Eur J Plant Pathol (2024) 168:527–540
https://doi.org/10.1007/s10658-023-02781-1

# Coffee fruit rot in Puerto Rico: distribution, ecology and associated fungi

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Accepted: 25 September 2023 / Published online: 17 October 2023


**Abstract** Anthracnose of coffee fruits caused by *Colletotrichum* species is responsible for major losses in coffee production worldwide. In most coffee-producing countries, this disease has been present for many years. Recently coffee fruit rot (CFR), a disease complex of *Colletotrichum* spp. and *Fusarium* spp. as casual agents, was described in Puerto Rico but it has not yet been quantified. This study surveys incidence and severity of CFR, correlates it to elevation, cultivation methods (full sun, shade or intercalated with taller plants) and the presence of the coffee berry borer (CBB), and identifies rot-associated fungi. We found that CFR is present throughout Puerto Rico at varying levels and that environmental and cultivation factors are associated with CFR incidence and severity. Specifically, increased altitude and shade correlated with lower CFR incidence. In addition, fruits damaged by the CBB were found to have significantly more disease than undamaged fruits. Lastly, we isolated 7 fungal genera associated with CFR, the most prevalent being *Colletotrichum* and *Fusarium*. Overall, this study suggests that planting coffee at higher altitude under shade and controlling CBB can reduce CFR.

**Keywords** Coffee fruit rot · Coffee berry disease · Anthracnose · *Coffea arabica* · *Colletotrichum* · *Fusarium* · Coffee berry borer

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## Introduction

Coffee is one of the most popular beverages in the world. In 2020 the global coffee market was valued at > $465 billion (Azoth Analytics, 2020). *Coffea arabica* L. (Gentianales: Rubiceae) supplies most of the world's coffee, and this plant is affected by pathogens and insect pests that can cause severe losses. Major coffee diseases include coffee fruit rot and anthracnose (Thurston, 1998; Serrato-Díaz et al., 2020). The most serious coffee pest is the coffee berry borer (or CBB) *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae).

**Coffee fruit rot** (CFR) is a type of **anthracnose** that can affect external or internal tissues, where *Fusarium* may also play a role in the disease (Serrato-Díaz et al., 2020). External symptoms are sunken, discolored lesions on green, yellow, and red coffee fruits, sometimes progressing to dry or mummified fruits (Fig. 1A). Internal symptoms are water-soaked lesions in the mesocarp and browning of the endosperm (Fig. 1B). Another type

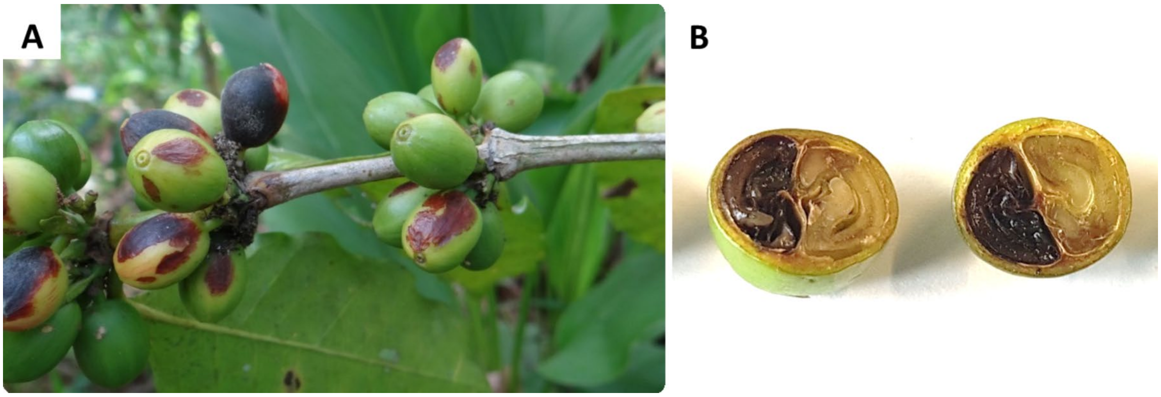


**Fig. 1** Symptoms of coffee fruit rot (CFR). **A** Coffee branch with fruits showing external CFR symptoms: sunken, discolored lesions on green, yellow and red coffee fruits, sometimes progressing to dry, mummified fruits. **B** Coffee fruit cut transversely showing internal rot symptoms: water-soaked lesions in the mesocarp and browning of the endosperm

of anthracnose that overlaps with CFR is **coffee berry disease** (CBD), which describes the disease of green coffee fruits at high altitudes in Africa, caused by *C. kahawae* sp. *kahawae*. Since the name CBD has been defined as coffee fruit rot exclusively in Africa (Serrato-Díaz et al., 2020), we use CFR here as a more general term for the disease in Puerto Rico.

Coffee fruit rot in Puerto Rico is reported to be caused by four species of *Colletotrichum* (*C. fructicola*, *C. siamense*, *C. theobromicola* and *C. tropicale*, Serrato-Díaz et al., 2020). Other fungi are also associated with CFR; *Fusarium* has been suggested to be important (Serrato-Díaz et al., 2020) but few data are available on fungi other than *Colletotrichum*. Because anthracnose reduces coffee yields, it has been studied in many parts of the world. In Puerto Rico, however, only two studies have examined coffee fruit rot and anthracnose (Mignucci et al., 1985; Serrato-Díaz et al., 2020). In 2021 coffee fruit rot became unexpectedly common and severe in Puerto Rico, reducing the crop by an estimated 5–10% and making front page news (Tolentino Rosario, 2021). A more complete understanding of the disease, its extent and its causes is urgently needed.

Coffee fruit rot caused by anthracnose has been studied, especially Africa and South America, but information about the disease and related pathogens is limited. For example, little is known about how the disease is influenced by environmental or biotic factors such as the presence of CBB (Motisi et al., 2022). Overall, a better understanding of this disease could lead to better control techniques that result in better, more sustainable crop production.

In this study we ask the following questions:

1. What is the incidence and severity of CFR in Puerto Rico? Surveys for CFR were conducted at the site, plant, branch, and fruit level. The hypothesis was that CFR would be found in almost all sites but there would be variations at the plant, branch, and fruit level (Mignucci et al., 1985).
2. Does the incidence or severity of CFR correlate with altitude and shade? Higher altitudes and shade are hypothesized to be less conducive to disease due to lower temperatures.
3. Does damage by CBB increase the occurrence of CFR? Because CBB was reported to act as a dispersal agent of fruit rot-causing fungi (Serrato-Díaz et al., 2020), we hypothesized that there will be an association between CFR and perforation of coffee fruits by CBB.
4. Does the identity and frequency of fungi on rotten tissue depend on the stage of maturation of coffee fruit (green, yellow, red) or the severity of rotting (initial, medial, final)? We hypothesize that frequency of fungi will depend on all these factors because they affect the microenvironment on the surface of the fruit, which in turn influences the sequence of colonization and the prevalence of some fungi over others.
5. Does internal rotting of coffee fruit correlate with external rotting? We hypothesized no correlation, based on previous studies that demonstrated

that species of *Colletotrichum* differ in ability to cause internal vs. external rots (Serrato-Díaz et al., 2020).

## Methods

### Definitions

**Incidence** is defined as "the number of plant units that are visibly diseased…usually relative to the total number of units assessed" (Madden & Hughes, 1995). It is expressed as a percentage or proportion of binary data. Here incidence is measured per site, plant, and branch (Fig. 2). Disease **severity** is defined as the proportion (or percent) of plant area destroyed by a pathogen (Agrios, 2007); in this case it is measured at the level of the coffee fruit. These are standard definitions in plant pathology, though the terms are used differently in other fields.

### Study area

In Puerto Rico coffee is grown mainly in the central-west region of the Cordillera Central (or central mountain range) (Flores, 2011). Surveys were conducted in ten municipalities within this region from August to November 2019,the season when coffee fruits are available. A total of 100 sites in 58 farms and rural areas were sampled,one sample per site. All sites sampled were planted with one or several of the following cultivars of *C. arabica*: Caturra, Borbón, Limaní, Catuaí, and Frontón. Because of the number of sites sampled, it was not possible to get weather and environmental data for every site. History of pesticide and fertilizer use was likewise unavailable.

All surveys were completed between 10am and 2 pm. The environmental variables listed in Table 1 are averages for all months of our survey: August, September, October, and November, 2019 (the period when fruits are susceptible to rot). During these months temperatures ranged from 19 °C to 33 °C, and rainfall from 62 to 74 mm (Table 1). Altitude ranged from 188 to 912 m.a.s.l. (meters above sea level). Sites were classified as full sun, shade and intercalated with taller crop plants. Shade refers to coffee growing under canopy of other trees. In Puerto Rico *Inga vera, Inga laurina, Andira enermis* and *Guarea guidonia* are the most important and abundant shade trees in the coffee agroecosystem (Arango, 2007). 'Intercalated' refers to coffee alternating with plantains or bananas and/or citrus, which provide partial shade. Coffee described as growing in full sun has no surrounding shade trees and is not intercalated with any other crop (Wilson, 1999).

### Incidence of coffee fruit rot at site level

Incidence at site level was defined as the proportion of sites with at least one diseased plant (Fig. 2). For each site, disease incidence was recorded for

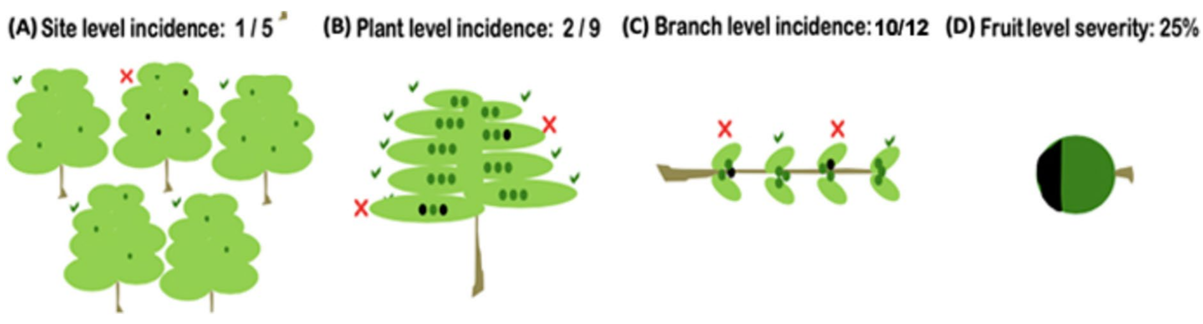


**Fig. 2 Sampling strategy used in this study.** Incidence is measured per site, plant, and branch; severity is measured per fruit. **A Site incidence** is the proportion of trees per site with at least one diseased fruit (shown in black) to the total number of trees per site. This study used 25–30 trees per site depending on availability. **B Plant incidence** is the proportion of branches per plant with at least one diseased fruit to the total number of branches sampled. This study sampled 10–15 branches per plant, 3–5 from the top, 3–5 from the middle, and 3–5 from the bottom of the plant. **C Branch incidence** is the proportion of diseased fruits to the total number of fruits per branch. Five branches from each site were counted. **D** Fruit severity is the percentage of rotting tissue per fruit. Fifty fruits per site were included

**Table 1** Altitude (meters above sea level) of sites sampled, average rainfall (mm) per month and low and high temperature (°C) from August to November 2019 of the surveyed areas. Rainfall and temperature data were obtained from weatherspark.com and do not reflect the variation among sites in each municipality

| Municipality | Number of sites visited | Altitude (m.a.s.l.) | Rainfall/month (mm) | Temperature range (°C) |
|---|---|---|---|---|
| Adjuntas | 23 | 430–681 | 65 | 29–33 |
| Ciales | 8 | 188–373 | 74 | 22–30 |
| Guayanilla | 3 | 343–370 | 62 | 22–32 |
| Jayuya | 21 | 558–887 | 69 | 19–27 |
| Lares | 11 | 312–588 | 71 | 21–29 |
| Maricao | 2 | 811–892 | 65 | 19–27 |
| Mayaguez | 1 | 199 | 66 | 23–31 |
| Orocovis | 2 | 432–521 | 71 | 19–27 |
| Utuado | 12 | 354–628 | 70 | 21–29 |
| Yauco | 17 | 655–912 | 62 | 22–31 |

thirty plants in an area of about 30 × 30 m. The thirty plants were selected by taking 20 paces and then selecting the first available tree. In some sites, adjustments to sampling were necessary because sites were smaller, or parts of the delimited area were inaccessible. Absence or presence of disease was noted for each plant, looking at all branches with coffee fruits. Only fruits were considered, even though anthracnose also occurs on leaves and stems (Gaitán et al., 2015). A total of 2946 plants were evaluated to determine disease incidence at site level.

### Plant level coffee fruit rot incidence

At the plant level, presence or absence of CFR was recorded for each plant by inspecting the fruits on all branches. Only external rot of coffee fruits was considered (since internal rot was not visible in field). Plant disease incidence was measured on ten randomly selected plants per site. For each plant, 10–15 branches (depending on availability) were selected: 3–5 branches from the top, 3–5 from the middle, and 3–5 from the bottom of the plant. A total of 963 plants (13,832 branches) were surveyed.

### Coffee fruit rot incidence at branch level

At the branch level, incidence was defined as the proportion of fruits with rot (internal or external). At each site five branches, each from a different plant, were selected randomly. Branches were bagged and transported to the lab in coolers with ice and evaluated the day after collection. For each branch, all fruits were detached and separated in two groups: fruits that showed external rot symptoms and fruits without external rot symptoms. Fruits were then separated by color (maturity) into four categories: green, yellow, red, and black (mummified, dried or raisin). Green, yellow and red fruits correspond to successive stages of ripening. Fruits with external rot of each color were counted for each branch, and 50 fruits were randomly selected to evaluate severity at fruit level for each site (see below). All remaining fruits (both with and without external rot) were cut in half to determine internal rot. Incidence at the branch level was measured in 940 branches (55,574 fruits). Because there is no way to visually distinguish the cause of mummification, culturing pieces of rotting tissue (described below) allowed for verification of the presence of fungi.

### Coffee fruit rot severity at fruit level

Disease severity per fruit was measured both externally and internally and was defined as the percentage of fruit area exhibiting signs of rot (Agrios, 2007). For each site, fifty fruits were selected randomly from all fruits, including those rotted and non-rotted, and perforated and not perforated by CBB. A visual scale from 0 to 100% was used to assess rot (Serrato-Díaz et al., 2020). Examples of internal and external rots are shown in Fig. 1. For internal rot, each fruit was cut in half and the exposed area was evaluated. For each fruit the following data were recorded: percent internal and external rot, color (green, yellow, red or raisin), the presence of the coffee berry borer (CBB) and presence of the fungus *Beauveria bassiana* (Bb). Presence of the CBB was indicated by a small, circular hole at the distal end of the fruit that is characteristic of CBB entry. Bb was identified by the presence of sporulating colonies and typical white mycelium growing on the CBB or near the hole bored by the CBB. Presence of CBB and Bb was determined since CBB has been proposed as a dispersal agent of pathogenic fungi, and Bb as a potential biological control agent for CFR (Serrato-Díaz et al., 2020). A total of 4,900 fruits were evaluated.

### Isolation of fungi

From every site 20 pieces (20–50 $mm^3$) for each of four categories of fruit rot (external initial, external medial, external final/ mummified, and internal rot) were sliced. Each piece was taken from a different fruit. For external fruit rot the following categories were defined: the initial stage ranged from 2–40% of total tissue with rot, medial stage from 41–75%, and final stage from 76–100%. Fruit pieces were surface sterilized in 70% ethanol for one minute, 1% sodium hypochlorite for one minute, rinsed in sterile distilled water for one minute, and then patted dry with a sterilized paper towel. The fruit pieces were then plated on potato dextrose agar (PDA) amended with 0.01 g/L streptomycin and 0.05 g/L ampicillin. Plates were incubated at approximately 23 °C with an 12:12 h photoperiod for two weeks. Fungal isolates that sporulated were identified to genus based on colony color, texture, and growth rate, and on presence, size and shape of reproductive structures such as conidia, pycnidia, acervuli, sporodochia, perithecia, asci and ascospores, if present (Barnett & Hunter, 1998). Isolates that were identified to genus were counted and the frequency of each genus found in each stage of rot (initial, intermediate, final/ mummified, and internal rot) was calculated.

### Data analysis

One-way ANOVAs and Tukey multiple comparisons of means tested the difference in disease incidence and severity between the different types of coffee cultivation (full sun, shade and intercalated). These tests were also used to determine significance of the difference in internal and external fruit rot severity in fruits with vs. without CBB, and to determine differences in frequencies of fungi isolated among the stages of fruit rot. All data from fruit rot analyzed by ANOVAs were transformed using the Bliss angular transformation (Bliss, 1937) to fulfill requirements of normality and homogeneity of variance. Untransformed data are presented in the figures.

Interval classes for altitude, incidence at plant, branch and fruit level were defined using the Jenks natural breaks classification, which reduces the variance within classes and maximizes the variance between classes (Jenks, 1967). Pearson's product-moment correlation was used to detect correlations between altitude and disease incidence, internal fruit rot and external fruit rot. Pearson's correlation was also used to test the relationship between internal rot and external rot. All analyses were done with R version 1.2.1335 (R core team, 2019). ANOVAs were performed with the aov() function and pairwise post-hoc tests with TukeyHSD () function with a confidence interval of 95%.

## Results

### Disease incidence at site level

Incidence per site ranged from 6% to 100;%. in 95% of sites it was greater than 20% (Fig. 3A). Significant differences were found among the three types of coffee cultivation ($F = 4.16$; $p = 0.0185$): coffee grown in full sun had 14% higher disease incidence than coffee grown under shade ($p = 0.035$) or intercalated with plantains and/or citrus ($p = 0.016$). No significant difference was detected in disease incidence between shade and intercalated sites ($p = 0.99$) (Fig. 3B). In sun coffee an average of 71% of coffee plants per site had CFR symptoms, while in shade and intercalated coffee an average of 60% of plants had symptoms.

A significant but weak negative correlation was found between altitude and CFR incidence at the site level ($r = -0.22$; $p = 0.03$): disease incidence at sites at higher altitudes (over 794 m.a.s.l) was 20% lower than at sites from 168 to 365 m.a.s.l. (Fig. 4). As altitude increased there was a reduction in how many plants per site had at least one fruit with rot.

### Fruit rot incidence at plant level

Disease incidence at plant level ranged from 7 to 100% of branches; in $> 80\%$ of plants it was $< 47\%$ (Fig. 5A). Disease incidence differed significantly among coffee in full sun, shade, and intercalated with other crops in ($F = 21.59$; $p < 0.0001$). Plants cultivated in full sun had 12% more diseased branches than those grown in shade ($p = 0.0002$), and plants grown in shade had about 6% fewer diseased branches than those intercalated ($p = 0.0145$). Branches of

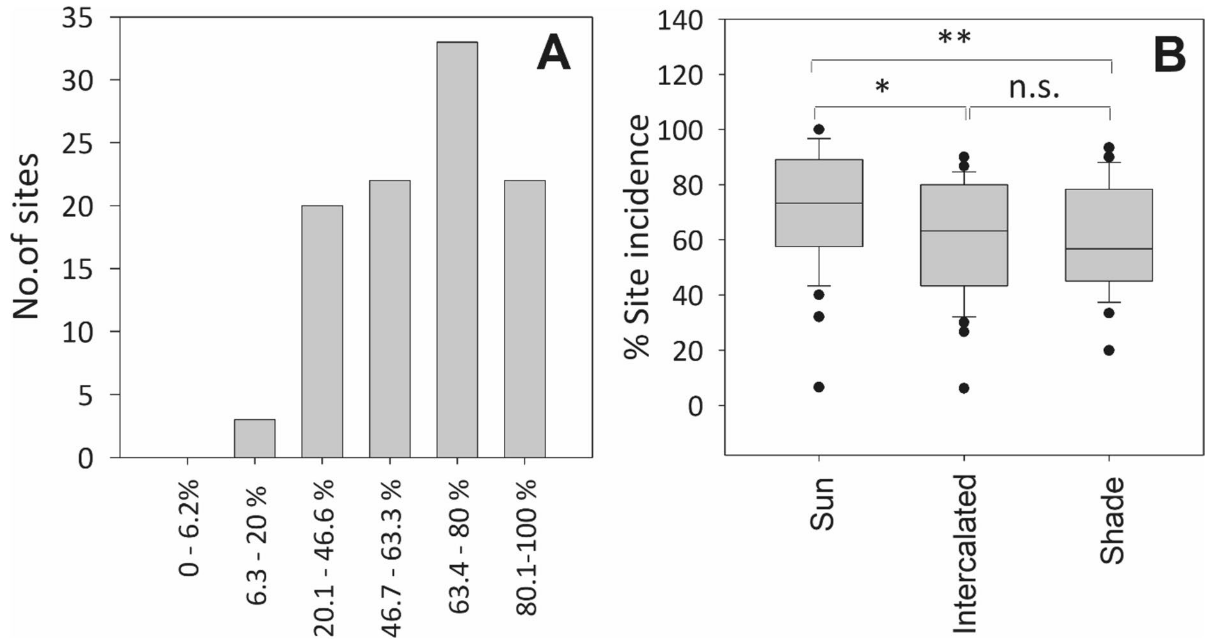


**Fig. 3** Site level distribution of incidence of coffee fruit rot (CFR) in Puerto Rico **A** CFR incidence per plot: average number of plants per site with at least one fruit with CFR. Bars represent the number of sites with a particular range of CFR incidence. Ranges were defined using the Jenks natural breaks classification. **B** incidence according to type of coffee cultivation. Significant differences from ANOVAs are shown by asterisks: *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$

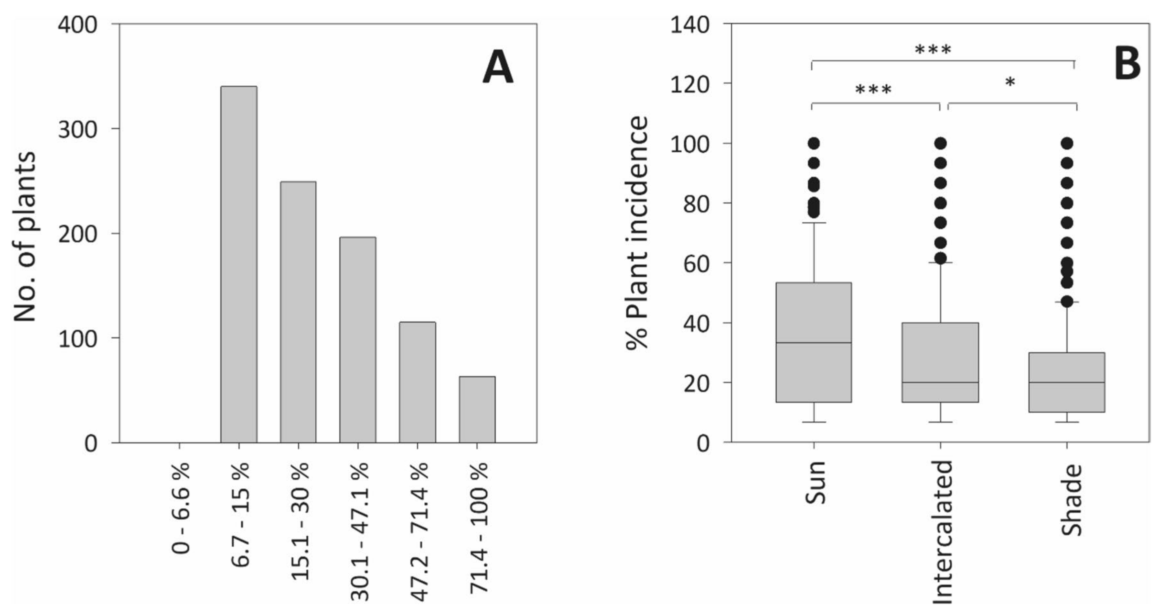


**Fig. 4** Plant level distribution of incidence of coffee fruit rot (CFR) in Puerto Rico. **A** CFR incidence per branch: Bars represent the proportion of branches with a particular range of CFR incidence **B** Incidence per plant according to type of coffee cultivation. Significant differences from ANOVAs models are shown by asterisks: *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$

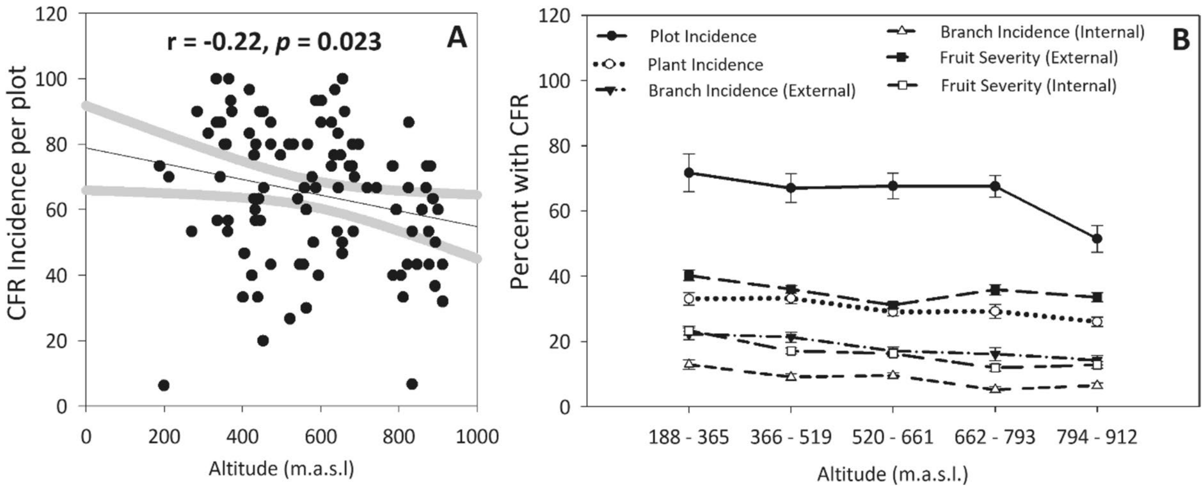


**Fig. 5** Altitude in meters above sea level (m.a.s.l.) vs. incidence and severity of coffee fruit rot (CFR) in Puerto Rico. **A** Linear regression of altitude vs. incidence per plot. The light grey lines are the 95% confidence interval of the regression line. **B** Altitude vs. site, plant and branch incidence and fruit severity. Mean ± 1 s.e. is shown

coffee in full sun had an average disease incidence of 35% whereas disease incidence in intercalated and shade and shade coffee was 29% and 23%, respectively (Fig. 5B). Altitude and disease incidence at the level of the plant had a significant but weak negative correlation (r = -0.10; p = 0.003); for sites located over 520 m.a.s.l. plant disease incidence was less than 30% (Fig. 5). As altitude increases there is a reduction in the number of branches per plant with at least one rotted fruit.

## Fruit rot incidence at branch level

The following results are based on all the fruits sampled (with and without external, visible CFR symptoms). Incidence of internal fruit rot ranged from 0–94% per branch, and external rot ranged from 0–100%. Average internal fruit rot incidence was 9%, while average external rot was 18%. Most branches had less than 15% of fruits with internal or external rot (80% and 60% of branches, respectively) (Fig. 6A) but 58.7% (552 of 940) of branches had one or more mummified fruits. Unexpectedly, green fruits had more internal rot than yellow fruits (F = 127.4; p < 0.0001) (Fig. 6B). Of the 55,574 fruits evaluated, 18% showed signs of penetration by CBB, including fruits with and without CFR. Significant but weak negative correlations were found between altitude and internal (r = -0.16; p < 0.0001) and external (r = -0.12; p = 0.0001) fruit rot incidence at the branch level. Internal rot incidence for sites located over 662 m.a.s.l. was less than half (5.2%) of incidence at sites from 188 – 365 m.a.s.l. (12.9%), while external rot was 8% less for sites located over 794 m.a.s.l. (Fig. 5).

## Fruit rot severity

The following results are based on samples of fruits that had visible (external) CFR damage. Most fruits had < 10% internal rot and external rot. However, 1259 fruits (> 25%) had more than 80% of surface area with external rot (Fig. 7A). Internal severity of fruit rot averaged 16%, and external fruit rot severity averaged 36%. Significant but weak negative correlations were found between altitude and internal ($r^2$ = -0.11; p < 0.0001) and external ($r^2$ = -0.04; p = 0.003) rot severity at fruit level. Internal rot severity at sites located over 794 m.a.s.l. was half that (12.8%) at sites 188 – 365 m.a.s.l. (23.3%), while external rot was reduced by 10% at sites located over 794 m.a.s.l. (Fig. 5). Additionally, there was a significant, moderate positive correlation between internal and external fruit rot ($R^2$ = 0.42; p = < 0.0001).

Considering all fruits evaluated, both internal and external rots were significantly more severe in fruits with CBB damage. Average internal rot for fruits without CBB was 12%, compared to 24% for fruits

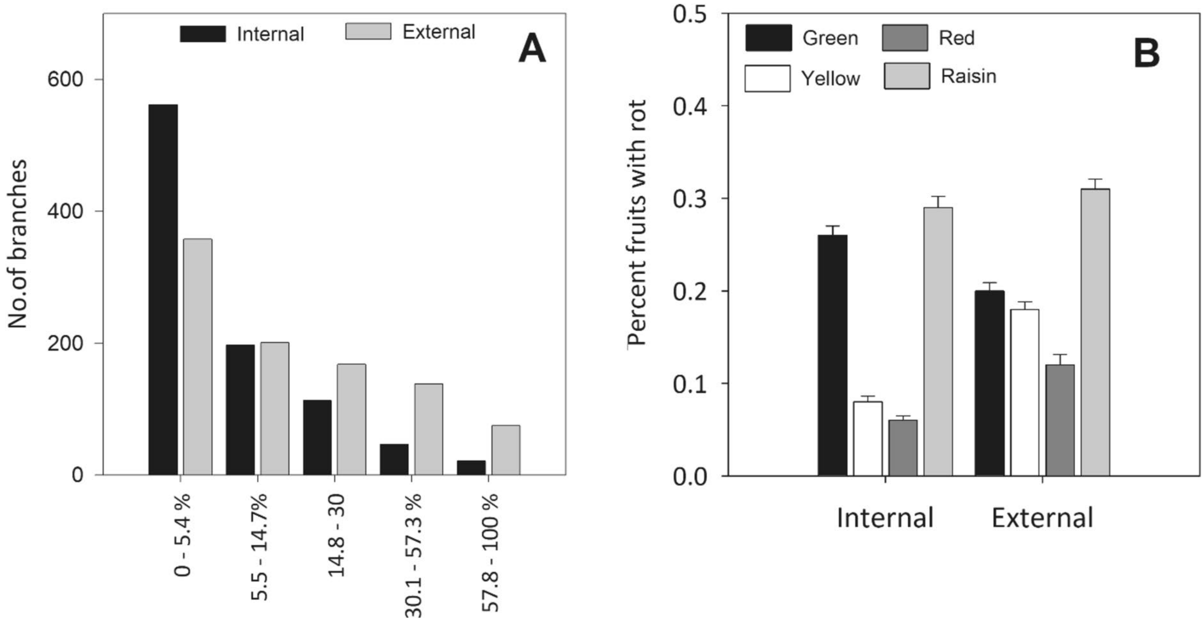


**Fig. 6** Distribution of incidence of fruit rot per branch of coffee fruit rot (CFR) in Puerto Rico **A** Number of branches in classes of fruit rot incidence **B** Incidence per branch according to fruit color. Bars with different letters are statistically different according to Tukey's HSD post hoc test (α = 0.05). Error bars indicate the standard error; comparisons according to fruit color were done separately for external vs. internal fruit rot

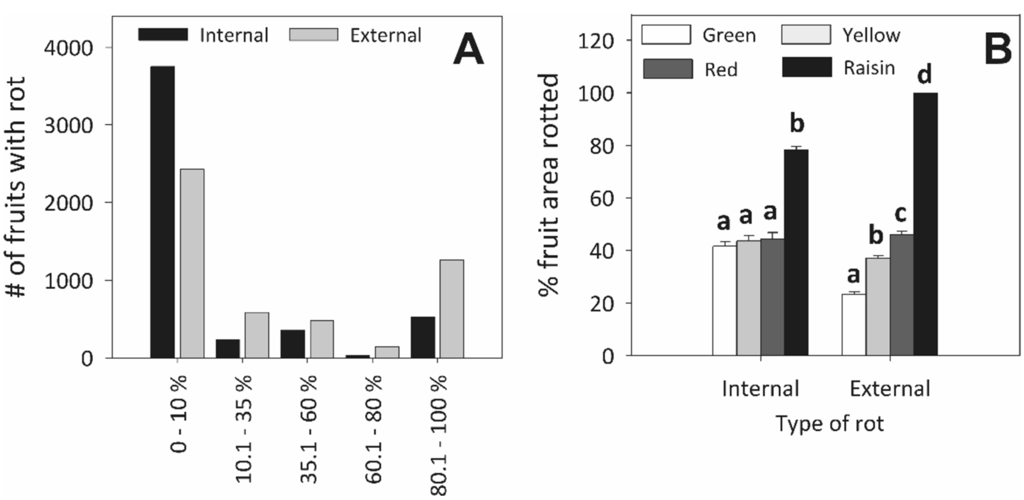


**Fig. 7** Coffee fruit rot (CFR) severity in Puerto Rico. **A** Percentages of severity per number of fruits sampled (internal and external). **B** Percent of fruit area rotted grouped by type of rot (internal or external) and maturity of fruit (green, yellow, red, raisin). Bars with different letters are statistically different according to Tukey's HSD post hoc test (α = 0.05). Error bars indicate the standard error; comparisons according to fruit color were done separately for external vs. internal fruit rot

perforated by the CBB ($F = 29.7$; $p < 0.0001$). External rot in fruits without CBB averaged 33%, while external rot in fruits infested by CBB averaged 38% ($F = 4.71$; $p = 0.0002$). Only 27 of the 4898 fruits had sporulating *B. bassiana* colonies, not sufficient to conduct meaningful statistical analysis.

Isolation and identification of fungi

From both internal and external fruit rot tissue six genera of fungi were isolated and identified: *Colletotrichum, Fusarium, Nigrospora, Phoma, Phomopsis* and *Pestalotiopsis*. *Colletotrichum* and *Fusarium* were the most common fungi isolated from all tissues.

Significant differences were found in the frequencies of these genera among the stages and location of fruit rot. *Fusarium* was most common genus in the final/ mummified stage of external rot (58% of isolates) and the least common genus in the initial stage (35% of isolates) (Fig. 8). *Colletotrichum* was most common genus in the initial stage of external rot (42%) and least common genus in the final/ mummified stage of external rot (27%). *Nigrospora* was also more common in initial stages of external rot than in later stages, while *Phoma* was more common in later stages.

For internal fruit rot, the frequency of *Fusarium* was significantly higher than *Colletotrichum* ($F = 204.1$; $p < 0.0001$). *Fusarium* was found at higher frequency in internal rot (64%) than in external rot (47%) (Fig. 8). The opposite was true for *Colletotrichum* which was higher in external fruit rot (35%) than in internal rot (21%).

## Discussion

Coffee fruit rot (CFR) causes losses which have a negative effect on the coffee industry, from farmers to consumers. The disease even changes the quality of coffee brewed from affected beans, making it less aromatic and more bitter (Ribeyre & Avelino, 2012). To control CFR we need to better understand its ecology. This study quantified the incidence and severity of CFR, because "without quantification of disease no studies in epidemiology, no assessment of crop losses and no plant disease surveys, and their applications would be possible" (Kranz, 1988).

Overall, CFR was found in 100% of sites, 65% of plants and 30% of branches sampled. External fruit rot was found in 17% of fruits on branches with at least one rotted fruit and internal fruit rot was found in 8% of fruits.

It is likely that these figures underrepresent the extent of CFR, for several reasons. Sites were evaluated just one time, so fruits that were healthy when surveyed could have developed rot later. Also, fruit rot is more likely to progress than heal once the rot begins, so more yellow fruits than green fruits would be expected to be rotten. However, we saw that green fruits had more internal and external rot than other, riper fruits. The most plausible

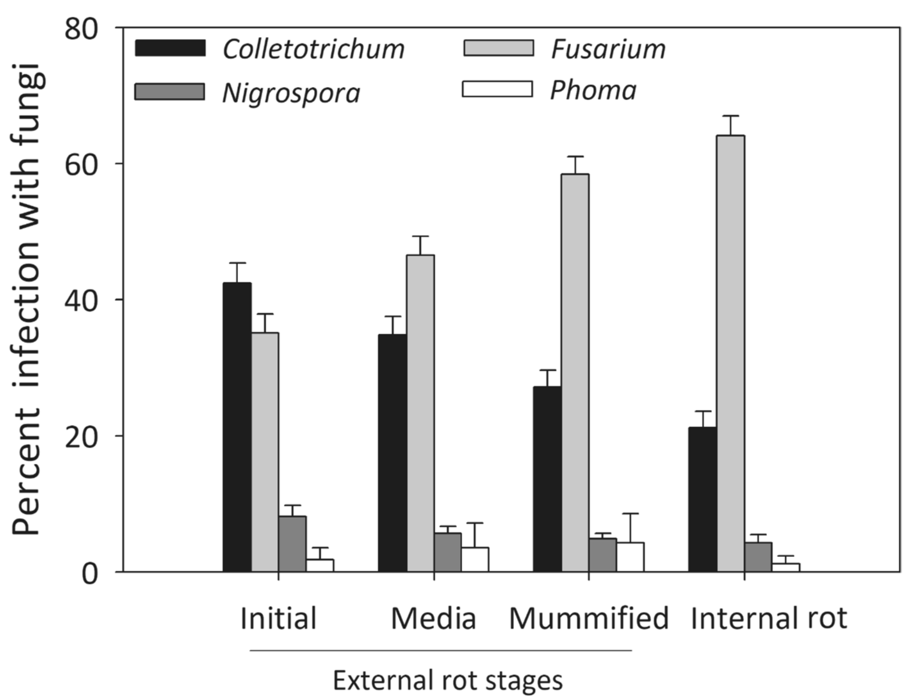


**Fig. 8** Frequency of the four most isolated fungal genera from three stages of external and internal rot of coffee fruits in Puerto Rico

explanation is that some green fruits with rot are abscised by the plant. While this has not been previously proposed for CFR, it is well known in coffee fruits infested with CBB (Vega et al., 2009). If this is true, these abscised fruits on the ground were overlooked and losses to CFR are even greater than reported here and in previous studies. Similarly, coffee berry disease is more common in green than in mature fruits (Motisi et al., 2022).

The amount of CFR found in Puerto Rico is similar to reports from other parts of the world. Prevalence, or the number of sites with coffee fruit rot, is commonly reported as 100%, but the number of plants, branches, or fruits infected does not exceed 75% even in the most extreme cases. In southern Ethiopia for example, the number of sites with CBD was reported as 100% and the number of plants with CBD as 49% (Mohammed & Jambo, 2015). In eastern Ethiopia the number of sites with CBD was also reported as 100%, but incidence was as high as 70% (Alemu et al., 2016). In Cameroon CBD was found in about 50% of coffee fruits surveyed (Mouen Bedimo et al., 2007). Aside from the scarce data available from Africa, there are no studies published that survey CFR or coffee fruit anthracnose in other parts of the world. This information would be a useful step towards establishing a defense against the disease.

## Coffee fruit rot and altitude

At all levels (site, plant, branch and fruit) a negative, significant correlations were found between CFR and altitude. Altitude over 794 m.a.s.l. had the least disease, and a slight reduction in disease was seen at 500 m.a.s.l. and higher. The negative correlation between CFR with altitude could be an effect of temperature (which decreases about 6.5°C per 1000 m altitude). Lower temperatures at higher altitude could increase resistance of coffee plants to fruit rot-causing fungi, since *C. arabica* is adapted to high-altitude equatorial regions and temperatures of 15–24°C (Wilson, 1999). This information is important when considering the location of new coffee farms, as establishing them at higher altitude could decrease the occurrence of CFR. However, higher altitude also tends to increase the frequency of damage by the coffee berry borer (Mariño et al., 2017).

## Coffee grown in full sun vs. shade

Coffee grown in shade and intercalated coffee had significantly less CFR than coffee in full sun. It has been reported that growing coffee under shade trees significantly reduces losses due to *Colletotrichum* spp. (Bedimo et al., 2008). A reason for this is that shade significantly reduces temperatures, about five to eight degrees (Mariño et al., 2016), and these cooler temperatures may not favor the development of fruit rot.

However, the relationship between CFR and shade is likely to be complex. Modeling of coffee berry disease showed that shade reduced pathogen transmission but also speeded up pathogen spore germination, with contrary effects (Motisi et al., 2022). Shade also slowed fruit maturation, prolonging the green stage which is most susceptible to the disease. A comparison of trees used for shade would be useful to determine which trees are least permissive to the development of fruit rots (Motisi et al., 2022).

A plethora of previous studies have reported other disadvantages of coffee cultivation in full sun for plant health. The amount of CBB was higher in sun coffee than in shade coffee (Mariño et al., 2016). Shade coffee preserves biodiversity by acting as a refuge for forest biota in locations where deforestation is high (Perfecto et al., 1996). In addition to strengthening local ecosystems, biodiversity of these groups has benefits for pest control. For example, many ant species have been found to be predators of the CBB but are most effective in shade during the wet season (Armbrecht & Gallego, 2007). Anything that reduces these natural predators eliminates a natural remedy for the CBB and could increase our dependence on insecticides.

These findings are especially relevant in Puerto Rico where shade trees were eliminated in many farms as a strategy to control coffee leaf rust (Borkhataria et al., 2012). Farming coffee without shade was encouraged since the 1960s, and farmers were even paid to adopt the suggested strategies. Between 1982 and 2007 there was about a 70% decrease in shade-grown coffee as many Puerto Rican farms converted to growing coffee in full sun (Fain et al., 2017). However, growing coffee in full sun has been reported to reduce coffee leaf rust, so further work is needed to determine a level of shade that might help mediate both diseases, CFR and coffee leaf rust.

### Coffee fruit rot and the coffee berry borer

The coffee berry borer (CBB) was recently shown to be a potential agent of dispersal for fruit rot-causing fungi in Puerto Rico (Serrato-Díaz et al., 2020), as has also been reported in Africa (Masaba & Waller, 1992). Fruits with signs of penetration by CBB had 12% more internal rot and 5% more external rot than those without CBB, and these differences were significant, suggesting that CBB might be involved to some extent in the dispersal of fungal spores. Serrato-Díaz et al. (2020) conducted detailed experiments that showed that CFR was more common in fruits penetrated by CBBs which had been inoculated with *Colletotrichum* spores. In addition, the CBB has been found to carry spores of *Colletotrichum* and *Fusarium*, among other fungi, on its head, abdomen and gut (Carrion & Bonet, 2004). This might explain why *Fusarium* species were found at a higher frequency in internal than in external rotted tissue (Serrato-Díaz et al., 2020), because they are directly introduced to the inside of the fruit by the CBB.

In addition to possibly introducing fungal spores into coffee fruit, CBB perforation is also thought to create a microhabitat that favors the establishment of these fungi (Carrion & Bonet, 2004). The microhabitat is formed by both the perforation of the fruit and modifications brought about by CBB larvae. After the CBB bores into fruit, the first fungi to appear include *Fusarium oxysporum* and *F. solani* (Carrion & Bonet, 2004).

### Coffee fruit rot-causing fungi

*Colletotrichum* was the most common fungal genus in CFR tissue and *Fusarium* to be the second most common. Similar findings have been reported elsewhere, specifically in Africa, Asia, Australia, South America and Puerto Rico (Waller et al., 1993; Serrato-Díaz et al., 2020). *Colletotrichum* has also been found to cause other coffee diseases including premature fruit drop, stem rotting, blossom blight and leaf anthracnose (Siddiqui & Ali, 2014). However, some *Colletotrichum* species are ubiquitous in coffee plant tissues as endophytes not causing disease (Santamaria & Bayman, 2005). A novel result in this study was that *Colletotrichum* was significantly more common in external rots and initial stages of fruit rot, whereas *Fusarium* was significantly more common in internal rots and later stages.

*Fusarium* has previously been reported to be associated with CFR (Serrato-Díaz et al., 2020), although it has not been as extensively studied as *Colletotrichum. Fusarium* has been found to naturally colonize green, ripe, and mummified coffee fruits (Mignucci et al., 1985). Although in some cases *Fusarium* can cause CFR alone (Baker and Guy, 1977), other studies have found that it is more commonly involved in mixed infections alongside *Colletotrichum* (Mignucci et al., 1985).

In addition, *Phoma* has also been previously reported to cause disease in coffee fruits (Mohammed & Jambo, 2015). This study found *Phoma* to be present at lower frequencies than *Colletotrichum* and *Fusarium* but present to some extent in almost all samples.

In general, the patterns of fungal frequency reported by this study suggest a possible succession of fungi during colonization of external fruit tissues in which *Colletotrichum* is replaced by *Fusarium* as the fruit matures and rot progresses. These patterns could also suggest antagonism between *Colletotrichum* and *Fusarium*, though this has not been explored.

Serrato-Díaz et al. (2020) described coffee fruit rot affecting all stages of maturity, as well as distinguishing between external and internal rot of coffee fruits. Both Serrato-Diaz et al. and the present study avoid the use of the term 'coffee berry disease' which by tradition is restricted to the disease caused by *Colletotrichum kahawae,* on green fruit and only in Africa at high altitude (Waller et al., 2007). The results presented here show the prevalence of CFR in Puerto Rico and reveal novel aspects of this disease. It is not clear if these aspects—the combination of *Colletotrichum*, *Fusarium* and other organisms, the combination of external and internal rots, and the relationship to altitude, shade, and the coffee berry borer—are unique to Puerto Rico, or if they exist in other coffee-producing countries but have not yet been reported.

## Recommendations and conclusions

This study provides evidence that altitude, cultivation conditions and prevalence of CBB, play a role in CFR incidence and severity. Based on these results, coffee farms at higher altitude and with shade trees or intercalated with other, taller crops such as plantain and citrus should generally have lower incidence of fruit rot. When looking for the location of new coffee farms, planners should consider placing them at higher altitudes to benefit from the disease reducing effect that these conditions offer. For farms that are already established, planting shade trees around coffee or planting other crops together with coffee may provide the same disease-reducing effect. It was also shown that fruit perforated by CBB had more rot, so measures such as biological control agents or artisanal traps to reduce or eliminate CBB (Mariño et al., 2016) should also lower CFR incidence. Considering that mummified fruits are a source of primary inoculum and that CBB can survive in fallen fruits (Ribeyre & Avelino, 2012), we also recommend clearing infected fruits from branches and ground throughout the growing season and more thoroughly at the end of the season. Another way to reduce damage by CBB is application of the fungus *B. bassiana* which acts as a biological control agent (De la Rosa et al., 1997) especially when local isolates are utilized (Bayman et al., 2021). Commercial products such as Mycotrol® are also effective at reducing the damage by CBB, and should reduce CFR as well.

A range of potential crop losses can be extrapolated from our data. We found that 98% of sites had at least 20% of plants with some CFR, implying that almost all coffee farms suffer some losses. At the branch level, an average of 9% of fruits had internal rots, while 18% had external rots. External rot affected 50% or more of surface area in about 34% of these fruits. It is unclear what effect these rots had on seed weight or quality. Internal rot, on the other hand, affected 50% or more of tissue in 18% of fruits. These fruits are major losses for farmers as the rot lowers harvest weight and the quality of the crop, producing off flavors (Ribeyre & Avelino, 2012). If half of fruits with > 50% internal rot do not contain usable seeds (assuming the levels of infection reported here are typical) it is possible that 9% of the crop is lost to CFR. Estimates of yield loss in Puerto Rico in 2021 are similar: 5–10% (Tolentino Rosario, 2021). These figures are probably underestimates, since they do not include undetected losses of green fruits to abscission, as mentioned above. The annual value of green coffee production is over $16,000,000,000 (FAO, 2015) so losses due to CFR probably total hundreds of millions of dollars. Yet the disease is largely overlooked, and many coffee farmers do not implement control measures.

Further research is needed to better understand CFR in relation to factors such as coffee variety, temperature, humidity, light, the species and location of shade trees or crops, and incidence and severity of other coffee diseases and pests. Studies could compare data from sites at different altitudes or monitor the progression of disease over time at the same sites with instruments to measure environmental variables such as temperature and humidity. This information could facilitate models that allow for trend predictions and strategies to combat CFR.

Our results also support previous claims of complex fungal communities on the surface and within coffee fruits (Mignucci et al., 1985), where increase of certain fungal genera is accompanied by the decrease of others. This could suggest competition between fungi. Elucidating these complex interactions will benefit our understanding of CFR and could help determine any potential biological control agents, including *B. bassiana*, that can compete with coffee fruit pathogens. It would also be useful to determine if the same fungi that cause rotting of coffee fruit also affect other parts of the plant such as the stem, leaves and roots.

Overall, this study provides a report of CFR incidence and severity at various levels throughout the major coffee-producing region of Puerto Rico. Altitude and shade appear to play a role in the incidence and severity of diseases. A better understanding of the CFR-causing organisms, as well as the ecological niche that they inhabit, is useful for controlling the negative effects they may have on coffee production. Coffee cultivation conditions 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, Asia, and Africa (Mignucci et al., 1985). This study is a significant step towards a better understanding of CFR and provides information that can lead to strategies that mediate resulting losses and damages.

**Acknowledgements** We are grateful to the growers for permission to sample on their farms. This work was supported by grants from the United States Department of Agriculture (Agreements 58-6090-2-005 and 2021-51181-35900) and the Puerto Rico Science & Technology Research Trust. The funding sources had no role in study design, collection and analysis of data, manuscript preparation or decision to publish. Mention of trade names or commercial products in this publication is

solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

**Declarations**

**Competing interests** None.



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