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Response of Ugandan common bean varieties to *Pseudocercospora griseola* and Angular leaf spot disease development in varietal mixtures

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ABSTRACT

Angular leaf spot (ALS) is one of the most economically important bean diseases in Africa. One promising control option for the disease is the use of mixtures of resistant and susceptible varieties. This research evaluated (1) the reaction of farmer preferred bean varieties to ALS in the screenhouse and on-station and (2) the effect of different spatial arrangements of resistant and susceptible bean varieties on ALS disease development. For the latter, five mixture combinations and two controls were laid out in a Randomized Complete Block Design having three replicates. Analysis of variance and least significant differences (LSDs) were used to compare disease levels in both studies above. Varietal screening showed significant differences in varietal reaction to ALS. Screenhouse disease scores ranged between 0 and 5, compared to 0.7–3.9 in the field. For the varietal mixture trial, the lowest disease levels and the highest mixture efficiencies were observed for the combination of equal proportions of the susceptible and resistant varieties randomly mixed (even mixture) prior to planting. We conclude that even mixtures reduce the amount of ALS disease that develops in the season.

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Angular leaf spot; evaluation; varietal mixtures; Africa; Uganda

1. Introduction

In East Africa, the common bean is a staple food, and is regarded as the second most important source of dietary protein (after maize) and the third most important source of calories (Pachico 1993; Johnson & Hodgkin 1999). According to Food and Agricultural Organization (FAO) stat (2013), 3.2 million hectares of dry beans were harvested in East Africa with Uganda accounting for 0.9 million hectares. The crop is mainly produced by small holder famers who use hand held tools and indigenous farming methods. Over 74% of the crop is grown as intercrops with mostly cereals and to a lesser extent bananas and root crops (Wortman et al. 1998). Okii et al. (2014) identified at least 100 bean varieties in Uganda alone including released varieties of which 51% were from the Andean and 49% from the meso-American gene pool. This number however is continuously growing due to on-going breeding efforts and introductions.

However, the profitability of bean production in East Africa has been greatly reduced by a number of constraints most importantly diseases, insect pests and soil infertility. According to Wortman et al. (1998), the most economically important biotic constraint to common bean production in East Africa and Africa in general is Angular leaf spot (ALS). The disease, caused by a fungus *Pseudocercospora griseola* (Sacc.) (Syn. *Phasaeiosariopsis griseola* Sacc.), is principally disseminated by wind driven rains and rain splash over short distances and by air currents over wider expanses (Correa-Victoria et al. 1989; Sengooba 1989; Allen et al. 1996). In this region, the disease is widely distributed across varying altitudes although prevalence starts to diminish significantly above 2000 m above sea level (Mwang'ombe et al. 2007). The disease is known to cause up to 70% losses depending on variety susceptibility, environmental conditions and pathogenicity of the isolates or pathotypes (Sartorato 2005). Yield losses occur as a result of defoliation, resulting in decreased green leaf area index (Bergamin et al. 1997; de Jesus et al. 2001). In Uganda, these losses are further aggravated by the fact that majority of farmers do not use fungicides on beans. This is partly because farmers believe that fungicide use is not profitable (Kisakye & Ugen-Adrogu 1990).

The high pathogenic variability among *P. griseola* isolates makes breeding for and maintaining resistance very difficult (Pastor-Corrales et al. 1998). For example, varieties resistant in one location and season can turn out to be susceptible in another. Besides, to date, available resistant varieties in most cases have limitations related to marketability, yield performance and taste, which limit their adoption. This makes farmers maintain their preferred varieties regardless of these being highly

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susceptible to ALS disease. It is also apparent that breeding for varieties with all farmer preferred attributes in addition to disease resistance is difficult and time consuming. The implication, therefore, is that farmers may for a long time maintain their preferred varieties regardless of their high susceptibility to ALS disease. This calls for exploration of alternative control options if farmers are to realize reduced crop damage and hence increased yields. One such option is the use of varietal mixtures, where varieties used have different levels of resistance to a particular disease or several diseases. The use of varietal mixtures to control disease has been extensively deployed in cereals (Mundt 2002) and on a more limited scale in other crops such as cassava (Sserubombwe et al. 2001), Caribbean stylo (Chakraborty et al. 1991) and bananas (Mulumba et al. 2012). At the farm level, this practice is not new in eastern and central Africa where it is common for farmers to grow more than one bean variety on a farm (Leakey 1970; Nantale et al. 2008). However, in these regions pure varieties fetch a slightly higher market price compared to mixtures. Since it is difficult to obtain clean seed from intra row mixtures (herein termed as even mixtures), this study sought to deploy both inter-row and intra-row mixtures in order understand the effect of both mixture combinations on ALS disease severity. This study was thus conducted to (1) evaluate the reactions of farmer preferred local bean varieties to ALS disease and (2) investigate the effect of different spatial arrangements of two varieties with varying levels of resistance to *P. griseola* on the infection and spread of ALS disease.

2. Materials and methods

2.1. Study site

All our studies were conducted at the National Crops Resources Research Institute (NaCRRI), located in Wakiso district, Uganda. The NaCRRI is located in the Lake Victoria crescent of Uganda in Wakiso district at latitude: 0.52119° N, longitude: 32.62671° E and altitude of 1150 m above sea level. It experiences bimodal rainfall with an annual rainfall of 1270 mm and temperature range of 15–29 °C, the first rains occur between March and June and the second between August and December. The selected site had fertile loamy soils which had been under fallow for two years and had moderate organic matter levels. No pesticides or fertilizers of any kind were used in the experiments. Hand weeding was done thrice beginning at three weeks then six weeks and finally at nine weeks after planting.

2.2. Screenhouse evaluation of farmer preferred bean varieties

Forty local bean varieties commonly cultivated in central and western Uganda, and obtained from farmers were sorted and cleaned prior to planting in the screenhouse. Pots containing the different varieties were arranged in a Completely Randomized Design. Each pot contained a single plant with each variety planted in three pots or replicates; in total 120 pots were used. The experiment was repeated once. Plants were watered regularly until the experiment was concluded.

P. griseola isolates for plant inoculation were obtained from diseased bean leaves at NaCRRI. The fungus was isolated and cultured on V-8 media (200 ml V-8 juice, 3 g $CaCO_3$, 18 g Bacto agar and 800 ml of distilled water) amended with rifamycin antibiotic and incubated in the laboratory at 24 °C. After seven days of growth, single spore cultures were established and allowed to proliferate for two weeks.

Plants were inoculated using the method by Mahuku et al. (2003), with minor modifications. Fungal spores were harvested from Petri dishes containing two-week-old single spore cultures, by brushing the colony surface with a soft brush into distilled water before stirring and sieving the resultant suspension through a cheese cloth. Ten isolates each from a different field were mixed and spore concentrations estimated using a haemocytometer. Final concentrations were adjusted to 2×10^4 spores ml^{-1} using sterile distilled water.

To inoculate plants with *P. griseola* spores, 21-day-old bean seedlings (of second or third trifoliate leaf stage) were sprayed on the lower and upper leaf surfaces with the spore suspension until run off. Inoculated seedlings were immediately placed in a humidity chamber at approximately 22 °C and 95% relative humidity with a 12 h dark/light cycle for five days. Upon removal from the humidity chamber, plants were placed on a raised platform in the screenhouse, where day temperatures were between 22 and 30 °C. The plants were watered regularly depending on the amount of moisture in the planting soil.

ALS disease was evaluated on the first trifoliate leaf 21 days after inoculation, when disease scores under these experimental conditions are expected to be maximum (Mahuku et al. 2004). A disease evaluation scale of 0–5 (Inglis et al. 1988) was used, where 0 = no disease symptoms, 1 = 1%–10% leaflet area with lesions, 2 = 11%–25% leaflet area with lesions, 3 = 26%–50% leaflet area with lesions and limited chlorosis, 4 = 50%–99% leaflet area with lesions and extensive chlorosis and 5 = complete defoliation.

2.3. On-station field evaluation of farmer preferred bean varieties

A field that had been planted to beans in the previous season was used for this trial. This was done to increase the chances of infection resulting from inoculum retained in the debris of beans produced in the previous season. Fields were dug, and harrowed two days to

planting. The first planting was done in September 2010 and the second in April 2011. The 40 varieties evaluated were planted in a Randomized Complete Block Design (RCBD). Since we had both bush and climbing bean varieties, the climbers were planted separately from the bush varieties so as to avoid effects of any microclimates that may be created by the highly leafy climbing types. Each variety was planted in a 6 m row, with inter- and intra-row spacing of 1 and 0.1 m, respectively, for bush varieties, and 1 and 0.2 m for the climbers. Each variety was replicated three times and the experiment repeated once.

Disease severity was assessed on each variety on the second trifoliate leaf at R8 (pod filling stage) using the scale by Inglis et al. (1988). ALS severity was assessed on six plants chosen from each row of a replicate at intervals of 1 m.

2.4. ALS disease development in varietal mixtures

Two market class bean varieties Shemererwa and Sugar 31, moderately resistant and susceptible to ALS, respectively, chosen from the studies in 2.2 and 2.3 above were used in this study. Fields previously used for the varietal screening experiments in 2.3 above were used. The experiment was conducted in September 2011 and repeated in April 2012.

Treatments consisted of plots where the two varieties were planted together using different row arrangements (referred to in this paper as mixtures) or in pure stands. The latter acted as controls. Mixture treatments (Figure 1) included (1) alternating rows of susceptible and resistant varieties (1S:1R), (2) two rows of susceptible variety followed by two rows of resistant variety (2S:2R), (3) two rows of susceptible variety followed by a single row of resistant variety (2S:1R), (4) a single row of susceptible variety followed by two rows of resistant variety (1S:2R) and (5) the resistant and susceptible varieties planted in the same rows (equal amounts of the two varieties mixed prior to planting – here termed as the even mixtures). The control treatments included pure stands of and susceptible varieties. The experiment was laid out on 19 m × 47 m field with an additional 2 m (four rows) of NABE 4 all around it as a guard. Treatments were laid out in 5 × 5 m plots with 2 m spacing between plots and between blocks; the whole experiment was arranged in a RCBD, with three replicates. Planting was done as in 2.3 above and there was no artificial inoculation (disease development relied on field inoculum).

ALS disease was assessed three times during the season at intervals of 14 days; at 50% flowering, pod initiation and pod filling. For disease assessment, four middle rows within a plot were used to avoid edge effects. Twenty plants (five per row) were selected at 1 m intervals. Disease severity was assessed on the second trifoliate leaves using the 0–5 scale (Inglis et al. 1988) described in 2.2 above.

2.5. Data analysis

Disease severity data were entered into Excel worksheets. Mean disease severity for every variety was then computed. This data was subjected to Analysis of variance (ANOVA), and treatment means compared using least significant differences (LSD), using the SAS/STAT ® software version, 9.1 (SAS Institute 2003).

For the varietal mixture experiment, Area Under Disease Progress Curves (AUDPCs) were calculated separately for each variety within the mixture, then they were combined and their weighted means calculated to determine the disease level for each mixture combination. The AUDPCs were calculated using the method by Campbell and Madden (1990), as defined below:

$$\text{AUDPC} = \sum_{1}^{n-1} \left(\frac{i_1 + i_2}{2} \right) (t_2 + t_1)$$

where i_1 = disease incidence at time t_1; i_2 = disease incidence at time t_2 and $\sum$ = summation.

The weighted means were calculated as follows: $M = ((n_a R) + (n_b S))/n_a + n_b$, where M is the mixture, R and S are the AUDPCs for the resistant and susceptible components, respectively, and n_x is the proportion of the component in the mixture.

The AUDPCs were then subjected to ANOVA using the SAS software. Means were compared using LSDs. For each mixture treatment, the mixture efficiency was calculated as the relative difference between disease severity of the mixture and that for the pure stand of susceptible variety. Mixture efficiency (expressed as a percent) was calculated using disease severity scores at the end of the season (final severity assessed at pod filling), using the following formula:

$$E = [1 + (\text{Sm}/\text{Ss})] \times 100$$

where E = mixture efficiency; Sm = severity in mixture and Ss = severity in susceptible variety.

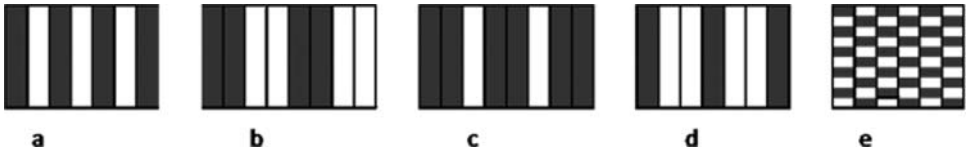

Figure 1. Illustration of varietal mixture arrangements in the order of listing above.

The percentage disease reduction by the mixture relative to the monocultures was also computed. This was calculated by comparing the AUDPC values observed in the mixture against the mean of the susceptible and resistant controls using the following formula:

$$R = \left[\frac{\pi(RS) - \mathrm{Sm}}{\pi(RS)}\right] \times 100$$

where R = reduction of disease in mixture relative to pure monocultures; Sm = severity in mixture and π(RS) = mean of pure resistant and susceptible control.

3. Results

3.1. Reaction of farmer preferred varieties to ALS disease in the screenhouse

Significant differences ($P < 0.0001$) were observed in the reaction of different bean varieties to ALS disease during the first screening experiment. The variety Mexico 54, which is the resistant check, did not develop any disease symptoms during the season. Of the varieties that showed disease symptom, Shemererwa had the least disease severity, 0.22 (Table 1). This was followed by Katosire with a mean disease severity score of 0.78. Whereas, Mexico 54 had the lowest disease severity score, this score was not significantly different from severity score for Shemererwa, Katosire and Nabe 10C. However, within the same experiment, the following varieties were completely defoliated by the disease: Nambale long, Nambale short, Gantagasize, Kadugala, Kahura short, Manyigamulimi, Shemeshenowa, Kakulungu, Rushare II, Nakaseke brown dotted, Kankuryembarukye Army Green, Nakaseke beauty, Nakaseke small red, Kasirira and Sugar 31 red. In the second screening experiment, there was also a significant difference in ALS disease severity ($P < 0.0001$) between varieties. Varieties Mexico 54, Katosire and Akeru short did not show any disease symptom (Table 1). While Nakaseke beauty, Nakaseke brown dotted and Kahura short were completely defoliated by ALS.

Table 1. Angular leaf spot severity on farmer preferred bean varieties 21 days after inoculation in the screenhouse.

Variety	Growth habit	Seed size	Mean disease severity ± SE Experiment 1	Mean disease severity ± SE Experiment 2
Kahura short	Bush	Medium	5.00 ± 0.00	5.00 ± 0.00
Nakaseke beauty	Bush	Small	5.00 ± 0.00	5.00 ± 0.00
Nakaseke brown dotted	Bush	Small	5.00 ± 0.00	5.00 ± 0.00
Kaki short	Bush	Small	4.78 ± 0.11	4.78 ± 0.11
Karolina	Bush	Small	4.78 ± 0.22	4.78 ± 0.22
Kadugala	Bush	Small	5.00 ± 0.00	4.56 ± 0.29
Kasirira	Bush	Small	5.00 ± 0.00	4.33 ± 0.33
Shemeshenowa	Climber	Large	5.00 ± 0.00	4.00 ± 0.00
Rushare II	Bush	Large	5.00 ± 0.00	3.67 ± 0.88
Kanyebwa short	Bush	Medium	4.56 ± 0.29	4.00 ± 0.00
Mahega long	Bush	Large	4.67 ± 0.33	3.56 ± 0.73
Gantagasize	Bush	Medium	5.00 ± 0.00	3.00 ± 1.15
Kakulungu	Bush	Medium	5.00 ± 0.00	2.78 ± 0.22
Nambale short	Bush	Medium	5.00 ± 0.00	2.33 ± 0.88
Kihura	Bush	Large	4.33 ± 0.51	2.78 ± 0.91
Kankuryembarukye army green	Bush	Medium	5.00 ± 0.00	2.00 ± 0.58
Manyigamulimi	Bush	Large	5.00 ± 0.00	2.00 ± 0.00
Nakaseke small red	Bush	Small	5.00 ± 0.00	2.00 ± 0.51
Nakawunde	Bush	Large	4.00 ± 1.00	3.00 ± 0.58
Sugar 31 red	Climber	Large	5.00 ± 0.00	2.00 ± 0.58
Nakyewogola	Bush	Large	4.22 ± 0.62	2.44 ± 0.29
Yellow long	Bush	Large	4.56 ± 0.44	2.00 ± 0.58
Yellow short	Bush	Medium	4.67 ± 0.33	1.67 ± 0.33
Kachwekano	Climber	Medium	4.89 ± 0.11	1.33 ± 0.33
Mamesha	Climber	Small	3.33 ± 1.07	2.89 ± 0.67
Nambale long	Bush	Large	5.00 ± 0.00	1.00 ± 0.00
Kanyebwa long	Bush	Large	3.00 ± 1.26	2.67 ± 0.88
Akeru long	Bush	Small	4.00 ± 0.58	1.00 ± 0.58
Bwiseri	Climber	Medium	3.78 ± 0.40	1.11 ± 0.95
NABE 13	Bush	Large	2.78 ± 0.91	1.67 ± 0.33
Rushare purple	Bush	Large	3.44 ± 0.73	1.00 ± 0.58
Akeru short	Bush	Small	4.33 ± 0.33	0.00 ± 0.00
Kankuryembarukye purple	Bush	Medium	2.33 ± 0.88	1.67 ± 0.67
Brown small	Bush	Small	2.56 ± 1.28	1.33 ± 0.33
Kayinja	Bush	Medium	3.67 ± 0.33	0.11 ± 0.11
Kishoga	Bush	Medium	2.11 ± 1.06	0.89 ± 0.59
NABE 10C	Climber	Small	1.00 ± 0.00	1.00 ± 0.00
Shemererwa	Climber	Large	0.22 ± 0.22	1.00 ± 0.40
Katosire	Bush	Small	0.78 ± 0.22	0.00 ± 0.00
Mexico54	Climber	Small	0.00 ± 0.00	0.00 ± 0.00
LSD ($\alpha = 0.05$)			**1.46**	**1.37**
% CV ($\alpha = 0.05$)			**23.06**	**31.6**

Note: SE = standard error, CV = coefficient of variation.

3.2. Reaction of farmer preferred varieties to ALS disease in the field

ALS disease severity was low in the first field screening experiment. However, significant differences were observed in ALS disease severity between varieties ($P < 0.0001$). The variety Nakaseke small red had the lowest disease severity followed by Rushare II and NABE 10C; however, there was no significant difference between the performance of Nakaseke small red and 36 other varieties (Table 2). Kakulungu followed by Kachwekano and Nakaseke beauty on the other hand had the highest disease severity scores (Table 2). In experiment 2, ALS disease severity was much higher than in experiment 1. Similarly, significant differences were observed in disease severity between varieties. For example, NABE 10C had the lowest disease severity (0.68), significantly lower ($P < 0.0001$) than all the other varieties except Shemererwa (Table 2). Kachwekano and Kahura short had the highest disease severities (3.85) in this experiment, although this was not significantly different from that of Kakulungu, Nakaseke beauty, Kanyebwa short and Shemeshenowa.

Overall, the varieties Shemererwa, NABE 10C, Katosire, Akeru Short, Akeru long, Bwiseri and Kaki short consistently had low disease severities in the field while Kakulungu, Nakaseke beauty, Kanyebwa short and Sugar 31 red on the other hand consistently registered high disease severities.

Table 2. Angular leaf spot severity on farmer bean varieties under natural field infestation at NaCRRI.

Variety	Growth habit	Seed size	Mean disease severity ± SE	
			Experiment 1	Experiment 2
Kakulungu	Bush	Medium	3.02 ± 0.21	3.74 ± 0.14
Kachwekano	Climber	Medium	2.63 ± 0.16	2.85 ± 0.20
Nakaseke beauty	Bush	Small	2.39 ± 0.2	3.80 ± 0.10
Gantagasize	Bush	Medium	2.32 ± 0.29	2.69 ± 0.19
Sugar 31 red	Climber	Large	2.28 ± 0.21	3.24 ± 0.25
Kanyebwa short	Bush	Medium	2.18 ± 0.18	3.82 ± 0.16
Nakaseke brown dotted	Bush	Small	2.11 ± 0.16	3.02 ± 0.18
Brown small	Bush	Small	1.96 ± 0.21	2.39 ± 0.28
Kaki short	Bush	Small	1.96 ± 0.17	1.98 ± 0.19
Shemeshenowa	Climber	Large	1.89 ± 0.13	3.50 ± 0.25
Mahega long	Bush	Large	1.87 ± 0.23	2.74 ± 0.19
Kanyebwa long	Bush	Large	1.86 ± 0.15	2.91 ± 0.26
Kankuryembarukye purple	Bush	Medium	1.85 ± 0.25	2.70 ± 0.21
Nakawunde	Bush	Large	1.85 ± 0.18	2.67 ± 0.16
Yellow short	Bush	Medium	1.83 ± 0.23	3.15 ± 0.15
Karolina	Bush	Large	1.76 ± 0.16	2.61 ± 0.20
Kihura	Bush	Medium	1.74 ± 0.19	3.20 ± 0.20
Nambale long	Bush	Large	1.65 ± 0.16	2.74 ± 0.15
Kankuryembarukye army green	Climber	Medium	1.63 ± 0.2	2.30 ± 0.20
Bwiseri	Climber	Medium	1.59 ± 0.21	1.33 ± 0.20
Manyigamulimi	Bush	Large	1.59 ± 0.17	2.44 ± 0.16
Rushare purple	Bush	Large	1.59 ± 0.21	2.44 ± 0.19
Akeru long	Bush	Small	1.52 ± 0.14	1.91 ± 0.20
Kishoga	Bush	Medium	1.52 ± 0.21	2.46 ± 0.22
Katosire	Bush	Small	1.5 ± 0.16	1.83 ± 0.22
Nakyewogola	Bush	Large	1.5 ± 0.21	2.48 ± 0.19
Yellow long	Bush	Large	1.46 ± 0.21	3.04 ± 0.14
Kadugala	Bush	Small	1.43 ± 0.24	2.48 ± 0.21
Mamesha	Bush	Small	1.42 ± 0.16	3.07 ± 0.32
Kahura short	Bush	Medium	1.41 ± 0.15	3.85 ± 0.15
Nambale short	Bush	Medium	1.39 ± 0.2	2.33 ± 0.24
Akeru Short	Bush	Small	1.19 ± 0.15	1.82 ± 0.20
Kasirira	Bush	Small	1.19 ± 0.23	2.06 ± 0.25
Shemererwa	Climber	Large	1.18 ± 0.26	1.09 ± 0.17
Kayinja	Bush	Medium	1.17 ± 0.14	2.11 ± 0.24
Mexico 54	Climber	Small	1.15 ± 0.18	3.24 ± 0.24
NABE 13	Bush	Large	1.15 ± 0.23	2.15 ± 0.22
NABE 10C	Climber	Small	1.11 ± 0.12	0.68 ± 0.06
Rushare II	Bush	Large	1.07 ± 0.13	2.75 ± 0.15
Nakaseke small red	Bush	Small	1 ± 0.17	2.48 ± 0.22
LSD ($\alpha = 0.05$)			**0.52**	**0.56**
CV ($\alpha = 0.05$)			**48.5**	**33.0**

3.3. ALS disease development in varietal mixtures

ALS disease was observed in the fields in both seasons 1 and 2 of the experiment. In season 1, the highest AUDPC value (53.53) was observed in pure stands of the susceptible variety and the lowest (21.54) in pure stands of the resistant one (Table 3). Mixture combinations had significantly reduced AUDPC values, compared to pure stands of the susceptible variety ($P < 0.0001$). However, significant differences were observed among mixture combinations. For example, the even mixture combination had the lowest overall AUDPC value, while the 2S:1R had the highest.

Looking at the susceptible component within a mixture, the even mixture treatment produced a significantly lower AUDPC value for the susceptible component, compared to the other mixtures (Table 3). This was followed by the 2S:1R arrangement. The 1S:1R arrangement had the highest AUDPC value for the susceptible component among all mixture combinations. However, the latter was not significantly higher than that for the pure susceptible stand.

The effect of the different mixture treatments on ALS disease development on the resistant component however was not very pronounced in most of the mixtures. Interestingly however the even mixture combination had lower AUDPC value compared to the pure stand of the resistant check.

Looking at mixture efficiencies, the even mixture combination had significantly higher mixture efficiency than any other mixture combination. It was distantly followed by the 2S:2R combination which had an almost 10% lower efficiency. The 2S:1R had the lowest mixture efficiency of all the mixture combinations.

Generally considering the disease reduction per mixture compared to the weighted mean of the controls, the even mixture followed by the 1S:2R combinations exhibited the highest disease reductions. However, the 2S:1R and the 1S:1R combinations exhibited higher disease levels compared to the weighted mean of the controls.

In the second trial, the susceptible variety still had the highest AUDPC value, with the resistant check having the lowest (Table 4). However, disease levels on the mixtures were substantially lower than the pure susceptible component unlike in the previous season. Furthermore, unlike in the first trial, non-significant differences were observed in ALS disease severity among mixture

Table 3. AUDPC values for angular leaf spot disease in susceptible and resistant bean varieties planted in different spatial arrangements under natural field infestation (season 1).

Mixture treatment[a]	AUDPC values			Mixture efficiency (%)	Disease reduction in mixture (%)
	Susceptible	Resistant	Mixture		
S	53.5	–		–	
2S:1R	44.5	22.6	37.2	27.1	−13.9
1S:2R	51.1	26.1	34.4	28.0	20.3
1S:1R	54.2	21.4	37.8	29.4	−0.8
2S:2R	47.9	21.1	34.5	35.6	8.0
Even mixture	42.2	16.3	29.2	45.3	22.1
R	–	21.5		–	
LSD		4.8			
CV		37%			

Note: [a]1S:1R = one row of resistant and one susceptible variety, 2S:1R = two rows of susceptible and one of resistant variety, 1S:2R = one row of susceptible and two of resistant variety, 2S:2R = two rows of susceptible and two of resistant variety, even mixture = equal proportions of resistant and susceptible varieties mixed prior to planting, *S* = pure stand of susceptible variety and *R* = pure stand of resistant variety.

Table 4. AUDPC values for angular leaf spot disease in susceptible and resistant bean varieties planted in different spatial arrangements under natural field infestation (season 2).

Mixture treatment[a]	AUDPC values[b]			Mixture efficiency (%)	Disease reduction in mixture (%)
	Susceptible	Resistant	Mixture		
S	60.7	–		–	
2S:1R	57.5	16.5	43.8	23.8	−13.47
1S:2R	43.6	22.5	29.5	32.3	6.42
1S:1R	46.3	31.0	38.7	16.5	7.25
2S:2R	51.4	20.2	35.8	30.4	4.15
Even mixture	46	18.5	32.25	29.9	14.25
R	–	16.5		–	
LSD		5.6			
CV		43%			

Note: [a]1S:1R = one row of resistant and one susceptible variety, 2S:1R = two rows of susceptible and one of resistant variety, 1S:2R = one row of susceptible and two of resistant variety, 2S:2R = two rows of susceptible and two of resistant variety, even mixture = equal proportions of resistant and susceptible varieties mixed prior to planting, *S* = pure stand of susceptible variety and *R* = pure stand of resistant variety.
[b]Values presented are means ± SE.

combinations. However, similar to the first trial, the even mixture combination had the lowest disease levels. Just like in the first trial, mixture combination 2S:1R still had the highest overall disease severity.

Looking at the susceptible variety, the lowest severity was observed in the mixture combination 1S:2R with AUDPC value of 43.6. This was followed by the even mixture combination. The highest severity for the susceptible variety was observed in the mixture combination 2S:1R.

Disease development on the resistant variety in the different mixture combinations had similarities to that observed for the susceptible variety. For example, the lowest disease severity for the resistant variety in the mixtures was observed in the even mixture combination and this was similar to disease severity in the pure resistant control. The second lowest disease severity for the resistant variety was also observed in the even mixture combination. Unlike the susceptible variety, the highest severity for the resistant variety in the mixtures was observed in mixture combination 1S:1R.

A trend similar to that observed in the first trial was noted for mixture efficiency in the second trial. Mixture combination 1S:2R had the highest mixture efficiency though this was not significantly different from the efficiency of the even mixture combination. The 2S:1R mixture combination still had the lowest efficiency.

Generally in the second season, almost all the mixture combinations apart from the 2S:1R exhibited reduced disease levels compared to the weighted mean of the controls. However, the even mixture combination showed the highest disease reduction followed by the 1S:1R.

4. Discussion

Our study recorded significant differences in the reaction of different bean varieties to ALS disease, both in the screenhouse and field.

In the screenhouse, there was a great disparity in the reaction of the varieties to ALS disease, with the highest (5) and lowest (0) severity scores both observed. We speculate that maximum disease developed because of the optimum conditions for *P. griseola* infection and disease development in the screenhouse. First, artificial inoculation ensured the availability of inoculum, and second, environmental conditions such temperature and humidity was conducive for infection and disease development. According to Inglis and Hagedom (1986), a temperature range of 16–28 °C, but an optimum of 24 °C is conducive for *P. griseola* infection of beans. Beebe and Pastor-Corrales (1991) reported that an optimum humidity of >95% is required for *P. griseola* infection. Only Mexico 54, a known resistant check for ALS disease (Sartorato et al. 1999), showed complete resistance in the screenhouse.

Two local varieties (Katosire and Shemererwa), and an improved variety (NABE 10C), showed reduced ALS disease severity in the screenhouse. The low disease levels exhibited by these varieties could mean that they are moderately susceptible to the *P. griseola* isolates they were exposed to. The varieties Nakaseke brown dotted, Kahura and Nakaseke beauty were defoliated by the disease in both experiments. Defoliation is important in ALS disease evaluation, as it denotes the most adverse effect of the disease, the reduction in photosynthetic area (Bergamin et al. 1997).

In the first field screening experiment (2010), disease severity levels were generally low throughout the study, with most varieties getting disease scores of 2 or less which generally falls within the resistant and moderately resistant boundaries using a comparative analogy with the International Center for Tropical Agriculture (CIAT) scale (van Schoonhoven & Pastor-Corrales 1987). However, higher disease severity scores were observed in 2011. These observations, together with the findings of the screenhouse experiment, show that the resistance exhibited by the varieties in the first field experiment was affected by uncontrolled factors. We attribute the low ALS disease levels to low field inoculum since the trial was planted in a new field (not previously planted to beans). This inference can be supported by the fact that more disease was observed in 2011 when beans were planted in a field previously planted to beans. This therefore indicates that crop rotation and fallowing

can be used as effective methods of managing ALS since they reduce inoculum levels in the field.

Mexico 54 the resistant check showed no disease symptoms in the screenhouse and a low disease score in the field in 2010. However, the variety registered a high ALS severity score in 2011. This is not uncommon with ALS disease, where varieties resistant in one location and season may become susceptible in another location or season (Pastor-Corrales et al. 1998; Silva et al. 2008; Wagara et al. 2011). This phenomenon is attributed to the high pathogenic variability within the pathogen population. The same reason explains the difference in reactions of the varieties to the disease in the field across the two seasons and between the screenhouse and the field. This is because although the same pathogen mixture was used in the screenhouse for both the first and second experiments, in the field there was a higher pathogenic variation whose composition probably changed from one season to the next due to inoculum movement and natural selection. We speculate that the difference in reaction of some varieties between the two screenhouse experiments was due to the difference in seed sets used.

The results of the current study show that varietal mixtures significantly reduce ALS disease compared to the weighted means of their pure components. This is similar to findings by Pyndji and Trutmann (1992) who obtained reduced ALS levels by supplementing farmer mixtures with resistant varieties. Generally, all the mixture combinations led to reduced ALS disease severity on the susceptible component, but certain mixture combinations were more effective than others. Disease reductions in mixtures (mostly intra-row varietal mixtures) for crops other than the common bean have been reported (Wolf 1985; Akanda & Mundt 1996; Ntahimpera et al. 1996). AUDPC, mixture efficiency and a comparison of the disease levels in the mixtures with the mixture means of the pure controls are important tools for comparing the effect of varietal mixtures on disease severity. The higher the mixture efficiency and the lower the weighted mean of the mixture, the more effective the mixture combination in reducing disease development. The reliability of the findings of our study is supported by the fact that in both seasons 1 and 2, the highest and lowest AUDPCs were observed for the susceptible and resistant varieties, respectively.

The even mixture combination consistently showed the highest disease reductions. Even mixtures are presumed to reduce disease in the following ways: first, varietal mixtures in which each hole has a different variety has a lower Genotype Unit Area (GUA) than mixtures consisting of alternating rows of different varieties. This reduction in the unit area occupied by an independent unit of susceptible tissue makes it difficult for spores to move from one susceptible plant to another due to increased distance between susceptible plants (Wolf 1985; Mundt 2002). According to Castilla et al. (2003), GUA is the most important factor determining mixture efficacy against pathogens that are spread by rain splash, like ALS. Second, the presence of resistant varieties between the susceptible plants acts as barriers to the free movement of spores and insect pests between the susceptible plants (Browning & Frey 1969; Ssekandi et al. 2016). Similar studies by Mundt et al. (1996) also showed that mixing varieties within rows considerably reduced yellow rust in wheat compared to alternating pure stands (mixing between rows). The other mixture combinations had very inconsistent reactions in the two seasons. Taking the 1S:2R combination for example, previous findings have shown that increasing the amount of resistant components in varietal mixture increases mixture efficiency (Castilla et al. 2003). In this study, however, the increased amount of the resistant variety in the 1S:2R arrangement was countered by the fairly large GUA of the susceptible components compared to the even mixture for example. Considering the small size of the plots, it is possible that the influence of external environmental factors could have been the determinant of which of the two factors above determined the reaction of the 1S: 2R mixture in each season. The same reason could be suggested for the inconsistent behavior of the other mixture arrangements. Small mixture plots are known to be adversely affected by exogenous and inter plot factors (Garrett et al. 2001; Mundt 2002; Skelsey et al. 2005). For example, in small plot experiments, more susceptible neighboring plots may produce large amounts of inoculum, thereby reducing the effect of the mixture. However, the effect of these factors may vary depending on external environmental conditions and the random location of the specific mixture combination in the experiment.

The results of this study have demonstrated that mixtures of moderately resistant and susceptible varieties are a viable management alternative for ALS disease of beans. While there are no bean varieties that are completely resistant to ALS in Uganda, the use of varietal mixtures especially the more effective even mixture combination should therefore be encouraged among resource poor farmers to manage the disease. It is important however to note that the introduction of exotic varieties as the resistant component in the mixture may lead to genetic erosion because of the reduced amount of the susceptible component in the varietal mixture. Hence, for purposes of bean biodiversity conservation, local farmer preferred resistant varieties are recommended.

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