EFFECTS OF ROOT TEMPERATURES AND NITROGEN CARRIERS ON NUTRIENT UPTAKE, GROWTH, AND COMPOSITION OF PINEAPPLE PLANTS, *Ananas comosus* (L.) Merr.

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By

Azeez Abdul Ravoof

Dissertation Committee:

Wallace G. Sanford, Chairman
Beatrice H. Krauss
James A. Silva
Goro Uehara
Hong Y. Young

We certify that we have read this dissertation and that in our opinion it is satisfactory in scope and quality as a dissertation for the degree of Doctor of Philosophy in Agronomy and Soil Science.

DISSERTATION COMMITTEE

William A. Stanford
Chairman

Beatrice H. Krauss

James A. Silva

Hong Yip Young

Goro Uehara

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ABSTRACT

The effects of four root temperatures (15, 20, 25 and 30 C) and three N-carriers [ammonium nitrate (AN), ammonium sulfate (AS), and sodium nitrate (SN)] on the uptake of nutrients, and the growth and composition of pineapple plants of the Smooth Cayanne variety, grown in complete nutrient solutions for 40 days were investigated.

The results on cumulative water and nutrient absorption indicate severe root injury at 15 C, and mild injury at 20 C root temperatures. Increases in root temperatures significantly increased water and nutrients absorbed. The optimum for N and K absorption was close to 25 C, but the optimum for water absorption probably is higher than 30 C. The optimum root temperature for the uptake of P, Ca, and Mg was different for different N-carriers.

The uptake of N and P was highest, and K, Ca, Mg and water was lowest in the AS culture; the reverse was true in the SN culture. Values for the absorptions of these ions in the AN culture were between those values for the AS and SN cultures. Calcium absorption was completely inhibited and K absorption severely inhibited with the use of AS. It appears that the presence of an easily absorbable anion such as NO_3, eliminates the inhibitory effect of NH_4 on K absorption (as happens with the use of AN) and K on Ca absorption (as happens with the use of SN). The ratio of NO_3 to K absorption was almost 1:1, and that of NO_3 to Ca 6:1. The absorption of NO_3 from AN culture was higher (60%) than that of NH_4 (40%), indicating possibly that pineapple plants "prefer" NO_3 to NH_4.

The absorption ratios of K:N, K:P and N:P and the total amount of alkali cations (K + Ca + Mg) absorbed were highest with the use of SN,

lowest with AS, with the values for AN between those for SN and AS. The total amounts of anions and alkali cations absorbed were nearly equal, regardless of the root temperatures and N-carriers.

Plant growth increased with increases in root temperatures; the use of AN and SN produced larger plants than AS regardless of root temperatures. At 15 C, there was a loss of plant weight with all three N-carriers probably due to leaf desiccation. Root weights in the SN cultures increased continuously up to 30 C. In the AN cultures root weights increased only up to 25 C, whereas, with the use of AS, root weights increased only up to 20 C with an actual decrease between 25 and 30 C; however, the capacity of roots to absorb nutrients was unaffected in the latter case. Stem weights were slightly greater with the use of AS than with AN and SN. The weights of leaves which constitute more than 75% of the total plant weight, increased continuously with increases in root temperatures; increases were higher with the use of AN and SN than with AS. The efficiency of nutrient utilization for growth was high with the use of SN and AN, but low with AS. Especially, N utilization efficiency with the use of AS was poor, probably as a result of "luxury consumption" of N or a lack of K for proper N metabolism or both.

A 20 C root temperature appeared to be high enough for absorption and assimilation of N in roots, but was not high enough for the translocation of these metabolites from roots.

The soluble, protein and total-N fractions in roots, stems and leaves, as well as the asparagine and total amide content of leaves were highest with AS, lowest with SN, and with values for AN lying between those for AS and SN. These results present additional evidence for the inefficient

utilization of N with the use of AS, probably due to insufficient available K.

In the leaf hydrolysates an unknown amino acid-type compound, which absorbed at 440 mμ wave length (similar to proline and its derivatives) was observed. This unknown showed a significant negative correlation with root temperatures and plant growth.

In conclusion, it appears that for better and more balanced nutrient absorption, a better utilization of these nutrients for growth, a nitrate or a mixed nitrate and ammonium source is desirable. Also, for better K and Ca absorption, especially where poor nitrification is suspected, the addition of an easily absorbable anion like NO_3 is essential.

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INTRODUCTION

In summing up the literature on plants in relation to inorganic salts, Steward and Sutcliffe (1959) observed that "plants grow because they can absorb and accumulate salts; they also accumulate because they can grow."

Nutrient uptake is not only dependent on the presence of different nutrient ions and their concentrations in the root environment but also on the preferential demand for various nutrients in specific quantities. Beside the external concentrations of salt solutions in which the roots are continuously bathed, the internal organization of plant components create complicated and often incompletely understood interactions dictating the quality and quantity of ionic species absorbed and accumulated.

Not only the absorption and accumulation but also the assimilation of salts is equally important for plants to grow. Temperature and light are the two most important external factors involved in plant growth. Light affects assimilation directly while its effect on absorption and accumulation are largely indirect. However, temperature is involved directly in all these three phases of plant growth, often times policing both speed and direction of "traffic".

Nielson and Humphries (1966) contend that "knowledge of how root temperature affects plant growth is woefully incomplete". The growth of root and shoot are mutually complimentary: root growth depends on the photosynthates translocated from the shoot while shoot growth depends on the supply of minerals from the roots. Yet, the effect of temperature around the root controls not only the growth and activity

of the root, but that of the shoot as well. Although the atmospheric temperature may be high enough to sustain good shoot growth, the temperature of roots in cold soils in temperate regions has overriding effects on shoot growth.

Different crop plants show variable responses towards the source of nitrogen. Those with large reserves of carbohydrates, such as potato and pineapple, are capable of utilizing NH_4-N while some others prefer NO_3-N (Kirkby and Hughes, 1970 and Nightingale, 1942b).

Pineapple, the most important cultivated Bromeliad, is at home in the tropical regions of the world, cultivated between 30° north and 30° south latitudes (Collins, 1960). The time from planting to fruit ripening is about 15 months on the Ivory Coast,[1] about 22 months in Hawaii, and about 32 months in Swaziland (Dodson, 1968). These differences are largely attributed to temperature differences in these areas. The work carried out at the Pineapple Research Institute of Hawaii has shown that NH_4-N is a better nitrogen source for pineapple than NO_3-N. However, at present due to low soil pH, fumigation, and foliar application of N, a mixture of NO_3 and NH_4-N is considered desirable.[1]

In the present study an attempt was made to assess the effects of root temperatures (15, 20, 25 and 30° C) and N-carriers (ammonium nitrate - AN, ammonium sulfate - AS, and sodium nitrate - SN) on nutrient uptake, growth, and composition of pineapple plants. Results of the experiment, supported by appropriate tables and figures, are presented in three chapters: Nutrient uptake in Chapter I, growth

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[1] W. G. Sanford (1973). Personal Communication.

characteristics of plants in Chapter II, and nitrogen composition of plant parts in Chapter III. A relevant review of literature with a discussion and summary of the results and conclusions drawn are also included.

REVIEW OF LITERATURE

The growth of plants is the product of extremely complicated, often poorly understood, mechanisms and their interrelationships involving certain inherent plant characteristics and the environment in which they happen to grow. The roots must absorb and accumulate certain nutrient ions discriminately, often in specific amounts, in order to supply the needs of shoots to assimilate and grow; and shoots, in turn, cater to the needs of roots for photosynthates and other metabolites to sustain continued root growth. Efficient and proper functioning of roots and shoots not only depends on the capacity of the root environment to supply the different nutrients but also the metabolic activity of the plants which largely depends on temperature and light, among other things. Thus, growth is the result or the end product of interrelated factors influencing absorption, accumulation and assimilation. Therefore, any attempt to single out any one of these factors and to relate it with growth is only artificial and incomplete, at best. Yet, only such an artificial separation is, perhaps, practical and manageable.

Root Temperature

Temperature is one of the most important environmental factors which affects almost every aspect of plant growth. There are some excellent books and reviews on this subject (Kramer, 1949 and 1969; Richards, et al. 1952; Went, 1953; Nielson and Humphries, 1966; and Philip, 1966). The temperature at which most physiological processes proceed normally range from 0 to 40 C (Went, 1953). Although there are

many references documenting the general effects of temperature on plant growth, the exact mechanism involved is still incompletely understood. This prompted Nielson and Humphries (1966) to conclude that "knowledge of how root temperature affects plant growth is woefully incomplete, partly because critical experiments are few and partly because of ignorance about root function". Also, in most studies the root and shoot temperatures were approximately the same, making it difficult to assess the effects of root temperature alone on the growth of the whole plant.

Effects on Absorption of Water

Absorption of water is partly metabolic and, therefore, influenced by temperature, and partly passive. Kramer (1949 and 1969) concluded that the principal cause of reduced intake of water by transpiring plants in cold soils was the physical effect of increased resistance to water movement across the living cells. The water uptake at 5 C was about one-fourth of that at 25 C, due to the additive effect of temperature on the viscosity of water and decrease in permeability of root protoplasm. Working with peas, Hylmo (1953) observed that at 0-2 C root temperature, water moved only about half as fast as at 20 C, and three-quarters as fast at 7-11 C. Growing tomato by a "divided root system", one-half placed in cold nutrient solution and the other in warm distilled water, Davis and Lingle (1961) concluded that, at low temperatures, metabolic effects influenced water uptake much more than change in viscosity.

Working with sugarcane, Mongelard and Mimura (1971 and 1972) concluded, from their own experiments and of that of their colleagues,

that water absorption progressively decreased with corresponding decreases in root temperatures. Unger and Danielson (1967) reported that beans grown in solution cultures at root temperatures of 10 to 32.5 C showed low water uptake and water stress at 15 C or less.

Effects on Nutrient Uptake

Absorption of nutrients, especially those accumulated against concentration gradients, requires the expenditure of metabolic energy which comes from root respiration and, therefore, on the temperature of the root medium. Zhurbitzkii and Shtrausberg (1958) suggested that at low temperatures plants lose their ability to absorb nutrients in the order of N>P>Ca>S>K. Ulrich (1941) showed that the ratio of cation to anion absorbed decreases with increasing temperatures. According to Walker (1969), uptake of nutrient ions increased with increasing soil temperature to maximal values between 26 and 34 C, dpending on the nutrient element.

a) Nitrogen absorption

In a review article Hewitt (1970) concluded that NH_4 absorption was highly temperature dependent with an optimum at 27 C at low pH, while with an increase in pH, temperature showed very little effect on the absorption of the predominant NH_4OH. In the absence of NH_4, the uptake of NO_3 showed a large temperature dependence, with no optimum up to 35 C. In the presence of NH_4, however, NO_3 absorption was highly temperature dependent above 20 C.

Working with barley, Williams and Vlamis (1962) observed that NO_3 absorption was much more affected by temperature than certain other

anions and cations, with almost no absorption of NO_3 at 13 C. The reduction in NO_3 absorption with decreasing temperatures was much more than for other ions; this prompted these authors to implicate very low nitrate reductase activity as the secondary mechanism in the inhibition of NO_3 absorption. Power, Grunes, Reichman and Willis (1970) also observed very low N uptake in barley at 9 C as compared to that at 15.5 or 22 C.

Frota and Tucker (1972) concluded that air and/or root temperature influenced NH_4 and NO_3 absorption by lettuce. Increases in temperature from 8 through 13, 18 to 23 C, increased the absorption of NO_3 more than of NH_4, resulting in a greater NO_3/NH_4 absorption ratio with increases in temperatures. Translocation of both ions to leaves also increased as temperature increased, with a maximum at 23 C for NH_4, and 18 C for NO_3. Absorption and N-content increased with increases in root temperatures from 12.5 C to 27.5 C in three forage crops (Ezumah, 1970).

b) Phosphorus absorption

The rate of absorption of P by tomato was greater at low than at high temperatures. Both absorption and translocation of P increased with increases in temperature in corn and oats (Zhurbitzkii and Shtrausberg, 1958; Knoll, Brady and Lathwell, 1964; and Case, Brady and Lathwell, 1964). Working with barley, Power, et al. (1964 and 1970) concluded that absorption of P from fertilizers was much more dependent on temperature than soil P and that temperatures around 11 to 15 C were optimum for P absorption.

c) Absorption of cations (K, Ca and Mg)

In a nutrient uptake study of barley and soybean at 12, 22 and 32 C, Wallace (1957) observed differential absorptions of K, Ca and Mg. The K content of plants progressively increased with increases in temperature in the case of soybeans, while in the case of barley, K content increased up to 22 C followed by a decrease. The absorption of Ca and Mg as related to temperature were opposite to that of K. Recently, Wallace (1966) showed that although Ca absorption was non-metabolic and non-temperature dependent, their translocation to the shoot was. Optimum K uptake by apple trees was observed (Gur and Shulma, 1971) at 25 C while at 35 C it is severely inhibited. Also, increased K uptake at 25 C increased Ca uptake, while at 35 C Ca uptake was inhibited. In an experiment with excised corn roots, Mass and Ogata (1971) observed inhibition of Mg uptake to be metabolic and, therefore, temperature dependent. Nielson and Cunningham (1964) observed only a slight increase in K uptake with increases in temperature while Mg and Ca absorptions increased considerably, due to enhanced NO_3 uptake.

Recently a soil temperature-dependent plant disorder resembling Ca deficiency has been reported in corn (Walker, 1969) and Tobacco (Chang, et al. 1968). Plants grown at 21 C produced normal leaves while those at higher temperatures up to 35 C showed progressively increasing leaf symptoms resembling Ca deficiency.

Effects on Morphology, Growth, Yield and Composition

Plant species exhibit differential temperature optimums for growth and yield: a tropical crop requires higher temperatures than one grown in the temperate zone. Within the same species the optimum temperature

for root growth is less than that for top growth, and higher for vegetative than for reproductive growth (Sanford, 1964 and Nielson and Humphries, 1966); these values decrease with advancing age and maturity (Radke and Bauer, 1969). Different optimal temperatures follow each other in succession, due to a succession of morphological and physiological processes, each with its own optimal temperature. Tomato plants showed that with diurnal thermoperiodicity, i.e., warm days and cool nights, there was better growth and dry matter accumulation (Went, 1953), Similar effects of thermoperiodicity have also been reported in alfalfa (Smith, 1969), rice (Chaudhury and Ghildyal, 1970) and many other crops, including sugarcane.

Root temperature has been shown to affect the morphology of plants. Working with apple and peach, Nightingale (1935) showed that at 19 C roots formed in large numbers but were white, very succulent and lacked mechanical strength. In general, a decrease in soil temperature resulted in brownish, thick and sparsely branched roots, sometimes lacking in root hairs (Nielson and Humphries, 1966). Pineapple has also been observed to form suberized roots under inclement water or temperature conditions (Sanford, et al., 1961). Working with rice, Chaudhury and Ghildyal (1970) recorded similar observations. Very low or very high temperatures are reported to inhibit the cell elongation in wheat (Burstrom, 1956) and corn (Beauchamp and Lathwell, 1966). Alfalfa was found to show adverse effects of root temperature in terms of leaf area, size, weight and anatomy (Bula, 1972). Even the direction of growth of seminal and nodal roots was affected by temperature: roots grew vertical at 36 C and horizontal at 18 C in corn (Mosher and Miller, 1972). Tiller production was also reduced at low root temperatures

in food crops such as barley (Mack, 1965), rice (Chaudhury and Ghildyal, 1970) and wheat (Smika and Ellis, 1971).

Effects of root temperature on shoot growth are very complex; growth may be inhibited more by cold soils than by cold air. A low temperature of roots exerts a greater control on shoot growth than does low temperature of shoots on root growth, (Went, 1953; and Nielson and Humphries, 1966) as shown from studies on tomato, sugarcane, clover and corn (Mongelard and Mimura, 1971; Sumner, et al., 1972; and Beauchamp and Lathwell, 1967a). Low root temperatures apparently delay the physiological maturity, with very little apparent injury or reduction in growth or yield potential in corn (Beauchamp and Lathwell, 1967) barley (Power, et al., 1970) or clover (Sumner, et al., 1972). These authors also showed that increases in root temperatures usually shortened the vegetative growth cycle.

Increasing root temperatures, especially at the young seedling stage, almost always improved root elongation and proliferation with an enhancement of nutrient absorption and translocation up to 20 to 25 C, depending on the crop. Low root temperatures nearly always resulted in poorer root growth, much less water absorption and translocation, and lower total dry matter production per plant at any given age, when compared to roots growing at higher temperatures. This led the researchers working with barley (Power, et al., 1963, 1964 and 1970), corn (Knoll, et al., 1964 a and b), tomato (Lingle and Davis, 1959), sugarbeets (Radke and Bauer, 1969) and sugarcane (Mongelard and Mimura, 1971 and 1972) to suggest that growth retardation at low root temperatures was largely due to inadequate water and nutrient absorption because of retarded root metabolism.

Effects of Nitrogen Carriers

On Pineapple Plants

Rather extensive research work has been carried on with pineapple nutrition, especially that of nitrogen, at the Pineapple Research Institute of Hawaii. Some of the results have been published in scientific journals, but most of them still remain unpublished except for compilations in the form of Technical Reports for Private Circulation (Sanford, 1959; Sanford and Fo, 1966; Gardner, 1966; and Sanford, et al., 1961).

Historically, most of the N nutrition studies included all-ammonium or all-nitrate sources, with a mixture of these two not tried until the 1950's. Sanford was the first one to compare soil as well as foliar application of Urea and ammonium nitrate; he showed that fruit yield and quality improved with ammonium nitrate (Sanford and Fo, 1966).

As early as 1923 sodium nitrate was found to be unsuitable for pineapple (Stewart, et al., 1925). Later, Sideris and his associates published a series of articles (1937, 1938, 1939, 1944, 1946a, 1947, 1950 and 1951) involving comparisons of all ammonium and all nitrate sources of nitrogen, only, in the form of soil applications (including sterile soils) and solution cultures. Their observations on plant growth characteristics can be summed up as follow:

Field grown plants supplied with ammonium-N were larger; more succulent, with greener leaves; lower in starch, sugars and organic acids; and with a larger leaf/stem ratio when compared to their counterparts receiving NO_3-nutrition. Their observations also showed that NH_4 was absorbed 2 to 3 times faster, and was more rapidly assimilated

than NO_3. The greater amount of NH_4 absorption was responsible for higher level of N in the tissues, increased succulence and greener plants. In solution cultures the results were somewhat different. At low N levels (28 ppm-N) both N sources produced approximately the same growth. But plants supplied with NO_3 had a somewhat higher chlorophyll content and greater chlorophyll/protein ratios. They also demonstrated that pineapples could be grown to maturity, i.e., to fruit harvest, with NH_4 as the sole N source; they suggested that NO_3 fertilizer was not indispensable for pineapple growth. In an experiment with sterilized soil, plants supplied with NH_4 showed poor growth with NH_4 toxicity; they therefore concluded that part of the NH_4 applied to the soil was nitrified and absorbed as NO_3.

In the solution culture experiments, Sideris, et al. (1946b and 1950) also observed that the total ash content of plants supplied with NO_3 was higher than in the case where the plants were supplied with NH_4; they attributed these results to the synergistic effects of anionic NO_3 and antagonistic effects of NH_4 on the absorption of ash constituents. In terms of the absolute amounts of nutrients absorbed, N and K were higher than others. High levels of K in solution suppressed NH_4 and Ca uptake, but favored NO_3 uptake. Leaf K levels were approximately the same with both low and high NO_3 levels in the solution, but at high NH_4 levels the K content was greatly reduced. Observations on other cations, such as Ca and Mg, were similar. Antagonism between NO_3 and P was also observed.

Reviewing the work on ionic interrelationships, Sanford, et al. (1961) concluded that in pineapple plants it is easier to establish

antagonistic effects because most of the fertilizers applied in pineapple culture (N as NH_4, K, Ca and Mg, as sulfates) are potentially antagonistic. Antagonistic effects of NH_4 and K on Ca and Mg have been observed, and increasing levels of these monovalent cations invariably decreased the divalent cation concentrations in the leaf; K was usually the most antagonistic.

In addition to the variations in plant growth and ash content, pineapple plants also showed variations in N metabolism and utilization depending on the source of N. Work by Sideris and his associates, previously referred to has repeatedly shown that amide-N fractions, especially asparagine than glutamine, were nearly always present in very high concentrations, with a slightly higher soluble and total-N content in plants supplied with NH_4 than when NO_3 was the N source. It was also observed that there was a steady increase in the asparagine content of plants from planting to floral differentiation, with an excellent correlation with the N supply. This led these workers to suggest that the concentration of asparagine reflected the N status of the plants, and that asparagine content might be a good index for determining the needs for N fertilization. They also suggested that asparagine formed a N pool in plants, and that there is a higher accumulation of this amide in plants in NH_4 than in NO_3 cultures due to a higher uptake of N from the former than the latter cultures. They also suggested that asparagine was the storage product following the conversion of NH_4 into organic N compounds. It was speculated that detoxification and storage of ammonium takes precedence over any other plant metabolic process, and that the carbohydrate reserve of the plant plays a vital role in this process. Their observation (1946a) also

showed that K deficiency caused accumulation of soluble-N fractions with the use of NH_4, being as high as 260% of that in the control.

Nightingale (1942 a and b), investigating nitrate nutrition in relation to carbohydrate reserves, and the effect of NO_3 on K and P absorption, developed the "Limiting factor theory" of pineapple growth. He concluded that plants need high amounts of NO_3 and sufficiently high carbohydrates for the efficient utilization of the NO_3. When the carbohydrate reserve was low, a low amount of NO_3 was sufficient for maximum growth and yield. However, when the carbohydrate supply was high, plants should be essentially filled to capacity with NO_3 for good yields. With a low carbohydrate reserve, NO_3 absorption was low, but that of P was high. When NO_3 was high, P absorption was inhibited and plant growth restricted due to a P deficiency. The absorption of K was related closely to NO_3 absorption. This author also contended that most of the N absorbed by the root was in the form of NO_3 as a result of excellent nitrification of NH_4 in the soil. He also showed that the presence of large amounts of NO_3 in the plant was necessary for active amino acid and protein synthesis.

Historically, ammonium sulfate was used almost exclusively as a N source in pineapple fields. A NO_3 source was considered to be undesirable for such reasons as its high cost and bulk per lb of N, its susceptibility to loss by leaching and denitrification, the possibility of accumulation of toxic levels of Na (from sodium nitrate), and low rate and slow absorption of NO_3 in comparison to the NH_4 source. A high rate of soil nitrification of NH_4 source was also considered to indicate that it was not necessary to apply a NO_3 source. However, this picture became complicated by the continued monoculture with pineapple leading

to a decrease in soil pH and the rate of nitrification; the introduction of soil fumigation causing partial soil sterilization, and resultant poor nitrification, and the increasing use of urea as foliar spray. These factors led Sanford and Fo (1966) to suggest using a mixed source of NH_4 and NO_3, in the form of ammonium nitrate, since the use of this N source would practically eliminate the injurious accumulation of an anion with an NH_4 source and a cation with a NO_3 source. They compared urea, ammonium nitrate, ammonium sulfate and sodium nitrate and concluded that ammonium nitrate gave the best results, ammonium sulfate the poorest, with urea and sodium nitrate lying between in terms of growth and yield. A comparison between urea and ammonium nitrate as N sources, also showed that, in terms of plant performance (growth, yields, etc.), the efficiency of N utilization was improved in that one part of ammonium nitrate N was equal to two parts of urea N.

Young (1965) also compared urea and ammonium nitrate nutrition in terms of the ethanol soluble N-fractions (including the amino acids) content of leaves. His observations showed that the supply of equal quantities of N produced higher amounts of protein-N, soluble-N and individual amino acids when ammonium nitrate was used than when urea was the N source. The N-carriers showed greatest accumulation of asparagine than any other N-fraction. He also found three unknown ninhydrin positive amino compounds in the unhydrolyzed samples, and seven in the hydrolyzed samples, as well as seventeen identifiable amino acids.

Subsequently, Connelly (1969) and Englerth (1969) also compared ammonium nitrate with all ammonium and all nitrate sources of N in relation to light intensity and nematode population, respectively.

Connelly (1969) reported that plants grown in ammonium nitrate were largest, followed by those in ammonium sulfate and least in sodium nitrate cultures. In regard to N-fractions, total soluble and protein-N levels were lowest with the use of sodium nitrate; on the other hand, total and protein-N were higher in ammonium sulfate cultures, with soluble-N almost equal in both the ammonium sulfate and ammonium nitrate cultures. Alkali cation (K, Ca and Mg) concentrations in leaves were higher with sodium nitrate, lower with ammonium sulfate and intermediate with ammonium nitrate sources. Plants supplied with sodium nitrate were stunted, chlorotic and low in carbohydrate while those supplied with ammonium nitrate and ammonium sulfate were similar, probably due to nitrification of NH_4 in the sand culture studies. Using ammonium nitrate, ammonium sulfate and calcium nitrate in soil culture studies Englerth (1969) came to essentially the same conclusions as Connelly (1969). Work carried out in other countries (Tay, et al., 1969; and Su, 1969) also showed that NH_4 was a better source of N for plant growth, but that NO_3 was better in so far as K absorption was concerned.

On Other Crops

Literature concerning the effect of N-carriers on absorption, growth, composition and N metabolism is abundant, but to determine the superiority of one over the other source in the reports is extremely difficult because of confusing and contradictory results published. The effect of any one source appears to be very distinctly related to carbohydrate supply, the plant species under investigation, soil reaction and the concentration at which these N sources are used (McKee, 1962; Kirkby and Hughes, 1970; DeKock, 1970). There is

overwhelming evidence to support the conclusion that, in the case of many crops, the use of NH_4 almost invariably results in a higher soluble-N content, especially a higher amide N content, than when a NO_3 source is used (Kretovich, 1965; and Morris and Giddens, 1963). Equally common to many crops is on the carrier effect nutrient interactions e.g., the use of an NH_4 source almost invariably suppresses the absorption of alkali cations, especially K, while enhancing P uptake; the reverse is true with a NO_3 source. Also, the absorption of NH_4 is much faster than NO_3: 3 to 5 times faster in tomato (Clark, 1936) and 5 to 20 times faster in rice (Fried, et al., 1965).

Working with tomatoes (Clark, 1936; Woolhouse and Hardwick, 1966; and Harada, et al., 1968), observed a higher soluble-N and amide-N content in plants supplied with NH_4-N than in those supplied with NO_3-N, with an indication of NH_4 toxicity in the former plants.

Investigations on corn (Leonce and Miller, 1966; Miller, et al., 1970; Blair, et al., 1970 and 1971) and on corn and soybean (Riley and Barber, 1971) showed that P absorption was enhanced when on NH_4-N source was used, primarily due to an increase in pH, resulting in an improved H_2PO_4/HPO_4 ratio at the soil-root interface. A specific role in transporting P across the root symplast has also been suggested for NH_4, but not for NO_3 source. At low concentrations of N, both NH_4 and NO_3 sources were equally good for growth of plants while at higher concentrations NH_4 cultures performed better and a combination of NH_4 and NO_3 was the best (Blair, et al., 1970; Power, et al., 1972 and Schrader, et al., 1972).

The rice plant has been considered to be more adopted for NH_4 than for NO_3 culture. Tanaka, et al. (1959) showed that at concentrations of

less than 20 ppm, both NH_4 and NO_3 gave equally good growth and yield but at high concentrations NH_4 was inferior, probably due to a poor root system and low K, Ca and Mg uptake. Karim and Vlamis (1962) showed that rice grew well in both NH_4 and NO_3 solutions when the pH was maintained by buffering with $CaCO_3$. However, higher rates of ammonium fertilization resulted in leaf tip burn due to NH_4 toxicity. Fried, et al. (1965) reported 5 to 20 times faster absorption of NH_4 than NO_3 from ammonium nitrate, and Harada, et al. (1968) found better growth and higher alkali cation concentrations in the plant with a NO_3 source.

De Kock (1970) and Rhoads (1972), using different individual N sources and combinations of these, showed that the quality and yield of tobacco leaves was better with a NO_3 than with an NH_4 source.

Wheat absorbed more NH_4 at a high pH while at a low pH, NO_3 was absorbed more readily. The highest total-N uptake and amide concentrations in the plant were associated with an NH_4 source. Also, N utilization efficiency was highest when a mixture of equal amounts of NO_3 and NH_4 was supplied (Weissman, 1950, 1951 and 1959).

Another important aspect of N nutrition is the organic acids metabolism of plants (Ulrich, 1941); this is related to both respiration and CO_2 fixation (Jacobson and Ordin, 1954). In an experiment involving sixteen plant species Noggle (1966) showed that an increase in organic acids content was positively correlated with growth and yield. He suggested that plant growth was, in part, regulated by the organic acids content. Kirkby and Mangel (1967), Kirkby (1968) and Leggett (1968) also showed that, irrespective of the ionic form of nutrients, a close balance existed between total cations (Ca + Mg + K + Na) and total anions

(NO_3 + PO_4 + SO_4 + Cl), and between total organic acids and inorganic acids. This was independent of the concentrations of nutrients used or dilution effects due to plant growth; a high organic acids content nearly always increased growth. Several workers reported similar results: Clark (1936) for tomato, Wadleigh and Shive (1939) for corn and Wallace (1966) for citrus. The use of NO_3-N improved the organic acids content while NH_4 decreased it. Many plants can apparently assimilate both the NH_4 and NO_3 forms of N but the growth and yield performance is best when both of these are supplied together (Street and Sheat, 1958; and McKee, 1962).

The univalent cations, NH_4 and K, antagonize each other very strongly (De Kock, 1970; and Craven, et al., 1971). Although K is not a component of plant tissues, it is the only univalent cation indispensable for plant growth, being required in large quantities; it is also considered to be essential in the activation of more than 46 enzymes (Evans and Sorger, 1966; Barber, et al., 1967; Hsiao, et al., 1970; and Craven, et al., 1971). Potassium is also required for protein synthesis, polymerization of amino acids and even as a protective agent against protein breakdown. High NH_4 and low K or a mild K deficiency have been implicated in many metabolic disorders, including accumulation of amides, soluble-N fractions, and ammonia toxicity leading to poor growth. A deficiency of K has also been known to cause an accumulation of amines putrescine and agamatine, in concentrations proportional to an insufficiency of K (Richards and Coleman, 1952; Coleman and Richards, 1956; Hackett, et al., 1965; and Hoffman and Samish, 1971). A mild K deficiency can severely retard stomatal opening, carbohydrate fixation,

and the amount and speed of photosynthate translocation in many crops (in cotton Ashley and Goodson, 1972; and in sugarcane, Hartt, 1969). Permeability of roots to water, translocation, succulence and accumulation of dry matter have also been shown to be affected severely by mild K deficiencies (Eaton, 1952; Hsiao, et al., 1970; and Graham and Ulrich, 1972). However, the uptake of NO_3 and K are mutually complementary and K is even shown to transport NO_3 as KNO_3 in plants; also, recirculation of K in plants is considered vital for such transport (Lips and Ben-Zioni, 1971).

Potassium also competes with polyvalent cations like Ca and Mg, and seriously inhibit their uptake. Potassium uptake does not depend on pH's above 4.5, whereas Ca uptake increases with increases in pH. Johansen, et al., 1968 and Laughlin, et al., 1973 suggested that K-induced Ca deficiency could be important in acid soils. Mass (1969), working with excised corn roots, also showed that K and H ions interfere and severely inhibit Ca absorption.

CHAPTER I

EFFECTS OF ROOT TEMPERATURES AND NITROGEN SOURCES ON CUMULATIVE AND TOTAL EVAPO-TRANSPIRATION AND NUTRIENT UPTAKE

MATERIALS AND METHODS

Materials

Fresh pineapple crowns of the Smooth Cayanne variety (courtesy of the Pineapple Research Institute of Hawaii) were weighed, and only those weighing between 175 to 225 gm were selected and "cured" in the shade for two weeks. A few whorls of basal leaves were removed and the crowns were allowed to root in tap water for four weeks. Plants with uniform, healthy root systems, weighing between 275 to 300 gm, were selected and grouped into four lots.

Within these groups, plants were randomly assigned to different treatments. The initial weights of these plants showed no statistical differences in the analysis of variance and were thus assumed to be reasonably uniform.

Methods

The solution culture experiment was carried out in the greenhouse, in water baths during the summer of 1971, for a period of 40 days.

Water Baths

Four constant temperature water baths were set up to provide 15, 20, 25 and 30 C root temperatures. The water baths were fitted with refrigeration and heating elements and temperature control was achieved by thermostats. The temperature was maintained within $\pm$0.5 C with the help of stirrers.

Nutrient Solutions

Stock solutions were prepared with demineralized water from analytical grade salts of ammonium sulfate, sodium nitrate and ammonium nitrate, representing the N-carriers, hereafter referred to as AS, SN and AN, respectively. The other plant nutrients were derived from potassium sulfate, magnesium sulfate, potassium dihydrogen phosphate, calcium chloride and sequestrene 330 for Fe. Micronutrients B, Mn, Mo, Zn and Cu were also provided for all treatments. The composition of the nutrient solution is presented in Table 1.

Table 1

The concentrations of nutrients in the solutions and their sources

Source	Nutrient Concentrations, ppm									
	NH_4-N	NO_3-N	P	K	Ca	Mg	Fe	S	Na	Cl
$(NH_4)_2\ SO_4$	105.0	-	-	-	-	-	-	120.0	-	-
$Na\ NO_3$	-	105.0	-	-	-	-	-	-	172.5	-
$NH_4\ NO_3$	52.5	52.5	-	-	-	-	-	-		-
$K_2\ SO_4$	-	-	-	67.2	-	-	-	27.6	-	-
$Mg\ SO_4$	-	-	-	-	-	19.4	-	26.0	-	-
$K\ H_2\ PO_4$	-	-	18.2	23.0	-	-	-	-	-	-
$Ca\ Cl_2$	-	-	-	-	21.8	-	-	-	-	38.0
Sequestrene 330	-	-	-	-	-	-	8.0	-	-	-

Growing the Plants

Two batches of 48 half gallon glass Mason jars were weighed and labelled with all treatment combinations (four root temperatures and three N-carriers with four replicates for each of the treatment combinations). One set of jars was filled with 1600 ml of complete nutrient solution each, with appropriate treatment combinations, preselected plants placed in the jars, and set out at random in the appropriate water bath. Water saturated air was bubbled through the solutions and the plants allowed to grow for 5 days. At the end of this period, the jars were removed from the water bath, the plants allowed to drain free of nutrient solution, and then transferred to an identical set of jars containing corresponding fresh nutrient solutions; the jars were then immediately returned to the appropriate water bath.

At the end of each five-day period, the jars containing nutrient solutions were wiped dry on the outside, weighed and brought back to the original volume of 1600 ml to determine the volume of water lost by evapo-transpiration. Then, these nutrient solutions were mixed thoroughly, filtered and a portion of filtrate from each jar stored separately for future analysis. This procedure was repeated for each 5 day period for the 40 day duration of the experiment.

Analysis of Nutrient Solutions

The nutrient solutions were analyzed to determine plant uptake of N, P, K, Ca and Mg.

a) Nitrogen

Nitrogen was determined by the standard macro Kjeldahl method. Since AS contained only NH_4-N, this solution was

distilled directly with five ml of 15 N NaOH and a few pieces of mossy zinc, the distillate absorbed in 4% boric acid with a mixed indicator of methyl red and bromcresol green, and titrated with standard $H_2 SO_4$.

The NO_3-N in the SN solutions was reduced to NH_4 in the presence of $H_2 SO_4$ and reduced iron powder; the resulting NH_4-N was determined after neutralizing excess $H_2 SO_4$ with 15 N NaOH, in the same manner as above.

Since AN originally contained equimolar concentrations of NH_4-N and NO_3-N, an attempt was made to determine the uptake of both ions separately. First, NH_4-N was determined directly as described with AS. To another aliquot, $H_2 SO_4$ and reduced iron powder were added to reduce NO_3 to NH_4 and the total N as NH_4 was determined. The amount of NO_3 was obtained as the difference between these two determinations.

b) Phosphorus

Phosphorus was determined colorimetrically at 430 mμ wavelength with a Coleman Spectrophotometer, using the Vanadate-Molybdate Yellow method of Barton (1948).

c) Potassium

Potassium was determined in a Beckman DU Flame photometer at 767 mμ wavelength.

d) Calcium and Magnesium

Calcium and magnesium were assayed in the Atomic Absorption Spectrophotometer, Model 303, fitted with a digital read out. Both nutrients were determined from the same aliquot, which contained 0.5% lanthanum to minimize interference, at 422.8 mμ

wavelength for calcium, and 285 mμ wavelength for magnesium.

The actual amounts of nutrients absorbed by plants were calculated as the difference between the actual concentrations of nutrients before and after the absorption by plants.

Statistical Analysis of the Data

The experimental lay-out was a split-plot design with four root temperatures as main plots, three N-carriers as sub-plots, with four reps within the main plots (temperature) [4 X 3 X 4]. Since root temperatures were not replicated, the error term used was the rep in temperature (twelve degrees of freedom) variance.

RESULTS

Results are presented in two sections (A and B) in this chapter. The first section (A) includes only the cumulative values at the end of successive time intervals. The second section (B) deals with the total values covering the entire 40 day period of investigation.

A. Cumulative evapo-transpiration and nutrient uptake

Results in the form of graphs are presented in Appendix Fig. 1, 2, 2a, 3, 4, 5 and 6. Examination of these figures show, in general, that the rate of uptake increased with successive increases in root temperatures for all N-carriers. The uptake patterns were nearly linear for all root temperatures except for 20 C. A steady rate of evapo-transpiration and nutrient uptake was attained in 5 days for plants grown at 30 C, 10 days at 25 C and 20 to 25 days at 20 C root temperatures; at 15 C, however, the evapo-transpiration and nutrient uptake remained low with very little change in the rates during the entire experimental period.

The slow initial rate of evapo-transpiration and nutrient uptake, which gradually increased over the initial period of 20 to 25 days followed by a more rapid rate of increase, may be due to a mild root injury at the beginning from which the plants recovered slowly. The plants grown at 25 and 30 C root temperatures apparently suffered no injury, while those at 15 C suffered serious root injury from which plants did not recover during the experimental period.

B. Total evapo-transpiration and nutrient uptake

Results of the statistical analysis of these data are presented as a summary of analyses of variance in Table 2. Pertinent data in support of Table 2 are also presented in Fig. 1, 2, 3, 4, 5 and 6 in

the text and in Appendix Table 1.

The interdependency of the uptake of nutrients and the growth of plants is well known. Nevertheless, an artificial separation of these two aspects is desirable, and also practical, to aid in an understanding of the interrelationships between different nutrient ions and their absorption. Accordingly, the aspects of nutrient uptake are dealt with in this section while the growth characteristics are considered in Chapter II. However, a short summary is given here in order to point out the relationships between nutrient uptake, and root growth, as well as the growth increase (ΔG) per plant.

Root temperatures, N-carriers and C X T interactions had highly significant effects on root weight. In the sodium nitrate (SN) cultures, root weights continued to increase almost uniformly with successive increases in root temperature. In the ammonium nitrate (AN) cultures, root weights also continued to increase, but the rate of increase declined with increases in root temperatures. In contrast, in the ammonium sulfate (AS) cultures, root weights increased sharply between 15 and 20 C, followed by a slight increase up to 25 C, followed by a significant decrease (Table 3, Fig. 11).

The growth increase (ΔG) of the plants was also very significantly affected by root temperatures, N-carriers and C X T interactions. The ΔG increased with successive increases in root temperatures, except at 15 C where losses in plant weight were observed. With AS as the N-carrier, there was significantly less ΔG than when AN and SN were the carriers (Table 3, Fig. 9).

Evapo-transpiration

Root temperatures and N-carriers significantly affected evapo-transpiration as shown in Table 2, Fig. 1 and Appendix Table 1. Successive increases in root temperatures resulted in corresponding significant increases in the rates of evapo-transpiration. The use of AS carrier significantly reduced evapo-transpiration when compared with the AN and SN carriers. This reduction with the use of AS was evident at all root temperatures except at 15 C; the reduction, however, was minor. The rate of increase in evapo-transpiration with successive increases in root temperature was essentially linear with all N-carriers.

Nitrogen Uptake

Root temperatures and N carriers had highly significant effects on N uptake (Table 2, Fig. 2, Appendix Table 1). The effects of C X T interactions were significant at $P = 0.05$. Successive increases in root temperatures resulted in correspondingly significant increases in N uptake at 15, 20 and 25 C. The rate of increase in N uptake above 25 C was small and not significant. The average amount of N absorbed by plants receiving SN was significantly less than when AN and AS were the N sources.

There were no significant differences in N uptake among the three carriers at 15 C, but at 20 C, N absorption was significantly higher in the AN culture than in the others. At higher root temperatures of 25 and 30 C, N absorption in the AN and AS solutions was not significantly different, whereas, the use of SN showed significantly lower N absorption than with AN and SN. The rate of N uptake with AN increased almost linearly as root temperatures were increased from 15 to 25 C.

Table 2

Summary of analyses of variance tests and Duncan's Modified LSD tests on the effects of root temperatures and N-carriers on evapo-transpiration (ml) and nutrient uptake (mg) per plant

TESTS		NUTRIENT UPTAKE						
		Evapo-transpiration	N	NH_4 and NO_3 from Ammonium Nitrate[1]	P	K	Ca	Mg
ANALYSES OF VARIANCE		SIGNIFICANCE OF F VALUES						
SOURCE	DF							
TEMPERATURE (T)	3	**	**	**	**	**	**	**
REP IN T.	12							
CARRIERS (C)	2	**	**	**	**	**	**	**
C X T	6	--	*	**	**	**	**	**
ERROR (b)	24							
DUNCAN'S MODIFIED LSD TESTS P=0.05†								
ROOT TEMPERATURE MEANS								
15		A	A	A	A	A	A	A
20		B	B	B	B	B	B	B
25		C	C	C	C	C	C	C
30		D	C	C	D	C	C	D
N-CARRIER MEANS								
AMMONIUM NITRATE (AN)		B	B	A	B	B	B	B
AMMONIUM SULFATE (AS)		A	B	B	C	A	A	A
SODIUM NITRATE (SN)		B	A		A	B	C	C

* = significant at 5% level

** = significant at 1% level

† means with same letters are not significantly different

[1] For the sub-plot treatment the DF will be form of N = 1, For X Temp. = 3 and Error (b) = 12. In the LSD Tests for Carrier Means the first is for NH_4 and the record is for NO_3.

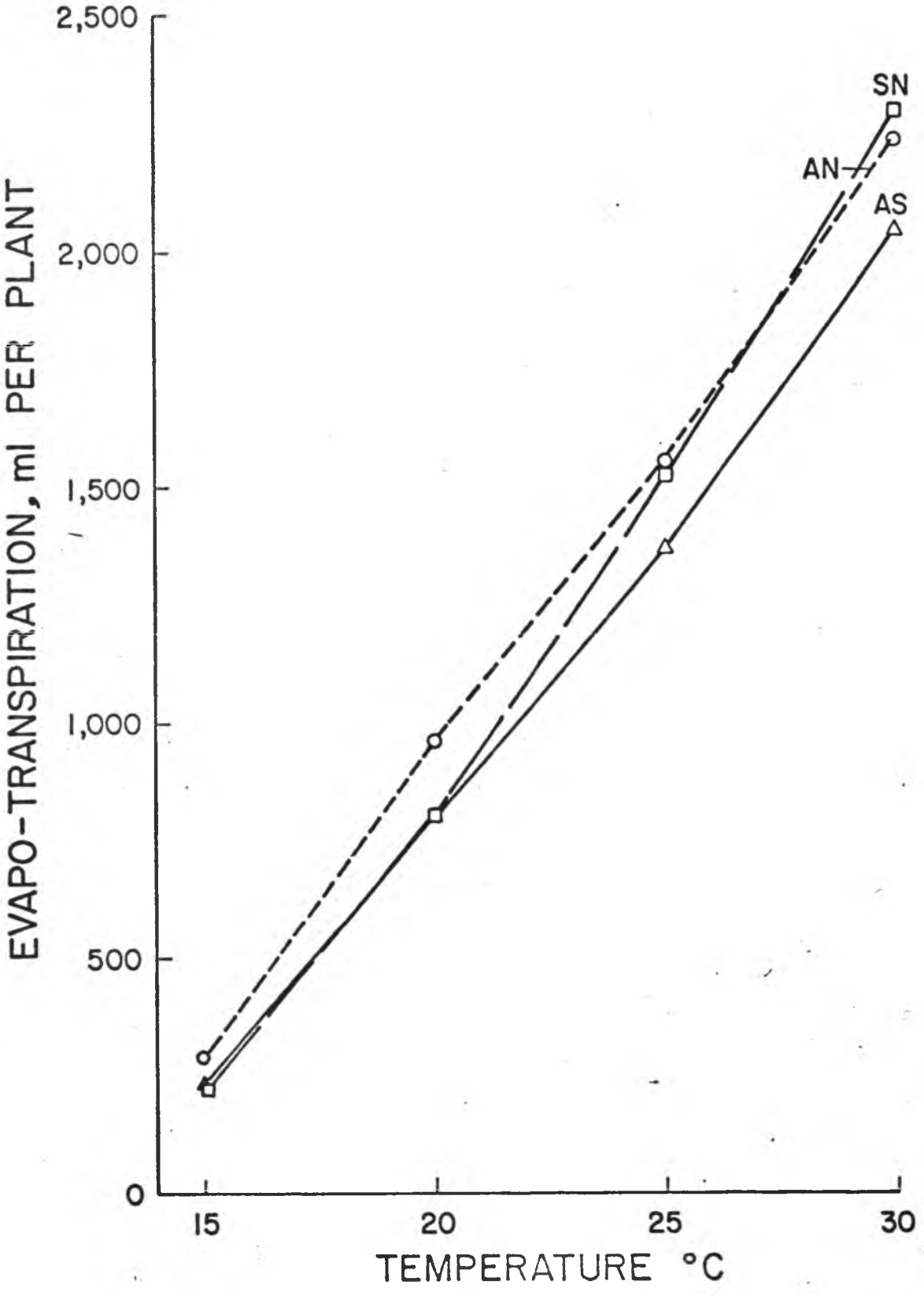

Fig. 1. Effects of root temperatures and N-carriers on total evapo-transpiration.

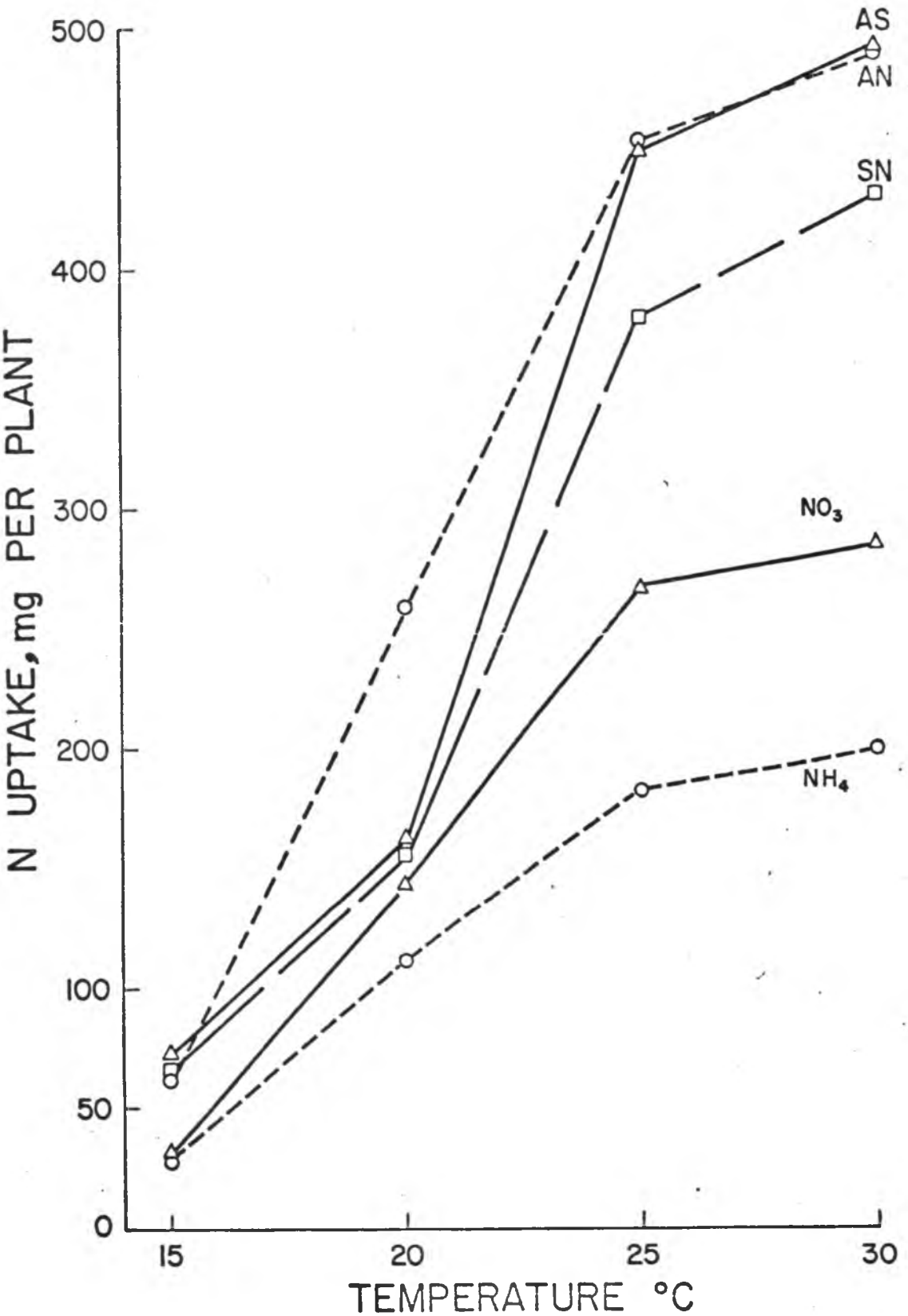

Fig. 2. Effects of root temperatures and N-carriers on total nitrogen uptake and NH_4-N and NO_3-N uptake from ammonium nitrate.

Since AN contains equimolar concentrations of NH_4 and NO_3 ions, an attempt was made to determine the uptake patterns of these two ions separately. These data are also shown in Fig. 2 and Table 2.

Root temperatures, ionic forms and their interactions had highly significant effects on the absorption of NH_4 and NO_3 ions. Successive increases in root temperatures showed corresponding significant increases in the uptake of both of these ions up to 25 C root temperature. These results are quite similar to those obtained for total N uptake, as reported above. In terms of ionic preferences, plants absorbed significantly higher amounts of NO_3 than NH_4 ions from the AN solution (Table 2 and Appendix Table 1). Examination of Fig. 2 shows that the uptake of these ions essentially increased in linear fashion up to 25 C, as was the case with the total N uptake pattern from the AN cultures. In the AN cultures, plants absorbed nearly 60% of the nitrogen in the form of NO_3-N with the rest as NH_4-N (Appendix Table 1). This was contrary to expectations since the total N uptake was higher from the all-ammonium (AS) source than from the all-nitrate (SN) source. This preferential NO_3 uptake may be expected to enhance the uptake of cations from the nutrient solution.

Phosphorus Uptake

Root temperatures and N carriers very significantly affected P uptake; the C X T interaction was also highly significant (Appendix Table 1 and Table 2). Successive increases in root temperatures showed corresponding significant increases in P absorption. There were significant differences in P uptake with the use of all three carriers. The uptake was highest with AS, followed by AN, with the least uptake with SN, at all temperatures except 15 C.

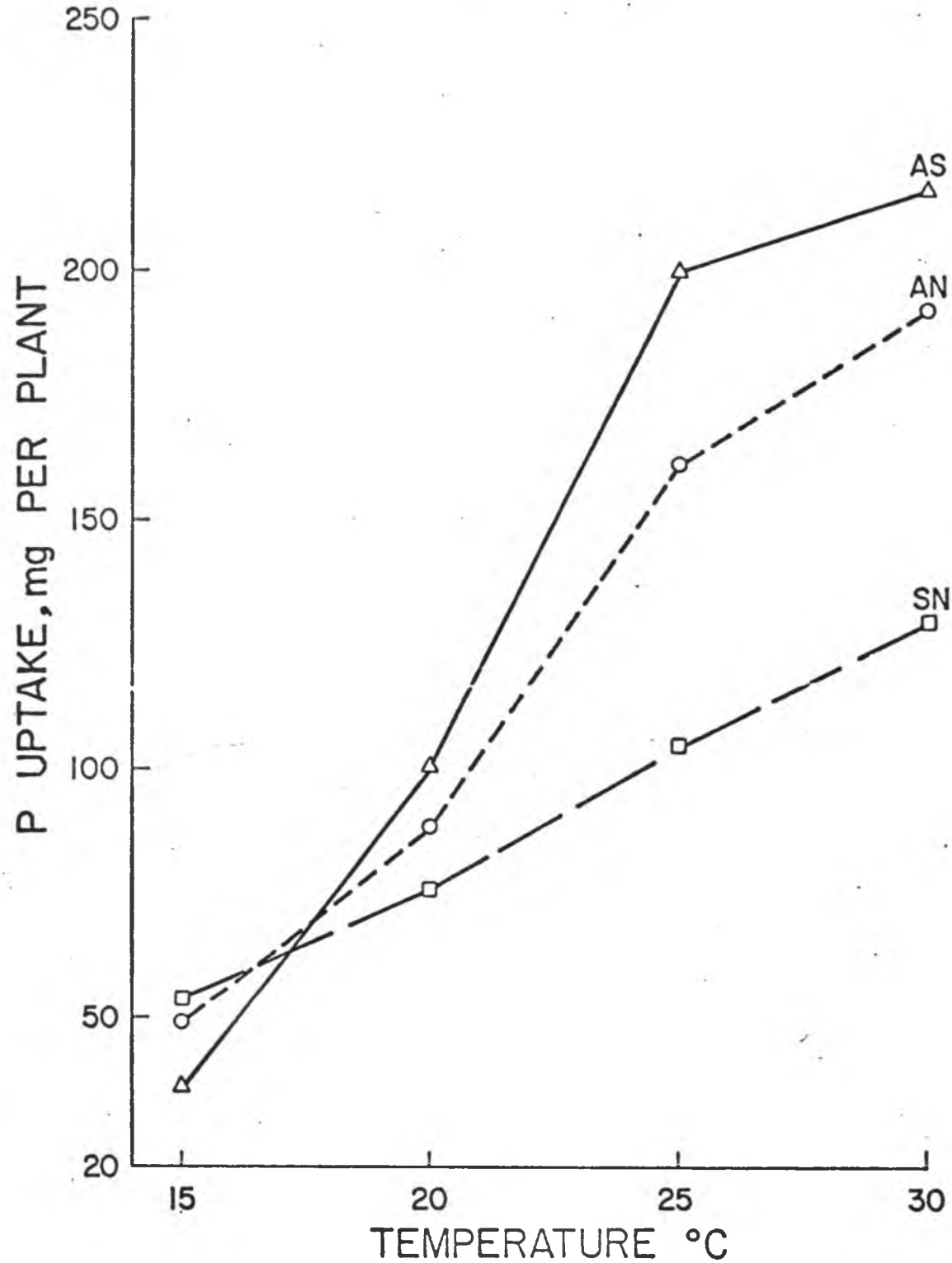

Fig. 3. Effects of root temperatures and N-carriers on total phosphorus uptake.

Examination of Fig. 3 shows a marked and almost linear increase in P absorption, up to 25 C, with the use of both the AN and AS sources. On the other hand, the absorption was moderate but nearly linear at all four root temperatures in the SN cultures. The data also indicates the possibility of the inhibition of P uptake with the use of SN.

Potassium Uptake

Root temperatures and N-carriers had highly significant effects on K absorption (Table 2, Fig. 4, Appendix Table 1). The C X T interaction was also highly significant. Root temperature increased K absorption significantly, up to 25 C, with all three N carriers. Above 25 C, K absorption decreased with the AN and AS sources of N, and increased only slightly with SN. The difference between 25 and 30 C, however, was not significant. It would, therefore, appear reasonable to assume that a root temperature of 25 C was optimum for K absorption, particularly with the AN and AS sources of N; this is also probably true in the case of SN. There were no significant differences in the amounts of K absorbed in the AN and SN cultures while K absorption in the AS culture was significantly less than in the other carrier treatments (Table 2). Examination of Fig. 4 and Appendix Table 1 shows that K uptake did not differ significantly from one root temperature to another when AN and SN were used. In contrast, significantly less K was absorbed from AS cultures than from AN and SN cultures at all root temperatures except 15 C, but even at this temperature it was less. In fact, K uptake with the AS carrier was only about 65% and 55% of the K uptake with SN at 25 C, and 30 C, respectively. These observations seem to indicate cationic antagonism between NH_4 (from AS) and K.

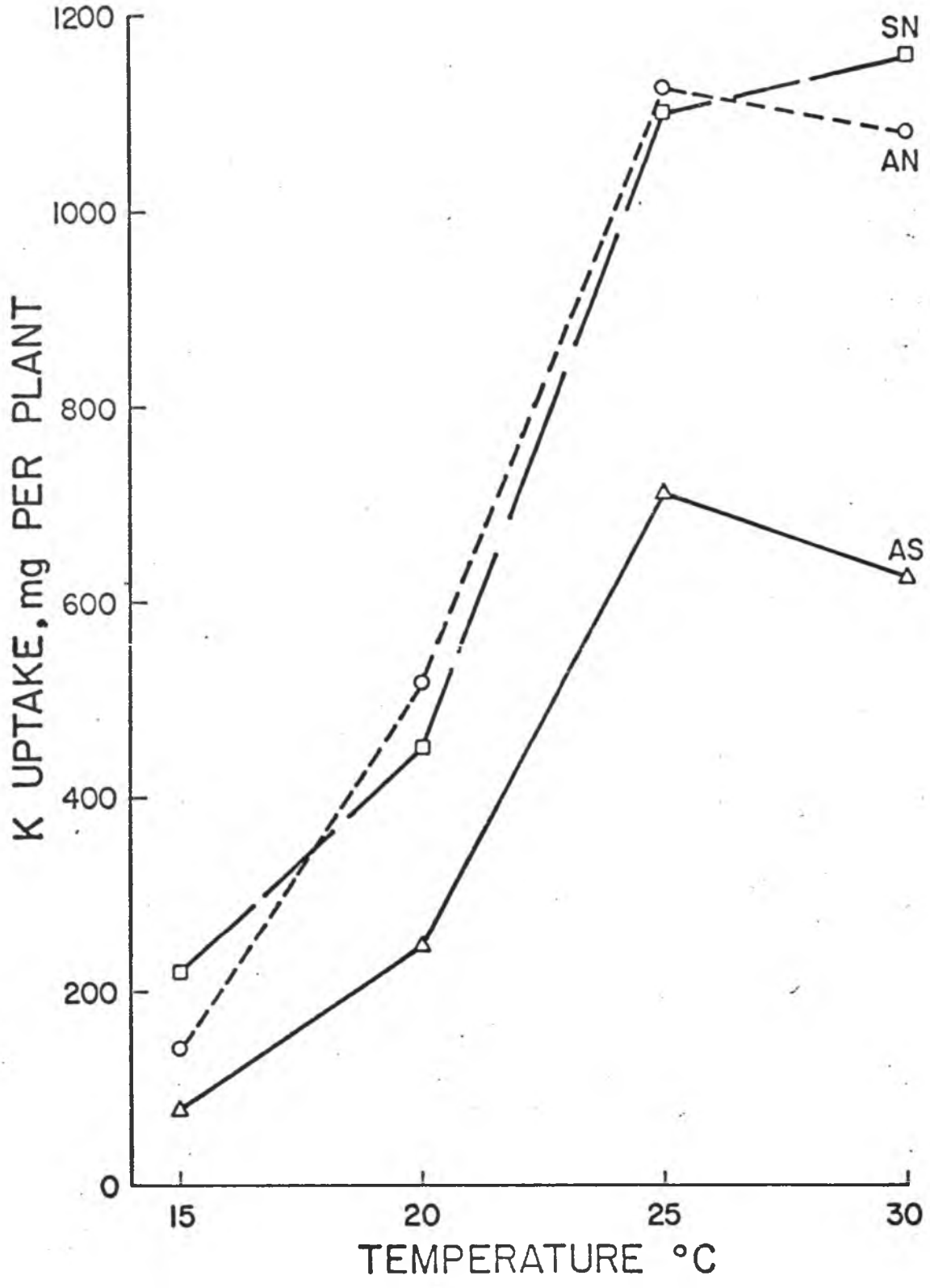

Fig. 4. Effects of root temperatures and N-carriers on total potassium uptake.

Calcium Uptake

There were great variations in the effects of N carriers on Ca absorption (Table 2, Fig. 5, Appendix Table 1). The differences associated with the three carriers were highly significant. The Ca uptake in SN cultures was quite high at all root temperatures; increases in Ca uptake from one temperature to the next were significant. With the use of the AN source, Ca absorption was only about 20% of that with SN, at all root temperatures except at 15 C; in the latter case there was a small increase in Ca in the nutrient solution. The Ca uptake from the AN cultures was linear from 15 to 25 C, followed by a slight decrease between 25 and 30 C. In sharp contrast to the absorption of calcium by plants in the SN and AN cultures, plants grown in the AS cultures lost a small amount of calcium at all root temperatures consistantly. Thus, the use of AS as the source of N not only completely inhibited Ca absorption by plants but also resulted in losses of plant calcium.

Magnesium Uptake

Root temperatures and N-carriers had very significant effects on Mg uptake; there was also a highly significant C X T interaction. Successive increases in root temperature showed corresponding significant increases in Mg absorption (Table 2, Fig. 6, and Appendix Table 1). The uptake of Mg also was significantly different for each of the three N-carriers; the largest amount of Mg was absorbed by plants grown in the SN solutions, followed by the AN and AS solutions (Table 2 and Appendix 1). Examination of Fig. 6 shows that the shape of the curves for Mg uptake in SN and AN cultures is similar at all root temperatures. Increases in the rate of Mg absorption in these two treatments for temperatures

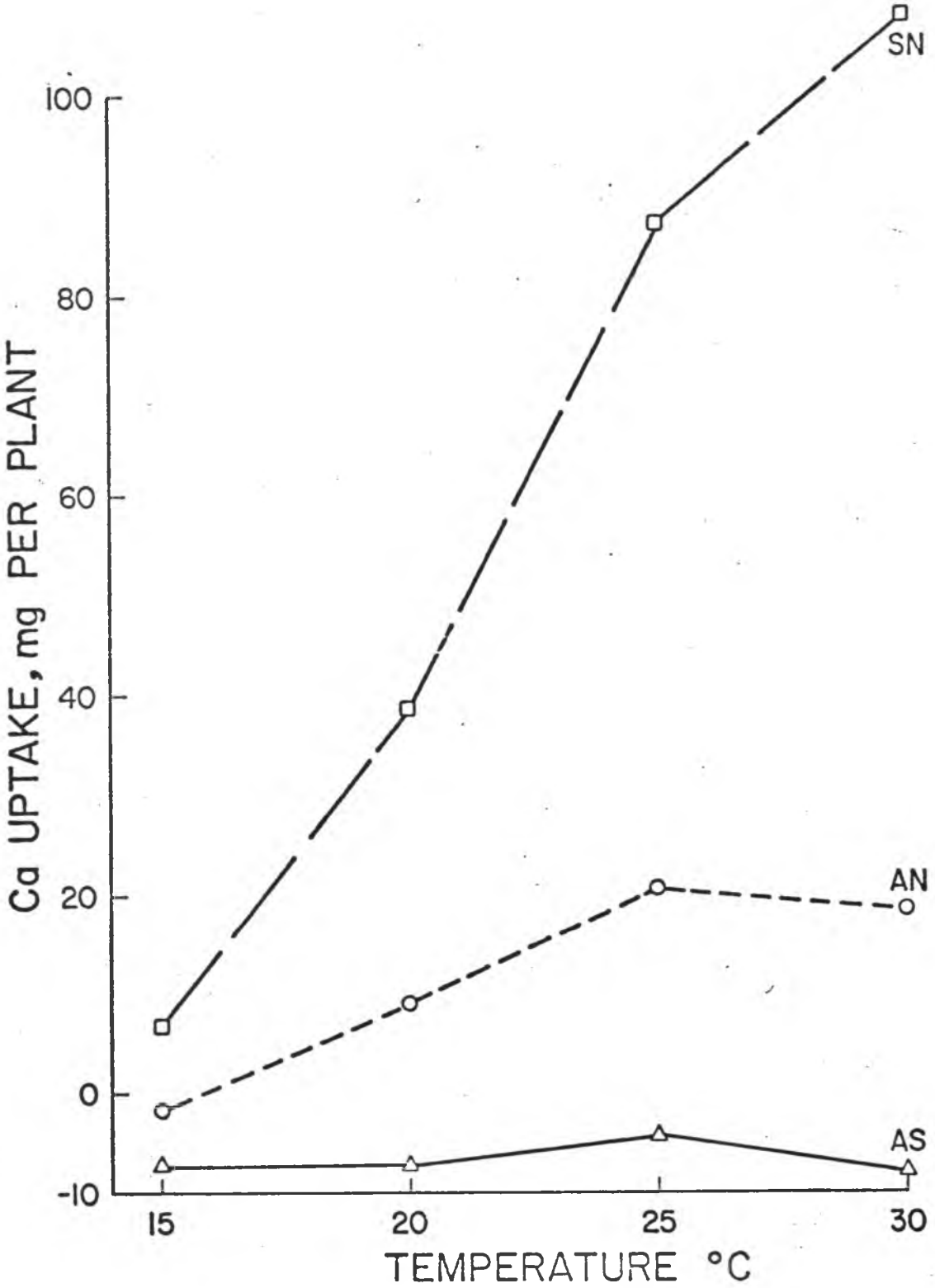

Fig. 5. Effects of root temperatures and N-carriers on total calcium uptake.

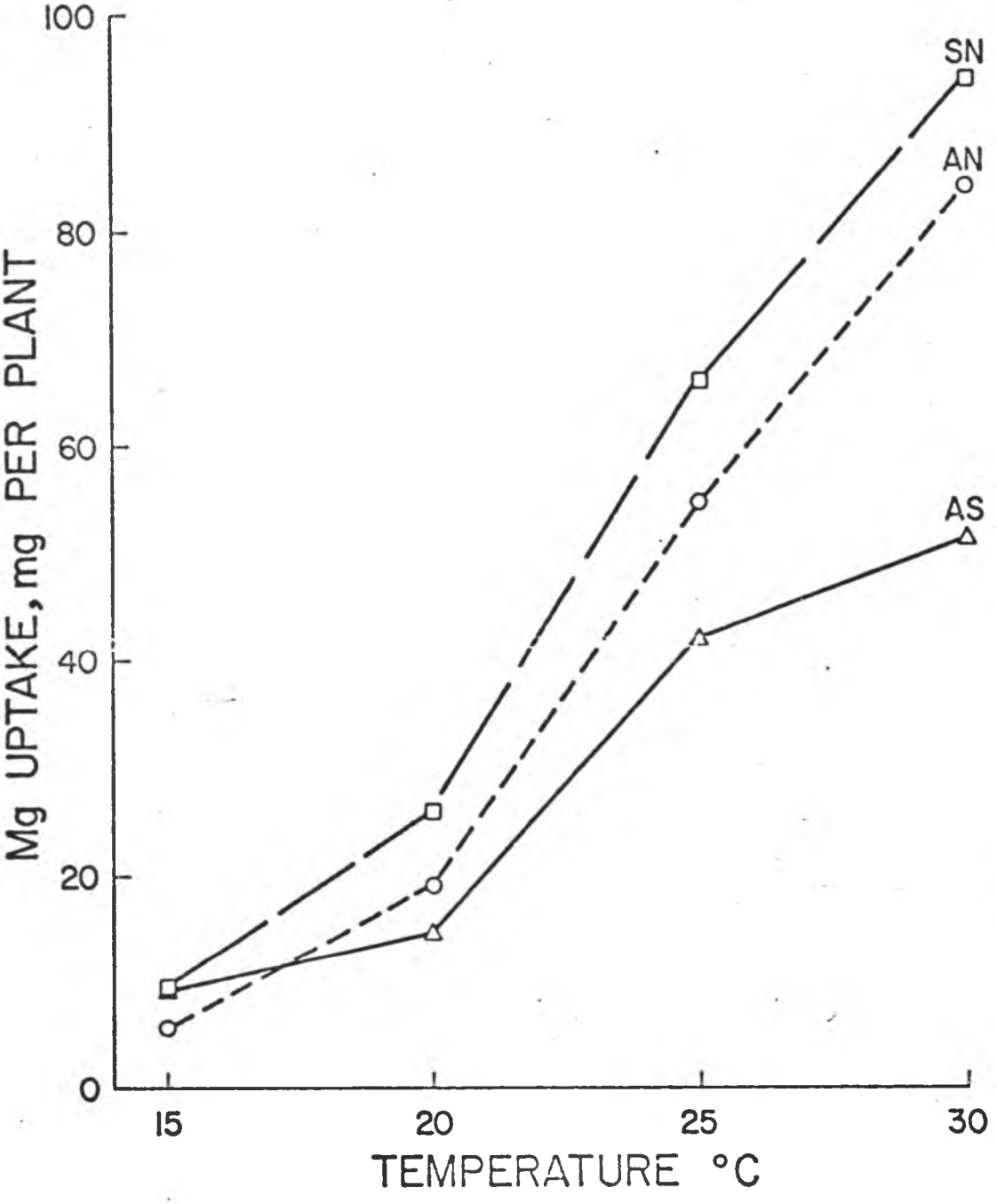

Fig. 6. Effects of root temperatures and N-carriers on total magnesium uptake.

ranging from 15 to 20 C, were low, followed by high and nearly linear increases at higher root temperatures from 20 to 30 C. In the case of the AS source, Mg absorption increased only slightly from 15 to 20 C, followed by a rapid increase up to 25 C; the rate of increase decreased, however, between 25 and 30 C. Consistantly high amounts of Mg were absorbed from SN solutions, followed closely by AN, and considerably less absorption from AS solutions, at all root temperatures except 15 C. The results were similar to Ca uptake, with only the magnitude different.

Nutrient Interrelationships

The original data were further studied to understand the inter-relationships of the following:

a) mechanism of nutrient uptake in relation to evapo-transpiration,

b) influence of major nutrients on the uptake of each other, and

c) total cation and anion uptake per plant.

a. Mechanism of nutrient uptake in relation to evapo-transpiration

The ratios presented in Appendix Table 2 were calculated to express the relationships between the actual uptake of nutrients and the theoretical values of nutrient uptake, based on the observed evapo-transpiration rates. These ratios were expected to indicate the mechanism of nutrient uptake viz. active or passive uptake.

Examination of Appendix Table 2 shows that the ratios are: more than 2.0 for N, between 4 and 8 for P, close to 5 or higher for K and consistantly better than 1.0 or nearly 2.0 in most cases for Mg, regardless of the root temperature and N carrier. The ratios for Ca

varied considerably, depending on the N carrier. In the SN cultures, the ratios were around 2.0 at all root temperatures except 15 C; on the other hand, all ratios were considerably less than 1.0 in the AN and AS cultures, showing an active inhibition of Ca uptake.

The ratios were, therefore, deemed to indicate a predominately active absorption mechanism against a concentration gradient for the nutrient studied.

b. Influence of major nutrients on the uptake of each other

The ratios between the major nutrients N, P and K, expressed in milliequivalents as K/N, K/P and N/P, are presented in Fig. 7 a, b and c, supported by Appendix Table 3.

In general, successive increases in root temperatures showed corresponding increases in the ratios, up to 25 C, followed by a slight decrease from 25 to 30 C. The ratios for SN cultures were consistantly high with the respective ratios for AN cultures only slightly less. Similar ratios for the AS cultures were always lower at comparable root temperatures than for the other two cultures, except for the N/P ratio at 15 C. It is particularly interesting to note that, despite a high N and P uptake in the AS cultures, the N/P ratios were always lower than corresponding ratios for SN and AN cultures.

c. The uptake of cations and anions per plant

The uptake of cations and anions, in m eq per plant, is presented in Table 3. Both cation and anion absorptions rapidly increased with increases in temperature, up to 25 C, followed by only a slight increase up to 30 C. The highest increase in absorption occurred between 20 and 25 C than the other temperature increases.

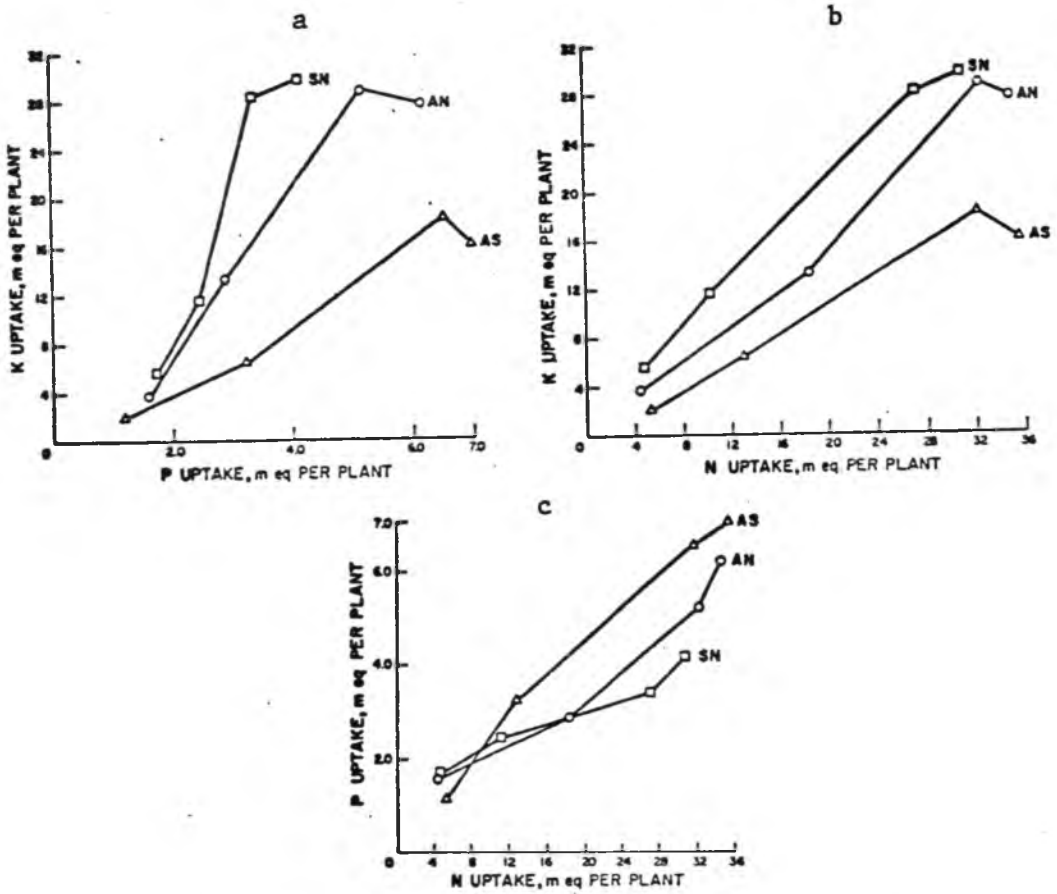

Fig. 7. Effects of root temperatures and N-carriers on the ratios of a) K/N, b) K/P and c) P/N.

Table 3

Uptake of cations and anions, m eq per plant

Carrier*	Root Temp. C	CATIONS NH_4	K	Ca†	Mg	Total	Total of K+Ca+Mg Only	ANIONS NO_3	PO_4	Total
AN	15	2.13	3.66	-0.08	0.48	6.19	4.06	2.37	4.78	7.15
	20	8.10	13.28	0.46	1.60	23.44	15.34	10.36	8.65	19.01
	25	13.14	28.83	1.04	4.57	47.58	34.48	19.11	15.65	34.76
	30	14.39	27.75	0.93	7.02	50.09	35.70	20.47	18.54	39.01
MEAN		9.44	18.38	0.59	3.47	31.83	22.40	13.08	11.90	24.98
AS	15	5.24	2.01	-0.37	0.76	7.64	2.40	--	3.50	3.50
	20	13.02	6.38	-0.36	1.24	20.28	7.26	--	9.71	9.71
	25	32.02	18.23	-0.21	3.50	53.54	21.52	--	19.64	19.64
	30	35.55	16.06	-0.40	4.29	55.50	19.95	--	21.04	21.04
MEAN		21.46	10.67	-0.34	2.45	34.24	12.28	-	13.47	13.47
SN	15	--	5.64	0.35	0.80	6.79	6.79	4.70	5.24	9.94
	20	--	11.57	1.94	2.17	15.68	15.68	11.21	7.40	18.61
	25	--	28.28	4.37	5.50	38.15	38.15	27.16	10.16	37.32
	30	--	29.68	5.40	7.85	42.93	42.93	30.79	12.57	43.36
MEAN		--	18.79	3.02	4.08	25.89	25.89	18.47	8.47	27.94

*AN = Ammonium Nitrate
AS = Ammonium Sulfate
SN = Sodium Nitrate

† negative (-) sign shows loss of calcium from the plant.

The total cation uptake was highest with AS, followed by AN and was lowest with SN. The results were just the opposite in the case of total anion absorption. A comparison between the absorption of total cations and total anions showed that absorption of cations was in excess of anions. However, the absorption of total alkali cations (K + Ca + Mg) was very nearly equal to that of total anions absorbed, regardless of root temperature and N-carrier. Also, the total alkali cation absorption was highest with SN, followed by AN and was lowest with AS.

DISCUSSION

Because of the rosette arrangement of pineapple leaves, the loss of water from solution cultures due to evaporation would be minimal, although such a loss would not be completely eliminated. Therefore, it is reasonable to assume that the major portion of measured evapo-transpiration would be due to transpiration. Consequently, the term water absorption to represent transpiration is used in this discussion in place of the more usual term evapo-transpiration.

Regardless of the N-carriers, sequential increases in root temperatures resulted in increased cumulative water and nutrient absorption with the exception of Ca absorption with AS. Absorption at the 15 C root temperature was very low, and almost uniform at all time intervals. At the higher temperatures of 25 and 30 C, the absorption attained a steady rate within a short period of 5 to 10 days, whereas to reach the same steady rate it took 20 to 25 days at 20 C. These differences may have been due to severe root injury at 15 C, a mild root injury at 20, with no apparent injury at 25 and 30 C. Transplantation into a slightly low temperature medium has been reported to result in a mild cold injury leading to a temporary inhibition of respiration, changes in membrane permeability and metabolism, with attendant low water and nutrient absorption in various crops (Nightingale, 1935; Lingle and Davis, 1959; Nielson and Humphries, 1966; Unger and Danielson, 1967; Power, et al., 1970; and Sumner, et al., 1972).

The measurements of total uptake of nutrients and water for the experimental 40 day period showed that sequential increases in root temperatures significantly increased uptake of nutrients up to 25 C,

with the exception of Ca. Between 25 and 30 C, the increases in uptake in most cases were not significant; in fact, K uptake was actually slightly decreased with AN and AS. This indicates that the optimum temperature for root absorption probably is somewhere near 25 C.

The individual N-carriers showed significant variations among themselves in regard to water and nutrient absorption; in all cases, the C X T interactions were also significant. Predictable ionic synergism and antagonism were also observed. The all ammonium source of AS resulted in the highest N uptake, showing a predictable enhancement of P uptake and the inhibition of cation uptake. On the other hand, the all nitrate SN showed the lowest N absorption but enhanced cation absorption (K, Ca and Mg) with a suppression of P uptake. The AN source showed results between those of AS and SN; there was equally good N uptake, a far amount of P uptake, but a desirably higher amount of cation uptake, compared to AS.

With AN as the source, a very interesting observation was made in regard to N uptake, as well as the influence of this latter ion on the absorption of other cations. The results showed that NO_3 was absorbed at a significantly higher rate than NH_4 at all root temperatures except 15 C. This is contrary to the expectation in light of various results of this and other experiments conducted with pineapple. The NH_4 uptake from AS was significantly higher, by as much as 20%, than NO_3 uptake from SN. Yet, with a mixed source of N, it appears as if NO_3 absorption accounted for about 60% of the total N uptake compared to a 40% of NH_4 uptake from AN. At 15 C, however, the NO_3 and NH_4 absorptions were almost equal; Nightingale (1942a) suggested that NO_3 absorption in pineapple may be inhibited at root temperatures below 20 C. Williams

and Vlamis (1962) indicated that NO_3 absorption is more temperature dependent than most other ions, and is completely inhibited at root temperature of 13 C or lower. This preferential or "excess" NO_3 absorption at higher temperatures probably affected the uptake of other nutrients as well, i.e., a slight decrease in P uptake and an enhancement of Ca and Mg uptake (similar to the effect of SN on these ions) with the use of AN compared to AS resulted.

Potassium absorption appears to show some interesting interactions with N sources. In the absence of easily absorbable anions (such as NO_3) to maintain electrical neutrality, the all ammonium source of AS severely inhibited K uptake, the mean K uptake with AS amounting to only 57% of that with SN. When SN was used, the absorption of NO_3 and K were very nearly equi-ionic, with absorption being in equimolar amounts. The ratio between NO_3/K absorption was 1.0 ± 0.05 at all temperatures except 15 C. At 15 C, K absorption was about 18% more than the NO_3 absorption, again indicating that NO_3 absorption was retarded more than K absorption at lower root temperatures. Sideris and Young (1950) also showed a similar relationship in pineapple, but with a wider ratio of 0.73 to 2.63, under varying levels of NO_3, K and Ca. Lutz, et al. (1972) showed the NO_3/K ratio to be close to 1.0 in tobacco.

From this experiment, it appears that the molar requirement or absorption of NO_3 or K can be calculated with reasonable accuracy if the value for one of the ions is known.

The relationship between AN and K appears to be more complicated. The absorption of K with AN was as much as with SN, and the presence

of 50% of N as NH_4 in AN does not appear to have inhibited K uptake. The possible explanation for the lack of an inhibition of K by NH_4 may be due to a preferential affinity of NH_4 to NO_3 or vice versa, leading to a virtual elimination of competition between NH_4 and K. In the absence of an easily absorbable anion such as NO_3, however, NH_4 absorption probably leads to an exchange with H and NH_4+ and/or H competes with K and inhibits its uptake as was observed with AS. This may have a practical implication in a pineapple fertilization program. For example, if AS is applied as the only N source to the soil, the rate of soil application of K may have to be increased or supplied by foliar means, or a mixture of ammonium and nitrate applied, depending on economic considerations and feasibility.

Absorption of P was higher with AS than with the other carriers. This was probably due to the direct effect of cationic NH_4 enhancing anionic PO_4, or was due to a reduction in pH at the solution-root interface, thus creating a favorable H_2PO_4-/HPO_4 = ratio, and increasing P absorption. Such effects were reported for corn and soybean (Leonce and Miller, 1966; Miller, et al., 1970; Blair, et al., 1970 and 1971; and Riley and Barber, 1971).

Calcium absorption also shows certain complex and interesting effects. Calcium absorption was completely inhibited by AS and actually Ca appears to have been lost by plants in this solution although the amount was quite small. This loss was not due to analytical errors or a simple exchange of Ca absorbed on the root surface since small but consistant gains of Ca in nutrient solutions were observed at all root temperatures and at all time intervals. This phenomenon is probably due to certain disruptions in plant metabolism, leading to

a loss of Ca in an internal plant adjustment due to NH_4 uptake and its inhibitory effect on K uptake or both. An inhibition of polyvalent cations (Ca and Mg) by monovalent cations (NH_4, K and H) has been reported in many crops, including pineapple (Sideris and Young, 1946; Connelly, 1969; Johanson, et al., 1968; Harada, et al., 1968; Mass, 1969; DeKock, 1970; Lutz, et al., 1972; and Laughlin, et al., 1973). However, Ca absorption was not inhibited by NH_4, temperature or pH in barley (Wallace, 1963) or by temperature in bean roots (Drew and Biddulph, 1969). In this experiment present the complete inhibition of Ca uptake does not appear to be entirely due to temperature or pH effects, since the absorption was inhibited at all root temperatures, and the pH changes at 15 and 20 C were very slight.

Calcium absorption in the SN cultures was high and showed continuous and near linear increases with increases in root temperature. However, the absorption was not equimolar as was the case between NO_3 and K, being approximately one mole of Ca for every six moles of NO_3. In the AN cultures some Ca was also absorbed at all temperatures except at 15 C, with the absorption linear up to 25 C, a trend very similar to that of NO_3 absorption with AN. A theoretical value of Ca absorption, based on the excess NO_3 absorbed over NH_4, with AN (NO_3 uptake - NH_4 uptake) on one hand and a factor of Ca/NO_3 uptake for SN on the other, showed a surprisingly close agreement with actual values of Ca absorption with AN, at all root temperatures except 15 C.

Considering the absorption of Ca in relation to N-carriers and K absorption, whenever an NH_4 source is used it appears desirable or even indispensable to supply an easily absorbable anion such as NO_3, preferably in equimolar concentrations, in order to avoid inhibitions of both K and Ca absorption. Under field conditions this may have a

greater importance in that the AS source may induce Ca deficiency in plants. The greatest effect of AS applied under field conditions has been a reduction in soil pH leading to the replacement of K, Ca and Mg ions on the soil colloids with H.

Absorption of Mg shows typical ionic inhibition in the presence of NH_4, and an ionic synergism with NO_3. With regard to the temperature effect, in the presence of NO_3 alone (SN), or a mixed source of NH_4 and NO_3 (AN), Mg absorption is nearly linear between 20 and 30 C, suggesting a temperature optimum for Mg absorption has not been reached. However, with the use of AS, the temperature maximum for Mg absorption probably is between 25 and 30 C, as indicated by the curvilinear response observed at these temperatures.

The data on nutrient uptake in relation to water absorption indicates an active rather than a passive absorption, since the ratios between actual uptake and theoretical uptake based, on evapotranspiration, nearly always were considerably different from 1.0.

The ratios of the major nutrients, K/N, K/P and N/P, expressed as meq, increased with increases in root temperatures up to 25 C, followed by a slight decrease, probably indicating that 25 C may be close to the optimum root temperature for nutrient absorption. The ratios were higher with SN, closely followed by AN, and was lowest with AS, despite a high N and P absorption with AS. This may also be an indication that a balanced nutritional relationship is more likely to occur with the nitrate source, or a mixture, than with an all ammonium source.

The absorption of total cations (NH_4 + K + Ca + Mg) and anions (NO_3 + PO_4) showed a rapid increase with increases in root temperatures from 15 to 25, followed by only a slight to moderate increase. Nearly

always the highest increase was observed between 20 and 25 C. The absorption of total cations was higher with AS, followed by AN, and was lowest with SN; the opposite was true in the case of the absorption of total anions. The lower absorption of NO_3 than of PO_4 at 15 C is probably due to generally poor NO_3 absorption (Nightingale, 1942a; and Williams and Vlamis, 1962) and high PO_4 absorption (Power, et al., 1964 and 1970) at this temperature. Comparisons between total cation and total anion uptake with AS and AN showed excess cation uptake. However, when total alkali cation uptake (K + Ca + Mg) was compared with total anion uptake, there was a close relationship between these two, showing an almost neutral electrical charge. The total alkali cation absorption was quite high with SN when compared to AS source; this could have a favorable effect on the organic acid status and growth of plants, as suggested by Sideris and Young (1954) and Kirkby and Mangel (1967).

SUMMARY AND CONCLUSIONS

Data on cumulative water and nutrient uptake show the possibility of a severe root injury at 15 C, with very little or no apparent injury at 25 and 30 C root temperatures. At 20 C, the roots probably sustained mild root injury but were able to recover slowly over a period of 20 to 25 days.

Increases in root temperatures showed consistent increases in water and nutrient uptake and greatest increases usually occurred between 20 and 25 C. A root temperature of 25 C was optimum for N and K absorption, whereas, the optimum for evapo-transpiration is probably higher than 30 C. Temperature optimum for the absorption of P, Ca, and Mg varied with the source of N; with SN, it was close to 30 or above, whereas, with AN and AS, 25 C was probably optimum with a possible exception of Ca with AS.

The absorption of water, K, Ca and Mg was lowest with the use of AS and highest with SN; whereas, the relationship of N and P absorption and N carriers was reversed. Absorption of Ca not only was completely inhibited by the use of AS, but plants even lost small amounts of Ca at all root temperatures. With the use of AN, rates of water and K, absorptions were equal to those rates with SN while N absorption rate was as much as that with AS. The rates of P, Ca and Mg absorptions were intermediate between those for AS and SN. Although there was a greater amount of NH_4 absorption from AS than NO_3 absorption from SN, the amount of NH_4 absorbed from the AN culture was lower (40%) than the absorption of NO_3 (60%).

Interactions between N, K, and Ca absorption and the interrelationships of these nutrients with the N carriers are interesting. In the presence of an easily absorbable anion such as NO_3, NH_4 appears to be absorbed along with NO_3, with very little effect of K absorption as with AN source. In the absence of NO_3, however, K absorption is severely inhibited by AS. With the use of SN, NO_3 and K are absorbed in an equimolar concentration, that is, in a 1:1 ratio. A complete inhibition of Ca with AS is probably due to primary inhibitory effects of NH_4 and/or K on Ca or due to a secondary inhibition of Ca absorption arising from NH_4 inhibited K or both. However, in the presence of easily absorbably anion such as NO_3, both NH_4 (as in AN) and K (as in SN) do not appear to inhibit Ca uptake. With the use of SN, the ratio of the absorption of Ca to that of NO_3 is approximately 1:6 meq. It is interesting to note that when AN is the carrier the amount of NO_3 absorbed in excess of the NH_4 absorbed also shows a $Ca:NO_3$ ratio of 1:6 similar to SN.

Although there were variations in the total amounts of N, P and K absorbed with the use of different N-carriers, the ratios between K:N, K:P and N:P were always highest with the SN carrier and lowest with AS; the ratios with AN carrier were close to those for SN.

Regardless of the root temperatures, the total cation uptake was highest with AS, followed by AN with absorption from the SN cultures being the lowest. The absorption of total anions was just the reverse in that the highest absorption values were highest with SN and lowest with AS with values for AN lying between. However, the amount of total alkali cations (K + Ca + Mg) was very nearly equal to that of the total anion uptake regardless of root temperatures and N-carriers. Also, the

highest alkali cation absorption was highest with SN, followed by AN and was lowest with AS.

Considering the results obtained for nutrient absorption, it appears that pineapple plants actually "prefer" NO_3-N over NH_4-N. To obtain a balanced nutrient absorption it appears that it is best to provide an all nitrate or a mixed source of N. It could also be pointed out that the use of an all ammonium source may induce K as well as Ca deficiency under field conditions, especially in those soils where nitrification is low. Ammonium nitrate application is particularly advantageous in that it will not leave any potentially harmful residues such as Na with the use of SN or an anion such as SO_4 with the use of AS.

CHAPTER II

EFFECTS OF ROOT TEMPERATURES AND N-CARRIERS ON PLANT GROWTH CHARACTERISTICS

MATERIALS AND METHODS

Pretreatment of crowns and other related procedures of growing plants in nutrient solutions are outlined in Chapter I under Materials and Methods.

Methods

At the end of the 40 day experimental period the plants were removed and nutrient solutions allowed to drain from roots which were then dried between paper towels. After the whole plant weights were recorded, plants were separated into: a) roots, b) stems and c) leaves.

a) Roots

Roots were thoroughly washed, with demineralized water, dried between paper towels and the fresh weight recorded. They were then finely chopped with a stainless steel knife, and a representative 25 gm sample (or less) was placed in a 250 ml Erlenmeyer flask containing 100 ml 95% ethanol. This was brought to a boil to inactivate enzymes, then cooled, stoppered and stored for future analysis.

b) Stems

The procedure adopted for stems was the same as described above with a sample of 25 gm stored in ethanol for future analysis.

c) Leaves

The weight of fresh leaves was obtained by difference (total plant weight - root fresh weight - stem fresh weight). All the green leaves were saved, washed free of dust, dust and

trichomes with a brush, and then dried between paper towels. The basal white portions were removed and discarded and the rest of the leaf material was finely chopped and thoroughly mixed. Three sets of 25 gm samples were taken, and two of them stored in 95% ethanol as already described. The third portion was dried in a forced draft oven at 70 C for 48 hours, and the dry weight then recorded.

Based on the data of final plant and plant parts weights, the following additional computations were made:

Increase in plant weight, hereafter referred to as Δgrowth or simply as ΔG, was obtained by the taking the difference between the final and initial plant weights.

Percent rate of increase was computed for each 5 day interval through the facilities available at the Computing Center. The computation was similar to the computation of compound interest rates, based on the formula:

$$\text{Percent rate of increase} = \left(\sqrt[8]{\text{FPW/IPW}} - 1\right) \times 100, \text{ where}$$

FPW = final plant weight

IPW = initial plant weight and

the number of 5 day time intervals = 8.

The weight of different plant parts, such as roots, stems and leaves, per plant was expressed as a percentage of the final plant weight.

Nutrient absorption capacity of the roots was expressed as the meq. of nutrients absorbed per 100 gm of fresh roots. The total nutrient uptake values reported in Chapter I were used for these calculations.

Nutrient utilization efficiency was calculated as meq. of nutrients utilized to produce 100 gm of ΔG. Since the 15 C root

temperature treatment resulted in a loss of plant weight, probably due to desiccation, the utilization efficiency could not be computed for this temperature treatment.

Statistical Analysis of Data

The data were analyzed as a split plot design, as already indicated in Chapter I under Materials and Methods.

RESULTS

The growth characteristics are summarized in Table 4 supported by relevant data in Appendix Table 4, and Fig. 8, 9, 10, 11, 12, 13, 14 and 15. All results are expressed on the gm fresh weight basis, unless otherwise indicated. Results on the efficiency of nutrient uptake and the utilization in terms of roots and growth are presented in Tables 5 and 6, respectively.

Initial weights of plants were not significantly different. Therefore, it was assumed that all the plants were reasonably uniform at the time of initiation of this experiment and that the differences in original weights are not expected to confound the experimental results.

Final Plant Weight

Root temperatures and N-carriers affected the final plant weight significantly; the C X T interaction was also highly significant (Table 4). Increasing root temperatures significantly increased plant weight. Plants grown in AS solutions were significantly smaller than those grown in AN and SN solutions but the weights of plants grown at 15 C were not significantly affected by N-carriers. At 20 C, plants receiving the AN source produced significantly heavier plants than the other two sources. At root temperatures of 25 and 30 C, there was no difference in weight of plants grown with SN or AN, but weight of plants in these two cultures were significantly greater than in the AS culture (Fig. 8 and Appendix Table 4). The photographs of the plants taken at the termination of the experiment (Plate 1) show visible overall increases in plant size with successive increases in root temperature.

Table 4

Summary of analyses of variance tests and Duncan's Modified LSD tests on the effects of root temperatures and N-carriers on plant growth characteristics (All results based on fresh weight unless indicated otherwise)

TESTS		PLANT GROWTH CHARACTERISTICS									
		Final Plant Wt., gm.	Δ Growth gm.	% Rate of Increase per 5 days	Weight of Plant Parts gm.			Leaf Dry wt.%	Proportion of Plant Parts (%)		
					Roots	Stem	Leaves		Root	Stem	Leaf
ANALYSES OF VARIANCE		SIGNIFICANCE OF F VALUES									
SOURCE	DF										
TEMPERATURE (T)	3	**	**	**	**	**	**	**	**	**	**
REP IN T	12										
CARRIERS (C)	2	**	**	**	**	--	**	*	*	*	--
C X T	6	**	**	**	**	*	*	--	**	*	--
ERROR (b)	24										
DUNCAN'S MODIFIED LSD TESTS P=0.05†											
ROOT TEMPERATURE MEANS											
15		A	A	A	A	A	A	A	A	C	A
20		B	B	B	B	B	B	B	B	B	A
25		C	C	C	C	B	C	B	B	B	A
30		D	D	D	C	B	D	C	B	A	B
N-CARRIER MEANS											
AMMONIUM NITRATE	(AN)	B	B	B	B	A	C	A	B	A	A
AMMONIUM SULFATE	(AS)	A	A	A	A	A	A	B	A	B	A
SODIUM NITRATE	(SN)	B	B	B	B	A	B	AB	B	B	A

* = significant at 5% level

** = significant at 1% level

† = means with same letters are not significantly different

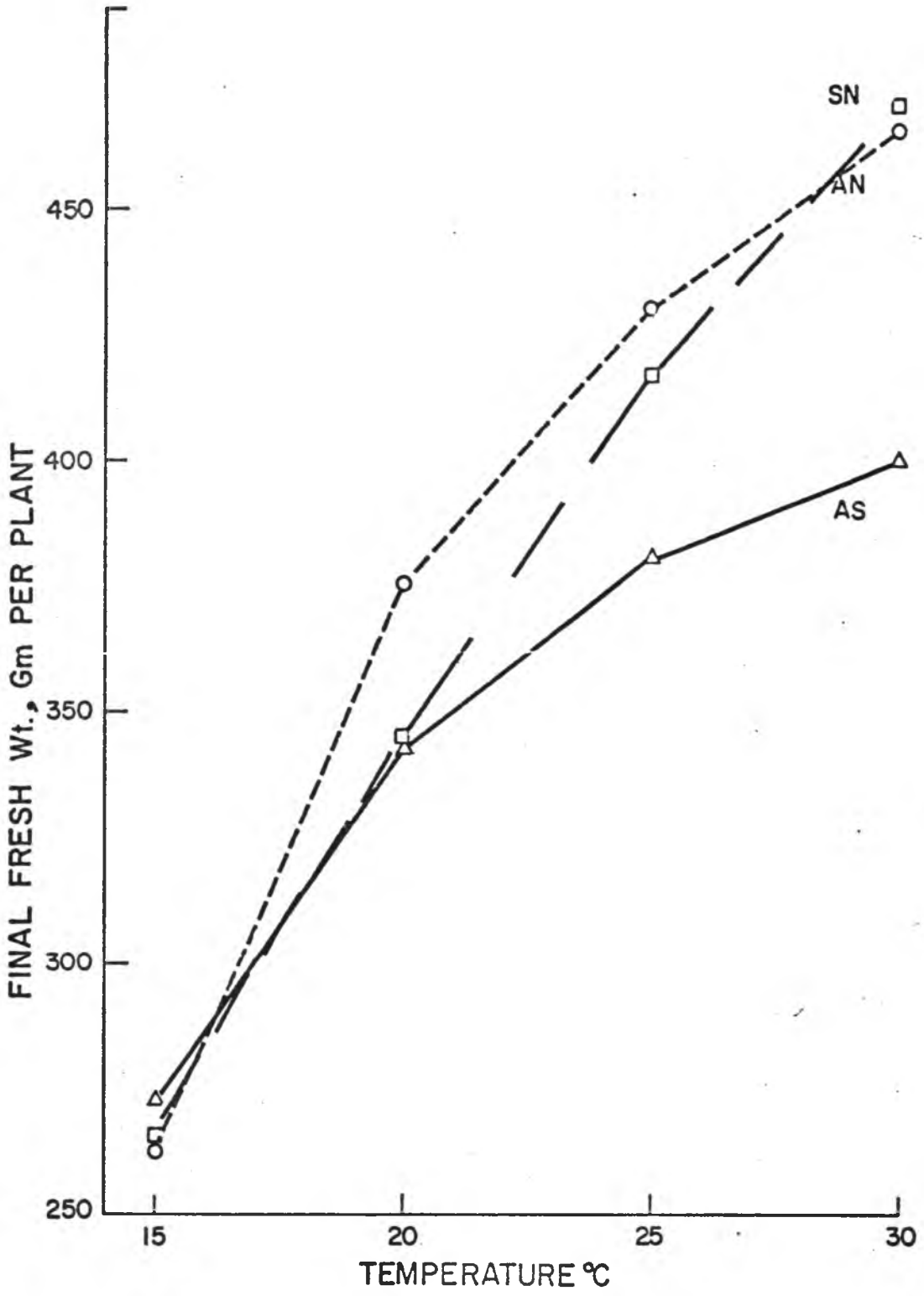

Fig. 8. Effects root temperatures and N-carriers on final weight.

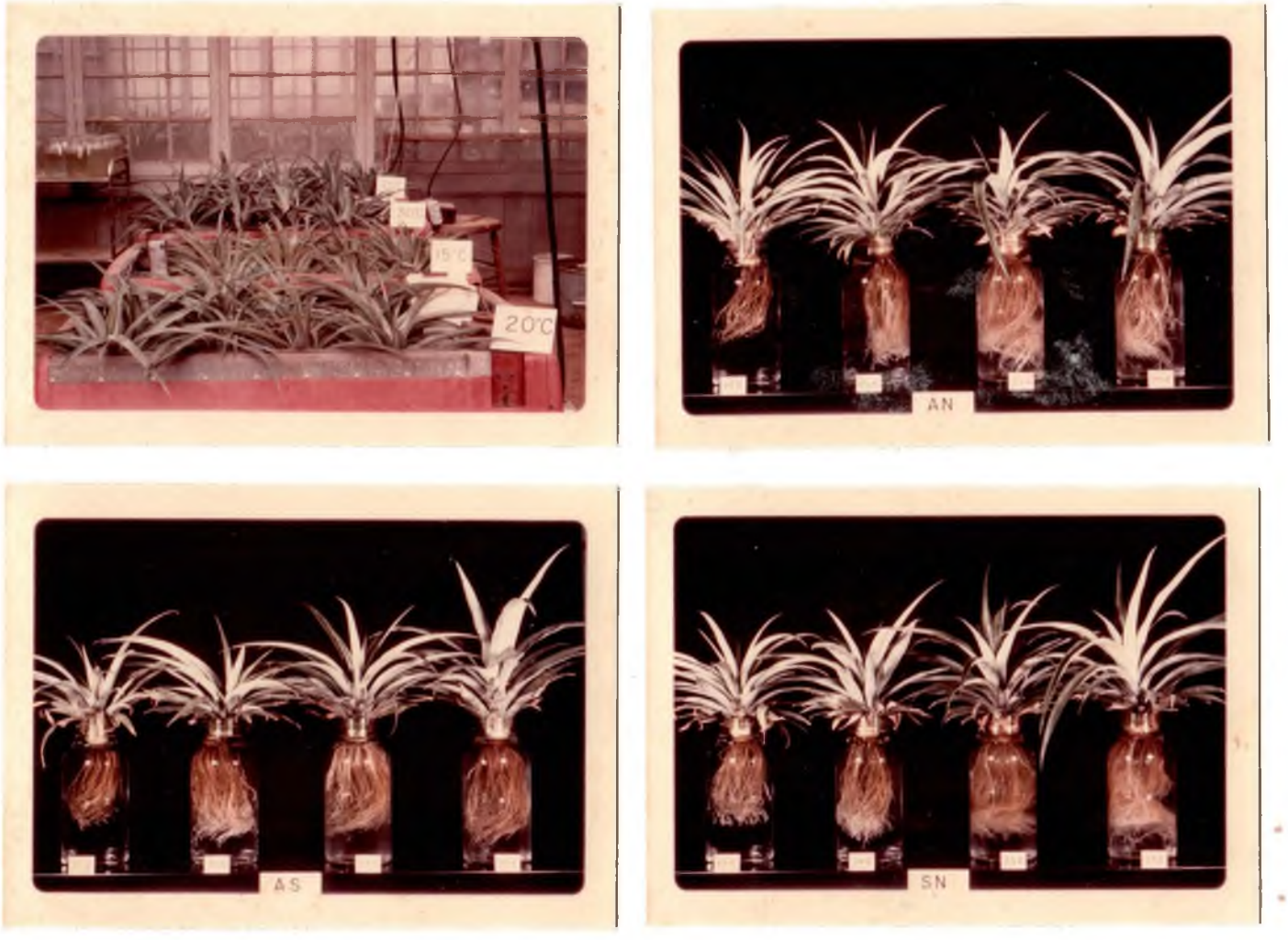

Plate 1. Effects of root temperatures and N-carriers on plant growth.

Growth of Plants (ΔG)

The ΔG (final plant weight - initial plant weight) was very significantly affected by root temperatures and N-carriers with a highly significant C X T interaction (Table 4). In fact, most of the responses were very similar to those of final plant weight. This was expected, since the initial plant weights were not significantly different and the differences in final plant weights were due to contributions of ΔG. It is interesting, however, that all plants grown at 15 C root temperature showed losses in weight at the end of the experimental period (Fig. 9 and Appendix Table 4). These losses were probably due to desiccation of leaves as a result of inadequate water uptake by roots. In fact, when this experiment was terminated, leaves of plants grown at 15 C showed symptoms of water deficiency regardless of the N-carrier used.

Percent Rate of Growth

Using ΔG values, the percent rate of growth per 5 day interval was calculated according to the law of exponential growth. The results showed that the effects of root temperatures and N-carriers on a percent rate of growth were similar to those of final plant weight and ΔG, as was expected (Table 4, Appendix Table 4 and Fig. 10). At 30 C root temperature, the rate of growth per 5 day interval was nearly 7.0 percent with the AN and SN carriers, compared to the significantly lower 4.5 percent with AS. The losses in plant weights at 15 C were as much as 0.65 percent of the initial plant weight (Appendix Table 4 and Fig. 10).

The percent total increase in plant weight $\left(\frac{\Delta G}{\text{Initial plant weight}} \times 100\right)$

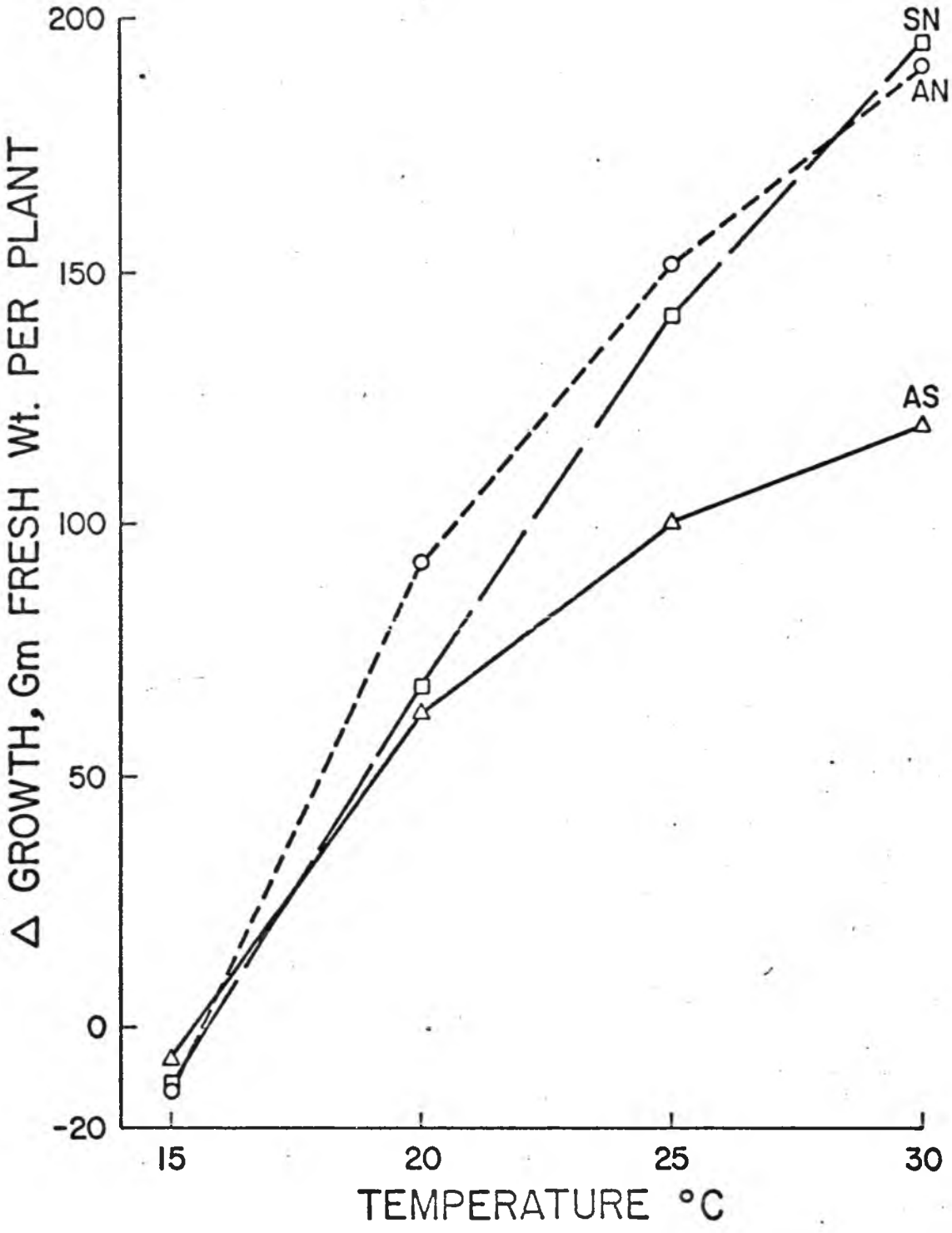

Fig. 9. Effects of root temperatures and N-carriers on growth (growth increase)

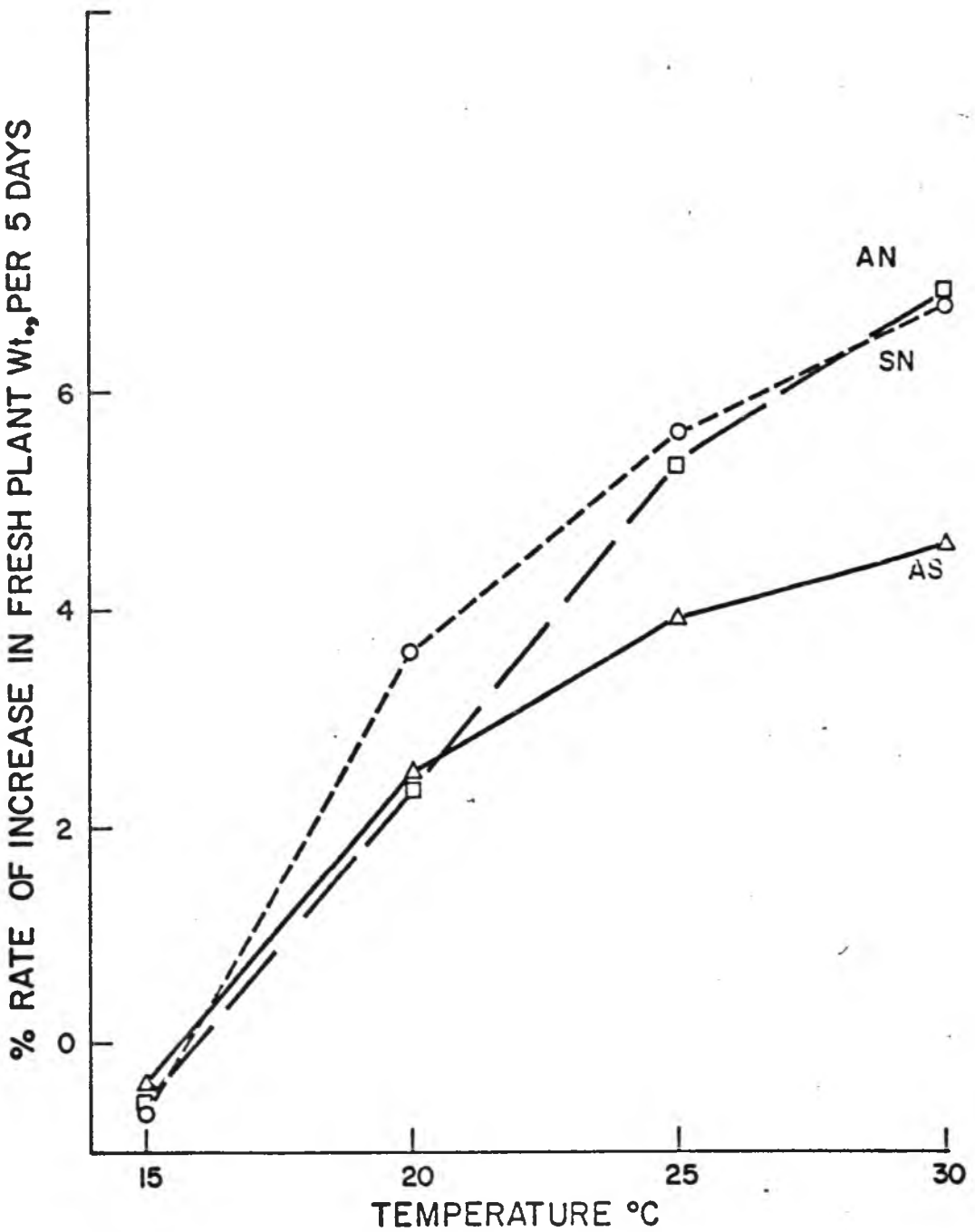

Fig. 10. Effects of root temperatures and N-carriers on percent rate of increase.

showed the same trend as that of the percent rate of increase per 5 day interval. For example, at 30 C root temperature the total increase was nearly 70 percent with AN and SN, compared to 42 percent with AS. The total increase was approximately 10 times as much as that of the percent increase per 5 days, regardless of N-carriers or root temperatures (Appendix Table 4).

Fresh weights of plant parts

Results on the weights of plant parts: a) roots, b) stem and c) leaves, are presented in Table 4, Fig. 11, 12 and 13 and Appendix Table 4.

a) Roots

Root temperatures and N-carriers had highly significant effects on root weight. C X T interaction was also highly significant (Table 4). Increases in root temperatures, up to 25 C, increased root weights significantly. Differences between 25 and 30 C were not significant. The AS cultures produced significantly less amounts of roots than the other two cultures. The increases in root weights were very high and nearly linear with the use of SN at all root temperatures. In the AN cultures, root weights increased greatly from 15 to 20 C, followed by small but continuous increases at all high temperatures. When AS was used as the N source, the root weight increased only from 15 to 20 C, with no increase up to 25 C, followed by a considerable decrease above 25 C. In fact, the root weight with AS, at 30 C, was only slightly more than that at 15 C (Fig. 11 and Appendix Table 4).

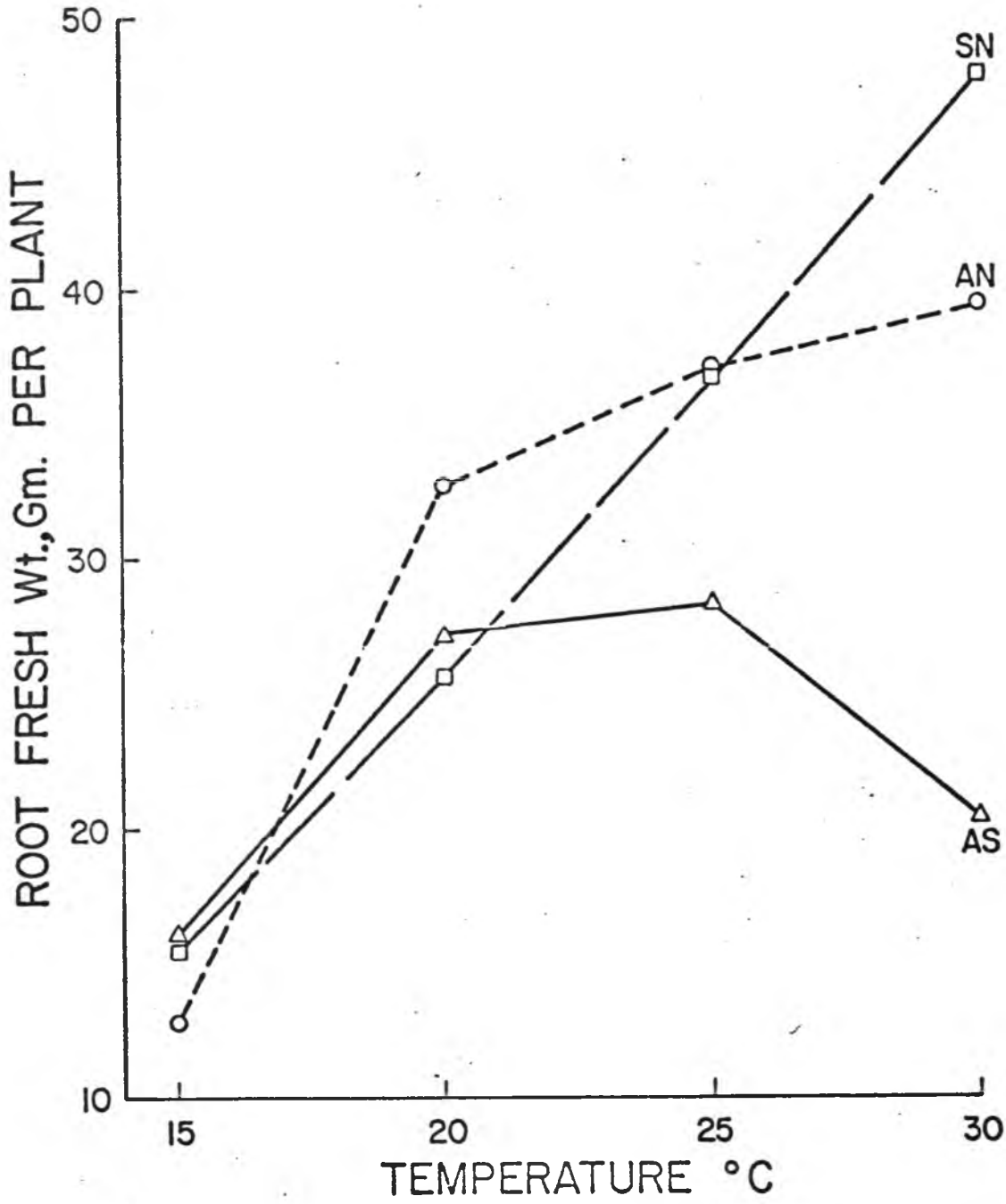

Fig. 11. Effects of root temperatures and N-carriers on root fresh weight.

Critical examination of Plate 1 shows very distinct effects of root temperatures and N carriers on root elongation and proliferation, and on the formation of new roots.

At 15 C root temperature there was very little root elongation, as indicated by short white root tips and little lateral root formation, especially in the AS and AN cultures. Also, very few new roots were formed from the root initials in leaf axils located at the base of the crown. The roots which were originally present at the beginning of the experiment turned brown, indicating suberization, by the end of the experiment.

At 20 C all three N-cultures showed reasonably good root elongation, with better lateral root formation and longer white root tips. Some of the new roots which originated from the axillary root initials also grew longer in all three N carriers. The roots were in general, succulent and healthy as opposed to the brown, suberized roots at 15 C. There were no visible differences in root characteristics between the N carriers.

At 25 and 30 C there were continued increases in root elongation and proliferation, and the formation of new roots when SN and AN were used as sources of N. With the use of AS, there were indications of root elongation and proliferation at 25 C, but not to the same degree as in the case of AN or SN. At 30 C, the total root mass was largest with the SN carrier, and smallest with the AS carrier with AN showing intermediate characteristics.

b) Stem

Root temperatures had highly significant effects on stem weight; N carriers did not show significant effects, with the C X T interaction significant at P = 0.05 (Table 4). At 15 C, there were no significant differences among the three N-carriers; however, at 25 C, the stem weight was significantly higher with SN source than with other N sources (Appendix Table 4). Examination of Fig. 12 shows that stem weights increased continuously at increasing root temperatures with the AS source. With SN, the weights increased rapidly up to 25 C, followed by a sharp decrease. In the AN cultures the stem weights increased only at temperatures of 15 to 20 C, followed by small decreases at all the higher root temperatures.

c) Leaves

Leaf weights were very significantly affected by root temperatures and N carriers. The C X T interaction was significant at P = 0.05. Successive increases in root temperatures showed corresponding significant increases in leaf weights. The mean leaf weights in the different N-carriers treatments were significantly different from each other. The greatest weight of leaves was produced with SN, followed by AN, and the least with AS (Table 4). There were no significant differences in leaf weights with the three N carriers at the 15 and 20 C root temperatures. On the other hand, at 30 C, the weight was significantly less with AS than with the other N-carriers. The rates of increases in leaf weights were very high and almost

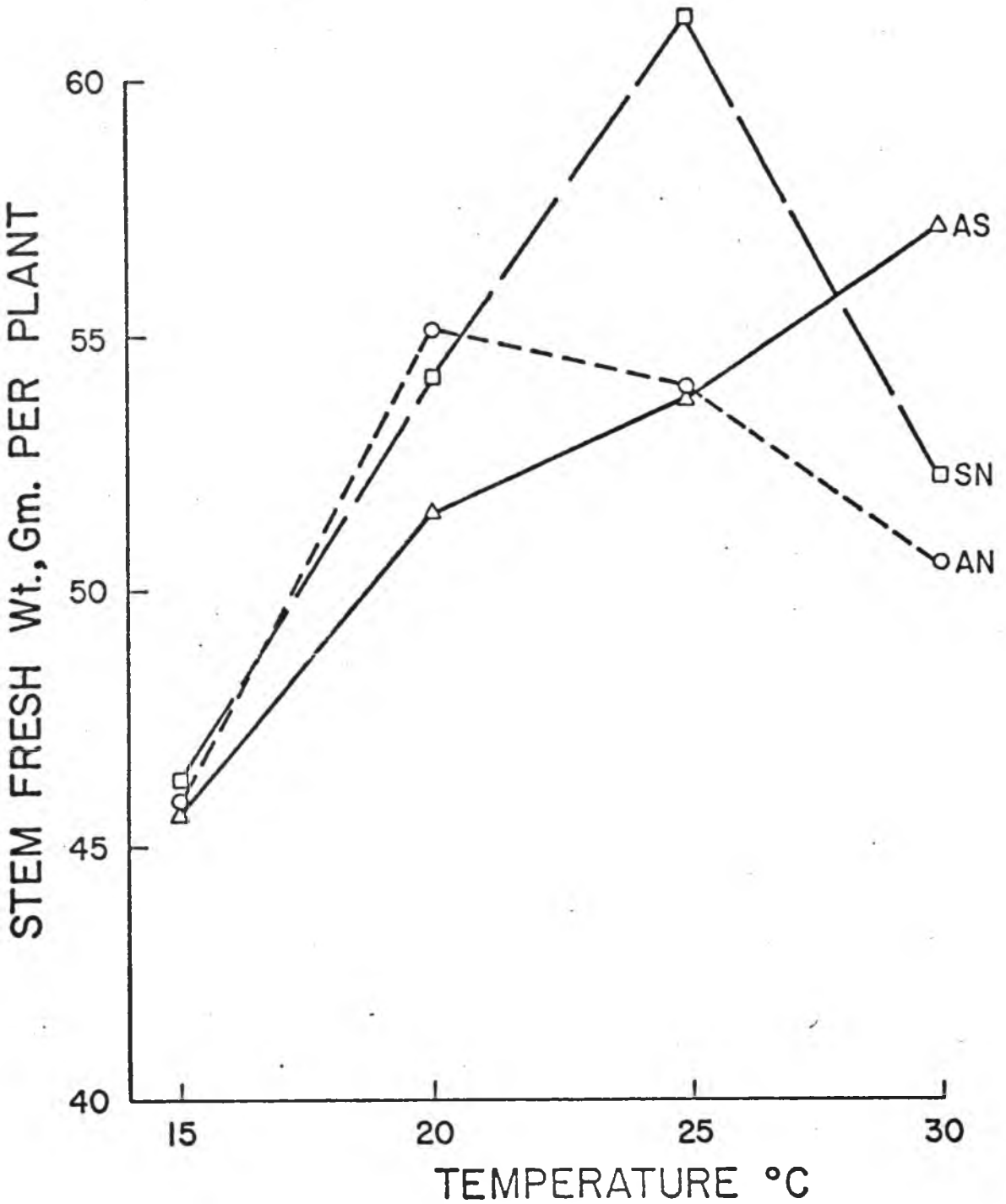

Fig. 12. Effects of root temperatures and N-carriers on stem fresh weight.

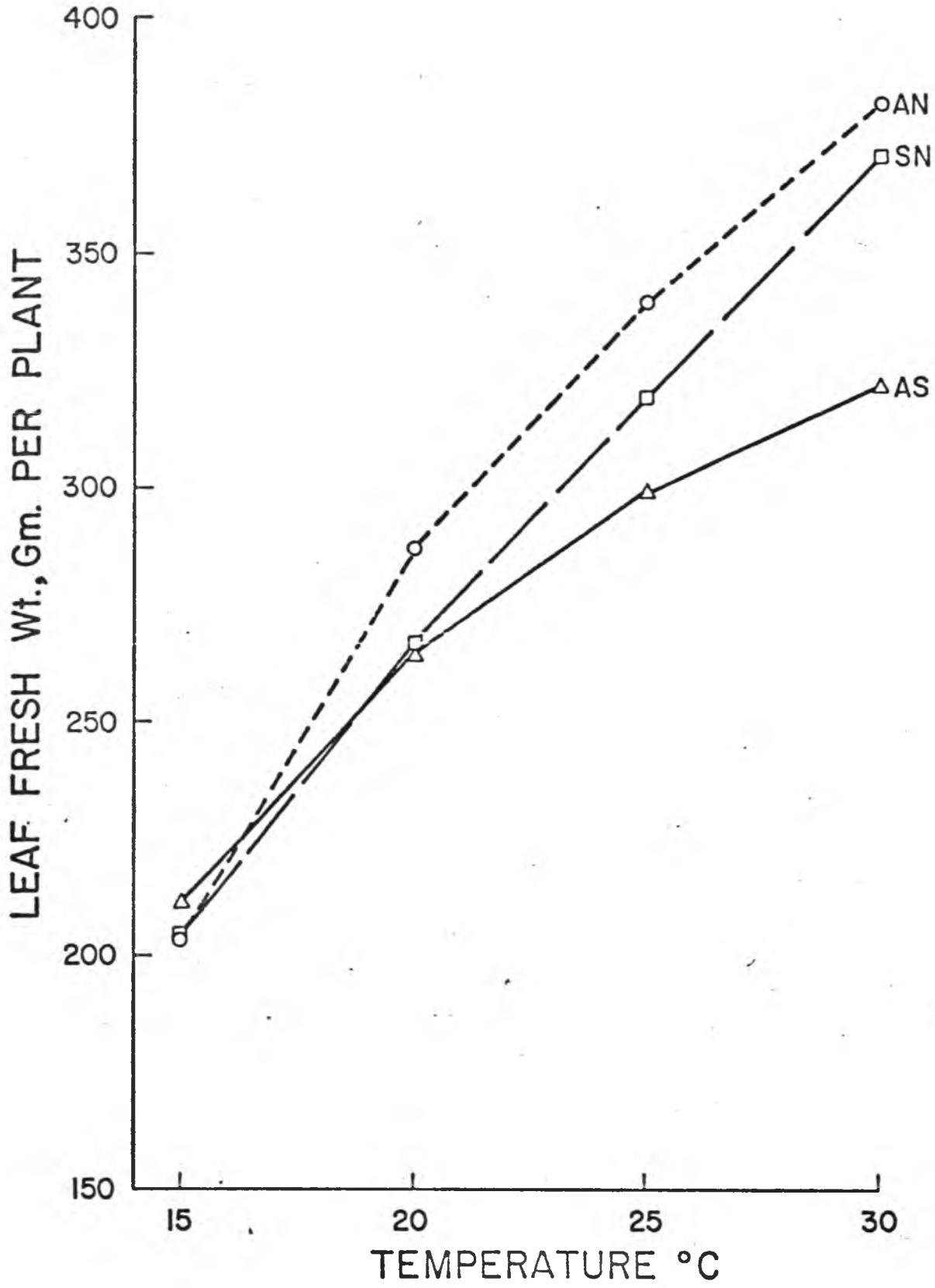

Fig. 13. Effects of root temperatures and N-carriers on leaf fresh weight.

linear between 20 and 30 C. The increases were highest with AN, followed by SN and the lowest with AS (Fig. 13 and Appendix Table 4).

Examination of Plate 1 shows that, in general, the size of leaves, considering both length and width, had visibly increased as root temperatures increased with all three N-carriers by the end of experimental period.

Percent Leaf Dry Matter Content

Data for percent leaf dry matter content indicate that this property was affected significantly (P = 0.05) by N carriers; however, the C X T interaction was not significant (Table 4, Fig. 14 and Appendix Table 4). Increases in root temperatures decreased the percent dry matter content. This was highest in plants grown in AS followed by those grown in AN and was lowest in SN. Examination of Fig. 14 also shows that the dry matter percent decreased with successive increases in root temperatures, regardless of N-carriers.

Relative Weights of Plant Parts

The weights of plant parts (roots, stem and leaves), contribute to the final plant weight (expressed on a percent fresh weight basis) are presented in Table 4, Appendix Table 4 and Fig. 15.

The data indicate that, in general, as root temperature was increased, the proportion of roots was inconsistently affected. On the other hand, the portion of stem decreased, while the proportion of leaves increased, with increases of temperature, especially above 25 C. The effects of N-carriers were very inconsistent, and are difficult to interpret.

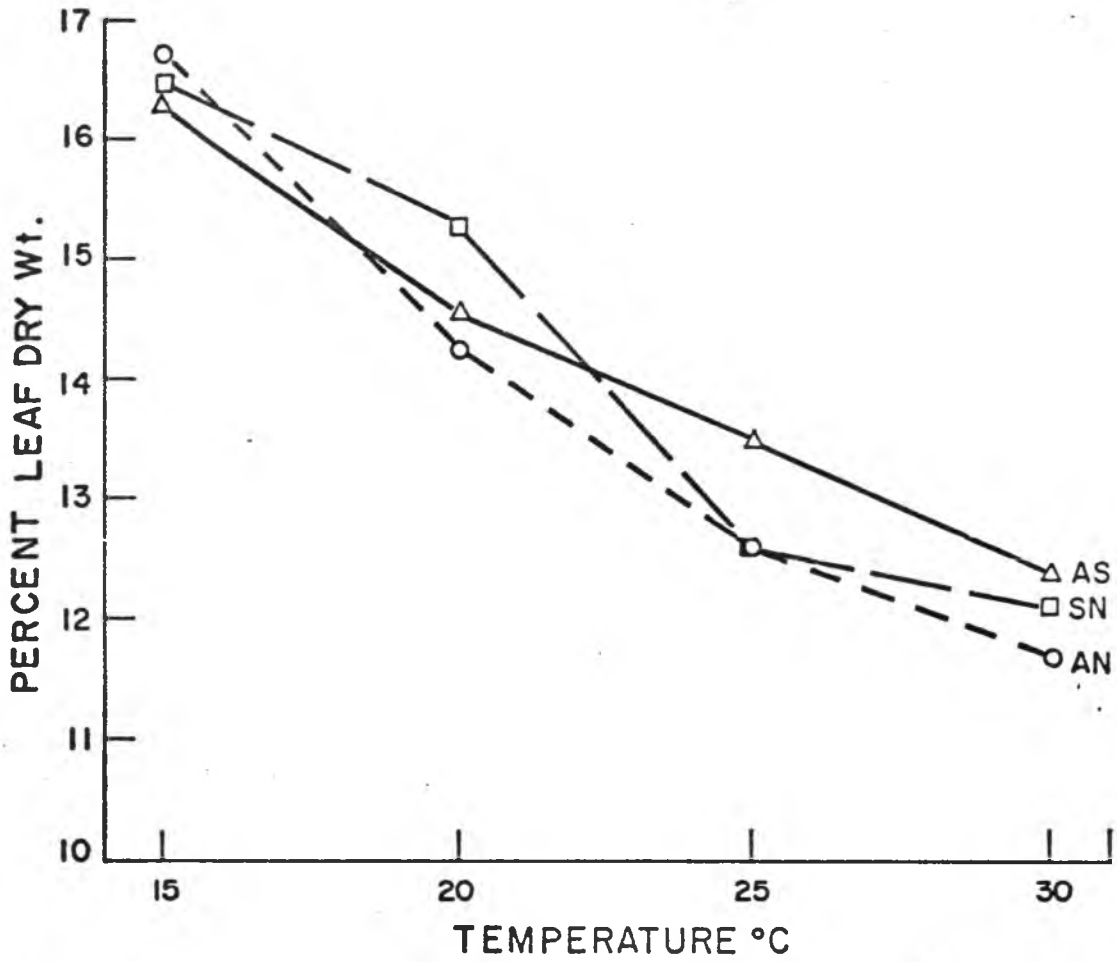

Fig. 14. Effects of root temperatures and N-carriers on percent leaf dry weight.

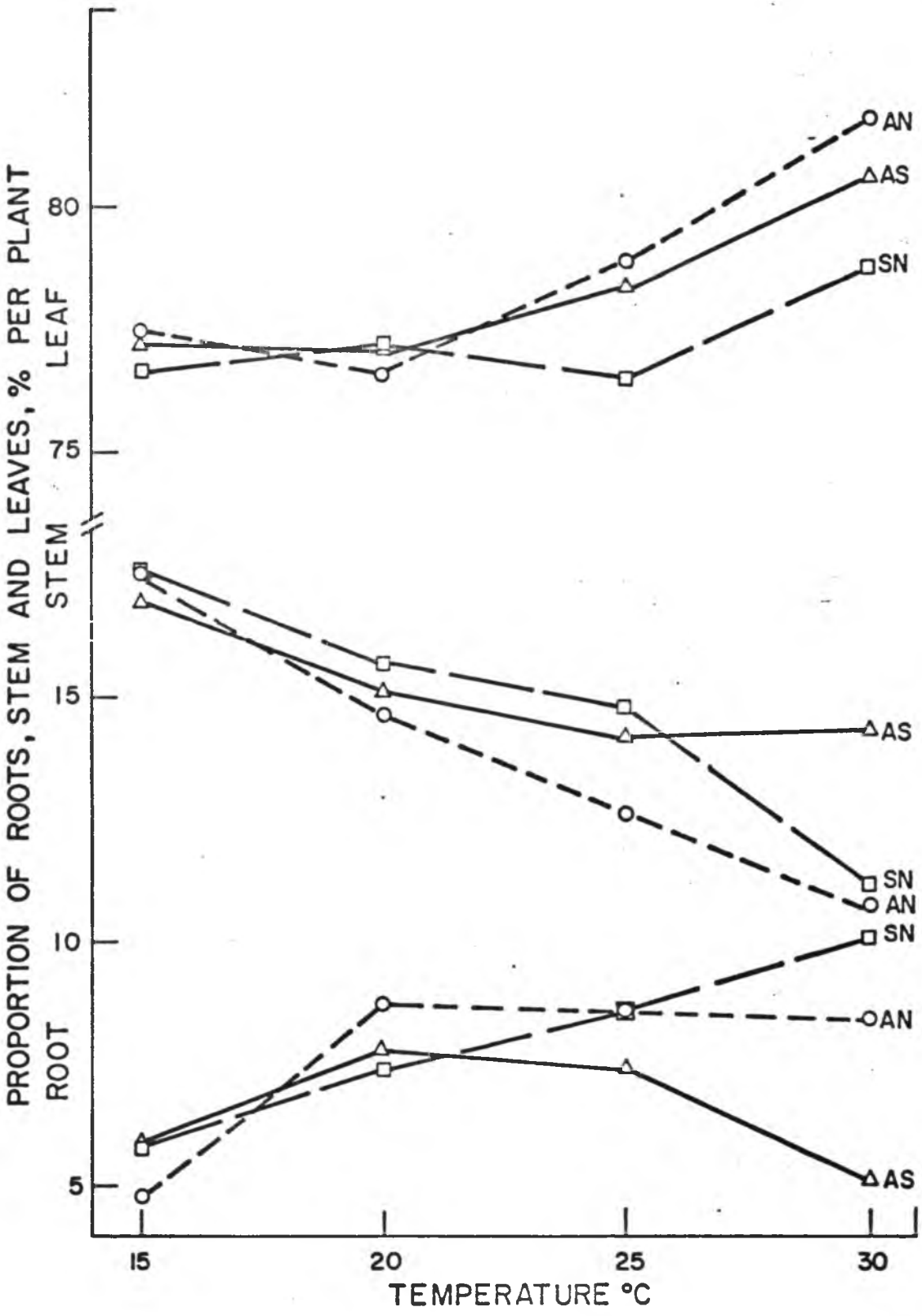

Fig. 15. Effects of root temperatures and N-carriers on proportion of roots, stem and leaves.

Nutrient Uptake and Utilization

1. Efficiency of nutrient uptake by roots

Data showing the capacity of nutrient uptake, expressed in milliequivalents per 100 gm of roots, are presented in Table 5.

The data show that the nutrient uptake increased as root temperatures were increased, the only exception being P uptake in the SN culture where P decreased with increases in root temperatures. The roots also showed higher absorption capacities at 25 and 30 C root temperatures than at lower temperatures.

The capacity of N and P absorption was highest in the AS cultures followed by the AN and SN cultures.

Potassium absorption capacity of roots supplied with SN was superior to those grown in AS; the effect of AN on K absorption was intermediate in relation to the other carriers.

The Ca uptake of roots varied tremendously, depending on the source of N in the culture medium. The Ca uptake was completely inhibited by AS, indicating an inability of roots to absorb Ca. In sharp contrast, roots grown in SN cultures absorbed Ca at a higher rate at all root temperatures. The AN cultures retained some ability to absorb Ca from the solution medium at all root temperatures, except at 15 C. The Ca absorption capacity in the AN cultures amounted to about one-fifth that of SN cultures.

Magnesium absorption capacity of the roots was almost the same regardless of root temperatures or N carriers, although those in SN cultures were slightly better, followed by those in the AS and AN cultures.

Table 5

Effects of root temperatures and N-carriers on the amount of nutrient uptake (m eq) per 100 gm of fresh root

N-CARRIER†	ROOT TEMP. C	N	NH_4-N	NO_3-N	P	K	Ca	Mg
AN	15	34.7	16.7	17.8	37.5	28.8	---*	3.7
	20	56.4	24.7	31.6	26.4	40.5	1.4	4.9
	25	86.9	35.4	51.5	42.1	77.7	2.8	12.3
	30	88.5	36.5	51.9	47.1	70.4	2.3	17.8
MEAN		66.6	28.3	38.2	38.3	54.4	1.5	9.7
AS	15	32.5			21.6	12.4	---*	4.7
	20	48.0			35.7	23.5	---*	4.5
	25	113.5			69.3	64.5	---*	12.4
	30	172.5			102.8	78.7	---*	21.0
MEAN		91.6			57.4	45.4	---*	10.6
SN	15	30.3			33.6	36.4	2.2	5.1
	20	43.5			28.7	45.0	7.5	8.4
	25	74.0			27.5	77.0	11.9	15.0
	30	64.2			26.1	61.9	11.3	16.4
MEAN		53.0			29.0	55.1	8.2	11.2

* Calcium was not absorbed

† AN = Ammonium Nitrate
AS = Ammonium Sulfate
SN = Sodium Nitrate

In the AS cultures, capacity of the roots to absorb N, P, K and Mg was consistently higher at 30 C than in the other cultures, whereas the fresh root weight showed the revenue relationship (See Fig. 11). These results indicate that, although high temperatures reduced root growth, the efficiency of nutrient absorption remained unaffected or increased with increases in root temperatures in the AS cultures.

2. Efficiency of nutrient utilization for growth

The efficiency of nutrient utilization, simply translated as the number of milliequivalents of nutrients utilized in the production of 100 gm ΔG, at all root temperatures, and N carriers are presented in Table 6.

The SN cultures showed the highest utilization efficiency for N and P (represented by the least meq. of these nutrients required to produce 100 gm of ΔG) at all root temperatures. Corresponding values for the AS cultures were low, with those for AN lying between.

The amount of K required to produce 100 gm of ΔG was almost the same with all N-carriers for corresponding root temperatures.

Since AS cultures showed an actual loss of plant Ca, rather than absorption at all root temperatures, no value can be reported for these cultures. The AN culture utilized less Ca than the SN culture, at all root temperatures. In fact, the SN cultures utilized 4.5 to 6.0 times as much Ca as that of the AN cultures for every 100 g of ΔG depending on root temperatures.

The amount of Mg required (meq) for the production of 100 gm ΔG was considerably lower than the corresponding values for N, P and K. The Mg utilization efficiency was high with the AN source, followed by the

Table 6

Effects of root temperatures and N-carriers on the efficiency of nutrient utilization (m eq) for growth per 100 gm of ΔG

N-CARRIER†	ROOT TEMP C.	N	NH_4-N	NO_3-N	P	K	Ca	Mg
AN	20	19.8	8.7	11.0	9.3	14.2	0.5	1.7
	25	21.2	8.6	12.5	10.3	19.0	0.7	3.0
	30	18.2	7.5	10.7	9.7	14.6	0.5	3.6
MEAN		19.7	8.2	11.5	9.8	16.1	0.5	2.8
AS	20	20.9			15.7	10.0	---*	2.0
	25	37.7			22.9	21.9	---	4.0
	30	29.3			17.7	13.4	---	3.5
MEAN		29.3			18.8	15.1	---	3.2
SN	20	18.4			11.5	18.8	3.0	3.4
	25	19.7			7.4	20.6	3.2	4.0
	30	15.8			6.6	15.4	2.7	4.0
MEAN		18.0			8.5	18.3	3.0	3.8

Note: Due to lack of growth at 15 C, values could not be calculated.

* Calcium was not absorbed

† AN = Ammonium Nitrate
AS = Ammonium Sulfate
SN = Sodium Nitrate

AS and SN sources.

Considering the effects of root temperatures alone, the efficiency of nutrient utilization was nearly always high at 20 C, root temperature was low at 25 C, and 30 C was only slightly less than at 20 C.

DISCUSSION

Growth, regardless of how it is expressed (Δgrowth or percent rate of increase per 5 day interval, assuming an exponential growth, or as the total increase from the initial to final plant weight) increased with increases in root temperatures; the largest increase usually occurred between 20 and 25 C, followed by a somewhat lesser rate of increase thereafter up to 30 C.

At the 15 C root temperature, the final plant weight was invariably lower than the initial plant weight, probably due to the desiccation of leaves resulting from nonabsorption of water. This desiccation of leaves at low root temperatures is in agreement with work on sugarcane in Hawaii (Mongelard and Mimura, 1971 and 1972); these authors showed that at 15 C or lower root temperatures the absorption of water was completely inhibited. Vaadia, et al. (1961) stated that plant growth does not depend on the amount of water absorbed but rather on a balance between the amount of water absorbed and that transpired.

The desiccation of leaves in this experiment would indicate a strong possibility that water absorption and translocation were impaired. According to Kramer (1959), water deficiency will severely inhibit growth and photosynthesis due to the desiccation of protoplasm and stomatal closure. Under these conditions, the carbohydrates would be hydrolyzed rapidly, and the enhanced respiration would deplete the carbohydrates reserves. It is probable that such a mechanism was responsible for the loss of plant weight at 15 C in this experiment. Water, being an excellent conductor of heat, would probably have kept

the apical meristem cooler than the atmospheric temperature which could have retarded both normal cell division and cell elongation as shown by Beauchamp and Lathwell (1967) for corn. Also, under these conditions production as well as translocation of certain metabolites such as "rhizocaulines", kinetins, gibberellins, etc. (Nielson and Humphires, 1966; Smika and Ellis, 1971; and Salisbury and Ross, 1967) would have been affected at low root temperature. As Kramer (1969) pointed out, low root temperatures may affect water absorption and translocation directly as a result of changes in root membrane structure and metabolism, resulting in increased root resistance to the entry of water.

Restricted nutrient uptake at low root temperatures has been shown to be associated with poor growth by many researchers. Smika and Ellis (1971) contend that attributing lower yields from plants grown in cold soils to nutrient deficiency is oversimplifying the problem. They believe that poor growth at low soil temperatures is not due to nutrient deficiencies due to an inadequate supply, as a result of low temperatures, of one or more metabolites produced in the roots which are essential for top growth. In pineapple, nutrient inadequacy alone does not appear to be entirely responsible for poor growth at low temperature. The crowns used in this experiment were fairly large and contained a considerable reserve of food material. Even those rooted crowns placed in demineralized water are capable of growing for a considerable period of time, probably up to 5 or 6 months. In fact, reserve non-experimental rooted crowns, held at "room" temperature, continued to grow in tap water, making fairly good growth even after the termination of this experiment. On the other hand, in this experiment

the plants which were kept at 15 C stopped growing completely and almost immediately regardless of N-carrier. This would indicate that factors other than nutrients were involved in the cessation of growth.

The fair amount of growth and the longer time it took to reach a steady rate of water and nutrient absorption at 20 C may indicate that there was initial cold injury, from which, however, the plants slowly recovered (see Chapter I). At high root temperatures, apparently there was no root injury, with growth proceeding normally.

Considering the effects of the N-carriers on growth, both SN and AN were equally good; on the other hand the AS plants showed poor growth despite a very high N and P uptake. Two possible limiting factors are K and Ca deficiencies. It is unlikely that Ca is limiting since it has been shown in many experiments by Wallace and his associates (1963, 1966 and 1971) and Jones and Lunt (1967) that the amount of Ca required for normal growth of plants is at micronutrient level as long as heavy metal ions are not present at toxic levels. The requirement of Ca by pineapple has also been shown to be very low by Sanford, et al. (1961), being in the range of about three percent that of K. In as much as heavy metal ions were supplied only at micronutrient levels in this experiment the chances of Ca deficiency are remote. This leaves only K as the possible limiting factor.

In an excellent review, Evans and Sorger (1966) showed that K is the most important monovalent cation; this is required in very large quantities to catalyze most of the 46 enzyme systems in plants and animals. Essentiality of K has been strongly implicated in important functions such as the absorption and utilization of N, protein synthesis

and its protection against degradation, and CO_2 fixation and translocation of photosynthates in plants (Evans and Sorger, 1966; Sideris and Young, 1945 and 1946). It has been shown that the rate of fixation and speed of translocation of photosynthates from the "source" to the "sink" is dependent on the concentration of plant K (Hartt, 1969 in sugarcane and Ashley and Goodson, 1972 in cotton). Often a drastic reduction in growth and metabolic rates occur at concentrations between those for optimum growth and those for visual deficiency symptoms. Even a very mild deficiency has been shown to affect water permeability of roots (Graham and Ulrich, 1972).

Some of these disorders were observed in this experiment with the use of AS; these disorders were the result of the competitive inhibition of K by NH_4. Water absorption also was significantly lower with AS than with the other two carriers (similar to the observations of Barker and Bradfield, 1963, in corn). Despite a high N uptake, growth was poor probably due to a poor utilization efficiency or a "luxury consumption" of NH_4 from AS. The absorption of K showed a very good relationship with growth in the case of all three carriers. Potassium utilization in terms of ΔG was nearly equal for all three carriers at the same root temperatures, showing that the carriers had no effect on utilization efficiency, but the amount of K uptake did affect growth. In other words a mild "functional" or "physiological" deficiency rather than a severe "visual" deficiency may have been operating when AS was used; this probably limited the growth of plants. On the other hand, with the use of the other carriers, the level of K did not limit growth. Although critical visual deficiency levels of K in the tissues has been shown to be 0.2% on fresh weight basis, both Nightingale (1942a) and

Sanford, et al. (1961) showed the optimum to be 0.35% or higher. MacLead (1971), working with corn, showed the need for higher K level with NH_4 than with NO_3. This author also observed outright death of corn seedlings when K deficient plants were fertilized with NH_4. Examining the data on nutrient absorption in this experiment, in the light of the observations made by these other authors, and "limiting factor theory" of Nightingale (1942a), it can be concluded that the poor growth with AS was most probably due to K deficiency since both N and P were absorbed in adequate amounts for normal growth.

Although the total cation uptake was higher with AS than with the other carriers (Chapter I), growth appears to be more closely related to the total alkali cation than to the total cation uptake. The total alkali cation (K + Ca + Mg) uptake was quite low with the use of As; this relationship has also been shown to be associated with low organic acids content and poor growth (Sideris and Young, 1954) in pineapple.

Root growth also showed variations with the use of various root temperatures, N-carriers and C X T interactions. There was an almost continuous increase in root growth with SN, up to 30 C and a continuous increase up to 25 C with AN. However, with AS, the amount of root growth increased only up to 20 C. There was no increase from 20 to 25 C, with an actual decrease in the weight of roots between 25 and 30 C. Root weight with AS at 30 C was only slightly more than that at 15 C. Roots are shown to be the "sink" for photosynthates (Nielson and Humphries 1966). It is shown that "detoxification" of ammonium takes precedence over any other plant function. NH_4 uptake increases almost continuously with increases in root temperatures, with K uptake inhibited.

Such high NH_4 uptake probably depletes all the plant carbohydrates reserve, in the process of the detoxification of NH_4. Also, due to a "physiological" or "functional" deficiency of K, photosynthetic fixation as well as translocation would be greatly reduced, and whatever CO_2 was fixed would have been preferentially used to detoxify the NH_4 absorbed by the roots. This would result in very little photosynthate being left for the growth of roots and there would, therefore, be no weight increase at the higher root temperatures.

The optimum temperature for root growth (and, therefore, indirectly nutrient absorption) has been shown to be somewhat less than that for top growth in pineapple and other crops (Sanford, et al., 1961; and Nielson and Humphries, 1966). Interesting differences in root growth in response to root temperatures and N-carriers were observed in this experiment. Root growth continued to increase uniformly in the SN cultures with increases in root temperature up to 30 C, suggesting an optimum higher than 30 C for root growth. In the case of AN, the optimum for root growth was 25 C. In the AS cultures, however, the optimum appears to be about 20 C, with temperatures higher than 25 C actually being detrimental to root growth, although the ability of the roots to absorb nutrients was not inhibited, but on the contrary increased, at higher root temperatures. In contrast, the top growth, especially leaf growth, did not follow the same trend as that of the roots. At low root temperatures the top growth closely followed root growth, whereas at higher temperatures the top growth was relatively independent of root growth.

Absorption of nutrients, especially N, P and K, nearly always showed an optimum at 25 C root temperature, regardless of N-carriers, with an

exception in the case of P with SN. Yet, top growth did not reflect the nutrient absorption maximum at 25 C and continued to grow fairly rapidly between 25 and 30 C, indicating an absorption maximum close to 25 C, and a top growth maximum close to 30 C or higher, regardless of the N-carriers.

Considering the weight of plant parts such as the root, stem and leaves, expressed as percentages, more than 75% was leaves and less than 10% was roots, the rest being stem, dependent on root temperatures and N-carriers. The root temperatures affected the proportion of plant parts to a greater extent than did N-carriers. These results also indicate that any factor detrimental to leaf growth would adversely influence the total weight of plants.

To reemphasize the effect of N-sources, growth characteristics indicate that an all nitrate source (SN), or a mixture (AN), is the best for the growth of both roots and the whole plant. Especially as a protective mechanism to tide over the adverse soil moisture conditions or soil borne pathogen (nematode) problems, a better root system would certainly be advantageous; to achieve this, it is necessary to utilize at least some NO_3 source in the fertilizer program. Inasmuch as an all ammonium source severely competes with and inhibits K uptake, foliar application of K under all ammonium cultures may be beneficial for plant growth. Although SN produced better root and plant growth, N uptake is poor in this culture, and a complete dependence on a nitrate source might eventually lead to N deficiency; in this contingency, foliar applications of N may be an advantage.

Considering the serious disadvantages with AS and the potential disadvantages with SN under field conditions, AN appears to be a very

promising N-carrier not only for solution cultures but for soils of low fertility. The use of AN has resulted in high N uptake, high K uptake and fair amounts of Ca and Mg uptake. The growth of roots, as well as that of the plant as a whole in this culture was sufficiently greater to indicate it to be the best choice, conditions of availability and economy permitting. The use of AN also gives an additional advantage in that it does not leave any acidic (as does AS) or alkaline (as does SN) residue in the soil, or on leaves when used as a foliar spray. Also, AN has a better N utilization efficiency compared to the conventional urea spray (Sanford and Fo, 1966).

SUMMARY AND CONCLUSIONS

Sequential increases in root temperatures, N-carriers and their interactions all very significantly affected plant growth. At 15 C root temperature, there was invariably a loss in plant weight probably due to insufficient water absorption by the roots through cold injury with a desiccation of leaves due to low or no water absorption. At 30 C, the increase in growth based on initial and final plant weights was a high 70% with the carriers AN and SN, compared to a low 42% with AS. The greatest increase in growth usually occurred between 20 and 25 C root temperatures, a relationship very similar to that of nutrient absorption at these temperatures.

Root growth showed distinct variations with root temperatures and N-carriers. At 15 C, root growth was very poor and roots were brown and suberized regardless of N-sources. With the use of SN, root weight increased continuously up to 30 C with the suggestion that the optimum temperature for root growth with SN was above 30 C. With the use of AN, root weight rapidly increased up to 25 C, followed by very little further increase in weight suggesting a root temperature of 25 C as optimum with AN. In contrast, with the use of AS, root growth increased only up to 20 C, with almost no increase up to 25 C and an actual decrease in growth above 25 C. It is probable that the lack of root growth with AS is due to a preferential utilization of carbohydrates in the detoxification of NH_4, on the one hand, and a possible "physiological deficiency" of K (thus limiting carbohydrate synthesis and translocation) on the other hand.

With regard to stem weight, the effects of root temperatures and N-carriers varied widely; stem weights were greater with the use of

AS than with AN and SN.

Leaf weights per plant tended to increase with increases in root temperatures, with no optimum up to 30 C. The total weight of the leaves at the higher root temperatures of this experiment was significantly lower with AS than with AN and SN.

The capacity of the roots to absorb nutrients tended to increase with increases in root temperatures. Although root growth did not increase (or even decrease) with AS, the capacity of the roots of these cultures to absorb nutrients was not affected; on the contrary, root absorption was improved at the higher root temperatures of 25 and 30 C. The amount of N and P utilized (nutrient utilization efficiency) in the production of 100 gm growth increase was lowest with SN, followed by AN; it was highest with AS indicating a poor nutrient utilization efficiency with the use of AS. The amount of K required for the greatest increase in growth was almost the same at any given temperature, regardless of the N-carrier; this suggests that the amount of K was the main limiting factor involved in poor plant growth with AS. Despite a high N and P uptake with AS, plant growth with AS was poor; this was probably due to either an inefficient utilization of N, a "luxury consumption", or both. The low K absorption and poor growth obtained with AS may also be due to a mild "physiological" N "functional" K deficiency which was not severe enough to visual symptoms. Also, the low total alkali cations absorption (K + Ca + Mg) may have been responsible for the poor growth with AS.

Regardless of the root temperatures and N-carriers, leaves constituted more than 75% of the total weight, with roots making up less than 10% and the stem the remainder.

Considering root growth or the total increase in plant weight, it is evident that a nitrate source of N or a mixture of nitrate and ammonium sources is better than an ammonium source. Also under adverse soil conditions, such as an inadequate water supply and/or the presence of soil pathogens it appears desirable to use a nitrate source or a mixed N source for a better and beneficial root growth.

CHAPTER III

EFFECTS OF ROOT TEMPERATURES AND N-CARRIERS ON THE COMPOSITION OF NITROGENOUS FRACTIONS IN VARIOUS PLANT PARTS

MATERIALS AND METHODS

Details on the composition of leaves are given in two sections: A and B.

A. Nitrogenous Fractions of Plant Parts

Material

Samples of fresh root, stem and leaf tissue preserved in 95% ethanol, as detailed in Chapter II under Materials and Methods, were used for the determinations of soluble and protein-N fractions.

Methods (PRI Method 14-6.1)[1]

The fresh tissue preserved in 95% ethanol was quantitatively transferred to a Waring Blendor, blended for about 3 minutes, filtered through Whatman No. 12 folded filter paper and the filtrate collected in a 800 ml Kjeldahl flask. The macerated tissue on the filter paper was washed at least five times with 80% ethanol.

Soluble nitrogen

The ethanol-soluble fraction collected in the Kjeldahl flask, containing soluble organic, ammoniacal nitrogen was converted to ammonia by digesting with concentrated sulfuric acid in the presence of sodium sulfate and selenium. During the process the organic matter was completely oxidized. The digest containing ammonia was subjected to standard macro-Kjeldahl distillation and N determination.

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[1] In PRI files

Protein nitrogen

The macerated residue containing crude protein nitrogen collected on the filter paper was transferred completely with the filter paper into a 800 ml Kjeldahl flask. The entire mass was digested in the presence of concentrated sulfuric acid, sodium sulfate and selenium as determined above. The digest was subjected to standard Kjeldahl distillation and N determination.

Total nitrogen

The total N fraction of different plant parts was determined as the sum of soluble + protein nitrogen.

Treatment of data

Data are presented as concentration (ppm) of N fractions in plant parts as well as the total amount of N present (mg per plant) in different plant parts. Only concentration data were analyzed by the standard AOV method as was discussed earlier.

B. Analysis of Amino Compounds Present in Leaves

Amino acid analyses were carried out in the Technicon Amino acid Autoanalyzer. A brief description of the equipment follows:

Chromatographic column is 150 cm long and filled with Technicon chromobead resin Type A. The column is surrounded by a water jacket to circulate hot water, and delivers eluate at the rate of 0.67 ml per minute. The column separates amino acids sequentially based on their respective retention times.

Water bath fitted with a pump to circulate hot water and temperature is controlled by immersion thermostat.

Autograd with nine buffer chambers to supply a gradient of buffer from pH 2.875 to 5.00.

Automatic manifold is power driven and connected to the chromatographic column, ninhydrin reagent (0.67%), hydrazine sulfate solution and N_2. This is connected to a glass coil in a heating oil bath where the color of ninhydrin-amino acid complex is developed. After color development the mixture goes to three colorimeters.

The three colorimeters are arranged sequentially and each sample is read in all three colorimeters.

Colorimeter I: Has a 15 mm cell and absorbs at 440 mμ wave length.

Colorimeter II: Has an 8 mm cell and absorbs at 570 mμ wave length.

Colorimeter III: Has a 15 mm cell and absorbs at 570 mμ wave length.

The colorimeters are connected to an automatic chart recorder which records the absorption in three different bands.

Material

The second batch of leaf samples stored in 95% ethanol, as described in Chapter II under Matherials and Methods, was used.

Method

The method of extraction of the ethanol-soluble fraction was very similar to the extraction procedures outlined for soluble N-fraction in Section A. The ethanol extract was quantitatively transferred to a flash evaporator flask where it was concentrated under vacuum at 40 C to a final volume of about 15 ml. This concentrate was transferred quantitatively (including three or four washings with small amounts of

water) to a 25 ml volumetric flask, made up to volume and mixed thoroughly by shaking. Then the concentrate was filtered through Whatman No. 2 filter paper, the filtrate collected in a 50 ml Erlenmeyer flask, stoppered and stored in the freezer for future analysis.

Sample Preparation for Amino Acid Analysis

Both soluble amino acid and amide-N determinations were made from composite samples obtained by mixing equal volumes of concentrates from rep 1 + 3 and 2 + 4. Thus, the total number of soluble amino acid and amide determinations were only 24 each (root temperatures 4 X N-carriers 3 X composite samples 2).

Soluble Amino Acids

An aliquot of a composite sample was mixed with 62.5% sucrose and norleucine (internal standard) and diluted to volume. A sample representing about 0.5 to 1.0 gm fresh tissue in 12.5% sucrose and 0.1 µm Norleucine was injected on top of the chromotographic column with a clinical syringe. The amino acids present in the sample were eluted with sodium citrate buffers with pH of 2.875, 3.80 and 5.00 stored in the autograd which delivered a pH gradient throughout the entire run. This "long" run took about 16½ hours and was terminated following the appearance of Arginine on the chromatographic chart. The different amino acids present were identified based on retention times from a standard chart, their net areas of absorbance calculated and the concentrations expressed in ppm of amino acid-N.

Amide-N

Asparagine and glutamine present in the sample were hydrolyzed to respective amino acids and then determined in a "short" 4 hour run using only the 2.875 pH buffer. The samples were hydrolyzed in 1 N HCl for one hour, cooled and the excess acid was neutralized with NaOH, made to volume, filtered and an aliquot prepared and injected as described for soluble amino acid. The run was terminated after the appearance of glutamic acid. Concentrations of aspartic and glutamic acids were determined as already described. After subtracting the values of these amino acids in the unhydrolyzed sample, the remainder was expressed as asparagine-N and glutamine-N.

The soluble amino acids, serine and threonine, resolved better in the short run and therefore, were calculated from this run.

Statistical Analysis of Data

Analysis of data was similar to that in other cases, except with amino acids the total number of observations were 24, instead of 48 as in all other cases.

RESULTS

The effects of root temperatures and N carriers on the composition of nitrogenous fractions are presented in two sections in this chapter: A, the composition of nitrogenous fractions of various plant parts (Table 7A, Appendix Tables 5 and 6 and Fig. 16, 17 and 18); and B, the amino compounds present in the ethanol extracts of fresh leaves (Table 7B, Appendix Tables 7 and 8 and Fig. 19, 20, 21 and 22).

A. Composition of Nitrogenous Fractions in Various Plant Parts

1. Nitrogenous fractions of roots

Results presented in Table 7A, Fig. 16 and Appendix Tables 5 and 6 showed that maximum concentrations of soluble, protein and total-N in the roots, with the exception of AS, always occurred at 20 C. Levels of protein and total-N fractions with the AS were slightly higher at 25 and 30 C root temperatures. The data also showed that the soluble-N fraction amounted to only 11.0 to 14.0 percent of the total-N depending on root temperatures and carriers.

2. Nitrogenous fractions of stems

Results presented in Table 7A, Fig. 17 and Appendix Tables 5 and 6 showed that, in general, as the temperature increases to 25 C, the concentration of all N fractions tend to decrease, the only exception being the soluble-N in the AN cultures. On the average, AN had the highest soluble-N levels, whereas AS had the highest protein and total-N levels in the stem. The proportion of soluble-N to total-N was also high, ranging from one-fourth to about one-third. At 25 and 30 C root temperatures, AS again showed higher concentrations of protein and total-N than the other two carriers.

Table 7A

Summary of analyses of variance tests and Duncan's Modified LSD tests on the effects of root temperatures and N-carriers on the composition of nitrogenous fractions (ppm fresh weight basis) of different plant parts

TESTS		NITROGENOUS FRACTIONS								
		ROOT			STEM			LEAF		
		Soluble -N	Protein -N	Total -N	Soluble -N	Protein -N	Total -N	Soluble -N	Protein -N	Total -N
ANALYSIS OF VARIANCE		SIGNIFICANCE OF F VALUES								
SOURCE	DF									
TEMPERATURE (T)	3	**	**	**	--	**	**	**	**	**
REP IN T	12									
CARRIERS (C)	2	*	**	**	**	**	--	**	**	**
C X T	6	**	**	**	*	--	*	**	**	**
ERROR (b)	24									
DUNCAN'S MODIFIED LSD TESTS P=0.05†										
ROOT TEMPERATURE MEANS										
15		A	A	A	A	C	C	A	A	A
20		C	B	B	A	B	B	A	AB	A
25		A	A	A	A	A	A	B	C	B
30		B	A	A	A	A	A	B	BC	B
N-CARRIER MEANS										
AMMONIUM NITRATE	(AN)	B	A	B	B	A	AB	B	B	B
AMMONIUM SULFATE	(AS)	B	B	C	A	C	B	B	B	B
SODIUM NITRATE	(SN)	A	A	A	A	B	A	A	A	A

* = significant at 5% level

** = significant at 1% level

† = means with same letter are not significantly different

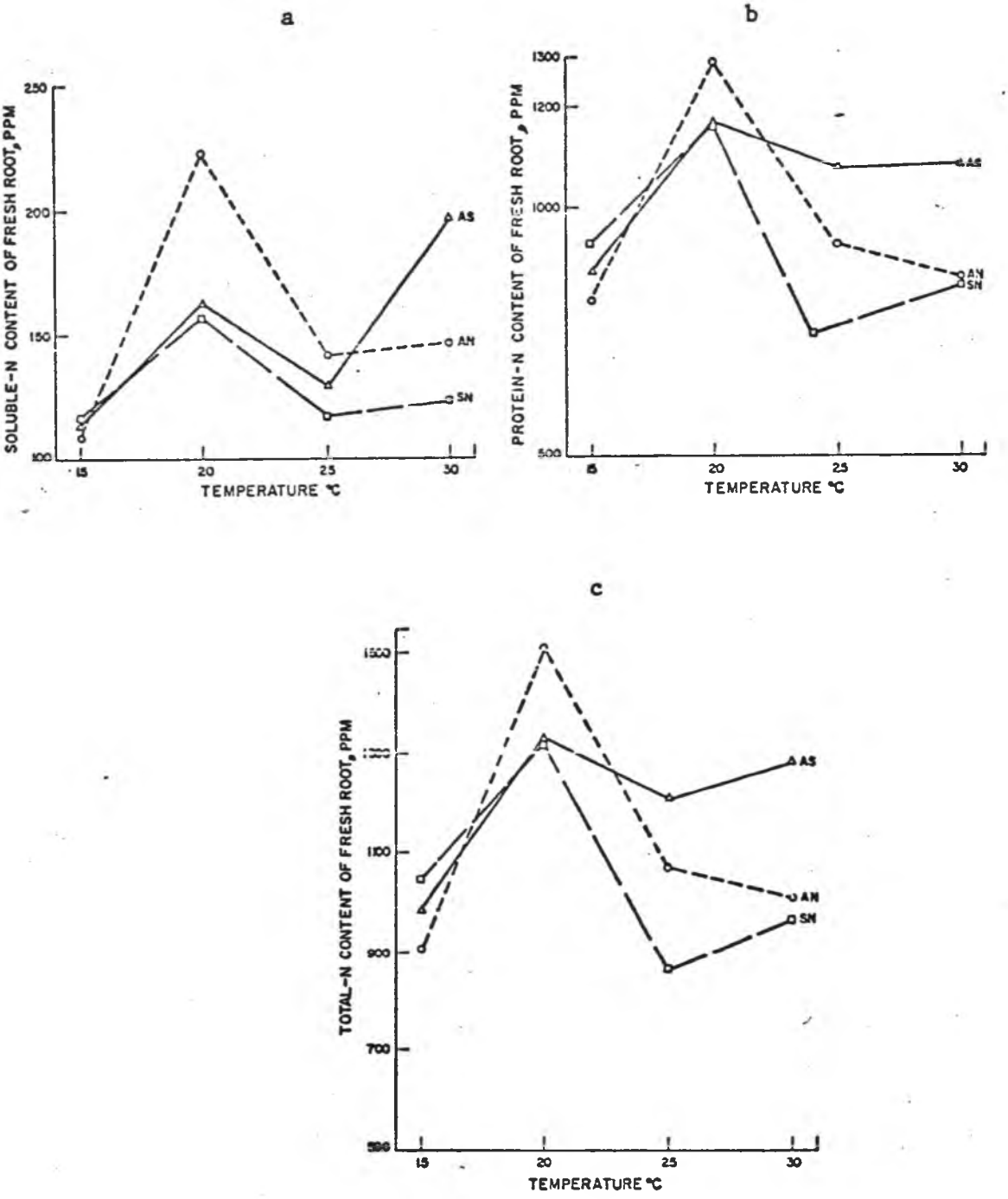

Fig. 16. Effects of root temperatures and N-carriers on (a) soluble-N, (b) protein-N and (c) total-N concentrations of roots.

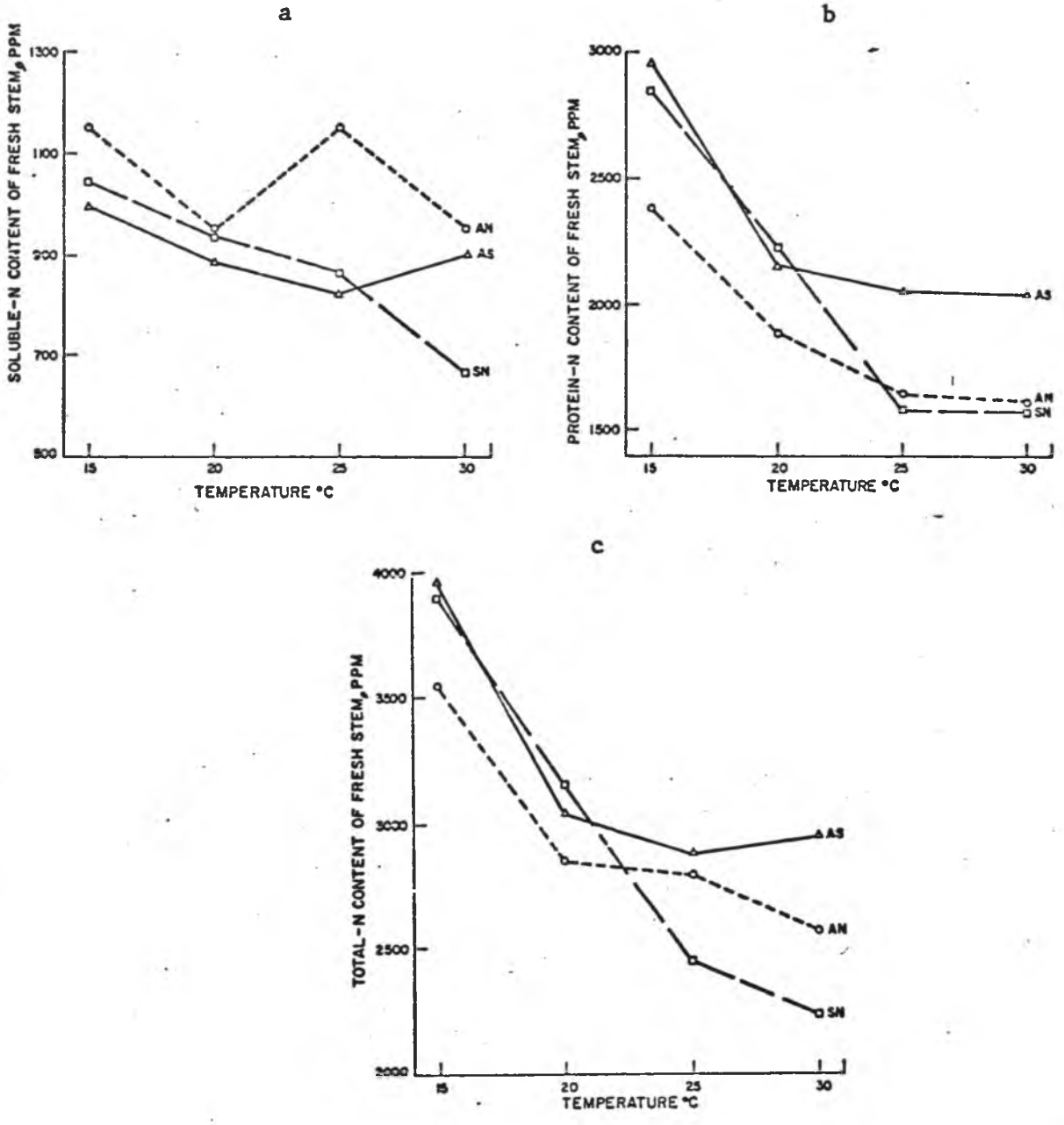

Fig. 17. Effects of root temperatures and N-carriers on (a) soluble-N, (b) protein-N and (c) total-N concentrations of stems.

3. Nitrogenous fractions of leaves

Amounts of nitrogenous fractions in leaves are presented in Table 7A, Fig. 18 and Appendix Tables 5 and 6. The data show that all the N fractions increased only slightly as temperature increased from 15 to 20 C, followed by a substantial increase to 25 C. Further increases in temperature to 30 C registered a slight increase in all N-fractions with AS, but showed a decrease with AN and SN; the only exception was a slight increase in soluble-N with AN. At 25 and 30 C, the leaves grown in the AS cultures showed higher concentrations of all N-fractions than in the other N cultures. Soluble-N constituted between 18 and 22 percent of the total-N, depending on the N-carrier and root temperature.

In general, the highest concentration (ppm) of N-fractions was in the stems followed by that in the leaves, with the least in the roots, whereas, the highest amount of N fractions (mg) per plant was in the leaves, followed by that in the stem with the least in the roots. The proportion of soluble-N to total-N was smallest in the roots, largest in the stem and intermediate in the leaves. At root temperatures of 25 and 30 C, almost all the N-fractions were higher in different parts of plants in the AS cultures than in the other N cultures.

B. Ethanol-Soluble Amino-N Content of Leaves

Two sets of chromatograms were developed in the Technicon Amino acid Autoanalyzer, one with the unhydrolyzed samples for soluble amino acids (long 16½ hour run) and the other using hydrolyzed samples for amides (short 4 hour run).

The fifteen soluble amino acids plus NH_4 which were found in the long run, when arranged in the order of increasing retention time were:

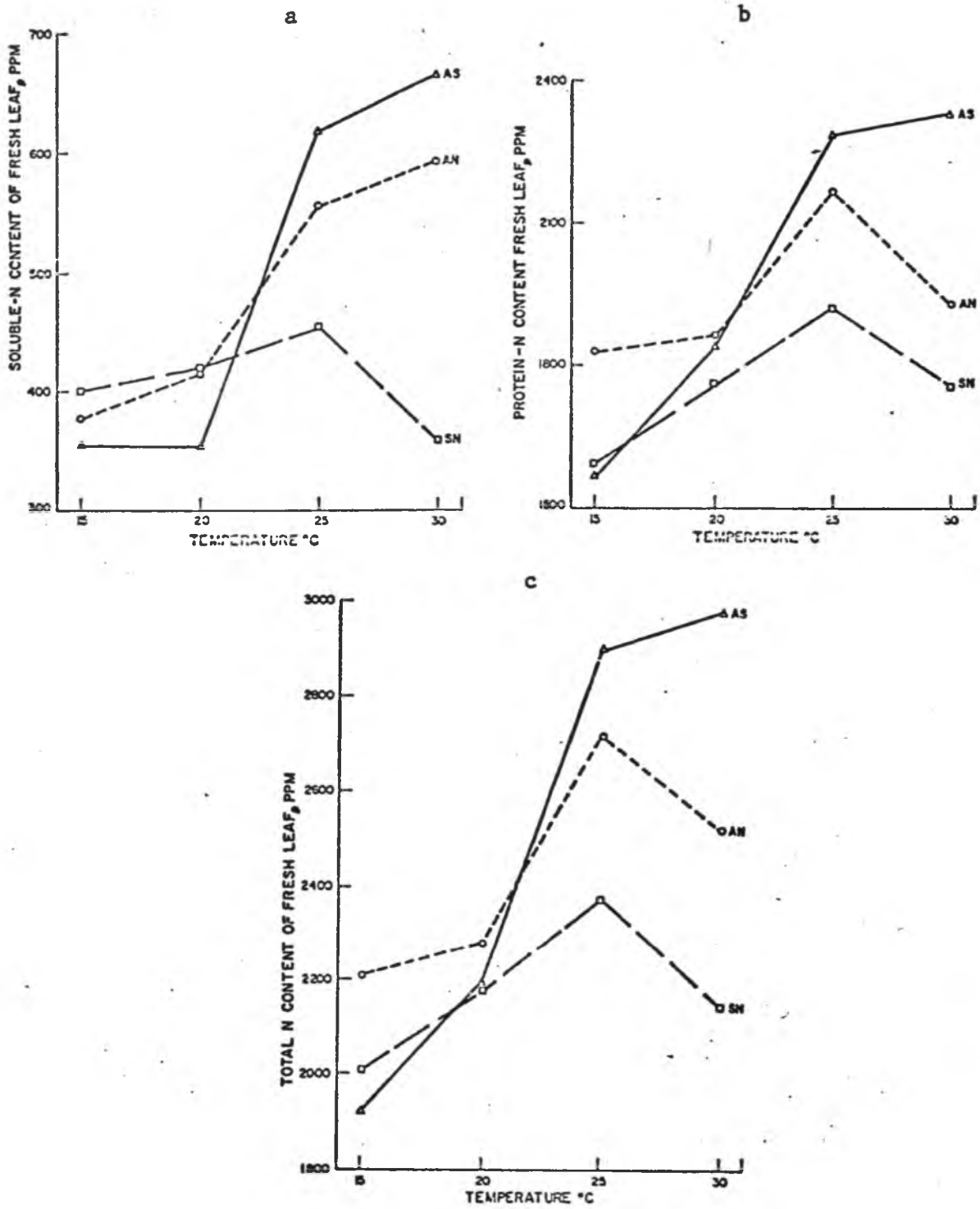

Fig. 18. Effects of root temperatures, N-carriers on (a) soluble-N, (b) protein-N and (c) total-N concentrations of leaves.

aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenyl alamine, γ-aminobutyric acid, ammonium, lysine and argenine. None of the sulfur amino acids were present in high enough concentrations to be detected in the Autoanalyzer (Appendix Table 7).

A brief summary is given below on some of the important individual amino acids in relation to the effects of root temperatures and N-carriers.

Ammonium-N was the largest contributor to the total amino acid-N pool, constituting about one-half to one-third of the total pool. With increases in root temperatures, NH_4 concentrations showed only slight changes in AN cultures, while AS and SN cultures showed moderate increases with temperature increases. The NH_4 content was slightly higher with the AN source than with the other sources.

Alanine-N was the most abundant amino acid, constituting about 12 to 25% of the total soluble amino acids-N, depending on the root temperature and N-carrier. In all three N-carriers, increases in root temperatures showed slight increases in alanine content. In the AN culture the concentration was slightly higher than in the other two cultures, especially at low root temperatures.

Serine-N showed slight increases with increases in root temperatures. The different N-sources did not cause sizable differences in serine content.

Threonine-N also showed slight increases in concentrations with successive increases in root temperatures. The threonine content was not affected by the source of N.

γ-Aminobutyric acid-N content was somewhat higher in the AN cultures than in the other two N source. Concentrations also increased with

corresponding increases in root temperatures.

Three other amino acids -- aspartic acid, proline and argenine -- were also present in moderate concentrations, ranging from 0.5 to 3.0 ppm-N, depending on the N-carriers and root temperatures. Leaves from AN cultures showed practically no increases in the concentrations of these amino acids with increases in root temperature. The AS and SN cultures, however, did show increases in aspartic acid and proline content with successive increases in root temperature.

The remaining eight amino acids were present in the leaves at relatively low concentrations.

Amide-N Content

a. Asparagine-N

Root temperatures and N-carriers affected the asparagine-N content of leaves very significantly. The C X T interaction was significant at $P = 0.05$. Concentrations of this amide did not differ significantly between 15 and 20 C, and between 25 and 30 C; however, the asparagine content in these two groups was significantly different. Asparagine content of the SN culture was significantly lower than that of the AS and AN cultures (Table 7B).

Examination of Fig. 19a and Appendix Table 8 shows very little change in concentrations between 15 and 20 C root temperature. There was a tremendous increase about 5 to 7 fold, when root temperature changed from 20 to 25 C. Between 25 and 30 C, there was a slight increase in the asparagine content in the AS culture, while in the other two cultures there was a slight decrease. At higher root temperatures, asparagine was greatest in the AS culture, followed by AN, with the least in SN

Table 7B

Summary of analyses of variance tests and Duncan's Modified LSD tests on the effects of root temperatures and N-carriers on amino-N fractions of leaves

TESTS		Amide-N			Total Soluble Aminoacid -N	"Unknown" -N
		Asparagine	Glutamine	Total		
ANALYSES OF VARIANCE		SIGNIFICANCE OF F VALUES				
SOURCE	DF					
TEMPERATURE (T)	3	**	**	**	*	*
REP IN T	4					
CARRIER (C)	2	**	--	**	*	--
C X T	6	*	--	--	--	--
ERROR (b)	8					
DUNCAN'S MODIFIED LSD TESTS P=0.05†						
ROOT TEMPERATURE MEANS						
15		A	A	A	A	B
20		A	A	A	AB	B
25		B	B	B	B	A
30		B	C	B	B	A
B-CARRIER MEANS						
AMMONIUM NITRATE	(AN)	B	A	B	A	A
AMMONIUM SULFATE	(AS)	B	A	B	A	A
SODIUM NITRATE	(SN)	A	A	A	A	A

* = significant at 5% level

** = significant at 1% level

† = means with same letters are not significantly different

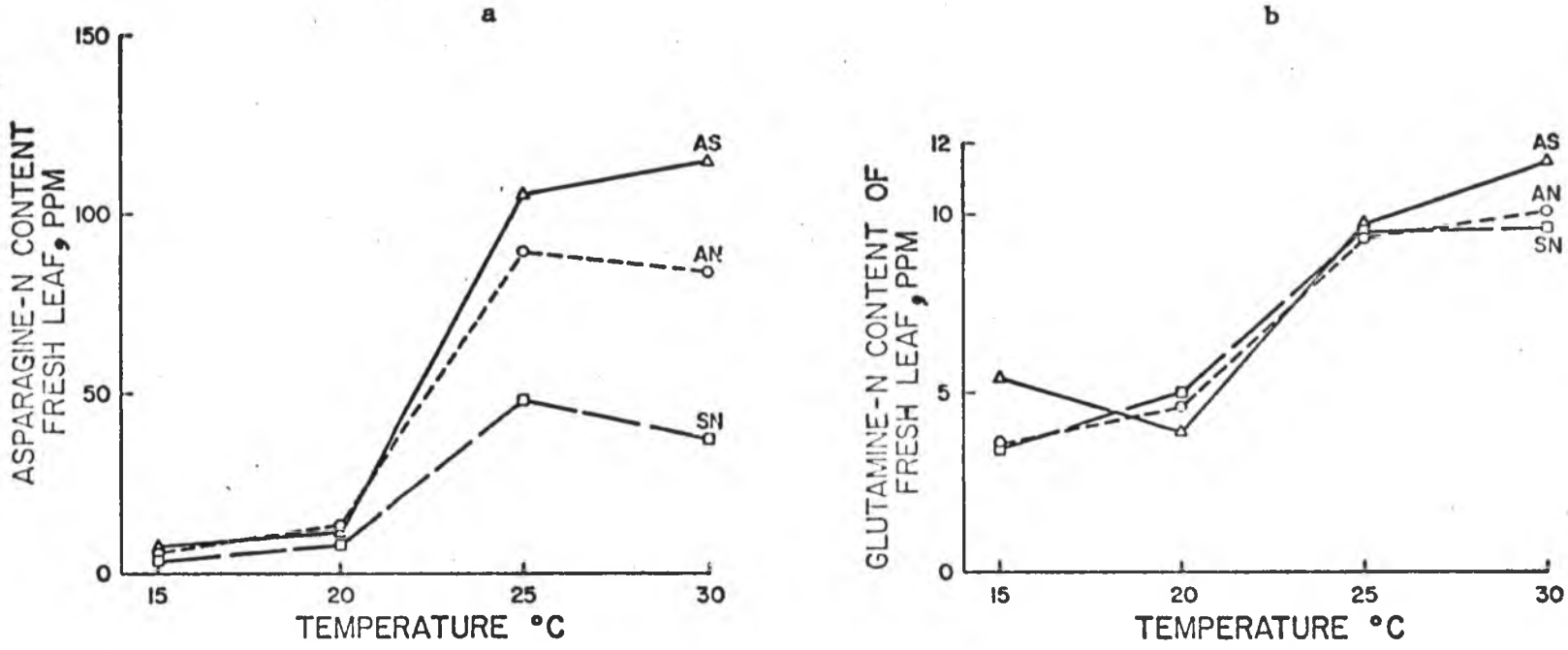

Fig. 19. Effects of root temperatures and N-carriers on amides-N content of leaves.

a. Asparagine-N
b. Glutamine-N

culture. In fact, the asparagine content of the SN culture at 25 C was around one-half and at 30 C about one-third as much as that of the AS cultures at comparable root temperatures.

b. Glutamine-N

Only root temperatures had significant effects on glutamine content of leaves. Glutamine content at 15 and 20 C was not significantly different. A further increase in root temperature to 25 and to 30 C caused significant increases in asparagine content (Table 7B). The highest increase in glutamine concentration occurred between 20 and 25 C with all three N-carriers. At 30 C, the glutamine content was higher than in the other cultures (Fig. 19b and Appendix Table 8).

In general, the mean asparagine content was 3.5 to 7.0 times as great as that of the glutamine content in the different cultures, with the SN culture showing the least difference between these two amides.

c. Total amide-N

The total amide-N (asparagine + glutamine) concentration was very significantly affected by root temperatures and N carriers (Table 7B). The effects of individual root temperatures and N sources on total amide content were quite similar to those on asparagine content (Table 7B, Appendix Table 8 and Fig. 19a and 20). This was expected since asparagine made up the greater part of the total amide content of the leaves.

Leaves from the AS cultures contained the greatest amount of total amide, followed by the AN cultures, with the least in the SN cultures.

Total Soluble Amino Acid-N

Total soluble amino acid (a total of 16 individual amino acids) content was significantly ($P = 0.05$) affected by root temperatures and

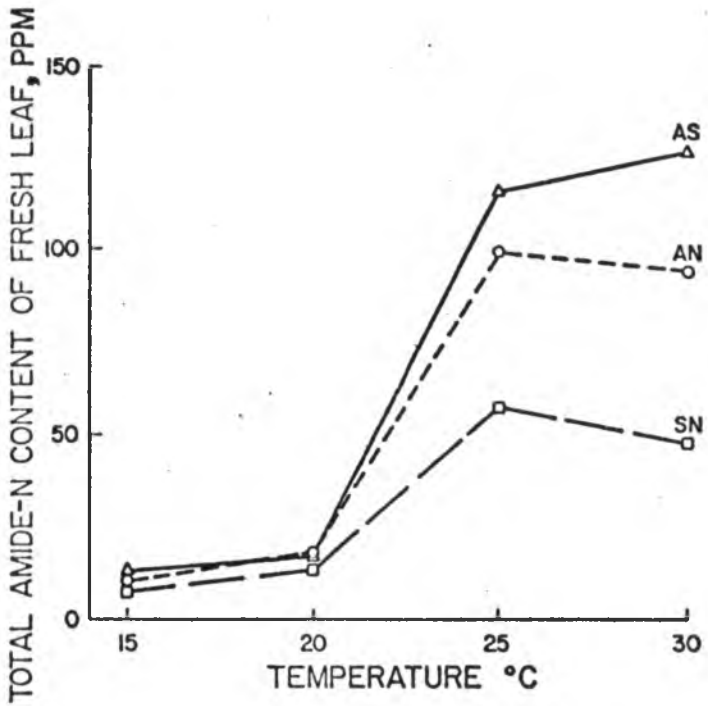

Fig. 20. Effects of root temperatures and N-carriers on total amides-N content of leaves.

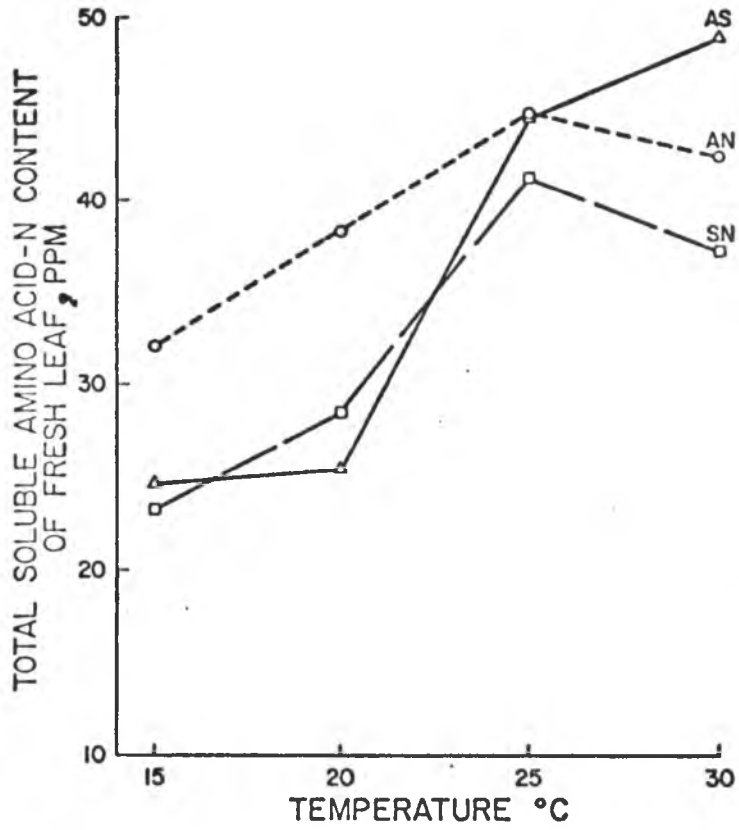

Fig. 21. Effects of root temperatures and N-carriers on total soluble amino acid-N content of leaves.

N carriers. The concentration of total soluble amino acids was significantly higher at 25 and 30 C root temperatures than at 15 C; at 20 C, there was no significant difference in the total soluble amino acids from that found at the other root temperatures (Table 7B and Fig. 21). The overall increase in the total amino acid content between 15 and 30 C amounted to about 50%; this indicates a relatively stable concentration which is less sensitive to change with changes in root temperatures. In contrast, the total amide content increased nearly 10 times when the root temperatures were increased from 15 to 30 C (Appendix 14, Fig. 20 and 21).

"Unknown" Amino Acid-Type Compounds

There were two "unknown" amino acid-type compounds which always occurred together in the hydrolysates. Each of these showed very distinct absorption wave lengths and retention times.

The first to emerge from the chromatographic column showed a retention time of about 90 minutes; produced a blue complex with ninhydrin; and was absorbed at 570 mμ wave length. The concentration of this compound varied from 1.0 to 4.6 ppm-N (Appendix Table 8).

The second "unknown" (hereafter simply referred to as the unknown) was always the second to emerge from the chromatographic column; had a retention time of about 100 minutes; and reacted with ninhydrin to produce a yellow complex which was absorbed at 440 mμ wave length. Because of certain very interesting relationships between this unknown and root temperatures, it was studied in detail.

Analysis of variance of the data showed that only root temperatures produced significant effects on the concentrations of the unknown (Table 7B). The concentrations of this unknown were not significantly

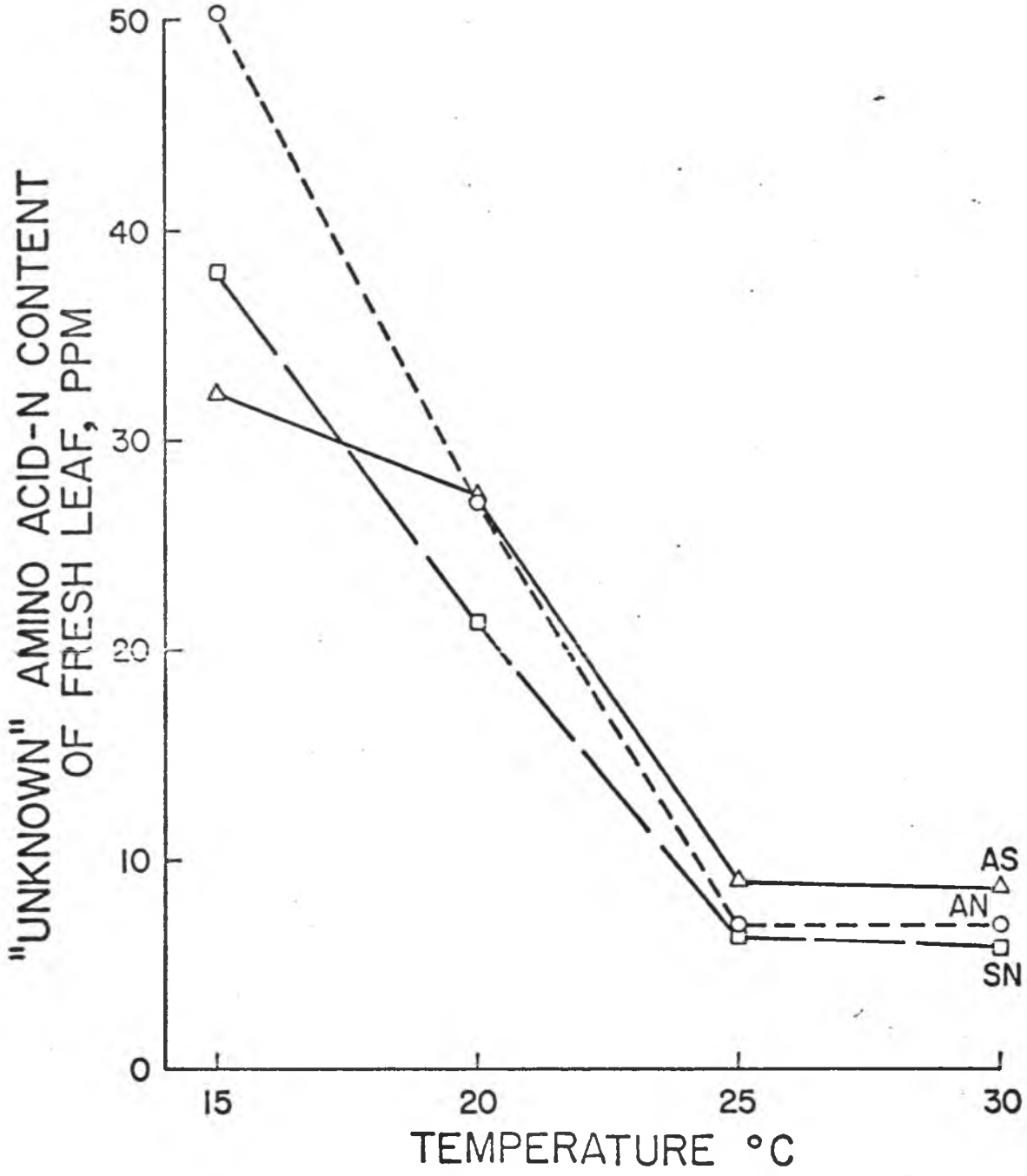

Fig. 22. Effects of root temperatures and N-carriers on "unknown" amino acid-N content of leaves.

different between 15 and 20 C, and between 25 and 30 C; however, differences between these two groups were significant (Table 7B).

Examination of Fig. 22 and Appendix Table 8 showed an inverse relationship between the concentration of the unknown and root temperatures. Increases in root temperatures from 15 to 25 C very sharply decreased the concentration with all three N carriers. A further increase in root temperature to 30 C produced practically no change in the concentration of the unknown. The final concentration of the unknown at 25 or 30 C root temperature amounted only to a fraction of the concentration at 15 C, that is, about 30% in the AS culture and about 15% each in the AN and SN cultures.

A comparison was made to show the relationships between the total amide-N content and ΔG (Fig. 23), and the unknown amino compound-N and ΔG (Fig. 24) at all four root temperatures. Both total amide-N content and ΔG showed positive relationships with root temperatures; both increased with increases in root temperatures. In contrast, the unknown amino-N content showed a negative relationship with both root temperatures and ΔG, decreasing with increases in root temperatures and ΔG.

Because of the interesting nature of the unknown amino compound, certain chromatographic tests were made to determine the nature of the compound. The results are given below:

The compound was ninhydrin positive and produced a yellow amino acid-ninhydrin type peak typical of the prolines. When a chromatogram was developed under standard conditions, just excluding ninhydrin, no peak appeared at the expected retention time. The unknown was not eliminated by filtering the hydrolysate through activated charcoal.

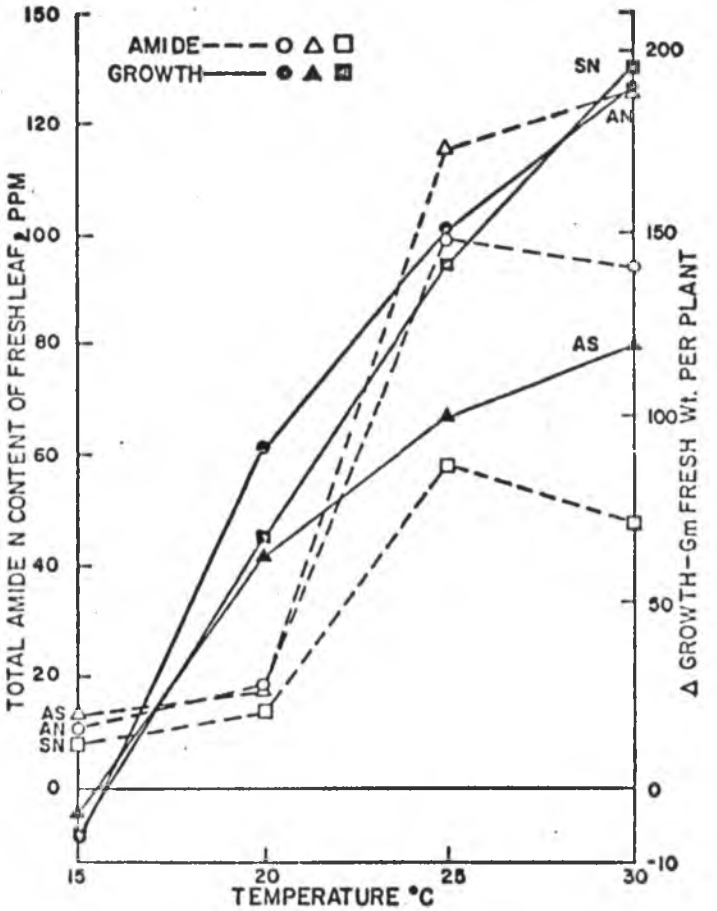

Fig. 23. Effects of root temperatures and N-carriers on total amides-N content of leaves and Δgrowth of plants.

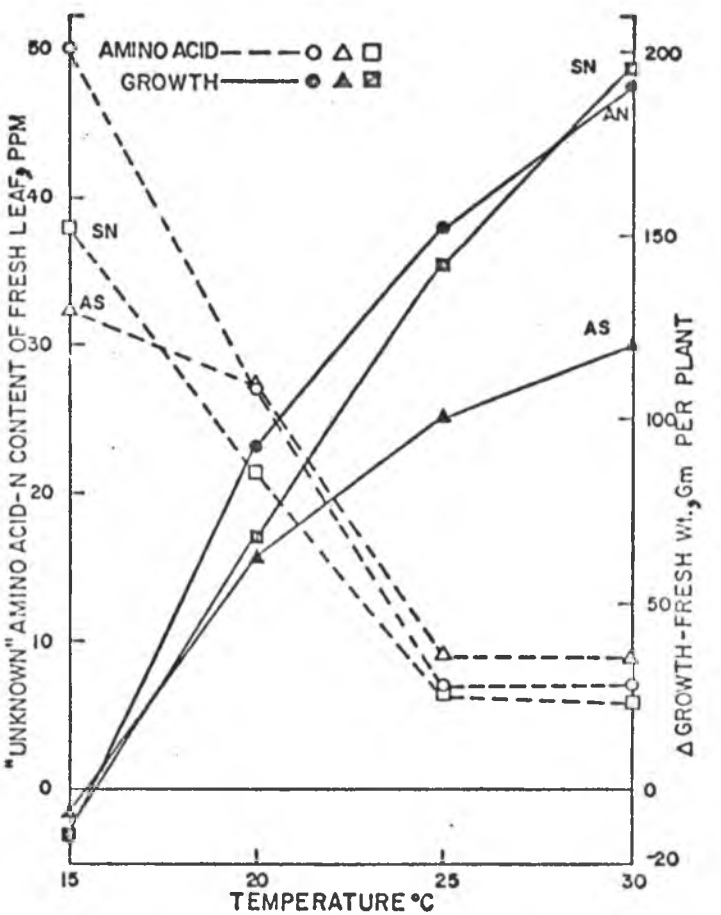

Fig. 24. Effects of root temperatures and N-carriers on "unknown" amino-N content of leaves and Δgrowth of plants.

These tests showed that the unknown was not a color pigment.

The retention time of the unknown did not coincide with the retention time of proline or its derivatives (hydroxy prolines including cis and trans isomers of 3 and 4 hydroxy proline). While proline and its derivatives were sensitive to change in buffer pH, the unknown was relatively insensitive.

The unknown was quite sensitive to concentrations of HCl during hydrolysis. Higher acid concentrations and/or prolonged hydrolysis (6 N, 20 hrs) completely destroyed the unknown. This particular observation is quite contrary to the stable nature of proline and its derivatives toward acid hydrolysis. Also, the fraction containing only the unknown, on further hydrolysis in 6 N HCl was completely destroyed giving rise to a large ammonia peak.

DISCUSSION

In roots, maximum concentrations of soluble, protein and total-N fractions always occurred at 20 C. The data seem to suggest that the 20 C temperature is probably high enough for N-metabolism in roots, but not high enough for translocation from roots to the stem and leaves, resulting in an accumulation in the roots. The different N-fractions in the stem were high at 15 C and tended to decrease with increases in temperature up to 25 C, probably due to an improved utilization of N fractions with growth, and, perhaps improved translocation from stem to leaves. The proportion of soluble-N to total-N was considerably greater in the stem than a similar relationship in the roots. This may also mean that the stem acts as a "conducting duct" and "reservoir" for N-fractions for later export to leaves as the case may be. In the leaves almost all the N-fractions increased slightly between 15 and 20, followed by a substantial increase up to 25 C. At higher temperature the concentration declined with AN and SN but slightly increased with AS. The proportion of soluble-N to protein-N in leaves was intermediate, compared to a low level in roots and a high level in stems.

Root temperatures and N-carriers showed certain interesting effects on the different N-fractions in the various plant parts. At 15 C, N absorption by and metabolism in the roots is poor. At 20 C the absorption as well as N metabolism is improved and the N metabolites are transported fairly freely from root to leaves through the stem. The amount and rate of transport improves with increases in root temperatures with the stem acting only as a temporary storage locale. The low levels of N fractions in leaves at 15 C and 20 C are probably due to a lack of N

uptake by the roots at 15 C, and a lack of N translocation at 20 C from the roots.

At 25 and 30 C root temperatures the effects of N-carriers is quite marked in that almost all N fractions in roots and leaves are high with AS, low with SN and intermediate with AN. This high accumulation of N in plants grown in the AS cultures is probably a result of the low rate of N metabolism and impaired protein synthesis in the presence of NH_4 ion and a possible K deficiency. Such an impaired N utilization and poor protein synthesis has also been reported for pineapple (Sideris, et al., 1946 and 1948; Connelly, 1969; and Englerth, 1969) as well as other crops (Evans and Sorger, 1966; Barber, et al., 1967; Ajayi, et al., 1970; Hsiao, et al., 1970). It is well to point out here that the low levels of N fractions at 25 and 30 C with the SN-carrier indicate a very efficient N utilization mechanism. However, a continued high rate of growth and low N absorption with SN may lead to a possible N-deficiency which induces poor growth. If indeed such a mechanism is operational with SN, the best N-carrier would be AN. Growth with this carrier was comparable to that with SN, and there was a higher N-uptake and equally good absorption of other nutrients.

The concentration of asparagine is very well related to the absorption of N from AS. Similar observations were made by Sideris, et al. (1947); this prompted these authors to suggest the possibility of using asparagine as the index of the N-status of the plant, and as a means of formulating fertilizer programs. The data from this present experiment suggests that an accumulation of asparagine in the leaves with AS may have been a manifestation of protective mechanism against NH_4 toxicity or improper utilization of N due to insufficiency of K or both. There is also

evidence in the literature to support this conclusion. Haghiri (1966) showed a negative correlation between asparagine and K contents of soybean leaves. In corn, it is shown that the general effect of K is to reduce the asparagine content of leaves at any given N level; that the accumulation of asparagine is related to detoxification and storage of NH_4 and a possible manifestation of nutritional disorder due to an excess of NH_4 absorption and/or of K deficiency; that the deficiency of K enhances the possibility of NH_4 toxicity; and that increased levels of K reduces asparagine content of the plants probably due to improved utilization of free amino acids (Barker and Bradfield, 1963). Poor plant growth in this experiment with a high asparagine content is, perhaps an index of "luxury consumption" or "inefficient utilization" of N with AS. In comparison, both AN and SN showed better growth, but a low asparagine content, with no apparent N-deficiency symptoms. Therefore, any attempt to fertilize pineapple plants, using asparagine content as an index but disregarding the source of N, might be very misleading, uneconomical or even lethal to plants.

A deficiency of K has been found to induce an accumulation of certain toxic amines such as agamatine and particularly putrescine (Richard and Coleman, 1952; Coleman and Richards, 1956; Hackett, et al., 1965 and Hoffman and Samish, 1971). These authors have indicated that putrescine levels in plants can be used as an effective index of K deficiency, well in advance of the appearance of visual deficiency symptoms.

Although such analyses were not carried out in this experiment, to do so in the future would help to understand whether the plants supplied with AS were really deficient in K.

Another useful test would be the estimation of the organic acids content of leaves from plants supplied with different N sources. It seems possible that such organic acids determination would have shown lower levels with AS than with AN and SN because the total uptake of K + Ca + Mg with AS was considerably lower than with the other carriers (Kirkby and Mangel, 1967).

When the hydrolysates of the ethanol soluble fraction of leaves were being analyzed for amino acids, an interesting pair of unknowns always appeared together. These two may have come from an ethanol soluble peptide. The unknown which appeared first was abosrbed at 570 m wave length while the other eas absorbed at 440 mμ. The second unknown showed an extremely interesting characteristic in that it decreased with increasing root temperatures. It occurred at highest concentration at 15 C; at 30 C the concentration was only about one-sixth to one-eight as much as at 15 C. It is not clear whether the presence of this second unknown was the cause or effect of low temperature induced inhibition of growth. It is also not possible to say whether this unknown was translocated from the roots as a result of low temperature or produced and accumulated within the leaf due to impaired metabolism.

Since proline and its derivatives are absorbed at 440 mμ wave length (Hamilton, 1963) and accumulation of free hydroxyprolines are inhibitory to plant growth (Cleland, 1967 and 1968; and Cleland and Karlsnes, 1967), attempts were made to determine if the second unknown is a proline derivative. Comparisons of retention times of standards of proline, 4-hydroxyproline and cis and trans isomers of 3 and 4-hydroxyprolines did not correspond to the retention time of the

unknown. Besides proline and its derivatives showed considerable changes in retention times with changes in the pH of the buffer, while the unknown was relatively unaffected. The unknown is also unlikely to be a putrescine or agamatine, the poisonous amines capable of retarding plant growth, since their absorption wave length is 570 mμ (Krikorian, 1973).[1]

Isolation and identification of this unknown and testing its effects on plant growth might prove useful in determining whether this material was indeed responsible for the complete cessation of growth at low root temperatures. Also, analyzing the stem exudate of plants grown in low root temperatures might show if the unknown was produced in the root and translocated to the top, or produced within the leaf as a result of desiccation and metabolic derangement.

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[1] Dr. A. D. Krikorian (1973). Personal Communication.

SUMMARY AND CONCLUSIONS

There was a rapid increase in concentrations of soluble, protein and total-N fractions in roots from 15 to 20 C. This increase was probably due to improved N absorption and assimilation in the roots with rise in temperatures, but a poor translocation from the roots. In the case of leaves, however, the concentrations of different N-fractions increased only very slightly from 15 to 20, followed by a very rapid increase up to 25 C. In the case of the stem, the different N-fractions generally tended to decrease with increases in root temperatures. These variations in the concentrations of N-fractions in various plant parts point up the differential effects of root temperatures on N absorption, assimilation and translocation from "source" to "sink".

The effects of N-carriers on different N-fractions of roots, stems and leaves become very marked at 25 and 30 C. Considering all plant parts, these fractions are highest with AS, followed by AN, with the lowest concentrations found with SN. These results again show that AS is a poor source of N, and that the greater concentrations of N-fractions found in plants in the AS culture are simply a result of the inefficient utilization of N.

Analyses of the ethanol fractions of leaves showed the presence of 15 detectable soluble amino acids. In the hydrolysates, two amides (asparagine and glutamine) besides two unknown amino acid type compounds were detected. The total amino acid pool appears to be stable, increasing only from 50 to 100%, depending on the N-carrier, and the root temperature between 15 and 30 C. However, the concentration of asparagine changed dramatically with increases in root temperature from 15 to 30 C; the

increase amounted to as much as 600 to 800%, depending on the N-carrier. Of all these N-fractions, asparagine showed both the greater concentration and change with temperature. Use of AS as the N source showed the largest amount of asparagine, being almost twice as much as when SN was the N source. This large accumulation of asparagine with the use of AS is probably an indication of an insufficient K content in leaves. There is also evidence in the literature to support this conclusion; it has been shown that a high NH_4 absorption, coupled with an inhibition of K, almost invariably results in the accumulation of higher amounts of asparagine. The amount of glutamine was also fairly large, but changes in quantities of glutamine were not as great as in the case of asparagine with different root temperatures and N-carriers. The concentration of glutamine was about one-sixth that of asparagine.

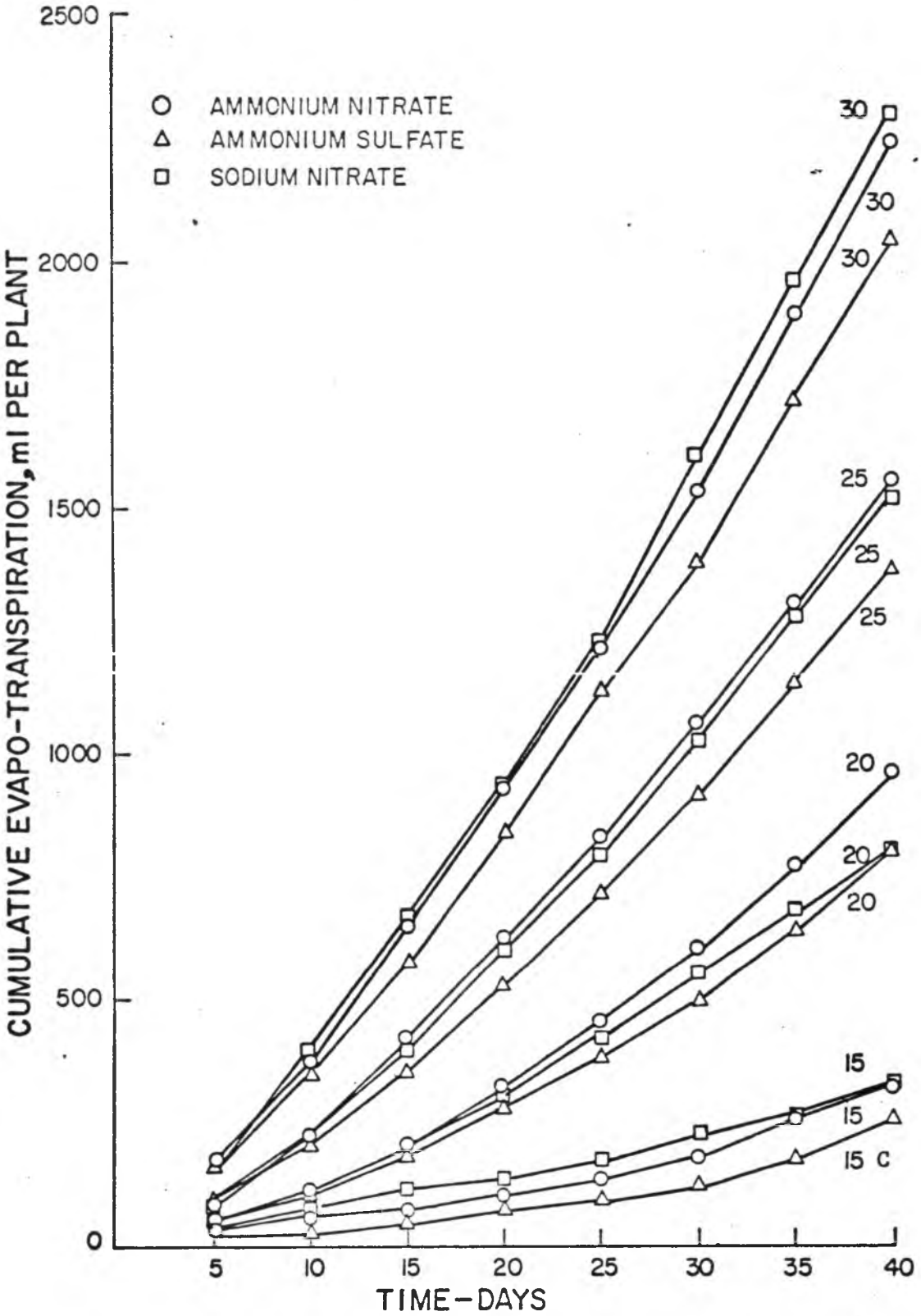
Of the two unknowns observed in the hydrolysates, the first one to appear was absorbed at 570 mμ wave length, and tended to increase in quantity with increases in root temperatures. The second one (hereafter simply referred as "unknown") produced a yellow compound with ninhydrin, and was absorbed at 440 mμ wave length, similar to proline and its derivatives. However, the retention times of this unknown did not correspond to the retention time of standards such as L-proline, 4-hydroxyproline, cis and trans isomers of 3 and 4-hydroxyproline. The retention time of the unknown was also relatively unaffected by changes in buffer pH.

The unknown has a very interesting relationship with root temperature and growth. While uptake of nutrients, growth and composition (including amino acids and amides) increased with increases in temperature, the unknown decreased with increases in temperature and, therefore, had a

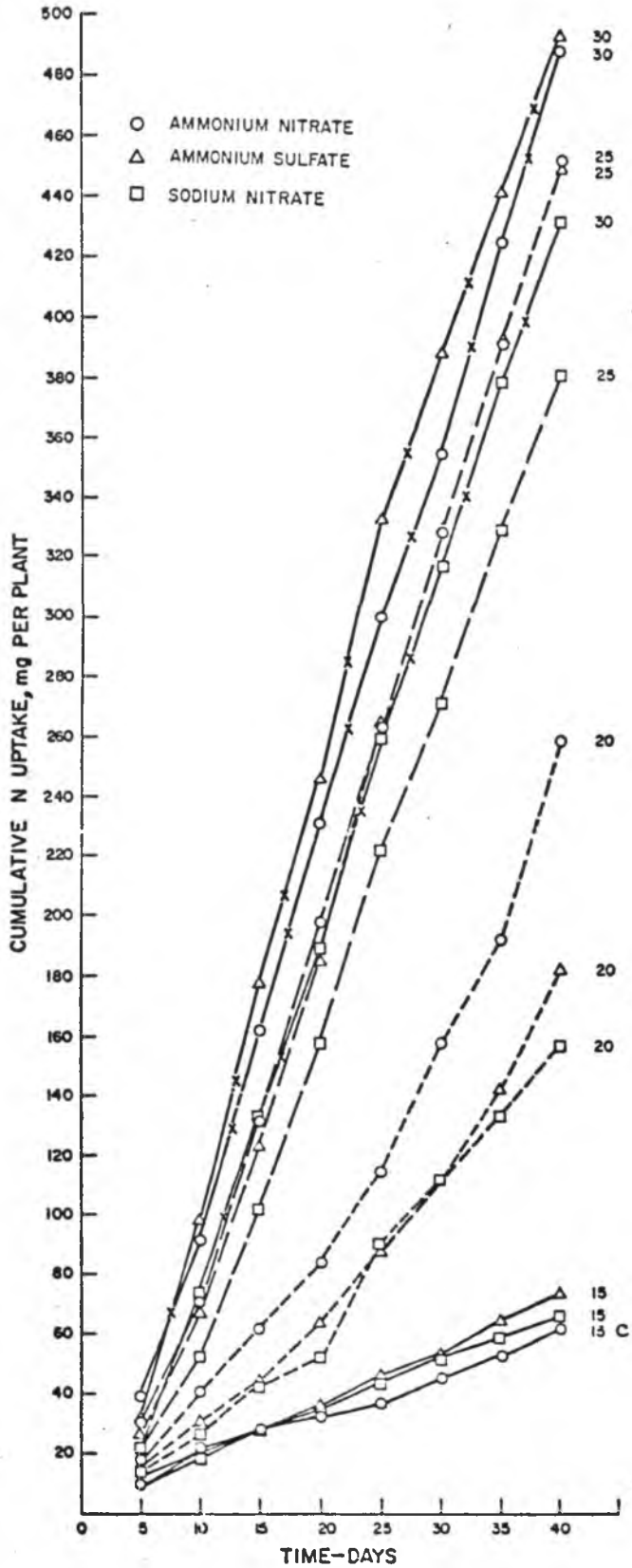
significant negative correlation with temperature and growth. It is not known whether this unknown is produced in the roots and transported to the leaves where it is accumulated, or whether it is produced within the leaves as a result of low root temperatures, or desiccation, or both.

The data on N-fractions and amide concentrations again suggest that AS is a poor source of N, either due to an inefficient N-utilization or inhibition of K absorption, or both. Also, the desirability of using a NO_3 or mixed source of N is indicated by the data collected in this experiment.

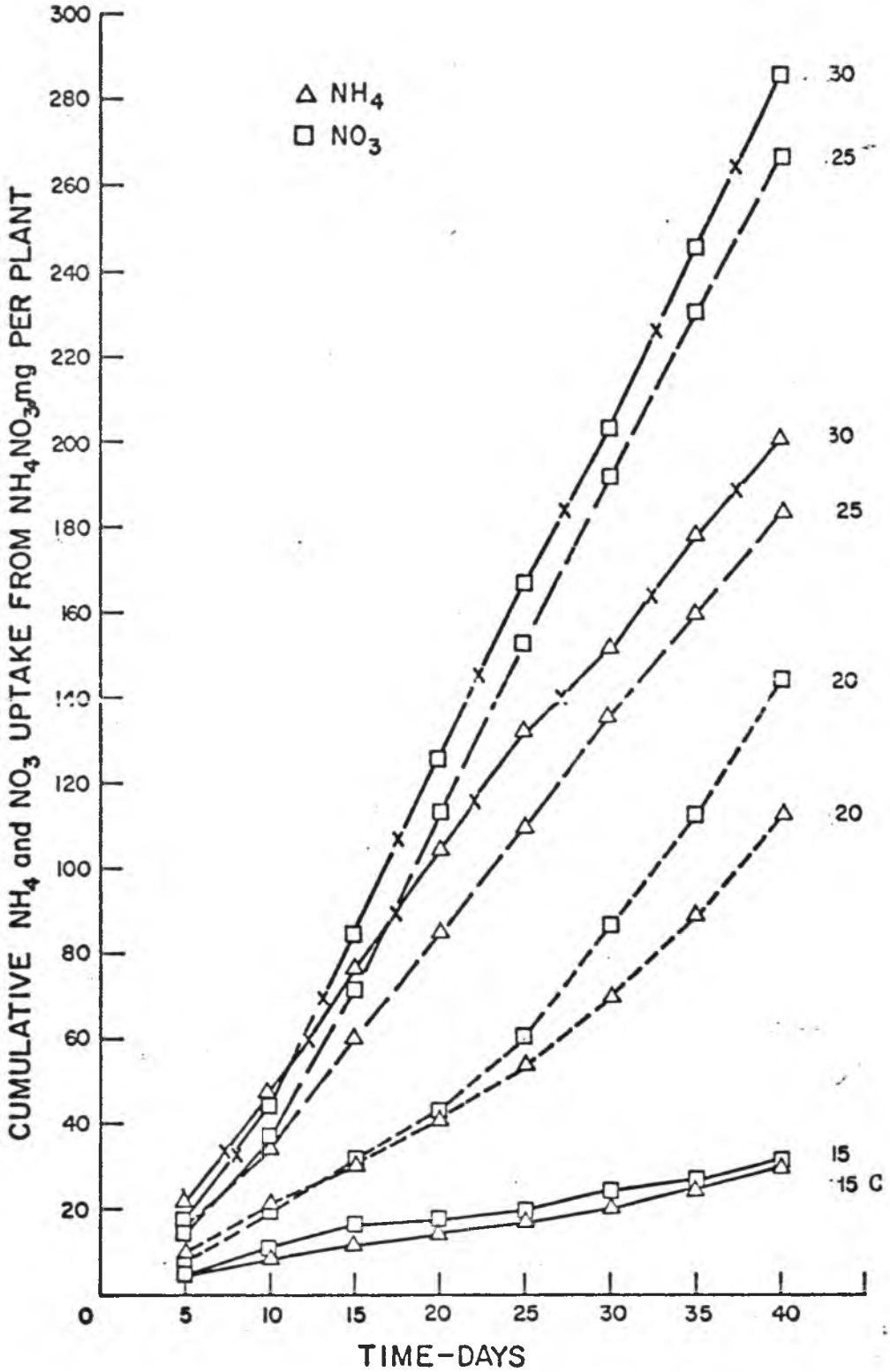
APPENDIX



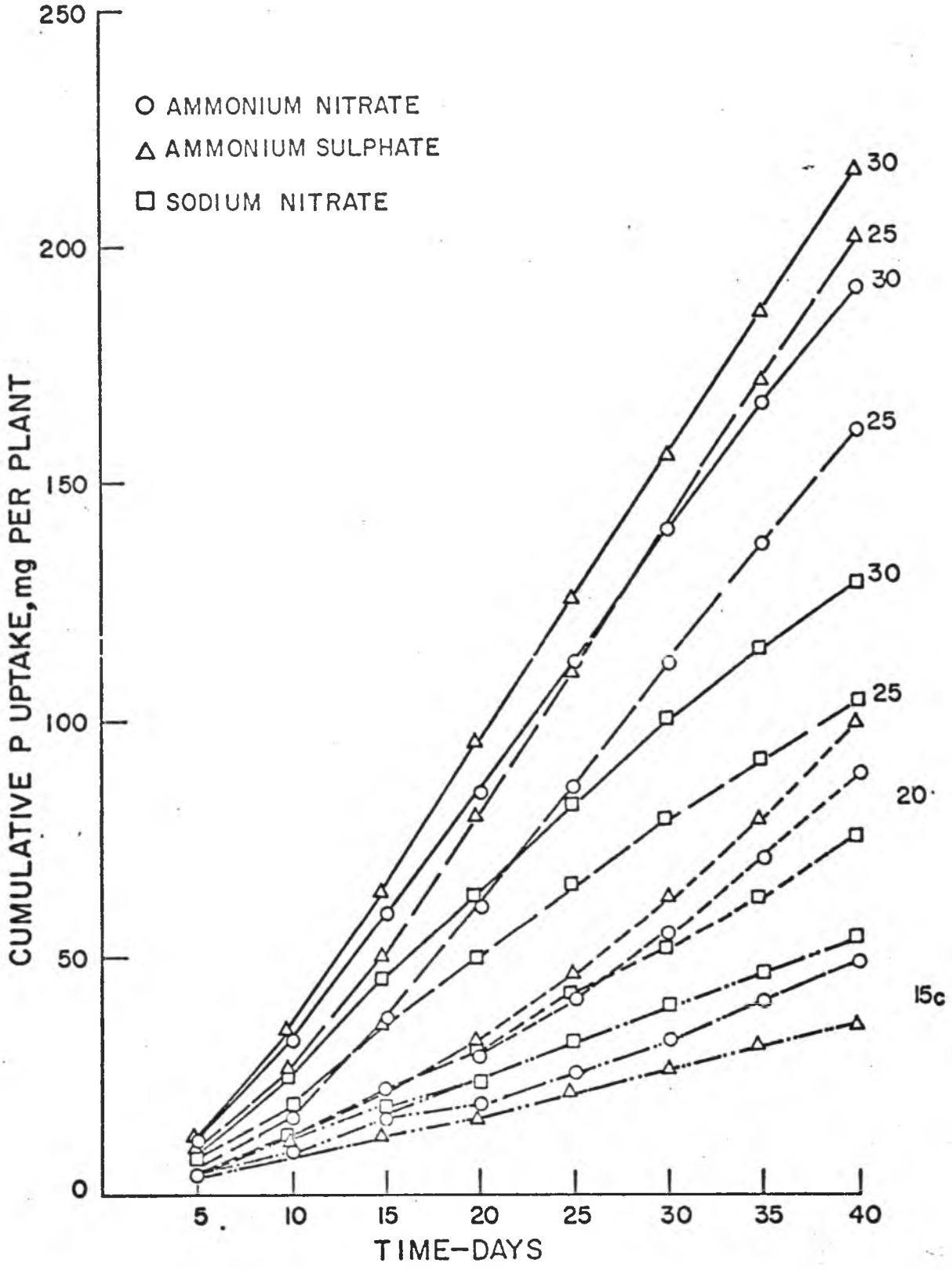
Appendix Fig. 1. Effects of root temperatures and N-carriers on cumulative evapo-transpiration.



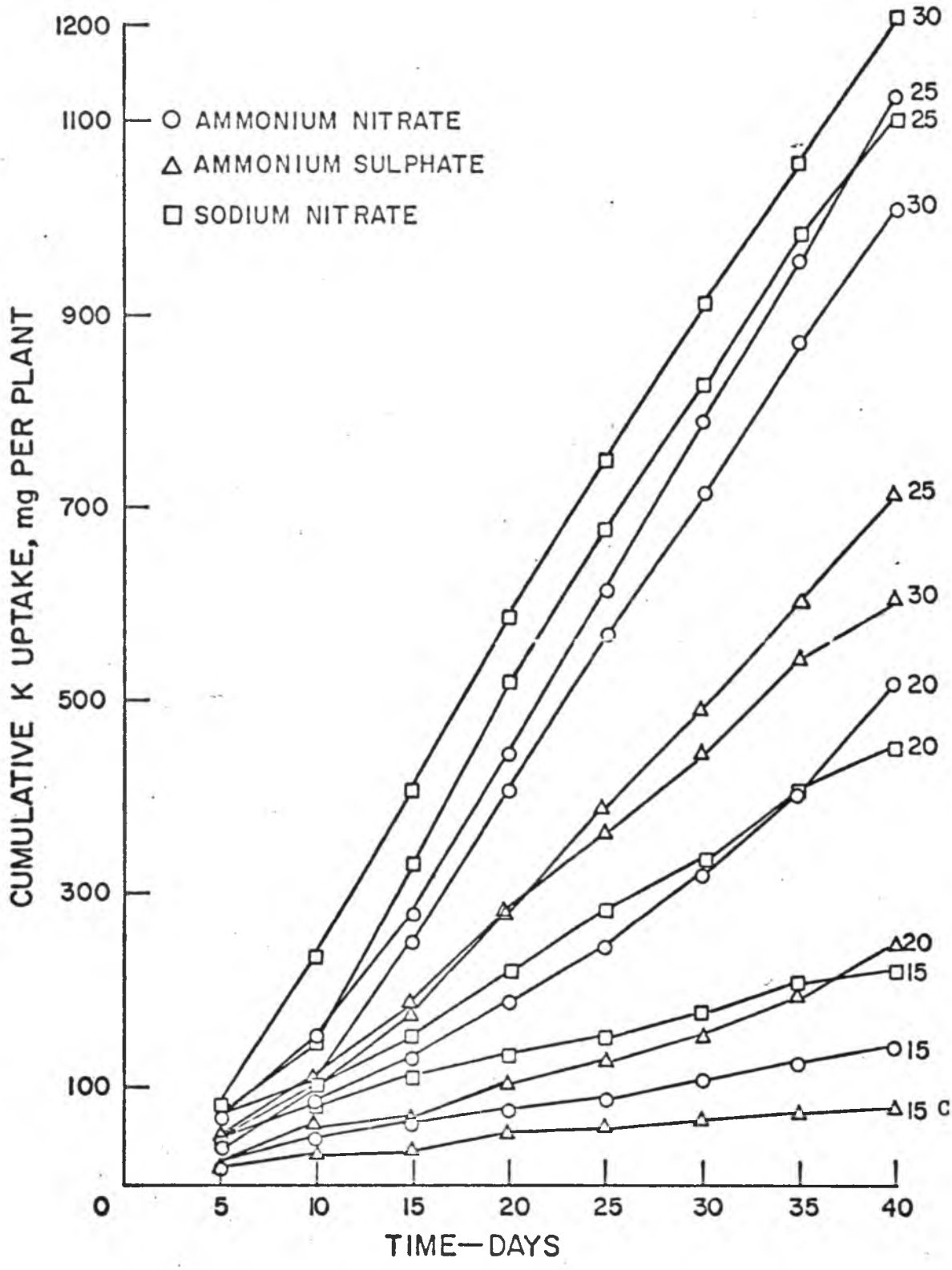
Appendix Fig. 2. Effects of root temperatures and N-carriers on cumulative nitrogen uptake.



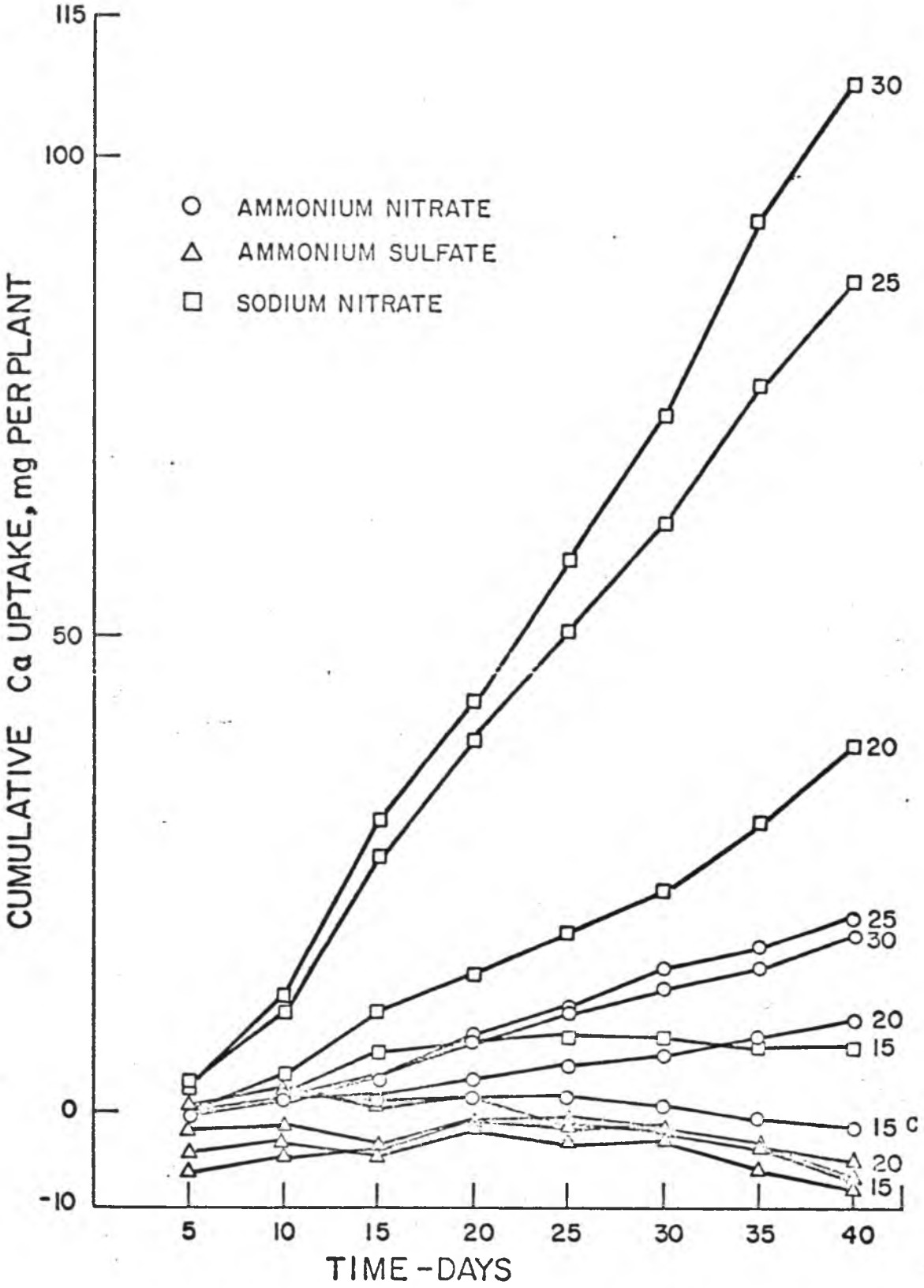
Appendix Fig. 2a. Effects of root temperatures and N-carriers on cumulative NH_4 and NO_3 uptake from ammonium nitrate.



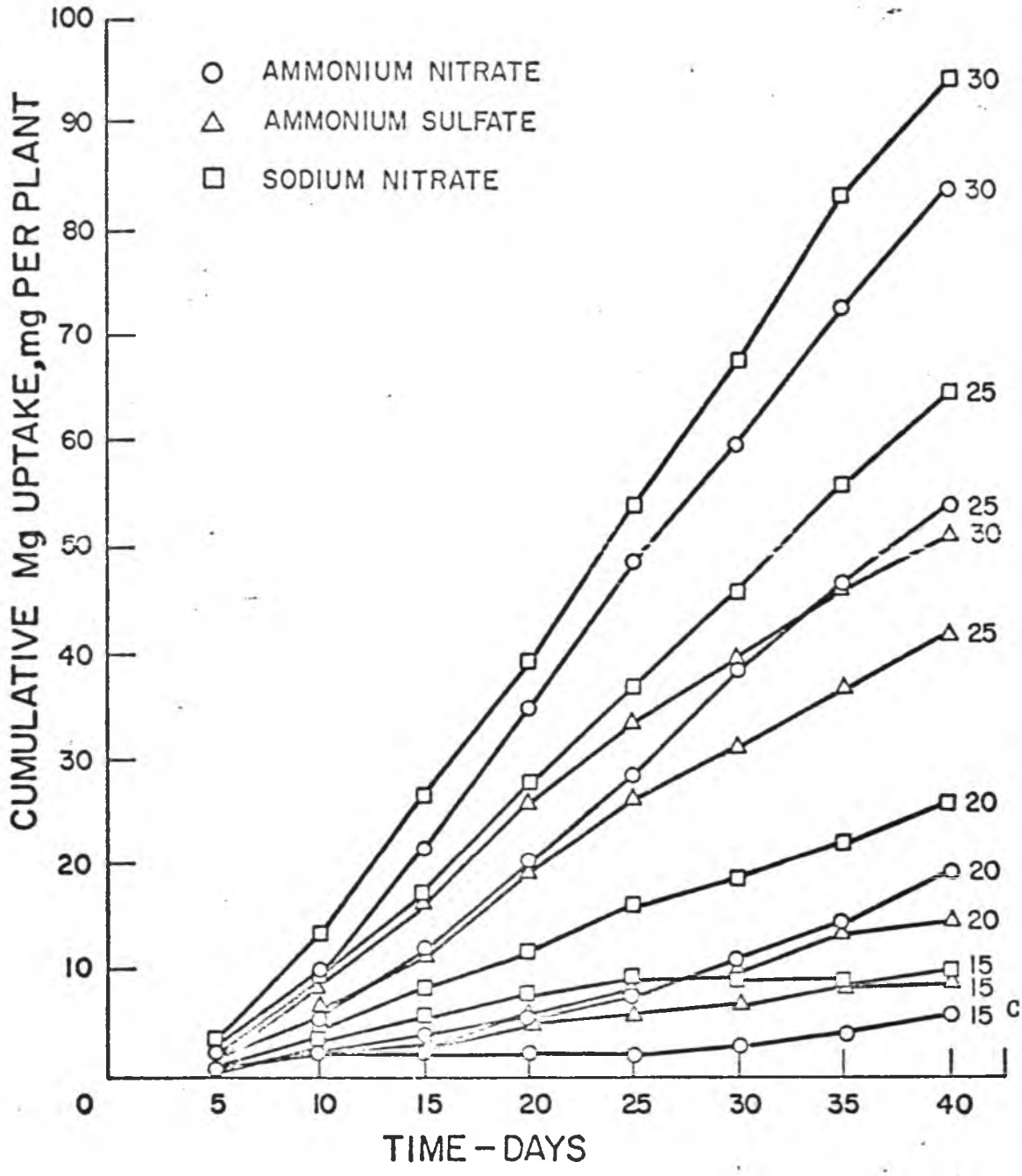
Appendix Fig. 3. Effects of root temperatures and N-carriers on cumulative uptake of phosphorus.



Appendix Fig. 4. Effects of root temperatures and N-carriers on cumulative uptake of potassium.



Appendix Fig. 5. Effects of root temperatures and N-carriers on cumulative uptake of calcium.



Appendix Fig. 6. Effects of root temperatures and N-carriers on cumulative magnesium uptake.

Appendix Table 1

Effects of root temperatures and N-carriers on evapo-transpiration (ml) and nutrient uptake (mg) per plant

Temperature and Carrier Means (average of 4 reps) tested by Duncan's Modified LSD test at P = 0.05

	N-CARRIER				N-CARRIER				N-CARRIER		
ROOT TEMP. C	AN	AS	SN	MEAN	AN	AS	SN	MEAN	NH_4	NO_3	MEAN
	Evapo-transpiration				N-uptake				NH_4 and NO_3 uptake from AN		
15	323	255	327	302^{A}	61.8	73.4	65.8	67.0^{A}	29.9	31.8	30.9^{A}
20	961	805	804	857^{B}	258.4	182.3	156.9	199.2^{B}	113.4	145.0	129.2^{B}
25	1556	1372	1525	1484^{C}	451.4	448.8	380.3	426.8^{C}	184.0	267.6	225.8^{C}
30	2238	2047	2298	2194^{D}	488.0	497.7	431.1	470.6^{C}	201.4	286.6	244.0^{C}
LSD FOR CXT INTERACTION = 156.5							51.6			12.9	
MEAN	1269^{B}	1120^{A}	1239^{B}		314.9^{B}	299.3^{B}	258.5^{A}		132.2^{A}	182.7^{B}	
	P Uptake				K uptake						
15	49.3	36.0	54.0	46.4^{A}	143	78	220	147^{A}			
20	89.1	100.1	76.2	88.5^{B}	518	249	451	406^{B}			
25	161.4	202.4	104.7	156.2^{C}	1124	711	1103	979^{C}			
30	191.9	216.7	129.5	179.4^{D}	1082	626	1158	955^{C}			
LSD FOR CXT INTERACTION = 17.2							91.8				
MEAN	122.9^{B}	138.8^{C}	91.1^{A}		717^{B}	416^{A}	733^{B}				
	Ca uptake				Mg uptake						
15	- 1.7	- 7.4	6.9	$- 0.7^{A}$	5.7	9.2	9.6	8.1^{A}			
20	9.1	- 7.1	38.7	13.6^{B}	19.2	14.8	26.0	20.0^{B}			
25	20.8	- 4.3	87.4	34.7^{C}	54.8	42.0	66.0	54.3^{C}			
30	18.6	- 8.0	108.0	39.6^{C}	84.2	51.4	94.2	76.6^{D}			
LSD FOR CXT INTERACTION = 11.4							10.0				
MEAN	11.7^{B}	$- 6.7^{A}$	60.3^{C}		41.0^{B}	29.4^{A}	48.5^{C}				

Appendix Table 2

Mechanism of nutrient uptake in relation to evapo-transpiration expressed as ratio of $\frac{\text{Actual nutrient uptake, mg}}{\text{Theoretical uptake based on evapo-transpiration, mg}}$ $\frac{(B)}{(A)}$

N CARRIER

NUTRIENT	ROOT TEMP. C	AN			AS			SN			MEAN
		THEORETICAL UPTAKE A	ACTUAL UPTAKE B	$\frac{B}{A}$	THEORETICAL UPTAKE A	ACTUAL UPTAKE B	$\frac{B}{A}$	THEORETICAL UPTAKE A	ACTUAL UPTAKE B	$\frac{B}{A}$	
N	15	33.9	61.8	1.82	26.8	73.4	2.74	34.4	65.8	1.92	2.16
	20	101.1	258.4	2.56	84.6	182.3	2.16	84.5	156.9	1.86	2.19
	25	163.3	451.4	2.76	144.1	448.8	3.12	160.1	380.3	2.38	2.75
	30	235.0	488.0	2.08	215.0	492.7	2.29	241.3	431.1	1.79	2.05
MEAN.				2.31			2.58			1.99	2.29
P	15	5.9	49.3	8.38	4.7	36.0	7.75	6.0	54.0	9.04	8.39
	20	17.5	89.1	5.08	14.7	100.1	6.81	14.7	76.2	5.20	5.70
	25	28.4	161.4	5.69	25.0	202.4	8.09	27.8	104.7	3.77	5.85
	30	40.8	191.9	4.70	37.3	216.7	5.80	41.9	129.5	3.09	4.53
MEAN				5.96			7.11			5.28	6.12

Appendix Table 2 (Continued)

Mechanism of nutrient uptake in relation to evapo-transipiration

expressed as ratio of $\frac{\text{Actual nutrient uptake, mg}}{\text{Theoretical uptake based on evapo-transpiration, mg}} \frac{(B)}{(A)}$

N-CARRIER

NUTRIENT	ROOT TEMP. C	AN			AS			SN			MEAN
		THEORETICAL UPTAKE A	ACTUAL UPTAKE B	$\frac{B}{A}$	THEORETICAL UPTAKE A	ACTUAL UPTAKE B	$\frac{B}{A}$	THEORETICAL UPTAKE A	ACTUAL UPTAKE B	$\frac{B}{A}$	
K	15	29.1	142.9	4.91	23.0	78.3	3.41	29.5	220.0	7.46	5.25
	20	86.7	517.8	5.97	72.6	248.9	3.43	72.5	451.1	6.22	5.21
	25	140.2	1124.4	8.01	123.7	711.1	5.75	137.5	137.5	8.02	7.26
	30	201.8	1082.3	5.36	184.6	626.3	3.39	207.2	207.2	5.57	4.77
MEAN				6.06			4.00			6.82	5.63
Ca	15	7.9	- 1.7	-	6.2	- 7.4	-	8.0	6.9	0.86	-
	20	23.6	9.1	0.39	19.7	- 7.1	-	19.7	38.7	1.97	-
	25	38.1	20.8	0.54	33.6	- 4.3	-	37.4	87.4	2.34	-
	30	54.8	18.6	0.34	50.2	- 8.0	-	56.3	108.0	1.92	-
MEAN				0.37						1.77	-
Mg	15	6.3	5.7	0.91	4.9	9.2	1.85	6.4	9.6	1.50	1.42
	20	18.7	19.2	1.03	15.6	14.8	0.95	15.6	26.0	1.67	1.22
	25	30.2	54.8	1.82	26.6	42.0	1.58	29.6	66.0	2.23	1.88
	30	43.4	84.2	1.94	39.7	51.4	1.29	44.6	94.2	2.11	1.78
MEAN				1.43			1.42			1.88	1.58

Appendix Table 3

Influence of major nutrients on the uptake of each other expressed as K/N, K/P and N/P ratio (m eq)

N-CARRIER

ROOT TEMP. C	AN	AS	SN	MEAN
		K/N		
15	0.83	0.38	1.20	0.80
20	0.72	0.49	1.03	0.75
25	0.89	0.57	1.04	0.83
30	0.80	0.45	0.96	0.74
MEAN	0.81	0.47	1.06	
		K/P		
15	0.77	0.57	1.08	0.81
20	1.54	0.66	1.57	1.26
25	1.85	0.93	2.79	1.86
30	1.49	0.77	2.37	1.54
MEAN	1.41	0.73	1.95	
		N/P		
15	0.93	1.51	0.90	1.11
20	2.14	1.34	1.52	1.67
25	2.07	1.64	2.68	2.13
30	1.88	1.70	2.46	2.01
MEAN	1.75	1.55	1.89	

Appendix Table 4

Effects of root temperatures and N-carriers on plant growth characteristics on fresh weight basis

Temperature and carrier means (average of 4 reps) tested by Duncan's Modified LSD test at P = 0.05

ROOT TEMP. C	N-CARRIER AN	AS	SN	MEAN	N-CARRIER AN	AS	SN	MEAN
	Final Plant Wt., gm				ΔGrowth per plant, gm			
15	262.5	273.0	265.5	267.0^{A}	-12.2	- 6.1	-11.2	-10.1^{A}
20	375.3	343.3	345.5	354.7^{B}	92.3	62.8	68.0	74.3^{B}
25	430.8	381.3	417.0	409.7^{C}	151.8	100.3	141.8	131.2^{C}
30	465.5	399.8	470.8	446.0^{D}	190.5	119.5	195.8	168.6^{D}
LSD FOR CXT INTERACTION = 27.2							28.9	
MEAN	383.5^{B}	349.3^{A}	374.7^{B}		105.6^{B}	68.9^{A}	98.6^{B}	
	% Rate of Growth per 5 day period				% Total Increase			
15	- 0.65	- 0.37	- 0.55	$- 0.53^{A}$	- 4.4	- 2.1	- 4.0	$- 3.6^{A}$
20	3.60	2.50	2.35	2.82^{B}	32.6	22.3	24.5	26.5^{B}
25	5.62	3.92	5.30	4.95^{C}	54.5	35.6	51.5	47.1^{C}
30	6.78	4.57	6.92	6.09^{D}	68.7	42,6	71.2	60.9^{D}
LSD FOR CXT INTERACTION = 1.04							10.4	
MEAN	3.83^{B}	2.65^{A}	3.50^{B}		37.9^{B}	24.5^{A}	35.7^{B}	

Appendix Table 4 (Continued)

Effects of root temperatures and N-carriers on plant growth characteristics on fresh weight basis

Temperature and carrier means (average of 4 reps) tested by Duncan's Modified LSD test at P = 0.05

ROOT TEMP. C	AN	AS	SN	MEAN	AN	AS	SN	MEAN	AN	AS	SN	MEAN
	Root wt., gm				Stem wt., gm				Leaf wt., gm			
15	12.7	16.1	15.5	14.8^{A}	45.9	45.7	46.3	45.9^{A}	203.5	211.3	203.8	206.2^{A}
20	32.7	27.1	25.7	28.5^{B}	55.1	51.5	54.2	53.6^{B}	287.3	264.8	267.0	272.8^{B}
25	37.1	28.3	36.7	34.3^{C}	54.0	53.8	61.2	56.3^{B}	339.8	299.0	319.0	319.3^{C}
30	39.4	20.4	47.9	35.9^{C}	50.5	57.1	52.2	53.2^{B}	382.5	322.3	371.0	358.7^{D}
LSD FOR CXT INTERACTION = 8.2						6.3				26.2		
MEAN	30.5^{B}	23.0^{A}	31.4^{B}		51.4^{A}	52.1^{A}	53.5^{A}		303.3^{C}	274.3^{A}	290.1^{B}	
	% Root weight per plant				% Stem weight per plant				% Leaf weight per plant			
15	4.8	5.9	5.8	5.5^{A}	17.5	16.9	17.6	17.3^{A}	77.5	77.2	76.7	77.1^{A}
20	8.7	7.8	7.4	8.0^{B}	14.7	15.1	15.7	15.1^{B}	76.6	77.1	77.2	77.0^{A}
25	8.6	7.4	8.6	8.2^{B}	12.6	14.1	14.8	13.8^{B}	78.9	78.4	76.5	77.9^{A}
30	8.4	5.1	10.1	7.9^{C}	10.8	14.3	11.2	12.1^{B}	81.8	80.6	78.8	80.4^{B}
LSD FOR CXT INTERACTION = 1.90						1.95				5.4		
MEAN	7.6^{AB}	6.6^{A}	8.0^{B}		13.9^{A}	15.1^{B}	14.8^{B}		78.7^{A}	78.3^{A}	77.3^{A}	

Appendix Table 5

Effects of root temperatures and N-carriers on the composition of nitrogenous fractions, (ppm fresh weight basis) of different plant parts

Temperature and carrier means (average of 4 reps)
tested by Duncan's Modified LSD test at P = 0.05

		N-FRACTIONS											
		SOLUBLE-N				PROTEIN-N				TOTAL-N			
ROOT TEMP. C	PLANT PART	AN	AS	SN	MEAN	AN	AS	SN	MEAN	AN	AS	SN	MEAN
15		109	114	117	113^{A}	802	873	931	868^{A}	908	986	1048	980^{A}
20	Root	222	163	157	181^{C}	1292	1170	1164	1209^{B}	1514	1333	1320	1389^{B}
25		141	130	117	129^{A}	929	1080	749	919^{A}	1073	1213	866	1050^{A}
30		147	197	123	155^{B}	865	1088	844	933^{A}	1012	1284	967	1088^{A}
LSD FOR CXT INTERACTION =			40.5				128.5				138.0		
MEAN		155^{B}	151^{B}	128^{A}		972^{A}	1052^{B}	922^{A}		1127^{B}	1204^{C}	1050^{A}	
15		1153	998	1045	1065^{A}	2393	2959	2852	2734^{C}	3546	3957	3897	3717^{C}
20	Stem	955	888	904	915^{A}	1897	2153	2252	2101^{B}	2852	3041	3156	3016^{B}
25		1154	823	865	948^{A}	1648	2056	1584	1763^{A}	2801	2880	2449	2710^{A}
30		955	901	668	841^{A}	1614	2048	1569	1744^{A}	2569	2949	2238	2585^{A}
LSD FOR CXT INTERACTION =			165.1				365.3				428.4		
MEAN		1054^{B}	903^{A}	871^{A}		1888^{A}	2304^{C}	2064^{B}		2942^{AB}	3144^{B}	2934^{A}	
15		378	354	413	382^{A}	1832	1567	1595	1665^{A}	2210	1922	2008	2047^{A}
20	Leaf	416	353	420	396^{A}	1865	1841	1760	1822^{AB}	2281	2194	2180	2218^{A}
25		554	618	454	542^{B}	2165	2285	1921	2124^{C}	2719	2902	2375	2666^{B}
30		593	665	388	549^{B}	1929	2314	1758	2000^{BC}	2522	2980	2146	2549^{B}
LSD FOR CXT INTERACTION =			91.0				231.9				264.9		
MEAN		485^{B}	498^{B}	419^{A}		1948^{B}	2002^{B}	1759^{A}		2433^{B}	2499^{B}	2177^{A}	

Appendix Table 6

Effects of root temperatures and N-carriers on the total amount of nitrogenous fractions, (mg fresh weight basis) per plant of different plant parts

Temperature and carrier means (average of 4 reps) tested by Duncan's Modified LSD test at P = 0.05

		SOLUBLE-N				PROTEIN-N				TOTAL-N			
ROOT TEMP. C	PLANT PART	AN	AS	SN	MEAN	AN	AS	SN	MEAN	AN	AS	SN	MEAN
15		1.4	1.8	1.9	1.7^{A}	10.3	14.1	14.9	13.1^{A}	11.7	16.0	16.7	14.8^{A}
20	Root	7.3	4.4	4.1	5.3^{B}	42.1	31.5	29.6	34.4^{B}	49.3	35.9	33.7	39.7^{B}
25		5.2	3.7	4.3	4.4^{B}	34.0	30.5	27.0	30.5^{B}	39.2	34.2	31.2	34.9^{B}
30		5.9	4.1	5.8	5.3^{B}	34.3	22.2	39.7	32.1^{B}	40.2	26.3	45.5	37.4^{B}
MEAN		4.9^{B}	3.5^{A}	4.0^{AB}		30.2^{B}	24.6^{A}	27.8^{AB}		35.1^{B}	28.1^{A}	31.8^{AB}	
15		52.0	45.4	48.6	48.7^{A}	109.9	134.4	132.1	125.5^{C}	161.9	179.7	180.7	174.2^{B}
20	Stem	52.2	45.8	49.0	49.0^{A}	105.0	111.0	120.8	112.3^{BC}	157.2	156.8	169.9	161.3^{B}
25		66.7	44.3	52.7	54.6^{A}	88.6	110.7	96.9	98.7^{AB}	155.3	155.0	149.6	153.3^{B}
30		48.2	51.6	34.7	44.8^{A}	81.0	116.9	82.1	93.3^{A}	129.2	168.4	116.8	138.1^{A}
MEAN		53.8^{B}	46.8^{A}	46.3^{A}		96.1^{A}	118.2^{B}	108.0^{AB}		149.9^{A}	165.0^{A}	154.3^{A}	
15		77.1	75.5	85.0	79.2^{A}	374.2	329.0	324.9	342.7^{A}	451.3	404.5	410.0	421.9^{A}
20	Leaf	119.7	94.2	111.3	108.4^{A}	536.5	489.4	469.5	498.4^{B}	656.2	583.6	580.8	606.8^{B}
25		188.9	183.8	145.3	172.7^{B}	734.2	683.1	607.2	674.9^{C}	923.1	866.9	752.6	847.6^{C}
30		226.2	215.3	145.8	195.7^{B}	738.6	744.7	651.3	711.5^{C}	964.7	960.0	797.1	907.2^{C}
MEAN		153.0^{B}	142.2^{AB}	121.9^{A}		595.9^{B}	561.6^{AB}	513.2^{A}		748.9^{B}	703.8^{B}	635.1^{A}	

Appendix Table 7

Effects of root temperatures and N-carriers on the composition of amino-N fractions of leaves (ppm fresh weight basis)

(Means of 2 composite samples)

N-CARRIER	ROOT TEMP. C	ASPARTIC	THREONINE	SERINE	PROLINE	AIANINE	Y-AMINO BUTYRIC	AMMONIUM	ARGENINE	OTHERS	TOTAL
AN	15	0.76	1.52	3.38	1.02	4.08	2.03	16.64	0.94	1.69	32.06
	20	1.00	2.27	3.45	1.35	5.25	1.74	15.97	1.94	5.36	38.33
	25	1.68	3.73	4.09	1.83	6.47	2.76	17.88	0.65	5.70	44.79
	30	1.76	2.96	4.75	1.27	6.03	2.28	16.70	1.35	5.41	42.51
	MEAN	1.30	2.62	3.92	1.62	5.46	2.20	16.80	1.22	4.29	39.92
AS	15	0.41	1.75	3.53	0.41	3.10	1.36	10.10	1.05	2.92	24.63
	20	0.48	1.81	3.20	0.70	3.45	1.28	9.44	1.02	4.14	25.52
	25	2.83	3.59	4.61	1.82	4.68	2.00	16.97	1.34	6.72	44.56
	30	2.15	4.55	4.64	2.37	5.51	1.90	17.99	2.72	7.04	48.87
	MEAN	1.47	2.93	4.00	1.33	4.19	1.64	13.63	1.53	5.21	35.89
SN	15	0.87	1.43	2.72	0.48	2.99	1.13	10.08	0.79	2.78	23.27
	20	0.76	2.01	3.32	0.59	3.88	1.68	10.12	1.45	4.70	28.51
	25	1.77	2.90	3.52	1.14	6.71	2.64	14.80	1.44	6.30	41.22
	30	1.62	3.11	4.45	1.29	5.97	2.39	12.99	0.97	4.41	37.20
	MEAN	1.26	2.36	3.50	0.88	4.89	1.96	12.00	1.16	4.55	32.55

Appendix Table 8

Effects of root temperatures and N-carriers on the composition of amino-N fractions of leaves (ppm fresh weight basis)

Temperature and carrier means (average of 2 composite samples) tested by Duncan's Modified LSD test at P =0.05

ROOT TEMP. C	AN	AS	SN	MEAN	AN	AS	SN	MEAN	AN	AS	SN	MEAN
			Asparagine-N				Glutamine-N				Total Amide-N	
15	6.85	7.61	4.13	6.19	3.66	5.47	3.39	4.17	10.51	13.09	7.53	10.37
20	13.74	13.39	8.39	11.83	4.59	3.88	5.00	4.48	18.33	17.27	13.38	16.32
25	89.66	105.65	48.19	81.16	9.30	9.71	9.55	9.40	98.96	115.36	57.75	90.69
30	83.67	114.61	37.75	78.67	9.98	11.41	9.59	12.05	93.66	126.02	47.34	89.01
LSD FOR CXT INTERACTION =		29.79				6.65					35.10	
MEAN	48.48[B]	60.31[B]	24.61[A]		6.88[A]	7.61[A]	6.79[A]		55.36	67.93	31.50	
			Total Soluble Amino acid-N				"Unknown" amino-N(1)				"Unknown" amino-N(2)	
15	32.06	24.63	23.27	27.32	1.64	0.96	4.24	2.28	50.3	32.2	37.9	40.2
20	38.33	25.52	28.51	30.78	2.74	4.64	1.83	3.07	27.0	27.2	21.4	25.2
25	44.79	44.56	41.22	43.52	1.53	1.78	1.10	1.47	6.9	9.0	6.4	7.4
30	42.51	48.87	37.20	42.86	1.06	1.40	0.75	1.07	7.0	8.7	5.8	7.2
LSD FOR CXT INTERACTION =		26.10				--					23.50	
MEAN	39.92	35.89	32.55		1.74	2.20	1.98		27.8	19.3	17.9	

"Unknown" 1 -- Has absorption wave length of 570 mμ. Calculations of concentrations based on Norleucine as standard.
"Unknown" 2 -- Has absorption wave length of 440 mμ. Calculations of concentrations based on L-proline as standard.

Appendix Table 9

Table of simple correlation (r) coefficients

	Ev. Tr	Growth	Root Wt.	Stem Wt.	Leaf Wt.	N	P	K	Ca	Mg	Temp.	NH_4	NO_3
Ev. Tr	--	.917	.707	.391	.926	.932	.814	.870	.470	.930	.971	-.064	.064
Growth		--	.850	.521	.968	.871	.696	.903	.560	.908	.909	-.166	.166
Root Wt.			--	.421	.819	.665	.434	.820	.633	.784	.674	-.304	.304
Stem Wt.				--	.421	.467	.401	.456	.318	.316	.478	-.103	.103
Leaf Wt.					--	.894	.732	.885	.489	.893	.906	-.104	.104
N						--	.910	.866	.317	.845	.929	+.097	-.097
P							--	.646	.003	.655	.852	+.319	-.319
K								--	.654	.923	.835	-.322	.322
Ca									--	.670	.423	-.729	.729
Mg										--	.891		.266
Temp.											--		.000
NH_4-N													
NO_3-N													

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