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Effect of Petiole Anoxia on Phloem Transport in Squash[1]

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ABSTRACT

Translocation of ^{14}C-labeled assimilates in Early Prolific Straightneck squash (*Cucurbita melopepo torticollis* Bailey) through a 15-centimeter oxygen-deficient zone of the petiole was studied as a function of varying periods of anaerobiosis (N_2 atmosphere). Initiation of anaerobic conditions caused an immediate and rapid decline in translocation to about 35 to 45% of the pretreatment rate within 30 to 40 minutes. This inhibition response (first inhibition response) was transient, however, and full recovery to the pretreatment rate occurred during the ensuing 60 to 90 minutes. Following this adaptation response to anaerobic conditions, translocation continued unimpaired for extended periods of time, approaching, and in some cases exceeding, 24 hours. The second inhibition response was permanent and could not be reversed by supplying air during a subsequent 20-hour period.

Studies on the oxygen requirement for phloem transport have been made by Curtis (3), Mason and Phillis (14), Bauer (1), Willenbrink (19), and Ullrich (17). The results of these studies are inconsistent and have led to different conclusions regarding the essentiality of oxygen in the transport process.

The extensive studies by Mason and Phillis (14) on cotton revealed that under certain conditions near normal rates of phloem transport may occur even after extended periods (up to 3 weeks) of stem anoxia. In only one series of experiments in which very drastic precautions were taken to ensure as completely anaerobic conditions as possible (including separating the phloem from lateral contact with the xylem, inserting a layer of waxed paper to prevent oxygen transfer from the transpiration stream to the phloem, and sealing off this stem region from the atmosphere by encasing it in a 20 cm long well of deoxygenated mineral lubricating oil), total inhibition of transport was achieved for a period of about 2 days. Even under these severe conditions, however, phloem transport partially recovered with time, reaching about 40% of the control rate at termination (162 hr) with evidence that recovery was still continuing. Mason and Phillis (14) attributed this recovery to gradual infiltration of atmospheric oxygen into the phloem through the oil seal. In general, they concluded that phloem transport does require oxygen and they postulated that this dependency is specified by the metabolic energy level required to maintain the requisite structural integrity of the sieve-tube cytoplasm.

Curtis (3) likewise concluded from experiments based on dry weight changes in bean leaves that phloem transport is oxygen-dependent. However, the differences between the treated and nontreated plants were small and of doubtful significance statistically.

By contrast, the extensive data compiled by Bauer (1), Willenbrink (19), and Ullrich (17) rather consistently revealed no effect of oxygen deficiency on phloem transport. These experiments, however, were of relatively short duration, in the range of 3 to 24 hr and were based in the main on the translocation of fluorescent tracers, principally fluorescein.

The present paper is a further contribution to the problem of the oxygen dependency of phloem transport. Advantage was taken in this investigation of the apparatus and methodologies developed in this laboratory in connection with earlier studies on phloem transport in relation to low temperatures (16). This method is based on measuring the rate of arrival at an active sink (young leaf) of labeled translocate of constant specific activity, and permits continuous, nondestructive rate measurements to be made.

MATERIALS AND METHODS

Early Prolific Straightneck squash plants (*Cucurbita melopepo torticollis* Bailey), pruned to a simplified source-path-sink system (Fig. 1A) were used in these experiments. Culture methods were essentially as previously described for sugar beets (5, 6), except that the plants were grown under a bank of water-filtered 300-w reflector-flood lamps for the final 9 to 10 days of their growing period to promote greater elongation of the source-leaf petiole.

The apparatus used for measuring translocation rates has been fully described in earlier papers from this laboratory (5, 6, 16). Briefly, the procedure was as follows: the blade of the source leaf was enclosed in a clear plastic cuvette and ^{14}C-labeled carbon dioxide, of constant concentration (500 μl liter^{-1} of air), and constant specific activity (1.34 to 1.56 μc ^{14}C ml^{-1} CO_2, the actual value varying with different experiments), was circulated through the cuvette throughout the total time course of the experiment (except in the long term experiments, where the protocol was modified as described below). Simultaneously, the influx of ^{14}C into the sink leaf was monitored with a thin window G-M detector connected to a rate meter and recorder. Following isotopic equilibration of the transport molecules with the ambient $^{14}CO_2$ (considered to obtain when the rate of ^{14}C influx into the sink leaf became constant, usually within 140 min from the start of labeling in these experiments), any variation in the translocation rate would be measured as a corresponding change in the ^{14}C-accumulation rate in the sink leaf.

Sink leaves, at the physiological stage used in these studies, do not export assimilates (18) and hence function as one-way

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traps. Thus the ^{14}C-accumulation data were not confounded by re-export of ^{14}C. To avoid possible errors arising from retranslocation of labeled translocate from the roots to the sink leaf, a 1-cm stem zone located about 1 cm below the insertion node of the source leaf was heat-killed (Fig. 1B) approximately 24 hr prior to an experiment. No wilting was evident in these plants for periods of up to several days.

Also prior to each experiment the source-leaf petiole (which is hollow in squash) was split lengthwise between the adaxial vascular bundles, and the petiole enclosed in a flattened configuration within a 15 cm long plastic cuvette. Anaerobic conditions for the enclosed portion of the petiole were established by perfusing water-saturated high purity N_2 gas (maximum impurities, 0.004%, manufacturer's rating) through this cuvette at a flow rate of 200 to 300 ml min^{-1} following an initial flush-out rate of 3000 to 4000 ml min^{-1} for 5 min. The petiole cuvette was covered during the experiments to exclude light.

For convenience of description we refer below to short term and long term experiments. In short term experiments, petiole anoxia was not initiated until after the attainment of steady-state labeling (*i.e.*, when the specific activity of the transport molecules was at saturation equilibrium with the $^{14}CO_2$ supplied). Any immediate effects of anaerobiosis on translocation would be readily discernible by this procedure because changes in the ^{14}C-accumulation rate in the sink leaf are readily measurable as long as the background count level of the sink is not excessively high. Given the usual specific activity of the labeled CO_2 employed in these studies and the usual rates of translocation encountered, the maximum time course of these measurements was restricted to about 8 hr. In long term experiments, therefore, petioles were subjected to anaerobic conditions for periods of varying duration prior to the start of labeling. This method, of course, precludes measurements of the pretreatment rate in the experimental plant, and hence the post-treatment rate must be referenced against nontreated plants for controls. In the short term experiments, by contrast, each plant served as its own control.

For purposes of this study the translocation rates were recorded simply as the average increase in cpm min^{-1} for $\Delta t =$ 10 min when the rate of change in slope occurred slowly and for $\Delta t = 5$ min when the rate of change occurred more rapidly. In short term experiments, the respective values for the pretreatment rates were normalized to 100 to facilitate interplant comparisons.

A more detailed description and analysis of these methods as applied to the present experiments is given by Sij (15).

RESULTS AND DISCUSSION

Figure 2 presents the results of four short term experiments. Following the start of N_2 treatment (time 0 for petiole anoxia), the translocation rate declined rapidly, reaching a value of 35 to 45% of the pretreatment or control rate in 30 to 40 min and then recovering fully over the next 60 to 90 min, even though the conditions for petiole anoxia were maintained. This response curve is remarkably similar to the low temperature response curve obtained in sugar beets, when a 2-cm zone of the source-leaf petiole was subjected to a temperature near 0 C (2, 16). The kinetics are also quite similar to those obtained for sink-leaf anoxia in sugar beet (4) and suggest that a common mechanism may operate during at least the initial stages of inhibition.

The "standard decay" curve given in Figure 2 is the average of three leaf-excision experiments, in which the source leaf, following the establishment of steady-state translocation, was excised at a position on the petiole corresponding to the basal end of the petiole cuvette. It appears that labeled translocate previously accumulated in the stem above the girdle and in the basal portion of the source-leaf petiole can be retranslocated in substantial quantities to the sink leaf when the source leaf is excised. If the same amount of retranslocation from these storage sites is induced by N_2 treatment, the actual inhibition resulting from anoxia would then be considerably greater than that shown in Figure 2. A correction based on these leaf-excision data was not attempted, however, because the specific activity of the translocate from these storage sites is not determinable, and furthermore it is not clear that an anaerobic block would necessarily function as the equivalent of actual excision of the source blade.

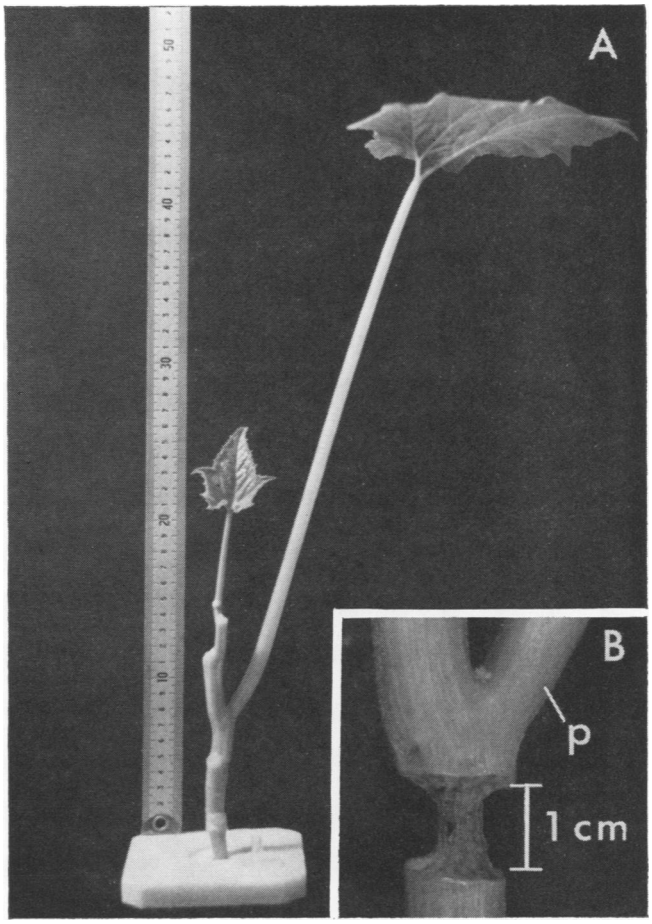

FIG. 1. A: A nongirdled 21-day-old squash plant trimmed to a simplified test system with a mature 4th leaf as the source and an immature 7th leaf as the sink. B: Close-up of heat-killed zone on stem about 1 cm below fourth leaf node; p: petiole of fourth leaf. Plants were girdled about 24 hr prior to experiment.

The cause of the transient inhibition response ("first inhibition response") is not known. Its occurrence has not been noted by previous investigators. As mentioned earlier, the time course of this response curve closely resembles the low temperature response curve in sugar beet (2, 16). It has been postulated that the inception of cooling imposes a transient water stress on the source-leaf blade, leading to a temporary closure of the stomates, reduced photosynthesis, and hence reduced translocation. No evidence, however, for such a transient reduction in photosynthesis was found in a recent study on sugar beets (2).

Table I presents the results of 10 long term experiments. These data show that the translocation capacity of the petioles, after recovery from the initial inhibition, was not significantly

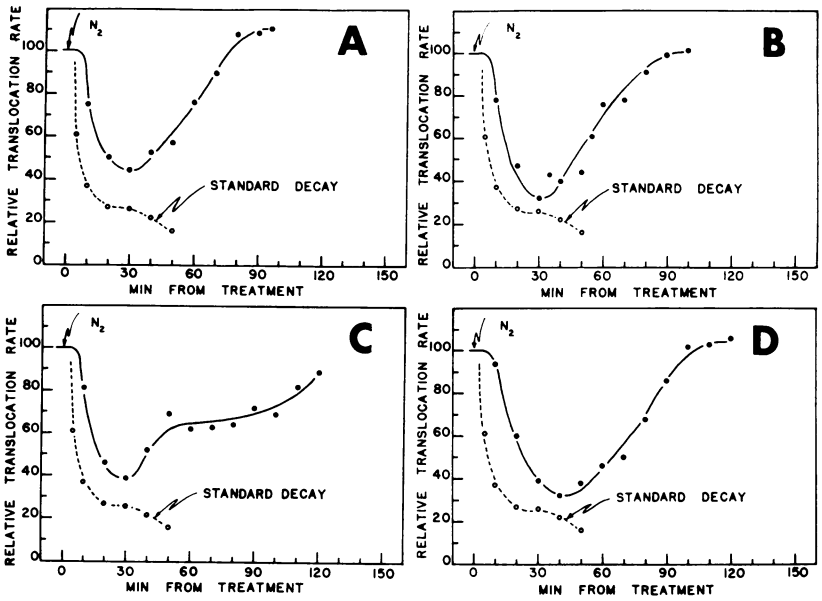

FIG. 2. Time course of relative translocation rates for four plants after initiating nitrogen treatment (short term experiments). The "standard decay" curve is an average of three experiments in which the source-leaf petiole was excised. A, B, C, and D: 100 equals respectively 236, 315, 252, and 236 cpm min^{-1}.

TABLE I. *Translocation Rates as a Function of Duration of Anaerobic Treatment Prior to Initiation of Labeling*

Results represent long term experiments.

Duration of Anaerobiosis[1]	No. of Plants	Rates
		Δ *cpm* min^{-1}
0 hr N_2 (control)	4	236, 236, 252, 315; Avg 260
18 hr N_2	4	178, 234, 240, 384; Avg 259
21 hr N_2	2	197, 238; Avg 218
24 hr N_2	3	0, 0, 71; Avg 24
37 hr N_2	1	0

[1] Prior to start of labeling. Total elapsed time including measurement period was 3 to 7 hr longer.

impaired again for at least another 18 to 21 hr of continuous anaerobic treatment. The duration of anaerobiosis required to effect total inhibition varied with different plants. An experiment of 37-hr duration revealed complete inhibition, as did two experiments of 24-hr duration. However, in a third 24-hr experiment, a moderate translocation rate was observed even after 3 hr of labeling, *i.e.*, after 27 hr total elapsed time of N_2 treatment. The two 21-hr experiments gave near normal translocation rates even after 7 hr of labeling or 28 hr total elapsed time. Because of this variability, efforts to define more precisely the maximum permissible duration of anaerobic conditions proved inconclusive. Inhibition resulting from prolonged anaerobiosis (the "second inhibition response") was permanent and could not be reversed by restoring the petioles to air for even a 20-hr period.

Although the first visible symptoms of damage to the petiole tissues due to anoxia (partial cell collapse and extrusion of cell liquids on surface and into intercellular air spaces) occurred well in advance of any measurable reduction in translocation, it is possible that this damage, at least initially, was mainly confined to the intervascular parenchyma. Ethanol and other fermentation products accumulate in plant parts during anaerobiosis (7, 9, 11, 14), but their accumulation to toxic levels may have been considerably delayed in the vascular tissues *per se* as a result of elution by either the transpiration stream or assimilate stream or both. If we postulate a toxicity threshold for transport inhibition, different elution rates resulting from different transpiration and translocation rates occurring in the several experiments may account at least in part for the variability noted above.

These data establish with reasonable certainty that phloem transport may occur without impairment for extended periods of time through a zonal region physiologically characterized by very low levels of aerobic metabolism. Of interest, therefore, is the finding by a number of investigators (10, 17, 19, 20) that carbohydrate transport in the phloem is markedly sensitive to cyanide inhibition. No clear explanation of this apparent contradiction is presently possible, but several possibilities may be suggested as follows:

1. Oxygen is continuously supplied to the phloem tissue by the transport of dissolved oxygen in either the transpiration stream or assimilate stream or both, thus preventing anoxic conditions within the sieve tubes *per se*. A rough calculation, however, indicates that this oxygen source would be very negligible. Assuming the dissolved oxygen content in the transpiration stream to be 5 μl O_2 ml^{-1} water (the saturation value in equilibrium with the partial pressure of atmospheric oxygen), a transpiration rate of 1 g hr^{-1}, and a respiration rate of 500 μl O_2 hr^{-1} g^{-1} fresh weight of fibrovascular tissue (12), an oxygen supply equal to about 0.6% of the potential aerobic demand would be maintained (the fresh weight of the fibrovascular tissue in a 15 cm length of squash petiole was estimated to be 1.5 g). Thus it appears highly probable that anoxic conditions obtained throughout all petiole tissues in the anaerobic zone, at least for a substantial portion of the time course of these experiments.

2. The rates of energy supply by fermentation may be less under conditions of cyanide-inhibited respiration (aerobic fermentation) at the concentration levels employed in these studies than under conditions of anoxia (anaerobic fermentation). Lindenmayer (13) reports that the half-value for cyanide inhibition of fermentation in yeast is about 10 mM (compared to about 5 μM for respiration). Ullrich (17) observed a comparable value for inhibition of fermentation in isolated seg-

ments of phloem tissue from *Heracleum spondylium*. This value is of the same order as the cyanide concentrations (1 to 10 mM) reported to inhibit translocation.

3. The cyanide molecule is readily translocatable and moves rapidly to the source leaf where it may be expected to inhibit photosynthesis or loading processes. By this argument cyanide inhibition of transport would be a secondary effect only. Definitive data for or against this view are lacking but evidence has accumulated that cyanide acts directly on the transport process or system within the phloem tissue (10, 20).

4. Cyanide may be more effective than anaerobiosis in decreasing the permeability of sieve-tube membranes. Ginsburg and Ginzburg (7), for example, showed that osmotic flow in *Zea mays* root tissues is severely inhibited by 3 mM cyanide; conversely, Glinka and Reinhold (8) reported no effect of anaerobiosis (100% N_2) on this process in roots of pea seedlings or in carrot root tissues and only moderate inhibition (18%) in sunflower hypocotyls. No data specific for phloem tissue have been reported.

5. Oxygenation of the phloem tissue is maintained by a cyanide-sensitive peroxidase-peroxide transport system. Some minor evidence supporting this hypothesis is reviewed by Mason and Phillis (14) and Ullrich (17).

Present data do not permit a more definitive resolution of this problem. Although the degree and duration of anoxia obtaining specifically in the phloem tissue in our experiments cannot be determined with certainty, our data strongly suggest that unimpaired transport is possible for an extended time period through a transport tissue maintained essentially by anaerobic metabolism. The eventual cessation of translocation under these conditions may be attributed to membrane denaturation and other moribund changes resulting from prolonged anaerobiosis. These data, particularly when considered in conjunction with the recent work of Coulson *et al.* (2) on sucrose transport in the sugar beet in relation to petiolar respiration, support the view that such energy requirements as exist for axial transport *within* the sieve tubes are linked mainly to protoplasmic maintenance.

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