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The Inhibition of Water Uptake in Sugar Beet Roots by Ammonia

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THE INHIBITION OF WATER UPTAKE IN SUGAR

BEET ROOTS BY AMMONIA

by

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INTRODUCTION

Various ammonium salts, urea, aqua ammonia, and anhydrous ammonia are important sources of nitrogen for the fertilization of agricultural crops. While ammonia and its compounds are useful as fertilizers, they can be, and often are, toxic to many plants (Willis and Rankin, 1930; Stout and Tolman, 1941; Raleigh, 1942; Stoll, 1954; Lorenz, 1955; Grogan and Zink, 1956; Allen, 1962; Cooke, 1962; Allred, 1963; Court et al. 1964; Hood and Ensminger, 1964). There are also products which have been designed to retard the oxidation of ammonia and its compounds (Goring, 1962). It is therefore important that the effects of ammonia and its compounds on plants, plant growth and plant metabolism be fully understood.

Ammonia (NH_3) has prevented the germination of many kinds of seeds and can also damage roots, causing them to turn dark and rot (Stout and Tolman, 1941; Hood and Ensminger, 1964). Ammonia (NH_3) injury also resembles various wilt diseases. Stoll (1954) observed that ammonia (NH_3) caused cucumbers to wilt and that the symptoms resembled those of cucumber wilt disease. Grogan and Zink (1956) found that although wilt disease symptoms in lettuce could be caused

by many chemicals, ammonium hydroxide and anhydrous ammonia
were the most toxic of those tested. Allen (1962) grew rough lemon
seedlings in sand and reported that the seedlings wilted within 24
hours and died in 21 days after the applications of ammonium hydroxide.

During 1960 and 1962, the author participated in an experimental
study in which it was observed that sugar beets grown in nutrient solu-
tion (pH 7, 6 - 8. 0) in the green house and out-of-doors with ammonium
sulfate as the nitrogen source, wilted strongly on hot summer days,
while sugar beets which received potassium nitrate as the nitrogen
source showed little, if any, wilting (Haddock and Stuart, 1963). On
cooler or cloudy days or at night no difference in wilting could be
observed between the treatments.

From these general observations it appeared that ammonia was,
in some unknown way, interfering with the movement of water through
the sugar beet plant. It seemed most likely that ammonia could either
inhibit the closing of stomates, causing them to lose water, or that
ammonia greatly reduced the absorption of water by the roots. Either
of these could cause wilting under conditions of high transpiration
demand.

OBJECTIVES

The objectives of the present investigation were as follows:

1. To measure the effect of ammonia (NH_3) on wilting, stomatal aperture, and the water content of leaf tissue under simulated field and greenhouse conditions.

2. To measure the effect of ammonia (NH_3) on the release of water from tissue disks and the movement of water through intact sugar beet root systems.

3. To find the region in the roots of the sugar beet where ammonia (NH_3) affects the uptake of water.

PART I

WILTING, STOMATAL APERTURE, WATER CONTENT

OF LEAF TISSUE, AND AMMONIA

The Experiments and observations reported in this section were undertaken to ascertain the effects of ammonia on the wilting, stomatal apertures and water content of the sugar beet leaves.

Literature Review

Sayre (1926) reported that the stomata of Rumex patientia open to 50% of their maximum value when exposed to ammonia vapor in the dark. Scarth (1926) floated sections of Zebrina pendula leaf epidermis in dilute solutions of ammonium hydroxide and found that by increasing the concentrations and pH the stomatal aperture continued to widen until the ammonium hydroxide became toxic to the tissue. A few years later Scarth (1932) observed that ammonia vapor would cause the stomata of Zebrina pendula to open in the dark at a guard cell pH of approximately 7.4. He also found that ammonia vapor even caused the stomata of wilted leaves to open.

Small (1939) and Alvim (1949) immersed epidermal strips in buffer solutions (M/10 or M/20) and found that the stomata increased

in aperture with increasing pH, but they did not take into consideration the effect of the relatively high concentrations of salts in the buffers. Arends (1926), as quoted by Amer (1954), obtained an increase in stomatal aperture when he placed epidermal strips in neutral salts. With pure bases, however, he observed stomatal closure, the direct opposite of that which Sayre (1926) and Scarth (1932) observed. Williams and Shipton (1950) found that stomata open when epidermal strips are placed in neutral unbuffered salt solutions and that the effect of the buffer may be due to salt and not to pH. Amer (1954) immersed strips from the lower epidermis of Pelargonium zonale in distilled water, 1/20 N and 1/50 N KOH and NH_4OH. He found that in distilled water the stomata gradually close, in KOH there was an initial opening followed by closure, in NH_4OH there was partial closing. He also found ammonia to be toxic to the guard cells at the concentrations used. Sayre (1926) and Scarth (1926, 1932) reported no toxicity with ammonia, possibly because they used more dilute solutions than those used by Amer (1954).

The work of Williams and Shipton (1950), Arends (1926), Amer (1954), Small (1939) and Alvim (1949) does not eliminate the possibility of ammonia causing the opening of stomata. In all cases, the concentrations of salts and bases used were much higher than the ammonia solutions of Sayre (1926) and Scarth (1932, 1926). (1/10, 1/20, and 1/50 N versus 1/500, 1/1000, and 1/2000 N).

1960 Study

This study was undertaken to show the effect of ammonia on the wilting and water content of sugar beet leaves.

Materials and methods

The sugar beets used in this study were a part of another experiment on the nutrient balance of sugar beets. (Haddock and Stuart, 1964). The treatments involved ten different nutrient cultures. These nutrient solutions were 1/2 strength Hoaglands No. 1 and modifications of 1/2 strength Hoaglands No. 1 nutrient solutions. Their composition is shown in Table 1. Ten replications of each treatment were arranged in a Latin Square.

Twenty sugar beet seeds of the commercial mono-germ variety S. L. 126 were planted April 15, 1960 in ten gallon cans (14 inches in daimeter and 15 inches deep) filled with No. 2 vermiculite. These were thinned June 29, to leave a final stand of three plants per can. 1/2 strength Hoaglands No. 1 nutrient solution was used until thinning time, after which the treatments were applied. The three beets in each pot had a total surface pot area of 1.07 square feet and an available top canopy of 11 square feet of which about four square feet were used.

The nutrient solutions were mixed in 55 gallon barrels using canal water. This water contained 1.0 ppm K, 6.0 ppm Na, 48 ppm Ca

Table 1. Composition of Nutrient Solutions

ppm	NO_3-N	NH_4-N	P	K	Ca	Mg	Na
1. Check (1/2 strength Hoagland's)	105	-	16	117	100	24	12
2. 1/2 K	105	-	16	59	130	24	12
3. OK	105	-	16	0	160	24	12
4. 1/2 N	53	-	16	117	100	24	12
5. 1/4 N	26	-	16	117	100	24	12
6. $NO_3 + NH_4$	105	53	16	117	100	24	12
7. 1/2 P	105	-	8	117	100	24	12
8. NH_4	-	105	16	117	100	24	12
9. 1/2 Ca + 1/2 Mg	105	-	16	117	50	12	115
10. Check-N same as No. 1 to Sept. 1 1/2N9/1-10/1NO-N10/1 to 10/15 harvest	105	-	16	117	100	24	12

Micro-nutrients were supplied to the dilute nutrient solution in the following concentrations: B, 0.5 ppm; Mn, 0.5 ppm; Zn, 0.05 ppm; Cu, 0.02 ppm; and Mo, 0.05 ppm.

Iron added as Fe - E. D. T. A. sodium complex at the concentration of 1.37 ppm.

Canal water contained 1.0 ppm K, 6.0 ppm Na, 48 ppm Ca, 16 ppm Mg, 2.0 ppm Cl, 9.0 ppm SO_4, 0.0 ppm CO_3, and 215 ppm HCO_3.

16 ppm Mg, 2.0 ppm Cl, 9.0 ppm SO_4, 0.0 ppm CO_3, and 214 ppm HCO_3. The canal water was pH 8.2 and the nutrient solutions varied from pH 7.6 - 8.0.

One gallon of solution was applied to each can daily except during the hot weather of mid-July to mid-August when one and one-half gallons were applied. The cans were buried in the soil to within one inch of the top in order to maintain temperatures comparable to those of the surrounding soil. Three inches of gravel were placed under each can and holes were punched in the bottoms to permit drainage. The moisture was kept at or near field capacity at all times.

On July 29th, 1960, a hot, dry day, (95°F and 15% relative humidity) recently matured blade samples were taken from each plant at 4:30 to 5:30 a.m. and again at 3:30 to 4:30 p.m.--periods of apparent maximum and minimum turgidity. The samples were placed in moisture-tight containers, weighed, dried in a forced draft oven at 60°C and reweighed. Moisture was then calculated as grams of water held per gram of dry material (Curtis and Clark, p. 257). At the time of the afternoon sampling, the wilting occurring in each pot of each treatment was rated on a scale of zero to ten (zero fully turgid and ten completely flaccid).

The temperature of each pot was taken at a depth of 15 centimeters daily.

Results and discussion

During the first month after different treatments were applied, the leaves of the ammonia-treated beets were a darker shade of green

than the other treatments. As the beets matured, however, the leaf color tended to be the same for all treatments except for those of the low-nitrate treatment which were small and had a pronounced yellowish color. Toward the latter part of the growing season, the ammonia-treated plants looked much like the others, except that they appeared slightly less thrifty, had thicker leaves and wilted less than they had formerly.

During the hot, dry days of July and August, the ammonia-treated sugar beets wilted faster and more severely, and recovered less quickly than did the beets from the other treatments even though the temperature of any pot at a depth of 15 centimeters never exceeded 22°F. In the mornings, at daybreak or before, there was no discernible difference in wilting between the ammonia treatment and any of the other treatments (Figure 1).

On July 29, 1960, the morning samples showed no significant difference (5% level) in the amount of water held per gram of dry material (see Figure 2) regardless of treatment. It can also be seen from Figure 2 that the ammonia treatment contained less water per gram of dry material than any of the other treatments. However, the water content of the ammonia treatment in the morning nearly matches that of the other treatments in the afternoon.

As seen in Figure 3, when the amount of water contained in the afternoon was subtracted from that contained in the early morning,
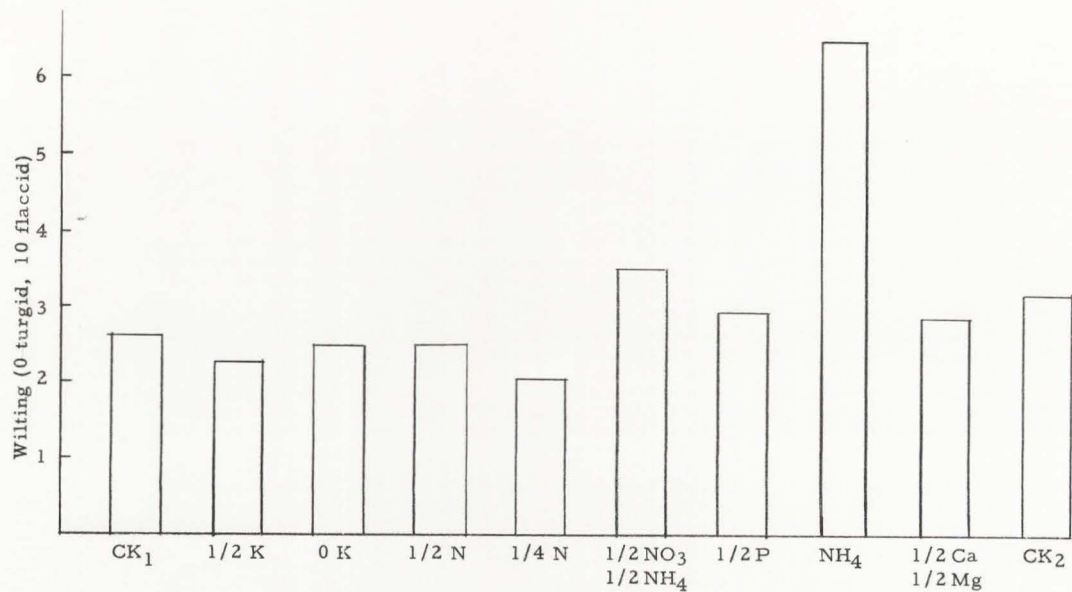
Figure 1.   Relative wilting of sugar beets in nutrient culture,   3:30 P. M. --July 29, 1960.
Each bar represents the mean of ten replications.
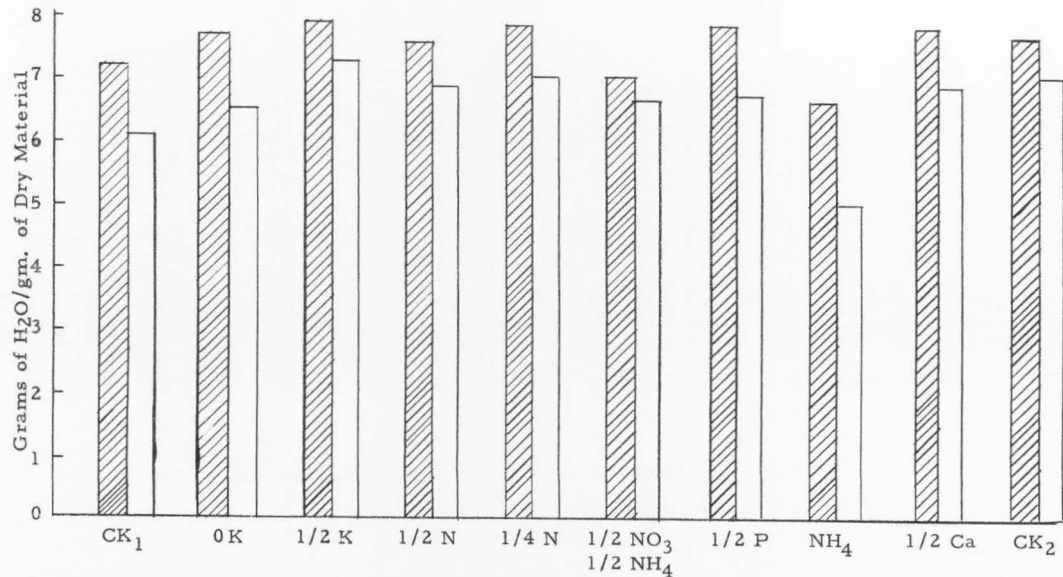
Figure 2. Water content of sugar beet leaves--July 29, 1960.
4:30 A. M. and 3:30 P. M.
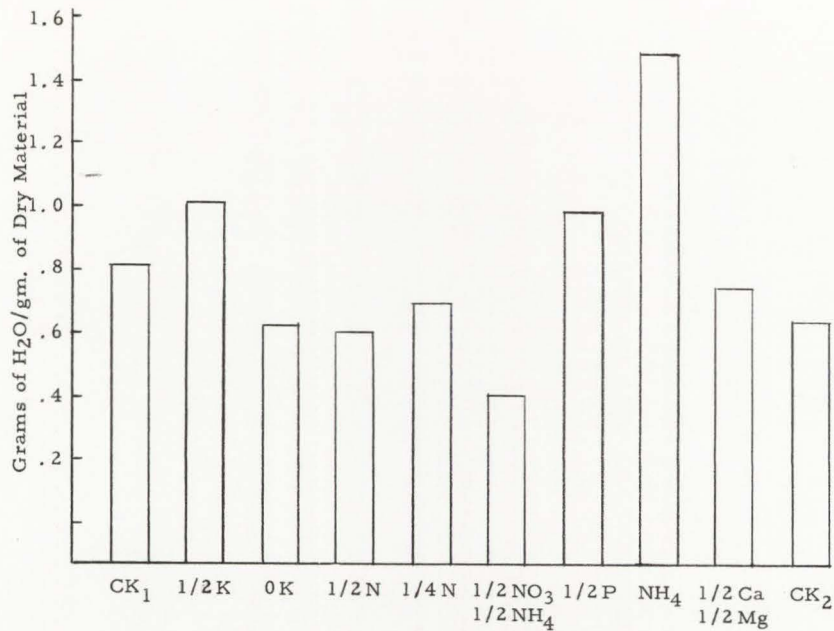
Figure 3. Amount of Water Lost A. M. -- P. M. July 29, 1960. A loss of 1.46 grams of water/gram of dry material or more is significant at the 5% level.

the blades from the ammonia treated plants lost more water than those

of the other treatments even though the ammonia treated plants con-

tained less water in their blades at 5:00 a. m. than did any of the

other plants at that time. While the ammonia treated plants showed

the greatest degree of wilting in the afternoon, by 6:00 p. m. they

had begun to regain their turgidity and shortly after sunset, little,

if any, difference among any of the treatments could be seen.

Conclusions

When ammonia (NH_3) is the sole source of nitrogen, it greatly

increases the degree of wilting by decreasing the amount of water held

by the leaf tissue of sugar beets, particularly on hot summer days.

While this phase of the over-all study did not answer the questions of

whether the cause of the wilting was that ammonia kept the stomates

open or that it reduced the flow of water through the roots, it did show

that ammonia (NH_3) affects the water relationship of sugar beets.

1962 Study

This study was undertaken to ascertain whether or not ammonia

could interfere with the function of stomata. As with the 1960 study,

it was conducted out-of-doors and unlike the 1960 study, also repeated

in the greenhouse.

Materials and methods

Out-of-doors. Monogerm variety S. L. 9140 MS mm sugar

beets were grown out-of-doors in nutrient culture using vermiculite

as the supporting media, as described in the 1960 study. In 1962,

however, only the 1/2 strength Hoaglands solution no. 1, the 1/2

NO_3 + 1/2 NH_4 and the NH_4 solutions were used (see Table 1).

Again the beets were planted in 10 gallon cans, as described

in the 1960 study, but on June 11 they were thinned to two plants per

can. The beets were watered with 1/2 strength Hoaglands no. 1 nutrient

(made with tap water) until June 11, at which time the 1/2 NO_3 + 1/2 NH_4

and the NH_4 treatments were begun. Each treatment consisted of ten

cans, for a total of 30 cans. Again the cans were buried in the soil

to within one inch of the top of the can for control of the root tempera-

tures, and holes were punched in the bottom with two inches of gravel

underneath for drainage. The sugar beets were again watered as in

the 1960 study.

Greenhouse. In the greenhouse study, the same kind of sugar

beet seed was used. The beets were started in vermiculite and watered

with nutrient solution as needed. After three weeks, the beets had

reached a suitable size (four leaf stage) for transferring to four gallon

ceramic pots containing Hoaglands 1/2 strength nutrient solution. The

pots were aerated continuously with aquarium bubblers. On June 11 the

1/2 NO_3 + 1/2 NH_4 and the NH_4 treatments were begun. Each treatment

consisted of ten pots with one beet per pot for a total of 30 pots. All

solutions, those used out-of-doors and in the greenhouse, were made

up with tap water and ranged in pH from 7.8 to 8.3. Since ammonia is

volatile at this pH and can be lost by aeration the solutions in the

ceramic pots were renewed every third day. The greenhouse was equip-

ped with an evaporative type cooler which held the air temperature

below 85°F.

On August 20th in the greenhouse and September 10th out-of-

doors, measurements were made of air temperature, relative humidity,

light intensity, stomatal aperture and the amount of water held per gram

of dry leaf tissue. These measurements were made at arbitrarily selec-

ted times during the day. Air temperature was taken with a regular

mercury thermometer suspended about five centimeters above the sugar

beet canopy. Relative humidity was measured with an Assmann Psych-

rometer placed about five centimeters above the sugar beet canopy.

Relative humidity was measured with an Assmann Psychrometer placed

about five centimeters above the sugar beet canopy. Light intensity

was measured with a Weston Model 130 photometer. Stomatal aperture

was measured by the infiltration method of Alvim and Havis (1954).

This method consists of making up a series of mixtures of xylene and

heavy mineral oil in 10% increments, zero being pure xylene and 10 pure

mineral oil. When a drop of one of these mixtures is placed on the leaf, xylene

enters the closed stomata and the heavy mineral oil enters the open stom-

ata. This method was only approximate, at best, but it gave a quick easy

check of stomatal aperture. The water content of the leaves was

taken by cutting a number of disks from the first mature leaf with a

cork borer. These samples, one centimeter in diameter, were

weighed, dried and weighed again. The results were reported as

grams of water held per gram of dry leaf tissue.

Results and discussion

Out-of-doors. The ammonia-treated sugar beet plants had

leaves that tended to be a slightly darker shade of green than those

receiving nitrates as the nitrogen source. The ammonia-treated

beets appeared to be about the same size as those used in the other

treatments but tended to be less vigorous. At the end of the season,

the roots of the ammonia-treated beets appeared to be as numerous as

the others and also as healthy.

As the plants became larger, the ammonia-treated beets wilted

during the day when the light intensity became high (6,000 - 9,000 foot

candles). The $1/2$ NO_3, $1/2$ NH_4 treated beets and the nitrate treated

beets wilted very little, if at all. As the light intensity decreased

towards evening, the wilting became less noticeable. By nightfall,

no discernible difference in wilting could be observed among any of

the treatments.

As the season progressed, the ammonia-treated beets which

had wilted strongly during the hot summer season began to show less

wilting. The leaves tended to become thickened and somewhat leathery,

while the leaf surface developed a distinct waxy feel. This may have been a result of adaptation to the droughty conditions induced by the ammonia treatment.

As can be seen in Figure 4, the ammonia-treated leaves contained less water at all times than did the other two treatments, but exhibited wilting only near midday. The amount of water held in the leaf tissue of the ammonia-treated beets appeared to correlate with light intensity but not with humidity, air temperature or stomatal aperture. The stomatal apertures increased very quickly as soon as direct sunlight struck them and no difference in aperture was detected between treatments for the rest of the day. However, the stomatal apertures of the leaves in direct sunlight were greater than those of the shaded leaves until about 2:00 p.m. when the apertures became about the same.

Greenhouse. The ammonia-treated sugar beets in the greenhouse also had leaves that tended to be a slightly darker shade of green than those receiving nitrates as the nitrogen source but toward the end of the season the color differences became less pronounced. The ammonia-treated beets were also slightly less thrifty than the others and the root systems appeared to be slightly smaller. However, toward the end of the season three of the ammonia-treated roots became infected with what appeared to be a black fungus while the other treatments remained healthy. This was not observed in any of the beets grown out-of-doors.
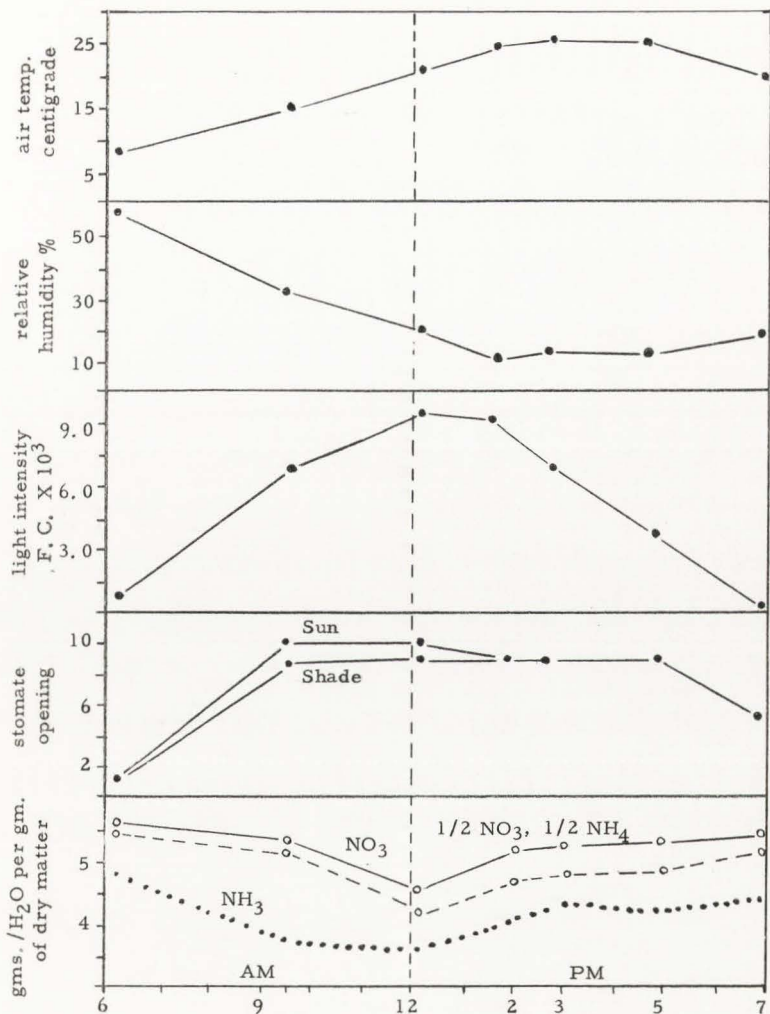
Figure 4. Water held by sugar beet leaf tissue, grown in nutrient solution out-of-doors September 10, 1962, Logan, Utah, as affected by stomate opening, light intensity, relative humidity, air temperature, and nitrogen source.

The ammonia-treated plants in the greenhouse also wilted during the day when the light intensity became great (about 7,000 foot candles). The other treatments, as was observed out-of-doors, also showed little or no wilting. As the light intensity decreased toward evening, the wilted plants became more turgid and by nightfall no difference in wilting among any of the treatments was observed. The ammonia-treated plants wilted strongly when the nutrient solution was fresh but when it became time to renew the nutrient solution they were wilting slightly.

Figure 5 shows that the ammonia treatment held less water than the other treatments near midday when wilting was strongest but at sundown the amount held was about the same as in the other treatments and no wilting was observed. As with the beets grown out-of-doors, the amount of water held in the leaf tissue of the ammonia-treated beets appeared to correlate with light intensity and not humidity, temperature or stomatal aperture. The humidity in the greenhouse was much higher than out-of-doors but this had no effect on the wilting.

Stomatal apertures increased very quickly as soon as direct sunlight struck them and no difference in the stomatal aperture between treatments was detected all day, except in the early afternoon when the light intensity was at its peak. At that time, the ammonia-treated plants were limp and flaccid and the stomatal apertures had decreased slightly due to the dehydrated condition of the leaf. As darkness
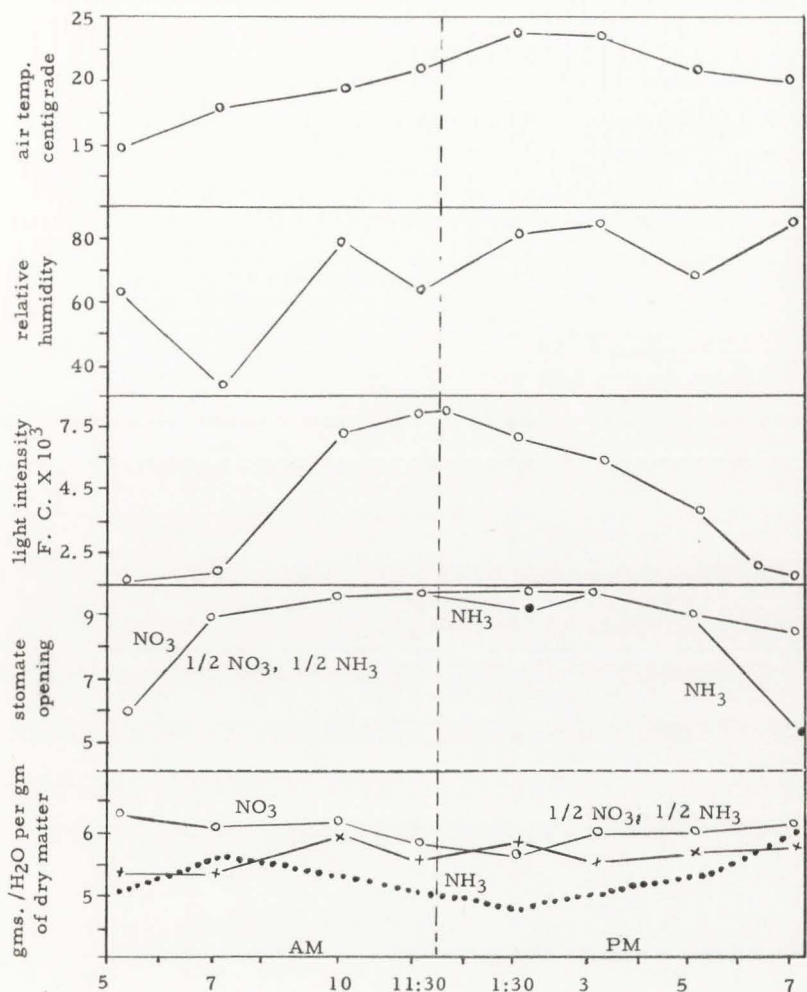
Figure 5.   Water held by sugar beet leaf tissue, grown in nutrient solution in a desert cooler equipped green house August 20, 1962, Logan, Utah, as affected by stomate opening, light intensity, relative humidity, air temperature, and nitrogen source.

approached, the ammonia-treated plants became turgid but the stomatal apertures appeared to decrease more rapidly than in the other treatments. Whether this early closing was directly due to the ammonia or to the severe wilting induced by the ammonia is not known.

Since little, if any, difference was observed in stomatal opening among any of the treatments, either out-of-doors or in the greenhouse, it is doubtful that ammonia had any effect on increasing or decreasing stomatal aperture in the leaves of the ammonia-treated sugar beets. Ammonia (NH_3) formed in the nutrient solution from ammonium sulfate at a pH greater than seven, probably caused the wilting by reducing the adsorption of water by the roots. How this occurs is not known.

However, if the roots were unable to adsorb water in sufficient quantity to keep pace with the transpiration demand of the leaves, wilting would occur. High light intensities cause an increase in transpiration because of the energy adsorbed by the leaf.

Conclusions

1. Ammonia (NH_3) causes sugar beets to wilt under conditions of high light intensity.

2. Ammonia (NH_3) applied to the roots has little, if any, effect on the size of stomatal aperture.

3. Ammonia probably reduces the absorption of water by sugar beet roots.

PART II

AMMONIA AND WATER MOVEMENT IN SUGAR

BEET AND POTATO STORAGE TISSUE AND

EXCISED SUGAR BEET ROOTS

The following series of experiments were designed to show the

effect of ammonia on the rate of water loss by disks of sugar beet and

potato storage tissue and the rate of water uptake by intact sugar beet

root systems.

Literature Review

Anything that interferes with the respiration of a cell or group of

cells also interferes with the rate of water movement in that cell or group

of cells. Reduced temperatures, lack of or reduced oxygen and chemi-

cal respiration inhibitors are all known to reduce the movement of water

into or out of a cell or group of cells. The literature review that follows

will discuss each of these factors in relationship to water uptake.

Temperature

Reduced water adsorption in plants due to low temperatures has

been observed by many investigators (Arndt, 1937; Bialoglowski, 1936;

Brown, 1939; Clements and Martin, 1934; Jensen and Taylor, 1961;

Kramer, 1949 and 1955; and Schroederer, 1939). Kramer (1940a, 1942,

1955) reported that water absorption increased with high root tempera-

tures and decreased with low temperatures. He claimed that this was

due to the increased viscosity of the water and protoplasm in the living

tissue at low temperatures. In dead tissue water adsorption was also

reduced with low temperatures but the reduction was not as great as in

living tissue. Low temperatures also reduced respiration but he claimed

this to be of secondary importance.

Jensen and Taylor (1961) measured the rate of water movement

through plants and found that the apparent activation energy for water

movement through roots was greater than that for the self-diffusion and

viscosity properties of water alone. They reasoned that another mech-

anism, besides the purely physical processes of diffusion and viscosity,

is involved in water uptake. Kuiper (1964) found Q_{10} values as high

as 3.8 for water movement through bean roots. This value is much

higher than that generally attributed to physical processes. When

Kuiper (1964) treated bean roots with dilute solutions of alkenylsuccinic

acids, the rate of water uptake increased and the Q_{10} of the process

was reduced to 1.18, which was in the range of a purely physical process.

He attributed the increased water uptake to the incorporation of alkenyl-

succinic acids into the lipid layer of the cytoplasmic membrane of the

cells, which changed the membrane from a phase characterized by a

high activation energy for water transport to a phase where only the effect of the viscosity of water was observed.

Since alkenylsuccinic acids change the temperature dependence of water transport, it is perhaps doubtful that increased cytoplasmic viscosity is of importance in reducing water transport at low temperatures. It is, perhaps, more plausible that respirational energy is used to maintain the structure of the cytoplasmic membranes.

Oxygen

Oxygen is an essential part of aerobic respiration and reducing or eliminating oxygen reduces respiration. Reduced oxygen tension has been shown to reduce the rate of water movement in plant roots (Kramer, 1940b; Lopushinsky, 1961; Mees and Weatherly, 1957; Roberson, 1964; and Rosene, 1950). Kramer (1940b) found that unaerated solutions reduced the uptake of water by roots. He attributed this effect to changes in the protoplasm of the cells brought about by the accumulation of carbon dioxide in the solution. Mees and Weatherly (1957) observed that tomato roots were less permeable when aeration was stopped that when the roots were being aerated. They bubbled nitrogen through the root media to expel carbon dioxide and other gases and found that the entire effect of reduced root permeability could be attributed to the lack of oxygen instead of an accumulation of carbon dioxide. Lopushinsky (1961) found a 69% reduction of water movement in tomato roots after stopping aeration.

Roberson (1964) also found that water uptake decreased in the

absence of aeration. Within two hours after measurements had begun,

the water uptake rate had decreased approximately 50 to 70 percent.

At this time aeration was begun and the water uptake rate increased

to 30 percent within ten minutes. Rosene (1950) observed that a lack

of oxygen or reduced amounts of oxygen diminished the rates of water

influx or outflux from onion root tissue and that this effect was rever-

sible if the lack of oxygen was not prolonged. However, Brouwer (1954)

found that reducing the supply of oxygen to plant roots had little, if any,

effect on water uptake.

Respiration inhibitors

Many investigators have shown that respiration inhibitors such

as cyanide, azide, 2, 4 dinitrophenol, flouride, iodoacetate, and phenyl-

urethane decrease the rate of water movement into disks of storage tissue,

segments of coleoptiles, segments of roots, and intact roots. Levitt

(1948) found that potassium cyanide, 10^{-4}M and 10^{-3}M, had no effect

upon the water uptake by disks of potato tissue placed in constantly

aerated solutions containing indoleacetic acid. He concluded that the

potassium cyanide may have hydrolized and resulted in the loss of

hydrogen cyanide from the solution. Hackett and Thiman (1950, 1953)

placed disks of potato tissue in shallow unaerated solutions and allowed

the tops of the disks to be exposed to the air. They found that water

uptake by the disks was inhibited by 2, 4 dinitrophenol, sodium azide,

sodium arsnite and flouroacetate.

Ordin and Bonner (1956) measured the permeability of Avena

coleoptile sections to water by the diffusion of duterium oxide and found

that metabolic inhibitors such as 2, 4 dinitrophenol and potassium cyanide

in non-lethal concentrations had only a slight, if any, effect on the rate of

duterium oxide diffusion. However, Ordin and Kramer (1956) using

duterium oxide diffusion rates found that non-lethal concentrations of

2, 4 dinitrophenol reduced water influx into sections of Vicia faba roots

by about 50 percent. Rosene (1947) observed that cyanide and sodium

azide reduced both the oxygen consumption and the water influx and

outflux from onion root tissue. She also found the sodium azide inhibi-

tion to be partly reversible.

Van Overbeek (1942) found that exudation from decapitated tomato

plants stopped when the plants were placed in dilute cyanide solutions.

Van Andel (1953) observed that salt uptake, salt secretion and water con-

ductivity in tomato plants were reduced or prevented by potassium cyanide,

sodium flouride, 2, 4 dinitrophenol, sodium arsenite, iodoacetate, and

phenylurethane. Poskuta (1961) grew corn under conditions of nitrogen

starvation and insufficient oxygen supply and found that potassium cyanide

at 10^{-3} and 10^{-4}M and 2, 4 dinitrophenol at 10^{-4}M and 10^{-5}M stopped

guttation.

Brouwer (1954) found that a 1 X 10^{-5}M solution of 2, 4 dinitro-

phenol and 1 X 10^{-4} and 1 X 10^{-5}M potassium cyanide did not inhibit

water uptake in Vicia faba roots. However, he did find that 1 X 10^{-3}M

potassium cyanide reduced water uptake 90 percent over a period of time and that 5×10^{-3}M potassium cyanide stopped water uptake immediately. Mees and Weatherly (1957) reduced the flow of water through tomato roots 90% by the use of potassium cyanide. They suggested that metabolic energy was used to maintain the structural features of the water pathways in plant roots. Lopushinsky (1961, 1964) reduced water movement through roots of tomatoes 90% with 1×10^{-3}M sodium azide.

Roberson (1964) found that after a three hour treatment with 2×10^{-4}M sodium azide, water movement through tomato roots was only 50 percent of the control rate. Increasing the concentration of azide to 1×10^{-3}M resulted in another 25 percent dedrease in water movement. When the azide solution was replaced with azide-free solution, water uptake increased until the initial rate was nearly reached. In unaerated solutions the effect of azide was even more pronounced. He also found that azide had no effect on water movement through dead roots. When he used 2, 4 dinitrophenol water movement was quickly reduced in living tomato roots but when used with an 1×10^{-2}M acetate buffer, it caused water movement to increase. He concluded that the increase was due to severe injury of the tissues by the buffer plus the 2, 4 dinitrophenol that ultimately resulted in death. Neither 2, 4 dinitrophenol or the acetate alone produced an inçrease in the rate of water movement. Adenosine triphosphate (ATP) in the presence of acetate buffer increased the rate of water movement, but without the

acetate buffer, ATP showed only a slight effect or perhaps caused a slight reduction in the rate of water movement.

Undissociated ammonia also acts as a respiration inhibitor. Altschul, et. al. (1946) treated cotton seeds with ammonia and inhibited respiration in both mature and immature seeds. Vines and Wedding (1959, 1960) studied the effect of ammonia on respiration. Oxygen uptake in a Warburg respirometer was used as an indication of the effect of gaseous ammonia and ammonium salts on respiration of excised barley roots and garden beet root disks, as well as leaf disks of spinach and sugar beets and garden beet root mitochrondria.

Vines and Wedding (1959, 1960) found that both gaseous ammonia and undissociated ammonia in equal concentrations inhibited respiration to the same degree in each of the tissues studied. They therefore concluded that undissociated ammonia caused the inhibition of respiration in plants. They found that pH was important because pH controlled the amount of undissociated ammonia present. They also reported that only succinate was partially able to overcome the ammonia-induced inhibition of respiration. This indicated a possible association with the electron transport system since succinate does not require diphosphopyridine nucleotide (DPN) as a cofactor. Further studies showed that the oxidation of reduced diphosphopyridine nucleotide (DPNH) by isolated beet root mitocondria was inhibited by ammonia treatment. It was suggested that the site of ammonia toxicity to plants was located in the electron transport system, specifically on the DPNH ⟶ DPN reaction.

Factors such as low temperature, reduced oxygen, chemical

respiration inhibitors (azide, cyanide, arsenite, etc.) have been found

to inhibit respiration and to reduce or inhibit water absorption of plant

roots and other plant tissue, as well. Ammonia (NH_3) has been found

to inhibit respiration; therefore, it is reasonable to expect that ammonia

(NH_3) will also reduce or inhibit water absorption in plant tissues. The

experiments which follow were designed to test this hypothesis.

Experiments with Disks of Sugar

Beet and Potato Storage Tissue

Materials and methods

A series of 0.1 molar potassium phosphate buffers pH 6.0, 7.0,

7.5, 8.0, 8.5, 9.0 and a series of Tris [tris (hydroxymethyl) amino

methane] buffers pH 7.0, 7.5, 8.0, 8.5 were prepared. Sulfuric acid

was used to adjust the pH of the tris buffers. A series containing 0,

2, 4, 6, 8, 10 X 10^{-3} molar ammonium sulfate was prepared for each

pH increment. Phosphate buffers were used for both potato and sugar

beet tissues and Tris buffers were used only for sugar beet tissues.

Uniform disks (14 mm in diameter and one mm thick) were cut

from sugar beet and potato storage tissues with the aid of a cork borer

and a hand-operated microtome. The disks were washed in tap water

until all of the starch and cell debris were removed from the cut

surfaces. Four disks of tissue were placed in 50 ml of buffer and

ammonium sulfate solution and allowed to stand for 12 hours. The

disks were then blotted on Whitman no. 1 filter paper (50 grams pressure for five seconds) and weighed to the nearest milligram. The potato disks were placed in a 13 atmosphere solution (90 gms/liter) of mannitol and the sugar beet disks were placed in a 29 atmosphere solution (180 gms/liter) of mannitol. The disks were gently shaken in a wrist action shaker for twenty minutes, removed from the mannitol, blotted and weighed. The loss in weight was recorded as percent initial weight. Initial weight was taken as the weight of the disks prior to placement in the mannitol solutions.

All operations were performed at room temperature.

Results and discussion

Disk thickness. An appropriate thickness of the disks was determined by cutting potato tissue 0.5, 1.0, and 1.5 millimeters thick and placing the disks in water rather than in buffer; otherwise, the procedure followed was as previously outlined, except that the disks were removed from the mannitol and weighed at ten minute intervals. The results are shown in Figure 6. Similar results were obtained for sugar beet disks. Water was removed most rapidly and in greatest quantities from disks 0.5 millimeter in thickness; however, they were very difficult to handle. Disks one millimeter in thickness were used for both the potato and sugar beet experiments because they were much easier to handle. An immersion time of 20 minutes in the mannitol solution was selected because the rate of water lost from the disks diminished at this point.
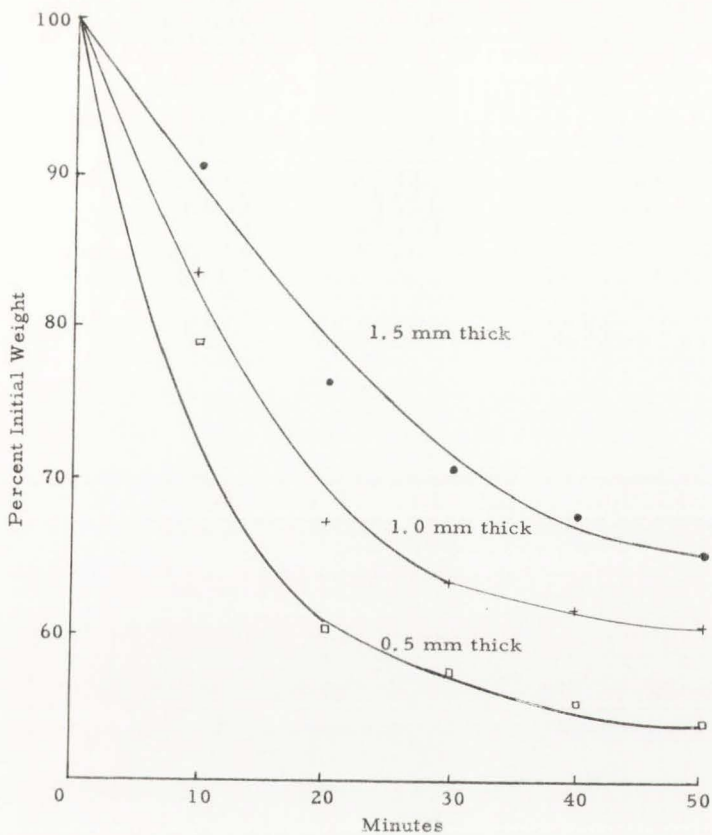
Figure 6.   Loss of water from potato disks of 0.5, 1.0, and 1.5 mm. thick, equilibrated with water, and then placed in a (90 gm./liter) solution of mannitol.

The thicker disks released water at a much slower rate; therefore, they were not used.

Potato. After the potato disks had remained in the buffer and ammonium sulfate solution for 12 hours they appeared to be normal in every respect. At pH 9.0 and at all ammonium sulfate concentrations they remained turgid and were not discolored. In one replication at pH 9.0 and 10 X 10^{-3} molar ammonium sulfate the disks developed small brown spots but the tissue remained turgid. The brown spots did not occur in any of the other replications.

Figure 7 shows that increasing pH had little, if any, effect upon water loss from potato disks, but that increasing both the pH and ammonium sulfate concentrations retarded the loss of water from the disks. The slight variations in the response surface may be due to experimental error.

An experiment was performed using potassium sulfate instead of ammonium sulfate but potassium sulfate had no effect on reducing water loss from the potato disks.

Sugar beet. After the sugar beet disks had remained in potassium phosphate and ammonium sulfate solutions for 12 hours they appeared to be normal. However, when the ammonium sulfate concentrations were increased to greater than 2 X 10^{-3} molar at pH 8.5 and 9.0 the tissue became flaccid and appeared to be dead. This was to be expected because at pH 8.5 and 9.0 the percentage of NH_3 present in the
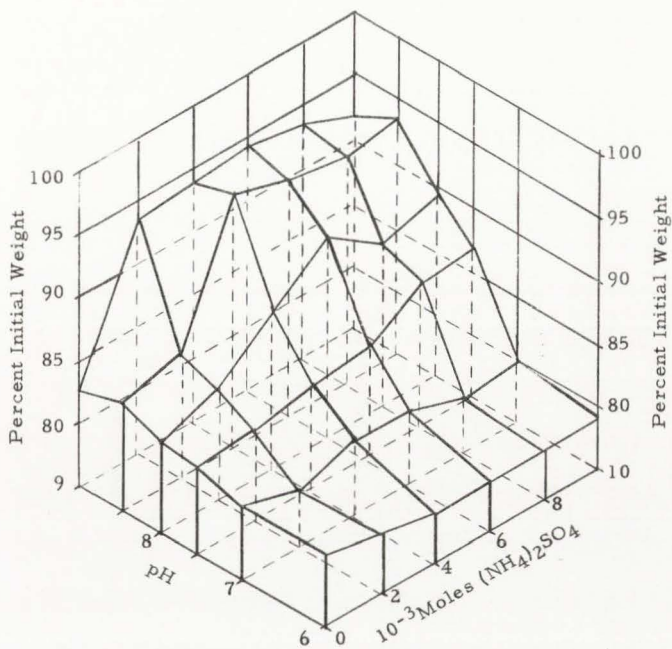
Figure 7.   Loss of water from potato tissue disks 1. 0 mm. thick,
            equilibrated in 0. 1 M potassium phosphate buffer, with
            added ammonium sulfate and then placed in a (90 gm. /
            liter) solution of mannitol. Represents an average of
            five replications.

solutions is quite high. When the flaccid disks were placed in the

mannitol solution, they actually gained weight rather than losing it as

they had a lower pH or concentration of ammonium sulfate.

Figure 8 shows that ammonium sulfate in phosphate buffer had

little or no effect at pH 6. 0 and 7. 0. However, at pH 7. 5 concentrations

greater than 6 X 10^{-3} molar ammonium sulfate retarded the loss of

water from the disks. It was apparent that pH in itself had little, if

any, effect on the loss of water when ammonium sulfate was absent from

the buffer. However, a slightly smaller amount of water was lost at

pH 8. 0 and 7. 0 than at the other pH values. This was also observed

for the potato disks but was not observed when tris buffer was used.

Again, small variations occured in the response surface and may be due

to experimental error. Figure 9 shows that sugar beet disks in Tris

buffer and ammonium sulfate reacted much the same as potato and sugar

beet disks in phsophate buffer and ammonium sulfate.

Extended immersion in mannitol solutions. Sugar beet and

potato disks treated with 0. 1 M pH 8. 0 phosphate buffer and 0, 4, and

8 X 10^{-3} molar ammonium sulfate solutions for 12 hours and then placed

in mannitol solutions for extended periods of time all lost about the same

percentage of water (see Figures 10 and 11). The procedure used was

the same as described previously, except that the potato disks were

removed from the mannitol solutions, blotted and weighed at 10 minute

intervals up to 60 minutes and at 20 minute intervals thereafter. Sugar

beet disks were treated similarly except that they were removed from

Figure 8.   Loss of water from sugar beet tissue disks 1. 0 mm. thick, equilibrated in 0. 1 M potassium phosphate buffer, with added ammonium sulfate, and then placed in a (180 gm. /liter) solution of mannitol. Represents an average of five replications.
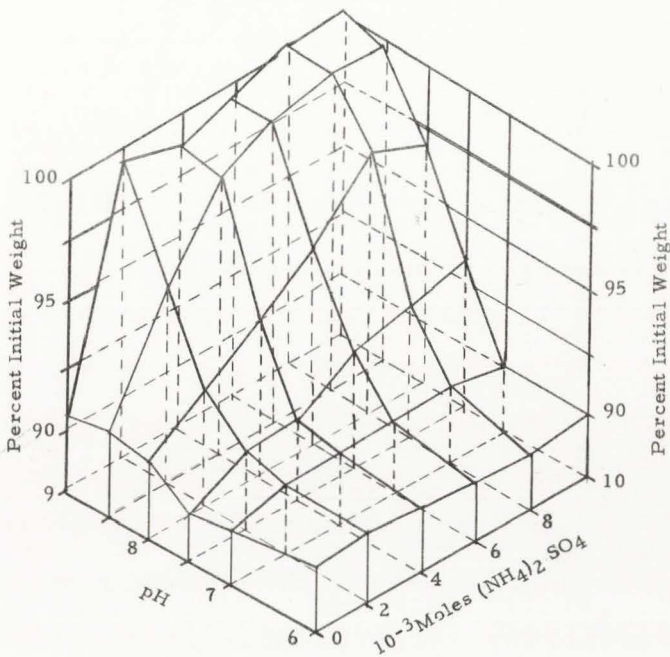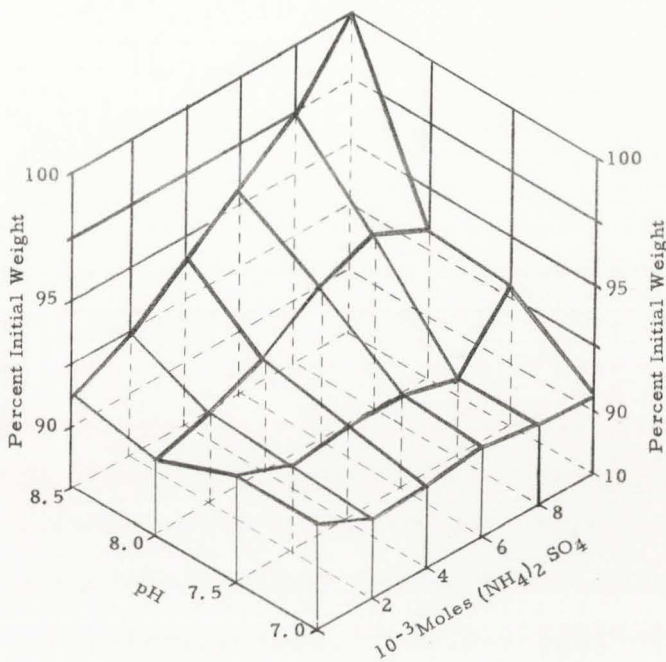
Figure 9. Loss of water from sugar beet tissue disks 1.0 mm.
thick, equilibrated in 0.1 M Tris buffer, with added
ammonium sulfate, and then placed in a (180 gm. /
liter) solution of mannitol. Represents an average
of four replications.

the mannitol solutions, blotted and weighed at 15 minute intervals.

Sugar beet disks treated with 0. 1 M pH 8. 0 tris buffer and 0, 4, and

8×10^{-3} molar ammonium sulfate (Figure 12) did not lose the same

percentage of water for the period of time they were observed. It was

likely they would have done so had they been observed for a longer

period of time.

Collander (1959) points out that there may be serious difficulties

in using plasmolysis and/or deplasmolysis methods. He claims that

the rate at which water is lost may not be due to membrane permeability

but rather to the rapidity with which the plasmolyizing substance diffuses

to the outer surface of the protoplast or from the suface to the bulk of

the bathing fluid. This error may be serious where tissue sections

rather than isolated cells are used. Thiman et al. (1960), working

with potato disks, found that mannitol containing C^{14} first enters the

apparent free space, entering and leaving very rapidly. Upon exposure

to mannitol for several days a small amount of mannitol was found to

actually enter the cell. They found that their results confirmed those

obtained by osmotic methods in showing that very little externally

applied mannitol enters the potato disks. It appears that Collander's

objections to plasmolysis and deplasmolysis methods may not be too

serious when mannitol is used as the plasmolyizing agent because it

diffuses readily and does not readily pass into the inside of the cell

(Thiman et al. 1960).
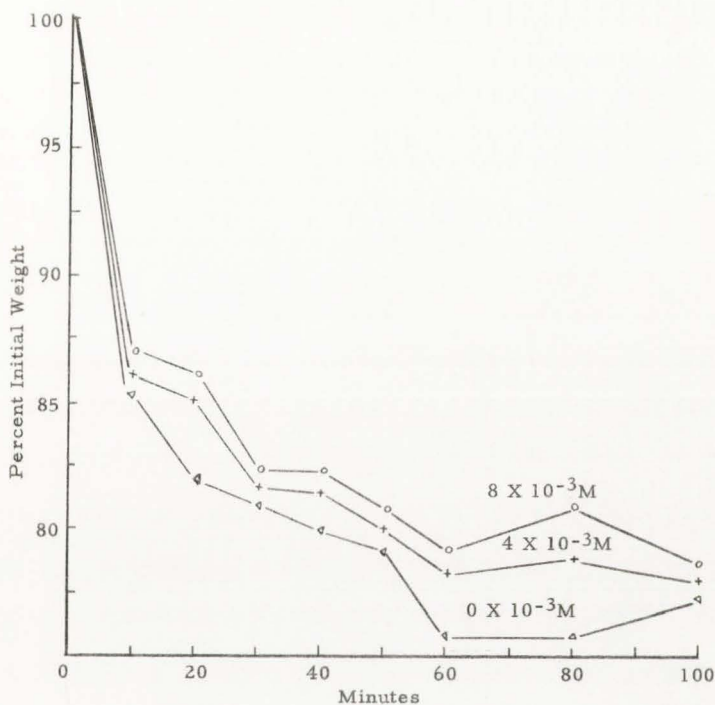
Figure 10.   Loss of water from potato tissue disks 1.0 mm. thick,
0.1 M phosphate buffer pH8 with three concentrations
of added ammonium sulfate with time.   Disks placed
in 90 gm./liter solution of mannitol.
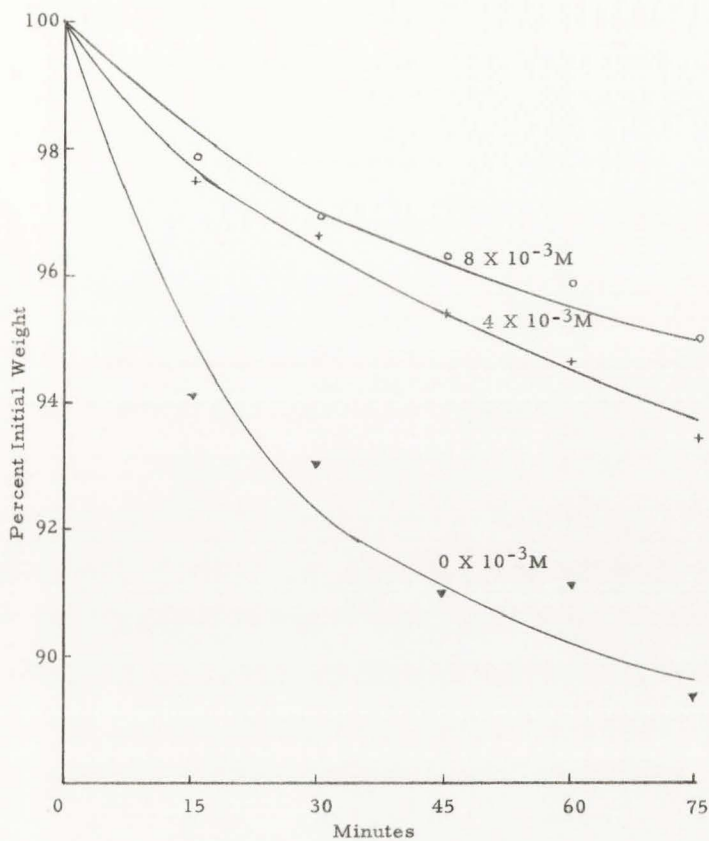
Figure 11.   Loss of water from sugar beet storage tissue disks 1.0
mm. thick, 0.1 M phosphate buffer pH 8 with three con-
centrations of added ammonium sulfate with time.   Disks
placed in 180 gm. /liter solution of mannitol.
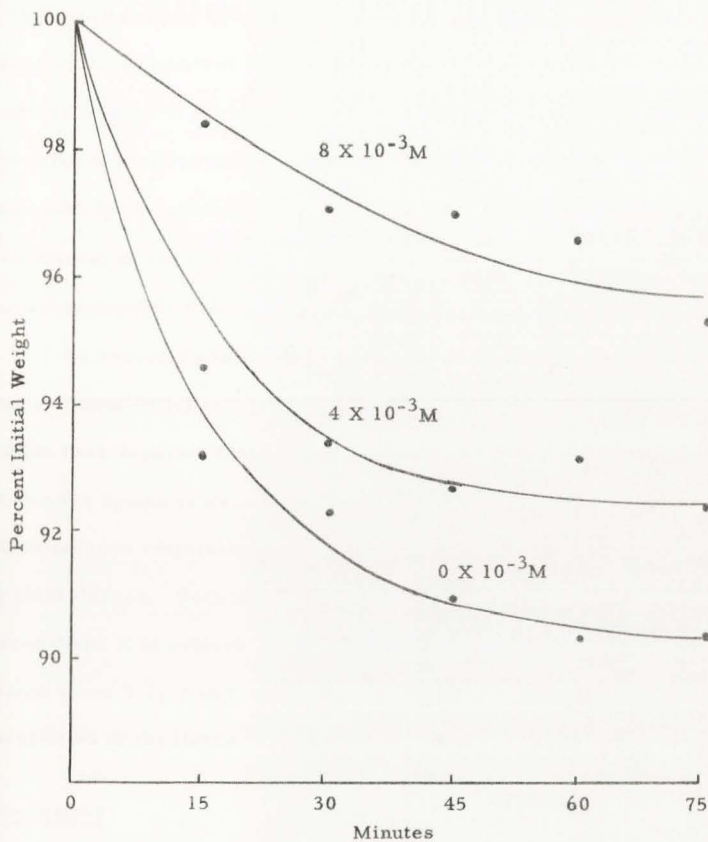
Figure 12.   Loss of water from sugar beet storage tissue disks
1. 0 mm. thick, 0. 1 M Tris buffer with three concentrations
of added ammonium sulfate with time.   Disks placed in
180 gm. /liter solution of mannitol.

Warren (1962) points out that the proportion of the total ammonia (NH_3) concentration which is ionized at any given pH is governed by the dissociation constant of the molecule. With the appropriate pH value and a knowledge of the total ammonia concentration and solution pH, the relative proportions of ionized and un-ionized ammonia can be calculated simply by applying the law of mass action. At a pKa of 9.0 the percentages of un-ionized ammonia at pH 6, 7, 8, and 9, respectively, are approximately 0.1, 1, 10 and 50 percent.

As reported previously, Vines and Wedding (1960) demonstrated that ammonia (NH_3) reduces the respiration of various plant tissues. Others have reported that other agents which reduce respiration also reduce the uptake of water by various plant tissues. Apparently, anything which reduces respiration also reduces the uptake or release of water by plant tissues. Because pH had no effect on water loss from the tissue disks it is evident that ammonia (NH_3), formed when the pH was raised above 7.0, reduced the rate of water loss by decreasing the respiration of the tissue.

Conclusions

1. pH alone has little, if any, effect on the release of water from disks of sugar beet and potato storage tissue.

2. Ammonia (NH_3) reduces the rate of water loss from disks of sugar beet and potato by possibly decreasing the respiration of the tissue.

Experiments with excised sugar beet roots, materials and methods

Culture of plants. Monogerm variety S. L. 9140 MS mm sugar

beet seeds were germinated in moist vermiculite and the seedling were

watered with 1/2 strength Hoagland's no. 1 nutrient solution (Hoagland

and Arnon, 1950) as needed. When the seedlings had developed three

or four leaves they were transferred to one gallon polyethylene contain-

ers filled with 1/2 strength Hoagland's no. 1 nutrient solution. One

plant was placed in each container. Distilled, deionized water was used

in the preparation of all solutions. The solutions were aerated contin-

uously and were renewed weekly.

The plants were grown in a greenhouse equipped with an evapora-

tive cooler that held the maximum temperature to 85°F. The temperature

usually varied from 60°F at night to 80°F during the day. During the

winter months, artificial light was used to increase the length of the

photoperiod and to increase the rate of growth. No bolting was observed

in any of the plants. Because plants of the same age were often different

in size, the plants were not used for the experiments until the roots were

3/8 to 1/2 inch in diameter.

Apparatus for measuring the rate of water movement through

sugar beet root systems. A diagram of the apparatus used can be

seen in Figure 13. A suction pump was used to supply the suction for

movement of water through the sugar beet root systems. The amount

of water moved in response to the suction applied was measured by

attaching the tube from the suction pump to a one millimeter bore
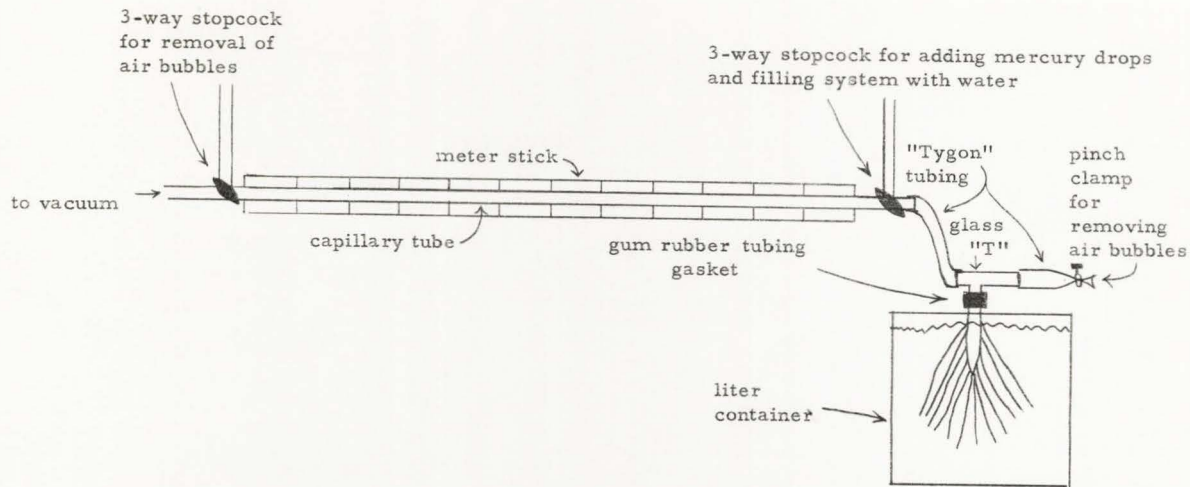
Figure 13. Apparatus for measuring the rate of water movement through excised sugar beet root systems.

capillary tube. The suction was regulated at 12" Hg by a mercury

Cartesian Diver Manostat. The capillary tubing was selected from

standard glass stock, was examined for irregularities in the diameter

of the bore and found to be accurate to within 5%. Each capillary tube

was attached to the surface of a one meter rule. A three-way stopcock

attached to either end of the capillary tube facilitated filling the system

with water and the introduction of a droplet of mercury. By following

the movement of the mercury droplet along the capillary tube after

suction was applied, the rate of water movement could be detected. By

disconnecting the tube to the vacumn pump from the capillary tube, the

rate of water movement without vacumn could also be measured. All

connections were made with 1/4 inch "Tygon" tubing (Plastics and

Synthetic Division, The U. S. Stoneware Co., Akron, Ohio). The appa-

ratus was set up in quadruplicate so that four root systems could be studied

at the same time.

The free end of the capillary tubing was connected directly to

the stump of a detopped sugar beet by use of a length of "Tygon" tubing.

To insure a water-and-air-tight seal, a short section of 1/2 or 3/8 inch

(depending upon the size of the root) gum rubber tubing was slipped

over the top of the root and then connected to the "Tygon" tubing through

a 1/4 inch diameter glass tee. A short section of tubing was placed on

the free end of the tee and clamped off. Air bubbles that collected in

the line could then be removed through the free end of the tube by

releasing the clamp. A faulty seal could be detected by holding the

connection above the solution surface and watching for the appearance

of air bubbles in the water-filled line above the connection after the

application of suction. If a faulty seal was detected, a short piece of

soft wire was placed around the gum rubber covering the stump and

tightened by twisting until the seal was effected.

Experimental procedure. An experiment was initiated by re-

moving a plant from the growth media and placing the roots into a one

liter beaker filled with aerated water (unless otherwise stated). The

plant was detopped just below the last leaf scar, the root pushed into the

gum rubber tubing and sealed. Vacuum (30 cm. Hg) was applied and the

line cleared of air bubbles; a mercury droplet was then introduced into

the capillary tube and the rate movement of the mercury droplet

recorded.

Readings were taken every 20 minutes until the flow tended to

even out or to diminish slightly. At the end of this time the roots were

lifted out of the water and various solutions of ammonium salts or

ammonia gas were added to the solution. The roots were then replaced

in the solution and the readings were continued as before.

By using this open beaker arrangement, solutions could be

changed or the salts or gases could be added directly to the control sol-

ution during the course of an experiment. Aeration of all solutions was

accomplished by bubbling air directly into the beaker. Approximately

1/4 to 1/2 hour was required to prepare a root system and start the

first measurement. This period is not included in the calculation of

time sequences in the data. Temperature measurements were made

periodically throughout the course of the experiments and were found

to vary less than one degree centigrade during the course of any one

experiment. Over a period of months, the temperatures of the solutions

varied from 25° to 21°C. The pH of the solutions was monitored by a

Leeds and Northrup model 7401 pH meter equipped with a glass electrode

and calomel cell.

Results and discussion

Root damage. Some difficulty was encountered because of dam-

aged roots. The roots had to be handled very carefully to avoid breaking

small roots and rootlets. In growing sugar beets in the greenhouse, the

levels of the nutrient solutions would sometimes fall below the top roots

and would expose them to the air. This happened on days when solar

radiation was intense and transpiration demand was high. The tips of

the young newly developing roots exposed to the air would dry out and

become brown or burned.

Adding ammonia at pH 9 or greater also damaged the roots,

causing them to turn a dark grey color. Not all of the root systems

were damaged by adding ammonia at pH 9; susceptibility to damage by

ammonia varied from root system to root system at this pH. However,

at a pH greater than 9 all roots were damaged. Raising the pH alone,

without adding ammonia, appeared to do little, if any, damage to the

roots.

When roots were damaged, whether from breakage, drying out or addition of ammonia, the rate of water movement appeared to increase in comparison to that of the undamaged roots. When ammonia was added to a damaged root system, there was little, if any, reduction in the rate of water movement.

Water movement in response to added ammonium carbonate. Except as noted, all of the following experiments were conducted in distilled water. The solutions used were aerated constantly and suction was applied in the manner indicated and maintained at a value of 30 centimeters of mercury.

The initial rate of water movement for these experiments was taken as the rate of movement for the first 20 minutes after an experiment had been initiated. All subsequent movement was reported as a percentage of the initial rate.

Figure 14 shows the effect of 1×10^{-3}, 5×10^{-4}, and 2.5×10^{-4} molar ammonium carbonate on the rate of water movement through the roots of sugar beets compared with the rate of water only. The results recorded here are for single root systems and the experiments were not replicated. Measurements were made every 20 minutes; however, after the ammonium carbonate was added they were made every five minutes. After 20 minutes a reading was taken at 10 minutes and one at 20 minutes.

The results show that ammonium carbonate, in each case, raised the solution to pH 8.3 - 8.4 and the movement of water was reduced to
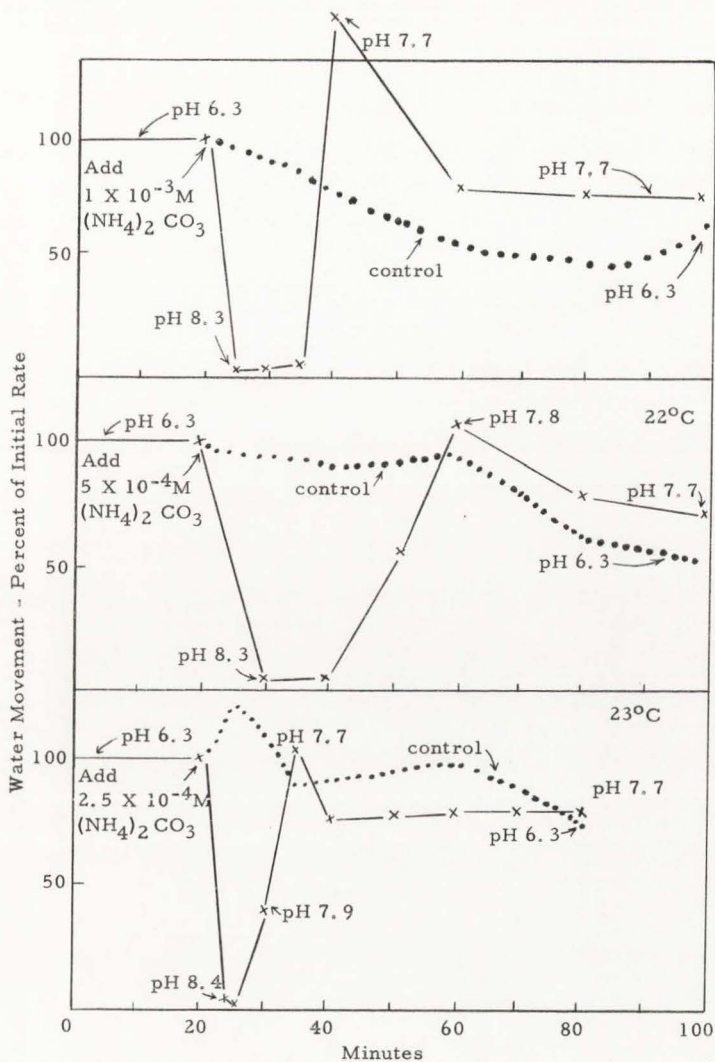
Figure 14. The effect of ammonium carbonate on the rate of water movement through sugar beet roots at 30 cm. Hg suction. Continuously aerated. Each figure represents one root.

five percent or less of the initial rate. At this pH, ammonia was removed from the solution by the air bubbling through it. In five to ten minutes the pH dropped to a value of 7.7 - 7.8 and water uptake resumed. The length of time it took for water uptake to resume depended upon the amount of air used to aerate the solutions. It was not possible to attain equal aeration for each root system. Upon recovery, the rate of water movement reached a peak and then declined. This is contrasted to that of water alone which showed a general decline in rate with time. Using tomato root systems, Roberson (1964) and Loposhinsky (1961) observed a similar phenomenon.

Figure 15 shows the effect of ammonium carbonate on water movement through sugar beet roots with no suction applied and the roots aerated constantly. The initial rates under these conditions were three to four times lower than those when suction was applied. However, the pattern of water movement was similar to that of the experiment where suction was applied. First there was a rapid inhibition of water movement when the ammonium carbonate was added, then as aeration removed the ammonia and the pH dropped to approximately 7.7, the movement of water resumed at a rate approximately equal to the initial rate. Again, the length of time required for the reversal of inhibition was related to the amount of aeration each root received.

Since ammonium carbonate inhibited water movement in roots placed in water, it was necessary to know if the same phenomenon would take place in nutrient solution.
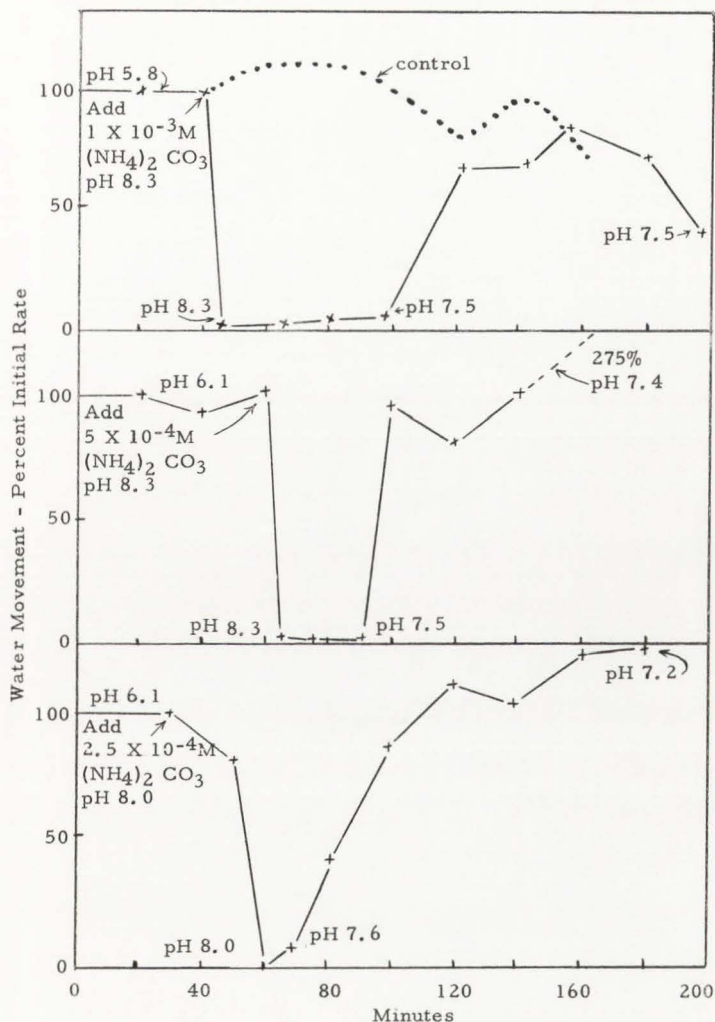
Figure 15.   The effect of ammonium carbonate on the rate of water movement through sugar beet roots at 0.0 cm. Hg suction. Continuously aerated. Each figure represents one root.

Figure 16 shows the effect of ammonium carbonate on the rate of water movement through sugar beet roots in a solution of 1/2 strength Hoagland's no. 1 nutrient solution. In this experiment, water movement through the roots was measured for sixty minutes before the ammonium carbonate was added. The initial rate for this experiment was taken as the rate of water movement in the last 20 minutes before the addition of ammonium carbonate. Just before ammonium carbonate was added, aeration was removed and was not replaced for 20 minutes. This prevented the removal of ammonia by aeration. Readings were taken every five minutes during this 20 minute period.

The pattern of water movement was much the same as in the other experiments. Upon the addition of ammonium carbonate, water movement practically ceased until aeration was resumed and enough ammonia was removed to drop the pH to approximately 7.7. At that time, water movement began again and quickly resumed at a rate near the initial rate.

Each graph represents the mean of two replications.

Raising the pH of the solution so abruptly could have caused a shock to the root system which inhibited water movement.

Effect with added potassium carbonate instead of ammonium carbonate. Figure 17 shows the effect of 2.5 X 10^{-3} molar potassium carbonate on the rate of water movement through sugar beet roots. The procedure was the same as that used in the last experiment. The pattern of water movement was quite unlike that found when ammonium
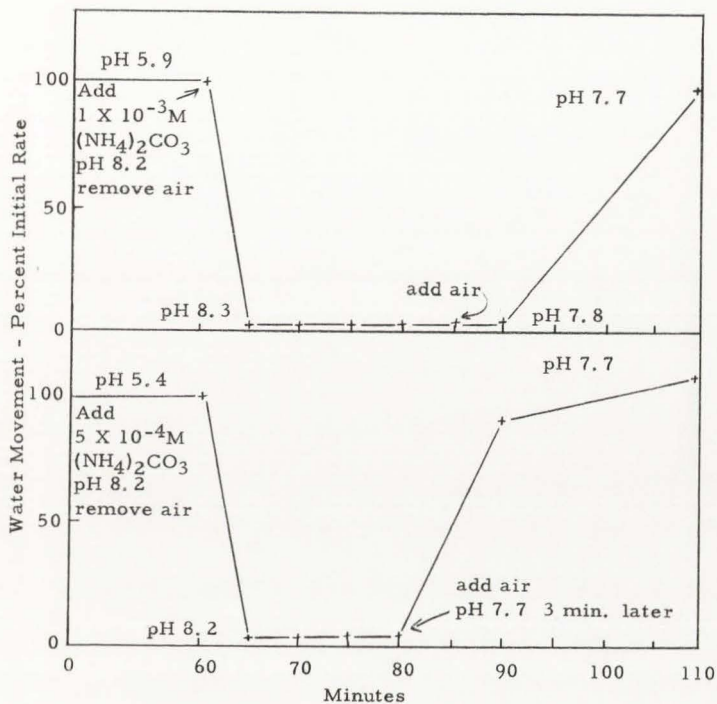
Figure 16. The effect of ammonium carbonate on the rate of water movement through sugar beet roots in 1/2 strength Hoaglands No. 1 nutrient solution at 30 cm. Hg suction. Average of 2 replications.
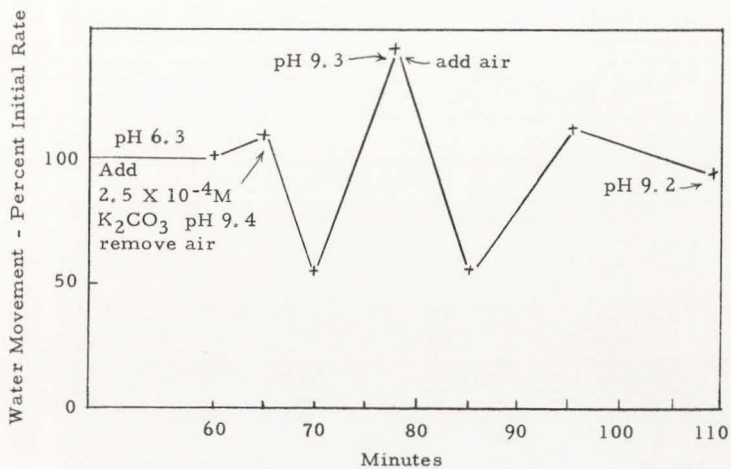
Figure 17. The effect of 2.5 X 10⁻⁴ K₂CO₃ on the rate of water movement through sugar beet roots at 30 cm. Hg. suction. Average of four replications.

carbonate was used. Upon addition of the potassium carbonate, the pH

of the solution increased to 9.4 and remained above 9 for the duration

of the experiment. Instead of inhibiting the movement of water through

the roots as did the ammonium carbonate, the rate of water movement

fluctuated; however, the rate did not fall below 50 percent of the initial

rate. Restoration of aeration had little or no effect on the movement of

water.

It was observed that the rate of water movement in sugar beet

roots under suction fluctuated when measured for short periods of time

(5 minutes); however, when the measurements were made over longer

periods of time (20 minutes) there was much less fluctuation. This

accounts for the fluctuations in water movement immediately after the

potassium carbonate was added and when the readings were taken at

five minute intervals. Roberson (1964), using suction to pull water

through tomato roots, also observed the same fluctuations in the rate of

water movement during short periods of time. When no suction was

used, the movement of water was smooth and relatively steady.

Apparently raising the pH without ammonia present did not

inhibit water movement through sugar beet roots.

Water movement in response to added ammonia gas. The same

technique was used as in the previous experiments except that ammonia

(NH_3) rather than ammonium carbonate, was introduced into the solu-

tions containing the roots. Ammonia gas was introduced by placing a

solution of about 1/2 molar ammonium hydroxide into a flask and passing

air over the top of the ammonium hydroxide. The regular aeration stream was withdrawn from the solution containing the roots and the air containing the ammonia was then introduced into the solution until the pH became 8.4 - 8.5. The air containing the ammonia was then withdrawn from the solution and the regular aeration stream was again introduced.

For these experiments, the initial rate was taken as the first 20 minute period when measurements were begun. Except as noted, suction was applied at 30 centimeters of mercury.

The result of this experiment is shown in Figure 18. In each of the four replications of this experiment, as soon as ammonia was introduced and the pH of the solution reached 8.4 - 8.5 water movement stopped until aeration had removed enough ammonia from the system to drop the pH to 7.7 - 7.8, at which point water movement resumed. The length of time the water movement was inhibited varied with the amount of air in the aeration stream. In three of the replications, a peak was noted after the recovery of water movement but in the last replication the rate increased rather than decreased at the end of the experiment. Whether this variation is due to individual root systems or to some other factor is not known.

When 0.0 centimeters Hg suction was applied, the water movement pattern looked very much like that in the experiments using ammonium carbonate and 0.0 centimeters Hg suction, but only at the beginning of the experiment (see Figure 18). When water movement

Figure 18. The effect of ammonia on the rate of water movement through sugar beet roots at 30 cm. Hg suction. Continuously aerated. Ammonia (NH_3) added until pH 8.3 - 8.5 was attained.

had resumed after the addition of ammonia, the recovery was only about

25 percent of the initial rate for two of the replications. In the other

replication, the water movement pattern was similar to that in the

ammonium carbonate experiments. The differences may have been due

to individual variations or the ammonia gas may have caused some slight

damage.

Water movement in response to buffers and added ammonium

sulfate. In the following experiments the roots were immersed in

either 1×10^{-2} molar potassium phosphate buffer or 5×10^{-2} molar

tris [tris (hydroxymethyl) amino methane] buffer, instead of water.

Otherwise, the experiments were conducted in the same manner as the

others. Ammonium sulfate instead of ammonium carbonate was used

as a source of ammonia. The initial rate was taken as the last 20

minutes before the addition of the ammonium sulfate. Suction was

applied at 30 centimeters of mercury.

The results of the experiments with 1×10^{-2} molar phosphate

buffer and ammonium sulfate are summarized in Figure 20. at pH 6

and pH 7 the water movement pattern is similar to the experiments in

which potassium carbonate was added instead of ammonium carbonate.

It is apparent that the added ammonium sulfate has little, if any, effect

on the rate of water movement at pH 6 and pH 7. However, when the

pH was raised to pH 8.0 the added ammonium sulfate acted in the same

manner as ammonium carbonate or ammonium gas, quickly reducing

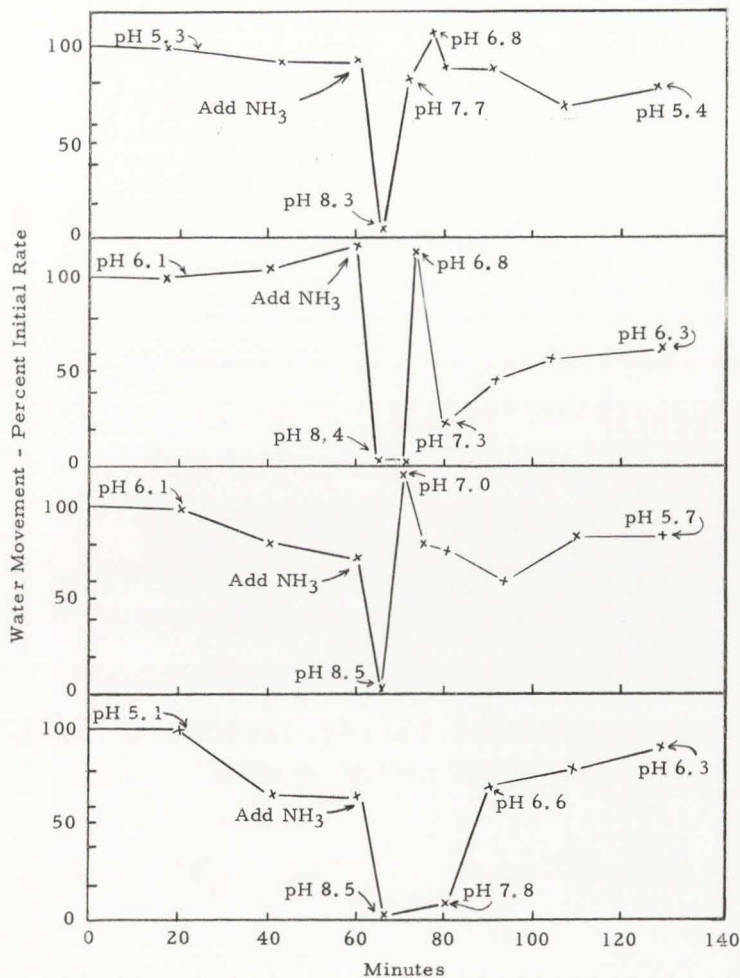the movement of water to five percent of the initial rate or less.
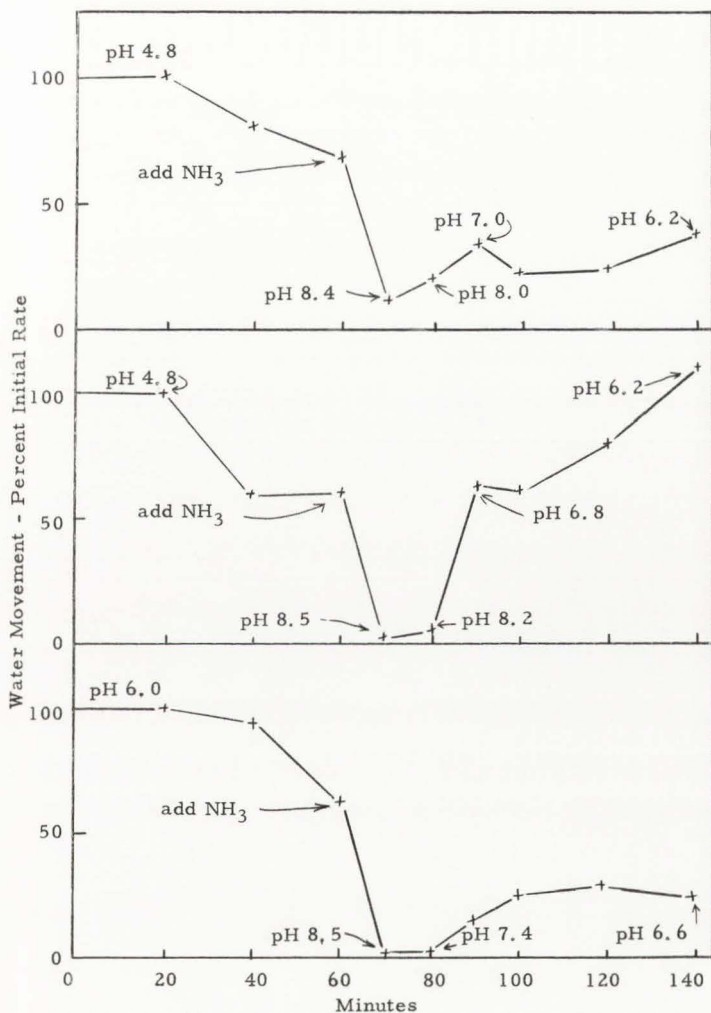
Figure 19. The effect of ammonia on the rate of water movement through sugar beet roots at 0.0 cm. Hg suction. Continuously aerated. Ammonia (NH_3) added until pH 8.3 - 8.5 was attained.
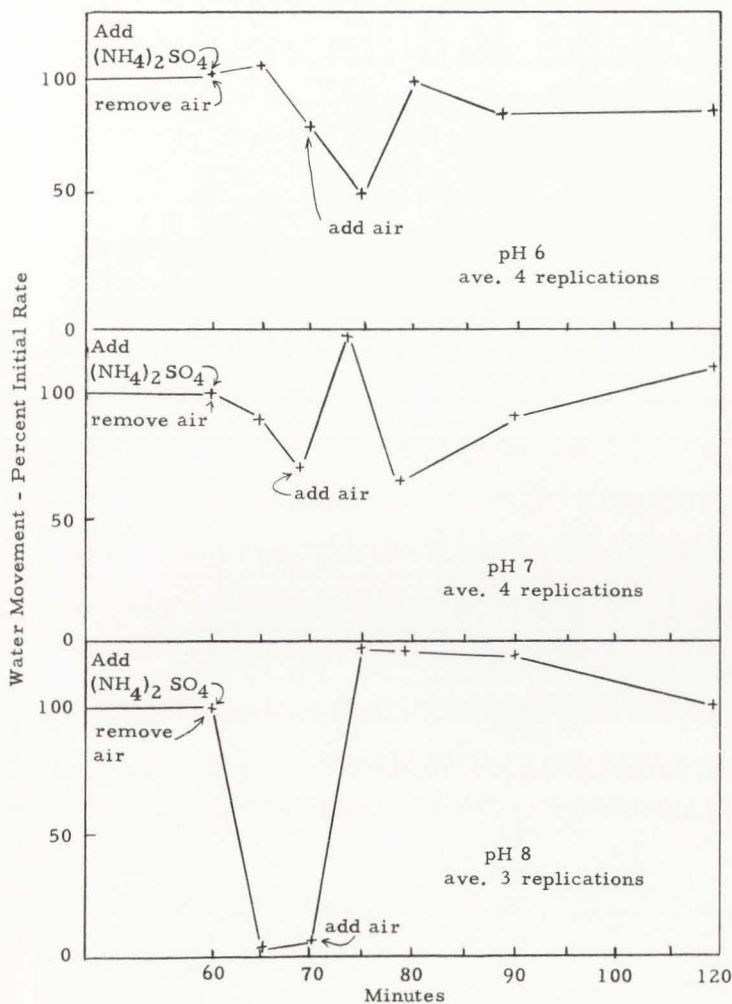
Figure 20. The effect of 1×10^{-3}M $(NH_4)_2$ on the rate of water movement through sugar beet roots in 10^{-2}M potassium phosphate buffer at pH 6, 7, 8 and 30 cm, Hg suction.

The results of the experiments with 5×10^{-2} tris buffer and ammonium sulfate are summarized in Figures 21 and 22. The experiments were conducted in the same manner as the phosphate buffer experiments. The tris buffer was adjusted to the appropriate pH with sulfuric acid. Ammonia was supplied as ammonium sulfate.

At concentrations of 5×10^{-4} molar ammonium sulfate and at pH 7 the water movement was much the same as in the potassium carbonate experiments and the pH 6 and pH 7 phosphate buffer experiments. However, at pH 8 and pH 9, the pattern of water movement was much the same as in the pH 8 phosphate buffer experiment. One difference was noted: the peak after water movement had resumed was 1.7 times greater than the initial rate at pH 8, and 2.36 times greater than the initial rate at pH 9. Toward the end of the experiment, the rate of water movement had returned to near the initial rate.

At concentrations of 2.5×10^{-4} molar ammonium sulfate, the pattern of water movement at pH 7 was the same as the others at that pH. At pH 8 and 9, the pattern was the same as that at the higher concentrations except that the rate of water movement after water movement had resumed was very high and at pH 9 did not return to near the initial rate. The author is unable to explain why the peak was so high only with this buffer.

The experiments with ammonium carbonate, ammonia gas and the buffers tend to implicate pH per se as the cause of the inhibition of water movement in sugar beet roots. However, the experiment
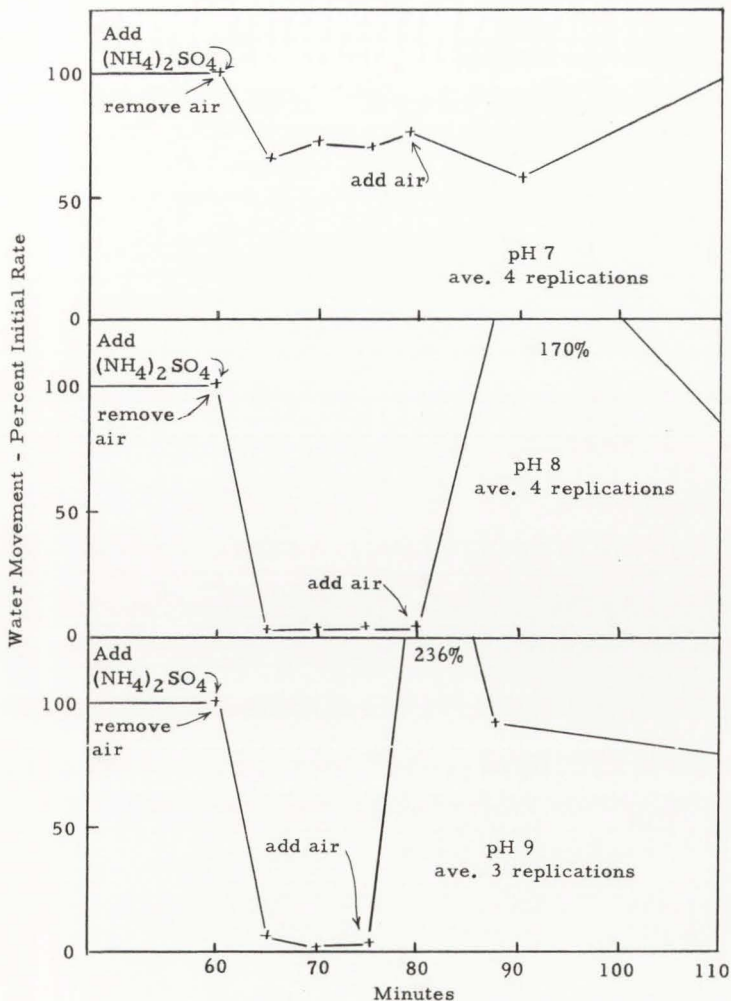
Figure 21.   The effect of 5 X 10^{-4}M $(NH_4)_2 SO_4$ on the rate of water movement through sugar beet roots in 5 X 10^{-2}M Tris buffer at pH 7, 8, 9 and 30 cm. Hg suction.
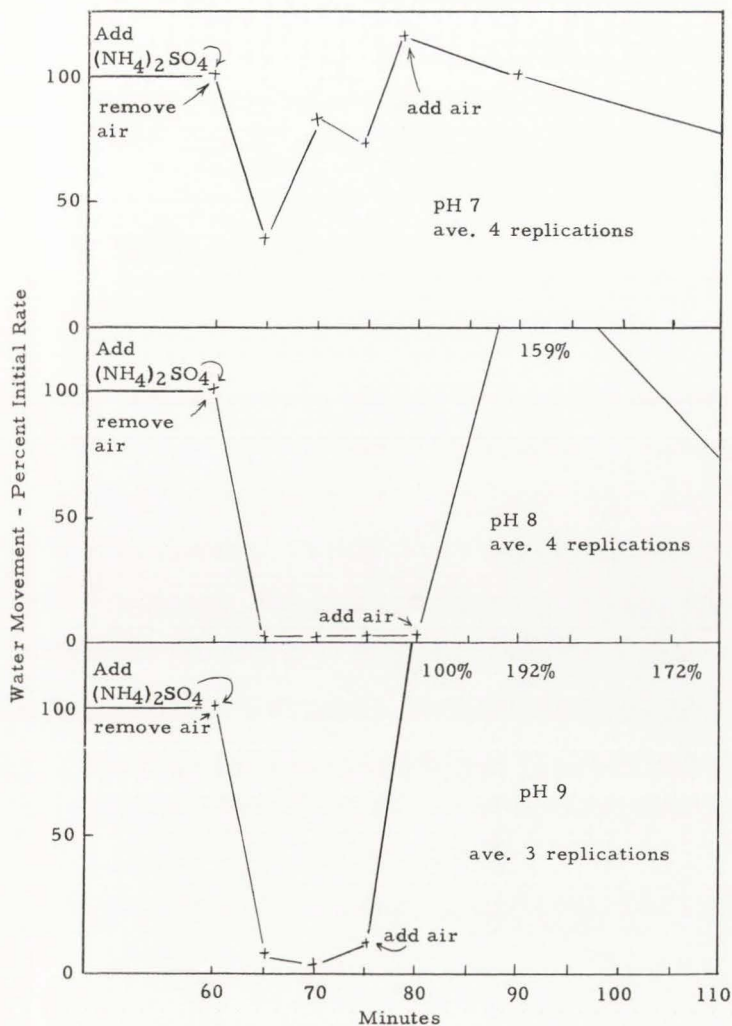
Figure 22. The effect of 2.5 X 10^{-4}M $(NH_4)_2SO_4$ on the rate of water movement through sugar beet roots in 5 X 10^{-2}M Tris buffer at pH 7, 8, 9 and 30 cm. Hg suction.

using potassium carbonate indicates that his was not the cause of the inhibition because no inhibition took place even at pH 9.4. Many workers (Court et al., 1964; Warren, 1962; Vines and Wedding, 1960; and Chipman, 1934) 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 (NH_3) present. Ammonia (NH_3), because of its small size, diffuses very rapidly across cell membranes and into the cytoplasm, while the ionized form (NH_4^+) of ammonia diffuses much more slowly (van den Honert and Hooymans, 1961; Warren and Nathan, 1958; Macmillian, 1956; and Hill, 1932).

Because the inhibition of water movement in the experimental sugar beet roots took place rapidly, within five minutes, and only at a pH greater than seven, ammonia (NH_3) appears to cause the inhibition of water movement. Ammonia (NH_3) could not cause inhibition of water movement at a pH less than seven because at this pH the concentration of undissociated ammonia is extremely low. Vines and Wedding (1960) showed that undissociated ammonia inhibits respiration and the literature review at the beginning of this section shows that anything that interferes with respiration. i.e., low temperatures, respiration inhibitors, reduced oxygen, etc., also reduces water movement in various plant tissues. It is concluded that ammonia (NH_3) is responsible for the inhibition of water movement in sugar beet roots and that it acts by reducing respiration.

Water movement in response to adenosinetriphosphate and ammo-
nium carbonate. The objectives of the following experiments were to
determine the effect of adenosinetriphosphate (ATP) on reducing the
inhibition of water movement by ammonia (NH_3). The experiments with
ATP were undertaken because ATP is a source of energy for many bio-
logical processes and is a prime produce of respiration. Kramer
(1955) and Slatyer (1962) both emphasize that respiration is closely
connected with the movement of water through various plant tissues.
It is important that we understand more of the functions of ATP and
its role, if it has one, in the movement of water through plant roots.

Each experiment was conducted in much the same manner as the
other experiments. 1×10^{-4} molar ATP (disodium salt) was added to
the water solution at the beginning of the experiment and another incre-
ment of 1×10^{-4} molar ATP was added 40 minutes later and twenty
minutes after this the ammonium carbonate was added. Aeration was
continuous except for the twenty minute period after the addition of the
ammonium carbonate. Suction was applied at 30 centimeters mercury.
Each graph represents four replications.

The results of these experiments are shown in Figure 23. The
pattern of water movement is quite unlike that when ATP was not added
to the solution and resembles the pattern of water movement in the pH 6
and pH 7 buffer experiments and in the experiments with potassium car-
bonate. The fluctuations in water movement can be explained in the
same way as those in the other experiments where inhibition of water
did not take place.
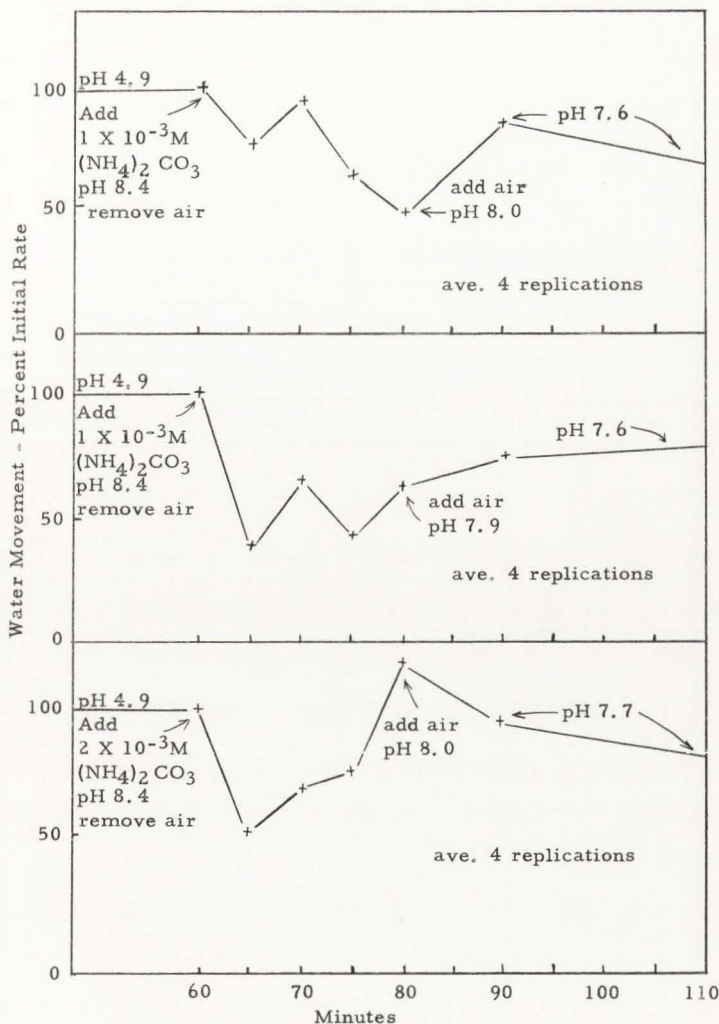
Figure 23. The effect of ammonium carbonate on the rate of water movement through sugar beet roots previously treated with 2×10^{-4}M ATP for one hour, at 30 cm. Hg suction.

Water movement was somewhat depressed towards the end of the experiment. Roberson (1964) found that ATP alone in 1/2 strength Hoagland's nutrient solution slightly depressed the movement of water but that ATP plus an acetate buffer at pH 4.5 increased the rate of water movement. He attributed the increase in the rate of water movement to possible injury by the acetate buffer. The pattern of water movement in these experiments also resembles the pattern of flow in damaged root systems. It is possible that the ATP may have injured the roots even though they did not behave like the acetate-treated roots in Robersons (1964) experiments. The ATP-treated roots appeared to be normal and no visible damage could be discerned.

Barring any injury by ATP, it appears that ATP can overcome, at least partially, the inhibition of water movement caused by ammonia (NH_3). It may be that ATP is directly involved in maintaining the porosity of various plant membranes to water.

Conclusions

1. Ammonia (NH_3) was responsible for the inhibition of water uptake by sugar beet roots.

2. ATP may overcome, at least partially, the inhibitors of water uptake caused by ammonia.

PART III

A POSSIBLE SITE FOR THE AMMONIA-INDUCED

INHIBITION OF WATER UPTAKE IN

EXCISED SUGAR BEET ROOTS

Since ammonia (NH_3) has been shown to inhibit water movement
through excised sugar beet roots, it would be of value if the site of the
inhibition were known. The following literature review will attempt to
show some of the pathways of water movement across roots. This
research indicates a possible site for the ammonia-induced inhibition
of water uptake.

Literature Review

As pointed out by Collander (1959), water must choose between
two alternate pathways when it moves across roots: (a) across the
protoplasts and (b) through the cell walls flanking the protoplasts.
Both paths will, of course, always be used simultaneously, and it
seems a priori clear that the intensity of each partial stream will be
inversely proportional to the resistance encountered along each path.

Mees and Weatherly (1957) concluded that most of the water movement they observed occurred external to the vacuole and probably took place in the cell walls. They increased pressure on the roots and observed increased root permeability; this was likely the result of increased pathway space either in the intercellular spaces, the cell walls, or in part of the cytoplasm.

Mees and Weatherly (1957) also found that metabolic inhibitors caused a 90 percent reduction in water movement through the roots and they did not find this inconsistent with the location of the main water pathways in the cell walls. This was not regarded as evidence that most of the movement took place elsewhere. Russell and Barber (1960) cite evidence that the endodermis may play an important role in water transport across the root.

Lebedev (1960) observed that D_2O was not readily exchanged with H_2O from the cytoplasm of Nitella but was exchanged rapidly from the outer part of the cell. Vartapetyan (1960) found that after plants were kept in H_2O^{18} over a long period of time (up to 75 hours) the concentration of H_2O^{18} in roots and leaves was only 70-80 percent of that in the external solution, but the O^{18} concentration in the stem tissues was almost the same as that of the external solution. This suggests that the endodermis may not play an important role in water transport across the root.

When Lopushinsky (1964) increased the pressure on tomato roots, he found an increase in the rate of flow of water. As the rate

increased, the concentration of salts in the transpiration stream decreased. The salt concentration of the exudates obtained under pressure usually was less than the concentration of the external solution. This indicates the existence of a barrier in the roots which prevents free movement of ions into the xylem. However, Veldstra and Booy, H. L. (1949) found that n-diamylacetic acid has a pronounced synergistic action with auxins and Burstrom (1949) found that n-diamylacetic acid ruptured the epidermal cells of wheat roots without any apparent damage to the endodermis. Sandstrom (1950) studied salt absorption in roots lacking epidermis (epidermal cells were removed with n-diamylacetic acid). He found that the concentration of salts in the transpirational stream of roots lacking epidermis approached that of the external media. This would indicate that the selective absorption of ions should be located in the epidermis. It could indicate that the site of respirational, including ammonia (NH_3), inhibition of water uptake is also located in the epidermis.

The following experiments using n-diamylacetic acid and ammonium carbonate were designed to validate the hypothesis that the site of the ammonia induced inhibition of water uptake occurs in the epidermis.

Methods and Materials

Sugar beets were germinated and grown as described in the previous experiments, Part II, except that the nutrient solution was

adjusted to pH 6 with K_2HPO_4. When the beets had grown to about

3/8 - 5/8 inch in diameter, n-diamylacetic acid was added to each

of 5 pots containing sugar beets until the concentration reached

$2 \times 10^{-5}M$, to another 5 pots until the concentration reached $4 \times 10^{-5}M$.

Nothing was added to another five pots to be used as controls.

After the n-diamylacetic acid had been added, all solutions

were changed daily for four days. Four sugar beets from each treatment

were then removed from the nutrient solutions, decapitated, and suction

applied to the cut surface of the roots as described previously (Section II).

After one hour $2.5 \times 10^{-4}M$ ammonium carbonate was added and the

resultant water uptake or lack of uptake was recorded.

One plant from each treatment was used to observe the action of

the n-diamylacetic acid on the roots. This same plant was allowed to

grow for an extra two weeks to observe the toxicity, if any, on the

continued growth of sugar beets.

Results and Discussion

Effect of n-diamylacetic acid

Each 24 hours the pH of the nutrient solutions containing the

n-diamylacetic acid, also those containing no acid, rose from pH 6

to pH 6.3 - 6.4 which is well within the active pH range of

n-diamylacetic acid (Burstrom, 1949).

When the roots had been treated with n-diamylacetic acid for

one day, very little, if any difference could be seen between the

treated and untreated roots, either visually or under a low-powered objective. After four days treatment, the roots treated with 4 X 10^{-5}M n-diamylacetic acid appeared to be less glossy than the non-treated roots. The newer rootlets had an occasional brown section on them that may have indicated local damage from the n-diamylacetic acid. Roots treated with 2 X 10^{-5}M diamylacetic acid were like the 4 X 10^{-5}M n-diamylacetic acid treated roots except the brown sections were much fewer.

Under a low powered microscope objective, the roots had a rough and ropy appearance and only an occasional sickle-shaped epidermal cell, as reported by Burstrom (1949) and Sandstrom (1950), could be seen. Ruptured root hairs were not observed because the sugar beet developed very few, if any, root hairs in the nutrient solution. The epidermis appeared to have been removed from the roots exposing the endodermis but 4 X 10^{-5}M diamylacetic acid appeared to have caused some damage to the new rootlets. If any damage were caused by 2 X 10^{-5}M n-diamylacetic acid it was not as apparent and was certainly much less than in the higher concentration.

During the n-diamylacetic acid treatment the plants appeared to be normal and exhibited no signs of distress. After four plants from each treatment were used to measure water uptake, the remaining plant from each treatment was placed in n-diamylacetic acid-free media. A week after the plants had been removed from the acid, the 4 X 10^{-5}M n-diamylacetic acid treated plant began to wilt and part

of the roots turned dark. At the end of the second week after removal

from the acid new roots began to grow, wilting ceased and growth

resumed. Treatment with $2 \times 10^{-5}M$ n-diamylacetic acid showed no

ill effects and the sugar beets appeared normal both during treatment

and afterward.

Effect of ammonium carbonate on water absorption
by roots treated with n-diamylacetic acid

The results of this experiment may be seen in Figure 24. With

roots that had been treated with n-diamylacetic acid, ammonium car-

bonate did not inhibit water absorption. With untreated roots ammonium

carbonate inhibited water absorption as was reported previously (Part II).

The $2 \times 10^{-5}M$ n-diamylacetic acid treated roots acted very much like

roots treated with potassium carbonate or ammonium sulfate at pH 7 or

less. Again there was some variation in water absorption over short

periods of time but there was no almost complete inhibition as occured

in the untreated roots.

The author cannot explain the large increase in the rate of

water absorption that occurred at the end of the experiment with

$4 \times 10^{-5}M$ n-diamylacetic acid treated roots, unless this was due to

injury caused by the higher concentration of acid. While the results

shown in Figure 24 are the mean of four replications, the individual

results for the 4×10^{-5} n-diamylacetic acid varied considerably.

These results resembled those roots that had sustained injury from

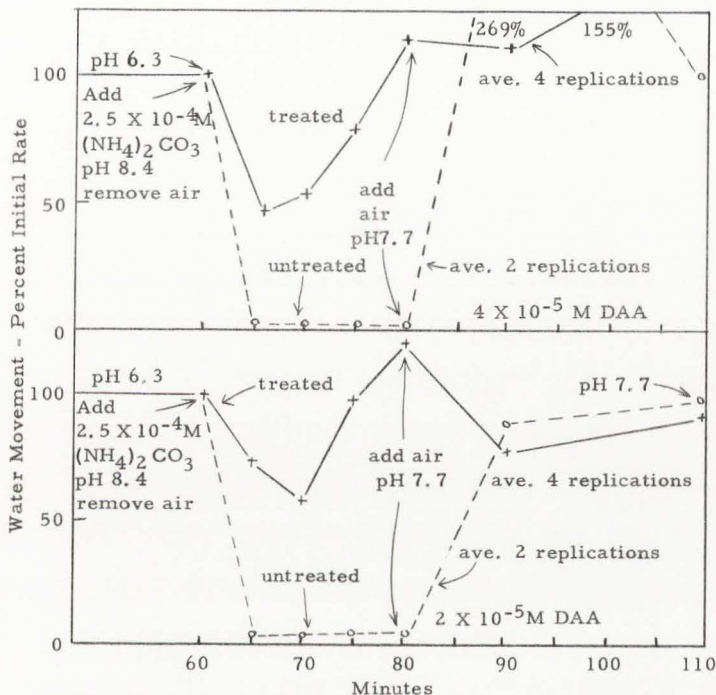high concentrations of ammonia or broken and otherwise damaged roots.

Figure 24. The effect of ammonium carbonate on water movement through sugar beet roots lacking epidermis. Epidermis removed with n-diamylacetic acid.

If the site of the ammonia induced inhibition of water uptake lies within the root epidermis, ammonia (NH_3) could not be expected to inhibit water absorption in roots that had had their epidermis removed. Conversely, if the site of the ammonia induced inhibition of water uptake is not within the epidermis then ammonia (NH_3) should inhibit the uptake of water; particularly if the pathway of water movement lies between the cells and in the cell walls but not through the cytoplasm.

Since ammonia (NH_3) did not inhibit water movement in roots that had had their epidermis removed, it may be concluded that the site of the ammonia induced inhibition of water uptake lies within the epidermis. However, the roots of the $4 \times 10^{-5}M$ n-diamylacetic acid treatment definitly appeared to be damaged. The roots of the $2 \times 10^{-5}M$ n-diamylacetic acid did not appear to be damaged but the possibility that root damage occured in this treatment cannot be ignored and casts doubt on the validity of the conclusions.

Conclusions

1. The epidermis of sugar beet roots may be removed by n-diamylacetic acid.

2. The endodermis of sugar beet roots may also be damaged by n-diamylacetic acid.

3. Ammonia (NH_3) does not inhibit water uptake in sugar beet roots that have been treated with n-diamylacetic acid.

4. The site of the ammonia induced inhibition of water uptake may be within the root epidermis.

PART IV

GENERAL SUMMARY

AND CONCLUSIONS

Ammonium salts, urea, aqua ammonia, and anhydrous ammonia
are important sources of fertilizer nitrogen but they are often toxic to
many crops. Aqua ammonia and anhydrous ammonia are the most toxic
because the high pH of these compounds in solution generates large
amounts of ammonia (NH_3). Ammonia toxicity symptoms oftem resemble
various wilt diseases.

In a previous study, sugar beets grown in nutrient solutions
(pH 7. 8) which contained ammonium sulfate as the nitrogen source wilted
strongly during periods of high transpiration demand. When nitrates
were used as the nitrogen source, little, if any, wilting was observed.
During periods of low transpiration demand (at night or on cloudy days)
no wilting was observed with either ammonium sulfate or nitrates. At
pH 7. 8 about eight percent of the total ammonia is in the NH_3 form.

These observations raised the following questions: Does
ammonia (NH_3) affect sugar beet stomata, causing them to remain

open, or does ammonia (NH_3) greatly reduce the absorption of water by the roots? Either could cause wilting under conditions of high transpiration demand. If ammonia (NH_3) affects the adsorption of water by the roots, what part of the root structure is affected - the epidermis or the endodermis?

In a series of experiments with sugar beets grown in nutrient solutions (pH 7.6 - 7.8) no difference in stomatal aperture was found between sugar beets supplied with ammonia as the nitrogen source during periods of high transpiration demand. However, severe wilting was observed with sugar beets supplied with ammonia as the nitrogen source during periods of high transpiration demand. The beets supplied with ammonia as the nitrogen source also contained significantly less water than those supplied with nitrates as the nitrogen source under the above conditions. During periods of low transpiration demand little, if any, difference was observed between the treatments. Apparently ammonia affects the absorption of water by the roots of sugar beets and has no effect upon stomatal opening or closing.

Disks of sugar beet and potato storage tissue were treated overnight with 0.00 to 0.01 molar ammonium sulfate in 0.1 molar potassium phosphate or Tris buffer. Water loss from the ammonia-treated disks, as measured by plasmolysis with concentrated mannitol solutions, was retarded only when the pH of the buffers was raised to pH 7.5 or higher. At this pH free ammonia (NH_3) was found. At pH 8.5 - 9.0 enough ammonia (NH_3) was formed to be highly toxic

to the sugar beet tissue but not to the potato tissue. In itself, pH had little, if any, effect upon the rate of water loss from the tissue disks. Ammonia (NH_3) was responsible for the reduction of water loss from the tissue disks because ammonia (NH_3) has been shown to be a respiration inhibitor. Respiration inhibitors or other factors which interfere with respiration reduce the absorption or desorption of water from various tissues.

Ammonia reduced the uptake of excised sugar beet roots by as much as 95 percent whether suction was applied to the cut end of the root or the root was merely allowed to bleed. Ammonia, whether added as ammonium carbonate, ammonium sulfate or ammonia gas was equally effective in inhibiting water uptake by sugar beet roots whenever the pH was sufficiently high to result in free ammonia. When the ammonia was removed, water uptake by the root returned to normal or above normal. Ammonia inhibited the uptake of water by reducing respiration.

Adenosine triphosphate (ATP) added to the roots in concentrations of 2×10^{-4} molar either wholly or partially overcame the inhibition of water uptake by ammonia. This indicates that ATP may be connected with the uptake of water by roots. Since water uptake is a passive process in plants the energy supplied by ATP may be used to maintain the integrity of cell membrane structures. Apparently ammonia interferes with the production of ATP in a cell by inhibiting respiration.

Ammonia did not inhibit water uptake in sugar beet roots which had had their epidermis removed with n-diamyl acetic acid. Root damage occurred at the concentrations 4×10^{-5} molar n-diamyl acetic acid but no damage was evident at the concentration of 2×10^{-5} molar. This indicates that the ammonia (NH_3)-induced inhibition of water uptake occurs in the root epidermis rather than the endodermis. However, because of the damage to the root caused by the n-diamyl acetic acid at the higher concentrations one cannot be certain that damage did not also occur at the lower concentration, casting some doubt on the validity of the results.

The following conclusions can be drawn from the preceding investigations.

1. Ammonia (NH_3) has no effect on the opening or closing of stomata, but lowers the water content of the leaf and causes sugar beets to wilt during periods of high transpiration demand.

2. Ammonia (NH_3) retards the loss of water from disks of sugar beet and potato storage tissue and inhibits the uptake of water by excised roots.

3. The ammonia (NH_3) induced inhibition of water uptake may be wholly or partially reversed by ATP.

4. The site of the ammonia (NH_3)-induced inhibition of water uptake may be within the epidermis of the root.

The ammonia induced inhibition of water uptake need further study, particularly with species other than sugar beet. Also, studies with ATP and ammonia need to be continued with other species and particularly with other respiration inhibitors such as dinitrophenol, azide, cyanide, etc. Some means of elucidating the mechanism or mechanisms of the inhibition of water uptake by respiration inhibitors should also be investigated.

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