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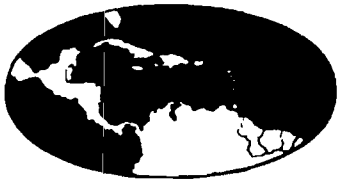

THIRD ANNUAL MEETING

RIO PIEDRAS, PUERTO RICO

AUGUST 9-13, 1965

VOLUME III

ABNORMAL ENZYME ACTIVITY AS AN EARLY INDICATOR OF NUTRIENT DEFICIENCIES IN PLANTS

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Investigators and growers are often called upon to diagnose nutrient deficiencies in plants and to recommend treatments for correction of these disorders. Yet regarless of the skill employed in the diagnosis, visual symptoms are but the end result of biochemical reactions initiated at an earlier time, with the result that the plant may have already acquired a weakened or stunted condition when the symptoms appear. Obviously it would be desirable to detect nutrient deficiencies before plants are expressing them visually.

The experiments herein reported were aimed at developing a technique whereby nutrient deficiencies could be detected prior to the appearance of visual symptoms. The method involved measurement of enzyme activity in plants receiving decreasing concentrations of specific nutrients. It was theorized that if a given nutrient was normally influencing the activity of a given enzyme, then a measurable deviation would appear in the activity of that enzyme as the plant passed from a normal nutritional status to one of approaching deficiency. These investigations were therefore aimed at revealing abnormal enzyme activity in near-deficient plants, as compared to the activity of the same enzymes in plants continually receiving adequate nutrient levels.

MATERIALS AND METHODS

Deficiencies of N, P, K, Ca, Mg, B and Fe [2/] were gradually induced over a period of 60 days in cauliflower plants of the variety Snowball Imperial. This was accomplished in a stepwise manner. All plants received adequate levels of all nutrients until 6 week of age. Thereafter, levels of the respective nutrients were lowered slightly at intervals of 10 days. For example, all plants received 11 meq/l of nitrate for 6 weeks at which time the decreasing N group was selected to receive 9 meq/l for the next 10 days, 7 meq/l for the following 10 days, etc., until the final 10 day period when only 1 meq/l of nitrate was provided. The other variable nutrients were gradually lowered in like manner (Table 1). Control plants received the original concentrations throughout the study. Decreasing nutrient levels were meant to be adequate for about 30 days, whereas visual deficiency symptoms were expected after 50 to 60 days of treatments.

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2/Abbreviations: Nitrate, N; phosphorus, P; potasium, K; calcium, Ca; magnesium, Mg; boron, B; and iron, Fe.

TABLE I

Decreasing concentrations of nutrients supplied to cauliflower plants in sand culture 1/

Nutrient	Days Following Initial Treatment						
	0 2/	0-10	10-20	20-30	30-40	40-50	50-60
	meq/l						
Nitrate	11.0	9.0	7.0	5.0	3.0	1.0	1.0
P	6.0	5.0	4.0	3.0	2.0	1.0	1.0
K	4.5	3.5	2.5	1.5	0.8	0.4	0.2
Ca	5.0	4.0	3.0	2.0	1.0	0.5	0.2
Mg	2.5	2.0	1.5	1.0	0.5	0.2	0.1
	ppm						
B	0.20	0.10	0.07	0.04	0.02	0.01	0.00
Fe	1.00	0.80	0.60	0.40	0.20	0.10	0.00

1/The following elements were held constant for all plants: S, 2.0 me/l; Cu, 0.02 ppm: Mn, 0.50 ppm; Zn, 0.05 ppm; Mo, 0.01 ppm.

2/Nutrient concentrations in this column were supplied to all plants until 6 weeks of age, and to control plants for the duration of the experiment.

Plants were grown in number 2 quartz sand contained in 2-gallon, glazed containers. Cauliflower seedlings were transplanted from flats of sterilized sand to the containers at 3 weeks of age. Here they continued to receive the complete nutrient solution for an additional 3 weeks. Leaf samples were harvested just before treatments were begun and samples were subsequently taken at the end of each 10-day period. The sand was leached thoroughly with tap water after each harvest to prevent accumulation of salts.

Two plants were taken at each sampling period for enzyme-activity and dry-weight measurements. From each plant, 2 or 3 of the youngest fully expanded leaves were placed in stoppered sugar tubes and immediately frozen in a mixture of dry ice and acetone. The leaves were lyophilized, weighed, ground to pass a 40-mesh screen, and stored in test tubes at -10°C. The remainder of the sampled plants was cut at the surface of the sand, dried in an oven, and weighed.

A completely randomized design was employed with 4 replicates for each of the 8 treatments. At each sampling period, variance between respective treatment and control means was measured according to Student's T test (5), with significance established at the 5-percent level.

Enzyme Analyses

Enzymes included peroxidase, polyphenol oxidase (tyrosinase), phosphatase, amylase, catalase, and cytochrome c oxidase. A general enzyme preparation was used for all analyses. Two hundred milligrams of freeze-dried tissue were extracted in 50 ml. of distilled water. The mixture was shaken for 1 hour and plant material was removed with no. 1 Whatman filter paper. The extract was clear, pale yellow to colorless, indicated strong enzyme activity, and was suitable for both colorimetric and manometric analyses.

Peroxidase and poly phenol oxidase were assayed colorimetrically with catechol serving as substrate. The technique of Bailey and McHargue (1) was modified for cauliflower peroxidase, while polyphenol oxidase was measured by the method of Ponting and Joslyn (3).

Phosphatase activity was determined by measuring the inorganic phosphorus released from beta-glycerophosphate. The technique is essentially that of Spencer (6), with the exception that extracts rather than tissue powder were used in the digests. Amylase was measured colorimetrically by determining the amount of maltose formed from soluble potato starch, according to the method of Bernfeld (2). The dinitrosalicylic acid technique of Summer (7) was used for maltose. Catalase was measured manometrically by the procedure of Bailey and McHargue (1).

The colorimetric procedure of Smith and Stotz (4) was used for cytochrome c oxidase. The substrate in this assay, reduced cytochrome c, is kept reduced by a leuco dye (2, 6 -dichlorobenzenoneindo-3-chlorophenol). Enzyme activity is indicated by the appearance of oxidized dye color.

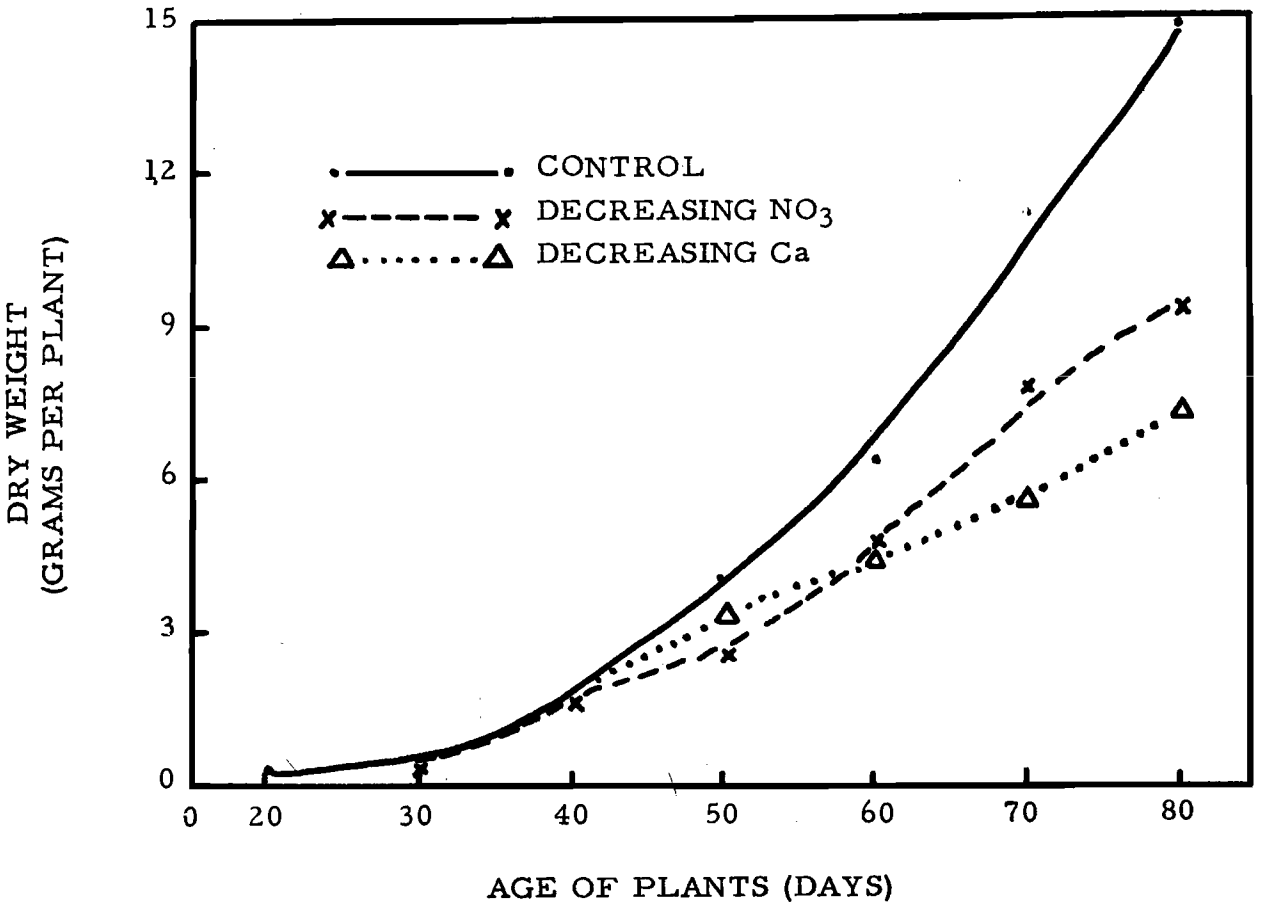

Fig. 1 - Relative growth rates of cauliflower plants receiving complete, low nitrate, and low calcium solutions in sand culture.

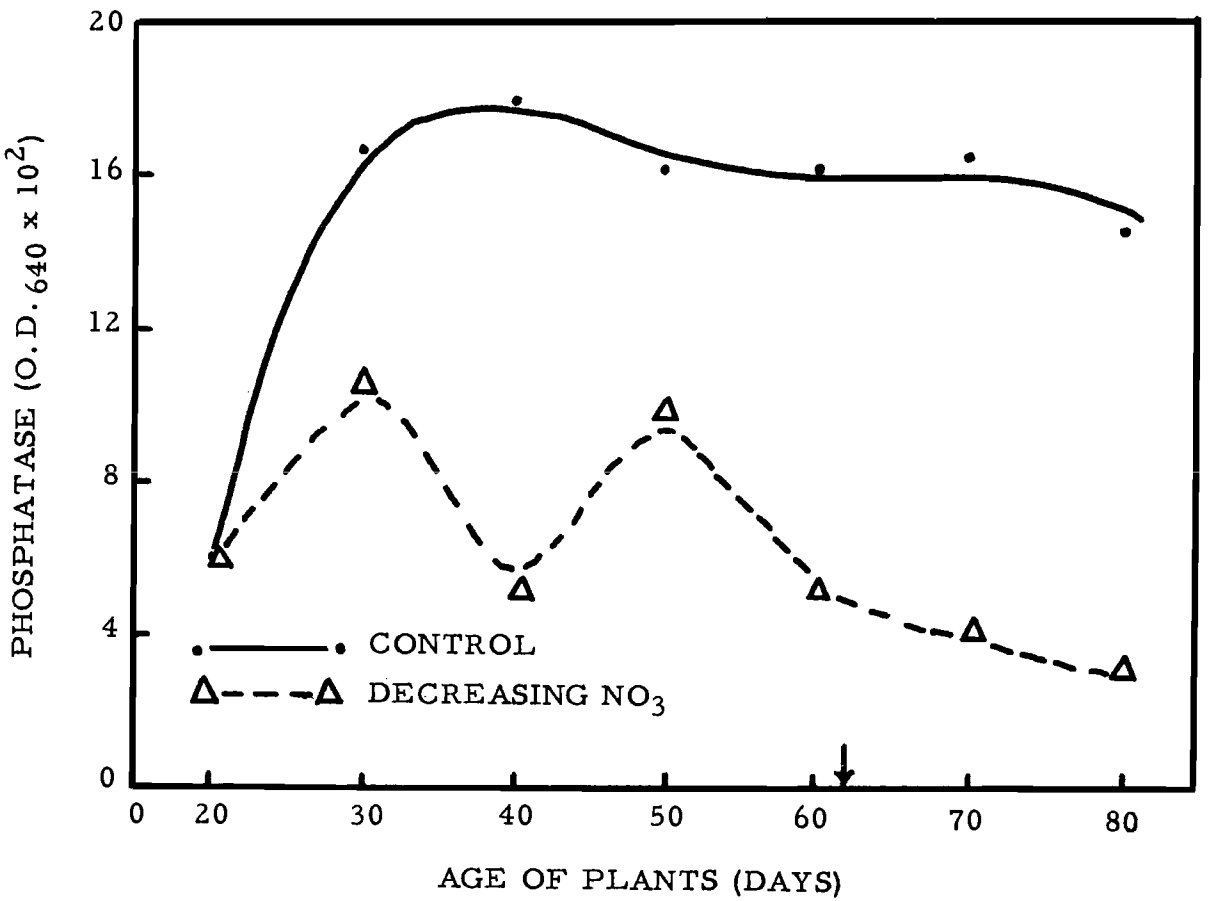

Fig. 2 - Decline of phosphatase activity in leaves of cauliflower supplied with decreasing nitrate in sand culture. Arrow on horizontal axis indicates appearance of first deficiency symptoms.

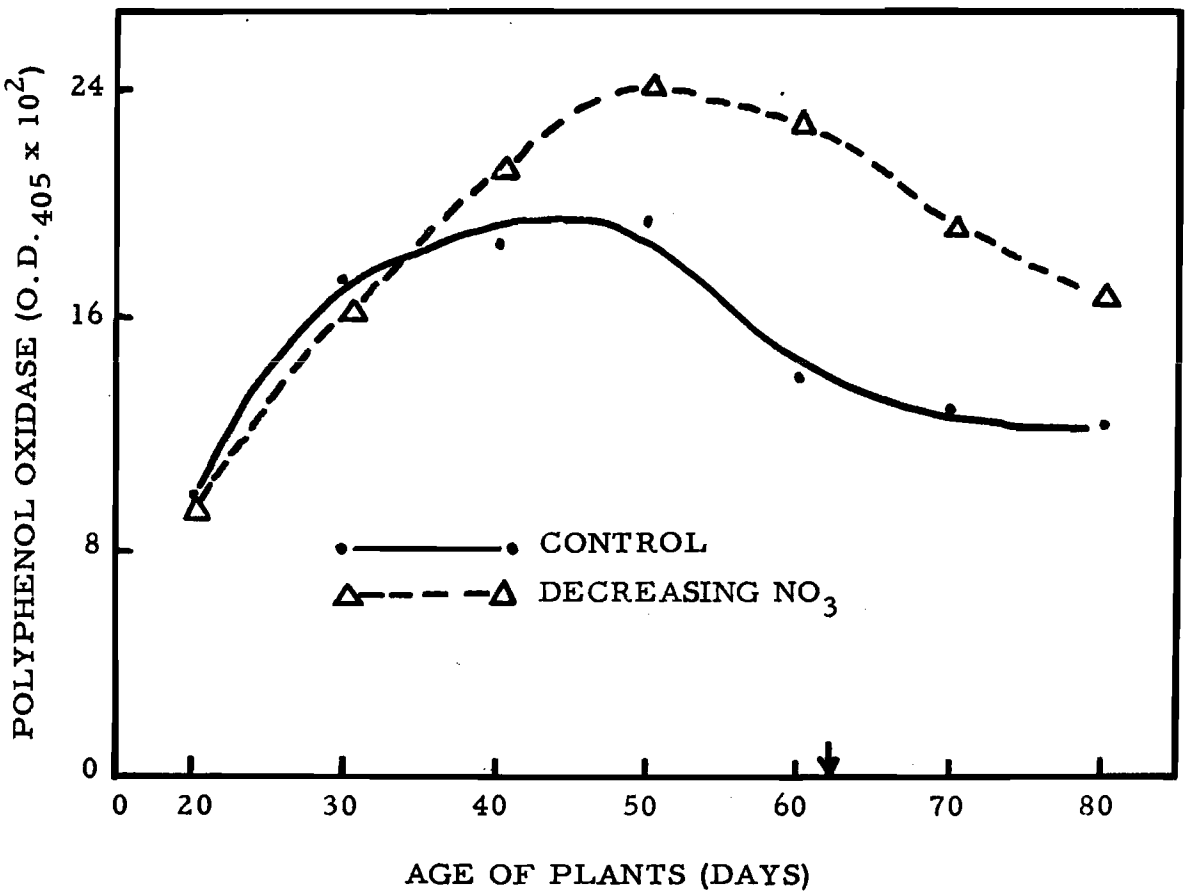

Fig. 3 - Effects of decreasing nitrate supply upon polyphenol oxidase of cauliflower.

TABLE 2

Dry weights and enzyme activities of cauliflower plants supplied with decreasing nutrient levels in sand culture 1/

Treatment	Harvest 1	2	3	4	5	6	7
Controls							
Peroxidase	.050	.130	.165	.114	.073	.035	.074
Polyphenol oxidase	.100	.173	.185	.194	.146	.137	.128
Phosphatase	.060	.165	.179	.160	.091	.164	.145
Catalase	5	203	125	19	32	29	93
Cyto. c oxidase	.100	.223	.284	.233	.156	.104	.096
Dry weight	.16	.58	1.92	4.38	6.35	11.29	15.33
Decreasing Nitrate							
Peroxidase	.050	.164	.096	.090	.059	.010	.025
Polyphenol oxidase	.100	.166	.213	.240	.220	.187	.165
Phosphatase	.060	.105	.049	.104	.051	.041	.032
Catalase	5	246	44	3	15	2	6
Cyto. c oxidase	.100	.119	.225	.120	.063	.024	.021
Dry weight	.16	.48	1.89	2.82	5.62	8.01	9.27
Decreasing P							
Peroxidase	.050	.060	150	.210	.060	.020	.040
Polyphenol oxidase	.100	.150	160	.210	150	.120	.130
Phosphatase	.060	.097	118	.129	.058	.068	.048
Catalase	5	92	266	268	55	28	38
Cyto. c oxidase	.100	.140	.230	.220	.090	.080	.070
Dry weight	.16	.54	1.68	4.07	6.78	7.37	11.03
Decreasing K							
Peroxidase	.050	.090	.100	.140	.070	.060	.040
Polyphenol oxidase	.100	.170	.150	.200	.140	.170	.170
Phosphatase	.060	.152	.128	.139	.122	.161	.076
Catalase	5	165	292	99	53	9	20
Cyto. c oxidase	.100	.180	.190	.190	.130	.090	.060
Dry weight	.16	.62	1.63	4.07	5.73	5.98	8.65

Treatment	Harvest 1	2	3	4	5	6	7
Decreasing Ca							
Peroxidase	.050	.170	.130	.140	.050	.010	.040
Polyphenol oxidase	.100	.160	.140	.190	.120	.120	.140
Phosphatase	.060	.096	.184	.230	.086	.090	.128
Catalase	5	98	167	94	41	10	17
Cyto. c oxidase	.100	.150	.320	.290	.100	.060	.030
Dry weight	.16	.54	1.78	3.72	5.01	5.77	7.77
Decreasing Mg							
Peroxidase	.050	.200	.150	.210	.070	.030	.030
Polyphenol oxidase	.100	.160	.180	.220	.140	.140	.140
Phosphatase	.060	.122	.195	.230	.116	.086	.132
Catalase	5	341	343	213	103	8	28
Cyto. c oxidase	.100	.240	.200	.270	130	.080	.050
Dry weight	.16	.68	2.28	3.87	5.51	5.86	10.04
Decreasing B							
Peroxidase	.050	.150	.120	.150	.050	.020	.050
Polyphenol oxidase	.100	.170	.160	.200	.130	.130	.160
Phosphatase	.060	.125	.159	.101	.068	.079	.100
Catalase	5	248	140	149	46	6	12
Cyto. c oxidase	.100	.244	.200	.185	.086	.032	.005
Dry weight	.16	.50	1.52	4.28	5.97	6.30	9.33
Decreasing Fe							
Peroxidase	.050	.123	.138	.097	.030	.016	.056
Polyphenol oxidase	.100	.160	.180	.200	.130	.130	.150
Phosphatase	.060	.108	.184	.140	.090	.104	.158
Catalase	5	26	99	54	17	4	36
Cyto. c oxidase	.100	.174	.255	.159	.095	.040	.013
Dry weight	.16	.56	1.85	3.31	4.97	5.57	9.10

1/ Enzyme activities are recorded in the following units: peroxidase, OD at 450 mμ; polyphenol oxidase, OD at 405 mμ; phosphatase, mg of inorganic P; catalase, cc of oxygen; cytochrome c oxidase, OD at 525 mμ. Dry weights are recorded in grams per plant.

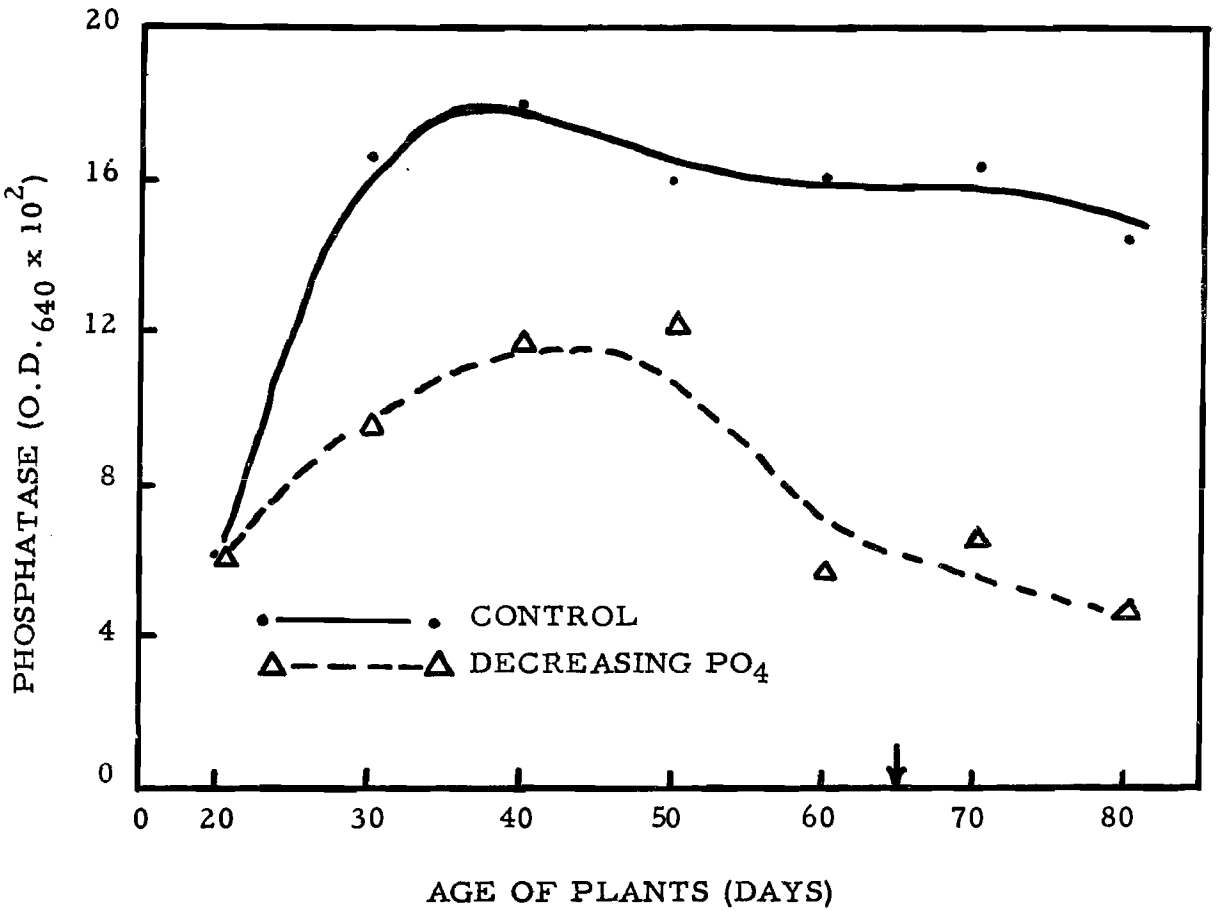

Fig. 4 - Decline of phosphatase activity in leaves of cauliflower supplied with decreasing phosphate in sand culture. Arrow on horizontal axis indicates appearance of first phosphate-deficiency symptoms.

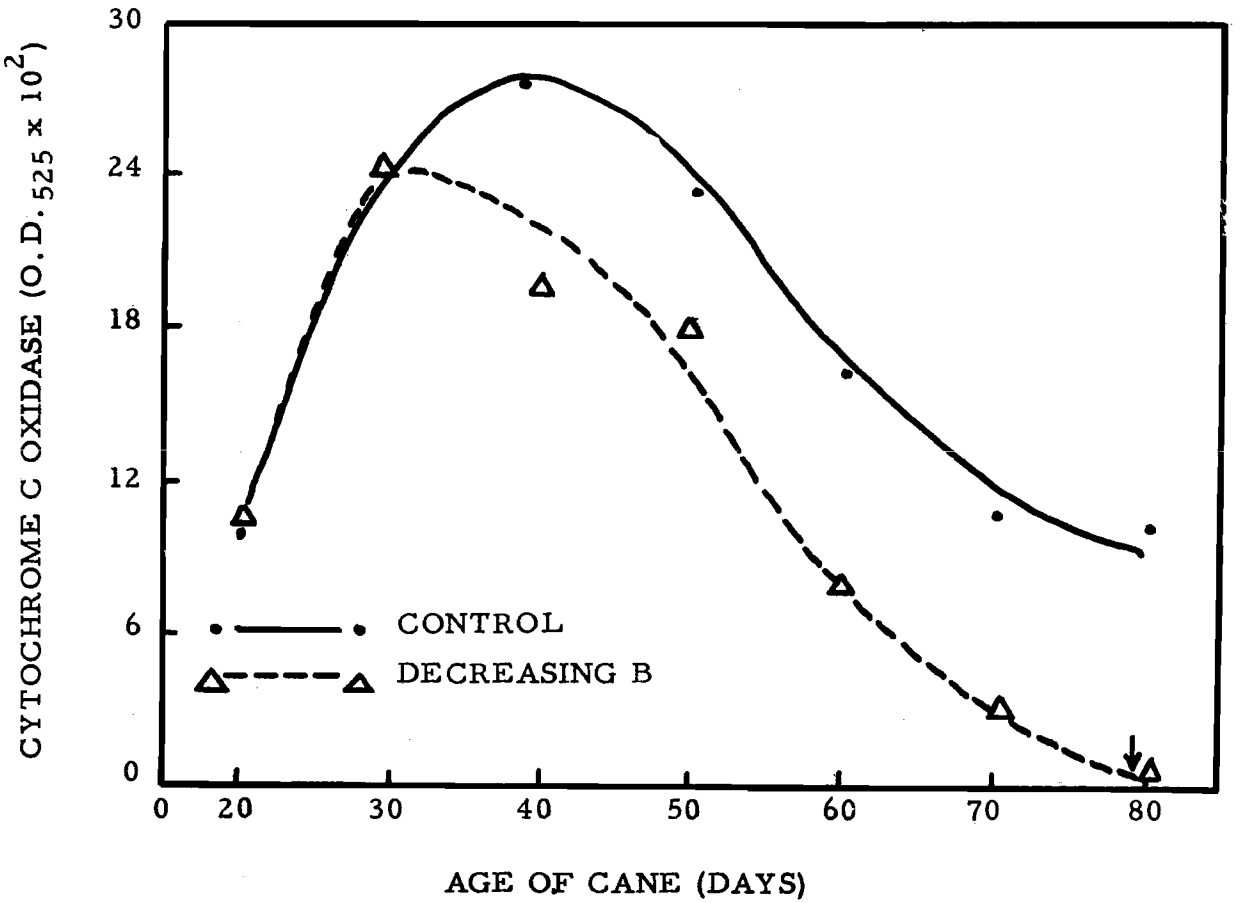

Fig. 5 - Decline of cytochrome c oxidase activity in leaves of cauliflower supplied with decreasing boron in sand culture. Arrow on horizontal axis indicates first appearance of boron-deficiency symptoms.

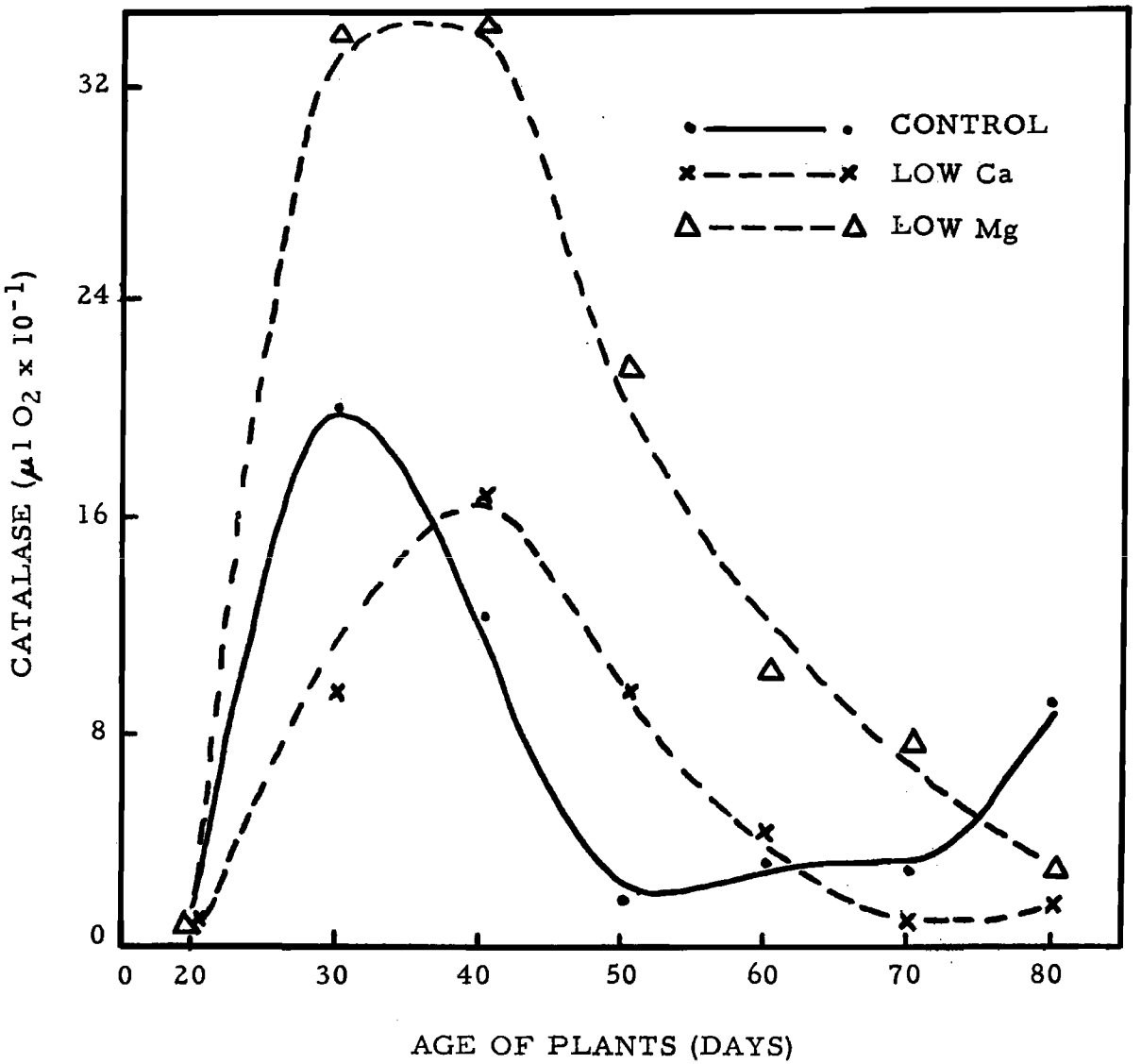

Fig. 6 - Effects of decreasing calcium and magnesium supply upon catalase of cauliflower grown in sand culture.

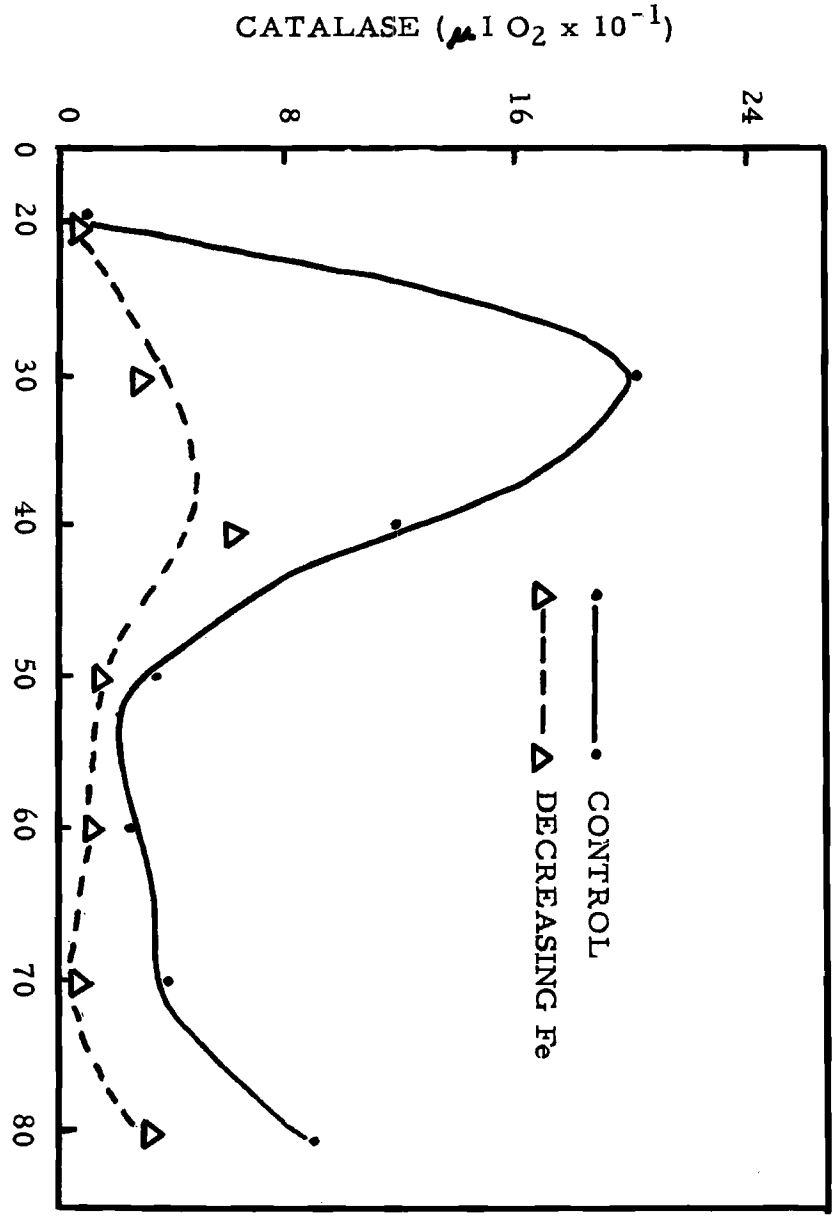

Fig. 7 - Decline of catalase activity in leaves of cauliflower supplied with decreasing iron in sand culture.

RESULTS AND DISCUSSION

The purpose of these experiments was to develop a technique by which approaching nutrient deficiencies could be detected prior to the appearance of visual symptoms. Results support the theory that approaching nutrient deficiencies can be detected by enzyme.

Two general responses were recorded with regard to dry weight and enzyme activity: (1) All plants slowly increased dry weight during the first 40 days of treatment (fig. 1). Thereafter, control plants made rapid gains in dry weight while plants receiving decreasing nutrient levels failed to make comparable increases. (2) Enzyme activity, regardless of treatment, was highest during the first 20 days when dry weight gains were slight. Activity declined after 20 days and reached its lowest levels during the period when plants were making their most vigorous growth.

It thus appears that the primary factor affecting the growth and enzyme activity of these plants was the stage of physiological development at the respective periods of harvest. Treatment effects were secondary. Treatments could cause deviations from trends established by control plants, but could not initiate trends characteristic only of themselves.

Responses to Decreasing Nitrate

Dry weights indicate that low N plants were unable to make growth comparable to the controls after 20 to 30 days of treatment (table 2). N - deficiency symptoms were first noted after 41 days, at which time the plants had just completed a 10-day treatment of 3 meq/l nitrate.

The initial lowering of nitrate from 11 to 9 meq/l resulted in a decrease in phosphatase and cytochrome c oxidase activity (fig. 2). Since these changes remained evident throughout the study, their appearance during the first 10 days of treatment signaled a deficiency at least 31 days in advance of visible symptoms.

At 7 meq/l nitrate, catalase activity decreased and polyphenol oxidase increased, as compared to controls (fig. 3). These responses were noted 21 days before deficiency symptoms appeared and were consistent through the final harvest.

Response to Decreasing B

Low B plants grew normally for 40 days of treatment. Dry weight was significantly reduced at 50 days and deficiency symptoms appeared at 57 days.

Lowering B from 0.2 to 0.1 ppm resulted in a significant suppression of phosphatase during the first 10 days of treatment. This effect was recorded at all but the third harvest. The phosphatase curve for the decreasing B treatment therefore anticipates B deficiency by at least 47 days. Yet unlike the phosphatase suppression

resulting from decreased N and P, the B curve more nearly resembles that of control plants, including a partial recovery of activity at the close of the study.

Cytochrome c oxidase was slightly stimulated as B was lowered from 0.2 to 0.1 ppm, but this response was reversed at 0.07 ppm B and activity remained below that of the controls for the duration of study (fig. 5). The almost complete absence of activity among B-deficient plants at the final harvest marked the end of a trend which began at least 37 days before B-deficiency symptoms appeared.

Response to Decreasing Fe

Dry-weight data reveals a slight depression of growth in low Fe plants after 30 days of treatment, and increasingly poor growth after 40 days. No Fe-deficiency symptoms were detected, however.

In spite of the apparent failure to reduce Fe to deficiency levels, the extremely low activity of Fe-requiring catalase and cytochrome c oxidase suggest that an abnormal Fe status did in fact exist toward the close of the study. An additional 10 or 20 days of low Fe treatment would likely have established visual deficiency symptoms. It is interesting to note that both catalase (fig. 6) and cytochrome c oxidase began to decline as soon as Fe was reduced. This suggests and Fe-enzyme sensitivity or balance whose expression needs not await an absolute Fe deficiency.

Responses to Decreasing Ca and Mg

Calcium and Mg treatments prevented normal growth after 30 days. Calcium deficiency symptoms appeared at 56 days, but Mg deficiency symptoms did not appear.

The effects of decreasing Mg on catalase was particularly striking (fig. 7). The first reduction of Mg, from 2.5 to 2.0 meq/l, initiated an almost twofold activity increase. Catalase activity continued nearly 3 times greater than controls up to the sixth harvest. At that time Mg was lowered from 0.5 to 0.2 meq/l and catalase activity dropped to less than a third of controls.

Both decreasing Ca and Mg had similar effects on phosphatase. Activity was initially depressed, then increased beyond control values, and again dropped below control activity during the final 20 days of treatment. Cytochrome c oxidase was affected in like manner by decreasing Ca.

Significance of Nutrient Enzyme Relationships

It is evident that certain nutrient deficiencies can be detected quite early by enzyme measurements. Yet several considerations must be delt with before this principle can be used in a practical manner.

First, a greater number of enzymes must be studied, preferably those most directly related to the roles of specific nutrients. The

response of a single enzyme may indicate that a nutritional disorder is in the making without identifying any specific nutrient. During the present study, phosphatase was significantly depressed by low concentrations of nitrate, P, and B. A detection of low phosphatase activity could mean that any one or all of these nutrients were approaching deficiency levels. However, as this work has also shown, low phosphatase combined with low cytochrome c oxidase and high polyphenol oxidase would specifically associate the disorder with low nitrate. A syndrome of enzyme behavior patterns would more clearly isolate the limiting nutrient.

Second, some practical system of controls would be needed for enzyme measurements in the field. The present experiments have shown that enzyme responses to treatments generally resemble control patterns. Treatment effects will be expressed in terms of degree rather than by any unique or predictable pattern. The problem of finding "normal" plants for controls would be crucial, particularly so because samples would be taken when all plants still look alike. Controls might be established by providing a single row, or even just a small patch, with a complete fertilizer mix based on the best available recommendations.

Third, it appears that enzyme-nutrient relationships need further study and classification if they are to be interpreted accurately. For example, two distinct types are already evident from work thus far completed. The responses of polyphenol oxidase to decreasing nitrate and of phosphatase to decreasing B seem to indicate a triggering effect of the nutrients rather than any relationship between enzyme and absolute nutrient concentration. Lowering these nutrients beyond some critical point initiated an enzyme shift which remained essentially constant regardless of how much lower the nitrate and B levels were dropped. On the other hand, cytochrome c oxidase was increasingly depressed as nitrate and B levels continued to be lowered. The former response suggests a triggering relationship at a critical nutrient level, the latter response suggests a direct relationship at any nutrient level.

Three significant advantages automatically befall an investigator dealing with nutrient-enzyme relationships: (1) He is working with active catalysts. He is in touch with reactions that will cause things to happen, that are causing things to happen, or have already caused things to happen because of the great work potential of the enzyme catalyst. (2) He is dealing with the how and why of plant nutrition simultaneously. Visible nutritional symptoms, and much of tissue-analysis data, only tell him what or how much. (3) He is at once working with that fraction of nutrient content actually taking part in biochemical reactions, not gross content of both the active and inert nutrient. Most important, the combined features of nutrient-enzyme relationships thereby enable him to study nutritional welfare while still in the making. When investigators have mastered the principles of nutrient-enzyme relationships, it will be possible to sample a field of still healthy young plants, and to predict their nutritional status for some seemingly distant date.

SUMMARY

A technique has been studied whereby approacning nutrient deficiencies in plants can be detected prior to ghe appearance of visual symptoms. Over a 60-day period, enzyme activity was measured at 10-day intervals in cauliflower plants receiving decreasing concentrations of nitrate, phosphorus, potassium, calcium, magnesium, boron, and iron in sand culture. It was theorized that if a nutrient was normally influencing the activity of an enzyme, then a measurable deviation would occur in the activity of that enzyme as the plant passed from a normal nutritional status to one of approaching deficiency.

Plants receiving decreasing nutrient levels were unable to make growth comparable to control plants after 40 days of treatment. Visual deficiency symptoms for nitrate, phosphorus, potassium, calcium, and boron appeared between 40 and 60 days of treatment. Enzyme activity was initially high, when plant growth was slight, and activity declined as growth became more vigorous.

Decreasing nitrate suppressed phosphatase and cytochrome c oxidase activity 31 days before nitrogen deficiency symptoms were recorded. Catalase decreased and polyphenol oxidase increased 21 days before the symptoms appeared. Decreasing phosphorus reduced phosphatase activity 35 days before phosphorus deficiency symptoms were observed. Lowering the boron supply caused a significant suppression of phosphatase and cytochrome c oxidase 47 days prior to boron deficiency symptoms.

Decreasing magnesium caused striking fluctuations in catalase activity, and to a lesser degree both magnesium and calcium caused fluctuations in phosphatase activity. Decreasing iron depressed cytochrome c oxidase after 20 days of treatment, and catalase after 40 days.

Results of this study support the theory that approaching nutrient deficiencies can be detected by enzyme measurements. Practical application of this principle is discussed.

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A REVIEW OF CURRENT RESEARCH ON FOOD, FIELD AND PHARMACEUTICAL CROPS AT THE TATE & LYLE CENTRAL AGRICULTURAL RESEARCH STATION

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INTRODUCTION

Imports of food into Trinidad and Tobago in 1962 were valued at $76,410,400. (1). Fruits, vegetables, cereals, and cereal preparations accounted for roughly 45 per cent of the food import bill. The enormity of this bill is one of the reasons why the Tate and Lyle Central Agricultural Research Station has devoted attention to research on food and field crops.

As the sugar industry becomes mechanized, and as yourger people are attracted to the cities, older employees in sugar are not always easily replaced. It is hoped that the research work on food and field crops will serve to motivate younger people to remain in agriculture and to grow a greater proportion of their own food. This would be of benefit to the nation, to the sugar industry, and to the agricultural community in general.

The importation and evaluation of new varieties of high quality food crops is an important facet of the research program. It is desirable that yields of vegetable and other crops be increased by growing more productive varieties. Accumulation of information on which varieties of a particular crop may best be grown in Trinidad at different times of the year, is also of great importance. Frequently, in the past, crops have not been grown successfully because the varieties were unsuited to the particular thermo- or photoperiods obtaining at different times of the year.

Research is also being undertaken on the growing of crops which are new to Trinidad. Seed or other forms of planting material of

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