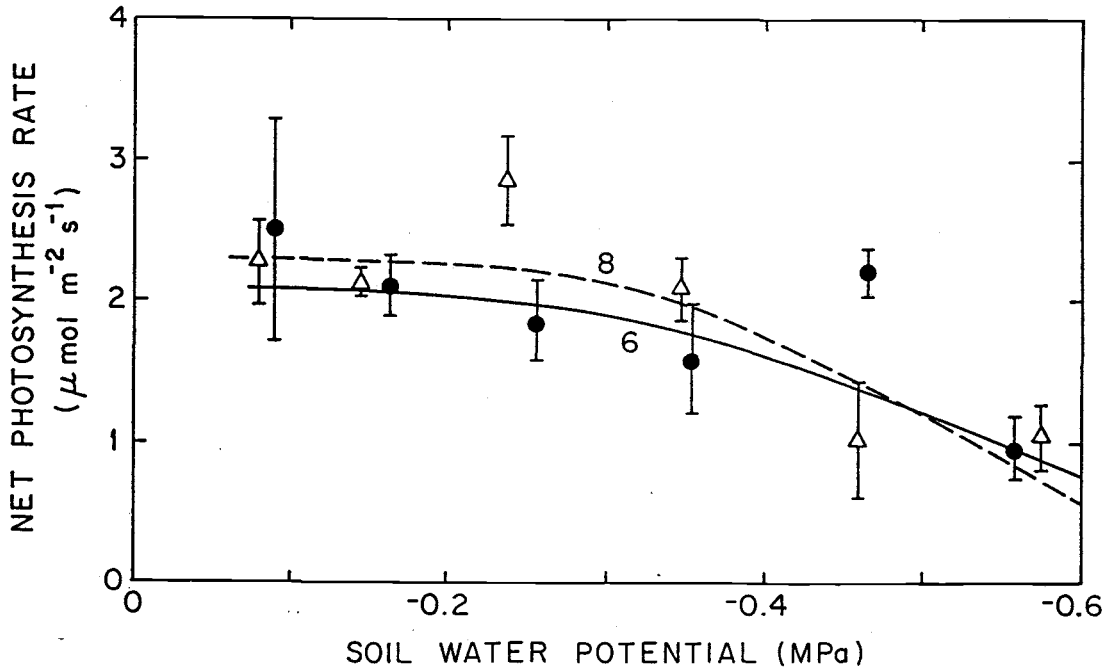


Figure 8. Net photosynthesis rate as a function of predawn soil water potential for 8-month-old nonmycorrhizal Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: transplanted at 6 weeks old (●); transplanted at 8 weeks old (△). Net photosynthesis rate was measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

potential in this figure is that of the bulk soil, measured at predawn, while net photosynthesis rates were measured late in the day. In this figure, the points represent means and their standard errors of observations made within each 0.1 MPa segment of the soil water potential range. Each line is a hand-fit curve representing all of the data. The details of the techniques used to fit the curves are described in the "METHODS" section on "Statistical Design and Data Analysis". This figure shows that net photosynthesis rate changed little in response to decreasing soil water potential from about -0.05 MPa to about -0.3 MPa soil water potential, then decreased more steeply to about 30% of the maximum rate at -0.6 MPa.

The influence of mycorrhizal colonization on net photosynthesis rate is shown in Figures 9 through 14. Laccaria colonization had no effect on net photosynthesis rate of 6-month-old seedlings in wet soil (Figure 9), but reduced that of 8-month-old seedlings over the soil water potential range of -0.05 MPa to at least -0.6 MPa (Figure 10). Hebeloma colonization also had no effect net photosynthesis rate of 6-month-old seedlings under wet soil conditions (Figure 9), but slightly increased net photosynthesis rates compared to nonmycorrhizal seedlings at 8 months old under drier soil conditions (Figure 10). Six-month-old Rhizopogon-colonized seedlings in wet soil had significantly higher net photosynthesis rates than nonmycorrhizal seedlings in the morning ($p=.05$), but this difference disappeared later in the day (Figure 11). However, at 8 months old, net photosynthesis rate late in the day was 1.36X that of nonmycorrhizal seedlings in wetter soil, a difference which gradually disappeared as the soil dried to about -0.6 MPa (Figure 12). Rhizopogon colonization gave a slightly different response in soil where no supplemental P fertilizer was applied. Six-month-old seedlings had significantly higher net photosynthesis rate than nonmycorrhizal seedlings late in the day under wet soil conditions ($p=.05$, Figure 13), and also tended to be higher for 8-month-old seedlings in drier soil (Figure 14).

Figures 15 and 16 show the effect of P fertilization on net photosynthesis rate for nonmycorrhizal seedlings. For 6-month-old seedlings in wet soil there was no effect of P fertilization on net

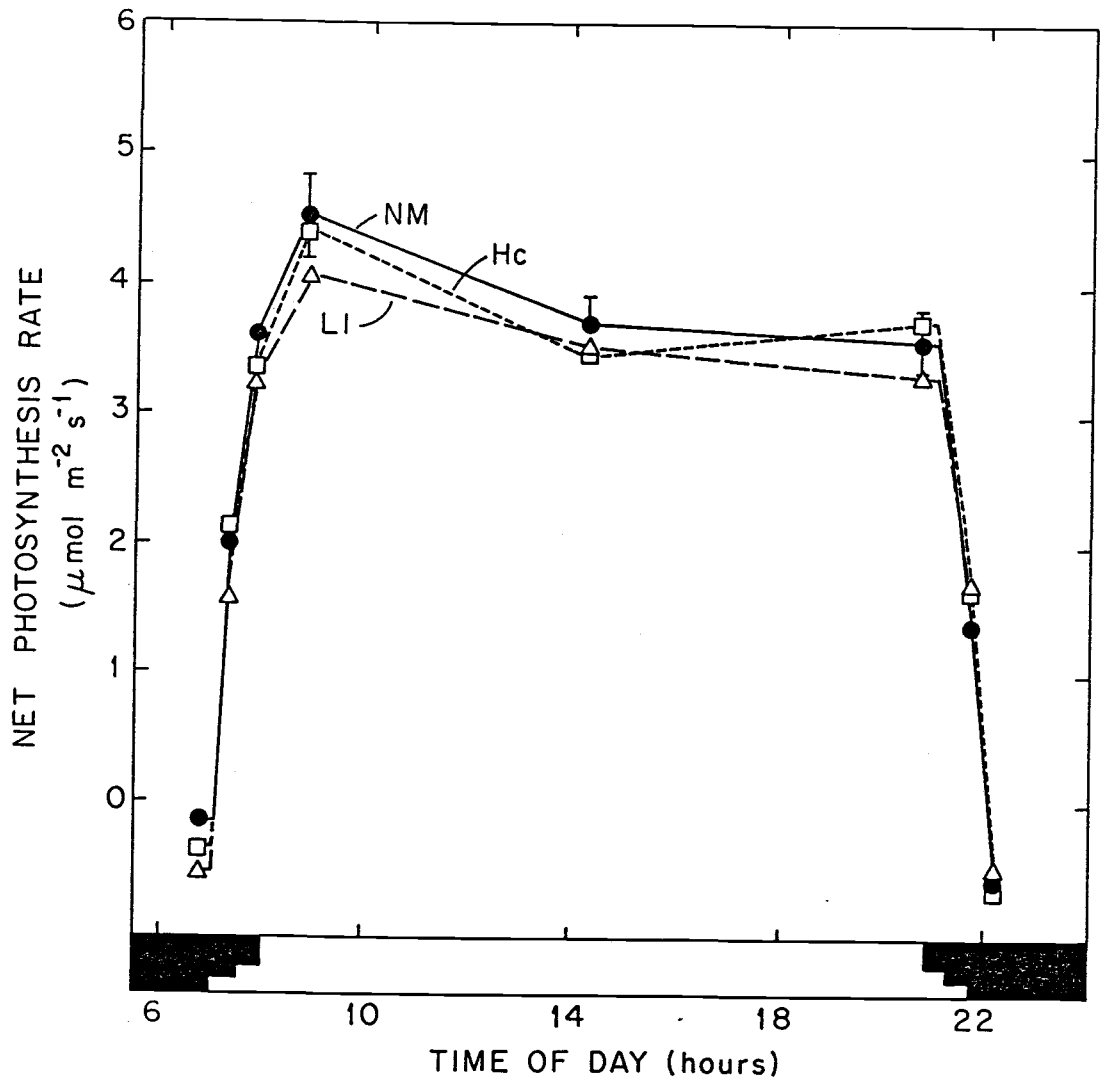


Figure 9. Net photosynthesis rate measured periodically over the course of one day under well-watered soil conditions for 6-month-old Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: nonmycorrhizal (NM, ●); colonized by Laccaria laccata (Ll, △); colonized by Hebeloma crustuliniforme (Hc, □).

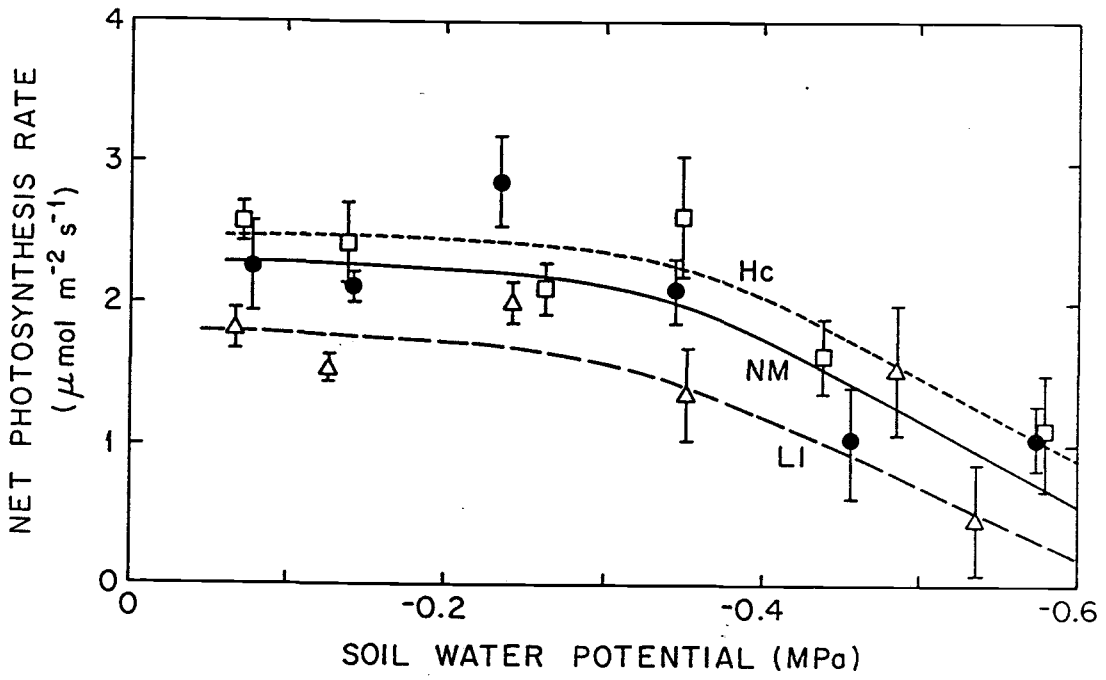


Figure 10. Net photosynthesis rate as a function of predawn soil water potential for 8-month-old Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: nonmycorrhizal (NM, ●); colonized by Laccaria laccata (Ll, △); colonized by Hebeloma crustuliniforme (Hc, □). Net photosynthesis rate was measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

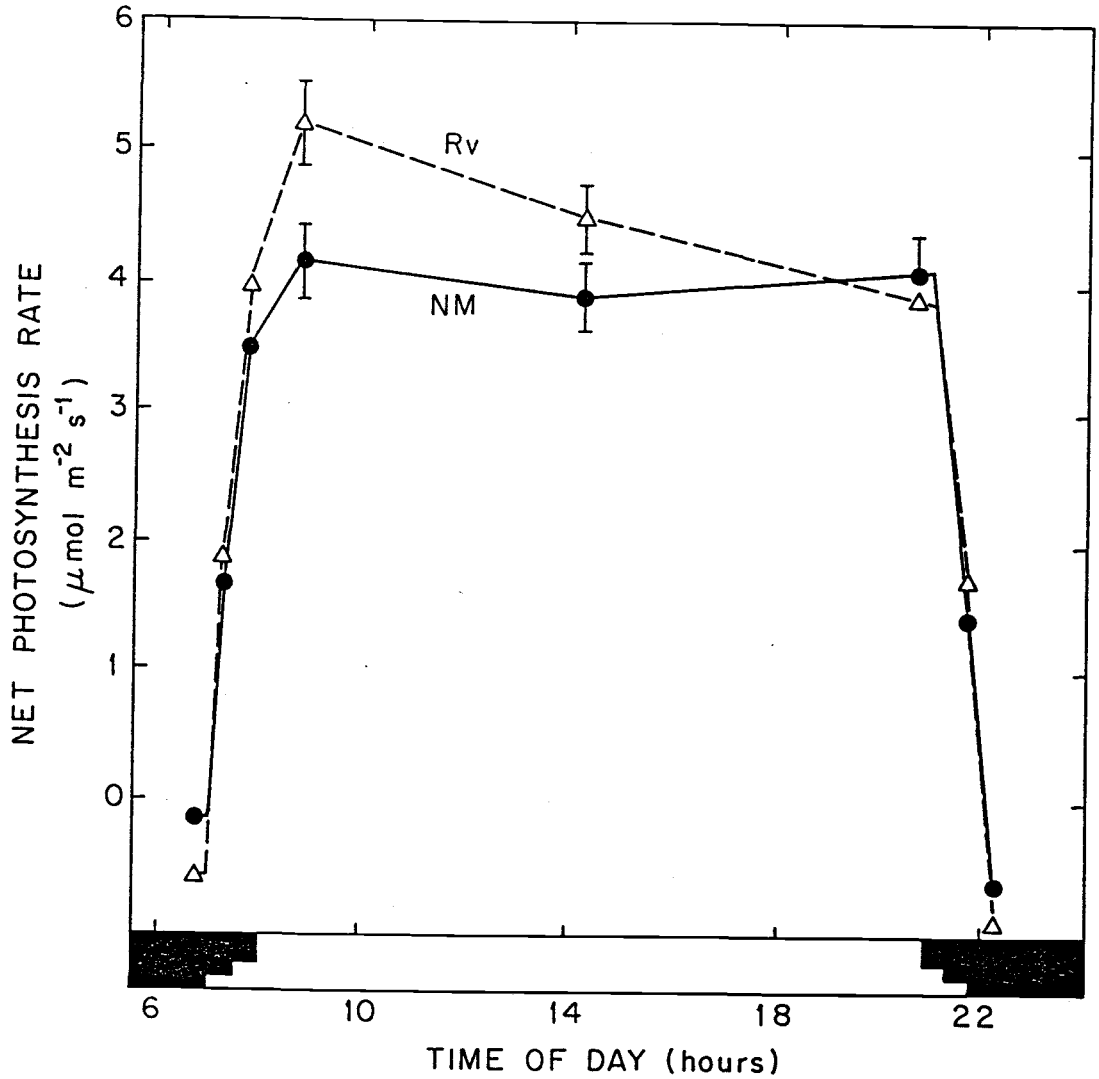


Figure 11. Net photosynthesis rate measured periodically over the course of one day under well-watered soil conditions for 6-month-old Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: nonmycorrhizal (NM, ●); colonized by Rhizopogon vinicolor (Rv, △).

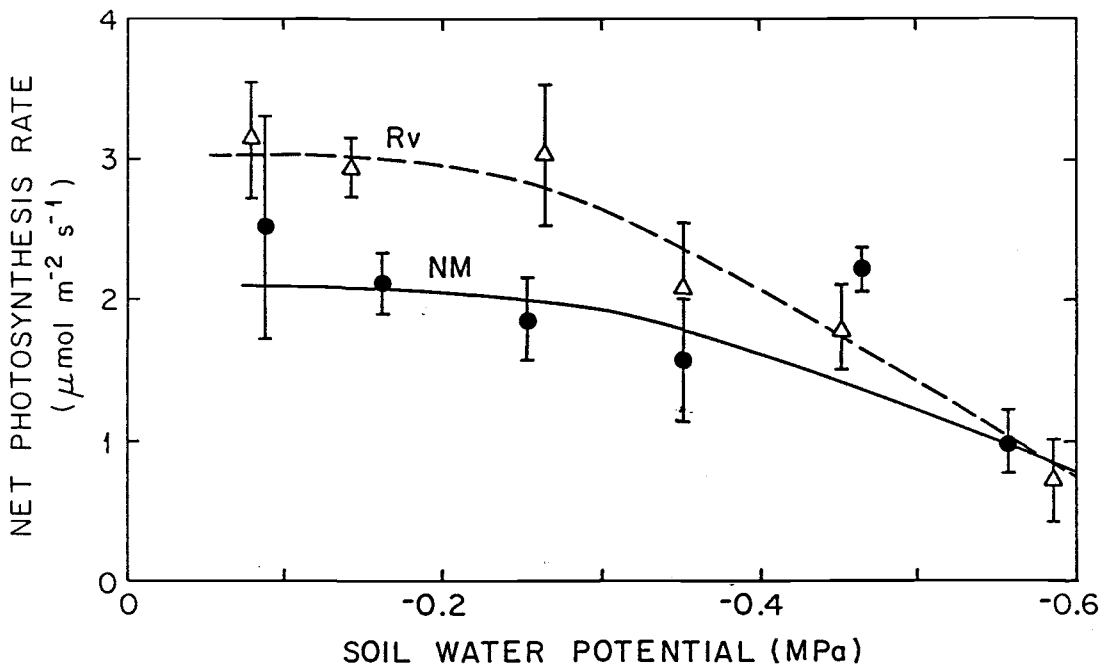


Figure 12. Net photosynthesis rate as a function of predawn soil water potential for 8-month-old Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: nonmycorrhizal (NM, ●); colonized by Rhizopogon vinicolor (Rv, △). Net photosynthesis rate was measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

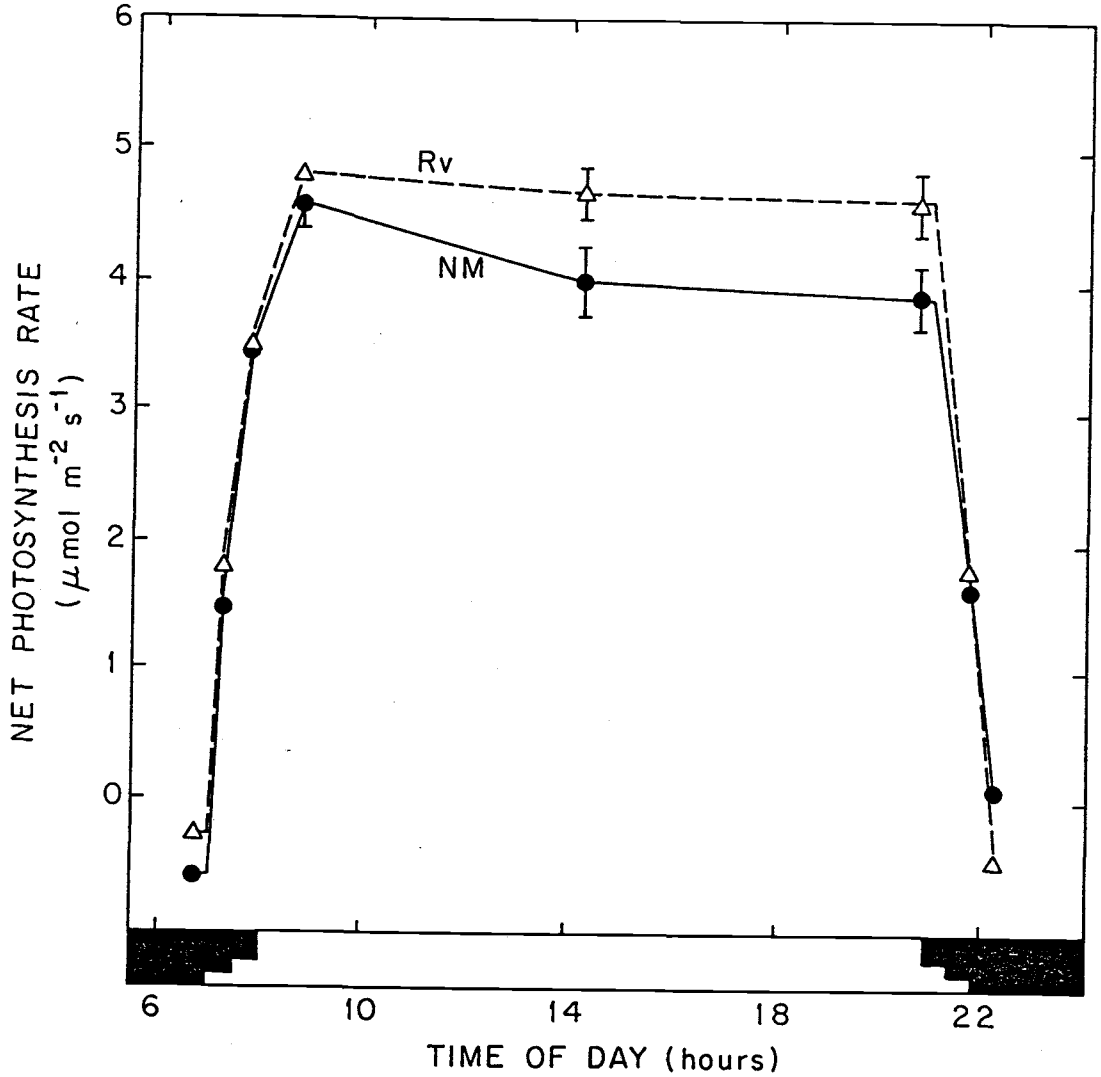


Figure 13. Net photosynthesis rate measured periodically over the course of one day under well-watered soil conditions for 6-month-old Douglas-fir seedlings from which P fertilizer was withheld: nonmycorrhizal (NM, ●); colonized by Rhizopogon vinicolor (Rv, △).

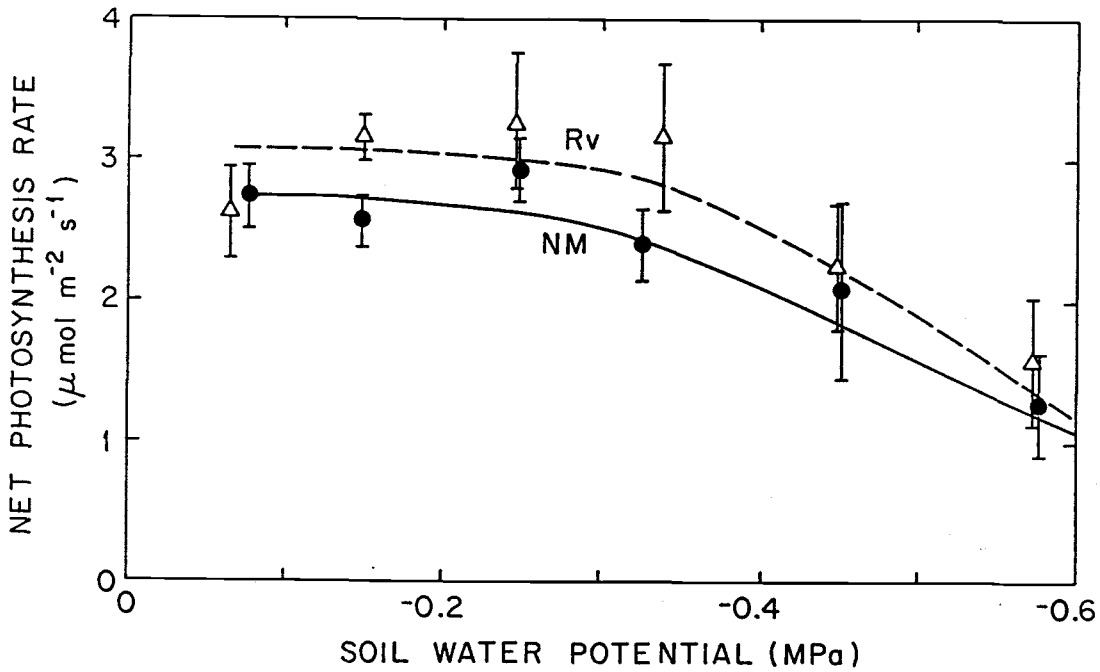


Figure 14. Net photosynthesis rate as a function of predawn soil water potential for 8-month-old Douglas-fir seedlings from which P fertilizer was withheld: nonmycorrhizal (NM, ●); colonized by Rhizopogon vinicolor (Rv, △). Net photosynthesis rate was measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

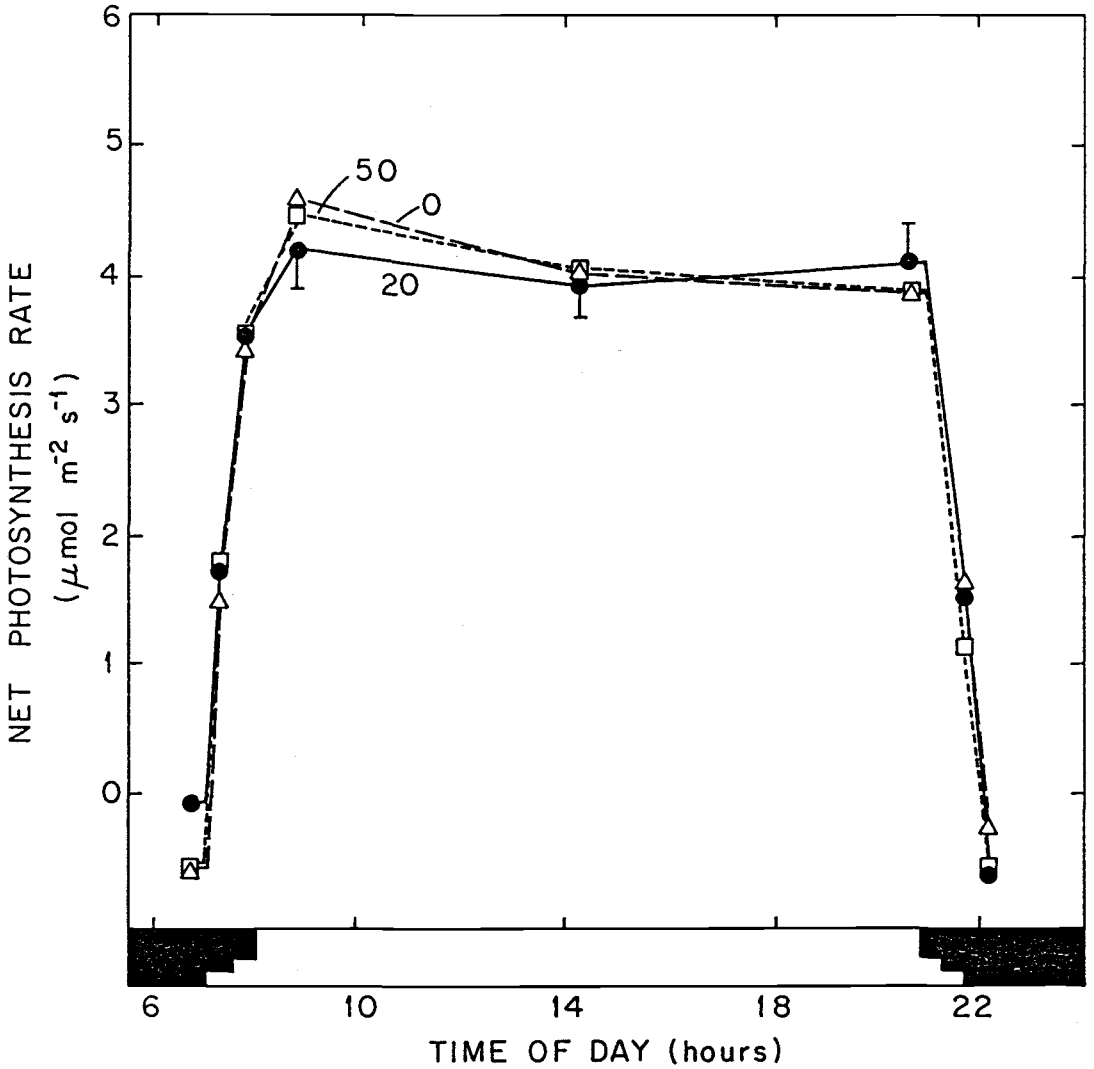


Figure 15. Net photosynthesis rate measured periodically over the course of one day under well-watered soil conditions for 6-month-old nonmycorrhizal Douglas-fir seedlings fertilized with solutions of 0 (△), 20 (●), and 50 (□) mg P kg^{-1}.

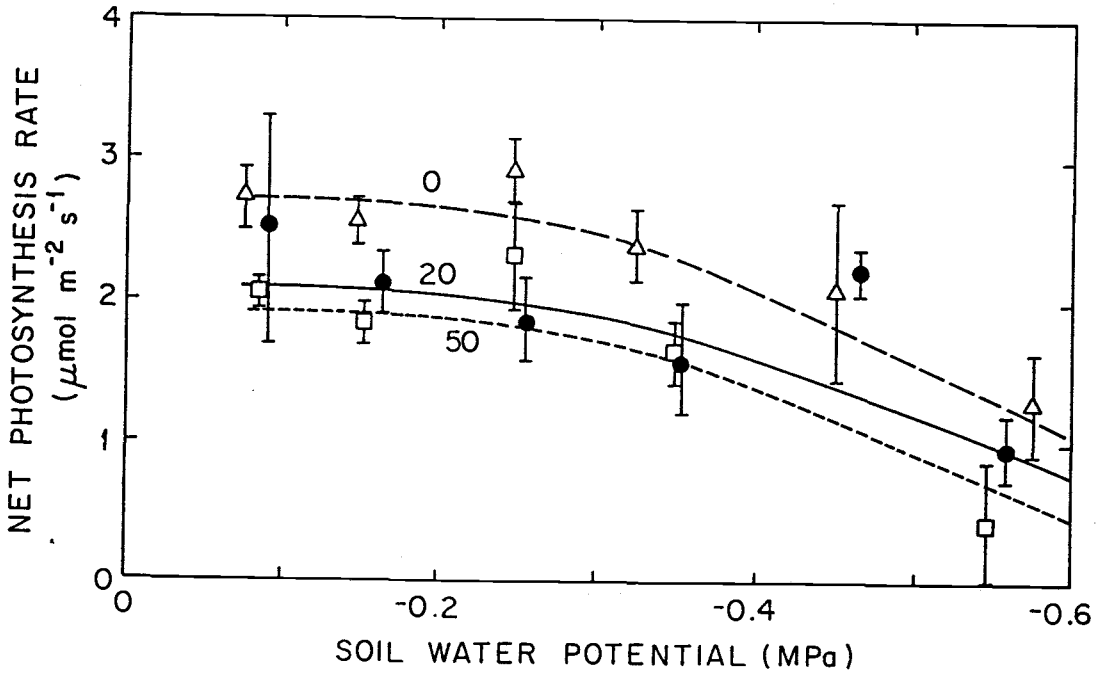


Figure 16. Net photosynthesis rate as a function of predawn soil water potential for 8-month-old nonmycorrhizal Douglas-fir seedlings fertilized with solutions of 0 (△), 20 (●), and 50 (□) mg P kg^{-1}. Net photosynthesis rate was measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

photosynthesis rate (Figure 15) in spite of significant effect on leaf P concentration (see Table 14). However, P fertilization tended to depress net photosynthesis rate of 8 month old seedlings over the entire range of soil water potential from about -0.1 to -0.6 MPa. Figure 16 shows that nonmycorrhizal plants which received no supplemental P fertilizer had a net photosynthesis rate of about 1.3X that measured on those which did receive supplemental P fertilizer. Withholding P fertilizer tended to have a similar effect on Rhizopogon-colonized seedlings (Figures 17 and 18). For 6 month old Rhizopogon-colonized seedlings in wet soil, net photosynthesis rate for those receiving no fertilizer P tended to be higher late in the day ($p=.05$, Figure 17), and at 8 months old, withholding P fertilizer resulted in enhanced net photosynthesis rate at soil water potentials below about -0.3 MPa (Figure 18). This occurred in spite of similar leaf nutrient concentrations (Table 14) and identical levels of apparent colonization (Table 13). This apparent decrease in net photosynthesis rate in response to P fertilization correlates with higher leaf P and K concentration which is opposite of what was expected, and lower leaf N (Table 14) which is consistent with expectations, although none of the differences in leaf N were statistically significant ($p=.05$). Based upon these correlations, it appears that net photosynthesis might have been limited to some degree by leaf N concentration in these experiments.

In all treatments, there was a large decrease in net photosynthesis rate per unit leaf area of seedlings from 6 months old to 8 months old. Since preliminary experiments clearly showed that net photosynthesis rate does not change over a soil dryness range of nearly saturated to at least as dry as -0.15 MPa soil water potential, net photosynthesis rates of 8-month-old seedlings measured late in the day at higher soil water potentials are directly comparable to values for 6-month-old seedlings measured at the same time of day under wet soil conditions. This comparison, shown in Table 21, reveals that net photosynthesis rate per unit leaf area for seedlings in wet soil decreased between 23 to 51% over this 2 month period. Since the measurement technique was the same throughout these experiments, and

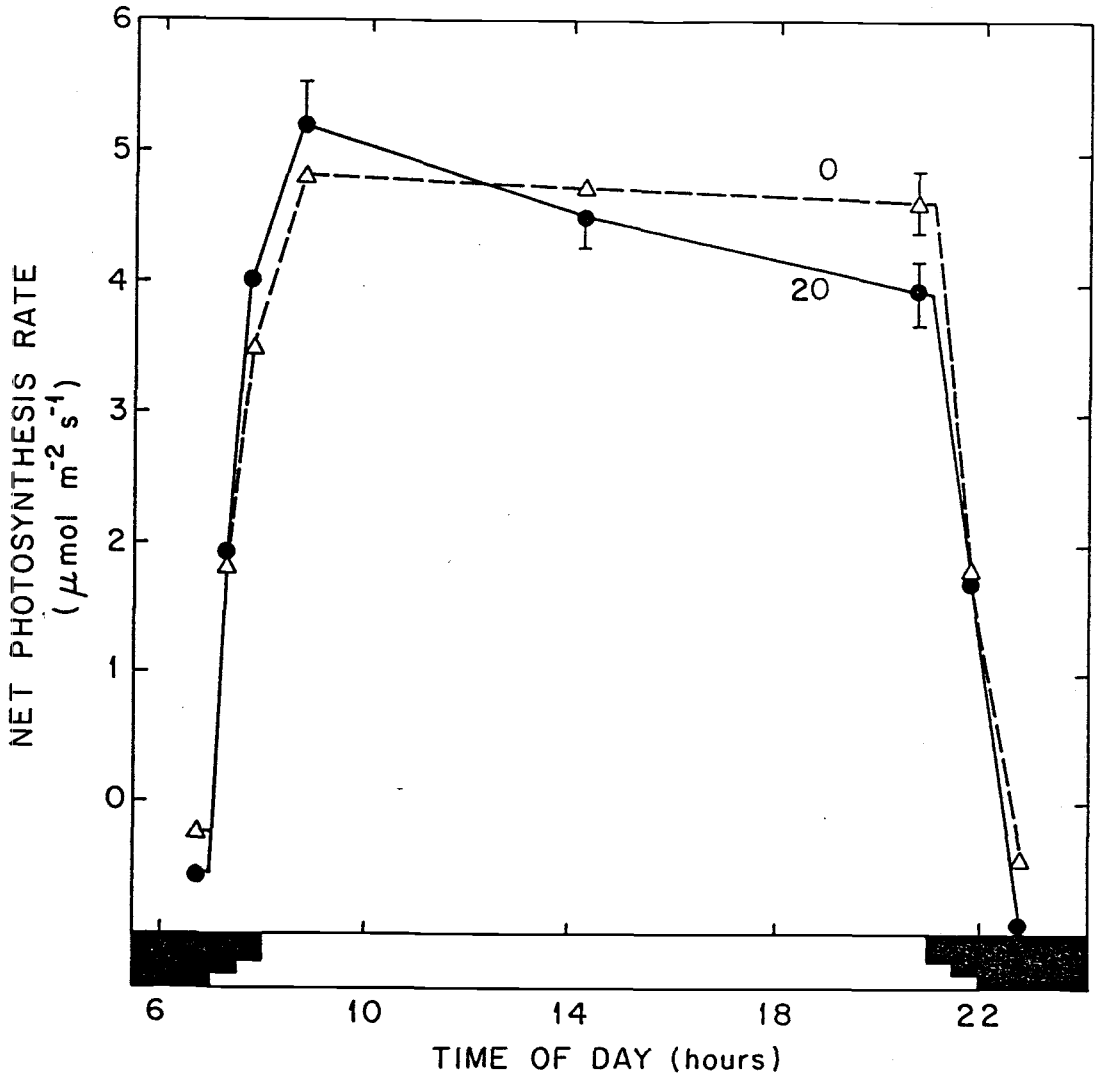


Figure 17. Net photosynthesis rate measured periodically over the course of one day under well-watered soil conditions for 6-month-old Rhizopogon vinicolor-colonized Douglas-fir seedlings fertilized with solutions of 0 (△) and 20 (●) mg P kg^{-1}.

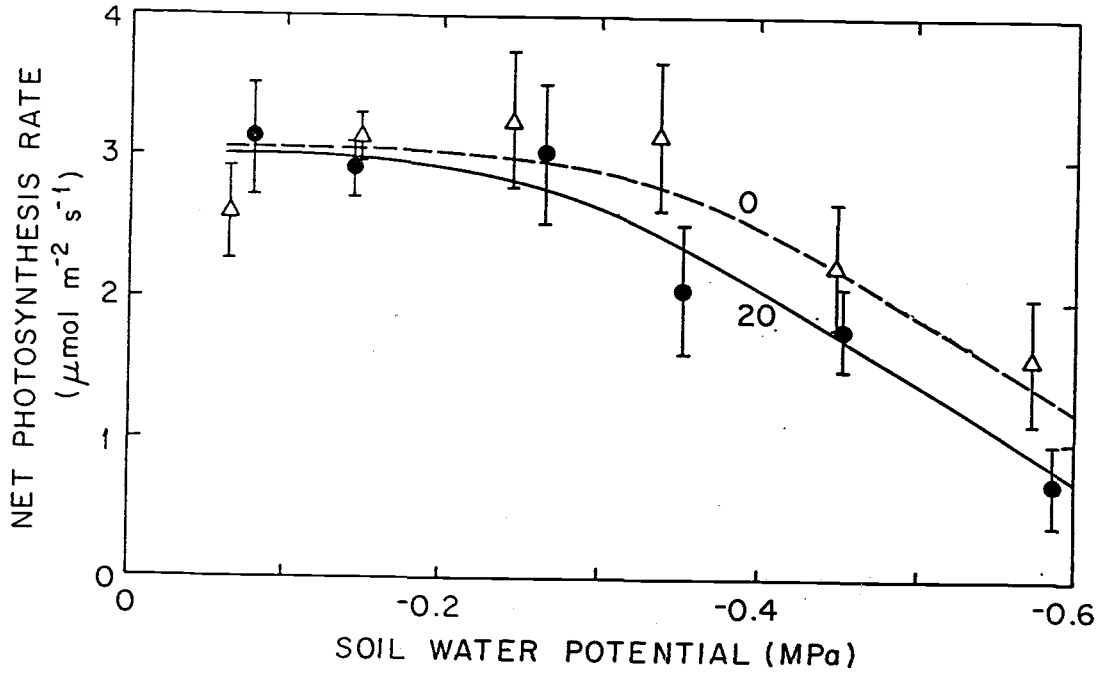


Figure 18. Net photosynthesis rate as a function of predawn soil water potential for 8-month-old Rhizopogon vinicolor-colonized Douglas-fir seedlings fertilized with solutions of 0 (△) and 20 (●) mg P kg^{-1}. Net photosynthesis rate was measured late in the day at about 1930 hours. The points are means and standard errors, and the lines are hand-fit to the data.

Table 21. Net photosynthesis rate of Douglas-fir seedlings under well-watered conditions at 6 months old and at 8 months old, and the relative change in rate from 6 months old to 8 months old, for the indicated mycorrhizal fungus treatment, fertilizer P concentration, and seedling age when transplanted. Relative change is expressed as the net photosynthesis rate at 8 months old divided by that at 6 months old. Net photosynthesis rate was measured late in the day at about 1930 hours.

Treatment			Net photosynthesis rate		
Age transplanted	Fungal treatment	Fertilizer P conc.	6 months	8 months	Relative change
(weeks)		(mg kg^{-1})	(nmol m^{-2} s^{-1})	(nmol m^{-2} s^{-1})	
6	NM[a]	0	3925 ± 251[b]	2700 ± 186[c]	.69
		20	4130 ± 315	2100 ± 249	.51
		50	3944 ± 248	1950 ± 184	.49
	Rv	0	4659 ± 233	3050 ± 199	.65
		20	3920 ± 270	3000 ± 210	.77
8	NM	20	3653 ± 252	2300 ± 144	.63
	Ll	20	3343 ± 243	1800 ± 99	.54
	Hc	20	3769 ± 194	2500 ± 346	.66

[a] fungal treatment abbreviations: NM, nonmycorrhizal; Rv, Rhizopogon vinicolor; Ll, Laccaria laccata; Hc, Hebeloma crustuliniforme

[b] measured mean ± one standard error

[c] estimated mean ± one standard error. Standard error values from data collected in the soil water potential range of -0.10 to -0.19 MPa.

leaf growth was relatively small over this period of time, this change in net photosynthesis rate is real. It appears that some physiological change had taken place in these seedlings between the ages of 6 and 8 months which slowed down the net photosynthesis rate. Seasonal change in net photosynthesis rate is well documented for field grown conifers and correlates with seasonal periods of plant growth and dormancy (Clark, 1961; Helms, 1965; Brix, 1971). Shoot growth dormancy was observed in seedlings used in this study, so the change in net photosynthesis rate observed here might reflect a seasonal-type change in seedling physiology. The magnitude of this decrease shows no consistent correlation with fertilizer or mycorrhizal treatments or with the amount of root growth (Table 20) that occurred over this period of time.

In summary, these results show that Rhizopogon colonization increased net photosynthesis rate, Hebeloma colonization slightly increased it, and Laccaria colonization appeared to reduce it. Supplemental P fertilization tended to decrease net photosynthesis rate. Colonization and P fertilization effects on net photosynthesis rate tended to be greater on 8-month-old seedlings.

Water Potentials

Expected Results

It is well established that for most plants, leaf water potential is lower during the daytime than at night. It normally decreases quickly to a constant level a few hours after the beginning of daylight (Joly, 1984; Al-Omran, 1986), then increases quickly after the lights go off (Al-Omran, 1986). This quick response of leaf water potential to light is a consequence of stomatal opening which increases water loss from the plant. Further decrease in leaf water

potential in response to soil drying results primarily from greater limitation to water uptake by roots (McCoy et al., 1983).

Osmotic potential of the leaf symplast should follow a diurnal trend similar to that for leaf water potential. Al-Omran (1984) showed osmotic potential of sudangrass decreased gradually to a steady level in response to light and then increased gradually after the lights went off. Joly (1984) showed a similar diurnal trend in Douglas-fir seedlings but the diurnal fluctuation was small, being only 0.2 to 0.3 MPa. Under increasing drought conditions, Joly (1984) found that osmotic potential of Douglas-fir seedlings remained relatively unchanged. Thus, it was expected that osmotic potential would not change much in these experiments. Since changes in osmotic potential results primarily from changes in the rate of photosynthetic production of sugars relative to their export rate to other parts of the plant (Turner and Jones, 1980), the lack of response to drought in Douglas-fir might result from rates of photosynthetic production and phloem translocation being affected to similar degrees by plant water stress.

Leaf cell turgor is the difference between leaf water potential and leaf symplast osmotic potential. Since it was expected that leaf water potential and osmotic potential would change similarly during the day, but leaf water potential would decrease much more than osmotic potential in response to daylight, leaf turgor was expected to follow a similar diurnal trend as leaf water potential. Turgor should decrease rapidly to a constant level in response to daylight and increase quickly in response to darkness. Turgor was also expected to decrease similarly to leaf water potential in response to soil drying.

The influence of plant nutrition on leaf water potential, symplast osmotic potential and turgor have received almost no attention in the literature. Radin and Parker (1979a) studied the influence of fertilization on osmoregulation in cotton, but could only suggest from their data that N nutrition had little effect. There are no other studies which directly address this question. Since water potentials throughout the plant are a result of balances between many processes in which mineral nutrients take part, such as root

permeability, stomatal behavior, photosynthesis and translocation, it is impossible to predict the effect of changing nutrition on these water potentials.

Only two studies have been made comparing water potentials of ectomycorrhizal conifers to those of nonmycorrhizal plants. In both of these studies, ectomycorrhizal seedlings had similar leaf water potential to nonmycorrhizal controls under wet soil conditions (Sands and Theodoreau, 1978; Parke et al., 1983). Under water-limiting soil conditions, Sands and Theodoreau (1978) measured lower leaf water potential in ectomycorrhizal pine seedlings than in nonmycorrhizal seedlings. No studies have been made of osmotic potentials or leaf cell turgor pressure of plants in response to ectomycorrhizal colonization. Some clues may be derived from several studies of crop plants and grasses colonized by VA mycorrhizae-forming fungi. Most of these studies show that VA mycorrhizal colonization did not affect leaf water potential compared to nonmycorrhizal controls in wet soil (Allen et al., 1981; Allen, 1982; Allen and Boosalis, 1983; Huang et al., 1984; Koide, 1985), in wet soil after a period of drought stress (Levy and Krikun, 1980; Allen and Boosalis, 1983), or under dry soil conditions (Allen et al., 1981; Stahl and Smith, 1984). However, there were exceptions to this observation (Nelson and Safir, 1982a; Sweatt and Davies, 1984). The only study of leaf osmotic potential response to VA mycorrhizal colonization showed no effect for well-watered plants, and lower osmotic potential compared to nonmycorrhizal controls for only one species of fungus out of two species tested under dry soil conditions (Allen and Boosalis, 1983). Leaf turgor of VA mycorrhizal plants has not been studied. Based upon all of this evidence, it is unlikely that ectomycorrhizae will significantly affect leaf water potential, osmotic potential or turgor pressure in Douglas-fir seedlings.

Results

Treatments were compared in 6 different combinations in order to determine the nature of the effect of ectomycorrhizae, P fertilizer level, and transplant age on the seedlings. These comparisons were outlined in the "METHODS" section covering "Statistical Design and Data Analysis", and are briefly reviewed here:

The effect of seedling age when transplanted:

1) The nonmycorrhizal treatments, 2 and 6 (see Table 6 for reference numbers), were used to exemplify "typical" seedling behavior, and to test for effect of transplant age on seedlings.

The effect of ectomycorrhizal colonization:

2) Hc and Ll (treatments 7 and 8) were compared with their nonmycorrhizal control, treatment 6, for moderate soil P levels.
3) Rv treatment 5 was compared with its nonmycorrhizal control, treatment 2, for moderate soil P levels.
4) Rv treatment 4 was compared to its nonmycorrhizal control, treatment 1, for seedlings where fertilizer P withheld.

The effect of P fertilization level:

5) For nonmycorrhizal seedlings, high P level (treatment 3) and the low P level (where fertilizer P was withheld, treatment 1) were compared with moderate P level (treatment 2).
6) For Rv, low P level (where fertilizer P was withheld, treatment 4) was compared with moderate P level (treatment 5).

In this section, comparisons are made in the above order. Furthermore, for each comparison made, 2 graphs are presented. The diurnal trend of water potentials for 6-month-old seedlings growing in wet soil is described first, followed immediately by the relationship between water potentials and predawn soil water potential found for 8-month-old seedlings.

Typical diurnal trends of leaf water potential, leaf symplast osmotic potential, and leaf turgor pressure in this experiment are shown in Figure 19. Leaf water potential declined rapidly, from about -0.5 MPa as the lights came on, to a steady level of about -1.0 MPa over most of the day, then increased rapidly as the lights went off. Leaf symplast osmotic potential generally decreased by about .3 MPa over the course of the daytime in these experiments. In most cases, osmotic potential rose between 0.1 and 0.2 MPa initially, then decreased steadily through the day. After the lights went off, osmotic potential would sometimes continue downward for at least 1.5 hours before rising again to predawn levels. Leaf cell turgor was calculated as the difference between measured values of leaf water potential and leaf symplast osmotic potential. In these experiments, leaf turgor decreased rapidly over about 2 hours after the lights came on, then rose gradually during the day. After the lights went off, turgor rose rapidly to levels 0.1 to 0.2 MPa higher than these measured at predawn. Leaf water potentials, leaf symplast osmotic potentials, and leaf turgor pressures found under well-watered conditions in this experiment are similar to those found for Douglas-fir seedlings in other studies (Parke et al., 1983; Joly, 1984).

The typical trend of leaf water potential, leaf symplast osmotic potential, and leaf turgor pressure in relation to soil water potential in this experiment are shown in Figure 20. Water potential of the bulk soil was measured at predawn, while leaves were measured late in the day. In this Figure, the points represent means and their standard errors of observations made within each 0.1 MPa segment of the soil water potential range. Each line is a hand fit curve representing all of the data. The details of the technique used to fit the curves are described in the "METHODS" section on "Statistical

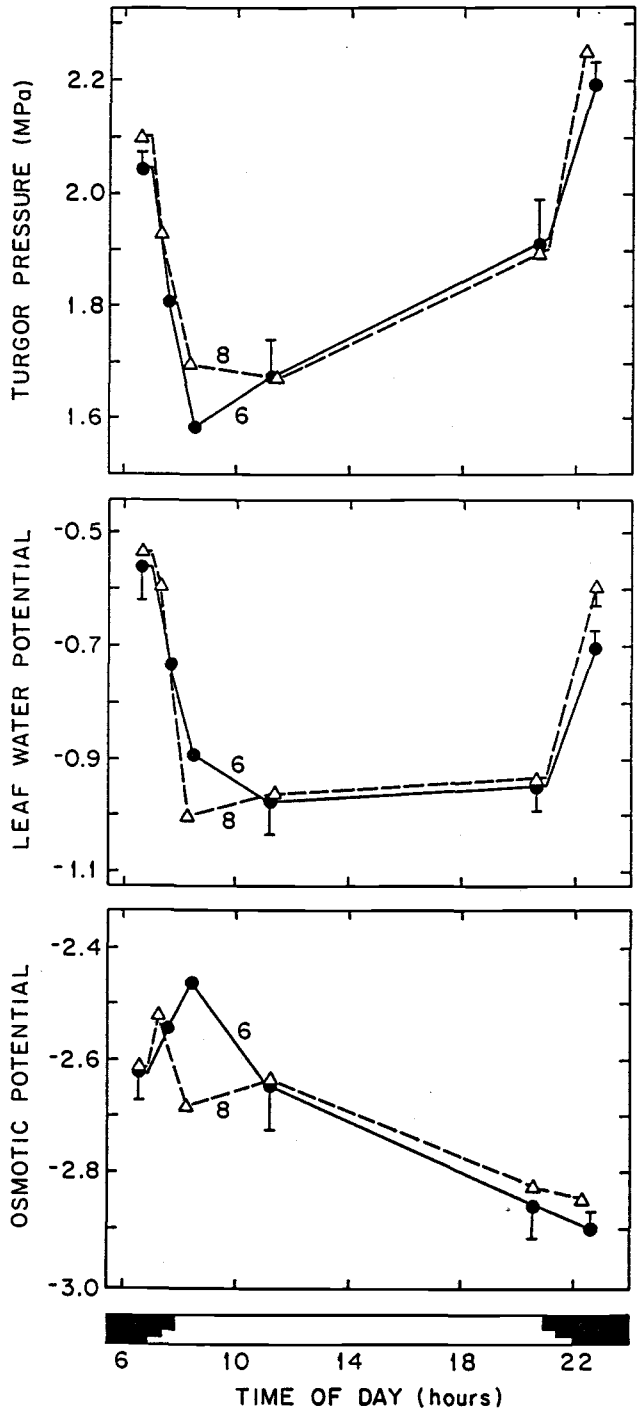


Figure 19. Leaf water potential, leaf symplast osmotic potential, and leaf turgor pressure measured periodically over the course of one day under well-watered soil conditions for 6-month-old nonmycorrhizal Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: transplanted at 6 weeks old (●); transplanted at 8 weeks old (△).

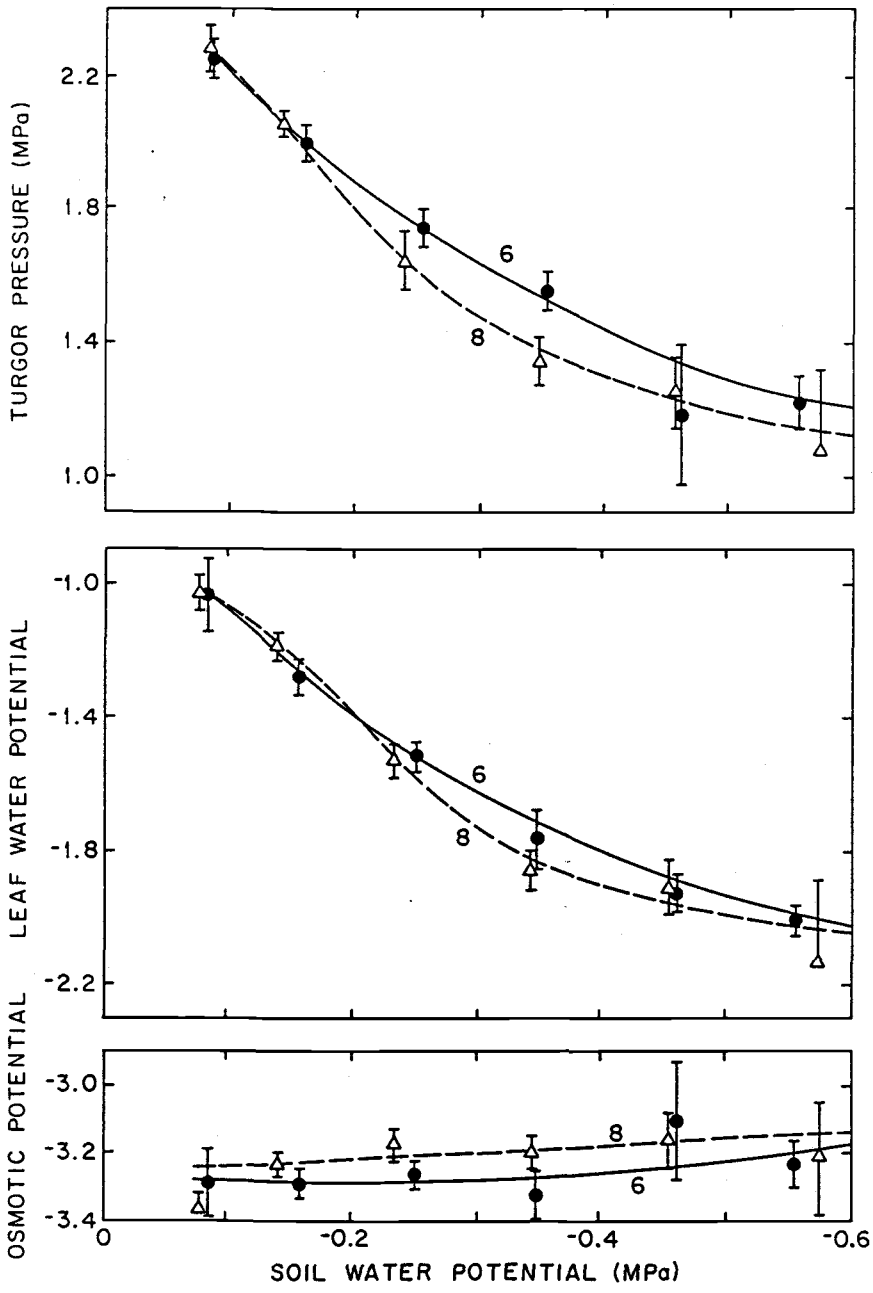


Figure 20. Leaf water potential, leaf symplast osmotic potential, and leaf turgor pressure as a function of predawn soil water potential for 8-month-old nonmycorrhizal Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: transplanted at 6 weeks old (●); transplanted at 8 weeks old (△). Water potentials were measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

Design and Data Analysis". Leaf water potential decreased steadily as the soil water potential decreased from about -0.075 to about -0.4 MPa and then began to level off. Leaf symplast osmotic potential tended to decrease, but only by about .1 MPa over the soil water potential range of -0.075 to -0.6 MPa shown here. Since osmotic potential changed very little relative to soil water potential, turgor decreased in a manner similar to that of leaf water potential as the soil water potential decreased.

The influence of ectomycorrhizal colonization on leaf water potential, leaf symplast osmotic potential, and leaf turgor pressure is shown in Figures 21 through 26. The general trends for mycorrhizal seedlings follow those described above for typical uncolonized seedlings. However, the magnitudes are influenced by colonization. Laccaria colonization had no effect on the daily cycle of leaf water potential, osmotic potential, or turgor pressure of 6-month-old seedlings under wet soil conditions (Figure 21). However, under drier soil conditions, 8-month-old Laccaria-colonized seedlings showed a clearer tendency toward higher turgor by about 0.1 MPa in soil dryer than about -0.25 MPa soil water potential, in spite of apparently negligible effect on leaf water and symplast osmotic potentials(Figure 22). On the other hand, Hebeloma colonization resulted in up to 0.2 MPa higher leaf water potential and up to 0.3 MPa higher symplast osmotic potential than both 6-month-old nonmycorrhizal and Laccaria-colonized seedlings under well-watered conditions, which resulted in somewhat lower turgor over the course of the entire day (Figure 21). Under drier soil conditions, leaf water potential of 8-month-old Hebeloma-colonized seedlings was not different from nonmycorrhizal and Laccaria-colonized seedlings, but symplast osmotic potential was clearly higher indicating lower turgor by about 0.1 to 0.2 MPa over the soil water potential range of about -0.05 to -0.6 MPa (Figure 22). Where fertilizer P was applied, Rhizopogon colonization resulted in lower leaf water potentials than nonmycorrhizal controls by as much as 0.15 MPa and similar or higher symplast osmotic potentials resulting in generally lower leaf turgor in well-watered 6-month-old seedlings (Figure 23). Under dryer soil conditions,

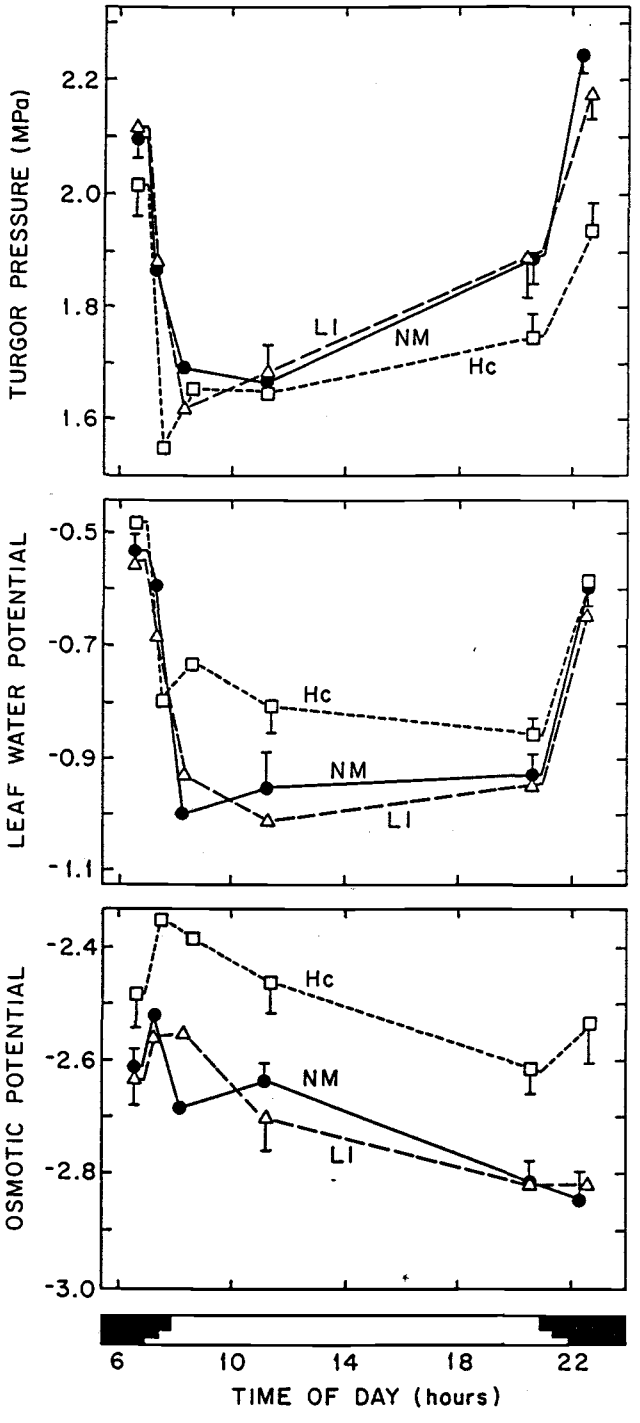


Figure 21. Leaf water potential, leaf symplast osmotic potential, and leaf turgor pressure measured periodically over the course of one day under well-watered soil conditions for 6-month-old Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: nonmycorrhizal (NM, ●); colonized by Laccaria laccata (Ll, △); colonized by Hebeloma crustuliniforme (Hc, □).

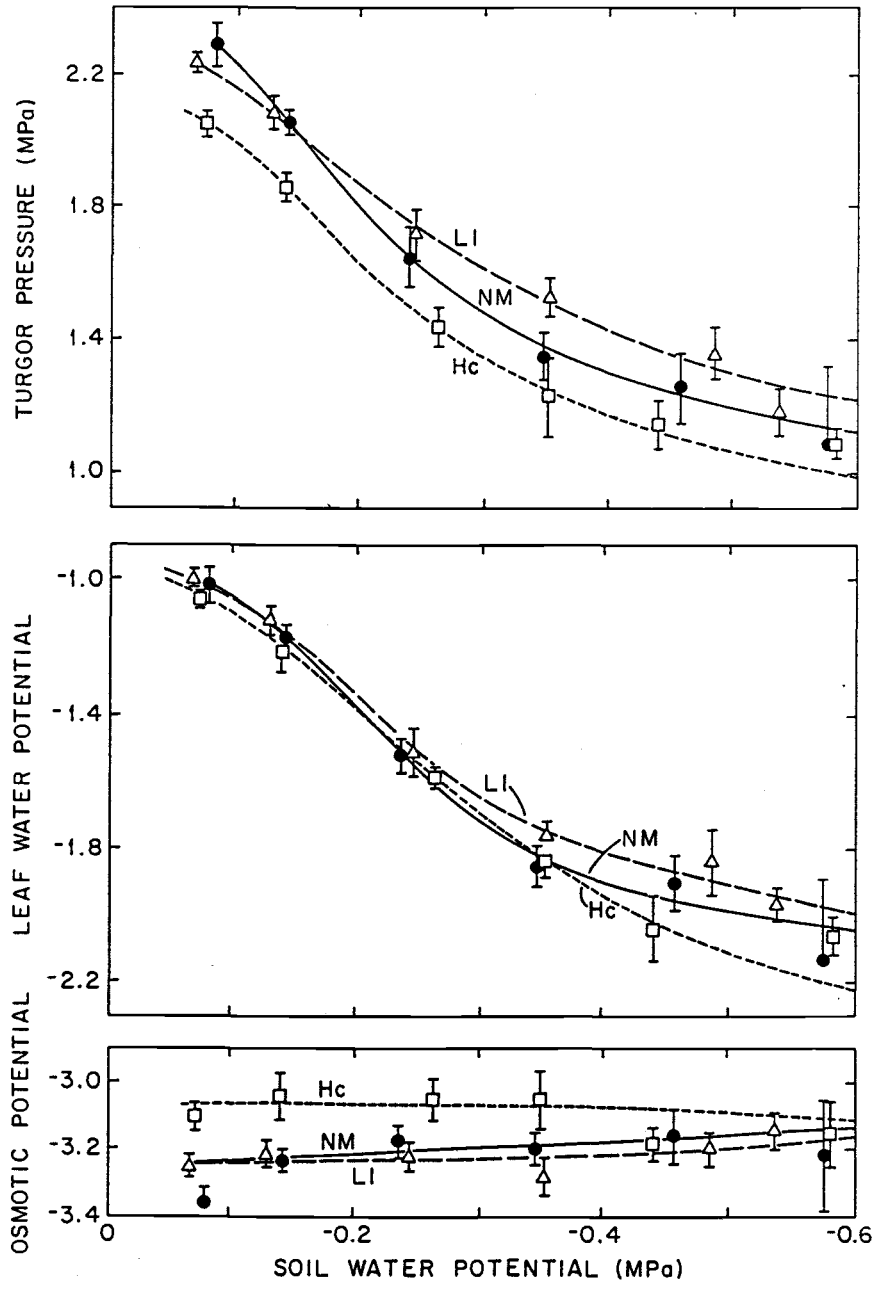


Figure 22. Leaf water potential, leaf symplast osmotic potential, and leaf turgor pressure as a function of predawn soil water potential for 8-month-old Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: nonmycorrhizal (NM, ●); colonized by Laccaria laccata (Ll, △); colonized by Hebeloma crustuliniforme (Hc, □). Water potentials were measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

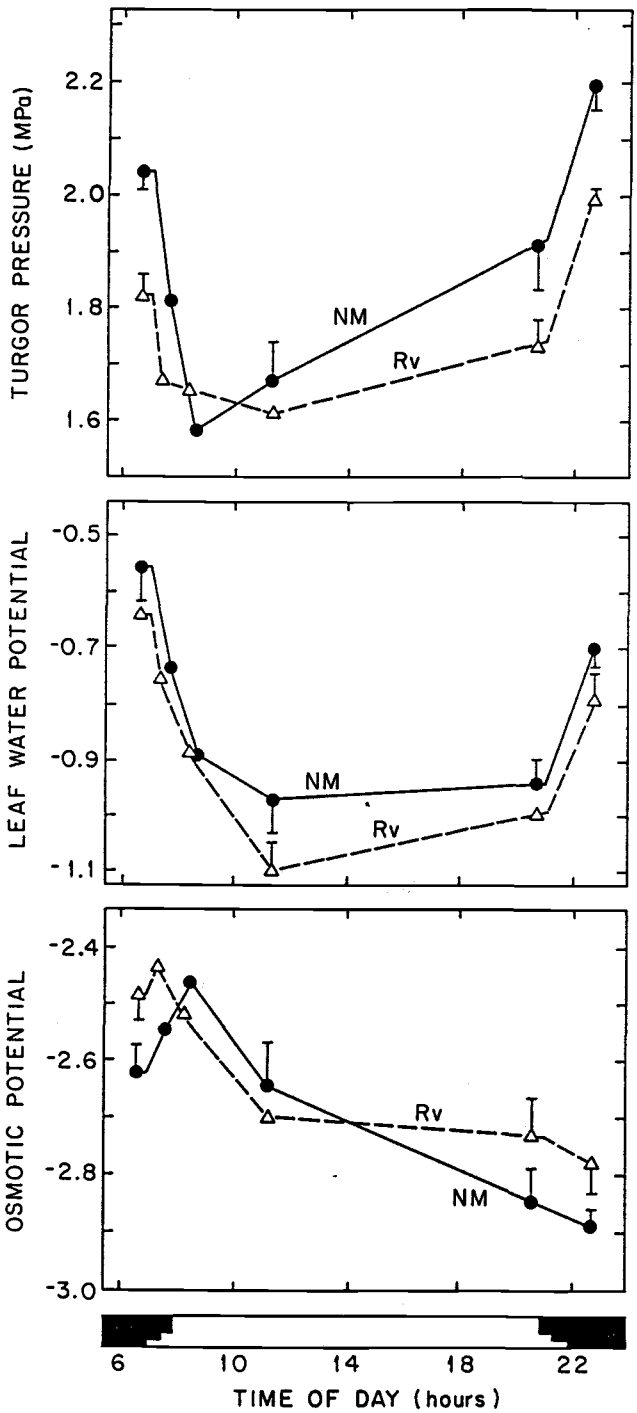


Figure 23. Leaf water potential, leaf symplast osmotic potential, and leaf turgor pressure measured periodically over the course of one day under well-watered soil conditions for 6-month-old Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: nonmycorrhizal (NM, ●); colonized by Rhizopogon vinicolor (Rv, △).

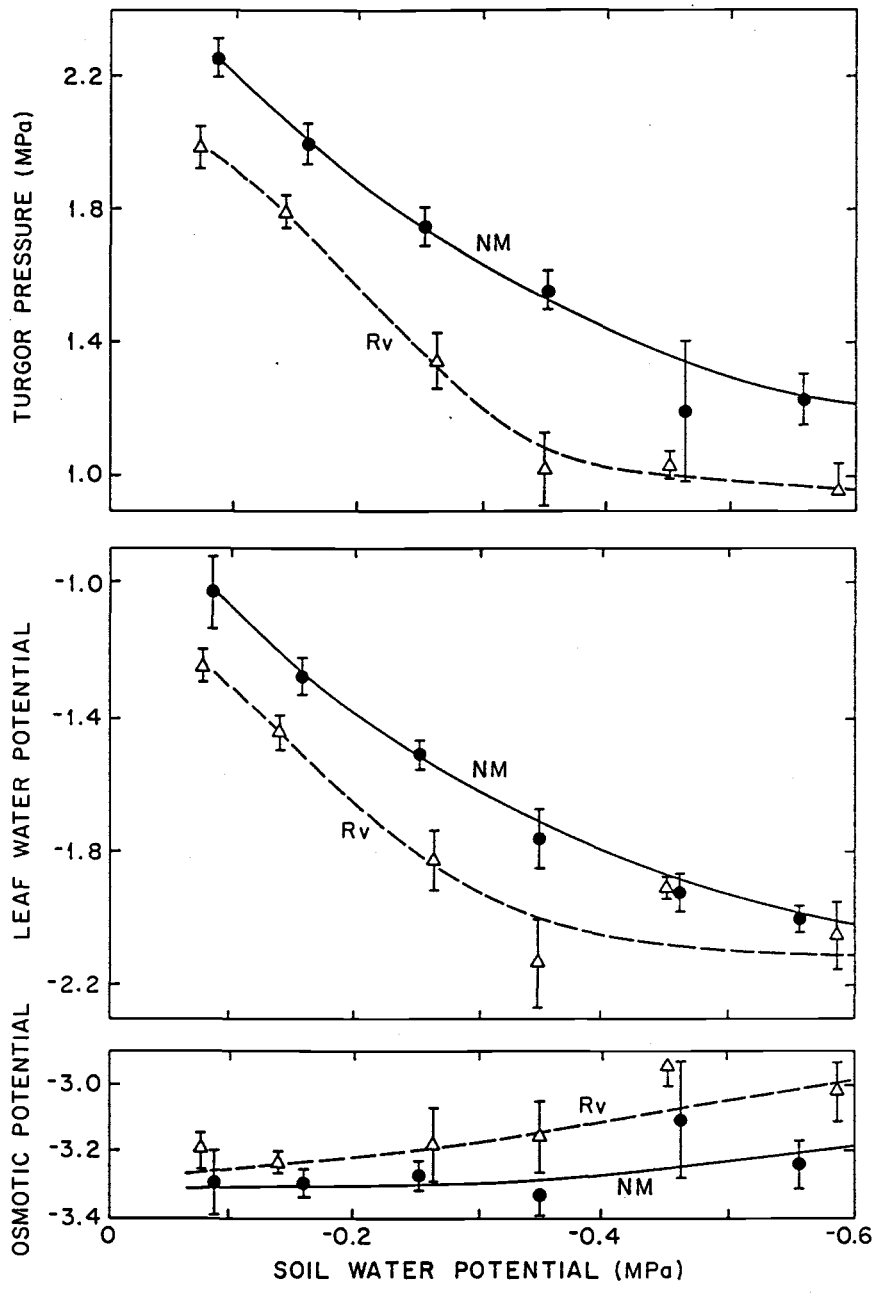


Figure 24. Leaf water potential, leaf symplast osmotic potential, and leaf turgor pressure as a function of predawn soil water potential for 8-month-old Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: nonmycorrhizal (NM, ●); colonized by Rhizopogon vinicolor (Rv, △). Water potentials were measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

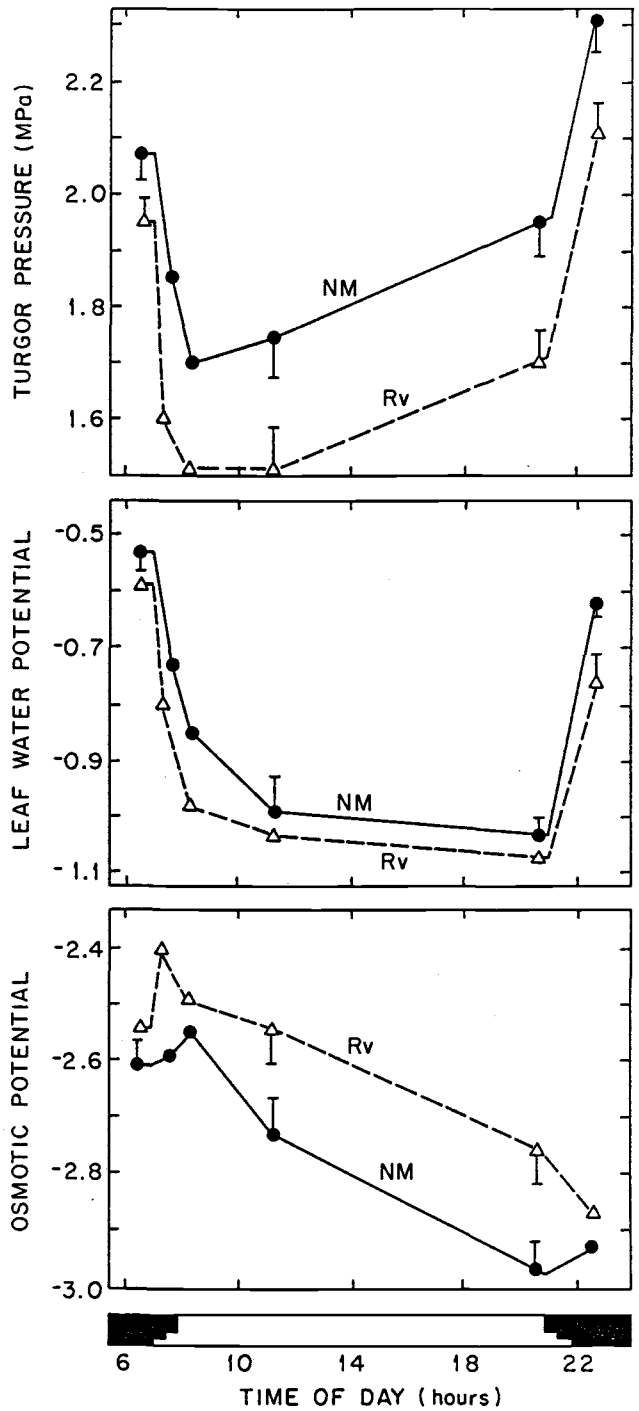


Figure 25. Leaf water potential, leaf symplast osmotic potential, and leaf turgor pressure measured periodically over the course of one day under well-watered soil conditions for 6-month-old Douglas-fir seedlings from which P fertilizer was withheld: nonmycorrhizal (NM, ●); colonized by Rhizopogon vinicolor (Rv, △).

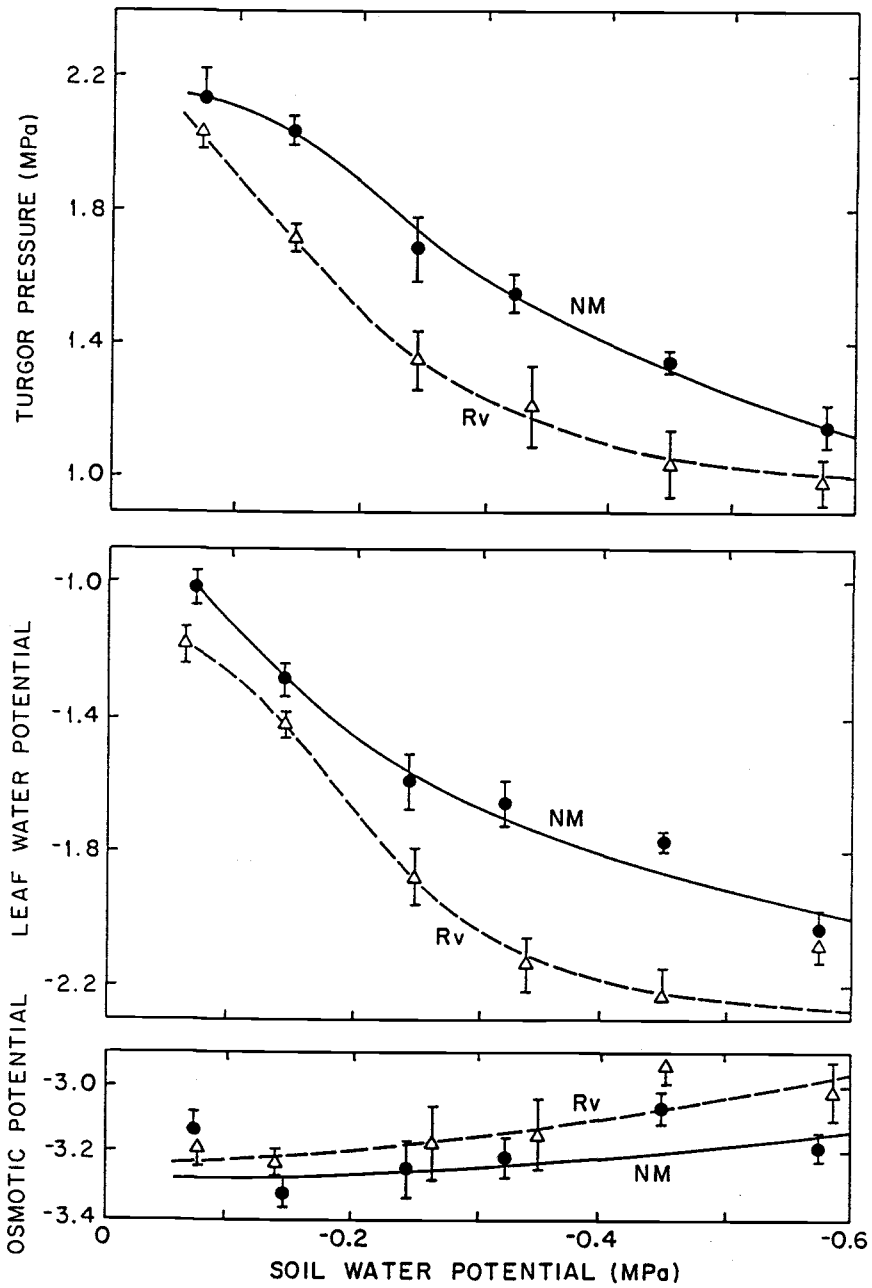


Figure 26. Leaf water potential, leaf symplast osmotic potential, and leaf turgor pressure as a function of predawn soil water potential for 8-month-old Douglas-fir seedlings from which P fertilizer was withheld: nonmycorrhizal (NM, ●); colonized by Rhizopogon vinicolor (Rv, △). Water potentials were measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

8-month-old Rhizopogon-colonized seedlings clearly had lower leaf water potentials than nonmycorrhizal controls by 0.2 to 0.3 MPa, and higher symplast osmotic potential, by 0.07 to 0.2 MPa, resulting in much lower turgor by 0.3 to 0.4 MPa over the entire soil water potential range of -0.05 to -0.6 MPa (Figure 24). Where fertilizer P was withheld, the effect of Rhizopogon colonization to lower leaf water potential, increase osmotic potential and reduce turgor pressure of 6-month-old seedlings was more exaggerated than where fertilizer P was applied (Figure 25), but similar to that where fertilizer P was applied for 8-month-old seedlings under drier conditions (Figure 26).

The influence of P fertilization on leaf water potential, leaf symplast osmotic potential, and leaf turgor pressure are shown in Figures 27 through 30. For 6-month-old nonmycorrhizal seedlings under well-watered conditions, there were no clear differences between fertilizer P treatments that were consistent with their ranking in terms of level of P fertilizer (Figure 27). There were also no statistically significant differences ($p=.05$) between any of these treatments at any time of day. The only apparent trends which were consistent with ranking in terms of the level of P fertilizer were lower leaf water potential during the daytime and higher turgor in darkness for seedlings from which P fertilizer was withheld. Under drier soil conditions, there were no differences in leaf water potential, symplast osmotic potential nor turgor for 8-month-old seedlings as the soil water potential decreased from about -0.075 MPa to -0.6 MPa (Figure 28). Phosphorus fertilization did not result in no clear water potential changes in Rhizopogon-colonized seedlings. The only notable trend for 6-month-old seedlings under wet soil conditions was lower turgor in predawn and evening darkness for seedlings which were fertilized with supplemental P (Figure 29). This difference was statistically significant at predawn ($p=.05$). Under drier soil conditions, 8-month-old Rhizopogon-colonized seedlings fertilized at different P levels did not exhibit differences in leaf water potential, symplast osmotic potential or turgor as the soil water potential decreased from about -0.05 to -0.6 MPa (Figure 30).

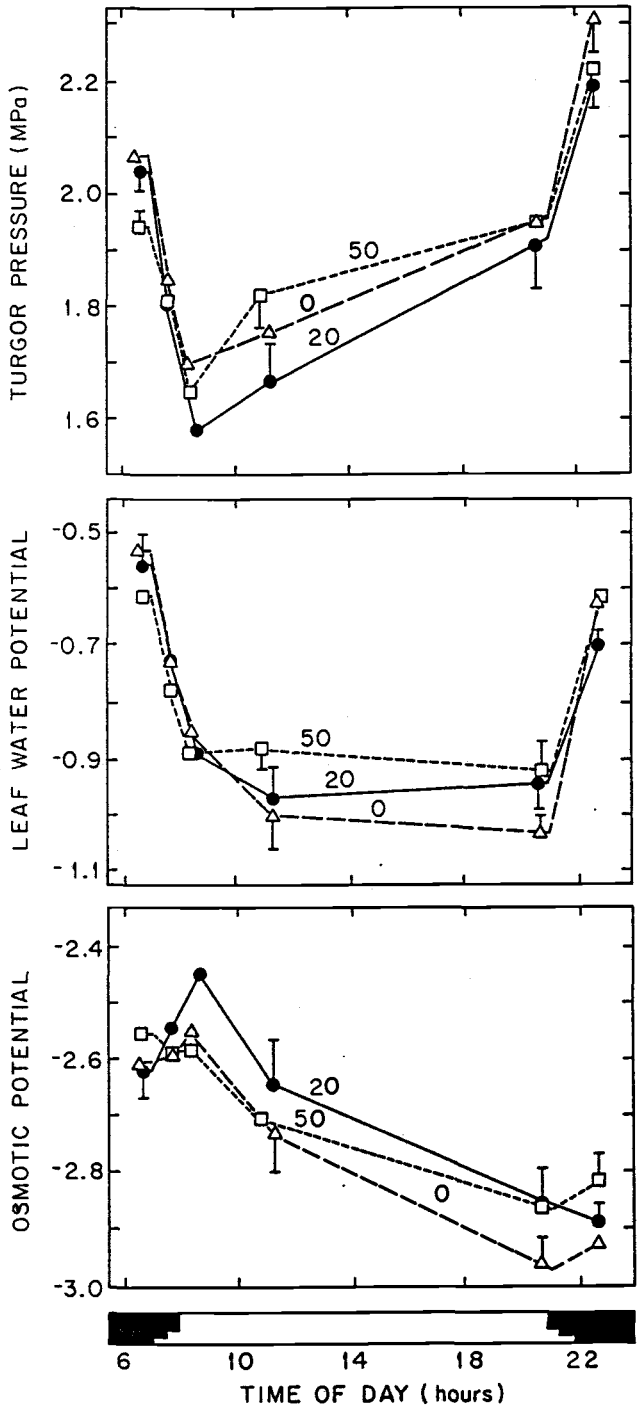


Figure 27. Leaf water potential, leaf symplast osmotic potential, and leaf turgor pressure measured periodically over the course of one day under well-watered soil conditions for 6-month-old nonmycorrhizal Douglas-fir seedlings fertilized with solutions of 0 (△), 20 (●), and 50 (□) mg P kg^{-1}.

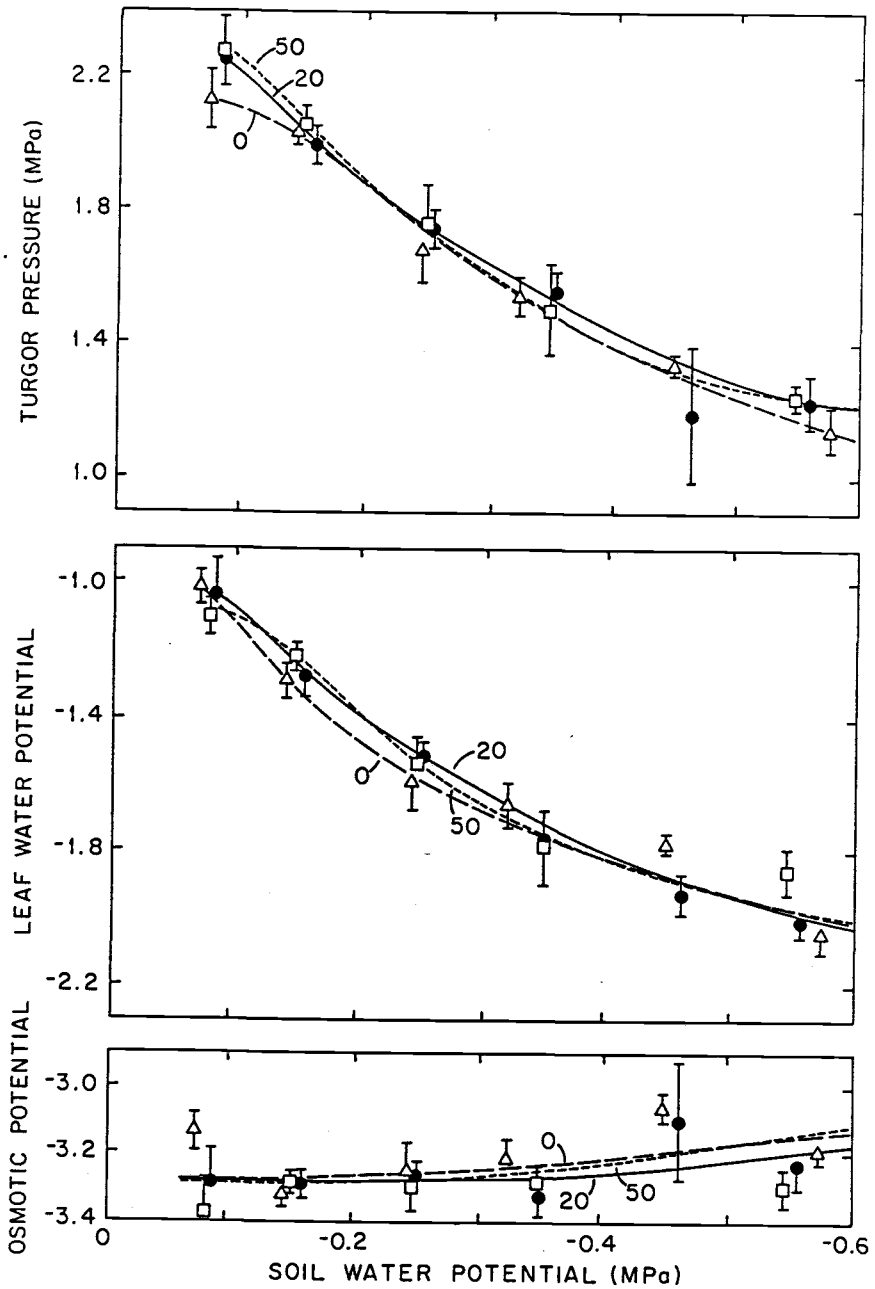


Figure 28. Leaf water potential, leaf symplast osmotic potential, and leaf turgor pressure as a function of predawn soil water potential for 8-month-old nonmycorrhizal Douglas-fir seedlings fertilized with solutions of 0 (△), 20 (●), and 50 (□) mg P kg^{-1}. Water potentials were measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

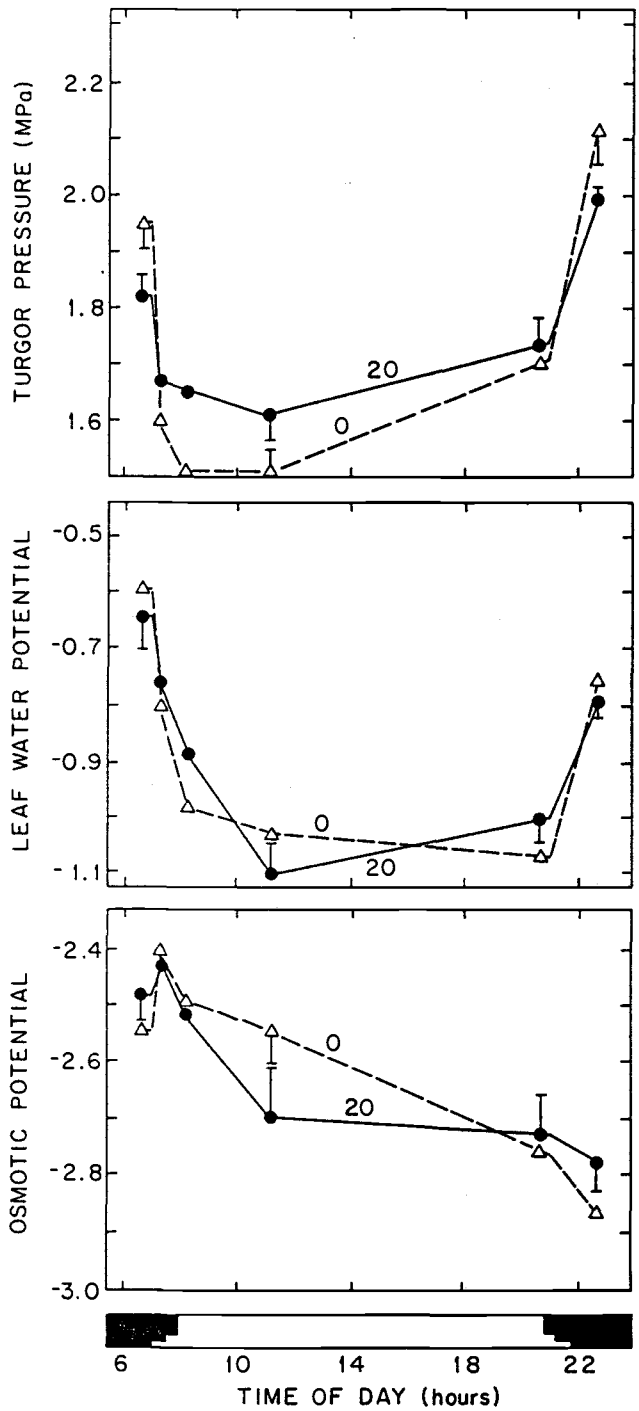


Figure 29. Leaf water potential, leaf symplast osmotic potential, and leaf turgor pressure measured periodically over the course of one day under well-watered soil conditions for 6-month-old Rhizopogon vinicolor-colonized Douglas-fir seedlings fertilized with solutions of 0 (△) and 20 (●) mg P kg^{-1}.

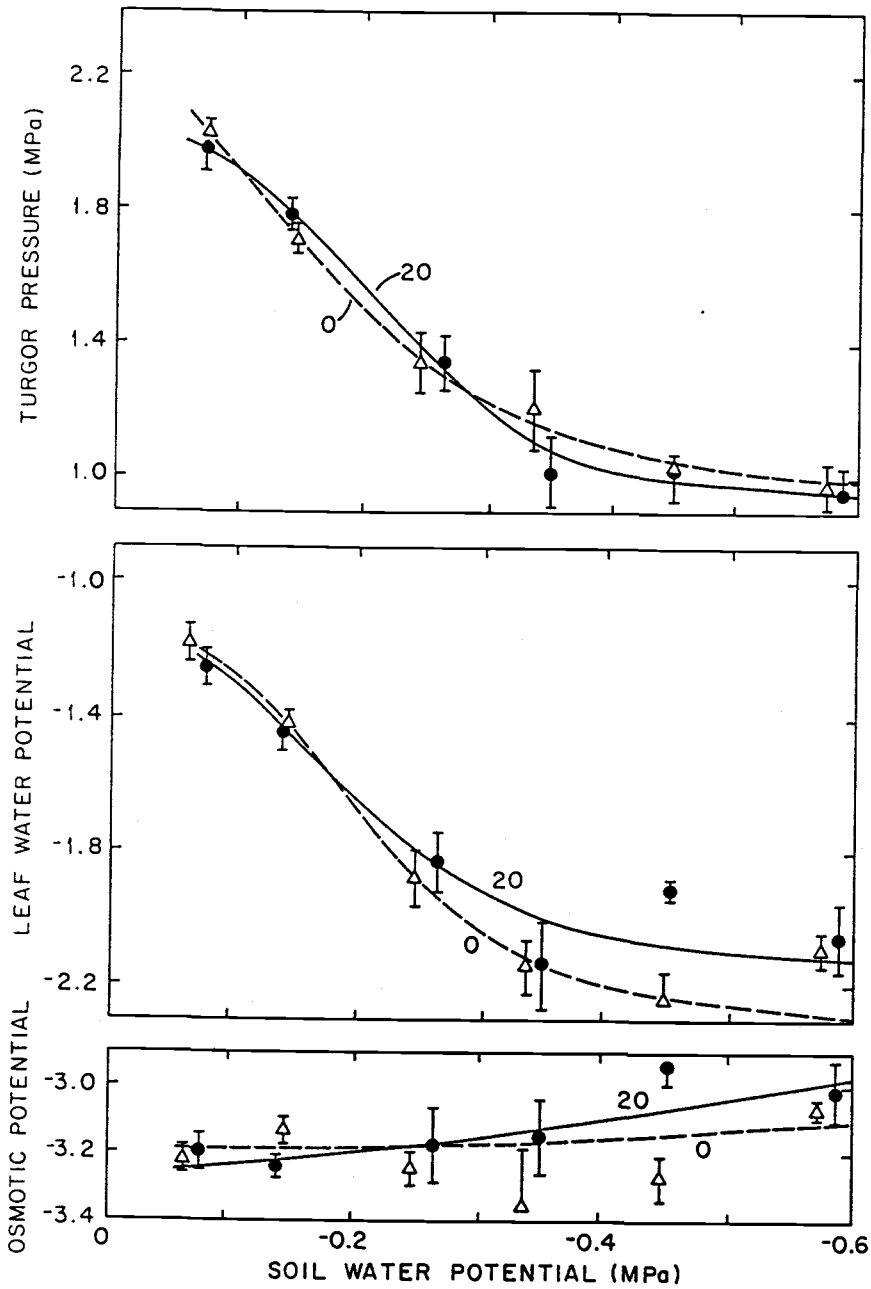


Figure 30. Leaf water potential, leaf symplast osmotic potential, and leaf turgor pressure as a function of predawn soil water potential for 8-month-old Rhizopogon vinicolor-colonized Douglas-fir seedlings fertilized with solutions of 0 (△) and 20 (●) mg P kg^{-1}. Water potentials were measured late in the day at about 1930 hours. The points are means and standard errors, and the lines are hand-fit to the data.

Stomatal Conductance and Transpiration Rate

Expected Results

It is well-established that stomata open in response to light and close in response to darkness. The opening response is usually quite rapid, requiring only a matter of several minutes (Meidner and Mansfield, 1968). Thus, under well-watered conditions, stomatal conductance is expected to increase rapidly when the lights come on and then decrease when the lights go off.

It is also well-established that stomatal conductance decreases in response to lower soil soil water potential, primarily through mechanisms associated with lower leaf water potential. Other conditions being equal, stomatal aperture decreases in response to lower leaf water potential, but there is a range of higher leaf water potentials over which there is no effect on stomata (Beadle et al., 1978; Ludlow, 1980). Thus, as the soil water potential decreases, stomatal conductance is expected to remain steady at first, and then decline after some limiting leaf water potential is reached.

Transpiration rate closely follows the stomatal conductance in these experiments. Transpiration rate is a function of stomatal conductance as well as boundary layer conductance and relative humidities in the stomatal cavity and the ambient atmosphere. Since in this experiment, boundary layer conductance and relative humidities were nearly constant, transpiration was influenced by light and soil water potential in the same manner as stomatal conductance.

The influence of mycorrhizal colonization on stomatal conductance have already been reviewed in the "Literature Review" section. Only a few pertinent points will be covered here. Most relevant studies report higher stomatal conductance in mycorrhizal plants, both VA and ectomycorrhizal, than in nonmycorrhizal controls. Only two of these studies compared ectomycorrhizal and nonmycorrhizal conifers. The data of Sands and Theodoreau (1978) suggest higher stomatal conductance

over a wide range of soil water potential for Pinus radiata seedlings colonized by Rhizopogon luteolus, while Parke et al. (1983) found that Laccaria laccata colonization had no effect and Rhizopogon vinicolor colonization reduced stomatal conductance of Douglas-fir seedlings in wet soil. Based upon these two studies, it appears that no generalization can be drawn about the influence of ectomycorrhizal colonization on stomatal conductance of Douglas-fir seedlings, except that each fungus type has a different effect. Based on the study of Parke et al. (1983) with Douglas-fir seedlings, Rhizopogon vinicolor is expected to reduce stomatal conductance, Laccaria laccata should not greatly effect it, and Hebeloma crustuliniforme could influence stomatal conductance differently than either of these fungi.

Mineral nutrient status of the plant might influence stomatal conductance. Koide (1985) found a correlation between higher leaf P concentration and higher stomatal conductance in sunflower, but only under conditions where low soil availabilty of P limited plant growth. Other studies reviewed in the Literature Review section show that extreme deficiencies of N, P and K correlate with reduced stomatal conductance. These studies suggest that if growth-limiting P deficiency occurs, it is expected that supplemental P fertilization will increase daytime stomatal conductance.

Results

Treatments were compared in 6 different combinations in order to determine the nature of the effect of ectomycorrhizae, P fertilizer level, and transplant age on the seedlings. These comparisons were outlined in the "METHODS" section covering "Statistical Design and Data Analysis", and are briefly reviewed here:

The effect of seedling age when transplanted:

1) The nonmycorrhizal treatments, 2 and 6 (see Table 6 for

reference numbers), were used to exemplify "typical" seedling behavior, and to test for effect of transplant age on seedlings.

The effect of ectomycorrhizal colonization:

2) Hc and Ll (treatments 7 and 8) were compared with their nonmycorrhizal control, treatment 6, for moderate soil P levels.
3) Rv treatment 5 was compared with its nonmycorrhizal control, treatment 2, for moderate soil P levels.
4) Rv treatment 4 was compared to its nonmycorrhizal control, treatment 1, for seedlings where fertilizer P withheld.

The effect of P fertilization level:

5) For nonmycorrhizal seedlings, high P level (treatment 3) and the low P level (where fertilizer P was withheld, treatment 1) were compared with moderate P level (treatment 2).
6) For Rv, low P level (where fertilizer P was withheld, treatment 4) was compared with moderate P level (treatment 5).

In this section, comparisons are made in the above order. Furthermore, for each comparison made, 2 graphs are presented. The diurnal trend of stomatal conductance for 6-month-old seedlings growing in wet soil is described first, followed immediately by the relationship between stomatal conductance and predawn soil water potential found for 8-month-old seedlings.

The typical diurnal trend of stomatal conductance for Douglas-fir seedlings in wet soil in this experiment is shown in Figure 31. As expected, stomatal conductance rose quickly to a maximum in response to lights coming on, and clearly decreased over the course of the day to a level only slightly greater than predawn levels. Stomatal conductance then decreased a little more steeply as the lights were

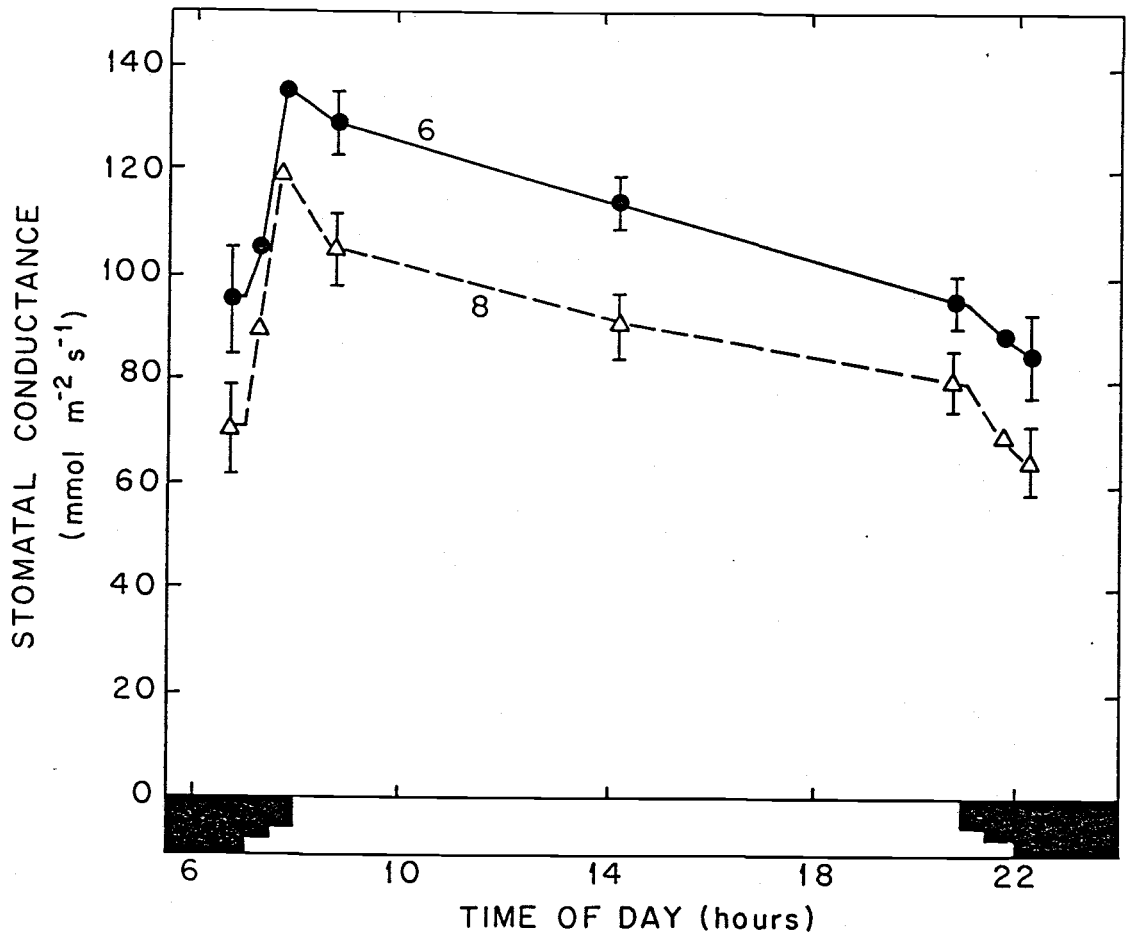


Figure 31. Stomatal conductance measured periodically over the course of one day under well-watered soil conditions for 6-month-old nonmycorrhizal Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: transplanted at 6 weeks old (●); transplanted at 8 weeks old (△).

turned off. In all treatments, the stomatal conductance at predawn was about 60% of the daytime maximum. Preliminary experiments showed that the nighttime minimum of about 40% of the daytime maximum occurred at about 4 hours before dawn. Nocturnal stomatal movements are commonly observed in crop plants, but also observed are much greater degrees of closure in darkness (Meidner and Mansfield, 1968). This rather narrow range of change in stomatal conductance suggests that stomata of Douglas-fir seedlings are only mildly responsive to light and darkness compared to many other kinds of plants.

Although stomatal conductance was not reduced greatly during dark periods, transpiration rate was greatly reduced compared to daytime rates due to much higher nighttime relative humidity (see METHODS section on Experimental Procedures) combined with the partial stomatal closure.

The typical trend of stomatal conductance in relation to soil water potential in this experiment is shown in Figure 32. Soil water potential is of the bulk soil, measured at predawn, while stomatal conductance was measured late in the day. In this figure, the points represent means and their standard errors of observations made within each 0.1 MPa segment of the soil water potential range. Each line is a hand-fit curve representing all of the data. The details of the technique used to fit the curves are described in the "METHODS" section on "Statistical Design and Data Analysis". As expected, this figure shows that there was little response as soil water potential decreased to about -0.15 MPa, then stomatal conductance gradually decreased to near total closure as the soil water potential decreased continued to dry to about -0.6 MPa.

The influence of mycorrhizal colonization on stomatal conductance of Douglas-fir seedlings is shown in Figure 33 through 38. Laccaria laccata colonization tended to lead to greater stomatal conductance in 6-month-old seedlings during the daytime under well-watered soil conditions (Figure 33). However, at 8 months, old stomatal conductances were lower for nonmycorrhizal controls over the soil water potential range -0.05 to -0.4 MPa (Figure 38). Stomatal conductance of 6-month-old Hebeloma colonized seedlings was relatively

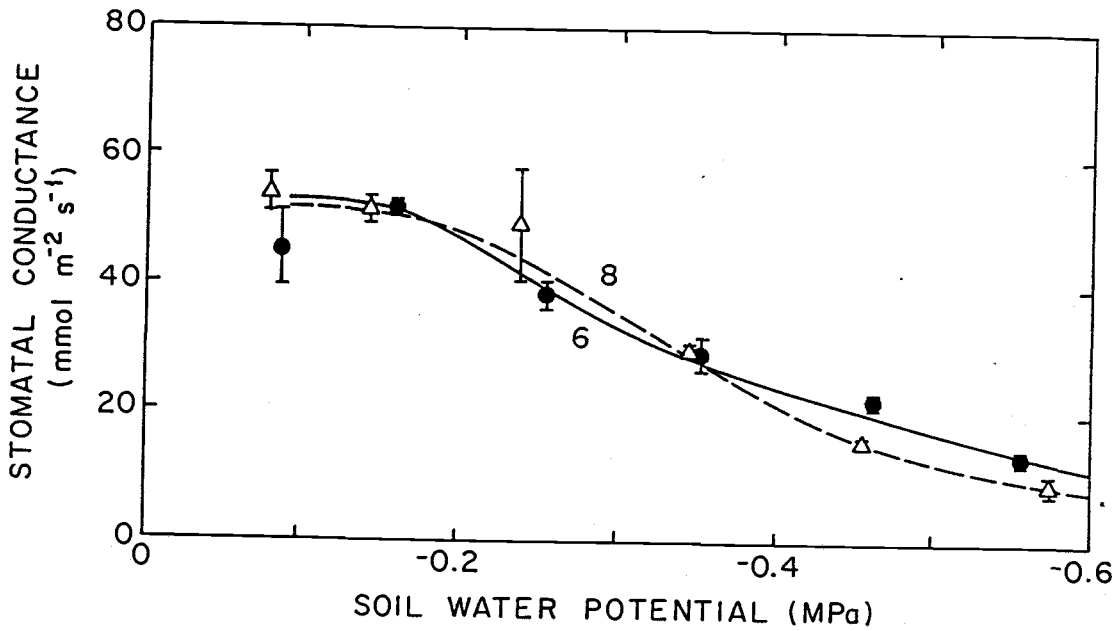


Figure 32. Stomatal conductance as a function of predawn soil water potential for 8-month-old nonmycorrhizal Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: transplanted at 6 weeks old (●); transplanted at 8 weeks old (△). Stomatal conductance was measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

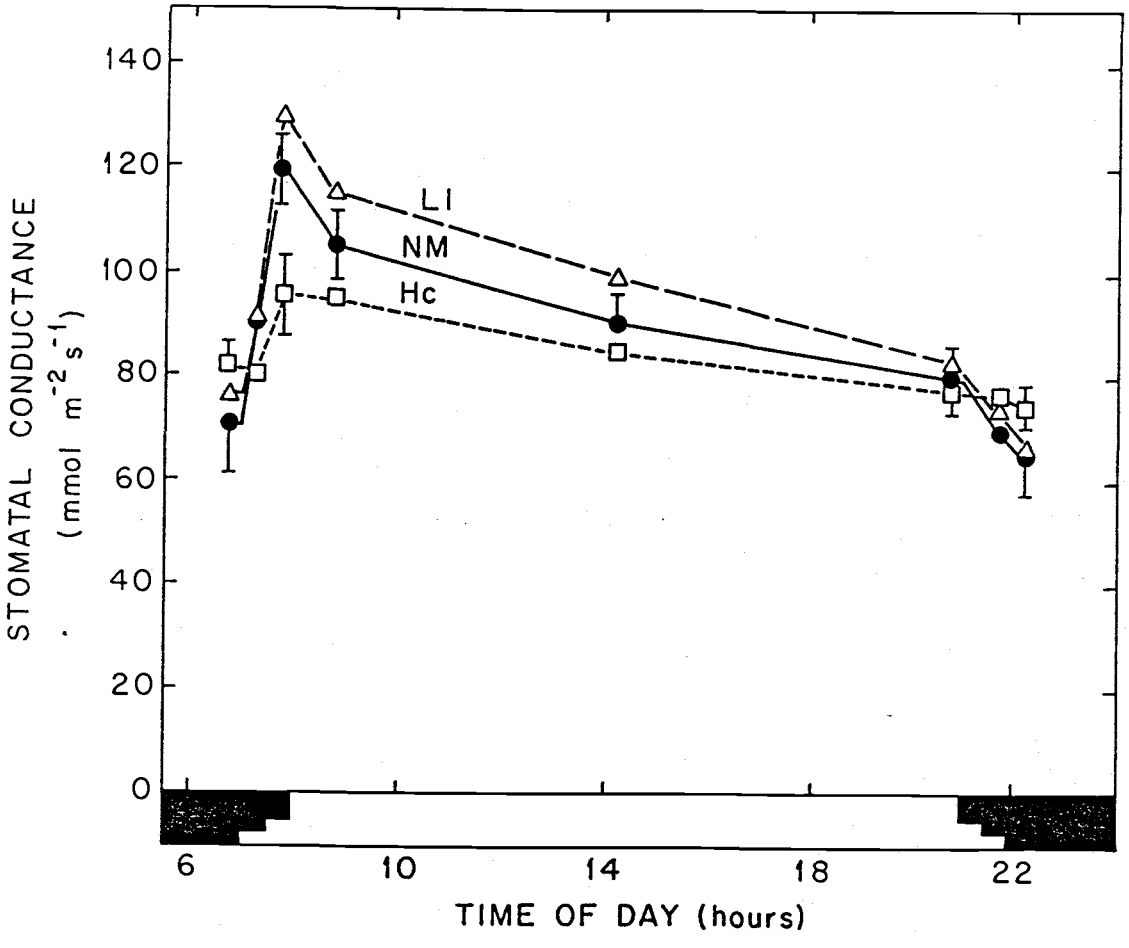


Figure 33. Stomatal conductance measured periodically over the course of one day under well-watered soil conditions for 6-month-old Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: nonmycorrhizal (NM, ●); colonized by Laccaria laccata (Ll, △); colonized by Hebeloma crustuliniforme (Hc, □).

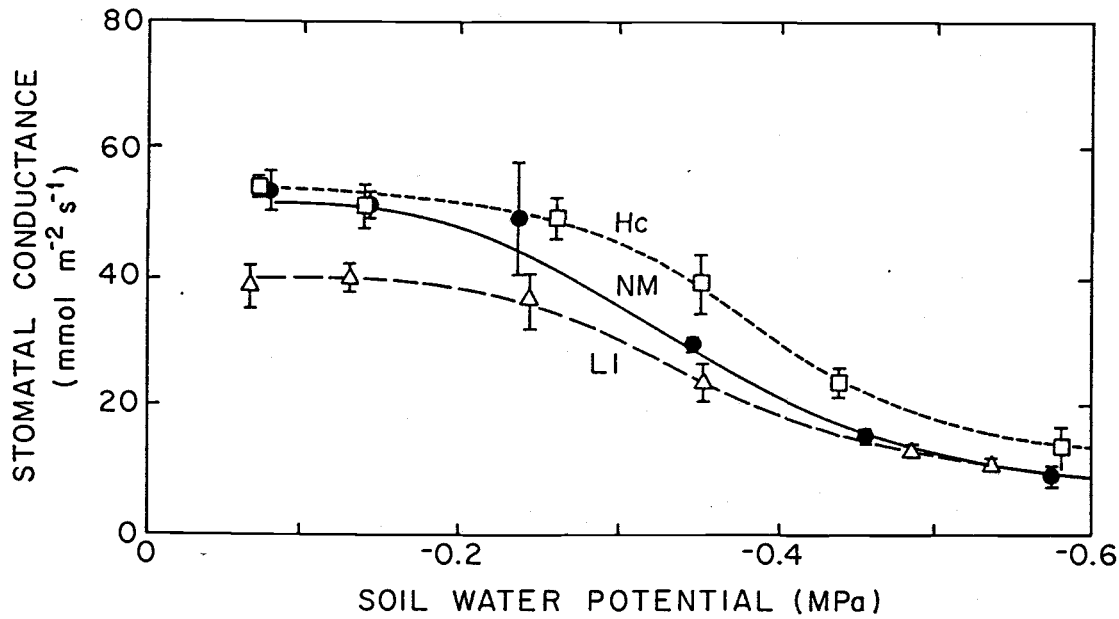


Figure 34. Stomatal conductance as a function of predawn soil water potential for 8-month-old Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: nonmycorrhizal (NM, ●); colonized by Laccaria laccata (Ll, △); colonized by Hebeloma crustuliniforme (Hc, □). Stomatal conductance was measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

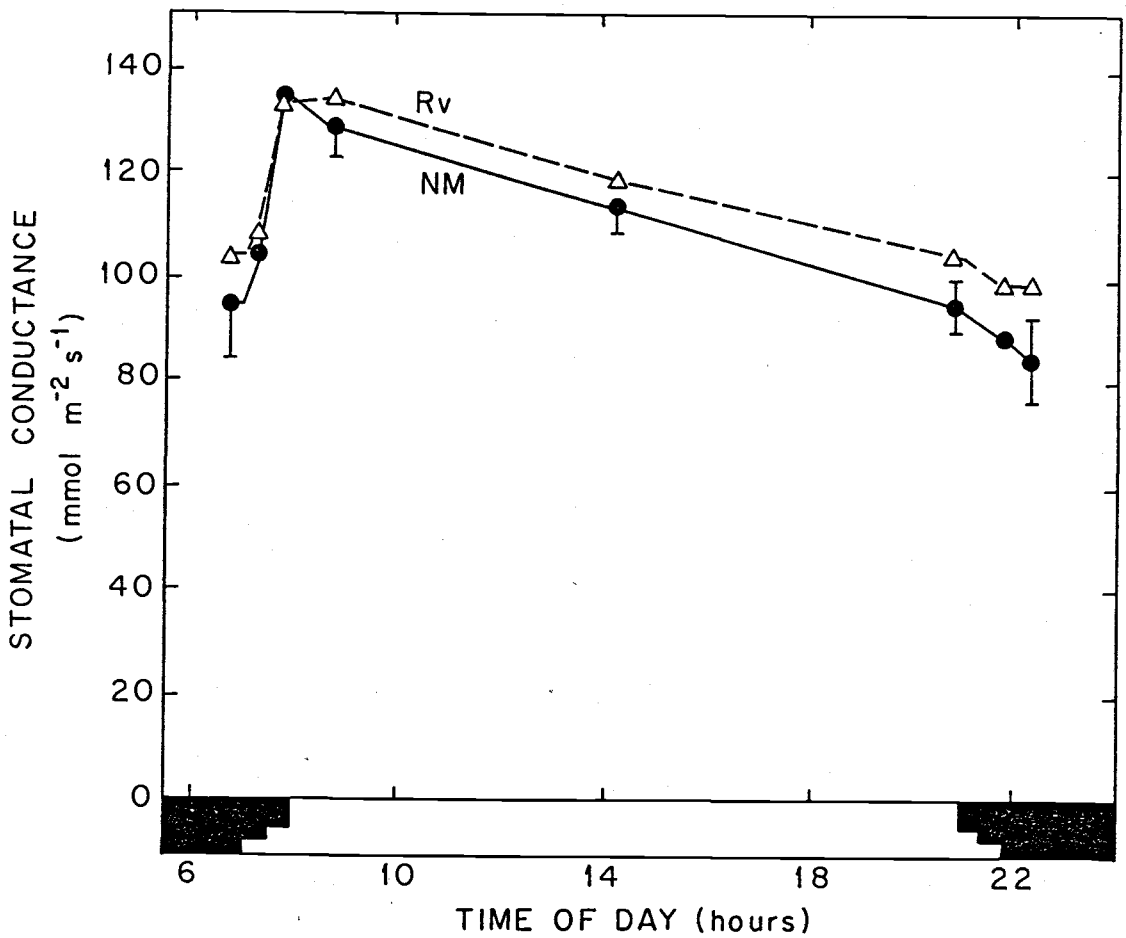


Figure 35. Stomatal conductance measured periodically over the course of one day under well-watered soil conditions for 6-month-old Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: nonmycorrhizal (NM, ●); colonized by Rhizopogon vinicolor (Rv, △).

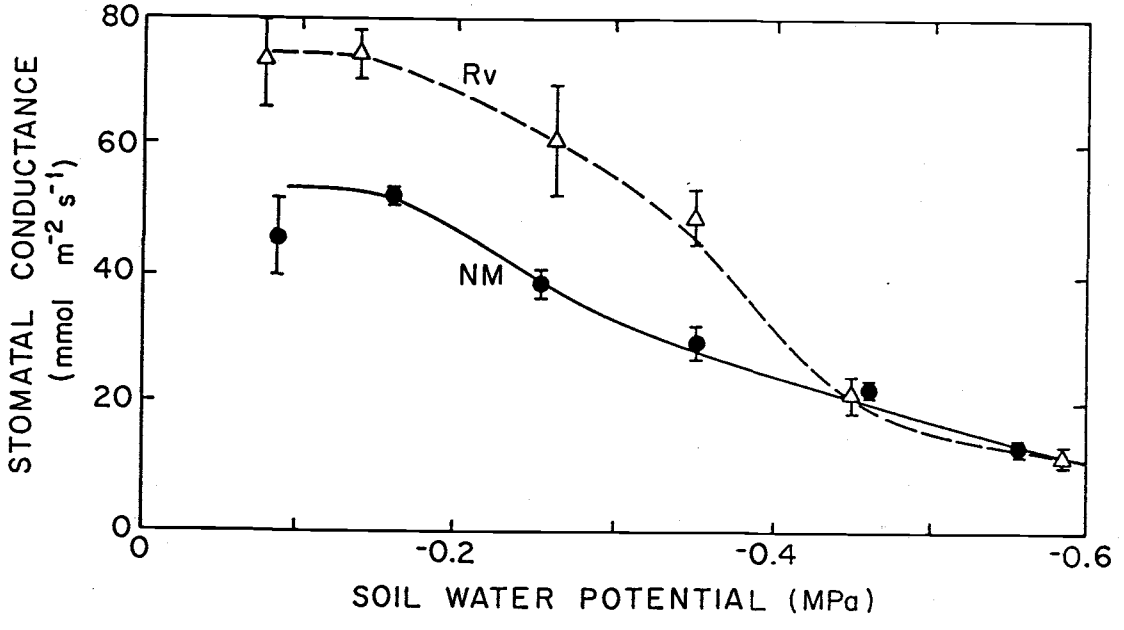


Figure 36. Stomatal conductance as a function of predawn soil water potential for 8-month-old Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: nonmycorrhizal (NM, ●); colonized by Rhizopogon vinicolor (Rv, △). Stomatal conductance was measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

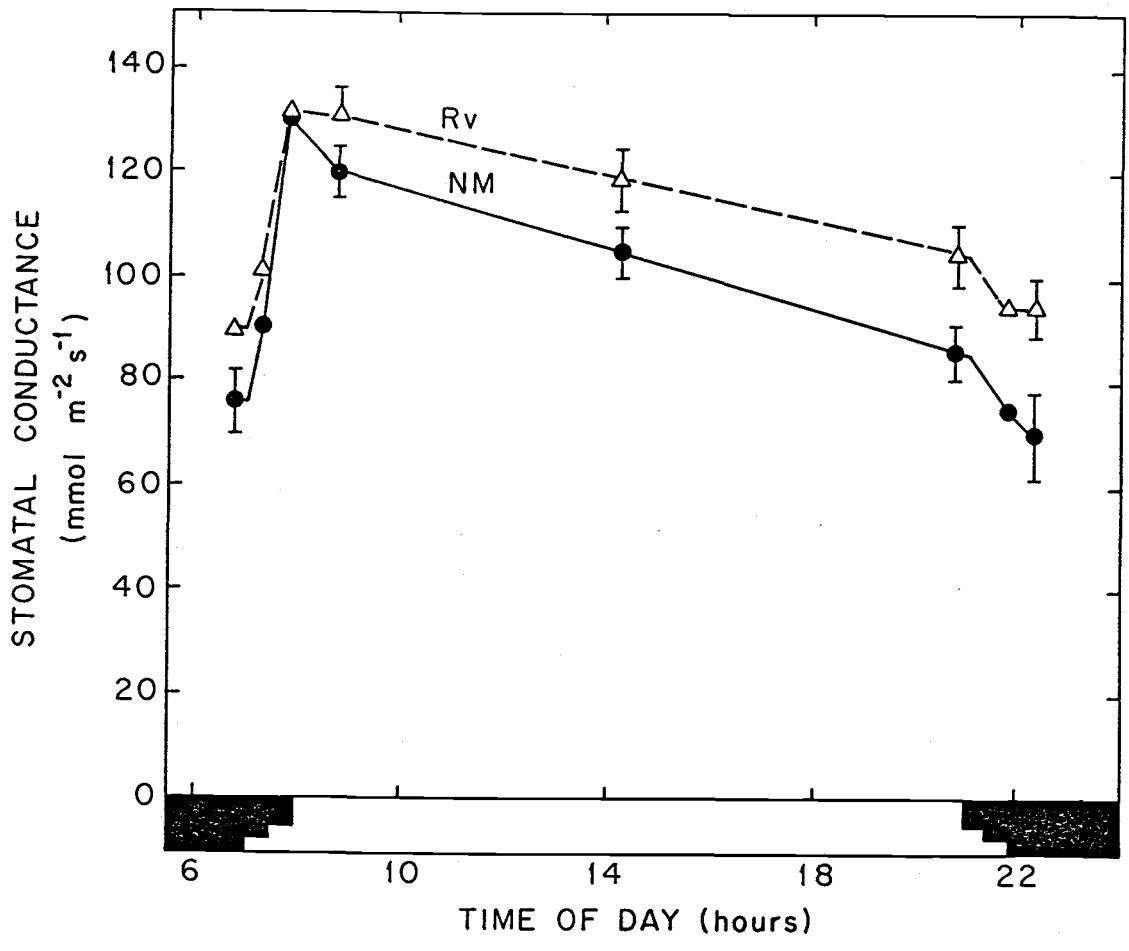


Figure 37. Stomatal conductance measured periodically over the course of one day under well-watered soil conditions for 6-month-old Douglas-fir seedlings from which P fertilizer was withheld: nonmycorrhizal (NM, ●); colonized by Rhizopogon vinicolor (Rv, △).

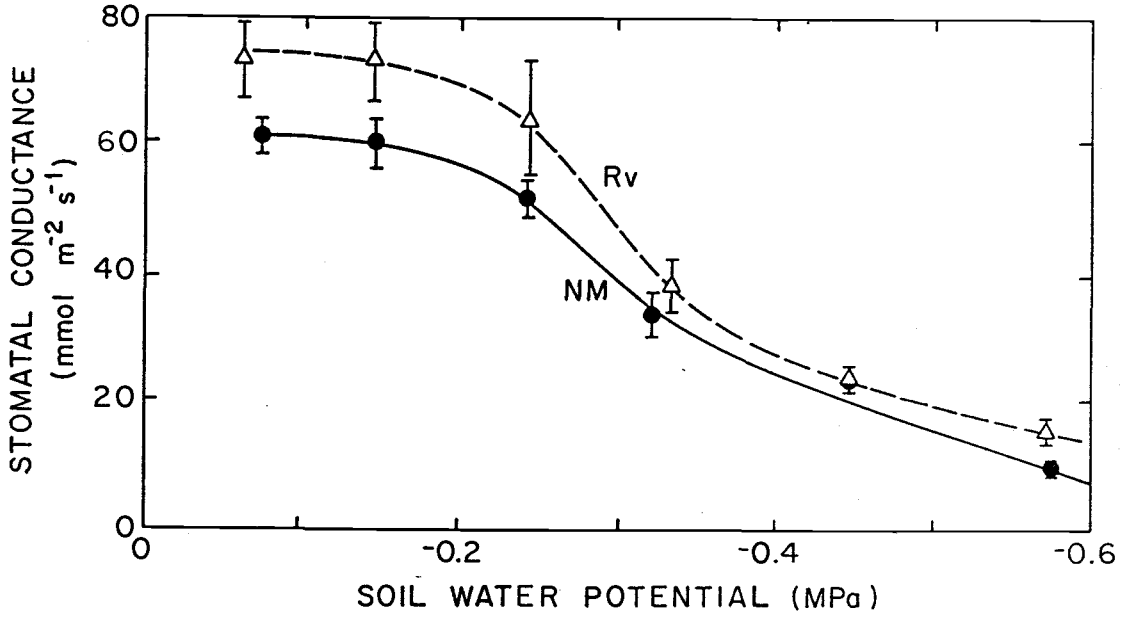


Figure 38. Stomatal conductance as a function of predawn soil water potential for 8-month-old Douglas-fir seedlings from which P fertilizer was withheld: nonmycorrhizal (NM, ●); colonized by Rhizopogon vinicolor (Rv, △). Stomatal conductance was measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

unresponsive to light, having lower stomatal conductance during the daytime than either nonmycorrhizal controls or Laccaria-colonized seedlings (Figure 33). Stomatal conductance of 8-month-old Hebeloma-colonized seedlings was similar to nonmycorrhizal seedlings in wet soil, but was higher as the soil water potential decreased from about -0.25 to -0.6 MPa (Figure 34). Rhizopogon colonization had little effect on stomatal conductance of 6-month-old seedlings under well-watered conditions (Figure 35), but it clearly increased stomatal conductance of 8-month-old seedlings over a wide range of soil water potential from about -0.075 to -0.4 MPa (Figure 36). Under conditions where P fertilizer was withheld, Rhizopogon colonization showed higher stomatal conductances of both 6-month-old seedlings in wet soil (Figure 37) and of 8-month-old seedlings as the soil water potential decreased to about -0.3 MPa (Figure 38).

There was no clear trend of stomatal conductance with P fertilizer level in 6-month-old nonmycorrhizal seedlings in wet soil since the intermediate P fertilizer treatment of 20 mg P kg^{-1} tended to have higher stomatal conductance (Figure 39), but these differences were not significant ($p=.05$). Under drier soil conditions, the only noticeable difference was that 8-month-old nonmycorrhizal seedlings from which P fertilizer was withheld had higher stomatal conductance at soil water potentials down to about -0.25 MPa (Figure 40). Fertilizer P level had no effect on Rhizopogon vinicolor-colonized seedlings at 6 months old under wet soil conditions (Figure 41) nor at 8 months old under drier soil conditions (Figure 42).

In all treatments, there was a large decrease in stomatal conductance, like that for net photosynthesis rate, of seedlings from 6 months old to 8 months old. Since preliminary experiments clearly showed that stomatal conductance does not change over a soil dryness range of nearly saturated conditions to at least as dry as -0.1 MPa soil water potential, stomatal conductance values of 8-month-old seedlings at higher soil water potentials are directly comparable to values measured late in the day on 6-month-old seedlings under wet soil conditions. This comparison reveals that under wet soil conditions stomatal conductance of 8-month-old seedlings was less by

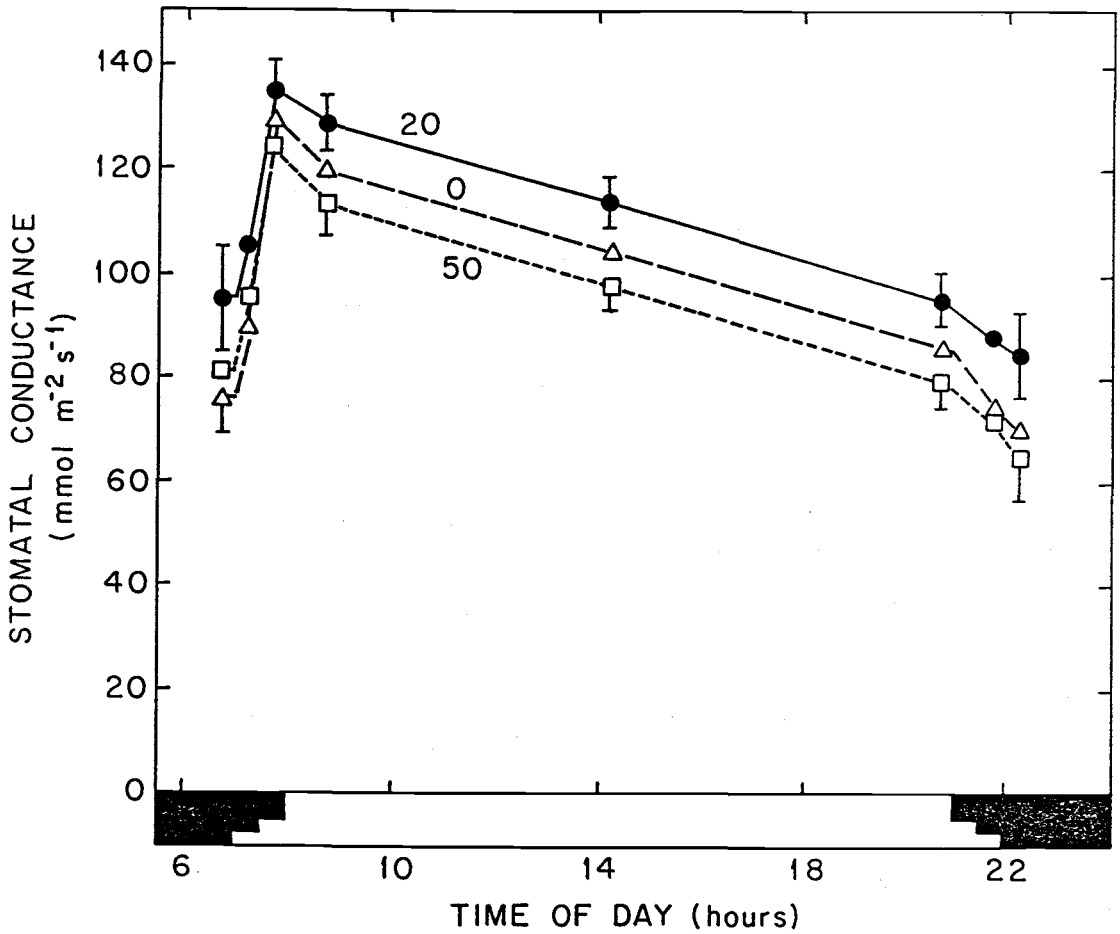


Figure 39. Stomatal conductance measured periodically over the course of one day under well-watered soil conditions for 6-month-old nonmycorrhizal Douglas-fir seedlings fertilized with solutions of 0 (△), 20 (●), and 50 (□) mg P kg^{-1}.

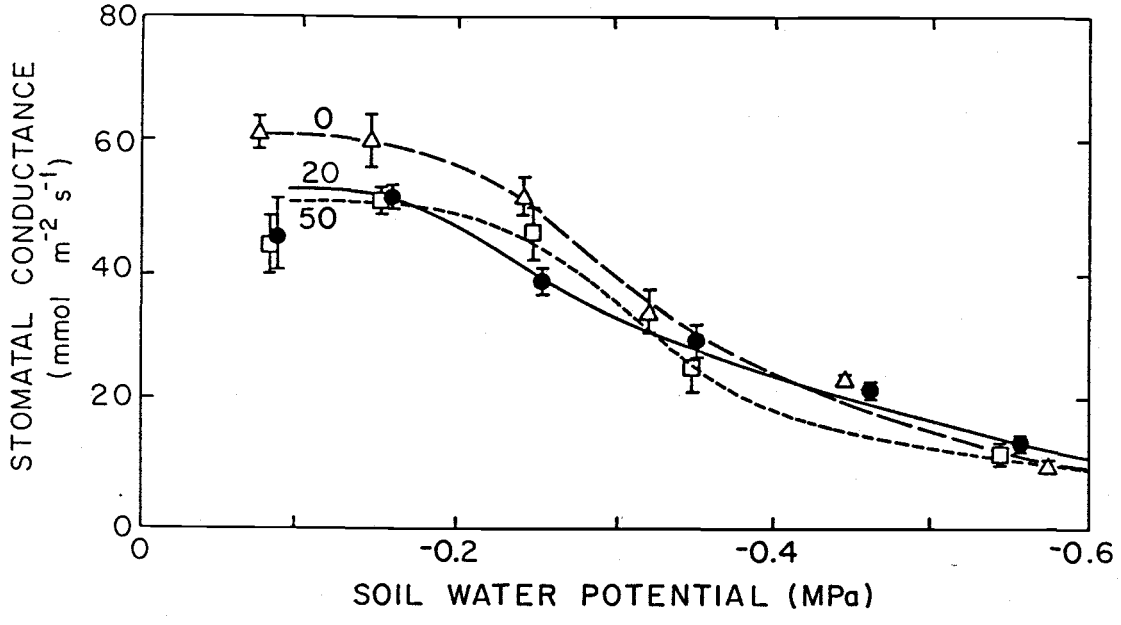


Figure 40. Stomatal conductance as a function of predawn soil water potential for 8-month-old nonmycorrhizal Douglas-fir seedlings fertilized with solutions of 0 (△), 20 (●), and 50 (□) mg P kg^{-1}. Stomatal conductance was measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

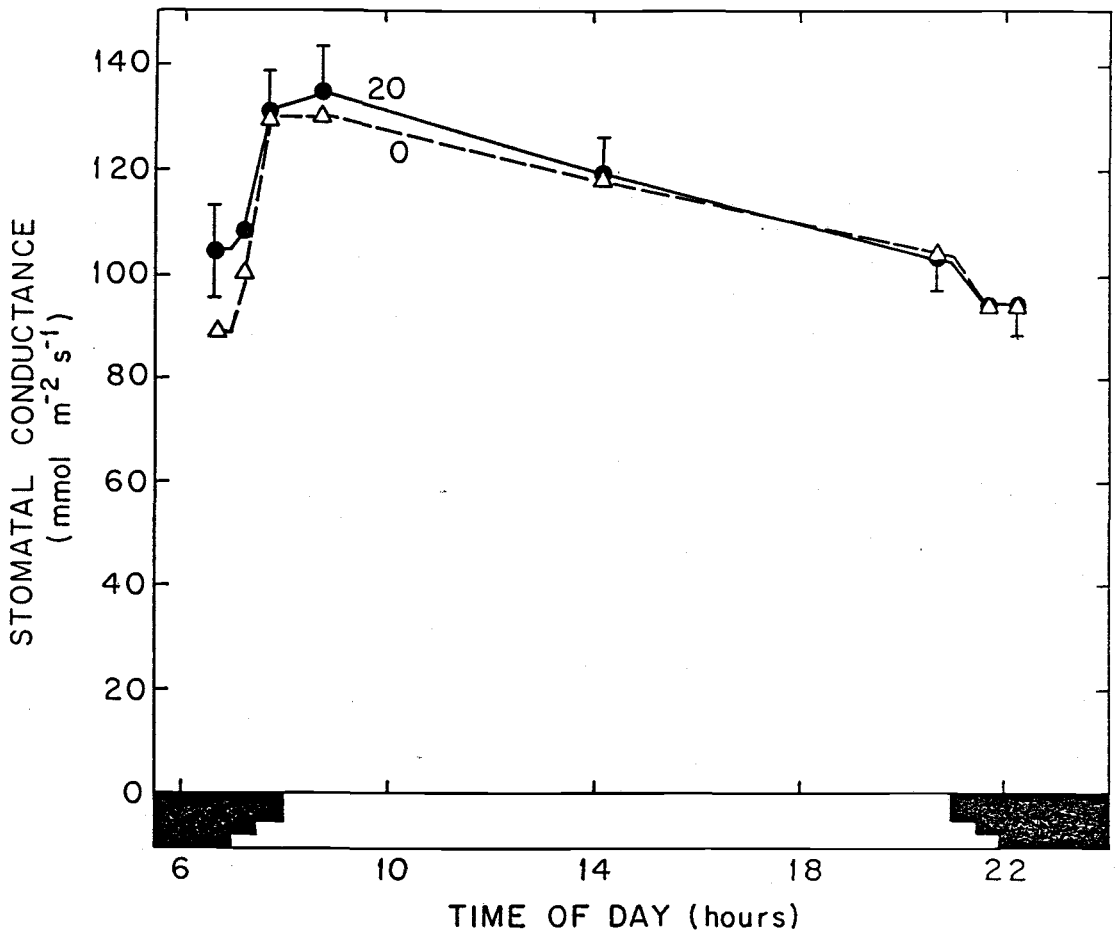


Figure 41. Stomatal conductance measured periodically over the course of one day under well-watered soil conditions for 6-month-old Rhizopogon vinicolor-colonized Douglas-fir seedlings fertilized with solutions of 0 (△) and 20 (●) mg P kg^{-1}.

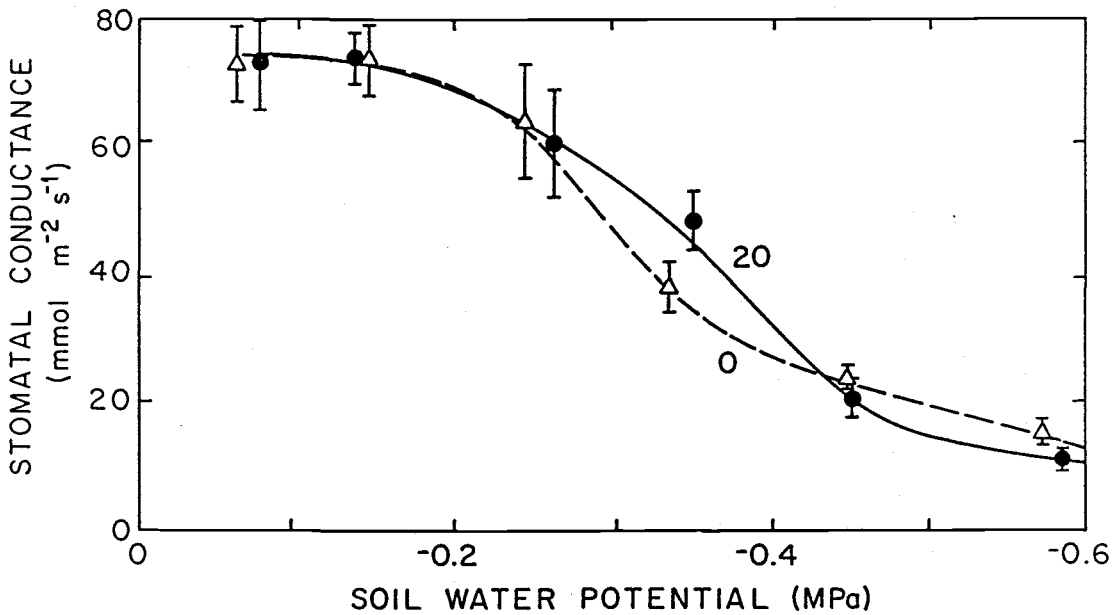


Figure 42. Stomatal conductance as a function of predawn soil water potential for 8-month-old Rhizopogon vinicolor-colonized Douglas-fir seedlings fertilized with solutions of 0 (△) and 20 (●) mg P kg^{-1}. Stomatal conductance was measured late in the day at about 1930 hours. The points are means and standard errors, and the lines are hand-fit to the data.

40% than for 6-month-old seedlings (Table 22). This change is similar to that found for net photosynthesis rate, and reflects some physiological change in these seedlings over the 2 month course of this study.

Water Uptake Conductance

Expected Results

Water uptake conductance refers to the ability of water to flow from the bulk soil to the leaves of plants. It describes the overall transmission characteristic of the entire water uptake system; soil, plant roots, and xylem. In this experiment it is calculated as the transpiration rate of the seedling (mol s^{-1}) per unit water potential difference between the bulk soil and the leaves (MPa). By calculating conductance in this way, it is best evaluated during times when water flow and water potentials through all segments of the water uptake and transpiration pathway are at steady state (van den Honert, 1948), such as nearly occurs for plants rooted in wet soil at predawn and late in the day. Under dry soil conditions, continual water uptake during the day decreases the water content and water transmission ability of soil adjacent to absorbing roots, thus continually decreasing water uptake rate through the day. Under these conditions, this expression of conductance is not strictly applicable (McCoy et al., 1983). However, since the influence of this drier soil layer on water uptake is itself a function of water uptake rate and bulk soil water potential, this expression remains a good index of conductance of the plant water uptake system in dry soil. Conceptually, higher water uptake conductance means that water can flow more easily from the bulk soil through the soil adjacent to absorbing roots, plant roots and xylem, to the leaves. In effect, higher water uptake conductance can result in both higher leaf water potential and water uptake rate.

Table 22. Stomatal conductance of Douglas-fir seedlings under well-watered conditions at 6 months old and at 8 months old, and the relative change in rate from 6 months old to 8 months old, for the indicated mycorrhizal fungus treatment, fertilizer P concentration, and seedling age when transplanted. Relative change is expressed as the stomatal conductance at 8 months old divided by that at 6 months old. Stomatal conductance was measured late in the day at about 1930 hours.

Treatment			Stomatal conductance		
Age transplanted	Fungal treatment	Fertilizer P conc.	6 months	8 months	Relative change
(weeks)		(mg kg^{-1})	(mmol m^{-2} s^{-1})	(mmol m^{-2} s^{-1})	
6	NM[a]	0	87 ± 5[b]	61 ± 4[c]	.70
		20	96 ± 5	53 ± 2	.55
		50	80 ± 5	51 ± 2	.64
	Rv	0	105 ± 6	75 ± 6	.71
		20	104 ± 7	75 ± 4	.72
8	NM	20	80 ± 6	51 ± 2	.64
	Ll	20	83 ± 6	54 ± 2	.65
	Hc	20	78 ± 5	40 ± 3	.51

[a] fungal treatment abbreviations: NM, nonmycorrhizal; Rv, Rhizopogon vinicolor; Ll, Laccaria laccata; Hc, Hebeloma crustuliniforme

[b] measured mean ± one standard error

[c] estimated mean ± one standard error. Standard error values from data collected in the soil water potential range of -0.10 to -0.19 MPa.

Characteristics of water uptake conductance and the influence of mycorrhizal colonization and plant nutrition on it is covered in the "Literature Review", so only a few pertinent points will be made here. For plants rooted in wet soil, water uptake conductance is limited primarily by the permeability of the roots to soil water. Root permeability is variable (Parsons and Kramer, 1974). It is higher during light periods than during dark periods. Based upon their structure, ectomycorrhizae could be less permeable than roots. Enhanced plant nutrient status could increase permeability of all absorbing tissues through greater plant membrane permeability (Radin and Eidenboch, 1984), whether enhanced by mycorrhizal colonization or fertilization. Under drier soil conditions the layer of soil near the root surfaces becomes the dominant limitation to water uptake conductance. As the soil dries, water uptake conductance becomes smaller as the ability of soil adjacent to the absorbing roots to conduct water to those roots declines rapidly. Water uptake by mycorrhizal hyphae from wetter soil only a few millimeters away from mycorrhizae and transport across the flow-limiting soil layer to the mycorrhizae surfaces could effectively increase water uptake conductance. Ectomycorrhizal colonization can lead to a smaller root system relative to the rest of the plant, which could offset any nutritional benefits to permeability in wet soil, and hyphal transport in drier soil.

Two studies on ectomycorrhizal conifers from which water uptake conductance estimates can be made indicate that conductance in wet soil is not altered by ectomycorrhizal colonization (Sands and Theodoreau, 1978; Parke et al., 1983). In drier soil, Sands and Theodoreau's data indicate lower water uptake conductance in ectomycorrhizal seedlings. Nutrition of these plants was not evaluated.

In view of this discussion, it is expected that water uptake conductance will decrease as the soil water potential decreases. In wet soil, it should be lower at predawn than late in the day, ectomycorrhizal colonization will have little effect, and P fertilization might improve it. In drier soil, ectomycorrhizal

colonization should increase water uptake conductance unless root growth more than offsets hyphal uptake and transport of soil water to the absorbing mycorrhizae.

Results

Treatments were compared in 6 different combinations in order to determine the nature of the effect of ectomycorrhizae, P fertilizer level, and transplant age on the seedlings. These comparisons were outlined in the "METHODS" section covering "Statistical Design and Data Analysis", and are briefly reviewed here:

The effect of seedling age when transplanted:

1) The nonmycorrhizal treatments, 2 and 6 (see Table 6 for reference numbers), were used to exemplify "typical" seedling behavior, and to test for effect of transplant age on seedlings.

The effect of ectomycorrhizal colonization:

2) Hc and Ll (treatments 7 and 8) were compared with their nonmycorrhizal control, treatment 6, for moderate soil P levels.
3) Rv treatment 5 was compared with its nonmycorrhizal control, treatment 2, for moderate soil P levels.
4) Rv treatment 4 was compared to its nonmycorrhizal control, treatment 1, for seedlings where fertilizer P withheld.

The effect of P fertilization level:

5) For nonmycorrhizal seedlings, high P level (treatment 3) and the low P level (where fertilizer P was withheld, treatment

1) were compared with moderate P level (treatment 2).

6) For Rv, low P level (where fertilizer P was withheld, treatment 4) was compared with moderate P level (treatment 5).

In this section, comparisons are made in the above order. Furthermore, for each comparison made, 2 graphs are presented. The diurnal trend of water uptake conductance for 6-month-old seedlings growing in wet soil is described first, followed immediately by the relationship between water uptake conductance and predawn soil water potential found for 8-month-old seedlings.

Typical values of water uptake conductance for 6-month-old Douglas-fir seedlings in wet soil in these experiments are shown in Figure 43. Water uptake conductance was calculated from transpiration rate and leaf water potential at predawn and at about 1.5 hours before the lights began to go off late in the day. At these times of day, levels of both of these parameters were reasonably steady. Water potential of wet soil is effectively zero. As expected, water uptake conductance was much higher late in the day than at predawn, indicating diurnal change in root permeability to water in these seedlings. Water uptake conductance was about 2X greater in the afternoon than at predawn.

Typical values of water uptake conductance late in the day for 8 month old Douglas-fir seedlings in relation to soil water potential in this experiment is shown in Figure 44. Soil water potential in this figure is of the bulk soil, measured at predawn, while water uptake conductance was calculated from transpiration and leaf water potential measurements made late in the day. In this figure, the points represent means and their standard errors of observations made within each 0.1 MPa segment of the soil water potential range. Each line is a hand-fit curve representing all of the data. The details of the technique used to fit the curve are described in the "METHODS" section on "Statistical Design and Data Analysis". This figure shows that water uptake conductance decreased rapidly to very low values as the soil water potential decreased to about -0.6 MPa. In some treatments,

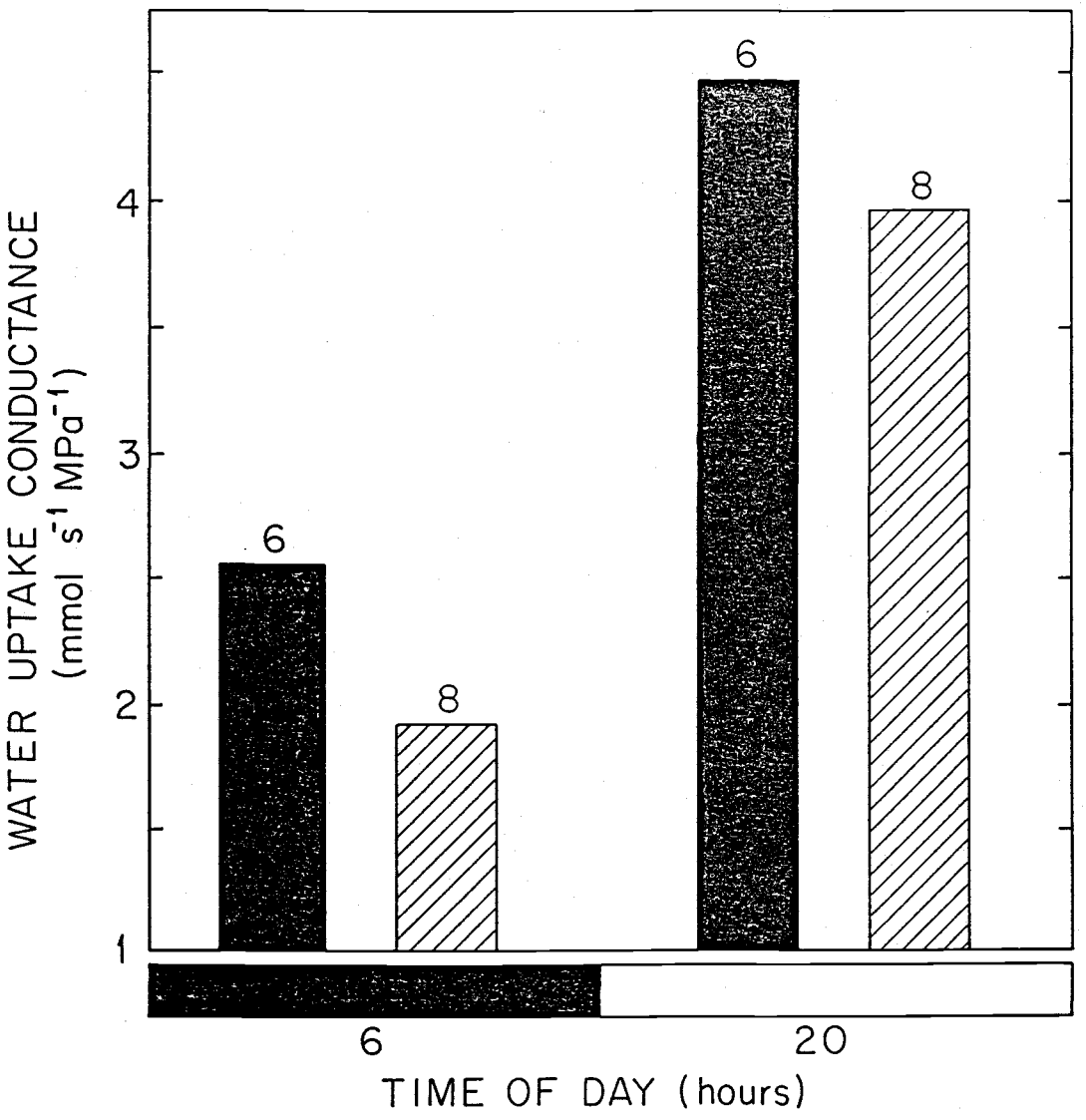


Figure 43. Water uptake conductance at predawn and late in the day under well-watered soil conditions for 6-month-old nonmycorrhizal Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: transplanted at 6 weeks old; transplanted at 8 weeks old.

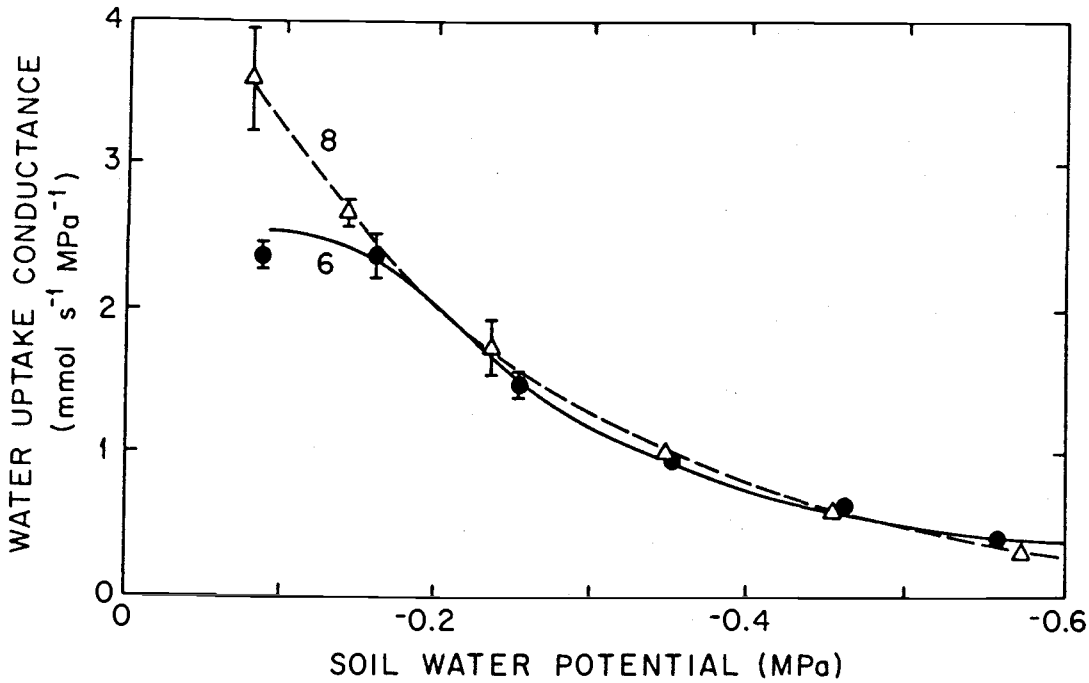


Figure 44. Water uptake conductance as a function of predawn soil water potential for 8-month-old nonmycorrhizal Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: transplanted at 6 weeks old (●); transplanted at 8 weeks old (△). Water uptake conductance was measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

water uptake conductance did not change much as soil water potential decreased to about -0.15 MPa, and then declined steeply as the soil water potential decreased further. However, in other treatments the data indicate declining conductance as soil water potential decreased from at least as wet as -0.075 MPa.

The influence of ectomycorrhizal colonization on water uptake conductance of Douglas-fir seedlings is shown in Figures 45 through 50. For 6-month-old seedlings in wet soil, Laccaria colonization had no effect on water uptake conductance at predawn or late in the day (Figure 45), but tended to reduce it compared to nonmycorrhizal seedlings in the wetter range of soil water potentials when the seedlings were 8 months old (Figure 46). Hebeloma colonization tended to reduce water uptake conductance of 6-month-old seedlings in wet soil late in the day (Figure 45), which also occurred at 8 months old (Figure 46). Neither Laccaria nor Hebeloma colonization influenced water uptake conductance in soil drier than about -0.2 MPa soil water potential. Rhizopogon colonization appeared to reduce water uptake conductance of 6-month-old P fertilized seedlings in wet soil (Figure 47), but not for 8-month-old seedlings over the soil water potential range of about -0.05 to -0.6 MPa (Figure 48). Where P fertilizer was withheld there was no difference between nonmycorrhizal and Rhizopogon-colonized seedlings in water uptake conductance in wet soil at 6 months old (Figure 49) or at 8 months old over the soil water potential range of about -0.1 to -0.6 MPa (Figure 50).

The influence of supplemental P fertilization level on water uptake conductance of Douglas-fir seedlings is shown in Figures 51 through 54. For 6-month-old nonmycorrhizal seedlings in wet soil, there was no consistent correlation between water uptake conductance and fertilizer P level because the seedlings fertilized with the intermediate 20 mg P kg^{-1} solution level tended to have higher water uptake conductance, than the other treatments at both times of day (Figure 51). For 8-month-old nonmycorrhizal seedlings under drier soil conditions, there were no clear differences between fertilizer P treatments, except an indication of higher water uptake conductance for seedlings from which P fertilizer was withheld in soil wetter than

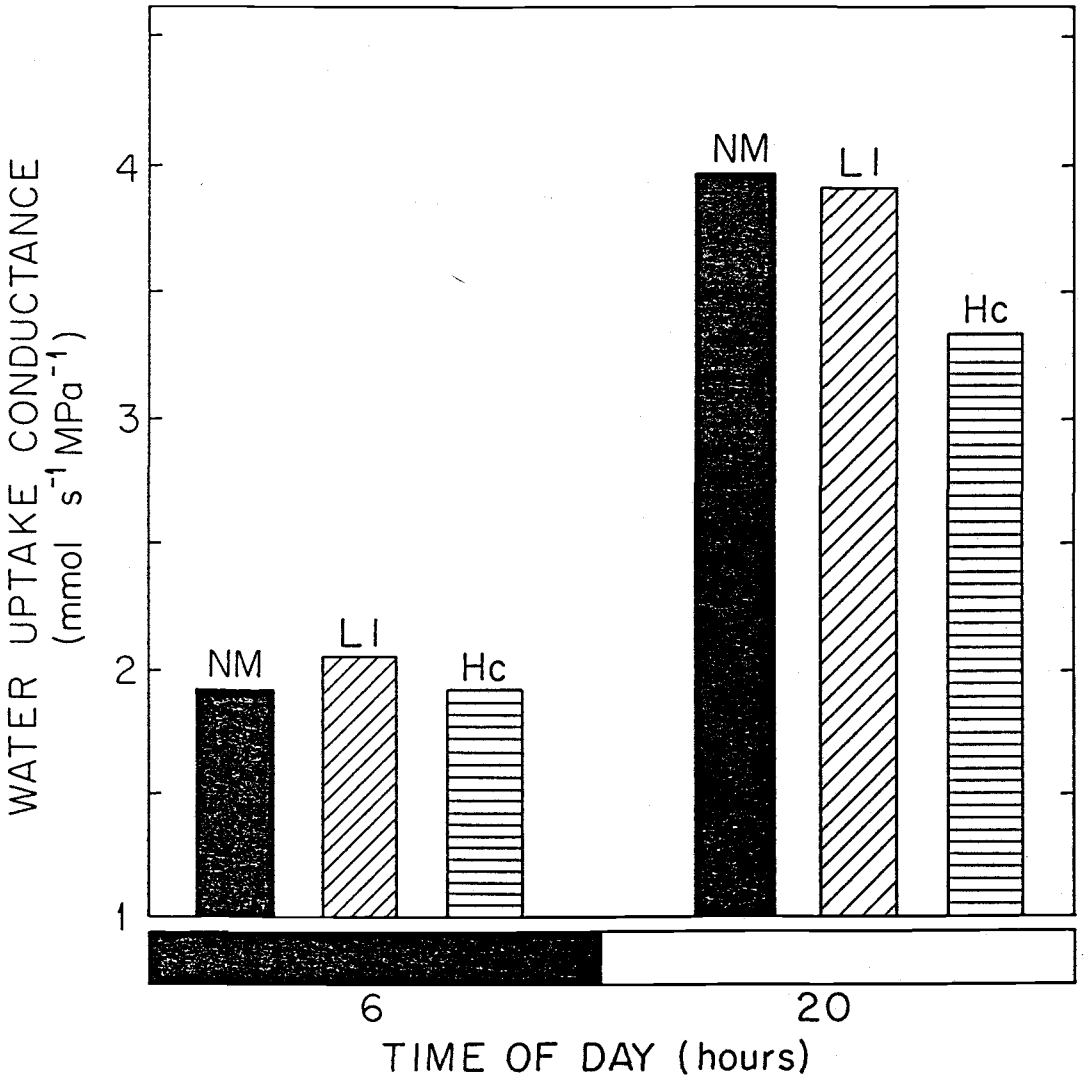


Figure 45. Water uptake conductance at predawn and late in the day under well-watered soil conditions for 6-month-old Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: nonmycorrhizal (NM); colonized by Laccaria laccata (Ll); colonized by Hebeloma crustuliniforme (Hc).

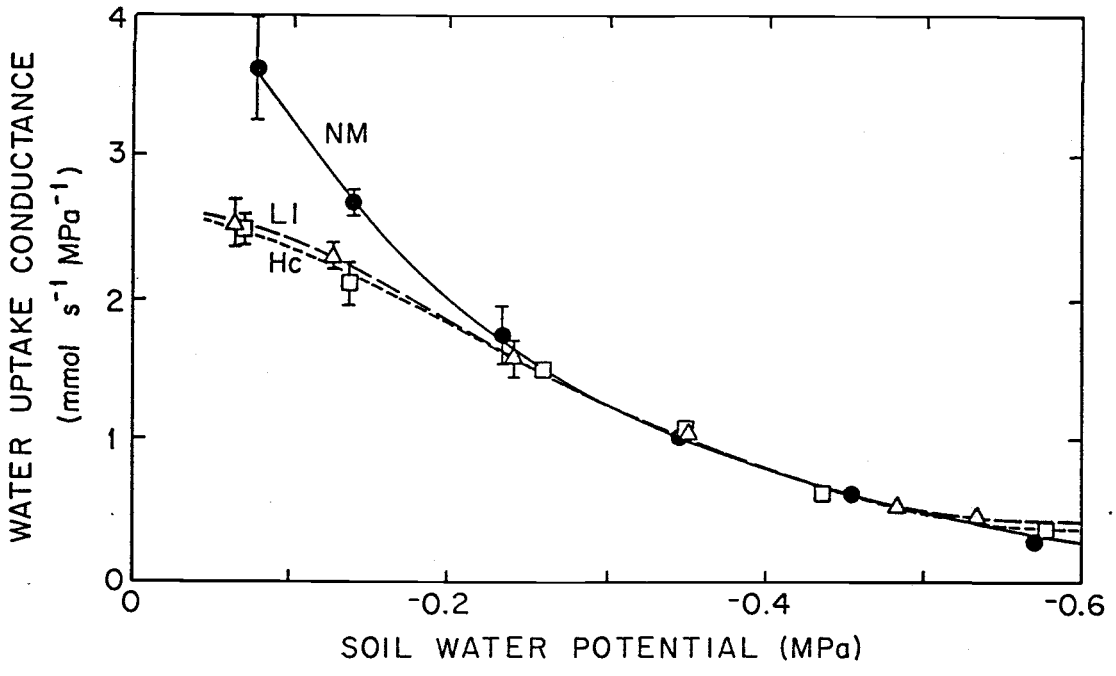


Figure 46. Water uptake conductance as a function of predawn soil water potential for 8-month-old Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: nonmycorrhizal (NM, ●); colonized by Laccaria laccata (Ll, △); colonized by Hebeloma crustuliniforme (Hc, □). Water uptake conductance was measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

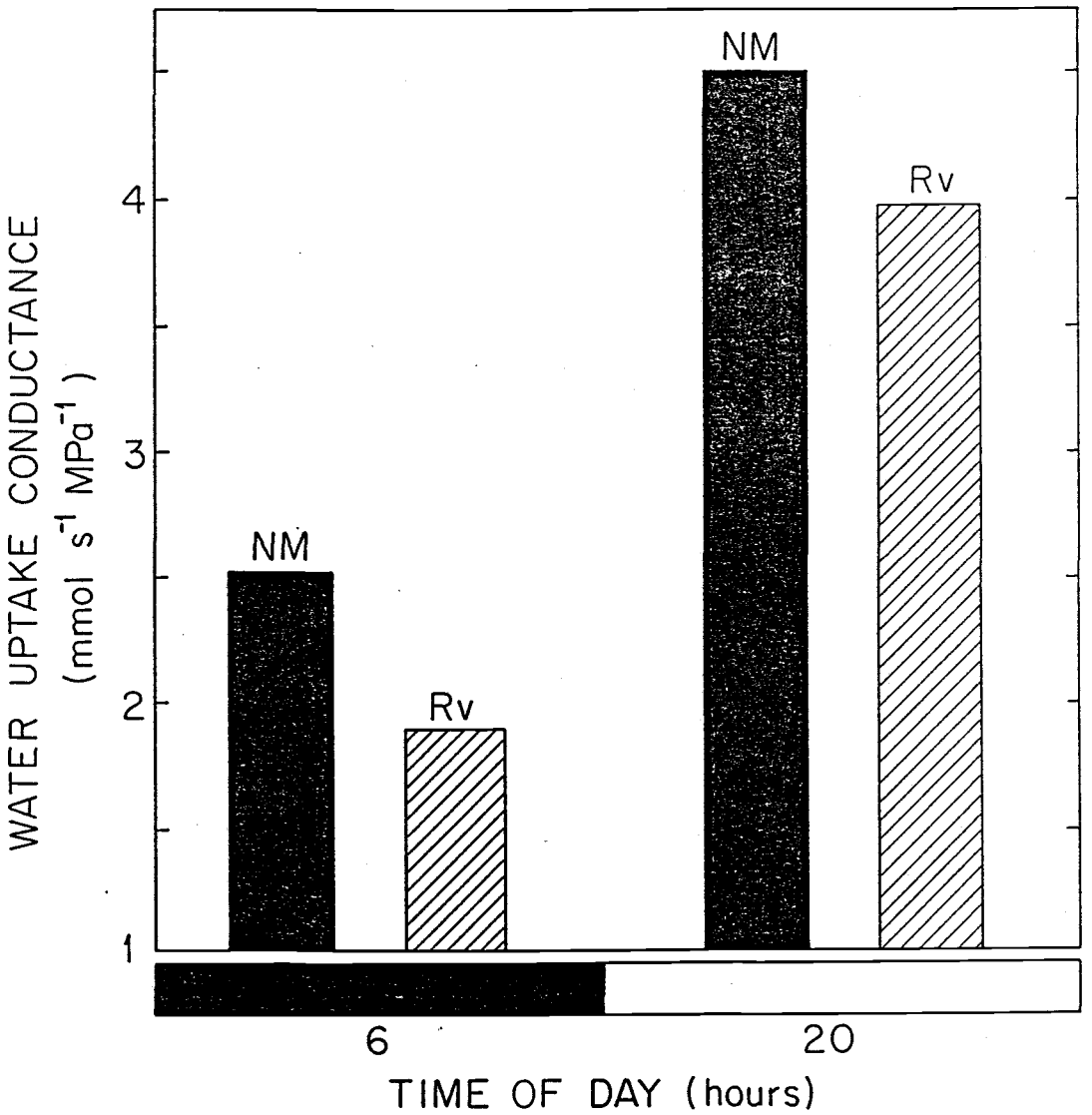


Figure 47. Water uptake conductance at predawn and late in the day under well-watered soil conditions for 6-month-old Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: nonmycorrhizal (NM); colonized by Rhizopogon vinicolor (Rv).

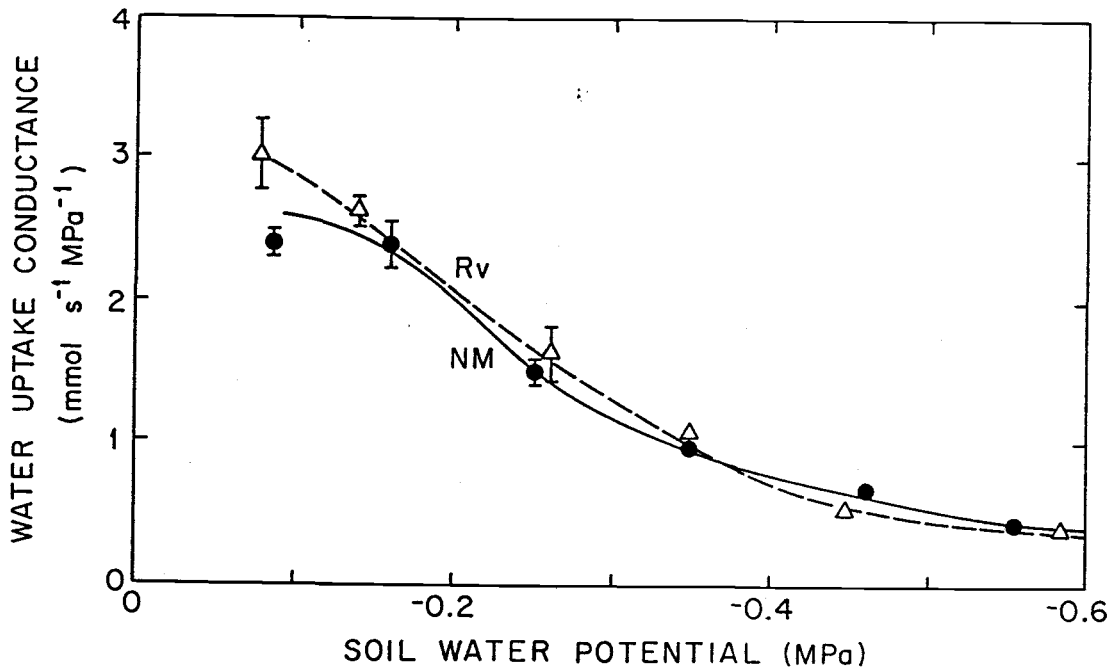


Figure 48. Water uptake conductance as a function of predawn soil water potential for 8-month-old Douglas-fir seedlings fertilized with a 20 mg P kg^{-1} solution: nonmycorrhizal (NM, ●); colonized by Rhizopogon vinicolor (Rv, △). Water uptake conductance was measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

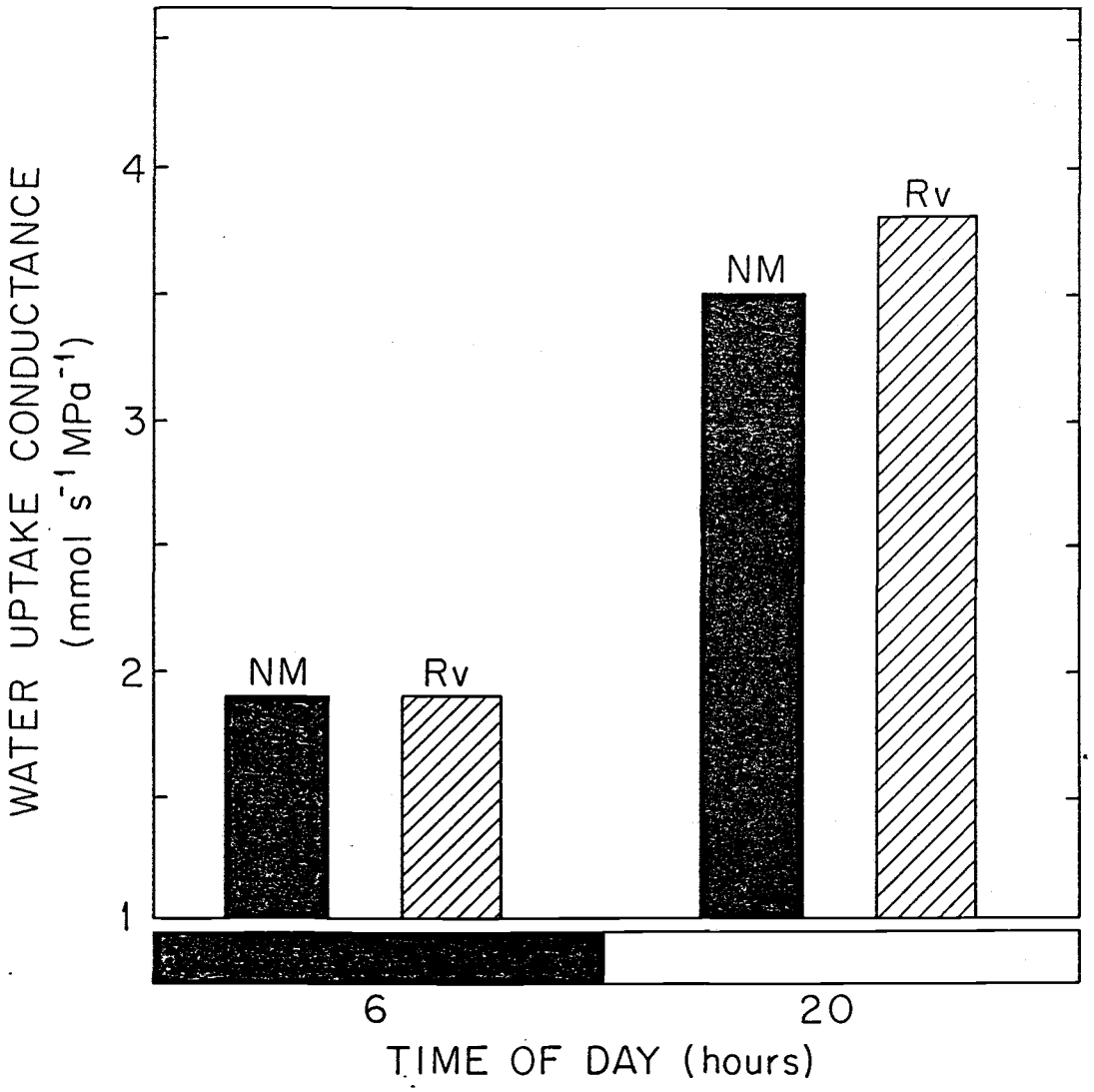


Figure 49. Water uptake conductance at predawn and late in the day under well-watered soil conditions for 6-month-old Douglas-fir seedlings from which P fertilizer was withheld: nonmycorrhizal (NM); colonized by Rhizopogon vinicolor (Rv).

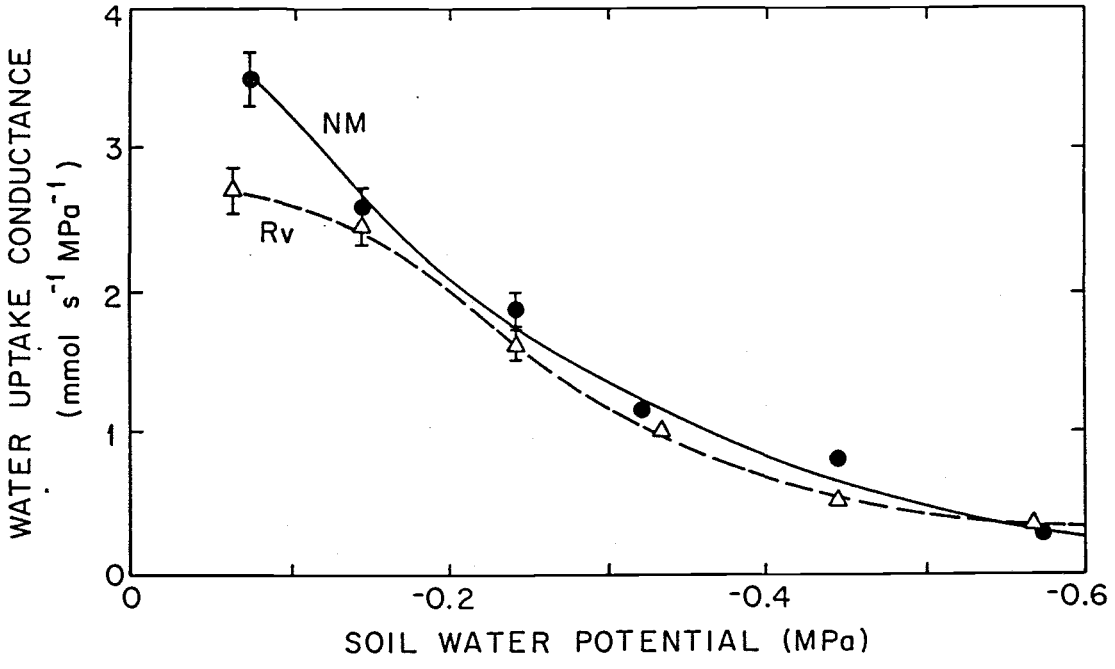


Figure 50. Water uptake conductance as a function of predawn soil water potential for 8-month-old Douglas-fir seedlings from which P fertilizer was withheld: nonmycorrhizal (NM, ●); colonized by Rhizopogon vinicolor (Rv, △). Water uptake conductance was measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

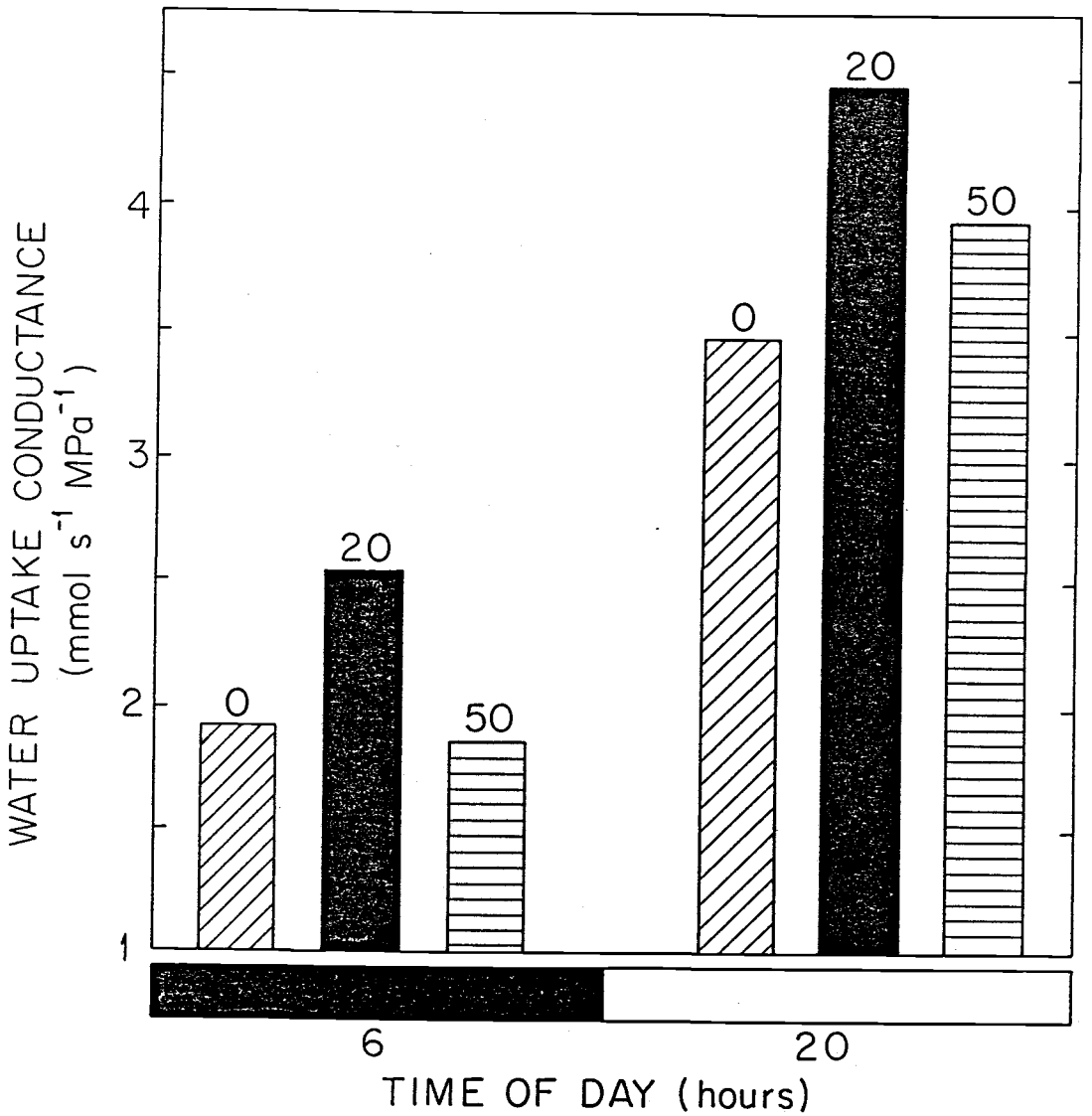


Figure 51. Water uptake conductance at predawn and late in the day under well-watered soil conditions for 6-month-old nonmycorrhizal Douglas-fir seedlings fertilized with solutions of 0, 20, and 50 mg P kg^{-1}.

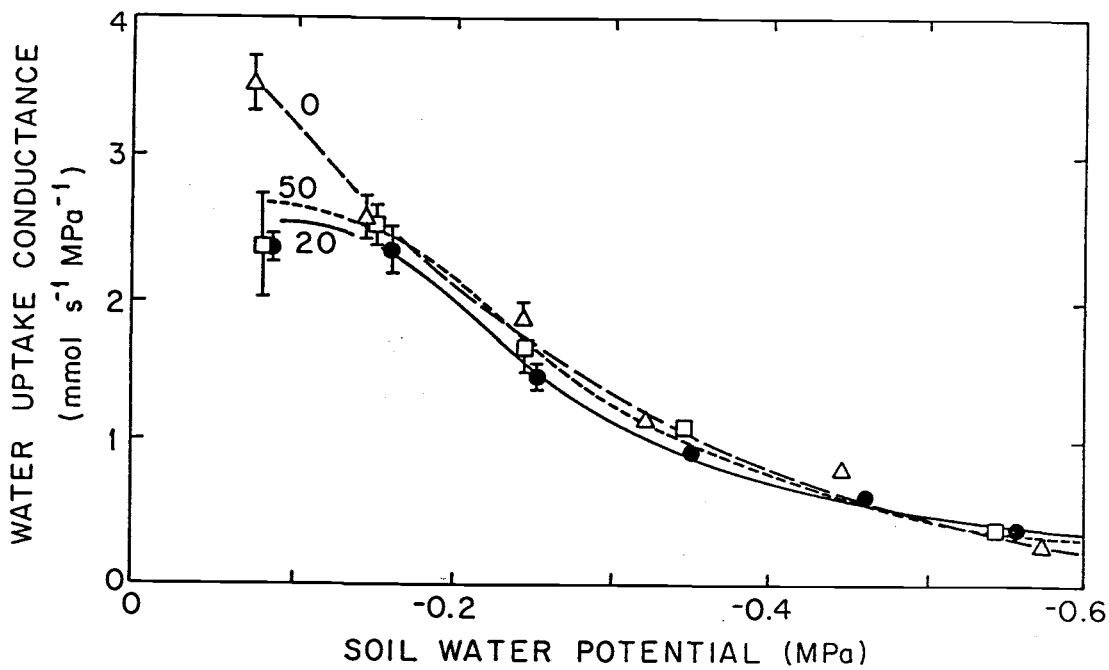


Figure 52. Water uptake conductance as a function of predawn soil water potential for 8-month-old nonmycorrhizal Douglas-fir seedlings fertilized with solutions of 0 (△), 20 (●), and 50 (□) mg P kg^{-1}. Water uptake conductance was measured late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

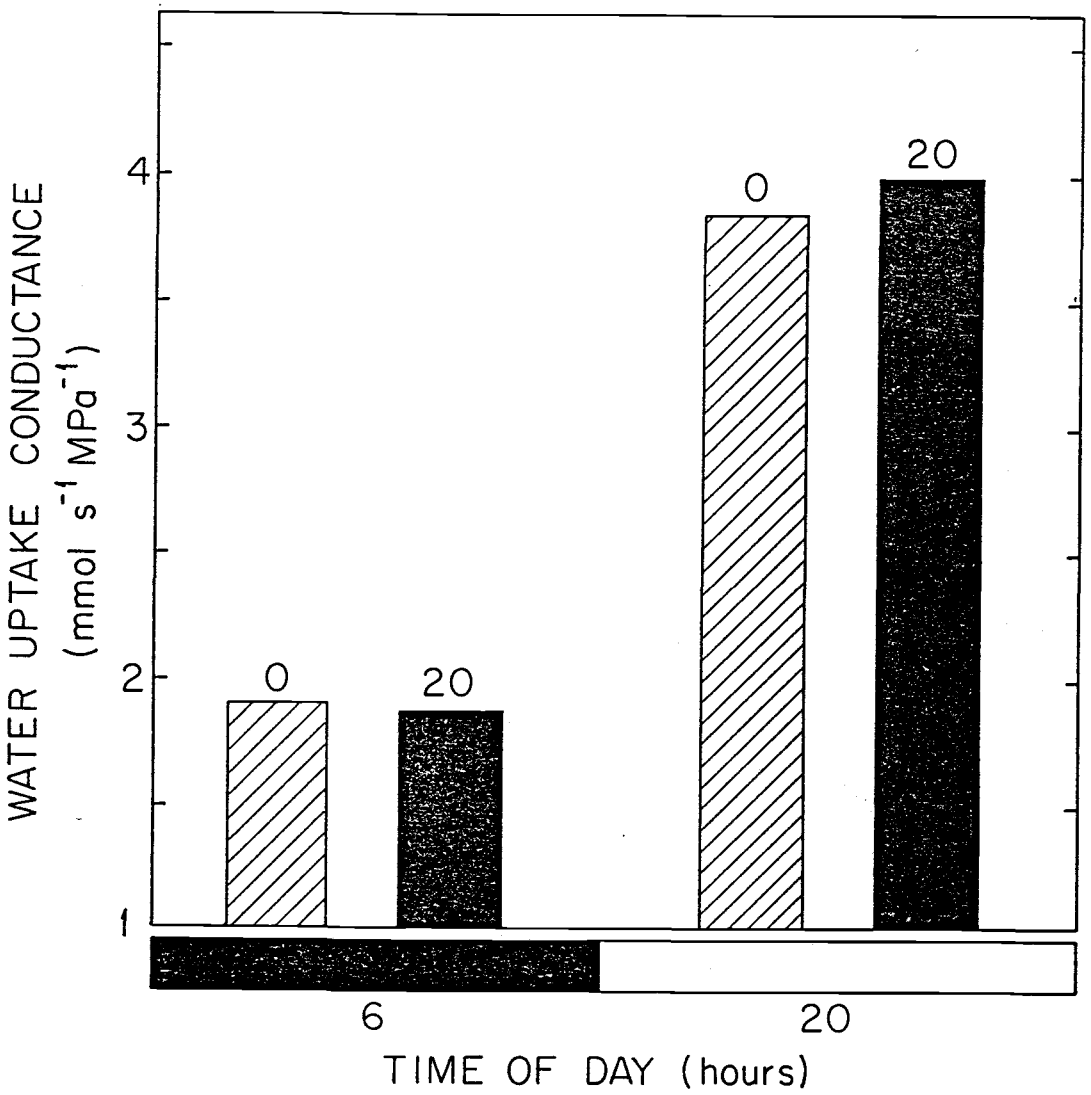


Figure 53. Water uptake conductance at predawn and late in the day under well-watered soil conditions for 6-month-old Rhizopogon vinicolor-colonized Douglas-fir seedlings fertilized with solutions of 0 and 20 mg P kg^{-1}.

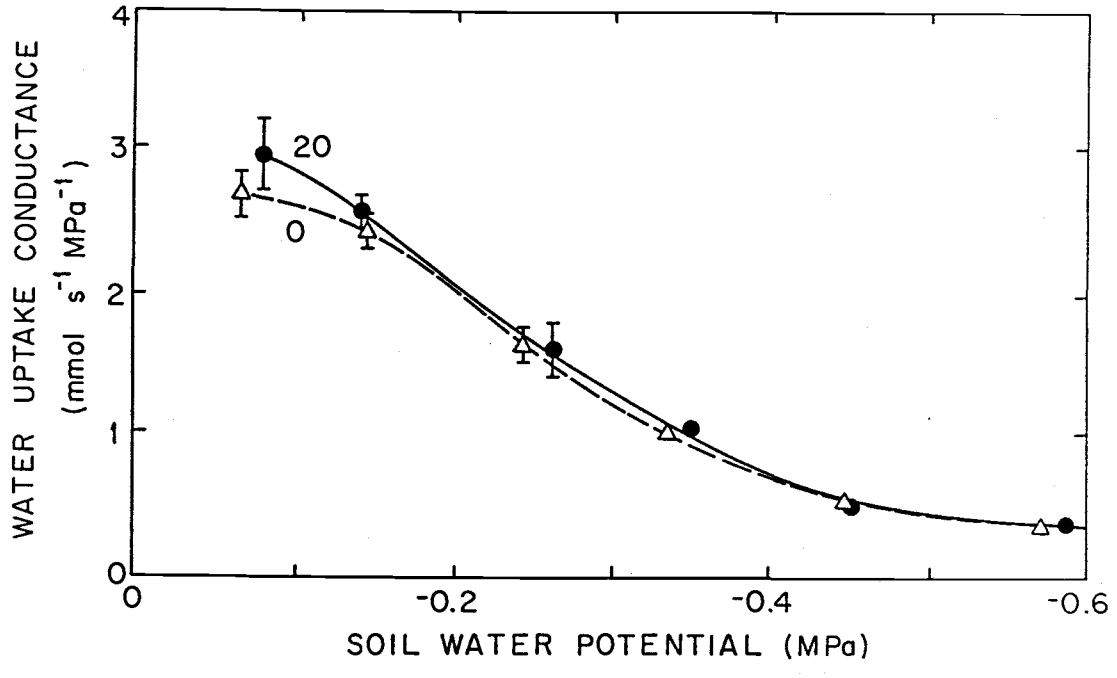


Figure 54. Water uptake conductance as a function of predawn soil water potential for 8-month-old Rhizopogon vinicolor-colonized Douglas-fir seedlings fertilized with solutions of 0 (△) and 20 (●) mg P kg^{-1}. Water uptake conductance was measured late in the day at about 1930 hours. The points are means and standard errors, and the lines are hand-fit to the data.

about -0.1 MPa soil water potential (Figure 52). Figures 53 and 54 clearly show lack of effect of P fertilization on water uptake conductance of 6-month-old Rhizopogon-colonized seedlings in wet soil or 8-month-old Rhizopogon-colonized seedlings in drier soil between about -0.05 and -0.6 MPa soil water potential.

DISCUSSION

Experimental Summary

Two questions were posed in this study:

1) Do ectomycorrhizae affect drought tolerance of Douglas-fir seedlings?
2) What mechanisms account for these changes?

In order to answer these questions, the influence of 3 ectomycorrhizal fungus species on net photosynthesis, stomatal conductance, and water potential components of Douglas-fir seedlings were evaluated under wet soil conditions and under progressive soil drought conditions in a growth chamber. Further interpretive information was derived from measurements of nutrition, growth, and colonization qualities of seedlings when grown under wet soil conditions in the greenhouse. The results of these experiments have been presented in the previous chapter.

Before addressing the thesis questions, the results can be simplified for easy reference. Recall that ectomycorrhizal effects were studied in two experimental blocks. In one block, Rhizopogon vinicolor-colonized plants, which were transplanted at 6 weeks old, were compared with nonmycorrhizal controls also transplanted at 6 weeks old (treatments 2 and 5, see Table 6). In the other block, Laccaria laccata- and Hebeloma crustuliniforme-colonized plants, which were transplanted at 8 weeks old, were compared to nonmycorrhizal controls also transplanted at 8 weeks old (treatments 6, 7, and 8). For the drought stress experiments, comparison of the results for the nonmycorrhizal controls from these two blocks (treatments 2 and 6) reveal that transplant age had no effect on net photosynthesis rate (Figure 8), water potentials (Figure 20), stomatal conductance (Figure 32), or water uptake conductance(Figure 44). Since there was no apparent experimental block effect, the results for Rhizopogon-colonized plants can be directly compared with those for

Laccaria and Hebeloma-colonized plants and their nonmycorrhizal controls. This simplification is shown in Figure 55.

For the wet soil experiments, comparison of nonmycorrhizal controls (treatments 2 and 6) shows that transplant age had no effect on net photosynthesis rate (Figure 7) and water potentials (Figure 19). However, there was an effect on stomatal conductance (Figure 31) which resulted further in differences in calculated water uptake conductance (Figure 43): seedlings transplanted at 6 weeks old had greater stomatal conductance, by about 20%, than seedlings transplanted at 8 weeks old. This effect could be real. However, certain observations suggest that it is due to sampling error:

1) The transplant age difference was observed only for stomatal conductance. If the change in stomatal conductance was real, it would be expected to have significant impact on other parameters such as leaf water potentials. The results clearly show that leaf water potentials were not affected by transplant age.

2) The transplant age difference was observed only in the wet soil experiment. If this effect was real, a change would also be expected in the drought stress experiments at high soil water potentials. The results clearly show that stomatal conductance was not affected by transplant age in the drought stress experiments.

3) Other nonmycorrhizal treatments, representing fertilizer P treatments at rates both greater (treatment 3) and less (treatment 1) than the control treatment 2, exhibited stomatal conductances which were not different from the control treatment 6. There is no apparent biological reason to believe that only the intermediate P-fertilized treatment (treatment 2) would exhibit unique stomatal behavior.

Based upon these observations, the apparent treatment age effect on stomatal conductance (Figure 31) and water uptake conductance (Figure 43) are interpreted here to result from sampling error and not from a real effect. Since there was no real experimental block effect, the results for Rhizopogon-colonized plants can be directly compared with those for Laccaria-colonized and Hebeloma-colonized plants and their nonmycorrhizal controls in the same manner as for the drought stress

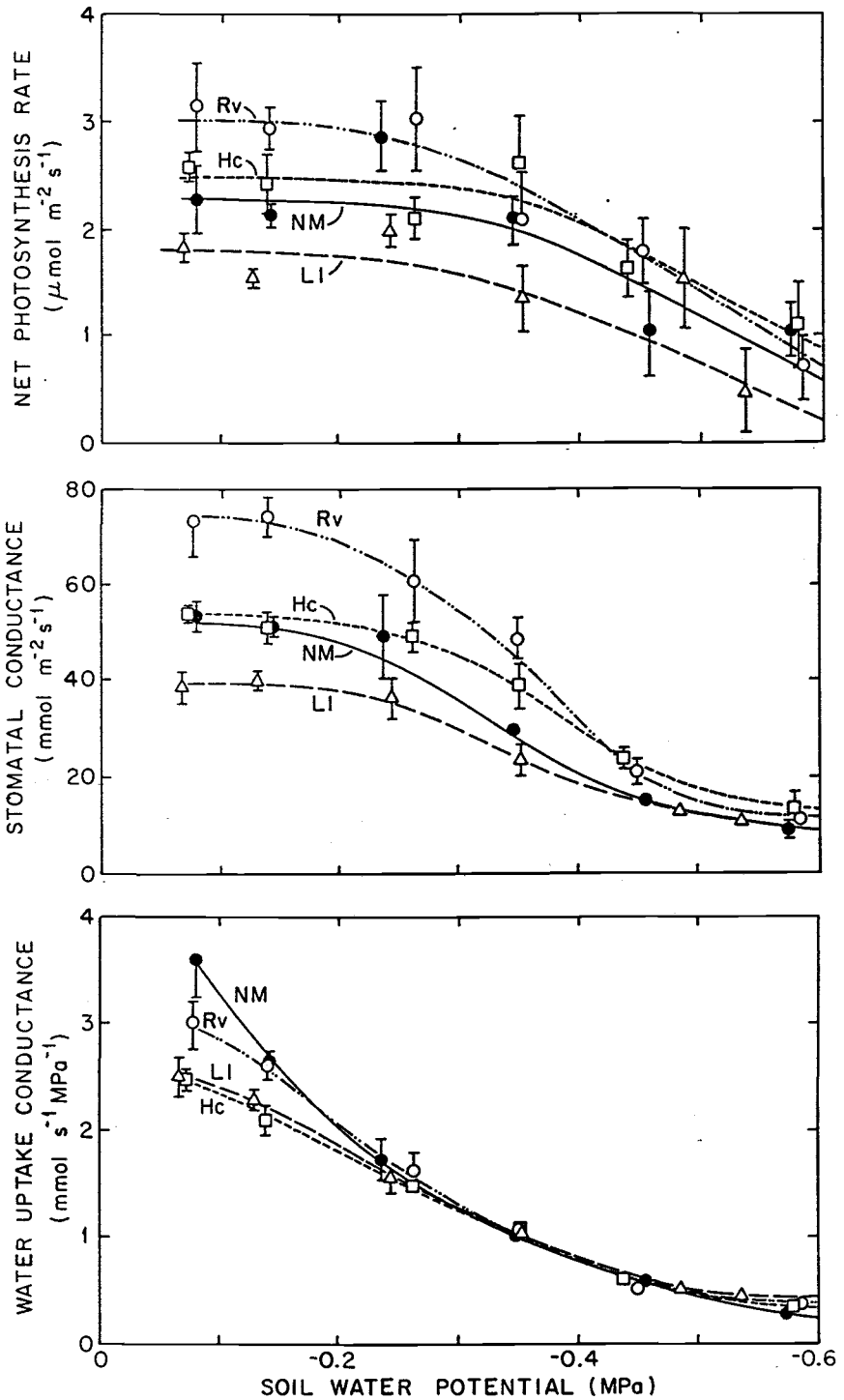


Figure 55. Net photosynthesis rate, stomatal conductance, water uptake conductance, leaf water potential, leaf sap osmotic potential, and leaf turgor pressure as functions of predawn soil water potential for Douglas-fir seedlings: nonmycorrhizal (NM, ●); colonized by Rhizopogon vinicolor (Rv, ○); colonized by Laccaria laccata (Ll, △); colonized by Hebeloma crustuliniforme (Hc, □).

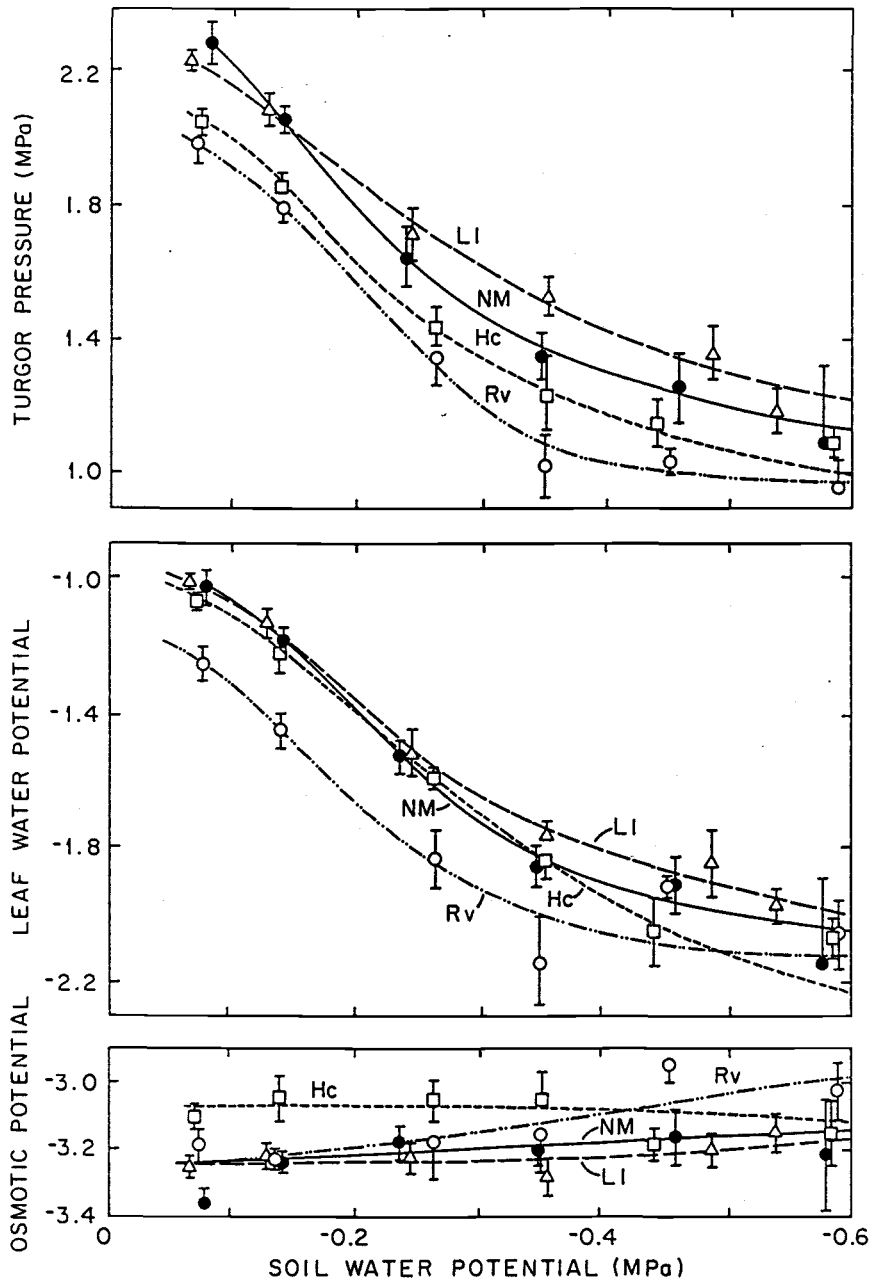


Figure 55 continued.

experiment. This simplification for the wet soil experiment is shown in Figure 56.

Referring to these figures, the questions posed will now be addressed.

Do Ectomycorrhizae Influence Drought Tolerance of Douglas-fir Seedlings?

In this study, drought tolerance is defined as the ability to maintain greater growth-process activity under drought conditions. Net photosynthesis rate was used as the measure of growth-process activity in seedlings, and soil water potential was used as the measure of drought. Figure 55 shows that net photosynthesis rate was clearly higher for Rhizopogon-colonized seedlings, slightly higher for Hebeloma-colonized seedlings, and lower for Laccaria-colonized seedlings than for nonmycorrhizal controls over most of the soil water potential range down to -0.6 MPa. By this measure, Rhizopogon and Hebeloma enhance drought tolerance of Douglas-fir seedlings, while Laccaria is detrimental.

The observed influence of ectomycorrhizae on net photosynthesis rate does not necessarily mean that Rhizopogon and Hebeloma-colonized seedlings will be larger or that Laccaria-colonized seedlings will be smaller when grown under drought conditions. For example, Rhizopogon also stimulated net photosynthesis rate under wet soil conditions (Figure 56), but biomass of these plants was less than for nonmycorrhizal controls (Table 16). This same effect might also be acting under drought conditions. This example illustrates that drought tolerance, as defined in this study, refers to greater growth-related activity under drought, and not to actual plant growth. This definition implies that net photosynthesis rate is related in some way to the ability of plants to minimize other detrimental effects of drought.

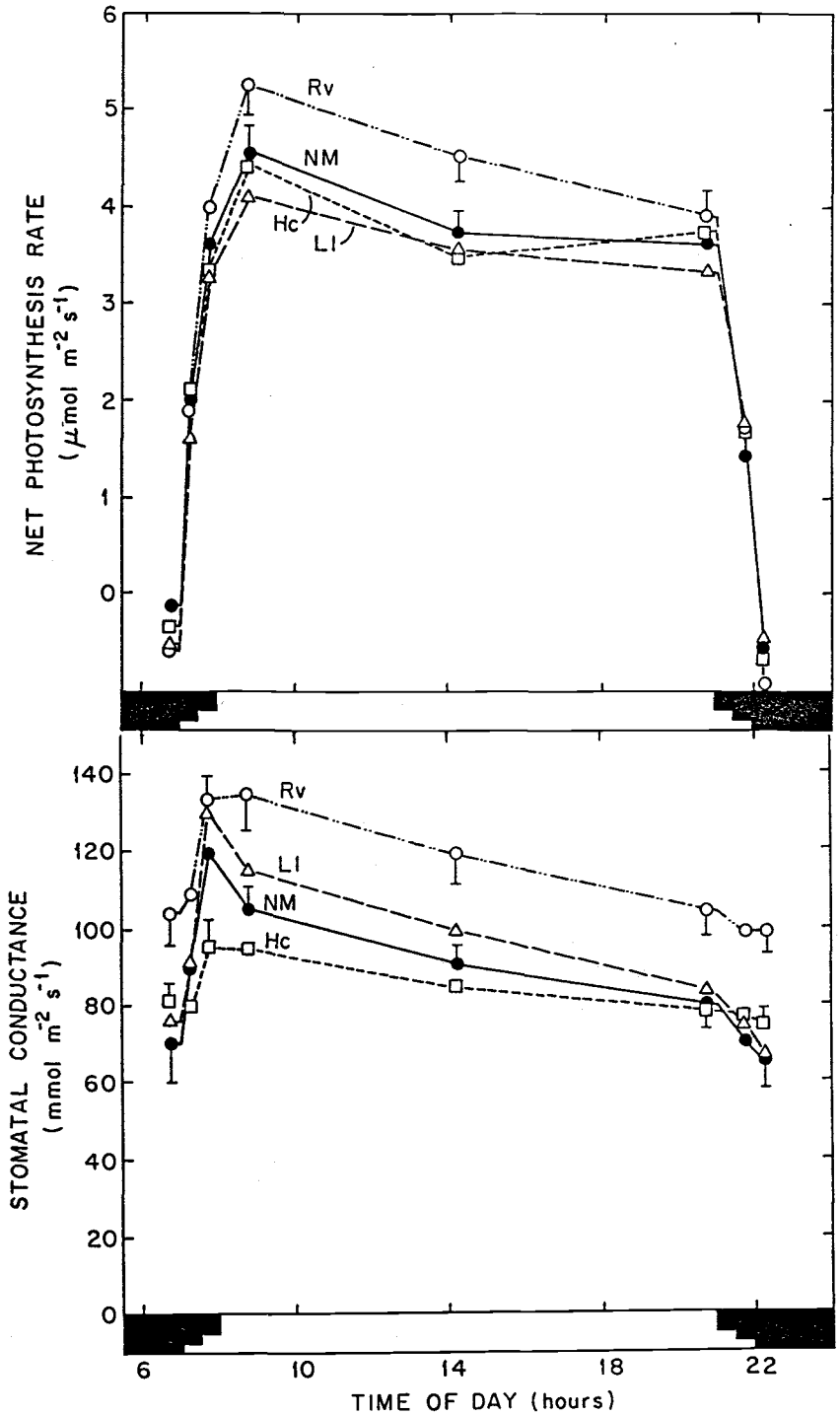


Figure 56. Net photosynthesis rate, stomatal conductance, leaf water potential, leaf sap osmotic potential, and leaf turgor pressure, and water uptake conductance, measured under well-watered soil conditions periodically over the course of one day for Douglas-fir seedlings: nonmycorrhizal (NM, ●); colonized by Rhizopogon vinicolor (Rv, ○); colonized by Laccaria laccata (Ll, △); colonized by Hebeloma crustuliniforme (Hc, □).

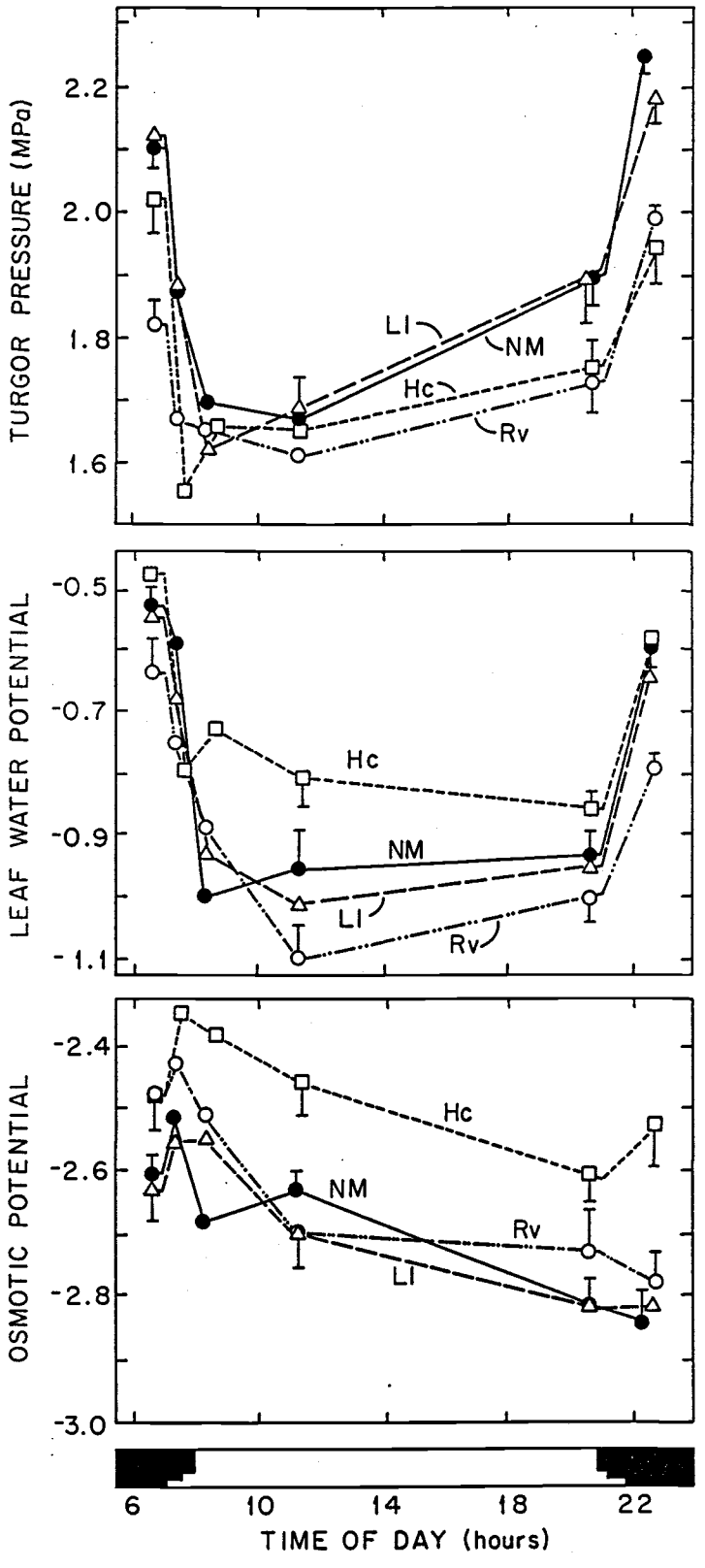


Figure 56 continued.

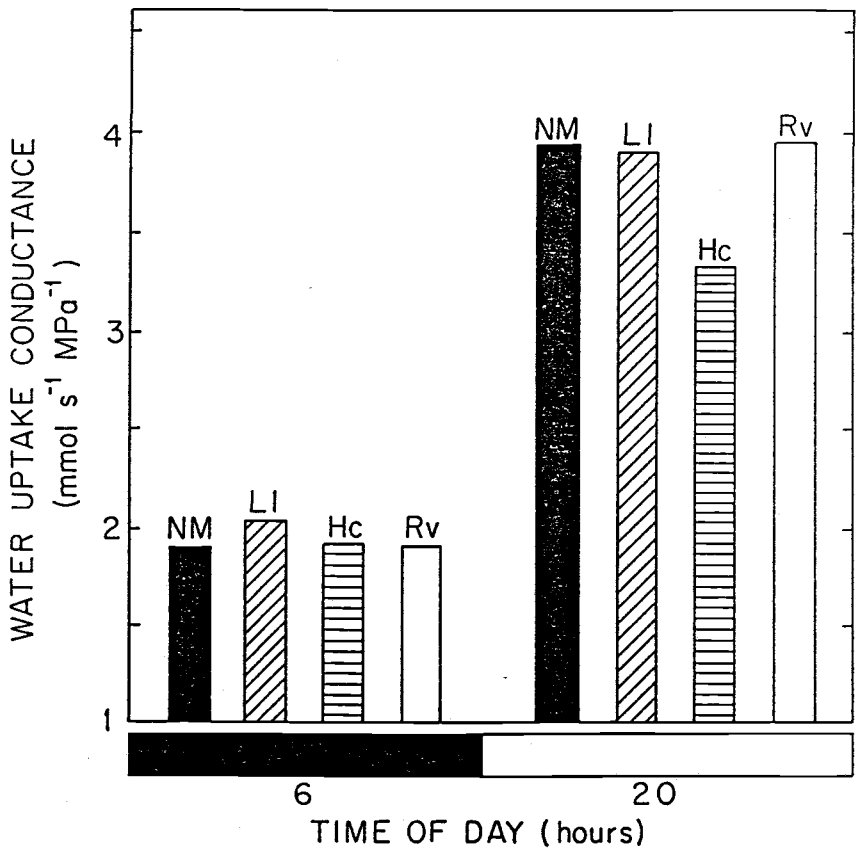


Figure 56 continued.

What Caused the Ectomycorrhizal Effect on Drought Tolerance of Douglas-fir Seedlings?

In order to determine mechanisms responsible for observed ectomycorrhizal influence on the drought tolerance of Douglas-fir seedlings, certain characteristics which are associated with drought tolerance were studied. They are:

1) the ability to minimize plant water stress during soil drought,
2) the ability to maintain greater growth-process activity under plant water stress.

In this study, leaf water potential was used as a measure of plant water stress, where lower leaf water potential is equivalent to greater plant water stress.

Examination of Figure 55 reveals that under limited soil water availability, none of the ectomycorrhizal species tested reduced plant water stress during drought. In fact, Rhizopogon vinicolor colonization lead to increased plant water stress, i.e., lower leaf water potential. Each ectomycorrhizal fungus species tested did, however, influence the relationship between growth activity and plant water stress. In general, Rhizopogon and Hebeloma stimulated net photosynthesis rate while Laccaria tended to suppress it, while leaf water potentials were similar or lower than for nonmycorrhizal seedlings over the entire range of soil water potential. This effect is more clearly visualized in Figure 57, where net photosynthesis rate is graphed as a function of leaf water potential. What is also very important, is that the mycorrhizal effect of each fungal species is evident at all soil water potentials (Figure 55) and leaf water potentials (Figure 57), indicating an overall mycorrhizal effect with similar response (slope) to drought rather than a fundamental change in the response of seedlings to drought.

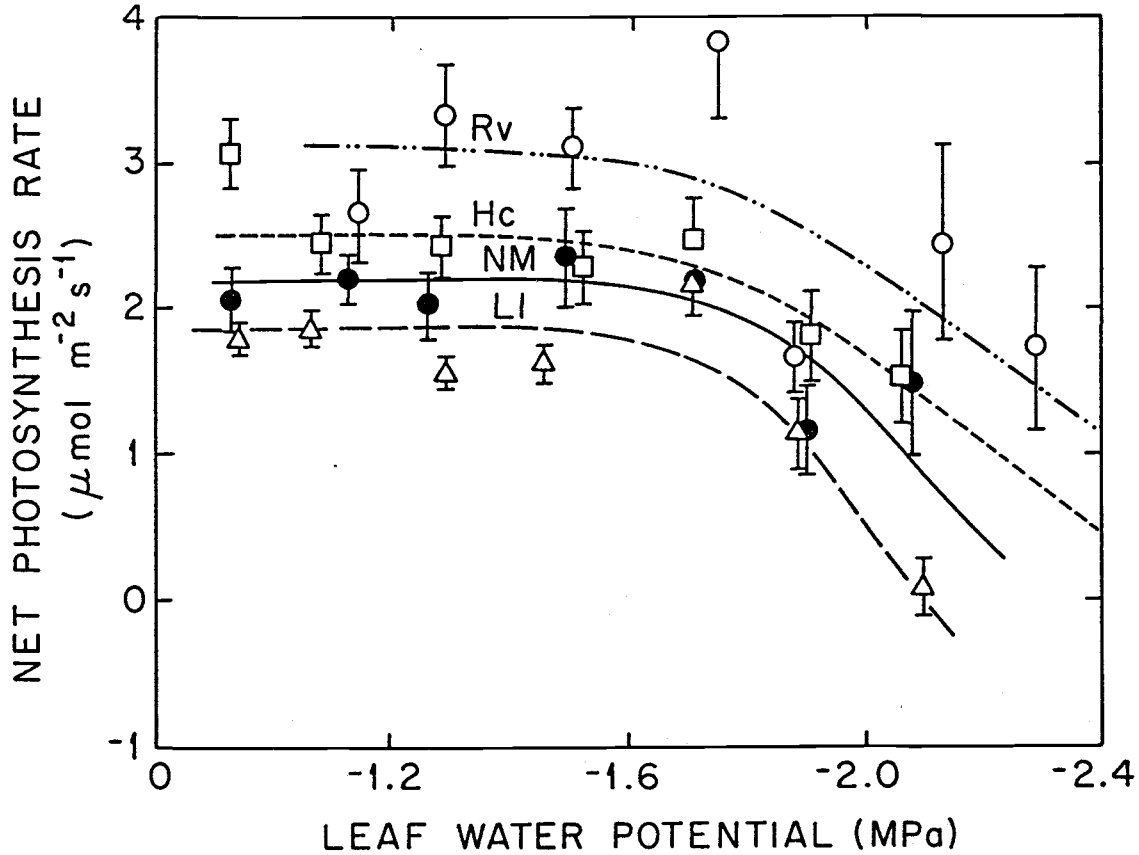


Figure 57. Net photosynthesis rate as a function of leaf water potential for Douglas-fir seedlings: nonmycorrhizal (NM, ●); colonized by Rhizopogon vinicolor (Rv, ○); colonized by Laccaria laccata (Ll, △) or Hebeloma crustuliniforme (Hc, □), . Measurements were made late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

How Did Ectomycorrhizae Change the Relationship Between Net Photosynthesis Rate and Plant Water Stress?

In this study, ectomycorrhizae caused an overall change in leaf net photosynthesis rate. In order for ectomycorrhizae to influence leaf activity there must be a stimulus transmitted between these two organs. Three possible contributors are:

1) mineral nutrient supply to the leaves,
2) plant hormones or other similar biochemical stimuli, and,
3) photosynthate source-sink relations between root system and leaves.

Evidence from this study suggests that ectomycorrhizae could alter photosynthate source-sink relations, and, under certain conditions, this could be the dominant contributor to the observed ectomycorrhizal effect on net photosynthesis rate. According to this hypothesis, ectomycorrhizal growth increases the root system sink for photosynthate from the leaves which, through decreasing sugar concentration throughout the phloem system, stimulates photosynthesis. This hypothesis is based upon three general observations from the literature:

1) mycorrhizal root systems are generally stronger sinks for photosynthate than nonmycorrhizal root systems,
2) photosynthate sink strength can exert control on photosynthesis rate,
3) the presence of mycorrhizae is correlated with higher net photosynthesis rate.

The evidence from this study supporting this hypothesis is more distinctive for Rhizopogon-colonized seedlings than for seedlings colonized by the other fungi, so the discussion will initially focus on Rhizopogon.

It has been shown that mycorrhizal root systems represent a stronger sink for photosynthate than nonmycorrhizal root systems (Harley and Smith, 1983). In the absence of reliable respiration and fungal biomass data, this conclusion was reached indirectly from

observations that net photosynthesis of mycorrhizal plants is higher without a proportional increase in plant biomass compared to nonmycorrhizal plants. Recent work shows that more photosynthate is diverted to mycorrhizal portions of a root system than to comparably sized nonmycorrhizal portions of root systems on the same plant (Koch and Johnson, 1984), thus confirming that mycorrhizal root systems are stronger sinks for photosynthate than nonmycorrhizal root systems. In this study under well-water conditions, Rhizopogon-colonized plants had less plant biomass (Table 16) in spite of higher net photosynthesis rate (Figure 56). These data suggest that Rhizopogon-colonized root systems require more photosynthate than nonmycorrhizal root systems. Since biomass tended to be smaller, it is suspected that the extra photosynthate produced by the plant was respired and/or accumulated into fungal biomass external to the mantel. Furthermore, the effect of each fungal species on net photosynthesis rate appears to correlate with its apparent relative abundance of hyphal growth into the soil surrounding the mycorrhizae. Both Rhizopogon and Hebeloma, which develop abundant hyphal growth, stimulated net photosynthesis rate, while Laccaria, which exhibited little hyphal growth beyond the mantel, did not.

Rate of translocation of photosynthesis to root systems is controlled by the pressure difference between shoots and roots according to the Münch hypothesis (Goeschl et al., 1976; Hall, 1982). The solute concentration of the phloem, which is primarily sucrose, and xylem water potential together determine the gradient in the phloem from the leaves to the roots (Canny, 1984). If mycorrhizal root systems represent a stronger sink relative to the photosynthate source, we would expect to find larger phloem pressure gradients in mycorrhizal plants. We would also expect to find lower solute concentrations throughout the phloem and cytosol system.

The hypothesis just stated can be tested using the experimental results. Rhizopogon-colonized seedlings had higher leaf sap osmotic potential over most of the day under wet soil conditions (Figure 56). From this observation it is deduced that Rhizopogon-colonized root systems represent a stronger sink for photosynthate than

nonmycorrhizal root systems. Since the rate of translocation is determined by pressure differences rather than by absolute pressure, the process remains little affected by plant water stress (Canney, 1984). Thus, we expect the photosynthate sink strength of Rhizopogon-colonized root systems to remain larger even under drought conditions, so long as mycorrhizal activity is maintained. Figure 55 confirms this for Rhizopogon, showing similar or lower leaf sap osmotic potential in spite of higher net photosynthesis rate over the soil water potential range -0.05 to -0.60 MPa.

Data in this study show that Rhizopogon colonization is correlated with higher net photosynthesis rate (Figures 55 and 56). Sink strength can exert control on rate of photosynthesis (Sweet and Waring, 1967; Herold, 1980). Herold (1980) discussed three ways in which high photosynthate sink strength of root systems could stimulate the rate of photosynthate in leaves. These are:

1) improved leaf mineral nutrition resulting from redistribution of nutrients within the plant, independent of root uptake,
2) enhanced production and translocation of hormones to leaves,
3) reduced sucrose concentration in leaves.

In this study, the data support the third alternative insofar as higher leaf sap osmotic potential in mycorrhizal seedlings reflects a reduction of sucrose concentration. No significant differences in leaf tissue concentration of N, P, K, or Ca were found between Rhizopogon-colonized seedlings and nonmycorrhizal controls (Table 14). In the experiment where supplemental P fertilization of nonmycorrhizal plants produced a doubling of leaf tissue P concentration, there was little change in rate of net photosynthesis (Figure 58). Thus, even if the small nutritional changes associated with Rhizopogon colonization were real, they probably had negligible effect on net photosynthesis rate. These observations suggest that altered nutrition was not involved here.

There remains the possibility that plant hormones synthesized in the roots may influence rate of photosynthesis. The synthesis of some hormones is stimulated by increased root metabolism rate, and most major hormone groups can directly affect photosynthesis rate (Herold,

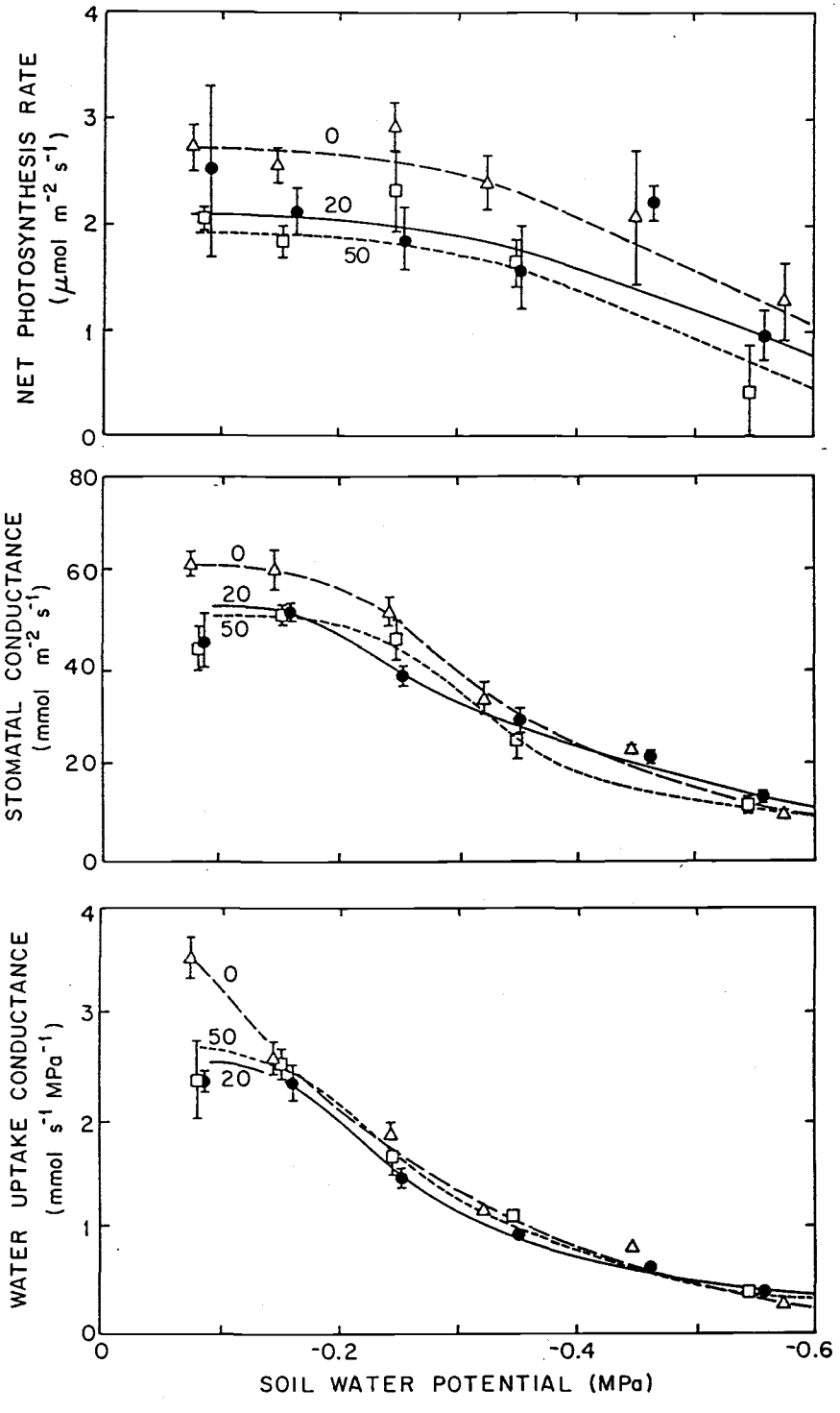


Figure 58. Net photosynthesis rate, stomatal conductance, water uptake conductance, leaf water potential, leaf sap osmotic potential, and leaf turgor pressure as functions of predawn soil water potential for Douglas-fir seedlings fertilized with solutions of 0 (△), 20 (●), and 50 (□) mg P kg^{-1}.

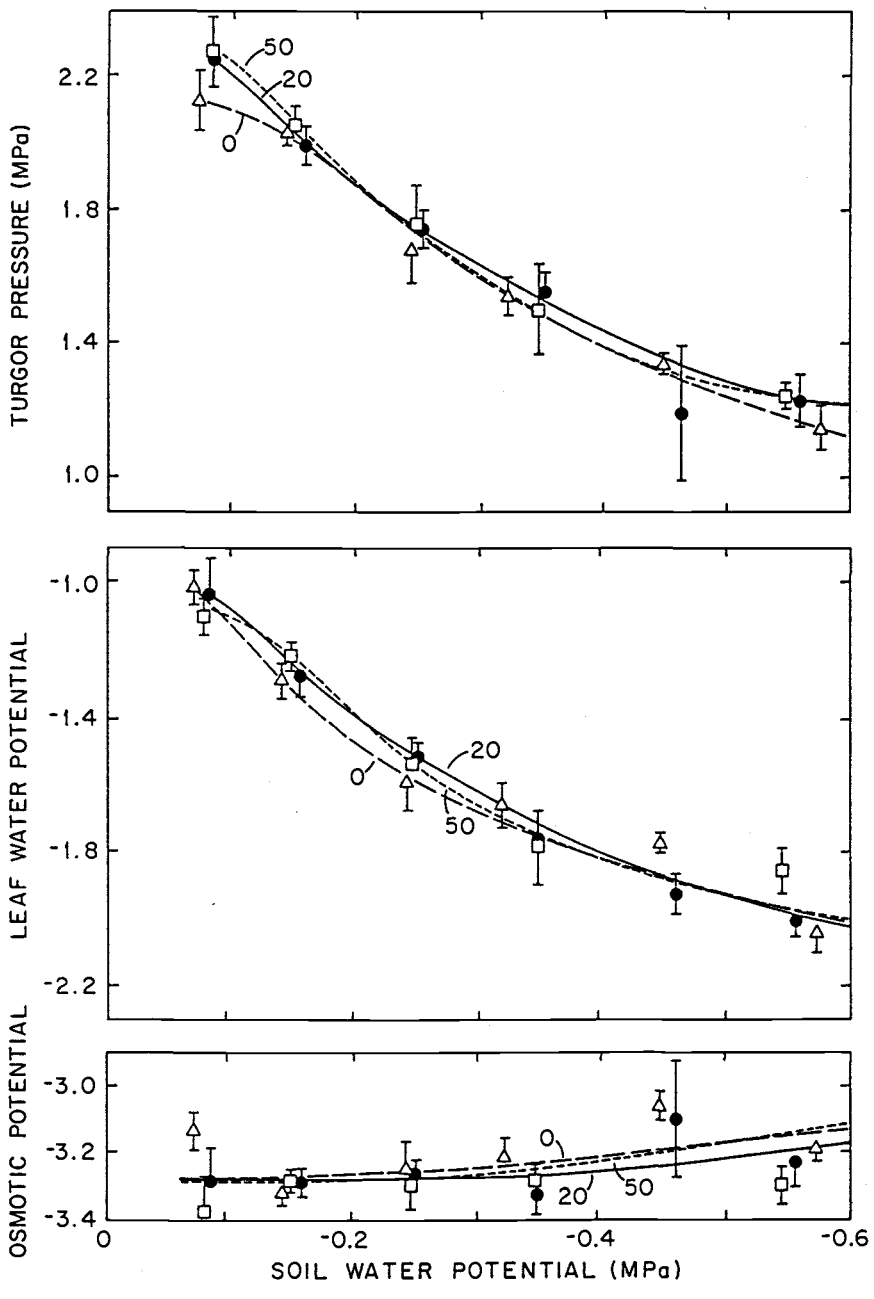


Figure 58 continued.

1980). However, there is not sufficient information to postulate that metabolism of root tissue is stimulated by mycorrhizal fungi. The possibility that phytohormones synthesized by the fungus may play a role has not been adequately investigated. Thus, it cannot be concluded whether our observations are due to hormonal regulation or directly to reduced photosynthate levels in leaves.

In the absence of a photosynthate sink mechanism, enhanced mineral nutrient uptake or hormone production by mycorrhizae could also influence net photosynthesis rate. Each of these latter two mechanisms alone, however, cannot account for our observations for Rhizopogon-colonized seedlings. If photosynthesis was stimulated directly without concurrent increase in sink strength, then we would expect to observe a higher net photosynthesis rate combined with lower leaf sap osmotic potential and greater plant biomass. Data from this study clearly shows higher leaf sap osmotic potential and lower plant biomass for Rhizopogon-colonized seedlings compared to nonmycorrhizal controls. Thus, these results are consistent with the hypothesis that Rhizopogon colonization stimulated net photosynthesis through the enhancement of photosynthate sink strength.

While evidence for a photosynthate sink mechanism is more clear for Rhizopogon, some elements of this mechanism are also observed for Hebeloma. Hebeloma colonization was also associated with a tendency for higher net photosynthesis rate for the same level of plant water stress (Figure 57). Under well-watered conditions, significantly less plant growth (Table 14) and higher leaf sap osmotic potential were observed in spite of negligible change in net photosynthesis rate (Figure 56). Under drought conditions, higher leaf sap osmotic potential was still observed in association with some stimulation in net photosynthesis rate (Figure 57). If the photosynthate sink mechanism is operating here as hypothesized, the smaller net photosynthesis stimulation at a greater increase in leaf sap osmotic potential for Hebeloma-colonized plants than for Rhizopogon-colonized plants, could result from:

1) variability in the correlation of leaf sap osmotic potential and soluble sugar, especially sucrose, concentration,

2) interaction of increased leaf nutrient concentration (Table 14) and the photosynthate sink activity of the fungus,

3) Interaction with some other factor such as plant hormones.

The first possibility is quite likely since the contribution of sucrose to total symplast osmoticum is much less than that in the phloem alone. The second possibility implies the interactive nature of nutrition and photosynthate source-sink relationships, since it appears that higher mineral nutrient concentration in leaves of Hebeloma-colonized seedlings resulted from suppressed growth due to photosynthate diversion to the fungus. In the experiment where nonmycorrhizal plants were fertilized at different levels of P, fertilization lead to higher leaf P concentration but lower net photosynthesis rate and no change in leaf sap osmotic potential (Figure 58) as though sink strength was reduced. If this enhanced nutrition effect is acting along with a photosynthate sink effect in Hebeloma-colonized seedlings, then we would expect lower net photosynthesis rate and higher leaf sap osmotic potential than if the photosynthate sink strength effect were acting alone. In this study, Hebeloma-colonized seedlings did show less net photosynthesis stimulation in spite of higher leaf sap osmotic potential compared to Rhizopogon-colonized seedlings. While the data from this experiment are consistent with this second hypothesis, it remains to be confirmed through more rigorous examination of the interaction between mineral nutrient and carbon economy of mycorrhizal plants.

Unlike Rhizopogon and Hebeloma, Laccaria colonization was correlated with lower net photosynthesis rate for the same level of plant water stress under drought conditions (Figure 57). At the same time, leaf sap osmotic potential remained unchanged relative to nonmycorrhizal plants. This suggests that Laccaria colonization represented a smaller photosynthate sink than the other fungi. Clearly, the Laccaria fungus did not affect the carbon balance of the host in the same way as Rhizopogon did. Laccaria-colonized plants had the lowest leaf N concentrations of all treatments in these experiments, although not significant statistically (Table 14). It might be that the Laccaria effect is due to the onset of a

photosynthesis-inhibiting N deficiency. This hypothesis remains totally unconfirmed.

How Did Ectomycorrhizae Change the Relationship Between Plant Water Stress and Soil Water Potential?

Figure 55 shows that xylem water potential was clearly lower for Rhizopogon-colonized seedlings than for nonmycorrhizal seedlings over a wide range of soil water potentials, while Hebeloma and Laccaria colonization did not result in any significant change. A change in water potential in the plant is the result of a change in the balance between the capability for water uptake and the ease of water loss. In this study, the ease of water loss was quantified by stomatal conductance, and the capability for water uptake was quantified by water uptake conductance, which was calculated as the transpiration rate per unit water potential difference between bulk soil and leaves. By evaluating these parameters we gain some insight regarding the cause of mycorrhizal effects on plant water stress.

Stomatal Conductance

In general, the ectomycorrhizal effect on stomatal conductance closely correlates with the mycorrhizal effect on net photosynthesis rate (Figures 55 and 56). Greater stomatal conductance correlates with higher net photosynthesis rate for Rhizopogon and Hebeloma-colonized plants and lower stomatal conductance correlates with lower net photosynthesis rate for Laccaria-colonized seedlings over the range of drought stress investigated in this study (Figure 55). Farquhar and Sharkey (1982) suggest a functional relationship between stomatal conductance and net photosynthesis rate. Based on the study of Wong et al. (1979) and other studies, the hypothesis was made that stomatal

conductance is controlled by net photosynthesis rate in such a way as to maintain nearly constant CO_2 concentration in the intercellular air spaces of the leaves. Theoretically, stimulation of net photosynthesis rate lowers CO_2 concentration in the stomatal cavity to a level where several possible CO_2 "sensing" metabolites would stimulate guard cells to adjust to a more open position in order to maintain stable internal CO_2 concentration. Data from this study are consistent with this concept in that the relationship between stomatal conductance and net photosynthesis rate is unchanged by mycorrhizal colonization (Figure 59). Through this mechanism, stimulated net photosynthesis rate could translate to greater stomatal conductance.

A hypothesis commonly put forth in the literature is that lower plant water potential and higher stomatal conductance could be due to osmotic adjustment. But this did not occur in this study. In fact, osmotic potentials were higher for mycorrhizal plants and not lower as the osmotic adjustment hypothesis would require, and did not change much in response to drought stress.

Water Uptake Conductance

For ectomycorrhizal seedlings, water uptake conductance under soil water-limiting conditions was not much different from nonmycorrhizal plants (Figure 55). For Rhizopogon and Hebeloma-colonized seedlings, this occurred in spite of significantly reduced root length. Higher transpiration rate and less root length would be expected to cause lower water uptake conductance under otherwise similar drought conditions (McCoy et al., 1983). Thus, it appears that these ectomycorrhizal root systems are more efficient at water uptake from soils with limited water availability. Hyphal transport of water to ectomycorrhizae is a likely mechanism for this increased efficiency (Harley and Smith, 1983), but on a whole plant basis, this just offsets lower root length and higher transpiration rate to yield no net benefit to water uptake. In wetter soil (Figures

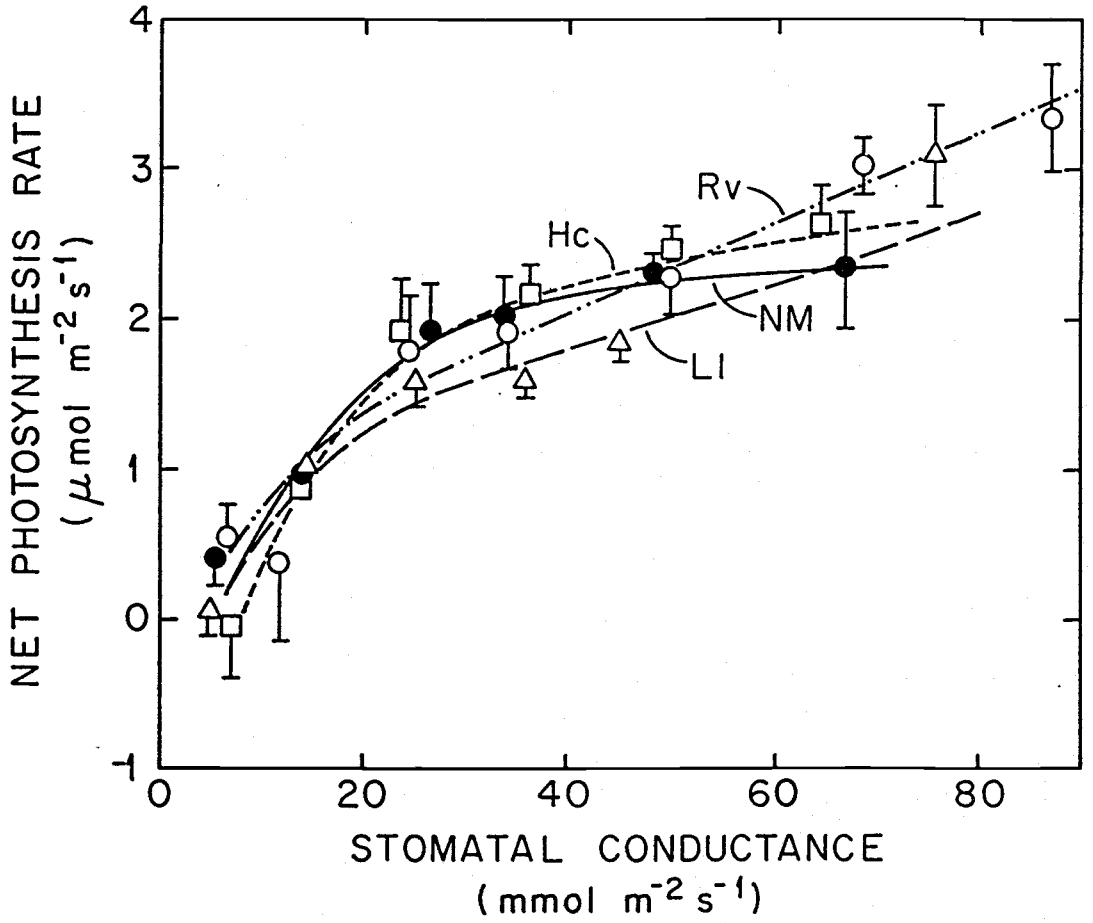


Figure 59. Net photosynthesis rate as a function of stomatal conductance for Douglas-fir seedlings exposed to various levels of soil dryness: nonmycorrhizal (NM,); colonized by Rhizopogon vinicolor (Rv,); colonized by Laccaria laccata (Ll,); colonized by Hebeloma crustuliniforme (Hc,). Measurements were made late in the day at about 1930 hours. The points are means with standard errors, and the lines are hand-fit to the data.

55, 56), less root length appeared to cause a trend toward lower water uptake conductance in ectomycorrhizal plants.

Reduced root length was a common attribute of Rhizopogon- and Hebeloma-colonized plants in this study when grown under wet soil conditions. Three requirements for root growth are:

1) photosynthate supply for root metabolism and structure,
2) turgor for elongation,
3) minimal resistant force to elongation.

Measurements in this study clearly indicate lower turgor in Rhizopogon- and Hebeloma-colonized seedlings (Figure 55 and 56). Both Rhizopogon and Hebeloma colonization showed signs of photosynthate diversion away from the roots. As a result, osmotic potential was high, and since xylem water potential was the same or lower, turgor was lower. Furthermore, a well-developed mycorrhizal mantel, such as observed for Rhizopogon, might provide a significant resistance to root elongation where it encloses root apices. Thus, all three of these factors could have contributed to suppressed root growth of mycorrhizal seedlings in this study.

Overall, the ectomycorrhizal effect on water uptake conductance from dry soil was negligible. Greater stomatal conductance without any net water uptake benefit lead to lower xylem water potential in Rhizopogon-colonized plants (Figure 55). This effect was smaller for Hebeloma-colonized seedlings and was not apparent for Laccaria-colonized seedlings.

Summary

This study shows that ectomycorrhizae can affect Douglas-fir seedling response to drought through its effect on water uptake, photosynthesis, stomatal conductance, and water potentials. There could be several independent mechanisms which act together to cause all of the observed changes. However, evidence presented here is consistent with the hypothesized mechanism that all of these effects are functionally connected and arise from an ectomycorrhizal effect on

photosynthate sink strength. An altered plant carbon economy could account for all of these observations through a reasonable sequence of causes and effects. It is hypothesized that:

1) ectomycorrhizal colonization represents a large additional photosynthate sink for the plant,
2) enhanced photosynthate sink strength simulates photosynthesis rate,
3) higher net photosynthesis rate stimulates stomata to adjust to a more open position,
4) higher transpiration rate, resulting from greater stomatal conductance, without any net benefit to water uptake, leads to lower xylem water potential.

This sequence appears to be most pronounced for Rhizopogon-colonized seedlings and occurs in the absence of any change in leaf mineral nutrition. It is less pronounced for Hebeloma, perhaps because of an interaction with a change in leaf mineral nutrition, which itself is a consequence of photosynthate diversion. Laccaria does not affect the carbon economy of the seedling in the way that Rhizopogon and Hebeloma do. The magnitude of this photosynthate sink effect between fungal species appeared to correlate with the visual abundance of hyphal growth which each of these fungal species developed.

How Does the Mycorrhizal Effect on Drought Tolerance Characteristics in This Study Relate to Seedling Drought Tolerance?

In The Laboratory

In this study, drought tolerance was evaluated by the magnitude of net photosynthesis rate. By this measure Rhizopogon clearly improved, Hebeloma slightly improved, and Laccaria was detrimental to the drought tolerance of Douglas-fir seedlings. Higher net photosynthesis rate is hypothesized here to result from photosynthate

diversion to the colonizing fungi. Since higher net photosynthesis rate due to this mechanism doesn't lead to greater plant growth, it is presumed that it is indicative of physiological activities which minimize other detrimental effects of drought stress on seedlings. Parke et al. (1983) found that higher net photosynthesis rates under drought stress correlated with quicker recovery from drought stress for Rhizopogon vinicolor-colonized Douglas-fir seedlings, lending some support to this presumption.

Factors which lead to mycorrhizal enhancement of drought tolerance in plants may have a temporal aspect. Since photosynthate is diverted into fungal structures of significant longevity, it may represent an investment which will yield plant growth-stimulating benefits at some future time. Although photosynthate might be continually diverted to the fungus, benefits such as enhanced water and nutrient uptake by fungal hyphae might increase as fungal biomass increases. In this way, mycorrhizae-derived benefits may lag behind a period of initial photosynthate investment. This implies that the net influence of mycorrhizae which we observe in the plants is an ever changing feature of these symbioses. This temporal concept might explain some of the widely varying and sometimes conflicting results among different studies regarding the mycorrhizal influence on plant growth and physiological responses to water stress.

In The Field

Results from this laboratory study are applicable to field conditions, but this must be done in a cautious manner. This study shows that certain characteristics of plant drought tolerance are influenced by mycorrhizal colonization. Conditions in this study fortuitously minimized the mineral nutrition factor and brought out nonnutrition-related effects of mycorrhizal colonization. Under different conditions, such as different fungi, soil, or fertilizer, it is likely that these nonnutritional effects would be brought out to

different degrees and interact with nutritional effects as well as other mechanisms to produce different net mycorrhizal effects. Field conditions present still other possibilities for mycorrhizal enhancement of drought tolerance which were not tested in this laboratory study. For example:

1. Conducting a pot study eliminated the possibility for long distance fungal transport of water and nutrients from distant sources such as rotten logs or deeper horizons of wet soil. This experimental system also eliminated any possibility of interplant connections whereby an established "nurse" plant could provide photosynthate to the growing fungus during initial colonization of the seedlings.

2. This laboratory study was conducted under lower light intensities than normally occur in the field. Higher light intensity could speed photosynthesis beyond the fungal needs, thereby reducing the relative impact of photosynthate diversion to the fungus.

3. As discussed in a previous section, many benefits of mycorrhizal colonization might not become clear until after mycorrhizae become well-established. This implies a period of initial photosynthate investment followed by many years, in the case of trees, of benefits derived from the symbiosis. This study was restricted to the first season of growth and colonization.

4. Tolerance of Douglas-fir seedlings to extreme water stress, as might occur during protracted drought in the field, was not tested in this study. Further work is needed to determine if mycorrhizal colonization leads to a change in susceptibility to damage from extreme water stress and damage repair mechanisms in the plant.

5. High temperatures are commonly associated with drought in the field and may interact with drought tolerance mechanisms. Temperature interaction was not examined in this study.

6. In this study, pasteurized soil was used to eliminate root pathogens. In the field, plant root pathogens might damage nonmycorrhizal roots. Greater damage to nonmycorrhizal root systems could lead to a different outward mycorrhizal effect relative to these damaged nonmycorrhizal plants.

These examples illustrate that the relative effect of mycorrhizal colonization on drought tolerance of seedlings in the field can be quite different from that found in this study. This can be attributed to additional conditions present in the field which mycorrhizae can exploit. Some of these conditions are exploited in predictable ways. However, fungal species probably differ in capacity to exploit them. In spite of these uncertainties, this laboratory study helps us understand the fundamental mechanisms acting in the mycorrhizal symbiosis. Such a basic understanding will give us some framework upon which we can build a more complete predictive model of ectomycorrhizal influence on Douglas-fir response to drought.

CONCLUSIONS

The objective of this study was to determine if ectomycorrhizae influence the drought tolerance of Douglas-fir seedlings, and if so, identify the mechanisms which are responsible for this effect. The conventional concept, drawn mainly from studies on VA mycorrhizae with crop plants, attributes enhanced drought tolerance to:

1) enhanced water uptake through the hyphal system,
2) improved mineral nutrition through hyphal uptake,
3) greater osmotic adjustment response to drought stress.

However, a growing number of observations, particularly from studies of ectomycorrhizal trees, cannot be explained by these mechanisms. These inconsistencies probably result from some other mycorrhizal factor which has not been considered. Pot experiments were conducted in a laboratory to test these concepts for ectomycorrhizal Douglas-fir seedlings. Three different species of ectomycorrhizal fungi were examined.

In this study, ectomycorrhizal colonization did effect drought tolerance of Douglas-fir seedlings as indicated by changes in net photosynthesis rate. Rhizopogon vinicolor greatly enhanced net photosynthesis rate of seedlings, Hebeloma crustuliniforme slightly enhanced it, and Laccaria laccata was detrimental. In all cases, the mycorrhizal effect on photosynthesis was general with respect to level of drought stress, since it occurred under wet and dry soil conditions, rather than as a response to dry soil.

It is hypothesized in this study, that enhanced net photosynthesis rate arises out of the additional photosynthate demand of the growing fungus. According to this hypothesis:

> The mycorrhizal root system represents a greater photosynthate sink than a similar but nonmycorrhizal root system. Faster photosynthate transport to this root system feeds back to the chloroplasts, perhaps through decreased sucrose concentration in the phloem system, to stimulate photosynthesis.

Other features of colonization which were observed could also arise out of the mycorrhizal demand for photosynthate. Extending the hypothesis:

> Stimulated photosynthesis rate leads to greater stomatal conductance and transpiration rate, probably through a corresponding stomatal opening adjustment in order to maintain a certain CO_2 concentration in the stomatal cavity. At the root system, the negative impact of faster transpiration rate and less root length on the ability of seedlings to take up water is offset by the greater efficiency of water uptake by mycorrhizae to yield no net change in water uptake ability. Faster transpiration without a corresponding improvement in water uptake capability leads to lower plant water potential.

This mechanism functions under wet soil and soil water-limiting conditions, and at a level which is dependent upon the magnitude of the additional photosynthate sink and which can interact with other consequences of mycorrhizal colonization.

The results of this study show that the effect of Rhizopogon colonization on Douglas-fir seedlings was consistent with this photosynthate sink strength mechanism, and occurred in the absence of any nutritional effect. Hebeloma colonization also showed evidence of a strong photosynthate demand, but there appeared to be an interaction with enhanced leaf mineral nutrition caused by growth stunting. Laccaria colonization, on the other hand, showed little evidence for altered photosynthate demand, or of nutritional changes which could account for suppressed net photosynthesis rate.

This laboratory study was designed to minimize the number of possible mycorrhizal factors which could influence plant response to drought. Applicability of these results to field conditions will require consideration of the influence of these other factors. The results of this study, however, provide a fundamental basis for this application, as it indicates the physiological ramifications of

mycorrhizal influence on the carbon economy of plants, particularly as they affect plant growth and response to drought stress.

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