

SPATIAL DISTRIBUTION OF SOYBEAN BACTERIAL LEAF PUSTULE IN BENIN AND IDENTIFICATION OF *XANTHOMONAS AXONOPODIS* PV. *GLYCINES* HOST PLANTS

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ABSTRACT

Soybean bacterial leaf pustule (SBLP) caused by *Xanthomonas axonopodis* pv. *glycines* (*Xag*) is one of the most economically significant soybean diseases. This study aims to determine the spatial distribution of SBLP in Benin and identify other potential host plants. Two surveys were carried out during the flowering and pod maturation periods in 175 fields across 24 districts belonging to six agro-ecological zones (AEZ) in Benin in 2019 and 2020. The disease incidence and severity were randomly assessed on 30 and 20 plants, respectively, selected on the diagonals of each field. Soybean and weeds leaves were sampled based on symptomatology. The disease occurred in 115 out of 175 fields within the prospected AEZs except the Zone of Depression. The average incidence ranged from 5 to 47.25% and from 17.22 to 44.87% in 2019 and 2020, respectively. The severity was 2.23 - 37.88% and 6.52 - 34.95% in 2019 and 2020, respectively. The Cotton Zone of North Benin is the most affected by the disease with Kandi, Parakou, Gogounou and Segbana the most vulnerable districts contrary to the West Atacora Zone and the Zone of «terre de barre». The disease-free districts of Kouande and Zogbodomey could be regraded for healthy soybean seeds production.

Finally, *Acalypha ciliata*; *Combretum molle*; *Christiana africana*; *Stylochaeton hypogeum* and *Amorphophalus dracontioides* are identified as *Xag* hosts. Better management of these host plants would contribute to more effective disease control and, as result, improve soybean output in Benin.

Key words: *Xanthomonas axonopodis* pv. *glycines*, distribution, host, soybean, Benin.

RESUME

DISTRIBUTION SPATIALE DE LA PUSTULE BACTÉRIENNE DU SOJA AU BÉNIN ET IDENTIFICATION DES PLANTES HÔTES DE *XANTHOMONAS AXONOPODIS* PV. *GLYCINES*

La pustule bactérienne du soja (PBS) causée par *Xanthomonas axonopodis* pv. *glycines* (*Xag*) est l'une des maladies les plus redoutables du soja. Cette étude vise à déterminer la distribution spatiale de la PBS au Bénin et identifier de nouvelles plantes hôtes. Deux prospections ont été conduites pendant les phases de floraison et de formation des gousses, dans 175 champs répartis dans 24 communes appartenant à six zones agro-écologiques (ZAE) au Bénin. L'incidence et la sévérité de la maladie ont été évaluées respectivement sur 30 et 20 plants de soja choisis au hasard sur les deux diagonales de chaque champ. Des feuilles de soja et de mauvaises herbes ont été échantillonnées sur la base de la symptomatologie. La maladie a été identifiée dans 115 des 175 champs prospectés.

En dehors de la Zone de la Dépression, l'ensemble des ZAE ont été infectées. L'incidence moyenne a varié de 5 à 47,25 % en 2019 et de 17,22 à 44,87 % en 2020. La sévérité quant à elle, a variée de 2,23 à 37,88 % en 2019 et 6,52 à 34,95 % en 2020. La Zone Cotonnière du Nord-Bénin est la plus affectée par la maladie, avec Kandi, Parakou, Gogounou et Ségbana les communes les plus vulnérables ; contrairement à la Zone Ouest-Atacora et la Zone de « terre de barre ». Les Communes de Kouandé et Zogbodomey exemptes de la maladie, pourraient être retenues pour la production de semences saines de soja au Bénin

Enfin, *Acalypha ciliata* ; *Combretum molle* ; *Christiana africana* ; *Stylochaeton hypogeum* et *Amorphophalus dracontioides* ont été identifiées comme hôtes du Xag. Une meilleure gestion de ces plantes hôtes contribuerait à une meilleure efficacité dans le contrôle de la maladie et ainsi, améliorer la production du soja au Bénin.

Mots clés : *Xanthomonas axonopodis* pv. *glycines*, distribution, hôte, soja, Bénin.

INTRODUCTION

Soybean (*Glycine max*) is one of the most important oilseed crops in sub-Saharan Africa (SSA) providing a range of economic, social, environmental (Sanginga and Bergvinson, 2015), nutritional (OECD and FAO, 2016) and agronomic (Karaboneye, 2013) benefits. It is cultivated by more than one million farmers in Africa. The huge increase in soybean' price worldwide, had affected national prices in SSA, making the crop production more profitable for farmers compared to other food and cash crops (Sanginga and Bergvinson, 2015). Soybean bacterial leaf pustule (SBLP), is one of the most economical important soybean disease in tropical and subtropical regions of the globe (Sinclair, 1999; Khaeruni *et al.*, 2007). *X. axonopodis* pv. *glycines* (Xag), the causal agent of SBLP, infects several legume species (Hartman and Hill, 2010). A part of *Glycine max*, Xag' hosts included vine (*Brunnichia cirrhosa*), kidney bean (*Phaseolus vulgaris*), lima bean (*Phaseolus lunatus*), horse gram (*Macrotyloma uniflorum*), and numerous *Phaseolus* and *Vigna* spp. (Kennedy and Sinclair, 1989). On soybean, the symptoms of the disease includes small, pale green spots with pustules, which grow into large necrotic spots leading to premature defoliation (Kennedy and Tachibana, 1973; Narvel *et al.*, 2001). The latter causes yield losses due to fall in seeds number and size, reduction and as result, grain production reductions. Such a severe defoliation has been reported in Nigeria and the Ivory Coast (Janse, 2005). The disease can affect all growth stages of the crop but may be severe on fully developed seedlings. Yield losses of 15.9 - 50% (Dirmawati, 2004) and then nearly 41% (Prathuangwong *et al.*, 1993), have been recorded in Indonesia and in some regions of the world, respectively. SBLP

was reported in some areas in Benin with yield losses ranging from 2.7 to 28.1% (Zinsou *et al.*, (2015a; b); Zinsou *et al.*, (2016)). The improvement of soybean production in Benin require the management of this devastating bacterial disease. However, recent data lacks as the last disease' diagnosis was carried in 2011 and 2012; prior to the extension of Jupiter and TGX 1910-14F; two soybean varieties currently adopted by farmers. In addition, to date, no study has been carried out in Africa to identify Xag host plants species, which may contribute to the spread of the disease. Hence, this study was designed to determine the spatial distribution of SBLP in Benin and explore alternate Xag host plants.

MATERIALS AND METHODS

STUDY AREA

Two surveyes were conducted in six Benin's agro-ecological zones (AEZ) namely AEZ II, III, IV, V, VI and VII.

As reported by MCVDD (2020), Chabi *et al.* (2019) and (MAEP, 2009), the AEZ II also called Cotton Zone of North Benin includes Banikoara, Kerou, Kandi, Segbana and Gogounou districts, and is located between a latitude of 10.5 and 12°. It is characterized by a cropping season which extends from May to September, a more or less sandy tropical ferruginous soil, a rainfall of 900-1000 mm and a temperature varying from 28 to 45°C.

The AEZ III (South Borgou Food Zone), is located between 1°10' - 3°45' E and 9°45' - 12°25' N. It includes the districts of Nikki, Perere, Kalale, Bembereke, N'Dali, Sinende, Pehunco and Kouande. AEZ III is characterized by a unimodal

rainfall distribution with an average annual rainfall less than 1000 mm and located in the Sudanese zone of Benin. The relative humidity ranges from 18 to 99% while the annual mean temperature fluctuates from 24 to 31°C. This AEZ is mostly covered by ferric and plintic luvisol (FAO, 2006).

The AEZ IV also called West Atacora Zone, covers Tanguieta, Cobly, Materi, Natitingou, Toucountouna, Boukoumbe, Copargo, Ouake and Djougou district. The annual rainfall is 800-1350 mm.

The Cotton Zone of Central Benin (AEZ V), located between 1°45' - 2°24' E and 6°25' - 7°30' N. The area is under the sudano-guinean zone also called transitional zone of Benin. The annual mean temperature is between 26 and 29°C and the average annual rainfall ranges from 1000 to 1400 mm. The relative humidity varies from 69 to 97%. The AEZ V is mostly covered by a ferric and plintic luvisol. Black and hydromorphic soils are found in the rivers' valleys.

Zone VI also called « Terre de barre » Zone due to the properties of the soils that cover it, is located in the southern part of Benin and includes 22 districts. Two rainy seasons (March-July and October-November) and two dry seasons (December-February and August) are

available in this zone with an annual rainfall of 1000-1400 mm.

The Depression Zone (AEZ VII), the smallest surveyed zone, includes the depressions of Tchi (District of Lalo), Lama (Districts of Toffo and Zogbodomey), and Issaba (Districts of Adja-Ouere and Pobe). It is characterized by a sub-equatorial climate with two rainy seasons with an annual rainfall of 800-1200 mm in its western part (Lalo) and 1000-1300 mm in its eastern part (Pobe). The mean relative humidity is 85%

SURVEYS

One hundred and thirty (130) and forty five (45) fields of at least 0.25 ha, located at a distance of 5 to 10 km, were visited in six agro-ecological zones of Benin in 2019 and 2020, respectively. Two to eighteen fields were surveyed per district for a total of 22 districts. Leaves of weeds, trees, and soybean crops suspected to be infected with *X. axonopodis* pv. *glycines* were collected in and around soybean fields and immediately placed in newspaper and then in press prior to further investigations. Global position systems (GPS) and geographic information systems (GIS) technologies help to map the surveyed sites (Figure 1).

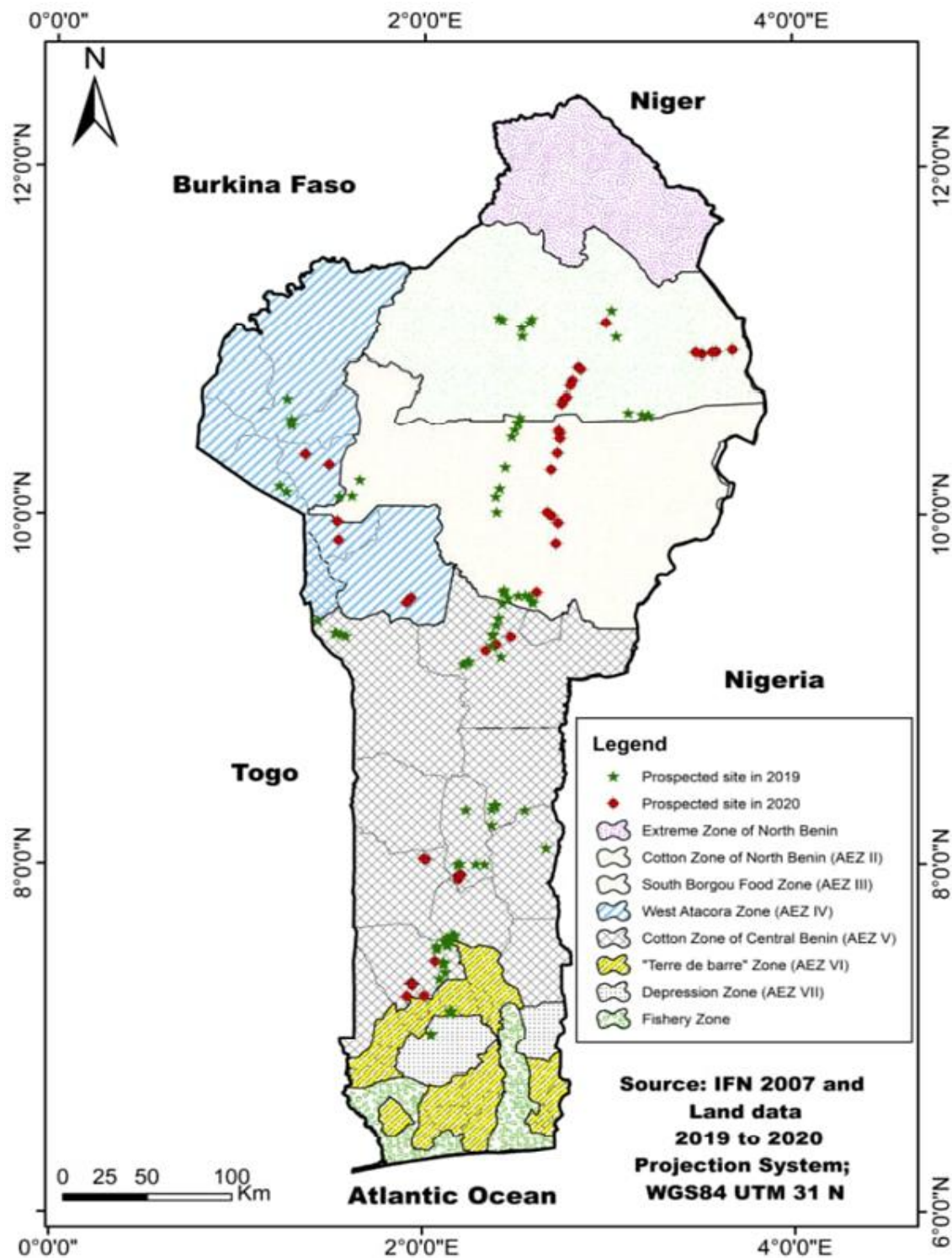


Figure 1: AEZ based distribution map of the surveyed sites in 2019 and 2020.

Carte de la distribution des sites prospectés en 2019 et 2020, suivant les ZAEs.

DATA COLLECTION

Incidence and severity assessment

The SBLP incidence was assessed on 30 plants randomly selected from each field using the diagonal method (Rwegasira *et al.*, 2011). It was then estimated from the ratio between the number of infected plants and the total number of plants assessed

The severity was assessed by putting a 4 x 7 cm stencil card with 9 circles of 1 cm in diameter on the infected leaflet surface randomly collected on low, medium and high portion of soybean crop showing typical symptoms of the disease (Prathuangwong *et al.*, 1993) at the rate of 20 plants per field along the diagonals (Rwegasira *et al.*, 2011). The number of lesions obtained was related to the leaf surface area.

Pathogenicity assay of *Xag* isolated from diseased soybean leaves and weeds

Xag was isolated from pustules that had been macerated in a small amount of sterile water onto Glucose Yeast Calcium carbonate Agar (GYCA; Difco Laboratories, Detroit, MI) plates and incubated at 30°C for 48h. Isolates were picked and repeatedly streaked on GYCA to establish pure cultures and then identified as described by Zinsou *et al.* (2005b) The suspending cells was dissolved in sterile distilled water to obtain aqueous cell suspensions (OD 600 = 0.2 $\approx 10^8$ CFU.ml⁻¹) that was inoculated to 3 weeks old soybean seedlings (variety TGX 1984-77F), maintained in greenhouse at 22.1-32.9°C and 54-95% relative humidity. Sterile distilled water was also inoculated to seedlings per variety and served as negative control. SBLP symptoms were recorded 3 to 7 days after inoculation.

Identification of *Xag* host plants

An examination of pictures and plants samples (other than soybean) collected during surveys was carried out at the « Laboratoire d'Ecologie, de Botanique et de Biologie Vegetale » of the University of Parakou, in order to identify the host plants of *Xag*. In this regards, the Benin Analytical Flora (Akoegninou *et al.*, 2006) was also exploited .

Statistical analysis

Incidence and severity data from surveys were subjected to a two ways analysis of variance (ANOVA) using RCore Team (2019) to compare the means at the 5% level. Tukey's test was then completed in order to separate the means in the case of a significant difference ($P < 0.05$). The values in the tables are the real means with their standard errors.

RESULTS

DISEASE INCIDENCE AND SEVERITY IN SIX DIFFERENT AGRO-ECOLOGICAL ZONES OF BENIN

The disease occurred in all surveyed AEZ except AEZ VII. The average incidence was 5 - 47.25% and 17.22 - 44.87%, in 2019 and 2020 while the severity ranged from 2.23 to 37.88 and from 6.52 to 34.95 in 2019 and 2020, respectively (Table 1). AEZ II is the most affected (47.25% and 37.88% in 2019; 44.87% and 34.95% in 2020) while the West Atacora Zone (7.41% and 2.71% in 2019; 17.22% and 6.52% in 2020) and the Zone of «terre de barre»(5.00% and 2.23% in 2019,) respectively were least affected. The Depression Zone (AEZ VII) was SBLP-free.

Table 1: SBLP incidence and severity in surveyed AEZs in 2019 and 2020.

Incidence et sévérité de la pustule bactérienne du soja suivant les ZAEs prospectées en 2019 et 2020.

Agro-ecological Zones	2019		2020	
	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
Cotton Zone of North Benin (AEZ II)	47.25 ± 2.21 ^c	37.88 ± 2.05 ^d	44.87 ± 2.52 ^c	34.95 ± 2.17 ^c
Cotton Zone of Central Benin (AEZ V)	22.82 ± 1.01 ^b	12.83 ± 0.52 ^c	30.00 ± 2.16 ^b	13.77 ± 1.26 ^a
South Borgou Food Zone (AEZ III)	21.17 ± 1.67 ^b	6.43 ± 0.69 ^b	27.27 ± 2.46 ^{ab}	23.30 ± 2.22 ^b
West Atacora Zone (AEZ IV)	7.41 ± 0.92 ^a	2.71 ± 0.29 ^a	17.22 ± 2.82 ^a	6.52 ± 1.20 ^a
"Terre de barre" Zone (AEZ VI)	5.00 ± 2.00 ^a	2.23 ± 0.97 ^a	-	-
Depression Zone (AEZ VII)	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	-	-
F	77.27	166.00	17.99	38.48
P	< 2e ⁻¹⁶	< 2e ⁻¹⁶	1.9e ⁻¹¹	< 2e ⁻¹⁶

Means marked with the same letter on the columns are not significantly different ($P < 0.05$)

Les moyennes marquées de la même lettre sur les colonnes, ne sont pas significativement différentes ($P < 0,05$)

DISEASE PREVALENCE, INCIDENCE AND SEVERITY IN DIFFERENT DISTRICTS OF BENIN

SBLP occurred in 65.71% (115 out of 175) of inspected fields. In addition, it was reported in 91.30% (21 out of 23) and 92.31% (12 out of 13) of the surveyed districts, in 2019 and 2020, respectively (Table 2). Zogbodomey (in 2019) and Kouande district (in 2019 and 2020) were SBLP-free. while Kandi, Gogounou, and

Segbana (AEZ II) and Parakou districts (AEZ V) were vulnerable to the disease as the highest incidence values were recorded in Gogounou (62.00%) and Segbana district (61.11%) in 2019, and Kandi (96.67%) in 2020 and the highest severity values recorded in 2019, in Parakou (44.29%); Kandi (41.47%); Gogounou (35.14%) and Segbana district (31.67%), and in 2020 in Kandi (71.43%). Bassila, Nikki, Copargo, Zatkota, Savalou and Tchaourou districts were less vulnerable to the disease.

Table 2: SBLP incidence and severity in the surveyed districts in 2019 and 2020.*Incidence et sévérité de la pustule bactérienne du soja suivant les communes prospectées en 2019 et 2020.*

Districts	2019		2020	
	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
Parakou	33.33 ± 8.75 ^{bet}	44.29 ± 2.82 ^h	-	-
Kandi	34.44 ± 2.90 ^{ce}	41.47 ± 3.28 ^h	96.67 ± 3.33 ^e	71.43 ± 3.30 ^f
Djidja	-	-	28.89 ± 3.39 ^{bc}	18.84 ± 2.68 ^{ac}
Gogounou	62.00 ± 3.98 ^g	35.14 ± 3.02 ^h	55.24 ± 3.44 ^d	37.63 ± 2.96 ^e
Segbana	61.11 ± 5.17 ^{fg}	31.67 ± 3.38 ^h	20.00 ± 3.28 ^{ac}	23.89 ± 3.21 ^{cd}
Glazoue	36.48 ± 2.07 ^e	19.46 ± 1.00 ^g	60.00 ± 4.49 ^d	20.00 ± 1.56 ^{ad}
Dassa	33.81 ± 3.27 ^{ce}	15.41 ± 1.10 ^{dg}	-	-
Save	28.89 ± 4.80 ^{cde}	15.36 ± 2.15 ^{etg}	-	-
Ouesse	10.60 ± 2.02 ^{abd}	13.33 ± 1.71 ^{df}	-	-
Perere	18.33 ± 5.04 ^{abe}	13.21 ± 3.71 ^{aetg}	-	-
Natitingou	7.33 ± 2.14 ^{ab}	13.14 ± 0.71 ^{abc}	14.44 ± 3.73 ^{ac}	4.82 ± 1.36 ^{ab}
Bembereke	30.00 ± 3.43 ^{ce}	8.93 ± 1.55 ^{cde}	35.00 ± 3.09 ^b	30.89 ± 2.79 ^{de}
N'Dali	27.14 ± 3.08 ^{ce}	6.07 ± 0.80 ^{bef}	10.00 ± 3.91 ^{ac}	4.55 ± 1.85 ^{ab}
Boukoumbe	12.38 ± 2.28 ^{abd}	5.41 ± 0.77 ^{be}	-	-
Bante	12.00 ± 1.88 ^{ab}	5.00 ± 0.77 ^{bef}	-	-
Tchaourou	10.00 ± 3.18 ^{abd}	4.40 ± 1.16 ^{bef}	8.89 ± 3.02 ^a	3.51 ± 1.23 ^b
Za-kpota	5.00 ± 2.00 ^a	2.23 ± 0.97 ^{abc}	-	-
Nikki	5.56 ± 2.43 ^a	2.02 ± 0.64 ^{abc}	-	-
Djougou	5.00 ± 1.41 ^a	1.92 ± 0.49 ^{abc}	25.00 ± 5.64 ^{ab}	8.75 ± 1.97 ^{abc}
Savalou	10.00 ± 5.57 ^{abc}	1.79 ± 0.76 ^{abc}	5.00 ± 2.84 ^a	1.52 ± 0.94 ^b
Bassila	6.11 ± 1.79 ^a	1.73 ± 0.41 ^{abc}	-	-
Copargo	5.24 ± 1.54 ^a	0.61 ± 0.17 ^{abc}	10.00 ± 5.57 ^{ab}	7.14 ± 4.50 ^{abc}
Kouande	0.00 ± 0.00 ^a	0.00 ± 0.00 ^{abc}	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
Zogbodomey	0.00 ± 0.00 ^a	0.00 ± 0.00 ^{abc}	-	-
Mean	21.31 ± 0.66	12.30 ± 0.43	31.93 ± 1.27	21.25 ± 1.00
F	32.91	52.20	26.00	21.92
P	< 2e ⁻¹⁶	< 2e ⁻¹⁶	< 2e ⁻¹⁶	< 2e ⁻¹⁶

Means marked with the same letter on the columns are not significantly different ($P < 0.05$).*Les moyennes marquées de la même lettre sur les colonnes, ne sont pas significativement différentes ($P < 0,05$).*

SYMPTOMS INDUCED AND HOST PLANTS OF XAG

The symptoms recorded in the field include small pale green spots with pustules or yellow spots

with brown and reddish centers and a pustule inside each center on the upper (Fig. 2a and b) and lower (Fig. 2c) soybean leaves. These spots then develop into large necrotic spots that can lead to premature defoliation (Fig. 2b).



Figure 2: Symptoms of bacterial leaf pustule on the upper (a and b) and lower (c) soybean leaf surface.

Symptômes de la pustule bactérienne sur les faces supérieure (a et b) et inférieures (c) des feuilles de soja.

A part from soybean, five plant species namely *Acalypha ciliata* Forssk (Fig 3a), *Combretum molle* R.Br. (Fig 3b), *Christiana africana* DC (Fig 3c), *Stylochaeton hypogeum* Lepr (Fig 3d) and *Amorphophalus dracontioides* (Fig 3e) have been

identified as Xag hosts. *Acalypha ciliata* Forssk infection has been reported in Bembereke (AEZ III), *Combretum molle* R.Br., *Stylochaeton hypogeum* Lepr., and *Amorphophalus dracontioides* (Engl.) N.E.Br.) in Kandi (AEZ II), and *Christiana africana* DC in Parakou (AEZ V).



Acalypha ciliata

Combretum molle

Christiana africana



Stylochaeton hypogeum

Amorphophalus dracontioides

Figure 3: *Xanthomonas axonopodis* pv. *Glycines* host plants.

Plantes hôtes de Xanthomonas axonopodis pv. *glycines*.

DISCUSSION

The results of the surveys indicate that the SBLP occurred in all surveyed AEZ except the Depression Zone (AEZ VII), and in 65.71% (115 out of 175) of inspected fields. Moreover, there is a very highly significant difference between these AEZs both for the disease incidence and severity in 2019 and 2020. The disease incidence values varied from 5.00 to 47.25% in 2019 and from 17.22 to 44.87% in 2020. The disease severity was 2.23 -37.88% in 2019 and 6.52 - 34.95% in 2020. These results corroborate those of Zinsou et al. (2015a; b) who reported the occurrence of the disease in 13 out of 18 fields in the Sudanese savannah and 33 out of 34 fields in the Guinean savanna of Benin. SBLP occurred mostly in the Cotton Zone of North Benin which is the hottest zone (H» 45°C) among the six surveyed agro-ecological zones. This result may be explained by climate factors (temperature, humidity, rain, radiation, etc...) that directly affect pathogens in the different stages of their development (survival, growth, reproduction and dispersion) (Desprez-Loustau et al., 2005). Rain and temperature would have influenced the SBLP expression as Narvel et al. (2001) showed that the bacterium survives in regions where temperatures and relative humidity are high and, accompanied by sporadic heavy rains. In addition, Hartmann et al. (2015) found that Xag thrives in the plant when the temperature is hot, the humidity is high, and after thunderstorms or hailstorms. Optimal growth of its bacteria occurs at temperatures ranging from 30 to 33°C with a maximum of 35°C and a minimum of 10°C (Hokawat, 1978).

The disease incidence in the surveyed districts ranged from 5.00 to 62.00% in 2019 and 5.00 to 96.67% in 2020. The severity was 0.61 - 44.29% and 1.52 - 71.43% in 2019 and 2020, respectively. These values are slightly higher than the incidence (15.82 - 70% and 2.50-52.50%) and severity (5.95 - 26.14% and 4.93 - 35.46%) reported in Benin Guinea savannah and Sudanese savannah, respectively, by Zinsou et al. (2015a; b). These high incidence and severity highlighted the spreading of virulent Xag isolates across Benin. Kouande and Zogbodomey districts were disease-free. This finding increases the number of potential safe soybean seeds production districts as Zinsou et al. (2015a) found Coby and Toucountouna districts SBLP-free in previous studies.

Finally, *Acalypha ciliata* Forssk, *Combretum molle* R.Br., *Christiana africana* DC, *Stylochaeton hypogeum* Lepr., and *Amorphophalus dracontioides* (Engl.) N.E.Br have been described as hosts of *X. axonopodis* pv. *glycines*. To the best of our knowledge, this is the first report of these weeds as Xag hosts.

CONCLUSION

Except the Depression Zone (AEZ VII), SBLP caused by Xag is reported in all surveyed agro-ecological zones. The disease is prevalent in the Cotton Zone of North Benin, with Kandi, Parakou, Gogounou, and Segbana being the most vulnerable districts while Kouande and Zogbodomey districts, SBLP-free, could be considered for healthy soybean seeds production. These discoveries take together with the detection of Xag's new hosts (*Acalypha ciliata* Forssk, *Combretum molle* R.Br., *Christiana africana* DC, *Stylochaeton hypogeum* Lepr., and *Amorphophalus dracontioides* (Engl.) N.E.Br.) open a path for a better management of SBLP in Benin.

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