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Persistence of *Xanthomonas axonopodis* pv. *vignicola* in weeds and crop debris and identification of *Sphenostylis stenocarpa* as a potential new host

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Abstract

The survival of *Xanthomonas axonopodis* pv. *vignicola*, incitant of cowpea bacterial blight and pustule, in residues of infested cowpea leaves was studied in the field in the forest savanna transition zone of South Benin and under variable controlled conditions. The pathogen survived for up to 60 days when placed on the soil surface, and up to 45 days buried at depths of 10 and 20 cm. In the glasshouse, bacteria survived in residue mixed with soil for at least 2 months in dry soil and less than 2 months in moist soil. The pathogen survived at least 30 days in the field after spray-inoculation on the weed species *Euphorbia heterophylla*, *Digitaria horizontalis* and *Synedrella nodiflora*; 20 days on *Panicum subalbidum*; 10 days on *Euphorbia hirta*; and 5 days on *Talinum triangulare*. After leaf-infiltration under glasshouse conditions, the pathogen was detected after 90 days in *D. horizontalis*; 75 days in *T. triangulare*, *P. subalbidum* and *S. nodiflora*; 60 days in *E. hirta*, and 30 days in *E. heterophylla*. Among 12 legume species tested as alternative hosts of *X. axonopodis* pv. *vignicola*, only *Sphenostylis stenocarpa* (African yam bean) showed typical symptoms of cowpea bacterial blight in a glasshouse experiment following artificial inoculation. This is the first time this legume species has been identified as a potential host of *X. axonopodis* pv. *vignicola*. Crop residue and weeds are likely sources of primary inoculum when planting two consecutive cowpea crops per year and they probably play a role in dissemination of the pathogen during the cropping season. The alternate host may form a bridge for primary inoculum between cropping seasons.

Introduction

Bacterial blight and pustule of cowpea, caused by *Xanthomonas axonopodis* pv. *vignicola* (Burkholder) Dye, occurs in cowpea (*Vigna unguiculata*) growing areas worldwide and can cause losses of up to 92% (Kishun, 1989). Seed losses of higher than 64% were observed in Benin (Sikirou, 1999). The disease develops as lesions and blight symptoms on leaves, but pustule symptoms occur on some cowpea genotypes (Verdier et al., 1998; Khatri-Chhetri, 1999; Khatri-Chhetri et al., 2003). Growing resistant varieties may be insufficient as a control measure due to high genotype×environment interactions (Sikirou et al., 2001; Wydra, 2002).

Seeds, crop debris, alternate hosts, and soil are the most likely sources of bacterial inocula for annual crops. Many bacterial species can establish large epiphytic populations without any apparent effect on the plant (Beattie and Lindow, 1999). Thus, weeds were reported to serve as reservoirs of bacterial pathogens, which may infect nearby susceptible crops (Cafati and Saettler, 1980). Xanthomonads are specifically known for their epiphytic survival capacity (Swings and Civerolo, 1993), but alternate hosts may also play a role in the

dissemination of the disease. Some strains of *X. axonopodis* have extended host ranges which usually include other members of the same plant family, with few exceptions. The host range of *X. axonopodis* also overlaps with hosts of *X. campestris* pathovars. In host range studies xanthomonad pathogens of leguminous plants were capable of colonizing a wide range of legume species (Bhatt and Patel, 1954; Zaumeyer and Thomas, 1957). Leguminous species are often grown in association with other crops and are frequent intercrops in traditional farming system. *X. axonopodis* pv. *phaseoli* is pathogenic on several species of the genera *Phaseolus*, and *Glycine* (Kleinhempel et al., 1989). However, little further information is available on its host range.

In addition to inoculum on infected seeds (Shekhawat and Patel, 1977; Sikirou, 1999) as inoculum source, other means of survival may play a role in the dissemination of the pathogen. The survival of *X. axonopodis* pv. *vignicola* under field conditions in Africa is not known. To develop sustainable control measures, knowledge of the role of infested leaf residue and weeds in survival of *X. axonopodis* pv. *vignicola* in the field under variable moisture conditions, as well as the identification of potential alternate hosts of *X. axonopodis* pv. *vignicola* are decisive.

Materials and methods

Marker strains of X. axonopodis *pv.* vignicola *and inoculum preparation*

X. axonopodis pv. *vignicola* strain GSPB 2509 (Göttinger Sammlung Phytopathogener Bakterien, Göttingen, Germany) was isolated from diseased cowpea leaves collected at the International Institute of Tropical Agriculture (IITA), Cotonou, Benin. The strain was identified to pathovar using the Biolog GN MicroPlate system (Biolog Inc., Hayward, CA, USA) and host plant inoculation. A marker strain resistant to rifampicin (100 ppm) and streptomycin (100 ppm) (GSPB 2509*rs*) was selected from strain GSPB 2509 and its pathogenicity verified.

Experimental site, climate and soils

Field experiments were conducted at the IITA station, located in the forest savanna transition zone of Benin. This zone is characterized by a bimodal rainfall distribution with an annual average of 1000–1400 mm. The rains fall from March to July and from September to October, interrupted by a small dry season with moderate temperatures in August. A long dry period extends from November to March. The mean temperature is about 27 °C with a low diurnal variation of 7 – 10 °C (Adam and Boko, 1993). The soil types are arenosols or acrosols (Maliki et al., 1997), with 2.62% organic matter, 5 ppm phosphor (Bray I), 1.52% C, 0.155% N, C7N ratio 13.6 and 0.77 meq K^+/100 g.

Survival in soil

Field experiments

Trial 1

Four-week-old plants of the susceptible cowpea cultivar IT84E-124 were spray-inoculated in the field 3 weeks after planting with *X. axonopodis* pv. *vignicola* strain GSPB 2509*rs* (10^7 CFU/ml in 0.01 M $MgSO_4$) in the evening hours. Leaves with intense and uniform spot and blight symptoms as well as wilted leaves were collected, mixed, and (i) kept on the soil surface, (ii) covered with a few soil particles, or (iii) buried at 20 cm depth in the soil, each in an area of 1 m^2 without soil shading.

Trial 2

The trial was repeated in the following year with few changes: ten grams of leaves with symptoms were mixed with 150 g of soil, enclosed in a net (500 cm^2, mesh size 5 mm) and (i) kept on the soil surface, or buried (ii) at 10 cm or (iii) 20 cm in the soil, with three replications in an area of 3 m^2. From each net, *X. axonopodis* pv. *vignicola* was quantified in two sub-samples. The soil was shaded with palm leaves at a height of 80 cm above the soil to avoid heating of the soil by the sun. The trials were conducted during the last third of the major rainy seasons in 1996 and 1997, respectively.

Glasshouse experiment

Forty grams of diseased leaves (see above) were mixed in alternate layers with about 2 kg of field soil (see above) per pot in two to four replications and subjected to four different water regimes: pots without watering, pots watered every 2 weeks, and pots watered every 3 days during 2 months and then kept dry. The trial was continued over 3 months. The glasshouse was shaded, with 28 ± 5 °C and about 65% relative humidity.

Quantification of bacterial survival

Quantification of *X. axonopodis* pv. *vignicola* was conducted on the day of deposit and then monthly from leaf residue samples in the glasshouse and from samples without nets from the field experiment. Bacteria were quantified every 15 days in samples in nets from the second field experiment starting the first day after deposit for up to 5 months. Samples in three replicates per treatment were homogenized and 10 g were assayed. The whole sample was assayed when nets were used. Samples were macerated in 0.01 M $MgSO_4$ solution, double-filtered through sterile cheesecloth and centrifuged at $5200 \times g$ at 15 °C for 20 min. The pellet was resuspended in 10 ml sterile $MgSO_4$ and aliquots of serial 10-fold dilutions were plated on two plates per dilution on rifampicin–streptomycin–cycloheximide–benomyl (RSCB) agar containing 8 g nutrient broth, 3 g yeast extract, 11 g glucose monohydrate, pH 7.2, 14 g Bacto agar and 1 l distilled water, supplemented with Benomyl (250 mg/l), rifampicin (100 ppm), streptomycin (100 ppm), and cycloheximide (250 mg/l). Bacterial colonies were counted after 3–5 days incubation at 30 °C.

Survival on weeds

Field experiments

Four un-weeded cowpea plots of 5×2.4 m in two replications were inoculated by spraying *X. axonopodis* pv. *vignicola* strain GSPB 2509*rs* at 10^8 CFU/ml water in the evening hours in September 1996. Leaves of the dominant weed species *Talinum triangulare* (Ceylon spinach), *Euphorbia hirta* (Asthma weed), *E. heterophylla* (wild Poinsettia), *Digitaria horizontalis* (Jamaica crabgrass), *Synedrella nodiflora* (nodeweed) and *Panicum subalbidum* (elbow buffalo grass) (Okezie Akobundu and Agyakwa, 1987) were collected every 5 days for quantification of the pathogen. Two to six leaves (2–5 g fresh weight), depending on leaf size, of weeds of four plots in two replications were macerated and isolation performed as described above.

Glasshouse experiments

The same weed species as used in the field trial were inoculated by leaf infiltration with *X. axonopodis* pv. *vignicola* strain GSPB 2509*rs* (10^7 CFU/ml), with two plants per pot and four pots per species. Leaf infiltration under pressure is a sensitive method simulating natural infection to test the susceptibility or resistance of cultivars and to identify hosts of pathogenic bacteria (Lozano and Ziegler, 1990; Hokawat and Rudolph, 1991). Glasshouse conditions were as described above. Two to six inoculated leaves per plant (0.2–3 g, according to species) were collected every 2 weeks for 90 days starting the first day after inoculation. Samples were treated as described above.

Host range

Legume species were obtained, if not otherwise stated, from IITA, Genetic Resources Unit, Ibadan, Nigeria as follows: *Arachis hypogaea* (groundnut; personal collection, Benin), *Canavalia ensiformis* DC. (jack bean; TCe2), *C. gladiata* (sword bean; TCg3), *Glycine max* Merr. (soybean; TGm1034), *Kerstingiella geocarpa* (Kersting's groundnut; TKg9), *Lablab purpureus* (lablab bean; TLn6), *Sphenostylis stenocarpa* (African yam bean; TSs60), *Vigna angularis* (rice bean; Tva1), *Vigna mungo* Hepper (mung bean; TVm1), *Vigna radiata* (green gram; TVr5), *Vigna subterranea* (Bambara groundnut; TVSu9), and *Vigna vexilata* (wild *Vigna*; TVNu64). *Vigna unguiculata* cultivar IT84E-124, susceptible to *X. axonopodis* pv. *vignicola* (Sikirou, 1999) was used as control. Two plants per pot and 2 pots per species were leaf-infiltrated at the age of 3–4 weeks as described above. Symptom development was recorded every 3–5 days during 64 days after inoculation in the glasshouse.

Statistical analysis

Area under survival curve at 15, 30, 45, 60 and 75 days after depositing samples in the soil was calculated in proportion-days with the calculus method of integration of area under a curve (Genstat for Windows, 1993), using original bacterial counts. Means and standard errors were calculated using the Excel program. Tukey test (level 5%) was used to determine significant differences between treatments.

Results

Survival in field soil

In unprotected leaf samples (trial 1, year 1996) *X. axonopodis* pv. *vignicola* survived less than 30 days in samples buried at a depth of 20 cm and at least 30 days, when residues were kept on the

soil surface (Figure 1). In trial 2 (year 1997) the bacteria survived at least 60 days, when leaf residues were kept on the soil surface, whereas burying in the soil caused a rapid reduction of the bacterial population to undetectable levels (Figure 2). Rains were rare during the trial period in year 1996 with 104 mm and frequent in year 1997 with 429.6 mm (Figures 1b, 2b). Comparing the

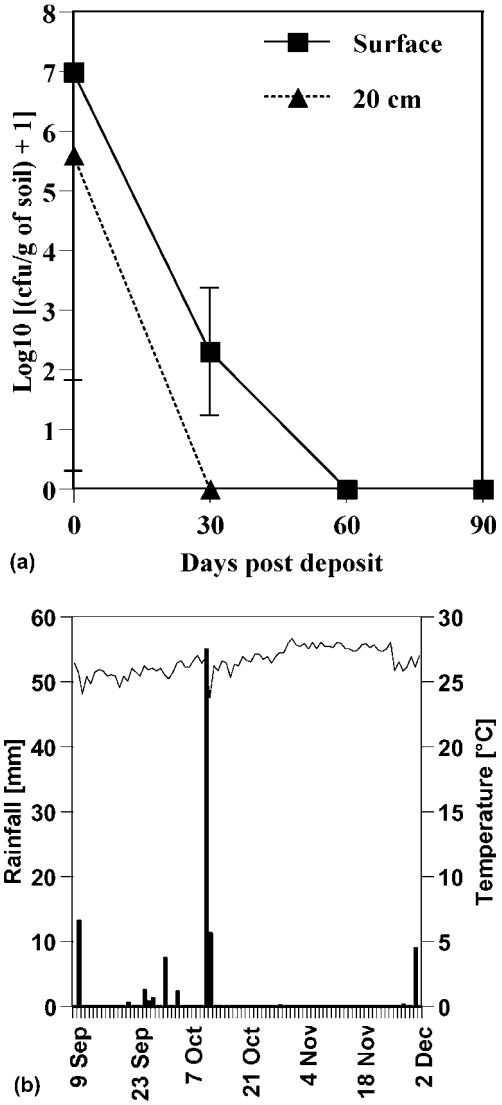

Figure 1. (a) Field survival of *X. axonopodis* pv. *vignicola* in infested cowpea leaves on the soil surface and at 20 cm soil depth in the forest-savanna transition zone of South Benin in 1996. Bars represent the standard error of the mean of *X. axonopodis* pv. *vignicola* colonies detected in three replications. Non-visible standard errors are within the sign margins. Leaves of cowpea plants were spray-inoculated with *X. axonopodis* pv. *vignicola* strain GSPB 2509*rs* (10^7 CFU/ml) and leaves with uniform, intense water-soaking and beginning blight symptoms selected for the trial. Samples in three replicates per treatment were homogenized and 10 g were assayed. (b) Daily precipitation and average temperature observed at the experimental site in 1996.

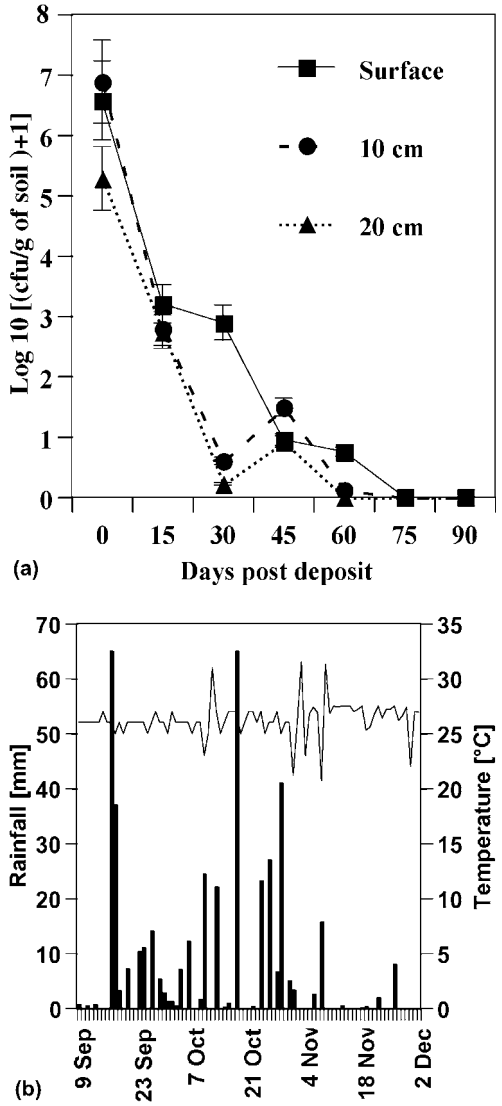

Figure 2. (a) Survival of *X. axonopodis* pv. *vignicola* in infested cowpea leaves mixed with soil and kept in a net in the field, under shade, on the soil surface, and at 10 and 20 cm soil depth in the field in the forest-savanna transition zone of South Benin in 1997. Bars represent the standard error of the mean of *X. axonopodis* pv. *vignicola* colonies detected in three replications. Non-visible standard errors are within the sign margins. Leaves of cowpea plants were spray-inoculated with *X. axonopodis* pv. *vignicola* strain GSPB 2509*rs* (10^7 CFU/ml) and leaves with uniform, intense water-soaking and beginning blight symptoms selected for the trial. The whole sample, e.g. residues and soil in nets, was used for detection. (b) Daily precipitation and average temperature observed at the experimental site in 1997.

area under the survival curves (AUSC) of the three residue treatments of the second year trial, a significant difference between AUSC values was not found (due to the low number of replicates) for the treatment of residues buried at 20 cm soil depth (AUSC 24.8 ± 12.6) compared to burying at 10 cm soil depth (AUSC 43.7 ± 18.2) or leaving residues on the surface (AUSC 70.1 ± 21.2) (data not shown). Population numbers at day of transfer were not included in the AUSC calculation due to differing starting populations between treatments.

Glasshouse experiments

In inoculated residues mixed with soil in pots and subjected to different water regimes, the pathogen survived longest in pots without watering, decreasing from $4.3\times10^6 \pm 3.0\times10^5$ CFU g^{-1} of soil at day 0 – $10^4 \pm 1.5\times10^3$ CFU g^{-1} of soil at day 60 and to undetectable level at day 90. The bacterium was detected only on the day of inoculation with $4.5\times10^5 \pm 5.0\times10^4$ CFU g^{-1} of soil, but not at 30 days after inoculation or later in treatments watered every 2 weeks, and on day 30 with $3.7\times10^2 \pm 7.0\times10$ CFU g^{-1} of soil, but not at day 60 or later, decreased from the initial population of $2.0\times10^5 \pm 5.6\times10^4$ CFU g^{-1} on day 0 after deposit in treatments watered every 3 days. The identity of the isolated bacteria from both soil trials was confirmed by a pathogenicity test on cowpea genotype IT84E-124.

Survival on weeds

Populations of *X. axonopodis* pv. *vignicola* in/on weeds in the field decreased to undetectable level for all weed species at 45 days after inoculation (Table 1). The bacterium survived longer (at least 30 days) on *E. heterophylla*, *D. horizontalis*. and *S. nodiflora*, than on *P. subalbidum* (at least 20 days), *E. hirta* (at least 10 days) and *T. triangulare* (at least 5 days).

The population development of *X. axonopodis* pv. *vignicola* in pathogen-infiltrated leaves in the glasshouse decreased to undetectable level for all weed species at 105 days post inoculation (dpi) (Table 2). The bacteria survived longer in *D. horizontalis* (at least 90 dpi) than in *T. triangulare*, *P. subalbidum* and *S. nodiflora* (at least 75 dpi), *E. hirta* (at least 60 dpi) and *E. heterophylla* (at least 30 dpi). Survival time in weeds after leaf-infiltration of the pathogen in the glasshouse was longer than in the field for all weed species. The identity of the isolated bacteria was confirmed by a pathogenicity test on cowpea genotype IT84E-124.

Host range of X. axonopodis *pv.* vignicola

All the five symptom types were observed on the susceptible cowpea cultivar IT84E-124: leaf spots (water-soaked spots) began after less than 8 dpi, beginning necrosis at 8 dpi, leaf blight (complete or advanced necrosis) at 16 dpi, stem/systemic symptoms (necrosis or canker on stems and/or blight of

Table 1. Development of populations of *X. axonopodis* pv. *vignicola* on/in weed leaves in the field in the forest-savanna transition zone of Benin

	CFU g^{-1} of leaf of weed species					
Dpi[a]	*T. triangulare*[b]	*E. hirta*	P. *subalbidum*	*E. heterophylla*	*D. horizontalis*	*S. nodiflora*
0	80.0 ± 0.6[c]	$2.6\times10^2 \pm 26.0$	$1.3\times10^2 \pm 0.5$	$1.8\times10^3 \pm 3.1\times10^2$	$2.0\times10^2 \pm 2.1\times10^2$	$1.0\times10^3 \pm 1.5\times10^2$
5	96.0 ± 6.0	$1.6\times10^4 \pm 1.0\times10^3$[d]	$2.5\times10^2 \pm 2.5\times10^2$	$3.0\times10^2 \pm 3.0\times10^2$	0	$1.1\times10^4 \pm 1.1\times10^4$
10	0	$8.1\times10^2 \pm 20.0$	$2.6\times10^2 \pm 50.0$	$2.7\times10^2 \pm 0.5$	$2.8\times10^2 \pm 47.0$	$1.1\times10^3 \pm 1.1\times10^3$
15	0	0	0	$9.0\times10^3 \pm 9.5\times10^2$	$2.8\times10^2 \pm 5.0$	$1.4\times10^4 \pm 0$
20	0	0	$1.2\times10^4 \pm 5.0\times10^2$	$1.0\times10^4 \pm 0$	$1.9\times10^3 \pm 4.7\times10^2$	45.0 ± 45.0
25	0	0	0	$1.2\times10^3 \pm 2.9\times10^2$	$1.2\times10^2 \pm 0$	$1.0\times10^2 \pm 0.5$
30[e]	0	0	0	$2.0\times10^2 \pm 7.0$	$2.7\times10^2 \pm 69.0$	$1.1\times10^3 \pm 2.5\times10^2$
45	0	0	0	0	0	0

[a]Days after spray-inoculation of *X. axonopodis* pv. *vignicola* marker strain (GSPB 2509*rs*), 10^8 CFU/ml in an un-weeded cowpea field.
[b]*T = Talinum*, *E = Euphorbia*, *P = Panicum*, *D = Digitaria*, *S = Synedrella*.
[c]Mean and standard error of the mean of bacteria on/in two to six weed leaves per plant of four plots in two replications.
[d]Some fluctuations of bacterial populations may be due to rainfall which may cause a reduction due to wash off, a later increase of the population due to availability of water, or to the sampling of new, non-inoculated leaves.
[e]30 dpi corresponded to the beginning of the dry season in year 1996.

Table 2. Development of populations of *X. axonopodis* pv. *vignicola* in weed leaves infiltrated with *X. axonopodis* pv. *vignicola* in the glasshouse

	CFU g^{-1} of leaf of weed species					
Dpi[a]	*T. triangulare*[b]	*E. hirta*	*P. subalbidum*	*E. heterophylla*	*D. horizontalis*	*S. nodiflora*
0	$7.9\times10^4 \pm 1.0\times10^3$[c]	$2.5\times10^4 \pm 0$	$1.3\times10^4 \pm 1.0\times10^3$	$5.1\times10^6 \pm 1.5\times10^5$	$4.4\times10^5 \pm 0$	$1.4\times10^5 \pm 4.0\times10^3$
15	$6\times10^3 \pm 1.0\times10^2$	$1.0\times10^3 \pm 0$	$1.0\times10^4 \pm 0$	$2.0\times10^5 \pm 3.0\times10^3$	$2.3\times10^5 \pm 4.5\times10^4$	$1.0\times10^4 \pm 0$
30	23.0 ± 0.5	0	$2.1\times10^2 \pm 20.0$	$3.0\times10^5 \pm 1.0\times10^5$	$4.9\times10^2 \pm 31.0$	$9.8\times10^3 \pm 6.8\times10^2$
45	85.0 ± 5.0	$3.0\times10^3 \pm 0$	$2.6\times10^3 \pm 0.5$	0	$7.8\times10^2 \pm 88.0$	$6.2\times10^3 \pm 8.9\times10^2$
60	33.0 ± 0	$1.1\times10^2 \pm 4.0$	$8.5\times10^2 \pm 48.0$	0	$1.0\times10^4 \pm 0$	$9.6\times10^3 \pm 4.2\times10^2$
75	$5.9\times10^3 \pm 1.4\times10^3$	0	$4.2\times10^3 \pm 250.0$	0	$2.7\times10^3 \pm 1.8\times10^2$	$2.8\times10^4 \pm 1.2\times10^4$
90	0	0	0	0	$2.7\times10^2 \pm 40.0$	0
105	0	0	0	0	0	0

[a]Days after leaf-infiltration of *X. axonopodis* pv. *vignicola* marker strain (GSPB 2509*rs*), 10^7 CFU/ml.
[b]*T = Talinum*, *E = Euphorbia*, *P = Panicum*, *D = Digitaria*, *S = Synedrella.*
[c]Mean and standard error of the mean of bacteria in two to six weed leaves per plant with two plants each in four pots.

non-inoculated leaves) at 44 dpi and plant death at 59 dpi, classifying this cultivar as highly susceptible. *Sphenostylis stenocarpa* developed clear and typical symptoms of cowpea bacterial blight, beginning as translucent water-soaked spots at 8 dpi, with first necrosis visible at 12 dpi, developing into large, brown necrotic lesions surrounded by a yellow halo at 16 dpi. One type of typical, yellow bacterial colonies were obtained on antibiotic-containing agar after re-isolation of the pathogen from symptomatic leaves. *Sphenostylis stenocarpa* was therefore classified as susceptible, and confirmed in repeated trials. Pustules which appear on some susceptible cowpea cultivars were not observed, and no symptoms were observed on stems. *Vigna angularis* and *Lablab purpureus* developed atypical symptoms with tiny water-soaked spots at 12 dpi which turned necrotic at 36 dpi, and transient symptoms of water-soaked spots surrounded by a chlorotic area at 8 dpi, respectively. In a repeated trial *Glycine max, Canavalia ensiformis*, *C. gladiata*, *Kerstingiella geocarpa, Arachis hypogaea* and *V. vexilata* showed a hypersensitive reaction, *V. subterranea* and *V. mungo,* a resistance reaction in the form of a red discoloration, while *V. radiata* showed no reaction.

Discussion

Survival in soil

X. axonopodis pv. *vignicola* survived longest in infested residues kept on the soil surface under field conditions and in dry residues, mixed with soil under glasshouse conditions. Bacterial populations in debris buried in the soil disappeared in the field trial at the beginning of the dry season in November. The area under survival curve showed a higher, though not significantly different value due to the low number of replicates, for the residues left on the soil surface. Nevertheless, the higher population number in unburied debris at the end of the trial is decisive to evaluate the treatments. An increase in the population of *X. axonopodis* pv. *vignicola* at one sampling date (day 45) might be due to the rainfall shortly before sampling.

Xanthomonas axonopodis pv. *manihotis* was reported to survive at least 60 days in debris on the soil surface compared to 30 days in buried debris in studies conducted in the same area (Fanou et al., 2001). Soil moisture was assumed to play a decisive role in survival of *X. axonopodis* pv. *manihotis*. Also high temperature may reduce survival as indicated by studies on the survival of *X. campestris* pv. *translucens* (Milus and Mirlohi, 1995), *X. axonopodis* pv. *vesicatoria* (Jones et al., 1986) and *Pseudomonas syringae* pv. *tomato* (McCarter et al., 1983) in plant debris. This supports our observations of a longer survival of the pathogen in the second year trial, where the soil was shaded. Nevertheless, survival in crop residues can be as long as 244 days, as observed in the case of *X. campestris* under field conditions (Schaad and White, 1974; Schaad and Dianese, 1981).

Also *X. axonopodis* pv. *vesicatoria* (Jones et al., 1986) and *X. axonopodis* pv. *citri* (Graham et al., 1987), as well as *Clavibacter michiganensis* subsp.

michiganensis (Gleason et al., 1991; Fatmi and Schaad, 2002) survived longer in crop residue placed on the soil surface than in buried debris. However, Duffy (2000) did not find a clear effect of burying residues on the decline of *X. axonopodis* pv. *dieffenbachiae* and attributed this to different soil properties. No infection of newly planted cowpea by *X. axonopodis* pv. *vignicola* beyond 7 days after deposit was observed by Khan (1999). But, he only evaluated symptoms on cowpea plants newly planted in the soil, used other cultivars and inoculum under different environmental conditions.

The population decrease to undetectable levels in infested leaf debris after 75 days suggests that infested cowpea debris is not an important inoculum source for the following cowpea cropping season in the next year. Also stem, pod and seed debris decompose after about 8 months when buried in soil. However, infested cowpea debris may serve indeed as an inoculum source for the following short cropping season one month after harvest (mid-September to mid-November) when double cropping is practiced. Also in case of double cropping, a survival of the pathogen over the dry season from November to March until the beginning of the next year's season in March seems unlikely. However, a survival might be possible, when stems, pods and seeds, which were not studied and should decompose slower than inoculated leaf debris, are left on the soil surface, but also in volunteers or in an alternative host growing late during the second cropping season and surviving part of the dry season. To enhance decomposition, plant debris, volunteers and an alternative host should therefore be incorporated into the soil. Ploughing is practiced by farmers in some West African regions and should be done immediately after harvesting cowpea fields.

Survival on weeds

The survival of the pathogen in or on weed species in the glasshouse (>90 days) was higher than in the field (>30 days), where epiphytic bacteria might have been washed from leaves by rain. Additionally, in the glasshouse, bacteria were infiltrated into the leaves, while in the field bacteria were sprayed on the leaf surface. In both cases, the bacteria did not multiply exponentially as they do in their natural host (Sikirou, 1999), nor did the plants show any symptoms. Therefore, the tested weeds are not considered host plants of *X. axonopodis* pv. *vignicola*. Moreover, the relatively short survival of the pathogen in or on weeds points to the minor role of weeds as primary inoculum source of *X. axonopodis* pv. *vignicola*. Nevertheless, weeds which harbour the pathogen for 30 days could form a bridge for pathogen carry-over when double cropping is practiced.

Fanou et al. (2001) observed the survival of *X. axonopodis* pv. *manihotis*, a pathogen of cassava, on weeds in the field up to 30 days under the conditions of South Benin. Conversely, Ikotun (1981) did not find survival of *X. axonopodis* pv. *manihotis* on alternate hosts or as epiphyte on weeds in Nigeria. Thus, it is concluded that survival depends on weed genera and species as well as environmental conditions. Weed species were also reported to play a minor role in the epidemiology of bacterial spot of tomato caused by *X. axonopodis* pv. *vesicatoria* (Jones et al., 1986), as well as in bacterial brown spot of bean (Ercolani et al., 1974), bacterial speck of tomato caused by pathovars of *P. syringae* (Schneider and Grogan, 1977), and bacterial wilt of Cucurbitaceae caused by *Erwinia tracheiphila* (Mackiewicz et al. (1998). Conversely, *X. campestris* pv. *campestris* was disseminated up to 12 m from infected cruciferous weeds to cabbage (Schaad and Dianese, 1981). The present data indicate that rain splash and insects (Zandjanjakou et al., 2001) could spread epiphytic populations of *X. axonopodis* pv. *vignicola* from weeds to cowpea plants and contribute to the build-up of an inoculum in the field during the cowpea growing period. Therefore, we recommend weeding of cowpea fields and burying or removal of the crop residues and weeds after harvest.

Host range

Xanthomonas axonopodis pv. *vignicola* was pathogenic to *Sphenostylis stenocarpa*, the African yam bean, evoking typical symptoms. The re-isolation of the inoculated marker strain confirms this result. This legume species was, therefore, for the first time identified as a host of *X. axonopodis* pv. *vignicola* after artifical inoculation. Further studies should confirm whether infection of African yam bean occurs under field conditions from a natural inoculum source.

The African yam bean is an under-exploited legume, grown in West Africa for both its edible seeds and its tubers (Porter, 1992). It is always found in association with other crops, and grown extensively as an intercrop in the traditional farming system. Usually, the last pods are harvested between December and January in the forest savanna transition zone. Therefore, this legume may serve as an inoculum source during the cropping season and as a bridge between seasons when practicing double cropping, or even allow pathogen survival over the long dry season in its debris.

The pustule symptom was not observed in this study, while we reported it in other studies on some susceptible cowpea genotypes which were not included in these experiments (Khatri-Chhetri, 1999; Khatri-Chhetri et al., 1999). *Vigna angularis* and *L. purpureus* only developed atypical symptoms and were classified as non-hosts. Jindal and Patel (1980) also reported that *V. angularis*, *V. mungo*, *G. max* and *V. radiata* were not hosts of *X. axonopodis* pv. *vignicola*. We could not confirm that *V. mungo* was susceptible to cowpea bacterial blight as reported by Rangaswami and Gowda (1963). This could be due to varietal differences in the susceptibility of *V. mungo* to *X. axonopodis* pv. *vignicola*. In general, it was reported that the pathogen may infect some other legume species, but is more aggressive on its natural hosts (Fang et al., 1964). Previously reported alternate hosts of *X. axonopodis* pv. *vignicola* include *Phaseolus lunatus* L. (Java bean) and *P. calcaratus* Roxb. (rice bean) (Jindal and Patel, 1980).

Our studies indicate that plant debris and weeds do not appear to be important sources of inoculum. However, weeds and debris may contribute *via* rain splash and vectors to the secondary spread of *X. axonopodis* pv. *vignicola* during the cropping season as well as to the primary infection of the next crop when growing cowpea in two seasons per year. The alternate host *S. stenocarpa* growing in or near cowpea fields may also serve as primary inoculum source. Nevertheless, contaminated seed should be considered the most important factor in the epidemiology of cowpea bacterial blight (Sikirou, 1999).

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References

Adam KS and Boko M (1993) Le Bénin. Flamboyant EDICEF, Paris

Beattie GA and Lindow SE (1999) Bacterial colonization of leaves: A spectrum of strategies. Phytopathology 89: 353–359

Bhatt VV and Patel MK (1954) Comparative study of species of *Xanthomonas* parasitising leguminous plants in India. Indian Phythopathology 7: 160–180

Cafati CR and Saettler AW (1980) Role of nonhost species as alternate inoculum sources of *Xanthomonas phaseoli*. Plant Disease 64: 194–196

Duffy B (2000) Survival of the anthurium blight pathogen, *Xanthomonas axonopodis* pv. *dieffenbachiae*, in field crop residues. European Journal of Plant Pathology 106: 291–295

Ercolani GL, Hagedorn DH, Kelman A and Rand RE (1974) Epiphytic survival of *Pseudomonas syringae* on hairy vetch in relation to epidemiology of bacterial brown spot of bean in Wisconsin. Phythopathology 64: 1330–1339

Fang CT, Chen HY and Chu CL (1964) A comparative study of the species of the genus *Xanthomonas* from leguminous plants. Acta Phytopathologica Sinica 44: 976

Fanou A, Wydra K, Zandjanakou M, LeGall P and Rudolph K (2001) Studies on the survival mode of *Xanthomonas campestris* pv. *manihotis* and the dissemination of cassava bacterial blight through weeds, plant debris and an insect vector. In: Akoroda MO and Ngeve JM (eds) Proceedings of the 7th Symposium of the International Society of Tropical Root Crops Africa Branch (ISTRC-AB) (pp 569–575) Cotonou, Benin, 1998

Fatmi M and Schaad NW (2002) Survival of *Clavibacter michiganenesis* subsp. *michiganensis* in infected tomato stems under natural field conditions in California, Ohio and Morocco. Plant Pathology 51: 149–154

Gleason ML, Braun EJ, Carlton WM and Peterson RH (1991) Survival and dissemination of *Clavibacter michiganenesis* subsp. *michiganensis* in tomatoes. Phytopathology 81: 1519–1523

Graham RL, McGuire RG and Miller JW (1987) Survival of *Xanthomonas campestris* pv. *citri* in citrus plant debris and soil in Florida and Argentina. Plant Disease 71: 1094–1098

Hokawat S and Rudolph K (1991) Variation in pathogenicity and virulence of strains of *Xanthomonas campestris* pv. *glycines*, the incitant of bacteria pustule of soybean. Journal of Phytopathology 131: 73–83

Ikotun T (1981) Studies on the host range of *Xanthomonas campestris* pv. *manihotis*. Fitopatologia Brasileira 6: 15–21

Jindal JK and Patel PN (1980) Variability in xanthomonads of grain legumes. Phythopathologische Zeitschrift 99: 332–356

Jones JB, Pohronezny KL, Stall RE and Jones JP (1986) Survival of *Xanthomonas campestris* pv. *vesicatoria* in Florida on tomato crop residue, weeds, seeds, and volunteer tomato plants. Phythopathology 76 : 430–434

Khan ANA (1999) Incidence, and perpetuation of bacterial blight of cowpea caused by *Xanthomonas campestris* pv.

vignicola. In: Plant Pathogenic Bacteria, Mahadevan A (ed), Centre for Advanced Study in Botany, University of Madras (pp 349–356) India

Khatri-Chhetri G (1999) Detection and characterization of *Xanthomonas campestris* pv. *vignicola* strains, incitant of cowpea bacterial blight and bacterial pustule, and studies on genotype/strain interactions. PhD thesis. University of Göttingen, Germany

Khatri-Chhetri G, Wydra K and Rudolph K (1999) Variability of strains of *Xanthomonas campestris* pv. *vignicola*, incitant of cowpea bacterial blight and bacterial pustule, collected in several African and other countries. In: Plant Pathogenic Bacteria, Mahadevan A (ed), Centre for Advanced Study in Botany, University of Madras, (pp 296–301) India

Khatri-Chhetri G, Wydra K and Rudolph K (2003) Metabolic diversity of *Xanthomonas axonopodis* pv. *vignicola*, causal agent of cowpea bacterial blight and pustule. European Journal of Plant Pathology 109: 851–860

Kishun R (1989) Appraisal of loss in yield of cowpea due to *Xanthomonas campestris* pv. *vignicola*. Indian Phytopathology 42: 241–246

Kleinhempel H, Naumann K and Spaar D (1989) Bakterielle Erkrankungen der Kulturpflanzen. VEB Gustav Fischer Verlag, Jena, Germany

Lozano JC and Ziegler R (1990) Methods to screen for resistance to foliar bacterial pathogens. In: Klement Z, Rudolph K and Sands DC (eds), Methods in Phytobacteriology (pp 334–338) Akademiai Kiado, Budapest, Hungary

Mackiewicz de D, Gildow FE, Blua M, Fleischer SJ and Lukezic FL (1998) Herbaceous weeds are not ecologically important reservoirs of *Erwinia tracheiphila*. Plant Disease 82: 521–529

Maliki R, Bernard M, Padonou E (1997) Combined effect of NPK and three different types of mulch on maize production in southern Benin. In: Renard G, Neef A, Becker K and von Oppen M (eds) Soil Fertility Management in West African Land Use Systems (pp 287–291) Weikersheim, Germany, Margraf Verlag, Germany

McCarter SM, Jones JB, Gitaitis RD and Smitley DR (1983) Survival of *Pseudomonas syringae* pv. *tomato* in association with tomato seed, soil, host tissue, and epiphytic weed hosts in Georgia. Phytopathology 73: 1393–1398

Milus EA and Mirlohi AF (1995) Survival of *Xanthomonas campestris* pv. *translucens* between successive wheat crops in Arkansas. Plant Disease 79: 263–265

Okezie Akobundu I and Agyakwa CW (1987) Handbook of West African Weeds. International Institute of Tropical Agriculture, Ibadan, Nigeria

Porter D (1992) Economic botany of *Sphenostylis* (Leguminosae). Economic Botany 46: 262–275

Rangaswami G and Gowda S (1963) On some bacterial diseases of ornamentals and vegetables in Madras State. Indian Phytopathology 16: 74–85

Schaad NW and Dianese JC (1981) Cruciferous weeds as sources of inoculum of *Xanthomonas campestris* in black rot of crucifers. Phytopathology 71: 1215–1220

Schaad NW and White WC (1974) Survival of *Xanthomonas campestris* in soil. Phytopathology 64: 1518–1520

Schneider RW and Grogan RG (1977) Bacterial speck of tomato: sources of inoculum and establishment of a resident population. Phytopathology 67: 388–394

Shekhawat GS and Patel PN (1977) Seed transmission and spread of bacterial blight of cowpea and bacterial leaf spot of green gram in summer and monsoon seasons. Plant Disease Reporter 61: 390–392

Sikirou R (1999) Epidemiological investigations and development of integrated control methods of bacterial blight of cowpea caused by *Xanthomonas campestris* pv. *vignicola*. PhD thesis. University of Göttingen, Gottingen, Germany

Sikirou R, Wydra K and Rudolph K (2001) Selection of cowpea genotypes resistant to bacterial blight caused by *Xanthomonas campestris* pv. *vignicola*. In: DeBoer S (ed), Plant Pathogenic Bacteria (pp 309–314) Kluwer Academic Publishers, The Netherlands

Swings JG and Civerolo EL (eds) (1993) Xanthomonas. Chapman & Hall, London

Verdier V, Assigbétsé K, Khatri-Chhetri G, Wydra K, Rudolph K and Geiger JP (1998) Molecular characterization of the incitant of cowpea bacterial blight and pustule *Xanthomonas campestris* pv. *vignicola*. European Journal of Plant Pathology 104: 595–602

Wydra K (2002) The concept of resistance, tolerance and latency in bacterial diseases: examples from cassava and cowpea. New Aspects of Resistance Research on Cultivated Plants, Beiträge für Züchtungsforschung, BAZ 8(3): 36–43

Zandjanakou M, Wydra K, Fanou A, Le Gall P and Rudolph K (2001) The role of the variegated grasshopper *Zonocerus variegatus* as vector of cassava bacterial blight in West Africa. In: DeBoer S (ed), Plant Pathogenic Bacteria (pp 353–358) Kluwer Academic Publishers, The Netherlands

Zaumeyer WJ and Thomas HR (1957) A monographic study of bean diseases and methods for their control. U.S. Department of Agriculture Technical Bulletin 868