Uptake of Dinitrophenol & its Effect on Transpiration & Calcium Accumulation in Barley Seedlings [1, 2]

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Introduction

There is still no general agreement as to the significance of transpiration in the overall process of ion accumulation in the shoots of intact plants. In a recent review, Russell and Barber (19) concluded that ions are first actively transported into the stele of the root and that thereafter transpiration may or may not affect their rate of movement to the shoot depending on the salt status of the root, the external ionic concentration, and the rate of transpiration. In contrast, Hylmo and his co-workers (12, 13, 14, 16), while acknowledging the existence of an active transport component, stress the importance of transpiration in the movement of ions to the shoot. Since the present work was completed, Falk (15) has shown, for K and NO_3, that active transport predominates over the concentration range generally found in soil solution and nutrient cultures, while at high concentrations (> 10 mM) mass flow in the transpiration stream becomes progressively dominant.

Moore et al. (17), concluded most, if not all, Ca was taken up passively. They showed with excised barley roots that 2,4-dinitrophenol (DNP) did not inhibit Ca uptake although it inhibited K and Mg uptake.

If their conclusion is correct, Ca must accumulate in the shoot independent of metabolism in the root either by movement into the xylem vessels through the symplast (this assumes that at least part of symplast is free space for Ca) or along cell walls. The route along the walls would quite probably be blocked by the suberized thickening of the endodermal cell walls. An alternative route would be directly up the root along the cortical cell walls, but this route would seem to be quite slow. If Ca is accumulated in the shoot passively, then the accumulation might be directly correlated with transpiration. However, in view of the work of Epstein (7), Crafts (6), and others, it seems more plausible that Ca moves across the plasmalemma by the expenditure of metabolic energy (like other ions), into the symplast and finally into the xylem.

In the present work, the Ca level, DNP level, O_2 level, root viability, and transpiration rates were varied to determine if Ca was actively accumulated by the barley plant.

Materials & Methods

Seeds of barley, *Hordeum vulgare* L. var. Arivat, were soaked in aerated, distilled water for 24 hours and then planted on cheesecloth supported by a stainless steel screen about 0.5 cm above the surface of an aerated 2×10^{-4} M $CaSO_4$ solution. The edges of the cheesecloth dipped into the solution. After 3 days in the dark at 25 C the resulting seedlings were sealed into modified screw cap jars each containing 220 ml of a one-fourth strength Hoagland solution (fig 1). Five seedlings were mounted in each jar and at least two jars of seedlings were used per treatment.

The jars were wrapped in black paper and placed in a growth chamber maintained at 25 C with 40 % relatve humidity and a 16-hour light period of 1,000 ft-c.

After 2 days the Hoagland solution was replaced by either 0.5 or 5.0 meq/liter $CaCl_2$ solution containing 10 μc/liter Ca^{45}, with or without DNP. The pH of the solutions was adjusted with HCl when pH 4 was required. The solutions were not buffered and the pH increased from 4.0 to about 5.5 when DNP was not present and to about 4.4 when DNP was present. However, it was shown in a preliminary experiment that Ca uptake to the shoot was not influenced by a nutrient solution pH between 4.0 and 8.0, but Ca taken up by the root was influenced, being only half as much at pH 4 as at pH 6.

In certain experiments the solution contained both C^{14} labeled DNP and Ca^{45}. Both radioisotopes were determined in the same tissue after weighing a constant amount of ground, dry tissue into a planchet. The tissue was glued to the planchet by moistening and drying at low temperature, counted (C^{14} + Ca^{45}) with a proportional counter, and then after the C^{14} was removed by cautiously heating the planchet over a gas flame the ash (Ca^{45}) was counted. Self-absorption corrections were made, and the cpm converted to weight units by using appropriate reference samples.

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Investigations were made of the rates of transpiration and Ca uptake of plants maintained in Hoagland solution containing Ca^{45} to determine if single-salt solutions were injurious to the plants and, therefore, influenced the results. The results did not differ significantly from those obtained with plants maintained in single-salt solutions.

Results

Brouwer (*2*) found that 10^{-5} M DNP, while inhibiting chloride accumulation in the shoot of *Vicia faba* seedlings, had no effect on water absorption—thus allowing the two processes to be separated. From our preliminary experiments, however, it became apparent that transpiration may be strongly inhibited and Ca accumulation in the shoot may be enhanced or inhibited in the presence of DNP, depending on the pH of the solution, the DNP concentration, the Ca concentration, and the period of uptake (1).

I. Transpiration & Calcium Uptake at High & Low Relative Humidity Levels: High and low rates of transpiration were obtained by using two growth chambers, one held at 40 % relative humidity and the other at 90 % relative humidity. Transpiration and Ca uptake by shoots and roots from 0.5 and 5.0 meq/liter $CaCl_2$ in the presence and absence of 10^{-5} M DNP at pH 4.0 were determined in replicated treatments.

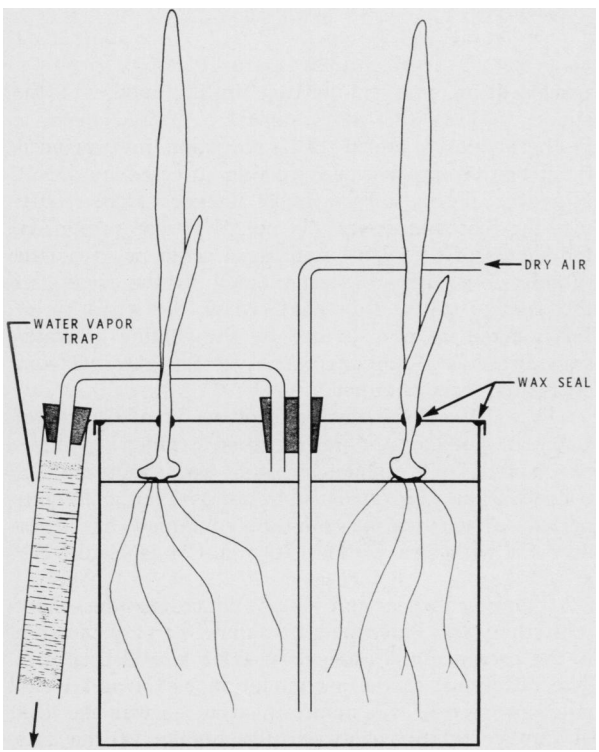

Fig. 1. Apparatus for determining the rates of transpiration and Ca uptake by intact barley seedlings growing in aerated solutions.

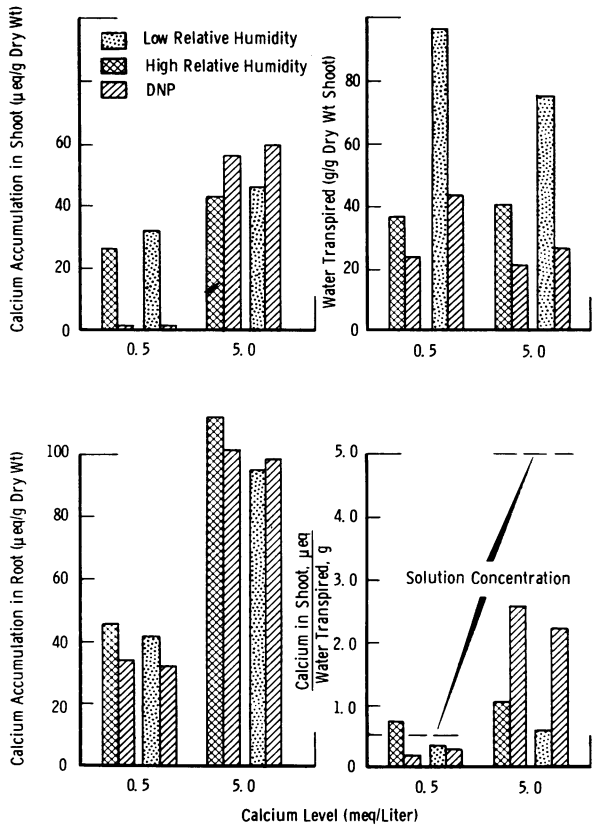

Fig. 2. The accumulation of Ca in the roots and shoots of intact barley seedlings from solutions containing 0.5 and 5.0 meq/liter $CaCl_2$, together with the amount of water transpired at high and low relative humidity levels, in the presence and absence of 10^{-5} M DNP at pH 4, over a 24-hour period.

In all cases transpiration was reduced in the presence of DNP (fig 2). Calcium uptake by the root was significantly reduced by DNP at the lower Ca level but not at the higher level. Calcium uptake in the shoot was reduced by DNP at the lower Ca level but increased at the higher level over the 24-hour period. Prior to our work, Chasson (5) found DNP increased Ca uptake by potato slices.

If Ca was accumulated in the shoot by the passive movement of ions in the transpiration stream, then more Ca should have been accumulated in the shoot at the high transpiration rate than at the low. Further, if Ca was accumulated in this manner, then the amount of Ca taken up, divided by the volume of water transpired, would give a ratio (in concentration units) that could be compared to the external solution. This value should be similar to the external Ca concentration and be independent of the transpiration rate. Values of the ratio of Ca accumulated to water transpired are shown in figure 2. Under all conditions, except at high relative humidity and low Ca, the concentration of Ca in the transpiration stream must have been considerably be-

Table I

Influence of pH on DNP Uptake in Shoot & on DNP-Induced Inhibition of Transpiration & Calcium Accumulation*

pH	Conc. DNP in shoot	Molarity of DNP required to inhibit transpiration & Ca uptake by 50 %	
	μg/g dry wt	Transpiration	Calcium, shoot
4	725	6.0×10^{-6}	1.9×10^{-6}
6	60	1.5×10^{-4}	3.8×10^{-5}

* From 0.5 meq/liter $CaCl_2$ solution over a 24-hour period.

low that of the external solution. At the low Ca level the ratio was twice as great at low as compared with high transpiration rates, and a tenfold increase in external Ca concentration, in the absence of DNP, caused less than a twofold increase in the internal concentration.

II. Dependence of Inhibitory Action of Dinitrophenol on pH & Concentration: The action of DNP was found to depend primarily on the pH of the solution (table I). The concentration of inhibitor required to reduce the rate of transpiration and Ca uptake to the shoot (from 0.5 meq/liter $CaCl_2$) by 50 % was 20 to 25 times as great at pH 6 as at pH 4. A 50 % reduction in the rate of Ca uptake was attained at only one-third the concentration of DNP necessary to reduce transpiration by the same amount, at either pH.

More C^{14}-labeled DNP was accumulated in the shoots from a 10^{-5} M solution at pH 4 than at pH 6 (table I). This accords with the findings of others (4, 9, 11, 21) that the rate of absorption of DNP is dependent on the concentration of unionized DNP molecules (pKa = 4.1) in the external solution.

The effect of DNP on transpiration appeared to be related to the accumulation of the inhibitor in the shoot since stomatal closure was induced within 45 minutes after adding 10^{-5} M DNP at pH 4, but not at pH 6. It seems unlikely that closure was due to a decrease in the water conductivity of the roots since in an unreported experiment with bean roots it was found that conductivity was increased in the presence of DNP.

The C^{14} activity in the shoot was not due to the accumulation of breakdown products of DNP since paper chromatographic separation of shoot and root extracts showed radioactivity only in a single spot, which corresponded to the inhibitor.

III. Transpiration & Uptake of Calcium & Dinitrophenol by Plants With Killed Roots: The uptake of Ca and C^{14}-labeled DNP, together with concurrent transpiration, were determined for plants which had their roots previously killed by immersion in boiling water. The external solution contained 5.0 meq/liter Ca and 10^{-5} M DNP at pH 4 (fig 3). As before, in the presence of DNP the control plants showed an accumulation of the inhibitor, a reduction in

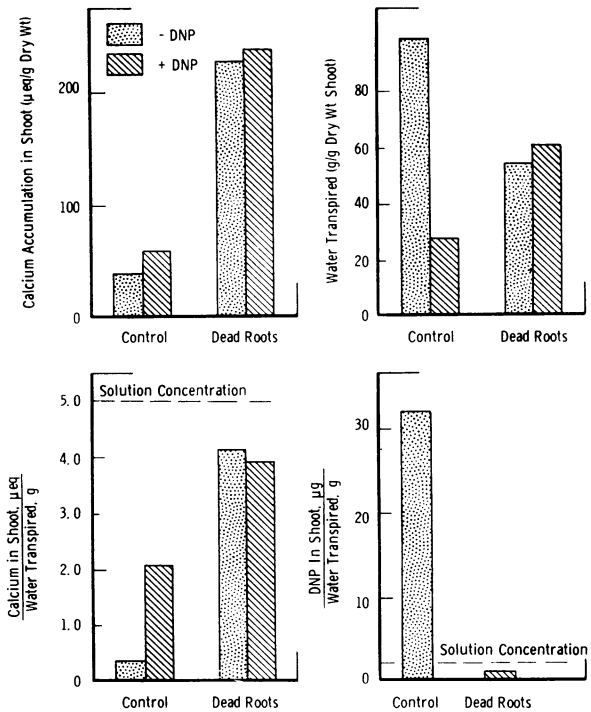

Fig. 3. The influence of living and dead roots on transpiration and the accumulation of Ca and DNP by the shoots of barley seedlings over a 24-hour period. The external solution contained 5.0 meq/liter $CaCl_2$ with and without 10^{-5} M DNP at pH 4.

transpiration, and an increase in Ca uptake to the shoot. Killing the roots caused a sixfold increase in the shoot Ca and a 45 % reduction in transpiration; neither process was further affected by DNP. However, the most remarkable feature of the results was the fact that essentially no DNP was present in the shoots of the plants with dead roots, whereas the inhibitor was greatly accumulated in the shoots of the control plants. In fact, when the quantity of DNP taken up was divided by the volume of water transpired, a 17 fold increase over the external concentration was obtained.

IV. Effect of Dinitrophenol on Time Course of Calcium Uptake & Transpiration: Determinations of Ca and DNP uptake by roots and shoots and of transpiration were made at intervals over a 24-hour period. The external solution contained 5.0 meq/liter Ca with and without 10^{-5} M C^{14} labeled DNP at pH 4.

Changes in Ca and DNP uptake to the shoot with time are illustrated in figure 4; transpiration in the presence and absence of DNP are also shown. The difference in the accumulation of Ca and DNP noted previously was again apparent as was the lack of any correlation between the uptake of the two substances and the rate of transpiration. The uptake of Ca was reduced in the presence of DNP over the first 12 hours and thereafter was increased

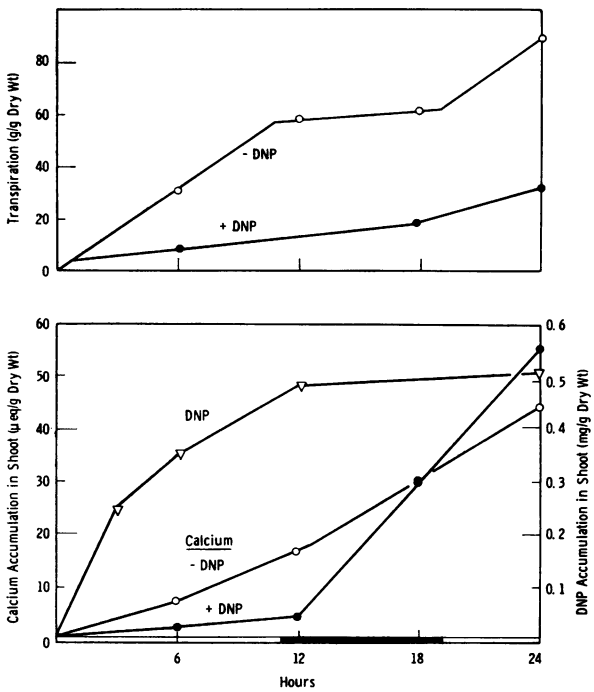

Fig. 4. Changes, with time, in transpiration and the accumulation of Ca from a solution containing 5.0 meq/liter $CaCl_2$ by the shoots of barley seedlings in the presence and absence of 10^{-5} M DNP at pH 4. DNP accumulation was also determined. The dark bar on the horizontal axis represents the 8-hour dark period.

markedly such that, in agreement with previous experiments, after 24 hours more Ca was accumulated in the shoots of treated plants than in the controls. Over the period when Ca uptake was depressed (first 12 hr) the rate of DNP uptake was at a maximum and thereafter fell to a very low level. The contrast in the patterns of uptake of Ca and DNP is illustrated in table II, where values are given for the ratios of Ca or DNP accumulated to water transpired and for the ratio

$$\frac{\text{DNP/Ca shoot}}{\text{DNP/Ca solution}}$$

for four consecutive six-hour periods (see fig 4 to visualize dark period). This latter fraction should have a value of 1.0 if Ca and DNP were taken up at the same relative rate. However, throughout the period of treatment the values were greatly in excess of 1.0 during the first two periods and slightly above 1.0 during the last two periods.

Since, in previous experiments in which uptake of Ca was determined for a 24-hour period there appeared to be a DNP induced decrease in Ca accumulation in the shoot with 0.5 meq/liter $CaCl_2$ and an increase from 5.0 meq/liter $CaCl_2$, the time course of Ca uptake from the lower concentration in the presence of DNP was investigated. It was apparent that at this Ca level also there was an initial decrease in Ca accumulation in the shoot (0.024 μeq Ca/g water) followed by a marked increase (0.22 μeq Ca/g water). However, the increased uptake during the last 12 hours did not completely compensate for the initial decrease, so that the total effect was a reduced accumulation of Ca over the 24-hour period.

V. Effect of Aeration on Transpiration & Uptake of Calcium & Dinitrophenol: Determinations were made of transpiration and the uptake of Ca and DNP at pH 4.0 in the shoot when plants were either aerated, not aerated, or when N_2 was bubbled through the solution at the same rate as air. When N_2 was used it was bubbled through the solution at a rapid rate for 1 minute to help clear the fresh solution of O_2. The external solution contained 0.5 meq/liter $CaCl_2$, with and without 10^{-5} M DNP labeled with C^{14}.

The results, expressed as percentage of controls (aerated), are given in table III. In the absence of aeration, Ca uptake and transpiration were reduced, and DNP uptake also was reduced when it was in-

Table II

Changes in Concentration of Calcium & Dinitrophenol in Shoot With Time

6-hr uptake periods*	Ca (Shoot)** / H_2O Transpired μeq/g***	DNP (Shoot) / H_2O Transpired μg/g***	DNP/Ca (Shoot) / DNP/Ca solution
0–6	0.23	39.8	475
6–12	0.42	25.4	163
12–18	4.85	2.88	1.6
18–24	2.31	1.00	1.2

* Plants in dark during 8-hour period between 11th and 19th hours.
** Solution contained 5.0 μeq/ml $CaCl_2$.
*** Solution contained 1.84 μg/ml DNP.

Table III

Effect of Nutrient Solution Aeration on Calcium & Dinitrophenol Uptake & Transpiration by Shoots of Barley Seedlings*

Treatment	% of Control: Calcium, shoot μeq/g dry wt	Water transpired g/g dry wt	DNP, Shoot μg/g dry wt
Air	100	100	
No air	84	86	
Nitrogen,	62	59	
Air, DNP	15	31	100
No air, DNP	28	38	68
Nitrogen, DNP	44	47	78

* From 0.5 meq/liter $CaCl_2$ solution over a 24-hour period.

cluded in the solution. When N_2 was bubbled through the solution Ca uptake and transpiration were further reduced, but DNP uptake was reduced to a lesser extent.

In the unaerated and N_2 treatments, DNP reduced both Ca uptake and transpiration, but this reduction was less than that obtained in the aerated, control plants. In other words, when DNP was present, aeration increased somewhat the inhibitory effect of DNP on both Ca accumulation in the shoot and transpiration.

Discussion

Handley and Overstreet (8) showed Ca uptake into vacuolated cells of *Zea mays* root to be strongly temperature dependent and, hence, largely metabolic. Prior to the above work, Moore et al. (17) suggested that Ca uptake by excised barley roots at pH 5 was largely non-metabolic, since Ca uptake was insensitive to low temperature and 10^{-5} M DNP. Our data regarding Ca uptake by roots were not conclusive as DNP only slightly affected Ca uptake by the root, especially at the high Ca level at pH 6 and 8. (DNP mainly affected Ca accumulation in the shoot, where Ca was decreased or increased depending on the Ca level of the solution and the time period.) However, our overall data indicated that metabolism was involved in the process by which Ca was eventually accumulated by the barley shoot. The following experimental results supported this conclusion:

I. By extrapolating water loss vs Ca shoot uptake curves (figure not included), approximately 70 % of the shoot Ca was taken up at zero transpiration.

II. There was no consistent correlation between Ca accumulation in the shoot and transpiration.

III. In an unreported experiment the Ca concentration of xylem exudate greatly exceeded the Ca concentration of the solution. (Also see Vaadia, 22.)

IV. DNP greatly inhibited Ca accumulation in the shoot during the first 12 hours.

V. N_2 bubbled through the solution bathing the roots reduced Ca accumulation in the shoot by 38 %.

VI. Killing the roots by heating or freezing increased Ca uptake to the shoot, but greatly reduced the uptake of lipid soluble DNP molecules.

No unequivocal correlation was apparent between transpiration and the accumulation of Ca in the shoot in plants with healthy roots. This was demonstrated by investigating the effect of DNP on the two processes, by determining the uptake of Ca at high and low rates of transpiration, and by comparing the Ca accumulation in shoots of plants with killed roots with that of control plants.

The rate of transpiration of plants with living roots was reduced with 10^{-5} M DNP at pH 4 under all conditions investigated. This reduction appeared to be caused by stomatal closure (determined by a porometer) induced by the accumulation of the inhibitor in the shoot. In contrast, Ca uptake to the shoots was first reduced and then increased in the presence of DNP, apparently due to the action of the inhibitor on root processes.

If Ca accumulation in the shoot is plotted against water lost by transpiration for the experiments in which the transpiration rate was varied, a slight increase in Ca uptake with increased transpiration is apparent and influx coefficients (12, 15, 18) may be calculated. The influx coefficient was 2.1 for the low Ca solution and 0.17 for the high. Extrapolation of the line shows that most of the Ca was accumulated at zero transpiration implying that most of the Ca was actively absorbed.

The present results accord with the hypothesis of Broyer and Hoagland (3), Russell and Shorrocks (20) and others that an increase in transpiration lowers the ionic concentration in the xylem thereby stimulating active uptake. The relative importance of these two possible effects of transpiration have been clearly demonstrated by Falk (15) who, from an examination of influx coefficients at various solution concentrations, showed that at low solution concentrations, as in the present experiments, transpiration stimulated the active transport of K and NO_3 and that only at high concentrations was mass flow in the transpiration stream of major importance.

Transpiration was hardly affected by either the H or Ca ion concentration of the root solution, but was markedly reduced by killing the roots, by including DNP in the solution, and by bubbling N_2 through the solution in place of air.

The low permeability or a barrier of the root to the passage of Ca to the shoot of undamaged plants is shown by the fact that the concentration of Ca accumulated in 24 hours (3 μeq/g fr wt) was small compared with the external concentration (0.5 μeq/ml) and, when the solution concentration was raised tenfold, there was only a twofold increase in accumulation (6 μeq/g fr wt).

Dinitrophenol is a widely used inhibitor of ion accumulation, yet it was accumulated by barley shoots to a concentration 17 times that of the solution bathing the roots. In the absence of aeration DNP uptake was reduced (table III). When changes in the uptake of Ca and DNP with time were determined, it was apparent that DNP was taken up mainly during the period when Ca uptake was slow; i.e., during the first 12 hours before prolonged exposure to the inhibitor had seriously damaged the root (table II & fig 4).

Maximum DNP accumulation was obtained in the shoots of plants with live roots, whereas Ca accumulation was greatest in plants with dead roots suggesting that living roots are highly permeable to DNP, but relatively impermeable to Ca. As cells have lipoprotein membranes, the lipophilic property of undissociated DNP molecules (9) may well have facilitated movement of DNP through such membranes. DNP uptake was so rapid initially that it may have moved predominantly along the lipid phase

of the plasmalemma directly to the vessels. Apparently an available supply of ATP is required for Ca (& Rb, 10) accumulation, and DNP quickly reduces this supply by enhancing the action of adenosine triphosphatase. Eventually the ATP deficit might result in disorganization of the membranes allowing Ca to penetrate freely but retarding lipid-soluble DNP molecules.

The data, while not permitting any definite conclusions to be drawn as to the nature of DNP accumulation, emphasize both the low permeability of the root to Ca and the necessity for the expenditure of metabolic energy as a prerequisite for Ca accumulation in the shoot, at least at the Ca concentrations studied.

Summary

The uptake of 2,4-dinitrophenol (DNP) and its effect on transpiration and the accumulation of Ca in the root and shoot from 0.5 and 5.0 meq/liter $Ca^{45}Cl_2$ solutions was determined.

DNP reduced transpiration and initially decreased Ca accumulation. However, when the roots were exposed to the inhibitor for more than 12 hours, the amount of Ca that accumulated in the shoots was increased over the controls.

There was considerable accumulation of DNP (C^{14} labeled) in the shoot and much more accumulated at pH 4 than at pH 6. In agreement with this, DNP was more effective in reducing transpiration and Ca accumulation at pH 4 than at pH 6. The effect on transpiration appeared to be determined by the presence of the inhibitor in the shoot, whereas Ca accumulation in the shoot was affected by the action of DNP on the roots.

When the integrity of the barrier (probably the plasmalemma) in the root was slightly altered by non-aeration or by short exposure to DNP, Ca uptake was reduced. When the barrier was drastically altered by heating, freezing, or prolonged exposure to DNP, Ca taken up by the shoot was markedly increased, and DNP uptake was markedly decreased. Under these drastic conditions the Ca barrier was essentially removed, allowing Ca to move freely with the transpirational water, and since the lipid phase of the barrier was apparently disrupted, the uptake by the shoot of the lipophilic and relatively large DNP molecules was reduced.

These and other data support the concept that an energy-requiring step is involved in the transport of Ca from the external solution to the xylem vessels of the root.

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