UNITED FRUIT COMPANY

DEPARTMENT OF RESEARCH
ANNUAL REPORT
1959

VOLUME II
DOMESTIC AND BASIC RESEARCH

CENTRAL RESEARCH LABORATORIES
NEW YORK LABORATORY
TRANSPORTATION AND GENERAL RESEARCH
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CENTRAL RESEARCH LABORATORIES

NORWOOD, MASS.

Norwood, Massachusetts

December 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 the 1959 Annual Project Reports from the Central Research Laboratories.

The year 1959 has been a period of growth - growth mainly in staff but also in facilities. Our staff at the end of 1958 - about 7 months after activation - consisted of 23 people of whom 13 were professional and technical. At the end of 1959 our staff now numbers 42 people of whom 27 are professional and technical. This increase in personnel has resulted largely from a broadening of our research program. Thus, three new laboratories have been activated - Anatomy-Morphology, Biochemistry and Entomology. With these additions to our staff we have been able to broaden our approach to the Fusarium wilt problem. Most of our efforts are still focused on this disease. We are now, however, also working on basic aspects of the Cercospora leaf spot and on some of the insect problems.

During the year, funds were made available to remedy the main weakness in our facilities - space for growing and experimenting with plants under controlled conditions. These facilities - a second greenhouse with four compartments and four small controlled environment chambers - will considerably broaden our potential contributions to Company problems when they are completed in the early part of 1960.

Sincerely yours,

G. R. Mandels

G. R. Mandels, Director
Central Research Laboratories

TABLE OF CONTENTS

TABLE OF CONTENTS (cont'd)

TABLE OF CONTENTS (cont'd)

TABLE OF CONTENTS (cont'd)

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

Annual Project Report 195

Technical Information Service

1. The banana abstracts file was kept up-to-date by continuously reviewing the journals, the abstracting journals, and miscellaneous publications received at the Norwood library.

2. Monthly distribution of a selected group of current abstracts was initiated to all members of the research staff, consultants, associated university scientists, and all other interested persons. The first issue was circulated November 1959.

3. The Company files on processed banana products was reviewed and summarized in six catagories: history of past ventures, patents and methods of manufacture, composition of products, merchandising and applications, economics, and research. This material still requires considerable organization, verification, and editing.

4. Prepared reports on special request for information on:
   a. The economics and agriculture of tropical spices
   b. Ultrasonic energy for killing nematodes
   c. Methods for distinguishing Antimycoin, Nystatin, Rimocidin, and Chromin
   d. A consideration of the equipment and techniques of the Bertuzzi Company
   e. The possibility of extending the shelf life of fresh bananas by irradiation
   f. Anthelminthic activity of Tanacetum vulgare

5. Other activities
   a. Established a file and correspondence on various aspects of the food technology of banana
   b. Assisted in bringing technical data on banana meal in animal diets to the attention of the Ralston Purina Company
   c. Traveled to and reported on: the 1959 Institute of Food Technologists' meeting (Philadelphia, Penn.), the activities of the Quartermaster Food and Container Institute (Chicago, Ill.), food technology at Purdue (Lafayette, Indiana), and the irradiation facilities of Curtis Wright (Quehana, Penn.)
   d. Assisted in some of the various considerations of the Chain Belt Company's (Wisconsin) banana powder.

Prepared by
R. C. Wornick
December 1, 1959

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BDF-1-0 (1959 Summary) Physiological-Microbiology Section
Annual Project Report 1959

The Role of Bacteria in the Fusarium Wilt Disease of Banana (1)

Two approaches have been considered in determining the role of the bacteria that are associated with the Fusarium wilt of banana. The first approach which appeared to offer a direct explanation was that of inoculating aseptic susceptible plants with an isolate of F. oxysporum f. cubense. This approach was found to be discouraging as attempts to grow aseptic abaca and banana (Musa bulbisiana) from seed met with only limited success. The time required to frow an aseptic plant was between 2-3 months and plants were not typical and showed a slow, stunted growth; thus would not be an unbiased sample for pathological study. A species of Botryodiplodia was isolated and identified as a common contaminant of both abaca and banana seed that were received by these Laboratories. A heat treatment proved to be effective in freeing abaca seed of this fungus. Abaca seedlings were maintained aseptic on aqueous agar in test tubes.

The second approach is that of applying an antibiotic screen in the roots of the plant. Vancomycin was tested as such a screen but did not fulfill that function. A number of other antibiotics are presently being tested. This approach may prove to be the method of choice in explaining the role of these bacteria.

1. Wilson, E. M. 1959. The role of bacteria in Fusarium wilt disease of banana. 2nd Quarter Reports, Central Research Laboratories.

Prepared by

Eugene M. Wilson
December 1, 1959

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BDF-1-10 Physiological-Microbiology Section
Annual Project Report 1959

Studies on the Behavior of Microconidia
of Fusarium oxysporum f. cubense.

Background

It is probable that the infection of banana plants by Fusarium oxysporum f. cubense takes place through wounded stelar tissue (Sequeira et al.). It is reasonable to suppose that germinated or ungerminated spores might be the causal agents and be taken up with the transpiration stream.

In order to develop methods for the control of Panama Disease it is important then that we understand the physiology and biochemistry of the spores. The following in vitro studies were undertaken to develop techniques and to provide some basic information.

Progress

Difficulties were encountered in obtaining pure suspensions of macroconidia or chlamydospores. Macroconidial suspensions always contained at least 20% microconidia and an efficient method of separation could not be found. The chlamydospores could only be obtained in aggregates joined by a considerable amount of mycelium and were not suitable for the type of experiments done.

For the preliminary experiments spores were taken from cultures that had originally been derived from a single spore culture of isolate 3 (yellow race, inodoratum from B. H. Waite). A supply of spores was kept refrigerated in distilled water for no longer than two weeks. A basic nutrient solution was used throughout all the studies and was made up as follows:-

$MgSO_4.7H_2O$, 2.22 g., Sucrose, 5.0 g., NH_4NO_3, 2.0 g., KH_2PO_4.3.4 g., K_2HPO_4 1.1 g., distilled water, 1000 ccs.

The pH ranged between 5.8 and 6.1.

If incubated in the above nutrient for 6 hours microconidia will swell to almost twice their original width before germinating. A suspension of macroconidia was prepared (this contained about 40% microconidia) and when incubated in nutrient they too increased in width but to a lesser extent than the microconidia. Neither the micro- nor macroconidia could be made to swell or germinate in tap, distilled or sterile water in 48 hours.

Germination rate in the nutrient solution varied with spore concentration, lower concentrations germinating much faster than the higher ones. This was probably due to insufficient nutrient and oxygen deficiency. When the higher concentrations were incubated in shake culture there was a marked increase in rate of germination (10% at 20×10^6 spores per ml.) At 1×10^6 spores per ml. at $25 \pm 1^\circ$ C. in shake culture, as much as 95% germination took place in 24 hours. Microconidia would not germinate under anaerobic conditions in 24 hours but 13% germination did take place in 3 days.

During the first 6 hours of incubation in nutrient solution there is a considerable increase in dry weight of microconidia. The constituents of the nutrient solution were varied in order to find their effect on this initial growth period. Spores for these experiments were taken from the Clone C isolate of Fusarium oxysporum f. cubense (ex. Stover). At the end of the 6 hour growth period microconidia were filtered off from the nutrient on Millipore filters. The filters were then dried overnight at 80° C and weighed, the dry weight being used as a measure of growth rate. Due to the fact that experiments only lasted six hours it was not necessary to use completely aseptic conditions.

The following results are more fully described in the 3d Quarterly Report.

Variations in the concentration of sucrose and ammonium nitrate had no effect on the growth rate of microconidia. The following amino acids were added to the nutrient solution, both individually and combined but with no effect:-

L-valine, L-aspartic acid, L-proline, D-alanine, Dl-isoleucine, L-Tryptophan.

The addition of 2.5 g. per liter Casein hydrolysate however, produced an 18% increase in dry weight. One mg. per liter of the following chemicals were added to the nutrient to provide additional trace elements:- zinc sulphate, copper sulphate, manganese sulphate, ferrous sulphate and boric acid. They were added individually and combined but there were no significant increases in dry weight. A gradual increase in pH, from 2.8 to 7.0 coincided with a gradual increase in dry weight and an addition of yeast extract (as low as 1/1000th %) also showed a marked stimulation.

Replacing the potassium phosphates in the solution with sodium phosphates had no effect, but removing the phosphates altogether and adding potassium as potassium chloride produced a dry weight 32% lower than the control. The growth rate decreased as each of the 5 constituents were removed from the basic nutrient solution.

The microconidia were provided with nitrogen either as ammonium chloride or as potassium nitrate. The spores grown in KNO_3 had a dry wt. of about 27% less than those grown in NH_4Cl or the control (NH_4NO_3). Steinberg (1936,1937) found that Aspergillis niger required more molybdenum when the medium contained nitrate rather than ammonium nitrogen. Experiments were done to see if additional molybdenum might have a similar effect with Fusarium oxysporum f. cubense.

There was no effect at all in the potassium nitrate treatment but in the ammonium chloride treatment there was a 13.3% increase in dry weight over the treatment containing molybdenum.

Conclusions

None.

Recommendations

None.

Prepared by

Alison M. Fletcher

December 1, 1959

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BDF-1-20

Soil Microbiology Section
Annual Project Report 1959

Investigations of Possible Methods for the Selective Isolation of Fusarium Oxysporum f. Cubense

Background

One factor that has handicapped studies on the distribution and relative frequency of F. oxysporum f. cubense in soils has been the lack of selective isolation procedures. Stover (7) considered the natural soil population of the pathogen too low for it to be accurately studied by dilution techniques, and Park (6) has reported similar findings.

A procedure which would permit the selective isolation of F. oxysporum would do much to further studies on the natural distribution of the fungus. The only method presently available for such studies is the method of incubating dilution plates in an atmosphere of CO_2, as suggested by Timonin (8). Since this method is not readily adaptable to field situations, other possible techniques have been investigated.

A. Investigations of extracellular oxidase enzymes of Fusaria.

The production of extracellular oxidase enzymes has been successfully demonstrated for a number of higher fungi (Nobles (4) and Bavendamm (1)) and particularly so in those fungi which cause white rots of wood. White rot fungi are distinguished from brown rot fungi in pure culture by the ability of the white rotters to produce a laccase type enzyme which results in a characteristic discoloration of certain phenolic compounds (Nobles (4)). The classic Bavendamm reaction, used to distinguish white and brown rot fungi, is the production of brown diffusion zone when the fungus is grown on an agar medium containing gallic or tannic acid. Nobles (4) has recently introduced a simplified test for the presence of oxidizing enzymes, which consists of applying an alcoholic solution of gum guaiac directly on the mycelium of the test organism. The production of a blue color is considered as a positive test. She found approximately 90% agreement between her test and the Bavendamm reaction.

To determine whether extracellular oxidase enzymes are produced by various pathogenic and saprobic fusaria, and whether production of such enzymes might be useful in characterizing pathogenic isolates, a series of cultures of Fusaria were tested by both Nobles' test and the Bavendamm reaction.

Thirteen isolates of Fusaria were tested by Nobles' method. All cultur gave a positive reaction, although variation in the speed and intensity of the reaction occurred among cultures. The same cultures were grown on malt agar co taining gallic or tannic acid and the cultures observed for a browning reaction.

Brown diffusion zones were produced by some of the isolates, but the results (in subsequent tests) were erratic and inconsistent. It was thus concluded that while the evidence indicated that certain Fusaria produced oxidase enzymes, the rapid test procedures would not be useful in identifying pathogenic strains.

More refined studies of possible oxidase enzymes were carried out by Dr. E. H. Buckley using culture filtrates from twenty-seven isolates of Fusarium. The filtrates were tested for enzymatic activity colormetrically on a variety of substrates. No characteristic or consistent pattern could be attributed to pathogenic species however, and it was concluded that such methods probably would not be useful for the identification of pathogenic species. Substrates oxidized by the filtrates of Fusaria included catechol, pyrogallol, protocatechuic acid, phloroglucinol, tannic acid, gallic acid, and DL-dopa. D (-) quinic acid, L-tyrosine and resorcinol were not oxidized.

Subsequent studies have indicated that enzyme production is influenced by the medium upon which the fungus is cultured. When Colones B, C, and D of F. oxysporum f. cubense were grown on Czapek's medium, the filtrates possessed no oxidase activity. When glutamic acid was substituted for KNO_3 in this medium, filtrates collected after ten days possessed some activity. In a recent experiment growth and oxidase enzyme production by Clone C were compared when the organism was grown on a basal mineral salts medium with glucose, on the basal medium with malt extract added, and on the basal medium with both malt and yeast extract added. Addition of malt extract produced a 15% increase in dry weight of mycelium; addition of both malt and yeast extract produced a 240% increase in mycelial yield. Results of Warburg studies using filtrates from these cultures are summarized in the following table:

	O_2 uptake after 90 min.
Basic medium	0 ± 3 ul
" " boiled	+2 ± 3 ul
Malt extract	19 ± 3 ul
" " boiled	+5 ± 3
Malt and yeast extract	44 ± 3 ul
" " " " boiled	+ 6 ± 3

Oxidase activity of the basal medium filtrate was negligible. Addition of malt extract resulted in increased enzymatic activity; addition of both malt and yeast extract resulted in 130% increase over the addition of malt extract alone. It is thus apparent that production of oxidase enzymes are influenced by culture conditions, and it seems likely that adaptive mechanisms may be involved. Qualitative and quantitative differences in nitrogen substrate, above minimal levels, apparently are not a factor in production of the oxidase enzyme.

Conclusions

Oxidase enzymes are produced by many isolates of Fusaria. Differences among isolates seem to be quantitative rather than qualitative, and it does

appear likely that production of such enzymes will be a useful tool in the identification of pathogenic Fusaria.

B. Alcohol culture medium.

Nokakavukaren and Horner (3) have recently reported that agar media in which ethyl alcohol is used as the carbon source permits the selective isolation of Verticillium from soil, since this medium promotes the development of microsclerotia characteristic of this species. Several clones of Fusarium oxysporum f. cubense were tested for their ability to grow on a medium in which alcohol was the sole carbon source. Growth occurred at levels up to 6%, but no growth was obtained at levels of 7.5% or above. At levels of 4% or above, growth of the fungus was markedly retarded. Sclerotial formation and production of dark blue pigments occurred after about one month. The pigment is pH sensitive, as was reported by Page (5), and becomes reddish purple when NaOH is added to the culture. Sporulation occurred at all levels where growth occurred.

Alcohol media, ranging from 1 to 4% alcohol content was used to prepare soil dilution plates to determine whether such media were selective for certain fungi. At the 1 and 2% levels abundant growth of bacteria and fungi occurred. Several species of fungi and many bacteria were present at the 3 and 4% levels also; and interestingly, a profuse development of nematodes occurred at the 4% level. No significance is attached to this latter observation, however, and there is no intent to imply that such a medium would be useful for isolating nematodes.

Conclusions

F. oxysporum f. cubense will grow on a mineral salt medium containing alcohol concentrations as high as 6%. Further study will be required to determine whether alcohol media will prove useful or selective in the primary isolation of soil fungi. From the preliminary results it appears essential that bacteriostatic agents be incorporated into such media to reduce the abundant development of bacteria that occurs on soil dilution plates.

C. Media with high osmotic pressure.

According to Lilly and Barnett (2), "an osmotic pressure higher in the fungus cells than in the surrounding host cells is apparently characteristic of the host-parasite relationship". This suggests that perhaps media with high osmotic pressures may be useful in selectively isolating plant pathogens from soil. Preliminary studies investigating this possibility have therefore been undertaken. Studies so far have been restricted to investigations regarding the ability of F. oxysporum f. cubense to tolerate high osmotic pressure. Results to date have indicated that spores of F. oxysporum f. cubense will germinat in three molal sucrose solutions. Growth is very limited. Germination has also occurred when the osmotic pressure of the medium was raised to over 100 atm. by increasing the salt content of the medium.

Conclusions

Preliminary investigations have indicated that F. oxysporum f. cubense will tolerate osmotic pressures of about 100 atm. The possibility that high osmotic pressure media will be selective in the primary isolation of soil fungi remains to be tested.

References

(1) Bavendamm, W. 1928. Uber das Vorbemmen und den Nochweiss von Oxydasen bei halzzerstarenden pilgen. Z. Pflanzenkrankh u. Pflanzenshutz. 38: 257-276.

(2) Lilly & Barnett. 1958. Physiology of Fungi.

(3) Nodakavukaren, M. J. and C. E. Horner. 1959. An alcohol agar medium selective for determining Verticillium micro-sclerotia in soil. Phytopath. 49: 527-529.

(4) Nobles, Mildred K. 1958. A rapid test for extracellular oxidase in cultures of wood-inhabiting hymenonycetes. Can. J. Botany 36: 91-99.

(5) Page, O. T. 1958. Annual Report.

(6) Park, D. 1959. Some aspects of the biology of F. oxysporum Schl. in soil Ann. Botany 28: 23-34.

(7) Stover, R. H. 1956. Behaviour of F. oxysporum f. cubense in different soils and soil extracts. Phytopath. 46: 27-28.

(8) Timonin. 1958. Annual Report.

Prepared by

R. D. Goos

December 1, 1959

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BDF-1-20

Soil Microbiology Section
Annual Project Report 1959

Isolation of Nutritionally Exacting Mutants of Fusarium oxysporum f. cubense.

Background

Cultures of F.o.c. were exposed to gamma radiation in order to determine the lethal dose for the fungus (cf. Irratiation of Button Seeds and F.o.c., Annual Report-1959). Advantage was taken of this study in that the irradiated cultures were examined for the presence of possible nutritionally exacting mutants. Since F.o.c. is a non-fastidious organism, i.e., it apparently has no specific growth requirements, auxotrophic mutants of this fungus would be useful in a variety of genetic and physiological studies.

Progress

Eighteen-day old cultures of F.o.c. (strain D) on PDA slants were irradiated with gamma rays from a Co-60 source, and serial dilution plates were prepared in order to count survivors after irradiation. Cultures which received a dose of 285 krad were used to isolate mutants, since this was the highest level at which sufficient survivors occurred. There were some survivors at the 570 krad level, but the variation between replicate dilution plates was great. One set of duplicate plates from this dose level was, however, included in this study. Dilution plates showing 10-20 discrete colonies were flooded with a small volume of sterile water, the surface of the plates gently scraped to dislodge spores, and the spore suspensions flooded onto plates containing a complete medium. A replicator, similar to that described by Roberts (Roberts, C.F., Jour. Gen. Microbiol. 20:540, 1959) and consisting of two leucite plates holding 41 dressmaker pins at regularly spaced intervals, was used to inoculate replicate plates. After good sporulation had occurred, the replicator pins were pressed into a flood plate and a new complete medium plate, the "charge plate", was inoculated. After the individual colonies had developed, a series of plates containing incomplete media were inoculated from the charge plate with the replicator. The media used were: minimal, Czapek's salts plus 1% glucose; complete, minimal

plus 0.5% vitamin-free casamino acids, 0.1% yeast ribonucleic acid, and various vitamins; complete minus sugar; complete minus amino acids; complete minus nucleic acid; and complete minus vitamins. After two days of incubation at 20°C, the plates were examined. If a colony was absent on any of the incomplete media, a transfer was taken from the replicated colony growing on complete medium and placed on a slant of complete medium. In order to verify the apparent nutritional requirements of these isolates, they were stab-inoculated onto the center of Petri dishes containing one of the following media: complete; minimal plus vitamins; minimal plus amino acids; minimal plus nucleic acids; minimal minus sugar; and minimal.

Thirty-five colonies which did not grow on one of the original incomplete media were isolated. When these colonies were inoculated into the second set of incomplete media, all colonies grew profusely and no difference in growth was observed between the apparent mutants and non-irradiated cultures.

A total of 984 colonies were tested for nutritionally exacting mutants in this study and not a single stable mutant was isolated. The irradiated colonies are now being tested for possible resistance to antibiotics (polymyxin B, actidione, and griseofulvin) and other irradiated cultures are being screened for nutritionally exacting mutants.

Conclusions

None

Recommendations

None

Prepared by

G. Stotzky
R. Goos

December 1, 1959

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BDF-1-30

Physiological-Microbiology Section
Annual Project Report 1959

The Density of Micro- and Macroconidia of Fusarium Oxysporum f. Cubense

Introduction

Obtaining homogenous preparations of spores, i.e. microconidia, macroconidia, and chlamydospores, has been a major concern in physiological studies of these spores. Microconidia may be obtained in homogenous preparations from colonies after 4-5 days' growth. These colonies are from isolates that tend to produce a prevalence of microconidia and generally produce macroconidia in sparse numbers after 10-14 days. Preparations of 80-85 per cent macroconidia may be obtained from pionnotal isolates of this fungus.

Conidia can be obtained free of chlamydospores by harvesting them from young cultures on rich medium before chlamydospores are formed. A method for obtaining a highly homogenous preparation of chlamydospores consists of washing the surface of colonies grown on V-8 juice agar and homogenizing the mycelia that can be peeled from the surface of the medium. The homogenate is filtered several times through a cloth filter. The resulting chlamydospores are then washed by centrifuging them three times in water.

As a method for separating microconidia from macroconidia has not been described, the present study was made to determine if these two spores could be separated on the basis of difference in spore density.

Experiments and Results

Aqueous suspensions of micro- and macroconidia were added as upper layers to aqueous solutions of sucrose (2-70 per cent) in 5.5 cm plastic tubes and then centrifuged for 1 hr. at 0°C., at c.a. 25,000 x g.

Conidia in the upper layer on 20 per cent aqueous solution of sucrose were forced to the bottom of the tubes. Conidia in the upper layer on 50 per cent aqueous solution of sucrose remained at or near the position where they were placed. Between concentrations of 20 and 50 per cent there was a decrease in the number of conidia that occurred in the bottom of the tubes and an increase in the number that occurred in the upper layer of the column (Fig. 1).

Microscopic examination of these conidia showed that micro- and macroconidia occurred in both the upper and lower layers of spores in these solutions.

Conidia germinated on a potato dextrose agar surface after exposure to the centrifugal force and to the osmotic pressures of 70 per cent sucrose.

Discussion

Based on the table of density and viscosity of aqueous sucrose solutions given by de Duve, et al.[1] the density of these spores varies between 1.09 and 1.24. Microscopic evidence indicates that both types of conidia vary within this range of densities.

These observations indicate that separation of microconidia from macroconidia on the basis of difference in densities is not possible.

Recommendations

A method of separation based on difference in shapes of the spores apparently merits consideration.

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1 de Duve, C., J. Berthet and H. Beaufay. 1959. Gradient centrifugation of cell particles. Theory and applications. Progress in Biophysics and Biophysical Chemistry 9: (P.357), 325-367.

Prepared by

Eugene M. Wilson

December 1, 1959

Figure 1. Schematic presentation of results from centrifuging aqueous suspensions of conidia layered at the top of various concentrations of sucrose in water.

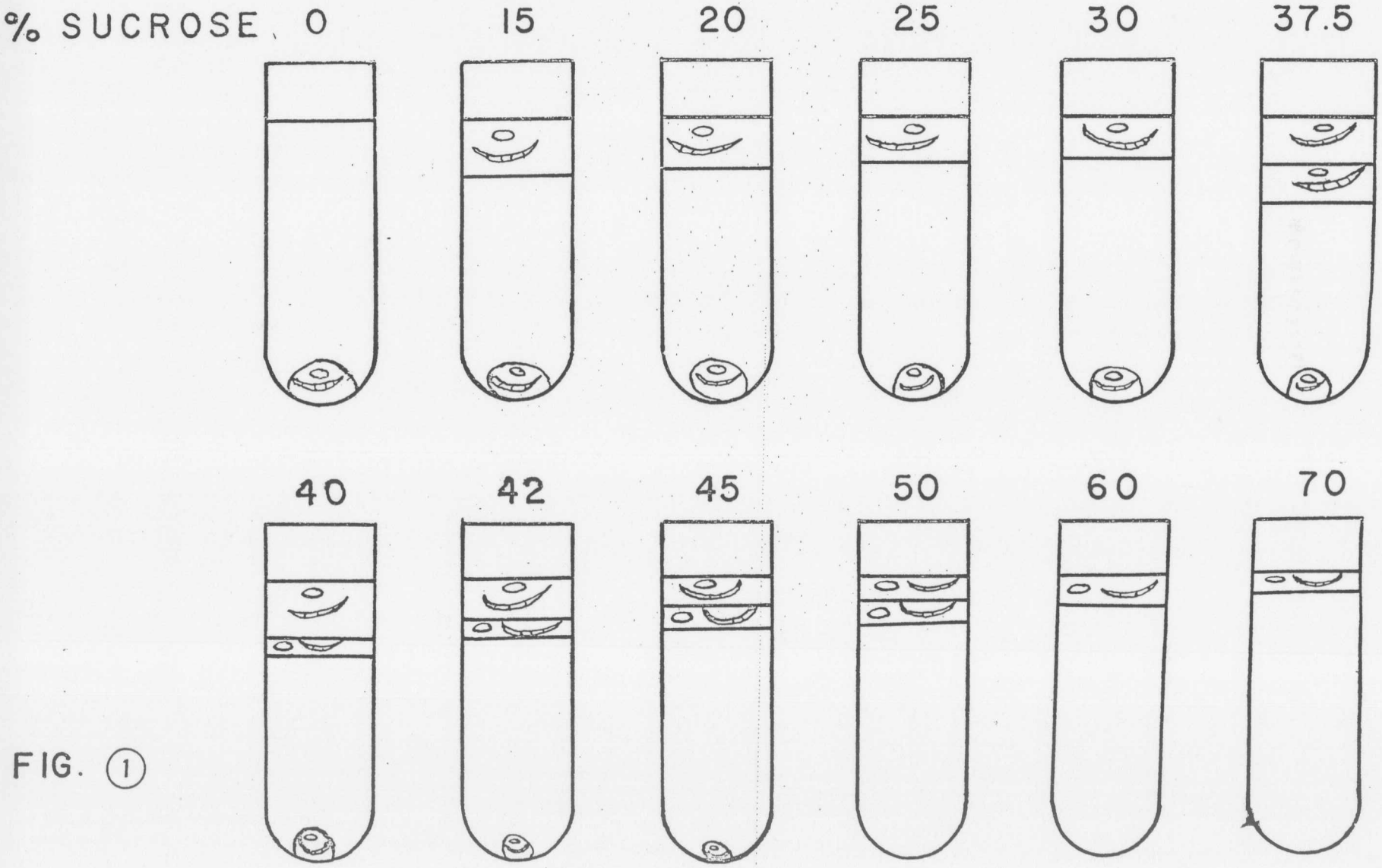

FIG. (1)

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

Physiological-Microbiology
Section
Annual Project Report 1959

BDF-1-40

Observations on Variation in *Fusarium oxysporum* f. *cubense*

Background

According to revisions proposed by Snyder and Hansen (Am. J. Botany 27: 64-67, 1940) 25 plant parasites of the Elegans fusaria were combined into one morphologic species, *Fusarium oxysporum* Schl., amended to agree with the description of section Elegans by Wollenweber (Phytopath. 3: 24-50, 1913). To conveniently systematize parasitic variation within *F. oxysporum*, *formae speciales* were then erected on the basis of pathogenicity *alone*. Thus, f. *cubense* comprises that genetic potential of *F. oxysporum* parasitic on *Musa* spp. In all respects other than pathogenicity, f. *cubense* coincides with *F. oxysporum* as well as all other *formae speciales* within *F. oxysporum*.

The foregoing concept does not resolve the classification of variants within *F. oxysporum* f. *cubense*. If f. *cubense* is culturally identical to *F. oxysporum*, and therefore to the 10 species, 18 varieties, and 12 forms of section Elegans, this admits to considerable potential cultural variation within f. *cubense*. While identification of the species, varieties and forms of section Elegans by cultural attributes generally has been abandoned in favor of host pathogenicity *alone*, the use of cultural features has persisted in the identification of variants of f. *cubense*. Thus, because of cultural differences in isolates of f. *cubense* variants are commonly recognized; subsequent tests of relative virulence of variants are then used to "prove" that the cultural variants were actually different in a pathologic or economic sense. As well, the organism is still commonly maintained and increased in culture as a step in subsequent proof of relative pathogenicity or of some other specific physiological aspect. During the past 40 years emphasis has been placed on odor, pigmentation, and the manner in which spores are borne, as criteria whereby strains of the banana-wilt *Fusarium* have been classified in culture. These criteria and others were examined in the present study in an attempt to clarify the dilemma of strain identification in *F. oxysporum* f. *cubense*.

Progress

Clones used in the present study were obtained from Dr. R. H. Stover, Honduras, and Mr. B. Waite, University of California, Berkeley. The clones obtained from Dr. Stover were designated A, B, C, D, and E, and were described in the Can. J. Botany 37: 245-255, 1959. The clones obtained from Mr. Waite were designated 1, 2, 3, 4, and 5. Clone 1 was a sporodochial type, that is a non-mutated type in which sporodochia were produced separately on the mycelium. Clone 2 was a "first stage" mutant in which sporodochia were less distinct and were produced in masses, if at all. Clone 3 was a white mycelial type without sporodochia and was considered to be very stable and does not mutate to clone 4 or 5. Clone 4 was a pigmented webby mutant, and clone 5 was a pionnotal type having little or no aerial mycelium, slimy in character, and produced readily from clone 4. Transfers of these cultures were maintained on 2% water (Bacto) agar by means of mycelial transfer taken from the margin of Petri-plate cultures.

Progress reported herein is concerned with observations on various cultural aspects used to "identify" clones or variants.

1. Pigment:- Snyder and Hansen (Am. J. Botany 27: 64-67, 1940) state that "Undoubtedly the striking nature of certain cultural characters, especially pigmentation, has overly influenced those who would classify these [fusaria] fungi." Pigmentation has been used in the classification of Elegans fusaria and thus f. cubense. In the present investigation pigmentation of rice agar by 10 variants of f. cubense was studied. Pigmentation on rice has been used in identification of variants of f. cubense during 40 years. When single spores are germinated on rice agar pigment production was first apparent in variants A, B, C, 4, and 5 within 3 days following incubation in darkness at 25°C; slight pigment production was apparent in variant 2 by the 7th day of incubation.

The color of the pigment was observed to vary from the center of an agar plate culture to the outside. The color ranged from blue in the center to purple, red-brown to pink toward the outside of a colony. This range in color was ascribed to pH. It was determined that the pigment in rice agar was red to pH 6.2 - 6.6 and blue at pH 7.0 - 7.2.

It was noted that the aerial mycelium was not visibly pigmented even though the rice agar substrate was darkly pigmented. Mycelium growing on steamed rice in flasks was observed to be stained blue, adjacent to the pigmented substrate, while other aerial mycelium may not be visibly pigmented. Since a sharply defined margin existed between some pigmented and non-pigmented hyphae, such hyphae were examined under oil immersion. Pigment was observed to be superficial and on the wall surface, particularly at the interface where two parallel hyphae were in contact. Although vascuoles in chlamydospores were observed to be pigmented other internal pigmentation of the organism on rice was doubtful.

On rice agar pigment is produced in the substrate, the walls of the rice kernel fragments appearing most darkly colored.

A red pigment was readily extracted from rice agar with acetic acid. Commonly the agar was gently melted and the pigment extracted with glacial acetic acid 2:1 (v:v), HAc:Agar. The red pigment was insoluble to very slightly soluble in water, ethanol, petroleum ether, acetone, chloroform, ethyl acetate, n-butanol, and dilute NaOH; the pigment was soluble in 6 N HCl. The crude pigment was found to contain a water extractable yellow pigment.

Mull and Nord (Archiv. of Biochem. 4: 419-433, 1944) considered that the pigment, rubrofusarin, produced by F. graminearum was either 2,8-dihydroxy-1-methoxy-7-methyl- or 2,3-dihydroxy-8-methoxy-7-methylxanthone. The possible structure was based on comparative spectroscopic studies with other xanthones.

Spectral analyses of the pigment in acetic acid showed absorption maxima at 270 mμ and at 485 mμ. Wolf (Bul. Torrey Bot. Club 82: 343-354, 1955) showed maxima at 265 and 500 mμ for a pigment isolated from F. oxysporum var. nicotianae which was considered to be in agreement with the spectrum obtained by Mull and Nord (1075 and 1350 fresnel) for rubrofusarin.

The intensity of pigmentation of rice agar varied with the variant producing the pigment.

Table I

Relative Density of Pigment Produced by Ten Variants of F. oxysporum on Rice

Variant	Optical density of pigment solution*	
	7 day culture**	21 day culture
Ck. (acid extract of rice agar)	.005	---
A	.91 +++	1.9 +++
B	1.0 +++	2.0 +++
C	1.5 +++	2.0 +++
D	.015 +	.365 +
E	.017 +	.57 +
1	.015 +	.335 +
2	.133 +	.470 +
3	.023 +	.285 +
4	.62 ++	1.2 ++
5	.435 ++	2.0 +++

*Determined at 500 mμ.

**Cultured on rice agar in darkness at 25°C.

It is apparent that variants A, B, C, and 5 are more darkly pigmented than variant 4, which is more darkly pigmented than variants 1, 2, 3, D, and E. While a dichotomy might be indicated if one were in possession of only variants 5 and 3, or C and D, for example, a range in pigmentation more closely characterizes the 10 variants examined.

Pigmentation was used to broadly characterize single-spore colonies developed on rice agar following inoculation with spore dilution taken directly from the host. The technique is limited unless one considers the extremes of pigment range, i.e., red vs. very little pigmentation. In spite of this, so-called white and red variants of F. oxysporum have been used elsewhere in studies of heterocaryon formation and recovery of "original" marked variants.

2. Odor:- The presence or absence of odorous volatiles has been used as a criterion whereby variants of f. cubense have been segregated dichotomously in vitro. If an odor was present attempts were made to define it, frequently by comparison with some familiar odorous substance. As a result, at least 12 odors have been referred to in culturally defining f. cubense.

Unsaturated volatile compounds decolorize aqueous solutions of $KMnO_4$, the rate of color loss depending on solubility and concentration. In these studies, a dilute $KMnO_4$ solution (0.001 N) was used and the gain in opacity to light (425 mμ) was measured colorimetrically. An "odoratum", D, and an "inodoratum", B, variants were cultured on steamed rice, 50 g/150 ml H_2O/500 ml flask at 30°C for 14 days. Flasks were inverted over tubes, each containing 25 ml $KMnO_4$ solution while the permanganate solution was constantly stirred by means of a magnetic stirrer.

Table II

Reduction of $KMnO_4$ by Volatiles Produced by "Odoratum" and "Inodoratum" Variants of F. oxysporum on Rice

Variant	Change in Opacity with Time (minutes)							
	0	15	30	45	60	75	90	105
Control	7.0	7.0	7.0	7.0	7.0	7.0	7.5	7.5
B	7.0	9.5	14.0	20.5	33.0	42.0	55.0	62.0
D	7.0	15.5	28.5	43.0	62.0	76.0	90.0	95.0

The results presented in Table II indicate that an oxidizable volatile compound was evolved by both variants. All 10 variants produced more or less odor which makes untenable the present basis for division of variants of f. cubense into 2 clonal groups or cultivars designated "inodoratum" and "odoratum". If odor could be conveniently characterized, qualitatively and quantitatively, it is apparent that it is an unsuitable criterion in distinguishing between 2, or more colonies in a single culture vessel. The inconvenience of this criterion was evident in assaying variation amongst numerous colonies, arising on dilution plates of spores obtained directly from infected plants.

3. Cultural Interaction:- The phenomenon of "cultural interaction" (Figure 1) has been further examined. Variant 2 was single spored successively 3 times on 2% water agar. The final colony was permitted to grow to approximately 1 cm in diameter and was then hyphal-tipped. Six hyphal-tip transfers were then arranged radially, equidistant from each other, and 35 mm distant from a central point of inoculation. A predictable pattern of "cultural interaction" was induced (Figure 2). Since this pattern is reproducible, i.e., between 12 pairings on each of 3 plates inoculated, it is indicative of similarity between colonies arising from a single spore of variant 2. Cultural interaction has also been demonstrable by pairing single spores of variant 4, and 4.

Cultural interaction may be rather sharply defined as illustrated, or it may be essentially indistinguishable, colonies overgrowing each other without apparent mycelial synergic response. "Cultural interaction" has been used as a diagnostic tool to distinguish between variants. In the example presented the response has been demonstrated within a variant. Since "cultural interaction" may occur when either like or apparently unlike variants are paired, its use as a diagnostic tool in distinguishing variants is questionable.

4. Ninhydrin Patterns:- The failure to satisfactorily classify variants by classical criteria led to the application of paper partition chromatographic technique in the assay of ninhydrin reactive metabolites. It was hoped that qualitative and quantitative differences in the production of such metabolites, i.e., free amino acids, might permit

differentiation of variants. From a previous investigation of fusaric acid production, it was apparent that quantitative differences existed among strains in the production of this organic acid. By examination of 8 or 10 substances simultaneously on a chromatogram, it was considered to be possible to "fingerprint" a variant.

Although culture techniques were modified from time to time, the technique to be described was found satisfactory. Because of the unequal evaporation losses from plugged flasks, and the time required for production of adequate quantities of amino acids in a relatively larger volume of culture solution, flask cultures were abandoned in favor of micro cultures. Accordingly, tubes 1.0 cm (O.D.) x 4.5 cm were charged with 0.5 ml of the following medium: 50 g d-glucose, 10 g NH_4NO_3, 5 g KH_2PO_4, 2.5 g $MgSO_4 \cdot 7H_2O$, 20 mg $FeCl_3 \cdot 6H_2O$ and distilled water to 1 liter. After autoclaving, tubes were inoculated with single spores of variants which had been maintained on 2% water agar. In a typical experiment, at least 10 replications of each of 10 variants were incubated in a water bath in darkness at 25°C for 10 days. Micro tubes were not plugged during incubation but were held erect in a covered vessel.

Ten microliter aliquots of neat filtrate from each culture were spotted on Whatman No. 1 filter paper. Chromatograms were irrigated with n-butanol:acetic acid: water (4:1:5, by volume) for 16 hours (descending), dried, and irrigated a second time. The usual precautions were taken to insure standard conditions. Circular, ascending and 2-dimensional chromatographic techniques were also used when convenient.

While admissably one-dimensional separation does not permit accurate qualitative evaluation, reproducible differences in ninhydrin reactive patterns are illustrated in Figure 3. Such patterns suggest that the variants are quantitatively different and identifiable. However, further research has indicated that while variants may exhibit quantitative differences, such differences are not sufficiently distinct to permit positive identification. This is illustrated in Figure 4 in which 9 single-spore isolates of a single variant, cultured under identical conditions, exhibit a range in ninhydrin pattern. By examination of

several hundreds of such patterns, it was considered that the range was such as to preclude positive separation of variants.

The free amino acids produced by the 10 variants examined appear to be qualitatively similar.

The free amino acids within the mycelium and in the filtrate are listed in Table III.

Table III

Free Amino Acids in Culture Filtrate and Mycelium of F. oxysporum

Amino Acid	Filtrate	Mycelium
Alanine	+++*	+++
Arginine		+
Aspartic acid	+	+
Cysteic acid	+	
Glutamic acid	++	+++
Glutamine		+
Glycine	+	+
Leucine (Isoleucine)	+	
Lysine	++	+
Phenylalanine		+
Proline	+	
Serine	++	
Threonine	+	++
Valine	++	+++
Unknown I	+++	
Unknown II	+	

*Relative to ninhydrin reaction spot.

The unknowns found in filtrates of all clones on heating with ninhydrin gave first a yellow color, then grey and finally purple as per peptides in which glycine carries a free amino group. Both unknowns fluoresced yellow under ultraviolet.

5. Miscellaneous:- Variant B exhibited a marked tolerance to zinc when grown on an agar medium with soluble starch as the principal carbohydrate source. Whereas the mean colony diameter of 9 other variants was 5.0 mm after 5 days' incubation, the colony diameter of the B variant was 33.0 mm on a level of 160 ppm $ZnSO_4$. There is no conclusive evidence that B variant is pathogenic to Gros Michel banana.

Conclusions

1. Odor was produced by all variants investigated making untenable the category "inodoratum".

2. "Cultural interaction" occurred when single-spore cultures of variant 2 were paired. "Cultural interaction" indicates similarity and is thus of doubtful value in differentiating pairs of supposedly different variants.

3. Assays of pigment and amino acids, diffused from variant thalli of single-spore origin, indicate that variants overlap markedly. It is not evident how isolates of F. oxysporum from banana can be placed into well-defined categories by any of the criteria investigated.

Recommendations

1. It is recommended that the classificatory system which incorporates the concept of degradative variation, from the sporodochial to the pionnotal condition, be used, until proven unsatisfactory.

2. Because of the importance of strain identification in f. cubense, it is urged that the study be continued.

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December 1, 1959

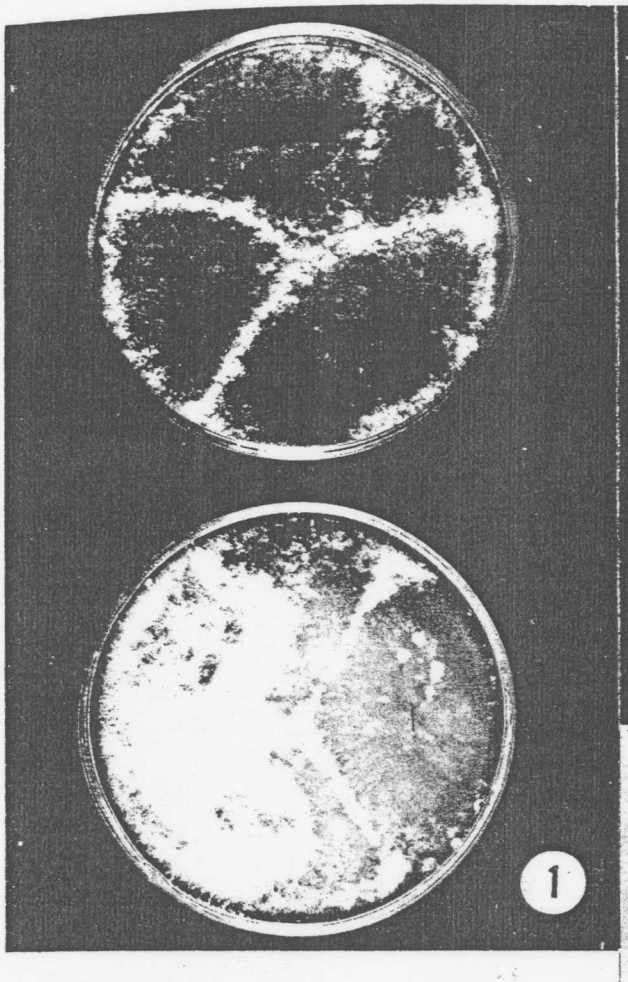
Legends to Plates

Figure 1. "Cultural interaction" occurring among colonies growing out of tracheary elements. Three tracheary elements were placed in each plate.

Figure 2. Predictable pattern of "cultural interaction" induced by arranging hyphal tips from a single-spore thallus of F. oxysporum.

Figure 3. Pattern of ninhydrin reactive substances obtained by application of duplicate volumes from single culture of each of 2 variants.

Figure 4. Chromatogram (blueprint) showing variation among single-spore isolates of variant 5 grown under identical conditions.



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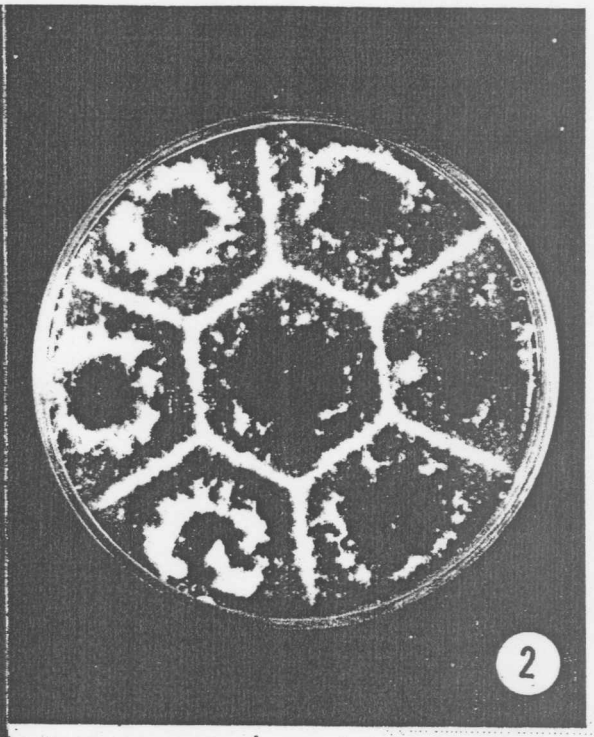

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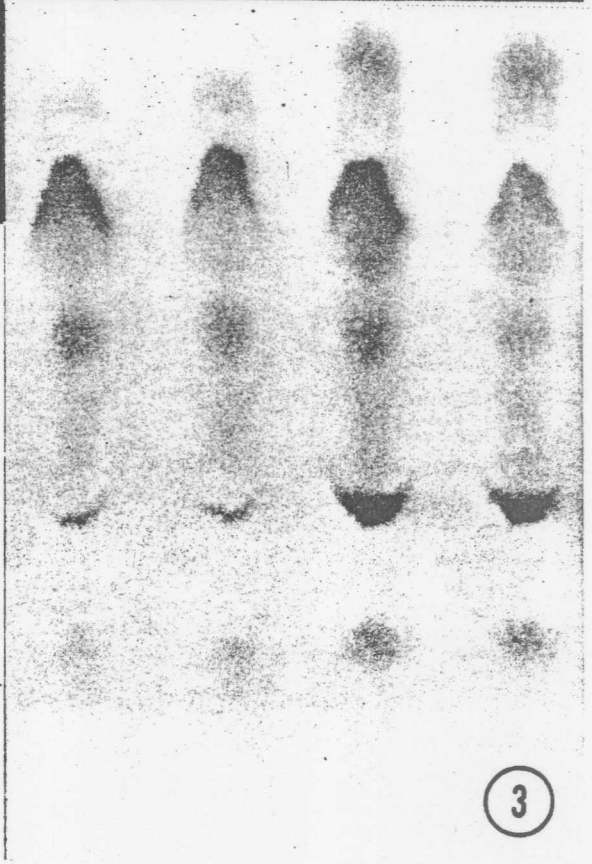

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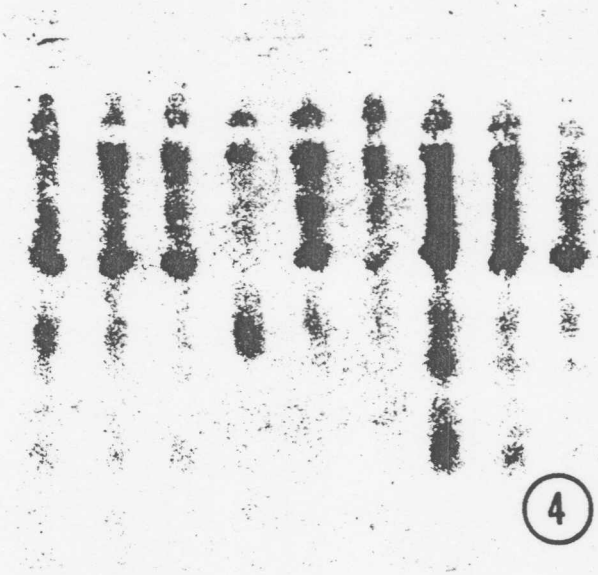

4

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BDF-1-50

Soil Microbiology Section
Annual Project Report 195

Antibiotic Screening Program

Background

A number of antibiotic compounds have been tested for activity against F. oxysporum f. cubense and Pseudomonas solanacearum using the assay disk method. The compounds tested were obtained from the Case Company, who supply the antibiotics as assay disks of "high" and "low" concentrations.

Progress

Tests with Fusarium were carried out as Czapek's agar, those with Pseudomona on a casein hydrolysate - glucose - peptone medium. Results with the "high" concentrations disk are summarized in Table I.

TABLE I

No.	Compounds	Conc. (High)	Diameter of Inhibition Zone* Fusarium	Pseudomonas
1	Streptomycin (dihydro)	10 mcg.	----	Slight
2	Kanamycin	30 mcg.	----	15 mm
3	Penicillin	10 units	----	-----
4	Novobiocin	30 mcg.	----	15 mm
5	Sulfamerazine	300 mcg.	Slight	35 mm
6	Isonicotinic Acid Hydrazide	25 mcg.	----	-----
7	p-aminosalicylic acid	100 mcg.	----	-----
8	Viomycin	10 mcg.	----	-----
9	Terramycin	30 mcg.	----	15 mm
10	Neomycin	30 mcg.	----	Slight
11	Erythromycin	15 mcg.	----	14 mm
12	Furoxone	100 mcg.	----	-----
13	Thiosulfil	300 mcg.	----	37 mm
14	Mandelamine	3 mg.	----	27 mm
15	Sulfathiozole	300 mcg.	----	40 mm
16	Sulfamethoxypyridazine	300 mcg.	Slight	38 mm
17	Mycostatin	100 units	----	Slight
18	Oleandomycin	15 mcg.	----	Slight
19	Carbomycin	15 mcg.	----	Slight
20	Aureomycin	30 mcg.	----	25 mm
21	Chloromycetin	30 mcg.	----	15 mm
22	Elkosin	300 mcg.	----	33 mm
23	Bacitracin	10 units	----	-----
24	triple sulfa	300 mcg.	Slight	35 mm
25	Ristocetin	30 mcg.	----	-----
26	Furadantin	100 mcg.	----	-----

No.	Compounds	Conc. (High)	Fusarium	Pseudomonas
27	Polymixin B	300 units	9 mm	-----
28	Furacin	100 mcg.	----	15 mm
29	Sulfadiazine	300 mcg.	Slight	30 mm
30	Tetracycline	30 mcg.	----	25 mm
31	Gantrisin	300 mcg.	Slight	35 mm

* Assay disk diameter equal 7 mm. Measurement is for diameter of zone across assay disk.

Conclusions

None.

Recommendations

None.

Prepared by

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CENTRAL RESEARCH LABORATORIES
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BDF-2-0

Soil Microbiology Section
Annual Project Report 1959

Survey of the Microorganism of Banana Soils: Use of Soil Immersion Tubes.

Background

Dilution plate methods of enumerating soil fungi isolate primarily species that sporulate abundantly or which are rapid growers. The species developing on dilution plates may not be physiologically active at the time of sampling, but may be present abundantly only as inactive spores, resulting in misinterpretation of the fungal ecology of soil.

Various techniques have been explored for the purpose of enumerating the fungi which are active in soil metabolic processes, and these have resulted in lists of species which are not frequently isolated from soils by dilution techniques (1, 2, 3). One such procedure employs tubes filled with an appropriate medium, and with small openings in the sidewalls (2). Fungi growing through soil grow into the holes and are isolated from the tubes by subsequent sub-culturing. This technique has shown some success in the isolation from soil of plant pathogens, such as Rhizoctonia solani, which, though present abundantly in some soils, are seldom isolated on dilution plates prepared from these soils (4).

As part of a general survey of the microflora of banana soils, soil immersion tubes were used in an attempt to 1) isolate fungal species not obtained on soil dilution plates, 2) derive some information on the species actively growing in these soils, and 3) determine if the type of fungi in the root zone differs with variety of bananas.

Progress

Bored plastic test tubes, 19 x 110 mm., covered with electrical tape (4), and containing one of the following agars: A) water, B) Martin's, and C) Harmsen's cellulose were placed at depths of (1) 0-4, (2) 6-8, (3) 14-18, (4) 28-32, and (5) 38-42 inches in the root zones of Lacatan, Grand Nain, and Gros Michel bananas located in the variety plots in Guaruma II Farm, La Lima. Only one plant of each variety was used due to the limited number of immersion tubes available. The tubes were left in contact with the soil for two days, then wrapped in aluminum foil and flown to the Norwood Laboratories. Prior to analysis the tubes were placed in a deep-freeze for 10 days. Exposed sections of the agar cores were sub-cultured on Martin's medium and the fungi identified. Soil adjacent to the immersion tubes was collected and sent to Norwood in polyethylene bags for analysis by dilution plate technique.

The fungi obtained by the latter procedure have not yet been identified, and a comparison of species isolated by the two techniques is not possible at this time.

Table 1 shows the species of fungi and the media on which they were isolated from the immersion tubes. Table 2 presents the total number of fungi isolated by the dilution plate method from the soils adjacent to the immersion tubes.

This was a preliminary experiment, not yet completed, and no conclusions can justifiably be drawn. Several observations, however, can be mentioned. No fungi not commonly found on soil dilution plates were isolated in the immersion tubes. On the other hand, Trichoderma, Gliomastix, Aspergilli, and Penicillia which were profusely present on the soil dilution plates were absent in the immersion tubes. This suggests that these species probably were not actively growing in the soils, but were present primarily as spores. Storage of the soils may have increased sporulation (cf. Fungi of virgin forest areas of Farm 55, Annual Rpt. 1958 and Microbiological investigations of "long" and "short" life soils, Annual Rpt. 1959). Identification of the fungi isolated from the soil dilution plates should clarify these assumptions. These tentative results agree in general with those obtained by Stover. (5).

Conclusions

None.

Recommendations

Further investigations are warranted.

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Table 1. Type and frequency of fungi isolated on various media from different depths of soils supporting three varieties of bananas.

VARIETY:	LACATAN					GRAND NAIN					GROS MICHEL				
DEPTH:	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
	Medium on which fungi were isolated														
FUNGUS															
Fusarium solani	AB*	A				AB				A	ABC		A		C
Mucor sp.	A	AB				C	A	B	C	A					
Cladosporium sp.								A							
Cephalosporium sp.	B									A					
Curvularia lunata								A	C						
Penicillium sp.						A									
Fusarium sp.											A				
Monilia sp.										C					
Unidentified	B												A		
No growth	C	C	ABC	ABC	ABC		BC	C	AB	B		ABC	BC	ABC	AB

* See text for definition of numbers and letters.

Table 2. Vertical distribution of fungi in soils supporting three varieties of bananas.

Depth	Lacatan	Grand Nain	Gros Michel
	No./g. soil		
0-4"	113,600	109,000	113,000
6-8"	131,000	8,600	192,900
14-18"	37,000	*	13,700
28-32"	5,100	4,300	**
38-42"	19,300	30,000	2,500

* All plates overgrown with colonies of Trichoderma.

** All plates overgrown with colonies of Trichoderma and Gliomastix.

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

Soil Microbiology Section
Annual Project Report 1959

BDF-2-10

Studies of Rhizosphere Fungi in Long-life and Short-life Soils.

Background

As a result of investigations into the relationships of soil types and the occurrence of Fusarium wilt, the concept of "long-life" (resistant) and "short-life" (susceptible) soils has developed. According to the review by Stover (4), various investigators have attempted to relate differences in these soils to such factors as soil pH, soil texture, drainage, or to other physical and chemical properties of the soil. The present investigation has been undertaken to determine, if possible, whether or not differences in these soils might not be reflected in, or related to, the microbial population present in such soils. The present report constitutes a preliminary summary of a study made in Omonita Farm.

The soils of Omonita Farm are classified as long-life soils. They are of alluvial origin, of sandy loam texture and excellent friability, even to depths of 40 inches. This farm has been in continuous banana production for many years. Omonita Farm was selected for this study since it seemed probable that the organism found in association with banana roots in such long established plantings may perhaps be considered typical of those normally associated with bananas. It is believed that a study of the fungal populations of an area such as Omonita Farm will provide data which will be of value in future comparative studies since it will aid in the characterization of the rhizosphere and root surface fungi in established banana plantings.

It should be noted that insects of the genus Scaptocoris were found in the vicinity of the roots used in this study, particularly in the case of roots collected below the 10 inch level. Timonin (5) has shown that volatile vapors produced by these insects have an adverse effect on soil fungi in culture, and it is possible that their presence may have had some effect on the activity of fungi in these soils.

Progress

Roots were collected from a mature healthy banana mat on July 15 and 16, 1958. A 4' x 4' excavation was made on one side of the mat, and as the roots were exposed, they were removed

with adhering soil and placed in sterile culture tubes. Roots were collected at four different levels as shown diagrammatically in Figure 1. At each level, collections were made of both fleshy main roots and of fibrous lateral roots in order that microbial populations around the two types of roots could be compared. At the surface and at the 10" level, it was possible to collect lateral roots from the same root from which a fleshy root sample had been taken. At the lower depths, however, this was not possible due to the scarcity of lateral roots. Therefore, collections of fibrous roots at 27" and 36" levels represent composite samples removed from several different fleshy roots. It was possible to determine the distance from the root apex at which the fleshy root samples were taken for collections at the upper layers, but such determinations could not be made for roots at deeper levels since it was not possible to follow these roots to their growing points.

The type of root, and the level at which collected, are summarized below:

A. Fleshy root segment taken approximately 10 cm. from the root tip, from surface layer.

B. Fibrous lateral rootlets of the fleshy root from which Sample A was taken.

C. Mature area of the same fleshy root from which Sample A was taken, removed approximately 1 foot from the rhizome.

D. Fibrous lateral roots, from the same fleshy root from which Sample C was taken, removed approximately 12 inches from the rhizome.

E. Fleshy root segment, from the 10" level, about 15 cm. from the root apex.

F. Fibrous lateral roots from the same fleshy root from which Sample D was taken.

G. Segment of a fleshy root collected at the 27" level.

H. Segment of a fleshy root collected at the 36" level.

I. Fibrous lateral roots collected at the 27" level.

J. Fibrous lateral roots collected at the 36" level.

Each sample was handled according to the following procedure: The roots with adhering soil were weighed, after which they were placed in 250 ml. flasks containing 100 ml. of sterile water and washed. Washing consisted of five minutes of shaking by hand. The roots were removed to sterile toweling, blotted dry, and weighed. Weight of the rhizosphere soil was determined by evaporating the soil suspension to dryness at 105° C., and subtracting the weight of the previously tared flask from that of the flask and dried soil. No correction was made for the soil removed in the preparation of the dilution series.

The washed, weighed roots were next placed in a 250 ml. flask containing 100 ml. of sterile water and 10 gms. of white sand. The roots were given a five-minute wash in the water-sand mixture by shaking gently with a circular motion by hand.

Following the sand wash, the roots were removed to sterile Petri dishes. The cortex was removed from the fleshy root segments by means of a flamed scalpel. The cortical and stelar tissue were then macerated separately in 100 ml. of sterile water in a Waring blender. Since the fibrous roots are too delicate to permit removal of the cortical tissue, these roots were macerated directly upon removal from the sand wash. The rhizosphere soil suspension, the sand wash suspension, and the macerated roots suspensions were used to prepare dilution plates.

Control soil samples were obtained at each level at which collections were made. These consisted of a sample of approximately 20 gms. in which no banana roots were growing. Samples were also collected for the purposes of determining soil moisture content at the different soil levels.

Dilution plates for fungal counts were prepared with Martin's rose bengal agar; plates for bacterial and actinomycete counts were prepared with soil extract agar (1% glucose, 1% yeast extract, 900 ml. water, 100 ml. soil extract, prepared by heating 1000 gm. soil in 1 liter of water, and filtering). All plates were incubated at 30° C. in the dark. Fungal counts were made after 4 days of incubation; bacterial and actinomycete counts after 1 week.

Fungal isolations were made from a dilution plate of each root or soil sample. A plate which contained 20-30 colonies, and which appeared to be representative of the fungi present in that sample, was selected, and all colonies present were transferred to agar slants. When several fungi of the same species were encountered in the same plate, and were readily identifiable as the same species a single isolation was made and the number of colonies of that type was recorded. Identification work was done with cultures grown on oatmeal agar, Czapek's agar, glucose-peptone agar or malt-peptone agar.

Results

The numbers of organisms occurring at the differing sampling sites is presented in Tables I, II, III, and IV. In general, they fall within the ranges reported for temperate soils (1). The number of organisms declines with the increasing depth of the sample, as is generally reported in studies of this type. By comparing the number of organisms present in Samples A and C, and B and D, it is readily apparent that the numbers are higher in older portions of the root. If Table I and Table IV are compared, it is also evident that higher counts were obtained from rhizosphere soil than from control soil.

One would have expected that few, if any, organisms would have been isolated from decorticated stelar tissue. However, rather high counts were obtained, and it seems probable that these tissues were contaminated during removal of the cortex.

A total of 268 fungi were isolated in this study; of these, 81 have been only tentatively identified or are forms which have failed to sporulate and cannot be identified. A list of the fungi identified and the sample or samples from which they were isolated is given in Tables V, VI, VII, and VIII.

It can be seen in Table V that certain fungi were found only on the root surface or in macerated roots, while others were found only in rhizosphere soil. Certain others, e.g. Cephalosporium, Cladosporium, were isolated from all three sample sources. The root surface, or rhizoplane fungi, and the possible root-inhabiting fungi are of particular interest, since these organisms occur in the environment in which pathogenic fungi must successfully compete if they are to invade the root. It is of interest that fungi such as Fusarium episphaeria and Cylindrocarpon were found only in the rhizoplane or in macerated roots, and were not found in rhizosphere soil. Other fungi, e.g. Penicillium lilacinum, Trichoderma viride, were found in the rhizosphere and rhizoplane samples, but were not found in macerated roots. Many of the organisms isolated from macerated roots (Phoma, Pyrenocheate, Periconia, Fusarium episphaeria) belong to genera which have been reported as root parasitic fungi. It is possible that they may be present as parasites on banana roots although no evidence was obtained to indicate that this is the case. It would appear, on the basis of their limited occurrence, that these fungi may be root-inhabiting fungi although further studies are required before such conclusions can be justified.

Certain fungi were isolated from all sampling depths. These include Absidia, Verticillium theobromae, and Phialophora. Phialophora sp. was particularly abundant at the lower levels.

Other fungi occurred in fairly large numbers at a single sampling site. Of particular interest is the isolation of large numbers of Schizophyllum commune from a single sampling site. Basidiomycetes are rarely isolated in dilution plates, but in this instance a large number of colonies of this particular fungus were found at the 10 inch level. Isolations were obtained from soil samples as well as from macerated roots.

Three species of Fusarium were isolated: F. roseum, F. episphaeria and F. solani, the latter being most abundant. Two fungi which appear to be identical with species considered by Wardlaw (6) as pathogens on aerial parts of banana were isolated: Verticillium theobromae (Syn. Stachlydium theobromae) has been reported to be pathogenic on banana fruit and Chloridium musae causes a leaf spot of banana, according to Stahel (3). No attempt has been made to determine pathogenicity of these isolates.

The majority of fungi isolated are common soil fungi, and are included in Gilman's Manual of Soil Fungi. Some have been found which are not reported by Gilman. Of the fungi listed, those which have not previously been reported from soil include: Chloridium musae, Pseudoarachniotus reticulatus, Schizophyllum, Verticillium theobromae, Fusicladium and Absidia sp. Absidia sp. is considered by Hesseltine (personal communication) to represent a new species. Pseudoarachniotus reticulatus has been described as a new species (2).

It was the intention of this study to determine the types of fungi present in the rhizosphere soil of long-established banana plantings. Most of the fungi isolated are ubiquitous in soil, but certain forms isolated do not appear to have been isolated from soil previously. Since most studies of soil fungi have been confined to temperate soil, this is not surprising. Doubtlessly, a detailed study of tropical soil fungi would reveal many new species. The isolation of fungi which have been reported as pathogens on the aerial parts of bananas indicates that these fungi may survive in the soil, and that soil and decaying organic matter may serve as an inoculum reservoir for certain of these pathogens.

Conclusions

None.

Recommendations

Additional studies of this type will be required to determine whether certain fungal species are typical banana root inhabitants. Since it is on the root surface that pathogenic organisms must successfully compete, further study of the root surface population seems desirable.

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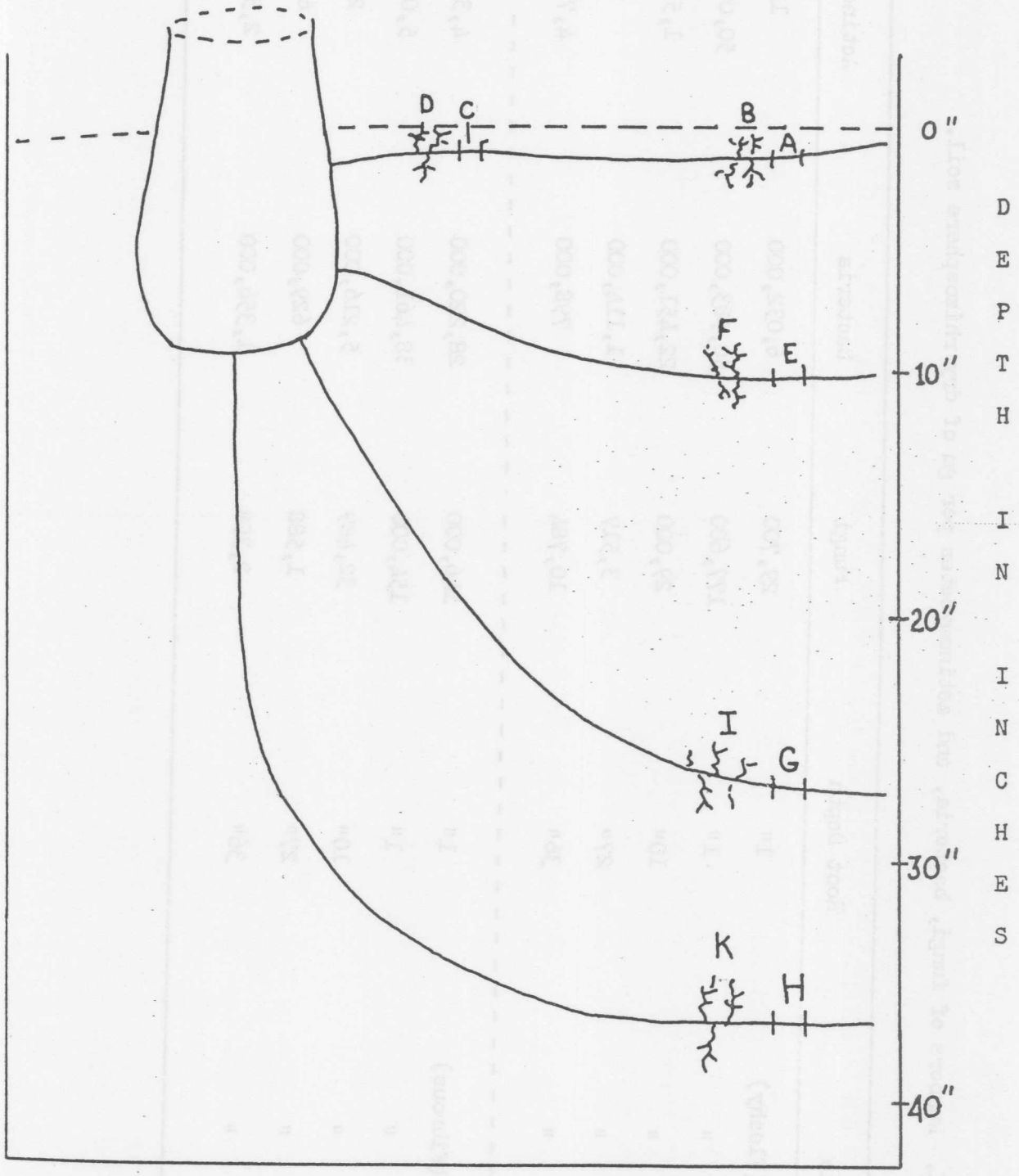

FIG. 1 ... SKETCH OF SAMPLING PROCEDURE

Table I. Numbers of fungi, bacteria, and actinomycetes per gm of dry rhizosphere soil.

Sample	Root Depth	Fungi	Bacteria	Actinomycetes
A (Fleshy)	1"	29,700	6,032,000	143,000
C "	1"	177,600	50,583,000	50,000,000
E "	10"	29,000	22,451,000	1,500,000
G "	27"	3,517	1,114,000	96,300
H "	36"	10,784	798,000	4,776,000
B (Fibrous)	1"	146,000	28,220,000	4,387,000
D "	1"	154,000	36,461,000	5,054,000
F "	10"	32,489	5,216,000	261,000
I "	27"	1,588	629,000	655,000
K "	36"	2,358	1,356,000	2,322,000

Table II. Number of fungi, bacteria and actinomycetes at the root surface (per gm root tissue).

Sample	Root Depth	Fungi	Bacteria	Actinomycetes
A (Fleshy)	1"	1,175	710,000	31,000
C "	1"	2,960	1,600,000	52,000
E "	10"	2,400	108,000	7,600
G "	27"	278	1,400	1,000
H "	36"	640	48,000	38,600
B (Fibrous)	1"	5,000	7,350,000	262,000
D "	1"	18,000	7,550,000	358,000
F "	10"	10,900	3,300,000	83,500
I "	27"	700	283,000	2,900
K "	36"	560	78,000	250,000

Table III. Numbers of fungi, bacteria and actinomycetes from macerated root tissue.

Sample	Root Depth	Root Weight (gms.)	Total			Cortex			Stele		
			Fungi	Bacteria	Actino-mycetes	Fungi	Bacteria	Actino-mycetes	Fungi	Bacteria	Actino-mycetes
A (Fleshy)	1"	4	180	8,600	0	150	4,600	0	30	4,000	0
C "	1"	6	2,460	9,605,000	0	1,760	860,000	0	700	2,945,000	0
E "	10"	3	10,600	467,400	0	5,740	455,000	0	4,880	12,400	0
G "	27"	3.9	480	263,500	0	480	260,000	0	0	3,500	0
H "	36"	3.7	2,434	683,700	28,800	2,337	682,500	26,000	97	1,200	2800
B(Fibrous)	1"	2	12,300	3,135,000	20,000	-	-	-	-	-	-
D "	1"	1.95	2,780	73,000	0	-	-	-	-	-	-
F "	10"	1.2	4,560	2,406,000	136,000	-	-	-	-	-	-
I "	27"	0.97	800	108,000	0	-	-	-	-	-	-
K "	36"	1.7	1,040	1,040,000	387,000	-	-	-	-	-	-

Table IV. Number of fungi, bacteria, and actinomycetes in control soils.

Depth	% moisture	Fungi	Bacteria	Actinomycetes
1"	27.9	47,660	3,600,000	850,000
10"	24.3	10,350	1,375,000	450,000
27"	23.7	3,830	1,275,000	116,000
36"	25.3	3,014	725,000	183,000

Table V. Fungi isolated from Omonita Farm, and their occurrence in rhizosphere soil, root surface and macerated roots.

	Rhizosphere soil	Root surface	Macerated roots
Absidia sp.	X	X	
Aspergillus flavipes	X	X	
Aspergillus terreus	X		
Aspergillus versicolor			X
Aspergillus sp.	X	X	
Cephalosporium sp.	X	X	X
Cladosporium sp.	X	X	X
Chloridium musae		X	X
Coniothyrium sp.	X		
Cunninghamella elegans	X		
Cylindrocarpon sp.		X	X
Fusarium episphaeria		X	X
Fusarium roseum	X		
Fusarium solani	X	X	X
Fusicladium sp.	X	X	X
Fusidium sp.		X	X
Geotrichum candidum	X	X	X
Gliocladium	X		
Gliomastix convoluta	X		
Gonytrichum macrocladum	X		
Graphium sp.	X		

	Rhizosphere soil	Root surface	Macerated roots
Acremoniella echinata		X	
Myrothecium sp.		X	
Nodulisporium			X
Penicillium lilacinum	X	X	
Penicillium sp.	X	X	X
Periconia sp.			X
Phialophora sp.	X	X	X
Phoma sp.	X		X
Pseudoarachniotus reticulatus	X		
Pyrenocheate		X	
Schizophyllum commune	X	X	X
Scopulariopsis brevicaulis			X
Spicaria sp.	X		
Sporotrichum sp.	X		
Stachybotrys atra	X		
Stagonospora sp.	X	X	
Torula sp.		X	
Trichoderma viride	X	X	
Verticillium theobromae	X	X	

Table VI. Fungi from rhizosphere soil.

	A	B	C	D	E	F	G	H	I	K
Absidia sp.	1	X	X	X			5		3	
Aspergillus terreus	X			1						
Aspergillus flavipes				X						
Aspergillus sp.			X	X						
Cephalosporium sp.	10	1	1	4	1					1
Cladosporium sp.		3	2	5	X					
Coniothyrium sp.		1		2						
Cunninghamella elegans		X		X						
Fusarium roseum	X									
Fusarium solani	1	1	1	1						
Fusicladium sp.	1	1	1							
Geotrichum candidum	1		1	1		1				
Gliocladium sp.	A									
Gliomastix convoluta	2									
Gonytrichum macrocladum			1							
Graphium sp.					3			1		
Penicillium lilacinum	X	3	5	5						
Penicillium sp.				1		2				
Phialophora sp.		1		1			2	1	3	1
Phoma sp.	X			2		1				
Pseudoarachniotus reticulatus										1

	A	B	C	D	E	F	G	H	I	K
Schizophyllum commune					3	5				
Spicaria sp.			1							
Sporotrichum sp.		1								
Stachybotrys atra				2						
Stagonospora sp.	X									
Trichoderma viride	X	X	X	X	X					
Verticillium theobromae		3	4			1				1
Unidentified	2		4	4	5	5	4	6	2	

The number given indicates the number of colonies of a particular species on the single dilution plate sampled. Colonies identified from other plates are designated with an X.

Table VII. Root surface fungi.

	A	B	C	D	E	F	G	H	I	K
Absidia sp.		X								
Aspergillus flavipes		X								
Aspergillus sp.						X				
Cephalosporium sp.	3	1			X					
Chloridium musae					1	1				
Cladosporium		1		3	2					
Cylindrocarpon						1				
Fusarium episphaeria		1	1							1
Fusarium solani	1	X								
Fusicladium sp.	2									
Fusidium	1									
Geotrichum candidum			1							
Memnoniella echinata	1									
Myrothecium sp.	1									
Penicillium lilacinum	X	1	1	1						
Penicillium sp.	X	4			1					
Phialophora sp.							5	13	1	
Pyrenocheate sp.	1			3						
Schizophyllum commune						8				
Stagonospora sp.			1							
Torula sp.	X									
Trichoderma viride		1								
Verticillium theobromae	X	1	2	1						
Unidentified	4			3	4	4	4	1		3

Table VIII. Fungi from macerated roots.

	A	B	C	D	E	F	G	H	I	K
Aspergillus versicolor			5							
Cephalosporium sp.				1						
Chloridium musae								X		
Cladosporium sp.	X		1	1						
Cylindrocarpon sp.				1	X					
Fusarium episphaeria			2							
Fusarium solani				1		1				
Fusicladium sp.	X									
Fusidium sp.			1							
Geotrichum candidum				1						
Nodulisporium sp.								5		
Penicillium sp.		43	2	1						
Periconia sp.			1							
Phialophora sp.			1					X		8
Phoma sp.					X					
Schizophyllum commune					X	1				
Scopulariopsis brevicaulis		11								
Unidentified	2			2	3	3	2	3	1	1

The frequency of isolation of different species of fungi from the four depths after storage is presented in Table 5. A total of 139 fungal colonies, representing 36 genera and 43 species, were isolated from soil dilution plates. With the exception of Stemphylium sp. and Heterosporium terreste, all the fungi identified have previously been isolated in this laboratory from tropical soils. The highest frequency of isolation was found with the heavily sporulating genera such as Aspergillus, Penicillium, and Trichoderma. The distribution of most species differed with depth in the profile, but the greatest variety in species was observed in the surface soil.

The distribution of bacteria with different nutritional requirements for maximum growth is presented in Table 6. Classification of the bacteria was based on the methods proposed by Lochhead et al. (8,9). The classification of the bacteria present in the soil after three months of storage is not yet complete, and the data on the distribution of the nutritional groups immediately after sampling are not available at the time of writing. Nevertheless, several observations should be mentioned. The large percentage of bacteria requiring amino acids for maximum growth is contrary to the results obtained with temperate region soils (9). This group of bacteria is usually small in non-rhizosphere soil, such as used in this study, but high in rhizosphere soil. On the other hand, bacteria requiring growth factors are high in non-rhizosphere temperate soils, whereas they were low in this soil. Relatively few bacteria requiring soil extract were isolated, and some depressive effect of soil extract was observed. Very few bacteria required Vitamin B_{12} for maximum growth. The number of bacteria which required yeast extract increased with depth. The significance of these observations cannot be determined from the limited data available. The peculiar distribution of nutritional groups may be the result of storage.

The type and distribution of algae in the stored soils is presented in Table 7. Primarily blue-green algae were isolated since the medium used was relatively specific for this class. Inaddition to the algae listed, fern prothalli and moss protonema developed in several of the dilution tubes prepared from all four depths. Spores of ferns and mosses were probably carried in and deposited with the original alluvium or else were leached through the soil during heavy surface water regimes. It was necessary to incubate the dilution tubes for three months under artificial and natural light in order to achieve the counts of algae presented in Tables 1-4. A diverse variety of blue-green algae was found. The high number present in the surface soil suggests that algae may be important in nitrogen fixation.

Soil dilution plates (soil extract medium and Martin's medium) were incubated for 18 days in 99.9% CO_2 in order to determine the number and types of microorganisms capable of developing under conditions of anaerobiosis and high CO_2 tension. After incubation in CO_2, the plates prepared from the stored soils were incubated aerobically for one week in order to de-

termine if the microflora was killed or only inhibited. Exposure to CO_2 killed approximately 95 % of the soil population, but the surviving 5% resumed growth after exposure to air. (Table 8). Actinomycetes did not develop in CO_2, but did grow when the plates were subsequently incubated in air. The fungi in the stored soils that were developed in CO_2 and those that were developed in air after exposure to CO_2 are presented in Tables 9 and 10, respectively. Several species of fungi were capable of growth in the environment of anaerobiosis and high CO_2 tension, in agreement with the work of Bisby et al. (2,3). These results suggest that the identification of Fusaria isolated from soils by incubation in CO_2 must be accompanied by morphological investigations. The fungi isolated after exposure to CO_2 were also found on the plates incubated aerobically (Table 5), with the possible exception of two species whose identification is tentative.

This study is not completed since a comparison of fungal species and bacterial groups between the two sampling times is yet to be made. However, certain conclusions can be suggested at this time. The marked decrease in most groups of microorganisms and the large increase in the number of actinomycetes during storage may explain the fungistatic properties of certain soils for Fusarium oxysporum f. cubense observed in the laboratory. Investigators concerned with biological control of soil-borne plant pathogens, organic matter or nitrogen transformations, or other studies involving the soil microflora should recognize the probability that the microflora is both qualitatively and quantitatively different in the laboratory than under natural field conditions. The increase in the number of fungi developing on soil dilution plates prepared with stored soils has been observed several times and is probably the result of increased sporulation caused by the environmental changes.

The reasons for changes during shipping and storage are not presently known. The loss of moisture during storage (Table 11) was small and probably not responsible since the soils after storage appeared moist and friable. The moisture constants for this soil have not yet been determined. The increase in the relative distribution of spore-forming bacteria and of bacteria capable of developing in CO_2 suggests that restricted gas exchange may be a causative factor. The decrease in the number of fungi capable of developing in CO_2 is not necessarily contradictory to this hypothesis since the spores of fungi are not as sensitive to changes in gas relationships as are vegetative cells. Fungal spores may not germinate and grow as rapidly in CO_2 as bacterial spores and cells and hence there may have been less opportunity for the selection of CO_2-tolerant fungi. Table 8 shows that a larger percentage of fungi than bacteria were capable of developing after exposure to CO_2, and Table 10 shows that a greater number of fungal species developed after than during exposure to CO_2. The diffusion rates of CO_2 and O_2 through a polyethylene film 1 mil thick were reported to be 2900 and 550 c.c/100 sq. in./24 hrs., respectively (11). These rates of dif-

fusion would be sufficient to insure adequate gas exchange during storage. Nothing is known about the gas diffusion rates through the plastic bags used to store these soils; they were not polyethylene and appeared to be slightly thicker than 1 mil.

Table 11. Change in moisture content of a Garcia virgin soil three months after sampling in the field.

Soil depth (inches)	Moisture content of soil (%)		Loss in moisture (%)	% Decrease
	original	After 3 months		
0 - 6	25.70	21.78	3.92	15.25
6 - 18	19.96	17.03	2.93	14.68
18 - 24	26.93	23.69	3.24	12.03
24 - 36	16.86	14.19	2.67	15.84

Another possible explanation for the observed changes is that substrates required for active microbial growth were depleted during storage (12). Under field conditions, the level of carbon in the soil is continually replenished by root secretions and sloughing, leaf drop, etc. Most of the undecomposed soil organic matter was removed from these soil samples during the screening prior to shipping. Once the available substrate in the stored soil was depleted, probably only those organisms capable of forming spores or of existing at a low metabolic level persisted. Experiments have been designed to test this possibility. Stored soils will be incubated with added banana trash, or bananas will be grown in these soils prior to microbial analysis in an attempt to restore the microbiological equilibrium. The microbial activity of soils prior to and after storage will be investigated. Studies will also be conducted on different methods of shipping and storing soils, e. g., air-drying prior to shipping, open vs. closed containers, temperature control.

Conclusions

The data show conclusively that marked changes occurred in the soil samples during storage. These changes and their implications are briefly discussed.

Recommendations

It is recommended that this type of experiment be repeated several times with both "short" and "long" life soils. Comparisons should be made of the types of changes occurring in soils

of different productive life.

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Table 1. Distribution of microorganisms in a Garcia virgin soil immediately after sampling in the field and after three months of storage. Profile depth: 0-6 inches.

Type of organism	Immediately after sampling		After 3 months storage		Changes (%)	
	No./g. soil	% Distribution	No./g. soil	% Distribution	Numbers	% Distribution
Total microflora	58,600,000	---	22,400,000	---	-62	---
Total bacteria	45,600,000	77.8 [a]	3,700,000	16.5 [a]	-92	-79
Spore-forming bacteria	8,100,000	17.8 [b]	1,600,000	43.1 [b]	-80	+142
Bacteria developing in CO_2	10,000	0.02 [b]	1,200	0.03 [b]	-88	+50
Total fungi	300,000	0.5 [a]	280,000	1.2 [a]	-7	+140
Fungi developing in CO_2	4,700	1.6 [c]	3,000	1.1 [c]	-36	-31
Actinomycetes	12,700,000	21.7 [a]	18,400,000	82.1 [a]	+45	+278
Cellulose decomposers	1,300,000	2.2 [a]	76,200	0.3 [a]	-94	-86
Pectin decomposers	---	---	1,600,000	7.0 [a]	---	---
Nitrate reducers	290,000	0.5 [a]	180,000	0.8 [a]	-38	+60
Denitrifyers	95,600	0.2 [a]	22,300	0.1 [a]	-77	-50
Nitrifyers (To NO^-_2)	47,400	0.1 [b]	23,600	0.6 [b]	-50	+500
Nitrifyers (To NO^-_3)	46,500	0.1 [b]	5,300	0.1 [b]	-89	0
Protozoa	207	---	14	---	-93	---
Algae (Blue-green)	---	---	402	0.002 [a]	---	---

[a] /% of total microflora [b] /% of total bacteria [c] /% of total fungi

Table 2. Distribution of microorganisms in a Garcia virgin soil immediately after sampling in the field and after three months of storage. Profile depth: 6-18 inches.

Type of organism	Immediately after sampling		After 3 months storage		Changes (%)	
	No./g. soil	% Distribution	No./g. soil	% Distribution	Numbers	% Distribution
Total microflora	11,000,000	---	6,500,000	---	-41	---
Total bacteria	7,500,000	68.1 [a]	1,400,000	21.5 [a]	-81	-68
Spore-forming bacteria	1,800,000	24.0 [b]	800,000	57.1 [b]	-56	+138
Bacteria developing in CO_2	4,700	0.06 [b]	550	0.04 [b]	-88	-33
Total fungi	5,200	0.05 [a]	12,600	0.2 [a]	+142	+300
Fungi developing in CO_2	110	2.1 [c]	50	0.4 [c]	-55	-81
Actinomycetes	3,400,000	30.9 [a]	5,100,000	78.4 [a]	+50	+154
Cellulose decomposers	62,000	0.6 [a]	21,500	0.3 [a]	-65	-50
Pectin decomposers	---	---	260,000	4.0 [a]	---	---
Nitrate reducers	35,600	0.3 [a]	75,000	1.2 [a]	+111	+300
Denitrifyers	23,600	0.2 [a]	23,600	0.4 [a]	0	+100
Nitrifyers (To NO^-_2)	9,700	0.1 [b]	2,100	0.2 [b]	-78	+100
Nitrifyers (To NO^-_3)	20,400	0.3 [b]	940	0.1 [b]	-95	-67
Protozoa	4	---	3	---	-25	---
Algae (Blue-green)	---	---	17	0.0003 [a]	---	---

[a] / % of total microflora [b] / % of total bacteria [c] / % of total fungi

Table 3. Distribution of microorganisms in a Garcia virgin soil immediately after sampling in the field and after three months of storage. Profile depth: 18-24 inches.

	Immediately after sampling		After 3 months storage		Changes (%)	
Type of organism	No./g. soil	% Distribution	No./g. soil	% Distribution	Numbers	% Distribution
Total microflora	5,700,000	---	9,900,000	---	+74	---
Total bacteria	4,600,000	80.8 [a]	2,000,000	20.2 [a]	-57	-75
Spore-forming bacteria	1,300,000	28.2 [b]	900,000	45.0 [b]	-31	+60
Bacteria developing in CO_2	3,500	0.08 [b]	1,900	0.1 [b]	-46	+25
Total fungi	3,000	0.05 [a]	6,700	0.1 [a]	+123	+100
Fungi developing in CO_2	74	2.5 [c]	17	0.3 [c]	-77	-88
Actinomycetes	1,100,000	19.2 [a]	7,900,000	79.7 [a]	+618	+315
Cellulose decomposers	80,000	1.4 [a]	28,400	0.3 [a]	-65	-79
Pectin decomposers	---	---	55,100	0.6 [a]	---	---
Nitrate reducers	44,400	0.8 [a]	63,900	0.6 [a]	+44	-25
Denitrifyers	13,600	0.2 [a]	14,500	0.1 [a]	+7	-50
Nitrifyers (To NO^-_2)	1,400	0.03 [b]	22	0.001 [b]	-98	-97
Nitrifyers (To NO^-_3)	1,700	0.04 [b]	85	0.004 [b]	-95	-90
Protozoa	9	---	0	---	-100	---
Algae (Blue-green)	---	---	10	0.0001 [a]	---	---

[a] / % of total microflora [b] / % of total bacteria [c] / % of total fungi

Table 4. Distribution of microorganisms in a Garcia virgin soil immediately after sampling in the field and after three months of storage. Profile depth: 24–36 inches.

Type of organism	Immediately after sampling		After 3 months storage		Changes (%)	
	No./g.soil	% Distribution	No./g.soil	% Distribution	Numbers	% Distribution
Total microflora	4,300,000	---	6,700,000	---	+56	---
Total bacteria	3,700,000	88.0 [a]	840,000	12.5 [a]	-77	-86
Spore-forming bacteria	150,000	4.0 [b]	200,000	23.8 [b]	+33	+485
Bacteria developing in CO_2	1,200	0.03 [b]	2,700	0.3 [b]	+125	+900
Total fungi	3,700	0.09 [a]	7,200	0.1 [a]	+95	+11
Fungi developing in CO_2	31	0.8 [c]	140	1.9 [c]	+352	+138
Actinomycetes	510,000	11.9 [a]	5,900,000	88.0 [a]	+1057	+640
Cellulose decomposers	21,000	0.5 [a]	28,900	0.4 [a]	+38	-20
Pectin decomposers	---	---	8,000	0.1 [a]	---	---
Nitrate reducers	130,000	3.02 [a]	57,400	0.9 [a]	-56	-70
Denitrifyers	10,400	0.3 [a]	4,200	0.1 [a]	-60	-67
Nitrifyers (To NO^-_2)	3,900	0.1 [b]	9	0.001 [b]	-100	-99
Nitrifyers (To NO^-_3)	1,500	0.04 [b]	24	0.003 [b]	-98	-93
Protozoa	1	---	1	---	0	---
Algae (Blue-green)	---	---	11	0.0002 [a]	---	---

[a] / % of total microflora [b] / % of total bacteria [c] / % of total fungi

Table 5. Vertical distribution and frequency of isolation of fungi in a Garcia virgin soil which was stored for 3 months after sampling in the field: Fungi developing aerobically on soil dilution plates.

Fungus	Depth in Profile (in.)			
	0-6	6-18	18-24	24-36
Acrostalagmus sp.	---	---	---	1
Alternaria sp.	---	1	---	---
Aspergillus luchuensis	1	---	4	1
Aspergillus niger	2	---	---	---
Aspergillus oryzae	---	3	2	10
Aspergillus sp.	7	1	3	1
Cephalosporium sp.	5	3	2	---
Chaetomium globosum	---	---	---	2
Chaetomium seminudum	---	1	---	---
Chloridium sp.	---	---	---	2
Cladosporium herbarum	1	---	2	1
Coniothyrium fuchelii	1	---	---	---
Cunninghamella sp.	2	2	1	---
Curvularia lunata	---	---	2	1
Cylindrocarpon sp.	---	2	---	---
Fusarium roseum	1	---	1	---
Fusarium solani	2	2	1	1
Geotrichum candidum	2	2	---	---
Gliocladium sp.	1	---	---	---
Gliomastix convoluta	1	---	---	1
Gonytrichum macrocladum	1	---	---	---
Graphium sp.	---	1	---	---
Heterosporium terrestre	1	---	---	---

Fungus	Depth in Profile (in.)			
	0-6	6-18	18-24	24-36
Humicola sp.	0	2	0	0
Illosporium sp.	1	0	0	0
Metarrhizum sp.	1	0	0	0
Myrothecium sp.	1	2	0	0
Mucor sp.	0	0	0	2
Penicillium lilacinum	5	5	5	1
Penicillium oxalium	0	0	1	3
Penicillium sp.	3	2	14	8
Phoma glomerata	1	0	0	0
Pseudobotrytis terrestris	1	0	0	0
Pyrenocheate sp.	1	0	0	0
Rhizopus stolonifera	1	0	3	2
Sporotrichum	0	0	0	1
Stagonospora	1	0	0	0
Stemphylium sp.	1	0	0	0
Stilbum (rhizomorphic)	0	0	2	1
Stilbum sp.	1	0	1	0
Trichoderma viride	1	2	6	3
Verticillium sp.	1	0	1	0
Volutella sp.	0	0	1	0
Unidentified	6	14	3	2

Table 6. Vertical distribution of bacteria in a Garcia virgin soil which was stored for 3 months after sampling in the field: Bacteria isolated from soil dilution plates and classified on basis of nutritional requirements for maximum growth.

Nutritional requirement for maximum growth	Depth in Profile (in.)			
	0-6	6-18	18-24	24-36
	% of total bacteria isolated			
Grow in basal medium (B)	15	9	9	not yet determined
Require amino acids(A)	31	29	26	
Require growth factors (G)	8	5	3	
Require amino acids and growth factors (AG)	23	26	11	
Require yeast extract (Y)	11	18	37	
Require soil extract (S)	0	6	4	
Require yeast extract and soil extract (YS)	12	6	7	
Require yeast extract and Vitamin B_{12} (YB_{12})	1	1	3	

Table 7. Vertical distribution and frequency of isolation of algae in a Garcia virgin soil which was stored for 3 months after sampling in the field: Algae isolated in Fogg's blue-green algae medium.

ALGA	Depth in Profile (in.)			
	0-6 (15)*	6-18 (10)*	18-24 (7)*	24-36 (5)*
	No. of tubes			
Blue-green				
Nodularia (filamentous)	5	7	6	2
Anabena (filamentous)	1	1	0	1
Nostoc (filamentous)	4	0	0	1
Chroococceus	0	0	0	1
Lyngbya	3	0	0	0
Plectonema	1	0	0	0
Symploca	4	0	0	0
Synechococcus	0	1	0	0
Gloeocapsa	1	0	0	0
Green				
Chlorella	4	0	0	1
Golden (diatom)	5	1	0	0

* Number of tubes showing positive growth at a dilution of 1:10 soil: water. Maximum possible number was 15.

Table 8. Vertical distribution of microorganisms in a Garcia Virgin soil which was stored for 3 months after sampling in the field: Microorganisms developing after an 18-day exposure of soil dilution plates to CO_2.

Type of organism	Measurement	Depth in profile (in.)			
		0-6	6-18	18-24	24-36
Bacteria	No./g. soil[a]	117,000	36,000	45,000	21,000
	% distribution	3.2	2.6	2.3	2.5
Fungi	No./g. soil[b]	16,000	350	320	260
	% distribution	5.7	2.8	4.8	3.7
Actino-mycetes	No./g. soil[c]	840,000	261,000	341,000	212,000
	% distribution	4.6	5.2	4.3	3.6

[a] % of total bacteria [b] % of total fungi [c] % of total actinomycetes

Table 9. Vertical distribution of fungi in a Garcia virgin soil which was stored for 3 months after sampling in the field: Fungi developing during an 18-day exposure of soil dilution plates to CO_2.

Fungus	Depth in profile (inc.)			
	0-6	6-18	18-24	24-36
Aspergillus sp.	+	-	-	-
Cladosporium herbarum	+	+	-	-
Cunninghamella sp.	-	+	-	-
Fusarium solani	+	+	+	+
Geotrichum candidum	+	-	-	-

Table 10. Vertical distribution of fungi in a Garcia virgin soil which was stored for 3 months after sampling in the field: Fungi developing after an 18-day exposure of soil dilution plates to CO_2.

Fungus	Depth in Profile (in.)			
	0-6	6-18	18-24	24-36
Acremonium sp. ? ***	-	- *	+ *	-
Aspergillus niger	+	-	-	-
Aspergillus oryzea	-	-	-	+
Aspergillus sp.	+ **	-	-	+
Chaetomium sp.	-	-	-	+
Cladosporium herbarum	+ **	+ **	-	-
Cunninghamella sp.	-	+ **	-	-
Fusarium solani	+ **	+ **	+ **	+ **
Geotrichum candidum	+ **	+	-	-
Penicillium sp.	+	-	+	+
Pseudobotrytis terrestris	+	-	-	-
Rhizopus sp.	-	-	+	-
Stagonospora sp.	+	-	-	-
Trichladium sp. ? ***	+	-	-	-

* +: presence; -: absence

** Species which developed in CO_2 (cf. Table 9).

*** Species not isolated on aerobic plates (tentative identification)

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Norwood, Mass.

BDF-2-10

Soil Microbiology Section
Annual Project Report 1959

Microbiological Investigations of "Long" and "Short" Life Soils: Clay Studies

Background

The correlation of effective banana production life with physical and chemical properties of soils has been attempted ever since Fusarium wilt became a major disease problem. Early investigators found some correlation between texture, pH, soil moisture and aeration, the content of soluble salts, especially potassium, and disease spread. These correlations were used with some degree of success as guides for the selection of new planting sites. In most cases, there were more exceptions than conformities to the correlations. Recently, the Cornell group again reported a high correlation between the extractable soil potassium content and soil life on a number of soils (Cornell Annual Report-1957-58). The relationship between soil potassium content and longevity is very often attributed to some effect of potassium on the relative resistance of the host or on the development of the pathogen. While potassium does appear to be associated with disease resistance in several plant species, data from both field and laboratory studies indicate that in short life soils potassium is not limiting for the active development of either the banana plant or the fungus. Therefore, since potassium does not appear to be involved directly in the host-parasite relationship in bananas, it is possible that the soil potassium level is only a reflection of the source, type, and amount of soil clay. The Cornell group recently subjected to mineralogical analysis, i.e., exchange capacity, differential thermal analysis, x-ray diffraction, petrographic analysis, several soils which showed a potassium to life relationship. The analyses separated the soils into two distinct groups: Group A had a relatively low exchange capacity, contained abundant mica, and the clays were primarily kaolinitic; Group B contained clays that were primarily of the hydrous mica type (illite-montmorillonite), had a higher exchange capacity than Group A, and a low content of mica but appreciable amounts of volcanic glass. The soils of Group A were short

life soils which were low in potassium; Group B soils were long life and high in potassium. These results confirmed the idea that perhaps the spread of F.o.c. in soils is related to the type of clay, of which potassium content is but an index.

Most, if not all, of the microbial activity in soils probably occurs within the water films that surround the individual soil particles and in the films formed in the smaller soil capillaries. The microbial activity is, therefore, strongly influenced by the amount and strength of the electrostatic forces arising from the soil clays in this environment. The effects of adsorption capacity of clays on microbial activity in soil are multifold; e.g., clays adsorb intact microorganisms, exoenzymes, substrates, reaction products, antibiotics or fungistatic materials. (See Esterman, E. F. and McLaren, A. D., Jour. Soil Sci. 10:64-78, 1959, for a review of the literature on this subject.) These reactions undoubtedly play an important role in the microbial ecology of soil.

As a result of the apparent correlation between soil life and the type of clay in these soils, and because of the marked influences of clays on microorganisms, a research project was initiated to study the effects of clays on the development and spread of Fusarium oxysporum f. cubense.

Progress

This project was just recently initiated and major emphasis so far has been directed primarily toward the development of techniques. To date, only pure, but not homoionic, kaolinite and bentonite clays have been used in these experiments. Spore suspensions of F.o.c. have been incubated in shake and static cultures with various concentrations of clay, and the effect of clay type and concentration on spore germination and growth of the fungus studied. The presence of clay does not appear to effect the germination of spores, but does effect growth of the organism. The relationship between clay concentration and growth is not linear; at certain concentrations, growth is enhanced, while at others it is restricted. Since only preliminary experiments have been conducted to date, further presentation of, and comments on, results are not warranted.

Conclusions

None

Recommendations

Based on the high correlation between soil-life and mineralogical characteristics of the few soils studied to date, it is strongly recommended that the validity of this correlation be thoroughly investigated. Mineralogical properties would probably be more reliable guides than pH, texture, potassium content, etc. of soils for the selection of new planting sites, and the differences between short and long life soils may be explainable on the basis of the soil minerals. Mineralogical analysis should, therefore, be made a part of the present soil sampling program. Analytical data of this type would greatly aid in studies, which are presently being conducted, of the relationship between the spread of Fusarium wilt and mineral composition of soil.

Prepared by

G. Stotzky

December 1, 1959

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Soil Microbiology Section
Annual Project Report 1959

Response of Fusarium Oxysporum f. Cubense when Grown in the Presence of Other Fungi

Background

The competitive and possibly antagonistic effects of the rhizosphere microflora upon pathogenic organisms has generally been regarded as being a significant factor affecting the spread and incidence of soil-borne pathogens. Eaton and Rigler (1946) found that maize plants grown in a sterile substrate were susceptible to Phymatotrichum root rot, while similar plants grown in non-sterile soil appeared to be resistant. They attributed the differences in susceptibility of the two groups of plants to the effects produced by rhizosphere organisms. Antagonisms between micro-organisms in pure culture are relatively common (DeVay, 1956); but whether such effects also occur in soil is at present a disputed point.

The present study was undertaken to determine the response of Fusarium oxysporum f. cubense when grown in association with fungi isolated from the rhizospher of bananas.

Progress

One hundred forty-eight isolates (not species) were tested for possible antagonism to Fusarium oxysporum f. cubense, Clone C, on glucose-peptone medium using the "T" streak method. The response of Fusarium to the test fungus appeared to fall into one of six categories:

1. The test fungus grew over the Fusarium
2. Fusarium overgrew the test fungus (Fig. 1)
3. No overgrowth by either fungus and no inhibitory response by either (Fig.
4. Mutual inhibition (Fig. 3)
5. Fusarium grows up to the test fungus but growth of lateral hyphae is inhibited (Fig. 4)
6. Fusarium does not grow up to the test fungus (Fig. 5)

Production of a distinct inhibitory zone between the test fungus and Fusarium (response 6) was considered as the highest degree of inhibition; however, inhibitory effects may be evident in other ways. For example, in Fig. 4 are shown cultures in which no inhibitory zone was produced, but lateral spread of Fusarium seems to have been prevented. This is also evident in plate C, Fig. 3, and in plate B, Fig. 5. This suggests that the production of an inhibitory zone is only one of several possible criteria by which to interpret the results of such studies.

The organisms eliciting response 5 & 6 from Fusarium were selected for further study. The "antagonists" were grown in liquid Czapek's solution for five or twelve days, depending on growth rate of the fungus. The filtrates were then collected, reduced to $\frac{1}{2}$ volume by evaporation, and the concentrated filtrates tested for inhibitory effects on Fusarium. Some filtrates produced a depression of growth, but none

were distinctly inhibitory. It thus appears doubtful that the inhibition noted in the "T" culture tests was due to the production of an antibiotic substance.

The same test fungi used for the filtrate tests were tested for competitive ability by growing them in association with Fusarium in soil. Twenty-five gms of unamended, sterile garden loam, moistened with 10 ml of water, was inoculated with a single conidium of Fusarium and with a mass transfer of the test fungus. The cultures were incubated for sixteen days, after which the growth of Fusarium was examined by dilution plate technique at a dilution of 1/10,000. Since the flasks were inoculated with a single conidium, multiple Fusarium colonies could be attributed to growth of the organism. Results of this study are presented in Table I.

TABLE I

Test Fungus	Range in Fusaria Numbers	Mean No. Fusarium	Relative Amt. of Test Fungus
B201	———	—	———
A127	27 - 112	60	xxxx
B101	3 - 24	12	x
B202	0 - 8	2	xxxx
A224	26 - 60	41	xxxx
D303	31 - 71	43	xxxx
A216	43 - 112	69	———
D110	22 - 60	41	xxxx
C106	25 - 77	49	x
D104	0	0	xxxx
C401	40 - 95	69	xxxx
E108	66 - 157	128	———
E113	15 - 48	29	xxxx
F307	70 - 130	104	———
E404	42 - 115	74	———
S301	17 - 88	45	xx
E112	0	0	xxxxx

Control (Fusarium only) 72 - 101

Dilution 1:10,000

From these results it appears that only cultures E112 (Aspergillus fischeri) D104 (Aspergillus terreus), B101, (phialophora sp.), and B202 (Absidia sp.) produce a significant competitive effect on Fusarium in soil. These results must be considered only as tentative, however, since it is possible that the single conidium inoculum may not have established a Fusarium colony. In several instances (A216, C106, E108, F307, and E404) Fusarium seems to have been very successful in competing with the soil isolate, since the soil fungus appears to have been greatly restricted in its growth.

Conclusions

The question can now be raised as to which aspect of competition may be more significant in soil ecology. Of the 17 cultures shown to be somewhat competitive or antagonistic to Fusarium in petri dish culture, only four appeared to have a significant inhibiting effect in soil while, in five instances, Fusarium appears to have overcome the test fungus. In the remaining eight trials both fungi appeared to have grown and multiplied. Definite conclusions are unwarranted on the basis of this limited study; but the results do suggest that inhibitive or competitive effects between fungi in soil may not be a primary factor affecting their distribution and occurrence in soil. Further studies are essential before such conclusions can be justified, however.

Recommendations

None.

Reference

Eaton, F. and N. Rigler. 1946. Jour. Agr. Res. (Engl.) 72: 137-161

Prepared by

R. D. Goos

December 1, 1959

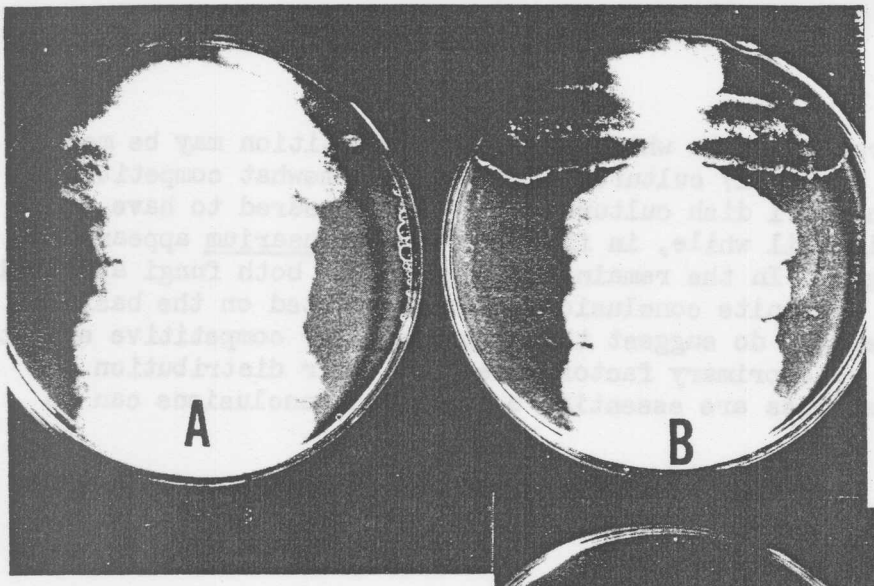

FIG. 1

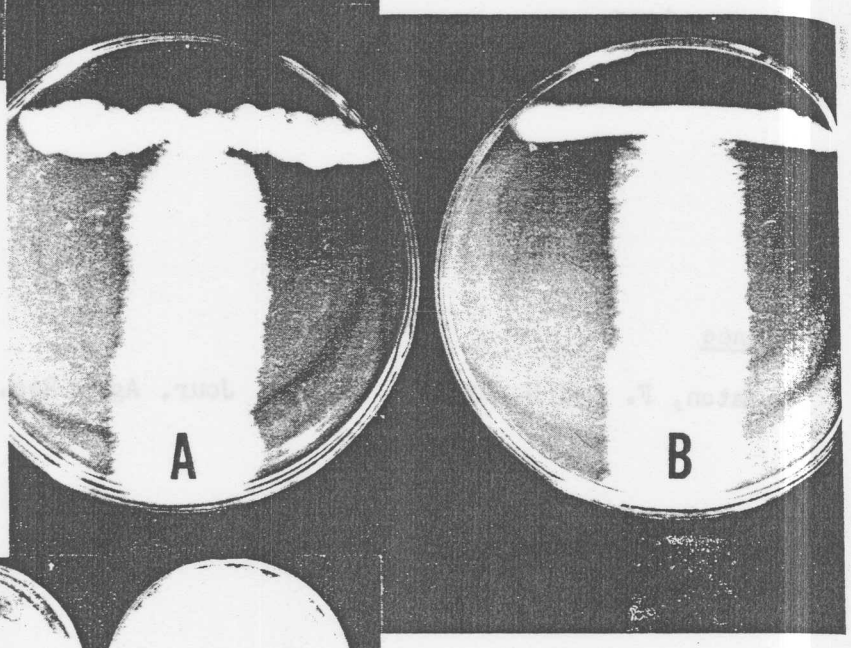

FIG. 2

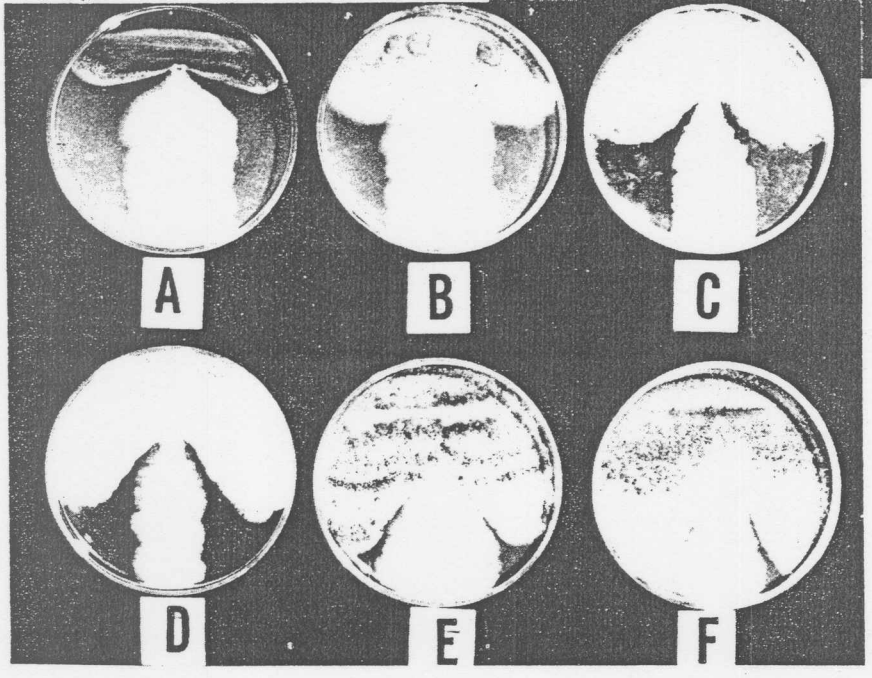

FIG. 3

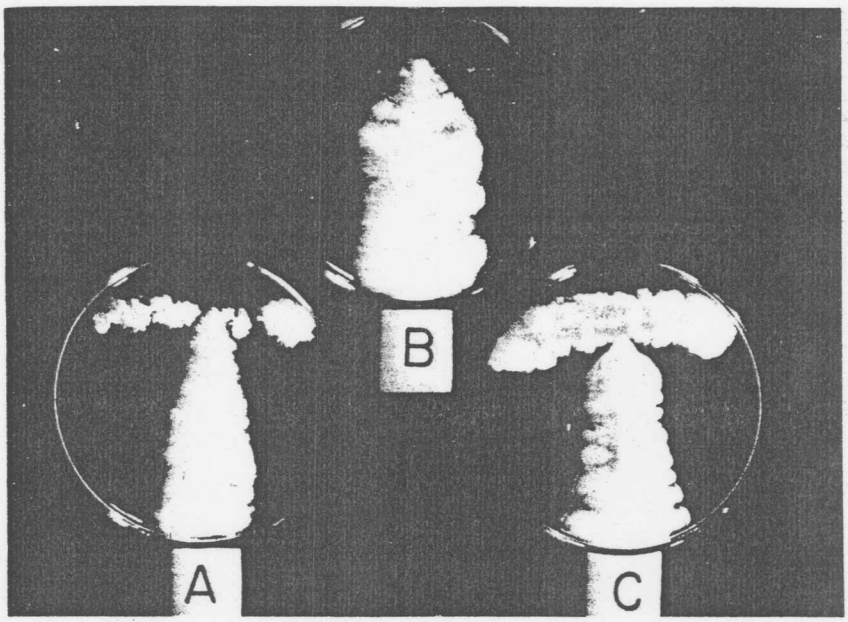

FIG. 4

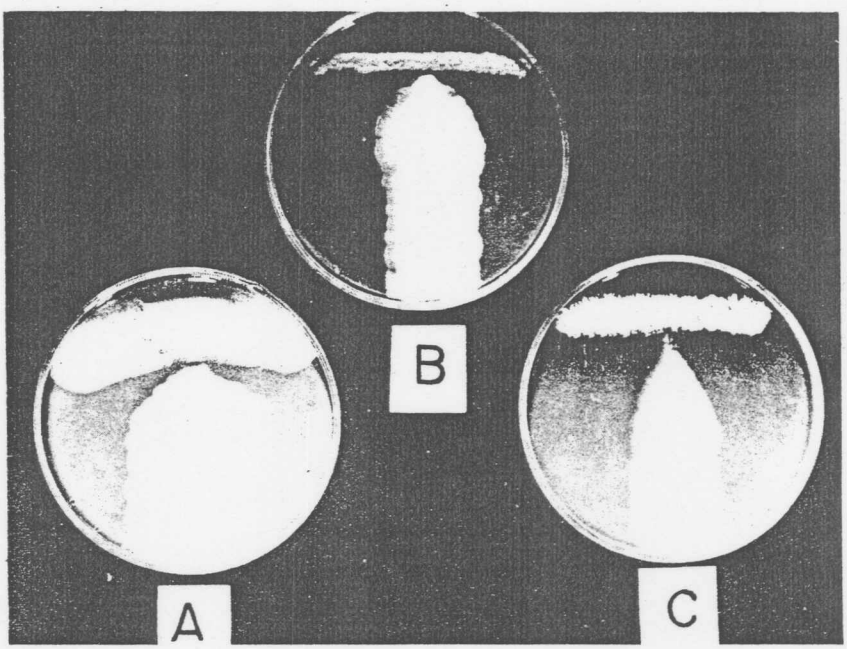

FIG. 5

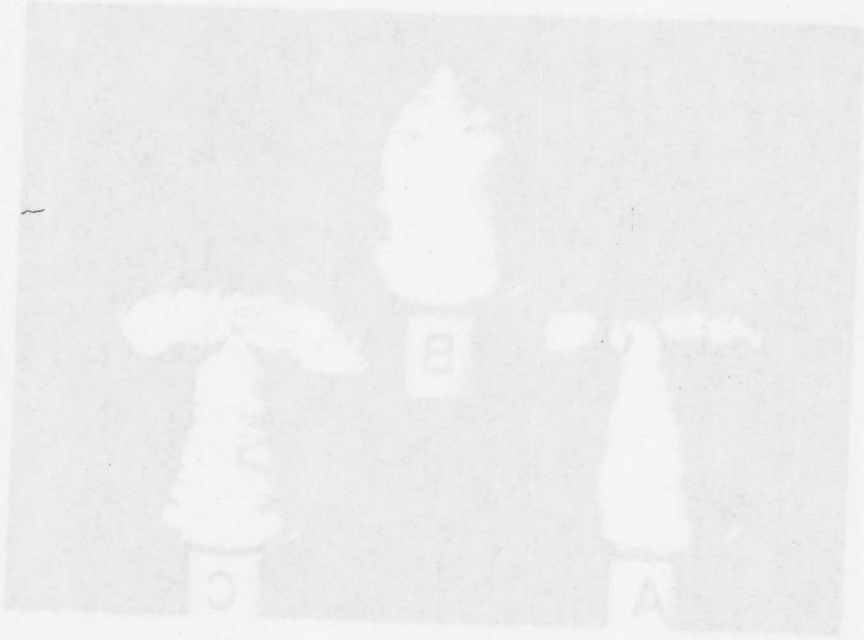

FIG. 4

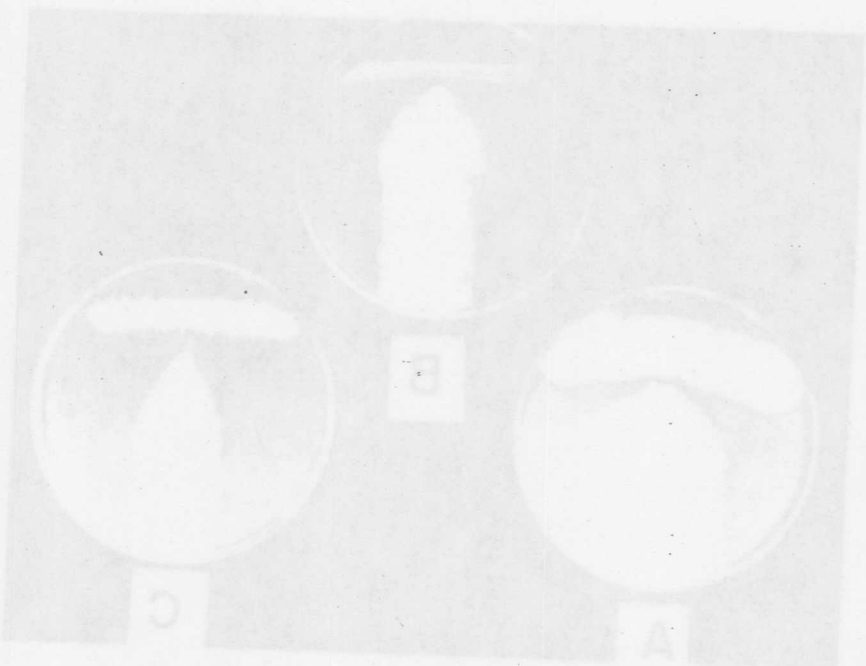

FIG. 5

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Soil Microbiology Section
Annual Project Report 1959

Rhizosphere Studies of Bananas: Root Excretions

Background

The dense mantle of microorganisms that surrounds the plant root probably results from the release of substrates from the root in the form of either excretions or sloughed cellular material (1,7). The nature of the compounds excreted by the roots determines the type and number of microorganisms in the rhizosphere (2). The resistance of some varieties of plants to root-invading pathogens may be the result, either directly or indirectly, of root excretions (8). The excretions may be toxic to the pathogen, or they may selectively stimulate a rhizosphere microflora which inhibits the development of the pathogen. The resistance of banana varieties to Fusarium Wilt may be related to the type of compounds excreted by the roots.

In order to conduct studies on the biochemical nature of root excretions, an apparatus is required for culturing plants with sterile root systems. The apparatus must meet several requirements besides maintaining sterility of the roots for long periods. The root system must be well aerated, and all CO_2 produced should be collected quantitatively in order to provide an index of root respiration. It must be possible to irrigate regularly and to sample periodically the liquid phase of the growth medium for analysis of the exudates. The volume of the sampled liquid must be small so that concentration prior to analysis is minimal, and yet the sample must be quantitatively representative of the excreted materials. The seed or embryo should be separated spatially from the root zone in order to eliminate contamination of the growth medium by organic compounds derived from the endosperm. Provisions must be made to permit introduction into the root zone of pure cultures of microorganisms or of other materials such as antibiotics. The apparatus must be compact and simple to operate.

Progress

An apparatus has been developed which meets most of the above requirements. The "growth unit" consists of 1) a growth tube in which the plant is germinated from either seed or embryo, 2) a root chamber, and 3) a nutrient solution reservoir. Nutrient solution is intermittently perfused through the root chamber by the compressed CO_2-free air which aerates the roots and flushes respired CO_2 into NaOH, wherein it is determined volumetrically. The perfused nutrient solution containing the root excretions is periodically removed from the solution reservoir and fresh solution is introduced under pressure. The individual growth units are compact and can be autoclaved in

one piece. The growth units are attached to two manifolds, one carrying the CO_2-free air stream and the other providing irrigation solution to the individual reservoirs. One-way check valves are used throughout the system to prevent spread of microbial contamination from one unit to another. At present, 12 growth units containing M. balbisiana plants, developed from excised embryos, are in operation. Some of these plants are approximately a foot in height, and all appear normal. The plants have well-developed root systems but, unfortunately, not all the plants are sterile.

In order to maintain sterility of the roots (the aerial portion of the plant is not maintained sterile) and to make the root zone air-tight so that respired CO_2 can be collected quantitatively, a sealer is placed around the stem of the plant. The type of CO_2-collectors presently in use require a seal that can withstand a continuous positive pressure of approximately 0.5 psig, but which will be sufficiently malleable to permit normal enlargement of the stem. A sealer which has these properties has not yet been found, but CO_2 collectors with lower hydraulic heads are now being tested. If these prove suitable, a lower positive pressure can be employed and some of the sealing compounds not now acceptable may be effective. Several types of sealing mixtures have been tested with tomato, corn, and banana seedlings. The hairiness of tomato stems resulted in leaks around the stem. With bananas and corn, leaks occurred at the sealer and growth-tube wall interface, but not around the stems. Scarifying the inner surface of the growth-tube by sandblasting, grinding, or invagination has not been too effective. Several of the sealers were toxic to banana seedlings. A few of the sealers so far tested, alone and in combination, are presented in Table 1. The best results have been obtained with layering one sealing mixture over another.

Several materials have been tested as potential growth media. The medium should be completely inert, free of organic materials, hold large quantities of water yet drain rapidly, and provide good aeration. Soil is not suitable due to its high exchange capacity, organic matter content, and unstable structure. Liquid culture is not desirable since the metabolism of roots is substantially modified in solution, and the results obtained would have limited ecological significance. The suitability of vermiculite, perlite, and sand, which conform to the above requirements, have been investigated by the following procedure: 1) standard amounts of an amino acid and sugar mixture were added to growth tubes containing the various materials; 2) a standard volume (usually 100 ml.) of water was perfused through the medium; 3) the perfusate was recovered quantitatively from the nutrient solution reservoir and analyzed for amino acid and sugar; 4) steps 2 and 3 were repeated until no more material was recovered. A mixture of amino acid and sugar was used in order to test both the chemical and physical adsorption capacities of the media.

Some preliminary results are presented in Figs. 1-3. The recovery of amino acid was less than that of sugar in all media except sand. This was probably the result of ionic adsorption of the amino acid on vermiculite and perlite. The cation exchange capacity of vermiculite is approximately 100 me./100 g. and that of non-separated perlite is approximately 1 me./100 g. Most of the exchange capacity of the perlite was apparently associated with the smaller particles, as indicated by the increased adsorption of amino acid by the 20 and 40 mesh fractions. Sand presumably had no adsorption capacity since all the added amino acid was recovered. The recovery of sugar was essentially complete from all materials except from the vermiculite. The high surface area of vermiculite apparently resulted in some physical adsorption of the sugar. When quantities of amino acids smaller than those used in these experiments were added to vermiculite, the percentage recovery was reduced, suggesting that the volume of material added is important in the recovery from vermiculite. This reduction in the amount of material recovered was not noted with perlite or sand. The highest and most rapid recovery of both amino acid and sugar was obtained with sand. Experiments in which the same volume of solution was perfused through the media several times prior to removal have also shown that the recovery from sand is better than from perlite and vermiculite.. The rate of gas diffusion through these various media is now being investigated.

The present method of obtaining M. balbisiana plants with sterile root systems involves a series of aseptic transfers. Embryos are explanted on agar medium, and when the plantlets are 2-3 inches tall, they are transplanted aseptically into growth tubes which are suspended in glass tumblers containing nutrient solution. The plants are allowed to develop until the roots have penetrated into the nutrient solution and have proliferated. When sufficient roots are present, the growth tubes are aseptically transferred to the root chambers of the growth units. Pieces of the roots and aliquots of the nutrient solution are cultured for microbial contamination when the growth tubes are transferred. Pieces of the leaves are cultured when the sealer is placed around the stem and the aerial portion of the plant is exposed to the air. The perfusate is checked for contamination each time it is removed from the growth unit for analysis. Experiments are currently being conducted wherein chipped seeds are planted directly in the growth tubes, hence eliminating the need for transfers and reducing the possibility of microbial contamination.

In order to obtain some idea of the quantity of material excreted by banana roots, the solution in several tumblers was analyzed for α-amino nitrogen (4), total carbon (9), and reducing sugars (6) after the plants were transferred to the growth units. The solutions were filtered through a bacterial filter in order to remove all cellular material, and then concentrated in a flash evaporator. The results of these analyses are presented in Table 2. The plants in the tumblers were grown under low light intensity (ca. 400 fc.) and were only 2-4 inches tall at the time of analysis. The root systems consisted of 2-5

roots, 3-6 inches in length. Despite the poor development of the plants, considerable quantities of organic material were found in the solutions. These materials presumably were excreted from the roots although soluble autolytic products of root cells were undoubtedly present. These preliminary results suggest that M. balbisiana roots excrete large amounts of organic materials, and that little difficulty should be encountered in obtaining measurable quantities of root excretions from more mature plants. The quantities of material excreted were larger than those reported for other plants (3,5). Techniques for the identification of these materials are presently being developed.

Conclusions

A technique for the culture of plants with sterile roots for use in studies on the biochemical nature of root exudates has been developed. The technique is simple and meets all the requirements necessary for this type of study. Some preliminary results are presented.

Recommendations

None.

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Prepared by: G. Stotzky, December 1, 1959

Table 1. The relative effectiveness and apparent toxicity of representative compounds tested, alone and in combination, as sealers for the culture of banana plants with sterile root systems.

Sealing compound	Relative effectiveness	Toxicity to banana seedlings
Beeswax (BW)	+	-
Petrolatum (P)	+	-
Household wax (W)	+	-
Latex (L)[1]	+	+ ?
Clay	-	-
Advawax M (AM)[2]	+	+
Advawax P (AP)[2]	+	+
Rhoplex (R)[3]	-	+ ?
Mercury (Hg)	+	- ?
Unknown composition (C)	+++	-
3:1 (P:W)	++	-
4:1 (P:W)	+	-
P + 3:1 (P:W), layered	+++	-
Polyurethane sponge (U)	-	-
U + 3:1 (P:W)	++	-
3:1 (P:AM)	++	+
3:1 (P:AM) + P, layered	+++	+
3:1 (P:W) + C, layered	+++	-
3:1 (P:W) + Hg, layered	+++	-

1 Non-cured latex, 68.5% solids; Naugatuck Chemical Company, Naugatuck, Conn

2 Polyisobutylenes dispersed in wax; Advance Solvents and Chemicals, New Brunswick, New Jersey.

3 Polyacrylic resin emulsion, Rohm and Haas, Philadelphia, Pa.

Table 2. Amount of material released by roots of M. balbisiana plants.

Plant Number	Age of plants[1] (Days)	Length of time roots were in nutrient solution[2] (Days)	Microbial contamination	Type of root system[3]	Total carbon (mg.)	∝-amino nitrogen (µl)	Reducing sugar (µl)
14	112	28	--	extensive	28	21	168
15	112	28	—	poor	38	40	36
17	112	28	—	extensive	13	not determined	78
18	115	28	—	poor	13	9	negligible
19	115	26	+	good	58	66	920
20	115	26	+	good	28	21	460
21	115	26	+	good	22	87	negligible

1 Age calculated from the day that the embryos were explanted to the time of analysis.

2 Length of time between transplanting into growth tube and transfer of growth tube to growth unit.

3 Relative evaluation of root system.

PERCENT RECOVERY

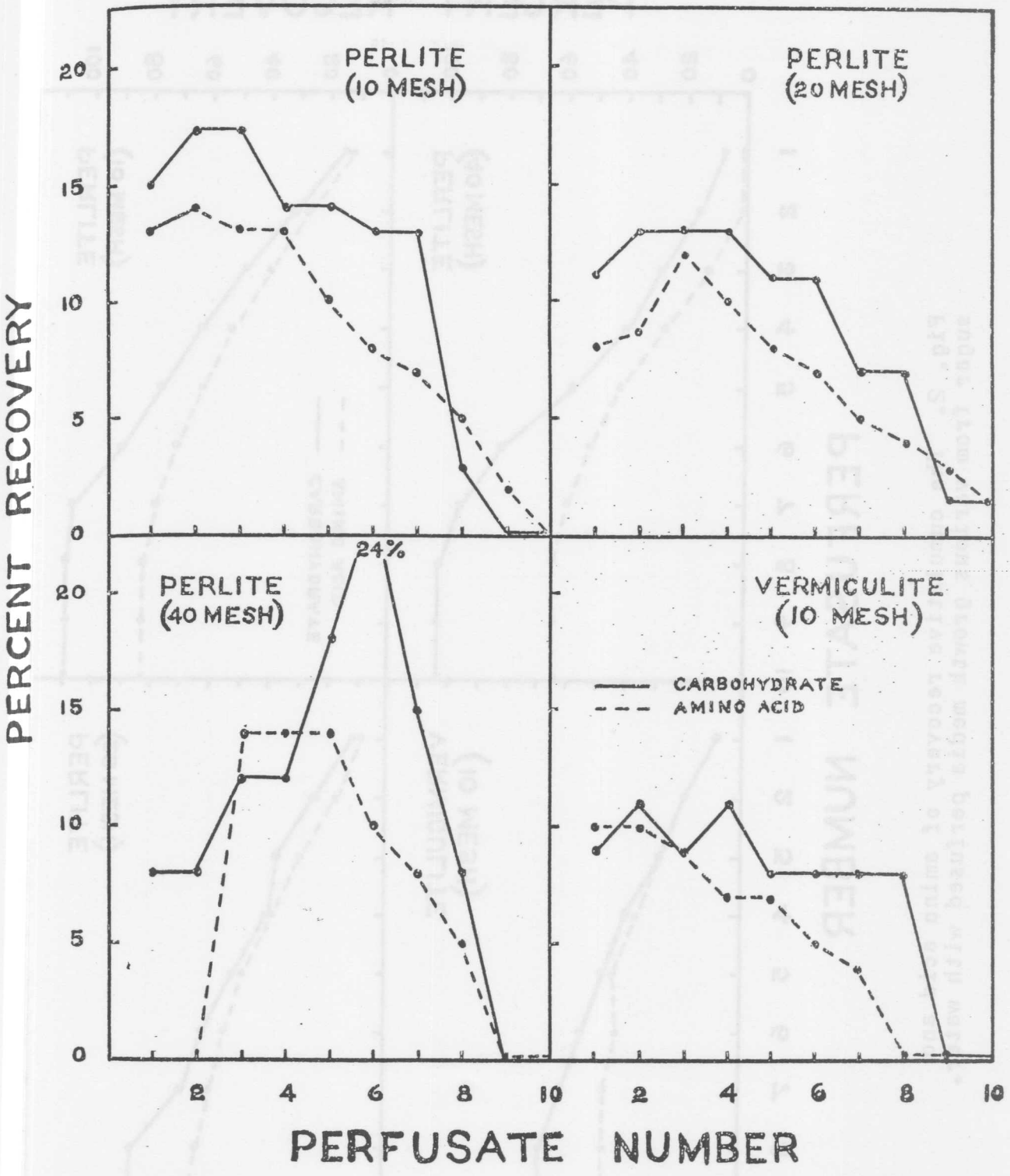

Fig. 1. The sequential recovery of amino acid and sugar from various growth media perfused with water.

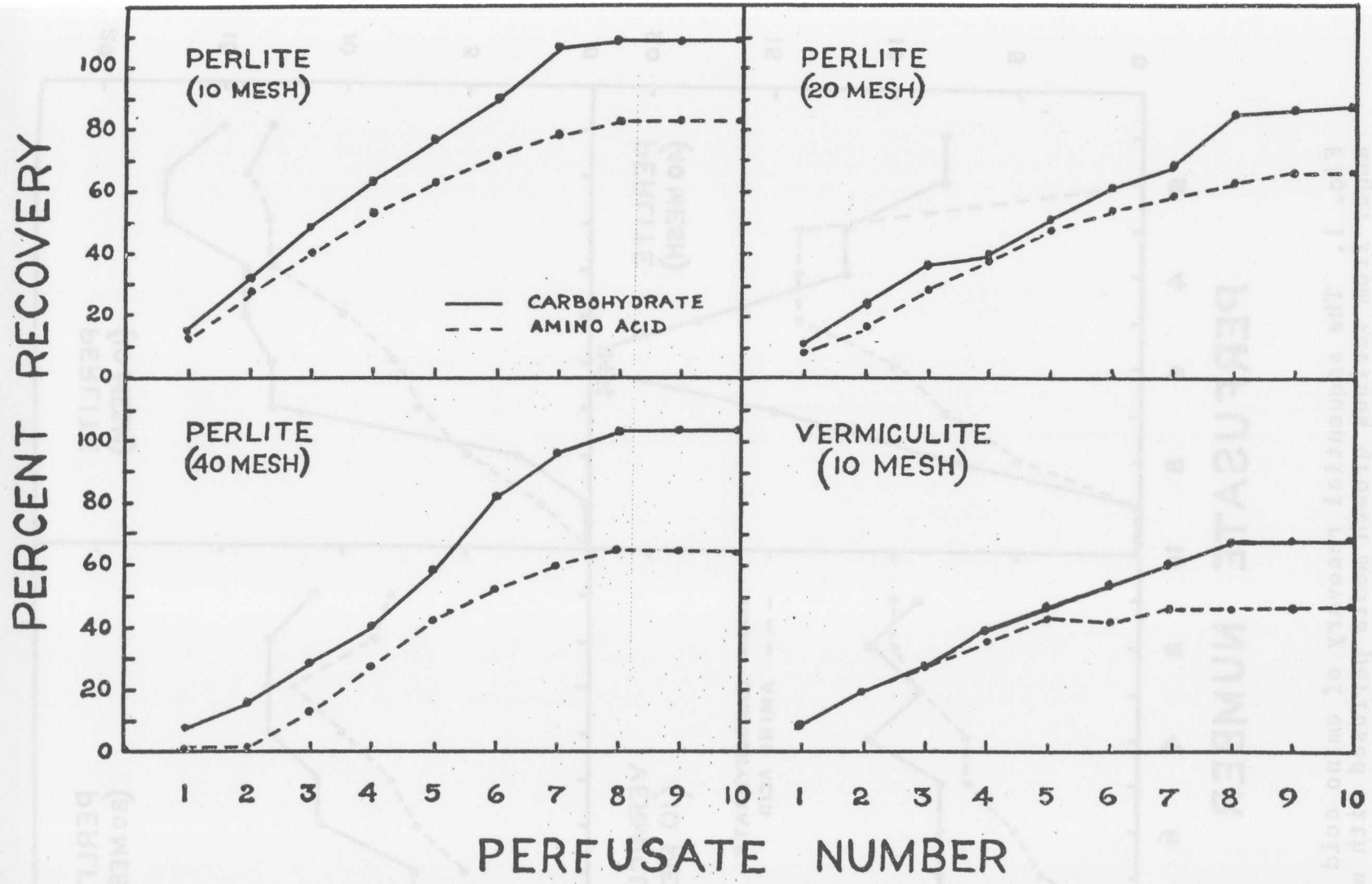

Fig. 2. The cumulative recovery of amino acid and sugar from various growth media perfused with water.

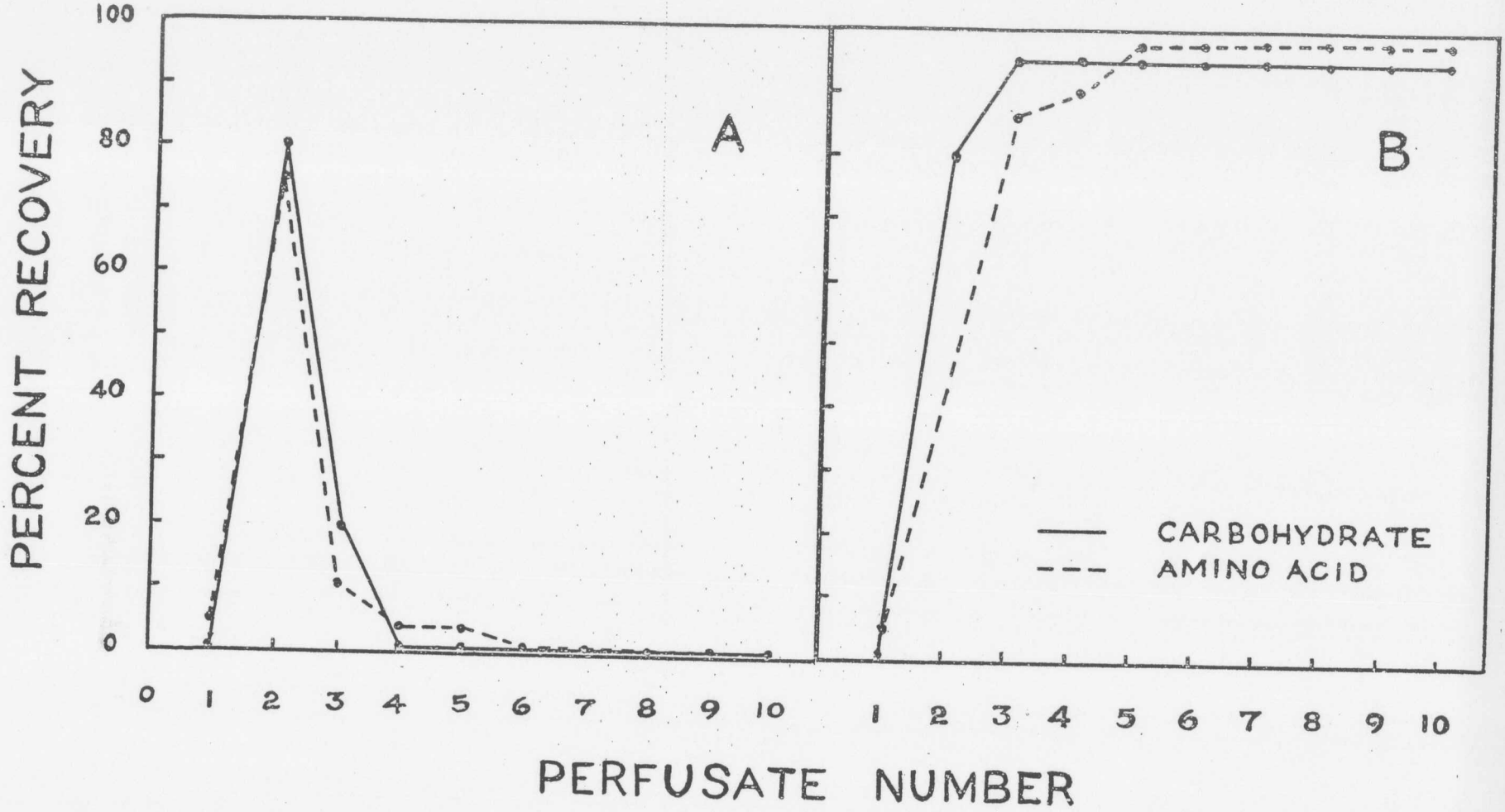

Fig. 3. The sequential (A) and cumulative (B) recovery of amino acid and sugar from sand (20-30 mesh).

A
B

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

DDF-2-20

Soil Microbiology Section
Annual Project Report 1959

Rhizosphere Studies of Bananas: Seed and Embryo Studies of M. balbisiana.

Background

Banana plants with sterile root systems are required for investigations of the biochemical nature of root excretions, microbiology of the rhizosphere, pathogenicity studies, etc. Aseptic plants of seeded species can usually be obtained by culturing surface-sterilized seeds under aseptic conditions. With parthenocarpic species, such as the commercial banana, propagation is vegetative, and to date it has not been possible to sterilize vegetative seed-pieces without deleterious effects. Until a technique is developed for obtaining sterile roots from rhizome pieces, studies requiring sterile roots are being conducted with the seeded banana variety, Musa balbisiana Colla.

The first attempts to obtain sterile plants from M. balbisiana seeds were generally unsuccessful. Studies were initiated to determine the factors affecting the germination and surface sterilization of seeds as well as the nutritional and environmental conditions necessary for optimum growth of the plants.

Progress

A. Age of seeds.

The Musa balbisiana Colla seeds used in these studies were kindly provided by Dr. R. H. Stover. Five lots of seeds are currently being used. Lots A, B, and C were collected near the Tropical Research Labs in La Lima, Lot D in Mopala Farm, and Lot E in the Lancetilla valley. The harvest dates were: Lot A, April 12, 1959; Lot B, Dec. 3, 1958; Lot C, unknown, but older than Lot B; Lot D, July 21, 1959; Lot E, fruit collected on August 27, 1959, and seeds removed from fruit on Sept. 7, 1959. Ages of the seeds at time of use are indicated in the graphs.

B. Depth of planting.

Seeds of Lots A, B, and C were planted in flats containing vermiculite or a sterilized soil and perlite (2:1) mixture at depths of 1 inch, ½ inch, and on the surface with a slight covering of the planting medium. In both media, seeds planted at a depth of 1 inch gave the highest percentage of germination, except

Lot A planted in the soil and perlite mixture (Fig. 1). Variable germination counts were obtained with this lot of seeds throughout all studies. Approximately 60 days after planting, 100 per cent germination was obtained in both media with seeds of Lot B planted at a depth of 1 inch. As a result of these experiments, all subsequent plantings have been at this depth. The effect of planting depths greater than 1 inch is being investigated.

C. Storage of seeds.

Seeds of Lot A were stored in glass sample bottles under the following conditions: at room temperature (ca. 23° C) over desiccant; at 6° C without desiccant; and at -4° C without desiccant. After three and six months, seeds were removed, subjected to a variety of treatments, and germination determined. The seeds stored for six months were only recently removed, and the data are not yet available. A portion of the stored seeds was surface sterilized with $CaOCl_2$ (cf. Section D) and soaked for 0, 24, 48, and 96 hours before planting in sterilized soil and vermiculite (2:1) mixture.

Figure 2 shows that storage of the fresh seeds (approximately 1½ months old at time of storage) markedly increased their germination (compare to Fig. 1). There appears to be a "primary dormancy" of M. balbisiana seeds since some aging is apparently necessary for increased germination. "Primary dormancy" is indicated by the results of this experiment and by the difference in the percentage of germination of Lots A and B (Fig. 1; cf. Section F). Apparently, further aging again lowers the germinability as indicated by Lot C in Fig. 1, suggesting a "secondary dormancy". The differences in germinability appear to be caused by factors in the seed coat and/or in the endosperm since there is essentially no difference in the "germination" of embryos excised from seeds of various ages.

Storage of seeds at low temperatures apparently affects the permeability of the seed coat. A higher germination was obtained with seeds stored at 6°C and soaked for 48 hours prior to planting than with seeds stored at 23° C. The germination of seeds stored at - 4° C decreased with increase in soaking time. The sub-freezing temperature may have increased seed coat permeability more than did storage at 6° C, thereby resulting in the more rapid removal during soaking of some factor(s) required for germination. No increase in germination was obtained as a result of soaking the seeds stored in the laboratory; the percentage germination was 20% lower than in the seeds stored in the cold. These results suggest that changes in the seed were not as marked in the laboratory-stored seeds. The decrease in germination of seeds soaked for 96 hours, regardless of the temperature of storage, will be discussed in Section F.

D. Surface sterilization.

Experiments on surface sterilization have so far been restricted primarily to the use of $CaOCl_2$. A $CaOCl_2$ solution is prepared by

adding 150 ml. distilled water to 10 g. dry $CaOCl_2$ (purified powder, 30-35% available Cl_2, The Matheson Co., Inc., Norwood, Ohio), shaking vigorously, and filtering. The filtrate has a pH of 11.5 and is relatively stable. The seeds are immersed in the filtrate and agitated for 35 minutes on a wrist-action shaker. Preliminary experiments included soaking seeds in $CaOCl_2$ under vacuum and immersing seeds in wetting agents, such as 70% alcohol and commercial detergents, prior to placing in the $CaOCl_2$. The use of vacuum did not increase the effectiveness of the hypochlorite, and wetting agents decreased its effectiveness. The use of ethanol reduced the viability of the seeds, and embryos excised from alcohol-treated seeds did not develop.

The use of $CaOCl_2$ alone has been effective in controlling most seed contaminants, except a few bacteria and the fungus, Botryodiplodia theobromae. This fungus does not appear to be present in older lots of seeds, but freshly harvested seeds are badly contaminated. The results of plating seeds of Lots A, B, C, and D on glucose-peptone medium after immersion in $CaOCl_2$ are summarized in Table 1 and show that B. theobromae is not eliminated by $CaOCl_2$. The fungus appears to be located deep within the reticulated seed coat and it does not develop until the surface organisms are eliminated.

Table 1. The effectiveness of $CaOCl_2$ as a surface sterilant of M. balbisiana seeds.

Seed Lot	No surface sterilization		Surface sterilization	
	Surface contaminants	Botryodiplodia theobromae	Surface contaminants	Botryodiplodia theobromae
	Relative degree of contamination			
A	heavy	none	none	heavy
B	heavy	none	slight	none
C	heavy	none	slight	none
D	heavy	slight	none	heavy

The surface sterilization of abaca (Musa textilis) seeds has also been attempted. All surface contaminants were eliminated by soaking for 50 minutes in $CaOCl_2$, but the seeds were 100% contaminated with B. theobromae after 3-5 days. Soaking seeds in concentrated H_2SO_4 for periods of time ranging from 4 to 60 minutes delayed the appearance of the fungus, but did not eliminate it. The failure of concentrated H_2SO_4 to eliminate the fungus again suggests that the organism resides within the seed coat where it

is not accessible to surface sterilants. Soaking seeds in water at 55° C for 5 minutes or at 58° C for 10 minutes did not eliminate the fungus, but 56% of seeds soaked for 10 minutes at 64° C were still uncontaminated after one month of incubation. No germination was obtained with the acid- or heat-treated seeds, and the effect of these treatments on seed viability is not known. The work with abaca seeds has been discontinued due to exhaustion of the seed supply.

Reduction of the pH of the $CaOCl_2$ solution from 11.5 to 7 by the addition of HCl increased the effectiveness of the solution. All contaminants, including B. theobromae, were eliminated from seeds immersed for 35 minutes in the low pH solution. No data are presently available on the effect of lowered pH on the viability of the seed.

Experiments designed to find effective surface sterilants are in progress. These studies involve screening 1) antibiotics, 2) mercurials, 3) surfactants, and 4) commonly used sterilants for their sterilizing effectiveness and innocuousness to the seed.

E. In vitro germination of intact seeds.

While it has been possible to germinate seeds in flats of soil in the greenhouse, the germinating of surface-sterilized intact seeds on agar has been unsuccessful. Procedures which have been attempted include presoaking seeds for 24 to 96 hours at various temperatures with and without wetting agents, momentary immersion of seeds in 30% or absolute alcohol or in carbon tetrachloride, heat treatment at 65° C in water for 10 minutes, incubation in the dark at 25, 30, 35, and 40° C, and stratifying the seed in agar at 28° C under continuous light.

Seeds presoaked for various periods of time ranging from 48 to 192 hours were also planted in sterile vermiculite or in sterile soil and vermiculite (2:1) mixture contained in 4 oz. glass sample bottles. Essentially no germination had occurred after 44 days, at which time the seeds were discarded. These experiments are now being repeated, since longer time may be required for germination in vitro.

F. Presoaking

In order to determine if the apparent dormancy of the seeds was the result of impermeability of the seed coat to water, the imbibition of water by seeds of Lots A, B, and C was measured. The seeds were pretreated in an attempt to increase the wettability of the seed coat: 1) no surface treatment; 2) soaking for 30 minutes in distilled water under vacuum from a water aspirator pump; 3) same as 2, except seeds were placed in 70% ethanol; and 4) same as 2, except seeds were placed in a 0.32% solution of Triton X-100. After pretreatment, the seeds were surface sterilized with $CaOCl_2$, rinsed with sterile distilled water, and placed

into plugged flasks containing 25-50 ml. of sterile water. The flasks were incubated at room temperature and seeds were removed after 6, 12, 24, 48, 96, 192, and 384 hours of soaking, blotted with paper towels, air-dried to constant weight (10-15 minutes), weighed, and planted in flats containing soil vermiculite (2:1) mixture. Some of the soaked seeds were not planted, but were retained for histological studies.

The results of this experiment are presented in Figs. 3, 4, and 5. Imbibition was greatest in the youngest seeds (Lot A) and least in the oldest (Lot C). Pretreatment designed to increase wettability of the seed coat did not increase imbibition, but rather, decreased it in most cases. There was no germination of the seeds pretreated with alcohol and, hence, the results obtained with these seeds were not presented. Several seeds of Lots B and C germinated in the flasks after 192 hours of soaking. The loss of endosperm as respired CO_2 during germination probably accounted for the decrease in weight observed in these two lots after 384 hours of soaking. Maximum imbibition in all 3 lots had occurred after 96 hours of soaking and only slight increases in weight were obtained after this time.

The germination of Lot A was only slightly increased by soaking. Soaking reduced the germination of Lot B. The germination of Lot C was markedly increased by soaking, regardless of pretreatment and, with one exception, the length of the soaking period. The time required to achieve maximum germination of Lots B and C, but not Lot A, was decreased by soaking. The decrease in germination of Lot B, as a result of soaking, suggests that some material necessary for germination was removed from these seeds. The increased germination of Lot A and especially of Lot C suggests that an inhibitor of germination may have been removed. The imbibition of water could not have been the only determining factor responsible for the increased germination of Lots A and C, since Lot C imbibed less water than did Lots A and B and yet exhibited the greatest increase in germination. There was poor correlation between the amount of imbibition and the rate and percent of germination, especially in Lot A, further indicating that factors other than water uptake are involved in the germination of these seeds.

Seeds of Lots A, B, C, and D were leached, without pretreatment, in running tap water at 21° C for the same periods of time as in the previous experiment. The results, presented in Figs. 6 and 7, show that the patterns of imbibition and rate and percent of germination were essentially the same as in the soaking experiment, even though the levels of imbibition and germination were slightly lower. The germination of leached seeds of Lot A was substantially lower than that of seeds not treated with water prior to planting. This was contrary to the increase in germination obtained with soaked seeds (Fig. 3), but, since the performance of Lot A was erratic throughout all investigations, not too much significance can be attached to this observation.

The results of these experiments on the soaking or leaching of seeds prior to planting strongly suggest that germination inhibitor(s), and possibly stimulator(s), are present in the seeds of M. balbisiana. The concentration of the inhibitor(s) appears to increase with maturation of the seeds, as evidenced by the lower level of germination of seeds of Lot C that were not treated with water. The inhibitor(s) appears to be water-soluble and can be removed by soaking or leaching of the seeds. As suggested in Section C, the inhibitor(s) appears to be associated with the seed coat and/or the endosperm since no dormancy is evident in excised embryos. Further evidence for the presence of an inhibitor-stimulator system is presented in Section G. The results also indicate that the age of seeds must be considered if soaking is used as means of increasing the amount and rate of germination.

G. Scarification.

In order to clarify whether or not the apparent dormancy of seeds was caused by impermeability of the seed coat, seeds of Lot B were scarified in various ways and cultured in Petri dishes on Randolph and Cox's medium containing 0.5% agar and 4% sucrose. All seeds were surface sterilized in $CaOCl_2$ prior to scarification. The seeds were treated as follows: 1) a small chip was removed from the side of the seed, exposing the endosperm, and the seed placed with the chipped side in contact with the medium; 2) seeds were split in half, exposing but not damaging the embryo, and placed either with the exposed embryo up, or with the exposed embryo in contact with the agar; 3) the chalazal mass (i.e., the bottom of the seed) was removed; and 4) the micropylar cap was removed. The seeds were incubated at $28 \pm 1^{\circ}$ C under 8-12 hours of light daily.

The results of this experiment are presented in Fig. 8. Two weeks after planting, germination had occurred. The seeds that were chipped on the side showed the highest percentage of germination; the embryos had enlarged, forcing out the micropylar cap, and growth of the radicle was apparent. Seeds from which the chalazal mass had been removed did not germinate in the typical manner; instead of forcing out the micropylar plug, the embryo emerged from the base of the seed as a result of the extension of the radicle, after which adventitious roots and a shoot developed. The germination percentage of the split seeds was markedly lower than that of the seeds from which only a small piece of the seed coat had been removed.

The highest and most rapid rate of germination, as well as the most vigorous seedlings, were obtained from seeds which had been chipped on the side. Since these seeds had the least amount of seed coat removed, the permeability of the seed coat to water is apparently not the only factor responsible for the apparent dormancy of M. balbisiana seeds (cf. Sections C and F).

The results of this experiment, in agreement with the experiments on soaking and leaching, suggest that the germination of M. balbisiana seeds is dependent upon a balance between an inhibitor(s) and a stimulator(s). Removal of a small part of the seed coat may have removed a sufficient amount of the inhibitor(s) to permit germination, but not enough of the stimulating material(s) to prevent it. The removal of substantial amounts of the seed coat and endosperm, as was done with the split seeds, may have deprived the embryo of sufficient quantities of the stimulator(s) necessary for germination. The germination of the split seeds which were placed with the embryo in contact with the agar was lower than that of the seeds placed with the embryo up. This may have been due to the loss of stimulating substances by diffusion into the agar. Seeds with the chalazal mass removed, which were positioned with only partial contact of the endosperm with the agar, had a higher percentage germination than the split seeds placed embryo-side down, further supporting this assumption. Excised embryos develop in the absence of possible stimulators; however, possible inhibitory factors are also removed by excision. Forcing out of the micropylar plug is the first visible evidence of germination in intact seeds (Fig. 9). The failure to germinate of the seeds from which the mycropylar plug had been removed indicates that water uptake and gas exchange are not the only factors involved in germination. Since the plug is situated immediately above the embryo and is separated from it by a membrane, its removal should not affect the balance of a stimulator-inhibitor system.

Further studies on the factors affecting germination of M. balbisiana seeds are in progress. Regardless of the mechanism involved in germination, it has been possible to obtain seedlings *in vitro* by the use of chipped seeds. Since plants obtained from seeds are more vigorous and easier to handle than plants derived from embryos, chipped seeds are being used as a source of sterile plants for rhizosphere studies.

H. Description and germination of M. balbisiana seeds.

Seeds of M. balbisiana are essentially spherical in shape and range in size from about 4-5 mm. (Fig. 9-1). They vary considerably in shape and frequently have flattened sides and angular edges, probably as a result of compression within the fruit during maturation. Except for the hilum region, the seeds are covered with a hard, thick, somewhat roughened seed coat which remains intact during the germination process. The hilum scar is the external surface of the micropylar plug.

Internally, the seed is divided into two chambers: the upper contains the embryo and endosperm, and the lower the chalazal mass. The embryo is mushroom shaped and ranges from 1-2 mm. in length. It consists of a cylindrical upper portion which extends into the neck-like canal formed at the junction of the integument and micropylar plug, and a discoid basal portion which is surrounded by the

endosperm (Figs. 10-10 and 10-11). The endosperm, especially in dried seeds, has a granular appearance and may not entirely fill the upper chamber. The lower chamber contains a brown gelatinous material, probably the remainder of the chalazal mass, and is separated from the upper chamber by a thickened wall which is complete except for a small opening near the center (Fig. 10-10). This opening is difficult to observe and is best seen after the contents of the lower chamber have been removed and the seed is viewed from the basal end.

The first sign of germination is the forcing out of the micropylar plug or cap by the elongating embryo (Fig. 9-2). The plug appears to be held in place in ungerminated seeds by a thin layer of cells which is easily broken. It can be removed readily with a flattened dissecting needle, and is occasionally absent in stored seeds, indicating that it can be dislodged by seed handling. After forcing out the micropylar plug, the elongating embryonic axis emerges (Figs. 9-3), and the primary root soon appears (Figs. 9-3 and 9-4). If the micropylar plug has been carried from the seed by the elongating embryonic axis, the primary root may push it some distance from the seed (Figs. 9-4 and 9-8). The epicotyl appears soon after the primary root emerges (Figs. 9-4, 9-5, and 9-6), followed by the development of adventitious roots (Figs. 9-6, 9-7, and 9-8). As in most monocots, primary root development is generally not extensive. The first two leaves that develop are closed sheathing leaves, the third being the first fully expanded leaf (Fig. 9-9). Plants 15-20 inches tall, and with 6-8 fully expanded leaves, still have the empty intact seed coat firmly attached.

I. Gamma-irradiated seeds.

Advantage was taken of the opportunity to expose seeds of M. balbisiana to gamma rays derived from a Co-60 source. Seeds of Lots B and D were placed in narrow test tubes and sealed with pliofilm. The choice of seed lots was conditioned by the fact that Lot B was 9 months old and was known to give high germination, and Lot D was the freshest lot (2 months old) available at the time. The seeds were irradiated by R. C. Wornick at doses of 0.096, 0.48, 0.72, 3.5, 11.8, 58, 114, 285, and 570 kilorads. (cf. Irradiation of button seed and F.o.c., BDF-6-10, 1959 Ann. Rpt.)

After irradiation, seeds were cultured on glucose-peptone agar. Bacteria and fungi developed profusely on seeds of Lot B which received doses below 285 krad, but only bacteria were apparent on seeds which received doses of 285 and 570 krad. No B. theobromae was noted, but, as previously mentioned (Table 1), Lot B was not contaminated with this fungus. Similar results

were obtained with seeds of Lot D, except that B. theobramae was evident in all but the seeds which received a dose of 570 krad. The fact that this fungus was able to develop in seeds exposed to more than 114 krad while other fungal contaminants were eliminated above this dosage, further indicates that the fungus is deep within the seed coat, since adsorption by the coat probably decreased the dose received by the fungus.

Irradiated seeds of both lots were surface sterilized in $CaOCl_2$, soaked for 0 and 8 days, and planted in flats. To date, there has been no germination of any seeds of Lot D nor of the seeds of Lot B that were soaked for 8 days. The germination percentages of the unsoaked seeds of Lot B were 27% for the seeds which received 0.096 krad, and 14% for the seeds which received 3.5 and 11.8 krad. These results are not final, since the seeds have only been planted for 5 weeks at the time of writing.

Embryos from the irradiated seeds were explanted on Randolph and Cox's medium. Since the embryos were only recently excised, the data from this phase of the study also are not complete at the time of writing. No development is evident in embryos derived from seeds which received doses greater than 114 krad. Only callus has formed on embryos from seeds exposed to doses between 11.8 and 114 krad. Plantlets have developed from embryos which received less than 11.8 krad. To date, more plants have developed from the seeds of Lot D than from Lot B. No abberations have been observed in the plantlets.

J. Embryo studies.

Since considerable difficulty was encountered in obtaining a steady supply of sterile M. balbisiana plants from intact seeds for rhizosphere studies, the possibility of obtaining plants from excised embryos was investigated.

The aseptic removal of embryos from seeds is relatively simple. The seeds are surface sterilized by agitating for 35 minutes in $CaOCl_2$ solution on a wrist action shaker, and placed in sterile Petri dishes. Removal of the embryo is accomplished with the aid of a dissecting microscope. The seed is punctured at the edge of the micropylar cap with a flamed sharp scalpel. A slight twist of the scalpel splits the seed and exposes the embryo (Fig. 10-10), which is lifted to a culture vessel with a flattened dissecting needle. If necessary, the excised embryo (Fig. 10-11) may be immersed in $CaOCl_2$ for 5 minutes without deleterious effect. This step is not necessary, however, with careful technique.

Preliminary studies were conducted with a modified Knudson's medium containing 2% sucrose and 1.2% agar. Although the embryos developed roots and a shoot, the roots failed to penetrate the medium. Growth of the plantlets ceased after approximately 6 weeks, resulting ultimately in death of the plants. When young plantlets were transferred from the Knudson medium to that of Randolph and Cox which contained only 0.7% agar, root development improved and the roots penetrated into the medium. In all current studies, the Randolph and Cox's medium is being used. The agar content of the medium has been further reduced to 0.5%, since more rapid growth and more vigorous plants have been obtained with this concentration. In early experiments, the embryo, after enlargement, was reoriented on the agar so that the cotyledon was placed in contact with the medium to provide a larger surface area for absorption. Repositioning was unnecessary when the low agar medium was used, since the weight of the embryo embedded the cotyledon into the soft agar. Callus grown generally is visible 5-6 days after excision, followed by the appearance of the coleoptile and roots (Fig. 10-12 and 10-13). Delicate hair-like growths often arise from the surface of germinating embryos prior to the appearance of the coleoptile and roots. Two weeks after excision, the first leaf and roots have developed (Fig. 10-13) and the second leaf follows shortly thereafter (Fig. 10-14). A six-weeks old plantlet is illustrated in Fig. 10-15.

In order to determine the best type and level of sugar for optimum growth, sucrose, glucose, and levulose (fructose) at concentrations of 1, 2, 4, and 8% were tested. The sugars were filter-sterilized and added to 4 oz. wide-mouth screw-cap jars containing 30 ml. of cooled autoclaved Randolph and Cox's medium. One embryo was placed in each jar and the jars incubated at 28±1° C under continuous light. In all embryo studies, "callus formation" has been used as an index of initial "germination". At this stage, the embryos have enlarged, and the external surfaces become darkened and hardened (Fig. 10-3). Of the three sugars tested, the highest percentage of germination was obtained with sucrose (Fig. 11). All embryos placed on agar containing 2 or 4% sucrose formed callus, but a greater number of plantlets developed with the 4% concentration. Two weeks after excision, plantlets which developed on 2 or 4% sucrose agar ranged from 3/4 to 2 ½ cm. in height.

One month after explantation, additional medium was added to all jars since the volume of the original medium had been reduced by drying. One half of the jars received nutrient agar minus sugar, and the other half received nutrients plus sugar at concentrations identical with those originally used. The height of the plants which received additional glucose or fructose increased less than did the height of the plants which did not receive additions of these sugars (Fig. 12). As the level of glucose or fructose added was increased, less increase in plantlet height was

observed, suggesting an adverse osmotic effect. A greater increase in height was observed in the plants which received additional sucrose than in those that received nutrients minus sucrose, in contrast to the results obtained with glucose and fructose. Since the plantlets which developed on sucrose were larger at the time of addition than those on glucose or fructose, most of the original sucrose had probably been utilized by the plants before the additions were made. Almost all of the plants which received additional sugar, regardless of type and concentration, turned yellowish, indicating that perhaps the presence of available sugar in the medium retarded the development of cholorophyll. Most of these plants eventually grew out of this necrotic state.

The effect on plant development of autoclaving the sucrose with the medium was compared to filter-sterilization of the sugar. Although the percentage of germination, as measured by "callus formation", was similar in both methods of sterilization, a higher percentage of plantlets developed with the autoclaved sucrose (Fig. 13). The best development was again obtained with the 4% level of sucrose, regardless of the method of sterilization.

Embryos excised from seeds of Lots C and D were incubated at 28±1° C under continuous light or continuous dark for 30 days. The embryos of Lot C germinated best in the dark, but the germination percentage of embryos of Lot D was higher in the light (Fig. 14). However, more plantlets developed in the dark from embryos derived from both lots of seeds. The plantlets which developed in the dark were etiolated, the pseudostems were weaker, leaf development was slightly retarded, and the leaves were elongated. After the etiolated plants were placed in light, they turned green and remained taller than the plants which were incubated entirely in continuous light.

Plants developed from mature embryos have been grown aseptically in the original culture vessels for 3 months, after which time they were transplanted to growth units (cf. Rhizosphere Studies of Bananas: Root Excretions, BDF-2-20, Ann. Rpt. 1959). These plants are approximately 12 inches tall and have 10 to 12 fully developed leaves and well developed root systems. The plants are smaller than seedlings of the same age which developed in soil under greenhouse conditions from intact seeds, but otherwise they appear to be similar in all respects. The difference in size may be the result of differences in light intensity, since the embryos were incubated under artificial light at an intensity of approximately 400 fc., while the plants in the greenhouse were exposed to sunlight.

Aside from providing a steady supply of sterile plants for rhizosphere investigations, embryo culture may have possible application in plant breeding studies.

A short note on the *in vitro* culture of embryos from *M. balbisiana* has been submitted for publication in *Nature*.

Conclusions

The results of these studies indicate that the apparent dormancy of *M. balbisiana* seeds may be the result of the presence of a germination inhibitor(s). Data on optimum depth of planting, storage, surface sterilization, and factors affecting germination are presented. Techniques for the culture of excised embryos are described. The application of these investigations to rhizosphere, and possibly plant breeding, studies are briefly discussed.

Recommendations

None.

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Conclusions

None.

Prepared by

G. Stotzky, R. D. Goos

December 1, 1959

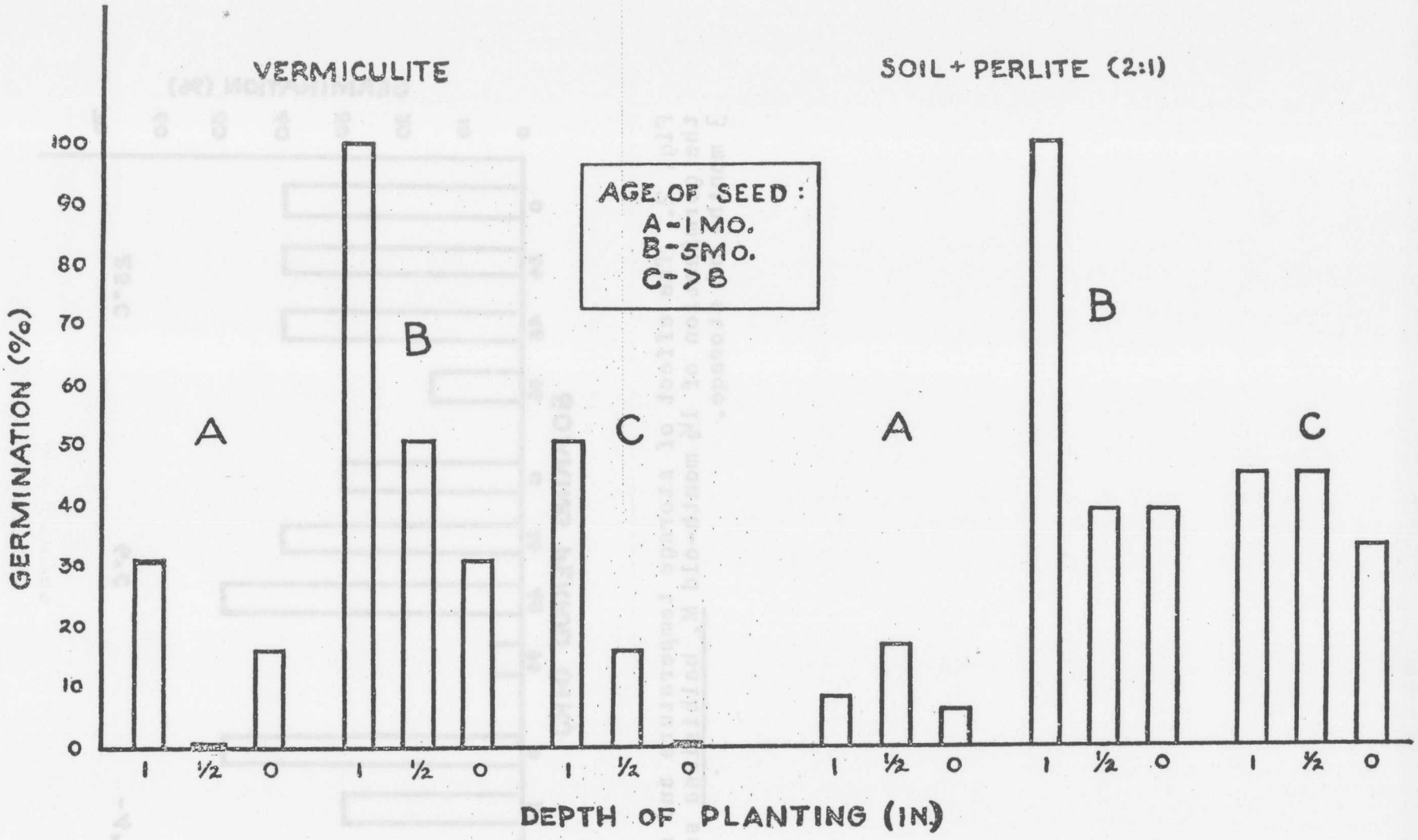


Fig. 1. The effect of planting depth on the germination of M. balbisiana seeds.

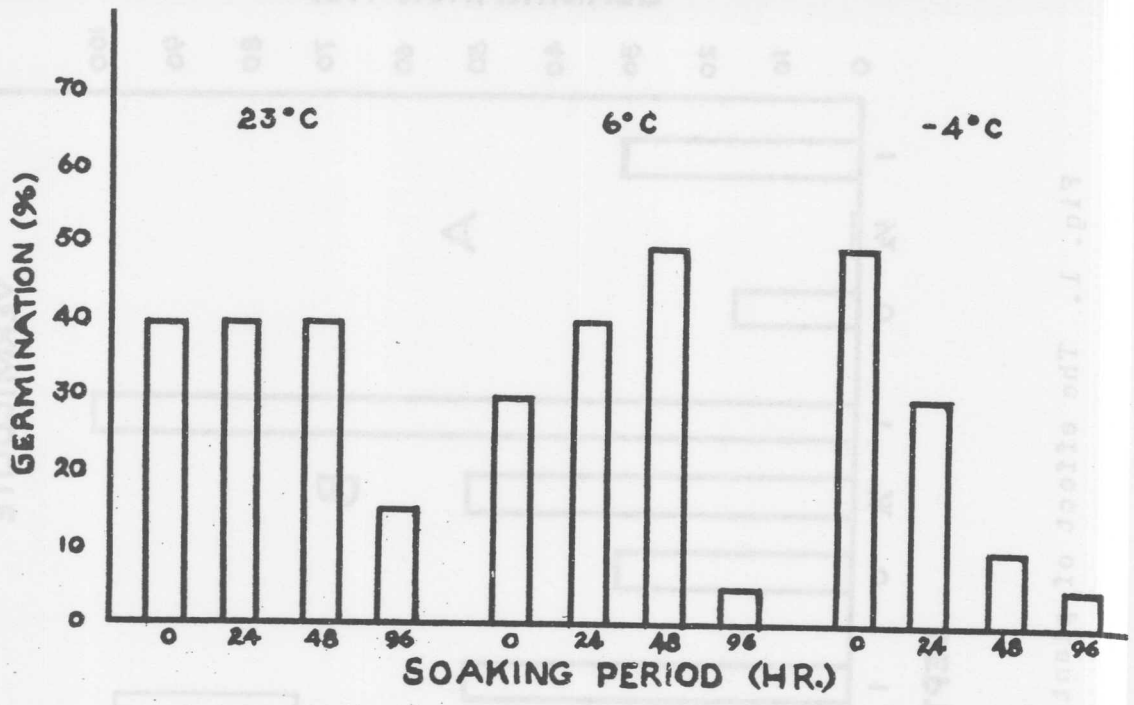


Fig. 2. The effect of storage temperature and soaking on the germination of 1½ month-old M. balbisiana seeds after 3 months of storage.

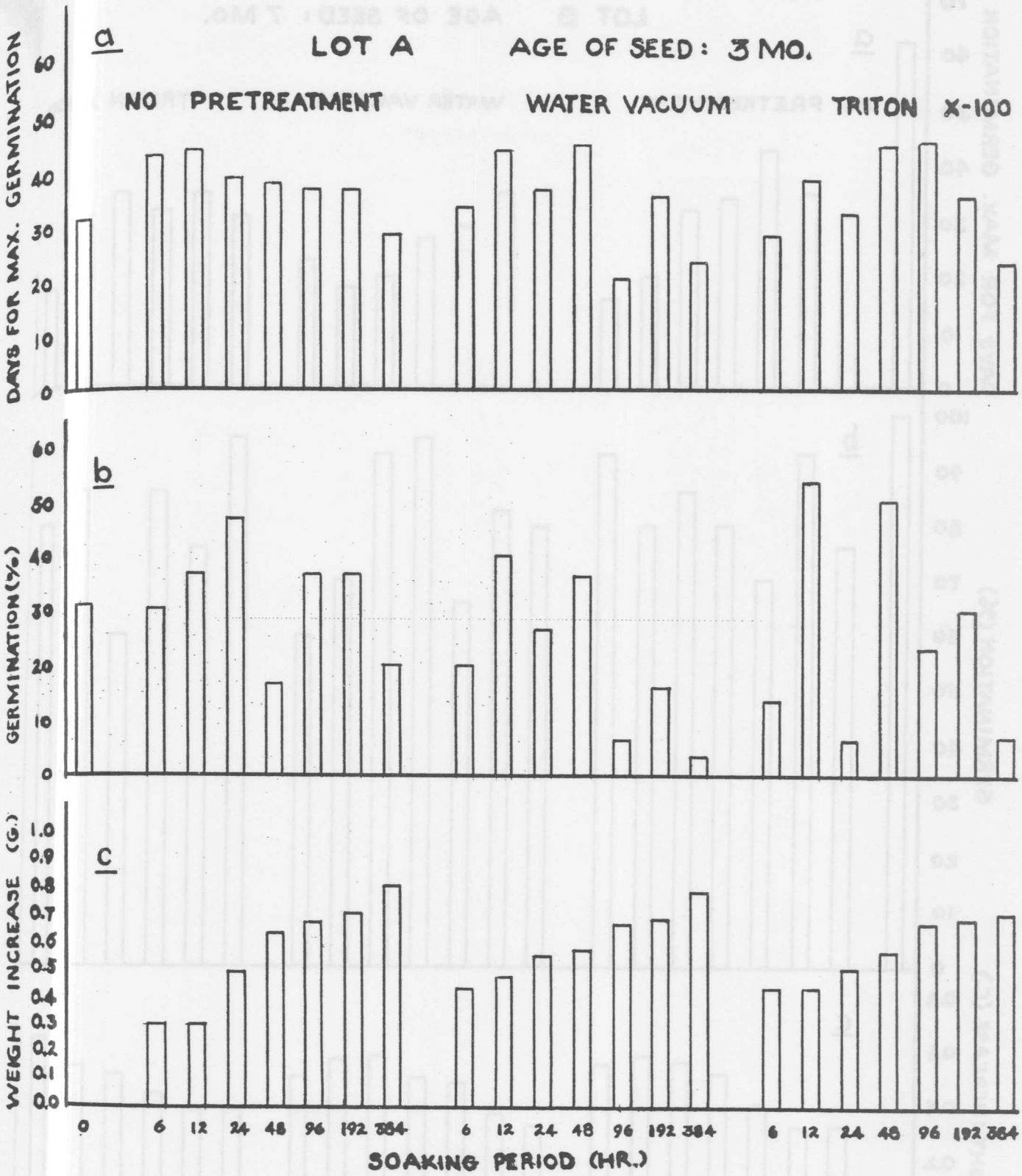


Fig. 3. The effect of soaking and pretreatment on the a: imbibition; b: germination; and c: length of time required to achieve maximum germination of 3 month-old M. balbisiana seeds.

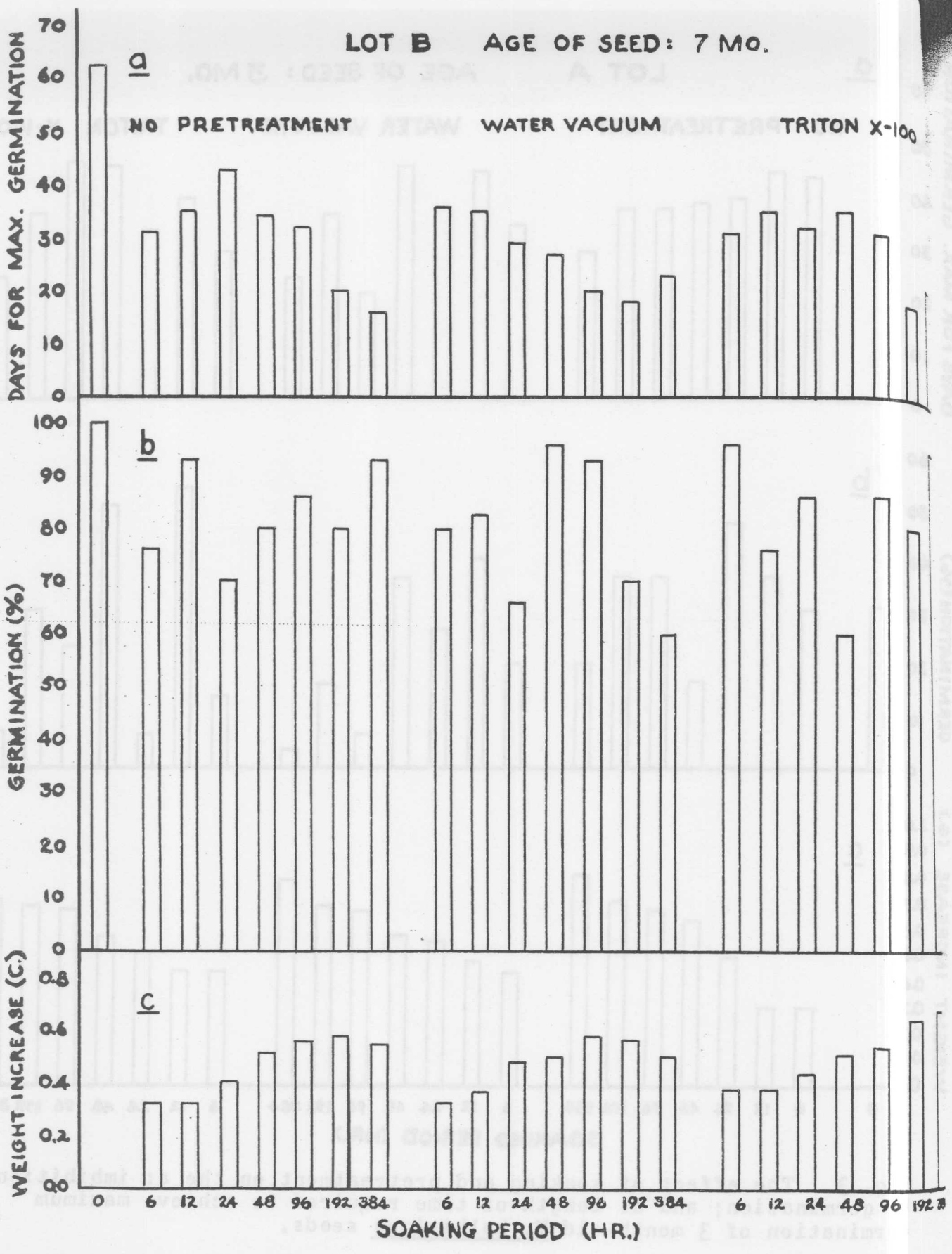


Fig. 4. The effect of soaking and pretreatment on the a:imbibition; b: germination; and c: length of time required to achieve maximum germination of 7 month-old M. balbisiana seeds.

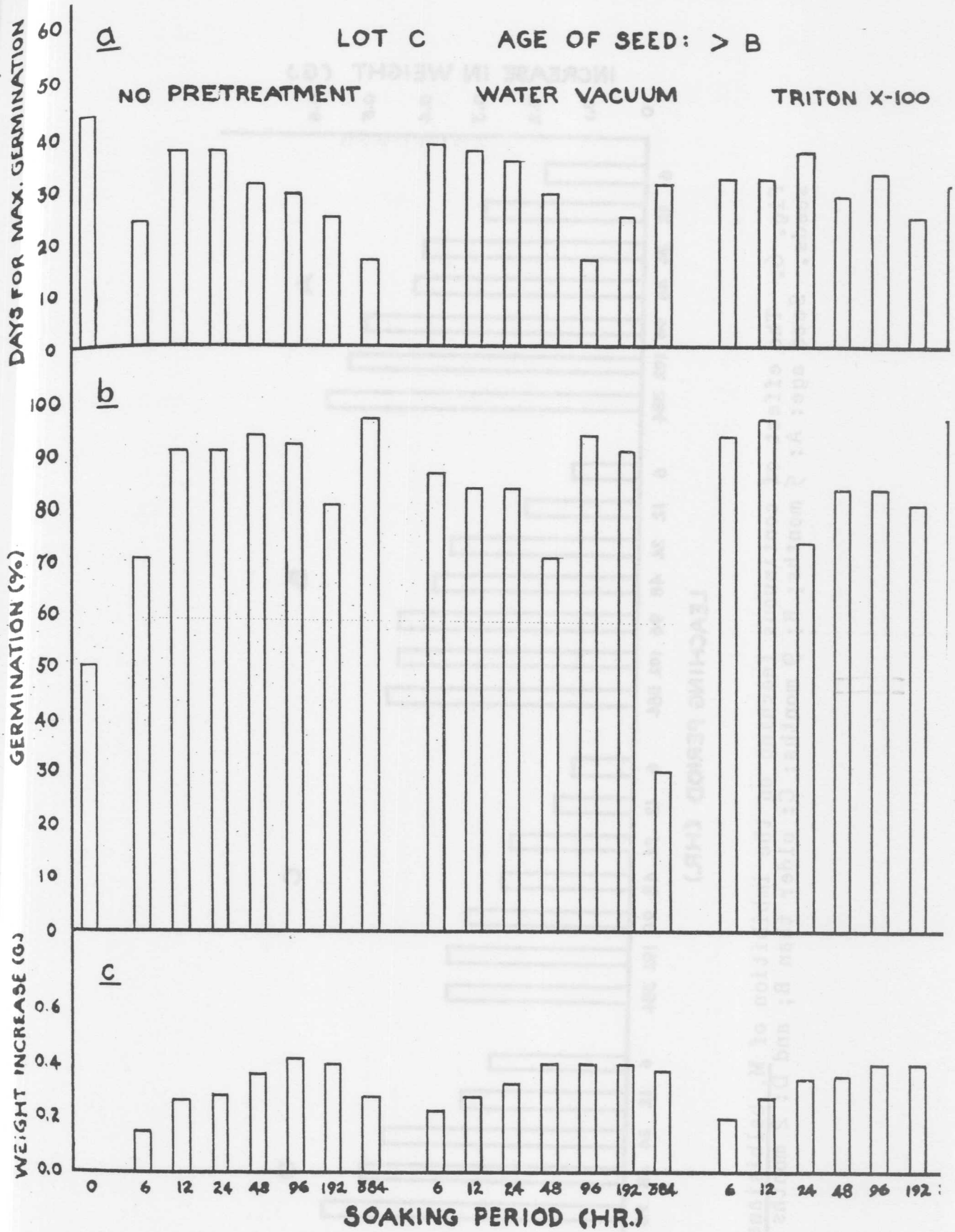


Fig. 5. The effect of soaking and pretreatment on the a: imbibitio b: germination; and c: length of time required to achieve maximum germination of M. balbisiana seeds of unknown age but known to be considerably older than 7 months.

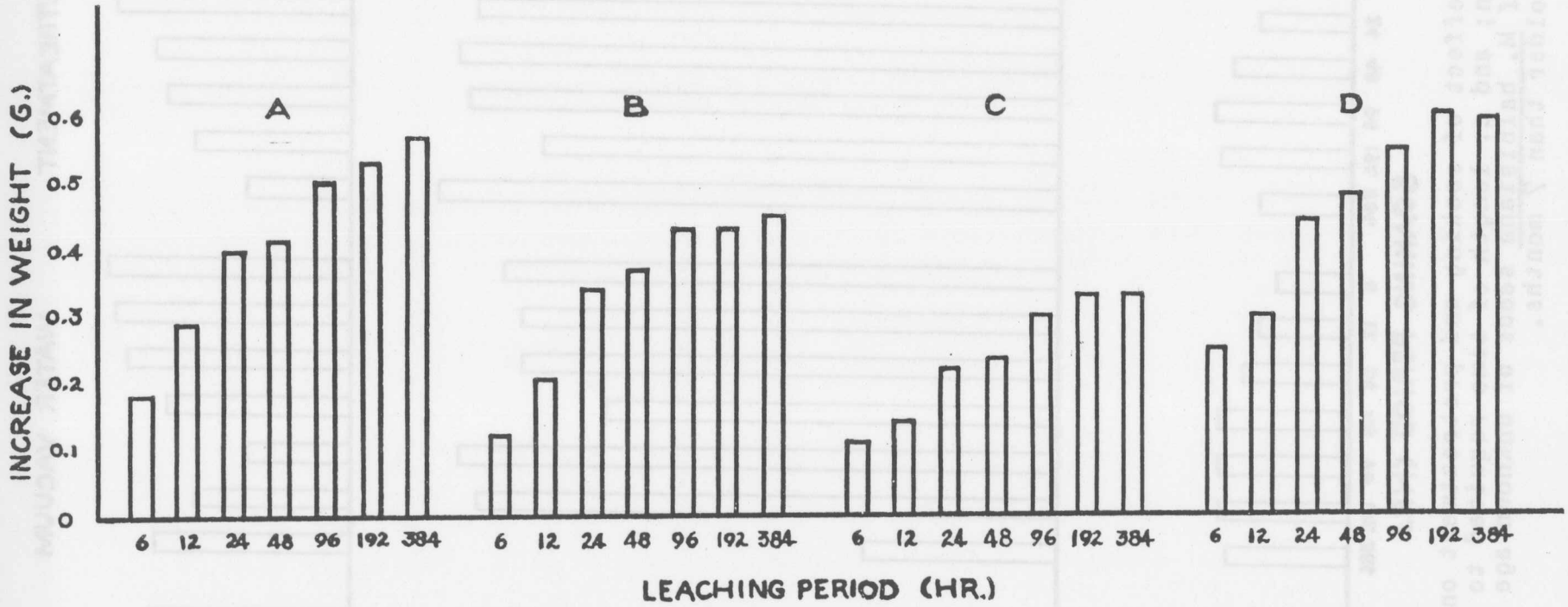


Fig. 6. The effect of continuous leaching on the imbibition of M. balbisiana seeds. Seed age: A: 5 months; B; 9 months; C: older than B; and D: 2 months old.

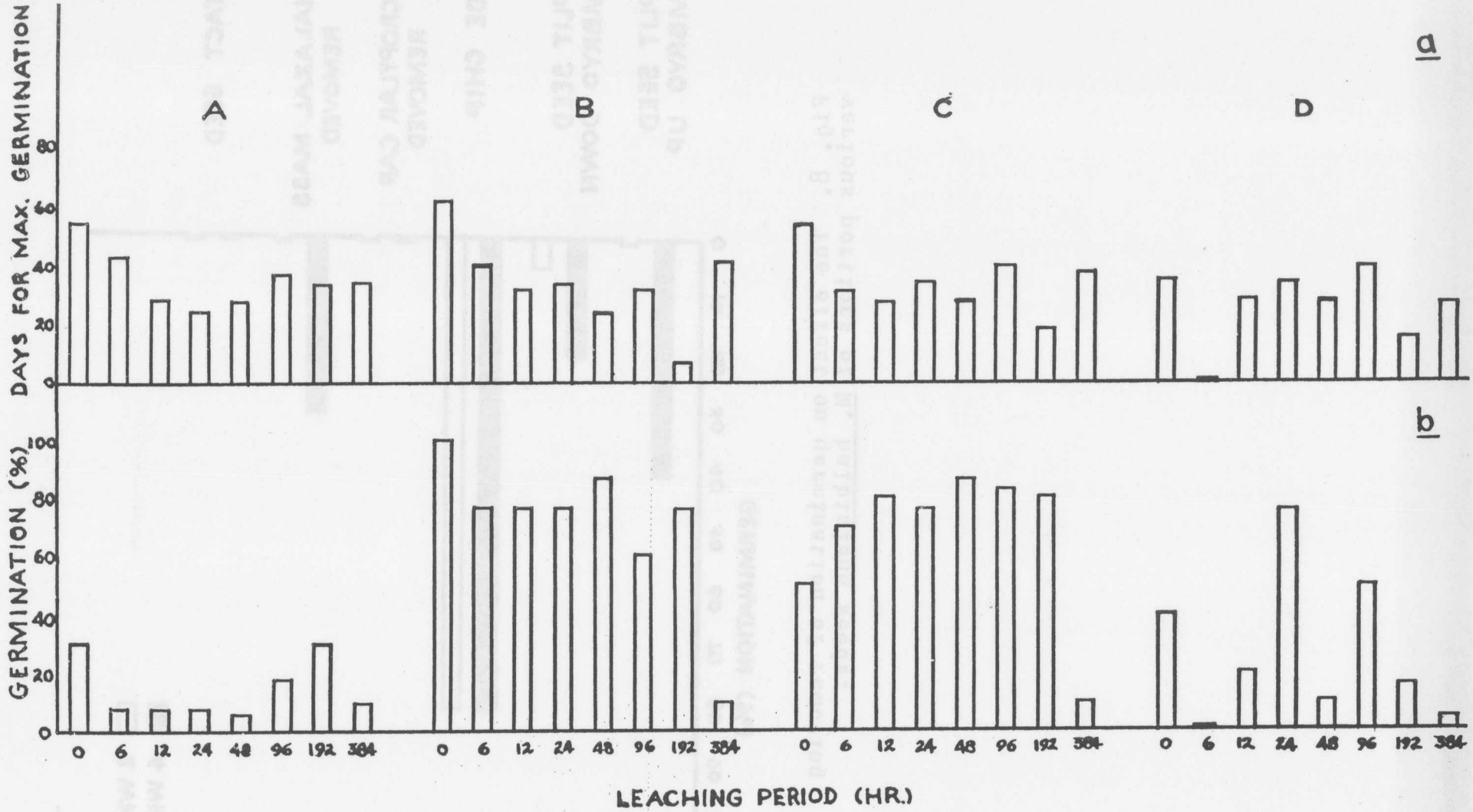


Fig. 7. The effect of continuous leaching on the germination (a) and length of time required to achieve maximum germination (b) of M. balbisiana seeds. Seed age: A: 5 months; B: 9 months. C: older than B; and D: 2 months old.

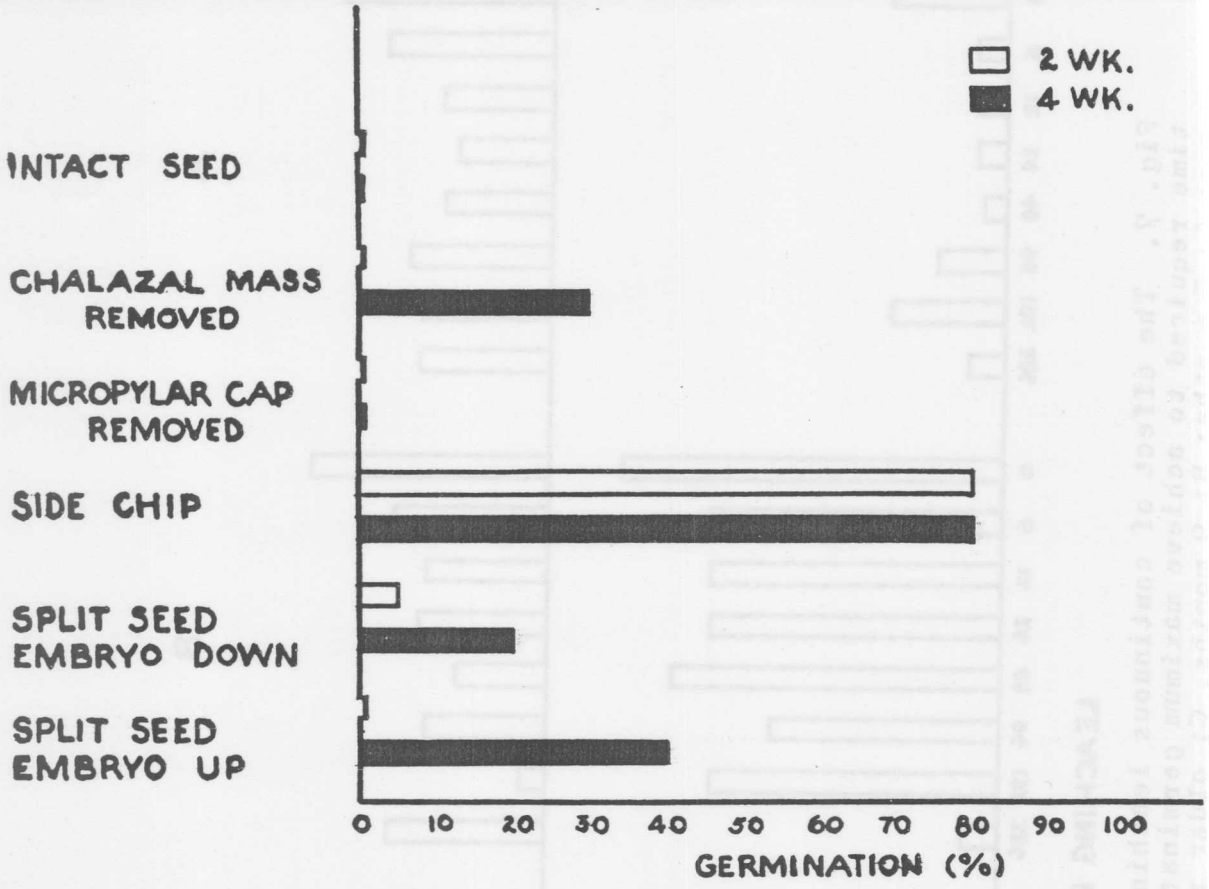


Fig. 8. The effect on germination of removing various portions of M. balbisiana seeds.

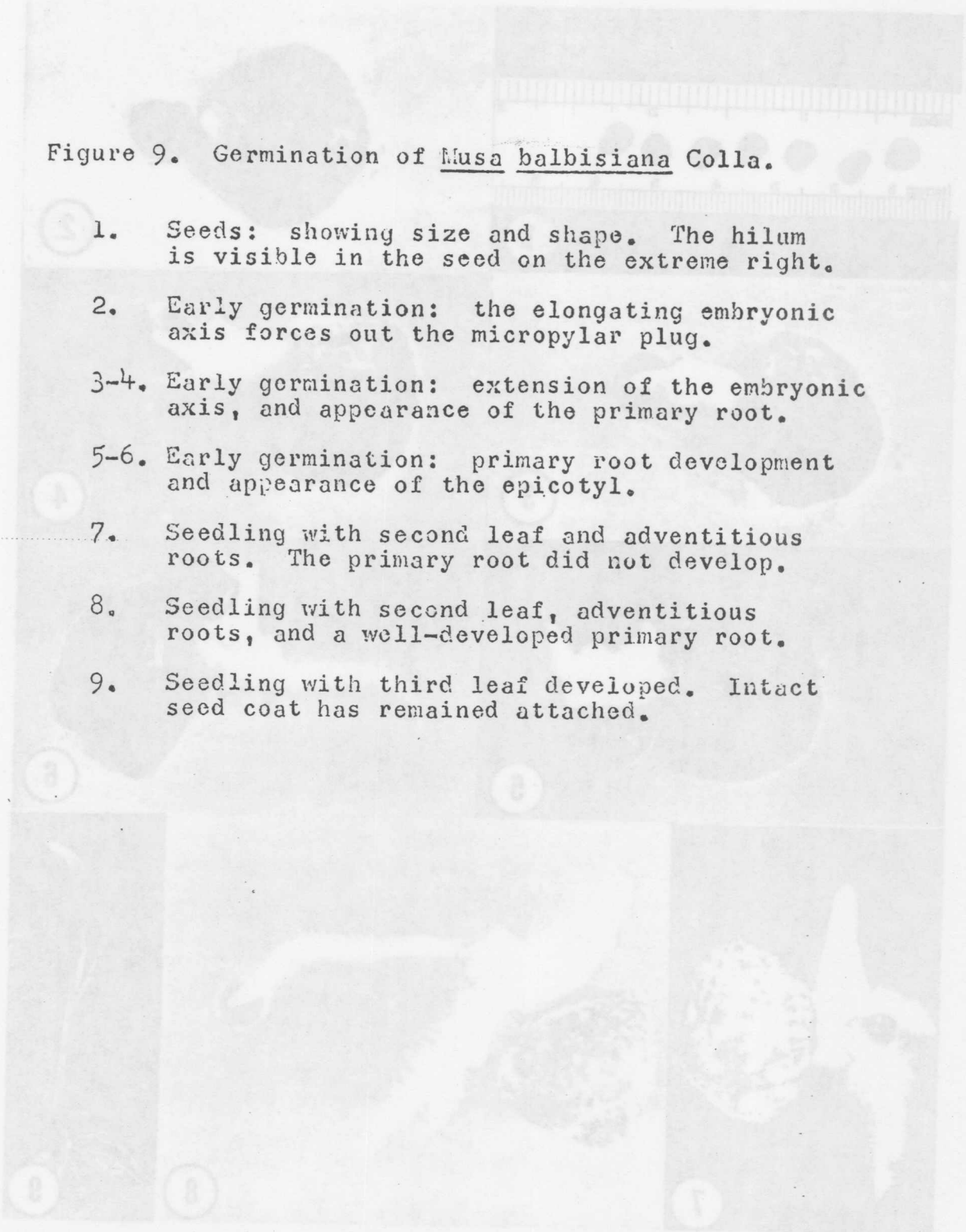


Figure 9. Germination of *Musa balbisiana* Colla.

1. Seeds: showing size and shape. The hilum is visible in the seed on the extreme right.

2. Early germination: the elongating embryonic axis forces out the micropylar plug.

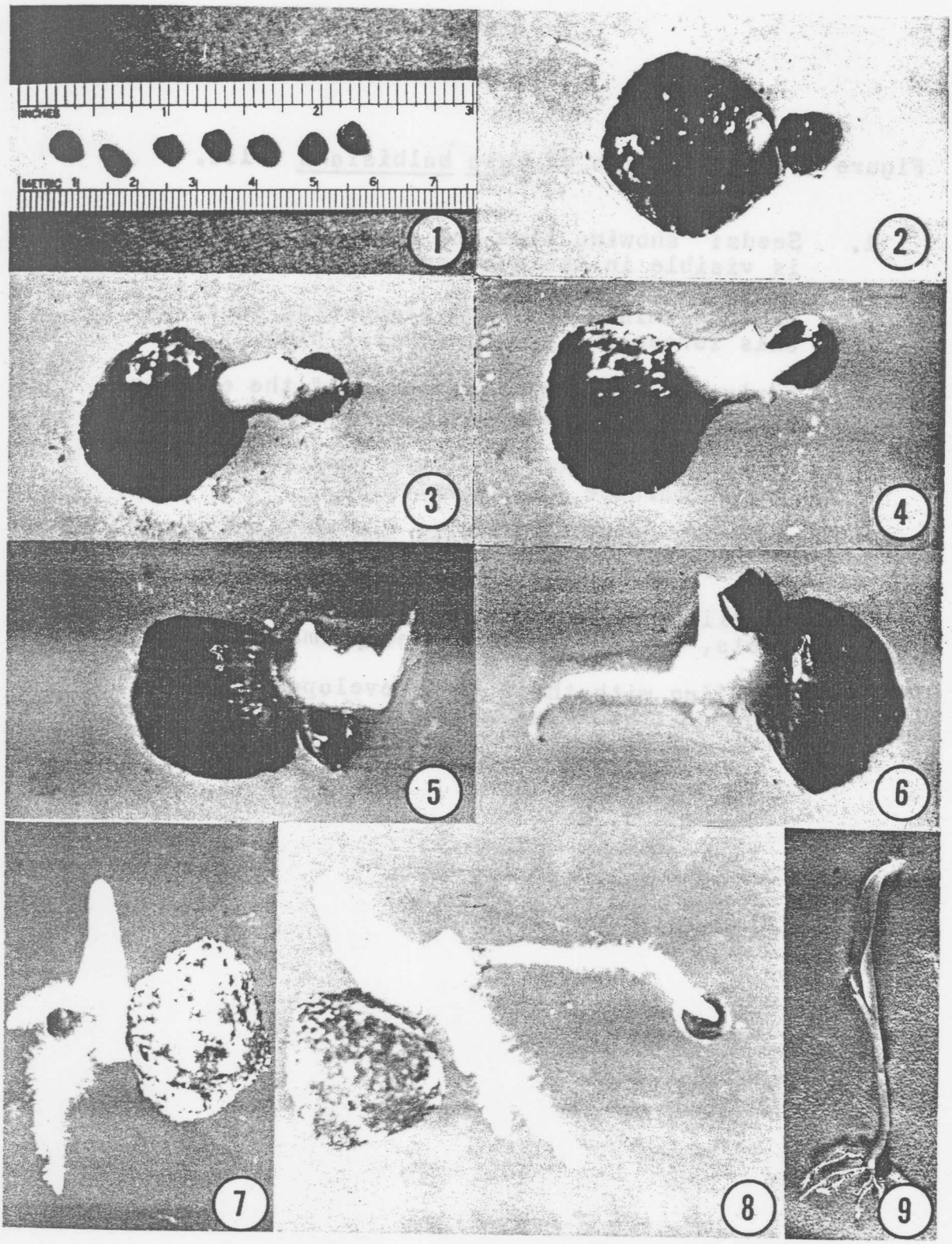
3-4. Early germination: extension of the embryonic axis, and appearance of the primary root.

5-6. Early germination: primary root development and appearance of the epicotyl.

7. Seedling with second leaf and adventitious roots. The primary root did not develop.

8. Seedling with second leaf, adventitious roots, and a well-developed primary root.

9. Seedling with third leaf developed. Intact seed coat has remained attached.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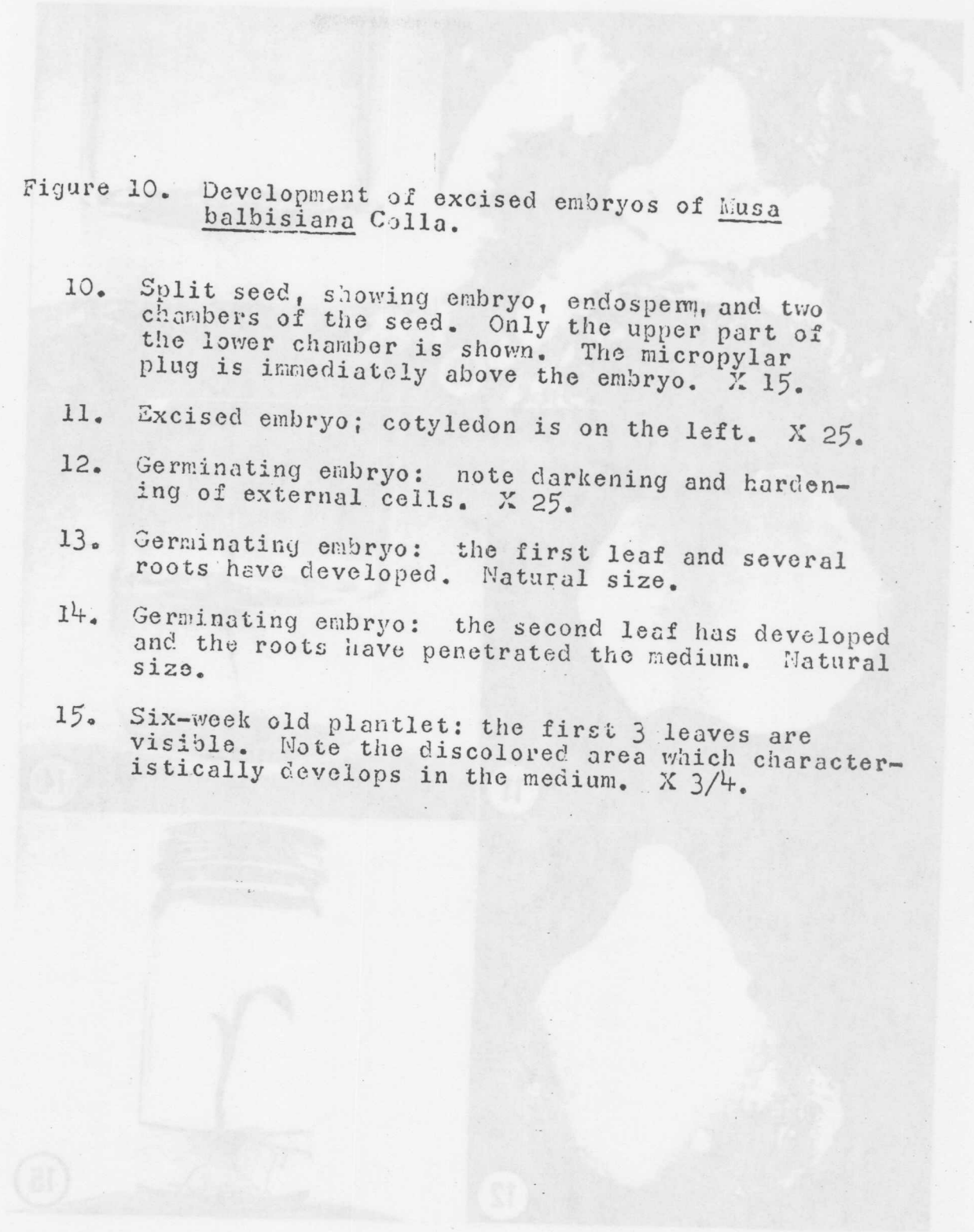


Figure 10. Development of excised embryos of Musa balbisiana Colla.

10. Split seed, showing embryo, endosperm, and two chambers of the seed. Only the upper part of the lower chamber is shown. The micropylar plug is immediately above the embryo. X 15.

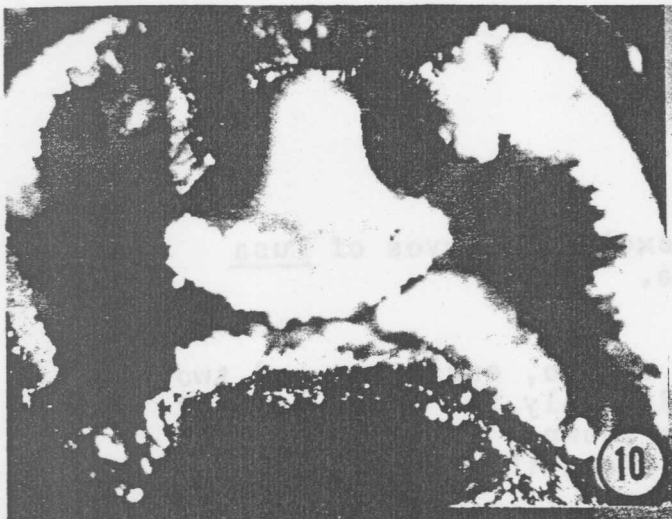
11. Excised embryo; cotyledon is on the left. X 25.

12. Germinating embryo: note darkening and hardening of external cells. X 25.

13. Germinating embryo: the first leaf and several roots have developed. Natural size.

14. Germinating embryo: the second leaf has developed and the roots have penetrated the medium. Natural size.

15. Six-week old plantlet: the first 3 leaves are visible. Note the discolored area which characteristically develops in the medium. X 3/4.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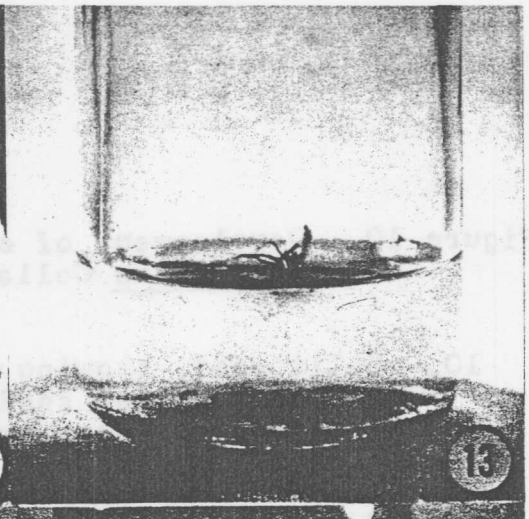
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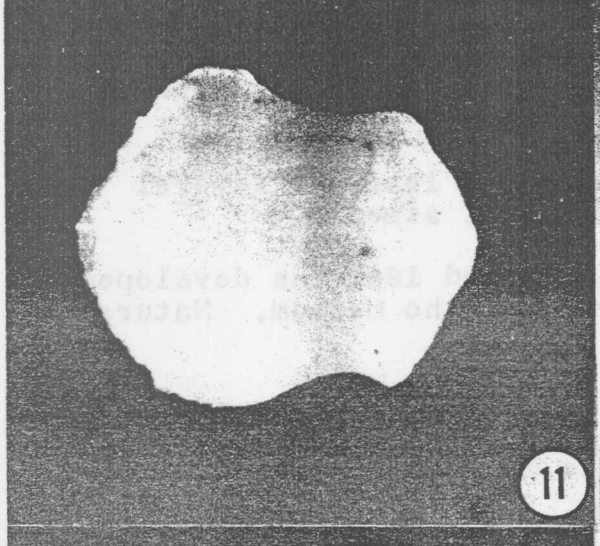
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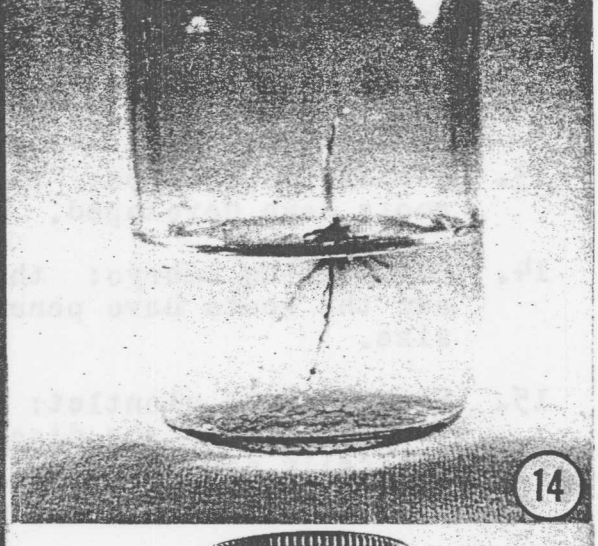
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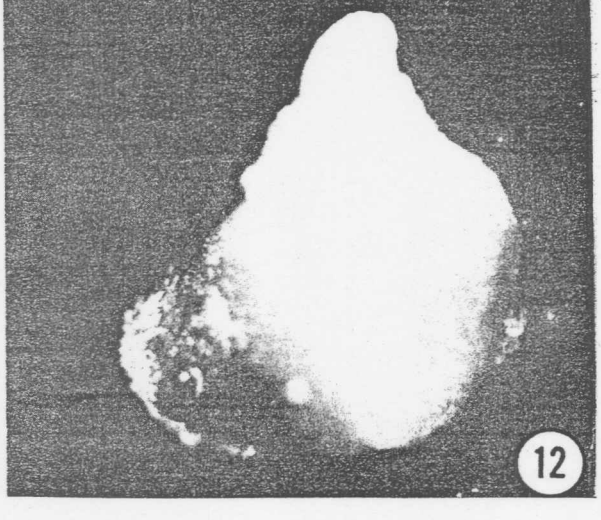
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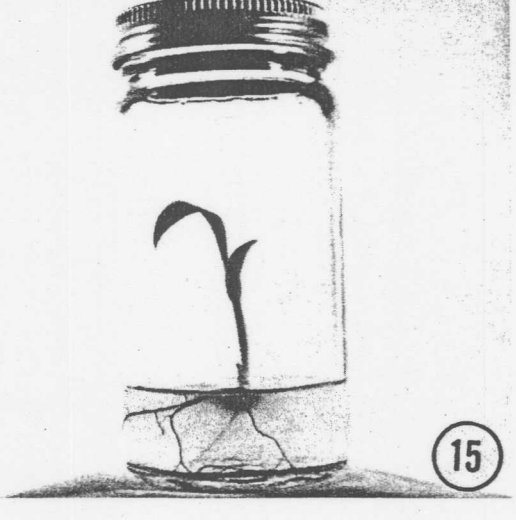
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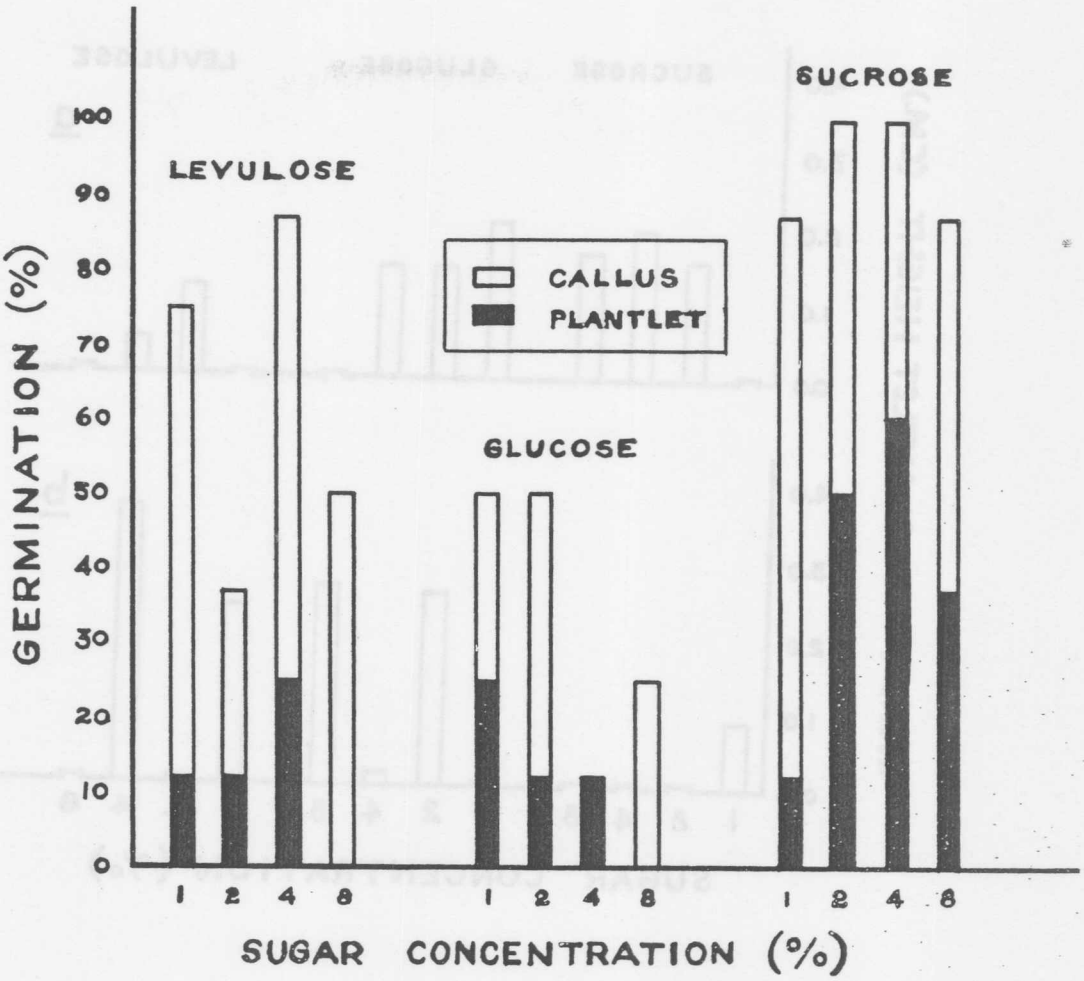


Fig. 11. The effect of type and concentration of sugar on the development of excised embryos of M. balbisiana.

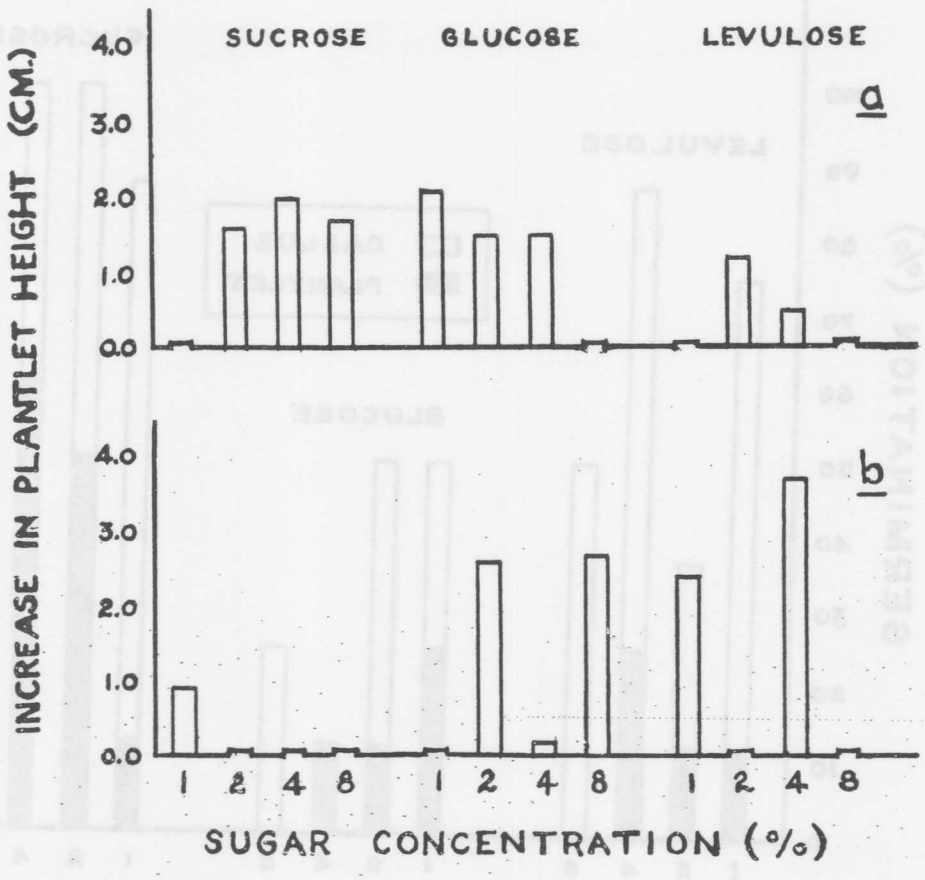


Fig. 12. The effect of additional sugar on the subsequent increase in plantlet height; a: sugars added; b: no additional sugar added.

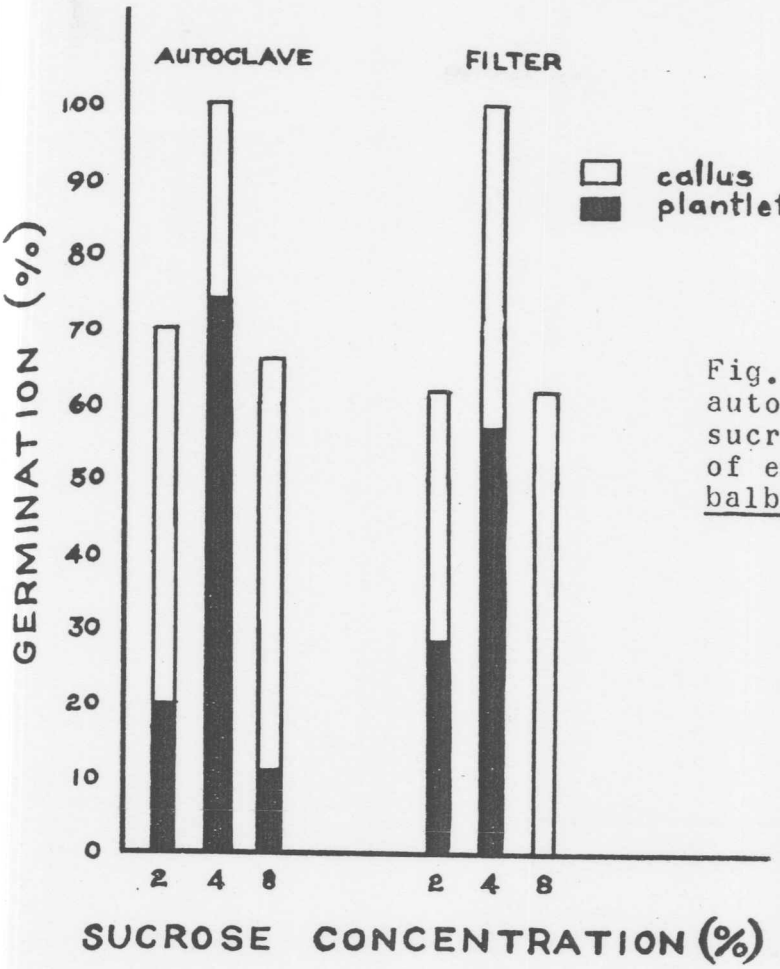


Fig. 13. The effect of autoclaved or filtered sucrose on the development of excised embryos of M. balbisiana.

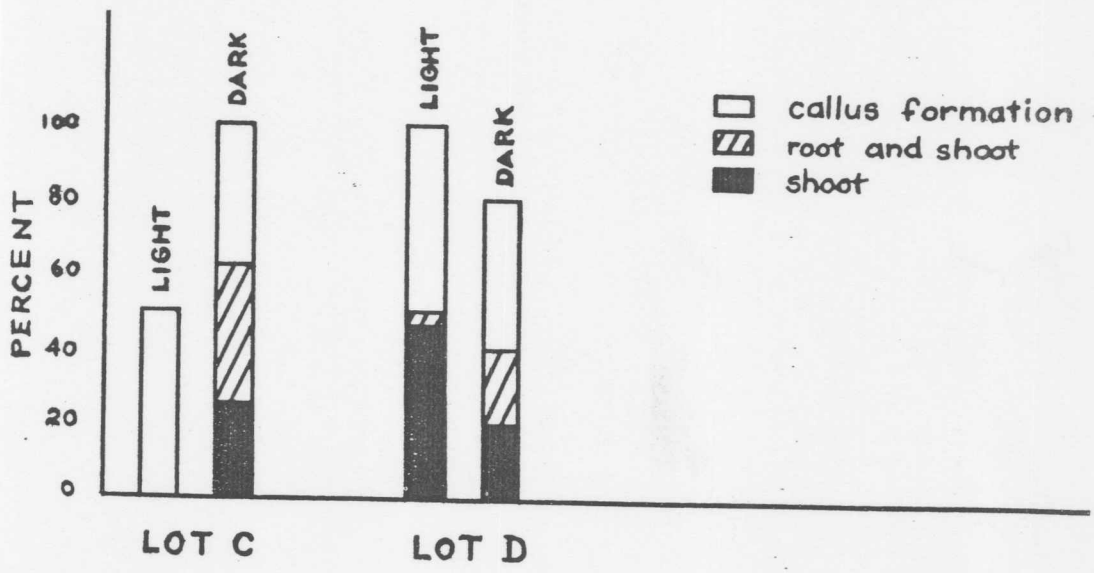
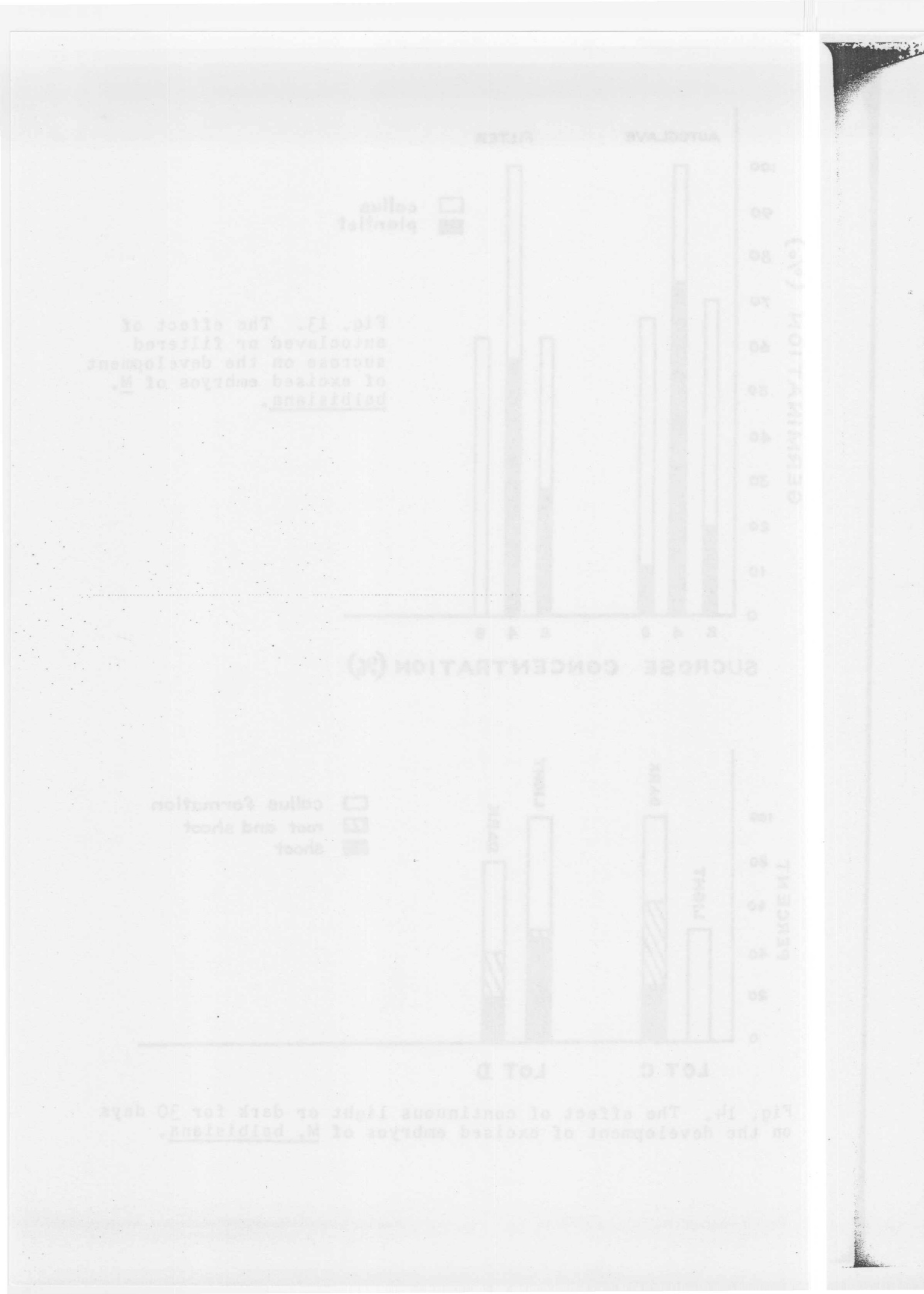


Fig. 14. The effect of continuous light or dark for 30 days on the development of excised embryos of M. balbisiana.



CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

Soil Microbiology Section
Annual Project Report 1959

BDF-2-20

The Effect of Root Injury on the Rhizosphere Flora.

Background

Sequeira et al (1) have presented evidence that root injuries may increase the incidence of Fusarium wilt of bananas. They also reported that the germination of conidia of Fusarium oxysporum f. cubense was markedly stimulated when the spores were placed in the proximity of wounded roots while those placed in the vicinity of intact roots appeared to be inhibited. Root injuries are considered important as portals of entry for many plant pathogens; however, little attention has been given to the effect of root injuries on the root microflora.

The root surface supports an abundance of micro-organisms. These organisms are undoubtedly stimulated by secretions released from root wounds and, hence microbial activity in the vicinity of wounds is probably more intense than in non-wounded areas. In order to invade this area, a pathogen must obviously overcome intense competition. Root secretions lost through wounds are probably attached rapidly and broken down by microorganisms, suggesting that it would be highly unlikely that such secretions would preferentially stimulate pathogenic organisms. If a pathogen is to enter a root injury, it must be present among the root surface organisms in position to attack the vulnerable tissue.

Since little is known about the response of root surface organisms when roots are wounded, investigations of root wound effects are being carried out.

Progress

Except for the developing of techniques, this project has been inactive for most of the year. Preliminary results (Annual Report 1958) have indicated that wounds result in a stimulation of bacterial activity, but that fungal activity is not greatly affected.

Root boxes, similar to those used by Sequeira, have been used for the studies so far carried out, and response of the microflora has been studied by dilution plate techniques. The results obtained by these methods have given rather inconsistent results and hence are not presented. There has been some difficulty in obtaining a constant supply of roots suitable for these studies, and consideration is being given to the possibility of

using peas for these experiments. Peas would offer several advantages in that (1) they form a tap root system, (2) the plants can be started from seeds and a large number of plants of similar age can be obtained, (3) the plants are smaller and easier to handle and (4) they are also susceptible to a Fusarium wilt.

Direct methods have also been investigated in an attempt to study the effects of roots on soil organisms. Fiber glass gauze strips, infiltrated with media of various types or without medium, have been placed in contact with roots. After various periods of incubation, these were examined microscopically. Sporulating actinomycetes and fungi have been observed in such mounts. Conidia of Fusarium oxysporum f. cubense have been enclosed between layers of cellophane and placed adjacent to roots. Abundant germination occurred, and numerous anastomosing germ tubes were seen (Figure 1). Glass wool pads, inoculated with conidia of Fusarium oxysporum f. cubense, were placed adjacent to injured roots, non-injured roots, and soil, and allowed to incubate for 2 weeks. Upon removal the pads were washed in 100 ml. of water and dilution plates prepared. Inconsistent results were again obtained, and there was no apparent affect of the root injuries on the growth of the pathogen.

Conclusions

It must be emphasized that studies reported here are of a preliminary nature, and conclusions are not warranted. Improvement of techniques and repetition of the experiments described are necessary in order to obtain meaningful results.

Recommendations

None

References: Sequeira, L., T. A. Steeves, M. W. Steeves, and J. M. Riedhart. Observations on the role of root injury in Panama disease infections. Nature 182: 309-311.

Prepared by

Roger D. Goos

December 1, 1959

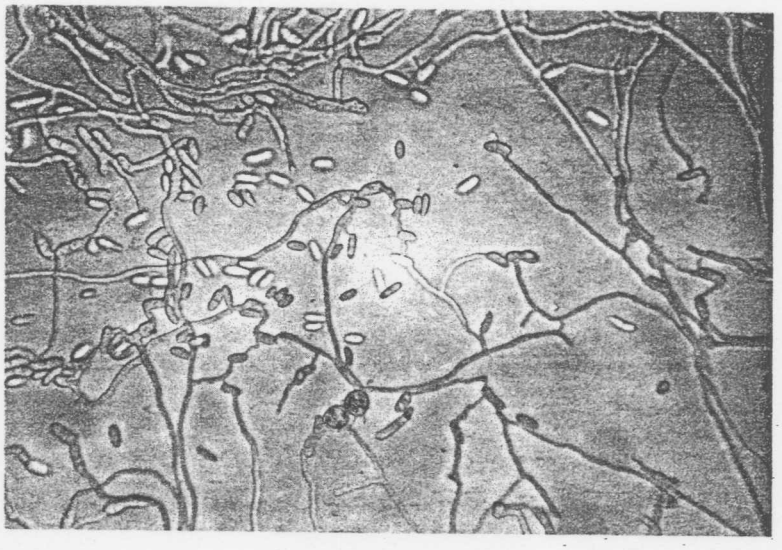
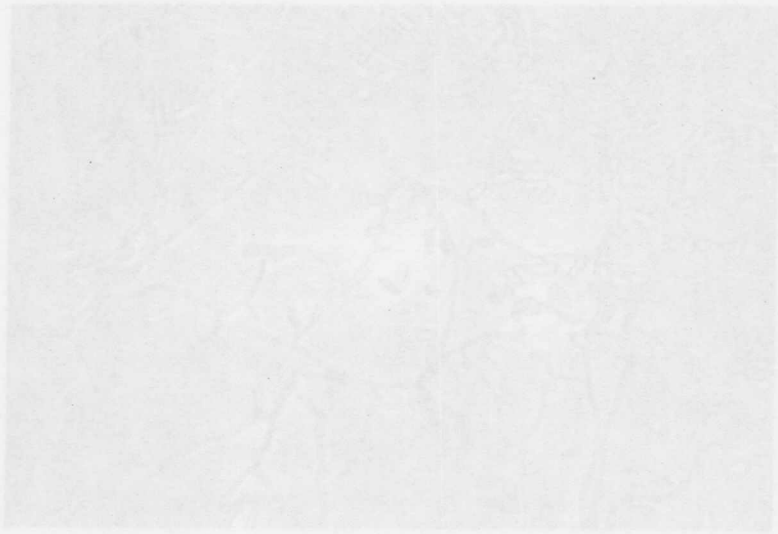


Figure 1. Spores, placed between layers of cellophane and incubated for 48 hours in contact with non-injured banana roots.



CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

Plant Pathology Section
Annual Project Report 1959

BDF-3-0

I. Inoculation Methods and Host Development in Relation to Infection and Disease Development.

A. Previous work

Infection following the puncture of roots under microspore suspensions of clone 1121 at various concentrations and under water was determined. Greatest infection, based on total extension of discoloration in the rhizome plexus (80 to 100 mm), resulted from spore concentrations of 10^2 to 10^3 per ml. of water. A somewhat lower infection (40 to 50 mm extension) resulted from spore loads of 10^{-2} to 10^1 per ml. These results are conditional on 2 counts. (a) Some discoloration (18 mm) occurred in water checks. Isolations from these areas generally yielded cultures of F. moniliforme Trichoderma sp., Myrothecium sp., Actinomyces sp. as well as bacteria were also isolated from these areas. Refined techniques are therefore essential to more definitive results. (b) Considerable variation in frequency of invasion of roots and plexus occurred in all spore-load treatments.

B. Unreported work

Primary emphasis has been given to the infection process because of the variation in infection (b) mentioned above. When a Gros Michel plant is split longitudinally, the peripheral area of the stele can be readily detected. In the upper portion of the rhizome, this tissue (when freshly cut) appears bluish or opalescent. In the lower portion this tissue is yellow. At times an intermediate white region is visible. It had been noted that infections into the plexus (joining root and rhizome stelar tissues) were common in the yellow region. These were seldom if ever observed in the upper (blue) region although the roots originating in this region might be well developed.

An experiment was set up to determine the extent of infection in roots originating in these 3 regions. Gros Michel plants approximately 6 to 8 feet tall and with rhizomes approximately 4 inches in diameter were used for this purpose. Portions of roots adjacent to the rhizome were washed free of soil with a jet of water and inoculated by means of a hypodermic needle. Results are given in the table which follows:

Region of origin of inoculated root	Infection confined to external root	Penetration of root stele to cortical region	Penetration to plexus
Blue	93%	7%	0%
White	20%	80%	0%
Yellow	0%	7%	93%

There was, then, a high incidence of plexal infection resulting from inoculations into lower roots arizing in the "yellow" plexal region. Infections in roots originating in the "white" region generally terminated in the cortical region of the rhizome, while in those roots originating in the "blue" region, invasion was generally confined to portions of the root outside the cortex. Similar results were obtained with 3 month-old plants, although the "yellow" region was often very limited.

The two most likely mechanisms of resistance to invasion appeared to be (1) biochemical, and (2) anatomical. Tissues from the blue and yellow plexal regions were finely ground, sterilized in 95 per cent ethanol, dried, and incorporated in Toussoun's (dextrose, inorganic salts) medium. Ethanol soluble fractions were separated into water and ethyl ether soluble fractions. These were added to absorbent discs and placed on Toussoun's agar. Cork-borer discs of these tissues were surface sterilized with alcohol and placed on agar. No inhibition of growth of F.o.c. (1121) could be detected through 6 days of culture on these media.

A series of experiments were conducted to determine if anatomical differences could be responsible for the above results. When upper or lower roots were severed under aniline blue (water soluble) dye, staining of vascular elements occurred throughout the rhizome and into the extremities of all but the youngest leaf. Transport to the youngest leaf was often limited or did not occur. Distribution from a single (upper or lower) severed root placed into a tube of dye solution was found to be general in the upper rhizome and pseudostem, except in the youngest leaf. These results indicate that a functional union, for water transport, exists between a single upper or lower root and all other parts of the plant.

Carmine red (water insoluble aluminum lake) was introduced in suspension into upper and lower roots to investigate the transport of particles in the vascular system. These particles

introduced into roots originating from the upper (blue) region were limited to the root in 7/7 instances, while particles introduced into roots originating in the lower (yellow) region penetrated the plexus in 7/8 instances and were confined to the root in the cortical region in 1/8 instances.

Finally, microspores were stained by mixing a heavy spore suspension (9 parts) with a strong solution of aniline oil in 50% ethanol (1 part). These spores were held for 3 days after which they were removed from the stain and washed by contrifugation. This spore suspension was introduced into 5 roots of a 3 month-old Gros Michel plant. After 1 hour this plant was dissected. Care was taken to make all cuts from distal portions of the rhizome toward the root treated. Blades were thoroughly washed between each cut to avoid the possibility of carrying spores into clean tissues mechanically. These tissues were then examined miscroscopically for the presence of spores. The results were as follows:

Origin of root	Spores found in stele of: Root	Cortical Region	Plexus
Blue	+ .5 cm	--	--
Blue	+ .25 cm	--	--
White	+ .5 cm	--	--
White	+ 4.0 cm	+	± (few)
Yellow	+ 6.0 cm	+	+ (many)

These results strongly indicate that differences in susceptibility to invasion of upper vs. lower roots of Gros Michel are dependent upon anatomical differences, probably associated with maturation or aging of roots.

Experiments are in progress to determine what these anatomical differences may be and whether resistance of other banana varieties may have an anatomical basis.

Conclusions

In Gros Michel plants spores of F.o.c. are carried by the transpiration stream through older roots into the rhizome-root plexus in a matter of minutes. They tend to lodge here to initiate plexal infections. Younger roots, though extensive and functional in water transport, appear to have anatomical obstructions which prevent passage of spores, as well as smaller particles, into the rhizome.

These results suggest that ungerminated spores may be the source of primary infection and that anatomical obstruction to passage of spores may be a primary source of resistance.

Recommendations

None

Prepared by

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M. E. Mace

December 1, 1959

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

Plant Pathology Section
Annual Project Report - 1959

BDF-3-0

II. Environmental Factors which Promote or Inhibit Disease Development.

Progress

Preliminary experiments indicate that a short (several days) pre-inoculation dark treatment or water deficiency treatment had little effect upon infection and establishment of F.o.c. in roots and rhizomes of small Gros Michel plants. No differences in extent of invasion resulted from 10 to 14-day post-inoculation light and dark treatment of small plants. An extended (10 week) pre-inoculation exposure of plants to various artificial light intensities appeared to have a considerable effect upon infection and establishment. With light intensities at 2200, 980, 680 and 420 f. c. successful plexal penetrations were 30%, 70%, 65% and 65%, respectively and total extension of plexal infections were 22, 92, 61, and 56 mm, respectively. No consistent differences in extent of root invasion resulted from low (22°C) or high (30°C) root temperatures following inoculations. In general, only a small percentage of root infections penetrated the rhizomes of these small (2-3 month-old) plants. When rhizome penetration did occur, however, extension of peripheral infection of the rhizome stele was considerably greater at the higher than at the lower temperature.

Conclusion

Results in the above experiments must be considered conditional on two counts, as discussed in the previous section. Since the environmental factors considered appeared to play a minor role in the infection process, further work on these factors in relation to establishment was discontinued during the final quarter until the infection process itself was more clearly understood. It is anticipated that these studies will be resumed shortly.

Recommendations

None.

Prepared by

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December 1, 1959

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

Plant Pathology Section
Annual Project Report - 1959

BDF-3-0

III. Fungal Metabolites in Relation to Disease Induction.

Progress

The production by F.o.c. of enzymes which may degrade host plant constituents have been studied. Pectin polygalacturnase, pectin methyl esterase and cellulase (cx) have been found. Production of the pectin hydrolyzing enzyme, polygalacturonase, in culture, has been established on the basis of viscosity loss, hypocodite titration tests and chromatographic identification of monogalacturonic acid as the reaction product when fungal filtrates are added to pectin solutions. Optimum production and activity of this enzyme were found at pH 3.0 and 4.0, respectively in the presence of the substrate. The presence of dextrose, with or without pectin, delayed or reduced enzyme production. Pre-conditioning inoculum with pectin enhanced P.G. production. PME production, as determined by titration of available methoxy groups in pectin, was found to be greatest between pH 6.5 and 7.5 in the presence of substrate. Results indicate an early production of these enzymes by germinating spores and young mycelium.

A comparison was made of polygalacturonase production by 9 clones of F.o.c. These were the Goos subcultures 1119, 1120, 1121, 1122, 1123, 1131, 1132, 1144, and 1145 of Stover's A-I clones, respectively. Spore suspensions prepared from mass-transfer or single-spore cultures were used as inoculum. Enzyme activity was determined by viscosity loss of standard pectin solutions 1 hour after the addition of 24 hour culture filtrates. The average viscosity loss of 18 replications per clone was as follows:

1119 (A) 15%	1122 (D) slight	1132 (G) slight
1120 (B) slight	1123 (E) 5%	1144 (H) 39%
1121 (C) 37%	1131 (F) slight	1145 (I) slight

Isolates which produced only a slight amount of enzyme in 24 hours showed a continued increase in enzyme titre at least through 72 hours of culture. A considerable variation in enzyme titre was found among cultures derived from single spores of clones 1119, 1121 and 1144. These results based upon 102 determinations, (Figure 1) suggest that clones 1121 and 1144 are similar in their capacity to produce a high titre of pectin polygalacturonase, but differ markedly from clone 1119 which has a rather low capacity for PG production. It should be noted, however, that these populations overlap.

Earlier results suggested that PG production by clone 1121 reisolated from banana plants was considerably greater than that held on PDA. These results appear to be dependent upon the "induction" of PG by the presence of the substrate. An induction period approximately 14 hours is indicated by data presented in Figure 2.

Conclusions

F.o.c. produces pectin polygalacturonase, pectin methyl esterase, and cellulase (cx). These enzymes might function in the host-parasite relationship to provide nutrients for the fungus and contribute to wilting of the host. That PG and PME are produced in the host is suggested by the fact that enzyme production was greater by sub-cultures which had been reisolated from banana than by ones which had been maintained on potato-dextrose agar. These differences in enzyme titre appear to be dependent upon induced production of enzymes in response to the presence of substrates. A lag period of 14 hours was found for induction of P.G. The mean P.G. titres for various clones differ considerably but the populations appear to overlap in this respect.

Recommendations

None

Prepared by

C. H. Beckman
M. E. Davies

December 1, 1959

fig. 1. Population distribution of single celled cultures of three clones of F.o.c. grouped according to P.G. production expressed as viscosity loss of pectin.

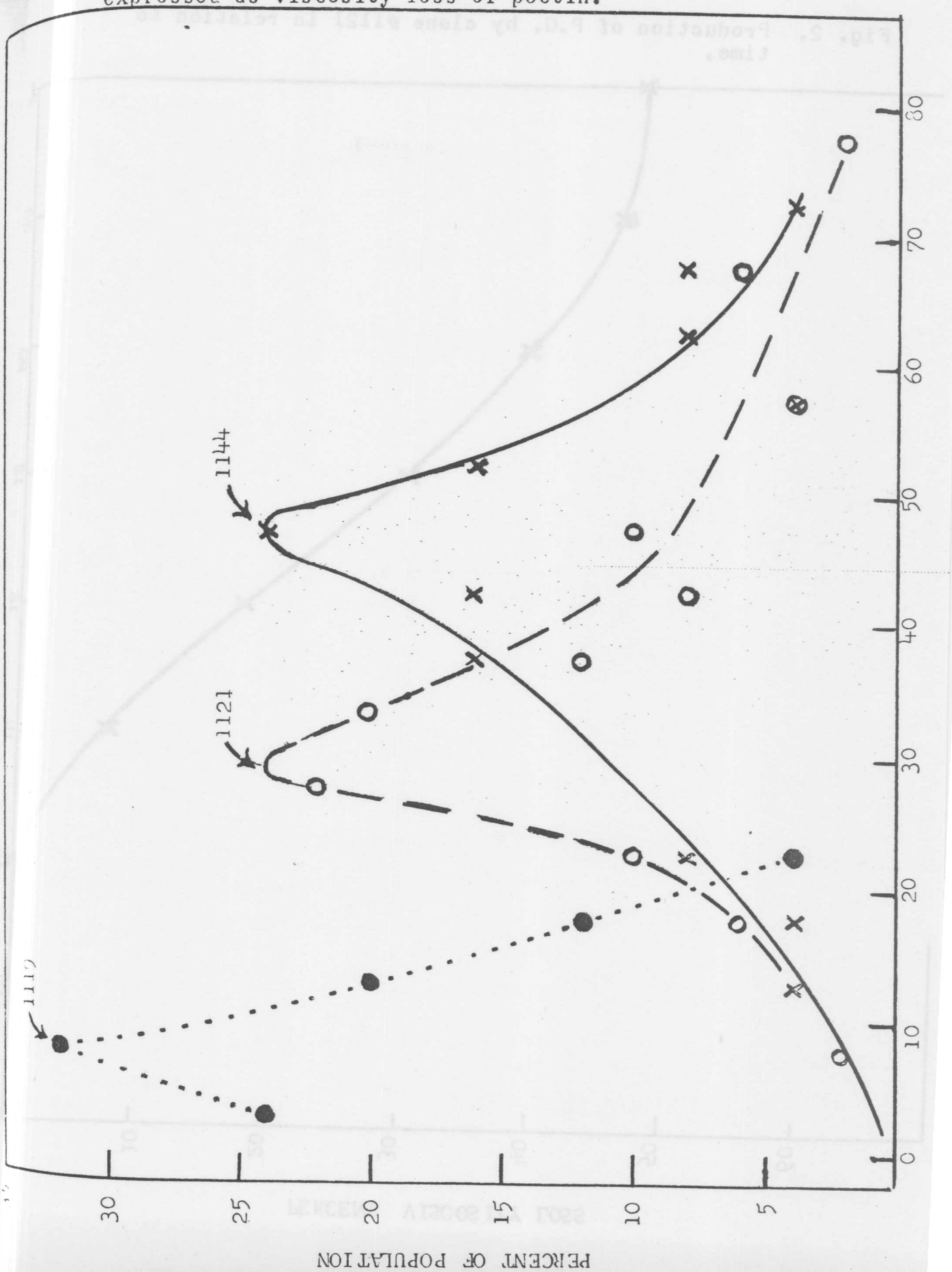
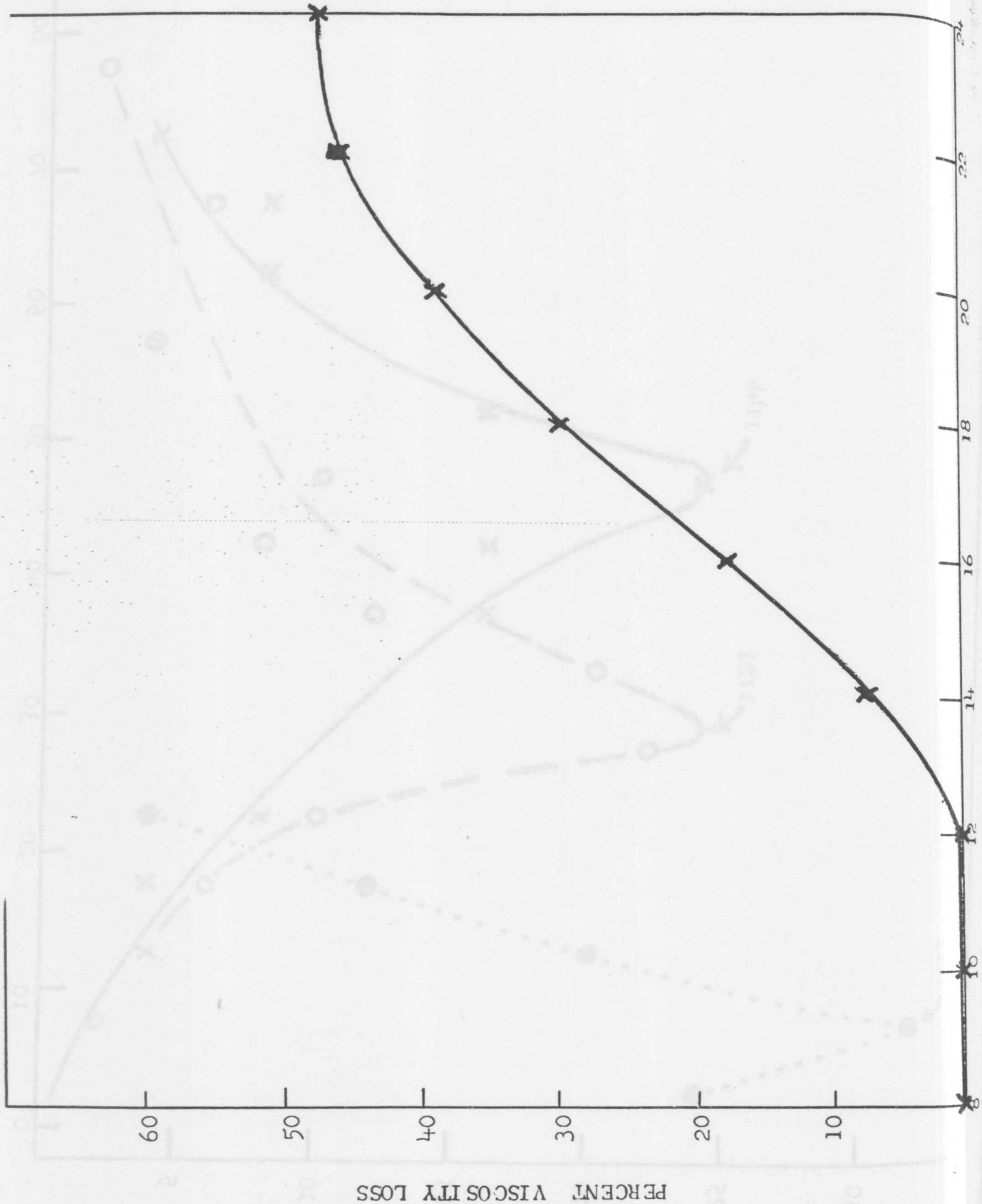


Fig. 2. Production of P.G. by clone #1121 in relation to time.



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Norwood, Mass.

BDF-3-0

Plant Pathology Section
Annual Project Report 1959

IV. Response of the Host to the Action of the Pathogen.

Progress

This project is essentially inactive until consistent infection and symptom development in small plants can be obtained.

Conclusions

None

Recommendations

None

Prepared by

Carl H. Beckman

December 1, 1959

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BDF-3-O

Physiological-Microbiology Section
Annual Project Report 1959

Transpirational Losses from Healthy and Fusarium Infected Plants (1)

Plants of about the same size and age have transpirational rates that vary between plants, but this variation does not appear to be too great to preclude using such a group of plants to determine the variation of transpirational rates of healthy and diseased plants.

Preliminary results indicate that transpirational rates may be controlled by varying either the light intensity or the moisture of the soil. A water deficit in the plant may be obtained through varying the moisture level of the soil under climatic conditions that favor an increased transpirational rate.

1. Wilson, E. M. 1959. Transpiration losses from healthy and Fusarium infected plants. 3rd Quarter Reports, Central Research Laboratories.

Prepared by

Eugene M. Wilson
December 1, 1959

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BDF-3-20

Plant Physiology Section
Annual Project Report-1959

Experimental Systems to Develop *Fusarium* Wilt Symptoms Without the Presence of *Fusarium oxysporum*.

Background

Active polyphenol oxidase systems have been demonstrated for *Fusarium oxysporum* f. *cubense* (Goos, First and Third Quarterly Reports, 1959) and for all organs of the banana plant (Freiberg and Greenberg, Second and Third Quarterly Reports, 1959). Furthermore, G. L. Farkas and G. A. Ledingham (Can. J. Microbiol. 5: 37-46, 1959) and Z. Kiraly (Phytopath. Z. 35: 23-26, 1959) indicated that in stem rust of wheat, the action of polyphenol oxidase systems (produced by both the pathogen and host) resulted in the formation of toxic polyphenols which kill the host cells. Resistance in wheat, therefore, can be increased by increasing the polyphenol oxidase activity of the host cells so that when disrupted by the pathogen, phenolics will be rapidly converted to a toxic form resulting in a sphere of dead cells about the infection, thereby isolating the obligate parasite, *Puccinia graminis*. If such a system operated in *Fusarium* wilt infections, the death of host cells would probably lead to susceptibility since *F.o.c.* is considered to be saprophytic and at best a weak parasite from a nutritional point of view.

Progress

As reported in the Second Quarterly Report-1959, methods to add the fungal polyphenol oxidase system to root tissues were devised. Limited dark (not reddish brown) discolourations extending some half inch up the vascular system were observed.

Studies are continuing with Dr. R. D. Goos to determine what nutritional or inductive factors are involved in the production and release of polyphenol oxidase systems into the culture medium by *F.o.c.*

Conclusions

None

Recommendations

None

Prepared by

E. H. Buckley

December 1, 1959

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BLF-3-20

Physiological-Microbiology Section
Annual Project Report 1959

Observations on the Fusarium Wilt Syndrome in Gros Michel Banana.

Background

Concurrent with investigations of cultural variations of the banana-wilt fungus, further observations were made on syndrome development following natural infection in the field. It was considered that by furthering an understanding of the Fusarium wilt syndrome one may be better able to evaluate disease severity and thus relative virulence of variants of f. cubense. Observations were largely confined to development of symptoms within the pseudostem and axis ("true stem") of Gros Michel plants during the first year after planting in Lake 1, Guaruma Farm.

Progress

The external symptoms of wilt usually consist of various graduations of chlorosis, leaf petiole failure, and death of the leaves. The relatively rapid development of symptoms and death of shoots of young plants in Lake 1 was in contrast with the slower development of symptoms often characteristic of older established plantings.

In general, the leaf sheaths of a Fusarium infected pseudostem were invaded in a more or less acropetal sequence, followed by ingress of the fungus into the bundles of the axis. Sampling of the fungus from a central vascular bundle of the axis represents then a sampling of a fungal colony of established persistence. The following symptom complexes have been observed to occur within the central region of a diseased axis at a height approximately 1 meter above the ground.

Commonly, the walls of tracheary elements, and contiguous xylem and ground parenchyma, are discolored and an amorphous brown material may be demonstrated in the lumen of tracheary elements. A technique was devised to permit a rapid assay of microorganisms from these diseased cells.

Concomitantly observations were also made on other host-fungus relationships at the cellular level.

Plugs, 1.5 cm in diameter by 5.0 - 6.0 cm in length were removed from the central part of a stem, broken and the halves carefully pulled apart to expose the helical secondary wall thickenings of tracheary elements (Figures 1 to 4). Individual helices were removed by sterile forceps and placed on an agar medium, or into a sterile water blank. As an alternative, a group of secondary wall thickenings were twisted together, then pulled from the broken halves of a plug and placed in a sterile water blank. Since the technique did not require the addition of antibacterial substances to suppress surface contaminants, bacteria as well as fungi were assayed.

Of 348 cells examined, 48 contained Fusarium alone, while 138 contained only bacterial (Table I). It is of interest that 47 cells from discolored vascular bundles were free of Fusarium and bacteria. Microscopic examination of sterile-water washings of tracheary cells showed that relatively large numbers of microconidia were present within some diseased tracheary cells.

Since spores of the pathogen could be isolated singly, directly from a diseased host cell, it was possible to survey rapidly in fungus colonies of demonstrable persistence within the host and so determine possible variation. Because of the lack of other suitable criterion, differences in pigmentation were used as an index of variation amongst colonies arising from incubation of spore dilutions on rice agar (15 g rice powder and 10 g Bacto Agar in 1 liter of distilled water).

Isolates producing little pigmentation on rice agar, and in this respect similar to the so-called "sporodochial" type, were obtained from 549 platings of individual cells, removed from the axis of 18 mats and assayed 410 days after planting. In this instance, considerable uniformity characterized the numerous colonies obtained from the cells examined. An examination of 100 cells from the axis of 10 mats in the same area (84' x 144') as above showed red pigment formers present, in addition to the above type, 500 days after planting. From the foregoing, it was established that more than one physiological variant of F. oxysporum, together with bacteria, may inhabit tracheary cells of an axis vascular bundle.

In addition to this microfloral complex, disease expression may vary among vascular bundles adjacent to each other in an axis. Tyloses have been observed within tracheary elements so as to occlude the element lumen. Such occlusion was shown to be non-restrictive to fungus hyphae in that Fusarium was isolated from metaxylem elements vertically above occluded elements in the same axis bundle.

Hypertrophy and hyperplasia of parenchyma, contiguous to metaxylem tracheary elements, have been observed to accompany collapse of metaxylem elements. Collapse of elements associated with hypertrophy is shown in Figures 5 to 10; hyperplasia is not evident in these illustrations. Fusarium has been isolated repeatedly from collapsed metaxylem elements of axis bundles.

In addition to pathologic changes in the vascular bundles, a marked decrease in starch stored in ground parenchyma cells has been observed in diseased axes. (Such decrease is not to be confused with the general absence of starch in xylem parenchyma in healthy bundles of an axis.) In severely diseased stems, very little starch may be detected in ground parenchyma cells.

The utilization of starch by F. oxysporum growing out of diseased bundles was demonstrated. Filter-paper discs, dipped in soluble starch, were placed on the transversely-cut surfaces of axes and held in place by inverted Petri plate halves. After a short incubation, the hydrolysis of starch was demonstrable by negative iodine reaction in areas of discs coincident with diseased bundles in the axes. It is not to be inferred that the decrease in starch in ground parenchyma is due to direct starch utilization by Fusarium, since it is possible that both soluble carbohydrate translocation and starch synthesis may be disrupted in a diseased axis. In addition to the foregoing host responses to Fusarium infection, imbalance in the water economy of leaf lamina, as well as the presence of a toxin has been reported (Page, O.T., Phytopath. 49:61-65; 49: 230. 1959).

The assistance of Mr. R. Lent, La Lima, in fixing, and of Dr. M. W. McCahan, Norwood, in sectioning and photographing the axes tissue illustrated in Figures 5 to 10 inclusive, is gratefully acknowledged.

Conclusions

The Fusarium wilt pathogen may induce several responses on the part of a Gros Michel host. These pathology responses include:

1. The commonly observed external symptoms which include chlorosis, leaf petiole failure, and death of leaves. An observed range in such external symptoms, together with the demonstrable presence of more than one variant of F. oxysporum f. cubense (and bacteria) makes untenable the division of the syndrome complex into two distinct categories.

2. Internal symptoms associated with F. oxysporum f. cubense in the axis include:

   (a) Browning of vascular bundles, with or without the immediate association of a pathogen.

   (b) The presence of an amorphous "plugging" substance of unknown composition within the lumen of tracheary elements.

   (c) Tyloses within the lumen of tracheary elements particularly metaxylem elements.

   (d) Hypertrophy with or without hyperplasia of parenehyma was associated with little to complete collapse of metaxylem tracheary elements. The general description of vascular and adjacent ground parenchyma has been shown.

   (e) Marked reduction in stored starch in ground parenchyma.

These preliminary observations indicate a complex host-parasite interaction in which the role of bacteria has not been elucidated.

TABLE I

NUMBER OF CELLS FROM FOURTEEN GROS MICHEL AXES INFECTED WITH FUSARIUM AND BACTERIA, LAKE 1, GUARUMA FARM 3

		BACTERIA		
		0	+	
FUSARIUM	0	47	738	
	+	48	115	163
			253	348 (Total)

Recommendations

To investigate host-parasite interactions following infection but before symptom expression. This would not involve further elucidation of observable host-parasite relationships, but would involve some more basic interaction possibly occurring before those reactions which result in observable pathologic host responses.

Prepared by

O. T. Page

December 1, 1959

Legends to Plates

Figure 1. Plug 1.5 cm in diameter removed by cork borer from the central part of an axis.

Figure 2. Plug broken and 2 portions pulled apart to expose the wall thickenings of tracheary elements. The wall thickening of an individual tracheary element can be removed and cultured, or a water suspension of spores can be made.

Figure 3. Conical apex of a secondary wall thickening removed from a tracheary element. Note hyphae of Fusarium.

Figure 4. A plug, as per Figure 2, with wall thickenings twisted to facilitate removal and a group of wall thickenings removed from an axis plug. Such group of wall thickenings may be immersed in sterile water to provide a spore suspension directly from diseased host cells.

Figure 5. "Normal" bundles. Phloem parenchyma and xylem parenchyma cells adjacent to tracheary element small, no evidence of hypertrophy.

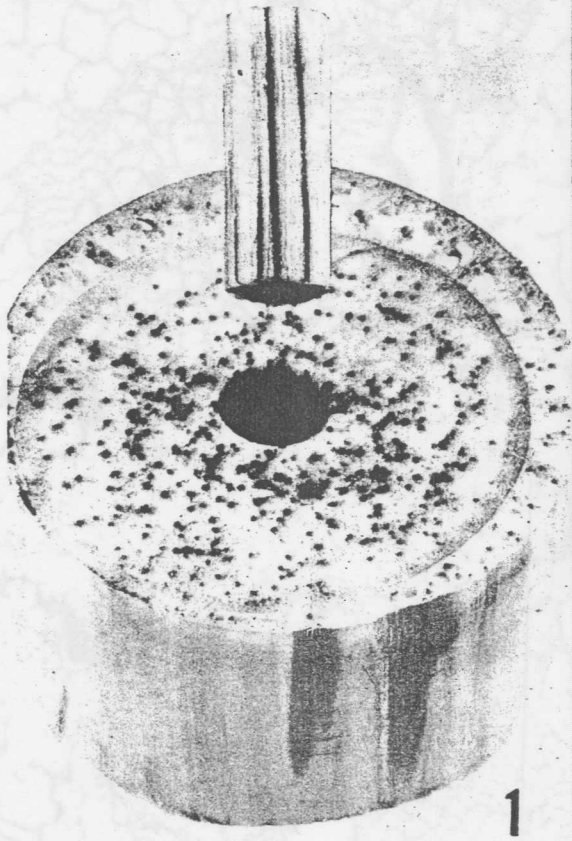
Figure 6. Hypertrophy of the phloem parenchyma has caused considerable collapse of the large metaxylem element.

Figure 7. Similar to Figure 6, but with virtually complete collapse of the metaxylem.

Figure 8. Unlike those bundles illustrated above. The partial collapse of the metaxylem element here has been brought about by essentially equal hypertrophy of the phloem parenchyma and the xylem parenchyma. (Note the tyloses in the collapsed element.)

Figure 9. An example of advanced hypertrophy in which the large metaxylem elements have collapsed as a result of hypertrophy of phloem, xylem and ground parenchyma. It would appear that phloem and xylem parenchyma cells, following their early hypertrophy, have become necrotic and have collapsed.

Figure 10. Further degeneration which has involved more than one bundle. Necrosis and collapse of parenchyma surrounding the affected bundle is general.



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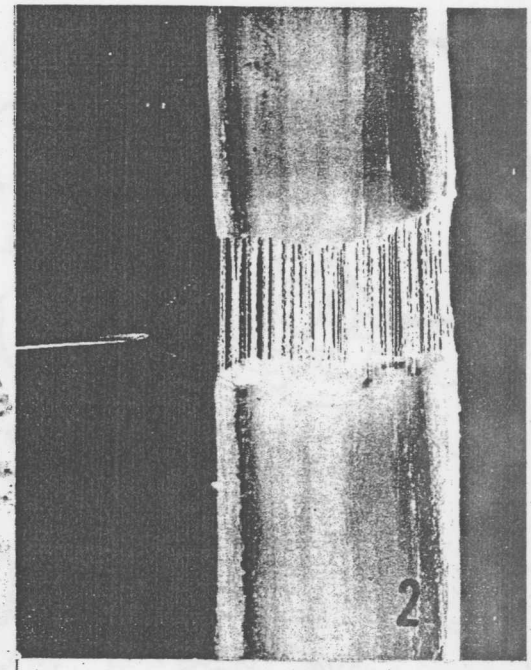

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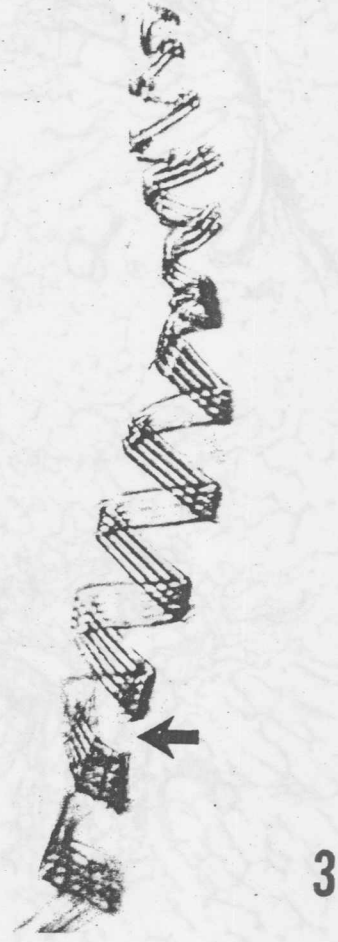

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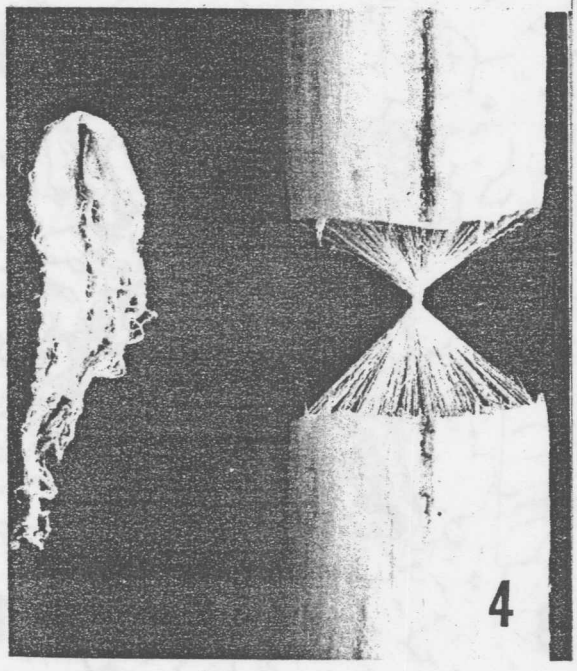

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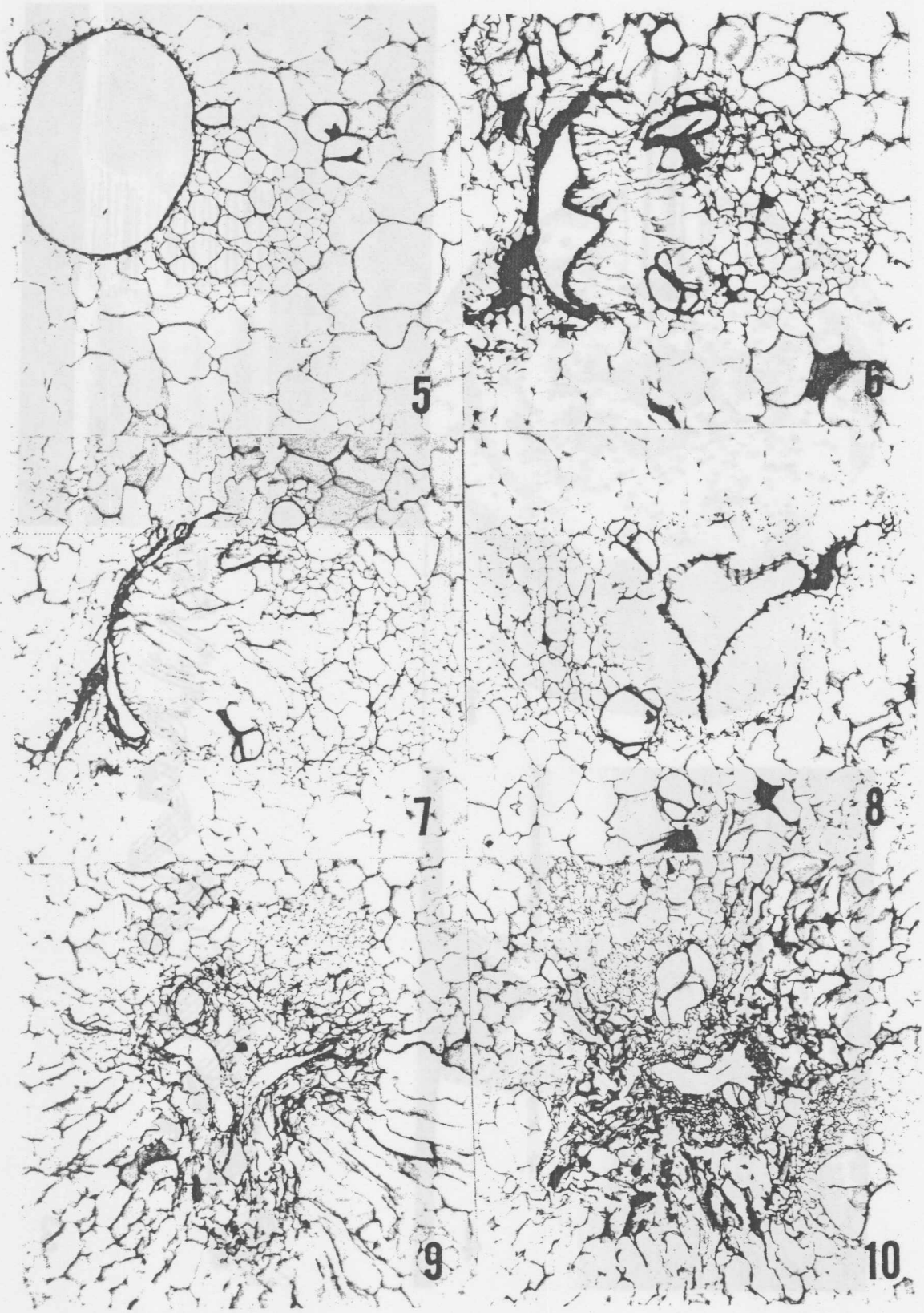
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CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BDF-3-30

Physiological-Microbiology Section
Annual Project Report 1959

The influence of foliar applications of zinc on the Fusarium wilt of banana.

Introduction

Sadasivan (1) reported that foliar applications of zinc as $ZnSO_4$ altered the susceptibility of cotton to Fusarium vasinfectum and thus controlled cotton wilt. This was explained to be due to a reduced substrate of pectin in the roots of the cotton plant. Zinc was found to be a limiting factor for fusaric acid production in vitro. This author also reported a characteristic vein-clearing symptom and a fluorescence in ultraviolet light (3,560 A) acropetally and histologically.

Attempts to observe similar effects of Zn on the Fusarium wilt of Gros Michel banana have been made. The vein-clearing symptom and the fluorescence in ultraviolet light were not observed in Fusarium wilt of banana.

Experimental Observations

Uniform Gros Michel plants were treated in the greenhouse with two foliar applications of $ZnSO_4$ in aqueous solution, one week apart. Two weeks after the first treatment they were inoculated with an isolate of Fusarium oxysporum f. cubense. Treatments were 1, 10, 100, 1,000 ppm $ZnSO_4$ and each treatment was replicated 3 times. Appropriate checks consisted of a treatment with water and a non-treated group.

All plants showed external symptoms of Fusarium wilt after 14 days from the time of inoculation, regardless of the treatment with $ZnSO_4$. Plants that had been treated with 100 and 1,000 ppm of $ZnSO_4$ showed a more severe symptom than those treated with lower concentrations of $ZnSO_4$.

Samish (2) reported that the level of leaf magnesium was raised in bananas by increasing the zinc supply. No attempt was made to confirm or reject this report.

Recommendations

The influence of trace elements on the growth and biochemistry of the banana plant needs further investigation.

(1) Sadasivan, T. S. 1959. In vivo changes in Fusarium wilted cotton. Abst. Proc. IX Int. Bot. Con. II: 338.
(2) Samish, R. M. 1959. Zinc nutrition in horitcultural practice. Abst. Proc. IX. Int. Bot. Con. II: 341.

Prepared by

Eugene M. Wilson
December 1, 1959

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BDF-5-O

Physiological-Microbiology Section
Annual Project Report 1959

The Influence of Soil Moisture upon Symptom Development of Fusarium Wilt of Banana.

Introduction

Under greenhouse conditions that are favorable for external symptom development of Fusarium infections, plants that are maintained in moist soil will not develop external symptoms; however, external symptoms appear if the plants are maintained in soil of low moisture content (1). The present study was made to determine the effect of water-logged soil on symptom development of infected plants. The temperature, per cent, relative humidity, and general condition of light were recorded, but will not be included in this report.

Experimental Observations

Plants were inoculated with an isolate of Fusarium oxysporum f. cubense by cutting off 4 roots, one inch from the rhizome. Roots near the crown of the rhizome were selected for this purpose. These plants were then repotted so there was a shallow layer of soil above the crown of the rhizome. Previous experiments have shown that this method of inoculation and post-inoculation culture resulted in early above-ground symptoms of Fusarium wilt.

One group (5 plants) was watered according to the schedule given in Table 1. Six days after inoculation the other group (5 plants) was placed in sixteen inch clay pots that had been plugged to prevent drainage. These pots only temporarily held water but by daily filling them with water there resulted a water-logged soil. Check plants were included for each condition.

Above-ground symptoms were apparent in both groups of inoculated plants within 14 days from the time of inoculation. Plants maintained in the water-logged soil showed more severe symptoms than those held under restricted soil moisture. Uninoculated plants in the water-logged soil appeared healthy. Root formation appeared to have been stimulated by this treatment.

---

1. Wilson, E. M. 1958. Gros Michel as a host in a quick pathogenicity test for Fusarium oxysporum f. cubense. Annual Report, Central Research Laboratories.

Symptoms in the inoculated plants that were held under restricted soil moisture were typical. Uninoculated plants appeared to have draught-hardened from this treatment, but showed no symptom of Fusarium wilt.

Discussion

These observations may possibly elucidate the conflicting observations on the seasonal occurrence of symptoms caused by this disease. Above-ground symptoms of this disease apparently occur under the two extremes of moisture conditions of the soil when other environmental factors are favorable. Whether light intensities, humidities, and temperatures, that are favorable for the development of symptoms under one soil moisture condition, will or will not also be favorable under the other soil moisture condition is yet to be determined.

Water-logging the soil as a method for obtaining above-ground symptoms in Fusarium infected plants may be used in studies on virulency of isolates of this fungus and in determining infection of experimental plants when dissection of the plant is not desirable.

Conclusions

Symptom development of Fusarium wilt is favored by either an excess or a deficit of soil moisture. This observation apparently elucidates the conflicting observation on the seasonal occurrence of this disease.

Recommendations

None.

Prepared by

Eugene M. Wilson
December 1, 1959

Table 1. Schedule of watering plants to obtain a moisture deficit in the soil which favored symptom development in Fusarium infected Gros Michel banana plants. (Weight of plant, pot, and soil was between 9 and 10 thousand grams).

Days after Inoculation	Date	Ml of water added
3	August 17, 1959	400
4	18	400
5	19	300
6	20	300
7	21	100
8	22	100
9	23	100
10	24	50
11	25	50
12	26	0
13	27	0
14	28	50
15	29	0
16	30	50
17	31	0
18	September 1	100
19	2	100
20	3	100

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BDF-5-0 Physiological-Microbiology Section
Annual Project Report 1959

A Correlation of Virulency of Isolates of Fusarium oxysporum f. cubense in Abaca and Gros Michel Banana

Introduction

Five isolates of Fusarium were found to show variation in virulence to abaca (1). These isolates were of the five culture types suggested by Mr. B. H. Waite. They have been described by Waite as follows:

1. Sporodochial type - slightly or moderate mycelia and pigmentation in agar, parental non-mutated.

2. Sporodochial type - no pigment in hyphal or agar, parental non-mutated type.

3. White mycelial mutant; a stable clone arising from 1 or 2, no pigmentation in agar.

4. Wooly mycelial mutant; unstable, readily going into type 5, pigmentation in coarse, heavy aerial hyphae is often very pronounced, arising from 1 or 2.

5. Slimy pionnotal mutant, arising from 4; with heavy pigmentation in submerged hyphae.

Further tests with these isolates have indicated that there is a definite correlation between virulency for abaca and Gros Michel (GM) banana plants within these five isolates.

Experimental

Abaca plants (variety Bungalanola) and banana plants (GM) were inoculated with these five isolates of Fusarium. Ten abaca plants (1-2 feet tall) were inoculated with each isolate. These tests were repeated 3 times with abaca and twice with banana. The severity of disease was recorded in terms of external symptoms based on an arbitrary scale. All groups of plants were compared against an appropriate check group about 20 days after inoculation (Table 1).

Discussion

Culture type or characteristic has been associated with syndrome and pathogenicity of isolates of F. oxysporum f. cubense (2); however, this association has not been reported from studies with potted plants in the greenhouse with similar soil and a uniform environment.

Stover and Waite (3) reported a correlation of Fusaria susceptibility in Musa bulbisiana and GM. Their observations were

based on root invasion. The present results show that susceptibility to Fusarium attack in abaca and GM banana are correlated. There is not enough evidence available to recommend abaca as an experimental host from which data could be obtained and extrapolated to GM banana.

The present results with these five isolates indicate that virulency of an isolate can be lost, e.g. isolate 4 arising from isolate 1. This observation with these isolates is inconsistent with the observation that virulency is retained in variants arising from a single microconidia regardless of culture type.

Conclusions

None.

Recommendations

An analysis needs to be made to determine if virulence is lost or gained in some variants arising from a specific isolate of Fusarium oxysporum f. cubense. Such an analysis should be made with a standardized pathogenicity test.

1. Wilson, E. M. 1959. Virulency of five isolates of Fusarium oxysporum f. cubense in abaca seedlings. 3rd Quarter Reports, Central Research Laboratories.
2. Stover, R. H. 1959. Studies on Fusarium wilt of bananas. IV. Clonal differentiation among wilt type isolates of F. oxysporum f. cubense. Can. J. Botany 37: 245-255.
3. Stover, R. H. and B. H. Waite. 1959. Studies on Fusarium wilt of bananas. V. Pathogenicity and distribution of F. oxysporum f. cubense Races 1 and 2. Manuscript submitted to Can. J. Botany.

Prepared by

Eugene M. Wilson
December 1, 1959

Table 1. Ratings of external symptoms resulting from inoculation of abaca and banana plants with five isolates of Fusarium.

Experiment Number	Isolates									
	1		2		3		4		5	
	A[a]	B[b]	A	B	A	B	A	B	A	B
1	10[c]	10	10	10	8	8	3	0	4	0
2	10	10	10	10	5	6	1	0	3	0
3	10		10		10		3		3	

a Abaca

b Banana

c External symptoms rated on an arbitrary scale of 0-10

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1

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4

P

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D

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BDF-5-0 (1959 Summary)

Physiological-Microbiology Section
Annual Project Report 1959

Pathogenicity Test

Resistance and susceptibility of banana varieties can be determined under certain greenhouse conditions, by external symptoms (1 and 3). Varieties may show a spectrum of resistance as was found with Gros Michel, Cocas, Variety 67, and Valery (1).

External symptoms of Fusarium wilt of Gros Michel banana plants are dependent upon environmental factors. The parameters of these factors have not been established due to the lack of climatically controlled growth chambers. Preliminary observations indicate that low relative humidities (20-50 per cent), high temperatures of soil and ambient air (25-35°C) and high intensities of light (5,000-10,000 foot candles) during the day, along with low soil moisture are necessary for the development of external symptoms of this disease in young growing plants. The formation of new roots at the crown of the rhizome may function to sustain the infected plant through these adverse climatic factors (2 and 3).

Virulency of five isolates of Fusarium oxysporum f. cubense to abaca (variety Bungalonon) and Gros Michel banana plants was found to be associated. An isolate which was extremely virulent to abaca was also extremely virulent to Grow Michel banana plants and a similar correlation occurred with less virulent isolates. External symptoms in both hosts appeared 14 days after inoculation when incubated in favorable conditions for the development of these symptoms (4).

Histological studies indicate that vascular tissue of a banana rhizome has a pH which is slightly higher (pH 5.6) than the surrounding parenchyma (less than 5.6). Sap from Valery plants had a pH of 4.25 and that from Gros Michel had a pH of 4.6 (1).

1. Wilson E.M. and C.H. Beckman. 1959. Pathogenicity of Fusarium oxysporum f. cubense. 1st Quarter Reports, Central Research Laboratories.
2. Wilson, E.M. 1959. Pathogenicity of Fusarium oxysporum f. cubense. 2nd Quarter Reports, Central Research Laboratories.
3. _____. 1959. Pathogenicity of Fusarium oxysporum f. cubense. 3rd Quarter Reports, Central Research Laboratories.
4. _____. 1959. Virulency of five isolates of Fusarium oxysporum f. cubense in abaca seedlings. 3rd Quarter Reports, Central Research Laboratories.

Prepared by

Eugene M. Wilson

December 1, 1959

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BDF-6-0

Plant Pathology Section
Annual Project Report 1959

The Possible Healing of Cut Button Seed Surfaces

During a study, earlier this year, (2nd Quarter Report 1959) on the germination of pieces cut from button seeds it was noted that the pieces survived longer when the cut surfaces were not allowed to dry out, i.e., cut seeds were held at high humidity prior to planting and during germination. An investigation of this phenomenon, in which quartered seeds were held at either 80% or 20-35% R.H. at 90°F, indicated that morphological differences, at the surfaces, were associated with relative humidity during storage.

The surfaces of pieces stored at high humidity showed a positive lignin reaction with phloroglucinol, whereas tissue stored at low humidity did not. At high humidity storage surface cells resembled normal cortical cells and appeared viable, whereas, at low humidity, loss of cell structure resulted in a fibrous, discontinuous surface layer.

Dr. M. McGahan expects to reactivate this project in the coming year.

Prepared by

Ronald C. Wornick

December 31, 1959

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

Plant Pathology Section
Annual Project Report 1959

BDF-6-0

Vacuum Impregnation of
Button Seeds with Fungicides

Attempts were made earlier this year (2nd Quarterly Report) to drive an aqueous solution of 2-mercaptobenzothiazole into button seed by the vacuum impregnation techniques. Penetration did not exceed the second centimeter from the surface. Furthermore, it was shown that compounds of this type may retard the metabolic processes of the plant. A prerequisite for this technique is, therefore, the selectivity of a lethal agent for the pathogen without threatening the host. For want of a material which might have these properties, the project has been inactive.

The possibility of improving penetration by using a gas, such as ethylene oxide, is currently being considered by American Sterilizer Company (Penn.), and the reactivation of this project will depend on their initial findings.

Prepared by

R. C. Wornick

December 1, 1959

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BDF-6-10

Plant Pathology Section
Annual Project Report 1959

Irradiation of Button Seeds and F. o.c.

Background

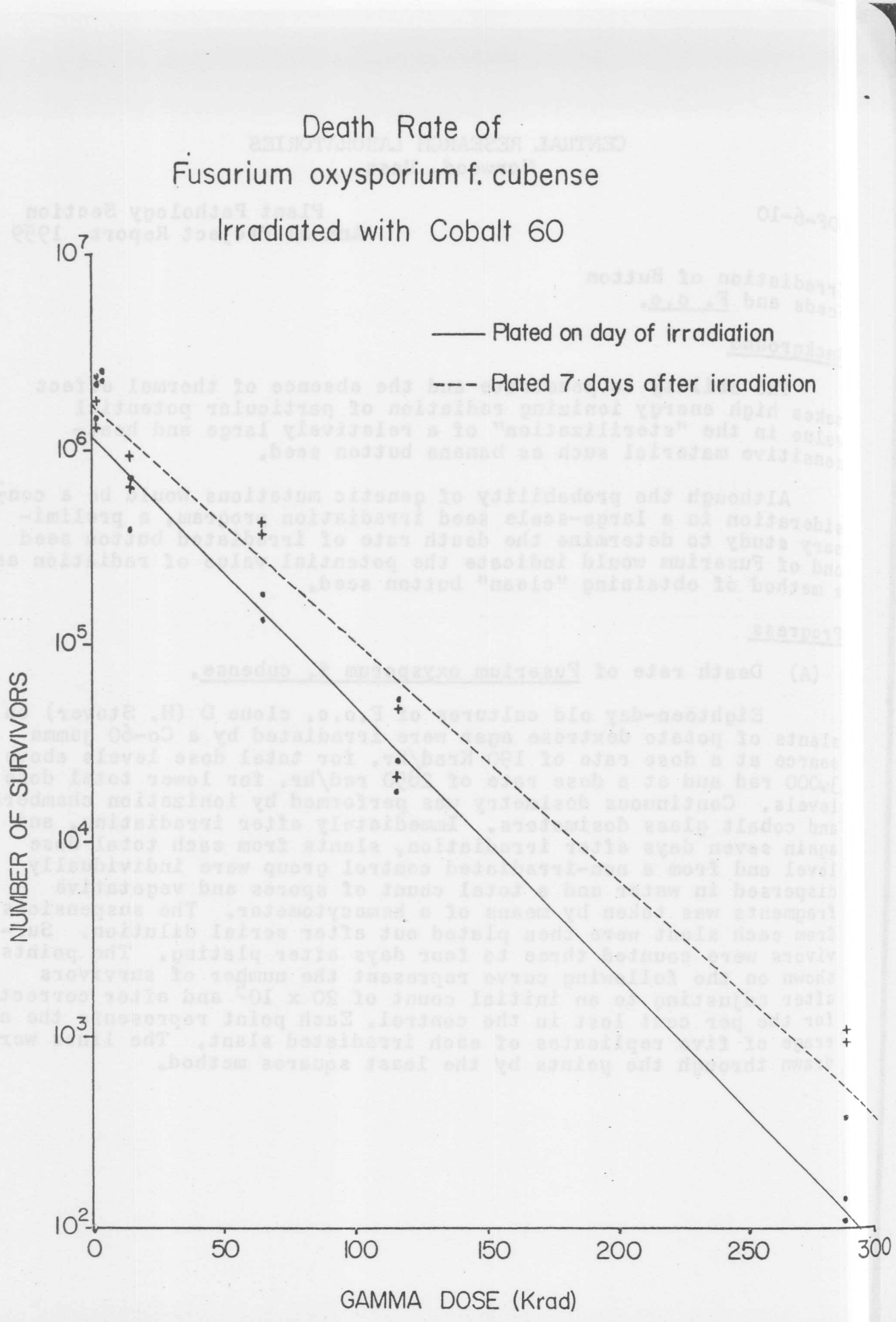
The ability to penetrate and the absence of thermal effect makes high energy ionizing radiation of particular potential value in the "sterilization" of a relatively large and heat-sensitive material such as banana button seed.

Although the probability of genetic mutations would be a consideration in a large-scale seed irradiation program, a preliminary study to determine the death rate of irradiated button seed and of Fusarium would indicate the potential value of radiation as a method of obtaining "clean" button seed.

Progress

(A) Death rate of Fusarium oxysporum f. cubense.

Eighteen-day old cultures of F.o.c. clone D (H. Stover) on slants of potato dextrose agar were irradiated by a Co-60 gamma source at a dose rate of 190 Krad/hr. for total dose levels above 3,000 rad and at a dose rate of 2850 rad/hr. for lower total dose levels. Continuous dosimetry was performed by ionization chambers and cobalt glass dosimeters. Immediately after irradiating, and again seven days after irradiation, slants from each total dose level and from a non-irradiated control group were individually dispersed in water and a total count of spores and vegetative fragments was taken by means of a hemacytometer. The suspensions from each slant were then plated out after serial dilution. Survivors were counted three to four days after plating. The points shown on the following curve represent the number of survivors after adjusting to an initial count of 20×10^5 and after correcting for the per cent lost in the control. Each point represents the average of five replicates of each irradiated slant. The lines were drawn through the points by the least squares method.


Death Rate of
Fusarium oxysporium f. cubense
Irradiated with Cobalt 60
Plated on day of irradiation
Plated 7 days after irradiation
NUMBER OF SURVIVORS
10^7
10^6
10^5
10^4
10^3
10^2
0
50
100
150
200
250
300
GAMMA DOSE (Krad)

(B) Germination of gamma irradiated banana button seed.

Button seed were given various gamma doses as shown below and were then planted in a moist sorgum-moss hot-bed at 90°F for germination. The results, forty days after irradiation, are shown in the following figure. Continuous dosimetry indicated that attenuation of the ionization intesity by the sample did not exceed 24% in the areas of lowest exposure.

Germination of Gamma Irradiated Button Seed

Dose (Krad)	Number of seeds	Number Germinated	Plant Height Average (in.)*	No. of firm ungerminated seed
Control	31	26	20	0
.096	15	14	16.8	0
.48	15	10	16.5	0
.72	15	10	14.9	0
3.5	15	6	8	5
11.8	15	1	1	5
58	15	0	-	4
114	15	0	-	1
285	15	0	-	0
570	15	0	-	0

* Crown to candle top

Conclusions

If 11,800 rad is taken as the approximate maximum gamma dose which button seed will survive, 70% of the Fusarium would be destroyed at that dose level.

Recommendations

None.

Prepared by

Ronald C. Wornick
Allison M. Fletcher

December 1, 1959

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BDF-6-10

Plant Pathology Section
Annual Project Report 1959

Clean Seed

Background

The possibility of subjecting button seeds to a thermal treatment for the purpose of reducing or eliminating various pathogens or pests has been considered previously. Using hot water bath methods, however, seed damage resulted from the long exposure made necessary by the low rate of heat conduction. This seemed to indicate that thermal "sterilization" must be limited to surface "sterilization".

A determination of the thermal death rates of both button seed and Fusarium chlamydospores (Norwood Annual Reports 1958) indicated that the pathogen would actually survive slightly more heat than the host. This difference was relatively small, however, and so the possibility of increasing the thermal resistance of button seeds and of developing a rapid and uniform seed-heating method were investigated in order to diminish this difference in death rates and to more nearly approach an internal "sterilization" as well.

Progress

(A) Effect of storage conditions on heat resistance of button seeds.

Button seeds were stored at 80°F and 65% R.H. (1st Quarter Report 1959) and at 60°F, 80°F and 90°F at 60% R.H. (2nd Quarter Report 1959). Periodically, tissue discs were cut by microtome from the meristem of seeds taken from the various storage conditions. Total 5 hr. respiration, and respiration rates, of samples heated 5 min. at 131°F and on unheated samples, were then determined manometrically at 86°F.

This investigation indicated that of the storage conditions studied the lowest storage temperature resulted in the most heat resistance. Under all conditions the seeds approached a maximum heat resistance which was maintained for a period of no more than two to three days and quickly lost. It was also shown that the time to reach the period of maximum heat resistance increases with diminishing storage temperature. The 18-20th day of storage at 60°F yielded seeds of maximum heat resistance.

(B) Geometrically uniform seed material.

The very irregular size and shape of button seed has considerably complicated the development of heat treatment methods since no two seeds are heated equally, whether by hot water or microwave energy techniques.

To determine if button seeds might be trimmed to a particular reproducible shape (2nd Quarter Report 1959) which would germinate well and also lend themselves to heat treatment, a large group of seeds was cut into various shapes, surface cured, and planted in moist sorgum moss. Cylinders 2½" (diameter) x 2" (length) which were cut to include the apical growing point at one end were far superior to all other shapes considered. Of the 24 cylinders of this size planted, 20 germinated and put out well-developed root systems. There were no incidences of rot in the germinated cylinders. All other shapes and sizes investigated either germinated poorly (smaller cylinders) or were prone to rot (slices). Ten weeks after germination, plants from 2½" cylinders and from whole button seed were equal in height.

(C) Heating button seed cylinders with microwave energy.

Earlier studies (Norwood Annual Report 1958) on microwave equipment, and microwave heated button seeds indicated that highly irregular heat patterns were set up within individual button seeds. This was due partly to the irregular geometry of the seeds and partly to irregular field patterns in the heating chamber. When the feasibility of geometrically uniform seed pieces was later demonstrated, it became desirable to reevaluate microwave heating.

To eliminate temperature differences caused by field irregularities a device for moving the sample through the field was constructed. When 2½" dia. cylinders were then exposed to 1600 watts of 2450 mc, heat patterns were much improved. However, a temperature gradient existed from the cylinder surface to its center. The possibility of utilizing a more penetrating wave length, 915 mc, was considered and equipment was made available through the courtesy of Dr. Robert Decareau, Raytheon Manufacturing Company. A series of trial and error exposure programs, utilizing both wave lengths and several wattages, resulted in a method for attaining a reasonably uniform temperature (±3°C) in the desired range of 50-65°C. The optimum exposure program consists of 1,000 grams/112 sec./800 watts with microwave exposure times in the ratio of three to one of 2450 mc and 915 mc in that exposure sequence. If the available energy is maintained at 800 watts, then the total exposure time is directly proportional to sample weight and is determined from the ratio given above.

An experiment to determine whether 2½" cylinders would survive such a treatment was performed. Upon receipt of button seeds from Coto, Costa Rica, each seed was cut into a cylinder by means of a 2½" cork borer and then trimmed to 2" in length. A total of 84 cylinders was used as follows: 30 cylinders - unheated controls, 30 cylinders - heated to 52°C ±3°C and cooled at room temperature (≈27°C), and 24 cylinders heated to 52°C ±3°C, held at room temperature for 1 minute (to equalize), then cooled in running tap water at ≈15°C for 5 minutes. All three groups were then planted in a moist sorgum-moss hot bed at 90°F for germination. The table which follows summarizes the results of this test.

Sample	No. of cylinders used	No. germinating	% germination	Aver. plant height[2] (in.)
Control[1]	30	20	67	15
Heated to 52°C±3°C equalized at room temp. and cooled in running water at ≈15°C	24	17	71	12
Heated to 52°C±3°C and cooled at room temp. ≈27°C	30	4	13	7

(1) Cylinders were not surface cured before heating and planting.

(2) Measured crown to top of candle.

Apparently one minute at 49-55°C is a safe treatment. If the heat is not then removed, however, severe damage will result as a consequence of the very slow cooling rate at room temperature.

Conclusions

Button seed cylinders 2½" (dia.) x 2" (length) with the apical growing point at one end will produce 70-80% as many plants as whole buttons and may be heated to 52°C±3°C by a microwave technique without interfering with germination.

Recommendations

The microwave technique decribed in this report should now be applied to button seed which are known to be infected with either nematodes, insects, Moko, or Panama disease or combinations of these pests or pathogens.

Prepared by

R. C. Wornick

December 1, 1959

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BMR-1

Anatomy-Morphology Section
Annual Project Report 1959

The Vascular Anatomy of the Rhizome of Banana.

Background

The relationship between the vascular system of the banana and vascular wilt diseases is apparent. The purpose of this investigation is to examine, in the greatest possible detail, the structure, arrangement and nature of the transport system of the plant. There will be essentially two phases to this study: 1) The nature and distribution of the tracheary elements; and 2) The patterns of vascular interconnections between leaves and roots.

There has been only one definitive study on the distribution of vessels and tracheids in the banana (1). In the two varieties reported (Musa paradisica L. and M. paradisica var. sapientum Kuntze), vessels were found only in the roots and in the inflorescence axis and "...apparently nowhere else in the shoot system". Less specific observations have been made incidental to other studies (2,3,4) and should be re-examined. The presence or absence of perforated cells (vessel elements) in the conducting system may be of critical significance in the spread of a pathogen in the host.

The exceedingly complex pattern of vascular bundle distribution in the rhizome has been described only in general terms by Skutch (3). He was able to trace the course of single bundles in mature, or nearly mature, rhizomes. A more complete and detailed picture is required if we are to understand the movements of materials in the plant.

Progress

1. The investigation of the nature and distribution of the tracheary elements is in its earliest stages. Various techniques, such as macerations, and dye infusion of fresh and killed material, are to be employed. One observation of interest is the occurrence of what appears to be perforations, ranging in diameter from 2 - 4 microns, in the common wall between two superposed tracheary elements. These perforations are distinctly not those usually associated with vessel elements.

Figure 1 shows these perforations (perf) in the primary wall material between the spirals of secondary wall material (sw) taken from a vascular bundle of the leaf sheath of a Lacatan plant. This fragment of wall material was removed from the plant by locating the individual tracheary element under a dissecting microscope and with fine forceps pulling out the spiral thickening, bringing with it fragments of the primary wall. Dr. Beckman had immersed the base of the pseudostem of this plant in a suspension of carmine red. (See Dr. Beckman's report for a fuller explanation of this.) The carmine red particles had moved up in certain bundles in the leaf sheaths and account for the darkened areas in Figure 1 (cr). If these elements are indeed tracheids, as suggested by Cheadle (1), then the presence of such perforations may explain the movement of the particles of dye. Insofar as I have determined at this time, however, such perforations have not been reported in tracheids except in the closing membranes of bordered pits in the Coniferales, and here the perforations are much smaller in diameter.

2. In order to determine the patterns of vascular interconnections, serial transverse sections of entire rhizomes must be made. These sections must then be photographed and each vascular bundle labelled as it joins the axis from the leaf base. By following the course of each bundle through successive sections, the entire pattern will eventually be revealed. It is necessary to begin with a seedling rhizome, since it is only at this stage that the pattern is likely to be sufficiently simple to understand. Once this has been accomplished, it will then be possible to undertake a study of the larger and decidedly more complex structure of the older rhizomes. It is axiomatic in gymnosperms and angiosperms that all primary vascular bundles of the stems are merely lower extensions of the leaf traces, with various, usually regular, patterns of interconnections. There is no reason to suspect that the banana plant will prove an exception. However, until this study is completed, it will not be known to what extent the various bundles are related to each other to any given leaf and to any given root.

At the present time, in the seedling rhizome of Musa balbisiana, the bundles have been traced from the level of the apical meristem to the level of the uppermost root primordium. At this latter level, most of the bundles are immature and consist only of procambium. There has been, furthermore, a surprising absence of bundle interconnections. At the level of the fifth node from the apex, there are about 75 or 80 separate bundles, each bundle having remained discrete in the area examined.

When the present series has been completed, we will continue the study using older seedlings and rhizomes derived from lateral buds.

Conclusions

None

Recommendations

This study should be continued as outlined above.

BMS-1

The Anatomy of the Embryo and Seedling of *Musa balbisiana*.

Background

In conjunction with the seed germination studies of *M. balbisiana* being conducted by the Soil Microbiology Section, an investigation of the morphology and anatomy of the seed, embryo and seedling has been initiated.

The seed and embryo have been described a number of times in the older literature. Wittmack (5) in 1867 and Greve (6) in 1909 described the seed of *M. ensete*; Humphrey (7) in 1896 described the seed of *M. rosea*; Gatin (8) in 1908 described the embryos of *M. religiosa* and *M. coccinea*; and Juliano and Alcala (9) in 1933 described the seed of *M. errans*. There has been no study of *M. balbisiana* and no detailed study of the embryo or seedling of any species or variety of banana.

Progress

To date, only a superficial anatomical study of the embryo has been made. Stages of late embryogeny and early germination must be studied to enable us to make a more valid interpretation of this rather unusual embryo.

Figure 2 is a drawing of a median longitudinal section of a mature embryo excised from the seed. The embryo is an obviously mushroom-shaped structure with the "cap" functioning as the cotyledon. This upper, rounded portion of the embryo remains appressed against the endosperm of the seed and acts as the absorbing organ. This cotyledonary region is profusely

vascularized by a radiating system of procambium strands. Figure 3 is a transverse section at the level indicated by A in Figure 2. For greater clarity, the procambium strands visible in this particular section have been outlined.

At a slightly lower level (B in Figure 2), the procambium is restricted to a small central region. This region, on the basis of morphology, must be considered a part of the cotyledon. The embryonic axis, consisting of epicotyl, hypocotyl and radical, forms an angle of approximately 90°. In Figure 2, this axis is indicated by a broken line. The only region that can properly be termed "hypocotyl" lies at the angle. Directly opposite the tip of the epicotyl there is a longitudinal fissure which Greve (6) termed the "cotyledonary split". It is apparently through this split that the epicotyl will emerge upon germination. The split can be seen in Figure 5 which is a transverse section taken at level C in Figure 1.

The epicotyl consists of one leaf (the coleoptile) and a dome-shaped apical meristem (Figure 6). The vascularization of these structures has not yet been determined. There is apparently a typical root tip organization at the apex of the radical.

In addition to the epicotyl and radical, there are several adventitious root primordia already present in the mature embryo (ar in Figures 1 and 5).

Conclusions

None

Recommendations

Continue the present study to complete the details of histology of the mature embryo and study the late embryogeny as well as germination stages up to the point where the study of the rhizome commences.

Prepared by

M. W. McGahan

December 1, 1959

(1) Cheadle, V. I., 1942. The occurrence and types of vessels in the various organs of the plant in the monocotyledonae. Am. J. of Botany 29: 441-450.

(2) Acquarone, P., 1930. The roots of Musa sapientum. United Fruit Co. Res. Bull. 26.

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(4) White, P. R., 1928. Studies on the banana. Z. Zellforsch. u mikroskop. Anat. 7: 673-733.

(5) Wittmack, L., 1867. Musa ensete. Ein Beitrag zur Kenntnis der Bananen. Linnaea xxxv.

(6) Greve, G., 1909. Beitrage zur physiologischen Anatomie von Musa ensete. Inaug. Dissertation der Kgl. Christian-Albrechts-Univ. zu. Kiel. 59 pp.

(7) Humphrey, J. E., 1896. The development of the seed in the Scitamineae. Ann. Botany 10: 1-40.

(8) Gatin, C. L., 1908. Recherches anatomiques sur l"embryon et la germination des Cannacees et des Musacees. Ann. Sci. Nat. Botan., Ser 1x, 8: 113-146.

(9) Juliano, J. B., and P. E. Alcala, 1933. Floral morphology of Musa errans (Blanco) Teodoro var. Botoan Teodoro. Philippine Agr. 22: 91-126.

LEGENDS TO PLATES

Figure 1. A fragment of the common wall between two elements from the leaf base of a Lacatan plant. Several perforations (perf) can be seen in the primary wall between the secondary wall thickenings (sw). X1000.

Figures 2 to 6. Mature embryo of Musa balbisiana.

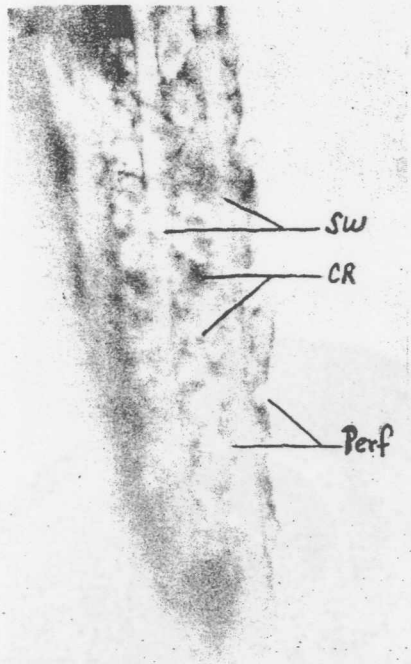
Fig. 2. A drawing of a near median longitudinal section. The broken line indicates the embryonic axis with the epicotyl at the right and the radical at the bottom. ar - adventitious root primordium. X50.

Fig. 3. A transverse section taken at level A in Figure 2. As in Figure 2, the procambium has been outlined. The apparent absence of vascularization on one side is due to a slight obliquity of the section. X100.

Fig. 4. Similar to Figure 3 taken at level B in Figure 2. At this level the procambium is restricted to the central portion. X100.

Fig. 5. Similar to Figure 3 taken at level C in Figure 2. The epicotyl, not in a median plane, projects laterally toward the cotyledonary split (cp). Three adventitious root primordia (ar) are visible in this section. X100.

Fig. 6. A transverse section about 40 microns below the level of Figure 5. This is approximately median for the epicotyl. Apical meristem - am; first leaf or coleoptile - col. X100.



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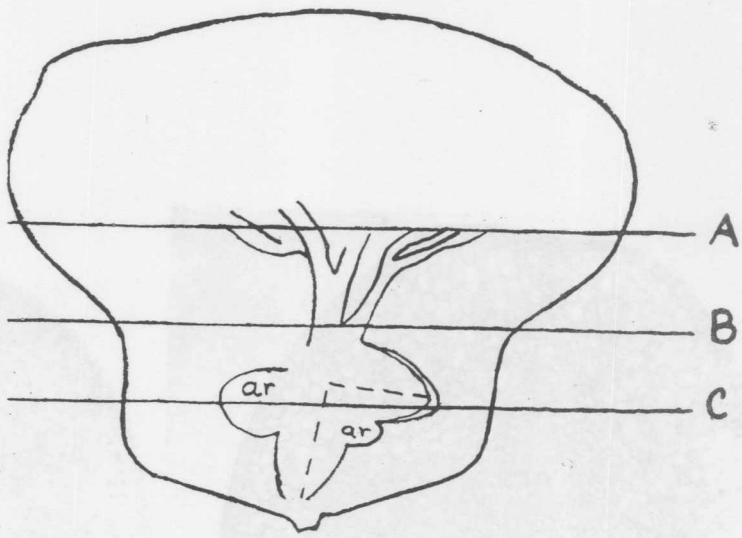

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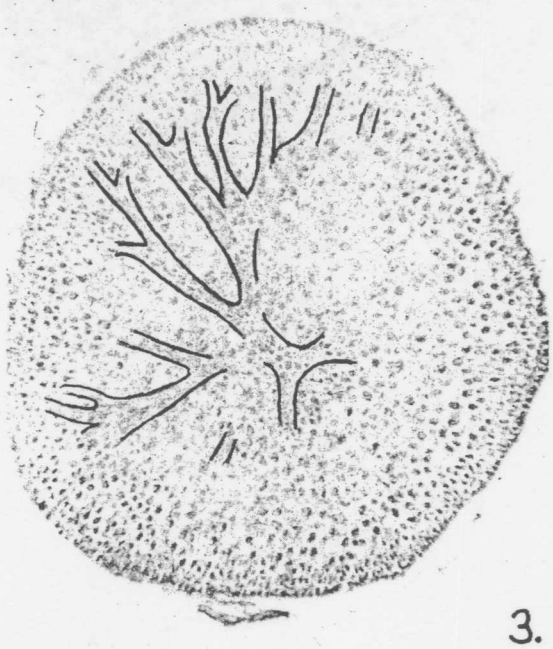

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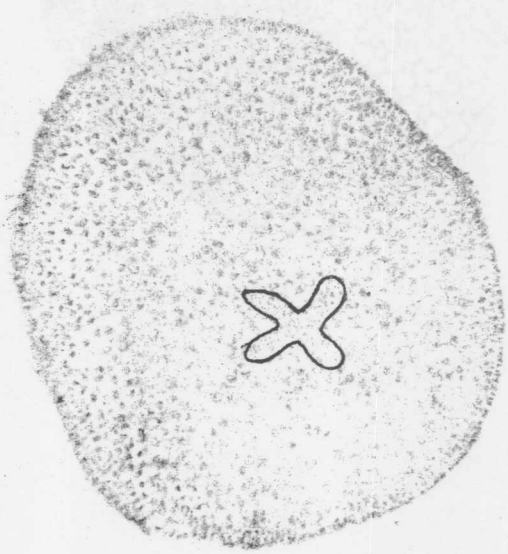

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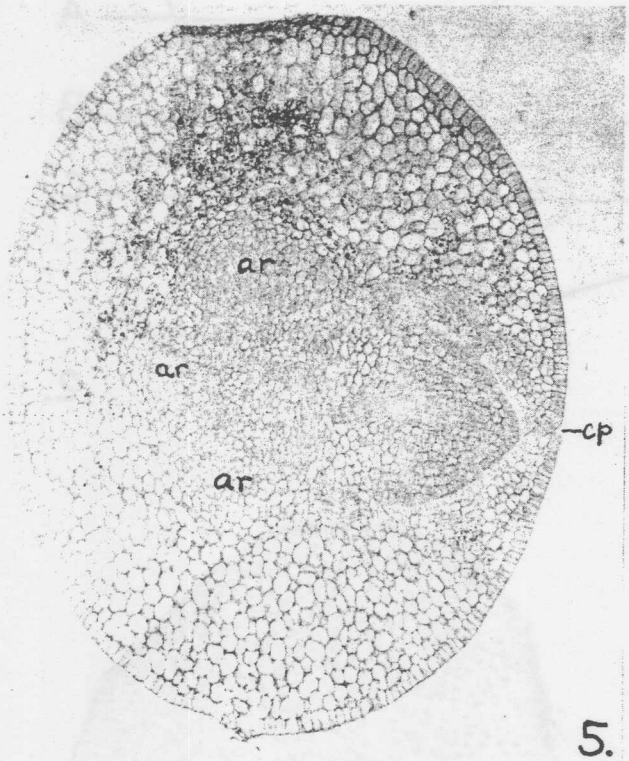

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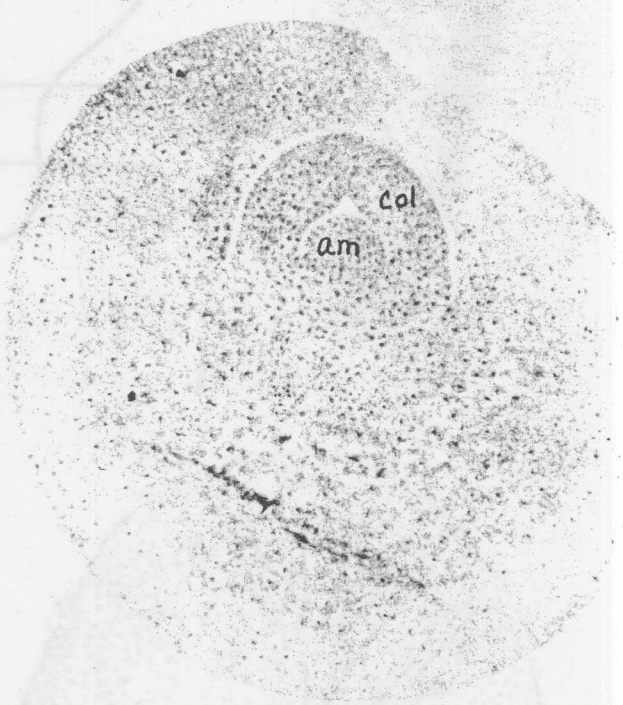


6.

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BPG-0-10
BPM-1-10
BDF-3-30

Plant Physiology Section
Annual Project Report 1959

Growth of Banana Plant as Affected by Environmental Factors, and Comparative Biochemistry of Banana Varieties in Relation to Resistance to Fusarium Wilt: Tannins

Background

Banana plants were grown at four different light intensities for the following reasons: First of all, to determine the optimum light conditions for growing banana plants since this is important in growing plants in controlled growth chambers for subsequent studies on the affects of other environmental factors, host-parasite interactions, metabolic changes, and floral initiation. Secondly, the tannins were determined since they havebeen implicated in resistance of other plants to infection (See Annual Report-1958). Thirdly, the affect of light intensity on infection was also determined in order to study its relation to tannin concentration.

Progress

Gros Michel banana plants were grown from button seed in 9 inch clay pots containing loam soil and were subjected to four different light intensities. The details of this phase of the study may be noted in the Second Quarterly Report-1959. Growth may be expressed as the change in length of successively new emerged leaves (Annual Report-1958). Figure 1 shows that growth at 2200 foot candles was comparable to banana plants growing under greenhouse conditions. Similar results were evident for plant height and width of leaves (Second Quarterly Report-1959). Figure 1 also shows that growth by the fifth week under uniform light intensity entered a lag phase suggesting a condition primarily affected by the reserve material of the rhizomes.

At the end of the experiment, the four plants from each light treatment were harvested and the tannins analyzed by methods described in the First Quarterly Report-1959. The results as shown in Figure 2 indicate that the highest light

intensity increased the tannin content significantly in the leaves and rhizomes. Light intensity did not, however, appear to alter the tannin content of the roots.

The tannin content and the growth of a banana plant appears to be affected by the size of the initial planted material. The average weight of the initial rhizome used for planting was 481.7, 360.0, 479.5 and 540.0 grams from the highest light intensity to the lowest light intensity, respectively. The size of one of the rhizomes planted and grown at 680 foot candles was the largest of all the rhizomes used, weighing 838 grams. The tannin content for this particular plant in the leaves and in the rhizomes was comparable to the tannin content found in those plants at the highest light intensity. This might help to explain the increasing order of tannin content in the rhizomes with the three lower light intensities.

Ten weeks after differential light, four plants in each treatment were inoculated by Dr. Beckman with a microspore suspension of Fusarium oxysporum f. cubense (See Third Quarterly Report-1959). Twenty-seven days after inoculation, plants containing the highest tannin content and at the highest light intensity had the lowest number of plexal infections (30%). The three lower light intensities had plexal infections of 70%, 65% and 65%, respectively, with decreasing light intensity. Furthermore, the total extension of discoloration within each treatment was also lowest for the highest light intensity.

Conclusions

Under the conditions prevailing in the experiment, the following conclusions may be made:

1. Banana plants appear to grow as well at 2200 foot candles artificial light as those grown under greenhouse conditions. Lower light intensities result in reduced growth. This suggests that light intensities approximately one-fifth of maximum sunlight intensity will be required to grow bananas in controlled growth chambers.

2. Approximately two months after planting, a lag phase appeared in growth of button seeds regardless of light intensity. This suggests that reserve materials of the rhizome had been depleted and that growth prior to the light phase was partly governed by the condition of the planting material. Thus, experiments with banana rhizomes will have to consider that superimposed upon a particular treatment prior to the light phase will be the growth due to the reserves of the planted storage organ.

3. The highest tannin content found was in leaves and rhizomes of plants growing at the highest light intensity (2200 f c). Tannin content of roots regardless of light treatment remained unaltered.

4. The tannin content of a banana plant (at least in the early stages of growth) may be in part related to the size of the initial planting material used. If there is a relation between tannin content and susceptibility to infection, this suggests that the size of the planting material used may be very important from the standpoint of incidence of disease.

5. Plexal infections of Fusarium oxysporum f. cubense in the rhizomes and the extension of vascular discoloration were found to be lowest in plants grown at the highest light intensity and containing the highest concentration of tannin in the rhizomes and leaves. This suggests that the tannin level of a banana plant may be correlated with the degree of susceptibility to infection by Fusarium oxysporum f. cubense. It also suggests that Gros Michel bananas under low light intensity or shade conditions may be more susceptible to Panama disease.

Prepared by

S. R. Freiberg
J. B. Greenberg

December 1, 1959

FIGURE 1.

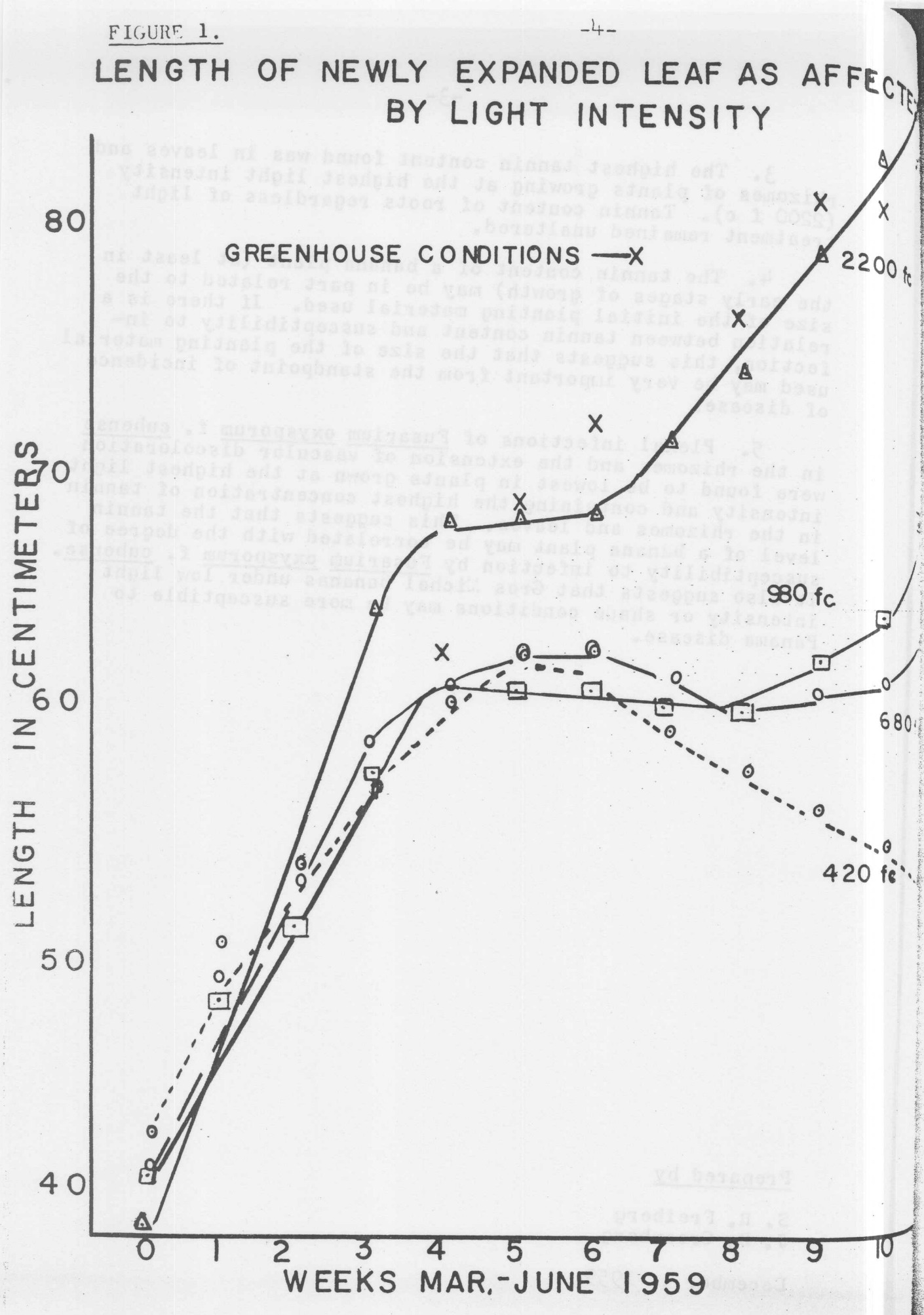
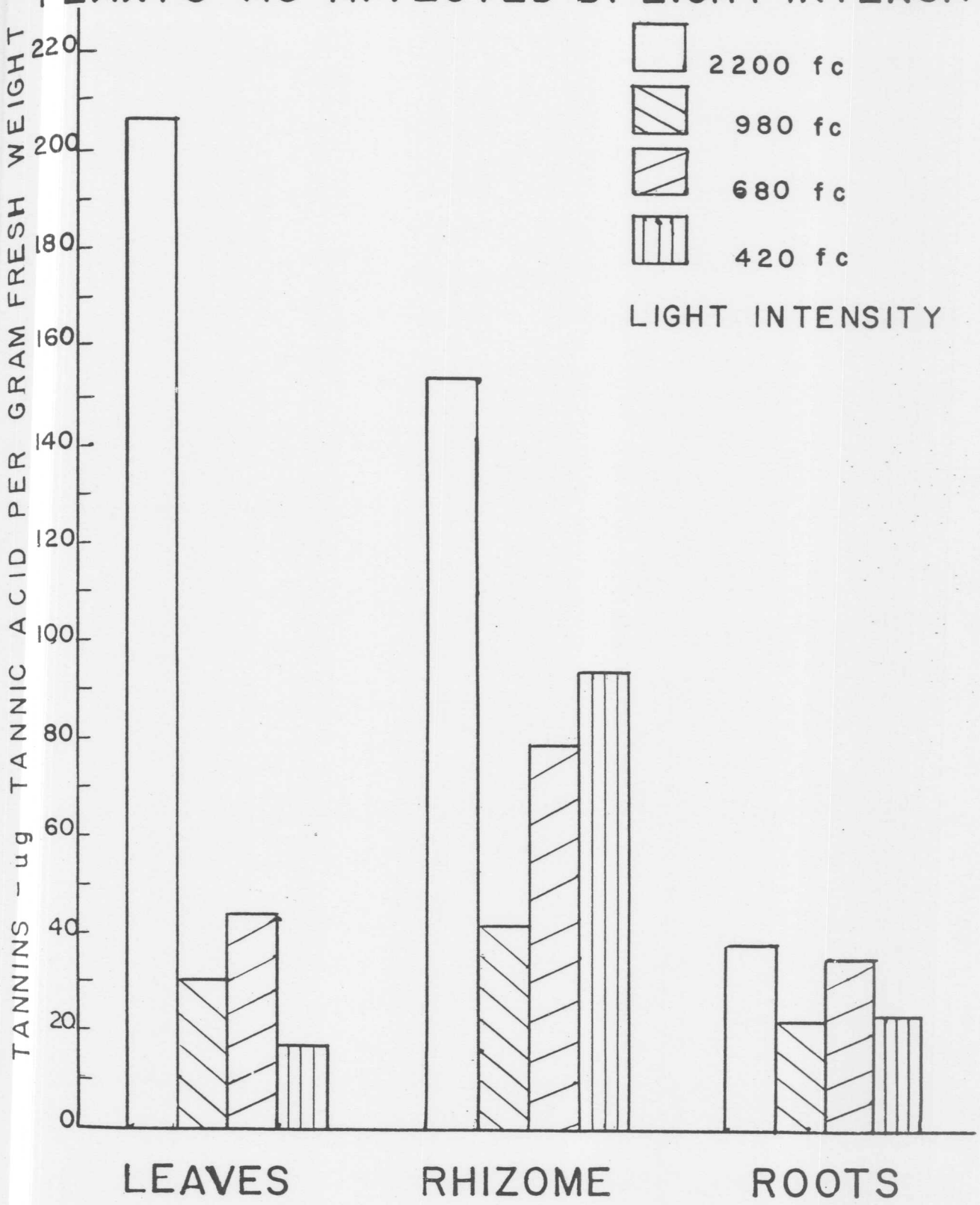
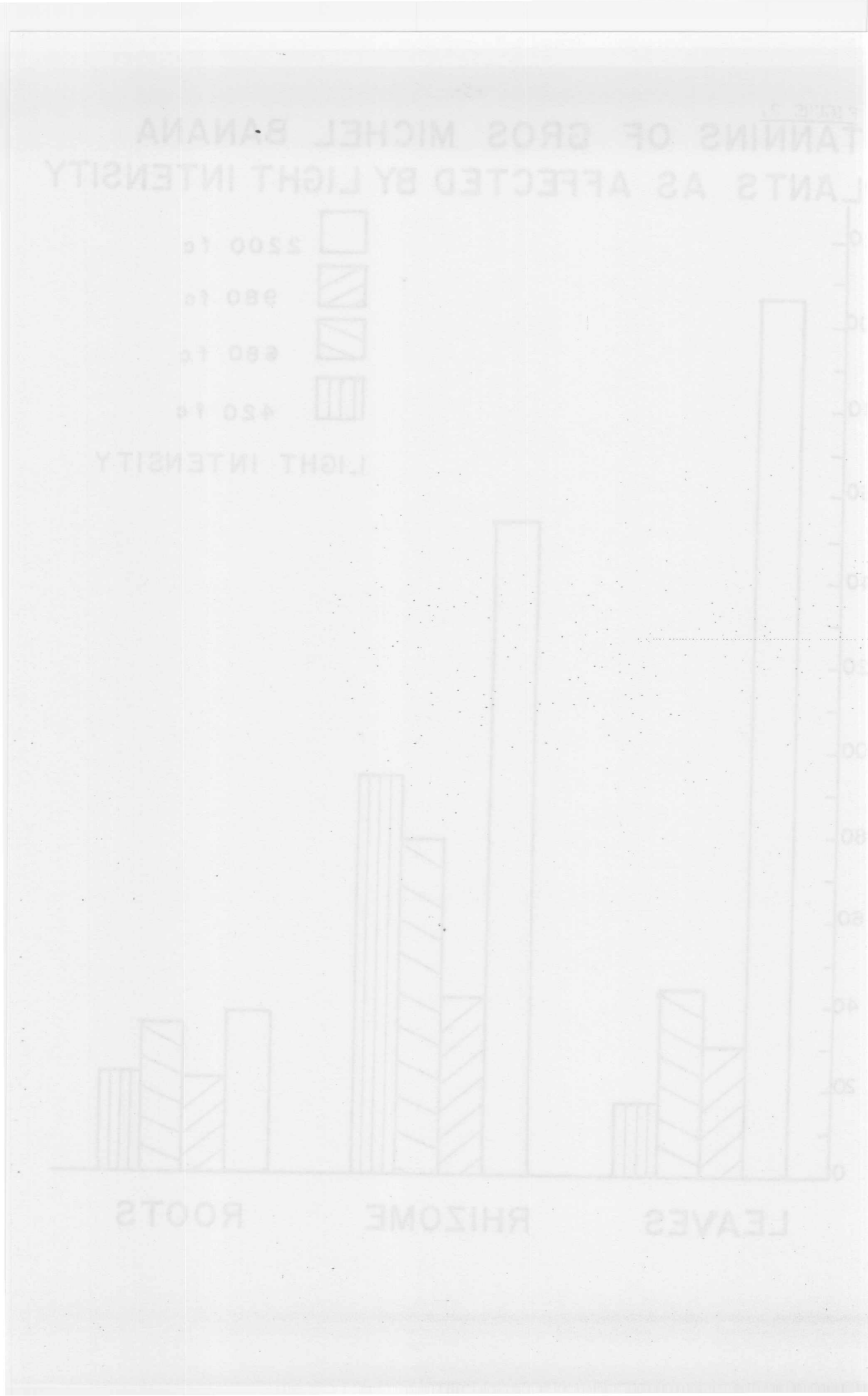

LENGTH OF NEWLY EXPANDED LEAF AS AFFECTE
BY LIGHT INTENSITY
GREENHOUSE CONDITIONS
2200 fc
980 fc
680
420 fc
LENGTH IN CENTIMETERS
80
70
60
50
40
0
1
2
3
4
5
6
7
8
9
10
WEEKS MAR.-JUNE 1959

FIGURE 2.

TANNINS OF GROS MICHEL BANANA PLANTS AS AFFECTED BY LIGHT INTENSITY





CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BPM-0-0 (1959 Summary)

Physiological-Microbiology Section
Annual Project Report 1959

Some Nutrients Found in the Vascular Sap of Banana Plants (1)

Preliminary results from a paper chromatographic analysis of the free amino acids in the untreated (raw) sap from young Gros Michel banana plants showed the presence of five distinct spots that reacted with ninhydrin. They were tentatively identified as aspartic, glutanic acid, alanine, isolucine, and valine. Plants held in droughty condition contained the same amino acids as those held in more favorable conditions of moisture (soil was kept moist) for the plant. Only minute quantities of sucrose appeared in the untreated sap of these plants and other sugars were not detectable by paper chromatographic.

1. Wilson, E. M. 1959. Some nutrients found in the vascular sap of banana plants. 1st Quarter Reports, Central Research Laboratories.

Prepared by

Eugene M. Wilson
December 1, 1959

CENTRAL RESEARCH LABORATORIES
Norwood, Mass.

BPM-1-10
BDF-3-30

Plant Physiology Section
Annual Project Report 1959

Comparative Biochemistry of Banana Varieties in Relation to Resistance to *Fusarium* Wilt: Polyphenol Oxidase

Background

As mentioned in the Second and Third Quarterly Reports-1959, there is evidence that the polyphenol oxidase of plants may be an important factor regulating the resistance of a plant to disease. This enzyme, by altering the level of oxidation of plant phenols, may control the level of toxicity of these phenols.

Progress

In the Second and Third Quarterly Reports-1959, evidence was presented for the existence of polyphenol oxidase activity in leaf and rhizome tissue of banana plants, and various characteristics of this activity were described. Since that time, preparations from pseudostem and from roots have also been shown to oxidize polyphenols.

Activity was measured with a Warburg manometer at 29.4°C. The center well contained 0.2 ml of 10% KOH and a filter paper wick. The side arm usually contained 1 ml of 1% substrate which was tipped into the main compartment at zero time. The main compartment contained 1.6 ml, including buffer (usually 0.8 ml of 0.1 M acetate or phosphate) and the source of enzyme activity. Treatments within an experiment were usually run in duplicate.

The source of enzyme was generally a tissue homogenate made by grinding fresh tissue with water or buffer. A Virtis homogenizer was used, and the flask was surrounded with a water-ice bath. The ground material was filtered through a single layer of cheesecloth and stored in the freezer. The homogenate was either assayed as such, or first separated into soluble and particulate fractions. This separation was effected by centrifuging the homogenate at 18,000 RPM for an hour at 0°C. The supernatant was poured off, and the particles resuspended in buffer, centrifuged again, and finally resuspended in fresh buffer to the original volume of homogenate.

For each tissue (i.e. leaf, pseudostem, rhizome, root), at least one experiment was run in which the following controls were included: 1) The homogenate was boiled for 20 minutes before assaying; 2) The substrate was replaced with water; in addition, 3) Each substrate was tested in the absence of homogenate. With all three types of controls, oxygen uptake was absent or negligible.

Substrates promoting oxygen uptake:-

Tables I and II of the Third Quarterly Report-1959 give data on the oxygen uptake observed with various substrates when leaf homogenate and rhizome homogenate, respectively, are used as enzyme source. With leaf homogenate, the most active substrates are: catechol, pyrogallol, phloroglucinol, chlorogenic acid, caffeic acid, and D-catechin. Gallic acid is less active, tannic acid much less active, and resorcinol and hydroquinone are essentially inactive. The different substrates appear to have different pH optima, even with the same leaf homogenate. For example, gallic acid is oxidized much more readily at around pH 4-5 than at pH's greater than 5. On the other hand, the oxidation of phloroglucinol by leaf homogenate drops markedly below pH 8. Some substrates are not as markedly affected by pH.

With rhizome tissue (See Third Quarterly Report-1959), catechol is quite readily oxidized, pyrogallol less so, and caffeic acid still less so. Gallic acid showed no activity, but the time interval might have been too short.

With pseudostem tissue homogenates, catechol and gallic acid were tested as substrates. Catechol was very active, gallic acid much less so. For example, in one experiment, catechol caused 22 microliters oxygen uptake per minute, while gallic acid caused about 1 microliter per minute.

With root homogenate, also, catechol and gallic acid were tested as substrates. In an experiment in which catechol caused 18 microliters oxygen uptake per minute, gallic acid was inactive.

Checking whether enzyme activity in soluble or particulate fraction:-

The Third Quarterly Report-1959 (Table III) gives data for both rhizome and leaf homogenates indicating that the polyphenol oxidase activity resides in the particulate rather than the soluble fraction. It will also be noted that with both

rhizome and leaf, the activity of the particulate fraction alone is greater than that of either the original homogenate or the particulate and supernatant fractions assayed together.

The data in Table I indicate that in pseudostem and root homogenates most of the activity resides in particulate fraction but there is also some in the supernatant. As with the rhizome and leaf homogenates, the activity of the particulate fraction alone is greater than that of the uncentrifuted homogenate or the particulate and supernatant fractions recombined. This is noteworthy in the case of pseudostem where the supernatant and particulate fractions assayed separately give a total activity of 12.9 + 3.8 = 16.7, whereas when assayed together the activity is 9.5. These data suggest that the supernatant contains some substance inhibitory to polyphenol oxidase.

Lack of proportionality between enzyme level and oxygen uptake:-

Table I also indicates that by diluting root extracts 10-fold, the activity is only reduced to about half, a marked departure from linearity. Data from an experiment with leaf homogenate also indicate this non-proportionality between oxygen uptake and enzyme level at the levels tested. It does not appear likely that substrate is limiting at the higher enzyme levels since the reaction mixtures contain 10 mg substrate (about 90 μ moles catechol). The rate of oxygen diffusion in the reaction mixture may be a limiting factor at higher enzyme levels. Perhaps at lower enzyme levels linearity may be observed, although the technical difficulties of accurately diluting a particle suspension must be considered.

Lack of proportionality between oxygen uptake and time of incubation:-

For preparations of all four tissues discussed, curves of oxygen uptake versus time of incubation are not linear, tending to bend towards the abscissa. However, up to about 10 minutes (taking readings every 3, 4 or 5 minutes), the readings usually come close to being linear. Thus, if activity is expressed as oxygen uptake per unit time, only values within the first 10 minutes should be used.

Loss of activity after storage:-

The tissue homogenates were stored in the freezer until ready to be used, at which time they were thawed and the remainder re-frozen. Experiments with pseudostem and root

homogenates, in which the same homogenate was assayed on two different days but under the same conditions, demonstrated a distinct loss of activity. For example, the root extract used to obtain the data in Table I lost about 75% of its activity as compared with a previous assay a week before. It would appear that most of the loss occurred during the thawing process and while it was in the thawed condition. The extract had been in the freezer for two months prior to being assayed for the first time, and yet it was very active. Several active pseudostem homogenates which were assayed on two succeeding days showed losses of from 23 to 71%. When the same extract is to be assayed on different days, it would seem advisable to freeze several aliquots separately to avoid the thawing and refreezing.

Acetone powder preparations:-

Enzymes are often extracted from biological materials by grinding with very cold acetone to precipitate the proteins, filtering, and drying the residue. The residue is then extracted with buffer to redissolve the proteins. Such acetone powder preparations were made from leaf tissue and pseudostem tissue to see whether by this means the polyphenol oxidase could be obtained in a soluble form. (Dr. Forsyth found this to be the case with cacao polyphenol oxidase.) The leaf acetone powder extract showed no activity. This was not unexpected because the filtering process was very slow, allowing the acetone to warm up and probably denature much of the protein. The pseudostem acetone preparation showed some activity. The extent of this activity seemed to be less than that of the pseudostem homogenates made by the usual procedure, but adequate controls are not available. Further work with acetone powders is anticipated, with care taken to keep the acetone from warming up during the preparation.

Conclusions

Leaf, pseudostem, rhizome and root tissue homogenates of the banana plant can be prepared which, when present in a mixture with a polyphenolic substrate, cause an uptake of oxygen as measured on a Warburg manometer. Various aspects of the nature of this polyphenol oxidase activity have been studied. Since there is evidence that the state of oxidation of polyphenols in plants may be involved in disease resistance, it is hoped that studies of this enzyme in resistant and susceptible varieties, as well as at various stages of Fusarium penetration, may give us more information on the response of the banana plant to Fusarium infection.

Prepared by

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S. R. Freiberg

December 1, 1959

Table I

Site of Polyphenol Oxidase Activity in Root and Pseudostem Tissue Homogenates

Fraction Tested	µl Oxygen uptake per minute*
Pseudostem - uncentrifuged homogenate	9.7
Pseudostem - resuspended particles	12.9
Pseudostem - supernatant fraction	3.8
Pseudostem - particles plus supernatant	9.5
A** - Root - uncentrifuted homogenate	7.4
A - Root - resuspended particles	9.3
A - Root - supernatant fraction	1.9
A - Root - particles plus supernatant	8.2
B** - Root - uncentrifuged homogenate	3.7
B - Root - resuspended particles	4.2
B - Root - supernatant fraction	0.3
B - Root - particles plus supernatant	3.7

*Based on 10 minute readings.
**B fractions are 1/10 dilutions of the A fractions. A and B were assayed in the same experiment.

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BPL-1-10
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Plant Physiology Section
Annual Project Report 1959

Comparative Biochemistry of Banana Varieties in Relation to Resistance to Fusarium Wilt: Tannins

Background

The tannins have been implicated in plant host-parasite relationships as indicated in the Annual Report-1958. Therefore, the tannin content of the various plant organs of a number of varieties are being studied to determine whether such a relationship exists in banana plants.

Progress

A technique suitable for the analysis of tannin content in various banana plant organs has been developed (First Quarterly Report-1959). An analysis of the tannin content of roots from field-grown bananas in Honduras (Table I, First Quarterly Report-1959) showed that there was little difference in the level between the various resistant and susceptible varieties. The one exception was Musa balbisiana which had a content considerably higher than in any of the other varieties. Analysis of the tannin content in the cortex and stele of roots from plants grown in solution culture in the greenhouse also showed no differences between resistant and susceptible varieties (Table I, First and Second Quarterly Reports-1959). These results suggest that if the tannins are at all important in explaining host-parasite relationships that the controlling mechanism does not reside in the roots.

Suggestive evidence has recently been reported (See Third Quarterly Report-1959) that tannin content may increase in the presence of the pathogen. Therefore, two experiments were initiated whereby banana tissues were kept in the presence of the Fusarium organism for a period of time and then analyzed for tannin content. The procedures used have been reported in the Third Quarterly Report-1959. At that time, the first preliminary results of the tannin content of banana plant tissues prepared for analysis at start of treatment were reported. The following table presents the complete data of that first experiment:

Table I

Tannin Content* of Excised Banana Plant Tissue as Affected by *Fusarium oxysporum* f. *cubense*

Variety	Surface Sterilization of Tissue	Plant Organ	Time After Treatment: 0 days (Control)	6 days (Control)	6 days (*F.o.c.* inoculated)
Cavendish #1	70% EtOH	pseudostem	271.8	135.0	125.2
Cavendish #2	"	"	214.4	129.9	129.0
Gros Michel #1	"	"	68.3	57.7	34.5
Gros Michel #2	"	"	58.7	49.5	31.9
Cavendish #1	sterile water	"	215.7	71.8	96.7
Cavendish #2	" "	"	173.7	103.1	109.3
Gros Michel #1	" "	"	55.1	63.6	34.6
Gros Michel #2	" "	"	52.4	30.8	24.5
Cavendish #1	70% EtOH	rhizome	1843.8	144.3	443.5
Cavendish #2	"	"	973.0	241.3	523.4
Gros Michel #1	"	"	531.8	167.3	229.1
Gros Michel #2	"	"	940.4	295.0	305.6

*"Tannin" expressed as micrograms tannic acid per gram fresh weight.

The results shown in the foregoing table indicate that the tannin content in most cases decreases with time after excision from the plant and placed in Erlenmeyer flasks in a nutrient media with or without the fungus. A major proportion of this loss can be accounted for by what has leached into the nutrient media. It is also evident that the Fusarium organism does not appear to have an effect on the tannin content of the tissues under the conditions of this experiment. The only possible exception is in the rhizomes of the Cavendish variety where tannin content was higher than the controls after 6 days, although much lower than at the start of treatment. It is also interesting to note that the tannin content of the Cavendish pseudostem tissue was significantly higher than that found in the Gros Michel tissues.

A second experiment was conducted with rhizome tissues excised from three varieties of banana plants. The procedures used were similar to those in the first experiment. Four plants each of the varieties Grand Nain, Vimama, and Gros Michel supplied the rhizome tissues for study. The tissues were harvested three days after inoculation with the Fusarium organism. The results are shown in the following table:

Table II

Tannin Content* of Excised Banana Rhizome Tissue as Affected by Fusarium oxysporum f. cubense

Variety	Time After Treatment		
	0 days (Control)	3 days (Control)	3 days (F.o.c. inoculated)
Grand Nain #1	306.4	276.8	239.2
Grand Nain #2	475.6	350.0	348.4
Grand Nain #3	683.6	375.6	297.6
Grand Nain #4	340.8	303.2	1144.8
Vimama #1	480.0	416.0	304.8
Vimama #2	700.8	385.6	393.2
Vimama #3	301.6	156.8	302.8
Vimama #4	423.2	402.4	400.8
Gros Michel #1	646.8	456.8	507.2
Gros Michel #2	1516.8	1184.8	-
Gros Michel #3	1349.6	657.6	600.0
Gros Michel #4	921.6	561.6	753.6

*"Tannin" expressed as micrograms tannic acid per gram fresh weight.

The results in the foregoing table show no affect of the fungus on the tannin content of the tissues under the conditions of the experiment. The only significant change noted was in Grand Nain #4 in which the tannin content increased approximately threefold, as a result of inoculation with F.o.c. Whether a difference would have been observed if a different time period was used is unknown. It is interesting to note that the two resistant varieties initially show less tannin content than the susceptible Gros Michel variety. This raises the question of just how important is the tannin content of the tissue in relation to host-parasite interactions when as much as five times the tannin content is found in this susceptible variety in contrast to the resistant variety. It may be, however, that the response of the resistant variety to the presence of the parasite cannot be determined except in the intact plant. Therefore, other experiments will be conducted with this in mind.

The above experiments were carried out with the cooperation of Dr. Beckman, who grew the fungus and carried out the inoculations on the excised banana plant tissues.

In view of the findings reported in the first table above, that the pseudostem tissue of the Cavendish contains much more tannin content than in the Gros Michel, further analyses were made. Samples of pseudostem tissue were obtained from mature plants growing in the greenhouse. A cork borer 2 cm in diameter was used to remove a portion from the pseudostem approximately four inches above the ground level. Analyses for eleven different varieties were obtained including the two susceptible Cocos and Gros Michel varieties. No differences between susceptible and resistant varieties were found as to tannin content. However, the cork borer sample generally did not contain more than the portions from three or four leaf bases. Since these were the outer leaf bases, they were from leaves which had already withered on the plant. Therefore, this experiment will be repeated, sampling pseudostem tissues connected to healthy green leaves. The tannin content of these pseudostem tissues ranged from a low of 226.4 µg per gram fresh weight in Grand Nain to a high of 626.0 µg per gram fresh weight in Cavendish.

Conclusions

None

Recommendations

None

Prepared by

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December 1, 1959

CENTRAL RESEARCH LABORATORIES
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Plant Physiology Section
Annual Project Report 1959

Comparative Biochemistry of Banana Varieties in Relation to Resistance to Fusarium Wilt: Polyphenolic Substances

Background

Polyphenols have been implicated in host-parasite interactions as indicated in the Annual Report-1958 (Freiberg). Previous studies have revealed a diversity of phenolic constituents in banana roots, but only one substance, compound no. 12, was found present in resistant plants and absent in susceptible plants (Freiberg, Annual Report-1958). The toxicity of compound no. 12 to Fusarium oxysporum f. cubense remained to be checked as did the toxicity of the other phenolic compounds.

Progress

Thirty-two compounds of probable phenolic nature were isolated from the roots of variety Valery (6-month-old potted plants) which were greenhouse grown. All compounds failed to inhibit F.o.c. cultured on Difco PDA agar (See Second Quarterly Report-1959) by cup bioassay method. Compounds (74.21) and (17.15) [i.e., compounds with R_f values in n-butanol, acetic acid, water (4:1:2.2) of 0.74 and 0.17, respectively, and in aqueous 2% acetic acid of 0.21 and 0.15, respectively, when chromatographed on Whatman No. 1 chromatographic paper] turned the culture media brown. Compound (17.15) does not react with any spray reagents for polyphenolics currently used. Compounds (44.72), (46.90) and (55.92) tint the culture reddish brown as do two compounds (92.82) and (92.90) which are primarily, if not entirely, artifacts of Whatman No. 1 filter paper.

The toxicity of compound no. 12 (isolated from twenty-six paper chromatograms of root extract from 6-month-old potted plants of Variety 67) were tested against F.o.c. grown on Difco PDA agar, through the cooperation of Dr. R. D. Coos. The concentration of compound no. 12 in the agar surrounding the bioassay cup, presumably was in excess of the concentration found in the roots, yet no zone of inhibition appeared in the F.o.c.

culture. An amount of compound no. 12, equivalent to that extracted from 17 grams of roots (ca. 2.1 grams dry weight) was added to each bioassay cup as a 0.50 ml aqueous solution.

Another compound, no. 38 (Freiberg, Annual Report-1958), identified as pyrogallol by paper chromatography, was found in the roots of both susceptible and resistant varieties. Subsequent experiments with commercially available pyrogallol (Baker analyzed reagent grade) have shown that pyrogallol is toxic to F.o.c. in low concentrations. Pyrogallol, 1.7×10^{-6} mole, was added to each bioassay cup as a 0.20 ml aqueous solution. Inhibition of both growth and germination of F.o.c. was observed in agar medium (3 gms. NH_4NO_3, 1 gm. KH_2PO_4, 0.5 gm. $MgSO_4$, 0.25 gm. KCl, 10 gms. glucose, 3 gms. yeast extract, 5 gms. malt extract, 15 gms. agar per liter). Within 24 hours a zone of inhibition 19 mm in diameter was apparent and the zone was not appreciably overgrown by the surrounding mycelium for a period of three days, at which time the experiment was terminated. The addition of 1.7×10^{-5} mole pyrogallol resulted in a zone of inhibition 28 mm in diameter under identical test conditions. Experiments are in progress to determine the ability of polyphenol oxidase systems from the banana plant to change pyrogallol to more toxic or less toxic forms. Preliminary data indicates that toxicity to F.o.c. is increased when amino acids are added to the reaction mixture. However, when chromatographed, R_f values of the toxic material(s) are identical with those of pyrogallol in water saturated 2-butanol solvent and aqueous 2% acetic acid solvent. Chromatographic evidence indicates that pyrogallol derivatives of different R_f values are formed but these compounds do not cause zones of inhibition in F.o.c. solid media cultures.

Phenolic constituents isolated from banana roots, however, have shown no toxicity against F.o.c. in the concentrations used. This suggested that the techniques used may play an important role in the isolation and nature of such materials. Therefore, a study was undertaken to determine the effect on the polyphenols of age of extract, number of extractions, storage of concentrate for chromatographic analysis and methods of concentrating extracts to dryness.

Age of Extract:- Ethanol extracts that were separated from the plant brei maintained their qualitative aspects regarding polyphenols (on the basis of paper chromatographic analysis) but substantial quantitative losses of these materials occurred within two weeks to a month. These losses were greatest in

root extracts and least in leaf extracts. When the plant brei was left in the extract, the quantitative losses were reduced but qualitative changes occurred which were associated with a visible darkening of the brei. This dark material, which is immobile on paper chromatograms when using acidic or neutral solvents, disrupts the discrete migration of other compounds. Analysis of such extracts can be made by passing the sample through a one inch cellulose column, eluting with n-butanol, acetic acid, water (4:1:2.2) reconcentrating at 40°C under vacuum and then chromatographing (See Second Quarterly Report-1959).

The addition of 10^{-3} M cysteine to the ethanol or methanol used for extraction stops discolouration of the brei for two to three weeks, after which time, darkening proceeds slowly. Nitrogen gas bubbled through the extract at the time of preparation has little effect. 0.1% to 1.0% conc. HCl (by volume) added to the extracting alcohol does reduce discolouration yet there is evidence that it hydrolyses some materials and, therefore, is not recommended.

Number of Extractions:- Repeated extractions (at least five) were found essential for the quantitative removal of soluble materials. Analysis of each consecutive extraction revealed not only the expected results of depletion of solubles from the brei, but also the appearance of entirely new compounds upon the second and third extractions. These new compounds may be artifacts of the extraction procedure. Contrast with another suitable technique would assist in substantiating the belief that the new compounds found in subsequent extractions are artifacts.

A technique for preparing tissues for analysis in many biochemical studies is that of freeze-drying the tissues. Therefore, with the cooperation of Dr. J. K. Palmer, we are determining how this technique contrasts with the present ethanol extraction procedure.

Storage of Concentrate for Chromatography:- Extracts sufficiently concentrated for application onto paper chromatograms cannot be stored satisfactorily even at 3°C or -20°C. Essentially, the changes that take place are quantitative rather than qualitative and are accompanied by a rapid increase in a black residue. On paper chromatograms, this residue is immobile in aqueous 2% acetic acid solvent and moves only slightly in alcoholic solvents (acidified or neutral).

The extracts can be stored for months however, as dried residues under vacuum in a vacuum desiccator.

Concentration of Extracts:- Fifty gram samples of tissue (fresh weight) extracted with ethanol can be concentrated to a volume of 30 ml at 40°C under vacuum without altering the extracted polyphenolics. The remaining solvent is best removed by quick freezing followed by sublimation in a vacuum system. The dried residue can be stored in an evacuated desiccator for months. Excellent chromatograms have been made from extracts concentrated and stored in this fashion.

Conclusions

1. All compounds of probable phenolic character which have been isolated from banana roots and tested against F.o.c. have not inhibited F.o.c. on PDA medium. This does not exclude the presence of a phenolic compound which becomes toxic to F.o.c. through the activity of a polyphenolic oxidase system (G. L. Farkas and G. A. Ledingham [Can. J. Microbiol. 5: 37-46, 1959] and Z. Kiraly [Phytopath. Z. 35: 23-26, 1959].)

2. Pyrogallol (Baker analyzed reagent grade) prevents both growth and germination of F.o.c. when 1.7×10^{-6} mole is added to a bioassay cup placed upon freshly inoculated solid media. There is chromatographic evidence that pyrogallol is present in roots of both susceptible and resistant varieties of banana.

3. The techniques currently employed for concentrating extracts, storing samples, and "cleaning" extracts through the use of short cellulose columns allow us to analyse any ethanol or methanol extract of a vegetative tissue for polyphenolic compounds as found in the original extract.

4. Five extractions of a tissue are the minimum that can be used for the quantitative solvation of the soluble polyphenolic compounds.

5. There is evidence that a few artifacts of phenolic compounds develop during the repeated extractions necessary for quantitative studies.

6. Neither methanol nor ethanol extracts can be stored. Immediate processing of extracts is the only good method. The addition of 10^{-3} M cystein to the methanol or ethanol used for extraction, however, will allow samples to be stored for 2 or 3 weeks before changes occur that can be detected

A PROGRESS REPORT ON RESEARCH

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Department of Horticulture

PURDUE UNIVERSITY
AGRICULTURAL EXPERIMENT STATION

Seed Bed Fumigation Study

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Seed bed fumigation for the control of soil pests, notably weeds, is widely practiced in the melon growing areas of southern Indiana. Methyl bromide has been used for many years. Newer materials that can be applied as drenches are very popular because it is believed a plastic cover is not needed. An experiment was initiated to determine the possible value of a plastic cover with materials applied as a drench.

Well-rotted manure was placed in veneer bands and the materials to be drenched were distributed with a sprinkling can and then leached with water. With methyl bromide, the manure was fumigated in a pile outside the seed bed. Materials studied in this experiment were: methyl bromide, Telone (1,3-dichloropropene), Vapam, and allyl alcohol. The plots were 3 x 6 feet in size with two replicates in a completely randomized block design. The fumigants were applied at rates of 400 and 800 pounds of active material per acre except for methyl bromide which was used at one pound per 100 cu. ft. Two seals were studied: water seal and a polyethylene cover. The polyethylene covers were removed five days after treatment and the beds allowed to aerate for three weeks. Melons were planted and weed counts were taken two weeks later.

The enclosed table presents the number of weeds per square foot. Although this data has not been statistically analyzed, it is evident that the polyethylene cover greatly improved weed seed control. In one instance Vapam at 400 pounds of active ingredient per acre sealed with polyethylene gave better weed control than Vapam at 800 pounds with a water seal.

Seed Bed Fumigation

Treatments	Lbs. active material/acre	Seal	No. weeds per sq. ft.	
			Grasses	Broadleaves
Check	0		215	30
Methyl Bromide	1 lb/100 cu. ft.	polyethylene	4	2
Telone (1,3-dichloropropene)	400	water	223	3
Telone	800	water	214	37
Telone	400	polyethylene	109	18
Telone	800	polyethylene	20	4
Vapam	400	water	198	36
Vapam	800	water	109	17
Vapam	400	polyethylene	5	6
Vapam	800	polyethylene	2	2
Allyl Alcohol	400	water	149	17
Allyl Alcohol	800	water	88	12
Allyl Alcohol	400	polyethylene	78	14
Allyl Alcohol	800	polyethylene	33	4

SUMMARY OF SEMI-ANNUAL REPORT OF RESEARCH SUPPORTED BY THE UNITED FRUIT COMPANY

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Investigations since July 1, 1959 have been concerned with (1) testing new fungicides against Fusarium oxysporum f. cubense (F.o.c.), (2) the effects of selected fungicides on the total soil micropopulation, (3) the pattern of reinfestation of thesoil following treatment with chemicals effective against F.o.c. and (4) the relation of wilt disease resistance to the nature of plant pectic substances

1. Testing new fungicides

In recent tests of new fungicides the most outstanding were 2 experimental materials produced by the Hercules Powder Co. (3944 and 4223), which had ED_{99} concentrations below 100 ppm.

2. Effects of selected fungicides on the total soil micropopulation

Techniques and experimental designs have been developed to investigate the effects of selected fungicides on the total soil micropopulation and to observe the pattern of reinfestation of the soil following treatment with chemicals effective against F.o.c. Vapam, Mylone, nabam, methylmercury oxinate, and CP 30249 (a nitrile from Monsanto Chemical Co.) were applied to freshly collected field soil at the ED_{99} concentration for F.o.c. in artificially infested soil. Soil samples were taken for dilution 1 day, 1 week, 2 weeks, and 1 month following treatment. The frequency of bacteria, streptomycetes, and most prevalent fungal genera was determined by standard plating techniques. Following are the significant results of each chemical treatment:

1. Vapam: Treatment with Vapam caused a general decrease in the total fungal population, but the bacterial and streptomycete populations were not greatly affected. Several phycomycetes were particularly sensitive to Vapam, and species of Penicillium, Chaetomium, Humicola and an unidentified basidiomycete were more resistant than the other fungi. No organisms built up following treatment.

2. Mylone: Mylone caused a general reduction in the fungal and streptomycete populations, but caused little change in the bacterial population. The native Fusaria and certain species of Penicillium were comparatively resistant to Mylone. As in the case of Vapam there was no build up of soil organisms following partial sterilization with Mylone.

3. Nabam: Following treatment with Nabam the fungal populations were greatly reduced with the exception of species of Trichoderma, which eventually built up to the original level of the total soil fungal population. The native Fusaria were also more resistant to Nabam than the other major fungal genera.

4. CP 30249: Following treatment with CP 30249 there was a general reduction in the fungal population with species of Fusarium and Trichoderma showing the greatest resistance. The bacterial population was relatively unaffected except for the build-up of a species insensitive to streptomycin. As with nabam, Trichoderma spp. built-up to a level equal to the total fungal population in the untreated soil. The streptomycete population was slightly reduced by treatment with CP 30249.

5. Methylmercury oxinate: The fungal population was almost completely elliminated from the soil very soon after treatment with this mercury compound. An unidentified basidiomycete was the most resistant of all the fungal genera. By the 8th day following treatment species of Penicillium began to build-up in the soil, and they eventually reached a level above the original total fungal population. The bacterial population was relatively little affected but the streptomycete population was greatly reduced.

Thus, only two fungal genera built-up following treatment: Trichoderma spp. in the nabam and CP 30249 treated soils and Penicillium spp. in the methylmercury oxinate treated soils. The only other build-up was by a bacterial species insensitive to streptomycin in the soil treated with CP 30249.

3. Reinvasion of treated soil by F.o.c. and normal flora

At the time samples of treated soils were collected for microflora analysis 2 sub-samples were taken. One sub-sample was infested with spores of F.o.c. and a small amount of the untreated field soil and the second sub-sample was infested with only the untreated field soil. The objectives of this phase of the experiment were to determine if F.o.c. could reinfest the soil following treatment, to determine the microflora changes associated with the establishment of F.o.c., and to determine if there was any difference in the build-up of soil organisms when the normal microflora was reintroduced as compared to the original samples in which build-up would have to come from organisms not killed by the chemical treatments. The following points were learned concerning the establishment of F.o.c. in treated soils.

1. Vapam: F.o.c. was easily established 1 day following treatment with Vapam, and on this and all subsequent infestation attempts F.o.c. became established at a higher level than in the untreated soil.

2. Mylone and Nabam: F.o.c. did not become established in the soil on the first day following treatment with these chemicals, but 1 week, 2 weeks, and 1 month following treatment F.o.c. became established at a higher level than in the untreated soil.

3. CP 30249: F.o.c. failed to become established in soil on the 1st day following treatment with CP 30249, but 1 week following treatment the soil could be infested at the same level as the untreated soil. Two weeks and 1 month following treatment the infestation was higher in the treated than in the untreated soil.

4. Methylmercury oxinate: Soil treated with methylmercury oxinate could not be infested with F.o.c. until 1 month following treatment, and at this time F.o.c. was established at a level far below that in the untreated soil.

These compounds with the exception of methylmercury oxinate had only a slight residual effect in the soil and when infestation was possible it occurred at a level above that of the untreated soil. However, the methylmercury oxinate treated soil resisted infestation up to 1 month following treatment.

4. Relation of wilt disease resistance to the nature of plant pectic substances

On July 1 of this year a project was initiated to study the role of pectic enzymes in Fusarium wilt syndromes and the relation of wilt disease resistance to the nature of plant pectic substances. Research is still in preliminary stages on this project, and will be reported in detail at a later date.

species and were inactive when drenched onto soil; but they were toxic to F.o.c. when mixed into infested soil. Class IV chemicals were toxic in agar but were inactive in all assays involving soil. Examples are given of compounds in each Class and it is suggested that Class I chemicals are the most promising soil fungicides with the potentialities of the Classes decreasing from Class II to III to IV.

Nabam, which has high fungitoxicity to F.o.c., is relatively inactive when applied as a drench. However, it was found that nabam, or its reaction product in the soil, could be washed down into the soil by water following treatment.

Preliminary results on the effects of soil temperature and moisture on the toxicity of several chemicals is reported. In general the chemicals are most toxic at moisture levels below field capacity. The volatile chemicals vapam and mylone gave maximum kill of F.o.c. at 15° C., and the other chemicals gave optimum kill at 20° C. However, these results may be confounded by the effects of temperature and moisture per se on the F.o.c. in the soil.

Because of their high innate toxicity to F.o.c. in soil, their ability to move into the soil when applied as a drench and their specificity for F.o.c. as versus the total soil fungal population, vapam, mylone and to a lesser extent CP 15986 were the most promising soil fungicides tested.

I. SUMMARY

Techniques have been developed to determine the effectiveness of selected fungicides in eliminating Fusarium oxysporum f. cubense (F.o.c.) from infested soil, and to investigate the effects of these fungicides on the total soil micropopulation. A method was developed for artificially infesting non-sterile field soil with the same fungal structures (mycelium, conidia and thick-walled resting spores) that exist in naturally infested soil, and without adding any culture substrates, which would greatly modify a natural soil.

A group of chemicals with toxicity to Fusarium species were tested in soil against F.o.c. by several methods. Vapam, mylone, nabam, methylmercury oxinate, Phaltan and 2 experimental fungicides CP 15986 and CP 30249 were most toxic to F.o.c., and they were selected for further testing.

When the selected chemicals were compared as a soil-mix and as a soil-drench it was found that several of the chemicals were virtually inactive as a soil-drench (Phaltan, methylmercury oxinate and CP 30249). Nabam was considerably less active as a drench than as a mix, but vapam, mylone and CP 15986 were only slightly less active as a drench.

Chemicals with a volatile activity were as effective mixed dry into the soil and watered afterwards as they were mixed wet into the soil. However, non-volatile chemicals were less active as dry-mixes than as wet-mixes.

Based on their activity in the various assays of fungitoxicity, the chemicals with high toxicity in agar to F.o.c. were divided into 4 Classes. Class I chemicals were active in all assays against all forms of inoculum and by mix or drench treatments. Class II chemicals were inactive when drenched onto the soil but were active in all other assays. Class III chemicals failed to penetrate organic matter to kill Fusarium

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July 1, 1958 - June 30, 1959
July 1, 1959 - December 31, 1959

THE EFFECTS OF FUNGICIDES ON FUSARIUM OXYSPORUM F. CUBENSE AND OTHER SOIL MICROORGANISMS

M. E. Corden
Roy A. Young

Department of Botany and Plant Pathology
OREGON STATE COLLEGE

3. Investigations on the nature of resistance to *Pseudomonas solanacearum*

Ellsworth Maine, Graduate Assistant
Arthur Kelman, Project Leader

In a survey of tobacco and eggplant varieties representing a wide range in levels of resistance to *Pseudomonas solanacearum*, no positive correlation was found between levels of chlorogenic acid and resistance. Evidence was obtained that the bacterium does not form chlorogenic acid oxidase. Rapid increase in levels of chlorogenic acid oxidase activity observed in tissues of diseased plants can, therefore, be attributed to changes in host metabolism.

Respiration of stem tissue of resistant and susceptible tobacco increased following infection by *P. solanacearum*. Evidence obtained by use of enzyme inhibitors and related studies suggest a relationship between increase in respiration and chlorogenic acid oxidase activity. Additional studies are underway on effects of various inhibitors on chlorogenic acid oxidase and ascorbic acid oxidase, effects of certain hydrolytic enzyme systems on respiration and tissue disintegration of resistant and susceptible varieties, and alteration of host resistance by chemicals.

2. Influence of host passage in solanaceous plants and mutagenic agents on virulence of the Moko bacterium.

Charles Averre, Graduate Assistant
Arthur Kelman, Project Leader

Cultures of Pseudomonas solanacearum from banana and tomato were passed through eggplant nine times. After the last host passage the final re-isolate was compared with the parent culture for pathogenicity on eggplant, tobacco, and tomato. The host passage resulted in an increase in pathogenicity of one banana isolate to eggplant and tomato, but there was no evidence of an increase on tobacco. A second isolate from banana and an isolate from tomato were not altered significantly by host passage. Nine serial passages of isolates through a susceptible and resistant tobacco variety have been completed and evaluation of effects of host passage will be made shortly using as test plants Musa balbisiana, tobacco, and tomato. These studies serve to complement work at Coto to determine whether isolates from Heliconia and solanaceous hosts with low virulence to banana have a potential to increase in pathogenicity following successive passage through banana.

In initial studies in which proportions of virulent and avirulent cells of the tomato strain were varied, it was found that pathogenicity did not decrease significantly as proportions of virulent to avirulent cells decreased; however, rate of initial symptom development was affected. Cultures from banana, Heliconia sp., and tomato have been irradiated and tested for virulence on tobacco and Musa balbisiana. In this initial work there was some evidence of changes in pathogenicity of the different isolates after irradiation although data was not conslusive. Evaluation of results of irradiation on pathogenicity are continuing.

genic on tobacco. Although less invasive initially than isolates from solanaceous and certain weed hosts, strains of the Moko bacterium from banana could be classified as moderate to highly pathogenic on tomato. Additional work on differentiation of strains of the Moko bacterium with respect to virulence on solanaceous hosts will be discontinued since the primary objectives of the project have been completed.

Report to Department of Research, United Fruit Company

of supported research on the Moko disease

at N. C. State College, Raleigh, N. C.

1. Pathogenicity of the Moko bacterium to solanaceous hosts.

Charles Averre, Graduate Assistant
Arthur Kelman, Project Leader

In greenhouse inoculations tests to evaluate pathogenicity, isolates of Pseudomonas solanacearum obtained from banana in Costa Rica were compared with isolates from plantain, Heliconia sp., tomato, and the following weed hosts: Solanum torvum, Solanum nodiflorum, Physalis angulata, and Eclipta alba. Stem inoculations in tobacco and tomato plants were made with the above isolates and cultures of P. solanacearum from North Carolina and Georgia. All of the isolates included in this test had been evaluated previously for virulence to banana at Coto and La Lima by Dr. Ivan Buddenhagen.

The results obtained with these isolates confirmed previous observations in greenhouse inoculations on solanaceous hosts. All isolates from Costa Rica were moderately to highly pathogenic on tomato; however, on tobacco, isolates from banana, plantain, and Heliconia were only very slightly pathogenic. In marked contrast, isolates from S. nodiflorum, E. alba, and P. angulata were highly pathogenic on tobacco. The cultures from tomato and S. torvum were moderately pathogenic on tobacco. In this respect, the tomato isolate from Georgia was similar to the tomato strain from Costa Rica in its high virulence on tomato and moderate to low virulence on tobacco. It was of interest to note that the isolate from E. alba with high virulence to tobacco was the only isolate from hosts other than banana or Heliconia that had been shown to be pathogenic to some degree to banana.

Further confirmation was given to previous data that none of the isolates of P. solanacearum capable of inducing "fast-wilt" symptoms on banana are highly patho-

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NORTH CAROLINA STATE COLLEGE

time, it would seem possible that soil organisms consistantly attracted to wound sites may be carrying spores to these areas. For example, in the case of enchytrid worms, their external surface is characterized by small tufts of bristles which inadvertently would be capable of carrying spores. In addition, many of these burrowing worms may be capable of carrying viable fungus spores through their digestive tract and depositing them in their castes.

Although the occurrence of E. minimus in the tropics has not been ascertained, the group Enchytraeidae is quite cosmopolitan with many members distributed in tropical and temperate regions. Further, it is quite likely that many other tropical soil organisms possess ecological habits similar to the enchytrid worm observed here.

J. L. Riopel

meristematic regions of the root. In making free-hand sections of wounded root material these worms have been found on rare occasions to be lodged in large vessels bordering the wound site. Reasons for their attraction to wound areas are at present not clear although evidence strongly suggests a parasitic relationship. Several members of the Enchytraeidae are thought to be parasitic on the roots of plants.[4] An early reference has been found concerning a worm, Enchytraeus parvulus, closely related to minimus, which was reported to burrow under the epidermis of Aster plant roots and ultimately result in death of the plant.[5]

If in fact wounds are significant in Fusarium penetration under field conditions, then the above observations become more than of academic interest for it is necessary to reconcile somehow the evidence presented by Goos[6] which suggests that under field conditions exudates from injured roots are rapidly decomposed by increased bacterial activity and have little stimulatory effect on rhizosphere fungi with the results of Sequeira et. al.[7] showing spore stimulation from wound exudates, while spores in contact with an intact root surface appeared to be inhibited. Thus successful invasion of Fusarium would seem to be dependent upon spores being present immediately at the wound site and thus stimulated before bacterial decomposition of organic exudates can occur. Clearly these circumstances are too haphazard to account for the success of the Panama Disease and although completely speculatory at this

[4]Storer, Tracy I. General Zoology. McGraw Hill Co. Inc. 1951. p. 449.

[5]Friend, Hilderic. The Zoologist. ser. 4. v. 1. 1897. p. 349.

[6]Goos, R. The Effect of Root Injury on the Rhizoshpere Flora. United Fruit Company Annual Project Report. 1958.

[7]Sequeira, L., T.A. Steeves, H.W. Steeves, & J.M. Riedhart. Observations on the role of root injury in Panama disease infections. Nature 182. p. 309-311.

Environmental Influences

In an effort to elucidate the mechanism of wound response and to find what environmental factors, if any, are of importance under field conditions, a study of a number of possible influencing variables is in progress. These studies are concerned with the effect of high concentrations of CO_2, Oxygen tension, the presence of soil organisms and auxin. Results of these studies at the present time are not sufficiently complete to allow conclusions. The contamination of control wounds by a number of soil organisms including species of root-knot nematodes, Meloidogyne, and a worm of the Class Oligochaeta has been a source of difficulty in carrying out this experimental work. Plants, surface sterilized and placed in steam sterilized soil or vermiculite, were found to be contaminated after three months. The use of isolation chambers was also unsuccessful in keeping wounds free from these organisms. Contamination has been kept at a minimum by maintaining large plants in nutrient solution with roots selected for experimental work growing in sterilized vermiculite surrounding the container, and results from these studies will be forthcoming.

The continued association of certain soil organisms with banana roots has prompted a study of their relationship and effects upon wound responses. This study has been especially concerned with the oligochaete worms mentioned earlier. With the cooperation of Dr. A. Weir Bell of Los Angeles City College, this worm has been identified as Lachytraeus minimus described by K. Bretcher in 1899.[3] Attempts to culture these worms have been quite successful and their effect on wound responses is now being studied.

Preliminary observations with soil grown plants have clearly shown a consistent association of these worms with vascular wounds and the tender

3Bretscher, K. Rev. Suisse Zool. V. 6. 1899. p. 402.

on several types of wounds including superficial and vascular gashes, punctures, scrapings, and apical damage. Briefly, the response of banana roots to injury is very similar irrespective of how the damage is inflicted. Superficial wounds result in cortical cell enlargements oriented at right angles to the wound surface. Although dependent upon the severity of the wound and its location on the root, superficial wound responses are localized and affect only the neighboring cells of the wound site. In contrast, wounds affecting vascular tissue may result in discolorations observable 3 - 4 centimeters below and above the wound. Correlated with vascular wounds is the occurrence of numerous tyloses which often completely plug the large metaxylem vessels. In addition, vascular wounds made in the immature regions of the root usually result in cell divisions in the stele oriented at right angles to the wound surface as in the cortex. It is interesting to note that the response to normal vascular wounds is in some respects similar to the response which in the past has been though of as diagnostic for the presence of Fusarium in vascular tissue. The presence of Fusarium in the vascular tissue is usually accompanied by cell divisions, tyloses and discolorations which are often at considerable distances from any normal vascular wounds which could provoke wound healing. Thus it may be possible to consider the cell proliferation effected by Fusarium as a wound response which is, in many ways, analogous to the response of artificially inflicted vascular wounds. There are some differences, however, for the cambiform layer as described by Wardlaw[2] is oriented around discolored vascular strands or the invading fungus, and the breakdown products from a normal wound do not appear to be the same as those of infected tissue.

[2]Wardlaw, C. W. Diseases of the Banana. Macmillan & Co. London. 1935. p. 62-85.

can be reinforced by the use of cytological chimeras induced by radiation or autoradiographic techniques. Unfortunately, in the present study preliminary attempts to use tritiated thymidine were unsuccessful and further use of this technique must await its refinement due to certain technical problems.

Studies of the developmental aspects of the root have been hindered by the refractory nature of banana roots. Although the roots may be treated with hydrofluoric acid to soften the lignified stele, considerable shrinkage of the phloem and other highly vacuolated cells occurs when the paraffin technique is used. Several attempts were made to obviate this difficulty including the use of a closely graded paraffin infiltration series, celloidin, celloidin-paraffin, carbowax and a freezing microtome. Of these methods only material embedded in celloidin or carbowax was free from shrinkage. As the celloidin method does not easily allow serial sections, carbowax was selected as the embedding medium. Since carbowax is water soluble, ribbons could not be floated on slides or handled in other ways customary to paraffin material and as a result, serial sections could be obtained only with difficulty. However, by introducing a few modifications in the carbowax technique it was possible to obtain serial sections quite readily. With this method, fixation, embedding, staining and mounting procedures are entirely aqueous and therefore preparations are free from shrinkage or distortion. At the present time serial sections of material embedded in carbowax are being prepared and will be used to complete the developmental study.

Normal Wound Responses

Studies of the normal response of roots to wounds in meristematic and mature regions are nearing completion. These studies include observations

Harvard Biological Laboratories
Cambridge, Mass.

Developmental Aspects and Wound Responses in Banana Roots

November 30, 1959

Annual Report

In view of the significant role of root injury in the Panama Disease, a study of wound responses and influencing factors was undertaken. In addition, a study of the root morphology has been in progress in order that the wounding studies be based upon a thorough understanding of the normal root development. The following reports in part the progress made with these studies and also contains suggestions for certain aspects of the problem which, though seemingly beyond the scope of the present study, may merit further attention within the research program of the United Fruit Company.

Morphology

A careful study of the banana root apex has been made and the earlier observations of Acquarone[1] considered in the light of modern concepts concerning apical organization. Evidence indicates that the idea of separate and clearly defined origins for the various tissue systems within the root is no longer tenable. Although superficial boundaries do exist between stele, cortex and root cap, these are due to differences in growth rates and planes of division. At their origin the stele, cortex and root cap are ultimately derived from a group of common initials which are indistinguishable from one another by conventional staining methods.

Admittedly studies of this nature become more meaningful if the evidence

[1]Acquarone, Paul. The Roots of Musa Sapientum, L. United Fruit Company Research Bulletin No. 26. August, 1930.

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Biological Laboratories

HARVARD UNIVERSITY

The possibility that soil attached to seed bits of banana could be spreading Fusarium oxysporum f. cubense was considered. Samples of soil remaining on the ready-to-plant rhizomes were collected at random and when plated showed the presense of the following Fusarium species. (Seed was selected from areas low in Panama disease incidence, washed, trimmed, and dipped in a 1% dithane solution.) Soil counts were made on Modified Martin's medium. Four plate counts were averaged per sample.

	Colonies per gram of wet soil			
Seed No.	F. solani	F. roseum	F. moniliforme	F. oxysporum
1	300	50	0	0
2	1650	4250	50	0
3	2250	1600	0	0
4	2200	3600	0	0
5	14700	0	0	0
6	750	1500	50	0
7	4500	3500	0	0
8	0	450	1100	0
9	0	1800	50	0

It was noticed that more than 30% of the seed pieces ready for planting had soil clumps attached to them.

These studies suggest that the actual procedures followed in the production of clean seed may not be adequate, although it is striking that F. oxysporum did not show up in these tests. Although Fusarium oxysporum was not found in any of the seeds sampled, spread of the organism causing Panama wilt could take place in this way.

A large number of the root cortex isolates were identical in morphological and growth characters to the pseudostem isolates. It is believed that the later colonization of the root system of a dead plant by the pathogen bears directly upon the rate of spread of the disease from plant to plant in the field. The literature related to the development of the banana root system in different soil types indicates that on light soils the radius of spread of the root system may average 14.5 ft. from the mat; and in heavy soils, 7.3 ft.

The extreme differences in the development of the banana root system according to the soil type, appear to be correlated with the rapid spread of Panama wilt in sandy soils("susceptible"),and its limited spread in clay soils("resistant"). The planting distances most commonly used in banana farming particularly favor the intermingle of the root systems of adjacent mats in light soils but not in heavy soils. Based on the evidence available, the proper planting distance, i.e. one which does not favor overlapping of root systems, according to the soil types, could be of importance in future plantings in virgin land.

Field inoculation tests showed that young wounded banana roots are resistant to invasion by Fusarium oxysporum f. cubense. Old heavily lignified roots did not show resistance when wounded and inoculated. Wound inoculations close to the apical meristem of old and young roots were not successful, thus the meristematic tissues of the roots of Gros Michel appear highly resistant to invasion by Fusarium oxysporum f. cubense.

Histological studies of the root systems of Gros Michel and Lacatan in relation to invasion by the fungus are in progress. A study of the pathological anatomy of the vascular elements of roots of Gros Michel has revealed a possible defense mechanism that manifests itself as a mechanical action. This defense mechanism present in the resistant section of the root is being sought in other parts of the plant in which there is meristematic activ

United Fruit Company - Progress Report - November 1959

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Techniques used to study Fusarium solani f. phaseoli in soils of the Salinas Valley, California, were used last summer in Honduras for a preliminary survey of Fusarium oxysporum in banana soils. Results from testing these soils revealed the possibilities of adapting similar techniques to the study of Fusarium oxysporum f. cubense and other Fusarium spp in banana soils.

The limited survey on the Honduras soils showed that there were field areas with a predominant population of Fusarium roseum, and other areas in which Fusarium solani was the predominant species. Fusarium oxysporum was generally found to be low in all soils tested. Although the presense of Fusarium oxysporum f. cubense was confirmed, there is strong evidence that the causative agent of Panama wilt is not uniformly distributed throughout the soil in the infested fields. Since these soils are not plowed nor cultivated, it is likely that the organism is found concentrated in the limited microenvironment of the decaying root system of the infected plant.

Investigations of the root systems of diseased plants having dead tops and rotted rhizomes in a state of organic decomposition indicated that at this stage the fungus progressively invades the cortex of the entire root system of an infected plant. Macerated and cultured cortex from the root system of five plants, sampled 5 feet and one foot, respectively, from the rhizome, showed a high count of Fusarium oxysporum present. Chlamydospore-like structures were observed by direct examination of the macerated root cortex. In four out of the five plants studied Fusarium oxysporum f. cubense was the predominant form of the fungus (Isolates have been tested for pathogenicity by Dr. R. Stover on M. balbisiana seedlings.)

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Virus disease of bananas

Further mechanical inoculations of the Central American banana virus were made into weeds and cultivated plants associated with banana plantations. Physalis spp., Isotoma longiflora, Ricinus, Honduran "Ayote", Honduran "Pepino", Vinca rosea, Crotalaria anagyroides, and Kudzu (Pueraria) were found to be susceptible to the virus. Isotoma and Comphrena sp. are symptomless hosts which produce local lesions when inoculated into cow pea and Chenopodium amaranticolor.

Host range and insect transmission studies with the virus disease are continuing.

Variability and pathogenesis in the Panama disease pathogen, Fusarium oxysporum f. cubense.

More isolates of F. oxysporum f. cubense were obtained from banana growing areas throughout the world. Using the single spore method, clones were cultured and studied in the laboratory. Plant inoculation studies were initiated to determine differences in virulence of the fungus clones and to determine also possible race differences. Most of the fungus clones are being tested in banana seedlings and large plants of plantain and banana at La Lima, Honduras.

An isolate from Heliconia latispatha, from Honduras, has proved to be a highly virulent clone of race 1.

Special efforts are underway to obtain isolates from bananas and plantains which may be race 2 or possibly other races not yet observed.

A publication dealing with nomenclature in Fusarium oxysporum is in preparation.

place very slowly, and the microconidia of the banana wilt organism rarely formed chlamydospores.

Once chlamydospores are formed in soil, they are not readily induced to germinate, and wetting of the soil may have little or no effect. The addition of solutions of such amino acids as glycine, proline, phenylalanine, or ornithine likewise did not induce germination. Aspartic acid brought about vigorous germination of F. oxysporum f. cubense within 24 hours, glutamic acid appeared to be somewhat slower acting. Glucose likewise gave good chlamydospore germination, but the hyphae appeared to lyse within a short period of time. Here again, the volume of soil used had a large bearing on the results obtained and, in a sufficiently large soil sample, differences were evident between the surface and the lower layers.

Plant tissue, such as excised banana roots, bean stems, and barley straw will stimulate chlamydospores to germinate apparently only if they are in direct contact with the plant part. To date, F. solani f. phaseoli chlamydospores have been found to germinate only in the presence of bean tissue. The chlamydospores of the banana wilt fungus do not appear to be as selective, but the end result of their germination may lead merely to the formation of new chlamydospores, to sporulation or to invasion, depending on the plant tissue and on the type and amount of soil used.

United Fruit Company - Progress Report-November 1959

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Studies are in progress on the behavior of F. oxysporum f. cubense and of F. solani f. phaseoli in soil. Relatively few conclusions can be drawn at this date and for this reason only the outlines of the work will be given in this report.

The introduction of the banana wilt Fusarium into soil eventually leads to the formation of chlamydospores by the organism. It appears, therefore, that this fungus exists in soil, in the absence of a host, and as a means of survival in soil, in the form of chlamydospores and that its behavior in this respect is similar to that found by Miss S. M. Nash for F. solani f. phaseoli, the cause of root and stem rot of bean.

Both fungi will grow through soil and sporulate on the soil surface if the soil is sterile. Very little growth occurs when they are added to non-sterile soil, especially if the inoculum is washed prior to its incorporation. The development of chlamydospores in sterile soil occurs only after active growth has ceased, a process which occurs gradually and sometimes over a period of several months. Chlamydospores are formed sooner in non-sterile soil within periods as short as two weeks or less. The rate of chlamydospore formation from macroconidia introduced into soil also depends on the fungus, the area of soil sampled, the mass of soil used, and the type of soil as well as the kind of plant tissue present. Aeration and moisture appear to have a strong bearing in the differences obtained. Spores of F. oxysporum f. cubense and F. solani f. phaseoli have been kept in sterile water blanks for more than one year. Under such conditions, chlamydospore formation took

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4. Marshall, K. C. 1959 Competition between the soil microflora and Fusarium oxysporum f. cubense (E.F.S.) Sny. and Hans., M. S. Thesis, Cornell University.

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The work dealing with the growth of F. cubense in the absence of atmospheric oxygen has been extended. Most soil fungi fail to develop under such circumstances, but the banana wilt fungus is capable of a slow but significant development following exclusion of gaseous O_2. Such growth requires the presence of some uncharacterized substance in yeast extract, and no single amino acid or a mixture of amino acids would permit equivalent development. Aerobic proliferation is likewise enhanced by a factor in yeast extract although growth proceeds readily in its absence. Partial replacement of yeast extract was achieved with several B vitamins but, although statistically significant, these effects vanished upon further cultivation - a variability inherent to the organism.

The stimulation reported by Nair of the metabolism of a related wilt fusarium could not be confirmed in the case of either maleic hydrazide or thiourea for the banana fungus. A possible influence of 2, 4-dinitrophenol has been observed.

F. cubense metabolizes most intermediates of the tricarboxylic acid cycle, but the oxidation is never complete. Acetate and glucose provided the highest respiratory rates, and the oxidation of citrate and isocitrate were negligible or even lacking. Enzyme studies using mycelium extracts have demonstrated the presence of succinic dehydrogenase, fumarase and aconitase, but isocitric dehydrogenase was apparently deficient.

Subproject 4. Physiological studies of Fusarium oxysporum f. cubense

Characterization of soil as a physico-chemical environment for the growth of F. cubense has been continued. These studies are carried out in conjunction with subproject 1 where the biological factor is reintroduced.

The physical and chemical composition of soil affects the rate of growth of the fungus, and proliferation varies appreciably between soils obtained from different localities. No correlation exists, however, between the growth in soil and the banana disease history. A bioassay technique has been developed in order to measure the potential nutrient delivery of these soils to the pathogen, and the adequacy of K, S, Mg and Zn but not of nitrogen and phosphorus is indicated. These results have a direct bearing on the investigations of competition reported in subproject 1. Potassium uptake from soil is correlated with chemically extractable K in the 12 Central American soils, but an anomalous growth suppression is observed with increasing potassium concentration.

The bioassay procedure utilized showed the presence of substances inhibitory and stimulatory to F. cubense. The greatest depressing effect was recorded in the three samples of varying texture for San Alejo. Evidence for the heat lability of this factor is presented. That more than a single chemical is implicated is suggested by the fact that the stimulation by soil extract is eliminated in one soil by montmorillonite but not in a second sample.

200 lb per acre in the presence of organic matter led to a substantial reduction in the numbers of fungi, the population being about ten-fold less than in parallel samples receiving no sulfur or sulfate. This is suggestive of sulfide toxicity being a major factor in the eradication of soil fungi by flood-fallowing.

III. Subproject 3. Ecology of the soil microflora and chemical changes as related to soil submergence

Microbiological studies were carried out during the flooding of Guaruma 2, lake 1 in Honduras. It was observed that flooding slowly reduced the population of bacteria and actinomycetes but was more active in reducing the fungus flora. When banana pseudostems were added to the flooded soil, the population of fungi was depressed still further at the 0-2 and 7-9 inch depths while the abundance of bacteria and actinomycetes did not decline as rapidly when compared to the normal flooding procedure. A preliminary trial of the application of the most probable number technique to flooded soil has shown that the procedure is readily adaptable to these conditions, and a single experiment in a flood fallow lake in Honduras failed to reveal a difference in rate of decline of bean fusarium between flooded and normal soil conditions.

Studies have been extended in order to characterize precisely the reasons underlying the effectiveness of flooding in the elimination of F. cubense. It has been noted that there is a distinct relationship between changes in oxidation-reduction potential and suppression of fungi in submerged soils, and both are decreased by the presence of readily available sources of organic matter. There occurs under such conditions of flooding a build-up of a factor or factors markedly detrimental to the fungus.

In addition to organic matter, the application of even small quantities of colloidal sulfur or sulfate at rates equivalent to

adding autoclaved soil-extracts to cultures reduced growth below that observed when filtered but not autoclaved soil-extracts were added.

The data obtained using autoclaved extracts also indicated that even though stimulating substances in soil extracts were destroyed by autoclaving, a clear indication of the presence of inhibiting substances in soil extracts was not obtained.

Third, in checking further on the occurrence of inhibiting substances in soil-extracts, it was shown that growth of Fusarium oxysporum f. cubense is stimulated by addition of yeast extract to cultures of this organism. The studies also showed that most of the soil extracts contained substances that inhibited the growth of this organism. This inhibition was especially pronounced in the case of extracts obtained from long-life soils.

Fourth, by making six (6) successive extracts of the same soil sample, it was possible to show that there are at least two groups of inhibiting substances. One of these groups of substances appears to be completely dissolved by water. The other either forms saturated solutions in water or partitions between soil and water and has a very high partition coefficient in favor of soil.

Fifth, the potassium relation to incidence of Fusarium wilt of bananas that was reported in 1957-58 was developed further. It has been found that the long-life soils contain appreciable amounts of volcanic ash and that the clays present in these soils are of the illite-montmorillonite group. Whereas, the short-life soils do not contain volcanic ash and the clays in these soils are of the kaolin, chlorite and/or vermiculite types.

II. Subproject 2. Fungitoxic substances in soil.

The development of a satisfactory assay procedure for antifungal substances in soil extracts was completed and published (Archiv für Mikrobiologie 33:182-185, 1959) by the end of the 1958-59 fiscal year. This method was then applied to the problem of determining whether or not antifungal substances occur in water extracts of soils from Central America.

First, it was shown that standing cultures of Fusarium oxysporum f. cubense that contained sterile extracts of short-life soils grew better than cultures that contained sterile extracts of long-life soils. It was then shown that cultures containing concentrated soil extracts differed from each other by much less than did cultures containing extracts that had not been concentrated.

Second, the effect of steam sterilization of soil extracts was studied. This work showed that shaking cultures of this organism resulted in much faster growth than that observed for standing cultures. Apparently, this was due to the sensitivity of the organism to oxygen level, which was affected by autoclaving. This work also showed that growth of the organism was less filamentous in cultures which were shaken than in the standing cultures. This difference in growth made it necessary to add aluminum hydroxide to the cultures prior to centrifuging them in the assay procedure. Three experiments were then done on the effect of autoclaved soil-extracts on growth of Fusarium oxysporum f. cubense. These experiments, conducted under appropriate conditions, showed that

competitive ability but (c) the effectiveness of bacteria is directly associated with their nutritional complexity, the active organisms being those with minimal requirements. This delineation of factors determining effectiveness holds only for the non-spore-formers, and an entirely different mechanism applies to Bacillus spp.

Investigations of the microbial interaction in various soil from Honduras and Panama has revealed a marked influence of non-biological factors, and differences in soil type are apparent. The suppression is negligible in some soils and appreciable in others; soil texture, organic matter and/or nitrogen content are tentatively implicated.

By use of a specific counting technique for Fusarium oxysporum f. conglutinans, microbiological influences on the pathogen in normal soil have been demonstrated. Whereas the fungus normally dies out in unamended or organic matter-treated soil, it will maintain itself provided adequate nitrogen is present. This suggests that the available nitrogen status is of importance to the resistance of the organism to microbial elimination and implicates nitrogen competition under normal circumstances in the field.

SUMMARY OF THE REPORT

I. Subproject 1. A study of the competition between the soil microflora and Fusarium

Studies of the basis of suppression of Fusarium oxysporum f. cubense by saprophytic microorganisms have been continued. The possibility of phosphorus deficiency being the cause of such inhibition has been demonstrated in culture solution, but evidence has not been obtained that this phenomenon is of importance in soil. The relative incidence of competitive bacteria has been examined in two soils from Honduras - one is a short-life soil and the other is still producing healthy bananas. None of the isolates from the former but 25% of the strains from the latter reduced the spread of F. cubense in the original soil. The lack of influence in the short-life soil from San Alejo may be due to its acidity.

The soil bacteria which reduce Fusarium growth were studied further to determine the mechanism of interaction. Antibiosis does not seem to be a factor in almost all of the effective cultures. In seven of eleven of the microorganisms tested, the detrimental influence resulted from nitrogen competition. In the remaining four, bacteria of the genus Bacillus, the inhibitory action is as yet unexplained.

Using 50 effective bacteria, the basis of competitive capacity against the pathogen was investigated. It has been shown that (a) the degree of effectiveness in culture medium is correlated with that in soil, (b) growth rate in culture solution or soil is not correlated with

Title

A Study of the Relation of Microbiological and Biochemical Properties of the Soil to Panama Disease of Bananas

Objective

To investigate the role of the microbial population of the soil and of certain biochemical characteristics on the persistence, growth and spread of the Panama disease fungus

A PROGRESS REPORT ON RESEARCH

Financed by

THE UNITED FRUIT COMPANY PANAMA DISEASE GRANT

1958-1959

N. C. Brady

M. Alexander
J. E. Dawson
K. C. Lu

Department of Agronomy

CORNELL UNIVERSITY

Tropical Agriculture in Trinidad and, to supplement this, collections have been made at stages throughout the development of fruits of both seeded and non-seeded varieties. The interpretation of the growth of the banana fruit must take account of growth which is stimulated in the wall of the loculus, tends to encroach upon the free space within the loculus, and it must also take account of the development which occurs in the placental regions within the loculus. These different growth responses may well be evoked by different stimuli. Work along these lines is proceeding.

The potentialities for growth of the different regions of the banana fruit have been investigated by tissue culture means. Explants from the fruit have been treated with different types of growth substances, singly and in combination. Different types of growth substance seem to stimulate different regions (the region of the wall of the loculus; the placentae, the funicular region of the ovule.) Some unexpectedly large responses have been obtained and described. On the basis of these responses observed in culture, some tests, with positive results, were made on the growth of banana fruits in the field; these tests were in cooperation with N. W. Simmonds in Trinidad. A start has also been made toward a study of the growth of banana cells in free cell culture. Again, very substantial growth -- leading to a range of cell forms -- has been observed. This work is being continued to determine the chemical stimuli which will unleash the inherent ability of the banana fruit cells to grow.

The vegetative shoot apex is being investigated with a view to a description and interpretation of the way the leaves originate on the apex and the way they grow to form the pseudostem and the laminae. This needs to be done in more intimate detail than hitherto. In view of their importance in vegetative propagation, the origin of buds, which in banana seems anomalous, is being investigated. Sections and illustrations have also been prepared of the apex of the floral shoot to show the differences between the organization of the vegetative and floral apex; the aim here is to describe exactly how floral induction occurs.

K. Stewart Dec 21. 55

The fruit of all these more or less resistant plants is distinguished by (a) low soluble nitrogen content, (b) a very low level of histidine, (c) very low level of glutamine, but within the low soluble nitrogen content asparagine is conspicuous

Since the fruit of these varieties has distinguishing characteristics, one needs to know whether these do, or do not, reflect an underlying metabolic pattern, which is associated with resistance in these varieties.

Work on Roots in Relation to Antifusarial Compounds.

(With Mr. R. A. Barr and Mrs. M. L. Connolly)

Investigation in this area has followed two lines of approach.

1. The detection of natural inhibitors (from Gros Michel and Cavendish roots) to the fungus;
2. Attempts to show translocation of an externally applied systemic fungicide, which is known to occur in nature, in Gros Michel.

Progress has been made in both of these approaches, and especially so in the latter

Both resistant and susceptible plants continue to yield substances which can inhibit the growth of fusarium on petrie plates. Differences are not, however, of the "all or none" kind, but tend to be quantitative, with the roots of resistant plants apparently richer in the substances toxic to the fungus.

Although at first success was not achieved, it now seems possible to apply a known systemic fungicide to the leaves and to detect its presence, or the presence of a closely similar compound in the roots when these are extracted and the extract appropriately fractionated and tested by ultraviolet absorption spectrometry.

Work on the Morphology and Development of the Banana Plant.

(With Dr. W. G. Barker, Dr. H. Y. Mohan Ram, and Dr. Manasi Ram)

The morphology and manner of growth of the banana fruit has been investigated Access was obtained to some unpublished material available in the School of

Biochemical Composition in Relation to Genetics and to Breeding of the Banana.

(With Mr. R. A. Barr and Mrs. M. L. Connolly)

The techniques and knowledge referred to above now permit plants representing stages in the breeding program being conducted in the B.W.I. to be analyzed; in this way one can see whether biochemical changes accompany the other characters for which selection is being made. The presumption is that it will be necessary to continue such work as the breeding work proceeds. Up to the present time tests have only been made on fruit which was sampled as far as possible at the same stage of ripening and in such a way that the general trend of the changes that occur during ripening, in both the inner and the outer pulp, may be detected. The first materials sampled were selected on the advice of N. W. Simmonds and were supplied by Mr. R. Osborne from Jamaica. The pattern of nitrogen metabolism characteristic of Gros Michel fruit is evident from the amide content (asparagine and glutamine) and the histidine contents of the inner and outer pulp respectively. Briefly, the Gros Michel characteristics tend to be impressed upon the fruit of hybrids in which Gros Michel was the female parent (3 of which were examined), but the nitrogenous composition also seems to be modified by the nature of the male parent. The observed deviation from the Gros Michel type of fruit was quite apparent in the hybrid Gros Michel X T27.

This work establishes the need to keep watch upon the composition of the banana plant as the breeding work proceeds, and attention should now be paid to sampling tissue other than that of the fruit and to other ranges of compounds.

Composition of Fruit of Certain Other Varieties.

Fruit of certain other varieties were obtained from Honduras and analyzed. The objective was to compare earlier results (in regard to general soluble nitrogen content) with those obtained on some of the members of the "Cavendish" group, which have resistance to infection by Fusarium oxysporum f. cubense.

BIOCHEMICAL AND MORPHOLOGICAL STUDIES OF THE BANANA PLANT

Cornell University, Department of Botany

F. C. Steward

Research in the Department of Botany has proceeded along several lines. Work on the biochemistry and physiology of the banana plant, started earlier, is being continued. Some work to relate the behavior of roots of Fusarium resistant and non-resistant plants to their biochemical characteristics has also been done. Work toward a better understanding of the growth and development of the banana plant with particular reference to the morphology of the fruit on the one hand and to the manner of growth of the vegetative and floral shoots on the other is being undertaken. A brief summary of each facet of the overall research appears below.

Biochemistry and Physiology of the Banana Plant.

A large body of biochemical data, obtained in prior years with the help of several investigators working in the Botany Department, has now been brought to the point of publication. The published account will appear in a series of papers in the Annals of Botany and submitted from the Department of Botany and the Research Department of the United Fruit Company. The papers have the following titles and will be bound together (approximately 70 pages) for circulation and use:-

PHYSIOLOGICAL INVESTIGATION ON THE BANANA PLANT

I. Biochemical Constituents Detected in the Banana Plant.

II. Factors Which Affect the Nitrogen Compounds of the Fruit.

III. Factors Which Affect the Nitrogen Compounds of the Leaves.

When published early in 1960, this material will add extensively to knowledge of the metabolism of the banana plant, especially in areas which had not previously been investigated; it will also form a background against which much other work may be viewed.

A PROGRESS REPORT ON RESEARCH

Financed by

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Department of Botany

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to evaluate results. In addition, there have been some complaints of refrigerator car shipments out of Baltimore. At the moment an investigation is under way to determine whether the cars themselves, the railroad handling of the cars or perhaps a handling procedure at the wharf is responsible for these complaints.

inquiries. This outline is advisory and more information will have to be compiled and studied before we can set up a standard to guide our jobbers. With continued increases in trailer loadings, it seems obvious that the quality of our fruit is seriously affected by some of the poor equipment in use.

Car Preparation

In an effort to reduce cost, we are making a study as to the feasibility of eliminating or reducing preheating services for refrigerator cars.

We are also reviewing all materials and services supplied by the company which are needed to prepare refrigerator cars properly for loading. This is being done with the view towards the reduction or elimination of those items and services which are not essential to the safe transportation of bananas in refrigerator cars.

As an example, we have recommended the elimination of papering refrigerator car doors and the reduction of door lumber for slatting from two per car door to one. Savings effected by the elimination of papering alone are estimated to exceed $10,000 per annum. Although these recommendations have not been put into effect yet, as they were only recently proposed, we do not feel that any serious difficulties will arise from these eliminations and reductions.

Rough Handling

The Association of American Railroads' Loss and Damage Prevention Section, and the Railroad Perishable Inspection Agency have reported to us that they were experiencing greatly increased claims for rough handling in banana cars to the Pittsburgh area on shipments from our Weehawken Terminal.

In meetings with us they asked our cooperation in exploring ways and means to ascertain how this damage is occurring, and also to try several new schemes in the method of bracing banana loads at the doorways of the cars.

It was agreed that the best way to study the problem initially was to institute heavy use of impact registers in cars consigned to Pittsburgh. When possible we are installing impact registers in every car consigned from Weehawken to Pittsburgh. A careful tabulation is also being kept on all shifted and damaged fruit whether an impact register is installed or not.

We have also cooperated on the AAR's suggestions for changes in the method of bracing fruit, although we feel that the tie-back method of bracing has been working very satisfactorily (see previous reports). To date, three tests of several cars each have been run with a paper tie-back across the front row bunches at the doorway instead of the method we are now employing of string tie-backs. It is too early

The few tests made, utilizing the modified Preco Cargotemp, have been successful.

We will follow the development of this system closely and intend to run some tests with this car. Air distribution from fans at only one end of the car may prove a serious problem. We hope to be able to work with the manufacturer to insure adequate circulation with our type of lading. Bananas are one of the few fruits or vegetables being shipped in bulk in railroad cars today.

Mechanically Refrigerated Cars

We have continued to study this type of car and our conclusions are still much the same as stated in the 1957 report. However, we are contemplating some tests with this type of car, as many industry authorities feel the mechanical refrigerator car will eventually replace all other types of cars, hauling perishables.

One western carline has on order 1025 mechanically refrigerated cars which will be delivered during 1960. These will be 50 foot mechanically refrigerated cars (Giants) especially designed not only for perishable commodities, but also to be utilized on the return haul for regular freight. These cars are very much in demand on the West Coast since they have a greater capacity which allows the shipper to take advantage of incentive rates.

It may be well to note here that there has not been any construction of the standard RS type car in the past year. There have only been 150 type RS cars constructed in the past two years. Obviously, the trend is away from the standard RS type car, and some consideration and study must be undertaken now or shortly to determine the requirements of a mechanically refrigerated car in relation to our product. At the moment our basic sale unit is in carlot size of approximately 20,000 to 22,000 pounds. The trend is toward construction of larger cars which would be only partially filled with 20,000 to 22,000 pounds of bananas. The size of ripening rooms has always been governed by carlot quantities. The trend toward large 50 foot mechanical cars is one worthy of attention for other reasons, including their inability to be served at platforms and wharf terminal facilities already built with proper clearances for 40 foot RS type cars. Many warehouses have door openings spaced for the 40 foot RS type cars.

Long Distance Trucking

The trend toward long distance trucking of bananas continues. Refrigerator car loadings are less than they were last year. In 1957, 74% of the fruit went by rail and 26% by truck, in 1958 60% went by rail and 40% by truck. The performance of long distance trucking is very much the same as stated in the 1958 report. There is much room for improvement before truck performance equals car performance.

An outline of minimum trailer qualifications and specifications for long distance trucking was drawn up as a result of jobber

TRANSPORTATION PROBLEMS

Many of the transportation problems involve cooperative work with other departments, particularly the Equipment Department and the Perishable Freight Inspection Department.

Alcohol Heaters

Due to the economic situation of carlines and railroads there was very little increase in the total number of alcohol heaters available for banana use. However, our Perishable Freight Inspectors are becoming more familiar with them and are using them to a greater degree than previously. Some difficulty has arisen in the use of the thermostat. After investigating the problem it was determined that the thermostat would have to be used as a modulating thermostat, since there is a considerable lag between air temperature and pulp temperature and thermostat setting. The direction in which the pulp temperatures are progressing would also determine where to set the thermostat.

We are now attempting to devise a formula which would guide the Perishable Freight Inspectors on the selection of thermostat setting. A statistical study is necessary to devise the formula. Although there were a few alcohol heater failures, none has been of a serious nature. The new mixed fuel of 2/3 methanol and 1/3 isopropanol is apparently working very well. We have not received any complaints attributable to this type fuel.

Propane Heaters

We have continued to work with manufacturers of propane heaters for transit heating of cars and trucks. One manufacturer we are working with has made a commercial shipment of bananas with a propane heater in refrigerator cars. Although the results were favorable in the test, the test was run under conditions from which no real conclusions can be drawn. This test was actually performed using competitive fruit.

During the year we have had several contacts with the manufacturers of the system known as the Preco Cargotemp method of temperature controlled shipments. However, none of the carlines has purchased this system for regular RS cars except for the prototypes.

During the year, this system was modified and shows great promise of being widely adopted by the railroad and carlines in an attempt to fill the gap between the continued increasing demand for mechanical cars and the regular RS type cars. This is mainly due to economics. The mechanically refrigerated car costs $26,000., over twice as much as a standard refrigerator car.

The Preco Cargotemp system has been modified by eliminating one of the end bunkers and including all of the air circulating fans in the opposite bunker. This provides much more loading area in the standard 40 foot car, and allows a shipper to take advantage of new incentive rates for increased loading.

Banding at retail store level is increasing at a faster rate than all other types of warehouse packaging combined. Pressure sensitive tape is sometimes employed because it is relatively easy to apply. Banding at store level is neither recommended nor discouraged. It is not recommended for the following reasons:

1. Banding at store level is more costly than warehouse banding because it is an inefficient operation accomplished without the production line methods available at the warehouse.

2. It is impossible to identify with our trademark.

3. Store level banding is performed on a spare time basis consequently during peak sales on Thursdays, Fridays and Saturdays when store personnel have a minimum of spare time, only a small percentage of fruit can be banded.

4. Fruit banded at store level is more likely to be bruised because of additional handling and because proper facilities and padded surfaces are not available.

The fact that banding is increasing at the retail level indicates that merchandising personnel are aware of its inherent advantages and would, therefore, purchase prepackaged fruit from their supplier if an acceptable package could be delivered to the store at a reasonable cost.

Banding at the warehouse level is not receiving more widespread acceptance due to the lack of consistently high quality fruit and due to present high labor costs.

Automatic machinery reduces the cost of actual package operation, but the cost of sectionizing the hands and grading the fruit is dependent on labor rates. When the high quality fruit required for packaging is segregrated, the remainder of the fruit on the average stem is downgraded. Thus the packaged fruit must be increased in price to compensate for the decrease in value of the downgraded fruit. If we ever box fruit in the tropics some thought should be given to retail units.

check the origin of the twine in question.

Some complaints of wrong applications of string have been received and we have noted this condition particularly on Dominican and Ecuador fruit. In the case of Ecuador we noted that, together with short tips, placement of string under the top hand was contributing to the high incidence of tip rot.

POLYETHYLENE WRAPPED FRUIT

During the early part of the year and in recent years, as we reported in the third quarter, the incidence of scarring was becoming increasingly serious. In the last quarter this condition has been somewhat improved. Mutilation of fingers is still quite serious on most varieties and is contributing to the latex problem.

During the year experimental shipments of locally extruded polyethylene have been received from Colombia, Guatemala, Chiriqui and Ecuador. At the present time locally produced film is available to all company areas except Honduras and Costa Rica. There were but two instances of defective film reported during the year. For the most part the quality and visibility of the film have been excellent. The light weight (1 mil) film has increased the visibility and greatly lowered the cost.

RETAIL PREPACKAGING

Greater interest is being shown in prepackaged bananas, but few new jobbers have undertaken this activity. More interest is evident at the store level than at the jobber level.

One large Eastern jobber is planning to increase his output of prepackaged fruit (clusters of fruit in trays with cellophane overwrap) from approximately 25% to 33% of total sales. During the month of January 1960 the jobber expects to move into a warehouse now under construction and the Jobber Service Department is presently designing a packaging layout capable of handling the increase noted above. The system is designed so that 50% of the total output can be packaged by adding another carton-forming machine and overwrap machine which the jobber is planning to purchase.

An independent jobber in the New York metropolitan area is making preliminary studies of packaging methods, equipment, and costs. There is a definite possibility that he may package clusters of fruit in cellophane overwrapped trays. He is now packing loose fingers in a window-type overwrapped carton.

One supermarket chain is packaging almost 100% of their output at the warehouse in polyethylene bags. An independent jobber has requested and received permission to use the "Chiquita" label on polyethylene bags and one jobber has been given permission to use our trademark on a retail carton-type package.

and to fill the wirebound crates to their 40 lb. capacities was responsible for the failure of these tomatoes to meet U. S. grade requirements. The chief defects reported were "puffy," "misshapen" fruits and "severe growth cracks" on some tomatoes. The affected fruits were not numerous and easily could have been thrown out. This would have improved the pack very greatly.

The quality of the tomatoes was generally considered excellent and even superior to the winter or early spring tomatoes on the northern market. It would seem for this reason that Dominican tomatoes properly graded and shipped to New York or Baltimore should do very well.

STALK TREATMENT

Our Third Quarter Report - 1959 included a brief resume of the stalk rot sanitation program since its inauguration in 1950. Attention to this program this year was stressed because a greater than usual incidence of Thielaviopsis rot was persisting in Pacific cargoes.

The treatment with P.E.P.S. paste on the butt and tip ends of the stalks gave satisfactory control on fruit from all divisions except Ecuador. While the degree of control has usually been less on Pacific fruit than on Central American fruit the difference has been insignificant.

Earlier in the year rather severe instances of stalk rot were reported on Ecuador cargoes discharging at West Coast ports. Investigations indicated that the rot continued to progress in the ripening rooms, but that the greatest loss due to Thielaviopsis rot was in top hand fruit. Thielaviopsis rot was also occurring on middle hands and bottom hands due almost entirely to mutilated fingers, broken hands and split crowns. Although butt rot was often found to be present after ripening (up to 50% of bunches affected) it had seldom progressed up the stalk to the first hand. Even in the case of the top hands much of the damage could be attributed directly to broken crowns and torn fingers. Short tips were found to be responsible for much of the top hand damage together with the misapplication of the string under the top hand. Obviously these conditions are not helped by P.E.P.S. application.

We recommended in July, and steps were taken immediately, to transfer spraying equipment in storage in Mobile to Seattle where it could be used to spray railroad cars used for long-distance rail shipments. At the same time we suggested that attention be given in Ecuador to trimming the tips and applying the string properly.

BANANA STRINGING

Locally produced stringing twine is being supplied in Guatemala, Costa Rica and Colombia. Other divisions are being supplied with Mexican twine. In general the twine has been satisfactory. Some complaints of twisting have been received but we have not yet been able to

that 4% was found on one shipment of blue marked polyethylene bags. In the previous nine shipments, the red lots averaged 2.8% and the blues 5.8% ripe and turning. No evidence of Chalcid wasp injury was noted in any of these subsequent red or blue marked shipments.

Santa Rosa Farm Experiment

Simultaneously with the above four additional shipments of treated stems and control stems, designated as brown or yellow marked bags, were shipped to Baltimore from Santa Rosa farm.

The ripe and turning percentages of the brown lots averaged 1.7% whereas the yellow lots averaged 2.4% ripe and turning. These results are not consistent with those of the previous nine shipments in which the brown lots averaged 6.1% and the yellow lots 2.8%. Differences in grade between these two lots were negligible. Chalcid wasp injury was fairly heavy on the yellow lots, averaging 10% as compared to an average 2.3% on the brown lots.

Klein Experiments

A special shipment of 261 stems labeled "Klein experiment" was discharged with a Fortuna cargo ex Santo Cerro-14 at Baltimore on November 2, 1959. This shipment announced the beginning of a new series of experiments from La Lima. The lots in this experiment were designated black and white marked bags. However, a subsequent shipment labeled "Klein fruit" in a Fortuna cargo ex Morazan, New Orleans, November 9, 1959 was marked in red and blue lots. We have not been told what the color codes mean nor have we been certain always as to which colors stand for the Klein experiment. A further example of the confusion existing at this end over the color designations may be seen in the shipment of a Fortuna cargo ex Santo Cerro-15, Baltimore, November 25, 1959. Eighty-five stems of this experimental fruit were marked with red or blue stripes and 155 other stems were marked with green or white tape on the butts.

In the cablegram from La Lima mention was made of an Agronomy experiment but no specific mention was made as to which colors represented the Klein experiment lots. Consequently, any descriptions of the belt checks on this experimental fruit must be given on an individual shipment basis rather than collectively, due to the color scheme confusion.

Reports on each of these shipments have been forwarded to Dr. Thornton in La Lima.

DOMINICAN TOMATOES

This project is discussed in greater detail in the Third Quarter Report - 1959.

Between February 16 and April 17, 1959 six shipments of tomatoes from the Dominican Republic were received and sold by the Fruit Dispatch Company. Inability to pack a uniform size and grade

OBSERVATIONS DURING COMMERCIAL RIPENING

For the fifth successive year special weekly reports have been made on the ripening behavior of the fruit. These reports were based on visits to jobbers' plants in the New York area. Through November 239 different lots of first-class fruit have been examined, mostly at the turning or hard-ripe stage, in course of boxing for delivery to the retail stores. Ninety-five of these lots have been Chiriqui, 34 Golfito, 29 Honduras and 26 West Coast Guatemala. Forty-one lots have been Ecuador, of which 28 have been competitive and 13 company fruit. Except for Changuinola, which has not been represented, the 14 remaining lots have included at least two or three examples of the other regular varieties.

Chiriqui has again been the most popular variety with the New York trade, exceeding all others in appearance and general excellence. Honduras ranked second in jobber appeal, despite a temporary lapse in March because of scarring, breakage and small top hands. Guatemala (West Coast) ranked third in popularity over the first five months but has seldom been available for observation in local rooms since that time. Condition was generally good but quality and appearance were frequently no better than fair. Golfito came fourth. This variety was briefly popular in March but has lost ground since because of inferior appearance attributable to scarring, "dirtiness," maturity stain and stem-end rot. Competitive Ecuador came next in line. Condition and quality have been quite good for this variety. Since February, however, appearance has become poorer. Company Ecuador has ranked somewhat inferior in quality and appearance to corresponding competitive fruit.

There remain four minor varieties too sparsely represented for definite ranking. Competitive Golden Beauty was seen on four occasions in September and October. With those jobbers who customarily handled it, this variety was seemingly quite popular. At its best it has reasonably good quality and fine color. Only two samples of Guatemala (East Coast) were seen in jobbers' rooms, one in April and one in May. This was quite good in all respects. Dominican fruit has been examined only five times, all prior to July. Condition and quality were quite good but appearance mostly fair to poor. Jobbers complained of "dirtiness," waste, "spots" (thrips) and "scraggliness." Santa Marta was seen only three times, all in February. Condition and quality were only fair. Appearance and jobber appeal were poor in these cases.

SPECIAL SHIPMENTS

Oil vs. Bordeaux

See the Third Quarter Report - 1959 for detailed descriptions of previous experiments from Mopala farm and Santa Rosa farm, Honduras.

As a continuation of this series, three additional shipments of oil vs. Bordeaux treated stems were sent to Baltimore from Mopala farm during the period from August 19 through September 28, 1959. No ripe and turnings were reported on any of the three shipments except

The percentage of wilted fruit has been rather formidable, ranging from 21% in February to 40% in July, after which it has gradually tapered off. Appearance has been mediocre with more than the normal amount of scarring, latex stain, maturity stain, tip rot and butt rot. Beginning with April, there has also been considerable stem-end rot of both the Santa Marta and the Thielaviopsis type.

Santa Marta cargoes were available for the first four months only. In February and March, when the grade was fairly high, ripe and turning fruit averaged 24% and 20% respectively. Bunch weights averaged from 60 lbs. to 65 lbs. In January and April, when the grade was rather light, it averaged 15% and 5% respectively. Appearance was rather poor, with heavy scarring and considerable leaf scar, speckling and Santa Marta type stem-end rot.

Changuinola was rather light in grade in March and May, fairly normal throughout the remainder of the year, about the same as for Chiriqui fruit. In January and February the ripe and turning and wilted percentages were rather high; otherwise they have been reasonable. Average bunch weights have ranged from 70 lbs. in February, April and May to 79 lbs. in October and November. Appearance has been about average.

Lacatan cargoes have been rather limited - one in January, four in February and three in April. Heavy grade in the January shipment was reflected in an excessive percentage of ripe and turning fruit (31%). Otherwise condition was fairly normal. Average bunch weights ranged from 65 lbs. to 74 lbs. Appearance was rather poor with the heaviest scarring for any variety and appreciable percentages of leaf scar, speckling and latex stain.

West Coast Guatemala was in rather limited supply from June to December; in August and September there were no shipments whatever. Although it was consistently reported light in grade except in October and November, the January and February cargoes averaged 13% and 15% ripe and turning respectively. Thereafter condition was not too bad. Average bunch weight ranged from 67 lbs. in March to 80 lbs. in November. Appearance was rather poor, with considerable scarring and latex stain.

East Coast Guatemala also showed excessive ripe and turning fruit in conjunction with a lighter than average grade during the first two months of 1959. Thereafter the grade was reported a little heavier, but nevertheless the percentage of ripe and turning fruit tapered off. Average bunch weights ranged from 68 lbs. in January and May to 75 lbs. in August. Appearance was rather better than average.

Dominican fruit was in supply during the first nine months of 1959. During most of this time it averaged heavier in grade than any other variety in regular supply. Because of the relatively short fingers, however, and the small number of hands per stem, monthly average bunch weight ranged from a maximum of only 68 lbs. to as low as 55 lbs. Ripe and turning ranged from 18% in January to 1% in May. Appearance was poor, with average scarring but considerable latex stain, thrips injury and Santa Marta type stem-end rot.

CARGO OUTTURN REPORTS

For the ninth consecutive year this department has maintained a systematic monthly record of cargo inspection data for each variety imported. Until May 1, 1959, the necessary information had been obtained from P.F.I. Form 101-A, now discontinued. Since that time it has come from the Wharf Department figures.

Chiriqui again has been the most acceptable variety throughout the year. In general it has attained a more uniform grade than any of the others. From January to March and from May to June inclusive, the average bunch weight equalled or exceeded 80 lbs. Only in August did it sink to as low as 75 lbs. The incidence of ripe and turning fruit has been closely correlated with grade and bunch weight.

Month	Grade (Index*)	Bunch Weight	Ripe & Turning
February	- 14	83 lbs.	10%
August	- 22	75 lbs.	3%

With regard to scarring and appearance in general, Chiriqui has averaged about as clean as any other major variety.

Honduras started the year considerably lighter, both in grade and in average bunch weight, than Chiriqui. By April it had caught up in grade; by June it had caught up in bunch weight; by July it had exceeded Chiriqui in both respects. Except in September it has maintained that advantage ever since. This improvement has been offset by a corresponding increase in ripe and turning fruit, ranging from 4% in January to 9% in July and 15% in October. One October cargo actually showed 74% ripe and turning. In general appearance Honduras did not suffer by comparison with Chiriqui.

Golfito started the year with a grade about equivalent to that of Chiriqui, but six pounds lighter in average bunch weight. For the first few months it declined more rapidly than Chiriqui in both of these respects, with a corresponding decrease in percentage of ripe and turning fruit. In May Golfito average weight was 67 lbs. compared to 80 lbs. for Chiriqui. About the first of June, however, both grade and bunch weight began a slow upward climb, culminating in an October spurt that sent bunch weights into the 80's accompanied by excessive percentages of ripe and turning. Much of the heavier grade fruit has since been eliminated from shipments of this variety but still it does not carry as well as it should. In most cases it has been somewhat more heavily scarred than Chiriqui fruit. During the latter half of the year maturity stain and Chalcid wasp injury have also detracted from its general appearance.

Ecuador started out with the lightest grade of any variety, and tended to become lighter still as the year went on. Average bunch weights have ranged from 74 lbs. in March and April to 64 lbs. from August through October and 63 lbs. in November. Through April the percentages of ripe and turning fruit were rather low. Beginning with May, however, although the grade was reported light, the monthly percentages of ripe and turning fruit were 9%, 15%, 15% and 12% in the order named. From September on, they have averaged from 6% to 7%.

* Index = F$\frac{3}{4}$ =0, F= plus 100, LF$\frac{3}{4}$ = 33.3, $\frac{3}{4}$ = -66.7, thin = - 100

Chiriqui and Golfito fruit ex Comayagua-6 were used also in the third test. Exposure time to ship conditions was 223 hours for Golfito and 201 hours for Chiriqui. No underpeel discoloration was noted at 320 and 298 hours when the simulated period was ended, but was evident on over half of the stems after 72 hours of ripening temperatures.

Microscopic Examination of Underpeel Discoloration

Microscopic comparisons were made of the damage to the peel structure occurring when underpeel discoloration was present at the time of discharge, when induced by a simulated extension of the ship voyage; also after fruit had been exposed to chilling temperatures (40 - 42°) for varying lengths of time. To date these studies seem to indicate that underpeel discolcration, incidental to mild cases of chilling, is indistinguishable from similar discoloration evident at discharge of certain cargoes or brought about by a simulated prolongation of the voyage period.

Miscroscopic examination of fruit, which had been exposed to 53-54° and showed medium underpeel discoloration, revealed that the contents of the latex tubes only had been affected.

Most commonly the latex globules had disappeared from solution and were replaced by fewer, larger, grayish, roughly spherical granules clustered in one end of the cells. Less frequently the entire contents of some cells coagulated into an orange colored amorphous mass.

Examination of fruit exposed to chilling temperatures (40-42°) for 18 hours showed a similar condition, except that some disintegration, but no discoloration of the plastids in parenchymatous cells was also noted. Longer exposures up to 42 hours resulted in the complete plugging of the latex tubes with a yellowish gel which contained clusters of coarse, grayish granules.

ranging from 800 to 4000 stems each. Outturns were as follows: ripes averaged 5.7%; turnings averaged 5.6%; and weight of nines averaged 68.9 pounds.

In all cases reports from interior jobbers indicate that the Lacatan fruit attained a fairly attractive color and stood up fairly well in the retail stores. In other respects the picture is not so good. Scarring and stem-end rot were quite severe. Shrinkage was said to be unduly heavy, although no specific figures were given.

UNDERPEEL DISCOLORATION

A summary of our observations and tests relating to underpeel discoloration was given in the Third Quarter Report. A resume of the test results is given below. We have confined our discussions of our observations of underpeel discoloration to the Chiriqui - Golfito cargoes discharged at Weehawken. We feel that by so doing we eliminate many of the variables involved. During the year there has been some underpeel discoloration reported in other cargoes. In no case, however, was this of a serious nature.

Of the Chiriqui - Golfito cargoes scheduled for Monday discharge at Weehawken through November 15th this year 14 were carried at 54° and were reported free of underpeel discoloration; 28 were carried at 53°, of which only 9 were free and 11 were affected. During this year only 4 cargoes were carried at 52° and all were affected.

During the period between August and the middle of November ten Thursday-Friday scheduled cargoes were discharged of which 5 out of 9 carried at 53° showed underpeel discoloration. One carried at 54° was not affected.

Tests to Induce Underpeel Discoloration

Three tests were conducted to induce underpeel discoloration by simulated extension of regular voyage time. All of the fruit selected for these tests was free of underpeel discoloration when moved to ripening rooms. Ship carrying and test room temperatures were 54°.

In the first test the fruit was Fortuna ex Tivives-6 which had been exposed to ship conditions for 134 hours. No indication of underpeel discoloration was noted on the fruit after extension of the exposure time to 220 hours. Also no evidence of this problem was noted after 287 hours. After ripening, however, all of the fruit was decidedly off-color and all showed varying degrees of underpeel discoloration.

The second test was conducted with Golfito and Chiriqui fruit ex Junior-6 which had been shipped at 54°. Golfito had been exposed to ship conditions for 218 hours, and Chiriqui exposed for 197 hours. Golfito showed no underpeel discoloration after 322 hours total exposure time.

Chiriqui also did not show evidence of this problem after 301 hours total elapsed time. However, underpeel discoloration did appear after the fruit had been subject to ripening temperatures for 67 hours and had reached color-3.

Perhaps the most outstanding single characteristic of these Tacana Sport stems was the large sturdy stalk. The average number of hands per stem increased from 10.5 to 11.9 at the end of March and then decreased to about 11 in April. Average weights followed closely the same trend. The average weight of the individual lots ranged from 70.8 pounds to 89.9 pounds.

New York reports indicate by the uniformity of the average weight per hand that finger size was uniform and medium. These reports also show that ripening shrinkage was high (16.4%) due to heavy stalks (12%). Scarring was heavy on all shipments and stem-end rot was severe on the open-handed stems. Misshapen hands, which at first seemed to be characteristic, were of minor importance in the recent series of shipments in which four stems with misshapen hands were noted. Oversized stalks, however, do seem to be characteristic. This is a disadvantage from the shrinkage point of view but may indicate a more sturdy plant which, in addition to its dwarf character, may make it more blowdown resistant.

Media Mata

A more detailed description of this variety has been given in the Third Quarter Report - 1959. Media Mata is a dwarf variety of bananas apparently native in the foothill regions of the Dominican Republic. The plant grows to about half the height of Gros Michel, hence the name. It is believed locally to be resistant to Panama Disease. It is said to resemble the Dwarf Cavendish but the fruit, in some respects, more nearly resembles the Lacatan. It seems possible that it may be a dwarf Lacatan. Between April and August 1959 some Media Mata bananas were regularly included in the Dominican cargoes to U. S. Ports, as well as at least two shipments which were made to England. Media Mata shipments totaling 3000 stems have been included twelve times in U. S. cargoes. The number of stems per shipment ranged from 57 to 504. The nines weighed 4 to 12 pounds heavier on the average than the average weight of nines in the same Dominican cargo. Slightly more hands per stem seem to account for this fact. Very limited data indicate that this variety tends to have shorter, plumper fingers of somewhat smaller but more uniform size than Gros Michel. Finger tips are well filled which tends to offset short-fingeredness as regards finger weight. Stalk diameters and stalk weights of Media Mata are less than even Dominican Gros Michel, but rather more comparable to Santa Marta.

Plump fingers tend to have long pedicels while the crowns are small due to the slender stalks which cause finger crowding in the hand arrangement. English reports have mentioned stem-end rot in this variety even though it has not been noted in most domestic shipments. Media Mata seems to resemble both the Cavendish and particularly the Lacatan in this characteristic. There have been rather strong indications that this variety, as Lacatan, is more sensitive to low temperatures than Gros Michel. While palatability and flavor are matters of personal preference, some indications are that Media Mata should not suffer on this score.

Lacatan Bananas

The Third Quarter Report - 1959 and Annual Report - 1958 present a more thorough coverage of this subject. Commercial scale shipments of Lacatan fruit, begun in the Spring of 1958, were continued on a gradually decreasing scale into 1959. There have been eight such shipments altogether,

A series of six special shipments of Cocos fruit in Fortuna cargoes were discharged at Baltimore between September 14 and November 2, 1959. During this period the ripes and turnings of Cocos ranged from 2.5% (Sept.) to 15% (Nov.). However, the ripes and turnings of the regular Fortuna cargoes were consistently higher during this same period, ranging from 3.5% (Sept.) to 21.5% (Nov.). The grade of Cocos was comparable to Fortuna during the period since the grade of both varieties fluctuated about equally between heavy and light for all six cargo outturns. Cocos showed less average open-handedness (5%) than did Fortuna (16.3%) for the six outturns. Short-fingeredness also ran lighter on the average for Cocos (1%) than it did for Fortuna (2.2%). Average weight of Cocos stems (81 lbs.) was heavier than the average weight of Fortuna stems (77 lbs.) during the period.

Severe scarring was also lighter on Cocos than on Fortuna. Cocos showed a heavier average percentage of latex stain during the last three cargo outturns than did Fortuna and there were more broken bunches of Cocos. Underpeel discoloration was reported on both the Cocos and Fortuna in the last cargo but it was more severe on the Fortuna.

Cocos Shipments - Chiriqui Cargoes

Over the period from September 14 to November 16, 1959 a series of eight special shipments of Cocos fruit were included in regular Chiriqui cargoes which were discharged at Weehawken, New Jersey. In addition, one cargo, originally destined for Weehawken, was diverted to New Orleans due to a longshoremen's strike but no inspections were made on the Cocos fruit. The first six of these shipments included a large proportion of plantilla fruit; the seventh and eighth consisted exclusively of second and third growth fruit.

The Cocos in the first six shipments was somewhat lighter in grade than the regular Chiriqui fruit. There was also two and one half times as much ripe and turning fruit as in the regular cargoes. The Cocos shipments also included much more very short-fingered fruit, as well as three times as much very open-handed fruit. Cocos was also much more subject to breakage and it weighed slightly less per stem than Chiriqui.

The Cocos stems in the seventh and eighth shipments continued somewhat lighter in grade and included even more very short-fingered fruit. These later shipments included not three but five times as much very open-handed fruit as the first six shipments. The Cocos still seemed more subject to breakage than the regular Chiriqui fruit.

While most of the foregoing comparisons must necessarily be considered unfavorable to the Cocos variety, many of the differences uncovered are matters of degree only. A high proportion of the initial six shipments consisted of plantilla fruit and many of their worst features-short-fingeredness, open-handedness and excessively heavy stalks-are common faults of such fruit. The seventh and eighth shipments which included only second and third growth Cocos fruit, comparatively speaking seemed no better than, or even slightly inferior to, the plantilla fruit.

Tacana Sport

No shipments of the Tacana Sport variety have been received since last reported in the Third Quarter Report - 1959.