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Analysis of Sclerotinia Expression Due to *Sclerotium rolfsii* Fungus in Market Gardening Crops in the Different Agroecological Zones of Côte d'Ivoire

Bolou Bi Bolou Antoine[1*], Abo Kouabenan[2], Doumbouya Mohamed[1], Kouamé Koffi Gaston[1], Kassi Koffi Fernand[1], Tuo Seydou[1], Nahoua Koné[1], Kouassi N'dri Pacôme[1] and Koné Daouda[1]

[1]Laboratory of Plant Physiology, Faculty of Biosciences, University Felix Houphouet Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire.
[2]Laboratory of Phytopathology and Plant Biology, Department of Training and Research Agriculture and Animal Resources (DFR-ARA), National Polytechnics Institute Felix Houphouet-Boigny (INP-HB), BP 1313, Yamoussoukro, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration between all authors. Authors BBBA and AK designed the study, performed the experiments and wrote the manuscript. Author DM carried out the experimental process. Author KKG was also responsible for data interpretation and statistical analysis. Author KKF helped in experiments and preparing the manuscript. Authors NK, TS and KNP participated in experiments, data collection, managed the analyses of the study and literature searches. Author BBBA also wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

In Côte d'Ivoire, the craze for market gardening is due, in large part, to the high demand of vegetables in urban markets especially during off-season periods. However, the yields are subject to the destructive action of dreadful enemies of such crops, including the pathogenic fungus

**Corresponding author: E-mail: bolantoine@yahoo.fr;*

Sclerotium rolfsii. A study on this pathogen was conducted during 12 months in the different agroecological zones (AEZ) of Côte d'Ivoire. Collections were made on pepper, tomato and eggplant crops in six agroecological zones and 93 isolates were obtained and kept in the fungal collection. The most infected zone was the humid forest zone so-called AEZ I where the attack rate was 48.38%. The other agroecological zones II, III, IV, V and VI had infection rates respectively of: 16.13%, 0%, 9.68%; 20.43% and 5.38%. Thus at the end of this survey, tomato was the most sensitive crop to the parasite compared with eggplant and pepper. And then, The AEZ I isolates had faster mycelial growth than those of the other AEZ. Finally, Of all isolates, eight morphological types were distinguished according to the production of sclerotia and air aspect of mycelium.

Keywords: Sclerotium rolfsii; characterization; morphological; agroecological zones; Côte d'Ivoire.

1. INTRODUCTION

Market gardening crops are grown in various parts of the globe for their nutritional and socio-economic importance. A multitude of market garden species provides a plurality of vegetables, fruits and herbs for human food. Favorable environmental and climatic conditions help to obtain good yields with such crops [1].

In Côte d'Ivoire, market gardening crops are grown in different agroecological zones (AEZ) spread all over the country. The area occupied by such market gardening crops is estimated at about 23,000 hectares and mainly concentrated around the shallows and peri-urban areas [2]. The major production areas are located close to major cities including Anyama, Songon, N'zianoua, Tiassalé, Zatta, Djébonoua, Sinfra, Souandala, Bakendenso and Gonzagueville. According to the same document, the average area per farmer varies from 1000 to 1500 m^2. The mainly cultivated species are, among others, tomato, sweet pepper, onion, eggplant, pepper, cabbage, lettuce, cucumber and okra. The craze for market gardening is due in large part to the high demand of vegetables in urban markets, especially during off-season periods from November to May. Market gardening is a source of jobs for more than 103,000 people of which one third are women, a majority in some areas.

However, market gardening is subject to biotic stresses caused by viruses, bacteria and fungi, causing various symptoms from nursery to storage after harvest. The attacks cause significant yield losses that significantly reduce the income of market gardeners through their actions on the quantity and quality of harvests. The different damage occur on plant organs at various stages of their growth [3]. One of the most dreadful enemies of these crops in tropical, subtropical and everywhere in warm regions is the *Sclerotium rolfsii* fungal pathogen [4]. This ubiquitous pathogen is a polyphagous that can attack over 500 plant species belonging to at least 100 families. Sclerotia are able to survive for more than 3 to 4 years in the soil. Thus, it becomes difficult to control such pathogen. The sclerotia can persist in crop debris and weed hosts. Indeed, the fungus is able to live as a saprophyte and can spread rapidly to the soil surface even without the presence of susceptible hosts [5]. They are spread by the wind, the rain and by farming practices. Sclerotia need good ventilation to germinate [6]. Thus, high temperature (25 -35°C), low or acidic pH (3-6) and high humidity stimulates mycelial growth on hosts [7].

The purpose of this study is to determine the distribution of the disease and characterize morphologically *Sclerotium rolfsii* isolates in the different agroecological zones of Côte d'Ivoire, on market gardening crops.

2. MATERIALS

2.1 Plant Material

This study took place both on-farm and in laboratory. Infected pepper, tomato and eggplant seedlings were used to isolate and purify the different *Sclerotium rolfsii* isolates. The varieties encountered during the different surveys, were, Safi F1 and Yolo Wonder F1 for peper. The varieties Lindo, Petomech, Caraïbo and UC 82 were the ones grown by tomato producers. Those used in eggplant production were, particularly, Ndrowa, Djamba and Kalenda.

2.2 Technical Material

For accurate location of collection sites, a GARMIN type GPS was used.

3. METHODS

3.1 Surveys and Sampling

The different areas of market garden crop production were surveyed from 2012 to 2014, in the months of February, May, July and October and in 2015, from March to 2 April 2015. The surveys were mainly carried out in the localities of high production, through the different agroecological zones (AEZ), as defined in Table 1 [8] and areas where severe impacts of wilting due to *Sclerotium rolfsii* were reported by market gardeners [9,10]. The information was provided by various agents of the National Agency for Rural Development (ANADER) of each zone. All the seven agroecological zones of Côte d'Ivoire have been visited (Fig. 1). During the surveys, the wilted seedlings of tomato, eggplant and pepper were collected on the basis of a seedling per infection outbreak. Weeds such as *Croton hirsutum* l'Hérit. (Euphorbiaceae), *Eleusine indica* (L.) Gaertner (Poaceae), *Imperata cylindrica* (L.) Raeuschel (Poaceae) and *Spigelia anthelmia* (L.) (Loganiaceae) were collected in case of attack by the pathogen.

The representation of sites was performed using GPS coordinates (North latitudes and West longitudes).

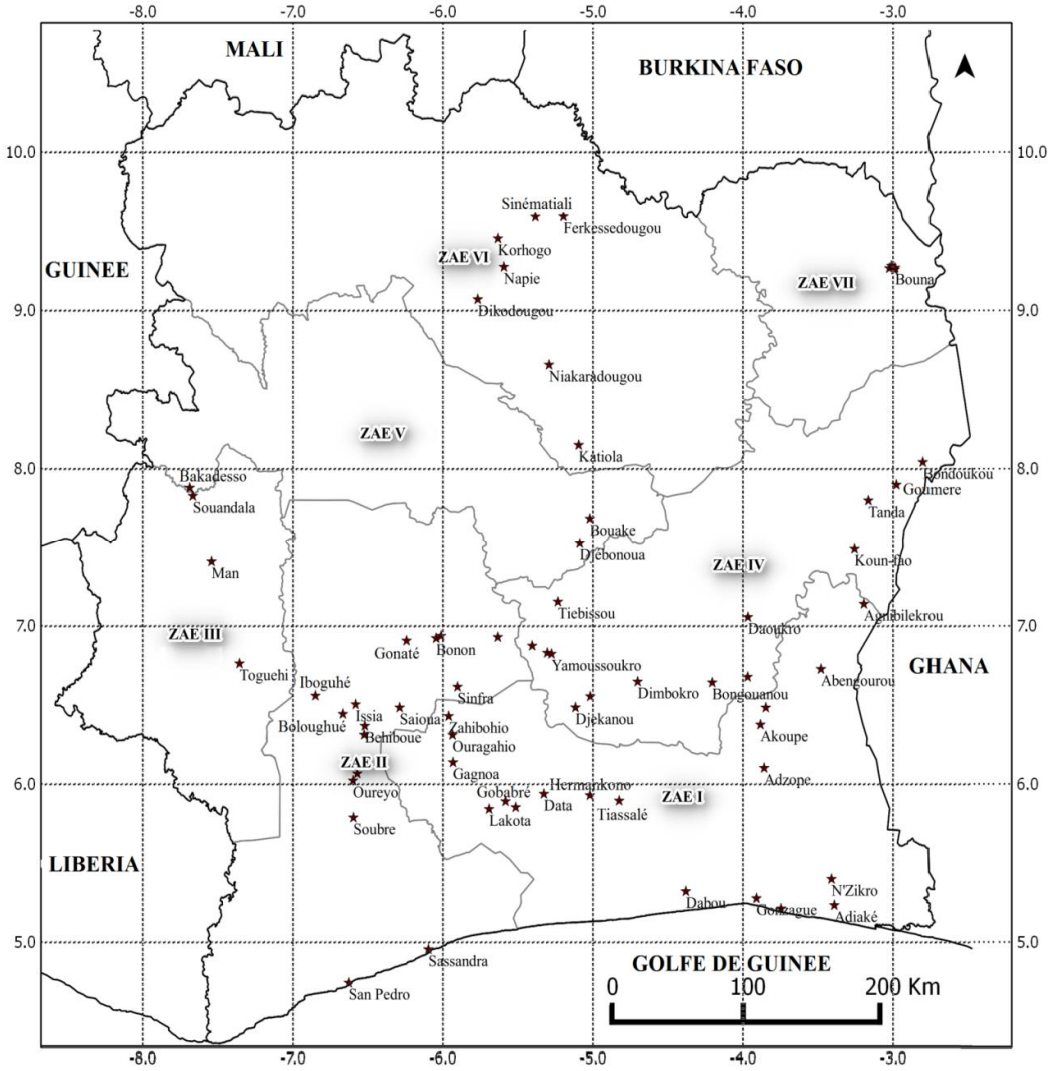

Fig. 1. Different localities visited during the study [8]

Table 1. Characteristics of the 7 agroecological zones (AEZ) in Côte d'Ivoire [8]

AEZ	Localities visited	Characteristics	Altitude (m)	Pluviometry (mm)	Average annual temperature in °C (Standard deviation)
I	Abidjan, Dabou, N'zikro, Adiaké, Akoupé, Adzopé, Abengourou, Tiassalé, Data, Hermankono, Lakota, Gobabré, Gagnoa, Ouragahio, Zahibohio, Agnibilekrou	Southern dense rainforest area	0-200	1400 - 2500 (bimodal)	29 (5.6)
II	Bonon, Gonaté, Sinfra, Behiboué, Iboguhé, Bolouguhé, Issia, Oureyo, Soubré, San-Pédro, Sassandra,	Western dense rainforest area	~1000 (Daloa)	1300- 1750	23.5 (13.4)
III	Duekoué (Toguehi), Man, Souandala, Bakadenso	Western semi-montainous forest area	> 1000 (Man)	1300- 2300	24.5 (7.7)
IV	Toumodi, Yamoussoukro, Djekanou, Tiébissou, Bongouanou, Daoukro, Koun-fao, Tanda, Goumeré, Bondoukou	Semi deciduous dense rainforest area	0-200	1300- 1750	23.5 (13.4)
V	Bouaké, Djebonoua	Transitional forest area	300-600	1300- 1750 (unimodal)	23.5 (13.4)
VI	Katiola, Niakaradougou, Dikodougou, Napié, Korhogo, Ferkessedougou, Sinématiali	Humid tropical savanna area	300- 500	1150- 1350 (unimodal)	26.7 (1.1)
VII	Bouna	Dry tropical savanna area	300-500	1150- 1350	26.7 (1.1)

3.2 Isolation and Purification of *Sclerotium rolfsii*

Sclerotium rolfsii was the main target fungal pathogen in this study. It was isolated from diseased tomato, eggplant, pepper seedlings and weeds collected in the different production areas showing symptoms of wilting, white collar mycelium or sclerotia cluster. The samples cut into small pieces (explants) underwent successive sterilisations in sodium hypochlorite diluted to 10%. The explants were drained on sterile filter paper. The PDA (Potato Dextrose Agar) culture medium was autoclaved at 121°C under a pressure of 1.5 bar for 30 min and then distributed in supercooled state in Petri dishes. After solidification of the culture medium, the Petri dishes were seeded with sclerotia or explants at a rate of five per culture dish.

Fungal colonies produced around the explant were sampled for transplanting into new Petri dishes. The end of the mycelium was removed and transferred to a new culture medium in sterile Petri dishes. Successive crops of the developed colony were made into new Petri dishes until a homogeneous and uniform colony was obtained.

3.3 Comparative Study of the Mycelial Growth of *Sclerotium rolfsii* Isolates

The PDA culture medium used was amended, supercooled, by streptomycin, which is a bactericide. The resulting mixture was distributed into Petri dishes of 90 mm diameter at a rate of 17 ml medium in each dish. Using a cookie cutter, slices of 5 mm diameter were taken from the growth front of *S. rolfsii* cultures older than 7 days; and then deposited in the center of each Petri dish. The inoculated dishes were incubated at 28°C and a photoperiod of 12 h for 7 days. The mycelial growth of colonies was measured every 24 hours over two perpendicular axes drawn at the base of each Petri dish and intersecting in the middle where the explant was deposited (Fig. 2). The fungi were arranged by area of origin. The experiment was repeated three times and each isolate was replicated in five Petri dishes for each experiment.

3.4 Morphological Characterization of *Sclerotium rolfsii* Isolates [12]

The characters were observed on thallus aged four to five days, originating from a clone directly derived from the initial isolation so as to avoid morphological changes due to successive transplanting. All cultures were subjected to identical conditions: temperature 28°C, with 12 hours photoperiod and 80% relative humidity. Two characters were selected for description of strains according to their stability in culture under the experimental conditions and their discriminating power to help identify elements specific to each individual or group of individuals. Data such as thallus color and sclerotia shape which mostly differ only by low grades have been intentionally excluded. The comparison of stems therefore focused on two characters that are qualitative and easily identifiable in culture: aspect of aerial mycelium and ability to sclerotia production [12].

4. RESULTS

4.1 Outward Signs of *Sclerotium rolfsii* in Agroecological Zones

A total of 93 isolates were collected during surveys. It appeared that tomato was the most attacked crop among the three solanaceae species. Samples of peppers were less attacked than tomato seedlings, but more attacked than eggplant. The samplings were carried out in 43 localities visited in six agroecological zones. Infection rates were 48.38%; 16.13%, 0%, 9.68%; 20.43%; 0% and 5.38% respectively, in agroecological zones I, II, III, IV, V and VI (Fig. 3). The fungus attacks were more pronounced in AEZ 1 where rainfall is important in Côte d'Ivoire, with an annual average temperature of 29°C. The fungus got to fit more easily in this zone since its biology and ecology matched exactly such zone compared to other zones. AEZ V and II were slightly attacked compared with AEZ I, with attack rates of 20.43% and 16.13% respectively. These two zones received less rainfall than AEZ I and had an average temperature of 23.6°C which is less favorable to mycelial growth and sclerotia conservation. AEZ IV had a low infection rate that was only 9.68%, but had the same features as the previous 2 zones. The wilted seedlings harvested in AEZ III were not caused by *Sclerotium rolfsii*, but rather *Fusarium* sp.

4.2 Outward Signs of *Sclerotium rolfsii* on Tomato, Pepper and Eggplant Crops

During the different surveys, tomato was the most cultivated and most attacked crop. An attack rate of up to 83.87% was recorded. All the

AEZ visited showed the presence of the pathogen in different proportions except AEZ III which showed no attack by *Sclerotium rolfsii*. AEZ I was the most infected with an attack rate of 41.02%. AEZ V was the second most infected area, with an attack rate of 24.78%. The least attacked area was AEZ VI. This area was seven times less attacked than AEZ I, the most infected.

The cultivation of pepper is most practiced in zone VI, but attacks due to *Sclerotium rolfsii* were more severe in AEZ I, just like tomato and eggplant. Pepper was more resistant to fungus attacks than tomato. The producers of the five other AEZ did not have any worries about cultivating pepper and eggplant faced with the aggression of *Sclerotium rolfsii*.

4.3 Mycelial Growth Speed in the Different Agroecological Zones

Mycelial growth speed was studied to know the pathogenicity, capacity or fitness that agro-ecological conditions play on isolates when they were putting in a same condition. Thus, the mycelial growth of *Sclrotium rolfsii* isolates of AEZ VI on the PDA culture medium after twenty four (24) hours was null. However, isolates of other infected areas have proliferated. Thus, the mycelial growth of AEZ I isolates was three times higher than that of AEZ II and AEZ IV. Furthermore, the mycelial growth of AEZ V isolates was twice greater than that of isolates originating from AEZ II and AEZ IV (Fig. 4).

On the second day, the mycelial growth in AEZ I was twice as large as those of AEZ II and AEZ IV. Moreover it was ten times greater than that of isolates from AEZ VI. AEZ VI which is the area where fungi do not grow quickly had a mycelial growth 5 times smaller than those of isolates from AEZ II and IV. AEZ II and IV had almost the same features and the fungus behaved in the same way during the first two days (Fig. 4).

On the third day of trial, the mycelial growths were identical in AEZ II and IV. However, the growth of isolates from AEZ VI was three times lower than that of the AEZ I and twice as high as AEZ V. On the fourth day, the situation was the same; the growth of AEZ I isolates remained always higher than that of isolates from other zones (Fig. 4).

On the fourth day, the last day of the experiment, many isolates were able to fill the Petri dishes. Almost all isolates from AEZ I invaded the Petri dish. The mycelial growth of isolates from zones AEZ II, AEZ IV and AEZ V was statistically identical and the radii varied from 3.52±0.10 cm and 3.84±0.04 cm. AEZ IV always had the lowest mycelial growth with an average radius of 3.20±0.08 cm (Fig. 4).

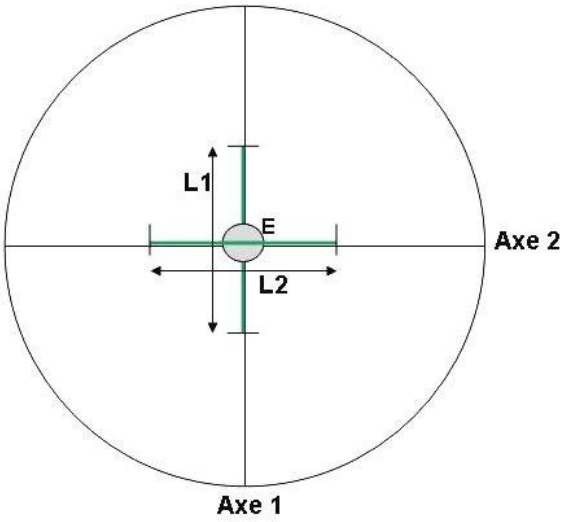

Fig. 2. Diagram illustrating the measurement method of fungal colonies growth in a Petri dish [11]

E: known average diameter of explant (= X) at time t_0, L1: diameter of the explant E according to axis 1 at time t_1, L2: diameter of the explant E according to axis 2 at time t_1

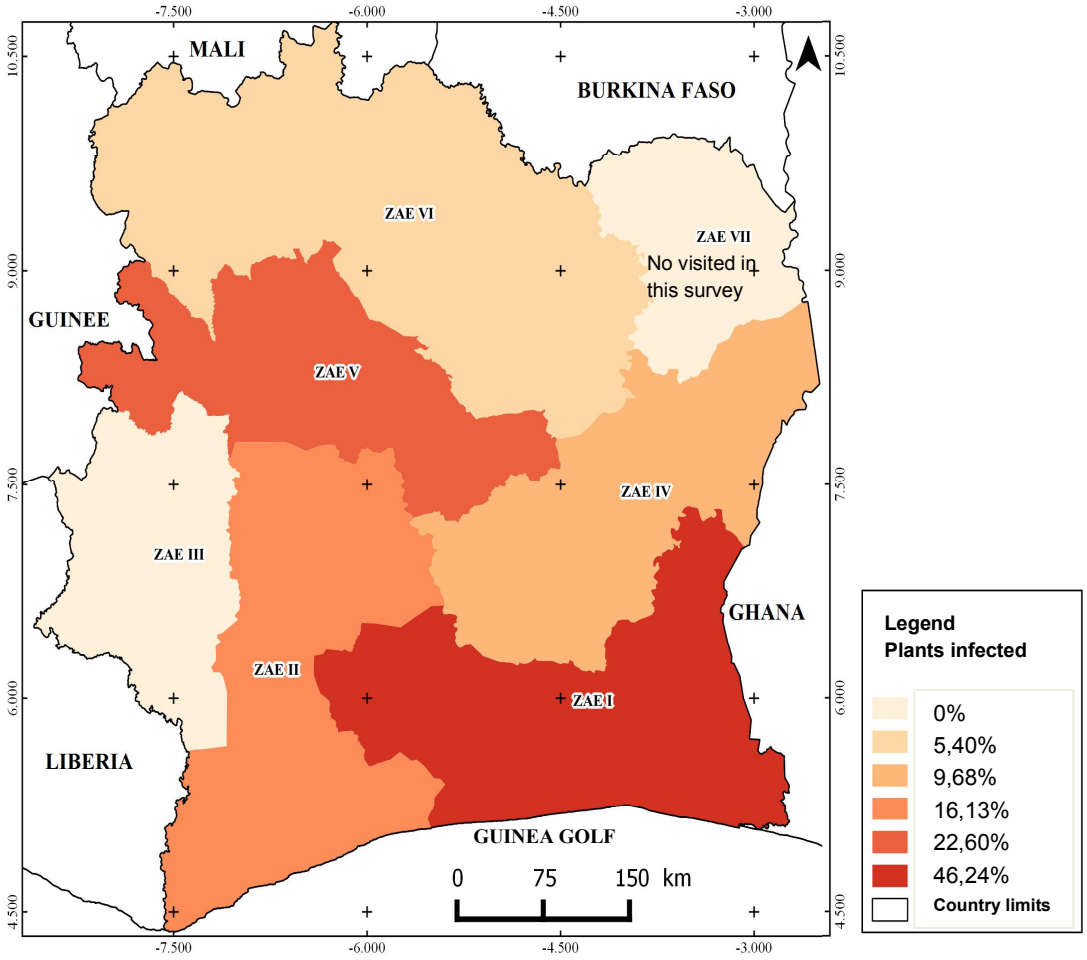

Fig. 3. Distribution of *Sclerotium rolfsii* in the different agroecological zones

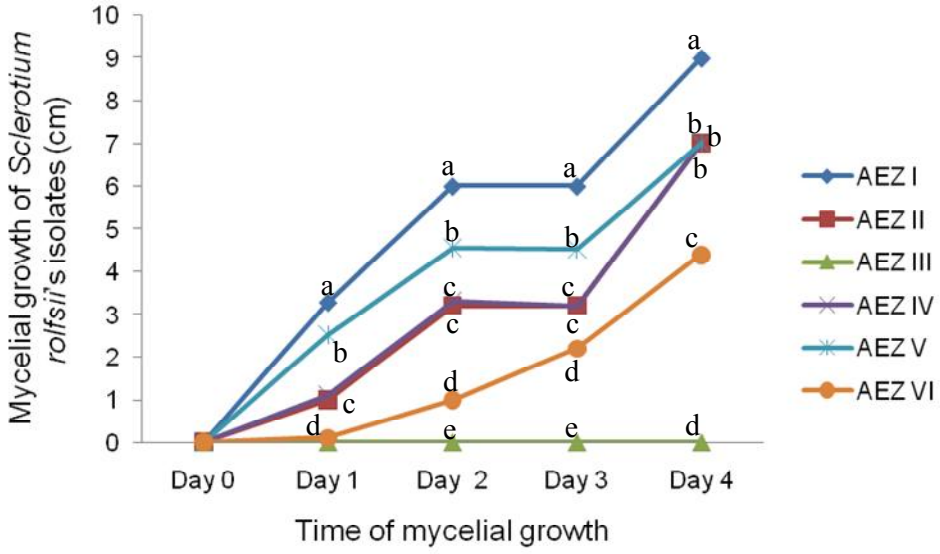

Fig. 4. Growth speed of *Sclerotium rolfsii* isolates from the different agroecological zones

4.4 Morphological Characterization of *Sclerotium rolfsii* Isolates

In pure culture, at young stage, the mycelium was first hyaline then appeared whitish sclerotia that became progressively brown to black. The texture and the abundance of aerial mycelium were also distinct among isolates, features which remain constant on fresh cultures and give them a particular facies. On this basis, was established the first classification that distinguishes two characteristics types within the whole: the downy type and luxuriant type.

The downy type had an average density aerial mycelium, with loose structure and forming tufts. Thirty isolates presented such facies. Isolates of this type were encountered in AEZ II (Sinfra) and AEZ I (Datta and Songon). As for the luxuriant type, there was a very dense and thick aerial mycelium, showing a very important development. Sixty three isolates belonged to such category. All agroecolgical zones showed the luxuriant mycelium type except AEZ II.

Thirty days after purification of the isolates, the number of sclerotia has been counted. It resulted in four groups of isolates. Group I consisted of isolates having difficulties to produce sclerotia. Twenty-one (21) isolates, that is, 22.58% failed to produce sclerotia (Figs. 5A and B). Isolates of this group originated from AEZ I and AEZ V, respectively, in the localities of N'guessankro located in the department of Divo and Kondoukro, not far from the city of Bouaké. In group II, the isolates produced a low number of sclerotia ranging between 1 and 20, with a size of 1 to 1.3 mm long and having a brown color; 16 isolates, that is, 17.20% belonged to such lot. Those isolates originated more from AEZ I and were characteristic of the locality of Songon (Figs. 5C and D). Thirty eight (38) isolates, that is, 41%, with an average number of sclerotia ranging between 21 and 75 sclerotia (Figs. 5E and F) belonged to group III. The sclerotia were dark brown and of medium size (0.5 mm to 1 mm). Such isolates were found in all agroecological zones. The last group, consisting of 18 individuals, that is, 19.35% produced a large number of black sclerotia, small in size ranging between µm to 0.5 mm (Figs. 5G and H). It was most encountered in AEZ I, precisely, in the locality of Songon- Mbraté.

5. DISCUSSION

Sclerotium rolfsii attacks are more important in humid agroecological zones and even in dry zones where market gardening is practiced in shallows. Market gardening crops (tomato, pepper and eggplant) and weeds are the targets of the fungus. Our works corroborate those of Goujon [13]. who demonstrated that *Sclerotium rolfsii* is a known telluric pathogen, attacking most little woody plant species such as market gardening crops. Garrett et al. [14], for this purpose, have shown that the occurrence of plant diseases depends on three factors: A host plant (market gardening crops and weeds), the presence of a pathogen (virulent, aggressive: *Sclerotium rolfsii*) and a permissive environment where the climate is a major determinant, as is the case of the humid agroecological Zone and shallows, in dry zones. These elements must be in place so that the disease is triggered and the synchronous interaction between host, pathogen and environment governs the development of the attack. Similarly, Krupinsky et al. [15] argue that the presence or severity of a disease is determined by the dynamic interaction of a susceptible plant, a specific pathogen and favorable environmental conditions. This interaction is known as the triangle of plant disease suitable for the different agroecological zones of Côte d'Ivoire. This would explain the attacks of market gardening crops that are sensitive to sclerotinia; the harmfulness of *Sclerotium rolfsii* itself being favored by environmental conditions.

Moisture is the key factor necessary for fungal growth. Water content of the air participates in the moisture content of materials. Thus, all the agroecological zones of Côte d'Ivoire according to their environmental characteristics are infected at different degrees by sclerotinia. AEZ V and II with a rainfall from 1300 to 1750 mm are moderately attacked by *Sclerotium rolfsii*, with respective attack rates of 20.43% and 16.13%. AEZ I remains the wettest zone compared to other zones and shows the highest attacks by sclerotinia, with 43% infection. The attacks by *Sclerotium rolfsii* are therefore important in wetter areas than dry ones. Our works are consistent with those of Fuhrer [16]. which showed that rainy seasons ensure, in general, better preservation of pathogens, thus increasing the amount of inoculum the following dry season. Further, Boland et al. [17] argued that the main bioclimatic factors influencing the development of diseases are temperature and humidity. Similarly, Bera et al. [18] found that *Sclerotium rolfsii* is a devastating pest of groundnut crops during periods of high humidity. Also, the correspondence of the average

temperature of 29°C±5.6 and rainfall from 1400 to 2500 mm of AEZ I, to the ecology of *Sclerotium rolfsii* which is an optimum growth temperature between 25°C and 35°C, is a favorable factor for the manifestation of this pathogen.

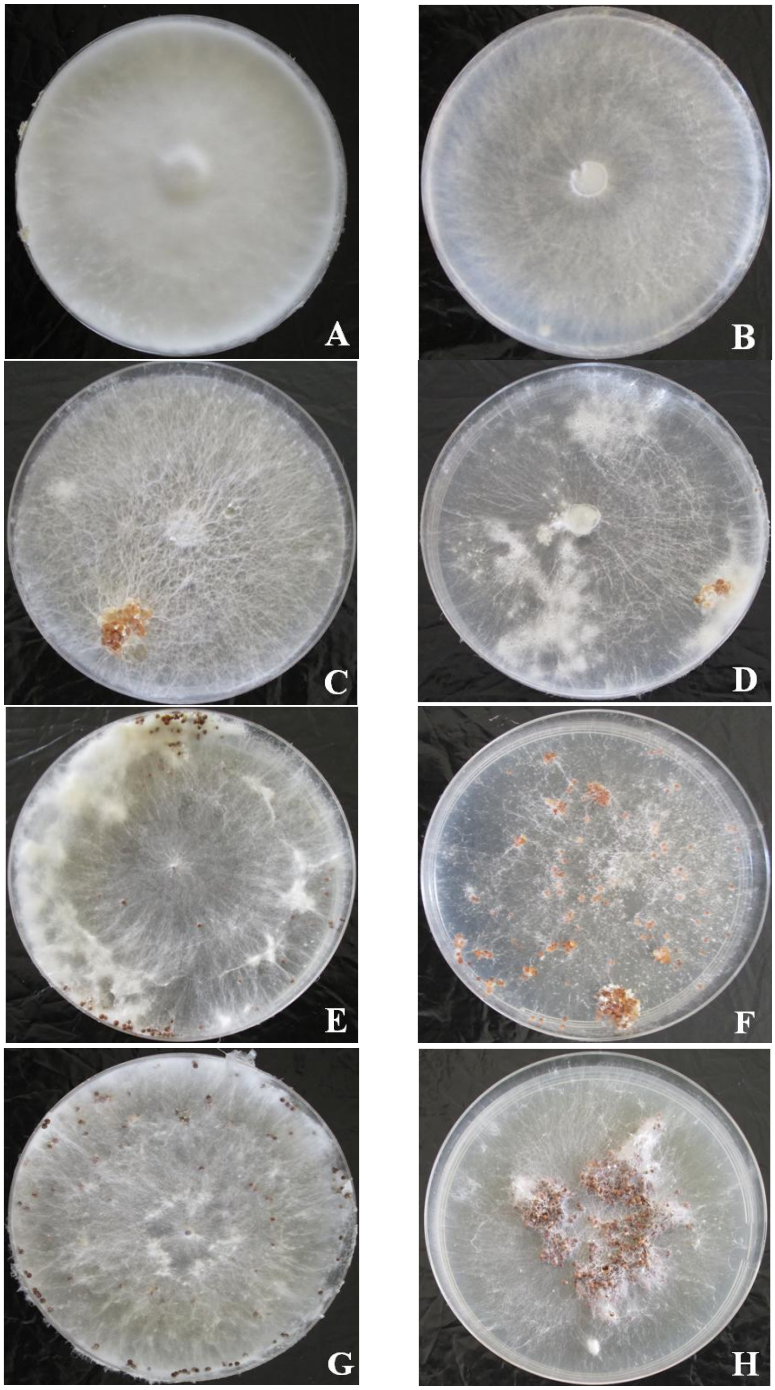

Fig. 5. Morphological features of *Sclerotium rolfsii* isolates

***A**: Luxuriant mycelium without sclerotia production **B**: Downy mycelium without sclerotia production **C**: Luxiriant mycelium with low sclerotia production, **D**: Downy mycelium with low sclerotia production*
***E**: Luxuriant mycelium with average sclerotia production, **F**: Downy mycelium with average sclerotia production, **G**: Luxuriant mycelium with high sclerotia production, **H**: Downy mycelium with high sclerotia production*

Attacks by *Sclerotium rolfsii* are low in northern Côte d'Ivoire where temperatures are warm and rainfall is low. Our works are in accordance with those of Fuhrer [14]; Ziska and Runion [19] who observed that in warmer weather, there is an increase of lignification that favors tolerance of host plants and limitation of entry of some pathogens. This would reflect the low attack rate in the localities of Korhogo, Sinématiali and Ferkessédougou, located in AEZ VI where the average temperature is higher and the rainfall lower.

AEZ III is the mountainous area of Côte d'Ivoire in which no attacks have been observed. The absence of *Sclerotium rolfsii* in this area is explained by the high elevation of land which does not keep water as long as possible. However, in AEZ I, which is a plateau zone where the infection is common, in the event of heavy rain, the plots can stay soaked in water over 4 days. Our results are consistent with those of Pauvert et Lamarque [20] who found that the presence of free water for at least 39 consecutive hours determines the success of the infection. The manifestation of sclerotinia due to *Sclerotium rolfsii* would depend thus on the environmental conditions of the different zones. That's what was shown by Coakley [21] indicating in his works that climatic factors added to environmental conditions can have a significant impact on the manifestation of plant diseases.

During the different samplings, tomato was the most attacked crop compared with the other crops. This attack rate amounted to 83.87%, which was 4 times higher than those of eggplant and pepper combined. The abundance of *Sclerotium rolfsii* varies depending on the crop rotation and crop type set up, especially tomato and its return in the plot. Indeed, in the plot where tomato was grown for four successive years, *Sclerotium rolfsii* was more abundant, which is demonstrated in the works of Tramier [22] who noted that the importance of telluric pathogens rises with the increasingly common monoculture practice.

As for morphological features of the pathogen, within the species, in addition to the more specific ones offered by molecular tools, they enable, above all, to make a classification of the isolates. According to the different morphological features observed, the study shows that there may be at least 8 groups of *Sclerotium rolfsii* in Côte d'Ivoire. Four (4) thallus groups belonging to the downy type and 4 others to the luxuriant type, according to sclerotia production on PDA culture medium. On both sides of the two other groups: downy and luxuriant, there were isolates that do not produce sclerotia, or which produce a small number of retention organs, or an average number of sexed spores and finally those which produce a great amount of sclerotia. These results are in accordance with those of Prabhu and Patil, [23] who identified three groups of isolates producing sclerotia of same size as ours. Furthermore and in the same order, Goujon [24] showed that the outbreak of initials (sclerotia formation start) and their maturation require the loss of trophic competition exerted on them by thallus margin during elongation. Their birth is only possible in the presence of a morphogenic factor of protein kind whose concentration in the thallus is above a certain threshold. Finally, their number and size are conditioned by the available quantity of this morphogenic factor (still unknown kind). The absence of sclerotia in isolates would be due to the inhibition of the synthesis of such morphogenic factor. The amount of sclerotia in isolates, would be explained by the importance of the morphogenic factor because the number of sclerotia formed is even larger than the morphogenic factor.

6. CONCLUSION

The objective of this study was, on the one hand, to understand the distribution of sclerotinia in market gardening crops in Côte d'Ivoire, across the different agroecological zones, and on the other hand, to determine the inspecific features of *Sclerotium rofsii*, from a morphological point of view.

It results from the expression of the disease that tomato is the most susceptible plant, among pepper and eggplant crops. The most attacked area includes the southern humid forest regions and shallow lands of the other regions, whose characteristics are conducive to the biology of the pathogen.

As for the morphological features of the fungus, it appears various growth rates, of which the most important are those of isolates originating from agroecological zone I. The thallus aspect lets appear two types: downy type and luxuriant type. The sclerotia production intensity enabled, moreover, to define four morphological groups. However, even if a higher number of isolates of a

type or group can be assigned to a given agroecological zone, this relationship morphological type - agroecological zone is not strict. Some types may be seen on more than one agroecological zone.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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