Effect of arbuscular mycorrhizal fungi (AMF) on development of brown spot disease of corn (*Zea mays* L.) due to *Physoderma maydis* in Far North Cameroon

Souleymanou Adamou[1], Alain Heu[2]. Serge B. Mboussi[3], Abdou N. Kone Nsangou[4], Paulin Sinama[5], Eloa Sesseumaga[5], Zaina T. Amawissa[5], *Jules P. Ngoh Dooh[5], Philippe Kosma[6] and Zachee Ambang[7]

[1]*University of Dschang, Faculty of Crop Sciences, Department of Plant Sciences, PO Box 67, Dschang, Cameroon*
[2]*Higher Technical Teacher's Training College, Department of Agriculture and Agropastoral, University of Ebolowa, Cameroon. Po Box 886 Ebolowa*
[3]*Laboratory of Quality Control, University Institute of Technology, University of Douala, Cameroon, PO Box 8698 Douala*
[4]*University of Dschang, Department of Plant Biology, Applied Botanic Research Unity, PO Box 67 Dschang*
[5]*Department of Biological Sciences, Faculty of Science, University of Maroua, PO Box 814 Maroua*
[6]*Higher National Polytechnic School of Maroua, University of Maroua, PO Box 1450 Maroua*
[7]*Laboratory of Biotechnologies, Phytopathology Unit, University of Yaounde I, PO Box, 812, Cameroon*
[*]*Corresponding author's email: ndjuliopat@yahoo.fr*

Abstract

Corn (*Zea mays* L.) is affected by a number of diseases that lead to yield loss. One of them is brown spot caused by the soil-borne fungus *Physoderma maydis*. In this study, the bioprotective potential of arbuscular mycorrhizal fungi (AMF) against this disease was carried out in the nursery and field. In a completely randomized block design, two maize varieties namely CMS9015 (V1) and CMS8704 (V2) were used. In the field, two mycorrhizal treatments (V1AMF, V2AMF) and two controls (CV1, CV2) were developed. In the nursery, the treatments consisted of sterilized and mycorrhized soil with disease inoculation (AMF+DI+SS:V1,V2), sterilized soil without mycorrhization with disease inoculation (DI+SS:V1,V2) and non-sterilized soil (NSS:V1,V2). Disease incidence and severity, plant height and leaf area, root density, root colonization, nitrogen and phosphorus levels were assessed. In the field, the root colonization by the fungus was 63% as compared to 33% for the mycorrhized plants and the controls. Disease incidence and severity were reduced by 33.7% and 35%, respectively, in the mycorrhized plots in the field. The highest levels of phosphorus (0.74 mg g^{-1}) and nitrogen (9.2 mg g^{-1}) were obtained in the mycorrhized field treatments. In the nursery, disease incidence and severity were 0% in pots containing sterilized soil with or without mycorrhizal fungi. In the nursery, the phosphorus level of 1.07 mg g^{-1} was recorded in the mycorrhizal treatments and the nitrogen level of 7 mg g^{-1} was recorded in the leaves of the pots on unsterilized soil. This study provides that AMF can be used for the control of *P. maydis* in corn.

Keywords: Arbuscular mycorrhizal fungi, Brown spot, *Physoderma maydis*, Varieties, *Zea mays.*

Introduction

Corn is the most widely grown cereal in the world, ahead of wheat (Raounet, 1984; Deffan *et al*., 2015). The International Grains Council (IGC) estimates its world production at 1510 million tonnes compared to 779.6 million tonnes for wheat (Erenstein *et al*., 2022). Corn occupies an important place in the world in general and in Africa in particular, not only for its energy contribution to the population's diet (Akanvou *et al*., 2006), but also for the place it occupies in certain African industries (brewery, oil mill, soap factory) as the main raw material (Akiyama, 2007). In Cameroon, corn production has a deficit of 0.6 million tonnes in high production regions such as the Western Region and the North of the country (MINADER, 2019). In the Far North Region, about one in five households is food insecure even though corn is mainly grown for subsistence purposes for self-consumption (Nzossie *et al.*, 2010).

The low level of corn yields and its production instability are a constraint to ensuring food security for the population (Nuss and Tanumihardjo, 2011). These concerns are partly linked to climate change, lack of mastery of ploughing techniques, soil degradation due to erosion and anthropogenic activities (Tsozue *et al.*, 2015). However, the presence of certain diseases, caused by microorganisms, can result in crop losses of more than 50%. Fungi are the most formidable and abundant microorganisms responsible for crop losses (Lepoivre, 2003). *Physoderma maydis* is a telluric fungus that causes brown spot and stalk rot in maize (Wise *et al.*, 2018; Ngoh Dooh *et al.*, 2021a). The impact of this disease on yield loss in 2013 was estimated at nearly 40.7% in the USA and Canada (Robertson *et al.*, 2015; Jackson, 2018). In Cameroon, the incidence of this disease has been estimated at over 50% (Ngoh Dooh *et al.*, 2021a).

Intensive agriculture focuses on the use of chemical pesticides, which are characterized by environmental pollution and the appearance of resistant pathogens, instead of favoring agro-ecological practices such as crop rotation, crop association or biological control, which can be achieved through the use of antagonistic microorganisms such as *Trichoderma* (Khan and Javaid, 2020; Khan *et al.*, 2021), *Aspergillus* (Khan and Javaid, 2021), *Penicillium* (Khan and Javaid, 2022) and plant growth promoting rhizobacteria (Sharf *et al.*, 2021) or plant extracts and biomass (Javaid and Khan, 2016; Jabeen *et al.*, 2021) to fight diseases and pests. To date, no control method has been registered against *P. maydis*. The use of certain bioprotective biological fertilizers such as arbuscular mycorrhizal fungi (AMF), which have a specific affinity with corn, may be a possible solution (Temegne *et al.*, 2017; Muthukumar and Karthik, 2021). These soil fungi form a symbiotic association with nearly 80% of plant species (Javaid and Khan, 2019; Zhang *et al.*, 2022). Mycorrhizal fungi improve water and mineral nutrition (Adamou and Ngakou, 2018) and resistance of plants against the pathogens (Ngoh Dooh *et al.*, 2021b). The fungus takes advantage of carbon resources synthesized by the plant via photosynthesis that are essential for its metabolism and fruiting (Nwaga *et al.*, 2013). In return, the fungal hyphae improve the water and mineral nutrition of the host plant through the increase in the volume of soil explored and the production of various extracellular enzymes (proteinases, phosphatases) that can mobilize nutrients from complex soil compounds (Strullu, 1991; Javaid, 2009). Furthermore, AMF have already shown their effectiveness against some pathogenic fungi such as *Fusarium* and *Phytophthora* (Adamou and Ngakou, 2018; Djenatou *et al.*, 2020). This efficiency translates into improved plant growth and increased resistance to many stresses (Adamou and Ngakou, 2018).

P. maydis is a soil-borne fungus that also penetrates through the roots. Mycorrhizal fungi can colonize the plant roots and could thus reduce the penetration of this agent into the plant. The aim of this study was to evaluate the bioprotective potential of AMF against the brown spot disease caused by *P. maydis* in the field and in the nursery.

Materials and Methods

Procurement of material

The maize varieties CMS 9015 (white color) and CMS8704 (yellow color) used in this study were obtained from the Agricultural Research Institute for Development (IRAD) in Maroua. The biofertilizers used were mycorrhizal fungi consisting of a mixture of species from two genera, *Glomus* and *Gigaspora*, both from the Nkolbisson Biotechnology Centre in Yaoundé 1, Cameroon.

Experimental design in nursery and field

The experimental design used was completely randomized block. In the nursery, five blocks with six treatments each were constituted: sterilized soil associated with mycorrhizal fungi and with each of the varieties and infected by *P. maydis* sporangia (SS+AMF+ DI +V1, SS +AMF+ DI +V2), sterilized soil without mycorrhizal fungi, with varieties and infected by *P. maydis* sporangia (SS + DI +V1, SS + DI +V2), no-sterilized soil with each of the varieties (NSSV1, NSSV2). Each treatment was repeated three times, for a total of 15 pots per treatment. Each pot contained two maize seeds. The substrate used in the nursery was soil from the experimental field where maize has been grown for more than 10 years in a continuous system and where brown spot disease is known to occur (Ngoh Dooh *et al.*, 2021b).

The field experiment consisted of three blocks with four treatments or plots. The plots received the mycorrhizal fungi (V1-AMF, V2-AMF) and the control plots (CV1, CV2). Each treatment was repeated three times. The blocks were separated by 1 m and the distance between the plots was 80 cm. Each plot measured 4.5 m² with 28 pits (hole), 80×50 row spacing. Each hole contained 2 seeds. The field was located in Maroua in the Far North Region of Cameroon. The soil of the site is sandy-clay and maize has been grown for more than 10 years in a continuous system.

Setting up the trial in the nursery

In the nursery, two types of substrate were used: sterilized soil and non-sterilized soil. This soil came from the experimental field. The substrates were placed in black polystyrene bags (45 cm long and 35 cm wide). The first substrate, no-sterilized soil, was placed in the pots with two grains of each of the varieties studied. The second substrate is the sterilized soil, *i.e.* heated for 24 h in the autoclave to reduce or even eliminate all microorganisms.

The diseased maize leaves showing symptoms of brown spot were collected and cleaned with 70 °C alcohol and rinsed with sterile distilled water three times. A longitudinal section of the midrib was made and 5 cm sections of the midrib were cut with a sterilized scalpel. The inside of each piece of vein was scraped with a needle in 5 mL of sterile distilled water to release the sporangia. Microscopic observation was made to confirm the presence of *P. maydis* sporangia (Fig. 1). Using a syringe, the solution was withdrawn and introduced into the different pots of the sterilized treatments during sowing.

Setting up the field trial

The field is an area used seasonally for corn cultivation. The land was prepared manually with a hoe. The ploughing depth is estimated at 10–15 cm. Sowing was done in rows, 4 to 6 cm deep with a spacing of 80 × 50 cm, i.e. 2 seeds per poquet with a density of 56 plants per plot.

Assessment of AMF impact in growth of corn in nursery and field

Measurements of height and leaf area were taken on 14 plants per plot every two weeks. The height of the plants was taken every two weeks for two months using a tape measure placed along the vertical stem of the plant from the collar (ground to stem). The leaf area was taken as a function of the maximum leaf area (LA max). It was calculated conventionally by multiplying the maximum length and width by a coefficient of 0.75 (Bonhomme *et al.*, 1982).

$$LA_{max} = (L_{max} \times W_{max}) \times 0.75$$

Assessment of root density

Root density between control and mycorrhized plants was observed after two months of growth, by extracting the whole plant, in order to obtain the whole root system of the plant (Adamou and Ngakou, 2018).

Impact of AMF on incidence of disease

Incidence was assessed every fortnight from day 26 after sowing (DAS) in the field and in the nursery, according to the formula (Ngoh Dooh *et al.*, 2021b).

$$DI\ (\%) = n/N \times 100$$

DI: Disease incidence; n: Number of diseased plants; N: Total number of plants per plot.

Effect of AMF on disease severity

In the field as well as in the nursery, the degree of disease severity on infected plants was assessed by estimating the leaf area occupied by brown spot disease symptoms using the following formula.

$$S\ (\%) = (\Sigma(xi \times ni))/Nm \times 100$$

xi: the disease score i; **ni**: the number of plants with score i; **Nm**: total number of diseased plants assessed per plot. Disease severity was monitored using a visual rating scale ranging from 0 to 4, according to Masood *et al.* (2010).

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

Assessment of AMF root colonization

Fine roots from 14 maize plants per plot were harvested after 8 weeks to assay the rate of root colonization by AMF.

Staining of root fragments

Roots were stained according to the method of Phillips and Hayman (1970) modified by Kormanik and McGraw (1982). The staining provides contrast for easy visualization of the fungal structures (mycelium, vesicles, arbuscular). Washed the fine root fragments previously preserved in 70 °C alcohol to be stained with large amounts of water and placed them in labelled test tubes. Introduce 10% KOH to immersion and heat in a water bath at 90 °C for 1 h. Then, remove the potash and wash the roots with plenty of water. Introduce 1% hydrochloric acid until the roots are immersed and leave at room temperature for 1 hour. Remove the hydrochloric acid and quickly wash the roots with water. Introduce the staining solution (lactic acid-glycerol-water with 0.05% trypan blue) until the roots are immersed and heat in a water bath at 90 °C for 1 h. Wash the roots with plenty of water and leave to decolorize for 24 hours in the lactic acid-glycerol-water decolorization solution. The stained root fragments were mounted in parallel on slides in groups of 10 and observed under the microscope at magnification 40 and 60. Three replicates were performed. The presence or absence of structures characteristic of mycorrhizae (mycelial filaments, spores, arbuscules and vesicles) was used to evaluate the percentage of root colonization according to the formula:

$$CR\ (\%) = (n/N)\ /100$$

n: number of root fragments observed with one or more mycorrhizal structures
N: Total number of fragments observed; **CR (%)**: Colonisation rate in percentage.

Total phosphorus and nitrogen determination

The aerial parts of the plants were oven-dried and ground in a mill. For mineralization, 0.3 g of dry powder from a sample was introduced into a matrass, to which 5 mL of H_2SO_4 and 0.5 g of mineralization catalyst were added. The whole was heated on a ramp until complete mineralization for 3 h. The volume of the mineralization mixture obtained is

adjusted to 50 mL with distilled water (Devani *et al.*, 1989; Okalebo *et al.*, 1993).

Determination of phosphorus

The reagent is obtained by mixing sulphuric acid (5 N), ammonium molybdate 4%, potassium antimony tartar 0.27% and ascorbic acid 1.8%. The procedure uses 5 mL of the mineralization mixture diluted in 20 mL of distilled water, then 10 mL of reagent is added and the whole is adjusted to 50 mL with distilled water. The mixture is well homogenized and left to stand for one hour before the optical density is read on a spectrophotometer at 880 nm. The amount of phosphorus in the sample is determined by reference to the calibration curve obtained with a 10 mg L^{-1} potassium hydrogen phosphate solution.

Nitrogen determination

The reagent consists of 37% formaldehyde and 7.8% acetyl acetone. For the 0.1 mL test sample procedure, 1.2 mL of sodium acetate and 1.6 mL of reagent are added. The mixture is heated to 97.5 °C for 15 min in a water bath and then reduced to approximately 30 °C with a stream of cold water. The volume of each mixture is adjusted to 10 mL with distilled water. The optical density of the yellow complex formed is read at 412 nm with a spectrophotometer. The amount of nitrogen in each sample is determined against a calibration curve obtained from a freshly prepared 0.4 mg L^{-1} ammonium sulphate solution.

Statistical analysis

Analysis of field and nursery data was carried out using SPSS 20.0 software. Duncan's test at the 5% level was used to compare means when the difference was significant.

Results

AMF effect on height and leaf area of plants in nursery

At the beginning of the season, the height and surface area of the plants in the sterilized and mycorrhized soil pots were smaller than those in the non-mycorrhized pots (Fig. 2A and B). A significant difference ($P \leq 0.001$) was recorded between these treatments. At the last week, the mycorrhized variety CMS9015 recorded the highest height, 1.70 m, which was 25.53% higher than the height on sterilized soil and 15.38% higher than the height on non-sterilized soil (Fig. 2A). The highest leaf area, 470 cm², was recorded in the mycorrhizal treatment (CMS8704), 46.38% compared to the sterilized soil treatment and 20.89% compared to the non-sterilized soil treatment (Fig. 2B). Height and area, irrespective of variety, were higher in sterilized and mycorrhized soils followed by non-sterilized soils and sterilized and non-mycorrhized soils.

AMF effect on height and leaf area of plants in field

Plant height and leaf area were higher in the mycorrhized treatments compared to the controls (Fig. 3A and B). At the last week of the growing season, a significant difference ($P \leq 0.001$) was obtained between the different treatments. The highest average heights ranged from 2±0.9 to 1.81±0.2 m and the lowest from 1.67±0.7 to 1.48±0.4 m respectively for the varieties CMS 9015 and CMS 8704 in the mycorrhizal treatments (Fig. 3A). The highest average plant leaf area, 610.3±0.09 cm², was obtained in the plots mycorrhized with CMS8704 and the lowest (396.6±0.44 cm²) in the control plots with the variety CMS9015 (Fig. 3B).

Effect of AMF in root density in nursery and field champ

Observation of the root system of the plants from the different treatments showed that the mycorrhized treatments had a large root area compared to the non-mycorrhized plants both in the nursery and in the field (Fig. 4).

The root system of the mycorrhized varieties (Fig. 4A) showed increased development of fine and secondary roots. However, the non-mycorrhizal treatments (Fig. 4B) showed less dense root development with less fine roots, or even none at all on some secondary roots.

Effect of AMF on incidence and severity of brown spot in nurseries

During the season a significant difference was recorded between treatments. Incidence and severity were zero (0%) in treatments that received soil with or without mycorrhizal fungi (Table 1). On the other hand, the disease was present, 100%, in all plants in the pots that received the unsterilized soil. The variety CMS 8704 showed the highest severity rate (75%) in the last week (Table 2).

Effect of AMF on the incidence and severity of brown spot in the field:

The application of mycorrhizal fungi in the field significantly ($P \leq 0.001$) reduced the incidence and severity of the disease throughout the season. At the end of the season, week 10, the incidence was 50.86±0.9 and 62.98±1.2% on the control plants and 32.55±0.8 and 41.21±1.1%, respectively, for the variety CMS9015 and CMS 8704 (Fig. 5A). The average severity was 56.9% in uninoculated plants compared to 36.88% in treated plants (Fig. 5B).

Root colonization in nursery and in field

In the nursery, the highest root colonisation rate, 43.33%, was obtained with plants from the mycorrhizal treatments SS+AMF+DI+V1 followed by plants from the SS+AMF+DI+V2 treatment, 36.66%. The non-sterilised soil treatments (NSSV1

and NSSV2) with field soil showed a low root colonisation rate, 13.33% and 16.66% respectively for CV1 and CV2 (Table 3).

In the field, the highest colonisation rate was obtained in the mycorrhised treatments V1AMF (63.33%) and V2AMF (43.33%). However, the colonisation rate of plants from non-inoculated plots was 33.33% and 40% respectively for CV1 and CV2 (Table 4).

Phosphorus and nitrogen rate of corn plants in nursery

A significant difference ($P \leq 0.001$) was obtained on the phosphorus level between the mycorrhizal treatments on sterilized soil (SS+AMF+DI) and the treatments on non-sterilized soil (NSS). The highest amounts of phosphorus 1.07±0.2 and 0.79±0.1 mg g^{-1} were obtained respectively with the varieties CMS9015 and CMS8704 mg g^{-1} in the mycorrhizal treatments on sterilized soil (Fig. 6).

On the nitrogen rate, a significant difference was also obtained in the mycorrhizal treatment on sterilized soil with the highest amount 7.01±0.4 mg g^{-1} (CMS9015) and the non-sterilized soil control 6.01±0.1 mg g^{-1} with the lowest amount (CMS8704). However, the variety CMS9015 absorbed more mineral elements than CMS8704 (Fig. 6). Nitrogen was found to be higher than phosphorus in the nursery.

Phosphorus and nitrogen rate of corn plants in field

A significant difference ($P \leq 0.001$) was recorded on the phosphorus rate between the mycorrhizal treatments and the controls (Fig. 7). The highest phosphorus rates 0.74±1.1 mg g^{-1} in variety CMS8704 and 0.66±0.4 mg g^{-1} in variety CMS9015 were obtained in the mycorrhized treatments (Fig. 7).

In case of nitrogen rate, a significant difference was obtained in the mycorrhized treatments, with the highest amount 9.2±0.2 mg g^{-1} (CMS9015) and the lowest 8.3±0.5 mg g^{-1} (CMS8704). Nitrogen level was higher in variety CMS9015 compared to variety CMS8704 (Fig. 7). Nitrogen was found to be higher than phosphorus in the nursery.

Discussion

The evolution of the height and leaf area of the plants in the field showed no significant difference between the mycorrhizal treatments and their controls at the beginning of the season. However, in the last week of growth, a significant difference was obtained between the mycorrhizal plants and the control plants. The AMF stimulated a 17.32% increase in the height of the mycorrhizal plants in the field. Several authors such as Fortin *et al.* (2015) have shown that the increase in the root system's absorption surface due to the presence of the AMF hyphal network favours water absorption, as the fungi's mycelium allows the plant to draw water from small interstices and aggregates in the soil that are usually not accessible to plant roots. Furthermore, many authors have shown that inoculation with mycorrhizal fungi can significantly increase the leaf area and root biomass of cowpea and lettuce plants in the greenhouse by 30 to 200% compared to controls (Cimen *et al.,* 2010); Aboubacar *et al.* 2013). In some pots, the mycorrhizal treatments showed a low average at the 2nd week of growth compared to the non-mycorrhized controls. This could be explained by the fact that in sterilized soil conditions the AMF-plant symbiosis was not yet established.

Results on plant height in the nursery showed a significant difference in the early weeks between the AMF-sterilized soils with the disease infection and the non-sterilized soils. It would appear that the unsterilized soils taken from the field may have the necessary fertilizers and indigenous mycorrhizal fungi which gives an advantage over the sterilized soil despite the presence of AMF, at the beginning of the season. It is also known that native strains of AMF have the great advantage of being well adapted to the environment in which they occur (Diaga *et al.* 2003); Plenchette *et al.,* 2005; James *et al*., 2020). However, at the final week, no significant difference was recorded, but a slight growth advantage of the mycorrhized plants was obtained over the controls. This result shows that, once the AMF-Plant symbiosis is established, plant growth is stimulated in sterilized plots with mycorrhizae (Ezawa *et al*., 2005; Veresoglou *et al.*, 2021; Zhang *et al*., 2022).

Although microorganisms are important for plant growth, their absence in sterilized and non-mycorrhized pots does not prevent the presence of minerals (obtained by mineralization through heat during soil sterilization). This result corroborates that of Groleau-Reneaud *et al.* (2000) who showed the influence of the sterility of the environment and the physical support on the morphology of maize. He considers that the sterility of the environment does not modify the development of the plant (leaf stage and root emission).

Although there is a functional compatibility between plant species and AMF determining the preferential association between symbionts, field trials of inoculating plants with a fungus have not always given the expected results, often due to competition from native AMF. Indeed, Cornet and Diem (1982) considered that the presence of other microorganisms in the field (native AMF) could influence the growth of mycorrhizal plants by inoculation. However, Alguacil *et al.* (2011) believe that mycorrhization by a highly efficient and competitive fungus does indeed allow significant gains in growth and production compared to mycorrhization of native AMF.

Root density was higher in both field and

nursery in mycorrhized plants compared to non-mycorrhizal plants. This result is in agreement with Garbaye (2013) who showed that the specificity of AMF with maize plants best develops their root system. Moreover, the maize root system is characterized by the presence of adventitious roots that allow nutrients to be drawn only from the superficial soil horizons. Thus, AMF are involved in the extensive development of roots over a wider soil surface, through the presence of fine roots while developing hyphae so that they can improve the uptake of water and mineral elements from the soil.

In addition, the root density of mycorrhizal plants not only allows them to colonise the soil in order to better absorb mineral elements that are sometimes difficult to access, but above all participates in protecting the plant against pathogens (Begoude *et al.,* 2016). Temegne *et al.,* 2017, Djenatou *et al.* (2020) believe that the colonization of plants by AMF can lead to changes in the plant's roots, in this case, greater lignification, which can create a physical barrier preventing the pathogen from penetrating the plant.

Disease incidence and severity were reduced in the plots that received the AMF in both the nursery and the field. Indeed, the AMF treatments showed a reduction in disease incidence and severity (33.7% and 35%). The levels of mineral elements, phosphorus and nitrogen (26.1% and 40.7% in the field; 7% and 58.6% in the nursery) in mycorrhizal plants could influence the effects of the pathogen. This result corroborates that of Gallou *et al*. (2011) who evaluated the bioprotective power of AMF associated with a potato seedling against *P. infestans*. They showed that a decrease in the foliar infection index (*P. infestans*) is due to a significant supply of minerals when the AMF is associated with the plant. In addition, many authors have shown the importance of minerals such as phosphorus and nitrogen in the resistance of plants to diseases (Olsen *et al*., 2003; Veresoglou *et al*., 2019). These minerals would thus strengthen the structure of the cell wall and make it difficult for pathogens to enter. In the case of Fusarium head blight in tomato, optimal phosphorus nutrition does not reduce the disease symptoms. This clearly indicates that other protective mechanisms are added to nutrition as a protective factor. Varietal genetics could provide an answer with the use of fungus-resistant hybrid varieties. Genetic resistance to fungal diseases varies greatly from one hybrid to another, although none of them have complete resistance, some are much less susceptible than others (Deffan *et al*., 2015). In this work, the yellow variety, CMS8704, was found to be more susceptible to the pathogen *P. maydis* despite the contribution of AMF. This result is in agreement with that of Ngoh Dooh *et al*. (2021b) who showed that the yellow maize variety CMS8704 was attacked faster in the field (3rd week) by *P. maydis* with very high incidence and severity rates compared to the white variety, CMS 9015.

Analysis of root colonization showed an average of 53.33 % for the mycorrhized treatments against 36.66% for the field controls, where the low percentage of contamination and pathogen evolution were observed on the mycorrhized plots. Several authors (Dugassa *et al.*, 1996) have shown that mycorrhizal colonization predisposes plants to react rapidly to pest attacks. Cordier *et al*. (1996) showed that mycorrhizal cells were not contaminated by the fungal pathogen. As the pathogen (*P. maydis*) is telluric as well as the mycorrhizae, it would seem that the latter are in competition for the colonization of the plant, which starts as early as the first weeks (7 to 9 days after sowing), during the absorption of the first nutrients by the plant (Djenatou, 2020). These results are also in agreement with those obtained by Johnson *et al.* (2013) who observed a variation in mycorrhization frequency from 45% at flowering to 60% at fruiting of cowpea while decreasing the influence of the pathogen. Muthukumar and Karthik (2021) believe that once mycorrhization is functional, the hyphae begin to produce and scatter in the surrounding soil molecules that repel, suppress and even kill fungal disease vectors (*Phytophthora*, *Pythium*, *Rhizoctonia*, *Fusarium*). In addition, specialized fungal cells accumulate around the root, building a form of armor to hold back any invasion and penetration into the plant.

In the nursery, inoculation of *P. maydis* sporangia on sterilized soil with fungus and sterilized soil without fungus did not result in disease symptoms, incidence and severity (0%). It seems that the sterilized soil is not favorable for the development of the pathogen. The pathogen could not develop in an environment devoid of other microorganisms and other unidentified elements that would be necessary for its proliferation. This can be justified by the presence of the disease in all maize plants (100% incidence), whatever the variety, on unsterilized soil taken in the field (NSS), from the 29th day after sowing. Ngoh Dooh *et al.* (2021b) suggested that the pathogen prefers moist environments rich in plant and animal debris. On the other hand, Edding (1933) dipped maize seeds in an aqueous suspension of sporangia and obtained no infection after 5 weeks of culture. It would seem that the mode of inoculation could be a very determining factor in the expression of this disease (brown spot) in nurseries.

The presence of 24.99% mycorrhiza colonization on unsterilized soil is due to pre-existing indigenous arbuscular mycorrhiza fungi (AMF) in the field soil (Cornet and Diem, 1982). However, the absence of this mycorrhiza colonization on the roots of the plants in the sterilized pots showed that the sterilization of the soil was effective, thus destroying all pre-existing microorganisms in the soil. However, Grünfeld *et al.*

(2022) showed that root colonization depends on the spatial distribution of host plants. Thus, Spatial distance between host plants may determine the root colonization strength.

Conclusion

The study focused on "The impact of AMF on the development of corn brown spot disease caused by *P. maydis* in Maroua in the Far North Region of Cameroon in the field and nursery. The mycorrhizal plants, both in the nursery and in the field, showed a very high rate of root colonization, with high root density, higher phosphorus and nitrogen levels than the non-mycorrhized plants, resulting in a 33.7% decrease in incidence and 35% decrease in severity of brown spot. Thus, AMF are an effective means of controlling this disease through a symbiosis that sets up a defense system and reinforces the plant's root walls to best prevent the entry and proliferation of the pathogen.

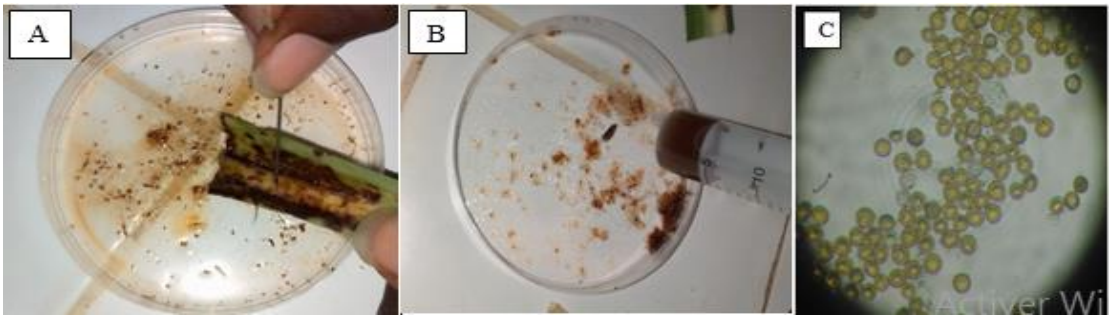

Fig. 1: Obtention of sporangia, **A:** Extraction; **B:** Sampling of the solution obtained; **C:** Microscopic view of sporangia of *Physoderma maydis.*

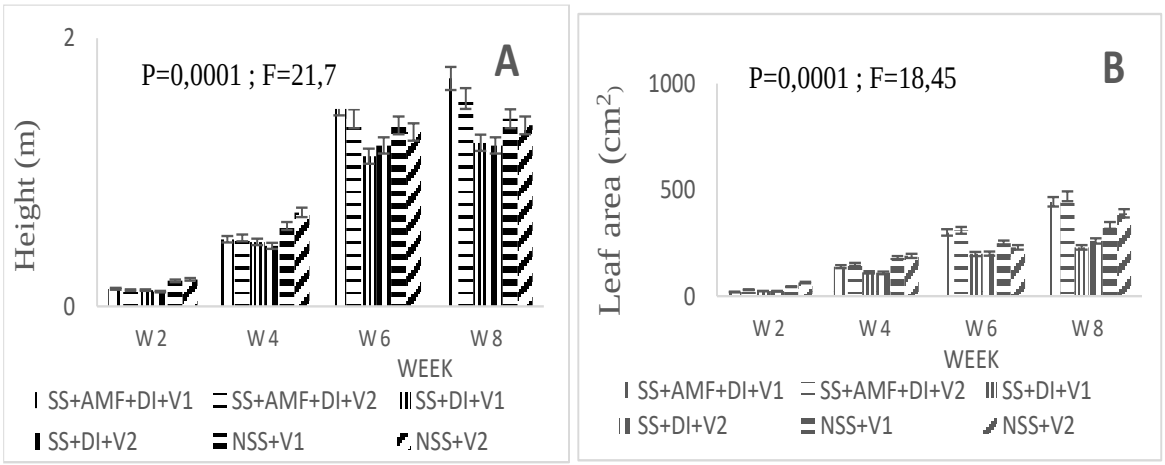

Fig. 2: Evolution of height (A) and leaf area (B) of plants in nursery.

Table 1: Incidence of brown spot disease due to *P. maydis* in different treatments in nursery.

Treat	Weeks after sowing											
	W4			W6			W8			W10		
	Variety											
	V1	V2	**Mean**	V1	V2	**Mean**	V1	V2	**Mean**	V1	V2	**Mean**
T1	0	0	0	0	0	0	0	0	0	0	0	0
T2	0	0	0	0	0	0	0	0	0	0	0	0
T3	100	100	100	100	100	100	100	100	100	100	100	100

V1 = CMS9015; **V2** = CMS 8704; **Treat** = Treatments; **T1** = CMA+MI+SS; **T2** = MI+SS ; T3 = SNS

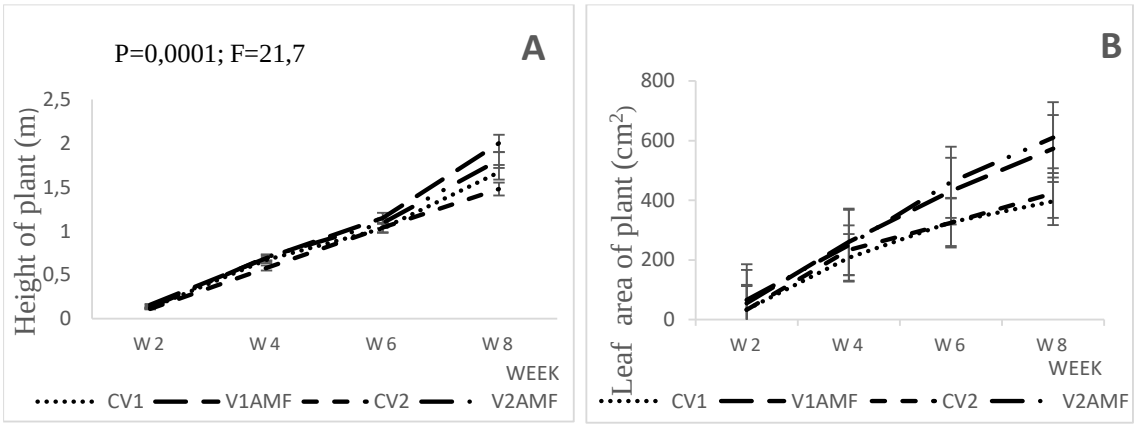

Fig. 3: Evolution of height (A) and leaf area (B) of plants in field.

Fig. 4: Effects of AMF on the development of the root system. A: Root of a mycorrhized plant, **B:** root of a non-mycorrhized plant in the field.

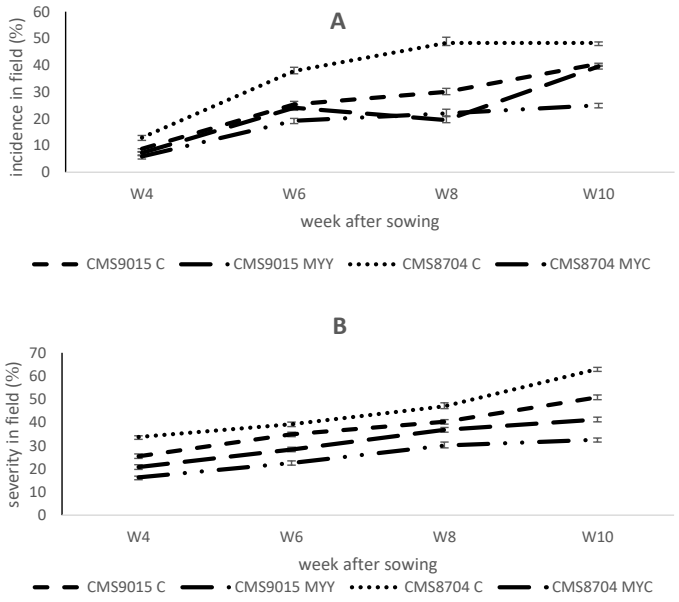

Fig. 5: Incidence (A) and severity (B) in the field.

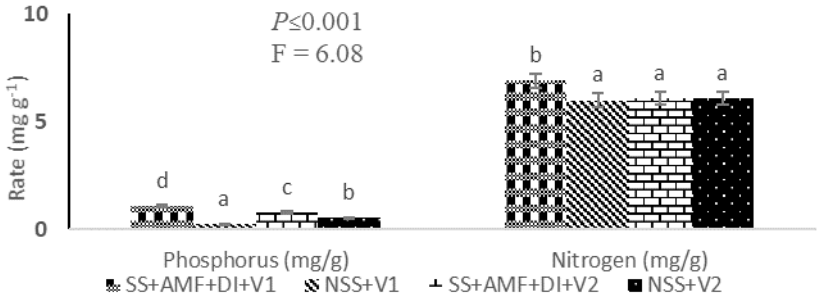

Fig. 6: Phosphorus and nitrogen rate at week 8 in the nursery. The bars with the same letters do not show a significant difference at the 5% level according to Duncan's test.

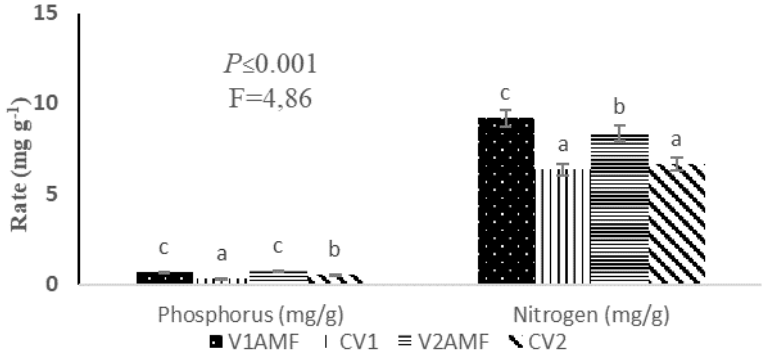

Fig. 7: Phosphorus and nitrogen rate at week 8 in field. The bars with the same letters do not show a significant difference at the 5% level according to Duncan's test.

Table 2: Severity of brown spot disease due to *P. maydis* on different treatments in nursery.

Treat	WAS											
	W4			W6			W8			W10		
	Variety											
	V1	V2	**Mean**	V1	V2	**Mean**	V1	V2	**Mean**	V1	V2	**Mean**
T1	0	0	0	0	0	0	0	0±0	0	0	0	0
T2	0	0	0	0	0	0	0	0±0	0	0	0	0
T3	25	25	25	25	50	37.5	50	75±0	62.5	50	75	62.5

V1 = CMS9015; **V2** = CMS 8704; **Treat** = Treatments; **T1** = CMA+MI+SS; **T2** = MI+SS; T3 = SNS

References

Aboubacar K, Ousmane ZM, Amadou HI, Issaka S, Zoubeirou AM, 2013. Effet de la co-inoculation du rhizobium et de mycorhizes sur les performances agronomiques du niébé au Niger. *J. Appl. Bioscie.*, **72**: 5846-5854.

Adamou S, Ngakou A, 2018. Morphological and structural diversities of indigenous endomycorrhiza communities associated to maize (*Zea mays*) in Northern Cameroonian soils. *J. Anim. Plant Sci.*, **38**: 6057-6073.

Akanvou L, Akanvou R, Toto K, Effet des variétés de maïs et de légumineuses dans la lutte contre *Striga hermonticaen* zone de savane en Côte d'Ivoire. *Agron. Afr.*, **18**: 13-21.

Akiyama K, 2007. Chemical identification and functional analysis of apocarotenoids involved in the development of arbuscular mycorrhizal symbiosis. *Biosci. Biotechnol. Biochem.*, **71**: 405-1414.

Alguacil MM, Torrecillas E, Kohler J, Roldan A, 2011. A molecular approach to ascertain the success of "in situ" AM fungi inoculation in

the revegetation of a semiarid, degraded land. *Sci. Total Environ.*, **409**: 2874-2880.

Begoude D, Sarr APS, Mpon TLY, Owona DA, Kapeua M, Araki N, 2016. Composition of arbuscular mycorrhizal fungi associated with cassava (*Manihot esculenta* Crantz) cultivars as influenced by chemical fertilization and tillage in Cameroon. *J. Appl. Biosci.*, **98**: 9270-9283.

Bonhomme M, Francoise RR, 1982. Estimation simple de la surface foliaire de plantes de maïs en croissance. *Agron. EDP Sci.*, **16**: 553-562.

Cimen I, Turgay B, Pirinç V, 2010. Effect of solarization and vesicular arbuscular mycorrhizal on weed density and yield of lettuce (*Lactuca sativa* L.) in autumn season. *Afr. J. Biotechnol.*, **9**: 3520-3526

Cordier C, Pozo MJ, Barea JM, Gianinazzi S, Gianinazzi-Pearson V, 1996. Cell defense responses associated with localized and systemic resistance to *Phytophthora parasitica* induced in tomato by an arbuscular mycorrhizal fungus: *Mol. Plant-microbe Interact.*, **11**: 10-17.

Cornet F, Diem H, 1982. Etude comparative de l'efficacité des souches de Rhizobium d'Acacia isolées des sols du Sénégal et effet de la double symbiose *Rhizobium-Glomus mosseae* sur la croissance de *Acacia holoserica* et *A. radiana*. *Boisci. For. Trop.*, **198**: 3-15.

Deffan KP, Akanvou L, Akanvou R, Nemlin GJ, Kouame PL, 2015. Evaluation morphologique et nutritionnelle des variétés locales et améliorés de maïs (*Zea mays*) produites en côte d'Ivoire. *Afr. Sci.*, **11**: 181-196.

Devani MB, Shishoo CJ, Shah SA, Suhagia BN, 1989. Microchemical methods; Spectrophotometric Methods for microdetermination of nitrogen in Kjeldahl digest. *J. Ass. Anal. Chem.*, **72**: 953-956.

Diaga D, Tahir AD, Ibrahima N, 2003. Actinorhizal, mycorrhizal and rhizobial symbioses: how much do we know? *Afr. J. Biotechnol.*, **2**: 1-7.

Djenatou P, Ngoh Dooh JP, P. Kosma, Ngonkeu EL, 2020. Evaluation of the inoculation effect of arbuscular mycorrhizal fungi on the growth of cocoa seedlings (*Theobroma cacao* L.) in the nursery. *Int. J. Sci.*, **9**: 6-13.

Dugassa GD, Vonalten H, Schönbeck F, 1996. Effects of arbuscular mycorrhiza (AM) on health of *Linum usitatissimum* L. infected by fungal pathogens. *Plant Soil*, **185**: 173-182.

Eddins AH, 1933. Infection of corn plants by *Physoderma zea-maydis* shaw. *J. Agric. Res.*, **46**: 241-253.

Erenstein O, Jaleta M, Sonder K, Mottaleb K, Prasanna BM, 2022. Global maize production, consumption, and trade: Trends and R&D implications. *Food Secur.*, **14**: 1295-1319

Ezawa T, Hayatsu M, Saito M, 2005. A new hypothesis on the strategy for acquisition of phosphorus in arbuscular mycorrhiza: upregulation of secreted acid phosphatase gene in the host plant. *Mol. Plant Microbe Interact.*, **18**:1046-1053.

Fagbola O, Osonubi O, Mulongoy K, Odunfa, 2001. Effects of drought stress and arbuscular mycorrhiza on the growth of *Glirincidia sepium* Jacq, Walp and *Leucaena leucocephala* Lam de Wit, in simulated eroded soil conditions. *Mycorrhiza*, **11**: 215-223.

Fortin JA, Piché Y, 2015. Les mycorhizes: l'essor de la nouvelle révolution verte, Québec. 2ième Éditions Multi Mondes. pp. 163.

Gallou A, Lucero-Mosquera HP, Cranenbrouck S, Suarez JP, Declerck S, 2011. Mycorrhiza induced resistance in potato plantlets challenged by *Phytophthora infestans*: *Physiol. Mol. Plant Pathol.*, **76**: 20-26.

Garbaye J, 2013. La symbiose mycorhizienne Une association entre les plantes et les champignons. Éditions Quae, collection synthèse. pp. 219.

Groleau-Reneaud S, Plantureux A, Tubeileh, Guckert A, 2000. Effet des dispositifs de culture sur la morphologie du système racinaire du maïs. Institut National Polytechnique de Lorrain.

Grünfeld L, Skias G, Rillig MC, Veresoglou, 2022. Arbuscular mycorrhizal root colonization depends on the spatial distribution of the host plants. *Mycorrhiza*, **32**: 387-395.

Jabeen N, Javaid A, Shoaib A, Khan IH (2021). Management of southern blight of bell pepper by soil ammendment with dry biomass of *Datura metel. J. Plant Pathol.*, **103**: 901-913.

James H, Ndakidemi PA, Mbega ER, 2020. effects of Selected Pesticidal Plants on Termites Affecting Maize Production in Arusha, Tanzania. American Journal of Plant Sciences, **11**: 510-527.

Jackson TP, 2018. *Physoderma* brown spot and stalk rot. Crop Watch. University of Nebraska-Lincoln. pp. 45.

Javaid A, 2009. Arbuscular mycorrhizal mediated nutrition in plants. *J. Plant Nutr.*, **32**: 1595-1618.

Javaid A, Khan IH, 2016. Management of collar rot disease of chickpea by extracts and soil amendment with dry leaf biomass of *Melia azedarach* L. *Philipp. Agric. Sci.*, **99**: 150-155.

Javaid A, Khan IH, 2019. Mycorrhizal fungi associated with mungbean. *Mycopath*, **17**: 45-48.

Johnson JM, Houngnandan P, Kane A, Sanon K, Neyra M, 2013. Diversity patterns of

indigenous arbuscular mycorrhizal fungi associated with rhizosphere of cowpea *Vigna unguiculata* L. Walp. in Benin, West Africa. *Pedobiologia,* **56**:121-128.

Khan IH, Javaid A, 2020. *In vitro* biocontrol potential of *Trichoderma pseudokoningii* against *Macrophomina phaseolina*. *Int. J. Agric. Biol.,* **24**: 730-736.

Khan IH, Javaid A, 2021. *In vitro* screening of *Aspergillus* spp. for their biocontrol potential against *Macrophomina phaseolina*. *J. Plant Pathol.,* **103**: 1195-1205.

Khan IH, Javaid A, Ahmed D, 2021. *Trichoderma viride* controls *Macrophomina phaseolina* through its DNA disintegration and production of antifungal compounds. *Int. J. Agric. Biol.,* **25**: 888-894.

Khan IH, Javaid A, 2022. DNA cleavage of the fungal pathogen and production of antifungal compounds are the possible mechanisms of action of biocontrol agent *Penicillium italicum* against *Macrophomina phaseolina. Mycologia,* **114**: 24-34.

Kormanik PP, McGraw A.C, 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. In: *Methods and Principles of Mycorrhizal Research*, N.C. Schenk (ed.), APS Press, Minneapolis.

Lepoivre P, 2003. Phytopathologie.De loece, les presses agronomiques de Gembloux. pp. 413.

Masood AS, Shafqat I, Naeem TM, Muhammad, Munawer RK, 2010. Methodology for the evaluation of symptoms severity of mango sudden death syndrome in Pakistan. *Pak. J. Bot.,* **42**: 1289-1299.

MINADER, 2019. Annuaire statistique du Cameroun, édition, 2019, pp. 243.

Muthukumar T, Karthik S, 2021. *Epipremnum aureum* (Araceae) roots associated simultaneously with Glomeromycotina, Mucoromycotina and Ascomycota fungi. *Bot. Complut.,* **45**: 1-9.

Ngoh Dooh JP, Bamle S, Djile B, Godswill NN, Gaye L, Tchoupou TDB, Boulga T, Kosma P, Ambang Z, 2021a. Impact of crop rotation and fertilizers (chemical, organic and bio) on diseases and yield of maize (*Zea mays*) in Far North Cameroon. *J. Agric. Crop Res.,* **9**: 40-49.

Ngoh Dooh J.P., Djile Bouba, Djongnang Gabriel, Tchoupou T. D. Brice, Yamagui Rita, Ntatsinda C. Durel, Heu Alain, Ambang Zachee, 2021b. Brown spot and stalk rot diseases of maize (*Zea mays*) and susceptibility of two varieties to *Physoderma maydis* in far north Cameroon. *Int. J. Phytopathol.,* **10**: 19-28.

Ngoh Dooh JP, Djongnang G, Tene TPM, Tenyang N, Deurnaye P, Mbou TPR, Yamagui R, Ntatsinda CD, Djile B, Ambang Z, 2021c. Efficacy and induction resistance of neem extract and mancozeb 80 wp on *Physoderma* brown spot and *Physoderma* stalk rot diseases of corn (*Zea mays*) caused by *Physoderma maydis* in Far North Cameroon. *Int. J. Phytopathol.,* **10**: 85-100.

Nuss TE, Tanumihardjo S, 2011. A quality protein for maize Africa: Closing the protein inadequacy gap in vulnerable populations. *Adv. Nutr.,* **2**: 217-224.

Nwaga D, Ngonkeu M, Adamou S, Fokom R, Tchameni N, Onguene N, Nana W, Chaintreuil C, Amougou A, Moulin L, Prin Y, Lebrun M, Dreyfus B, 2013. Diversité des Champignons mycorhiziens arbusculaires du Cameroun. Institut de recherche agricole pour le développement, Université de Yaoundé I. B.P 812. Yaoundé Cameroun. pp. 143-144.

Nzossié ÉJF, Ndamè JP, Temple L, Dury S, Ndjouenkeu R, Kamdem MS, 2010. "L'émergence du maïs dans la consommation alimentaire des ménages urbains au Nord-Cameroun" Économie Rurale. Agricultures, Alimentations, Territoires. pp. 65-79.

Okalebo, J. R., Gathua, K. W. and Woomer, PL, 1993. Laboratory methods of soil and plant analysis: A Working Manual. Nairobi, Kenya: Tropical Soil Biology and Fertility Programme, UNESCO and EPZ Publishers.

Olsen JE, Jorgensen LN, Petersen J, Mortensen JV, 2003. Effects of rates and timing of nitrogen fertilizer on disease control by fungicides in winter wheat. 2. Crop growth and disease development. *J. Agric. Sci.,* **140**: 15-29.

Plenchette C, Clermont-Dauphin C, Meynard JM, Fortin JA, 2005. Managing arbuscular mycorrhizal fungi in cropping systems. *Can. J. Plant Sci.,* **85**: 31-40.

Robertson AE, Jesse G, Munkvold E, Salaau-Rojas, Mueller DS, 2015. Physoderma brown spot and stalk rot of corn caused by *Physoderma maydis* in Iowa. Plant Health Brief BR-15-0003. *Plant Health Prog.,* **16**: 90-92.

Rouanet G, 1984. Le maïs: Le technicien d'Agriculture tropicale. Édition Maisonneuve et Larose, Paris, France. pp. 142.

Sharf W, Javaid A, Shoaib A, Khan IH, 2021. Induction of resistance in chili against *Sclerotium rolfsii* by plant growth promoting rhizobacteria and *Anagallis arvensis. Egypt. J. Biol. Pest Control,* **31**: 16.

Strullu, DG, 1991. Les mycorhizes des arbres et des plantes cultivées. Techniques et Documentation Lavoisier. Paris. pp. 242.

Temegne NC, Wakem GAl, Taffouo VD, Mbogne TJ, Onguene AN, Youmbi E, Ntsomboh-Ntsefong G, 2017. Effect of phosphorus fertilization on arbuscular mycorrhizal fungi

in the Bambara groundnut rhizosphere. *Afr. J. Microbiol. Res.*, **11**: 1399-1410.

Tsozue D., Nghonda JP, Mekem DL, 2015. Impact of land management system on crop yields and soil fertility in Cameroon. *J. Solid Earth*, **7**: 1761-1796.

Veresoglou SD, Grünfeld L, Mola M, 2021. Micro-landscape dependent changes in arbuscular mycorrhizal fungal community structure. *Appl. Sci.*, **11**: 5297.

Veresoglou SD, Verbruggen E, Makarova O, 2019. Arbuscular mycorrhizal fungi alter the community structure of ammonia oxidizers at high fertility via competition for soil NH_4^+. *Microb. Ecol.*, **78**: 147-158.

Wise K, Kennedy B, Mehl K, Bradley CA, 2018. Physoderma brown spot. University of Kentucky. https://plantpathology.ca.uky.edu/

Zhang S, Tsuruta M, Li C, 2022. Estimation of the most suitable nitrogen concentration for sporocarp formation in *Laccaria japonica* colonizing *Pinus densiflora* seedlings through *in vitro* mycelial culture. *Mycorrhiza*, **32**: 451-464.