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Assimilation, Distribution, and Root Exudation of ^{14}C by Ponderosa Pine Seedlings under Induced Water Stress[1]

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ABSTRACT

The effect of specific levels of induced water stress on the root exudation of ^{14}C from 9-month-old and 12-month-old ponderosa pine (*Pinus ponderosa* Laws.) seedlings was examined. Polyethylene glycol (PEG-4000) was used to decrease root solution water potentials by 0, −1.9, −2.6, −5.5, −9.6 and −11.9 bars in either aerated 0.25X Hoagland's nutrient solution or aerated distilled water.

Assimilation of $^{14}CO_2$ by plants under stress and subsequent translocation of ^{14}C label to the roots were both inhibited by a decrease in substrate water potential. Six days after $^{14}CO_2$ introduction essentially no ^{14}C was detected in the roots of plants maintained at solution potentials of −5.5 bars or below. In subsequent studies $^{14}CO_2$ was introduced 4 days prior to induction of stress. This allowed sufficient time for distribution of ^{14}C label throughout the root system.

Root exudation of ^{14}C-labeled sugars, amino acids, and organic acids from plants in nutrient solution showed an increase from 0 to −1.9 bars, a decline from −1.9 to about −5.5 bars, and then an increase again from −5.5 to −11.9 bars. As substrate potential decreased, sugars as a percentage of total exudate increased, organic acids decreased and amino acids showed a slight decrease. Marked changes in percentages occurred between 0 and −2.6 bars. The exudation of sugars, amino acids, and organic acids from plants in distilled water showed similar trends in response to water stress as those in nutrient solution, but the quantity of total ^{14}C exuded was greater.

The importance to plant growth of microorganism populations in the rhizosphere is certainly recognized. However, the complexity of the interaction between the rhizosphere microorganisms and the organic compounds which are exuded from roots is poorly understood. Harley (9) points out that the explanations of high activity and specificity in rhizosphere populations involve the release of organic nutrients and accessory growth factors which control or affect the microorganisms. Specialized associations of microorganisms and roots which result in the formation of composite organs having an integrated physiology are very common (*e.g.* nitrogen-fixing nodules and mycorrhizas) (9).

There are numerous reports on the exudation of organic compounds from roots (28). The quantity and quality of root exudates have been found to change with plant age and stage of development (7), presence of disease (13), and nutrient status of the root environment (2). Very few investigations, however, have examined the effects of water stress on root exudation of organic compounds.

The purpose of this study was to investigate the effect of specific levels of induced water stress on the movement of ^{14}C-labeled compounds in tree seedlings and the resultant exudation of ^{14}C from the roots. Carbohydrates were considered of special importance because of their role in the formation of ectomycorrhizas (8, 9).

MATERIALS AND METHODS

The effect of water stress on the distribution and exudation of ^{14}C was examined in plants at the time of $^{14}CO_2$ introduction, and with plants which had assimilated $^{14}CO_2$ several days prior to subjection to stress.

Two sets of experimental plants were used. The first consisted of 9-month-old ponderosa pine (*Pinus ponderosa* Laws.) seedlings maintained in aerated distilled water, while the second consisted of 12-month-old seedlings maintained in aerated 0.25× modified Hoagland's nutrient solution (11). Both sets had been previously grown in a vermiculite-nutrient solution medium (1) under greenhouse conditions. Root systems were removed from the media, rinsed with distilled water, and carefully cleaned to remove root debris. Each plant was then placed in 300 ml of aerated solution at least 24 hr prior to treatment. All studies were conducted in a controlled environment growth chamber maintained at 17 C, 60% relative humidity, a 6-hr photoperiod of fluorescent and incandescent light, and an 18-hr dark period. A short photoperiod was used to retard starch accumulation in the shoot. Radioactive CO_2 was introduced to the seedling shoot by acidifying 10 μc of $NaH^{14}CO_3$ in a closed assimilation chamber. The release of $^{14}CO_2$ was started 1 hr after the onset of the photoperiod. After 2 hr the assimilation chamber was removed. Temperature in the assimilation chamber was 23 C, and the total radiant energy in a 400 to 800 nm spectrum, as measured by an International Light Plant Growth Photometer, was 1 mw cm^{-2}.

Water stress treatments consisted of decreasing the water potential of the root bathing media to 0, 1.9, 2.6, 5.5, 9.6, and 11.9 negative bars by using PEG,[2] mol wt 4000. Actual water potential of the solutions was determined by use of a Richards-Ogata type thermocouple psychometer (3). The 0.25× Hoagland's nutrient solution had a potential greater than −0.2 bar. Estimates of the shoot water potential of the treated seedlings at the end of the experiments were obtained by measuring the xylem sap pressure with a pressure chamber (18).

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[2] Abbreviation: PEG: polyethylene glycol.

Seedlings Stressed before $^{14}CO_2$ Introduction. Individual seedlings were placed in each of the aerated distilled water treatment solutions (0 to −11.9 bars). Each treatment was replicated once. After an equilibration period of 24 hr, $^{14}CO_2$ was applied and the seedlings were maintained in solution 6 days. At the end of the period the plants were harvested and oven-dried. Plants were then autoradiographed and sampled for quantitative determinations of ^{14}C in various plant parts. The root bathing solution medium from each plant was separated into amino acid, sugar, and organic acid fractions.

Seedlings Stressed after $^{14}CO_2$ Introduction. Radioactive CO_2 was introduced to seedlings growing in aerated nutrient solution and seedlings in aerated distilled water. After 4 days, individual nutrient solution maintained plants were randomly selected for transfer to specific treatment nutrient solutions containing PEG (replicated once). After the same period, individual distilled water maintained plants were randomly selected for transfer to treatment solutions of PEG added to distilled water. All plants were then maintained in the appropriate treatment solutions (0 to −11.9 bars) for 6 days. A period of 3 to 4 days without stress had been previously determined to be suitable for allowing adequate translocation of ^{14}C-labeled compounds into the root system. At the end of the treatment period the plants were harvested, oven-dried, and sampled for quantitative distribution of ^{14}C. The root bathing solution from each plant was separated into amino acid, sugar, and organic acid fractions.

Quantitative Determination of ^{14}C. The quantity of radioactivity in experimental material was determined by use of a liquid scintillation spectrometer (Packard Instruments Co., Inc., Model 2420, Tri-Carb). Tissue samples were digested in counting vials with 30% H_2O_2 and 60% $HClO_4$ at 75 C and then counted in a scintillation solvent mixture of toluene-cellosolve (25).

Aqueous samples from the root bathing solution fractions were counted directly in a commercial scintillation solution Insta-gel (Packard Instruments Co., Inc.). Sample radioactivities were corrected for efficiency using automatic external standardization procedures based on quench curves prepared for the two scintillation solutions used. Only net count rates of samples significantly (0.01 level of significance) above background were used.

Fractionation of Root Bathing Solutions. Ion-exchange resins were used to separate the root solutions into various fractions (30). The solution was first passed through a Dowex 50W-X4(H+) resin column. The eluant passing through the column was then passed through a Dowex 1-X8 (formate) resin column. The resulting eluant contained the neutral fraction (sugars). Amino acids were removed from the Dowex 50W column by use of 50 ml of 2 N HCl followed by 25 ml of concentrated HCl and then 25 ml of distilled water. Organic acids were removed from the Dowex 1 column by use of 60 ml of 8 N formic acid followed by 25 ml of distilled water. All fractions were evaporated *in vacuo* at 40 C. Aliquots of each fraction were then counted for ^{14}C by liquid scintillation.

RESULTS

Plants Stressed before $^{14}CO_2$ Introduction. Table I presents quantitatively the decrease of ^{14}C specific activities in various shoot and root parts as stress was increased. As the substrate (root bathing media) water potential decreased, the total amount of $^{14}CO_2$ assimilated also decreased. When the radioactivity in the roots and in the shoots is expressed as a percentage of the total radioactivity in the plant, it can be seen that the quantity of ^{14}C translocated from the shoot to the root also decreased with decreasing water potential. The decrease in ^{14}C in the shoot and in the root is vividly illustrated by the autoradiographs of one of the treatment replications (Fig. 1). Because of the effect of stress on the translocation of ^{14}C to the root system, no ^{14}C was detected as root exudate at treatment substrate potentials lower than −2.6 bars. There was, however, sufficient activity in the root bathing solutions having water potentials as low as −2.6 bars to allow separation of sugars, amino acids and organic acids (Fig. 2).

Table I. *Distribution of Radioactivity in Plant Parts of Ponderosa Pine Seedlings Subjected to Substrate Water Stress*

Nine-month-old ponderosa pine seedlings were stressed in aerated distilled water solutions containing PEG. After a 24-hr equilibration period in solution, 10 μc of $^{14}CO_2$ were introduced to each seedling for a 2-hr assimilation period. Plants were harvested 6 days after $^{14}CO_2$ introduction. Values represent averages of two replications.

Substrate Water Potential	Specific Radioactivity of Plant Parts						Total Plant Radioactivity	Distribution of Total Radioactivity	
	Terminal needles	Mature needles	Stem	Primary roots	Lateral roots	Short roots		Shoot	Roots
-bars	*cpm/g dry wt × 10⁻³*						*cpm × 10⁻³*	*%*	
0	3111	3477	1910	925	207	42	1234	93	7
1.9	1888	1860	610	73	88	83	1425	96	4
2.6	358	832	197	10	0	5	139	97	3
5.5	124	20	1	0	0	0	22	100	0
9.6	8	18	9	0	0	0	2	100	0
11.9	42	9	1	0	0	0	7	100	0

Plants Stressed after $^{14}CO_2$ Introduction. Figure 3 demonstrates that relatively high quantities of ^{14}C were present in the roots of the 12-month-old seedlings maintained in nutrient solution at the time of harvest. This is also supported by the data on the appearance of ^{14}C in the root bathing media of the same seedlings maintained in solution for the 4 days prior to PEG treatment (Fig. 4). A similar plant distribution pattern was observed in the 9-month-old seedlings in distilled water. Root exudation as related to substrate water potential of seedlings in distilled water and those in nutrient solution is presented in Figure 5. Root exudation is expressed as a percentage of total radioactivity in the root system, rather than as total radioactivity in the root bathing medium. This is believed appropriate since the amount of ^{14}C reaching the root system after $^{14}CO_2$ assimilation is somewhat variable even when the plants are not under stress treatment. It was of primary interest to know the effect of stress on the loss of radioactivity from the roots in relation to how much was there, presumably at the time of stress induction. By examining the quantity of ^{14}C in various parts of the root system and relating it to the amount of root exudation through means of linear regression, it was found that the regression of root exudation at 72 hr (before stress) on root radioactivity at time of harvest was best correlated by use of total cpm in the total root system ($r = 0.77$; level of significance = 0.01). This was slightly better than using specific radioactivity of the total root system.

The distribution of ^{14}C-labeled sugars, amino acids, and organic acids in the root exudations is presented in Figure 6 for the distilled water seedlings and the nutrient solution seedlings. Although the water stress in the roots is of primary interest here and would be expected to be similar to the potential of the substrate, the water potential of the shoot was estimated at the various treatment potentials by use of the pressure chamber

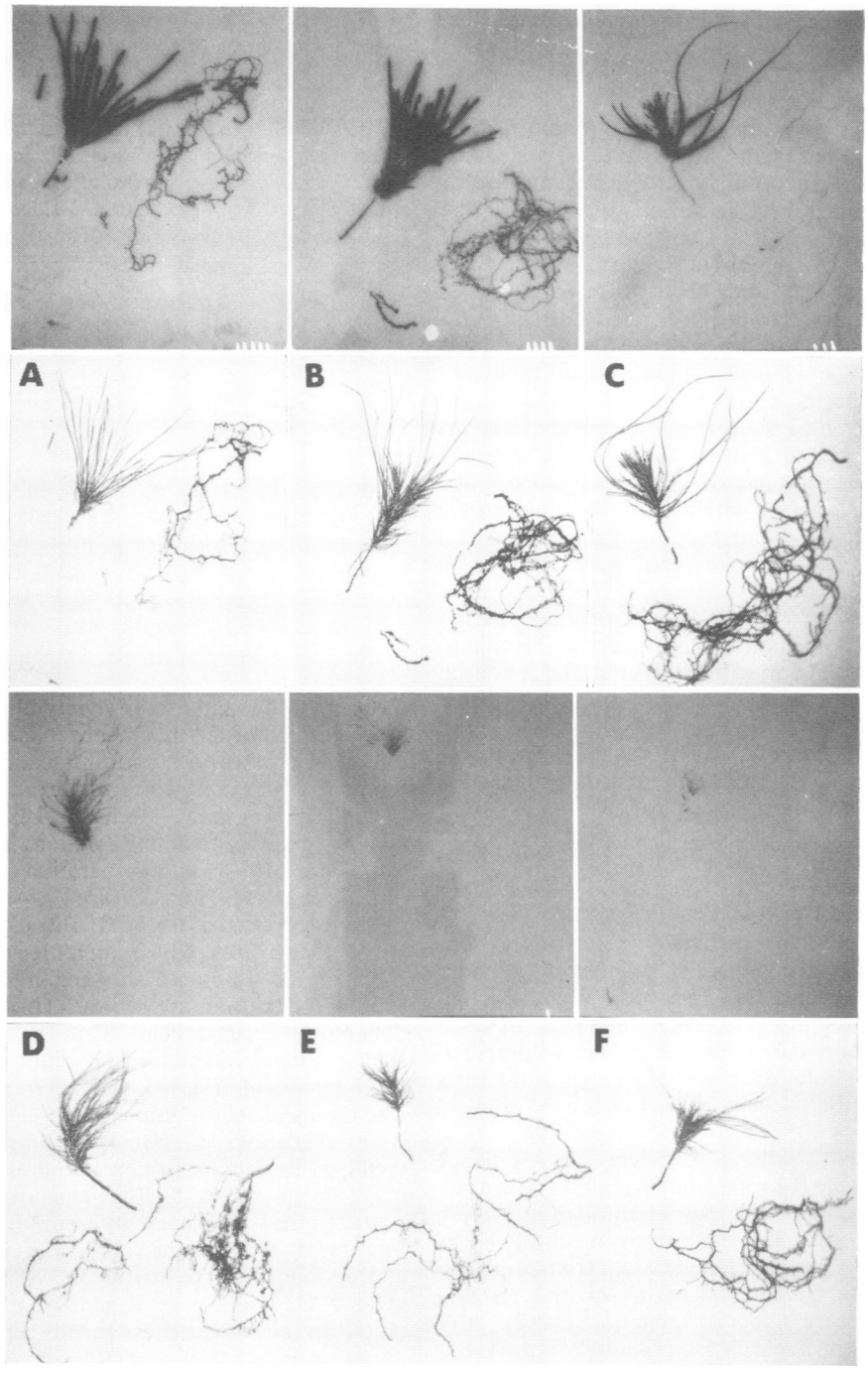

FIG. 1. Autoradiographs of 9-month-old ponderosa pine seedlings subjected to water stress and harvested 6 days after a 2-hr assimilation period with 10 μc of $^{14}CO_2$. Water potential of root bathing solution: A: 0.0 bars; B: −1.9 bars; C: −2.6 bars; D: −5.5 bars; E: −9.6 bars; F: −11.9 bars. Stress treatment was begun 24 hr before $^{14}CO_2$ introduction.

technique (Fig. 7). The values in Figure 7 represent the average of three replicates and should be used only as estimates of the actual potential.

As reported in the literature (20) roots should not be readily permeable to PEG 4000 because of its large molecular size. However, PEG 4000 can penetrate into the plant where root damage allows breaks in the endodermis or other suberized areas. Although care was taken in cleaning and transplanting root systems, undoubtedly some damage occurred. Therefore, analyses of PEG in the needles of the nutrient solution-grown seedling treatment replications were conducted according to the techniques reported by Hyden (12) and Lawlor (20). Studies with pine needle tissue using known amounts of PEG 4000 and ^{14}C-labeled PEG 4000 yielded variable recoveries ranging between 2.4 and 9.8%. Adjustment of the quantity of PEG detected in foliage samples by both the low and high recovery percentages gave a range of concentration between 0.15 to 2.63 of PEG as percentage of dry weight of tissue for the 12 plants. There appeared to be no consistent relationship between the concentrations of PEG in tissue and in the root bathing solution. If the largest PEG concentration found in needles (2.63% of dry weight) is converted to a concentration based on water

content of the needles, the concentration of PEG is 1.1% (w/w). Based on data from Mexal and Reid (22), this would be a potential greater than −0.7 bars.

DISCUSSION

The marked reduction in $^{14}CO_2$ uptake at substrate potentials −2.6 bars or below as evidenced by the autoradiographs (Fig. 1) and by the quantitative data in Table I is very likely a result of stomatal closure. This would agree with findings of Morris and Tranquillini (23) for 2-year-old *Pinus contorta* seedlings, that generally, photosynthesis was greatly reduced at a nutrient solution osmotic potential of −3 bars and ceased at −9 bars. Lopushinsky and Klock (21) reported that transpiration rates of *P. contorta* and *P. ponderosa* were 33 to 42% of maximum when soil water potential was −5 bars. At −10 bars, transpiration rates were only 9% of maximum. Unpublished data by this author have also shown that transpiration rates of 2-year-old *P. contorta* seedlings drop markedly when substrate water potential is lowered from about −0.5 bars to −5.5 bars. Brix (4) reported a decrease in the net rate of photosynthesis when loblolly pine needles reached a leaf water potential of −4 bars and a net rate of zero when at −11 bars. According to Figure 7 the substrate potentials of −1.9 and −2.6 bars used in these experiments correspond approximately to a shoot water potential of −4 bars. A plateau in the shoot potential curve beginning at a substrate potential of about −6 bars closely agrees with findings by Lopushinsky and Klock (21).

The net reduction in translocation of ^{14}C-labeled compounds from the shoot to the roots as stress increased supports the observations of previous investigators (10, 24, 27, 31). The almost complete absence of ^{14}C in the roots at substrate potentials below −5.5 bars (Table I) is somewhat surprising, especially since 6 days elapsed between the time of $^{14}CO_2$ introduction and plant harvest.

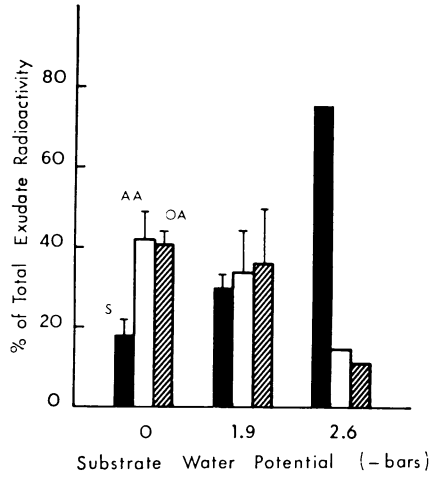

FIG. 2. Sugars (S), amino acids (AA), and organic acids (OA) as percentages of total radioactivity of root exudates from 9-month-old ponderosa pine seedlings subjected to substrate water stress. Root exudates were collected for 6 days in aerated distilled water containing PEG after introduction of $^{14}CO_2$ to foliage. Vertical bars represent one-half the range of values about the mean. Total cpm in exudate from each treatment potential was: 0, 634; 1.9, 337; 2.6, 438.

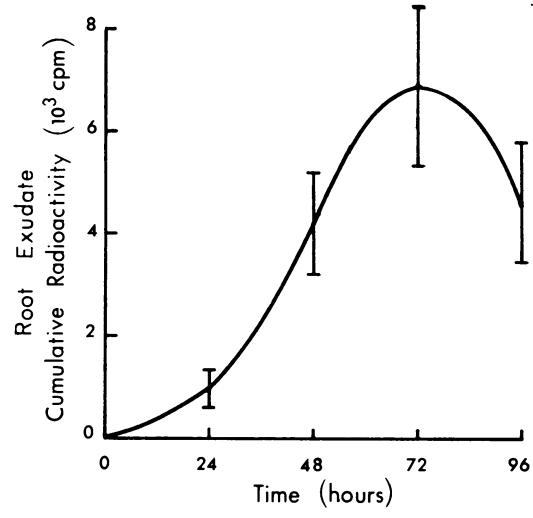

FIG. 4. Cumulative loss of ^{14}C from roots of 12-month-old ponderosa pine seedlings maintained in aerated 0.25× Hoagland's nutrient solution after introduction of 10 μc of $^{14}CO_2$ to the foliage. Vertical bars represent the SE of the mean of 12 plants.

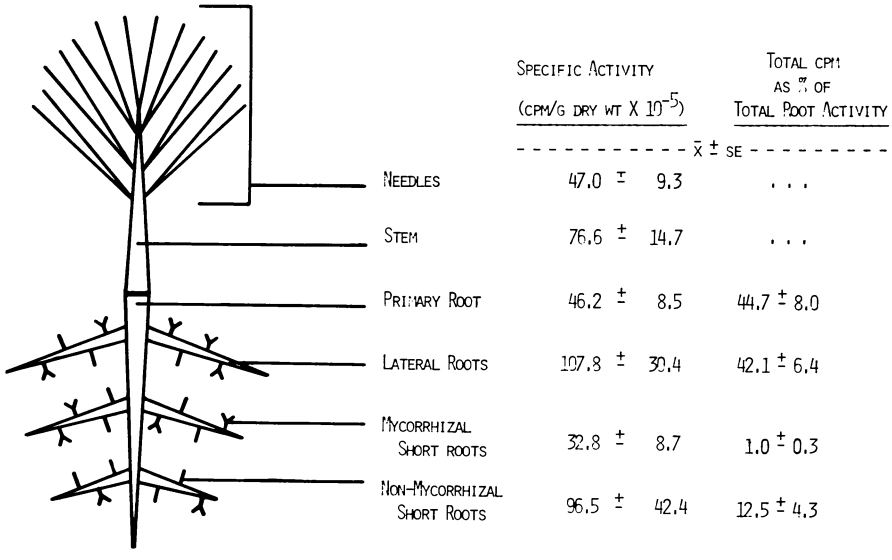

FIG. 3. Distribution of ^{14}C in 12-month-old ponderosa pine seedlings 10 days after introduction of 10 μc of $^{14}CO_2$ to the foliage. Mean specific activities (cpm/g dry wt) ±SE are presented for 12 plants. Plants were maintained in aerateted 0.25× Hoagland's nutrient solution.

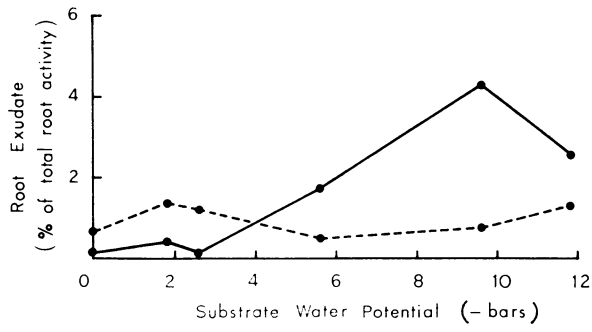

FIG. 5. Total root exudation of ^{14}C (as percentage of total root radioactivity) from ponderosa pine seedlings subjected to substrate water potentials (0 to −11.9 bars) for 6 days. $^{14}CO_2$ was introduced to the foliage 4 days before induction of stress with PEG. Nine-month-old seedlings in aerated distilled water containing PEG (●——●); 12-month-old seedlings (average of 2 replications) in aerated 0.25× Hoagland's nutrient solution containing PEG (●- - -●).

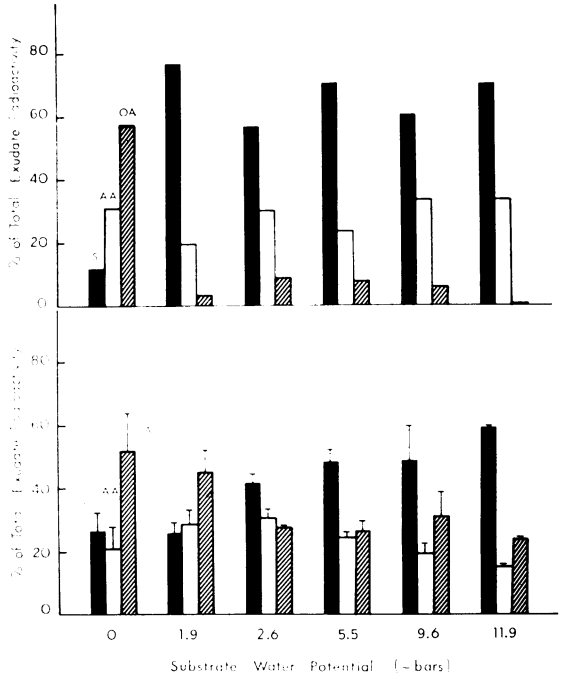

FIG. 6. Sugars (S), amino acids (AA), and organic acids (OA) as percentages of total radioactivity of root exudates from ponderosa pine seedlings subjected to substrate water stress for 6 days. $^{14}CO_2$ was introduced to the foliage 4 days before induction of stress. Nine-month-old seedlings (upper graph) were maintained in aerated distilled water containing PEG. Twelve-month-old seedlings (average of two replications) (lower graph) were maintained in 0.25× Hoagland's nutrient solution containing PEG. Vertical bars represent one-half the range of values about the mean. Total cpm in exudate from each treatment potential of the 9-month-old plants (upper graph) was: 0, 119; 1.9, 3156; 2.6, 2998; 5.5, 2184; 9.6, 5040; 11.9, 4280. Mean total cpm in exudate from 12-month-old plants (lower graph) was: 0, 8058; 1.9, 8386; 2.6, 1758; 5.5, 5547; 9.6, 9142; 11.9, 4616.

In examining the distribution of ^{14}C in the roots of the nutrient solution plants 10 days after $^{14}CO_2$ assimilation (Fig. 3), it is interesting that mycorrhizal roots had a significantly lower ^{14}C specific radioactivity than did nonmycorrhizal short roots. It has been proposed that mycorrhizal roots, relative to noninfected roots, act as a metabolic sink for photosynthetically fixed ^{14}C (8). The roots classified as mycorrhizal were the dichotomously branched short roots. These were not histologically examined for confirmation of the Hartig net.

The total ^{14}C lost by root exudation in response to substrate potential (Fig. 5) is especially difficult to interpret because of few replications. Under these circumstances it would be difficult to demonstrate statistically significant differences between treatments, and data can be used to indicate trends at best. The greater loss of ^{14}C from the roots of plants maintained in distilled water at potentials below −2.6 bars might be attributed to the absence of inorganic salts in the media and their effect on permeability. Low levels of both calcium and phosphate have been shown to affect root loss of organic compounds (2, 29). Even for plants in nutrient solution, the trend toward an increase in quantity of exudate at lower water potentials might be partially attributed to respiration-linked permeability responses. The decline in respiration of root tissue subjected to water stress has been shown (6, 17, 26). Certainly, water stress induced changes in permeability could affect the exudation of compounds as demonstrated by Resnik and Flowers (26) for beet root discs and Greenway (6) for vacuolated root tissues of *Zea mays*.

Although data collected from plants maintained in distilled water might be considered questionable, it is important to note

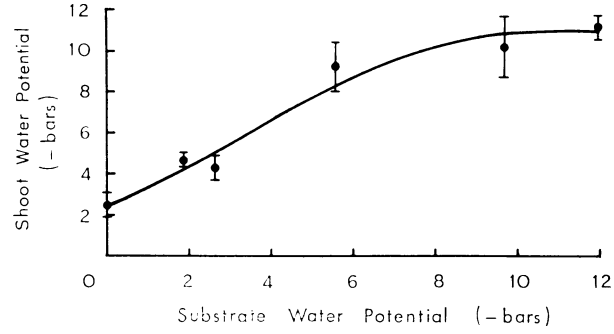

FIG. 7. Shoot water potential of ponderosa pine seedlings as related to substrate water potential. Vertical bars represent the SE of the mean of three plants measured by the pressure chamber technique.

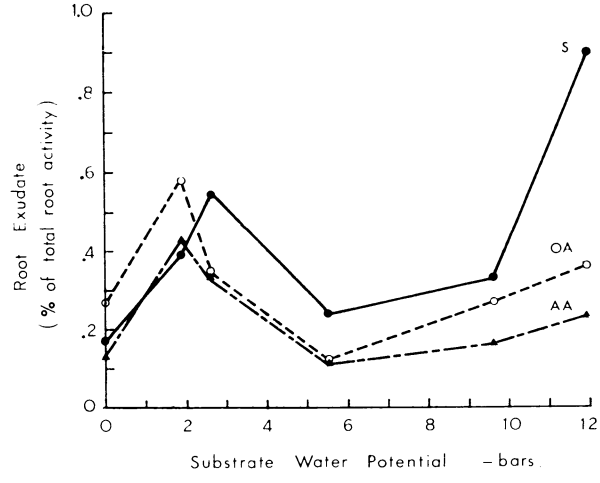

FIG. 8. Total exudation of sugars (S), amino acids (AA), and organic acids (OA) from roots of 12-month-old ponderosa pine seedlings subjected to substrate water stress for 6 days. $^{14}CO_2$ was introduced to the foliage 4 days before stress induction. Exudate values expressed as percentage of total root radioactivity are average of two replications. Plants were maintained in 0.25× Hoagland's nutrient solution.

that when all treatment responses are considered together, there appeared to be a definite trend of an increasing proportion of sugars in the exudate as stress increased, with a marked change between 0 and −2.6 bars (Figs. 2 and 6).

When the total amount of radioactivity for each compound class at each treatment is expressed on the basis of total root radioactivity, the quantity of all three classes of compounds for the nutrient-grown seedlings increases near −1.9 to −2.6 bars, shows a decline at −5.5 bars and then an increase again at −9.6 to −11.9 bars (Fig. 8). A similar trend was indicated for the seedlings maintained in distilled water. Although the quantity of exudate was quite variable between replicates at any particular treatment level and must be considered as only indicative of trends, the relative distribution of organic compound fractions was much less variable as shown by the small range in replicate values in Figures 2 and 6.

The marked shift in relative percentages of sugars, amino acids, and organic acids and the apparent peak of exudate quantity of these same components at −1.9 to −2.6 bars is intriguing. What changes in the root occur at these particular levels of stress? Although there are too few replicates to conduct a rigorous statistical analysis in these experiments, the observed responses at these levels of stress seem to be supported by other reports examining various physiological responses to water stress. Reports of shifts in carbohydrate metabolism at −2.5 bars and protein inhibition at −3.2 bars (6), stimulation of root growth in nutrient solution at −1.2 to −1.3 bars (15), peaks in net assimilation rate and relative growth rate at a mean soil water potential of about −1.3 bars (16), and peak germination of ponderosa pine seeds at −3 bars (19) suggests that significant changes in the plant occur near −2 bars substrate potential.

The responses observed here are not believed to be a result of toxic effects of PEG 4000. Studies by Lawlor (20) indicate that inorganic contaminants in commercial PEG 4000 are unlikely to cause plant damage. He did show that if PEG 4000 is permitted to enter the plant through damaged roots it can cause damage to the foliage, presumably by desiccation resulting from blockage of the water pathway. The highest concentration of PEG found in pine needles after 6 days (1.1%) in this study is similar to Lawlor's finding that plants with undamaged roots grown in −10 bars PEG 4000 solution for 8 days showed an uptake of PEG less than 1% (10 mg/g fresh weight). Other studies have demonstrated the lack of apparent toxicity to plant tissue using various PEG's (see *e.g.* refs. 5 and 14). The fact that $^{14}CO_2$ assimilation was markedly reduced by decreased substrate water potential after the root systems had been in PEG solutions for only 24 hr would also suggest that PEG accumulation in tissue was an unlikely cause of observed responses.

Since few investigations have examined the relationship between water stress and root exudation, possible explanations for the responses reported here would have to be based on available evidence related to water stress effects on plant constituents. This would certainly be speculative since the observed responses in levels of particular compounds in the leaves or even the roots are not necessarily indicative of a similar response in root exudation (28).

If root exudation responses to water stress as observed here are indicative, conflicting reports on the levels of various organic constituents in plants as affected by water stress might partially be reconciled by examining more carefully the potential of the tissue during stress treatment. As suggested here, levels of particular compounds may increase or decrease depending on the particular level of stress.

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