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First report of Okra leaf virus (OLCV) in Benin its distribution and farmers' cultural practices for the disease management

Martine ZANDJANAKOU-TACHIN[1*], Valerien Amegnikin ZINSOU[2], Elie DANNON[3], Martina Akouele TOWANOU[2], Daouda KONE[4], Fatogoma SORHO[4], Leonard Antoine Chaffara AFOUDA[2].

[1]Ecole d'Horticulture et d'Aménagement des Espaces Verts (EHAEV), Université Nationale d'Agriculture (UNA), Kétou

[2]Laboratoire de Phytotechnie, d'Amélioration et de Protection des Plantes (LaPAPP), Université de Parakou (UP), BP 123 Parakou, Benin.

[3]Ecole Normale Supérieure (ENS), Université Nationale des Sciences, Technologies, Ingénierie et Mathématiques d'Abomey, Natitingou, Bénin

[4]Université Felix Houphouët-Boigny, Abidjan, Côte d'Ivoire.

* Corresponding author: mtachin@yahoo.com

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ABSTRACT

Objective: Okra leaf curl virus (OLCV) transmitted by *Bemisiatabaci* strongly compromises Okra production throughout the world, and particularly in tropical and subtropical regions with yield losses of up to 70%. This study aims to assess the distribution and level of endogenous knowledge of producers in different localities in Benin.

Methodology and Results: A Survey was conducted on OLCV during the flowering and fruit development stage on 76 okra production sites of 24 communes in Benin in order to determine the incidence and severity index of the disease. In addition, the endogenous knowledge of the producers on the disease was assessed. Seventy five(75) % of the field survey were infected with OLCV and confirmed via DNA amplication by PCR. The incidence varied from 5 to 100% and the severity index from 2.5 to 73.13%per commune. There is a significant interaction between incidence, severity index and field maintenance on one side, and between incidence and crop association with okra on the other.

Conclusions and application of findings: Recent complaints from consumers and producers were related to quality and quantity of okra. Many factors can be the cause but the presence of Okra leaf curl Virus (OLCV) was discovered in the farmers' fields. Therefore, important is to get knowledge on this virus through its characterization. Okra leaf curl virus was confirmed in the collected leave samples using Polymerase chain reaction. This finding is a prerequisite for any control strategy. Moreover, knowing the effect of intercropping and field maintenance on the spread of the disease will help advise the farmers on which type of crop to consider and which behaviour to adopt to keep Okra a valuable crop and source of income to farmers with its nutritional role in Benin.

Keywords: Benin, okra, Okra leaf curl virus, begomovirus, *Bemisiatabaci*.

INTRODUCTION

Okra (*Abelmoschus* sp.) is one of the most important vegetable plants in the tropics. Cultivated in all tropical and warm temperate regions of the world for its fruits and seedpods but also for its ornamental value (Amadi *et al.,* 2014). In West Africa, it is the second most important vegetable crop after tomato (Hamon & Charrier, 1983; Hamon *et al.,* 1991). World production of okra in 2019 occupied an area of 2,729,811 ha with a total production of 9,953,537 tons. In Africa, the production of okra in 2019 covered an area of 2,153,010 ha with a production of 3,289,395 tons. The largest producers are Nigeria, Cote d'Ivoire, and Egypt (FAOSTAT, 2020). In Benin the production of okra was 40,403 tons in 2017, 63,264 tons in 2018 and 60,341 tons in 2019(FAOSTAT, 2020). Generally, okra is used for various purposes. This is the case, for example, in Nigeria, where unripe pods are consumed as boiled or dried vegetables, or used as soup thickeners or stews (Yadev & Dhankhar, 2002). Fibres extracted from the stems are used in Mali and Niger for making twine and nets (Siemonsma, 1982). In Guinea, okra sauce is very popular and goes well with rice, fonio, yam and cassava in the form of foufou (USAID, 2006). It can be used to cure diseases such as cardiovascular diseases by reducing blood cholesterol, diabetes in Malaysia (Indah, 2011), intestinal ulcers and haemorrhoids by Yoruba in Nigeria (Akinyele & Temikotan, 2007). The fruit is rich in carbohydrate in form of mucilage (7 to 8% of dry matter), protein (1.8% of dry matter), with an average content of vitamin A (300 IU). The survey by Sawadogo *et al.* (2009) showed that the production and sale of okra provides substantial income ($8 /plant/producer and $3.73/day/seller) to the various stakeholders. Despite its multiple uses, okra remains a crop facing enormous problems and pays the heaviest price to diseases and pests causing the wilting of the seedlings, foliage and fruit alterations by virus infections, fungi, nematodes and insect damage thus reducing its yield. These pests and diseases can totally compromise success in okra production if no phytosanitary measures are undertaken (Doumbia & Seif, 2008). Among all, the Okra leaf curl virus (OLCV) transmitted by whitefly (*Bemisiatabaci)* is recognized as a major constraint for okra production in tropical regions (Tiendrebeogo *et al.,* 2010). This disease can stunt growth or stop the development of the whole plant when infection is early and severe (Doumbia and Seif, 2008). Yield losses caused by this disease are up to 70% (Atiri & Fayoyin, 1989). In Africa, it has recently been shown that OLCV is associated with a complex of begomoviruses: Cotton leaf curl Gezira virus (CLCuGV) (Idris& Brown, 2002; Shih *et al.,* 2009); Okra yellow crinkle virus (OYCrV) (Shih *et al.,* 2007), Hollyhock leaf crumple virus (HoLCrV) (Bigarre *et al.,* 2001; Idris *et al.,* 2002) and satellite beta DNA (Idris *et al.,* 2002; Kon*et al.,* 2009). In Benin, the work carried out on okra has been devoted to the study of okra diversity and ethnobotany in the south-west of the country (Gnawe, 2015). Given the importance of okra, the losses caused by this disease, the fact that the consumers are complaining of the early rooting of the vegetable, it is therefore necessary to investigate whether this disease is present in Benin. In addition, for the development of effective integrated disease management adapted to the needs of local farmers, okra leaf curl disease diagnosis and endogenous knowledge on management practices contributing to prevent or control the disease infection are needed. Thus, having a good knowledge of the presence, distribution, as well as the level of endogenous knowledge of the producers on the development of the disease is prerequisite.

MATERIAL AND METHODS

Study area: Benin distinguishes three main types of climate: humid tropical or sub-equatorial climate in the south, humid tropical transition climate in the centre and dry tropical climate in the north with an average temperature varying between 25 and 29°C and relative humidity between 69 and 97% (Akabassi *et al.,*2020). This study was carried out in August 2016 in precisely 76 villages (Fig. 1) in 24 communes (Abomey-Calavi, Adjaouere, Athieme, Banikoara, Bante, Bembereke, Bonou), Cobly, Come, Copargo, Dassa, Djougou, Gogounou, Houeyogbe, Kandi, Kouande, Malanville, N'Dali, Nikki, Ouinhi, Perere, Pobe, Savalou and Tanguieta), all of which were brought together in the 8 agro-ecological zones during the vegetative period of okra in August. The choice of these sites was made according to their agronomic potential.

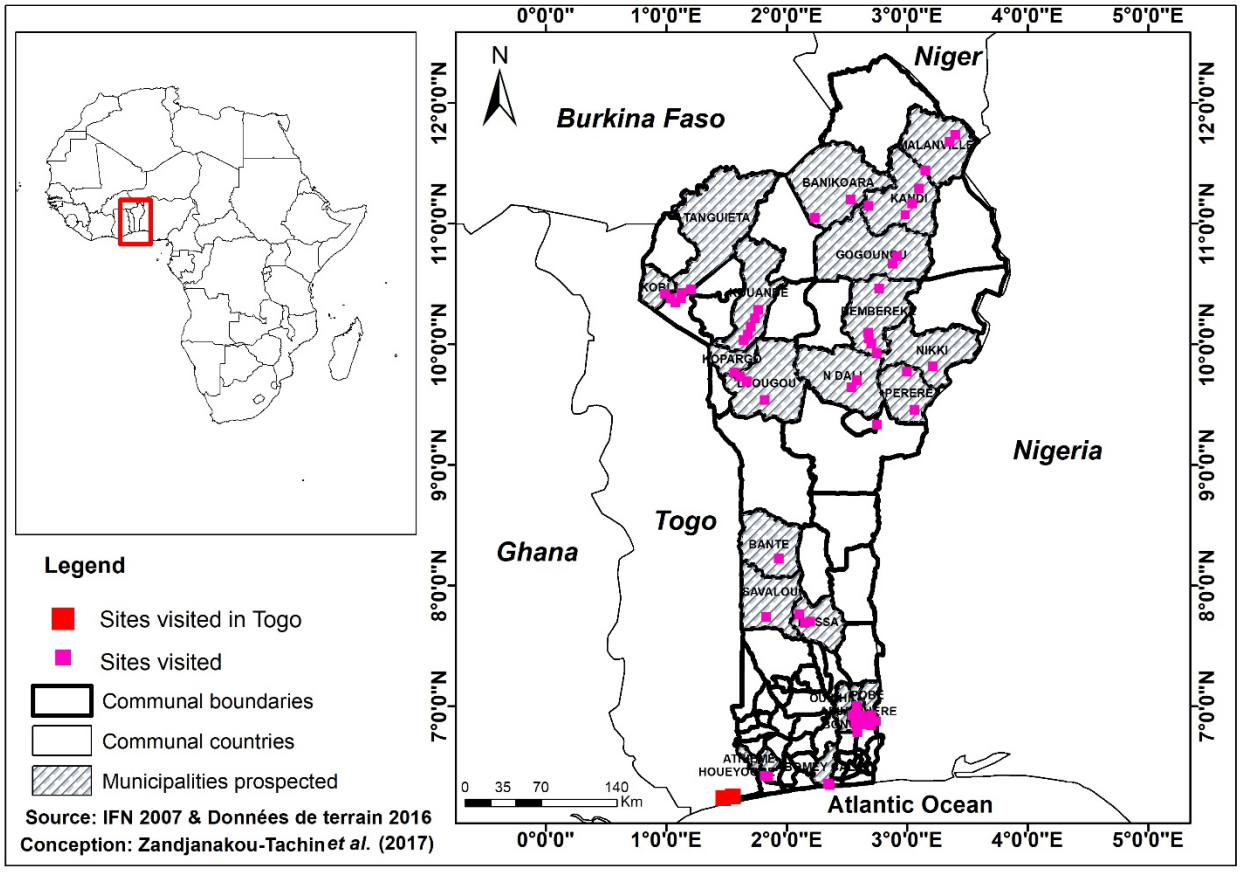

Figure 1: Map showing location of survey sites in Benin

Disease survey: For the representativeness of the study, 76 fields distributed in 24 communes, based on their agronomic potential in Benin (Fig. 1) were surveyed on okra in August 2016. One to ten fields at least 100m^2 and 2km apart were selected per commune. Leaf samples showing visible symptoms of okra leaf curl virus (Fig.2) were collected for DNA extraction and virus indexation. At each site, latitude and longitude were recorded with geographical positioning system (GPS) (Garmin).

Figure 2: Okra leaf curl disease symptom from our survey

Data collection, processing and analysis: To assess the incidence and severity of okra leaf curl virus, 20 plants were randomly selected from the two central field lines during the survey. The incidence was noted based on the presence/absence of symptoms characteristic of the disease. The disease incidence at each site was calculated using the equation proposed by Cooke *et al.* (2005).

$$IM = \frac{n}{N} \times 100$$

With ***IM,*** the incidence of the disease at the site, ***n,*** the number of plants on which the disease is present at the site and ***N***, the total number of plants surveyed at the site.

For severity, the visual scale of 1 to 4 developed by Alegbejo (1997) and slightly modified was used (Table 1). The disease severity index per site was calculated according to Galanihe *et al.* (2004).

DSI = (Sum (P XQ) / (MXN)) x 100

P= severity value, Q= number of infected plants with the same score, M= total number of plants observed, N= maximum number on the rating scale.

Table 1 Scale for assessing the severity of okra leaf curl disease severity

Score	Description of symptoms
1	No symptoms.
2	Upper leaves rolled up and stunted plant.
3	All leaves rolled up and stunted plant.
4	All leaves are severely rolled with stunted plant and whitefly proliferation.

Source: Alegbejo, 1997 slightly modified.

The data were entered with Excel 2007 software and then analysed with the software R version 3.3.2. on the transformed incidence and severity index values on the one hand and on the general endogenous knowledge level (field size, field maintenance, weeding, field age and crop association) on the other hand. For the analyses in R a Generalized Linear

Model (GLM) test was used. The Student-Newmann-Keuls test was used to compare average incidence and severity index.

Total genomic DNA extraction from okra leaves and Polymerase chain reaction

DNA extraction: DNA extraction and PCR were performed on 60 samples collected from60 fields. The DNA extraction was performed according to the protocol described by Edwards *et al.* 1991 and slightly modified*;* Approximately 1mg of dried leaf was crushed using a mortar and pestle in 100µl of extraction buffer (200mMTrisHcl pH7.5, 250mMNacl, 25mM EDTA. 0.5% SDS) stored at room temperature. The mixture transferred to a 2 ml eppendorf tube was added to 1 ml of phenol-chloroform-isoamyl (25:24:1), mixed well and centrifuged at 13,000rpm for 5 min. The supernatant was transferred into a new 2 ml eppendorf tube using a propipette to which 1 ml of chloroform-isoamyl (24:1) was added, mixed again and centrifuged at 13,000 rpm for 10 min. The supernatant was recovered in a 1.5 ml Eppendorf tube. Precipitation was done using a volume of approximately 500µl of isopropanol stored at -20°C (in the freezer) to the supernatant (at equal volume) and mixed by inversion for 1 min. The isopropanol was used cold in order to induce a thermal shock, which may promote DNA precipitation. The DNA is thus precipitated in the form of filaments called pellets. The mixture is centrifuged at 13,000 rpm for 5 min to recover the pellet as a DNA pellet. The DNA pellet was cold cleaned with 70% ethanol to remove impurities (mineral salts). The mixture was centrifuged and the DNA pellet was dried at room temperature for about 30 min until total evaporation of the alcohol. The extracted DNA was rehydrated in 200µl sterile water and stored at -20°C for direct use in PCR (polymerase chain reaction). For the Conventional PCR amplification of DNA, 3µl of diluted DNA was collected (concentration of approximately 10ng/µl) and amplified in a total reaction volume of 25µl following the protocol of Tiendrebeogo *et al.* (2010). The primers used are: Okra-F318:5'-AATTATGTCGAAGCGACCAG-3' and –R1004:5'-GCATTCTCCGTATGATTCTC-3'.In general, this amplification protocol is summarized in 3 essential steps of denaturation, hybridization and elongation followed by the visualization after electrophoresis.

RESULTS

Incidence and severity of okra leaf curl disease in the communes and agro-ecological zones: From the survey, Okra leaf curl virus disease was present on 57 of the 76 sites surveyed as shown in the map (Fig. 3).Comparing the distribution of the LOCV at agroecological zone; it appears that the pathogen is well established. The highest incidence were recorded in the communes of Come (100%), Savalou (97.5%) and Dassa (70%) (Table 2) while no disease was found in the communes of Athieme, Bonou and Perere. In term of the severity index, the highest values were obtained in the communes of Savalou (73.13%), Come (50%) and Dassa (45.94%); the lowest values were recorded in the communes of Copargo, Djougou and N'Dali (2.5%) (Table 2). Statistical analysis of the mean incidence and severity index per commune revealed a variability of 5 to 100% for the incidence and 2.5 to 73.13% for the severity index (significant value at the 5% threshold). The distribution of incidence of okra leaf curl disease is shown in Figure3.The highest incidence values were observed at 17 sites in 9 communes. On the other hand, low incidence values were recorded on the 27 sites of 16 communes (Fig. 3). Distinct leaf symptoms were used to classify plant severity levels into four intervals [1; 2[, [2; 3[, [3; 4] and [4; 5[. The percentages of plants per severity class and per agro-ecological zone

were represented in Figure 4. The analysis of variance of the class severity means showed a very highly significant difference from one class to another (P=2e-16 ***) (Table3). Thus class [1; 2] predominates in all four agro-ecological zones surveyed (Coastal Savannah= 53.33%, Southern Guinean Savannah = 50.06%, Northern Guinean Savannah = 77.75% and Sudanian Savannah = 80.97%). Classes [2; 3 [and [3; 4] were present in all agro-ecological zones with a predominance of class [2; 3] in the Coastal Savannah and [3; 4] in the South Guinean Savannah. Concerning the last class [4; 5[, it is absent in all the zones surveyed (Fig.5).

Table 2: Incidence and average severity index of okra leaf curl disease by commune and agro-ecological zone in Benin.

Agro-Ecological Zone	Commune	Incidence (%)	Severity index (%)
Sudanian Savannah		19,03±6,17a	11,10±3,41a
	Gogounou	53,75±26,88b	27,19±13,29b
	Cobly	38,75±16,63b	25,63±13,17b
	Tanguieta	13,75±5,54b	8,75±3,89b
	Malanville	11,67±11,67b	6,25±6,25b
	Kouande	11,00±6,60b	7,25±4,23b
	Kandi	10,00±5,77b	6,67±3,63b
	Banikoara	8,33±3,33b	4,58±1,50b
	Copargo	5,00±2,89b	2,50±1,44b
Northern Guinean Savannah		22,25±11,62a	11,69±6,18a
	Bembereke	51,25±23,46b	28,44±13,06b
	Nikki	50,00±35,00b	25,00±17,50b
	N'Dali	5,00±3,54b	2,50±1,77b
	Djougou	5,00±2,89b	2,50±1,44b
	Perere	0,00±0,00b	0,00±0,00b
Southern Guinean Savannah		49,23±11,55a	32,09±8,96a
	Savalou	97,50±2,50a	73,13±1,87a
	Dassa	70,00±18,82b	45,94±13,27b
	Bante	50,00±50,00b	37,50±37,50b
	Pobe	50,00±11,95b	32,75±9,12b
	Ouinhi	48,33±26,19b	14,67±11,57b
	Adja-ouere	28,75±23,84b	20,63±18,15b
	Bonou	0,00±0,00b	0,00±0,00b
Coastal Savannah		39,17±21,39a	20,31±10,64a
	Come	100,00±0,00a	50,00±0,00b
	Abomey Calavi	31,67±6,67b	18,75±2,17b
	Houeyogbe	25,00±0,00b	12,50±0,00b
	Athieme	0,00±0,00b	0,00±0,00b
	F	1,88	1,82
	P	0,03	0,04

The average incidence and severity index values with the same lowercase letters in the columns are not significantly different at the 5% threshold.

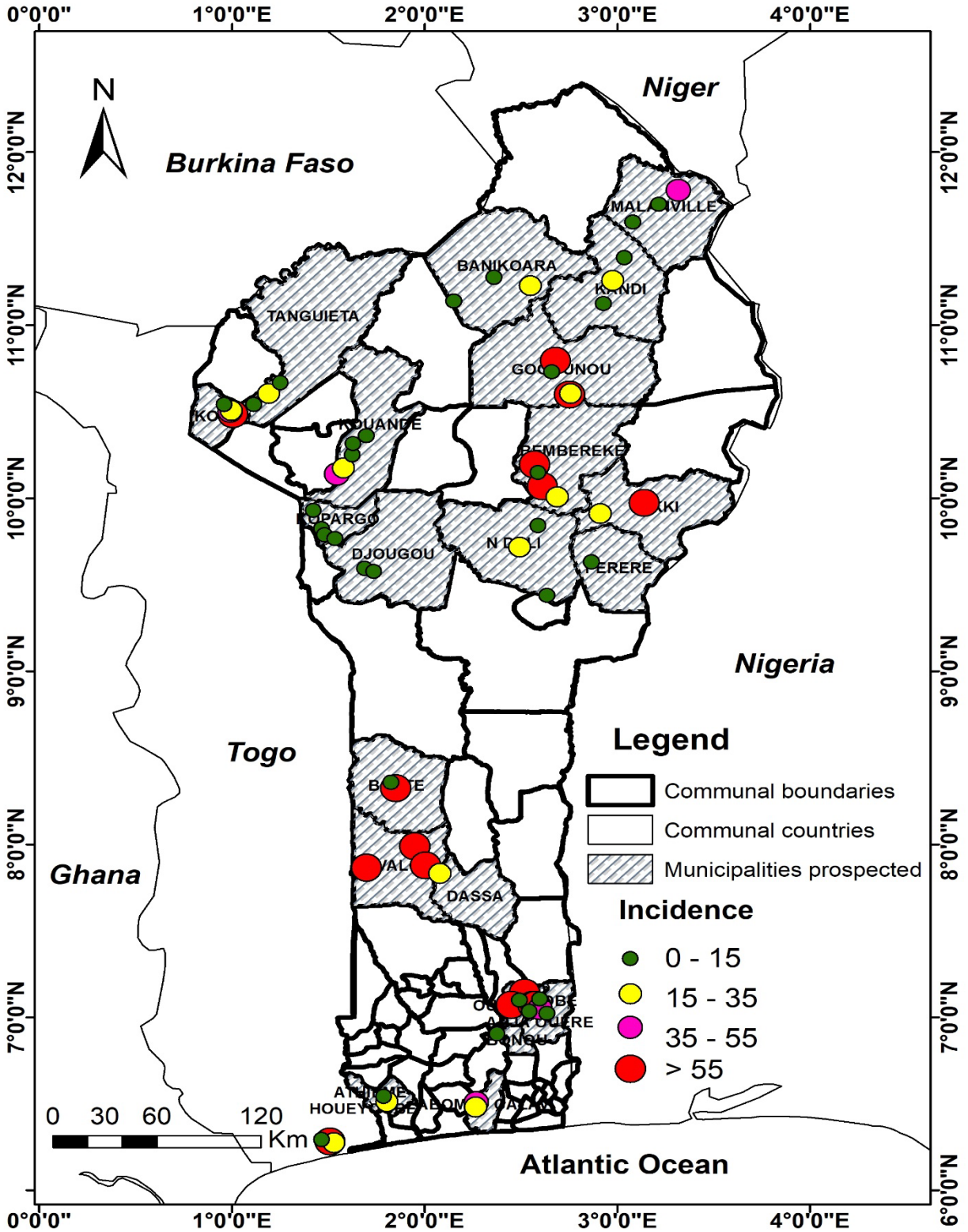

Figure3: Map of the distribution of the incidence of okra leaf curl virus disease in the different areas surveyed in Benin in 2016

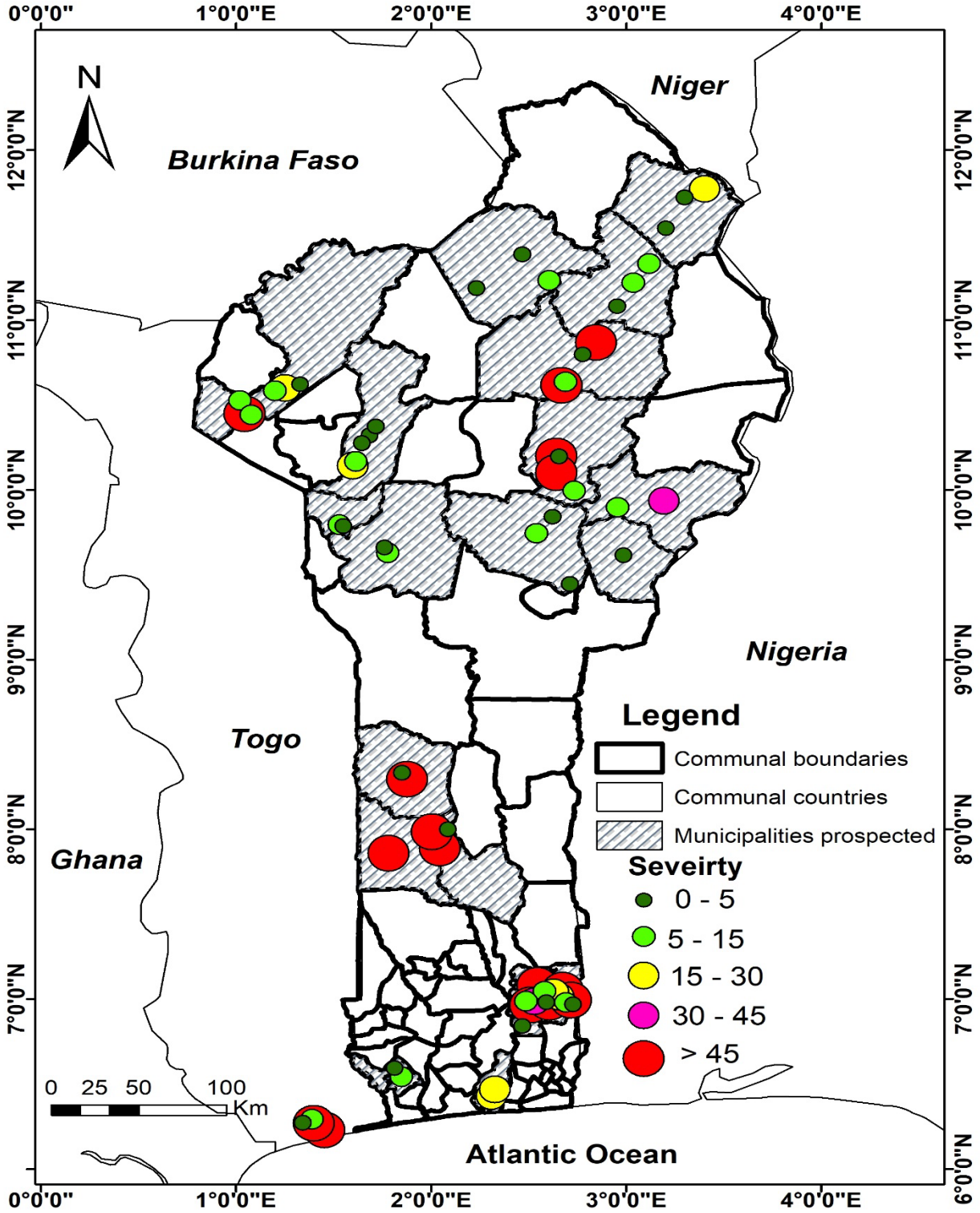

Fig. 4: Map of the distribution of *okra leaf curl virus* disease severity index (severity values) in the different areas surveyed in Benin in 2016

Table 3: Average severity-by-severity class of okra leaf curl disease in Benin

Severity class	Average severity (%)
[1,2[	65.6625±3,56c
[2,3[	18.3175±2,60b
[3,4[	16.1550±4,14b
[4,5[	0.0000 ±0a
P	2e-16 ***
F	87.67

Mean field values with the same lowercase letters in the columns are not significantly different at the 5% threshold, ***= Very highly significant at the 0.05 threshold.

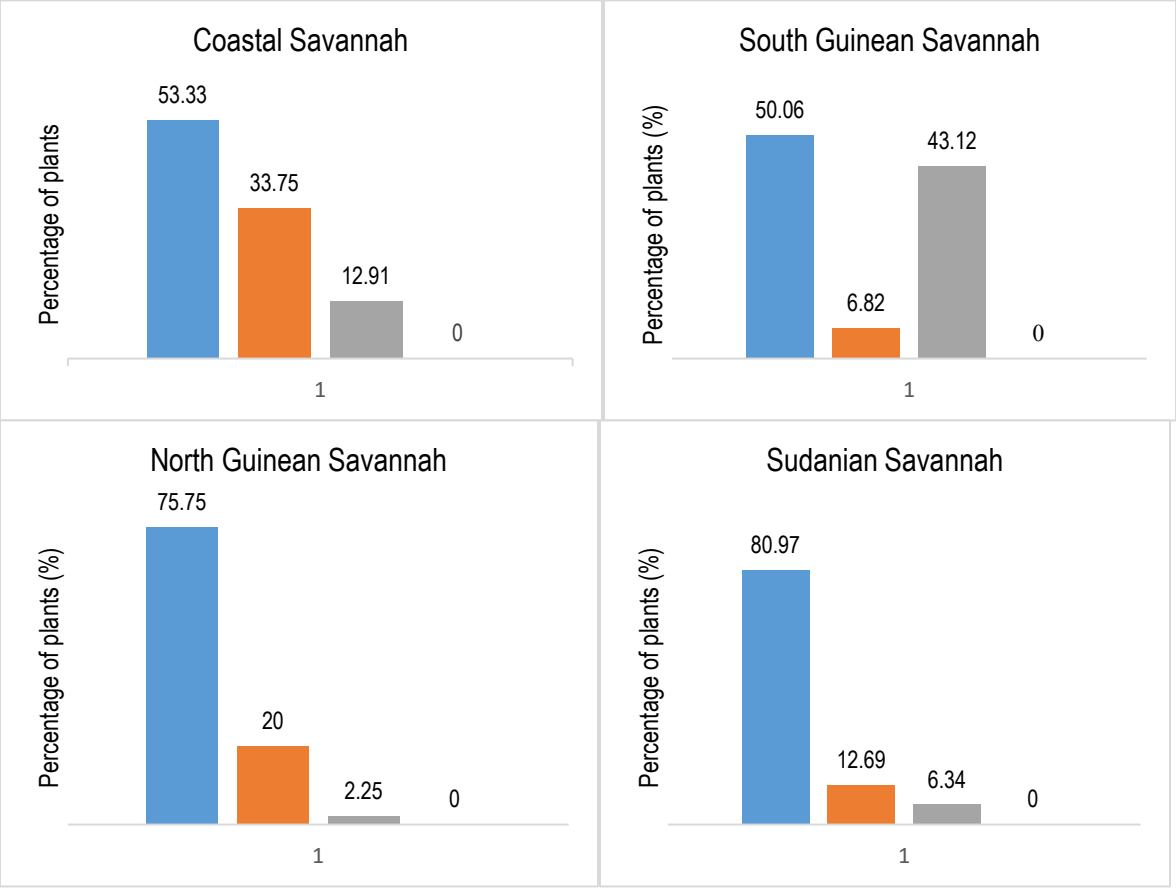

Figure 5: Percentages of plants according to the severity classes of okra leaf curl disease by agro-ecological zones surveyed.

The Sudanian Savannah has 80.97% of the plants in the class 12, followed by the North Guinea Savannah where 77.75 % of the plants fall in the class, the Coastal Savannah (53.33%) and the South Guinea Savannah (50.06%).

The general endogenous knowledge evaluated during our survey: The results showed that 34 fields had crops associated with okra (44.74%), 33 fields were weeded (43.42%) and 43 fields were maintained (56.58%). Table 4 shows that no maintenance was observed in the fields of Bonou and Come

communes, unlike in Ouinhi, Calavi, Athieme, Houeyogbe, Perere, Malanville, Banikoara, Djougou, Cobly, Copargo and Dassa, where more than half of the prospected fields were maintained (>50%). With regard to weeding, we found that producers in the communes of Adja-Ouere, Come, Nikki, Malanville, Banikoara, Copargo and Dassa have strongly adopted this cultivation operation in their fields because more than half of the fields were weeded in these communes, in contrast to the communes of Bonou, Athieme, Houeyogbe and Savalou where no weeding of the fields was observed. Finally, with regard to crop association, more than half of the fields surveyed in the communes of Pobe, Adja-Ouere, Ouinhi, Come, Nikki, Gogounou, Malanville, Cobly, Savalou and Dassa are associated fields. There was no crop association in the fields of the communes of Bonou, Athieme, Houeyogbe, Perere, Djougou, Tanguieta, Kouande and Copargo.

Table4: Percentage of farming practices by communes and agro-ecological zones surveyed

Communes	Field Maintenance (%)	Weeding (%)	Cultural association (%)
Abomey-Calavi	100	33.33	33
Adja–Ouere	50	75	75
Athieme	100	00	00
Banikoara	66.67	66.67	33.33
Bante	50	50	50
Bembereke	50	25	50
Bonou	00	00	00
Cobly	75	50	75
Come	00	100	100
Copargo	66.67	66.67	00
Dassa	75	75	75
Djougou	100	33.33	00
Gogounou	50	50	75
Houeyogbe	100	00	00
Kandi	33	33.33	33
Kouande	20	40	00
Malanville	100	66.67	66.67
N'Ddali	50	25	25
Nikki	50	100	100
Ouinhi	66.67	33.33	66.67
Perere	100	50	00
Pobe	40	30	60
Savalou	50	00	100
Tanguieta	25	50	00

*= Significant at the 0.05 threshold;
***= Very highly significant at the 0.05 threshold.

Influence of the level of general endogenous knowledge of producers on the incidence of the disease: The analysis in Table 5 shows that there is a very highly significant interaction between incidence and associated crops (p=2.759e-05), and a significant interaction between incidence and field maintenance (p=0.01885). No significance was observed

with other variables such as field age, weeding, field size and agro-ecological zones. Figure 6 shows the level of disease incidence in the case of a crop combination and in the case of field maintenance. This incidence is higher in fields where the crop association is carried out (56.45%) as opposed to fields where there was no crop association (13.16%). In terms of field maintenance, we find that when the field is maintained, it reduces the chance of disease occurrence from 46.57% to 23.67%.

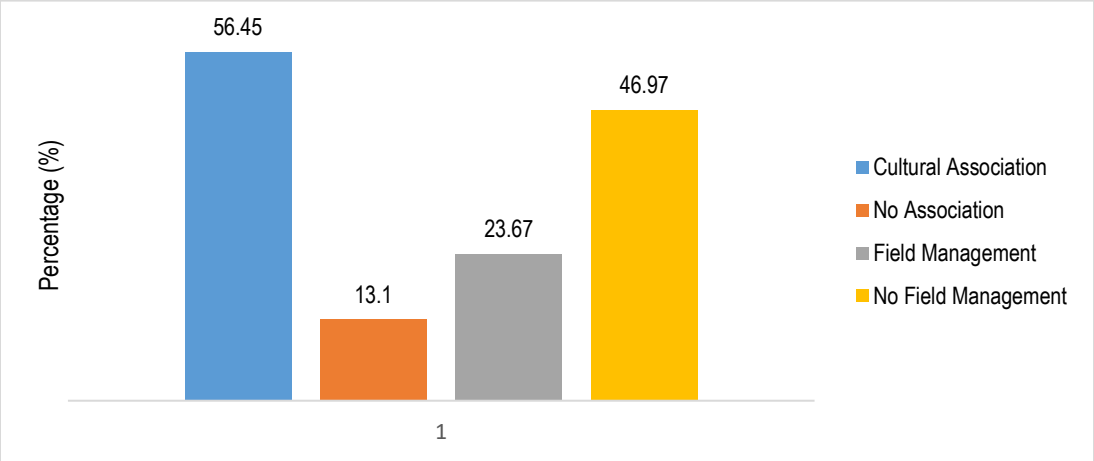

Figure 6: Evolution of incidence as a function of crop combination and field maintenance

Influence of the level of general endogenous knowledge of producers on the disease severity index: Analysis of Table 6 revealed a significant influence only between field maintenance and the disease severity index (p=0.04607). As for the other explanatory variables (intercropping, weeding, field size and agro-ecological zone), no difference was recorded with the severity index. The level of disease severity according to whether the field is maintained or not is shown in the figure 7. When the field is maintained, the degree of disease attack would be halved from 27.19% to 14.61%.

Table6: Generalized linear model test between the severity index and the different explanatory variables (associated crops, field maintenance, weeding, field size and agro-ecological zone).

	Df deviancy residence	**Df residence deviancy**	**P (>khi 2)**
None	79	89,974	
Field Maintenance	1 3,9792	78	85,994 0,04607 *

*= Significant at the 0.05 threshold.

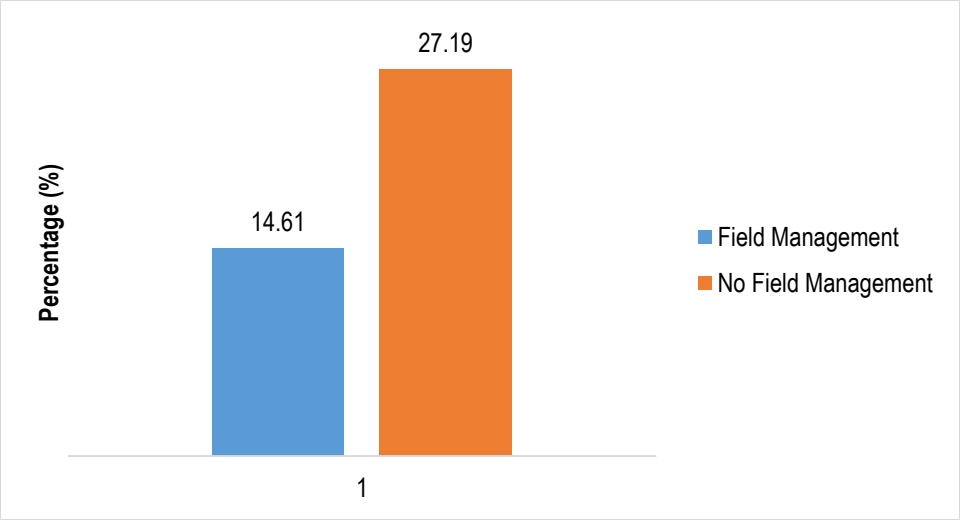

Figure 7: Evolution of the severity index as a function of field maintenance

Molecular characterization of okra leaf curl disease: Analysis of the profile from the DNA extraction of the samples showed that only 5 samples (29, 31, 38, 46 and 48) did not revealed any visible DNA in the 60 samples (Figure 8). The PCR using DNA primers led to an amplification around 700 base pairs (bp). Marker M is 1Kb in size. Figure 9 does not show any bands for the target virus which should normally be around 700Pb for the 36 samples of the first gel. However, a band was observed in samples 37, 52 and 60 of gel 2, while in sample 59; a band was not found exactly where we expected it to be (700 base pairs) but rather around 507 base pairs.

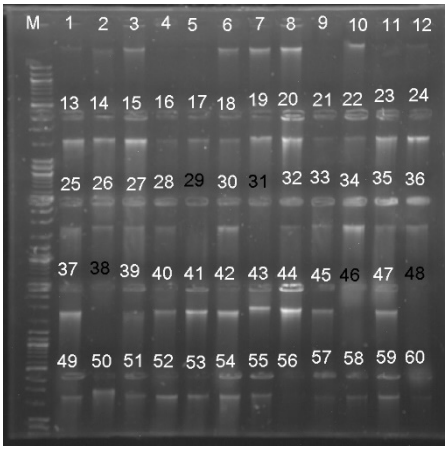

Figure 8 DNA extraction on 1% agarose, gel red staining, migration 50min, 100vagarose gel

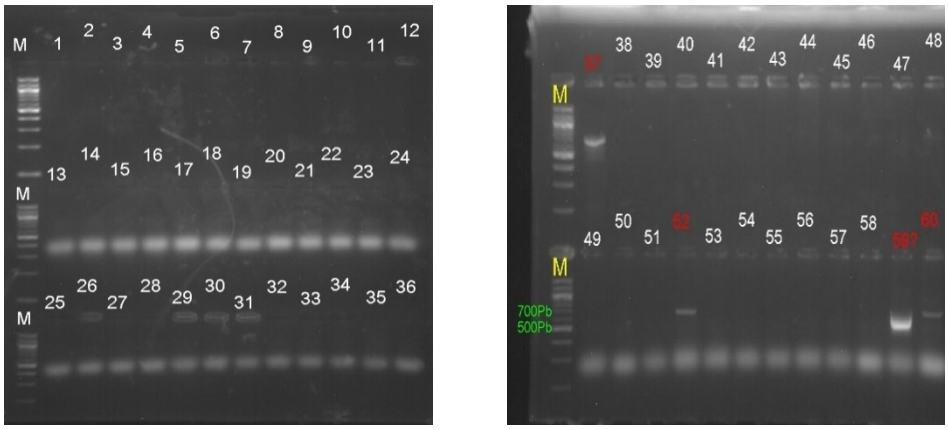

Figure 9: Amplification of OLCV DNA from naturally infected leaves of okra gel agarose 2%, gel red staining, migration 60 min, 100v

DISCUSSION

Incidence and severity index of okra leaf curl disease: The okra leaf curl virus was present on 57 of the 76 sites surveyed. The mean incidence and severity index showed a variability of 5 to 100% for the incidence and 2.5 to 73.13% for the severity index per commune. These results show that the okra leaf curl virus is an important viral disease in Benin, which corroborates reports by some authors that OCLD is a serious constraint to the cultivation of certain okra varieties in West Africa (Haruna and Jabil, 2017; Tiendrébéogo *et al.,* 2010; Kumar *et al.,* 2010; N'guessan *et al.,* 1992). In addition, the incidence and average severity have varied from one agro-ecological zone to another. According to Makhlouf *et al. (*2015), cotton is also a host plant of *Bemisiatabaci* as well as okra, both crops belong to the same botanical family of malvaceae, in addition to vegetable crops (tomato and pepper) and finally cassava, which is also host, plants of the whitefly (SPV-Guyane, 2004; Nzi *et al.,* 2010). All these different crop groups are grown in the different communes where these high incidence and severity values were obtained. This explains the results obtained in these communes where the incidence and severity index of the disease were found to be very high.

Influence of the level of general endogenous knowledge of producers on the incidence and severity index of the disease: There was a very highly significant interaction between incidence and associated crops ($p=2.759e-^{5}$), and a significant interaction between incidence and field maintenance ($p=0.01885$). This incidence was lower in non-associated fields (13.16%) in contrast to fields where there was crop association with okra (56.45%). This means that if okra were associated with certain crops that are unfavourable to it, this would increase the chances of disease occurrence. As for field maintenance, we found a reduction of the disease in the fields that were maintained (23.67%), in contrast to the fields where no maintenance was carried out (46.57%). Statistical analyses showed a significant influence between field maintenance and the disease severity index ($p=0.04607$). When the field is maintained, this would halve the degree of disease attack from 27.19% to 14.61%. Indeed, when a field is maintained (tended), it limits all the conditions necessary for the development of diseases and pests. These results were in line with those of Alegbejo *et al.* (2008), who carried out similar work on okra mosaic and Tahir *et al*. (2015) on weed begomoviruses with the potential of contamination for crops. In terms of crop association, cotton, tomato, chilli pepper, and cassava were also host plants of *Bemisiatabaci* (Devaraja *et al.,* 2005; Gnankine *et al.,* 2007;

Sattar *et al.,* 2013; Pitrat &Trottin-Caudal, 2015). During the survey, these same crops were associated with okra. Thus, by associating these crops with okra, the chances of occurrence of this vector transmitting the leaf curl disease on okra plants in these associated fields increased, hence this result. These results are consistent with those of Muqit*et al*. (2008), and Smith and McSorley (2000). This influence of field maintenance on the severity of the disease could be explained by the fact that when the disease is already present in the field and no control method (field maintenance) against these vectors is adopted, it will favour a higher severity of the disease. These results are in line with those of Alegbejo *et al.* (2008) on okra mosaic in Nigeria.

Identification of okra leaf curl virus by PCR: The analysis of the profile resulting from the DNA extraction of the samples shows that only 5 samples (29, 31, 38, 46 and 48) did not reveal visible DNA out of the 60, i.e. 55 samples were successfully extracted. With this DNA extraction result, we expected to have, after DNA amplification, a significant number of samples with the desired virus, which was not the case as we only had 4 samples out of the 60 with viruses. This result could be explained by the fact that okra contains a high amount of polysaccharides, the substance that is responsible for the sticky texture of okra. According to Fang *et al.* (1992), the polysaccharides have a viscous glue and make the DNA ingestible in pipetting, which is inappropriate for PCR because they inhibit the activity of the *Taq*DNA polymerase. These polysaccharides and polyphenols are compounds that are difficult to separate from isolated DNA and obstruct polymerases, ligases and restriction enzymes during their activity (Michaud *et al.,* 1995; Porebski *et al.,* 1997; Csaikl *et al.,* 1998; Tribounch *et al.,* 1998; Schlink&Reski, 2002). According to Doyle &Doyle (1987), the observation of soft (less clear) bands indicates a high level of polysaccharides and polyphenols in the samples. Increasing the volume of the DNA extraction buffer and decreasing the amount of plant material helps to remove the majority of polysaccharides with a high salt concentration. The use of salt during the DNA precipitation phase increases the solubility of polysaccharides in ethanol, thus preventing co-precipitation with DNA (Murray &Thompson, 1980; Paterson *et al.,* 1993; Lodhi *et al.,* 1994). Thus, all of the reasons noted above would explain the results obtained. As for the virus found, it is indeed a begomovirus because the band was found at the expected position (700 base pairs) for 3 samples: samples 37, 52 and 60. These results corroborate those of Sayed et al. (2014) and Tiendrebeogo *et al. (*2010) who have done similar work on begomoviruses of okra in India and on the molecular diversity of viruses responsible for cotton leaf curl and their DNA linked to disease of okra leaf curl in Burkina Faso, respectively.

CONCLUSIONAND APPLICATION OF RESULTS

The okra leaf curl disease (OLCD) is present in all agro-ecological zones of Benin (southern Guinean savannah, coastal savannah, northern Guinean savannah and Sudanian savannah). However, some agro-ecological zones are more vulnerable to the disease than others, such as the southern Guinean savannah where we noted the highest percentage of infected plants (49.94%), followed by the coastal savannah (46.66%). As for the Sudanian savannah, it is the zone least susceptible to the disease because we recorded the lowest infestation rate (19.03%) at this level. The same applies to the North Guinean savannah where the infestation level is also low (22.5%). The communes most infected by okra leaf curl disease are Come (incidence=100%, severity=50%), Savalou (incidence=97.5%, severity=73.13%) and Dassa (incidence=70%, severity=45.94%). As for the general endogenous knowledge of the producers, on the 76 sites (fields) surveyed, we found that 34

producers had grown crops associated with okra, i.e. a percentage of (44.74%), 33 others weeded their fields properly (43.42%) and finally 43 went a little further (56.58%) by carrying out the maintenance of their fields (weeding, phytosanitary treatments, daily watering, etc.). These cultural practices carried out by these producers had an impact on the chances of the disease appearing and on the level of severity of the disease. Indeed, the combination of cultivation and lack of maintenance of the fields increased the incidence of the disease. The lack of field maintenance also had a negative impact on the degree of disease attack (severity). Laboratory tests revealed that the causal agent of the disease in Benin is a virus, more precisely a begomovirus of the germiviridae family.

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