

Changes in K, Rb, and Na Transport to Shoots after Anoxia¹

Received for publication May 27, 1986 and in revised form September 4, 1986

DAVID BRAUER*², J. EVERETT LEGGETT, AND DENNIS B. EGLI
Agronomy Department (D.B., D.B.E.) and United States Department of Agriculture, Agricultural Research Service (J.E.L.) Agricultural Science Center-North, Lexington, Kentucky 40546-0091

ABSTRACT

The effect of anoxia on subsequent uptake and transport of K, Rb, and Na was examined with seedlings of barley (*Hordeum vulgare* L.), corn (*Zea mays* L.), and tall fescue (*Lolium* × *Festuca* hybrid derivative) to further our understanding of xylem loading. Roots were incubated in solutions depleted of O₂ by flushing with N₂ gas. After 1 hour exposure, plants were returned to aerated solutions for 16 hours prior to measuring uptake and transport. For each species, anoxia pretreatment significantly enhanced Na transport to the shoot. The rate of Na accumulation into roots, however, was not affected. There was no enhancement of either K or Rb accumulation in shoots, indicating specificity for Na transport. A minimum exposure to anoxia of 30 minutes and a minimum of 12 hours elapsed time was necessary to achieve the maximum rate of Na transport to the shoot in barley seedlings. Accumulation of Na in the shoot of both the control and anoxia pretreated barley plants was inhibited by anoxia and by addition of the proline analog, L-azetidine-2-carboxylic acid, during the uptake period. Enhancement of Na transport was associated with a proportional increase in the rate of synthesis of a membrane bound protein with a molecular weight of 78,000 daltons.

There is increasing interest in substituting Na salts for K fertilizers as a means of reducing the hypomagnesemias potential of some pastures. Hypomagnesemias or grass tetany is a metabolic disorder of ruminants characterized by low serum Mg, *i.e.* less than 1.8 mg ml⁻¹ (22), and has been associated with high K concentrations in the ingested herbage (24, 31). Sodium additions can reduce K accumulation and the critical concentration of K required to achieve maximum yields (12, 17, 30).

Sodium accumulation in shoots of several grass species is determined by the amount exported from the roots to the shoots by xylem loading (18, 29). Crafts and Broyer (7) proposed that xylem loading resulted from leakage of solutes from root cells into the ascending transpirational stream. However, more recently, active transport processes have been advocated for xylem loading (15). Our knowledge of the processes involved in xylem loading is quite limited, in part, because there are few treatments that affect the rate of xylem loading without producing confounding effects on uptake, respiration, and transpiration. Pitman (25) and Pitman *et al.* (26) demonstrated that xylem loading was reduced by inhibitors of functional protein synthesis prior to decreases in uptake and respiration. Leggett and Stolzy (21)

reported that the transport of Na from roots to shoots of barley (*Hordeum vulgare* L.) seedlings was 2- to 3-fold greater when the roots were exposed to anoxia treatment the previous day. The purpose of this study was to further characterize the effect of anoxia pretreatment on root uptake of K, Rb, and Na and transport to shoots to gain further understanding of xylem loading that may aid the development of cultural practices substituting Na for K.

MATERIALS AND METHODS

Growing Conditions. Barley (*Hordeum vulgare* L. cv 'Barsoy') seeds were allowed to imbibe water overnight in 0.2 mM CaSO₄ and were incubated for 2 d between moistened layers of germination paper. After radicle and coleoptile emergence, 5 seedlings were placed on a stainless steel grid (3.0 cm diameter) inserted into a plastic retention ring and covered with moistened perlite. Twenty-four retention rings were suspended over 10 L of aerated 0.2 mM CaSO₄, and held in the dark at 22°C for 2 d. On d 5, the CaSO₄ solution was replaced with 0.1 strength Hoagland solution (16) and a 12 h day/night regime was imposed.

On d 8, after 6 to 8 h illumination, roots of half of the plants were placed in an anaerobic environment created by bubbling N₂ gas through the nutrient solution. Except where indicated, plants were returned to an aerated nutrient solution after 1 h of treatment. With minor exceptions, uptake experiments were conducted 15 to 18 h after the anaerobic pretreatment.

Maize (*Zea mays* L. cv 'Pioneer 3369A') seedlings were similarly grown. Two-week-old seedlings of tall fescue (*Lolium* × *Festuca* hybrid derivative, selection '78-5') were cultured in a similar fashion except that the incubation between moistened towels was omitted and the illuminated growth period was increased to 11 d. Buckner *et al.* (4) have described the breeding program that produced the population of hybrid derivatives from which '78-5' was selected.

Transport Experiments. Sixteen sets of 5 seedlings were incubated in 2.5 L of 0.2 mM CaSO₄ containing 1.0 mM chloride salts of K, Na, or Rb with minor exceptions as noted. The pH of the solution at the beginning of the absorption period was adjusted to 6.0 by addition of Ca(OH)₂. Uptake of radionuclides was terminated by incubating roots in solutions lacking radioisotopes for 5 min. Roots and shoots were excised from the seedlings and placed in the bottom of 1 cm diameter, preweighed, plastic scintillation tubes.

The transport of Na and Rb into roots and shoots was monitored by the accumulation of ²²Na and ⁸⁶Rb, as determined by γ scintillation counting. Sodium and Rb accumulation were calculated on the basis of the specific activities of the solutions at the beginning of the uptake period and the radioactivity of the sample. Sodium and Rb values determined by radiotracer techniques were comparable to those found by measuring changes in Rb and Na content by atomic absorption spectrophotometry (D Brauer, JE Leggett, DB Egli, unpublished data). After counting, tissue samples were dried at 40°C *in vacuo*. The mass of the tube

¹ Contribution from Department of Agronomy and United States Department of Agriculture, Agricultural Research Service, University of Kentucky. This paper (No. 86-3-67) is published with approval of the Director of the Kentucky Agricultural Experiment Station.

² Present address: USDA/ARS/ERRC, 600 E. Mermaid Lane, Philadelphia, PA 19118.

plus sample was determined so that tissue mass could be obtained by difference. Potassium transport was estimated by measuring changes in the K content of the dried tissue. Plant tissue was digested with 9:1 (v/v) nitric-perchloric acid, and the resulting residue was dissolved in 1.0 N HCl and 0.2 g SrCl₂/100 ml and K was determined by atomic absorption spectrophotometry. Accumulation of cations in root and shoot material is expressed as μmol per g of DW³ of tissue. Data from 3 sets of plants were averaged and results from one experiment were confirmed in duplicate experiments. Bars representing the Standard Deviation appear where the error exceeds the size of the data point.

The intracellular distribution of Na was assessed following the method of Balke and Hodges (1) by measuring the efflux of ²²Na from roots into an external solution of 0.2 mM CaSO₄ and 1.0 mM NaCl. Prior to measuring exchange, roots were incubated for 3 h in 0.2 mM CaSO₄ and 1.0 mM NaCl labeled with ²²Na. Rates of exchange and sizes of intracellular compartments were determined by the method of Cram (8). The relative radial distribution of Na in roots was assessed by the appearance of ²²Na into external solution containing 0.1 N HCl after loading the tissue for 3 h in 1 mM NaCl and 0.2 mM CaSO₄ in a manner similar to that of Leggett and Gilbert (20).

Double Labeling Experiments. Ten g of roots from controls as well as from plants pretreated 15 to 18 h earlier with 1 h of anoxia were labeled for 3 h in 20 ml of 0.2 mM CaSO₄ containing either 20 μCi of [¹⁴C]Leu (0.34 Ci mmol⁻¹) or 100 μCi of [³H]Leu (115 Ci mmol⁻¹). After the labeling period, roots from both control and anoxia pretreated plants were combined, rinsed with water, and homogenized by mortar and pestle in the presence of 1 g of insoluble PVPP and 80 ml of extracting buffer containing 50 mM Tris-Hepes (pH 7.5), 3 mM EDTA, 3 mM DTT, 0.25 M sucrose, and 100 μg BHT ml⁻¹. The brei was filtered through Miracloth and centrifuged for 5 min at 1,000g. The supernatant was then separated by differential centrifugation into three fractions: mitochondria, microsomes, and soluble proteins. A crude mitochondrial pellet was collected by centrifugation at 13,000g for 15 min. Microsomes were collected from the 13,000g supernatant by centrifugation at 80,000g for 35 min. The soluble proteins remaining in the supernatant were precipitated by the addition of 50% (w/v) TCA solution to a final concentration of 10% (w/v) and collected by centrifugation at 10,000g for 10 min. Both crude membrane pellets were suspended in 5 ml of extracting buffer containing 0.2 M NaCl and then collected by centrifugation. Each fraction was suspended in a sufficient volume of SDS sample buffer to yield 1 mg of protein ml⁻¹, and incubated for 15 min at 80°C. The SDS sample buffer contained 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol, 2.3% (w/v) SDS, 60 mM Tris-HCl (pH 6.8), and 10 μg BHT ml⁻¹. As found by Booz and Travis (2), the addition of BHT was necessary to obtain separation of proteins upon electrophoresis. Fractions were either subjected to SDS electrophoresis immediately, or frozen at -20°C. Protein concentrations were determined by the method of Bradford (3).

Electrophoresis. Proteins were separated by SDS electrophoresis on horizontal slab gels at a constant current of 10 mamp slab⁻¹ by the method of Laemmli (19) using polyacrylamide concentrations of 5 and 11% in the stacking and running gels, respectively. Proteins were fixed in TCA and visualized by Coomassie brilliant blue R-250 staining. Lanes were excised from the gel and sliced into 1 mm segments. Each segment was digested overnight with 0.4 ml of 70% H₂O₂ at 65°C in a 20 ml scintillation vial. After the addition of 15 ml of scintillation fluid, the amount of ¹⁴C and ³H was determined by liquid scintillation

counting using an appropriate setting for simultaneous ³H and ¹⁴C counting.

RESULTS

Effect of Anoxia. In agreement with Leggett and Stolzy (21) shoot accumulation of Na by barley seedlings was greater when roots were previously exposed to an anaerobic environment (Fig. 1). After a 3 h incubation, barley seedlings from the anaerobic pretreatment had approximately 4 μmol Na g⁻¹ DW shoot compared with 2 μmol g⁻¹ DW in the control plants. Enhanced accumulation of Na in shoots did not correspond to an increased accumulation of Na in roots. Roots from both controls and anoxia pretreated plants contained slightly more than 8 μmol Na g⁻¹ DW after 3 h. From the amounts of Na and dry weights of roots and shoots, Na accumulation in the whole plant per g⁻¹ DW of roots was calculated. The data in Figure 1 indicated that control and anoxia pretreated plants accumulated 15.2 and 16.3 μmol Na g⁻¹ DW of roots, respectively, with a SD of 1.6 and 1.8, respectively. A student *t* test testing the hypothesis that total Na uptake by control plants equaled that of anoxia pretreated was confirmed (*t* = -0.79). Additionally, the total Na uptake into both roots and shoots after 3 h expressed per g of roots for control versus anoxia pretreated plants in four other experiments were: 13.6 versus 13.3, 14.7 versus 14.1, 17.0 versus 17.0, and 13.3 versus 15.0 μmol Na g⁻¹ DW of roots, respectively. Across the five experiments, total Na accumulation at 3 h averaged 14.7 and 15.1 μmol g⁻¹ DW of roots for control and pretreated plants, respectively, with SD of 1.5 and 1.6, respectively. A student *t* test

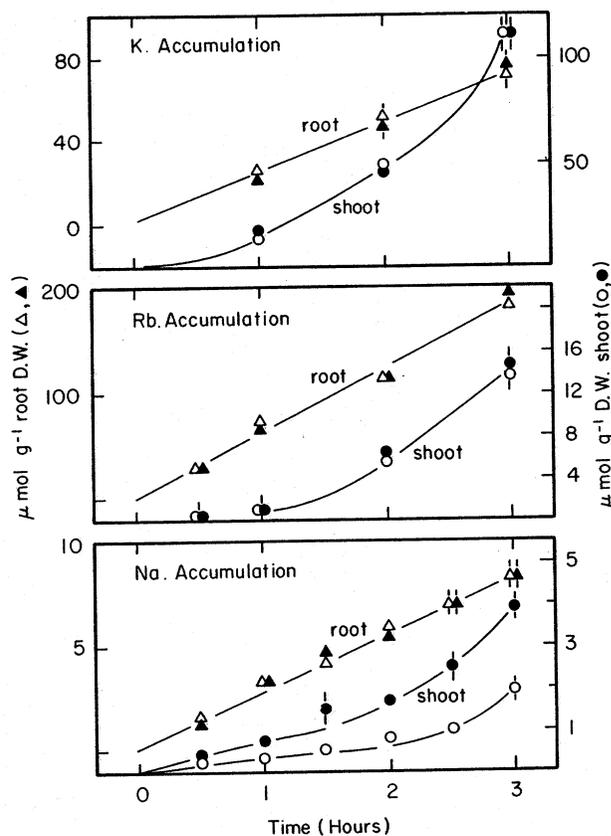


FIG. 1. Accumulation of K, Na, and Rb in roots and shoots of barley seedlings as effected by anoxia pretreatment. Uptake solutions contained 1 mM NaCl, KCl, or RbCl in 0.2 mM CaSO₄. Data for control and plants pretreated for 1 h with anoxia 16 h earlier are represented by open and closed symbols, respectively. Values for root and shoot accumulation per g⁻¹ DW are represented by triangles and circles, respectively.

³ Abbreviations: DW, dry weight; AZ, L-azetidine-2-carboxylic acid; BHT, butylated hydroxytoluene.

between these means equaled -0.47 , again confirming the hypothesis that total plant Na accumulation was unaltered. Therefore, there was only a slight tendency for anoxia pretreated plants to accumulate more Na on a whole plant basis. A slight enhancement of Na uptake by plants exposed to anoxia may have occurred in order to maintain root Na levels in the presence of enhanced translocation to the shoot by relieving feedback inhibition on the uptake mechanism (13).

Even considering the extreme limits of the data, enhancement of uptake would have been less than 25% by the anaerobic treatment, whereas transport to the shoot was increased nearly 100%. Therefore, transport of Na to the shoot was preferentially affected by anoxia pretreatment.

Similar trends were found with seedlings of both maize and tall fescue (data not shown). Roots from both control and pretreated maize plants contained $60 \mu\text{mol Na g}^{-1} \text{ DW}$ after 3 h incubation. Shoots from pretreated seedlings contained over 6 times more Na than control seedlings, 0.25 and $0.04 \mu\text{mol g}^{-1} \text{ DW}$ shoot, respectively. Similarly, leaves of tall fescue seedlings pretreated with anoxia contained 50% more Na than controls (0.73 versus $0.50 \mu\text{mol g}^{-1} \text{ DW}$) after 3 h uptake period. Roots from tall fescue had about $25 \mu\text{mol Na g}^{-1} \text{ DW}$ after 3 h regardless of the pretreatment.

Barley seedlings were used in further studies because the rate of Na accumulation in shoots was greater, while the response to anoxia was intermediate among the three species examined.

Location of Effect. Enhancement of Na accumulation in barley shoots by anoxia pretreatment could be due to increased cytoplasmic levels of Na in roots, a faster rate of radial transport across the root or a more rapid rate of xylem loading. There was no apparent difference between controls and anoxia pretreated roots with respect to the distribution of Na between the cytoplasm and vacuole as determined by the time course of Na efflux from roots (Fig. 2). Approximately 10% of the absorbed Na was in a compartment with a half-time of 10 min, presumably the cytoplasm, independent of treatment. The remainder of the tissue Na was in a compartment that exchanged very slowly with the external solution, presumably the vacuole.

Radial distribution of Na in roots after 3 h incubation was estimated by measuring the appearance of ^{22}Na into 0.1 N HCl (20). The acid should rupture root cells in successive concentric cylinders as it diffused inward. There was no difference between controls and anoxia pretreated roots with respect to the rate of Na loss after exposure to acid. Quadratic equations relating the percentage of the absorbed Na remaining in the roots as a

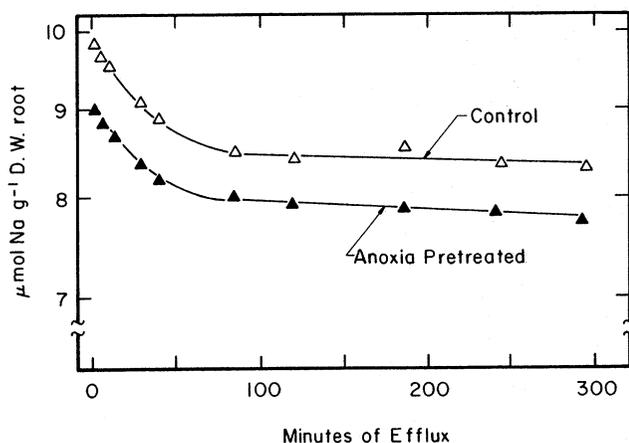


FIG. 2. Exchange of Na from roots of control and anoxia pretreated barley plants presented in semilog plot. Prior to measuring exchange between root pools and an external solution of 1.0 mM NaCl and 0.2 mM CaSO_4 , plants were incubated 3 h in a similar solution labeled with ^{22}Na . Data are expressed per g DW of roots.

function of time in 0.1 N HCl were: % remaining = $100.49 - 3.90 (\text{min}) + 0.015 (\text{min})^2$ and % remaining = $100.31 - 3.84 (\text{min}) + 0.013 (\text{min})^2$ for pretreated and control plants, respectively. This indicates that there was no dramatic change in the rate of radial penetration of Na following anoxia. Anoxia pretreatment must, therefore, have affected the rate of xylem loading.

Characteristics of the Effect of Anoxia Pretreatment. Time courses for the accumulation of Na, Rb, and K by roots and shoots from 1 mM chloride salts are presented in Figure 1. For each cation, uptake by roots was linear with time during a 3 h incubation and there was no treatment effect. By the end of the 3rd h, roots accumulated 8, 180, and $75 \mu\text{mol g}^{-1} \text{ DW}$ of Na, Rb, and K, respectively.

Cation accumulation in shoots was a curvilinear function of time. With Na, anoxia pretreatment increased accumulation by almost 100% from $2.01 \mu\text{mol g}^{-1} \text{ DW}$ of shoot with controls to $3.83 \mu\text{mol g}^{-1} \text{ DW}$ of shoot. The enhancement of Na transport to the shoot was not associated with a difference in the length of the lag period. This supports the hypothesis that radial transport and intracellular distribution of Na were not important factors. Transport of both K and Rb to the shoots was not affected by pretreatment, indicating that the xylem loading of Na was selectively increased.

The time course of the development of the enhanced xylem loading of Na was followed by measuring Na accumulation in roots and shoots during 3 h incubation at 4 h intervals after anoxia (Fig. 3). Sodium accumulation in shoots of control seedlings decreased slightly with time from 1.87 to $1.60 \mu\text{mol g}^{-1} \text{ DW}$ (Fig. 3). From time 0 to 4 h, accumulation of Na in pretreated shoots was slightly less than that found with controls. Thereafter, the ability of pretreated plants to accumulate Na in shoot tissue increased as a sigmoidal function of time after anoxia from 1.43 to $3.42 \mu\text{mol g}^{-1} \text{ DW}$, reaching a maximum between 12 and 16 h. The time required for enhanced Na transport and the magnitude of the enhancement appeared to be independent of either the light regimes and nutrient status of the plant before or after anoxia (data not shown).

The persistence of increased xylem loading of Na was determined over a 120 h period by measuring Na accumulation during 3 h incubation at 24 h intervals (Fig. 3). The Na accumulation in shoots of pretreated plants increased during the first 48 h and

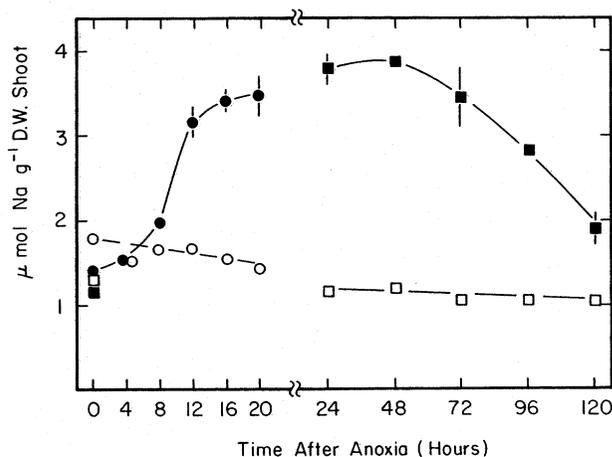


FIG. 3. Effect of time between the end of the anoxia pretreatment and the beginning of the transport experiment on Na accumulation in barley shoots. Sodium accumulation determined after 3 h incubation in 1 mM NaCl and 0.2 mM CaSO_4 . Data for control and anoxia pretreated plants per $\text{g}^{-1} \text{ DW}$ are represented by open and closed symbols, respectively. Data from 24 and 120 h experiments are represented by circles and squares, respectively.

then began to decline. There were no differences between control and pretreated seedlings in the rate of dry matter accumulation in roots and shoots (data not shown).

Exposure of roots to anoxia for a period as short as 5 min significantly increased Na accumulation in the shoots from 2.13 ± 0.2 to $2.64 \pm 0.1 \mu\text{mol g}^{-1}$ DW during 3 h incubation 15 h after treatment (Fig. 4). The capacity to transport Na increased rapidly as the anoxia period was increased to 15 min and reached a maximum with 30 min.

Effect of Inhibitors on Transport. The effects of anoxia and of protein synthesis inhibitors during uptake were compared between control and anoxia pretreated seedlings to see if these treatments affected xylem loading independent of pretreatment. In the following experiment, both controls and anoxia pretreated plants were transferred to 10 mM NaCl in 0.2 mM CaSO₄ for 16 h from the end of the anoxia treatment to the start of the uptake period. Half of the plants were exposed to anoxia during the uptake period by bubbling N₂ gas into the solution. Root accumulation of Na from 10 mM solution was not effected by pretreatment or by anoxia during the uptake period (Fig. 5). Transport of Na to the shoots, however, was completely inhibited by anoxia in both controls and anoxia pretreated plants.

Inhibitors of the synthesis of functional proteins reduce the rate of xylem loading prior to effecting uptake (25). Pitman *et al.* (26) were the first to report that the proline analog, AZ, selectively inhibited xylem loading. In agreement with these findings, the addition of 0.2 mM AZ significantly reduced the rate of Na accumulation in shoots (Fig. 6). Under similar experimental conditions, the addition of AZ prevented the incorporation of previously absorbed proline into TCA insoluble material within 30 min without effecting Leu incorporation (data not shown). After 30 min exposure to AZ, the logarithm of the hourly rate of Na accumulation in shoots declined as a linear function of time, indicative of a first order decay. The decay constants estimated from the slopes of the regression equations relating the logarithm of the rate of shoot accumulation to time after exposure to AZ were approximately the same for anoxia pretreated plants and controls, 0.0060 and 0.0067 min⁻¹, respectively. The decay constant for Rb transport also was 0.0060 min⁻¹.

Double Labeling Experiments. Double labeling experiments were conducted to identify proteins which were being synthesized at different rates following anoxia. The rate of protein synthesis

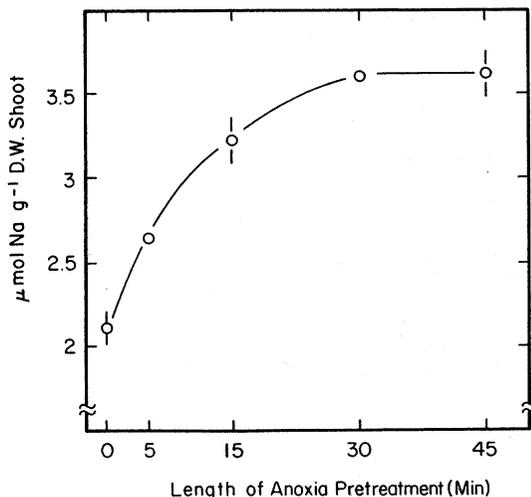


FIG. 4. Effect of duration of anoxia pretreatment on Na accumulation in barley shoots. Sodium accumulation per g⁻¹ DW was determined after 3 h incubation in 1 mM NaCl and 0.2 mM CaSO₄. Plants were exposed to the anoxia pretreatment 15 h earlier.

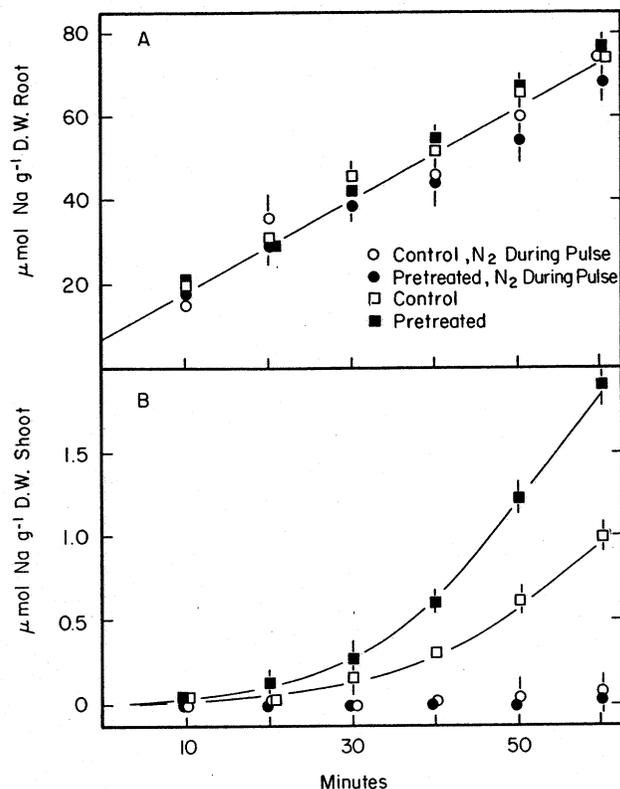


FIG. 5. Effect of anaerobiosis during the uptake period on Na accumulation in barley roots (A) and shoots (B). After anoxia pretreatment, all plants including controls were transferred to 10 mM NaCl and 0.2 mM CaSO₄ for 16 h. Open and closed symbols have the same meaning as in Figure 3. Data from the anaerobic treatment during the uptake experiment are represented by circles. Data are expressed per g⁻¹ DW.

as assayed by the incorporation of labeled amino acids into TCA insoluble material was not significantly different between anoxia pretreated plants and controls (data not shown). Five times more ³H was added to roots from anoxia pretreated plants as compared with the amount of ¹⁴C used to label controls. Thus, a deviation in the (³H/¹⁴C) ratio of a protein band from 5.0 indicated that its rate of synthesis was different following anoxia. Only one faintly stained protein band from the microsomal fraction had a (³H/¹⁴C) ratio that significantly deviated from 5.0. In Figure 7, this protein was associated with segment 19 and had an isotopic ratio in excess of 9.0. A similar peak was observed in each of four double labeling experiments. The mol wt of this protein was estimated to be 78,000 D with a standard deviation of 2,000. From the isotopic ratio, it was estimated that the net synthesis of this protein was proceeding at a rate that was 90% greater than that found with the controls.

DISCUSSION

Sodium Transport following Anoxia. The rate of delivery of Na to the shoots of barley, maize, and tall fescue was greater when the roots were exposed to 1 h of anoxia 15 to 18 h before the absorption period. This enhancement in transport occurred without an increase in the amount of Na present in the roots. The enhanced rate of Na transport to the shoots appeared to be due to an increase in the rate of xylem loading. There was no difference between roots from anoxia treated plants and controls in the intracellular and radial distribution of Na. The similarity in the time course (Fig. 1) and the greater steady state rate of Na accumulation in anoxia pretreated shoots (Fig. 6) also support the conclusion that only xylem loading was influenced. The

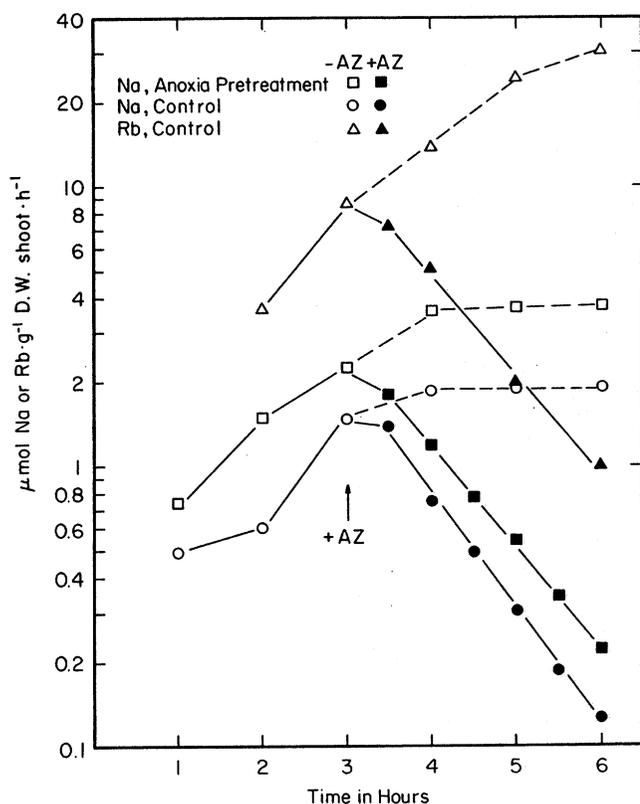


FIG. 6. Effect of 0.2 mM L-azetidine-2-carboxylic acid (AZ) on the rate of Na and Rb accumulation in barley shoots. Uptake solutions contained 1 mM NaCl or RbCl in 0.2 mM CaSO_4 with and without 0.2 mM AZ. Closed symbols indicate data from plants treated with AZ. Rubidium and Na accumulation by controls and Na accumulation by anoxia pretreated plant are expressed per g^{-1} DW and are represented by triangles, circles, and squares, respectively.

effect of anoxia appeared to be specific for Na transport since the accumulation of K and Rb was not affected (Fig. 1).

The length of exposure to anoxia required to induce an increase in the xylem loading of Na was relatively short (Fig. 4). Placing roots of plants in a solution bubbled with N_2 gas for as short as 5 min significantly increased the rate of shoot accumulation 16 h later (Fig. 4). No more than a 30 min exposure was necessary for a maximum effect. During the first 8 h after anoxia, plants did not exhibit an increase in transport Na (Fig. 3). Between 8 and 16 h after anoxia treatment, the rate of Na transport to the shoots increased. With respect to the above characteristics, anoxia-accelerated Na transport was similar to certain phytochrome-mediated responses that are photoreversible for only a short period, but require longer intervals for expression (11). The rates of Na transport to shoots by anoxia treated plants declined after 2 d. One possible explanation for this decline is the proportion of the root with the enhanced rate of Na transport is diluted by growth. However, the possibility that maintenance of the enhanced rate of transport requires exposure to anoxia cannot be ruled out.

Sodium accumulation in shoots by both controls and pretreated plants was equally inhibited by anoxia (Fig. 6) and by AZ (Fig. 7) during the uptake period. These results suggest that a similar transport system is operating in both control and anoxia pretreated roots. This proposal is supported by the observation that the rate of net synthesis of the 78,000 D protein was of the same magnitude as the increase in the rate of Na transport. From Figure 6, the steady state rate of Na accumulation in shoots was approximately 1.8 and $3.6 \mu\text{mol g}^{-1}$ DW h^{-1} for control and

anoxia pretreated plants, respectively, which represents 100% increase in rate. Anoxia pretreatment increased the isotopic ratio ($^3\text{H}/^{14}\text{C}$) of the 78,000 peptide from an average of 5.4 to 9.2 (Fig. 7) which represents a 90% increase in the net rate of synthesis. The function of this protein is hypothesized to be that it mediates the transport of Na from the symplasm of the roots to the xylem fluid and anoxia increases the amount of the transport protein present.

The enhancement of Na transport to the shoots following anoxia may explain why the Na concentration in spring herbage of the fescue cultivar 'Kenhy' was greater than that found in summer and fall (6). Intermittent, anaerobic soil conditions that occur most frequently in the spring may have favored the xylem loading of Na. The results in this study suggest that the substitution of Na salts for K fertilizers would be an effective method for increasing the Na content of forage grown in poorly drained soils. Since the mineral composition of forages grown in low Na, water logged soils is reported to be high in K and low in Mg (14), the use of Na salts in substitution for K fertilizers should be beneficial to producers. Further research is needed to determine how low the O_2 tension of the solution must be to enhance Na transport.

This research also demonstrates that the physiology of the plant is changed by exposure to anoxia. An anaerobic root environment altered the activity of an aerobic process, presumably by altering protein synthesis. Modulation of protein synthesis by anaerobiosis has been previously demonstrated. Maize grown in an anaerobic environment for 5 to 72 h synthesized a unique set of proteins (28) and had higher levels of alcohol dehydrogenase (27). Similar results have been found with germinating rice embryos (23). It is of interest that one of the 11 major proteins synthesized by maize under anaerobic conditions identified by Sachs *et al.* (28) had a mol wt similar to the one identified here.

Comparison of K and Rb Transport. An interesting observation in this study was that the time course of accumulation of K in roots and shoots differed from that of Rb (Fig. 1). The shoot to root ratio of these seedlings varied between 1 and 2. Using the accumulation data in Figure 1 and assuming a shoot to root ratio of 2, seedlings absorbed approximately 290 and $240 \mu\text{mol g}^{-1}$ DW root of K and Rb, respectively. However, the distribution of these two cations between root and shoot tissue was quite different. After a 3 h incubation, over two-thirds of the absorbed K had accumulated in shoots, whereas less than 20% of the Rb was in the shoots (Fig. 1). This difference suggests that transport of Rb does not mimic that of K and the validity of following K transport by ^{86}Rb accumulation needs further examination. The reduced rate of xylem loading of Rb might be due to the fact that transport of Rb into the vacuoles competed with xylem loading. It is hypothesized that the relative flux of K into the vacuole was less because of a greater initial concentration; therefore, a greater portion of the absorbed K was available for transport out of roots. Accordingly, increasing the Rb concentration of the roots by preincubation in RbCl would increase the rate of Rb transport to the shoot. Such results have been found in preliminary experiments.

Effect of Inhibitors. Transport of Na to shoots was inhibited by anoxia during the absorption period (Fig. 5). The reduction in Na transport out of the root did not result from a decrease in transport into the root, when the uptake solution contained 10 mM NaCl and plants were previously incubated at this NaCl level. Similar results were obtained for Rb transport under analogous conditions (data not shown). However, when experiments were conducted under conditions in which one would suspect active uptake into roots (*i.e.* low external concentrations), anaerobiosis during the uptake period inhibited cation accumulation in both roots and shoots (data not shown). Therefore, it appears

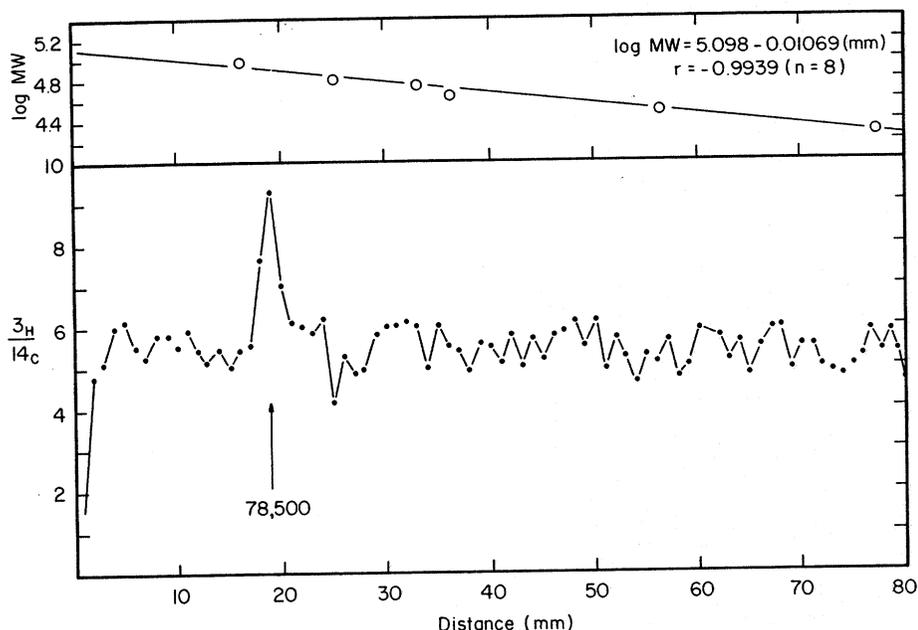


FIG. 7. Ratio of ($^3\text{H}/^{14}\text{C}$) of microsomal proteins that were separated by SDS electrophoresis. Proteins from barley roots of anoxia pretreated plants were labeled for 3 h with $100 \mu\text{Ci } ^3\text{H Leu}$ while control roots were labeled with $20 \mu\text{Ci } ^{14}\text{C LEU}$.

that xylem loading *per se* required energy from respiration. This conclusion is in agreement with the interpretation of electropotential data by Davis and Higinbotham (9). These authors argue that xylem loading of cations must require energy because of the magnitude of the positive potential between the symplasm of the root cells and the xylem fluid. Similar conclusions were recently made by Clarkson *et al.* (5) and deBoer *et al.* (10).

Xylem loading requires continuous protein synthesis and is affected by inhibitors of protein synthesis prior to decreases in uptake and respiration (26). In the current study, the decay constants describing the inhibition of Na and Rb transport in control and Na transport in anoxia pretreated plants by AZ were alike (Fig. 6), and similar to those found for a variety of anions and cations by others (25). This suggests that the protein affected by AZ involves a process common to the loading of all ions, and warrants further investigations.

Acknowledgments—We are thankful to Dr. Paul Lin for his help with the double labeling experiments and electrophoresis, and to Dr. R. C. Buckner for providing seeds of tall fescue hybrid selection 78-5.

LITERATURE CITED

- BALKE NE, TK HODGES 1979 Effect of diethylstilbestrol on ion fluxed in oat roots. *Plant Physiol* 63: 42-47
- BOOZ ML, RL TRAVIS 1980 Electrophoretic comparison of polypeptides from enriched plasma membrane fractions from developing soybean roots. *Plant Physiol* 66: 1037-1043
- BRADFORD MM 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254
- BUCKNER RC, HD HILL, PB BURRUS 1961 Some characteristics of perennial and annual ryegrass \times tall fescue and of the amphiploid progenies of annual ryegrass \times tall fescue. *Cro Sci* 1: 75-80
- CLARKSON DT, L WILLIAMS, JB HANSON 1984 pH Regulation of the xylem sap in onion roots: the role of stelar proton pump. *Plant Physiol* 75: S-250
- COALE FJ 1983 Effect of N, Na and K fertilization on mineral composition, potential incidence of grass tetany and yield of tall fescue. MS thesis. University of Kentucky, Lexington
- CRAFTS AS, TC BROYER 1938 Migration of salts and water into the xylem of the roots of higher plants *Am J Bot* 25: 529-535
- CRAM WJ 1968 Compartmentation and exchange of chloride in carrot root tissue. *Biochim Biophys Acta* 163:339-353
- DAVIS RF, N HIGINBOTHAM 1976 Electrochemical gradients and K^+ and Cl^- fluxes in excised corn roots. *Plant Physiol* 57: 129-136
- DE BOER AH, HBA PRINS, PJC KUIPER 1984 Fusaric acid stimulated proton excretion into the xylem of tap roots of *Plantago maritima*, L. *Plant Physiol* 75: S-223
- FROSCH S, H DRUMM, H MOHN 1977 Regulation of enzyme levels by phytochrome in mustard cotyledons: multiple mechanisms? *Planta* 136: 181-186
- GAMMON N 1953 Sodium and potassium requirements of pangola and other pasture species. *Soil Sci* 76: 81-90
- GLASS ADM 1978 The regulation of potassium influx into intact roots of barley by internal potassium levels. *Can J Bot* 56: 1759-1764
- GRUNES DL 1983 Uptake of magnesium by different plant species. In JP Pontenot, GE Bunce, KE Webb, VG Allen, eds, *The Role of Magnesium in Animal Nutrition*. Virginia Polytechnic Institute and State University, Blacksburg, VA pp 23-28
- HANSON JB 1978 Application of the chemiosmotic hypothesis to ion transport across the root. *Plant Physiol* 62: 402-405
- HOAGLAND DR, DI ARNON 1950 The water culture method for growing plants without soil. *Calif Agric Exp Stn Bull* 347
- HYLTON LO, A ULRICH, DR CORNELIUS 1967 Potassium and sodium inter-relationships in growth and mineral content of Italian ryegrass. *Agron J* 59: 311-314
- JESCKHE WD 1983 Cation fluxed in excised and intact roots in relation to specific and varietal differences. *Plant Soil* 72: 197-212
- LAEMMLI UK 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage. *Nature* 227: 680-685
- LEGGETT JE, WA GILBERT 1967 Localization of the mediated apparent ion selectivity in the cross-sectional volume of soybean roots. *Plant Physiol* 42: 1658-1664
- LEGGETT JE, LH STOLZY 1961 Anaerobiosis and sodium accumulation. *Nature* 192: 991-992
- LITLEDIKE ET, JA STUEDEMAN, SR WILKINSON, RL HORST 1983 Grass tetany syndrome. In JP Pontenot, GE Bunce, KE Webb, VG Allen, eds, *Role of Magnesium in Animal Nutrition*. Virginia Polytechnic Institute and State University, Blacksburg, VA, pp 173-196
- MOCQUOT B, C PRAT, C MOUCHES, A PRADET 1981 Effect of anoxia on energy charge and protein synthesis in rice embryo. *Plant Physiol* 68: 636-640
- NEWTON GL, JP FONTENOT, RE TUCKER, CE POLAN 1972 Effects of high dietary K intake on the metabolism of Mg by sheep. *J Anim Sci* 35: 440-445
- PITMAN MG 1977 Ion transport into xylem. *Annu Rev Plant Physiol* 28: 71-88
- PITMAN MG, RA WILDES, N SCHAEFER, D WELFARE 1977 Effect of azetidine 2-carboxylic acid on ion uptake and ion release to the xylem of excised barley roots. *Plant Physiol* 60: 240-246
- SACHS MM, M FREELING 1978 Selective synthesis of alcohol dehydrogenase during anaerobic treatment of maize. *Mol Gen Genet* 161: 111-115
- SACHS MM, M FREELING, R OKIMOTO 1980 The anaerobic proteins of maize. *Cell* 20: 761-767
- SHONE MGT, DT CLARKSON, T SANDERSON 1969 The absorption and translocation of sodium by maize seedlings. *Plant* 86: 301-314
- SMITH FW 1974 The effect of sodium on potassium nutrition and ionic relations in Rhodes grass. *Aust J Agric Res* 25: 407-414
- TOMAS FM, BJ POTTER 1976 Effect and site of action of K upon Mg absorption in the sheep. *Aust J Agric Res* 27: 873-880