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STUDIES ON COFFEE ROOT DISEASE IN PUERTO RICO

I. A Coffee Fusarium Wilt

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INTRODUCTION

Among the numerous diseases of coffee (*Coffea arabica* L.) reported throughout the coffee-growing areas of the world, those causing root troubles are the least understood. Very little research, comparatively speaking, has been conducted on these root maladies and in many instances no experimental evidence has been presented in the literature as to the pathogenicity of the organisms reported associated with them. In the case of the *Rosellinia* or black rot, primary attention has been paid to mycological details. Most of the information gathered in regard to this malady is

substratum and under a high relative humidity, symptoms of damping-off were predominant. The lower part of the stem near the substratum level at first became water-soaked, later turned dark and soft, and finally deteriorated. The pathogen developed a vigorous growth in the affected tissues and ascended the stems up to the leaves; in some cases the latter also being invaded.

Most of the seedlings showing damping-off wilted even before the yellowing symptoms developed. When affected seedlings were pulled up they disclosed an advanced deterioration of the cortex tissues. Affected roots were tinged black. Internal symptoms of the disease included the necrosis of vascular elements, particularly the xylem vessels. Some of these vessels were enlarged as compared with healthy ones, and some were filled with an ochreous substance. No indication of mycelial penetration was observed in such vascular elements. The roots showed a similar symptomatology.

MATERIALS AND METHODS

Isolates.

Isolations were made by the writer in July 1939, from diseased coffee trees which were found at Mr. A. Sastre's farm in Utuado, and which showed the characteristic symptoms of black rot. The usual precautions with respect to the technique of isolation were taken. A *Verticillium* sp. was repeatedly isolated from the bark of diseased trees. Morphologically, this fungus resembles *V. lycopersicii* Pritchard.

In making isolations from the innermost tissues of the wood of stem and roots the following technique was followed: the cortex was peeled off and the exposed woody tissue with its characteristic vascular strands was flamed. From the deeper, unburnt tissues of the wood, pieces of infected material were cut off and placed on potato dextrose agar. After a week or two a slow growing fungus developed in many of the plates. A microscopic examination revealed it to be a *Fusarium* sp. belonging to the section Elegans. This Fusarium resembles morphologically *F. bulbigenum* var. *batatatis*, as pointed out by Dr. Sherbakoff, to whom the organism was sent for identification. This Fusarium was found, as will be shown later in this work, to be responsible for coffee black rot and wilt. A species of *Fusarium Solani* occurred as a contaminant. See fig. 1.

Growing coffee seedlings

In growing coffee seedlings for our experimental work, efforts were made to avoid natural contaminations and infections from root rot agents. Fresh coffee seeds were surface disinfected with a 1 to 1000 mercuric bichloride solution and then rinsed throughly in distilled water to remove

the toxic salt. The treated seeds were left soaking overnight in distilled water, and then sown on flats in soil sterilized for four hours at 15 pounds pressure, then left for a week. The seed started germinating within a month. All coffee seedlings have been kept growing under a lath half

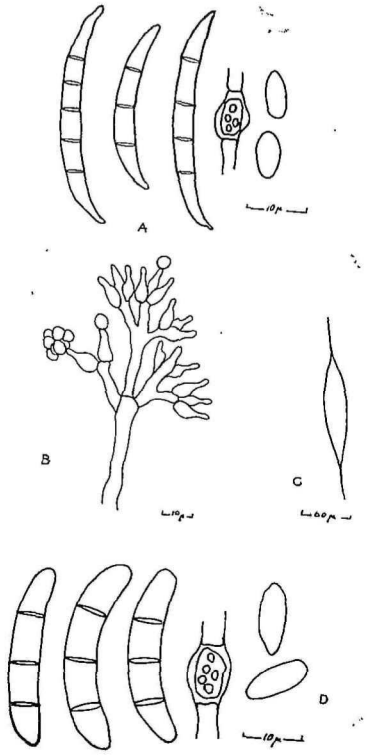

FIG. 1. Camara lucida drawings of isolates. A. Conidia and chlamydospore of *Fusarium bulbigenum* var. *coffeae* grown on potato-dextrose-agar; B. Conidiophore and conidia of *Verticillium* sp. grown on potato-dextrose-agar; C. Conidia and chlamydospore of *Fusarium Solani* sp. grown on potato-dextrose-agar; D. Ascospore of *Rosellinia bunodes* from perithecium found on dead coffee wood.

shade and are watered regularly with boiled or sterile water. In no instance have symptoms or signs of disease been observed among the seedlings.

METHOD OF INOCULATION

Our repeated trials for inducing coffee black rot by planting coffee seedlings in naturally contaminated soil met with failure. The new isolates, as well as the *R. bunodes*, were tested for pathogenicity using Wellman's (75) technique for "studying host resistance and pathogenicity in

tomato fusarium wilt." The method consists in immersing recently pulled seedlings, without particular care for root pruning, in a mycelial and spore suspension of the inoculum, before transplanting to sand cultures.

PREPARATION OF THE INOCULUM

Coon's solution was found satisfactory for the growth of the isolates as well as for the development of coffee seedlings; consequently, it was decided to use it in our work (fig. 2.) The medium consists of: saccharose, 7.2 grams; dextrose, 3.6 grams; potassium nitrate, 2.02 grams; magnesium sulphate, 1.23 grams; potassium acid phosphate, 2.12 grams; and water, 1 liter. Electrometric determination of the pH of the solution showed it to be 4.53.

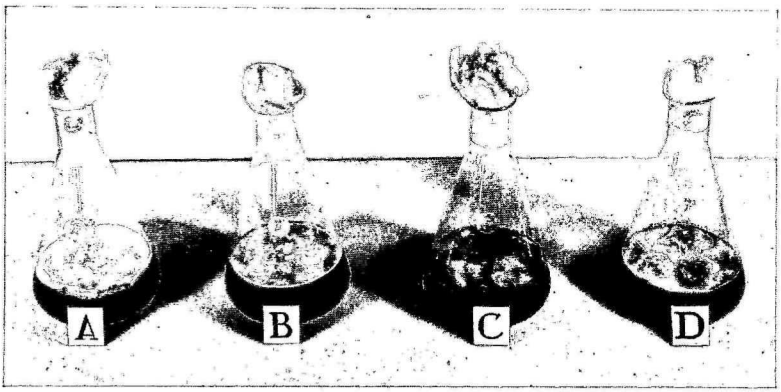

FIG. 2. Coffee root isolates growing in Coon's solution at a temperature of 28°C. A. *Fusarium bulbigenum* v-*coffeae*; B. *F. solani*; C. *Rosellinia bunodes*; D. *Verticillium* sp.

One hundred cubic centimeters of the solution were poured into 250 cc. flasks and autoclaved for thirty minutes at 15 pounds pressure. Cultures of the isolates were made by seeding the sterile solution with pieces of agar cultures of the different organisms. The cultures were agitated twice during the second day and thereafter left undisturbed. They were incubated at 28°C. for 5 days.

In the preparation of the inoculum, care was taken to decant the liquid from each culture. The mycelial mat was then transferred to another flask containing 100 cc. of fresh, sterile Coon's solution. The mat was well shaken to break it up into small fragments. One hundred cc. of the inoculum were found sufficient to inoculate 100 seedlings.

EXPERIMENTAL RESULTS: THE PATHOGEN; CULTURAL CHARACTERS

Fusarium bulbigenum Cke. et. Mass. v. *coffeae* v. n. This organism was found to be the cause of a coffee wilt and black rot in Puerto Rico.

It resembles, morphologically, the fungus *F. batatatis* var. *vanilla*, reported by Tucker (72) as the pathogen responsible for vanilla wilt in Puerto Rico. Tucker has shown that the vanilla wilt parasite is not pathogenic to coffee.

Our work has demonstrated that the *Fusarium* causing the coffee wilt and black rot is not pathogenic to vanilla.

The coffee wilt Fusarium grew well in many kinds of culture media.

On potato dextrose agar the mycelium grew abundantly, was white at first and turned with age to reddish or vinaceous. Chlamydospores were produced in old cultures. Macroconidia were abundant, produced in white or colored sporodochia, sickle-shaped, pedicellate, slightly constricted at the septa and densely granular. They were generally three-septate, and varied in length from 18 to 36 by 3.0 to 4.0μ in width. Two, four and five-septate spores were less frequently produced. Microconidia, generally non-septate, were produced in false heads.

On potato plugs the mycelium was white, abundant, and constricted at the septa, later forming either terminal or intercalary chlamydospores, the latter measuring from 5 to 12μ by 6 to 12μ. The substratum was not changed in color. Macroconidia were formed abundantly, and were granular, pedicellate, slightly constricted or not. The spores were rather large and with one or more intercalary chlamydospores when old; they were generally three or four-septate and sometimes five-septate. Three-septate spores measured from 30 to 42μ by 3.0 to 3.7μ, four septate spores from 30 to 45μ by 3.0 to 3.7μ, and five-septate spores from 33 to 45 by 3.0 to 3.5μ. Microconidia were abundant, the great majority being non-septate and from 6 to 15 by 3.0 to 3.5μ.

On 5 per cent dextrose agar the mycelium grew rapidly changing with age to a reddish or vinaceous color. No chlamydospores were observed in cultures fifteen days old. Macroconidia are borne in sporodochia or in false heads, abundantly three-septate, sickle-shaped, densely granular, pedicellate and measuring from 16.75 to 33μ by 3.0 to 3.5μ. One, two, four, five and six-septate spores were relatively few. Non-septate microconidia were abundant and measured from 3 to 12 by 2.0 to 3.0μ.

On steamed rice the aerial growth was white. The substratum changed gradually to reddish or vinaceous, particularly at the bottom of the flask. In older cultures the mycelium turned a deep blue. Macrospores were born abundantly on reddish or white sporodochia, generally three-septate, with a few six-septate spores. Three-septate spores measured from 30 to 45 by 3.0 to 3.5μ, four-septate spores from 36 to 45 by 3.0 to 3.5μ. A pleasant aroma was emitted by the cultures.

On oatmeal agar the mycelium was less abundant, with abundant chlamydospores intercalary or terminal, single or in chains, the larger measuring from 6 to 12μ by 6 to 12μ, thick-walled, densely granular.

Microconidia were produced in salmon-colored masses or were brown in sporodochia at the bottom of the slant, where the substratum was tinged slightly with lilac. Abundant three-septate macroconidia were produced and measured from 18 to 39μ by 3.0 to 3.5μ. Few four-septate spores were formed. These, as well as other cultures studied, were incubated at 28°C.

Fusarium bulbigenum Cke. et Mass. v. *coffeae* v. n.

"Macroconidiis is sporodochiis salmonis colore instratis, 3–6 septatis, falcatis, pedicellatis, 3-septatis: 16.75–45.0 x 3.0–3.7 μ, microconidiis 6–12 x 2.0–3.5 μ. Chlamydosporis terminalibus it intercalaribus, singularis vel catenulatis."

Macroconidia sickle-shaped, hyaline, pedicellate, not constricted when young, usually three-septate and measuring from 16.75 to 45 μ by 3.0 to 3.7 μ. Occasionally one and two-septate conidia formed. Four and five or six-septate conidia produced. On potato plugs four-septate conidia were relatively abundant. Microconidia abundantly non-septate, oval or ellipsoidal, measuring from 6 to 12 by 2.0 to 3.5 μ. Chlamydospores formed in old cultures either terminal or intercalary, single or in chains. Substratum in oatmeal agar tinged lilaceous; in potato dextrose agar reddish to vinaceous to bluish. Spores formed in salmon colored masses or on sporodochia. Sporodochia either white or tinged; observed in potato-dextrose agar, oatmeal-agar and steamed rice cultures.

Growth temperature relations

The relation between growth of the isolate and temperature was observed in order to provide further elucidation concerning the effect of the environment on the development of coffee wilt and black rot. Five millimeter disks containing mycelium from each respective isolate were removed with a sharp-edged steel tube from five-day-old cultures of the fungi grown on potato dextrose agar. The cultures were kept at the temperature at which the growth rate was to be determined. The mycelial disks were cut just back of the growing point of each colony so as to describe a concentric circle. In this manner the greatest uniformity is secured not only in size, shape and age, but also in the amount of mycelium taken. Petri dishes containing 15 cc. of 2 per cent dextrose agar, of pH 6.14, were planted with the respective mycelial disks of the islate. The disks were placed in the center of the medium. Triplicates of each isolate were immediately incubated at temperatures of from 10 to 36°C. Measurements of daily increments of radial growth were taken, as well as the total growth in a period of seven days. The results are shown in table 1.

It may be seen that the pathogen is benefited, as far as development is concerned, by high temperatures. The limits for optimum growth are from 24° to 30°C. This temperature range is more or less that prevalent in Puerto Rico.

TABLE 1

Seven-day growth temperature relation of culture of the isolates grown on potato dextrose agar, pH 6.14. Average daily increment in cm. of three cultures in each treatment

Degrees C.	Average Daily Growth in Cm. of F. bulbigenum var. coffeae
10	0.20
12	0.37
16	0.46
20	0.85
24	1.24
26	1.45
28	1.45
30	1.24
32	0.70
34	0.39
36	0.00

Relation between hydrogen-ion concentration of the substratum and growth

The growth of the pathogen on a substratum with varying pH values was considered of interest, not only from the point of view of the physiologic phenomenon itself, but also with respect to the relation between its behavior at each pH value and the development of the disease.

TABLE 2

Average daily increment of growth of isolates when grown on 2 per cent potato dextrose agar for seven days at 28°C., at different initial pH values of the substratum. Growth in cm.

Initial pH	Average Daily Growth in Cm. of F. bulbigenum var. coffeae
3.51	0.64
4.00	0.94
5.12	1.20
6.14	1.45
6.47	1.20
7.32	1.21

These studies, as well as others conducted in our work, have been made with virulent and monosporial cultures of the pathogen.

Petri dishes with 15 cc. of 2 per cent potato dextrose agar medium, adjusted to varying pH values either by the addition of the necessary amounts of dilute sodium hydroxide or hydrochloric acid solutions, were

seeded as previously explained, with five mm. mycelial disks removed from five-day-old cultures of the isolate. Triplicates were incubated at 28°C. for seven days and daily increments of radial growth recorded. A solid culture medium was selected. The disadvantage of using dry weight in the determination of fungal growth has been shown by Brown (18). The results are presented in table 2. These results show that the pathogen develops without much difference in growth rate within pH values of 5.00 to 7.00. It is pertinent to mention here that this range is much wider than that for the coffee soils in Puerto Rico, which vary from pH 4.50 to pH 6.00, though a great number of coffee soils are closer to the lower pH value. Growth of the isolates in Coon's solution, adjusted to pH values of 4.53, 5.55, 6.00, 6.49, 7.32 and 7.95; was abundant.

Effect of filtrates on cut coffee seedlings

At the end of four weeks, cultures of the pathogen grown in Coon's solution of pH 4.53 were filtered through a Chamberlain bacterial filter and the filtrates checked for contamination. One hundred cubic centimeters of each filtrate were poured into parallel flasks. One set of each filtrate was then boiled for fifteen minutes and allowed to cool to room temperature. Young coffee seedlings with four pairs of opened leaves were then cut under water and immersed in each boiled and untreated set respectively, and left for two days for observation. Other seedlings (placed in the unboiled filtrate of *F. bulbigenum* var. *coffeae*) showed wilting and chlorotic leaf symptoms similar to those observed in diseased coffee trees. The seedlings in the boiled and sterile Coon's solution were turgid and showed no indication of physiologic disturbance.

In 1932, Picado (49) obtained similar results with filtrates from four-week cultures of *F. anisophylum* grown in Richard's solution. He inferred that wilting of the coffee seedlings was in all probability due to a toxic action effective at a distance and capable of inducing a chlorotic condition and shedding of the leaves. Brandes (17) found that *F. oxysporum* Schl. var. *cubense*, responsible for the banana wilt, when grown for two weeks on Richard's and Uschinsky's solutions yielded filtrates producing wilt of buckwheat plants, banana leaves, and bean plants. The filtrates were thermolabile.

Pathogenicity

On November 29, 1939, a mixture of one part of well-rotted manure and two parts of river-bottom soil was potted in clay pots and autoclaved for four hours at 15 pounds pressure. The soil was left for a week, before it was used. The pH of the mixture was found to be around 6.00.

Young coffee seedlings, variety "Puerto Rico," of approximately 3 months growth were inoculated with each of the isolates, following the technique used by Wellman. Seedlings were transplanted in groups of ten, immediately after dipping the roots in the inoculum and while still dripping. Thereafter, the seedlings were kept under a capillary water dripper so as to maintain a wet substratum at all times. The seedlings stayed under these conditions for three months and in no instance were symptoms of disease produced. Control plants similarly treated but not inoculated, were growing inside the greenhouse under a temperature which fluctuated between 20 and 30°C., as recorded by a thermograph.

On February 2, 1939, the same procedure in testing for pathogenicity of the isolates was followed. This time, however, the soil was saturated at the time of transplanting of the seedlings and allowed to evaporate gradually, but not to the extreme of becoming too dry. Water was then again added to saturate the soil and the process was repeated regularly. The controls and inoculated seedlings were kept in this manner for two months and during this time only 12 per cent of the seedlings inoculated with the *Fusarium bulbigenum* var. *coffeae* displayed symptoms of disease. The affected seedlings became chlorotic and eventually wilted. The air temperature fluctuated between 20 and 32°C. Repetition of this work, however, gave erratic results.

In view of these results with plants grown on soil, it was decided to conduct further experiments with coffee seedlings kept growing this time in sand culture, to which Coon's solution was added as a source of nutrients. In this manner, variability in soil characters, contaminations with other organisms, and other factors affecting the coffee seedlings and the pathogen are materially avoided.

Coffee seedlings with one pair of leaves well opened, were pulled from the flats, dirt washed from the roots, and the seedlings immediately immersed in the respective inoculum and transplanted into sand cultures. The pH of the sand substratum was determined electrometrically and found to be 5.53 after the addition of the Coon's solution. These seedlings were inoculated on December 15, 1939, and kept growing on a saturated sand substratum, an automatic water feeder being used for the purpose of saturation. Under these conditions, the incidence of disease was very low, only 10 per cent of the seedlings inoculated with the *F. bulbigenum* var. *coffeae* showing symptoms of disease. (The symptoms of disease.) The symptoms appeared 21 days after inoculation and were initiated with a root and stem rot of the seedlings.

Coffee seedlings when inoculated and planted on a and substratum of pH 5.53, and saturated only at the time of planting, showed black root and crown rot within 10 days after inoculation with *F. bulbigenum* var.

coffeae. The seedlings were kept under bell jars at all times to prevent excessive drying of the sand as well as to provide a high relative humidity around them. None of the other isolates were capable of inducing disease.

The results of this and other greenhouse experiment have shown that inoculated coffee seedlings when grown on a very wet substratum are less liable to become infected than when kept growing on a moderately wet substratum or on one with a fluctuating water content. Wardlaw (74) found a similar behavior with respect to the infection of banana roots with *F. oxysporum* var. *cubense*.

FIG. 3. Damping-off of coffee seedling inoculated with *F. bulbigenum* v. *coffeae*. Seedlings grown in sand culture.

Succulent coffee seedlings with one or two pairs of leaves become infected a short time after inoculation. The typical symptom of disease is the rotting of the stem at the substratum level. Damping-off is produced on a moderately wet substratum and at a high relative humidity (fig. 3).

Picado (49), working with a Fusarium wilt in Costa Rica, indicated his failure to produce infection with coffee seedlings under one year of age. He attributed the lack of infection to the high content of caffein found in small seedlings. Our work has shown that with our pathogen the percentage of infection is very high under suitable conditions for its development. Pathogenicity studies with plants over one year of age will be conducted in the future.

A combination of the isolates grown independently in Coon's solution, was made to determine to relation between their behavior when in association and the incidence of disease. The same procedure followed for other experiments also was carried out in this one. In every combination where the pathogen *F. bulbigenum* var. *coffeae* was present, symptoms of disease appeared within a short time.

Effect of pH of the substratum on the production of the disease

The relation between the hydrogen-ion concentration of the substratum and the development of disease has been studied for other *Fusarium* wilts. Scott (53) has shown that the incidence of tomato *Fusarium* wilt due to *Fusarium lycopersicii* Sacc. is less frequent at pH values between 6.4 to 7.0, and that a maximum for disease manifestation lies on each side of the pH range.

On March 13, 1940, several experiments were undertaken to establish the relation of the hydrogen-ion concentration of the substratum to the incidence of coffee *Fusarium* wilt. Parallel tests were conducted with seedlings of the same age. The coffee seedlings were kept growing under identical conditions of substratum moisture content, relative humidity, substratum and air temperatures and amount of inoculum present. The pH value of the substratum of each set of plants under observation obviously was different. Coon's solution was used after being modified to give different pH values by the addition of potassium acid phosphate and potassium dibasic phosphate in various proportions; but always keeping chemical equivalent quantities as that supplied by the potassium acid phosphate in the original solution. Dilute hydrochloric acid and sodium hydroxide were used whenever necessary.

To each of four pots of one gallon capacity filled with washed, white granitic sand, was added one liter of the respective solutions, whose pH had been determined electrometrically. This amount of solution per pot was found to be enough to saturate the sand. After two hours of incorporating the solutions, the pH of the substratum was again determined and recorded as the initial pH. The plants were then inoculated and transplanted as explained previously and kept growing under bell jars inside the greenhouse. The temperature of the substratum fluctuated between 20 and 32°C., the air temperature between 20 and 38°C.

Table 3 indicates that coffee *Fusarium* wilt is more likely to occur at low than at high pH values of the substratum, provided all other conditions for infection are maintained. Whether the high acidity has a bearing on the virulence of the pathogen or is lowering the resistance of the host, is something that cannot be explained from these data. The range for infection

and that for growth of the causal agent are not identical, as the organism is able to grow abundantly within a wider range of pH values. Its growth was profuse within the range of pH 4.5 to 8.68. The limits for growth were not determined.

TABLE 3

Incidence of coffee Fusarium wilt on seedlings grown at varying pH values of the substratum for two months

(Each figure represents date of 20 plants per pot)

Initial pH of Substratum	Final pH of Substraum	Average Time for Expression of Symptoms	Amount of Diseased Plants by the End of the Experiment
		days	*per cent*
4.53	6.08	7	100
5.53	6.37	11	65
5.57	6.37	15	60
5.97	7.91	7	100
6.00	6.64	21	40
6.49	8.17	0	0
7.32	8.10	0	0
7.95	8.17	0	0
Controls for all pH values.....		0	0

Relation between temperature and disease

In previous experiments it was observed that the incidence of *Fusarium* wilt is very high in inoculated coffee seedlings kept growing within an enviroment of fluctuating substratum and air temperatures of from 20 to 32, and 20 to 38°C., respectively, provided that the seedlings were planted in a moderately wet and acid substratum and surrounded by a high relative humidity. High temperature is, therefore, to be considered a factor necessary for the production of infection and disease.

In order to observe the effect of constant soil and air temperatures on the incidence of the malady, 20 coffee seedlings with two pairs of opened leaves were inoculated and transplanted into sand cultures adjusted to pH 5.53 and saturated at the time of transplanting. The seedlings were then kept at all times under bell jars. Control plants were treated similarly but not inoculated. The potted plants were placed in an air-conditioned room and the temperature adjusted to 24°C.

The inoculated plants showed typical symptoms of root and crown rot within seven days. In the experiment begun February 17, 1941, all check plants remained in perfect condition while all the inoculated plants became diseased.

Picado (49) in his work with *Fusarium anisophyllum*, reported a decrease in the percentage of coffee wilt at temperatures from 18° to 22°C. Due to lack of facilities for this particular phase of the work, no further trials were conducted to determine the rate of infection on either side of the 24 to 30°C. temperature range.

The development of the disease coincides with the temperature range for optimal growth of coffee seedlings and of the organism. Our work in Wisconsin showed that coffee seedlings develop better at temperatures above 20°C.

Testing coffee varieties for resistance

Two varieties of coffee, "Columnaris" and "Puerto Rico", were compared for resistance to the malady. The plants were three months old, and were inoculated and transplanted to sand cultures as formerly explained. The results are given in table 4.

TABLE 4

Development of coffee fusarium wilt in two varieties of coffee

(Each figure represents data for 60 plants)

Variety	Initial pH of Substratum	Per Cent of Wilted Plants
PuertoRico	5.5	100
Columnaris	do	100
Puerto Rico	7.32	0
Columnaris	do	0
Controls		0

On March 24, 1942, small coffee seedlings with one pair of cotyledonous leaves opened were tested for resistance. The seedlings were inoculated following Wellman's technique and transplanted immediately to a large iron pan painted on the inside with a chemical-proof paint and filled with granitic sand to a height of about one inch from the top. The pan was placed inside a glass box with adjustable side doors and with inside shelves for keeping the soil and air thermograph and a hygrograph. (fig. 4) The initial pH of the substratum was electrometrically determined to be 5.63. After the third day from transplanting the pH had changed to 7.15. The air temperature inside the glass box fluctuated between 22 and 40°C. The substratum temperature fluctuated between 22° and 28°C. The relative humidity was around 80 per cent except when the box was opened for examination of the seedlings. During these short periods of time the relative humidity dropped to 35 per cent.

The substratum was saturated at the time of transplanting and thereafter lost water gradually. The results are shown in table 5.

Examination of the roots of the inoculated plants showed that all the coffee varieties are susceptible to the attack of the pathogen. Owing to the fact that the pH of the substratum had changed within three days after inoculation, some of the varieties were able to recover. New roots were then formed from tissues above those diseased. These roots apparently were healthy and no infection, therefore, took place under the new alkaline pH condition in which they were thriving. Acidity of the substratum is

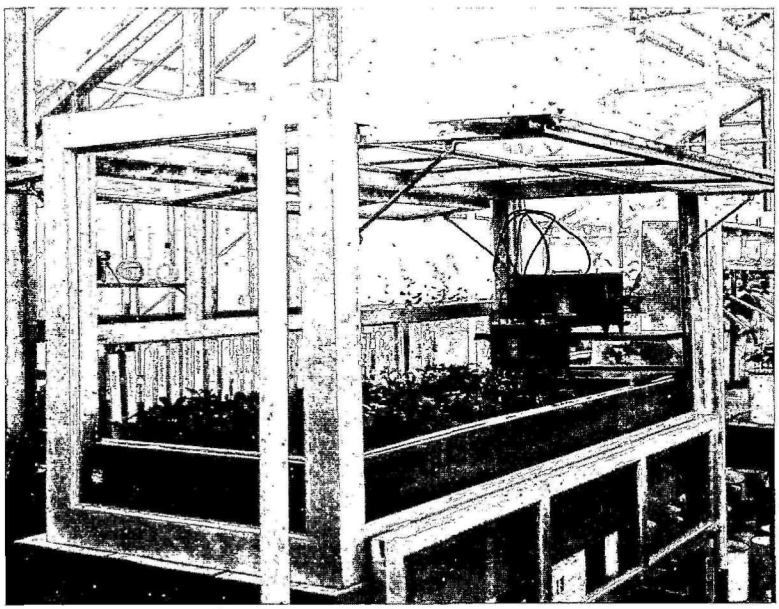

FIG. 4. Testing coffee varieties for resistance to infection with *F. bulbigenum* v. *coffeae*.

an important factor in determining whether infection will occur. The recovery of some infected plants can be explained as a result of the selective absorption of ions either by the pathogen or the plants, leaving those ions with marked alkaline reaction, unabsorbed.

Testing for the resistance of the 28 varieties of coffee was repeated in order to obtain more elucidation on this important matter of acidity. This time the pH of the substratum was always maintained on the acid side by the use of ammonium sulphate as a partial source of nitrogen. In this manner, the selective absorption or use of the ammonium radical left the

sulphate acid radical remaining in the medium. The initial pH of the substratum as determined electrometrically was 4.44. By the end of the experiment it had changed to 5.29 This acid range lies within the pH

TABLE 5

Results of testing twenty-eight varieties of coffee of the Arabian, Robusta and Liberian Groups for resistance to coffee wilt

(Each figure represents data for 100 plants)

	Time for Expression of Symptoms	Wilted Plants in 30 Days
	days	*per cent*
Moca	15	40
Guadalupe	15	20
Carmelita 80	10	10
Padang	10	20
Preanger	15	20
Uganda hybrid	10	40
Murta	11	10
Ceylon hybrid	9	10
San Ramón	10	30
Erecta	9	40
Bourbon	9	40
Congensis	9	40
Excelsa	9	40
Liberica	10	30
Dewevrei	9	20
Maragogipe	10	40
Java Moca	10	30
Robusta	10	50
Congensis Chalotti	9	80
Blue Mt. Jamaica	9	80
Pantgoer	11	30
Robusta Canephora	10	70
Robusta Quillour	10	50
Marasan	11	20
Columnaris	9	40
Puerto Rico	9	30
San Kruense	9	40
Arnoldiana	9	70

range previously determined to be favorable for the development of coffee wilt.

Infected plants formed new roots above the points of infection, but these were in turn attacked. Therefore, recovery under this condition is less likely to occur. The data are presented in table 6.

TABLE 6

Results of testing for resistance to coffee wilt of twenty-eight varieties of coffee of the Arabian, Robusta and Liberian Groups, the inoculated seedlings being kept on an acid substratum

(Each figure represents data for 50 plants)

	Time of Expression of Symptoms	Wilted Plants by the End of the Experiment
	days	*per cent*
Moca	7	100
Guadalupe	7	100
Carmelita 80	7	93
Padang	7	86
Preanger	7	73
Uganda hybrid	7	60
Murta	9	06
Ceylon hybrid	9	20
San Ramón	10	73
Erecta	7	46
Bourbon	10	66
Congensis	7	66
Excelsa	7	80
Liberica	7	86
Dewevrei	7	20
Maragogipe	7	36
Java Moca	7	36
Robusta	7	80
Congensis Chalotti	7	66
Blue Mt. Jamaica	7	33
Pantgoer	7	33
Robusta Canephora	7	40
Marasan	7	46
Puerto Rico	7	33
Columnaris	7	33
Robusta Quillou	7	46
San Kruense	7	66
Arnoldiana	7	40

DISCUSSION AND SUMMARY

The association of the fungus *Rosellinia bunodes* (Berk & Br.) Sacc. with black root rot and wilt of coffee in Puerto Rico, and probably elsewhere, has heretofore been based merely on mycological details. In the literature consulted, there is not a single instance in which the pathogenicity of the organism has definitely been demonstrated. Its host range is still widening and includes herbs, all sorts of weeds, shrubs and trees. The fungus was abundantly found in decaying organic matter.

In the course of our investigation, the fungus *R. bunodes* has failed, in every instance and under varying enviromental conditions, to induce the

disease. However, under the same enviromental conditions, a *Fusarium* sp., named for the sake of identification *F. bulbigenum* var. *coffeae* var. nov., and isolated from infected coffee wood showing the markings of the so-called Rosellinia root rot; was found to induce coffee black root rot and coffee wilt independently or in association with other isolates. None of the other isolates proved to be pathogenic. It seems, therefore, that, at least under our experimental conditions, the *R. bunodes* and other isolates mentioned previously in the course of this work are mere saprophytes.

The association of this and other species of *Rosellinia* with root rots of plants in the tropics have been questioned. Petch (48) working in Ceylon and Malaya, along with other workers, has indicated that among the fungi found associated with the root diseases of tea, *R. bunodes* is the least common. The cause of the red root disease of limes in Dominica is ascribed by some authors to *R. bunodes* and *R. pepo*. Other workers believe it to be due to the fungus *Sphaerostilbe repens* Berk & Br., while Ashby (10) attributes it to the extremely wet conditions of the environment. Carruther (19) associated a *Rosellinia* species with the root disease of tea in Ceylon, but he failed to induce the disease by planting tea plants on soil contaminated with ascospores of the fungus. In the Kew Gardens in England, apple roots and seedling beeches became infected when planted in soil contaminated with diseased material from which *R. radiciperda* Mass. had been isolated; but there is doubt as to whether some other organism present in the infected material might not be responsible for the infection. Averna Saccá ((5, 6) found *Fusarium pallens* associated with *Rosellinia bunodes* in diseased coffee trees in Brazil and in plant beds attacked by a *Colletotrichum* sp. and nematodes.

Pathogenicity studies conducted with the *F. bulbegenum* var. *coffeae* have shown that the incidence of the disease is favored by high temperatures and by substrata, medium to wet, or with fluctuating moisture content. Temperatures fluctuating between 20° and 36°C., as recorded by a thermograph, were favorable for the development of the disease. A constant temperature of 24°C. also was found to be adequate for the development of the disease. A substratum kept wet all times was less favorable for the development of the malady than one with a fluctuating water content. Very dry and very wet conditions seemed to be unfavorable for disease development. A high relative humidity of from 70 to 80 per cent favored the development of damping-off symptoms. In atmospheres of low relative humidity or with fluctuating humidity, the characteristic symptom is wilting with or without chlorosis. These findings conformed with field observations. Fawcett (29) found that "the disease often does most harm amongst the best trees, the sun-exposed slopes of the poor coffee plantations remaining quite free from trouble . . . the only things which retard or stop its progress seem to be excessively dry or excessively wet soils,

natural barriers, such as brooks, and the scarcity of food material (decaying vegetation) in the soil." The author had the opportunity to observe severe cases of coffee wilt at Mr. Luis Vilella's farm at Lares and at Mr. Sastre's farm at Utuado. Other coffee groves in the neighborhood around Adjuntas-Lares also were found to be heavily infected. The conditions resembled those already quoted from Fawcett.

The question of soil pH reaction and the incidence of the disease had not been elucidated previously. Coffee seedlings under one year of age were very susceptible to infection when grown on substrata with the pH varying between 4.50 and 6.50, and provided that such other factors as humidity of the substrata, temperature, and relative humidity of the atmosphere were considered. Our coffee soils fall under a pH range of from 4.50 to 6.00, though more frequently closer to the lower limit of acidity. Steinmann (63) states, however, in relation to the black root rot of tea in Java; attributed to *Rosellinia bunodes* and *R. arcuata*, that: "in contrast to most other root fungi, these species of *Rosellinia* occur almost exclusively in neutral, young, volcanic soils where the exchange acidity does not exceed 1.0 or the degree of hydrolytic acidity 55.0." He recommends that "infected areas should be isolated by trenches dug on porous soil to a depth of at least 1 m. and further. In order to increase the degree of exchange acidity in neutral soil 2.0 the limit for *Rosellinia* infection it would be necessary to apply 100 gm. of aluminum sulphate per sq. m. of soil. A more economical and probably more effective method could be treatment of the soil with alum or colloidal sulphur."

The question of the relation between the pH of the soil and the incidence of *Fusarium* wilt has promoted many debates among investigators. In the case of the Panama disease or banana wilt, the consensus of opinion favors the idea that the disease may be best reduced in soils well supplied with lime, and with a pH value of the soil above 6.00. However, there have been reports of serious outbreaks in soils rich in lime and with a high pH value. The coffee wilt organism is able to grow within a wide pH range, as was mentioned before, thus leaving the question open as to whether the organism loses virulence in an alkaline substratum, or whether the plants are less susceptible. Coffee wilt in Puerto Rico is more liable to occur in the acid soils of our highlands. Incidence of coffee wilt is less in neutral or slightly alkaline soils.

CONTROL

The control for coffee wilt, as it appears in Puerto Rico and elsewhere, has not been woked out as yet. Such recommendations as the eradication of diseased trees and contaminated material followed by the application of lime to the soil, scraping of contaminated material and then liming, pruning

of shade trees and underbrush, with pruning of lower branches of the coffee trees to allow for air circulation, and isolation of contaminated areas by deep trenches, are some of the preventive measures of control recommended. So far, however, none of these practices have been shown to be effective.

Chemical control has been attempted, but the results obtained are far from being practicable; in many instances their high cost invalidates such measures. Fawcett (29) experimented with lime, sulphur, copper sulphate, potassium permanganate, chloronaphtholeum and potassium bisulphite. Small coffee plots in contaminated areas were treated with lime and sulphur, respectively, at the rate of 500 grams per square meter. Observations over a three-year period showed no incidence of disease in the treated lots. Untreated plots revealed 5 per cent of diseased trees. Plots treated with 150 grams of sulphur and 50 cc. of a 5 per cent solution of chloronaphtholeum per square meter, respectively, showed 3 per cent of diseased trees. Choloronaphtholeum applied at the rate of 450 cc. per square meter, was found effective.

Copper sulphate solution sprayed at the rate of 20 grams per square meter was reported sufficient to check and exterminate the fungus.

In Martinique, Bordoz (16) reported very good results with carbon bisulphide emulsion. The same measure was reported inefficient from Dominica in controlling root diseases.

Those who have dealt with the question of chemical control ascribed their good results, if any, to the fungicidal properties of the substances used. The question of the acidity of the soil and its possible relation to the root rots has sometimes been mentioned but not elucidated. Nowell (45) pointed out the possibility of inhibition of the pathogen by the application of lime to the soil with the resulting neutralization of the soil acidity. It can be recalled that in the work by Fawcett, already mentioned, successful results were obtained when using 500 grams of either sulphur or lime per square meter of soil. The former substances tends to lower the pH of the soil, while the latter tends to raise it. However, it is still a matter of doubt whether each chemical in itself was sufficient to alter materially the pH value of the soil, considering the buffer capacity of the latter.

Our work revealed, other factors considered, that for the incidence of coffee wilt, the pH value of the substratum is important, at least under greenhouse conditions. The application of lime in substantial amounts to change the pH of the soil for a relatively long period of time, and beyond pH 6.50, is an advisable control measure, in addition to other practices mentioned in the course of this report.

According to Guiscafré and Gómez (33, 34), 95 per cent of the coffee root system develops within the first 12 inches of the top soil, with an average spread of from 3 to 4 feet from the tree trunk. They also found

that the best root development occurs in soils with reaction of from pH 6.5 to 7.5. In soils with pH values of from 4.0 to 4.50 root development is lessened. Nutman (46) presents evidence on the favorableness to root development of neutral or slightly acid soils of approximately pH 5.8 to 6.0, which he considers to be the acid limit for better root growth. Nowel (45) also indicates that in all probability, the benefit of liming the soil is not to be attributed exclusively to the neutralization of the soil acidity, but also to the possible increase in the rate of decomposition of the organic matter of the soil in addition to the correcting action of the lime. Smith (56) in discussing this question, has indicated that in Jamaica the incidence of banana wilt has been decreased considerably by the addition of lime to soils in which the organic matter has been collected and buried in trenches. In our coffee plantations it is customary to pile organic matter around and close to the tree trunks. In this manner there is provided an ideal environment for the growth of fungi. Although the organic matter from leaves and decayed wood is a fine source of nutrients, why accumulate it close to the tree trunks? The absorbing area of the roots is far away from the trunk itself, and much better use of the decomposing organic matter can be made by the tree if such nutrients are adjacent to the feeding areas. Among the varieties of coffee tested for resistance to coffee *Fusarium* wilt, the "Murta" showed the highest tolerance to the disease. Other varieties, such as the "Ceylon Hybrid" and "Dewevrei," were somewhat tolerant, but not much more than the "Puerto Rico" and the "Columnaris". The latter variety is very promising. The noncommercial variety of coffee *Stenophylla*, has been reported tolerant to the malady in Dominica. Tolerant varieties could be planted in contaminated areas and could possibly be used for grafting.

It is well to remember that laboratory and greenhouse conditions are adjusted to create an adequate environment for the incidence of the disease and that all our experiments were conducted with coffee seedlings under one year of age. What may be the response of plants over one year of age and grown under field conditions is something else to consider. The variety "Columnaris" has been planted on several farms where coffee wilt is known to exist. So far, there have been no reports of the appearance of the malady on such plants. Further observations will demonstrate the good characteristics of this variety when grown under field conditions.

RESUMEN

Por el nombre de "podredumbre negra" es conocida en Puerto Rico una enfermedad de la raíz de los cafetos muy difundida en nuestras plataciones e informada en otras islas del Caribe y países productores de café en Centro y Sud América. En Puerto Rico la enfermedad reviste gran importancia

económica, considerándosele un factor limitativo en el cultivo de este producto agrícola. Basta echar una ojeada a muchas de nuestras plantaciones para reconocer los graves perjuicios producidos.

La enfermedad se conoce en las Antillas desde el 1840 y a pesar de creerse al hongo *Rosellinia bunodes* (Berk. & Br.) Sacc., responsable de ella, no hay evidencia experimental que lo pueda constatar. En nuestra Isla se apunta también al *R. bunodes* como al posible agente causante de la podredumbre negra, sin que se haya demostrado categóricamente esa relación.

Ante esta situación creímos pertinente comprobar si el *R. bunodes* o igualmente otros organismos aislados de material infecto, tendrían que ver con la enfermedad. Ensayos de patogenicidad efectuados en la Universidad de Wisconsin durante el año escolar de 1938 al 1939 con el susodicho organismo y con una especie de *Verticillium*, demostraton que dentro de las condiciones de temperatura y humedad en que se hicieron las pruebas, el *R. bunodes* o igualmente el *Verticillium*, eran incapaces de producir, *independientemente* o conjuntamente, la podredumbre negra. Experimentos subsiguientes realizados en esta Estación durante los años comprendidos entre el 1939 y 1942, en condiciones variables de humedad y acidez del substrato y dentro de un ambiente más o menos uniforme, ratificaron la incapacidad patogénica de los mencionados organismos.

El hecho de encontrar un gran número de cafetos con infecciones radicales con o sin mostrar los signos o síntomas característicos de la podredumbre negra, esto es, estriaciones vasculares oscuras; hizo pensar en la posibilidad de que fuese otro organismo, y no el *R. bunodes*, el responsable de la enfermedad y, que este organismo, al igual que otros aislados de material infecto, fuera únicamente uno de tantos saprófitos que toman incremento en cafetos enfermos.

En efecto, se encontró al hacer ensayos subsiguientes de patogenicidad, que entre los varios organismos aislados de cafetos enfermos, había uno del género *Fusarium*, capaz, por sí solo, de producir la enfermedad. Sin embargo, el leño de las plantas afectadas por este organismo no presenta los signos internos característicos de la podredumbre negra y atribuidos al *R. bunodes*. El *Fusarium* aislado pertenece a la sección *Elegans* y, de acuerdo con Sherbakoff, a quién se la envió para su clasificación; es morfológicamente idéntico al *F. bulbigenum*. Pudiendo ser una nueva raza o forma de esta especie se la denomina aquí para fines de identificación, como *F. bulbigenum* var. *coffeae* var. nov. Su similaridad con el *F. batatatis* var. *Vanilla* Tucker es notable. Este último no ataca al café. El nuestro no ataca a la vainilla como hemos podido probarlo.

El organismo crece bien en un sinnúmero de substratos nutritivos, especialmente en aquellos ricos en azúcar; como límites de crecimiento, temperaturas de 8 y 36 grados centígrados y con un optimum entre los 26

y los 28 grados centígrados. Puede crecer abundantemente en substratos con acidez entre los pH 2.19 y 8.5, aunque su mejor crecimiento es entre los pH 4.5 al 6.00. Las macrosporas son generalmente triseptadas, fluctuando en longitud entre 16.75 a 45 micras y en grosor entre 3.0 a 3.7 micras. Ocasionalmente se producen esporas de menor o mayor septación. Las microconidias son abundantes y por lo general sin tabiques.

Encontrándose que el organismo crecía bien en la disolución de Coon, formulada para este género de hongos, se utilizó dicha disolución para los ensayos de patogenia con plantas pequeñas de menos de un año de crecimiento. Primeramente se encontró que el hongo producía un producto metabólico, termolábil a 100°C., descartándose, consecuentemente, el filtrado de los cultivos al tiempo de prepararse el material inoculante a usarse para las pruebas. Al hacerse anteriormente pruebas de patogenia con los diversos organismos aislados, se notó cuan pocos eran los casos de infección cuando el patógeno se incorporaba en tierra esterilizada a vapor, siendo los resultados erráticos en muchas ocasiones. Para evitar las posibilidades de contaminaciones usando tierra, y asimismo otros factores de carácter fisicoquímico sobre los cuales no podíamos tener dominio alguno, fué que se decidió hacer todas las pruebas en un substrato de arena granítica, lavada, a la cual se la añadía la disolución de Coon de composición y acidez conocidas.

En efecto, se condujeron ensayos con plantas de café mostrando tres a cinco pares de hojas completamente formadas, inoculándolas según la técnica de Wellman (75). Dicha técnica consiste en la inmersión de las raíces de las plantas recién arrancadas de los semilleros en una suspensión de esporas y micelio del organismo en cuestión. En vista de que el patógeno produce una substancia tóxica, se lavó el micelio y se hizo luego la suspensión en solución estéril de Coon. Las plantitas de café así inoculadas y sembradas inmediatamente en arena, saturada ésta con la disolución de Coon y revelando un pH de 5.53, mostraron síntomas característicos de la infección en un plazo de diez días.

El ambiente mantúvose cálido, fluctuando entre los 24° a los 36°C. y relativamente saturado por estar los cafetos inoculados bajo campanas de cristal.

Los síntomas se iniciaron con la aparición de una clorosis, siguiendo primeramente a lo largo de las nerviaciones de las hojas y luego distribuyéndose en forma de un moteado amarillo por el parénquima. Más tarde, las hojas se tornaron completamente amarillas, permaneciendo así algún tiempo, después del cual se ennergrecieron y secaron, cayéndose finalmente. En algunos tiestos de ensayo los cafetos infectados en vez de mostrar la clorosis descrita, se marchitaron. En condiciones de gran humedad relativa las plantitas mostraron síntomas de podredumbre del

tallo, estado conocido vulgarmente por "salcocho". El hongo en este ambiente húmedo crece profusamente sobre las partes infectas y asciende en muchos casos por los tallos hasta invadir los pecíolos de las hojas. Una particularidad notada entre los cafetos enfermos fué su lento desarrollo.

Un examen microscópico de las plantas afectadas mostró algunos vasos conductivos del xilema necróticos, un tanto hipertrofiados y, en otras ocasiones, llenos de una substancia ocre. Cuando la infección está muy adelantada, la necrosis de los vasos se extiende hasta bien arriba del tallo. Las raíces muestran también un cuadro histológico similar. La corteza se deteriora: como se notó al arrancar las plantitas de los tiestos de arena. Las raíces y tallos mostrábanse desnudos de corteza.

Nuestros experimentos demostraton que en un substrato completamente saturado de humedad, en todo tiempo las plantas se enfermaban menos que en uno en que la humedad fluctuaba entre el punto de saturación y grados relativamente secos, aunque sin llegar hasta el límite en que las plantas sufrieran por falta de agua.

Las temperaturas altas comprobaron ser importantes para la manifestación de la enfermedad. En las pruebas realizadas a temperaturas fluctuando entre los 24° y los 46°C.; el porcentaje de infecciones fué muy crecido. Asimismo se comprobó que a una temperatura sostenida de 24°C., las infecciones occurrían frecuentemente.

Ensayos relacionados con la acidez del substrato y su posible efecto en las infecciones demostraron que entre pH 4.53 y 6.50, el porcentaje de infecciones es altísimo, mientras que a pH mayores de 6.50 las plantas no manifestaron en ningún momento síntomas de infección. También pudo verificarse que cuando el pH del substrato cambia en pocos días hacia un punto neutral o ligeramente alcalino, algunas plantas pueden recuperar de la enfermedad. Sosteniendo el pH siempre bastante ácido, el por ciento de plantas enfermas es mucho más crecido.

Al hacerse varias pruebas sobre la propensión o posible tolerancia y resistencia de 28 variedades de café de los grupos Arabica, Robusta y Libérica, se encontró que unas eran más o menos propensas que la Puerto Rico. La variedad "Murta" demostró ser bastante tolerante a la enfermedad.

El estudio sobre esta relación de la acidez, entre otros factores, indica la posibilidad de aminorarse los casos de infecciones en los cafetales, ya neutralizándose la acidez de los terrenos dedicados a la siembra de cafetos o bien con el uso de algunas de las variedades que presentaron mayor grado de tolerancia a la enfermedad.

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