

DISTRIBUTION OF CASHEW TREE BACTERIAL BLIGHT (*ANACARDIUM OCCIDENTALE* L.) IN THE CASHEW NUT PRODUCTION AREAS OF COTE D'IVOIRE

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ABSTRACT

Bacterial blight is one of the main constraints of cashew trees that can hamper cashew yield. The objective of this study was to update the distribution map of bacterial blight in cashew production areas in Côte d'Ivoire.

Surveys conducted in 2019 during all phenological stages of cashew tree in all production regions made it possible to assess the average infection rate and the average severity index of bacterial blight in 358 orchards. A sampling of symptomatic organs were also carried out.

Cashew tree bacterial blight is present in all cashew nut producing regions of Côte d'Ivoire. Bacterial blight symptoms have been observed on cashew tree organs. The infection rates and severity index per region revealed a variability from 25.28 % (Moronou) to 99.39 % (Marahoué) and from 2.15 % (Indénié-Duablin) to 48.87 % (Worodougou), respectively.

Bacterial blight is present in all cashew-growing regions, with varying levels of infection. The average rates above 90 % indicate the severity of the disease.

Key words : Cashew tree, Bacterial blight, Infection rate, Severity index, Côte d'Ivoire.

RESUME

DISTRIBUTION DE LA BACTERIOSE DE L'ANACARDIER (ANACARDIUM OCCIDENTALE L.) DANS LES ZONES DE PRODUCTION DE LA NOIX DE CAJOU EN CÔTE D'IVOIRE

La bactériose est l'une des principales contraintes de l'anacardier susceptibles de freiner le rendement de la noix de cajou. L'objectif de cette étude a été d'actualiser la carte de distribution de la bactériose dans les zones de production de l'anacardier en Côte d'Ivoire.

Des prospections conduites en 2019 durant tous les stades phénologiques de l'anacardier dans toutes les régions de production ont permis d'évaluer le taux moyen d'infection et l'indice moyen de sévérité de la bactériose dans 358 vergers. Des échantillonnages d'organes symptomatiques ont été également effectués.

la bactériose de l'anacardier est présente dans toutes les régions de production de noix de cajou en Côte d'Ivoire. Les symptômes de la bactériose ont été observés sur les organes de l'anacardier. Les taux d'infection et les indices de sévérité par région ont révélé une variabilité respective de 25,28 % (Moronou) à 99,39 % (Marahoué) et de 2,15 % (Indénié-Duablin) à 48,87 % (Worodougou).

La bactériose est présente dans toutes les régions productrices de l'anacardier, avec des niveaux d'infection variables. Les taux moyens, au-delà de 90 % dénotent de la gravité de la maladie.

Mots clés : Anacardier, Bactériose, Taux d'infection, Indice de sévérité, Côte d'Ivoire.

INTRODUCTION

Cashew (*Anacardium occidentale* L., Anacardiaceae) is a plant native to northeast Brazil. It has been introduced in several African countries to combat deforestation and soil erosion (Lacroix, 2003). This plant is mainly cultivated for its fruit: the apple and the cashew nut (Agboton *et al.*, 2014). Cashew nuts are used in several fields, including agribusiness, cosmetology, medicine and the automotive industry (RONGEAD, 2010; Ricau, 2013; Catarino, 2015). Indeed, the kernel contained in the nut after shelling is consumed in the salted or Chili form. The food industry is used to manufacture cakes, chocolate, confectionery, ice cream, and oil. Cashew balsam, a blackish and corrosive oily liquid extracted from the shell surrounding the almond, can be transformed into resins used to manufacture brake linings and clutch discs. It is also used to modify rubber properties and protect soils from chemicals (Kouassi *et al.*, 2017).

In Côte d'Ivoire, following the decline in the price of cotton, the primary cash crop of the North on the international market, and the increase in the purchase price of cashew nuts, several farmers have become interested in cashew nut cultivation to improve their socio-economic level to meet their needs (Nugawela *et al.*, 2006). Indeed, cashew cultivation participates in the socio-economic development of Côte d'Ivoire where it occupies a prominent place in the national economy to the tune of 8.8% of GDP (Sinan and Abou, 2016). In a decade, it has become one of the most important cash crops in the savannah regions of Côte d'Ivoire, thus offering hope for the populations of these regions and for the country growth in agriculture worldwide (Sinan and Abou, 2016).

During these years, cashew nut production in Côte d'Ivoire has recorded a significant increase from 235,000 tons in 2006 to 792,678 tons in 2019 (FAOSTAT, 2019). However, this increase in production is mainly due to an expansion of the harvested area: 650,000 hectares in 2006 to 1,913,073 hectares in 2019, in contrast to the production per hectare, which still remains low. This yield was estimated at 414.3 kg/ha in 2019 compared to 1000 to more than 2000 kg/ha for countries like Gambia, Vietnam, Mexico (FAOSTAT, 2019). This low yield would be due to the use of unimproved plant material, still traditional production techniques, especially to

the persistence of phytosanitary constraints, including insect pests and diseases (Soro *et al.*, 2015; Akessé *et al.*, 2015; Silué *et al.*, 2017). Among these diseases, we have cashew bacterial blight, which is the subject of our study. This emerging disease is caused by *Xanthomonas citri* pv *anacardii* and *Xanthomonas citri* pv *mangiferae indicae* (Zombre *et al.*, 2016).

Bacterial blight symptoms on cashew start with small oily angular spots on the leaves, usually on the veins, surrounded mainly by a yellow halo before turning brown. On cashew nuts, black oily spots are observed that become necrotic, and to a lesser extent, stems may show some exudate (Wonni *et al.*, 2017).

The bacterium responsible for this disease is most easily transmitted from one infected plot to another through already contaminated plant material (young plant), natural pathways (hydathodes, lenticels, stomata) or wounds in plant organs. Infected irrigation water and heavy rainfall with high winds can also favour their spread and insects, nematodes and certain cultural practices (Kerr and Gibb, 1997; Gagnevin and Pruvost, 2001).

The geographical distribution, incidence and severity of bacterial blight have been studied in Latin American and African countries. The results of these studies showed that bacterial blight is one of the most important diseases of cashew. Indeed, Viana *et al.*, (2006) showed that the monitoring of cashew orchards in some Brazil cities showed that the genus *Xanthomonas* was capable of causing considerable losses since the still green cashew nuts infected from cashew plants to give fruits unfit for marketing. The same is true for the studies of Gama *et al.*, (2013), which revealed that cashew production could be limited by diseases caused by plant pathogenic bacteria of the genus *Xanthomonas*. The incidence of the disease on fruit is strongly correlated with the severity of the leaves, observed six months before fruiting. In Tanzania, bacterial blight is among the diseases responsible for significant damage to cashew (Otuonye *et al.*, 2014). Bacterial blight of cashew in Benin had the highest average severity: 18.19% (Afouda *et al.*, 2013). In Côte d'Ivoire, very few data exist on cashew bacterial blight distribution at the national level.

Nevertheless, a preliminary study to establish the cashew nut health map showed that bacterial blight is a threat in the zones of Séguéla and Odienné with an incidence of 57.24% (Soro *et al.*, 2015). Yet, this disease has proven to be

serious for its yield losses in orchards worldwide. Thus, knowledge of the incidence and severity of the disease by production area is a prerequisite for estimating the production losses caused to anticipate and guide the implementation of appropriate means of control of the pathogen. In this perspective, the general objective of our study is to update the sanitary map of bacteriosis in Côte d'Ivoire. Specifically, it is to study the symptomatology of bacteriosis on cashew organs, isolate and identify the bacteria's morphologically, evaluate the infection rate, and establish the distribution map of the severity of this disease.

MATERIAL AND METHODS

SURVEY SITE

Survey missions were conducted from February 2019 to December 2019 in the 19 cashew producing regions of Côte d'Ivoire (Bounkani, Gontougo, Indénié-Duablin, Hambol, Gbêké, Béliér, Iffou, Moronou, N'Zi, Poro, Bagoué, Tchologo, Folon, Kabadougou, Bafing, Marahoué, Béré, Haut-Sassandra, Worodougou) (Figure 1). A total of 358 orchards were visited from the pre-floral to the fruiting phase of cashew trees.

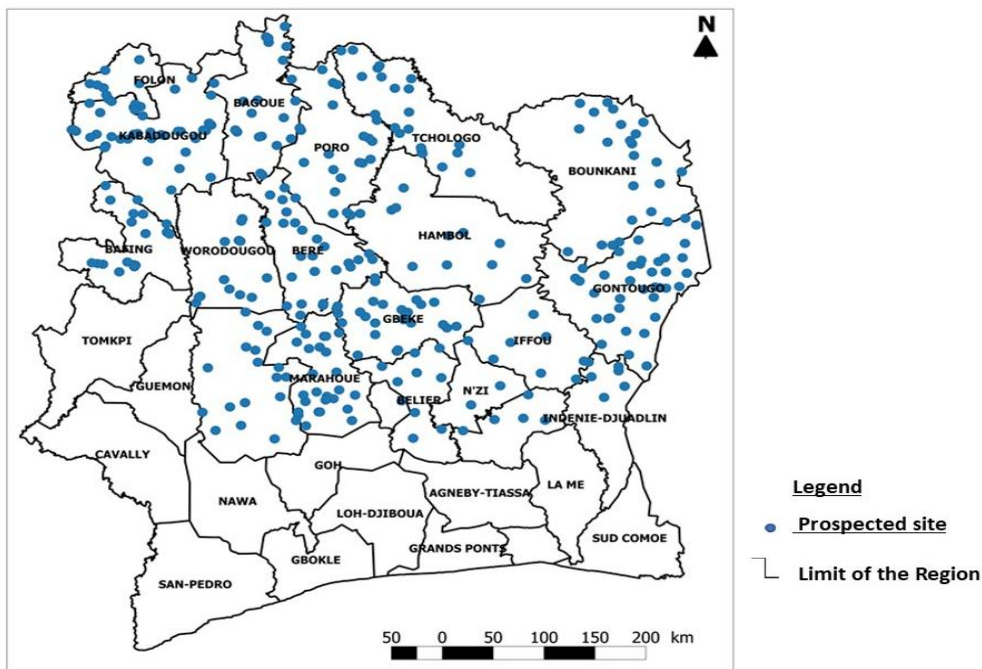


Figure 1 : Distribution map of surveyed locations in all cashew producing regions of Côte d'Ivoire.

Carte de distribution des localités prospectées dans toutes les régions productrices de noix de cajou de Côte d'Ivoire.

MATERIAL

The plant material used consisted of cashew trees of the «all new» genotype which were evaluated in the different orchards visited. A GARMIN GPS was used to record the geographic coordinates of the orchards, a SONY digital camera was used to take pictures, khaki envelopes, a refrigerated cooler for the conservation of samples and a data collection sheet constituted the main technical equipment.

METHODS

Survey and sampling

Surveys were conducted in randomly selected plantations in the 19 cashew producing regions of Côte d'Ivoire.

The plantations surveyed were at least 25 km apart. A survey form developed in the form of a questionnaire allowed us to collect information

on the producers and their orchards. The geographical coordinates of the different plots were recorded on the survey forms. The evaluation of the sanitary state of the plots visited was done on the basis of the symptoms described in the literature and observed in the field.

During these surveys, samples of organs (leaf, twig, flower, apple and cashew nut) showing symptoms of bacterial blight were described according to their colour, size and distribution on the different organs. These samples were then collected, placed in persimmon envelopes and stored in a refrigerated cooler to be transported to the laboratory for pathogen isolation.

Parameters evaluated

The parameters evaluated were the infection rate of the disease and the severity of its damage. The evaluation of these parameters was done on ten (10) cashew trees randomly selected along two diagonals in each plantation. These estimates were made on the east and west sides of the crown of each tree.

Bacterial blight infection rate was determined by the ratio of the number of diseased buds to the total number of buds in a 1 m² dial delineated on the crown of each side of the tree (Cooke modified, 2006).

$$I (\%) = \frac{A_i}{A_{ti}} \times 100$$

A_i : number of diseased buds ; A_{ti} : total number of buds

As for the severity, it was done on the first 5 leaf stages of three buds taken at random in the 1 m² frame according to the scoring scale of Moral and Trapero (2009) ranging from 1 to 9 :

0 = Absence of symptoms ;

1 = 1 - 5% ;

3 = 6 – 10% ;

5 = 11 - 25% ;

7 = 26 – 50% ;

9 > 50% of the leaf area infected. From these scores, the disease severity index was determined in each orchard from the following equation (Kranz, 1988) :

$$I_s (\%) = \frac{\sum (X_i \cdot n_i)}{NZ} \times 100$$

X_i : disease severity i on the tree;

n_i : number of trees of severity i;

N : total number of trees observed;

Z : highest severity scale

Isolation and morphological characterization

In the laboratory, the isolations were carried out on the organs presenting the symptomatic characters of bacteriosis. Indeed, each of the explants (section of diseased tissues) was disinfected respectively for 30 seconds in 70% alcohol, a 1% sodium hypochlorite solution (bleach 12°) for 1 minute and then rinsed three times in sterilized distilled water. These explants were then dilacerated in sterile distilled water. The product obtained after dilaceration underwent a series of dilutions. Then 50 µl of each of these dilutions were spread on YPGA (Yeast extract, Peptone, Glucose, Agar) standard culture medium of bacteria amended with 40% cephalixin, 20% kasugamycin, and 50% propiconazole previously prepared. The different media were incubated for 72 h at 30°C. Isolates obtained after 72 h of incubation showing typical characters of the genus *Xanthomonas* were subsequently purified on new YPGA media and incubated. After 48 h of incubation, the obtained isolates were characterized morphologically and compared to a reference strain of the genus *Xanthomonas* (Wonni *et al.*, 2014).

STATISTICAL ANALYSIS

Statistica 7.1 software was used to perform a series of analyses. The data obtained from the different surveys were recorded with the Excel spreadsheet and then subjected to a one-criteria analysis of variance classification (ANOVA 1). The Fischer LSD test was used to classify the means for determining the different homogeneous groups in case of difference at the 5% threshold.

The bacterial disease map was developed with QGIS software version 2.18.9 where the surveyed areas were projected.

RESULTS

SYMPTOMOLOGY OF CASHEW BACTERIAL BLIGHT ON CASHEW ORGANS

Symptoms of cashew bacterial blight can be seen on cashew leaves as well as on apples and cashews.

On the leaves, the disease appears on the upper

surface as black or brown spots. These spots are angular, translucent, often surrounded by a yellow halo and located at the veins level (Figure 2 A and 2 B), but also in the leaf blade (Figure 2 C).

On apples, these are cracks in three forms: vertical and oblique (Figure 3 A), vertical and horizontal (Figure 3 B).

On nuts, two types of symptoms have been observed. They are translucent brownish oily spots (Figure 4 A and 4 B) and necrosis (Figure 4 C).

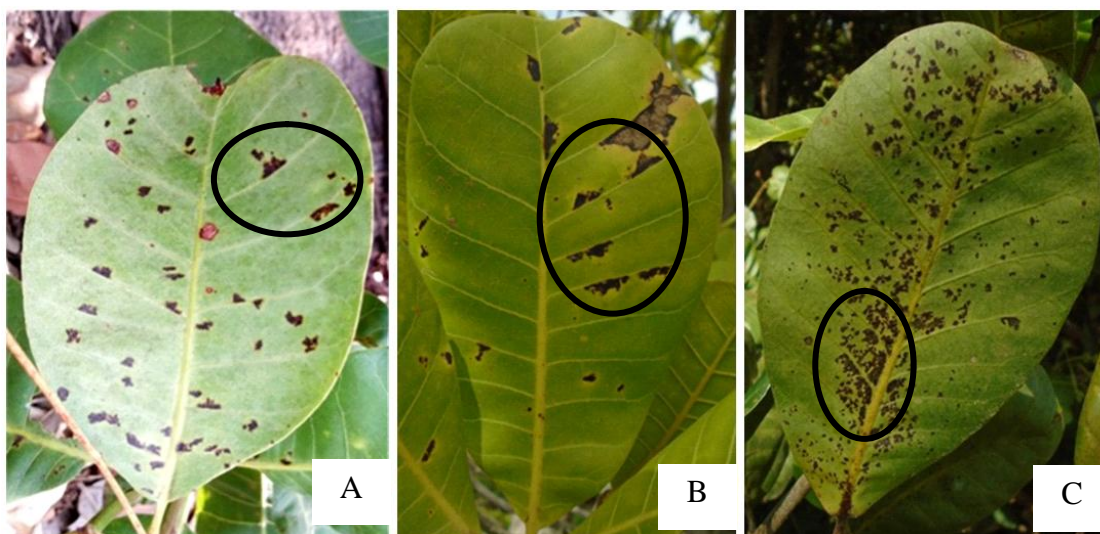


Figure 2 : Angular translucent spots observed on cashew leaves in a plantation.

Taches translucides anguleuses observées sur feuilles d'anacardier en plantation.

A : brown spots at the level of the veins ; B : brown spots surrounded by a yellow halo at the level of the veins;
C : brown spots in the leaf blade

A : taches brunes au niveau des nervures ; B : taches brunes entouré de halo jaune au niveau des nervures ;
C : taches brunes dans le limbe

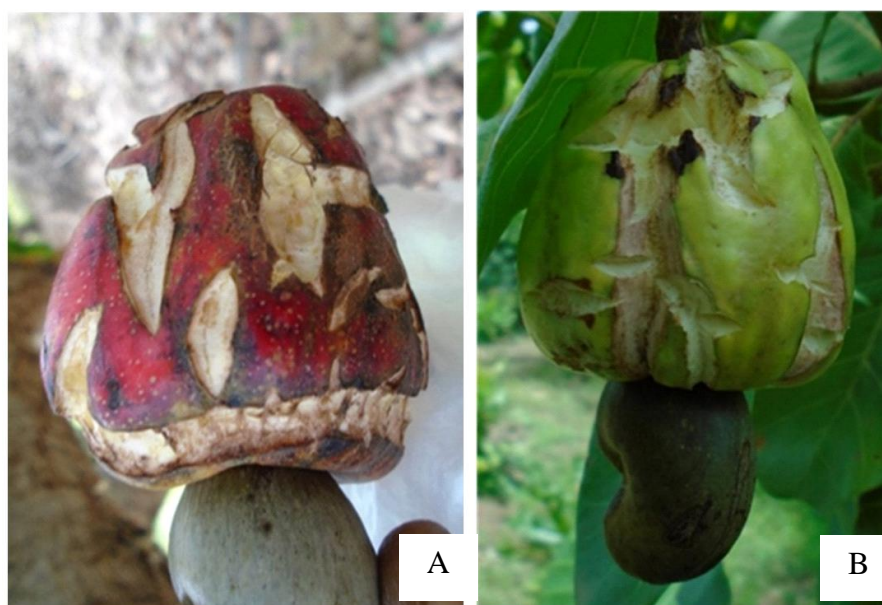


Figure 3 : Symptoms observed on cashew apples in plantation.

Symptômes observés sur pommes de cajou en plantation.

A : vertical and oblique cracks ; B : vertical and horizontal cracks

A : fissures verticales et obliques ; B : fissures verticales et horizontales

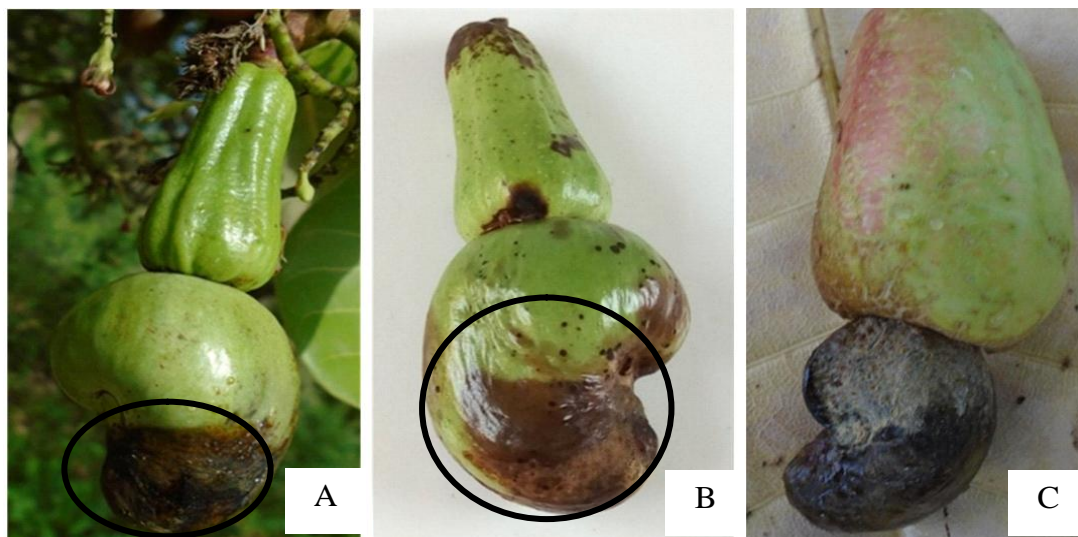


Figure 4 : Symptoms observed on cashew nuts in plantations.

Symptômes observés sur noix de cajou en plantation.

A and B: translucent brown spot ; C : necrotic nut

A et B : tache brune translucide ; C : noix nécrosée

ISOLATION AND MORPHOLOGICAL IDENTIFICATION OF BACTERIA

Characteristic bacterial colonies of the genus *Xantho-*

monas were obtained on YPGA medium. These colonies are translucent ivory-white in colour, spherical and convex in shape with regular contours and have a smooth and viscous appearance (Figure 5).



Figure 5 : Purified isolate of *Xanthomonas* sp 72 h after incubation on YPGA medium.
Isolat purifié de Xanthomonas sp 72h après incubation sur milieu YPGA.

RATE OF INFECTION OF BACTERIOSIS

Bacteriosis was found in most orchards visited, thus in all cashew production regions in Côte d'Ivoire. However, infection rates varied from one region to another and ranged from 25.28 to 100%. Statistical analyses showed a significant difference between the mean infection rates of these different regions

($F = 57.10$; $P < 0.05$) (Table 1).The highest infection rates were obtained in the Marahoué, Haut-Sassandra, Worodougou, and Béré Regions and were 99.39, 98.60, 98.51, and 96.30%, respectively. These different values are statistically identical to each other according to the Fisher LSD statistical test. The lowest rate of infection of the disease was recorded in the Moronou region at 25.28%.

Table 1 : Average infection rate of bacterial blight in all cashew producing regions of Côte d'Ivoire.

Taux moyen d'infection de la bactériose dans toutes les régions de production de la noix de cajou de Côte d'Ivoire.

Regions	Average infection rate (%)	F	P
Marahoué	99,39 ± 0,40 ^a		
Haut-sassandra	98,60 ± 0,79 ^a		
Worodougou	98,51 ± 0,95 ^a		
Béré	96,30 ± 1,09 ^a		
Bagoué	77,32 ± 2,88 ^b		
Kabadougou	73,20 ± 2,41 ^b		
Poro	72,79 ± 3,37 ^b		
Bounkani	71,84 ± 3,67 ^b		
Gbêkê	61,10 ± 3,06 ^c		
Tchologo	54,90 ± 3,63 ^{cd}	57,10	0,00
Folon	44,96 ± 5,70 ^e		
Gontougo	43,08 ± 2,33 ^e		
Bélier	42,41 ± 3,89 ^e		
Bafing	42,34 ± 3,65 ^e		
N'Zi	40,69 ± 4,72 ^{ef}		
Hambol	38,44 ± 4,03 ^{ef}		
Iffou	36,76 ± 4,10 ^{ef}		
Indénié-Duablin	28,41 ± 3,55 ^f		
Moronou	25,28 ± 4,56 ^f		

The numbers assigned to the different letters are significantly different according to Fisher's LSD test at the 5% threshold.

Les chiffres attribués aux différentes lettres sont significativement différents selon le test LSD de Fisher au seuil de 5 %.

AVERAGE SEVERITY OF BACTERIAL BLIGHT IN ALL CASHEW GROWING AREAS OF CÔTE D'IVOIRE

The average severity indices of the different cashew production regions ranged from 2.15 to 48.87%. The highest average severity index was obtained in the Worodougou region: 48.87% while the lowest were noted in Hambol, Bagoué, Bélier, Bafing, Folon, Bounkani, Poro, Tchologo, Gontougo, Moronou, N'Zi, Iffou, and Indénié-Djuablin regions and were respectively; 7,99 ; 7,71 ; 7,50 ; 7,08 ; 6,84 ; 6,64 ; 6,34 ; 5,32 ; 2,90 ; 2,81 ; 2,77 ; 2,15%. Statistical analyses showed a significant difference between the mean severity indices of these different regions (F = 57.69; P < 0.05) (Table 2).

Thus, within each region, the majority of the surveyed locations had high severities. However, the severity of bacterial blight varied among locations in different regions. Almost all localities in Gontougo, Bounkani, Indénié-Duablin, Poro, Bagoué, Tchologo, Hambol, Gbêkê, Bélier, N'Zi, Moronou, Iffou, Folon, Bafing, and Kabadougou regions showed severity indices ranging from 0 to 25%. Almost all localities in the Haut-Sassandra, Béré, Worodougou, Mrahoué and some localities in the Gbêkê and Kabadougou regions had severities ranging from 25 to 50%. However, the highest severities ranging from 50 to 75% were observed in several localities in Worodougou, Haut-Sassandra, Marahoué, Béré, and a few localities in Kabadougou region (Figure 6).

Table 2 : Average severity index of bacterial blight in all cashew producing regions of Côte d'Ivoire.

Indice moyen de sévérité de la bactériose dans toutes les régions de production de la noix de cajou de Côte d'Ivoire.

Regions	Average severity index (%)	F	P
Worodougou	48,87 ± 3,03 ^a		
Haut-sassandra	45,46 ± 2,79 ^{ab}		
Marahoué	44,55 ± 1,89 ^{ab}		
Béré	42,14 ± 2,45 ^b		
Kabadougou	18,66 ± 2,64 ^c		
Gbêkê	14,55 ± 2,18 ^c		
Hambol	8,34 ± 1,70 ^d		
Bagoué	7,99 ± 0,89 ^d		
Béliér	7,71 ± 1,54 ^d		
Bafing	7,50 ± 1,10 ^d	57,69	0,00
Folon	7,08 ± 2,91 ^d		
Boukani	6,84 ± 1,04 ^d		
Poro	6,64 ± 0,73 ^d		
Tchologo	6,34 ± 0,70 ^d		
Gontougo	5,32 ± 0,80 ^d		
Moronou	2,90 ± 0,87 ^d		
N'Zi	2,81 ± 0,93 ^d		
Iffou	2,77 ± 1,01 ^d		
Indénié-Duablin	2,15 ± 0,41 ^d		

The numbers assigned to the different letters are significantly different according to Fisher's LSD test at the 5% threshold.

Les chiffres attribués aux différentes lettres sont significativement différents selon le test LSD de Fisher au seuil de 5 %.

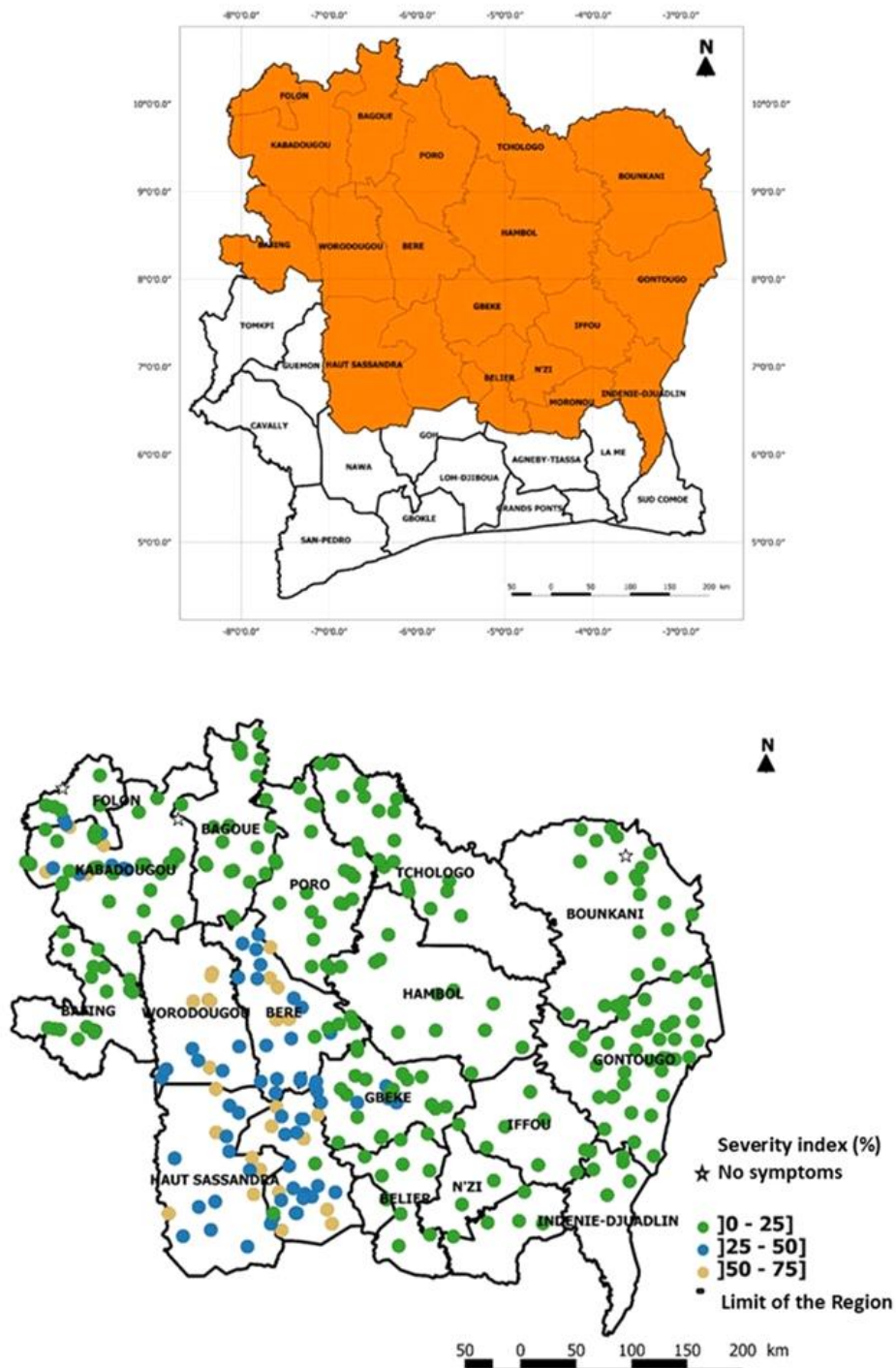


Figure 6 : Distribution map of the bacterial blight severity index observed on cashew leaves in the cashew nut production areas of Côte d'Ivoire.

Carte de répartition de l'indice de sévérité de la bactériose observée sur les feuilles d'anacardier dans les zones de production de noix de cajou de Côte d'Ivoire.

DISCUSSION

Surveys of cashew orchards in all cashew production areas have revealed that bacterial blight is present in all cashew production areas in Côte d'Ivoire. Therefore, it is considered one of the major cashew diseases in view of its infection rate and its high severity in almost all the orchards of the country. Bacteriosis is a real threat to production since it has had a high infection rate in all production areas, especially since it infects both the leaves and the fruiting bodies (flowers and fruits). The characteristic symptoms of bacterial blight observed on the leaves and fruits of cashew were similar to those described in the literature of other African and Latin American countries (Afouda *et al.*, 2013; Wonni *et al.*, 2017; Viana *et al.*, 2006; Gama *et al.*, 2013). These different authors have described similar symptoms in cashew leaves and cashew nuts in their works. These appear as translucent spots characteristic of bacterial attacks. Regarding the cracks observed in cashew apples, they would resemble the gaps observed in mango, which are also characteristic symptoms of bacterial blight in mango fruits (Pruvost *et al.*, 2011). These results would indicate that cashew leaves and fruits are subject to bacterial blight.

Isolation allowed the identification of bacteria with the specific characteristics of *Xanthomonas* isolates. The bacteria obtained are white-ivory and translucent in colour, spherical and convex in shape, with regular contours, viscous and smooth in appearance. These morphological characters are similar to the specific characteristics of *Xanthomonas* isolates. (Affery *et al.*, 2018).

The distribution of bacterial blight varied among regions. These results are similar to those of Garrett *et al.*, (2009), who showed that environmental conditions strongly influence plant pathogen attacks. The Worodougou, Marahoué, Haut-Sassandra, and Béré regions had the highest infection rates and severity of bacterial blight. The microclimate of the localities could explain these results in these regions, which are, for the most part, humid tropical zones with higher rainfall than the other areas surveyed. Indeed, the humidity would be favourable to the development and propagation of the agent responsible for bacteriosis. Thus, the alternation of rainy and dry seasons would lead to humidity favourable to bacteriosis development (Sud Arbo

Fiches Techniques, 2011). The high distribution of bacterial blight in these regions could be because the splashing of water drops, caused by rain, would move the pathogen from a healthy tree to a diseased tree (IITA, 2000), leading to a spread to neighbouring trees. However, the N'Zi, Moronou and Iffou regions, although also humid tropical areas with high rainfall, had the lowest infection rates. This could be explained by the fact that cashew cultivation in these regions is still very recent, so the pathogen would not have had time to spread rapidly, unlike in other older areas. Although infection rates of bacterial blight are high in the Poro, Bounkani, Folon, Gontougo, and N'Zi regions, their severity index is lower than that of the other Regions. This would indicate that the bacterial strains in these areas are less aggressive than those in other regions. These results corroborate those of Soro *et al.*, (2015), who showed in their work that bacterial blight was present in almost all cashew growing areas, but with low incidence in the vast majority of orchards surveyed. However, the present study revealed an increase in the infection rate and severity of the disease in these different production areas. These results obtained are in agreement with those of Afouda *et al.*, (2013). Indeed, these authors revealed bacteriosis in all cashew production areas in Benin, but with the highest severity, which is 18.19%.

CONCLUSION

This study showed cashew bacterial blight in most of the orchards in the cashew producing regions. However, the infection rates and severity of the disease are variable from one area to another with high severity (48.87%).

Infection rates above 50% were observed in ten surveyed regions: Marahoué, Haut Sassandra, Worodougou, Béré, Bagoué, Kabadougou, Poro, Bounkani, Gbêkê and Tchologo. The severity was more pronounced in the regions of Worodougou, Haut-Sassandra, Marahoué and Béré.

Typical colonies of the genus *Xanthomonas* were isolated from symptomatic leaves and fruits (walnut and apple).

Thus, control strategies against bacteriosis should be considered to increase production.

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