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RED RUST DISEASE OCCURRING IN SOME FRUITS SPECIES IN CAMEROON

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Abstract

The knowledge of the red rust disease remains limited in Cameroon, with a view to developing a control method. This work consisted in studying red rust on some fruit species such as *Annona muricata* (soursop), *Dacryodes edulis* (safou), *Psidium guajava* (guava) and *Theobroma cacao* (cocoa).

Diseased leaves were collected in the field in the Maham site, in west region of Cameroon.The symptomatology of disease (colour, number and diameter of lesions) was studied. Coefficient of variation (%) was calculated. The incidence and severity of the disease was assessed in the different orchards surveyed. The measurement of the different structures of the thallus (length, width of sporangia and sporangiophores) was carried out using a microscope with a micrometer.

The disease is characterized by circular orange to orange-brown spots on the upper surfaces and rarely on the lower surface. Number of lesions, varied from 245–510 respectively with *D. edulis* and *T cacao.* Lesion diameters varied from 0.1–1 cm, 0.1–7 cm, 0.1–1.5 cm in safou (African pear), guava and soursop respectively. The length and width of sporangiophores varied from 280.5–714×10.2–25.5 µm for *A. muricata*, 408–612×15.3–25.5 µm for *Dacryodes edulis*, 433.3–663×15.3–20.4 µm for *P. guajava* and 484.5–612×20.4–35.7 µm for *T. cacao.* The number of sporangiophores varied from 1 to 11 at the maximum threshold. But, number of sporangia was the same in the four species, 1–9. The pathogenicity test was negative.

The data measurements show that the specie observed is *Cephaleurus virescens* which is a parasitic alga.

The data obtained are a basis for the development of an integrated control strategy against this emerging disease.

Keywords: red rust, *Cephaleurus virescens*, algae, characteristics, sporangiosphore, sporangia, *Annona muricata*, *Dacryodes edulis*, *Psidium guajava* and *Theobroma cacao*.

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1. Introduction

In Africa, fruit species provide local people with food and medicinal products to meet their primary needs. Their use is most increased in rural areas where many people depend on them for their livelihood needs [1]. In addition, woody plant products provide substantial income for many households [2]. Indeed, in addition to wood that is exploited to cover energy and construction needs in rural areas [2], the fruit and leaves are well traded in local, national, regional and international markets [3] and are essential natural resources for human nutrition and care [4–6].

In some regions of Cameroon, agro-ecological conditions allow for abundant fruit production and almost permanent availability of fruit at all times of the year. These agro-ecological conditions are favourable to the cultivation of several fruit species such as mango, guava, soursop, cocoa and safoutier [7].

But unfortunately, several constraints hinder the development of these fruit species. The most important are the control of good cultivation practices and diseases and pests [8]. These cause considerable economic losses (20 to 100 %) due to the adverse effects on both quality and quantity. These constraints have led to the abandonment of fruit orchards.

Many diseases such as anthracnose, downy mildew, die-back attack many fruit species. Their impact is more or less low thanks to the existence of appropriate control methods. However, some emerging diseases such as red rust do not yet have an approved control method. Many *Cephaleurus* species have been associated with red rust in many countries and on several species: *Cephaleurus virescens* on *Mangifera indica* [9] in Brazil, *Cephaleurus virescens* on *Swietenia* sp. [10] in Brazil, *Cephaleurus parasiticus* on *Camellia sinensis* [11].

In Cameroon, studies have shown the presence of red rust due to *C. virescens* only in cashew (*Anacardium occidentale*) and mango (*Mangifera indica*) [12, 13]. However, the diversity of the genus *Cephaleurus* is not yet established in Cameroon. This study therefore aims to establish the presence of red rust due to *Cephaleurus* spp. on certain species in order to better characterize it and to have a database necessary for the future development of a control strategy against this disease in the production areas of these species in Cameroon.

2. Materials and methods

2. 1. Sampling and description of disease symptoms

Sampling was carried out on trees bearing leaves showing typical symptoms of red rust along the diagonal of each orchard visited. The samples were collected early in the morning and packed in plastic bags containing cotton soaked in sterile distilled water and transported to the laboratory.

The description was made on leaves collected in the field and showing the characteristic symptoms of red rust. Twenty leaves (20) of each species were used. Identification keys were used for this description [9–14]. These symptoms were described by visual observation and parameters such as colour, shape, lesion distribution on the leaf, number of lesions, coefficient of variation and lesion diameter were evaluated.

2. 2. Morphological characterization and identification of the pathogen

This was carried out using samples of diseased leaves. To obtain these structures, the rust spots were scraped with a needle and placed on a slide containing a drop of sterile distilled water for microscopic observation. For each parameter an average of 10 structure measurements was taken and one measurement was retained. The length, width, number of sporangia and sporangiophores were recorded using an OMAX optical microscope with a micrometer.

The identification of the causal agent of red rust on the different species was done using the identification keys [10–16].

2. 3. Assessment of incidence and severity

2. 3. 1. Incidence

The assessment of disease incidence required sampling of at least 10 trees per orchard at each site. It was calculated according to the formula of [17]:

$$I\ (\%)=X_i/X_t\times 100,$$

I – incidence; X_i – number of infected trees per locality; X_t – total number of trees collected per locality.

2. 3. 2. Severity

The degree of disease severity on infected plants in the field was done according to the scale of [17]:

0=no symptoms;
1= 25 %: [0–1/4] of leaf and fruit areas showing symptoms;
2=50 %: [1/4–2/4] of leaf and fruit areas showing symptoms;
3=75 %: [2/4–3/4] of leaf and fruit areas showing symptoms;
4=100 %: [3/4–4/4] of leaf and fruit areas showing symptoms.

The Severity Index (percentage of diseased leaf area) was calculated according to the formula of [17]:

$$SI=\sum n_i/(X_t\times 4)\times 100,$$

SI – severity index; n_i – individual score for red rust symptoms on each leaf; X_t – total number of leaves observed; 4 – highest score on the scale.

2. 4. Data analysis

Data analysis was performed using SPSS 20.0 software. Means were compared using Duncan's test 5 %.

3. Results

3. 1. Symptoms of red rust on leaves and fruits of the different species studied

Symptoms (lesion) of red rust disease occurred in different organs (leaf, fruit) according to specie surveyed:

1. Annona muricata (Soursop) leaves.

Observation made on soursop leaves showed numerous circular spots varying from 0.1 to 1 cm in diameter. These lesions have an average diameter of 0.44 cm. These spots are grey-brown in colour for the larger spots and orange for the smaller ones **Fig. 1, *a***. The lesions tend to form on the midrib **Fig. 1, *b***.

2. Dacryodes edulis (Safou) leaves.

Red rust symptoms observed on safou leaves show the presence of numerous circular lesions (**Fig. 1, *c***) of different diameters (**Table 1**) but much larger than those of other species. The leaf spots are iron-grey in colour. They are distributed on the leaf blade between the secondary veins and sometimes on the secondary veins.

3. Psidium guajava (Guava) leaves and fruit.

They initially appear as tiny dark-brown spots which enlarge into a roughly circular lesion with a dark-brown colour **Fig. 1, *d***. These lesions usually form between the secondary veins. Lesion diameters ranged from 0.1 to 0.7 **cm** with an average diameter of 0.28 **cm (Table 1)**.

4. Theobroma cacao (Cocoa) leaves and fruits.

Observation of cocoa leaves shows numerous white spots **Fig. 1, *e*** ranging from 0.1 to 0.3 cm in diameter (**Table 1**), white **Fig. 1, *e*** and with a diameter varying from 0.1 to 0.3 cm **(Table 1)**. On the other hand, on the fruits these lesions are rather orange-brown in colour **Fig. 1, *f***.

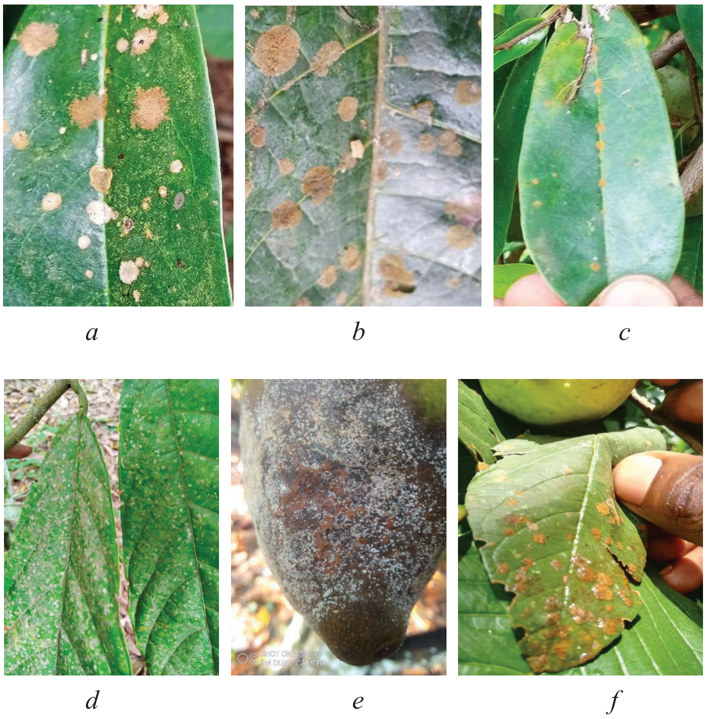

Fig. 1. Spots of red rust in different organs of species survey: *a* – spot on the blade of soursops; *b* – spot on the midrib of soursop; *c* – spots on safou leaf; *d* – spots on guava leaf; *e* – spots on fruit of cocoa; *f* – spots on leaf of cocoa

Table 1

Average diameter and number of lesions in species studied

Hosts	Average diameter (cm)	Coefficient of variation (%)	Number of lesions
Theobroma cacao	0.14a	46.43	510
Psidium guajava	0.28b	39.48	248
Annona muricata	0.44c	48.55	208
Dacryodes edulis	0.45c	46.08	245

Note: Values followed by the same letter in each column of the same parameter show no significant difference according to Duncan's test at the 5 % threshold.

3. 2. Micro-morphological characteristics

After measuring the algal structure, the sporangia have a length of 17.85 to 25.5 µm and a width of 20.4 to 25.5 µm for soursop, a length of 15.3 to 25.5 µm and a width of 20.4 to 25, 5 µm for safou, 15.3 to 30.6 µm in length and 15.3 to 20.4 µm in width for guava and 20.4 to 30.6 µm in length and 20.4 to 30.6 µm in width for cocoa (**Table 2**). The total number of sporangia produced by each sporangiophores varies from 1 to 9 for all the different species.

The number of sporangiophores produced varies from 1 to 11 for soursop, from 1 to 10 for safoutier and cocoa, from 1 to 13 for guava. The length of the sporangiophores varies from 280.5 to 714 µm and the width from 10.2 to 25.5 µm, for soursop, a length of 408 to 612 µm and a width of 15.3 to 25.5 µm for safou. In guava, the length of the sporangiophores varied from 433.5 to 663 µm and the width from 15.3 to 20.4 µm, while in cocoa, the length varied from 484.5 to 612 µm and the width from 20.4 to 35.7 µm (**Table 2).**

Table 2

Morphological characteristics of the thallus structures of the red rust pathogen on the different species studied

Hosts	Pathogens	Sporangiophore (µm)			Sporangia (µm)		
		Nb/C	Length	Width	Nb/C	Length	Width
Annona muricata	*Cephaleurus virescens*	1-11	280.5–714	10.2–25.5	1–9	17.85–25.5	20.4–25.5
Dacryodes edulis	*Cephaleurus virescens*	1-10	408–612	15.3–25.5	1–9	15.3–25.5	20.4–25.5
Psidium guajava	*Cephaleurus virescens*	1-9	433.5–663	15.3–20.4	1–9	15.3–30.6	15.3–20.4
Theobroma cacao	*Cephaleurus virescens*	1-10	484.5–612	20.4–35.7	1–9	20.4–30.6	20.4–30.6

3. 3. Identification of the agent

The results of microscopic observations on soursop, safou, guava and cocoa showed that the sporangiophores were either isolated (**Fig. 2, *a***) or clustered (**Fig. 2, *b***). Each sporangiophore had more than one septum with one (**Fig. 2, *c***) or more sporangia clustered at the head of the sporangiophore as a crown (**Fig. 2, *d***). The sporangium attached by a cell suspensor contains zoospores inside which can be released after rupture of the sporangium membrane. The symptoms and characteristics thus described show that the parasitic alga is *Cephaleurus virescens* (Kunze).

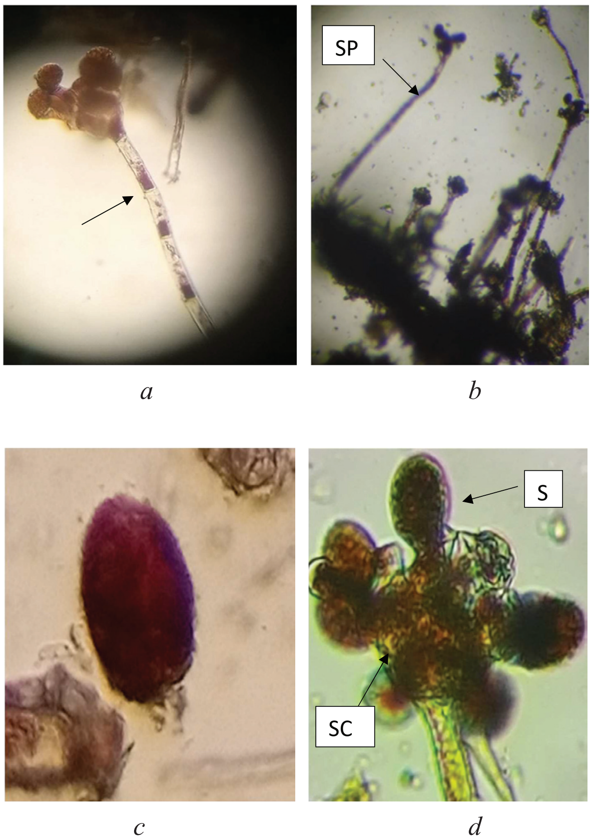

Fig. 2. Micromorphological characteristics of C. virescens: *a* – single sporangiophore (narrow indicates septum in sporangiophore) x200; *b* – clumped sporangiophores (*SP*=sporangiophore) x100; *c* – single sporangium; *d* – head of sporangiophore (*SC*=suspensor cell, *S*=sporange)

3. 4. Incidence and severity

A highly significant difference (P=0.0001) was obtained in the evolution of the incidence and severity of red rust disease in the surveyed sites. Incidence remained high in all species 100±0.0 %, 80±0.0 %, 70±0.8 %, 60±0.7 % for soursop, safou, guava and cocoa respectively. Severity was high (75±0.9 %) for *Annona muricata* and *Psidium guajava*, (60±0.7 %) for *Theobroma cacao* and low (40±1.4 %) for *Dacryodes edulis* (**Table 3**).

Table 3

Incidence and severity of red rust on different species studied

–	*Annona muricata*	*Dacryodes edulis*	*Psidium guajava*	*Theobroma cacao*
Incidence (%)	100±0.0	60±0.7	80±0.0	70±1.8
Severity (%)	75±0.9	40±1.4	75±0.9	60±1.7

4. Discussion

The present work identified red rust due to *C. virescens* in soursop, safou, guava and cocoa in the surveyed sites. Several authors have identified red rust due to *C. virescens* in other species [18, 19]. The presence of the alga is favoured by average monthly temperatures and rainfall of 23 °C and 127 mm respectively [15]. During August and September, rainfall was very high at the sites, which favours the opening of the membrane envelope of the sporangia that release zoospores by the wind [20].

Little or no work has been done on the size of algal structures affecting guava, soursop, safou and cocoa. However, based on the morphological characteristics of the alga, measurements of sporangiophores and sporangia of the algal species are 280.5×714 µm and 17.85×25.5 µm respectively on *A. muricata,* 408×612 µm and 15.3×25.5 µm on *D. edulis*, 433.5×663 µm and 15.3×30.6 µm on *P. guajava* and 484.5×612 µm and 20.4×35.7 µm on *T. cacao* in this study and are close to *C. virescens* identified by [10, 15], *Ficus benghalensis* and *Citrus sinensis* respectively However, these results do not corroborate, mainly for the length of sporangiophores, those obtained by [11] on *Camellia sinensis* (880 to 1256 µm) who rather identified *Cephaleurus parasiticus*. Similarly, [21] identified *C. parasiticus* on guava instead. Although *C. virences* and *C. parasiticus* have similarities in shape and coloration, the sporangiophores of *C. parasiticus* are larger in length and width [11].

Sporangiophores size is therefore the most reliable measure to distinguish the two species, as [22] demonstrated that sporangia size is identical for *C. virescens* and *C. parasiticus*. The sporangiophores size results in this study are in agreement with those of [9] who obtained measurements of 245.5 to 545.6 µm.

Measurement variability was obtained from sporangia and sporangiophores of *C. virescens*, where coefficients of variation of 37 and 13 % for sporangiophore length and 20 % for sporangium length were observed. There is little relationship to the variability of the measurements found in *C. virescens*. These results show that the length of the sporangiophores is more variable than that of the other measured structures. It should be noted that the homogeneity obtained in the measurement of micro-morphological structures helps in the characterization and standardisation of the measurement of the structure of *C. virescens*. [15] stated that there is a higher incidence of red rust on shaded leaves in the lower third of the plant. Interestingly, the attacked leaves showed an average of 90 to 100 lesions depending on the type of species. This result is close to the one obtained by [23] on *Mycosphaerella citri*, which showed an average of 131 lesions per leaf. On safflower, soursop, guava and cocoa, the attacked leaves showed an average of 13.8, 16.3, 16.5 and 16.6 lesions per leaf, which corroborates the results of [10] who obtained an average of 19.4 and 11.4 lesions of different diameters per leaf on cashew nut in Brazil (*S. macrophylla*).

Moreover, 98 % of the lesions were smaller than 1 cm in diameter. This demonstrates a greater ability of the pathogen to reach different points of the leaf blade [15]. According to [24], the number of lesions is an important fact as it is a variable that is strongly correlated with the severity of the disease.

Direct isolation and pathogenicity test of the pathogen from agar media did not yield any results (results not shown). This corroborates with [1–27] who showed that solid agar media such as PDA do not support the growth of the parasitic alga *C. virescens* because this medium is rich in sugar and starch and therefore insufficient for their growth.

Study was undertaken in one region. Thus, survey of other plants is necessary in all area of our country to characterize this disease. Molecular identification is also needed to identify accurately all *Cephaleurus* spp species occurring in our environment.

5. Conclusions

The symptomatological study on these species in the different sites showed the presence of characteristic symptoms of red rust whose causal agent is a parasitic alga, *C. virescens*. Molecular studies are needed to confirm the nature of this pathogen in Cameroon.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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Data availability

Manuscript has no associated data.

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