Research Article

Effect of Angular Leaf Spot Disease on Bean Inter-cropped with Maize in Foumbot and Bambili, Cameroon

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ABSTRACT

The ability to increase beans output at local levels is often hindered by farmer's ability to control angular leaf sport disease caused by *Phaeoisariopsis griseola*, in the field. Studies were carried out in Foumbot and Bambili, using a Randomized Complete Block Design (RCBD) with four treatments and four replications in order to assess the impact of intercropping on angular leaf spot disease. Two varieties of bean were used (white dwarf and red dwarf marked beans). Treatments one and two consisted of mono-cropping of white dwarf and red dwarf marked bean varieties while treatments three and four consisted of the same varieties of beans inter-cropped with maize respectively. The data on disease incidence, disease severity, number of pods and pods weight were recorded in mono-cropped and inter-cropped fields. Pathogenicity assessment was carried out in the screen house by inoculating healthy plants with fungal isolate (3×104 spores / ml), during which the lesion diameter was measured. Beans varieties inoculated with spores' suspension of *P. griseola* in the screen house showed symptoms identical to that of infected plants in the fields. The results obtained for disease severity and yield revealed that there was a significant difference (Pd"0.05) on the different treatments and in the different fields. The best yields and least disease severities of beans were observed in fields inter- cropped with maize because wind and rainfall splashes were intercepted by the plant which is not a host to *P.gliseola* thus a reduction of disease in the inter-cropped fields' plant which is not a host to *P.gliseola* thus a reduction of disease in the inter-cropped fields. This shows that intercropping beans with maize can be used as a best management option to control angular leaf spot disease for the two varieties of beans used in this study.

Key words: Angular leaf spot disease, beans, intercropping, Maize, Bambili Foumbot, Cameroon.

Received: 04/07/2023

Accepted: 26/09/2023

DOI: https://dx.doi.org/10.4314/jcas.v19i3.2

RESUME

La possiilité d'augmenter la production de haricots au niveau local est souvent entravée par la capacité des agriculteurs à contrôler la maladie de la tache angulaire causée par *Phaeoisariopsis griseola*, dans le champ. Des études ont été menées dans les champs de Bambili et Foumbot pour évaluer l'impact de la culture intercalaire sur la maladie des taches angulaires. Les champs expérimentaux ont été conçus selon un plan en blocs complets randomisés de quatre traitements différents répétés quatre fois. Les traitements un et deux étaient constitués de monoculture de haricots nains blancs et de haricots nains rouges marqués. Les traitements trois et quatre étaient constitués de haricots nains blancs et de variétés de haricots nains rouges marqués en culture intercalaire avec du maïs. Les données sur l'incidence de la maladie, la sévérité de la maladie, le nombre de gousses et le poids des gousses ont été enregistrées dans les champs en monoculture et les champs en culture intercalaire. L'évaluation de la pathogénicité a été effectuée en inoculant des plantes saines dans la serre avec un isolat fongique (3×104 spores / ml) et en mesurant le diamètre des lésions. Les variétés de haricots inoculées avec une suspension de spores de *P. griseola* dans la serre ont présenté des symptômes identiques à ceux des plantes infectées dans les champs. Les résultats obtenus à partir de l'analyse de la variance sur la sévérité de la maladie et le rendement ont révélé qu'il y avait une différence significative (Pd''0.05) sur les différents traitements dans les différents champs, à l'exception de l'incidence de la maladie. Les meilleurs ont été observés dans les champs cultivés en association avec du maïs parce que les éclaboussures de vent et de pluie ont été interceptées par la plante de maïs qui n'est pas un hôte de *P. gliseola*, donc une réduction des maladies dans les champs entre cultures Cela indique que la culture intercalaire de haricots et de maïs devrait être utilisée pour contrôler la maladie des taches foliaires angulaires des haricots pour la variété naine de haricots blancs et les haricots rouges marqués dans le champ.

Mots clés : Maladie des taches angulaires, haricots, culture intercalaire, maïs, Bambili, Foumbot, Cameroun.

INTRODUCTION

Bean (*Phaseolus vulgaris*) is a herbaceous plant which originated from Meso-America and later on spread to other parts of the world including Africa (Martha *et al.*, 2017). Beans are widespread leguminous plants throughout the world occupying a cultivation area of approximately 12.5 million hectares (FAO, 2006). In Cameroon, the sales of beans provide income to farmers and traders in the Northwest and Western Regions. Bean is consumed by man and it is a good source of protein, carbohydrates, vitamins and minerals, such as iron and zinc (Broughton *et al.*, 2003; Suárez-Martínez *et al.*, 2015).

The global total production of dry bean in the world was 22.8 million metric tons in 2014, out of which 17 % was produced in Africa (FAOSTAT, 2014). The Institute of Agricultural Research for Development (IRAD) has developed and introduced many improved bean varieties in Cameroon which led to an increase in production from 198000 tons in 2005 to 265000 tons in 2010 (Siri *et al.*, 2016). These beans varieties are moderately susceptible to angular leaf spot disease.

Reduction in yields of baens can be attributed to many factors such as adverse climatic conditions, low soil fertility, pest and diseases (Mwangombe *et al.*, 2007). Angular leaf spot caused by *Phaeoisariopsis griseola* is one of the most destructive diseases of beans in Cameroon, the tropics and sub-tropics (Stenglein *et al.,* 2003). The disease is of great economic importance in Eastern and Central African countries (Sengooba and Mukiibi, 1986; Pastor-Corrales *et al.*, 1998). Angular leaf spot causes up to 80 % yield loss of beans in susceptible genotypes under severe conditions of infection (Singh and Schwartz, 2010). Disease occurrence, epidemic development and severity are influenced by cropping systems and production practices, topographical features, crop genotypes, altitudinal ranges, cropping season and field management practices under a given environment (Zhu *et al*., 2000).

Several management practices such as growing resistant variety, fungicides application and intercropping have been used to control the disease (Mahuku *et al*., 2002). Resistant varieties are very effective in managing the disease at low cost in small scale farms (Mahuku *et al*., 2009). Intercropping has many advantages, such as perfect utilization of environmental factors, soil protection and production of different varieties of crops (Yayeh, 2014). However, losses associated with high angular leaf spot (ALS) disease epidemics still prevail and have been shown to vary in different cropping systems (Schoeny *et al*., 2010). Angular leaf spot disease in beans causes serious economic losses to the farmers of the North West and Western Regions of Cameroon. Thus, this research work was conducted to determine how when inter-cropped with maize, the disease develops and affects yield of bean in IRAD Foumbot and Agricultural School Bambili, in Cameroon.

MATERIALS AND METHODS

Experimental sites

Two field sites selected for this experiment were the Institute of Agricultural Research for Development (IRAD),Foumbot and Agricultural School research farm, Bambili. IRAD Foumbot is located between latitude 5° 25'- 5° 35' N and longitude 10° 33'-10° 44' E with a mean altitude of 1100 m above sea level. The Agricultural School research farm Bambili lies between latitudes 5 ° and 6 ° North of the equator and longitudes 10 ° East of Greenwich Mean Time and at an altitude of between 1600 to 2000 m above sea level. Laboratory analysis and screen house experiments were conducted in the Catholic University Laboratory, Bamenda**.**

Climate dynamics of Foumbot and Bambili.

The two locations fall under the Western Highlands Plateau Agro ecological zone of Cameroon. The climate is tropical, characterised by two seasons, a long rainy season which starts from mid-March and ends in mid-November and a dry season from mid-November to mid-March. The average monthly temperatures and rainfall are 21˚C and 1713 mm respectfully (Gwanfobe *et al.,* 1983).

Effect of angular leaf spot disease on beans inter-cropped with maize

Two varieties of beans, white dwarf bean (BGG) and the red marked bean (GLP) and one variety of maize (Cameroon maize selection (CMS)) were used for this study. The fields' layout was a Randomised Complete Block Design (RCBD) with four treatments and four replications. Treatments one and two consisted in mono-cropping of white dwarf bean (BGG) and red dwarf marked bean (GLP) varieties planted at a distance of 25 cm x 25 cm between plants and within rows. Treatments three and four were BGG and GLP inter-cropped with maize at inter-row spacing of 100 cm for maize and 25 cm for bean. The fields were planted on the 17 July 2020. Two seeds were planted per hole for all the treatments. Weeding was done manually with hands and hoe at two weeks' interval till maturity. After the first weeding, the plants were molded 16 days after planting (DAP).

Assessment of disease incidence and severity (*Pheaosariopsis griseola*)

Disease incidence and severity were recorded at weekly intervals starting from the first appearance of the symptoms in the field. Disease incidence was assessed by counting the number of infected plants in the middle ridges at 7 days' interval.

Determination of Percentage Disease Incidence

The percentage disease incidence was calculated using the following formula:

$$Disease\ incidence = \frac{\text{Number of infected bean plants}}{\text{Total number of bean plants}} \times 100$$

Determination of disease severity

The severity symptoms of each bean variety were scored using the scale from 1-5 (Fokunang *et al.*, 2000) according to which:

1.= No symptom

2. = Few angular leave spots

3.= Many leaf spots covering 50% of the leaf infected with angular leave spot

4.= Leaf spots covering over 80 % of affected leave

5.= Complete defoliation and plant damage

Yield Assessment of beans

The beans planted in the two different locations (Foumbot and Bambili) were harvested at 81 DAP. The yield was assessed by counting the number of pods per plant. The pods were then weighed using an electronic scale.

Pathogencity assessment of *Pheaosariopsis gliseola* from infected plant materials

Bean leaves showing lesions of angular leaf spot disease were collected from Foumbot and Bambili fields, each preserved in separate paper bags and transported to the Catholic university (CATUC) laboratory in Bamenda. The leaves were cut into small fragments of 2 mm from the edges having blight symptoms. The fragments were surface-sterilized in 5 % dilute solution of sodium hypochlorite for 20 seconds and rinsed four times in sterilized distilled water in Petri dishes for 5 minutes. The leave fragments were then dried on sterilized filter paper and three fragments placed on solidified cool V_8 culture medium. These dishes were labeled and placed at room temperature (22-24°C). Four days later, they were subculture three times to obtain a pure culture' (Fig. 1) (Manju *et al.*, 2020).

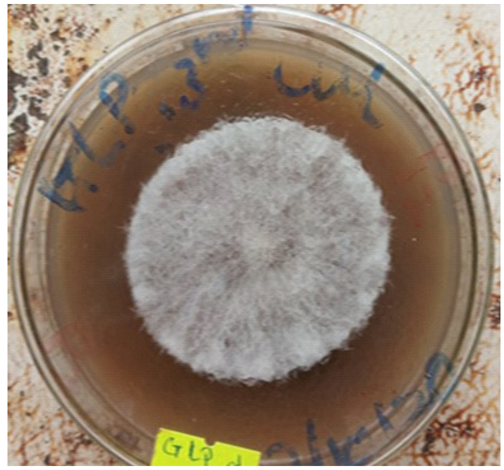

Figure 1. Pure culture of *Pheaosariopsis gliseola*.

Preparation of inoculums

The pure culture obtained was used to prepare a spore suspension. Five ml of sterile distilled water were poured into a beaker. The spores in each Petri dish were brushed into two separate beakers. The spores were adjusted with a haemocytometer to a spore density of 3×10^4 spores / ml of distilled water and transferred into two separate syringes for inoculation in a screen house.

Screen house test

The two beans varieties (white dwarf bean) and (red marked bean) were planted in plastic pots filled with steam sterilized soils in a screen house. These plants were arranged in a Completely Randomized Design (CRD) with two replicates of four plants per replicate. The beans were inoculated 20 days after planting with a spore suspension of *Pheaosariopsis gliseola*. Inoculation was done by using a syringe to inject the spore suspension on one spot on each leaf. Observations were made and lesion area was measured using a ruler. Data for average lesion area was recorded for 9 days by multiplying the length and the width of the infected area (Manju *et al.*, 2020)

Statistical analysis

Data collection for disease incidence, severity, yield parameters, and area of lesion of *Pheaosariopsis gliseola* for the two varieties of beans were subjected to analysis of variance (ANOVA) using statistical software (JUMP11). Their treatment means were separated using Turkey HSD and least significant difference (LSD) at statistical significance of 95% confident interval (Pd"0.05). Mean data were used to plot graphs for appropriate representation of the results.

RESULTS

Effect of angular leaf spot disease incidence (*Pheaosariopsis gliseola*) on beans mono-cropping and beans and maize intercropping

Angular leaf spot disease symptoms appeared in Foumbot field at 17 DAP while in Bambili, it appeared at 24 DAP with small water-soaked lesions on the leaves. Disease incidence of 100 % was recorded on the Foumbot field at 31 DAP for white dwarf bean variety (BGG) and 33 DAP for the red marked dwarf bean variety (GLP). For the Bambili field, 100 % disease incidence was recorded at 38 DAP for the white dwarf bean variety and 41 DAP for the red marked bean variety. Mono-cropping and intercropping showed no significance difference in the mean disease incidence at 4-7 weeks in both fields (Foumbot and Bambili) (Fig. 2).

Effect of angular leaf spot disease severity (*Pheaosariopsis gliseola*) on mono-cropped beans and on beans inter-cropped with maize

Disease severities for the different treatments in the two locations were different. Angular leaf spot lesions on the white dwarf bean variety were wider and less spotted than on the red marked variety for both the mono-cropped and inter-cropped patterns. The leaves of the white dwarf bean variety were highly infected for the mono-cropped fields compared to those of red marked dwarf beans for the same treatment. For inter-cropped fields, both varieties of beans showed less infection rate in the inter-cropped fields for the two locations (Foumbot and Bambili).The highest mean disease severity of 3.7 and 5.0 were recorded on white dwarf bean variety for mono-cropped fields compared to the mean disease severity of 2.1 and 3.0 on beans inter-cropped with maize for the two locations (Foumbot and Bambili) at week seven respectively (Fig. 3). Generally, there was a

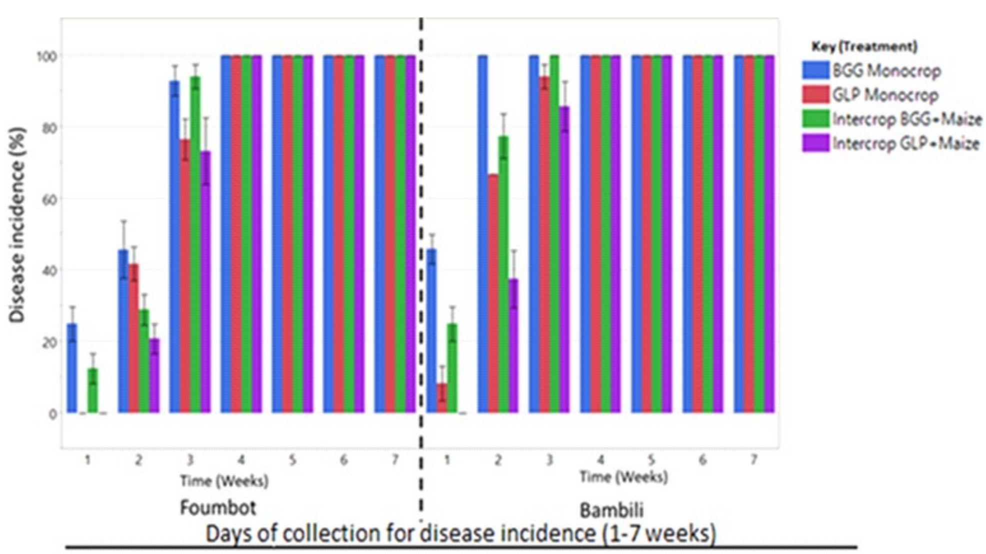

Fig 2: Disease incidence on beans mono-cropped and beans and maize inter-cropped at 1-7 weeks in Foumbot and Bambili respectively.

Bars represent means of disease incidence with standard errors significant difference (P ≤ 0.05) in disease severity on the beans varieties for both mono-cropped and inter-cropped fields in Foumbot and Bambili (Fig. 3).

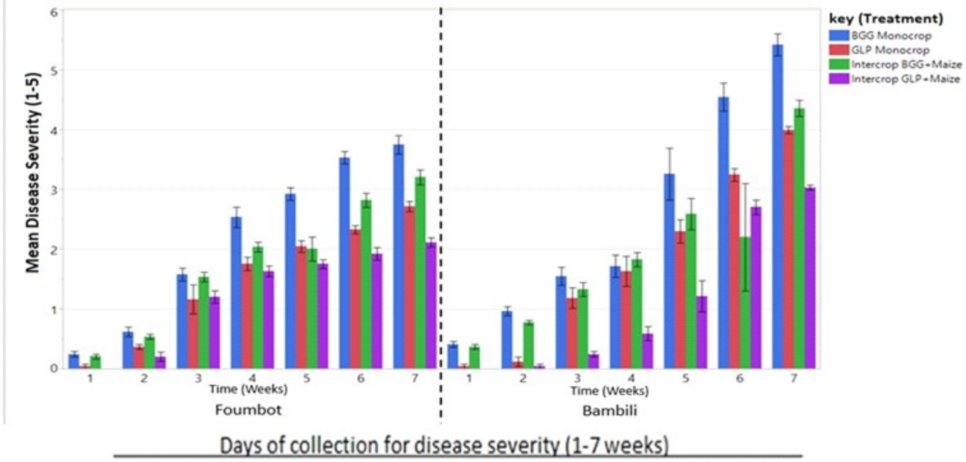

Figure 3: Disease severity on mono-cropped beans and on beans inter-cropped with maize at 1-7 weeks in Foumbot and Bambili.

Bars represent means of disease severity with standard errors.

Assessment of number of pods per plant for monocropping and intercropping of beans and maize

Crops in Foumbot got matured earlier at 67 DAP compared to those in Bambili which got matured at 81 DAP. The number of pods per plant was different for the two locations and in the different cropping patterns. Number of pods per plant was higher for the inter-cropped pattern compared to the mono-cropped pattern. Fields inter-cropped with white dwarf bean variety and maize had the highest mean pods number of 10 and 8 in Foumbot and Bambili respectively. The least mean number of pods of 3 was recorded in the mono-cropped treatment of white dwarf bean variety in Bambili (Fig. 4). Generally, there was a significant difference (Pd"0.05) in the mean number of pods per plant in the mono-cropped compared to the inter-cropped patterns for both locations (Fig. 4).

Assessment of the weight of beans pods in mono-cropped and inter-cropped fields in Foumbot and Bambili

The highest mean weights of 28g and 23g was recorded on the pods of red marked beans in the inter-cropped fields in Foumbot and Bambili respectively, while the lowest mean weights of 8g and 13g were recorded on the pods of white dwarf bean in the mono-cropped fields in Bambili and Foumbot respectively. Comparing the weight of the pods from the two varieties of bean, a significant difference (Pd"0.05) was observed in the mean weights of beans for both mono-cropped and inter-cropped fields in Foumbot and Bambili (Fig. 5).

Pathogenicity testing of *Pheaosariopsis gliseola*

Two days after disease inoculation in the screen house, the disease appeared on the leaves with higher rate of infection observed in dwarf white bean variety compared to the red marked bean variety (Fig. 6). Lesion area increased from day one to seven and became stable up to day nine for all the beans varieties tested. The highest lesion area of 0.67mm^2 was recorded in white dwarf bean variety and the least lesion area of 0.05mm^2 was recorded in red marked beans variety at two days of inoculation.

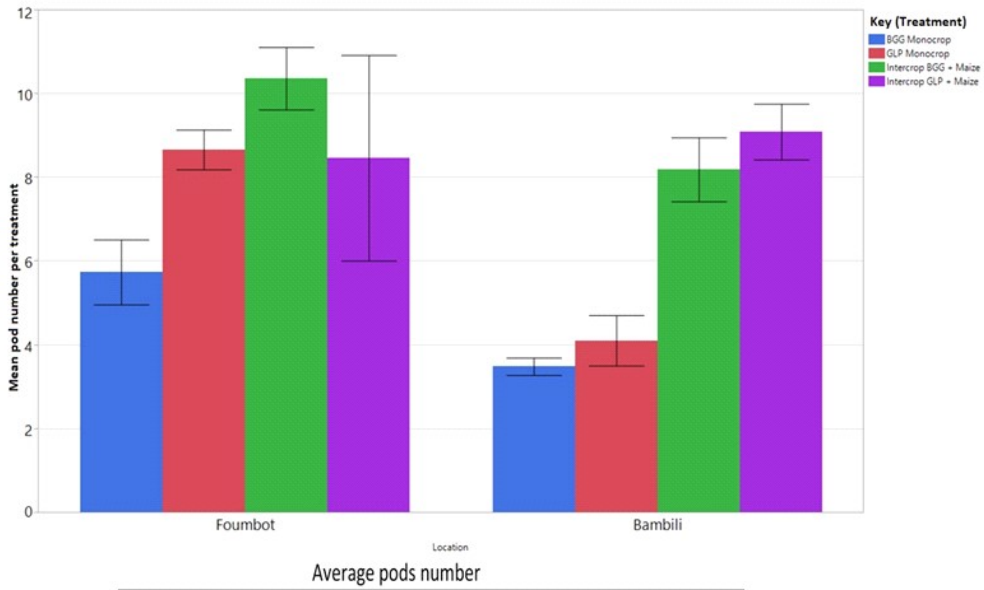

Figure 4: Mean number of pods per plant for beans in mono-cropping and beans and maize in inter-cropping in Foumbot and Bambili

Bars represent mean pod numbers with standard errors.

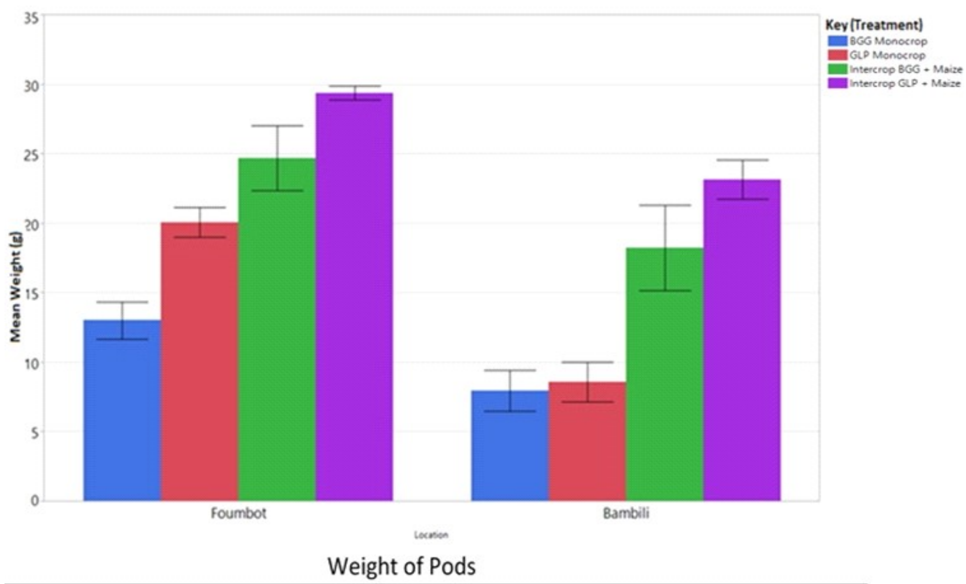

Figure 5: Pods weight of beans in mono-cropped and inter-cropped fields in Foumbot and Bambili

Bars represent mean weight per treatment with standard errors.

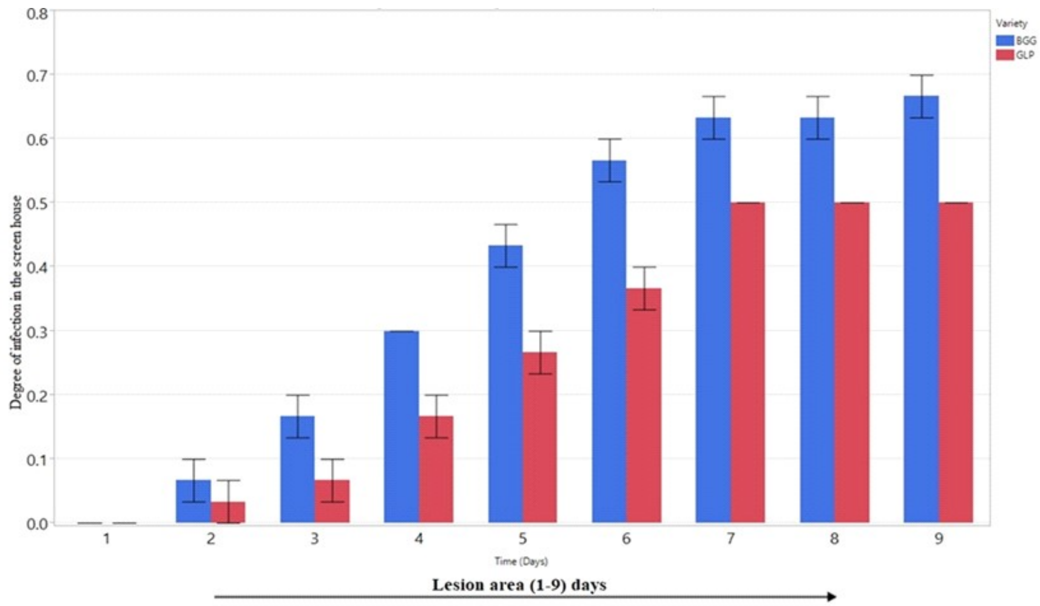

Figure 6: Effect of spores' inoculation on the leaves of BGG and GLP plants in the screen house-

Bars represent mean lesion area with standard errors.

DISCUSSION

Disease incidence occurred earlier in the Foumbout field with warm climate than the Bambili field with cold climate. This could be due to high levels of humidity in Foumbot as compared to Bambili which favoured disease development. Kanz and Rotem (2012) reported that there is high survival of fungi in the warmer environment (Foumbot) compared to a cool environment (Bambili). There was no significant difference in the disease incidence in Foumbot and Bambili fields. Bambili and Foumbot belong to the same agro-ecological zones with the same climatic factors such as temperature and rainfall which favour disease development. Ddamulira *et al*. (2014) reported that environmental conditions of similar agro-ecological zones are known to have same disease occurrence pattern.

Angular leaf spot disease severity was higher in Bambili compared to Foumbot for both mono-cropped and inter-cropped fields. There was a low mean disease severity observed in inter-cropped fields for both Foumbot and Bambili. The spores of *P. gliseola* that were blown by wind and rainfall splashes were intercepted by the plant which is not a host to *P.gliseola* thus a reduction of disease in the inter-cropped fields' plant which is not a host to *P.gliseola* thus a reduction of disease in the inter-cropped fields. This could also be due to an increase in spatial distance between host plants, which might inhibit free dispersal of pathogen and suppress weeds responsible for the build-up of high humidity under the canopy. This is confirmed by Boudreau (2013) who reported that mechanisms by which inter-cropped plants affect disease dynamics include alteration of wind, rain, and vector dispersal; modification of microclimate, especially temperature and moisture; changes in host morphology and physiology; and direct pathogen inhibition.Crops grown simultaneously enhance the abundance of predators and parasites, which in turn prevent the build-up of diseases, thus minimizing the need of using expensive and dangerous chemical pesticides. Langer *et al.* (2007) also reported that when the environment of a neighboring plant and microclimate is altered, the host plants and natural content is also altered. Inter-cropped plants are often less damaged by various pests and disease pathogens than when grown as sole crops. Mixed crop species can also delay the onset of diseases by reducing the spread of disease carrying spores and by modifying environmental conditions so that they are less favourable to the spread of certain pathogens (Risch, 1983).

Fajinmi (2006) stated that intercropping had effect on the population buildup of diseases and reduced disease incidence and severity in cases where the crops used in intercropping are not hosts of the same pathogen. Cropping system employed might have an influence on overall disease development and epidemics (Stenglein *et al.,* 2003; Mwangombe *et al*., 2007).

High mean disease severity was observed in mono-cropped fields. This might be associated to the use of same plant type which is a host to the same pathogen that permit rapid spread of pathogen from neighbouring plants during favourable environmental conditions for disease development (Kijana *et al.,* 2017).

The study also revealed that mean disease severity was higher in Bambili field located at an altitude of 1558 m above sea level than Foumbot at 1118m above sea level. This could be partly due to the high rainfall and relative humidity common in the higher altitude compare to lower altitude, which could favour angular leaf spot infection and development. Mwangombe *et al.* (2007) also obtained high disease incidence and severity in the higher altitude compare to lower altitude due to the cool temperature in high altitude areas that may inhibit angular leave spot development. Angular leaf spot severity is high in the higher altitudes than lower altitude areas (Kijana *et al*., 2017). In addition to this, variation in disease severity for both locations would be attributed to prevailing environmental conditions and crop management practices, which is similarly reported by Sceuemann *et al.* (2012).

Results obtained for number of pods and weight of pods per plant show that there was high number of pods and weight of pods in the inter-cropped fields for both varieties of beans compared to the mono-cropped fields. Number of pods and weight of pods of beans were higher from the Foumbot field than in Bambili field for both cropping systems. This could be due to higher soil nutrient content and lower amount of pathogen inoculum in Foumbot than in Bambili. Higher altitude is characterized by higher rainfalls which courses erosion of soil nutrients leading to a decrease in crop output (Suiven and Zephania, 2018). The amount of inoculum present in each field, and type of cropping system employed might also have an influence on overall crop output (Mwangombe*et al*., 2007). There were significant differences in pods weight in inter-cropped fields compared to mono-cropped fields. The inter-cropped systems had higher yield than the mono-cropped systems. These results are confirmed by Niringiye *et al.* (2005) who reported that sole-cropped bean yields were less than inter-cropped bean yield. Reduction in yield for mono-cropping may be due to competition among the plant population density (Niringiye *et al*., 2005, Mbong *et al*., 2016).

Results obtained after inoculation of *P. gliseola* spores on beans leaves in the screen house show that all the plants were infected by angular leaf spot disease. Angular Leaf infection rate on the white dwarf bean variety was higher than the marked dwarf red variety after inoculation in the screen house. This may be due to genetic variations of the individual bean variety. This result is confirmed by Leung *et al.* (1993) who reported that large pathogenic variation could have been associated with mutation, recombination and migration. Invasion of wounded leaves by the fungus resulted in severe or slight disease development, depending on the bean variety.

CONCLUSION

The study indicates that inter-cropping both varieties of beans with maize reduces *P. gliseola* infection in the field and increases yield for both beans varieties in the Foumbot and Bambili fields .The intercropping of red marked dwarf bean (GLP) with maize was more resistant than white dwarf bean (BGG) inter-cropped with maize. Thus inter-cropping can be used to control angular leaf spot disease in Foumbot and Bambili.

The results obtained on pathogenicity of *P. gliseola* on the different beans varieties revealed that all the two varieties were infected by *P. gliseola* spores of 3×10^4 spores / ml of distilled water.

The white dwarf variety showed a stronger sensitivity to disease infection in the screen house than the red marked dwarf bean (GLP) variety.

ACKNOWLEDGMENTS

The authors wish to thank Catholic University of Bamenda, Agricultural School Bambili and Institute of Agricultural Research for Development Foumbot Cameroon for their technical support and laboratory facilities to aid this study.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author MEB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MGA, KSM and TRK managed the study design, statistics and the literature searches. All authors read and approved the final manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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