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THE EFFECTS OF POTASSIUM ON AMMONIUM NUTRITION

IN TOMATO (LYCOPERSICON ESCULENTUM, MILL.,

HEINZ 1350)

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> Department of Plant and Soil Sciences University of Massachusetts September 1969

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DEFINITION OF PROBLEM

A great deal of work has been done by scientists to determine the functions of nitrogen in plants, since the first concrete proposal of its essentiality was reported by De-Sausseur in 1804. Nitrogen can be applied to the soil for plant use in a variety of forms, i.e., nitrate, ammonium and organic (15).

In practical farming, the application of the nitrate and organic forms are relatively expensive. Recently, the availability of nitrogen in the inexpensive ammonium form has generated considerable interest and has become very popular among farmers. For example, approximately 80 per cent of the fertilizer nitrogen used is in the ammonium form (68). However, it has been found that the prolonged fertilization or absorption of ammonium salts causes ammonium toxicity in most cultivated plant species (42, 43, 45).

Potassium is very important in the regulation of nitrogen metabolism. It influences the amounts of protein and soluble nitrogenous compounds present in the plants (8, 33, 66) and also restricts plant injuries caused as a result of ammonium toxicity (7, 9, 32).

It is the aim and purpose of this thesis to determine the possibility of recovery from ammonium toxicity, i.e., the healing of stem lesions by supplemental potassium in the tomato plant (<u>Lycoversicon</u> <u>esculentum</u>; Heinz 1350). Experiments were also conducted to determine the extent of lesion development which occurred before the damage was irreversible.

REVIEW OF LITERATURE

History of the Tomato Plant

The tomato is a native of tropical America and is reported to have been eaten by the native tribes of Mexico, who called it "tomati" (67). It is one of the most important and popular vegetables grown in the world and the first known record of it as a plant was made by Matthiolus in Italy in 1554 (67).

Taxonomy

The tomato belongs to the nightshade or Solanaceae family and the genus Lycopersicon. It is a herbaceous plant, annual where frost occurs and perennial in the tropics. The stems are round, soft, brittle and hairy when young, but become angular, hard and almost woody when old. The leaves are alternate and glandular, secreting greenish yellow juice. The flowers occur in clusters along the stem between the nodes. The fruit is a two- to many-celled berry with fleshy placentae and many hairy, kidney-shaped seeds. The root system is wide and deep, lacking extended tap root. It is self-pollinated. Two distinct species, <u>Lycopersicon esculentum</u> and <u>Lycopersicon</u> <u>pimpinellifolium</u> are generally recognized by most authorities with five varieties listed under the former (67).

The tomato is a warm-season crop and is sensitive to frost. High humidity with high temperature favors the development of foliage diseases. Hot drying winds often cause the dropping of the blossoms, but irrigation will lower the temperature, raise the humidity and prevent much of the blossom drop (77). Tomatoes grow on nearly all kinds of soils from

light sand to heavy clays and can tolerate fairly acid soils (80). For high production, it is essential that the soil be well-drained and retentive of moisture.

Tomato Production in the United States

Tomatoes are produced commercially in almost every state in the United States, with Florida and California ranking first, as the most important producers for the fresh market (68). In 1965, Massachusetts ranked first in New England with a yield of 185 cwt per acre followed by Rhode Island with 165 cwt per acre and Connecticut with 140 cwt (74). Tomato Diseases

Generally, the diseases are of two types - parasitic and non-parasitic (70). Unfavorable environmental conditions, such as excessive moisture or drought, extremes of temperature, and lack or excess of certain mineral elements in the soil may cause the non-parasitic disease. The parasitic diseases are caused by living organisms, such as bacteria, fungi and viruses, and they are the most serious (70).

Blossom-end rot, a non-parasitic disease is a physiological disorder of tomato fruits (17, 42, 50, 57). Some of the symptoms are the appearance of dark, irregular, watersoaked areas at or near the blossom-end of the fruit, followed by the coalescing of these discolorations to form a depressed, leathery area at the blossom-end. Fruits are more frequently affected at the immature green stage.

Some of the factors causing blossom-end not disease are unfavorable weather conditions, (17, 44, 57) high osmotic pressures of nutrient solutions (42, 50), applications of fertilizers high in K^{+} and

 NH_4^+ (17, 42, 50, 57, 69), and insufficient amount of Ca⁺⁺ (17, 42, 50, 57). Satisfactory control of blossom-end rot lies in the adequate supply of Ca⁺⁺ and the avoidance of excesses of K⁺, NH_4^+ and Mg^{++} fertilizers.

Forms of Nitrogen Supplied by Fertilizers

The increase in the use of nitrogen fertilizers has enhanced the production of agricultural crops, by increasing yields with reduced use of land and manpower. The bulk of the nitrogen used by plants is in the combined forms of ammonia, ammonium ions, nitrate and amine (15). Anhydrous ammonia supplies nitrogen as gaseous ammonia (NH_3), while ammonium sulfate, aqua ammonia, ammoniated solutions and ammonium nitrate supply ammonium ion (NH_4^+) (23). The nitrate ion (NO_3^-) is supplied by nitrate salts, e.g., KNO_3 and the amine form is supplied by urea (15, 23).

Black (15) discussed two methods by which nitrogen fertilizers could be incorporated into the soil, i.e., by direct application and indirect application by cultivation operations. Anhydrous ammonia and ammonium hydroxide are usually placed below the soil, because of the volatility of ammonia. Solid nitrogen fertilizer is applied to the surface of the soil and urea may be absorbed by the foliage after spraying.

Forms of Nitrogen in the Soil

Elemental nitrogen is present in the soil in three main forms; gaseous, inorganic and organic. Nitrous oxide (N_2^0) , nitric oxide (NO), nitrogen dioxide (NO_2) , and ammonia (NH_3) are the gaseous forms, and ammonium (NH_4^+) , nitrite (NO_2^-) and nitrate (NO_3^-) are the inorganic forms in which nitrogen exists in the soil (23). The organic nitrogen is largely proteinaceous, (60) and is derived from plant and eminal residues.

Some effects of nitrogen fertilizers on the soil pH have been reported. Donald <u>et al.</u> (23), observed that ammonia and aqua ammonia application to the soil raises the pH in the area of application, because they are basic. On the other hand, the application of ammonium nitrate, calcium nitrate and sodium nitrate have little immediate effect on pH, because they are neutral salts. pH of the soil varies with different nitrogen carriers (23). Nitrogen carriers containing metallic cations either increase or have a neutralizing effect on acidity, while those carriers with NH_4^+ as the cations lower the pH, an example of which is ammonium sulfate, which carries the acidic sulfate radical.

Forms of Nitrogen Absorbed by Plants

It is generally known that nitrogen occurs in both inorganic and organic forms in plants. The organic forms predominate over the inorganic (15). Nitrate is absorbed by plants as an inorganic form, assimilated by reduction to ammonium, and later incorporated into the organic forms. Most of the organic nitrogen is present in the form of protein (60), which includes enzyres (15).

Nitrogen deficiency results in a reduction in leaf size and the number of lateral shoots (23), stems are thin and there is a general reduction in plant growth. Leaves have a pale-green color due to a reduction in chlorophyll content (49), with older leaves often becoming yellow - a condition associated with severe proteolysis (35, 49).

Morsening conditions may bring about a predominance of anthocyanin pignants, which are usually present in the petioles and veins of tomato plants (35).

Functions of Ammonium-nitrogen in Plants

As earlier stated, the ammonium ion constitutes one of the most important sources of nitrogen utilized by plants. Experiments with a number of plant species have shown that ammonium exerts a pronounced effect on both the growth and chemical composition of plants, when supplied as a nitrogen source (3, 9, 13, 20, 44, 79).

Working with tobacco plants and using isotopic N¹⁵H₃, Vickery <u>et al</u>. (75), demonstrated the incorporation of ammonia into amides, amino-acids and proteins. Similar findings were reported by Cocking <u>et al</u>. (20) with barley seedlings, and also Austin (3), working with nitrogen starved wheat seedlings. Syrett (63) observed that the basic amino acids (arginine, lysine, and ornithine), represented a large proportion of the free amino acids, formed in the first stage of ammonia assimilation in <u>Chlorella vulgaris</u>. Deficiency and excess amounts of ammonium-nitrogen were found to increase the amino-acid contents, (aspartic acid, arginine and histidine) in the tops of soybean plants, while in the roots, the amino-acid contents increased with increases in the nitrogen concentrations in the root medium (32).

Studies conducted by Maynard <u>et al</u>. (9, 44) linked some severe tomato injuries, with the accumulation of ammonium in their tissues. Barker <u>et al</u>. (13) noted that bean plants were just as sensitive to ammonium nutrition. William <u>et al</u>. (79) showed that a heavy fertilization of ammonium salts in orchard grass could cause a stress in their

metabolism, by causing an accumulation of asparagine, while reducing the potassium content below 1.5 per cent of the dry weight.

One of the direct results of ammonium accumulation is ammonium toxicity. Ammonium toxicity is caused by the prolonged fertilization or absorption of ammonium salts, as a source of nitrogen by most plant species. (9, 13, 44). It results in chlorosis, restricted growth and in some cases, the death of the plant. In tomato plants one of the chief morphological symptoms of ammonium toxicity is the development of stem lesions (9, 44), spreading to the petioles and leaf-blades upon continuous ammonium application.

Anatomically, Barker <u>et al</u>. (10) showed that cellular injury localized within the epidermal and cortical regions of the affected stems appeared evident, while the vascular and pith tissues remained intact. Damaged cells showed evidence of cellular collapse and thickening of the cell walls.

Physiologically, there is a high accumulation of soluble nitrogenous compounds, including considerable amounts of inorganic ammonium in tomato plants, under ammonium toxicity conditions (9, 44). Barker et al. (13) noted similar effects of ammonium toxicity in bean plants.

Utjee (73) observed the greatest incidence of root rot and corkiness in tomato roots, when ammonium application was highest. He suggested that ammonium accumulation could be a possible cause of corkiness or root rot in tomatoes, or could provide conditions suitable enough for the successful invasion of the roots, by soil-borne organisms.

Other deleterious effects have been observed in protein synthesis, photosynthesis, respiration and carbohydrate metabolism. Yerm and

Willis (81) and later Barker <u>et al.</u> (14) suggested a decline in protein synthesis along with an increase in soluble nitrogen compounds, possibly due to proteolysis. In concentrations of 0.6mM., ammonium ions inhibited ATP formation by 50 per cent (39) and also caused a reduction in CO_2 fixation within the chloroplast (71). With ammonium accumulation, chlorophyll loss and a decrease in photosynthesis were observed in the leaves (49).

Many workers (4, 5, 12) have studied the effects of the addition of ammonium salts to nitrogen-starved algae and higher plants. Increases were found in the respiratory ratio of such tissues. (29, 34, 63). Continuous supply of ammonium brings about reactions which incorporate ammonium into organic compounds at the expense of other vital growth processes such as protein and cell-wall syntheses (4). Carbohydrate and metabolic energy may therefore be diverted to the synthesis of nitrogenous storage compounds, such as amides (4).

Evidence of the effect of ammonium ion on the absorption of other ions has also been noted (30, 37, 41, 48). Prianishnikov (48), concluded that the inadequacy of ammonium fertilizers was due to their strongly acidic nature, and Gouny (30) in his work agreed that H^+ ions generated during ammonium uptake competed directly with other cations for absorption by plant roots. Kirby, (37) working with white mustard plants observed that plants supplied with ammonium-nitrogen generally did not grow well and contained lower concentrations of inorganic cations such as Ca^{++} , Kg^{++} and K^+ , and higher concentrations of anions, such as sulfate, phosphate and chloride, when compared with tissues supplied with nitrate-nitrogen. MacLand and Carson, (41) noted that under high arconium source and low K^+ , there was a depression in yield,

reduction in tillering and significant changes in the per cent K^+ , Ca^{++} , Mg^{++} and P in grasses.

One of the environmental factors that govern the utilization of ammonia by plants is pH. High pH favors the uptake of ammonia (2). Wall (76) reported that the injuries in tomato leaves became much worse under K^+ deficient conditions and when the pH was below 6.0. Sheat <u>et al.</u> (56) concluded that ammonium served as an effective nitrogen source for the growth of excised tomato roots, only when acidity was maintained in the range of pH 6.8 - 7.4. Barker (7) agreed with this concept and showed an increased incorporation of ammonium into organic compounds in the roots of bean plants, under neutral conditions.

The age of the plant also influences nitrogen assimilation (16, 65). Thus rice (16) and similarly wheat and oat seedlings (65) assimilate ammonia better than nitrate when young, and later attain the ability to assimilate nitrate equally well upon maturation.

The ability to utilize ammonium as a source of nitrogen varies among different plant species (19). Very few species develop better on ammonium nutrition than on nitrate nutrition (78). The ericaceous plants (blueberries, rhododendrons) are well-known in this respect (19, 22). Cain (19) and Colgrove <u>et al</u>. (22) pointed out that these plants often became chlorotic on nitrate nutrition and under alkaline culture conditions. On the other hand, ammonium nutrition would permit their normal growth. Ericaceous plants seem to thrive in acidic soils and their survival is apparently related to their ability to use ammonium nitrogen in an acidic environment (19, 22). Within species, plants are known to differ with respect to ammonium tolerance. The

resistance of certain tomato varieties to stem lesions illustrated an example of variation within species (45).

Potassium has been recognized as an essential plant requirement for over 100 years. It is one of the most important macronutrients and is present in greater quantity than any other inorganic ions in plants, with the possible exceptions of nitrogen and hydrogen (15). Forms of Potassium In Soils

Potassium is widely distributed in the soil and most of it is present in the mineral forms of feldspar and mica (54). The most important of these mineral forms are the orthoclase and microline feldspars, the biotite and muscovite micas and the micaceous clays called illite (54). All of these minerals occur in the sand, silt and clay fractions of the soil (15). Chemically, the soil potassium is classified into three groups - nonexchangeable, exchangeable and water-soluble (18).

On the basis of solubility, Black (15), categorized the K^+ -bearing minerals into two groups, one group with moderate to high solubility in water and another with extremely low solubility. The low solubility group includes principally the feldspars and micas. Although, they contain considerable amounts of K^+ , they must be treated in order to increase K^+ solubility for fertilizer purposes (15). However, the cost of treatment makes their use uneconomical in comparison with the soluble minerals (15). The potassium sources used in fertilizer production are soluble salts (18).

Forms of Potassium Supplied by Fertilizers

Potassium is supplied to the soil as fertilizer such as murinte

of potash (KCl) which contains 51 per cent of water-soluble K^{+} (58). Other K^{+} fertilizers are potassium sulfate, (44.87 per cent K^{+}) potassium-magnesium sulfate (18.26 per cent K^{+}), potassium phosphate (33.09 per cent K^{+}) and potassium nitrate (38.66 per cent K^{+}) (15, 58). Potassium Movement in the Plant

With reference to the distribution of potassium in plants, K⁺ is found in the individual cells, most commonly in the vacuole and cytoplasm (62). It is very mobile and therefore, readily redistributed under stress conditions (62). Generally, potassium is transported to the metabolically active portions - young buds, root-tips and young shoots, of the plants (6), hence its deficiencies are first noticed in the older portions (35). Due to its mobility and the fact that it exists as free ions (58), the determination of its location in plants is difficult.

Potassium has been indicated to be absorbed both passively and actively by plant roots (25, 26, 62). Passive absorption, which is non-metabolic and reversible consists of diffusion of ions into the free space of the cell-wall, and the exchange of K^+ cation for the cations held on the root surface (26, 58, 62). Active uptake, which makes use of metabolic energy (25, 26, 62) is irreversible and requires ion-binding compounds called carriers (21, 25, 26). The carrier combines with the K^+ at the root surface, transports it through the plasma membrane, and then releases it inside the cell (58, 62).

There is selectivity in the nature of the ion uptake process, and this has been attributed to the types of carriers which are present on the root, during the transport of the ions into the plant (27, 62).

Working on excised root, Epstein and Hagen (27) showed that K^+ , Rb^+ and Ca^+ competed for the same carriers, while Na^+ and Li^+ did not compete for these sites.

Some of the important functions of K⁺in agronomic crops are in increasing the yields of corn, alfalfa, sugar-cane, soybeans, and other crops (32), improving the quality of sugarbeets and sugar-cane (55), decreasing the amount of lodging in corn (38), and increasing disease resistance in peanut (24), and Bermuda-grass (1).

Functions of K⁺ in Plant Metabolism

The physiological functions of K^+ in plants are various (28, 31, 64, 72). Although some important roles in plant metabolism have been attributed to it, some doubt still exists as to the exact mechanism in which it is involved (8). Potassium is essential for the activity of the enzyme pyruvic kinase, which is responsible for the transformation of carbohydrate intermediates (28). Gregory and Richards (31), found that K^+ deficiency increased the rate of respiration in barley leaves, and decreased their rates of photosynthesis, resulting in a depressed rate of dry matter production. Barker <u>et al</u>. (13) reported similar findings in mature bean leaves, but associated the depression in photosynthesis, with chlorophyll degradation as well as potassium loss from the tissue. As a result of the increased respiration, and decreased photosynthetic rates, carbohydrate depletion (21, 61) and reduction in growth rates (47, 66) were observed in potassium deficient plants.

Potassium plays a chief role in the regulation of nitrogen metabolism. Plants deficient in potassium usually contain a lower protein content (33, 52, 61, 66) and higher amounts of soluble nitrogenous

compounds, like amino-acids, (52, 72) and amides (47, 64), then those adequately supplied with K^+ . Richards and Templeman (53) suggested that proteolysis might occur more rapidly in K^+ deficient plants, and that this might account for the high amino-acid and amide contents of such plants. Barker <u>et al.</u> (13) arrived at a similar conclusion in bean plants, and that proteolysis accounted for 60 per cent of the accumulating free amino-acids when ammonium was supplied as the nitrogen source. Accumulation of these soluble nitrogeneous constituents was accentuated under K^+ deficiency (13, 52).

The production of toxic amines under conditions of potassium stress in wheat and barley plants was reported by Coleman and Richards (21). It was evident that normal nitrogen metabolism was severely affected, and also that putrescine was produced. The production of putrescine was enhanced in the presence of ammonium ions.

Severe injury found under conditions of ammonium toxicity was reduced in the presence of K^+ (51). Increased accumulation of total and soluble nitrogen content of tomato plants decreased with increases in K^+ concentrations, (49), thus lessening the chances of ammonium toxicity. This result was also supported by the work of MacLeod and Carson (41), who found that the total-protein, non-protein and nitrate-N in alfalfa, bromegrass, orchard-grass and timothy-grass grown in soil culture decreased with increasing concentration of K^+ .

MATERIALS AND METHODS

Preliminary experiments were conducted in the spring of 1968, and Factorial experiments were conducted in 1969 with tomato plants (Lycopersicon esculentum, Mill e.v. Heinz 1350) in the greenhouse in order to achieve the following objectives:

1. To study the effects of potassium on ammonium accumulation and the development of ammonium toxicity symptoms manifested by stem lesions with time.

2. To determine the possibility of recovery from ammonium toxicity i.e., the healing of stem lesions by supplemental potassium application, and to determine the extent of lesion development which must occur before damage is irreversible.

The tomato plant was used because of its rapid development of stem lesions when supplied with ammonium-nitrogen. This is a manifestation of its susceptibility to ammonium toxicity (45).

Greenhouse Culture Technique

1. Time Study on Ammonium Accumulation

Soil culture: Experiment I. - Eight-week old tomato plants were transplanted from flats to six-inch clay pots filled with a mixture of soil, peat, and sand mixed in the ratio of 7:3:2. Treatments were initiated two weeks after transplanting on March 13, 1969. Each treatment was replicated four times; each pot represented a single-plant plot.

The plants received solutions of $0.08N (MH_4)_2SO_4$ with concentrations of potassium at 0, 0.04N, and 0.08N KCl applied to the soil surface at the rate of 250 ml. per pot daily for 24 days. Plants were

harvested at 0, 4, 8, 12, 16, 20 and 24 days respectively after application of the treatments.

The fresh weights of the plant shoots were taken and the incidence of stem lesions was noted using 0-3 scale, i.e., 0 - no lesion; 1 - slight; 2 - moderate; and 3 - severe. The pH of the soil samples before and after the conclusion of the experiment was also recorded.

Soil Culture: Experiment II. - Eight week-old tomato plants were transplanted from flats to six-inch clay pots, filled with a mixture of soil, peat and sand mixed in the ratio of 7:3:2, as in the previous experiment. Treatments were initiated on March 13, 1969, two weeks after transplanting. Each treatment was replicated four times; each pot represented a single-plant plot.

The plants received equinormal concentrations of ammonium sulfate and potassium chloride at 0, 0.01N, 0.02N, 0.04N, and 0.08N, at the rate of 250 ml. per pot daily for a period of three weeks. Plants were harvested at 0, 7, 14 and 21 days after the application of treatments.

The fresh weights of the plant shoots were noted and the incidence of stem lesions was recorded using the 0-3 scale. The pH of the soil samples before and after the conclusion of the experiment was recorded.

2. Ammonium Reversibility

Sand culture: Experiment I. - Seven-week old tomato plants were transplanted from flats to six-inch plastic pots, containing approximately 1,000 gms. of 1:1 mixture of fine and coarse pure quartz sand. In one aspect of this experiment designed to show the effect of liming on lesion formation, two groups of these plants were transplanted to pots of sand containing 10 gm. (10 tons/acre) of limestone (28.5 per

cent Ca⁺⁺ and 4.5 per cent Mg⁺⁺) per pot. The resulting pH was 6.9.

On November 20, 1968, all the plants were treated with normal Hoagland's solution (36) - 250 ml./ plant daily for a period of two weeks. This treatment was replaced with a modified Hoagland's solution containing $0.02N (NH_4)_2SO_4$ and applied at 250 ml per pot daily, until slight severity (No. 1 rating) lesions were developed. Later, treatments to reverse this effect of ammonium were commenced with the application of Hoagland's solution modified with additions as follows:

- 1. 0.02N KCl at 250 ml per pot daily.
- 2. 0.02N KCl at 250 ml per pot daily + CaCO3.
- 3. One application of 10-g CaCO₃ + 250 ml of deionized water per plant daily.
- 4. 0.02N KCl + 0.02N $(NH_4)_2SO_4$ at 250 ml per plant daily.

5. $0.02N (NH_4)_2SO_4$ at 250 ml per plant daily. (Control). Each treatment was replicated four times with each pot representing a single-plant plot.

The experiment was carried out for three weeks and plants were harvested at 0, 7, 14 and 21 days respectively, after the initiation of treatments at the various K^+ levels. The fresh weights of the shoots just before and during the treatments were recorded. The disappearance of lesions was also observed.

 Composition of Nutrient Solution

 Nutrients
 Concentration

 CaCl₂
 .10.0 meq/l

 MgSO₄
 .00 meq/l

 MgSO₄
 .00 meq/l

 KH₂PO₄
 .00 meq/l

 NaNO₃
 .00 meq/l

 NaNO₃
 .00 meq/l

 KNO₃
 .00 meq/l

 Minor elements
 1.00 ppm

 Minor elements
 .1.0 ml

 Modified Hoagland's (NH₄⁺) Solution

 Nutrients
 Concentration

 $\begin{array}{c} CaCl_2 \\ MgSO_4 \\ MgSO_4 \\ MaH_2PO_4 \\ MaH_2PO_4 \\ MaH_2SO_4 \\ MaH_2SO_$

Modified Moagland's	s (K ⁺) Solution
Nutrients	Concentration
CaCl ₂ · · · · · · · ·	•••• 10.0 meg/1
Mg50 ₄	•••• 4.0 meg/1
NaH ₂ PO ₄ · · · · · ·	•••• 1.0 meq/1
KCl	•••• 20.0 meq/1
Iron	•••• 1.0 ppm
Minor elements (Hoagland and Arnon	(36)). 1.0 ml

<u>Soil culture</u>: <u>Experiment II</u>. - Eight-week old tomato plants first grown in flats were later transplanted to six-inch clay pots containing a mixture of soil, peat and sand mixed in the ratio of 7:3:2. Treatment with 0.04N $(NH_4)_2SO_4$ at the rate of 250 ml per plant was applied until lesions were formed at the 1, 2 and 3 degrees of severity. On October 4, 1968, this treatment was replaced with the following treatments, at the rate of 250 ml per plant daily:

- 1. 0.04N KC1
- 2. 0.04N KCl + 0.04N (NH₄)₂SO₄
- 3. Deionized water
- 4. 0.04N (NH₄)₂SO₄ (Control)

This application was continued for a three-week period and plants were harvested at 0, 7 and 14 days respectively after the application of the above-mentioned treatments. Each treatment was replicated four times.

The fresh-weights of the shoots at lesion formation and also during the application of treatments to reverse this effect were recorded, and the appearance of lesions was observed.

Analytical Procedures

Ten gram samples of fresh leaf materials from each treatment of the experiments on the time study of ammonium accumulation and ammonium reversibility were selected and stored at -20°C until they could be prepared for analyses.

The samples were homogenized in 70% ethanol and extracted several times, using suction filtration. The extract was evaporated to dryness under the hood, and then dissolved in 70% ethanol with small amounts of chloroform, in order to facilitate solution. This was transferred into Kjeldahl flasks, evaporated under a stream of air and later analyzed for ammonium-nitrogen, and amide-nitrogen (measured in mg/g fresh weight), using the modified Kjeldahl method (11).

The remaining fresh samples of the leaves and petioles of the shoots were oven-dried at 80°C. These dried samples were then ground in Wiley Intermediate Mill, using a 20-mesh screen. A 200 mg dry-weight sample was analyzed for total-nitrogen (measured in per cent dry-weight), using a Micro-Kjeldahl method (19). One-hundred milligrams was weighed and digested using $HNO_{3-}H_2O_2$ procedure described by Lagerwerff and Peech (40).

The digested solutions were quantitatively transferred to 25 ml volumetric flask with distilled water. The solution was further diluted 1:50 prior to K⁺ determination, using the Perkin-Elmer 290 Atomic Absorption Spectrophoto-meter.

Soil pH was measured by placing 50 g of soil or sand into a beaker and adding 50 ml of 0.01M CaCl₂. After stirring the mixture

thoroughly, the pH of the supernatent was measured using a Beckman Expandomatic pH meter.

Statistical analyses of variance, and Duncan's multiple range tests for all the experiments were performed by the methods described by Steel and Torrie (59). A complete partitioning of treatment factors and interactions have been calculated in all analyses but only the Duncan's multiple range tests are reported therein.

RESULTS

Experiment 1. The Effects of K⁺ on the Occurrence of Ammonium Toxicity and on Plant Composition

The results of a greenhouse soil experiment designed to show the effects of concentrations of K^{\dagger} with time on ammonium nutrition are shown in Table Ia - If.

Stem Lesions

Ammonium toxicity, as measured by the severity of stem lesions, (Figure 1) was evident four days after the initiation of treatments and was severe after twelve days, when $(NH_4)_2SO_4$ was supplied alone (Table Ia). Stem lesions also appeared after four days, when K⁺ was supplied at 0.04N, but the severity did not increase greatly with time. The occurrence of stem lesions was completely prevented for the entire duration of the experiment, when K⁺ was supplied at 0.08N (Table Ia). Fresh Weight

Growth, as measured by the fresh weight of tomato shoots (Table Ib), was not enhanced with time, when $0.08N (NH_4)_2SO_4$ was supplied alone. Substantial growth was observed however, when K⁺ was supplied at 0.04N and 0.08M from eight days to twenty-four days after the initiation of treatments. There was no significant difference between the 0.04N and 0.08N K⁺ treatments with time.

$\mathbb{NH}_4^+ - \mathbb{N}$

There was a significant increase in the concentration of MH_4^+-N in tomato leaves with time (Table Ic) when NH_4^+ was supplied to the



Figure 1. Normal tomato stem (right) and tomato stem showing ammonium-induced lesions (left).

plants without K^{\dagger} . Although, the concentration of $NH_4^{\dagger}-N$ increased significantly with time at $0.04NK^{\dagger}$, there was a lower $NH_4^{\dagger}-N$ concentration twenty-four days after the initiation of treatments, when compared to the $0.08N(NH_4)_2SO_4$ treatment.

Amide-N

Significant increases in the amide-N concentration occurred in four days, after the initiation of treatment with 0.08N $(NH_4)_2SO_4$. Amide-N concentrations at 0.04N K⁺ and 0.08N K⁺ treatments also showed substantial increases with time, but these concentrations were significantly lower than those obtained with the 0.08N $(NH_4)_2SO_4$ treatment, in the absence of K⁺.

Total-N

The absence of K^+ enhanced the percentage of total-N in the leaves of tomato plants supplied with NH_4^+ , with time (Table Ie). Smaller increases in the total-N concentrations were observed with 0.04N and 0.08N K^+ treatments, than with 0- K^+ ; the percentage total-N was least in the 0.08N K^+ treatment.

Potassium

The K^+ concentrations in the leaves of tomato plants treated with 0.08N (NH₄)₂SO₄ alone (Table If), showed a sharp decline after four days, and then declined more slowly, until the conclusion of the experiment. The 0.04N K⁺ treatment slowly increased the K⁺ concentration of the leaf tissue, throughout the experiment. At 0.08N K⁺ more rapid increases in K⁺ concentration were observed.

Soil pH

The pH of the soil samples (Table Ig) treated with OKCl, 0.04NKCl

and 0.08NKCl in the presence of 0.08N $(NH_4)_2SO_4$, decreased in 4 days, but remained constant 8, 12, 20 and 24 days after the application of treatments. There was a general decrease in the soil pH in all three treatments 16 days after treatments. The soil pH of all three treatments showed no significant differences when compared with one another.

EFFECTS OF K⁺ TREATMENTS ON THE DEVELOPMENT OF AMMONIUM TOXICITY, AS EVIDENCED BY STEM LESIONS, IN TOMATOES GROWN ON 0.08N (NH_A)₂SO₄ IN SOIL KCl Concentration Days 0 0.04N 0.08N Stem Lesion Rating (o; none - 3; severe) 0 0a 0a 0a **1.75c 1.00**b 4 0a 1.00b 8 2.00c 0a 3.00d 1.00b 0a 12 16 3.00d 1.00b 0a 3.00d 1.00b 0a 20 3.00d 1.75c 0a 24

Means within a sub-table followed by the same letter are not significantly different at the 5 per cent level of probability.

TABLE la

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EFFECTS OF K⁺ TREATMENTS ON GROWTH, AS MEASURED BY THE FRESH WEIGHT OF TOMATO SHOOTS GROWN ON $0.08N (NH_4)_2SO_4$ IN SOIL

Days	KCl. O	Concentration 0.04N	0.08N
	Fresh Weight of Plant	Shoot (gms)	
gan 115 000 000 000 or			
0	29. 8a	29. 8a	29. 8a
4	37.0ab	38.8ab	29.5 a
8	49.0ab	54.2b	41.5ab
12	50. 9ab	61.0bc	64.0bc
16	52.0ab	72.2bc	71.8bc
20	47.8ab	85.3c	78.5c
24	36.5ab	90.5c	84 .7c

Means within a sub-table followed by the same letter are not significantly different at the 5 per cent of prohability.

•	TABLE IC			
EFFECTS OF 1	K ⁺ TREATMENTS O	N THE AMMONIUM-1	J	
CONCENTRA	ATION IN TOMATO	LEAVES GROWN		
ON (0.08N (NH ₄) ₂ SO ₄	IN SOIL		
	•			
Days	KCl (Concentration 0.04N	0.08N	
Ammonium-N concentration of plant leaves (mg/g fresh weight)				
0	0.03a	0.03a	0.03a	
4	0.17ab	0.09ab	0.12ab	
8	0.54cd	0.34bc	0.19b	
12	0.58d	0.43c	0.21b	
16	0.66de	0.48cd	0.41c	
20	0.73e	0.7le	0.42c	
24 .	1.48g	1.00f	0.57d	

Means within a sub-table followed by the same letter are not significantly different at the 5 per cent of probability.

EFFECTS OF K ⁺ TREATMENTS ON THE AMIDE-N					
	CONCENTRATI	ION IN TOKATO	LEAVES GROWN		
	ON O.C	0311 (17H ₄) 250 ₄	IN SOIL		
Days	;		Oncentration 0.04N	0.0811	
		ncentration of			
	(1	ng/g fresh-wei	got)		
0	0.	. 04a	0.04a	0.042	
4	0.	2 8b	0.25b	0.265	
8	0.	32 b	0.365	0.325	
12	0.	,7 4d	0.395	0.33b	
16	1.	,24de	0.385	0.385	
20	1.	,44e	0.56c	0.38b	
24	1.	.54e	0.59c	0.42bc	

Means within a sub-table followed by the same letter are not significantly different at the 5 per cent of probability.

TABLE Id

	TABLE	Ie		
EFF	PECTS OF K ⁺ TREATHE	NTS ON THE TOTA	L-1!	
CC	NCENTRATION IN TOM	ATO LEAVES GRO.	MC	
	ON 0.08N (NH4)2	S) ₄ IN SOIL		
Days		Cl Concentratio		
Total-N concentration of tomato leaves (% dry weight)				
0	1.01a	1.01a	1.01a	
4	3.936	3.47cd	2. 58b	
8	4.47e	3.68cd	3.24c	
12	4.74ef	3.96d	3.24c	
16	4.99f	4.14de	3.33c	
20	5.021	4.43e	3.84d	
24	5.43fg	4.53ef	4.04d	

Means within a sub-table followed by the same letter are not significantly different at the 5 per cent of probability.

TABLE If

EFFECTS OF K^+ TREATMENTS ON THE K^+ CONCENTRATION OF TOMATO LEAVES GROWN ON 0.08N (NH₄)₂SO₄

IN SOIL

			4.7 4.4 au en 62 1.7 4.2 au en pa 14.
Days	K O K ⁺ concentration o: (% dry we:		0,08N
0	3.60c	3.60c	3.60c
4	2.10b	3.80c	4.50cd
8 .	2.10b	4.10cd	4.70d
12	1.70ab	4.20cd	5.00d
16	1.40ab	4.40cd	5.00d
20	1.40ab	4.70d	5.30e
24	1.10a	4.80d	6.90f

Means within a sub-table followed by the same letter are not significantly different at the 5 per cent of probability.

TABLE Ig

EFFECTS OF K⁺ TREATMENTS ON THE SOIL PH

SUPPLIED WITH 0.03N (NH4)2SO4

	85 88 m² nº as de as en as es as es as		
DAYS	О	l Concentration 0.04N	0.08N
0	5.30bc	5.30bc	5.30bc
4	4.90a	5.00ab	5.10b
8	5.40cd	5.50cd	5.50cd
12	5.50cd	5.60d	5.50cd
16	5.10b	5.00ab	5.00ab
20	5.60d	5.70d	5.60d
24	5.70d	5.70d	5.70d
	40 41 40 40 41 40 41 51 21 43 41 40 31		

Means within a sub-table followed by the same letter are not sifnificantly different at the 5 per cent level of probability. Experiment II. The Effects of Equinormal Concentrations of NH₄⁺ and K⁺ at Different Sampling Dates on the Ammonium Toxicity of Tomatoes

The results of a greenhouse soil experiment with tomatoes grown at four equinormal concentrations of NH_4^+ and K^+ is shown in Tables IIa - IIf. Stem Lesions

The occurrence of stem lesions was prevented at all four levels of equinormal concentrations of $(NH_4)_2SO_4$ and KCl (0.01N, 0.02N, 0.04N and 0.08N) (Table IIa). In the absence of KCl, the severity of stem lesions, in twenty-one days was moderate at 0.01N and 0.02N $(NH_4)_2SO_4$, but very severe lesion conditions were manifested at 0.04N and 0.08N $(NH_4)_2SO_4$. Without supplemental K⁺, the average occurrence of lesions increased with time.

Fresh Weight

Equinormal concentrations of NH_4^+ and K^+ (0.01N to 0.04N) increased the fresh weight of tomato shoots with time (Table IIb). At 0.08N NH_4^+ + K^+ , no significant increases in fresh-weight were observed after seven days of treatment. The application of 0.01N and 0.02N $(NH_4)_2SO_4$ alone, respectively, raised the fresh weight, while slight growth occurred at 0.04N $(NH_4)_2SO_4$ and further growth occurred at 0.08N $(NH_4)_2SO_4$. The application of equinormal concentrations of $NH_4^+ + K^+$ at 0.01N and 0.02N resulted in better growth of tomato shoots, when compared with NH_4^+ applied alone. However, lower equinormal concentrations (0.01N and 0.02N) of NH_4^+ + K^+ enhanced plant growth more than the higher concentrations at 0.04N and 0.08N.

Nitrogen Fractions

The application of $(MH_4)_2SO_4$ without KCl at concentrations of 0.01N to 0.08N, progressively increased the MH_4^+ - N, amide-N and

total-N concentrations with time (Table IIc; IId; IIe). The concentrations of NH_4^+ -N, amide-N and total-N were slight at 0.01N and 0.02N equinormal concentrations of NH_4^+ and K^+ , while concentrations of these soluble nitrogen compounds at 0.04N and 0.08N NH_4^+ and K^+ showed greater increases with time.

Potassium

The K⁺ concentration of tomato leaves was substantially lowered, when NH_4^+ was supplied alone (Table IIf). Gradual increases in K⁺ concentration with time were noticed from 0.01N to 0.08N equinormal concentrations of NH_4^+ and K⁺.

Soil pH

The soil PH of samples treated with 0.01N $(NH_4)_2SO_4$ without KCl, decreased significantly with time. The soil pH of the samples supplied with 0.02N, 0.04N and 0.08N $(NH_4)_2SO_4$ remained generally constant from 0-21 days. Soil samples supplied with 0.01N equinormal concentration of $NH_4^++K^+$ were not statistically different from those supplied with 0.01N $(NH_4)_2SO_4$ in the absence of K^+ . Similar trends were observed with the equinormal concentrations of 0.02N, 0.04N and 0.08N $(NH_4)_2SO_4$ + KCl when compared with 0.02N, 0.04N and 0.08N $(NH_4)_2SO_4$ respectively.

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EFFECTS OF EQUINORMAL CONCENTRATIONS OF AMMONIUM AND POTASSIUM AT DIFFERENT SAMPLING DATES

ON AMMONIUM TOXICITY AS MEASURED BY STEM LESION RATING IN TOMATOES IN SOIL CULTURE*

ഗ Means within a sub-table followed by the same letter are not significantly different at the per cent level of probability.

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FART F	

EFFECTS OF EQUINORMAL CONCENTRATIONS OF AMMONIUM AND POTASSIUM AT DIFFERENT SAMPLING DATES ON GROWTH, AS MEASURED BY THE FRESH WEIGHT OF TOMATO SHOOTS IN GRAMS, IN SOIL CULTURE

0.08N (NH ₄)2S04 + 0.08N KC1	28 50a	54.00b	62 . 25bc	400°11	at the 5
0.08N (NH4)2SO4	28 . 50a	37 . 00a	40•54b	33 . 61a	
0.04N (NH ₄)2S0 ₄ + 0.04N KC1	28 . 50a	62 . 75bc	98 . 00c	131. 50d	are not significantly different
0.04N (NH4)2SO4	28 . 50a	45 - 20b	45 . 70b	45•45b	letter are not
0.02N (NH ₄)2 ^{SO} 4 + 0.02N KCL	28 . 50a	63 . 75bc	106.25c	149.25de	same
0.02N NH4)2504	28 . 50a	69 505	91 . 75c	126.25d	Means within a sub-table followed by the
0.01N (NH ₄)2 ^{SO} 4 + 0.01N KCl	28 . 50a	62 . 25b c	126.50d	155 . 75e	la sub-table
0.01N (NH4,)2504	28 . 50a	64 • 50bc	131 . 50d	148 . 50de	Means within
DAYS	0	7	14	12	

per cent level of probability.

	·		LEAVE:	UN TEL ANMONTON-NITHOGEN CONCENTRATION NIG/ 9 FRESH WEIGHT) OF LOWING	VING 9 FILL	TO / TUOTAM L	OTUDOT	
DAYS	0,01N 0,01N 0,2SO	0.01N (NH ₄)2 ^{SO} 4 + 0.01N KCl	0•02N (NH ₄)2 ^{SO} 4	0.02N (NH ₄)2504 + 0.02N KC1	0.04N (NH ₄)2SO4	0.04N (NH ₄)2S0 ₄ + 0.04N KCL	0.08N (NH ₄)2504	0.08N (NH4)2504 + 0.08N KC1
0	0.01a	0 . 01a	0 . 01a	0.01a	0 . 01a	0.01a	0 . 01a	0.01a
7	0.12c	0 . 03ab	0.24e	0.12c	0 . 39£	0 . 22d	0•45g	0.24e
14	0.14c	0.055	0 . 39£	0.18ed	0•43g	0 . 22d	0.67h	0.44g
21	0 . 22d	0.06b	0.72h	0.21d	0.84î	0.43g	0.90i	0. 70h
per ce	Means within a sub-table cent level of probability.	n a sub-table probability.	Means within a sub-table followed by the nt level of probability.	•	same letter are not significantly different at the 5	: significant	:ly different	at the 5

TABLE IIC

EFFECTS OF EQUINORMAL CONCENTRATIONS OF AMMONIUM AND POTASSIUM AT DIFFERENT SAMPLING DATES

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EFFECTS OF EQUINORMAL CONCENTRATIONS OF AMMONIUM AND POTASSIUM AT DIFFERENT SAMPLING DATES

ON THE AMIDE-NITROGEN CONCENTRATION (mg/g FRESH WEIGHT) OF TOMATO LEAVES

GROWN IN SOIL CULTURE

0.08N (NH ₄)2S04 + 0.08N KC1	0 . 04a	0 . 09c	0.14d	0.33f	the 5
0 + 50 + 5 + HN 5 • O	0.0	0•0	0.1	•	ц. Б
0.08N (NH4)2SO4	0 . 04a	0.32f	1.21j	1•43k	significantly different
0.04N (NH ₄)2 ^{SO} 4 + 0.04N KC1	0 . 04a	0.10c	0.22e	0•31£	
0.04N 0.504	0 . 04a	0.39g	0.42h	0.501	letter are not
0.02N (NH ₄)2S0 ₄ + 0.02N KC1	0•04a	0.02d	0.13d	0.15d	same
0.02N (NH4)2504	0 . 04a	0.12d	0.29£	0•44h	Means within a sub-table followed by the nt level of probability.
0.01N (NH ₄)2 ⁵⁰ 4 + 0.01N KCl	0•04a	0 . 04a	d80-0	0.13d	n a sub-table probability.
0.01N (NH ₄)2504	0 . 04a	0.21e	0.21e	0 . 22e	Means within a sub-table per cent level of probability.
SYAG	0	7	14	77	per co

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EFFECTS OF EQUINORMAL CONCENTRATIONS OF ANMONIUM AND POTASSIUM AT DIFFERENT SAMPLING DATES

ON THE TOTAL-N CONCENTRATION (% DRY WEIGHT) OF TOWATO LEAVES GROWN IN SOIL CULTURE

0.08N (NH ₄)2S0 ₄ + 0.08N KC1	1.33a	4.84ef	5 • 24£	5.62£g
0.08N (NH4)2 ^{SO} 4	1. 33a	5.91g	5 . 95gh	6.05h
0.04N (NH ₄)2504 + 0.04N · KC1	1.33 a	4.81e	5。08ef	5.26£
0.04N (NH4)2S04	1. 33a	4.85ef	5 . 80g	e-00h
0.02N (NH ₄)2SO ₄ + 0.02N KCL	1 • 33a	3.205	4.41d	5 • 05ef
0.02N (NH ₄)2S04	1 • 33a	5.32£	5 . 54£g	5 . 79g
0.01N (NH4,)2S04 + 0.01N KC1	1 . 33a	3 . 53bc	3 . 75c	4. 35d
0.01N (NH4,)2 ^{SO} 4	1 .33a	4.24d	4.80c	5.07ef
DAYS	0	2	すて	21

Means within a sub-table followed by the same letter are not significantly different at the 5 per cent level of probability.

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EFFECTS OF EQUINORMAL CONCENTRATIONS OF AMMONIUM AND POTASSIUM AT DIFFERENT SAMPLING DATES

ON THE K CONCENTRATION (% DRY WEIGHTS) OF TOMATO LEAVES GROWN IN SOIL CULTURE

0.08N (NH ₄)2S0 ₄ + 0.08N KCl	2.87c	3.97de	4.31e	4 . 38e	
0_08N (NH4)2SO4	2 . 87c	2.32bc	d09.1	1. 05a	
0.04N (NH ₄)2SO ₄ + 0.04N · KCl	2.87c	3 . 94d	4.06de	4.13de	
0_04N	2 . 87c	2.45bc	2 . 00b	1.19a	
0.02N (NH ₄)2 ^{SO} 4 + 0.02N KC1	2 . 87c	2.78c	3.66d	3.97de	
0.02N (NH2)4504	2.87c	2 . 34bc	1.97b	1.19a	
0.01N (NH ₄)2 ^{SO} 4 + 0.01N KCL	2 . 87c	2 . 03b	3 . 75d	3.97de	
0.01N (NH ₄)2 ^{SO} 4	2 . 87c	2 . 05b	1.945	1.25 a	
DAYS	0	7	14	21	

Means within a sub-table followed by the same letter are not significantly different at the 5 per cent level of probability.

TABLE IIG

EFFECTS OF EQUINORMAL CONCENTRATIONS OF AMMONIUM AND

POTASSIUM AT DIFFERENT SAMPLING DATES ON THE SOIL PH

0.08N (NH ₄)2 ^{SO} 4 + 0.08N KC1	5 . 22d	5 . 50e	5.70e	5 . 52e	
0.08N (NH4)2504	5.22d	5.42de	5.08cd	5.40de	
0.04N (NH ₄) ₂ S0 ₄ + 0.04N KCL	5 . 22d	5 . 18d	5.12cd	5 . 05cd	
0.04N (NH ₄)2504	5 . 22d	5 . 20d	5.ldcd	5.10cd	
0.02N (NH ₄) ₂ S0 ₄ + 0.02N KC1	5 . 22d	4.90bc	4.70ab	4 . 58a	
0.02N (NH ₄)2504	5 . 22d	4.82b	4.98c	5 . 08cd	
0.01N (NH ₄)2S0 ₄ + 0.01N KC1	5 . 22d	4.95bc	4.65a	4.58a	
0.01N 0.01N 04,2504	5 . 22d	4.98c	4.94bc	4.72ab	
SXVQ	0	7	1.1	21	

Means within a sub-table followed by the same letter are not significantly different at the 5 per cent level of probability.

Experiment III. The Effects of K⁺ and CaCO3 on

Ammonium Toxicity Reversal on Tomatoes Grown

on 0.02N (NH₄)₂SO₄ in Sand Culture

In the experiment designed to show the effects of K^+ and $CaCO_3$ on the reversal of ammonium toxicity symptoms, in sand culture, tomato plants were first treated with 0.02N (NH₄)₂SO₄, until stem lesions appeared at the first degree of severity.

Stem Lesions

Although visible signs of stem lesion healing were noticed in some plants treated with 0.02N KCl or $CaCO_3$, this reversal was not statistically significant after seven days of treatments (Table IIIa). However, a remarkable disappearance of stem lesion was noticed, after fourteen days with 0.02N KCl + $CaCO_3$ treatment. Stem lesions became more severe with time, in plants receiving the 0.02N (NH₄)₂SO₄ treatment alone.

Fresh Weight

Growth, in terms of fresh weight (Table IIIb) showed significant increases in the 0.02N KCl, and 0.02N KCl + $CaCO_3$ after fourteen days, and $CaCO_3$ treatment, in seven days, while 0.02N (NH_4)₂SO₄ + 0.02N KCl was not significantly increased until twenty-one days of treatment. No improvement in growth was observed in plants subjected to 0.02N (NH_4)₂SO₄ treatment alone, after seven days.

Nitrogen Fractions

The application of 0.02N $(NH_4)_2SO_4$ in the absence of K⁺ increased the NH₄⁺-N, amide-N and total-N concentrations with time (Tables IIIc; IIId; IIIe). The replacement of 0.02N $(NH_4)_2SO_4$ with 0.02N KCl, almost completely eliminated the NH_4^+ -N and amide-N in seven days (Tables IIIc; IIId), while the total-N concentration (Table IIIc), gradually decreased with time over a period of twenty-one days. A combination of 0.02N KCl + CaCO₃ (previously added to the sand culture) gave a similar trend as 0.02N KCl alone. Treatment with CaCO₃ alone reduced the NH_4^+ -N concentration sharply in seven days (Table IIIc), while total-N concentration was gradually decreased with time (Table IIIc). The amide-N concentration (Table IIId) decreased rapidly in seven days, but rose slightly in fourteen days and twenty-one days. When 0.02N KCl + 0.02N (NH_4)₂SO₄ treatment was supplied, there was a decrease in NH_4^+ -N in seven and fourteen days, with a rapid increase in twenty-one days (Table IIIc).

Treatment with 0.02N KCl or 0.02N KCl + $CaCO_3$ showed no significant difference between the two treatments in the concentration of amide-N (Table IIId), but the 0.02N KCl + $CaCO_3$ treatment appeared to have a lower concentration of $NH_4^{+}-N$ (Table IIIe) in twenty-one days. Total-N concentration was reduced more by 0.02N KCl than by the 0.02N KCl + $CaCO_3$ treatment (Table IIIe). Plants treated with 0.02N KCl generally contained lower concentrations of $NH_4^{+}-N$, amide-N and total-N, than those of the 0.02N KCl + 0.02N (NH_4)₂SO₄ treatment (Tables IIIc; IIId; IIIe). Potassium

The percentage concentration of K⁺ found in plants treated with $0.02N (NH_4)_2SO_4$ decreased significantly with time, while the K⁺ content of plants subjected to 0.02N KCL, $0.02N KCL + CaCO_3$ and $0.02N (NH_4)_2SO_4$ + 0.02N KCl treatments respectively, increased considerably (Table IIIIf). However, in the CaCO₃ treatment, a sharp drop in K⁺ content was observed after fourteen days. There was a higher K⁺ concentration in plants with 0.02N KCI + CaCO3 at twenty-one days, than 0.02N KCl treatment.

TABLE IIIa

EFFECTS OF DIFFERENT CONCENTRATIONS OF POTASSIUM AND CALCIUM CARBONATE ON

AMMONIUM TOXICITY REVERSAL AS MEASURED BY STEM LESION RATINGS

AT 0.02N (NH₄)₂SO₄

0.02N (NH ₄)2 ^{SO} 4 ⁺ 0.02N KC1	000 • T	0.62ab	0 . 75b	1.12bc	
CaCO ₃	1.00b	0.50ab	0.25ab	0.25ab	0 8 0 0 1 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8
0.02N KC1 + CaCO ₃	1.00b	0.38ab	0 . 25ab	0 . 12a	
0.02N KC1	d00 - 1	0.50ab	0.25ab	0.25ab	
0_02N (NH ₄)2 ^{SO} 4	1.00b	1. 25bc	1.50c	1.50C	
DAYS	0	7	マト	21	

Means within a sub-table followed by the same letter are not significantly different at the 5 per cent level of probability.

TABLE IIIb

EFFECTS OF DIFFERENT CONCENTRATIONS OF POTASSIUM AND CALCIUM CARBONATE ON

GROWTH, AS MEASURED BY FRESH WEICHT OF TOMATO SHOOTS GROWN IN SAND

CULTURE AT 0.02N (M_A) $_2$ SO $_4$

0.02N (NI1 ₄)2 ⁵⁰ 4 ⁺ 0.02N KCI	49.50a	54.75ab	59.50ab	67.75bc	
CaCo_3	49.50a	79 . 25c	72 . 25bc	66 . COb	
0_02N KCL + CaCO ₃	49 . 50a	55.50ab	74.75bc	89 . 75 c	
0.02N KC1	49 . 50a	52.75ab	74.25bc	83 . 00c	
0.02N (NH ₄)2 ^{SO} 4	49 - 50a	62 . 75b	53 . 00ab	51.2 5ab	
DAYS	0	2	ΤŢ	51	

Means within a sub-table followed by the same letter are not significantly different at the 5 per cent level of probability

TABLE IIIC

DEFECTS OF DIFFERENT CONCENTRATIONS OF POTASSIUM AND CALCIUM CARBONATE ON THE AMMONIUM - NITROGEN CONCENTRATION (mg/g FRESH WEIGHT) OF TOMATO

LEAVES GROWN IN SAND CULTURE AT 0.02N (NH $_{4})_{2}\mathrm{SO}_{4}$

0.02N (NH _A) ₂ SO _A + 0.02N KC1	0.180	0.13d	0.15d	0.22cf	
caco ₃	0.18c	0.05c	0•04p	0.02a	
0.02N KCL + CaCO ₃	0.18c	d10.01b	0.03ab	0 . 02a	
0.02N KC1	0.180	0.03ab	0.03ab	d⊉0•0	
0.02N (NH ₄)2 ⁵⁰ 4	0.18c	0.22ef	0.27f	0 . 32g	
SXVQ	0	2	ЪĄ	21	

Means within a sub-table followed by the same letter are not significantly different at the 5 per cent level of probability.

TABLE IIId

EFFECTS OF DIFFERENT CONCENTRATIONS OF POTASSIUM AND CALCIUM CARBONATE ON THE AMIDE-NITROGEN CONCENTRATION (mg/g FRESH WEIGHT) OF TOMATO LEAVES GROWN IN SAND CULTURE AT 0.02N (NH₄) $_{2}$ SO₄

0.02N (NH ₄) ₂ SO ₄ + 0.02N KC1	0.100	0.100	0.13d	0.14d	
caco3	0.10c	0 . 04a	0 - 07b	0.06b	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8
0.02N KCl + CaCo ₃	0.10c	0.04a	0.01a	0.03a	
0 • 02N KC1	0.10c	0•04a	0.04a	0.04a	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
0_02N	0.10c	0.12d	0.12d	0.13d	
DAYS	0	2	77	21	1 1 1 2 1 8

Means within a sub-table followed by the same letter are not significantly different at the 5 per cent level of probability.

TABLE IIIe

EFFECTS OF DIFFERENT CONCENTRATIONS OF POTASSIUM AND CALCIUM CARBONATE ON THE TOTAL-NITROGEN CONCENTRATION (% DRY WEIGHT) OF TOMATO LEAVES GROWN

IN SAND CULTURE AT 0.02N (NH_4) $_2$ SO $_4$

0.02N (NH4)2504 0.02N KC1	3.21de	3.45e	3 . 82g	4.10fg	
caco ₃	3.21de	3 . 27de	2.47bc	2.47bc	
0.02N KCL + CaCO3	3 . 21de	3.05đ	2 . 63c	2 . 38b	
0.02N KC1	3.21de	2.82c	2.48bd	1. 92a	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
0.02N (NH ₄)2 ^{SO} 4	3.21de	3 . 76f	3 . 80£	4.31g	
DAYS	0	7	74	21	1 1 1 1 1 1

Means within a sub-table followed by the same letter are not significantly different at the 5 per cent level of probability.

TABLE IIIf

EFFECTS OF DIFFERENT CONCENTRATIONS OF POTASSIUM AND CALCIUM CARBONATE ON THE POTASSIUM CONCENTRATION (% DRY WEIGHT) OF TOMATO LEAVES GROWN IN

SAND CULTURE AT 0.02N (NH₄) $_{2}$ SO₄

0.02N (NH ₄)2 ^{SO₄+ 0.02N KCI}	2.25b	3 . 72d	3 . 81d	4. 49C	
CaCO ₃	2.255	3 - 00c	2.11b	1.04a	
0.02N KCl + CaCO ₃	2 . 25b	4.44e	4 . 56e	1-94£	
0_02N KC1	2.25b	4.00de	4. d.6e	4 . 66e	
0_02N (NH4)2S04	2 . 25b	3 . 72d	2.41b	2.19b	
, SYA	, 0	2	14	21	

Means within a sub-table followed by the same letter are not significantly different at the 5 per cent level of probability.

Experiment IV. The Effects of K⁺ at Different

Stem Lesion Ratings and Sampling Dates, on

the Reversal of Ammonium Toxicity of

Tomatoes Grown on 0.04N (NHA) SOA

in Soil Culture

A second experiment on the reversal of ammonium toxicity by K^+ was carried out in soil culture. A 0.04N $(NH_4)_2SO_4$ solution was supplied at 250 ml per plant daily until lesions were formed at 1 (after three days), 2 (after six days), and 3 (after eight days) degree-ratings. The results are reported in Tables IVa - IVe.

Stem Lesions

Ammonium toxicity reversal, as measured by the disappearance, or healing of stem lesions was evident at ratings1 and 2, fourteen days after the initiation of treatment with 0.04N KCl alone (Table IVa). No significant lesion disappearance was noticed at lesion rating 3 with 0.04N KCl treatment. Treatment with 0.04N KCl + 0.04N $(NH_4)_2SO_4$ did not bring about a reversal of lesions at any level of lesion formation. The severity of stem lesions in all cases increased with time, with 0.04N $(NH_4)_2SO_4$ application.

Fresh Weight

The fresh weight (Table IVb) of tomato shoots supplied with 0.04N KCl at degrees 1, 2 and 3 lesion ratings indicated substantial gains seven days, after the initiation of treatments and these increases continued until fourteen days. When stem lesions were slight, or moderate, plant growth showed substantial improvement with time, with the application of 0.04N KCl + 0.04N (NH_4)₂SO₄. Under severe lesion conditions, with the same treatment, significant increases in fresh weight of the shoots were observed after only seven days. Plants supplied with deionized water showed smaller gains in fresh weight with time, at the various degrees of lesion severity than those receiving 0.04N KCl. Continuous application of 0.04N $(NH_4)_2SO_4$ with time (Table IVb), resulted in continuous plant growth at lesion ratings 1 and 2, but caused a decrease with time at the 3 lesion rating. Nitrogen Fractions

At each degree of lesion severity, 0.04N KCl significantly reduced NH_4^+ -N (Table IVc) and amide-N (Table IVd) in seven days after treatment; the total-N concentration steadily decreased over the period of fourteen days (Table IVe). The application of 0.04N $(\text{NH}_4)_2\text{SO}_4 + 0.04\text{N}$ KCl at 1, 2 and 3 degrees of lesion severity had no significant effects on NH_4^+ -N (Table IVc), but resulted in a reduction of amide-N (Table IVd) and total-N (Table IVe) concentrations in the tomato leaves with time. The reversal of NH_4^+ -N (Table IVc), amide-N (Table IVd) and total-N (Table IVe) concentrations with time, with the application of deionized water was as effective as KCl at the lowest lesion rating, but its effect on the amide-N (Table IVd) was less pronounced. At the 2 and 3 levels of lesions, deionized water was generally less effective than KCl in reversing NH_4^+ -N, amide-N and total-N concentrations, than it was at the first lesion rating.

Potassium

The K^+ concentrations were significantly increased in all degrees of lesion severity with the 0.04N KCl treatment with time (Table IVf). The concentration of K^+ found in plants treated with 0.04N

 $(NH_4)_2SO_4^{-} + 0.04N$ KCl (Table IVf) increased with time at the moderate and severe lesion ratings, but increased significantly only during the first seven days at the first lesion rating. The K⁺ concentrations in the plants treated with 0.04N KCl and 0.04N $(NH_4)_2SO_4 + 0.04N$ KCl (Table IVf) showed no significant difference between each other at 1 lesion rating. However, higher K⁺ concentration was found in the 0.04N KCl treatment, after seven days of treatment at 2 and 3 lesion ratings, than the 0.04N $(NH_4)_2SO_4 + 0.04N$ KCl treatment.

TABLE IVa

EFFECTS OF POTASSIUM AT DIFFERENT LESION RATINGS (1-3)

SAMPLING DATES ON AMMONIUM TOXICITY REVERSAL MEASURED BY THE DISAPPEARANCE OF <u>STEM LESIONS</u> ON TOMATOES IN SOIL CULTURE PREVIOUSLY TREATED WITH 0.04N (NH₄)₂SO₄

an na an an dir 64 45 r	. Fi An an an go tr DD or an an an an an an an an		a din dife dala ank dina ura bit nas ank dina ang ank ank	a and the and the every day and day are and and and		
	TREATMENTS					
			0.04N (NH ₄)2504			
DAYS	0.04N (NH ₄)2 ^{SO} 4	0.04N · KCl	+ 0.04N KCl	Deionized Water		
aar aa ro aa aa a o o	n an an an go re an dd nn de be dd ne dd an de de d	10 24 56 <u>36</u> 24 28 0 9 94 94 86 56 96 96 56 4	. 202 010 111 011 011 011 010 010 010 011 011 011 011			
			<u>1 Le</u>	esion (slight)		
0	1.00b	10 0b	1.00b	1.00b		
7	1.25b	1.00b	1.00b	1.00b		
14	2.50e	0.3 8a	1.00b	1.25bc		
			2° Lesi	on (Moderate)		
0	2.00d	2.00d	2.00d	2.00d		
.7	2.50e	2.00d	2.00d	2.25e		
14	3.00f	1.50c	2.00d	2.75ef		
			<u>3</u> ° Le	esion (Severe)		
0	3.00f	3.00f	3.00f	3.00f		
7	3.00f	3.00f	3.00f	3.00f		
11	3.00f	2.75ef	3.00f	3.00f		

Means within the sub-table followed by the same letter are not significantly different at the 5 per cent level of probability. TABLE IVb

EFFECTS OF POTASSIUM AT DIFFERENT LESION RATINGS (1-3) AND SAMPLING DATES ON GROWTH, AS MEASURED BY <u>FRESH WEIGHT</u> OF TOMATO SHOOTS (gms) PREVIOUSLY TREATED WITH

0.04N (NH_4)₂SO₄ IN SOIL CULTURE

	TREATMENTS					
			0.04N (NH ₄)2504			
DAYS	0.04N (NH ₄)2SO4	0.04N KCl	+ 0.04N KCl	Deionized Water		
an an do do tro no ro n			<u>l° Les</u>	sion (Slight)		
0	66.50ab	66.50ab	66.50ab	66.50ab		
7	· 99.75bc	130.25cd	116.75cd	96.50bc		
14	71.75b	151,25d	195.75e	142.25cd		
			2° Lesic	on (Moderate)		
0	62.25ab	62.25ab	62.25ab	62.25ab		
7	93.00bc	122.50cd	113.75c	95.00bc		
1.4	41.25ab	160.00d	178.75de	143.75dc		
			3° Les	sion (Severe)		
0	60.75ab	60.75ab	60.75ab	60.75ab		
7	89.64bc	117.00cd	121.00cd	93.00bc		
14	31.50a	138.00cd	137.00cd	123.73cd		

Means within the sub-table followed by the same letter are not significantly different at the 5 per cent level of probability.

TABLE IVC

EFFECTS OF POTASSIUM AT DIFFERENT LESION RATINGS (1-3) AND SAMPLING DATES ON THE <u>AMMONIUM-NITROGEN</u> (mg/g FRESH WEIGHT) CONCENTRATION IN THE LEAVES OF TOMATO PLANTS PREVIOUSLY TREATED WITH 0.04N (NH₄)₂SO₄ IN SOIL CULTURE

	n na an All an 62 an 69 an an an an 29 an 29 an 49 an a			77 Aug 487 AVE 107 AVE 498 AVE 415 AUE 408 AUE 417 \$10			
		TREATMENTS					
		0.04N (NH ₄) ₂ SO ₄					
DAYS	0.04N (NH ₄) ₂ SO ₄	0.04N KCl	+ 0.04N KCl	Deionized Water			
ga an da an 64 as 64 a		a da an an 900 90 90 90 90 90 90	<u>1° Le</u>	sion (Slight)			
0	0.31bc	0.31bc	0.31bc	0.31bc			
7	0.53d	0 . 05a	0.18b	0.07a			
14	0.76e	0.04a	0.16ab	0 . 03a			
			2° Lesi	on (Moderate)			
0	0.38cd	0.38cd	0.38cd	0.38cd			
7	0.44cd	0.05a	0.36cd	0.185			
14	1.17f	0.04a	0.21bc	0.07a			
			3 ⁰ Le	sion (Severe)			
0	0.46cd	0.46cd	0.46cd	0.46cd			
7	0.81e	0.05a	0.33c	0.21bc			
14	1.20f	0.05a	0.35cd	0.21bc			

Neans within the sub-table followed by the same letter are not significantly different at the 5 per cent level of probability.

TABLE IVd

EFFECTS OF POTASSIUM AT DIFFERENT LESION RATINGS (1-3) AND SAMPLING DATES ON THE <u>AMIDE-NITROGEN</u> (mg/g FRESH WEIGHT) CONCENTRATION IN THE LEAVES OF TOMATO PLANTS PREVIOUSLY TREATED WITH 0.04N (NH_4)₂SO₄ IN SOIL CULTURE

40 4 , 4 , 5 , 5 , 5	n an an an Mil An 68 48 an 68 m 68 an 68 an 68 an 6		an 60 an an 60 pn an n 60 an as as at 6.2			
	TREATMENT					
			0.04N (NH ₄)2SO4			
DAYS	0.04N (NH ₄)2 ^{SO} 4	0.04N KCl	+ 0.04N KCl	Deionized Water		
	3 0* 00 PT 0* 0* 0* 00 0* 00 0* 00 0* 00 0* 00 00		<u>l° Le</u>	esion (Slight)		
0	0.32b	0.32b	0.32b	0.32b		
7	0.33b	0.09a	0 . 17a	0.19a		
14	0.48b	0.05a	0.16a	0 .].3a		
			2° Lesi	.on (Koderate)		
0	0.44b	0.44b	0.44b	0.44b		
7	0.65b	0.11a	0.31b	0.28a		
14	2.34c	0.09a	0.19a	0.16a		
			3° LC	esion (Severe)		
0	0.52b	0.52b	0.52b	0.52b		
7	0.85b	0.11a	0.24a	0 . 26a		
14	3.63d	0.11a	0.38a	0 . 26a		
		and any data and and and and any or a diffe data for				

Means within the sub-table followed by the same letter are not significantly different at the 5 per cent level of probability.

TABLE IVe

 \mathbf{z}

EFFECTS OF POTASSIUM AT DIFFERENT LESION RATINGS (1-3) AND SAMPLING DATES ON THE TOTAL <u>NITROGEN CONCENTRATION</u> (% DRY WEIGHT) IN THE LEAVES OF TOMATO PLANTS PREVIOUSLY TREATED WITH 0.04N (NH₄)₂SO₄ IN SOIL CULTURE

gas ann ann dait ann dhà ma n-	. 29 84 24 86 94 86 84 94 24 24 25 28 74 94 94 94 26 27 24					
	TREATMENT					
DAYS	0.04N (NH ₄)2 ^{SO} 4	0.04N KCl	0.04N (NH ₄)2 ^{SO} 4 + 0.04N KCl	Deionized Water		
			l [°] Le.	sion (Slight)		
0	5.63d	5.63d	5.63d	5.63d		
7.	6.05de	3.91bc	5.08cd	4.60c		
l	6.34e	2. 15a	4.59c	3.08d		
			2° Lesi	on (Moderate)		
0	5.88de	5.88de	5.88de	5.88de		
7	6.82e	4.44C	5.68d	5.54d		
14	6.83e	2.75ab	4.83c	3.86bc		
			<u>3° Le</u>	sion (Severe)		
0	6.00de	6.00de	6.00de	6.00de		
7	6.24de	4.01bc	5.72d	5.68d		
14	7.22e	3.50bc	5.74d	5.16cd		

Means within the sub-table followed by the same letter are not significantly different at the 5 per cent level of probability.

TABLE IVF

EFFECTS OF POTASSIUM AT DIFFERENT LESION RATINGS (1-3) AND SAMPLING DATES ON THE <u>POTASSIUM CONCENTRATION</u> (% DRY WEIGHT) IN THE LEAVES OF TOMATO PLANTS PREVIOUSLY TREATED WITH 0.04N (NH₄)₂SO₄ IN SOIL CULTURE

	1 an 22 62 44 an an de 65 ar 54 65 69 ar 60 14 ar 6	n dah ari dali ma din dar an an un das ka dar an		10			
		TREATMENT					
		0.04N (NH ₄) ₂ SO ₄					
DAYS	0.04N (NH ₄)2 ^{SO} 4	0.04N KCl	+ 0.04N KCl	Deionized Water			
		11 (112 (112 (112 (112 (112 (112 (112 (an an an an dd er an an an Yn en eu dd r	ne als nin win fan det win sep wa win das det der det			
			l [°] Le:	sion (Slight)			
0	3,34de	3.34de	3.34de	3.34de			
7	3.12de	4.06f	4.22fg	2.34c			
14	2.37c	5.19gh	5.06g	1.88b			
			2° Lesi	on (Moderate)			
0	2.97d	2.97d	2.97d	2. 97d			
7	2.06bc	4.91g	3.72ef	2.41c			
14	2.00bc	6.22i	4 .99g	2.00bc			
			3° Le	sion (Severe)			
0	2.19bc	2.19bc	2.19bc	2.19bc			
7	2.16bc	4.78g	3.56e	1.82b			
14	2.13bc	6.44i	5.53h	1.60a			
		200 was date was dithe was date date date date was date are					

Means within the sub-table followed by the same letter are not significantly different at the 5 per cent level of probability.

DISCUSSION

The data presented in Tables I to IV show that excessive application of NH_4^+ , as a nitrogen source to tomato plants (Heinz 1350) could cause ammonium toxicity. This is in agreement with the findings from several experiments (9, 13, 44). Continuous NH_4^+ supply, in the absence of K⁺ could hinder plant growth at 0.04N and 0.08N (Tables 1b, IIb), induce tomato stem lesions at 0.01N, 0.02N, 0.04N and 0.08N (Tables Ia, IIa, IIIa, and IVa), and caused a general increase in the concentrations of NH_4^+ -N, amide-N and total-N (Tables I - IV) with time.

Lower concentrations of $(NH_4)_2SO_4$ at 0.01N and 0.02N (Table IIb) had a less deleterious effect on growth and gave lower internal concentrations of the soluble nitrogenous compounds, than the higher concentrations at 0.04N and 0.08N (Tables IIc, IId, IIe). This was probably due to the less toxic effects of NH_4^+ ions at 0.01N and 0.02N, than at 0.04N and 0.08N.

Barker et al. (10) have pointed out the injurious effects of NH_4^+ toxicity on the epidermal and cortical cells of the affected stems. It seems possible that the external manifestation of excess NH_4^+ , in the appearance of stem lesions may be due to the collapse and death of these cells.

In healthy plants, NH_4^+ supplied at low concentrations and in limited quantity is generally incorporated into non-toxic organic compounds (75). Using ¹⁵N labelling, Barker <u>et al.</u> (14) found that the major portion of the ammonium and free amino-acids in NH_4^+ toxic plants were of endogenous origin. Hence, he suggested that protein degradation

might have occurred. The high concentrations of total-N observed as a result of $(NH_4)_2SO_4$ supply (Tables Ie, IIe, IIIe, IVe), when K⁺ was withheld may be due to protein degradation. Increases in the NH_4-N concentrations in the tomato leaves, may have been caused either by the presence of uncombined NH_4^+ absorbed by the plants, or from protein breakdown.

In plants suffering from ammonium toxicity, the amides and especially asparagine have been detected in considerable amounts in the soluble organic nitrogen concentration (3, 13, 20). Steward and Preston (61) proposed that amides served as nitrogen storage compounds, ready to donate N to synthesis reactions. The high concentration of amide-N under NH_4^+ -toxic conditions may be made possible by the fact that the availability of amino-acids and uncombined NH_4^+ accelerated the synthesis of more amides. Or, amide synthesis and the synthesis of amino-acids proceeded at a rate that made some amino-acids unavailable for protein synthesis. Barker (7) seemed to support the latter concept.

The application of 0.04N KCl, along with 0.08N $(NH_4)_2SO_4$ reduced the severity of stem lesions (Table Ia), improved plant growth (Table Ib) and decreased the concentrations of NH_4^+ -N (Table Ic), amide-N (Table Id) and total-N (Table Ie) in tomato leaves with time, relative to $(NH_4)_2SO_4$ treatment without K⁺. This indicated that K⁺ might prevent or reduce NH_4^+ toxicity symptoms. This indication was further confirmed, by the observation that the addition of 0.08N KCl to 0.08N $(NH_4)_2SO_4$, completely prevented stem lesion formation (Table Ia) and further reduced the concentrations of NH_4^+ -N (Table Ic), amide-N (Table Id) and total-N (Table Ie). Similar actions of K⁺ have been observed (7, 9, 14).

The application of various levels of equinormal concentrations of $\text{NH}_4^+ + \text{K}^+$ at 0.01N, 0.02N, 0.04N and 0.08N showed that stem lesions were completely prevented (Table IIa), growth, as measured by fresh weight was enhanced (Table IIb), and there was a general reduction in the concentrations of NH_4^+ -N (Table IIc), amide-N (Table IId) and total-N (Table IIe), when compared with plants fed with similar concentrations of $(\text{NH}_4)_2\text{SO}_4$ without K⁺ respectively.

In assessing the relative reduction of the soluble nitrogenous compounds, by comparing the concentrations of the various levels of equinormal concentrations of $\mathrm{NH}_4^+ + \mathrm{K}^+$, with $(\mathrm{NH}_4)_2\mathrm{SO}_4$ treatments without K^+ , it may be concluded that the lower equinormal concentrations of $\mathrm{NH}_4^+ + \mathrm{K}^+$ at 0.01N and 0.02N brought about a greater reduction of $\mathrm{NH}_4^+ - \mathrm{N}$, amide-N and total-N (Tables IIc, IId, ITe), and a better plant growth (Table IIb), than the higher concentrations at 0.04N and 0.08N. Despite the fact that equal concentrations of K^+ and NH_4^+ were present, NH_4^+ ions might have caused toxicity symptoms at a rate too fast for the K^+ ions to neutralize their effects, or the salt concentration may have been too high for growth.

The finding that K^+ imparts a particular configuration to certain enzymes, e.g., pyruvic kinase (28, 31) has given attention to its possible role in maintaining the structure of proteins. NH_4^+ and K^+ ions are similar in ionic radii and may substitute for each other in the isomorphic series (9). These two ions differ in that NH_4^+ will form H-bonds with the oxygen-containing, or other groups of the protein molecule. Hydrogen bonds are very important to the tertiary structure of proteins (9), and if broken, the protein may be subjected to

61. -

proteolytic action. Hence, the action of NH_4^+ could possibly be to cause a disruption of the protein structure to a sufficient extent as to enhance proteolysis (7, 9). One way to counter NH_4^+ action, would be to increase the K⁺ supply. The function of K⁺, therefore, could be in the maintenance of the protein molecule configuration. This may explain the growth improvement and the reduction in the concentrations of NH_4^+-N , amide-N and total-N. Barker and Bradfield (8) further explained that with higher K⁺ nutrition, more of the absorbed NH_4^+ was being used for the synthesis of insoluble organic N compounds. As a result, a smaller concentration of N was accumulating in the amides.

From the data on Table III, it is evident that 0.04N KCl, 0.04N KCl + $CaCO_3$ and $CaCO_3$ respectively, caused reductions in lesion ratings (Table IIIa), improved plant growth (Table IIIb), and decreased the concentrations of NH_4^+ -N, (Table IIIc), amide-N (Table IIId) and total-N (Table IIIe) with time, after the tomato plants had been subjected to an initial $(NH_4)_2SO_4$ treatment until lesions were formed at 1 lesion rating. This result suggests that these three treatments were capable of reversing the ammonium toxicity effects in tomato plants.

Calcium carbonate has been known to facilitate K^+ absorption into plant roots (30). Barker (7) found more K^+ in the roots of bean plants, when CaCO₃ was added to the nutrient medium, than when it was withheld. CaCO₃ is very effective in neutralizing the acidity caused by the absorption of NH_4^+ from a nutrient medium, without the formation of alkaline conditions (7, 30). A result similar to that obtained in Table III was observed in bean plant, with regards to the CaCO₃ effect. CaCO₃ reduced IH_4^+ content of the shrots and prevented protein degradation (7). Thus, it was postulated that the presence of $CaCO_3$ in the culture medium induced the stabilization of the plant proteins and caused the deceleration of protein degradation, which occurred under conditions of prolonged NH₄⁺ nutrition (7). It is also possible that the presence of $CaCO_3$ reduced the transport of NH₄⁺ to the tomato leaves and enhanced amide synthesis in the roots as reported by Maynard and Barker (42).

The treatment of plants with 0.04N (NH4)2SO4 + 0.04N KCl had a very slight effect in reversing NH4 toxicity, i.e., stem-lesion rating (Table IIIa) and $NH_{A}^{+}-N$ (Table IIIc), amide-N (Table IIId), and total-N (Table IIIe) concentration. After seven days of treatment, ammonium toxicity symptoms again became apparent. Two reasons could be advanced for this observation. (a) The application of more NH_{2}^{+} ions, after the initial treatment with (NH₄)₂SO₄ to develop lesion 1 degree rating, might have prevented or inhibited K^+ effect, possibly by NH_4^+ ions competing with K⁺ ions for absorption by plants or competition within the plants. (b) K^+ might have brought about NH_A^+ toxicity reversal to its fullest capacity, for a short time and the presence of more NH_A^+ ions might have countered the reversal effect of K⁺. The first explanation seems justified, because the K⁺ concentrations in Table IIIf, at 0.04N (NH₄)₂SO₄ + 0.04N KCl treatment, after pretreatment with NH₄⁺ were lower than the same concentration of K^+ in 0.04N KCl applied alone. The second explanation, also merits further consideration, judging by the fact that $NH_{A}^{+}-N$ (Table IIIc), amide-N (Table IIId) and lesion rating (Table IIIa) were depressed in the first seven days of treatment, and later worsened in fourteen and twenty-one days of treatment. It appears that both processes might take place in the plant.

 $\rm NH_4^{++}$ toxicity reversal could be effected by KCl treatment at 1, 2 and 3 lesion ratings (Tables IVa - IVe). Deionized H₂O was also effective in reversing (Tables IVb, IVc, IVd, IVe) $\rm NH_4^{++}$ toxicity symptoms, although H₂O was generally less effective than K⁺. In the presence of deionized H₂O, nitrification might have converted $\rm NH_4^{++}$ to $\rm NO_3^{--}$, and since no additional $\rm NH_4^{++}$ was supplied, the $\rm NH_4^{++}$ level eventually declined to nothing.

No differences in soil pH were observed when different concentrations of $(NH_4)_2SO_4$ were compared with the different concentrations of $(NH_4)_2SO_4$ + KCl supplied to the soil (Tables Ig, IIg).

SUMMARY

The results of the investigations conducted in this thesis revealed that prolonged fertilization or absorption of NH_4^+ ions supplied as $(NH_4)_2SO_4$, to tomato plants at 0.01N, 0.02N, 0.04N and 0.08N, in the absence of K⁺ caused ammonium toxicity. Ammonium toxicity was manifested in the appearance of stem lesions, which increased in severity with time, upon continuous NH_4^+ application. Other ammonium toxicity symptoms are growth restriction, as measured by the fresh weight of shoot, and high concentrations of NH_4^+ -N, amide-N and total-N in the plant tissue.

The presence of K^+ , in equinormal concentrations of $NH_4^+ + K^+$ at 0.01N, 0.02N, 0.04N and 0.08N completely prevented the formation of stem lesions, enhanced plant growth and considerably reduced the concentrations of NH_4^+ -N, amide-N and total-N in tomato leaves. Increasing concentrations of K^+ supplied to the plants, brought about increases in the concentrations of K found in the leaves.

When ammonium toxicity was induced by pretreating tomato plants with $(NH_4)_2SO_4$, it was observed that either K⁺ or $CaCO_3$ or a combination of both K⁺ and $CaCO_3$ could cause ammonium toxicity reversal. Deionized H₂O brought about some reversal, but it was not as effective as K⁺.

Supplying the soil with $(NH_4)_2SO_4$ alone compared with $(NH_4)_2SO_4$ + KCl did not effect any significant change in the soil pH.

LITERATURE CITED

- 1. Adams, W. E. and M. Twersky. 1960. Effect of soil fertility on winter killing of Coastal Bermuda-grass. Agron. J. 52: 325-326.
- Arrington, L. and J. Shive. 1935. Rates of absorption of ammonium and nitrate nitrogen from culture solutions of ten-day-old tomato seedlings at two pH levels. Soil Sci. 39: 431-435.
- Austin, A. 1959. The assimilation of inorganic nitrogen by nitrogen-starved wheat seedlings. Oyton, Gaspar, Campos 841, Vicente Lopez, FNGBM, Argentina. 12 (1): 43-57.
- Austin, A. 1960. The effect of inorganic nitrogen on the respiration of excised wheat roots, supplied with organic carbon.
 I. The effect of nitrate and some reduced forms of inorganic nitrogen on the endogenous and exogenous respiration. Indian J. of Plant Physiol. Vol. III, No. 2.
- Austin, A. 1960. The effect of inorganic nitrogen on the respiration of excised wheat roots supplied with organic carbon.
 III. Anomalous effects of high concentration of pyruvic acid on respiration Indian J. Plant Physiol. Vol. III. No. 2.
- Bange, G. G. T. and E. V. Vliet. 1961. Translocation of potassium and sodium in intact maize seedling. Plant and Soil 15: 312-328.
- 7. Barker, A. V. 1965. Effect of calcium carbonate and potassium on ammonium assimilation of bean plants. Agron. Abstr., 57th Ann. Meeting, Am. Soc. Agron., Columbus, Ohio. p. 100.
- Barker, A. V. and R. Bradfield. 1963. Effect of potassium and nitrogen on the free amino-acid content of corn plants. Agron. J. 55: 465-470.
- 9. Barker, A. V., D. N. Maynard and W. H. Lachman. 1967. Induction of tomato stem and leaf lesions, and potassium deficiency by excessive ammonium nutrition. Soil Sci. 103. 319-327.
- Barker, A. V., D. N. Maynard, W. H. Lachman and G. S. Puritch. 1967. Anatomical studies of ammonium-induced stem lesions in tomato. Hort. Sci. 2: 159-166.
- 11. Barker, A. V. and R. J. Volk. 1964. Determination of ammonium-, amide-, and total-nitrogen in plant extracts, by a modified Kjeldahl method. Analytical Chem. 36: 439-441.
- Barker, A. V., R. J. Volk and W. A. Jackson. 1965. Effects of ammonium and nitrate nutrition on dark respiration of excised bean leaves. Crop Sci. 5: 439-444.

- 13. Barker, A. V., R. J. Volk and W. A. Jackson, 1966. Growth and nitrogen distribution patterns in bean plants subjected to ammonium nutrition. Soil Sci. Soc. Am. Proc. 30: 228-232.
- Barker, A. V., R. J. Volk and W. A. Jackson. 1966. Root environment acidity as a regulatory factor in ammonium assimilation by the bean plant. Plant Physoil. 41: 1193-1199.
- 15. Black, C. A. 1968. Soil Plant Relationships, 2nd edition, John Wiley and Sons, Inc. New York. pp. 405-543; 654-763.
- 16. Bonner, J. 1945. The role of organic matter, especially manure, in the nutrition of rice. Botan. Gaz. 108: 267-278.
- Brooks, C. 1914. Blossom-end rot of tomatoes. Phytopath.
 4: 345-373.
- 18. Buckman, H. O. and N. C. Brady, 1960. The nature and properties of soils. 6th edition. Macmillan Co. p. 452.
- 19. Cain, J. C. 1952. A comparison of ammonium and nitrate nitrogen for blueberries. Proc. Am. Soc. Hort. Sci. 59: 161-166.
- 20. Cocking, E. C. and E. W. Yemm. 1961. Synthesis of amino-acids and proteins in barley seedlings. New Phytologist 60: 103-116.
- 21. Coleman, R. G. and F. J. Richards. 1956. Physiological studies in plant nutrition. XVIII. Some aspects of nitrogen metabolism in barley and other plants in relation to potassium deficiency. Ann. Bot. N. S. 20: 397-402.
- 22. Colgrove, M. S., Jr. and A. N. Roberts. 1956. Growth of the Azalea as influenced by ammonium and nitrate nitrogen. Proc. Am. Soc. Hort. Sci. 68: 522-536.
- 23. Donald, L., H. J. Stangel and J. T. Pesek, Jr. 1963. Advances in knowledge of nitrogen fertilization in the USA since 1950. In: Fertilizer technology and usage, McVickar, M. H. Bridger, G. L., Nelson, L. B. eds. Soil Sci. Soc. Amer., Madison, Wisc. pp 75-87.
- 24. Dubey, H. D. 1959. Influence of nitrogen, phosphorus and potassium fertilizer on the mortality of peanut caused by root rot disease. Agron. J. 51: 369-370.
- 25. Epstein, E. 1956. Mineral nutrition of plants: mechanism of uptake and transport. Ann. Rev. Plant Physiol. 7: 1-24.
- 26. Epstein, E. 1960. Space barriers and ion carriers. Ion absorption by plants. Am. J. Bot. 47: 393-399.
- 27. Epstein, E. and C. E. Hagen. 1952. A kinetic study of the

absorption of alkali cations by barley roots. Plant Physiol. 27: 457-474.

- 28. Evans, H. J. 1963. Effect of potassium and other univalent cations on activity of pyruvate kinase in Pisum Sativum. Plant Physiol. 38: 397-402.
- 29. Folkes, B. F., A. J. Willis and E. W. Yemm. 1952. The respiration of barley plants VIII. The metabolism of nitrogen and respiration in seedlings. New Phytologists 51: 317-341.
- 30. Gouny, P. 1955. Role de calcaire dans l'assimilation de l'ozote ammoniacal. C. R. Acad. Sci. 241: 95-97.
- 31. Gregory, F. G. and F. J. Richards. 1929. Physiological studies in plant nutrition: I. The effect of manurial deficiency in the respiration and assimilation rate in barley. Ann. Not. (London) 43: 119-161.
- 32. Haghiri, F. 1960. Influence of macronutrient elements on the amino-acid composition of soybean plants. Agron. J. 58: 609-612.
- 33. Hartt, C. E. 1934. Some effects of potassium upon the growth of sugar cane and upon the absorption and migration of ash constituents. Plant Physiol. 9: 399-452.
- 34. Hattori, A. 1958. Studies on the metabolism of urea and other nitrogenous compounds in Chlorella Ellipsoidea II. Changes in levels of amino-acids and amides during the assimilation of ammonia and urea by nitrogen-starved cells. J. Biochem (Japan). 45: 57-64.
- 35. Hewitt, E. J. 1963. The essential nutrient elements: Requirements and intractions in plants: In: <u>Plant Physiology</u>, a treatise, F. C. Steward, ed. Academic Press, New York. 1963.
- 36. Hoagland, D. R. and D. I. Arnon. 1950. The water-culture method for growing plants without soil. Calic. Expt. Sta. Cir. p. 347.
- 37. Kirby, E. A. 1958. The influence of ammonium and nitrate nutrition on the cation-anion balance of nitrogen and carbohydrate metabolism of white mustard plants grown in dilute nutrient solution. Dept. of Agric. Chem. The Univ. of Leeds 2. England.
- 38. Krantz, B. A. and W. V. Chandler. 1951. Lodging, leaf composition, and yield of corn as influenced by heavy applications of nitrogen and potassium. Agron. J. 43: 547-552.
- 39. Krogman, D. W., A. T. Jagendorf and M. Avron. 1959. Uncouplers of spinach chloroplast photosynthesis phosphorylation. Plant Physiol. 34: 272-277.

- 40. Lagerweff, J. V. and M. Peech. 1961. Relation between exchange absorption and accumulation of calcium and rubidium by excised barley roots. Soil Sci. 91: 84-93.
- 41. MacLeod, L. B. and R. B. Carson. 1966. Influence of potassium on the yield and chemical composition of grasses grown in hydroponic culture, with 12; 50 and 70% of the nitrogen supplied as ammonium. Agron. J. 58: 52-57.
- 42. Maynard, D. N., W. S. Barham and C. L. McCombs. 1957. The effect of calcium nutrition on tomatoes as related to the incidence and severity of Blossom-end rot. Proc. Amer. Soc. Hort. Sci. 69: 318-322.
- 43. Maynard, D. N. and A. V. Barker. 1969. Studies on the tolerance of plants to ammonium nutrition. J. Amer. Soc. Hort. Sci. 94: 235-239.
- 44. Maynard, D. N., A. V. Barker and W. H. Lachman. 1966. Ammonium-induced stem and leaf lesions of tomato plants. Am. Soc. Hort. Sci. 33: 516-520.
- 45. Maynard, D. N., A. V. Barker and W. H. Lachman. 1966. Variation among lines with respect to ammonium tolerance. J. Amer. Soc. Hort. Sci. 1: 17-18.
- 46. Ogihara, T. 1958. Studies on potash deficiency in paddy rice. Fotash Re., Sub. 5. 9th Suite.
- 47. Otto, H. J. and H. L. Everett. 1956. Influence of nitrogen and potassium fertilization on the incidence of stalk rot of corn. Agron. J. 48: 301-305.
- 48. Prianishnikov, D. N. 1951. Nitrogen in the life of plants (Translated by S. A. Wilde). Kramer Business Service, Inc., Madison, Wisc. p. 2.
- Puritch, G. S. and A. V. Barker. 1967. Structure and function of tomato leaf chloroplasts during ammonium toxicity. Plant Physiol. 42: 1229-1238.
- 50. Raleigh, S. M. and J. A. Chucka. 1944. Effect of nutrient ratio and concentrations on growth and composition on tomato plants and on the occurrence of blossom-end rot of the fruit. Plant Physiol. 19: 671-678.
- 51. Richards, F. J. 1941. Physiological studies in plant nutrition. XI. The effect on growth of rubidium with low potassium supply and modification of this effect by other nutrients. Part I. The effect on total dry weight. Ann. Bot., N. S. 5: 263-296.

- 52. Richards, F. J. and E. Berner, Jr. 1954. Physiological studies in plant nutrition. XVII. A general survey of the free aminoacids of barley leaves as affected by mineral nutrition, with special reference to potassium supply. Ann. Bot. N. S. 18: 15-33.
- 53. Richards, F. J. and W. G. Templeman. 1936. Physilogical studies in plant nutrition. Ann. Bot. 50: 100-106.
- 54. Russell, E. W. 1961. Soil Condition and Plant Growth. 9th edition. John Wiley and Son, Inc., New York. p. 65.
- 55. Samuels, G. and L. J. Pable. 1955. The influence of potassium on the yield and sucrose content of sugar cane. Soil Sci. Soc. Am. Proc. 19: 66-68.
- 56. Sheat, D. E. G., B. H. Fletcher and H. E. Street. 1959. Studies on the growth of excised roots. VIII. The growth of exised tomato roots supplied with various inorganic sources of nitrogen. New Phytol. 58: 128-141.
- 57. Spurr, A. R. 1959. Anatomical aspects of blossom-end rot in the tomato with special reference to calcium nutrition Hilgardia: J. Agric. Sci. 28: 1-15.
- 58. Stanley, A. B. and R. P. Humbert. 1963. Advances in knowledge of potassium relationships in the soil and plant. In: <u>Fertilizer</u> <u>technology and usage</u>, McVickar, M. H., Bridger, G. L. and Nelson, L. B. eds. Soil Sci. Soc. Amer., Madison Wisc. pp. 231-234.
- 59. Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York.
- 60. Stevenson, F. J. 1965. Amino-acids. Agron. 9: 1437-1451.
- 61. Steward, F. C. and C. Preston. 1941. The effect of salt concentration upon the metabolism of potato discs and the contrasted effects of potassium and calcium salts, which have a common ion. Plant Physiol. 16: 85-116.
- 62. Sutcliffe, J. F. 1962. Mineral salt absorption in plants. Pergamon Press. p. 121.
- 63. Syrett, P. J. 1953. The assimilation of ammonia by nitrogenstarved cells of Chlorella Vulgaris. Part I. The correlation of assimilation with respiration. Ann. Not. N. S. 17: 1-19.
- 64. Teel, M. R. 1962. Nitrogen-potassium relationships and biochemical intermediates in grass herbage. Soil Sci. 93: 50-55.
- 65. Thelin, G. and A. Beaumont. 1934. The effect of some forms of nitrogen content of wheat and rice plants. J. Amer. Soc. Agron. 26: 1012-1017.

- Thomas, W. H. and R. W. Krauss. 1955. Nitrogen metabolism in Scenedesmus as affected by environmental changes. Plant Physiol. 30: 113-122.
- 67. Thompson, H. 1949. Vegetable Crops. 4th edition. McGraw-Hill Book Co., Inc. pp. 479-506.
- 68. Tomato. 1958. Extension Service, University of Massachusetts, Amherst, Mass. Commodity ref. manual. Nos. 5. pp. 1-14.
- 69. <u>Tomato</u>. 1962. Vegetable Production Guide. University of Florida, Agriculture Ext. Service. Gainesville, Florida. Cir. 98B. pp. 1-10.
- 70. Tomato Diseases. 1957. Farmers Bulletin. U. S. Dept. Agric. No. 1934. pp. 6-19.
- 71. Trebst, A. V., M. Lasada and D. I. Arnon. 1960. XII. Inhibitors of carbon-dioxide assimilation in reconstituted chloroplast system. J. Biol. Chem. 235: 840-844.
- 72. Tso, T. C. and J. E. McMurtrey, Jr. 1960. Mineral deficiency and organic constituents in tobacco plants. II. Amino-acids. Plant Physiol. 35: 865-870.
- 73. Uljee, A. H. 1964. Ammonium-nitrogen accumulation and root injury to tomato plants. New Zealand J. Agr. Res. 7: 343-356.
- 74. Vegetable for Fresh Market, Acreage, Production and Value. 1959-1965. Statistical Bulletin No. 412. U. S. Dept. Agric. 1967.
- 75. Vickery, H. B., G. Pucher, R. Shoenheimer and D. Rittenberg. 1940. Assimilation of ammonia nitrogen by tobacco plant: A preliminary study with isotopic nitrogen. J. Biol. Chem. 135: 531-539.
- 76. Wall, M. E. 1940. The role of potassium in plants. III. Nitrogen and carbohydrate metabolism in potassium deficient plants supplied with either nitrate, or ammonium nitrogen. Soil Sci. 49: 393-409.
- 77. Ware, G. W. and J. P. McCollum. 1959. <u>Raising Vegetables</u>. The Interstate Printers and Publishers, Inc., Danville, Illinois. pp. 370-384.
- 78. Webster, G. C. 1959. <u>Nitrogen Metabolism in Plants</u>. Row, Peterson and Co., Evanston, Illinois, White Plains, New York. pp. 59-70.
- 79. William, G. R., M. R. Teel and H. E. Parker. 1964. Influence of nitrogen and potassium on the yield and chemical composition of Orghardgrass. Agron. J. 56: 473-475.

- 80. Work, P. 1945. Vegetable Production and Marketing. John Wiley and Sons., Inc. p. 351.
- 81. Yemm, E. W. and A. J. Willis. 1953. The respiration of barley plants. XI. The metabolism of roots during the assimilation of nitrogen. New Phytologist 55: 229-252.

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