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REMOVAL OF SYMPTOMATIC CASSAVA LEAVES AS CULTURAL PRACTICE TO CONTROL CASSAVA BACTERIAL BLIGHT

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A B S T R A C T

The cassava landrace BEN 86052, susceptible to cassava bacterial blight (CBB), was chosen for the field experiments conducted in the forest savannah transition zone at Abomey-Calavi, Benin Republic. One month old plants were spray-inoculated with *Xanthomona axonopodiss* pv. *manihotis* (Xam) strain GSPB 2506 to achieve a homogenous infection across all plots. Disease development and cassava growth parameters were monitored over 12 months. Diseased leaves were removed four times at an interval of three weeks. A detailed symptom evaluation of percentage of spots, blight, wilt and dieback revealed significant reduction of blight and wilt symptoms in removed leaves plots. Disease severity was reduced by 71% in plots with leaf removal compared to non-removed leaves plots. Growth parameters leaf, stem and root weight at 6 and 12 months after planting were not significantly different between removed leaves and non-removed leaves plots. In fact, removal of infected leaves had no significant effect on root yield and reduced the epidemic potential of cassava bacterial blight in the field in the same season and the transfer of the infection by contaminated cuttings to the next season. Removal of diseased leaves should be highly effective in cassava fields with low disease incidence and specifically recommended for moderately resistant or resistant cultivars as part of an integrated management to control the cassava bacterial blight.

Keywords: Disease, *Xanthomona axonopodiss* pv. *manihotis*, Cassava, Symptoms, Growth parameters.

INTRODUCTION

In most of the producing areas of the world cassava (*Manihot esculenta* Crantz) is affected by a high number of diseases (Lozano *et al.,* 1981, Théberge, 1985). Among the foliar diseases, cassava mosaic disease in its various forms, cassava bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) and brown leaf spot caused by *Cercospora henningsii* are undoubtedly the most important factors limiting cassava production (Lozano and Booth, 1976; Otim-Nape *et al.,* 1992; Fanou, 1999; Alabi et al. 2011). Root and stem rots are also serious constraints in all ecozones (Lozano et al. 1981, Banito et al. 2010a). Cassava bacterial blight has been reported from all ecozones in several West-African countries (Banito et al. 2001, Wydra and Verdier 2002), though with variable severity. Characterizing the genetic diversity of the pathogen population is determinant to develop effective control strategies (Trujillo *et al.,* 2014 a and b). This disease causes not only severe root yield loss but also affects the market value of cassava leaves. However, cassava leaves are important in human and animal diet because they are a rich source of protein, vitamins and minerals and are widely consumed in Africa and Asia (Osiname and Landu, 1992; Dahniya, 1980; Mahungu *et al.,* 1992; Lutete *et al.,* 1992). In Zaire, selling cassava leaves as a vegetable is a major source of income for farmers and urban gardeners (Lutete *et al.,* 1992). The use of cassava leaves to feed livestock is a common practice in Africa. A total loss of leaves due to CBB can cause a deficit in the protein diet of low-income communities. Thus, healthy cassava plants with a high number of leaves of good quality are desired.

Various control measures have been suggested, such as use of tolerant and resistant genotypes (Zinsou *et al.,*

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2006; Banito *et al.,* 2010b; Wydra *et al.,* 2007), intercropping, soil amendment and shift of planting time (Fanou, 1999; Zinsou *et al.,* 2004) and control of vectors (Zandjanakou-Tachin *et al.,* 2007), but they should be part of a package of integrated measures to sustainably reduce the disease (Wydra *et al.,* 2001).

As additional control measure, pruning of diseased plant parts was recommended for various crops. Thus, pruning was used to control fungal diseases in orchards (Uddin and Steveson, 1998; Noriega-Cantú *et al.,* 1999), but few reports exist on successful pruning to control bacterial diseases. Thus, pruning of infected twigs coupled with copper compound spray to control *Xanthomonas axonopodis* pv. *mangiferae indicae*, cause of bacterial black spot of mangoes, was proposed to reduce inoculum (Pruvost, 1993), and early cut of *Prunus* buds avoids transmission of *Xanthomonas axonopodis* pv. *pruni*, causal agent of prunus bacterial spot (Goodman and Hattingh, 1988). Pruning the young cassava shoots (25 to 30 cm), which are more susceptible to CBB, to reduce inoculum and delay spread of the disease was recommended as means to control CBB (NRCRI, 1984; Lozano, 1986). However, this method had little effect on susceptible cassava cultivars, because plants develop rapidly new shoots that become infected (Lozano, 1986; Arene, 1990). Studying the effect of harvesting cassava leaves on the epidemiology of CBB, Lutete et al. (1992) reported that the ablation of cassava shoots to a length of 25 cm increased the severity of the disease and reduced root yield. Since chemical control of CBB is neither suitable nor possible, a feasible, alternative method without negative impact on yield is needed. This study was undertaken to evaluate the effect of removal of symptomatic leaves as a control strategy for CBB leading to healthy leaves' production and consequently increase cassava health and root yield.

MATERIALS AND METHODS

Experimental Design and Planting: The research was performed at the International Institute of Tropical Agriculture (IITA), Abomey-Calavi, Benin in the coastal forest savannah transition zone. The average annual rainfall ranges between 1200 to 1400 mm spread from March to July and from September to October, intercalated by a small dry season in August. The long dry period extends from November to March. The soil is a sandy loam with pH 6.9. The experimental design was a randomized complete block design with three replications. Plots were 10 m x 10 m and contained ten ridges each of 10 m with a distance of 1 m between them. The two treatments included (T1) removal of symptomatic leaves and (T2) a non-removed leaves. Stems of the susceptible cassava cultivar BEN 86052 were collected from one year-old cassava field from CBB-free plants at the IITA station. Cuttings of about 20 cm length were planted at begin of the rainy season in June (Table. 1) with a distance of 1 m on ridges giving 10,000 plants ha^{-1}. A total of 100 stakes were planted per plot. Three weeks after planting, dead cuttings were replaced. The experimental plots were kept free of weeds by monthly weeding with hoes during the rainy period.

Table 1. Weather data recorded at the experimental site.

Month	Rainfall (mm)	Temperature (°C)	Relative humidity (%)
June*	406.6	25.8	86.9
July	89.5	24.9	85.5
August	70.6	24.7	86.5
September	162	25.8	83.1
October	239.5	26.2	81.1
November	25.2	22.2	80.1
December	23.7	26.6	77.9
January	4.1	24.9	69.3
February	1.6	28.7	72-9
March	25.4	29.2	73.7
April	27.4	28.9	71.2
Mai	192.6	27.5	72.1
June	250.1	26.2	75.1

* Data from years 1997/1998

Inoculum and Spray Inoculation: The strain GSPB 2506 (Göttinger Sammlung Phytopathogener Bakterien, Göttingen, Germany) of *Xam* was isolated from a naturally infected cassava stem collected from the field at IITA, Benin. An antibiotic-resistant (streptomycin and rifampicin, 100 ppm) marker strain was selected and conserved on Glucose Yeast Carbonate Agar (glucose 5g/l, yeast 5 g/l, $CaCo_3$ 10 g/l, agar 15 g/l) (Dye, 1962) at 16 ºC. Inoculum deriving from single colonies was produced on Nutrient Glucose Agar (NGA) (nutrient broth 8 g/l, glucose 11 g/l, yeast extract 3 g/l, agar 15 g/l, pH = 7.2) incubated at 30 ºC (Lozano and Sequeira, 1974a) for 48 h. The inoculum suspension (10^7 cells/ml) supplemented with few drops of Tween 80 was spray-inoculated one month after planting with a motorized sprayer (type Solo, Germany) onto the lower surface of the leaves in the evening. All six plots were sprayed. This sprayer was well calibrated to spread uniformly the bacterial suspension across plots.

Symptom Evaluation and Removal of Leaves: One week after spraying first symptoms appeared. Disease symptoms were assessed at three-weekly intervals until symptoms disappeared during the dry season. The last evaluation was carried out at the second harvest 12 months after planting. Symptoms were observed on ten plants selected at random. The percentage of leaves bearing angular leaf spots, blight or wilt was determined. When leaves showed more than one symptom type, they were classified under the more severe symptom type: a leaf with both spot and blight was counted as blighted leaf only. The percentage of dieback was assessed by relating the number of shoot tips with dieback to the total number of shoot tips. Each symptom was evaluated by a percentage scale divided in classes: <5%, 5-10%, 10-20%, 20-50%, 50-80% and 80-100%. Disease severity (Ds) was calculated according to the following formula:

$$Ds = (1 \times S + 2 \times B + 3 \times W + 4 \times D)/10$$

where S, B, W and D represent the percentage of spot, blight, wilt and dieback, respectively.

After each evaluation, from plants of one plot per replication leaves which had developed cassava bacterial blight symptoms were removed (T1), while in the second plot of the same block infected leaves were not removed (T2). The time interval of three weeks was chosen for leaf removal because new leaves appear on cassava plants within two weeks (El-Sharkawy, 2004). A total of four removals were performed.

Harvests: Six months after planting, five plants selected at random were harvested per plot. Plant height, dropped leaves, total number of leaves, number of spotted leaves, blighted leaves and wilted leaves, root fresh weight, stem fresh weight and leaf fresh weight were recorded. Dry weight of roots, stems and leaves was determined (105 ºC for at least 72 h). A second harvest was conducted 12 months after planting.

Statistical Analysis: The data were subjected to statistical analysis using Mixed Model of SAS software (Statistical Analysis System Institute Inc., Cary, N. C. 1997). Symptoms and severity data were square-root transformed, while the root yield and plant growth parameters data were log transformed to stabilize variances. The means were compared using LSD test. The means in Table 2 were square-root back-transformed, and the means in Table 3 and Table 4 were 10^x back-transformed.

RESULTS

Development of Symptom Types and Disease Severity during the Growing Season: In plots with diseased leaf removal and in non-removed leaves plots, the development of angular spots on leaves followed a similar trend (Figure 1). However, the percentage of leaves with spot recorded after the first removal of leaves (day 21) was lower in the removed leaves plots than in those of the non-removed leaves until the 6th evaluation. The general decrease of the percentage of symptomatic leaves from 105 days after inoculation was due to the start of the dry season during which CBB symptoms on leaves were not observed.

After the first removal of diseased leaves, the number of leaves with blight symptoms decreased significantly until the second and third leaf removals, 42 and 63 days after inoculation, while this value increased in the non-removed leaves plots (Figure 2). The percentage of leaves with blight was generally reduced in removed leaves plots until the start of the dry season.

The disease severity was reduced by removing diseased leaves over the whole growing period, with a percentage of disease severity lower than 7% in removed leaves plots during the first five months after planting and of 13% at the second harvest, while in non-removed leaves plots, disease severity ranged between 7% and 21%, and increased up to 30%, respectively (Figure 3).

Severity of Cassava Bacterial Blight Symptoms and Disease Severity over the Growing Period: The percentage of blight and wilt was lower ($p > 0.05$) in removed leaves plots than in those of non-removed leaves (Table 2). Disease severity, a combined value from spot, blight, wilt and dieback symptoms, was reduced by leaf removal by 71% ($p = 0.0028$), with 4.0% severity in removed leaves plots and 13.6% in the non-removed ones. The percentage of leaves with angular spots and dieback were not significantly reduced by leaf removal.

Growth and Yield Parameters of Cassava Six and 12 months after Planting: The difference between removed and non-removed leaves plots was not significant (p = 0.05) for all parameters (root fresh and dry weight, leaf fresh and dry weight, stem fresh and dry weight) at six months (Table 3) and 12 months (Table 4) after planting. Six months after planting, the root fresh and dry yield were slightly higher in the leaf removal variant, while 12 months after planting root yield was approximately the same in both variants.

Table 2. Effect of removal of leaves on cassava bacterial blight symptoms and disease severity.

Treatment	Spot [x]	Blight	Wilt	Dieback	Disease severity [y]
T1Leaf removal [w]	11.6a[z]	7.7a	0a	0.9a	4.0a
T2[w]No removal	11.4a	13.5b	23.4b	0.3a	13.6b

[W] Cassava leaves presenting CBB symptoms were removed at three-weekly intervals. The removal was performed four times. [x] Spot, blight, wilt and dieback symptoms were rated in percentage. [y] Disease severity in percentage was calculated according to the formula: Ds = (1xS + 2xB + 3xW + 4xD)/10 where S, B, W and D represent the percentage of spot, blight, wilt and dieback, respectively. [Z] Means followed by the same letter within a column are not significantly different at p = 0.05 according to the least significant difference test. Means are the average of seven evaluations repeated each three times.

Table 3. Cassava root, leaf and stem weights (t/ha) six months after planting in plots with (T1) and without (T2) removal of diseased leaves.

Treatment	Root		Leaf		Stem	
	FW	DW	FW	DW	FW	DW
T1 Leaf removal	12.6*	3.7	5.6	1.5	10.4	2.8
T2 No removal	16.3	4.8	7.2	1.8	13.1	3.7

FW = fresh weight, DW = dry weight

* Means within a column are not significantly different at p = 0.05 according to the least significant difference test. Means are the average of three replications.

Table 4. Cassava root, leaf and stem weights (t/ha) 12 months after planting in plots with (T1) and without (T2) removal of diseased leaves.

Treatments	Root		Leaf		Stem	
	FW	DW	FW	DW	FW	DW
T1 Leaf Removal	17.4*	5.	3.1	0.8	17.3	4.6
T2 No removal	16.7	4.3	3.5	0.8	23.5	6.0

FW = fresh weight, DW = dry weight

* Means within a column are not significantly different at p = 0.05 according to the least significant difference test. Means are the average of three replications.

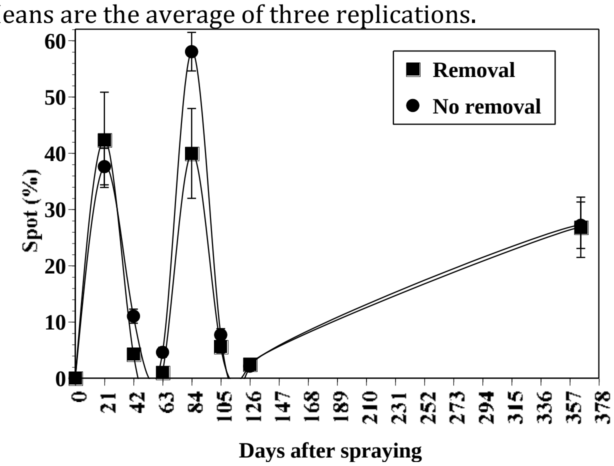

Figure 1. Development of angular leaf spots after removal and no removal of symptomatic leaves in cassava cropping patterns in the forest savannah transition zone at the IITA, Abomey-Calavi.

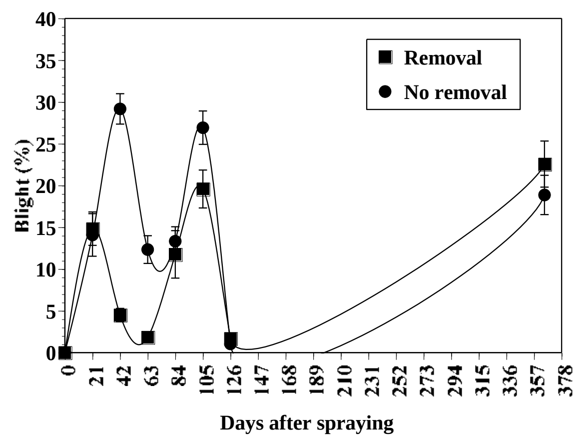

Figure 2. Development of blight symptoms after removal and no removal of symptomatic leaves in cassava cropping patterns in the forest savannah transition zone at the IITA, Abomey-Calavi.

Each point is the mean of three replications. Vertical bars indicate standard error of the mean.

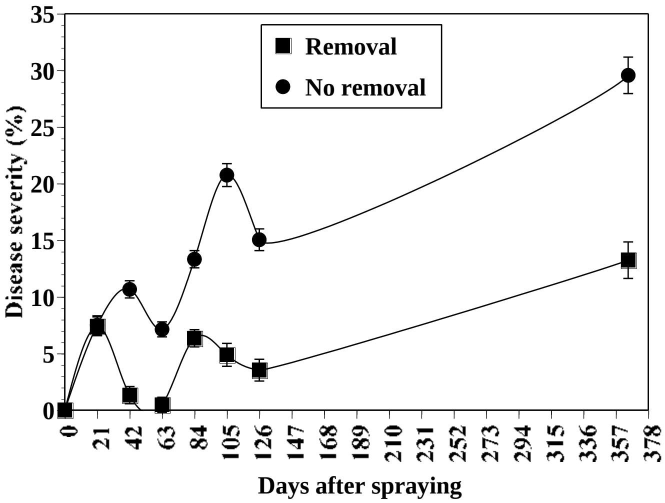

Figure 3. Development of disease severity after removal and no removal of symptomatic leaves in cassava cropping patterns in the forest savannah transition zone at the IITA, Abomey-Calavi. Each point is the mean of three replications. Vertical bars indicate standard error of the mean.

DISCUSSION

In this study an alternative method to standard pruning of whole plants recommended by NRCRI (1984) and Lozano (1986) for controlling CBB was evaluated. The removal of infected leaves four times at intervals of 21 days during crop growth resulted in a lower disease severity and percentage of blight and wilt symptoms. The interval of 21 days between successive removals may have inhibited the development of spots into blight symptoms before the following removal was performed. Thus, though blight and wilt were reduced, a similar development of angular leaf spots was obtained in both removed and non-removed leaves plots leading to a rapid contamination of the newly formed leaves. Under humid conditions, high epiphytic activity of phytopathogenic bacteria occurs on the phyllosphere (Romantschuk *et al.,* 1996). Large populations of *Pseudomonas syringae* were correlated to intense rainfall events (Cross *et al.,* 1983; Martins, 1982; Smitley and McCarter, 1982; Hirano *et al.,* 1987), and also the epiphytic growth of *Xam* on cassava plants (Persley, 1978; Daniel and Boher, 1985) and disease epidemics (Wydra and Verdier 2002) were related to rainfall patterns. A higher epiphytic development linked with favourable weather conditions from August to November in our study may have contributed to the rapid development of spots on the rest of the leaves and the newly formed leaves of the treated plants. The short distance of 2 m between removed and non-removed leaves plots of the same block was favourable for the transport of bacterial cells from the non-removed leaves plots to those where leaves were removed. It was reported that plant-colonising epiphytic and pathogenic bacteria are dislodged as aerosol from plants (Lindemann *et al.,* 1982) or within rainsplash (Romantschuk *et al.,* 1996). Those dislodged within rainsplash do not move on great distances (Butterworth and McCartney 1991), but their spread to neighbouring leaves and plants could induce the disease (Romantschuk *et al.,* 1996). This fact may have also influenced the increase of spots in removed leaves plots. Studying the spread of *Xanthomonas axonopodis* pv. *translucens* in wheat, Tubajika et al. (1995) detected the pathogen at 0, 2, 4, and 6 m distance interval in the susceptible wheat variety.

Contrary to our results Lutete et al. (1992), who used pruning of 25 cm of shoots repeated three times to control CBB, found a higher severity of cassava bacterial blight and African cassava mosaic disease for all of the five cultivars tested in three different agroecozones. The same authors observed a reduction of 49.2% of cassava fresh root yield in the pruned variant.

In our investigations, the root yields were not significantly different comparing both variants at six and 12 months. After removal of leaves, the plants quickly developed new leaves and compensation occurred. These results are supported by the physiological observations of Cock and El-Sharkawy (1988), in which short periods of stress had little effect on the overall growth. Cock (1978) reported that premature leaf fall has little effect on vigorous varieties and that cassava is rather tolerant to defoliation.

The effect of disease reduction in defoliated plots was not reflected by a significant difference in cassava root yields after six months. Nevertheless, the slightly lower root yields obtained in the treated plots may have been due to the effect of removal of leaves. Due to the lowest disease severity in the treated variant and the phenomenon of physiological compensation of losses by the plants through growth of new leaves which might be photosynthetically more active, root yields in the defoliation variant were slightly higher than in the untreated plots.

It is supposed that the still relatively high disease severity in the removed leaves plots compared to those where leaves were not removed was due to the proximity of both plots in each block. The ability of the highly susceptible cultivar BEN 86052 to develop quickly CBB symptoms may also explain the early reappearance of new symptoms despite the successive removals of infected leaves. Lozano (1986, 1992) and Arene (1990) also reported that the success of pruning depends on the susceptibility of the cultivar and observed that results of pruning of cassava whole plants were best with resistant and moderately resistant cultivars, with regular and extensive pruning not even being necessary. In the present studies, the removal of infected leaves of the susceptible and vigorous cultivar BEN 86052 with a disease incidence of plants per plot of 100% was time consuming.

In conclusion, the removal of diseased leaves is recommended to control adequately cassava bacterial blight during the cropping season, especially where the disease pressure is low, to reduce infection of the cuttings needed for the next planting season and thus, to prevent that the disease becomes endemic. Burying of infected leaves is recommended (Fanou, 1999). Early removal of infected leaves will be successful in a cassava plantation far away from other plantations with CBB. In zones with higher cassava bacterial blight pressure, the application of this method may only be sustainable on moderately resistant or resistant cultivars and in combination with other methods of integrated control, such as burying of infected plant material in soil, use of healthy cuttings and mixed or intercropping (Fanou, 1999).

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