Leaf spot of *Telfairia occidentalis* incidence and severity influenced by altitude and planting date in the West Region of Cameroon

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Abstract

Leaf spot disease of *Telfairia occidentalis*, caused by *Phoma sorghina*, represents one of the major biotic constraints to *T. occidentalis* production under small scale farming systems in West Africa. Currently this critically important seed and leaf vegetable is cultivated under varying altitudinal levels at different planting dates. In this framework, a field study was conducted in 2019 and 2020 cropping seasons between March and July in the localities of Dschang and Santchou to determine the influence of altitude and planting date on the incidence and severity the disease.

A 2 by 4 factorial randomized complete block design (RCBD) with three replications and four planting dates were used. Data for disease incidence and severity documented fortnightly, were submitted to analysis of variance using SPSS version 23, and the means were separated by least significant difference (LSD) at a 95% confidence interval.

Statistical analysis revealed that, the low altitude recorded a significantly ($p < 0.05$) lower incidence than high altitude while disease severities between both altitudes were not significantly different. Moreover, the initial three planting dates at low altitude and planting date three in Dschang recorded significantly lower incidences than other planting dates investigated in the study.

We established that, the initial three planting dates at low altitude and planting date three at high altitude could be helpful in reducing leaf spot incidence and severity of *T. occidentalis.*

Keywords: Altitude; incidence; leaf spot; planting date; severity; *Telfairia occidentalis.*

Introduction

Telfairia occidentalis Hook. f. (fluted pumpkin) is one of the highly prized vegetable crops in Cameroon. Growing *T. occidentalis* strongly enhances the livelihoods of poor resource base farmers because it can be harvested and sold throughout the year at weekly intervals compared to other locally cultivated vegetable crops. The crop plant has inherent immense nutritional and medicinal values (Odiaka and Schippers 2004; Kayode and Kayode 2011), with potentials of being used industrially as a food supplement (Odiaka and Schippers 2004).

However, sustainable production is greatly constrained by various diseases each year, of which leaf spot (causal agent: *Phoma sorghina*) in the field is the most important (Annih et al. 2020; Mbong et al. 2021). Leaf spot disease is one of the most important limiting factors for the cultivation of the seed and leaf vegetable crop in tropical and subtropical areas (Bassey and Opara 2016).

In the field, leaf spot appears within three weeks after emergence and continues throughout the life of the plant in the field. The translucent white spots enlarge, turn brown and shatter, leaving the leaves with perforations. The pathogen attacks the leaves of the crop and produces localized lesions of dead or collapsed cells with the consequent effect of reducing the leaf lamina. Under severe attack, the entire leaf dies (Udo et al. 2013). This has the effect of limiting production and degrades its quality, thereby reducing its market value and profitability. Farmers therefore often face substantial plant losses before harvesting with severe economic losses. In addition, infection of leaves by the pathogen also significantly reduces the nutrient content (Udo et al. 2013).

Subsistence farmers in developing countries, including Cameroon, have very few options on the control of leaf spot disease on their crops. Synthetic fungicides can be used in the management of the disease under field conditions. Biweekly spraying of cocktails of synthetic fungicides significantly reduced the disease incidence in the field (Nwufo and Ihejirika 2008). However, the fungicides threaten farmers' health and environment and lead to resistance development of the pathogen against the pesticides while they also impact non-target and beneficial organisms. The chemicals are also not cost-effective (Godwin-Egein et al. 2015). In addition, the poor farmers even lack expertise and other accessories for the proper application of these chemicals (Udo et al. 2013). Improper use of such chemical has led to residue accumulation *on T. occidentalis* (Mbong et al. 2019), which may have intoxicating effects in the body when the vegetable is consumed. With limited preferences as such, the subsistence farmers therefore rely mainly on cultural practices as an important aspect of leaf spot disease control. The shortcomings and the growing desire for organic produced food devoid of synthetic chemicals necessitate the need for alternative disease control that are applicable, inexpensive, safe and ecologically friendly.

In production zones across Cameroon, the cultivation of *T. occidentalis* usually begin at each farming season, commencing in March. Naturally, these production areas are located at different altitudes. Of recent, there has been growing demand for the vegetable due to its nutritional values, pharmacotherapeutic properties and industrial potentials. To meet this challenge, there is need to address the constraints on *T. occidentalis* production. The main objective of this study was to investigate how altitude and planting dates during the growing season impact leaf spot incidence and severity to identify optimal planting dates and at different altitudes for *T. occidentalis*.

Material and methods

Study sites

The study was conducted in the cropping seasons of 2019 and 2020 in the localities of Dschang and Santchou, both in the Menoua Division between March and July of each year. Menoua Division is one of the eight Divisions in the West Region of the Republic of Cameroon. The Division is unique in having localities with two different tropical climatic conditions under which *T. occidentalis* is cultivated mostly by poor resource based farmers. These climatic conditions fall within identical climatic belts of the North West and South West Regions of Cameroon, where *T. occidentalis* is extensively cultivated. The study area constitutes one of the main crop-producing areas in the Western Highlands agro-ecological zone of Cameroon. Farming in this area is dominated by small-scale farmers and the agricultural population is estimated at over 72 % with about 160,000 households. The terrain of the Western Highlands agro-ecological zone consists mainly of plateaus and depressions stretching from 300 to 3000 m above sea level (Kome et al. 2017).

In Dschang, the field study was conducted at the Faculty of Agronomy and Agricultural Sciences (FASA) teaching and research farm in the main campus of the University of Dschang, some 200 m from the University Lake. Dschang lies on latitude 5°26′38″ N and longitude 10°03′11″ E. (Mbong et al. 2021) with an equatorial monsoon climate (PCD Dschang, 2015). The town is located at an altitude of between 1300 - 1400 metres, almost twice to that of Santchou. This altitudinal difference is a principal element which tints this locality from Santchou. The mean annual rainfall is 1872 mm with a relative humidity comprised between 64.3 % and 97 % (Bamou et al. 2021). The climate of the region is of Sudano-Guinean type influenced by the altitude (Tamungang et al. 2016). The average annual temperature is 20 °C with February being the hottest month (Bamou et al. 2021). The region has one rainy season, which lasts from mid-March to mid-November and one dry season from mid-November to mid-March (Tamungang et al. 2016). The climatic conditions and terrain of Dschang are similar to those in the North West Region of Cameroon where *T. occidentalis* is cultivated extensively by local farmers.

In Santchou, The field study was set up at IRAD (Institute for Agricultural Research and Development) research and seed multiplication field. Santchou is located between 5°16′N and 9°58′E. It has an altitude of 786 m with a surface area of 95.05 km^2. The annual average temperature in Santchou is 22.5 °C. Its annual average precipitation is 1364.4 mm with a relative humidity of 92% (Bamou et al. 2021). Santchou has very complex vegetation, and its climate is equatorial to the Guinean type (Santchou council development plan, 2015), similar to the Littoral and Southwest regions, which are hosted to several cultivators of *T. occidentalis* (Bamou et al. 2021). The study area is characterized by two main seasons: the dry season, which extends from mid-November to March, and the rainy season, which runs from March to November.

Experimental design

The experiment for the two years was laid out in a 2 by 4 factorial randomized complete block design with 36 experimental units laid out in three blocks. The factors were two altitudes (low and high altitudes) and four planting dates. Within each block, three experimental units were selected randomly and sowed for each of the four separate planting dates. The experiment was laid out over a surface area of 121 m^2. Each experimental unit measured 2.25 m^2. The experimental units and blocks were both separated by passageways of 0.4 m. The experimental layout for each growing season and study area was identical.

Planting materials

Intact and mature *T. occidentalis* fruit pods for seeds were harvested from senescent shoots from an intercropped farm in the town of Dschang (Mbong et al. 2021). A minimum number of fruit pods of the same cultivar required for the research were harvested. To extract the seeds, pods were cut open with the aid of a knife, and the seeds were carefully isolated from the pulp manually. The seeds were air-dried for two days, given that they are recalcitrant, to prevent decay before planting. The seeds used in the subsequent year were of the same cultivar. The experimental research and field studies of the cultivated crop plant including the collection of planting material was within institutional, national and international guidelines and legislation.

Field preparation

During each investigation, a parcel of land measuring 121 m^2 was manually cleared of the weeds. The cleared debris was removed and dumped beyond the field experimental site. A hoe was later employed to uniformly plow the field to facilitate the construction of regular experimental units. Other equipment including a decameter, pegs and cords were employed to demarcate the tilled field into experimental units, and hoe was again made use of to set up mounds of 1.5 m x 1.5 m separated by alleys of 0.4 m.

Planting of seeds and treatments

The topsoil was used as substrate for planting. Healthy air-dried seeds were taken to the field and planted by direct seeding at a depth of 3 - 4 cm and covered with topsoil on each experimental unit at a rate of 1 m x 1 m. Four seeds were sown per experimental unit.

Four planting dates each were chosen and staggered seven days apart for the selected experimental units to determine the most appropriate time that planting the crop produce minimal leaf spot disease incidence and severity. The first planting date was on the 21st of March while the second, third and fourth planting were on the March 28th, April 4th and 11th of April in both study areas.

Crop maintenance in the field

After the four-week plant string, the field was constantly monitored for weed removal and staking with advancing growth. The removal of weeds commenced two weeks after emergence in the field. This was achieved manually once every fortnight to ensure optimal growth voids of other plant competitors and for better monitoring of disease parameters in the field. The field was secured with a barrier to keep local domestic animals out of reach and to check the indiscriminate movement of passers-by into or across the experimental field.

Staking with the aid of pegs locally harvested in the neighborhood of the field was initiated at three weeks after emergence and continued for an additional period of two weeks. The pegs were trimmed to a height of 1 m. The staked stands were tied with robes drawn from plantain stems. This was meant to train the clambering vines to the trellis and to facilitate their creeping pattern. Bamboo trellises were constructed for each experimental unit to serve as a supporting platform for optimal crop growth and for ideal disease assessment.

Data Collection

Disease Assessment in the Field

Disease incidence in the field was established by visual observations of symptoms of *T. occidentalis* leaf spot on the leaves. The data for disease incidence and severity commenced three weeks after emergence and were documented for a period of eight weeks. In the process, diseased leaves and the totality of leaves for each stand were counted, and the information was methodically recorded. The data was collected at weekly intervals as presented in the disease severity scale in Table 1.

Calculations for disease incidence

The information recorded in the field was used to calculate the percentage of the proportion of leaves infected per plant within the speculated period using the following formula for disease incidence (DI):

$$\textbf{Percentage DI} = \frac{\textbf{Number of leaves infected}}{\textbf{Total numberof leaves sampled}} \textbf{x100}$$

Determination of Disease Severity

The disease severity commenced immediately after the assessment of the disease incidence, and different treatments were the same as for disease incidence. The disease severity was assessed based on proportion of diseased leaves per plant following the modified disease severity scale of Orji et al. (2015), (Table 1).

Table 1: Disease severity scale of leaf spot (scored at weekly intervals)

Severity Scale	Numerical Rating	Description of symptom
0	0	No disease.
1 – 20	1	Infection of the leaves with small spot lesions.
21 – 40	2	Moderate infection of leaf with spot lesions spreading on the surface of the leaves.
41 – 60	3	Severe infection of the leaves with leaf spot lesions almost found in all the leaflets.
61 – 80	4	Very severe infection on all the leaves with spot lesions spreading in all the leaflets and coalescing.

81 – 100	5	The entire plant is completely infected with all leaves having leaf spot disease, some of the leaves having holes and there is leaf tearing.

Statistical Analysis

The information documented on the total number of leaves and number of diseased leaves from each stand at the corresponding planting dates was submitted to analysis of variance (ANOVA), and the means were separated by least significant difference (LSD) at a 95% confidence interval. The disease incidence was calculated using the Microsoft Excel program while SPSS was used for ANOVA. The scored data for disease severity was subjected to ANOVA. Data for disease incidence and severity were documented fortnightly, commencing five weeks after planting (WAP).

Results and discussion

Results

Disease assessment at weekly intervals in Dschang and Santchou

In the trial study in Dschang and Santchou, very high leaf spot disease incidences were observed at 11 WAP contrary to a minimal incidences that were mostly witnessed at 5 WAP. Considering the evolution of disease incidence at weekly intervals in planting date one, the very high incidence observed at 11 WAP in Dschang was significantly different ($p < 0.05$) from the incidences registered in the fifth, and seventh weekly intervals at both sites including the ninth weekly interval in Santchou (Table 2).

With regard to the weekly development of leaf spot incidence at planting date two, the seventh week after planting in Dschang registered a very high incidence which differed significantly from the incidences recorded in all weekly intervals in Santchou as well as the fifth week after planting in Dschang (Table 2).

Taking into consideration the progress of disease incidence at weekly intervals in the third planting date, the eleventh week after planting in Dschang revealed a significantly very high leaf spot incidence which differed from the incidences registered in all weekly intervals investigated in the study at both sites (Table 2).

In view of the evolution of disease incidence at weekly intervals in the fourth planting date, an extremely low incidence was documented at the fifth week after planting in Dschang which was significantly different from all disease incidences recorded at weekly intervals in the study except for the seventh weekly interval in Dschang (Table 2).

Table 2: Disease incidence at WAP in both study sites

Site	**WAP**	**PD 1, DI (%) ± SE**	**PD 2, DI (%) ± SE**	**PD 3, DI (%) ± SE**	**PD 4, DI (%) ± SE**
Dschang	Five	7.19 ± 2.00^{bc}	5.59 ± 2.00^{bc}	7.77 ± 2.00^{bc}	3.15 ± 2.00^{bd}
	Seven	14.08 ± 2.00^{b}	18.62 ± 2.00^{a}	6.89 ± 2.00^{bc}	6.93 ± 2.00^{bd}
	Nine	17.35 ± 2.00^{a}	15.35 ± 2.00^{a}	12.45 ± 2.00^{b}	17.89 ± 2.00^{bc}
	Eleven	19.73 ± 2.00^{a}	17.46 ± 2.00^{a}	19.40 ± 2.00^{a}	28.60 ± 2.00^{a}
Santchou	Five	8.99 ± 2.00^{b}	12.06 ± 2.00^{b}	9.19 ± 2.00^{bc}	13.56 ± 2.00^{bc}
	Seven	5.17 ± 2.00^{bc}	12.60 ± 2.00^{b}	9.83 ± 2.00^{bc}	14.27 ± 2.00^{bc}
	Nine	8.42 ± 2.00^{bc}	6.92 ± 2.00^{bc}	7.75 ± 2.00^{bc}	14.36 ± 2.00^{bc}
	Eleven	16.39 ± 2.00^{a}	8.50 ± 2.00^{bc}	10.27 ± 2.00^{b}	22.33 ± 2.00^{b}

$^{a, b, c, d}$ Means in the same column with the same superscript are not significantly different at $p > 0.05$ (LSD).
SE = Standard error.
PD = Planting date
WAP = Weeks after planting.
DI = Disease incidence
% = Percentage.

Considering disease severity at weeks after planting in the study sites, very high leaf spot disease severities were generally registered at 11 WAP in both sites while the least severities were recorded at 5 WAP in the survey areas (Table 3).

Taking into account the evolution of leaf spot at weekly intervals in planting date one, the very high severity observed at 11 WAP in Dschang was significantly different ($p < 0.05$) from the disease severities recorded in the initial three weekly intervals in Santchou including the first two weekly intervals in Dschang (Table 3).

With regard to the weekly progress of disease severity in the second planting date investigated during the study, the 11 WAP in Dschang registered a very high severity which differed significantly ($p < 0.05$), from the severities documented in the first two weekly intervals in Santchou including the first week after planting in Dschang (Table 3).

Looking at the development of disease severity at weekly intervals in the third planting date, the eleventh week after planting in Dschang revealed a significantly ($p < 0.05$), very high leaf spot severity which differed from the leaf spot severities registered in the fifth, ninth and eleventh weekly intervals in Santchou and the leading two weekly intervals in the study site in Dschang inclusive (Table 3).

Considering the progress of disease severity at weekly intervals planting date four, an extremely low severity was registered at 5 WAP in Dschang which was significantly ($p < 0.05$), different from all leaf spot severities recorded at weekly intervals during the study except for the seventh weekly interval in Dschang (Table 3).

Table 3: Disease severity at WAP in Dschang and Santchou

Site	**WAP**	**PD 1, DS ± SE**	**PD 2, DS ± SE**	**PD 3, DS ± SE**	**PD 4, DS ± SE**
Dschang	Five	0.47 ± 0.12^b	0.28 ± 0.12^b	0.72 ± 0.12^b	0.28 ± 0.12^b
	Seven	0.92 ± 0.12^b	1.22 ± 0.12^a	0.78 ± 0.12^b	0.56 ± 0.12^b

	Nine	1.22 ± 0.12^{a}	1.22 ± 0.12^{a}	1.11 ± 0.12^{a}	1.25 ± 0.12^{a}
	Eleven	1.33 ± 0.12^{a}	1.33 ± 0.12^{a}	1.39 ± 0.12^{a}	1.75 ± 0.12^{a}
Santchou	Five	0.81 ± 0.12^{b}	1.06 ± 0.12^{a}	0.81 ± 0.12^{b}	1.14 ± 0.12^{a}
	Seven	0.67 ± 0.12^{b}	1.11 ± 0.12^{a}	1.03 ± 0.12^{a}	1.22 ± 0.12^{a}
	Nine	0.78 ± 0.12^{b}	0.72 ± 0.12^{b}	0.92 ± 0.12^{b}	1.17 ± 0.12^{a}
	Eleven	1.14 ± 0.12^{a}	0.92 ± 0.12^{b}	1.03 ± 0.12^{b}	1.56 ± 0.12^{a}

[a, b] Means in the same column with the same superscript are not significantly different at $p > 0.05$ (LSD).
SE = Standard error.
PD = Planting date
WAP = Weeks after planting.
DS = Disease severity.

Disease assessment at planting dates in both study areas

Considering the two study sites, the disease incidences observed at the initial three planting dates in Santchou were generally lower than the incidences recorded at similar planting dates in Dschang. The very high leaf spot incidence recorded at the fourth planting date in the study site of Santchou differed significantly ($p < 0.05$), from the incidences registered at leading three planting dates in Santchou including the third planting date in Dschang. Significantly low leaf spot incidences were registered in the initial three planting dates in at low altitude including planting date three at high altitude compared to the disease incidences documented at other planting dates in the investigation (Table 4).

Regarding disease severity, significantly ($p < 0.05$) low leaf spot severities were recorded within all planting dates in study areas except for planting date four at low altitude (Table 4).

Table 4: Disease assessment parameters at planting dates in the study areas

Planting date (days)	Study site	Disease incidence (%) ± SE	Disease severity ± SE
One	Dschang	14.59 ± 1.00^{a}	0.99 ± 0.06^{b}
	Santchou	9.74 ± 1.00^{b}	0.85 ± 0.06^{b}
Two	Dschang	14.25 ± 1.00^{a}	1.01 ± 0.06^{b}

	Santchou	10.02 ± 1.00^{b}	0.95 ± 0.06^{b}
Three	Dschang	11.63 ± 1.00^{b}	1.00 ± 0.06^{b}
	Santchou	9.26 ± 1.00^{b}	0.94 ± 0.06^{b}
Four	Dschang	14.14 ± 1.00^{a}	0.96 ± 0.06^{b}
	Santchou	16.13 ± 1.00^{a}	1.27 ± 0.06^{a}

[a, b] Means in the same column with the same superscript are not significantly different at $p > 0.05$ (LSD).
SE = Standard error.
% = percentage.

Disease assessment between Dschang and Santchou

In the study, the disease incidence in Santchou (at low altitude) was established significantly lower than the incidence recorded in the study area of Dschang (at high altitude). However, there was no significant difference between the leaf spot disease severities observed between the two study sites (Table 5).

Table 5: Disease incidence and severity between the study areas

Study site	Disease incidence (%) ± SE	Disease severity ± SE
Dschang	13.65 ± 0.50^{a}	0.99 ± 0.03^{a}
Santchou	11.28 ± 0.50^{b}	1.00 ± 0.03^{a}

[a, b] Means in the same column with the same superscript are not significantly different at $p > 0.05$ (LSD).
SE = Standard error.

Discussion

Leaf spot disease incidence was significantly low at the low altitude (in Santchou) compared to the incidence registered at the high altitude (in Dschang). The leaf spot pathogen of *T. occidentalis* is essentially known to be carried from one host plant to another by air current. Therefore, it is probable that wind could have played a significant role in the prevalence of the disease between the study sites. This observation is consistent with the findings of Waller et al. (2002), who reported that, the transmission of fungi spores by air current is greater at high altitude. In addition, Helen and Michele (1997), opined that a plant disease can be avoided entirely by planting a crop in different sites or regions or at different altitudes from those in which it normally grows. In this study, conducted in Menoua Division, the study site of Dschang is located at a higher altitude, almost twice that of Santchou. This disparity in altitude, as it naturally exist, could have facilitated the dispersal of the small, lightweight spores of *Phoma sorghina* in the field by air current leading to further spread of infection and higher leaf spot disease incidence. Another study (Kassaw et al. 2021) reported contradictory results.

The planting dates within which *T. occidentalis* leaf spot incidences were significantly low could have coincided with growing stages of the crop that were less susceptible, more resistant, to infection and spread of the leaf spot pathogen, resulting in leaf spot disease avoidance. The results in this study are also in agreement with previous investigations conducted and reported by Akhileshwaria et al. (2012). The researchers affirmed that, adjustment of planting dates is one of the important cultural practices that can be exploited to minimize crop losses due to disease. The authors intimated that, there was a decrease in powdery mildew severity in sunflower following strategic manipulation of planting dates. The authors went on to ascertain that, such a cultural technique avoided coincidence with susceptible stage of the crop, consequently, resulting in disease escape. Subsequent reports by Apeyuan et al. (2017), confirmed that, strategic alteration in planting dates was effective in the control of some plant diseases. Previous reports (Mbong et al. 2010; Jitendiya and Chhetry 2014), established that sowing dates significantly influenced the epidemiology of crop diseases under field conditions. In addition, the results in this study further confirms the earlier emphatic viewpoints of Helen and Michele (1997), who explained that many crop plants tend to be more susceptible to attacks by various parasites at certain stages of their development. The authors added that, changing the usual planting time of a crop can exploit weather conditions which are not favourable for the spread of pathogens and reduce crop losses.

Furthermore, in this study, *T. occidentalis* leaf spot disease incidence and severity were significantly high at planting date four in the Dschang (high altitude). This could be due to the fact that the inoculum density within the field was very high due to accumulation from previous planting dates coupled with the fact that the weather conditions could have been more favourable for infection. Therefore, the more conducive microclimate together with conceivable high initial inoculum population could have encouraged the proliferation of the already populated fungal spores, their germination and rapid multiplication, which favoured new and rapid infections, resulting in extremely high leaf spot disease incidences and severities. This result corroborates with previous investigations of Kone et al. (2017), who suggested that warm and humid weather conditions favoured the propagation of disease in cucurbits under field conditions. In addition, Ilondu (2013), earlier acknowledged that leaf spot diseases are favoured by humid weather conditions, where they destroy a greater portion of the foliage. With such humid conditions, the spores readily germinated within a brief period resulting in further spread of the disease among the stands.

Conclusion

In this study it was revealed that, the leading three sowing dates at low altitude (in Santchou) and the third planting date in Dschang were crucial in reducing leaf spot disease incidence and severity under field conditions. In addition the low altitude recorded a significantly low leaf spot disease incidence compared to the high altitude. The first three sowing dates in Santchou including the third planting date in Dschang could therefore be vital in minimizing the prevalence leaf spot disease of *T occidentalis*.

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