

Colletotrichum Gloeosporioides Species Complex: Pathogen Causing Anthracnose, Gummosis and Die- Back Diseases of Cashew (*Anacardium Occidentale* L.) In Ghana

A. Muntala, P. M. Norshie, K. G. Santo, and C. K. S. Saba

Abstract — A survey was conducted in twenty-five cashew (*Anacardium occidentale*) orchards in five communities in the Dormaa-Central Municipality of Bono Region of Ghana to assess the incidence and severity of anthracnose, gummosis and die-back diseases on cashew. Cashew diseased samples of leaves, stem, inflorescences, twigs, flowers, nuts and apples showing symptoms (e. g. small, water-soaked, circular or irregular yellow, dark or brown spots or lesions on leaves, fruits and flowers, sunken surface, especially on the apples, blight, gum exudates) were collected for isolation of presumptive causative organism. The pathogen was isolated after disinfecting the excised diseased pieces in 70% ethanol, plated on potato dextrose agar (PDA) and incubated at 28 °C for 3 to 7 days. The identity of the putative pathogen was morphologically and culturally confirmed as belonging to *Colletotrichum gloeosporioides* species complex using standard mycological identification protocols. The pathogen had varied conidia sizes of between 9-15 up to 20 µm in length and diameter of 3-6 µm. The conidia were straight and cylindrically shaped with rounded or obtuse ends. The septate mycelium was whitish-grey, velvety and cotton-like in appearance from the top. The results confirmed the presence of the pathogen in the orchards with incidence ranging from 6.9% and 14.0% for gummosis and averaged 22.9% for anthracnose infected orchards. The result of the pathogenicity test confirmed the isolates to be pathogenic on inoculated cashew seedlings and were consistently re-isolated, thereby establishing the pathogen as the true causal agent of the said diseases in cashew trees and thus completed the Koch's postulate.

Index Terms — Cashew tree, *Colletotrichum gloeosporioides*, anthracnose, die-back, gummosis pathogenicity, Ghana.

I. INTRODUCTION

Cashew (*Anacardium occidentale* L.), a commercial golden fruit tree, plays an important socio-economic role in the Ghanaian agricultural sector. Cashew production is carried out in most parts of Ghana where the average yearly rainfall ranges between 1000-1500 mm. The production of the crop has largely increased in the past decades and it is changing the land use system not only in Ghana, but also in other West African countries. Farmers who were previously

into the production of cocoa and other cash crops are shifting their attentions to this golden crop of 21st century, due to domestic and international demands. Small-scale farmers who constitute about 88% of cashew producers [1] have organized themselves into small cooperative societies for easy access to inputs. The tree, which is native to tropical provinces of Brazil, was first taken to India and then to Mozambique in the second half of 16th century from where it spread to other parts of world [2]-[4]. Per country break down, the two major producers, namely Vietnam and Nigeria, produced 30% and 21% of cashew nuts, respectively, followed by Brazil, and appreciable yields in Benin, Côte d'Ivoire and Guinea Bissau [5].

In the market trade, cashew is one of the major horticultural export cash crops, cultivated on commercial basis in several other countries of the tropics [6], [4], with world total production of the nuts being estimated at one million metric tonnes [7]. In three states of Northeastern Brazil, cashew contributes to annual income of about 230 million dollars with high export of about 90% of cashew products such as shell liquid and nuts [5]. Cashew production plays an important role in the agriculture sector of the economy of African countries such as Guinea-Bissau and Ghana. The sector contributed over 35% of their gross national product (GNP) in 2010 [8], [9]. About 98% of Ghana's cashew is being exported as raw cashew nuts (RCN), with the remaining 2% being processed locally into products like raw cashew kernels. In 2011 for instance, Ghana exported 280,834 MT of RCN priced at US\$ 379 million. These exports contributed about 6.1% to the GDP and 18.2% to the agricultural GDP [10], [1].

The economic gains from cashew production are threatened by invisible foes called plant pathogens, which limit the nut yield potential of the crop. Cashew trees are susceptible to the threat of more than 10 fungi diseases [11]. The most serious diseases that have been reported to attack and cause severe damages to cashew trees in the cashew producing regions of the world include anthracnose caused by *Colletotrichum gloeosporioides*, formerly known as *Vermicularia gloeosporioides* Penz. This pathogen has been

Published on November 16, 2020.

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singled out as the most important disease of cashew, causing substantial nut yield losses (40% in Brazil) and across the globe [12]-[15]. The second disease is gummosis of the tree trunk and twigs caused by *Lasiodiplodia theobromae* [16].

Colletotrichum gloeosporioides was first reported in Brazil in 1937 on *Swietenia humilis*, a species of tree in the family *Meliaceae* and on coffee by Butler in 1918, in India. The disease is not only widespread in tropical regions, but also in sub-tropical and temperate regions as well [17], [18]. *Glomerella cingulate* is the teleomorph form (sexual phase), while *C. gloeosporioides* is considered as anamorph phase (asexual phase) of the fungus [19]. The pathogen thrives well at the temperature range of 25-28 °C and pH of 5.8-6.5. It is usually not active during dry spells, but switches to active mode when the environmental conditions become favourable. It has hemibiotrophic means of infection, where both necrotrophic and biotrophic stages occur in succession. Anthracnose disease of cashew has been reported to be caused by several species of *Colletotrichum* (*C. fructicola*, *C. asianum*, *C. theobromicola*, *C. siamense sensu lato*, *C. tropicale* and other undesignated taxon of *Colletotrichum* sp.) belonging to *C. gloeosporioides* species complex [20].

There are several species under *Colletotrichum* genus, but only four species such as *C. higginsianum*; *C. graminicola*; *C. orbiculare* and *C. fructicola* have their genome completely sequenced [21], [22]. Even though there is little study on the genomic sequence of *C. gloeosporioides*, a number of genes that are involved in host defence mechanism and pathogenesis have been identified. *Colletotrichum gloeosporioides* is not only a pathogen of cashew nut fruit, but also affects a large number of other host plants at any stage of their development including flowers, leaves and fruits [23]. Die-back and stem gummosis caused by *C. gloeosporioides* and *L. theobromae* have also been reported to cause significant damages to cashew plants [15]. Other diseases of less importance on cashew include powdery mildew (*Oidium anacardii*), black mold (*Pilgeriella anacardii*) etc. [12], [16].

Traditionally, identification of this pathogen relies on the morphological characteristics such as colony colour, shape and size of conidia and appressorium among other features [24]. Owing to the morphological and genetic multifarious nature of this species [25], in recent times, molecular methods such as Amplified Fragment Length Polymorphism (AFLP); Microsatellites; Restriction Fragment Length Polymorphism (RFLP); Random Amplified Polymorphic DNA (RAPD) and Internal Transcribed Spacers (ITS) have all been employed in the identification and characterization of this pathogen to species level, which is paramount in solving issues of species delimitation [26], [27].

The cultivation of this crop comes with a myriad of challenges, among which are diseases caused by numerous biological agents. Farmers in this zone are well aware that there is a serious problem of pests and diseases affecting their crops, but helplessly have little knowledge about the exact causes of these problems. With the few exceptions [28], there is extremely very little published information about diseases of cashew in Ghana. Most available information on cashew diseases is found in newsletters, echoed by politicians (especially ministry of Agriculture) to achieve their political ambitions. Other information on the diseases of this crop are

also typed in hard copies either as student projects or private works and packed on the shelves, which are beyond the reach of international partners in cashew production.

The objective of this study was, therefore, to isolate, identify and evaluate the incidence and severity of *C. gloeosporioides* causing anthracnose, gummosis and die-back diseases of cashew in the Bono Region of Ghana.

II. MATERIALS AND METHOD

A. Collection of Samples for Fungi Isolation

During 2019 and 2020, a survey was conducted in twenty-five (25) cashew orchards in five major cashew-growing communities, namely Antwirifo, Agyemankrom, Badukrom, Kyeremansu and Tonasuano in the Dormaa Central Municipality of Bono Region of Ghana - West Africa (Fig. 1) to assess the incidence and severity of anthracnose, die-back and gummosis diseases following complaints from the cashew farmers. Five orchards were randomly selected from each community. Cashew diseased samples such as leaves, stem bark, inflorescences, twigs, flowers, nuts and apples showing varied degrees of symptoms (small initially water-soaked, circular or irregular yellow, dark or brown spots or lesions on leaves, fruits and flowers, depressed or sunken surface, especially on the apples, blighted, dead leaves and twigs, parts with gum exudates) were collected and packaged in brown envelopes and sent to the Central Laboratory of the University of Energy and Natural Resources – Sunyani, for isolation of the putative pathogen. The isolation was done according to a modified method described by [29]. The pathogen was isolated after disinfecting the excised diseased plant pieces in 70% ethanol for a minute and passed through three exchanges of sterile distilled water and blotted dry on Whatman's cellulose filter paper. The disinfected tissues were then plated on potato dextrose agar (PDA) using flamed forceps and replicated three times in each Petri plate at equidistance, parafilmmed and incubated at 28°C for 3 to 7 days for observation of the colony growth. The isolation was repeated 5 times using fresh samples taken from each field visit. The isolated putative pathogen was further purified through hyphae tip technique to obtain pure culture, which was then stored at -20 °C for further studies.

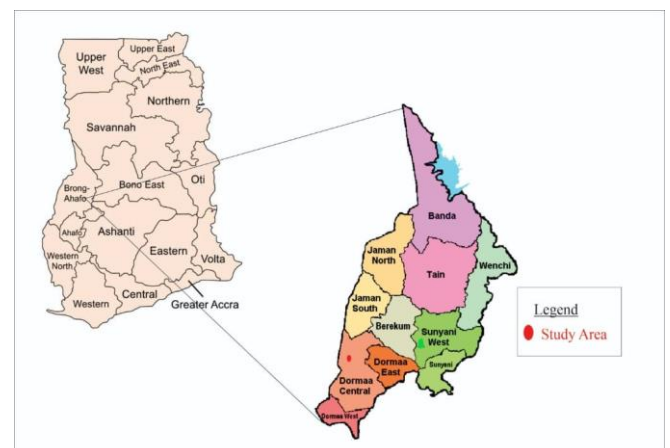


Fig. 1. Map of Ghana showing the study area (Dormaa Central Municipality).

B. Morphological Assessment and Identification of the Pathogen

The purified pathogen was examined for morphological and cultural characteristics using various parameters such as hyphae form, conidial shape and size, colour, colony margin, aerial mycelium and sporulation. Preparation of the pure cultures for observation of outgrowth of the fungi was done under compound light microscope (Olympus CX23, 40X/0.25 ∞ /- FN20) using both dry and wet mounts techniques. For the dry mount, Cello-tape was used to pick the mycelium by ticking it on fungi outgrowth on the petri dish and then placed on the microscopic slide and mounted on the microscope for observation (40X). In the second wet mount method, 5 μ l (microlitre) of sterile distilled water was pipetted severally on the petri dish, containing the fungi outgrowth for the release of the spores into the water, a drop of this water-spore's mixture was then pipetted on the slide, covered with slide slip and mounted on the microscope stage for examination (40X). The identification was based on standard identification protocols as described by [30]-[38]. The shape and the sizes (μ m) of the conidia were captured and recorded using calibrated Image View, version: x64, 3.7.9229.2 017- 0607.

C. Pathogenicity Assay

For pathogenicity trial, the stored pure cultures at -20 °C were further cultured on PDA for 7 days at 28 °C. The harvesting of the fungi spores for inoculation was done using autoclaved (121 °C for 15 minutes) water in order to avoid contamination with other pathogens. Spore suspension was prepared by flooding the Petri dishes with autoclaved water and scraping the media surface gently with a spatula tip and the concentration adjusted to 1×10^6 conidial suspensions per ml, after which two months' healthy cashew seedlings were sprayed according to the method described by [39]. The control treatment was sprayed with sterile distilled water. Before the spray was done, the seedlings were slightly bruised as per the pin prick method before the conidial suspensions were introduced onto the plants using a small hand atomizer. The inoculated seedlings were covered with plastic sheets over night to provide relative high humidity necessary for fungi conidial germination and infection before being maintained in a control environment. The experiment was replicated thrice. The setup was monitored daily for disease symptoms in order to confirm the Koch's postulate and establish *C. gloeosporioides* species complex as the causal agent of the same disease found in the various orchards in the Bono Region.

D. Incidence and Severity of Diseases

The incidence of anthracnose/die-back and gummosis suspected to be caused by *C. gloeosporioides* on cashew were assessed and expressed in percentage. For anthracnose disease incidence, a maximum of 100 leaves per tree were evaluated. The evaluation followed a protocol designed for cashew powdery mildew by [40]. For gummosis, the presence of critically examined gum exudates on the number of infected trees were assessed using a formula described by [41]. The disease incidence (D.I) was expressed as per formula 1 below:

$$D.I. (\%) = \frac{\text{Number of infected cashew trees}}{\text{Total number of cashew trees}} \times 100 \quad (1)$$

The severity assessment was carried out only on anthracnose /die-back infected cashew trees. The severity of the disease was based on percentage of necrotized leaf area symptoms observed on the cashew trees and scored using a scale (5-point score) as described by [41] (Table 1).

TABLE 1: DISEASE SEVERITY MEASUREMENT BASED ON A 5-POINT SCORE (0–4 SCALE)

Scale	Severity score range	Interpretation
0	< 1 =	No infection
1	1 – 25 =	Low infection
2	26 – 50 =	Moderate infection
3	51 – 75 =	High infection
4	>75 =	Very high infection

E. Data Analysis

The data on the incidence and severity of the diseases as observed on cashew trees across the visited orchards were square root transformed prior to analysis of Variance (ANOVA) using statistical software, GenStat release 12th Edition [42]. The mean variability among the incidence and severity of the diseases from each of the orchards were evaluated. The treatment means were separated using Tukey's Test and standard error at 0.5% level of significance. The results were presented in the form of tables and graphs. Pictorial frames of the diseased samples are shown. The morphological data on the pathogen characteristics were determined using standard mycological identification protocols established in the literature and mycological identification guide.

III. RESULTS

A. Symptoms on the Cashew Trees

During the survey in the various cashew orchards, data on number of symptoms observed on the cashew trees suspected to be caused by *C. gloeosporioides* were collected. The symptoms were visible on the leaves, stem, tree trunk, inflorescences, twigs, flowers, nuts and apples in the form of small, circular or irregular yellow, dark or brown water-soaked spots or lesions on leaves, fruits and flowers, depressed or sunken surfaces, especially on the apples, slow tip die-back on tree branches, which later resulted in complete drying of the tree, withering of twigs from the base, defoliation, abortion of cashew flowers, blighted leaves and exudation of gum. The exudates were brown or reddish in colour (later became black), oozing from the cracks of branches and stems or trunk. The affected inner cavities and the space between the bark and the trunk were filled with brown or reddish fluid. The symptoms on the panicle or lateral branches were characterized by longitudinally expanded, sunken resinous lesions or wounds. Longitudinally splitting the infected branches showed necrotic brown streaking vascular tissues. The developed symptoms are shown in Fig. 2-8 below.



Fig. 2. Anthracnose symptoms on cashew leaves in orchards in the Bono region of Ghana.

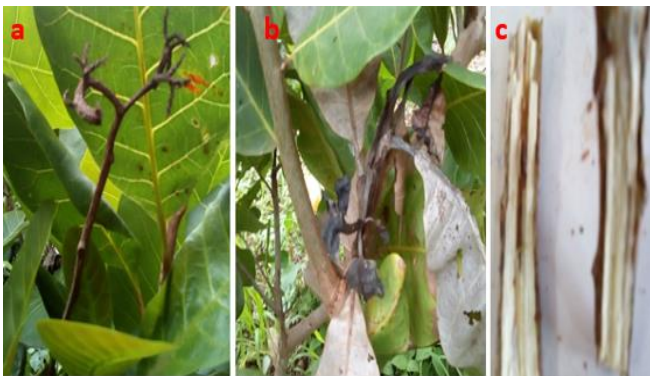


Fig. 3. a: Dead twig of cashew; b: blighted leaves; c: longitudinally split infected branch showing necrotic brown streaking vascular tissue.



Fig. 4. a: anthracnose inflorescence; b: abortion of cashew flowers. c: longitudinally expanded, sunken resinous wounds on lateral branch.



Fig. 5. a: Gummosis on cashew tree b: longitudinal sectional area of tree bark showing dark necrotic cambium layer with watery brown exudate.



Fig. 6. Farmers showing cashew die-back in their orchards in Atesikrom and Amomaso communities.



Fig. 7. a: Healthy cashew apples and nuts; b: Anthracnose symptoms on cashew apples with sunken alligator skin effect surfaces.



Fig. 8. Anthracnose symptoms on cashew showing complete death of nuts and apples.

B. Morphological Evaluation and Identification of the Pathogen

The cultural and morphological characteristics of the pathogen as observed on the media, showed a greyish-white, cottony or woolly colonies. The pathogen was successfully isolated from spotted leaves (Fig. 9 a), dead twigs (Fig. 9 b), necrotic inflorescence /flowers (Fig. 9 c), exudated tree bark (Fig. 9 d), dead nuts and apples (Fig. 9 e) on PDA, aseptically, under laminar hood. All the morphological and cultural characteristics of the fungus described in this study are in accordance with standard mycological description reported by [34], [37], [38]. Apart from gummosis infected samples in which two pathogens (*L. theobromae* - data not shown, and *C. gloeosporioides*) were isolated, *C. gloeosporioides* was consistently found to be associated with the all the diseased samples collected from different orchards within the municipality.



Fig. 9 a. Isolation from cashew leaves; b: a greyish-white, cottony or woolly colonies of *C. gloeosporioides* on PDA.



Fig. 9 b. Isolation from cashew dead twigs with corresponding colony on PDA.



Fig. 9 c. Isolation from inflorescence/ flowers with corresponding colony on PDA.

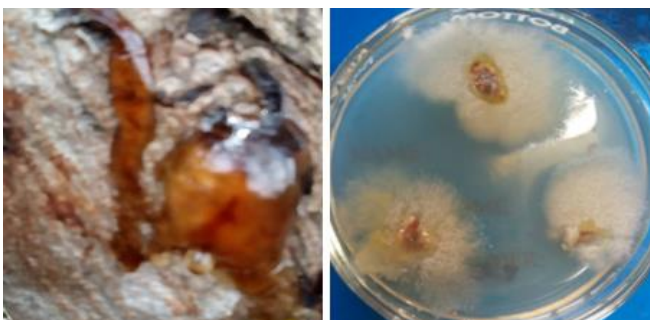


Fig. 9 d. Isolation from exudated tree bark with corresponding colony on PDA.



Fig. 9 e. Isolation from dead nuts and apples with corresponding colony on PDA.

The growth of the fungi as observed under the microscope showed branched, hyaline and septate mycelium (Fig. 10). The observed conidia also showed straight, hyaline cylindrically shaped structures with round or obtuse ends produced on distinctive hyaline conidiophores. Some of the conidia were also slightly curved. There were also variations in the conidia sizes as taken from different locations with some being longer than others, but in all, the conidia sizes ranged between 9 -15 up to 20 μm in length and diameter of 3-6 μm (Fig. 11). Both dry and wet mount methods produced the same results with regards to the spores' examination under the microscope, but the dry mount method produced intact conidia on the conidiophore as shown in Fig. 11 following standard mycological identification protocols by [30]-[38]. The isolated putative pathogen with all the features described above was identified as *C. gloeosporioides* species complex, which was responsible for causing anthracnose, gummosis and die-back diseases of cashew trees in the study area.

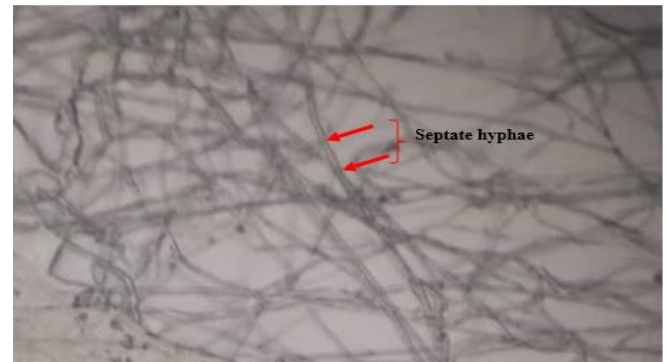


Fig. 10. Branched, hyaline and septate mycelium of *C. gloeosporioides*.

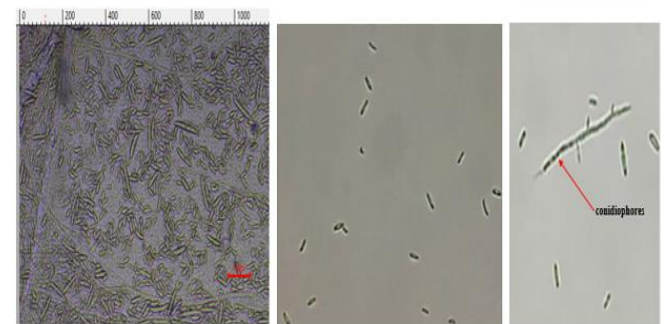


Fig. 11. Varied hyaline cylindrical conidia of *C. gloeosporioides* (40X) from different locations.

C. Koch's Postulate Confirmation and Pathogenicity Test

The symptoms elicited by *C. gloeosporioides* on the inoculated cashew seedlings in the course of pathogenicity test were recorded. The pathogen was able to infect the seedling and produce symptoms on the inoculated plants that included initial small, circular or irregular yellow, dark or brown water-soaked lesions or spots on leaves, limited expansion of the lesion with ageing plant, leading to shoot-hole appearance, lesions on the leaves coalesced together with time to form blight and complete death of the seedlings (Fig. 12). Leaf defoliation was also observed. All the symptoms, except exudation were produced from the inoculated seedlings. The sterile distilled water-inoculated seedlings, which served as the control, remained healthy and symptomless until the experiment ended. The symptoms

produced on the inoculated seedlings were similar to those observed in all the visited orchards as described by [43]-[45], [12]. The pathogen was consistently re-isolated, thereby confirming *C. gloeosporioides* as the causal agent of anthracnose and die-back disease of cashew.



Fig. 12. Pathogenicity test showing healthy and diseased seedlings after inoculation with sterile distilled water and *C. gloeosporioides*.

D. Anthracnose Incidence and Severity

The incidence of anthracnose in the twenty-five orchards averaged 22.9% and differed significantly ($P < 0.001$) among the orchards (Table 2). The lowest and highest infections of 3.0% and 45.8%, respectively were recorded at orchards Ant4FARM(Antwirifo) and Kye2FARM (Kyeremansu), respectively. Ant4FARM also had lower infections than Bad1FARM, Ton2FARM, and Ton4FARM. The infection at Agye5FARM was significantly lower than that of Kye2FARM. The disease severity also differed significantly among the orchards ($P = 0.005$) and correlated positively with the disease incidence ($r^2 = 0.6412$; $P < 0.001$). It averaged 1.69 and ranged from the least severity of 0.8 at Ant4FARM to the most severity of 2.60 at Kye2FARM. No other significant differences were seen.

E. Incidence of Gummosis on Cashew

Figure 13 shows the incidence scores for gummosis of cashew in the communities investigated. Results showed that the incidence scores were within a range of 6.9% at Antwirifo to 14.0% at Agyemankrom communities, but no significant differences ($P = 0.105$) were found among the locations.

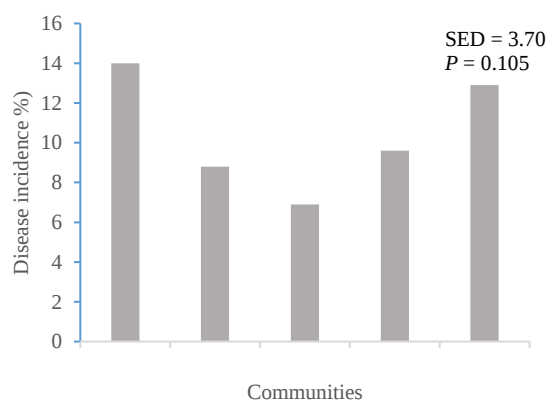


Fig 13. Incidence of gummosis of cashew in five communities in Dormaa-Central Municipality of the Bono Region.

TABLE 2: INCIDENCE AND SEVERITY OF ANTHRACNOSE OF CASHEW IN 25 ORCHARDS DURING A DISEASE SURVEY IN THE FIVE COMMUNITIES IN THE 2019/2020 CROPPING SEASON IN THE DORMAA CENTRAL MUNICIPALITY. THE DATA IS PRESENTED AS A SQUARE ROOT TRANSFORMATION OF THE RAW DATA SHOWN IN PARENTHESES

Orchard	Disease Incidence (%)			Disease Severity		
Ant1FARM	4.71	(25.4)	abcd	1.35	(2.00)	ab
Ant2FARM	4.37	(19.4)	abcd	1.00	(1.00)	ab
Ant3FARM	2.63	(9.8)	abc	0.88	(1.00)	ab
Ant4FARM	1.32	(3.0)	a	0.80	(0.80)	a
Ant5FARM	3.62	(13.8)	abcd	1.00	(1.00)	ab
Agye1FARM	5.64	(35.8)	bcd	1.58	(2.60)	b
Agye2FARM	4.18	(18.2)	abcd	1.25	(1.60)	ab
Agye3FARM	3.98	(16.8)	abcd	1.49	(2.40)	ab
Agye4FARM	3.97	(18.8)	abcd	1.15	(1.40)	ab
Agye5FARM	2.40	(8.0)	ab	1.25	(1.60)	ab
Bad1FARM	6.23	(40.4)	cd	1.46	(2.20)	ab
Bad2FARM	3.68	(14.0)	abcd	1.08	(1.20)	ab
Bad3FARM	4.45	(22.2)	abcd	1.35	(2.00)	ab
Bad4FARM	3.44	(12.0)	abcd	1.00	(1.00)	ab
Bad5FARM	3.95	(16.6)	abcd	1.17	(1.40)	ab
Kye1FARM	4.91	(25.8)	abcd	1.46	(2.20)	b
Kye2FARM	6.64	(45.8)	d	1.60	(2.60)	ab
Kye3FARM	5.70	(33.8)	bcd	1.38	(2.00)	ab
Kye4FARM	3.03	(11.1)	abcd	1.31	(1.80)	ab
Kye5FARM	4.21	(22.3)	abcd	1.40	(2.00)	ab
Ton1FARM	4.04	(19.7)	abcd	1.17	(1.40)	ab
Ton2FARM	6.27	(40.0)	cd	1.33	(1.80)	ab
Ton3FARM	6.16	(38.8)	cd	1.23	(1.60)	ab
Ton4FARM	5.49	(30.4)	bcd	1.17	(1.40)	ab
Ton5FARM	4.95	(30.0)	abcd	1.43	(2.20)	ab
Grand mean	4.40	(22.9)		1.25	(1.69)	
CV%	34.8			25.6		
S.E.D.	0.968			0.20		
P- value	<.001			0.005		

CV: Coefficient of variation; SED: Standard error of the differences of means; Ant: Antwirifo; Agye: Agyemankrom; Bad: Badukrom; Kye: Kyeremansu; Ton: Tonasuano.

IV. DISCUSSION

Disease symptoms caused by *C. gloeosporioides* have been reported to occur on all aerial parts of cashew [12] but are more commonly noticed on the leaves [46]. The observed symptoms on different parts of cashew tree in the various orchards in Dormaa Central Municipality are in line with similar ones reported by [12] and [43] who in their separate submissions, indicated that the pathogen has the ability to infect cashew inflorescences, young fruits and apples, twigs and leaves, resulting in the production of angular lesions, blight, leaf drop in severe cases and sunken sub-circular symptoms among others. They, including other researchers-[44], [45], [12], also reported the role of different environmental factors such as rainfall, wind, temperature and humidity on the dispersal of these diseases. Similar trends have also been observed in this study, where some symptoms appeared at the outset of the rains, increased or decreased with fluctuating temperature, humidity and heavy rains. In

most of the orchards visited, it was observed that, the cashew trees produced a lot of flowers, but later on recorded high numbers of flower abortion due to the effect of anthracnose and in most cases the infected flowers get aborted or blown away with any slight rain or wind, leaving exposed skeletal twigs (Fig. 4 b), thereby compromising fruit setup and nut yield. This observation was corroborated by [44] and [14] who reported that when there is high humidity and rainfall conditions favouring the pathogen development during the flowering stage and cashew nut set, the quality of the kernel and the apple (Figs. 7 b; 8) are seriously affected and thereby drastically reducing cashew productivity. Also, in line with our findings in terms of flower abortion, [47] reported that dropping of flowers and young fruits were the main measurable damage caused by anthracnose to cashew yield. Defoliation, death of inflorescences, necrotic tissues and immature nuts abortion have also been reported in severely infected adult trees [46]. Others [12] and [14] described cashew anthracnose symptoms to include initial grayish necrotic lesions on young leaves, which later become reddish on the older leaves. They also reported blackening of young leaves, which become twisted and fall off under severe situations, wilting, falling of flowers and blackened or mummified cashew apples. Similar observations were made in this study.

On gummosis, the exudates observed on some of the diseased cashew trees in the orchards are in agreement with other reports such as [48], [49] and [15], who reported the production of resin exudate on cashew trees incited by *C. gloeosporioides*.

On die-back, our findings are supported by [50] who reported symptoms on the cashew twigs to include dark brown to black and sunken, extended lesions that finally result in regressive die-back. Recurrent die-back has also been reported to trigger stag headed symptoms that will finally lead to the death of cashew trees, especially at the younger stage of plant development [48]. Cashew seedlings and shoots die-back have been reported to cause an indirect economic loss [48].

Culturally, the isolated pathogen produced greyish-white, cottony or woolly colonies on the PDA media with septate hyphae (Fig. 10). This characteristic, typical of this fungus has been observed by [51], who reported narrow, sparsely septate hyphae that was initially hyaline and turned slightly dark with age. Also, grey coloured mycelial have been associated with *C. gloeosporioides* isolates on strawberry [52], apple, pecan and peach [53] and mango [54]. The examined conidia produced straight, hyaline cylindrically shaped structures with obtuse ends connected to hyaline conidiophores when in an undisturbed state. The size of the conidia was diverse among the municipalities and were between 9 -15 up to 20 µm in length and diameter of 3-6 µm. These sizes are well within ranges that have been reported in the literature. In their work [55], reported variable conidia sizes of *C. gloeosporioides* to be 11-16 x 4-6 µm and 13.8 x 4.8 µm, with rounded ends and 11.9-17.0 x 3.6-5.8 µm plants include 8.3 to 27.4 µm in length and 2.0 to 6.6 µm in width [31], 8-20 x 5-7 µm [57], 6.0-10 x 2.0-2.5 µm [24], 9.0-24 x 3.0-4.5 µm [58], 12.5- 17.5 x 3.8-7.5 µm [59], 12.1-18.1 x 3.6-8.2 µm [60], 12-16 x 4-6 µm [51], 14.5 - 17 x 4 - 4.5 µm [52], 13.6 x 5.1 µm [53], 13.2 x 6.0 µm [54], 13-16

µm x 4-5 µm [61], 17.09 x 7.74 µm, 16.23 x 7.81 µm, 15.94 x 7.51 µm [62]. It was observed in this study that the sizes of the conidia could vary depending on several factors such as the type of media used, the composition of the media and how it is prepared, the sample location per the prevailing weather conditions, as well as, the temperature used in growing the cultures. The *C. gloeosporioides* formae speciales has also been reported by [24], who recorded the existence of heterogeneity among the groups with huge variation in terms of morphology. The variability that exists in conidia architecture between *C. gloeosporioides* isolates in terms of morphology and physiology has also been reported by [30], [63], [64]. [65] in his work defined the key for 22 species of the genus *Colletotrichum* established on cultural characteristics. At present, around 40 species within this genus are recognized based on more comprehensive studies on morphological, pathogenic dexterity and cultural characteristics [66].

In terms of the shape, we observed conidia that were straight, cylindrical, or oblong, had obtuse ends and hyaline in nature. Similar features were reported by [67], [68], [32], [62], [54], [53], [52]. The morphological characteristics as reported here, with supporting literature, provide enough evidence to identify *C. gloeosporioides* species complex as the causal agent of cashew diseases in the Dormaa Central Municipality of the Bono Region. This assertion was supported by the infectivity of cashew seedlings by *C. gloeosporioides* in the pathogenicity test. The pathogen induced symptoms such as water-soaked, small circular to irregular dark brown or reddish necrotic lesions from the initial greyish colour on the leaves, necrotic spots of varied sizes on various parts of the leaves, especially from the tip, coalesced to form blight, which progressed downwards until the whole seedling became dried and died off. Defoliation of the leaves and stem lesions was also recorded. Shoot-hole appearance (Fig. 12) due to the restricted expansion of the lesions was observed and this is in line with [49] who reported cracks and holes on cashew leaf limbs as a result of delimited expansion of the lesion as the plant ages. [69] in their pathogenicity trial on mango seedlings using pathogenic isolates of *C. gloeosporioides*, observed 85 - 90% mortality within a period of 2 to 3 months. [70] observed pathogenic symptoms development effects of *C. gloeosporioides* on various detached leaves of fruits and vegetables in pathogenicity experiment. Also, in a related pathogenicity tests on avocado, tomato, strawberry and olive, using conidial suspensions (1000x 10 µl droplet and 10⁵) of *C. gloeosporioides*, [71], [72], reported the development of necrotic lesions on leaves, stems, petioles and other parts of all the inoculated fruits. The symptoms, as a result of the infection, have been reported to occur on leaves, fruits and seedlings in all stages of plant development and could result in seedlings necrosis and death as well as rot in nuts [73], [74].

Also, in agreement with our findings, in terms of symptoms development on the inoculated seedlings, is [75] who reported the occurrence of lesions of variable sizes on the various parts of cashew leaf apex and limbs. Under severe infection conditions, the individual spots have been reported to coalesce, covering over half of the infected leaf, from the tip and progress downwards [49], [75]. Again, infected tender

tissues may become crinkle, dry up and drop off with blight symptoms [75], [50]. All the symptoms, except exudation were produced from the inoculated seedlings in this study. The inability of the pathogen to produce exudate on the young seedling does not mean the identified pathogen was not one of the causal agents of gummosis observed on the infected trees in the visited fields. The possible explanation could be that, for exudates to form, it may require sufficient time. This is evident, during the survey as it was observed that more than 90% of the trees that produced the exudates were older plants, with only very few of the younger plants producing it. It is, therefore, not surprising that the inoculated seedlings failed to produce exudate before the seedlings died off during the experiment. *C. gloeosporioides* has been described as a complex species with variable physiology and pathogenicity [46]. The pathogen was consistently re-isolated from the inoculated cashew seedlings and thereby confirming the pathogenic role of *C. gloeosporioides* as the causal agent of anthracnose and die-back diseases of cashew, thus completing Koch's postulate.

The disease incidence and severity scores has established that the disease is widespread in the municipality. This should be valuable information to guide farmers and other stakeholders, in particular, to having interventions in place to mitigate effects on crop yields. Despite the incidence of gummosis in all the municipality, anthracnose has by far been considered the most dreadful disease of cashew elsewhere [12], [76], [14], thus, may require immediate attention in the municipality. Indeed, it registered an incidence of 22.9% across the orchards. This is within a range of incidence reported India [77], [15]. The variability observed in incidence is attributable to varying rainfall, temperature and humidity differential that may favoured the pathogen growth and development. There is already literature to support environmental impact on the pathogenesis of *C. gloeosporioides*. The pathogen has been reported [73] to trigger severe damage and seriously decrease peduncle and nuts production potential under favourable environmental conditions. Reduction in the marketability value of the cashew products as a result of damage caused by anthracnose at storage has also been reported [68]. In a study on the incidence and impact of diseases associated with cashew in Nigeria, [78] have implicated *C. gloeosporioides* as one of the many fungi causing the disease, together with the evidence of cashew gummosis [79], [80].

There is a ghost or little information on the occurrence of cashew diseases in Ghana and in most African countries. We believe that this work presents the first extensive insight of *C. gloeosporioides* species complex causing number of diseases on cashew and its products. The cashew plant might have evolved alongside the diseases when the plant was first introduced into the country, or as reported by others, it could also be attributed to expansion in the cultivation areas, monoculture practices, planting of genetically uniform plants and an increase in international trade, which could lead to overwhelming epidemics in the agroecosystems [81], [82], [83]. Anthracnose and the causal agent, *C. gloeosporioides* have been reported to have a wide-reaching distribution (incidence), especially in cashew growing areas or regions, namely Brazil, Argentina, India, Pakistan, Indonesia, Philippines, Florida and Hawaii in the USA, Argentina, Peru,

Trinidad, South Africa among other countries of the world with high rainfall and relative humidity [51], [44], [14]. The outbreak of these diseases in cashew orchards could lead to both social and economic consequences in the near future [84].

The severity of anthracnose was 1.69 (on a 0-5-point score) and could be considered as low across the orchards for the 2019/2020 cropping season. Nonetheless, the disease severity correlated positively with the disease incidence in our study. This may imply that higher incidence was associated with higher severity and may point to pathogenic virulence of the *C. gloeosporioides* isolates on cultivars currently grown in the orchards visited. Thus, farmers may be encouraged to grow other lesser known varieties. Also, high severity can be due to favourable environmental conditions, particularly high humidity. Indeed, we observed during our field visits, that a greater number of orchards were planted at closer spacing than the recommended planting spacing of 3 meters by 3 meters. Closer spacing may create dense canopies, a likely precursor for high humid conditions, which may favour a more aggressive pathogenesis. Perhaps, adherence to recommended agronomic practices may help reduce disease severity in the municipality. A number of publications have reviewed the relationship that exist between incidence and severity of many pathosystems [85], [86]. In line with the relationship that exists between incidence and severity, anthracnose leaf incidence has been found to be consistently connected with leaf severity [14].

V. RECOMMENDATION

In view of the little information on the diseases of cashew in Ghana, there is the need to conduct wider surveys, covering the entire cashew growing regions of the country to identify different species of pathogens that are limiting cashew production, using both morphological combined with the molecular tools for more in-depth and accurate identification of the pathogens to species level.

Morphologically and culturally, the pathogen was identified as *C. gloeosporioides* species complex, meanwhile, this complex consists of at least six distinct species. Although many of the species in this group could not be reliably distinguished using ITS region of the pathogen, identification using both molecular (e.g. using specific primer and RAPD) and morphological methods, will help to segregate this complex to species level as well as determine the diversity that exists among the numerous isolates that will be collected from different locations, and this will warrant application of specific management strategies for their control in order to reduce cost.

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