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Identification of pathogens and monitoring methods for leaf spots disease of shea tree (*Vitellaria paradoxa* Gaertn. C. F.) in the cropping systems of Komki Ipala (Burkina Faso)

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ABSTRACT

Vitellaria paradoxa is a multipurpose plant species which unfortunately is under multiple pressures causing damages to the plant. This study was to develop methods for monitoring causative agents of shea trees leaf spots in Burkina Faso. An inventory of shea tree individuals and a characterization of leaf spots were performed in Tampoussoumdi, Burkina Faso. Thus, twelve (12) plots of 100 m x 50 m were set up in fallows and fields for identifying all shea trees. Pathogens were also identified by isolated infected leaves fragments of on PDA medium. Isolates were purified and observed under a microscope (X40). As to develop pathogens monitoring methods, three mycorrhizal fungi (yac, fada, Ga) were used to inoculate shea trees cultivated in greenhouse. After two months, inoculated plant leaves were sprayed with prepared isolates of identified pathogens and infection occurrence was checked daily. Results revealed that infection occurred only in unburned fallows and concerned 65% of the shea tree communities. Fungi were found to be responsible for these infections and three species were identified including *Fusarium moniliforme*, *Pestalotia guepini* and *Phoma sorghina*. Results also showed that mycorrhizae can inhibit pathogens growth partially or totally. Their potential use could help controlling the leaf spots disease in shea parks.

Keywords: Burkina Faso, spots, mycorrhizae, pathogenic fungi, *Vitellaria paradoxa*

INTRODUCTION

Biodiversity protection is a concern of national and international interest. Within this biodiversity, it was found several woody species including shea tree (*Vitellaria paradoxa* Gaertner C. F.) which is typical of the Sudanese savannas in Africa (Baggnian et al., 2014; Ahamidé et al., 2017). This plant is protected because of its ecological and economic importance (Sanou and Lamien, 2011). In Burkina Faso, this plant thrives in association with other species in its habitat and occupies nearly 70% of the country area, but it faces multiple pressures (parasitic, bacterial

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and viral attacks ...), which threaten its survival (Gnanglè et al., 2012). A Cumulative effect of these pressures is increasingly affecting the plant and causing a decreased production (Soro et al., 2011) which needs to be addressed by developing methods to fight against these diseases.

Nevertheless, attacks of *V. paradoxa* communities by Loranthaceae, which appeared to be the most widespread and damaging agent, have been already studied and even control methods were developed (Djekota, 2014; Asare et al., 2019; Azongnide et al., 2019). However, fungal, bacterial and viral diseases which were previously rare, are becoming common on the shea trees. This seems to be the leading cause of the encountered spots in shea parks in the central region of Burkina Faso. These infections affect shea leaves by showing up spots called leaf spots. Therefore, it is necessary to evaluate the extent of these infections and to identify the causative pathogens in order to help to develop control methods. Thus, this study was initiated as to evaluate the occurrence of these leaf spots and related pathogens in the shea parks located in the rural municipality of Komki-Ipala, while developing effective control methods to better manage these disease issues.

MATERIALS AND METHODS

Study site

This study was carried out in Tampoussoumdi (12˚11'36 "North 1˚41'13.9" West) in the rural municipality of Komki-Ipala, Kadiogo province, Central region. This site is under tropical climate, with two seasons which are rainy and dry ones. The rainy season is from June to September and characterized by the wet monsoon winds. The annual rain fall ranged from 600 and 900 mm and with temperatures reaching 23°C. With regards to the dry season, it lasts less than 8 months (from October to May) with temperatures reaching 48°C in the mid-season. The dry season is longer than the rainy one and dominated by Harmattan winds. Temperatures vary between 17 and 42°C, depending on the season. Relief consists largely of a low peneplain, with two majors topographic: a plain with an average altitude of 300 m and shallows with an average altitude of 200 m. Soils are tropical, ferruginous, lateritic-clayey type, resting on a large mass of cracked granites. In general, the vegetation is subject to anthropogenic actions and to bush fires caused by the galloping population which was estimated to be 1,459,198 inhabitants in 2007 and projected to be 3,080,375 inhabitants in 2020 (INSD, 2009). The ethnic groups are mainly Mossis and Fulanis.

Methods

An inventory was performed to evaluate the importance of leaf spots in the shea tree communities. However, a part of the park has been burned by bushfires and the extent of leaf infections appears to be different between the burned and unburned parts. Thus, the inventory took into account these two parameters, as well as that of anthropogenic actions (field). This helped to set up a completely randomized system made of three types of plots (field = PC, unburned fallow = JNB and burned fallow = JB). For each plot, an area of two hectares (2 ha) was considered, in which rectangular plots (100 m x 50 m) were set (Agbogan et al., 2017; Djekonbe et al., 2018). Twelve (12) randomized and letter-assigned plots, A to D (burned fallow), E to F (unburned fallow) and I to L (fields) were used for data collection (Figure 1). This allowed the counting of all diseased shea tree individuals (presence of typical symptoms) and healthy ones (absence of typical symptoms). Symptoms are characterized primarily by the presence of typical leaf spots. Direct observations on the color and shape of the leaf spots allowed the identification of the individuals exhibiting symptoms. The impact of leaf spots was assessed by calculating the infection rate and the spots density in each plot. This infection rate (T) as used by Djekonbe et al. (2018) represents the percentage of diseased shea trees and was calculated following the formula below:

$T = \frac{NP}{NT} x100$, where NP = number of infected shea trees; NT = total number of observed shea trees.

An isolation of pathogens followed by their identification allowed to determine the leaf spots causative pathogens. Thus, isolation was performed on infected shea leaves randomly taken from 20 shea tree individuals. Fragments of infected leaves were isolated in Petri dishes on PDA medium. After isolation, Petri dishes were incubated at 22°C under 12 h of alternating cycle of near ultra violet light and darkness for 5 days. After one week of successive subcultures, different isolates were purified, followed by their macroscopic and microscopic observations. Each species was identified based on fungal determination keys as recommended by Mathur and Kongsdal (2003). After one week of successive subcultures, different isolates were purified, followed by their macroscopic and microscopic observations. Each species was identified based on fungal determination keys as recommended by Crous and Groenewalds (2014). After identification, isolates of pathogens were maintained on PDA media and stored at 4°C in order to develop control methods. For that, shea trees were obtained in greenhouse after germination using 5-liter plastic bags containing 5 kg of a soil-based culture medium. Then, efficacy tests using mycorrhizal fungi were carried out on these plants. Three mycorrhizal fungi including yac (yac 2 mix), fada (fada mix) and Ga (*Glomus aggregatum*) were also obtained from the laboratory of Forest Microbiology / Department Environment and Forest and used as inoculum. Thus, eight months old plants were first inoculated with mycorrhizal fungi (yac, fada and Ga). This consisted of depositing inoculums on these plants (10 g of inoculum/plant). Three months later, the isolates of pathogens, described above were prepared and used to spray the leaves of inoculated plants to determine infection occurrence. For that, a completely randomized system consisting of three treatments with three replicates and a control was set up. Pathogens were first coded as (1) for *Fusarium moniliforme*, (3) for *Pestalotia guepini* and (4) for *Phoma sorghina*. Thus, treatments were inoculated plants of which leaves are sprayed with pathogens and defined as follow: yac1, fada1, Ga1 corresponding to inoculated plants with arbuscular mycorrhizal fungi (AMF) yac, fada, Ga respectively and sprayed with *Fusarium moniliforme*; yac3, fada3, Ga3 are those inoculated with AMF yac, fada, Ga respectively and sprayed with *Pestalotia guepini*; treatments Yac4, Fada4, Ga4 are inoculated plants with AMF yac, fada, Ga respectively and sprayed with *Phoma sorghina*. Three controls were used and noted as TO1, TO3 and TO4 which are non-inoculated plants and sprayed with *Fusarium moniliforme*, *Pestalotia guepini* and *Phoma sorghina* respectively.

In the inoculation process, about 1 ml of pathogen isolates was sprayed on the leaves of each plant. The plants were then kept in a white plastic (polyethylene) micro-greenhouse to maintain a relatively high humidity (95-100%). The high humidity was maintained in order to create a microclimate (around 20°C) which is favorable to the growth of mycoses (Silué et al., 2018). Finally, a regular monitoring was performed on the plants through direct observations of the leaves and for recording the number of infected leaves. Thus, the efficiency of mycorrhizal fungi was determined by calculating the infection rate which is the ratio between number of infected plants and the total number of plants.

Data analysis

Data were entered using Excel version 2018 to calculate average values of density of infected plants. Infection rates were calculated and analyzed using XLSAT Software, version 2018.

Figure 1: Experimental set up for the inventory in Komki-Ipala (Google Earth 2020).

RESULTS

Infection rates of leaf spots in the shea tree communities

Observations of leaves presenting spots during the inventory of shea tree in the rural municipality of Komki-Ipala showed the extent of the infection (Table 1). Adults individual, with a size ranging from 2 to 3 m (Figure 2 a and b), as well as the regeneration (0.6 m) (Figure 2 c), were attacked. Results showed that all infected individuals were found only in unburned fallows (UF). No apparent leaf spot was recorded in burned fallows (BF) and in fields (F).

Morphological characterization of leaf spots

Results showed the occurrence of many external signs that characterize infected shea tree leaves. The color and shape of the spots were found to be the most visible signs of infection. Symptoms appeared as black or brown, red-brown, pale-brown, or brown-black spots that are more or less rounded or circular to irregular shapes (Figure 3 a), with variable size (small, medium and large). These spots are characterized by concentric browning, with flat lesions or perforations (Figure 3 b). Results also showed the presence of necrotic and oval spots of brown or yellowish color having a purple brown halo on the border (Figure 3 c). These spots which appear on different parts of the shea tree leaves (veins, edges and the center), represent the cells death in a limited area.

Observations also showed that the infected leaves start wilting and yellowing (Figure 4) and eventually wither and fall off.

Identification of pathogens

Results of the identification of pathogens in PDA culture medium after purification showed the characteristics of isolates which are particularly distinguished by their color and shape. Macroscopic observations of isolates showed a white color of the mycelium, with circular layers which were characteristic to *Fusarium* (Figure 5 a). It was also observed a white background mycelium with small black circular spots above, which characterizes *Pestalotia* (Figure

5 b). As shown in Figure 5 c, the observed light pink color isolates, with circular shape indicate the features of *Phoma*.

Through microscopic observations and the use of identification keys, three species with different characteristics were identified. Thus, it was observed an elongated spindle-shaped and septate macroconidium, with a small spindle-shaped microconidium corresponding to the characteristic of *Fusarium moniliforme* (Figure 6 a). It was also found oval shaped conidia, with 2 to 3 septa, and 2 flagella which were attributed to *Pestalotia guepini* (Figure 6 b). From the Figure 6 c, it was observed an oval to elliptical conidia representing the characteristic of *Phoma sorghina*.

Effect of mycorrhizal fungi on shea tree leaf spots disease

Results from the inoculation of plants showed different infection rates which also differ among treatments. No infection (0%) was found in treatments fada1, yac1, fada3, yac3, yac4 and Ga4 when pathogens were applied on plant leaves. Results showed that regardless of the pathogen, yac completely inhibited the development of symptoms (Figure 7 a, b and c). In treatments Ga1, Ga3 and fada4, plant leaves were partially infected (33 to 66.66%).

Table 1: Infection rate and density of diseased shea trees in the cropping systems.

Cropping system	Infection rate (%)	Density (Number of infected individuals / ha)
Unburned fallows	65	65
Burned fallows	0	0
Fields	0	0

Figure 2: Infected shea tree individuals (Figure 2a and b), and regeneration (Figure c).

Figure 3: Colors (a), forms (b), circular purple margins (c) of leaf spots.

Figure 4: *Y*ellowing of diseased shea tree leaves.

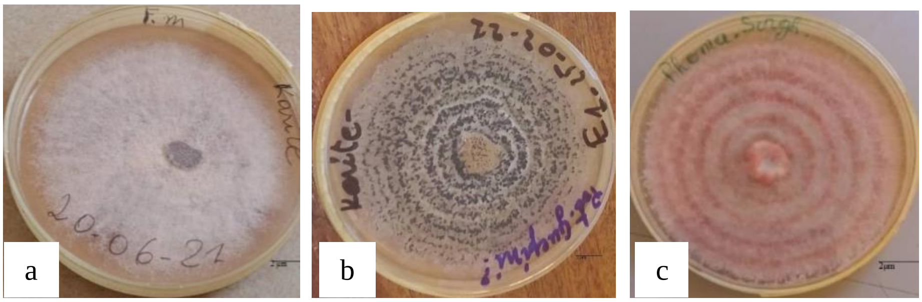


Figure 5: Macroscopic appearance of the mycelium of *Fusarium* (a), *Pestalotia* (b) and *Phoma* (c) isolates in PDA medium.

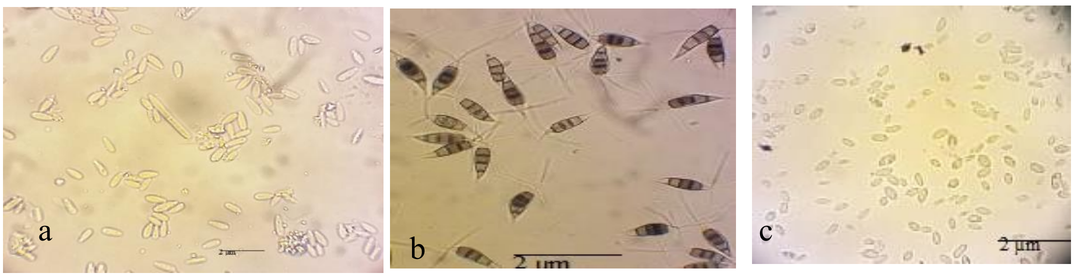
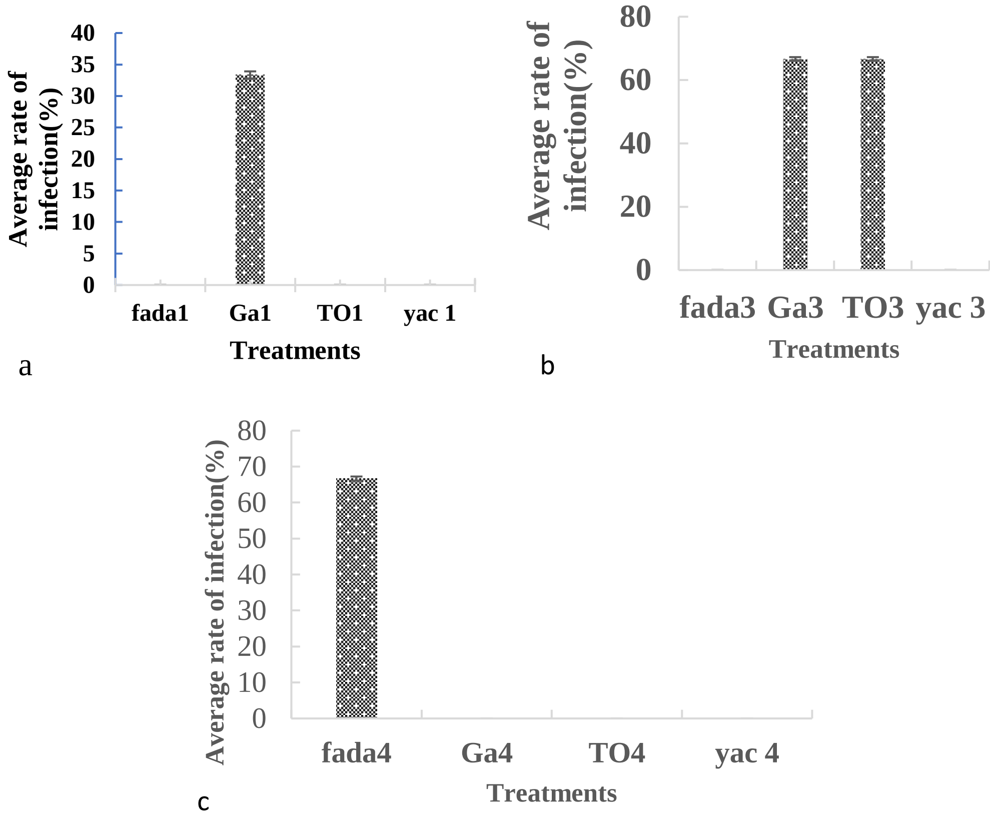


Figure 6: Microscopic characteristics of *Fusarium moniliforme* (a), *Pestalotia guepini* (b) and *Phoma sorghina* (c) conidia observed on shea tree leaves at Komki Ipala (X40).



Mycorrhizal fungi: yac= yac 2 mix, fada= fada mix, Ga= *Glomus aggregatum*
1= sprayed with *Fusarium moniliforme, 3*= sprayed with *Pestalotia guepini, 4*= sprayed with *Phoma sorghina*

Figure 7: Infection rate of leaf spot disease in shea trees inoculated with mycorrhizal fungi.

DISCUSSION

Evaluation of leaf spots

The evaluation of the importance of shea leaf infection revealed a high rate of diseased shea trees (65%), with an apparent absence of infection in other individuals in the same plot (35%). This apparent lack of the pathogens spreading among some shea tree individuals suggests that these trees are resistant to the disease. This confirms the results of Fontaine et al. (2004), Akrofi and Amoah (2009) which showed the existence of disease resistance within the shea tree communities due to genotypic and phenotypic variations. However, the high number of infected individuals / ha was more justified as even young shea trees were attacked. These results were similar to those of Akrofi and Amoah (2009), showing the occurrence of fungi induced disease on shea tree leaves in Ghana. We could then hypothesize that the pathogens causing these attacks come from Ghana and the contamination was possible because of the transhumance (migration of animals from Ghana to Burkina Faso) as also explained by Dr Souleymane GANABA in the case of the municipality of Leo which is a bordering locality between Ghana and the central region of Burkina Faso (Personal communication).

In addition, the observed apparent absence of leaf spots on shea trees in JB and PC could be explained by the presence of bush fires and anthropogenic actions which contribute to significantly reduce the disease spreading in regulating the density of the plant species community. In these plots (BF, F), it was observed that the density of trees was low compared to that in other plots (UF) (Semdé et al., 2021). This confirms the results of Djekonbe et al. (2018), which pointed out that low density of trees reduced the vulnerability of the shea tree to various threats or contributed to protect individuals against some pathogens. These results corroborate with those of Ahamidé et al. (2017), Djekonbe et al. (2018) which demonstrated that the impact of pathogens was more important in undisturbed natural environments than it was in agricultural systems. Opposite results were found by Glèlè Kakaï et al. (2011), showing that, although these factors regulate the density of species, it could make the plant vulnerable to parasites and cause physiological disorders. However, this could depend on the type of encountered parasites.

Identification of pathogens and control method

Results from the isolation of the parasites associated with shea leaf spots showed that no sample was free of microorganisms. It was observed from the culture media, three genus of pathogenic fungi responsible for the shea tree leaf spots. These are *Fusarium*, *Pestalotia* and *Phoma*, which were identified by colors differentiation. The observed color for these isolates can vary for the same genus according to Pažoutová (2009). So, for each given genus, one species of pathogenic fungi was identified on the shea tree leaves through microscopic observations and the use of identification keys. Then, three pathogenic species were identified as the leading cause of leaf spots which were observed for the shea trees in Komki-Ipala. These species were *Fusarium moniliforme*, *Pestalotia guepini* and *Phoma sorghina*. These pathogenic fungi were found to be diverse and this could complicate a subsequent control of the leaf spot diseases.

However, to control these pathogens on shea tree leaves, an analysis of the effectiveness of mycorrhizal fungi in the occurrence of the symptoms was performed. Results appeared to be interesting as it showed little apparition (33.33%) or apparent absence (0%) of typical symptoms (spots) on the leaves of shea trees. Indeed, among all used mycorrhizal fungi, yac completely inhibited the development of the disease regardless of the type of pathogen. This could suggest that yac is the most effective mycorrhizal fungus in terms of regulating the spreading of the leaf spots disease. Therefore, the use of mycorrhizal fungi could be considered in order to reduce the intensity of shea leaf spot disease. These results corroborate with those of Silué et al. (2018) which showed that mycorrhizal fungi were useful in fighting against fungal pathogens. Similar results were found by Manga et al. (2017) and Slezack (2021) showing that the use of mycorrhizal fungi was active in protecting forest trees against some diseases caused by fungi. However, in this study, the fact that few mycorrhizal treatments were infected and the controls were not infected could be due to handling errors or the virulence of the pathogens.

Conclusion

This study allowed to determine the intensity of leaf spot infection on shea tree communities by considering plots. These infections were more important in natural environments than it was in disturbed areas. This shows that bush fires and human actions, even though detrimental to the environment, contribute in one way or another to regulate some disasters. The study was conclusive because it also allowed the identification of the causative pathogens for the leaf spots in shea trees. These agents were the pathogenic fungi which caused leaf spots, a new disease of shea tree leaves in Burkina Faso. Thus, with regards to control methods, this study recommends the use of mycorrhizae as a means of biological control or an integrated monitoring system for a better management of the leaf spots disease.

COMPETING INTERESTS

The authors declare that there is no competing interests for this article.

AUTHORS' CONTRIBUTIONS

IS, SG, HH and SB revised the manuscript; KS designed the experimental set up, data collection, the preparation and writing of the manuscript.

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