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Colletotrichum species causing cassava (*Manihot esculenta* Crantz) anthracnose in different eco-zones within the Recôncavo Region of Bahia, Brazil

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Abstract

A survey to investigate the occurrence of cassava anthracnose disease (CAD) and distribution of *Colletotrichum* spp. in cassava plantations in different eco-zones of the Reconcavo Region in Bahia, Brazil, investigated during the rainy season of 2014. A total of 50 cassava fields distributed among 18 municipalities were visited and intensity of anthracnose evaluated. The highest disease incidence (DI) (83.3%) was in samples collected in São Félix, and the lowest (34.4%), in Varzedo. Municipalities that presented the highest values for DI were located within the '*Af*' Köppen–Geiger eco-zone, also presenting the highest values for the estimated McKinney disease index. Based on previous studies of multilocus phylogeny, seven different species of *Colletotrichum* were identified (*Colletotrichum fructicola*, *Colletotrichum tropicale*, *Colletotrichum gloeosporioides* s.s, *Colletotrichum theobromicola*, *Colletotrichum siamense*, *Colletotrichum brevisporum* and *Colletotrichum plurivorum*) and a new approach based on ERIC-PCR was used aiming to group the 82 isolates according to these findings. The highest percentage of genetic variance (> 78%) was among isolates within fields. Based on the survey and genetic analysis, *C. fructicola* is probably the main causal agent of cassava anthracnose in the Recôncavo Region, since this species was present with highest incidence in all eco-zones, 47.61, 42.86 and 57.14% for *Af* (tropical rainforest climate), *As* (tropical dry savanna climate) and *Aw* (tropical wet savanna climate), respectively. This study is the first report of *C. fructicola* lineages as the most likely pathogen causing anthracnose disease of cassava in Brazil, and these findings may be used to guide the selection of resistant varieties.

Keywords Disease severity · Disease survey · Species diversity · ERIC-PCR

Cassava is one of the most important food resources and major staple food for humans and animals worldwide (Suppakul et al. 2013). However, its production is strongly limited by many pests where cassava anthracnose disease (CAD), caused by *Colletotrichum* spp., is considered one of the most destructive cassava leaf diseases especially in main cassava producing countries worldwide (William et al. 2012; Sangpueak et al. 2018)

CAD is characterized by cankers on stems, branches, fruits, leaf spots, and diebacks on aerial parts of diseased plants (Kunkeaw et al. 2010; Silva et al. 2018). Despite the recent reports of different *Colletotrichum* species associated with cassava antracnose in Brazil (Bragança et al. 2016; Oliveira et al. 2016, 2018; Silva et al. 2019), little is known about its distribution, incidence, prevalence and disease severity across different production regions. Furthermore, there are no data regarding the relationship of CAD incidence and severity and agroecological practices and/or climatic zones in Brazilian conditions. From a practical point of view, this information could drive the adoption of disease management strategies prior to the occurrence of pandemic events decreasing the need of curative interventions (Plantegenest et al. 2007).

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The study of genetic diversity of plant pathogens and species to identify and characterize causal agents of diseases is a key point for selection of adequate isolates for screening of resistance in plant breeding programs (Ramdial and Rapersad 2015). More recently, Silva et al. (2019) demonstrated that enterobacterial repetitive intergenic consensus-polymerase chain reaction markers (ERIC-PCR) can be used as a complementary tool for discrimination of species within the *Colletotrichum gloeosporioides* complex associated with CAD in Brazil with a very high correlation with multilocus phylogenetic analysis.

The objective of the present work was to evaluate the occurrence of CAD in cassava plantations in the Recôncavo Region of the Bahia State by quantifying disease incidence and severity, as well as analyzing the genetic diversity of *Colletotrichum* spp. and putative species distribution over the different eco-zones and municipalities.

A survey was conducted by Silva et al. (2019) during the rainy season in 2014 (April–July), and diseased leaves and stems of cassava were collected in different production areas in the Recôncavo Region of the State of Bahia, Brazil. A total of 50 cassava fields distributed among 18

municipalities were visited and evaluated for the incidence and severity of anthracnose. The municipalities assayed are presented in Fig. 1.

Locations were chosen according to information of CAD outbreaks provided by the plant disease station at 'Embrapa Mandioca and Fruticultura', cassava growers and previous history of CAD incidence in experimental and/or commercial areas. These municipalities comprise of three different eco-zones according to the Köppen–Geiger climate classification system, being: *Af* = tropical rainforest climate, *As* = tropical dry savanna climate and *Aw* = tropical wet savanna climate.

Thirty plants were randomly selected and evaluated in each selected location by scouting the area using a Z-pattern layout in the field. Disease evaluation was based on the rate scale described by Muimba (1982).

Disease incidence was calculated based on the percentage of plants showing symptoms of disease. The scores according to the scale in the literature were then transformed into disease index (DI) based on McKinney (1923) using the following equation:

$$DI = \frac{\sum(\text{disease score} \times \text{number of plants with a certain disease score}) \times 100}{(\text{number plants evaluated per plot} \times \text{the highest score adopted in the scale})}$$

Fig. 1 Location and number of cassava fields surveyed in 18 municipalities located in the Recôncavo Region of the State of Bahia, Brazil. Blue-color shades relate to three different eco-zones based on the Köppen–Geiger climatic classification: *Af* = tropical rainforest climate, *As* = tropical dry savanna climate, *Aw* = tropical wet savanna climate. *ARA* Aratuípe, *CPA* Cabaceiras do Paraguaçú, *CAC* Chachoeira, *CAV* Castro Alves, *COF* Conceição da Feira, *CRZ* Cruz das Almas, *DMC* Dom Macêdo Costa, *GMA* Governador Mangabeira, *LAJ* Laje, *MFE* Muniz Ferreira, *MUR* Muritiba, *NAZ* Nazaré, *SAJ* Santo Antônio de Jesus, *SFE* São Félix, *SMM* São Miguel das Matas; *SAP* Sapeaçú and *VZO* Varzedo
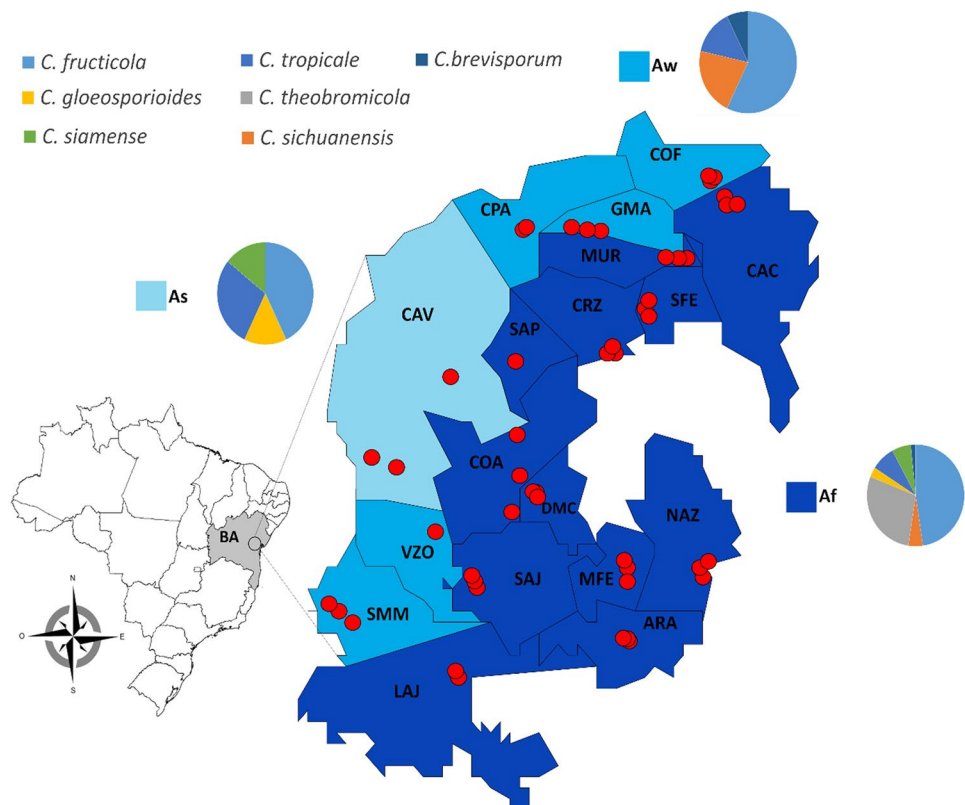
whereas 0 = no symptoms; 1 = development of shallow cankers on the stems and lower down on the plant; 2 = development of successive cankers higher up on the plant with the cankers on older stems becoming larger and deeper; 3 = development of dark-brown lesions on the green shoots, petioles and leaves, while the young shoots collapse and appear distorted; 4 = wilting, drying up of shoots and young leaves, and death of part or of the entire plant.

Monosporic isolates were obtained and ERIC-PCR amplifications followed the protocol described in Silva et al. (2019), based on the growth of the isolates in sucrose and yeast extract broth and DNA extraction using the CTAB method. Pathogenicity was confirmed based on the in vitro detached leaf assay method proposed by Kunkeaw et al. (2010) for pre-screening resistant genotypes.

Species diversity was estimated by measures of richness, diversity, and evenness for the ERI-PCR binary data and errors due to different sample sizes corrected according to Grünwald et al. (2003). The estimate of the species diversity in each of the locations was generated based on the H' (Shannon–Wiener) and G (Stoddart and Taylor) index. Confidence intervals for H' and G values were also calculated based on 1.000 permutations by resampling the frequency of the species using the 'vegan' and 'vegetarian' packages implemented in the R software (R Development Core 2018). Similarly, the genotypic evenness was estimated using the E_5 index (Grünwald et al. 2003).

Multiple correspondence analysis (MCA) was used to identify association of disease severity in each eco-zone using the 'FactoMineR' package in the R software (R Development Core 2018).

A total of 82 *Colletotrichum* spp. *gloeosporioides* sensu lato isolates within 18 municipalities were identified by Silva et al. (2019) associated with anthracnose symptoms of cassava. The number of isolates per municipality ranged from two to 24 for 'Muritiba' and 'Nazaré', respectively. The municipalities surveyed, geographical location of each collection site and the three different climatic zones are presented in Fig. 1.

Anthracnose disease was found in all the different municipalities and eco-zones. The highest disease incidence (83.3%) was found for samples from 'São Félix' and the lowest (34.4%) from 'Varzedo' (Fig. 2a). Differences were also found for the climatic zones where the lowest incidence was noticed for the municipalities within the driest eco-zone ('As') according to the Köppen–Geiger classification (Fig. 2a).

Regarding disease index obtained from the transformation of the disease scores, there was a large difference among properties within the municipalities with index values varying from 12.5 to 80.0% of severity (Fig. 2b). For the comparison of the disease index between eco-zones

the same behavior was noticed, being the lowest severity value associated with the driest ('As') eco-zone (11.7%).

The MCA analysis confirmed the positive association between three severity class defined as "low" (ID < 30%); "medium" (30% ≤ ID ≤ 70%) and "high" (ID > 70%); and the eco-zones, and a moderate association between these three variables (severity; incidence and eco-zones) (Fig. 3). There was no association among the other factors.

Despite the differences in sample size for the municipalities and eco-zones, the species diversity was quite similar for the entire region, since the number expected for multilocus genotype (eMLG's, or putative species) was almost the same when scaling the population size based on the lowest number of isolates ($n = 7$). No significance was found for the values obtained using the Shannon (H') and Stoddart and Taylor (G) indices where the same richness was expected, as well as the same evenness for species distribution in the different eco-zones (Table 1).

The severity of anthracnose in cassava plantations has been evaluated in some parts of the world where the crop plays a key role in sustainability, feed and nourishment (Morgan and Choct 2016; Zippora et al. 2016). Wydra and Verdier (2002) evaluated cassava anthracnose disease severity and incidence in planting material in Benin and Ghana, and the authors concluded that there was a positive correlation between the CAD severity and more humid eco-zones, which is in agreement with our findings. However, the lack of association between intercropping and mixture of cassava genotypes with the reduction in disease severity, is in contrast with data reported by Wydra and Verdier (2002), where the presence of plant diversity within the field seemed to negatively influence the development of CAD.

The municipalities that had the highest values for disease incidence were located within the 'Af' eco-zone, which also presented the highest values for disease severity, considering the calculated disease index. This could be due to the fact that a more humid climate favors the spread and incidence of the disease. Despite this association between humidity and high incidence, there were some municipalities within humid eco-zones with low disease incidence/severity, and the most likely explanation for these differences could be related to inoculum availability and conidia dispersion, since plant variety, plant age or agronomic practices, seem to not affect the disease in the conditions of the survey.

Our results show that disease incidence and severity are higher in more humid locations rather than dry ones, which is in agreement with Onyeka et al. (2008). The 'As' Köppen–Geiger eco-zone is less conducive to cassava anthracnose disease in comparison to the other two eco-zones studied. *C. fructicola* was the most frequent putative species found among the municipalities and eco-zones. This pathogen should therefore, be considered in the control strategies as shown in many related pathosystems whose
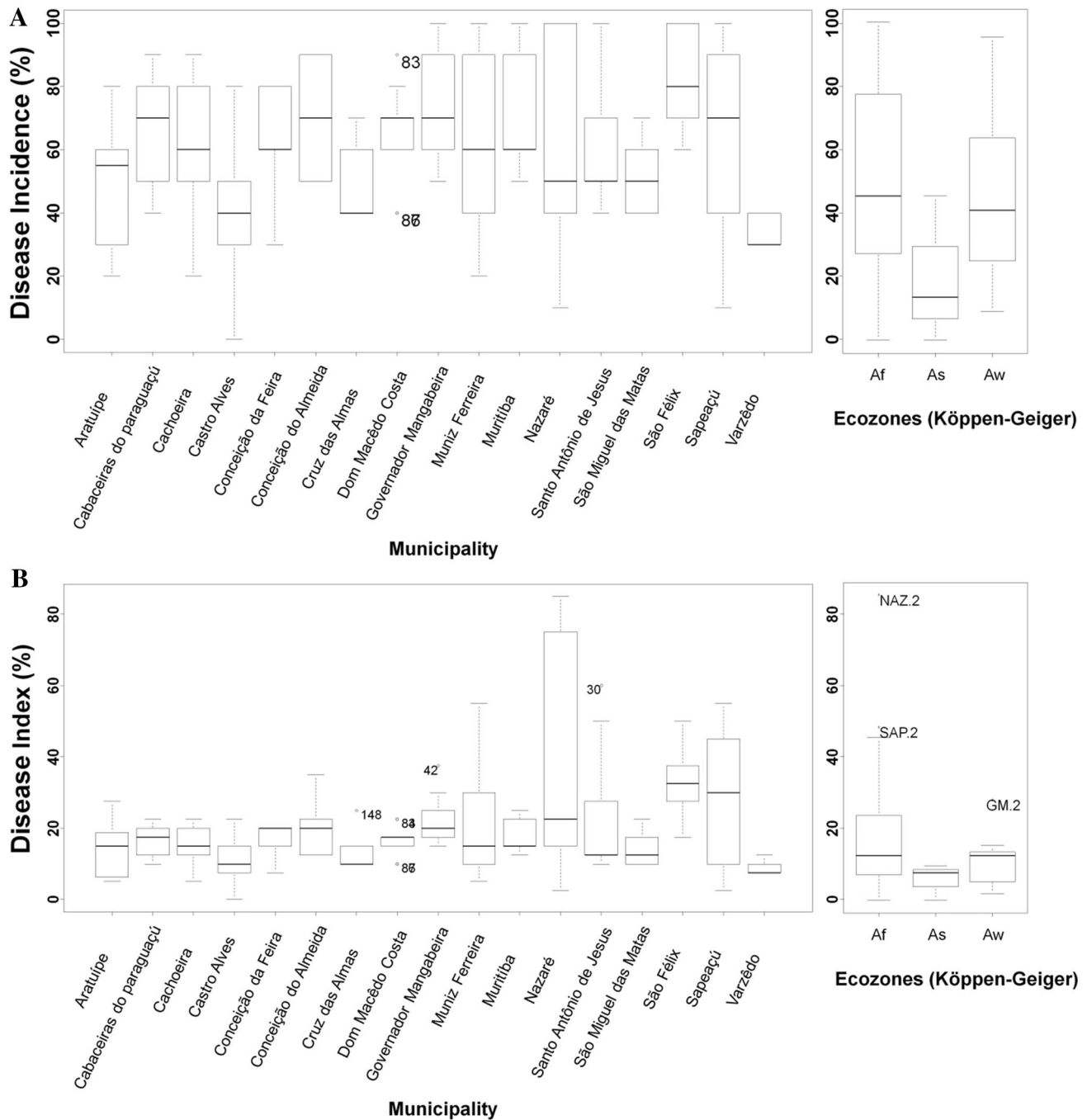
Fig. 2 Box plot analysis of disease incidence (**a**) and disease index (**b**) for the 18 municipalities surveyed for cassava anthracnose disease (CAD) according to reported outbreaks. Each box plot displays the observations between the first and the third quartile in the rectan-gle, with a line for the median. Circles together with number and/or names represent outliers beyond the end of the whiskers. Eco-zones (Köppen–Geiger): *Af* = Tropical rainforest climate, *As* = tropical dry savanna climate, *Aw* = tropical wet savanna climate

previous etiology was attributed to *C. gloeosporioides* since different species may have different behavior as to patho-genicity aspects, such as fungicide sensitivity and responses to environmental conditions (Xu et al. 2014).

Management of anthracnose in cassava is a relevant prob-lem mainly due to diversity of pathogenicity, virulence, aggressiveness and the different species that make up the *Colletotrichum* sp. complex. Knowledge of the diversity and different species of pathogens are key to the decision-making process when it comes to control measures (Talinhas et al. 2015) once different species respond differently to the use of fungicides (Ramdial and Rapersad 2015).

Fig. 3 Multiple correspondence analysis (MCA) for association of the qualitative data for incidence and severity classes (low, medium or high) of cassava anthracnose disease; Köppen–Geiger climate classification (*Af*, *As* or *Aw*), agroecosystem characteristics (Monoculture or Intercropping); Previous Crops (monocot or dicot) and presence of previous Crop Rotation
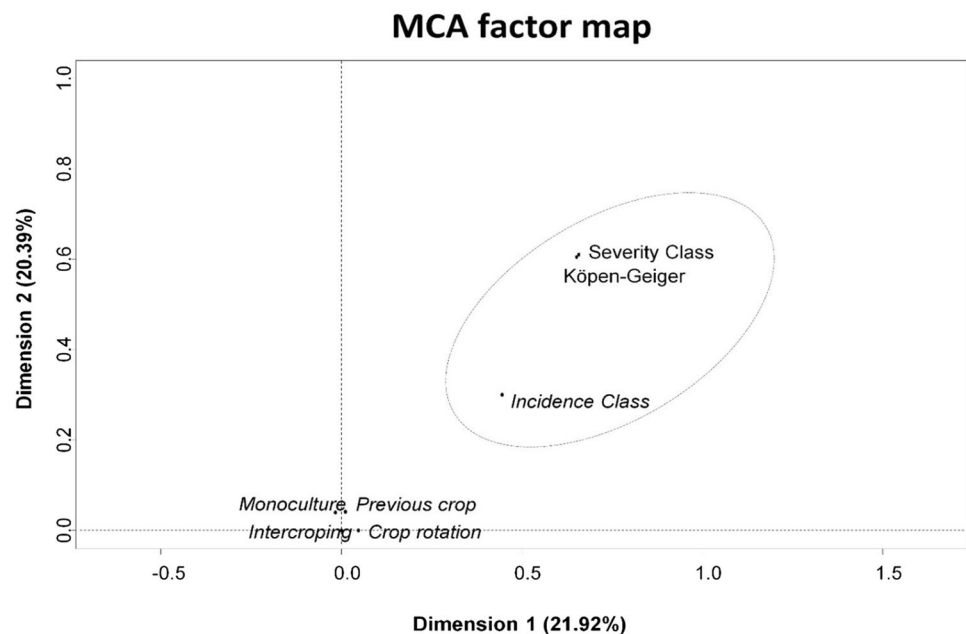

Table 1 Species diversity, richness and evenness of *Colletotrichum* spp. populations in different Köppen–Geiger eco-zones

[a]Eco-zone	N	MLG	[b]eMLG[7]	H'	G	E_5
Af	62	7	3.37	1.41	3.10	0.68
Aw	13	4	3.17	1.17	2.51	0.67
As	7	4	4.00	1.28	3.27	0.87
Total	82	7	3.55	1.48	3.27	0.67

[a]*Af*=tropical rainforest climate, *As*=tropical dry savanna climate, *Aw*=tropical wet savanna climate. *N*: total of isolates obtained for the hierarchical unity studied, *MLG*: multilocus groups richness (syn=phylogenetic lineages)

[b]eMLG: number of expected multilocus groups (MLGs) for the population, when scaled using rarefaction curves, based on the smallest population size ($n=7$); *H'*: Shannon index of diversity; *G*: Stoddart and Taylor index of diversity; *E₅*: evenness index estimated using the expression: $E_5 = \frac{(1/\lambda)-1}{e^{H'}-1}$, as described by Grünwald et al. (2003)

Compliance with ethical standards

Conflict of interest The authors confirm that the manuscript has been prepared in accordance to the COPE ethical guidelines and there is no conflict of interest.

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