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Effect of Path or Sink Anoxia on Sugar Translocation in Roots of Maize Seedlings

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ABSTRACT

After feeding the scutellum of young maize seedlings with a labeled analog of glucose, 2-deoxy-D-glucose, the progress of radioactivity along the root was followed when either 70% of the path or the whole root were in strict anoxic conditions, and was compared with the translocation pattern of aerobic seedlings. Special care was taken to suppress the internal O_2 transport and to control its occurrence.

In air, the radioactive compounds accumulated from 30 minutes in the root tip mainly as an analog of sucrose. When the whole root was anoxic, the progress of the radioactivity was very slow and never reached the tip which did not survive more than 8 hours. When 70% of the path was in strict anoxia and the sink (root tip) in air, the translocation was not impaired and the radioactivity accumulated in the tips as fast as in aerobic controls. The addition of 3 millimolar NaF, which inhibits the fermentative energy production, did not modify these results. It is concluded that long distance transport in maize sieve tubes has no special energy requirements and is controlled by source-sink relationships. The inhibition of sugar supply in anoxic root tips is attributed to an effect on unloading processes rather than on sink metabolism.

The roots of young maize seedlings have no significant carbohydrate reserves and the metabolic activity of the tip is highly dependent on the flux of carbohydrate imported from the seed (15). In the absence of limiting factors acting on the source (seed) or on the translocation path, the amount of sugar transported is adjusted to the needs of the sink (root tips). Conversely, a limitation of sugar delivery to the tip will limit its activity almost immediately.

In natural conditions, the root systems are often submitted to large fluctuations of O_2 partial pressure in the rhizosphere and eventually to long periods of anoxia in flooded soils which might limit either the flux of carbohydrates translocated in the phloem or the metabolic activity of the sink. Under these conditions both the path and the sink are submitted to anaerobiosis and it is difficult to ascertain a precise site for the inhibition, if any, of sugar supply in the absence of O_2.

The effect of anoxic treatment on the translocation of carbohydrates in roots has received little attention and most of the studies are done on aerial organs which is meaningless to an agronomical point of view. A recent study (19) suggests that inhibition of sugar supply in anaerobiosis might operate on phloem unloading processes in the maternal seed coat of developing soybean seeds.

In general, the inhibition of the energy metabolism of sink regions results in decreased translocation (6) but in tissues other than developing seeds, it is difficult to discriminate between the effects on the portion of the path included in the sink region, the unloading process itself and the subsequent utilization or accumulation of assimilates by the tissues. In addition, except for the work of Mason and Phillis (11), no precautions are mentioned in the more recent studies focusing on sugar supply in anoxic conditions to control and limit the internal O_2 transport which may offset in many plants the absence of O_2 in the external medium (17).

These considerations and the fact that no ubiquitous mechanism exists for translocation and phloem unloading (7) might explain why anoxic treatment of both path and sink regions have led to inconsistent results and conflicting conclusions (6, 18).

The present study has been designed to separate the effects of anoxia on sugar transport in path and sink regions of maize roots. Taking advantage of the methodology developed previously (17), special care has been taken in these experiments to control internal O_2 transport.

MATERIALS AND METHODS

Maize seeds (*Zea mays* L. INRA 508) were first infiltrated under vacuum with the nutrient medium described in Saglio and Pradet (15) and germinated for 4 d at 25°C between sheets of filter paper soaked with the nutrient medium and inclined at an angle of 45° in order to obtain straight seminal roots. Just before the experiment, the coleoptile, secondary roots, and the endosperm were removed from each maize seedling in order to obtain a simplified source sink system composed of the scutellum and the main seminal root (10–12 cm long).

The Experimental Device. The seedling was inserted through a hole made in a plastic cap and through the luer end of a disposable 5-ml syringe (Fig. 1). The scutellum was held by a gasket of sealing compound (terostat 9010). When needed, a circular incision was made under a microscope in the root cortex (girdle) at the level where the root extruded from the luer end of the syringe. This incision was made in order to fill the cortical air spaces with sap to prevent internal O_2 transport from the aerobic toward the anaerobic region. For anoxic treatments of the path, some sealing compound was pushed around the girdled root in the luer end of the syringe, with the help of a sawn-off cone, cut off from a disposable pipetting tip. The separation between the translocation path compartment (8 cm long) and the sink compartment (root tip) was waterproof and gas tight. The 5-ml syringe was fitted onto another 10-ml disposable syringe (sink compartment) and the scutellum (the source) was covered by a disposable 5-ml pipetting tip. All the compartments, source, path, and sink, were lined with wet filter paper and could be flushed separately or not as desired, by air or N_2 saturated with water. The gas flow was about 100 ml/min and the N_2 contained less than 50 μl O_2/l.

Translocation Studies. Translocation was followed by measuring the progress of radioactivity along the root, after feeding

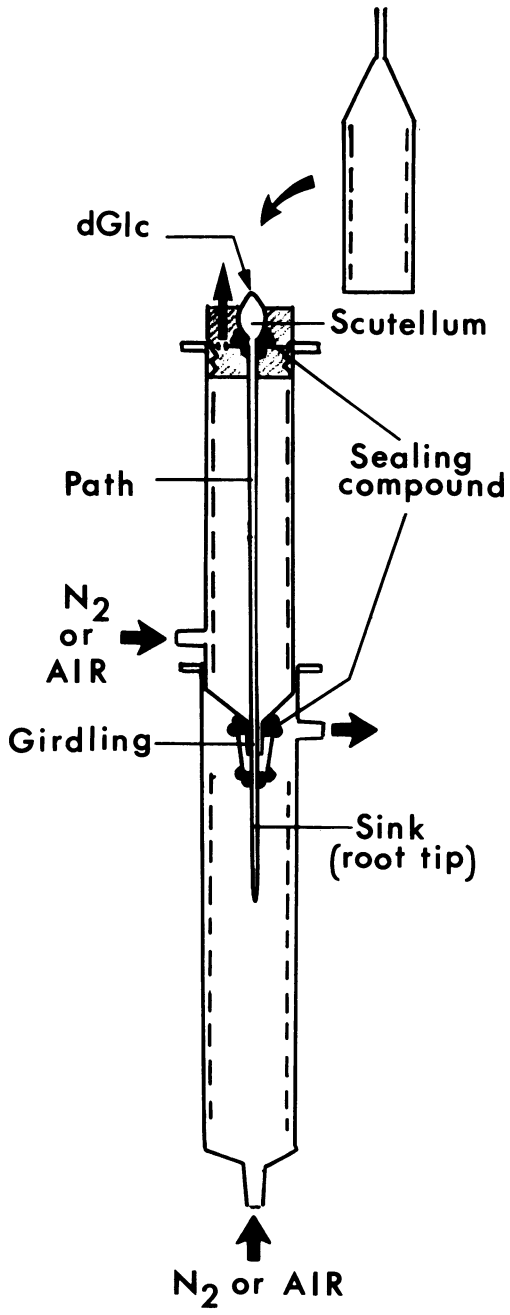

FIG. 1. Experimental device permitting the maintenance of the source, path and sink compartments at different oxygen partial pressure during the translocation studies.

the scutellum with a labeled analog of glucose, dGlc[1]. The scutellum was coated with a very thin layer of cotton wool which was subsequently soaked with 20 μl (1 μCi) of either [1-^{14}C]dGlc (50 mCi/mmol, CEA, France) or [1-^{3}H]dGlc (20 Ci/mmol, CEA, France). The cotton wool permitted a close contact between the surface of the scutellum and the labeled solution for periods of time lasting from 10 min to several hours depending on experiments. At the end of the feeding period, the cotton wool was removed and the scutellum was rinsed three times each with 1 ml of deionized H_2O. After various times, adjacent 1-cm root segments or the root tip alone were cut off and added to 2 ml of aqueous counting scintillant (ACS II, Amersham corporation) for the determination of the radioactivity in a liquid scintillation spectrophotometer (SL 30, Intertechnique, France).

Anoxic Path Treatment. To assess that the stele has not been hurt and that the translocation was not affected by the circular incision made to stop the internal O_2 transport from the aerobic apex to the anoxic path, we have used a double labeling method.

We first added [1-^{3}H]dGlc to the scutellum of incised maize seedlings secured on the experimental devices as for anoxic path treatment, but maintained in air for 2 h. Then, the scutellum was rinsed 3 times, wrapped in new cotton wool and [1-^{14}C]dGlc added. After 10 min in air to permit the loading of the [1-^{14}C]dGlc, the scutellum was rinsed again and both path and sink compartment were flushed with N_2 in order to prevent any possibility of internal O_2 transport from the scutellum. After 2 h, the 1-cm root tip was excised and the radioactivity counted using two preselected windows of the spectrophotometer to differentiate the ^{3}H from the ^{14}C radioactivity. The remaining seedling was saved for control of internal O_2 transport as described below.

So, each seedling has its own control of translocation in air, consisting of the accumulation or the absence of accumulation of [1-^{3}H]dGlc in the tip of the incised root. The build up of [1-^{3}H]dGlc in the tip will mean that the translocation path was intact and, therefore, conclusions could be drawn about the effect of path anoxia on the translocation of [1-^{14}C]dGlc. On the contrary, the absence of ^{3}H radioactivity in the tip would mean that the cortical incision had stopped the translocation and the experiment could be discarded.

Control of the Internal O_2 Transport. At the end of the experiment, the O_2 transport was quantified essentially as described in Saglio *et al.* (17). Briefly, the path compartment was filled with liquid medium (15) supplemented with 0.1 M glucose and 3 mM NaF. Both path and source were flushed for 1 h with air and then with N_2. After 90 min of anoxic treament, the seedlings were quickly frozen by transfer into liquid N_2. The contact with air lasted less than 2 s. Then, a 2-cm segment between 3 and 5 cm from the base of the scutellum was excised from the frozen root. This segment, which corresponded to the middle of the translocation path, was assayed for adenine nucleotides as in Saglio and Pradet (15). When a value lower than 0.5 was found for ATP/ADP ratio, it was assumed that no O_2 transport occurred. Values higher than 0.5 were interpreted to mean some internal O_2 transport and the experiment was discarded.

Analysis of the Translocated Labeled Compounds. The [1-^{14}C]dGlc was added, as described, on the scutellum of 20 maize seedlings. After 4 h of incubation in air at 25°C, the root tips (1 cm) were excised and boiled together twice in 10 ml ethanol (80% in water, v/v) for 10 min. The extract was then dried under vacuum and resuspended in 200 μl H_2O. An aliquot (5 μl corresponding to about 30,000 cpm) was spotted on TLC aluminum sheets of cellulose (F_{254} Merck). The chromatogram was run in *n*-butanol-acetic acid-water (2/1/1, v/v/v) for 6 h at 20°C. The radioactive compounds were located on Fuji medical x-ray film after a 3-d exposure period.

To characterize the spot expected to correspond to 2-deoxysucrose, 50 μl (300,000 cpm) of the tip extract were chromatographed as described. The corresponding spot was recovered and incubated for 10 min at 100°C in 100 μl of 0.1 N HCl. A standard of [1-^{14}C]dGlc was treated in a similar way. The sample was pelleted for 5 min at 12,000 rpm in an Eppendorf microcentrifuge and, an aliquot (about 25,000 cpm) was chromatographed with the treated dGlc standard (about 15,000 cpm) as described above.

RESULTS

Control of O_2 Transport. The results of Table I show the effect of various treatments on the value of ATP/ADP ratios in the 2-

[1] Abbreviation: dGlc, 2-deoxy-D-glucose.

Table I. *Occurrence of Internal Oxygen Transport in the Translocation Path of Seminal Roots of Maize Seedlings*

The path was immersed in liquid medium maintained anoxic by continuous N_2 bubbling. The source and the sink were maintained either in air or in N_2.

Environmental Conditions			Girdling[a]	ATP/ADP Values[b]
Source (seed)	Path (root)	Sink (root tip)		
air	N_2	N_2	no	2.58, 3.01, 4.04, 1.18, 2.16, 2.48
N_2	N_2	N_2	no	0.39, 0.26, 0.41, 0.93, 0.52, 0.37
N_2	N_2	air	no	1.95, 0.95, 1.67, 2.53, 3.88, 2.57, 2.33, 3.52, 1.56, 3.87, 2.92, 3.61
N_2	N_2	air	yes	0.33, 1.06, 0.27, 0.31, 0.27, 0.32, 0.34, 4.38, 2.81, 0.56, 4.29, 0.26, 0.26, 0.22, 0.23, 0.25, 0.70, 1.99, 0.41, 0.86, 0.35, 0.40, 1.32, 0.25, 1.56, 1.09, 0.74, 0.26

[a] The girdle, defined as a circular incision of the root cortex, was situated between the path and the sink.

[b] Each value corresponds to a 2-cm path segment excised from a single seedling. In air, the ATP/ADP ratio was higher than 5.

cm path segments assayed for adenine nucleotides. When the source (scutellum) was in air and the root in N_2, the value of ATP/ADP ratios indicated some respiratory activity due to internal O_2 transport as previously reported (17). A similar internal O_2 transport occurred in the absence of girdling when the sink was maintained in air and the path and source region were in N_2. When a circular cortical incision was made between the sink and the path compartments, the ratio of 17 among the 28 tested seedlings dropped to values close to that of the control maintained in N_2, indicating that internal O_2 transport had been prevented in 17 of the 28 girdled seedlings tested.

Determination of the Minimum Loading Time in Air. When the seed or the scutellum is in air, there is significant internal O_2 transport down the root (Ref. 17; Table I). In anoxic treatment of path and sink, the easiest way to avoid this transport was to maintain the scutellum in N_2 during the experiment. However, phloem loading processes, which are now relatively well understood (2, 7), are usually reported to be energy dependent (9) and inhibited by anoxia. Although it has been reported that phloem loading in maize leaves is unaffected by the absence of O_2 (20), it is not known that these findings also apply to the scutellum. Furthermore, the interference of internal O_2 transport which was not controlled in these experiments may have masked the inhibitory effect of anoxia. To be sure that enough label had been loaded in the sieve tubes before starting the anoxic treatment, the minimum loading time required in air to detect some radioactivity in the 1st cm of the root near the scutellum was determined (Table II).

After a 10-min loading period, radioactivity was detected in the 1st cm below the scutellum, some in the 2nd cm, but very little or no radioactivity lower than that. After 20 min, the radioactivity had already reached the tip in which some accumulation of labeled compounds could be observed after 30 min. These data indicate a translocation velocity of about 60 cm/h which falls in the range of the most commonly accepted values (10). Unless otherwise noted, a 10-min loading period in air was chosen in all subsequent experiments.

Aerobic or Anaerobic Treatments of Both Path and Sink. Typical translocation patterns obtained after several hours are reported in Table III. These experiments were repeated several times. Significant differences could be noticed in the total amount of radioactivity recovered in the root which varied by a factor of 5 from seedling to seedling and also in the percentage of radioactivity accumulated in the tip which ranged between 20% and 50% for all the translocation time tested in air. However, all these experiments gave a similar pattern. In air, there was a quick and strong accumulation of labeled compounds in the tip. In anoxia, the translocation was almost completely stopped and after 6 h of anoxic treatments the radioactivity was hardly detectable in the tips, which were still alive as indicated by the amount of adenine nucleotides found in the tissues (2 nmol/tip) and by the turgescence of the tips. After 8 h of anaerobiosis, the tips were soft and the absence of measurable amounts of adenine nucleotides, even after transfer for 1 h in air, indicated that the tissues were dead.

Anaerobic Path Treatments. The results of the measurement of ^3H and ^{14}C radioactivity in the 1-cm root tips after 2 h of anoxic path treatment and the value of ATP/ADP ratios in the corresponding 2 cm segment of the path are shown in Table IV. There was no radioactivity accumulated in the tip of 14 seedlings of 29 assayed, indicating that in these 14 plants, the path had been damaged by the incision made to stop the internal O_2 transport. The remaining plants which had accumulated [^3H] dGlc compounds and therefore had an intact translocation path, also accumulated [^{14}C]dGlc compounds. Of these plants, six still maintained some internal O_2 transport as shown by a ratio of ATP/ADP higher than 1, two were doubtful, and seven plants in which ATP/ADP value was lower than 0.5 had no internal O_2 transport. Nevertheless, they accumulated [^{14}C]dGlc very actively in the root tips.

Identification of Translocated Labeled Compounds. As shown on the chromatogram presented in Figure 2, the radioactivity accumulated in the tip was distributed among four compounds. One of them (spot no. 3) is dGlc which represents 27% of the total radioactivity. Two minor spots (nos. 1 and 4) are not identified. The major one (spot no. 2: 61% of the total radioactivity) has been identified as 2-deoxysucrose on the basis of its acidic hydrolysis which produced a single labeled compound spotting at the same R_F as that of the reference dGlc (Fig. 2).

Effect of NaF on Translocation. To suppress the metabolic energy provided by the fermentative processes which are active in maize tissues in the absence of O_2 (16), the roots of some seedlings were preincubated for 1 h in aerated nutrient solution containing 3 mM NaF. Then, the nutrient solution was removed and the translocation of dGlc compound was studied as described. At the end of the experiment, a 2-cm segment of the anoxic path was analyzed for nucleotides in order to check both the energetic status of the tissues during the experiment and the occurrence of internal O_2 transport. As shown in Table V, preincubation with NaF did not significantly modify the translocation pattern when both path and sink were maintained in air or in N_2 (compare *cf.* Table V and Table III). There was still a quick and strong accumulation of radioactive compounds in the tip in aerobic conditions, whereas in anoxia, the translocation was almost stopped and no radioactivity accumulated in the tip. In anoxic path treatment (the sink being maintained in air), the translocation and accumulation of labeled compounds in the tip were not prevented by NaF treatment (Table VI). Yet the fermentative energy metabolism of at least four of the 11 plants tested was drastically impaired as shown by the very low values of ATP/ADP found in the path segments. These low values also

Table II. *Determination of the Minimum Loading Time of [1-^{14}C]dGlc*

Each result represents the counts recorded during 10 min in 1-cm root segments, from the 1st cm below the scutellum (first line) to the root tip. The background was 250 ± 20 counts/10 min.

Loading Time								
10 min			20 min			30 min		
1[a]	2	3	1	2	3	1	2	3
3,205	51,900	10,485	10,520	9,802	16,025	30,990	9,157	20,365
817	2,500	485	2,842	4,462	4,550	18,542	4,527	4,295
343	762	305	992	1,522	2,242	10,270	2,795	1,805
ND[b]	ND	ND	ND	ND	ND	5,605	1,240	920
ND	ND	ND	ND	ND	ND	3,025	675	612
ND	ND	ND	ND	ND	ND	2,997	620	427
ND	ND	ND	ND	ND	ND	2,395	595	475
ND	ND	ND	ND	ND	ND	2,200	2,485	370
260	355	297	762	1,505	1,055	4,955		3,257

[a] These numbers correspond to individual seedlings. Three plants were tested for each loading time.
[b] Not done.

Table III. *Translocation Pattern of [1-^{14}C]dGlc Compounds in Whole Maize Seedlings Maintained in Air or in N_2*

After a 10-min loading time, the scutellum was rinsed and the whole intact seedling (no cortical incision) was flushed either with air or with N_2. These results are expressed as in Table II. Numbers in parenthesis are the percentages of the total counts recovered in the root, corrected for the background which was 250 ± 20 counts/10 min. The seedlings chosen in this table were selected among 9, 6, and 3 seedlings, as representative of the average pattern obtained respectively after 2, 4, and 6 h of translocation for each treatment.

Translocation Time					
2 h		4 h		6 h	
Air	N_2	Air	N_2	Air	N_2
5,668 (20)	125,827 (89)	10,508 (6)	22,127 (51)	13,492 (4)	125,660 (24)
3,559 (12)	13,289 (9)	6,437 (3)	9,261 (20)	6,027 (2)	105,600 (20)
2,389 (8)	1,839 (1)	6,004 (3)	5,610 (10)	6,960 (2)	30,350 (6)
2,037 (7)	563 (0.2)	7,931 (4)	3,257 (7)	8,307 (2.5)	86,080 (16)
1,411 (4)	417 (0.1)	9,954 (5)	1,908 (4)	12,595 (4)	82,870 (15)
919 (2)	487 (0.1)	12,726 (7)	1,307 (2)	11,647 (3)	42,620 (8)
697 (1)	300 (0)	12,966 (7)	829 (1)	11,925 (3)	20,490 (4)
852 (2)	280 (0)	17,884 (10)	648 (0)	13,480 (4)	15,540 (3)
623 (1)	315 (0)	17,593 (10)	292 (1)	19,165 (6)	7,150 (1)
575 (1)	276 (0)	78,485 (44)	232 (0)	17,740 (5)	4,660 (0.8)
10,306 (38)			276 (0)	89,017 (28)	1,170 (0.1)
				110,095 (35)	210 (0)

indicate that no internal O_2 transport occurred during the experiment.

DISCUSSION

In the seminal roots of our maize seedlings there was no lysogenic cortical aerenchyma that would indicate some adaptation to hypoxic conditions (4). Gaseous porosity consisted only of a thin network of intercellular gas spaces, as found in most differentiated plant tissues. Our plant material was therefore not particularly well adapted to internal O_2 transport. Nevertheless, as illustrated in this study as well as in a former paper (17), the internal diffusion of O_2 along the path can overcome to a large extent the absence of O_2 in the external medium. Energy production by oxidative phosphorylation is so efficient as compared to fermentation (36 ATP/mol of glucose instead of 2 in anaerobiosis), that even a very low respiratory rate may significantly mask the effects of external anoxic conditions. To avoid erroneous conclusions in studies dealing with anoxia, it is therefore absolutely necessary to eliminate any possibility of internal O_2 transport. Vacuum infiltration of gas spaces by liquid medium, which is very efficient in preventing internal O_2 transport in short term experiments (17), unfortunately seems to disturb the transport of sugars (unpublished results). The method of root girdling used in this study, insofar as it did not hurt the stele, was quite harmless to the root tip which accumulated labeled dGlc and grew in air at the same rate as intact root (0.2 cm·h^{-1} for 24 h, results not shown). However, root girdling did not always completely prevent internal O_2 transport either because the incision of the cortex was not complete or because some air spaces were present in the stele. The use of ATP/ADP ratio as an indicator of the respiratory rate (14, 17) permitted a check of these points and determined with a very high degree of certainty whether or not there was some internal O_2 transport during a given experiment.

As already emphasized (13, 21) the use of dGlc, a synthetic analog of D-glucose, seems particularly suitable for studies of translocation processes. Because it is readily absorbed by the cells and converted to 2-deoxysucrose, this analog of sucrose can be subsequently loaded into the sieve tubes and transported to distal sinks where it accumulates, undergoing only a limited catabolism. In contrast, another analog of glucose, 3-*O*-methyl-D-glucose, which is also readily absorbed by the cells, cannot be

Table IV. *Effect of Anaerobic Path Treatment on the Translocation of dGlc Compounds in Girdled Maize Seedlings. Control of Internal O_2 Transport*

The ^3H radioactivity accumulated during 2 h while the path was in air, and the ^{14}C radioactivity, after 10 min loading time in air, was allowed to translocate during the next 2 h but while 70% of the path was maintained in N_2.

Plant No.	Radioactivity Accumulated in 1-cm Root Tip[a]		Control of Internal O_2 in the Path[b]	
	^3H	^{14}C	ATP/ADP	Internal O_2 transport
	counts/10 min		*ratio*	
1	5,594	6,295	0.34	no
2	42	141		
3	43	151		
4	41	131		
5	17,287	39,881	4.38	yes
6	7,761	24,186	2.81	yes
7	30,900	38,575	0.56	?
8	22,379	17,685	4.29	yes
9	1,115	1,054		
10	19,497	77,990	1.56	yes
11	72,867	58,426	1.09	yes
12	110,057	106,744	0.74	?
13	59	155		
14	31,717	55,449	0.26	no
15	46	165		
16	48	162		
17	47	162		
18	46	146		
19	48,834	62,373	0.26	no
20	63,336	69,514	0.26	no
21	82	149		
22	29,529	34,916	0.22	no
23	67	136		
24	67,695	50,265	0.23	no
25	185	220		
26	101,737	175,128	0.25	no
27	155	241		
28	77	135		
29	105	173		

[a] Each result represents the counts recorded in 10 min.

[b] The ATP/ADP ratio was determined on a 2-cm segment in the middle of the translocation path maintained in N_2.

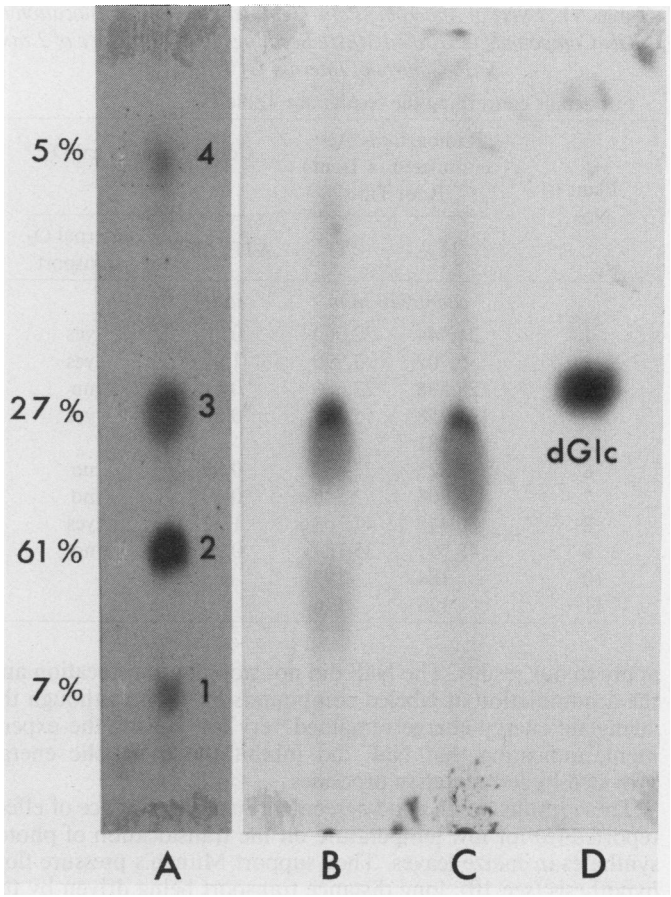

FIG. 2. TLC of the labeled compounds accumulated in the root tip. A, ethanol extract from 1 cm root tips. B, spot 2 after 10 min in 0.1 N HCl at 100°C. C, standard dGlc after a similar treatment. D, untreated standard dGlc.

Table V. *Translocation Pattern of [1-^{14}C]dGlc Compounds in Whole Maize Seedlings Maintained in Air or in N_2 in the Presence of 3 mM NaF*

The results are expressed as in Table III. The seedlings were intact (no cortical incision) and were chosen as representative of the averge pattern obtained with three seedlings for each time and each treatment. The percentage of radioactivity accumulated in the tip ranged between 5 and 20% after 2 h and between 18 and 27% after 4 h in air.

Translocation Time			
2 h		4 h	
Air	N_2	Air	N_2
3,150 (15)	1,046 (20)	58,157 (26)	27,358 (39)
1,373 (6)	1,406 (29)	50,944 (23)	15,470 (22)
1,680 (7)	1,266 (26)	25,404 (11)	11,627 (16)
1,627 (7)	769 (13)	10,727 (5)	7,679 (11)
1,439 (6)	489 (6)	6,493 (3)	4,425 (6)
1,267 (5)	362 (3)	7,548 (3.5)	2,788 (4)
1,355 (5.5)	280 (0.7)	6,399 (3)	1,293 (1.5)
1,098 (4)	247 (0)	5,184 (2)	541 (0.4)
1,122 (4)	273 (0)	4,721 (2)	333 (0)
1,062 (4)	260 (0)	6,303 (3)	305 (0)
2,901 (14)	258 (0)	39,585 (18)	246 (0)
	239 (0)		265 (0)

phosphorylated and converted to the corresponding sucrose analog. It is not loaded into the sieve tubes and its migration down the root is extremely slow (unpublished results), resembling that obtained with dGlc when both path and sink are maintained in anoxia. This pattern is characteristic of a simple diffusion process and contrasts sharply with that obtained with the anaerobic treatment of the path alone. Provided that the sink is maintained in air, a strict anoxia of 70 to 80% of the translocation path does not significantly modify the translocation profile of the labeled compounds as compared with the aerobic controls. These results show unequivocally that in our plant material, the long distance phloem transport does not depend on respiration in the path tissues. Neither does it depend on fermentation: anoxic incubation of the path in the presence of NaF does not impair the quick accumulation of dGlc compounds in the root tips. We are aware of the difficulties in the interpretation of results obtained from the use of metabolic inhibitors in phloem transport studies (22). These difficulties arise mainly in the case of positive responses which can easily be ascribed to secondary effects. They do not

Table VI. *Effect of Anaerobic Path Treatment on the Translocation of dGlc Compounds in Girdled Maize Seedlings in the Presence of 2 mM NaF. Control of Internal O_2 Transport*

For details concerning the results, see Table IV.

Plant No.	Radioactivity Accumulated in 1-cm Root Tip		Control of Internal O_2 Transport	
	^3H	^{14}C	ATP/ADP	Internal O_2 transport
	counts/10 min		*ratio*	
1	21,944	22,060	0.70	yes
2	5,707	3,990	1.99	yes
3	28,598	22,680	0.41	no
4	121,258	55,789	0.86	yes
5	181	177		
6	48,825	27,637	0.35	no
7	4,806	2,546	0.40	no
8	60,449	47,708	1.32	yes
9	48,797	35,784	0.25	no
10	184	197		
11	128	176		

apply to our results. The NaF did not stop the translocation and the accumulation of labeled compounds in the tip although the adenylate energy charge remained very low during the experiment, indicating that NaF did inhibit the metabolic energy provided by fermentative processes.

These results are in good agreement with the absence of effect reported (5) for low temperature on the translocation of photosynthates in maize leaves. They support Munch's pressure flow hypothesis (see 10), long distance transport being driven by the osmotic and pressure gradient controlled by source-sink processes.

The arresting of translocation when both path and sink were in N_2 implies that the inhibition operates at the sink level. In a former paper (16), we have shown that under strict anoxia, maize root tips had an active fermentative metabolism and were able to use their cytoplasmic soluble sugars at a rate comparable to that found in aerobiosis (15). Therefore, the sink 'strength' for sugar utilization remains potentially unimpaired in anoxia as long as sugars are accessible to the metabolic compartment. These considerations lead us to conclude that the inhibition of translocation by sink anoxia was not the result of the inhibition of cell metabolism but more likely operated on the unloading mechanism itself (8).

Sink unloading mechanisms are not very well understood and may vary depending on plant and organs. However, data based on anatomical and metabolic observations support the hypothesis of a symplastic route for sucrose unloading in young growing organs (7). Investigations on pea root tips (3), and more recently on maize root tips (8), indicate a symplastic route in these tissues also.

If this is the case in our material, our results suggest that the entry of sucrose into the sink cell through the plasmodesmata can be stopped by anaerobiosis. Since the metabolic activity of the sink which maintains the diffusion gradient between the phloem and sink cell is unaffected by anoxia, it is tempting to assume that the absence of O_2 induced a reversible discontinuity in the interconnected cytoplasms at the level of the plasmodesmata. This hypothesis is supported by ultrastructural observations made on maize (1) and tomatoes (12) showing dramatic but reversible modifications of the ER involved in plasmodesma structure induced by treatments such as anoxia or an uncoupler of oxidative phosphorylation which impair energy production.

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