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EFFECT OF CERTAIN NUTRIENT CONDITIONS ON ACTIVITY OF OXIDASE AND CATALASE

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THESIS

Engineering

Effect of Certain Nutrient Conditions on Activity of Oxidase and Catalase

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THESIS

INTRODUCTION

Enzymes have long been known to be important constituents of plant cells. During recent years more and more importance has been attributed to their activities. Certain plant physiologists have termed the enzyme activity of a plant as "the strategic center of vital phenomena". This might lead one to suppose that when any factor, such as disease or nutrient supply, operates to retard or increase the growth of a plant the direct results may possibly be due to a change in the enzyme content.

The available experimental evidence shows that the active enzyme content of the plant is not always a fixed constant but subject to variation through the influence of such conditions as disease, changes in the reaction of the tissue fluids, etc. In view of the fact that nutrient substances, when properly applied, increase the tone and growth response of the plant, it may be supposed that they too are effective by causing favorable stimulation of the essential enzymes. Since little or no work has been done in determining the effect of plant nutrients on enzyme activity in herbaceous vegatation, this investigation was undertaken as a study of that relationship.

REVIEW OF LITERATURE

In reviewing the literature the work of Sullivan (21) stands out as bearing directly on the effect of various

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salts on enzyme activity. He found that potassium sulphate even in dilute solutions had a retarding effect on the oxidizing action of wheat roots, while sodium nitrate and mixtures of $Ca\ H_4(P\ O_4)_2$, $Na\ N\ O_3$ and $K_2\ S\ O_4$ had an accelerating effect. In testing the action of these salts in solutions with concentrations of 100 parts per million of P_2O_5, $N\ H_3$, and $K_2\ O$, used singly and also in combination, on commercial malt diastase in starch paste he observed that $CaH_4(PO_4)_2$ increased the diastatic activity of the enzyme from 8 to 16%; that a mixture of equal parts of $Ca\ H_4(PO_4)_2$, $Na\ No_3$ and $K_2\ SO_4$ increased the diastatic activity from 6 to 16%. while $K_2\ SO_4$ alone uniformly retarded the enzymatic action. $Na\ NO_3$ alone made little change in the rate of conversion of the starch to reducing sugar. Plants growing in solutions containing 100 parts per million of either P_2O_5, NH_3 or K_2O, or a mixture of equal parts of these and 200 mgm. of starch paste, had the power to convert the starch to sugar. As a rule, the diastatic activity was retarded by K_2SO_4 and $Ca\ H_4(PO_4)_2$ but was increased by either $Na\ NO_3$ or a mixture of the three salts.

Heinicke (12) found that nitrogenous fertilizers increased catalase activity in leaves from apple trees growing in sod, also that leaves from trees growing in sod showed less activity than leaves from trees under cultivation, and leaves from trees growing in sandy soil less than those growing in clay loam. In general his work showed that the presence of growth producing substances (nutrients) favors catalase activity while substances that tend to inhibit vegetative growth have a retarding influence on its activity. As a result he suggested the use of the catalase test as an indicator of the physiological responses of apple tree tissue to cultural treatment or conditions. He (13) obtained similar results from the bark of dormant trees. Auchter (4) working with trees and shrubs to which nitrate had been applied to one side found increased activity only in the foliage of the side on which the fertilizer was applied.

Weiss and Harvey (23) working on potatoes infested with potato wart disease found that the wart tissue had greater catalase activity and also a greater pH concentration than the healthy tissue. With other plants the diseased tissue showed increased activity in every case but the pH concentration was less in the diseased tissue. In all plants studied the pH values were greater than pH 7.0.

Bunzel (7) concluded that the oxidase content was low in a normal plant but increased greatly if the growth was interfered with by drouth, excessive moisture, diseases or any other factor that tends to retard growth. With the potato plant grown normally, he found that the young shoots showed great oxidase activity which later began decreasing in amount and continued falling until the fortieth day after planting, after which time it began increasing again as the plant came to maturity.

Moore and Whitley (16) demonstrated that the distribution of oxidases varies widely in the same plant, being present in the largest quantity in the region containing the greatest abundance of "respiratory vessels". Woods (24) found that the oxidase content of tobacco suffering from mosaic was greater in the diseased areas than in normal ones: the oxidase content being inversely proportional to the chlorophyll present as judged by color. Rose (19) has shown that the oxidase content of apple tree bark affected with Illinois canker is greater than in normal bark.

Cook and Taubenhaus (8) found oxidase abundant in young fruits, and gradually decreasing as the fruit ripened. This loss of enzyme activity seemed to be accompanied by increased susceptibility of the fruit to disease. The authors suggested that the oxidase content of the fruit might be directly related to disease resistance through the production of tannin by the oxidase. Appleman (1) found a great varietal difference in the oxidase content of potato tubers. The Mc Cormick variety (grown in Maryland) had an oxidase activity four times greater than that of Carmen No. 1 (grown in New York). When specimens of the two varieties had been kept in storage under the same conditions for a period of two months. Bunzel (6) found that large healthy leaves contained less oxidase than small healthy leaves and that leaves collected at sundown showed much greater oxidase activity than leaves collected at sunrise. If any normal function (such as failure to develop seeds in the normal way) was inhibited in any manner oxidase activity was increased. He also discovered a general parallelism between oxidase activity and green color of the green part of the plant (beets). True and Stockberger (22) working with the opium poppy plant (Papaver somniferum) found an increased activity from base to summit of plant with greatest activity in floral structures, capsules and all actively growing parts.

The available experimental evidence does not warrant a definite conclusion regarding the relation of nutrient substances or other growth-promoting factors to the activity of oxidase and catalase. Some investigations, particularly those of Heinicke and Auchter (previously cited) yielded data showing that plants whose growth was stimulated by certain nutrients showed increased catalase content, while other investigations have resulted in the accumulation of facts which show as conclusively that the activity of both oxidase and catalase was increased when normal growth was in any way prevented or interrupted. In the work to be reported herein herbaceous plants, free from disease, grown in a great variety of nutrient and environmental conditions were used. Numerous determinations were made and the data obtained therefrom may serve as a contribution towards the solution of this important problem.

METHOD

Data were taken from four different experiments (described later), essentially the same methods for measuring enzymic activity having been used in each instance. The manner of the preparation of the samples was similar to that used by Heinicke (12) for catalase determinations. By means of a leaf punch two disks per leaf were taken from two leaves at the same level on the opposite sides of each of several plants. The disks, one centimeter in diameter, were taken from the opposite sides of the midrib and about two-thirds the way to the tip. The leaves from which samples were taken were just reaching vegetative maturity. The disks were weighed immediately and mixed with an equal amount of dry calcium carbonate and enough water to cause the calcium carbonate to spread evenly over the material. After the disks were thoroughly coated with the calcium wash they were ground gently in a mortar until a uniform creamy mixture was obtained. Distilled water was then added so that one gram of the fresh tissue was suspended in 25 cc. The solution was then placed in a tightly stoppered bottle and kept until ready for use.

In testing enzyme activity use was made of Harvey's modification of the simplified Bunzell oxidase apparatus (11) suspended in a DeKhotinsky thermostatic bath. The temperature of the bath was held constant at 35°C; fluctuations not exceeding 0.1°C. Wire baskets were used to hold the oxidase tubes while immersed in the bath and to allow uniform shak-

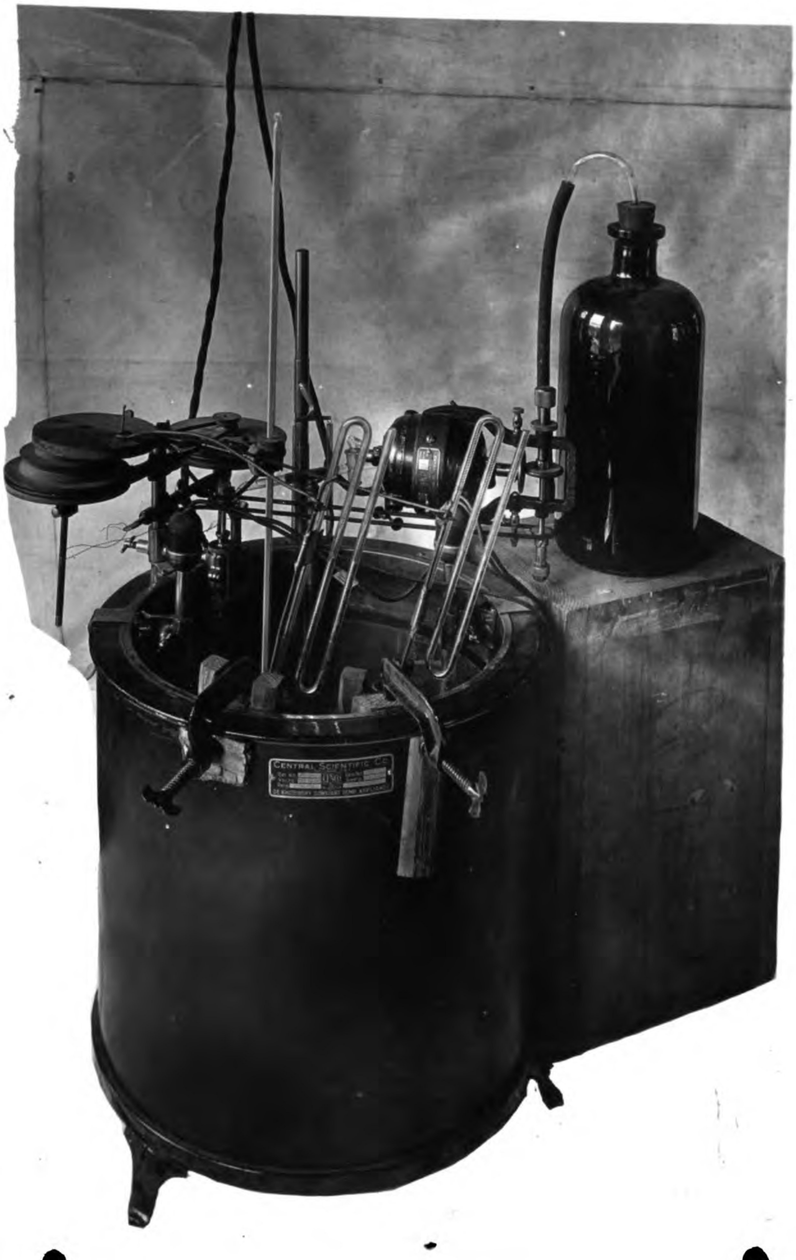

Fig. 1. Apparatus Used for Enzyme Determinations

ing to be accomplished by means of a mechanical shaker. The shaker was driven by the motor used to run the stirrer of the bath (Fig. 1). The rate of shaking was such as to cause the solution to flow from one end of the tube to the other 40 times per minute. In making a determination of oxidase activity, the suspension of macerated tissue was first thoroughly mixed by shaking the bottle gently and then taking 2 cc. of the content and placing this in the short arm of the reaction tube. In the other arm of the tube 5 cc. of a fresh one percent pyrogallol solution were introduced. The alkali tube containing 1 c.c. of normal solium hydroxide was put in place, the manometer adjusted and the tube placed in the bath. As soon as the contents came to the temperature of the bath, the tube was closed, shaking started and continued for one hour. The reading of the manometer at the end of one hour was taken as a measure of oxidase activity. In case positive pressure was developed at the beginning correction for this was made at the end.

For catalase the same sample was used as for oxidase, but diluted to give one gram of fresh tissue to 100 c.c. of water. Catalase determinations were then made as for oxidase except that 5 c.c. of 3 per cent hydrogen peroxide, previously neutralized with sodium carbonate, were substituted for the pyrogallol solution and the readings of the manometer taken at the end of one, two and three minutes. For determining the catalase activity in Experiment 4 the water displacement method as described by Heinicke (13) was used and the leveling tube adjusted so that a constant pressure of one atmosphere was maintained. The amounts of water displaced at the end of one, two and three minutes were taken as measures of the activity of the enzyme.

Preliminary tests showed that from the time of preparation of the sample oxidase activity increased for about six hours, when the sample was kept at room temperature. After reaching a maximum there followed a period of stability lasting 10 to 12 hours, after which there was a slow decline. Data showing this are presented in Table 1.

Table 1. -- Effect of Time on the Oxidase Activity of Prepared Samples of Lettuce Tissue.

Time, after preparation	Oxidase Activity in 1 hr. (Cm. of Hg.)
Immediately	2.45
1.5 hrs.	2.80
6 "	3.50
18 "	3.50
30 "	3.20
48 "	3.10
27 days.	2.40

Consequently, the oxidase determinations were made between the sixth and eighteenth hours following the preparation of the samples. Catalase is less unstable than oxidase and retains its original activity for a much longer time. This being true the determinations of catalase were made the day following the oxidase tests. All determinations were run in duplicate and discarded and repeated, when not in close agreement. All results shown in Tables are averages of the duplicates.

EXPERIMENTAL

Experiment 1. -- Lettuce.

The soils used in this experiment were (1) muck (2) a mixture of muck and sand, and (3) a good greenhouse potting soil. The lime requiredment of the muck was 34,200 lbs. calcium oxide, and that of the mixed soil 15,000 lbs. calcium oxide per acre according to the Jones method of determination (14). The potting soil was neutral to litmus. A basic treatment of commercial sodium nitrate and potassium chloride (the former at 1000 lbs. per acre, the latter at 2500 lbs. per acre) was given to both the muck and the mixed soil several weeks before the plants were set and the experiment started. Hydrated lime and commercial acid phosphate were used in various amounts and proportions as shown in Tables 2 to 4. These treatments were also applied at the same time, as the basic applications mentioned above, the soil being kept moist during the interval.

Lettuce of the Grand Rapids Forcing variety was used. The seeds were germinated in flats of soil, pricked off once, and later carefully selected for uniformity of size and vigor and transplanted to the experimental flats. Six plants were grown in each flat, the flat being 12 x 21 x 3 inches, two flats being used per treatment. The flats were placed on the boards of the bottom of a greenhouse bench in order to confine the roots. Environmental conditions were kept optimum for the growth of lettuce and uniform for all the flats. Watering was watched closely and the soil moisture held as near as possible at the apparent optimum for each soil. The duration of the experiment was 80 days. The data are set forth in tables 2 to 4 inclusive.

The data in Table 2 show little if anything regarding catalase, while decreases in oxidase activity where the plants were treated are quite evident. The differences for lots 2, 3 and 4 as compared with lot 1 were 20, 8 and 12 percent respectively. Clearly, positive correlation between the growth effect of the soil treatments and enzyme activity is wholly lacking.

As seen in Table 3 the soil treatments fall into three series: increasingly large applications of both lime and acid phosphate, increasingly large applications of lime with amount of phosphate constant, gradually larger amounts of lime with no phosphate. The results show that neither the lime nor the phosphate affected oxidase activity very significantly. In Series 1 the plants were not significantly altered in size as the amounts of lime and acid phosphate increased, and the oxidase activity remained essentially the same as compared with the check. In Series 2 the same thing prevailed, while in Series 3 there is some indication that the additions of lime increased oxidase activity; decreasing at the same time the growth of the plants. This is in agreement with Bunzell (6) (7) who found that anything which checked normal growth tended to accelerate the action of oxidase.

Table 2 -- Oxidase and Catalase Activity of Lettuce Plants Grown in Muck Soil, Experiment 1.

Lot	Lime Application (P.ct.)[1]	Acid Phosphate per acre (tons)	Green wt. per plant, tops (gms.)	Water per gm. of dry wt. of tops (gms.)	Oxidase, (cm. of Hg. at end of 60 mins.)	Catalase (cm. of Hg.) Time in minutes 1	2	3
1	None	None	3.1	7.3	1.25	1.95	2.55	2.85
2	0	15	.6	5.0	1.00	----	----	----
3	50	15	61.8	14.4	1.15	1.80	2.45	2.75
4	0	5	4.7	7.4	1.10	1.95	2.55	3.05

1. Percentage of total lime requirement of soil.

Table 3 -- Oxidase and Catalase Activity of Lettuce Plants Grown in Mixed Soil, Experiment 1.

Lot	Lime application (P. ct.)[1]	Acid Phosphate per acre (tons)	Green Wt. per plant tops (gms.)	Water per gm. of dry Wt. of tops (gms.)	Oxidase Cm. of Hg. at end of 60 mins.	Catalase (Cm. of Hg.) Time in minutes 1	2	3
Series 1								
5	Check	Check	74.4	16.6	1.00	1.65	2.00	2.20
6	33	2	72.8	19.0	.80	1.15	1.70	1.85
7	66	4	77.1	18.5	1.10	.90	1.30	1.60
8	100	6	65.8	20.4	1.10	.85	1.20	1.35
9	133	8	60.4	17.6	1.00	.95	1.25	1.35
Series 2								
10	25	8	92.3	17.1	1.00	1.70	1.90	2.15
11	50	8	82.5	16.5	.90	1.35	1.90	2.30
12	75	8	76.5	14.0	1.00	1.40	1.70	2.00
13	100	8	69.1	15.4	1.00	1.40	2.00	2.30
14	125	8	83.2	16.2	.75	1.10	1.40	1.60
Series 3								
15	25	0	80.5	15.6	.90	1.40	1.75	1.95
16	50	0	76.4	15.2	1.20	1.35	1.70	1.90
17	75	0	44.2	15.5	1.30	1.40	2.00	2.20
18	100	0	29.2	15.8	.95	2.00	2.60	3.00
19	125	0	17.2	12.5	1.10	2.15	2.75	3.15

1. Percentage of total lime requirement of soil.

Table 4 -- Oxidase and Catalase Activity of Lettuce Plants Grown in Potting Soil, Experiment 1.

Lot	Lime applications per acre (tons)	Acid Phosphate per acre (tons)	Green Wt. per plant, tops (gms)	Water per gm. dry Wt. of tops (gms)	Oxidase cm. of Hg. at end of 60 minutes.	Catalase (cm. of Hg.) (Time in minutes) 1	2	3
Series 1								
20	None	None	25.9	8.5	.95	1.25	1.55	1.80
21	0	2	24.3	8.3	1.05	2.30	2.80	3.00
22	1	2	26.0	8.9	1.30	1.80	2.20	2.40
23	2	2	28.2	10.8	1.25	2.05	2.50	2.85
24	3	2	24.7	9.8	1.10	2.35	2.70	2.95
25	4	2	18.7	10.0	1.40	1.85	2.10	2.30
Series 2								
26	1	0	26.3	10.7	1.20	1.35	1.70	1.95
27	2	0	20.0	12.5	1.05	1.40	1.70	1.90
28	3	0	12.6	14.8	1.10	1.25	1.70	1.90
29	4	0	10.2	15.3	1.10	1.40	1.80	1.95

As regards catalase the data are somewhat definite. Where growth was reduced as in Series 3 (lime only) the activity of the enzyme was increased, while in Series 1 (lime and acid phosphate) the size remained practically the same with a decrease in catalase.

As may be observed from Table 4 there were two series of soil treatments: increasingly large applications of lime with the amount of phosphate the same; no phosphate with larger and larger amounts of lime. In both series the activity of oxidase and catalase was increased over that of the check by the soil treatments. In Series 1 the size of the plants was not consistently influenced in either direction. In Series 2 growth was gradually diminished.

Experiment 2 -- Lettuce and Radish.

In this experiment two soils were used (1) a good soil consisting of five parts loam and one part sandy gravel (Series 1), and (2) a poor soil consisting of five parts sandy gravel and one part loam (Series 2). Fertilizer treatments were applied as shown in Tables 5 and 6, one gram of each nutrient substance (c.p.) being applied per pot. Where cumulative doses were applied one-fourth of the total amount was applied at the beginning and the remaining one-fourth portions at successive intervals during the experiment.

The lettuce grown was of the Grand Rapids Forcing variety. The seedlings were treated as in Experiment 1 but were grown in six inch pots instead of in flats, a single plant per pot. Twenty pots were used for each treatment. The pots were filled uniformly with the soil and kept plunged in sand on a greenhouse bench. The pots were systematically distributed to offset any place effect and to give equal exposure to the individual plants of each series. Environmental conditions were similar to those in Experiment 1. The plants were grown for 94 days, or from November 7 to February 9.

The radish grown was the Scarlet Globe variety. The seed was planted in flats on December 15 and transplanted to six inch pots January 13. The soil was the poor soil (Series 2) mentioned above, and the fertilizer treatments those shown in Table 6. Environmental conditions were similar to those surrounding the lettuce plants. The plants were grown for 37 days.

A study of Table 5 shows that the size of the plants was increased by the nutrient treatments in both series except in lots 36 and 43 where potassium was used. Changes in oxidase activity were not significant in either series, and did not follow the variations in the development of the plants. The same was true regarding catalase; the determinations varying considerably but not consistently nor at all positively with the increases in the growth of the plants from lot to lot.

Table 6 shows that while the size of the tops of the radish plants was affected by the size of the fertilizer treatments the oxidase content remained unchanged except

Table 5 -- Oxidase and Catalase Activity of Lettuce Plants, Experiment 2.

Lot	Fertilizer	Green Wt. per plant tops (gms)	Water per gm. dry Wt. of tops (gms)	Oxidase (cm. of Hg. at end of 60 minutes)	Catalase (cm. of Hg.) Time in minutes 1	2	3
Series 1							
30	None	61.0	13.7	1.05	1.55	1.95	2.10
31	$Ca(NO_3)_2$	96.6	14.6	1.05	1.60	1.90	2.15
32	$CaH_4(PO_4)_2$	63.4	13.5	1.00	1.10	1.45	1.65
33	$Ca(NO_3)_2$ $CaH_4(PO_4)_2$	107.0	15.4	1.10	1.40	1.75	1.95
34	$Ca(NO_3)_2$ $CaH_4(PO_4)_2$ Cum.	99.6	14.2	1.05	1.40	1.85	2.10
35	$Ca(NO_3)_2$ Cum.	89.6	17.2	1.20	1.30	1.75	2.05
36	KCl	61.8	12.0	1.05	1.10	1.40	1.50
Series 2							
37	None	63.5	14.9	1.05	1.50	1.85	2.15
38	$Ca(NO_3)_2$	100.0	15.9	1.15	1.50	1.85	2.00
39	$CaH_4(PO_4)_2$	74.1	14.4	1.05	1.50	2.00	2.35
40	$Ca(NO_3)_2$ $CaH_4(PO_4)_2$	108.9	16.3	1.05	1.40	1.80	2.05
41	$Ca(NO_3)_2$ $CaH_4(PO_4)_2$ Cum.	113.8	17.3	1.10	1.30	1.65	1.85
42	$Ca(NO_3)_2$ Cum.	87.9	17.9	1.15	1.15	1.45	1.60
43	KCl	51.0	14.4	1.00	1.10	1.40	1.60

Table 6 -- Oxidase and Catalase Activity of Radish Plants.

Experiment 2.

Lot	Fertilizer	Green wt. per plant tops (gms.)	Water per gram dry of tops (gms.)	Oxidase (cm. of Hg. at end of 60 mins.)	Catalase (cm. of Hg.) Time in minutes.		
					1	2	3
44	None	3.2	11.8	1.10	1.75	2.50	2.90
45	$Ca(No_3)_2$	5.6	12.7	.95	1.70	2.55	3.00
46	KCI	2.8	8.5	1.10	1.85	2.55	3.05
47	$Ca(No_3)_2$t KCI	3.6	11.6	1.15	1.60	2.05	2.30
48	$CaH_4(Po_4)_2$	4.2	11.6	1.10	1.50	1.90	2.15

in the nitrogen lot where the oxidase activity was lessened. The catalase activity was increased slightly over the check by nitrogen and by potassium when applied alone, but lowered when both were added together and also lowered when phosphorous was added. There was no positive correlation between size of plants and catalase activity, but rather a tendency towards negative correlation.

Experiment 3 -- Lettuce and Spinach.

Spinach plants of the Savoy type and lettuce plants of the Grand Rapids Forcing variety were used. The seedlings were grown from select seeds and transplanted to six inch pots on Feb. 24, 1925. One plant was grown in each pot with seven plants for each series or treatment. The length of the growth period was 40 days.

The soil was a mixture, consisting of two-thirds medium coarse sand and one-third fine sandy loam. One-half of the total amount of each fertiliser treatment was mixed in the dry state into the soils of the several series just before potting; the remainder was added in solution on March 12, sixteen days after potting. The nutrient salts were all of c.p. quality. The pots were filled uniformly with the soil and kept plunged in sand on a greenhouse bench. The sand about them was kept moist. Water was applied in exactly equal quantities per pot during the period of growth. The pots were systematically distributed as in Experiment 2. Development within each

Fig. 2

Fig. 3

Treatments Reading From Left to Right

Check	
$Ca(NO_3)_2$	0.3 gms.
$Ca(NO_3)_2$	0.8 gms.
$Ca(NO_3)_2$	1.6 gms.
$\{Ca(NO_3)_2$	1.6 gms.
$\{CaH_4(PO_4)_2$	0.8 gms.
$\{Ca(NO_3)_2$	1.6 gms.
$\{KCI$	0.8 gms.
$\{Ca(NO_3)_2$	1.6 gms.
$\{KCI$	0.8 gms.
$\{CaH_4(PO_4)_2$	0.8 gms.

Table 7 -- Oxidase and Catalase Activity of Spinach Plants,
Experiment 3.

Lot	Fertilizer Kind	Fertilizer gms. per pot	Green wt. per plant tops (gms.)	Water per gm. dry wt. tops (gms.)	Oxidase cm. of Hg. at end of 60 mins.	Catalase (cm. of Hg.) Time in minutes 1	2	3
49	Check	---	3.7	12.2	.95	2.35	2.75	2.95
50	$Ca(NO_3)_2$	0.3	4.9	12.3	.90	2.10	2.60	2.70
51	$Ca(NO_3)_2$	0.8	6.5	11.8	.95	2.85	3.60	4.00
52	$Ca(NO_3)_2$	1.6	10.4	10.2	1.00	2.40	2.95	3.20
53	$Ca(NO_3)_2$ $CaH_4(PO_4)_2$	1.6 0.8	10.1	11.8	.90	2.75	3.40	3.65
54	$Ca(NO_3)_2$ KCl	1.6 0.8	12.0	11.6	.95	2.80	3.25	3.55
55	$Ca(NO_3)_2$ KCl $CaH_4(PO_4)_2$	1.6 0.8 0.8	11.9	11.8	.80	2.15	2.65	2.90

series was uniform. The differences between the plants of the various lots due to the treatment were very definite and consistent (see Figs. 2 and 3). The results are given in Table 7.

Table 7 shows consistent increases in the size of the plants, following the nutrient treatments. The activity of oxidase was not materially affected nor different within the several lots. In general, the catalase content was greater where the nutrients were applied, but the increases do not consistently follow the size of the plants nor were they correlated in detail with changes in growth.

Table 8 shows that the nutrient treatments resulted in increased growth of the plants. Oxidase activity did not vary with increases in size of plants but rather in the opposite direction. Again, as with the spinach plants of this experiment catalase activity was greater in general in the fertilized plants, but the variations are not at all closely parallel.

Experiment 4 -- Lettuce.

In this experiment the effect on enzyme activity of lengthening and of shortening the normal day in conjunction with nutrient treatments was studied. Grand Rapids Forcing lettuce plants were grown singly in six-inch pots of fine sand low in fertility. Various nutrient substances were applied to the soil and the periods of illumination of the plants controlled. Three series varying

Table 8 -- Oxidase and Catalase Activity of Lettuce Plants.

Experiment 3.

Lot	Fertilizer Kind	Gms. per pot.	Green Wt. per plant tops (gms.)	Water per gm. dry Wt. tops (gms.)	Oxidase cm. of Hg. at end of 60 mins.	Catalase (cm. of Hg.) Time in minutes 1	2	3
56	Check	---	7.4	9.5	1.30	2.60	3.30	3.70
57	$Ca(NO_3)_2$	0.3	14.6	11.2	.95	3.25	4.60	5.30
58	$Ca(NO_3)_2$	0.8	14.5	13.3	1.00	2.70	3.55	4.00
59	$Ca(NO_3)_2$	1.6	20.4	12.7	.95	3.25	4.35	5.00
60	$Ca(NO_3)_2$	1.6						
	$CaH_4(PO_4)_2$	0.8	36.5	11.2	1.00	2.95	4.10	4.70
61	$Ca(NO_3)_2$	1.6						
	KCI	0.8	22.7	13.3	1.25	3.75	4.30	4.60
62	$Ca(NO_3)_2$	1.6						
	KCI	0.8						
	$CaH_4(PO_4)_2$	0.8	33.9	10.4	.85	3.10	3.65	3.90

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in the amount of illumination given were used. Series 1 (short day) was given daylight illumination from 9 A.M. to 3 P.M. and covered with specially built light-tight boxes the rest of the time. These boxes were 10 feet long, 5 feet wide and 3 feet high, with light-tight ventilators arranged in each end so that circulation of the air within might take place. Series 2 consisted of check plants grown in normal daylight (Nov. 5 to Jan. 3), and Series 3 (long day) received illumination from daylight in the morning until 11 P. M. The extra illumination of these plants was accomplished by means of Madza light bulbs (1000 Watts each) socketed in porcelain enameled steel dome reflectors suspended at a height of 4.5 ft. above the bench. Three of these bulbs, evenly spaced apart were used over a bench 21 ft. long and 5 ft. wide, the pots containing the plants being distributed so as to receive equal illumination, and being systematically shifted about each week. These plants were grown in the winter months (Nov. 5 to Jan. 3) so the daily period of illumination was lengthened about 6 hours. The periods of illumination of the different series were approximately 6, 10 and 16 hours respectively. The pots were kept plunged in sand on a greenhouse bench and moisture and temperature conditions kept favorable for growth. From 15 to 22 plants were used for each treatment in the different series, and plant nutrients supplied as shown in Tables 9 and 10, one gram of c.p. material being added to each pot.

In each series the fertilized plants were larger than the check in the same series. In Table 9, Series 1 there is no change in the oxidase activity with the different treatments. Series 2 consisted of a normal day check only, while in Series 3 fertilizer treatments and growth were accompanied by increased oxidase activity, and also by some increases in catalase activity. In Table 10 Series 1 there was a decrease in oxidase activity with the fertilizer treatments and growth. The same is true in Series 3 with the exception of Lot 69 which showed increased oxidase activity and lowered catalase activity in comparison to the other lots in same series. The last application of fertilizer was made ten days before the samples were taken, so the alteration of the enzyme activity was perhaps not due to this application. The catalase of the other lots in Series 3 was not materially affected; in Lot 71 where nitrogen and phosphorous was applied cumulatively the catalase activity was slightly higher.

Differences in the length of day had but slight effect on the activity of catalase, while with the short day there was a tendency towards increased oxidase activity.

With oxidase the samples taken at different times during the growth period showed little difference, four out of the nine lots showed slightly greater activity on December 4 than they did on December 21, the other five showed slightly greater activity on December 21. The catalase activity was greater in every case on the latter

Table 9 -- Oxidase and Catalase Activity of Lettuce Plants on December 4, Experiment 4.

Lot	Fertilizer	Green Wt. per plant tops[1] (gms.)	Water per gram dry weight tops (gms.)	Oxidase Cm. of Hg. at end of 60 mins.	Catalase (cc. of water displaced) Time in minutes		
					1	2	3
Series 1							
63	Check	8.2	23.6	3.55	----	----	----
64	$Ca(NO_3)_2$	15.1	29.3	3.40	----	----	----
65	$Ca(NO_3)_2$ Cum.	13.4	18.3	3.55	1.65	2.10	2.30
Series 2							
66	Check	11.0	16.5	2.45	1.85	2.25	2.50
Series 3							
67	Check	11.2	10.9	2.20	1.70	2.10	2.35
68	$Ca(NO_3)_2$	49.8	15.6	2.10	2.05	2.60	2.95
69	$Ca(NO_3)_2$ Cum.	54.2	27.5	2.95	1.90	2.35	2.65
70	$Ca(NO_3)_2$ $CaH_4(PO_4)_2$	58.8	20.4	3.90	2.00	2.50	2.80
71	$Ca(NO_3)_2$ $CaH_4(PO_4)_2$ Cum.	75.2	18.0	3.25	1.70	2.25	2.60

[1] Weights of plants when harvested the first week in January.

Table 10 -- Oxidase and Catalase Activity of Lettuce on December 21, Experiment 4.

Lot	Fertilizer	Green Wt. per plant tops 1 (gms.)	Water per gram dry weight tops (gms.)	Oxidase cm. of Hg. at end of 60 mins.	Catalase c.c. of water displaced, Time in minutes: 1	2	3
Series 1							
63	Check	8.2	23.6	3.90	2.10	2.70	3.10
64	$Ca(NO_3)_2$	15.1	29.3	2.95	2.25	2.90	3.30
65	$Ca(NO_3)_2$ Cum.	13.4	18.3	2.80	2.20	2.70	3.10
Series 2							
66	Check	11.0	16.5	3.05	2.30	2.80	3.20
Series 3							
67	Check	11.2	10.9	2.90	2.15	2.70	3.10
68	$Ca(NO_3)_2$	49.8	15.6	2.70	2.10	2.60	3.00
69	$Ca(NO_3)_2$ Cum.	54.2	27.5	3.45	1.95	2.50	2.90
70	$Ca(NO_3)_2$ $CaH_4(PO_4)_2$	58.8	20.4	2.60	2.20	2.85	3.25
71	$Ca(NO_3)_2$ $CaH_4(PO_4)_2$ Cum.	75.2	18.0	2.80	2.35	2.90	3.30

1 Weights of plants when harvested first week in January.

date. It is also noteworthy that in the plants of this experiment oxidase activity on the whole was far greater than in the plants of any previous experiment conducted under different conditions.

DISCUSSION

The obvious tendency in the results that have been presented is for greater activity of the enzymes to be associated with diminished growth and size of the plants. In order to test, and confirm or else disprove this observation coefficients of correlation were determined respecting average green weights of the tops of the plants of all the lots of all experiments and the activity of both oxidase and catalase, using the manometer readings at 3 minutes for catalase. The Pearsonian formula $\frac{\Sigma(xy)}{n(\sigma_x \sigma_y)}$ was used for calculating the coefficient of correlation, and the formula $\frac{1-r^2}{\sqrt{n}}.6745$ for obtaining their probable errors. The results are shown in Table 11.

Table 11 shows clearly that correlation existed between the average size of the tops of all plants of the several experiments and enzyme content of their tissues, that the correlation was highly significant with both oxidase and catalase, ~~being nearly perfect respecting the latter~~, and further that the correlation was negative in character. This means that reduced activity of the enzymes (oxidase and catalase) was associated with greater growth of the plants. In addition it tends logically to

Table 11 -- Coefficients of Correlation for Average Size of Tops and Enzyme Content, all Plants of all Experiments.

Number of lots	Means			Coefficients of Corr.	
	tops (gms.)	Enzyme		Oxidase	Catalase
		Oxidase	Catalase		
77	42.4 ± 22.16	1.45 ±.553	2.64 ±.554	-.246 ± .072	.503 ± .085 -.~~939~~ ± .~~009~~

the conclusion that since nutrient substances resulted in increased growth of the plants their effect relative to these enzymes was to diminish their content and activity. This reasoning which would establish the following chain of antecedents: increased nutrients -- decreased oxidase and catalase activity -- increased growth, assumes, of course as a premise that growth is directly subject to changes in the amount or properties of the enzymes under consideration. It also, neglects taking account of any effect of nutrient substances which may be exerted otherwise than indirectly through affecting these enzymes.

It is true that Heinicke (12) (13) and also Auchter (4) have reported finding increased catalase activity associated with greater vigor of fertilized trees and shrubs. Perhaps the facts are not the same for woody plants as for herbaceous ones. In an effort to duplicate the results of the above mentioned workers determinations of catalase activity were made during the summer of 1925 (July 15) on apple trees in an orchard where a fertilizer test has been under way for the past five years. The orchard has been in sod for many years. The trees, set 35 feet apart, have been lightly pruned; kept well sprayed, and retained in a very productive state. Differences as to vigor between the plots are obvious at once when the orchard is viewed during the growing season. The plot treatments together with the yields from the plots sampled for enzyme determinations are shown in Table 12. In sampling, discs were taken between 10 and 11 A. M. from the leaves to the outside of 3 trees of the record row of the plot, and from the same side at the same approximate vertical level of the trees of each plot. Only leaves of equal size and apparently equal vigor on non-bearing spurs of the same length (1 to 2 inches) were used. Necessary equipment was taken to the orchard so that upon collecting a sample it was immediately weighed, macerated and fully prepared for keeping and being made use of in the laboratory. The method of determining the enzyme content was the same as described on page 6.

The yields shown in Table 12 make it evident that the several plots varied significantly in productivity and hence in vigor as measured by yield. The enzymatic activity of the samples from plots 2 and 3 composed of the more vigorous and productive trees was 62% less in each case than in plot 1.

Deductively, the conclusion that decreased activity of oxidase and catalase is associated with increased growth and size in plants, which has been reached in this work and other work where disease etc. has been a factor in checking growth or rendering it abnormal particularly the works of Bunzel (6) (7), Woods (24) and Rose (19) would seem to be pertinent and correct. These two enzymes are effective in oxidation processes within the cells. Through processes of this nature compounds

Table 12 -- Yield and Catalase Activity of Trees in Farrand Orchard.

Plot	Treatment (lbs. per tree per yr.)	Yield average (lbs. per tree per yr.) 1921-1925	Catalase Activity (cm. of Hg.) Time in minutes		
			1	2	3
1	None	195	3.10	3.70	3.85
2	5 lbs. $NaNO_3$	491	1.10	1.30	1.45
3	4 lbs. $(NH_4)_2SO_4$	499	1.10	1.30	1.40

already synthesized and either assimilated or prepared for assimilation are broken down to simpler substances, even to carbon dioxide and water, and the energy they contained released for the activities of the organism. In other words, oxidase and catalase are primary in the catabolic phase of metabolism. Since growth or increase in mass is the resultant of the products of anabolism being in excess of the amounts of elaborated materials destroyed through catabolism, it must follow that it can take place to a greater degree only as the activity of those agencies concerned in catabolic processes is diminished relatively. Hence if it be true that growth-promoting substances (nutrients) are effective through the enzymes oxidase and catalase it is not surprising to find that they bring about a reduction in the activity of the two catalysts.

The fact that catalase activity in seeds is higher during germination as has been demonstrated by Shull (20) and others does not conflict with the results and conclusions reached herein. It appears of course that this increased activity of the enzyme is concomitant with the beginning and acceleration of growth of the seed embryo, but the situation is not closely analogous to that which obtains in the green leaf or stem. Synthesis of foods from raw materials is not taking place in the seed and it is not exposed ordinarily to light and the other energizing factors of aerial environment. Yet the need for energy is high and the supply of it must come from the decomposition of stored materials through oxidation by means of these oxidizing enzymes. Perhaps other enzymes, concerned in hydrolytic reactions, are more positively correlated with the nourishment and actual increase of the embryo, and here as in the green leaf the increased activity of oxidase and catalase in meeting the requirement of energy tend at the same time to reduce the amount of assimilable growth-promoting material. Where the stored supply of food is large relative to the requirement of the embryo prior to the appearance and functioning of the green leaves as in most seeds, the diminution of this supply occasioned through oxidations affected by oxidizing enzymes would not be critical nor very apparent as a check on growth. However it is very noteworthy that experimental data available on the rate of increase in growth from the time of the beginning of seed germination show that there intervenes a period at the start during which there is either no increase in mass or even an actual reduction. Growth curves with increase in size plotted against time show this as a common characteristic. For example the publication of Briggs, Kidd and West (5) wherein they have analyzed and presented graphically the data compiled by Kreuster et.al. respecting the growth rate of maize from the time of sowing the seed. On the generalized form of the growth rate curve (page 121) there is an initial portion covering the

first three week period "before the assimilatory organs are able to counter balance the loss in dry weight due to respiration, and the rate of growth is consequently negative or nil". Quoting further from page 114, the authors say: "In following the curves in Fig. 3 from the date of sowing there is seen to be an initial phase lasting for about three weeks during which the rate of growth is negative, or in other words the plant is actually losing in weight. This phase of negative growth persists until a point in the development of the plant is reached at which approximately four leaves have appeared. During the time occupied by germination, before the appearance of these leaves, the negative rate of growth is clearly to be attributed to a loss of carbohydrate through respiration. The order of magnitude of the loss in dry weight through respiration in germinating seeds is 3 to 6 percent of their dry-weight per day at 16°C (Garreau (5))". Of equal interest is the fact that the generalized form of the growth-rate curve (page 121) shows two minima which precede two subsidiary maxima, these maxima coinciding "with the time of the record of the appearance of the male and female flowers respectively". The comment of the authors on this is as follows, the parenthesis having been inserted: "The minima x, y (indicating a decrement in growth) which precede these maxima, correspond with the earliest stages of flower development, and are possibly due to increased respiration (which predicates increased oxidase and catalase activity) during that period."

As additional evidence in support of the existence of a reverse relation between growth and the activity of respiratory enzymes the work of Priestley and Evershed (17) may be mentioned. They studied the root growth of Tradescantia and Lycopersicum, based on increase in the dry and wet weights of the roots from cuttings. The grand period curves which were obtained proved to be series of S-shaped curves. The subsidiary maxima of the curves corresponded with the times of appearance of successive crops of roots subordinate in the order of branching. These maxima were preceded by minima which corresponded it would seem to the periods of the origin and differentiation of these branches in the pericycle region, and consequently were coincident to the times of accelerated respiration in that same region.

Lest it be supposed that the rise in the growth-rate curves following the depressions discussed above, particularly that which marks the early stages of germination, be due not to the actual decrease of the activity of the enzymes under consideration and hence of respiration intensity but wholly to the initiation and rapid increase in the assimilatory functions of the leaves relative to an unchanged respiratory rate attention should be called to the fact that there are considerable data which show that the intensity of respiration declines with increased age of the plant. A single citation, that of the work of Kidd, West and Briggs (15) on Helianthus annuus both in the laboratory and in the field, will be sufficient. Using the respiratory index, which they define as the respiration, measured by the rate of carbon dioxide emitted, per gram of dry weight at 10°C when the amount of respirable material is not limiting and where the external concentration of oxygen is that of the atmosphere, they conclude from numerous experiments that the respiratory index of the entire plant declines continuously as age increases. A typical specimen of their results is the instance where entire plants at 2 days after germination gave 3 mgm. of carbon dioxide per gram of dry weight per hour, while plants 136 days old produced only 0.39 mgm. of carbon dioxide per gram of dry weight per hour. The stem, leaves and flowers separately considered showed decreases with age similar to that of the entire plant.

The fact that growth requires the energy released in the oxidations accomplished by the enzymatic action is the thing which underlies any supposition that growth and the activity of oxidase and catalase are positively correlated. It should be remembered however that the processes of growth are catabolic as well as anabolic and too, are not the only ones taking place in the plant which use up energy, and furthermore that there is no evidence to support any theory which would embody the concept of there being an exact quantitative balance between the necessary amount of energy, and the activity of energy producing agencies. This would attribute to plant organisms a degree of economy and an inherent capacity for conservation of energy which they may or may not and most likely do not possess.

Investigators have endeavored for some time to determine whether catalase or oxidase is the more closely connected with respiration. Perfect agreement on the question has not been reached, the chief difficulty seeming to have been the fact that the two enzymes are so closely related that their separation for purposes of experimentation is beyond our methods at present. What evidence there is favors the conclusion that catalase bears a closer relationship to respiration than oxidase does. Reed's (18) data showed that the two enzymes are independent of each other and that in the ripening of fruit catalase increased while oxidase stayed constant, or practically so. Crocker and Harrington (9) found catalase activity ran parallel to respiration in the fruits of <u>Andropogan halepenses</u>, but did not do this in seeds of <u>Amaranthus</u>. Appleman (2) determined the comparative activity of these enzymes in the expressed juice of the potato tuber and found a close positive correlation between catalase content and respiration intensity but no such relation as regards oxidase. The data presented in

Table 11 confirm the results of these investigators though the reasoning employed is necessarily somewhat indirect, since direct determinations of respiratory intensity were not made. The coefficient of correlation between average size of plant and oxidase activity was $-.246 \pm .075$, while with catalase it was $-.$~~$959 \pm .009$~~$503 \pm .085$. The difference is very great and also wholly significant. The negative character of these coefficients may indicate that as the activity of the enzymes was lowered by the nutrient treatments of the soil, growth was accelerated. Remembering that these particular enzymes seem to be the principal ones effective in respiration it means that as their activity was reduced the intensity of respiration declined as well, this decrease being of such magnitudes that growth was increased because of a rise in the ratio of anabolism to catabolism. Hence it follows that since the coefficient of correlation for catalase activity and growth was ~~nearly four times~~ twice as great as that for oxidase and growth, catalase appears to stand in a closer relationship to respiration than oxidase. In other words, a greater depression of catalase than of oxidase was antecedent theoretically at least to lowered respiration and consequently to increased growth and size.

SUMMARY

In these investigations the effect of certain nutrient conditions of the soil on the activity of oxidase and catalase in lettuce, radish and spinach plants and the relation of this effect to growth have been studied. The results were as follows:

1. As between size of plant and oxidase activity correlation was slightly but significantly negative in character.

2. Correlation between size of plant and catalase activity was strongly negative, ~~being almost perfectly so.~~

3. In so far as the soil treatments affected growth and size of plant they were antecedent to decreased oxidase and catalase activity when accelerating growth and increased activity when growth was retarded.

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LITERATURE CITED

1. Appleman, C. O. Relation of catalase and oxidase to respiration in plants. Md. Agri. Exp. Sta. Bul. 191. 1915.

2. Appleman, C. O. Relation of oxidases and catalase to respiration in plants. Amer. Jour. Bot. 3:223. 1916.

3. Appleman, C. O. Respiration and catalase activity in sweet corn. Am. Jour. Bot. 5:207. 1918.

4. Auchter, E. C. Is there normally a cross transfer of foods, water and mineral nutrients in woody plants? Md. Agri. Exp. Sta. Bul.257. 1923.

5. Briggs, G. E., Kidd, F. and West, C. A quantitative analysis of plant growth. Ann Appl. Biol. 7:103. 1920.

6. Bunzel, H. H. A biochemical study of curly-top of sugar beets. U. S. D. A. Bur. of Plant Ind. Bul. 277. 1913.

7. Bunzel, H. H. Oxidases in healthy and curly-dwarf potatoes. Jour. Agri. Res. 2:373. 1914.

8. Cook, M. T., and Taubenhaus, J. J. The relation of parasitic fungi to the contents of the cells of the host plants. Del. Agri. Exp. Sta. Bul. 97. 1912.

9. Crocker, Wm. and Harrington, G. T. Catalase and oxidase content of seeds in relation to their dormancy, age, vitality and respiration. Jour. Agri. Res. 15:137. 1918.

10. Harvey, R. B. Relation of catalase, oxidase and H^+ concentration to the formation of over-growths. Amer. Jour. Bot. 7:211. 1920.

11. Harvey, R. B. An apparatus for measuring oxidase and catalase activity. Jour. Gen. Physiology 2:253. 1920.

12. Heinicke, A. J. Factors influencing catalase activity in apple-leaf tissue. Corn. Univ. Agri. Exp. Sta. Mem. 62. 1923.

13. Heinicke, A. J. Catalase activity in dormant apple twigs: its relation to the condition of the tissue, respiration and other factors. Corn. Univ. Agri. Exp. Sta. Mem. 74. 1924.

14. Jones, C. H. Method for determining the lime requirement of soils. Jour. Assoc. of Official Agri. Chemists 1:43. 1915.

15. Kidd, F., West, C. and Briggs, G. E. A quantative analysis of the growth of Helianthus annus. Part 1. The respiration of the plant and of its parts throughout the life cycle. Proc. Roy. Soc., London, B, 92:368. 1921.

16. Moore, B. and Whitley, E. The properties and classification of the oxidizing enzymes, and analogies between enzymic activity and the effects of immune bodies and complements. Biochemical Jour. 4:136. 1909.

17. Priestley, J. H. and Evershed, A. F. C. H. A quantitative study of the growth of roots. Ann. Bot. 36:225. 1922.

18. Reed, G. B. The relation between oxidase and catalase in plant tissues. Bot. Gaz. 62:409. 1916.

19. Rose, D. H. Oxidation in healthy and diseased apple bark. Bot. Gaz. 60:55. 1915.

20. Shull, C. A. and Davis, W. B. Delayed germination and catalase activity in Xanthium. Bot. Gaz. 75:268. 1923.

21. Sullivan, M. X. The action of salts used as fertilizers on plant enzymes. Jour. Biol Chem. 6:XLIV. 1909.

22. True, R. H. and Stockberger, W. W. Physiological observations on alkaloids, latex and oxidases in Papaver somniferum. Amer. Jour. Bot. 3:1. 1916.

23. Weiss, F. and Harvey, R. B. Catalase, hydrogen-ion concentration and growth in the potato wart disease. Jour. Agri. Res. 21:589. 1921.

24. Woods, A. F. Observations on the mosaic disease of tobacco. U. S. D. A. Bur. of Plant Ind. Bul. 18. 1902.

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