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Correlation Between Populations of *Xanthomonas Axonopodis* Pv. *Vignicola* Builds Up *In Vivo* and Symptoms Manifestation in Infected Cowpea Plant.

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Abstract.
From the perspective of plant disease epidemiology, pathogen spread and multiplication is important in assessing the likelihood of disease outbreaks. Many plant pathogenic bacteria multiply quite successfully in association with susceptible tissues without causing lesions. In this paper we aimed to understand the dynamics of Xanthomonas axonopodis pv. vignicola population sizes in regard to symptom development on cowpea plant. Seven cowpea genotypes were use for the investigation. The seed lots were obtained from seed companies, research institute and open markets within Zaria environment. These were so selected to reflect the various ways farmers usually obtain their seeds. The seed samples were grouped into treated and inoculated and non-treated inoculated seed lots. The treated seed lots were treated with three different types of fungicides (Apron star, Dress force, and Team fungicides) before inoculation with bacteria suspension. Since Xanthomonas axonopodis pv. vignicola is a seed-borne pathogen, the inherent bacteria were estimated from which the population builds up were determined. The seeds were inoculated with bacteria suspension adjusted ca. 4.7 x 10^7 cfu/ml and were also spray inoculated at 14 days after sowing. The result shows that there were no correlation between bacteria disease incidence, severity and population with respect to foliar lesion development. The usual practice of taking disease incidence and severity should be discouraged. I would recommend pathogen population assessment should be undertaken in conjunction with disease incidence not disease severity. Fungicides used had no significant effect on the pathogen's growth and pathogenicity. Although, seed treatment has been adjudged to be the best method of control soil and seed-borne diseases, seed treatment chemical must be pest specific.
Keywords; correlation, population, pathogenicity, symptom.

1. Introduction

Production of cowpea (*Vigna unguiculata* L. Walp) is hampered by pests among which is the bacterial blight induced by *Xanthomonas axonopodis* pv. *vignicola* (*Xav*). Both internally and externally infected seed have been mentioned as important source of inoculums (Cafati and Saettler, 1980).When describing the epidemiology of plant diseases, we often say that a disease has spread when new disease occurs. The occurrence of new disease implies that the pathogen has successfully dispersed, to a new habitat, a susceptible host, and that growth on the host has occurred, leading to disease (Upper *et al.*, 2003). Much of the classical epidemiology of plant diseases has been developed for disease in which disease development can be equal to pathogen population. Many pathogenic bacteria multiply quite successfully in association with susceptible tissues without causing lesions (Hirano and Upper, 1983). Attempt to fit classical epidemiological model to such diseases are often less successful and clearly have less biological relevance (Upper *et al*., 2003). In this system, the likelihood of disease occurring on a given leaf is a function of some other factors rather than merely pathogen population size on that leaf (Rouse *et al*., 1985). Most gram-negative bacteria pathogens harbor a T3SS (type III secretion system) that allow the delivery of effectors directly into the plant cytoplasm, leading to a hypersensitive reaction (HR) in incompatible interactions and symptoms development in compatible ones (Bruning and Gabrial, 2003; Gurlebeck *et al*., 2006). The plant immune system is based on the recognition of pathogen-associated molecular patterns (PAMPs), resulting in the activation of defense responses (Niruberger and Lipka, 2005). From the perspective of plant disease epidemiology, pathogen spread and multiplication is important in assessing the likelihood of disease outbreaks. In this paper we aimed to understand the dynamics of *Xav* population sizes in regard to symptom development on cowpea plant

2. Materials and methods

Three Ife-brown seed lots were collected from different cowpea seed companies and were already treated with different fungicides; Alheri seed treated with Apron star (Alheri A), Premier seed treated with Dress force (Premier D), Masalaha seed treated with Team (Masalaha T). Three 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 and IT98-503-1 obtained from International Institute of Tropical Agriculture (IITA). Five seed lots of SAMPEA-7 and one Local Ife-brown were obtained from Institute for Agricultural Research and open market respectively. These were 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. The initial populations of *Xav* were quantified by ground up four hundred seeds each of the seed lot and plated on YDCA media before sowing from which population build were monitored. The inoculated Ife-brown was as a result of its moderate resistance to *Xav*, and inoculated SAMPEA-7 was as a result of its known susceptibility to *Xav* to serve as positive control. These non treated but inoculated seeds and the control were first of all soaked in ethanol for twenty minutes to remove inherent pathogens after which, the seeds were washed three times in sterile distilled water. 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 %). These were carefully selected to reflect the various sources farmers usually obtained seeds. Seeds were inoculated by soaking hundred seeds in 100 ml of bacteria suspension adjusted to *ca* 4.7 x 10^7 cfu/ml for four hours before fungicide treatment. The fungicidal treatment was done at the rate of 2 g/kg of seeds. 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. Disease incidences were taken by counting the number of infected plants and severity were scored using a modified CIAT 1-9 scale (Opio *et al*., 1993).

After which the plants were observed for a typical blight symptoms on cotyledons, stem, leaves and general seedling mortality for two weeks. 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. At 14, 28, 42, and 56 DAS, leaf and stem discs measuring about 2 mm^2 were taken from the plants using cork borer. The discs were teased in drops of SDW and a loopful of the resulting suspensions was mixed in 10 ml of SDW from which a serial dilution of up to 10^{-5} were prepared. Ten micro milligrams of the suspensions were placed on YDCA in Petri-dishes and spread using glass spreader and incubated at 28 ^{0}C for 48 h.

To determine the number of infected pods and consequently aborted pods, ten plants were randomly chosen in pots and tagged accordingly at 42, 49, 56, 63 and 70 DAS.

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 infected aborted flower and pods were calculated. The experiments were laid out using CRD. Data collected were analyzed statistically using ANOVA and means were separated using New Duncan's multiple Range Tests (NDMRT). The trials were repeated once.

3. Results

Table 1; shows the incidence; severity and population build-up on germinating and emerging seedling at 14 DAS. There was significant difference ($P \leq 0.05$) between the treatments in terms of germination though the control treatments appeared to be the lowest (60 %) which was as a result of soaking the control seed in ethanol for 20 minutes. There was significant difference between the treatments in terms of seedling establishment. While the control treatments were recording 100 % establishment Masalaha seed treated with Team fungicide had 50 % seedling establishment. There was high disease incidence (67 %) in local variety obtained from Sabon Gari market compared to the control seedlings (0.00). The disease severity was higher on all the SAMPEA-7 varieties (both treated and untreated) which were statistically higher than other treatments and there was however, significant difference between all other treatments and the control. The population build up *in vivo* was higher in SAMPEA-7 seeds treated with Apron star (4.5x 10^8) and was statistically difference from other SAMPEA-7 treatments, variety IT98K-503-IT and IT86D-721A. IT98K-503-I T Masalaha T, and Alheri A had lower *in vivo* population build up (2.0x10^8) but were statistically difference from Premier D, MasalahaT, IT86D-721A, local W, Local SG and Local S. Although initial *Xav* population on the non-treated inoculated seeds was higher (4.7x10^7), there was no statistics difference in terms of the effect of pathogen on the seedling parts and the population builds up *in vivo* compared to other initial populations and their effects on seedling parts and the resultant population builds up *in vivo*. The result demonstrated the non significance effect of fungicides on *Xav*.

Fig.1. shows the disease incidence and severity after inoculation. At 14 DAS the disease incidence was higher in SAMPEA-7I followed by SAMPEA-7D, SAMPEA-7T and SAMPEA-7A. There was no statistical difference between all other treatments except the control. Similar results were also obtained in the disease severity, where the severity was higher in all SAMPEA-7 varieties except the control, LIfbI which is statistically comparable to

IT98K-503-IT, IT86D-721A, MasalahaT, Premier D and Alheri A. There was no statistical difference between Local W, Local SG, and Local S. At 28 DAS disease incidence was higher in SAMPEA-7I, SAMPEA-7 D and SAMPEA-7T followed by SAMPEA-7A. There was no statistical difference between all other treatments except the control. The disease severity was however high in SAMPEA-7A followed by Masalaha T, IT98K-503-IT and Local W and was statistically comparable to all the other treatments except the control. At 42 DAS, there was no statistical difference between all the treatments except Alheri A. In disease severity however, SAMPEA-7D had the highest severity and were comparable to SAMPEA-7I, SAMPEA-7T, SAMPEA-7A, Local S, MasalahaT, Premier D.f and Alheri A. The lowest severity was obtained in IT86D-721A but was comparable to IT98K -503-IT, Local W, Local SG and LIfbI. At 56 DAS, the disease incidence was higher on all SAMPEA-7 variety. There was no statistical difference between all other treatments except Alheri A. The disease severity was higher in SAMPEA-7A which was comparable to SAMPEA-7T, SAMPEA-7D and SAMPEA-7I followed by AlheriA which was comparable to Premier D Masalaha T, Local W and Local SG. The lowest severity was obtained in Local S and LIFbI.

Table 2 shows the severity of *Xav* on floral parts. The percentage of flower aborted at 42 DAS was higher on IT98K-503-IT (29.85 %), the least was SAMPEA-7C which was the control. There was statistical difference between all the treatments. There was statistical difference between the percentage of flower aborted on all the treatments and the control at 49 DAS, similar trend of results were obtained at 56, and 63 DAS. At 63 DAS, however, the highest pod abortion was recorded on IT86D-721A (46.40 %) and there was statistically difference between all other treatments and the control (10.40 %). At 70 DAS, the severity on pod was higher (4.65 %) on MasalahaT and there was statistical difference between all other treatments and the control.

4. Discussion

Cowpea bacterial blight is a seed-borne disease (Cafati and Saettler, 1980) and seed treatment is an effective method of controlling seed-borne diseases (Sikirou, 1999). But the result of this work showed that all the fungicides used for the treatment did not have significant effect on the pathogen. These results confirm the report of McMullen and Lamey (2000) and Shenge (2007) that most fungicides do not control bacterial pathogens and most will not control all types of fungal diseases. The seed treatment chemical must be pest specific. The percentage germination of control treatments was lower (60 %) than the other treatments. This was as a result of soaking control seeds in ethanol for 20 minutes. Gilbert and Peaden (1987) and Taormina *et al*. (1999) reported that ethanol was very effective in killing naturally occurring microorganisms in/on seeds but it inhibits seed germination. The variable effect of *Xav* on the seedling establishment may be partly due to variation in the location of the pathogen on the seeds (Shoaga, 1996). The internally located pathogen (especially on the embryo can cause seedling mortality while the pathogen located on the surface of the seed will contaminate the soil (Murty and Devadath, 1984). Similarly the variability in disease incidence, severity and population of *Xav in vivo* could be attributed to the differential susceptibility of the different varieties. Varietal susceptibility or other wise of cowpea to bacterial blight have been reported (Singh and Monoz, 1999), while Shoaga (1996) reported the variability in pathogenicity of different isolates of *Xav* and Okechukwu *et al*. (2010) reported the variability in pathogenicity of different isolates of *Xav* due to different agro ecological differences. From (Table 1), there was no clear correlation between initial pathogen population and disease incidence and severity. The result corroborate the report Hirano and Upper, (1983) that many pathogenic bacteria multiply quite successfully in association with susceptible tissues without causing lesions .Spread of the pathogen often occur independent of lesion occurrence (Upper *et al*., 2003) and when pathogen population remain modest in size, host and pathogen coexist without disease..

Lesions on the other hand, occur when extensive multiplications require the bacterial to rely on expression of T3SS (Darsonval *et al*., 2003). In compatibles interactions, defense responses are suppressed by T3SS effectors (DebRoy *et al*., 2004; Boureau *et al*., 2006). This result corroborate the report of Rouse *et al*. (1985) that as population sizes increase, so does the probability of disease development. While the disease incidence remain fairly constant, disease severity steadily declined at 28 and 42 DAS. There was general increase in flower abortion from 42-49 DAS and there was no apparent varietal difference in CoBB effect on flower. Similar results were also observed in pod abortion. Most yield losses, however, presumably result from high flower and pod abortion (Goodwin, 1992). The result of this work is completely at variance with the report of Leith and Reynolds (1988) that the effect of a phytophagous pest on plant growth was considered to be equivalent to the amount of defoliation it caused, that is reduction in radiation interception (RI). However, this will not be appropriate for bacterial blight of cowpea, because photosynthesis is reduced by more than merely a loss of radiation interception (RI) due to blight lesions (visibly destroying green leaf tissue) but reduce radiation use efficiency (RUE) (Goodwin, 1982; Upper *et al*., 2003). Similar to other bacterial leaf spot pathogens, *Xav* may colonize cowpea plants without inducing visibly symptoms. Symptomless systemic infection of cowpea could also result in seed contamination in absence of foliar symptoms (Barak *et al*., 2002). In such case, field

inspection of seed crops would fail to reveal this source of seed infection.

5. Conclusion

Fungicides used for the treatment of seeds had no significant effect on the pathogen growth and pathogenicity. Although, seed treatment has been adjudged to be the best method of control soil and seed-borne diseases, seed treatment chemical must be pest specific. Population build up did not correlate to foliar symptoms (lesion) developments. Visibly lesion free cowpea field is not a guarantee to get bacterial free seed especially for the seed companies. Thus, it appears advisable to perform seed assays to confirm that seed lots are not contaminated with *Xav* particularly if such seed lots are to be planted in areas where conditions are favorable for development of bacterial leaf blight disease. The usual practice of taking disease incidence and severity should be discouraged. I would recommend pathogen population assessment should be undertaken in conjunction with disease incidence not disease severity. Experiment to evaluate cowpea resistance genotype should be conducted on cowpea plant from germinating to production stage since resistance varies from vegetative to reproduction stage (flower and pod abortion).

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Table 1: Incidence, Severity and Population build-up of *Xav* on Germinating Cowpea Seedling 14 DAS in 2011 Two Trials Combine Analysis

Source/Variety	Initial Population	Germination %	Establishment %	Incidence %	Severity	Population cfu/ml
Alheri A	$1.3x10^{5}$	65.0c	56.30f	27.7j	2.0i	$2.0x10^{8}$j
Premier D	$1.0x10^{5}$	70.0b	58.40c	50.0i	2.4e	$2.0x10^{8}$j
Masalaha T	$1.3x10^{5}$	65.0c	50.0h	50.0i	2.1h	$2.9x10^{8}$g
IT86D-721A	$1.3x10^{5}$	70.0b	51.0g	49.8j	2.4e	$3.2x10^{8}$f
IT98K-503-1T	$1.0x10^{5}$	75.0a	57.0e	52.1g	2.1h	$3.4x10^{8}$e
Local W	$1.9x10^{5}$	70.0b	58.3d	64.0b	2.3f	$3.5x10^{8}$d
Local SG	$1.4x10^{5}$	70.0b	57.0e	63.4c	2.3f	$2.3x10^{8}$h
Local S	$3.3\ x10^{5}$	70.0b	57.0e	67.3a	2.2g	$2.2x10^{8}$i
SAMPEA-7A	$2.7\ x10^{5}$	70.0b	43.0f	52.4f	4.6b	$4.5x10^{8}$a
SAMPEA-7T	$3.1\ x10^{5}$	70.0b	58.3d	50.0i	4.8a	$3.3x10^{8}$e
SAMPEA-7D	$1.0\ x10^{5}$	65.0c	57.0e	50.3h	4.3d	$3.2x10^{8}$g
SAMPEA-7I	$4.5\ x10^{7}$	65.0c	57.3e	62.4e	4.4c	$3.8x10^{8}$b
Ife-brown I	$4.5\ x10^{7}$	70.0b	64.0b	62.9d	4.3d	$3.6x10^{8}$c
SAMPEA-7C	0.00	60.0d	100a	0.00k	0.0j	0.00k
S.E		0.74	0.93	0.84	0.92	0.91

Means in a column followed by the same letter are not significantly different at $p \leq 0.05$ level of significance using NDMRT test.

Table 2: Flower and Pod aborted and Severity of *Xav* on Pod in 2011 Two Trails Combine Analysis

	% Flower Aborted	% Pod Aborted	Severity on Pod		
Source/ Variety	**42**	**49**	**56**	**63**	**70 DAS**
Alheri A	20.60l	30.65j	53.95j	43.65h	4.05g
Premier D	24.85h	30.65j	60.15a	42.95k	4.00h
Masalaha T	29.00c	33.60c	59.10b	45.90c	4.65a
IT86D-721A	23.85 i	32.10g	57.45d	46.40a	4.60b
IT98K-503-1T	29.85a	31.10i	54.45i	45.60d	4.20f
Local W	24.30k	32.20f	54.60h	43.05j	4.25d
Local SG	24.95h	31.40h	55.60g	45.10e	3.85j
Local S	25.45g	32.80d	55.60g	43.30j	4.05g
SAMPEA-7A	29.10b	34.10b	57.75c	44.35f	3.95i
SAMPEA-7T	26.20f	31.40h	55.60g	43.45i	4.21e
SAMPEA-7D	26.60e	33.60c	56.15e	46.20b	4.30c
SAMPEA-7I	27.10d	53.10a	55.75f	43.80g	4.00h
Ife-brown I	24.40j	32.45e	54.45i	42.25l	4.20f
SAMPEA-7C	3.50m	5.40k	10.10k	10.40m	0.00k
S.E	0.70	0.67	1.22	1.34	1.37

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

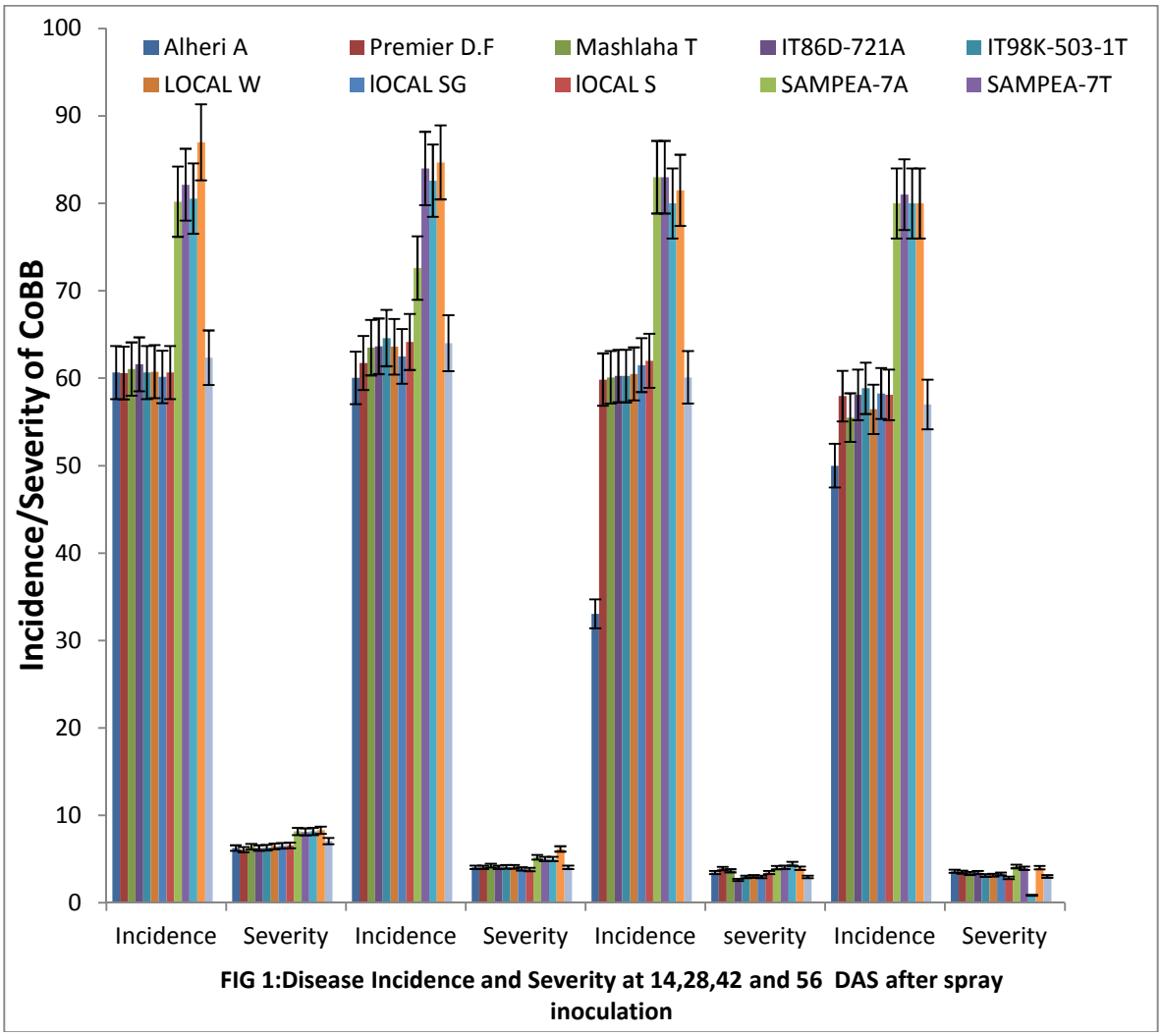

FIG 1:Disease Incidence and Severity at 14,28,42 and 56 DAS after spray inoculation