

Epiphytic Survival of *Xanthomonas axonopodis* pv. *phaseoli* (E. F. SM)

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Key Words

Xanthomonas phaseoli, bean, host, nonhost, epiphytic population.

1 SUMMARY

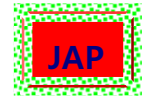
Common bacterial blight, caused by *Xanthomonas axonopodis* pv *phaseoli*, is a major disease in beans (*Phaseolus vulgaris*) throughout the world. Field and greenhouse experiments were conducted to determine epiphytic *X. phaseoli* populations of bean varieties and nonhost plants. Leaves of three bean varieties grown in the field were sampled for *X. phaseoli* populations at two-week intervals from two weeks after crop emergence while stems, flowers and pods were assessed for the pathogen at eight weeks after crop emergence. Weeds and maize growing in the vicinity of the bean fields were also assessed for the pathogen. Epiphytic pathogen populations of up to 3.75×10^4 cfu/ml were detected on bean varieties (Mkuzi, Cranberry and Michigan C20), while on *Zea mays*, *Oxalis latifolia*, *Cyperus rotundas*, *Bidens pilosa*, and *Amaranthus hybridus*, the pathogen population was between 1.02×10^4 and 2.14×10^4 cfu/ml. Leaves of *Amaranthus hybridus* and *Zea mays* hosted pathogenic strains of *X. phaseoli*, but those of *Oxalis latifolia*, *Bidens pilosa* and *Setaria spp* had nonpathogenic xanthomonads. Both plant age and bean variety had no effect on pathogen population, and bacterial populations in the nonhost plants were not significantly different from each other ($p=0.345$). The study confirmed the existence of *X. phaseoli* on nonhost plants and on the stems, flowers and pods of bean varieties. The epiphytic bacteria have potential to act as inocula reservoir during and between growing seasons. The nonhost plants must be controlled as part of common bacterial blight management.

2 INTRODUCTION

Xanthomonas axonopodis pv. *phaseoli* (E.F.SM) (synonym *Xanthomonas phaseoli*) is one of the major constraints to common bean (*Phaseolus vulgaris* L) production in the world (Opio *et al.*, 1996; Coyne *et al.*, 2003). The pathogen incites common bacterial blight, one of the five most important biotic constraints to bean production in sub-Saharan Africa (Gridley, 1994). According to Allen (1995), 19 of the 20 bean producing countries in Eastern and Southern Africa have reported the presence of the disease. Giga (1989) reported the disease as one

of the major constraints to bean production in Zimbabwe. Worldwide, the pathogen causes between 10 and 40% yield loss depending on cultivar susceptibility, environmental conditions and stage of infection (Saettler, 1989). The quantitative yield losses specific to Zimbabwe have not yet been determined.

Common bacterial blight usually occurs together with fuscous blight (caused by *Xanthomonas axonopodis* pv. *phaseoli* var *fuscans*), making it difficult to estimate yield loss by each pathogen. Diseased pods have water-soaked



areas that enlarge with time, becoming brick red and slightly sunken. Bacterial exudates can be seen covering the lesions. On white seeds, brown spots can be seen on the hilum or seed coat. The spots are not apparent on dark-colored seeds. Leaf symptoms initially appear as water-soaked spots on the underside of leaflets. The spots then enlarge irregularly, and adjacent lesions frequently coalesce causing the plant to appear burnt. Lesions can be found at the margin and in interveinal areas of the leaf node. Infected areas appear flaccid, and are encircled by a narrow zone of lemon-yellow tissue which later turns brown and necrotic (Hall, 1994).

In Zimbabwe, common beans are grown by both large-scale commercial farmers and communal farmers. Large-scale commercial farmers grow beans mainly for export, with only 5% sold in local supermarkets as snap beans or mixed frozen vegetables. Communal farmers mainly grow beans (dry pulses) as a cash crop. Some bean varieties mature in two months, thus providing farmers with quick cash returns. The dried pulses, with 22% protein, are consumed as a substitute for the expensive animal protein amongst low-income families (Iranga *et al.*, 1985).

X. phaseoli has been reported to survive on crop residues (Arnaud-Santana *et al.*, 1991). It is also seedborne (Schaad, 1982), and can survive in the seed for as long as the seed remains viable (Hirano and Upper, 1983; Ridout and Roberts, 1997). From contaminated and/or infected seed, the pathogen is capable of colonizing the surface of shoot tissues (epiphytic development) or invading the developing seedling (endophytic development). The pathogen is particularly well adapted to epiphytic survival in the phyllosphere because it is able to aggregate in biofilms that protect it against environmental stresses (Darrasse *et al.*, 2007). Epiphytic populations of *X. phaseoli* are an important phase in the epidemiology of

common bacterial blight. While large epiphytic populations have been associated with times of disease onset and with increased disease severity (Weller and Saettler, 1980a); large epiphytic populations can also develop in the absence of the disease (Hirano and Upper, 1983). The pathogen is capable of surviving epiphytically on both bean hosts and non-host plants (Cafati and Saettler, 1980a). In the Dominican Republic, Angeles-Ramos *et al.* (1991) detected *X. phaseoli* on *Euphorbia heterophylla* (L), *Acanthospermum hispidum* (DC) and *Portulaca oleracea* (L). In Tanzania and Uganda, Saettler (1989) reported the pathogen on *Chenopodium album* (L), *Solanum nigrum* (L), *Echinochloa crusgalli* (L) Link, *Beta vulgaris* (L) and *Amaranthus retroflexus* (L). The alternative epiphytic hosts for Zimbabwe are yet to be determined.

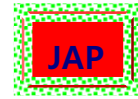
All above-ground parts of host and non-host plants are capable of supporting epiphytic populations of the bacterium. The epiphytic populations serve as reservoirs for inoculum. Symptoms appear when environmental conditions are favourable (i.e. high relative humidity and warm temperature) and when endophytic pathogen population size reaches 10^6 cfu/g of fresh weight (Jacques *et al.*, 2005). Epiphytic populations enable the pathogen to survive between growing seasons when the true hosts are absent.

The objective of this experiment was to demonstrate epiphytic survival of *X. phaseoli* on different bean varieties and non-host plants. In the case of Zimbabwe, knowledge of alternative hosts would be useful in disease management. The study also looked at the effects of host plant age on epiphytic populations of the pathogen. No work has been done on this aspect. The study also sought to investigate the relationship between epiphytic populations and symptom appearance.

3 MATERIALS AND METHODS

3.1 Research site: The field trial was carried out at the University of Zimbabwe (UZ) Department of Crop Science Field at latitude 17°

48'S, longitude 31° 03'E at an altitude of 1506 meters. The area is in agroecological region IIa characterized by mean annual rainfall of 800mm.



Mean summer temperatures range from 23°C in December to 18°C in April. The site has a gentle slope and red clay soils, classified as 5E.2 on the Zimbabwean classification system, Rhodic Paleustalf (USDA classification, or Chromic Luvisol on the FAO system).

3.2 Determining the epiphytic population of *X. phaseoli*

3.2.1 Trial layout and Bean varieties plant sampling: Three bean varieties (Mkuzi, Cranberry and Michigan C20) were grown in solarized plots at UZ Crop Science Departmental Field. Plots measuring 5m x 4.5m each were marked out and planted with the bean varieties at spacing of 45cm x 10cm. To each plot, 200 kg/ha chopped common blight infected bean residues with 4.3 x 10⁶ cfu/g dry weight (*X. phaseoli* dose) were incorporated into furrows at planting. The trial was laid out as a complete randomized design with five replications.

Ten grams of leaves for each bean variety were sampled from each plot for epiphytic populations at 2-week intervals from two weeks after crop emergence (WACE) up to pod filling. A 10g composite leaf sample for each variety was used to determine epiphytic bacterial populations as in section 3.2.3 below. For each variety and replicate, 10 grams of each of stems, flowers and pods were sampled at eight WACE and epiphytic bacterial populations determined as in Section 3.2.3 below.

3.2.2 Non-host Plants Leaf Sampling : A total of 36, 10-grams each leaf samples equally representing eight weed species and *Zea mays* L. were collected at random from both within and outside borders of a 337.5m² common blight infected bean field at eight WACE. Each sample was placed in a separate sterile plastic bag. Ten grams of a composite sample for each weed species and *Z. mays* leaf samples were weighed out and examined for epiphytic *X. phaseoli* population in the laboratory as in Section 3.2.3 below.

3.2.3 Bacterial Isolation: Epiphytic bacteria were isolated by adding 50ml of phosphate buffer saline (PBS), pH 7.4 to each 10g weighed out composite sample in a conical flask and shaking the contents for 20 minutes on a shaker. The 10g samples were not ground prior to the addition of PBS. Ten-fold dilutions were prepared from each sample by taking one milliliter of each working

plant sample supernatant. These were serially diluted by first transferring into a bottle holding 9ml sterile distilled water (SDW) to obtain a one-tenth dilution of the original supernatant. One milliliter of the one-tenth dilution was transferred to another bottle containing 9ml of SDW to obtain a one-hundredth dilution. The above process was repeated until a 1/10 000 dilution was obtained. One milliliter of the 1/1 000 and 1/10 000 dilutions were plated onto nutrient agar (NA) and incubated at 26°C for 2-3 days. Characteristic, starch-hydrolyzing, yellow colonies were counted after the incubation period. The bacteria were purified by transferring two single colonies three times onto NA.

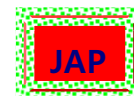
3.3 Pathogenicity Tests for Bacteria isolated from weeds and *Z. mays*: Michigan C20 bean plants were grown under greenhouse conditions in 2.6 kg plastic pots filled with sterilized UZ red clay soil. Sterilization was done by autoclaving the soil at 120°C for two hours. There were ten pots each containing two plants for each bacterial isolate, giving a total of 90 pots. Trifoliate leaves of 14-day old plants were inoculated with 7.3 x 10⁶ cfu/ml bacteria isolated from *Z. mays* and weeds (Section 3.2.3) using the cotton swab inoculation method. The inoculated plants were kept at 28-30°C and relative humidity at 80% to create conditions ideal for blight development. The high relative humidity was achieved by spraying the plants with two liters of SDW daily for 21 days. Control leaves were inoculated with PBS, pH 7.4. Inoculated leaves were evaluated for common bacterial blight symptoms at 24 days after inoculation. Disease incidence and severity were not measured- only the capacity of the strains to incite disease symptoms was noted.

3.3 Data Analysis: Mean bacterial colony counts were log (x+1) transformed to reduce variation. The transformed values were subjected to an analysis of variance (ANOVA) using MSTAT-C package. Mean separation was done using Tukey's Honestly Significant Difference Test at P=0.05 significance level. Results were presented as untransformed bacterial colony counts on tables and a line graph.

4 RESULTS

4.1 Epiphytic populations of *X. phaseoli* on leaves of bean varieties: Neither bean variety

(p=0.18) nor plant age (p=0.43) affected the epiphytic populations of *X. phaseoli* and



xanthomonads. The pathogen was detected on field grown beans as early as the second week after crop emergence, showing that there was early colonization of the bean varieties by the pathogen. Epiphytic population ranged from 2.25×10^3 to 3.75×10^4 cfu/ml. *X. phaseoli*-induced symptoms were first observed at five WACE on Michigan C20, and a week later on Cranberry and Mkuzi.

Symptom expression coincided with the epiphytic populations of 3.16×10^4 cfu/ml on Cranberry, 1.78×10^3 cfu/ml on Mkuzi and 1.78×10^4 cfu/ml on Michigan C20. Epiphytic pathogen population on all the varieties increased after the eighth week. The greatest increase occurred on Mkuzi, the least on Michigan C20, and Cranberry showed a marginal increase (Figure 1).

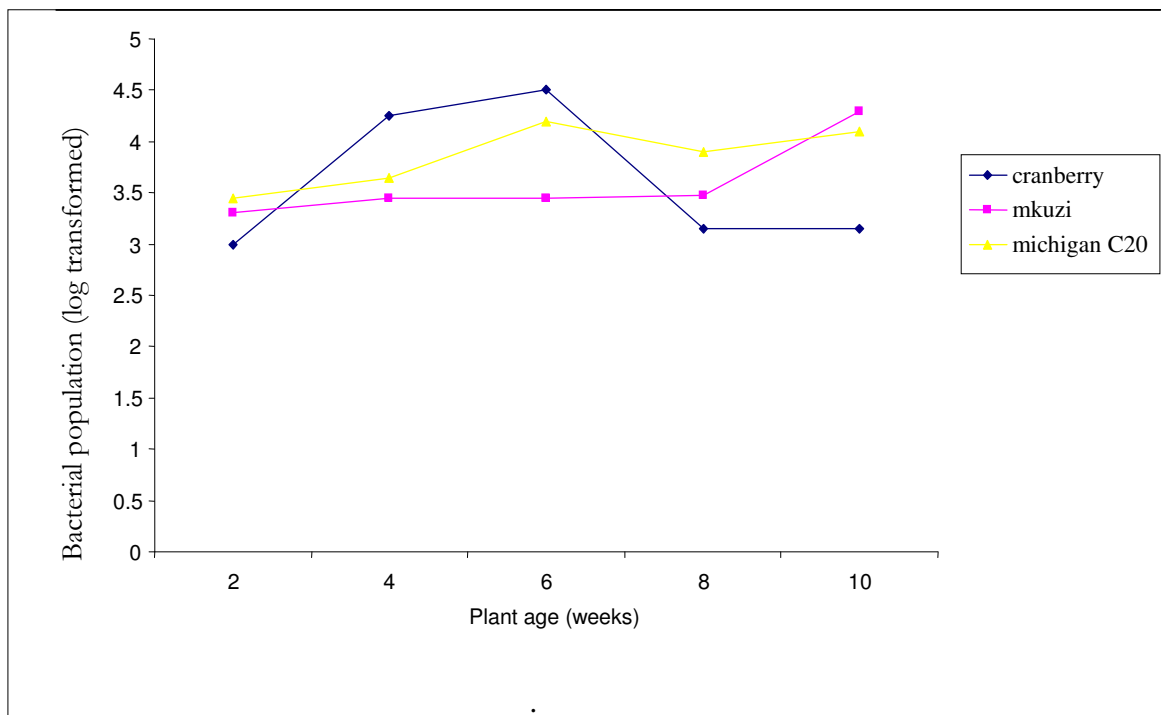


Figure 1: Epiphytic *X. phaseoli* and xanthomonads population on bean varieties

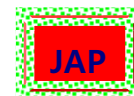
4.2 Epiphytic population of *X. phaseoli* on bean stems, flowers and pods.

Table 1: Epiphytic populations of *Xanthomonas phaseoli* on bean flowers, stems and pods

Bean Variety	Bacterial Population (cfu/ml)		
	Stems	Flowers	Pods
Mkuzi	2.08×10^4	3.75×10^4	3.75×10^3
Michigan C20	1.05×10^4	2.93×10^4	2.75×10^3
Mkuzi	1.50×10^4	1.33×10^4	2.25×10^3
N	20		
CV(%)	8.63		
HSD _{0.05}	4.02×10^4		

The bacterium was detected on bean stems, flowers and pods. Epiphytic bacterial population size was not influenced by plant part ($p=0.10$), nor by bean

variety ($p=0.91$). The population ranged between 2.25×10^3 cfu/ml (Mkuzi pods) to 3.74×10^4 cfu/ml (Cranberry flowers) (Table 1).



4.3 Epiphytic survival of *X. phaseoli* and xanthomonads on nonhost plants

Table 2: Epiphytic *X. phaseoli* populations on nonhost plants

Weed Species	Family	Bacterial Population (cfu/ml)
<i>Oxalis latifolia</i>	Oxalidaceae	1.78 x 10 ⁴
<i>Nicandra phylasoides</i>	Solanaceae	Not detected
<i>Zea mays</i>	Poaceae	1.02 x 10 ⁴
<i>Setaria spp</i>	Poaceae	Not detected
<i>Tagetes minuta</i>	Asteraceae	Not detected
<i>Cyperus rotundus</i>	Cyperaceae	2.01 x 10 ⁴
<i>Amaranthus hybridus</i>	Amaranthaceae	2.14 x 10 ⁴
<i>Bidens pilosa</i>	Asteraceae	1.12 x 10 ⁴
<i>Eleusine indica</i>	Poaceae	Not detected
N		36
CV (%)		10.35
HSD _{0.05}		1.21 x 10 ⁴

The epiphytic bacterial population ranged from 0 to 2.14 x 10⁴ cfu/ml (Table 2). Epiphytic bacteria were detected on the leaves of *Oxalis latifolia* (Kunth), *Zea mays* L., *Amaranthus hybridus* (L.) and *Bidens pilosa* L. Neither *X. phaseoli* nor pectolytic xanthomonads were detected on *Nicandra phylasoides* (L.) Gaertn., *Setaria spp*, *Tagetes minuta* (L.) and *Eleusine indica* (L.) Gaertn. None of the weed samples assessed for epiphytic *X. phaseoli* showed symptoms of pathogen infection. The detected bacterial populations were not significantly different from each other (p=0.09).

4.3 Pathogenicity tests with bacterial strains recovered from nonhost plants

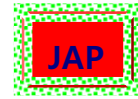
Only nonpathogenic strains were recovered from samples of *Oxalis latifolia*, *Bidens pilosa* and *Cyperus*

rotundus. When tested for pathogenicity, the nonpathogenic xanthomonads induced a weak, incompatible response on Michigan C20. Small, light brown spots were observed at seven days after inoculation. These spots did not develop further. Leaf samples of *Amaranthus hybridus* and *Zea mays* had both pathogenic and nonpathogenic strains of the pathogen. When pathogenicity tests were done, the pathogenic strains induced a small water-soaked zone around the inoculation point after six days. After ten days, the zone became light yellow, irregular, and surrounded a light brown centre. By the fourteenth day, the chlorotic zone had intensified. The tissue around the inoculation site became necrotic and collapsed.

5 DISCUSSION

5.1 Epiphytic pathogen populations on bean varieties: Results obtained showed that leaves, stems, flowers and pods of all the bean varieties supported epiphytic survival of *X. phaseoli*. Cafati and Saettler (1980b), Weller and Saettler (1980) and Ishmaru *et al.* (1991) have also reported that *X. phaseoli* possesses an epiphytic/resident phase. On leaves, the pathogen was detected at two WACE, showing that there was early colonization of the bean varieties. The residues introduced in the furrows at planting are a possible source of inocula that colonized the bean varieties. According to Cafati and Saettler (1980b), the pathogen is capable of systemic movement. There is a strong possibility that the pathogen introduced through the residues at planting was systemically translocated in the plant as the seeds germinated and the seedlings grew.

Wind-driven rain also helped the spread of bacteria in the expanding plant canopy. Up to 740 mm of rain fell between January and April, the normal growing season for beans in Zimbabwe. This amount was more than double the 300 mm seasonal requirement of beans (Olivine Industries, 1998/99). Of this amount, 190 mm fell during the flowering period (in March), and so facilitated further pathogen spread and build-up in the bean varieties. There is a strong likelihood that the aphids, foliage beetles (*Ootheca spp*) and pollen beetles (*Mylabris spp*) that were observed during the growing season also contributed to pathogen spread. These insects can transmit the bacteria as they crawl over or feed on the different plant parts. It is important therefore, that, as one disease management option, the insects be controlled. Also, when spraying bactericides, there should be thorough coverage of all above-



ground plant parts so that the pathogen is killed or prevented from infecting the plant.

Bean variety had no effect on epiphytic population of *X. phaseoli*. However, large epiphytic populations have been associated with times of disease onset and with increased amounts of disease for common blight (Weller and Saettler, 1980). Susceptible cultivars generally host higher epiphytic and endophytic populations than resistant cultivars. It has also been demonstrated that endophytic populations are more directly responsible for disease induction (Beattie and Lindow, 1995), and these must achieve a threshold level of 1×10^5 to 1×10^6 cfu/g leaf tissue (Jacques *et al.*, 2005). In this study, endophytic populations were not measured, but an epiphytic population of 1.78×10^3 cfu/ml was recorded on Michigan C20 at the onset the disease. On Cranberry and Mkuzi, 3.16×10^4 cfu/ml and 1.78×10^3 cfu/ml respectively, were recorded at symptoms appearance.

Plant age had no effect on epiphytic *X. phaseoli* population. For other epiphytic pathogenic and nonpathogenic bacteria, meteorological factors like temperature and moisture have a greater influence on population buildup (Lindow and Brandl, 2003) than the age of the plant. This seems to be true also for *X. phaseoli*.

5.2 Epiphytic populations of the bacteria on nonhost plants: *X. phaseoli* and other xanthomonads were recovered from *Bidens pilosa*, *Zea mays*, *Oxalis latifolia*, *Amaranthus hybridus* and *Cyperus rotundus*. The fact that the bacteria were recovered from these plants indicated that the pathogen spread from the bean varieties to the nonhost plants. These nonhosts, therefore, can serve as reservoirs of inocula which can be disseminated to uninfected plants during the growing season. The nonhosts can also provide

6 CONCLUSION

The study confirmed the existence of epiphytic populations of *X. phaseoli* on host and nonhost plants. Epiphytic population size was not dependent on bean variety or plant age. The study concluded

7 ACKNOWLEDGEMENTS

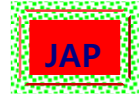
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refuge to the pathogen during unfavorable conditions and re-infect the host when the environment becomes favourable or when susceptible hosts become available. Alternatively, the pathogen may overwinter on the nonhosts and provide inocula for the next bean crop. This has been supported by findings from Hirano and Upper (1983), and Ishmaru *et al* (1991).

Pathogen spread from the bean varieties to the weeds and *Z. mays* was most likely by insects and wind-driven rain. That insects can spread *X. phaseoli* was reported by Kaiser and Vakili (1978). Leaf chewing insects were reported to be more efficient in disseminating the pathogen than sucking insects. As part of common bacterial blight management, the insects have to be controlled. Also, the bean crop should be kept weed-free so as to deprive the bacteria of alternative hosts. Because *X. phaseoli* and other xanthomonads were recovered from outside the field, destruction of weeds around the field would protect a disease-free bean crop from infection by inocula from weeds. These control measures could be augmented by the introduction of bean varieties like Mkuzi which are tolerant to common blight. The fact that the pathogen exists epiphytically on *Z. mays* may have a bearing on intercropping and rotation of beans with maize (Gent *et al.*, 2005). In the case of intercropping *Z. mays* and beans, it means that maize plants can act as reservoirs of the pathogen which will infect the bean crop. As for beans-maize rotation sequence, it simply means the pathogen will be carried over from one crop to the other. The pathogen's ability to survive asymptotically on *Z. mays* negates some of the benefits associated with intercropping and beans-maize sequential rotation as cultural practices of common bacterial blight management.

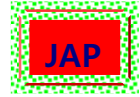
that *Zea mays* and *Amaranthus hybridus* supported pathogenic strains of *X. phaseoli*, and *Oxalis latifolia*, *Bidens pilosa* and *Cyperus rotundus* supported non-pathogenic strains.

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