

# Assessment and diagnostic of cashew seedling diseases in nurseries in Benin

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## ABSTRACT

Cashew plantations are established mainly from young seedlings from nurseries. These seedlings can be sources of disease contamination in plantations. In this paper, the perception of cashew nursery producers on the diseases associated with cashew seedlings and their management methods, the inventory and distribution of these diseases in three agroecological zones (AEZ) of Benin were investigated. Thus, a survey was conducted in the three AEZs to collect data on nursery management and samples of diseased seedlings. The results revealed anthracnose development caused by *Colletotrichum gloeosporioides*, bacterial leaf spot caused by *Xanthomonas axonopodis* pv. *anacardii* and black spot disease caused by *Phyllosticta* sp. on cashew seedlings in nurseries in the three AEZ. Significant differences, high disease incidence and severity were recorded across AEZs, municipalities and sites ( $p < 0.05$ ). The highest incidence and severity were 19.33 and 8.8%, respectively for anthracnose, 23.10 and 8.9% for bacterial leaf spot, 18 and 2.76% for black spot disease.

**Keywords:** Cashew seedlings, production system, diseases, management.

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## INTRODUCTION

The cashew (*Anacardium occidentale* L.) cultivation is of social and economic importance in the development of several countries worldwide (Martin, 2003). It originated from Brazil (Trevian et al., 2005), and was introduced into East Africa and India in the 16th century by the Portuguese (Martin et al., 1997). The plant seeds were introduced in Benin around 1960 for environmental protection purposes (Lemaître et al., 2003). The first organization of cashew cultivation in Benin was held in 1967 and was planted in eight departments over the twelve composing the country (Matthess et al., 2008).

In 2017, the cashew cultivation covered 455,704 ha for a production estimated to be 151,836 tons (FAO, 2019). It is one of Benin's strategic cultures of economic importance (Yabi et al., 2013). However, the average

cashew nut yield of 386 to 544 kg/ha remains low in Benin (Tchétingni et al., 2016), due to several factors among which diseases are the main constraints. Some diseases that attack plantations are derived from nurseries' young plants. Most of the time the diseases in the cashew seedlings are due to seed infection or grafting practices. The transmission of the pathogen from the seeds of grafted organs was reported (Thind et al., 1984; Sikirou and Wydra, 2011). Also, the development of technologies in cashew production is done with less consideration to producers (Röling and Fliert, 1994). Several studies revealed the importance of integrating producer knowledge and its implication in developing modern disease management technologies (Schreinemachers et al., 2015; Traoré et al., 2015; Islam

et al., 2020).

To reduce the dissemination of diseases in cashew plantations in Benin, the perception of the nursery producers on cashew diseases and management, and the inventory of cashew nursery diseases must be evaluated. This study aimed to (i) evaluate the perception of nursery producers on cashew seedling diseases and the management methods, (ii) diagnostic the diseases associated with cashew seedling, their incidence and severity, and (iii) establish their geographical distribution in Benin.

## MATERIALS AND METHODS

### Description of the zones of the study

The study was carried out in Benin's three agroecological zones (AEZ): AEZ IV, AEZ V and AEZ VI. The sites selected for the study were Djidja (Department of Zou), Dassa, Glazoué, Savalou, Savè, Bantè and Ouessè (Department of Hills) in AEZ IV; Kouandé, Natitingou (Department of Atacora); Ouaké, Djougou, Bassila (Department of Donga); Tchaourou (Department of Borgou) in AEZ V, and Parakou, N'Dali, Nikki (Department of Borgou); Sinendé and Bembèrèkè (Department of Alibori), Péhunco (Department of Atacora) in AEZ VI (Figure 1). The AEZ IV is characterized by a subequatorial climate with two rainy seasons alternated with a Sudanese climate with one rainy season, with an average annual rainfall from 1000 to 1200 mm and an average annual temperature from 24 to 29°C (Tonon et al., 2017). The soils are leached tropical ferruginous ones, whereas the vegetation is dominated by clear forest, shrub savannah and wood savannah. The AEZ V is characterized by a hot tropical Sudanese-type climate with one rainy season and one dry season, and an average annual rainfall of 1100 mm. The soils are mainly ferruginous with ferritic and hydromorphic soils somewhere. The vegetation is dominated by clear forest, shrub savannah and wood savannah. The AEZ VI is characterized by a Sudan-Sahelian climate with one rainy season and one dry season, and an average annual rainfall of around 900 mm. The soils are hydromorphic and tropical ferruginous. The vegetation is wood savannah and shrub savannah with small and sparse species of more than 66% of the zone (Dagbénonbakin et al., 2003).

### Sampling

The nursery producers visited in 2018 were selected from the recent list of cashew plantations of INRAB Center of Save (CRA-Center/INRAB). Based on the production capacity and the diversity of the AEZ 27 producers out of 37 were selected for the study.

For each producer, 2- to 3-month-old nurseries were delimited into simple plots of 250 seedlings. A total of 4 to 16 plots were obtained depending on the nursery area. 50 seedlings per simple plot were randomly selected and evaluated following the two diagonals or medians.

### Data and samples collection

The selected producers were interviewed using semi-structured questionnaire on their perception of the nursery production methods, the phytosanitary status of the seedlings and the control measures used. The recorded information included the socio-demographic characteristics of the nursery producers, the seedling production, the diseases of the cashew seedlings in their exploitations, the various methods of managing these diseases, the production costs and their income. For the disease knowledge, a series of color pictures of cashew seedlings with disease symptoms were shown to producers.

For disease evaluation on the nursery plants, the incidence and severity of each type of disease were assessed following the method described by Tonon et al. (2017). The severity was recorded per seedling on the last five leaves from the apex using 0 to 4 disease scale as follows:

0: no leaf spots (0%); 1: leaf spots on 1% to 10% of the leaf surface; 2: leaf spots on 11% to 25% of the leaf surface; 3: leaf spots on 25% to 50% of the leaf surface; 4: leaf spots on more than 50% of the leaf surface.

Cashew seedlings showing disease symptoms on the leaves were carried with their pots to the INRAB laboratory of plant protection.

### Isolation of pathogens and pathogenicity test

#### *Isolation, identification and pathogenicity test of fungi*

Fungi were isolated from blight and black spots symptom leaves and stems of seedlings using the method described by Walker et al. (1998). The samples were washed with running tap water and surface disinfected with ethanol 70%. After that, sections of about 25 mm<sup>2</sup> showing fresh disease symptoms were cut, soaked for 1 min in a 1% sodium hypochlorite solution, rinsed with sterile distilled water and dried on sterile filter paper under a laminar hood. The dried sections were plotted on PDA in Petri dishes and incubated at 28°C for 5 to 7 days.

For the pathogenicity test, a fungal suspension of 10<sup>6</sup> conidia/ml of each isolate was prepared with a solution of 0.05% of Tween 20. Six-week-old cashew seedlings were

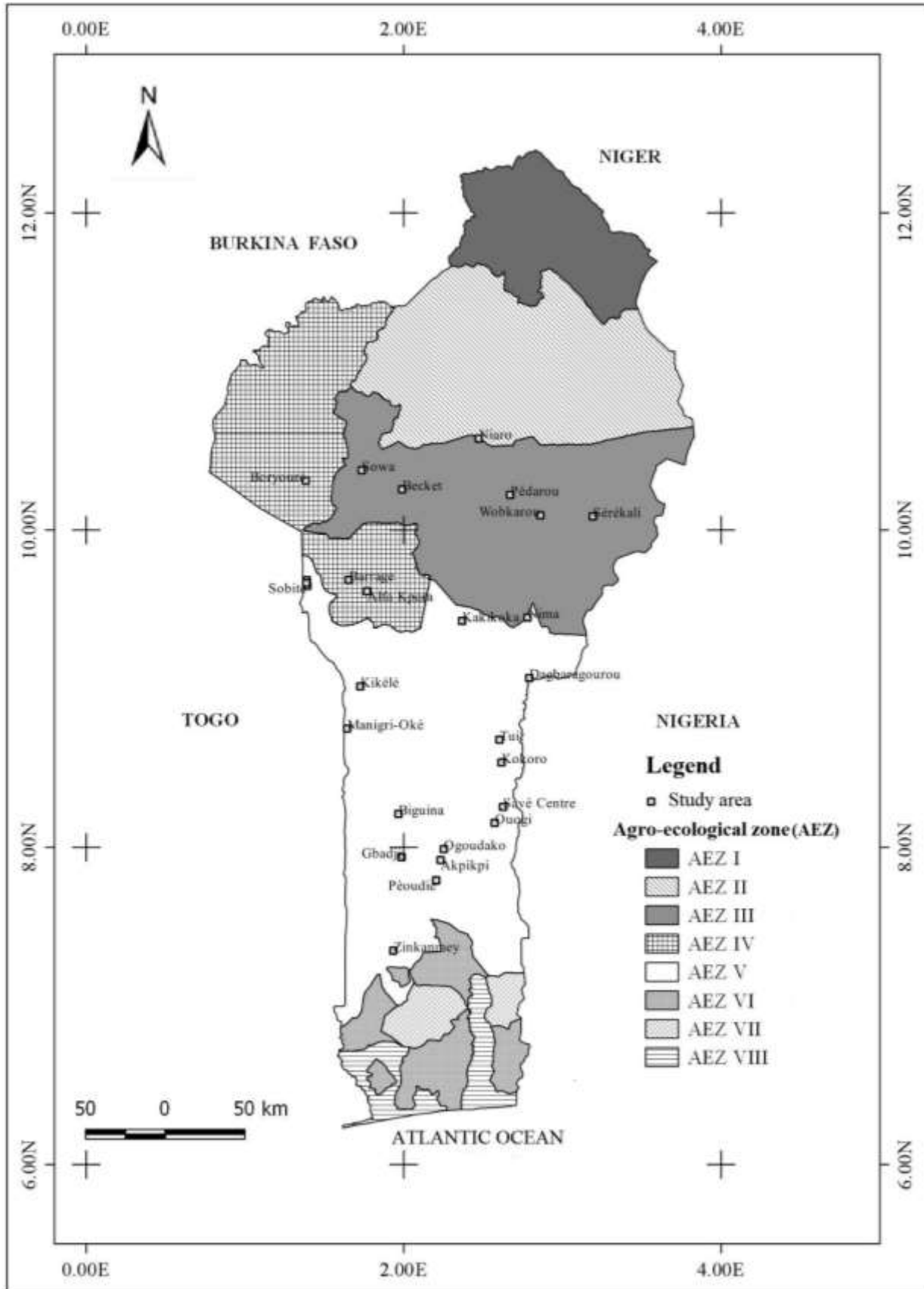


Figure 1. Agroecological zones and sites visited in Benin. AEZ Agroecological Zone.

inoculated with 10 ml of the suspension per plant using the Hary Brand Sprayer of 0.5 l t. Five seedlings were inoculated per isolate. Control seedlings received a solution of 0.05% Tween 20 in sterile distilled water. Each inoculated seedling was covered with black oilcloth and kept under shelter.

Fungi which positive pathogenicity results were identified using macroscopic characteristics of the structures, microscopic and the fungi identification key (Diguta, 2010).

### ***Isolation and pathogenicity test of bacteria***

Bacteria were isolated from angular spots of cashew seedlings leaves samples, following the method used by Sikirou (1999) and Banito (2003). The leaves were rinsed with tap water and surface sterilized with 70% ethanol. Then, small sections showing fresh angular spots were cut and macerated in mortar in drops of sterile distilled water. The macerate was incubated under a laminar hood for 3 min, then mixed. Loopfuls of the suspension were streaked on nutrient agar medium in Petri dished. The cultures were incubated at 30°C for 3 days and specific colonies were purified.

The 24-hour-old bacterial suspension was prepared from each pure culture bacteria isolates. The suspension was then collected in a sterile syringe and inoculated to tobacco leaf on the plant. The inoculated plants were incubated under natural conditions, and evaluation of symptoms was carried out daily until the development of the first necrosis of the inoculated parts of the leaves.

A pathogenicity test was performed with positive hypersensitivity reaction isolates. Thus, four isolates from different nursery sites were tested. Three young leaves per seedling were inoculated by puncture and abrasive with a bacterial suspension of  $10^8$  CFU/ml. Five plants were inoculated per mode of inoculation and per isolate. The control plants received sterile distilled water. Each plant was covered with oilcloth and kept under shelter (Touré et al., 2017).

### **Data analysis**

Citation frequency matrices of (i) substrates for setting up nurseries, (ii) water sources for watering and (iii) methods of protecting nurseries were submitted for principal component and specific weights analysis as described by Qannari et al. (2000) to highlight the main characteristics of each agroecological zone. The same procedure has been used for disease symptoms found in nurseries and for disease management methods used by producers. For each of the identified diseases, the variance analysis was performed for interactions between agroecological zone, district and village, and disease incidence and severity (Goodnight, 1978). The analysis was run in the R

version 3.5.1 statistical environment (R Core Team, 2018).

## **RESULTS**

### **Demographic and socioeconomic characteristics of cashew nursery producers**

The results revealed that the people surveyed were 40% and 100% farmers in AEZ IV and AEZ V, respectively, whereas in AEZ VI 66% were specialized in cashew seedlings production. In AEZ IV, 20% of visited people surveyed had cashew seedlings production as their primary activity. The nursery producers had an average of 44 to 50 age-old in the three zones, with an average seedling production of 5,440 and 7,550 plantlets. The cashew seedling producers had 8 years of experience in AEZ IV and V and more than 10 years in AEZ VI. For the three zones, 89.3% of producers set up the nurseries with nuts from their plantations, whereas 10.7% used the selected nuts (Table 1). The average price of an ungrafted seedling was 248 FCFA, while the grafted one was 587 FCFA.

### **Nursery management methods**

The results revealed precision of the principal component and specific weight analysis that explained 94.44% of the starting data, thus sufficient to guarantee the accuracy of the interpretation (Figure 2). On Axis 1, AEZ V nursery producers with agriculture as the main activity, use sand, baukaski (mixture of potting soil, sawdust, corn bran and spoiled milk) and the mixture of potting soil and sand as substrate, and were distinguished from producers in AEZ IV and VI, who used preferentially potting soil as a substrate for the nurseries. In Axis 2 of the component, nursery producers were compared according to the water source for watering and the type of shelter for the nurseries' installation. In AEZ IV, nurseries were under shelter, and borehole and dam water were mainly used for watering the seedlings. In contrast, in the AEZ VI, the nursery producers used wells' water for watering preferentially and had their nurseries installed under trees (Figure 2).

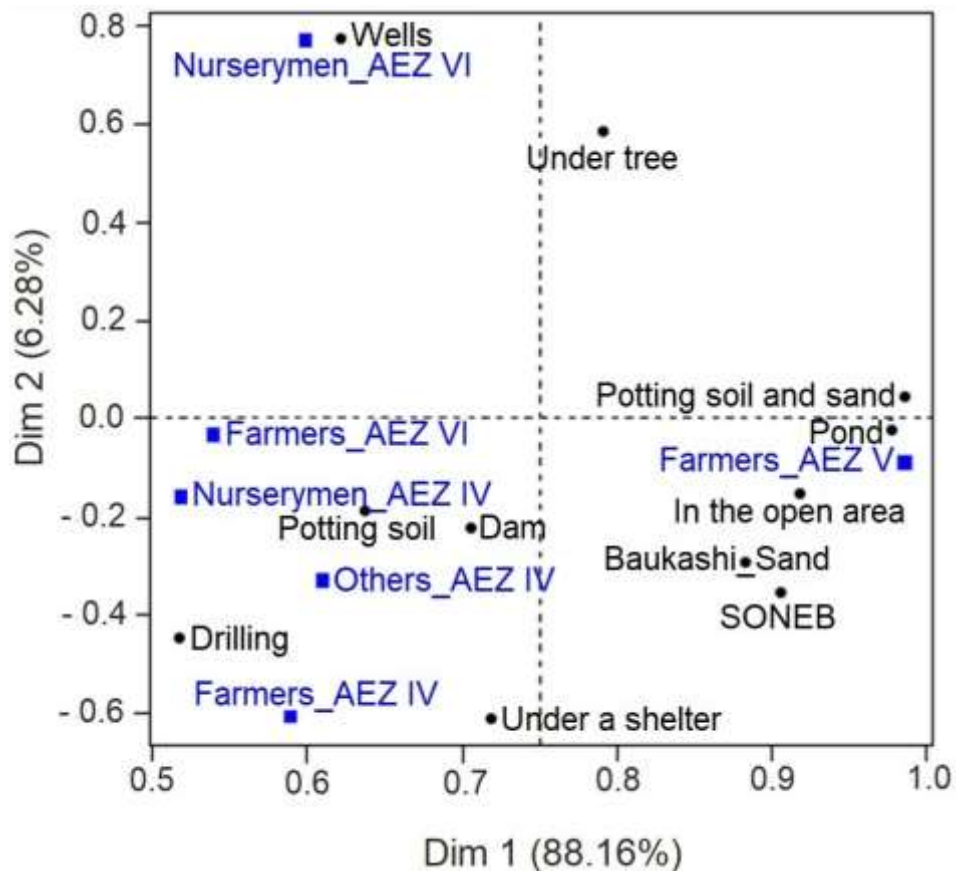
### **Diseases of cashew seedlings and control methods applied**

Diseases attacked more than 78% of nurseries inspected. The most frequent disease pointed out by the producers was the leaf black spot disease of cashew seedlings. The results revealed precision of the principal component and specific weight analysis explained 91.11% of the starting date on the diseases and the

**Table 1.** Demographic and socioeconomic characteristics of surveyed producers.

AEZ	Variables	Activities	Frequency (%)	
IV	Principal activity	Seedling producers	20	
		Farmers	40	
		Others*	40	
	Age	44.10 ± 12.75	-	
	Experience	8.60 ± 7.73 <sup>1</sup>	-	
V	Principal activity	Farmers	100	
		Age	50.50 ± 12.21 <sup>1</sup>	-
		Seniority in the trade	7.92 ± 4.29 <sup>1</sup>	-
	Average number of seedlings	7633.83 ± 5809.31 <sup>1</sup>	-	
	VI	Principal activity	Nurserymen	66.67
Farmers			33.33	
Age		45.83 ± 9.43 <sup>1</sup>	-	
Seniority in the trade		10.83 ± 4.36 <sup>1</sup>	-	
Average number of seedlings		7550 ± 4555.77 <sup>1</sup>	-	

\* Other categories workers (Land management, forestry, sellers, salariers).

**Figure 2.** Management of cashew nurseries in three agroecological zones of Benin.

control methods applied by the producers (Figure 3). The first axis discriminated the AEZs with black spot disease caused by *Phyllosticta* sp. as a primary disease from AEZs with anthracnose and bacterial infections as major ones. Thus, AEZ VI was characterized mainly by the leaf black spot disease whereas anthracnose and bacterial diseases were most observed in the AEZ IV

and AEZ V. The second axis discriminated the zones regarding the control measures used by the producers. The analysis revealed sanitation as the main method used by the producers to control bacterial disease in AEZ V, whereas synthetic chemical pesticides and botanical extracts such as neem oil were in AEZ IV (Figure 3).

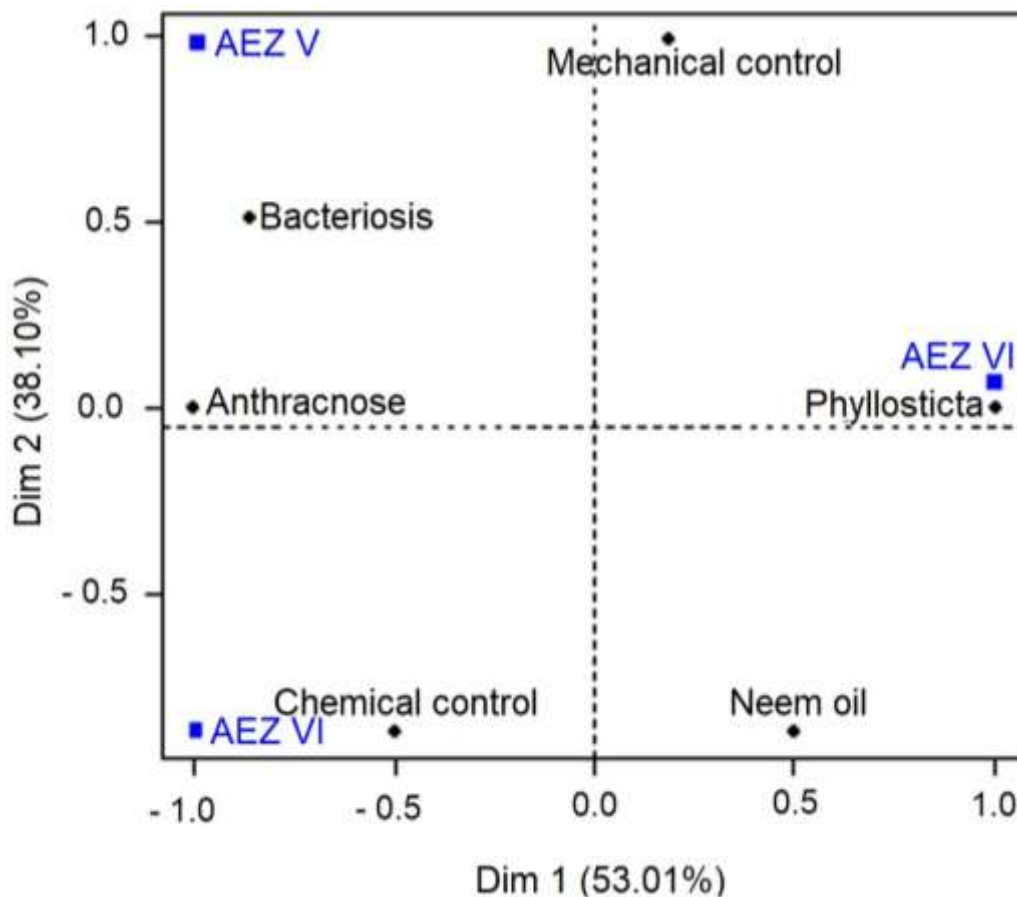


Figure 3. Dominant diseases of cashew seedlings and control methods used by nurserymen.

## Diseases associated with cashew seedlings in nurseries

### Black spot disease

Laboratory work allowed isolating and identifying *Phyllosticta* sp. causing the leaf black spot disease on cashew seedlings. The disease is characterized by large and round black spots generally in leaf interveinal. The affected leaves usually turn yellow.

The mycelium from the spots caused by this fungus was abundant on PDA, whitish black and bearing globular-shaped ascospores with variable size. The conidia were hyaline, unicellular, non-septate, irregular shapes with oval to cylindrical, and rounded tips. All

these characteristics are specific to *Phyllosticta* sp. The pathogenicity test performed on the plantlets confirmed these characteristics and the disease symptoms.

### Anthracnose

Symptoms of anthracnose caused by *C. gloeosporioides* are characterized by wilting, deformation and blighting of young leaves of cashew seedlings. The mycelium from a 5-day culture on PDA medium was a slightly bushy, greyish white and formed lot of concentric orange-colored acervuli layers which turn black brown. The conidia were hyaline, unicellular, non-septate, elongated, oval to cylindrical shape with rounded tips. The pathogenicity

test confirmed these characteristics.

### Bacterial leaf spot

From the angular leaf spotted cashew leaves samples, *Xanthomonas axonopodis* pv. *anacardii* (Xaa) was isolated. After 72 hours of incubation, whitish to cream-white, opaque and regular round-shaped and concave colonies were observed on nutrient agar medium. The positive pathogenicity test confirmed the description and the identification.

### Incidence of anthracnose, bacterial leaf spot and black spot of cashew seedlings

Component analysis revealed low variability in disease incidence between AEZs and between municipalities, whereas high variability in incidence was observed between sites for anthracnose and between municipalities within AEZ for bacterial and black spot diseases. The variance model analysis revealed

significant variability in disease incidence between each group of components the sites for leaf black spots where no variation was observed (Table 2).

Significant differences in disease incidences were observed between AEZs, districts and nursery sites ( $P < 0.05$ ). For anthracnose, the highest incidences were recorded in the sites of Peoudie and Gbadji with 19.33 and 19%, respectively in the AEZ IV, Sonoholou and Djougou barrage with 24.67 and 19.40%, respectively in the AEZ V. Generally low incidence of anthracnose was recorded in the AEZ VI. For bacterial leaf spot, the highest incidences were recorded in the sites of Sinkanme Centre and Ouoghi with 17, 22.8 and 23.40%, respectively in the AEZ IV, Kilele with 18.40% in the AEZ V and Pédarou in the AEZ VI. For black spot, the significant high incidences were observed in the Zinkanme, Centre and Ouoghi, ranging from 12 to 18% in the AEZ IV, whereas the disease incidence was generally low in the sites of the AEZ V except for anthracnose (Table 3).

The prevalence of the diseases was 67, 59 and 41% for anthracnose, bacterial leaf spot and black spot, respectively.

**Table 2.** Variability of disease incidence in cashew nurseries in components.

Components	Anthracnose	Bacterial leaf spot	Black spot
AEZ	12.07 (17.64%)***	8.02 (13.31%)***	6.93 (20.96%)***
District (AEZ)	17.09 (24.98%)***	35.35 (70.18%)***	26.12 (79.04%)***
Site (District)	39.26 (57.38%)***	8.70 (15.51%)*	0 (0%) <sup>ns</sup>

\* : significant at 5%, \*\* : significatif au seuil de 1%, \*\*\* : significatif au seuil de 0,1%, ns : non significatif at 5% of Student-Newman-Keuls test.

**Table 3.** Incidence (%) of anthracnose, bacterial leaf spot and black spot of cashew nurseries in three agroecological zones (AEZ) of Benin.

AEZ	Anth	Bact	Phyl	District	Anth	Bact	Phyl	Sites	Anth	Bact	Phyl
IV	4.62 <sup>b*</sup>	9.72 <sup>a</sup>	6.39 <sup>a</sup>	Bantè	19.33 <sup>a</sup>	3.67 <sup>c</sup>	-	Peoudie	19.33 <sup>ab</sup>	3.67 <sup>c</sup>	-
				Dassa-Zoumé	1.20 <sup>b</sup>	2.80 <sup>c</sup>	-	Ayédèro	1.20 <sup>c</sup>	2.80 <sup>c</sup>	-
				Djidja	5.57 <sup>b</sup>	17.00 <sup>ab</sup>	18.00 <sup>a</sup>	Zinkanme	5.57 <sup>c</sup>	17.00 <sup>ab</sup>	18.00 <sup>a</sup>
				Glazoué	0.50 <sup>b</sup>	5.25 <sup>c</sup>	1.00 <sup>c</sup>	Akpikpi	0.50 <sup>c</sup>	5.25 <sup>c</sup>	1.00 <sup>c</sup>
				Savalou	19.00 <sup>a</sup>	5.40 <sup>c</sup>	-	Gbadji	19.00 <sup>ab</sup>	5.40 <sup>c</sup>	-
				Ouèssè	-	-	-	Kokoro	-	-	-
				Tui	-	-	-	Tui	-	-	-
				Savè	0.10 <sup>b</sup>	23.10 <sup>a</sup>	14.80 <sup>ab</sup>	Center	0.20 <sup>c</sup>	22.80 <sup>a</sup>	17.00 <sup>a</sup>
				Ouoghi	-	23.40 <sup>a</sup>	12.60 <sup>a</sup>	Ouoghi	-	23.40 <sup>a</sup>	12.60 <sup>a</sup>
				Bassila	4.07 <sup>b</sup>	10.27 <sup>bc</sup>	0.13 <sup>c</sup>	Kikele	7.80 <sup>c</sup>	18.40 <sup>ab</sup>	-
V	9.27 <sup>a</sup>	3.34 <sup>b</sup>	0.40 <sup>b</sup>	Biguina	0.80 <sup>c</sup>	9.60 <sup>bc</sup>	-	Biguina	0.80 <sup>c</sup>	9.60 <sup>bc</sup>	-
				Manigri oké	3.60 <sup>c</sup>	2.80 <sup>c</sup>	0.40 <sup>c</sup>	Manigri oké	3.60 <sup>c</sup>	2.80 <sup>c</sup>	0.40 <sup>c</sup>
				Djougou	17.43 <sup>a</sup>	1.86 <sup>c</sup>	-	Alfa kpara	12.50 <sup>bc</sup>	-	-
				Djougou barrage	19.40 <sup>ab</sup>	2.60 <sup>c</sup>	-	Djougou barrage	19.40 <sup>ab</sup>	2.60 <sup>c</sup>	-
				Kouandé	1.20 <sup>b</sup>	-	-	Sowa	1.20 <sup>c</sup>	-	-

**Table 3.** Continues.

					Natitingou	9.60 <sup>ab</sup>	0.40 <sup>c</sup>	-	Boyouré	9.60 <sup>bc</sup>	0.40 <sup>c</sup>	-
						15.57 <sup>a</sup>	-	-	Ouaké centre	6.18 <sup>c</sup>	-	-
					Ouaké				Sonoholou	24.67 <sup>a</sup>	-	-
									Wekete ouekete	6.80 <sup>c</sup>	-	-
					Tchaourou	-	3.30 <sup>c</sup>	2.20 <sup>c</sup>	Dagbaragourou	-	6.60 <sup>c</sup>	4.40 <sup>bc</sup>
									Kakikoka	-	-	-
					Bembèrèkè	0.20 <sup>b</sup>	19.00 <sup>a</sup>	10.60 <sup>b</sup>	Pédarou	0.20 <sup>c</sup>	19.00 <sup>ab</sup>	10.60 <sup>ab</sup>
					Parakou	-	4.73 <sup>c</sup>	1.82 <sup>c</sup>	Nima	-	4.73 <sup>c</sup>	1.82 <sup>c</sup>
VI	0.49 <sup>c</sup>	4.91 <sup>b</sup>	2.42 <sup>b</sup>		Pehunco	2.60 <sup>b</sup>	-	-	Becket	2.60 <sup>c</sup>	-	-
					Sinendé	-	6.33 <sup>c</sup>	2.00 <sup>c</sup>	Niaro	-	6.33 <sup>c</sup>	2.00 <sup>c</sup>
					N'dali	-	-	-	N'dali centre	-	-	-
					Nikki	-	-	-	Sérékali	-	-	-

Anth = Anthracnose; Bact =Bacterial leaf spot; Phyl = Phyllosticta ; AEZ = Agroecological Zone; - = no disease. \* Means followed by the same letter in a column are not significant different at 5% of Student-Newman-Keuls test.

### Severity of anthracnose, bacterial leaf spot and black spot in cashew nurseries

Significant variability was observed between each group of the components ( $P < 0.05$ ). High variability in anthracnose severity was observed between municipalities within AEZ and between sites within the municipality, with a rate of about 45% in both components. A significant variation of 59% in bacterial leaf spot was also observed between sites within the municipality and between municipalities within AEZ for black spot disease with a rate of 76%. The lowest variability in disease severity was observed between

AEZs (Table 4).

Significant differences in disease severity were observed within the different AEZs, districts and sites ( $P < 0.05$ ). The highest severity of anthracnose was recorded at Djougou barrage with 8.86 in the AEZ V, followed by that at Gbadi with 6.54 in the AEZ IV, whereas it was observed only in two sites of the AEZ VI with the severity index of 0.08 and 0.54. For the bacterial disease, the highest severity was observed at the centre with 8.96 in the AEZ IV, followed by that at Kilele (5.37) and Pedarou (5.39), in the AEZ V and AEZ VI, respectively. The black spot severity was generally low in the 3 AEZs with a maximum index of 2.76 (Table 5).

**Table 4.** Variability of disease severity in cashew nurseries in components.

Components	Anthracnose	Bacterial leaf spot	Black spot
AEZ	0.51 (10.45%)***	0.58 (10.41%)***	0.14 (15.53%)***
District (AEZ)	2.21 (45.14%)***	1.69 (30.43%)***	0.68 (76.43%)***
Site (District)	2.17 (44.40%)***	3.28 (59.15%)***	0.07 (8.04%)***

\* : significant at 5%, \*\* : significatif au seuil de 1%, \*\*\* : significatif au seuil de 0,1%, ns : non significatif at 5% of Student-Newman-Keuls test.

**Table 5.** Severity of anthracnose, bacterial leaf spot and black spot in cashew nurseries.

AEZ	Anth	Bact	Phyl	District	Anth	Bact	Phyl	Sites	Anth	Bact	Phyl
				Bantè	1.54 <sup>cd</sup>	0.84 <sup>de</sup>	-	Peoudie	1.54 <sup>def</sup>	0.84 <sup>def</sup>	-
				Dassa-Zoumé	0.11 <sup>d</sup>	0.21 <sup>e</sup>	-	Ayédero	0.11 <sup>f</sup>	0.21 <sup>f</sup>	-
				Djidja	2.50 <sup>c</sup>	3.79 <sup>c</sup>	2.27 <sup>a</sup>	Zinkanme	-	3.79 <sup>c</sup>	2.27 <sup>b</sup>
IV	1.32 <sup>b*</sup>	2.40 <sup>a</sup>	0.88 <sup>a</sup>	Glazoué	0.30 <sup>d</sup>	0.48 <sup>e</sup>	0.08 <sup>c</sup>	Akpikpi	0.30 <sup>ef</sup>	0.48 <sup>ef</sup>	0.08 <sup>df</sup>
				Savalou	6.54 <sup>a</sup>	1.37 <sup>de</sup>	-	Gbadji	6.54 <sup>b</sup>	1.37 <sup>def</sup>	-
								Kokoro	-	-	-
				Ouèssè	-	-	-	Tui	-	-	-



Table 3. Continues.

				Savè	0.09 <sup>d</sup>	6.56 <sup>a</sup>	2.22 <sup>a</sup>	Center Ouoghi	0.18 <sup>f</sup> -	8.96 <sup>a</sup> 4.15 <sup>c</sup>	2.76 <sup>a</sup> 1.67 <sup>c</sup>
				Bassila	0.99 <sup>d</sup>	1.88 <sup>d</sup>	0.02 <sup>c</sup>	Kikele Biguina Manigri oké	2.41 <sup>cd</sup> 0.02 <sup>f</sup> 0.54 <sup>ef</sup>	5.37 <sup>b</sup> 0.20 <sup>f</sup> 0.07 <sup>f</sup>	- - 0.05 <sup>df</sup>
				Djougou	7.21 <sup>a</sup>	0.34 <sup>e</sup>	-	Alfa kpara Djougou barrage	3.06 <sup>cd</sup> 8.86 <sup>a</sup>	- 0.48 <sup>ef</sup>	- -
V	1.98 <sup>a</sup>	0.66 <sup>c</sup>	0.05 <sup>c</sup>	Kouandé Natitingou	0.75 <sup>d</sup> 3.64 <sup>b</sup>	- 0.08 <sup>e</sup>	- -	Sowa Boyouré	0.75 <sup>ef</sup> 3.64 <sup>c</sup>	- 0.08 <sup>f</sup>	- -
				Ouaké	1.63 <sup>cd</sup>	-	-	Ouaké centre Sonoholou Wekete ouekete	1.82 <sup>def</sup> 2.00 <sup>de</sup> 0.69 <sup>ef</sup>	- - -	- - -
				Tchaourou	-	0.88 <sup>de</sup>	0.30 <sup>bc</sup>	Dagbaragourou Kakikoka	- -	1.76 <sup>de</sup> -	0.60 <sup>d</sup> -
				Bembèrèkè Parakou Pehunco	0.08 <sup>d</sup> - 0.54 <sup>d</sup>	5.39 <sup>b</sup> 1.20 <sup>de</sup> -	2.23 <sup>a</sup> 0.50 <sup>bc</sup> -	Pédarou Nima Becket	0.08 <sup>f</sup> - 0.54 <sup>ef</sup>	5.39 <sup>b</sup> 1.20 <sup>def</sup> -	2.23 <sup>b</sup> 0.50 <sup>de</sup> -
VI	0.11 <sup>c</sup>	1.39 <sup>b</sup>	0.55 <sup>b</sup>	Sinendé N'Dali Nikki	- - -	2.04 <sup>d</sup> - -	0.61 <sup>b</sup> - -	Niaro N'dali centre Sérékali	- - -	2.04 <sup>d</sup> - -	0.61 <sup>d</sup> - -

Anth = Anthracnose; Bact =Bacterial leaf spot; Phyl = Phyllosticta ; AEZ = Agroecological Zone; - = no disease. \* Means followed by the same letter in a column are not significant different at 5% of Student-Newman-Keuls test.

## DISCUSSION

The present study revealed Anthracnose, bacterial leaf spot and black spot caused by *C. gloeosporioides*, *X. axonopodis* pv. *anacardii* and *Phyllosticta* sp., respectively as the major diseases observed in cashew nurseries in the three agroecological of Benin. Anthracnose and bacterial leaf spot were the most common diseases observed among these. In Mozambique, anthracnose disease was one of the most critical threats in cashew nurseries (Uaciquete, 2013). A high incidence of bacterial leaf disease was observed and revealed the importance of cashew nurseries in the visited AEZs. High prevalent of the bacterial leaf disease caused by *X. axonopodis* pv. *anacardii* in Anacardiaceae was reported by Ah-You et al. (2007). Previous investigations revealed the distribution of anthracnose and bacterial leaf diseases in cashew plantations in Benin (Afouda et al., 2013), and could be explained by the development of these diseases on seedlings in cashew nurseries as observed in the present study. Black spot disease caused by *Phyllosticta* sp. was never reported before in Benin. Thus, the present study is important as a database on this pathogen affects the cashew seedlings in nurseries and other plants. Black spots caused by *Phyllosticta* sp. have been reported on

citrus leaves and fruits (Wulandari et al., 2009) and mango (Glienke-Blanco et al., 2011). Wikee et al. (2012) reported *P. ophiopogonis* causing black spots on the leaves of Japanese lily of the valley (*Ophiopogon japonicus*).

For each disease, the variation in incidence and severity was low between AEZ and high within municipalities and sites. The three AEZ in which the nurseries have been installed have variable climate characteristics. AEZ IV is more humid than AEZ V and VI. Based on this natural condition and relative humidity and temperature which are favorable factors for developing these diseases on the cashew seedlings, the severities were expected to be significantly higher in EAZ IV than in the other AEZs. The low variability in disease severity between AEZ revealed that the climate factors could not be responsible for expressing disease symptoms on nursery plants. Furthermore, the seedlings were evaluated in the dry season with a mean temperature of 40°C and low moisture. Thus, the diseases observed may not be related to climate factors. However, the differentiation in disease severity observed could be related to diversity in practices and the system of production implemented by nursery producers in each site. The survey revealed a diversity in the substrates, source of the seeds, watering source and the type of

manure used by producers. In all the AEZs visited, the results showed that some producers used seeds from their plantation instead of the selected and certified seeds from appropriate companies, thus could explain the bacterial disease observed on seedlings. The genus *Xanthomonas* is known to be seed transmissible bacteria (Punina et al., 2009), thus can be carried in cashew seeds from the plantation to the nursery when used. Most of the disease-free nurseries found were those where producers used seeds from selected disease-free plants previously selected. The soils used to make up the nurseries were taken from the existing cashew plantations. These soils may harbor pathogens, thus may be the source of contamination for seedlings once installed. Whatever the disease developed the cashew seedlings affected lose their market value, thus reducing the producers' income.

## Conclusion

The present study revealed anthracnose, bacterial leaf spot and black spots caused by *C. gloeosporioides* X. *axonopodis* pv. *anacardii* and *Phyllosticta* sp., respectively are the primary diseases associated with cashew nurseries seedlings in the AEZ IV, V and VI of Benin. High and differential disease incidences and severities were observed across ecozones, districts and sites visited. Thus, bacterial and fungal diseases are becoming more severe in cashew nurseries in the three AEZs. Therefore, measures such as strengthening producers' capacity in disease identification and management must be taken to avoid possible epidemics and prevent losses and reduction of income.

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