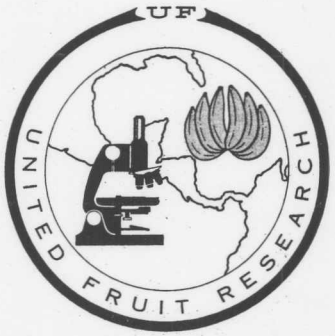
UNITED FRUIT COMPANY

DEPARTMENT OF RESEARCH

ANNUAL REPORT

1958



VOLUME II

DOMESTIC AND BASIC RESEARCH

ANNUAL REPORT

1958

UNITED FRUIT COMPANY

DEPARTMENT OF RESEARCH

VOLUME II

DOMESTIC RESEARCH

CENTRAL RESEARCH LABORATORIES
NEW YORK LABORATORY
TRANSPORTATION AND GENERAL RESEARCH - NEW YORK
UNIVERSITY AND EXPERIMENT STATION GRANTS
BANANA BREEDING RESEARCH

Norwood, Mass.

January 15, 1959

Dr. Jesse E. Hobson
Vice President and Director of Research
United Fruit Company
Boston, Massachusetts

Dear Dr. Hobson:

I am pleased to submit our 1958 Annual Project Reports from Central Research Laboratories.

As you know, 1958 was a year of organization and orientation at the new Laboratories which we have occupied only since last April. Our research efforts during this period have been directed entirely towards the Fusarium Wilt problem. Substantial progress has been made in initiating our basic approach to the control of this disease.

Indicative of the scope of our approach are the following areas in which work has started: The types of materials within the plant that may be responsible for resistance or susceptibility to disease; the microorganisms found around roots as influenced or determined by conditions known to affect the progress of disease; physiological characteristics of reproduction in Fusarium; study of methods for freeing seed of pests. Of particular practical implication are certain experiments which indicate that a rapid pathogenicity test can be developed by using small plants from button seed.

Sincerely yours,

G. R. Mandels, Director
Central Research Laboratories

CENTRAL RESEARCH LABORATORIES

ANNUAL PROJECT REPORTS

1958

Comparative Biochemistry of Banana Varieties in Relation to Resistance to Panama Disease

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Observations from Attempts to Lyophilize Spores of Fusarium oxysporum f. cubense

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Annual Project Reports - Cont'd.

Gros Michel as a Host in Quick Pathogenicity Test for *Fusarium oxysporum* f. *cubense*

Inoculation Methods and Host Development in Relation to Infection and Rate of Symptom Development

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Growth of the Banana Plant as Affected by Environmental Factors

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BPM-1-10
BDP-3-30

Plant Physiology Section
Annual Project Report 1958

Comparative Biochemistry of Banana Varieties in Relation to Resistance to Panama Disease

Background

The inherent ability of certain varieties of banana plants to resist infection and parasitism by Fusarium oxysporum cubense to a greater degree than other varieties suggests a distinction which may be detected by physiological, morphological or biochemical means. This distinction, if it exists, may be studied by a comparative survey of biochemical constituents in various organs of banana varieties. It may also be studied by metabolic reactions of the susceptible and resistant varieties in response to altered environmental or nutritonal conditions and in the presence or absence of the parasite. For a comparative biochemical study, the polyphenolic substances and the tannins of the banana plant organs and particularly the roots are being investigated. The polyphenolic substances have been implicated in the resistance of plant tissues to microbial attack as may be noted by a few of the number of published studies shown below[1]. Similarly, the tannins which contain polyphenolic constituents, have also been related to plant resistance[2]. The tannins are not believed to be particularly toxic to microorganisms. It is believed that the effect of tannins is due to their ability to insolubilize protein and to inactivate the extra-cellular enzymes of the parasite.

Progress

A. Polyphenolic substances

Roots in alcohol and rhizomes from different banana varieties growing in Honduras were collected with the efforts of the Division of Tropical Research and by Dr. W. G. Barker in particular and shipped to Norwood. The rhizomes were prepared for growth in solution culture and grown first on tap water and then on full nutrient solution. The roots were extracted with alcohol and then concentrated to a small volume. Small quantities of these extracts were then analyzed by paper chromatography for polyphenolic substances. Table 1 shows a number of substances which have been detected in the roots of these banana varieties.

1 Link et.al., J.B.C. 81,369-75 (1929); Rubin et.al. Biokhim. 12, 141-52 (1947); Schaal and Johnson, Phytopath. 45,626-28 (1955); Kuc et.al, J.Am.Chem.Soc.78,3123-25 (1956); Kirkham, J.Gen.Microbiol.17,491-504 (1957).

2 Rubin et.al,Dok.Akad.Nauk.79,303-6(1951);Nienstadt,Phytopath. 43,32-8(1953).

The greatest proportion of these substances are as yet unidentified and probably are not all polyphenolic in nature. These substances have been detected by their position in the two solvent systems used, by their reactions to various reagents, and their appearance in visible and ultraviolet light. Based upon this information a tentative identification has been made of some of these substances and fairly positive identification for one of them, chlorogenic acid. Table 2 lists the various substances which have been so identified. Of particular interest are the substances designated No. 12 and 19. No. 12 which is tentatively believed to be and may be phloroglucinol carboxylic acid (a phloroglucinol type substance) has been found in all varieites of the banana plant resistant to Panama disease, whereas in the varieties Cocos and Gros Michel it could not be detected. This does not mean that substance 12 may not be present in the suceptible varieties but that if it is present then it is so low that it can not be detected by the technique concentrations used. Substance No. 19, chlorogenic acid, has only been found in the variety Musa balbisiana and was the major polyphenolic constituent detected. It is also interesting to note that Musa balbisiana was the only seeded variety analyzed. Whether there is a relationship between the presence of seeds and chlorogenic acid remains to be determined. Some general observations may be made regarding the rather large number of constituents which have been detected. This is based upon the chromatographic behavior of the substances[3]. First of all, it is possible that some of the substances may be glycosidic in nature. This is based on the fact that the Rf position of many of these substances in the aqueous acetic acid solvent is quite high, and that this solvent has the property of moving glycosides and leaving the aglycones near the origin. Furthermore, when these extracts are acid hydrolyzed to break the glycosidic linkages, then fewer of these substances can be detected on the chromatograms. Another generalization which might be made is that due to their high Rf position in the butanol-acetic acid-water solvent, it might be expected that most of these substances have less than 4 hydroxyl groups in their ring structures since additional substitution of hydroxyl groups tends to decrease the movement of the substances in this particular solvent. At the present time and with the instrumentation available, efforts are being made to further characterize the various substances found by paper chromatograph. Particular attention is being given to

[3] Bate-Smith and Westall, Biochem.Biophys.Acta 4, 427-40 (1950).

TABLE 1 (cont'd)

Substances detected in Ethanol Extracts of Banana Roots by 2-Directional Chromatograms

Without NH_3		With NH_3		Ammonia-cal $AgNO_3$	$FeCl_3$ $K_3Fe(CN)_6$	Diazotized P-nitro Aniline	Varieties									
I	UV[2]	VL	UV				Cocos	Grand Nain	Gros Michel	Congo	Variety 67	Lacatan	Vimama	Musa Balbisiana	Tumoc	Giant Fig
	Bl	C	Bl	-	-	-	XXX	-	-	-	-	-	-	-	-	-
	Bl	C	Bl	-	-	-	X	X	X	-	X	XX	X	-	XX	X
	C	C	Bl	-		-	-	-	-	-	-	-	-	X		-
	Bl	Y	Bl	-	Bl	-	-	-	-	-	-	-	-	XXX	-	-
3	pBl	pB	pBl	BGr	Bl	Y	XX	X	X	X	X	X	X	X	XX	X
	Bl	C	Bl	-	-	-	X	X	XX	XX	XX	XX	X	X	X	X
	Bl	Y	G	Gr	Bl	-	-	-	-	-	-	-	-	XXXX	-	-
1	C	Pi	C	-	-	-	-	-	XX	-	-	-	-	-	-	-
	Bl	C	Bl	-	-	-	X	XX	X	-	XX	XX	XX	-	XX	-
	Bl	C	Bl	-	Bl	-	X	-	-	-	-	-	-	X	-	-
	Bl	C	Bl	-	-	-	-	-	-	X	-	-	X	-	-	X

TABLE 1 (cont'd)

Substances detected in Ethanol Extracts of Banana Roots by 2-Directional Chromatograms

Without NH_3		With NH_3		Ammonia-cal $AgNO_3$	$FeCl_3$ $K_3Fe(CN)_6$	Diazotized P-nitro Aniline	Varieties									
	UV	VL	UV				Cocos	Grand Nain	Gros Michel	Congo	Variety 67	Lacatan	Vimama	Musa Balbisiana	Tumoc	Giant Fig
	Bl	C	Bl	-	-	-	XXX	-	-	-	-	-	-	-	-	-
	Bl	C	Bl	-	-	-	X	X	X	-	X	XX	X	-	XX	X
	C	C	Bl	-		-	-	-	-	-	-	-	-	X		-
	Bl	Y	Bl	-	Bl	-	-	-	-	-	-	-	-	XXX	-	-
	pBl	pB	pBl	BGr	Bl	Y	XX	X	X	X	X	X	X	X	XX	X
	Bl	C	Bl	-	-	-	X	X	XX	XX	XX	XX	X	X	X	X
	Bl	Y	G	Gr	Bl	-	-	-	-	-	-	-	-	XXXX	-	-
	C	Pi	C	-	-	-	-	-	XX	-	-	-	-	-	-	-
	Bl	C	Bl	-	-	-	X	XX	X	-	XX	XX	XX	-	XX	-
	Bl	C	Bl	-	Bl	-	X	-	-	-	-	-	-	X	-	-
	Bl	C	Bl	-	-	-	-	-	-	X	-	-	X	-	-	X

TABLE 1 (cont'd)

Substances detected in Ethanol Extracts of Banana Roots by 2-Directional Chromatograms

Without NH_3		With NH_3		Ammonia-cal $AgNO_3$	$FeCl_3$ $K_3Fe(CN)_6$	Diazotized P-nitro Aniline	Varieties									
I	UV[2]	VL	UV				Cocos	Grand Nain	Gros Michel	Congo	Variety 67	Lacatan	Vimama	Musa Balbisiana	Tumoc	Giant Fig
	Bl	C	Bl	-	-	-	XxX	-	-	-	-	-	-	-	-	-
	Bl	C	Bl	-	-	-	X	X	X	-	X	XX	X	-	XX	X
	C	C	Bl	-		-	-	-	-	-	-	-	-	X		-
	Bl	Y	Bl	-	Bl	-	-	-	-	-	-	-	-	XXX	-	-
3	pBl	pB	pBl	BGr	Bl	Y	XX	X	X	X	X	X	X	X	XX	X
	Bl	C	Bl	-	-	-	X	X	XX	XX	XX	XX	X	X	X	X
	Bl	Y	G	Gr	Bl	-	-	-	-	-	-	-	-	XXXX	-	-
1	C	Pi	C	-	-	-	-	-	XX	-	-	-	-	-	-	-
	Bl	C	Bl	-	-	-	X	XX	X	-	XX	XX	XX	-	XX	-
	Bl	C	Bl	-	Bl	-	X	-	-	-	-	-	-	X	-	-
	Bl	C	Bl	-	-	-	-	-	-	X	-	-	X	-	-	X

TABLE 1

Substances detected in Ethanol Extracts of Banana Roots by 2-Directional Chromatogram

Sub-stance	R f BuOH-HAc-H_2O 4:1:2.2	HAc 2%	Without NH_3 VL[1]	Without NH_3 UV[2]	With NH_3 VL	With NH_3 UV	Ammonia-cal $AgNO_3$	$FeCL_3$ $K_3Fe(CN)_6$	Diazo-tized P-nitro Aniline	Varieties: Cocos	Grand Nain	Gros Michel	Congo	Variety 67	Laca-tan	Vi-mama	Musa Balbi-siana
1	.16	.93	C	Bl[3]	C	Bl	Gr	-	-	4	-	-	XX	-	-	-	-
2	.19	.83	C	C	C	C	pGr	GBl	-	X	X	X	XX	XX	XX	XX	-
3	.19	.91	C	Bl	C	Bl	-	-	Y	X	X	X	XX	X	XX	XXX	X
4	.28	.86	C	C	C	C	Gr	-	-	-	-	X	-	-	-	-	-
5	.30	.95	C	C	C	C	-	-	Y	-	-	-	-	XX	-	-	½
6	.33	.86	C	C	C	C	Gr	GBl	-	-	-	-	-	X	-	-	-
7	.34	.95	C	Bl	C	Bl	-	-	-	X	-	XX	X	XX	XX	XX	-
8	.42	.80	C	C	C	C	-	Bl	-	XX	-	-	-	-	-	X	-
9	.43	.00	pPi	Pi	pPi	Pi	-	-	-	X	XX	-	-	XX	XX	XXXX	XXX
10	.43	.90	C	Bl	C	Bl	-	-	-	-	-	-	-	-	XX	-	X
11	.46	.72	pY	C	pY	C	YGr	Bl	-	X	XX	XXX	XX	XXX	X	X	-
12	.49	.05	C	C	C	Bl	-	-	-	-	XX	-	XX	XX	XX	X	X

TABLE 2

Tentative identification of substances shown in Table 1 based on Chromatographic behavior, and reactions in visible and ultra violet light.

Substance Number	
9	Anthocyanidin (probably Delphinidin)
12	Phloroglucinol type (probably phloroglucinol carboxylic acid)
19	Chlorogenic acid
20	Anthocyanidin (probably malvidin)
24	Esculin
26	Anthocyanidin (probably cyanidin)
28	Phloroglucinol
29	Gallic acid
37	Coumaryl quinic acid
38	Pyrogallal
39	4-hydroxycoumarin
40	Apigenin

fluorimetric measurements using an attachment to the Beckman spectrophotometer. Ten varieties of banana plants have been grown in solution culture and the roots and leaves have recently been harvested. Healthy roots from these plants have been separated into cortex and stele in order to determine whether the polyphenolic substances and tannins are found mainly in the cortex or the tissues (stele) to which the parasite is restricted.

B. The Tannins

An analytical method is being devised by Dr. Greenberg whereby the tannins may be detected in the various organs of banana plant tissue. The method is based on precipitation of the tannins by lead and subsequent determination using a color reagent[4]. Preliminary results show that the major proportion of tannin content is found in the roots and rhizomes and to a lesser extent in the leaves, as shown in Table 3.

Table 3

Tannin Content of Banana Plant Tissue (Gros Michel)

Plant organ	Amount of "Tannin" expressed as mgms. tannic acid per gm. dry weight	
	Plant A	Plant B
Leaf Blade	1.17	1.36
Leaf Sheath (pseudostem)	1.70	2.45
Rhizome	7.15	7.40
Root	6.60	6.70

C. Optical density of root extracts

It has been reported that the tannin content of a particular plant tissue may be correlated with the optical density of an extract at wave length 280 mu[5]. Therefore, the optical density of some root extracts have been determined using a Beckman Ratio Spectrophotometer. Table 4 presents preliminary information on the optical density of banana root extracts at

4 Official Methods of Analysis of the Assoc. Off. Agric.Chem. 1955; Diemair et.al.,Z.Analyt.Chem.133,346-52 (1951); Snell & Snell, Colorimetric Methods of Analysis (1953).

5 Roux, J.S.L.T.C. 35,322-37 (1951).

TABLE 4

Optical Density of Banana Root Extracts at 280 mu

Variety	Optical Density
Gros Michel	.295
Cocos	.300
Variety 67	.330
Giant Fig	.350
Vimama	.375
Congo	.325
Grand Nain	.345
Lacatan	.375
Tumoc	.420
Musa Balbisiana	.600

280 mu and show that the optical density of the so-called resistant varieties to Panama disease tend to have higher values than for the two susceptible varieties tested, Gros Michel and Cocos. When these root extracts are acid hydrolyzed then the optical density at 280 mu in some of these extracts tends to increase. When these hydrolyzed extracts are placed on chromatograms it is found that the region on the paper in the butanol acetic acid solvent corresponds to between .75 to .84 and may be substance number 36 listed in Table 1. Of all the strips cut out and eluted from the paper and measured in the Beckman Spectrophotometer this particular region gives the greatest absoprtion at 280 mu.

Conclusions

None.

Recommendations

None.

Prepared by

S. R. Freiberg

January 5, 1959

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BCD-0-0

Annual Project Report
Special Projects
July - December, 1958

Button Seed Propagation Experiments

SUMMARY

Various methods were tried in a preliminary study to determine the optimum medium and conditions for successful button seed development.

Sphagnum moss proved to be a very satisfactory experimental medium in which to germinate this type seed. Vermiculite did not prove suitable as a bed material when used in pots. Water logging and compacting were the apparent drawbacks.

Temperatures most favorable for maximum germination appears to run between 27-32° C. Temperatures above 35° C. are detrimental to seed survival. From approximately 27° C., every increase in temperature (during rooting and germination) caused a reduction in root development. Germination of the growing point can be hastened by increased temperatures (up to 32° C.) but is done so at the expense of root development.

Germination in the high 90's is possible with good seed material and proper conditions. Seed germinated in a hot bed with a controlled temperature between 28-32° C. produces utilizable plants (2-2.5' tall) in 30-40 days.

A trial fumigation using 2-3/4 lbs./1,000 cu. ft. of bromide under various conditions proved fatal to all button seed.

Various methods of forcing the dormant lateral buds into activity have proved somewhat fruitful. In most all cases where the growing point has been removed from healthy seed, two or more additional points of growth are activated. From such studies it may be possible to greatly reduce the introduction of infected material into a clean seed bed while utilizing fully all potential growth. Such a practice may be applicable in the field as well as laboratory.

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BCD-0-0

Annual Project Report
Special Projects
July - December, 1958

Button Seed Propagation Experiments

In view of the Norwood Installation and the very type of research that would be conducted here, it was thought advisable to look into the possibility of propagating our own plant material for laboratory work. As there is always the possibility of having the Plant Quarantine Division restrict the importation of banana seed pieces, any means developed that would permit maximum utilization of our present, as well as future, plant propagating material would be an asset to the overall program.

While it does not appear as though any difficulty will be encountered in importing sufficient seed for our purposes, certain regulations concerning the transhipment of this seed stock have already been imposed by the government agencies involved. Such a move restricts our ability to furnish material to cooperating organizations and could slow down the overall efficiency of the program.

It looks as though this restricted movement of seed from our locality to the warmer sections of the States is due primarily to the infestation of some button seeds by the borer Castniomera humboldti. With a complete knowledge of the life cycles of this insect, particularly its larval stages and its feeding habits, it is easy to see that survival of any larvae inhabiting a button would not be possible. The rate of feeding by this larva is so rapid, even in the first instars, that consumption soon depletes the seed's vigor to a point where little or no germination is possible. Our practice of planting seeds in individual pots upon germination makes the transfer of any larva from one seed piece to another impossible. Any plant displaying symptoms of sickness after germination are inspected and destroyed if necessary.

In order to reduce the cost of importing large quantities of seed from the tropics and to enable personnel to have on hand sufficient and adequate plants for an extended range program, various tests have been conducted. Button seed have been subjected to various temperatures to check the effect of heat on germination. Other methods, later described, were tried in an effort to divert meristematic activity from the central growing point to the semi-dormant lateral buds in an attempt to obtain a rapid, maximum production from each seed.

Experiment No. II

Exploratory test to check the effects of:

1. Perimeter trimming (cutting away all material on the sides of buttons until an arbitrary diameter of 8.5 cm was obtained).

2. Growing point removal (passing a 1.7 cm cork borer completely through seed - to include growing point).

3. Planting with growing point upright.

4. Planting with growing point upside down.

Various combinations of the above treatments, as well as single treatments were employed. All buttons after treatment were planted in vermiculite with clay pots and placed on the greenhouse bench. Data on this test follows:

Table Number I

Treatment	Number Seed Used	Number Seed Germinated	Per Cent Germination
1. Trimmed and planted down	65	7	10.9
2. Trimmed, cored and planted down	65	12	18.4
3. Planted down	20	3	15.0
4. Planted up	20	0	0

It will be noticed immediately that germination on the whole was very low. This is accounted for by the fact that the buttons received too much water. The planting medium (vermiculite) displays certain characteristics which are not the most suitable for seed germination. Water tends to accumulate in the lower half of the pot while the upper half will be dry due to evaporation. There is apparently little or no capillary action in vermiculite and appearances of the surface may cause over-watering, with a resultant rot of the seed material.

In this particular test, it is interesting to note that the only treatment to produce multiple growth was the one which had the growing point cored out. Of the twelve seeds that germinated, nine had two or more points of aerial growth; or a multiple germination per cent of 75.

Experiment No. III

The purpose behind this test was to duplicate the previous test and at the same time to check the effects of heat treatment on germination. One half of all the seeds used in each treatment were immersed in a hot water bath of 65° C. for five minutes.

Due to the Plant Quarantine Division's notice that they are finding stalk borer larvae (Castniomera humboldti) in the button seed along with various other miscellaneous insects (flies, thrips, and larvae of flies) all seeds of this shipment were gassed with Parathion. This was done for two reasons. One, principally as a precautionary measure, and two, to test the effects of this chemical on germination and borer larvae. Unfortunately, and by mistake, all seeds were treated instead of only a part as planned.

Seeds for all treatments were randomly selected and when ready were again individually potted in vermiculite. This time watering was closely regulated but the results were not entirely satisfactory.

Again germination was found to be very low but just how much influence the fumigation may have exerted is not known. It is felt though that the gassing did have some pronounced effect and caused a reduction in the overall germination. Such thoughts are substantiated by tests conducted by the Plant Quarantine people on seeds used in the next test. Fumigation with methyl bromide reduced germination to zero. Fumigation in this case preceded all other treatments.

Table No. II shows the results obtained from this experiment. Germination again is very low; being comparable to that obtained in the first test. From these tests, it appears as though the hot water bath prior to planting did not adversely affect the overall germination. In fact, there is ever so slight an indication that heating may have stimulated multiple sprouting. Removal of the principal growing point is another practice that appeared to warrant further attention. Germination as well as increased multiple shooting was induced in both tests where this practice was employed.

Despite the fact that both shipments arrived in good condition, with some buttons just beginning to root, it took between 28 and 85 days for the first and last seed to germinate in the first trial and between 5 and 118 days in the second test. Mean germination time for both trials was approximately 60 days.

Both of these tests were run in greenhouses with controlled temperatures and humidity. Humidity was maintained at 60% and temperature at 80. There was some fluctuation of both during July, August and September but they were controlled as close as possible.

Table Number II

BUTTON SEED GERMINATION TRIALS

Treatment	Number Seed Used	Number Germinated	Number with Multiple Shooting	% Germination	% Multiple Germination
1. Seed trimmed to 8.5 cm diameter plugged with cork borer, heat treated and planted with growing point down.	33	5	3	15.1	60.0
2. Same as above, but not heat treated.	33	7	4	21.2	57.1
3. Same as #1, except the growing point was not plugged.	33	3	1	9.1	33.3
4. Seed trimmed, not plugged not heat treated, planted down.	33	3	0	9.1	
5. Seed not trimmed, not plugged, not heat treated, planted down.	10	0	0		
6. As #5, but heat treated.	10	0	0		
7. Seed not trimmed, not plugged, not heat treated, planted with growing point up.	10	0	0		
8. As #7, but was heat treated.	10	0	0		

Experiment No. IV

With the knowledge of this basic information and added facilities, the next step was to test the influence of temperature on seed development. For this test, a walk-in constant temperature room was used. Temperature was regulated to a steady 32° prior to planting.

In view of the difficulties with moisture accumulating in the vermiculite and the possibility of frequent watering altering the temperature around the seed, it was decided to shift to a more suitable planting medium. For this series, sphagnum moss was to be used as the bed material in place of vermiculite. This material was chosen because of its pliability when moist and its great water holding capacity when saturated. Both of these features made the moss appear very suitable. First, during root development little or no resistance from the moss would be met. Secondly, its high water retention would provide adequate moisture over a long period without restricting aeration. This avoided frequent watering, often times with cold water, and its insulation value would aid in maintaining a more constant bed temperature.

To facilitate handling and to use as little space as possible, plastic trays 12 x 14 x 4 inches were used. To prevent the seed from coming into contact with any stagnant free water, one inch of blue stone pebbles was placed in the bottom of the trays. The rest of each tray was then filled with saturated sphagnum. Button seed were then nestled in the moss so that they were nearly covered. Over each tray a sheet of perforated aluminum foil was placed, the sides tightly tucked about the trays. Limited watering was necessary when the moss dried out.

Eleven days after rooting had begun the plants were placed in clay pots filled with sterile loam. The pots were then placed in a heated room at 30° C. for germination. After one month, they were transferred to the greenhouse.

Table No. III gives a resume of the treatments and results obtained from this test.

Table Number III

Treatment	Number Seed Used	Number Rooted	Number Plants Produced	Per cent Rooting	Per cent* "take"
1. Seed planted with growing point up.	60	58	49	96.6	84.5
2. Seed planted with growing point down.	54	53	48	98.1	86.8

*Per cent "take" indicates per cent of seeds rooted that produced plants.

In the first treatment two buttons failed to root altogether. One of the 58 seed that did root failed to germinate because it contained a feeding larva of the stalk borer.

The one seed that failed to root in the second treatment likewise was infested with a borer.

From this experiment, it became evident that a germination per cent in the eighties may be expected from a normal shipment of button seed and that this figure might be raised even higher under proper conditions. Plants produced in this manner, without exception, were extremely vigorous.

Only eleven days were needed from time of planting until sufficient rooting had occurred to enable transplanting of the seed. Twenty-eight days after planting, the plants had attained a height of approximately 2.5 feet and were suitable for inoculation studies.

During the passage of this shipment through Plant Quarantine, 50 seed were selected for a fumigation trial. These were divided into 5 groups with 10 seeds per group. The treatments employed were:

A. 2-3/4 lb./1000 cu. feet for 2 hours
26" sustained vacuum - 75° F.

B. 2-3/4 lb./1000 cu. feet for 2 hours
15" sustained vacuum - 75° F.

C. Same as B.

D. 2-3/4 lb./1000 cu. feet for 2 hours
normal atmosphere pressure - 75° F.

E. No treatment.

None of the treated seed rooted. The control seed, however, had a germination of 80%. Methyl bromide at this concentration and exposure, even at normal atmospheric pressure, is too severe for banana button seed.

From these results, it has become evident that certain moisture and temperature requirements must be met before maximum germination can be expected. The effects of both these factors need further study and the next experiment was attempted with this in mind. While excessive still water tends to cause poor germination and rapid seed decay, the lack of adequate moisture prolongs seed development. Temperature about the rhizome evidently has a good deal of influence on the rate of chemical changes governing the plant's rate of metabolism.

Experiment No. V

A section of one bench in the greenhouse was utilized to construct a hot bed for future seed and propagation studies. Two lengths of heating cable were installed on top of one inch of blue stone pebbles and one inch of sterile loam to form two beds 6 feet long. Four more inches of sterile loam were added on top of the cable to finish the bed. Two inches of sphagnum moss were placed as a cover on the soil bed. Regulation of the temperature was accomplished by the use of an adjustable soil thermostat. After the bed had been brought up to nearly constant temperature of 30-32° C. with the soil in a moist condition, the buttons were planted. Planting was done so that approximately only one third of the button was in contact with the soil; the upper remaining portion being covered with the moss which was kept moist at all times.

181 seeds were planted in the hot bed on November 10. Formation of roots began in two days and fourteen days after planting the buttons were germinated sufficiently to begin transplanting. By December 1, or twenty-one days after planting, 97.2% of the seed had germinated and, with the exception of a few left in the beds for experimental purposes, had been successfully transplanted. This group of seed was selected for condition and apparent germination potential before planting.

Experiment No. VI

Three groups of seed comprised of 6 treatments with 5 button seed per treatment were selected for size and quality for a temperature/germination/fusarium inoculation study. Treatments of each are as follows:

1. 5 seed incubated at 35° C.
   5 seed inoculated with F.o.c. and incubated at 35° C.

2. 5 seed incubated at 40° C.
   5 seed inoculated with F.o.c. and incubated at 40° C.

3. 5 seed incubated at 45° C.
   5 seed inoculated with F.o.c. and incubated at 45° C.

Incoulations of the seeds with a spore suspension of F.o.c. was done by removing, with a cork borer, a plug 3/8" in diameter from the rhizome tissue just below the principal rooting zone. The plug was broken in half; 1/4" from the internal end of one of the halves was removed; this half of the plug was replaced. Two milliliters of the spore suspension were placed in the cavity; the other plug replaced and both external ends were sealed with melted paraffin.

Plastic trays with sphagnum moss were used as bed material to check development.

Eleven days were needed before germination was sufficient to transplant. All seed, including those inoculated, placed in incubators at 35° C. germinated but it was noticed that rooting was very weak at this point. Only one inoculated and one non-inoculated seed at 40° C. germinated. Rooting of these two was even weaker than those developing at 35° C. At 45° C. no germination nor rooting was accomplished. All seeds at this temperature rotted after eleven days of incubation.

Experiment No. VII

In previous tests the ability of some buttons to produce two shoots at once was noticed. This was undoubtedly due to the stage of development and varied greatly among all buttons. However, the knowledge that more than one potential growing point was available on each seed piece led to the foregoing test. It was thought that if the meristematic activity could be diverted from the central growing point, it may be directed to the lateral buds thereby forcing them out of dormancy.

In order to test this theory, 10 buttons were selected for trial. Each seed was quartered vertically through the growing point. The portion of the growing point remaining was removed from each quarter before it was placed in moist moss and incubated at 32°.

Planting was done November 24, 1958. Rooting began the second day, and transplanting began eight days later. These figures compare favorably to whole button seed without alteration. To date, 17 of the quarters have been germinated and have been transplanted, 5 have been discarded due to decay and 18 remain in a solid but semi-dormant condition.

The value of this particular test cannot be over emphasized. Two very favorable points have developed when considering the importance of securing disease-free seed. Quartering permits the inspection of 8 internal surfaces of each seed piece without sacrificing any planting material; in fact, this procedure would permit maximum utilization of all potential growth.

It is true that plants developed from small pieces such as button seed quarters may be weak and slower in developing than plants from say a maidenhead or a sucker, but after rooting has been established it should grow equally well. The chances of its being planted free of insects and disease is much greater than for a larger piece.

Prepared by

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December 24, 1958

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BDP-1-O

Physiological Microbiology
Section
Annual Project Report 1958

The Role of Bacteria Associated with Panama Disease

Background

The role of the bacteria commonly isolated from banana plants infected with Fusarium oxysporum f. cubense has not been studied in detail. Hartley and Rands[1] reported, "...in this chronic disease there is an interesting secondary flora, consisting largely of Fusaria and bacteria."

Progress

A gram negative rod-shaped bacterium was found associated with diseased tissue from a banana plant. In a preliminary experiment when this bacterium and Fusarium were used as inoculum either together or separately there resulted vascular discoloration in the plants. With the Fusarium alone, or with the Fusarium plus the bacterium as the inoculum there resulted above-ground symptoms in 10-14 days, whereas with the bacterium alone above-ground symptoms did not become pronounced until after 30-40 days. In the latter plants Fusarium solani was isolated from the discolored vascular tissue.

On rhizome tissue from button seeds this bacterium and Fusarium oxysporum f. cubense produced more decay when together than did either of them when in pure culture. On a sodium polypectate gel with Marsh's salts, they were more effective in liquefying the gel than either of them in pure culture. This synergistic reaction appeared to be favored by slight increases of pH above pH 6.1. The above observations indicate that this bacterium and Fusarium oxysporum f. cubense are synergistic in biochemical activity. As results with their combination in the limited study on infection would not lead one to consider them synergistic, this may be explained in terms of inoculum potential. Studies with this combination in which the concentration of Fusarium is low may show that there is a synergistic relationship between these two organisms in Panama disease.

Characteristics of this bacterium, its appearance on a potato dextrose-peptone-agar medium, its ability to rapidly decay tissue of potato tubers, and its morphological characteristics, indicate that it is Erwinia aroideae (Townsend) Holland. To support this indication further studies need to be made.

1 Hartley, C., and R. D. Rands. 1924. Plant Pathology in the Dutch East Indies. Phytopathology 14:11.

Conclusions

The *Fusaria* and bacteria associated with tissue of plants suffering from Panama disease may act synergistically in causing symptoms of this disease. The water economy of the host may also be associated with the action of these organisms.

Recommendations

Further studies need to be made on the role of this bacterium in association with Panama disease.

Prepared by

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December 31, 1958

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BDP-1-10

Physiological Microbiology
Section
Annual Project Report 1958

Growth of Fusarium oxysporum f. cubense in Controlled Atmospheres

Background

The soil in which Fusaria are capable of existing and carrying on saprophytic growth and sporulation has a variable atmosphere. The influence of this atmosphere upon these fungi has not been sufficiently studied.

Hollis[1] reported that Fusarium oxysporum survived for a longer time as a saprophyte in soil than did F. eumartii and that both grew at extremely low O_2 tensions. At an oxygen tension of less than 1 per cent F. oxysporum survived, but F. eumartii did not survive. Concentrations of Co_2 above 0.03% has been reported to increase the multiplication of Fusarium in soil, whereas low oxygen tensions did not greatly reduce sporulation in soil until it was below 5 per cent in sandy loam and between 8 and 10 per cent in loam.

Progress

Spore of Fusarium oxysporum f. cubense on the surface of potato-dextrose agar germinated and formed short hyphae when in an atmosphere of 1 per cent O_2 in nitrogen. These hyphae produced few spores and apparently have a limited growth (Table 1). As the O_2 concentration increased in a nitrogen atmosphere, there was an increase in the number of microconidia produced per unit of mycelium. Colonies which developed under low O_2 concentration produced macroconidia earlier, after being returned to a normal atmosphere, than did those colonies held at relatively higher O_2 concentrations.

Experiments with different media indicate that this Fusarium is neither anaerobic nor aerobic per se, since this characteristic is related to the substrate to which it is exposed. With a chemically defined substrate little or no growth was apparent whereas on the same medium supplemented with 0.2 per cent yeast extract, considerable growth took place in a nitrogen atmosphere. In an atmosphere of Co_2 microconidia germinated and formed germ tubes which bore chlamydospores. Growth of the fungus appeared to be limited, and not stimulated by the yeast extract.

[1] Hollis, J. P., 1948. Oxygen and carbon dioxide relations of Fusarium oxysporum Schlecht and Fusarium eumartii Carp. Phytopathology 38:761-775.

Button seed tissue supported more abundant growth of F. oxysporum f. cubense when it was held at low oxygen tensions in a nitrogen atmosphere. At O_2 concentrations of 5, 2.5, or 1 per cent and in a nitrogen atmosphere there resulted less discoloration of the cut surfaces of the tissue and a deeper decay into the tissue than at higher O_2 concentrations.

Conclusions

The need for a more detailed investigation into soil environments upon the survival and infection of banana plants by Fusaria is apparent. This Fusarium produces abundant macroconidia when first grown in an atmosphere of low concentration of O_2 and then placed in a normal atmosphere. When it is grown in a normal atmosphere few macroconidia are produced.

Recommendations

None.

Prepared by

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December 31, 1958

Table 1. The influence of the concentration of O_2 in a nitrogen atmosphere upon the growth and sporulation of Fusarium oxysporum f. cubense.

Per cent Oxygen	mm. of Growth[a]	Microconidia[b]	Macroconidia[c]
15	59	5	0
10	60	4	0
5	56	4	1
2.5	41	3	2
[illegible]	[illegible]	2	5
[illegible]	[illegible]	1	5
Check (In Air)	72	5	0

a. Diameter of growth in millimeters.
b. Microconidia per unit of mycelium, based on an arbitrary scale of 0-5.
c. Macroconidia per unit of mycelium, based on an arbitrary scale of 0-5, two days after removing from controlled atmosphere.

CENTRAL RESEARCH LABORATORIES
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BDP-1-10

Physiological Microbiology Section
Annual Project Report 1958

Respiration of spores of a clone of *Fusarium oxysporum* f. *cubense*

Background

Thick-walled chlamydospores of fungi are considered as being in a dormant or "resting" stage. This has been accepted as being true for chlamydospores of *Fusarium* species although there is little evidence to support this. The magnitude of O_2 uptake during aerobic respiration is assumed to be an indication of relative dormancy.

Progress

Preparations of micrconidia, macroconidia, and chlamydospores were studied in chemically defined media of Marshs salts, sucrose, and ammonium nitrate. Oxygen-uptake was determined by conventional Warburg techniques. Oxygen-uptake was an indicator of the magnitude of aerobic respiration.

Oxygen-uptake by either chlamydospores, macroconidia, or microconidia varied from spore harvest to spore harvest. This variation may have been due in part to differences in homogeneity or the preparation of spores, the substrate from which the spores were grown, and the age of the spores. Results of a number of experiments are present in Tables 1, 2, and 3.

Oxygen-uptake by microconidia was greater than by either macroconidia or chlamydospores. The magnitude of O_2-uptake after five hours was directly proportional to the dry weight of the microconidia. Yeast extract increased the amount of O_2-uptake. The influence of pH upon O_2-uptake by microconidia was only slight in the range of 4.4 and 6.4 (Table 4). This was also true for chlamydospores (Table 5).

Macroconidia had higher Qo_2 values than did chlamydospores. In water chlamydospores had lower Qo_2 values than either micro- or macroconidia. The addition of yeast extract to these media increased the Qo_2 values for both microconidia and chlamydospores. Macroconidia were not studied with these media.

Microscopic observation of these three morphological forms of *Fusarium oxysporum* f. *cubense* showed that the macroconidia and chlamydospores germinated within the first five hours in the Warburg flask at 29.7° C. Microconidia produced germ tubes only after 9-14 hours at this temperature. Evidence that respiration was correlated with synthesis in these spores was a doubling and tripling in their size during incubation.

Table 1. Oxygen-uptake by microconidia as indicated by Qo_2 values during incubation in different media.

Experiment No.	Spore Source	Qo_2 Sucrose[a]	Yeast Extract[b]	Water[c]
1	water agar	26.7-187.4		5
2	V-8 juice	36 - 71		5
3	PDA	30.7- 58.4	34 - 71	13.3-8.18
4	PDA	23 - 41	24.8- 56.4	0 -7.6
5	PDA	48 -135	46 -198	13.1-6.6

a In a medium of Harsh's salts with sucrose as the carbon source.
b Same as in [a] with 0.2 per cent yeast extract.
c Endogenous respiration.

Table 2. Oxygen-uptake by macroconidia as indicated by Qo_2 values during incubation in different media.

Experiment No.	Spore Source	Qo_2 Sucrose[a]	Qo_2 Water[b]
1	V-8 juice Agar	18	5
2	V-8 juice Agar	32	5
3	V-8 juice Agar	14-25	12

[a] In a medium of Marsh's salts with sucrose as the carbon source.

[b] Endogenous respiration

Table 3. Oxygen-uptake by chlamydospores as indicated by Qo_2 values during incubation in different media.

Experiment No.	Spore Source	Qo_2		
		Sucrose[a]	Yeast Extract[b]	Water[c]
1	PDA	10 -12	----------	1.5
2	PDA	17.3	----------	11.3
3	PDA	10.9	----------	1.45
4	PDA	9 -13.3	----------	5.3
5	PDA	4 -18	----------	3.4
6	V-8 Juice Agar	10 -23.2	----------	1.8-5.4
7	PDA	8.5-15.3	----------	-------
8	PDA	9.8-18.5	11.7-25	1.8-2.4
9	PDA	6.6-13.6	8 -19	1.2-2.8

[a] In a medium of Marsh's salts with sucrose as the carbon source.

[b] The same medium as in [a] with 0.2 per cent yeast extract.

[c] Endogenous respiration.

Table 4. Qo_2 values of microconidia during incubation in media of Marsh's salts, 5 g/l sucrose, and 1 g/l NH_4NO_3. The pH of each medium was obtained by varying the concentrations of KH_2PO_4 and K_2HPO_4 in the basal salt solutions.

	Qo_2 Values during Incubation Intervals							
pH of Media[a]	0.5[b]	1.5	2	2.5	3.5	4	4.5	5
4.4	21.6	33.6	38	41.7	50	56	61.4	67.6
4.8	33	38.6	43.6	47	54	59	64	69.5
5.25	29.6	35.4	40	44	52	58	63	69.5
5.92	43.2	39.5	45	47.5	54	60	64.5	71.5
6.4	37	41.5	45	47	55.6	61.5	67	----
7.2	36.4	39.3	43	45	53.2	59.2	64.5	71.3
Basal salts[c]	34	39.4	43.5	42.8	48.5	51.5	52.8	54.4
Water[d]	0.9	4.8	3.4	3.9	4.3	4.9	4.9	5

a The pH of these media at time of seeding them.
b The interval is given in units of hours.
c Basal salts consisted of Marsh's salts with a pH of 5.5.
d Endogenous oxygen-uptake.

Table 5. Qo_2 values of chlamydospores during incubation in media of the same composition as those mentioned in Table 4.

	Qo_2 Values During Incubation Intervals				
pH of Media[a]	1[b]	2	3	4	4.5
4.4	10.3	13	16.6	20	23.2
4.8	11.7	13.3	17.3	21.6	25.0
5.25	9.2	12.4	17.1	20.9	24.0
5.92	11.6	14.0	18	21.6	24.6
6.4	11.4	13.6	17.6	21.6	24.6
7.2	10.8	13.5	16.8	20.5	23.3
Basal salts[c]	9.15	12.5	16.8	20.5	23.0
Water[d]	1.8	2.1	3.4	5.6	5.4

[a] The pH of these media at time of seeding them.
[b] The interval is given in units of hours.
[c] Basal salts consisted of Marsh's salts with a pH of 5.5.
[d] Endogenous oxygen-uptake.

When the same medium and cultural conditions were used, the greater the number of microconidia per unit volume of medium the lower was the percent germination and increase in spore volume. In distilled water these spores germinated only when in very low concentrations, whereas macroconidia and chlamydospore germinated when in high concentration. Whether self-inhibitors or a limited nutritional substrate caused this has not been determined.

Conclusions

Chlamydospores appear to have a lower Qo_2 than micro- or macroconidia.

Recommendations

Studies of respiration of this Fusarium under controlled atmospheres of CO_2, O_2, and nitrogen may help elucidate the behavior of this fungus in its role as either a saprophyte or a pathogen.

Prepared by

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January 5, 1959

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BDP-1-10

Physiological Microbiology
Section
Annual Project Report 1958

Sporulation of Fusarium oxysporum f. cubense in Relation to Carbon and Nitrogen in Chemically Defined Media.

Background

Increasing the carbon and nitrogen concentration of soil has been reported to influence the population of Fusarium and incidence of Panama disease. Whether this influence may be related to the effect of the carbon to nitrogen ratio (C/N ratio) on the sporulation and growth of Fusarium or to other factors has not been determined. Experiments were conducted to determine the effect of C/N ratio upon the sporulation of Fusarium in chemically defined media of Marsh's salts and various concentrations of sucrose and ammonium nitrate.

Progress

Production of microconidia appeared to be favored by certain levels of nitrogen (0.087 g/l) and not significantly influenced by the C/N ratio (Table 2). High concentrations of carbon did not favor microconidial formation when the nitrogen level was 0.349 g/l. However, no microconidia were formed unless there was carbon present in the medium.

Macroconidia occurred in these cultures only sparsely and without correlation with the carbon and nitrogen levels or C/N ratio.

In standing- or shake-cultures chlamydospores production was favored either by low levels of carbon or a low C/N ratio (Tables 1, 2 and 3). It is noted from these data that on media of a high level of nitrogen chlamydospore production was favored by a low C/N ratio, whereas on similar media of a low nitrogen level chlamydospore production was favored by relatively higher C/N ratio.

In Petri dishes on the surface of agar media the production of chlamydospores and microconidia was favored by high levels of carbon in the medium. On low levels of nitrogen (0.022 g/l) there were not as many of these spores produced as on a medium of higher nitrogen concentration when the concentration of carbon was high. With any of the media tested in Petri dish cultures, macroconidia production was sparse and was not correlated with the relation of carbon and nitrogen.

Table 1. The production (based on an arbitrary scale of 0-10) of chlamydospores per unit of mycelium of F. oxysporum f. cubense as related to the concentration of nitrogen and the carbon to nitrogen ratio of the medium.

	Carbon to Nitrogen Ratio									
Nitrogen (g.l.)	386.24	192.67	96.34	48.17	24.06	12.03	6.02	3.01	1.50	0.75
0.349	-----	-----	-----	-----	0	0-1	6	6	8	10
0.175	-----	-----	-----	0	0-1	5	7	10	10	----
0.874	-----	-----	0	0-1	5	8	10	10	----	----
0.044	-----	0-1	0-1	6	6	10	10	----	----	-----
0.022	0-1	1-2	3	4	5	10	----	----	----	-----

Table 2. The production (based on an arbitrary scale of 0-10) of microconidia per culture and chlamydospores per unit of mycelium of F. exysporum f. cubense as related to the carbon and nitrogen ratio of the medium.

Nitrogen (g/l)	Carbon (g/l)						
	8.42	4.21	2.10	1.05	0.53	0.26	0
0.349	0[a] 0[b]	0 0-1	0 6	1 6	0 8	2 10	0 10
0.175	4.5 0	0-1 0-1	0-1 5	0-1 7	5 10	2 10	0 10
0.087	5 0	5 0-1	3 5	5 8	5 10	3 10	0 10
0.044	10 0-1	8 0-1	0-1 6	1-2 6	2 10	4 10	2 10
0.022	1 0-1	1 1-2	3 3	2 4	5 5	5 10	0 10

[a] Microconidia

[b] Chlamydospores

Table 3. The production (based on an arbitrary scale of 0-10) of chlamydospores per unit of mycelium of F. oxysporum f. cubense as related to the concentration of carbon and the carbon to nitrogen of the media.

	Carbon to Nitrogen Ratio									
Carbon (g/l)	386.24	192.67	96.34	48.17	24.06	12.03	6.02	3.01	1.50	0.75
8.42	1 [a] 0-1 [b]	10 0-1	5 0	4.5 0	0 0	--- ---	--- ---	--- ---	--- ---	--- ---
4.21	---- ----	1 1-2	8 0-1	5 0-1	0-1 0-1	0 0-1	--- ---	--- ---	--- ---	--- ---
2.10	---- ----	--- ---	3 3	0-1 6	3 5	0-1 5	0 6	--- ---	--- ---	--- ---
1.05	---- ----	--- ---	--- ---	2 4	1-2 6	5 8	0-1 7	1 6	--- ---	--- ---
0.53	---- ----	--- ---	--- ---	--- ---	5 5	2 10	5 10	5 10	0 8	--- ---
0.26	---- ----	--- ---	--- ---	--- ---	--- ---	5 10	4 10	3 10	2 10	0 10

[a] Microconidia

[b] Chlamydospores

Conclusions

On chemically defined media chlamydospore production by Fusarium is correlated with the C/N ratio. On media of high C/N ratio there were relatively fewer chlamydospores formed than on media of low C/N ratio. The production of chlamydospores decreased as the C/N ratio increased when this fungus was grown on either a low or high nitrogen concentration in the medium. The higher the nitrogen concentration of the medium the lower was the C/N requirement for chlamydospores production. At low levels of carbon chlamydospore production was greater when the C/N ratio was low than when it was high. These relationships were observed with either standing- or shake-cultures, but not with cultures on surfaces of similar media solidified with 2 per cent Bacto-agar in Petri dishes.

Recommendations

Whether these relationships occur in soil needs to be determined in order that some application could be made of them.

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December 22, 1958

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BDP-1-10

Physiological Microbiology
Section
Annual Project Report 1958

The Influence of Ultraviolet Light upon the Formation of Macroconidia by Fusarium oxysporum f. cubense

Background

Ramsey and Bailey[1] showed that exposure to ultraviolet radiation increased macrospore production, and Harter[2] found that cultures of Fusarium spp. grown in the light showed increased macrospore length. More recently, Carlile[3] reported that light promoted the formation of macroconidia by Fusarium oxysporum f. gladioli.

Some isolates of Fusarium oxysporum f. cubense produce an abundance of macroconidia, whereas other isolates produce few of these conidia only after a long period of incubation. With an isolate that had the latter characteristic, experiments with different exposures of ultraviolet light were conducted to determine the influence of this light upon the formation of macroconidia.

Progress

At an equal distance (ca 5 ft.) from a Hanovia 94A1, sterilization lamp one-day-old cultures of Fusarium oxysporum f. cubense were subjected to exposures of 2 to 128 minutes in duration. These cultures were made by adding a suspension of conidia to unsolidified PDA. From time of seeding these media, these cultures developed in the dark, and after the exposure to light, they were placed in the dark with the unexposed cultures, the checks.

[1]Ramsey, G. B. and A. A. Bailey. 1930. Effects of ultraviolet radiation upon sporulation in Macrosporum and Fusarium. Bot. Gaz. 89:113.

[2]Harter, L. L. 1939. Influence of light on the length of the conidia in certain species of Fusarium. Amer. J. Bot. 26:234.

[3]Carlile, M. J. 1956. A study of the factors influencing non-genetic variation in a strain of Fusarium oxysporum. J. Gen. Microbiol. 14:643-654.

In some experiments, increased production of macroconidia occurred after the culture had been exposed to ultraviolet light for 4 to 16 minutes (Table 1).

Some cultures of the same clone of this Fusarium produced almost as many macroconidia in the dark as it did after an exposure to ultraviolet light. This variation within the spores themselves along with the influence of light on the mechanism which determines whether a culture will produce macroconidia or not may be found to be related.

Conclusions

Ultraviolet light appears to stimulate the production of macroconidia by some isolates of Fusarium oxysporum f. cubense.

Recommendations

None.

Prepared by

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January 15, 1959

Table 1. The effect of ultraviolet light upon the macroconidia formation of Fusarium oxysporum f. cubense.

Minutes of Exposure	Macroconidia[a]
2	5.3
4	55.0
8	49.0
16	40.3
32	9.0
64	25.0
128	3.0
unexposed check	4.8

a. Macroconidia per culture given in arbitrary units.

CENTRAL RESEARCH LABORATORIES
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BDP-1-10

Physiological Microbiology
Section
Annual Project Report 1958

Observations from Attempts to Lyophilize Spores of *Fusarium oxysporum* f. *cubense*

Background

The survival of the spores of *Fusarium* may play an important role in the control of Panama disease. Although strains of this fungus has been found to survive for many years in soil, the means of survival has not been completely determined.

In a laboratory study attempts were made to dry the different spore forms of *Fusarium* from the frozen state, a technique known as lyophilization. This method has been used in preserving cultures of fungi and bacteria for extended periods of time.

Progress

Chlamydospores of *Fusarium* survived lyophilization whereas conidia lost their viability. Freezing conidia of this fungus at -62° C. was not apparently deleterious to their potential for germination. Only eight to ten per cent of the conidia germinated after vacuum-desiccation for 7 hours in an unfrozen state.

Conclusions

Controlled vacuum-desiccation of conidia apparently destroyed conidia whereas the thicker-walled chlamydospores withstood this treatment.

Recommendations

Further investigations into the loss of viability of conidia due to vacuum-desiccation may show some physical-chemical change which may add to our understanding of survival of this fungus in soil over long periods of time.

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December 31, 1958

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BDP-1-20

Physiological-Microbiology Section
Annual Project Report 1958

Synthesis of Oligosaccharides by an Isolate Fusarium oxysporum f. cubense

Background

A number of fungi including some of those which are capable of inciting a wilt disease of vascular plants synthesize exocellular polymers of sugars.[1,2] Whether these are produced in the vessels and other tissues of the infected plant has not been determined. Until there is sufficient evidence to accept or reject the hypothesis that they are involved in the biochemistry of a wilt disease, one may want to consider them in exploratory experiments along with pectolytic enzymes and fusaric acid in explaining the mechanism of pathogenesis in the Panama disease.

Progress

Media consisting of Marsh's salts at a pH of 5.9, NH_4NO_3 (1 gm/l), and sucrose at a concentration of either 20, 10, 5, 2.5, 1.25, 0.625 or 0.0 gm/l were seeded with Fusarium oxysporum f. cubense. Some cultures were aerated by continuous shaking while others remained stationary. Small quantities of the culture medium were removed during the 6-10 day incubation period. These were analyzed for sugars by paper chromatography by a method previously described[2].

Results from these experiments are given in Table 1.

The results with shake-cultures were similar to those with standing-cultures. One experiment indicated that sucrose and the oligosaccharide were not present after 6 days in media of shake-cultures whereas, the oligosaccharide (Rf. 0.0515) and fructose were present in media of standing-cultures.

If glucose were present in these media, it was not detected by the methods of chromatography used in these experiments.

1 Le Tourneau, D. 1957. The production of oligosaccharides by Verticillium alboatrum. Phytopathology (Abst.) 47:527.

2 Wilson, E. M. and V. G. Lilly. 1958. The utilization of oligosaccharides by some species of Ceratocystis. Mycologia 50: 376-389.

Table 1. Rf. values of the saccharides in media surrounding mycelium of Fusarium oxysporum f. cubense (3 days growth; standing-culture).

Gram Sucrose/Liter	Rf. Values		
20	.0515	0.131	0.216
10	.0485	0.126	0.230
5	-----	0.124	0.221
2.5	-----	0.122	0.231
1.25	-----	-----	-----
0.625	-----	0.131	0.231
0.0	-----	-----	-----
Sucrose[a]	-----	0.126	-----
Fructose[b]	-----	-----	0.240
Sucrose and Fructose[c]	-----	0.119	0.221

[a] Two per cent sucrose in water.

[b] Two per cent fructose in water.

[c] Spotted with both [a] and [b].

Conclusions

An unidentified oligosaccharide is synthesized by *Fusarium oxysporum* f. *cubense* from chemically defined media with NH_4NO_3 as the nitrogen source, and sucrose as the source of carbon.

Recommendations

Whether this oligosaccharide occurs in the vessels of infected banana plants remains undetermined. It may be involved in the mechanism of pathogenesis of the Panama disease. If this is true, this mechanism may be of importance in the chemotherapy of this disease.

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January 20, 1959

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BDP-2-10

Soil Microbiology Section
Annual Project Report 1958

Soil Fungi from Virgin Forest Areas of Farm 55, Golfito

Background

During June, 1958, Farm 55, Golfito, was being prepared for banana planting operations. Native jungle had been felled on this area about five years previously and the secondary growth had been chopped and burned within the preceding six months. Since planting operations were about to be undertaken on this farm, it seemed an opportune time to obtain samples of virgin forest soils, for the purpose of studying the fungal population in such areas, and comparing it with that in established plantations. Samples were taken from four different sites in the last section of Farm 55, to the West of the railroad line, and adjacent to Farm 56. Sampling sites have been marked, in the event that future studies of this area should be carried out.

Progress

The soil samples were collected on June 11, in sterile cotton plugged test tubes. The tubes were then covered with pliofilm to aid in retention of soil moisture, and were maintained in this condition at laboratory temperatures until September 26, when dilution plates were prepared. Considerable drying of the soil occurred during the interval between sampling and planting, doubtlessly accompanied by alteration of the soil microbial population.

Conclusions

On dilution plates prepared from these soil samples, it was observed that frequently a single organism predominated. This was doubtlessly a reflection of the sporulating ability of certain fungi. In general, two types of fungi appeared to predominate among the isolates obtained: (1) Heavy sporulators, such as Gliomastix, Trichoderma and Penicillium, and (2) Chlamydospore producers, such as Humicola. No isolates were obtained of Phycomycetes, which may indicate that the long storage period may be detrimental to organisms belonging to this group.

Soils maintained in storage in this way would not appear to be favorable material for quantitative studies, inasmuch as rather high counts were obtained by dilution methods, with generally a single organism present in high numbers. For example, in a single 1/10,000 dilution plate, 59 colonies of Gliomastix were counted. In other instances where high counts were obtained, Penicillium or Trichoderma was predominant.