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THE PENNSYLVANIA STATE COLLEGE

The Graduate School

Department of Agricultural and Biological Chemistry

The Catalase and Oxidase Activity of Pennsylvania Cigar-Leaf Tobacco As Modified by Fertilizer Treatment

A Thesis

by

Philip Regan Tarr

Submitted in partial fulfillment

for the degree of

Master of Science

June 1933

Approved:

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TABLE OF CONTENTS

INTRODUCTION

The most difficult kind of tobacco to produce in perfection is the leaf used in the manufacture of cigars of the finest quality. Fertilizer experiments have accomplished a great deal in the efforts to produce a better cigar-leaf tobacco, but attention must also be given to the curing and sweating processes.

Curing of tobacco involves far more than merely drying the leaf. A marked loss of dry matter as well as a loss of water occurs during the process. It is probably a life process, due chiefly (if not wholly) to the activity of the cells of the leaf.

The sweating or fermentation of tobacco is the last process before the tobacco is made into cigars. It is usually packed tight and as the weather grows warmer, the sweating begins and continues for a long time. The sweating process is to tobacco what fermentation is to wine; it ripens and prepares it for use, perfecting its color and improving its flavor. The acrid or pungent taste is subdued while the burning qualities are increased. It also gives the tobacco a shiny, oily surface, which is called "satin face". The loss of weight often amounts to 10 or 15 per cent.

Since fermentation is a measure of tobacco quality, it is altogether logical that the enzymes concerned with respiration should prove to be an index of tobacco quality. With that in mind, this investigation was initiated. Cured tobacco will be used and its catalase and oxidase activity will, if possible, be correlated with the fertilizer treatment received. This work will be followed by similar determinations on the fermented tobacco.

REVIEW OF THE LITERATURE

Loew (1) in 1901 discovered catalase in tobacco and suggested that it acts in a protective capacity, destroying the accumulation of hydrogen peroxide formed in the cell as a result of intense oxidation processes. This theory is generally accepted today although Reed (2) has contrasting views. Reed points out that some bacteria are without catalase which would indicate its protective capacity is small, if any really exists.

The term "oxidizing ferment" was first introduced in 1877 by Traube in his later writings on fermentations. According to him, the oxidizing ferments are those which combine loosely with atmospheric oxygen, forming unstable compounds which give up their oxygen to easily oxidizable substances. Bertrand (3) was led to believe that the oxidizing ferments are more or less specific in their action, so he proposed the name "oxidases" as a group name for these ferments.

Onslow (4) claims that an oxidase is a system consisting of three components, viz. (1) an enzyme, oxygenase, (2) an aromatic substance containing an ortho-dihydroxy grouping as in catechol, and (3) a peroxidase. The oxygenase catalyzes the oxidation of the catechol substance with the formation of a peroxidase. The universally present peroxidase decomposes the peroxide with the formation of atomic oxygen. The atomic oxygen which the system forms from molecular oxygen brings about oxidation which otherwise wouldn't occur. This viewpoint is now generally accepted.

Appleman (5) found the oxidase content of potato juice gives no

indication of the intensity of respiration in the tubers. However, he found the catalase activity in the potato juice showed a very striking correlation with respiratory activity in the tubers.

Reed (6) believed oxidases must be universally distributed if they are related to biological oxidation. He pointed out that most plants free from oxidases are of an acid or alkaline nature, and that the oxidases may be present in a protected form which masks the reaction in vitro. Indeed he proved the presence of oxidases in citrus fruits, which were supposed to be free from the enzyme.

A year later Reed (7) proved that oxidases played the chief role in biological oxidation, repudiating Lillie's (8) theory that the chief role is assigned to intracellular surfaces or phase boundaries.

In working with germinating corn, Lantz (9) failed to show a close correlation between catalase activity and respiration. He concluded by stating there is no evidence for believing that catalase is the enzyme chiefly concerned in physiological oxidation. The evidence more nearly supports the theory that catalase prevents excessive oxidation.

Some interesting work by Burge (10) at Illinois showed the catalase activity of pine needles was greater in the summer than in the winter. Catalase measurements were made on the blood of rabbits and the activity was greater in the winter than in the summer. It is known that a fall in temperature decreases the oxidative processes in plants and increases them in warm blooded animals, and that the reverse is true for a rise in temperature. By comparing Burge's (10)

results, it may be seen that a fall and rise in temperature has the same effect on catalase that it has on the oxidative processes.

Morgulis (11) has found the optimum reaction of catalase to be from pH 6 to pH 8, although catalase prepared from beef kidney showed a strong activity between pH 5 and pH 9.

The optimum temperature, according to Morgulis (11), is between 0° and 10°C. The loss of activity between 10° and 20°C. is much smaller than between 20° and 30°C., while between 30° and 40°C. the loss is so rapid that the catalase reaction is very quickly exhausted.

Knott (12) found that phosphorus and nitrogen, when supplied to the plant through the roots, resulted in increased catalase activity.

As early as 1901, Loew (1) noticed that potassium salts retard the catalase reaction in vitro, and that the nitrates of the alkali group retard more than the other salts.

Working with the oxidase of apple tissue, Cruess and Overholser (13) found the organic peroxide to be much more susceptible to heat than the peroxidase. The former was inactivated at 73.5° to 78°C., while the latter was inactivated at 90° to 100°C.

Cruess and Fong (14) found the effect of pH on the inactivation temperature of oxidase to be very significant. The resistance to heat was greatest in the range of pH 5 to pH 7. These same workers (15) found that H_2O_2 greatly affects the intensity of the oxidase reaction. The optimum is naturally affected by the relative activity of the catalase which tends to rapidly destroy the H_2O_2.

Rose and his co-workers (16) found that N/10 solution of all

chlorides tested decreased oxidase activity while N/10 solution of all sulfates tested slightly increased the activity. Potassium, sodium, and magnesium nitrates had no effect on oxidation, while nitrates of barium, calcium, manganese, and iron decreased it. They concluded that the chlorides which retard the combustion of tobacco at high temperatures also retard the oxidase action at low temperatures.

OBJECT OF THE INVESTIGATION

The object of these investigations were to make a study of the catalase and oxidase activities of Pennsylvania cigar-leaf tobacco as related to different fertilizer treatments.

PLAN OF THE EXPERIMENT

A. Materials Used

The tobacco came from the new tobacco experimental field, one mile northeast of Lancaster, Pa., and was of the Swarr-Hibshman strain. It was grown on the three-year rotation field on plots that are 1/21 of an acre in area. The tobacco was harvested, air-cured, and sent to The Pennsylvania State College where it was dried, stripped, and the leaves ground in a Wiley mill for analysis.

B. Fertilizer Treatment Received

The fertilizers used include:

1. Varying amounts of nitrogen, 1/3 in nitrate of soda and 2/3 in cotton seed meal.
2. Varying amounts of phosphoric acid in 16% superphosphate.
3. Varying amounts of potash in sulphate of potash.
4. Three rates of application of a 6-8-12 (N-P-K) fertilizer; namely, 500, 1000, and 1500 pounds per acre, both broadcasted and in the row.
5. The position of manure, whether applied alone or as a supplement application.

The different treatments are listed in the following table.

Table 1

The Plot Treatment, the Yield, the Fire-holding Capacity and Elasticity of the Cured Tobacco

Plot	Treatment	Application	Time of Burn	Elasticity	Yield
		Lbs. per Acre	Minutes		Lbs. per Acre
1	6-8-4	1000	5	fair	1783
2	6-8-8	1000	5	good	1911
3	6-8-12	1000	5	good	1925
4	6-8-16	1000	9	good	1827
5*	0-8-12	1000	6	fair	1281
6	3-8-12	1000	7	good	1596
7	9-8-12	1000	6	good	1596
8	6-0-12	1000	6½	poor	1554
11	6-8-12	500	5	good	1734
12	6-8-12	1500	6	good	1806
16	6-8-12	500 in row	5	fair	1869
18	8-8-12	1500 in row	7	fine	2583
19	none	none	3	none	1050
20*	0-8-12	1000	4	fair	1680
23	3-8-8	1000 plus manure	4	good	2058
27	10 Tons Manure		5	fair	1869

*Check Plots

EXPERIMENTAL METHODS

A. Estimation of Catalase Activity

The method for determining catalase activity is the one used by Knott (12).

One gram of the ground tobacco sample was mixed with an equal weight of calcium carbonate (to regulate the acidity), transferred to a 50 cc. volumetric flask and filled to the mark with water.

The catalase activity was determined by withdrawing 2 cc. in a pipette immediately after thoroughly shaking the flask. The sample was placed in one arm of a dry reaction tube, and in the other arm was placed 2 cc. of fresh "Dioxogen" (= 26 cc. O_2) which had been previously neutralized with a slight excess of $CaCo_3$.

The apparatus was of the same type of shaking device as used by Knott (12). By adjusting the rheostat, the number of swings per minute could be controlled. For this work, a rate of 60 complete swings was found to be the minimum at which the liquids would be thrown from one arm to the other sufficiently to give good mixing.

The reaction tube was placed in a bath of water at 15°C. for three minutes before mixing took place, and remained submerged in this bath during the reaction.

Since the supernatant liquid in the catalase preparation is low in activity, there is always the possibility of error in drawing the 5 cc. sample if one has failed to mix the preparation well or does not measure the 5 cc. quickly before the heavier material begins to settle out of the pipette. Failure to get close checks between dup-

licates can be largely laid to error in this operation.

Since the catalase activity as determined here is only used as a relative measure, it was not deemed necessary to carry the reaction to completion. Northrop (18) says that the rate of decomposition of the peroxide is proportional at any time to the concentration of the catalase present. This view is now generally accepted. In this work, we shall express catalase activity as the cubic centimeters of oxygen liberated in thirty minutes.

A blank determination was run, using 5 cc. of the boiled preparation. As expected, it was very low.

The liberated oxygen was corrected for the blank and changed to standard conditions. The obtained values were multiplied by ten to give the catalase activity of one gram of the tobacco.

B. Estimation of Oxidase Activity

The iodimetric method of Guthrie (17) was used for determining oxidase activity. Glucose reduces iodine in acid solution and is a suitable substrate for the oxidase enzyme. This is the foundation of this method which answered our purpose well.

Preparation of substrate: Forty grams of glucose were added to 400 cc. of N. sodium hydroxide, placed in a 500 cc. Florence flask and immersed in a water-bath at 80°C. for fifteen minutes; removed and neutralized at once with 10 cc. of 85% phosphoric acid. Twenty-five grams of decolorizing charcoal (Norit A was used) were added and allowed to stand over night. A small portion of the filtrate was filtered and diluted one to five and the pH value determined

(Lamotte method was used). If not close to pH 6.5, it was adjusted to this pH with N sodium hydroxide or N hydrochloric acid. The addition of 2 cc. either to 100 cc. of the filtrate shifts the acidity about 0.1 pH. The iodine value for 25 cc. should be equal to about 60 cc. of N/50. Before using, the filtrate should be diluted with an equal volume of water.

The method: Pipette 10 cc. of the diluted substrate into Van Slyke-Cullen aeration tubes. Add 5 cc. of the tobacco preparation (as used in catalase) containing the enzyme. Add five drops of paraffin oil to each tube and aerate for one hour. Wash into 300 cc. Erlenmeyer flasks containing 10 cc. of 10% trichloracetic acid, adding in all about 25 cc. of water. Add 10 cc. of N/50 iodine in N/10 potassium iodide and allow to stand for 30 minutes. Titrate with N/100 sodium thiosulfate, using 1 cc. of 1% starch paste as an indicator. The difference between this and a blank titration is a measure of the oxidase activity of the sample.

The blank was relatively high as we would ordinarily expect. As in catalase, the results are multiplied by 10 to give us the values for a 1 gram sample. Oxidase activity will be expressed as the "iodine value" of the preparation.

PRESENTATION OF DATA

All catalase and oxidase results are expressed for a 1 gram sample of cured tobacco.

The catalase values are the cubic centimeters of oxygen liberat-

ed from hydrogen peroxide by the above sample at 15°C. The gas volumes were corrected for the blank, and changed to standard conditions.

The oxidase values correspond to the iodine value (N/50) of the tobacco preparation, i.e., if the sample has an oxidase activity of 25.0, it is equivalent to 25.0 cc. of N/50 iodine as an oxidizer.

Table 2

Total Results of Catalase and Oxidase Activity of Cured Tobacco
As Modified by Fertilizer Treatment

Plot	Treatment	Application	Catalase**	Oxidase***
		Lbs. per Acre	cc. O_2 Liberated	Iodine Value (cc. N/50)
1	6-8-4	1000	38.0	24.8
2	6-8-8	1000	33.9	25.5
3	6-8-12	1000	28.8	13.8
4	6-8-16	1000	31.1	23.9
5*	0-8-12	1000	24.3	43.3
6	3-8-12	1000	43.4	15.4
7	9-8-12	1000	14.8	13.5
8	6-0-12	1000	31.8	16.3
11	6-8-12	500	44.3	37.9
12	6-8-12	1500	36.8	34.1
16	6-8-12	500 in row	17.3	25.3
18	8-8-12	1500 in row	34.2	37.9
19	None	none	41.2	36.7
20*	0-8-12	1000	19.9	43.3
23	3-8-8	1000 plus manure	22.4	40.1
27	10 Tons Manure		27.1	46.4

*Check plots
**1 gram sample used at 15°C.
***1 gram sample used

Table 3

Catalase Activity of Cured Tobacco as Altered by Mode of Application of 6-8-12 Fertilizer

Plot	Application	Catalase*
		cc. O_2 Liberated
16	500 lbs. in row	17.3
3	1000 lbs. broadcasted	28.8
12	1500 lbs. broadcasted	36.8
11	500 lbs. broadcasted	44.3

*1 gram sample used at 15°C.

Table 4

Oxidase Activity of Cured Tobacco as Altered by Mode of Application of 6-8-12 Fertilizer

Plot	Application	Oxidase*
		Iodine Value (cc. N/50)
3	1000 lbs. broadcasted	13.8
16	500 lbs. in row	25.3
12	1500 lbs. broadcasted	34.1
11	500 lbs. broadcasted	37.9

*1 gram sample used

Table 5

Catalase Activity of Cured Tobacco as Altered by Varying the Nitrogen Content of the Fertilizer Application*

Plot	Treatment	Catalase**
		cc. O_2 Liberated
20	0-8-12	19.9
5	0-8-12	24.3
6	3-8-12	43.4
3	6-8-12	28.8
7	9-8-12	24.8

*The fertilizer application was 1000 lbs. broadcasted
**1 gram sample used at 15°C.

Figure 1

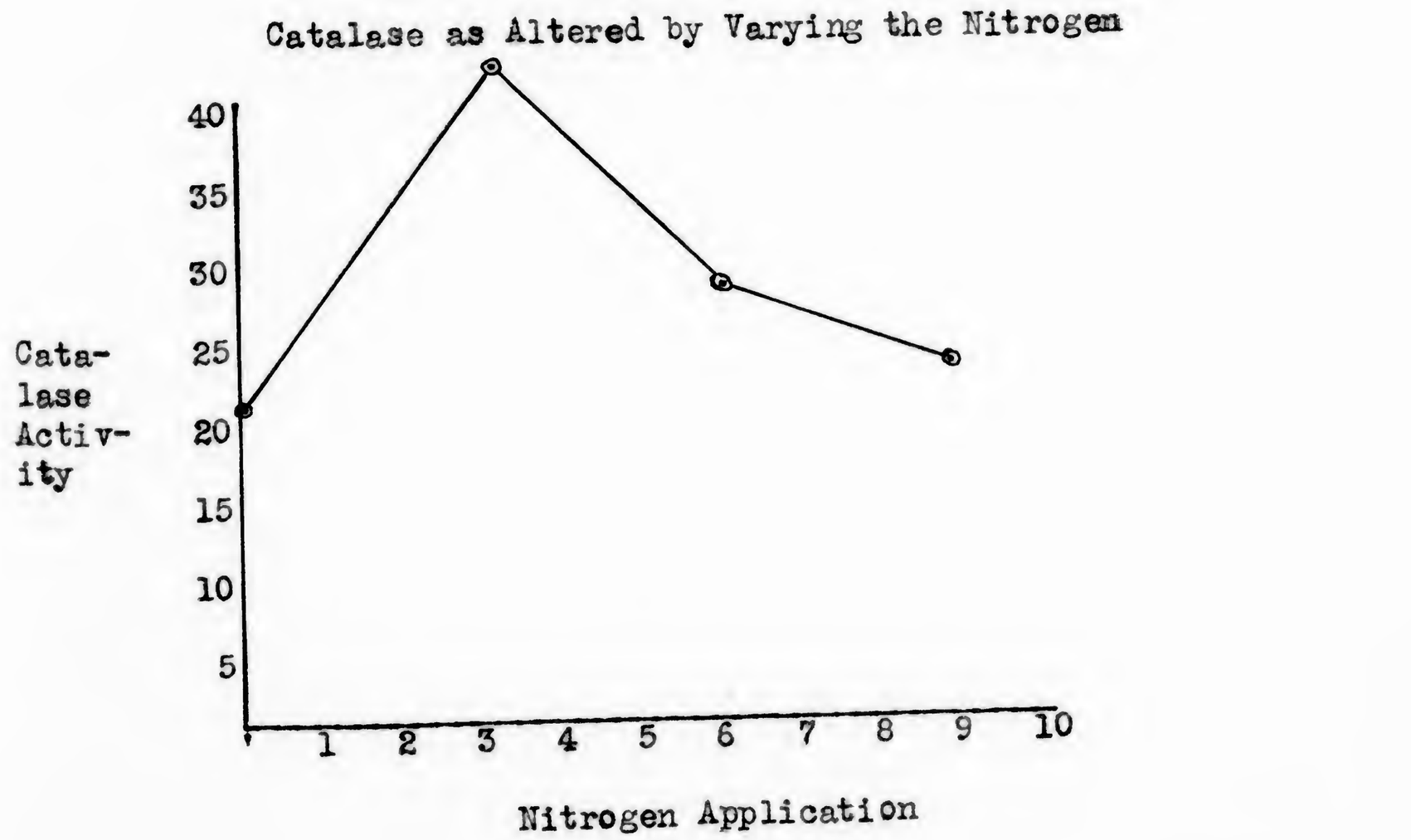

Table 6

Oxidase Activity of Cured Tobacco as Altered by Varying the Nitrogen Content of the Fertilizer Application*

Plot	Treatment	Oxidase**
		Iodine Value (cc. N/50)
20	0-8-12	43.3
5	0-8-12	43.3
6	3-8-12	15.4
3	6-8-12	13.8
7	9-8-12	13.5

*The fertilizer application was 1000 lbs. broadcasted
**1 gram sample used

Figure 2

Oxidase as Altered by Varying the Nitrogen

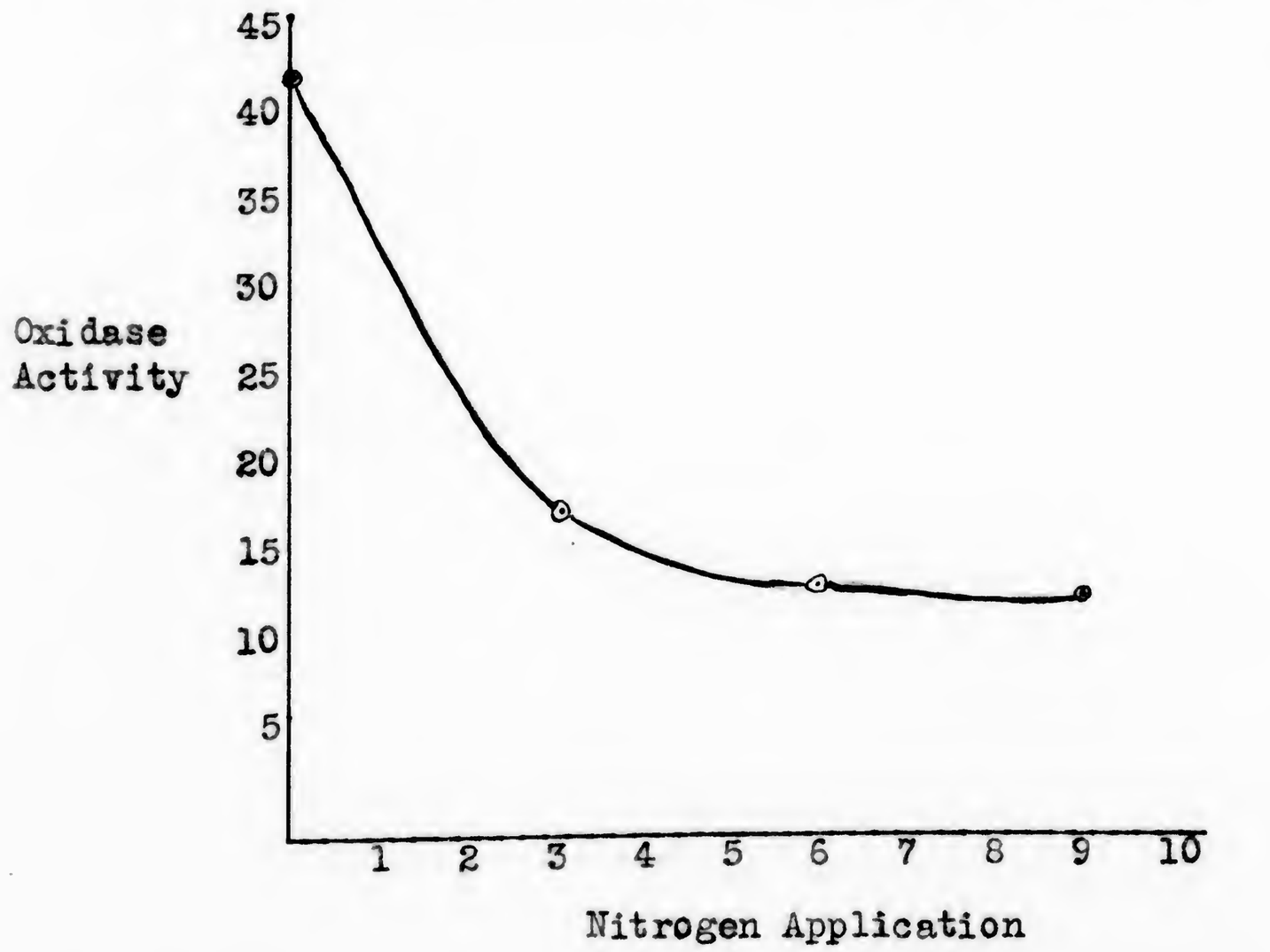

Table 7

Catalase Activity of Cured Tobacco as Altered by Varying the Phosphorus Content of the Fertilizer Application*

Plot	Treatment	Catalase**
		cc. O_2 Liberated
8	6-0-12	31.8
3	6-8-12	28.8

*The fertilizer application was 1000 lbs. broadcasted
**1 gram sample used at 15°C.

Figure 3

Catalase as Altered by Varying the Phosphorus

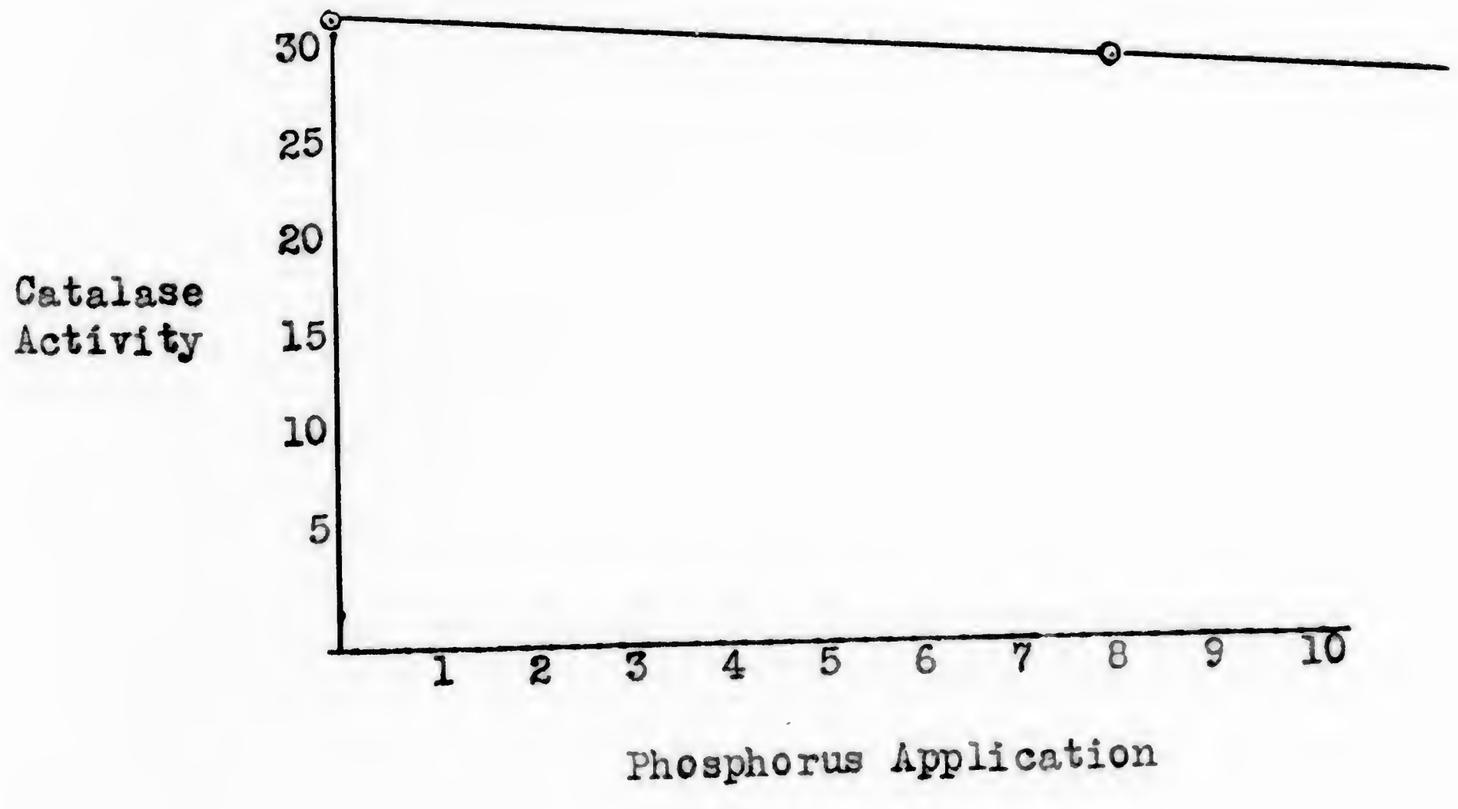

Table 8

Oxidase Activity of Cured Tobacco as Altered by Varying the Phosphorus Content of the Fertilizer Application*

Plot	Treatment	Oxidase**
		Iodine Value (cc. N/50)
8	6-0-12	16.3
3	6-8-12	13.8

*The fertilizer application was 1000 lbs. broadcasted
**1 gram sample used

Figure 4

Oxidase as Altered by Varying the Phosphorus

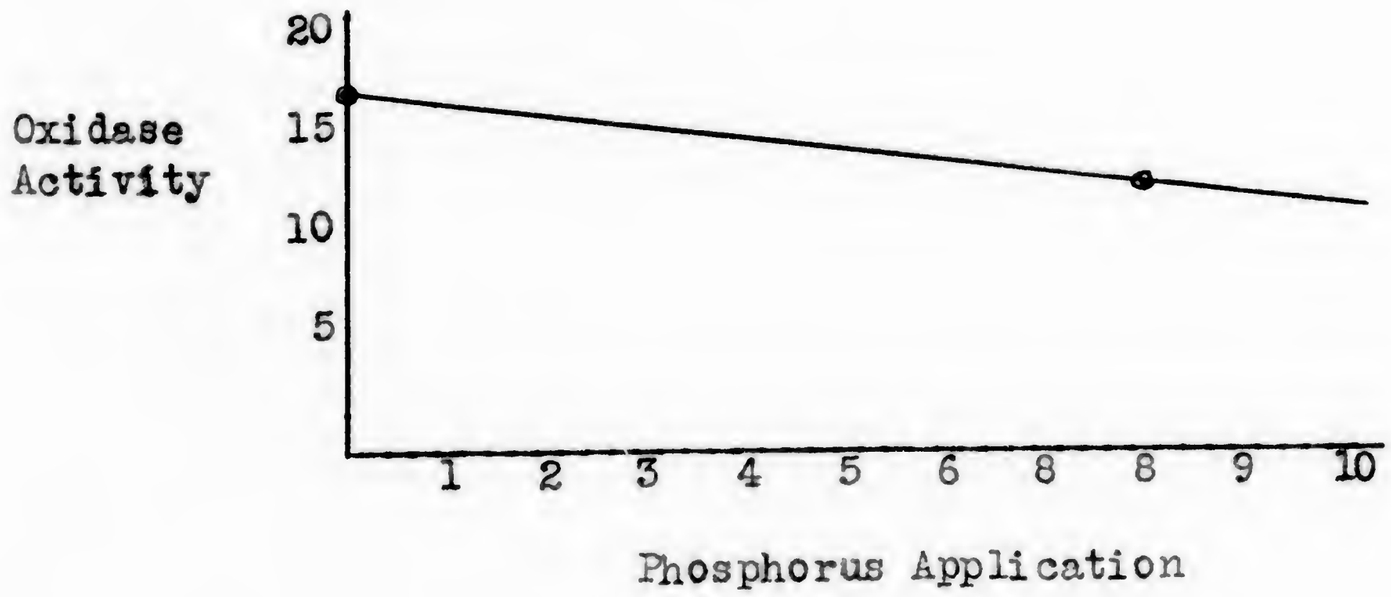

Table 9

Catalase Activity of Cured Tobacco as Altered by Varying the Potassium Content of the Fertilizer Application*

Plot	Treatment	Catalase**
		cc. O_2 Liberated
1	6-8-4	38.0
2	6-8-8	33.9
3	6-8-12	28.8
4	6-8-16	31.1

*The fertilizer application was 1000 lbs. broadcasted
**1 gram sample used at 15°C.

Figure 5

Catalase as Altered by Varying the Potassium

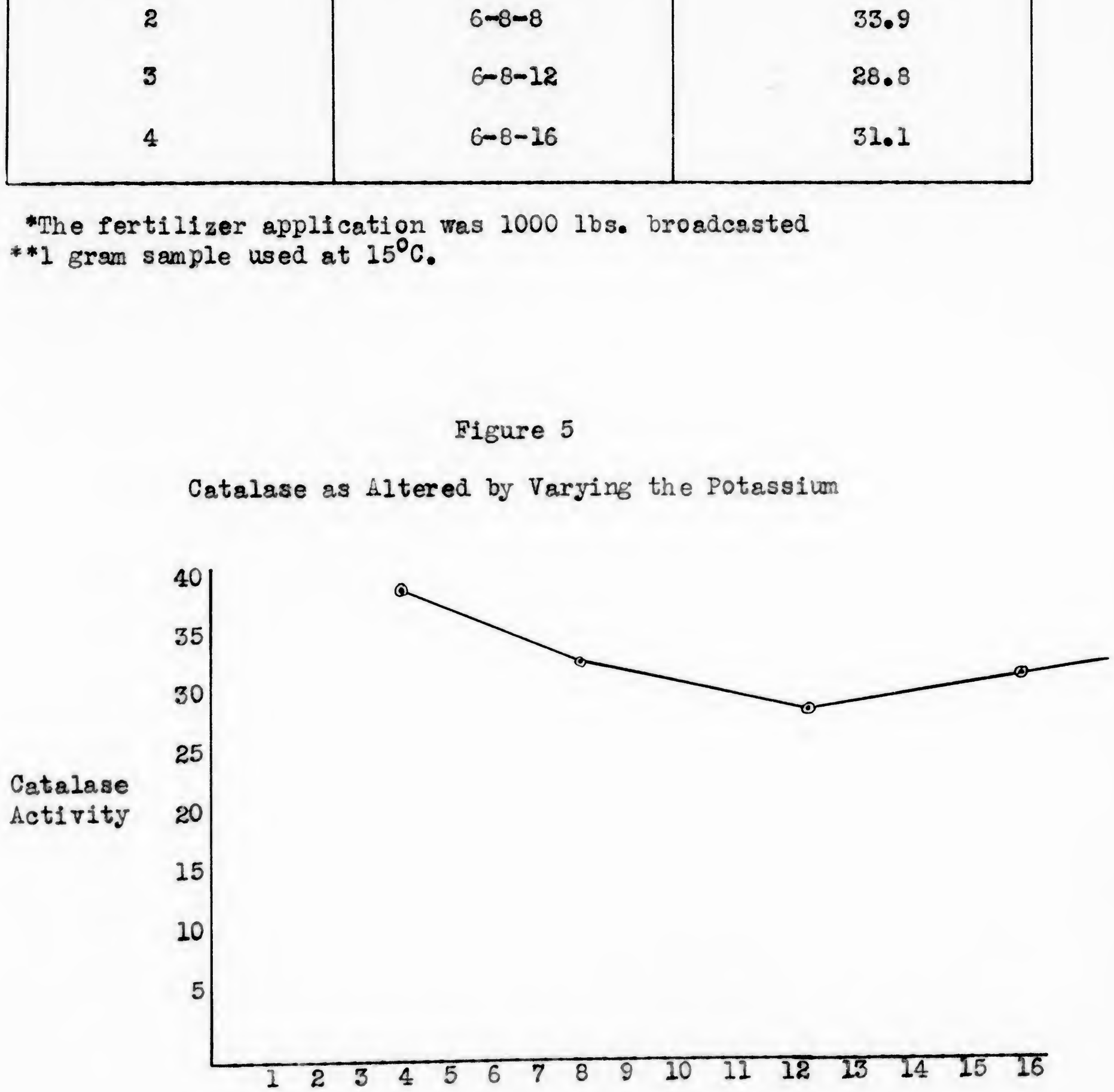

Table 10

Oxidase Activity of Cured Tobacco as Altered by Varying the Potassium Content of the Fertilizer Application*

Plot	Treatment	Oxidase**
		Iodine Value (cc. N/50)
1	6-8-4	24.8
2	6-8-8	25.5
3	6-8-12	13.8
4	6-8-16	23.9

*The fertilizer application was 1000 lbs. broadcasted
**1 gram sample used

Figure 6

Oxidase as Altered by Varying the Potassium

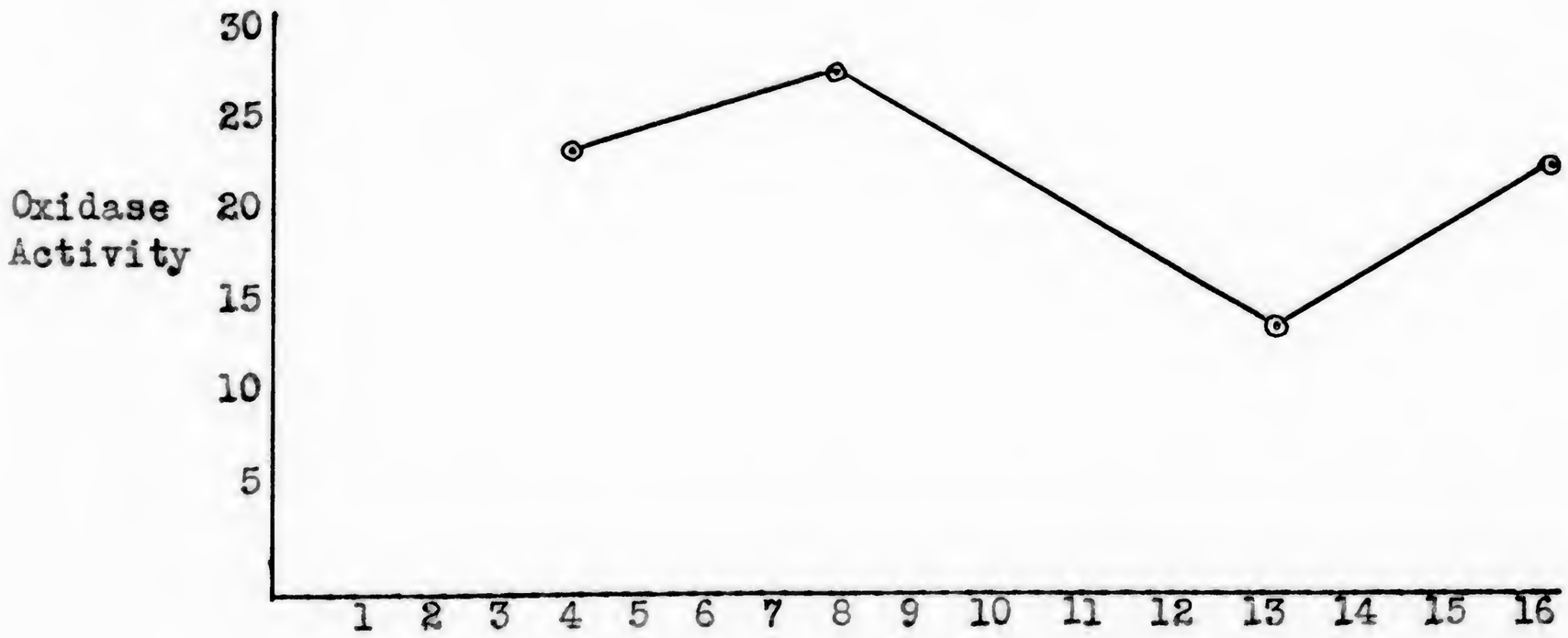

Table 11

Catalase Activity of Cured Tobacco as Altered by Varying the Nitrogen-Potassium Content of the Fertilizer Plus the Addition of Manure

Plot	Treatment	Application	Catalase*
			cc. O_2 Liberated
23	3-8-8	1000 lbs. plus manure	22.4
3	6-8-12	1000 lbs. broadcasted	28.8

*1 gram sample used at 15°C.

Table 12

Oxidase Activity of Cured Tobacco as Altered by Varying the Nitrogen-Potassium Content of the Fertilizer Plus the Addition of Manure

Plot	Treatment	Application	Oxidase*
			Iodine Value (cc. N/50)
23	3-8-8	1000 lbs. plus manure	40.1
3	6-8-12	1000 lbs. broadcasted	13.8

*1 gram sample used

Table 13

Catalase Activity of Cured Tobacco as Altered by Varying the Nitrogen Content of the Fertilizer as well as the Mode of Application

Plot	Treatment	Application	Catalase*
			cc. O_2 Liberated
18	8-8-12	1500 lbs. in row	34.2
3	6-8-12	1000 lbs. broadcasted	28.8
7	9-8-12	1000 lbs. broadcasted	24.8

*1 gram sample used at 15°C.

Table 14

Oxidase Activity of Cured Tobacco as Altered by Varying the Nitrogen Content of the Fertilizer as well as the Mode of Application

Plot	Treatment	Application	Oxidase*
			Iodine Value (cc. N/50)
18	8-8-12	1500 lbs. in row	37.9
3	6-8-12	1000 lbs. broadcasted	13.8
7	9-8-12	1000 lbs. broadcasted	13.5

*1 gram sample used

Table 15

Catalase Activity of Cured Tobacco as Altered by Using A Manure Application Only

Plot	Treatment	Application	Catalase*
			cc. O_2 Liberated
27	10 Tons of Manure	10 Tons of Manure	27.1
3	6-8-12	1000 lbs. broadcasted	28.8

*1 gram sample used at 15°C.

Table 16

Oxidase Activity of Cured Tobacco as Altered by Using A Manure Application Only

Plot	Treatment	Application	Oxidase*
			Iodine Value (cc. N/50)
27	10 Tons of Manure	Ten Tons of Manure	46.4
3	6-8-12	1000 lbs. broadcasted	13.8

*1 gram sample used

Table 17

Catalase Activity of Cured Tobacco as Altered by Using No Treatment at All

Plot	Treatment	Application	Catalase*
			cc. O_2 Liberated
19	No treatment	No treatment	41.2
3	6-8-12	1000 lbs. broadcasted	28.8

*1 gram sample used at 15°C.

Table 18

Oxidase Activity of Cured Tobacco as Altered by Using No Treatment at All

Plot	Treatment	Application	Oxidase*
			Iodine Value (cc. N/50)
19	No treatment	No treatment	36.7
3	6-8-12	1000 lbs.	13.8

*1 gram sample used

DISCUSSION OF RESULTS

Table 1 shows a great variation in both catalase and oxidase activity between the samples analyzed. In this discussion, the variation will be correlated with the N-P-K ratio of the fertilizer applied, as well as the amount and mode of application of the specific fertilizer.

Plot 3 was broadcast with a 6-8-12 fertilizer to the extent of 1000 lbs. This plot is used as a standard for the comparison of the other plots regardless of the treatment. It will be noted that both catalase and oxidase are low, especially the oxidase.

Using the standard 6-8-12 fertilizer but varying the applications, we find extreme changes in the catalase activity of the tobacco (Figure 1). Using 500 pounds of fertilizer applied in the row, the lowest catalase results. By broadcasting the same amount, the highest catalase is found. By raising the application to 1500 pounds, the catalase is raised just as if it were lowered 500 pounds.

The oxidase activity varies with the catalase with one exception. The lowest oxidase value is for the standard application of 1000 pounds. The 500 pounds in row treatment is again much lower than the 1500 and 500 pounds broadcast applications (Figure 2).

By raising the nitrogen from 0 to 3 units, the catalase activity is doubled. However, by raising the nitrogen more in this same proportion we find the catalase decreasing (Figure 1). The decrease from raising the nitrogen 3 to 6 units is much greater than the decrease resulting from an increase of nitrogen from 6 to 9 units. It

is seen that the catalase with 0-nitrogen and 9-nitrogen are both slightly lower than the standard 6-nitrogen, but the 3-nitrogen makes for an enormous increase in the activity.

Regarding oxidase activity, 0-nitrogen shows a great value. An increase to 3-8-12 depresses the activity nearly three times. The activity slowly drops (Figure 2) when increasing the nitrogen to 6 and 9 units respectively. The latter increase, however, is very slight showing the oxidase activity to be at a minimum. In fact the 9-8-12 formula showed the lowest oxidase activity recorded.

Phosphoric acid content was varied in only one plot, but the change from 6-0-12 to 6-8-12 should tell us in which way the oxidase and catalase should change, if at all.

The catalase activity shows a slight inverse variation (Figure 3) with the amount of phosphoric acid in the fertilizer applied to the plot. It is so slight, however, that we may assume phosphorus has little or no effect on catalase activity.

Oxidase, similarly to catalase, is changed very little by variations of phosphoric acid in the fertilizer. Just as catalase, it is very slightly lowered by raising the P in N-P-K from 0 to 8 (Figure 4).

Slight changes in the catalase activity of the tobacco are caused by changing the potash content of the fertilizer. An inverse variation is seen when the potash is varied from 6-8-4 to 6-8-12 (Figure 5). However, by raising the potash to 6-8-16, the catalase is proportionally raised. This indicates the lowest catalase is when the potash has a value of 12.

By altering the potash, the oxidase activity varies in an unorthodox way as shown by Figure 6. Very little change occurs on application of potash values of 4, 8, or 16. But on a 6-8-12 fertilizer, the oxidase shows a decided drop. Just as catalase, the 6-8-12 fertilizer has the lowest oxidase with the activity increasing when the potash is increased or decreased. However, by decreasing the value from 8 to 4 we find a corresponding oxidase decrease.

By using a 3-8-8 fertilizer, we are lowering the nitrogen and potassium proportionally from the standard 6-8-12 application. Both of these treatments should tend for higher catalase as was shown by Figures 1 and 5. But here the catalase is lowered(Figure 3). This lowering in catalase must be due to the addition of manure to the fertilizer treatment.

The lowering of both nitrogen and potash from 6-8-12 to 3-8-8 should make for higher oxidase, but the enormous increase, as shown by Figure 4, must be partly due to the addition of manure to the above treatment.

The 8-8-12 fertilizer should produce a lowered catalase from the 6-8-12 treatment, according to Figure 1. However, the 1500 pounds in row application is responsible for a higher catalase activity (Figure 5).

The enormous increase in oxidase caused by the 1500 pounds in row application can be seen in Figure 6. Just as in catalase, the 8-8-12 treatment should lower the oxidase theoretically. (Figure 2). This verifies the results shown in Figure 1, that 1500 pounds application greatly raises catalase and oxidase activity.

By substituting ten tons of manure for 1000 pounds of 6-8-12 fertilizer, scarcely any difference in catalase activity is noted (Figure 5). However, the value for the manure application is slightly lower. This verifies Figure 3, in that manure lowers catalase.

The great increase in oxidase caused by manure was shown in Figure 4. Figure 6 substantiates this, in that plot 27 had the highest oxidase activity recorded. This plot receiving no treatment at all showed both oxidase and catalase to have a high value.

By comparing Tables 1 and 2, we see little correlation between catalase and time of burn. However, as a general rule, it can be noted that the average catalase activity makes for the longest burning time, while low and high catalase tend to cause low values for the burn.

Regarding oxidase, with few exceptions, the longest burn goes hand in hand with the lowest oxidase, and vice versa.

The elasticity varies with the time of burn except in plots 5 and 8 where nitrogen and phosphorus were respectively omitted. In these plots, the burns were good but the elasticities were bad.

Comparing catalase with yield, it is seen that the mediocre catalase shows the higher yields. Higher or lower activities go along with the lower yields.

With several exceptions, a high yield of tobacco varies directly with a high oxidase.

SUMMARY AND CONCLUSIONS

1. The purpose of this investigation was to study the catalase and oxidase activities of Pennsylvania cigar-leaf tobacco as modified by different fertilizer treatment.

2. The manner of applying the same quantity and quality of fertilizer greatly affects the catalase and oxidase content. A 500 pounds in row application shows a much lower catalase and oxidase than a 500 pounds broadcast application does.

3. The amount of fertilizer applied also shows a great variation. Both catalase and oxidase are greatly increased when an application 1000 pounds of 6-8-12 fertilizer is either lowered or raised to the extent of 500 pounds.

4. By varying the nitrogen from 0-8-12 to 9-8-12, both catalase and oxidase are decreased with one exception, viz., the catalase is greatly raised when the nitrogen is increased from 0 to 3 units.

5. Increasing the phosphoric acid content from 6-0-12 to 6-8-12 finds both catalase and oxidase decreasing slightly.

6. Catalase decreases slightly from 6-8-4 to 6-8-12 increase in potash, but when it is increased to 6-8-16 the catalase raises proportionally.

Oxidase behaves in an unorthodox manner by modifying the potash. Increasing the potash from 6-8-4 to 6-8-8 causes a slight increase in oxidase activity. But a raise from 6-8-8 to 6-8-12 shows an enormous decrease, while a raise from 6-8-12 to 6-8-16 causes a

great increase again.

7. When manure is substituted for fertilizer, we find the catalase slightly lowered while the oxidase reaches its maximum value of this investigation.

8. The addition of manure to a 3-8-8 fertilizer causes the catalase to be decreased. Without the manure it would be increased. Oxidase is greatly increased by this addition of manure, although 3-8-8 should show a slight increase over 6-8-12.

9. By increasing the nitrogen from 6-8-12 to 9-8-12, both catalase and oxidase should be slightly lowered. But if 500 pounds additional fertilizer are used and it is applied directly in the row, we find both catalase and oxidase considerably increased.

10. No treatment at all on the plot produces tobacco with a high catalase and oxidase activity.

11. Several very general statements were discussed regarding burn, elasticity, and yield of tobacco as compared with its catalase and oxidase activities.

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End of Title