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FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

of

BHARAT SINGH

B.Sc., Banaras Hindu University,
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M. Sc., Ranchi University, Ranchi, India, 1961

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COMMITTEE IN CHARGE

Chairman: Dr. I. McT. Cowan

A. Gronlund	G.H.N. Towers
D.P. Ormrod	D.J. Wort
G.E. Rouse	S.H. Zbarsky

External Examiner: Dr. Albert Ulrich
Department of Soils and Plant Nutrition
University of California
Berkeley, California

Research Supervisor: D.J. Wort

CHEMICAL CONTROL OF GROWTH IN SUGAR BEET (BETA SACCHARIFERA L.)

ABSTRACT

Metabolic inhibitors and growth regulators were used in an attempt to control the growth of sugar beet plants at the time of "ripening" of the roots. Maleic hydrazide (MH), pyrocatechol (PC), and vanadium sulfate (VS) were found to be most effective in controlling growth regardless of the age of the plants.

The solutions containing MH, PC, or VS were applied to the foliage of 4.5-month-old plants and the effects on leaf expansion and content of sucrose, reducing sugars, nitrite, nitrate, ammonia, amino acid, protein and total nitrogen were determined 7, 14, and 21 days after treatment. The rate of photosynthesis and respiration and the activity of nitrate reductase, transaminase, invertase, adenosine triphosphatase (ATP-ase), glucose-1-, glucose-6-, fructose-6-phosphatase, uridine diphosphate glucose pyrophosphorylase (UDPG-pyrophosphorylase), sucrose synthetase and sucrose phosphate synthetase was measured.

Compared with untreated plants, with few exceptions, all treatments affected the growth; the chemical composition; the rate of photosynthesis and respiration, and the activities of enzymes measured, in a similar manner. Growth of the plants was determined by measuring the leaf area. MH, PC, and VS significantly inhibited growth of leaves under both "summer" and "fall" conditions.

In the treated plants, the percentage reducing sugars, based on fresh weight of the root, decreased and percentage sucrose increased steadily.

Application of MH, PC, and VS resulted in a significant decrease in nitrite and an increase in nitrate content of roots. Ammonium nitrogen of the plants treated with MH was more than that of the

untreated plants on the 7th, 14th, and 21st day after treatment. Plants reated with PC and VS had a lower ammonium content on the 7th and the 14th day but more on the 21st day. The soluble amino acid content of the roots of MH-treated plants was higher than that of the controls. PC-treated plants had a lower amino acid content on the 7th day but a higher content on the 14th and 21st day. VS caused a reduction in amino acid content of the roots on all dates of harvest.

The rate of photosynthesis was measured by infrared technique. MH and VS caused a stimulation in the rate of net CO_2 assimilation, however, PC inhibited the rate of net CO_2 assimilation on the 7th day after treatment. The rate of respiration of the storage roots, measured by the Warburg technique, was lower than that of the control plants in the case of MH- and VS-treated plants and it was higher in the PC-treated plants.

The results indicated that the application of MH, PC, and VS caused significant reduction in the activity of nitrate reductase, transaminase, invertase, ATP-ase, glucose-1-, glucose-6-, and fructose-6-phosphatase. These treatments also resulted in the stimulation of the activity of UDPG-pyrophosphorylase, sucrose synthetase and sucrose phosphate synthetase.

The inhibition of growth by MH, PC, and VS is discussed on the bases of the reductions in the activities of invertase, nitrate reductase, and transaminase. The increase in sucrose content of the roots is explained on the bases of low invertase and high sucrose synthetase and sucrose phosphate synthetase activities in the treated plants. The possible participation of the phosphatases in the regulation of sucrose biosynthesis is indicated by the negative correlations between the activities of phosphatases and sucrose phosphate synthetase.

GRADUATE STUDIES

Field of Study: Botany (Plant Physiology)

Plant Physiology	D.J. Wort
Plant Biochemistry	G.H.N. Towers
Radioisotopes in Botany	E.B. Tregunna

Other Studies:

Plant Responses to Controlled Environment	D.P. Ormrod
Agricultural Climatology	V.C. Brink
Biochemistry	Biochemistry Faculty
Advanced Statistical Methods	A. Kozak

CHEMICAL CONTROL OF GROWTH IN SUGAR BEET

(*Beta saccharifera* L)

by

BHARAT SINGH

B.Sc., Banaras Hindu University, Varanasi, India, 1958
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We accept this thesis as conforming to the

required standard

THE UNIVERSITY OF BRITISH COLUMBIA

May, 1968

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Department of Botany

The University of British Columbia
Vancouver 8, Canada

Date May 31, 1968

ABSTRACT

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I. THE SUGAR BEET PLANT

1. Discovery

The sugar beet (Beta saccharifera) is unique among crop plants because it is a modern development with a well documented history. A chemist A.S. Marggraff (1747) of the Royal Academy of Science in Berlin proved that the "white mangold", the so-called sugar root, and red mongold or red beet (called by Marggraff Beta rubra) all contained a sugar identical with that from sugar cane.

About 1799 experimental and promotional work was done by Franz Karl Achard in Germany and by Delessert in France. Meanwhile, the development of the white Silesian beet root, from which all modern strains have come, was carried out by Moritz von Koppy.

2. Production

The sugar beet is the source of 40 percent of world's sugar. The principal sugar beet growing areas are the U.S.S.R. (European and Asiatic), United States, France, Poland, Germany, Czechoslovakia, Canada and the United Kingdom.

The main climatic requirements for sugar beet growth are rain or other frequent application of water during the growing season, mean temperatures near 70°F, and cool dry weather at the end of the season. A succession of warm days and cool nights give the best growth. The plant will grow as long as temperature and water conditions are favorable. A long growing season means higher tonnage.

Sugar beets grow in soils ranging from deep clay to silt loam or sometimes in fine sandy loam or muck soils which are alkaline to slightly acid, well drained, and free from hard pan. The crop is very tolerant to salinity and responds to available phosphorus and to abundant nitrogen.

3. Gross Morphology

The sugar beet, a member of the family Chenopodiaceae, is a herbaceous, biennial dicotyledon. The mature beet root is an elongate pear-shaped body composed morphologically of three regions - the crown, the neck, and the root. The crown bears a tuft of large succulent leaves and leaf bases. Adjoining the crown, is the neck, which constitutes ontogenetically, the thickened hypocotyl. The root region, which forms by far the bulk of the beet tissues, is cone shaped and terminates in a slender tap root.

The leaves are arranged on the crown in close spiral with the divergence of 5/13. The lamina of the leaf is elongate triangular with rounded tip and undulate margin, the base is cordate and decurrent on the petiole.

4. Anatomy

The anatomy of sugar beet has been described in very great detail by Artschwager (1926). The young seedling root has a central strand of vascular tissue enclosed by a cortex and bounded at the periphery by an epidermis.

Secondary growth of the root involves the activity of a primary cambium and of secondary cambium. The cambium arises apparently as a continuous band, but forms more or less separate

bundles, bands of conjunctive parenchyma developing between the vascular strips. The position of each new cambium, as it arises in the pericycle, is such that it encloses a few layers of pericyclic cells. These rapidly multiply and build up a parenchymatous layer as rapidly or even more rapidly than the cambium builds up the vascular layer. Alternate bands of proliferated pericycle and of vascular bundles are thus formed.

The bundles are themselves largely parenchymatous. Growth continues through all layers - in the bundles apparently both by cambial activity and by proliferation of the parenchyma of the xylem and phloem. Thus, the beet root increases in diameter by growth throughout its layers.

The epidermis of the lamina of the leaf is similar on both surfaces. The stomates are of a simple type. The pores are surrounded by a pair of specialized guard cells. There are no accessory cells. Stomates are found on both upper and lower surfaces, but are more numerous on the lower. The mesophyll of the leaf is normally divided into palisade tissue and spongy parenchyma.

Brovchenko (1965) found that the thin vascular bundles of sugar beet leaves contained 3-4 times more sugar than the adjacent assimilating tissues. Thus, movement from the mesophyll to the thin bundles occurred against concentration gradient, which was an indication of the active character of the process.

Brovchenko also observed that sucrose synthesis took place along the whole conducting system with the rate of synthesis of the disaccharide being particularly high in the thin bundles of the leaf blade. He has suggested that partial or complete hydrolysis of sucrose precedes its entrance into the conducting bundles of the sugar beet leaves.

II. NITROGEN METABOLISM OF THE SUGAR BEET AS RELATED TO LATE SEASON GROWTH WHICH RESULTS IN A LOW SUCROSE CONTENT OF THE ROOT

1. Introduction

Nitrogen is an essential element for the growth of all plants. Plants can utilize nitrogen sources such as ammonia, nitrite, and amino acids but in general nitrate will support growth equal to, or better than other sources (Ghosh and Burris, 1950).

There has been a great increase in the rate of application of nitrogen fertilizers on sugar beets during the last few decades. Associated with increased yields, which follow the use of nitrogen fertilizers, there has been a decline in quality. Processing losses have increased. The depressing effect of high nitrogen nutrition on sugar beet quality was reported by Headden as early as 1912. Later studies by Gardner and Robertson (1942) showed that excessive nitrogen reduced the sugar percentage and the purity. These authors estimated that the reduction in sugar percentage was an approximately linear function of nitrate nitrogen in the beets at harvest and that each 0.025 percent nitrogen in the beet reduced the sugar percentage by approximately one percent.

In his experiments with nutrient solutions, Ulrich (1942) found that whenever the nitrate content of the petioles was high, the sugar percentage of the beets was lower than in corresponding beets in which the nitrate content was low. When the nitrate

content of the beets was high, and the growing conditions favorable, rapid top growth took place, and continued until the nitrogen supply was depleted. Thereafter the growth of the tops, as measured by their fresh weight, decreased gradually while root weights increased rapidly at first, and then more slowly until the time of the last harvest. He maintained that at the harvest time, the sugar content of the beets would be a function of growth, leaf area, light intensity, temperature, etc.

Ogden et al. (1958) found that an excessive application of nitrogen fertilizer resulted in a decreased percentage of sucrose and an increased percentage of non-sugar carbohydrates in three different varieties of sugar beets. Several other investigators from different parts of the United States and Canada reported that the excessive nitrogen lowered the sucrose content of the beets (Ulrich, 1950; Loomis and Ulrich, 1959; Tolman and Johnson, 1958; Stout, 1961; Baldwin and Davies, 1966; Hale and Miller, 1966)

Baver (1964) gave an account of the work done in Germany. The studies at the Institute of Sugar Beet Investigations at Gottingen established two rather significant points. In the first place, there was an increasing concentration of unassimilated nitrogenous compounds in the beet juice as the amount of nitrogen applied as fertilizer was increased. This nitrogen, given the name "harmful nitrogen", consists primarily of amino acids and related compounds. In the second place, increased nitrogen fertilization raised the amount of ash in the juice. Both these factors had a deleterious effect upon the thin juice purity and the extraction of the sugar.

Adam (1960) and Tinker (1965) from Great Britain, Okanenko (1959) from Russia, Anita et al. (1963) from Romania and Noda Kenji et al. (1963) from Japan, noted that increase in supply of N gave more vigorous top growth. The shift from root growth to root "ripening" was retarded by high N.

Thus, the extensive studies in Europe, United States and Japan on the influence of nitrogenous fertilizers applied to sugar beets have resulted in the general conclusion that excessive quantities of nitrogen depress the sugar content of the beets, delay "ripening" at harvest time, cause excessive top growth, and increase the "harmful nitrogen" content of the beets. To explain the above mentioned facts the following sections underline the requirements for growth and the relationships of N-metabolism to carbohydrate metabolism.

2. Requirements of growth and differentiation

(a) Cell expansion as a process of growth:-

(i) changes in water content — The changes in the volume of the cell is due primarily to an increase in water content which may be twenty-fold.

(ii) changes in the dry weight and the cell wall mass — The increase in dry weight of the expanding cells is due primarily to the growth of the wall. The wall of the meristematic cell is probably composed principally of polygalacturonides. Cellulose becomes incorporated into the primary wall and the proportion of this increases with time. The synthesis of polygalacturonides and of cellulose involves utilization of sugars, either stored in the root, as in case of sugar beet, or formed in the process of photosynthesis.

(iii) changes in the protein content. Heyes (1960) recorded that in the pea root apex, the protein content increased about five fold during the expansion phase of growth. Protein synthesis requires amino acids and energy (ATP). The production of both is at the expense of stored sugar or newly formed sugar diverted for this purpose.

(iv) changes in the nucleic acid content. The RNA content follows closely that of protein. Heyes (1960) reported a constant RNA-protein ratio in expanding cells of pea root apex. During expansion, DNA content also increased. Jansen (1956) found that with progressing age of the cells of the root tip of Vicia faba an initial increase, an intermediate short phase of constant DNA content, and a final stage of increasing content occurred.

(v) expansion of the metabolic system of the cell. Growth also involves an active metabolic system. This might indeed be expected from the fact of protein increase, but is shown by a variety of determinations of which the most instructive possibly are some on the rate of oxygen uptake. An increase in the rate of respiration has been found in the expanding cells of root and shoot apex (Sanderland and Brown, 1956).

(b) Cell differentiation. In cell differentiation one can note:-

(i) changes in enzyme activities and respiration rates,

(ii) changes in protein and nucleic acid composition.

Robinson and Brown (1952) recorded the activity of invertase, phosphatase and dipeptidase and the protein content in broad bean (Vicia faba) roots for different distances from apex. They found the maximum cell volume and protein content at about 8 mm behind

the tip. After the peak values, protein decreased. The activities of invertase and phosphatases follow the protein curve very closely. They increased during the phase of expansion and decreased during that of early maturity. The enzyme activities showed that the growing cell is a metabolically expanding system and that this is a consequence of greater enzyme activity, attributed, at least in part, to an accumulation of protein.

The base composition of RNA and DNA from the apex and 8.00 mm from it, has been determined in pea roots (Robinson and Brown, 1952). In DNA there is no change in composition with increase in distance from the apex, the base composition of RNA differed only slightly. It is suggestive that the material from the older tissue had a higher purine to pyrimidine ratio than that from the younger. The changes in the composition of the RNA suggests a corresponding change in the composition of the protein.

3. Carbon and nitrogen sources for protein synthesis and growth of sugar beet leaves

The above discussion brings out the fact that for satisfactory growth of young leaves, abundant supplies of carbon and nitrogen are required. Joy (1964) pointed out that the young leaves of sugar beet import carbon as photosynthate from older leaves, but become increasingly independent until at about two-thirds maximum size their own photosynthetic processes are able to supply all their requirements. In 1967 Joy further noted that carbon from autonomous photosynthesis and from translocated sucrose is utilized for synthesis of most amino acids, with a considerable preference for the former source. While much nitrogen arrives in

young leaves in inorganic form, an important contribution is also made by glutamine/glutamic acid translocated in the phloem from roots. Using $C^{14}O_2$, Joy demonstrated that carbon fixed in autonomous photosynthesis contributed a greater proportion to the protein increase than to the insoluble carbohydrate increase in younger leaves. When sucrose from older leaves is available there is a tendency for it to contribute more carbon to insoluble carbohydrate than to protein.

Labelled sucrose was supplied directly to roots of beet seedlings, and after 18 hours various parts of the seedlings were analyzed (Joy, 1967). Radio carbon had been translocated and greatest activity was found in the youngest leaves. Glutamic acid was the predominantly labelled amino acid in both root and leaf. In young leaf protein glutamic acid was again the main labelled component. Joy (1967) concluded that fibrous roots must therefore synthesize considerable amounts of glutamic acid (or glutamine) from the sucrose, normally translocated to them from mature leaves, and sucrose carbon is then re-exported in this form to young leaves.

Siskajan and co-workers in Russia (1948, 1951, 1952) made a very different approach to the study of the metabolic changes in the sugar beet, especially with respect to sucrose storage. Their investigations have concentrated on change in the activity of leucoplasts extracted from roots at various stages of development. They regard the leucoplasts as constituting a depot of absorbed enzymes which can enter the metabolism of the cell when this is called for by the ontogenetic development of the plant. Leucoplasts in autolysing tissue liberated much invertase.

In storage roots of sugar beet, the leucoplasts become progressively less dense and less granular, but this is associated with decreasing invertase activity. During the vegetative period of the sugar beet plant, the invertase activity of leucoplasts in the root increased greatly, concurrently with a decrease in the invertase activity of the chloroplasts of leaves. This implies that when growth of new leaves or new fibrous roots is going on, stored sucrose is hydrolyzed to supply the carbon skeleton of the proteins and the insoluble carbohydrates.

4. Interrelationships of nitrogen metabolism and carbohydrate metabolism

Nitrate is the principal source of nitrogen utilized by higher plants. Nitrate uptake and metabolism require energy derived at the expense of sugar accumulation.

(a) Nitrate uptake. Energy is required for the intake of nitrate into the roots, where its concentration is usually many times greater than in the soil solution (Lundergardh, 1950). The energy for this accumulation of ions against a concentration gradient is derived from the respiration of stored food, principally sugar.

(b) Nitrate reduction. Nitrate must be reduced before it can be incorporated into usable compounds. The first step in nitrate reduction is conversion of nitrate to nitrite which is catalyzed by nitrate reductase (NRase). NRase was isolated from soya bean and Neurospora by Evans and Nason (1953) and later from many plants including bacteria, algae, fungi, and higher plants. Nitrate reductase from sugar beet was isolated

by Yang (1964). This enzyme system utilizes a reduced pyridine nucleotide as the H-donor and contains flavin adenine dinucleotide (FAD) as the prosthetic group. Molybdenum has been shown as an essential component of the nitrate reductase system (Nicholas and Nason, 1954; Nicholas et al. 1954). The sulfhydryl nature of the enzyme was indicated by the reversal of p-chloromercuribenzoate inhibition by the sulfhydryl reagents glutathione and cysteine, and also by the strong inhibition by iodoacetate and cupric sulfate (Nicholas and Nason, 1954; Nicholas et al. 1954; Yang, 1964).

Enzyme systems capable of catalyzing the reduction of nitrite have been prepared from both Neurospora and soybean (Nason and Evans, 1954). Medina and Nicholas (1957) demonstrated in Neurospora that hyponitrite is a product of nitrite reduction. The nitrite reductase of Neurospora is an $NADPH_2$ - dependent flavoprotein containing FAD, Fe, and Cu. Copper may be involved in the transfer of electrons from reduced FAD to nitrite; the role of iron is unknown.

An enzyme has been found in Neurospora that will catalyze the conversion of hyponitrite to hydroxylamine (Medina and Nicholas, 1957). Frear and Burell (1955) have shown that plant preparations are capable of reducing labelled hyponitrite to ammonia. Studies with Neurospora extracts indicate that iron and copper are required for the activity of hyponitrite reductase (Medina and Nicholas, 1957).

The final step in the sequence is the conversion of hydroxylamine to ammonia. The enzyme catalyzing this reaction hydroxylamine reductase, has been found in Neurospora by Zucker and Nason

(1955) and in higher plants by Frear and Burell (1955). Mn^{++} has been found to be associated with hydroxylamine reductase.

The overall reaction for the reduction of nitrate may be represented:

$$\underset{+5}{NO_3^-} \xrightarrow{Mo} \underset{+3}{NO_2^-} \xrightarrow{Fe,\ Cu} \underset{+1}{HNO} \xrightarrow{Fe,\ Cu} \underset{-1}{NH_2OH} \xrightarrow{Mn} \underset{-3}{NH_3}$$

Nitrate Nitrite Hyponitrite Hydroxylamine Ammonia

The above reaction sequence involves 2-electron changes at each step. It means that before nitrate can be incorporated into usable compounds, it requires eight hydrogen ions for each nitrate ion. The energy is derived at the expense of sugar accumulation, whether it is from the direct metabolism of sugar or from the photolysis of water.

5. Nitrate reduction in relation to respiration and photosynthesis

The main sources of reducing power in intact plants are the respiratory breakdown of carbohydrate and the photolysis of water in the chloroplasts. It is known that the ultimate H-donors in the initial step and in the subsequent steps of nitrate reduction are the reduced pyridine nucleotides. This would be true whether nitrate reduction were coupled to respiration or to photolysis of water.

A number of enzymes involved in plant respiration such as glucose-6-phosphate dehydrogenase, malic dehydrogenase, glutamic dehydrogenase, glyceraldehyde phosphate dehydrogenase and isocitric acid dehydrogenase are known to be linked to pyridine nucleotides. The oxidative reactions catalyzed by these enzymes generate reduced pyridine nucleotides, and in this way

the energy for the reduction of nitrate is derived directly from the oxidative breakdown of carbohydrates. In this respect, many investigators have observed that under conditions of high nitrate reduction and assimilation in the dark, carbohydrate levels in the plant are significantly lowered. Lower sucrose percentage and high "harmful nitrogen" and continued growth are the common observation in the sugar beet under the high nitrate fertilization in the field. The decrease of carbohydrate content of the cells can be explained on the bases of nitrate reduction and incorporation into amino acids and protein.

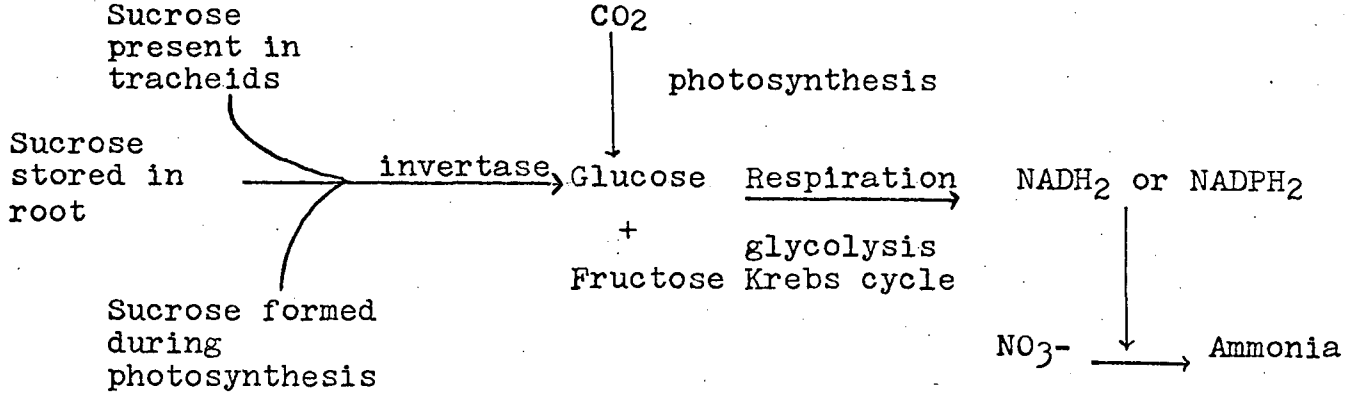
In photosynthetic tissues in the light, another potent source of reducing power is available, i.e., the formation of reduced NADP. Thus, Evans and Nason (1953) showed that the combination of NRase and grana from soybean leaves could reduce nitrate to nitrite in light if catalytic amounts of NADP were present. Ramirex et al. (1964) reported the findings of non-cyclic photosynthetic electron flow, in which flavin nucleotides mediate the direct transfer of electrons from illuminated grana to nitrate with the aid of NRase. Yang (1964) found the coupling of photoreduction of FAD with the reduction of nitrate by NRase and suggested the probability of participation of NRase in a flavin nucleotide - catalyzed enzymatic photoreduction of nitrate.

When nitrate reduction is coupled to respiration, the nitrate replaces oxygen as the terminal oxidant or H-acceptor. Extra carbohydrate must be broken down to provide energy for the reduction and extra carbon dioxide is released without a corresponding increase in O_2 consumption, i.e., R. Q. increases. Thus, it was observed by Hamner (1935) that nitrogen deficient plants had large carbohydrate reserves. When they were supplied

with nitrate they exhibited an increased rate of carbon dioxide production (measured in the dark), a depletion of carbohydrate reserves, and the temporary formation of nitrite in the leaves.

Cramer and Myers (1948) showed that nitrate reduction in the dark by chlorella in a glucose medium is accompanied by an increase in R. Q. from 1.2 to 1.6 caused by an increased rate of carbon dioxide production. During photosynthesis at low light intensity, nitrate utilization in this organism is accompanied by a decrease in assimilation quotient from 0.9 to 0.7 owing to the decreased CO_2 uptake (Van Niel et al. 1953). Under these conditions presumably carbon dioxide and nitrate are alternative acceptors of the reduced H-carriers generated by the photolysis of water.

Evidence, then, suggests that photosynthesis and respiration supply the necessary reducing power for nitrate reduction. Photosynthesis, however, plays its part in all probability through its photosynthates via respiration (Beevers et al. 1964). In sugar beet, the formation of $NADH_2$ for the reduction of nitrate to ammonia and the depletion of sugars for it, can be summarized as follows:



(c) The utilization of ammonia

The ammonia formed by the reduction of nitrate enters into organic combination by three main reactions:

(i) as an ∝-amino group

(ii) as the amide group of asparagine and glutamine

(iii) as carbamyl phosphate, an intermediate in the synthesis of citrulline and pyrimidines.

Formation of amino acids

Knoop and Osterlin (1925) postulated the formation of amino acids from ∝-keto acids and ammonia by reductive amination. In 1937 Gregory and Sen discussed the interrelationship of carbohydrate and nitrogen metabolism. They regarded some carbon from carbohydrate as entering into the constitution of protein. Krebs and Johnson (1937) suggested that the citric acid cycle plays a major role in carbohydrate oxidation in animals. Chibnall (1939) realized its importance and suggested that the citric acid cycle occupies the central and key position, in the carbohydrate, protein, and fat metabolism of plant cells.

The importance of the Krebs cycle was further emphasized when Euler and Heyman (1938) showed that the dihydrocodehydrase reduces iminoglutaric acid to glutamic acid. Thereafter the following reactions have been proposed by which ammonia, derived from nitrate can form the ∝-amino group of the amino acid.

(i) Fumarate + NH_3 ⟷ aspartate

This reaction is catalyzed by the enzyme aspartase which is present in bacteria, but its presence in higher plants is doubtful.

(ii) Pyruvate + NH_3 + $NADH_2$ ⟷ alanine + NAD + H_2O

This reaction has been demonstrated in bacteria and liver mitochondria.

(iii) α-Ketoglutarate + NH_3 + $NADH_2$ ⟷ glutamate + NAD + H_2O

This reaction is generally regarded as quantitatively the most important reaction. Evidence to support this view has been provided in the case of Torulopsis utilis (Folkes, 1959). The reaction is reversible and proceeds in two steps.

$$\underset{\text{glutarate}}{\alpha\text{-Keto}} + NH_3 \rightleftharpoons \underset{\text{glutarate}}{\alpha\text{-imino}} \xrightarrow[\text{glutamic dehydrogenase}]{NADH_2} \text{glutamate} + NAD$$

The first step probably proceeds spontaneously, but the second reaction is catalyzed by the enzyme glutamic dehydrogenase and requires the presence of reduced NAD. Because of the central importance of glutamate in the synthesis of other amino acids and because of the higher proportion of glutamate formed in this manner by the plant, this reaction represents the "port of entry" into the metabolic system for inorganic nitrogen. However, it has been suggested by Smith et al. (1961) that the synthesis of alanine during photosynthesis does not involve transamination from glutamic acid. It is suggested by the authors that alanine is formed by the reductive amination of phosphenol pyruvate.

The discovery of transamination, i.e., the transfer of the amino group from an amino acid to a keto acid, by Braunstein and Kritzman (1937) also placed the keto acids in a position such that they might receive amino groups from another source and produce both primary and secondary amino acids. The cofactor of transaminases is pyridoxal-5-phosphate or pyridoxamine-5-

phosphate, which is tightly bound to the enzyme but may be, at least partly, removed during precipitation of the protein with ammonium sulfate.

A survey of transaminase activity in various plants has been made by Leonard and Burris (1947). They found that the transamination rate per unit of tissue decreased with age in the growing plant. Wilson et al. (1954), have used C^{14}-α-ketoglutarate to study transamination in plants. Although transamination reactions involving glutamic acid are by far the most prevalent in the plant, other transamination reactions have been found. For example, transamination reactions involving aspartic acid and alanine has been found in higher plants (Wilson et al. 1954).

It is generally accepted now that reductive amination of α-keto acids followed by transamination is a major pathway of amino acid formation. These processes account for formation of the dicarboxyl amino acids, and of alanine from which all the other naturally occurring amino acids except proline and hydroxyproline may be derived.

The formation of amides:— These compounds are present in plants as part of protein and as soluble amides. Glutamine synthetase, requiring ATP for its operation, is responsible for the amidation of glutamic acid and has been extracted and purified from tissue of higher plants (Elliot, 1953; Varner and Webster, 1955). The synthesis proceeds by the overall reaction:

$$\text{glutamate} + \text{ATP} + NH_3 \xleftrightarrow{Mg^{++}} \text{glutamine} + \text{ADP} + P_i$$

The synthesis of asparagine probably occurs by reactions similar to those involved in glutamine synthesis.

$$\text{aspartate} + \text{ATP} + NH_3 \longleftrightarrow \text{asparagine} + \text{ADP} + P_i$$

An asparagine synthetase has been isolated and purified from lupine seedlings (Webster and Varner, 1955).

Joy (1967) suggested that in sugar beets, glutamine is synthesized mainly in the roots and the carbon source is sucrose. Glutamine was also found to be present in the tracheids. Perhaps glutamine or glutamate is transported from the roots to the leaves.

The lower sucrose content in the root along with increased leaf area and increased amino acids with added nitrogen, suggested that in the sugar beet plant the synthesis and metabolism of sucrose are influenced by available nitrogen. To test this possibility, Snyder and Tolbert (1966) exposed mature sugar beet plants grown on a complete nutrient solution, to $C^{14}O_2$ in sunlight. After one hour of photosynthesis, the blades of the - N plants had a significantly greater percentage of C^{14} in sucrose and smaller percentage in citric, malic, and amino acids than the blades of the + N plants. After 24 hours, the - N plants retained approximately 40% less C^{14} in the blades and proportionately more in roots than the + N plants. The roots of the - N plants contained 39% of the C^{14} while those of the + N plants contained only 19%. Of the C^{14} retained in the blades of the - N plants, 36% resided in sucrose and 21% in citric plus malic plus amino acids, whereas the blades of the + N plants contained 16% of the C^{14} in sucrose and 44% in citric, malic, and the amino acids.

The above data suggest that plants having a continuous and adequate supply of nitrogen may preferentially snythesize the citric acid cycle products and their amino acid counterparts and thus produce less sucrose. Also with adequate nitrogen,

photosynthetic products may be channelled preferentially into new growth at the expense of sucrose being synthesized and transported to the root (Fig. 1).

Protein synthesis - The current concept of protein biosynthesis has been summarized by Davies, Giovanelli and AP Rees (1964) as follows:

(1) Activation of amino acid

Enzyme + amino acid + ATP $\longleftrightarrow$ Enz - AMP - AA + PPi

(2) Formation of aminoacyl - RNA

Enz - AMP - AA + s-RNA $\longleftrightarrow$ AA - s-RNA + AMP + Enz

(3) Formation of peptide bonds

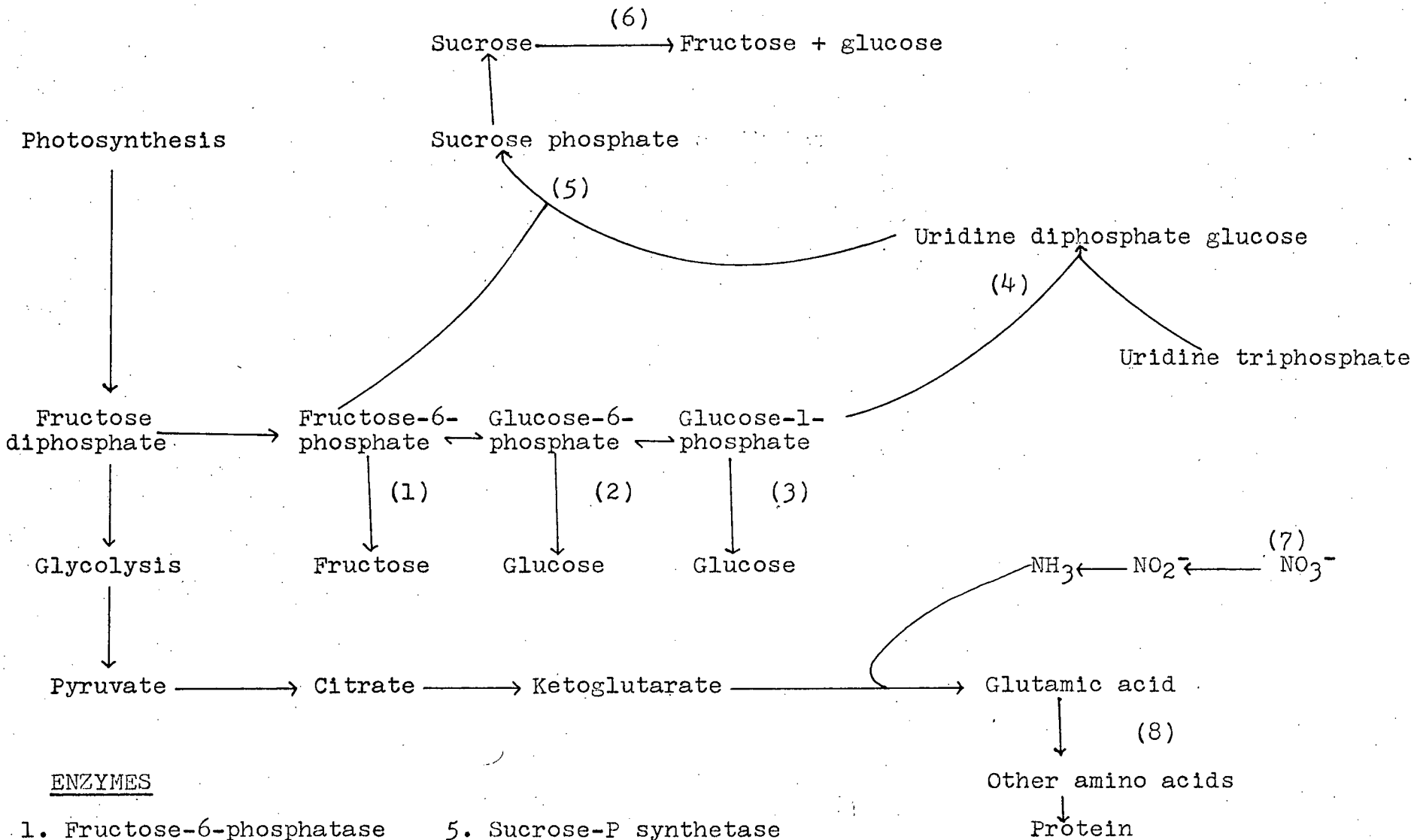
n (AA - s-RNA) + ribosome $\longleftrightarrow$ polypeptide on ribosome + n (s-RNA)

(4) Release of polypeptide

Polypetide on ribosome $\longrightarrow$ polypeptide + ribosome

This scheme indicates that for protein synthesis amino acids and ATP are required. Since respiration furnishes the stores of usable energy (as ATP), it is to be expected the respiration will be intimately connected to protein synthesis. Since the Krebs cycle can operate as a route of synthesis, producing keto acids as "ports of entry" for nitrogen, respiration is envolved here also as a means of mobilizing carbon from carbohydrate metabolism.

Formation of nucleotides for nucleic acids - In an earlier section, the work of Heyes (1960)was quoted in which he found that the RNA content of the growing cell closely followed that of protein. Steward and Durzan (1965) drew attention to the similar fact that agents like coconut milk which promote growth and protein synthesis do so, in part, because they affect the



ENZYMES

1. Fructose-6-phosphatase
2. Glucose-6-phosphatase
3. Glucose-1-phosphatase
4. UDPG-pyrophosphorylase
5. Sucrose-P synthetase
6. Invertase
7. Nitrate reductase
8. Transaminase

Fig. 1. Interrelationships of Nitrogen Metabolism and Carbohydrate Metabolism

effectiveness of the RNA in those cells for protein synthesis.

The formation of pyrimidine bases involves ATP, aspartic acid, NH_3,CO_2 and phosphoribosyl pyrophosphate. The synthesis of nucleotides containing adenine bases involves amino acids, for example, glycine, glutamine and aspartic acid, and also CO_2, formate and phosphoribosyl pyrophosphate. The ultimate source of carbon for amino acids, ATP and phosphoribosyl pyrophosphate are the products of carbohydrate metabolism. Thus, increase in nucleic acid content of the growing cells will again involve the depletion of sucrose in sugar beet.

The above discussion outlines the main causes of the low sugar content in roots of sugar beets under continued supply of nitrate fertilizer. The following tabulation will summarize the events from nitrate uptake to its assimilation, and also the involvement of carbohydrate metabolism.

Physiological process	Depletion of sucrose or diversion of sucrose synthesizing system provides:
(1) Nitrate uptake	Usable energy as ATP
(2) Nitrate reduction to ammonia	
(a) Nitrate to nitrite	Reduced NAD (or NADP)
(b) Nitrate to hyponitrite	Reduced NAD
(c) Hyponitrite to hydroxylamine	Reduced NAD
(d) Hydroxylamine to ammonia	Reduced NAD
(3) Formation of amino acids	
(a) Reductive amination	Carbon skeleton as keto acids, and reduced NAD
(b) Transamination	Carbon skeleton as keto acids
(4) Formation of amides	Carbon skeleton as amino acids, and usable energy as ATP
(5) Protein synthesis	Carbon skeleton as amino acids
(6) Formation of nucleotides	
(a) Pyrimidine containing nucleotides	Carbon skeleton as aspartate, phosphoribosyl pyrophosphate, and usable energy as ATP
(b) Purine containing nucleotides	Carbon skeleton as glycine, formate, phosphoribosyl pyrophosphate, and usable energy as ATP

III CONTROL OF GROWTH IN SUGAR BEET

The growth and development of sugar beet plants appear to be closely related to the sucrose economy of the beet plants, namely sucrose formation and utilization. An adequate supply of nitrogen stimulates growth of new leaves and fibrous roots. The conversion of large amounts of sugar to top and root growth would result in a storage root with a low sucrose concentration.

The inverse relationship between the nitrogen status of the plant and the sucrose concentration of the beet root has been observed many times and has led to the suggestion that for a period before harvest, nitrate supply should be greatly reduced to prevent the reinvestment of sucrose in the production of surplus foliage and fibrous roots. The following discussion will give some of the prevalent cultural practices to reduce the nitrogen supply at the time of "ripening" of sugar beet. It will also present the outline of the more reliable and definite control of growth by the use of metabolic inhibitors.

1. Cultural practices

(a) The use of optimum amount of nitrate:- This method was advocated by Ulrich (1942). The method generally used is to estimate the optimum amount of nitrogen to apply earlier in the season so that nitrate will be depleted to the proper extent in the root zone at the right time. In an average year it is assumed that 80 to 100 pounds per acre of actual nitrogen will be utilized by the crop and the beets will run out of nitrogen by the end of growing season.

(b) The critical level of nitrate fertilizer:— Ulrich (1950) found that nitrate nitrogen of the petioles of the sugar beet leaves inversely correlated to the sucrose content and could be used for the estimation of critical level of nitrate fertilizer in the field. Thus, the method being used is to test the nitrate nitrogen content in the beet petiole and when it drops below 1000 ppm, wait three weeks and then harvest. This method works fairly well in areas where there are long harvest seasons.

(c) Smaller beets:— Loomis and Ulrich (1959) investigated the response of sugar beets to nitrogen depletion in relation to root size. Starting from a high nitrogen status, small beets increased faster in sucrose concentration with the onset of nitrogen deficiency than did large beets. The authors suggested that until sugar beet varieties are available that will "ripen" naturally to a high sucrose concentration under high nitrogen conditions, it may be possible for the grower to take advantage of the knowledge that small beets respond more readily than large beets to changes in nitrogen status. It would not be practicable for a grower to reduce the mean root size by delaying planting date. In that case two possibilities remain: (1) reduce the average plant spacing, thus increasing the plant population, and (2) alter the length of the period of nitrogen deficiency prior to harvest (Loomis and Ulrich, 1959).

(d) N-fertilization and date of planting:— Schmehl et al. (1963) determined that interactions of the rate of nitrogen fertilization, date of planting, and plant spacing in the row, on yield and quality of the beet. They found that early planted beets produced higher yields and more sucrose than late planted

beets. They suggested the need to adjust the rate of nitrogen fertilization with date of planting in climatic areas where the harvest date cannot be extended.

(e) Irrigation schedules:— Wolley and Bennet (1962) found that the use of moderate amounts of nitrogen fertilizer with an irrigation schedule that allowed the soil moisture to be maintained near field capacity produced the highest yield of roots and sucrose. The authors believed that for any given irrigation regime there is a nitrogen level best calculated to give maximum sugar production.

(f) Redistribution of nitrate in the soil:— Stout (1961, 1964) drew attention to the fact that the nitrate ion moves very freely with moisture in soils. He suggested that according to the relative pattern of distribution of nitrate, one can develop cultural practices to help nature put the nitrate where the plants cannot get it. For example, planting beets so close as to give enough foliar protection to the soil near the root zone in case of rain before the harvest, avoiding sprinkler irrigation, and the use of some product which might reduce the mobility of nitrogen nutrients either by reducing the rate of nitrification of ammonium salts or by reducing solubility of nitrate by incorporating it into more slowly soluble forms. Stout (1964) further suggested that any supplemental nitrate added to row crops after the first irrigation should be placed below the bottom of irrigation furrows in order to lengthen the period of availability to plants before it reaches the dry surface layer of soil.

2. Selection and the breeding of the new varieties

Payne et al. (1961) suggested the possibility of improving the quality of sugar beet by plant breeding in association with fertilizer practices. In their chemical genetic studies the above authors found that genetic variability was associated with total nitrogen, betaine and glutamic acid. On the bases of their data, they claimed that increases in percentage sucrose can be obtained on high fertility soils by breeding populations of sugar beets adapted to growing under these conditions. They found two hybrids which were capable of producing high percentage sucrose at higher fertility levels.

3. Disadvantages of the prevalent methods

The above outline methods are common in sugar beet fields but they have some obvious disadvantages. The major disadvantage of the use of optimum amount of N-fertilizer is the dependency on 'average' weather condition to ensure complete use of nitrogen. Tolman (1960) very well points out another disadvantage of this method. The greatly increased number of micro-organisms competes with the beet for the available nitrate when the beets need it most. Late in the summer, the micro-organisms begin to die and release nitrate at a time when the supply to the beets should be greatly reduced.

The disadvantage of the method of estimation of critical level of nitrate in the petioles of the beet, is the gradual supplying of nitrate from the soil in the areas where there are short growing seasons and there is no cutting off this nitrate supply (Russel, 1965).

Stout (1964) studied the nitrate content of irrigation and drainage waters and found that, under normal conditions, irrigation practices for leaching of nitrate from soils have been overestimated.

The breeding of a new variety is the best answer to the problem, but the process is very slow. In the sugar beet, the breeding on the line of Vilmorin takes at least 8-10 years. And also the performance and stability of a new variety depend on the variables like climatic conditions etc.

Thus, it is very difficult or impossible to control the depletion of sucrose by the above mentioned methods under the conditions of high nitrogen fertilization, because uncertainty is always associated with them.

4. Chemical control of growth in sugar beet

Alternatives to the foregoing methods might involve the control of growth of the sugar beets at the time of "ripening" (two to three weeks before the harvest) of the roots by the use of metabolic inhibitors or growth regulators.

In plants, four types of growth regulators have been recognized: auxins, gibberellins, kinins and inhibitors. The term auxin includes two types of materials: the growth hormones, which are natural plant constituents and which regulate cell enlargement in the manner of indoleacetic acid, and synthetic materials, which can also stimulate cell enlargement in the manner of indoleacetic acid.

The gibberellins also regulate growth, but through a type of action which is distinctive in the sense that they do not

stimulate growth of roots, and their translocation is not in a polar fashion. The kinins regulate growth, at least in part, by stimulating cell division. It seems likely, however, that gibberellins and kinins require the presence of auxin for their growth effects.

The inhibitors include a wide array of chemical entities which may inhibit growth or developmental functions or may inhibit some component reactions relating to the growth regulators. Examples of natural inhibitors are the various phenolic compounds, such as benzoic acid, cinnamic acid, chlorogenic acid, caffeic acid, p-coumaric acids, etc. The synthetic inhibitors are compounds like maleic hydrazide, aminotriazole, cycocel and various metallic ions.

A depression of growth and the control of the depletion of sucrose can result from a great variety of inhibitory mechanisms in sugar beets. Some of the likely mechanisms are as follows:

(a) Inhibition of systems important for the synthesis of precursors necessary for a process. For example, (i) inhibition of nitrate reductase, the enzyme responsible for the reduction of nitrate to nitrite which on further reduction gives rise to ammonia required for amino acid synthesis (hence protein synthesis). (ii) inhibition of transaminases, the systems necessary for the synthesis of most of the amino acids. (iii) inhibition of 3-deoxy-D-arabinoheptulosonic acid 7-phosphate (DAHP) synthetase, the first enzyme of the shikimic acid pathway for the synthesis of aromatic amino acids.

(b) Inhibition of the systems which supply the substrates for respiration; e.g., inhibition of invertase activity which

hydrolyzes sucrose to glucose and frutose and makes available the hexoses for utilization in respiration.

(c) Inhibition of respiratory pathways and energy generating system. Intermediates of the Krebs cycle may be involved in the synthesis of lipids, proteins, nucleic acids etc. Respiration also provides usable energy as ATP for the synthetic reactions and also the reduced coenzymes for nitrate reduction and reductive amination.

(d) Inhibition of synthesis of some growth regulators, e.g., inhibition of tryptothan synthetase which will result in the inhibition of the synthesis of indoleacetic acid.

(e) Inhibition of some steps in protein or nucleic acid synthesis. For example, use of chloramphenicol which binds with 50S ribosomal particle and inhibits the protein synthesis; Actinomycin D which binds to guanine in the minor groove of DNA and blocks RNA synthesis.

(f) Incorporation of an abnormal analogue into a synthetic pathway resulting in a metabolic block if one of the abnormal substances formed cannot undergo further metabolism or is unable to function as its normal analogue. For example, use of 5-bromouracil, which replaces normal bases, causing H-bonding errors and mistakes in incorporation and replication of DNA. The result is the change in codon for amino acids. Similarly, the use of benzimidazole or 2-aminopurine which cause mistakes in incorporation of adenine.

(g) Direct disturbance of the mitotic sequence (such as inhibition of spindle formation, or of protoplasmic movements with cleavage or the breakdown of chromosomes e.g., by maleic

hydrazide).

(h) Inhibition of auxin activity, e.g., the use of an anti-auxin such as p-phenylbutyric acid, trans-cinnamic acid or maleic hydrazide.

The present investigation is an attempt to control the growth of sugar beet at the time of "ripening" of roots by the use of metabolic inhibitors or growth regulators. It is consistent with the discussion presented in the preceding sections, that the growth and development of plants are the products of the metabolic systems mediated by the enzymes. It is also consistent with the fact that the enzyme activity can be affected by interaction with a small molecule — inhibitor or activator.

Since nitrate is considered to be the prime source of nitrogen available to sugar beet for continued growth, emphasis has been given the use of inhibitors of nitrate reductase. Nitrate reductase has a major role in regulating nitrogen metabolism in plants. Its regulatory nature is evident in the sense that it is (a) the first enzyme in the pathway of nitrate reduction; (b) inducible by substrate (NO_3^-) (Beevers et al. 1965); labile in vivo under environmental stress (Mattas and Pauli, 1965); (c) variable in level diurnally (Yang, 1964); (d) sensitive to the inhibitors of protein synthesis e.g., cycloheximide (Shrader and Hageman, 1967); (e) index of the total protein producing potential of the grain in wheat (Croy, 1967); (f) and regulated by hormones as indicated by the differential affects of 2,4-D on mono- and dicotyledonous plants (Beevers et al. 1965).

The following other inhibitors have also been used:
Benzimidazole - A synthetic analogue of adenine and found to

inhibit the growth of the plants (Rebstock et. 1955).

Chloramphenicol - Inhibits protein synthesis and auxin-induced growth in plants (Noodén and Thimann, 1965).

Caffeic acid - A phenolic acid and naturally occurring growth inhibitor in plants (Leopold, 1964)

Cycocel (2-chloroethyl trimethylammonium chloride) - is a growth retarding chemical which exerts its influence on plant growth processes through the enhancement of auxin destruction (Kuraishi and Muir, 1963).

Maleic hydrazide - Inhibits growth of the plants by reacting with several plant constituents (Leopold and Klein, 1962; Noodén, 1967).

The sequence of steps in the overall investigation is as follows:

(1) Various inhibitors were applied to the leaves of one-month-old plants. The inhibition of growth has been determined by measuring the leaf area, seven days after treatment.

(2) The effect of the three most effective inhibitors of the growth of leaves of one-month-old plants were determined, 7, 14, and 21 days after the treatment of 4.5-month-old plants on:

(a) the growth of the leaves

(b) the chemical composition of the roots

(3) To explain the observed effects on the growth of leaves and the changes in the chemical composition of the roots due to the treatments, effects on metabolic processes have been determined.

IV MATERIAL AND METHODS

1. Growth of the plants

Beta saccharifera seed, S.K.E. R-11, obtained from the British Columbia Sugar Refining Co., Vancouver, B.C., was sown in wooden flats filled with vermiculite. They were watered once every day with nutrient solution containing 0.005 M $Ca(NO_3)_2$, 0.0005 M KH_2PO_4, 0.002 M $MgSO_4$, 0.05 M KNO_3, 0.5 ppm Fe as FeEDTA, 0.04 ppm of Cu as $CuSO_4$, 0.25 ppm of Mo as Na_2Mo_4, 2.0 ppm of boron as Na_3BO_4, 0.02 ppm of Zn as $ZnSO_4$ and 0.5 ppm of Mn as $MnCl_2$. When the seedlings were nine days old (early 2-leaf stage), they were transplanted to tin cans of 5" diameter containing vermiculite or to one-gallon crocks containing sandy loam.

The plants were grown in either a controlled environment room in a greenhouse at the University of British Columbia. The conditions in the controlled environment room were: photoperiod, 16 hours; light intensity 1800 foot-candles at the top of the plants; temperature, day 21-26°C, night 18-22°C and relative humidity, day 62-70%, night 65-80%. These conditions are designated as "summer" growth conditions.

Three series of growth room experiments were conducted: the first, in which the effects of various chemicals on the expansion of leaves of one-month-old plants grown in vermiculite in 5" diameter tin cans, were determined; the second, in which the effects of maleic hydrazide, pyrocatechol and vanadium sulfate (the three most effective inhibitors of leaf expansion of one-month-old plants) on the inhibition of leaf expansion of 4.5-month-old plants grown in one-gallon crocks, were determined; and

the third, in which the effects of maleic hydrazide, pyrocatechol and vanadium sulfate on the photosynthesis and respiration of 4.5-month-old plants were determined.

Three series of greenhouse experiments were conducted with 4.5-month-old plants to find out the effects of maleic hydrazide, pyrocatechol and vanadium sulfate on (1) the nitrogenous constituents of the roots and on the respiration of the root discs; (2) the hydrolytic enzymes of roots and leaves; (3) the reducing sugar and sucrose percentage of the roots, and the enzymes of nitrogen metabolism and of sucrose synthesis.

In three series of growth room experiments, 4.5-month-old plants were transferred to a controlled environment room with the following conditions; photoperiod 12 hours; light intensity at the top of the plants 1800 foot-candles; temperature day 14-16°C, night 5-7°C; and relative humidity, day 55-70%, night 75-80%. These conditions are designated as "fall" growth conditions. The effects of maleic hydrazide, pyrocatechol and vanadium sulfate under the fall conditions on (1) leaf area; (2) sucrose and reducing sugar percentage and respiration of root discs and (3) photosynthesis and respiration of whole plants were determined.

2. Mode of application of chemicals

The aqueous solutions of the inhibitors were sprayed to wet the leaf surfaces of the sugar beet plants. Vanadium compounds were applied, however, with 0.2% Tween 20 (a wetting agent).

3. Determination of leaf area

Leaf area was determined by the use of the formula BL x BW/

1.645. Here BL = blade length from the base of the blade to the tip and BW = maximum blade width. The factor 1.645 was obtained by a preliminary experiment to account for the difference of the actual area of the blade from the value obtained by BL x BW.

A grid-dot sheet was used to determine the actual area (BA) of the tracings of the blades of 7 leaves from each of 7 plants. The maximum width (BW) and length (BL) of each of the leaf blades were measured, and BW x BL calculated. The formula BW x BL/BA was used to obtain the correction factor which could be employed to calculate blade area from BW and BL measurement.

Plant	A	B	C	D	E	F	G
Average true area (cm^2)	55.51	76.47	72.12	33.14	42.75	67.33	39.37
Average of BL x BW (cm^2)	85.14	126.69	123.68	52.06	74.59	113.45	62.90
BL x BW/BA	1.545	1.657	1.715	1.571	1.745	1.685	1.598
Average of BL x BW/BA of 49 leaves from 7 plants	1.645						

To test the accuracy of the method, the area of a leaf taken from each of 10 different plants was determined by the grid-dot sheet method and also by the formula. The following data show the correspondence of area values obtained by the two methods.

	BA	BL x BW/1.645	Difference	% difference
	(cm^2)	(cm^2)		
(1)	43.24	41.91	1.33	3.1
(2)	61.92	63.01	2.19	3.5
(3)	47.20	45.12	2.08	4.5
(4)	20.48	18.85	1.63	7.8
(5)	18.56	19.95	1.39	6.9
(6)	38.36	37.20	1.16	2.9
(7)	69.23	71.80	2.67	3.8
(8)	24.96	26.37	1.41	5.7
(9)	48.12	45.48	3.64	7.4
(10)	62.24	59.41	2.83	4.4
			Avg.	5.63

4. Determination of the chemical composition of the root

Preparation of the samples

The beets were trimmed of leaves and small roots, washed, and the crowns removed. The entire remaining portion was finely chopped and blended in a Waring blendor for 3-4 minutes. Three 50-gram aliquots of the blended material were used for sucrose and reducing sugars determinations, and another three 50-gram samples were heated at 110°C for five minutes to stop enzymatic changes, dried at 85°C to a constant weight, and then ground, redried and stored over calcium chloride in desiccators.

(a) Determination of reducing sugars and sucrose

Preparation of the extract

Fifty grams of the freshly blended beet root was added to 250 ml boiling 95% ethanol in a 600 ml beaker, boiled for 10 minutes on a water bath, and then cooled. The liquid was filtered through dry filter paper into a 500 ml volumetric flask.

The residue was covered with 80% ethanol, heated gently for 30 minutes on a water bath and then allowed to cool to room temperature. The liquid was filtered into the flask containing the first extract. This process was repeated eight times for two days. After the final addition of the liquid through the filter, the volume was made to 500 ml with 80% ethanol.

One hundred ml of the above extract was reduced to about 5 ml by heating on a hot water bath. Then 50 ml of water was added. The resulting solution was warmed to soften the gummy residues and bring the sugars into solution. A rubber-tipped rod was used vigorously to break up the gummy residues. The hot water extract was transferred quantitatively to a 250 ml volumetric flask.

The solution in the flask was cooled. Two ml of saturated lead acetate was added to remove tannins and other reducing impurities. The volume was made to 250 ml, mixed thoroughly and filtered through dry filter paper into a 500 ml erlenmeyer flask containing about 0.3 g anhydrous potassium oxalate. By adding a drop of dilute lead acetate, the deleaded solution was tested for excess of oxalate. If a heavy precipitate did not appear, more oxalate was added. Toluene was added and mixed well. The flask was stoppered and allowed to stand overnight. The solution was then decanted through a dry filter into a dry flask. This solution was used for reducing sugars and sucrose determinations (Loomis and Shull, 1937).

Determination of reducing sugar

Reducing sugar was determined by the arsenomolybdate reagent method of Nelson (1944). To 1 ml of the sugar solution was added 1 ml of the copper reagent. The solution was then heated for 20 minutes in a boiling water bath.

At the end of this time, the tubes were cooled, and 1 ml of arsenomolybdate reagent was added. The mixture was diluted, if required, and the optical density was measured at 520 mu with a Beckman Model B spectrophotometer. The amount of reducing sugar was caluculated from a standard curve and expressed as percentage of the fresh weight.

The copper reagent was a mixture of 25 parts of reagent A and one part of reagent B. Reagent A was prepared by dissolving 25 g of Na_2CO_3 (anhydrous), 25 g Rochelle salt, 20 g of $NaHCO_3$ and 200 g of Na_2SO_4 (anhydrous) in about 800 ml of water and diluting to one liter. Reagent B was 15% copper sulfate containing one or two drops of concentrated sulfuric acid per 100 ml.

The arsenomolybdate reagent was prepared by dissolving 25 g ammonium molybdate in 450 ml of distilled water. To this was added 21 ml of concentrated sulfuric acid and 3 g of sodium arsenate dissolved in 25 ml of H_2O. The contents were mixed and placed in an incubator at 37°C for 48 hours.

Determination of sucrose

In order to determine sucrose, 25 ml of the cleared and deleaded extract was pipetted into a 400 ml beaker. Then two drops of methyl red, 4 drops of invertase solution and 5 drops of 10%

acetic acid were added. The contents were mixed and allowed to stand overnight at about 25°C. The total reducing sugar was determined by the arsénomolybdate reagent. The amount of sucrose present was calculated by subtracting the value of reducing sugars before hydrolysis of sucrose, from the total reducing sugars and multiplying the remainder by 0.95. Sucrose was expressed as percentage of fresh weight of the roots.

(b) Determination of ammonium content

Ammonium content of the root was determined by an adaptation of the method of Vickery and Pucher (1929), on the alcohol extract made for reducing sugars and sucrose determinations. The volume of 100 ml of alcohol extract was reduced to about 20 ml on a boiling water bath. Then 50 ml of water was added to it and the volume was reduced for the second time. The concentrated alcohol-free extract was quantitatively transferred to a distillation flask. A few glass beads, a small piece of paraffin and 1 to 2 g of magnesium oxide were added. The flask was fitted for distillation.

The distillation tube was dipped beneath the surface of 3 ml of 0.1 N HCl containing a drop of methyl red solution. The contents of the distillation flasks were mixed and heated to boiling with a micro-burner at such a rate that steam began to rise in 3 minutes. Distillation was continued for 5 minutes. The end of the distillation tube was washed with a few drops of water. The tube was then removed.

The distillate was allowed to cool and then transferred to a 100-ml flask containing 3 g permutit together with enough water

to make about 15 ml. The flask was shaken for 5 minutes and laid on its side for one minute. The fluid was then decanted. The permutit was washed as above three more times.

The permutit was then rinsed to the bottom of the flask with about 5 ml of water. Thereafter 1 ml of 10% NaOH was added and shaken for 3 minutes. About 65 ml water was added, the flask agitated, and then 5 ml of Nessler's reagent added. The contents were diluted to 100 ml and the optical density was read at 450 mu. Ammonium sulfate was used to make the standard curve.

(c) Determination of nitrite, nitrate and amino acid content

Preparation of extract

Nitrite, nitrate and amino acid contents were determined on an aqueous extract prepared from the dry powder. To 0.5 g of dried powder was added 35 ml of distilled water and boiled for 5 minutes. The extract was then cooled, made to 50 ml volume, centrifuged and finally filtered through glass wool.

Determination of nitrite content

Nitrite content of the aqueous extract was determined by the method of Wolley et al. (1960). To 1 ml of the extract was added 9 ml of 20% acetic acid. By the use of a measuring scoop, 0.8 g of an intimate mixture of 100 g of barium sulfate, 75 g of citric acid, 4 g of sulfanilic acid and 2 g of 1-naphthylamine was added. The sample was shaken for about 15 seconds and was similarly shaken 3 minutes later. After 3 minutes more, the sample was shaken for the third time and centrifuged for 3

minutes at 1000 x g. The supernatant solution was poured through a small loose plug of borosilicate glass wool. The light absorbance was measured at a wavelength of 520 mu and the amount of nitrite was calculated from a standard curve using potassium nitrite.

Nitrate content

Nitrate content was measured by the method of Wolley et al. (1960). Nine ml of 20% acetic acid containing 0.2 ppm of copper as copper sulfate was added to 1 ml of the aqueous extract. To this was added 0.8 g of an intimate mixture containing 10 g of manganous sulfate dihydrate, 2 g of powdered zinc, 100 g of barium sulfate, 75 g of citric acid, 4 g of sulfanilic acid and 2 g of 1-naphthylamine. The sample was shaken for about 15 seconds and was similarly shaken three minutes later. The sample was shaken for the third time after another three minutes and centrifuged for three minutes at 1000 x g. The supernatant solution was poured through a small loose plug of borosilicate glass wool. The light absorbance was measured at a wavelength of 520 mu and the amount of nitrate was determined from a standard curve using potassium nitrate - allowance being made for the nitrite content.

Determination of amino acid content

The amino acid content of the aqueous extract was determined by the method of Rosen (1957). One ml of the sample was heated for 15 minutes in a boiling water bath after the addition of 0.5 ml of cyanide - acetate buffer (0.0002 M NaCN in sodium acetate - acetic acid buffer, pH 5.4) and 0.5 ml of 3% ninhydrin in methyl cellosolve (ethylene glycol monoethyl ether).

Immediately after removing from the water bath, 5 ml of isopropyl alcohol - water (1:1) diluent was added, shaken vigorously, and allowed to cool to room temperature. The intensity of color was read at 570 mu. The concentration of amino acids was calculated from a standard curve prepared from L - leucine.

(d) Determination of total nitrogen

Total nitrogen was determined by the standard Kjeldahl method described by Loomis and Shull (1937). Two 1.00-gram samples of the dry powder and a 1.00 gram of sucrose were weighed on 7-cm filter papers. Filter papers were folded and placed in 800 ml Kjeldahl flasks. A selenized crystal was added to each flask. Then 25 ml sulfuric - salicylic acid mixture was poured in and allowed to react in the cold for 30 minutes. Thereafter 5 g of sodium thiosulfate was added. The flasks were warmed slightly for 5 minutes and then cooled. By the use of a measure 8 g sodium sulfate - copper sulfate mixture was added to the flasks. The flasks were allowed to heat gently on the digestion rack until danger of frothing was over and then strongly until clear. The heating was continued for another 30 minutes. Then the flasks were allowed to cool in a fume closet.

Several pieces of zinc, 250 ml of water and 100 ml of 33% NaOH were added and the flasks fitted for distillation. The distillation tube was dipped beneath the surface of 50 ml of 0.1 N HCl containing a drop of methyl red in a wide mouth 500-ml Erlenmeyer flask. After 150 ml was distilled, the receiver flasks were lowered, distillation was continued for another 5 minutes, and the outside of the tube was rinsed with water.

Unused acid was titrated with 0.1 N NaOH using methyl red-methylene blue indicator. Sucrose was used in place of the plant material for the blank. "Net titration" was determined (blank minus the nitrogen titration). Total nitrogen for the "net titration" was calculated on the basis that 0.01 N NaOH equals 1.4 mg of nitrogen.

(e) Determination of total protein

Preparation of the TCA insoluble extract

Samples for the determination of protein of root were prepared according to the method of West (1962), by grinding 25 g of fresh root material with 100 ml of cold demineralized water in a Waring blendor for four minutes at 0^{o}-4^{o}C. The homogenate was centrifuged at 3800 x g for five minutes to remove cell walls, and debris. Aliquots of the supernatant which had been cleared by centrifugation were added to equal volumes of 10% TCA to precipitate the TCA insoluble protein. The precipitate was sedimented at 500 x g for five minutes and dissolved in 0.1 N NaOH. The volume was made to 20 ml.

Determination of protein

Protein was determined by the method of Lowry et al. (1951). To 0.4 ml of the sample was added 2 ml of alkaline copper solution. (This solution was composed of 50 ml of 2% Na_2CO_3 in 0.1 N NaOH, and 1 ml of 0.5% $CuSO_4$ in 1% sodium potassium tartarate). The contents were mixed. After 10 minutes 0.2 ml of 1 N Folin - Ciocalteau phenol reagent was added, the contents were mixed, and

the optical density was read at 500 mu. Protein content was obtained by comparison with a standard curve prepared by the use of crystalline bovine albumin and was expressed as mg per g of fresh weight.

5. Determination of the rate of respiration of root

The rate of respiration of root was determined by the method of Wort and Shrimpton (1959). After the roots had been cleaned and trimmed, a trans-section 3.75 cm in height was cut from each root just below the region of greatest diameter. One-mm thick trans-slices were cut from these cylinders by a Spencer hand microtome. Discs one cm in diameter were cut by a steel cork borer from the trans-slices, avoiding the periphery and the core of the beet. The discs were rinsed with distilled water for 30 minutes.

Twenty discs were blotted dry, quickly weighed and placed in a Warburg vessel containing a total of 4.0 ml of a solution whose composition was 0.4 M sucrose, 0.05 M phosphate buffer, pH 6.8, and 0.04 M KCl.

After 15 minutes equilibration, the oxygen consumption at 25°C was determined at 20 minute intervals for periods up to two hours. The rate of shaking was 120 strokes per minute. Respiration rates were expressed as microliters of oxygen consumed per hour per gram fresh weight of the discs.

6. Determination of photosynthesis and respiration of whole plants

The rate of CO_2 uptake was measured in an open system with Beckman infrared analyzer IR 215 and a Heath Built Servo -

Recorder, model EUW-20A. The analyzer was connected to a plant chamber by tygon tubing. The plant chamber was a 20-lb capacity polythene bag of 3 mil thickness, the open end of which was sealed by making pleats and tied around a three-holed rubber stopper with a string. The soil surface of the potted plant was covered with silicone rubber for the duration of the measurement.

The air containing about 300 ppm of CO_2 was passed into the chamber from a tank through a tygon tubing with a Matheson Co. flow meter of tube size R-2-15-B, with a regulated rate of 1 liter per minute. Temperature in the chamber with the plant remained from 28-31°C during the measurements, and was monitored by a telethermometer from the Yellow Spring Instrument Co. The light intensity inside the chamber was 1600 foot-candles. All the measurements were made in the controlled environment room where the plants were grown.

At the start of each measurement, CO_2 gas in the analyzer sample chamber was flushed out by nitrogen gas and the instrument was brought to zero. The CO_2 concentration in the tank was determined before connecting the tank to the chamber. In the illuminated system, a drop in the CO_2 concentration compared with the tank, was considered as due to the CO_2 fixation (apparent photosynthesis). In the dark system the increase in CO_2 concentration was considered as due to the CO_2 liberation by the plant (dark respiration).

The product of flow rate by the difference in the CO_2 concentration of the air before and after passing through the chanber gave the rate of CO_2 exchange (fixation in an illuminated system and liberation in a dark system) of the enclosed

plant, which was expressed as microliters per hour per dm^2 of the leaf area.

7. Determination of the activities of nitrate reductase and transaminase

Preparation of the crude extracts

Crude extract was prepared by grinding one weight of finely chopped leaf blades or root material with 4 weights of cold 0.1 M phosphate buffer, pH 7.8, containing 10^{-3} M reduced glutathione, in a Waring blendor (at full speed) for 1 to 2 minutes, at 0-4°C. The homogenate was strained through 4 layers of cheesecloth. For transaminase, the homogenate was diluted 5 times by the addition of cold distilled water and was used for assay. For nitrate reductase, it was centrifuged in a Servall centrifuge at 20,000 x g for 20 minutes at 0-4°C and the resulting green, cell free supernatant solution was used for the assay of the activity.

Assay of nitrate reductase (NRase) activity

The activity of NRase was measured by a modification of the method of Evans and Nason (1953). A mixture containing 0.2 ml of crude enzyme, 0.1 ml 0.1 M KNO_3, 0.05 ml 2 x 10^{-5} M FAD, 0.05 ml 2 x 10^{-3} M DPNH, and 0.1 M phosphate buffer pH 7.0 in a total volume of 0.5 ml was incubated at 30°C for 30 minutes. The reaction was stopped by the addition of 1 ml of H_2O and 1 ml of 1% (w/v) sulfanilamide reagent. One ml of 0.22% (w/v) N-(1-naphthyl)-ethylene diamine hydrochloride reagent was added and the contents mixed by inverting the tubes. The color was allowed to develop 15 minutes and the optical density was determined with a

Beckman Model B spectrophotometer against a blank solution (complete but containing boiled enzyme) at 540 mu. Nitrite content was obtained from a standard curve. The specific activity was defined as mμmoles of nitrite formed per mg protein per 30 minutes. Protein in the enzyme preparation was determined by the method of Lowry et al. (1951)

Assay of alanine-glutamate transaminase activity

Alanine-glutamate transaminase activity was assayed by an adaptation of the method of Reitman and Frankel (1957). One ml of α-ketoglutarate - alanine substrate (100 μM each) was pipetted into a test tube and placed in a water bath at 37°C for 10 minutes. Upon the addition of 0.2 ml of the crude extract, the contents were mixed and incubated for 30 minutes at 37°C.

Immediately after removing the tubes from the water bath, 1.0 ml of 2,4-dinitrophenylhydrazine reagent (made by dissolving 19.8 mg of 2,4-dinitrophenylhydrazine in 100 ml of 1 N hydrochloric acid), was added. This reagent stops further transaminase activity. After the tubes were allowed to stand at room temperature for 20 minutes, 10 ml of 0.4 N sodium hydroxide was added. A clean rubber stopper was inserted to each tube and the contents were mixed by inversion. At the end of exactly 30 minutes, the color intensity of the solution was measured by a Klett-Summerson colorimeter equipped with a green filter.

While the samples were incubating, a control for each homogenate, was prepared. One ml of the substrate, 0.2 ml of the homogenate, and 1 ml of 2,4-dinitrophenylhydrazine reagent were mixed in a test tube. After 20 minutes 10 ml of 0.4 N sodium

hydroxide was added and after a further 30 minutes the color intensity was measured as above.

The difference in transmittance between the incubated tubes and the appropriate control was determined, and the concentration of pyruvate formed was calculated from a standard curve. Specific activity was expressed as µm pyruvic acid per mg of protein per 30 minutes under the conditions of the assay.

8. Determination of invertase activity

Preparation of crude extract

The crude extract for the determination of invertase activity was prepared by a modification of the method of Pressy (1966), by grinding 100 g finely chopped root or leaf with 100 ml of cold distilled water at 0-4°C in a Waring blendor for 1 to 2 minutes. The slurry was squeezed through 4 layers of cheesecloth and the homogenate obtained was clarified by centrifugation at 10,000 x g for 20 minutes at 0-4°C in a Servall centrifuge. Two milliliters of sodium sulfite, 0.01 M, was added to prevent darkening of the supernatant solution. The solution was then dialyzed against 20 volumes of 0.01 M NaCl at 0-4°C with 5 changes, for 24 hours. The small amount of precipitate formed during dialysis was removed by centrifugation.

Invertase assay

The incubation mixture for invertase assay contained 400 µmoles sodium acetate buffer, pH 4.7, 730 µmoles sucrose, and a suitable aliquot of invertase preparation, in a total volume of

5 ml.

The sample and a heated enzyme control, were incubated at 37°C for one hour. The reaction was terminated by addition of 5 ml of 0.5 M dibasic sodium phosphate and heating in a boiling bath for 5 minutes. One ml of the solution was then analyzed for reducing sugars by heating with 1 ml of copper reagent in a boiling water bath for 20 minutes. The solution was cooled and 1 ml of arsenomolybdate reagent was added. The sample was diluted and the optical density was measured at 520 mu.

Enzyme activity was expressed as mμmoles of reducing sugars formed per hour per mg of protein under the conditions of assay.

9. Determination of activities of phosphatases

Preparation of the extract

Determinations of the activities of phosphatases were made by an adaptation of the method of Hinde and Finch (1966). The leaf or root material was cut into small pieces and ground in a small volume of medium A (sucrose, 0.35 M; $KHCO_3$, 0.035 M; KCl, 0.025 M; $MgCl_2$, 0.004 M) for 1-2 minutes at 0-4°C. The homogenate was squeezed through 4 layers of cheesecloth and then centrifuged in a Servall centrifuge at 15,000 x g for 15 minutes at 0°C. The supernatant was again centrifuged at 105,000 x g for 60 minutes in a Model L Preparative Spinco ultra centrifuge. The supernatant of this second centrifugation was the extract used for enzyme assay.

Assay of the activity of the enzyme

The phenylphosphatase and adenosine triphosphatase (ATP-ase)

activities were assayed by incubation of 0.6 ml of enzyme preparation made up to 1.0 ml with a solution which was 100 mM with respect to sodium acetate - acetic acid buffer, pH 5.1, and 3 mM with respect to phenylphosphate or ATP. The time was 15 minutes and the temperature 27°C.

Glucose-1-, glucose-6-, and fructose-6-phosphatase activities were determined by the incubation of 0.6 ml of enzyme preparation with a solution containing glucose-1-, glucose-6-, or fructose-6-phosphate, 3 mM, and 100 mM tris (hydroxymethylamino) methane- HCl (tris-HCl) buffer at pH 8.2 in a total volume of 1.0 ml, at 27°C for 15 minutes.

The amount of phosphate originating from the enzyme preparation was determined by the use of the appropriate amount of buffer, water and 0.6 ml of enzyme preparation in a total volume a 1 ml, incubated at 27°C for 15 minutes. The reaction was stopped by the addition of 3 ml of cold 10% trichloracetic acid to the incubation mixture.

Inorganic phosphate was determined by the method of Fiske and SubbaRow (1925). Prior to analysis, the TCA precipitated protein from the incubation mixture was removed by filtration. One ml of the filterate was diluted to 8 ml with water. To this was added 1.0 ml of 2.5% ammonium molybdate in 5 N sulfuric acid and 0.4 ml of 0.25% amino-naphthol sulfonic acid reagent. (prepared by dissolving 0.5 g of aminonaphthol sulfonic acid, 28.5 g $NaHCO_3$ and 30 ml of 10% sodium sulfite in 90 ml of distilled water and making the volume 200 ml). Water was added to make the final volume 10 ml. Color was allowed to develop for 5 minutes and read in a colorimeter (equipped with a red filter)

which had been adjusted to zero optical density on the blank solution prepared by diluting 5 ml of TCA to 8 ml and adding to it 1.0 ml of ammonium molybdate reagent and 0.4 ml of amino-naphthol sulfonic reagent in a total volume of 10 ml. The concentration of inorganic phosphate was determined from a standard curve prepared from KH_2PO_4. Enzyme activity was expressed as mμm Pi formed per mg of protein per 15 minutes

10. Determination of the activities of sucrose synthetase, sucrose phosphate synthetase and UDPG-pyrophosphorylase

Preparation of the extract

Crude extracts were prepared by the method of Rorem et al. (1960). One hundred grams of fresh and thoroughly washed root or leaf materials was finely chopped and blended in a Waring blendor with 100 ml of 0.05 M phosphate buffer, pH 7.2, for 1-2 minutes, at 0-4°C. The homogenate was then squeezed through 4 layers of cheesecloth. The homogenate obtained was centrifuged at 13,000 x g for 15 minutes. The supernatant was gradually taken to pH 4.9 with acetic acid and immediately centrifuged at 13,000 x g for 15 minutes. The precipitate was discarded and the clear yellowish supernatant was left overnight at 4°C. The flocculent precipitate which then formed was collected by centrifugation and washed several times with 0.02 M, pH 4.9 acetate buffer. The recentrifuged precipitate was then dissolved in sufficient 0.05 M phosphate buffer, pH 7.2, to make a thick slurry and this final fraction was then dialyzed against 0.02 M, pH 7.2 phosphate buffer. This enzyme fraction was used to determine the activity of sucrose synthetase, sucrose phosphate

synthetase, and UDPG-pyrophosphorylase.

Assay of activities of sucrose synthetase and sucrose phosphate synthetase

The complete reaction mixture for sucrose synthetase contained 1.5 µM UDPG, 4 µM fructose, 0.1 ml enzyme preparation, 0.002 ml of 0.1 M $MgCl_2$ and 0.01 ml of 1 M tris-HCl buffer in a total volume of 0.2 ml. The reaction mixture used for the determination of sucrose phosphate synthetase activity was identical to that used for sucrose synthetase except that 4 µM fructose-6-phosphate was substituted for the fructose, and in addition 0.01 ml of 1 M KF was present as a phosphatase inhibitor.

After 2 hours incubation at 37°C, the tubes containing the enzyme and substrate were placed in a boiling water bath for 5 minutes and then cooled. In order to eliminate interference by reducing sugar, 0.8 ml of a solution of 0.025 N NaOH containing 15 mg sodium borohydrate was added to each tube. This was followed by a few drops of ethanol as a foam retardant. These tubes were left for 1 hour at room temperature and were then covered with glass stoppers and placed in a boiling water bath for 5 minutes to complete the reduction of the hexoses to their corresponding sugar alchols.

The mixtures were cooled and acidified with a few drops of acetic acid. The sucrose was determined by the method of Roe (1934). To each tube, 2 ml of resorcinol solution (0.1%, w/v in absolute ethanol) and 6 ml of 30% HCl were added. The tubes were heated for 30 minutes in a water bath adjusted to 80°C.

After cooling, the colors were measured at 490 mu and sucrose content was calculated from a standard curve. The enzyme activity was expressed as mμmoles of sucrose or sucrose phosphate formed per mg of protein per 2 hours under the conditions of assay.

Assay of the activity of UDPG-pyrophosphorylase

The enzyme assay was carried out by a modification of the method of Gander (1966) as follows: 5 μmoles of UDPG, 1 μmole of inorganic pyrophosphate, 100 μmoles of tris-HCl buffer, pH 7.5, and 1 ml of enzyme preparation in a total volume of 2 ml were allowed to react for 15 minutes at room temperature. The reaction was stopped by heating the tubes for 5 minutes in boiling water, followed by rapid cooling in cold running water.

One ml of the digest was analyzed for glucose-1-phosphate in a 3-ml quartz cuvette by addition of 0.1 μmole of 2,6-dichlorophenol indophenol, 0.3 μmole of phenazine methosulfate, 0.5 μmole of NADP, 100 μmole of tris-HCl buffer, pH 7.5, one international unit of phosphoglucomutase and glucose-6-phosphate dehydrogenase in a total volume of 3 ml. The absorbance at 600 mu was compared with that of a blank prepared from the inactivated enzyme plus the other reagents. The concentration of glucose-1-phosphate was calculated from a standard curve. The enzyme activity was expressed as mμmoles of glucose-1-phosphate formed per mg of protein per 15 minutes.

V EXPERIMENTAL RESULTS

In the text, reference to an increase or decrease is to be taken to mean an increase or decrease compared with the appropriate value found in untreated plants, unless specificially stated otherwise.

1. Growth of the leaves

The effects of the various inhibitors on growth of one-month-old plants 7 days after the treatment are given in Table I. More than 30% inhibition was achieved by maleic hydrazide (MH), mercuric chloride, amino triazole, iodoacetate, vanadium sulfate (VS), pyrocatechol (PC) and copper sulfate.

The effect of 8×10^{-3} M MH, 10^{-2} M PC and 10^{-2} M VS on the growth of 4.5-month-old sugar beet plants is given in Table II and Fig. 2. Under both summer and fall conditions MH, PC, and VS caused significant inhibition of the leaf growth. The analysis of variance and comparison of the means by Student-Newman-Keuls' test (Steel and Torrie, 1960) revealed that all the three treatments resulted in a highly significant decrease in the leaf area.

Under summer conditions the maximum inhibition of the leaf area by MH, 65%, was evident on the 7th day after treatment. PC and VS caused maximum inhibition of the leaf area on the 14th day after treatment. The inhibitions were 72% and 60% respectively.

Under fall conditions, the maximum inhibitions by MH, PC, and VS were 49%, 48%, and 46% respectively. Evidently the treatments were more effective when applied under summer growth

TABLE I

Effect of inhibitors on the growth of leaves of one-month-old sugar beet plants

Compound	Type of action	Concentration used	Reduction in leaf expansion, 7 days after treatment
A. Nitrate reductase (NRase) inhibitors			
1. Aminotriazole	(a) NRase inhibitor (b) Inhibitor of chlorophyll synthesis	1×10^{-2}M	45.0%
2. Copper sulfate	NRase inhibitor	1×10^{-2}M	30.9%
3. Iodoacetate	Inhibitor of NRase and dehydrogenases	1×10^{-4}M	44.6%
4. Mercuric chloride	Inhibitor of NRase and invertase	1×10^{-4}M	51.9%
5. Pyrocatechol (PC)	NRase inhibitor	1×10^{-2}M	33.0%
6. Vanadium	NRase inhibitor		
(a) Ammonium vanadate	"	1×10^{-2}M	12.5%
(b) Vanadium sulfate (VS)	"	1×10^{-2}M	36.1%
7. PC + Copper sulfate	"	1×10^{-2}M	27.9%
8. PC + VS	"	1×10^{-2}M	31.2%
9. VS + Copper sulfate	"	1×10^{-2}M	37.1%
B. Other Inhibitors			
1. Benzimidazole	A synthetic analogue of adenine. Inhibits nucleic acid synthesis and growth	1×10^{-4}M	29.0%
2. Caffeic acid	A phenolic acid, action not known	5×10^{-3}M	24.0%
3. Chloroamphenicol	Binds with 50S ribosomal particle, interferes in protein synthesis	1×10^{-2}M	21.6%
4. Cycocel	"Growth retardant"	2×10^{-2}M	24.0%
5. Maleic hydrazide	Growth inhibitor	8×10^{-3}M	61.0%
6. p-phenylenediamine	Transaminase inhibitor	1×10^{-2}M	20.7%

TABLE II

Effect of maleic hydrazide (MH), pyrocatechol (PC), and vanadium sulfate (VS) on the growth of the leaves of 4 1/2-month-old sugar beet plants

Growth conditions	Days after treatment	Percentage Increase in leaf area				*Q		T/C (%)		
		**CK	MH	PC	VS	.05	.01	MH	PC	VS
Summer conditions	7	46.32	15.79	19.62	29.40	1.08	1.22	34.09	42.40	63.63
"	14	64.15	23.03	17.72	25.69			35.79	27.62	40.05
"	21	88.70	39.70	28.30	49.39			44.75	31.90	55.69
Fall conditions	7	62.35	37.93	32.33	33.54	1.63	1.92	60.83	51.85	53.79
"	14	65.50	41.46	36.17	40.18			63.29	55.22	61.34
"	21	73.85	45.45	45.66	47.73			61.54	61.82	64.63

*Q - Significant difference according to the Student - Newman-Keuls test.
**CK - Control

FIG. 2. EFFECT OF MALEIC HYDRAZIDE (MH), PYROCATECHOL (PC) AND VANADIUM SULFATE (VS) ON LEAF GROWTH OF SUGAR BEET

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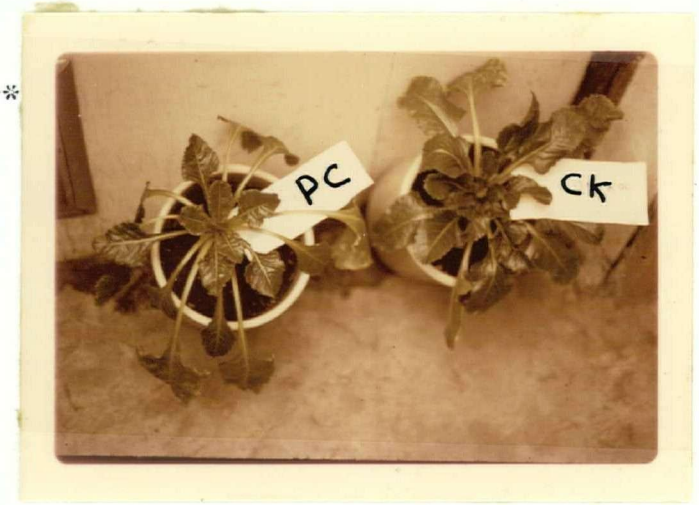

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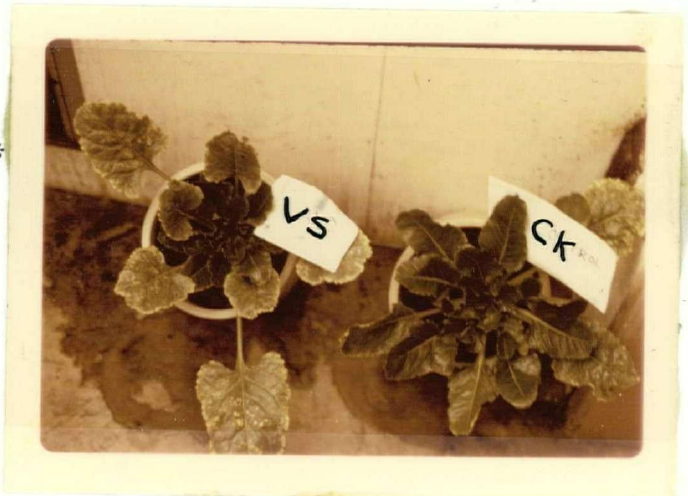


*8 days after treatment of one-month-old plants
**21 days after treatment of 4.5-month-old plants

conditions.

The inhibitory effects of each three compounds were found to decline by the 14th day after treatment under fall conditions (Fig. 4). Under summer conditions, the effect of MH on growth of the leaves followed the same pattern as under fall conditions, while in case of PC and VS, the maximum inhibitions were on the 14th day. By the 21st day the effects were found to decline (Fig. 3).

2. Sucrose content of the roots

The percentage of sucrose in the roots of treated beets were significantly higher than in the roots of untreated beets. Of significance, too, was the interaction between treatments and the time of harvest. The data are given in Table III and Figs. 5, 6, and 7.

The maximum sucrose percentage, 28%, more than control, under summer conditions was recorded 7 days after treatment when VS was used. Under fall conditions also, the maximum increase, 27%, in sucrose percentage was due to VS treatment. This was measured on the 14th day after treatment.

The maximum in sucrose percentage under summer condition, induced by MH and PC was 22% and 10% respectively. The maximum increase induced by MH under fall conditions was 11.5% on the 21st day and by PC 16% on the 14th day after treatment.

3. Reducing sugars content of the roots

A significant decrease in percentage reducing sugars was found due to each treatment under both, summer and fall conditions

FIG. 3. EFFECT OF MALEIC HYDRAZIDE (MH), PYROCATECHOL (PC) AND VANADIUM SULFATE (VS) ON LEAF GROWTH OF SUGAR BEET UNDER SUMMER GROWTH CONDITIONS

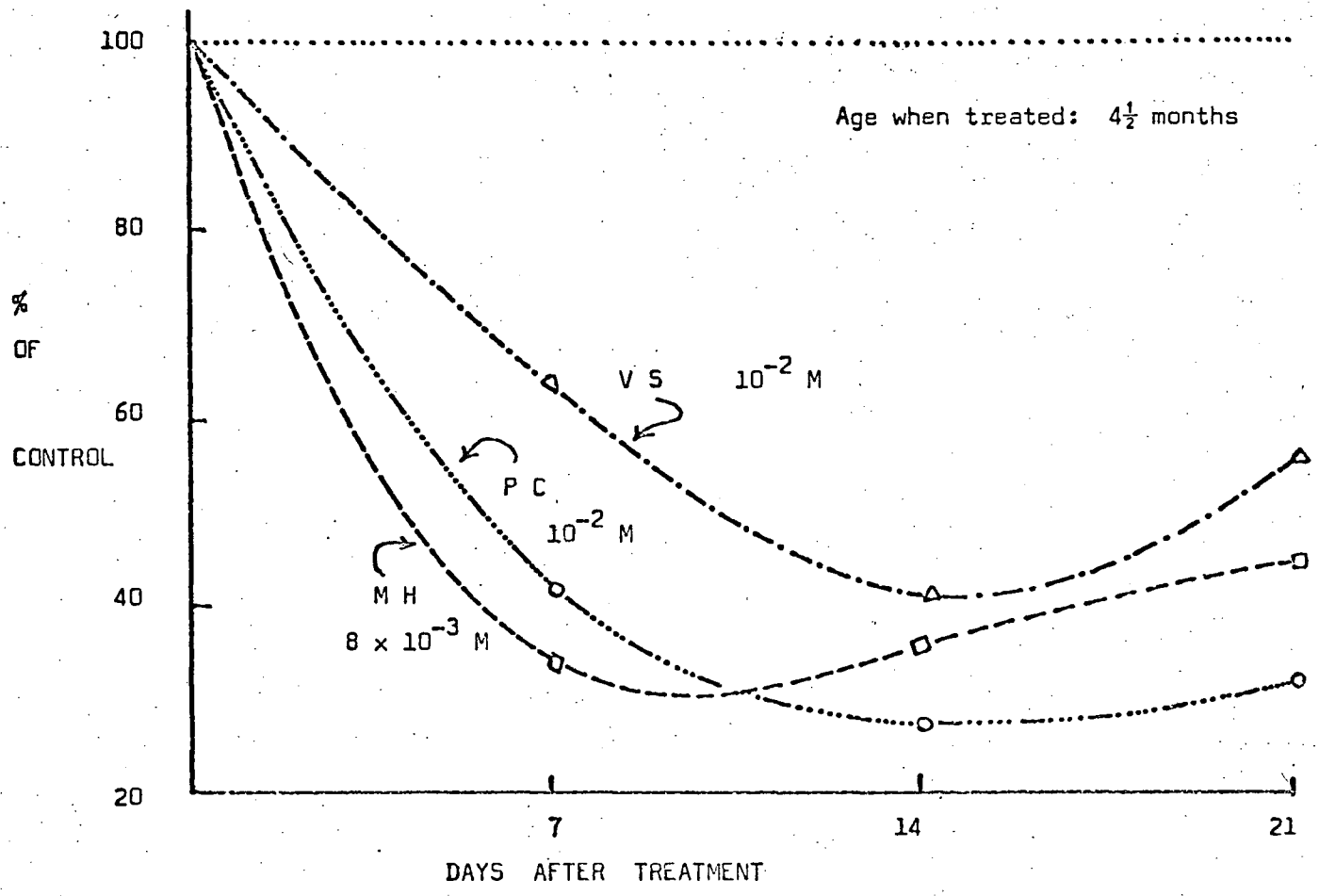


FIG. 4. EFFECT OF MALEIC HYDRAZIDE, PYROCATECHOL, AND VANADIUM SULFATE ON LEAF GROWTH OF SUGAR BEET UNDER FALL GROWTH CONDITIONS

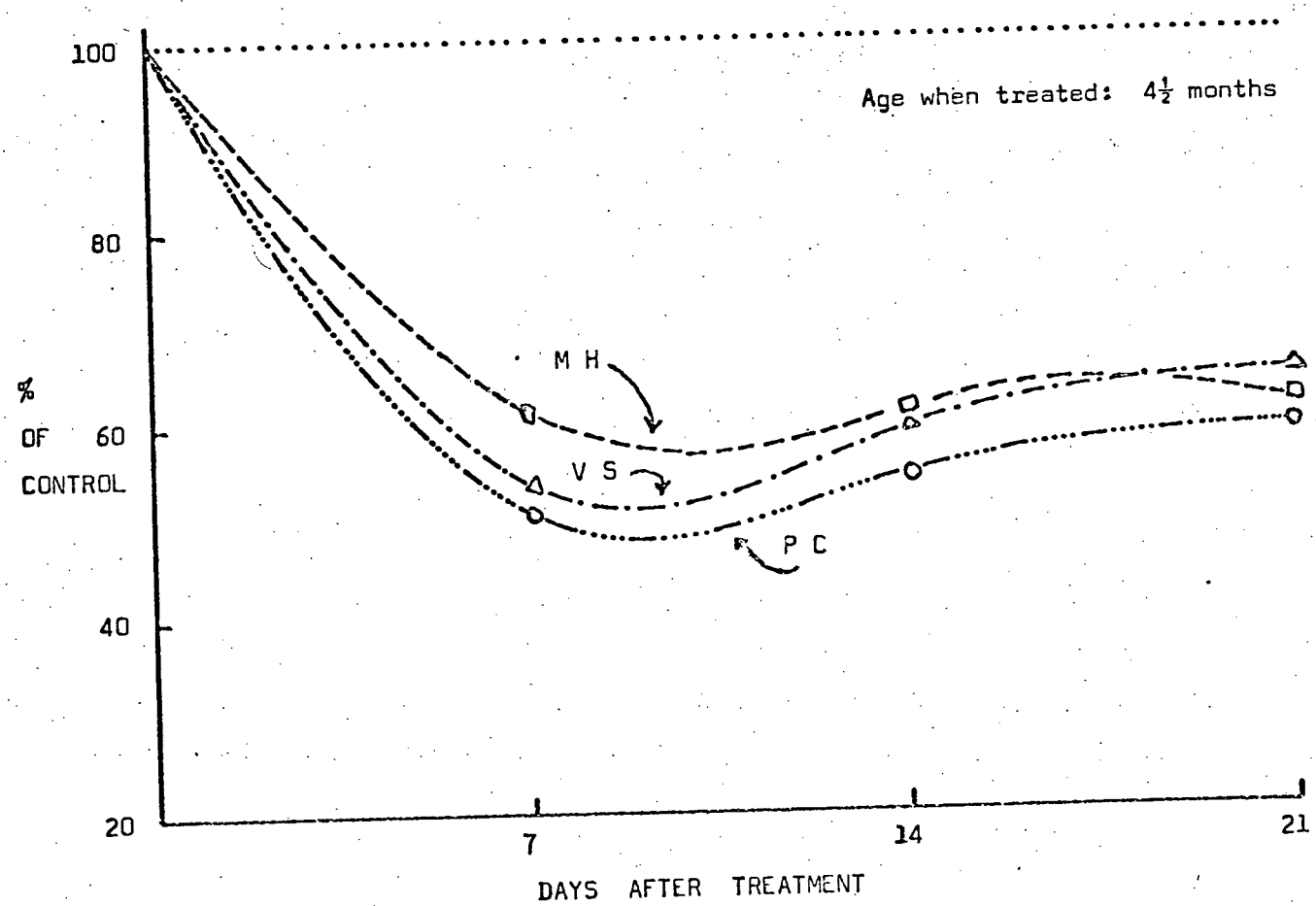


TABLE III

Effect of maleic hydrazide (MH), pyrocatechol (PC), and vanadium sulfate (VS) on the sucrose content of the roots of 4.5-month-old sugar beet plants

Growth condition	Days after treatment	Sucrose content as percent of the fresh weight				*Q		T/C (%)		
		**CK	MH	PC	VS	.05	.01	MH	PC	VS
Summer	0	14.3	...	...	...	0.15	0.17			
"	7	14.7	17.2	16.2	18.8			117.46	110.90	128.40
"	14	17.3	18.1	18.4	19.8			104.43	105.97	114.35
"	21	13.9	17.0	15.1	17.3			122.14	108.50	123.87
Fall	0	14.6	...	...	...	0.55	0.62			
"	7	14.3	15.9	16.2	17.9			110.75	112.88	124.72
"	14	13.6	14.5	15.9	17.3			106.57	116.80	127.29
"	21	13.1	14.6	14.2	15.8			111.51	108.48	120.67

*Q - Significant difference according to the Student-Newman-Keuls test.
**CK - Control

FIG. 5. EFFECT OF MALEIC HYDRAZIDE ON SUCROSE AND REDUCING SUGAR CONTENT OF ROOT OF SUGAR BEET GROWN UNDER SUMMER AND FALL CONDITIONS

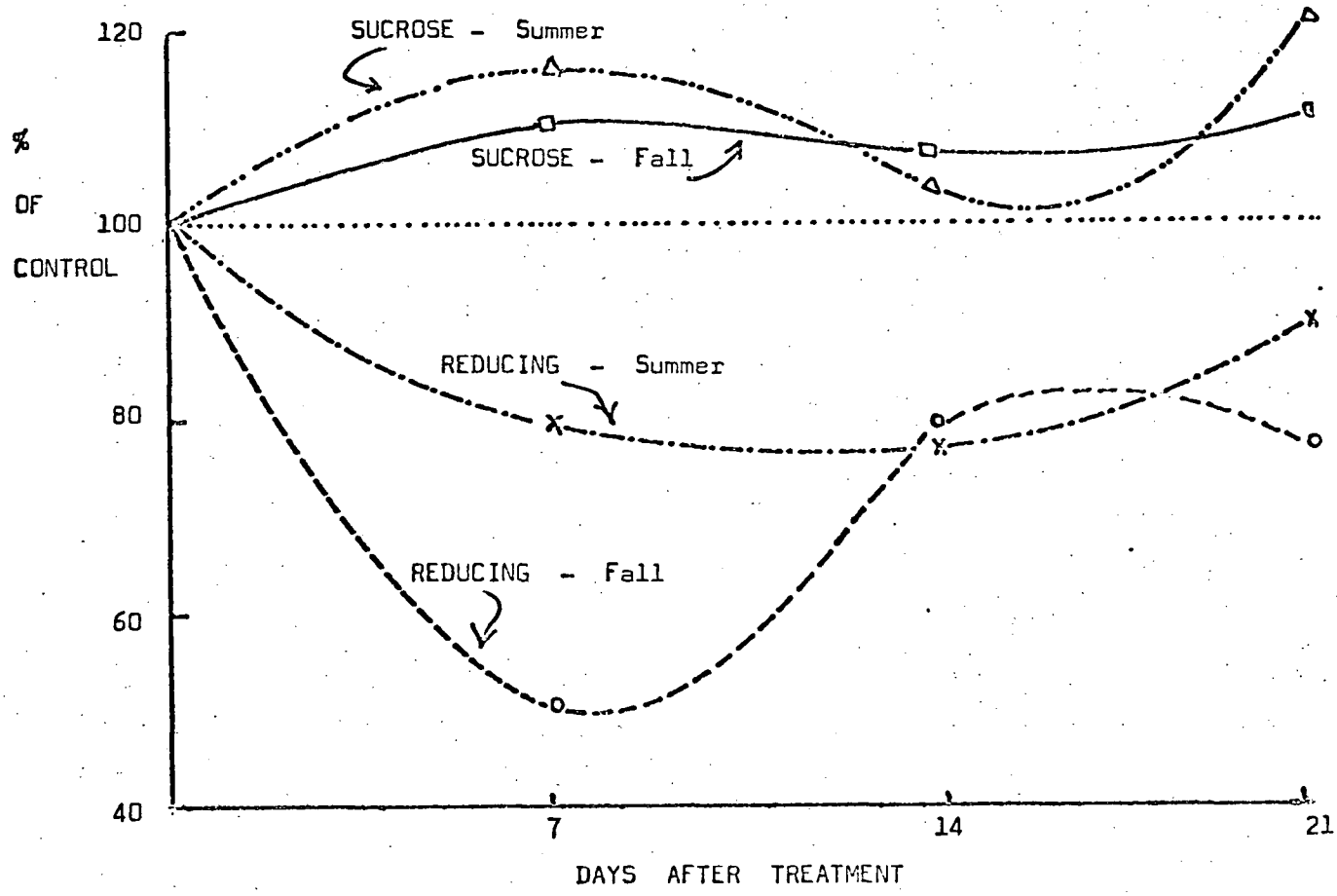


FIG. 6. EFFECT OF PYROCATECHOL ON SUCROSE AND REDUCING SUGAR CONTENT OF ROOT OF SUGAR BEET GROWN UNDER SUMMER AND FALL CONDITIONS

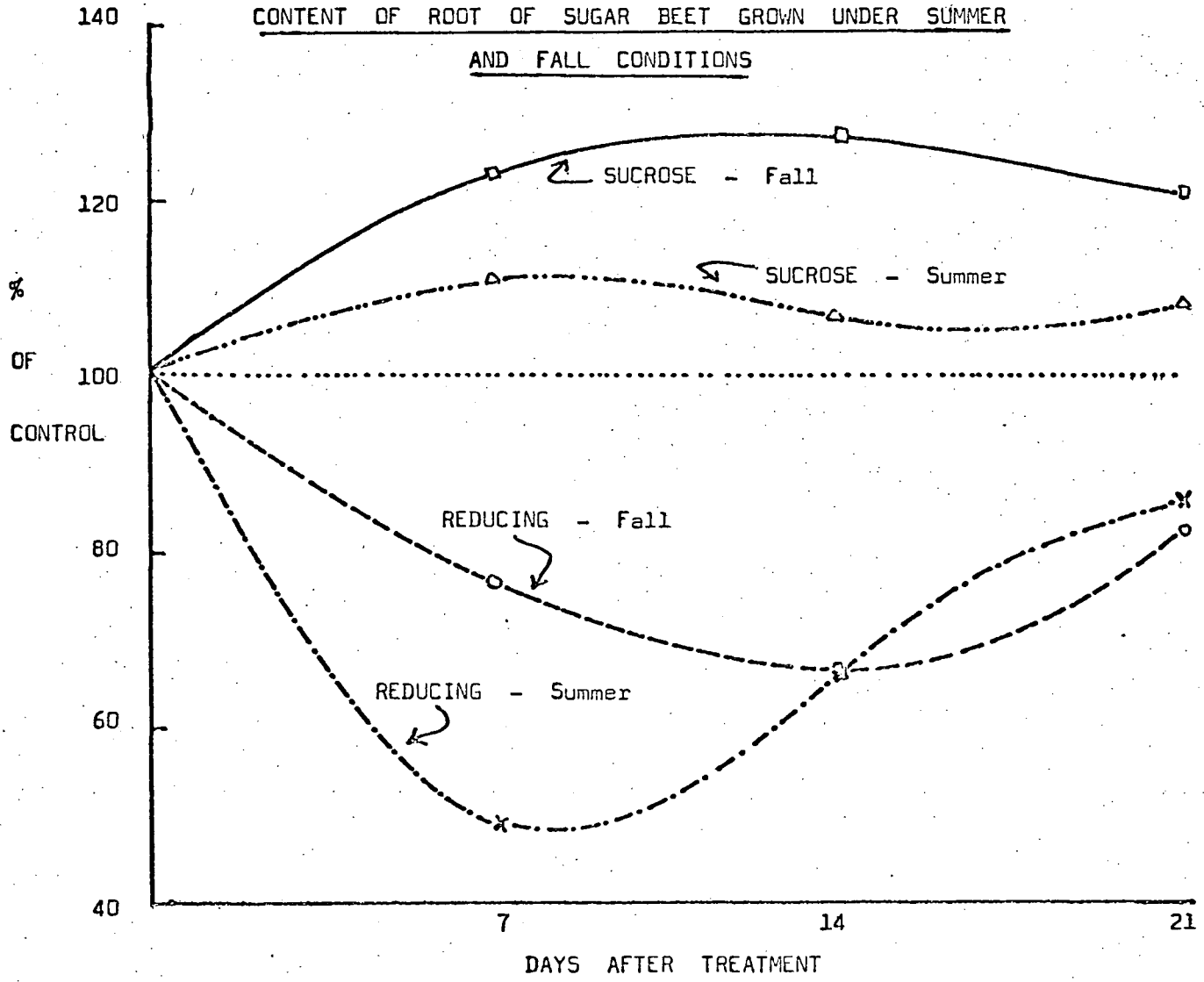
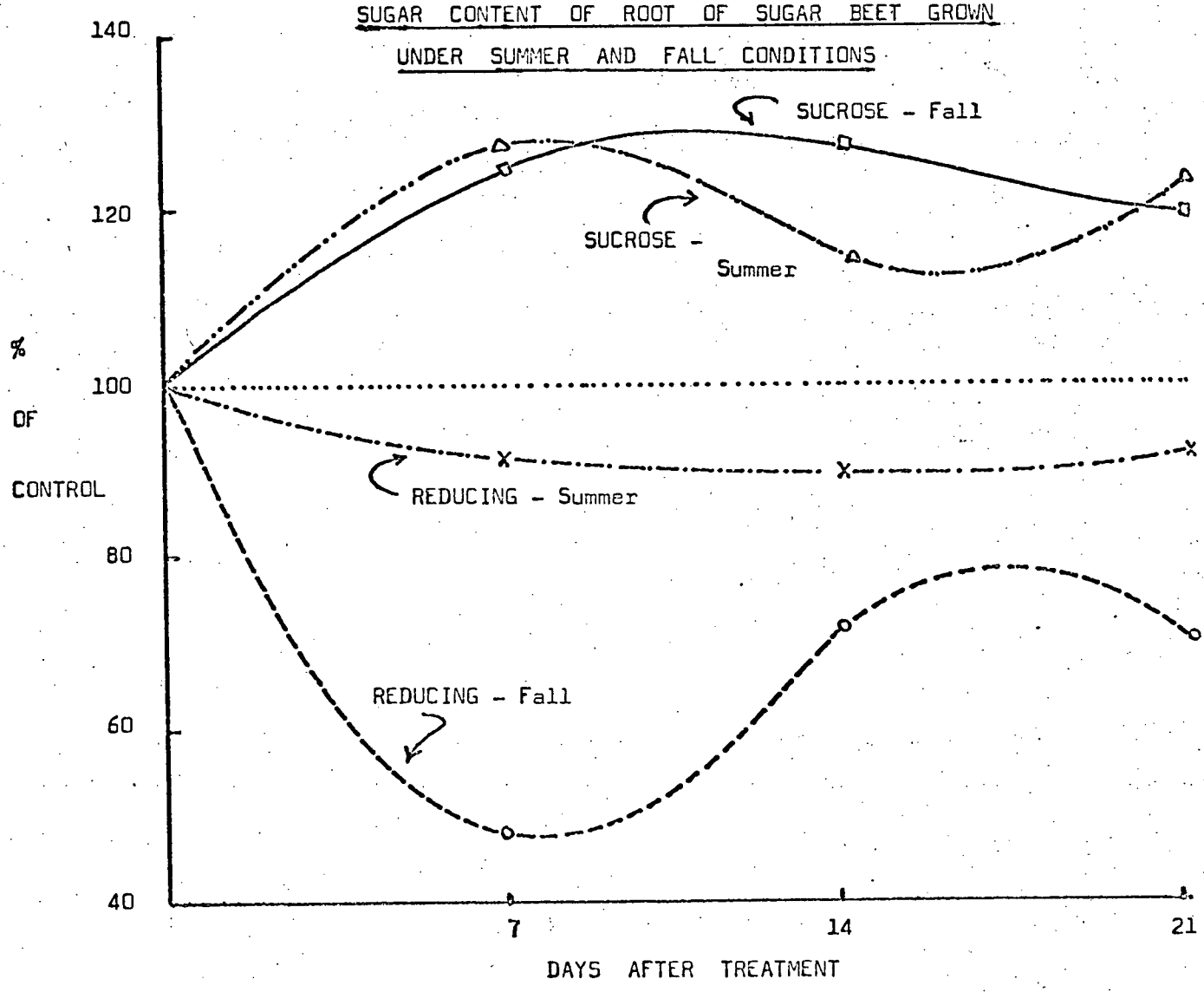


FIG. 7. EFFECT OF VANADIUM SULFATE ON SUCROSE AND REDUCING SUGAR CONTENT OF ROOT OF SUGAR BEET GROWN UNDER SUMMER AND FALL CONDITIONS



(Table IV and Figs 5, 6, 7). Under summer conditions, the maximum reductions in reducing sugars were on the 14th day after treatment. These reductions were 22%, 34%, and 10% for MH, and VS respectively.

The relative effects of MH, PC, and VS on leaf growth, reducing sugars and sucrose content of the roots have been summarized for summer and fall conditions in Figs. 8 and 9 respectively.

4. Nitrogenous constituents of the roots

Nitrate N - The treated plants had significantly higher (0.01 level) nitrate content in their roots than in the untreated plants. The data are given in Table V. In general, the maximum increase in nitrate was found to be on the 7th day after treatment. The content values on the 7th day after treatment were 89, 121, 100, and 125 µg/gm of the dry weight of the root for control, MH-, PC-, and VS-treated beets respectively.

Nitrite N - Nitrite content of the roots is given in Table V. The analysis of variance showed that treatments caused significant reduction in nitrite content of the roots. Comparison of the means with those of the control plants by Q test indicated that except on the 7th day after treatment in PC-treated beets, all the treatment means were significantly lower (0.01 level). The maximum reduction in the nitrite content of the roots, up to 59%, was caused by MH on the 7th day after treatment.

Ammonium N - In MH-treated plants ammonium N was significantly more than in the control on all dates of harvest. The

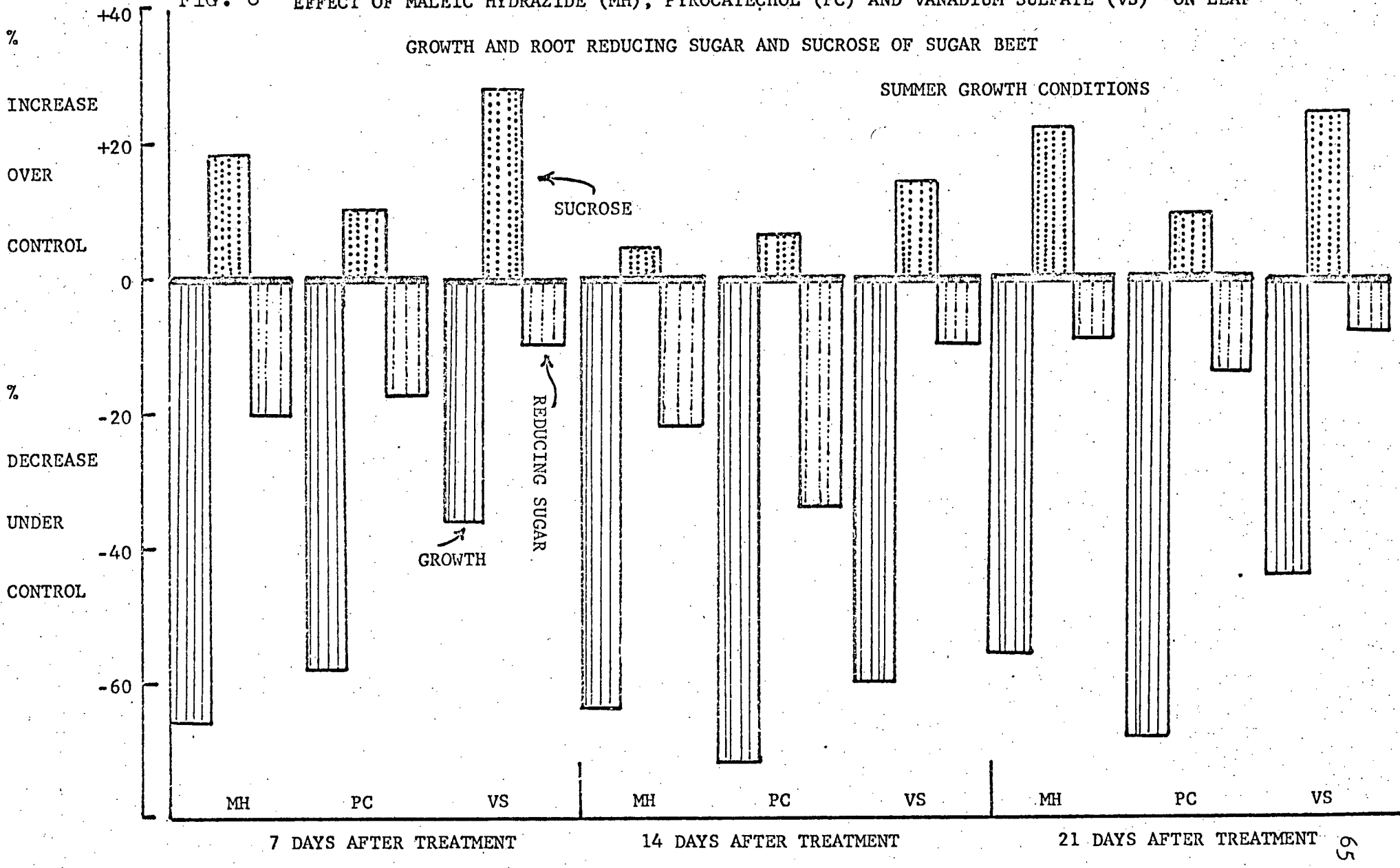
TABLE IV

Effect of maleic hydrazide (MH), pyrocatechol (PC), and vanadium sulfate (VS) on reducing sugar of the roots of 4.5-month-old sugar beet plants

Growth condition	Days after treatment	Reducing sugars as percent of the fresh weight				*Q		T/C (%)		
		**CK	MH	PC	VS	.05	.01	MH	PC	VS
Summer	0	0.17	...	...	...	.0081	.0092			
"	7	0.21	0.17	0.16	0.19			80.09	77.38	91.92
"	14	0.19	0.15	0.13	0.17			78.48	65.82	90.18
"	21	0.24	0.22	0.21	0.22			91.66	86.63	91.66
Fall	0	0.63	...	...	...	.051	.059			
"	7	0.71	0.36	0.34	0.35			50.26	47.63	48.47
"	14	0.77	0.61	0.52	0.56			79.86	67.30	72.89
"	21	0.83	0.65	0.69	0.57			77.69	83.04	68.45

*Q - Significant difference according to the Student-Newman-Keuls test.
**CK - Control

FIG. 8 EFFECT OF MALEIC HYDRAZIDE (MH), PYROCATECHOL (PC) AND VANADIUM SULFATE (VS) ON LEAF GROWTH AND ROOT REDUCING SUGAR AND SUCROSE OF SUGAR BEET



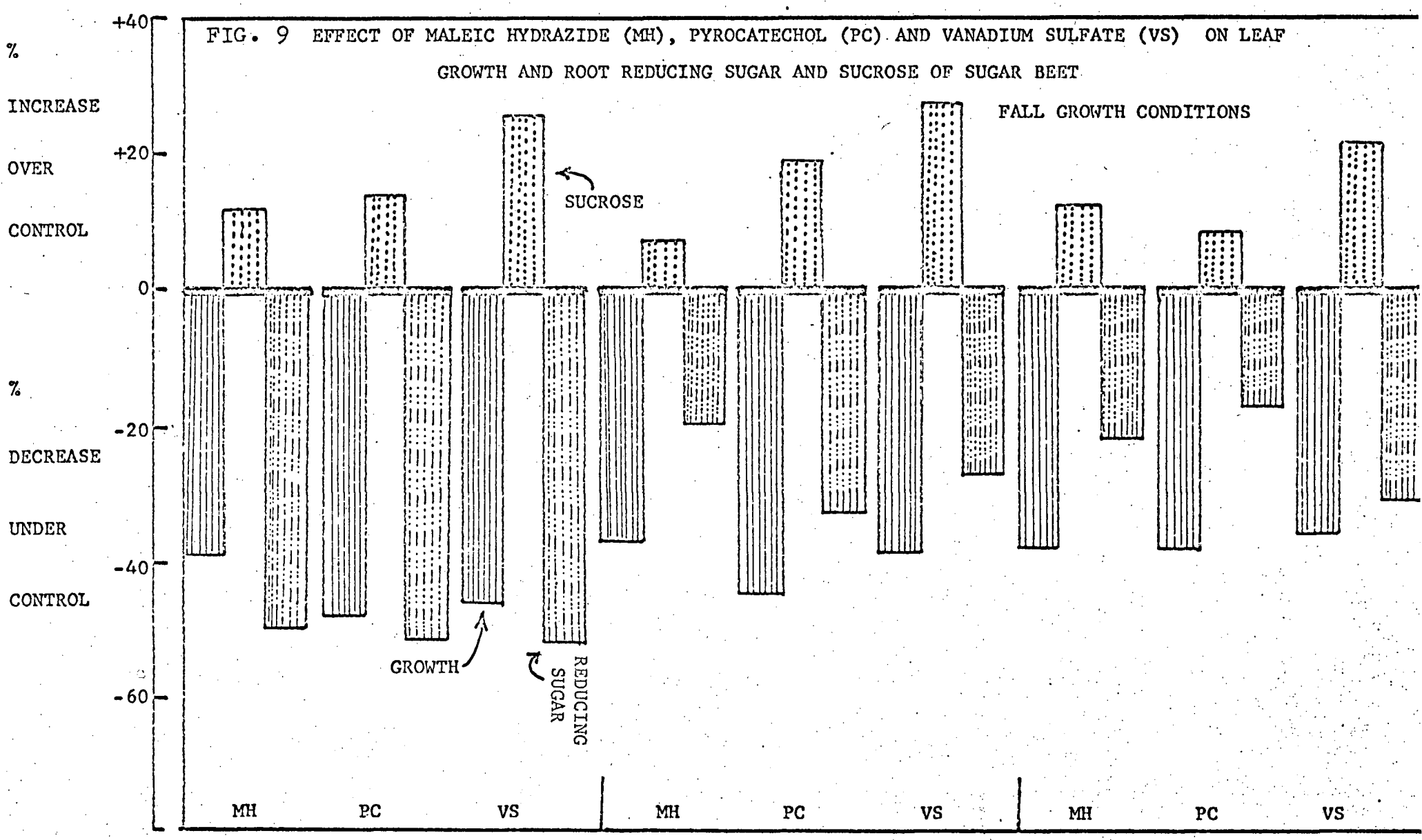


FIG. 9 EFFECT OF MALEIC HYDRAZIDE (MH), PYROCATECHOL (PC) AND VANADIUM SULFATE (VS) ON LEAF GROWTH AND ROOT REDUCING SUGAR AND SUCROSE OF SUGAR BEET
FALL GROWTH CONDITIONS
% INCREASE OVER CONTROL
% DECREASE UNDER CONTROL
+40
+20
0
-20
-40
-60
SUCROSE
GROWTH
REDUCING SUGAR
MH
PC
VS
MH
PC
VS
MH
PC
VS
7 DAYS AFTER TREATMENT
14 DAYS AFTER TREATMENT
21 DAYS AFTER TREATMENT

TABLE V

Effect of maleic hydrazide (MH), pyrocatechol (PC), and vanadium sulfate (VS) on the nitrogenous constituents of the roots of 4.5-month-old sugar beet plants

Days after treatment	Kind of nitrogen	Nitrogen content as µg/N gram of dry weight				*Q		T/C (%)		
		**CK	MH	PC	VS	.05	.01	MH	PC	VS
0	Nitrate	95	...	...	...	7.86	8.92			
7	"	89	121	100	125			135.61	111.93	139.72
14	"	108	126	119	149			116.47	110.09	132.36
21	"	118	148	130	119			126.17	110.86	100.86
0	Nitrite	19	...	...	...	1.31	1.48			
7	"	31	13	30	19			40.77	97.84	62.84
14	"	32	20	19	14			63.49	61.51	44.40
21	"	45	29	25	28			65.73	55.57	62.06
0	Ammonia	143	...	...	...	7.94	9.01			
7	"	163	183	127	145			112.28	77.86	89.16
14	"	125	182	117	112			146.06	94.22	89.89
21	"	98	199	125	105			130.36	127.85	107.15
0	Amino	1850	...	...	...	70.90	80.44			
7	"	2325	2175	2050	2053			93.55	88.17	88.30
14	"	1875	2800	2425	1604			149.33	129.33	85.56
21	"	1725	2700	2100	1492			156.52	121.74	86.47
0	Total N	11427	...	...	...	1458.1	1654.4			
7	"	12127	12000	9395	12750			98.95	76.77	105.10
14	"	14980	12250	12040	12850			81.77	80.37	85.70
21	"	18190	14140	12340	13967			77.73	67.84	76.70

*Q - Significant difference according to the Student-Newman-Keuls test.
**CK - Control

TABLE V

Effect of maleic hydrazide (MH), pyrocatechol (PC), and vanadium sulfate (VS) on the nitrogenous constituents of the roots of 4.5-month-old sugar beet plants

Days after treatment	Kind of nitrogen	Nitrogen content as µg/N gram of dry weight				*Q		T/C (%)		
		**CK	MH	PC	VS	.05	.01	MH	PC	VS
0	Nitrate	95	...	...	...	7.86	8.92			
7	"	89	121	100	125			135.61	111.93	139.72
14	"	108	126	119	149			116.47	110.09	132.36
21	"	118	148	130	119			126.17	110.86	100.86
0	Nitrite	19	...	...	...	1.31	1.48			
7	"	31	13	30	19			40.77	97.84	62.84
14	"	32	20	19	14			63.49	61.51	44.40
21	"	45	29	25	28			65.73	55.57	62.06
0	Ammonia	143	...	...	...	7.94	9.01			
7	"	163	183	127	145			112.28	77.86	89.16
14	"	125	182	117	112			146.06	94.22	89.89
21	"	98	129	125	105			130.36	127.85	107.15
0	Amino	1850	...	...	...	70.90	80.44			
7	"	2325	2175	2050	2053			93.55	88.17	88.30
14	"	1875	2800	2425	1604			149.33	129.33	85.56
21	"	1725	2700	2100	1492			156.52	121.74	86.47
0	Total N	11427	...	...	...	1458.1	1654.4			
7	"	12127	12000	9395	12750			98.95	76.77	105.10
14	"	14980	12250	12040	12850			81.77	80.37	85.70
21	"	18190	14140	12340	13967			77.73	67.84	76.70

*Q - Significant difference according to the Student-Newman-Keuls test.
**CK - Control

ammonium content of the PC-treated plants was significantly lower at the 0.01 level on the 7th day and at the 0.05 level on the 14th day after treatment. On the 21st day after treatment, the ammonium content of PC-treated beets was significantly higher than the control plants at the 0.01 level. In VS-treated beets, the ammonium content was lower by 11 and 10% respectively on the 7th day and the 14th day. The increase of the ammonium content of the VS-treated beets on the 21st day after the treatment was not statistically significant (Table V)

Amino N - Amino N was decreased in the roots of all the treated plants on the 7th day after treatment. These decreases, significant at the 0.01 level, were 6.5, 11.8 and 11.6% for MH-, PC-, and VS-treated beets respectively.

On the 14th and 21st day after treatment, there was a significant rise in the amino acid content of the MH- and PC-treated beets. This rise in amino acid content of the roots was 49 to 56% in MH-treated beets and 21 to 29% in the PC-treated beets. VS-treated beets had always a significantly lower amino acid content in their roots (Table V).

Total N - There was no significant difference between the total N content of the treated and of the untreated plants on the 7th day after treatment. However, the total N content of the treated beets was significantly lower on the 14th and 21st day after treatment. The maximum reduction in the total N content in the roots of the treated beets was on the 21st day after treatment. The values were 18.19, 14.14, 12.34, and 13.96 mg per gram of the dry weight of the roots, for the control, MH-, PC- and VS-treated plants respectively (Table V).

Protein - There was a significant reduction in the protein content of the roots of the treated plants. The maximum reduction in the protein content of the roots was on the 7th day after treatment by PC, the 21st day by MH and the 14th day by VS. In general VS caused the maximum reduction. The protein content was 69.60, 66.84 and 69.15% of the control on the 7th, 14th and 21st day after treatment respectively in the roots of the VS-treated beets (Table VI).

5. Photosynthesis and respiration

Photosynthesis and dark respiration of leaves

The effect of MH, PC and VS on CO_2 fixation, CO_2 liberation (dark respiration), and the true photosynthesis (CO_2 fixation + dark respiration) under summer and fall conditions are given in Table VIII and Figs. 13, 14, 15, 16, and 17.

The rate of net CO_2 fixation, under summer conditions in the MH-treated plants was lower by 8%, on the 7th day after treatment. The difference was not significant statistically. A significant decrease in the rate of net CO_2 fixation, in PC-treated plants was measured on the 7th day after treatment. VS enhanced the rate of net CO_2 fixation by 6.6% on the 7th day, 9.8% on the 14th day and 30.0% on the 21st day after treatment. The difference between the values in control and VS-treated plants was significant at the 0.01 level on all the three dates of observation. MH and PC also stimulated the rate of net CO_2 fixation significantly on the 14th and the 21st day after treatment. The stimulation by MH were 41 and 49% and by PC 34 and 8%

TABLE VI

Effect of maleic hydrazide (MH), pyrocatechol (PC), and vanadium sulfate (VS) on the protein content of the roots of 4 1/2-month-old sugar beet plants

Days after treatment	Protein content (mg/gm of the fresh weight				Q		T/C (%)		
	CK	MH	PC	VS	0.05	0.01	MH	PC	VS
0	9.17	...	...	...	0.880	0.980			
7	9.03	7.33	6.23	6.27			81.45	69.14	69.60
14	8.23	7.12	5.84	5.50			86.64	70.98	66.84
21	8.57	6.65	6.39	5.93			77.60	74.63	69.15

Q - significant difference according to Student-Newman-Keuls' test

The effects of each treatment on the various nitrogenous constituents of the roots have been summarized separately Figs. 10, 11 and 12.

The effects of all the three treatments on the chemical composition of the roots of sugar beet are summarized in Table VII.

TABLE VII

The summary of the effects of maleic hydrazide (MH), pyrocatechol (PC), and vanadium sulfate (VS) under summer conditions on the chemical composition of the roots of 4 1/2-month-old sugar beet plants

Item	* # Effect of treatment with								
	MH			PC			VS		
Sucrose	+	+	+	+	+	+	+	+	+
Reducing sugar	-	-	-	-	-	-	-	-	-
Nitrate N	+	+	+	+	+	+	+	+	+
Nitrite N	-	-	-	n.s.-		-	-	-	-
Ammonium N	+	+	+	-		-	-	-	n.s.
Amino N	-	+	+	-	+	+	-	-	-
Total N	n.s.-		-	n.s.-		-	n.s.-		-
Protein N	-	-	-	-	-	-	-	-	-

The symbols + and - refer to increase and decrease respectively, compared to the control plants. Three symbols in each treatment correspond to harvests made 7,14, and 21 days after treatment.
n.s. - not significant at .05 and .01 level.
* Significant only at .05 level.

FIG. 10. EFFECT OF MALEIC HYDRAZIDE ON THE NITROGEN CONTENT OF ROOT OF SUGAR BEET

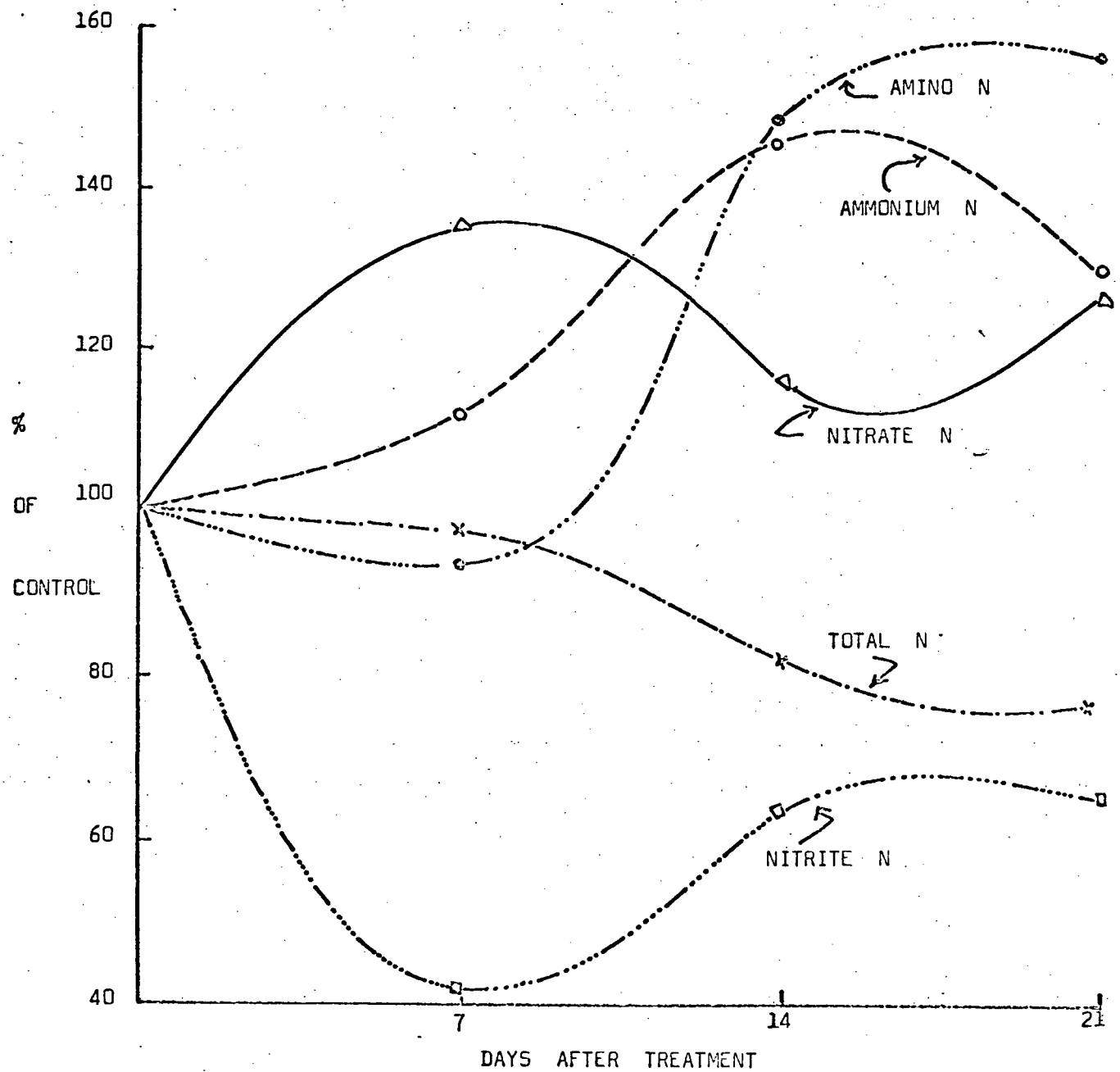


FIG. 11. EFFECT OF PYROCATECHOL ON THE NITROGEN CONTENT OF ROOT OF SUGAR BEET

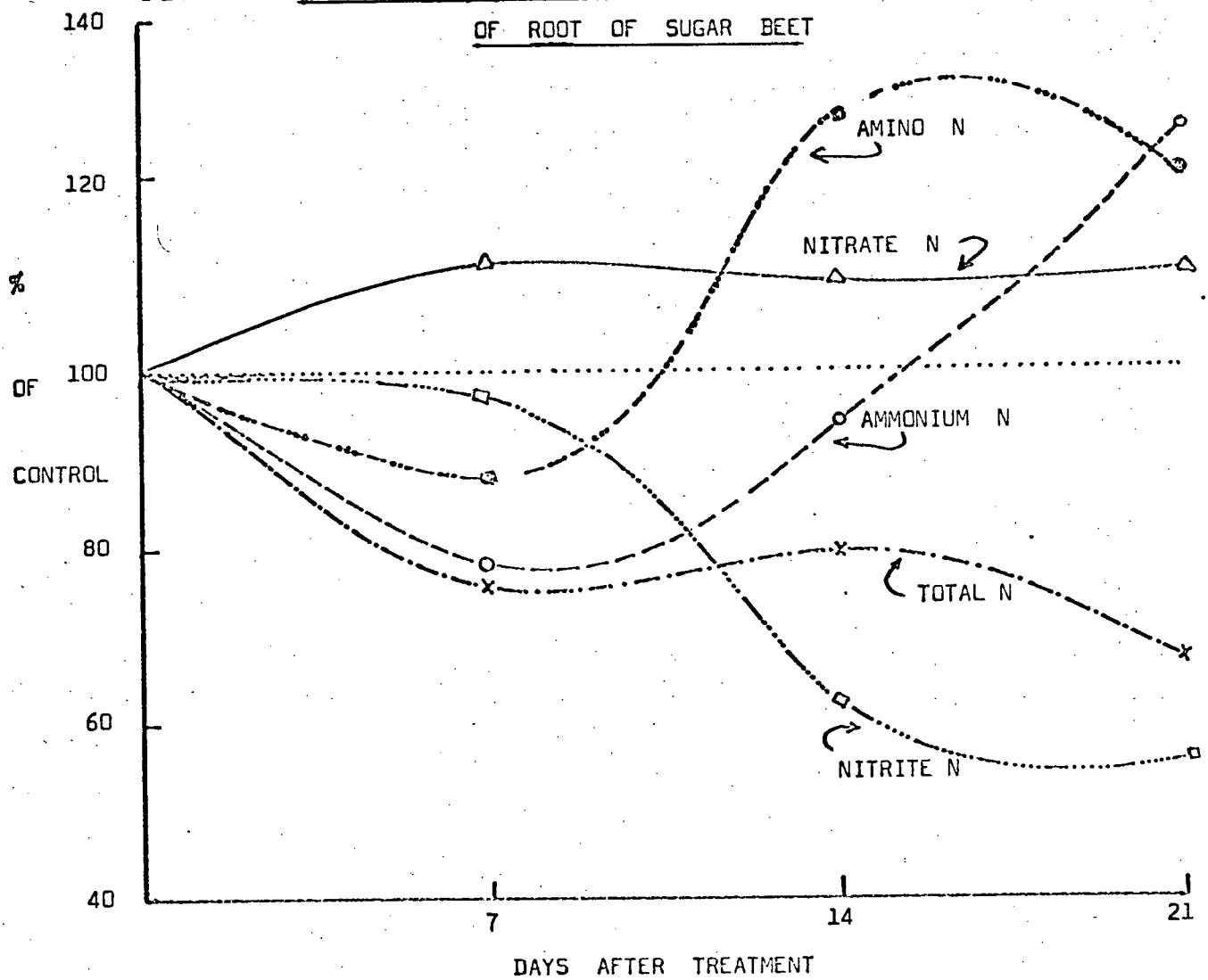


FIG. 12. EFFECT OF VANADIUM SULFATE ON THE NITROGEN CONTENT OF ROOT OF SUGAR BEET

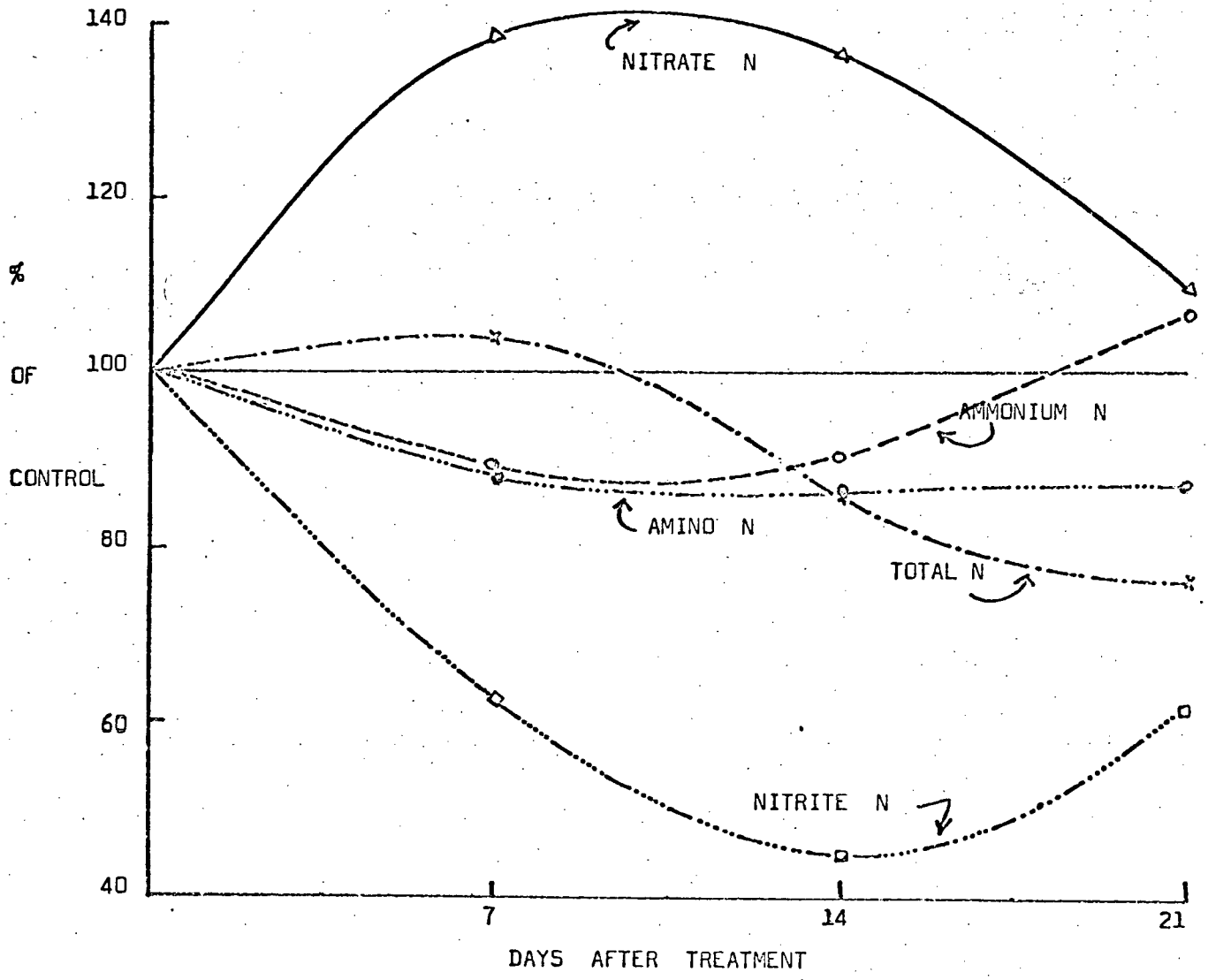


TABLE VIII

Effect of maleic hydrazide (MH), pyrocatechol (PC), and vanadium sulfate (VS) on photosynthesis and respiration of 4.5-month-old sugar beet plants

Growth condition	Days after treatment	Rate of net CO_2 fixation ($\mu l\ CO_2/dm^2/hr$ of the leaf area)				*Q		T/C (%)		
		**CK	MH	PC	VS	.05	.01	MH	PC	VS
Summer	0	1434	1386	1385	1426	55.92	63.45			
"	7	1412	1373	670	1506			92.23	46.54	106.65
"	14	1012	1433	1360	1112			141.57	134.32	109.89
"	21	949	1414	1033	1238			149.00	108.89	130.41
		Rate of dark respiration ($\mu l\ CO_2/dm^2/hr$ of the leaf area)								
	0	444	470	392	430					
	7	610	444	879	493			72.77	144.12	80.84
	14	517	315	455	376			60.80	87.98	72.71
	21	352	349	474	201			99.13	134.76	65.28
		Rate of true photosynthesis ($\mu l\ CO_2/dm^2/hr$ of the leaf area)								
	0	1878	1857	1773	1856					
	7	2023	1817	1552	1999			89.85	76.74	97.21
	14	1530	1748	1815	1446			114.25	118.64	98.85
	21	1301	1763	1508	1141			135.51	115.86	87.70
		Rate of net photosynthesis ($\mu l\ CO_2/dm^2/hr$ of the leaf area)								
Fall	0	1510	1495	1452	1445	68.18	74.24			
	7	1562	1931	1514	2183			123.57	98.63	139.70
	14	1652	1849	1748	2192			111.94	105.81	132.65
		Rate of dark respiration ($\mu l\ CO_2/dm^2/hr$ of the leaf area)								
	0	569	567	576	606					
	7	962	748	902	872			77.76	93.76	90.58
	14	994	635	957	554			63.86	96.22	55.76

*Q - Significant difference according to the Student-Newman-Keuls test
**CK - Control

TABLE VIII (cont'd)

Effect of maleic hydrazide (MH), pyrocatechol (PC), and vanadium sulfate (VS) on photosynthesis and respiration of 4.5-month-old sugar beet plants

Growth condition	Days after treatment	Rate of net CO_2 fixation (μl $CO_2/dm^2/hr$ of the leaf area)				*Q		T/C (%)		
		**CK	MH	PC	VS	.05	.01	MH	PC	VS
Fall		Rate of true photosynthesis (μl $CO_2/dm^2/hr$ of the leaf area)								
	0	2079	2062	2028	2051					
	7	2525	2679	2416	3054			106.12	95.71	121.01
	14	2868	2484	2705	2745			93.13	101.36	102.93
Summer		Rate of respiration of root (μl O_2/gm fresh wt/hr)								
	0	82	..	..	..	1.33	1.51			
	7	82	49	84	64			59.24	101.58	78.25
	14	75	47	75	51			62.97	100.12	67.77
	21	66	46	68	55			69.48	101.59	83.98
Fall	0	65	..	..	..	1.88	1.96			
	7	66	44	56	43			66.07	84.88	64.43
	14	73	52	57	46			71.15	78.76	62.59
	21	73	54	60	48			73.98	81.63	65.95

*Q - Significant difference according to Student-Newman-Keuls' test

**CK - Control

FIG. 13. EFFECT OF MALEIC HYDRAZIDE ON PHOTOSYNTHESIS AND RESPIRATION OF LEAVES AND ON RESPIRATION OF ROOT OF SUGAR BEET GROWN UNDER SUMMER CONDITIONS

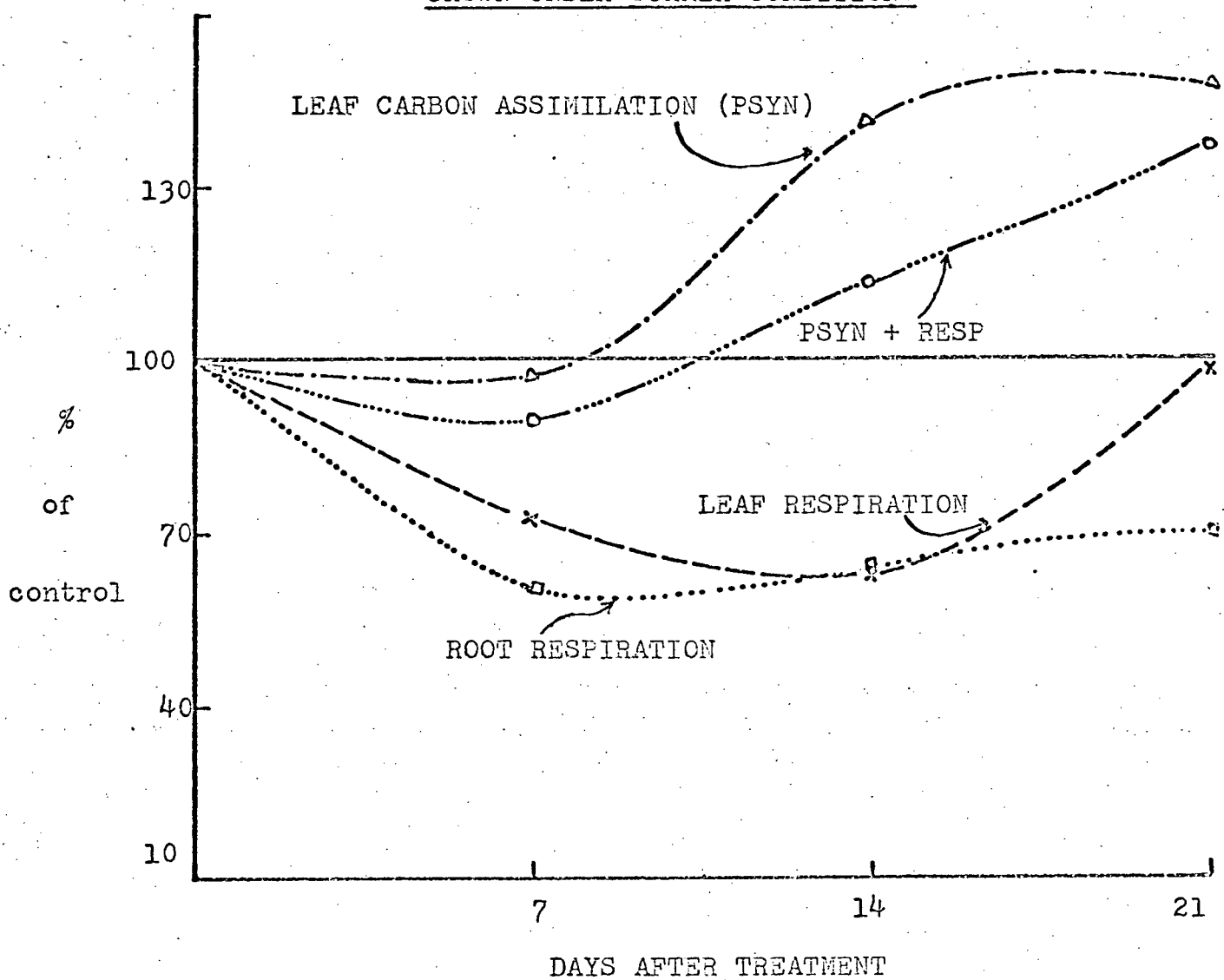


FIG. 14. EFFECT OF MALEIC HYDRAZIDE ON PHOTOSYNTHESIS AND RESPIRATION OF LEAVES AND ON RESPIRATION OF ROOT OF SUGAR BEET UNDER FALL CONDITIONS

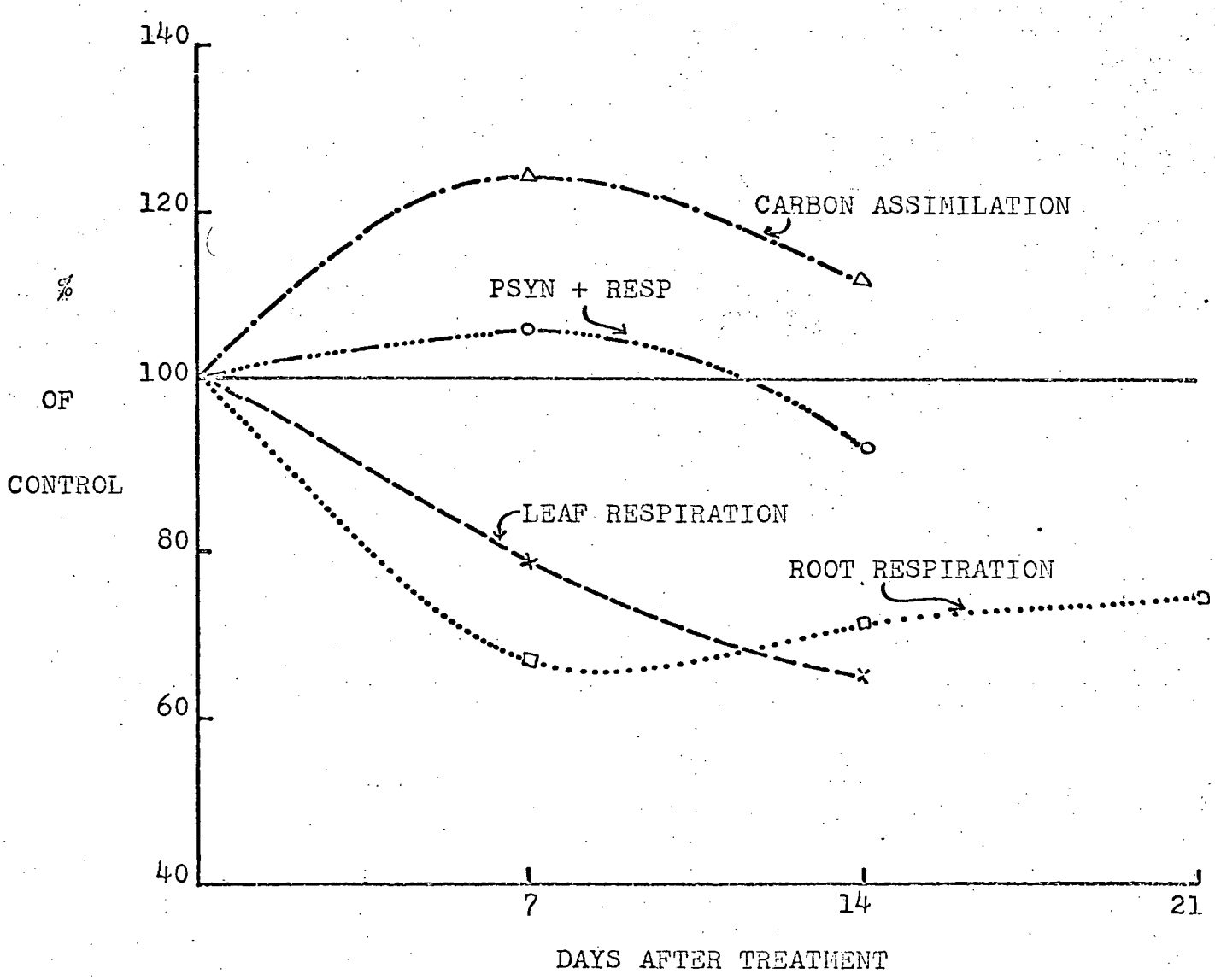


FIG. 15. EFFECT OF PYROCATECHOL ON PHOTOSYNTHESIS AND RESPIRATION OF LEAVES AND ON RESPIRATION OF ROOT OF SUGAR BEET GROWN UNDER SUMMER CONDITIONS

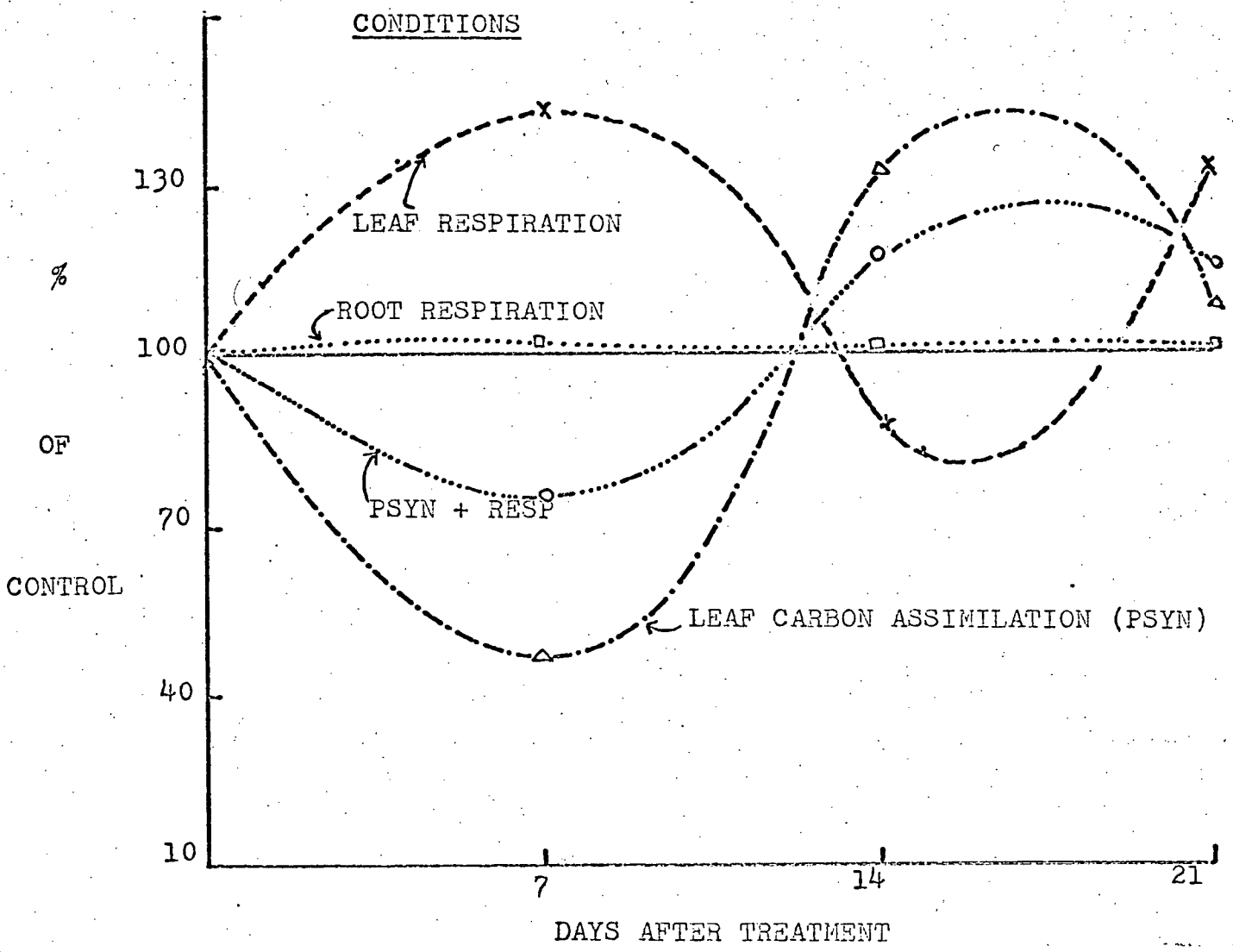


FIG. 16. EFFECT OF VANADIUM SULFATE ON PHOTOSYNTHESIS AND RESPIRATION OF LEAVES AND ON RESPIRATION OF ROOT OF SUGAR BEET GROWN UNDER SUMMER CONDITIONS

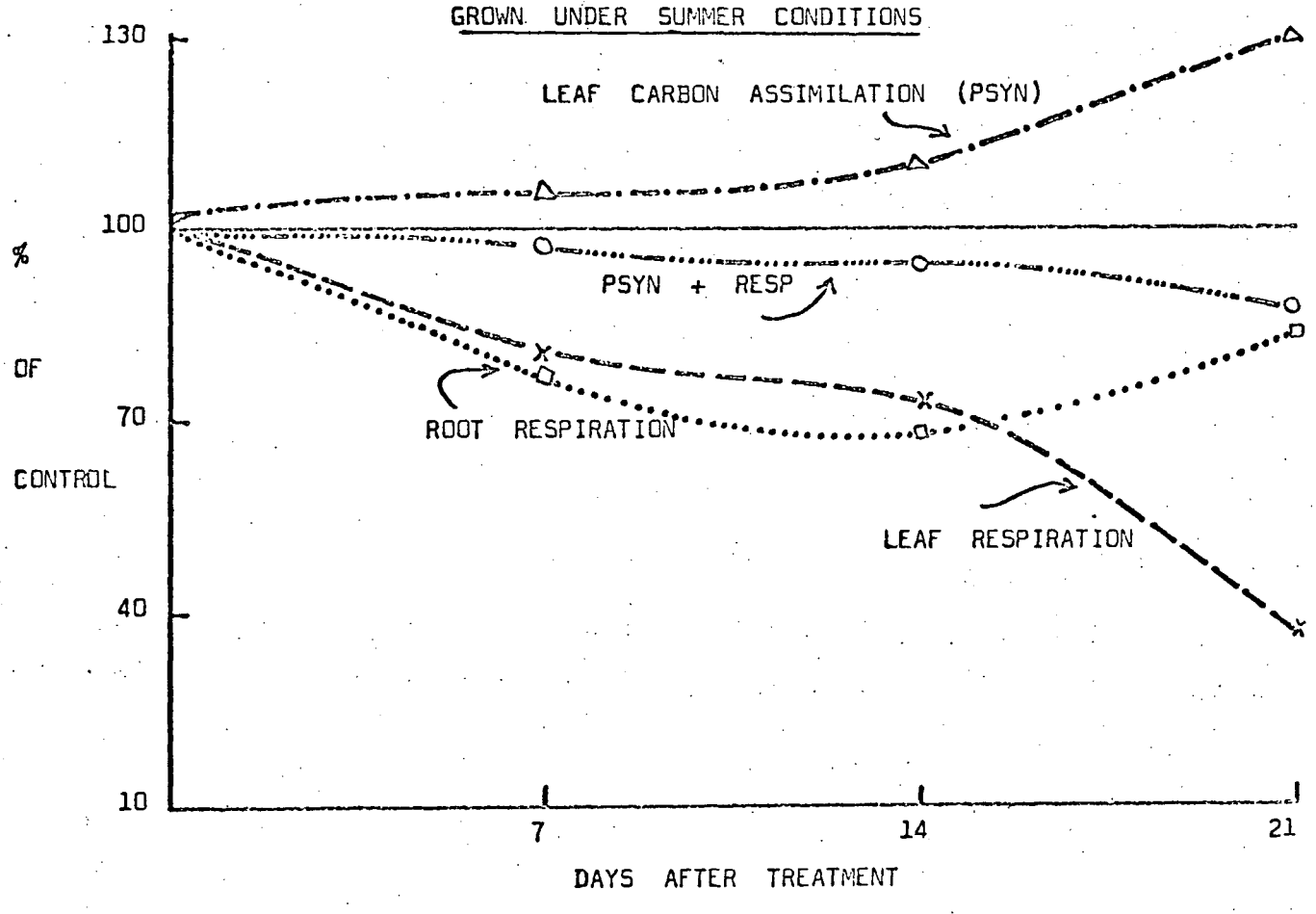
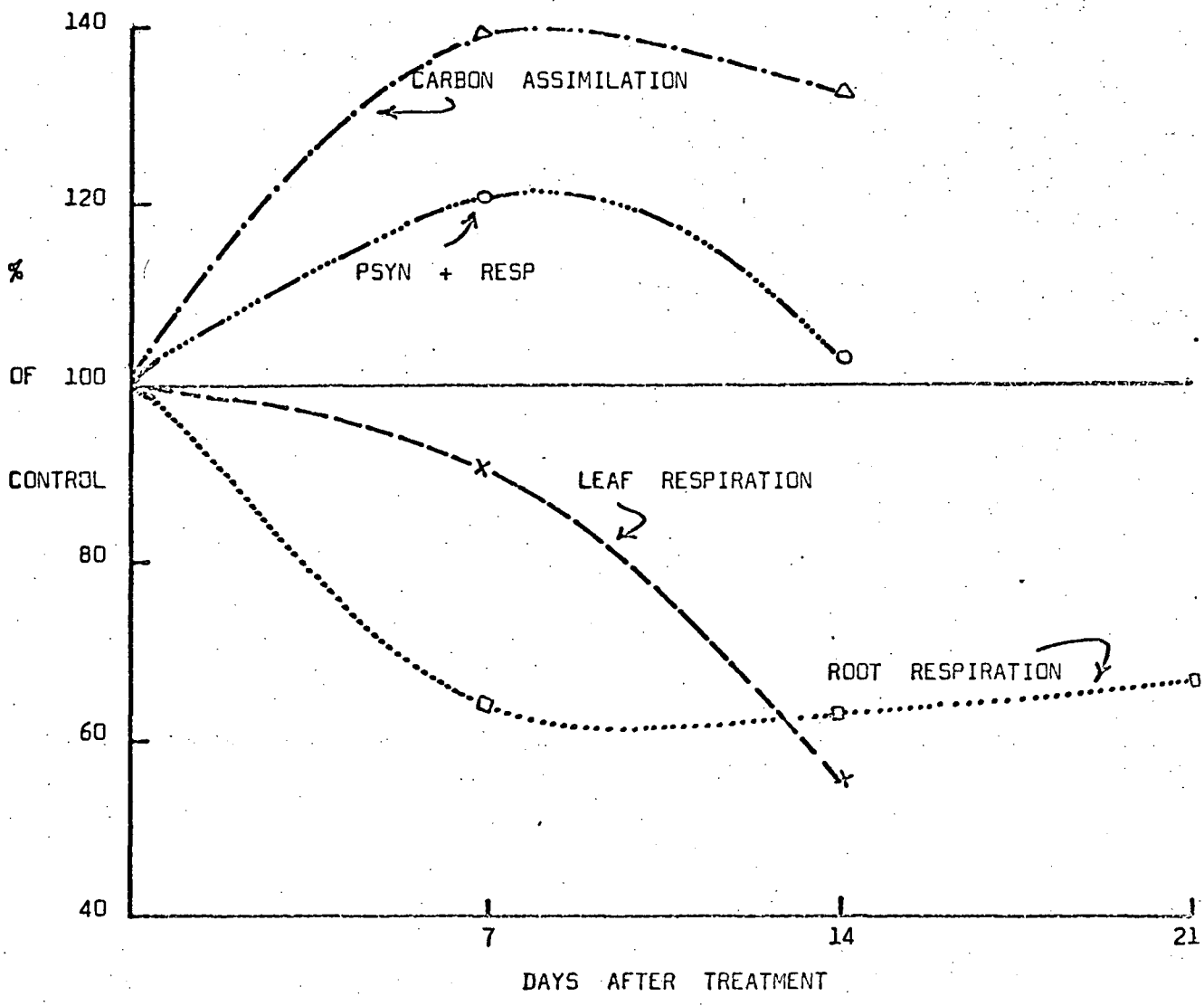


FIG. 17. EFFECT OF VANADIUM SULFATE ON PHOTOSYNTHESIS AND RESPIRATION OF LEAVES AND ON RESPIRATION OF ROOT OF SUGAR BEET UNDER FALL CONDITIONS



on the 14th and 21st day after treatment, respectively.

The rate of dark respiration under summer conditions in MH-treated plants was slowed down. It was 72.7, 60.8, and 99.1% of the control values on the on the 7th, 14th and 21st day after treatment, respectively. There was a stimulation in the rate of dark respiration by PC on the 7th and the 21st day of 44.1 and 34.7% respectively. VS caused a consistent reduction in the rate of dark respiration on all the three dates of observations.

The rate of net CO_2 fixation under fall conditions was stimulated by MH and PC on the 7th and the 21st day after treatment. PC also stimulated the rate of net CO_2 fixation on the 14th day. The maximum stimulation, 32 to 39%, was by VS under fall conditions.

The dark respiration was slowed down by all three treatments under fall conditions. Due to MH, the reduction in the rate of dark respiration on the 7th day was 22.2% and on the 14th day, 36.0%. PC caused the reduction in the rate of dark respiration under fall conditions on the 7th day by 6.2% and on the 14th day by 3.7%. The reductions in the rate of dark respiration under fall conditions by VS were 9.4 and 44.3% on the 7th and the 14th day respectively.

The rate of true photosynthesis was calculated by addition of net CO_2 fixation and CO_2 liberation in the dark. The rate of true photosynthesis under summer conditions was lower than the control on all the three dates of observations in the VS-treated plants. In the MH- and PC-treated plants it was lower than the control on the 7th day but higher on the 14th and 21st day of observations.

The rate of true photosynthesis under fall conditions was higher on the 7th day and lower on the 14th day after treatment in MH-treated plants. PC-treated plants had a lower rate of true photosynthesis on the 7th day but higher on the 14th day after treatment. VS-treated plants had a higher rate of true photosynthesis on both days of observations.

Respiration of the roots

The oxygen uptake by the beet tissue slices were determined by the Warburg method and is given in Table VIII. MH and VS inhibited the rate of oxygen uptake under summer and fall conditions. The inhibition by MH was 38% under summer and 34% under fall conditions on the 7th day after treatment. The inhibition by VS was 33% under summer and 38% under fall conditions. The differences between the values for respiration on the MH- and PC-treated plants and the control plants were significant statistically.

PC stimulated the rate of oxygen uptake under summer conditions. However, the differences between control and the treated plants were not significant except on the 7th day after treatment. Under fall conditions, PC inhibited the rate of respiration up to 20% and this inhibition was significant at the 0.01 level.

The effects of MH, PC, and VS on photosynthesis and respiration are summarized in Table VIII(a).

6. Nitrate reductase and transaminase activity

MH-treated and the control plants showed a steady increase in nitrate reductase (NRase) activity from 7 days to 21 days after

TABLE VIII (a)

Summary of the effect of maleic hydrazide (MH), pyrocatechol (PC), and vanadium sulfate (VS) on photosynthesis and respiration of sugar beet

Item	Growth condition	*Effect of treatment with								
		MH			PC			VS		
Net photosynthesis	Summer	ns	+	+	-	+	+	+	+	+
	Fall	+	+	N	ns	+	N	+	+	N
Dark respiration of leaf	Summer	-	-	-	+	-	+	-	-	-
	Fall	-	-	N	-	-	N	-	-	N
True photosynthesis	Summer	-	+	+	-	+	+	-	-	-
	Fall	+	-	N	-	+	N	+	+	N
Respiration of roots	Summer	-	-	-	x	ns	ns	-	-	-
	Fall	-	-	-	-	-	-	-	-	-

*The symbols +, - and N refer to increase, decrease, and not determined, respectively. Three symbols for each treatment correspond to the harvests made 7, 14, and 21 days after treatment.

ns - not significant at 0.01 level

x - Significant only at 0.05 level.

treatment in the root and the leaf. In PC- and VS-treated plants the lowest specific activity recorded was on the 14th day after treatment. All three treatments caused significant inhibition of specific NRase activity in the root and in the leaf on all the dates of harvest (Table IX and Figs. 18, 19 and 20).

The specific activity values for the leaves of MH-treated plants were 78.8, 69.5 and 59.1% of the control values, respectively on the 7th, 14th and 21st day after treatment (Fig. 18). In PC-treated plants, the specific activity values for leaves were 72.1% on the 7th day, 62.1% on the 14th day, and 44.4% on the 21st day, of the control values (Fig. 19). The maximum inhibition of the activity of NRase was due to VS and occurred in the leaves. These inhibitions were 42, 51, and 55% respectively on the 7th, 14th and 21st day after treatment (Fig. 20). VS also caused the maximum inhibition of the root NRase activity.

The activity of transaminase in the root and the leaf was inhibited significantly by all three treatments. The maximum inhibition in the leaf was due to VS. The values for transaminase activity in the leaf of VS-treated plants were 40.5% on the 7th day, 43.5% on the 14th day, and 52.2% on the 21st day, of the control values. The maximum reductions of transaminase activity in the leaf of MH- and PC-treated plants were on the 14th day after treatment.

The maximum reductionsin transaminase activity in the root of MH- and VS-treated plants were on the 7th day after treatment. These reductions were about 40 and 44% by MH and VS respectively. The maximum reduction in the transaminase activity of the root of the PC-treated plants was about 25% on the 14th day after treatment.

TABLE IX

Effect of maleic hydrazide (MH), pyrocatechol (PC), and vandium sulfate (VS) on nitrate reductase and transaminase activity of the root and the leaf of 4 1/2-month-old sugar beet plants

Enzyme	Plant part	Days after treatment	*Specific activity value				**Q		T/C (%)		
			CK	MH	PC	VS	0.05	0.01	MH	PC	VS
Nitrate reductase	Leaf	0	33.40	...	...	...	2.39	2.71			
"	"	7	20.88	15.63	15.05	12.05			78.83	72.06	57.72
"	"	14	20.76	15.83	12.12	11.15			69.57	62.05	48.99
"	"	21	33.32	19.70	14.79	14.99			59.14	44.41	45.01
"	Root	0	24.30	...	...	...	0.46	0.53			
"	"	7	14.54	12.90	12.12	7.47			88.75	83.39	51.38
"	"	14	17.33	14.37	7.88	6.46			82.90	46.46	37.31
"	"	21	19.80	16.26	13.06	10.15			82.13	66.03	51.22
Trans-aminase	Leaf	0	45.47	...	...	...	0.86	1.56			
"	"	7	69.23	54.01	40.19	28.03			78.02	58.05	40.48
"	"	14	63.22	40.27	36.03	27.53			63.69	56.99	43.54
"	"	21	78.28	57.63	39.79	40.85			73.61	50.83	52.18
"	Root	0	80.20	...	...	...	.031	.078			
"	"	7	93.98	56.78	88.05	53.35			60.42	93.68	56.76
"	"	14	82.22	76.09	62.22	48.13			92.54	75.68	58.54
"	"	21	96.34	69.66	87.32	76.33			72.30	90.63	79.23

*Specific activity of NRase - mμm NO_2/mg of protein, specific activity for transaminase - μm pyruvic/mg protein

**Q- significant difference according to Student-Newman--Keuls' test

FIG. 18. EFFECT OF MALEIC HYDRAZIDE ON NITRATE REDUCTASE AND TRANSAMINASE ACTIVITY IN LEAF AND ROOT OF SUGAR BEET

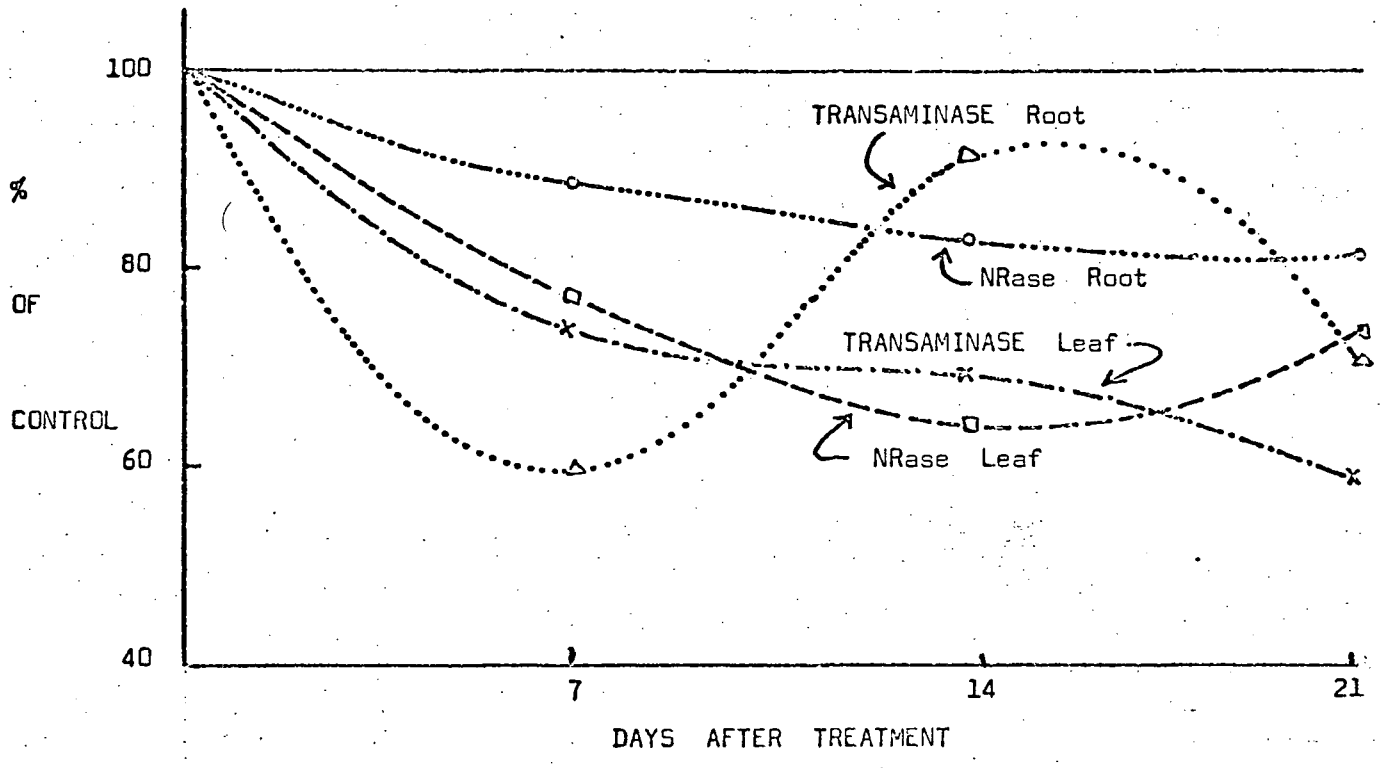


FIG. 19 EFFECT OF PYROCATECHOL ON NITRATE REDUCTASE AND TRANSAMINASE ACTIVITY IN LEAF AND ROOT OF SUGAR BEET

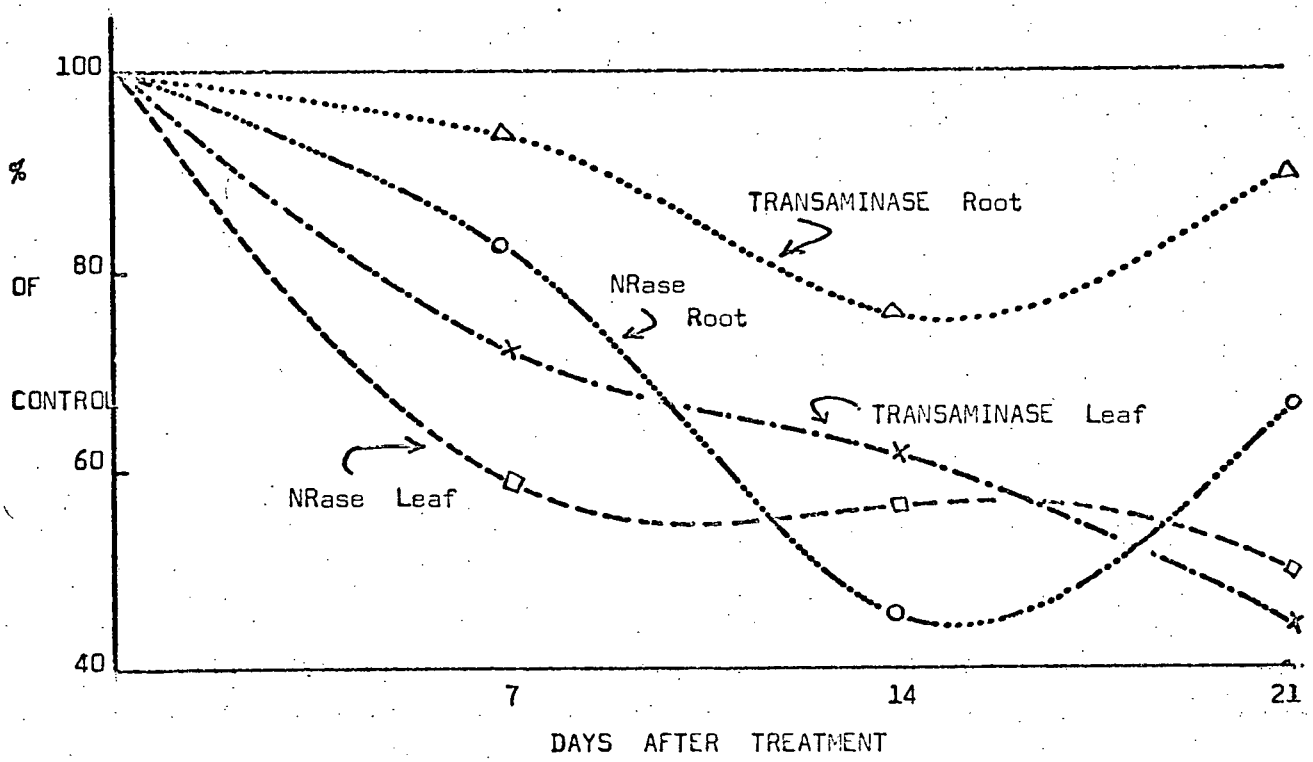
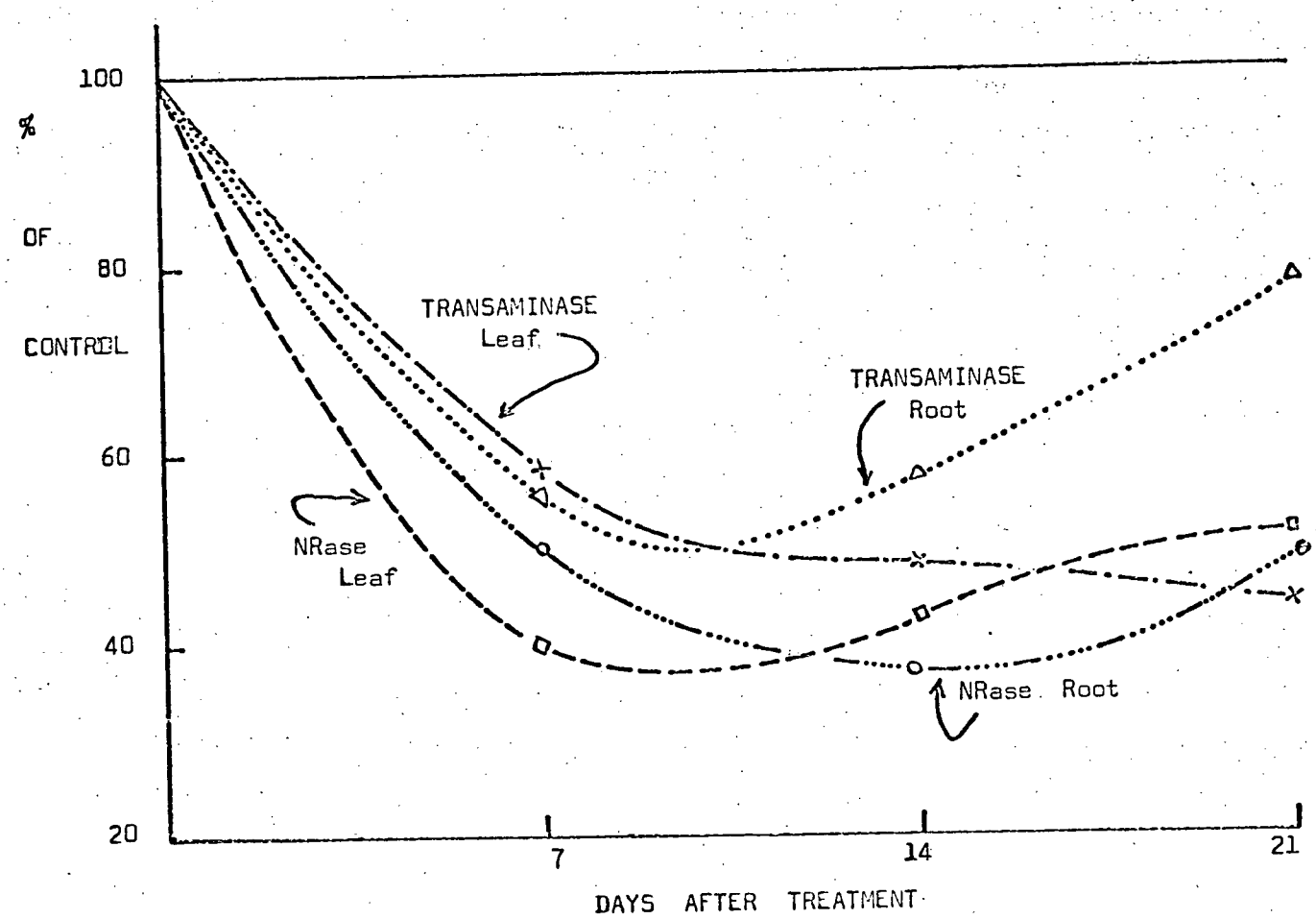


FIG. 20. EFFECT OF VANADIUM SULFATE ON NITRATE REDUCTASE AND TRANSAMINASE ACTIVITY IN LEAF AND ROOT OF SUGAR BEET



7. Invertase activity

The invertase activity of the leaves of the treated plants was reduced significantly. Maximum inhibition of the leaf invertase was achieved through PC treatment. These inhibitions were 55.7% on the 7th day, 78.3% on the 14th day and 60% on the 21st day after treatment. Root invertase activity was also inhibited most by pyrocatechol. The root invertase activity of the PC-treated plants was 71%, 62% and 75.7% of the control activity on the 7th, 14th, and 21st day after treatment. The root invertase activity of the MH-treated plants was not significantly different from those of the untreated plants on the 7th day after treatment, but on subsequent days of harvest the differences were highly significant.

The invertase activity is given in Table X. The inhibition by MH and VS increased progressively in the leaf (Fig. 21 and 23). Figure 22 shows that maximum inhibition by PC was on the 14th day after treatment. The maximum inhibition of invertase activity of the root by MH and PC was also on the 14th day (Figs. 24 and 25) and by VS on the 21st day after treatment (Fig. 26).

8. Phosphatases

Table XI indicates that phosphatases in general were inhibited by the treatments. Phenylphosphatase activity of the roots and the leaves was significantly inhibited by all the three treatments. The maximum inhibition of phenylphosphatase activity of leaf by MH, PC, and VS was on the 14th day after treatment. The values on this day were 35.7% for MH, 75.4% for PC and 57.7% of

TABLE X

Effect of maleic hydrazide (MH), pyrocatechol (PC) and vanadium sulfate (VS) on the invertase activity of the root and the leaf of 4 1/2-month-old sugar beet plants

Plant part	Days after treatment	(mμm reducing sugar/mg protein)				*Q				
		CK	MH	PC	VS	0.05	0.01	MH	PC	VS
Leaf	0	9955	...	...	...	463.86	526.21			
	7	7373	6501	3268	5920			88.17	44.32	80.29
	14	7150	4045	1556	4695			56.57	21.70	65.66
	21	9283	4633	3716	4185			49.90	40.03	45.08
Root	0	7843	...	...	...	167.19	189.70			
	7	6089	6047	4294	5559			99.96	70.98	91.29
	14	3936	3402	2441	3648			86.43	62.01	92.68
	21	5122	4907	3877	4407			95.80	75.68	86.04

*Q - significant difference according to Student-Newman-Keuls' test

FIG. 21. EFFECT OF MALEIC HYDRAZIDE ON ENZYMES OF SUCROSE SYNTHESIS AND HYDROLYSIS IN THE LEAF OF SUGAR BEET

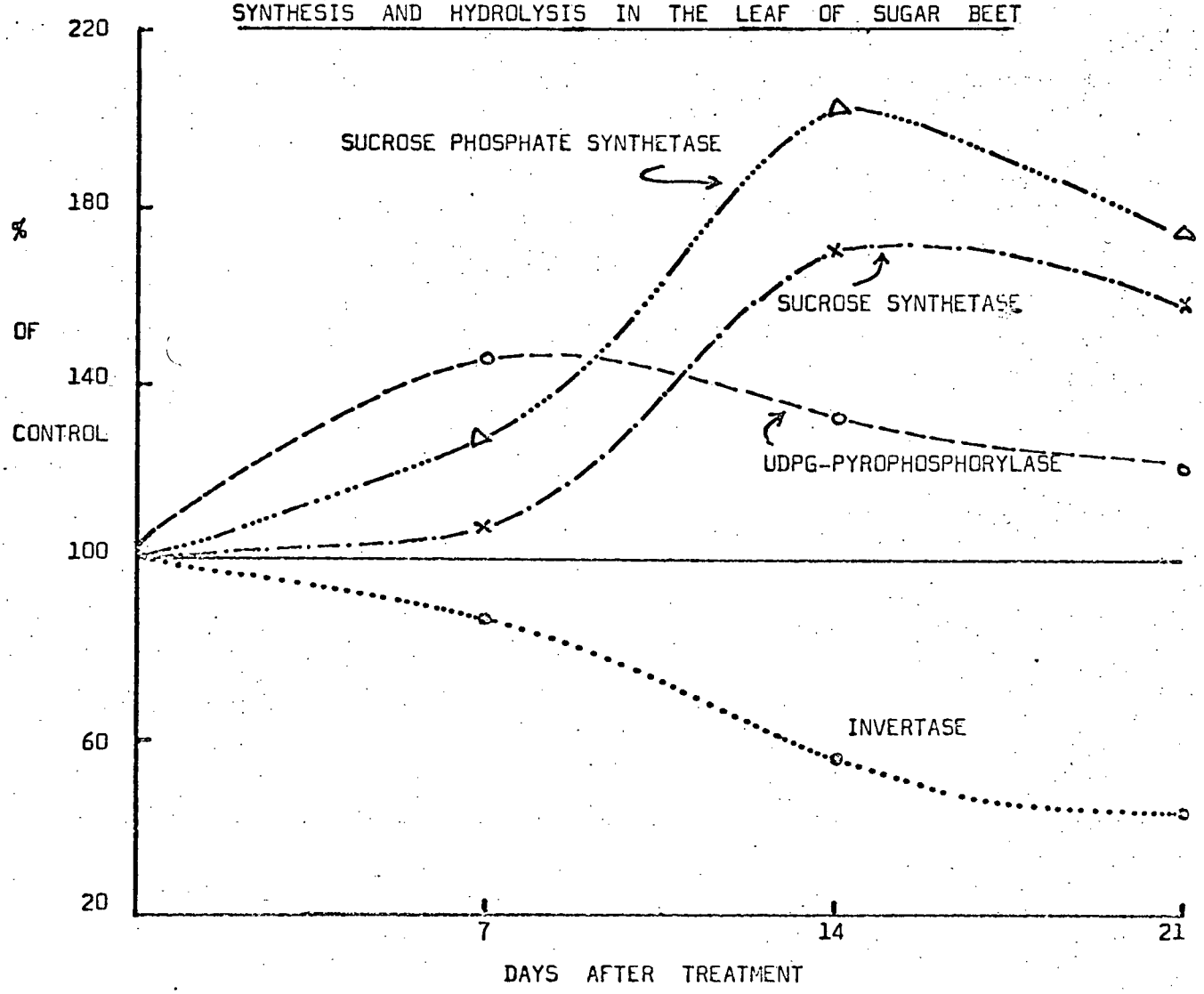


FIG. 22. EFFECT OF PYROCATECHOL ON ENZYMES OF SUCROSE SYNTHESIS AND HYDROLYSIS IN THE LEAF OF SUGAR BEETS

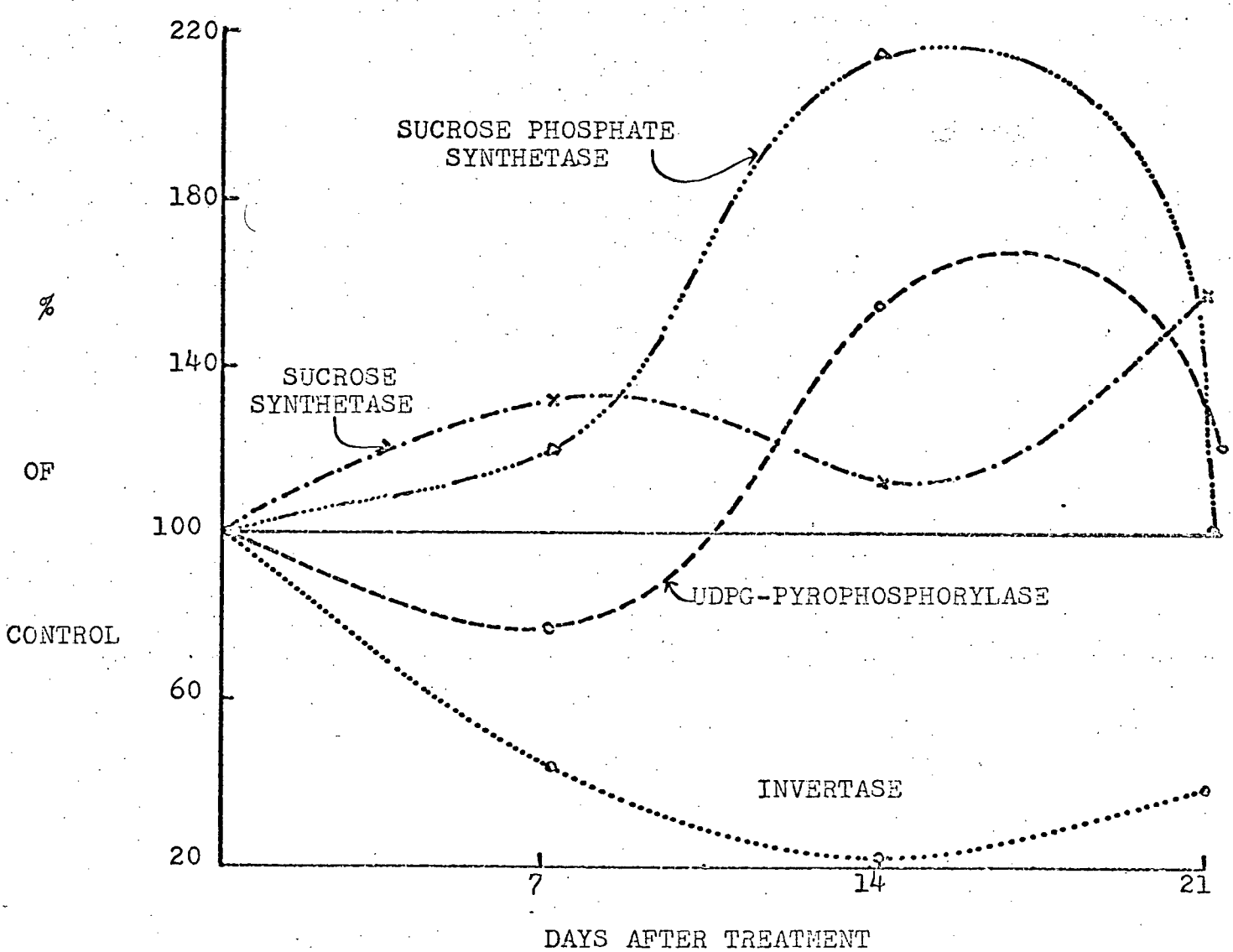


FIG. 23. EFFECT OF VANADIUM SULFATE ON ENZYMES OF SUCROSE SYNTHESIS AND HYDROLYSIS IN THE LEAF OF SUGAR BEET

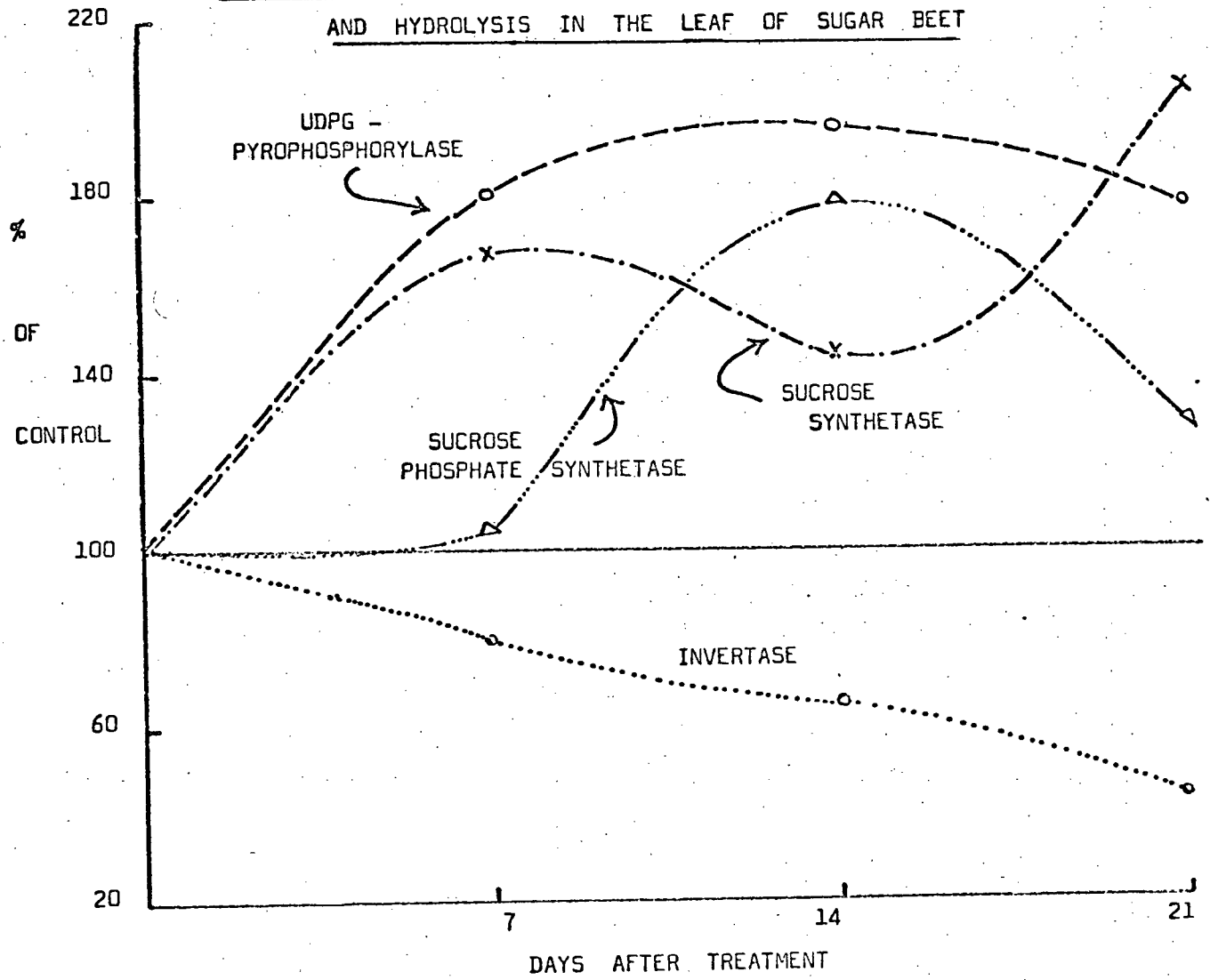


FIG. 24. EFFECT OF MALEIC HYDRAZIDE ON ENZYMES OF SUCROSE SYNTHESIS AND HYDROLYSIS IN THE ROOT OF SUGAR-BEET

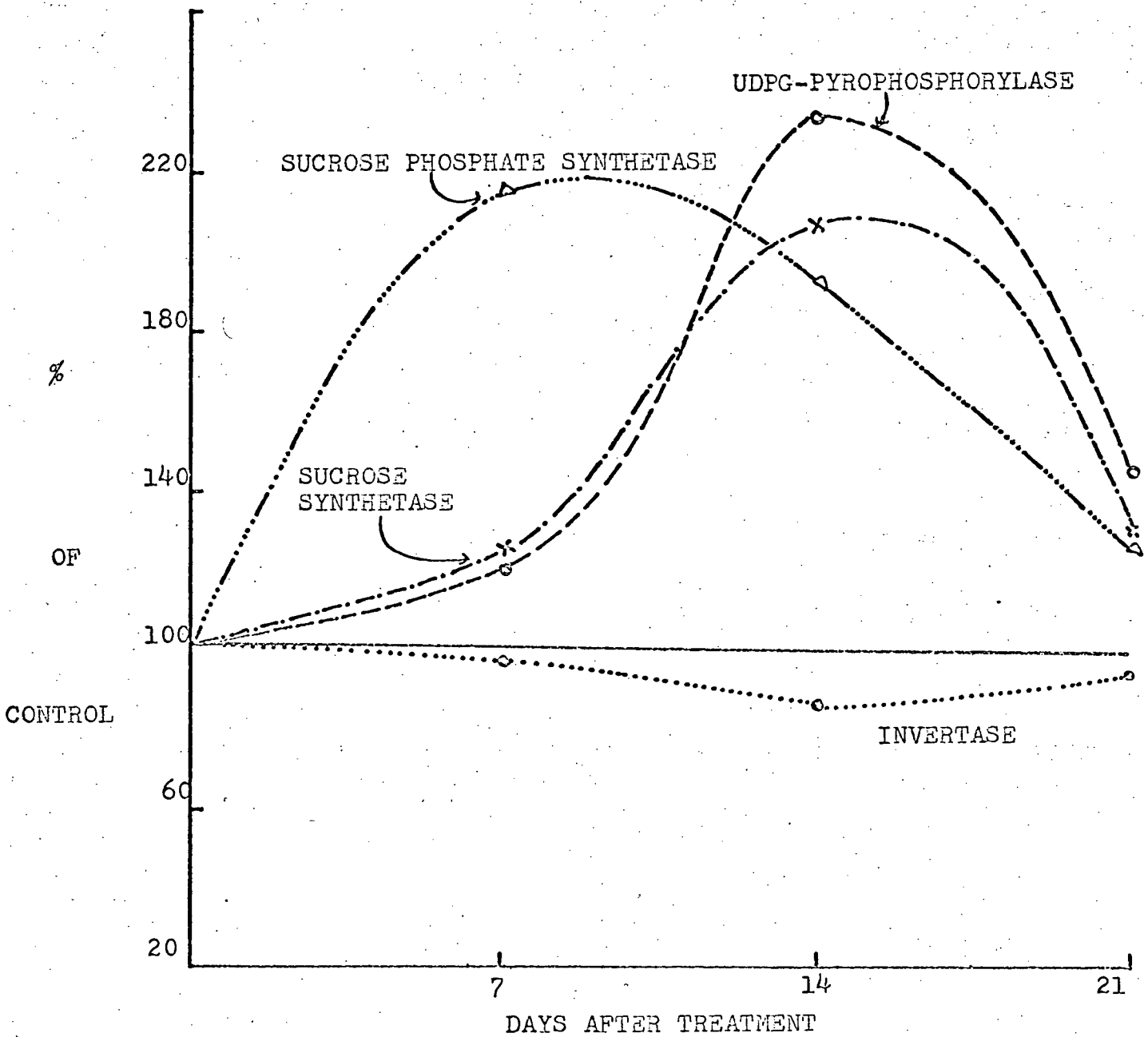


FIG. 25. EFFECT OF PYROCATECHOL ON ENZYMES OF SUCROSE SYNTHESIS AND HYDROLYSIS IN THE ROOT OF SUGAR BEET

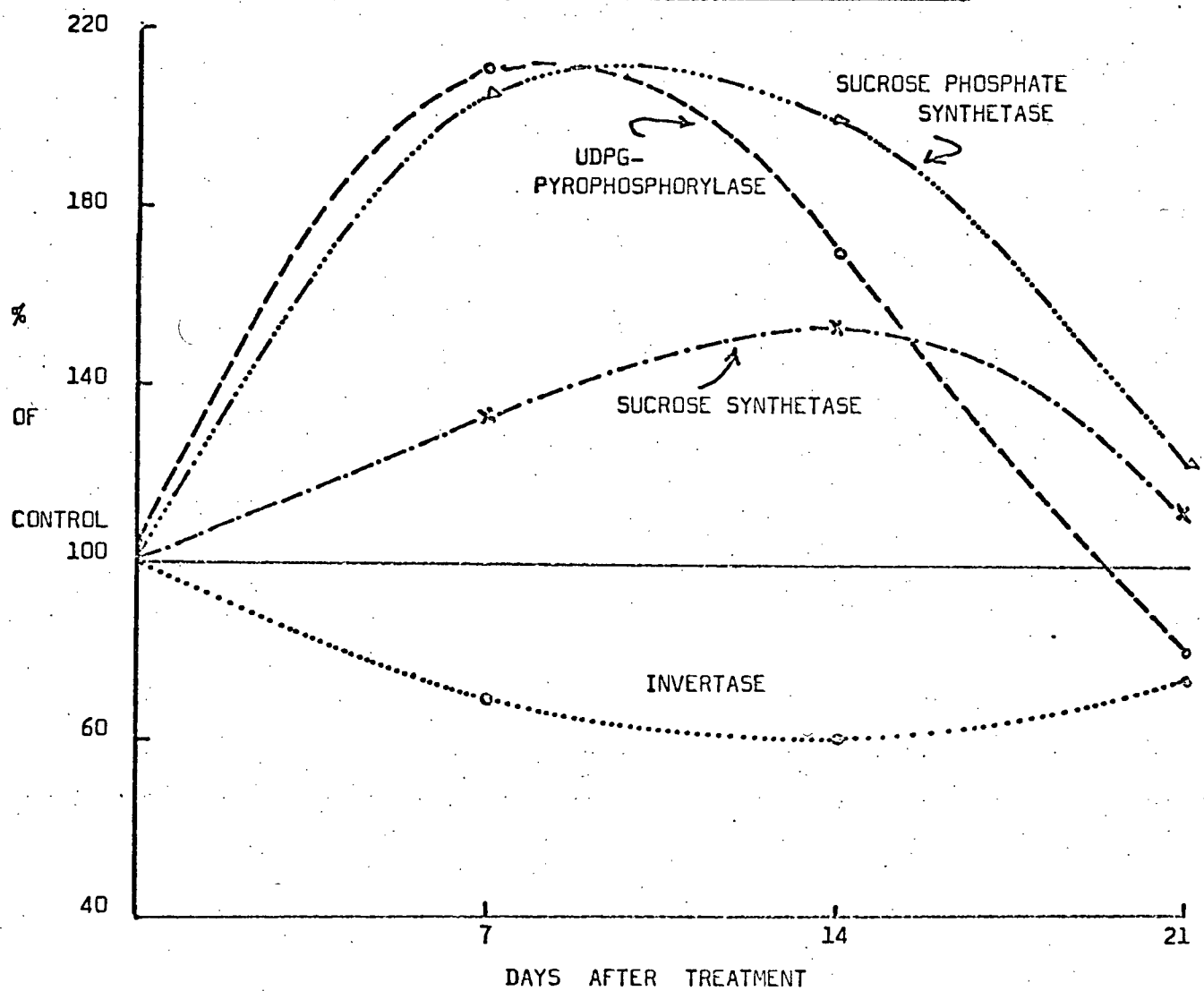


FIG. 26. EFFECT OF VANADIUM SULFATE ON ENZYMES OF SUCROSE SYNTHESIS AND HYDROLYSIS IN THE ROOT OF SUGAR BEET

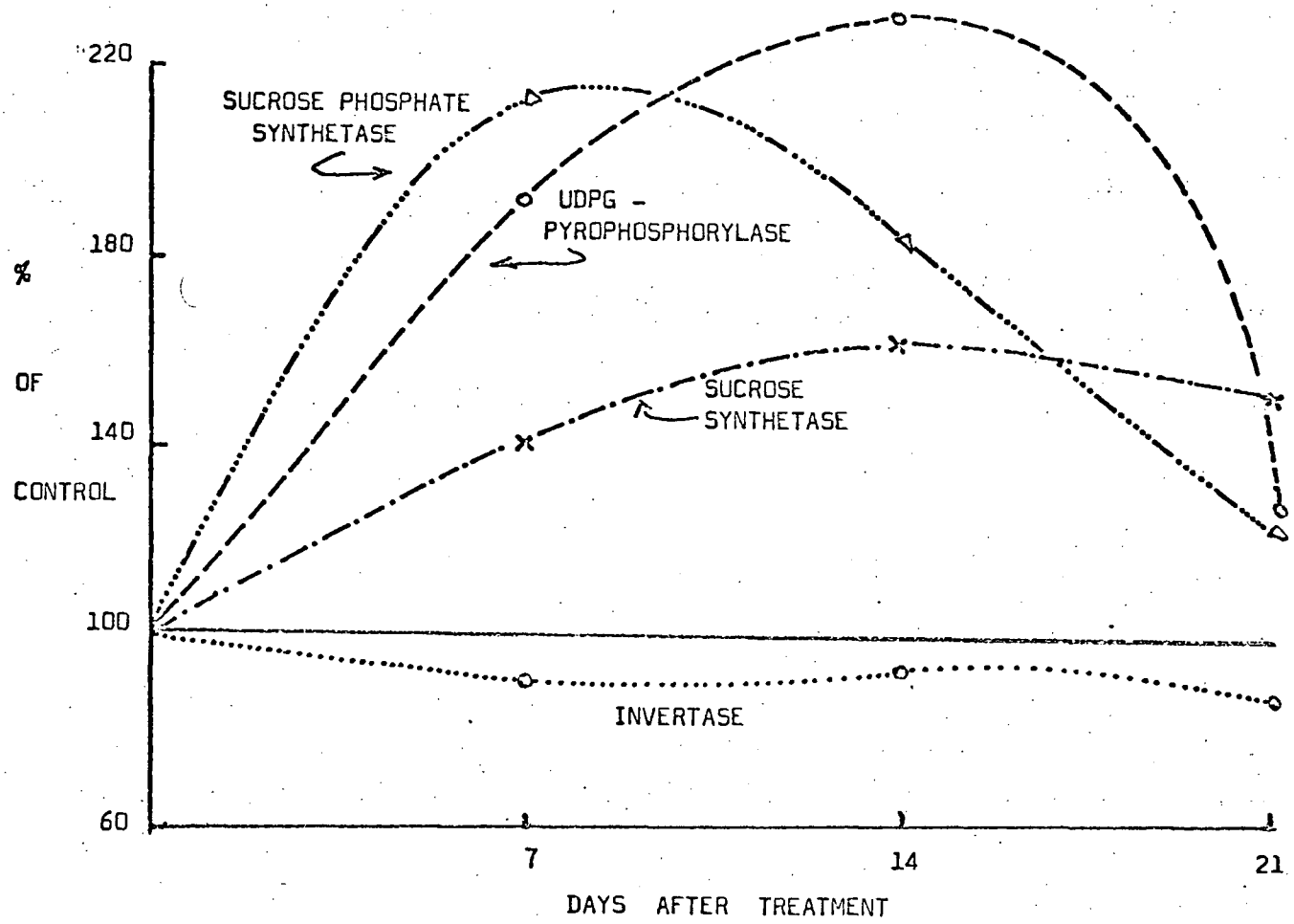


TABLE XI

Effect of maleic hydrazide (MH), pyrocatechol (PC), and vanadium sulfate (VS) on the activity of phosphatases of leaf and root of 4.5-month-old sugar beet plants

Enzyme	Plant part	Days after treatment	Specific activity (mμmoles Pi/mg protein)				*Q		T/C (%)		
			**CK	MH	PC	VS	.05	.01	MH	PC	VS
Phenyl-phosphatase	Leaf	0	312.24	...	...	...	13.58	15.41			
"	"	7	195.80	148.69	181.02	122.47			75.94	92.44	62.81
"	"	14	293.64	104.76	221.44	151.53			35.67	75.41	57.72
"	"	21	275.75	245.10	248.00	165.68			88.78	92.02	60.25
"	Root	0	61.53	...	...	...	1.95	2.21			
"	"	7	42.35	26.17	29.99	35.30			61.79	70.81	83.36
"	"	14	36.84	34.13	34.37	32.42			92.65	93.28	87.99
"	"	21	40.31	29.32	13.37	33.29			72.74	33.16	82.60
Adenosine tri-phosphatase	Leaf	0	538.70	...	...	...	14.20	16.11			
"	"	7	249.20	243.65	147.84	237.86			97.77	59.17	95.53
"	"	14	525.22	206.34	117.73	427.35			39.28	22.41	81.37
"	"	21	570.15	508.24	427.35	466.03			89.14	74.94	81.76
"	Root	0	46.74	...	...	...	1.94	2.19			
"	"	7	15.73	13.50	14.47	11.85			85.87	92.01	75.38
"	"	14	11.61	10.85	8.27	8.80			93.43	71.23	75.81
"	"	21	21.05	23.21	13.78	14.33			110.25	65.44	68.70
Glucose-1-phosphatase	Leaf	0	361.50	...	...	...	30.50	34.62			
"	"	7	391.25	104.64	72.98	133.48			32.77	22.85	41.82
"	"	14	138.50	132.03	138.85	84.07			95.32	100.24	60.70
"	"	21	109.68	93.32	76.95	108.92			85.07	70.16	99.38

*Q - Significant difference according to Student-Newman-Keuls' test.
**CK - Control

TABLE XI (cont'd)

Effect of maleic hydrazide (MH), pyrocatechol (PC), and vanadium sulfate (VS) on the activity of phosphatases of leaf and root of 4.5-month-old sugar beet plants

Enzyme	Plant part	Days after treatment	Specific activity (mμmoles Pi/mg protein)				*Q		T/C (%)		
			**CK	MH	PC	VS	.05	.01	MH	PC	VS
Glucose-1-phosphatase	Root	0	64.26	...	...	...	2.32	2.63			
"	"	7	49.90	26.51	48.36	50.26			53.12	96.91	100.71
"	"	14	48.36	21.54	36.27	47.31			44.55	62.60	97.84
"	"	21	39.25	18.44	19.06	38.52			46.99	48.56	98.29
Glucose-6-phosphatase	Leaf	0	343.80	...	...	...	20.39	23.13			
"	"	7	260.77	122.19	100.36	186.63			46.85	42.38	71.57
"	"	14	145.25	162.11	141.01	90.43			111.16	97.07	62.26
"	"	21	119.09	105.85	125.18	73.62			88.28	105.12	61.82
"	Root	0	48.02	...	...	...	1.54	1.75			
"	"	7	26.45	27.07	17.07	17.91			102.33	64.54	61.72
"	"	14	29.90	26.08	12.04	18.73			87.22	40.27	62.64
"	"	21	45.53	18.63	28.08	36.77			40.93	63.80	78.10
Fructose-6-phosphatase	Leaf	0	186.19	...	...	...	25.91	29.40			
"	"	7	197.58	189.90	113.99	138.95			96.11	57.61	70.33
"	"	14	155.49	133.42	137.60	118.35			85.80	88.49	76.14
"	"	21	360.00	320.01	388.11	300.63			88.89	107.80	83.51
"	Root	0	50.09	...	...	...	2.59	2.94			
"	"	7	12.47	12.56	10.82	7.01			100.73	86.76	56.29
"	"	14	17.38	13.86	13.10	10.87			79.65	75.38	62.58
"	"	21	13.47	10.86	15.12	8.33			80.64	112.28	61.83

the control for VS. The maximum inhibition of the root phosphatase was on the 7th day by MH and on the 21st day by VS and PC.

The activity of ATP-ase was significantly lower in the leaves of the treated plants except 7 days after treatment in MH-treated plants where the activity was not significantly different from the control plants. The inhibition caused by MH was 61% and by PC, 78% on the 14th day after treatment. The activity of root ATP-ase in the treated plants was also significantly lower than the control plants except on the 14th and 21st day in MH- and the 7th day in PC-treated plants.

The activity of glucose-1-phosphatase in the leaf of MH-treated plants was decreased. However, the decreases were not significant statistically on the 14th and the 21st day. The difference between glucose-1-phosphatase activity in the leaves of PC-treated and the control plants was not significant on the 14th day. PC-treated plants had lower glucose-1-phosphatase activity in their leaves on the 7th and the 21st day. VS-treated plants had significantly lower activity of glucose-1-phosphatase on the 7th and the 14th day. The maximum inhibition of glucose-1-phosphatase activity was about 77% by MH on the 7th day after treatment. The root glucose-1-phosphatase activity was significantly lower in MH-treated plants on all the dates of harvest. In PC-treated beets, the root glucose-1-phosphatase was inhibited significantly on the 14th and the 21st day after treatment. VS-treated plants had no significant effects on glucose-1-phosphatase activity of the roots.

There was a significant decrease in glucose-6-phosphatase activity in the leaves of the VS-treated plants. The values for

glucose-6-phosphatase activity were 71.6%, 62.3% and 61.8% of the control values in the leaves of VS-treated plants. There was considerable decrease in the glucose-6-phosphatase activity in the leaves of MH-, and PC-treated plants 7 days after the treatment but on the subsequent days the values in the treated plants were not significantly different from the control plants.

The glucose-6-phosphatase activity in the root of the MH-treated plants was significantly lower than the control plants on the 14th and the 21st day after treatment. The maximum inhibition of glucose-6-phosphatase activity, 60% was caused by PC on the 14th day after treatment.

VS inhibited fructose-6-phosphatase activity in the root and the leaf on all the dates of harvest. The maximum decrease of the fructose-6-phosphatase activity in the leaf of VS-treated plants was 30% on the 7th day.

PC also caused the inhibition of the activity of fructose-6-phosphatase in the leaves and the roots. The maximum inhibition in the leaf was 42.4% on the 7th day and in the root 25% on the 14th day in PC-treated plants.

MH significantly inhibited the activity of fructose-6-phosphatase in the leaf on the 14th day only and in the root on the 14th and 21st day. The maximum inhibition of the leaf fructose-6-phosphatase by MH was 11.1% on the 21st day after treatment. Inhibition in the root up to 20% was achieved by MH on the 14th and 21st day after the treatment.

9. UDPG-pyrophosphorylase

UDPG-pyrophosphorylase activity was stimulated significantly

by MH and VS on all days of harvest (Table XII). PC-treated plants showed low UDPG-pyrophosphorylase activity in the leaf on the 7th day and in the root on the 21st day after treatment. Maximum stimulation, up to 48.7%, of UDPG-pyrophosphorylase activity of the leaf by MH was on the 7th day. The maximum stimulation of the leaf UDPG-pyrophosphorylase in PC- and VS-treated plants were 56.3 and 95.7% respectively on the 14th day after treatment. Compared to untreated, more than double activity was recorded in the root on the 14th day in case of MH-treated and VS-treated plants, and on the 7th day in PC-treated plants.

10. Enzymes of sucrose synthesis

The presence of the enzymes sucrose synthetase and sucrose phosphate synthetase in the sugar beet leaf and root has been shown by Rorem et al. (1960) and Dutton et al. (1961). According to the results obtained in the present investigation the specific activity of the sucrose synthesizing enzymes, sucrose synthetase and sucrose phosphate synthetase, was greater in the leaf than in the root of both control and treated sugar beet plants (Table XIII)

All three treatments stimulated the activity of both sucrose synthetase and sucrose phosphate synthetase very significantly.

The activity of sucrose phosphate synthetase in the leaf of all treated plants was approximately double that of the control plants on the 14th day after treatment, the actual stimulation percentages being 104, 116 and 79 for MH, PC and VS respectively.

TABLE XII

Effect of maleic hydrazide (MH), pyrocatechol (PC), and vanadium sulfate (VS) on UDPG-pyrophosphorylase activity of root and leaf of 4.5-month-old sugar beet plants

Plant part	Days after treatment	Specific activity (mμmole G-1-P/mg of protein)				*Q		T/C (%)		
		**CK	MH	PC	VS	.05	.01	MH	PC	VS
Leaf	0	137.72	...	...	...	12.78	13.08			
	7	110.54	164.25	85.99	199.72			148.68	77.79	180.67
	14	74.17	98.93	115.94	145.16			133.38	156.29	195.69
	21	98.59	120.95	116.12	177.28			122.67	117.78	179.81
Root	0	168.89	...	...	...	16.97	19.26			
	7	79.28	98.78	168.43	151.52			124.59	212.45	191.12
	14	60.72	142.50	103.39	140.05			234.66	170.27	230.62
	21	98.17	142.28	79.40	119.69			145.13	80.88	121.92

*Q - Significant difference according to Student-Newman-Keuls' test

**CK - Control

TABLE XIII

Effect of maleic hydrazide (MH), pyrocatechol (PC), and vanadium sulfate (VS) on sucrose-P-synthetase and sucrose synthetase of root and leaf of 4.5-month-old sugar beets

Enzyme	Plant part	Days after treatment	Specific activity (mμmoles sucrose/mg protein)				*Q		T/C (%)		
			**CK	MH	PC	VS	.05	.01	MH	PC	VS
Sucrose-P-synthetase	Leaf	0	207.78	...	...	...	4.59	5.21			
"	"	7	202.41	256.98	242.03	213.86			126.96	119.57	105.65
"	"	14	126.19	258.83	273.15	226.46			204.95	216.46	179.46
"	"	21	113.86	196.09	114.54	144.92			172.21	100.59	127.32
"	Root	0	19.62	...	...	...	2.09	2.39			
"	"	7	11.31	24.45	23.31	24.24			216.24	206.01	214.34
"	"	14	13.40	25.90	26.99	24.35			193.04	201.37	181.68
"	"	21	18.37	22.92	22.32	22.02			124.78	121.52	119.88
Sucrose synthetase	Leaf	0	61.69	...	...	...	7.01	7.96			
"	"	7	61.62	67.27	82.95	97.87			109.17	134.61	158.78
"	"	14	72.05	123.49	81.65	104.50			171.39	113.33	145.04
"	"	21	57.67	92.80	90.92	118.02			160.68	157.64	204.63
"	Root	0	47.60	...	...	...	0.92	1.04			
"	"	7	27.43	33.89	33.65	38.55			123.59	122.69	140.54
"	"	14	55.49	109.74	85.77	89.73			197.78	154.82	161.71
"	"	21	47.07	61.64	51.95	70.00			130.94	110.37	148.87

*Q - Significant difference according to Student-Newman-Keuls' test

**CK - Control

The maximum stimulation of root sucrose phosphate synthetase activity, 116%, was induced by MH on the 7th day after treatment.

The stimulated sucrose synthetase activity in the leaves and roots of MH-treated plants followed the same pattern as that of sucrose phosphate synthetase, reaching a maximum level on the 14th day (Fig. 21 and 24). Although both PC and VS treatment significantly stimulated the activity of sucrose synthetase in the leaves, the maximum occurred on the 21st day, and the curves for the activity of the two enzymes differed. (Fig. 22 and 23). The activities of sucrose synthetase in roots of VS-treated plants are shown in Fig. 26, and of PC-treated plants in Fig. 25.

The effects of MH, PC, and VS on the activity of various enzymes have been summarized in Table XIV.

11. Simple correlation coefficients

The simple correlation coefficients have been determined to find out the relationship between the growth of the leaf and the various parameters measured after the treatment. These are given in Table XV. The data indicate that growth of the leaf in MH-treated plants was positively and significantly correlated with the reducing sugars, nitrite, nitrate, amino acids, protein and the total N content of the roots. It was also positively correlated with photosynthesis, respiration of the root, nitrate reductase (leaf and root), transaminase (root) and invertase activity (root and leaf).

In PC-treated plants the growth was correlated significantly and positively with reducing sugars, nitrite, nitrate and protein content of the root and nitrate reductase, transaminase

TABLE XIV

Summary of the effects of maleic hydrazide (MH), pyrocatechol (PC) and vanadium sulfate (VS) on enzyme activities

Enzyme		*Effects of treatment with MH			PC			VS		
Nitrate reductase	Leaf	-	-	-	-	-	-	-	-	-
"	Root	-	-	-	-	-	-	-	-	-
Transaminase	Leaf	-	-	-	-	-	-	-	-	-
"	Root	ns	-	-	-	-	-	-	-	-
Invertase	Leaf	-	-	-	-	-	-	-	-	-
"	Root	-	-	-	-	-	-	-	-	-
Phenyl-phosphatase	Leaf	-	-	-	-	-	-	-	-	-
"	Root	-	-	-	-	-	-	-	-	-
Adenosine tri-phosphatase	Leaf	-	ns	-	-	-	+	-	-	-
"	Root	-	-	+	ns	-	-	-	ns	-
Glucose-1-phosphatase	Leaf	-	ns	ns	-	ns	-	-	-	ns
"	Root	-	-	-	ns	-	-	ns	ns	ns
Glucose-6-phosphatase	Leaf	-	ns	ns	-	ns	+ns	-	-	-
"	Root	ns	-	-	-	-	-	-	-	-
Fructose-6-Phosphatase	Leaf	ns	-	-	ns	-	+	-	-	-
"	Root	ns	-	-	-	ns	-	-	+	-
UDGP-pyro-phosphorylase	Leaf	+	+	+	-	+	+	+	+	+
"	Root	+	+	+	+	+	-	+	+	+
Sucrose synthetase	Leaf	ns	+	+	+	+	+	+	+	+
"	Root	+	+	+	+	+	+	+	+	+
Sucrose-P-phosphetase	Leaf	+	+	+	+	+	+	+	+	+
"	Root	+	+	+	+	+	+	+	+	+

*The symbols +, -, refer to stimulation and inhibition. Three symbols for each treatment correspond to the harvest made 5, 14, and 21 days after treatment.
ns - means not significantly different from control at 0.05 and 0.01.

TABLE XV

Relationship of growth to different variables

Variables	*Correlation coefficients (r) MH	PC	VS
Growth	1.00	1.00	1.00
Sucrose	n.s.	n.s.	n.s.
Reducing sugar	0.627	0.577	0.878
Nitrite	0.612	0.583	0.579
Nitrate	0.981	0.872	n.s.
Ammonia	-0.691	-0.756	-0.659
Amino acids	0.828	n.s.	n.s.
Protein	0.946	0.844	0.815
Total N	0.874	n.s.	0.990
Photosynthesis	0.905	n.s.	n.s.
Respiration (root)	0.851	n.s.	0.791
Nitrate reductase (leaf)	0.698	0.907	0.817
" " (root)	0.628	0.585	0.727
Transaminase (leaf)	n.s.	n.s.	n.s.
" " (root)	0.646	n.s.	n.s.
Phenyl phosphate (leaf)	n.s.	-0.779	n.s.
" " (root)	-0.890	-0.906	-0.856
ATP-ase (leaf)	n.s.	n.s.	n.s.
" (root)	-0.618	-0.917	-0.791
Glucose-1-phosphatase (leaf)	-0.849	-0.946	-0.886
" " (root)	-0.893	-0.898	-0.925
Glucose-6-phosphatase (leaf)	-0.869	-0.575	-0.728
" " (root)	-0.913	-0.670	n.s.
Fructose-6-phosphatase (leaf)	-0.868	-0.760	-0.875
" " (root)	-0.841	-0.887	-0.875
Invertase (leaf)	0 803	0.866	0.755
" (root)	0.800	0.725	0.765

n.s. - not significant at .05 level

*Significant correlation coefficient (.05) = 0.576

and invertase activity of the root and the leaf.

In VS-treated plants, the growth was positively and significantly correlated with reducing sugars, nitrite, protein, the total N content and respiration of the root. The growth was also positively correlated with the activity of the enzymes nitrate reductase and transaminase (Table XV).

The sucrose concentration in the root of MH-treated beets was significantly and positively correlated with the nitrate and amino acid contents of the root, photosynthesis, and sucrose phosphate synthetase activity. It was significantly and inversely correlated with the protein content and the rate of respiration, ATP-ase, glucose-1-phosphatase, glucose-6-phosphatase, fructose-6-phosphatase, invertase, and nitrate reductase activity of the root and the leaf (Table XVI).

The sucrose concentration of the root of the PC-treated plants showed significant positive correlations with the activity of sucrose phosphate synthetase and negative correlations with protein, reducing sugars and ammonium content of the root. There was also a significant correlation between the sucrose content of the root and the activity of the enzyme, ATP-ase, glucose-6-phosphatase, fructose-6-phosphatase, invertase, nitrate reductase and transaminase (Table XVI).

VS-treated plants had significant positive correlations between sucrose and the nitrate content of the root and sucrose phosphate synthetase activity of the root and the leaf. The sucrose content also had significant negative correlations with protein content and the respiration of the root, all the phosphatases measured (except fructose-6-phosphatase), invertase,

nitrate reductase and transaminase activity of the root and the leaf (Table XVI).

TABLE XVI

Relationship of sucrose to different variables

(Storage root)

Variables	*Correlation coefficients (r) MH	PC	VS
Sucrose	1.00	1.00	1.00
Reducing sugar	n.s.	n.s.	n.s.
Nitrite	n.s.	n.s.	n.s.
Nitrate	0.780	n.s.	0.966
Ammonia	n.s.	-0.839	n.s.
Amino acids	0.838	0.913	n.s.
Protein	-0.809	-0.737	-0.892
Total N	n.s.	n.s.	0.587
Photosynthesis	0.723	n.s.	n.s.
Respiration (root)	-0.960	n.s.	-0.855
Phenyl phosphatase (leaf)	-0.880	n.s.	-0.834
" " (root)	-0.836	n.s.	-0.905
ATP-ase (leaf)	-0.758	-0.845	-0.666
" (root)	-0.977	-0.656	-0.947
Glucose-1-phosphatase (leaf)	-0.919	n.s.	-0.909
" " (root)	-0.945	n.s.	-0.632
Glucose-6-phosphatase (leaf)	-0.881	-0.798	-0.787
" " (root)	-0.859	-0.883	-0.972
Fructose-6-phosphatase (leaf)	-0.859	n.s.	n.s.
" " (root)	-0.782	-0.682	-0.907
Invertase (leaf)	-0.927	-0.822	-0.877
" (root)	-0.915	-0.826	-0.856
Nitrate reductase (leaf)	-0.973	-0.672	-0.912
" " (root)	-0.903	n.s.	-0.954
Transaminase (leaf)	n.s.	-0.765	-0.940
" " (root)	-0.902	-0.815	-0.938
Sucrose-P-synthetase (leaf)	-0.578	-0.692	-0.738
" " (root)	-0.930	-0.910	-0.689

*Significant correlation coefficient (.05) = 0.576

n.s. - not significant at .05 level

VI Discussion

MH inhibited the growth of the leaves and caused them to become narrower and curled. The effects appeared to be permanent. A similar result has been noted by Peterson and Naylor (1953) in tobacco. The authors suggested that possibly the marginal meristematic activity was more easily inhibited by MH treatment than the earlier developing mid-rib meristematic cells, the result being development of narrow leaves.

The inhibitory effect on NRase and transaminase activity in vivo, coupled with lower protein and higher amino acid content, suggest that either protein hydrolysis, or inhibition of the protein synthesis, or both, occur under the conditions of MH inhibition. Table XV lists the correlation coefficients of several variables measured with growth. Root and leaf nitrate reductase shows a significant positive correlation coefficient with growth. Root transaminase also has a significant positive correlation coefficient with growth. In view of the fact that glutamic acid or glutamine is synthesized mainly in the root of sugar beet (Joy, 1967),it is probable that transamination reactions are more important for amino acid synthesis in the root than in the leaves.

The NRase activity is related to protein producing potential of the plants and is important for the maintenance of the continued growth, have been demonstrated by several workers. For example, Croy (1967) found that NRase activity was linearly related to the total grain protein production in wheat, within a given genotype. Filner (1966) demonstrated that the repressors

of NRase of tobacco cells grown in suspension culture, inhibited the growth of the tissue and derepressors prevented this inhibition. The variation in the level of NRase with the age of the tissue (Shrader and Hageman, 1967; Wallace and Pate, 1967) and with the plant maturation (Zieserl et al. 1963; Yang, 1964; Wallace and Pate, 1967) also suggested the role of NRase in the regulation of growth.

The reduction in sugar beet NRase activity by MH during the present investigation can be explained on the basis of the possible interaction with the – SH groups of the protein (Isenberg, 1951). On the basis of his studies with C^{14}-MH, Noodén (1967) reported that MH can bind with proteins very tightly. Thus, inhibition of NRase by MH is also possible by the binding of MH with the enzyme protein.

Nitrate reductase is an inducible enzyme (Beevers et al. 1965; Filner, 1966; Shrader and Hageman, 1967). Beevers et al. (1965) found that the inhibition of DNA-dependent RNA synthesis resulted in the inhibition of the induction of NRase. Then, the reduction in the activity of NRase could also be possible by the inhibition of its synthesis by MH, because MH is known to inhibit DNA synthesis in whole intact corn roots in about 16 hours, and RNA synthesis a few hours later (Noodén, 1967)

Repression of NRase synthesis is possible by ammonium ions (Syrett and Morris, 1963) and amino acids (Filner, 1966). A larger content of ammonium and amino acids after MH treatment was recorded during the present investigation. The progressive increase in the soluble amino acid content of the roots and the corresponding decrease in the nitrate reductase activity of the

leaves and the roots suggest the possibility that amino acids might be taking part in the inhibition of nitrate reductase synthesis.

However, it is premature to suggest that inhibition of growth is only due to the effect of MH on nitrate reductase and transaminase activity in sugar beet. Several other types of significant effects have been noted during this investigation, and also by the others.

MH was discovered to have significant effects on the rate of plant respiration. The rate of respiration of beet storage root and of leaves during this investigation was reduced by 30 to 40% and 40% respectively. The respiration of the storage root was closely correlated with the growth. A significant correlation coefficient, 0.851, was found between the rate of respiration of the root and the growth of the leaves of sugar beet (Table XV).

Inhibition of the rate of respiration following MH treatment has been noted also by several workers. The respiration of root tips of a number of plant species has been found to be reduced, following MH treatment, and Brian (1964) concluded that MH competes for active sites of an enzyme concerned with respiration. MH interacts with the thiols, thus, the inhibition of succinic dehydrogenase is possible (Brian, 1964) and, in fact partial inactivation of one or more dehydrogenase has been suggested by Isenberg et al. (1951) as a result of studies on onion.

Invertase activity of leaf and root of sugar beet was inhibited by MH. Since invertase activity was found to be positively correlated with growth of the leaves (Table XV), the observed growth inhibition by MH during this investigation may also

be attributed in part to the inhibitory effect of MH on invertase. That invertase activity was associated with growth was also demonstrated by Wort and White (1956). In the work of these authors the tops of mature field grown sugar beets were frozen or removed by knife and the leaves allowed to regrow. In the extensive studies of the metabolic changes in sugar beet leucoplasts, Sisakjan and co-workers (1948, 1951, 1953) found that during the vegetative period of the sugar beet plant invertase activity of leucoplasts in the root increased greatly, while in the storage roots the invertase activity in leucoplasts decreased.

Hatch and Glasziou (1963) found that rate of elongation of internodes in sugar cane remained correlated with acid invertase activity irrespective of whether the independent variable was age of tissue, temperature, or water regime. Under enviornmental conditions which gave rapid growth of immature internodes, there was no net sugar storage in the mature internodes of the same stalk. The reverse was also true, indicating that growth and storage are reciprocally related, presumably because of competition for available photosynthates. In immature storage tissue of sugar cane, availability of carbohydrate in the cell cytoplasm appears to be regulated by the level of two acid invertases, one located in the outer space (which includes the cell wall) and the second in the storage compartment (Sacher et al. 1963). Control of invertase level appeared to these authors to be mediated through auxin, which may increase or decrease the level depending on concentration, and by a feed back system involving the level of sugar present in the metabolic compartment (cell cytoplasm).

Work with tissue slices from rapidly expanding internodes of sugar cane indicates that the level of invertase is a function of the balance between synthesis and destruction of m-RNA (Glasziou et al. 1966). From studies with inhibitors of protein and RNA synthesis, Glasziou and his coworkers (1966) concluded that auxin increases the rate of synthesis and glucose increases the rate of destruction of messenger RNA required for the production of invertase. Maleic hydrazide might then, cause a decrease in the level of invertase (i.e., its synthesis) acting as an anti-auxin. That MH has an anti-auxin property, has been demonstrated by Leopold and Klein (1951, 1952). It has also been reported to accelerate the oxidation of indoleacetic acid (Kenten, 1955).

Kaufman et al. (1968) found that the increase in invertase activity closely paralled the growth promotion that was caused by gibberellic acid (GA_3) in Avena stem segments incubated in the dark at 23°C. Cycloheximide, an inhibitor of protein synthesis abolished all GA_3-promoted growth and invertase activity in Avena stem segments. This again reveals the fact that invertase activity is associated with the growth and MH can inhibit it by its anti-auxin property or through the inhibition of DNA-dependent RNA synthesis required for protein synthesis.

MH can inhibit growth perhaps through several other sites. For example, Ito and Yoshinaka (1964) found that RNA and protein content in MH-treated onion was reduced. Noodén (1967) found that MH was bound strongly to protein within the plant. This might explain the inhibition of several enzymes by maleic hydrazide. Povolotkaya (1961) observed that uracil restored normal growth and development of MH-treated plants. He suggested that

MH functions as a uracil antimetabolic. Mcleish (1953) reported that mitosis in all actively growing tissue in a variety of plant species was suppressed by MH. This observation is consistent with the fact that DNA synthesis is inhibited by MH (Noodén, 1967). Thus MH can inhibit growth by inhibiting cell division and also auxin-induced cell elongation.

Phenylphosphatase, ATP-ase, glucose-1-phosphatase, glucose-6-phosphatase, and fructose-6-phosphatase were inhibited by MH, Moreover, significant negative correlations exist between growth and these activities. Thus, the hydrolysis of these substrates (phenylphosphate, ATP, glucose-1-phosphate and glucose-6-phosphate) does not favor growth in maleic hydrazide-treated plants. Maevskya and Alekseeva (1964) observed increased ATP-ase activity in apical buds of boron deficient sunflower plants before signs of deficiency were visible. Hinde and Finch (1966) noted increased activity of phenylphosphatase and ATP-ase in boron deficient bean roots. These works demonstrate that the increase in activity of these enzymes is associated with the decrease in the growth. This is understandable, because hexose phosphates are active intermediates of the respiratory pathways. Their removal from the pathway might cause an inhibition of the respiration through glycolysis and the Krebs cycle. The inhibition of respiration will lead to cessation of growth. ATP is required for the activation of amino acids for protein synthesis. High rate of the hydrolysis of ATP by ATP-ase may cause an inhibition of the protein synthesis and hence growth.

The inhibition of phosphatases by maleic hydrazide is possible either by its binding with the protein of these enzymes

or by diminution of the DNA and RNA required for the synthesis of the enzyme proteins.

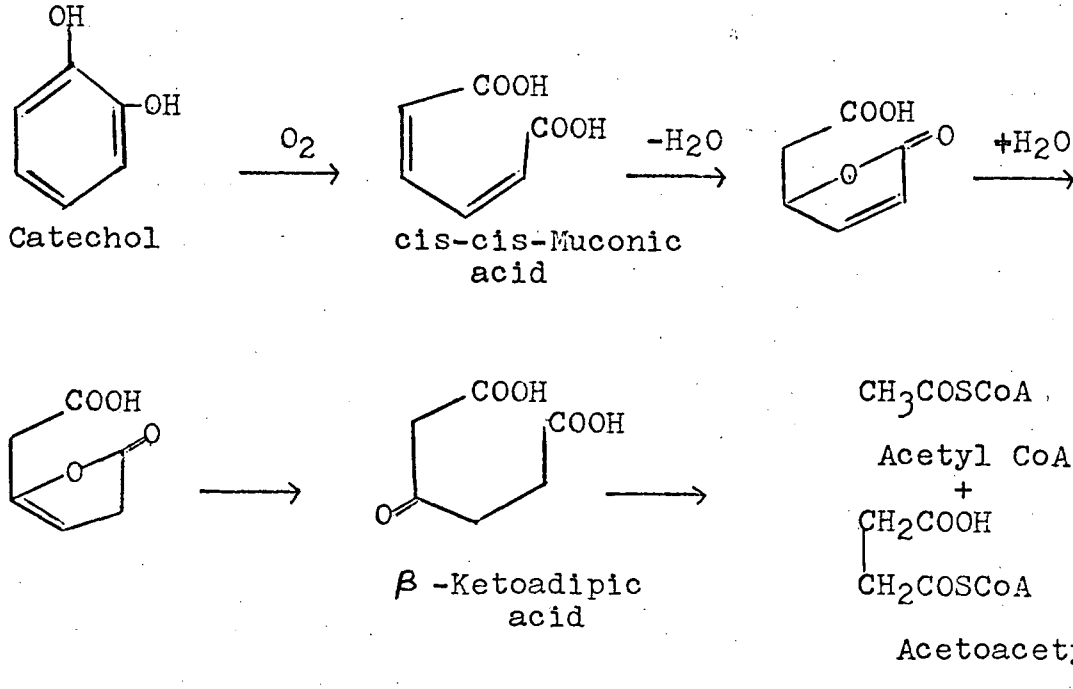
PC was found to inhibit growth of leaves more effectively than MH and VS. Like MH it also inhibited nitrate reductase, transaminase, invertase and phosphatases, but not respiration, rather it stimulated the respiration of leaves (dark respiration) by 44% on the 7th day after treatment. The respiration of root was also slightly stimulated. The growth in PC-treated plants was significantly correlated with reducing sugars, nitrite, amino acids and protein content of the root and activity of invertase, nitrate reductase and transaminase. In these respects, results of PC treatment closely resemble those obtained by MH.

Yang (1964) demonstrated, in vitro, that sugar beet nitrate reductase was inhibited completely by PC at a concentration of 1×10^{-4} M. Nitrate reductase is a sulfhydryl containing enzyme. Inactivation of this, and other enzymes responsible for protein synthesis and growth regulation is possible, if one considers PC to be an o-diphenol, easily oxidized by o-diphenol: O_2 oxido-reductase (o-diphenol oxidase) to quinone. O-diphenol oxidase is very commonly found in higher plants (Shiroya et al. 1955; Clayton, 1964; Pierpoint, 1966). That tannins and quinones inhibit enzymes through the -SH group was shown by Mason, 1955; Young, 1965; and Slack, 1966. Anderson and Rowan (1967) found that thiols or other reducing agents protect enzymes containing -SH group from inactivation by quinones and tannins. Transaminase, invertase and phosphatase may be inhibited by PC either through the oxidation of their sulfhydryl groups or through the binding of PC or its polymers to the protein.

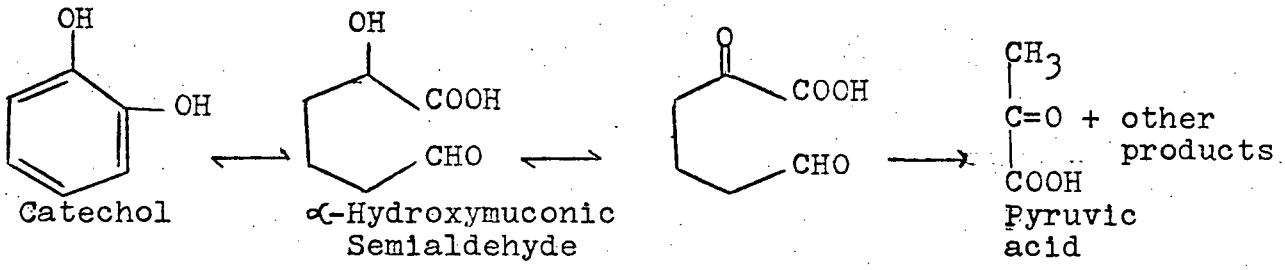
The inhibition of nitrate reductase synthesis in vivo by PC is perhaps possible on the basis of the facts that various plant phenols inhibit protein synthesis in excised plant tissues, (Parupus, 1967) and some of the phenolic compounds have been found to inhibit the induction of nitrate reductase in corn (Shrader and Hageman, 1967). The possibility of repression of nitrate reductase by ammonium and amino acid also exists on the basis of increased ammonium and amino acid content of the roots and a concomitant decrease in NRase activity following PC treatment, recorded during the present investigation.

The high rate of respiration in PC-treated plants may be explained by PC's participation in respiratory metabolism in plants and micro-organisms. Towers (1964) has recently reviewed the role of PC as the key intermediate in the breakdown of benzene and simple phenols especially in micro-organisms in the following two ways:

(1)

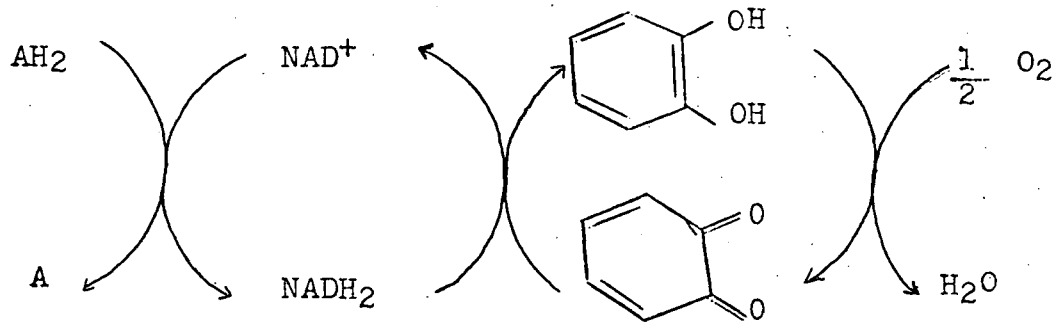


(2) an alternate pathway is also possible:



However, participation of PC as a respiratory substrate in higher plants has not been demonstrated in the above manner.

Wort and Shrimpton (1959) in their experiment with mature sugar beet root discs, observed that the addition of catechol to the medium resulted in a very considerable increase in respiration. Thus, the proposal that has received considerable attention is that o-diphenol together with various phenol oxidases, functions as a terminal oxidase as illustrated below with catechol:



As long as the o-quinone is rapidly reduced, before it can polymerize or be degraded by other reactions, such a system will remain cyclic. o-Diphenol oxidase mediates the aerobic oxidation of pyrocatechol to o-quinone. Quinone reductase catalizes the reduction of quinones with NADH.

While it cannot be denied that the capacity of this type of oxidation exists in many plants, the utilization of these enzymes in this fashion in the intact plant seems negated by a number of observations (Nakabayashi, 1954). It must also be pointed out

that it has not been possible to demonstrate coupled oxidative phosphorylation with the above oxidase system, and until this is achieved it seems preferable to assign the respiratory role of plants to the cytochrome-containing systems (Hanson and Zucker, 1967). It appears then, that although rate of CO_2 evolution and oxygen consumption was increased by PC during the present investigation, it was not at the expense of sugar stored or sugar newly formed in the leaves. High sucrose percentage of the roots supports this contention. This increase in rate of respiration (CO_2 evolution) was, perhaps, unable to generate ATP necessary for growth, and that may be the reason that rate of respiration in PC-treated plants was not significantly correlated with the growth of the leaves (Table XV).

The respiration under fall conditions was inhibited by PC. It would seem that the action on respiration of this compound is temperature dependent. Perhaps one of the enzymes necessary for the active participation of PC in terminal oxidation was inactivated under low temperature conditions. Possibly it was quinone reductase, because in the absence of the activity of this enzyme quinone may actively participate in the inactivation of the enzymes of Krebs cycle containing - SH groups, for example, α-ketoglutaric dehydrogenase and succinic dehydrogenase. Inhibition of respiration may then be possible, under such conditions.

Vanadium belongs to the transition group metals along with titanium, chromium, manganese, and iron. Little information is available on the effects of vanadium in biological systems. Previous investigations have indicated a role for this metal in: (a) replacing molybdenum in microbial nitrogen fixation pathway

(Burk, 1934). (b) in effecting alteration in mammalian lipid oxidation and synthesis (Curran and Costello, 1957), (c) in counteracting the effect of Mn, and possible interaction with it in flax, soybean and oat (Warrington, 1957), (d) in inhibiting the growth of Mycobacterium tuberculosis being competitive with Mn and chromium (anatagonistic) (Costello and Hedgecock, 1959), (e) in inhibiting nitrate reductase very effectively in wheat embyro (Spencer, 1959) and sugar beet (Yang, 1964), (f) in the uncoupling of oxidative phosphorylation in mitochrondria isolated from liver of chicks (Hathcock et al. 1961).

In the present investigation, VS was found to inhibit the growth of sugar beet leaves under both summer and fall conditions. The inhibition of growth was accompanied by the inhibition of activity of the enzymes nitrate reductase, transaminase, invertase and phosphatases. Respiration was also inhibited. Significant correlation of the growth with nitrate reductase, invertase and respiration might explain the possible cause of growth inhibition by vanadium.

Vanadium being a heavy metal may inhibit nitrate reductase through its action on sulfhydryl groups. It can also interact with molybdenum, the prosthetic metal for nitrate reductase, or may bind with the enzyme protein as Rockold and Talvitie,(1956), demonstrated.

Inhibition of respiration rate by VS may be explained on the basis of its possible interaction with Mn (Costello and Hedgecock, 1959; Warrington, 1951). Mn is required for the activity of various enzymes of the glycolytic pathway and Krebs cycle, viz. hexokinase, isocitric dehydrogenase, malic dehydro-

genase, malic enzyme, oxaloacetate decarboxylase and condensing enzyme. It is thus possible for vanadium to inhibit the activity of the above mentioned enzymes, especially isocitric dehydrogenase and malic dehydrogenase where Mn is the absolute requirement, by counteracting the effect of Mn.

Mn is also part of some of the enzymes of nitrogen metabolism. A Mn flavoprotein was associated with nitrite reductase, the enzyme responsible for the reduction of nitrite to hydroxylamine (Nason et al. 1954). Thus, nitrite reductase may also be inhibited by vanadium.

Alexander (1965) found in sugar cane that active acid invertases are protein - sugar - Mn complexes, in which the protein constituent is virtually inactive in the absence of Mn or sugar. If that is true for sugar beet invertase, vanadium inhibition of invertase could be interpreted as a counteraction of Mn in the active invertase system.

Increase in sucrose content of the root

Increase in sucrose content of the root of sugar beet was recorded after the treatment of plants with MH, PC and VS during the present investigation. Increase in percent sucrose following to MH application to sugar beet has been noted by many other workers (Wittwer and Hansen, 1952; Kalinin et al. 1965). Excessive sucrose accumulation has been noted to result from MH application to other plant species also (Greulach, 1953; Samborski and Shaw, 1957; Peterson and Naylor, 1953; Alexander, 1965)

The larger sucrose content observed in the root of sugar beet may be the result of the following: (a) stimulation of

photosynthesis, (b) inhibition of invertase activity, (c) inhibition of respiration, (d) stimulation of sucrose synthesizing enzymes (e) inhibition of the enzymes which hydrolyze the substrates of the sucrose synthesizing enzymes e.g., phosphatases. These mechanisms will involve the inhibition of many processes and stimulation of others. This was encountered in the present investigation.

Stimulation of the rate of photosynthesis was observed in treated plants. Perhaps this was associated with the inhibition of nitrate reductase. Cramer and Myers (1948) showed that during photosynthesis by chlorella at low light intensities nitrate utilization was accompanied by a decrease in the assimilation quotient from 0.9 to 0.7, owing to a decreased carbon dioxide uptake. Yang (1964) found that nitrate reductase activity was high during the day in the leaves of sugar beet. Presumably, photosynthesis supplies reduced coenzymes toward nitrate reduction. The channeling of reduced coenzymes toward nitrate reduction might cause a reduction in the rate of photosynthesis in the plants when nitrate reductase activity is very high. Inhibition of nitrate reductase then, might result in a stimulation of carbon reduction in photosynthesis. This may have happened in the treated plants during this experiment.

Photosynthesis of red kidney beans has been found to be increased by maleic hydrazide (Sorensen, 1956). Callaghan and Norman (1956) also observed an increase in the rate of photosynthesis in Swiss chard and tobacco. They explained the increased rate of photosynthesis on the basis of higher chlorophyll content per unit area of leaves after treatment with higher concentrations

of maleic hydrazide.

Maleic hydrazide and vanadium inhibited the growth of the leaves. Mutual shading of the leaves, in that situation will be minimized, which will result in the higher efficiency of the leaves to photosynthesize. This could be another reason for the observed increase in the rate of photosynthesis per unit of leaf area.

Observed stimulation of the enzymes of sucrose synthesis may also explain the increased percentage of sucrose in the treated plants.

It might seem paradoxical for an enzyme inhibitor to increase the rate of some phase of metabolism, but actually such stimulation is not uncommon. "It is probably justifiable to say that the greater the number of interrelated enzymes in the total system, and greater the complexity and organization of the metabolic pathways, the more likely will it be that stimulation can occur" (Webb, 1963). Examples of such a phenomenon are many. Arsenite, although a general enzyme inhibitor has been found to stimulate papain, malic dehydrogenase (Green, 1936), uricase (Mahler et al. 1955), and the Pi - ATP exchange enzyme (Plant, 1957). In the present experiment also, we note similar phenomena. Although maleic hydrazide, pyrocatechol and vanadium sulfate inhibit the activity of nitrate reductase, transaminase, invertase and phosphatase, they stimulate the activity of sucrose phosphate synthetase, sucrose synthetase and UDPG-pyrophosphorylase.

Webb (1963) suggests some of the ways by which stimulation of the enzyme could be achieved by an enzyme inhibitor:

(a) removal or inactivation of some inhibiting substance, (b) alteration of metabolic flow in multienzyme e.g., in a divergent polylinear chain when one pathway is inhibited, another is stimulated. There the appearance of stimulation will depend on the aspect of the total system one is examining. This mechanism is actually a diversion of metabolism rather than a true stimulation. (c) removal of a dynamic equilibrium (d) depression of a reaction-controlling mechanism. The ability of a living cell to adjust its metabolism according to its functional activity makes it necessary for the principal metabolic pathways to be under some sort of controlling or regulating mechanism. The removal of the restricting control will manifest itself as stimulation. (e) the inhibition of an enzyme that destroys some important metabolic substance may increase the steady state level of this substance and accelerate reactions with which it is concerned. Inhibition of ATP-ase, for example can secondarily influence many reactions dependent on ATP. The inhibitor may also increase permeability in some manner and thus stimulate reactions whose rates are limited by the access of the substrate, or it may either directly or indirectly damage the membrane so that the structural disorganization will allow reactions to be released from their normal control, which may be simply a spatial separation of reactant.

The inhibitors applied in this experiment with sugar beet have effects which suggest alteration of metabolic flow in multienzyme systems. One pathway was inhibited, the other was stimulated. The pathways of protein synthesis, sucrose hydrolysis and carbohydrate breakdown (respiration) were inhibited. The pathway

for the synthesis of sucrose was stimulated. The interrelationship of these systems is given in Fig. 1, page 20.

Nitrate reductase and sucrose relationship

The inverse relationship of sucrose concentration and the activity of nitrate reductase is evident from the results obtained during the present investigation. It has been pointed out earlier that the conversions of nitrate to nitrite, hydroxylamine and ammonia are energy-requiring processes which must be coupled to carbohydrate breakdown. This was clearly demonstrated by Hamner (1935) who found that the application of nitrate to nitrogen starved tomato plants resulted in the formation of nitrite, the depletion of carbohydrate reserves, and a marked increase in respiration. The data from the experiment with sugar beet by Snyder and Tolbert (1966) suggest that if the nitrogen supply is not cut down plants may preferentially synthesize the citric acid cycle products and their amino acid counterparts, and thus produce less sucrose. Inverse relationship between nitrate content of the petiole and the sucrose percentage was shown by Ulrich (1950) in sugar beet. It seems likely that this relationship requires hexose degradation both for the supply of energy needed for nitrate reduction and as a source of material for organic acid synthesis. Thus, the low nitrate reductase activity coupled with weak invertase and phosphatase (hexose monophosphatases and ATP-ase) activities would favor sucrose accumulation. This agrees well with the experimental results.

The situation which is encountered in the treated plants is that perhaps the carbohydrates or other energy yielding

substrates are abundant but amino nitrogen is absent due to inhibition of nitrate reductase and transaminase (perhaps glutamic dehydrogenase also). The resultant inability of the cell to synthesize protein and carry out energy-requiring growth processes would result in accumulation of ATP. Inhibition of ATP-ase activity would further favor the accumulation of ATP. This compound is a negative effector of citrate synthetase which catalyzes the entry of acetyl CoA into the Krebs cycle, thus under these conditions the cycle will compete weakly for acetyl CoA (Atkinson, 1965).

When the concentration of ATP is higher, the AMP concentration is necessarily low and isocitric dehydrogenase will be inhibited (Hathway and Atkinson, 1963). AMP modulates the catalysis by phosphofructokinase of fructose-6-phosphate to fructose diphosphate. ATP is the negative effector of phosphofructokinase. Thus, in a cell with high ATP and low AMP concentrations the kinetic behavior of phosphofructokinase will be such as to lead to high concentrations of fructose-6-phosphate and its precursor glucose-6-phosphate. The reversal of Krebs cycle and glycolytic pathway will be favored in energy rich cells. The result will be less utilization and more storage of the carbohydrates and lipids (sucrose in case of sugar beet, starch or fats in other plants, glycogen or fats in animals). This kind of control mechanism by ATP and AMP (or ADP) is designated by Atkinson as "regulation by adenylates." ATP, ADP or AMP are termed "regulatory effectors."

Phosphatases and sucrose biosynthesis

The studies of Leloir and co-workers (1953, 1955) with wheat germ, Rorem et al. (1960) with sugar beet leaf, Dutton et al. (1961) with sugar beet root, Hatch et al. (1963) with sugar cane storage tissue, Bird et al. (1965) with tobacco leaf chloroplasts, and Haq and Hassid (1965)with sugar cane leaf chloroplasts have all provided convincing evidence which is consistent with the synthesis of sucrose by following overall reactions:

(1) ATP + UDP $\xrightleftharpoons[\text{nucleoside diphosphate kinase}]{}$ UTP + ADP

(2) Glucose-6-phosphate $\xrightleftharpoons[\text{phosphoglucomutase}]{}$ Glucose-1-phosphate

(3) UTP + glucose-1-P $\xrightleftharpoons[\text{UDP-glucose pyrophosphorylase}]{}$ UDP-glucose + pyrophosphate

(4) Fructose-6-phosphate + UDP-glucose $\xrightleftharpoons[\text{sucrose-6-phosphate synthetase}]{}$ sucrose-P+UDP

(5) Sucrose - P + H_2O $\xrightarrow[\text{sucrose-6-P phosphatase}]{}$ sucrose + H_3PO_4

An alternate system would substitute sucrose synthetase for reaction (4) and (5)

(6) Fructose + UDP - glucose $\xrightleftharpoons[\text{sucrose synthetase}]{}$ sucrose + UDP

The result of the present investigation indicates that sucrose-6-phosphate synthetase is more active than the sucrose synthetase in the leaf of the sugar beet. The results obtained by Burma and Mortimer (1956) with sugar beet leaves also indicated that sucrose-6-phosphate synthetase was the enzyme mainly

concerned with sucrose production. Bird et al. (1965) suggested that synthesis of sucrose as a result of photosynthesis in leaves proceeds mainly, if not solely, via sucrose phosphate in chloroplasts. Furthermore a consideration of the equilibrium constants for the two synthetases shows that sucrose phosphate rather than sucrose synthesis is favored (Mendicino, 1960). A specific sucrose phosphatase from the stem tissue and leaves of sugar cane which catalyses the hydrolysis of sucrose-6-phosphate to sucrose and phosphate was isolated by Hawker (1966). Sucrose-6-phosphatase according to Hawker is situated near or at the tonoplast membrane of the vacuole. It is possibly concerned with movement and accumulation of sucrose.

Hexose phosphatases and ATP-ase were inhibited by MH, PC, and VS during the present investigation. The influence of these phosphatases on sucrose biosynthesis is evident from the involvement of hexose phosphates and ATP in the overall scheme of the synthesis. ATP could enter the scheme outlined above via its ability to phosphorylate UDP to UTP (Reaction 1). Glucose-6-phosphate and glucose-1-phosphates are involved in reactions, (2) and (3). Fructose-6-phosphate supplies the fructosyl moiety of the sucrose molecule. The enzymes ATP-ase, glucose-1-phosphatase, glucose-6-phosphatase and fructose-6-phosphatase would be able to alter the equilibrium of Reactions (1), (2), (3) and (4) and may be able to inhibit the sucrose synthesis as a whole. The inhibition of these during the present work, thus favors sucrose biosynthesis.

The correlation coefficient values for different phosphatases with sucrose concentrations of the root are given in

Table XVI. All the phosphatases had a negative correlation with the sucrose. The following correlation coefficient values for ATP-ase, glucose-1-phosphatase, glucose-6-phosphatase and fructose-6-phosphatase with sucrose phosphate synthetase provide evidence that the phosphatases were negatively correlated to the enzyme sucrose phosphate synthetase.

CORRELATION COEFFICIENTS (r)

Variables	ATP-ase	Glucose-1-phosphatase	Glucose-6-phosphatase	Fructose-6-phosphatase
	1.00	1.00	1.00	1.00
Sucrose-P-synthetase (MH)	-0.871	-0.340	-0.288	-0.636
" " (PC)	-0.886	-0.531	-0.302	-0.838
" " (VS)	-0.810	-0.302	-0.116	-0.914

Significant r (.05) = 0.57

Alexander (1965) reported that the inhibitions of phosphatase by molybdenum resulted in the higher sucrose content in the sugar cane. He also noted the inverse relationship between ATP-ase activity and the sucrose content in the leaves of sugar cane (Alexander, 1965a).

Nitrogenous constituents of the roots

High nitrate and low nitrite content of the treated plants could be logically interpreted on the basis of low nitrate reductase activity due to inhibition of MH, PC and VS.

Increase in nitrate nitrogen in the molybdenum deficient plants was noted even before the actual discovery of nitrate reductase (Leeper, 1941; Hewitt and Jones, 1947). The vanadium, supplied through the roots to flax, oats and soybeans, caused a

rise in nitrate nitrogen was noted by Warrington (1951). A greater nitrate content in MH-treated tobacco plants was recorded by Peterson and Naylor (1953). All these results could be explained on the basis of inhibition of nitrate reductase activity.

MH treatment resulted in an increase in the ammonium content of the roots. High ammonia content suggests that either ammonia was not incorporated into the amino acids, possibly by the inhibition of glutamic acid dehydrogenase, or some of the amides are hydrolyzed and give rise to more free ammonia.

Amino acid content of the root of MH-treated plants was lower than the control 7 days after the treatment but higher on the 14th and 21st day. That the interval from treatment to harvest is one of the important factors in determining changes within the plant, is well illustrated by the above results. Also Fults and Payne (1956) and Livingston (1954) noted a similar trend in MH-treated sugar beet plant, where they found that 5 days after treatment the content of alanine, lysine, tyrosine and glutamic acid was lower in the roots of treated plants, but 60 days after spraying all of these amino acids were present in larger amounts in the treated roots.

The increase in free amino acids after MH application was also noted by Peterson and Naylor (1953) who found that treated tobacco plants had more soluble nitrogen including free ammonia, glutamine, asparagine, uncombined amino acid, and nitrate nitrogen but less protein. An increase in alcohol-soluble N was noted in wheat by Samborski and Shaw (1957) after MH treatment.

In the Pc-treated plants ammonia was less than in the control plants up to 14 days after treatment, but it was more on the 21st day. The possibility of increased formation of ammonia through nitrate reductase is very small, because 21 days after treatment, PC-treated plants had the lowest rate of nitrate reductase activity in the leaf. Then, the increase in free ammonia might be due to a greater breakdown of amides by 21 days after treatment.

The amino acid content of the PC-treated beets was lower than the control on the 7th day after treatment but higher on the 14th and 21st day. The lower rate of nitrate reduction and of transamination which was measured, suggests a slowing of new amino acid synthesis. Any increase in amino acid content might be accounted for on the basis of protein degradation. Low total nitrogen and protein content of the roots support this suggestion.

In VS-treated plants amino acid, ammonium, and protein content was lower than in the control plants. The lower protein content and low activity of nitrate reductase and transaminase provide the evidence that protein formation was inhibited by vanadium through nitrate reductase.

It has been shown by Klotz (1954) that Mn stabilizes the peptidases and the amidases and by Warrington (1951) that vanadium counteracts with Mn. If that be true, vanadium could cause an inhibition of the enzymes responsible for amide and peptide hydrolysis, hence would exclude the possibility of an increase in ammonia and amino acids by degradation of protein or the amides. Perhaps this is why an accumulation of the ammonia and amino acids

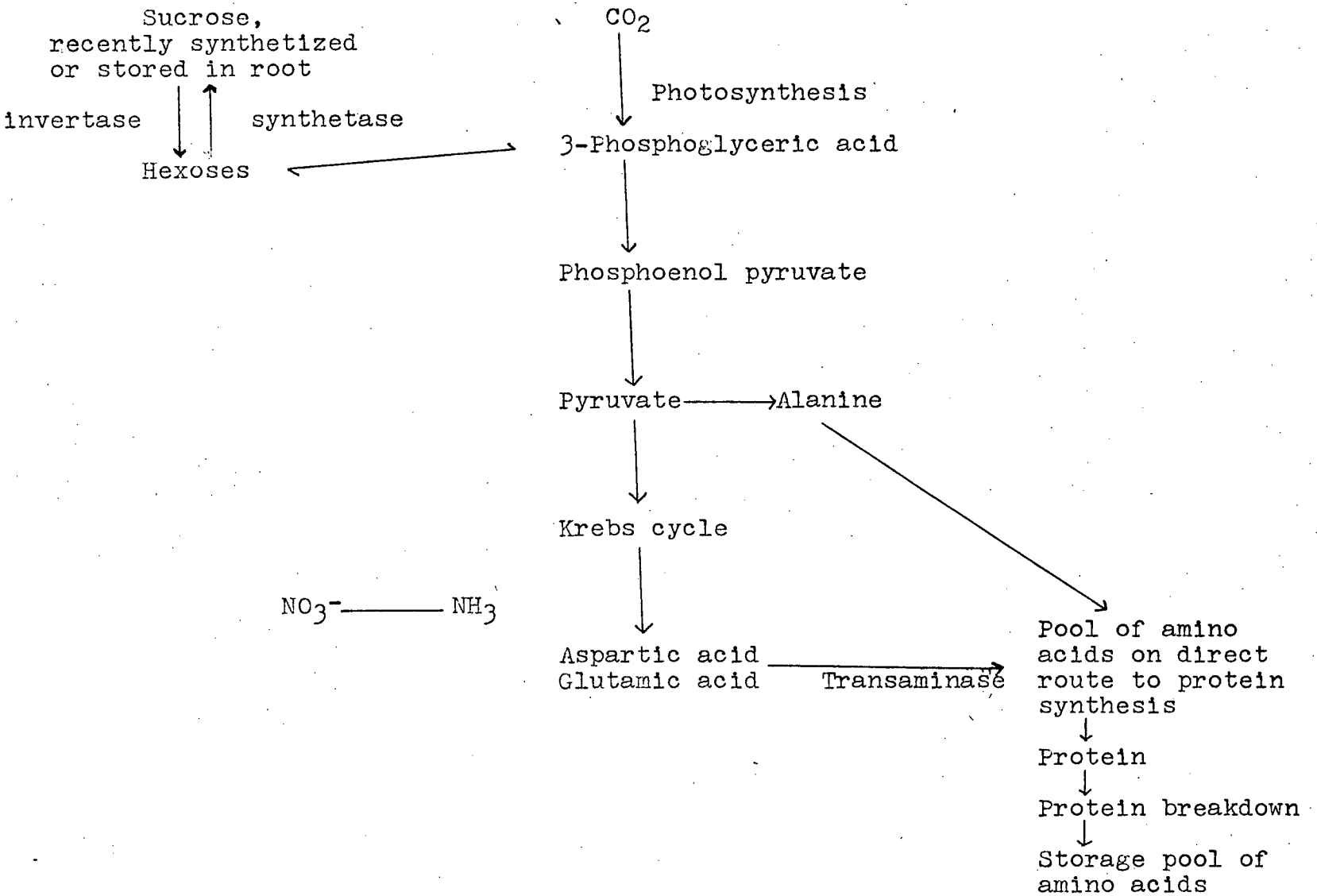
in the VS-treated plants during the present investigation was not recorded.

That amino acids derived from protein breakdown may accumulate and may not be utilized for protein synthesis was noted long ago by Gregory and Sen (1937). Steward and Bidwell (1965) point out that (a) the mere presence of free amino acids in the cell is not sufficient to ensure their direct incorporation into the protein, in fact they may well be debarred from this. (b) only if the protein precursors are generated at the right site, or alternatively from the right source (i.e., one which reaches this site) may they be incorporated directly into the protein, and they can do so without mingling with the free amino acid pool. (c) These situations can prevail only in a highly organized system with discrete compartments, the identity and separate functions of which can be physically or chemically maintained and subjected to some form of regulatory control.

Hellebrust and Bidwell (1963) found that in wheat leaves, the protein-bound serine and glycine were derived by a route which bypassed the bulk of soluble pools of amino acids. Bidwell et al. (1964) have presented considerable evidence for the existence of two types of amino acid pools in carrot tissue, one being a "protected pool of amino acids en route to protein" into which carbon from sugars could pass, and the other being a storage pool into which exogenously supplied amino acids passed and which is mainly the product of breakdown of proteins. Davies and Cocking (1967) found that amino acids labelled after supplying C^{14} bicarbonate pass more readily into proteins, than amino acids exogenously supplied. It was suggested by these authors

that different types and pools of amino acids exist in the locule cells of tomato.

The above considerations suggest that in MH- and PC-treated plants the excess amino acids (soluble) are the degradation products of proteins and they are not taking part in the synthesis of new proteins. The following diagram based on the discussion presented in the preceding paragraphs will illustrate the position of the two amino acid pools in sugar beet.


Sucrose,
recently synthetized
or stored in root
invertase
synthetase
Hexoses
CO_2
Photosynthesis
3-Phosphoglyceric acid
Phosphoenol pyruvate
Pyruvate
Alanine
Krebs cycle
NO_3^-
NH_3
Aspartic acid
Glutamic acid
Transaminase
Pool of amino
acids on direct
route to protein
synthesis
Protein
Protein breakdown
Storage pool of
amino acids

CONCLUSIONS

The results show that the foliar application of MH, PC, and VS to sugar beet evoked a number of similar responses by the plants: (1) leaf growth inhibition (2) reduction in the content of reducing sugars, nitrite, and protein in root tissue (3) increase in the content of sucrose and nitrate in root tissue (4) inhibition of the activity of nitrate reductase, transaminase, invertase and phosphatases in leaf and root (5) stimulation of the enzymes of sucrose biosynthesis in leaf and root.

Some responses were dissimilar: (1) while MH and PC caused an increase in the content of ammonia and amino acids of the storage roots, VS caused a decrease in the content of these constituents (2) the rate of respiration of the storage roots and the foliage was reduced by MH and VS but not by PC (3) the rate of net CO_2 assimilation by intact plants was increased by VS and MH. PC also caused a significant increase, except on the 7th day after treatment.

On the basis of the results obtained, and their statistical analysis, it seems reasonable to conclude that:

(1) The growth of the sugar beet leaves was positively correlated with the reducing sugar, nitrite, and protein content, and with the activity of nitrate reductase, transaminase and invertase. The reduction in each of these items, following the application of MH, PC, and VS to the plant, may then be a causal factor in the reduction of growth.

(2) the sucrose content of the roots was negatively correlated with invertase and phosphatase activity and positively correlated with sucrose phosphate synthetase and sucrose synthetase activity. The increase in sucrose, resulting from use of the three regulators may have been the result of their inhibition of invertase and phosphatases and their stimulation of the enzymes of sucrose synthesis (sucrose synthetase and sucrose phosphate synthetase)

(3) although MH, PC, and VS apparently acted at the same sites in several instances, the magnitude of their effects on the chemical composition or metabolic processes was different

(4) for each chemical, the interval from treatment to the day of observation was one of the important factors in determining the nature and magnitude of changes within the plant (growth, chemical composition or metabolic processes)

(5) the general enhancement of sucrose percentage of the roots by MH, PC, and VS indicated their importance as agents for the control of growth and the induction of"ripening" of the roots of sugar beet. However, VS exhibited most suitable properties from the standpoint of practical sucrose production in that it also decreased the content of ammonia and amino acids of the roots.

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