

# The role of nonhost cereal seeds in the epidemiology of cowpea bacterial blight (*Xanthomonas axonopodis* pv. *vignicola* (Burkholder) Dye)

## Abstract

Cowpea (*Vigna unguiculata* (L) Walp.) production in Nigeria is mostly undertaken under mixed cropping systems with cereal crops. To determine the role of nonhost cereal seeds in the epidemiology of cowpea bacterial blight, farmer-saved maize, millet and sorghum seeds were collected from nine cowpea producing states and assessed for ability to harbor and transmit *Xanthomonas axonopodis* pv. *vignicola*. The pathogen was extracted consistently from millet seed samples from all the states, except Kebbi state at concentrations ranging from 0.2–3.3x10<sup>2</sup>CFU/mL–1. All maize and sorghum seed samples tested negative for the pathogen. By comparing direct seed plating, seed washing alone and seed blending pathogen extraction assays, it was found that pathogen extraction efficiency was significantly ( $P < 0.05$ ) enhanced when seeds were blended.

Emerging maize, sorghum, millet and cowpea seedlings harbored populations of the pathogen up to 3.3x10<sup>7</sup>CFU/mL–1 in the first week of seedling emergence. There was no significant variability in the pathogen population sizes on cowpea and nonhost seedlings, indicating that tissue colonization at this stage of growth was epiphytic. Pathogen population kinetics over a 49-day sampling period showed a gradual decline over time to levels that did not differ from those of untreated control in maize and sorghum. However, pathogen populations in millet tissues remained high on Day 49. *Xanthomonas axonopodis* pv. *vignicola* was transmitted systemically from inoculated cowpea, millet and sorghum (but not maize) seeds to seedlings, to harvested seeds at maturity. Susceptible cowpea plants grown together with the inoculated cereal crops were not cross-infected. The results of this study demonstrated that seeds of nonhost cereal crops could serve as passive reservoirs for *Xanthomonas axonopodis* pv. *vignicola*. Such epiphytic pathogen populations could serve as inoculum sources for bacterial blight in cowpea, leading to significant economic losses.

**Keywords:** *xanthomonas axonopodis* pv. *vignicola*, cowpea; bacterial blight, nonhost plants, seeds, sorghum, millet, maize, Nigeria

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## Introduction

Efficiency of transmission is an important requirement for pathogen fitness.<sup>1-3</sup> In their quest for success, pathogens use plant seeds as an efficient means for dissemination and survival.<sup>3-5</sup> Transmission through seeds occurs when pathogenic microorganisms successfully spread from infected or contaminated seeds to the emerging seedlings. Developing infected seedlings could become symptomatic within a few days or grow asymptotically for variable periods of time. In addition, pathogens could survive perpetually in association with infected plants, spreading from seeds to plants and from plants to seeds without causing symptoms.<sup>5,6</sup> Plant seeds could also harbor and disseminate a diverse microbial community that may include human pathogens like serovars of *Salmonella enterica* and *Escherichia coli*.<sup>7,8</sup> Some human pathogens establish themselves endophytically in plants and may be inherited from one generation of its host to the next.<sup>9-11</sup>

On the behavior of seed-inhabiting bacterial plant pathogens during seed germination, Hirano et al.,<sup>11</sup> reported that *Pseudomonas*

*syringae* pv. *syringae* did not behave like a parasite during bean seed germination, as its Hrp Type III secretion system (T3SS) did not seem to play a major role during seed germination and seedling colonization. According to Darrase et al.,<sup>1</sup> experiments to determine the efficiency of pathogen transmission from flowers to seeds, and from seeds to seedlings in incompatible, compatible and null pathogen–host combinations involving *Xanthomonas campestris* pv. *campestris*–bean, *Xanthomonas campestris* pv. *citri*–bean, and *Escherichia coli*–bean combinations, respectively, showed that bacterial population dynamics were similar in all three host–pathogen combinations, and that no defense responses were induced at seedling emergence. These results were similar to those of Dutta et al.,<sup>4</sup> who reported that *Acidovorax citrulli*, *Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomonas syringae* pv. *tomato*, *Xanthomonas euvesicatoria*, and *Pseudomonas syringae* pv. *glycinea* were transmitted efficiently in compatible, incompatible and null combinations with watermelon, tomato, pepper and soybean plants. Collectively, these reports demonstrated that bacterial pathogens could be transmitted efficiently

from flower-to-seeds, and from seeds to seedlings without associated defense response induction.<sup>1</sup> The reported transmission of plant-pathogenic bacteria to, from, and by nonhost seeds indicates that seeds of nonhost plants could be inoculum sources for important plant and human pathogens.<sup>1,12,13</sup> In view of this possibility, and in consideration of the cropping systems in Northwestern Nigeria, where cowpea is often cultivated in mixed-cropping systems, the current study aimed at determining the possible role of nonhost cereal crops as a reservoir for the cowpea bacterial blight pathogen in Nigeria. A quantitative assessment of the traditional cropping systems revealed the existence of up to 43 crop mixtures in the Sudan savanna of Nigeria with a millet-cowpea mixture being predominant, representing 22% of the fields.<sup>14</sup> Since plant seeds are reported to be colonized commensally by bacterial pathogens,<sup>1</sup> the possibility of *Xanthomonas campestris* pv. *vignicola* being carried on the seeds of maize, sorghum, and millet was examined in this study. Seed borne bacterial pathogens are of major concern globally as strategies to manage bacterial disease are limited and often ineffective.<sup>3</sup> Information on the transmission mechanisms of cowpea bacterial blight is critical to efforts aimed at developing effective strategies for controlling the disease.

## Materials and method

### Collection of nonhost seeds

Seeds of maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and foxtail millet (*Setaria italica* L.) were purchased from the open market in nine cowpea producing states in Nigeria. Three states were selected from each of North-Eastern, North-Central and North-Western Nigeria. In Borno state, seeds of the three crops were purchased from local farmers at Bama. Other locations where seeds were purchased were Akor (Gombe state), Mubi (Adamawa State), Duchin Kura (Sokoto State), Jega (Kebbi State), Saminaka (Kaduna State), Keffi (Nassarawa State), Mokwa (Niger State) and Idah (Kogi State). At each location, seeds of each crop weighing 2.5kg were purchased from local farmers. The purchased seeds came from farmer-saved stocks and had no visible disease symptoms.

### Detection of *Xanthomonas axonopodis* pv. *vignicola* on cereal grains

The occurrence of *Xanthomonas axonopodis* pv. *vignicola* (*Xav*) on maize, sorghum and foxtail millet seeds was determined using seed washing, seed plating and seed destruction assays.<sup>8,15</sup> For each crop, 400 seed-samples were drawn and washed thoroughly in running tap water to remove soil and debris. The washed seeds were surface-disinfected by soaking them in 2% sodium hypochlorite for three minutes, after which they were rinsed with sterile distilled water (SDW) and blended using a Philips blender model HR 2167/40 (Royal Philips, Amsterdam, The Netherlands). The paste was suspended in 250mL of 0.85% sterile saline solution, from which serial dilutions up to 10<sup>-5</sup> were prepared. From each dilution, 100μL were spread on two Petri-dishes containing Yeast Dextrose Carbonate Ager (YDCA) using a Drigalski spatula.<sup>16,17</sup> The plates were there after incubated at 27°C for 7days, after which colonies with typical *Xav* characteristics<sup>18</sup> were enumerated and selected for pathogenicity testing.

Putative isolates were tested for their Gram reaction, and other biochemical tests,<sup>19,20</sup> including Kovac's oxidase test,<sup>21</sup> arginine dihydrolase, catalase activity, gelatin hydrolysis, starch hydrolysis and levan production. Putative bacterial pathogens were tested for induction of a hypersensitive reaction in tobacco leaves,<sup>22,23</sup> and their

pathogenicity confirmed by spaying suspensions of the pathogens (standardized at 107 CFU/ml) on 35-day-old cowpea cv. Ife Brown.

### Washing assay for externally-borne pathogens

The occurrence of *Xav* on the surfaces of maize, sorghum and millet seeds was determined by randomly selecting 400seeds from each seed lot. The seeds were transferred into a 250mL flask containing sterile distilled water (SDW) and shaken on an orbital shaker at 250rpm for 5min, after which the rinsate was filtered through three layers of cheese cloth. The filtrate was dispensed into two 50-mL centrifuge tubes and centrifuge at 12,000g for 10min (approx. 10,000rpm) to pellet bacteria. One ml of the pelleted bacteria from each seed lot was transferred into McCartney bottles containing 9ml of SDW. From these, serial dilutions up to 10<sup>-5</sup> were prepared in SDW, from which 10μL were spread on Petri-dishes containing YDCA using a Drigalski spatula. The treatments were incubated at 28°C for 7days, after which colonies with typical *Xav* characteristics<sup>14</sup> were enumerated and selected for pathogenicity testing.

### Direct seed plating assays

Four hundred (400)seeds from each of cereal seed lots were washed with SDW, after which 10seeds were selected randomly and plated on YDCA in ten replicates. Seeds bearing *Xav* colonies growth were recorded as positive and those without *Xav* growth were recorded as negative. Colonies that were mucoid, yellow, Gram-negative and hydrolyzed starch on YDCA were selected for pathogenicity testing. The trial was repeated twice.

### *Xanthomonas axonopodis* pv. *vignicola* population kinetics in cowpea and cereal seedlings following seed inoculation

Four hundred (400) seeds from each of the cereal seed lots and cowpea (cv. Ife brown) were inoculated with *Xav* by soaking them in a suspension of the bacteria adjusted to ca. 4.5x107CFUml<sup>-1</sup> for 4h. After 4h of soaking, the bacteria suspension was drained out and three seeds from each treatment in a 25cm-diameter pot filled with heat sterilized soil. Emerging seedlings were watered as required and fertilized following fertilizer requirements for maize, sorghum, foxtail millet and cowpea. Seeds soaked in SDW for 4h served as control. Leaf and stem discs measuring ca. 2mm<sup>2</sup> were taken from the seedlings at 7, 14, 21, 35 and 49days after inoculation, using a cork borer. The discs were teased apart in 1-2drops of SDW, from which a loop ful was transferred into 1 mL of SDW, from which serial dilutions up to 10<sup>-5</sup> were made and plated on YDCA. Emerging *Xav* colonies were enumerated after 48 h. The experiments were laid out on a bench in the screen house using a completely randomized design with three replications. Data collected were analyzed statically using ANOVA models in MSTAT-C and treatment means separated using Student-Newman-Kuels (SNK) tests. The trials were repeated twice.

### Transmission of *Xanthomonas axonopodis* pv. *vignicola* to nonhost seeds via vascular and floral systems

Direct infection of seeds by *Xanthomonas axonopodis* pv. *vignicola* through the vascular system: Direct systemic infection of seed by *Xav* was investigated using artificial inoculation methods, followed by grow-out assays and seed sampling. The cereal seeds were divided into 3 portions of 40seeds each. One of the three portions was soaked in a *Xav* suspension adjusted to ca. 4.5x107CFU/ml-1 before planting. The second portion of seeds was spray-inoculated with a bacterial suspension adjusted to ca. 4.7x107cfu ml<sup>-1</sup> at 25days after sowing,

while third portion was soaked in sterile distilled water and served as control. Three seeds from each of the three seed portions were planted in 25cm-diameter pots filled with sterilized soil. Emerging seedlings were thinned to two plants per pot after seedling establishment. After harvest, 100seeds from each treatment were placed on YDCA and emerging *Xav* colonies enumerated following incubation at 28°C for 48h. Another batch of three seeds from each treatment was planted in soil-filled pots in the screen house and tissue 2-mm<sup>2</sup> tissue samples from emerging seedlings assayed for the presence of *Xav* as described previously.

### Indirect systemic *Xanthomonas axonopodis* pv. *vignicola* infection via floral parts

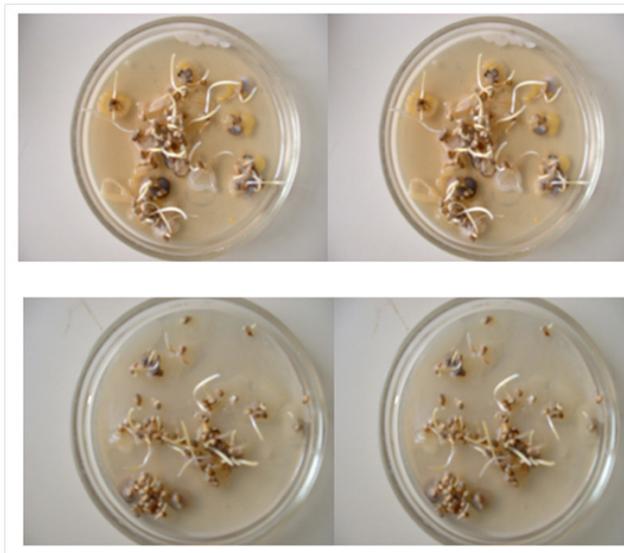
To determine indirect *Xav* systemic infection in the floral parts, cowpea plants inter-cropped with maize, sorghum and foxtail millet were sprayed with a bacterial suspensions adjusted to ca.  $4.5 \times 10^7$  cfu/ml at flowering stage using an atomizer. Cowpea pods were harvested at maturity and 100seeds each from cereal crops were placed on YDCA media. The seeds were also planted in the screen house and examined for the presence of *Xav* after seedling emergence using sampling and processing assays described previously.

## Results

*Xanthomonas axonopodis* pv. *vignicola* was not detected on all the maize and sorghum seed sample. Results of detection assays for detection of *Xav* on millet seeds are summarized in Table 1. The results showed that efficiency of detection of the pathogen varied with detection assay used. Using washing assays, the pathogen was detected on millet seed samples from Gombe, Kaduna, Nassarawa, Kogi and Niger states at concentration levels ranging  $1.0^{-1} - 4 \times 10^2$  CFU/mL. However, millet seeds from Borno, Adamawa, Sokoto and Kebbi were free of the pathogen. Apart from the millet sample from Gombe state (North-Eastern Nigeria), which tested positive for the pathogen,

all the samples that tested positive came from states in North-Central Nigeria. With seed plating assays (Figure 1), *Xav* was detected on millet seed samples from Borno, Nassarawa, Kogi and Niger states, but not on seed samples from the other states. Seed destruction assays produced more consistent pathogen recovery from the seeds; all the millet sample (except those from Nassarawa State) tested positive for the pathogen, with recovered concentrations of the pathogen ranging from  $0.2^{-3} - 3 \times 10^2$  CFU/mL. All the nine putative *Xav* isolates of the pathogen recovered from positive millet samples tested positive for *Xanthomonas axonopodis* pv. *vignicola* on the basis of determinative biochemical, phenotypic, pathogenicity and hypersensitivity (HR on tobacco) tests (Table 2), (Table 3). Microscopic examination showed that the isolates possessed a unipolar flagellum. Isolates did not fluoresce under UV light; they were oxidase-negative, catalase-positive and positive for oxidative metabolism.

Results of *in vivo* *Xav* population dynamics on 7, 14 and 21 DAS showed that *in vivo* rhizosphere and phyllosphere populations of the pathogen in seedlings arising from treated seeds were significantly higher ( $P < 0.05$ ) than those in seedlings emerging from control plants, regardless of the host status of the crop and the plant part assayed (Table 4). At 35 and 49 DAS, *in vivo* assays recovered the pathogen from the leaves and stems of cowpea (cv. Ife Brown), and foxtail millet, but not in sorghum, maize and the untreated control (Table 5). Seed blending, seed washing and seed plating assays to determine direct vascular transmission and indirect transmission of *Xav* in non host seeds recovered the pathogen from millet seeds but not in maize seeds. However, inoculation of maize and millet floral systems resulted to transmission of the pathogen from the contaminated floral systems to seeds formed there from. Pathogen concentration in maize seeds was  $2.0 \times 10^2$  CFU mL<sup>-1</sup>, while that in millet seeds, as detected using seed destruction and seed washing assays was  $1.0 \times 10^2$  CFU mL<sup>-1</sup> and  $1.4 \times 10^3$  CFU mL<sup>-1</sup>, respectively (Table 6).



**Figure 1** Growth of *Xanthomonas axonopodis* pv. *vignicola* colonies on millet seeds in Yeast Dextrose Carbonate Agar (YDCA) plates.

**Table 1** Detection of *Xanthomonas axonopodis vignicola* from millet seeds in Nigeria ‡ ‡The pathogen was not detected on sorghum and maize seeds. \*+=Xav. Detected; - =Xav. Not detected

Source	Pathogen extraction assay		
	Seed washing (CFU mL <sup>-1</sup> )	Direct seed plating*	Plating blend seeds (CFU mL <sup>-1</sup> )
Borno (Bama)	-	+	0.2x10 <sup>2</sup>
Gombe (Akor)	1.0x10 <sup>2</sup>	-	1.3x10 <sup>2</sup>
Adamawa (Mubi)	-	-	1.0x10 <sup>2</sup>
Sokoto (Duchin Kura)	-	-	1.4x10 <sup>2</sup>
Kaduna (Saminaka)	1.2x10 <sup>2</sup>	-	3.3x10 <sup>2</sup>
Kebbi (Jega)	-	-	-
Nasarawa (Keffi)	1.1x10 <sup>2</sup>	+	1.2x10 <sup>2</sup>
Kogi (Idah)	1.0x10 <sup>2</sup>	+	2.4x10 <sup>2</sup>
Niger (Mokwa)	1.4x10 <sup>2</sup>	+	1.0x10 <sup>2</sup>

**Table 2** Morphological characteristics of *Xanthomonas axonopodis vignicola* isolates from millet seeds in Nigeria. \*B, bright yellow; LY, light yellow; CY, creamy yellow C, extracellular polysaccharide †+ = positive

Source of isolates	Pigment color*	EPS <sup>†</sup>	Starch hydrolysis <sup>†</sup>	Time of colony emergence (days)
Borno (Bama)	B	+	+	3
Gombe (Akor)	B	+	+	3
Adamawa (Mubi)	B	+	+	3
Sokoto (Duchinkura)	B	+	+	4
Kaduna (Saminaka)	CY	+	+	4
Kebbi (Jega)	B	+	+	3
Kogi (Idah)	B	+	+	3
Nassarawa (Keffi)	LY	+	+	4
Niger (Mokwa)	CY	+	+	4

**Table 3** Reaction of susceptible cowpea cultivars and tobacco plants to *Xanthomonas axonopodis* pv. *vignicola* isolates from millet seeds +=Compatible/ positive reaction

Source of isolates	Cowpea varieties				Time of symptom development (Day)
	IT86D-721	SAMPEA-7	Ife brown	Hypersensitive reaction on tobacco	
Borno (Bama)	+	+	+	+	15-21
Gombe (Akor)	+	+	+	+	15-21
Adamawa (Mubi)	+	+	+	+	12-21
Sokoto (Duchinkura)	+	+	+	+	10-21
Kaduna (Saminaka)	+	+	+	+	10-21
Kebbi (Jega)	+	+	+	+	8-21
Kogi (Idah)	+	+	+	+	7-21
Nassarawa (Keffi)	+	+	+	+	7-21
Niger (Mokwa)	+	+	+	+	9-21

**Table 4** Concentration of *Xanthomonas axonopodis* pv. *vignicola* (CFU mL<sup>-1</sup>) in the rhizosphere and phyllosphere of cowpea, sorghum, maize and millet seedlings. Means in a column followed by the same letter are not significantly different at P<0.05 by SNK test

Treatment	Sampling point (Days after sowing)					
	7		14		21	
	Roots	Shoot	Root	Shoot	Root	Shoot
lfe brown	3.20×10 <sup>7</sup> a	1.6×10 <sup>7</sup> a	3.4×10 <sup>8</sup> a	2.7×10 <sup>8</sup> a	2.4×10 <sup>8</sup> a	3.1×10 <sup>8</sup> a
Samsorg-12	2.5×10 <sup>7</sup> b	1.0×10 <sup>7</sup> c	2.2×10 <sup>7</sup> ab	1.2×10 <sup>7</sup> b	1.9×10 <sup>6</sup> b	1.0×10 <sup>6</sup> c
Millet	3.3×10 <sup>7</sup> a	1.4×10 <sup>7</sup> ab	3.2×10 <sup>7</sup> b	2.4×10 <sup>7</sup> b	2.2×10 <sup>6</sup> ab	2.1×10 <sup>6</sup> b
Maize	2.4×10 <sup>7</sup> b	1.3×10 <sup>7</sup> b	2.1×10 <sup>7</sup> b	1.1×10 <sup>7</sup> b	1.4×10 <sup>6</sup> c	0.3×10 <sup>7</sup> d
lfe brown	0.00c	0.00d	0.00c	0.00c	0.00d	0.00e
Millet control	0.00c	0.00d	0.00c	0.00c	0.00d	0.00e

**Table 5** Concentration of *Xanthomonas axonopodis* pv. *vignicola* (CFU mL<sup>-1</sup>) in leaf and stem tissues of older cowpea, sorghum, maize and millet seedlings. Means in a column followed by the same letter are not significantly different at P<0.05 by SNK test

Treatment	Sampling point (Days after sowing)			
	35		49	
	Leaves	Stem	Leaves	Stem
lfe brown	2.3×10 <sup>8</sup> a	1.5×10 <sup>8</sup> a	2.4×10 <sup>8</sup> a	1.5×10 <sup>8</sup> a
Samsorg- 12	0.00c	0.00c	0.00c	0.00c
Millet	1.5×10 <sup>4</sup> a	0.5×10 <sup>4</sup> b	1.2×10 <sup>4</sup> b	0.6×10 <sup>4</sup> b
Maize	0.00c	0.00c	0.00c	0.00c
lfe brown	0.00c	0.00c	0.00c	0.00c
Millet control	0.00c	0.00c	0.00c	0.00c

**Table 6** Direct vascular and indirect floral transmission of *Xanthomonas axonopodis* pv. *vignicola* by non host cereal crops. \*+= Detected; - =Not detected

Treatment	Direct vascular transmission*			Indirect floral system transmission*		
	Planting blend seeds (CFU mL <sup>-1</sup> )	Seed washing (CFU mL <sup>-1</sup> )	Direct seed plating	Planting blend seeds (CFU mL <sup>-1</sup> )	Seed washing (CFU mL <sup>-1</sup> )	Direct seed plating
Maize	-	-	-	2.0×10 <sup>2</sup> (cfu)	-	-
Millet	2.3×10 <sup>4</sup> (cfu)	-	+	1.0×10 <sup>3</sup> (cfu)	1.4×10 <sup>3</sup> (cfu)	+

## Discussion

The current study represents a systematic approach to examining the role of nonhost seeds in the transmission and epidemiology of the cowpea bacterial blight pathogen in Nigeria. The non-detection of *Xanthomonas axonopodis* pv. *vignicola* on maize and sorghum from all samples collected across the cowpea producing states in Nigeria indicated that seeds of these crops probably did not have a role in the transmission of *Xav* from and to cowpea, and that they may therefore, not contributors to introduction of primary inoculums of cowpea bacterial blight to non-infested fields.

The results of this study demonstrated that efficiency of pathogen recovery from contaminated seeds can vary widely, depending on seed lot and extraction method employed. These findings were congruent with reports by Gitaitis et al.<sup>3,24</sup> The finding in this study

that selective media assays involving blending of the seeds were more sensitive than seed washing and direct seed plating assays were similar to those of Hadas et al., Fatimi and Schaad,<sup>24,25</sup> who reported that blending tomato seeds for 10–15min resulted in an increase in the extraction of *Clavibacter michiganensis* ssp. *michiganensis* from the seeds by 100% or more over washing for 72hr. The inconsistency in detecting *Xav* on some of the millet seed samples could also be due to low incidence of the disease in the field of origin or stress imposed on the pathogen by seed treatment.<sup>26,27</sup> Another reason could be that the bacteria were firmly attached to the seeds in biofilm, such that that washing assay could not dislodge them.<sup>26</sup> Fett et al.,<sup>28</sup> reported that bacterial in biofilm are notoriously resistant to washing and other antibacterial treatments.

This study showed variations in the time it took *Xav* colonies to produce typical yellow pigmentation on YDCA media and disease

symptoms on susceptible cowpea plants. These findings were in agreement with those of Alexander et al.<sup>15</sup> Okechukwu et al.,<sup>29</sup> who also reported variability in the time it took *Xanthomonas* isolates to induce symptoms in susceptible hosts.

Bacterial population buildup at the early crop seedling stage is enabled by seed exudates released during seed germination.<sup>20,30,31</sup> The finding of this study that *Xav* populations were significantly higher in the roots of cowpea and cereal seedlings at 7 DAS than in the shoot system were similar to those of Gilbertson et al.,<sup>32</sup> who reported that seed borne bacteria, especially *xanthomonads* colonized the surfaces of seedlings without an early endophytic development phase. The findings are also similar to those of Hirano et al.,<sup>11</sup> who reported that nutrients were not limiting factors in the bean spermosphere, enabling the rapid colonization of seedling surfaces. The steady decline in *Xav* populations observed in this study over time in all the cereal crops suggested a corresponding decline in nutrient availability for the bacteria during the exponential growth phase of the seedlings.

Seed infestation with plant pathogenic bacteria and subsequent transmission of pathogens by infected seeds has been reported.<sup>3,33,34</sup> However, the role of nonhost crop seeds in the epidemiology of plant bacterial diseases is not well understood. The finding in the current study that *Xanthomonas axonopodis* pv. *vignicola* was able to colonize the seeds of some nonhost crops, and to be transmitted systemically from floral systems of millet and sorghum to the developing seedling is in agreement with previous reports by Darasse et al.,<sup>1</sup> Dutta et al.<sup>4</sup> and Darsonval et al.,<sup>26</sup> Similar observations were also reported by Dutta et al.<sup>4</sup> However, this study also demonstrated that sorghum and millet plants arising from contaminated seeds were unable to serve as reservoirs of the pathogen, and to subsequently cross-contaminate cowpea plants with the pathogen when the crops were grown together.

## Conclusion

This study demonstrated the asymptomatic colonization of nonhost cereal seeds by *Xanthomonas axonopodis* pv. *vignicola*. The study also showed that the pathogen was capable of being transmitted to the developing seeds of nonhost crops through direct vascular and floral pathways. Although these results point to the possibility that nonhost cereal crops could serve as passive reservoirs of the cowpea bacterial blight pathogen, their role in the epidemiology of the pathogen could not be established, since susceptible cowpea plants intercropped with pathogen-contaminated nonhost cereal crops were not cross-contaminated. However, epiphytic populations of the pathogen in cowpea fields could potentially infect cowpea crops and cause bacterial blight epidemics. These findings emphasize the need for cowpea bacterial management strategies under mixed cropping systems to apply the same preventative measure on the seeds of nonhost cereal crops as they do on cowpea seeds. The results of this study also demonstrated the need for cowpea seed certification standards in mixed cropping systems to take into account the possibility of nonhost seed borne inoculums of cowpea bacterial blight.

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## Conflict of interest

The author declares no conflict of interest.

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