

## Comparative virulence of bacterial blight (*Xanthomonas campestris* pv. *vignicola*) isolates and their effect on yield components of cowpea (*Vigna unguiculata*)

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### ABSTRACT

The virulence of Ibadan, Ikenne and Kano isolates of bacterial blight pathogen and their effect on yield components were compared in cowpea cultivars Ife Brown, TVx 3236 and 86D-721 artificially inoculated in the field. The isolates differed significantly ( $p \leq 0.05$ ) in their virulence in each cultivar causing 6.3 - 33.8% disease incidence with associated disease severity ratings of 1.4 - 9.4. A significant area under the disease progress curve (AUDPC) was associated with each isolate/cultivar combination. The isolates caused significant reductions of 23.6-56.5% in number of pods/plant across the cultivars. Significant reductions of 7.6-13.8% and 12.1 - 20.8% were also recorded for pod length and number of seeds/pod, respectively. Yield depressions of 62.4%, 25.8% and 36.5% in Ife Brown; 64.9%, 13.1% and 29.4% in TVx 3236; and 62.3%, 5.4% and 38.1% in 86D-721, were associated with infections by Ibadan, Kano and Ikenne isolates of bacterial blight, respectively. Overall, Ibadan isolate was more virulent than Kano and Ikenne isolates which had comparable virulence in each of the cultivars.

**Key words:** Virulence, *Xanthomonas campestris* pv. *vignicola*, cowpea, disease severity, yield

### INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp is a cheap and major source of protein for the urban and rural population of West Africa (Bressani 1985; Alghali 1991). It is cultivated in about 12.5 million hectares with an annual production of over 3 million tonnes worldwide (FAO 1996), with Nigeria accounting for 70% of the world's production (Balde *et al.* 1997).

Cowpea production is constrained by a number of factors. Yield reduction and crop failure occur when the rains end too soon (Raheja 1986), or when there is heavy rainfall (Jain and Mehra 1980), and competition with weeds (Emechebe *et al.* 1991). In addition insect attack (Jackai and Daoust 1986) and diseases caused by fungi, bacteria and viruses (Patel 1985) may cause considerable yield reductions.

Cowpea bacterial blight caused by *Xanthomonas campestris* pv *vignicola* (Burkholder) Dye is of economic importance (Prakash and Shivashanker, 1982); the disease can cause complete defoliation of susceptible cowpea cultivars in heavy epiphytotic (Ekpo 1978; Emechebe and Shoyinka 1985) and pre-and post-emergence seedling mortality (Kishun 1989). There is extensive information on ecological and pathological effects of the pathogen but literature is lacking on effects of the pathogen on cowpea yield, in response to infection by different bacterial isolates from different agro-

ecologies. This study compared the virulence of three cowpea isolates of bacterial blight from different ecological zones, and their effect on yield components of three cowpea cultivars artificially inoculated in the field.

### MATERIALS AND METHODS

Seeds of three cowpea cultivars namely: Ife Brown, TVx 3236 and 86D-721 and *Xanthomonas campestris* pv *vignicola* isolates from Kano, Ikenne and Ibadan were obtained from International Institute of Tropical Agriculture, Ibadan, Nigeria. The isolates were sub-cultured and maintained on nutrient-calcium carbonate agar at 28 °C. Bacterial suspensions were prepared from 24 hour cultures and adjusted to  $10^8$  colony forming units per ml (CFU ml<sup>-1</sup>).

The experiment was conducted in a 32m x 12m plot at the University of Ibadan Teaching and Research Farm on a site used for cowpea breeding trials in the early planting season (March-July, 1997). The experimental design was a split-plot randomized complete block with three replications. Cowpea varieties were the main plots and the sub-plot treatments were the bacterial isolates. The sub-plots were 3m long and consisted of 4 rows spaced 60 cm apart. Seeds were sown on September 5, 1997 at the rate of two seeds per hole at in-row spacings of 30 cm. Seedlings were thinned to one per hole, 7 days after emergence. Plots were weeded manually 3 and

**Abbreviations:** AUDPC: Area under disease progress curve, CFU: Colony forming units

8 weeks after seedling emergence. Plants were sprayed twice with Cymbush ( $2\text{g l}^{-1}$ ) at three weeks interval after flowering to protect against insect damage.

Test plants of each cowpea cultivar were spray-inoculated separately, three weeks after seedling emergence with Kano, Ikenne and Ibadan isolates of *X. campestris* pv. *vignicola*. The second and third trifoliolate leaves of each plant were held against the palm and inoculated on the abaxial surface from distance of about 2 cm using a plastic sprayer. The treatment produced visible water-soaked spots.

The plants were rated for disease incidence and severity 28 days after inoculation and subsequently at 7 day intervals for 4 weeks in the two middle rows of each plot. The rating was based on visible diagnostic symptoms which were necrotic lesions surrounded by yellow halos. To determine the disease incidence, all plants in the two middle rows of each plot were examined for the presence of symptoms. Disease severity was scored on the first three trifoliolate leaves above the primary leaves of ten plants randomly selected from the two middle rows of each subplot. The disease severity was rated on a scale of 1-5 where, 1 = no symptoms, 2 = visible necrotic lesions with halos on 10% of leaf area affected, 3 = symptoms on 10-30% of leaf area, 4 = 31-50% of leaf area affected, and 5 = > 50% of leaf area affected. The area under the disease progress curve (AUDPC) was calculated for each subplot based on disease index, a product of disease incidence and severity ratings using the formula:  $\text{AUDPC} = \sum_{i=1}^n (X_i + 1 - X_{i+1}) (t_{i+1} - t_i) / 2$ , where  $x_i$  = disease index at the  $i^{\text{th}}$  observation,  $t_i$  = time (days) at the  $i^{\text{th}}$  observation, and  $n$  = total number of observations (Shaner and Finney 1977).

At maturity, all pods were harvested manually from the two middle rows of each plot. The number of pods per plant was determined from three replicate counts of pods on 20 plants selected at random from each sub-plot. Twenty pods selected at random from each sub-plot were used to determine mean pod length and the number of seeds per pod.

Pods were threshed by hand, 100 seeds selected at random and their weight was measured. Total seed yield from each subplot was converted to yield per hectare.

The data were subjected to analysis of variance (ANOVA), and the means were compared using Duncan's Multiple Range Test at 5% level.

## RESULTS

The bacterial isolates were pathogenic in all tested cowpea cultivars but showed significant virulence variation on individual cultivars. Disease incidence varied significantly ( $p \leq 0.05$ ) among cowpea cultivars (Table 1). Disease Incidences of 33.8-60.5% associated with Ibadan isolate of bacterial blight were significantly ( $p \leq 0.05$ ) higher than those recorded for Kano isolates (7.6-20.3%) and Ikenne isolates (6.3-17.9%) in comparable cowpea cultivars. Kano and Ikenne isolates were associated with similar disease incidences in each of the cultivars. Natural infection in uninoculated control plants gave rise to 1.7%, 2.4% and 1.6% disease incidences in Ife Brown, TVx3236 and 86D-721, respectively (Table 1).

Disease severity varied significantly ( $p \leq 0.05$ ) among cultivars (Table 1). In all cultivar/isolate combinations, severity scores associated with Ibadan isolate (6.7-9.4) were significantly higher than those associated with either Kano (1.2-3.5) or Ikenne (1.4-3.5) isolate of bacterial blight. There were no significant differences in disease severity scores associated with Kano and Ikenne isolates. The disease severity scores of 0.1-0.3 recorded for natural infection in uninoculated plants were significantly ( $p \leq 0.05$ ) lower than those associated with each of the bacterial isolates in comparable cultivars.

The AUDPCs, differed significantly ( $P \leq 0.05$ ) among cultivars in different isolate/cultivar combinations (Table 1). In Ife Brown, Tvx3236 and 86D-721, the AUDPCs associated with Ibadan isolate were 197.7, 142.7 and 163.5, respectively. These AUDPCs were significantly ( $P \leq 0.05$ ) higher

Table 1. Effect of *X. campestris* pv. *vignicola* isolates on cowpea bacterial blight incidence, severity and AUDPC in the field.

Bacterial Isolates	Disease incidence*			Disease severity**			AUDPC***		
	Ife Brown	Tvx3236	86D-721	Ife Brown	Tvx3236	86D-721	Ife Brown	Tvx3236	86D-721
Ibadan	51.7a	60.5	33.8b	9.4a	6.7b	7.7ab	197.7a	142.7b	163.5ab
Kano	20.3c	7.6de	15.9c	3.4cd	1.2d	3.5c	51.4cd	27.2ef	75.3c
Ikenne	10.7d	6.3de	17.9c	2.0d	1.4d	3.5c	42.8de	25.3ef	74.7c
Control (Natural Infection)	1.7e	2.4e	1.6e	0.3e	0.2e	0.1e	6.6f	3.5f	1.6f

\*Percentage of diseased plants in the two centre rows of each plot. Mean of three replicates.

\*\*Percentage of diseased leaf area on the first three trifoliolate leaves of 10 randomly selected plants. Mean of three replicates.

\*\*\*AUDPC = Area under the disease progress curve

Means with the same letters in each parameter are not significantly different according to Duncan's Multiple Range Test ( $P \leq 0.05$ ).

Table 2. Effect of inoculation of cowpea cultivars with *Xanthomonas campestris* pv *vignicola* isolates on seed yield and components.

Bacterial Isolates	Pod plant <sup>-1</sup>			pod length (cm)			Seed pod <sup>-1</sup>			100 seed weight (g)			Seed yield (kg ha <sup>-1</sup> )		
	Ife	TVx	86D-721	Ife	TVx	86D-721	Ife	TVx	86D-721	Ife	TVx	86D-721	Ife	TVx	86D-721
	Brown	3236		Brown	3236		Brown	3236		Brown	3236		Brown	3236	
Ibadan	5.2e (27.8*)	5.4e (56.5)	5.1e (53.3)	9.2h (9.8)	10.8ef (9.2)	11.2de (13.8)	5.7h (20.8)	9.9bc (14.7)	7.3efg (18.9)	10.1b	8.9b	10.6b	47.7h (62.4)	147.1f (64.9)	126.3f (62.3)
Kano	5.0e (30.6)	6.5cd (47.6)	6.8cd (40.4)	9.9g (2.9)	10.9e (8.4)	12.5ab (3.8)	6.9fg (4.2)	10.2b (12.1)	8.3de (7.8)	9.8b	9.0b	11.2a	94.2g (25.8)	364.3b (13.1)	317.1c (5.1)
Ikenne	5.5e (23.6)	6.0de (51.3)	5.9de (48.2)	10.0g (2.0)	11.0e (7.6)	11.7cd (10.0)	6.4gh (11.1)	9.4bc (19.0)	7.6ef (15.6)	12.4ab	9.0b	11.8ab	80.7g (36.5)	295.9d (29.4)	207.6e (38.1)
Control	7.2c	12.4a	11.4b	10.2fg	11.9bc	13.0a	7.2efg	11.6a	9.0cd	14.2ab	11.5ab	16.1a	127.0f	419.2a	3353bc

\*Figures in parentheses are percentage reduction in respective parameters

Means with the same letters within each parameter are not significantly different at  $P < 0.05$  using Duncan's Multiple Range Test.

than those recorded for Kano isolate (51.4, 27.2 and 75.3) and Ikenne isolate (42.8, 25.3 and 74.7) in Ife Brown, TVx3236 and 86D-721, respectively. A significantly ( $P \leq 0.05$ ) lower AUDPC (1.6-6.6) was recorded for naturally infected cultivars compared to artificially inoculated plants. Overall, the virulence of the isolates was in the order Ibadan > Kano > Ikenne while the susceptibility of cultivars was in the order Ife Brown > 86D-721 > TVx3236.

The number of pods/plant in uninoculated control plants was significantly ( $p \leq 0.05$ ) higher than those of inoculated plants in all tested cultivars (Table 2). In Ife Brown, comparable percentage reductions of 27.8, 30.6 and 23.6 in pod number were associated with Ibadan, Kano and Ikenne isolates, respectively. In TVx3236, percentage reduction of 56.5% in pod number caused by Ibadan isolate was significantly ( $P \leq 0.05$ ) higher than comparable percentage reductions of 47.6% and 51.3% caused by Kano and Ikenne isolates, respectively. In cultivar 86D-721, a reduction of 53.3% in pod number associated with Ibadan isolates was similar to 48.2% reduction caused by Ikenne isolates but significantly ( $p \leq 0.05$ ) higher than 40.4% reduction recorded for kano isolates.

The effect of the bacterial isolates on pod length varied with the cultivars. Significant reductions were recorded for Ibadan isolate in Ife Brown, all isolates in TVx3236, and Ibadan and Ikenne isolates in 86D-721. In general, the reductions in pod length varied from 2.0-13.8% across the cultivars. Significant percentage reductions in number of seeds/pod, were recorded for Ibadan isolate in Ife Brown (20.8%), all isolates in TVx3236 (12.1-19.0%) and Ibadan and Ikenne isolates in 86D-721 18.9% and 15.6, respectively (Table 2).

One hundred seed weights of Ife Brown and TVx3236 inoculated with each of the bacterial isolates were similar and slightly lower than that of uninoculated plants. Also in 86D-721, 100 seed weight of plants inoculated with Kano and Ikenne isolates was similar to that of control plants. However, a significant reduction of 34.1% in 100 seed weight was recorded for Ibadan isolate in 86D-

721 (Table 2).

Seed yields were generally low and varied significantly ( $p \leq 0.05$ ) among isolate/cultivar combinations. Seed yield in inoculated cultivars varied from 47-94 kg/h in Ife Brown, 147-364 kg/h in TVx3236, and 126-317 kg/h in 86D-721. The three isolates caused significant ( $p \leq 0.05$ ) yield depressions across the cultivars, the highest being associated with Ibadan isolate. On the average, Ibadan isolate induced yield depressions of 62.4%, 64.9% and 62.3% in Ife Brown, TVx3236 and 86D-721, respectively. Kano isolate caused yield depressions of 25.8%, 13.1% and 5.4% in Ife Brown, TVx3236 and 86D-721, respectively. Ikenne isolate was associated with yield depressions of 36.5%, 29.4% and 35.1% in Ife Brown, TVx3236, and 86D-721, respectively.

## DISCUSSION

The cowpea cultivars varied in their response to artificial infection with Ibadan, Ikenne and Kano isolates of bacterial blight, thus indicating the existence of virulence variation among isolates of bacterial blight. The blight incidence varied from 6.3% in the least compatible TVx3236/Ikenne isolate combination to 60.5% in the most compatible TVx3236/Ibadan isolate combination. In all cultivars, the virulence of Ikenne and Kano isolates was similar and significantly ( $P \leq 0.05$ ) lower than that of Ibadan isolate. The observed variation was similar to pathogenic variation reported in isolates of *Xanthomonas campestris* pv. *phaseoli* (E.F. Smith) Dows. in beans (Ekpo and Saettler 1976).

A significant ( $P \leq 0.05$ ) variation in AUDPC was also observed among cultivars and was cultivar/isolate combination dependent. In general, the AUDPCs associated with cultivars infected with Ibadan isolate were significantly ( $P \leq 0.05$ ) higher than those recorded for infections with Kano and Ikenne isolates in corresponding cultivars. In all cases, artificial inoculations with each of the isolates gave rise to significantly higher disease incidence, disease severity, and AUDPC compared to natural

infection, thus confirming the success of water-soaking spray-inoculation procedure and the effectiveness of insecticide, Cymbush in reducing spread of bacteria from inoculated to uninoculated plants.

The adverse effect of bacterial infection was generally more pronounced on number of pods/plant and number of seeds/pod than on pod length and 100 seed weight. The significant reductions of 27.8%, 30.6% and 23.6% in number of pods/plant associated with Ibadan, Kano, and Ikenne isolates, respectively, were similar to the 30% reduction reported for Ife Brown in a related study (Ekpo 1982). Also, the yield reduction of 26.4% recorded in Ife Brown (Ekpo 1982) was similar to the 25.8% yield loss reported herein for infection by Kano isolate but lower than 62.4% and 36.5% losses associated with infection by Ibadan and Ikenne isolate of bacterial blight in the same cultivar. Overall, our observed yield depressions of 5.4-64.9% across different cultivar/isolate combinations fell within the range 2.7-92.2% reported by Prakash and Shivashanker (1982).

In general, the susceptibility ranking of cultivars was in the order Ife Brown > 86D-721 > TVx3236 while the yield potential was in the order TVx3236 > 86D-721 > Ife Brown. The observed susceptibility of the cowpea cultivars to bacterial blight disease in artificial infection was similar to the variation in response of cowpea cultivars to natural infection reported by Ekpo (1997a, 1997b).

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