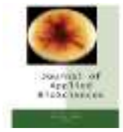

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Morphological characterization of the causal agent of mango red rust in two production basins (Noun and Lékié) in Cameroon

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ABSTRACT

Objective: In Cameroon, mango fruit is of vital importance as it is essential for food, health, nutritional and economic security of many villages and small cities. Unfortunately, its yield is reduced by numerous diseases such as red rust, for which there is no appropriate control method yet. The aim of this study was to contribute to the control of mango red rust through morphological characterization of the causal agent in two production basins.

Methodology and Results: The state of the disease and its symptoms were assessed at the two study sites (Noun and Lékié). The pathogen was characterized morphologically, the incidence and severity assessed and finally a pathogenicity test was carried out on the laboratory. The results show that the incidence was 100% in both sites, while the severity ranged from 58.23 % to 10.75% in Noun and Lekié respectively. The development of red rust on mango was therefore significantly different at the two sites Typical rust symptoms are characterized by rusty brown-orange spots of circular shape, with leaves showing an average of 65 lesions with an average diameter of 2.32 mm in Noun, while in Lekié there was an average of 87 lesions with an average diameter of 2.43 mm. Microscopic observation showed that the sporangiophores were single or grouped, septate (with an average of 6 partitions). At the Noun site, observation showed an average of 4 sporangiophores per thallus with an average size of 325 µm/19.3 µm and each sporangiophores produced an average of 3 sporangia with an average size of 32.15 µm/22.15 µm. For Lekié, microscopic observations showed an average of 3 sporangiophores per thallus with an average size of 253.55µm/16.05µm and each sporangiophore produced an average of 3 sporangia with an average size of

26.45µm/15.35µm. The pathogenicity test carried out showed no characteristic symptoms of red rust on the leaves inoculated with the algal suspensions.
Conclusion and application of results: The symptomatological study, microscopic observation and study of the micro-morphological characteristics of the strains associated with the red rust symptoms enabled us to identify the *Cephaleurus virescens* species (which is the highest among the different species of the *Cephaleurus genus*) as responsible for the disease in the two study sites.
Key words: Algae; *Cephaleurus virescens;* Red rust; sporangium; sporangiophore.

INTRODUCTION

Mango is the seventh most cultivated fruit in the world. In 2018, global mango production was 55.4 million tonnes, dominated by India with 39% (22 million tonnes) of the total world production. China and Thailand followed. After Asia, Africa is the world's second largest mango fruit producer with a production of 3.6 million tonnes (FAO, 2019). Appreciated for its gustatory qualities, the mango fruit is an interesting food resource with recognised energy, aromatic and nutritional qualities (Provitamin A, Vitamin C and Potassium) (Brustel, 2018). Because of their nutritional properties in terms of quantity and quality, mango fruits are consumed in various forms (fresh, dried, fruit processed into juice, syrups, jams or incorporated into pastries). The fruit, particularly when depreciated, can be used as animal feed (De Laroussilhe, 1980). In addition to the nutritional role of the fruit, other parts of the mango plant have medicinal properties or other uses. For example, the seed powder is recommended for treating diarrhoea and haemorrhoids. The flowers are used to heal the heart and combat worms and dysentery, while the leaves are diuretic and are used to treat fever (De Laroussilhe, 1980). In addition, the high tannin content of the leaves means they can be used to treat sore throats, mouth and teeth, asthma, gonorrhoea, dysentery and bronchitis. The stem bark, which also has many medicinal properties, is used to treat a number of illnesses (dysentery, gonorrhoea, rheumatism, haemorrhoids, etc.) (De Laroussilhe, 1980). Cameroon has exceptional potential in terms of diversity of local mango variety. This is undoubtedly, why the government has decided to become an exporter of this fruit to the European market by 2025 (Mballa, 2015). Local demand is constantly increasing as well as the sub-regional markets (Gabon, Equatorial Guinea and Congo). Moreover, in many villages and secondary cities in the regions of Cameroon, the fruit gives an essential contribution to the food, health, nutritional and economic security. Unfortunately, mango trees in Cameroon are threatened by numerous biotic and abiotic constraints that lead to significant yield losses. Among the biotic constraints, diseases and pests are the most damaging and compromising yield quantity and quality. Numerous diseases such as anthracnose, downy mildew and dieback attack many fruit species, including mango trees. Their impact is low thanks to the existence of appropriate control methods. However, certain emerging diseases such as red rust do not yet have appropriate registered control methods. Many species of the *Cephaleurus* genus have been associated with red rust in many countries, notably *Cephaleurus virescens* on mango leaves (Ponmuringan *et al.,* 2010). The alga of the genus *Cephaleurus* has a better reputation in the scientific community, because it is widely distributed in tropical and subtropical regions throughout the world (Gokhale and Shaikh, 2012). The damage caused by *Cephaleurus* on the reduction of the photosynthetic surface of leaves, which can represent an economic loss, particularly during periods of high humidity and temperature (Malagi *et al.,* 2011; Piccinin *et al.,* 2005). In this context, the knowledge of plant diseases is important when considering management measures and increasing productivity. Consequently, correct identification of the pathogen is necessary as the first step towards further control of the disease. In Cameroon, no study has been carried out on the characterization of the causal agent of mango red rust although Asta (2019) and Ngoh *et al.* (2021) reported the presence of red rust on cashew. Consequently, this study was designed with the general objective of contributing to the control of mango red rust through characterization of the causal agent in the two major mango production basins in Cameroon.

MATERIAL AND METHODS

Presentation of the study sites: This study was carried out in the Noun and Lékié Divisions, two of Cameroon's major mango production basins. The Noun Division is located in the agroecological zone of the High Plateaux. The geographical coordinates are 5°18'15"Latitude North and 10°35'04" Longitude East. The Lékié Division is located in the humid forest zone with bimodal rainfall. The geographical coordinates are 4°12'0" Latitude North and 11°24'0" Longitude East. Laboratory experiments were carried out at the Research Unit of Phytopathology and Agricultural Zoology (UR_PHYZA) of the Faculty of Agronomy and Agricultural Sciences (FASA) at the University of Dschang, Cameroon.

Collection of samples of leaves showing red rust symptoms: In each study site, two orchards were selected and in each orchard, a quadrat of 10 mango trees lengthwise by 10-mango trees width wise was delimited. The disease parameters (incidence and severity) of red rust were assessed on 10 trees along the diagonal. A total of 40 trees were surveyed.

Assessment of incidence: The impact was assessed using the formula of Masyahit *et al.* (2009):

$$I\ (\%) = Xi / Xt \times 100$$

I: incidence; Xi: number of infected trees per locality; Xt: total number of trees sampled.

Severity assessment: The severity of the disease on infected plants in the field was assessed by observation of mango plants using the scale adapted from Masood et al (2010):

- 0 = no symptoms;
- 1= 25%:] 0- 1/4] of the foot has symptoms;
- 2 = 50%:] 1/4-2/4] of the foot has symptoms;
- 3=75 %] 2/4-3/4] of the foot has symptoms;
- 4=100 %] 3/4 -4/4] of the foot has symptoms.

Sampling of infects leaves: Sampling was carried out on trees bearing leaves showing typical symptoms of red rust. In order to better observe the spatial distribution of the disease in the study sites, 10 samples of infected leaves were taken from each tree chosen along the diagonal of each of the quadrats in each site. A total of 400 leaves were used. The samples were collected early in the morning and packed in plastic bags containing cotton soaked in distilled water before being transported to the laboratory. For each sample, details such as the date of collection and the name of the locality visited were noted.

Assessment of the number of lesions and lesion diameter: The number of red rust lesions was assessed by counting the leaves sampled at each site. The diameter of the lesions was assessed using a littlegrasseu analogue caliper. For each leaf sampled at each study site. The mean and coefficient of variation were calculated using the following formulae:

$$\mu = \Sigma\ Xi / N$$

with μ = mean
Xi = number of lesions per leaf
N = total Numbers of leaves

$$\sigma = \sqrt{\Sigma\ (Xi-\mu)^2/N}$$ σ: standard deviation

$$CV = \sqrt{\sigma}$$ CV: coefficient of variation

Characterization of the pathogen: Characterization was carried out using samples of diseased leaves from the two study areas. Red rust spots were scraped off with a needle and placed on a slide containing a drop of sterile distilled water, then covered with a coverslip. The whole slide was observed on an OMAX optical microscope with a micrometre. For each locality, an average of 10 measurements of the structures of each parameter was taken, namely the number of sporangiophores, the length and width of sporangiophores, the number of sporangia, the length and width of sporangia, and the number of partitions.

Pathogen culture and pathogenicity test: A direct method was used to culture the pathogen, which involved isolating strains of the pathogen from small fragments of leaves showing symptoms of red rust. Brought back to the laboratory, these fragments were disinfected with 5% sodium hypochlorite (bleach) for 5 minutes, and rinsed three times with sterile distilled water for 5, 10 and 15 minutes respectively to eliminate traces of the disinfectant, and dried for 2 minutes. Using a scalpel, the infected parts of the leaves were removed and then placed in Petri dishes containing the different media in a microbiological hood near a Bunsen burner flame. The Petri dishes were closed, sealed with cling film and then incubated at a temperature of 18-20°C. Seven days after inoculation, the fungal colonies visible around the inoculated leaf portions were identified under the microscope. Various culture media were prepared to purify the pathogen, including PDA (potato dextrose agar) medium, agar water (AG) medium and mango leaf-based aqueous medium. Algae spots were scraped from a petri dish containing distilled water and drops of inoculum were placed on the upper surface of each leaf. The dishes were then labelled (name of isolate and date of inoculation). The leaves were incubated for 7 days at room temperature under a photoperiod of 12/12.

Data analysis: The data collected on the incidence, severity, symptomatology and microscopic characteristics of the pathogen were entered into Microsoft Excel 2013 and then subjected to analysis of variance (ANOVA). Means were separated using Duncan's test at the 5% probability threshold. SPSS (Statistical Package for Social Science) version 22.0 was used for this purpose.

RESULTS

Incidence and severity: Incidence was 100% in both orchards at both sites, so there was no significant difference in incidence between the two sites. Despite the very high incidence in both study sites, the severity remained low regardless of the site, although it was much higher in the Noun (58.23%) than in the Lekié (10.75%). The evolution of red rust on mango was significantly different in the two sites according to Duncan's test at the 5% probability threshold.

Table 1: Incidence and severity in the Noun and Lekié sites.

	Incidence (%)	**Degrees of severity (%)**
Lekié	100 a	10,759 b
Noun	100 a	58,233 a

Numbers followed by the same letter are not significantly different according to Duncan's test at the 5 % threshold.

Symptomology: Symptoms of red rust were observed on leaf surfaces at both sites. At the Noun site, symptomatic leaves showed numerous circular lesions ranging from 50 to 100 with an average of 64.9. The coefficient of variation was 15.96% and lesion diameters on the leaves ranged from 1.12 to 3.57 mm with an average of 2.32 mm. The coefficient of variation for lesions diameters was 25.94%. At the Lekié site, the number of lesions per leaf ranged from 40 to 190, with an average of 86.89 and a coefficient of variation of 22.85%. Lesion diameters in Lekié ranged from 1.17 to 2.9 mm, with an average of 2.43 mm and a coefficient of variation of 12.91%. Leaf spots were rusty orange-brown in colour and generally appeared on the upper surface of the leaves (Figure 1a), although a few samples showed spots on the lower surface at the Lekié site (Figure 1c). Coalescing lesions occurred in the vein (figure 1b). Infected leaves were mainly found in the basal part of attacked plants and generally on old leaves.

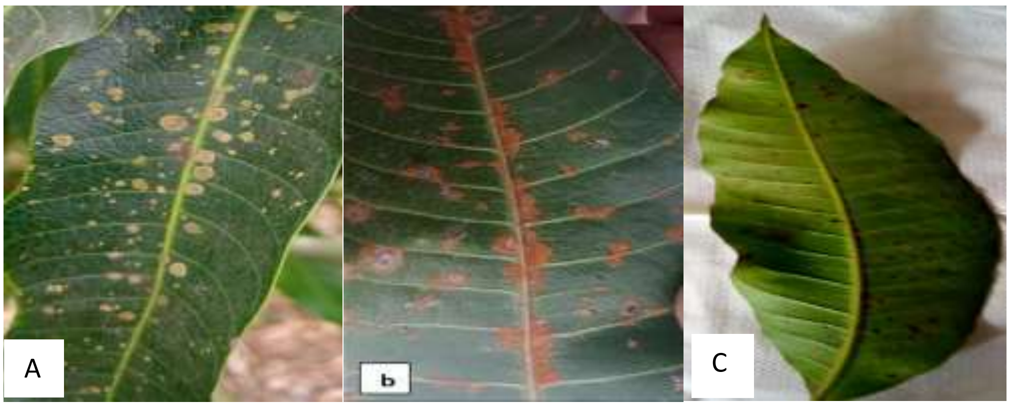

Figure 1: Symptoms of red rust on mango leaves;
A: Symptoms on the upper surface; B: Symptoms on the midrib; C: Symptoms on the lower surface.

Statistical analysis of the values showed no significant difference in lesion diameter between the Noun and Lekié sites. On the other hand, there was a significant difference between the two sites erms of the number of lesions (Table 2).

Table 2: Symptomatology characteristics of the thallus structures of the pathogen responsible for red rust on mango in the Noun and Lekié sites

	Average number of lesions	**Coefficient of variation (%)**	**Average lesion diameter (mm)**	**Coefficient of variation (%)**
LEKIE	86,89±22,85 a	22,85	2,43±0,31 a	12,91
NOUN	64,9±10,36 b	15,96	2,32±0,6 a	25,94

Numbers followed by the same letter are not significantly different according to Duncan's test at the 5% threshold.

Characteristics of the identified pathogen: Microscopic observations showed that the sporangiophores were single (Figure 2a) or grouped (Figure 2b). Each (more than one septa) (Figure 2c) and sporangia attached by a suspensor cell (Figure 2d). The sporangia appeared to form a crown at the head of the sporangiophores (Figure 2d). The sporangium (Figure 2e) was fixed by a cell suspensor that contained zoospores inside that could be released after rupture of the sporangium membrane. The characteristics described show that the parasitic alga is *Cephaleurus virescens* (kenze) (Vasconcelos *et al.*, 2019).

Figure 2: Microscopic characteristics of *Cephaleurus virescens*

After measuring the structure of the algae, the results show that in Noun the average number of sporangiophores produced per thallus was 4 with a coefficient of variation of 45.32 %. In Lekié, the average number of sporangiophores produced per thallus was 3 with a coefficient of variation of 57.14 %. The length of the sporangiophores varied from 255 to 375µm with an average of 325µm and a coefficient of variation of 13.19 %. The width varied between 15 and 25µm with an average of 19.3µm and a coefficient of variation of 25.40 % in the Noun site, while the length of the sporangiophores varied between 200 and 300µm with an average of 253.55µm and a coefficient of variation of 11.75 %. The width ranged from 10 to 20 µm with an average of 16.05 µm and a coefficient of variation of 16.50 % at the Lekié site. The results of microscopic observation also showed that the average number of sporangia produced by each sporangiophore was 3 at both sites, but with a coefficient of variation of 69.17 % at Noun and 36.19 % at Lekié. Measurements (length and width) of sporangia gave a length varying between 15 and 45µm with an average of 32.15µm and a coefficient of variation of 20.95 %; a width varying between 15 and 25µm with an average width of 22.15µm and a coefficient of variation of 18.43 % in the Noun site. In the Lekié site, the length of the sporangia was between 20 and 35µm with an average of 26.45µm and a coefficient of variation of 16.99 %, their width varied between 10 and 20µm with an average of 15.35 and a coefficient of variation of 16.61 %. For the number of septa, the average was 6 with a variation coefficient of 19.04 % in Noun. On the other hand, at the Lekié site, the average number of septa per sporangiophore was 4, with a variation coefficient of 14.29 %. According to Duncan's test at the 5% probability threshold, there was no significant difference between the Noun and Lekié sites as concerns the number of sporangiophores, the width of sporangiophores, and the number of sporangia and the length of sporangia. On the other hand, there was at least one significant difference between the two sites for the length of sporangiophores, the width of sporangia and the number of partitions (Table 3).

Table 3: Comparison of morphological parameters of *Cephaleurus virescens* thallus structures in the two study areas

	Number Sporangiophores	Length sporangiophores	Width sporangiophores	Number of sporangia	Length of sporangia	Width Sporangia	Number of partitions
Noun	4.14±1.87a	325.00±8.57a	19.28±0.97a	2.71±1.87a	32.14±1.34a	22.14±0.81a	5.571±1.06a
Lekie	3.00±1.71a	253.57±5.95b	16.07±0.53a	2.143±0.77a	26.43±0.89a	15.35±0.51b	4.00±0.57b

The numbers followed by the same letter in the columns are not significantly different according to Duncan's test at the 5% threshold.

Pathogenicity test: After seeding leaves showing symptoms of red rust disease on the various culture media and incubating them at room temperature for 7 days, no development of the pathogen *(Cephaleurus virescens*) was observed. The results of the pathogenicity test carried out on detached healthy leaves enabled us to discover that the pathogen cannot be transmitted by simple contact, as the leaves inoculated with sporangial suspensions did not cause any contamination.

DISCUSSION

The aim of this study was to contribute to the control of mango rust by characterizing the pathogen in two mango production basins in Cameroon. According to the results of our study, the evaluation of the mango disease in the main production sites in Cameroon, i.e. the Noun and Lekié Divisions, revealed red rust disease, the pathogen of which is *Cephaleurus virescens*. Although the incidence is very high at these sites, the severity of the disease remains very low. This study was carried out in April, a period during which rainfall is not abundant, which could be the reason why the severity obtained was low. This low severity is contrary to the results obtained by Vasconcelos *et al.* (2019) who identified red rust on mango in Brazil, as well as the results obtained by Majune *et al.* (2018); Afouda *et al.* (2013); Khatoon *et al.* (2017) and Wonni *et al.* (2013) who all identified red rust on cashew. According to the works of Malagi *et al.* (2011), the presence of algal blotch would be favoured by average monthly temperatures around 23°C and average monthly rainfall of 127mm. During the months of July and August, rainfall is very high, which would be ideal for the membrane envelop of the sporangia to break down which will facilitate the dispersal of zoospores by the wind (Duarte *et al.*, 2005). This is corroborated by the works of Muthukumar, Uma and Priyadharsini (2014) who report the presence of algae and lesions restricted to the rainy season and high temperatures in several plant species, suggesting that optimal environmental conditions may be critical for disease development in some susceptible hosts. However, our study was carried out during the month of April, a period during which rainfall is very scanty and consequently does not facilitate rupture of the sporangial membrane and hence the absence of dispersal of zoospores, hence this very low severity. The severity obtained in the Noun was 58.23, close to the results obtained by Amawissa *et al.* (2021) on *Theobroma cacao* in Cameroon, whereas it was 10.76 in the Lekié, a result close to those obtained by Ngoh *et al.* (2021) on *Anacardium occidentale* in Cameroon. There was therefore a significant difference in severity between the Noun and Lekié sites, and this was probably due to the very advanced age of the orchards studied in the Noun where, over the years the disease has spread to almost all the mango trees. The incidence obtained in these studies was 100% in both sites. Similar results were found by Ghini *et al.* (2011) who obtained an incidence of 73 to 90% on cashew in Benin. This study also revealed that the pathogen mainly attacks older leaves. The results also showed that disease incidence and symptom severity are influenced by orchard microclimates. Attacked leaves showed an average of 65 lesions in the Noun, which may be considered a low value compared with *Mycosphaerella citri*, which showed an average of 131 lesions per leaf (Silva *et al.*, 2008). On the other hand, in the Lekié, attacked leaves showed an average of 87 lesions, which corroborates the results obtained by Ngoh *et al.* (2021) who obtained an average of 100 lesions on cashew nuts in 2021. According to Keller *et al.* (2000), the number of lesions is an important fact because it is a variable strongly correlated with severity. Duncan's test at the 5% probability threshold showed that there is a significant difference between the two sites in the number of lesions, and this was due to the microclimate or the age of the orchards. In both sites, a high percentage of lesions (83% for the Noun and 100% for the Lekié sites) had a diameter of less than 3 mm, demonstrating a greater capacity of the pathogen to reach different points of the leaf blade. Similar results were obtained by Ngoh *et al.* (2021), who obtained 98% of lesions with diameters less than 3 mm. In both sites, there was little variation between the sizes, (coefficient of variation of 25.90% in the Noun and 12.91% in the Lekié) which suggests a typical symmetry of the algal spots. On the basis of micro-morphological characteristics, it was found that the measurements obtained for algal sporangiophores and sporangia (255.0-375.0µm × 15.0-25.0µm) and (25.0-45.0µm ×15.0-25.0µm) in the Noun ; (200.0-300.0µm × 15.0-20.0µm) and (20.0-35.0 × 12.5-20.0) in the Lekié found on mango trees were close to those obtained for *Cephaleurus virescens* identified by Pereira *et al.* (2020) on *Swietenia* sp, Magali *et al.* (2011) on *Ficus benghalensis* and Han *et al.* (2011) on *Citrus sinensis*. However, these measurements are different, mainly about the length of *Cephaleurus parasiticus* sporangiophores, obtained by Ponmurugan *et al.* (2010) on *Camellia sinensis* (800 to 1256 µm). Although *Cephaleurus virescens* and *Cephaleurus parasiticus* have similarities in shape and coloration, the sporangiophores of *Cephaleurus parasiticus* are larger in length and width than *Cephaleurus virescens* (Ponmurugan *et al.*, 2010). It is important to mention that the measurements of sporangia obtained in this work (20.0-45.0µm × 12.5-25.5µm) were larger those of *C. parasiticus* sporangia found in *Camellia sinensis* (17.4-27.5µm × 17.4-20µm) by Ponmurugan *et al.* (2010). Nevertheless, it can be argued that sporangiophores size is a more reliable feature to distinguish the two species, as Suto *et al.* (2014) stated that sporangia size is the same for *C. virescens* and *C. parasiticus.* Several authors have reported the occurrence of the genus *Cephaleurus*. However, *C. virescens and C. parasiticus* are the most common species worldwide. In several morphological characterization studies, comparisons have been made between the morphological characteristics of the species, *C. virescens*, *C. parasiticus and C. diffusus*, being species of greater morphological similarities (Suto *et al.*, 2014; Pitaloka *et al.*, 2015). Although there are similarities between *C. virescens* and *C. diffusus*, particularly in the coloration of the sporangiophores and the size of the sporangia (length and width), the main distinguishing features between the two species is the length of the sporangia and the width of the sporangiophores, which are both smaller in *C. diffusus* than in *Cephaleurus virescens.* In addition, *C. diffusus* bears a greater resemblance to *C. parasiticus*, to the extent that they can only be distinguished by morphology (Thomas *et al.*, 2016). The other species of the genus Cephaleurus less present, but which has some similarity with the species *Cephaleurus virescens is C. biolophus* (Suto and Othani, 2009). More broadly, a distinctive feature that separates *C. virescens* from *C. biolophus* is the termination of the main cell, which is only terminal in *C. virescens*, whereas in *C. biolophus* it is terminal and lateral. (Suto and Othani, 2009). Duncan's 5% test showed that there were significant differences between the production sites in sporangiophores length, sporangia width and number of septum. This could be due to the age difference between the orchards at the two sites, as these parameters are all higher at the Noun site where the orchards were much older. These differences could also be due to the microclimate of the orchards. Direct isolation of the pathogen from samples from both sites in the different agar culture media (PDA medium, agar water medium and mango leaf aqueous extract medium) did not yield any results. This is in line with Vasconcelos et al (2018), Ren et al (2013) and Ponmurugan et al (2010) who showed that solid agar media such as PDA do not allow the parasitic alga *C. virescens* to grow because this medium is rich in sugar and starch and therefore insufficient for their growth. Culture may only be possible after isolation in liquid media due to the progressive adaptation of algae to artificial growth media, first liquid then solid, and/or due to abundant filamentous cell growth, which could facilitate transfer of the micro-organism to solid culture (Vasconcelos *et al.*, 2018). This result can be explained by the pathogenicity test carried out, which gave no results. The absence of mycelium in the solid culture media used meant that the healthy samples could not be reinfected.

CONCLUSION AND APPLICATION OF RESULTS

At the end of this study, which was initiated with the aim of contributing to the control of mango red rust through the characterization of the causal agent in two agro-ecological zones of the western Highlands of Cameroon. It emerged that the symptomatological study of mango trees at the various sites showed the presence of characteristic red rust symptoms. These symptoms are characterized by rusty brown-orange spots of circular shape. The results showed that red rust was present on mango leaves at both study sites. Disease incidence was 100% in both orchards at both sites. Symptom severity was significantly different at the two sites (58.23% and 10.75% respectively in the Noun and Lekié). The evolution of red rust on mango was therefore significantly different at the two sites. The results also showed that the severity of the disease is influenced by microclimates and the age of the orchards. Microscopic observation and study of the micro-morphological characteristics of the strains associated with red rust symptoms made it possible to identify the *Cephaleurus virescens* species (which is the highest among the different species of the Cephaleurus genus) in the two study sites. The pathogenicity test carried out showed no characteristic symptoms of red rust on leaves inoculated with *Cephaleurus virescens*. This observation shows that aggressiveness does not depend on locality, nor on environmental factors, but on characteristics specific to this species. Characterization and knowledge of the pathogen responsible for rust in the two production basins will enable us to envisage environmentally-friendly control methods that will boost production in these basins.

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