THE ROLE OF ECTOMYCORRHIZAS IN DROUGHT TOLERANCE OF DOUGLAS-FIR SEEDLINGS*

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(*Accepted 11 April 1983*)

SUMMARY

Experiments were conducted to test the relative ability of mycorrhizal and non-mycorrhizal Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seedlings to tolerate and recover from drought conditions, using reduction in CO_2 fixation as an overall indicator of plant moisture stress. Seedlings were watered daily or conditioned to cyclic drying and re-wetting of the soil. Net photosynthetic rates of mycorrhizal and non-mycorrhizal seedlings watered daily did not differ significantly; however, drought-stressed mycorrhizal seedlings fixed CO_2 at a rate ten times that of non-mycorrhizal seedlings. Total leaf water potentials of mycorrhizal plants were lower (more negative) than those of non-mycorrhizal plants but they recovered more rapidly.

Non-mycorrhizal seedlings and seedlings inoculated with four ectomycorrhizal fungus species were allowed to become desiccated, then were rewatered and compared for their ability to tolerate and recover from drought. Seedlings inoculated with *Rhizopogon vinicolor* were less affected by drought than any of the other mycorrhizal or non-mycorrhizal treatments. Net photosynthetic rate of *Rhizopogon*-inoculated seedlings 24 h following re-watering was seven times that of non-mycorrhizal seedlings. The transpiration rate of *Rhizopogon*-inoculated seedlings was low before desiccation, declined rapidly during the drought period and, after re-watering, quickly resumed a rate higher than that for other treatments.

INTRODUCTION

In southern Oregon and northern California, container-grown or bare-root conifer seedlings are planted on reforestation sites in the winter and spring. First-year mortality of these seedlings can approach 100% on harsh sites (Herman, 1965). Plant moisture stress is probably the single most important cause in southern Oregon (Hermann, 1965, 1977; Cleary, 1971; Heiner and Lavender, 1972; Froehlich, 1977; Cleary, Greaves and Hermann, 1978; Williamson and Minore, 1978; Gratkowski, Jaszowski, and Armstrong, 1979; Hobbs *et al.*, 1980), owing to low water-holding capacity of some soils, shallow root systems, infrequent summer precipitation and warm temperatures (Hobbs *et al.*, 1989; Johnson and

* Technical Paper No. 6638 of the Agricultural Experiment Station, Oregon State University, in cooperation with the Agricultural Research Service, US Department of Agriculture.

0028-646X/83/090083+13 $03.00

Beschta, 1980; Kandiko, Timmis and Worral, 1980). Considerable effort and expense has been directed towards reducing plant competition for limited soil water through elimination of non-conifer species using herbicides and manual brush control. At the time of outplanting, conifer seedlings are generally non-mycorrhizal or colonized by fungi not necessarily adapted to forest soils. Inoculum density and viability of native ectomycorrhizal fungi may be reduced as a result of timber harvest and soil disturbance (Parke, Linderman and Trappe, 1983a; Worley and Hacskaylo, 1959).

Although ectomycorrhizas are commonly assumed to enhance water uptake by their hosts (Trappe, 1977; Trappe and Fogel, 1977; Ruehle and Marx, 1979), few researchers have addressed this experimentally (Reid, 1979). Some mycorrhizal fungi grown *in vitro* grow or at least survive at water potentials below the permanent wilting point of their host (Uhlig, 1972; Theodorou, 1978) although tolerance to low water potentials varies widely among species (Mexal and Reid, 1973; Theodorou, 1978). Cromer (1935) found that mycorrhizal seedlings of *Pinus radiata* resumed growth more quickly than non-mycorrhizal seedlings following re-watering after drought stress. Theodorou and Bowen (1970) observed that outplanted *P. radiata* seedlings inoculated with *Suillus granulatus* or *Rhizopogon luteolus* survived a particularly dry summer better than uninoculated seedlings. Maronek and Hendrix (unpublished data as cited in Maronek, Hendrix and Kiernan, 1981) reported that greenhouse-grown non-mycorrhizal oak seedlings wilted more readily than seedlings inoculated with *Pisolithus tinctorius*. Dixon *et al.* (1980) showed that, although *Quercus alba* seedlings inoculated with *Pisolithus tinctorius* and subjected to drought conditions had xylem potentials lower than non-mycorrhizal seedlings, root growth during the drying period was greater. Following re-watering, xylem potential recovered to normal levels more rapidly among mycorrhizal seedlings. In a pot experiment in which water was withheld from mycorrhizal and non-mycorrhizal *Pinus radiata* seedlings, Sands and Theodorou (1978) found that resistance to water flow from the soil through mycorrhizal plants was greater than for non-mycorrhizal plants, due largely to differences in root geometry. Leaf water potentials of mycorrhizal seedlings were lower than for non-mycorrhizal seedlings.

It is well established that net photosynthesis declines in response to plant water stress, mainly owing to stomatal closure that accompanies loss of turgor (Boyer, 1976). The present experiments were conducted to test the relative ability of mycorrhizal and non-mycorrhizal container-grown Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seedlings to tolerate and recover from drought conditions.

MATERIALS AND METHODS

Experiment 1: *Effect of native ectomycorrhizal fungi on growth and tolerance of Douglas-fir seedlings to periodic desiccation.* In this experiment, net CO_2 fixation by mycorrhizal and non-mycorrhizal Douglas-fir seedlings conditioned to cyclic drying and re-wetting of the soil was compared with that of mycorrhizal and non-mycorrhizal seedlings watered daily.

Forest soil known to contain an abundance of ectomycorrhizal propagules was collected near Roseburg, Oregon (Boomer Hill study site). The site is an old-growth mixed conifer stand dominated by *Pseudotsuga menziesii* (Douglas fir). Soil was sieved through 1 cm^2 mesh to remove pebbles and large organic debris and mixed

1:1 (volume basis) with coarse-grade vermiculite. A portion of soil–vermiculite mix was left untreated; the remaining portion was treated with areated steam (65 °C/30 min) to eliminate mycorrhizal fungal propagules without altering soil nutrient status. Both soils were sown with surface-sterilized (30% H_2O_2/30 min) Roseburg area Douglas-fir seed in 50 cm^3 tubes (Ray Leach Cone-tainer Nursery, Canby, Oregon). Plants were maintained in the greenhouse under high-pressure sodium vapour lamps (16 h photoperiod) with a 22 °C day/18 °C night temperature. All seedlings were watered daily for 4 months and received 3 ml Long-Ashton nutrient solution (Hewitt, 1966) at 1/4 phosphorus levels (11 p.p.m.) every 5 days to supplement the low phosphorus availability in the soil–vermicuite mixture (7 p.p.m. Olsen available phosphorus). Four months after planting, randomly selected seedlings from each treatment were harvested and examined for mycorrhizal fungus colonization. Seedling shoot length, root and shoot dry weight were also recorded. The remaining seedlings were randomly assigned to one of two watering regimes: watered daily or watered every fifth day. At 7 months, net photosynthetic rates, leaf water potential, seedling growth and percentage mycorrhizal colonization were recorded.

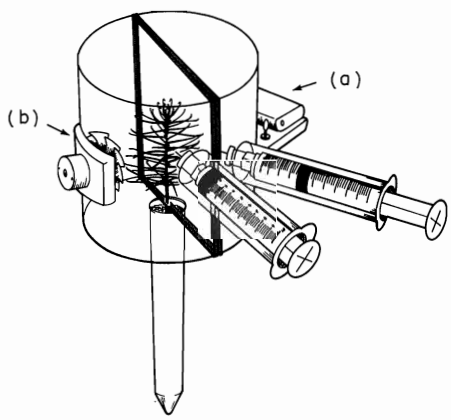

Fig. 1. Hand-held plexiglass photosynthesis chamber for measuring *in situ* CO_2 fixation rates of Douglas-fir seedlings. (a) Battery-operated, (b) fan circulates air during fixation periods.

Net CO_2 fixation was used as an overall indicator of physiological responses associated with plant water stress and recovery (Odening, Strain and Oechel, 1974). A portable plexiglass chamber modified from Cary (1977) was constructed to enclose individual Douglas-fir shoots *in situ* (Fig. 1). Syringes inserted into the sealed chamber permitted collected of air samples at the beginning and end of 2·5 min fixation periods; these were analysed for CO_2 concentration using an infrared gas analyser (Clegg, Sullivan and Eastin, 1978) calibrated daily with CO_2 of a known concentration. Differences in the two CO_2 concentrations were considered to represent net photosynthetic fixation and were expressed on a needle area basis as mg CO_2 dm^{-2} h^{-1}. In this experiment, photosynthetic rates of plants watered daily were measured on 5 successive days in the greenhouse under saturating conditions (irradiance 220 to 260 μE m^{-2} s^{-1}, initial CO_2 concentration 320 to 340 p.p.m.) (Doehlert and Walker, 1981). Plants watered every fifth day were allowed to reach maximum stress of the drought cycle and then re-watered daily during the 5-day fixation period. Temperature in the chamber was 23 °C and varied

less than ± 1 °C during the sampling periods. Photosynthetic rates were averaged over the 5-day period to compensate for variability in greenhouse light intensity.

Leaf potential was measured before and after water was withheld using an isopiestic thermocouple psychrometer (Boyer and Knipling, 1965); five needles per seedling were sacrificed for each measurement.

At the time of harvest, root systems were rinsed free of soil and examined for percentage colonization by ectomycorrhizal fungi. Needles were removed and the area of one side of the needles was measured using a LiCor Area Meter. Needles, shoots and roots were dried 72 h at 70 °C and weighed.

Experiment 2: Effect of four ectomycorrhizal fungi on drought tolerance and recovery of Douglas-fir seedlings. In this experiment, non-mycorrhizal seedlings and seedlings inoculated with one of four species of ectomycorrhizal fungi were allowed to become desiccated and then re-watered. Their ability to tolerate and recover from drought was compared.

Soil from the same source as for Experiment 1 was used. All soil was sieved and treated with aerated steam (95 °C/30 min) to eliminate mycorrhizal fungus propagules and most other soil microorganisms and was mixed 1:1 with modified Melin–Norkrans medium, vermiculite mycelial cultures of ectomycorrhizal fungi, or with autoclaved cultures for non-mycorrhizal controls and the treatment involving spore inoculum. Mycelial inocula included *Laccaria laccata* S238A, *Pisolithus tinctorius* S471, and an unidentified native fungus isolated from mycorrhizal roots grown in Boomer Hill soil in previous studies (Parke *et al.*, 1983a, b). Three sporocarps of *R. vinicolor* from Mary's Peak near Corvallis, Oregon, collected and identified by Dr J. M. Trappe, were macerated in a Waring blender in sterile distilled water to make 1 l spore suspension and stored at 4 °C for 12 weeks. Soil–vermiculite mixtures were placed in 50 cm^3 tubes and sown with surface-sterilized (30% H_2O_2, 30 min) Douglas-fir seed. Spore suspension (3 ml) was pipetted into tubes of the *R. vinicolor* treatment. In an attempt partially to restore soil microbial populations, an extract free of ectomycorrhizal fungus propagules was prepared from natural soil and added to all treatments. This was made by mixing 500 cm^3 soil with 2 l water, allowing the suspension to sit overnight, filtering through a series of mesh sizes and ultimately through a nucleopore membrane (3 μm). Extract (3 ml) was pipetted on to each tube of steam-treated soil.

Seedlings were grown in the greenhouse for 6 months under the same conditions as in Experiment 1. They were then moved to a growth chamber (16 h photoperiod, irradiance 240 $\mu E\ m^{-2}\ s^{-1}$, 22 °C day/18 °C night temperature, 35% relative humidity). Two weeks later, seedlings were divided into two groups: those to be watered daily as before, and those allowed to dry out for 7 days before being re-watered. Day 1 was the last day in which all seedlings were watered. The stressed seedlings were not re-watered until day 8 but were watered daily thereafter during the recovery period (days 9 to 13).

Photosynthetic rates were determined for all seedlings on days 1, 8 and 13. Photosynthetic rates of stressed plants were also measured daily during the recovery period (days 9 to 13). The technique was the same as that described for Experiment 1. Daily means were determined for each experiment.

Transpiration rates, calculated from hourly change in weight, were measured daily after sealing the tops of tubes around stems with Permagum, a non-toxic sealant, and covering basal drainage holes with plastic film. Weighings were made

hourly for 3 h each day (10.00 to 13.00 h) to yield a mean rate of water loss (mg H_2O dm^{-2} h^{-1}). This rate was no different than hourly rates determined during a single 3 h period, so transpiration of seedlings was not significantly changed by manipulations during weighing and handling.

Needle areas, shoot dry weight, root dry weight and percentage mycorrhizal roots were measured following plant harvest.

Table 1. *Experiment 1: Growth data for 16-week-old mycorrhizal (M) and non-mycorrhizal (NM) Douglas-fir seedlings*

	Percentage mycorrhizas	Root dry weight (mg)	Shoot dry weight (mg)	Shoot:root dry weight
M	66·3 ± 5·4 a	147 ± 16 a	167 ± 13 a	1·14 a
NM	0 ± 0 b	111 ± 7 a	96 ± 7 a	0·86 a

Values are means ± s.e. Values not followed by the same letter are significantly different at the $P < 0{\cdot}05$ level.

All seedlings watered daily.

Results

Experiment 1. As shown in Tables 1 and 2, data for the early harvest (16 weeks), before any plants were subjected to water stress, indicate no statistically significant difference in shoot length, shoot or root dry weight between seedlings grown in natural *vs* pasteurized soil, although mycorrhizal plants were consistently larger than non-mycorrhizal. Mycorrhizal colonization was 66 and 0% for seedlings grown in natural and pasteurized soil respectively. At 7 months, mycorrhizal and non-mycorrhizal seedlings watered daily showed no significant difference in growth parameters; however, those conditioned to cyclic drought were smaller than seedlings watered daily. Stressed mycorrhizal plants had greater needle areas, root dry weight and shoot dry weight than stressed non-mycorrhizal plants.

Net photosynthetic rate did not differ significantly between mycorrhizal and non-mycorrhizal seedlings watered daily. However, with stressed mycorrhizal seedlings it was approximately ten times greater than with stressed non-mycorrhizal seedlings. Photosynthetic rate of stressed mycorrhizal seedlings did not differ significantly from that of plants watered daily.

Minimum leaf potential of mycorrhizal and non-mycorrhizal seedlings watered daily did not differ significantly. For stressed treatments, however, minimum leaf potential measured at the peak of the drying cycle was lower for mycorrhizal (−19·8 bars) than for non-mycorrhizal seedlings (−12·8 bars). Leaf potentials measured after re-watering of stressed seedlings did not differ significantly between treatments.

Mycorrhizal plants were colonized at high levels (> 90% of root tips colonized), predominantly (> 85%) by unidentified fungus forming thick white mantles and large ropy dark rhizomorphs. Present at much lower levels (< 5%) were brown, smooth, swollen mycorrhizas. *Cenococcum geophilum* mycorrhizas occurred only occasionally (< 1%). Among the mycorrhizal seedlings subjected to periodic drought, a black, slender mycorrhiza was common (20% of tips) although the white rhizomorphic fungus still predominated.

Table 2. *Response of 7-month-old mycorrhizal (M) and non-mycorrhizal (NM) Douglas-fir seedlings watered daily or subjected to cyclic drought (watered every fifth day).*

Treatments	Net photosynthesis (mg CO_2 dm^{-2} h^{-1})	Leaf potential (bars)		Needle area (cm^2)	Root dry weight (mg)	Shoot dry weight (mg)	Shoot:root dry weight
		Max	Min				
M, watered daily	11·07±0·95 a	—	−10·7±0·6 a	10·2±1·32 a	408±23 a	287±20 a	0·72±0·058 ab
NM, watered daily	7·55±1·07 a	—	−10·6±0·6 a	11·2±2·04 a	337±32 ab	268±44 a	0·76±0·075 ab
M, watered every fifth day	8·95±1·48 a	−12·0±0·50 a	−19·8±1·3 b	8·4±0·87 ab	253±27 bc	237±20 ab	0·96±0·041 b
NM, watered every fifth day	0·87±1·02 b	−12·3±0·31 a	−12·8±0·8 a	3·3±0·31 b	182±14 c	118±10 b	0·65±0·025 a

Values are means of five daily readings for 11 (watered daily) or seven (watered every fifth day) seedlings. ±s.e. Values not followed by the same letter differ significantly at the $P < 0·05$ level (Scheffe's Multiple Range Comparison).

Table 3. *Experiment 2: Comparison of stressed and non-stressed seedlings either non-mycorrhizal or inoculated with ectomycorrhizal fungi*

	Needle area (cm^2)	Total dry weight (mg)	Shoot:root dry weight
Stressed			
Rhizopogon vinicolor	17·4 ± 2·0 bc	582 ± 48 a	1·25 ± 0·21 a
Laccaria laccata	14·5 ± 2·5 abc	988 ± 47 b	0·84 ± 0·13 a
Native fungus	13·9 ± 2·5 abc	681 ± 51 a	0·98 ± 0·18 a
Pisolithus tinctorius	8·5 ± 0·5 a	640 ± 57 a	0·73 ± 0·06 a
Non-mycorrhizal	9·5 ± 1·3 ab	607 ± 36 a	0·90 ± 0·20 a
Non-stressed			
Rhizopogon vinicolor	16·2 ± 1·4 abc	629 ± 33 a	1·31 ± 0·10 a
Laccaria laccata	14·9 ± 0·6 abc	930 ± 49 b	0·81 ± 0·03 a
Native fungus	20·6 ± 3·6 c	925 ± 133 b	1·12 ± 0·12 a
Pisolithus tinctorius	13·8 ± 2·0 abc	909 ± 82 b	0·73 ± 0·07 a
Non-mycorrhizal	11·7 ± 0·8 ab	675 ± 44 a	0·83 ± 0·07 a

Values are means ± s.e. Values within a column not followed by the same letter are significantly different at the $P < 0·05$ level (Student–Newman–Keuls' Test).
See text for explanation of treatments.

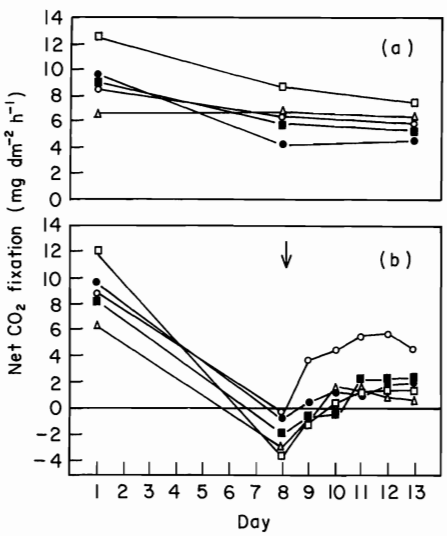

Fig. 2. Comparison of net CO_2 fixation by (a) non-stressed and (b) stressed Douglas-fir seedlings either non-mycorrhizal or inoculated with one of four ectomycorrhizal fungi. Arrow indicates time of re-watering for stressed seedlings. ○, *Rhizopogon vinicolor*; ■, *Laccaria laccata*; △, native fungus; □, *Pisolithus tinctorius*; ●, non-mycorrhizal.

Experiment 2. Comparison between non-stressed Douglas-fir seedlings and seedlings suddenly subjected to drought stress is shown in Table 3. For both watering treatments, *Rhizopogon*-inoculated seedlings had the largest needle areas, largest shoot:root dry weight ratios, but the smallest total dry weight. This indicates that there was a greater proportion of total dry weight in the needles and stems compared to other treatments; however, most of these differences were not statistically significant.

Rates of CO_2 fixation during initial stages of drought were similar for stressed

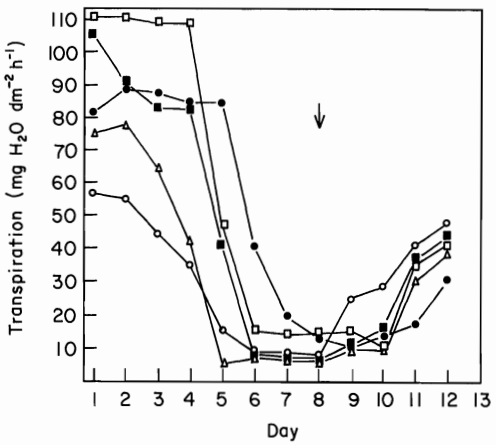

Fig. 3. Transpiration rate of mycorrhizal and non-mycorrhizal Douglas-fir seedlings subjected to drought. Arrow indicates time of rewatering. Symbols as for Figure 2.

and non-stressed seedlings (Fig. 2). When water was not limiting, seedlings inoculated with *Pisolithus tinctorius* fixed CO_2 at a rate higher than that of other treatments. However, after seedlings were allowed to dry for a week, *Rhizopogon*-inoculated seedlings fixed CO_2 at the highest rate. On day 8, all treatments showed a negative net CO_2 fixation rate, i.e. respiration exceeded photosynthesis, but *Rhizopogon*-inoculated seedlings were least affected by drought and resumed a positive net CO_2 fixation rate more quickly and at significantly higher levels ($P < 0{\cdot}05$, Student–Newman–Keuls' Test) than other treatments during the recovery period (days 9 to 13). Net photosynthesis by *Rhizopogon*-inoculated seedlings 24 h after re-watering was approximately seven times greater than that by non-mycorrhizal seedlings. It is not clear why net CO_2 fixation among non-stressed seedlings showed a downward trend during the course of the experiment.

The transpiration rate of seedlings inoculated with *Rhizopogon* was the lowest of any treatment when water was not limiting on day 1 (Fig. 3). With initiation of drying, transpiration by seedlings inoculated with *Rhizopogon* or the native fungus rapidly declined. Non-mycorrhizal seedlings and seedlings inoculated with either *Laccaria* or *Pisolithus* responded more slowly to the onset of drought and continued to lose water at a rapid rate until day 4 or 5. After re-watering on day 8, *Rhizopogon*-inoculated seedlings resumed higher transpiration rates more quickly than seedlings in any of the other treatments.

DISCUSSION

The effects of water stress on plant growth include loss of turgor resulting in stomatal closure, reduction of photosynthetic surface, decreased biomass, reduction in photosynthetic rate and diversion of photosynthate to roots at the expense of shoot growth (Kramer, 1969). Results from Experiments 1 and 2 strongly indicate that certain ectomycorrhizal fungi can help plants to tolerate and recover from soil water deficits.

Net photosynthetic rate as measured here provided a sensitive and rapid assessment of the overall effect of plant moisture stress in intact Douglas-fir

seedlings. Optimal photosynthetic rates of non-stressed seedlings correspond to those reported elsewhere for Douglas fir (Brix, 1979; Doehlert and Walker, 1981). The increase in photosynthetic rate among mycorrhizal seedlings is similar to that reported by Allen *et al.* (1981) for the vesicular–arbuscular (VA) mycorrhizal grass, *Bouteloua gracilis*.

In Experiment 1, during drought imposed on seedlings conditioned to cyclic stress, mycorrhizal seedlings had a net CO_2 fixation rate (on a unit area basis) ten times that of non-mycorrhizal seedlings. This occurred even though mycorrhizal seedlings had a larger transpirational surface and might be expected to become more desiccated than non-mycorrhizal seedlings. Rate of net photosynthesis of stressed mycorrhizal seedlings did not differ significantly from rates for seedlings watered daily. In addition to a reduction in photosynthetic rate, cyclic drought reduced root and shoot dry weight, needle area and shoot:root ratio of non-mycorrhizal seedlings more than those of mycorrhizal seedlings. In Experiment 2, in which non-mycorrhizal Douglas-fir seedlings and seedlings inoculated with one of four ectomycorrhizal fungi were suddenly subjected to drought, one fungus in particular (*R. vinicolor*) appeared to lessen the severity of drought effects on its host. Rates of net CO_2 fixation during drought were reduced less, and recovery to near-normal CO_2 fixation levels occurred more rapidly after re-watering among *Rhizopogon*-inoculated seedlings that among other treatments. Interestingly, the native ectomycorrhizal fungus in this experiment did not enhance host drought tolerance as it did in Experiment 1; perhaps conditioning to stress is necessary for this fungus to develop water-absorption or water-transport mechanisms beneficial to its host during drought.

In Experiment 1, leaf water potential of stressed mycorrhizal seedlings became lower than non-mycorrhizal seedlings; however, total leaf water potential alone can be a misleading measure of prolonged plant moisture stress. Non-mycorrhizal seedlings had such a low mean net photosynthetic rate for the recovery period that sugar reserves may have been depleted, leading to a reduction in osmotic potential and a higher total leaf potential. Osmotic potential measurements, in addition to total leaf potential, would have been helpful in evaluating these effects. The probable depleted state of leaf solutes might help to explain the results of others (Sands and Theodorou, 1978; Dixon *et al.*, 1980), who found mycorrhizal plants with total leaf or xylem potential lower than in non-mycorrhizal plants and accepted this as an indicator of increased plant moisture stress.

Transpiration data provide an additional measure of response to and recovery from drought. The fact that *Rhizopogon*-inoculated seedlings transpired at a rate lower than other treatments when photosynthesis was not limited by water availability suggests that *R. vinicolor* might somehow influence stomatal regulation. The unusual pattern of biomass allocation among *Rhizopogon*-inoculated seedlings also warrants further investigation, especially as it relates to drought tolerance. It may be that *Rhizopogon*-inoculated seedlings allocate a greater proportion of total dry weight to stem and photosynthetic tissue because the mycelial network makes the relatively small root system more efficient.

Mycorrhizas may help to reduce plant moisture stress by increased water uptake through (*a*) decreased resistance to water flow from soil to roots, (*b*) increased absorptive surface or (*c*) potential for fungal hyphae to penetrate smaller soil pores than root hairs (Reid, 1979). Additional mechanisms proposed for the role of VA mycorrhizas in host water relations are indirect benefits as a result of improved host nutrition (Safir, Boyer and Gerdemann, 1971, 1972), perhaps through

changes in membrane permeability due to increased phosphorus availability (Nelsen and Safir, 1982), or altered hormonal regulation of stomatal closure (Levy and Krikun, 1980). Hardie and Leyton (1981) found that larger water flow rates through roots of mycorrhizal clover could not be explained by phosphorus nutrition alone. Mycorrhizal roots were longer and larger in diameter, which together led to a 26 to 86% increase in absorptive surface compared with non-mycorrhizal root systems. When soil water was not limiting, transpiration rates of mycorrhizal plants were higher than for non-mycorrhizal plants, indicating lower root and possibly also lower leaf diffusion resistance. When soil water became limiting, however, mycorrhizal plants developed a higher leaf diffusion resistance and transpiration rates lower than that for non-mycorrhizal plants. They concluded that VA mycorrhizas somehow enabled plants to develop a lower leaf potential under stress conditions through osmoregulation. Lower leaf potential and a root system with higher conductivity would explain how mycorrhizal plants recover from stress more quickly than non-mycorrhizal plants.

It is quite possible that VA- and ectomycorrhizas have similar modes of action in host water relations. In our study, growth of mycorrhizal and non-mycorrhizal treatments was similar except under prolonged conditions of cyclic drought (Experiment 1) indicating that host nutrition was not a factor. Increased water uptake would seem to be the best explanation for our results, although the role of mycorrhizas in stomatal regulation should also be investigated.

Rhizomorphs, or mycelial strands, were well developed among seedlings inoculated with *R. vinicolor* (Fig. 4) or the native ectomycorrhizal fungus, and may be important in water conductance. Rhizomorphs have been shown to transport phosphorus and zinc (Skinner and Bowen, 1974a, b); mechanisms for water transport are thought to function similarly (Sanders and Tinker, 1973). Duddridge, Malibari and Read (1980) demonstrated transport of ^3H_2O through rhizomorphs of *Suillus bovinus* to *Pinus sylvestris* seedlings, and the structure of rhizomorphs has been recently described (Duddridge *et al.*, 1980; Foster, 1981). In mature strands, two types of thin-walled cells, one type containing cytoplasm and the other type ('vessels') devoid of cell contents, are surrounded by a ring of small thick-walled hyphae. A polysaccharide gel is found between and surrounding the hyphal strands. Reid (1979) states that it is unlikely that mycorrhizal sheaths themselves function in water absorption but it is conceivable that hydration of spongy fungus mantles, mycelial strands and gels could prevent rapid water loss through soil as after a sudden summer rainfall. It is interesting that mycorrhizal fungi confer benefits on their hosts even when soil volume is artificially limited and hyphal growth is restricted; this suggests that absorption and/or transport by fungi is more effective than by roots.

It is of interest that growth of mycorrhizal plants differed from non-mycorrhizal only when seedlings were subjected to water stress, indicating that when water is available, photosynthate is diverted to mycorrhizas at the expense of root and shoot growth; this is compensated for by increased absorptive capacity, more rapid recovery of photosynthetic activity and increased chances for survival during drought.

We found that *in vitro* growth of these ectomycorrhizal fungi over a range of nutrient solutions osmotically adjusted with polyethylene glycol was a poor indication of effectiveness in reducing plant moisture stress *in vivo*, suggesting that these studies are of little value in interpreting the role of ectomycorrhizas under drought conditions (Parke and Linderman, unpublished results). *In vitro* growth

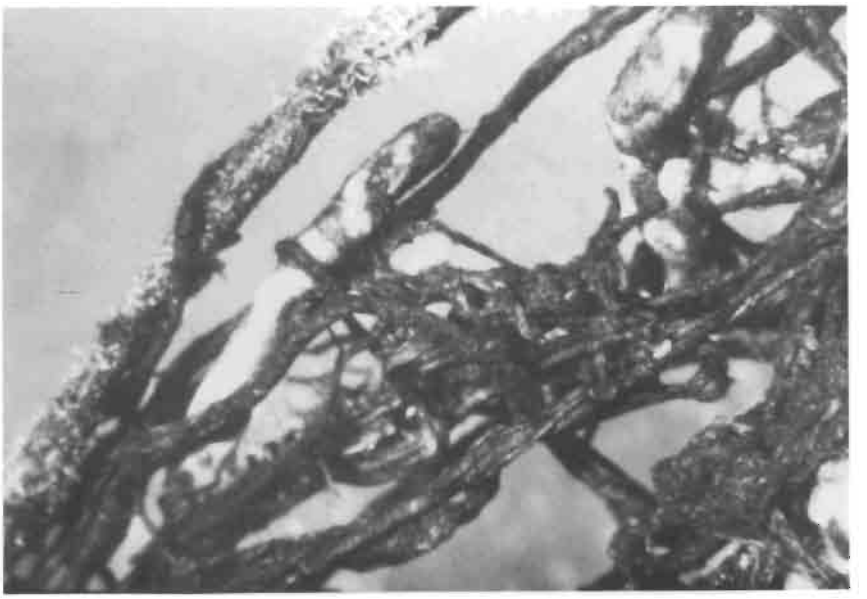

Fig. 4. Root colonization by *R. vinicolor* showing mycorrhizas and rhizomorphs.

of ectomycorrhizal fungi may be impaired nutritionally even when no 'toxic' substance is found in the medium (Reid, Bowen and McCleod, 1978; Luard and Griffin, 1981), O_2 diffusion rates may be limiting (Mexal *et al.*, 1975) and host-mediated effects are eliminated. This is in agreement with Theodorou and Bowen (1970), Theodorou (1978) and Mexal and Reid (1973).

In the Pacific Northwest, cessation of root growth of conifers in the late spring limits water uptake during the summer to the upper soil regions, which are rapidly depleted of moisture. Mycorrhizal colonization can be viewed as a kind of 'drought avoidance' in that dry soil conditions are avoided spatially through hyphal penetration of deeper zones. Seasonal drought avoidance could be achieved by mycorrhizal fungi able to grow and colonize roots at cool soil temperatures when moisture is not limiting (Parke, Linderman and Trappe, 1983b). Differences in host response to inoculation with various ectomycorrhizal fungi suggest that drought tolerance should be considered one of the more important criteria for selection of fungus species and ecotypes suitable for nursery inoculation.

Acknowledgements

Financial support for this research was provided by the following public agencies and private forest organizations cooperating in the California–Oregon Plantation Studies (COPS): Boise Cascade Corp., Champion International Co., Fruitgrowers Supply Co., International Paper Co., Medford Corp., Southern Pacific Land Co., Roseburg Lumber Co., Sun Studs Corp., the US Bureau of Land Management, and the USDA Forest Service, Pacific Northwest Forest and Range Experiment Station. We thank Dr John R. Potter and Mrs Juanita M. Rasmussen of the USDA-ARS Horticultural Crops Research Laboratory, Corvallis, for the helpful advice and the generous use of the infrared gas analyser. Butler County Mushroom

Farm provided ectomycorrhizal inoculum for Experiment 2. We also wish to thank Mr Jack Martin, USDA–ARS Agricultural Engineering, Corvallis, for skillful construction of the portable photosynthesis chamber.

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