Annual Research & Review in Biology



36(1): 53-65, 2021; Article no.ARRB.65515 ISSN: 2347-565X, NLM ID: 101632869

Identification of Susceptible Weed Hosts of *Phytophthora* spp. in Cocoa Trees in the Nawa Region, South-West of Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration between all authors. Author YRB wrote the protocol, collected, analyzed the data and wrote the manuscrit. Author IJP designed and supervised the work. Authors DKK and DCN supervised also the work and further analyzed data. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2021/v36i130333 <u>Editor(s):</u> (1) Dr. Rishee K. Kalaria, Navsari Agricultural University, India. <u>Reviewers:</u> (1) S. Elain Apshara, ICAR- CPCRI, India. (2) Bilal Saeed Khan, University of Agriculture, Pakistan. Complete Peer review History: <u>http://www.sdiarticle4.com/review-history/65515</u>

> Received 10 December 2020 Accepted 13 February 2021 Published 12 March 2021

Original Research Article

ABSTRACT

The cocoa tree, the mainexport crop in Côte d'Ivoire is frequently attacked by a disease: brown pod rot, caused by Phytophthora spp. which causes a considerable drop in production. This soil-borne pathogen attacks on so-called weeds when environmental conditions are favourable. The presence of these susceptible weed hosts can amplify this scourge of brown rot by transmitting the pathogen to the crop plant. In order to improve the yield of this crop, a study was conducted in the Nawa region to identify the weed hosts through the characterization of the pathogen. This study consisted first in the inventory of susceptible host weeds of *Phytophthora* spp. and

This study consisted first in the inventory of susceptible host weeds of *Phytophthora* spp. and second in the morphological characterization of the pathogen.

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Concerning the host weeds inventory we proceeded determining all symptomatic weeds located within 3 m of the cocoa plants affected by brown rot.

We identified 48 susceptible host species, divided into 41 genera and 22 families and we observed brown spots on the leaves of *Oplismenus burmannii* and *Laportea aestuans*; on the leaves of *Clerodendrum splendens* and *Xanthosoma mafaffa*, brown necroses surrounded by a yellow halo were noticed. Oily brown spots were examined on the leaves of Ageratum conyzoides. On the proximal, lateral and distal parts of the pods symptoms of brown spots covered with whitish mycelia were observed. *Phytophthora* strains obtained from the pods and weeds on PDA culture medium were characterized by matted, white mycelial colonies with a cottony appearance. Microscopic features revealed the presence of sporocysts, oospores and chlamydospores of different shapes.

Keywords: Cocoa tree; weeds; phytophthora; symptoms; morphological characterization.

1. INTRODUCTION

Côte d'Ivoire is an agricultural country which based its economy on coffee and cocoa culture. During the 2015-2016 farming operation, the Ivory Coast, the leading cocoa producer, provided 35% of world production with an annual production of 1.7 million tones [1]. Crops such as cotton and cashew nuts occupied 7% of the land each. Oil palm and rubber trees covered 5% and 3% of the land respectively. Other cash crops (coconut, dessert banana, etc.) accounted for 3% of the land area overall [1]. Thus, coffee and cocoa play a key role in the Ivorian economy. The cocoa economy involves nearly 700,000 farmers and provides a livelihood for 4 million people, or 25% of the Ivorian population [2].

The world production of cocoa which is 3.6 millio n tons [3], position Côte d'Ivoire at the forefront of the world supply. This performance is partly due to the sanitary state and especially to the maintenance provided by producers on cocoa plantations. Today, beyond the attacks of harmful insects such as Mirides [4], the cocoa crop that forms the basis of the Ivorian economy is facing other constraints. Indeed, it is subject to numerous parasitic attacks, in palmivora particular Phytophthora and Phytophthora megakarya, which cause "brown pod rot" [5]. This disease directly results in yield losses as affected pods produce cocoa beans that are unfit for consumption. The disease is spread by spores of this pathogen dispersed by rain, wind, insects, and some mammals such as rats and bats [6]. Apart from these sources of spread, some weeds or weeds found in cocoa farms could be considered as reservoirs. These may show symptoms similar to those observed on diseased cocoa trees. Indeed, weeds have attributes that make them difficult to control [7] both in cocoa and other

crops. They also serve as hosts for many pests, including insects, viruses and fungi. Weeds are therefore a real problem for agriculture in general and for cocoa farming in particular.

The objective of this study was to contribute to the improvement of cocoa crops by identifying susceptible host weeds of *Phytophthora* spp. in order to alert cocoa industry stakeholders to the impact of these weeds on cocoa crop production.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted in the southwestern part of Côte d'Ivoire in the Nawa region. It is located between 6°12' and 7°08' West longitudes and 5°19' and 6°34' North latitudes. This region, born from the split of the former Lower Sassandra Region, has Soubré as its chief town. It is bordered to the North by the Regions of Guémon and Haut -Sassandra; to the South by the Regions of Gbôklè and San-Pedro; to the East by the Regions of Goh and Loh Djibouo and to the West by the Region of Cavally. The region includes the Departments of Soubré, Buyo, Méagui and Guéyo

2.2 Study Material

The biological material consisted of cocoa trees plant and weeds likely to be hosts of *Phytophthora* spp. and fungal material, *i.e. Phytophthora* strains isolated from pods and symptomatic weeds.

2.3 Methods

2.3.1 Sampling of pods and host weeds

One or two symptomatic pods are removed from each cocoa tree affected by brown rot. Weed sampling was carried out 3 m from the diseased

cocoa trees, covering an area of 28.26 m². From the symptomatic plants in this area, tissue samples were taken from the diseased organs.

2.3.2 Isolation from surface flaming pods and weeds

Pods showing symptoms of brown rot were rinsed with tap water. The cortex was cleaned with alcohol at 70°C with paper towels and then placed under a laminar flow hood (CRUMA model CR870FL series VTR571316). They were then soaked in alcohol and flambeed. Using a sterile punch, tissue fragments were removed from the subcortical tissues at the growth fronts of the brown rot. Washers were placed on poor medium (agar water culture medium) in 90 mm diameter petri dishes.

For weeds, the necrotic leaf and stem organs were washed with tap water and placed in jars after removing fragments of these organs at the growth front of the necrosis. Disinfection was carried out by putting 70% alcohol in the jars containing the fragments of these necrotic organs for 3 minutes. They were then soaked in a 5% sodium hypochlorite solution (NaOCI) for 1 minute. After disinfection, these fragments were rinsed three times successively with sterilized distilled water and dried for 60 seconds before being transferred to the petri dishes containing the poor medium (agar water culture medium). After mycelium formation, the isolates obtained were successively transferred to the PDA culture medium.

2.3.3 Identification of the pathogen

Macroscopic observations focused on cultural traits such as thallus texture and color, and growth contour. *Phytophthora* strains were observed under optical microscope at X400 magnification. Microscopic observation was performed by removing with a sterile needle a mycelial fragment which was put between slide and lamina for observation

2.3.4 Data analysis

The data collected made it possible to establish the list of host weeds susceptible to *Phytophthora* and to carry out the analysis of the diversity of the flora. The diversity of the flora makes it possible to determine the richness of the flora of a given environment. According to Ake-Assi [8], a flora is all the more diversified as it includes less large families and large multispecies genera. It is determined using the index of floristic diversity (lds) and is expressed as follows:

$$Ids = \frac{E}{G}$$

Ids = the floristic diversity index E = Number of species G = Number of gender

3. RESULTS

3.1 Flora of Weed Hosts Susceptible to Phytophthora

The weed inventory identified 48 susceptible host species of *Phytophthora* associated with cocoa (Table 1). These species are divided into 41 genera and belong to 22 families (Table 2). Among these families, the most notable (in bold) are Asteraceae, Fabaceae, Poaceae (Gramineae), Solanaceae and Verbenaceae. The taxa inventoried belong mainly to the class of Broadleaf (85.06%) than to the class of Monocotyledons (14.94%).

3.2 Specific Diversity Index (Ids)

The index of specific diversity allowed to note that Asteraceae (Ids = 1.12) and Fabaceae (Ids = 1.2) are the most diversified families. The Table 3 shows that these two families have the lowest specific diversity index. The Solanaceae and Verbernaceae, each with the highest species diversity indices (Ids = 3), are the least diverse.

3.3 Characteristics of Symptoms Caused by Phytophthora in the Field

Various symptoms caused by *Phytophthora* were observed on pods and weeds. On the proximal, lateral and distal parts of the pods symptoms of brown spots were observed (Fig. 1). The brownish parts of the pods were covered with whitish mycelia. In addition, the brown spots on the surface of the pods were hard to the touch.

No	Species	Families
1	Acroceras zizanioides (Kunth) Dandy	Poaceae
2	Ageratum conyzoides Linn.	Asteraceae
3	Albizia zygia (DC.) J.F. Macbr.	Mimosaceae
4	Alchornea cordifolia (Schum. & Thonn.) Müll.Arg.	Euphorbiaceae
5	Aneilema beniniense (P. Beauv.) Kunth	Commelinaceae
6	Axonopus compressus (Sw.) P. Beauv.	Poaceae
7	Baphia bancoensis Aubrév.	Fabaceae
8	Bidens pilosa Linn.	Asteraceae
9	Ceiba pentandra (Linn.) Gaerth.	Bombacaceae
10	Celtis milbraedii Engl.	Ulmaceae
11	Centrosema pubescens Benth.	Fabaceae
12	Chromolaena odorata (L.) R. M. King & H. Rob.	Asteraceae
13	Clerodendrum splendens G. Don	Verbenaceae
14	Clerodendrum umbellatum Poir.	Verbenaceae
15	Clerodendrum volubile P. Beauv.	Verbenaceae
16	Coccinia barteri (Hook.f.)Keay	Cucurbitaceae
17	Crassocephalum biafrae (Oliv. & Hiern) S.Moore	Asteraceae
18	Croton hirtus L'Hérit.	Euphorbiaceae
19	Cyathula prostrata (Linn.) Bl. Var prostrata	Amaranthaceae
20	Desmodium adscendens (Sw.) DC. var. adscendens	Fabaceae
20	Desmodium adscendens (Sw.) Des. val. adscendens Desmodium scorpiurus (Sw.) Desv.	Fabaceae
22	Emilia coccinea (Sims) G. Don	Asteraceae
22	Emilia sonchifolia (Linn.) DC.	Asteraceae
23 24	Ficus exasperata Vahl	
24 25	Ipomoea involucrata P. Beauv.	Moraceae Convolvulaceae
26	Justicia flava (Forssk.) Vahl	Acanthaceae
27 28	Laportea aestuans (Linn.) Chew Merremia Sp	Urticaceae Convolvulaceae
29	Mikania cordata (Burm.f.) B.L. Robinson var. cordata Morinda lucida Benth.	Asteraceae
30		Rubiaceae
31	Mucuna flagellipes Hook.f.	Fabaceae
32	Oplismenus burmannii (Retz.) P. Beauv.	Poaceae
33	Oplismenus hirtellus (Linn.) P. Beauv. Subsp. Fasciculatus U. Scholz	Poaceae
34	Phaulopsis barteri (T. Anders.) Lindau	Acanthaceae
35	Porrophylum ruderal	Asteraceae
36	Pouzolzia guineensis Benth.	Urticaceae
37	Pueraria phaseoloides (Roxb.) Benth. var javanica (Benth) Baker	Fabaceae
38	Ruthalicia eglandulosa (Hook.f.) Jeffrey	Cucurbitaceae
39	Solanum distichum Thonn. var. distichum	Solanaceae
40	Solanum erianthum D. Don	Solanaceae
41	Solanum rugosum Dun.	Solanaceae
42	Solenostemon monostachyus (P. Beauv.) Briq. subsp.	Lamiaceae
	monostachyus	
43	Spathodea campanulata P. Beauv.	Bignoniaceae
44	Spermacoce latifolia Aubl.	Rubiaceae
45	Spigelia anthelmia Linn	Loganiaceae
46	Synedrella nodiflora Gaertn	Asteraceae
47	Telosma africanum (N.E. Br.) Colville	Asclepiadaceae
48	Xanthosoma mafaffa Schott	Araceae

 Table 1. List of Phytophtohra susceptible weed hosts inventoried in cocoa farms

Necrosis was observed on the leaves and stems of the weeds sampled (Fig. 2). Brown spots were observed on the leaves of *Oplismenus burmannii* and *Laportea aestuans*. Oily brown spots were also observed on the leaves of Ageratum conyzoides. On the leaves of *Clerodendrum*

splendens and *Xanthosoma mafaffa*, brown necroses surrounded by a yellow halo was noticed.

3.4 Macroscopic Characteristics of Isolated *Phytophthora* Strains

Phytophthora strains obtained from the pods were characterized by white thallus with a cottony appearance. These strains present lined mycelial colonies that diffuse into the PDA culture medium (Fig. 3). These mycelial colonies without pigments have regular and irregular contours.

Concerning weeds, the strains of *Phytophthora* obtained are characterized by airy white thallus without pigments, regular and fluffy in appearance. Some strains, however, showed white mycelial colonies (Fig. 4).

3.5 Microscopic Characteristics of Isolated Phythophthora Strains

At the microscopic level, all *Phytophthora* strains showed an unsegmented mycelium. These strains were distinguished by the presence of sporangia, clamydospores and different forms of Oospore (Fig 5 and Fig 6).

4. DISCUSSION

Analysis of the floristic richness of susceptible weed hosts of *Phytophthora* spp. revealed 48 species in 41 genera belonging to 22 families. The number of species obtained is the result of the specificity of our study. Indeed, our inventories were carried out only in cocoa plantations and targeted a specific category of hosts plants, namely, susceptible to Phytophthora. This specificity reduces the type and number of species to be counted, even if the plantations offer a higher range of weed species. This has been confirmed by the National Agricultural Research Centre of Côte d'Ivoire [9]. Their study consisted in identifying the host species of swollen shoot virus in cocoa production. During this study, 47 species divided into 26 genera and 15 families were inventoried. In addition to the work of CNRA, studies conducted by Singo [10] on the inventory of wild hosts of Achaea catocaloides in cocoa trees in Toumodi and Taabo Departments also confirmed this finding. This author has identified during his work, 61 species divided into 55 genera belonging to 34 families. These different results are thus quite close to ours. Contrary to the above-mentioned results, the results obtained during the work of

Table 2. Families of weed hosts susceptible to Phytophthora spp. in cocoa trees

Families	Number of genera	Number of species
Acanthaceae	2	2
Amaranthaceae	1	1
Araceae	1	1
Asclepiadaceae	1	1
Asteraceae	8	9
Bignoniaceae	1	1
Bombacaceae	1	1
Commelinaceae	1	1
Convolvulaceae	2	2
Cucurbitaceae	2	2
Euphorbiaceae	2	2
Fabaceae	5	6
Lamiaceae	1	1
Loganiaceae	1	1
Mimosaceae	1	1
Moraceae	1	1
Poaceae	3	4
Rubiaceae	2	2
Solanaceae	1	3
Ulmaceae	1	1
Urticaceae	2	2
Verbenaceae	1	3

Kpangui [11] in cocoa trees in the sub-prefecture of Kokumbo in central Côte d'Ivoire differ from ours. He inventoried 384 species divided into 231 genera and 91 families. The same applies to the results obtained by Adou [12], whose studies were conducted in the cocoa and coffee plantations around the village of Moussadougou in the classified forest of Monogaga. During his work, he identified 309 species belonging to 106 families. Indeed, the work of these different authors covered all the flora of cocoa and coffee trees.

In this floristic diversity, five families are the most represented. These are Asteraceae, Fabaceae, Poaceae, Solanaceae and Verbenaceae. Most species of the Asteraceae and Poaceae family proliferate rapidly by wind (anemoria) when they reach the fruiting stage [13]. According to Mangara [14], this mode of spread by wind allows them to colonize cultivated environments very guickly. The predominance of families such as Asteraceae, Fabaceae, Poaceae and Solanaceae has been observed by Bakayoko [15]. The dominance of these four families was also noted by Boraud [16] and Traoré and al. [17]. These families can be found on the list of ten families considered by Akobundu [18], as "major world weeds", which are: Poaceae, Asteraceae. Cyperaceae, Amaranthaceae.

Fabaceae, Convolvulaceae, Euphorbiaceae, Malvaceae, Solanaceae and Polygonaceae [18]. Work carried out by Kouakou [19] in maize cultivation in M'bahiakro Department showed that species of the Asteraceae, Poaceae and Fabaceae families are in the majority. These observations were also made in the work carried out in the Mediterranean region by Maillet [20] and Loudyi [21]. Indeed, these authors found in the course of their work that the Poaceae, Asteraceae and Fabaceae are the most representative families. The predominance of broadleaf trees (85.06%) in this work, was also observed pineapple cultivation in in Morocco by Bouhache and Chougrani [22], Taleb and Maillet [23], Tanji and Boulet [24], with respectively 82.3%, 87% and 84% of their flora. This high representativeness of the broadleaved trees has also been mentioned in several works such as: the work of Kouamé Kra [25] in the District of Yamoussoukro in rice cultivation and that of Kouakou [19] in maize cultivation in the Department of M'bahiakro. In addition to these results, the strong presence of broadleaf trees was also noted in northern Côte d'Ivoire in sugar cane cultivation by Boraud [16]. According to Déat [26], there is no weed flora specific to a given crop, but rather to ecological parameters and agronomic factors.

Table 3. Indices of specific diversity of weed families

Family	G	E	lds	
Asteraceae	8	9	1,12	
Fabaceae	5	6	1,2	
Poaceaa	3	4	1,33	
Solanaceae	1	3	3	
Verbernaceae	1	3	3	

Legend: G = genre; E = species; Ids = Specific index

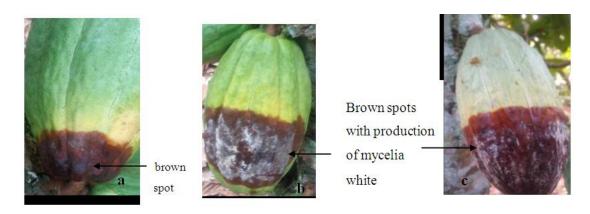


Fig. 1. Symptom of brown rot observed on the pods in the field a: proximal infection; b: lateral infection; c: distal infection

Baka et al.; ARRB, 36(1): 53-65, 2021; Article no.ARRB.65515

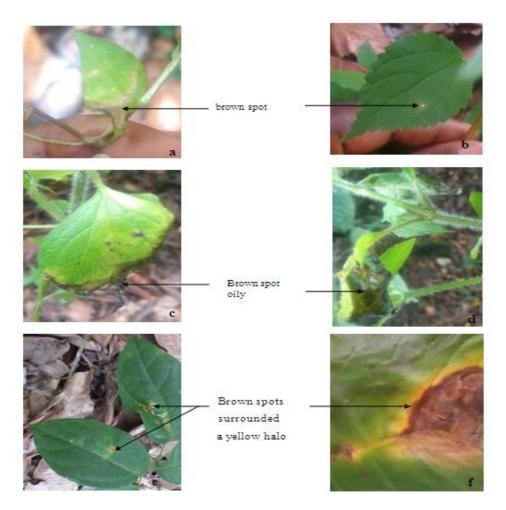


Fig. 2. Different types of symptoms observed on cocoa weeds in the field a: leaf of Oplismenus burmannii (Poaceae); b: leaf of Laportea aestuans (Urticaceae);

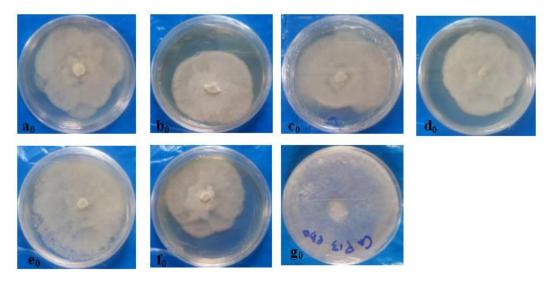


Fig. 3. Morphological colonies of Phytophthora isolated from pods on PDA media

Baka et al.; ARRB, 36(1): 53-65, 2021; Article no.ARRB.65515

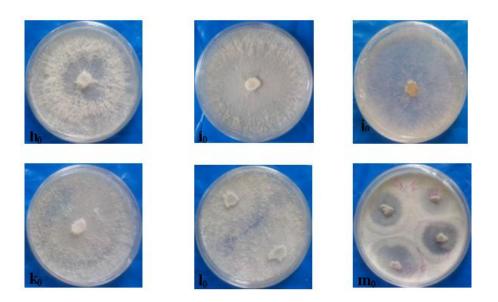
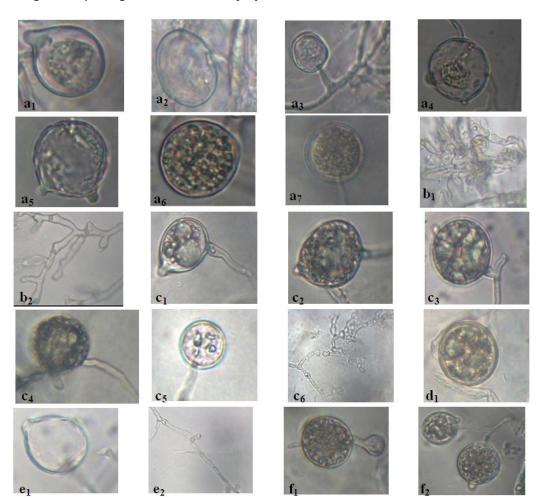


Fig. 4. Morphological colonies of Phytophthora isolated from weeds on PDA media



Baka et al.; ARRB, 36(1): 53-65, 2021; Article no.ARRB.65515

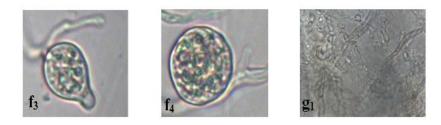


Fig. 5. Microscopic structures of *Phytophthora* spp. formed on PDA medium isolated from pods

a1 and e1: Ovoid Sporangium with rounded papilla; a2 and a3: Clamydospores; a4: Spherical Sporangium with pointed papilla and long pedicel; a5: Spherical Sporangium with two rounded papillae; a6: Ovoid Sporangium; a7, c4, c5 and f4: Spherical Clamydospores; b1, b2, c6, e2 and g1: Uncompartmentalized Mycelium; c1 and f2: Spherical Sporangia with rounded papillae and long pedicels; c2: Ovoid Sporangia with pointed papilla and long pedicel; c3 and d1: Spherical Oospore; f1: Spherical Sporangia with pointed papilla and long f3: Ovoid Sporangia with rounded papilla and short pedicel; f4: Spherical Sporangia with rounded papilla and short pedicel

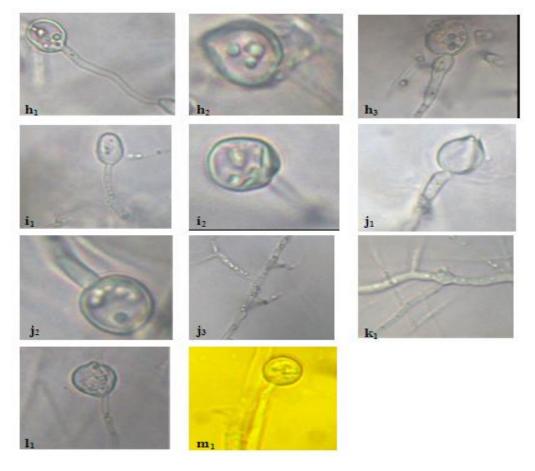


Fig. 6. Microscopic structures of *Phytophthora* spp. formed on PDA medium isolated from weeds

h1: Ovoid Sporangium without papilla; h2: Semi rounded ovoid Sporangium; h3: Spheroid Sporangium without papilla; i1: Ovoid Sporangium without papilla; i2: Spherical Sporangium without papilla; j1: Ovoid sporangium without papilla; j3 and k1: Uncompartmentalized mycelia; l1: Rounded semi-papillated spheroid sporangium; m1: Spherical clamydospore

Based on observations of infected pods collected from cocoa fields, symptoms of brown rot appeared as brown spots. These brown spots developed at the peduncular, lateral and distal ends of the pods. The surface of the infected pods was covered with whitish mycelium. In addition, pods with these brown spots are hard to the touch. These results would indicate that the symptoms observed are characteristic of Phytophthora palmivora or P. megakarva. These symptoms are similar to those described on pods by Adabe and Ngo-Samnick [27] and [28] as those of brown rot of cocoa pods. In addition to the symptomatic remarks made by these authors, [29] and [30] observed lesions in their studies that initially had a translucent appearance. According to these authors, these lesions develop into brown rot which are hard to the touch and in the final stage become covered with a sporiferous creamv-white coating. Concerning the observations made on weeds, brown necroses were observed on the leaves of Oplismenus burmannii, and Laportea aestuans. On the leaves of Clerodendrum splendens and Xanthosoma mafaffa, brown necrotic lesions surrounded by a yellow halo were noticed. Oily brown necrotic lesions were examined on the leaves of Ageratum conyzoides. These observations are similar to those made by Kerroum [31] on leaves and stems of Solanum tuberosum and Solanum lycopersicum. This author revealed brown necrotic lesions surrounded by a pale to yellowish ring on the infected areas of these two species. These brown necroses were also observed inside the fruit s of Solanum tuberosum [32]. According to [31] and [32], these symptoms are due to Phytophthora infestans which causes late blight disease in Solanaceae. In addition to these observations, [33] noted the appearance of discoloured spots with an oily appearance and then brown with a light green border that appear on the upper surface of the leaves of Solanum tuberosum. And on the underside of these leaves, a white down that appears on the circumference of the necrotic zone. On the petioles and stems of this same species, brown spots are also observed by this author. Tubers of Solanum tuberosum mildewed show purplish to brown spots on the epidermis [33]. In addition to these observations, studies by Jackson and Wright [6] showed that lesions on the leaves generally have a brown or greyish center surrounded by a yellow halo. On leaves, the center of these lesions sometimes punctures, leaving a round hole [6]. According to [34] and

[35] cited by Pohe and al. [36], these *phytophthora* rots are rain-related. Indeed, rain favours the installation and development of Phycomyces diseases such as brown pod rot due to *Phytophthora*. It creates favorable conditions for their reproduction and the dissemination of their propagules. Thus, water appears to be the indispensable element, especially in the initiation and maintenance of the epidemic [37].

Seven strains of phytophthora were isolated from weed symptoms during this study. This result reveals that the cocoa crop of Côte d'Ivoire contains plant species that are potential hosts for Phytophthora. According to [38], Phytophthora species are able to survive several years in the soil and can attack plants when conditions become favourable. Indeed, the pedoclimatic conditions of cocoa trees being favourable, Phytophthora strains could live there for several years in its preservation structures such as chlamydospores, sporangia and oospores on weeds. These Phytophthora species isolated from the weeds could be potential sources of infection of pods with brown rot and cause considerable losses.

The macroscopic and microscopic characteristics of the observed Phythophthora strains were described by Yao [39] and Jung and al. [40] on PDA culture medium. *Phythophthora* strains obtained from the pods were macroscopically characterized by white thallus with a cottony appearance. These strains of Phythophthora showed lined mycelial colonies that diffuse into the culture medium. These mycelial colonies without pigments have regular and irregular contours. These same macroscopic features were also observed on Phythophtora strains obtained from weeds. These remarks were also made by Yao [39] during his work on the diversity of fungal flora in soils of the cocoa rhizosphere in Côte d'Ivoire. Indeed, this author indicated during his observations that Phythophtora have fibrous, aerial, fluffy white Thalles with irregular growth whatever the zone of origin of pods and soils of cocoa trees. At the microscopic level, sporangia of different forms with or without papillae and pedicels or not, formed on PDA culture media were observed. Microscopic structures such as Spherical Clamydospores Spherical and Oospore were also examined in this study. The same microscopic observations were made by Yao [39] and Pohe [41] on PDA culture media. These authors highlighted during their study the

presence of ovoid sporangia with rounded apex, ovoid sporangia with reduced pedicel, spheroid sporangia with pointed apex and long pedicel, spherical terminal Chlamydospore, spherical Oospore and branched and non-partitioned mycelia. In addition to the observations made by these authors, Jung and al. [40] made the same remarks on V8 culture medium.

5. CONCLUSION

The inventory carried out in cocoa farms identified 48 weeds that could be hosts for Phytophthora. The study of the morphological characterization of these weed hosts susceptible to Phytophthora showed that among them, five are potential hosts of Phytophthora. They are: Ageratum conyzoides (Asteraceae), Clerodendrum splendens (Verbenaceae), Laportea aestuans (Urticaceae), Oplismenus burmannii (Poaceae), Xanthosoma mafaffa (Araceae). The Phytophthora strains obtained from these weeds were characterized by airy, pigment-free, regular and fluffy looking white thallus. However, some strains present white mycelial colonies with a lined appearance. At the microscopic level, all Phytophthora strains showed an unsegmented mycelium. This study shows that there are potential host plants for Phytophthora in cocoa plantations in the Nawa Region of South- Western Côte d'Ivoire. Therefore, regular weeding of the plantations should be carried out by farmers to eliminate potential Phytophthora weed hosts responsible for brown rot. Weeding should also be carried out before the application of fungicide against the pathogen. In addition, growers often have to prune cocoa trees to allow sunlight to penetrate the plantations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history: The peer review history for this paper can be accessed here: http://www.sdiarticle4.com/review-history/65515