Inhibition of Water Uptake in Sugar Beet Roots by Ammonia

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Abstract. Ammonium sulfate, ammonium carbonate or ammonia gas inhibited water uptake in sugar beet roots whenever the pH was sufficiently high to cause the production of ammonia. When ammonia was removed by aeration, inhibition of the water uptake by roots was rapidly reversed. ATP at 0.2 mM appeared to either wholly or partially prevent the ammonia-induced inhibition of water uptake by roots. ATP may be involved in maintaining the structure of water pathways through the root. In roots lacking epidermis, ammonia did not inhibit water uptake by the roots. This may indicate that the site of the inhibition lies within the root epidermis.

During a previous study we observed that sugar beets grown in the greenhouse or out-of-doors in a nutrient solution (pH 7.6-8.0) using ammonium sulfate as the nitrogen source wilted severely on hot summer days. Sugar beets receiving potassium nitrate as a nitrogen source showed little, if any, wilting. On cloudy days or at night, no difference in wilting was observed between the treatments. It appeared that ammonia may have been interfering with the movement of water through the sugar beet plant.

Several investigators have reported that respiration inhibitors and/or uncouplers such as cyanide (2, 3, 15, 16, 17, 19), azide (2, 13, 19), 2,4-dinitrophenol (16), fluoride (2), iodoacetate (2), arsenate (2), phenylurethane (2), and lack of oxygen (15, 20) decrease the movement of water into or out of coleoptile segments, root segments or intact roots. Ammonia (NH₃) and/or undissociated ammonium hydroxide, hereinafter called ammonia, may also act as respiration inhibitors. Altschul et al. (1) showed that treating cotton seed with ammonia inhibited respiration in both mature and immature seeds. Vines and Wedding (24, 26) found that ammonia inhibited respiration in excised har' ey roots, garden beet disks, leaf disks of spinach and sugar beets and garden beet root mitochondria. They found that the degree of inhibition was related to pH, since pH controls the amount of ammonia present in solutions containing ammonium ions (NH₄⁺).

Since ammonia acts as a respiration inhibitor, one might expect that ammonia would also reduce or inhibit water uptake by sugar beet roots.

Materials and Methods

Materials. Sugar beet (Beta vulgaris L. var. monogerm) seed was germinated in moist vermiculite and moistened with one-half strength Hoagland no. 1 nutrient solution as needed. At the 3- to 4-leaf stage the seedlings were transferred to 4-liter polyethylene containers filled with one-half strength Hoagland no. 1 nutrient solution with full strength minor elements and iron added as sodium ferric ethylenediamine di- (o-hydroxyphenylacetae) (8). One seedling was placed in each container. The solutions were aerated constantly and renewed weekly. Deionized water was used for all solutions.

Plants of the same age varied in size; therefore, the plants were used for experimental purposes when the top of the main root reached 8 to 12 mm in diameter.

Methods. A modification of Kramer’s apparatus (10) was used to measure water uptake by the sugar beet root (fig 1). An experiment was initiated by removing a plant from the nutrient solution

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Fig. 1. Apparatus for measuring the rate of water uptake by excised sugar beet roots.
and placing the roots into a liter beaker filled with deionized aerated water, nutrient solution, or a dilute buffer solution. By using this open beaker arrangement, solutions could be changed and ammonia or ammonium salts conveniently added. Aeration was accomplished by bubbling air through an aquarium stone directly into the beaker. Temperature varied from 21° to 24° during the course of all the experiments.

The plant was topped just below the last leaf scar, and the root was gently slipped into the gum rubber tubing and sealed by tightening a piece of soft wire around the tubing. Thirty centimeters of mercury suction or, in some experiments, no suction was applied to the end of the capillary tube and after a few minutes the system was checked for leaks and cleared of bubbles. A drop of mercury was introduced into the capillary tube through the right-hand stopcock and the rate of its movement was recorded. Readings were taken every 20 minutes until the rate of water uptake became steady, usually about 1 hour. The roots were then lifted out of the water and an ammonium salt or ammonia gas was added to the solution. The roots were replaced in the solution and readings were continued.

The roots used varied in size, weight, surface area, and amount of water moved per root per unit of time; because of this, all movement of the mercury droplet was recorded as percent initial rate. The initial rate was taken as the rate of movement just before the treatment was applied.

In measuring water uptake in Hoagland's one-half strength nutrient solution with added ammonium carbonate and dilute buffers with added ammonium chloride, aeration was stopped just before the ammonium salt was added and was not resumed for 20 minutes. This prevented the removal of ammonia from the nutrient solution until aeration was resumed.

When ammonia gas rather than ammonium carbonate or ammonium chloride was used the ammonia gas was introduced into the aeration stream by placing a solution of about 0.5 M ammonium hydroxide into a flask and passing air over the top of the solution. The regular aeration stream was withdrawn from the water containing the roots, and the air containing the ammonia was introduced into the water until the pH reached 8.4 to 8.5. The air containing the ammonia was then withdrawn from the solution and the regular aeration stream was again introduced.

Adding ammonia to pH 9.0 injured some of the roots, causing them to turn dark. When the roots turned dark water uptake greatly increased and then became erratic. At greater than pH 9.0 all roots were damaged when ammonia and/or ammonium salts were present in the concentrations we used. Roots that had been broken or mechanically damaged showed water uptake similar to the ammonia-damaged roots.

Results

Inhibition of Water Uptake by Ammonia. In each of the experiments the addition of ammonium carbonate or NH₄ raised the pH to 8.2 to 8.4 and water uptake dropped to less than 5% of the initial rate (fig 2, 3, 4, 6, 7). As ammonia was removed from the water or nutrient solution by the aeration stream, the pH dropped to 7.5 to 7.7, or when NH₄ was added to 7.3 to 7.4, and water uptake again resumed. After resumption, the rate of water uptake reached a peak and then decreased (fig 2, 3). In nutrient solution this decrease was not noted (fig 4). Results with 0.5 and 0.25 mM ammonium carbonate, not shown, were similar. Roots placed in water without added ammonium carbonate showed a general decrease in water uptake with time.

When suction was not applied, the initial rates were much lower than when suction was applied. The heights of the water uptake peaks after ammonia had been removed were somewhat and unaccountably variable (fig 3). When ammonia was added instead of ammonium carbonate the peak was much smaller and returned to only 25 to 50% of the initial rate (fig 7).

To eliminate the possibility that high pH alone inhibited water uptake by sugar beet roots, 0.25 mM potassium carbonate was used instead of ammonium carbonate (fig 5). Upon addition of the potassium carbonate, the pH of the water increased to 9.4 and remained above 9.0 for the remainder of the experiment. Instead of inhibiting the uptake of water, as did ammonium carbonate, the rate of water uptake fluctuated unaccountably, but did not fall below 50% of the initial rate. Restoration of aeration had little or no effect on water uptake.

With the dilute buffers, the added ammonium sulfate had little, if any, effect on the rate of water uptake at pH 6 and 7 (fig 8, 9). At these pH's the water uptake was similar to that with water at pH 6.3 or with potassium carbonate at pH 9.4. However, when the pH was raised to 8.0 or 9.0 the added ammonium sulfate acted in the same manner as ammonium carbonate or ammonia gas, rapidly reducing water uptake to less than 5% of the initial rate. Water uptake returned to the initial rate or greater when ammonia was removed by the aeration stream. In contrast to the phosphate buffers (fig 8), the tris buffers (fig 9) showed a much increased water uptake rate after the removal of the ammonia.

Water Uptake in Response to Added Adenosinetriphosphate (ATP) and Ammonium Carbonate. Ammonia has been shown to inhibit both the photosynthetic formation (11) and the oxidative formation (6, 18, 24, 26) of ATP. Perhaps, ATP is involved in maintaining the structure of water pathways in various tissues. Kramer (10) and Slattery (21) both emphasize that respiration is closely connected with the uptake of water by various plant tissues.
The effect of ATP on water uptake was determined in much the same manner as the other experiments. The sugar beet roots were immersed in 0.1 mm ATP (disodium salt) at the beginning of the experiment and another increment of 0.1 mm ATP was added 40 minutes later. Ammonium carbonate was added 20 minutes after the last addition of ATP. Aeration was continuous except for a 20-minute period after the addition of ammonium carbonate. Suction was applied at 30 cm Hg.

The pattern of water uptake by sugar beet roots with added ATP and ammonium carbonate is quite unlike the pattern where ammonia was added and ATP was not (fig 10). However, it does resemble the pattern of water uptake in experiments with buffers at pH 6 and 7, where ammonia was ex-

Fig. 2. (top, left) Ammonium carbonate and the rate of water uptake by sugar beet roots in water at 30 cm Hg suction. Continuously aerated. A typical replication.

Fig. 3. (middle, left) Ammonium carbonate and the rate of water uptake by sugar beet roots in water at 0.0 cm Hg suction. Continuously aerated. A typical replication.

Fig. 4. (bottom, left) Ammonium carbonate and the rate of water uptake by sugar beet roots in one-half-strength Hoagland no. 1 nutrient solution at 30 cm Hg suction. Average of 2 replications.

Fig. 5. (top, right) Potassium carbonate and the rate of water uptake by sugar beet roots in water at 30 cm Hg suction. Average of 4 replications.

Fig. 6. (middle, right) Ammonia (NH₃) and the rate of water uptake by sugar beet roots in water at 30 cm suction. Continuously aerated. Ammonia (NH₃) added until pH 8.3 to 8.5 was attained. A typical replication.

Fig. 7. (bottom, right) Ammonia (NH₃) and the rate of water uptake by sugar beet roots in water at 0.0 cm suction. Continuously aerated. Ammonia (NH₃) added until pH 8.3 to 8.5 was attained. A typical replication.
teremely low because of the low pH, and with potassium carbonate (compare fig 10 with fig 5, 8, 9). ATP, alone, did not increase the initial rate of water uptake, but it did appear to have reversed, partially at least, the ammonia induced inhibition of water uptake in sugar beet roots.

**Water Uptake in Roots Lacking Epidermis.** Veldstra and Booy (23) observed that n-diamyl acetic acid (DAA) has a pronounced synergistic action with auxins, causing root cells to swell and burst. Burstrom (4) found that 10 μM DAA ruptured epidermal cells of wheat roots without any apparent damage to the endodermis. Sandstrom (21), using wheat roots with their epidermis removed by DAA, found the concentration of the xylem sap to be about the same as the nutrient solution in which they were placed. It may be that the root epidermis contains the site of the ammonia induced inhibition of water uptake.

Sugar beets were grown as previously described and Sandstrom’s method (21) was used to remove the epidermis with 20 μM DAA. Water uptake of roots was measured as before in the presence of 0.25 mM ammonium carbonate with and without the DAA treatment. Microscopic and visual observations showed that 40 μM DAA treatment had damaged the sugar beet roots, causing dark necrotic spots to appear on the small rootlets. Because of this damage, results of the 40 μM DAA treatment are not included in this report. Damage in the 20 μM DAA treatment was not apparent but this does not eliminate the possibility of damage.

Ammonium carbonate did not inhibit water uptake by DAA treated roots (fig 11). In the untreated roots, however, ammonium carbonate inhibited water uptake as in the other experiments. Roots treated with 20 μM DAA behaved very much like roots that had been treated with ammonium.
sugar beet roots. However, both the experiments using potassium carbonate and buffers indicate that this was not the cause since the high pH obtained with potassium carbonate or buffers, in the absence of ammonia, did not inhibit water uptake. Many workers (5, 22, 25) have shown that as pH increases above pH 7, the toxicity of ammonium compounds increases and that this is due to an increase in the percent of ammonia present. Ammonia because of its small size, diffuses very rapidly across cell membranes and into the cytoplasm, while the ammonium ion (NH₄⁺) diffuses much more slowly (7, 9, 14, 25).

Ammonia appears to be the cause of the inhibition of water uptake in sugar beet roots since at a pH greater than 7 water uptake was inhibited in less than 5 minutes, whereas NH₄⁺ did not cause any inhibition of water uptake at pH 7 or less. At this pH the concentration of NH₄⁺ is very high and that of ammonia extremely low. Vines and Wedding (24) reported that ammonia increased rather than decreased the permeability of the red beet root disks as measured by the loss of anthocyanin. However, owing to the high concentrations of ammonia 2.0 to 8.0 mM that they used, damage to the tissue undoubtedly occurred. At a pH of about 9.2, half of the ammonia is in the NH₃ form. In this study sugar beet roots were damaged with 1.0 mM ammonium sulfate at pH 9.0 and some roots still showed damage with 0.5 mM ammonium sulfate at pH 9.0.

It would appear that ATP successfully reversed the ammonia-induced inhibition of water uptake in sugar beet roots. However, we cannot eliminate the possibility that ATP may have damaged the sugar beet roots. Damaged roots had a pattern of water uptake similar to the ATP-treated roots in that ammonia did not reduce water uptake in either case, but the initial rate of uptake was much slower with ATP-treated roots than with damaged roots. The ATP-treated roots appeared to be normal and showed no visible damage.

Barring any injury by ATP, it appears that ATP can overcome, at least partially if not completely, the ammonia-induced inhibition of water uptake. Admittedly, the evidence is not overwhelming, but it may indicate that ATP or similar compounds are directly involved in maintaining the porosity of various tissues to water.

Ammonium carbonate did not inhibit water uptake by DAA-treated roots (Fig. 11). Roots treated with 20 μM DAA behaved very much like roots that had been treated with ammonium sulfate buffered at pH 6 or 7 or potassium carbonate, except that the initial rate was higher. Again, water uptake varied somewhat over short periods of time, but there wasn’t the almost complete inhibition that occurred in the untreated roots.

**Discussion**

The experiments with ammonium carbonate and ammonia gas would not eliminate pH, per se, as the cause of the inhibition of water movement in

Fig. 10. Ammonium carbonate and the rate of water uptake by sugar beet roots in water at 30 cm Hg suction. Average of 4 replications.

Fig. 11. Water uptake in sugar beet roots treated with 20 μM n-diamylacetic acid and 0.25 mM ammonium carbonate in water at 30 cm Hg suction. Average of 4 replications.
Literature Cited


