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## BROWN SPOT AND STALK ROT DISEASES OF MAIZE (*ZEA MAYS*) AND SUSCEPTIBILITY OF TWO VARIETIES TO *PHYSODERMA MAYDIS* IN FAR NORTH CAMEROON

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### ABSTRACT

Corn (*Zea mays* L.) is one of the most widely grown cereals in the world. However, in Cameroon, particularly in the Far North Region, yields remain low because of diseases increasing due to farming practices and climate change. This work aimed to characterize Brown Spot and Stalk Rot of corn and evaluate the susceptibility of two maize varieties to *Physoderma maydis* in field conditions. Two maize varieties, CMS9015, and CMS8704 were evaluated in a randomized block design. Disease symptoms were observed and described using identification keys. Microscopic characteristics of pathogens were performed using a microscope. Incidence, severity, and areas under disease curve progress (AUIPC and AUSiPC) were calculated. Brown spot occurred 3 or 4 weeks after sowing (WAS) at the growing stage in CMS8704 variety first, and stalk rot 7 WAS. Brown spot appeared in leaves and is characterized by oval yellowish spots in the leaf blade and brownish spots in the midrib. Stalk rot infects stem nodes and is characterized by brownish spots, leading to the broken stem. The incidence of brown spot increased with time and environmental conditions (rainfall). The highest incidence of *Physoderma* Brown Spot was recorded with CMS8704 variety 53.06 % against 51.72 % with CMS9015. Severity was respectively 54.40 % and 47.12 % with CMS8704 and CMS9015. The incidence and severity of stalk rot were almost identical in both varieties. AUIPC of PBS and PSR was higher in CMS9015 than in CMS 8704, 63 DAS. PBS and PSR infect maize production, and both varieties are susceptible to *P. maydis*.

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### INTRODUCTION

Corn is an annual, monoecious herbaceous plant native to South America (Maybelline *et al.*, 2012). It is a crop with high economic potential and can solve food deficit problems if well managed (Rouanet, 1984; Kambire *et al.*, 2010). Maize is the world's most cultivated plant and the first cereal produced, ahead of wheat and rice (Li *et al.*,

2018). Maize is mainly used for food, animal feed, and raw material for many industrial applications (FAO, 2019).

World maize production in 2019 was 1445 million tons, compared to 763 million tons for wheat. Maize is agricultural speculation that is intensifying in Africa because of this crop's economic stakes, which has become increasingly important (Régine and Coderre,

2000). Cameroon is classified as Africa's thirteenth largest maize producer (FAO, 2019). This crop is the third most important food commodity produced and the first cereal in Cameroon.

In Cameroon, demand is still higher than supply (0.6 million tons' deficit) (IRAM/MINADER, 2017), and it is more marked in the West and North of the country, where maize is used in various culinary compositions (Njomaha, 2003). In the Far North Region of Cameroon, this deficit is due to poor farming practices by farmers, inability to buy fertilizers, lack of land, biotic constraints (diseases and pests, choice of seed varieties), and abiotic constraints (climate change, scarcity of rainfall, soil poverty) (IRAM/MINADER, 2017).

To solve this problem, corn growers are looking for a sustainable alternative by using green and organic fertilizers that promote microbial activity in the soil (Biaou *et al.*, 2017). Such as animal dung (chicken droppings and cow dung) because many farmers combine animal husbandry with crop production. However, other work has also shown that these organic fertilizers (cow dung) also promote and maintain the development of microorganisms that are harmful to crops (Tompkins *et al.*, 1992; Reid *et al.*, 2001; Veresoglou *et al.*, 2012; Le poivre, 2003). Many diseases have been reported in Cameroon on maize. Apart from streak disease, which is the most widespread viral disease, physoderma brown spot appears to be the most common fungal disease during the agricultural seasons. Bamle (2018) had shown that brown spot of leaves and stem rot of maize caused by *Physoderma maydis* developed more subsoil amended with organic fertilizers in the Far North. Worldwide as well as in the Far North region Cameroon *Physoderma maydis* is responsible for Physoderma Brown Spot and Physoderma Stalk Rot of corn (Robertson *et al.*, 2015). This disease leads to a reduction in leaf area and stems breakage (Tisdale, 1919). In 2013, yield loss was estimated for this disease to more than 13 million plants in the United States and Canada (Mueller and Wise, 2014). In addition, disease frequency in the field is reported at 80% in southern Iowa (Robertson, 2008; Robertson *et al.*, 2015).

Currently, despite the low reported impact on yield and the lack of studies on this pathology in the world, agronomic, genetic, and chemical control remain the most adopted means. In Cameroon, and particularly in the Far North, no study has been undertaken to evaluate existing maize varieties' resistance to *P. maydis*. However, the

lengthening rainy season (from 3 to 4 months) in recent years in the Far North zone is a favorable condition for developing this disease (Burns and Shurtleff, 1973; Robertson *et al.*, 2015). The poverty of the population thus implies a less expensive method of control for farmers. Therefore, this work aimed to study Brown Spot and Stalk Rot of corn in Far North Cameroon and evaluate the susceptibility of two varieties of maize (*Zea mays*) to *Physoderma maydis* in field conditions.

## MATERIALS AND METHODS

### Plant material

The plant material consisted of two maize varieties, CMS 8704 and CMS 9015, purchased in IRAD Maroua. CMS 8704 is yellow in color and has 110-115 days of cycle duration. CMS 9015 is white in color and has 90 days of cycle duration. Both varieties are resistant to *Striga hermonthica*.

### Experimental design and sowing

The experimental design at the main site was in completely randomized blocks. Three blocks with an area of 500 m<sup>2</sup> were separated by 1.5 m. Each block consisted of three plots (treatments) spaced by 1 m. The corn spacing was 80×40 cm. The planting depth of the corn was about 1.5 cm with two seeds per pack. About 40 plants were used per plot (variety) and 80 per block. Insecticide-fungicide, CALTHIO (Chlorpyrifos-ethyl 25%+Thiram 25%) was used to coat (mix) the seeds during sowing to protect maize seeds against insects and soil fungi. About 3 g of calthio were used to treat 1 kg of seeds.

### Identification of symptoms and pathogens

According to the phenological stage of plants, symptoms were observed and described in the field, and sporangia of fungi were observed in the laboratory with a microscope (Omax). Identification keys (Harvey *et al.*, 1955; Robertson *et al.*, 2015; CIMMYT, 1978; Jakson, 2018) were used. The occurrence of symptoms on the corn organs was checked every two days in the field, followed by microscopic observations to detect the stage of sporangia appearance.

Isolation of the fungus was performed from infected leaves and stalks, showing symptoms of Physodermatosis. These organs were washed several times with tap water, disinfected with alcohol at 95 °C, and then flame sterilized. The leaves were then cut longitudinally at the midrib. The sporangia were released by scraping off the midrib's black

part and were placed in a Petri dish containing a few drops of sterile distilled water for microscopic observation. Measurements of sporangia diameter were performed using a micrometric ocular. Some sporangia were introduced in Petri dishes containing PDA medium for mycelia development and obtention of pure culture. These Petri dishes were incubated at 12/12 photoperiod.

### Assessment of incidence and severity of *Physoderma brown spot* and *Stalk Rot* of maize

#### Assessment of incidence of both diseases

The incidence of the disease was evaluated every seven days. The incidence of diseases was evaluated using the following formula (Le poivre, 2003):

$$I (\%) = \frac{n \times 100}{N}$$

I have incidence; there are n number of plants showing symptoms per plot; and N total number of plants in the plot.

#### Assessment of the severity of both diseases

The severity of the disease on infected plants in the field was assessed using a visual rating scale (score) ranging from 0 to 4. The number of leaves attacked by brown spot disease and the number of infected internodes and nodes by stalk rot were counted. The following index score was used (Cooke, 2006).

- 0= no symptoms;
- 1= 25%: [0- 1/3] of leaves or internodes infected;
- 2= 50%: [1/4-2/4] of leaves or internodes infected;
- 3= 75%: [2/4-3/4] of leaves or internodes infected;
- 4= 100%: [3/4 -4/4] of leaves or internodes infected

The disease severity index was calculated according to the formula:

$$S = (\sum(xi \times ni)) / N \times 100$$

Where, xi: is the (i) score of the disease; ni: the number of plants with (i) score; N: the total number of diseased plants assessed per plot.

#### AUDPC "area under disease progress curve"

The areas of disease progression were calculated using the incidence and disease severity index. Thus, the area under the disease progress curve (AUIPC) for disease incidence was calculated using the formula described by Muengula-Manyi *et al.* (2013) and Kone *et al.* (2017):

$$AUIPC = \frac{\sum_{i=n}^n (X1 + X (n + 1))}{2(t)}$$

Xi is the incidence of disease at the time i, Xi + 1 is disease incidence recorded at the time i + 1, n is the number of registration on the incidence, and t, days between the registration of Xi and Xi + 1.

The Area under severity index progress curve (AUSiPC) for disease severity was calculated using the formula described by Shaner and Finney (1977) as below:

$$AUSiPC = \sum_{i=1}^{n-1} (DS1 + DS2) / 2 \times (t2 - t1)$$

Where DS1 is disease severity recorded in time 1 (t1) and DS2 the disease severity recorded in time 2 (t2).

#### Rainfall

The evaluation of rainfall provides information on the degree of precipitation that can influence crop planting and disease progress. The data were collected using rain gauges installed in the middle of the fields. Data were recorded each morning.

#### Statistical analysis

Data collected were subjected to analysis of variance (ANOVA), and means were separated using Duncan's multiple range test (5%). SPSS 20.0 software was used to perform the statistical analysis

## RESULTS

### Evolution of the symptoms of *Physoderma Brown Spot* (PBS)

Four WAS, PBS was already visible in the leaves in some plots in the two varieties. But, CMS8704 was the first to receive symptoms. The disease starts with the appearance of small elongated yellow spots (oblong) towards the edge of the leaves (Figure 1a). Microscopic observations reveal no sporangia at this stage. Afterward, the coloration of these spots turns dark yellow (Figure 1b). Microscopically, some sporangia can already be seen on the leaf blade. Forty-nine days after sowing, the midrib blackens with the presence of many sporangia (Figure 1c), the leaves become almost entirely yellowish. In this stage, symptoms of eyespot (*Kabatiella zae*), which are similar to PBS symptoms, are visible and can be confused with those of PBS. The leaf blade color changes from yellow to brown to red after fruiting (Figure 1d). Some leaves wilt and dry out.

The sheaths, spathes and stalks of some corn cobs were infested, causing them to rot (Figure 2). PBS appears in the vegetative stage and evolves until the reproductive and maturation stage of the corn cobs.



Figure 1. symptoms of PBS in the leaves according to the growing stage. (a) 28 DAS. (b) 35 DAS. (c) 49 DAS. (d) 66 DAS.



Figure 2. sheaths (A), spathes (B) and stalks showing the symptom of Physoderma Bown Spot (PBS).

#### Evolution of the symptoms of Physoderma Stalk Rot

The stalk rot disease appeared one week before the beginning of flowering (43 days after sowing). The symptomatic colour observed was black to brown (Figure 3B, C). Lesions appeared at the nodes and internodes

(Figure 3A, D). The black spots observed represent clusters of sporangia. The nodes rot and cause a break of the stalks of some plants (Figure 3E). A cross-section of a node infected by *Physoderma maydis* shows numerous sporangia towards the stalk edges (black) (Figure 4).



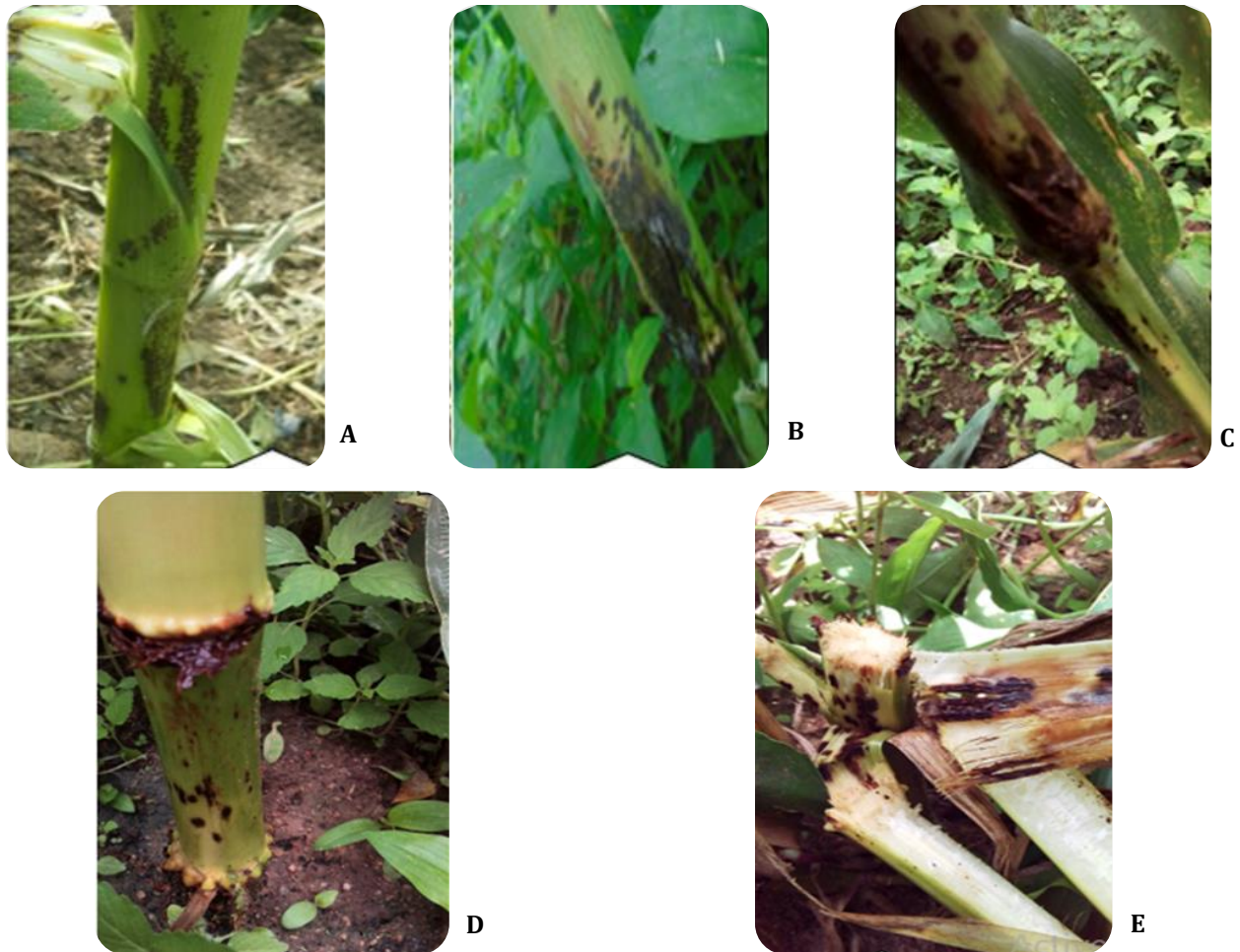


Figure 3. Symptoms of Stalk Rot. In the internodes (A, B, C and D) ; in the node (E).



Figure 4. cross-section of the infected node. Arrow indicates presence of sporangia at the edge of stalk.

#### Characteristics of *P. maydis*

*Physoderma maydis* is characterized by many sporangia (Figure 5B) yellowish (dark in the field) in all parts of infected organs. The diameter of sporangia ranged from

20.66-25.82  $\mu\text{m}$ . Microscopic observations showed hyphae with many septae. (Figure 5A). Whether the conditions are favorable, these sporangia release zoospores (Figure 5C), which can swim and infect other leaves or stalks.

**Incidence of both diseases**

The incidence of PSB expressed as AUIPC showed a significant difference ( $P < 0.05$ ) among the varieties at 35, 42, and 49 days after sowing (DAS). CMS 9015 variety had the highest AUIPC values than CMS 8704. However, at the 56 and 63 DAS, no significant difference was observed ( $P = 0.58$ ), suggesting that both varieties

were affected by *P. maydis* (Figure 6A).

ANOVA for the AUIPC values based on disease incidence of PSR revealed a significant difference ( $P < 0.05$ ) among the varieties 42, 49, and 63 DAS. CMS 9015 had the highest AUIPC value, 63 DAS, while CMS 8704 variety had the lowest (Figure 6B).

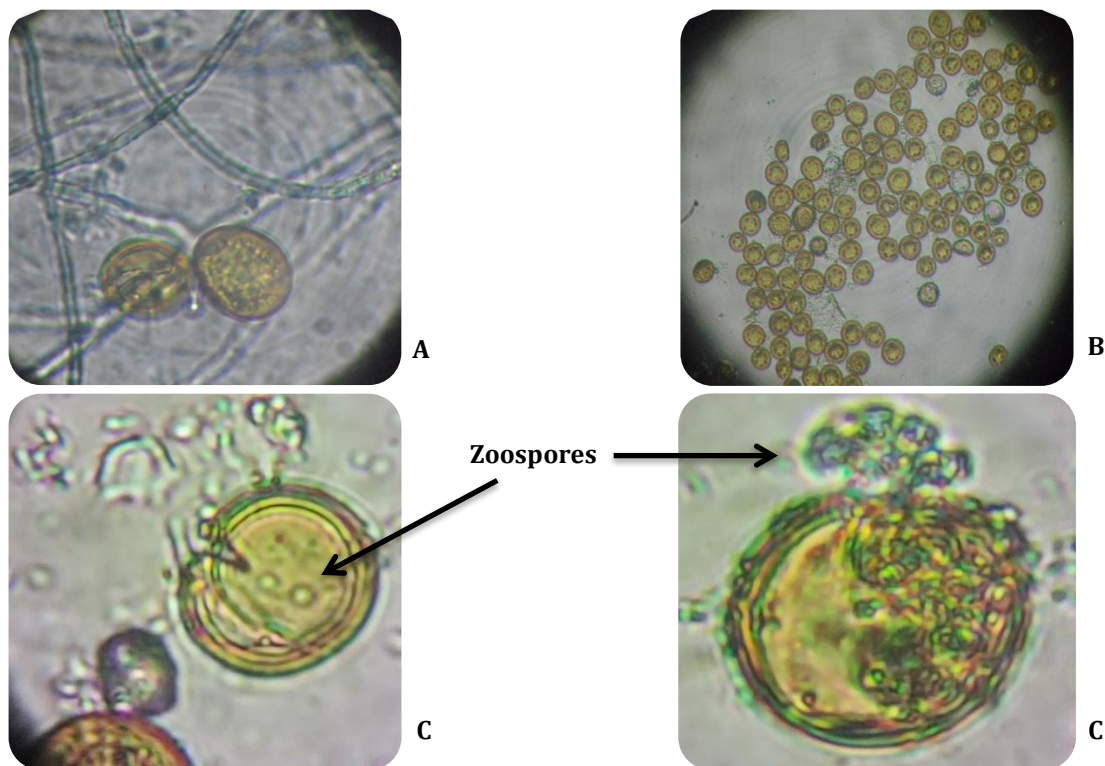


Figure 5. Micro characteristics of *Physoderma maydis* : (A) filamentous ; (B) fil sporangia; (C) sporangia releasing zoospores.

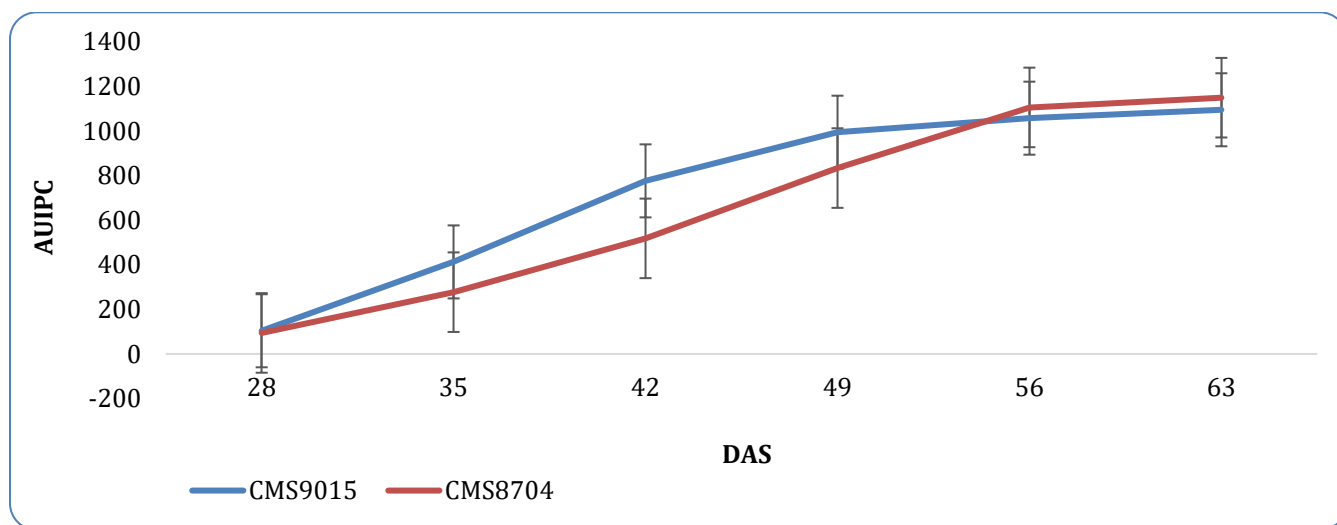


Figure 6A. Incidence of PSB expressed as Area Under Disease Incidence Progress Curve on corn varieties.

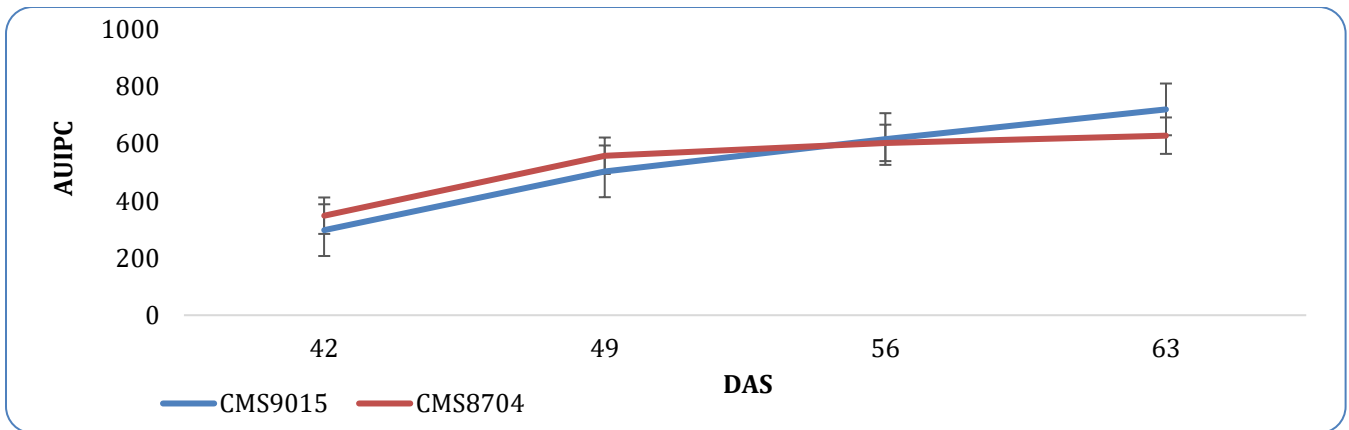


Figure 6B. Incidence of PSR expressed as Area Under Disease Incidence Progress Curve on corn varieties.

**Disease severity**

PBS AUSiPC value of CMS9015 was significantly highest than AUSiPC values of CMS 8704 during 28, 35, 42, and 49 DAS. However, AUSiPC values recorded for CMS8704 at 63 DAS, was significantly higher ( $P < 0.05$ ) than that of

CMS9015 (Figure 7A).

No significant difference was observed ( $P > 0.05$ ) among both varieties with PSR AUSiPC values at 56 and 63 DAS. AUSiPC values of CMS9015 were significantly higher than the AUSiPC value of CMS8704 at 49 DAS (Figure 7B).

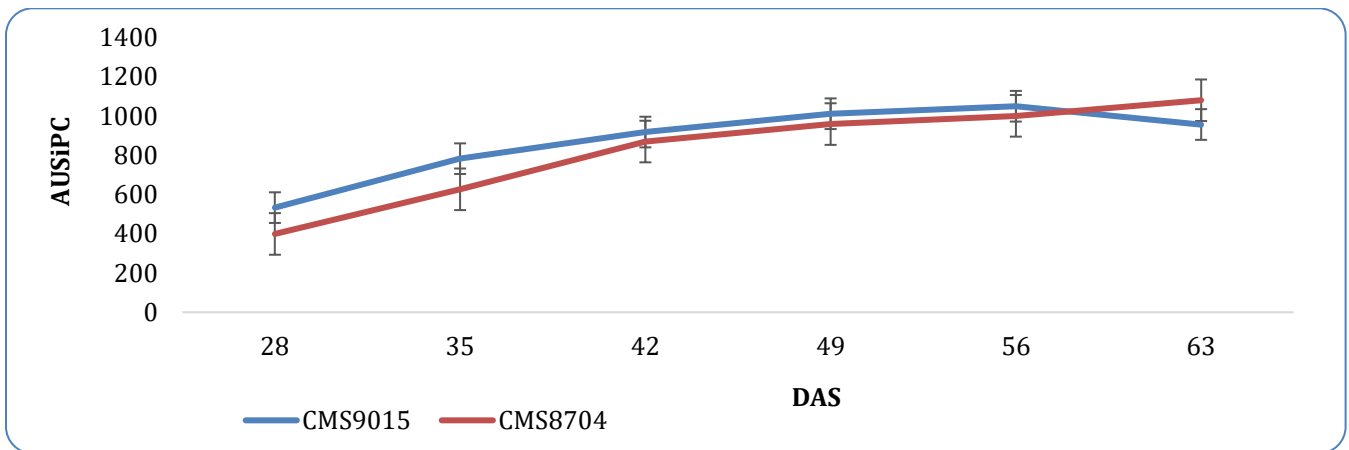


Figure 7A. Severity of PBS expressed as Area Under Disease Severity Progress Curve on corn varieties.

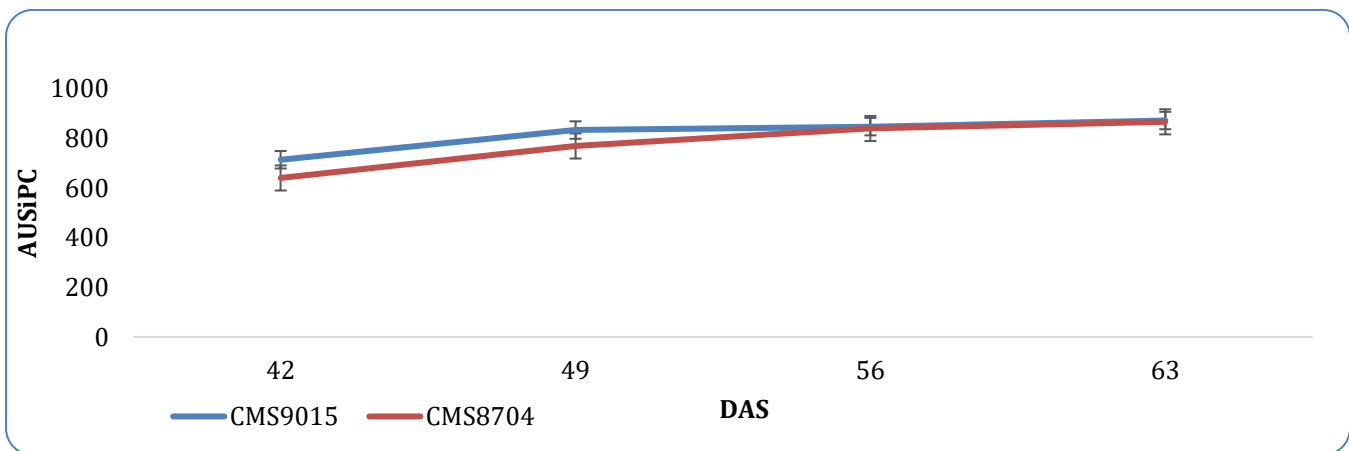


Figure 7B. Severity of PSR expressed as Area Under Disease Severity Progress Curve on corn varieties.

## Rainfall

During the growing season, 903 mm of water was recorded. July and September were the highest months

of precipitation, with an average of 75 mm of water per month. The first two weeks of September were the weeks with the highest rainfall (Table 1).

Table 1. rainfall recorded during the growing season.

week	Months													
	June		July				August				September			
	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12	W14	W15
R*	80	70	0	80	78	60	50	30	40	70	175	135	80	25

\*Rainfall (mm)

## DISCUSSION

There are few studies on *Physoderma* Brown spot and stalk rot diseases due to *Physoderma maydis*. However, *Physoderma* Brown Spot and Stalk Rot infect leaves and stalk organs of corn. These diseases are characterized by the yellowish colour of leaves and black spots of a stalk. These results corroborate that of Tisdale (1919); Eddins (1933); Robertson *et al.* (2015) and Wise *et al.* (2018), who have described the same symptoms on maize.

Both varieties are infected in the three blocks almost at the same growth stage. Sporangia's presence can explain this produced one or more seasons previously in the soil (Eddins, 1933; Wise *et al.*, 2018). The experiment has been carried out in the field where maize is continuously sown (no rotation) for 10 years. Eddins (1933) and Tisdale (1919) have shown that sporangia kept in a bottle in the laboratory for one or two years gave very high percentages of infection than those who remain after three years which lost their power to cause disease. Moreover, farmers use cow dogs or chicken dropping as fertilizers. Many studies (Tompkins *et al.*, 1992; Reid *et al.*, 2001; Veresoglou *et al.*, 2012) have reported that organic manure can keep spores of many diseases for several years.

On the other hand, sandy soil is favourable for sporangia transport. Far North Cameroon is in Soudano Sahelian Zone with sandy clay soil. Crop rotation is then one management strategy against *P. maydis*. Li *et al.* (2010) and Li *et al.* (2018) have also shown that tebuconazole can manage the spatial distribution of corn brown spots and other diseases.

First symptoms occurred in CMS 9015 variety almost three to four weeks after sowing. This result is in agreement with that of Eddins (1933), who has shown that the first symptoms occurred 24 days after sowing. Moreover, precipitation was favourable for sporangia to

release zoospores Abendroth *et al.* (2011). Symptoms occur after the firsts precipitations of June (Robertson *et al.*, 2015; Hatfield *et al.*, 2011). Burns and Shurtleff (1973) and Tisdale (1919) have shown that PSB and PSR are more severe when precipitations increase.

Incidence (AUIPC) and severity (AUSiPC) of both diseases increased with time in both varieties (CMS9015 and CMS 8704). During this last decade, PSB and PSR are more effective in Maroua area. Precipitations were only in June, July, and August in the past. But, since 2016 (4 years), the last rainfalls in October. Climatic change is the reality in Far North Cameroon. Climatic changes increase the incidence of some diseases (mainly PSB and PSR). Robertson (2008) and Robertson *et al.* (2015) have shown that increase of PBS and PSR in Iowa is likely related to wet springs, crop production practices, and hybrid genetics.

## CONCLUSION

*Physoderma* Brown Spot and Stalk Rot of corn due to *Physoderma maydis* infect all maize varieties. The incidence increases with rainfall. This work is a baseline in the Far North for managing strategy against *Physoderma maydis*.

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#### CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

#### AUTHORS CONTRIBUTIONS

This article is the result of the combined efforts of a multidisciplinary team of phytopathologists (NGOH DOOH JP, HEU ALAIN, TCHOUPOU TDB, DJILE BOUBA, DJONGNANG G, YAMAGUI Rita, NTATSINDA CD, and AMBANG Z) who contribute to elaborate the protocol; experimental design and sampling (NDJP, DG, NCD, YR TTDB), diseases and pathogen identification (NDJP, HA, DB, and AZ), disease parameters (NDJP, DG, NCD, YR). NGOH DOOH drafted the manuscript, which all co-authors have reviewed.

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