Population Dynamics of *Xanthomonas axonopodis* pv. *vignicola* (Burkholder) Dye in/on growing Cowpea Plant

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ABSTRACT

With very few management options available, it is clear that a better understanding of the population structure of the highly diverse bacteria pathogen is needed in order to develop targeted and, possibly, genotype targeted management practices. Understanding the population growth of *Xanthomonas axonopodis* pv. *vignicola* (*Xav.*) in Cowpea plant parts and at different stages of growth would provide the necessary basis to set up new management approach. The objective of the study therefore was to determine the population dynamics of *Xav.* in/on the growing cowpea plant. Seed samples were collected from various sources to reflect the various ways farmers obtain seeds. Each seed lots were planted at the rate of 3 seeds per pot but thinned to 2 plants per pot after seedling establishment, with 5 replications. Two weeks after, the plants in the pots were spray inoculated with the suspension of bacterial blight pathogen adjusted to *ca*.4.5 x 10^7 cfu/ml. Two mm^2 discs of leaf and stems were taken from both symptomatic and non symptomatic cowpea plant at 14, 28, 42 and 56 days after inoculation (DAI) to quantify *Xav* population. The discs (stem/leaves) taken were washed with SDW. The washed suspension were serially diluted and plated accordingly to determine phyllosphere population of *Xav*. To determine the number of infected flower and pods and consequently the fallen flower/pods and aborted flower/pods, ten plants were randomly chosen in pots and tagged. There was significant difference between the treatments in terms of disease incidence and severity on the vegetative parts. There was no significant difference between all the treatments in terms of *Xav* population build up on the floral parts. Population build up did not correlate to folial symptoms development, indicating the latent debilitating effect on the plant by the pathogen.

INTRODUCTION

Cowpea (*Vigna unguiculata* L.[Walp]) is an important protein source in the tropics and subtropics. It is also an important cash crop that makes up part of the export for the countries cultivating it (Rajapakse and van Eden, 1997). In Africa, cowpea is a traditionally considered as a legume of the poor and mostly cultivated by small scale farmers as a subsistence crop (Ahmed *et al*., 2010). It is a drought tolerant and can be cultivated in poor soils (Singh *et al*., 1997). Cowpea is able to fix nitrogen in the soil efficiently in its nodules through symbiosis with bacteria (Ahmad *et al*., 2010). There is a growing awareness that the protein deficiency is the most critical and complex aspects of the total food problem. Indeed, this problem is more pronounced in the developing nations, where agricultural production and purchasing power are generally low (Ahmed *et al*., 2010). Low yield of 240-300 kg/ha is considered to be a significant attribute of cowpea production in Africa (Jackai and Singh, 1988; Adipala *et al*., 1995). In tropics and sub-tropics, where 80% of the cowpea production takes place, bacterial blight induced by *Xanthomonas axonopodis* pv. *vignicola* (*Xav.*) (Burkholder) Dye is the most important bacterial disease that limits cowpea production (Alabi and Emechebe, 2004). Yield loss caused by *Xav* is between 38-68% (Opio *et al*., 1996; Singh and Munoz, 1998; Okechukwu *et al*., 2010). Symptoms elicited by *Xav* include large, irregular foliar lesions with yellow margins; stem cankers, flower and pod abortion, leaf defoliation and both pre-emergent and post-emergent seedling mortality (Kishun, 1989; Alabi and Emechebe, 2004). Management options such as sowing pathogens free seeds, crop rotation, and chemical control have not adequately managed the disease. With very few management options available, it is clear that a better understanding of the population structure of the highly diverse bacteria pathogen is needed in order to develop targeted and, possibly, genotype targeted management practices (Ivey *et al*., 2007). The effective management of the disease depends on the knowledge of *Xav* movement and population in/on the growing cowpea plant. However, the movement of bacteria in the host plant has been studied (Barak *et al*., 2002). Understanding the population growth of *Xav* in Cowpea plant parts and at different stages of growth would provide the necessary basis to set up new management approach. The objective of the study therefore is to determine the population dynamics of *Xav* in/on the growing cowpea plant.

Materials and Methods

Three Ife-brown seed lots from different cowpea seed companies were treated with different fungicides, Alheri seed treated with Apron star (Alheri A), Premier seed treated with Dress force (Premier DF), Masalaha seed treated with Team (Masalaha T). Three untreated seed lots of local varieties were purchased from open markets within Zaria; these were Local Wusasa (Local W), Local Sabon-Gari (Local SG) and Local Samaru (Local S). Two seed lots, IT86D-721 treated with Apron star, IT86D-721A and IT98-503-1 treated with Team (IT98K-503-IT) were obtained from International Institute of Tropical Agriculture (IITA) and SAMPEA-7 divided into four seed lots; SAMPEA-7 treated with Apron star (SAMPEA-7A), SAMPEA-7 treated with Team (SAMPEA-7T), SAMPEA-7 treated with Dress force (SAMPEA-7D, untreated SAMPEA-7 as control (SAMPEA-7C), inoculated SAMPEA-7 (SAMPEA-7I), and inoculated Ife-brown (IFB I) making a total of fourteen seed lots, that were used in the trial. These were carefully selected to reflect the various sources farmers usually obtained seeds and effects this might have on the bacterial population build up in/on the host. The different fungicides used by the seed companies were Apron-star (tiamethoam 20 % + metalaxyl-m 20 % + difenocoazole 20 % w/w), Dress force (imidacloprids 20 % + metalaxyl-m 20 % + tebuconazoles 20 %) and Team (carbendazin 12 % + mancozeb 63 %). Seeds from each seed lot were planted in plastic pots of 25 cm diameter filled with sterile

soil. Each seed lots were planted at the rate of 3 seeds per pot but thinned to 2 plants per pot after seedling establishment, with 5 replications. The seeded pots were placed randomly in the screen house and observed for germination. Two weeks after, the plants in the pots were spray inoculated with the suspension of bacterial blight pathogen adjusted to *ca*.4.5 x 10^7 cfu/ml. Two mm^2 discs of leaf and stems were taken from both symptomatic and non symptomatic cowpea plant at 14, 28, 42 and 56 days after inoculation (DAI). The discs (stem/leaves) taken were washed with SDW. The washed suspension were serially diluted and plated accordingly to determine phyllosphere population of the pathogen. To determine the number of infected flower and pods and consequently the fallen flower/pods and aborted flower/pods, ten plants were randomly chosen in pots and tagged. The number of flower fallen without the pod initiation was counted (aborted flower). The total number of flowers and pods produced by the tagged plants were recorded, from which, the percentage of aborted flower and pods were calculated. The experiments were laid out using complete randomized design (CRD). Data (*Xav)* population (cfu/g), percentage of aborted flower and pod, and fallen flower collected were analyzed statistically using ANOVA and means were separated by New Duncan's Multiple Range Test (NDMRT). The trial was repeated once. The experiment was carried out in the screen house of Institute for Agricultural Research (IAR), Ahmadu Bello University Zaria.

Results

In terms of disease incidence, there was no significant difference between cowpea inoculated with bacterial inoculum and non-inoculated cowpea seed lots. Similarly, fungicides used for the treatment of seed lots by seed companies had no significant effects on bacterial diseases incidence. The population of *Xav* isolated both symptomatic and non symptomatic cowpea plants were generally high (10^8-10^9 cfu/2mm^2) of leaf/stem and the population growth did not reflect lesion severity on the host plant. Figure 1 shows the population dynamics of *Xav* in /on cowpea plant after spray inoculation. At 14 DAI, the population of *Xav* build up in cowpea plant was higher in SAMPEA-7A but statistically similar to SAMPEA-7T, SAMPEA-7DF and Local W. The population of *Xav* was lower in SAMPEA-7I and was statistically similar to other treatments and the lowest population of *Xav* was recorded in Ife-brown. There was no statistical difference between all the treatments in terms of phyllosphere *Xav* population. After 28 DAI, the population of *Xav in vivo* was higher in the ground assay on SAMPEA-7A; this was followed by SAMPEA-7T but was statistically similar to all other treatments. There was no statistical difference between the phyllosphere populations of *Xav* on all the treatments 42 DAI. Similar results also were obtained from the ground assay 56 DAI where the *in vivo* population of *Xav* was higher in SAMPEA-7 DF but was statistically similar to all other treatments except the control. The phyllosophere Population of *Xav* was higher in SAMPEA-7I and Local SG followed by IT98K-503T which was statistically similar to other treatments except Alheri A which had the population of *Xav*. At 56 DAI, the population of *Xav in vivo* was higher in Alheri A followed by IT98K-503-IT which was statistically similar to all other treatments except SAMPEA-7T. There was high phyllosophere population of *Xav* on SAMPEA-7T but was statistically comparable to all the treatments except the control.

The distribution of *Xav* population on plant part at flowering (70 DAS) is outlined in Figure2. The population of *Xav* was highest on flower cushion and flower of all the varieties while the lowest population was observed on stem and on the root. There was however no statistical difference between population of *Xav* on the stalk and leaf. There was relative varietal resistance observed in the distribution of *Xav* in plant parts except in the flower cushion and the flower.

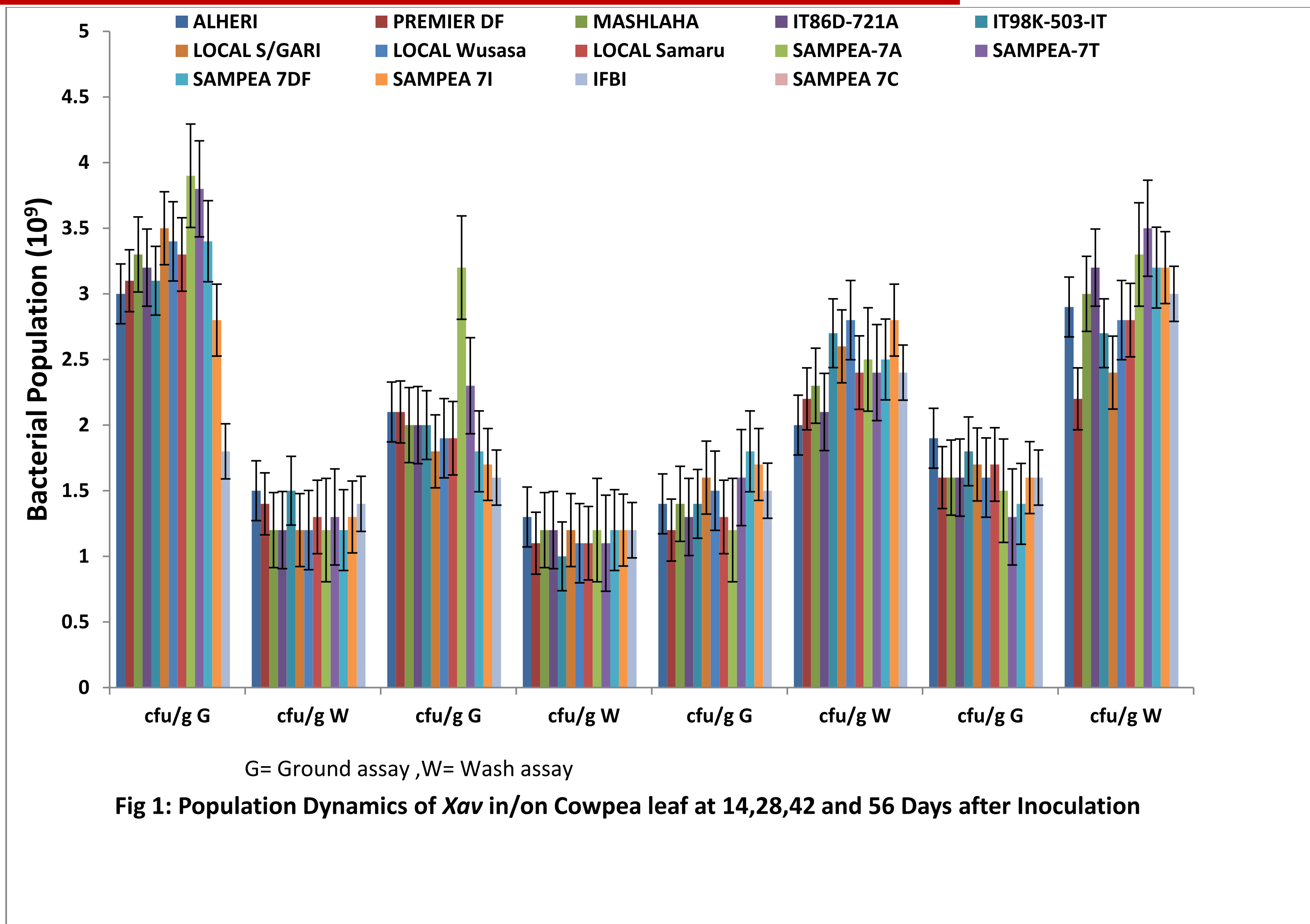

Fig 1: Population Dynamics of *Xav* in/on Cowpea leaf at 14,28,42 and 56 Days after Inoculation

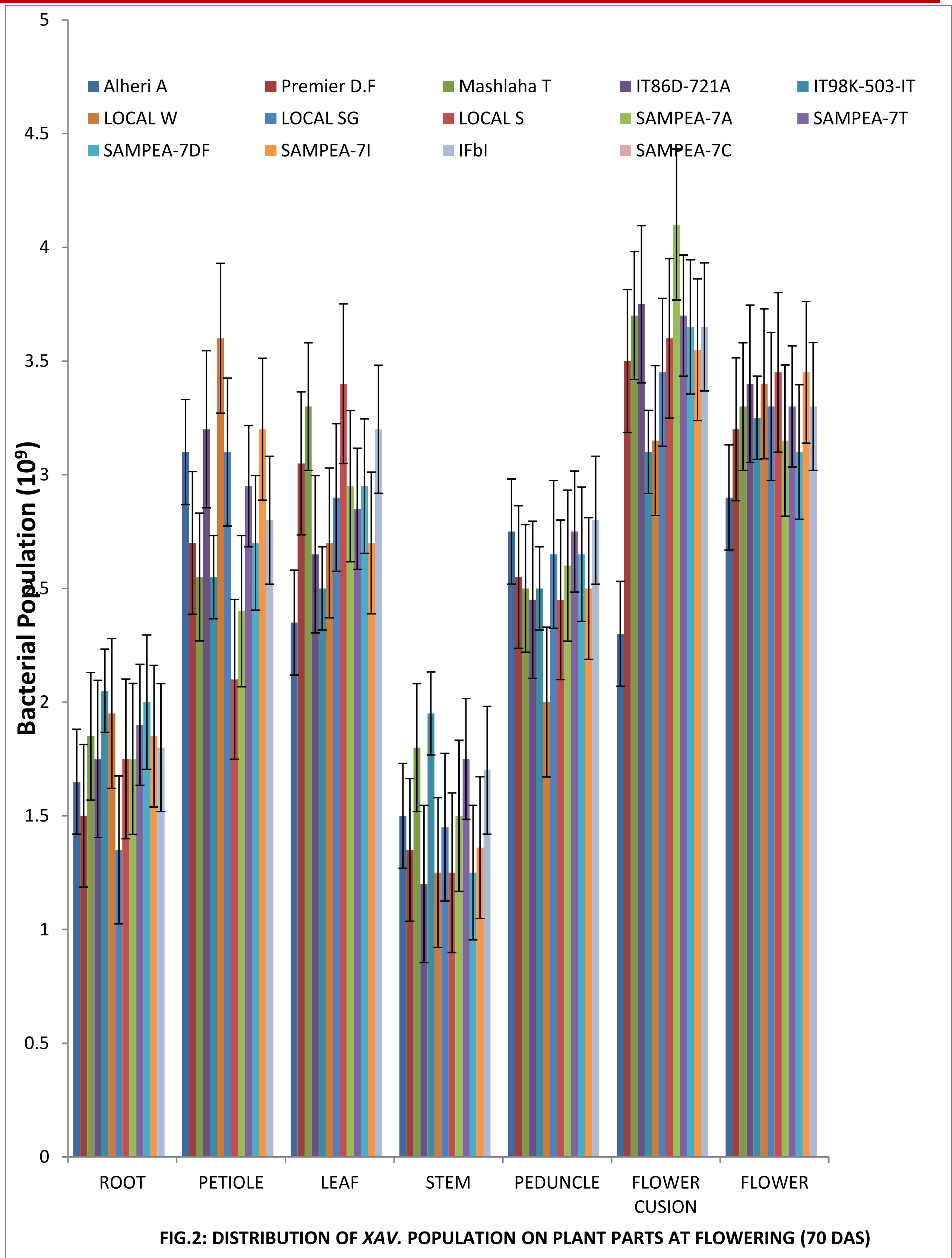

FIG.2: DISTRIBUTION OF *XAV.* POPULATION ON PLANT PARTS AT FLOWERING (70 DAS)

Table 1 shows *Xav* populations on shed flowers, aborted flower and aborted pods. Population of *Xav* in shed flower ranges from 10^2-10^8 while the aborted flower and aborted pod had high population of *Xav* (10^9). At 70 DAS, the highest population of *Xa.* isolated from shed flower was on Alheri A which is statistically similar to SAMPEA-7DF. Also there was no statistical difference between IT98k-503-IT, Local S and Ife-brown, Premier DF and Local W, IT86D-721A and SAMPEA-7T, Masalaha T, Local S and SAMPEA-7I. At 77 DAS the highest population of *Xav* was recorded Premier D (10^9) which is statistically similar to SAMPEA-7A and SAMPEA-7DF. Similarly, there was no statistical difference between IFBI. SAMPEA-7I, SAMPEA-7T, Local S, Local SG, Local W, IT86D-721A, Masalaha T and Alheri A. At 84 DAS, however, the highest population of *Xav* isolated from aborted pod was on SAMPEA-7T which was statistically similar to Local W. This was followed by SAMPEA-7I which is statistically similar to SAMPEA-7A, Local SG and MasalahaT. There was no statistical difference between all other treatments except the control.

Table 1: Population of *Xav.* (cfu/ g) in Shed, Aborted flower and Pods in 2011 Combines Analysis of Two Trials

	70 DAS	77 DAS	84 DAS
Source/variety	**cfu/g shed flower**	**cfu/g arborted flower**	**cfu/g aborted pod**
Alheri A	$1.5x10^8$a	$1.2x10^9$b	$1.0x10^9$c
Premier D.F	$1.3x10^6$c	$1.3x10^9$a	$1.1x10^9$c
Masalaha T	$1.2x10^2$f	$1.1x10^9$c	$1.2x10^9$b
IT86D-721A	$1.3x10^4$d	$1.2x10^9$b	$1.1x10^9$c
IT98K-503-1T	$1.5x10^7$b	$1.3x10^9$a	$1.0x10^9$c
Local W	$1.3x10^6$c	$1.1x10^9$c	$1.3x10^9$a
Local SG	$1.5x10^2$f	$1.2x10^{9b}$	`$1.2x10^9$b
Local S	$1.4x10^7$b	$1.1x10^9$c	$1.1x10^9$c
SAMPEA-7A	$1.0x10^3$e	$1.3x10^9$a	$1.2x10^9$b
SAMPEA-7T	$1.2x10^4$d	$1.0x10^9$c	$1.3x10^9$a
SAMPEA-7D	$1.5x10^8$a	$1.3x10^9$a	$1.1x10^9$c
SAMPEA-7I	$1.4x10^2$f	$1.2x10^9$b	$1.2x10^9$b
Ife brown I	$1.3x10^7$b	$1.2x10^9$b	$1.0x10^9$c
SAMPEA-7C	0.00g	0.00d	0.00d
S.E	1.03	1.22	1.12

Means in a column followed by the same letter are not significantly different at 5 % level of significance NDMRT test.

Discussion

The high population of pathogen usually associated with leaves (Phyllosophere colonization) could be a survival strategy (Darsonval *et al*., 2009). The genus *Xanthomonas* is particularly well adapted to epiphytic survival in the phylosphere because it is able to aggregate in biofilm that protect it against environmental stresses (Jacques *et al*., 2005). Functional T3SS is essential for inplanta colonization but not the epiphytic survival. There was general dramatic increase in population of *Xav* both in/on the surface of the plant from 42-56 DAI. This was as a result of the pathogen getting full adaption to the new environment (Upper *et al*., 2003; Feil *et al*., 2007). Canopy provides conducive environment for the pathogen to grow and form biofilms. This result is in agreement with the report of Ramey *et al*. (2004) that, aerial plant surface support large population of bacterial epiphytic, including plant pathogen that multiply on the leaf surface before initiating disease. The leaf surface is partitioned into preferred microhabitats along veins, near trichomes and stomata (Beattie and Lindow, 1999; Monier and Lindow, 2004). Spread of the pathogen often occur independent of lesion occurrence (Upper *et al*., 2003). Lesions on the other hand, occur when extensive multiplications require the bacterial to rely on expression of T3SS (Darsonval *et al*., 2008). Foliage and stems of bean are known to harbor relatively high population of plant pathogenic bacteria without exhibiting discernable symptoms (Mabagala, 1997; Barak *et al.,* 2002). The differences in population of *Xav* observed indicated the differences in varietal susceptibility to the pathogens (Okechukwu *et al*., 2010). The gradual decrease in population of *Xav* in the ground assay was as a result of plant growing more cellulose and lignifications of tissues, thereby creating challenge for pathogen growth and development (Ramey *et al.,* 2004). Many phytopathogenic bacteria multiply quite successfully in association with susceptible tissues without causing lesion (Upper *et al.,* 2003). Bacterial pathogen can reduce phosphorylation by causing a loss of chloroplast structure and function (Kosuge and Kimpel, 1982). Plant pathogenic bacteria produce Extracellular Polymeric Substances (EPS) that cause water soaking of intercellular spaces of leaves. Water soaking is a result of altered plant membrane functions, which will also cause a loss of compartmentation and possibly a disruption of chloroplast function (Kosuge and Kimpel, 1982). Also, the pathogens secrete effectors into the apoplast and/ or cytoplasm of the host plants to suppress host defense. These effectors may include the T3SS or enzyme whose products act as elicitor (Hogenhout *et al*., 2009). The pathogens that infect green aerial tissues (photosynthetic tissues) are more likely to affect plants productivity. The pathogen, once established in host tissue, redirect the host nutrients for their own use. In most diseases, the water flow through the xylem is reduced to a mere 2-4% of that flowing through stems of healthy plants (Goodwin, 1992; Chaube and Pundhir, 2005).

There was increase in population of *Xav* from the root having the lowest population to flower and flower cushion having the highest population. This was as a result of the available nutrient in plant moving towards the reproductive parts such as peduncle, flower cushion and flower. The pathogens also move in that direction. The low population of *Xav* may be due to poor nature of root extract as the plant matures (Yaryura *et al*., 2008; Green *et al*., 2007). The low population of *Xav* in the mature stem could be due to morphological and biochemical factors that regulate the pathogen population in plant at this stage. The morphological factor is as a result of maturing stem producing more waterproof substance called suberin. The suberin is deposited in the cell walls in the form of b and s called suberin strips (Taylor *et al*., 1997). Plants are known to produce varieties of biochemical substance that acts as defense against the invading pathogen. The high population of *Xav* in leaf, petiole, Peduncle, flower cushion and flower could be due to

high nutrients available in these parts of the plant at maturity. This result was in line with Goodwin (1992) who reported that photosynthetic rate in cowpea increase at flowering to a maximum rate at pod set, and the amount of this increase is positively correlated with yield. The high population of *Xav* in both the flower cushion and the flower is consistent with the fact that flowers are considered excellent microbial habitats, being well supplied with nutrients (Ngugi and Schem 2006) and offering protecting habitats against abiotic stresses (Darsonval *et al*., 2009). Furthermore, flowers provide short lived but non hostile environment to microbes (Stockwell, 2005). Most importantly, flowers do not induce defense responses (Ngugi and Scherm, 2006) and this accounts for the high population of *Xav* isolated in this environment. Most yield losses, however, presumably result from high flower and pod abortion (Goodwin, 1992). This heavy flower and pod abortion could be due to the high population of *Xav* in the floral parts and flower does not induce defense response making the pathogen population often grow explosively (Ngugi and Scherm, 2006). Disruption of membrane permeability is an important cause of flower and pod abortion (Van Alfen, 1989).

There was high population of *Xav* in flowers and pod even on the normally shed flowers. This high population of *Xav* in the aborted flowers and pod could lead to serious soil contamination (Murty and Devadath, 1984).

Conclusion

A major step toward effective management of this disease would require an understanding of bacterial population dynamics in and on cowpea plant. Population build up did not correlate to folial symptoms development, indicating the latent debilitating effect on the plant by the pathogen. Spread of the pathogen often occur independent of lesion occurrence. The symptoms of *Xav* did not only manifest as leave lesions but also cause leaves defoliation, aborted flowers and aborted pods. Measurement of disease severity based on leave lesions alone could be misleading as most of the defoliated leaves did not have extensive lesions, and most importantly, flower and pod aborted were not showing obvious symptoms. I would recommend pathogen population assessment should be undertaken in conjunction with disease incidence not disease severity.

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