Anthracnose and Berry Disease of Coffee in Puerto Rico[1]

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ABSTRACT

A survey revealed that Anthracnosis (*Glomerella cingulata* asex. *Colletotrichum gloeosporioides*) was the principal aboveground disease of field coffee in Puerto Rico. Isolates of *C. gloeosporioides* from both diseased soybeans and coffee caused typical branch necrosis in coffee after in vitro inoculation. Noninoculated checks showed no symptoms of branch necrosis or dieback. Necrotic spots on coffee berries collected from the field were associated with the coffee anthracnose fungus (*C. gloeosporioides*), the eye spot fungus (*Cercospora coffeicola*) and the scaly bark or collar rot fungus (*Fusarium stilboides*). Typical lesions were dark brown, slightly depressed and usually contained all three fungi. Fascicles of *C. coffeicola* conidiophores formed a ring inside the lesion near its periphery. Acervuli of *C. gloeosporioides* and the sporodochia of *F. stilboides* were mixed in the center of the lesions. Monthly fungicide sprays (benomyl plus captafol) and double normal fertilization (454 g 10-5-15 with micronutrients/tree, every 3 months) partially controlled berry spotting. Double normal fertilizer applications alone appeared to reduce the number of diseased berries by approximately 41%, but fungicide sprays gave 57% control. Combining high rate of fertilization and fungicide applications resulted in a reduction of approximately 85% of diseased berries.

INTRODUCTION

Coffee (*Coffea arabica*) is a major crop in Puerto Rico, particularly on the humid northern slopes of the western central mountains. The 1979–80 crop was harvested from about 40,000 hectares yielding over 11,350,000 kg with a value of $44 millions. Although the commodity is heavily subsidized by the government, coffee imports are needed to satisfy the Island's demand. One of the factors most detrimental to Puerto Rican coffee growers is low yield. During the last 50 years, coffee yields have stagnated at nearly 182 kg/ha. Studies at the Puerto Rico Agricultural Experiment Station (7, 23, 24) revealed that implementation of improved practices can result in 9 to 15 times higher yields and in a net income of more than $1,334/ha.

There are two worldwide foliar diseases of major concern affecting coffee bearing trees: rust (*Hemileia vastatrix*) and anthracnose (*Colletot-*

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richum gloeosporioides). Both diseases are known to cause substantial yield losses. At present, rust is absent in coffee plantations in Puerto Rico. It was introduced on coffee seedlings, but it was eradicated before spreading.[3] Anthracnose, besides reducing yield, also causes fruit spotting and mummification, thereby reducing coffee quality and yield. Anthracnose symptoms also include branch dieback, defoliation and retention of mummified berries.

The anthracnose disease is caused by *C. gloeosporioides*, the asexual stage of *Glomerella cingulata*. In Africa, sunken spots on green berries, fruit mummification, and branch dieback have been attributed to a particular *Colletotrichum* strain. There the disease has been called coffee berry disease (CBD) and the causal fungus has been named *C. coffeanun* Noack.

Coffee berry disease is known to cause up to 80% yield loss in Africa and has caused the abandoning of many plantations on that continent. In Kenya, great efforts have been made toward the development of fungicide spray schedules for controlling this disease (6, 9, 10, 16, 21). Among arabica cultivars, Geisha and Blue Mountain have shown some resistance to CBD whereas Harrar, SL selections and Bourbon are particularly susceptible (11).

Coffee anthracnose has probably been in Puerto Rico for many years. One of the first accounts on coffee diseases in Puerto Rico is that of Fawcett in 1915 (2). He mentions isolates of *Colletotrichum* (*Gloeosporium*) causing twig and berry necrosis. Farmers in Puerto Rico call the disease "paloteo," which signifies defoliated branches, and have claimed that heavy fertilization controls the disease.

The overall goal of our investigations is to determine the role that diseases play in low coffee yield and quality in Puerto Rico. We report on the effects of anthracnose, fungicide spray and fertilizer application on coffee quality.

MATERIALS AND METHODS

ANTHRACNOSE SURVEY

From 1979 to 1981, plantations of 4- to 8-year-old coffee trees grown without shade were surveyed to evaluate the incidence and severity of anthracnose from January to July. All farms surveyed were located in the coffee growing zone which includes the municipalities of Adjuntas, Ciales, Guayanilla, Jayuya, Lares, Las Marias, Maricao, Mayagüez, Orocovis, Utuado and Yauco. Disease incidence (number of trees with anthracnose) and severity (percentage of tree area with anthracnose)

[3] Stevenson, John A., 1975. Fungi in Puerto Rico and the American Virgin Islands. Braun-Brumfield, Inc., Michigan, USA.

were determined at 6 to 8 random sites in which 25 trees were rated in each site. A farm in Adjuntas was selected to evaluate berry losses at harvest time by determining the percentage of mummified berries harvested and those that floated during the washing process. At the UPR Experiment Substation at Limaní, Adjuntas, dry coffee beans were classified as double embryos, broken, mummified, clean, and evaluated for losses associated with anthracnose.

MYCOFLORA SURVEY

Plant parts (green, ripened and mummified berries, healthy appearing branches with lesions, dead branches, green and dead nodes) were assayed for microorganisms. Samples were placed in culture media (potato dextrose agar) or on moistened cellulose pads to determine the organisms associated with them. Before being placed in either substrate, samples were surface disinfested with NaOCl (10% Clorox) for 3 to 5 minutes and incubated at 28° C. Those on cellulose pads were incubated in a germinator providing 10 hr of light and 100% RH; those in PDA were put in the dark in an incubator. After 7 or more days of incubation, plant parts were examined under the stereo-microscope to identify colonies of microorganisms and, with the aid of the compound microscope, genera and species were determined. Tissue samples were periodically examined to determine the succession of organisms. Fungi were grown and kept on PDA for further studies and purification.

INOCULATIONS WITH COLLETOTRICHUM

Detached branches and whole plants of *C. arabica* cv. Bourbon were inoculated with either of two isolates of *C. gloeosporioides*. One of the isolates came from soybean leaves showing symptoms of anthracnose, and the other isolate came from spotted coffee berries. Plants were incubated in a greenhouse at 22 to 33° C and 80% RH. Branches were placed on cellulose pads inside a cabinet at 22° C at night and 28° C during the day with a photoperiod of 12 hours of light.

SITE SELECTION

A private farm in Adjuntas (900 m above sea level) with 8-year-old trees of the Bourbon cultivar grown without shade were used to measure the effects of anthracnose, fertilization and fungicide sprays on coffee quality. The site was selected for its high incidence and severity of coffee anthracnose. Main plots consisted of 16 experimental trees. Each subplot (8 trees) was separated by one border row on each side and by three nontreated trees in the row.

TREATMENTS

Fungicide mixture of benomyl plus captafol (228 g a. i. of each) diluted in 100 gal of water was applied monthly from flowering to berry fill (six times from February to July). The fungicide mixture was sprayed with a Hardi backpack sprayer (20L capacity) at approximately 30 lb/in^2 until runoff. Every 3 months, from February to August, fertilizer 10-5-15 with micronutrients was applied three times, 227 or 454 g per tree each time. Treatments replicated four times included: 1) high fertilizer application + fungicide; 2) low fertilizer application + fungicide; 3) high fertilizer application without fungicide; and 4) low fertilizer application without fungicide (check).

DISEASE ASSESSMENT

At the beginning of the test, anthracnose severity was estimated by visual inspection of each tree. The percentage of branch area with dieback symptoms was recorded. Berries were harvested three times from August 1 to September 20. At each picking, 400 berries were sampled from each plot and the number of mummified, spotted and healthy berries per tree were determined. Samples of 100 berries of each type were placed on wet cellulose pads, incubated at 95% RH and 28° C for 7 days. The mycoflora associated with each type of berry were identified at the end of the incubation period. Counterpart samples of both branches and berries were surface disinfested with 10% Clorox, plated on oatmeal agar media and incubated in the dark at 28° C. Isolated fungi were then identified under a microscope.

RESULTS

The disease survey conducted throughout the coffee growing region in Puerto Rico (12) revealed anthracnose of coffee to be widespread and the most destructive disease of coffee in the Island. Of 26 surveyed farms, 50% were found to have trees with severe anthracnose symptoms. Disease severity of trees ranged from 1 to 70% (table 1). The survey also revealed that farmers had not been aware of the pathological nature of this condition and that they did not use any control measures for this or any other foliar diseases on bearing trees in Puerto Rico.

Microscopic examination (table 2) of diseased berries revealed colonies of *Glomerella cingulata* and its asexual stage *C. gloeosporioides* and *Mycosphaerella* sp. (sexual stage of *Cercospora coffeicola*). *Fusarium stilboides* was also found colonizing the berries. Fungi sporulation on branches and on dead nodes on wet cellulose pads was heavy and yielded *C. gloeosporioides* and its sexual stage (*G. cingulata*) and *Phomopsis* sp. From all plant parts, except from green nodes (table 2), *C. gloesporioides* was isolated on artificial media. *C. coffeicola* was isolated from all berry

TABLE 1.—*Estimated anthracnose severity of coffee trees grown without shade in Puerto Rico during 1980 to 1982*

Farm number	Municipality	Severity[1]	Farm number	Municipality	Severity[1]
		%			%
1	Adjuntas	30	16	Maricao	15
2	Adjuntas	—[2]	17	Maricao	20
3	Adjuntas	4	18	Maricao	25
4	Adjuntas	15			
5	Adjuntas	20	19	Mayagüez	10
6	Adjuntas	70	20	Mayagüez	25
7	Guayanilla	15			
8	Jayuya	—	21	Utuado	1
9	Lares	—	22	Utuado	—
10	Lares	8	23	Utuado	5
11	Lares	50	24	Utuado	20
12	Lares	40			
13	Lares	—	25	Yauco	15
14	Las Marías	3	26	Yauco	2
15	Las Marías	1			

[1] Severity = estimated tree area showing dieback symptoms.

[2] Values were not recorded because damage from anthracnose was not distinguishable from damage caused by harvesting practices.

TABLE 2.—*Percentage of fungi recovered from different plant parts of coffee trees*

Fungi	Green berries	Ripe berries	Mummies	Green healthy branches	Green branches with spots	Dead branches	Green nodes	Dead nodes	$\bar{X}$
Colletotrichum gloeosporioides	62	50	38	62	0	37	13	25	35.9
Aspergillus sp.	37	63	50	0	0	0	50	13	28.3
Cercospora coffeicola	25	25	75	0	25	0	0	0	18.8
Nigrospora sp.	37	13	25	0	0	37	13	13	17.3
Phomopsis sp.	0	0	13	0	25	25	13	25	12.6
Fusarium stilboides	25	38	13	0	0	0	0	0	9.5
Trichoderma sp.	0	0	0	0	0	0	25	0	4.8
Nectria haematococca	0	0	0	0	0	0	25	0	3.1
Cylindrocladium sp.	0	0	0	0	0	0	25	0	3.1
Pyrenochaeta sp.	0	0	0	0	0	0	0	0	3.1
Fusarium semitectum	13	0	0	0	0	0	0	0	1.6
Pestalotia sp.	0	0	0	0	0	13	0	0	1.6
Fusarium oxysporum	13	0	0	0	0	0	0	0	1.6

types and also from spots on green branches, but not from other plant parts. *F. stilboides* was prevalent on all berry types but not on other plant parts assayed. Necrosis and dieback symptoms developed on branches inoculated with both the soybean and coffee isolates of *C. gloeosporioides* but not on inoculated greenhouse coffee plants nor check branches in the laboratory.

Mummified berries are less dense than uninfected ones and often float during the washing process. The farm selected to evaluate mummification of the berries had about 30% of the 8-year-old trees with severe anthracnose dieback symptoms, and a severity range of 40 to 70% per tree. Forty percent of the seed from these trees were mummified and floated during washing. At the Limaní Experiment Substation, about 7% of all coffee berries harvested in a particular day were mummified and floated during washing. Of the seed that sank, 33% was discarded by hand for being sterile, having double embryos or for being broken or deformed. Eighty-two percent of the selected seed had germinated during the first 60 days when tested on cellulose pads, in comparison to the discarded and mummified ones that had 52% and 1% germination, respectively. Those

TABLE 3.—*Overall effects of fungicide and fertilization levels on the incidence of diseased coffee berries*

Factor	Percentage of diseased berries			Factor means[1]
	Harvest dates			
	August 1	August 26	September 20	
Fungicide[2]	15.1	21.3	7.5	14.6
No fungicide[2]	41.5	36.9	12.3	30.2
Low fertilization[3]	37.4	29.1	7.8	24.8
High fertilization[3]	19.3	29.0	7.8	18.7
Date means[4]	28.3	29.0	8.8	
FLSD .05	16.1	NS	NS	
.01	23.0	NS	NS	

[1] FLSD for factor means 11.6 and 16.0 for P = 0.05 and P = 0.01, respectively.
[2] Over high and low fertility regimes.
[3] Over fungicide and no fungicide regimes.
[4] FLSD for date means 10.1 and 13.8 for P = 0.05 and P = 0.01, respectively.

mummified berries were infected by *G. cingulata*, *Fusarium* spp., *Penicillium sp.*, *Myscosphaerella coffeicola* and *Gliocladium* sp.

At the beginning of the field experiment, branch dieback on individual trees ranged from 20 to 48.4% having an overall mean severity of 34% for the experimental site. Twenty-eight, 29, and 9% of diseased berries were recorded for the August 1, August 26, and September 20 pickings, respectively (table 3). In the first picking fungicide had a highly significant effect (P = 0.01) in reducing berry spotting or mummification from 41.5% without treatment to 15.1% with treatment. Best control was found at high fertilization plus fungicide application (7.5% diseased berries) and the worst was found at low fertilization without fungicide (52% diseased berries, table 4). In the second and third pickings, fungicide-treated trees tended to have less mummified berries but the fertilizer effects were no longer evident. In general, fungicide reduced diseased berries by 57%, high fertilization by 41% and the two practices combined resulted in 85% control.

DISCUSSION

Wellman (27) commonly isolated *C. gloeosporioides* as the dominant fungus on dying coffee branches. We have consistently isolated *C. gloeosporioides* from diseased branches. Isolates of *C. gloeosporioides* from both coffee and soybean caused twig lesions and dieback when coffee was artificially inoculated. Workers in Africa (5, 14) have considered all strains of *C. gloeosporioides* other than the coffee berry disease strain as "saprophytic." Small (20) and Hocking (8) found that both the coffee berry disease fungus and coffee anthracnose fungi had large and overlapping host ranges. Researchers (5, 14) have noted the extreme variation among *Colletotrichum* isolates from coffee. Although *C. acutatum* can easily be separated from *C. gloeosporioides* on the basis of distinctive conidial size and shape, isolates of *C. coffeanum* and *C. gloeosporioides* do not differ in conidial morphology and are separated by their appearance in culture and their ability to cause lesions on green berries. We believe that giving a species designation to the coffee berry strain of *C. gloeosporioides* found in East Africa may be misleading since the difference in host range and cultural appearance falls in the normal intraspecific variation of *C. gloeosporioides*. Frossard (3) has noted the polymorphic and unstable nature of *C. gloeosporioides*. We consider that the coffee berry disease pathogen is a physiological race of *C. gloeosporioides*

TABLE 4.—*Incidence of diseased berries from coffee trees under four regimes of fertilization and fungicide*

Treatment	Percentage of diseased berries		
	Harvest dates		
	August 1	August 26	September 20
Fungicide[1] + low fertilizer[2]	22.8	21.5	7.5
Fungicide + high fertilizer	7.5	21.0	12.3
No fungicide + low fertilizer	52.0	26.8	7.8
No fungicide + high fertilizer	31.0	37.0	7.8
FLSD .05	22.6	NS	NS
.01	32.5	NS	NS

[1] Fungicide = six monthly sprays of Benlate 50 W plus Difolatan 4F at 227 g a.i. of each per acre.

[2] Fertility = low application 10-5-15 with Mg and micronutrients applied every 3 months at 227 g per tree; high fertility, the same fertilizer applied at 454 g per tree.

and that infections that occur in the berry are part of the disease syndrome (anthracnose) caused by the same fungus.

Although *C. gloeosporioides*, *C. coffeicola*, or *F. stilboides* were sometimes found individually associated with berry lesions, more commonly, mixed infections occurred. Each fungus alone is reported as berry rot pathogen (1, 11, 13, 19 and 25). Our finding of mixed infections suggests further studies are needed not only to test the individual and combined effects of these fungi on berry spotting but also to determine the succession of microorganisms on the coffee berry during its development and senescence. Berry lesions and mummification may well be caused by a pathogen complex and not just by the action of a single pathogen. Mixed infection of *Colletrotrichum*, *Cercospora*, and *Fusarium* has also been noted on coffee berries in Costa Rica and Guatemala (18).

Eye spot (*C. coffeicola*), anthracnose (*C. gloeosporioides*) and scaly bark disease (*F. stilboides*) are all reported to increase when plants are under stress of low fertility (1, 11, 13). Puerto Rican farmers believe that high rates of balanced fertilizer effectively control paloteo or coffee anthracnose and berry necrosis. Foliar applications of superphosphates were found to greatly reduce coffee berry disease in Kenya (21, 22) and anthracnose in Brazil (17). In our studies, at the first picking, berry spotting and mummification was reduced approximately 41% by use of higher fertilizer applications alone. A synergistic effect of fungicide and fertilizer applications was detected by the extreme reduction in berry lesions and mummification when the two practices were combined.

Fungicide applications reduced berry lesions and mummification by 57%. When recommending a fungicide program for control of coffee diseases, one must carefully weigh the benefits and risks. Furtado (4) found that copper fungicides increased the populations of the coffee berry disease pathogen after continued long term use. Benzimidazoles, although they were initially very effective against coffee berry disease, led to the development of benzimidazole resistant races of coffee berry disease pathogen (10, 16). For protectant fungicides to be effective they must have long residual action and resistance to the high humidity and rainfall of coffee production zones. While captafol has been successful under these conditions, less success has been found with chlorothalonil (9). In our studies of yam anthracnose a similar reaction of these fungicides has been noted. To achieve the advantages of both the systemic and protectant fungicides, tank mixes of the two or alternate applications are suggested (10). These schemes can prevent the population build-up of the pathogen strains resistant to systemic fungicides, which, when uncontrolled can cause major economic losses. Gibbs (6) found that long harvest periods make control of coffee berry disease more difficult. In our studies the best control was detected in the first picking. Although coffee berry disease is effectively controlled in early pickings, it seems that control in later pickings is reduced through fungicide erosion. Fungicides are not usually applied during the harvest season because of possible residue developing in the berries and because labor is concentrated in harvest activities. Use of hormones to synchronize flowering and shorten the harvest season may be helpful to make fungicide control programs more effective. Fertilizers which help reduce disease severity may be useful when applied just before or during the harvest season because they could reduce disease severity of later harvest without harmful fruit residues.

The type of system which can be used to control coffee diseases in Puerto Rico must be tailored to the Island's small size and high population density quite unlike coffee areas in other parts of Latin America and in Africa. Airplane application may not be wise because of the small field sizes, irregular terrain, and the high human population which could be affected by pesticide drifting. Nutman et al. (15) stressed the need for better coverage in the program for fungicide control of coffee berry disease because conidia in the upper canopy were most effective in spreading coffee berry disease and systemic fungicides, while moving readily in herbaceous plants do not readily move in woody species such as coffee. For these reasons, complete coverage of the coffee tree, particularly the top, is recommended. In conducting a spray program, auxiliary practices such as pruning and adoption of dwarf varieties may be warranted. Use of microdroplet applicators which use low volumes of water may make fungicide spray programs more workable. Spraying by helicopter should also be considered.

Nutman et al. (15) stressed the variation of population of the coffee berry disease pathogen over the season. Although this could be used to decrease the number of sprays needed to control coffee berry disease, Waller (26) noted that rainy season conditions are generally favorable for the reproduction of the pathogen, and spray schedules are safer than predictive systems. We believe that there are critical periods of coffee susceptibility that are important in developing a fungicide program. Coffee berries appear to be most susceptible to coffee berry diseases during flowering and in berry senescence. Therefore, application of systemic fungicides should be timed to give adequate protection during these critical periods. In stages of berry fill when berries are less susceptible, use of protectant fungicides alone may provide excellent control and reduce fungicide costs and the problems of development of resistant strains.

Our studies indicate that coffee anthracnose is widespread and severe in Puerto Rico and that integration of cultural practices such as fertilizer and fungicide applications can be used for their control. Since the study indicates a synergism between these practices, it suggests that further work should look deeper into the integration of other practices such as coffee cultivar selection, pruning, and fungicide application techniques for developing an effective disease control program.

RESUMEN

La antracnosis del cafeto, conocida en Puerto Rico popularmente como "paloteo", es la enfermedad más importante de cafetos en producción en la Isla. Esta enfermedad se caracteriza por la muerte regresiva de las ramas, defoliación, manchas y momificación de las bayas. El hongo *Colletotrichum gloeosporioides* y su estado perfecto, *Glomerella cingulata*, es el hongo más comúnmente asociado con ramas enfermas. Dos cepas de *C. gloeosporioides*, una aislada de hojas enfermas de soya y la otra aislada de bayas de café, causaron la necrosis típica en ramas de café inoculadas *in vitro*. Las ramas sin inocular permanecieron saludables bajo las mismas condiciones del experimento. De las manchas necróticas en bayas de árboles con antracnosis, se aislaron los hongos *C. gloeosporioides*, *Cercospora coffeicola* (causante del "ojo de sapo") y *Fusarium stilboides* (causante del mal de la corteza). Las lesiones típicas en las bayas son marrón oscuro, a veces con el centro deprimido y usualmente contienen las tres especies de estos hongos. Fascículos de conidióforos de *C. coffeicola* forman un anillo dentro de la lesión bordeando la periferia de la mancha. Acérvulos de *C. gloeosporioides* y los esporodoquios de *F. stilboides* comparten la parte central de la mancha. Se logró una reducción de 41% en el número de bayas enfermas al aumentar la cantidad de abono; el fungicida contribuyó a disminuir el número de bayas enfermas en un 57%. La combinación de abonamiento intenso y la aplicación de fungicida redujo hasta 85% el número de bayas afectadas.

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