

Effect of Water Stress on Turgor Differences and ^{14}C -Assimilate Movement in Phloem of *Ecballium elaterium*^{1, 2}

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ABSTRACT

The effects of water stress on pressure differences and ^{14}C -assimilate translocation in sieve tubes of squirting cucumber *Ecballium elaterium* A. Rich were studied. Water stress was induced by transfer of plants from culture solution to a polyethylene glycol 6,000 solution having an osmotic potential of -18.2 atm. Sieve tube turgor, turgor differences between source and sink, and translocation rate were decreased. After 260 minutes of translocation, only 19% of the total fixed $^{14}\text{CO}_2$ had moved out of the leaf, compared to the control value of 62% after the same period of time. The results suggest that water stress slows translocation by lowering sieve tube turgor differences, which are essential for the pressure flow mechanism of conduction.

MATERIALS AND METHODS

Plant Material. Squirting cucumber (*Ecballium elaterium* A. Rich) plants were grown under greenhouse conditions until they were 5 to 8 cm tall. Then they were transferred to $1 \times$ Hoagland solution and cultured about 60 days to provide plants 30 to 35 cm tall. Three days before application of $^{14}\text{CO}_2$, they were transferred to a growth chamber under a 16-hr light period and 28 C temperature. In order to have more uniform plants, each plant was trimmed to five leaves 48 hr prior to the application of $^{14}\text{CO}_2$, the younger leaves at the top grading to the most mature at the base. Six uniform plants were selected for each experiment. Just prior to the $^{14}\text{CO}_2$ application, the culture solution of the first set of three control plants was replaced with fresh $1 \times$ Hoagland solution. The three plants in the second set were transferred to a polyethylene glycol solution "PEG 6,000³" with a water potential of -18.2 atm. The solution was prepared by adding 400 g PEG (average mol wt 6,000-7,500) to 1000 g distilled H_2O (in the range 0.057-0.066 molal). Water potential was measured with a vapor pressure osmometer, Wesco model 5130. Air was bubbled into the solution to provide O_2 for the roots.

Because water in the plant exists as a continuum, change in hydration level in any tissue or organ affects the total water balance. Thus, the sieve tube system can be considered to be regulated in its functioning by the availability of water. Weatherley *et al.* (13) found that in the case of *Salix* phloem exudate collected from aphid stylets, wilting of the leaves caused a marked decrease in exudation rate and an accompanying increase in osmotic concentration. Hall and Milburn (3) examined phloem exudation from *Ricinus* plants subjected to water stress. When water was temporarily withheld, the rate of exudation decreased sharply and this was accompanied by an increase of solute concentration in the sap. Upon relieving the water stress, the rate increased rapidly with a corresponding fall in sap concentration.

The effect of water stress on translocation of ^{14}C -assimilates was studied by Hartt (5), Pallas (6), Plaut and Reinhold (7), and Roberts (9). They all reported that moisture stress reduced the rate of translocation. Additional evidences are reported by Crafts and Crisp (2). Since transport is dependent on turgor gradients in sieve tubes, reduced translocation rates may result from a direct disturbance of turgor gradients through lowering of tissue water potential, but there have been no direct studies relating water deficits, sieve tube turgor levels, and rates of photosynthate translocation.

The purpose of this research was to measure the sieve tube pressure in stems of water-stressed and well hydrated plants over a period of time and to follow the corresponding rates of ^{14}C -assimilate translocation.

Radioactive Tracer Technique. Radioactive CO_2 was prepared from ^{14}C -sodium bicarbonate solution as described in an earlier paper (10). Forty μCi $^{14}\text{CO}_2$ were applied to about half of leaf 3 (from the top), and 10 min fixation time in sunlight (outdoors) was allowed. Then the plants were transferred to a growth chamber with a 16-hr light period and 2400 ft-c light. After 80, 140, and 260 min of ^{14}C -assimilate translocation, phloem pressures were measured with the needle technique (4, 10) in the tip, middle, and lower part of the stem, two measurements in each location, in a random order. Translocation was terminated after each pressure measurement by cutting the plants quickly into segments: the five leaves individually, stem, and roots. The segments were dried at 80 C for 48 hr, then ground in a Wiley mill and counted using a Nuclear-Chicago model 1042 counter. A 30-mg sample from each portion was counted for 10 min, twice. Initial and final background counts were averaged and subtracted from the average gross count. Net counts less than 5% of the background were considered to be zero.

The amount of ^{14}C -assimilate in each plant part was expressed as a percentage of the total radioactivity taken up by the plant, including the treated leaf. Tracer movement out of the treated leaf was evaluated by adding together the percentages of radioactivity in all of the plant parts except for the leaf that was fed $^{14}\text{CO}_2$. The data reflected the effects of two factors: (a) water balance; and (b) duration of assimilate translocation. A three-way analysis of variance was used. This method was selected because it permits not only the establishment of significant differences among the means of more than one factor, but also

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³ Abbreviation; PEG: polyethylene glycol.

interactions between different levels of each factor.

In order to study the effect of water stress on phloem pressure gradients, the pressures were measured at three locations in the plant as described before. The pressure differences between the source region and the upper or lower parts of shoots were calculated in controls and water-stressed plants after 80, 140, and 260 min of ^{14}C -assimilate translocation. A mean \pm SE for each combination of treatments was calculated. Two groups of three uniform plants served as controls and treated plants, and these were replicated three times. All of the calculations for analysis of variance were made with a Burroughs B6700 computer.

RESULTS AND DISCUSSION

Water stress decreased the hydrostatic pressure in the phloem as measured with the phloem needle (Table I). Differences in phloem pressure between the source region and other parts of the shoot were lower in water-stressed plants compared to controls. Table II shows that pressure differences between the source region and the upper and lower parts of the control plants were higher than in water-stressed plants after the different periods of translocation. Signs of slight wilting were observed in the leaves 1 hr after plants were placed in the PEG solution. The total $^{14}\text{CO}_2$ fixation in these plants was essentially the same as that in well watered plants, because the $^{14}\text{CO}_2$ was applied before water stress developed. Translocation of radioactive material occurred under water stress but at a much lower rate than in controls. After 80 min of translocation in stressed plants, only 3.1% of the total radioactive assimilate moved out of the treated leaf and became distributed in the other parts of the plant, compared to 19.1% in control plants (Fig. 1). After 140 min of translocation, the percentages of radioactive distributions were 13.9 and 50.4% in water-stressed and well watered plants,

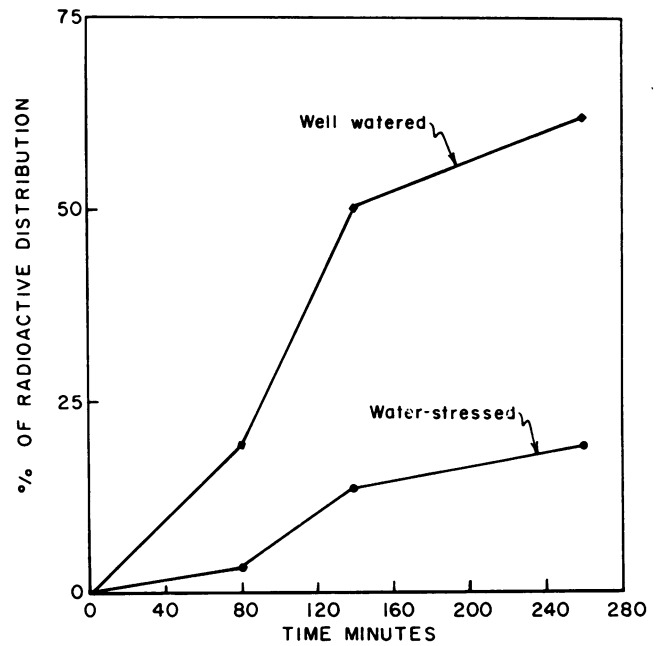


FIG. 1. Per cent of radioactive distribution in well watered and water-stressed *Ecballium elaterium* plants after different periods of translocation.

respectively. A transport time of 260 min allowed movement of 19.1% radioactive material out of the treated leaf of stressed plants compared to 62% out of the treated leaf of control plants.

Table III shows analysis of variance of radioactive distribution in well watered and water-stressed plants after certain periods of $^{14}\text{CO}_2$ translocation. Water stress had a significant effect in

Table I. Effect of water stress on phloem turgor measured in three locations of the stem after certain periods of translocation

Translocation time (min)	Replicate	Pressure in atmospheres					
		Control			Water-stressed		
		P ₁	P ₂	P ₃	P ₁	P ₂	P ₃
80	1	5.9	8.7	3.4	4.0	4.3	2.3
	2	4.0	5.4	2.3	4.6	5.1	3.3
	3	4.9	7.6	3.0	4.1	4.6	2.8
	Mean	4.9	7.2	2.9	4.2	4.6	2.8
140	1	6.5	8.7	4.6	2.5	3.5	1.4
	2	5.1	6.0	2.6	1.2	2.8	1.5
	3	5.2	7.7	3.2	1.9	2.4	1.5
	Mean	5.6	7.5	3.5	1.9	3.0	1.5
260	1	5.0	6.3	4.0	2.5	3.1	2.3
	2	2.8	4.0	2.5	2.1	2.8	1.3
	3	3.2	5.1	2.9	1.3	2.6	2.1
	Mean	3.7	5.1	3.1	2.0	2.8	1.9

Table II. Effect of water stress on phloem turgor gradients between source leaf and upper or lower parts of the shoot after certain periods of translocation

Translocation time (min)	Replicate	Pressure in atmospheres			
		Control		Water-stressed	
		Upper P ₂ -P ₁	Lower P ₂ -P ₃	Upper P ₂ -P ₁	Lower P ₂ -P ₃
80	1	2.8	5.3	0.3	2.0
	2	1.4	3.1	0.5	1.8
	3	2.7	4.6	0.4	1.8
	Mean	2.3 \pm 0.45	4.3 \pm 0.64	0.4 \pm 0.05	1.8 \pm 0.06
140	1	2.2	4.1	1.0	2.1
	2	0.9	3.4	1.6	1.3
	3	2.5	4.5	0.5	0.9
	Mean	1.8 \pm 0.49	4.0 \pm 0.32	1.0 \pm 0.31	1.4 \pm 0.35
260	1	1.3	2.3	0.6	0.8
	2	1.2	1.5	0.7	1.5
	3	1.9	2.2	1.3	0.5
	Mean	1.4 \pm 0.14	2.0 \pm 0.25	0.8 \pm 0.21	0.9 \pm 0.29

Table III. Calculations used in the three-way analysis of variance. Comparisons of means of % of radioactive distribution in well-watered and water-stressed plants after 80, 140, 260 min of translocation

Analysis of variance table for r=2 treatments, t=3 different time periods with s=3 observations for each combination of rt.				
Source of variation	d.f.	Sum of squares	Mean square	F
Between treatments	1	4541.0450	4541.0450	**** 70.228
Within treatments	4	258.6475	64.6619	
Between times	2	2750.6681	1375.3340	**** 115.397
Interaction of treatments and times	2	590.0425	295.0213	**** 4.754
Within interaction of treatments and times	8	95.3460	11.9183	
Total	17	8235.7491		

**** Significant level, 0.005

decreasing both phloem pressure differences and translocation. Although transport increased with time from source to sinks, there was always much less movement in water-stressed compared to control plants.

Water deficits were induced by the polyethylene glycol solution, which contained no nutrients. This unbalanced solution might have had some effect on the metabolic activity of the plants. Because the time periods for the experiments were short, any toxic effects of the polyethylene glycol solution were considered to be negligible.

Since phloem transport is thought to depend on turgor pressure gradients, suppressed translocation is considered to result from a direct lowering of phloem pressure through reduction in tissue water potential. After 280 min, the pressure difference between source region and lower shoot was as low as 0.9 ± 0.29 atm, and as low as 0.8 ± 0.21 atm between source and the upper shoot, of the water stressed plants; still, 19.1% of labeled assimilates moved from source to sinks during the translocation period.

Weatherley and Johnson (12) calculated that 0.56 atm of pressure/m is required for assimilate translocation in *Salix* sieve tubes at a rate of 100 cm/hr. Based on Crafts and Crisp's (2) calculations, even a lower pressure difference, about 0.23 atm/m, would be sufficient for flow through the sieve tubes of well watered plants. Hall and Milburn (3) demonstrated that when *Ricinus* plants were subjected to water stress, the rate of phloem exudation decreased sharply and this was accompanied by a rise in the osmotic concentration of the sap. On relieving the water stress, the rate increased rapidly with a corresponding fall in sap concentration. They concluded that exudation from plants under considerable water stress might be considered evidence for the operation of a "sugar pump" capable of maintaining a high turgor pressure in sieve tubes at the source.

Water stress had a highly significant effect in decreasing the percentage of radioactive distribution. Although the percentage

of tracer translocation increased with time from source to sinks, there was always much less in water-stressed compared to control plants. These results are in agreement with those of Reinhold and Glinka (8), Roberts (9), Wardlaw (11), and Weatherley *et al.* (13), and the interpretations are the same as made by Crafts (1) in his review. Our observations lead to the conclusions that the sieve tube system is regulated in its function by the availability of water and that a sieve tube turgor gradient is the driving force for assimilate distribution.

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