Journal of Science and Sustainable Development (JSSD), 2024, 12(2), 12-28

ISSN: 2304-2702 (print); 2414-4479 (online)
DOI: https://doi.org/10.20372/au.jssd.12.2.2024.0517 [12]

RESEARCH PAPER

Importance of seed-borne inoculum level on the development of common bean bacterial blight disease and associated yield loss of common bean in the central rift valley of Ethiopia

Ararsa Leta[1]*, Fikre Lamessa[2] and Getachew Ayana[3]

[1]*Ambo University Guder Mamo Mezemer Campus School of Agriculture, P.O.Box 19 Ambo, Ethiopia;*
[2]*Jimma University College of Agriculture and Veterinary Medicine, P.O.Box 307 Jimma, Ethiopia;*
[3]*EIAR, Malkassa Agricultural Research Center, Crop Protection Department, P.O.Box 436 Adama, Ethiopia*
***Corresponding Author:** Email: ararsaleta@gmail.com*

Abstract

*Common bacterial blight (CBB), caused by Xanthomonas axonopodis pv. phaseoli (Xap) is one of the major constraints to common bean production worldwide, with up to 40% yield loss. Xap is a seed-borne pathogen, and the worldwide distribution of the disease it causes can be attributed to transport on or within the seed. However, the relationships between seed inoculum level and disease developed in the field and associated yield loss have not been investigated so far in Ethiopia. Therefore this study aimed to determine the relationships of seed inoculum level with seedling infection, disease intensity and yield loss. The experiment was carried out during the 2015 main growing season at two sites of Malkassa Agricultural Research Center trial site. The experimental design was a split-plot, with the seed infection type as the main plot and the infection level as a subplot. Treatments were sown onto 2m * 2m (4m2) plots and each treatment was replicated three times. Germination and seedling infection were recorded 10 and 21 days after sowing respectively. After that, disease incidence and severity records were taken at 35, 49, 63 and 77 days after planting. At harvest, yield component and yield data were recorded from the net plot. All the data were subjected to ANOVA using SAS and treatment means were separated using the LSD test. The results of the study revealed significant differences among seed infection-type treatments for most of the parameters measured. Seed infection levels were also significantly different for all disease and yield and yield component parameters at both locations. From the results, it can be concluded that infected seeds were effective sources of initial inocula for common bacterial blight disease development in the field. Hence the production and use of disease-free seeds can be implemented as effective disease management strategies where environmental conditions permit common blight outbreaks.*

Keywords: CBB, common bean, inoculum threshold, seed transmission, yield loss

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume for human consumption both as dry and snap bean because of its health benefits (Willet et al., 1995). Dry beans are a key source of proteins, with high contents of lysine and methionine and have 22% protein, while the green snap bean has 6.1% protein (Purseglove., 1988). In developing countries, dry beans are consumed as an animal protein substitute by low-income families, while immature pods are grown mainly for export. Common bacterial blight (CBB), caused by *Xanthomonas axonopodis pv. phaseoli* (*Xap*) is one of the major constraints to common bean production worldwide where it causes up to 40% yield loss (Iacobellis et al., 2004; Mutlu et al., 2005).

Xap is a seed-borne pathogen, and its worldwide distribution is attributed to transport on or within the seed (Gilbertson and Maxwell, 1992; Lopez et al., 2006). Contaminated and/or infected seeds are an important primary

inoculum source and can lead to extensive colonization of common bean seedlings and plants (Weller and Saettler, 1980a, b; Darrasse et al., 2007). In tropical and sub-tropical areas infected common bean debris is also an important inoculum source allowing spatial and temporal dispersal of these pathogens (Arnaud-Santana et al., 1991; Fininsa and Tefera, 2001; Fininsa and Yuen, 2002) even though the survival period is the subject of controversy. Gilbertson *et al.* (1988) showed that *Xap* can survive in dry leaves under laboratory conditions for at least six years. Karavina *et al.* (2008) isolated *Xap* from bean debris kept in a greenhouse for 12 months in Zimbabwe, while Opio *et al.* (1994) reported that the pathogen survived for more than 18 months in dried leaves kept in a laboratory in Sudan. Santana *et al*. (1991) reported that pathogen survival occurs in bean debris placed on topsoil, but not 20 cm below the soil surface. Moreover, Torres *et al*. (2009) reported that high rainfall and warm temperatures were shown to limit *Xap* survival in residues left on the soil surface.

However, *Xap* has recovered from three, ten and fifteen-year-old bean seed (Schuster and Coyne, 1974; Rideout and Roberts, 1997). The recovered seed-borne isolates were viable and virulent. Thus, contaminated seeds are the primary source of inoculum (Gilbertson *et al*., 1990; Grum *et al*., 1998), and is an extremely efficient means of both local and widespread dissemination of the pathogen. The inoculum threshold of a seed-borne pathogen is the amount of seed infection or infestation with plant pathogens that will cause a disease in the field under a conducive environment and lead to economic loss (Kuan, 1987). In this regard, it is important to establish the inoculum threshold level when clean seed is used as a disease control measure for seed-borne pathogens in general and common bacterial blight in particular. Opio et al. (1993) have reported a positive correlation between *Xanthomonas* campestris pv. Phaseoli seed infection, seed transmission and disease incidence.

The inoculum load and contamination rate of seed lots required to initiate disease varies according to the environmental conditions in which the crop is growing. According to studies made in Canada, in southern Ontario infection of approximately 1 in 10,000 seeds was capable of causing an outbreak of CBB (Sutton and Wallen, 1970). Another study in the same country indicated a 0.5 % seed infection level has been shown to lead to disease epidemics (Zaumeyer and Thomas, 1957). Furthermore, a report from Uganda indicated a 0.2 % seed infection level resulted in a serious disease outbreak (Opio et al., 1993). Hence it is imperative to study the level of inoculum for seed-borne pathogens in general and CBB in particular. However, such studies have not been done so far for CBB in Ethiopia; as a result, information is lacking concerning inoculum levels, and subsequent disease development and associated yield losses. Therefore the purpose of this study was to determine the relationships of seed inoculum level with seedling infection, disease intensity and associated yield loss under field conditions in major common bean growing areas.

Materials and methods

Description of Study Areas

The experiment was carried out during the 2015 main growing seasons at two trial sites of Melkassa Agricultural Research Center (MARC); Malkassa and Arsi Negele. Melkassa is located 15 km southeast of Adama in the semi-arid region of Central Rift Valley at 8o24’ N latitude, 39o 12’ E longitude and an altitude of 1550 m.a.s.l. The area receives an average of 763mm annual rainfall and the maximum and minimum annual mean temperatures are 28oC and 14oC, respectively. The soil type of the site is Andosol which was cultivated for a long period of time (MARC, 1997).

Arsi Negelle is also one of the sub-centers of MARC, situated north of Shashmane, Western Arsi zone at 7o 25’ N latitude, 38o 31’ E longitude and an elevation of 1900 m.a.s.l. The area receives an average annual rainfall of 1100 mm and the maximum and minimum annual mean temperatures are 25oC and 10oC, respectively. The soil type of the site is Nitosol (MARC, 1997).

Experimental Materials and Treatments

To study the correlation between the extent of seed infection and disease transmission to seedlings in the field, and the resulting yield loss, different levels of infected seeds from different infection types were prepared by mixing naturally infected bean seeds of the Awash -1 variety with their respective seed lots obtained from disease free healthy crops of the same cropping season. Diseased seeds were visually inspected for symptoms of common bean blight, which include darkening spots confined mostly to the hilum region and butter-yellow discoloration on the seed coat. The seeds were grouped into three infection types (type1: symptomless, no lesion or discoloration on seeds; type 2: slight to moderate symptoms, seeds with less than 10% discoloration or with discoloration in the hilum region; type 3: severe symptoms, seeds with greater than 10% discoloration with partial shrivelling). The infection level of the seeds in each category was examined before use. Disease free seeds were also examined in the laboratory to confirm that they were free from *Xap* infection. Then one hundred seeds of 0, 1, 2, 4, and 8% disease infection level were prepared from each infection type for planting.

Experimental Design and Management

The experimental design was a split plot, with seed the infection type as the main plot and the infection level as a subplot. Treatments were sown on to $4m^2$ plots and each treatment was replicated three times. Planting was performed on July 15, 2015, at Arsi Negele and on July 18, 2015, at Malkassa. Main plots were separated by 2m and a row of maize variety MH 130 plants was sown between each block and plot to reduce inter-plot interference. Moreover, a dense row of maize was also planted in the whole surroundings of the experimental fields as a natural barrier. The experimental field was isolated by 10m from other common bean fields to avoid splashing bacterial cells from other sources. The experiment was conducted in fields where beans are not grown for at least for two consecutive years. Cultivation and weeding were performed manually.

Data Collection

Germination and seedling infection were recorded 10 and 21 days after sowing respectively. The infected seedlings were identified by the typical water-soaked spots on the underside of the leaf. Seedling infection was determined as the percent infected seedling per plot and seed-to-seedling transmission efficiency (TE) of Xap was calculated using the formula developed by Carmona *et al.*, (1999) $TE = \frac{c}{s} \times 100$ where, C is the percentage of infected seedlings, while S is the percentage of seed infection. Disease incidence and severity were recorded at 35, 49, 63 and 77 days after planting. Disease incidence was determined as the number of plants affected per plot and expressed as a percentage. Disease severity was assessed as the modified CIAT 0 – 9 scales CIAT (1998) where 0 = no infection, 1= 1%, 2=2 - 5%, 3=6-10%, 4=11 - 15%, 5= 16 -30%, 6=31 - 50%, 7=51-75%, 8=75 - 855% and 9= >85% lesion area on the infected leaves. The severity grade was converted into percentage severity index (PSI) with the following formula: $PSI = \frac{Snr}{Npr \times Mss} \times 100$, Where Snr = the sum of numerical ratings, Npr = number of plants rated, Mss = the maximum score of the scale. Disease severity was assessed on 10 randomly selected and tagged plants per plot. PSI was fitted to the disease progress curve to see the progress of disease epidemics at different growth stages of the crop. The area under the disease progress curve (AUDPC) was calculated according to Shaner and Finney (1977). $AUDPC=\Sigma^{n}_{i=1}$ 1/2[(Yi+1+Yi)] [(X i+1-Xi)] where Yi=disease severity score at time i, and Xi=time of scoring (days after planting).

At harvest, the two border rows of each plot were discarded to give a net plot size of $2.4m^2$. For yield and yield components, the mean number of pods per plant from each treatment was recorded at harvest by counting the number of pods from 10 plants randomly taken from the net plot and computing the average. The mean number of seeds per pod was computed

as the average number of seeds from randomly sampled 10 pods. Grain yield was measured as grain weight from the net plot. The hundred seed weight (Hswt) was measured as the weight of 100 randomly sampled seeds. Percent seed discoloration was determined from randomly sampled 100 seeds from the total seed yield per plot. The relative percent yield loss (RPYL) from each plot was calculated in relation to the yield of control treatment of each seed infection category using the formula: $\text{Relative yield loss}(\%) = \frac{Yct - Yit}{Yct}$ where Y_{ct} is the yield of the control treatment (treatment with 0% seed infection level) and Y_{it} is yield of infected treatments (treatments with 1, 2, 4 & 8 % seed infection level).

To analyze and relate prevailing weather conditions meteorological data for each experimental site was obtained from Melkassa Agricultural Research Center, Meteorology and Geospatial Research Program.

The rainfall distributions, and minimum and maximum temperatures and relative humidity for the 2015 main growing season for Melkassa are shown in Figure 1 whereas meteorological information for Arsi Negele was unavailable due to recording problems at the site. The seasonal rainfall was 318 mm and the total amount received after sowing (after July 18) was 210.6 mm. The maximum monthly rainfall for the growing period (80.8 mm) was received in August which lies in the vegetative growth of the crop while the minimum rainfall (8.2 mm) was in October.

Results

Weather Data

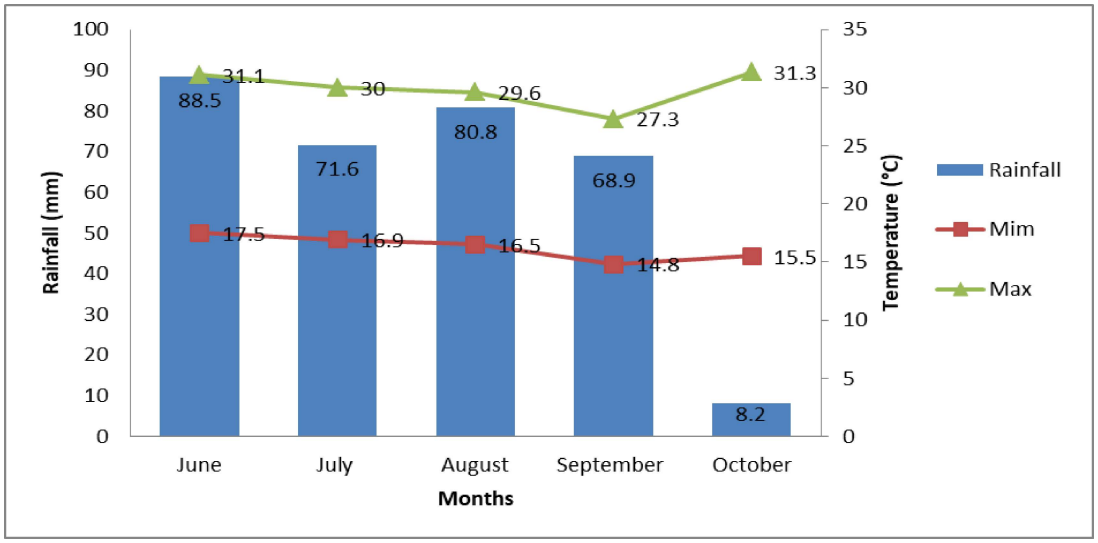

Figure 1. Total monthly rainfall and mean monthly maximum and minimum temperatures for Melkassa
Source: Meteorological and Geospatial Research Program Department of MARC, 2015

The minimum and maximum temperatures for the site are also given in Figure 1. The temperature ranges were narrower at the beginning of the season and slowly became wider at the end. The maximum temperature ranged from 27.3°C to 31.3°C in the trial period while the minimum temperature ranged between 14.8°C to 17.5°C. The relative humidity data also show that maximum humidity (63%) occurred in August while the minimum humidity (48%) occurred in October (Figure 2).

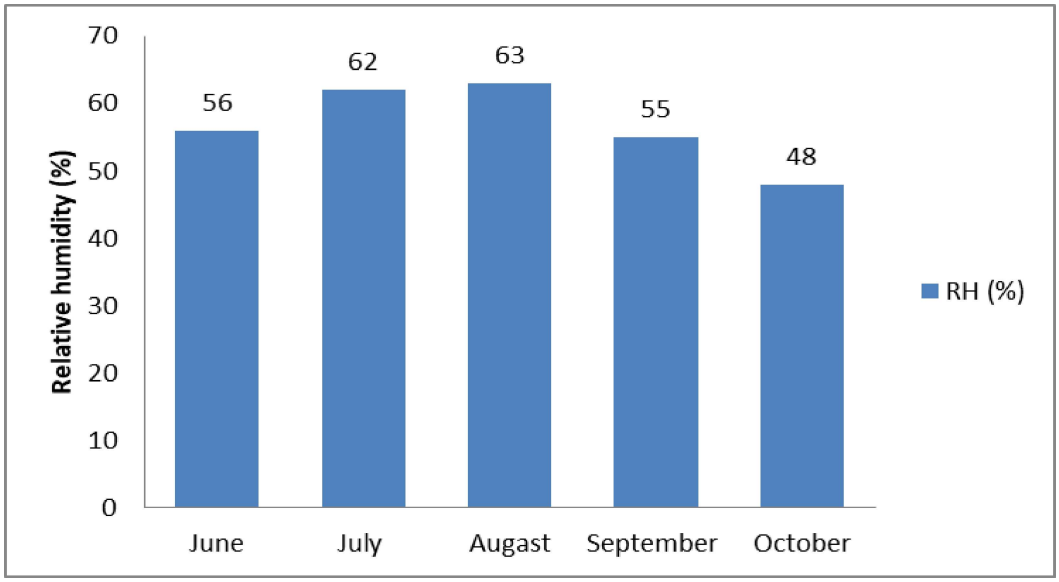

Figure 2. Mean monthly relative humidity for Mellkassa during main growing season

Source: Meteorological and Geospatial Research Program Department of MARC, 2015

Germination percentage

There was no interaction effect of seed infection type and seed infection level on seed germination percentage. However, the main factors, infection type and % seed infection levels significantly affect seed germination at both trial locations. For seed infection type treatments, low seed germination or crop stand was recorded for infection type-3 both at Arsi Negele and Melkassa and high germination was observed in infection type-1(Figure 3). However, infection type 2 was comparable with both infection-type treatments in both sites (Figure 3). For percent seed infection level treatments, a higher seed germination percentage was observed in the control (0% infection level) at both locations and lower seed germination was obtained from 4% and 8% seed infection level treatments at Arsi Negele and 8% seed infection at Melkassa trial sites (Figure 4).

Seed to seedling transmission efficiency (TE)

At both locations, seedling infection was observed at 21DAP for higher seed infection levels, but no infection was observed in plots planted with disease-free seeds and seeds with 1% seed infection level at this time. The Xap seed-to-seedling transmission ratio or transmission efficiency (TE) was not significantly different among the seed infection type treatments at both locations but there was a significant difference in TE among seed infection level treatments. At Arsi Negele higher transmission efficiency (65.421%) was recorded in 8% followed by 4% and 2% infection-level treatments. At Melkassa higher TE (62.742%) was recorded in 2% followed by

4% and 8% infection-level treatments. Low TE was obtained in 0 and 1 % seed infection level treatments at both locations (Table 2).

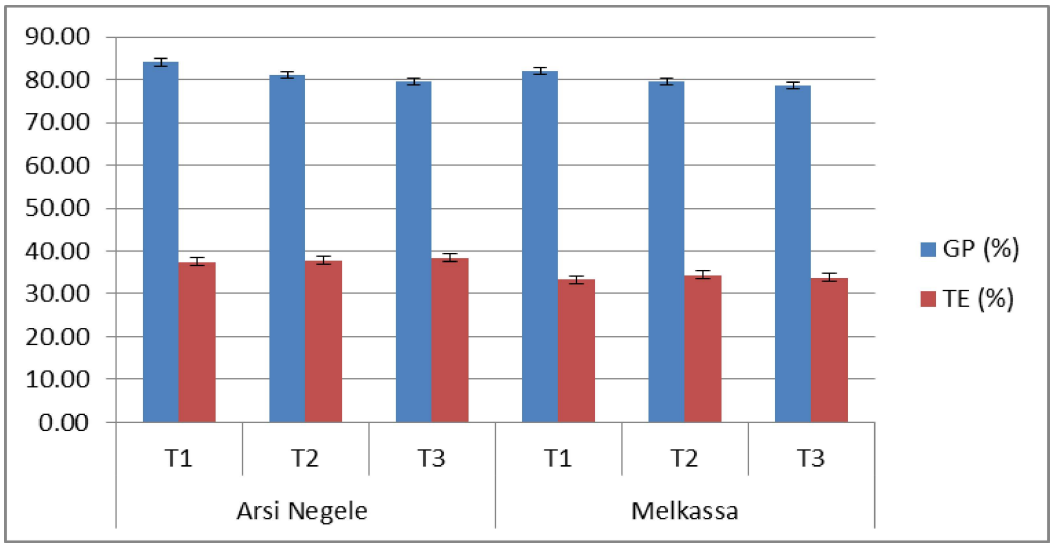

Figure 3. Effect of CBB seed infection type on germination percentage (GP) and seed-to-seedling transmission efficiency (TE) at Arsi Negele and Melkassa

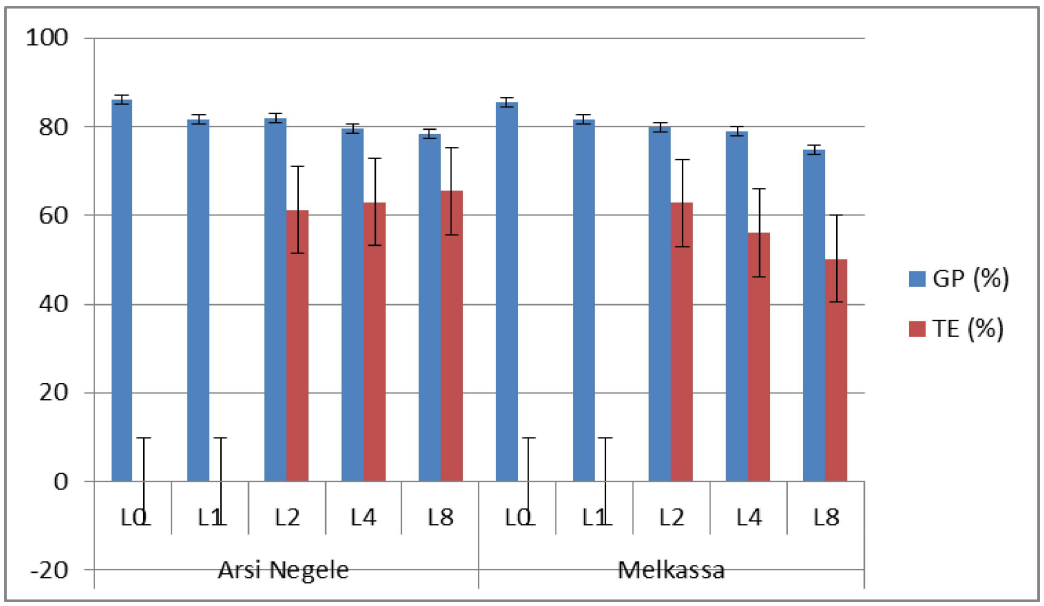

Figure 4. Effect of CBB seed infection level on germination percentage (GP) and seed to seedling transmission efficiency (TE) Arsi Negele and Melkassa

Disease incidence

In both sites, Xap symptoms were observed on bean plants at the early stage of crop development for higher initial seed infection. At all the crop growth stages, there were no significant differences among seed infection type treatment means for disease incidence; but significant differences among treatment means were obtained among seed infection level treatments. At the initial disease assessment, 21DAP no disease was observed for 0 and 1 % seed infection and lower disease incidence were recorded in treatments with 2% initial seed infection while higher incidence was recorded with 8% seed infection treatments at both locations. In general treatments with higher seed infection levels consistently gave rise to higher disease incidence throughout the plant growth stage at both trial sites and lower disease incidence was observed in the control treatments (Table 1). Generally, disease incidence increased with time and lower incidence was observed at Melkassa than at Arsi Negele trial site.

Disease severity

Like for disease incidence significant differences in disease severity were not observed among seed infection-type treatments. However, seed infection levels were significantly different in terms of disease severity index at all disease assessment times. High seed infection levels resulted in higher disease severity scores than did low seed infection levels treatments at both sites in all disease assessment times (Table 2). At the initial disease assessment time (21DAP) the control (0% seed infection level) and 1% seed infection level resulted in no infection symptoms and lower severity resulted in treatment with 2% seed infection while the higher severity was associated with treatment with 8% seed infection at both locations. At the final date of disease assessment, the control treatment resulted in a lower severity of 42.592% and 31.481% at Arsi Negele and Melkassa respectively, while the higher seed infection level (8%) treatments resulted in 79.62% and 69.26% severity at Arsi Negele and Melkassa respectively. Generally, the trend in PSI shows that disease severity increased as seed infection level increased and time progressed (Table 2).

Table 1. Effect of CBB seed infection level on disease incidence (DI) at different days after planting at Arus Negele and Melkassa

% Seed infection level	Arsi Negele					Melkassa				
	21D AP	35D AP	49D AP	63D AP	77D AP	21D AP	35D AP	49D AP	63D AP	77D AP
0	0.00 0d	0.000 e	7.497 e	31.14 6e	50.09 8e	0.00 0d	0.000 e	3.637 e	17.67 0e	34.79 3e
1	0.00 0d	9.650 d	27.24 3d	47.83 3d	71.14 2d	0.00 0d	5.301 d	18.35 0d	30.90 3d	55.97 7d
2	1.22 2c	22.70 9c	40.49 9c	61.47 2c	84.11 7c	1.25 4c	10.57 2c	27.37 3c	39.22 3c	69.05 8c
4	2.51 8b	27.21 8b	48.36 4b	68.86 1b	89.27 7b	2.24 2b	12.96 6b	34.05 3b	45.02 6b	74.44 2b
8	5.23 2a	37.23 6a	61.29 3a	81.98 9a	99.00 1a	4.01 8a	18.90 0a	40.32 8a	53.01 7a	82.84 3a
CV%	20.1	14.2	10.0	7.0	4.4	23.4	12.6	10.6	9.7	4.4
LSD	0.4	2.7	3.6	4.0	3.4	0.3	1.2	2.5	3.5	2.7

Means with the same letter in the same column are not significantly different

Table 2. Effect of CBB seed infection on disease severity index at different days after planting at Arsi Negele and Melkassa

% Seed infection level	Arsi Negele					Malkassa				
	21D AP	35D AP	49D AP	63D AP	77D AP	21D AP	35D AP	49D AP	63D AP	77D AP
0	0.00 0d	0.000 e	8.150 e	30.49 4c	42.59 2d	0.00 0d	0.00 0d	5.680 e	16.66 8e	31.48 1d
1	0.00 0d	9.013 d	27.03 7d	54.56 8b	70.74 0c	0.00 0d	5.06 1c	25.06 2d	37.53 0d	60.49 3c
2	1.11 0c	19.38 3c	33.95 2c	57.90 1b	74.19 7b	1.11 0c	8.39 7b	30.86 4c	45.92 7c	63.82 6b
4	2.34 3b	22.59 2b	38.64 2b	62.84 0a	75.92 6b	1.97 3b	10.8 63a	35.06 2b	50.74 1b	62.92 6b
8	4.44 1a	25.06 2a	44.81 4a	62.71 8a	79.26 0a	3.33 0a	10.7 40a	38.76 6a	54.93 9a	69.26 0a
CV%	21.0 3	15.88	9.09	92.16	4.96	24.9 8	9.75	13.62	8.94	4.99
LSD	0.32 3	2.349	2.697	4.816	3.309	0.31 2	0.66 5	3.589	3.582	2.828

Means with the same letter in the same column are not significantly different

Area Under Disease Progress Curve (AUDPC) and Disease Progress Rate

At both trial sites AUDPC was not significant for the interaction effect of seed infection type and level. A significant difference in mean AUDPC among seed infection-type treatments was only observed at Arsi Negele. Lower AUDPC was observed in treatment with symptomless seed infection type (type 1) whereas in treatments with slight and heavy visual seed infection, the AUDPCs were significantly the same and greater (Table 3). For infection levels, a higher AUDPC resulted in higher seed infection level treatments whereas lower values of AUDPC were observed in lower infection level treatments at both trial sites. In general, the AUDPC increased with increasing seed infection levels among the treatments for both trial sites (Table 4). Higher AUDPC values were recorded at Arsi Negele than at Melkassa.

Table 3. Effect of CBB seed infection type on AUDPC and disease progress rate at Arsi Negele and Melkassa

Seed infection type	Arsi Negele		Melkassa	
	AUDPC	r	AUDPC	r
1	1829.85b	0.103a	1469.48a	0.108ab
2	1912.81a	0.104a	1460.67a	0.107b
3	1906.07a	0.105a	1479.85a	0.110a
CV%	4.883	5.444	7.207	2.929
LSD(0.05)	80.1	ns	ns	0.0018

Means with the same letter in the same column are not significantly different

For disease progress rate, there was a significant difference among seed infection level treatments at both locations while seed infection type treatments were significantly different only at Melkass site trial. A higher infection rate was observed in the infection type3 (seed with heavy visual infection symptoms) than infection type2. For seed infection level treatments, a higher disease progress rate resulted in the control treatments at both locations while a lower rate was obtained in treatments with 2, 4 and 8 % seed infection levels at Arsi Negele and with 4% seed infection level at Melkassa.

Table 4. Effect of CBB seed infection level on AUDPC and disease progress rate at Arsi Negele and Melkassa

% Seed infection level	Arsi Negele		Melkassa	
	AUDPC	r	AUDPC	r
0	839.14e	0.2669a	533.21e	0.255a
1	1763.83d	0.0768b	1370.62d	0.080b
2	2084.44c	0.0593c	1647.16c	0.071c
4	2284.94b	0.0570c	1828.64b	0.066d
8	2442.22a	0.0582c	1970.37a	0.070c
CV%	4.883	5.444	7.207	2.929
LSD	89.457	0.0055	103.080	0.003

Means with the same letter in the same column are not significantly different

Yield Components

An interaction effect was not significant for any of the yield component parameters either Arsi Negele or Melkassa. For the seed infection type treatments, only mean pod per plant (ppplt) and seed discoloration percentage (SDP) varied considerably among treatments at Arsi Negele. PPPlt was higher in seed infection type1 treatment compared to type3 treatment. SDP was lower in seed infection type1 treatment while seed infection type2&3 were statistically the same and had higher SDP. At Malkassa, only seed per pod (SPP) and seed discoloration percentage were significantly different for seed infection-type treatments. Seed infection type1 resulted in higher seed per pod than seed infection type3 while seed infection type2 was statistically comparable with both treatments. SDP is lower in infection type1 and higher in infection type3 (Table 5). For seed infection level, treatments were significantly different in all yield component parameters. Control treatments resulted in higher mean pods per plant (PPPlt), seed per pod (SPP) and hundred seed weight (Hswt) at both locations except mean Hswt was not significant at Melkassa. Higher seed infection level treatments were associated with lower PPPlt and SPP both at Arsi Negele and Melkassa. For SDP, the lowest seed discoloration percentage r occurred in the control treatment and the highest was obtained in treatment with the highest (8%) seed infection level at both site trials (Table 6).

Table 5. Effect of CBB seed infection type and on yield and yield components at Arsi Negele and Melkassa

Seed infection type	Arsi Negele				Melkassa					
	PPPlt	SPP	HSwt	SDP	PPPlt	SPP	HSwt	SDP	yield	RYLP
1	13.527 a	4.55 3a	15.86 7a	8.267 b	10.62 0a	4.133 a	15.80 0a	7.93 3c	1.589 a	23.225 ab
2	13.160 ab	4.54 7a	15.80 0a	9.667 a	10.56 7a	3.867 ab	15.73 3a	8.40 0b	1.574 ab	24.587 ab
3	12.893 b	4.34 0a	15.80 0a	10.26 7a	10.40 7a	3.793 b	15.53 3a	8.66 7a	1.541 ab	25.128 a
CV%	3.036	3.64 5	4.639	8.829	5.388	7.702	4.482	13.5 65	3.843	11.724
LSD(0.05)	0.6	ns	ns	1.229	ns	0.324	ns	0.26 2	ns	ns

Means with the same letter in the same column are not significantly different

Table 6. Effect of CBB % seed infection level on yield and yield components at Arsi Negele and Melkassa

% Seed infection level	Arsi Negele				Malkassa					
	PPPlt	SPP	HSwt	SDP	PPPlt	SPP	HSwt	SDP	yield	RYLP
0	16.58 9a	5.26 7a	16.222 a	6.111 e	12.98 9a	4.367 a	15.778 ab	5.111 e	2.07 1a	0.000 e
1	13.60 0b	4.60 0b	16.111 ab	7.556 d	11.16 7b	3.989 bc	16.111 a	7.111 d	1.73 7b	16.23 7d
2	12.33 3c	4.33 3c	15.778 ab	9.111 c	10.20 0c	4.056 b	15.778 ab	8.222 c	1.62 0c	21.81 1c
4	11.97 8c	4.16 7d	15.444 b	10.66 7b	9.900 c	3.722 cd	15.333 b	9.778 b	1.49 8d	27.65 6b
8	11.46 7d	4.03 3d	15.556 ab	13.55 6a	8.400 d	3.522 d	15.444 ab	11.44 4a	0.91 3e	55.86 2a
CV%	3.036	3.64 5	4.639	8.829	5.388	7.702	4.482	13.56 5	3.84 3	11.72 4
LSD	0.389	0.15 9	0.714	0.807	0.552	0.295	0.684	1.099	0.05 9	2.773

Means with the same letter in the same column are not significantly different. PPPLt = number of pods per plant, SPP =number of seeds per plant, HSwt = hundred seed weight, SDP =seed discoloration percentage, yield = grain yield of the crop, RYLP = relative yield loss percentage

Seed yield

There was a significant difference among treatment combinations at the Arsi Negele trial site. Control treatment i.e. treatment with 0% bean seed bacterial blight infection level had the highest seed yields regardless of the bean infection type. However, at 1% seed infection level higher seed yield was observed in bean seed from type1 (symptomless) seed infection type followed by treatment from slight disease symptom seed infection type. The lowest seed yield was obtained from treatment with 8% infection level from seed infection type 3 (Table 7). At the Melkassa site experiment, no significant differences among seed infection-type treatments were observed. However, all seed infection levels show significant differences. The highest yield was obtained from 0% seed infection treatment followed by treatment with 1% seed infection level and the lowest seed yield was from treatment with the highest seed infection (8%) level in this experiment (Table 8). Generally, trends in seed yields among treatments across both sites were

similar and there were low seed yields at the Melkassa experimental site (Table 8 & 9).

Relative yield loss

Similar to seed yield, there were significant differences in mean relative yield loss among treatment combinations at Arsi Nagele. The highest yield loss (35.827%) resulted in treatment with a higher seed infection level (8%) from seed infection type3 followed by treatment with a similar seed infection level from infection type2 and type1 (Table 7). The lowest yield loss (6.493%) resulted from a lower infection level of type 1 seed infection type. At Melkassa, mean yield losses were significantly different among seed infection levels regardless of their infection type sources. The highest yield loss (55.862%) resulted from the highest seed infection level and the lowest yield loss (16.237%) was obtained from the lower infection level (Table 6). In general, the relative yield losses have similar trends at both trial sites. However, the relative yield loss is much more at Melkassa than at Arsi Negele.

Table 7. Effect of CBB seed infection type and % infection level on yield and relative yield loss at Arsi Negele

seed infection type	% seed infection level	yield	RYLP
1	0	2.667a	0.000j
1	1	2.493b	6.493i
1	2	2.170e	18.743f
1	4	2.030gh	23.903de
1	8	1.807j	32.167b
2	0	2.650a	0.000j
2	1	2.340c	11.693h
2	2	2.103f	20.450f
2	4	1.987h	25.057d
2	8	1.763j	33.340b
3	0	2.670a	0.000j
3	1	2.240d	15.967g
3	2	2.057g	22.803e
3	4	1.887i	29.367c
3	8	1.713k	35.827a
CV%		1.318	5.463
LSD		0.046	2.2037

Means with the same letter under the same column are not significantly different

RYLP = relative yield loss percentage, CV = coefficient of variation, LSD = least significant difference

Relation between parameters

The interactions between parameters were generally the same in both locations. At both locations, all yield parameters i.e. mean crop stand per plot (germination percentage), pod per plant, seed per pod and hundred seed weight were positively correlated with seed yield and negatively correlated with relative yield loss percentage (Table 8 and 9). In contrast, all the disease parameters except disease progress rate; disease incidence, percent severity index, AUDPC, disease transmission efficiency (TE) and seed discoloration percentage (SDP) were negatively correlated to seed yield and positively correlated to yield loss (Table 8 and 9). Moreover, disease parameters i.e. disease incidence, disease severity, AUDPC, disease transmission efficiency and SDP were

positively correlated to each other. Yield attribute parameters: germination percentage, pod per plant, seed per pod and hundred seed weight were also positively correlated with each other (Table 8 and 9). Hundred seed weight (HSwt) was weakly correlate with all parameters at both site and significantly correlated ($p<0.05$) only with seed discoloration percentage (SDP) and disease transmission efficiency (TE) at Melkassa.

Table 8. Correlations between disease parameters; yield and yield component variables at Arsi Negele

Variables	TE	DI	PSI	AUDPC	rate	GP	PPPlt	SPP	Hswt	SDP	yield	RYLP
TE												
DI	.881**											
PSI	.723**	.903**										
AUDPC	.839**	.975**	.971**									
rate	-.678**	-.881**	-.967**	-.939**								
GP	-.602*	-.754**	-.775**	-.801**	.700**							
PPPlt	-.837**	-.964**	-.960**	-.987**	.937**	.819**						
SPP	-.830**	-.946**	-.945**	-.968**	.897**	.829**	.975**					
Hswt	-.675**	-.669**	-.511^{ns}	-.636*	.529*	.583*	.652**	.560*				
SDP	.792**	.879**	.736**	.837**	-.645**	-.860**	-.840**	-.863**	-.622*			
yield	-.882**	-.973**	-.869**	-.950**	.811**	.836**	.951**	.948**	.691**	-.949**		
RYLP	.883**	.972**	.868**	.949**	-.811**	-.833**	-.951**	-.950**	-.692**	.948**	-1.000**	

Table 9. Correlation relation of disease, yield and yield component variables at Melkassa

Variables	TE	DI	PSI	AUDPC	rate	GP	PPPlt	SPP	Hswt	SDP	yield	RYLP
TE												
DI	.826**											
PSI	.695**	.923**										
AUDPC	.788**	.983**	.973**									
rate	.648**	-.877**	-.973**	-.936**								
GP	-.665**	-.881**	-.823**	-.853**	.720**							
PPPlt	-.764**	-.978**	-.901**	-.956**	.828**	.930**						
SPP	-.519*	-.783**	-.766**	-.778**	.660**	.929**	.820**					
Hswt	-.520*	-.469^{ns}	-.229^{ns}	-.372^{ns}	.173^{ns}	.490^{ns}	.452^{ns}	.494^{ns}				
SDP	.746**	.956**	.856**	.927**	-.757**	-.944**	-.973**	-.889**	-.547*			
yield	-.627*	.899**	.785**	-.854**	.681**	.921**	.954**	.839**	.454^{ns}	.950**		
RYLP	.627*	.900**	.785**	.855**	.683**	.920**	.954**	.838**	.450^{ns}	.950**	1.000**	

Discussion

There were significant variations between seed infection types and seed infection level treatments on seed germination at both sites. Low seed germination was resulted from treatment for seed infection type3 (seed with severe discoloration). For seed infection level treatments, higher seed infection level treatments in all infection types resulted in low seed germination. This result was in agreement with the finding of Hall (1994). Weller and Saettler (1980b) also reported that heavily infected seeds fail to germinate resulting in reduced crop stand. Agrios (1988) also reported that seedling mortality resulting from heavy seed infection may reach up to 60%. Even if the seed emerges and the seedling does not die, the bean plant will be infected with common blight at an earlier stage in the season resulting in both quantitative and qualitative yield losses.

Different Xap infection levels also resulted in significant differences in seed-to-seedling transmission efficiency. High transmission efficiency (TE) is obtained from higher infection-level treatments. The control treatment and low seed infection (1%) level treatment does not show seedling infection at 21DAP at both trial sites. As disease symptoms at this stage i.e. early crop stage were only confined to higher seed infection levels, this observation suggested that the lower seed infection levels had not attained the minimum bacterial numbers required for symptom initiation. Similarly, Weller and Saettler (1980a) reported that Xap displayed epiphytic growth during early inoculation stages, whereby it multiplies and survives on the bean canopy without showing visible symptoms. This allows bacterial populations to attain quantities that permit disease development under favorable environments (Hirano and Upper, 1983). Then after, the incidence and severity of common bean blight infection in the

field gradually increased with time both at Arsi Negele and Melkassa. This is typical of seed-borne diseases because they develop relatively slowly, and disease increase is logarithmic with time and the rate of increase is unaffected by initial inoculums (Hewett, 1978). Bowen (2003) also reports that disease development increases in time and weather, particularly temperature and moisture, influences the rate of disease development. However, in this study, the amount of disease was also affected by initial inoculum i.e. lower seed infection levels generally resulted in lower proportions of plants infected with common bean blight, and higher seed infection levels resulted in higher disease incidences at both sites at all disease assessment times.

These trends suggest that seed infection levels, (number of infected seeds) are important in determining the number of plants that can potentially become diseased under suitable conditions for common bean blight development although they may not directly influence the rate at which the disease spreads. On the other hand, as the infection levels directly affected disease incidence, this, in turn, influenced disease severity. High disease incidences caused by high infection levels corresponded to high disease severity at all assessment times at both sites. This is characteristic of most diseases, where disease incidence and severity are positively correlated (Bowen, 2003).

As CBB is seed-borne, the initial inoculum occurs on infected seed and the epidemic starts as these seeds germinate to yield seedlings bearing lesions (Weller and Saettler, 1978). Bacteria from the lesions could then be transmitted by wind and splashing rain throughout the period of crop development (Hirano et al., 1996). Weller and Saettler (1980b) reported the incidence and severity of common bean blight in bean fields as being closely related to the stage of plant development. In this study, although common bean blight symptoms were first observed at 21DAP for higher seed infection levels (2%, 4% and 8%) only both at Arsi Negele and Melkassa; later on disease developed in all treatment plots even on plants from control plot at both locations and disease incidence and severity were at a peak at maturity (77DAP). Finisa and Yuen (2001) also reported a high severity of common bean blight on bean plants at crop maturity. During the vegetative stages of plant development, the foliage would be rapidly expanding and therefore disease severity, as measured by the area of diseased tissue (James, 1974), would be very low (Imhoff et al., 1982; Weller and Saettler, 1980b), because new leaves got zero or very low ratings in disease severity assessments.

Variation in common bean blight incidence and severity and its relation to yield between the two locations trials seems to have been influenced by differences in seasonal weather between the locations. Although the weather data was not obtained from the metrological station for Arsi Negele because of an instrument problem, it was observed that the total seasonal rainfall and the amount received after sowing was much better both in intensity and distribution than that received at Melkassa and it was expected being within the range (350-500mm) required for optimal bean growth and development (EARO, 2004). However, at Melkassa both total seasonal rainfall and the amount received after sowing were less than the optimal range for crop development and the relative humidity was also fairly low (63%), for that a relative humidity above 80 % for some time is required to allow sufficient bacterial multiplication (Darrasse et al., 2007). The maximum temperature ranges from 27.3 to 31.3°C which is in the range (28–32 °C) of optimal temperature for CBB disease development (Opio et al., 1992). For the fact that there were more conducive weather conditions observed at Arsi Negele, common bacterial blight was more severe at Arsi Negele than at Melkassa for all seed infection treatments in the trial. However, better seed yield and low relative yield loss under these conducive conditions for common bean blight development were observed at Arsi Negele. A comparison of the seed yield differences between the two locations indicates that the general reduction in seed yield at Melkassa was highly influenced by rainfall shortage and aggravated by the disease which resulted in

much more relative yield loss (55.86%) than at Arsi Negele (35.82).

Conclusion and recommendations

The study revealed that X. axonopodis pv.phaseoli (Xap) was seed borne and therefore, infected seed was effective sources of initial inocula for common bacterial blight disease development in the field. Hence production of disease free seed in dry high land areas and seed certification and use of disease free seed can be implemented as effective disease management strategies where environmental conditions permit common blight outbreak. Furthermore a careful examination of seed lots following a suitable seed health testing method is of prerequisite to check the spread of disease from disease endemic to disease free areas.

Acknowledgment

The authors would like to thank Jimma University for financial support. We also acknowledge Melkassa Agricultural Research Center for their research material and facility support.

Conflicts of interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

Agrios, G N. 1988. Plant Pathology. Third Edition. Academic Press.

Arnaud-Santana, E. Pena-Matos, E. Coyne, D.P. and Vidaver, A.K. 1991. Longevity of Xanthomonas campestris pv. phaseoli in naturally infested dry bean (Phaseolus vulgaris) debris. Plant Disease 75: 952-953.

Bowen, K.L., 2003. Development of stem rot (caused by Sclerotium rolfsii) in peanut in Alabama. Peanut Science, 30(2), pp.120-128.

CIAT (Centro International de Agricultura Tropical) 1998. Annual report. International centre for Tropical Agriculture, CIAT, Cali, Colombia, 39p.

Darrasse, A. Bureau, C. Samson, R. Morris, C. and Jacques, M.A. 2007. Contamination of bean seeds by Xanthomonas axonopodis pv. phaseoli associated with low bacterial densities in the phyllosphere under field and greenhouse conditions. European Journal of Plant Pathology, 119, 203-215.

EARO (Ethiopian Agricultural Research Organization) 2004. Directory of Released Varieties and Their Recommended Cultural practices. EARO, Addis Ababa, Ethiopia.

Fininsa, C. and Yuen, J. 2002. Temporal progression of bean common bacterial blight (Xanthomonas campestris pv. phaseoli) in sole and intercropping systems. European Journal of Plant Pathology, 108, 485-495.

Fininsa, C. and Tefera, T. 2001 Effect of primary inoculum sources of bean common bacterial blight on early epidemics, seed yield and quality aspects. International Journal of Pest Management. 47 (3): 221-225.

Finney, R. and Shanner, G. 1977. The effect of nitrogen fertilization on the expression of slow mildewing resistance in Knox wheat. Phytopathology 67: 1051-1056

Gilbertson, R. L. R and, R.E., Carlson, E. and Hagerdon, D.J. 1990. Survival of Xanthomonas campestris pv. phaseoli and pectolytic strains of X. phaseoli in bean debris. Plant Disease 74: 322-327.

Gilbertson, R. L. R and, R.E., Carlson, E. and Hagerdon, D.J. 1988. The Use of Dry Leaf Innoculum for Establishment of Common Bacterial Blight of Beans. Plant Disease 77:385-389

Gilbertson, R. L., and Maxwell, D. P. 1992. Common bacterial blight of bean. In H. S. Chaube & J. Kumar (Eds.), Plant diseases of international importance. Diseases of vegetables and oil seed crop Vol. II (pp. 18–39). Englewood Cliffs, NJ: Prentice Hall.

Grum, M., Camloh, M., Rudolph, K. and Ravnikar, M. 1998. Elimination of bean seed-borne bacteria by thermotherapy and meristem culture. Plant Cell, Tissue and Organ Culture 52: 79-82.

Hall, R. 1994. Compendium of Bean Diseases. The American Phytopathological Society. New York, USA.

Hewett, P.D. 1978. Epidemiology of seed borne diseases. In: Scott, P.R. and Bainbridge, A. (eds). Plant Disease Epidemiology. Blackwell Scientific Publications, Oxford, UK. Pp 163-173.

Hirano, S.S. and Upper, C.D. 1983. Ecology and epidemiology of bacterial plant pathogens. Annual Reviews of Phytopathology. 21: 243-269

Hirano SS, Baker LS, Upper CD. 1996. Rain drop momentum triggers growth of leaf-associated populations of Pseudomonas syringae on field-grown snap bean plants. Appl Environ Microbiol 62:2560–2566

Iacobellis, N.S. Bazzi, C. Stafani, E. and Lo cantore, P. 2004. Problemi emergenti nella difesa delle colture: mallatie batteriche. Informatore Fitopatologico, 7, 50-69.

Imhoff, M.W. Leonard, K. J. and Main, C.E. 1982. Analysis of disease progress curves, gradients, and incidence-severity relationships for field and phytotron bean rust epidemics. Phytopathology 72: 72-80.

James, W.C. 1974. Assessment of plant diseases and losses. Annual Review of Phytopathology 12: 27-48

Karavina, C. Chihiya, J and Tigere T.A. 2008. Detection and characterization of Xanthomonas phaseoli (e. f. sm) in common bean (Phaseolus vulgaris L) seeds collected in Zimbabwe. Journal of Sustainable Development in Africa, 10 (1): 105 – 119.

Kuan, T.L. 1987. Inoclum Threshold of Seedborne Pathogens: overview. Prsented at the 76th Annual Meeting of American Phytopathology, University of Guelph, Ontarion, American Phytopathology Society,vol.1.No.6. 1988

Lopez, R. Asensio, C. and Gilbertson, R. L. 2006. Phenotypic and genetic diversity in strains of common blight bacteria (Xanthomonas campestris pv. phaseoli and X. campestris pv. phaseoli var. fuscans) in a secondary center of diversity of the common bean host suggests multiple introduction events. Phytopathology, 96,1204–1213.

MARC (Melkassa Agricultural Research Center), 1997. Melkassa Agricultural Research Center Profile, Melkassa

Mutlu, N. Miklas, P.N. Steadman, J.R. Vidaver, A.V. Lindgren, Reiser, D. J. and Pastor-Corrales, M.A. 2005. Registration of pinto bean germplasm line ABCP-8 with resistance to common bacterial blight. Crop Sci. 45:806–807.

Opio, A.F. Teri, J.M. and Allen, D.J. 1992. Assessment of yield loss caused by common blight of beans in Uganda. Annual Report of the Bean Improvement Cooperative, 35, 113-114.

Opio, A.F., Teri, J.M. and Allen, D.J. 1993. Studies on seed transmission of Xanthomonas campestris pv. phaseoli in common beans in Uganda. African Crop Science Journal 1: 59-67.

Opio, A.F.; Teri, J.M. and Allen, D.J. 1994. Survival of Xanthomonas campestris pv phaseoli in Uganda. African Crop Science Conference Proceedings Volume 1: 255-259.

Purseglove, J. W. 1988. Tropical Crops. Dicotyledonous Plants. Longman, London, UK.

Rideout, M.S. and Roberts, S.J. 1997. "Improving quality control procedures for seedborne pathogens by testing sub-samples of seeds." Seed Science and Technology 25: 195-202.

Santana, A.E. Pena-Matos, E. Coyne, D.P. and Vidaver, A. 1991. Longevity of Xanthomonas campestris pv. phaseoli in naturally infested dry bean debris (Phaseolus vulgaris). Bulletin, University of Nebraska. 7pp.

Schuster, M.L. and Coyne, D.P. 1974. Breeding and genetic studies of tolerance to several beans (Phaseolus vulgaris L.) bacterial pathogens. Euphytica, 23: 651-656.

Sutton, M.D. and Wallen, V.R. 1970. Epidemiological and ecological relations of Xanthomonas phaseoli and Xanthomonas phaseoli var. fuscans on beans in south western Ontario, 1961-1968. Can. J. Bot., 48(7): 1329-1334.

Torres, J.P. Maringoni, A.C. and Silva TAF, J.R. 2009. Survival of Xanthomonas axonopodis pv. phaseoli var. fuscans in common bean leaflets on soil. Journal of Plant Pathology, 91, 195–198.

Weller, D.M. and Seattler, A.W. 1980b. Evaluation of seedborne Xanthomonas phaseoli and Xanthomonas phaseoli var fuscans as primary inoculum in bean blights. Phytopathology 70: 148-152.

Weller, D.M. and Saettler, A.W. 1980a. Colonisation and Ditribution of Xanthomonas phaseoli in field grown navy beans. Phytopathology 70: 500-506.

WELLER, D. M., and A. W.. SAETTLER. 1978. Rifampin-resistant Xanthomonas phaseoli \ar. hiiscans and Xanthomonas phaseoli: Tools for field study of bean blight bacteria. Phytopathology 68: 778-781.

Willet WC, Sacks F, Trichopoulous T, Drescher G, Ferroluzzi A, Helsing F, Trichopoulous D. 1995. Mediterranean diet pyramid: A cultural model for healthy eating. Am. J. Clin. Nutr. 61: 1402 – 1406.

Zaumeyer, W.J. and Thomas, H.E. 1957. A monographic study of bean diseases and methods for their control. US Department of Agriculture Technical Bulletin, 868, 255 pp.