Plant Physiol. (1988) 87, 794–796
0032-0889/88/87/0794/03/$01.00/0

Communication

Root Excision Decreases Nutrient Absorption and Gas Fluxes[1]

Received for publication March 1, 1988

ARNOLD J. BLOOM* AND RICHARD M. CALDWELL
Department of Vegetable Crops, University of California at Davis, Davis, California 95616

ABSTRACT

The roots of barley plants (*Hordeum vulgare* L. cv Steptoe) were monitored before and after excision for net uptake of carbon dioxide, oxygen, ammonium, potassium, nitrate, and chloride and for their content of sucrose, glucose, fructose, and malic acid. All fluxes began to attenuate within 2 hours after excision. Net potassium uptake returned to control levels 6 hours after excision, but carbon dioxide, oxygen, ammonium, and nitrate fluxes continued to diminish for the remainder of the observation period. The addition of 0.1 molar glucose or 0.1 molar sucrose to excision medium had no significant effect on these changes in ion and gas fluxes. Net chloride uptake was negligible for all treatments. Sugar and malic acid content of the root declined after excision. Sucrose and glucose levels remained depressed for the entire observation period, whereas fructose and malic acid returned to control levels after 9 hours. These results indicate that excision has profound, adverse effects on root respiration and the absorption of mineral nitrogen.

Although excision of plant tissue often produces severe changes in metabolic processes (23), many studies of nutrient absorption have been based on data obtained from excised roots. Use of excised roots was justified in some cases. For example, differences in $^{86}Rb^+$ influx between excised and intact barley or corn roots became insignificant after 2 to 4 h of washing (11, 12, 15). Calcium and phosphate absorption also was relatively unaffected by excision (7, 13). In contrast, excised barley roots absorbed less NO_3^- (1) and excised barley and corn roots developed smaller pH gradients (9, 12) than intact roots. These results suggest that the effects of excision may be ion specific.

Our research has compared absorption of NH_4^+ and NO_3^- (2, 3, 5) and determined the influence of different nutrient solutions upon respiration (4) in intact roots. In the following, we examined whether excised roots are appropriate material for such studies.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Barley seeds, *Hordeum vulgare* L. cv Steptoe, were sprouted on wet germination paper. After 3 to 4 d, the seedlings were suspended by foam plugs around the stem above light-tight root boxes. These boxes contained one-tenth strength modified Hoagland solution (10) that was replenished every 4 d. The plants were grown in an environmental chamber set for a 16 h, 25°C day and a 8 h, 15°C night with 70% RH day and night. Light levels at plant height were 450 μmol $m^{-2}s^{-1}$ PAR.

[1] Supported in part by National Science Foundation grant BSR-84-16893.

Ion and Gas Fluxes. About 2 weeks after germination, when the third leaf had just emerged and the roots were over 15 cm in length, a plant was transferred to a measurement system. Teflon tape was wrapped around the root about 1 cm below the endosperm. A slotted rubber stopper was placed around the wrapped section of root, and the remaining 12-cm portion of root was placed into a Plexiglas/stainless steel cuvette (3). On top of the cuvette, a bath 3 cm deep was fabricated from putty and filled with nutrient solution or nutrient solution plus 0.1 M sucrose or 0.1 M glucose; the upper 1 cm of root sat in this bath, and the shoot was held erect above it. To permit recovery from any transplant shock, this plant was kept for at least 8 h in the dark and 3 h in the light at 500 μmol $m^{-2}s^{-1}$ PAR before experimental data were taken. The temperature of the root cuvette was 20°C and that of the shoot was 25°C.

Ion-selective electrodes simultaneously and continuously monitored depletion or addition of CO_2, NH_4^+, K^+, and NO_3^-, and a miniature polarographic electrode monitored depletion of O_2 from a nutrient solution flowing through the root cuvette (3, 4). This solution initially contained 1 mM Na_2SO_4 to adjust ionic strength, 0.1 mM $CaSO_4$ to maintain membrane integrity, 0.5 μM K_2PO_4 to avoid phosphate deficiency, and 50 μeq each of NH_4^+, NO_3^-, K^+, and Cl^-. The solution was saturated with air at 35°C to set the initial concentrations of dissolved gases. To estimate total dissolved CO_2, a computer-based controller maintained the proton concentration of the solution flowing past the CO_2 electrode at pH 4.5 with small additions of 0.1 N H_2SO_4.

In the control treatment, fluxes from the roots of an intact plant were monitored for the entire experimental period. For the excision treatments, gas and nutrient fluxes from the intact roots were allowed to reach a steady rate for over 4 h; the roots were then excised just below the endosperm at the section of root that was immersed in the bath above the cuvette. The excision medium contained either nutrient solution, nutrient solution and 0.1 M glucose, or nutrient solution and 0.1 M sucrose.

Sugar and Malic Acid Contents. The plants used for measurements of sugar and malic acid contents were kept in a nutrient solution without any glucose or sucrose. Three plants were harvested before excision and at 3-h intervals after excision. Roots of the harvested plants were submerged in liquid nitrogen for 1 min, dried overnight, and weighed. The remaining sample preparations were conducted over ice or in a cold room. The roots were ground with a tissue homogenizer in 1 ml of 60% ethanol. The homogenizer was washed with an additional 0.5 ml of 60% ethanol, and the total solution was centrifuged at 4,000*g* for 8 min. Finally, 20 μl of the supernatant were injected through a 0.4-μm filter into an HPLC system. The column in this system was an Aminex HPX- 87H from Bio-Rad. It was eluted with 0.05 N H_2SO_4 at a flow rate of 0.4 ml min^{-1}. Sugars were detected by a differential refractometer and malic acid by UV absorbance at 210 nm.

Statistics. The fluxes were analyzed according to a one-way

ANOVA with repeated measures (General Linear Model Procedure, SAS): excision medium was the main factor and time after excision was the repeated measure. Because the results with 0.1 M glucose and 0.1 M sucrose in the excision solution were indistinguishable, these data were pooled. The data for sugar and malic acid contents were analyzed with a Student's *t*-test.

RESULTS

Net Cl^- uptake was negligible under all treatments (-0.05 ± 0.03 μmol g^{-1} dry weight root min^{-1}, mean ± SE). In control plants, fluxes of the gases and nutrients did not vary significantly over a 10-h period. Net CO_2, O_2, NH_4^+, NO_3^-, and K^+ uptake mean ± SE were, respectively, -3.31 ± 0.18, 3.24 ± 0.13, 1.40 ± 0.01, 0.98 ± 0.06, and 0.15 ± 0.07 μmol g^{-1} dry weight root min^{-1}.

With excision, net K^+ uptake rapidly became negative and returned to control levels only 6 h later (Fig. 1). Net fluxes of CO_2, O_2, NH_4^+, and NO_3^- began to decline significantly within 2 h after excision, continued to diminish for the next 4 h, and then remained steady at the depressed levels for the last 2 h of monitoring (Fig. 1). The respiratory quotient (CO_2 efflux/O_2 influx) was uniform throughout the experiment (-0.91 ± 0.02, mean ± SE). The addition of sugar to the excision medium did not significantly alter the effects of root excision (Figs. 1 and 2).

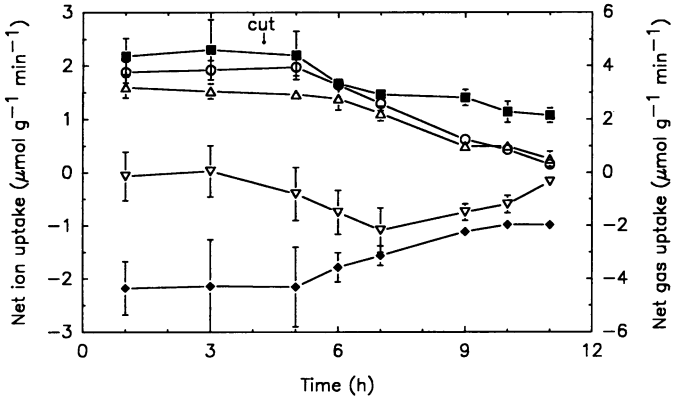

FIG. 1. Gas and ion fluxes from barley roots before and after excision when the excision medium contained only nutrient solution. Shown is the time dependence for net uptake of CO_2 (♦), O_2 (■), NH_4^+ (○), K^+ (▽), and NO_3^- (△) in μmol g^{-1} dry weight root min^{-1}. Excision occurred at 4.25 h. Means and SE are indicated with small SE incorporated into the symbols.

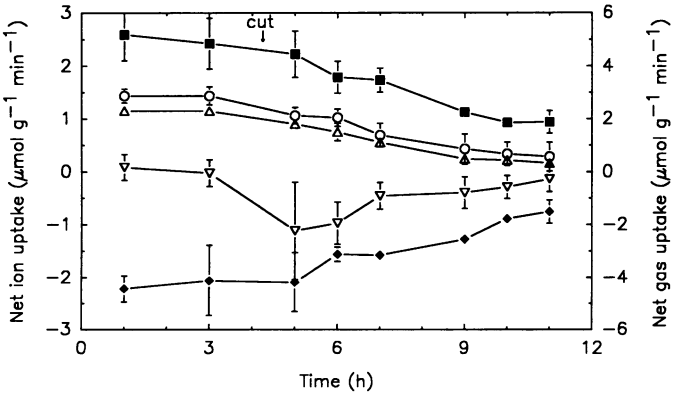

FIG. 2. Gas and ion fluxes from barley roots before and after excision when the excision medium contained nutrient solution and 0.1 M glucose or 0.1 M sucrose. Shown is the time dependence for net uptake of CO_2 (♦), O_2 (■), NH_4^+ (○), K^+ (▽), and NO_3^- (△) in μmol g^{-1} dry weight root min^{-1}. Excision occurred at 4.25 h. Means and SE are indicated with small SE incorporated into the symbols.

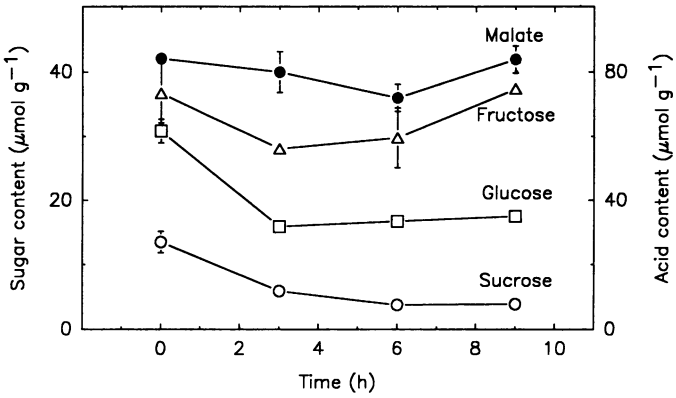

FIG. 3. Soluble sugar and malic acid contents in excised barley roots. Excision occurred at 0 h. Shown are the means and SE for fructose (△), glucose (□), sucrose (○), and malic acid (●) in μmol g^{-1} dry weight root. Small SE are incorporated into the symbols.

The root contents of sucrose, glucose, and fructose decreased significantly within 3 h of excision (Fig. 3). Sucrose and glucose contents remained at about half their initial levels for the rest of the observation period, whereas fructose content returned to the initial level after 9 h. Malic acid content decreased slightly during the first 6 h after excision, but recovered to the initial level after 9 h (Fig. 3).

DISCUSSION

Within a few hours of excision, root respiration and mineral nitrogen absorption decreased to less than 50% and 25%, respectively, of their control levels. This rapid and dramatic decline might result from either direct effects of excision such as a wounding response or more indirect effects such as reduction of water movement through the roots and limitations to the root carbohydrate supply.

Roots are very sensitive to perturbations: literally, rubbing roots the wrong way can substantially reduce phosphate absorption and energy charge (13), calcium absorption (18), or root pressure and exudation (16). We tried to minimize the extent of wounding in these experiments by cutting only a small amount of tissue that was located over 2 cm outside of the root cuvette where the fluxes were actually measured. We cannot, however, discount the possibility that the 2-h delay in the response to excision might have been related to the distance between the site of excision and the roots in the cuvette.

Excision reduces water movement through the roots. Not only does the passive movement of ions through the transpiration stream cease, but active translocation of ions to the shoot is disrupted. As a consequence, ions may accumulate in the roots and this, in turn, may inhibit uptake (21). On the other hand, under similar experimental conditions, NH_4^+ and NO_3^- absorption were independent of large changes in water movement (20); therefore, the decline in fluxes observed here were probably unrelated to water flow.

Carbohydrate supply in the roots should influence NH_4^+ and NO_3^- absorption because these processes require substantial amounts of energy (6). We found that mineral nitrogen absorption, root respiration, glucose content, and sucrose content decreased with time after excision in a similar fashion (Figs. 1–3). This indicates that excision inhibited the uptake of NH_4^+ and NO_3^- through carbohydrate limitations.

These data are consistent with those from several other studies. Clement *et al.* (8) reported that defoliation of perennial ryegrass quickly produced a severe depression in root NO_3^- absorption and proposed that the energy limitations were responsible. Clarkson *et al.* (7), Saglio and Pradet (19), Aslam and Huffaker (1),

and Davidian *et al.* (9) showed that soluble sugar content of roots declined significantly after excision.

Providing glucose or sucrose to the excised root surfaces had essentially no effect (Figs. 1 and 2). The rates of NH_4^+ and NO_3^- absorption were slightly lower for the sugar-treated roots; this probably relates more to variability among individuals than to treatment effects. In any case, the decline in root respiration and mineral nitrogen absorption was similar for both treatments. These results contrast with those of Saglio and Pradet (19), Aslam and Huffaker (1), and Davidian *et al.* (9) who found that exogenous glucose alleviated the effects of excision on barley roots. Their nutrient solutions contained from 0.01 to 0.2 M glucose. We added 0.1 M glucose or 0.1 M sucrose only to the excision medium, not to the nutrient solution in the root cuvette; this was to reduce the possibility of a serious bacterial and fungal infection in the root cuvette. Probably, the short section of root in the excision solution could not absorb or translocate sufficient amounts of sugar to meet the requirements for the remainder of the root.

Rates of K^+ absorption observed here were relatively low presumably because equimolar amounts of NH_4^+ were present in the solution (5). Nonetheless, the response of K^+ absorption to excision—a rapid decline followed by a slow recovery—was consistent with other studies (11, 12). This response was distinct from those of the gases, the other ions, glucose, and sucrose but similar to those of fructose and malic acid (Figs. 1–3). This may suggest a relationship among fructose content, malic acid content, and K^+ absorption: fructose, through its high energy phosphate forms, provides both substrate and control of glycolysis and dark carbon fixation (14); malic acid, a product of both processes, plays a key role in cellular pH regulation (22); K^+ flux into roots is dependent upon pH (17). To establish causality among these processes would, however, require additional evidence.

In light of these findings, we advise caution in the use of excised roots for studies of root respiration and mineral nitrogen absorption.

Acknowledgments—We thank Jeffrey P. Mitchell for assistance in analyzing carbohydrates and organic acids, Donald Nevins for use of the HPLC system, and William J. Lucas for comments on the manuscript.

LITERATURE CITED

1. ASLAM M, RC HUFFAKER 1982 *In vivo* nitrate reduction in roots and shoots of barley (*Hordeum vulgare* L.) seedlings in light and darkness. Plant Physiol 70: 1009–1013
2. BLOOM AJ 1985 Wild and cultivated barleys show similar affinities for mineral nitrogen. Oecologia 65: 555–557
3. BLOOM AJ, FS CHAPIN III 1981 Differences in steady-state net ammonium and nitrate influx by cold and warm adapted barley varieties. Plant Physiol 68: 1064–1067
4. BLOOM AJ, E EPSTEIN 1984 Varietal differences in salt-induced respiration in barley. Plant Sci Lett 35: 1–3
5. BLOOM AJ, J FINAZZO 1986 The influence of ammonium and chloride on potassium and nitrate absorption by barley roots depends on time of exposure and cultivar. Plant Physiol 81: 67–69
6. CHAPIN FS, III, AJ BLOOM, CB FIELD, RH WARING 1987 Plant responses to multiple environmental factors. BioScience 37: 49–57
7. CLARKSON DT, M SHONE, A WOOD 1974 The effect of pretreatment temperature on the exudation of xylem sap by detached barley root systems. Planta 121: 81–92
8. CLEMENT CR, MJ HOPPER, LHP JONES, EL LEAFE 1978 The uptake of nitrate by *Lolium perenne* from flowing nutrient solution. II. Effect of light, defoliation, and relationship to CO_2 flux. J Exp Bot 29: 1173–1183
9. DAVIDIAN J, A SOLER, C GRIGNON 1984 Development of H^+ extrusion by barley roots after their excision. Physiol Veg 22: 163–170
10. EPSTEIN E 1972 Mineral Nutrition of Plants: Principles and Perspectives. John Wiley & Sons, New York, pp 412
11. GLASS AD 1978 Influence of excision and aging upon K^+ influx into barley roots. Recovery or enhancement? Plant Physiol 61: 481–483
12. GRONEWALD JW, JB HANSON 1980 Sensitivity of the proton and ion transport mechanisms of corn roots to injury. Plant Sci Lett 18: 143–150
13. GRONEWALD JW, JB HANSON 1982 Adenine nucleotide content of corn roots as affected by injury and subsequent washing. Plant Physiol 69: 1252–1256
14. HUBER SC 1986 Fructose 2,6-Bisphosphate as a regulatory metabolite in plants. Annu Rev Plant Physiol 37: 233–246
15. KOCHIAN LV, WJ LUCAS 1982 Potassium transport in corn roots. I. Resolution of kinetics into a saturable and linear component. Plant Physiol 70: 1723–1731
16. MILLER DM 1987 Errors in the measurement of root pressure and exudation volume flow rate caused by damage during the transfer of unsupported roots between solutions. Plant Physiol 85: 164–166
17. NEWMAN IA, LV KOCHIAN, MA GRUSAK, WJ LUCAS 1987 Fluxes of H^+ and K^+ in Corn Roots. Characterization and stoichiometries using ion-selective microelectrodes. Plant Physiol 1177–1184
18. RINCON MAGALY, JB HANSON 1986 Controls on calcium ion fluxes in injured or shocked corn root cells: Importance of proton pumping and cell membrane potential. Physiol Plant 67: 576–583
19. SAGLIO PH, A PRADET 1980 Soluble sugars, respiration, and energy charge during aging of excised maize root tips. Plant Physiol 66: 516–519
20. SCHULZE E-D, AJ BLOOM 1984 Relationship between mineral nitrogen influx and transpiration in radish and tomato. Plant Physiol 76: 827–828
21. SHENNAN CS 1981 Salt tolerance in *Aster tripolium* (L.). PhD thesis, University of Cambridge, England
22. SMITH FA, JA RAVEN 1979 Intracellular pH and its regulation. Annu Rev Plant Physiol 30: 289–311
23. VAN STEVENINCK RFM 1975 The "washing" or "aging" phenomenon in plant tissues. Annu Rev Plant Physiol 26: 237–258