OPEN ACCESS Int. J. Phytopathol. 13 (01) 2024. 25-41 DOI: 10.33687/phytopath.013.01.4712

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Available Online at EScience Press
International Journal of Phytopathology
ISSN: 2312-9344 (Online), 2313-1241 (Print)
https://esciencepress.net/journals/phytopath

INVENTORY AND DISSEMINATION OF CITRUS FUNGAL DISEASES IN BENIN

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ARTICLE INFO

Article History
Received: July 27, 2023
Revised: March 19, 2024
Accepted: April 07, 2024

Keywords
Benin
Citrus
Distribution
Epidemiology
Fungal diseases

ABSTRACT

Fungal diseases are a major constraint for the intensification of citrus production in Benin. The aim of this study was to identify the main citrus fungal diseases and to assess their distribution, prevalence, and severity). A total of 315 orchards were surveyed in the four agro-ecological zones (AEZ V, VI, VII, and VIII) where citrus is produced in Benin. During the surveys, samples of diseased fruits were collected for isolation in the laboratory. The results revealed four main fungal diseases including black spot caused by *Phyllosticta* sp., anthracnose caused by *Colletotrichum* sp., brown rot disease caused by *Curvularia* sp., and fruit rot caused by *Fusarium* sp. Among these diseases, black spot is the most distributed with 76.69% infected plants as compared to anthracnose, brown rot disease and fruit rot which infected 32.4%, 6.3% and 1.9% of plants respectively. The diseases severity was 2.88, 1.46, 1.08 and 0.60 for black spot, anthracnose, brown rot and fruit rot respectively. Of the three cultivated varieties, Pineapple and Valencia were susceptible to the four diseases while the variety Tangelo was less susceptible. The highest severity (3.51) was recorded in the AEZ VI and the lowest (0.81) in the AEZ VII. This study showed that citrus tree was susceptible to several diseases in Benin with black spot disease caused by *Phyllosticta* sp., as the most important. Any effort to increase qualitatively and quantitatively citrus productivity should be based on sustainable management of diseases.

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INTRODUCTION

Citrus is an economically important fruit crop in the world (Manner *et al.*, 2006). To date, they have grown in more than 140 countries. The main commercial species are oranges (*Citrus sinensis* L. Osbeck), mandarins (*Citrus reticulata*), lemons (*Citrus limon*), limes (*Citrus aurantiifolia*) and grapefruits (*Citrus maxima*), belonging to the genus Citrus of the *Rutaceae* family. Subtropical regions are the main commercial production areas where the best quality of citrus fruits is produced (Manner *et al.*, 2006; Liu *et al.*, 2012). World citrus production was estimated to more than 161 million tons in 2021 (FAOSTAT, 2022). According to TrendEconomy (2022), the world exports of citrus were estimated to 16.2 billion dollars in 2021. In tropical Africa, citrus is largely grown by small farmers for local consumption and export (Mohammed, 2002). Annual production in Africa that more than 21 million tons was harvested in 2021 from an area of 1896562 hectares of citrus, i.e., 11.33 tons/ha. Egypt was in first place with more than 4.3 million tons followed by Nigeria (4112301.47 tons), and South Africa with more than 3.2 million tons

(FAOSTAT, 2022). In Benin, citrus production is about 14772 tons coming from 6443 ha with no exports in 2021 (FAOSTAT, 2022). This leads to a yield of 2.29 tons/ha. Most parts of citrus fruits are rich sources of high economic value contents, such as different types of flavonoids, dietary fibre, polyphenols, carotenoids, essential oils, sugars, ascorbic acid and significant levels of some trace elements. The high amount of sugar in citrus fruits is much more used for bioethanol production by fermentation (Khan *et al.*, 2021).

Although the main products derived from citrus fruits are fruit juices and essential oils, they are also used in alcoholic and non-alcoholic beverages, aperitifs, liqueurs and pastries. Through the export of fruit, cooperation links between countries are established. In the farmer countries, citrus is a source of employment (FAOSTAT, 2021). Although citrus production is an important activity in Benin, its export is limited to border countries such as Nigeria, Burkina Faso, Togo and Niger (Lokossou *et al.*, 2009). The low yield recorded in citrus production and the non-export are related to various production constraints, including insect pests, diseases and climate constraints. The damages caused by diseases associated with climate change is huge and directly affects food security. Food stability has also been repeatedly affected in recent years by food crises, caused by climatic variations and biotic agents (Gregory *et al.*, 2005). This phenomenon has been observed in recent decades with the expansion of the most destructive crop diseases (Gilligan *et al.*, 2009). It is the case of the citrus bacterial canker epidemics in Florida (Gottwald *et al.*, 2001), recent severe epidemics of the UG99 strain of wheat rust that spreading in Africa and threatening other continents (Singh *et al.*, 2011) and bacterial wilt of solanaceous crops that has spread throughout West Africa (Sikirou *et al.*, 2019). Among the most devastating cases was the appearance of a new strain of *Phytophthora infestans* (Mont.) de Bary in Ireland following monoculture practices. This disease caused famine resulting in the death of one million people (Agrios, 2004).

Citrus industry in Benin is confronted with the fungal diseases emergency, which were unknown a decade ago. Complaints from farmers reported by extension workers to Crop Protection Laboratory led researchers to investigate these diseases. A field survey allowed noticing a disappearing or abandoning of several orchards due to rapid development of fungal diseases which conducted to plants death. In Benin, apart from photos of two suspected diseases such as greasy spot and *Alternaria* disease, without symptoms description in the literature (Lokossou *et al.*, 2009) no data is available on citrus diseases. Information on the inventory and extent of diseases damages is lacking. In Benin, no study has yet addressed these diseases epidemiology related to climate variability which are bases of a sustainable management strategy.

MATERIALS AND METHODS

Study Area

The study was conducted in four agro-ecological zones (AEZ) in south of Benin including zones V (cotton region of middle Benin), VI (zone dominated by ferralitic soils), VII (zone of depressions) and VIII (zone of fisheries) characterized by two rainy seasons with rains from March to July and from September to October (Figure 1). Average rainfall varies from 1300 to 1800 mm per year and is spread over 80 to 111 rainy days (NAPA, 2008). The soils in this area are characterized by ferralitic soil, tropical ferruginous, vertisol, alluvial and sandy soils. which have good physical characteristic and appropriate vegetation for citrus production (PUASA, 2009). The 4 AEZ includes the districts of Kétou, Djidja and Aplahoué in AEZ V, that of Allada, Tori-Bossito, Zè, Toviklin, Dogbo, Klouékanmè, Agbangnizoun, Covè, Zakpota, Zagnanado, and Sakété in AEZ V, that of Toffo, Lalo, Pobè and Zogbodomey in AEZ VII and that of Athiémé, Bopa, and Lokossa in AEZ VIII.

Sampling and Data Collection

Sampling was carried out in the 21 citrus-producing districts belonging to the four (4) agro-ecological zones. In each district, 3 citrus producing villages were randomly selected. In each village, 5 orchards were randomly selected. A total of 315 citrus orchards were surveyed to assess the prevalence, incidence and severity of fungal diseases associated with citrus in Benin.

These orchards were selected on different axes at a minimum distance of 10 km. Within each orchard, 20% of representative citrus plants were randomly selected on the diagonals for incidence assessment while five citrus trees were considered for disease severity determination. Five citrus fruits per tree with diseases symptoms were collected from all four sides of each tree. Data on geographic coordinates including latitude and longitude were taken at each sampling point using a GPS (Global Positioning System).

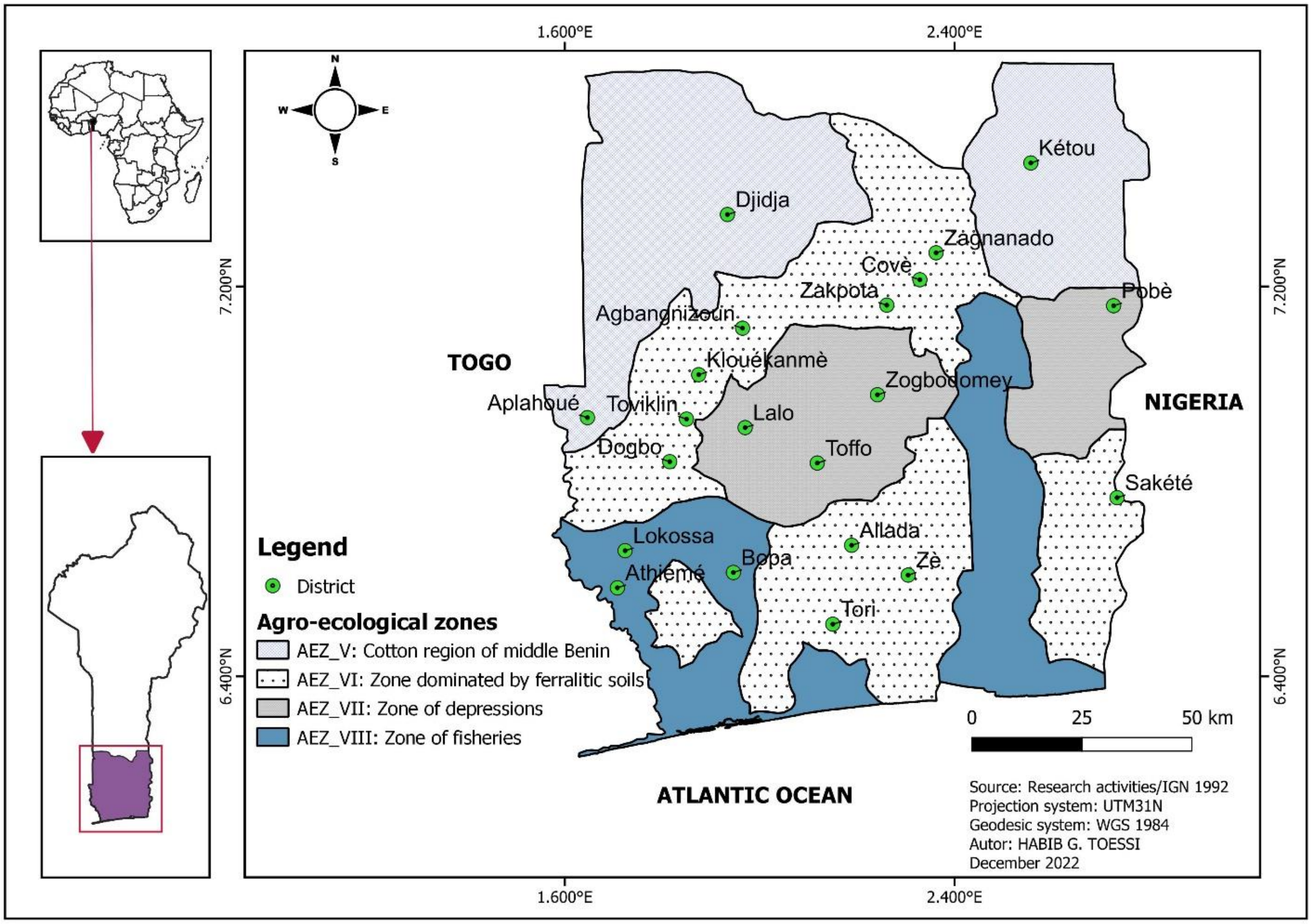

Figure 1. Geographical location of the study sites in agro-ecological zones.

Evaluation of Frequencies and Infection Rates of Fungal Species

Incubation of fungal pathogens from orange fruits using Whatman paper

To isolate the pathogens, the blotting paper method of Ikechi-Nwogu *et al.* (2021) was used with a slight modification. Each orange fruit was washed using tape water. From each fruit four peel fragments of 20 mm^2 with symptoms were cut and successively disinfected by soaking them in 70% ethanol for 1 minute, then in 1% sodium hypochlorite for 1 minute and rinsed twice with sterilized distilled water. Disinfected fragments were incubated onto a blotting paper disc previously sterilized (at 120°C for 30 mn), moistened with sterile distilled water and deposited in 9 cm diameter Petri dishes. Petri dishes were then incubated at 25 ± 2 °C for 12 hours of photoperiod during 3 to 7 days. Each Petri dish was coded with information (origin, variety and incubation date) related to the sample. Observations were made with a binocular (x40 resolution) and a microscope (x40 resolution) from 3 to 15 days after incubation. The number of occurrences of a fungal species on a given fragment was recorded. The frequency of observed fungus species appearance and the infection rate of the observed fungus species were calculated.

Isolation of fungi using PDA (Potato Dextrose Agar) medium

For isolation and identification of the fungi, the single spores obtained from incubated samples were cultured using the protocol of Kinge and Besong (2021) with a slight modification. The distinction of the single spores of the different fungal species was realized using a binocular and a microscope (x40 resolution). Single spores were then transferred into PDA plates, sealed with parafilm and incubated for 72 hours at 25 ± 2°C with a 12-hour photoperiod to promote mycelial development. The purification was carried out using the successive subculture technique to obtain pure isolates. Per isolate, the purified isolates were kept cold (-80 °C) in 2 ml Eppendorf tubes containing a glycerol solution (a mixture of 1ml glycerol and 0.5ml glucose) for morphological, molecular and pathogenic characterization.

Diseases Prevalence, Incidence and Severity

To evaluated disease incidence per orchard in each agro-ecological zone, five density squares of variable shapes regrouping 20 orange plants were considered. In each density squares, the incidence of each disease was calculated as the ratio between the number of diseased plants and the total number of assessed plant per density square as follow Arain *et al.* (2012):

$$Disease\ incidence\ (\%) = \frac{Number\ of\ diseased\ plant}{Total\ number\ of\ assessed\ plant} \times 100$$

The disease prevalence was calculated as follow (Amata *et al.* 2009):

$$P = \frac{I}{N} \times 100$$

Where: P = prevalence, I = number of infected orchards, N = total number of prospected orchards

The diseases severities were determined on the fruits using the scoring system of (Seif *et al.* 1999) where: 0 = healthy fruit; 1 = 1 to 25% affected fruit surface; 2 = 26-50% affected fruit surface; 3 = 51-75% affected fruit surface and 4 = above 75% affected fruit surface. The following formula was used to calculate disease severity:

$$SI = \frac{(0 \times a) + (1 \times b) + (2 \times c) + (3 \times d) + (4 \times e)}{N}$$

Where: SI = severity index; 0, 1, 2, 3 and 4 = scales of severity; a, b, c, d and e = number of fruits examined in each category of severity; N = total number of fruits assessed.

Data Analysis

Data were analyzed using R version 4.0.2 (Team, 2013) and Excel software. Geographic information systems (GIS) were used to map the prevalence, incidence and severity of the inventoried diseases across the agro-ecological zones. Fisher's exact test was used to determine whether there was an association between zones and citrus species. Logistic regression was used to determine the interactive effect of orchard age on disease incidence. A linear mixed effect model was used to test the difference in severity for each disease between Agro-ecological zones and to study the effect of orchard age on disease severity.

RESULTS

Identification of Citrus Fungal Diseases

The visual examination of the symptoms of *Citrus sinensis* on fruits and leaves in the surveyed orchards made it possible to recognize 4 different fungi namely, *Pyllosticta* sp., *Collectotricum* sp., *Curvularia* sp. and *Fusarium* sp. respectively responsible for black spot, anthracnose, brown rot and fruit rot diseases (Figure 2 and 3). Among these diseases, black spot and anthracnose were the most important diseases causing enormous damage to citrus production in Benin.

Symptom of black spot disease   Symptom of anthracnose

Figure 2 (a). Symptoms of different citrus fungal diseases observed during the survey in the agroecological zone.

Symptom of curvulariosis Symptom of fruit rot

Figure 2 (b). Symptoms of different citrus fungal diseases observed during the survey in the agroecological zone.

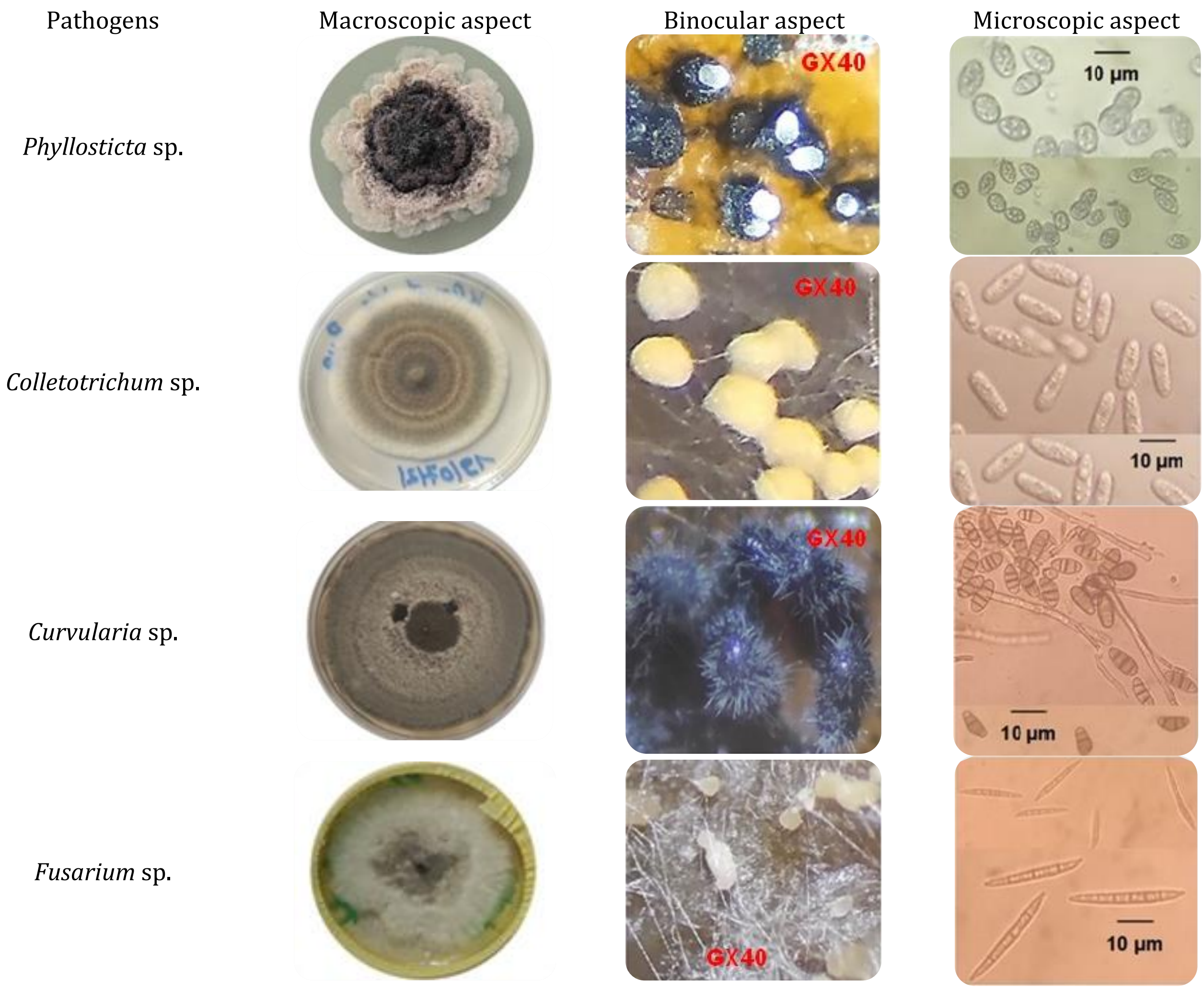

Figure 3. Pure cultures on PDA, binocular observation and conidia of pathogens.

Black Spots

The black spot disease was characterized by the appearance of numerous tiny to middle-sized dark brown to black spots, ranging in size from 3 to 10 mm in diameter. The tiny spots had a grey center with a dark brown to black border surrounded by a green or yellow halo, depending on the maturity of the fruit. In cases of severe infection, the fruits were completely covered with black spots, and rot. The leaves also had numerous black spots when severely infected.

Anthracnose

Anthracnose disease was characterized by the appearance of circular to irregular dark brown spots measuring about 1.5 mm in diameter on the fruit surface. The margins of the lesions on young fruits were surrounded by yellow halos. Leaves symptoms were characterized by light brown or greyish circular spots with purple margins and prominent yellow halos.

Brown Rot

Symptoms of brown rot on fruit were characterized by diffuse, yellowish spots that became brown. Infected fruits subsequently rotted. It progressively colonizes the entire surface of the fruit.

Fruit Rot

The disease was expressed as a dry rot that becomes soft and wet, brown and spongy with a light brown halo, causing the infected fruit to drop. Advanced lesions were generally surrounded with masses of white mycelium.

Prevalence of Fungal Diseases by Agroecological Zone

Citrus fungal diseases were significantly dependent on agroecological zones ($p<0.05$). Considering all AEZ, the disease prevalence was 100% for black spot disease, 31.75% for anthracnose, 6.98% for brown rot and 1.9% for fruit rot. Black spot was found in all orchards visited, i.e. a prevalence of 100%. Anthracnose was prevalent at 26.66%, 38.18%, 26.50% and 20% in the orchards of AEZ V, VI, VII and VIII respectively. Brown rot was observed in the orchards of three agro-ecological zones including AEZ V, VI, VII with prevalences of 4.33% 9.18% and 8.25% respectively. As for fruit rot, it was encountered only in the orchards of AEZ V, with a prevalence of 3.64% (Figure 4). Regarding citrus species, all identified diseases were significantly observed in orange orchards than in Tangelo orchard. The prevalence of black spot was 100% in orange orchards and 23.08% in Tangelo orchards. The prevalences of anthracnose, brown rot and fruit rot were 27.47%, 8.40% and 2.88% respectively in Pineapple orchards and 31.19%, 11.51% and 6.12% in Valencia orchards. Fruit rot was recorded in Tangelo orchards, i.e. 5.38% (Table 1).

Table 1. Prevalence of diseases (%) by citrus species.

Diseases	Varieties			*P*	Overall
	Pineapple	Valencia	Tangelo		
Black Spot	100^{a}	100^{a}	100^{a}		100
Anthracnose	24.47^{b}	31.19^{b}	-	< 0.05	31.75
Brown rot	3.40^{d}	7.49^{c}	-		6.98
Fruit Rot	0.85^{d}	1.00^{d}	0.89^{d}		1.9

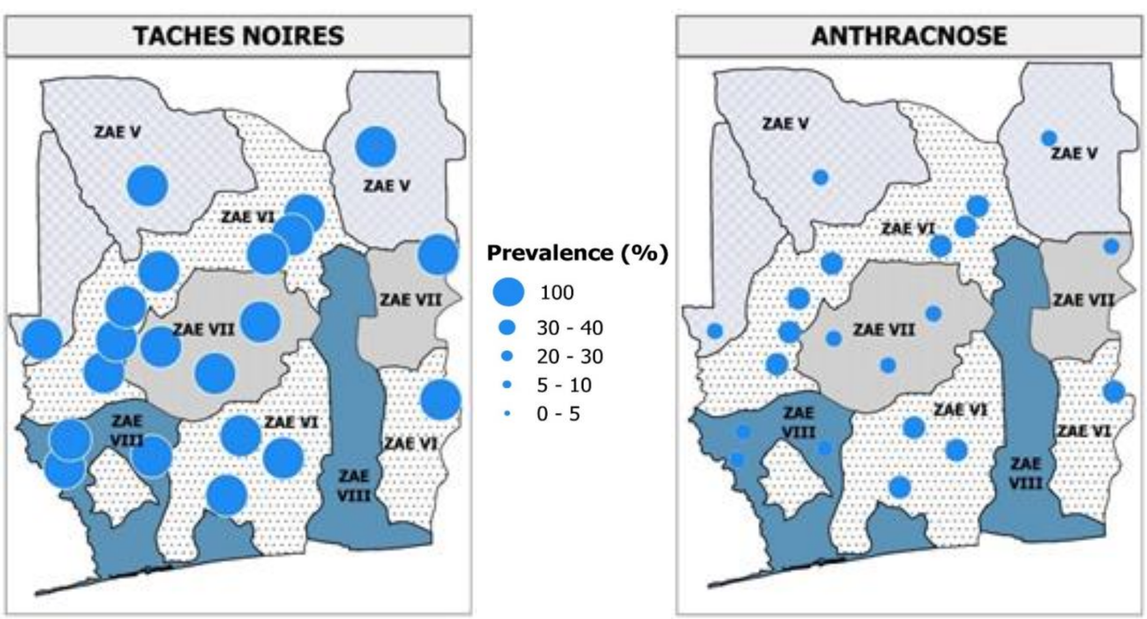

Figure 4 (a). Prevalence of fungal diseases by agro-ecological zone.

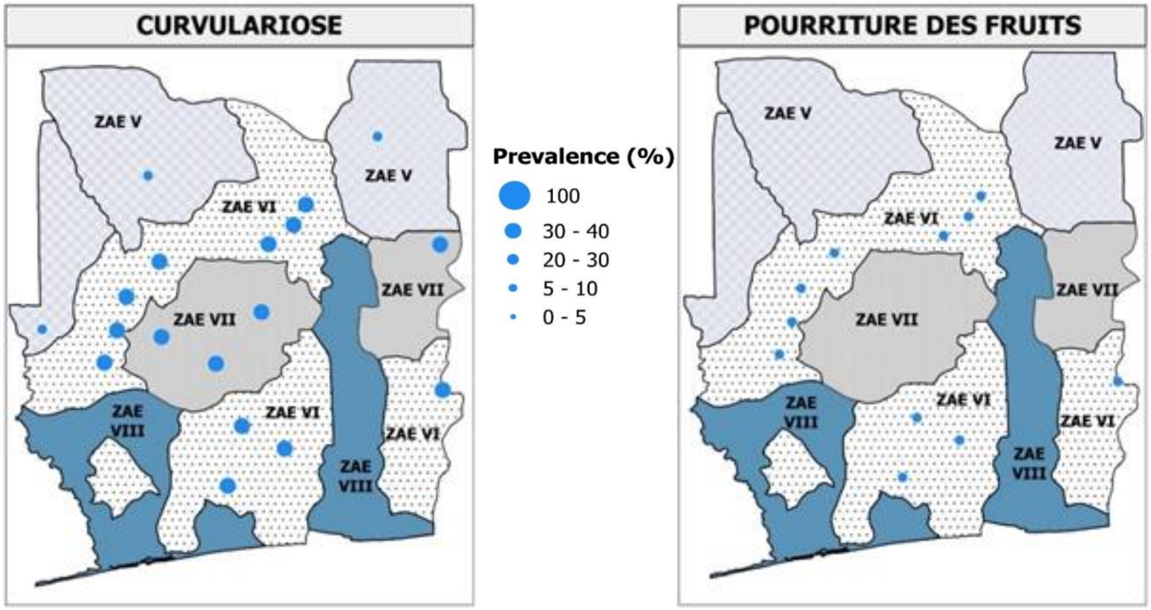

Figure 4 (b). Prevalence of fungal diseases by agro-ecological zone.

Incidence of Fungal Diseases by Agro-Ecological Zone

Statistical analysis revealed significant difference in disease incidences between AEZ and varieties ($p < 0.05$). Black spot disease presented the highest incidence, 76.68% significantly higher than anthracnose, brown rot and fruit rot which recorded 18.67%, 4.75% and 1.55% respectively. The incidences of black spot were 62.67%, 83.40%, 74.44%, and 65.33% in AEZ V, VI, VII and VIII respectively. Anthracnose recorded incidences of 8.67%, 23.20%, 15.56% and 16% in AEZ V, VI, VII and VIII respectively. Incidences of brown rot were recorded in three agro-ecological zones including AEZ V, VI and VII with 4.67%, 3.40% and 10.56% respectively.

As for fruit rot, it was observed only in AEZ VI with an incidence of 2.80% (Figure 5). The incidences of black spot, anthracnose, brown rot and fruit rot were respectively 81.40%, 20.48%, 7.14% and 1.67% on Valencia variety and 76.9%, 20%, 3.13% and 1.20% on Pineapple variety of orange species. Tangelo species recorded black spot and fruit rot diseases with incidences of 41.67% and 2.86% respectively (Table 2).

Severity of Fungal Diseases by Agroecological Zone

The severity was significantly different ($p<0.001$) between the agro-ecological zones for all diseases. The highest severity scale (2.85) was recorded in zone VI and caused by black spot. In contrast, the lowest severity (0.77) was observed in zone VI and caused by fruit rot (Figure 6). Black spot was the most severe disease with a severity scale of 2.62. The severity scales for anthracnose, brown rot and fruit rot were 1.14, 0.84, and 0.51 respectively.

The severity of fungal diseases was significantly higher for the orange varieties (Pineapple and Valencia) than for the Tangelo species. According to the results, the severity was significantly different between Valencia and Pineapple varieties. Black spot and Anthracnose recorded severity scales of 3.10 and 1.49 for Valencia and 2.74 and 1.43 for Pineapple respectively (Table 3).

Table 2. Incidence (%) of fungal diseases by citrus species.

Diseases	Varieties			*P*	Overall
	Valencia	Pineapple	Tangelo		
Black spot	81.4^{a}	76.9^{b}	41.67^{c}		76.69
Anthracnose	20.48^{d}	20^{d}	-	< 0.05	18.56
Brown rot	7.14^{e}	3.13^{ef}	-		4.53
Fruit rot	1.67^{f}	1.20^{f}	2.86^{ef}		1.55

p = p-value from Fisher's exact test. The value in bold is significant at 0.05 probability level.
Means with the same subscript are not statistically different at 5% probability level.

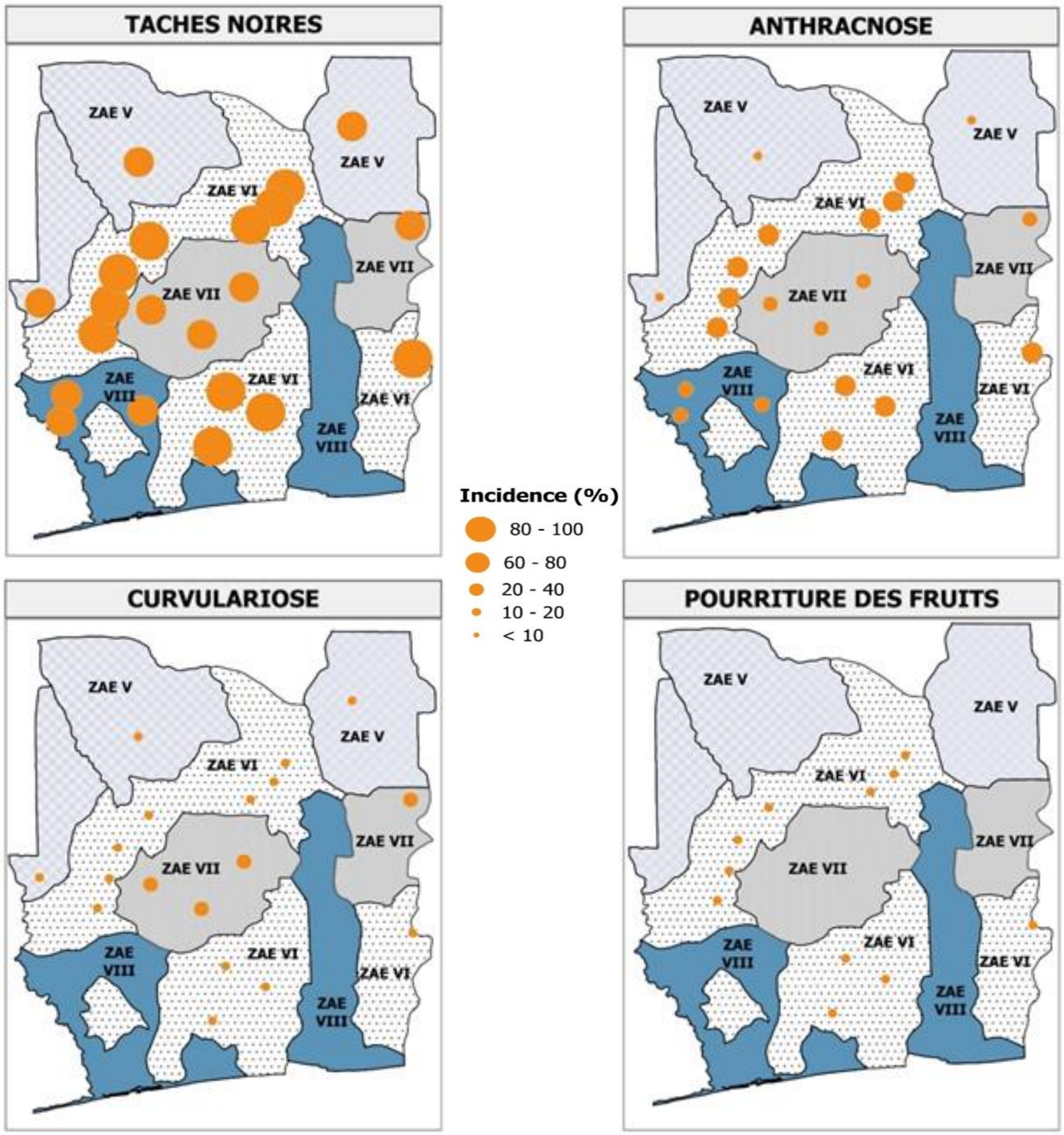

Figure 5. Incidence of fungal diseases by agro-ecological zone.

Table 3. Severity of fungal diseases by citrus species.

Diseases	Varieties			*P*	Overall
	Valencia	Pineapple	Tangelo		
Black spot	3.10[a]	2.74[b]	1.55[c]		2.62
Anthracnose	1.49[cd]	1.43[d]	-	< 0.05	1.14
Brown rot	1.08[e]	1.07[e]	-		0.84
Fruit rot	0.65[f]	0.55[g]	0.2[h]		0.51

p = p-value from Fisher's exact test. The value in bold is significant at 0.05 probability level
Means with the same subscript are not statistically different at 5% probability level.

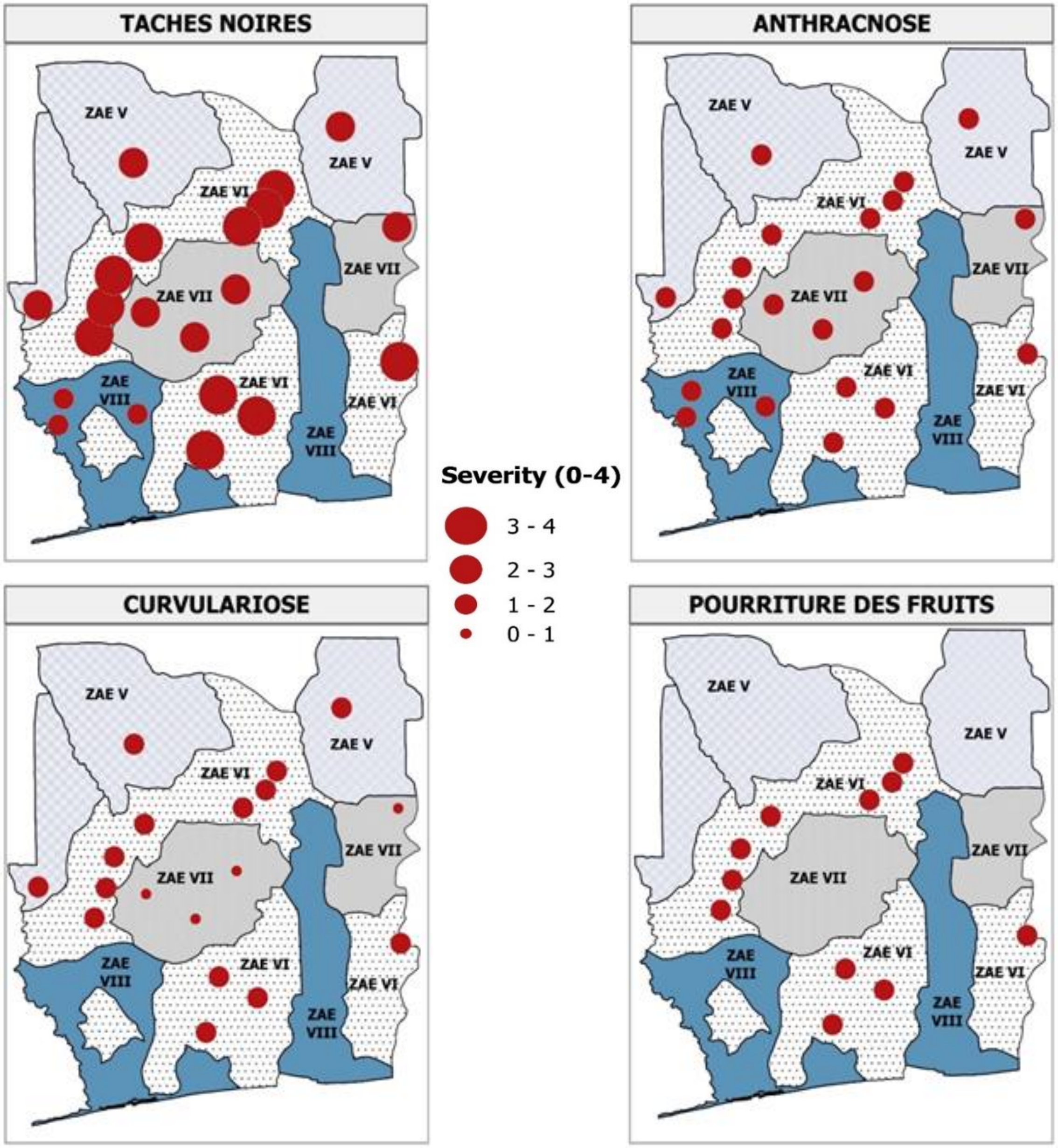

Figure 6. Severity of fungal diseases by agro-ecological zone.

Effect of Tree Age on the Incidence and Severity of Citrus Fungal Diseases

The incidence and severity of the identified fungal diseases were higher correlated with trees age ($p<0.05$). The correlation was significantly positive for each disease (Figure 7 and 8). The general trend based on the Pearson correlation test showed that disease incidence and severity increased linearly with tree age (Figure 9 and 10). Older trees (≥ 10 years) had greater disease incidence than younger trees (< 10 years) in each agro-ecological zone. Disease incidences on older trees were 81.31%, 13.75%, 2.62% and 1% for black spot, anthracnose, brown rot and fruit rot respectively (Table 4). Disease severity on fruit from older trees (≥ 10 years), was significantly higher than on fruit from younger trees (< 10 years), with mean scale severities of 2.6, 0.3, 0.05, and 0.02 for black spot, anthracnose, brown rot and fruit rot respectively (Table 5).

DISCUSSION

This study showed that several fungal diseases of citrus fruits and leaves such as black spot, anthracnose, brown rot and fruit rot caused by the fungi *Phyllosticta* sp., *Colletotrichum* sp., *Curvularia* sp. and *Fusarium* sp.

respectively are present in the orchards of agro-ecological zones producing citrus in Benin. Black spot and anthracnose were the main diseases causing damage and significant economic losses (Toessi *et al.*, 2023).

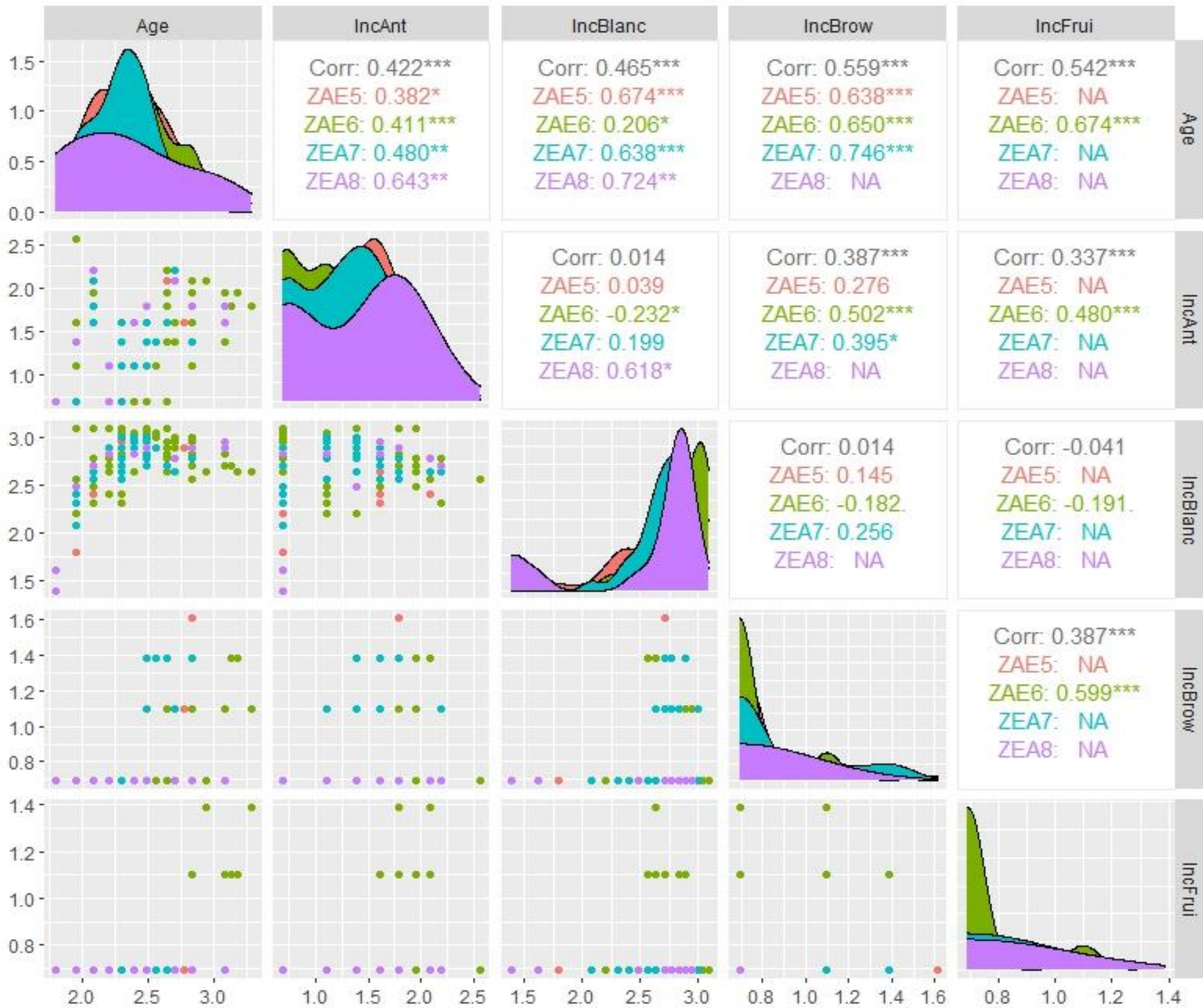

Figure 7. Correlation of tree age and disease incidences in agro-ecological zones.
IncBlanc = Black spot Incidence ; IncAnt = Anthracnose Incidence ; IncBrow = Brown rot Incidence ; IncFrui = Fruit rot Incidence

Table 4. Logistic regression testing the effect of age, orchard size, and their interaction on fungal disease incidence.

Tree age (year)	Agro-ecological zones	Black spot	Anthracnose	Brown rot	Fruit rot
< 10	AEZ V	17.19	7.5	0	0
	AEZ VI	40.1	5.83	0	0
	AEZ VII	26.04	6.67	0	0
	AEZ VIII	24.44	7.22	0	0
≥ 10	AEZ V	82.5	13.57	2.5	0
	AEZ VI	82.69	12.88	2.11	1.63
	AEZ VII	75	14.17	6.25	0
	AEZ VIII	79.17	20.83	0	0

Table 5. Interactive effects of orchard age, orchard size, and tree orientation on fungal disease severity.

Tree age (year)	Agro-ecological zones	Black spot	Anthracnose	Brown rot	Fruit rot
< 10	AEZ V	1.00	0.12	0	0
	AEZ VI	1.47	0.09	0	0
	AEZ VII	1.39	0.10	0	0
	AEZ VIII	1.02	0.09	0	0
≥ 10	AEZ V	2.14	0.30	0.04	0
	AEZ VI	2.84	0.28	0.04	0.03
	AEZ VII	2.07	0.28	0.12	0
	AEZ VIII	2.48	0.44	0	0

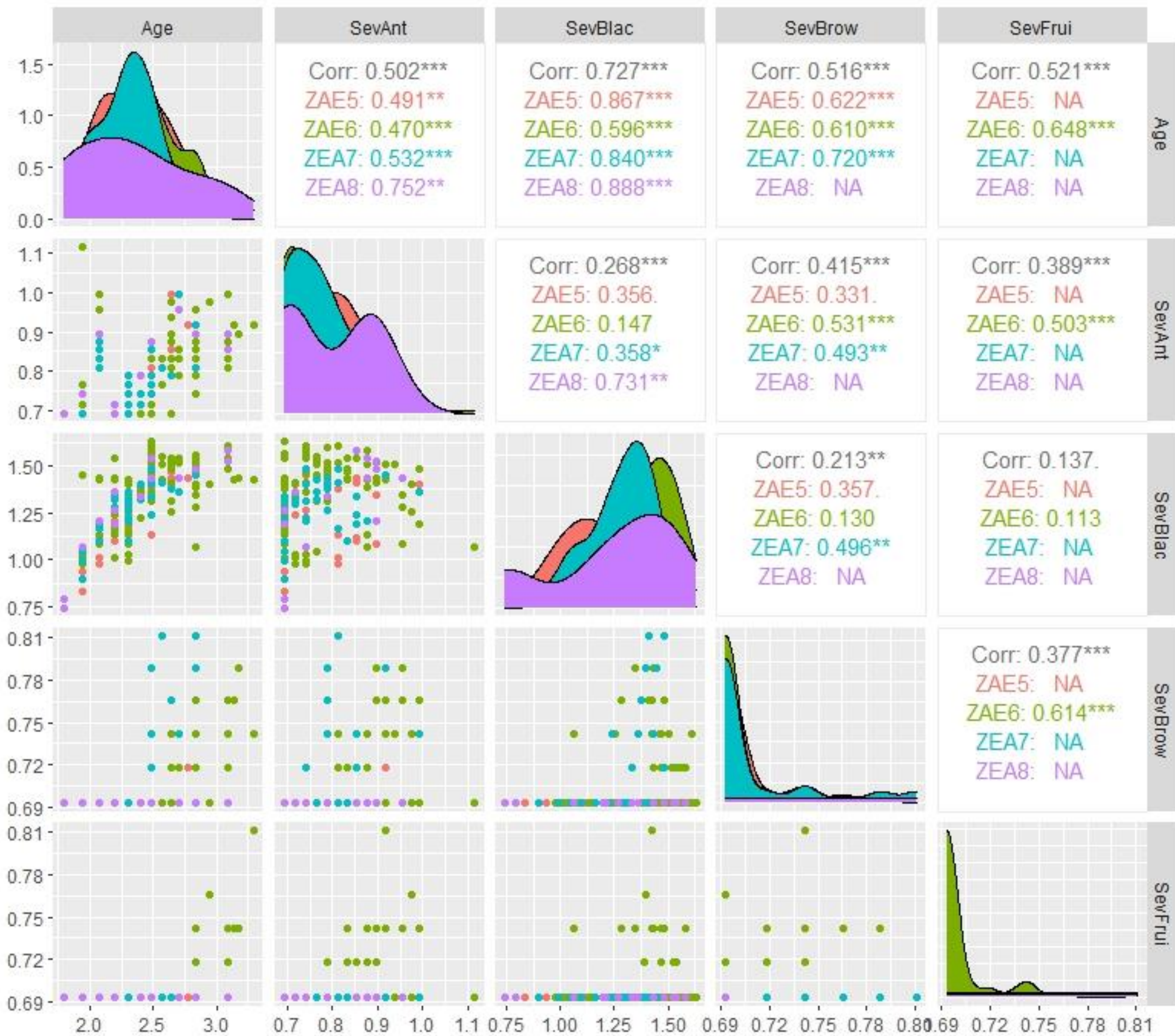

Figure 8. Correlation of tree age and disease severities in agro-ecological zones.

SevBlac = Black spot Severity ; SevAnt = Anthracnose Severity ; SevBrow = Brown rot Severity ; SevFrui = Fruit rot Severity

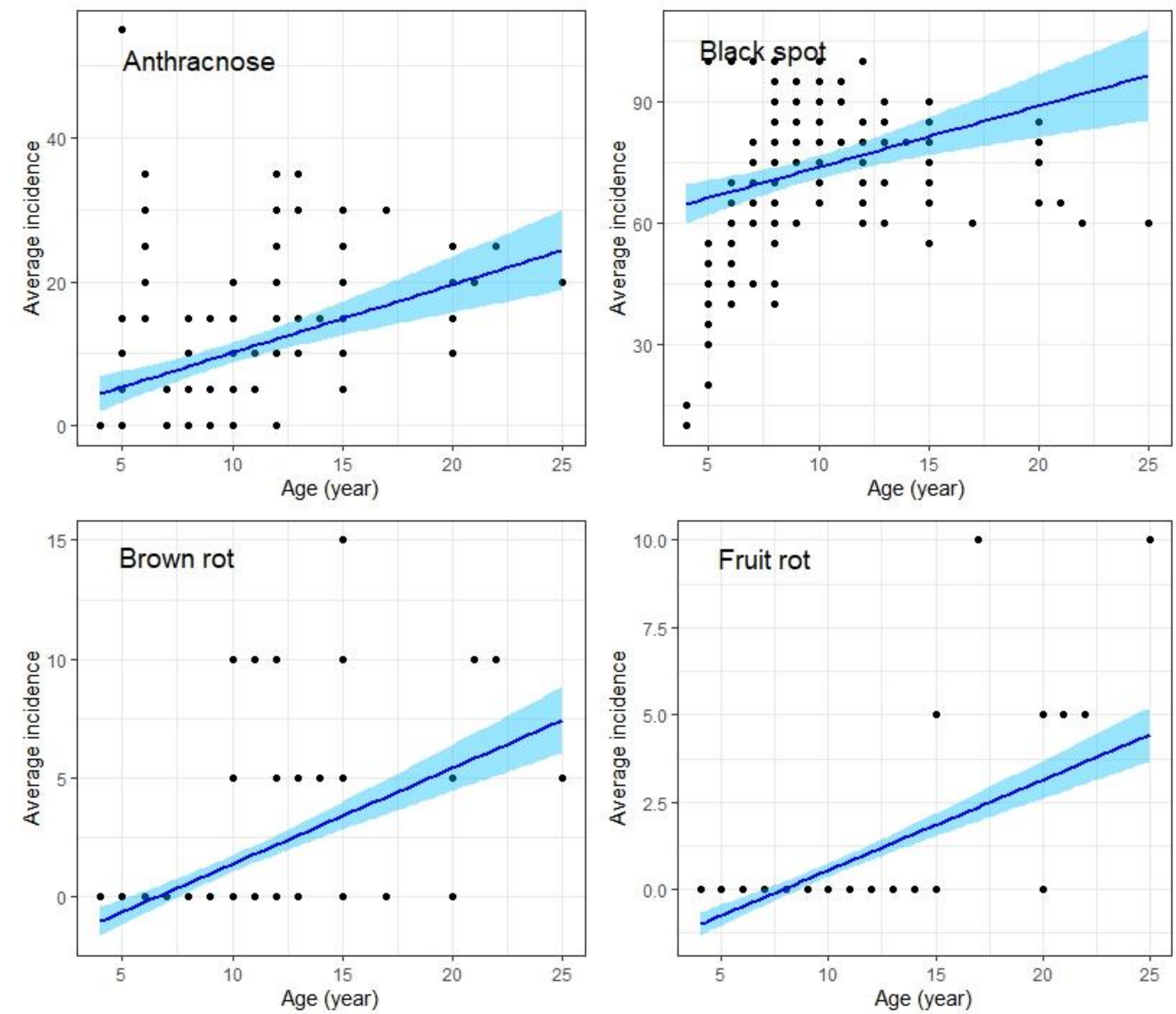

Figure 9. General trend of correlation between trees age and disease incidences.

Although these diseases are recorded for the first time on citrus in Benin, most of them had already been reported on citrus in several countries in Africa. Black spot has been reported on citrus in Tunisia, Angola, Ghana, Uganda, Kenya, Mozambique, South Africa, Zambia and Zimbabwe (EFSA, 2020). Anthracnose has been reported on citrus in Ghana (Honger *et al.*, 2016), Tunisia (Rhaiem and Taylor, 2016) and South Africa (Douanla-Meli *et al.*, 2013). Citrus fruit rot had also been reported in Nigeria (Olaniran *et al.*, 2014), Morocco, Tunisia and Egypt (Ezrari *et al.*, 2021).

The symptoms of black spot disease observed on fruits in the visited citrus orchards and the morphological characteristics of the pathogen obtained in the laboratory are typical to those of *Phyllosticta citricarpa* described by several authors worldwide (Baldassari *et al.*, 2006; Spósito *et al.*, 2008; Junior *et al.*, 2016). The morphological characteristics and expression of anthracnose described in this study are similar to those of *Colletotrichum gloeosporioides* observed and described by Honger *et al.* (2016); Rhaiem and Taylor (2016). As for *Curvularia* sp. and *Fusarium* sp., symptoms similar to those described in this study have already been described by many authors (Kunta *et al.*, 2015; Sandoval-Denis *et al.*, 2018). However, the pathogenicity test carried out with isolates of *Curvularia* sp. and *Fusarium* sp. on fruit gave a negative result. This means that *Curvularia* sp. and *Fusarium* sp. may not be responsible for brown rot and fruit rot diseases, respectively. According to Leslie and Summerell (2008), *Curvularia* sp. and *Fusarium* sp. are the fungi generally present in soil and plant tissues that acts as a saprophytic colonizer.

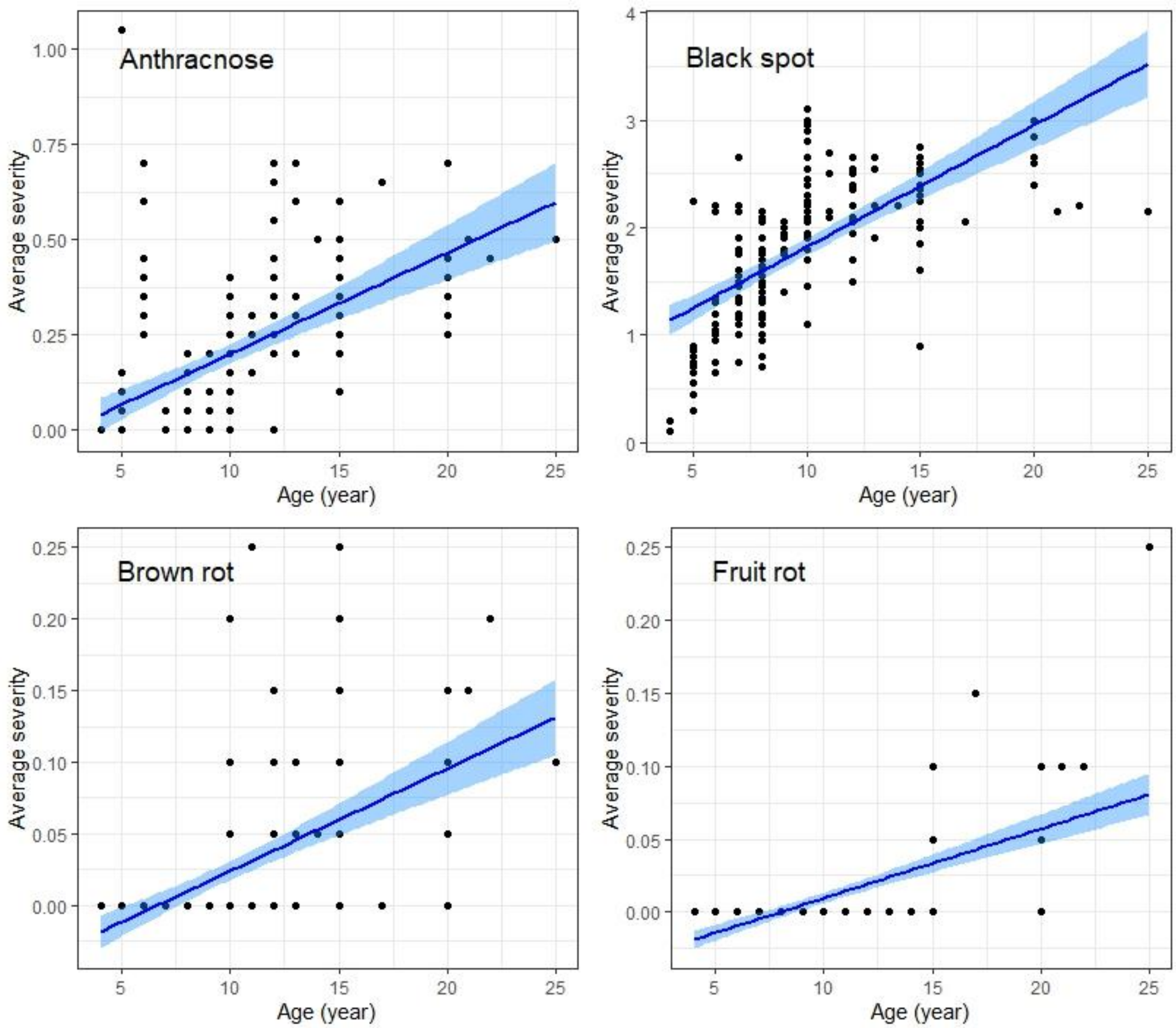

Figure 10. General trend of correlation between trees' age and disease severities.

Silva Junior *et al.* (2022) revealed that, among all the fungal inventoried diseases from an untreated citrus orchard in São Paulo (Brazil), black spot was the most significantly prevalent and causing severe damage to citrus yield and production. The four agro-ecological zones studied are generally humid zones characterized by annual rainfall ranging from 800 to 1400 mm and temperatures from 14 to 31 °C. According to Hendricks *et al.* (2020) and Magarey *et al.* (2015), infection of *Phyllosticta* sp. occurs when daily temperatures are between 10 and 35 °C, combined with relative humidity above 50% and daily rainfall above 0.2 mm. The agroclimatic conditions of the study areas are therefore very favorable to the development of black spot and few favorable to anthracnose disease, which clearly justify their high incidence level of 76.69% and 18.56% respectively. Agroecological zone VI, characterized by a Sudano-Guinean climate with a highest rainfall of 800 to1400 mm and a temperature between 25.3 °C and 29.7 °C, has recorded the highest incidence of citrus fungal diseases in general and black spot in particular. These results could be explained by the fact that in the agroecological zone VI, citrus is largely produced with orchards of high density, dominated by the older ones (Lokossou *et al.*, 2009). This type of cropping facilitates the development of diseases and the colonization of healthy neighbored citrus orchards by pathogens. *Phyllosticta* sp. and *Colletotrichum* sp. are among the fungi that sporulate extensively, with one ascospore capable of releasing over 3,000 lesions (Guarnaccia *et al.*, 2017). In terms of severity, analyses showed that black spot was the most severe (>80%) of all fungal diseases in the agroecological zones. Moreover, many studies reported that black spot, is a severe and destructive disease of citrus worldwide (Brentu *et al.*, 2012; Hendricks *et al.*, 2020; Fialho *et al.*, 2022). According to Wulandari *et al.* (2009) the fungus *Phyllosticta* is a harmful pathogen of citrus. Spósito *et al.* (2008), Wikee *et al.* (2013) and Glienke *et al.* (2011) works have also reported the severity of this disease on citrus and other crops (lily of the valley, mango) worldwide. Studies have shown that black spot disease can cause yield losses of more than 80% (Baldassari *et al.*, 2006; Brentu *et al.*, 2012). According to our results, the severity of black spot and anthracnose were high in agro-ecological zone VI recording the highest scale. Regarding citrus species, the varieties Pineapple and Valencia were very susceptible to all diseases, while the Tangelo species was less susceptible. However, of the two orange varieties, Valencia is the more susceptible orange variety to fungal diseases. Our finding confirms those of Timmer *et al.* (2000) who reported that of all orange varieties produced Valencia is the most susceptible to black spot disease. According to Dewdney *et al.* (2021), black spot disease can affect all commercial citrus species and cultivars, but sweet oranges, which are late-ripening varieties such as 'Valencia', are highly susceptible. According to the same author, Tangelo is moderately susceptible.

The present study also reported that diseases incidence and severity were higher on older trees (≥ 10 years) than on younger (< 10 years) and mainly for black spot disease in all surveyed agroecological zones. The high incidence and severity observed in older trees could be explained by the fact that the organs (leaves, fruits, twigs, stems) of these trees were exposed to diseases infection for a long time, thus causing a significant accumulation of inoculum. In addition, older trees contain more dead or senescent tissues which are the main sources of inoculum. Diseased leaves represent a permanent inoculum source for a new coming fruit. However, the low incidence and severity recorded could be due to a low concentration of inoculum in the tissues of young trees. These results confirm those of Hendricks *et al.* (2020) who observed that young trees bearing fruit for the first time in Florida citrus orchards showed a lower incidence and severity of black spot disease than older trees. Brentu *et al.* (2012) also confirmed these findings by reporting that the incidence and severity of fungal diseases are higher on mature citrus trees than on young ones. According to de Oliveira Silva *et al.* (2017) any activity that leads to tissue senescence of fungal disease infected plants, whether physiological or pathological, can lead to the release of viable inoculum within 45 days, when climatic conditions are favorable.

However, the damage caused by these diseases is a major concern for the farmers and special attention needs to be given to the fungal diseases in order to ensure better production.

CONCLUSION

This study has identified 4 fungal diseases under agroecological zones appropriate for citrus production in Benin: black spot disease caused by *Phyllosticta* sp., anthracnose caused by *Coletotrichum* sp., brown rot caused by *Curvularia* sp. and fruit rot caused by *Fusarium* sp. Black spot disease are the most severe and disseminated disease of all fungal diseases. The incidence and severity of diseases varied within agroecological zones. These results provide essential information that should be taken into consideration for developing an appropriate control method.

ACKNOWLEDGEMENTS

The authors thank the Partnership for Skills in Applied Sciences, Engineering and Technology (PASET) and Regional Scholarship and Innovation Funds (RSIF) programme, the Université Félix Houphouët-Boigny of Côte d'Ivoire, the Institut National des Recherches Agricoles du Bénin (INRAB) and the African Centre of Excellence of Climate Change, Biodiversity and Sustainable Agriculture (CEA-CCBAD/Côte d'Ivoire) for financial and technical support and providing working facility of the research study.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORS CONTRIBUTION

All authors contributed equally to this research.

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