GROWTH AND BIOCHEMICAL RESPONSES OF THE TOMATO (*Lycopersicum esculentum* var. Bonny Best) to K NAPHTHENATES

by

SOONG-MING CHU

B.Sc., New Asia College, Chinese University of Hong Kong, Hong Kong, 1966

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the Department of

Botany

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

April, 1969

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and Study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Botany

The University of British Columbia
Vancouver 8, Canada

Date April 18, 1969

ABSTRACT

Recent reports, especially those of Russian scientists, have emphasized that application of stimulatory concentrations of naphthenates (Naps) induced greater and better growth and productivity of a number of species of plants. This stimulatory action of Naps has been found to result from seed soaking and spraying seeds or developing plants once or repeatedly. However, no systematic attempt has so far been made to investigate physiological and biochemical changes induced in a relative short period following immediately these treatments. A correlation of such changes with final improvements in growth and yield may provide a better understanding of the mechanism of action of Naps. It was therefore essential and significant to investigate these aspects.

Seeds of tomato (Lycopersicum esculentum var. Bonny Best) were germinated in wooden flats containing sterilized soil and were transplanted when 10 days old to plastic pots of 6 inches diameter containing sterilized soil. The plants were grown in a growth room. In separate experiments, potassium naphthenate (KNap) aqueous solutions, 2,500 ppm and 5,000 ppm, were sprayed onto tomato leaves when plants were 2, 3, and 4 weeks old.

Measurements of vegetative growth, based on fresh and dry weights of plant tops, indicated that maximum stimulation was induced by the 5,000 ppm KNap solution applied to plants when

3 weeks old. It was then decided to investigate the biochemical and physiological responses of the tomato plants to 5,000 ppm KNap when treated at the age of 3 weeks.

Determinations of pigment content, intensities of photosynthesis and respiration, activity of enzymes involved in nitrogen metabolism, such as nitrate reductase (NRase) and glutamic-pyruvic transaminase (transaminase), and of enzymes involved in carbohydrate metabolism, such as succinic dehydrogenase, phosphorylase, and phosphoglyceryl kinase were made three times at 2-week intervals, beginning 2 weeks after the spraying. Number and fresh weight of tomato fruits, quality of tomato fruits in terms of sugars, titratable acidity and ascorbic acid were also investigated at scheduled intervals.

Results indicated the following: (1) In the treated plants, the content of the pigments chlorophyll a and b, and especially carotenoid, in the leaf blades was higher than in control plants, (2) Measurements made with intact plants using an infrared CO_2 analyzer revealed increases in intensities of photosynthesis and respiration of the aerial portions 4 weeks after treatment but the opposite was true 2 weeks after treatment, (3) Under the influence of KNap, of the 5 enzymes examined only phosphorylase activity was found to be stimulated at all three observation times. Transaminase activity was greater 6 weeks after treatment. Activities of succinic dehydrogenase, NRase,

and phosphoglyceryl kinase were all reduced by treatments, (4) In a subsequent experiment, leaf blades of plants treated when 2 weeks old were analyzed for succinic dehydrogenase activity 4, 8, 12, 16, 20, and 24 days after spraying. The effect on succinic dehydrogenase activity fluctuated with the age of the plant. Parallel changes in the protein content of the enzyme extract could not be detected, (5) Tomato fruit yield, based on number and fresh weight, was decreased by 2,500 ppm KNap treatment but increased by 5,000 ppm KNap. In addition, 5,000 ppm KNap-treated plants were more resistant to blossom-end rot and showed better and quicker recovery when the deficiency disease was treated with $CaCl_2$. Earlier maturity was found in 5,000 ppm KNap-treated plants, (6) The mature tomato fruits from 5,000 ppm KNap-treated plants contained larger amounts of sugars (reducing sugar and sucrose) than the controls, and the sugars in mature tomato fruits were lost at a lower rate during the storage period. The treatment resulted in decreased titratable acid and ascorbic acid content. It afforded no protection against loss of titratable acid and ascorbic acid during storage.

ACKNOWLEDGEMENT

The author wishes to take this opportunity to extend her deepest thanks and appreciation to Dr. D.J. Wort who not only suggested the original idea for this study, but who has given his guidance, advice, encouragement, and patience to the author throughout the progress of this investigation. Furthermore, the author wishes to thank him for his constructive review of the entire manuscript.

Sincere gratitude is also due to Dr. G.H.N. Towers, Dr. D.P. Ormrod, and Dr. B.A. Bohm for their moral support and advice through this study.

Particular appreciation is owed to Dr. G.W. Eaton for his generous help in statistical analysis for this thesis.

Cordial thanks go to Mr. K. Patel who has helped the author in many ways.

Finally, acknowledgement is expressed to the Department of Botany for the use of all available facilities and equipment to make the experiments possible.

TABLE OF CONTENTS

TABLE OF CONTENTS (cont'd)

TABLE OF CONTENTS (cont'd) Page

LIST OF FIGURES

LIST OF FIGURES (cont'd)

LIST OF FIGURES (cont'd)

LIST OF TABLES

I. NAPHTHENIC ACIDS

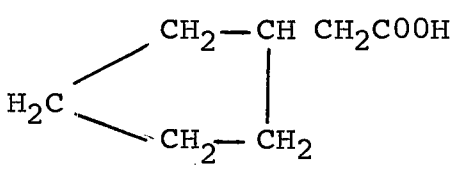
A. Introduction

The name, naphthenic acids, was suggested in 1883 by Markovnikoff and Ogloblin for the $C_{11}H_{20}O_2$ acids of unknown structure which Hell and Medinger had discovered from Rumanian oil.

Currently, the term is used to denote the carboxylic acids occurring in and recovered from petroleum. They are frequently termed "petroleum acids", because subsequent work has shown that phenols and aliphatic acids are also present in some crudes. Commercial naphthenic acid (HNap) is a product which contains all the acidic components of the crude, and varying amounts, usually less than 10%, of "oil", that is to say nonacidic compounds mostly hydrocarbons.

B. Composition

Chemically, HNaps are monocarboxylic acids of naphthene (alicyclic) series of hydrocarbons. Their general formula may be written $R(CH_2)_nCOOH$, where R is a cyclic nucleus composed of one or more rings. These rings are usually 5-membered (cyclopentane) and may be alkylated. The simplest acid conforming to this definition when n= 1 is cyclopentane-acetic acid,



The literature on the composition of the acids occurring in petroleum leads to the following generalizations:

1. They are natural components of the crude and not formed during refining.

2. They are predominantly monocarboxylic acids.

3. Generally, the carboxyl group is not directly attached to the ring but through a methylene group or a chain containing up to 5 or more methylene groups.

4. Cyclopentane rings predominate but cyclohexane rings are present in some cases. Aromatic rings or fused aromatic-naphthene rings may be expected in the high molecular weight acids.

5. The types of acids in petroleum and the approximate carbon range in which they have been found is as follows:

acids	n
aliphatic, $C_nH_{2n}O_2$	< 7
monocyclic, $C_nH_{2n-2}O_2$	7-12
bicyclic, $C_nH_{2n-4}O_2$	12-20
tricyclic, $C_nH_{2n-6}O_2$	> 20
polycyclic, $C_nH_{2n-8}O_2$ to $C_nH_{2n-14}O_2$	> 20

C. Properties

Naphthenic acids are oily liquids of a characteristic odor which varies with the acid source and degree of refinement. Phenols and sulfur compounds are responsible for most of the odor.

D. Uses

Naphthenic acid can be used in lubricants, driers and

catalysts, perservatives, emulsifiers, corrosion, inhibitors, and fungicides. Besides, HNaps and their salts of sodium, potassium, nickel, and copper have been found to act as growth stimulators. More detailed information about the HNaps was given by Jolly (1967) in Encyclopedia of Chemical Technology Vol 13.

II. EFFECTS OF NAPHTHENATES ON LIVING ORGANISMS

A. Introduction

It has been reported by a number of workers, most of which are Russians, that HNaps or their salts (Naps) can act as growth stimulators. Nap has been found to stimulate or promote growth, yield, and biochemical and physiological activities of a number of species of animals and plants.

B. Effects on animals

Glushkov and Yakovlev (1963) reported that the bee group which received the NaNap fed in a dose of 200 g/day/family for 3 weeks showed 18.30% more growth than controls. Probosces were 1.97% longer than those of the controls.

There are several additional reports which bear out the stimulative effect on growth of animals. Aliev (1963) reported that NaNap fed to swine, cattle, and calves at the rate of 2 to 20 mg/kg of feed resulted in an increase in body weight over that of control animals. Starkova et al. (1963) reported that 3 mg NaNap/kg of feed raised the weight increments of pigs by 10 to 17%,

and that of poultry by 15%. Dimitrov and Popoff (1966) reported that Naps fed to hens showed encouraging results in increased egg-laying.

C. Effect on plants

Some of the literature concerning the stimulatory effect of Nap on different species of plants is summarized as follows.

It has been shown by Burachevskii (1965) that the application of NaNap to cultures of Aspergillus usamii could promote both growth and amylase formation by the mold.

Cotton plants treated with NaNap have shown to be increased in the rate of photosynthesis, content of chlorophyll and ascorbic acid of the leaves. It has also been demonstrated that the effects of NaNap on cotton plants appeared to interact with the amount of applied fertilizers (Agakishier and Bazanova, 1965; Bazanova and Akapova, 1966; and Naghibin, 1966).

Under the influence of NaNap, corn has been observed to be stimulated in the development of vegetative and generative organs, and increased in yield. (Starkova and Sevast'yanova, 1963; Popoff et al., 1966; and Eyubov and Issaeva, 1966).

The effect of Nap on potatoes has been examined by different workers including Starkova et al. (1963), Abolina and Ataullaev (1966), and Ladygina (1965). The latter investigator demonstrated that higher chlorophyll content in leaves stimulated photosynthetic and respiratory intensities, more protein and starch content in

the tuber, and increased yield were found in potatoes soaked in 0.0005% Nap for 1 hour before planting.

Promotive effects of Nap on the growth and development of tobacco plants were noticed by Zamanov (1966) and Popoff et al. (1966). However, no clear effect was established with respect to the quality of the tobacco leaves.

Different investigators postulated that Nap has a favorable influence on both the quality and quantity of a number of different fruits. For instance, Kulieva (1964) showed that when olive trees were sprayed with a 0.01% solution of the petroleum growth-promoter NaNap during fruit formation, fruit yield was increased approximately 70%. The average number of fruits which matured increased 1.50 to 2.60%. Work of Abolina and Ataullaev (1966) indicated that Nap spraying gave a considerable yield increase of different sorts of melons up to 10 to 15 tons per ha. The sugar content of the fruit was also increased by the treatment. The application of petroleum growth-promoting substance to the grape plant led to increased photosynthesis, increased catalase activity, and increased sugar content in grapes (Kolesnik, 1965).

Extensive studies on the effect of Nap on tomato plants have been made by several investigators. Székely (1966) demonstrated that the crop yield of tomato was increased significantly by seed treatment with Nap and Nap + trace elements.

Pakhomova (1965) showed that spraying tomato plants with 0.005% aqueous petroleum growth-promoting stubstance caused changes in proteins and nucleic acids in leaves. Besides, an increase of 18.80% in tomato yield was found. Aliev (1965) accelerated the development of tomato plants by the application of NaNap in combination with mineral fertilizers. He suggested that the most effective ways of application of NaNap were into the soil and as a spray. An increase of 30 to 37% in yield was found. In addition, the composition of the tomato fruit changed sharply, increased amounts of sugars, dry substances and ascorbic acid were observed. Popoff (1966) obtained early ripeness by spraying tomato plants with Nap.

Other investigators at the University of British Columbia also got enhanced growth in Nap-treated plants. For example, maize, sugar beet, sunflower, radish, spinach, tomato and tobacco (Wort, unpublished), and bush bean (Wort, Patel and Fattah, unpublished) were found to be stimulated by Nap treatment. Patel and Fattah (unpublished) observed higher pigment contents, greater photosynthesis and respiration intensities, and increased activities of transaminase, phosphoglyceryl kinase, and phosphorylase in leaf blades of KNap-treated bush bean plants.

III. MATERIALS AND METHODS

A. Plant material

A single variety of tomato (Lycopersicum esculentum var. Bonny Best) was used in all the experiments. Seeds were obtained from Buckerfield's Limited, New Westminster, British Columbia. The seeds were placed,equally spaced,in the wooden flats containing sterilized garden soil to germinate. The seeds, and later the plants, were watered daily with tap water. When the seedlings were about two weeks old, one or two uniform seedlings were transplanted into each of the plastic pots, of 6 inches diameter, containing sterilized garden soil. The transplantation was conducted very carefully to minimize damage to roots of the seedlings. The pots were put into a growth room wherein the photoperiod was 16 hr, the light intensity was 1,400 foot candles at the tops of the plants. The temperature was 20 to 25°C, and the relative humidity was 55 to 72% during light period. In the dark period, the temperature was maintained at 18 to 22°C, and the relative humidity 70 to 85%. The pot locations were changed at intervals in order to average local environmental variability.

Plants which were raised for mature growth were transplanted into a bench of soil in a green house. The seedlings were spaced 16 inches apart. In the green house the environmental factors were somewhat variable and difficult to control.

Soluble fertilizer, N:P:K,20:20:20, at the concentration

of 0.5 g/l was applied weekly to the soil at the rate of 50 ml/ plant beginning when plants were 3 weeks old.

B. Preparation of the potassium naphthenate aqueous solution from naphthenic acid (HNap)

KOH solution (2.1 g KOH dissolved in 17 ml distilled water) was added to a flask containing 5 g HNap and shaken vigorously for 15 minutes. The solution was allowed to stand and then the upper portion was made to a volume of 24 ml with distilled water. The solution obtained was the stock solution which contained 25o mg of KNap/ml.

By diluting 1 ml of the stock solution to 100 and 50 ml with distilled water containing the wetting agent Tween 20, 0.3%, 2,500 ppm and 5,000 ppm KNap solutions were obtained.

The pH value of the diluted solution was adjusted to about 10 with dilute HCl.

C. Spray treatments

The 2,500 ppm and 5,000 ppm KNap solutions at the rate of 2 ml/plant were sprayed onto the tomato leaves when the plants were 2, 3, and 4 weeks old, in different experiments. The solutions were allowed to dry on the surface of the leaves and thereafter the pots were transferred to the growth room again.

D. Experimental design

Five experiments were carried out. For the first two experiments, the treated and controlled plants were randomized in each of three blocks. In the last three experiments, the

layout was a complete randomized design.

E. Measurement time

For the first experiment, harvests were made 2 and 5 weeks after treatment. There were three harvests made on the 14th, 28th and 90th days after spraying in the second experiment. Plants were harvested when 33 and 47 days old in the third experiment. All these experiments were concerned primarily with the fresh and dry weights of leaves and stems of the plants.

In the fourth experiment, all measurements of juvenile growth, chemical composition, photosynthesis, respiration, and enzyme activities were made 2, 4, and 6 weeks after treatment. The fourth harvest, for mature tomato analysis, was made when the plants were 100 to 110 days old.

In the last experiment, determinations of succinic dehydrogenase activity were made 4, 8, 12, 16, 20, and 24 days after the application of KNap to 2-week-old plants.

F. Vegetative growth measurement

The stems of each plant were cut just above the soil surface. The leaf blades from each plant were grouped together to get the fresh weight of leaves. Stem and leaf petioles were weighed together to give the fresh stem weight.

After fresh weights were recorded, the plant materials were dried at 75°C for 24 hours.

G. Determination of pigments

Chlorophyll a and b, and carotenoid contents of leaf blades were determined spectrophotometrically (Beckman Model B). The pigments were extracted according to the procedure suggested by Frank and Kenny (1954). Leaf materials were cut into small pieces and mixed thoroughly. One-gram aliquots were blended in 80 ml of 85% acetone in a Waring flask for 2 minutes. The homogenate was filtered through Whatman No. 1 filter paper using a Buckner funnel, and made to 100 ml volume with 85% acetone. The optical density (O.D.) of each pigment solution was read at 663 mμ, 645 mμ, and 440.5 mμ against 85% acetone as blank in a 1-cm cell. The concentrations of chlorophyll a and b were calculated following formulas of McKinney (1940) and that of carotenoid using the equation of von Wettstein (1957). The results were expressed as mg of pigment/g fresh leaf blade.

The formulae used were as follows:

$$C_a = \frac{(12.3\ D_{663} - 0.86\ D_{645})\ V}{d \times 1000 \times w}$$

$$C_b = \frac{(19.3\ D_{645} - 3.6\ D_{663})\ V}{d \times 1000 \times w}$$

where c = concentration in mg/g fresh weight

a = chlorophyll a

b = chlorophyll b

D = optical denisty at wavelength indicated

d = length of light path in cm

V = final volume of extract

W = fresh weight of leaf material used in extraction

$$C_c = 4.695\ D_{440.5} \times \frac{V}{1000W} - 0.268\ C_{(a + b)}$$

where C_c = concentration of carotenoid in mg/g fresh weight

H. Determination of photosynthesis and respiration

The same two potted control and the same two treated plants were used for photosynthesis and respiration measurements 2 and 4 weeks after treatment.

The rate of CO_2 exchange was measured in the growth room using an open system with a Beckman infrared analyzer IR215, and a Heath Built Servo-Recorder, Model EUW-20A.

The pot of the plant was enclosed in a 2-mil polythene bag sealed around the plant stem to prevent CO_2 escape from the soil. The potted plant was set carefully into the chamber, which consisted of a 20-lb capacity polythene bag of 3-mil thickness and the opening of the chamber was sealed tightly around a three-holed rubber stopper which provided inlet and exit for the gas and the thermistor probe. This chamber was connected to the analyzer by tygon tubing. The air in a tank containing about 300 ppm of CO_2 was passed into the chamber through tygon tubing at a constant rate of 2,000 ml/min.

For photosynthesis measurement the chamber was exposed to light of 1,400 f.c.. For determination of the rate of respiration, the chamber was covered with three layers of black cloth to exclude light. In the illuminated system, a drop in the CO_2 concentration compared with that in the tank was considered to be due to the CO_2 fixation in apparent photosynthesis. In the dark system the increase in CO_2 concentration was considered as due to the CO_2 liberation by the plant in dark respiration. The sum of the CO_2 consumed in apparent photosynthesis and the CO_2 evolved during dark respiration gave an approximation of true photosynthesis.

The product of the flow rate by the difference in the CO_2 concentration of air before and after passing through the chamber gave the rate of CO_2 exchange. The results were expressed as microliters CO_2/hour/dm^2 of leaf blade area.

Sensitized paper was used to determine the leaf area. Since the shapes of tomato leaves were very irregular, use of the relation between weight and area of the sensitized paper, gave the approximate leaf area of tomato leaf.

I. Determination of enzyme activities

The enzymes which were investigated were nitrate reductase (NRase), glutamic-pyruvic transaminase (transaminase), phosphoglyceryl kinase, succinic dehydrogenase, and phosphorylase. The first two enzymes are involved in N metabolism while the last

three relate to carbohydrate metabolism.

The above enzyme activities were assayed 2, 4, and 6 weeks after spraying. Succinic dehydrogenase activity was determined 4, 8, 12, 16, 20, and 24 days after treatment in Experiment V. Leaf blades were detached from petioles and washed with cold distilled water. The enzyme activities were determined in homogenates prepared from freshly harvested tissue.

1. Preparation of Tris-HCl buffer solution

Distilled water, 0.2 M trihydroxmethylamine methane (Tris), and 0.1 N HCl were mixed in the ratio 7:8:5 by volume. The pH value of this solution, 0.05 M, was adjusted to 7.4 to 7.5.

2. Preparation of the crude extract

The crude extract was prepared by grinding one weight of finely chopped leaf blades with 4 weights of cold 0.05 M Tris-HCl buffer of pH 7.4 to 7.5 in a Waring blendor at full speed for 2 minutes in a cold room at 0 to 4°C. The homogenate was decanted through four layers of cheesecloth and then centrifuged in a Servall centrifuge at 2,000 x g for 20 minutes at 0 to 4°C. The resulting cell-free supernatant solution was used for the assay of the enzyme activities.

The protein content in the enzyme preparation was determined by the method of Lowry et al. (1951).

3. Nitrate reductase (NRase)

A modification of the procedure of Evans and Nason (1953) was followed to determine the NRase activity. At zero time, 0.4 ml of the enzyme preparation was added to a reaction mixture containing 0.1 ml of 0.1 M KNO_3, 0.05 ml of 2 x 10^{-5} M FAD, 0.05 ml of 2 x 10^{-3} M DPNH, and 0.1 M phosphate buffer, pH 7.0, to give a total volume of 0.5 ml. Incubation was continued for 30 minutes in a constant temperature water bath at 30°C, after which 1 ml of H_2O, 1 ml of 1% (W/V) sulfanilamide, and 1 ml of 0.22% (W/V) N-(1-naphthyl)-ethylene diamine hydrochloride reagent were added and the contents mixed by inverting the tube. Fifteen minutes were allowed for the development of the pinkish color. Finally, the optical density of each solution and its blank (complete except for DPNH) was measured at 540 mμ by the Beckman Model B spectrophotometer. The actual amount of the nitrite formed was determined from a standard curve (see Appendix Fig. 24) prepared in advance with known quantities of nitrite.

The specific activity was defined as μg of nitrite formed/mg protein/hour.

4. Glutamic-pyruvic transaminase (transaminase)

Transaminase activity was measured by following the method of Reitman and Frankel (1957). One ml of -keto-glutarate-alanine substrate was pipetted into a dry, clean test tube and this was 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 exactly 30 minutes in the water bath.

One ml of 2,4-dinitrophenylhydrazine reagent (prepared by dissolving 19.8 mg of 2,4-dinitrophenylhydrazine in 100 ml of 1 N HCl) was added to the tubes immediately after being removed from the water bath. This reagent stopped further transaminase activity. After the tubes were allowed to stand at room temperature for 20 minutes, 10 ml of 0.4 N NaOH was added. A clean rubber stopper was inserted in 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. The only difference in control tubes was that the 2,4-dinitrophenylhydrazine reagent was added to the reaction mixture before incubation.

The specific activity was defined as an increase of 0.05 in optical density/mg protein/hour.

5. Phosphoglyceryl kinase

The method described by Axelrod and Bandurski (1953) was employed. 3-Phosphoglyceric acid in the presence of ATP, was used as the substrate. The diphosphoglycerate was trapped with hydroxylamine, and the anhydride thus formed measured

colorimetrically by the hydroxamic test of Lippman and Tuttle (1945).

Reactions were run in 10 ml test tubes. To each tube were added 1 ml of 0.1 M succinate buffer (succinic acid, 4.724 g, was dissolved in distilled water and made to a volume of 100 ml. Twenty-five ml of the succinate and 46 ml of 0.4 M NaOH were mixed and adjusted to pH 6.2 with NaOH solution), 1 ml of 2 M hydroxylamine hydrochloride (equal volumes of 4% hydroxylamine-HCl and 3.5% NaOH were mixed and adjusted to pH 6.2), 0.5 ml of 0.01 M ATP, 0.25 ml of the crude enzyme preparation, 1 drop of NaF and 1 drop of 0.01 M $MgCl_2$, in the above order. The tube was shaken after the addition of each reagent. The control tube at this time received 2 ml of $FeCl_2$-TCA-HCl reagent to stop the enzyme activity. (The $FeCl_2$-TCA-HCl reagent was prepared by dissolving 9.3 g $FeCl_3.6H_2O$ in 42 ml conc. HCl and then 20 g TCA was added. The mixture was made to a volume of 500 ml with distilled water.) The reaction in the experimental tube was then initiated by the addition of 1 ml of 0.01 M 3-phosphoglyceric acid (barium salt). The reaction was allowed to continue for 1 hour at 30°C in a constant temperature water bath and was terminated by the addition of 2 ml of $FeCl_3$-TCA-HCl reagent to the experimental tubes. Thirty minutes was allowed for color development. The optical density of the solution from each experimental tube, and its blank, was determined by a Beckman Model B spectrophotometer

at 430 mμ.

Specific activity was defined as the increase of 0.05 in optical density/mg protein/hour.

6. Phosphorylase

Sumner's (1950) method, which is a modification of Fiske and Subbarow's method (1925), was employed to assay the enzyme phosphorylase by running the reaction in the direction of starch synthesis and measuring the amount of inorganic phosphate liberated. The reaction mixture consisted of 1 ml of enzyme preparation of 2 ml buffered substrate (the buffered substrate was prepared by dissolving 1 g G-1-P in 50 ml distilled water. The solution was shaken with dry $Ca(OH)_2$ or CaO to remove inorganic phosphate and then was filtered. The filtrate was neutralized with drops of HCl. Equal volumes of filtrate and citrate buffer, pH 6.0, were mixed. This buffered substrate was kept in a refrigerator. Before use, the buffered substrate was mixed with an equal volume of 1% potato starch solution, and a crystal of thymol is added). The test tubes were kept in a 30°C water bath for one hour. At the end of the incubation period, the reaction was terminated with 5 ml of 6.66% ammonium molybdate. Addition of 5 ml of 7.5 N H_2SO_4 followed by 5 ml of 4% acidic $FeSO_4$ developed a deep blue color. The solution was diluted with 10 ml of distilled water and the optical density of each solution and its corresponding blank (ammonium molybdate was added before the addition of substrate) was read in a Klett-

Summerson colorimeter equipped with a red filter. The actual amount of phosphate formed was read from a standard curve (see appendix Fig. 25) prepared with known amounts of phosphate using the same reagents as above.

Specific activity was defined as µg of inorganic phosphate liberated/mg protein/hour.

7. Succinic dehydrogenase

The procedure of Kun and Abood (1949) was followed essentially, with the modification that the incubation period was prolonged to 20 hours, as suggested by Isenberg et al. (1951). Into a 15 ml test tube were pipetted 0.5 ml 0.1 M phosphate buffer of pH 7.4 (prepared by mixing 81 ml 0.1 M Na_2HPO_4 and 19 ml of 0.1 M NaH_2PO_4 and adjusting the pH to 7.4), 0.5 ml 0.2M sodium succinate, 2 ml of homogenate and finally 1 ml of freshly prepared 0.1% triphenyltetra-zolium chloride solution. After shaking, the tubes were stoppered and were kept in a constant temperature water bath at 38°C. The incubation was allowed to continue for 20 hours, at the end of which 7 ml of pure acetone was added to each tube. The contents were shaken vigorously to let the red precipitate dissolve in the acetone. The solutions were then centrifuged for 3 minutes at 2000 x g and the clear red solution was decanted off. The optical density of each test solution, and its blank (with homogenate heated at 80°C for 5 minutes before assaying) was determined using a Klett-Summerson colorimeter

equipped with a blue filter. A standard curve was prepared in advance by using known amounts of formazan dissolved in 10 ml of acetone. The amount of formazan formed in each experimental tube was read from the standard curve (see appendix Fig. 26).

Specific activity was defined as the ug of formazan formed/mg protein/hour.

In the fifth experiment, the activity of succinic dehydrogenase was assayed by using the method described by Ziegler and Rieskie (1967). However, the specific activity was modified and expressed as 0.05 optical density increased/mg protein/hour.

J. Determination of protein

The method of Lowry et al. (1951) was employed to determine the protein content of all the enzyme preparations mentioned above. To 0.4 ml of the enzyme was added 2 ml of alkaline copper solution (prepared by mixing 50 ml 2% Na_2CO_3 in 0.1 N NaOH and 1 ml 0.5% $CuSO_4.5H_20$ in 1% sodium potassium tartarate). The contents were mixed. After 10 minutes 0.2 ml of 1 N Folin-Ciocalteau phenol reagent (Fisher Scientific Co., Fairlawn, N.J.) was added and the contents were mixed and allowed to stand for 30 minutes for color development. The optical density of each experimental solution and its blank (phenol reagent was replaced by distilled water) was read at 500 mμ with a Beckman Model B spectrophotometer. Protein content as mg/ml was obtained by comparison with a standard curve which was prepared with known

amounts of Bovine albumin. The values expressed as mg/ml were then converted to mg protein/g fresh leaf blade.

K. Pollination

As the plants were grown in either growth room or green house, artificial pollination was achieved by flipping the plant body lightly.

L. Blossom-end rot

Some of the tomato fruits exhibited blossom-end rot. This rot is caused by undesirable environmental conditions, such as a sudden shortage of water and fluctuating humidity, or by calcium deficiency. The leaves, flowers, and developing fruits of the plants were sprayed with $CaCl_2$ aqueous solution at a rate of 0.5 g $CaCl_2$/plant.

M. Yield measurement

For the third harvest in Experiment II, and the fourth harvest in Experiment IV, number and weight of both green and mature tomato fruits were recorded.

For chemical composition determination at the fourth harvest of Experiment IV, tomato fruits were picked as soon as they were ripe, that is, red and firm. After being weighed they were stored in a refrigerator at 5°C.

N. Quality of tomato fruits

The measurements of sugars, titratable acid, and ascorbic acid of the ripe tomato fruits were made. The tomato fruits were

stored at 5°C for 10, 4, and 1 day before the actual assays were carried out in order to check the effect of KNap on the loss of these compounds during storage.

1. Determination of reducing sugars and sucrose

Soxhlet extraction in ethanol, clearing and deleading of extracts were done according to the method of Loomis and Shull (1947).

Nitrophenol (DNP) reagent was prepared by dissolving 7.145 g sodium 2,4-dinitrophenol in 230 ml 5% NaOH. The mixture was heated on a water bath until the DNP dissolved completely. Then 2.5 g phenol was added. If the solution did not remain clear, it was heated further. One hundred g sodium potassium tartarate was dissolved in ca 500 ml distilled water. The two above-mentioned solutions were transferred to a 1-liter volumetric flask and made to a volume of 1-liter.

For sucrose determination, 25 ml of the cleared and deleaded extract was pipetted into a 400 ml beaker. Two drops methyl red, 5 drops of 10% acetic acid and 4 drops of invertase solution were added to each beaker. The mixture was rotated and was allowed to stand overnight at room temperature. The reducing sugars of both hydrolyzed and unhydrolyzed solutions were measured colorimetrically using the dinitrophenol reagent method. A standard curve was prepared with graded solutions of glucose. The difference between hydrolyzed and unhydrolyzed extract, after

multiplication with the correction factor 0.95 gave the amount of the sucrose.

The DNP reagent method for the measurement of reducing sugar was as follows: six ml DNP reagent was pipetted into 3 large test tubes. Two ml distilled water was pipetted in tube 1, and 2 ml of clear deleaded extract was pipetted into tubes 2 and 3. The tubes were kept in cold, running water until all samples were ready. All tubes were stoppered loosely with glass stoppers and were placed in boiling water for exactly 6 minutes. The tubes were then transferred to cold,running water again. The absorbance of each solution was read in a Klett-Summerson colorimeter with a red filter, 3 to 20 minutes later.

Sugar contents were expressed as percentage of dry weight of tomato fruit.

2. Titratable acid

The methods described by Lepper et al. (1945) for preparation of the extract and analysis of titratable acid were followed.

The fruits were cleaned, dried, chopped, and quickly blended in a Waring blendor. One hundred and fifty g of the sample and 400 ml of distilled water were put into a 2-liter beaker. The mixture in the beaker was boiled for one hour and water lost by evaporation was replaced at intervals. The solution was made to a volume of one liter and filtered. Twenty-five ml

of the above-mentioned solution was diluted to 250 ml with recently boiled water. The diluted solution was titrated with 0.1 N KOH using 0.3 ml of 1% phenolphthalein solution (prepared by dissolving 1 g phenolphthalein in 50 ml of 95% ethanol and then 50 ml water were added) for each 100 ml of solution being titrated. The results were reported as ml of 0.1 N KOH/100 g of fresh tomato fruit.

3. Ascorbic acid

The extraction procedure used was essentially the same as described by Loeffler and Ponting (1942) except that 0.5% oxalic acid was used rather than metaphosphoric acid. Twenty g of tomato in 150 ml 0.5% oxalic acid was blended in a Waring blendor. The extract was decanted through two layers of cheesecloth. The method for determination of ascorbic acid in the extract followed the indophenol reduction technique as modified by Schuster (1952) for use with a Klett-Summerson colorimeter. One ml of tomato extract was pipetted into each of the two colorimeter tubes. Nine ml of dye solution was pipetted into a colorimeter tube. (The dye solution was prepared by dissolving 14 mg Na-2, 6-dichlorophenol indophenol in warm distilled water. The solution was filtered and made to a volume of one liter with cold distilled water.) The solution was mixed by inversion and read within 20 seconds with a Klett-Summerson colorimeter equipped with a green filter. The blank was prepared by mixing 1 ml 0.5% oxalic acid, 9 ml dye solution and a tiny

crystal of pure ascorbic acid (Nutritional Biochemical Corporation, Cleveland). A standard regression line (see appendix Fig. 27) was drawn from readings obtained with graded solutions of pure ascorbic acid.

Results were expressed as mg of ascorbic acid/100 g of fresh tomato fruit.

IV. 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 (or control) plants. Results, unless specifically stated otherwise, were subjected to statistical analysis. In most instances, no statistically significant difference was found. The results did show that there was a positive tendency of KNap to stimulate growth, yield, and some of the biochemical and physiological aspects observed in tomato plants, if the general concept that 10% changes was regarded as of some significance was considered.

A. Vegetative growth

Data on fresh and dry weights of leaves, and petioles and stem are given in Tables Ia to Ie, and also illustrated in Figures 1 to 5. In the text, stem includes the petioles, while "leaves" means leaf blades.

1. Experiment I

Results are given in Table 1a and Fig. 1. No statistically significant difference was found in this experiment.

For the first harvest, plants were harvested 2 weeks after treatment. Based on dry weight magnitude, plants sprayed with 2,500 ppm KNap showed a slight increase, 0.91%, in stem weight, but decreases of 4.68% and 1.30% in leaf and total weights. In the 5,000 ppm KNap-treated plants, both the leaves and stem and therefore the total were increased by 3.28%, 10.77%, and 7.80% respectively. When dry weight was under consideration, 2,500 ppm KNap caused an increase of 2.31% in stem but inhibited growth of leaves 4.11%, consequently the total weight was reduced 1.30%. In the case of 5,000 ppm KNap-sprayed plants, increases of 5.70%, 13.21% and 8.30% were observed in leaves, stem and total respectively.

The second harvest was done 5 weeks after treatment. Fresh weight results revealed decreases of 0.21%, 3.62%, and 3.50% in leaves, stem and total in the 2,500 ppm KNap-treated plants. In 5,000 ppm KNap-treated plants, only a slight drop of 0.61% was observed in stem. The leaves and total showed increases of 4.84% and 1.10% respectively. Effect of the treatment on dry weight followed closely the pattern of the fresh weight changes. Leaves, stem, and also the total weight were all decreased by the

2,500 ppm KNap to the extent of 5.07%, 9.14%, and 5.70% respectively. Increases of 6.15% and 1.10%, and a decrease of 1.57% were found in leaves, total and stem of 5,000 ppm KNap-treated plants.

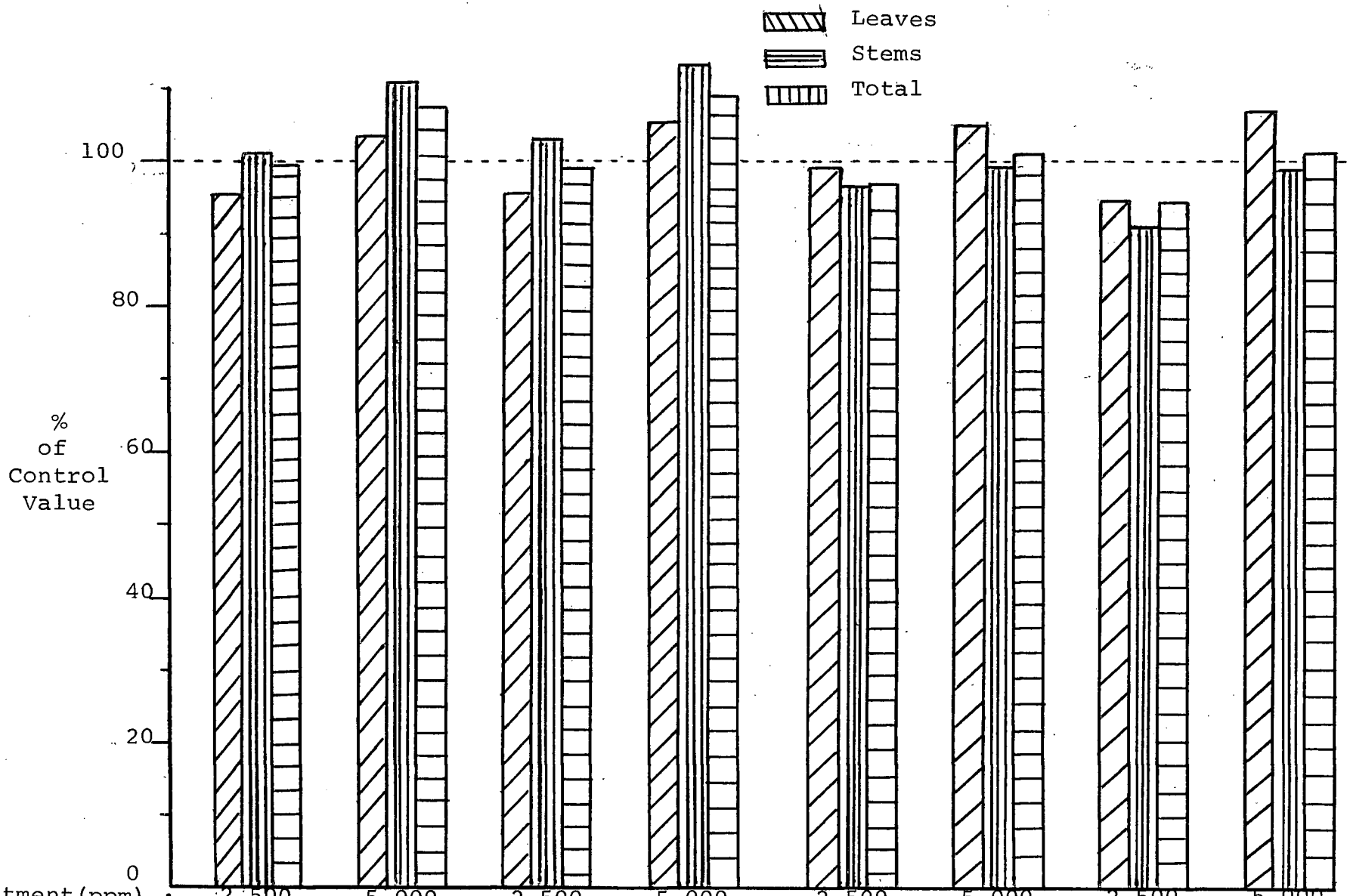
TABLE Ia

The effect of KNap on fresh and dry weights of leaves and stems of tomato plants (Experiment I).

		Treatment (ppm)	Weight (g)			T/C%		
			Leaves	Stems	Total	Leaves	Stems	Total
First Harvest[x]	Fresh Weight	C	317.04[±]	476.12	793.16			
		2,500	302.19	480.43	783.62	95.32	100.91	98.70
		5,000	327.45	527.40	854.88	103.28	100.77	107.80
	Dry Weight	C	47.91	36.80	84.71			
		2,500	45.94	37.65	83.59	95.89	102.31	98.70
		5,000	50.68	41.66	92.34	105.78	113.21	108.30
Second Harvest	Fresh Weight	C	466.70	1001.10	1476.80			
		2,500	451.70	964.90	1416.60	99.79	96.38	96.50
		5,000	489.30	995.00	1484.30	104.84	99.39	101.10
	Dry Weight	C	87.75	128.60	216.35			
		2,500	83.30	120.70	204.00	94.93	90.86	94.30
		5,000	93.15	125.60	218.80	106.15	98.43	101.10

[x]: In the first harvest, plants were harvested 2 weeks after treatment or at the age of 6 weeks. Each reading is the pooled value of 24 plants/treatment. In the second harvest, plants were harvested when 9 weeks old or 5 weeks after treatment. Each value is the pooled value of 15 plants/treatment.

[±]: No statistically significant difference was found in this experiment.



Treatment (ppm) :	2,500	5,000	2,500	5,000	2,500	5,000	2,500	5,000
Weight :	Fresh	Fresh	Dry	Dry	Fresh	Fresh	Dry	Dry
Weeks after Treatment :	2	2	2	2	5	5	5	5

Fig. 1 : Effect of 2,500 ppm and 5,000 ppm KNap on fresh and dry weights of leaves, and stems of tomato plants (Experiment I).

2. Experiment II

The results are given in Table Ib and illustrated in Fig. 2. Statistically significant differences were not found in Experiment II.

On the first harvest, both 5,000 ppm and 2,500 ppm KNap treatments enhanced growth of plants 2 weeks after spraying, that is when 6 weeks old. In the sequence of leaves, stems, and total, increases of fresh weight of 8.32%, 4.34%, and 6.00% were observed in 2,500 ppm KNap-treated plants, whereas 5.02%, 0.22%, and 2.00% increases resulted from the application of 5,000 ppm KNap. Increases of 7.16%, 10.05%, and 8.30% were observed in the dry weights of plants which had been sprayed with 2,500 ppm KNap. Less pronounced increases of 1.31%, 2.85% and 1.90% were observed in the 5,000 ppm KNap-treated plants.

The second harvest was carried out 2 weeks after the first. The 2,500 ppm KNap induced augmentation in fresh weights of leaves, stem, and total by 4.40%, 2.52%, and 3.20% respectively. The 5,000 ppm KNap stimulated growth of stems only, 2.48% in fresh weight, but decreased those of leaves and total by 5.62% and 0.80%. The effect of 2,500 ppm on leaf dry weight production was found to be inhibitory, though not significantly so, by showing a slight decrease of 0.42%, while the weights of the stem and total were increased by 1.50% and 0.50% respectively. Under the influence of 5,000 ppm KNap treatment, leaves and total dry weights were decreased by 4.85% and 1.60% respectively. The stem

TABLE Ib

The effect of KNap on fresh and dry weights of leaves and stems of tomato plants (Experiment II).

		Treatment (ppm)	Weight (g) Leaves	Stems	Total	T/C % Leaves	Stems	Total
First[x] Harvest	Fresh Weight	C	303.47±A	472.40	775.87			
		2,500	328.72 B	492.90	821.62	108.32	104.34	106.00
		5,000	318.70 AB	473.49	792.15	105.02	100.22	102.00
	Dry Weight	C	37.41	27.12	64.21			
		2,500	40.09	30.12	70.20	107.16	110.05	108.30
		5,000	37.90	28.15	66.05	101.31	102.85	101.90
Second Harvest	Fresh Weight	C	475.25	688.25	1163.50			
		2,500	496.15	705.60	1201.75	104.40	102.52	103.20
		5,000	448.55	705.30	1153.80	94.38	102.48	99.20
	Dry Weight	C	82.50	78.85	158.35			
		2,500	82.15	77.00	159.15	99.58	101.52	100.50
		5,000	78.50	77.20	155.70	95.15	101.78	98.40

[x]: In this experiment, plants were sprayed at the age of 4 weeks. The first harvest was done 2 weeks after treatment. The second harvest was done 4 weeks after treatment. Each value in this experiment is the pooled value of 9 plants/treatment.

±: In Tables Ia-e to III, and VII, means differing significantly from the respective control mean at the 0.01% level are indicated by **, and sample means sharing the same letter do not differ significantly according to Duncan's New Multiple Range Test at the 5% protection level.

$$\frac{T}{C} = \frac{\text{Value in treated plants}}{\text{Value in control plants}} \times 100$$

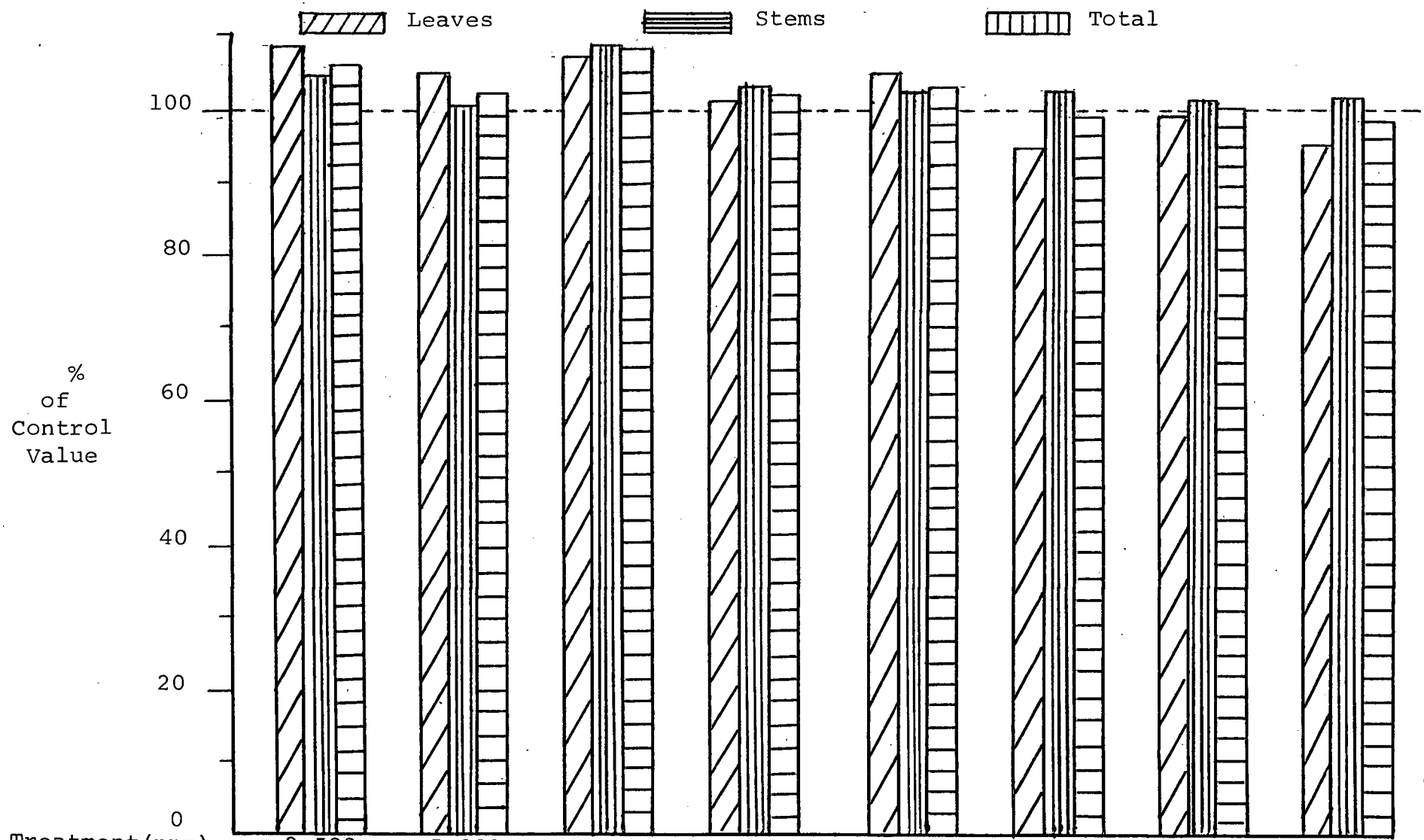


Fig. 2 : Effect of 2,500 ppm and 5,000 ppm KNap on fresh and dry weights of leaves and stems of tomato plants (Experiment II).

dry weight was increased by 1.78%.

3. Experiment III

In this experiment, the plants were divided into 5 groups. One of these groups was untreated; the second group received 2,500 ppm KNap spray at the age of 12 days; and the third group received 5,000 ppm KNap spray at the same age of 12 days. The other two groups were treated 7 days later, that is when 19 days old, with 2,500 ppm and 5,000 ppm KNap respectively. Half of each group was harvested when plants were 33 days old, and the other half two weeks later at which time the plants were 47 days old.

The results of the first harvest are tabulated in Table Ic and represented graphically in Fig. 3. The results indicated that treatment fresh weight means were significantly different from the respective control means at 5% or 1% level, but no statistically significant difference among treatment means was found according to Duncan's New Multiple Range Test. Fresh weights greater by 3.85% in leaves, 7.64% in stems, and 6.11% in total were induced by 5,000 ppm KNap applied to plants when 19 days old. The growth of the other three groups was inhibited by the KNap. Differences in percentage are given in the order of leaves, stems, and total in the following statements. The fresh weights of plants treated at the age of 12 days by 2,500 ppm KNap were decreased by 7.02%, 10.63%, and 9.24%, while

the fresh weights of plants which received 5,000 ppm KNap at the same stage, were less by 3.33%, 5.99%, and 4.91%. The plants which were sprayed with 2,500 ppm KNap when 19 days old had weight values which were lowered by 3.78%, 7.20%, and 5.82%. Results based on dry weight showed that both the 2,500 ppm and 5,000 ppm KNap, sprayed either when plants were 12 or 19 days old, increased the dry weights of leaves, stems and the total top weights. The increases range from 0.83% to 9.92% but no statistically significant difference was observed.

Data on the second harvest are included in Table Id and Fig. 4. The effect on fresh weights of 2,500 ppm KNap applied to 12-day old plants showed 9.94% decrease in leaves, 2.56% increase in stem and 4.40% decrease in total weight, while that of 5,000 ppm showed a decrease of 7.37% in leaves, but 9.18% and 1.70% increases in stem and total weight respectively. The plants which received 2,500 ppm KNap at 19 days of age showed 1.11% decrease, 12.29%, and 6.20% increases in leaves, stems and total weight, while those under the influence of 5,000 ppm KNap at the same age showed increases of 8.66%, 24.03%, and 17.70% in leaves, stems, and total weight respectively. The differences in dry weights of leaves and stems are statistically significant at 5% level. Increases of 20.13%, 8.66%, and 14.95% in leaves, stems, and total dry weight were observed in plants treated with 2,500 ppm when 12 days old. Increases of 26.79%, 18.18%, and 5.20% in

leaves, stems and total dry weights were observed in plants sprayed with 5,000 ppm when 12 days old. Higher dry weights of 36.11%, 18.83%, and 9.90% were found in leaves, stems, and total tops in plants sprayed with 2,500 ppm KNap when 19 days old. More striking increases of 43.63%, 32.47%, and 18.70% in leaves, stems, and total dry weights were found in plants sprayed with 5,000 ppm KNap when 19 days old.

4. Experiment IV

In this experiment 5,000 KNap was applied to 3-week-old plants. The results obtained from the first three harvests made 2, 4, and 6 weeks after treatment are summarized in Table Ie and shown in Fig. 5. No statistically significant difference was found in this experiment.

For the first harvest, decreases in fresh weight of 4.52%, 4.93%, and 4.70% in leaves, stems, and total were found. Increases of 0.48%, and 2.55% and 0.64% of decreases of dry weight were found in leaves, stems and total top respectively.

The 5,000 ppm KNap resulted in an inhibitory effect on both fresh and dry total top weights when plants were harvested 4 weeks after spraying. The decreases of 6.82%, 6.45%, and 6.60% on fresh weight, and 7.34%, 3.28%, and 5.50% on dry weight were observed in leaves, stems, and total respectively.

In the third harvest, a lessening of 4.07% in leaf fresh weight, and increases of 2.74% and 0.25% in stem and total

TABLE Ic

The effect of KNap on fresh and dry weights of leaves and stems of tomato plants (Experiment III, first harvest).[x]

	Treatment (ppm)	Weight (g) Leaves	Stems	Total	T/C% Leaves	Stems	Total
	C	144.20 A*	214.55 B	358.00 C			
	2,500±1	133.85 A	191.75 B	325.60 C	92.98	89.37	90.76
Fresh	5,000-1	139.40 A	201.70 B	341.10 C	96.67	94.01	95.09
Weight	2,500-2	138.75 A	199.10 B	337.85 C	96.22	92.80	94.18
	5,000-2	149.75	230.95	380.70	103.85	107.64	106.11
	C	19.40	12.10	31.50			
Dry	2,500-1	20.60	12.20	32.80	106.19	100.83	104.12
Weight	5,000-1	21.00	12.50	33.50	108.25	103.31	106.34
	2,500-2	20.70	12.65	33.35	106.70	104.55	105.85
	5,000-2	21.20	13.30	34.50	109.28	109.92	109.52

x: Each value is the pooled value of 12 plants/treatment and plants were harvested when 33 days old.

±: In Tables Ic and Id, 2,500-1 = 2,500 ppm KNap applied when plants were 12 days old; 5,000-1= 5,000 ppm KNap applied when plants were 12 days old; 2,500-2= 2,500 ppm KNap applied when plants were 19 days old; and 5,000-2= 5,000 ppm KNap applied when plants were 19 days old.

*: In a column, values not followed by a common letter are significantly different at the 0.05 level.

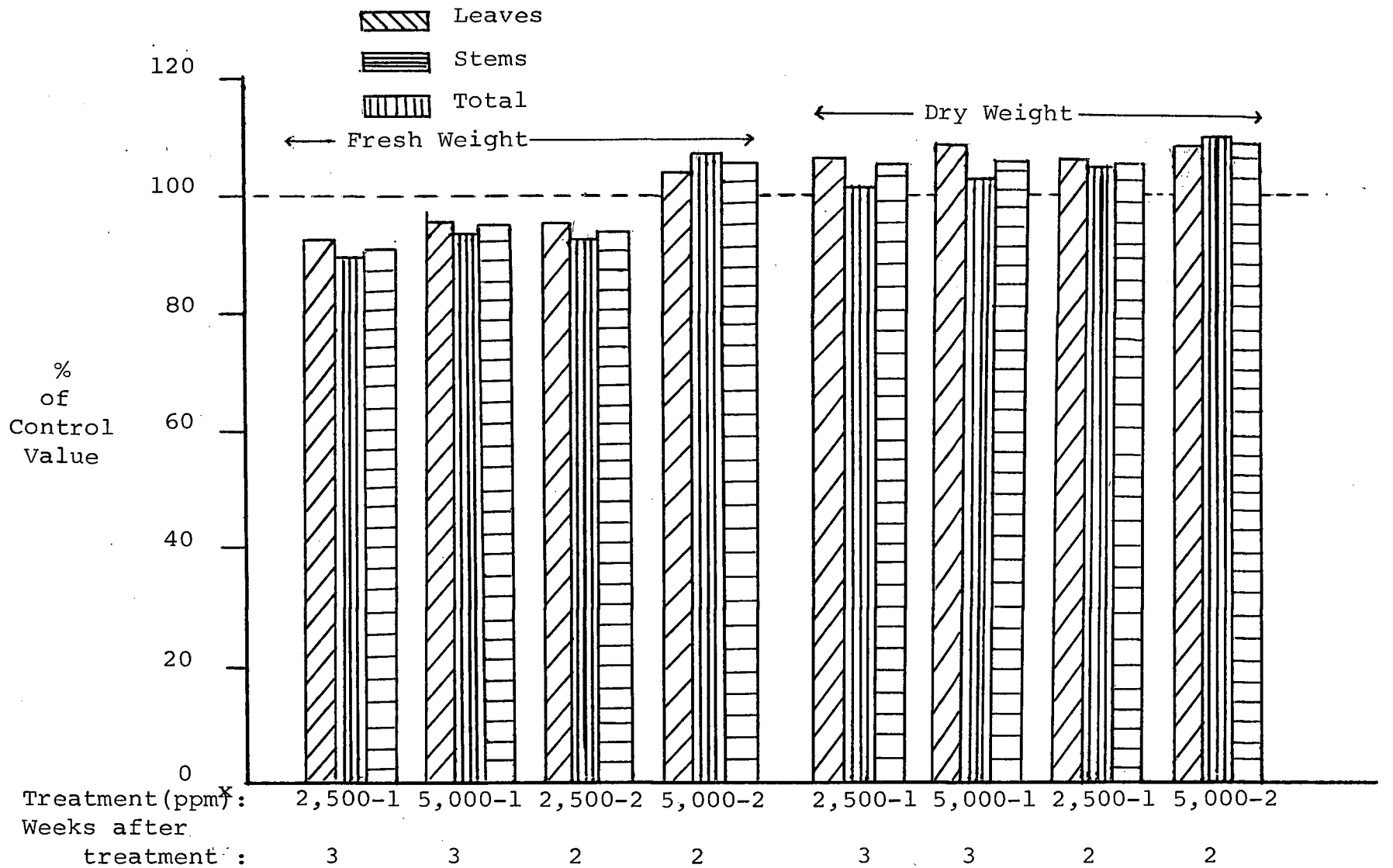


Fig. 3: Effect of 2,500 ppm and 5,000 ppm KNap on fresh and dry weights of leaves, and stems of tomato plants (Experiment III, first harvest).

x: -1= sprayed on 12 days old tomato plants; and -2= sprayed on 19 days old tomato plants.

TABLE Id

The effect of KNap on fresh weights and dry weights of leaves, petioles, and stems of tomato plants (Experiment III, second harvest).[x]

	Treatment (ppm)	Weight (g)			T/C%		
		Leaves	Stems	Total	Leaves	Stems	Total
Fresh Weight	C	194.10	234.25	428.35			
	2,500-1	174.80	240.25	415.05	90.06	102.56	95.60
	5,000-1	179.80	255.75	435.55	92.63	109.18	101.70
	2,500-2	191.95	263.03	454.90	98.89	112.29	106.20
	5,000-2	210.90	290.55	501.45	108.66	124.03	117.70
Dry Weight	C	30.05 A	23.10 C	53.15			
	2,500-1	36.10 A	25.10 C	61.20	120.13	108.66	114.95
	5,000-1	38.10 AB	27.30 C	65.40	126.79	118.18	105.20
	2,500-2	40.90 AB	27.45 CD	68.35	136.11	118.83	109.90
	5,000-2	43.16 B	30.66 D	73.76	143.63	132.47	118.70

[x]: Each value is the pooled value of 6 plants/treatment. Plants were harvested when 47 days old.

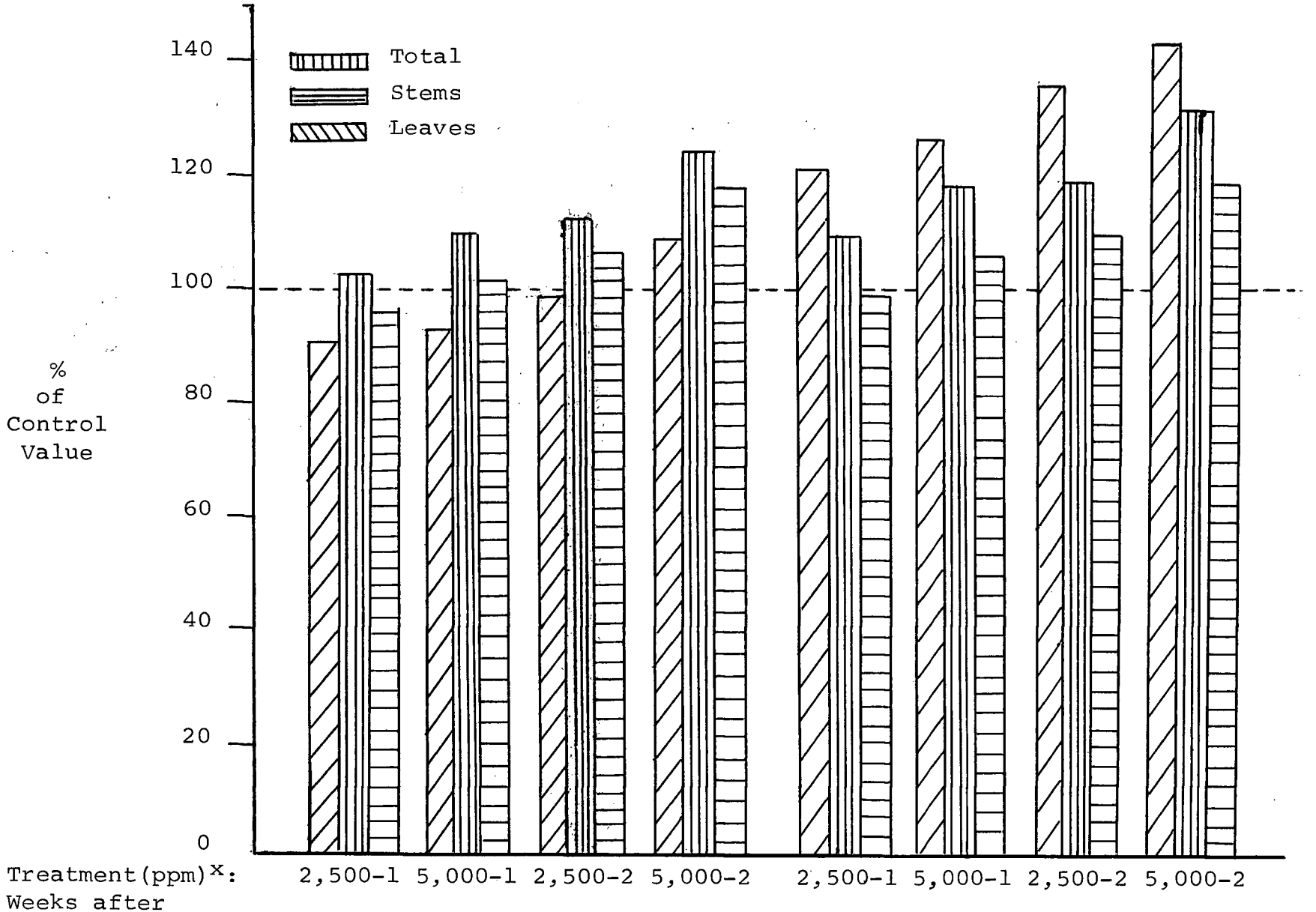


Fig. 4 : Effect of 2,500 ppm and 5,000 ppm KNap on fresh and dry weights of leaves and stems of tomato plants (Experiment III, second harvest).

TABLE Ie

The effect of KNap on fresh and dry weights of leaves and stems of tomato plants (Experiment IV).[x]

		Treatment (ppm)	Weight (g)			T/C%		
			Leaves	Stems	Total	Leaves	Stems	Total
First Harvest	Fresh Weight	C	105.00	130.90	230.90	—		
		5,000	100.25	124.45	224.70	95.48	95.07	95.30
	Dry Weight	C	20.80	11.75	32.55			
		5,000	20.90	11.45	32.35	100.48	97.45	99.36
Second Harvest	Fresh Weight	C	146.70	235.50	382.20			
		5,000	136.90	220.30	357.00	93.18	93.55	93.40
	Dry Weight	C	36.80	30.50	67.30			
		5,000	34.10	29.50	63.60	92.66	96.72	94.50
Third Harvest	Fresh Weight	C	3240.50	5650.00	8890.50			
		5,000	3108.50	5805.00	8913.50	95.93	102.74	100.25
	Dry Weight	C	78.90	91.00	169.90			
		5,000	73.50	94.35	167.85	93.16	103.68	98.80

[x]: Values in first and second harvests are pooled values of 6 plants/treatment, and in the third harvest 10 plants are pooled in each value.

±: Plants were 5, 7, and 9 weeks old (or 2, 4, and 6 weeks after treatment) in first, second, and third harvest respectively.

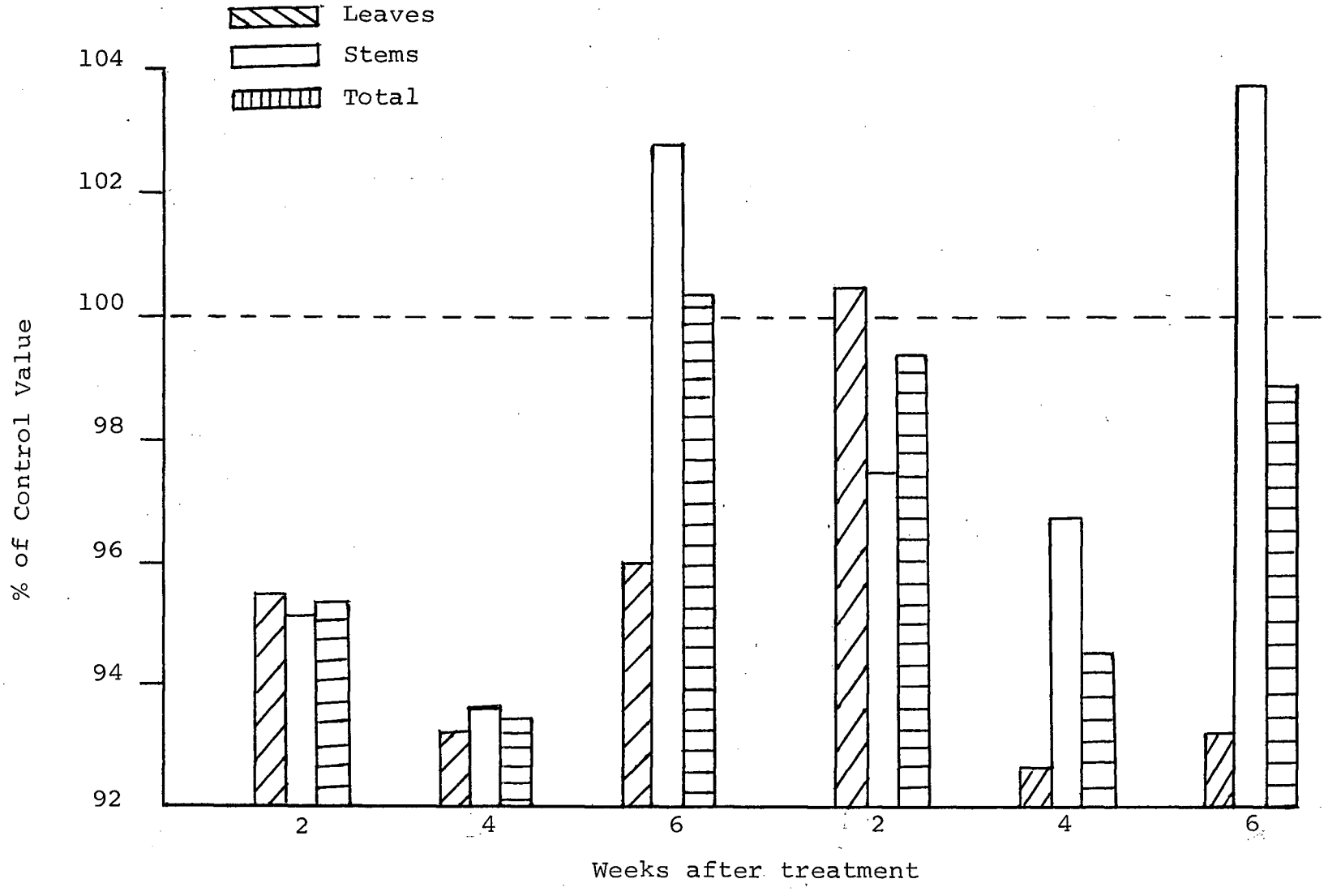


Fig. 5: Effect of 5,000 ppm KNap on fresh and dry weights of leaves and stems of tomato plants (Experiment IV).

fresh weights were caused by the 5,000 ppm KNap treatment. On a dry weight basis, 6.84% and 1.20% decreases, and a 3.68% increase were found in leaves, total and stems respectively.

B. Chlorophyll and carotenoid content

Plants were sprayed with 5,000 ppm KNap when 3 weeks old. Three measurements of chlorophyll a and b, and carotenoid content of leaf blades were conducted 2, 4, and 6 weeks after treatment. Results are shown in Table II and Fig. 6 and 7. No statistically significant difference was found.

In all the 3 observations, both chlorophyll a and b contents were increased rather ineffectively in the leaf blades by the KNap spray. Chlorophyll a content was found to show small increases of 2.70% and 1.00% in the treated plants in the first and third measurements. Chlorophyll b content was found to be increased by the 5,000 ppm KNap to 2.10% and 1.80% in the first and second observations, but a decline of 1.50% was noticed in the third measurement. The stimulatory effect of KNap on carotenoid content was more pronounced. All observations showed increases in carotenoid content in treated plants, 6.50%, 13.80% and 1.40% increases were detected for the three measurements.

C. Photosynthesis and respiration

The rates of photosynthesis and respiration are presented in Table II and also represented graphically in Fig. 7 and 8. Five thousand ppm KNap applied to 3-week-old plants resulted in a

TABLE II

The effect of 5,000 ppm KNap on the rates of photosynthesis and respiration of tomato plants, and on the pigment contents of tomato leaf blades.

Unit		Harvest time (weeks after treatment)	Rate or content		T/C %
			C	5,000 ppm	
µl CO_2 exchanged/hr/ dm^2 of leaf blade area	Apparent photosynthesis	2	1740.00[x]	1500.00	86.20
		4	945.00	985.00	104.20
	Respiration	2	1640.00	1500.00	91.50
		4	1345.00	1475.00	109.70
	True photosynthesis	2	3380.00	3000.00	88.50
		4	2290.00	2460.00	107.40
mg/g of fresh leaf blade	Chlorophyll a	2	0.75	0.77	102.70
		4	0.84	0.84	100.00
		6	0.96	0.97	101.00
	Chlorophyll b	2	0.47	0.48	102.10
		4	0.55	0.56	101.80
		6	0.66	0.65	98.50
	Carotenoid	2	0.61	0.65	106.50
		4	0.65	0.74	113.80
		6	0.73	0.74	101.40

[x]: Rates of photosynthesis and respiration are the average of two measurements. Each value of pigment content is the average value of 6 samples.

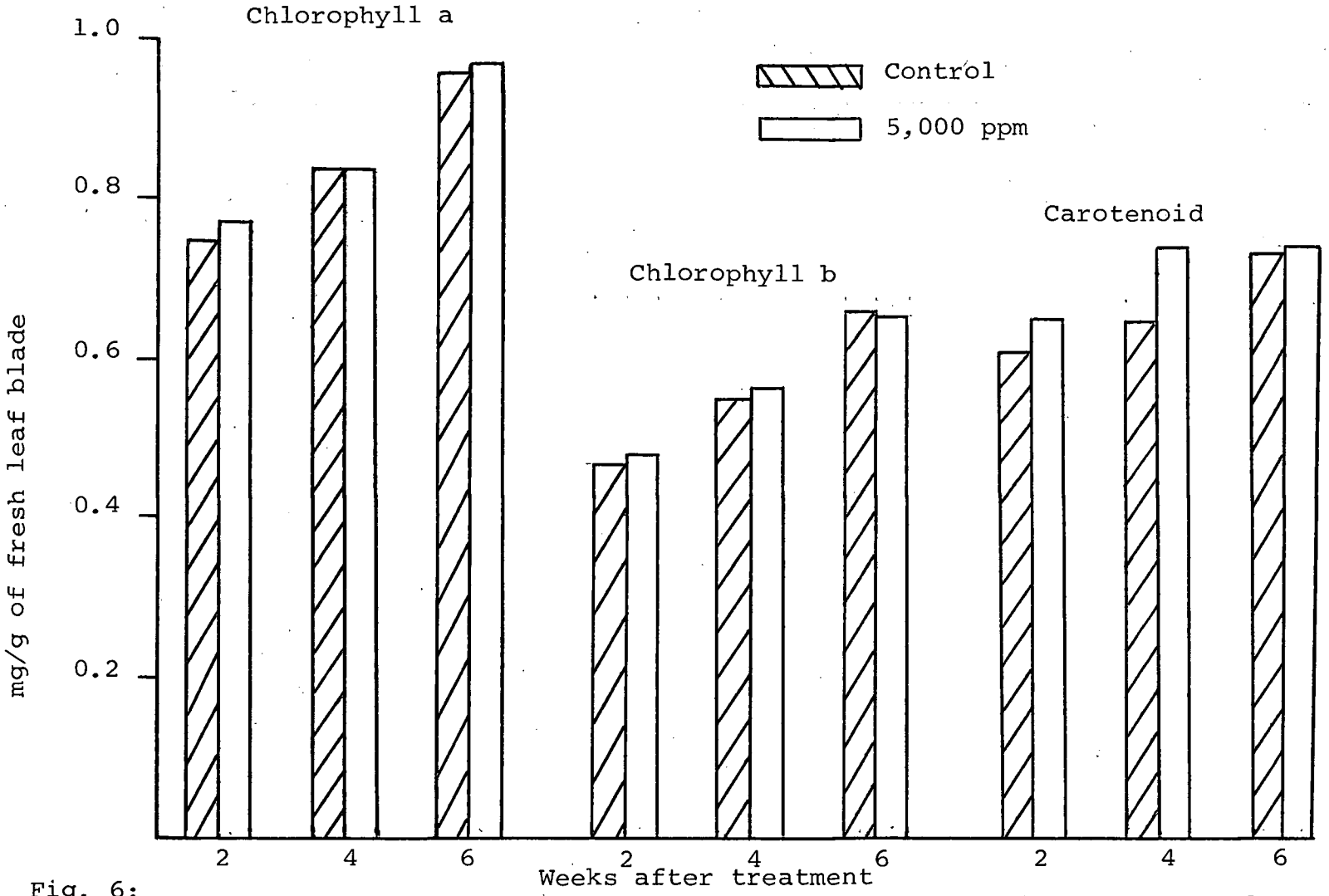


Fig. 6: Effect of 5,000 ppm KNap on the contents of chlorophylls a and b, and carotenoid of tomato leaf blades.

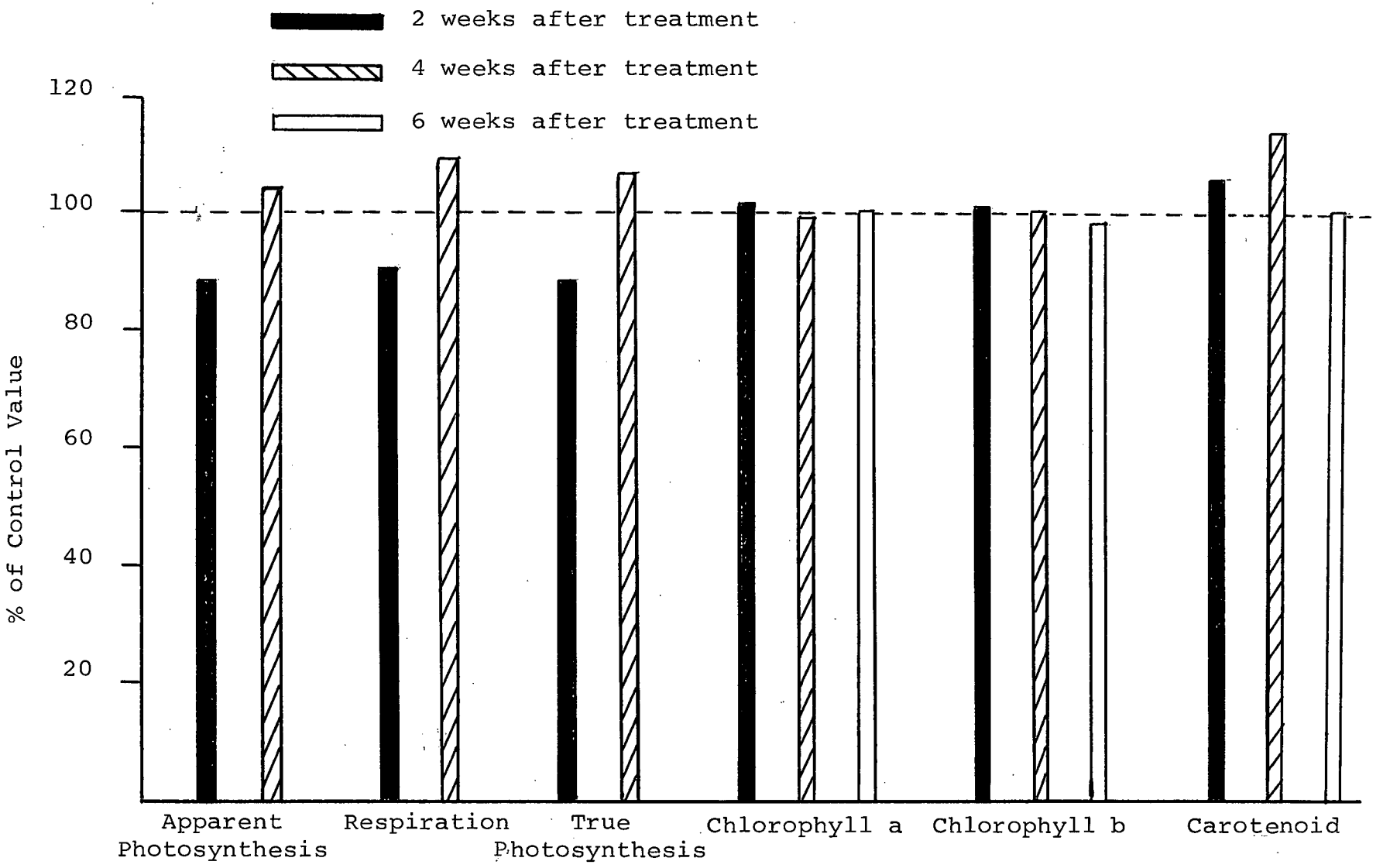


Fig. 7: Effect of 5,000 ppm KNap on the rates of photosynthesis and respiration of tomato plants, and on the contents of pigments of tomato leaf blades.

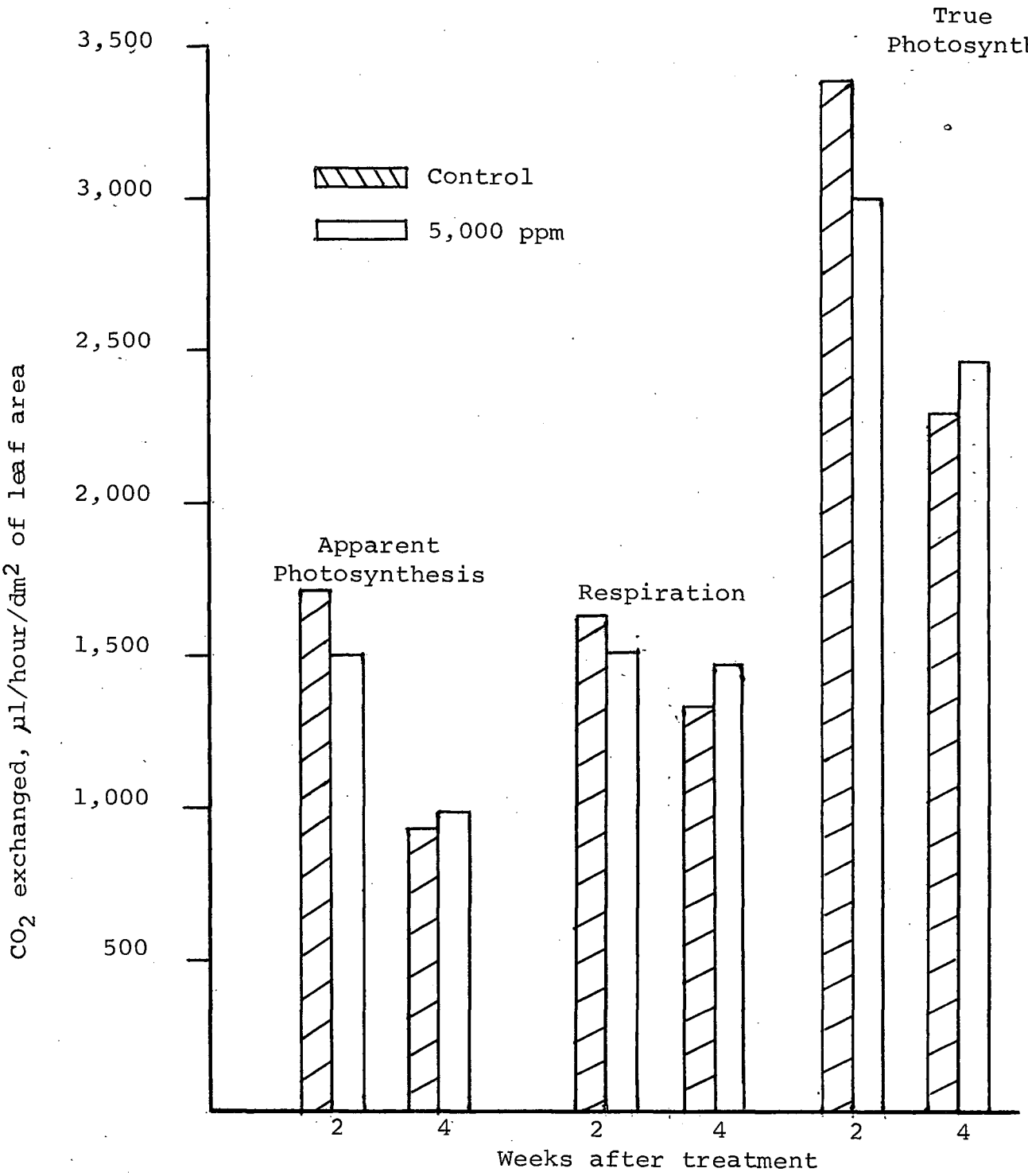


Fig. 8: Effect of 5,000 ppm KNap on the rates of apparent photosynthesis, respiration and true photosynthesis of tomato plants.

slowing down of both apparent photosynthesis and respiration at the early stage, that is 2 weeks after treatment, and the values were 13.80% and 8.50% below those of the untreated plants. However, the treatment resulted in higher rates in both apparent photosynthesis and respiration 4 weeks after treatment by showing 4.20% and 9.70% increases respectively.

In treated plants, the rate of true photosynthesis, equal to the sum of net CO_2 fixation and CO_2 liberation in the dark, showed an increase of 7.40% 4 weeks after treatment, whereas a decrease of 11.40% was detected 2 weeks after spraying. However, no difference in photosynthesis, or in respiratory rates, was found to be significant statistically.

D. Enzyme activities

The five enzymes under study were NRase, transaminase, phosphoglyceryl kinase, phosphorylase and succinic dehydrogenase. The activities of these five enzymes were determined in cell-free homogenates of freshly harvested leaf blades 2, 4, and 6 weeks after spraying with 5,000 ppm KNap, applied when plants were 3 weeks old. Additional measurements of the succinic dehydrogenase were also made 4, 8, 12, 16, 20 and 24 days after the treatment which was applied to plants 14 days old in another experiment. The specific activities of the enzymes are presented in Tables III and IV and also by Fig. 9 to 16. All data, excluding those for succinic dehydrogenase activities examined in Experiment V, were

subjected to statistical analysis and no difference was found to be significant statistically.

The 5,000 ppm KNap treatment had different effects on the activities of different enzymes and also had different effects on the activity of an enzyme in plants at different ages. A stimulation was found in the case of transaminase 6 weeks after treatment, and phosphorylase at all three times of observation. Inhibitory effects on enzyme activities of other enzymes studied were recorded.

1. Nitrate reductase (NRase)

Results are shown in Table III and Fig. 9 to 14. NRase activities in both control and treated plants measured 4 weeks after treatment were very similar, but a decrease in activity in the leaf blades of treated plants of 24.80% was observed 6 weeks after treatment. Incorrect analytical procedures were used in the assay carried out 2 weeks after treatment; hence the results have not been tabulated.

2. Glutamic-pyruvic transaminase (transaminase)

Results included in Table III and Fig. 12 and 14 show that the transaminase activity was retarded most effectively in the leaves harvested 2 weeks after spraying and a decrease of 54.80% occurred. A decrease of 7.70% was observed 4 weeks after treatment. On the other hand, 6 weeks after application, an appreciable increase, 20.40%, resulted from treatment. Both the

TABLE III

The effect of 5,000 ppm KNap on the activities of succinic dehydrogenase, phosphoglyceryl kinase, glutamic-pyruvic transaminase, nitrate reductase, and phosphorylase of tomato leaf blades.

Enzyme	Unit	Weeks after treatment	Specific activity[x] C	Specific activity[x] 5,000 ppm	$\frac{T}{C}$ %
Succinic dehydrogenase	µg formozan formed/ mg protein/ hour	2	5.820	0.900	18.00
		4	1.640	1.480	90.25
		6	1.740	1.150	66.10
Phosphoglyceryl kinase	An increase of 0.05 optical density/ mg protein/hour	2	0.024	0.018	75.00
		4	0.039	0.036	92.30
		6	0.067	0.066	98.50
Glutamic-pyruvic transaminase	An increase of 0.05 optical density/ mg protein/hour	2	0.376	0.843	45.20
		4	3.900	3.600	92.30
		6	4.760	5.730	120.40
Nitrate reductase	µg of nitrite formed/ mg protein/hour	4	0.556	0.554	99.70
		6	1.010	0.760	75.20
Phosphorylase	µg of inorganic phosphate formed/ mg protein/hour	2	33.920	41.400	122.05
		4	5.040	5.670	114.00
		6	10.660	19.070	178.89

[x]: Each value of specific activity is the average value of 6 samples.

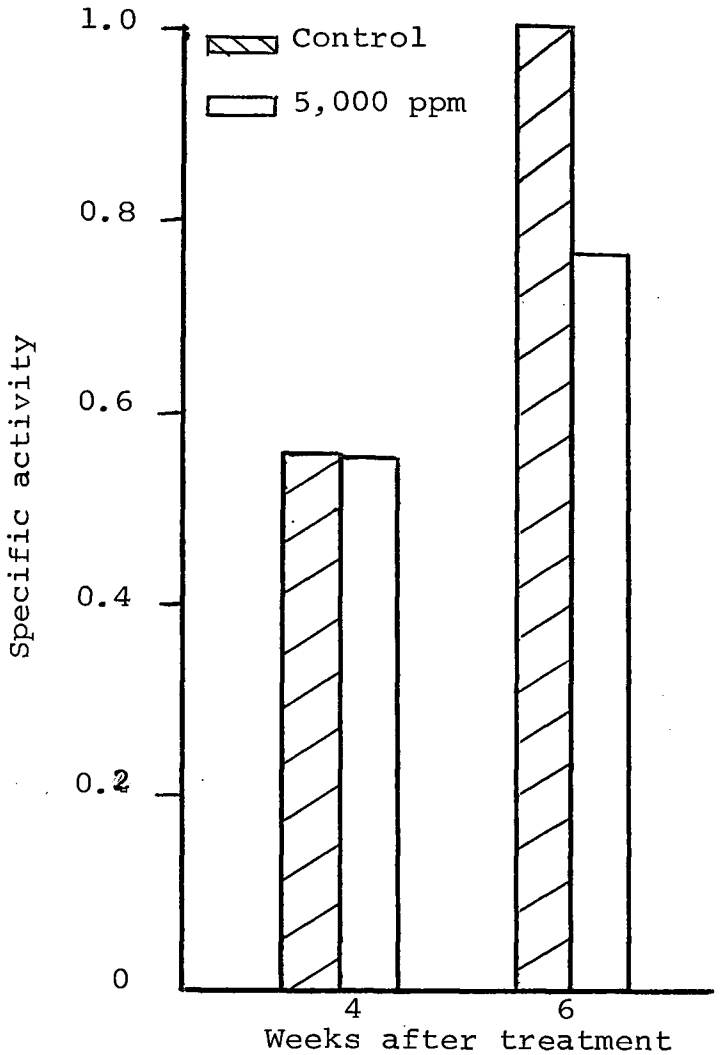


Fig. 9: Effect of 5,000 ppm KNap on the activity of nitrate reductase in tomato leaf blades.

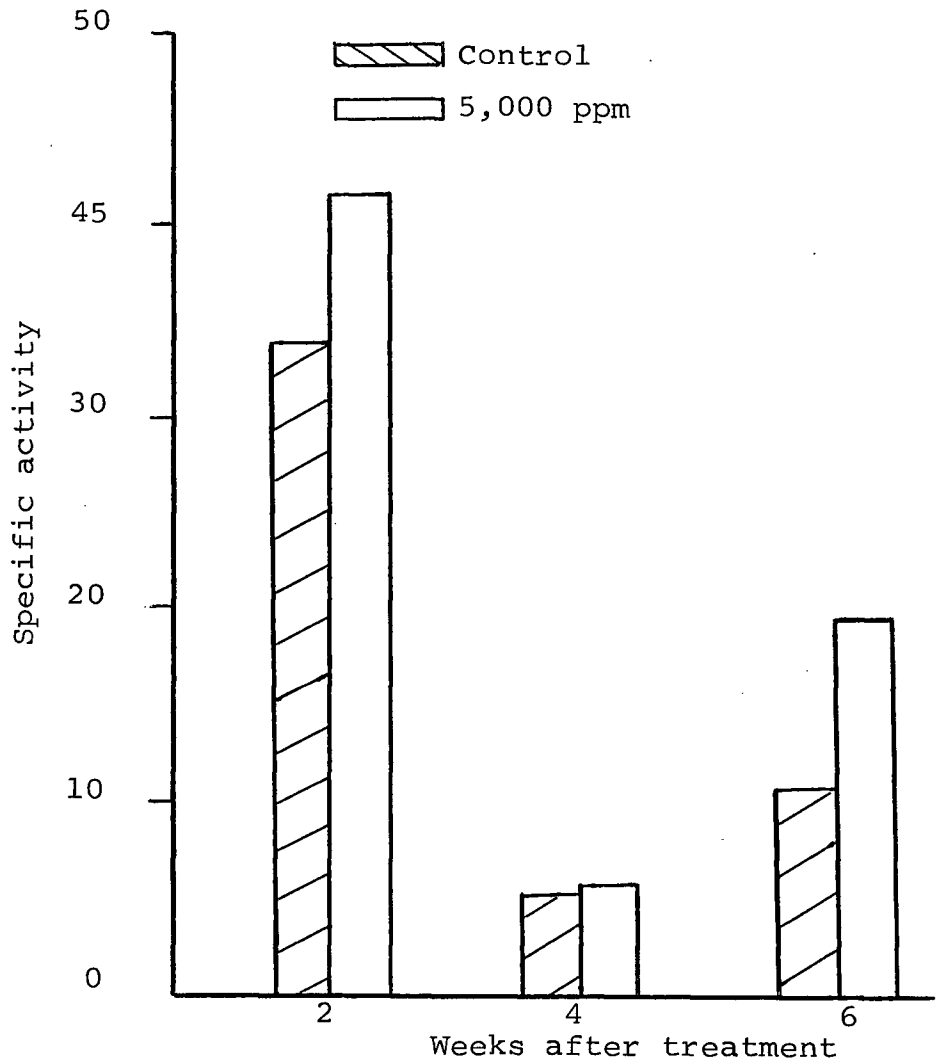


Fig. 10: Effect of 5,000 ppm KNap on the activity of phosphorylase in tomato leaf blades.

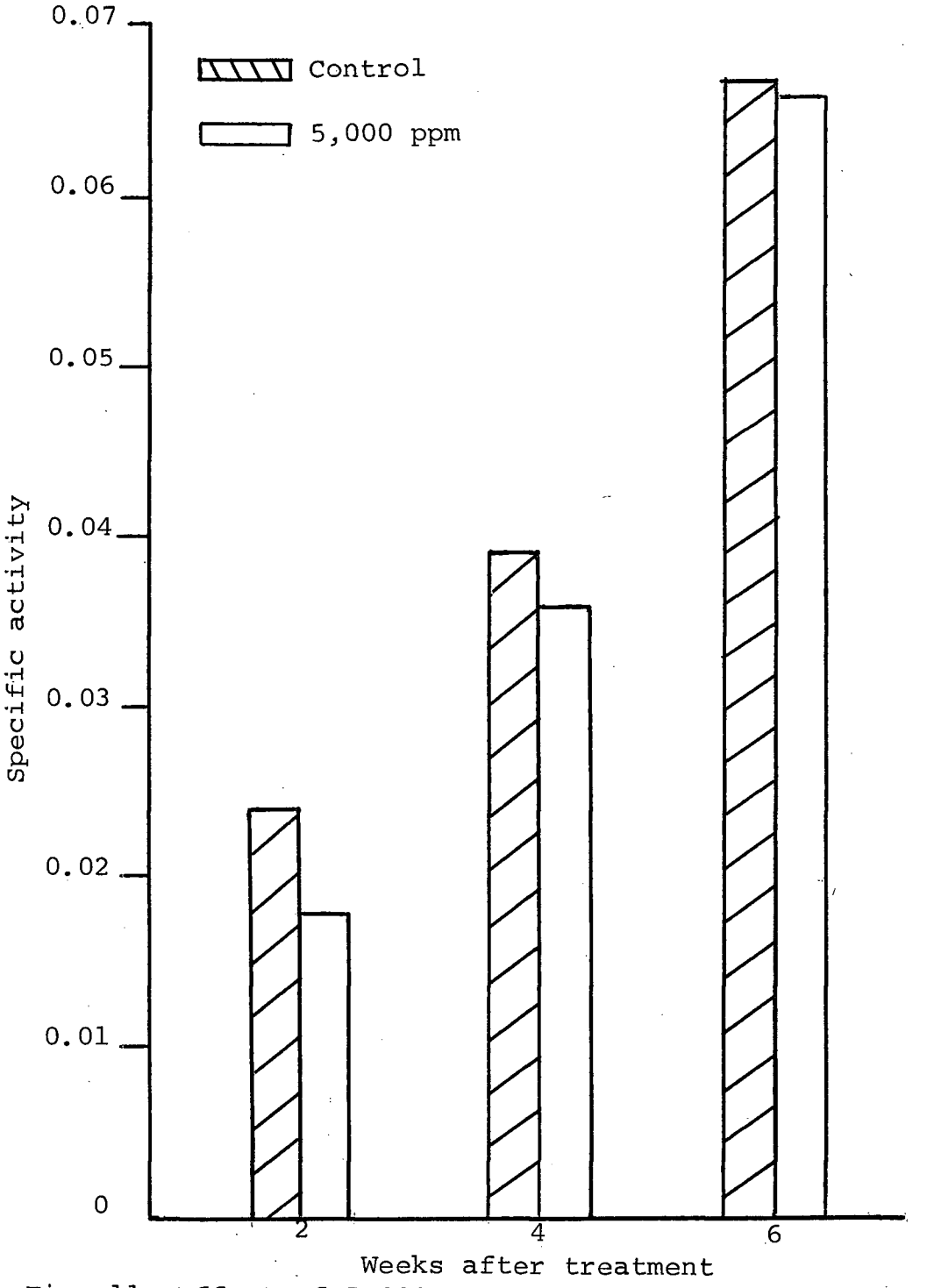


Fig. 11: Effect of 5,000 ppm KNap on the activity of phosphoglyceryl kinase in tomato leaf blades.

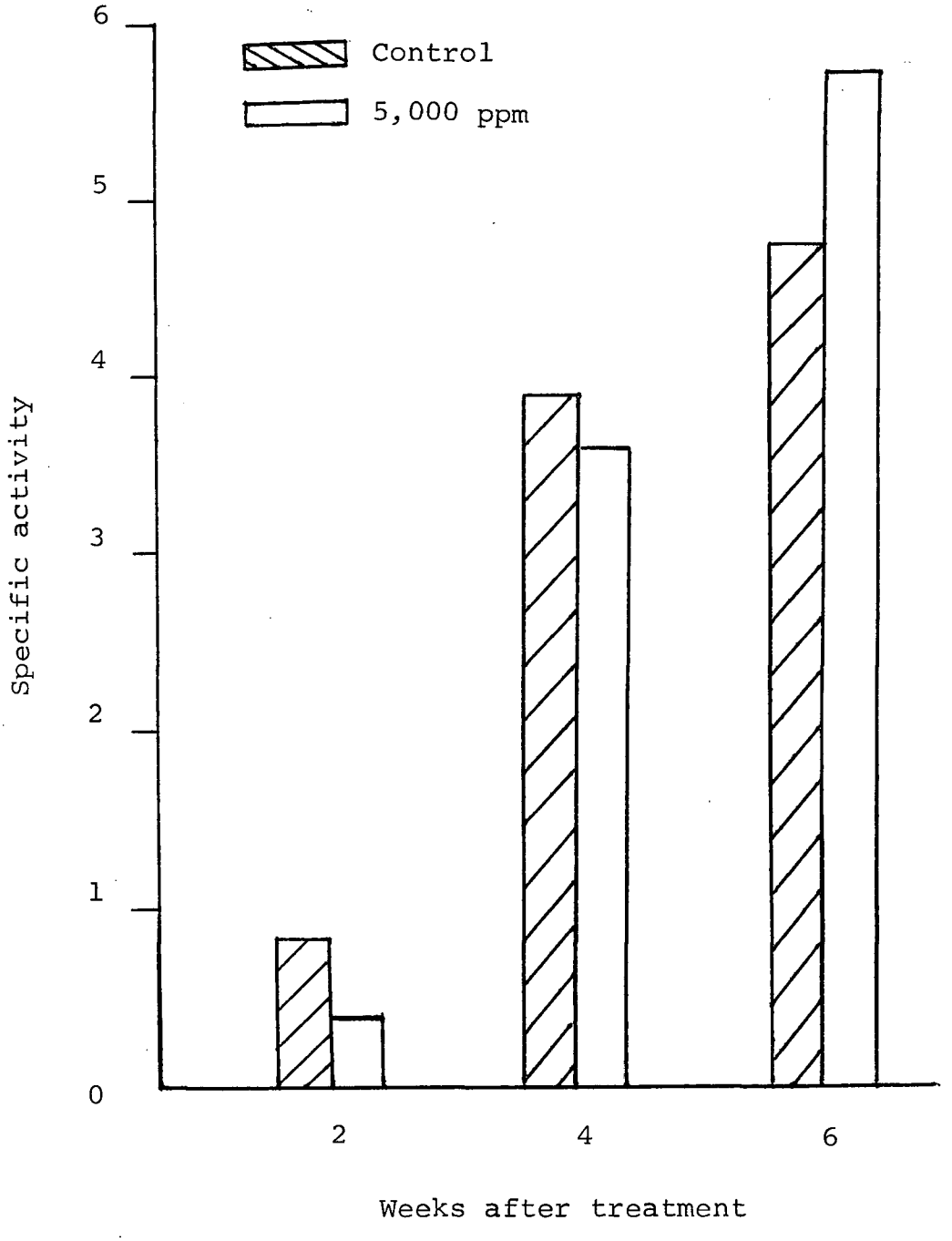


Fig. 12: Effect of 5,000 ppm KNap on the activity of glutamic-pyruvic transaminase in tomato leaf blades.

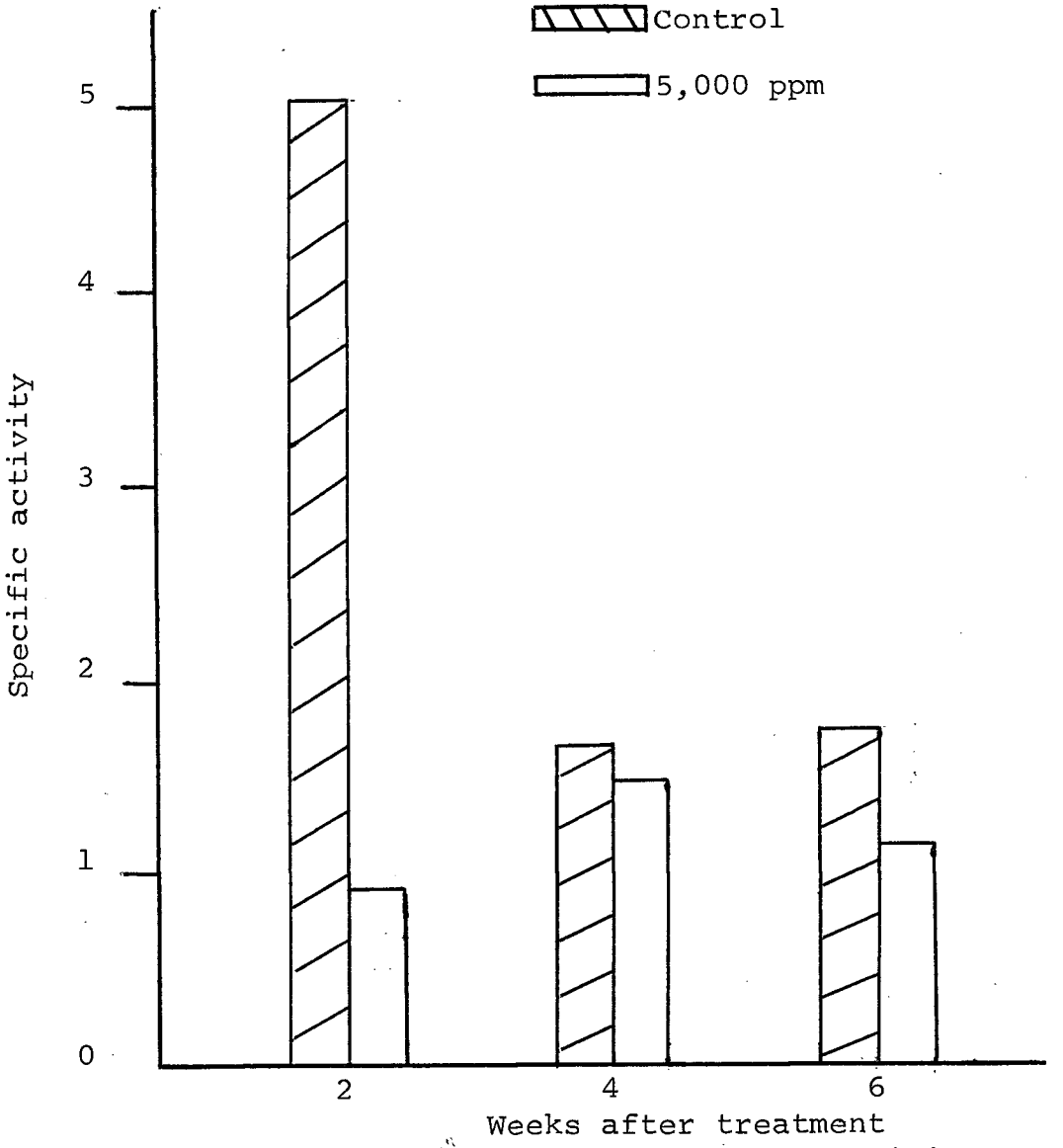


Fig. 13: Effect of 5,000 ppm KNap on the activity of succinic dehydrogenase in tomato leaf blades (Experiment IV).

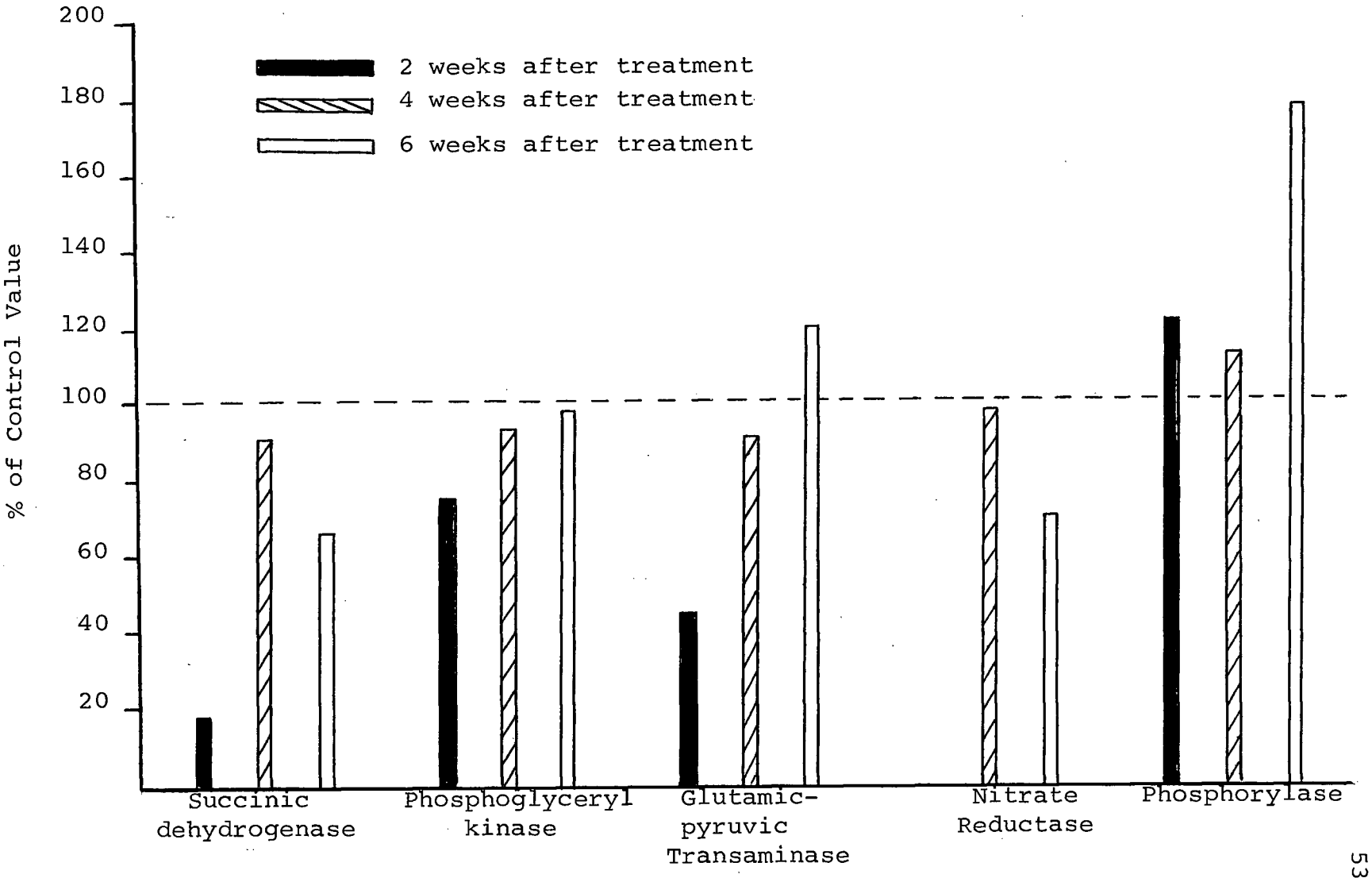


Fig. 14: Effect of 5,000 ppm KNap on the enzyme activity of tomato leaf blades.

treated and control plants exhibited a steady increase in transaminase activity over a period of 4 weeks beginning when plants were 3 weeks old.

3. Phosphoglyceryl kinase

Results are listed in Table III and Fig. 11 and 14. The phosphoglyceryl kinase activity in leaf blades of treated plants was decreased by 25.00%, 7.70% and 1.50% 2, 4, and 6 weeks after the treatment.

4. Phosphorylase

Data are given in Table III and Fig. 10 and 14. This was the only enzyme found to be stimulated in activity by the 5,000 ppm KNap spraying in the three observations made, although the extents of stimulation were different. Increases of 22.05% and 14.00% were found in the treated plants in the first and second observations. A sharp increase of 78.89% was detected in the last assay made 6 weeks after the treatment.

5. Succinic dehydrogenase

Results given in Table III and Fig. 13 and 14 show that the activity of succinic dehydrogenase was inhibited markedly by the 5,000 ppm KNap treatment. The maximum reduction, 72.00%, was found 2 weeks after treatment. As the plants became older, the inhibition was less, being 9.35% and 33.90% at 4 and 6 weeks after the application of KNap. In the subsequent experiment (Experiment V), a different technique was employed to determine the activity of succinic dehydrogenase in order to shorten the

20-hour incubation period. Plants sprayed when 2 weeks old were analyzed for succinic dehydrogenase activity 4, 8, 12, 16, 20, and 24 days after treatment. The results showed that succinic dehydrogenase activity in treated plants was lower than in the controls in the first 4 measurements. The reductions measured at these times were 35.11%, 36.32%, 41.53%, and 17.14%. However, in the last two determinations, enhanced succinic dehydrogenase activity of 6.90% and 14.23% were obtained.

E. Protein content

Protein content of each enzyme extract was determined. Only those of Experiment V are tabulated in Table IV and presented graphically in Fig. 17 and 18. Increases due to treatment, 48.48%, 12.72%, and 29.79%, were measured 4, 12, and 20 days after application of KNap. However, treatment resulted in drops of 14.82%, 4.20%, and 23.41% as determined 8, 16, and 24 days after treatment. These quantitative changes in enzyme protein suggest that the growth stimulation may affect the synthesis of the enzyme at the ribosomal level.

F. Tomato Yield

Data of Experiment II, in which the plants were sprayed when 4 weeks old, are recorded in Table V and illustrated in Fig. 19. In this experiment, malformation and/or darkened distal end of the tomato fruit were observed. Up to the age of 111 days, results based on fresh weight of fruit showed that the treated

TABLE IV

The effect of 5,000 ppm KNap on the specific activity of succinic dehydrogenase and the protein content of the enzyme extract of the leaf blades of tomato plants over a period of 20 days beginning when 18 days old.

Age of plants (day)	Succinic dehydrogenase specific activity‡			Protein content[x]		
	C	5,000 ppm	$\frac{T}{C}$ %	C	5,000 ppm	$\frac{T}{C}$ %
18	8.38	5.44	64.89	4.95	7.35	148.48
22	4.68	2.98	63.68	4.86	4.14	85.18
26	1.43	0.83	58.47	5.11	5.76	112.72
30	2.94	2.44	82,86	7.62	7.30	95.80
34	5.47	5.85	106.90	8.46	10.88	129.79
38	1.82	2.07	114.23	7.05	5.40	76.59

[x]: Protein content: expressed as mg protein/100 mg of fresh leaf blades.

‡: Specific activity: a 0.05 optical density increase/mg protein/hour.

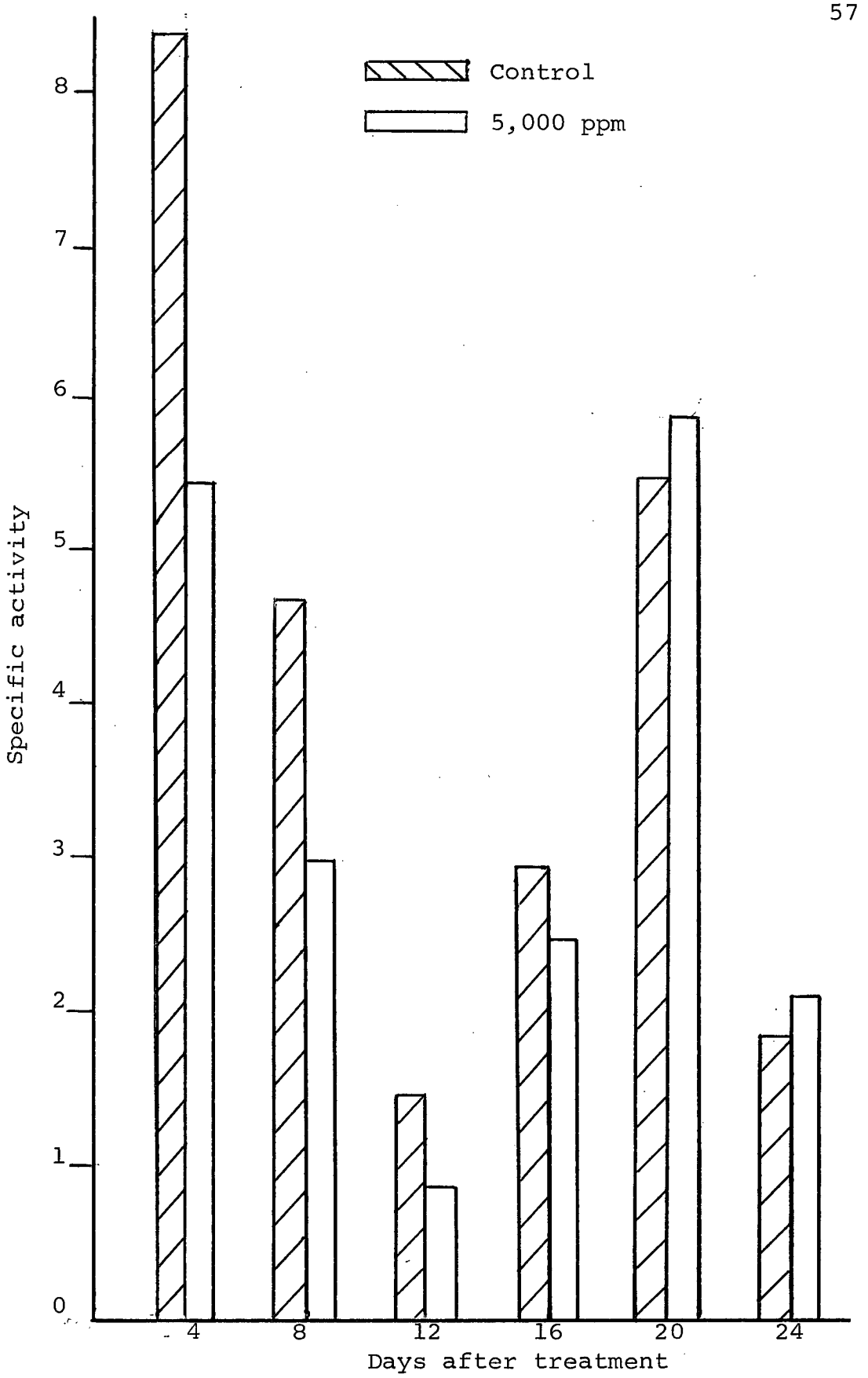


Fig. 15: Effect of 5,000 ppm KNap on the activity of succinic dehydrogenase in tomato leaf blades (Experiment V).

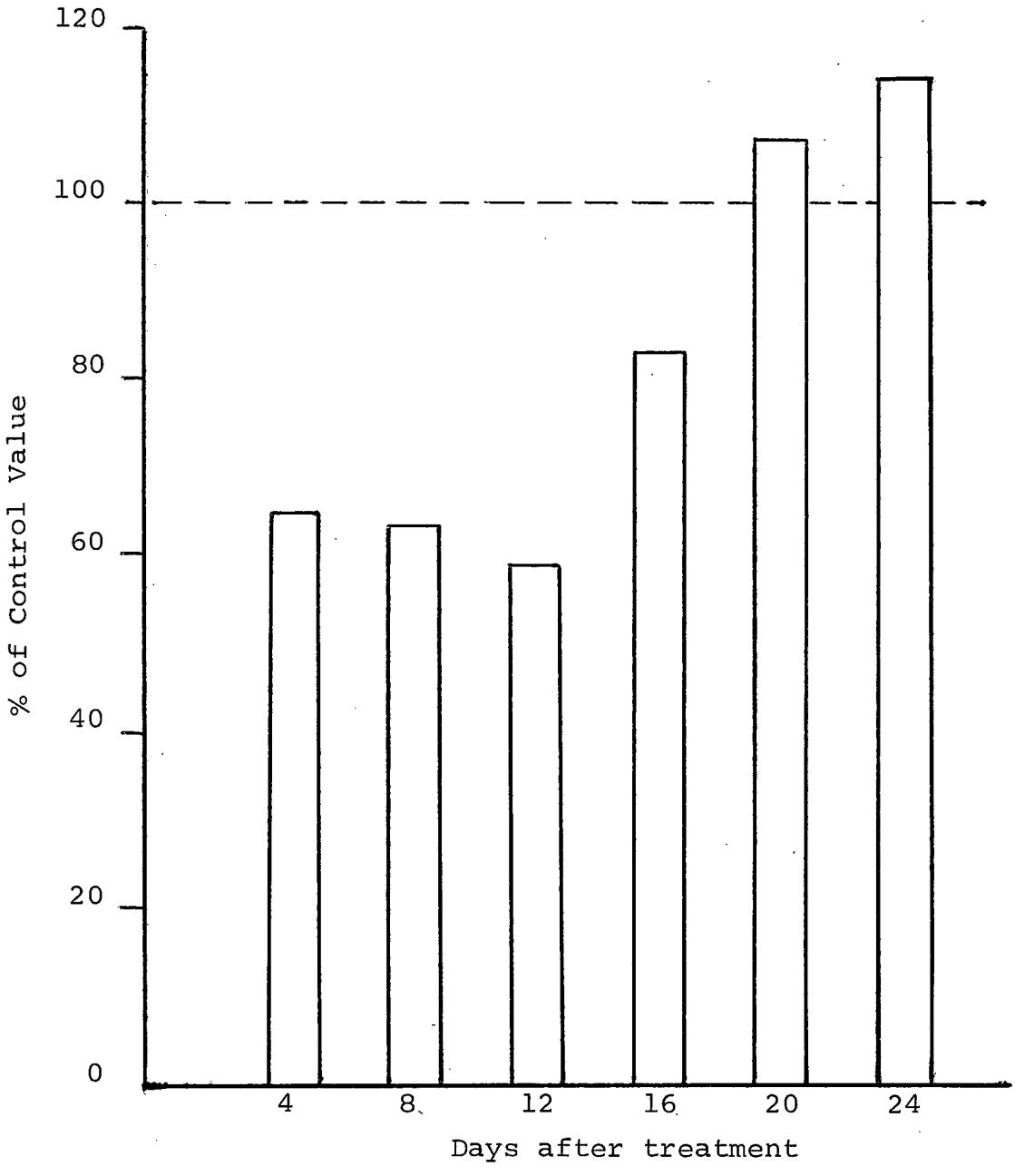


Fig. 16: Effect of 5,000 ppm KNap on the activity of succinic dehydrogenase in tomato leaf blades (Experiment V).

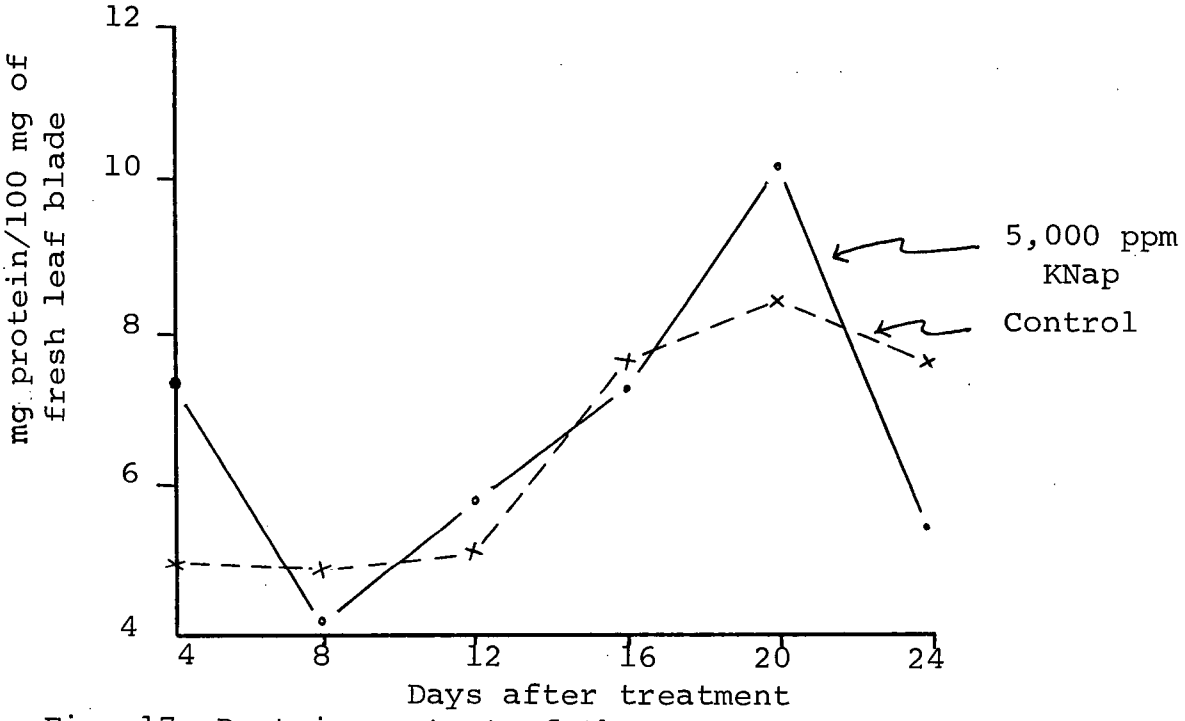


Fig. 17: Protein content of the enzyme extracts of the leaf blades of KNap-treated and control tomato plants.

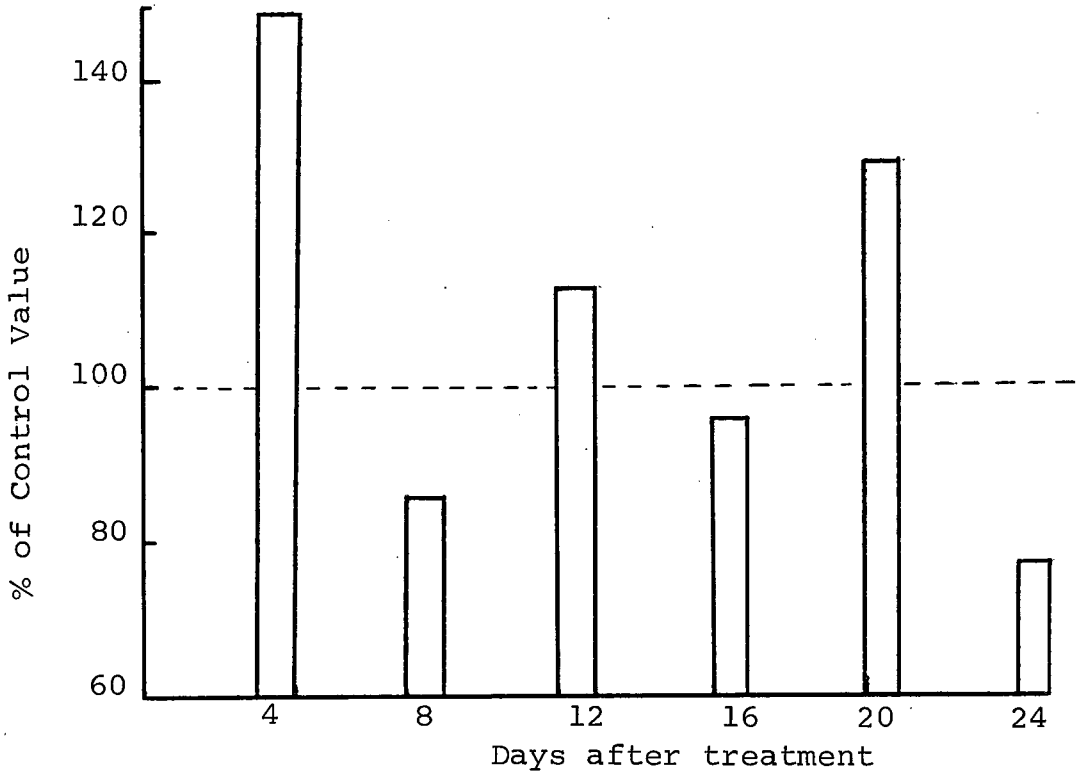


Fig. 18: Effect of 5,000 ppm KNap on protein content of enzyme extracts of leaf blades of tomato plants.

plants were less susceptible to this disease. On 2,500 ppm-and 5,000 ppm-treated plants, the ripe tomatoes free of blossom-end rot were 7.70% and 62.50% more than those of the control plants by weight. Results based on fruit number showed that good tomato fruits from 2,500 ppm KNap-treated plants were 16.00% fewer but were 40% more in the case of 5,000 ppm KNap-treated plants. In the case of the diseased tomato fruits, an increase of 16.13% and a decrease of 35.48% in number were induced by the 2,500 ppm and 5,000 ppm KNap treatments.

For Experiment IV, the total number and fresh weight of both green and ripe tomato fruits are listed in Table VI and illustrated in Fig. 19. There were 67 ripe tomato fruits in both control and treated plants, although the fresh weights of ripe tomato fruits from 5,000 ppm KNap-treated plants were 24.60% more than that of the controls. The fresh weight of green tomato fruits from the 5,000 ppm KNap-treated plants was almost identical with that of the controls. The number of green tomato fruits from the treated plants was decreased by 38.00%. If all green and ripe tomatoes were considered together, the treatment appeared to result in a favorable effect on both number and fresh weight of tomatoes, by showing 16.92% and 14.71% promotions respectively. Each of the values here represented the pooled values of 10 plants/treatment.

Statistical analysis for fruit yield has not been done due

TABLE V

The effect of KNap on number and fresh weight of ripe and rotten tomato fruits (Experiment II).[x]

Tomato Fruit	Treatment ppm	Number	Fresh Weight (g)	T/C% Number	T/C% Fresh Weight
Ripe	C	50	847.20		
	2,500	42	712.50	84.00	107.70
	5,000	70	1376.90	140.00	162.50
Rotten	C	31	346.50		
	2,500	36	752.80	116.13	188.30
	5,000	20	173.50	64.52	50.40
Ripe + Rotten	C	81	1193.70		
	2,500	78	1465.30	96.29	122.74
	5,000	90	1550.40	111.11	121.48

[x]: All tomato fruits were harvested at the same time when plants were 111 days old. Each value is the pooled value of 9 plants per treatment.

TABLE VI

The effect of KNap on number and fresh weights of ripe and green tomato fruits (Experiment IV).

Tomato Fruit	Treatment (ppm)	Number	Fresh Weight (g)	T/C % Number	T/C % Fresh Weight
Ripe	C	67	5635.65		
	5,000	67	7022.28	100.00	124.60
Green	C	63	3882.64		
	5,000	87	3908.17	138.00	100.66
Ripe + Green	C	130	9518.29		
	5,000	154	10930.45	116.92	114.71

[x]: Ripe tomato fruits were picked as soon as they were firm and red. Green tomato fruits were picked when plants were 110 days old. Each value represents the pooled value of 10 plants.

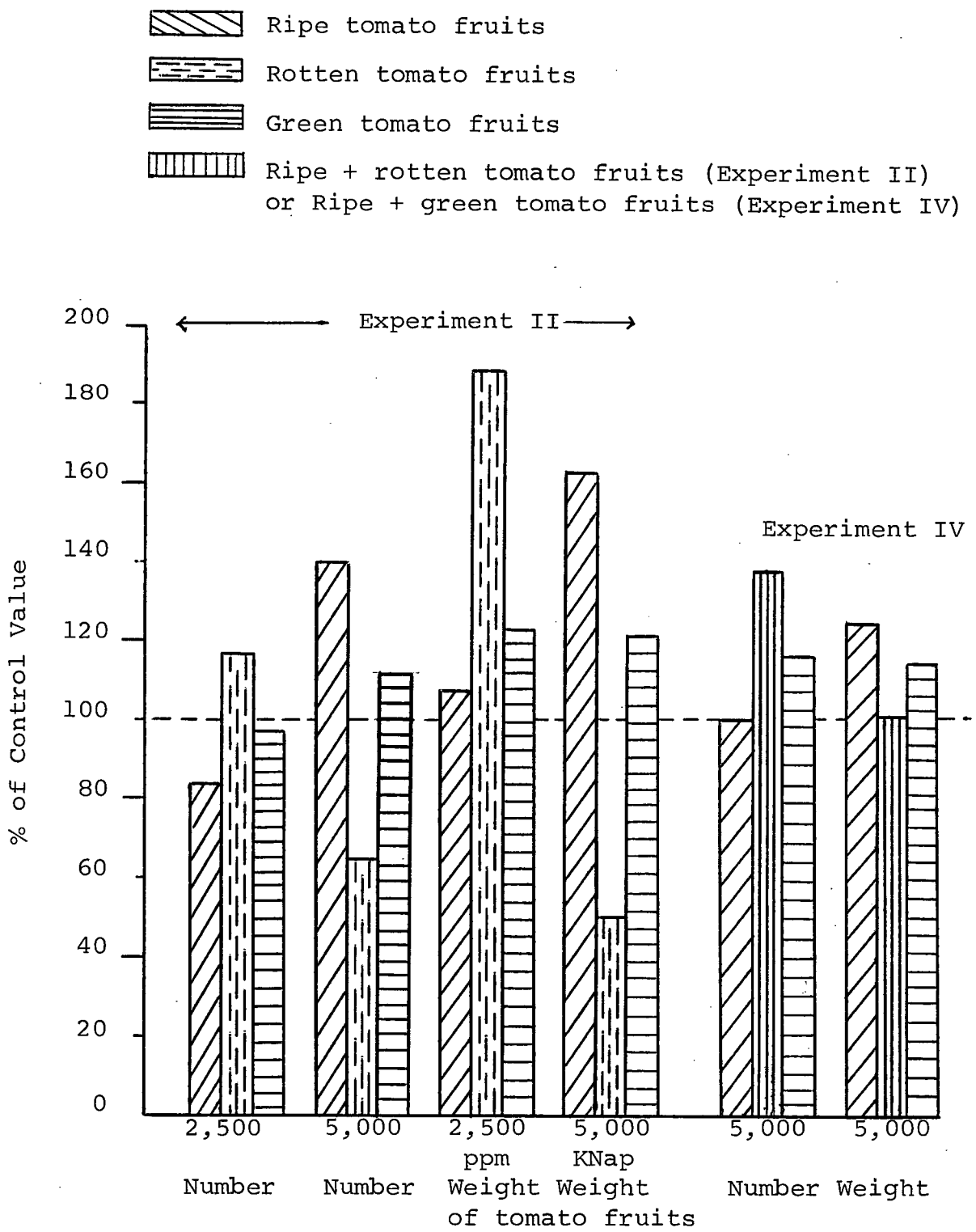


Fig. 19: Effect of KNap on number and fresh weight of tomato fruits (Experiments II & IV).

to the fact that there were not enough replicates in the experiments. However, the results showed that there was an evident promotive effect of KNap on fruit yield.

G. Quality of tomato fruits

Reducing sugar, sucrose, total sugars, titratable acid, and ascorbic acid (vitamin C) of the mature tomato fruits were determined to see if the KNap treatment affected the chemical composition of tomato fruits.

1. Sugars

Sugar content data are summarized in Table VII and illustrated graphically in Fig. 20 and 23.

The mature tomato fruits from the treated plants had higher sucrose content, being 7.91%, 20.75%, and 5.48% in fruits stored for 10, 4, and 1 day. Of course, total sugars behaved in a similar pattern and were increased by 11.08%, 23.64% and 4.33% in tomato fruits stored for 10, 4, and 1 day respectively.

2. Titratable acid

At the end of 10 days of storage, the titratable acid content of fruits of treated plants was lower than that of the controls by 4.30%, but an increase of 5.60% in titratable acid was found when the storage period was shortened to 4 days. These results can be seen in Table VII and Fig. 21 and 23.

3. Ascorbic acid

The results tabulated in Table VII and illustrated in

TABLE VII

The effect of 5,000 ppm KNap on the content of sugars, ascorbic acid, and titratable acid in mature tomato fruits.

	Days of storage after harvest	Content[X]		T/C %
		C	5,000 ppm	
Sucrose	10	10.07[xx]	12.25	121.60
	4	10.17	13.58	125.40
	1	11.87	11.97	100.85
Reducing sugar	10	35.40	38.20	107.90
	4	37.10	44.80	120.70
	1	38.30	40.40	105.50
Total sugars	10	46.00	51.10	111.08
	4	47.80	59.10	123.60
	1	50.80	53.00	104.30
Ascorbic acid	10	34.40	25.30	73.60
	4	32.60	29.60	90.80
	1	33.50	28.90	86.30
Titratable acid	10	115.00	110.00	95.70
	4	118.00	125.00	105.60

X: Content of sugars: Percentage of dry tomato fruit weight.

Ascorbic acid content: mg/100 g of fresh tomato fruit
Titratable acid content: ml of 0.1 N KOH/100 g of fresh tomato fruit

xx: Each value is the average value of 6 samples.

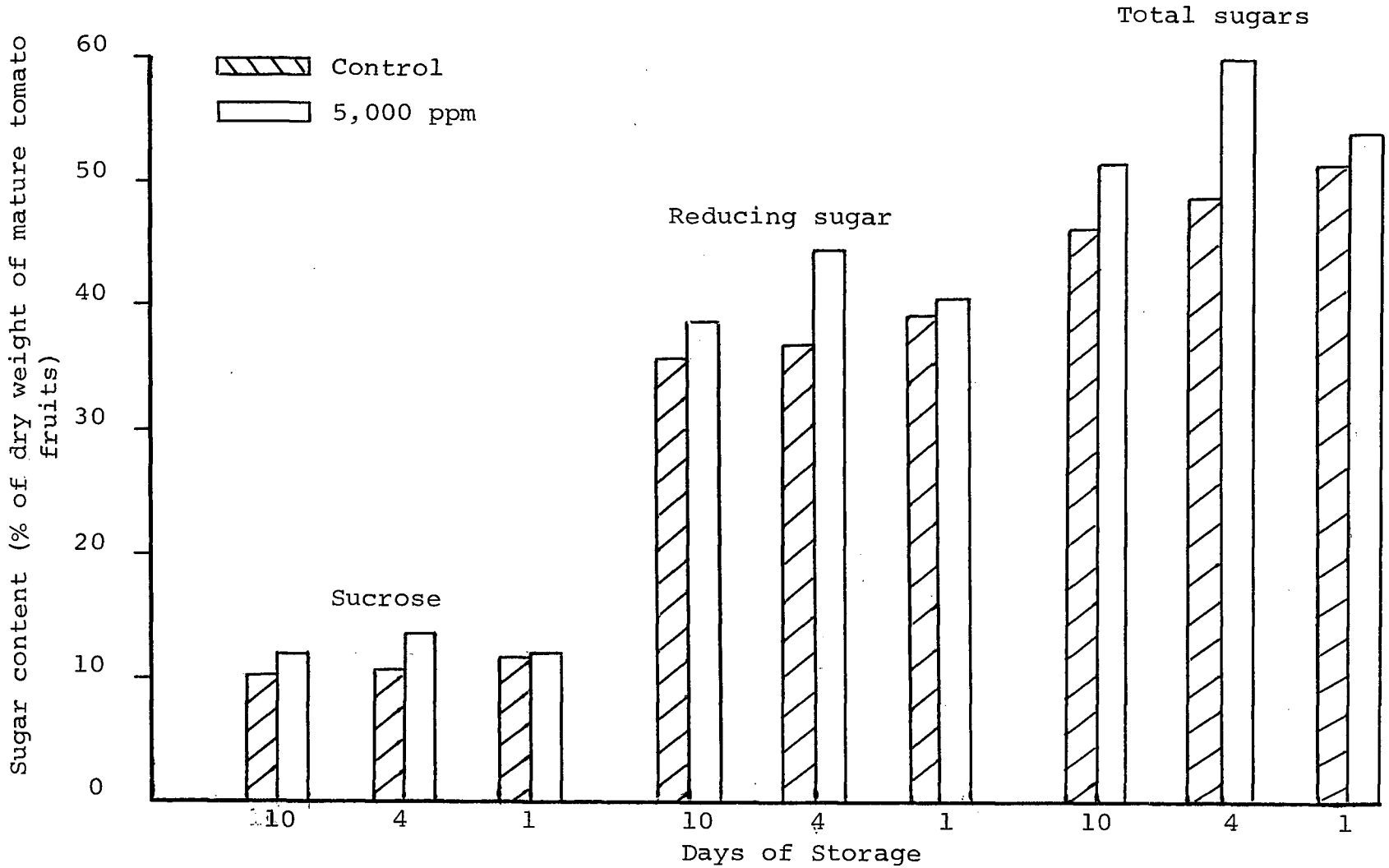


Fig. 20: Effect of 5,000 ppm KNap on content of sucrose, reducing sugar, and total sugars in mature tomato fruits.

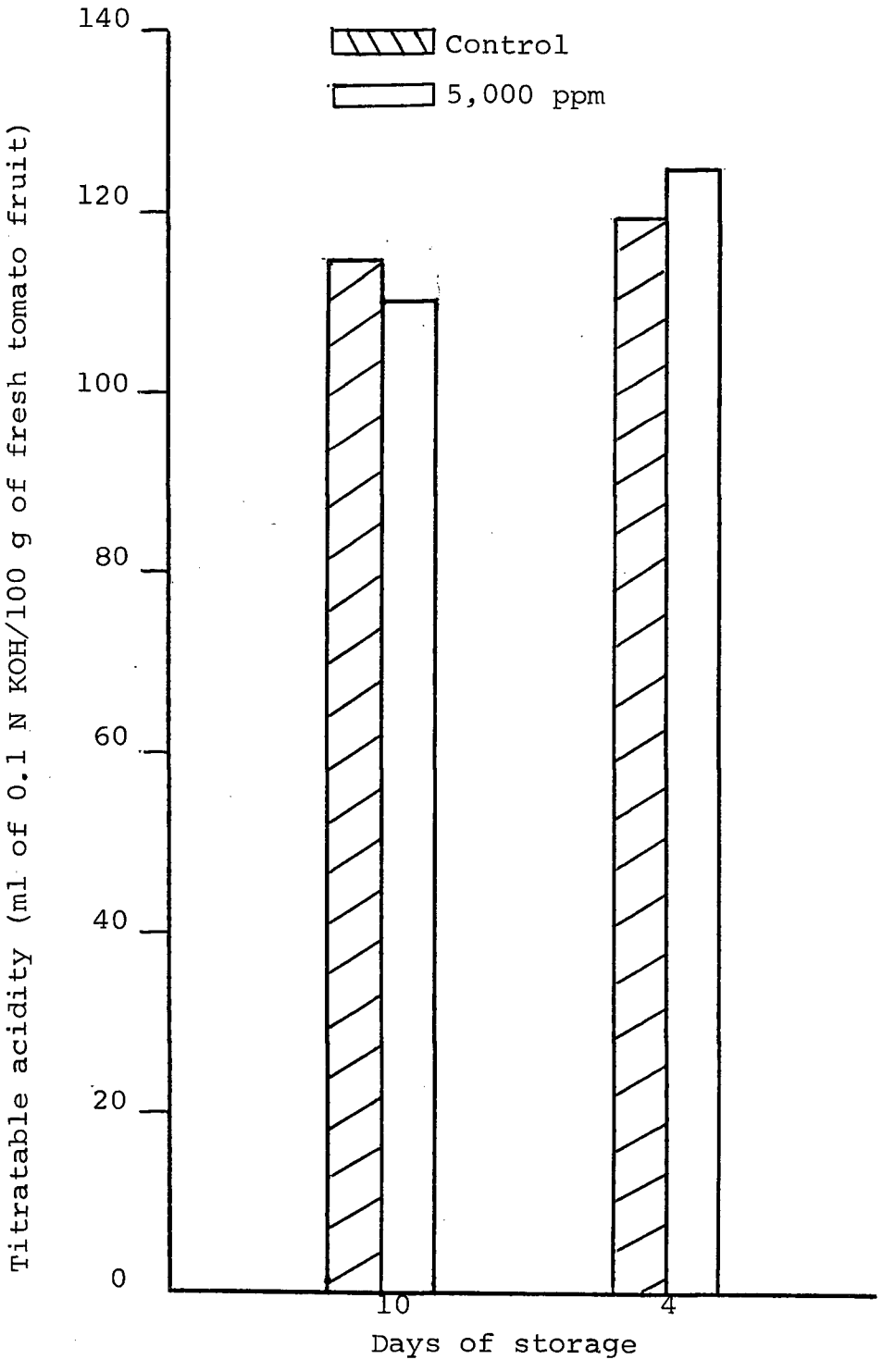


Fig. 21: Effect of 5,000 ppm KNap on the content of titratable acid in mature tomato fruits.

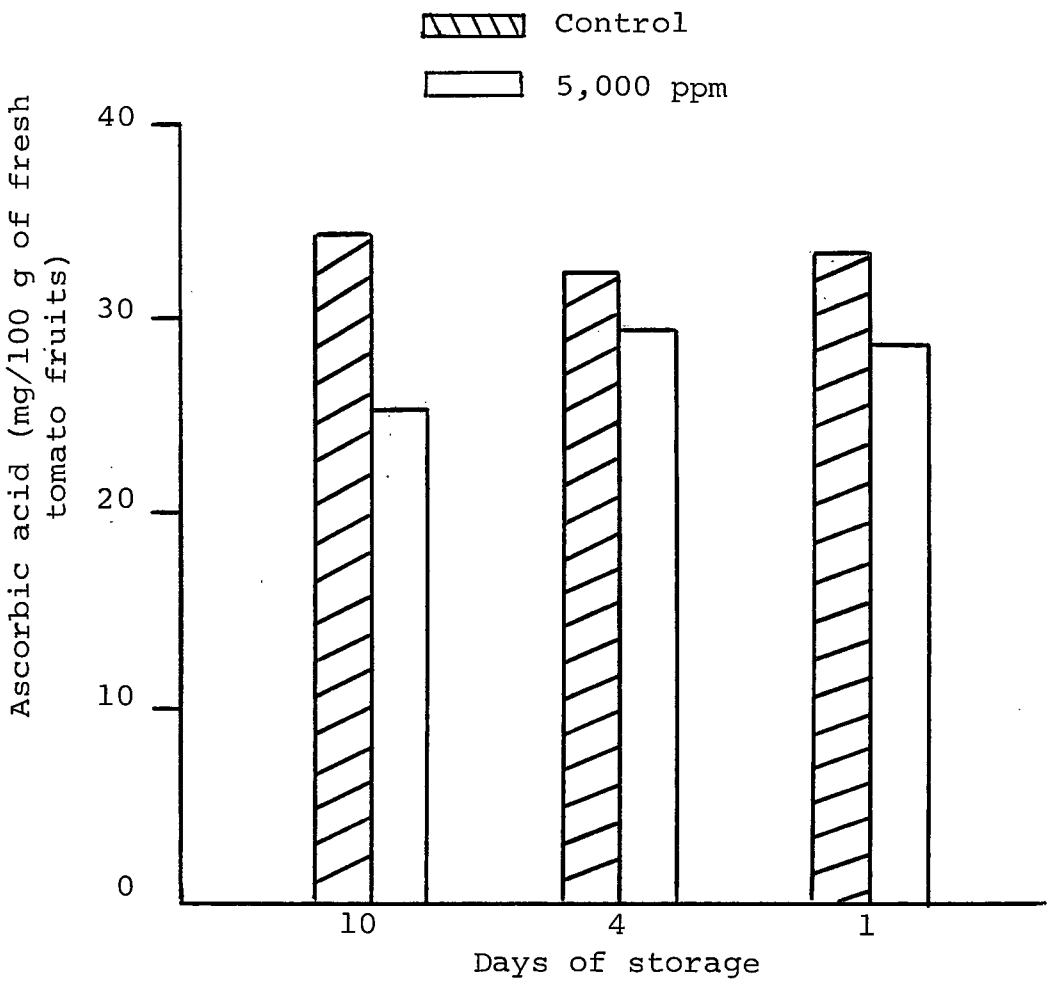


Fig. 22: Effect of 5,000 ppm KNap on content of ascorbic acid in mature tomato fruits.

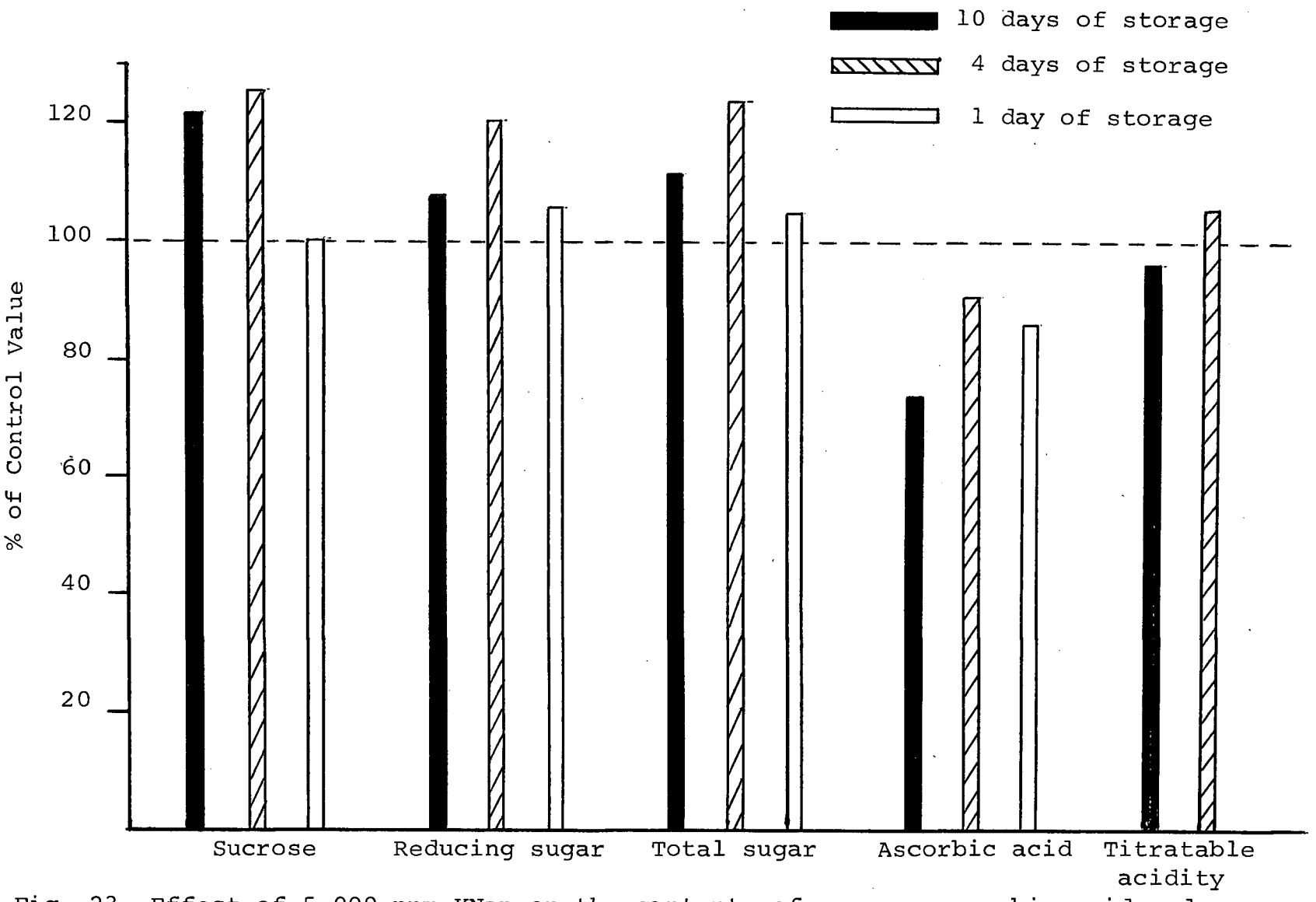


Fig. 23: Effect of 5,000 ppm KNap on the contents of sugars, ascorbic acid and titratable acid in mature tomato fruits.

Fig. 22 and 23, indicated that the ascorbic acid content was decreased by the treatment. Decreases of 26.40%, 9.20% and 13.73% were detected in tomato fruits stored for 10, 4, and 1 day respectively.

Although there was no statistically significant difference found in sugars, titrable acidity, and ascorbic acid content, a distinct tendency toward change caused by KNap treatment is evident.

V. DISCUSSION

A. Vegetative growth

It has been shown by Wort (unpublished) that the foliar application of aqueous solutions of KNap and NaNap, 2,500 and 5,000 ppm, to 2-week-old bush bean plants (Phaseolus vulgaris var. Top Crop) resulted in a 20% increase in juvenile growth and in yield of green pods and ripe seeds. In the experiments described in this thesis KNap solutions, 2,500 and 5,000 ppm, were applied to tomato (Lycopersicum esculentum var. Bonny Best) plants.

In the first two experiments tomato plants were treated at the age of 4 weeks and no definite and consistent growth responses were obtained. It was thought that the age of the plants at the time of treatment, 4 weeks old, might have been responsible for the inconsistent responses.

The third experiment was designed to examine the

interaction between concentration of KNap and the treatment time and the tomato plant growth. The results suggested that the 5,000 ppm KNap applied to tomato plants when 19 days old gave encouraging results in terms of fresh and dry weights of plant tops. The application of 5,000 ppm KNap to 12-day-old plants and of 2,500 ppm KNap to plants when 12 and 19 days old gave inconsistent results.

Data of the fourth experiment in which plants were sprayed when 3 weeks old with 5,000 ppm KNap indicated that the effects of treatment again on fresh and dry weights measured 2, 4, and 6 weeks after treatment had no statistical significance.

Based on the results of the four experiments, it appears that both the 2,500 ppm and the 5,000 ppm KNap treatment resulted in inconsistent effects on growth of tomato plants.

Székely (1966) reported that HNap could increase growth of tomato plants. Yuréva (1965) proved that Nap favorably affected the growth of corn plants when applied at strength of 0.005%. More concentrated solutions were inhibitory. Members in the team working with KNap at the University of British Columbia observed considerable stimulation of growth of a number of species of plants such as maize, sugar beet, sunflower, bush bean, radish, spinach, tobacco, and tomato (Wort, unpublished). The growth of tomato plants was found to be increased by 12.50% by 2,500 ppm KNap sprays, in terms of fresh and dry weights of plant tops.

Fattah and Patel (unpublished) got enhanced growth and yield of bush bean plants by the application of KNap. Bazanova and Akopova (1966) reported that effects of HNap interact with level of N-P fertilizers applied at the same time, therefore the growth appeared to depend on the amount of applied fertilizer. Popoff, Dimitrov, and Stefanova stated that Naps markedly stimulated the growth of wheat and corn. However, most of the above-named investigators studied the effect of Nap on species of plants other than tomato and for this reason a direct comparison of the present data with above-mentioned reports would not seem very valid.

B. Chlorophyll and carotenoid content

It was noticed that the contents of chlorophyll a and b, and carotenoid, increased as both the control and treated plants became older. However, when the pigment content in leaf blades of treated and control plants of the same age were compared, the chlorophyll content (both a and b) was increased to a small extent but the carotenoid content was increased more. This might be the reason for the increased photosynthetic rate observed simultaneously.

Bazanova et al. (1966) reported that Nap could either increase or decrease the chlorophyll content of the cotton plant depending on the level of applied N-P fertilizer. Higher chlorophyll content was found in the Nap-treated plants when the usual amount of N-P was applied. Under the influence of Nap, increases in chlorophyll and/or carotenoid content have been

found in other species of plants, for example, potato (Lydygina, 1965, Abolina and Ataullaev, 1966), corn, sugar beet (Yureva, 1965), and bush bean (Fattah, and Patel, unpublished).

In the experiments, soluble N:P:K, 20:20:20, fertilizer was applied weekly to the soil during the growth period of the tomato plants. The slight increases in chlorophyll content observed in this experiment might have resulted from the interaction of the fertilizer applied and the effect of KNap.

C. Photosynthesis and respiration

The data suggest that the 5,000 ppm KNap-treated plants proceded more energetically in both photosynthesis and respiration 4 weeks after treatment, but the opposite was observed 2 weeks after treatment. This suggests that a rather long time (for example, 4 weeks in this experiment) is needed for KNap to exhibit its stimulatory effect on photosynthesis and respiration of tomato plants. The higher photosynthetic rate was found to be accompanied by increases in pigment content and both fresh and dry weights of plant tops.

The stimulatory effect of Nap on the intensities of photosynthesis and respiration has been reported by several investigators in different species of plants, for example, potatoes (Ladygina, 1965; Abolina and Ataullaev, 1966); grape plants (Kolesnik, 1965); melons and other vegetables (Abolina and Attaullaev, 1966). Besides, Fattah (unpublished) observed

similar effects in bush bean plants under different light intensity regimes.

D. Enzyme activities

The effects of Nap on activities of several enzymes have been reported by some scientists. Nap was found to promote both activity and formation of amylase in the mold Asperigillus usamii (Burachevskii, 1965). Activities of ascorbic acid oxidase and peroxidase in leaves of Nap-treated cotton plants were found to be higher than the controls (Agakishier and Bazanova, 1965). Effects of Nap on catalase activity of cotton (Akopova & Bazanova, 1966) and grape plant (Kolesnik, 1965) were found to be stimulative. Fattah (unpublished) showed that activities of transaminase, phosphoglyceryl kinase, and phosphorylase were higher in the leaf blades of KNap-treated bush bean plants.

The results of the present experiment indicated that the effect of KNap on enzyme activities depended on the specific enzyme and the age of plants. Among the five investigated enzymes, only phosphorylase in all three observations, and transaminase in the last observation made 6 weeks after treatment, were increased in activity by the treatment. However, NRase, succinic dehydrogenase, and phosphoglyceryl kinase were decreased in activities by the KNap application.

Experiment V was done to follow more closely the immediate effect of KNap on succinic dehydrogenase activity and the protein

content of the corresponding enzyme extract. The results indicated that there was no parallelism between the changes of protein content and specific activity of succinic dehydrogenase.

The activities of phosphoglyceryl kinase, succinic dehydrogenase and NRase were lessened by the KNap treatment. This might be because the KNap solution used was too concentrated. Indeed, catalase activity was found to be decreased by high concentrations of Nap or double spraying with a lower concentration by Kolesnik (1965).

E. Protein content

The effect of KNap on protein content in leaf blades of tomato plants was found to be quite inconsistent. Maximum amount of protein was measured 4 days after spraying by showing 50.00% increase.

Higher protein content in corn and sugar beets treated by Naps was reported by Yureva (1965), and similar results were obtained by Abolina and Ataullaev (1966) on different sorts of potatoes. Subbotina (1965) found that 0.1% Naps increased protein content in apple tree leaves 1 month after treatment, but 1.5 months after the application the concentration of N compound decreased. Furthermore, Pakhomova (1965) reported that the protein and nucleic acids contents in Nap-treated tomato plants were different from the controls.

F. Fruit Yield

Several investigators in the past have reported the effect of Naps on fruit quantity and quality of different species of plants. Kulieva (1964) reported that olive fruit yield was enhanced by approximately 70.00% under the influence of NaNap. Marshaniya, Sharashenidze, and Dumbadze (1965) found that 0.05% Nap treatment increased the tangerine fruit weight. The fact that Naps accelerated the ripening of the cotton plant and opening of bolls was confirmed by Naghibin (1965). Working on the same plant, Bazanova and Akopova (1966) discovered that the maturation and crop yield of cotton depended on the amount of applied fertilizer.

More valuable information stating that an increase of 6 to 13% in total yield of Nap-sprayed tomato plants was contributed by Popoff and Boikov (1962). Increased yield up to 18.80% in Nap-treated tomato plants was reported by Polikarpova (1965).

Results of Experiments II and IV indicated that the KNap at the concentrations of 2,500 ppm or 5,000 ppm could favor the tomato fruit number and fresh weight and also earlier maturity.

In Experiments II and IV, tomato fruits were found to be rotten at the distal end. This blossom-end rot may be due to the poor ventilation system of both the growth room and green house. Besides, the plants were placed quite close together due to the limitation of space. It was noticed that the 2,500 ppm

KNap treatment induced a greater incidence of the disease, but the 5,000 ppm treatment seemed to induce resistance to the rot, and also the plants reacted more favorably to the $CaCl_2$ spray.

It was suggested that Ca deficiency, rather than water shortage was the primary cause of the disorder. In view of this, and the experimental results, KNap at the strength of 5,000 ppm might influence the calcium uptake from the soils and/or facilitate the translocation of the rather immobile Ca to the leaves and consequently decrease the blossom-end rot to a certain extent.

G. Quality of tomato fruits

Different workers have claimed that Nap treatment could affect the chemical composition such as sugar, ascorbic acid and titratable acid of different plants and plant organs. Agakishier and Bazanova (1965) reported that KNap heightened the ascorbic acid content of cotton plant leaves. Bazanova and Akopova (1966) observed that Nap increased the ascorbic acid and free carbohydrate in leaves of cotton plants only if low concentration or no fertilizer was applied. Sugars and vitamin C content were raised in cabbage plants by Nap in the experiment done by Asadov (1965). In tangerine fruits of Nap-treated plants as stated by Marshaniya and Sharashenidze (1965), increasing sugar content accompanied by decreasing acid content were observed. Higher sugar content in grapes (Kolesnik, 1965), higher sugar content in melon fruits, and higher vitamin C in cabbage, carrots,

and onions (Abolina and Ataullaev, 1966) were observed under the influences of Naps. Aliev (1965) reported that with the introduction of NaNap, the composition of the tomato fruits changed sharply; increased amount of sugar and ascorbic acid.

In the present experiment, 5,000 ppm KNap did exert a positive effect on reducing sugar, sucrose, and total sugars in the mature tomato fruits and also the loss of sugar content during the course of storage was less. Titratable acid of the mature tomato fruit under the influence of KNap was higher at the end of 4 days storage but lower as the duration of storage was longer. Apparently, 5,000 ppm KNap increased titratable acid in tomato fruits, but during storage the acid content disappeared quicker and more. Ascorbic acid content was lessened by the treatment which also did not exhibit favorable effect on the protection against ascorbic acid loss during storage.

H. Overall statement

In view of the above-mentioned experimental results, it seems too early to propose any mechanism for the action of KNap on plants. The mechanism concerning how the KNap treatment affects different biochemical and physiological aspects of the tomato plants is still up in the air and therefore further studies are essential to gain more knowledge about this. In fact, radioactive KNap labelled at the carboxylic C was proved to form conjugates with glucose and aspartic acid (Severson, unpublished).

However, it can be pointed out that since the activities of NRase and transaminase were suppressed the quantity of NH_3, and synthesis of glutamic acid, and in turn other amino acids synthesized by transamination would be less. Acids involved in the Kreb Cycle then would not be drawn away for amino acid and protein synthesis. In addition, succinic dehydrogenase activity declined. Under these situations, sugar content would be accumulated more and perhaps this was the reason why higher sugar content in mature tomato fruits was detected under the influence of 5,000 ppm KNap. Higher pigment content was found to associate with the increased photosynthetic rate in KNap-treated plants. The KNap treatment might increase the absorption and/or translocation of Ca and/or water and consequently protected tomato plants from blossom-end rot which was supposed to result from Ca deficiency and sudden water shortage. How the KNap affected the enzyme activities could not be explained by the results obtained.

VI. CONCLUSIONS

KNap aqueous solution of the concentrations 2,500 ppm and 5,000 ppm were sprayed on tomato leaves to study the effects on the growth, productivity, and metabolism of tomato (Lycopersicum esculentum var. Bonny Best) plants. Specifically, effects pertaining to growth, yield, enzyme activity, pigment content, photosynthesis, respiration and quality of tomato fruits

in terms of sugar, titratable acid and ascorbic acid were studied.

Based on the results obtained, it is possible to draw the following conclusions:

(1) Contents of pigments including chlorophyll a and b, and carotenoid were increased in the leaf blades of the 5,000 ppm KNap-treated plants.

(2) Higher photosynthesis and respiration rates were found under the influence of KNap.

(3) Increase in photosynthetic rate might be the cause of promoted growth and higher yield of tomato fruits. Besides, the 5,000 ppm KNap treatment also hastened the maturity of tomato fruits, protected them against blossom-end rot to a certain extent and also increased the recovery frequency after receiving $CaCl_2$ spray.

(4) The treatment induced considerable alterations in chemical composition of tomato fruits. Reducing sugar, sucrose, and total sugars were more in mature tomato fruits of the treated plants. Titratable acid and ascorbic acid, however, were lessened under the influence of the KNap.

(5) Only phosphorylase up to 6 weeks after treatment and NRase at 6 weeks after treatment were found to be increased in activity. Other enzymes, succinic dehydrogenase, phosphoglyceryl kinase and transaminase were found to have lower activities caused by the treatment.

VII. REFERENCES

Abolina, G., and N. Ataullaev.. 1966.
Influence of naphthenic growth substance (NGS) upon the physiological and biochemical progresses and the productivity of potatoes, melons and vegetables in the conditions of Uzbekistan. Symposium on Plant Stimulation. Sofia, Bulgaria. Oct. 25-30. Abstracts.

Aliev, A.A.. 1963.
Petroleum nutrients, Quoted from Chem. Ab. 64:10158a.

Aliev, A.Y.. 1965.
Effect of NRV (sodium naphthenate) on growth, development, and composition of tomatoes. Quoted from Chem. Ab. 66:45849n.

Ali-Zade, M.A., and Z.B. Guseinov. 1965.
Effect of petroleum growth-promoting substances, gibberellin, and heteroauxin on the growth and development of egg plants. Quoted from Chem. Ab. 67:20847t.

Agakishier, D., and T.B. Bazanova. 1965.
Effect of some growth stimulants on cotton plant at different degrees of soil salinity. Quoted from Chem. Ab. 64:20538h.

Asadov, Sh.D.. 1965.
Effect of new types of fertilizers on growth, development, and yield of vegetables. Quoted from Chem. Ab. 66:45850f.

Asadov, Sh.D.. 1967.
Effect of petroleum growth-promoting substance on the cabbage crop. Quoted from Chem. Ab. 67:20835n.

Axelrod, B., and R.S. Bandurski. 1953.
Phosphoglyceryl kinase in higher plants. Jour. Biol. Chem. 204:939-948.

Bailey, J.L.. 1962.
Techniques in Protein Chemistry. Acad. Press Inc., N.Y.

Bazanova, T.B., and K.M. Akopova. 1966.
Effect of naphthenic acids on the metabolism of cotton plants and yield of fine cotton fibers in various states of nutrition. Quoted from Chem. Ab. 67:2315p.

Burachevskii, J.J.. 1965.
Activation of the synthesis of amylase by Aspergillus usamii. Quoted from Chem. Ab. 62:16920h.

Chi, C.H.. 1964.
Effect of 2,4-dichlorophenoxyacetic acid on the yield and metabolism of the bean plant. M.Sc. Thesis, Univ. of British Columbia.

Dimitrov, D.I., M. Popoff, and A.D. Stefanova. 1966.
Testing the physiological activity of synthetic acids obtained from naphtha and other surface-active substances. Symposium on Plant Stimulation. Sofia, Bulgaria. Oct. 25-30. Abstracts.

Dixon, M., and E.C. Webb. 1964.
Enzymes. Acad. Press Inc., N.Y.

Eul'fugarly, D.I., D.M. Guseinov, and A.M. Ataullaev.. 1967.
Biological action of Ni, Mn, Cu naphthenate. Quoted from Chem. Ab. 68:94771f.

Evans, H.J., and A. Nason. 1953.
Pyridine nucleotide-nitrate reductase from extracts of higher plants. Plant Physiol. 28:233-254.

Eyubov, R., and F. Issaeva. 1966.
Influence of naphthenic growth substances upon the growth, development and yield of maize and lucerne. Symposium on Plant Stimulation. Sofia, Bulgaria. Oct. 25-30. Abstracts.

Fiske, C.H., and Y. Subbarow.1925.
The colorimetric determination of phosphorus. Jour. Biol. Chem. 66:375-400.

Frank, S., and A.L. Kenney. 1955.
Chlorophyll and carotenoid destruction in the absence of light in seedlings of Zea mays L.. Plant Physiol. 30:413-418.

Glushkov, N.M., A.S. Yakovlev. 1963.
Bee feeding with growth substances. Quoted from Chem. Ab. 63:60806

Guseinov, D.M., and F.G. Isaeva. 1965.
Effect of combined delivery of petroleum growth-promoting substances and radioactive phosphorus on the alfalfa crop. Quoted from Chem. Ab. 67:20836p.

Hubert, G.. 1966.
Treatment of naphthenic oil to sugar cane to increase sugar content. Quoted from Chem. Ab. 65:17655a.

Isenberg, F.M.R., M.L. Odland, H.W. Popp, and C.O. Jensen. 1951.
The effect of maleic hydrazide on certain dehydrogenases in tissue of onion plants. Science. 113:58-60.

Jolly, S.E.. 1967.
"Naphtenic acids". in Enclyclopedia of Chemical Technology 13 edited by Mark, H. et al. John Wiley and Sons, Inc. pp 727-734.

Kosobokov, V.I.. 1965.
Effect of petroleum growth-promoting substance on growth and development of feed cultures. Quoted from Chem. Ab. 67:20839s.

Kazakova, I.I.. 1965.
Effect of growth stimulants on the germination and growth of weed seeds. Quoted from Chem. Ab. 20837q.

Kolesnik, Z.V.. 1965.
Effect of petroleum growth-promoting substance on the biochemistry of the grape plant. Quoted from Chem. Ab. 67:20838v.

Krasnova, E.M., V.I. Kotyashkina, and G.S. Ilichev. 1965.
Biological characteristics of petroleum growth-promoting substances as plant activators. Quoted from Chem. Ab. 67:20840K.

Kulieva, N.A.. 1964.
Effect of petroleum growth promoters on the olive fruit drop. Quoted from Chem. Ab. 66:27894h.

Kun, E., and L.G. Abood. 1949.
Colorimetric estimation of succinic dehydrogenase by triphenyltetrazolium chloride. Science. 109:144-146.

Ladygina, E.A.. 1965.
Effect of petroleum growth-promoting substance on growth, development, and yield of potatoes. Quoted from Chem. Ab. 67:20833K.

Lepper, H.A.. 1945.
Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists. Published by The Association of Official Agricultural Chemists. 6th Edition.

Lipmann, I., and L.C. Tuttle. 1945.
A specific micromethod for determination of acyl phosphates. Jour. Biol. Chem. 159:21-28.

Loeffler, H.J., and J.D. Ponting. 1942.
Ascorbic acid- rapid determination in fresh frozen, or dehydrated fruits and vegetables. Ind. Eng. Chem. Anal. 14:846-849.

Loomis, W.E., and C.A. Shull. 1937.
Methods in Plant Physiology. McGraw Hill, Inc. N.Y.

Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951.
Protein measurement with Folin phenal reagent. Jour. Biol. Chem. 193:265-275.

Mans, R.J.. 1967.
Protein synthesis in higher plants. Ann. Rev. Plant Physiol. 18:127-146.

Markovnikoff, W., and W. Ogloblin. 1883.
Naphthenic acids. J. Russ. Phys. Chem. Soc. 13:34.

Marshaniya, I.I., M.G. Sharashenidze, and A.I. Dumbadze. 1965.
Effect of petroleum growth-promoting substances on the tangerine crop and quality. Quoted from Chem. Ab. 67:20834m.

McKinney, G.. 1940.
Criteria for purity of chlorophyll preparations. Jour. Biol. Chem. 132:91-107.

Naghibin, Ia.D.. 1966.
Influence of naphthenic growth substances (NGS) upon the development and productivity of the cotton-plant in the conditions of Tadjikistan. Symposium on Plant Stimulation. Sofia, Bulgaria. Oct. 25-30. Abstracts.

Pakhomova, G.I.. 1965.
Effect of treatment with petroleum growth-promoting substance on biochemical processes in tomato leaves. Quoted from Chem. Ab. 67:20841m.

Popoff, Mih.D., and Al.D. Boikov. 1966.
Testing the stimulation effect of naphthenic growth substances (NGS) on plants. Symposium on Plant Stimulation. Sofia, Bulgaria. Oct. 25-30. Abstracts.

Popoff, M.D., D. Dimitrov, and M.A. Stefanova. 1966.
Testing of the stimulating effect of naphthenates obtained by oxidation of Bulgarian naphtha. Quoted from Chem. Ab. 66:10095Z.

Popoff, M.D., and D. Kolev Hristozov. 1966.
Effect of naphthenic growth substances on the yield and quality of tobacco. Symposium on Plant Stimulation. Sofia, Bulgaria. Oct. 25-30. Abstracts.

Rathore, V.S.. 1968.
Physiological and biochemical aspects of growth and yield stimulation of bean (Phaseolus vulgaris L.) plants by 2,4-D-mineral sprays. Ph.D. Thesis, Univ. of British Columbia.

Reitman, S., and S. Frankel. 1957.
A colorimetric method for the determination of serum glutamic-oxalacetic and glutamic-pyruvic transaminases. Amer. Jour. Clin. Patho. 28:56-63.

Saberova, G.V., N.K. Mn'Kovskaya, and G.V. Porutskii. 1966.
Plant growth stimulation. Quoted from Chem. Ab. 66:36751X.

Singh, B.. 1968.
Chemical control of growth of sugar beet. Ph.D. Thesis, Univ. of British Columbia.

Steel, R.G.D., and J.H. Torrie. 1960.
Principles and procedures of statistics. McGraw-Hill Book Co., New York.

Steward, F.C.. 1963.
Plant Physiology, III. Inorganic nutrition of plants. Acad. Press, New York and London.

Subbotina, N.V.. 1965.
Physiological reaction of fruit tree to petroleum growth-promoting substance. Quoted from Chem. Ab. 67:20845r.

Sumner, J.B., T.C. Chou, and A.T. Beever. 1950.
Phosphorylase of the Jack bean. Its purification, estimation, and properties. Arch. Biochem. 26:1-5.

Webster, G.. 1961.
Protein synthesis. Ann. Rev. Plant Physiol. 12:113-132.

Wittwer, S.H., and A.E. Murneek. 1946.
Further investigations on the value of hormone sprays and dusts for green bush snap beans. Proc. Amer. Soc. Hort. Sci. 47:285-293.

Wittwer, S.H... 1964.
Foliar absorption of plant nutrients. Adv. Frontiers Plant Sci. 8:161-182.

Yurèva, K.V.. 1965.
Results of using petroleum growth-promoting substances in plant growing. Quoted from Chem. Ab. 67:20846S.

Zamanov, P.. 1966.
Influence of naphthenic growth substances upon the growth and productivity development of tobacco plant. Symposium on Plant Stimulation. Sofia, Bulgaria. Oct. 25-30. Abstracts.

Ziegler, D., and J.S. Rieske. 1967.
Preparation and Properties of Succinate Dehydrogenase-coenzyme Q Reductase (Complex II) in Methods in Enzymology X. edited by Estabrook and Pullman, Academic Press, New York and London. pp 231-235.

VIII. APPENDICES

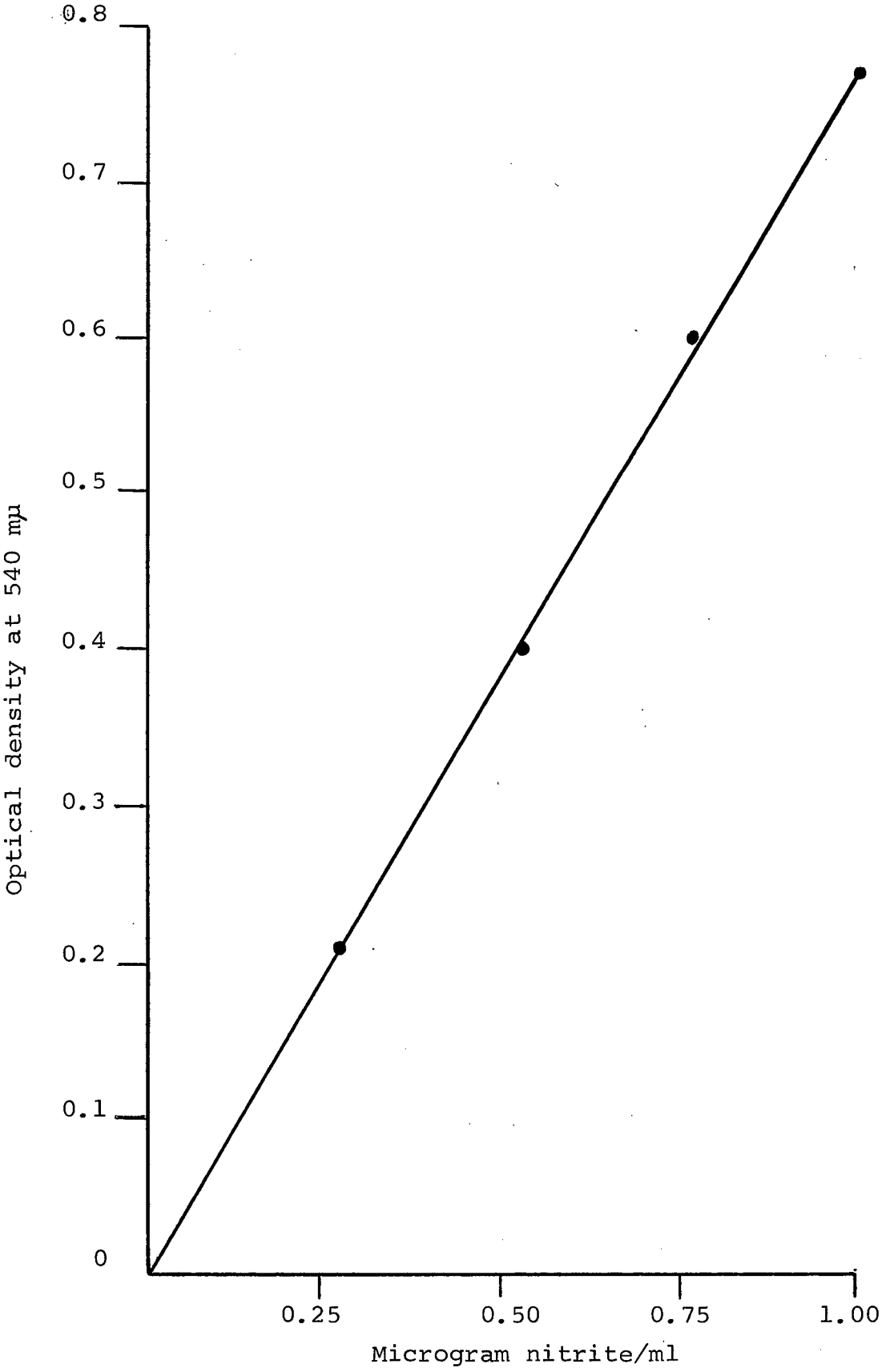


Fig. 24: Standard chart for nitrate reductase.

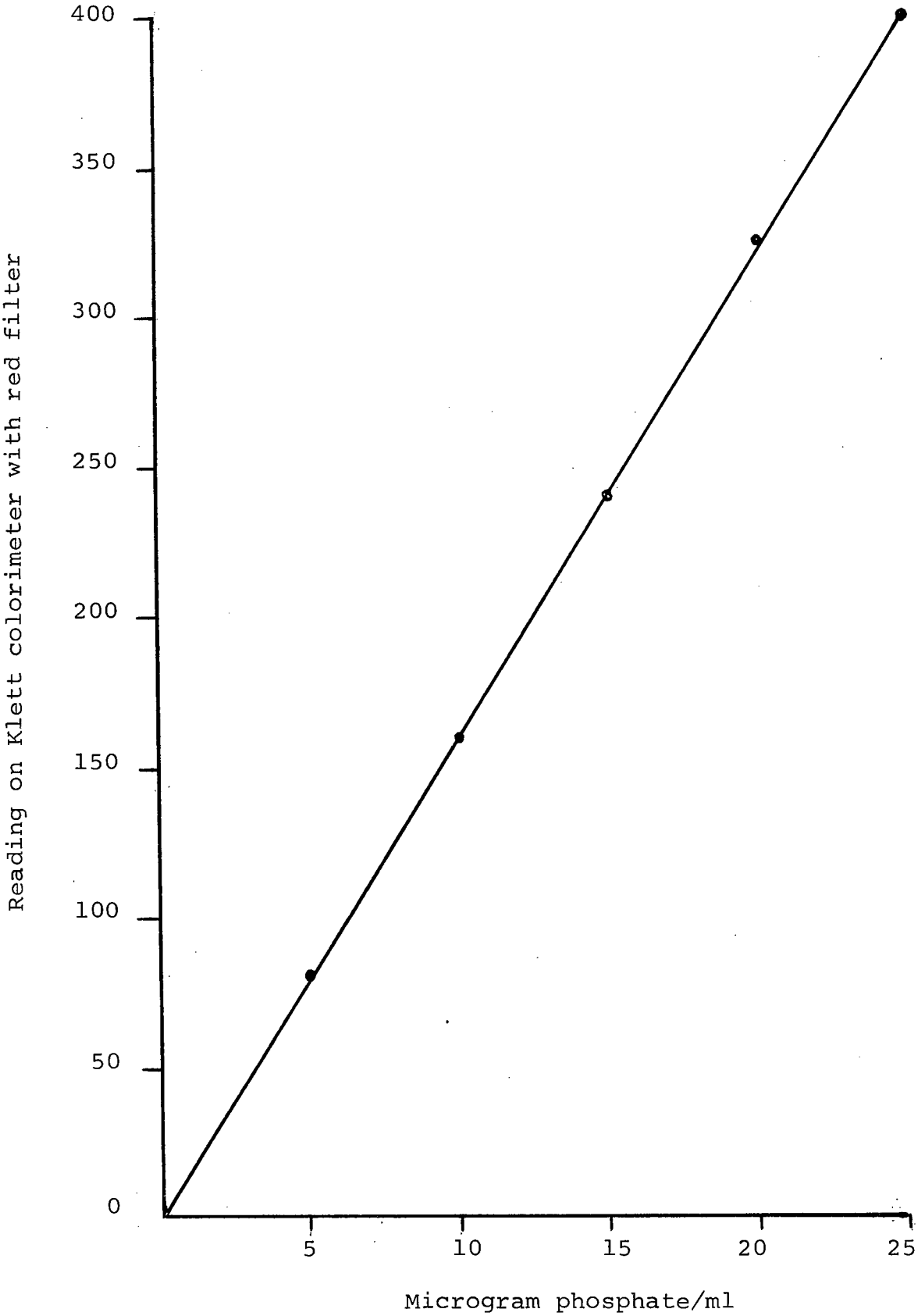


Fig. 25: Standard chart for phosphorylase.

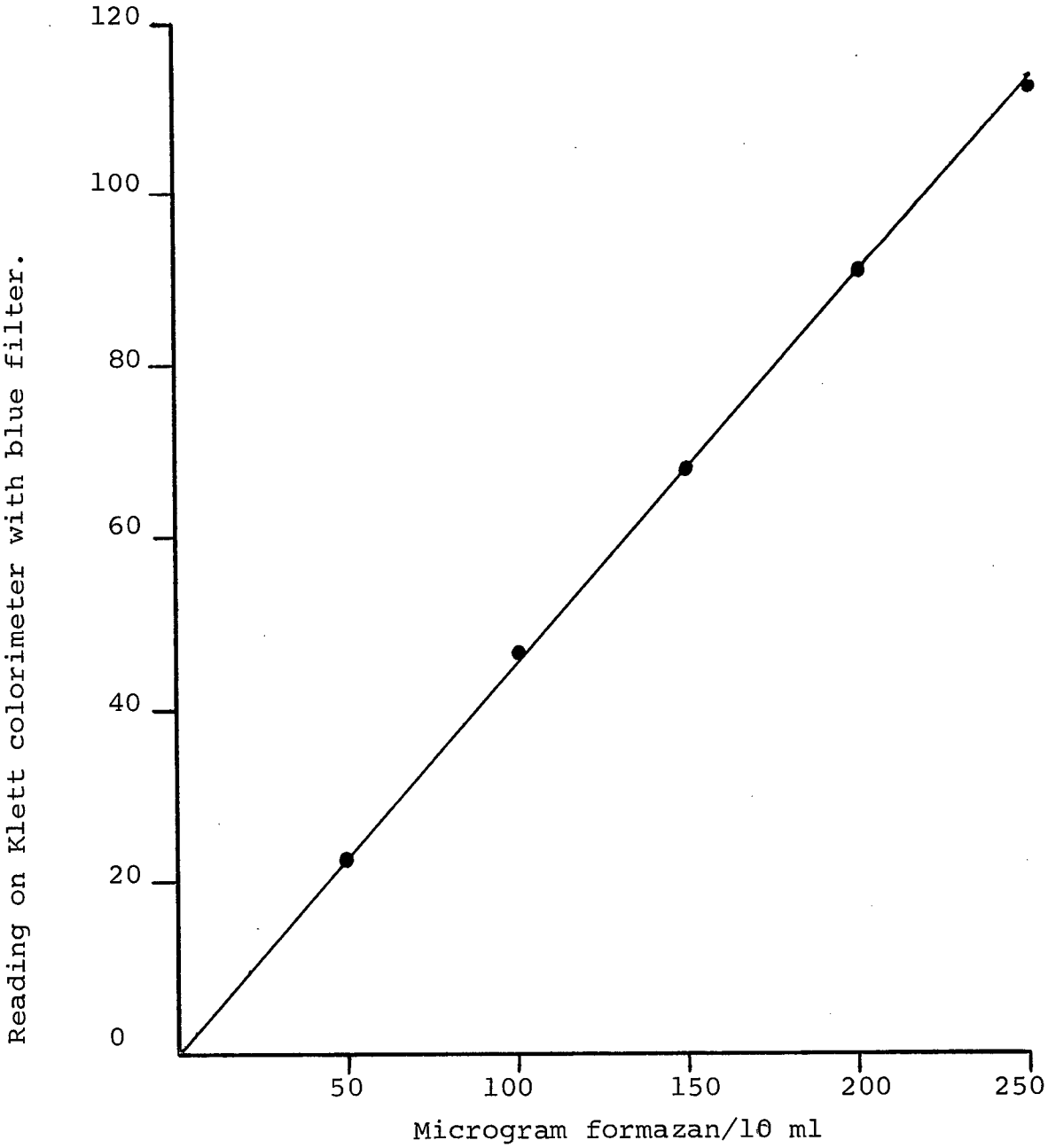


Fig. 26: Standard chart for succinic dehydrogenase.

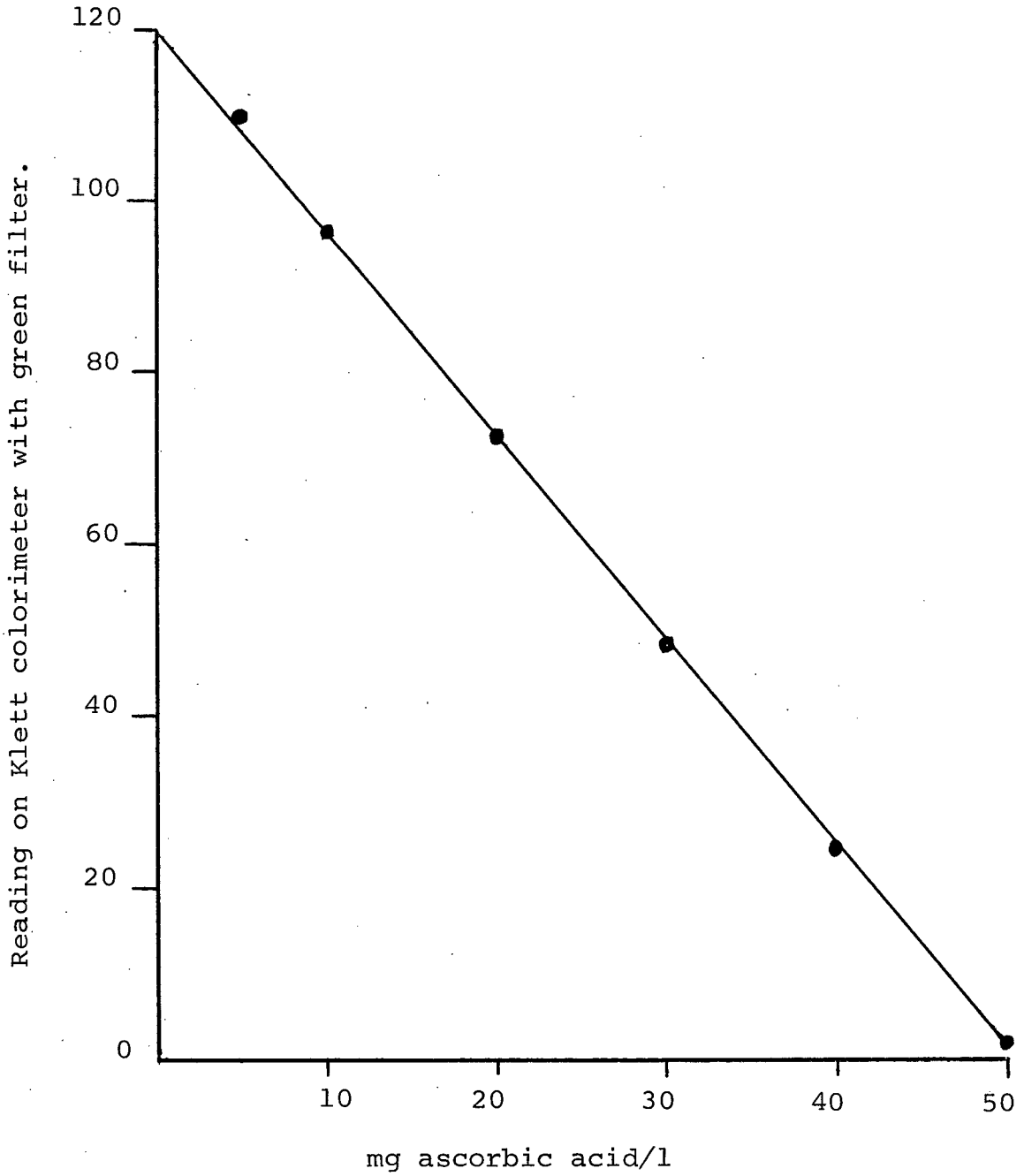


Fig. 27: Standard chart for ascorbic acid.