PHYSIOLOGICAL STUDIES ON GLOEOSPORIUM MUSARUM COOK. ET MASS., THE CAUSAL ORGANISM OF BANANA ANTHRACNOSE

1. Changes in the carbohydrate composition of banana pulp with reference to the adaptive secretion of amylase

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Introduction

As have already been pointed out by many investigators that an extended development of the typical symptoms of banana anthracnoce is usually delayed until when banana fruits reach the full ripen stage. This so-called dormant infection may have an important bearing on the hydrolysis of pulp starch, since this process is an important factor involved in fruit maturity. The fact that the organism is able to assimilate the sugars which are brought about by simultaneous hydrolysis of pulp starch indicates that a study on the changes in the carbohydrate composition of the anthracnose-affected banana pulp is of immense importance.

It is not exaggerated to say that a list of studies describing such changes in plant tissues caused by plant pathogenic fungi is long and cumulative. It is, however, regretful to say that this area of study, particularly of the anthracnose-affected banana fruits, has not yet been thoroughly made, so far as the writer is aware.

In attempting to throw more light on the solution of this problom, the present experiments were undertaken. In addition, special emphasis was laid on the question as to whether the fungus in question could secrete amylase *in vivo*, which, according to the pioneer work made by Kervegent (1935), was not significant.

Before going further, the writer wishes to acknowledge his indebtedness to Prof. Matsumoto, under whom this work was performed, for valuable criticism as well as kindly help in the preparation of this paper. Thanks are also due to JCRR for the appropriation of the subsidy and to Dr. H. W. Li of Academia Sinica for his good offices in the publication of this paper.

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Materials and Methods

Each hand, 3/4 ripe, was removed from a bunch by means of a flamed knife and the cut end was covered with vaseline. The calyxes were also removed and the fruit surface was wiped several times with 95% alcohol. Three fingers were then placed in a large Petri dish under aseptic conditions. Three square holes were then made in each fruit skin. Both sides of the holes were inoculated with spores and mycelia obtained from a pure culture of *Gloeosporium musarum* (strain A of our laboratory) and middle one was left as a control.

After a given length of time, the fruits were longitudinally divided into two parts and sampling was made by selecting the sound and diseased portions derived from the corresponding parts of fruit of the same hand.

For moisture measurement, about twenty grams of samples were collected in glass-stoppered weighing bottles. They were immediately dried at 80–85°C. for one hour to inhibit the enzyme action, then the temperature was lowered to about or below 60°C. to carry out further desiccation until a constant weight is secured. The difference in moisture content between the fresh and dry matter is expressed in percentage. The dried samples were finely powdered and stocked in wide-neck bottles for the determination of carbohydrates. In this connection, it is worthy to refer to Loesecke's paper (1950), in which a destruction of invertase was pointed out to be absolutely necessary prior to analysis.

For carbohydrate determination, the total carbohydrate including the reducing sugars and most of the acid hydrolyzable carbohydrates with the exception of the true cellulose was measured. In the total carbohydrate analysis, hydrolysis of the powdered samples was accomplished by heating the mixture of the samples and appropriate amounts of 2.5 per cont HCl at the temperature of 100°C for 3 hours. The insoluble residue was then filtered off. The hydrolyzed solution was neutralized, cleared with basic lead acetate, and deleaded with 70 per cent sulphuric acid. The reducing power of the cleared filtrate was then determined with Fehling's solution and the residual copper was determined by Bertrand's method excepting that the centrifugarization was applied to collect the precipitates of Cu_2O instead of filtration as was stated in the original paper.

For the free sugar determination, the weighed samples were covered with a sufficient amount of redistilled alcohol, to which sufficient precipitated calcium carbonate (1 gram per a liter) was added to neutralize the acidity and heated close to b.p. in a water bath for 80 minutes after which time they were left to stand for a few hours with frequent agitation, and restore to volume with 80 per cent alcohol. A desired amount of this alcoholic solution was neutralized and distilled in vacuum. The reducing power of the aqueous solution which

was cleared, and deleaded as done in the foregoing procedure was determined in the same manner as already mentioned. The results were expressed in the percentage of glucose.

The hydrolysis of total sugar was effected by the presence of 0.1 per cent HCl when heated at 100°C. for 30 minutes. The result was expressed as percentage of invert sugar. Further details of methods will appear in the appropriate ensuing sections.

Experimental Results of Analysis

1. Changes of moisture

The results obtained from the determination of moisture content are given in Table 1. In general, the moisture content of the diseased portion is greater than that of the normal one, though the difference between them are not so remarkable. In this connection, it is noteworthy to mention that the outermost surface of the matorials to be dried may undergo dextrinization, preventing further removal of moisture from the innermost portions. Therefore, it is suggested that somewhat modified procedures must be followed up in the course of determining the moisture contents of starchy materials.

Table 1. Showing the moisture and dry matter contents of the pulp of anthracnose-infected banana fruits in comparison with those of normal ones

Sample No.	Percentage moisture		Percentage dry matter		Ratio of moisture between the dis'd and healthy (%)
	Healthy	Diseased	Healthy	Diseased	
1	83.46	84.97	16.54	15.03	102
2	82.57	85.86	17.43	14.14	104
3	82.95	85.87	17.05	14.13	103
4	82.95	85.56	17.05	14.44	103
5	86.04	87.84	13.96	12.16	102
6	85.31	87.31	14.69	12.69	102
7	85.89	87.77	14.11	12.23	102
8	85.74	87.64	14.26	12.36	102

Remarks: Readings were made after 10-day incubation at 28°C.

2. Changes of carbohydrates

As is shown in Table 2, reducing sugar, total sugar and total carbohydrate of the diseased pulp tissues are appreciably lower than those of normal portion derived from the same hand. It was also found that the invert sugar which was computated from the differences between the total sugar and free reducing sugar was greatly reduced in amounts by the fungus. As will be clear from

the data given in Table 3, dissacharides are rapidly attacked by the fungus, while monosaccharides are much slowly reduced.

Determination of starch were not followed in this experiment, because both the diseased and healthy pulp tissues revealed no demonstrable amounts of starch, if any, by the histochemical method at the termination of incubation period. This result is comparable to Barnell's work (Loesecke 1950), in which it was experimentally verified that the percentage of starch in the healthy Gros Michel pulp decreased below 0.5% at the full ripen stage. Barnell, however, suggested that a distributional pattern of the starch grains in the pulp tissues might be heterogenous and that the starch might be more quickly lost from the outward. If this is to be the case, a trace amount of starch so far reported may be deeply seated in the innermost portions, to which the fungus never be able to gain access during the course of infection. Under such circumstances, it is hardly possible to differentiate whether a dissolution of the starch is brought about by the host amylase or by in vivo secretion of the fungus amylase.

Table 2. Showing the soluble reducing sugar, total sugar and total carbohydrate contents of anthracnose-infected banana pulp tissues in comparison with those of normal ones
(Based on dry matter)

Exp. No.	Reducing sugar calculated as glucose %		Total sugar calculated as invert sugar %		Invert sugar calculated from the difference %		Total carbohydrate calculated as invert sugar%		Ratio of total carbohydrate Dis'd: Healthy (%)
	Sound	Diseased	Sound	Diseased	Sound	Diseased	Sound	Diseased	
1 Max.	18.07	14.28	22.31	15.44	4.02	1.10	28.31	16.16	57
Min.	11.94	6.67	16.20	6.84	4.04	0.16	18.91	10.63	56
Ave.	14.38	9.22	18.56	9.95	3.97	0.69	24.46	14.31	58
2 Max.	11.10	5.56	13.52	5.80	2.99	0.22	19.80	12.89	65
Min.	5.29	2.12	7.55	2.11	2.14	—	9.59	9.59	100
Ave.	7.57	3.61	8.77	3.68	1.14	0.06	14.69	11.24	76

Remarks: Triplicated determinations were averaged.

3. *Paper chromatographic studies on the changes of soluble carbohydrates in the anthracnose-affected pulp*

In parallel with the quantitative studies, it was intended in this experiment to demonstrate clearly the qualitative changes of soluble sugar occurring in the diseased pulp of banana. The matured fruits, about 3/4 ripe, were inoculated in the similar manner as already mentioned. After 6-day incubation at 28°C, both the healthy and diseased pulp tissues were taken from the same hand. Each of the collected pulp tissues was then separately blended in a Waring blendor with about 50 times (W/V) of 5% ethanol. Each tissue suspension

thus obtained was placed in a separate Erlenmeyer flask (250 ml) stoppered with a glass cock, and the vessels were kept stored in a refrigerator for about 24 hours with frequent agitation.

These tissue suspensions were subsequently filtered, and the filtrate was concentrated by vacuum distillation (30 mm Hg; 55°C) until about 3 ml. of syrupy solution were obtained. These concentrated samples were stored in a refrigerator until when spottings were made on Toyo No. 50 filter paper (30× 30 cm; 9 spots). An ascending method and butanol-acetic acid-water (4:1:1) as solvent were used in the study. Development of sugar spots was attempted by spraying aniline-phthalic acid in water-saturated butanol on the air-dried filter paper followed by heating at 105°C for 5 minutes.

In view of the fact that Rf-values of different sugars obtained under different experimental conditions may fluctuate to a certain extent, a 1% aqueous solution of sucrose, glucose, fructose, lactose and raffinose was separately spotted on a test paper for the purpose of identification. The resultant data are briefly given in Table 3 and Figs. 5 and 6.

Table 3. Paper chromatographic analysis of the soluble carbohydrate fractions both in the diseased and healthy banana pulps

Materials and conc.	Amount spotted	Rf-values and color densities of different spots (*)			
		No. 1	No. 2	No. 3	No. 4
Lactose 1%	10 μl	0.08 ++ (0.09)**			
Sucrose 1%	10 μl		0.13 ++ (0.14)**		
Glucose 1%	10 μl			0.19 +++ (0.18)**	
Fructose 1%	10 μl				0.23 ++ (0.23)**
Diseased pulp 3.6 gm/3.0 ml	5 μl	0.075 ±	0.12 +	0.18 +	0.24 ++
	10 μl	0.075 ±	0.12 +	0.18 ++	0.23 ++
Healthy pulp 2.0 gm/2.0ml	5 μl	0.075 ±	0.12 ++	0.18 ++	0.23 +~++
	10 μl	0.075 ±	0.12 ++	0.17 ++~+++	0.22 ++

Footnote: (*) Average of 5 determinations.
(**) Rf-values given in parentheses were quoted from Lederer et al "Chromatography" Elsevier Co., 1954.

It is inferrable from the above experimental data that at least four different soluble sugars are present both in the diseased and healthy pulp tissues, so far as the methods and materials studied are concerned. Since the Rf-values of four sugar spots show fitness with those of standard ones, it is pertinent to say that these sugar spots may correspond with those of lactose, sucrose, glucose and fructose in an ascending order named (see Figs. 5 and 6).

From the color densities of these sugar spots it can be said that sucrose and glucose fractions of the healthy pulps are appreciably higher in concentration than those of the comparable diseased ones. While, no significant changes in the content of fructose and lactose were noted between the diseased and healthy tissues of comparable ones. These results are practically in harmony with those of the macrochemical analysis shown in Table 2.

Poland and his associate (Loesecke 1950) found that sucrose, glucose and fructose comprised the major part of soluble sugars in the ripe Gros Michel. Maltose was also claimed to be present in very small amounts. While, mannose, galactose, sorbose, mannoheptose, raffinose, melibitose and rhamnose were not identified in the same materials. It is interesting to note that lactose may be present, though very low in concentration as is judged from the color density observed, in pulps of the native variety so far studied.

4. *Acidity and carbohydrate changes in the banana decoction inoculated with* ***Gloeosporium musarum***

In consideration of the fact that no demonstrable amounts of starch can be seen both in the healthy and diseased pulp tissues at the time of analysis, it remains still open to question whether the diastatic enzyme of the causal organism is able to attack the starch grains of banana pulp *in vivo*.

To provide more complete information on this line the following experiments are undertaken. Two hundred grams of 3/4 matured banana pulp were extracted in 1000 ml of distilled water for one hour at 100°C, and after filtration 50 ml of the decoction were distributed to each 200 ml Erlenmeyer flask. The media are then autoclved at 15 lbs for 15 minutes. They were then inoculated by a few loopfuls of spore-suspension and the cultures were kept at 28°C. At desired intervals, three flasks were removed from the incubator and acidity, reducing sugar and total sugar determinations were made in the same way as mentioned before. Presence of starch was demonstrated with IKI solution.

The triplicated results are shown in Table 4 accompanied with two figures (Figs. 1 and 2).

It is apparent from the resultant data that the reaction of banana decoction inoculated with the fungus in question was shifted to the alkaline side during the course of the fungus growth. It was also confirmed that the total sugar content in the inoculated media exhibited a gradual decrease in concentration. At the termination of these experiments about 40 or more per cent of the total sugar originally present in the decoction were exhausted.

It is, however, interesting to note that the reducing sugar, chiefly consisting of glucose and fructose as is judged from paper chromatographic analysis, are gradually increased and the highest quantity was attained about 7 days after incubation accompanied with the gradual disappearance of starch reaction.

After 14-day incubation at 28°C, the amounts of reducing sugars were more or less same as those of uninoculated ones. It can be said that diastatic enzymes of the causal fungus are capable of acting upon the starch grains in the decoction prepared.

Table 4. Showing the changes of acidity and carbohydrates in the banana-pulp decoction inoculated with *Gloeosporium musarum* at 28°C.

Incubation Period (day)	(***) Growth of fungus	Acidity		(*) Reducing sugar as glucose mg/10 ml	(*) Total sugar as invert sugar mg/10 ml	Starch	(**) Index of total sugar (%)
		pH	N/10 NaOH per 10 ml filtrate (ml)				
Opening day	−	4.5	1.08	127.90	261.50	+++	100
2-day	+	5.7	0.30	122.70	257.80	++	99
5-day	++	7.3	0.10	148.10	201.80	+	77
7-day	+++	7.3	0.12 ****	157.60	188.70	+	72
10-day	+++	7.7	0.06 ****	134.30	150.30	+	58
14-day	+++	8.1	−	131.10	145.20	±	56

Footnote: (*) Average of three determinations.
(**) Index number of total sugar is based upon the calculation in which that of the opening day is expressed as 100.
(***) − No growth; + Poor mycelial mats formed; ++ Aerial mycelia abundant; +++ Sporulation predominant.
(****) Determined by N/100 NaOH.

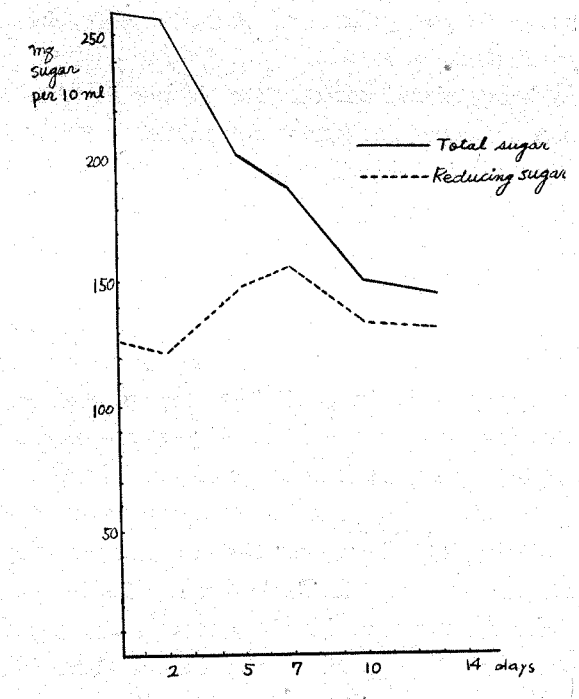

Fig. 1. Changes of total sugar and of reducing sugar in the matured pulp decoction inoculated with *Gloeosporium musarum* at 28°C.

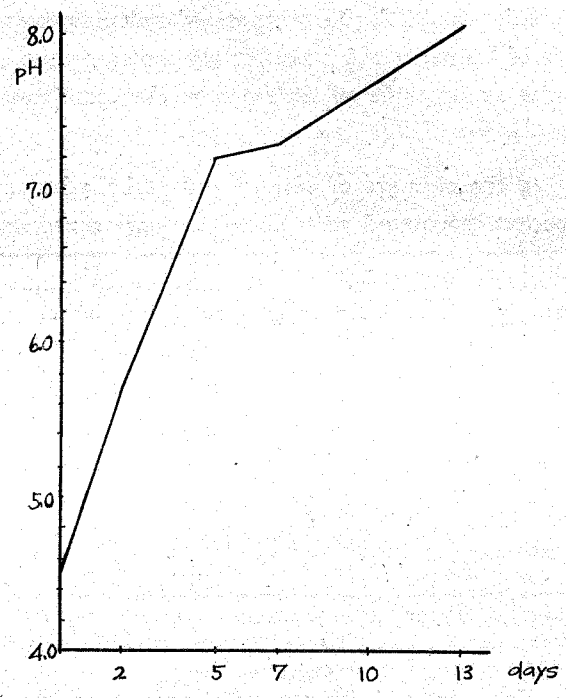

Fig. 2. Changes of pH in the matured pulp decoction inoculated with *Gloeosporium musarum* at 28°C.

5. *Changes occurring in the green and matured pulp decoctions inoculated with Gloeosporium musarum*

As has already been pointed out that the fungus is able to infect green, immature fruits, but this may not extent any further within the tissues until they become matured. It is inferred that this so-called dormant infection may have some bearing on the hydrolysis of pulp starch, since this process is an important factor involved in fruit maturity.

In order to elucidate whether starch grains obtained from the green immature banana pulp can be attacked by the diastatic enzyme of the fungus the following experiments were made. A decoction was made from 200 grams of green immature banana pulp in a similar way as was mentioned earlier. In parallel with this decoction some other fingers originated from the same bunch were kept at 31°C for 1 week in order to hasten the maturing process, then a decoction was made from them in the same manner as stated above. Determinations of acidity, reducing sugar and total sugar are same as before. The triplicated data are presented in Table 5 with two figures (Figs. 3 and 4).

It is obvious from the result so far obtained that the decoction of mature pulp is more favorable to the growth of the fungus than that of green immature pulp. As is clear from Table 5, neither invert sugar nor reducing sugar was demonstrated in the decoction made from the immature pulp even

after ixtended heating. Under such circumstances, the fungus could initiate the vegetative growth and brought about a slight accumulation of the readily-assimilable sugars in the decoction at the expense of starch grains after 5-day incubation. After then the limited amounts of sugars are becoming progressively less and practically no trace of sugar can be found after 14-day incuba-

Table 5. Showing the growth of the fungus and chemical changes occurring in the green and mature pulp decoctions inoculated with *Gloeosporium musarum*

Age of culture	Growth of fungus		Sporulation		Acidity				(*) Reducing sugar as glucose mg/10 ml		(*) Total sugar as invert sugar mg per 10 ml		Starch Reaction	
					pH		Titratable							
	Gr'n	Ma'd	Gr'n	Ma'd	Gr'n	Ma'd	Gr'n	Ma'd	Gr'n	Ma'd	Gr'n	Ma'd	Gr'n	Ma'd
Opening day	—	—	—	—	5.8	4.6	0.64	1.22	—	167.3	—	246.5	+	+
5-day	+	++	++	+	7.2	5.6	—	0.39	15.58	177.5	32.5	197.6	+	+
8-day	++	+++	+++	+	8.1	6.0	0.20 (HCl)	0.24	trace	139.3	trace	159.0	+	+
14-day	mg 59.73 (**)	mg 248.7 (**)	+++	++	8.2	7.0	0.23 (HCl)	—	—	105.0	—	121.0	—	—

Footnote: (*) Average of three determinations.
(**) Average weight of three cultures.

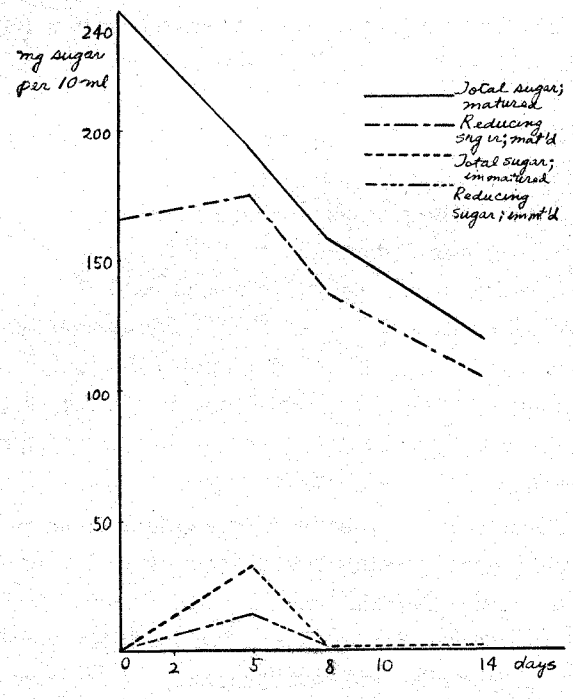

Fig. 3. Changes of different sugars in the immature and mature pulp decoctions inoculated with *Gloeosporium musarum* at 28°C.

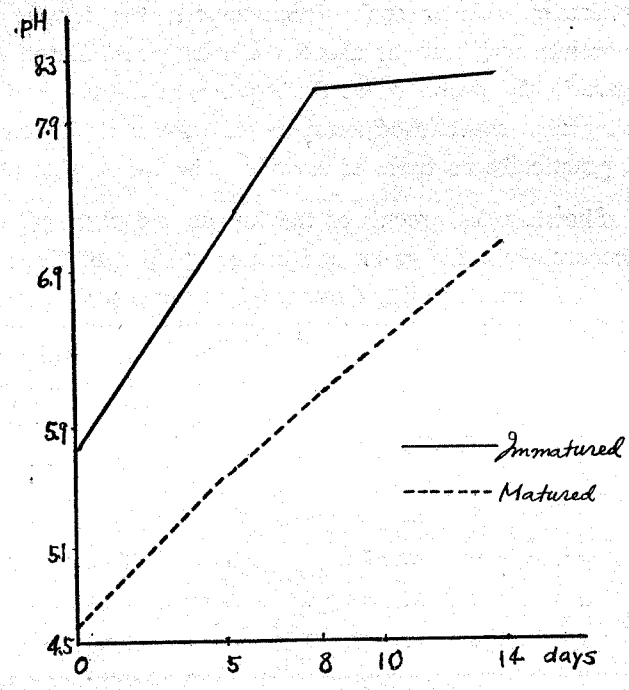

Fig. 4. Changes of pH occurring in the immature and mature pulp decoctions inoculated with *Gloeosporium musarum* at 28°C.

tion at 28°C. It is intersting to note that the starch grains found in decoction of both immature and mature pulps disappeared equally at the termination of this experiment.

It is reasonale to assume that the exogenous diastatic enzyme secreted by the fungus *in vitro* is capable of attacking the starch grains derived from the immature banana pulp. These experimental evidence is comparable to that of Liu's (1951), in which it was demonstrated that the fungus could use soluble starch as a carbon source in the synthetic media used.

It was also demonstrated that pH of the non-inoculated immature fruit decoction (5.8) was higher than that of the comparable mature one (4.6), both being shifted to more alkaline side after 14-day incubation at 28°C. It will be interesting to quote here that the pH of the pulp varies from 5.02 to 5.6 in green bananas, to from 4.20 to 4.75 in the ripe one (10).

6. Adaptive production of amylase by ***Gloeosporium musarum***

In the foregoing experiments, it was demonstrated that the fungus was apparently able to secrete diastatic enzyme when banana decoction was used as a substratum. In general, the species of fungi exhibit more or less marked differences both qualitatively and quantitatively in respect to secretion of enzymes uunder different conditions. To provide such information it is intended in this experiment to make a brief study on the effect of certain environmen-

tal factors, especially pH and temperature, on the adaptive secretion of amylase by the fungus, and, at the same time, its activity was compared with that of other fungus pathogens, viz., *Ceratostomella paradoxa* and *Botryodiplodia theobromae*, the causal organisms of the fruit-rots of banana.

Twenty grams of well-washed polished rice grains were placed in 250 ml Erlenmeyer flasks and were autoclaved in a routine way. In this experiment no attempt was made to use spore suspension as the inocula because our stock culture of *Botryodiplodia theobromae* failed to produce spores on the culture media. As an inoculum the mycelial mat grown on PDA for 7 days at 28°C was cut out with a biscuit cutter and transferred to the substrata.

Ten days after inoculation, they are removed from an incubator and were extracted with 100 ml of phosphate buffer (pH 7.17) to which 0.5 ml of toluol was added as an antiseptic. The extraction was effected under the ordinary room-temperature conditions for 24 hours with frequent agitation. After a given length of time, the fluid was filtered through Seitz filter and the filtrates obtained were separately kept in a sterile flask to which 0.5 ml of toluol was added as an antiseptic.

The substrata used in this experiment are formulated as follows:

(1) 10 ml of 3% soluble starch+10 ml of buffer solution+2.5 ml of enzyme extract+0.4 ml of toluol

(2) For control, 10 ml of 3% soluble starch+5 ml of N/10 NaOH+5 ml of distilled water+2.5 ml of enzyme extract+0.4 ml toluol

Twenty hours after incubation at a given temperature, 10 ml of 10% NaOH were added to the flasks to stop the enzyme action and the substrata were then diluted to proper containers with distilled water. Ten ml were required for the determination of reducing sugar as did in the former case. McIlvaine's citrate-phosphate buffer solution was used throughout this experiment.

Experimental Results

(a) *Optimum hydrogen ion concentration*

The triplicated data pertaining to the amylase activities at different pH values are briefly tabulated in Table 6.

Table 6. Showing the relation of diastatic activities of different organisms to the pH of the reaction-mixture

pH-value	$KMnO_4$ (ml)*		
	Gloeosporium musarum	*Ceratostomella paradoxa*	*Botryodiplodia theobromae*
4.0	20.50	34.10	31.50
4.6	22.00	33.00	31.50
5.0	23.50	32.00	32.00
5.6	21.50	32.00	30.00
6.0	21.50	30.00	29.00
Control	9.00	21.00	9.00

Reading was made after 20-hour incubation at 20°C.
(*) Average of three determinations.

From the result it is tentatively concluded that the amylase of *Gloeosporium musarum* and that of *Botryodiplodia theobromae* is most active at pH 5.0 or thereabout, while more acid reaction is favorable for the amylase activity of *Ceratostomella paradoxa.*

(b) *Comparison of the diastatic activities of the above-named three organisms*

This experiment is made in attempting to compare the amylase activity of the three organisms named above at different tomperatures. For comparison, influence of pH must be taken into consideration. Therefore, pH 5.0 was temporarily selected for this purpose. The triplicated data so far obtained are presented in Table 7.

Table 7. Showing the quantitative study of the amylase activity of three fungi at different temperatures (28 and 37°C.)

Names of orgamisms	$KMnO_4$ ml (*)				
	Total Reducing Sugar		Control	Reducing sugar actually converted	
	28°C	37°C.		28°C.	37°C.
Gloeosporium musarum	23.50	30.70	9.00	14.50	21.70
Ceratostomella paradoxa	31.50	40.00	22.00	9.50	18.00
Botryodiplodia theobromae	31.50	40.00	9.00	22.50	31.50

(*) Average of three determinations. Readings were made after 20-hour incubation at a given temperature.

As is shown in Table 7, the amylase of *Botryodiplodia theobromae* is the strongest among the three fungi studied, and was followed by *Gloeosporium musarum* and *Ceratostomella paradoxa* in a descending order named, so far as the conditions studied are concerned.

Discussion

The present experiment clearly indicated that *Gloeosporium musarum* was capable of secreting amylase in the decoction made from either green immature or mature banana pulps. It remains, however, insignificant whether the enzyme in question is able to attack the starch grains *in vivo*, inasmuch as the total absence of starch grains was noted on the outer layers of both authracnose-affected and healthy pulp tissues at the termination of incubation period. As has already been pointed out before, a list of experiments which report the failure in demonstrating *in vivo* secretion of amylase in plant tissues is long and cumulative. However, some of the representative examples are worthy to be reviewed here.

According to Hawkins (1916), *Fusarium oxysporum, F. radicicola,* and *F. coerukeum* were unable to attack the starch of potato tubers, showing no action

upon it even after one week, although extracts of these fungi rapidly digested soluble starch. In the meantime, mention must be made on the amylase of *Phytophthora infestans*. Lepik (1929) found that in the latter stages of the disease a superficial corrosion and gradual dissolution of the starch could be detected under the microscope. Whether such dissolution was caused by the fungus in question or by a possible existence of some other secondary putrefying organisms was not mentioned.

While, Sukhorukoff et al (1935), in reply to Lepik's paper, emphasized that no amylase could be demonstrated in the fungus mycelia under aseptic conditions and that the fungus consistently failed to grow in media containing starch as the source of carbon. They further stated that substances capable of inhibiting amylase activity (sistoamylase) were contained in the potato varieties apparently resistant to the late blight. In this connection, Sakai (1957) commented that starch was poor source of carbon for the mycelial growth of the fungus, but the growth in the synthetic media added with dextrin was quite comparable to that of maltose. In his subsequent study on the physiological properties of amylase, he experimentally demonstrated that alpha-amylase comprised the majority of diastatic enzyme, though beta-amylase might be present, if any, in an exceedingly low concentration.

In *Gloeosporium musarum*, an extensive cultural work was done by Liu (1951). He found that soluble starch was less favorable for the growth of the fungus than that of di- or monosaccharides. In this connection, depolimerization of the starch subjected to heat sterilization must not be overlooked. In his further experiment (1954), the fungus was demonstrated to be capable of inducing localized lesions on the green immature fruits when dense masses of conidia were used as inocula. In consideration of the fact that these localized lesions do not extend any further until the fruits become ripen, it is pertinent to say that the causal fungus, presumably in a mycelial form, may not be able to attack the adjoining starch grains by the reasons which are discussed below.

First of all, it seems likely that amylase activity of conidia and of mycelia may act differently on the starch grains *in situ*. Whether such is found to be the case in the fungus under consideration must be thoroughly investigated in the near future.

Recently, Tomiyama and his associate (1959) have conducted an important experiment on the mechanism of resistance of potatoes to *Phytophthora infestans*. They found that in both resistant and susceptible tissue penetration was similar. However, a great difference rests in the fact that the metabolic activity of cells surrounding the invaded cell is much greater in resistant than in susceptible tissue and in the reactive resistant tissues phenol becomes more

prominent followed by rapid necrosis. Whether such peculiar metabolic activity may be noted in the tissues surrounding the localized lesions in the green immature banana fruits is still open to question, so far as the writer is aware.

The next point to be considered is the role of antiamylase substances, such as "sistoamylase" mentioned by Sukhorukoff et al (1938) in plant tissues. A survey of the literature indicated that indole nucleus containing plant hormones were effective anti-amylases when added to refined starch in concentration as low as 0.01 per cent (Volker 1950). Eyster (1950), however, was of opinion that *in vitro* auxin retardation of diastase was solely due to pH effect, while he presumed that *in vivo* inhibition might occur in nature. Anyhow, it still remains obscure whether these plant hormones are actually contained in banana fruits to such an extent that they exhibit a marked *in vivo* inhibition of the amylase.

In this connection, mention must be made on the earlier works done by many investigators on the study of banana amylase. The foregoing studies (see Loesecke 1950) indicated that some workers successfully demonstrated amylase, while other failed. As to this contradictory evidence, Loesecke (1950) referred to Barnell and Barnell's work which postulated that banana diastase was found to be either precipitated or inactivated in the presence of tannin. As a matter of fact, Sastri and Row (Loesecke 1950), based on their histochemical works, suggested that ripening of the fruit was controlled by the presence or disappearance of tannins as early as 1934. Liu (1954) stated that the contents of tannins remained constant during the course of ripening process. Harris and Poland (see Loesecke 1950) pointed out that free tannin decreased as the fruit ripen because the tannins are slowly bound in insoluble, supposedly inert, "vegetable tannate".

If it is true, it must be scrutinized whether the so-called free tannin found in the immature banana fruit tissues are capable of exhibiting *in vivo* inhibition of the diastatic enzymes produced by *Gloeosporium musarum*, inasmuch as the enzymes of different nature may differ qualitatively or quantitatively in the reaction to the external agents. It is hoped that this area of study will be explored in the near future.

Summary

1. Chemical analysis of the artificially-inoculated banana pulps exhibited an appreciable decrease in the amounts of reducing sugar, total sugar and total carbohydrate when comparison was made to the healthy tissues derived from the same hand. Moisture contents were also slightly increased in the diseased pulp than in the healthy ones. Starch was not found, if any, by the histochemical test in the outer layers of both the inoculated and healthy pulps.

2. Paper chromatographic analysis of soluble sugars in both anthracnose-affected and normal pulp tissues was also attempted. Besides sucrose, glucose and fructose already reported, lactose was also found in very small amounts in the experimental materials studied. From the color densities of these sugar spots, it was observed that both glucose and sucrose of the diseased pulps were markedly lowered in concentration, while no marked changes were seen in the amounts of fructose and lactose during the course of infection.

3. Changes of acidity, reducing sugar and total sugar occurring in the banana pulp decoction inoculated with *Gloeosporium musarum* were also studied. pH of both matured and immatured pulp decoctions was markedly shifted to alkaline side by the fungus after 14-day incubation at 28°C.

There was ample evidence that the concentration of total sugar was progressively lowered, while reducing sugar was somewhat increased after 5- to 7-day incubation, thereafter a slow decrease in concentration was demonstrated in the decoctions studied. Starch was completely disappeared in the decoctions after 14-day incubation at 28°C.

A decoction of the non-inoculated immature pulp contained neither reducing sugar nor invert sugar after prolonged heating in an autoclave. Growth of the fungus on the immature pulp decoction was less favorabe than that of the mature pulp.

4. *In vitro* production of the exogenous amylase (saccharifying) was found in *Gloeosporium musarum*. The optimum pH for the amylase activity was found to be in the vicinity of pH 5.0. Attempts were also made to compare the diastatic activity of the fungus with that of ***Ceratostomella paradoxa*** and ***Botryodiplodia theobromae.***

香蕉炭疽病病原菌之生理學的研究

(1) 病原菌之糖化酵素之適應性的分泌與香蕉果肉組織內碳類成分的變化

王　明　樟

1. 遭受香蕉炭疽病病原菌 (*Gloeosporium musarum*) 爲害之果肉組織 (Pulp tissues), 其全炭水化物 (Total carbohydrate)，非還元糖 (Invert sugar) 以及還元糖 (Reducing Sugar) 之含量皆顯著地減少。反之，被害組織之水分則稍見增加。接種後約經十天，在成熟果肉組織之上層部，不拘健全與罹病者，用組織化學法 (Histochemical Methods) 檢查後，發現皆已無澱粉顆粒存在。Barnell 等曾報告 Gros Michel 品種之健全果肉在成熟後期尙留有少量澱粉 (約 0.5%)，此澱粉顆粒似指存在於果肉之內部者。因而此病原菌是否在被害組織內可分泌糖化酵素尙無法提出有力證據。

2. 本試驗所用香蕉品種，其果肉組織所含之可溶性糖類 (Soluble sugars) 由 Paper Chromatography 法作定性分析後發現共有四種，即爲葡萄糖 (Glucose)，果糖 (Fructose)，蔗糖 (Sucrose) 與乳糖 (Lactose) 等。遭受該病原菌爲害後，葡萄糖與蔗糖的含量顯然減少。然果糖及乳糖之含量，則採自同一果指上之健病兩果肉組織間，並無顯著差異。

3. 爲了要究明該病原菌是否在被害組織外可分泌外生糖化酵素 (*in vitro* production of exogenous amylase)，將未成熟及已成熟之果肉作成煎汁 (Decoction)，而以此病原菌作人工培養。經人工培養後，知非還元糖，即蔗糖，其濃渡在第 14 天約減少一半。然還元糖在接種後約七天時稍見增加，嗣後乃漸告減少。成熟果肉煎汁所含之澱粉在 14 天內完全消失。用未成熟果肉組織所作成之煎汁，未接種前完全無可溶性糖類。然接種後第五天，由於外生糖化酵素之作用而產生少量之蔗糖及還元糖。在第八天所有可溶性糖類僅殘留微量，至第十三天澱粉反應亦消失。由此可知，此炭疽病病原菌在人工作成之煎汁內確可分泌糖化酵素。

不問香蕉果肉已成熟或未成熟，由該項果肉所作之人工煎汁，其 pH 經人工接種後，皆變成爲弱鹼性 (Alkaline side)。然未成熟者其 pH 較由同一果房所採之成熟果肉者更稍偏於鹼性。

4. 炭疽病病原菌之外生糖化酵素其作用的最適的 pH 約爲 pH 5.0。如以 pH 5.0 作爲糖化酵素比較之基準點時，其作用較 *Botryodiplodia theobromae* 爲稍低，然較 *Ceratostomella paradoxa* 爲稍高。(摘要)。

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Explanation of Plate

Fig. 5: Paper chromatographic analysis on the soluble sugars of the anthracnose-affected and of healthy pulps of banana fruits: 1. Glucose (10 μl), 2. Fructose (10 μl), 3. Sucrose (10 μl), 4. Diseased pulp (5 μl), 5. Diseased pulp (10 μl), 6. Sound pulp (5 μl), 7. Sound pulp (10 μl), 8. Sucrose (10 μl), 9. Glucose (10 μl).

Fig. 6: An aniline-phthalic acid-developed paper to serve to show the identity of the lowest sugar spot. 1. Lactose (10 μl), 2. Raffinose (10 μl), 3. Diseased pulp (5 μl), 4. Diseased pulp (15 μl), 5. Lactose (20 μl), 6. Raffinose (20 μl), 7. Diseased pulp (5 μl), 8. Diseased pulp (10 μl), 9. Sucrose (10 μl).

Fig. 7: The healthy and artificially-inoculated fruits incubated at 28°C for 7-days, showing negative starch reaction of the pulp tissues.

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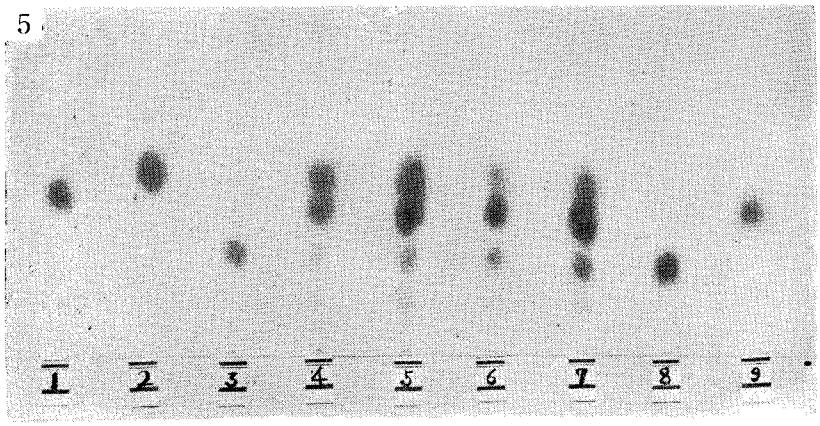

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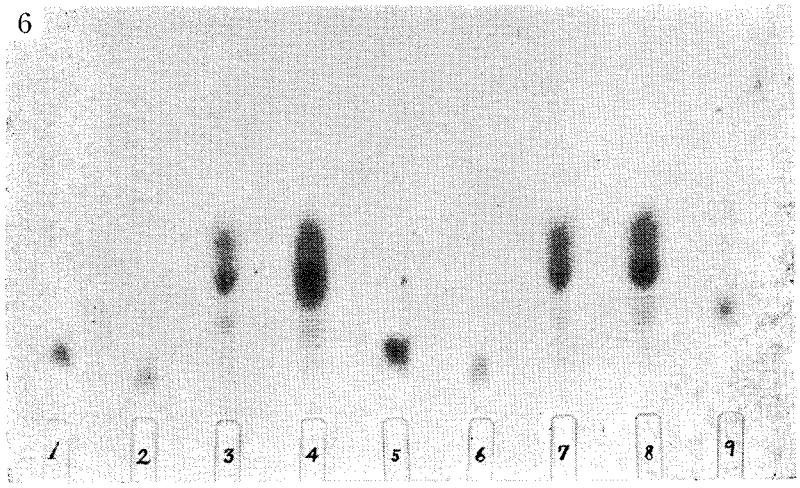

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