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Cross-Infectivity of *Colletotrichum* Isolates, Causal Agent of Anthracnose on Fruits in Three Regions of Cameroon

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ABSTRACT

Anthracnose, caused by Colletotrichum spp., is one of the main constraints to the commercialisation and exportation of fruits in Cameroon. The present study was designed to test the ability of the pathogen isolates from five fruits; avocado, banana, mango, pawpaw and plum to cause disease by cross-infectivity between fruits crops. Isolates of the pathogen were cultured in Potato Dextrose Agar (PDA). Pathogenicity and cross-infectivity of Colletotrichum spp. were carried on each of the five apparently healthy fruits collected from market in Dschang, West Region of Cameroon using wound inoculation method and dropping inoculation method. The results showed that lesion areas induced by different Colletotrichum isolates, varied with the fruit, the isolate as well as the origin of the isolates. Isolates of Colletotrichum formed larger lesion on fruits with the wound inoculation method compared to dropping inoculation method. Based on those lesions, three groups of isolates were found with the two methods. AVIS1, BAIS1, MAIS1, PAIS and SAIS isolates produced larger lesions, AVIS2 and MAIS2 induced smaller lesions and BAIS2 did not form any lesion except on banana, the original host. These results suggest further characterisation for a better identification and development of control strategies for the fungus.

Key words: *Anthracnose, Colletotrichum isolates, Cross-infectivity, Fruits, Lesion areas*

INTRODUCTION

Fruits are important items of high commercial and nutritional value. Fruits constitute an essential part of the human diet because of their nutrients' contents. They also contribute to the improvement of the social well-being of populations by providing the organism with vitamins, minerals and fibres essential for health[1]. Cameroon has a high potential for fruit production and the main production areas are Centre, Littoral and West regions[2]. Fruit sector; which has been neglected in the past, is currently in expansion with an increasing production over the last two decades[3]. This increase can be justified by the fact that fruit marketing has become an important part of the Cameroonian economy as it generates large income for the populations[4].

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Fruits are sold in different markets of Economic and Monetary Community of Central Africa (CEMAC), European Union (EU) and America[5]. In 2010, Cameroon were avocados (834.4 tons), bananas (262 890 tons), mangoes (1560.4 tons), pawpaw (17 611 tons) and plums (473.4 tons) were the main exported fruits which yielded about 76 billion francs CFA[4].

In Cameroon, the constraints that hinder the development of the fruit sector and its maintenance at a level that could respond to national and international demands in terms of quantity and quality of products concerns the depreciation of the quality due to many pests and to the packaging, transportation and storage deficits. Deterioration of fruit is mostly due to post-harvest diseases of fungal origin. Among those fungal diseases, anthracnose caused by *Colletotrichum* spp. is the most frequent one that degrades the quality and quantity of fruits, reduces the market value leading to post-harvest fruit loss varying between 20 % and 50 %[6]. The works of Keuete[7] showed that *Colletotrichum gleoesporioides* was the most frequent pathogen in avocado fruits.

Anthracnose is a disease that develops on plants belonging to highly varied families and is responsible for damages affecting mainly the aerial parts of plants: leaves, flowers and fruits[8,9,10]. Similarly, those of Shanti *et al*.[11] showed that isolates of *Colletotrichum gloeosporioides* after injury on mangoes, pawpaw and rambutans induced lesions of anthracnose. *Colletotrichum gloeosporioides* isolates inoculated on avocado and mango fruits produced symptoms of anthracnose at significantly identical degrees[12]. Sanders and Korsten[13] reported that cross-inoculation of *Colletotrichum gleoesporioides* isolated from mango and avocado fruits showed large lesions on the hosts fruits and produced lesions on other fruits except on lemon fruits. Isolates of *Colletotrichum gloeosporioides* isolated from mango fruits produced symptoms of anthracnose in pawpaw, avocado and guava fruits[14]. However, species such as *Colletotrichum kahawae* infects only coffee berries, *C. coccodes* can produce symptoms of anthracnose only in tomato fruits and potato tubers, *C. falcatum* infects only sugar-cane and *C. musae* produces symptoms of anthracnose only in bananas[8,15,16,17,18,19].

The aims of the study are to test the ability and level of host preference of different isolates of *Colletotrichum* from various fruits to cause disease on others, to provide information on post-harvest disease management.

MATERIAL AND METHODS

Isolation of *Colletotrichum* species

Fruits (avocado, banana, mango, papaya and plum) with symptoms of anthracnose, were collected in markets of four Divisions in West Cameroon; namely Bamboutos, Mbam and Inoubou, Mougo and Noun on July 2016. These fruits were putted in appropriate plastic bags labelled and transported into the Research Unit of Phytopathology and Agricultural Zoology (UR_PHYZA) of the University of Dschang for isolation of *Colletotrichum* spp. The fruits were primarily washed separately with tap water and fragments of disease tissues of about 2 mm² excised from diseased lesions. The cut pieces were surface sterilized in 5 % sodium hypochlorite solution for five minutes and rinsed three times in sterile distilled water, placed on hydrophilic paper to remove excess water and plated on Potato Dextrose Agar (PDA) medium amended with chloramphenicol (20 mg/L) to control bacterial contamination. The plates were incubated at at 24 ± 2° C. Fine hyphae that grew from diseased tissue on the culture media were sub-cultured on fresh PDA. Colonies of *Colletotrichum* sp., were morphologically identified and subjected to further sub-culturing on PDA. Pure cultures of the isolates were sealed in Petri dishes with parafilm paper and stored in a refrigerator at 4° C for further studies.

Pathogenicity test

Apparently healthy and freshly harvested mature fruits of avocado, banana, mango, pawpaw and plum collected from the market in Dschang, were washed under running tap water and surface sterilized for five minutes in 5 % sodium hypochlorite solution. This was immediately followed by three rinses in sterile distilled water and dried with sterile tissue paper. The sterilized fruits were placed on plastic box in which humidity was maintained using sterile hydrophilic cotton pads soaked with sterile distilled water. Two methods of inoculation; wound inoculation method and dropping inoculation method were used.

In wound inoculation method, each fruit species was inoculated using the method of Lin *et al.*[20], which included pin-pricking the fruits to a 5 mm diameter with a sterile needle in the middle portion of fruit and then placing 5 mm diameter each of mycelia fragments from each pure culture of *Colletotrichum* isolates from different fruit species onto the wound. In dropping inoculation method, each of the fruits received a drop of 50 µl of conidial suspension of different *Colletotrichum* isolates, previously prepared and adjusted to $5x10^4$ conidia/ml by a haemocytometer[21]. Control fruits did not receive any inoculum. Each inoculated fruit species was incubated in the containers at room temperature (24 ± 2° C) in an experiment arranged in a completely randomized design with *Colletorichum* isolates (table 1) as treatment and five replications.

After five days of incubation, each incubated fruit was examined, and the lesion areas induced were measured using a graph paper.

Table 1: Origin of *Colletotrichum* spp. isolates used for the study

Isolate code	Host fruit	Scientific name of fruit
AVIS1	**Avocado**	***Persea americana***
AVIS2	**Avocado**	***Persea americana***
BAIS1	**Banana**	***Musa sapientum***
BAIS2	**Banana**	***Musa sapientum***
MAIS1	**Mango**	***Mangifera indica***
MAIS2	**Mango**	***Mangifera indica***
PAIS	**Pawpaw**	***Carica papaya***
SAIS	**Plum**	***Dacryodes edulis***

Statistical Analysis

Data collected on lesion areas were subjected to analysis of variance (ANOVA) using SPSS software version 21.0, and mean values were separated using Duncan's Multiple Range test (DMR) and LDS Fisher test at a 5 % probability level.

RESULTS

Species of *Colletotrichum* develop on various fruits, inducing necrosis in the form of black spots. Cross-infection test showed variability in lesion formation of the different *Colletotrichum* isolates on the host fruit and other fruits. Lesion areas induced by the different isolates of *Colletotrichum* on fruit, varied with the fruit and the isolate as well as its origin. The controls were however, not infected any fruits. The lesion area observed on the different fruits inoculated by the wound inoculation method was greater than that observed on the fruits inoculated by the dropping inoculation method. The BAIS2 isolates did not develop any lesions on the other four fruits, except on the banana whatever the method used for inoculation.

The lesions areas induced by the different isolates of *Colletotrichum* on avocado fruits, varied with respect to the isolate of *Colletotrichum*, as well as the method of inoculation. The analysis of lesions developed by different *Colletotrichum* spp., showed that the isolates of BAIS1, MAIS1, PAIS and SAIS had developed the significantly identical surfaces lesion than those produced by AVIS1 (isolated from avocado). These lesion areas were greater than those induced by the other *Colletotrichum* isolates and varied between 19.1 and 26.3 cm² for the wound inoculation method and between 2.49 and 2.77 cm² with the dropping inoculation method (table 2). The AVIS2 isolate isolated from avocado produced a lesion area similar to that produced by isolate MAIS2. The lesions developed by these isolates were the smallest. The BAIS2 isolate did not induce lesions on the avocados.

The results also showed that the lesion areas developed by the different isolates with the wound inoculation method was significantly higher than those developed by the same isolates with the dropping inoculation method.

Table 2: Lesion areas developed by different *Colletotrichum* isolates on avocado (cm²)

	Divisions							
	Bamboutos		Noun		Mbam and Inoubou		Mungo	
Isolate	1st method	2nd method	1st method	2nd method	1st method	2nd method	1st method	2nd method
AVIS1	**23.12aA***	**1.65aB**	**26.34aA**	**1.69aB**	**20.9aA**	**1.91aB**	**21.09aA**	**2.17aB**
AVIS2	**2.72bA**	**0.8bB**	**2.49bA**	**0.79bB**	**2.7bA**	**1.12bB**	**2.53bA**	**1.23bB**
BAIS1	**25.76aA**	**1.71aB**	**25.15aA**	**1.75aB**	**24.63aA**	**1.91aB**	**26.11aA**	**2.01aB**
BAIS2	/	/	/	/	/	/	/	/
MAIS1	**26.63aA**	**1.84aB**	**22.96aA**	**1.74aB**	**25.01aA**	**2.05aB**	**22.41aA**	**2.05aB**
MAIS2	**2.88bA**	**0.71bB**	**2.64bA**	**0.74bB**	**2.77bA**	**0.92bB**	**2.64bA**	**1.23bB**
PAIS	**23.78aA**	**1.65aB**	**25.42aA**	**1.69aB**	**25.22aA**	**1.91aB**	**24.77aA**	**2.04aB**
SAIS	**21.99aA**	**1.78aB**	**21.88aA**	**1.72aB**	**19.1aA**	**1.99aB**	**20.51aA**	**2.15aB**

***Means in columns followed by the same small letter are not significantly different by Duncan's Multiple Range test at 5 % probability level and means in the line followed by the same capital letter are not significantly different by LDS Fisher test at 5 % probability level. / signify that the isolates did not produce a lesion.

The lesion areas developed by the different *Colletotrichum* spp. on banana, varied with the isolate, origin of isolates and the method used for inoculation. The BAIS2 isolate from banana developed a lesion area was significantly greater than those induced by other isolates of *Colletotrichum*. The isolates AVIS1, BAIS1 (obtained from banana), MAIS1, PAIS and SAIS produced similar lesion areas, which were intermediate. The lesions produced by AVIS2 isolates from avocado and MAIS2 from mango were the smallest (table 3).

With the wound inoculation method, *Colletotrchum* spp. induced on banana the larger lesions than those observed on bananas inoculated with dropping inoculation method.

Table 3: Lesion areas developed by different *Colletotrichum* isolates on banana (cm²)

	Divisions							
	Bamboutos		Noun		Mbam and Inoubou		Mungo	
Isolate	1st method	2nd method	1st method	2nd method	1st method	2nd method	1st method	2nd method
AVIS1	**4.63bA***	**1.56bB**	**4.22bA**	**1.52bB**	**4.35bA**	**1.8bB**	**4.56bA**	**1.95bB**
AVIS2	**0.94cA**	**0.48cB**	**0.93cA**	**0.51cB**	**0.9cA**	**0.94cB**	**0.85cA**	**0.95cB**
BAIS1	**4.76bA**	**1.54bB**	**6.12bA**	**1.53bB**	**4.44bA**	**1.82bB**	**5.2bA**	**2.06bB**
BAIS2	**15.17aA**	**2.62aB**	**15.91aA**	**2.51aB**	**16.13aA**	**3.1aB**	**12.1aA**	**3.15aB**
MAIS1	**5.55bA**	**1.56bB**	**5.73bA**	**1.51bB**	**4.64bA**	**1.84bB**	**5.36bA**	**1.9bB**
MAIS2	**0.93cA**	**0.51cB**	**1.11cA**	**0.53cB**	**1.05cA**	**0.96cB**	**0.99cA**	**1.02cB**
PAIS	**4.25bcA**	**1.56bB**	**5.19bA**	**1.52bB**	**4.16bA**	**1.85bB**	**3.89bA**	**1.96bB**
SAIS	**3.98bcA**	**1.55bB**	**5.72bA**	**1.53bB**	**4.82bA**	**1.91bB**	**3.92bA**	**1.89bB**

*****Means in columns followed by the same small letter are not significantly different by Duncan's Multiple Range test at 5 % probability level and means in the line followed by the same capital letter are not significantly different by LSD Fisher test at 5 % probability level; / signify that the isolates did not produce a lesion.

On mango, the different *Colletotrichum* spp. induced lesions that varied with respect to the isolates and their origin. In general, lesion areas observed with the wound inoculation method were significantly larger than those observed with the dropping inoculation method. The results showed that MAIS1 isolated from mango, produced on the same fruit lesion surfaces were similar to those produced by AVIS1, BAIS1, MAIS1, PAIS and SAIS. These isolates produced the largest lesions. Similarly, MAIS2 from mango and AVIS2 induced significantly identical lesion surfaces, except that these lesions were smaller. The BAIS2 isolate from the banana did not cause injury to the mangoes.

However, the lesion areas developed by the different isolates of *Colletotrichum* following inoculation through wounds created on the fruit, were significantly higher than those induced by these same *Colletotrichum* spp. following inoculation with conidial suspension deposits

Table 4: Lesion areas developed by different *Colletotrichum* isolates on mango (cm²)

	Divisions							
	Bamboutos		Noun		Mbam and Inoubou		Mungo	
Isolate	1st method	2nd method	1st method	2nd method	1st method	2nd method	1st method	2nd method
AVIS1	**26.33aA***	**2.17aB**	**25.01aA**	**2.14aB**	**27.6aA**	**2.21aB**	**25.22aA**	**2.22aB**
AVIS2	**3.15bA**	**1.4bB**	**2.62bA**	**1.54bB**	**2.39bA**	**1.64bB**	**2.9bA**	**1.61bB**
BAIS1	**24.03aA**	**2.14aB**	**27.66aA**	**2.09aB**	**21.82aA**	**2.1aB**	**25.86aA**	**2.06abB**
BAIS2	**/**	**/**	**/**	**/**	**/**	**/**	**/**	**/**
MAIS1	**26.54aA**	**2.18aB**	**24.67aA**	**2.25aB**	**27.57aA**	**2.17aB**	**24.54aA**	**2.32aB**
MAIS2	**3.16bA**	**1.65bB**	**3.15bA**	**1.63bB**	**2.6bA**	**1.54bB**	**2.86bA**	**1.62bB**
PAIS	**22.91aA**	**2.06abB**	**27.98aA**	**2.05aB**	**26.43bA**	**2aB**	**26.51aA**	**2.07abB**
SAIS	**23.94aA**	**2.13aB**	**25.2aA**	**2.07aB**	**24.64aA**	**2.01aB**	**19.09aA**	**2.15aB**

*****Means in columns followed by the same small letter are not significantly different by Duncan's Multiple Range test at 5 % probability level and means in the line followed by the same capital letter are not significantly different by LSD Fisher test at 5 % probability level. / signify that the isolates did not produce a lesion.

Cross-infection test of the different isolates on papaya showed that *Colletotrichum* isolate expressed different lesion areas (Table 5). In Bamboutos, the AVIS1, BAIS1, MAIS1 and PAIS isolates induced similar lesion areas that were significantly greater than those induced by the other isolates. Similarly, the AVIS2, MAIS2 and SAIS isolates expressed significantly similar lesion areas. In all divisions, the BAIS2 isolates did not induce any lesion.

Table 5: Lesion areas developed by different *Colletotrichum* isolates on papaya (cm²)

	Divisions							
	Bamboutos		**Noun**		**Mbam and Inoubou**		**Mungo**	
Isolates	1st method	2nd method	1st method	2nd method	1st method	2nd method	1st method	2nd method
AVIS1	**34.25aA***	**3.13aB**	**40.99aA**	**3.09aB**	**40.49aA**	**3.31aB**	**35.95aA**	**3.26aB**
AVIS2	**11.19bA**	**1.69bB**	**6.38bA**	**1.69bB**	**7.44bA**	**1.83bB**	**6.55bA**	**1.91bB**
BAIS1	**40.38aA**	**2.99aB**	**40.24aA**	**2.98aB**	**40.08aA**	**3.35aB**	**38.73aA**	**3.35aB**
BAIS2	/	/	/	/	/	/	/	/
MAIS1	**40.55aA**	**3.25aB**	**31.51aA**	**2.94aB**	**39.9aA**	**3.17aB**	**40.23aA**	**3.25aB**
MAIS2	**11.87bA**	**1.68bB**	**9.05bA**	**1.63bB**	**10.87bA**	**1.91bB**	**10.8bA**	**1.84bB**
PAIS	**30.18abA**	**2.94aB**	**40.54aA**	**3.09aB**	**35aA**	**3.2aB**	**40.22aA**	**3.25aB**
SAIS	**17.47bA**	**3.03aB**	**24.27abA**	**2.97aB**	**21.79abA**	**3.27aB**	**20.83abA**	**3.29aB**

***Means in columns followed by the same small letter are not significantly different by Duncan's Multiple Range test at 5 % probability level and means in the line followed by the same capital letter are not significantly different by LDS Fisher test at 5 % probability level. / signify that the isolates did not produce a lesion.

On plum, the results showed that AVIS1, BAIS1, MAIS1, PAIS and SAIS isolates produced the largest lesions, which more significantly greater than those produced by other *Colletotrichum* isolates. AVIS2 and MAIS2 isolates developed small lesion areas. BAIS2 did not induce any lesion on plum. In Bamboutos Division with the wound inoculation method, the isolates AVIS1, BAIS1, MAIS1, PAIS and SAIS, produced larger lesion areas, which were significantly identical. The AVIS2, BAIS1, MAIS2 and SAIS isolates, induced small lesions areas and were significantly identical.

The lesion areas induced, by the different *Colletotrichum* isolates with the wound inoculation method were significantly greater than those obtained with the dropping inoculation method.

Table 6: Lesion areas developed by different *Colletotrichum* isolates on plum (cm²)

	Divisions							
	Bamboutos		**Noun**		**Mbam and Inoubou**		**Mungo**	
Isolates	1st method	2nd method	1st method	2nd method	1st method	2nd method	1st method	2nd method
AVIS1	**11.52aA***	**0.52aB**	**8.14aA**	**0.59aB**	**9.14aA**	**0.5aB**	**7.97aA**	**0.52aB**
AVIS2	**3.68bA**	**0.4bB**	**0.51bA**	**0.45bB**	**0.82bA**	**0.27bB**	**0.83bA**	**0.31bB**
BAIS1	**6.52abA**	**0.56aB**	**8.58aA**	**0.54aB**	**8.35aA**	**0.53aB**	**7.39aA**	**0.49aB**
BAIS2	/	/	/	/	/	/	/	/
MAIS1	**9.26aA**	**0.48abB**	**9.32aA**	**0.53aB**	**8.83aA**	**0.47aB**	**9.88aA**	**0.52aB**
MAIS2	**2.8bA**	**0.44bB**	**0.69bA**	**0.44bB**	**0.69bA**	**0.5aB**	**0.76bA**	**0.29bB**
PAIS	**10.68aA**	**0.51aB**	**9.56aA**	**0.59aB**	**9.35aA**	**0.47aB**	**10.3aA**	**0.49aB**
SAIS	**6.43abA**	**057aB**	**7.67aA**	**0.56aB**	**6.75abA**	**0.54aB**	**6.45aA**	**0.5aB**

***Means in columns followed by the same small letter are not significantly different by Duncan's Multiple Range test at 5 % probability level and means in the line followed by the same capital letter are not significantly different by LDS Fisher test at 5 % probability level; / signify that the isolates did not produce a lesion.

Dendrogram resulting from the hierarchical cluster analysis of lesion areas induced by different *Colletotrichum* spp. isolates on various fruits showed three groups (figure 1). The first Group consisted of AVIS2 and MAIS2 isolates produced smaller lesions on different fruit. The second group composed of AVIS1, PAIS, BAIS1, MAIS1 and SAIS isolates induced the larger lesions on different fruits except on banana, though the lesion areas were intermediate. The third group comprised of BAIS2 formed lesion only on banana fruit, the original host.

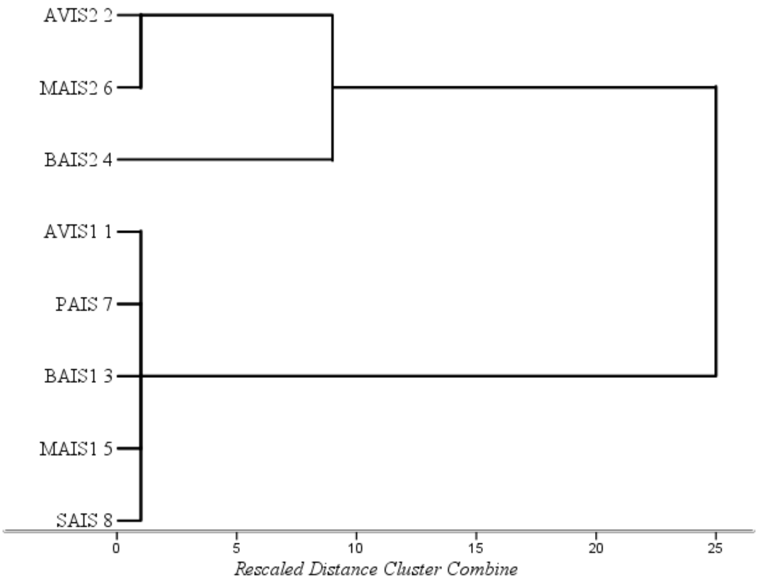

Figure 1: Cluster analysis based on the lesion areas produce on different fruit by *Colletotrichum* spp.

DISCUSSION

The results of this study showed that *Colletotrichum* spp., isolated from the fruits of avocado, banana, mango, pawpaw and plum showed variation in lesions formation. These lesion areas varied with the fruit and the isolates as well as its origin. The ability of *Colletotrichum* spp., to develop on a large variety of fruits and causing significant post-harvest losses has been reported by several authors[19,22,23,24].

Lesion areas produced on avocado, banana, mango, pawpaw and plum fruits irrespective of the method used by the various isolates in general, made it possible to rank the species of *Colletotrichum* into three groups; The first group consisting of AVIS1, BAIS1, MAIS1, PAIS and SAIS, the second group composed of AVIS2 and MAIS2 and the third group consisting of BAIS2. These results are in accordance with those of Keuete *et al.*[25], who, on a study on the Cultural and morphological variations of the fungus also found three groups with the same isolates. The difference in the lesion areas developed by these different isolates on fruits could be due to the existing diversity within this *Colletotrichum* spp. These results corroborate with those reported by Shanti *et al.*[11] who showed that the spores of *Colletotrichum gloeosporioides* inoculated after injuring mangoes, pawpaw and rambutans fruits induced similar anthracnose lesions. The same, Giblin and Coates[12] showed that *Colletotrichum gloeosporioides* inoculated on avocado and mango fruits produced symptoms of anthracnose in significantly different degrees. Phoulivong *et al.*[26] showed that *Colletotrichum asianum*, *C. fructicola*, *C. siamense* and *C. simmondsii* produced almost identical lesion areas on guava, mango, pawpaw and apple fruits. On the other hand, these results are different from those reported by Lakshmi *et al.*[27] which found that isolates of *C. gloeosporioides* produced larger lesions on the leaves of the original host compared to the lesion areas developed on the leaves of alternative hosts. This difference could be due to the high sensitivity and the high-water content of fruits compared to the leaves. Other authors reported that larger lesions of *Colletotrichum gloeosporioides* isolates produced on their original host compared than the alternative host[14,28].

The BAIS2 isolates following inoculation on the different fruits caused lesions only on banana, which was its original host fruit. Bananas contain substances that could condition the development of BAIS2 isolates unlike other fruits. These results corroborate those of several authors who stated that some species of *Colletotrichum* develop only in a single host. This is the case of *C. kahawae*, which infects only coffee, *C. coccodes*, which grows only on tomatoes and potatoes, *C. falcatum* which attacks sugar cane and *C. musae* which infects banana[15,17,19,29,30]. Similar cross infection tests carried out on *Colletotrichum* spp. isolates from cashew, mango, papaya and passion fruit produced necrotic and depressed lesions on fruits, except on passion fruit, which was susceptible to its isolates only[31]. BAIS2 isolate produced large lesions than those developed by other isolates, suggesting that BAIS2 species could be the most aggressive on banana.

The lesions developed on the wounded fruits were larger than those developed on fruits that received a conidial suspension of the different isolates. Wounds created might have stressed the fruits, causing a gradual reduction in the elements involved in the fruit's defence mechanism.

CONCLUSION

Cross-infectivity of *Colletotrichum* isolates from fruits could produce anthracnose symptoms not only on original but also on non-original hosts. It also showed the existence of three groups within *Colletotrichum* isolates. In addition, wound inoculation method produced larger lesions than spraying inoculation method. These results suggest a molecular characterisation of *Colletotrichum* isolates for better identification and development of control strategies for the fungus.

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