SURVIVAL OF *XANTHOMONAS CAMPESTRIS* PV. *VIGNICOLA* IN INFESTED SOIL, COWPEA SEED AND COWPEA DEBRIS

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ABSTRACT

Field and laboratory experiments were conducted in the Sudan savanna site of IITA's experimental Station, Minjibir, Kano State, to study the survival of *Xanthomonas campestris* pv. *vignicola* in infested soil, cowpea seed, and debris. The experiment was conducted in a randomised complete block design with five cowpea varieties over three cropping seasons (from 1996 to 1998). The results showed that the bacterium survived in infected plant debris, seed, and soil but after 8 months off-cropping season, the bacterium was not recovered from the soil. This implies that seeds and plant debris are the major means by which *X. campestris* pv. *vignicola* survives through seasons.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important grain legume throughout the tropics and subtropics. Cowpea is eaten in the form of grain, green pods, and leaves (Adejumo, 1997). The roots are eaten in Sudan and Ethiopia, and the peduncles and stems are used as fibres in Nigeria (Adejumo, 1997).

Cowpea production is constrained by bacterial blight caused by *Xanthomonas campestris* pv. *vignicola* (Burkholder), other problems from insects and fungal diseases that often affect the plant throughout its life cycle and the seed in storage (Singh and Allen, 1979; Emechebe and Shoyinka, 1985).

Programmes for the control of the bacterial pathogen may be designed to reduce the multiplication rate at the site of infection on the host plant or to eliminate the inoculum at its source. Control measures that affect pathogens at primary sources are more effective when the rate of multiplication of the pathogen is low, rather than high (Green, 1994). To control a pathogen at its source, it is necessary to ascertain the relative importance of the source in relation to the survival of the pathogen and the initiation of epidemics. The contributions of cowpea debris and bacteria-infested soil to the carry-over of bacterial blight inoculum across seasons are an aspect that has not been scientifically investigated to quantify the contributions of each of these sources of inoculum to the incidence of bacterial blight disease of cowpea.

The objective of this study, therefore, was to study the survival of *Xanthomonas campestris* pv. *vignicola* in infested soil, cowpea seed and plant debris to ascertain the relative importance of each

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source of inoculum to disease carry-over from season to season.

MATERIALS AND METHODS

Three field experiments were conducted at the IITA's experimental research station in Minjibir, Kano State, 1996–1998. Minjibir is in the arid/semi-arid agroecology, longitude 08° 31' E, latitude 12° 03' N, altitude of 1500 masl (Kowal and Knabe, 1972). The soil is coarse textured, 11.2% clay, 76.5% silt, 7.9% fine sand, 4.1% coarse sand, pH $CaCl_2$ (3.95), organic carbon (0.14%), total N (0.02%), exchangeable Ca (0.80 Meq/100g), Mg (0.25 Meq/100); K (0.11 Meq/100g), Na (0.03 Meq/100g), and effective CEC (1.34 Meq/100g).

The experiment was conducted in a randomised complete block design with two replications. Five cowpea varieties were used, two susceptible to bacterial blight (IAR-48 and IT82D-889), two moderately susceptible (IT81D-985 and IT92KD-357-2), and one resistant (IT93K-2332-16). The resistant variety was used at two levels. In each replication there was a Macuprax®-sprayed IT93K-2332-16 (identified as IT93K-2332-16Tr) and a non-sprayed -IT93K-2332-16. Macuprax®, is a copper-based bactericide (2 g/l of SDW); the active ingredient is Copper sulphate (Adegbola and Filani, 1982). Macuprax® was introduced to prevent infection and depict a truly resistant variety. Spray regimes on the resistant variety were at 7, 15 and 22 days after seedling emergence.

In each year (1996–1998) the same methods were used for land preparation (manual weeding and ridging). Fertilizer application was N:P:K 15:15:15 at 200 kg/ha. The total land area was 1530

m^2 and consisted of four fields, with 12 plots of 20.25 m^2 each. A 1-m alley on all sides separated the plots and were sown with three rows of millet on 9 July 1996, 1 July 1997, and 7 July 1998. The millet was sown to prevent inter-plot interference.

Seeds of test varieties were manually (three per station) sown on 24 July 1996, 15 July 1997, and 21 July 1998 at about 2.5 cm depth at in-row spacing of 0.25 m. A mixture of pre-emergence herbicide (Gramoxone® + Galex®) was applied immediately after planting at a concentration of 100 ml herbicide /20 L water at a rate of 250 g a.i./ha. Subsequent weed   control was carried out manually at 3-week   intervals after plant emergence. A week after emergence, seedlings were thinned to two/ station (36 plants/row; 216 plants/plot).

Each year, all test seedlings except IT93K-2332 -16Tr were artificially inoculated with *X. campestris* pv. *vignicola* (10^6 CFU/ml), 14 days after emergence by spraying the abaxial surface of two youngest fully expanded trifoliate leaves (4-leaf-stage) until water-soaked spots were obtained, using a hand-operated atomiser. IT93K-2332-16Tr was sprayed with sterile distilled water (SDW). There were two sprays at 2-week intervals of Sherpa plus® (a combination of Dimethoate and Cypermethrin) at a concentration of 80 ml of insecticide/20 L water at a rate of 250 g a.i./ha using a knapsack sprayer against attack by flower thrips (*Megalurothrips sjostedti*) and pod borers (*Maruca testulalis*).

At maturity, all pods were manually harvested from the two centre rows of each plot and left to sun-dry for 5 days, then manually threshed. The seeds collected per plot were subjected to bacterial seed test in the laboratory.

At the end of 1996 and 1997 experiments, markers were put in the field to enable the exact positions of the plots to be traced in the next season. Soil and debris samples were   collected randomly from five stations in each plot for laboratory analysis. A quadrat 75 x 25 cm was marked around each of the five stations, so that each quadrat had as its centre the spot where the plants stood. The plants in each quadrat were pulled out by hand. Debris (stems, leaves, and pods) on the soil surface within each quadrat was collected. With the aid of a hand trowel, the topsoil in each quadrat was taken to the depth of 1 cm. The soil from each of the five quadrats within a plot was bulked in a polythene bag and rolled and shaken vigorously to create a bulk sample. From this sample, two aliquots (80 ml cup) of soil were taken. A month before the next planting season began, soil and debris samples were also collected from each plot for laboratory analysis.

Bacterial colony counts in seed samples: To ascertain the quantity of bacterial blight pathogen in the seedlot collected from the field, washing method assay and direct plating method were used (Klement *et al.*, 1990).

Fifty seeds from each plot were placed in sterile conical flasks and 100 ml of SDW was added. The flasks were mechanically shaken vigorously for 10 min. A 10-fold serial dilution of the suspension was thereafter carried out. Ten µl dilution of 10^{-8} was plated using the pour plate technique on Difco® Nutrient Agar. The plates were incubated at 30 °C for 48 h. Colony counting was done by means of a Gallenkamp colony counter. Calculation of colony forming units (CFU) per ml of sample was based on the formula:

$$\text{CFU/ml of sample} = \text{no. of colonies x dilution factor x 100}$$

The direct plating method involved surface sterilization of fifty seeds from each of the plots in 5% NaOCl for 5 min. The seeds were then plated on nutrient agar and incubated at 30 °C for 48 h.

In both methods, only round, smooth, entire, domed, and yellowish bacterial colonies were counted (Klement *et al.*, 1990). The number of bacterial counts per sample was log transformed using (log x+1) because of normalisation (Gomez and Gomez, 1984). Transformed data were then subjected to analysis of variance using SAS (1996) statistical software, version 6.12.

Bacterial colony counts in soil samples: A preliminary experiment was undertaken to determine the optimum dilution level of soil and debris suspension concentration required to give distinct colonies of bacteria when soil suspension or debris suspension was plated onto media. Suspensions were prepared of 1 g, 2 g, and 5 g of soil in 10 ml of SDW in test tubes. From each of the stock suspensions, a 10-fold dilution series was carried out.

From each dilution (10^{-1} to 10^{-10}), 10 µl was pipetted aseptically and transferred in triplicate onto dry nutrient agar in Petri dishes. The suspension was spread on the surface of the agar by means of a sterile bent glass rod and allowed to dry before the plates were sealed with parafilm. Plates were incubated at 30 °C. The plates were placed upside down during incubation to prevent contamination of the culture by condensation moisture. Plates were examined after 24 h. Only round, smooth, entire, and yellowish bacterial colonies were counted. Colony counting was done by means of a Gallenkamp colony counter. Dilution of 10^{-8} from the suspension of 1 g of soil in 10 ml SDW gave consistent bacteria colony counts of between 10 and 30 colonies and was chosen to be used in the full bacterial population counts in soil samples.

Bacterial colony counts in debris samples: Cowpea debris consisting of leaves, stems, pods, and seeds was ground into powder using a blender. Stock suspensions in SDW were prepared: 1 g of debris in 10 ml; 1 g of debris in 20 ml; 1.5 g of debris in 20 ml; and 0.5 g of debris in 20 ml. From each of the stock suspensions, a 10-fold dilution series was carried out. The stock suspension of 0.5 g of debris in 20 ml of SDW at dilution 10^{-8} was selected for bacterial colony count using Nutrient agar.

RESULTS

The populations of *X. campestris* pv. *vignicola* in debris, and soil at the end of 1996 planting season (Nov. 1996), beginning of the 1997 planting season (Jun. 1997), and the end of the 1997 planting season (Nov. 1997) are shown (Table 1). Varying populations of *X. campestris* pv. *vignicola* were recorded in plant debris and soil under different cowpea varieties. The bacterial populations in debris ranged from 1.4×10^8 to 7.6×10^8 CFU in 1996 and from 1.8×10^8 to 8.0×10^8 CFU in 1997. In the soil, the population of *X. campestris* pv. *vignicola* was much lower than that in the   debris and ranged from 0.1×10^8 to 1.5×10^8 in 1996 and from 0.5×10^8 to 1.8×10^8 CFU in 1997 at the end of the season. During the off-season, at the beginning of 1997 planting season, after the first rains, a much lower   number of *X. campestris* pv. *vignicola* was detected. In the debris, the population ranged from 0.4×10^8 to 3.0×10^8. No detectable population was found in the soil.

There was a significant (P≤0.01) difference between populations of *X. campestris* pv. *vignicola* found at the end of the 1996 season (Nov. 96) and the beginning of the 1997 season (June 97) in both cowpea debris and soil. Similarly, there was also a significant difference (P≤0.01) in the bacterial population observed in cowpea debris between the beginning of 1997 season (June 97) and the end of the 1997 season (Nov. 97). Overall, in the soil and debris samples, bacterial populations were   signifi-

cantly higher (P≤0.01) at the end of the season than at the beginning of the season.

The population of *X. campestris* pv. *vignicola* found on seeds using the washing method ranged from 5.8×10^8 to 19.8×10^8 CFU/ml in 1996 and from 9.8×10^8 to 25.0×10^8 CFU/ml in 1997 (Table 2). Using the direct plating method of seed test, seed infection by *X. campestris* pv. *vignicola* was recorded in 12.3–28.5% of seeds harvested at the end of the 1996 growing season and in 17.5–42.5% of seeds harvested at the end of the 1997 season. There was a significant difference (P≤0.05) between the populations of bacteria found in seeds at the end of the 1996 season and at the end of 1997 season, using both the washing and direct plating assays. The highest bacterial population was obtained from IT82D-889 debris, seed, and the soil in which it grew. The lowest bacterial population was observed in variety IT93K-2332-16Tr.

DISCUSSION

Since *X. campestris* pv. *vignicola* does not form resting spores as do fungi (Coyne and Schuster, 1974) the inoculum source is very important in the survival of the bacteria. Nebane (1980) shares this opinion and adds that *X. campestris* pv. *vignicola* also depends upon its ability to escape adverse environmental conditions. It remains hypobiotic during the quiescent period in association with animate or inanimate agents.

X. campestris pv. *vignicola* survived in the soil, cowpea debris, and seeds. The longevity of survival in these media varied; at 8 months after harvest, the bacteria were not found in the soil. Since the bacterium was successfully isolated in this study from cowpea debris and seeds at the beginning of a new season, 8 months after the last season, but not from the soil, it implies that seeds and plant debris are the major means by which *X. campestris* pv. *vignicola* survives through seasons. Similar results have been reported by Schuster and Coyne (1975) who also isolated the bacterium from cowpea seeds and residue in Columbia. Vakili *et al*. (1975) made similar isolations in subtropical Texas.

Table 1. Populations of X. campestris pv. vignicola in cowpea debris and soil under different cowpea varieties.

	Population (x 10^8 CFU)					
	Nov 1996		Jun 1997		Nov 1997	
Variety	Debris	Soil	Debris	Soil	Debris	Soil
IT82D-889	7.63	1.50	3.00	0.00	8.00	1.75
IAR-48	3.88	0.88	1.63	0.00	6.50	1.25
IT81D-985	2.75	0.13	1.25	0.00	4.50	1.50
IT92KD-357-2	2.75	0.50	1.25	0.00	5.00	0.75
IT93K-2332-16	1.88	0.25	0.88	0.00	3.25	0.50
IT93K-2332-16Tr	1.38	0.13	0.38	0.00	1.75	0.50
LSD	1.59	0.65	0.68	0.00	3.27	1.04
CV	27.21	38.98	26.01	-	18.87	20.94

Table 2. Population of *X. campestris* pv. *vignicola* in cowpea seeds from different varieties

	Washing method (x 10^8 CFU)		Direct- plating method (%infection)	
Variety	Nov 1996	Nov 1997	Nov 1996	Nov 1997
IT82D-889	19.75	25.00	28.50	42.50
IAR-48	18.38	23.75	21.88	32.50
IT81D-985	12.63	16.00	16.88	22.50
IT92KD-357-2	12.63	13.75	19.38	22.50
IT93K-2332-16	10.50	11.75	17.25	20.00
IT93K-2332-16Tr	5.75	9.75	12.25	17.50
LSD	2.53	4.41	10.03	11.86
CV	15.87	15.51	11.59	14.70

Significantly higher ($P \leq 0.05$) bacterial populations observed at the beginning of the planting season in the seeds compared to the lower populations at the end of the previous season suggest that the pathogen thrived, multiplied, and had colonised most seed tissues during the interval between the end of the previous season and the beginning of the next planting season. Seeds thus, are the major source of bacterial blight inoculum carryover in fields. The ability of bacteria to survive in seeds influences seed transmission (Veena *et al.,* 1996).

In the seedlot tested in this study, 30% was infected with *X. campestris* pv. *vignicola*. When such seeds are planted, epigeal germination will favour transmission of the pathogen to aerial parts of the plant (Shekhawat and Patel, 1977; Kaiser and Ramos, 1979; Emechebe and McDonald, 1979; Nebane, 1980). Crop debris on or near the soil surface is also a favourable site for bacterial survival (Nebane, 1980). In this study, successful isolations of *X. campestris* pv. *vignicola* were made from 8-month-old cowpea debris located on the soil surface.

Seed and plant debris transmission of *X. campestris* pv. *vignicola* is important for the initiation of primary infections. Insect transmission and dispersal of inoculum by rain splash are implicated in secondary infections (Walker and Patel, 1964; Williams, 1975; Nebane, 1980).

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