CATALASE ACTIVITY, GROWTH AND SPORE PRODUCTION OF SPHAEROPSIS MALORUM AS INFLUENCED BY TEMPERATURE

by

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INTRODUCTION

The Problem

The nature and function of catalase in both plant and animal tissues have been studied by previous investigators and interpreted in the light of experimental data of various kinds. Catalase activity has been used as a measure of relative metabolic activities and physiological conditions after the organisms employed have been cultured under known conditions and definite stages of development. The plants used for experimental purposes have ranged from anaerobic and aerobic bacteria to cereal crops and conifers.

Previous investigators have shown the presence of catalase in mycelial mats of fungi, and with other plant tissues they have shown that the changes in metabolism which accompany reproduction are attended by changes in catalase activity. This suggests to me that with changes in metabolism accompanying spore production of fungi there would also be changes in catalase activity.

The fungus chosen for experimental purposes was Sphaeropsis malorum Pk, a spore-producing strain isolated Oct. 30, 1915 from a rotting pear by A. J. Mix, and a non-sporulating strain, a mutant of a sporulating strain obtained from the laboratories of the Imperial College of Science and Technology, London, England. These two strains afforded an opportunity to study catalase activity in relation to sporulation.

The metabolic activity of plants increases with an increase in temperature up to a certain optimum and catalase activity might be expected to be similarly influenced. With these ideas in mind, it is the purpose of this paper to present data showing the effect of temperature upon the catalase activity, sporulation and growth of Sphaeropsis malorum.

Earlier Studies of Catalase and its Activity

The literature dealing with catalase is by no means consistent. Widely differing opinions have been offered as to its nature and function. Only brief mention is made here of some of the literature.

Lowe (21) (1901) suggests that catalase protects the cell against toxic concentrations of hydrogen peroxide. He concludes that there does not exist a group of organisms, an organ, a plant or animal cell that does not contain some catalase. Shaffer (39) (1905) believes

that catalase protects the cell against toxic concentrations of hydrogen peroxide if molecular oxygen is liberated in the process but he adds that if nascent oxygen is liberated its effect would be more harmful than hydorgen peroxide. He reaches the conclusion, however, from his experiment that molecular oxygen is liberated. Rosenberg (36) (1910) reports that catalase acts as an aerobic ferment. In her studies of catalase, she found that the autolysis of Lupinus in water destroys catalase. Palladin (31) (1910) believes catalase to be an anaerobic enzyme. Waentig (44) (1915) and Winkler (46) (1915) found catalase to be protein-like in nature. Von Euler and Borgenstam (42) (1920) conclude that no proportionality is to be taken for granted between catalase quantity and catalase activity, since simply a brief warming or drying, or a trace of poison can increase activity by 20 to 30 times, 300 to 600 per cent in the case of yeast cells. In their work with red blood corpuscles they found that by warming horse blood up to 57° activation was raised 170 per cent. Oxidase and catalase reactions, Reed (33) (1916a) believes, occur separately in the living cell and he also states in another paper (34) (1916b) that catalase is not universally present in living cells. Haber (13) (1928) also reached the

conclusion that oxidase and catalase activity are apparently independent of each other.

On the contrary Callow (6) (1923) found no traces of catalase in the nine anaerobes examined, but it was present in all the aerobes tested. When the anaerobes were exposed to the air they did not show traces of hydrogen peroxide nor could they live aerobically even when inoculated into broth containing catalase. Hagan (14) (1924) and McLeod and Gordon (22) (1923a) have tried growing anaerobes on heated blood agar in test tubes and the results obtained are regarded as indications that traces of hydrogen peroxide were given off.

Catalase activity has been found to vary with stages of growth. The catalase activity of grass seeds, Crocker and Harrington (7) (1918) found rises rapidly as their germination progresses. In connection with this study it was also found that a fall in catalase activity was accompanied by a fall in respiration. Sherman (40) (1921) noted an increase in catalase activity under after-ripening and germinating conditions of Crataegus, characteristic of seeds with dormant embryos. In Acersaccharum and Juniperus virgiana both catalase activity and respiration intensity increased as dormancy ended and germination began. In Crataegus catalase activity increased continuously up to the twelfth day in the germinator, but the increase was not uniform.

Respiration intensity increased up to the sixth day. In Amaranthus, the respiration as well as the catalase activity is maintained at a relatively unifrom rate for some time, but fluctuations in the one are not coincident with fluctuations in the other, and at times may be in an opposite direction. Eckerson (10) (1913) also noted an increase in the activity of catalase during the after-ripening period of Crataegus seeds. Working with wheat seed, Choate (5) (1921) detected the presence of catalase in all parts of the grain both before and during germination. The amount of catalase present increased during the first seven days at a rate corresponding to the rate of increase in the respiratory activity. Through his studies, Gracinin (12) (1926) found that in the case of Pisum sativum, Avina sativa, Hordeum vulgare, Zea mays, Pisum arvense and Sinapis alba the germination of the seed was attended by a rapid increase in the activity of catalase, the maximum activity being reached in five days. The increase began on the first day in all but Pisum sativum and Sinapis where the activity fell on the first day, after which the activity rapidly rose. The curves of catalase activity were practically the same in all cases.

The catalase was included in the embryo in seeds of Pisum sativum, Lupinus angustifoliua, Sinapis alba and Citrus nobilis. In Zea mays it was in both embryo and

endosperm. The testas of the seeds studied had an insignificant amount of catalase, from which fact the conclusion was drawn that catalase has a definite function in the catabolism of complex molecules during the germination of seeds and the development of the plant. It was found that in the case of the cotyledons, roots and stems during the germination of Pisum sativum and Pisum arvense catalase activity rose in the cotyledons up to the fifth day and then fell; in the roots catalase activity began at once on their production and kept on at a certain rate through a definite period; in stems the activity rose on the first day of germination with a more rapidly ascending curve than in the case of the roots, following pretty closely the curve of the cotyledons. Experiments with fully-developed plants of Lupinus angustifolina, Medicago sativa and Zea mays showed that an accumulation of catalase occurred principally in the stems, and the conclusion was drawn that catalase has a physiological function in those organs especially whose cells are most active physiologically.

Appleman (1) (1910) found that catalase activity of the potato decreased under the same conditions as did respiration. The catalase activity of different strains of ungerminated corn decreased when germination started according to Lantz (18) (1927), though it increased in

the later stages of germination to a maximum which was followed by a decrease as the reserve food of the grain was utilized. Respiration increased slowly in the early stages of germination and then more rapidly, while catalase activity decreased during the early stages of germination and then increased as germination progressed. Respiration increased rapidly with a temperature increase, catalase failing to show a corresponding increase. Some correlation between catalase activity and respiration was apparent during the germination of the different strains of corn at 20° but not at 10° or 30°. No warrant was found for concluding that catalase is the enzyme chiefly concerned in physiological oxidation. Schmieder (38) (1927) found that during the process of germination the catalase content of seeds passed through a grand period reaching a maximum and then declining rapidly. The course of catalase was said to be correlated with that of growth and was in relation to the other factors concerned in germination. Deleano (8) (1909), working with Pinus communis, obtained the same results for catalase activity during germination. He believed that the chief function of catalase was to saponify fats. Rhine (35) (1924) noted a sudden drop in the amount of catalase contained in the dry seed at the start of germination, with a subsequent rise, and interpreted this phenomenon to mean the sudden production

of hydrogen peroxide or some unknown substance which destroyed the catalase at first and then acted as a stimulant to its production. She also drew attention to the gradual decrease in catalase during the ripening of seed. Morinaga (27) (1925) germinated rice under both aerobic and anaerobic conditions. His conclusions were that in anaerobic germination of rice seeds catalase does not increase, but in a medium with a reduced amount of oxygen catalase increases slowly. Aerobically-grown seedlings with high catalase activity use much more oxygen than do anaerobically grown seedlings with low catalase activity. Catalase activity once increased by aerobic conditions decreases during anaerobiosis while the growth of the plant continues. Knott (16) (1927) noted a difference in the catalase content of leaves of spinach and celery. The younger and older leaves of spinach and celery are usually low in catalase activity, while the leaves of spinach and celery intermediate in age have a higher and approximately equal activity. In spinach the catalase content decreases in the tip of the stem on which the flowers are born in response to a longer daylight period.

With regard to the effect of heat and light upon catalase, Burge and Burge (4) (1924) found that a fall in temperature produced a decrease in catalase content

of Spirogyra, and a rise in temperature caused an increase in keeping with the fact that a fall in temperature decreases and a rise in temperature increases the respiratory metabolism of the plants. Increase in light also produced an increase in the catalase of Spirogyra less extensive, than that brought about by a rise in temperature, in keeping with the fact that increase in light is less effective than a rise in temperature in increasing the metabolism of plants. The conclusion which Morgulis (26) (1921) reached from his study of metabolism was that the amount of catalase is not a measure of metabolic activity. Crocker and Harrington (7) (1918) decreased the catalase activity of Johnson grass seed by a large percentage by heating to 81° C. and destroyed all catalase by heating to 100° C. for five hours. When untreated Johnson grass seeds were retained for one year in an incubator at 20° C. the catalase activity was greatly reduced, but the germination of Johnson grass seeds at 20° C. almost doubles catalase activity. Morgulis (25) (1929) claims that the velocity and extent of heat inactivation of catalase depends upon the hydrogen ion concentration of the enzyme solution. It was concluded that 65° was the critical temperature for the enzyme preparation since at this temperature catalase was instantly inactivated at all P_H values. Ultra-violet

rays were found to be the most effective portion of the spectrum in inactivating catalase by light. Inactivation by light was found to be greater in an alkaline than in either an acid or neutral medium. Von Euler and Laurin (43) (1919) reached the conclusion that standing in sunlight weakened the catalast activity of living cells. Batelli and Stern (2) (1910) believe catalase to be destroyed by light, no difference in the rate of destruction being evident whether it is in media containing oxygen or not. Taking the opposite view, Begeman (3) (1914) says that various lengths of light rays acted specifically upon catalase and gradually increased its amount. An increase in temperature increased the catalase content of seedlings experimented upon. The observations of Doyle and O'Connor (9) (1930) show that cold weather decreases and hot summer weather increases the catalase of pine needles. Overholzer (29) (1928), working with four varieties of pears, found that the effect of the storage temperature upon catalase activity varied with the duration of the storage period and the temperature at which they were stored.

Methods and Procedure

The Maintained Temperatures

For a low temperature an electric refrigerator was employed which maintains a temperature of 6-10° C. For medium temperature a 20° incubator was used. For the high a 30° incubator was employed. The last two showed variations of less than two degrees.

The cultures were grown in 150 c.c. Erlenmeyer flasks protected from light by black paper wrappers. Each flask contained 80 c.c. of sterilized nutrient solution.

The Nutrient Solution

Coon's culture solution was selected as the nutrient solution to be used in this experiment because it contains all the necessary elements for fungous growth and contains nothing which could combine with any of the other contents in the solution and form a colloid which might interfere with the results. Coon's culture solution contains the following ingredients:

$MgSO_4$----------1.23 gms.

KH_2PO_4----------2.72 gms.

KNO_3----------2.02 gms.

Maltose----------7.2 gms.

H_2O----------1,000 c.c.

The flasks containing the solution were sterilized in an arnold steam sterilizer.

The Fungus Used

Sphaeropsis malorum (a sporulating strain and a non-sporulating strain), was selected for this study because this fungus can grow at a rather high temperature and in either light or dark. The main reason for selecting this fungus was to compare the effect which sporulation has upon catalase activity. The sporulating strain will sporulate in either light or dark but the non-sporulating strain never produces spores under any known conditions.

The Experiment Periods

Both sporulating and non-sporulating strains were tested for catalase at time intervals of 14, 21 and 28 days. Cultures which were grown to check up on growth were also dried and weighed at the same time intervals.

Procedure for Catalase Tests

After the solution had been filtered off, the mycelium was superficially dried with filter paper. Five c.c. of hydrogen peroxide was used to test 0.25 gm. of mycelium. Sufficient calcium carbonate was used to neutralize the hydrogen peroxide and any acids which might have been present in the mycelium. The mixture was shaken continuously for 10 minutes in a water bath maintained at a temperature of 24° C. Every minute an accumulative reading was taken of the amount of oxygen

given off in the gas burette. The speed of the catalase apparatus was 180 complete excursions per minute.

Apparatus and Procedure For Measuring Growth

Gooch filters were prepared and dried for several days in an electric oven. The filters were carefully weighed after they had been allowed to cool to room temperature in dessicators. After the weighings had been made, the nutrient solution was filtered off through the Gooch filters with a suction pump and the mycelial mats placed in the electric oven to dry for several days. The filters plus the dry mycelial mats were carefully weighed. The differences in the weights of the filters gave the dry weights of the mycelial mats.

General Plan of the Experiment

It was planned to arrange the tests in such a way that the relation of catalase to sporulation, growth and temperature could all be tested at one time. Three flasks of the sporulating and three flasks of the non-sporulating strain were started to grow in the refrigerator, the same number in the 20° room and the same number in the electric incubator, at the same time. After 14 days one flask of the sporulating and one of the non-sporulating strain were removed from the refrigerator, as well as two from the 20° room and two from the electric incubator, and tested for catalase. This procedure was repeated at

21 and at 28 days. This experiment was repeated six times. This same plan was carried out when tests were made to check up on growth.

Results and Discussion

The averaged results of the tests for catalase are presented in tables 1, 11, and 111. The three main vertical sections of the tables correspond to the three periods used and the two horizontal sections represent the two strains of *Sphaeropsis malorum*. Each table represents a different temperature. Each group of values represents a single experiment preformed six times.

The highest values of either strain are for the cultures subjected to 30° C. on the 14th and 21st days (see table 1 and fig. 1 and 2).

At 30° C. the values of both strains decrease after 14 days (table 1 and figs. 1 and 2). At 20° C. the values of the sporulating strain decrease after 14 days (table 11 and fig. 1) but the values of the non-sporulating strain increase at 21 days and decrease at 28 days (table 11 and fig. 11). There is no decrease in the values of either strain at 6-10° C. (table 111 and figs. 1 and 2).

The values of the sporulating strain are slightly higher than the values of the non-sporulating strain at all temperatures except 20° C. At 20° C. the values

of the non-sporulating strain are slightly higher than those of the sporulating strain on the 21st day.

TABLE 1

Average Results for Six Separate Tests of the Catalase Activity of Sphaeropsis malorum Grown at 30° C.

	Temperature	14 days		21 days		28 days	
		Min.	c.c. of O_2 given off	Min.	c.c. of O_2 given off	Min.	c.c. of O_2 given off
Sporulating Strain	30°C.	1	4.33+	1	3.33	1	2.46+
		2	5.03+	2	4.68+	2	3.36+
		3	5.3	3	5.28+	3	3.6
		4	5.56+	4	5.41+	4	3.86+
		5	5.73+	5	5.05	5	3.9
		6	5.9	6	5.65	6	3.61+
		7	6.03+	7	5.71+	7	3.93+
		8	6.13+	8	5.73+	8	3.93+
		9	6.2	9	5.75	9	3.95+
		10	6.26+	10	5.75	10	3.96+
Non-sporulating Strain	30°C.	1	1.93+	1	2.8	1	1.3
		2	2.73+	2	3.75	2	2.35
		3	3.8	3	4.55	3	2.85
		4	4.26+	4	4.95	4	3.22+
		5	4.8	5	5.1	5	3.52+
		6	5	6	5.25	6	3.65
		7	5.13+	7	5.35	7	3.7
		8	5.2	8	5.35	8	3.75
		9	5.33	9	5.35	9	3.9
		10	5.4	10	5.35	10	3.95

TABLE 11

Average Results for Six Separate Tests of the Catalase Activity of *Sphaeropsis malorum* Grown at 20° C.

	Temperature	14 days		21 days		28 days	
		Min	c.c. of O_2 given off	Min	c.c. of O_2 given off	Min	c.c. of O_2 given off
Sporulating Strain	20°C.	1	1.41+	1	1.33 +	1	1.3 +
		2	2.41+	2	2.26 +	2	2.1 +
		3	2.96+	3	2.9	3	2.8 +
		4	3.43	4	3.41 +	4	3.17+
		5	3.9	5	3.71+	5	3.5
		6	4.23	6	4.03+	6	3.56+
		7	4.38	7	4.23+	7	4.17+
		8	4.56+	8	4.4	8	4.4
		9	4.80+	9	4.52	9	4.5
		10	4.96+	10	4.66 +	10	4.65+
Nonsporulating Strain	20°C.	1	.86+	1	1.83+	1	1.11+
		2	1.01+	2	2.73+	2	1.9
		3	2	3	3.29+	3	2.58+
		4	2.46+	4	3.76 +	4	3.04+
		5	2.79+	5	4	5	3.35
		6	3.05+	6	4.16+	6	3.71+
		7	3.28+	7	4.33+	7	3.90+
		8	3.43+	8	4.46+	8	4.08+
		9	3.61+	9	4.58+	9	4.2
		10	3.76+	10	4.73+	10	4.26+

TABLE 111

Average Results for Six Separate Tests of the Catalase Activity of Sphaeropsis malorum Grown at 6-10° C.

	Temperature	14 days		21 days		28 days	
		Min	c.c. of O_2 given off	Min	c.c. of O_2 given off	Min	c.c. of O_2 given off
Sporulating Strain	6-10°C.	1	.06+	1	.79+	1	.56+
		2	.14+	2	1.2	2	1.03+
		3	.16+	3	1.38+	3	1.29+
		4	.19+	4	1.53+	4	1.6
		5	.21+	5	1.63+	5	1.76+
		6	.24+	6	1.7	6	1.93+
		7	.26+	7	1.73+	7	1.96+
		8	.28+	8	1.83+	8	2.04+
		9	.28+	9	1.93+	9	2.09+
		10	.28+	10	1.99+	10	2.19+
Non-sporulating Strain	6-10°C.	1	.4	1	.36+	1	.2
		2	.46+	2	.58	2	.48+
		3	.46+	3	.8	3	.69+
		4	.46+	4	.86+	4	.93+
		5	.46+	5	1	5	1.15
		6	.46+	6	1.1	6	1.38+
		7	.46+	7	1.15+	7	1.48+
		8	.46+	8	1.21+	8	1.59+
		9	.46+	9	1.25+	9	1.69+
		10	.46+	10	1.31+	10	1.76+

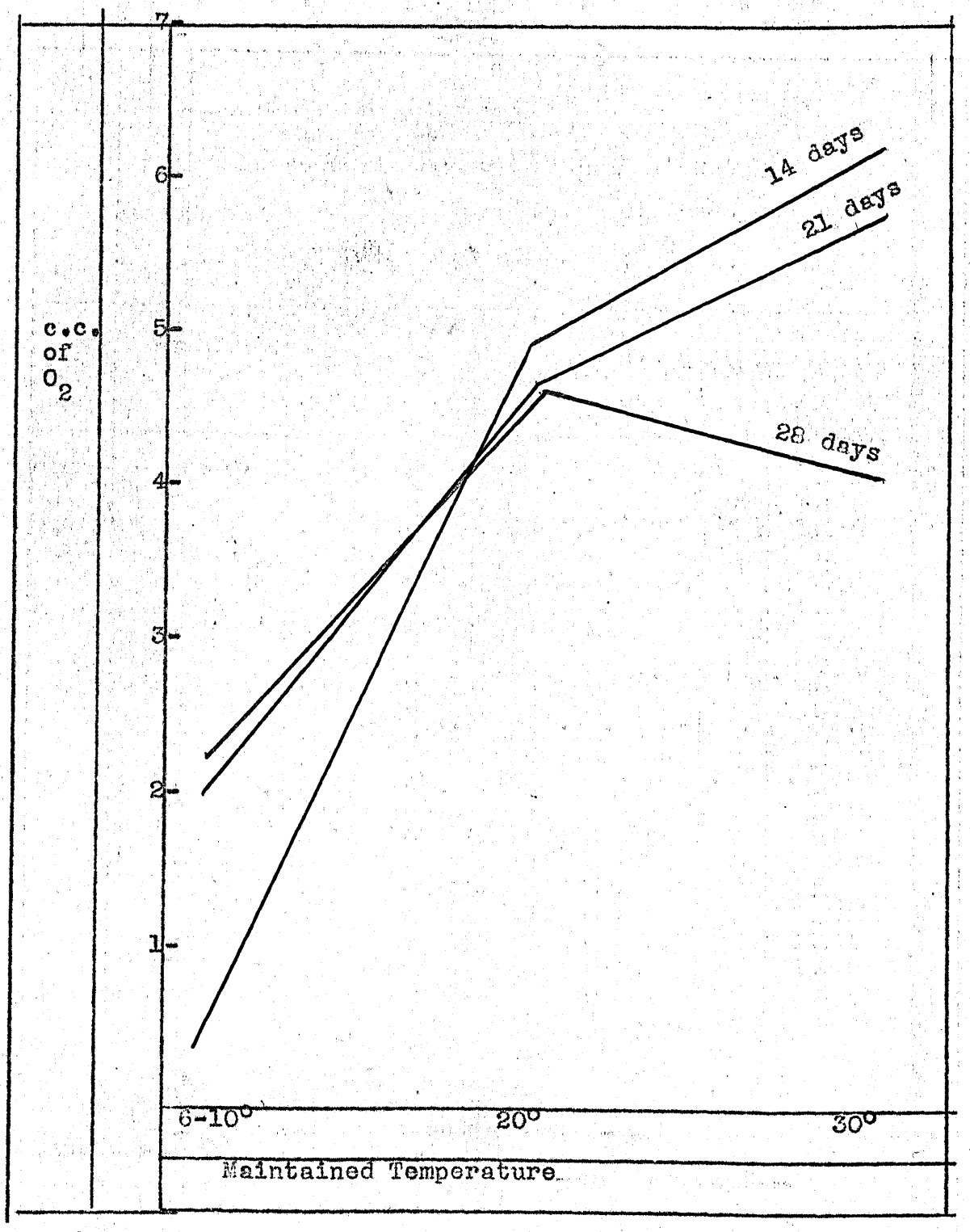

FIGURE 1. Representative temperature graphs of catalase activity of Sphaeropsis malorum (sporulating strain), grouped according to periods of growth.

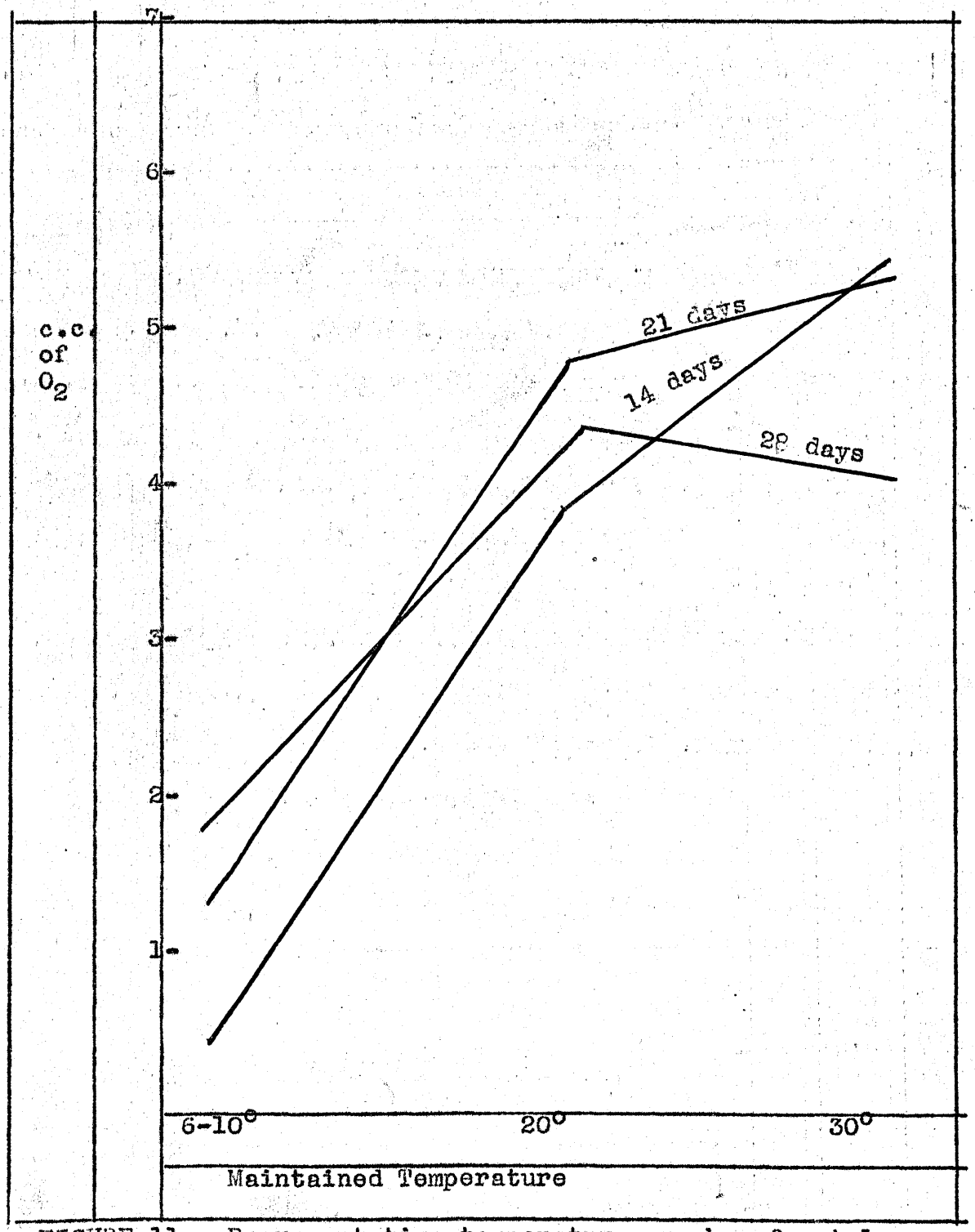

FIGURE 11. Representative temperature graphs of catalase activity of Sphaeropsis malorum (non-sporulating strain), grouped according to periods of growth.

The results of tests for growth are presented in table IV. The three main vertical sections of the table correspond to the three temperatures used and the two horizontal sections represent the two strains of Sphaeropsis malorum. The dry weights of the mycelium are given in the three subdivisions of each main vertical section. The dry weights of the mycelial mats of the sporulating strain are except at 20° C. higher than those of the non-sporulating strain. The weights of both strains are lowest at 6-10° C., highest at 20° C. and decrease at 30° C.

TABLE IV

	6-10°C.			20°C.			30°C.		
	14days	21days	28days	14days	21days	28days	14days	21days	28days
Sporulating Strain	.0030	.0087	.0258	.1235	.1207	.1097	.1025	.0876	.0826
Non-sporulating Strain	.0056	.0060	.0107	.1173	.1413	.1303	.0923	.0879	.0826

Summary and Conclusions

The results of an experimental study of the catalase activity of Sphaeropsis malorum (a sporulating and a non-sporulating strain) are herein reported. In each catalase test the fungus was transferred to a culture flask containing 80 c.c. of sterilized Coon's culture solution. Three temperatures (6-10°, 20° and 30°) were employed for growth periods of 14, 21, and 28 days. At the end of each growth period the mycelial mats were tested for catalase. The amount of oxygen given off was used as the measure of catalase activity.

The results of growth measurement are also presented. The dry weights of the mycelial mats were used as measures of growth. The conditions for growth study were the same as for the study of catalase activity.

The growth of both strains of Sphaeropsis malorum is greatest at 20° C. At 6-10° C. the dry weights of the mycelial mats of both strains increase after 14 and 21 days. The dry weight of the mycelial mats of the sporulating strain decrease after 14 and 21 days at 20° and 30° C. The weights of the non-sporulating strain also show a decrease after 14 and 21 days at 30° C. but at 20°C. there is an increase at 21 days followed by a decrease at 28 days. The decrease in weights is due to autolysis. Autolysis of the sporulating strain at

20° C. begins before the 21st day while that of the non-sporulating strain does not begin until after 21 days. The catalase activity of both strains is lessened by autolysis as shown by the fact that the catalase values decrease as the dry weight of the mycelial mats decrease. While the vegetative growth is not so great at 30° C. as at 20° C., the fungus sporulates better at 30° C. Evidently there is an increase of catalase activity accompanying sporulation, since the catalase activity of the sporulating strain is greatest at the temperature most favorable to sporulation and the catalase activity of the sporulating strain is also higher than that of the non-sporulating strain at all temperatures.

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