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The absorption of potassium and several organic compounds by barley roots: Effect of siduron

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Absorption of ^{42}K by excised roots of barley (*Hordeum vulgare* L.) grown in 0 or 5 ppm siduron (1-(2-methylcyclohexyl)-3-phenylurea) was a linear function of time for at least 60 minutes with transport being unidirectional. Absorption of siduron was a function of the external concentration to the limits of its solubility (0.09 mM). However, the siduron-^{14}C absorbed by roots grown in either 0 or 5 ppm siduron was in a readily exchangeable form and desorption for 4 hr exchanged 80% of the label. Glucose-^{14}C, adenine-8-^{14}C and leucine-^{14}C were actively absorbed with 70 to 85% of the label being absorbed in 24 hr. Although roots grown in siduron absorbed less ^{42}K, glucose-^{14}C, adenine-^{14}C and siduron-^{14}C, and more leucine-^{14}C than similar roots grown in water culture, it is probable that these differences were not large enough to account for the noted reduction (60%) in root growth.

Siduron (1-(2-methylcyclohexyl)-3-phenylurea) has been shown to decrease growth and disrupt the metabolism of barley roots (*1*). Siduron, unlike the other phenylureas (*2*), does not inhibit photosynthesis (*3*) and a root based action has been suggested for this herbicide (*1*, *3*). Mineral elements and various organic compounds are absorbed by roots from the soil and this absorption is a primary process by which essential ions are removed from the inorganic environment. Similarly, to be an effective herbicide, siduron must also be absorbed from the surrounding environment. The effect of siduron upon absorption is of interest in this regard.

The results of an investigation of the absorption of ^{42}K, glucose-^{14}C, adenine-^{14}C, leucine-^{14}C and siduron-^{14}C by excised barley roots grown in solution culture are presented in the present paper.

Material and methods

Plant material

Barley (*Hordeum vulgare* L., variety Trial) was grown in solutions of water or 5 ppm siduron by a modification of the method of EPSTEIN (*4*).

Seeds were allowed to germinate 24 hr in 1 liter of aerated water or siduron solution in the dark at 22°. The germinating seeds were removed and spread on a layer of cheesecloth. The cheesecloth was supported by a polyethylene screen about 1 cm above the surface of 2 liters of 0 or 5 ppm siduron solution with 2×10^{-4} M $CaSO_4$ (*4*) in a 2 liter polyethylene beaker. A second cheesecloth was spread over the seeds. The corners of the cheesecloth were dipped into the solution. This assembly was placed in the dark and aerated continuously for 7 days. The solutions were changed after 5 days with care taken to insure sterile conditions. The roots were excised, rinsed in distilled water and lightly blotted dry.

Radioactive materials

D-Glucose-U-^{14}C, L-leucine-U-^{14}C or adenine-8-^{14}C were dissolved in distilled water to give a 1 μmole solution having 7.5 μc of radioactivity in 0.05 ml. Siduron-carbonyl-^{14}C was supplied by E. I. Du Pont de Nemours and Company and used at 5 ppm in uptake studies or diluted with technical siduron to a constant specific activity in experiments with various concentrations. ^{42}K was supplied by the Reactor Lab at the University of Illinois as $^{42}K_2CO_3$ and converted to ^{42}KCl before use.

Incubation procedure

Duplicate 1.5 g samples (replicated twice) of excised roots grown in 0 or 5 ppm siduron were placed in No. 15 medium fritted glass filter funnels containing 0.05 ml of glucose-^{14}C, adenine-^{14}C or leucine-^{14}C and 10 ml of 0.1 M potassium phosphate, pH 6.7 and 100 μg of tetracycline to prevent bacterial contamination (*5*). The system was closed and air was passed through a 50% KOH scrubber and then through the base of the filter funnels to aerate the tissues suspended in solution. Respired $^{14}CO_2$ was carried in the air stream and bubbled through 10 ml of 20% KOH in a 50-ml centrifuge tube. The absorbed CO_2 was converted to $BaCO_3$, filtered and the filter paper counted for radioactivity. The radioactivity in the CO_2 was included in the total uptake.

Siduron-^{14}C is not metabolized by barley roots (*1*) and $^{14}CO_2$ was, therefore, not a factor in its uptake. In studies with siduron-^{14}C and ^{42}K, the excised roots grown in 0 or 5 ppm siduron were weighed and transferred to a single layer of cheesecloth. The edges of the cheesecloth were gathered together, making a "teabag" (*6*). The teabag was transferred to a solution of 0.10 mM ^{42}KCl or various concentrations of siduron-^{14}C at 28° and pH (unbuffered) 6.4. The volumes were such that the decline in the concentration of siduron or K in the experimental solutions, due to withdrawal by the roots, did not exceed 2% of the initial concentration.

Analytical methods

At predetermined times, the tissues in solutions of glucose-^{14}C, leucine-^{14}C or adenine-^{14}C were removed, rinsed with deionized water to remove

any non-absorbed compounds, and transferred to 50 ml of boiling 100 % ethanol and boiled for 3 min. The ethanol was decanted and the tissues were ground with a mortar and pestle. The residues were successively extracted in boiling 80 % ethanol, 50 % ethanol and then again in 80 % ethanol. The extracts were combined and taken to dryness at 35° under reduced pressure. An aliquot of this extract and the insoluble residue was taken and counted for radioactivity.

After absorption, the roots in siduron-^{14}C and ^{42}KCl were placed in a solution of 5 mM of cold siduron or KCl for 30 min at 8° to remove the radioactive material in the outer space of the tissues. Siduron-^{14}C was extracted from the tissues with 80 % acetone and aliquots assayed for radioactivity. Tissues which absorbed ^{42}KCl were ashed at 500° for 1 hr before assaying for label (*4*).

Results

Fig. 1 shows the effect of varying the concentration of siduron upon the amounts of siduron absorbed in a 12 hr period. The amount of siduron absorbed was linear with respect to concentration up to 20 ppm, the

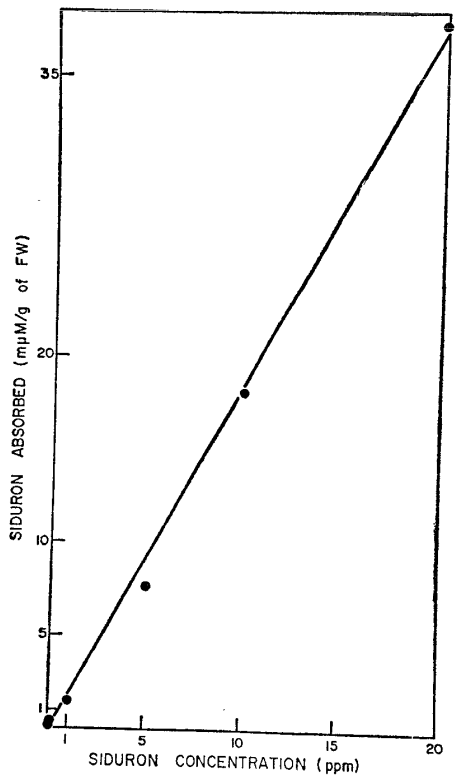

Fig. 1. The rate of absorption of siduron-^{14}C as a function of the concentration of siduron.

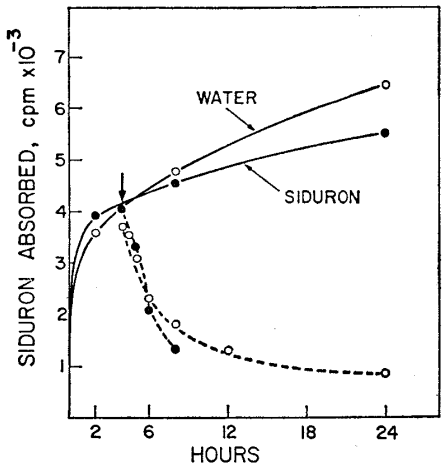

Fig. 2. Absorption of siduron-^{14}C from a 5 ppm solution by barley roots grown in water or 5 ppm siduron, as a function of time, and the effect of transferring the tissue to a chemically identical solution in which the siduron was unlabeled. Solid line, tissue in siduron-^{14}C; dotted line, tissue in unlabeled siduron. "Siduron absorbed" refers to labeled siduron.

maximum solubility of siduron. The growth of barley roots was reduced 60% in 7 days by 5 ppm of siduron and this concentration was selected for uptake inhibition studies.

The results of an experiment in which absorption of siduron-^{14}C was followed as a function of time is shown in Fig. 2. Absorption of siduron was rapid during the first 3 hr and then slowly increased over the next 21 hrs. Over 50% of the siduron absorbed by roots grown in either water or 5 ppm siduron solution occurred during the first 3 hr of uptake. After 4 hr, 1 set of root samples was transferred to a second solution, identical in chemical composition with the solution in which the samples were kept before, but the siduron was unlabeled. At intervals, the samples were removed. A nearly linear rapid loss of siduron to the unlabeled solution occurred over the next 4 hr, although siduron-^{14}C continued to be absorbed (Fig. 2). Siduron-^{14}C continued to be lost to the unlabeled solution and after 20 hr of desorption the roots placed in unlabeled siduron contained only 23% as much siduron-^{14}C as they had initially absorbed. Of the siduron-^{14}C retained by the roots, one-third of the ^{14}C was incorporated into the insoluble fraction (*1*) and, therefore, unavailable to be exchanged. Thus, the amount of siduron-^{14}C available to be lost to the unlabeled solution was even less than that shown in Fig. 2. Roots grown in water absorbed 21% more siduron-^{14}C than those grown in 5 ppm siduron.

To study the effect of siduron upon ion absorption, roots grown in water or 5 ppm siduron solutions were allowed to absorb ^{42}K for various times (Fig. 3). The absorption of ^{42}K was linear for the first 60 min by roots grown in siduron and 90 min by roots grown in water. The rate

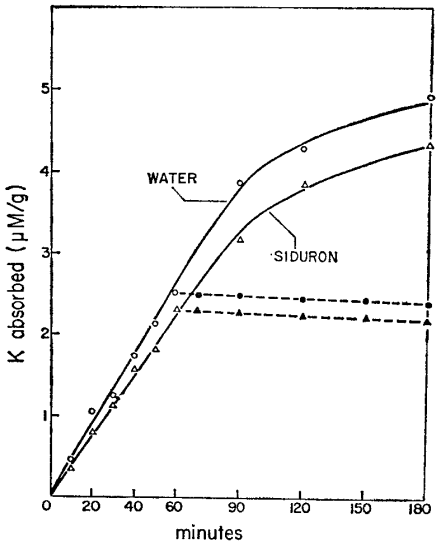

Fig. 3. Absorption of ^{42}K from 0.1 mM ^{42}KCl by barley roots grown in water or 5 ppm siduron, as a function of time, and the effect of transferring the tissue to a chemically identical solution in which K was unlabeled. Solid line, tissue in ^{42}K; dotted line, tissue in unlabeled K. "K absorbed" refers to labeled K.

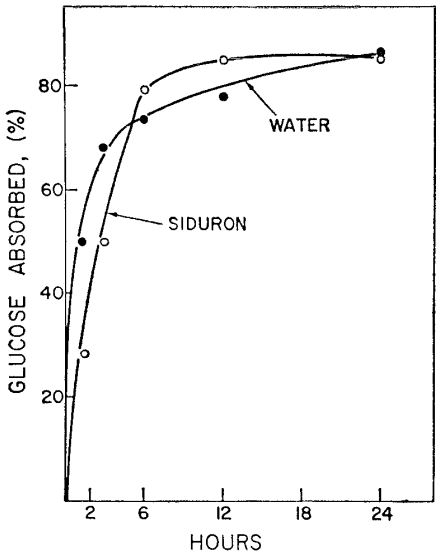

Fig. 4. Rate of absorption of glucose-U-^{14}C by roots grown in water or 5 ppm siduron as a function of time.

of uptake then decreased. Roots grown in siduron absorbed less ^{42}K at all times than those grown in water. When roots were removed from the ^{42}KCl solutions and placed in cold KCl similar to the siduron-^{14}C uptake studies (Fig. 2), only a very slight fraction of the previously absorbed

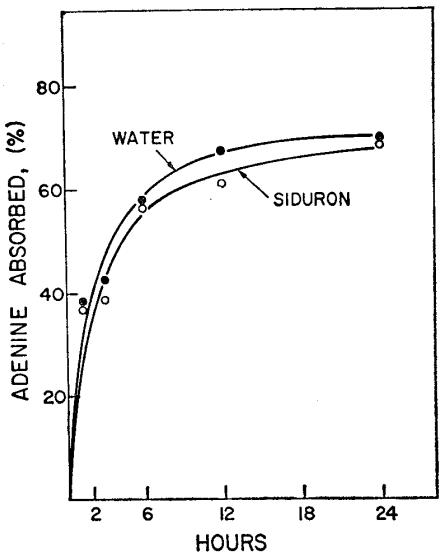

Fig. 5. Absorption of adenine-8-^{14}C with time by roots grown in water or 5 ppm siduron.

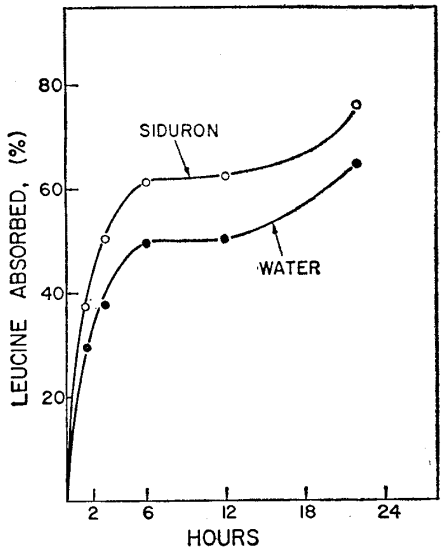

Fig. 6. Absorption of leucine-U-^{14}C with time by roots grown in water or 5 ppm siduron.

labeled K was lost to the unlabeled solution.

In 6 hr, 80 % of the available glucose-^{14}C was absorbed by barley roots (Fig. 4). The rate of uptake was linear for 3 hr by roots grown in water and 6 hr for roots grown in siduron. The roots grown in siduron absorbed 20 % less glucose at early times but by 24 hr both these roots and those grown in water had absorbed 85 % of the available substrate.

The absorption of adenine (Fig. 5) approximated that of glucose absorption. Nearly 60 % of the adenine was absorbed in the first 6 hr and an additional 10 % was absorbed over the next 18 hr. Roots grown in siduron absorbed less adenine at all times than those grown in water although by 24 hr the amounts were nearly equal.

Roots grown in siduron absorbed more leucine at all times than those grown in water (Fig. 6). Contrary to these results, the absorption of other substances (Fig. 2-5) was reduced when the roots were grown in siduron. At the end of the 24 hr absorption period, siduron grown roots had absorbed 75 % of the available leucine and 10 % more than water grown roots.

Discussion

Kinetic and other experiments on the absorption of ions by higher plant tissues have led to the recognition of two different mechanisms of absorption (*7*). EPSTEIN (*7*) has reported evidence which indicated that the outer cytoplasmic membrane, the plasmalemma, was the site of mechanism 1 and the tonoplast as the site of mechanism 2. In the present investigation, the absorption of K was studied in experiments in which the K concentration never exceeded 0.10 mM so that only mechanism 1 should be operative. The K absorption curves (Fig. 3) are similar to those observed for Cl absorption by barley roots (*8*) and it would appear that equal molar amounts of K and Cl were absorbed.

When the concentration of siduron was varied from 0.1 ppm to the limits of its solubility (20 ppm), the rate of its absorption was found to be a function of the external concentration (Fig. 1). Less than 0.3 % (7 mμM) of the available siduron was absorbed in 24 hr (Fig. 2). This is in contrast to K absorption which was absorbed at the rate of 40 mμM/min (Fig. 3). Nearly 30 % of the available pyrazon (5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone) applied to tomato foliage was absorbed in 1 hr (*9*).

The large loss of previously absorbed labeled siduron, when unlabeled siduron was substituted for labeled siduron in the solution, shows that siduron transport is not unidirectional (dotted line of Fig. 2), as was the case in K absorption (dotted line of Fig. 3). The slight loss of K observed may have occurred from the xylem, via the cut end. It is not probable that the quantity of siduron exchanged was lost in a like manner, nor was it metabolized (*1*). Rather, the tissue contained, in addition to a small fraction of siduron which was actively accumulated (and very sluggishly exchanged), a large fraction that was held in readily exchangeable form. As K and the organic substances studied were actively absorbed and siduron was not actively absorbed, siduron should not compete with these substances for carrier sites. The data do not permit one to determine if the labile (nonaccumulated) siduron was bound or free within the cell wall, absorbed on the cation exchange capacity of the roots or actually moved across the cell membranes by diffusion or ion carriers.

Glucose uptake from dilute solutions (Fig. 4) proceeded at a linear rate and although most of the glucose absorbed was metabolized to CO_2, this metabolism was important only as it utilized the entering sugar and not that it prevented the internal concentration from rising above that in the medium. In the case of adenine (Fig. 5) and leucine (Fig. 6), the criteria for active transport against a gradient was satisfied and the internal level was higher than that in the medium.

Siduron inhibits the growth of barley roots (*1*) and this inhibition was relieved when the roots were transferred to a water solution. It appears that relief of this inhibition was due to the desorption of exchangeable siduron, and although the effect of siduron lasts a considerable time after the roots have been removed from the siduron solutions (Figs. 2-6), the siduron previously absorbed is readily removed (Fig. 2) from the roots.

Although siduron reduced the uptake of K, glucose, adenine and siduron itself, and stimulated the absorption of leucine, these differences in uptake do not appear to be great enough to cause the noted reduction in root growth. Rather, it is probable that siduron disrupts the metabolism of these compounds once they are absorbed.

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