SEVERITY AND DISTRIBUTION UDAPTED OF ANTHRACNOSE ON CASHEW TREES (*ANACARDIUM OCCIDENTALE* L.) IN COTE D'IVOIRE

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ABSTRACT

This study aim to update the health map of anthracnose, one of the main diseases of cashew trees in Côte d'Ivoire. Sanitary surveys were carried out from 22 October to 22 December 2019 in 290 orchards in 19 Regions of the country. In each orchard visited, 10 cashew trees were surveyed. The assessment consisted to determinate the incidence and severity index of anthracnose on the foliage. Leaves symptomatic of anthracnose were sampled for laboratory analysis. During the pre-flowering period of cashew trees, anthracnose manifests itself on the leaves as necrotic spots in the form of dotted lines or a grey patch. The highest average incidence was obtained in the region of Marahoué (100%) and the lowest in the region of Moronou (29.82%). The average severity index at national level was 28.48%. 85 isolates of *Colletotrichum gloeosporioides* were obtained from all the samples collected. The study revealed that anthracnose is present in all cashew orchards and its incidence varies from one region to another in Côte d'Ivoire. The region of Haut-Sassandra showed the highest severity.

Key words: Anthracnose, Cashew tree, Incidence, Severity, Côte d'Ivoire

RESUME

SEVERITE ET DISTRIBUTION ACTUALISEE DE L'ANTHRACNOSE DE L'ANACARDIER (ANACARDIUM OCCIDENTALE L.) EN CÔTE D'IVOIRE

Cette étude vise à actualiser la carte sanitaire de l'anthracnose, l'une des principales maladies des anacardiers en Côte d'Ivoire. Des prospections sanitaires ont été effectuées du 22 octobre au 22 décembre 2019 dans 290 vergers répartis dans 19 Régions du pays. Dans chaque verger visité, 10 anacardiers ont été prospectés. L'évaluation a consisté à déterminer l'incidence et l'indice de sévérité de l'anthracnose sur le feuillage. Des feuilles symptomatiques à l'anthracnose ont été échantillonnées pour des analyses au laboratoire. Pendant la période pré-florale des anacardiers, l'anthracnose se manifeste sur les feuilles par des taches nécrotiques sous forme de pointillés ou par une plage grise. L'incidence moyenne la plus élevée a été obtenue dans la Région de la Marahoué (100 %) et la plus faible a été obtenue dans celle du Moronou (29,82 %). L'indice de sévérité moyen au niveau national s'élève à 28,48 %. 85 isolats de Colletotrichum gloeosporioides *ont été obtenus sur l'ensemble des échantillons collectés. L'étude a révélé que l'anthracnose est présente dans tous les vergers*

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d'anacardiers et son incidence varie d'une Région à l'autre en Côte d'Ivoire. La Région du Haut-Sassandra a présenté la plus forte sévérité.

Mots clés : *Anthracnose, Anacardier, Incidence, Sévérité, Côte d'Ivoire*

INTRODUCTION

The cashew tree, *Anacardium occidentale* L. (Anacardiaceae) is a perennial plant cultivated in several countries of the world. In West Africa, cashew nut is the main product of cashew tree and has become one of the most important export products and the main cash crop along with cotton in a broad Sahelo-Sudanese climatic band which stretch from Senegal to Nigeria (Ricau, 2013).

In Côte d'Ivoire, cashew was introduced in the north in the years 1959-1960 with the objective of reforestation and soil protection against erosion (Lebailly *et al*., 2012). However, today, cashew trees are cultivated for their fruit and their cultivation in Côte d'Ivoire constitutes an important sector, particularly for the population of the savannah areas. Indeed, several factors have aroused the enthusiasm of the population of these regions for cashew cultivation. Firstly, the advent of cashew nut processing and consumption at the international level (Nugawela *et al*., 2006). This was followed by the fall in the price of cotton, the main cash crop in northern Côte d'Ivoire. Finally, the rise in the purchase price of cashew nuts (Tuo, 2017). Since 2015, Côte d'Ivoire has been the world's leading cashew nut producer. Its national cashew production has continued to increase, reaching 792 678 tons of raw cashew nuts in 2019 (FAOSTAT, 2019).

However, despite this high production and Côte d'Ivoire's ranking, the crop faces enormous difficulties. Indeed, orchards are largely made up of «all-purpose» plant material and are exposed to continuous insect and disease attacks. This situation is partly responsible for the low yield of Ivorian orchards, estimated at 547 kg of nuts/ha in 2018, compared to 1 tonne or even 2 tonnes in some countries (Akessé *et al*., 2015; Soro *et al*., 2015; FIRCA, 2018). in effect, previous studies conducted in cashew orchards in Côte d'Ivoire have identified several pathogens and insect pests infested with this crop (Akessé *et al*., 2015; Soro *et al*., 2015; Kra *et al*., 2017). Recent plantings are made with research-recommended material but this material has not been tested for its susceptibility to diseases and pests. Regarding cashew diseases, anthracnose is one of the main diseases of the cashew orchard in Côte d'Ivoire (Soro *et al*., 2015; Silué *et al*., 2017). This disease, caused by the fungus *Colletotrichum gloeosporioides*, causes much damage to cashew trees. It occurs on the leaves, flower panicles and even on the fruits of cashew trees (Soro *et al*., 2013; Silué *et al*., 2017). The survey carried out by the research team (Soro *et al*., 2015) in all cashew production areas in Côte d'Ivoire allowed the distribution map of the main cashew diseases to be established. However, for a better management of diseases it is important to follow the spatio-temporal distribution in order to determine the appropriate periods of intervention according to agro-ecological zones. Therefore, data on the distribution and severity of different diseases need to be updated. It is in this context that this study was conducted. Its general objective was to update the health map of cashew tree anthracnose in Côte d'Ivoire. Specifically, the aim was to (i) determine the level of anthracnose infection in cashew tree orchards throughout the cashew tree growing area after the last surveys in 2017, (ii) update the distribution map of anthracnose in cashew in Côte d'Ivoire and (iii) morphologically characterize the fungus responsible for anthracnose.

MATERIAL AND METHODS

MATERIAL

The surveys were carried out in plantations with the aim of covering all cashew growing areas in Côte d'Ivoire from 22 October to 22 December 2019. Samples of diseased leaves taken during these surveys were kept in khaki envelopes and then sent to the laboratory for isolation of the causal agent. The isolation and characterisation of isolates of the fungus *Colletotrichum gloeosporioides* were carried out at the laboratory of the African Center of Excellence on Climate Change, the Biodiversity and Sustainable Agriculture (CEA-CCBAD) located in the Félix Houphouët Boigny University scientific center (Bingerville, Côte d'Ivoire). The equipment used

in the laboratory is made up of the fixed equipment which are essentially the autoclave, the laminar flow hood, the oven and the optical microscope and the consumables which we can summarize by alcohol, sodium hypochlorite, 90 mm diameter Petri dishes, slides and lamellae and culture media.

METHODS

Sanitary surveys and collection of samples of organs symptomatic of anthracnose

The sanitary surveys were carried out in plantations during the pre-flowering stage of cashew trees. They focused on the health level of anthracnose on the foliage and were carried out on cashew tree genotypes known as «all-purpose» in production. In each orchard, the health assessment was carried out on ten (10) cashew plants chosen at random along the two diagonals and at least 20 metres apart. Leaves of cashew trees were observed on two opposite sides of each tree following the method described by Soro *et al.* (2015) modified. This method consisted, after the selection of the tree, placing two quadrates of one square metre on each side at the level of the cashew tree foliage, one quadrate per side. Subsequently, the total number of buds and the number of diseased buds in each quadrate were counted. Finally, three (3) buds were randomly selected from each quadrate on which the evaluation was done on the last five (5) mature leaves starting from the apex of the bud at its base. The level of anthracnose infection in the surveyed orchards was determined through the infection rate or incidence and severity.

The infection rate in an orchard was calculated from the average ratio of the number of diseased buds to the total number of buds in the quadrate.

As for severity, it was assessed using a rating scale that includes 6 grades ranging from 0 to 9 (Table 1) (Groth *et al.,* 1999; Cardoso *et al.,* 2004).

Finally, the anthracnose severity index was determined in each orchard taking into account these grades from the equation of Kranz (1988):

$$Is = \frac{\Sigma(xi.ni)}{N.Z} X\,100$$

Is: Disease severity index; xi: Severity i of disease on the leaf; ni: Number of leaves of severity i; N: Total number of leaves observed and Z: Highest score on the scale, i. e. 9.

During these surveys, samples were taken from leaves showing symptoms of anthracnose as described in the literature. Then, each sample is placed in an envelope and sealed for storage. The type of symptoms and the location are written on the envelopes and the envelopes were transported in refrigerated coolers to the laboratory for analysis.

Table 1 : Anthracnose severity rating scale according to Groth *et al.* (1999) and Cardoso *et al.* (2004).

Échelle de notation de la sévérité de l'anthracnose selon Groth et al. (1999) et Cardoso et al. (2004).

Grade	Infected leaf surface	Interpretation
0	0 %	No disease
1	1-5 %	Low
3	6-10 %	Moderated
5	11-25 %	Slightly severe
7	26-50 %	Severe
9	> 50 %	Very severe

Isolation and characterisation of the fungus responsible for anthracnose

Isolation of the causal agent of anthracnose was carried out by direct seeding of leaves explants on PDA medium (Potato Dextrose Agar) following the method described by Silué *et al*. (2017). This method consisted to removing 4-6 mm of the infected leaf at the symptom front by using a sterile scalpel. Then, the explants were successively sterilised in 70% alcohol for one (01) minute, in 3% sodium hypochlorite for three (3) minutes and then rinsed three (3) times with sterilised distilled water under a laminar flow hood. They were dried on sterile filter paper (Whatman grade 42) before being placed in Petri

dishes containing the solidified PDA medium (20 ml per dish) (Silué *et al.,* 2017). Petri dishes were sealed with stretch film and incubated at room temperature (25-28°C) (Dembélé *et al.,* 2020). 48-72 h after incubation, the growing mycelia colonies were taken from the growth front and transferred to a new PDA medium and the Petri dishes were incubated at the same room temperature to obtain pure cultures. Finally, isolates of the fungus *Colletotrichum gloeosporioides*, responsible for anthracnose, were identified by macroscopic (colour and appearance of the mycelium) and microscopic (shape of conidia) observations using the identification key of Barnett & Hunter (1972).

DATA ANALYSIS

The data collected during these surveys were analysed using Statistica 7.1 software. The means of incidence and severity index of anthracnose on cashew trees were subjected to an ANOVA test. When a significant difference was observed, the Newman-Keuls post hoc test was used to separate the means into homogeneous subsets at a significance level of 5%.

The distribution map of the severity index of anthracnose in cashew nut production areas was produced using QGIS software version 2.18.9.

RESULTS

SYMPTOMS OF ANTHRACNOSE ON CASHEW TREE IN CÔTE D'IVOIRE

The surveys carried out in plantations during the pre-flowering stage of the cashew trees have allowed us to observe the presence of anthracnose in the orchards. During these surveys, anthracnose symptoms were observed in different form on the foliage of the cashew trees (Figure 1).

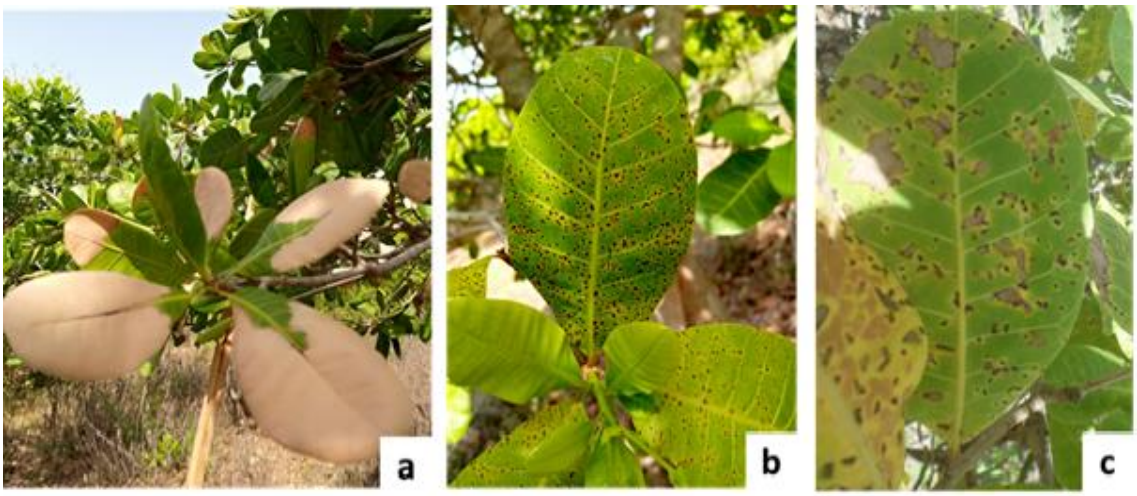

Figure 1: Symptoms of anthracnose observed on cashew trees in plantations.

Symptômes de l'anthracnose observés sur le feuillage des anacardiers en plantation.

a : Grey area of the leaf blade encompassing the veins ;
b : Necrotic spots as dotted lines on the leaf blade ;
c : Necrotic spots with small grey patches forming on the veins.

a : Plage grise du limbe englobant les nervures ;
b : Taches nécrotiques sous forme de pointillés sur le limbe ;
c : Taches nécrotiques avec formation de petites plages grises au niveau des nervures.

ANTHRACNOSE INFECTION LEVEL ON CASHEW TREES IN COTE D'IVOIRE

The sanitary surveys were carried out in 290 plantations revealed that the average incidence of anthracnose on the foliage varied from one region to another. Statistical analysis of the incidence obtained revealed a significant difference between the different regions. Indeed, the highest incidence was observed in the region of Marahoué (100%) and the lowest incidence was observed in the region of Moronou (29.82%). This result also shows that the regions in the central area (Béliér, Gbêkê and Hambol) and

central-eastern area (Iffou, Moronou and N'Zi) of Côte d'Ivoire had the lowest incidence (Table 2).

Table 2: Average incidence of anthracnose on cashew tree foliage by cashew nut production region in Côte d'Ivoire.

The incidence means with the same letters are not significantly different according to the Newman- Keuls test at the 5% significance level.

Incidence moyenne de l'anthracnose sur le feuillage des anacardiers par Région de production de la noix de cajou en Côte d'Ivoire.
Les moyennes d'incidence affectées des mêmes lettres ne sont pas significativement différentes selon le test de Newman-Keuls au seuil de significativité de 5 %.

Region	Incidence (mean ± standard error %)	F	P
Bafing	96,00 ± 4,00^{ab}		
Bagoué	83,14 ± 5,65^{abc}		
Bélier	62,07 ± 8,99^{c}		
Béré	96,97 ± 1,76^{ab}		
Bounkani	81,54 ± 4,13^{abc}		
Folon	94,98 ± 3,53^{ab}		
Gbêkê	72,88 ± 6,44^{abc}		
Gontougo	71,96 ± 5,01^{bc}		
Haut-Sassandra	98,94 ± 1,05^{ab}		
Hambol	61,98 ± 7,70^{c}	13,055	0,00
Iffou	36,07 ± 7,65^{d}		
Indenié-Djuablin	76,71 ± 11,56^{abc}		
Kabadougou	94,15 ± 2,99^{ab}		
Marahoué	100,00 ± 0,00^{a}		
Moronou	29,82 ± 5,58^{d}		
N'Zi	42,68 ± 5,89^{d}		
Poro	84,35 ± 4,06^{abc}		
Tchologo	89,33 ± 3,68^{ab}		
Worodougou	98,30 ± 0,95^{ab}		

The average severity index at national level was 28.48%. It varied between 0.44 and 85.18%. The highest severity index was obtained in the region of Haut-Sassandra (51.96%) and the lowest in the region of Iffou (4.52%) (Table 3). Regarding the distribution of the disease severity index, 147 orchards out of the 290 surveyed or 50.68% of the plantations surveyed presented a severity index less than or equal to 25% while only three orchards located in the region of Kabadougou (north-western of Côte d'Ivoire) showed severity index greater than 75% (Figure 2).

Table 3: Average severity index of anthracnose on cashew tree foliage by cashew nut production region in Côte d'Ivoire.

The means of severity index with the same letters are not significantly different according to the Newman-Keuls test at the 5% significance level.

Indice de sévérité moyen de l'anthracnose sur le feuillage des anacardiers par Région de production de la noix de cajou en Côte d'Ivoire

Les moyennes de l'indice de sévérité affectées des mêmes lettres ne sont pas significativement différentes selon le test de Newman-Keuls au seuil de significativité de 5 %.

Region	Severity index (mean ± standard error %)	F	P
Bafing	29,83 ± 3,14^{cd}		
Bagoué	18,11 ± 2,58^{cde}		
Bélier	8,44 ± 1,47^{e}		
Béré	47,93 ± 2,28^{a}		
Bounkani	8,59 ± 1,09^{e}		
Folon	32,97 ± 4,86^{bc}		
Gbêkê	18,87 ± 3,30^{cde}		
Gontougo	10,96 ± 1,99^{e}		
Haut-Sassandra	51,96 ± 2,40^{a}		
Hambol	15,86 ± 2,31^{de}	30,78	0,00
Iffou	4,52 ± 1,62^{e}		
Indenié-Djuablin	6,50 ± 2,23^{e}		
Kabadougou	43,19 ± 4,03^{ab}		
Marahoué	50,10 ± 2,53^{a}		
Moronou	5,02 ± 2,25^{e}		
N'Zi	6,11 ± 1,88^{e}		
Poro	19,44 ± 2,63^{cde}		
Tchologo	21,44 ± 2,48^{cde}		
Worodougou	48,99 ± 3,02^{a}		

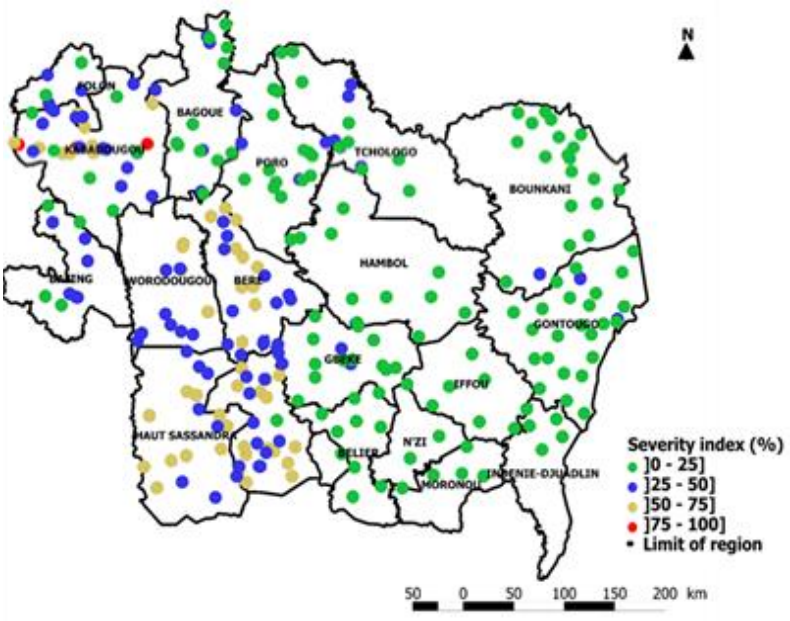

Figure 2: Distribution of cashew tree anthracnose severity index in Côte d'Ivoire.

Distribution de l'indice de sévérité de l'anthracnose de l'anacardier en Côte d'Ivoire.

ISOLATION AND MORPHOLOGICAL CHARACTERIZATION OF THE FUNGUS RESPONSIBLE FOR ANTHRACNOSE

The isolations carried out in the laboratory from the anthracnose symptoms observed on cashew trees in orchards were used to obtain isolates of *Colletotrichum gloeosporioides*. A total of 85 isolates of *C. gloeosporioides* were isolated and identified from anthracnose symptomatic samples collected in cashew trees orchard. The figure 3 shows the morphological characters (macroscopic and microscopic) of the *C. gloeosporioides* isolates obtained.

Macroscopically, all isolates showed a cottony mycelium. The colour of the isolates varied from dark grey to white on the seventh day of incubation and on this basis three different groups were formed. The first group is characterised by isolates with a dark grey colour on the surface of the mycelium and a black underside (Figures 3a and 3b). The second group is characterised by isolates with a dark grey centre with a white outline on the surface of the mycelium and a black underside with a white outline (Figures 3d and 3e). Finally, the third group is characterised by isolates with a white coloration on the surface of the mycelium and a white underside with the black inoculation point (Figures 3g and 3h).

Microscopic observation of the isolates one month after culture on the PDA medium showed conidia of cylindrical shaped (Figures 3c, 3f and 3i).

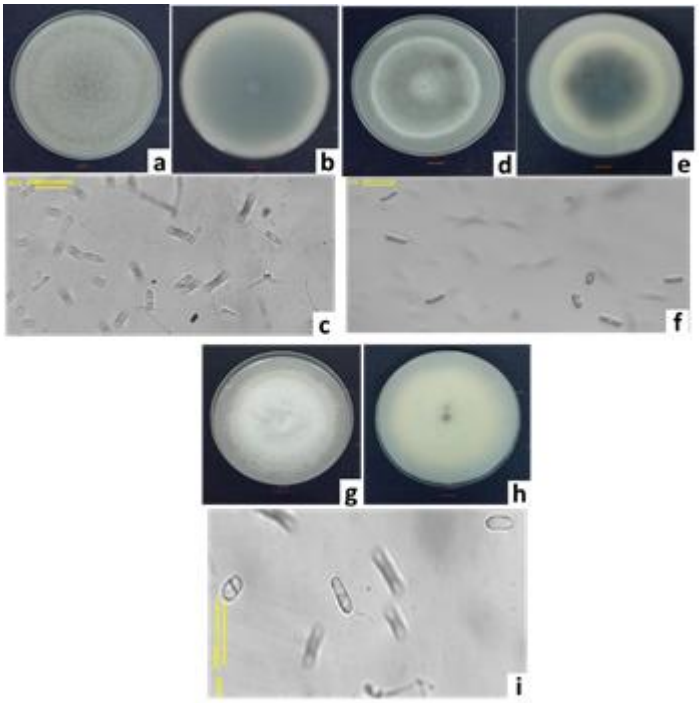

Figure 3: Macroscopic and microscopic characteristics of *Colletotrichum gloeosporioides* isolates obtained.

Caractères macroscopique et microscopique des isolats de Colletotrichum gloeosporioides obtenus.

View of the surface (a, d, g**)** and underside (b, e, h) of the mycelium in the Petri dish. Cylindrical conidia of the different isolates of *C. gloeosporioides* obtained (c, f, i).
Vue à la surface (a, d, g) et au revers (b, e, h) du mycélium en boite de Pétri. Conidies cylindriques des différents isolats de C. gloeosporioides obtenus (c, f, i).

DISCUSSION

Anthracnose symptoms observed during these surveys are identical to those described in previous years in cashew orchards in Côte d'Ivoire by Soro *et al.* (2013) and Silué *et al.* (2017). The presence of anthracnose on the foliage level in all orchards and on all the trees visited would indicate its impact on cashew nut yield. This would further justify the assertion by Silué *et al.* (2017) that anthracnose caused by *Colletotrichum gloeosporioides* is the most important fungal disease of cashew trees in Côte d'Ivoire. This disease also occurs in cashew orchards in many countries such as Ghana, Benin and Burkina Faso, where it manifests itself with virtually the same symptoms. It is therefore described as the most important disease of cashew trees in these countries (Afouda *et al.*, 2013; Wonni *et al.*, 2017; Muntala *et al.*, 2020).

The low incidence observed in the central and central-eastern areas of Côte d'Ivoire could be explained by the high presence of red rust observed on the leaves in these areas with higher humidity levels compared to other areas. Thus, by competition and with its more accelerated spread due to its powdery appearance, red rust could prevent the expression of anthracnose symptoms on cashew leaves. The low incidence in the central area corroborates with the work of Soro *et al.* (2015). Indeed, these authors revealed that of the five (5) cashew nut production areas in Côte d'Ivoire, Bouaké area (located in the centre) had the lowest incidence of anthracnose.

Concerning the severity of anthracnose, this study revealed a variation in the severity index from one region to another. This variation could be linked to the virulence of the fungus responsible for the disease, to the cultural practices adopted but also to the resistance capacity of the different plants existing in each region. According to Yao *et al.* (2017) the virulence of the fungus once present, would be influenced not only by the microclimate of the area but also by the plant's preference as a preferred host. Also, the study revealed that more than half of the orchards visited (50.68%) had a

severity index of 25% or less. This same finding was observed in 2015 by Soro *et al*.. However, contrary to these authors, anthracnose was observed in the regions of Gontougo and Indénié-Djuablin where it did not exist in 2015. Thus, this disease would have spread to the entire cashew tree growing area in Côte d'Ivoire over the last four (4) years. Furthermore, it has been noted that severities above 50% were observed in the central-western and north-western areas of the country. The forest island present in these areas would create optimal environmental conditions which would be the basis for increase in the disease severity index in these areas.

The processing of the samples in the laboratory resulted in isolates of *Colletotrichum gloeosporioides* that varied in thallus colouration on PDA medium ranging from dark grey to white. This difference in colouration could be related to the environmental conditions of the cashew tree growing areas but also to the management and agronomic practices prevailing in cashew tree orchards. Furthermore, the cylindrical conidia observed are characteristic of the species *C. gloeosporioides* (Silué *et al.,* 2017; Dembélé *et al.,* 2020). However, although the literature defines the species *C. gloeosporioides* as the only species responsible for anthracnose in cashew trees (Afouda *et al.,* 2013; Soro et *al.,* 2013; Wonni et *al.,* 2017), the identification of *Colletotrichum* species based of these morphological characteristics remains difficult because of the enormous variations within this genus. Therefore, in order to complete this traditional morphological identification, sequence analyses are still required to confirm the identity of isolates of this *Colletotrichum* species.

CONCLUSION

This study on the severity and distribution of anthracnose in cashew tree orchards in Côte d'Ivoire were aimed to determine the level of anthracnose infection in cashew tree orchards, to map the distribution of anthracnose in cashew trees in Côte d'Ivoire and to characterize the fungus responsible for this disease. The study revealed that anthracnose is a disease present in all cashew tree orchards and occurs during the pre-flowering period on the leaves of trees. Its incidence varied from region to another and ranged from 29.82 to 100%. The average severity index at national level was 28.48%. It varied between 0.44% and 85.18%. In addition, 85 isolates of *Colletotrichum gloeosporioides* were obtained from all samples collected. These isolates are characterised by a dark grey and/ or white colouration on the surface of the mycelium and a black or white underside with cylindrical-shaped spores. However, for an even more accurate classification of the isolates obtained, other characteristics such as the length and width of spores and growth rate of the isolates could be taken into consideration. More importantly, sequence analysis should confirm the identity of the isolates.

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