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THE CONTRIBUTION OF SOIL AND CROP DEBRIS INOCULA TO THE OUTBREAK OF BACTERIAL COMMON BLIGHT IN FIELD BEANS (*Phaseolus vulgaris* L) UNDER ZIMBABWEAN CONDITIONS

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Abstract

*An experiment was carried out to assess the contribution of soil and crop debris inocula to the outbreak of bacterial common blight in field beans (**Phaseolus vulgaris L**) in Zimbabwe. A field, in which beans had been grown the previous season, was planted with three bean varieties on solarized and unsolarized plots with and without infected bean residues. The trial was laid as a 3 × 4 factorial experiment in a complete randomized design. Disease incidence and severity, grain yield, and seed-borne bacterial population were assessed for on growing plants and harvested seed. Disease incidence and severity were not influenced by inocula source. The bean variety had significant effects on the incidence of outbreak, but not on the severity of the disease, with Michigan C20 and Cranberry having higher disease incidences than Mkuzi. Grain yield of the three varieties were significantly different from each other, with the Cranberry variety producing the highest yield of 2.008 t/ha compared to 1.33 and 0.956 t/ha for Mkuzi and Michigan C20, respectively. Inocula source affected yield, with the solarized soil minus debris treatment, giving the highest yield of 2.068 t/ha. Harvested seed bacterial population was influenced by variety, with Michigan C20 having the highest population of 6.91×10^4 cfu/ml. The experiment showed that **Xanthomonas phaseoli** survives in the soil and crop debris in Zimbabwe. However, soil and crop debris inocula had no effect on disease incidence and severity, but provided inocula for disease development. Mkuzi was more tolerant to common bacterial blight than Michigan C20 and Cranberry.*

Introduction

Bacterial common blight (CBB), caused by *Xanthomonas phaseoli* (E. F. Sm), is one of the major constraints to dry bean production in the world, with up to 40% yield loss (Opio *et al*., 1996). Dry beans are a key source of proteins, with high contents of lysine and

methionine. The dry pulse bean has 22% protein, while the green snap bean has 6.1% protein (Purseglove, 1988). In developing countries, dry pulses are consumed as an animal protein substitute by low-income families, while immature pods are grown mainly for export.

Xanthomonas phaseoli is seed-borne, and contaminated seed is one of the primary sources on inoculum (Gilbertson *et al*., 1990; Hall, 1994). Therefore, planting a pathogen-free seed is one of the primary methods of disease control. Because the disease still occurs in fields planted with certified seed, it means there are alternative sources of inocula present. Bean debris and the soil are alternative sources, but studies conducted to determine their importance have yielded conflicting results (Saettler *et al*., 1986). *X. phaseoli* has been reported to over-winter in bean debris under field conditions in Nebraska, but not in Michigan (USA) or Victoria (Australia) (Opio *et al*., 1994). Survival of the pathogen in soil or plant debris is influenced by geographical area, climate, cultural practices, host genotypes, and bacterial strains.

Although a lot of research has been done on common blight epidemiology and ecology in temperate, tropical, and subtropical countries, no such work has been done in Zimbabwe, despite the nutritional and economic importance of dry beans to the country. Results and recommendations from these countries are not directly transferable and applicable to Zimbabwe. This is because pathogen isolates may be different, and thus, behave differently in Zimbabwe's climatic conditions. The objective of this experiment was to assess the role of soil and crop debris as over-wintering sites for *X. phaseoli*, and their contribution to the outbreak of common blight under Zimbabwean conditions.

Materials and Methods

Estimating X. phaseoli population levels in soil and crop debris: The estimation of ***X. phaseoli*** population levels in soil and crop debris was determined using the dilution plate method. Contaminated soil was sampled from a field on which beans had been grown the previous season. A quantity of fifty milliliters (ml) of the soil sample was thoroughly mixed with 200 ml of phosphate buffer saline (PBS), pH 7.4 ($CaCl_2$ scale), placed on a swirling table for 20 minutes, and allowed to stand for three hours. One millilitre of the supernatant was serially diluted ten-fold by first transferring it into a bottle holding 9 ml of sterile distilled water (SDW) to obtain a one-tenth dilution of the original supernatant. One millilitre of the one-tenth dilution was transferred to another bottle containing 9 ml of SDW to obtain a one-hundredth dilution. The above process was repeated until a 1/10,000 dilution was obtained. One millilitre aliquots of the 1/1,000 and 1/10,000 dilutions were plated onto four replicates on MXP (a semi-selective medium for *Xanthomonas Phaseoli*); the colonies that developed were counted after the culture plates had been inoculated at 25^0C for three days.

Ten grams of *X. phaseoli* contaminated crop residues collected from the previous season's bean crop were ground into a fine powder using a mortar and pestle. The resultant powder was mixed with 50 ml of PBS, placed on a swirling table for 20 minutes, and allowed to stand for three hours. After this, serial dilutions were made to obtain 1/1,000 and 1/10,000 dilutions (as above). One millilitre aliquots of these dilutions were plated on MXP and incubated at 25^0C. The pathogen colonies that developed after three days were counted and recorded.

Land preparation and crop management: A field where beans had been grown the previous season was disced and plots measuring 5m × 4.5m marked out. Some plots were

solarized before planting, while others were not. *X. phaseoli* contaminated bean residues collected from the previous season's crop and stored in the glasshouse were chopped into small pieces before incorporation into the soil prior to planting. A basal fertilizer of Compound D (8N: $14P_2O_5$: $7K_2O$) was applied at a rate of 200 kilograms/hectare (kg/ha) to all plots.

One bean seed was planted per station at a spacing of 45cm × 10cm, giving a plant population of 222,222 plants/ha. A top dressing fertilizer of ammonium nitrate (34.5% N) was applied at a rate 51.75 kg/ha at four weeks after crop emergence (WACE). Weeds were controlled by hoe weeding at three and seven weeks, respectively, post crop emergence. An insecticide, Diazinon (32.2% a.i. m/v), was applied at 1.2 ml/l water to control bean stem maggots (*Ophyiomyia phaseoli* Tyron), while another insecticide, Dimethoate 40 EC (40% a.i. m/v), was applied at 1.0 ml/l water to control both sucking and leaf-eating insects. The first dimethoate spray operation was applied at three WACE to control against aphids (*Aphis fabae* Scopoli). A second dimethoate spray operation was carried out a week later. Benomyl (50% a.i. m/m) sprays (a fungicide) were carried out at the sixth, eighth, and tenth WACE, at a rate of 1g per 2 liters of water to control rust. The beans were harvested at physiological maturity. The two outer rows, one on either side of every plot, were discarded as border rows. Four outer plants at both ends in each net row were also discarded as border plants.

Design of experiment and treatments: The trial was laid out as a 3 × 4 factorial experiment in a complete randomized design with three replications. Three bean varieties: Cranberry, Mkuzi, and Michigan C20 were planted. There were four sources of bacterial inocula: solarized soil plus crop debris, solarized soil minus crop debris, unsolarized soil plus crop debris, and unsolarized soil minus crop debris.

Disease Assessment: Disease incidence was determined by counting the number of diseased plants in the net plot and expressing these as a percentage of the total plot plant population. Disease severity ratings were obtained from 5 randomly selected plants for each cultivar in each net plot. The number of diseased trifoliate leaves and leaflets per plant were recorded. Plants were visually rated for disease incidence, based on the scale developed by Gilbertson *et al*. (1988):

0: No symptoms on the plant

1: Very scattered infections covering less than 10% of the foliage

2: Scattered infections covering between 11 and 25% of the foliage

3: Moderately severe infections covering between 26 and 50% of foliage

4: Severe infections covering between 51 and 75% of the foliage

5: Very severe infections covering more than 75% of the foliage).

The disease infection on harvested seed was determined based on seed color. Seed color was rated on a scale (developed by Gilbertson *et al*., 1988):

1: Spotless/clean seed

2: Darkening of the hilum (least visible infection)

3: Few butter-yellow discoloration and shriveling of seed

Isolation of X. phaseoli from harvested seed: Harvested seeds were first disinfected with 1% sodium hypochlorite for 30 seconds to remove contaminant bacteria. They were rinsed with SDW to remove traces of sodium hypochlorite before being dried on multi-wipe tissue paper. The seeds were ground with a blender in PBS, pH 7.4. The resultant supernatant from the ground seed was plated on MXP growth media, after serial dilutions as above. The

plates were then incubated at 25ºC for three days. The resultant bacterial colonies on the growth medium were counted.

Data Analysis: For each of the variables (grain yield, disease incidence and severity, and seedborne bacterial population), an analysis of variance was performed on mean replicate values incorporating the effects of bacterial inocula source, bean variety, and replication. Data for disease incidence was arcsine transformed, while that for disease severity was square root transformed. The logarithmic transformation was performed on data for bacterial population in harvested seed. Where the F-statistic was significant, means were separated by Fischer's Isd test.

Results

Determination of bacterial population in soil and crop debris: Viable bacterial colonies were recovered from both the soil and crop debris nine months after the previous season's bean crop had been harvested. The population of *X. phaseoli*, determined by plating out dilutions of dry leaf inoculum and the soil, averaged 4.3×10^4 colony forming unit/gram (cfu/g) in the debris and 3.85×10^3 cfu/g in the soil.

Leaf and pod symptoms: X. phaseoli induced symptoms were first observed on the lower leaves of the Michigan C20 variety at five WACE. The symptoms consisted of water-soaked spots on the leaf underside. A week after, the spots enlarged to cover about a tenth of the leaf. On severely infected leaves, the spots enlarged and coalesced. Infected leaves had spots encircled by a narrow zone of yellow tissue; the infected portions later turned brown.

No common blight symptoms were observed on bean pods or stems in the field. However, epiphytic *X. phaseoli* populations were detected on all the varieties at 8 WACE on flowers, pods, and stems.

Disease incidence and severity: The incidence and severity of bacterial common blight was not influenced by sources of inocula of *Xanthomonas phaseoli* ($p<0.05$). Bean variety had an effect on the incidence of, but not on the severity of, common blight ($p<0.05$). There was no interaction between inocula source and bean variety on common blight incidence and severity.

Common blight incidence on Cranberry and Michigan C20 were statistically the same, but different from that of Mkuzi. Mkuzi had the lowest common blight incidence (13.20%), compared to 29.40 and 29.50% for Cranberry and Michigan C20, respectively (Table 1).

There were also significant differences in yield among the varieties ($p<0.05$). Cranberry had the highest yield of 2.008 tons per hectare (t/ha), compared to 1.33 t/ha for Mkuzi, and 0.956 t/ha for Michigan C20 (Table 5.1). Michigan C20 was the most susceptible to common blight and rust (Table 5.1), with a disease score of 1.5.

Table 1: Effect of Bean Varieties on Yield, Common Blight Incidence and Severity

Variety	*Yield (t/ha)*	*Common Blight Incidence*	*Severity*
Michigan C20	0.956a	29.50a	1.5
Mkuzi	1.330b	13.20b	1.2
Cranberry	2.008c	29.40a	1.4
Mean	1.431	15.95	
Isd (0.05)	0.324	9.71	
CV (%)	16.70	19.50	

Pairs of means followed by the same letter in column are not significantly different ($p <0.05$).

Effect of soil solarization, crop debris, and bean variety on yield: Both the source of pathogen inocula and bean variety had significant effects on bean yield. There was no interaction between source of inocula and bean variety. The highest bean yield of 2.068 t/ha was realized where no debris was added to solarized soil. This yield was significantly different from the 1.402 t/ha realized where debris was added to solarized soil. There were no significant differences in yield between solarized plots with debris and solarized plots without debris ($p<0.05$) (Table 2). The lowest grain yield of 0.919 t/ha was obtained where debris was added to unsolarized soil. The severity of common blight had a score of between 1.3 and 1.4, meaning that between 11 and 25% of the foliage was infected. Disease severity had no effect on yield ($p<0.05$).

Table 2: Effect of Source of Inocula on Bean Yield and Disease Severity

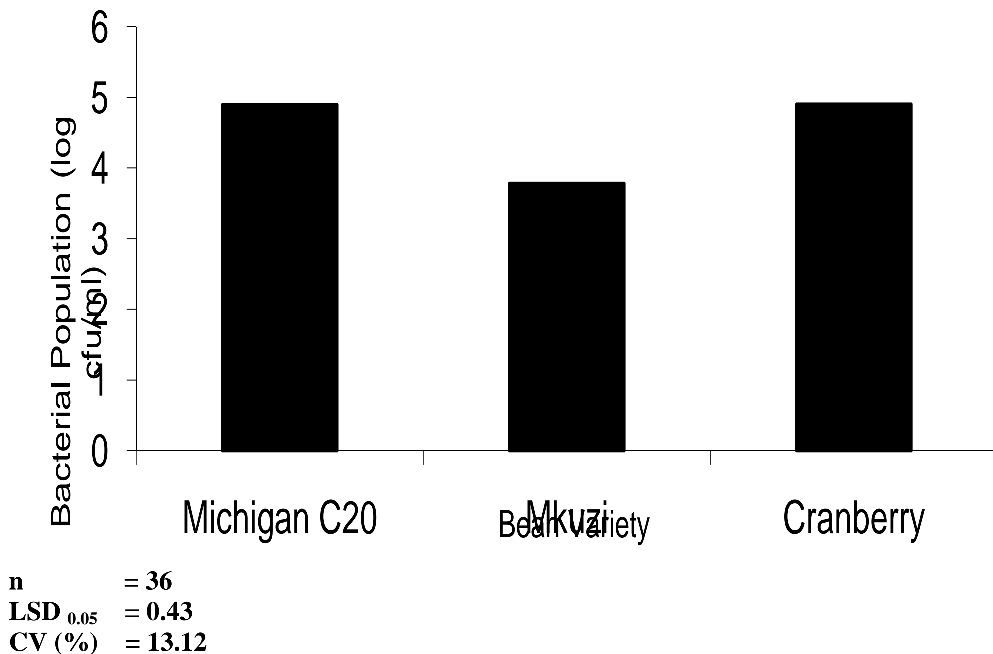
Source of Inocula	*Yield (t/ha)*	*Severity of Common Blight*
Fumigated soil – debris	2.068a	1.4
Fumigated soil + debris	1.402b	1.3
Unfumigated soil – debris	1.337b	1.4
Unfumigated soil + debris	0.910c	1.4
Mean	1.431	
CV (%)	16.70	
$LSD_{0.05}$	0.374	

Means in the same column followed by the same letter are not significantly different ($p<0.05$).

Bacterial population in harvested seed: The source of inocula had no effect on the bacterial population in the harvested seed. Bean variety, however, had an effect on bacterial population. There was no interaction between source of inocula and bean variety on bacterial population in the seed. The highest bacterial population of 6.91×10^4 cfu/ml was recovered

from Michigan C20 seed, although this was not significantly different from the 5.50×10^4 cfu/ml in Cranberry ($P<0.05$). Mkuzi seed had the lowest bacteria population of 4.15×10^3 cfu/ml, and this was significantly different ($p<0.05$) from that of Michigan C20 and

Figure 1: ***Xanthomonas phaseoli*** **Population in Harvested Seed**



n = 36
$LSD_{0.05}$ = 0.43
CV (%) = 13.12

Cranberry (Figure 1).

Disease Status of harvested Seed: Evaluating the disease status of harvested seed on the basis of seed color was not easy, especially for colored seeds of the varieties Mkuzi and Cranberry. It was however, easy with Michigan C20, a white seeded variety. Internal seed infection by *X. phaseoli* did not cause visible external symptoms. Some Michigan C20 seeds had brown discolorations, but this was not due to *X. phaseoli*, as confirmed by the direct plating procedure which did not detect the pathogen. The hilum region of the seeds did not have the butter-yellow discolorations, characteristic of severely infected seeds. Michigan C20

seeds, which were spotless, were infected with up to 4.33×10^3 cfu/ml bacteria, while those with brown discolorations had 2.66×10^3 cfu/ml.

Discussion

X. phaseoli over-wintered in both bean debris and in the soil. This confirms findings by Gilbertson *et al* (1990) that the pathogen can survive in dry leaf inoculum for at least six years. The pathogen has the ability to survive in an infected seed for up to fifteen years (Neergard, 1989). In Nebraska, Saettler *et al*. (1986) recovered *X. phaseoli* in standing debris on the soil surface, but not in standing debris in Michigan.

Lower populations of the pathogen were recovered from the soil compared to that from the stored debris. This is because the pathogen is a poor saprophyte (a parasitic plant on fungi) (Agrios, 1988). *X. phaseol*i is mainly found in association with the bean plant or plant material (like seeds and debris). The pathogen population tends to decline rapidly where infested debris is incorporated into soil. The pathogen, therefore, survives longer in standing debris than when debris is incorporated into the soil. The findings that *X. phaseoli* could be recovered from both infested debris, stored for nine months after harvest, and from debris mixed with the soil showed the importance of infested debris in pathogen survival under conditions in Zimbabwe.

The leaf debris, used for inoculum preparation at planting, was reported to have been severely infected with common blight (Mwashaireni, personal communication). Leaf inocula applied to the seeds at planting enable rapid colonization of the emerging seedling, but this did not cause seedling mortality. It was not necessary to collect leaves with severe symptoms for inoculum preparation because the bacterium can survive epiphytically on bean leaves, so

even leaves with mild symptoms can be heavily colonized by the pathogen. The bacterium also has a short generation time which allows for a small amount of primary inocula surviving in the debris to initiate disease development under favorable environmental conditions.

The results showed that the source of bacterial inocula had no effects on the incidence and severity common blight. This meant that solarizing and residue removal or incorporation did not significantly increase or reduce the chances of disease outbreak. Environmental conditions seem to contribute to the incidence or severity of common blight. The weather conditions that were prevailing during the course of the experiment were not very favorable for common blight development. An outbreak of common blight requires high relative humidity of at least 60% and high temperatures of between 28 and 32ºC for at least two weeks (Saettler, 1989).

The removal of debris and soil fumigation helped to increase bean yield, though these treatments had no effects on common blight disease incidence and severity. This was because the disease is weather-induced and polycyclic, and the quantity of primary inoculums, either from the soil or crop debris is, therefore, not important in disease epidemiology. Besides eliminating *X. phaseoli*, solarization can control nematodes, weed seeds, soilborne fungi, and other soilborne bacteria. This could have attributed to the improved growing environment for the bean crop, hence the high yields realized.

The results on bacterial population in harvested seed showed that Michigan C20 had a higher population of *X. phaseoli* than Cranberry seed. Mkuzi had the lowest pathogen population of the three varieties. It also had the lowest incidence and severity of common blight. Mkuzi was the most tolerant variety to common blight. In areas where common blight

is a problem, farmers are encouraged to grow Mkuzi because of its tolerance to the disease. Even in areas where farmers use retained seed from season to season, planting a variety like Mkuzi ensures low pathogen build-up in the seed, thereby reducing the chances of high seedling mortality.

Even though seeds with visible symptoms of infection are the main source of primary inocula (Gilbertson *et al*., 1990), those with no visible symptoms are also a source of *X. phaseoli*. This was indicated by the presence of the pathogen in hilum-spotless seed. The bacteria may have invaded the plant systemically, passed into the developing seed through the vascular system without producing visible symptoms. The bacteria may also have entered the pod cavity, either via the stomata or breaking through the vascular tissue of the pod suture (Weller & Saettler, 1980). The bacteria then passed into the funiculus and the raphe or the micropyle leading into the seed. This suggests that seed infection is a sequel of colonization. Inocula introduced at planting via crop debris and from soil may also have contributed to internal seed infection.

Conclusion

The study showed that *X. phaseoli* can over-winter between crops in residues and soil under conditions in Zimbabwe. Infected soil and residues can, therefore, be sources of inocula for common bacterial blight. Growing tolerant cultivars and soil solarization significantly increased yield and can be implemented as effective disease management strategies where environmental conditions permit common blight outbreak.

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