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Full Length Research Paper

Analysis of combined strategies for the management of Asian soybean rust

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Asian soybean rust, if not well managed, can reduce grain yield up to 90%. Due to the severe damage caused by the disease, especially in Brazil, it is important to evaluate management strategies combined with fungicides, to minimize the losses. The aim of the work was to evaluate the effect of adopting different combined management strategies on the severity of soybean rust and its impact on the performance of the crop. Research was carried out at 2016/2017 and 2017/2018 growing seasons. Soybean cultivar NA 5909 RG® (susceptible to Asian rust), LG 60163 IPRO® (highly tolerant) and TMG 7062 IPRO® INOX® (resistant) were evaluated with different combinations of fungicide, copper and potassium phosphites foliar fertilizers applications, in two development stages, R_1 (early flowering) and $R_{5.1}$ (early grain filling). Disease severity assessments were performed at R_1 and repeated every 14 days (up to R_5 + 14 days) to determine the area under the Disease Progress Curve (AUDPC). The genetics of resistance to Asian soybean rust contributed to the less progress of the disease in plants. Even with differential responses between cultivars and treatments applied to the severity of Asian soybean rust, the disease did not compromise grain yield in both growing seasons.

Key words: *Phakopsora pachyrhizi*, fungicide, foliar fertilizers, genetic resistance.

INTRODUCTION

Asian rust (*Phakopsora pachyrhizi*) is present in most countries that commercially exploits the soybean crop. The disease can have a high potential for damage, due to early defoliation, which has affected the formation and weight of grains. In environmental conditions favorable to the disease, and without the correct management, losses can be 80 to 90% (Hartman et al., 2015, Godoy et al., 2016, Dalla Lana et al., 2015).

The most efficient management strategy for controlling Asian rust has been the use of fungicides. Since the disease entered Brazil in 2001, many products have been tested. Over the years, some products previously very efficient, have ceased to be recommended, especially in isolated use; while others have shown promise, especially in mixtures (Aguiar et al., 2016; Reznikov et al., 2019; Zuntini et al., 2019). These differential responses of fungicide efficiency, during the crop years, are due to the variation of the fungus in the environment, with the appearance of less sensitive populations and even resistant to the currently available fungicides

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(Klosowski et al., 2016; Simões et al., 2017), which requires constant research and the adoption of combined strategies.

The use of foliar fertilizers and resistance inducers associated with fungicides, for the management of soybean diseases, especially Asian rust, is a strategy that has been explored by research (Oliveira et al., 2015) and, also, commercially by companies. These technologies aim to reduce disease severity through better plant nutrition, activation of the pathogen defense system, and, consequently, increased fungicide efficiency (Andrade Júnior et al., 2015; Neves and Blum, 2014).

Allied to the use of fungicides and foliar fertilizers for the management of Asian rust, is the use, by farmers, of cultivars tolerant or resistant to the pathogen (Godoy et al., 2017). When compared with susceptible and rust-resistant cultivars, lower disease progress is observed on resistant ones (Hoffmann et al., 2019). Thus, cultivars that have genetic resistance require differentiated management in fungicide applications (Melo et al., 2015).

The aim of this study was to evaluate different strategies for the Asian soybean rust management by applying fungicide, copper and potassium phosphites foliar fertilizers, as well as genetic resistance to disease severity over its yield components and grain productivity.

MATERIALS AND METHODS

The trials in the 2016/2017 and 2017/2018 growing seasons were conducted, at the Experimental Station of the Universidade Tecnológica Federal do Paraná, Campus Dois Vizinhos, latitude 25° 41' 52" S, longitude 53° 03' 94" W, and altitude of 509 m above the sea level. Soil has been managed in a no-tillage system since 2001. According to the Köppen classification, the climate is Cfa, with mean annual rainfall of 2000 mm and an annual average temperature between 20 to 22°C (IAPAR, 2009). Soil at the experimental site is classified as Red Latosol, with a smooth relief and a very clayey texture, according to Embrapa (2006).

Each trial (growing season) was organized in a bifactorial scheme, with factor 1 represented by soybean cultivars and factor 2 by the product combinations applied for the management of Asian rust (Table 1), in the stages of soybean development R_1 (early flowering) and $R_{5.1}$ (early grain filling). The soybean cultivars tested were: NA 5909 RG® (susceptible to Asian rust), LG 60163 IPRO® (highly tolerant) and TMG 7062 IPRO® INOX® (resistant) cultivars. Fungicide used was based on azoxystrobin (300 g kg^{-1}) and benzovindiflupir (150 g kg^{-1}), applied at the dose of 300 g ha^{-1} of commercial product (c.p.). In the applications, adjuvant based on soybean methyl ester at a concentration of 72.0% w/v was used.

Potassium phosphite A (PPh A) has a concentration of 25% K_2O + 35% P_2O_5 w/v (% weight by volume) and 500 ml ha^{-1} of commercial product (c.p.); Potassium phosphite B (PPh B) has a concentration of 33.6% P_2O_5 + 29.0% K_2O w/v, and 500 ml ha^{-1} of c.p.; Copper (Cu) foliar fertilizer contains 50% Cu w/v by applying 50 mL ha^{-1} of c.p. The application model followed the agronomic recommendation of the technology holders.

Soybean cultivars were sowed in the last week of October, for both growing seasons. Seed number per linear meter followed the traits of each cultivar and the recommendations of the breeding companies; 14 seeds m^{-1} (about 311,108 seeds ha^{-1}) for cultivar NA 5909 RG®, and 11 seeds m^{-1} (about 244,442 seeds ha^{-1}) for cultivars LG 60163 IPRO® and TMG 7062 IPRO® INOX®.

Applications were carried out with a CO_2 pressurized costal sprayer, coupled to a 2 m spray bar, consisting of four application spray nozzles, 0.5 m apart, using Teejet XR 11002 tip, working with a pressure 42 lbs in^{-2}, and applying a spray volume of 200 L ha^{-1}.

First assessment of Asian rust severity was performed at the R_1 stage, by visual diagnosis and the use of a portable magnifying glass, with 20x magnification, evaluating the abaxial side of three trefoils from the lower, middle and upper canopies of three plants per plot, at random using the diagrammatic scale proposed by Godoy et al. (2006). Subsequently, the evaluations occurred every 14 days, being the last one performed 14 days after the second application of the treatments, more specifically 14 days after $R_{5.1}$, totaling four evaluations in each growing season. The values of Asian rust severity were used to obtain the area under the disease progress curve (AUDPC), which takes into account the disease severity and progress over time according to the equation:

$$AUDPC = \sum_{i=1}^{n}\{(Y_{i+1}+Y_i) \times 0,5) \times (T_{i+1}-T_i)\};$$

where Y_{i+1}= disease severity at the time of assessment (i+1); Y_i= disease severity at the time of assessment i (i = 1...n); T_{i+1}= evaluation season i+1; T_i= evaluation season i (whereas the number of days after the emergence of the plant is considered; n = number of observations).

At harvest, five plants were randomly collected per experimental plot and subsequently evaluated for the following yield components: plant height (PH), first pod height insertion in the main stem (FPH), number of pods per plant (NPP), number of grains per plant (NGP) and number of grains per pod (NGPo).

Soybean cultivar samples from the three central rows, each with 2 m long (sample area 2.7 m^2 per point) per plot were manually harvested, threshed by a stationary combine harvester and cleaned. Moisture content of the seeds was evaluated using portable automatic equipment and thousand grain weight (TGW) and final yield (kg ha^{-1}) were adjusted to a moisture content of 12%. Additionally, the TGW was assessed by manual counting and weighing 8 samples of 100 grains. The experimental design used was a randomized block, with three replicates. Data were subjected to analysis of variance and compared by the Duncan test, at 5% probability using the Genes Statistical Program (Cruz, 2008).

RESULTS

Analysis of variance for AUDPC indicated interaction between soybean cultivars × Asian rust managements for the lower, middle and upper canopy of soybean plants, as well as for the general average considering the whole canopy, in both growing seasons (Table 2).

In general, the AUDPC of Asian soybean rust was higher in NA 5909 cultivar (susceptible to the pathogen) and in the lower part of the plants, also differing on the treatments applied (Tables 3 and 4).

The highest level of rust expressed as AUDPC at the lower canopy were found at the soybean susceptible cultivar (NA 5909) treated only with phosphites (PPh A in R_1 and PPh B in $R_{5.1}$). For the tolerant cultivar (LG 60163), the highest values were found in treatments PPh A + Cu in R_1 and PPh B in $R_{5.1}$. For the rust resistant cultivar (TMG 7062) the highest values were found in treatments PPh A in R_1, PPh B + Cu in $R_{5.1}$ and control. The same trend for both growing seasons (2016/17 and 2017/18) was noticed for the middle canopy in relation to the isolated and combined use of fungicide and foliar

Table 1. Product combinations applied to three soybean cultivars, at the R_1 and $R_{5.1}$ development stages, for the management of Asian rust, at the 2016/2017 and 2017/2018 growing seasons.

Product combinations (PC)	Development stage R_1	Development stage $R_{5.1}$
1	Fungicide	Fungicide
2	Fungicide + PPh A*	Fungicide + PPh B**
3	Fungicide + PPh A + Cu	Fungicida + PPh B
4	Fungicide + PPh A	Fungicida + PPh B + Cu
5	PPh A	PPh B
6	PPh A + Cu	PPh B
7	PPh A	PPh B + Cu
8	Control, without application	Control, without application

*PPh A: Potassium phosphite A; **PPh B: Potassium phosphite B; Cu: Copper foliar fertilizer.

Table 2. Variance analysis of the Area Under the Disease Progress Curve (AUDPC) of Asian soybean rust for the lower, middle and upper plant canopies and the plant canopy average, of the three soybean cultivars through eight product combinations for the management of disease, at the 2016/2017 and 2017/2018 growing seasons.

SV	DF	Mean square			
		AUDPC 2016/2017 growing season			
		Lower canopy	Middle canopy	Upper canopy	Plant canopy
Blocks	2	638.01	4775.07	184.91	173.92
PC (F1)	7	89055.36**	36524.52**	3391.69**	28893.68**
Cultivars (F2)	2	261188.70**	39274.78**	3333.11**	57684.07**
Int. F1×F2	14	36045.12**	2681.37**	1157.66**	4763.94**
Residue	46	1945.68	930.72	65.59	397.25
CV (%)		15.50	26.87	30.06	14.18
		AUDPC 2017/2018 growing season			
Blocks	2	8.51	9.71	0.055	2.33
PC (F1)	7	19283.79**	6272.85**	339.61**	6071.78**
Cultivars (F2)	2	186521.88**	48853.31**	4712.87**	58207.03**
Int. F1×F2	14	12780.91**	3590.1**	167.54**	3463.74**
Residue	46	10.24	7.28	1.86	1.09
CV (%)		4.25	6.59	9.78	2.39

SV: Sources of variation; DF: degrees of fredom; PC: product combinations; CV: coefficient of variation. **Significant at 1% level of probability ($p< 0.01$).

fertilizers for all three cultivars, where the absence of fungicide increased the severity of the disease (Table 3).

On the other hand, the lowest AUDPC values at the lower canopy were noticed at product combination PC1 (only fungicide at R_1 and $R_{5.1}$) and PC3 (Fungicide + PPh A + Cu in R_1 and Fungicide + PPh B in $R_{5.1}$) for the susceptible (NA 5909) and highly tolerant (LG 60163) soybean cultivars (Table 3). Considering the lower values of AUDPC from the treatments that received fungicide combined or not with phosphites (PC1 to PC4) it is possible to infer that phosphites (foliar fertilizer) and Cu are not effective for rust management when used alone, even for resistant cultivars (Table 3).

Regarding to the AUDPC considering the whole canopy of soybean plants, it is noticed that resistant cultivar (TMG 7062) showed lower values followed by highly tolerant cultivar (LG 60163) and susceptible cultivar (NA 5909). In general, the severity results were similar when only fungicide (PC1) or fungicide combined with phosphites (PC2, PC3 and PC4) was used, for the three cultivars (Table 4).

Differences among studies comparing rust disease management may differ substantially due to environmental conditions. Due to these variations in environmental conditions, it is important to relate disease pressure to climatic conditions (Figure 1). In the months when the Asian rust is more aggressive (December, January and February), at the 2016/2017 growing season,

Table 3. Soybean Asian rust AUDPC from the lower, middle and upper canopy of soybean plants in relation to three soybean cultivars subjected to eight product combinations for disease management, at the 2016/2017 and 2017/2018 growing seasons.

PC*	2016/2017 growing season			2017/2018 growing season		
	NA 5909	LG 60163	TMG 7062	NA 5909	LG 60163	TMG 7062
1	368.7^{bA}	147.0^{dB}	6.0^{dC}	40.8^{fA}	22.2^{dB}	14.0^{cC}
2	340.7^{bA}	295.2^{cA}	15.2^{dB}	88.7^{dA}	15.2^{eB}	16.30^{bcB}
3	149.3^{dB}	336.0^{bcA}	85.8^{cdB}	61.8^{eA}	23.3^{dB}	14.0^{cC}
4	240.3^{cA}	305.7^{bcA}	29.3^{dB}	86.3^{dA}	14.0^{eB}	14.0^{cB}
5	539.0^{aA}	506.3^{aA}	120.2^{cB}	326.7^{aA}	42.0^{bB}	16.3^{bcC}
6	418.8^{bA}	474.8^{aA}	272.2^{bB}	210.0^{cA}	36.2^{bcB}	21.0^{abC}
7	380.3^{bA}	381.5^{bA}	394.3^{aA}	280.0^{bA}	35.0^{cB}	22.2^{abC}
8	357.8^{bA}	274.2^{cB}	390.8^{aA}	320.8^{aA}	63.0^{aB}	25.7^{aC}
CV (%)	15.50	-	-	4.25	-	-
			AUDPC - middle canopy			
1	92.2^{cdA}	43.2^{eAB}	10.9^{bB}	59.5^{eA}	9.3^{dB}	11.7^{abcB}
2	56.4^{deAB}	89.8^{deA}	31.5^{bB}	36.2^{gA}	12.8^{dB}	7.0^{cdC}
3	19.8^{eB}	133.0^{cdA}	28.1^{bB}	44.3^{fA}	12.8^{dB}	2.3^{dC}
4	51.3^{deAB}	75.8^{deA}	22.2^{bB}	39.7^{fgA}	10.1^{dB}	9.3^{bcB}
5	211.2^{aA}	180.8^{bcA}	128.3^{aB}	155.2^{bA}	25.7^{bcB}	11.7^{abcC}
6	197.2^{abB}	252.0^{aA}	106.2^{aC}	77.0^{dA}	21.8^{cB}	9.3^{bcC}
7	150.1^{bcB}	205.3^{abA}	113.2^{aA}	108.5^{cA}	28.0^{bB}	14.0^{abC}
8	200.7^{abA}	210.8^{abA}	114.8^{aA}	221.7^{aA}	38.9^{aB}	16.3^{aC}
CV (%)	26.87			6.59		
			AUDPC - upper canopy			
1	6.7^{cA}	8.3^{cA}	1.4^{dA}	18.7^{fA}	7.0^{dB}	0.0^{cC}
2	7.9^{cA}	11.7^{cA}	13.4^{cdA}	21.0^{efA}	7.0^{dB}	4.7^{abC}
3	3.0^{cB}	9.3^{cAB}	18.8^{bcA}	18.7^{fA}	7.0^{dB}	0.0^{cC}
4	10.7^{cA}	14.6^{bcA}	5.4^{cdA}	23.3^{deA}	7.0^{dB}	0.0^{cC}
5	52.5^{bA}	60.7^{aA}	38.5^{aB}	59.5^{aA}	9.3^{cdB}	4.7^{abC}
6	81.7^{aA}	29.7^{bB}	36.2^{aB}	28.0^{cA}	12.5^{bB}	0.0^{cC}
7	87.1^{aA}	16.3^{bcB}	8.6^{cdB}	25.7^{cdA}	11.7^{bcB}	2.3^{bcC}
8	74.7^{aA}	16.3^{bcC}	33.8^{abB}	41.2^{bA}	18.7^{aB}	7.0^{aC}
CV (%)	30.06			9.78		

Means followed by distinct lowercase letters in the column and uppercase in the row, differ from each other by the Duncan Test at 5% probability. *PC (Product combinations) 1: Fungicide – Fung (R_1) and Fung ($R_{5.1}$); 2: Fung + Potassium phosphite A - PPh A (R_1) and Fung + Potassium phosphite B - PPh B ($R_{5.1}$); 3: Fung + PPh A + Copper foliar fertilizer – Cu (R_1) and Fung + PPh B ($R_{5.1}$); 4: Fung+ PPh A (R_1) and Fung + PPh B + Cu ($R_{5.1}$); 5: PPh A (R_1) and PPh B ($R_{5.1}$); 6: PPh A + Cu (R_1) and PPh B ($R_{5.1}$); 7: PPh A (R_1) and PPh B + Cu ($R_{5.1}$); 8: control, without application.

the maximum average temperature verified was 24°C and the relative humidity of the air 85%. In the 2017/2018 growing season, the maximum average temperature in this period was 23°C and the humidity of the air 84%. That is, in both growing seasons the disease found favorable environmental conditions.

At the 2017/2018 growing season, there was a high precipitation at the end of December, exceeding 190 mm, when the soybean crop was in the vegetative phase. In the second half of January, beginning of the reproductive phase of the crop, precipitation exceeded 110 mm, exposing the plants for a long period of leaf wetness, as in February.

For the evaluated yield components, the analysis of variance indicated interaction between soybean cultivars versus Asian rust treatments for the variables: number of pods per plant (NPP), and number of grains per plant (NGP), at the 2016/2017 growing season, and thousand grain weight (TGW), in both growing seasons (2016/2017 and 2017/2018) (Table 5).

Table 4. Soybean Asian rust AUDPC in the canopy average of soybean plants in relation to three soybean cultivars subjected to eight product combinations for disease management, at the 2016/2017 and 2017/2018 growing seasons.

PC*	2016/2017 growing season			2017/2018 growing season		
	NA 5909	LG 60163	TMG 7062	NA 5909	LG 60163	TMG 7062
1	155.8^{bA}	66.2^{dB}	5.4^{cC}	$43.4f^{A}$	12.4^{deB}	8.6^{deC}
2	142.3^{bcA}	128.7^{cA}	18.3^{cB}	49.4^{eA}	$10.5^{e}f^{B}$	9.3^{cdeB}
3	53.5^{dB}	193.7^{bA}	31.7^{cB}	$44.7f^{A}$	14.4^{dB}	7.4^{eC}
4	110.6^{cA}	127.6^{cA}	30.7^{cB}	$45.9f^{A}$	10.1^{eB}	7.8^{eC}
5	228.3^{aA}	249.9^{aA}	106.2^{bB}	180.4^{bA}	23.3^{cB}	10.9^{bcC}
6	231.0^{aA}	241.1^{aA}	166.1^{aB}	104.6^{dA}	22.9^{cB}	10.1^{cdC}
7	216.7^{aA}	185.9^{bAB}	154.4^{aB}	142.3^{cA}	32.7^{bB}	12.8^{bC}
8	211.9^{aA}	158.4^{bcB}	158.8^{aB}	194.8^{aA}	40.8^{aB}	16.3^{aC}
CV (%)	14.18	-	-	2.39	-	-

Means followed by distinct lowercase letters in the column and uppercase in the row, differ from each other by the Duncan Test at 5% probability. *PC (Product combinations) 1: Fungicide – Fung (R_1) and Fung ($R_{5.1}$); 2: Fung + Potassium phosphite A - PPh A (R_1) and Fung + Potassium phosphite B - PPh B ($R_{5.1}$); 3: Fung + PPh A + Copper foliar fertilizer – Cu (R_1) and Fung + PPh B ($R_{5.1}$); 4: Fung+ PPh A (R_1) and Fung + PPh B + Cu ($R_{5.1}$); 5: PPh A (R_1) and PPh B ($R_{5.1}$); 6: PPh A + Cu (R_1) and PPh B ($R_{5.1}$); 7: PPh A (R_1) and PPh B + Cu ($R_{5.1}$); 8: control, without application.

Table 5. Variance analysis of the variables plant height (PH), first pod height insertion in the main stem (FPH), number of pods per plant (NPP), number of grains per plant (NGP), number of grains per pod (NGPo), thousand grain weight (TGW) and grain yield of three soybean cultivars subjected to eight product combinations for the management of Asian rust, at the 2016/2017 and 2017/2018 growing seasons.

SF	DF	Mean square						
		PH (cm)	FPH (cm)	NPP	NGP	NGPo	TGW (g)	Grain yield (kg ha^{-1})
Blocks	2	54.86	67.77	57.85	706.15	0.0013	73.06	4332.4
PC (F1)	7	44.54^{ns}	8.65^{ns}	109.99^{ns}	511.35*	0.014ns	135.41**	173579.5^{ns}
Cultivars (F2)	2	5875.96**	560.35**	63.60^{ns}	1969.16**	0.365**	13147.48**	1289486**
F1×F2	14	34.10^{ns}	15.40^{ns}	133.28*	560.57**	0.0064^{ns}	99.97**	249025.6^{ns}
Residue	46	22.35	15.60	57.13	220.79	0.0086	32.49	132296.5
CV (%)		4.09	14.21	13.67	11.82	4.04	3.28	7,58
SF	DF	PH	FPH	NPP	NGP	NGPo	TGW (g)	Grain yield
Blocks	2	23.13	9.74	6.72	166.61	0.021	145.85	7905.5
PC	7	24.24^{ns}	18.73^{ns}	97.83*	426.8*	0.01^{ns}	1319.80**	444640.4**
Cultivars	2	3841.6**	1466.8**	327.5**	871.1*	0.11**	26624.08**	819895.8**
Int. F1×F2	14	7.32^{ns}	13.20^{ns}	41.45^{ns}	280.7^{ns}	0.02^{ns}	141.20*	136366.9^{ns}
Residue	46	12.10	13.55	33.25	178.33	0.0139	66.05	73638.1
CV (%)		2.96	13.51	13.10	13.91	5.45	4.24	6.70

SV: Sources of variation; DF: Degrees of fredom; PC: Product combinations; CV: Coefficient of variation. ** Significant at 1% level of probability ($p < 0.01$); * significant at 5% level of probability ($0.01 \leq p < 0.05$); ns not significant ($p \geq 0.05$).

Plant height (PH), first pod interaction (FPI), number of grains per pod (NGp) and yield (Kg ha^{-1}) variables differed among cultivars in both growing seasons (Table 6).

Also, during the 2017/2018 growing season, some variables such as NPP, NGP and yield differed among rust management treatments.

The PH was higher in the cultivars LG 60163 and TMG 7062 compared to NA 5909 in both growing seasons (Table 6). This trait, despite being influenced by the environment, is intrinsic to the genotype. Longer cycle cultivars tend to have taller plants.

Usually, FPH follows the trend of PH. In this case, this occurred only for the cultivar TMG 7062, where taller plants conditioned higher FPH. Cultivar LG 60163 presented similar results to the cultivar NA 5909, both

Table 6. Plant height (PH), first pod height insertion (FPH) and number of grains per pod (NGPo) of three soybean cultivars at 2016/2017 and 2017/2018 growing season.

Cultivar	2016/2017 growing season			2017/2018 growing season		
	PH (cm)	FPH (cm)	NGPo	PH (cm)	FPH (cm)	NGPo
NA 5909	97.5^{b}	26.3^{b}	2.2^{b}	103.0^{b}	26.6^{b}	2.1^{a}
LG 60163	123.6^{a}	23.9^{b}	2.4^{a}	124.5^{a}	19.7^{b}	2.1^{a}
TMG 7062	125.4^{a}	33.2^{a}	2.4^{a}	125.3^{a}	35.5^{a}	2.1^{a}
CV (%)	4.09	14.21	4.04	2.96	13.51	5.45

Means followed by different letters differ from each other by the Duncan Test at 5% probability.

Table 7. Number of pods per plant (NPP) and number of grains per plant (NGP) of three soybean cultivars submitted to eight product combinations for the management of Asian rust, at the 2016/2017 and 2017/2018 growing seasons.

PC*	NPP			NGP		
	NA 5909	LG 60163	TMG 7062	NA 5909	LG 60163	TMG 7062
			2017/2018 growing season			
1	63.5^{aA}	53.8^{abcA}	54.4^{aA}	131.3^{aA}	125.2^{abcA}	121.7^{aA}
2	47.9^{aB}	66.1^{aA}	51.2^{aAB}	105.2^{aB}	147.9^{abA}	121.3^{aAB}
3	54.1^{aA}	49.3^{abcA}	55.2^{aA}	119.2^{aA}	111.1^{bcA}	131.5^{aA}
4	55.0^{aA}	53.8^{abcA}	51.5^{aA}	115.3^{aA}	130.1^{abcA}	128.3^{aA}
5	58.3^{aA}	65.6^{abA}	65.3^{aA}	121.8^{aB}	156.8^{aA}	152.4^{aA}
6	61.3^{aA}	46.1^{bB}	56.3^{aAB}	121.9^{aA}	111.8^{bcA}	128.4^{aA}
7	51.6^{aAB}	43.5^{cB}	62.8^{aA}	110.6^{aB}	106.3^{cB}	146.5^{aA}
8	49.6^{aA}	51.4^{abcA}	58.9^{aA}	104.3^{aB}	125.4^{abcAB}	143.5^{aA}
CV (%)		13.67			11.82	
			2016/2017 growing season			
	1	2	3	4	5	6	7	8	CV (%)
NPP	44.7^{a}	43.7^{a}	48.3^{a}	40.1^{a}	40.9^{a}	41.1^{a}	44.7^{a}	48.8^{a}	13.10
NGP	98.2^{a}	96.4^{a}	106.4^{a}	86.9^{a}	89.8^{a}	89.3^{a}	98.1^{a}	102.8^{a}	13.91

Means followed by distinct uppercase letters in the row and lowercase letters in the column differ from each other by the Duncan Test at 5% probability. *PC (Product combinations) 1: Fungicide – Fung (R_1) and Fung ($R_{5.1}$); 2: Fung + Potassium phosphite A - PPh A (R_1) and Fung + Potassium phosphite B - PPh B ($R_{5.1}$); 3: Fung + PPh A + Copper foliar fertilizer – Cu (R_1) and Fung + PPh B ($R_{5.1}$); 4: Fung+ PPh A (R_1) and Fung + PPh B + Cu ($R_{5.1}$); 5: PPh A (R_1) and PPh B ($R_{5.1}$); 6: PPh A + Cu (R_1) and PPh B ($R_{5.1}$); 7: PPh A (R_1) and PPh B + Cu ($R_{5.1}$); 8: control, without application.

inferior however than TMG 7062 (Table 6). The NGp was higher during the 2016/2017 growing seasons for the cultivars TMG 7062 and LG 60163, differing from the cultivar NA 5909. At the 2017/2018 growing season, there were no differences between cultivars for this response variable.

For the LG 60163 cultivar, in general, the treatments presented similar results when compared with the control. There was difference only between PC2 (Fungicide + PPh A in R_1 and Fungicide + PPh B in $R_{5.1}$) and PC6 and PC7 (only foliar fertilizers), which showed lower values. However, these treatments did not also differ from the control (Table 6).

During the 2016/2017 growing season, number of pods per plant (NPP) of the cultivar NA 5909 and TMG 7062 did not differ among rust management strategies, even in relation to the control (Table 7). In the same way, during the 2017/2018 growing season, there were no differences among cultivars tested or even with respect to rust management strategies for the NPP variable. These similarities among cultivars and treatments for the NPP may explain, therefore, the lack of difference on soybean grain yield.

When analyzing each treatment tested for Asian rust among the cultivars, it was found that, in general, the cultivars did not differ among each other for the NPP, as the treatments with PC1, PC3, PC4, PC5 and PC8 showed no statistically significant differences (Table 7).

Number of grain per plant (NGP) showed the same trend observed for the NPP, in which, in general, there

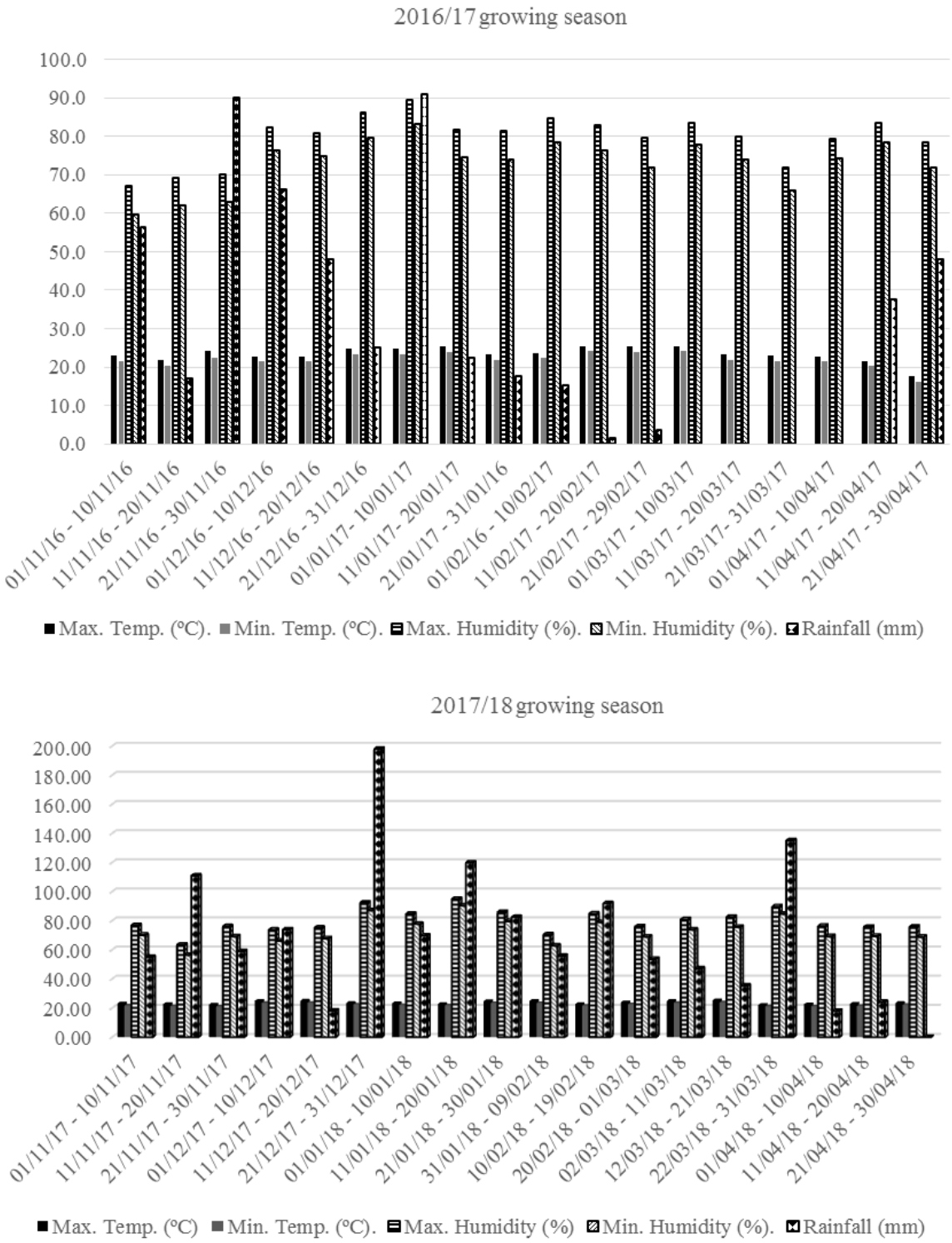

Figure 1. Maximum and minimum average temperature, maximum and minimum average air humidity and rainfall at Dois Vizinhos/PR, in the 2016/2017 and 2017/2018 growing season.
Source: Adapted INMET (2018).

was no difference between Asian rust managements (similar in both 2016/2017 and 2017/2018 growing seasons), as well as between cultivars for each treatment. For the cultivars that differed, it was not possible to establish a pattern, as they did not differ from the control (Table 7).

Table 8. Soybean thousand grain weight (TGW) of three soybean cultivars submitted to eight product combinations for the management of Asian rust, at the 2016/17 and 17/18 growing seasons.

PC*	2016/17 growing season			2017/18 growing season		
	NA 5909	LG 60163	TMG 7062	NA 5909	LG 60163	TMG 7062
1*	148.7^{abcC}	193.2^{abA}	175.9^{aA}	164.3^{aC}	232.5^{aA}	212.9^{aB}
2	156.2^{abC}	203.7^{aA}	179.0^{aB}	165.3^{aC}	227.3^{aA}	207.3^{aB}
3	153.5^{abC}	200.3^{aA}	174.7^{aB}	165.0^{aC}	238.3^{aA}	217.5^{aB}
4	158.7^{aC}	198.2^{aA}	176.1^{aB}	165.0^{aB}	223.2^{abA}	212.7^{aA}
5	147.6^{abcB}	183.1^{bA}	172.3^{aA}	143.7^{aB}	213.0^{abA}	197.6^{aA}
6	144.9^{abcC}	194.8^{abA}	173.2^{aB}	145.2^{aB}	185.7^{cA}	198.0^{aA}
7	143.0^{bcC}	196.7^{abA}	183.4^{aB}	142.0^{aC}	217.7^{abA}	197.9^{aB}
8	138.1^{cB}	191.7^{abA}	186.2^{aA}	142.3^{aB}	197.8^{bcA}	192.9^{aA}
CV (%)		3.28			4.24	

Means followed by uppercase letters in the row and lowercase letters in the column differ from each other by the Duncan Test at 5% probability. [1]*PC (Product combinations) 1: Fungicide – Fung (R_1) and Fung ($R_{5.1}$); 2: Fung + Potassium phosphite A - PPh A (R_1) and Fung + Potassium phosphite B - PPh B ($R_{5.1}$); 3: Fung + PPh A + Copper foliar fertilizer – Cu (R_1) and Fung + PPh B ($R_{5.1}$); 4: Fung+ PPh A (R_1) and Fung + PPh B + Cu ($R_{5.1}$); 5: PPh A (R_1) and PPh B ($R_{5.1}$); 6: PPh A + Cu (R_1) and PPh B ($R_{5.1}$); 7: PPh A (R_1) and PPh B + Cu ($R_{5.1}$); 8: control, without application.

Relating soybean cultivar NA 5909 thousand grain weight (TGW) with rust managements, it is possible to observe (2016/17 growing season) that the treatment with only fungicide (PC1) or its association use (PC2, PC3, PC4), did not differ from the treatments that received only foliar fertilizers (PC5, PC6); except for PC7, which differed only from PC4. Comparing to the control treatment, TGW was lower than the treatments that received fungicide and potassium phosphite fertilizers (PC2, PC3 and PC4) (Table 8).

For the cultivar LG 60163, treatments that combined applications of fungicide and foliar fertilizers (PC2, PC3, PC4) showed higher TGW than the treatment that received only phosphites (PC5- PPh A in R_1 and PPh B in R_5). However, all these treatments did not differ from the others, including the control (PC8). For cultivar TMG 7062, TGW did not vary among the treatments tested for Asian rust management (Table 8) which might be explained due to the higher tolerance of this cultivar to the Asian rust disease.

Comparing the TGW among cultivars within the Asian rust managements, it was possible to verify that, in general, the cultivar LG 60163 presented the highest values for most treatments (PC2, PC3, PC4, PC6, PC7), while similar results were observed in the 2017/2018 growing season.

Despite some differences between cultivars, for some treatments, the genetic factor prevailed for TGW in which the NA 5909 cultivar has lower TGW compared to TMG 7062 and LG 60163, which showed similar results, and are in accordance with seed breeders. It was possible to establish that the TGW was not particularly affected by the treatments, since, the control value was similar to most of the others treatments (Table 8). Similarity of soybean yield components probably resulted in similar grain yield too (Table 9).

DISCUSSION

The AUDPC of Asian soybean rust was lower in treatments that received only fungicide, with similar results when it was combined with phosphites and copper-based foliar fertilizer. Silva et al. (2013), when evaluating different sources of phosphites compared to the use of fungicides, with regard to the AUDPC of the disease, found results similar to those observed in the present study; indicating that the use of phosphites for the management of Asian rust in general is not necessary, since the combined use of phosphites with the fungicide did not increase the efficiency of the latter. However, the results obtained by Andrade Júnior et al. (2015), using foliar fertilizer at a concentration of 35.1% P_2O_5 and 25.7% K_2O, in a program combined with fungicides, for the management of Asian soybean rust, proved to be promising. The application combinations occurred in stages R_1, R_3, $R_{5.1}$ and $R_{5.3}$, in the south of Mato Grosso State, Brazil, resulting in a reduction of the disease severity and an increase in grain yield.

Meneghetti et al. (2010), in studies carried out in a greenhouse, obtained results that indicated that the potassium phosphite used in isolation did not induce the manifestation of defense mechanisms, not decreasing the severity of Asian rust and the AUDPC, regardless of the cultivar used. When fungicides were applied to control the disease, the AUDPC was reduced in relation to the control, to a value between 58 and 63%. The use of potassium phosphites did not result in significant data when associated with fungicides, similar to the results of the present study.

With the closure of the canopy at the beginning of soybean flowering, it is expected that the severity of Asian rust will be higher in the lower part of the plants, and lower in the upper part, due to the fact that conditions

Table 9. Soybean grain yield (Kg ha^{-1}) of three cultivars at two growing season and in relation to eight product combinations for the management of Asian rust, at 2017/18 growing season.

Cultivar	2016/2017 growing season	2017/2018 growing season
	Grain yield (kg ha^{-1})	Grain yield (kg ha^{-1})
NA 5909	4728.2[a]	3849.1[a]
LG 60163	5056.0[a]	4211.5[a]
TMG 7062	4608.1[a]	4093.5[a]
CV (%)	7.58	6.70

	2017/2018 growing season							
PC*	1	2	3	4	5	6	7	8
Grain yield (kg ha^{-1})	4248[a]	4215[a]	4252[a]	4285[a]	3778[a]	3845[a]	3993[a]	3796[a]

Means followed by distinct letters differ from each other by the Duncan Test at 5% probability. CV (%) of 6.7 at the 2017/18 growing season. ¹*PC (Product combinations) 1: Fungicide – Fung (R_1) and Fung ($R_{5.1}$); 2: Fung + Potassium phosphite A - PPh A (R_1) and Fung + Potassium phosphite B - PPh B ($R_{5.1}$); 3: Fung + PPh A + Copper foliar fertilizer – Cu (R_1) and Fung + PPh B ($R_{5.1}$); 4: Fung+ PPh A (R_1) and Fung + PPh B + Cu ($R_{5.1}$); 5: PPh A (R_1) and PPh B ($R_{5.1}$); 6: PPh A + Cu (R_1) and PPh B ($R_{5.1}$); 7: PPh A (R_1) and PPh B + Cu ($R_{5.1}$); 8: control, without application.

favorable to the disease are created in this microenvironment, such as longer leaf wetting time, less air circulation and less incidence of solar radiation. These factors combined end up impairing the efficiency of the application of fungicides for the management of the disease, especially in the lower part (Cunha et al., 2016; Moura et al., 2017; Weber et al., 2017). The time of exposure to light directly affects the spore germination and the growth of the *P. pachyrhizi* germ tube. In a study by Blum et al. (2015), it was found that spores submitted to the dark had a higher germination rate. Therefore, as the leaves of the upper part (and even of the middle part, depending on the cultivar and leaf area index) are more exposed to the sun, they tend to suffer less than the leaves of the lower part.

In the comparison between cultivars, the lowest AUDPC value of Asian soybean rust was found in the TMG 7062 cultivar, resistant to the pathogen, followed by the cultivar considered tolerant (LG 60163 cultivar). These data corroborate those reported by Almeida et al. (2013). When evaluating the behavior of soybean lines and cultivars in Rolim de Moura, Rondônia, regarding the susceptibility and resistance to leaf diseases, Almeida et al. (2013) found that genotypes without genetic resistance are more susceptible to the attack of soybean rust; culminating, also, in a greater source inoculum in the field. In research by Hoffmann et al. (2019), to evaluate the effect of genetic resistance and fungicide management on Asian soybean rust, it was found that the resistant cultivar contributed to reducing the severity of the disease, compared to the susceptible cultivar, which increased efficiency of the applied fungicides; evidencing the importance of the association of strategies.

According to Maphosa et al. (2013), the development of genotypes resistant to Asian rust has been a challenge, due to the instability associated with vertical resistance, that is easily broken, and the difficulties associated with the identification of horizontal resistance. Therefore, different methods have been adopted for plant breeding, such as the study of genetic tolerance (Inayati and Yusnawan, 2016). Even with the evolution of soybean breeding in Brazil, inadequate management strategies favor the development of more resistant pathogens. The application of fungicides is still the most efficient measure to control diseases, according to Oliveira et al. (2015).

Evaluating 17 soybean genotypes in a study carried out in a greenhouse, Glanesapp et al. (2015) found that the lowest averages of AUDPC in plants inoculated with *Phakopsora pachirhizi*, occurred in resistant cultivars. In general, grain yield was not influenced by the treatments tested, in both growing seasons, supported by the similarity in the NPP and NGP components. Melo et al. (2015), seeking to evaluate the tolerance of soybean genotypes to Asian rust, succeeded to discriminate cultivars regarding this trait of interest, verifying that the BRS 239 cultivar was tolerant to the fungus, since its productivity did not change due to the presence or absence of chemical management, showing productive stability in the face of the disease.

When assessing the timing of application of fungicides on soybeans, in different crops, based on the detection of Asian soybean rust in the area, Nascimento et al. (2018) also did not observe the effect of disease management on grain yield, in one of the evaluated crops, even though there was a greater mass of a thousand grains and less defoliation in treatments that received fungicide. In Argentina, in a trial carried out on three growing seasons (2014/2015, 2015/2016 and 2016/2017) aiming to evaluate different fungicides and application times, for the management of Asian soybean rust, it was found that the results varied depending on the crop year and the fungicides tested (Reznikov et al., 2019). In general, the

sequential application in R3 + R5 and the isolated application in R5 were promising in comparison to the control, without fungicide.

A possible explanation for the absence of difference in grain yield between cultivars, even if they differed for TWG and NGP, with lower values for cultivar NA 5909, is that such a cultivar should be cultivated in a population of plants greater than that recommended for the cultivars TMG 7062 and LG 60163. For the cultivar NA 5909, its cycle and height are smaller, its leaves are triangular and its architecture is more compact. Thus, the use of greater population density induces greater height, with a compensatory effect.

According to Debortoli et al. (2012), the differential plant architecture between soybean cultivars, with regard to their size, branching, type of leaf and foliar angle, can be a complicating factor to the correct penetration of products in the canopy at the time of application. In addition, the leaf area index is also of fundamental importance in the process (Barbosa et al., 2019). In the soybean adaptation region where the study was conducted, the three cultivars tested, especially LG 60163 and TMG 7062, are considered to have a longer cycle. Even so, in both crop years, the severity of the disease in the most affected treatments was not more than 30% in the development stage $R_{5.3}$, the last severity assessment carried out.

Thus, in regions with climatic conditions similar to those of the research, the excessive use of fungicides for Asian rust in crops sown until the end of October, with cultivars of maturation groups around 6.2, is not justified. Under these conditions, the control of Asian soybean rust must be managed with fewer applications of fungicides and more rational use of them, according to the careful monitoring of the crop.

Despite all Asian rust management strategies aiming not to compromise grain yield, it is also important to consider reducing the inoculum source in the area, which can contribute to less fungus pressure and, thus, minimize possible breaks in resistance to fungicides and resistant cultivars, maintaining the sustainability of technologies for a longer period of time. Thus, the use of foliar fertilizers based on phosphites, combined with fungicides, can be considered.

Conclusions

The genetics of resistance and tolerance to Asian soybean rust contributed to less progress of the disease in plants.

The severity of Asian rust was higher in the susceptible cultivar, as well as in the lower part of the plants, of the three cultivars.

Even with differential responses between cultivars and treatments applied to the severity of Asian soybean rust, the disease did not compromise grain yield in both growing seasons.

Under the conditions of this study, the control of Asian soybean rust should be managed with fewer applications of fungicides and more rational use of them, according to the careful monitoring of the crop.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Aguiar RA, Cunha MG, Araújo FG, Carneiro LC, Borges EP, Carlin VJ (2016). Efficiency loss of recorded fungicides for the control of asian soybean rust in central region of Brazil. Revista de Agricultura Neotropical 3(2):41-47.

Andrade Júnior ER, de Cassetari Neto D, Machado AQ, Villela PMC de A (2015). Programs of foliar fertilization using silicon and potassium associated with fungicides to control asian rust in soybeans. Bioscience Journal 31(1):18-24.

Almeida FA, Bruscke EL, Polizel AC, Petter FA, Hamawaki OT, Alcantara Neto F de (2013). Desempenho agronômico de linhagens e cultivares de soja frente a doenças foliares. Revista de Ciências Agrárias 56(2):88-94.

Barbosa GF, Centurion MAPDC, Ferreira MDC, Alves PLDCA (2019). Fungicide spray coverage and deposition in soybean Asian rust management. Bioscience Journal 35(5):1422-1431.

Blum MMC, Reis EM, Vieira FT, Carlini R (2015). In vitro effect of substrate, temperature and photoperiod on Phakopsora pachyrhizi urediniospore germination and germ tube growth. Summa Phytopathologica 41(2):101-106.

Cunha JPRC, Marques RS, Alves GS (2016). Deposição da calda na cultura da soja em função de diferentes pressões de trabalho e pontas de pulverização. Ceres 63(6):761-768.

Cruz CD (2008). *Programa Genes:* Diversidade Genética. Editora UFV: Viçosa. 278p.

Dalla Lana F, Ziegelmann PK, Maia AHN, Godoy CV, Del Ponte EM (2015). Meta-Analysis of the Relationship Between Crop Yield and Soybean Rust Severity. Phytopathology 105(3):307-315.

Debortoli MP, Tormen NR, Balardin RS, Dalla Favera D, Stefanello MT, Frigo Pinto F, Uebel JD (2012). Espectro de gotas de pulverização e controle da ferrugem-asiatica-da-soja em cultivares com diferentes arquiteturas de planta. Pesquisa Agropecuária Brasileira 47(7):920-927.

Embrapa (2006). Centro Nacional de Pesquisas de solo. Sistema brasileiro de classificação de solos – 2.ed. – Rio de Janeiro: Embrapa solos. 306p.

Glanesapp JS, Sediyama T, Cruz CD, Matsuo E, Brommonachenkel SH, Oliveira R de CT, Hamawaki OT (2015). Diversidade de características agronômicas e moleculares em cultivares de soja com diferentes graus de resistência à *Phakopsora pachyrhizi.* Bioscience Journal 31(1):25-36.

Godoy CV, Seixas CDS, Soares RM, Meyer MC, Costamilan LM, Adegas FS (2017). Boas práticas para o enfrentamento da ferrugem-asiática da soja. Comunicado Técnico 92. Embrapa Soja, Londrina, PR.1-6. Available at:

http://acacia.cnpso.embrapa.br:8080/cferrugem_files//1568669683/ComTec92.pdf
Godoy CV, Seixas CDS, Soares RM, Marcelino-Guimarães FC, Meyer MC, Costamilan LM (2016). Asian soybean rust in Brazil: Past, present, and future. Pesquisa Agropecuária Brasileira 51(5):407-421.
Godoy CV, Koga LJ, Canteri MG (2006). Diagramatic scale for assessment of soybean rust severity. Fitopatologia Brasileira 31(1):63-68.
Hartman GL, Sikora EJ, Rupe JC (2015). Rust. In: Hartman GL, Rupe JC, Sikora EJ, Domier LL, Davis JA, Steffey KL (eds.), Compendium of soybean diseases and pests. 5th ed. St. Paul: American Phytopathological Society pp. 56-58.
Inayati A, Yusnawan E (2016). Characterization of soybean genotypes for Asian soybean rust reaction under screen house condition. Biodiversitas 17(2):609-613.
Hoffmann LL, Roehrig R, Boller W, Forcelini CA (2019). Chemical control of Asian soybean rust as a function of cultivar, row spacing and spray bar support systems. Engenharia Agrícola 39(4):504-511.
IAPAR (2009). Cartas climáticas do Estado do Paraná. Londrina: IAPAR.
INMET - Instituto Nacional de Meteorologia (2018). Estações automáticas, Dois Vizinhos. Available at: http://www.INMET.gov.br
Klosowski AC, May de Mio LL, Miessner S, Rodrigues R, Stammler G (2016). Detection of the F129L mutation in the cytochrome b gene in *Phakopsora pachyrhizi*. Pest Management Science 72:1211-1215.
Maphosa M, Talwana H, Tukamuhabw AP (2013). Assessment of comparative virulence and resistance in soybean using field isolates of soybean rust. Journal of Agricultural Science 5(5):249-257.
Meneghetti RC, Balardin RS, Dalla Corte G, Dalla Favera D, Debona D (2010). Avaliação da ativação de defesa em soja contra *Phakopsora pachyrhizi* em condições controladas. Ciência e Agrotecnologia 34(4):823-829.
Melo CLP, de Roese AD, Goulart ACP (2015). Tolerância de genótipos de soja à ferrugem asiática. Ciência Rural 45(8):1353-1360.
Moura B, Deuner CC, Visintin G, Boller W (2017). Use of a canopy opener in fungicide applications to improve Asian soybean rust control. Transactions of the ASABE 60(6):1819-1825.
Nascimento JN, Gavassoni WL, Bacchi LMA, Oliveira JL, Laborde MC, Pontin BCA, Mendes MP (2018). Manejo da ferrugem asiática da soja com aplicação de fungicidas iniciadas na detecção do patógeno ou posteriores. Revista Agrarian 11(39):42-49.
Neves J da S, Blum LEB (2014). Influência de fungicidas e fosfito de potássio no controle da ferrugem asiática e na produtividade da soja. Revista Caatinga 27(1):75-82.
Oliveira GM, de Pereira DD, Camargo LCM, de Saab OJGA (2015). Fosfito e silicato de potássio no controle da ferrugem asiática da soja (*Phakopsora pachyrhizi* Syd. & P. Syd). Agrária - Revista Brasileira de Ciências Agrárias 10(1):60-65.
Reznikov S, Lisi V, de Claps P, Gonzáles V, Devani MR, Castagnaro AP, Ploper DL (2019). Evaluation of the efficacy and application timing of different fungicides for management of soybean foliar diseases in northwestern Argentina. Crop Protection 124(104844):1-8.
Simões K, Hawlik A, Rehfus A, Gava F, Stammler G (2017). First detection of a SDH variant with reduced SDHI sensitivity in Phakopsora pachyrhizi. Journal of Plant Diseases and Protection 125:21-26.
Silva OC, Santos HAA, Deschamps C, Dalla Pria M, May de Mio LL (2013). Fontes de fosfito e acibenzolar-S-metílico associados a fungicidas para o controle de doenças foliares na cultura da soja Tropical Plant Pathology 38(1):72-77.
Weber NC, Santos EM, Russini A, Silva FF (2017). Deposição de calda ao longo do dossel na cultura da soja utilizando pulverizador equipado com controlador de fluxo. Engenharia na Agricultura 25(5):459-468.
Zuntini B, Alvarez R de CF, Faria TG, de Zuffo AM (2019). Effect of adding fungicide to mixtures of triazoles and strobilurins in the control of downy mildew and Asian soybean rust. Pesquisa Agropecuária Tropical 49(e53688):1-9.

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Races of *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara in major bean growing regions in Tanzania

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Breeding for resistant varieties has been shown to be the most suitable method to control bean anthracnose caused by *Colletotrichum lindemuthianum* though the method is challenged by the existence of many races of the pathogen. This work focused on characterizing races of *C. lindemuthianum* from potential bean agro ecological zones of Tanzania using a set of differential bean cultivars. From 144 anthracnose infected bean samples collected, 50 pure isolates were obtained and characterized whereby 42 races were identified. The most virulent race identified was race 3610 from the Southern Highland zone of Tanzania while the least virulent was characterized as race 0. Race 2 was the most widely spread (4.2 %) found in Northern, Southern highland and in Eastern zones of Tanzania. The work confirms that G2333 can still be used as a potential donor of resistant genes to varieties that are to be grown in Northern, Eastern and Lake zones but not for the varieties from Southern Highland and Western zones of Tanzania since isolates from these zones overcame resistant genes *Co-4*^{2}*, Co-5, Co-7* in G2333. 95% of all races which were identified are new and were not specific to either Mesoamerican or Andean origin common bean.

Key words: *Colletotrichum lindemuthianum,* races, resistant genes, differential cultivars, Anthracnose.

INTRODUCTION

Bean anthracnose is one of the most serious diseases of common bean caused by the fungal pathogen *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara in areas where common bean is grown. The disease has been reported to cause losses of up to 100% if not well controlled (Opio et al*.,* 2001; Markell et al., 2012). In Tanzania, the work of Allen et al*.* (1998) in Northern Tanzania reported that, for each 1% increase in anthracnose incidence, seed yield decreases by 9 kg/ha while Shao and Teri (1985) and Mwalyego (1991) reported loss due to anthracnose to be up to 100% if the disease is not well controlled. According to Opio et al*.* (2001), the effective control measures to reduce losses caused by bean anthracnose is the use of resistant varieties. This has been proven to be cost effective and requires less skill for farmers to apply it (Mahuku et al.,

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2002; Abraham and Mashilla, 2018).

Several genes from both Meso-Americans and Andean bean origin have been shown to offer resistance to anthracnose disease of common bean to anthracnose disease (Méndez-Vigo et al*.,* 2005). The Mesoamerican genes include *Co-2, Co-3* (and its alleles *Co-3*^{2}*, Co-3*^{3}*, Co-3*^{4}*,* and *Co-3*^{5}), *Co-4* (and it alleles *Co-4*^{2}*, Co-4*^{3}), *Co-5* (and its *allele Co-5*^{2}), *Co-6, Co-11, Co-16, Co-17, Co-u,* and *Co-v* while the Andean genes include: *Co-1* (and its alleles *Co-1*^{2}*, Co-1*^{3,} *Co-1*^{4}, and *Co-1*^{5}), *Co-12, Co-13, Co-14, Co-15, Co-x, Co-w, Co-y,* and *Co-z* (Kelly and Vallejo, 2004; de Lima Castro et al*.,* 2017). But the effective choice and introgression of resistant genes to the host (common bean) in order to develop a resistant variety depends on the knowledge of the variability of *C. lindemuthianum*.

Mahuku and Riascos (2004), Kelly and Vallejo (2004), Silva et al*.* (2007), and Vidigal et al*.* (2007) reported that *C. lindemuthianum* has a high rate of evolution resulting in emergence of the new race of the pathogen from time to time. This causes resistance breakdown for the already developed resistant varieties because the resistance of common bean to *C*. *lindemuthianum* is very specific, so that a resistance gene from a bean genotype confers protection against specific isolates or races of *C. lindemuthianum.*Therefore, before breeding for anthracnose resistant varieties, it is important to study and identify the available races of *C. lindemuthianum* which is helpful in planning suitable breeding strategies for varieties with durable resistance and to design effective gene pyramids and deployment of resistant cultivars over time and space.

In Tanzania, although some of the works to develop resistant varieties to bean anthracnose have been done, information on *C. lindemuthianum* variability and the race identification is still limited for effective breeding for the anthracnose disease. Therefore, the present work focused on studying the variability of *C. lindemuthianum* in major bean growing regions of Tanzania and identifying races of *C. lindemuthianum* available in such areas using differential bean cultivars as proposed by Pastor-Corrales (1991).

MATERIALS AND METHODS

Collection of bean sample infected with anthracnose

Collection of common bean samples infected with anthracnose disease was done in five bean growing zones in Tanzania (Figure 1) during 2016/2017 bean growing season. Selection of zone was based on potential common bean production. In each zone, the distance between sampling fields was separated by a distance of 2 to 5 km. A minimum of five fields were sampled per zone. In the field, quadrants of 3 m x 3 m were used to randomly collect anthracnose symptomatic bean plants. A total number of four subplots identified by quadrants per field were established and, in each subplot four to nine plants were sampled. The collected infected plant parts were pods, leaves and stems. The pod samples collected were pressed well and stored in the herbarium, while other bean pods of the same plant were preserved in silica gel. Leaves and stems were pressed well in the herbarium before they were taken to the plant pathology laboratory at Sokoine University of Agriculture, Morogoro for isolation and characterization of *C. lindemuthianum*.

Pathogen isolation and inoculum preparation

Pathogen isolation was performed from the well-developed anthracnose diseased bean pods and leaves. Selected samples were sterilized for two minutes using 2% sodium hypochlorite (NaOCl) solution and rinsed well using sterile distilled water. Small cuts of 5cm long were made on the symptomatic areas and the cut placed on the prepared V8 growth media (17 g micro agar, 3 g of calcium carbonate, 200 ml of V8 juice and 800 ml of distilled water mixed together and autoclaved at 120°C and 2 bars of pressure for 15 min). The culture media were then incubated in the dark room at 22 to 24°C for seven days. From well grown isolates, mono-conidial isolates were obtained by picking pin-point colonies from the media using a sterile needle, and then sub-cultured on fresh V8 medium for purification of cultures using the single spore technique; and then single spore isolates were incubated aseptically. The standard spore suspensions for each obtained fungal isolate were prepared following the procedure of (Pastor-Corrales et al*.,* 1995) and were stored at -4°C before inoculation to the differential bean cultivar.

Raising differential cultivars and inoculation

A set of differential cultivars as described by Pastor-Corrales (1991) (Table 1) obtained from the Bean Improvement Program at Sokoine University of Agriculture (SUA) were planted in pots in the screen house at the Department of Crop Science and Horticulture, SUA. At fourteenth days after sowing, each differential cultivar seedling was inoculated separately by standard *C. lindemuthianum* inoculum prepared from the obtained isolate. The spore concentration used were quantified and adjusted to spore concentrations of 1.2×10^{6} spores/ml (Pastor-Corrales et al*.,* 1995) using the hemocytometer (Neubauer-Preciss Chamber) and the light microscope. The inoculation was done using the hand sprayer and the experiment was replicated three times.

In order to maintain the relative humidity of approximately 95% for the infection to take place, each plant was covered by the transparent polythene sheets for four days after inoculation followed by regular management such as watering and fertilizer application.

Data collection and data analysis

Symptom evaluation and scoring of anthracnose disease severity on inoculated bean plants was done eight days after inoculation using a scale of 1-9 previously used by Balardin et al. (1990). The average disease scores were obtained and the disease reaction type categories were determined. Cultivars with scores of 1-3 were regarded as resistant while cultivars with scores of 4-9 were regarded as susceptible (Barladin et al*.,* 1990). Race identification was done as the sum of binary numbers of all differential cultivars showing susceptible reaction to the particular isolate.

RESULTS

Phenotypic characterization of *C. lindemuthianum* done by using set of differential cultivars as proposed by Pastor-Corrales (1991) was able to identify 50 isolates

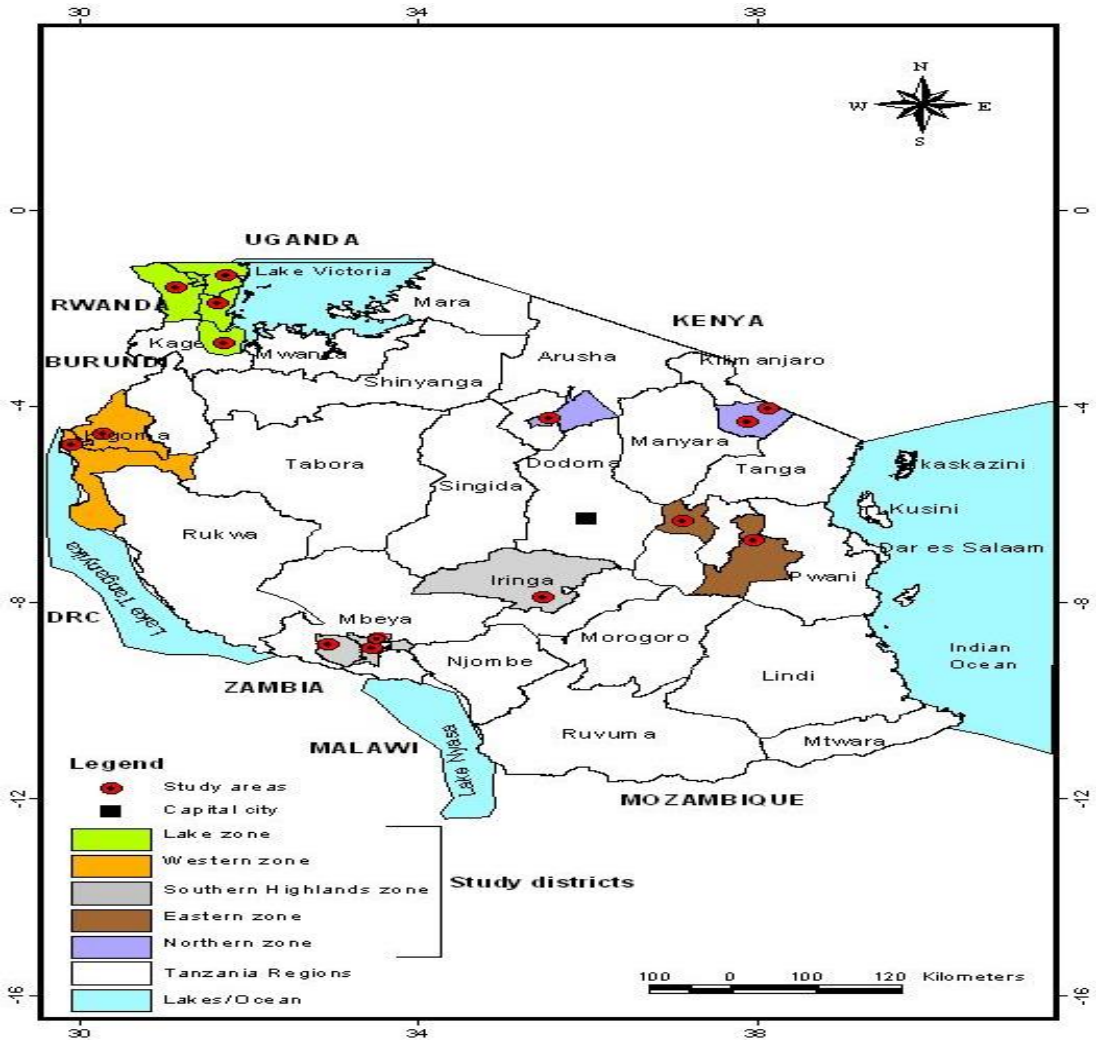

Figure 1. The map of Tanzania showing regions where isolates where collected.

Table 1. Anthracnose differential series, resistance genes, host gene pool, and the binary number of each cultivar used to characterize races of anthracnose in common bean.

Differential cultivar	Host genes	Place of cultivar	Gene pool	Binary number	Growth habit
Mitchelite	-	0	MA	1	II
Michigan Dark Red Kidney	*Co1*	1	A	2	II
Perry marrow	*Co-1*^{3}	2	A	4	II
Cornel 49242	*Co-2*	3	MA	8	II
Widusa	*Co-9*	4	A	16	II
Kaboon	*Co-1*^{2}	5	A	32	II
Mexico 222	*Co-3*	6	MA	64	II
PI	*Co-4*^{3}, *Co-9*	7	MA	128	III
TO	*Co-4*	8	MA	256	I
TU	*Co-5*	9	MA	512	III
AB136	*Co-6*, *Co-8*	10	MA	1024	IV
G2333	*Co-4*^{2}, *Co-5, Co-7*	11	MA	2048	IV

MA= Middle American gene pool; A= Andean gene pool of Phaseolus vulgaris. Binary numbers: 2n, where n is equivalent to the place of the cultivar within the series (0-11). Growth habit: I = Determinate; II = Indeterminate bush; III = Indeterminate bush with weak main stem and prostrate branches; IV = Indeterminate climbing habit.
Source: Pastor-Corrales (1991).

Table 2. The number of isolates and races of *Colletotrichum lindemuthianum* identified in each zone by phenotypic characterization.

Zone	Number of isolates	Races identified
Eastern zone	6	2, 31, 39, 129, 716, 770
Northern Zone	10	0, 2, 62, 91, 167, 277, 344, 524
Western Zone	15	0, 105, 166, 191, 274, 398, 661, 832, 849, 1510, 1805, 1891, 2061, 2614, 3068
Lake zone	8	0, 9, 101, 112, 128, 133, 1176, 1271
Southern Highland Zone	11	0, 2, 128, 316, 776, 944, 1696, 2434, 2566, 3264, 3610

from 144 bean samples collected. Of these 50 isolates obtained, 15 were from Western zone, 8 from Lake zone, 10 from Northern zone, 11 from Southern highland zone and 6 isolates were from Eastern zone (Table 2). Further it was possible to identify 42 races out of 50 isolates obtained (Tables 2 and 3). The most virulent race was race 3610 which was collected from the Southern Highland zone while the least virulent isolate was characterized as race 0, found in the Northern, Lake, Western and Southern Highland zones (Tables 2 and 3). From the Eastern zone, six isolates of *C. lindemuthianum* were identified out of all samples which were collected (Table 2). No isolate from the Eastern zone was able to infect the differential cultivar G2333 while differential cultivar Michigan Dark Red Kidney was the most affected differential cultivar with the four isolates. From the Eastern zone, the least virulent isolate was race 2 while the most virulent was characterized as race 770 which affected the differential cultivars Michigan Dark Red, TO, TU and AB136 (Table 3). From the Northern bean growing zone of Tanzania, among a total of 10 isolates collected, 8 races of *C. lindemuthianum* isolates were characterized (Table 2). These isolates affected almost all the differential cultivars except AB136 and G2333. Differential cultivars Michigan dark red kidney, Perry Marrow, Cornell and Widusa were highly infected with four isolates from the northern zone. The most virulent isolate was characterized as race 524 while the least was characterized as race 0 (Table 3). From the western zone, 15 isolates were obtained (Table 2). All differential cultivars were affected by these isolates including the most resistant differential cultivar G2333, which was affected by two isolates while Perry Marrow was the most affected differential cultivar. From this zone, the most virulent race was race 3068 while the least virulent race was race 0 (Table 3).

A total of eight isolates of *C. lindemuthianum* were obtained from the Lake zone (Table 2). These isolates could not affect differential cultivars G2333, TO and TU while Michelite was the most affected differential cultivar by four isolates (Table 3). The most virulent isolate from the Lake zone was characterized as race 1271 while the least virulent isolate from this zone was characterized as race 05 (Tables 2 and 3). From the Southern highland zone, 11 isolates were obtained in which (Table 2). Michelite was the only differential cultivar not affected by any isolate from this zone; while TU was the most affected differential cultivar affected by four isolates (Table 3). Isolates from this zone were able to infect the differential bean cultivar G2333 (Table 3). The most virulent isolate from the Southern Highland zone was characterized as race 3610 which infected differential bean cultivars Widusa, TU, AB136 and G2333; while the least virulent isolate was characterized as race 0 (Table 3).

Among the differential bean cultivars used, Perry Marrow was the most susceptible differential cultivar and it was infected by 42% of all *C. lindemuthianum* isolates collected while differential bean cultivar G2333 was the most resistant cultivar to most of the collected isolates; it was affected by only 14 % of isolates collected, and these were from the Western and Southern Highland zones (Tables 2 and 3).

Of all the races identified in bean growing zones of Tanzania, race 2 was the most common with high frequency of 4.8 % and widely distributed within bean growing zones; and it was distributed in Northern, Southern Highland and in Eastern zones, while race 128 was only distributed in Lake and Southern Highland zones. Race 3610 collected from the Southern Highland zone was the most virulent isolate compared to all isolates collected from bean growing zones while the least virulent race identified was characterized as race 0.

DISCUSSION

The phenotypic characterization of *C. lindemuthianum* in major bean growing areas in Tanzania revealed the existence of races and the variability of *C. lindemuthianum*. This study reveals new races of *C. lindemuthianum* which were not reported before. Earlier studies by Ansari et al. (2004) identified five races (0, 2, 12, 38 and 192) of *C. lindemuthianum*in Tanzania whereby only races 0 and 2 were also reported by this work distributed in all five zones.

This study confirms the evolution of *C. lindemuthianum* with time in Tanzania. The work of Mwalyego (1991) identified races 6, 28, 60, 63, 98, 155, 182, 287, 618, 958, 1478, 1515 and 1678 whereby out of them no races were reported in this study.

In this study 96% of the races identified from the

Table 3. Reaction of differential cultivars to the isolates of *Colletotrichum lindemuthianum* and identification of races.

Isolate number	Differential cultivars												
	MT	MDR	PM	CL	WD	KB	Mex	PI	TO	TU	AB 136	G2333	Race name
LZ02	R	R	R	R	R	R	R	R	R	R	R	R	0
WZ06	R	R	R	R	R	R	R	R	R	R	R	R	0
NZ06	R	R	R	R	R	R	R	R	R	R	R	R	0
NZ07	R	R	R	R	R	R	R	R	R	R	R	R	0
SHZ01	R	R	R	R	R	R	R	R	R	R	R	R	0
NZ09	R	R	R	R	R	R	R	R	R	R	R	R	0
NZ10	R	S	R	R	R	R	R	R	R	R	R	R	2
SHZ09	R	S	R	R	R	R	R	R	R	R	R	R	2
EZ02	R	S	R	R	R	R	R	R	R	R	R	R	2
LZ07	S	R	R	S	R	R	R	R	R	R	R	R	9
EZ03	S	S	S	S	S	R	R	R	R	R	R	R	31
EZ06	S	S	S	R	R	S	R	R	R	R	R	R	39
NZ05	R	S	S	S	S	S	R	R	R	R	R	R	62
NZ08	S	S	R	S	S	R	R	R	R	R	R	R	91
LZ03	S	R	S	R	R	S	S	R	R	R	R	R	101
WZ01	S	R	R	S	R	S	S	R	R	R	R	R	105
LZ05	R	R	R	R	S	S	S	R	R	R	R	R	112
LZ08	R	R	R	R	R	R	R	S	R	R	R	R	128
SHZ08	R	R	R	R	R	R	R	S	R	R	R	R	128
EZ01	S	R	R	R	R	R	R	S	R	R	R	R	129
LZ01	S	R	S	R	R	R	R	S	R	R	R	R	133
WZ10	R	S	S	R	R	S	R	S	R	R	R	R	166
NZ01	S	S	S	R	R	S	R	S	R	R	R	R	167
WZ09	S	S	S	S	S	S	R	S	R	R	R	R	191
WZ05	R	S	R	R	S	R	R	R	S	R	R	R	274
NZ03	S	R	S	R	S	R	R	R	S	R	R	R	277
SHZ10	R	R	S	S	S	S	S	R	S	R	R	R	316
NZ04	R	R	R	S	S	R	S	R	S	R	R	R	344
WZ03	R	S	S	S	R	R	R	S	S	R	R	R	398
NZ11	R	R	S	S	R	R	R	R	R	S	R	R	524
WZ11	S	R	S	R	S	R	R	S	R	S	R	R	661
EZ04	R	R	S	S	R	R	R	S	R	R	R	R	716
EZ05	R	S	R	R	R	R	R	R	S	S	R	R	770
SHZ03	R	R	R	S	R	R	R	R	S	S	R	R	776
WZ07	R	R	R	R	R	R	S	R	S	S	R	R	832
WZ13	S	R	R	R	S	R	S	R	S	S	R	R	849
SHZ07	R	R	R	R	S	S	R	S	S	S	R	R	944
LZ04	R	R	R	S	S	R	R	S	R	R	S	R	1176
LZ06	S	S	S	R	S	S	S	S	R	R	S	R	1271
WZ12	R	S	S	R	R	S	S	S	S	R	S	R	1510
SHZ06	R	R	R	R	R	S	R	S	R	S	S	R	1696
WZ15	S	R	S	S	R	R	R	R	S	S	S	R	1805
WZ14	S	S	R	R	R	S	S	R	S	S	S	R	1891
WZ04	S	R	S	S	R	R	R	R	R	R	R	S	2061
SHZ11	R	S	R	R	R	R	R	S	S	R	R	S	2434
SHZ02	R	S	S	R	R	R	R	R	S	S	R	S	2566
WZ08	R	S	S	R	S	S	R	R	R	S	R	S	2614
WZ02	R	R	S	S	S	S	S	S	S	S	R	S	3068
SHZ04	R	R	R	R	R	R	S	S	R	R	S	S	3264

Table 3. Contd.

SHZ05	S	R	R	R	S	R	R	R	R	S	S	S	3610

NZ = Northern Zone, SHZ = Southern Highland Zone; LZ = Lake Zone, WZ = Western zone and EZ = Eastern Zone; R = resistance reaction, S = Susceptible reaction; MT = Michelite, MDR = Michigan Dark Red Kidney, PM = Perry Marrow, CL = Cornell 49242, WD = Widusa, KB = Kaboon, Mex = Mexico 222, PI = PI 207262.

collected isolates have not been reported before in Tanzania. The previous work done by Ansari et al*.* (2004) to study the variability of *C. lindemuthianum* of isolates from Tanzania who also identified five races (0, 2, 12, 38 and 192) out of 8 isolates which were characterized and race 0 and 2 were common races which were also identified from this work. This could be due to the extensive collection of isolates which was done during this study; it covers large area growing common bean. Some new races could have evolved from when the last study was conducted in the country till now when this study was conducted. Most of the virulent isolates were found in areas where the weather condition favors the growth and development of the pathogen (Mohamed, 2013). For example, the most virulent isolate characterized as race 3610 was found in the Southern Highland zone. This zone was characterized by very cool and humid conditions to most of its areas during the bean growing season (Tanzania Meteorological Agency, 2017). Such conditions favor growth and developments of *C. lindemuthianum* which may influence its variability as it have been reported by Tu (1983) and Mohammed (2013).

This study shows that *C. lindemuthianum* in Tanzania is not specialized to either Meso-American or Andean common bean gene pool only. The collected isolates were able to affect differential bean cultivars of both Meso-American and Andean gene pool. For example isolate SHZ05 from the Southern Highland zone was able to overcome both Andean resistance gene *Co-9* in Widusa and Meso-American resistance genes (*Co-4*^{2}*, Co-5, Co*-7) in G2333, *Co-6* and *Co-8* in AB136 and *Co-5* in TU*.* Similar work of Balardin et al*.* (1990) and Balardin et al*.* (1997) indicated that some races contain virulence factors to both Meso-American and Andean gene pools and they infect both Andean and Meso-American bean gene pools. In the areas with large host diversity of both Mesoamerican and Andean at the same time it highly influences the pathogen diversity such as isolates collected from Western zone. Therefore, in order to develop the durable resistance variety, it is important to incorporate more than one resistant gene by gene pyramiding technique as it was also proposed by Balardin and Kelly (1998), Kelly and Vallejo (2004), Pastor-Corrales et al*.* (1995) from both Meso-American and Andean sources so as to provide the broader range and durable resistance variety.

Out of 12 differential cultivars used, G2333 was the most resistant differential bean cultivar affected by only 7 isolates from the Western and Southern Highland zones only. Previous work reported that the presence of three resistant genes (*Co-4*^{2}*, Co-5* and *Co-7*) in G2333 offer broader resistance to a wide range of *C. lindemuthianum* races (Young et al*.,* 1998; Mahuku et al., 2002). Therefore based on races identified in Northern, Eastern and the Lake zones, G2333 can be used as a potential donor for resistance genes in bean breeding program for the varieties to be grown in those areas, according to Mwalyego (1992a) who reported 15 separate isolates and none of them was pathogenic to G2333 bean cultivar. But varieties that are to be grown in the Southern Highland and Western zones should be incorporated with genes for resistance from other sources. de Lima Castro et al*.* (2017) reported Paloma cultivar as a new source of resistance gene to common bean anthracnose. This cultivar has a new dominant gene conferring resistance to anthracnose, which is independent of *Co-1, Co-2, Co-3*^{4}*, Co-4, Co-4*^{2}*, Co-4*^{3}*, Co-5, Co-6, Co-12, Co-13, Co-14, Co-15* and *Co-16* resistance genes identified previously; this gene is named *Co-Pa*. Therefore, in the future Paloma cultivar can be included in the list of differential cultivars before being recommended as the donor source of resistant genes to the cultivars grown in the Western and Southern Highland zone. Also more characterization work should be done on *(Co-Pa)* resistant genes in Paloma.

Conclusion

This study indicates that the pathogen, *C. lindemuthianum* which causes anthracnose disease, *C. lindemuthianum* shows variability in major bean growing areas of Tanzania. The pathogen seems to be widely distributed in all bean growing areas with the most virulent isolate from the Southern Highland zone. The best control method for the disease in these potential bean growing areas is using the host resistance. It is recommended to use *Co-4*^{2}*, Co-5* and *Co-7* from G2333 to develop resistant varieties to be grown in the all zones except in Southern and Western zone. The research should also focus on characterizing other genes that can offer resistance to races from Western and Southern Highland zones that were able to overcome resistance genes in G2333 such as *Co-Pa* in cultivar Paloma.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abraham N, Mashilla D (2018). Effect of integrating variety, seed treatment, and foliar fungicide spray timing on managing common bean Anthracnose at Bako, western Ethiopia. East Africa Journal of Science 12(2).

Allen DJ, Burachara RA, Smithson JB (1998). Diseases of common bean. In. Allen DJ, Lenne JM (Eds.), The Pathology of Food and Pasture Legumes. CAB International: Wallingford, UK, pp. 179–265.

Ansari KI, Palacios N, Araya C, Langin T, Egan D, Doohan FM (2004). Pathogenic and genetic variability among *Colletotrichum lindemuthianum* isolates of different geographic origins. Journal of Plant Pathology 53:635-642.

Balardin RS, Kelly JD (1998). Interaction between *Colletotrichum lindemuthianum*races and gene pool diversity in *Phaseolus vulgaris.* Journal of American Society 123:1038-1047.

Balardin RS, Jarosz AM, Kelly JD (1997). Virulence and molecular diversity in *Colletotrichum lindemuthianum* from South, Central and North America. Phytopathology 87:1184-1191

Balardin RS, Pastor-Corrales MA, Otoya MM (1990). Variabilida de patogênica de *Colletotrichum lindemuthianum* no Estado de Santa Catarina. Fitopatologia*.* Brasilia 15:243-245.

de Lima Castro SA, Gonçalves-Vidigal MC, Gilio TA, Lacanallo GF, Valentini G, Martins VD, Song Q, Galván MZ, Hurtado-Gonzales OP, Pastor-Corrales MA (2017). Genetics and mapping of a new anthracnose resistance locus in Andean common bean Paloma. BioMed Central Genomics 18(306):1-13.

Kelly JD, Vallejo VA (2004). A comprehensive review of the major genes conditioning resistance to anthracnose in common bean: Crop and soil sciences. Journal of Horticultural Science 39(6):1196-1207.

Mahuku GS, Jara CE, Cajiao C, Beebe S (2002). Sources of resistance to *Colletotrichum lindemuthianum* in the secondary gene pool of *Phaseolus vulgaris* and in crosses of primary and secondary gene pools. Plant Disease 86:1383-1387.

Mahuku SG, Riascos JJ (2004). Virulence and molecular diversity within *Colletotrichum lindemuthianum* isolates from Andean and Mesoamerican bean varieties and regions. European Journal of Plant Pathology 110:253-263.

Markell S, Wunsch M, Del Río L (2012). *Anthracnose of Dry Beans.* State University Extension Publication, Fargo. 33p.

Méndez-Vigo B, Rodriguez-Suarez C, Pañeda A, Ferreira J, Giraldez R (2005). Molecular markers and allelic relationships of anthracnose resistance gene cluster B4 in common bean. Euphytica 141:237-245.

Mohammed A (2013). An Overview of Distribution, Biology and the Management of Common Bean Anthracnose*.* Journal of Plant Pathology and Microbiology 4:193-199.

Mwalyego FM (1991). Progress in bean anthracnose research in Tanzania. In. Buruchara R (eds.), Proceedings of the First Pan-African Working Group Meeting on Anthracnose of Beans, Ambo, Ethiopia African Workshop. Centro Internacional de Agricultura Tropical, Cali Colombia. pp. 61–65.

Mwalyego F (1992a) Progress report on studies of the bean anthracnose pathogen *(Colletotnchum lmdemuthianum)* in Tanzania. Unpublished report Uyole Agncultural Centre Mbeya Tanzania Cited in Allen DJ, CIAT African Workshop Senes No 27 211 236.

Opio F, Ugen MA, Kyamanywa S, David S, Mugisa-Mutetika M (2001**)**. Beans. In. Mukiibi K (ed.), Agriculture in Uganda Crop. (National Agricultural Research Organization, Uganda, pp. 56- 72.

Pastor-Corrales MA (1991). Standardization of differential varieties and designation of races of *Colletotrichum lindemuthianum*. Phytopatholoy 81(6):694.

Pastor-Corrales MA, Otoya MM, Molina A, Singh SP (1995). Resistance to *Colletotrichum lindemuthianum* isolates from Middle America and Andean South America in different common bean races. Plant Disease 79:63-67.

Shao FM, Teri JM (1985). Yield losses in Phaseolus beans induced by anthracnose. International Journal of Pest Management 31(1):60-62.

Silva KJD, Souza EA, Ishikawa FH (2007). Characterization of *Colletotrichum lindemuthianum* isolates from the state of Minas Gerais, Brazil. Journal of Phytopathology 155:241-247.

Tanzania Meteorological Agency (2017). Tanzania weather condition. [www.meteo.go.tz] site visited on 4/9/2018.

Tu JC (1983). Epidemiology of anthracnose caused by *Colletotrichum lindemuthianum* on white bean (*Phaseolus vulgaris*) in Southern Ontario: Survival of the pathogen. Plant Disease 67:402-404.

Vidigal FPS, Goncalves-Vidigal MC, Kelly JD, Kirk WW (2007). Sources of resistance to anthracnose in traditional common bean cultivars from Parana, Brazil. Journal of Phytopathology 155:108-113

Young R, Melotto A, Nodari MRO, Kelly JD (1998). Marker assisted dissection of oligogenic anthracnose resistance in the common bean cultivar. Theoretical and Applied Genetics 96:87-89.

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The *in vitro*, *ex vivo*, and *in vivo* experimental findings of *Nephrolepis exaltata*

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***Nephrolepis exaltata* (L.) Schott decreases the heartbeats of cockroaches and it was postulated that the plant could be an anticholinesterase agent and could have effects. It was performed: (a) *In vitro:* hydroalcoholic extract of *N. exaltata* was pharmacognostically characterized, the cholinesterase activity determined with 1.0 and 3.0 mg/ml, comparing to positive control and negative control, and the preliminary toxicity was evaluated with 5 mg/plate through *Salmonella*/microsome assay using TA100 strain; (b) *Ex vivo:* 2, 5, or 10 mg of extract was assayed on mouse phrenic nerve-diaphragm preparation using conventional myographic technique; and (c) *In vivo*: 2.0, 1.0 or 0.5 g of extract was exposed to *Allium cepa* root cells, using onions bulbs for further measuring and microscopic analysis. The cholinesterase activities (U/L, n=3) of 1.0 and 3.0 mg/mL fern extract were of 2,866.6 ± 200.7 and 3,092.9 ± 214.2, respectively, *versus* 87.1 ± 58.1 ($p<0.05$) for positive control. The extract showed the absence of micronucleus and inhibited the root growth reaching 100% at 2 mg. The plant has no anticholinesterase activity, it is not toxic on bacterial reverse mutation or nerve-muscle parameters and is not genotoxic on *A. cepa* assay, but inhibits the root growth of *A. cepa*.**

Key words: *Allium cepa*, cholinesterase, fern, pharmacognosy, *Salmonella*.

INTRODUCTION

Nephrolepis exaltata (L.) Schott, family Nephrolepidaceae, is an ornamental and perennial plant (Figure 1S), terrestrial or epiphytic in its native state, and is originally from the south of the USA – popularly known as Boston fern, Central and South America. It is also naturalized in Canary Islands, Africa, Asia, India, Polynesia, and New Zealand (Large and Farrington, 2016). The plant has been studied for its soil

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phytoremediation properties (Sultana et al., 2015) against arsenic and other metals (Osusu-Dentaah, 2017 : Rao and Khan, 2017) as well as the antimicrobial effects of its volatile oil such as 2,4-Hexadien-1-ol (16.1%), nonanal (14.4%), β-Ionone (6.7%) and thymol (2.7%) (El-Tantawy et al., 2016). It has also been studied for its hormonal and cytotoxic effects on human cancer cells (Thomas-Charles and Fennell, 2019); its air-purifying capabilities (Chauhan et al., 2017), and its ability in hydrolyzing the ester linkages of phenol and aromatic and aliphatic alcohol acetates in biotransformation reactions (Mironowicz et al., 1994).

Faced with the crescent interest in studying this plant by the above advantages, Sanchez et al. (2018) described an insect-based model, in which aqueous extract of *N. exaltata* caused a decrease in heartbeats of a semi-isolated heart preparation of *Nauphoeta cinerea*. Researchers have postulated a possible insecticidal activity mediated by parasympathetic nerves at heart, where acetylcholine causes bradycardia due to blockade of postjunctional muscarinic receptors (Pace and Serpell, 2015). It is known that the standard battery for determining genotoxicity includes the assessment of mutagenic damage, using bacterial reverse gene mutation assay to detect relevant genetic changes as well as most genotoxic carcinogens for rodents and humans (Maron and Ames, 1983; Mortelmans and Zeiger, 2000). Thus, aiming to secure safety to *N. exaltata* plant, genotoxic studies were carried out by *Allium cepa* assay, an ideal bioindicator for the first screening of genotoxicity (Ramos et al., 2020), and also toxicity determination by *Salmonella*/microsome assay.
Besides, since organophosphate insecticides inhibit the plasmatic cholinesterase enzyme (ChE) causing bradycardia, and inhibit the twitches of a nerve-muscle preparation, the insecticide effect of hydroalcoholic *N. exaltata* extract was evaluated through ChE determination and on neuromuscular twitches. The entire study was preceded by a quality control analysis of *N. exaltata* to authenticate either ashes or humidity content in its powder leaves.

MATERIALS AND METHODS

Plant

Nephrolepis exaltata specimens were commercially purchased from Kashima Flores®, box 30 of CEAGESP Sorocaba, SP, Brazil. The plant was initially identified by R.Y.H. Miura from Biological Sciences Course, Health Sciences Institute, Paulista University (UNIP), Sorocaba, SP, Brazil, and further confirmed by L. C. Bernacci from Instituto Agronômico de Campinas, where a voucher specimen was deposited (IAC number 57451).

In vitro studies

Hydroalcoholic extract from leaves of *N. exaltata* and solubilization. The obtained vegetable drug mass was 938.7 g, when dried its yield was of 594.05 g (63.3% yield). An amount of 126.68 g of leaves powder was taken and macerated along with 2.5 L of 70% ethanol. The obtained solvent was evaporated (Buchi rotavapor®, Valinhos, SP, Brazil) until dryness, and the dried extract (Thermo Fisher Scientific lyophilizer®, Australia) was then protected from light and humidity at room temperature until the assays.

Quality control assays of the vegetal drugs – the ash and humidity tests

To observe their elementary physical and chemical characteristics, the powder obtained from the *N. exaltata* leaves was subjected to the ash and humidity tests (Brazilian Pharmacopoeia, 1998). Briefly, 100 g of powder specimen were placed in six calibrated melting pots, which were warmed until total powder carbonization. The melting pots were kept at 650°C and the ashes were weighed. Results were presented in grams of ashes/100 g of the sample. The humidity test was performed by placing 1g of powder specimen in each one of six calibrated porcelain capsules, which were warmed at 105°C for 4h and then weighed.

Cholinesterase (ChE) inhibition assay

The extract was solubilized in polyethylene glycol 400 (PEG 400) (Sinth®, Diadema, SP, Brazil) according to Cintra-Francischinelli et al. (2008) to be used in the enzymatic reaction (ChE activity). The ability of the *N. exaltata* extract to inhibit ChE activity was assessed using a colorimetric assay (Labtest Diagnóstica S.A., Lagoa Santa, MG, Brazil) that was standardized with a human serum calibrator of known ChE activity (BioControl N), according to the manufacturer´s recommendations. BioControl N (Bioclin Quibasa), a pool of normal human serum, was used as internal quality control, according to the manufacturer´s recommendations. The procedure was carried out according to Werner et al. (2015). The ChE activity was determined spectrophotometrically (UV-M51 spectrophotometer, BEL Engineering, Rio de Janeiro, RJ, Brazil) at 405 nm. The percentage of enzyme inhibition was calculated by comparing the enzymatic activity in the presence of 1.0 and 3.0 mg/mL of *N. exaltata* extract or with PEG 400 (20 µL) used as solubilizer or with 1 mg/mL concentration of neostigmine (Sigma®) used as a cholinesterase inhibitor. The assays were done in triplicate.

Preliminary toxicity

A preliminary toxicity assay without metabolization by the preincubation method (Maron and Ames, 1983) for future Ames test of *N. exaltata* extract was carried out using TA100 *S. typhimurium* strain (kindly provided by B.N. Ames, Berkeley, CA). the TA100 strain, a histidine dependent was selected as, all other *Salmonella* tester strains, and also contains a deletion mutation through the uvrB-bio genes, a mutation (rfa) that leads to a defective lipopolysaccharide (LPS) layer (Ames et al., 1973), and presence of plasmid pKM101 (Ames et al., 1975). In theory, mutagens can cause a base-pair substitution in TA100 as a reversion event. For a while, we measured the toxicity on TA 100 as a need to know enough concentration able to kill prokaryotic cells (Oliveira et al., 2019). The TA100 strain from frozen culture was grown overnight for 12 to 14 h in Oxoid Nutrient Broth No. 2. The recommended maximum concentration of 5 mg/plate (5,000 µg/plate) *N. exaltata* extract (volumes of 25, 50, and 100 mL) were added to 0.5 ml of 0.2 M sodium phosphate buffer (pH 7.4), with 0.1 mL of bacterial culture and then incubated at 37°C for 20 min. Next, 2 mL of top agar (0.6% agar, histidine and biotin 0.5 mM each, and 0.5% NaCl) was added and the mixture was poured into a plate containing minimal glucose agar (1.5% Bacto-Difco agar and 2% glucose in Vogel- Bonner medium). The plates were incubated at 37 °C for 48 h and the His+ revertant colonies were counted manually. All

experiments were performed in triplicate. For TA100, the standard mutagen used as a positive control in experiments without S9 mix was sodium azide (2.50 µg/plate). Dimethylsulfoxide (DMSO) was used as a negative (solvent) control (50 µL/plate). Toxicity was evaluated either as a reduction in the number of His+ revertants or as an alteration in the auxotrophic background (Mortelmans and Zeiger, 2000; Yoshida et al., 2016).

Ex vivo pharmacological experiments

Animals

Eighteen male white Swiss mice (25-30 g) were purchased from Anilab (Laboratory Animals, Paulinia, SP, Brazil), and only 12 animals were used. The animals were housed at 25 ± 3°C (77 ± 3°F) in a light/dark cycle of 12 h and had access to food and water *ad libitum*. This study was approved by the Animal Ethics Committee of Sorocaba University (protocol n° 144/2019), and the experiments were carried out according to the international guideline - ARRIVE (Animal Research: Reporting of *In-Vivo* Experiments (Kilkenny et al., 2010).

Mouse phrenic nerve-diaphragm muscle (PND) preparation

The diaphragm and its phrenic nerve branch were obtained from mice anesthetized with Halothane (Cristália®, Itapira, SP, Brazil) and sacrificed by exsanguination. Hemidiaphragms were mounted under a tension of 0.5 g in a 5 mL organ bath (Bülbring, 1997) containing Tyrode solution, and aerated with 95% O_2 and 5% CO_2. Tyrode solution maintains the physiological conditions of the neuromuscular preparation at pH 7.0 and consists of (in mM): NaCl 137; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 0.49; NaH_2PO_4 0.42; $NaHCO_3$ 11.9 and Glucose 11.1. The preparation is indirectly stimulated through the phrenic nerve (ESF-15D double physiological stimulator), using supramaximal stimuli and a frequency of 0.06 Hz with a duration of 0.2 ms. Isometric twitch tension was recorded with a force-displacement transducer (cat. 7003, Ugo Basile, Italy) coupled to a digital recorder system (Data Capsule, cat. 17400, Ugo Basile) containing a Basic Preamplifier (cat. 7080, Ugo Basile), coupled to a computer via a USB interface for the data store. PND could stabilize for at least 20 min before the start of experiments. After recording under control conditions during 10 min stabilization of the preparation, the pharmacological protocols were performed using 2, 5, and 10 mg of PEG 400-solubilized *N. exaltata* extract added into the bath (n=6, each).

In vivo study

Allium cepa test

This test has been widely used since it allows the evaluation of the effects or damages that mutagenic agents might cause. For the sample, it is necessary to be in constant mitotic division, seeking to identify the toxic effects and alterations during all cell cycle (Silva et al., 2018). Either the mitotic index as the replication index was used as indicators of adequate cell proliferation (Bonciu et al., 2018).

Briefly, scraped onion bulbs at the root (to promote the emergence of new roots) were exposed during 48 h for the root growth. They were divided into 5 groups for testing *N. exaltata* extract (at 2, 1 and 0.5 g solubilized in 1 L of water, n=6 each), positive control (paracetamol, 750 mg/L, n=6) (Bezerra et al., 2016), and negative control (potable tap water, n=6), in a total of 30 onion bulbs. After 48 h, the number of roots grown/bulb was counted and further removed and measured. Bulbs with minor root growth were excluded from each group (Sumitha et al., 2016). The ends of major root grown were immersed in Carnoy's solution fixative (ethanol: acetic acid, 3:1), maintained at refrigerator for 24 h. Afterwards, two ends of each bulb were isolated and immersed in a heated HCl 1 N solution. Furthermore, they were transferred to microscopic slides and stained with toluidine solution 1.5 % and covered by a glass slip under slight pressure (squash technique). Quantification was made through optical microscopy in the objective of 10 or 40 giving increases of 100 or 400 X. A range of 50 - 85 cells per bulb to examine the presence of micronucleus.

Data analysis

The data were expressed as the mean ± SEM (triplicate or n=6 depending on the experimental protocol). Results were analyzed by Student's *t*-test, with $p<0.05$ indicating significance. The *Salmonella*/Microsome results were analyzed using the Salanal statistical software package (U.S. Environmental Protection Agency, Monitoring Systems Laboratory, Las Vegas, NV, version 1.0, from Research Triangle Institute, RTP, North Carolina, USA), adopting the Bernstein et al. (1982) model. The data (revertants/plate) were assessed by analysis of variance (ANOVA) followed by linear regression. All data analyses were done using Origin© v.8.0 (OriginLab Corporation, Northampton, MA, USA).

RESULTS AND DISCUSSION

N. exaltata extract was investigated on three approaches – a) *in vitro* (ashes and humidity; cholinesterase determination, and preliminary toxicity by *Salmonella*/Microsome); b) *ex vivo* (pharmacological profile on mammalian nerve-muscle synapse); and c) *in vivo* (genotoxicity using *Allium cepa* model), aiming to cover altogether pharmacognostic, biochemical, pharmacological and genotoxic parameters.

The elementary physical and chemical characteristics of *N. exaltata* such as ashes and humidity were carried out as important quality control of the plant, since excess water in herbal materials will encourage microbial growth, the presence of fungi, or insects, and deterioration following hydrolysis (Liu, 2019). Limits for water content should, therefore, be set for every given herbal material, being 11.7 ± 0.04 g/100 g in case of *N. exaltata* extract used here. On the other hand, ash values help determine the quality and purity of crude drugs in powder form, to remove all traces of organic matter resulting only inorganic residue (Pal et al., 2018), which measure is important, because mineral matter may be the cause of a pharmacological effect (Arraiza et al., 2017). *N. exaltata* extract showed 8.8 ± 0.11 g/100 g of ash content, like those found in leaves' powder of *Sesbania rostrate* (Momin and Kadam, 2011), an important dietary nutritive (amino acid, minerals, and antioxidants vitamins) source in countries of Southeast Asian.

Previous results obtained with *N. exaltata* were carried out using an aqueous solution from macerated leaves of *N. exaltata* fern, an ornamental plant considered to be safe (Popovici et al., 2018). When added to semi-isolated

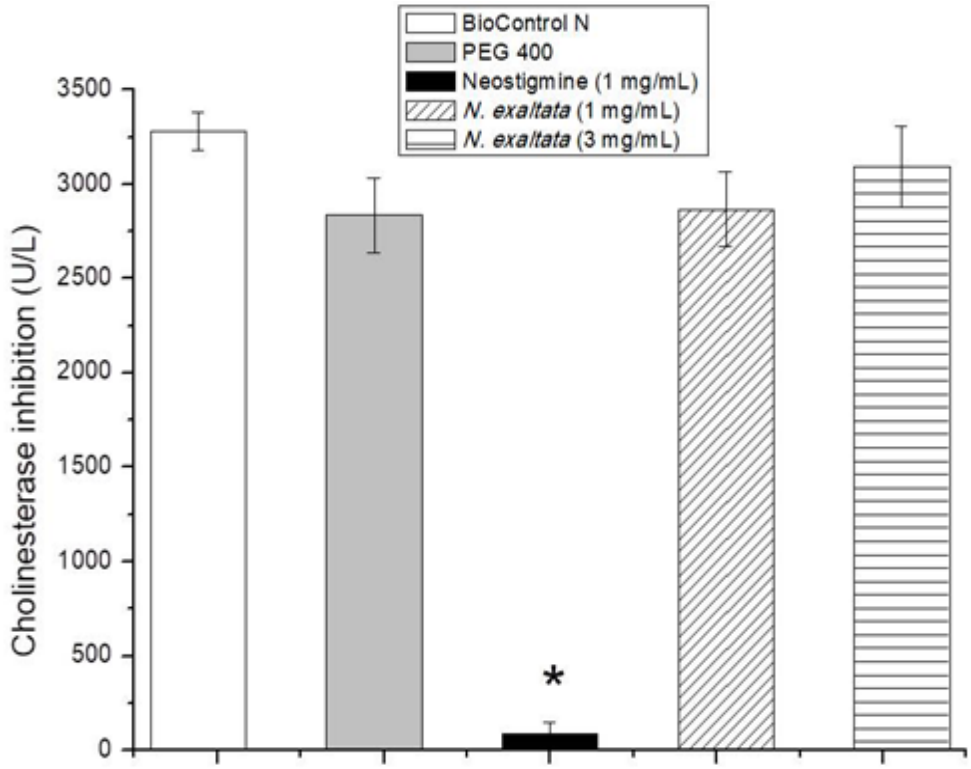

Figure 1. Cholinesterase inhibition (U/L). The activity of *N. exaltata* extract at 1 and 3 mg/ml was compared with the known anticholinesterase agent neostigmine (1 mg/mL, positive control) and BioControl N (negative control). Notice that the plant extract has no inhibitory effect on cholinesterase enzyme activity.

heart preparation of *Nauphoeta cinerea* the aqueous solution was cardiotoxic to cockroaches in a dose-dependent manner (Sanchez et al., 2018). The authors postulated an insecticide activity, as organophosphates are able since the plant exhibited an effect similar to that produced by acetylcholine (ACh) at the parasympathetic system.

Here, a validated protocol was used (Werner et al., 2015) to clear this question comparing the results with normal serum, negative control (BioControl N), and a known anticholinesterase agent, positive control, neostigmine (Table 1S). Figure 1 shows the contrasting results among negative control, the solubilizing PEG 400, and neostigmine. The mechanism by which aqueous *N. exaltata* inhibits the heartbeats was not caused by an anticholinesterase effect by the inhibition of the enzyme acetylcholinesterase. It is known that, in consequence of enzyme inhibition, the increased acetylcholine concentration at nerve terminals in areas other than the neuromuscular junction, several muscarinic receptor-mediated side-effects are seen, in the parasympathetic system, including abdominal cramps, diarrhea, increased gastric and bronchial secretions, salivation, lacrimation, nasal discharge, sweating, increased urination, and vagal effects as bradycardia (Kalla et al., 2016), prolonged QT interval (Winter et al., 2018), and asystole (Nkemngu, 2017).

Ex vivo experiments using mouse phrenic nerve-diaphragm preparations are used to obtain the pharmacological effects of different compounds, a technique validated since 1946 by Bülbring (1997). The first effect showed by any anticholinesterase agent (acetylcholinesterase inhibitors) is a facilitatory effect showed by an increase of amplitude due to an increase of acetylcholine (ACh) release at synapses (Werner et al., 2015). Observing the Figure 2A none dose (2, 5 or 10 mg, n=6 each) of *N. exaltata* extract exhibited any facilitatory effect significantly different from Tyrode control. At the end of experiments at 60 min (Figure 2B), all concentrations were not different from control showing that the plant extract is not toxic to biological preparation since the basal response was maintained during the experiment.

Also investigated was the preliminary toxicity using *Salmonella*/microsome parameter, widely accepted short-term bacterial assay for identifying substances that can produce genetic damage that leads to gene mutations (Mortelmans and Zeiger, 2000), using a standard limit of 5 mg/plate (5,000 µg/plate), that is the maximum recommended dose for routine testing (Hamel et al.,

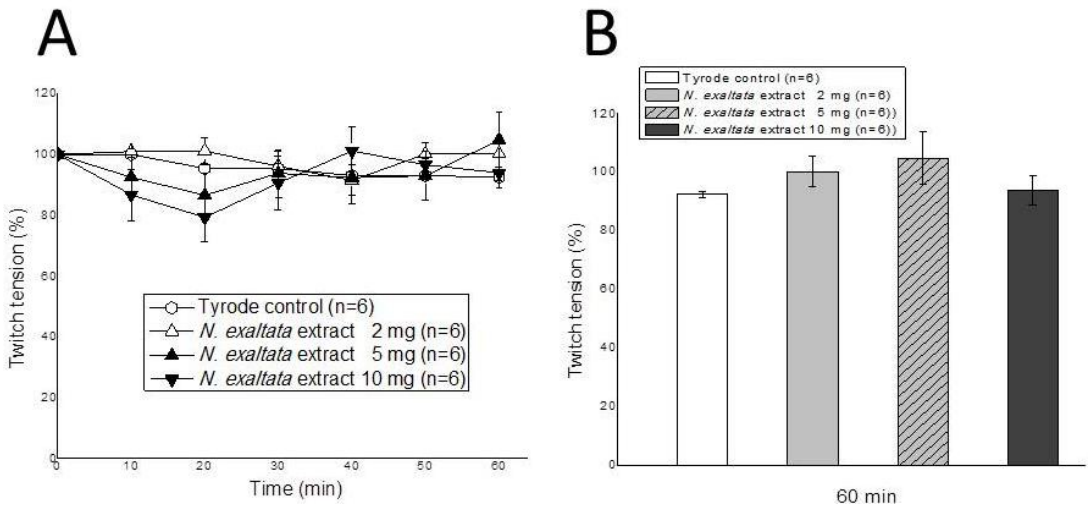

Figure 2. Mouse phrenic nerve-diaphragm preparation (indirect stimuli). Kinetic concentration-response curve of 2, 5, and 10 mg of *N. exaltata* extract (A) and at the end of the experiment (B), at 60 min. The number of experiments (n) is shown in the legend of the figure. No significant difference was observed when compared to Tyrode control ($p>0.05$).

2016). Table 2S shows the revertant colony counts after the *N. exaltata* extract exposure to TA100 (without metabolic activation, -S9) compared to negative and positive controls. Evaluation of toxicity is confirmed under the partial or complete absence of a background lawn or a substantial dose-related reduction in revertant colony counts, a fold response of less than 0.6-times compared to the negative control (Hamel et al., 2016). Figure 3 shows the absence of toxicity in *N. exaltata* extract visualized by colony counts in all tested concentrations (range of 625- 5,000 µg/plate). Preliminary toxicity is mandatory before the *Salmonella*/microsome achievement according to OECD Guideline for Testing of Chemicals, Bacterial Reverse Mutation Test (1997).

Tables 3S and 4S show the root growth and the altered cells count, respectively, of *N. exaltata* extract (2, 1 and 0.5 g), in comparison to negative (potable tap water) and positive (750 mg/L Paracetamol) controls. The major concentration of *N. exaltata* extract inhibited 100% of *A. cepa* root growth, being more effective as an inhibitor than the positive control paracetamol (Table 3S). Concerning the altered cell count (Table 4S) the presence of micronucleus in the selected areas was not observed neither in the positive control nor in *N. exaltata* extract. Figure 4 shows images of negative control, which in turn, did not differ from *N. exaltata* extract. Notice the absence of micronucleus in the selected parameter to visualize genotoxicity. In a study recorded by Informative Geum Bulletin (2015), the presence of micronucleus was found using 125 (0.16 ± 0.05%), 250 (0.10 ± 0.05%), and 500 (0.06 ± 0.02%) µg/mL paracetamol, although the values were not statistically different from negative control (distilled water; 0.12 ± 0.03%).

The *A. cepa* test allows the test of any compound (pure or in the mixture) able to cause damage to the DNA of eukaryotes (Bauer et al., 2015), and in an *in vivo* model, to secure safety a certain substance. The lack of genotoxicity in *N. exaltata* justifies its classification as a non-toxic plant even if aqueous extract causes the decrease dose-dependently the heartbeats of *Nauphoeta cinereal* (Sanchez et al., 2018). This pharmacological behavior and also the ability of *N. exaltata* extract in inhibiting the root growth of *A. cepa* would be explained by the presence of allelochemicals, as phenolics and terpenoids, in the plant. Allelochemicals are present in almost all plant tissues (leaves, flowers, fruits stems, roots, rhizomes, seeds, and pollen), and are released to the environment through volatilization, leaching, root exudation, and decomposition of plant residues (Sangeetha and Baskar, 2015).

The mechanisms by which allelochemicals act to inhibit germination, shoot, and root growth of other plants, involves the nutrient uptake destroying the plant's usable source of nutrients (Hassan et al., 2018), among others such as a generalized reduction in mitotic activity in roots and hypocotyls, hormone activity, rate of ion uptake, photosynthesis, respiration, protein formation, the permeability of cell membranes and/or enzyme action (Chang and Cheng, 2015). The presence of terpenes was identified, but not flavonoids, in *N. exaltata* aqueous extract (Sanchez et al., 2018), and it is known that terpenes inhibit the nitrification of soil, influencing the productivity of a plant community (Coskun et al., 2017). Corroborating these findings, the ethanol and aqueous extracts showed low cytotoxic effects on both *Triticum aestivum* roots and *Artemia franciscana* nauplii (Popovici et al., 2018). *N. exaltata* fern must be differentiated frombracken fern *Pteridium aquilinum* (L) Kuhn

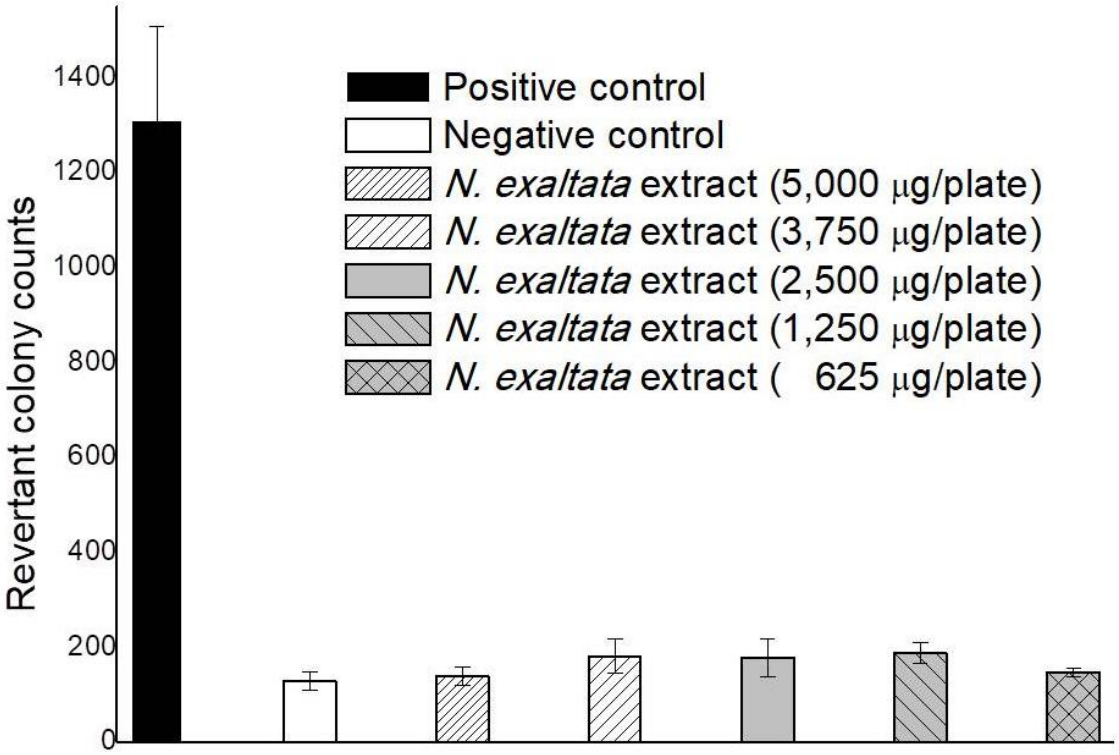

Figure 3. Evaluation of toxicity (*Salmonella*/microsome assay). Revertant colony counts face to several concentrations of *N. exaltata* extract submitted to TA100 strain.

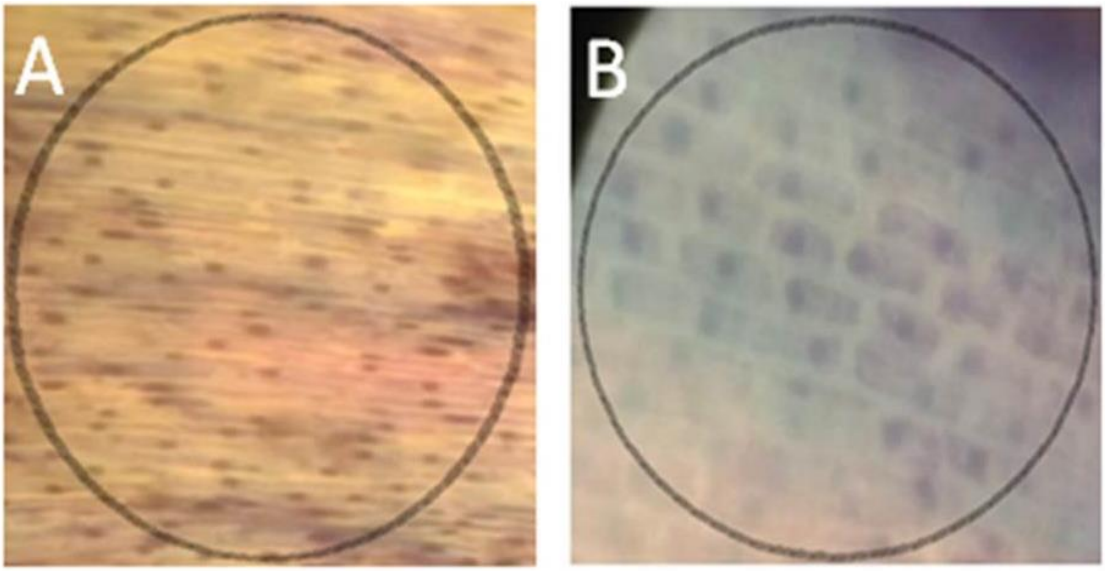

Figure 4. Characteristic photographs of negative control in two magnifications: A, 100 X; B, 400X. Micronucleus are absent in these images.

(Polypodiaceae) (Figure 2S), a specie that causes neoplasms of the urinary bladder and upper digestive tract related to the ingestion of bracken fern in cattle, due to the presence of ptaquiloside (Agarwal et al., 2018) or in horses, by the toxic agent thiaminase (Reed et al., 2017).

Conclusion

The plant has no anticholinesterase activity, is not toxic on bacterial reverse mutation or nerve-muscle synapses, neither on *A. cepa* assay, but inhibits the root growth of *A. cepa,* suggesting that *N. exaltata* does it as a mechanism of self-defense.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Agarwal K, Haldar S, Boland W, Venkatesan R (2018). Chemical ecology of Bracken Ferns. In: Ferns. Nova Science Publishers, Inc 2:57-96.

Ames BN, Lee FD, Durston WE (1973). An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proceedings of the National Academy of Sciences USA 70(3):782-786.

Ames BN, McCann J, Yamasaki E (1975). Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test. Mutation Research 31(6):347-364.

Arraiza MP, Gonzáles-Coloma A, Burillo J, Calderón-Guerrero C (2017). Frontiers in horticulture. Medicinal and Aromatic Plants: The Basics of Industrial Application. Bentham Science Publishers pp. 92-102.

Bauer NC, Corbett AH, Doetsch PW (2015). The current state of eukaryotic DNA base damage and repair. Nucleic Acids Research 43.21:10083-10101.

Bernstein L, Kaldor J, McCann K, Pike MC (1982). An empirical approach to the statistical analysis of mutagenesis data from the *Salmonella* test. Mutation Research 97(4):267-281.

Bezerra CM, Dinelly CMN, Oliveira MAS (2016). Avaliação da toxicidade, citotoxicidade e genotoxicidade do infuso de malva-santa *Plectranthuys barbatus* (Lamiaceae) sobre o ciclo celular de *Allium cepa.* Brazilian Journal of Pharmaceutical Sciences 13(3):220-228.

Bonciu E, Firbas P, Fontanetti CS, Wusheng J, Karaismailoğlu MC, Liu D, Menicucci F, Pesnya DS, Popescu A, Romanovsky AV, SchiffS, Ślusarczyk J, Souza CP, Srivastava A, Sutan A, Papini A (2018). An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay. Caryologia 71:3, 191-209.

Bülbring E (1997). Observation on the isolated phrenic nerve diaphragm preparation of the rat. British Journal of Pharmacology 120(Suppl 1):1-2.

Chang F, Cheng Z (2015). Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. Frontiers in Plant Science 6:1020.

Chauhan P, Rawat NMS, Gauba NP (2017). Role of Plants in Indoor Air Remediation. International Journal of Engineering Technology Science and Research 4(9):749-756.

Cintra-Francischinelli M, Silva MG, Andréo-Filho N, Cintra ACO, Leite GB, Cruz-Höfling MA, Rodrigues-Simioni L, Oshima-Franco Y (2008). Effects of commonly used solubilizing agents on a model nerve-muscle synapse. Latin American Journal of Pharmacy 27(5):721-726.

Coskun D, Britto DT, Weiming S, Kronzucker HJ (2017). Nitrogen transformations in modern agriculture and the role of biological nitrification inhibition. Nature Plants 3:17074.

El-Tantawy ME, Shams MM, Afifi MS (2016). Chemical composition and biological evaluation of the volatile constituents from the aerial parts of *Nephrolepis exaltata* (L.) and *Nephrolepis cordifolia* (L.) C. Presl grown in Egypt. Natural Product Research 30(10):1197-2013.

Hamel A, Roy M, Proudlock R (2016). The bacterial reverse mutation test. In: Proudlock R, editor. Genetic Toxicology Testing. Elsevier Inc. pp. 79-138.

Hassan G, Rashid HU, Amin A, Khan IA, Schehzad N (2018). Allelopathic Effect of *Parthenium hysterophorus* on Germination and Growth of Some Important Crops and Weeds of Economic Importance. Planta Daninha 36:e018176372.

Informative Geum Bulletin (2015). Evaluation of toxicity, cytotoxicity, mutagenicity and genotoxicity of dipyrone sodium and paracetamol in meristematic cells of *Allium cepa* roots. Informative Geum Bulletin 6(4):7-15.

Kalla M, Herring N, Paterson DJ (2016). Cardiac sympatho-vagal balance and ventricular arrhythmia. Autonomic Neuroscience 199:29-37.

Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010). Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. Plos Biology 8(6):e1000412.

Large M, Farrington L (2016). The *Nephrolepis* Boston fern complex (including *Nephrolepis exaltata* [L.] Schott), Nephrolepidaceae, naturalised in New Zealand. Perspectives in Biosecurity 2:1-7.

Liu K (2019). Effects of sample size, dry ashing temperature and duration on determination of ash content in algae and other biomass. Algal Research 40:101486.

Maron DM, Ames BN (1983). Revised methods for the *Salmonella* mutagenicity test. Mutation Research 113(3-4):173-215.

Mironowicz A, Kromer K, Pawlowicz P, Siewínski (1994). Abilities of some higher plants to hydrolyze the acetates of phenols and aromatic-aliphatic alcohols. Acta Societatis Botanicorum Poloniae 63(1):43-48.

Momin R, Kadam VB (2011). Determination of ash values of some medicinal plants of genus *Sesbania* of Marathwada Region in Maharashtra. Journal of Phytology 3(12).

Mortelmans K, Zeiger E (2000). The Ames *Salmonella*/microsome mutagenicity assay. Mutation Research 455(1–2):29-60.

Nkemngu NJ (2017). Asystole Following Neuromuscular Blockade Reversal in Cardiac Transplant Patients. Annals of Cardiac Anaesthesia 20(3):385-386.

OECD (1997). Guideline for Testing of Chemicals, Bacterial Reverse Mutation Test. http://www.oecd.org/chemicalsafety/risk-assessment/1948418.pdf. Accessed 08 October 2019.

Oliveira ICF, de Paula MO, Lastra HCB, Alves BB, Moreno DAN, Yoshida EH, Amaral Filho J, Cogo JC, Varanda EA, Rai M, Santos CAD, Oshima-Franco Y (2019). Activity of silver nanoparticles on prokaryotic cells and *Bothrops jararacussu* snake venom. Drug and Chemical Toxicology 42(1):60-64.

Osusu-Dentaah E (2017). Phytoremediation of Heavy Metal Contaminated Soil Using *Thelypteris accuminata* (Houtt.) C.V. Morton and *Nephrolepis exaltata* (L.) Schott. 2017. A thesis submitted to the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology. Available at: http://ir.knust.edu.gh/handle/123456789/10027.

Pace N, Serpell M (2015). Core topics in basic anaesthesia: prepare for the FRCA. Elsevier in Health Sciences, British Library Cataloguing in Publication Data, pp. 279.

Pal RS, Pal Y, Wal P, Wal A (2018). Pharmacognostic Evaluation of Roots of *Benincasa hispida* (Thunb.) Cogn. (Cucurbitaceae). The Open Plant Science Journal 11:1-6.

Popovici PC, Ancuceanu VR, Olaru TO, Stoicescu C-S, Dinu M (2018). Toxicity assessment of *Nephrolepis exaltata* (L.) Schott, Fam. Nephrolepidaceae. Acta Biologica Marisiensis 1(1):26-35.

Rao RAK, Khan U (2017). Adsorption studies of Cu (II) on Boston fern (*Nephrolepis exaltata* Schott cv. Bostoniensis) leaves. Applied Water Science 7:2051-2061.

Ramos LPN, Leite DM, Macedo WA, Farias CBM, Oliveira AS, Dahmer N, Karsburg ISV (2020). Evaluation of the cytotoxic and genotoxic effect of *Allium cepa* L. (Amaryllidaceae) root cells after exposure in water samples of five lakes of Alta Floresta, State of Mato Grosso. Revista Ambiente e Água - An Interdisciplinary Journal of Applied Science 15(1):e2463.

Reed SM, Byly WM, Sellon DC (2017). Equine Internal Medicine. 4th Edition. Elsevier. Saunders. pp.1488.

Sanchez DME, de Souza J, Oshima-Franco Y, Rostelato-Ferreira S (2018). Pharmacological effects of *Nephrolepis exaltata* L. (fern) aqueous extract on an insect-based model (*Nauphoeta cinerea*). Journal of Plant Science *and* Phytopathology 2:031-036.

Sangeetha C, Baskar P (2015). Allelopathy in weed management: A critical review. African Journal of Agricultural Research 10(9):1004-1015.

Silva MAS, Yamashita OM, Rossi AAB, Concenço G, Carvalho MAC, Felito RA (2018). Cytotoxic and genotoxic effects *Macroptilium lathyroides*. Planta Daninha 36:e0181166574.

Sultana MS, Jolly YN, Yeasmin S, Islam A, Tarek SM (2015). Transfer of heavy metals and radionuclides from soil to vegetables and plants in Bangladesh. In: RehmanK, Muhammad H, Münir S, Ahmet Ö, Mermut R, editor. Soil Remediation and Plants: Prospects and Challenges. Massachusetts: Academic Press.

Sumitha KV, Thoppil JE (2016). Genotoxicity assessment of two common curing weeds: *Hyptis suaveolens* (L.) Poir. and *Leucas indica* (L.) R. Br. Cytotechnology 6:1513-1527.

Thomas-Charles C, Fennell H (2019). Anti-prostate cancer activity of plant-derived bioactive compounds: A review. Current Molecular Biology Reports 5:140-151.

Werner AC, Ferraz MC, Yoshida EH, Tribuiani N, Gautuz JA, Santana MN, Dezzotti BA, de Miranda VG, Foramiglio AL, Rostelato-Ferreira S, Tavares RV, Hyslop S, Oshima-Franco Y (2015). The facilitatory effect of *Casearia sylvestris* Sw. (guaçatonga) fractions on the contractile activity of mammalian and avian neuromuscular apparatus. Current Pharmaceutical Biotechnology 16(5):468-481.

Winter J, Tipton MJ, Shattock M (2018). Autonomic conflict exacerbates long QT associated ventricular arrhythmias. Journal of Molecular and Cellular Cardiology 116:145-154.

Yoshida EH, Tribuiani N, Sabadim G, Moreno DAN, Varanda EA, Oshima-Franco Y (2016). Evaluation of betulin mutagenicity by *Salmonella*/Microsome test. Advanced Pharmaceutical Bulletin 6(3):443-447.

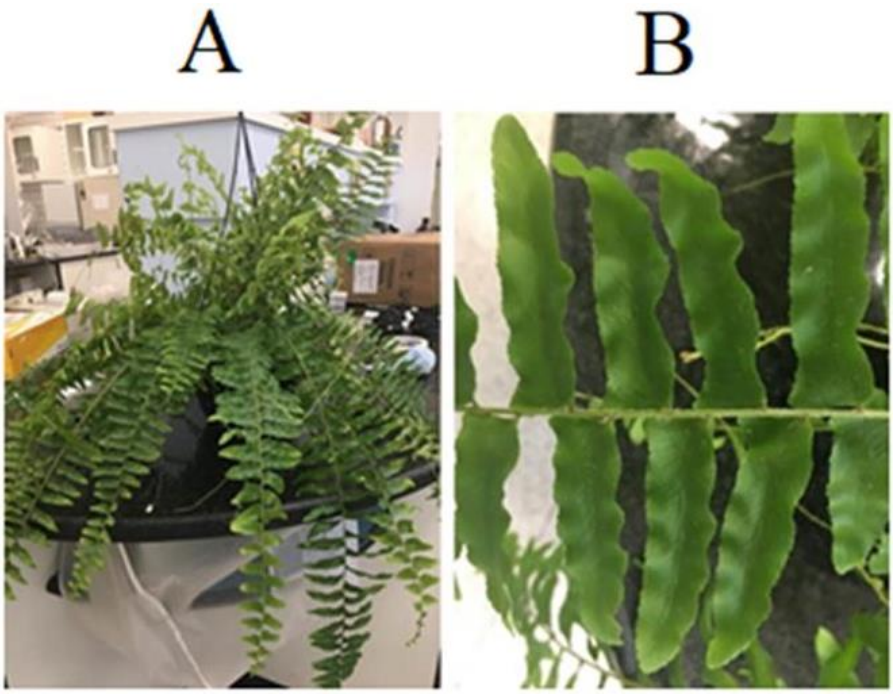

Figure 1S. Ornamental *N. exaltata* (L.) Schott. (A) The appearance of a commercially obtained specimen. (B) Details of leaves.

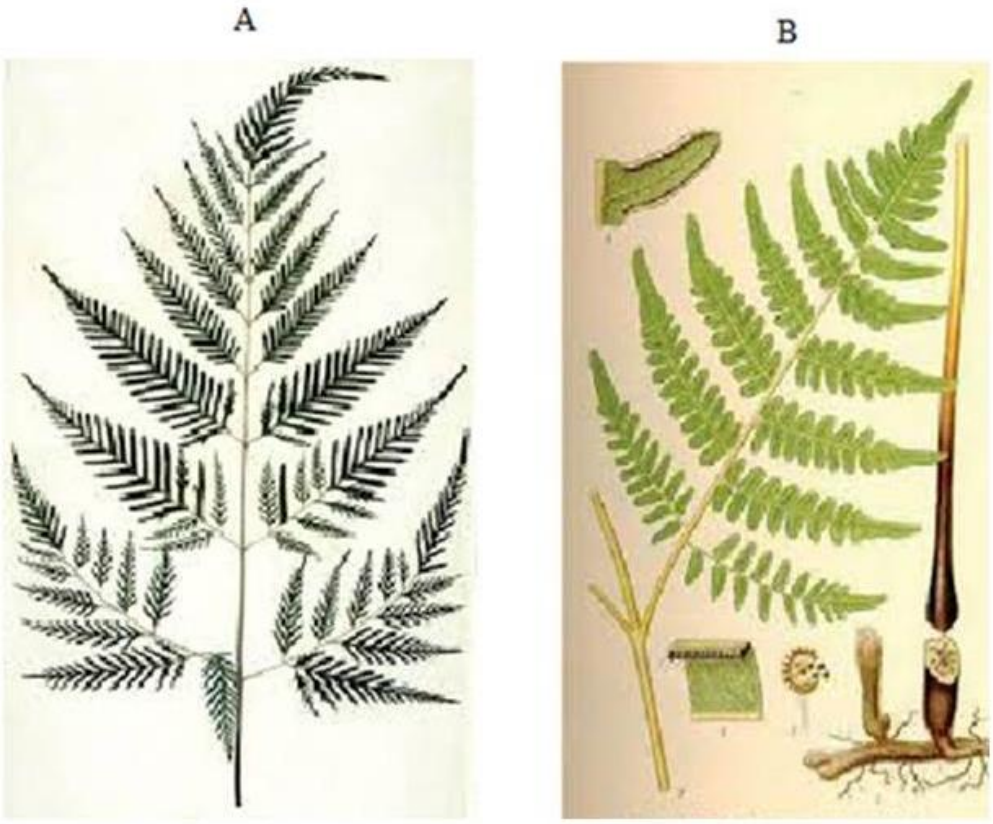

Figure 2S. Images from the botanical illustration (A, pinterest.com) and exsiccate (B, figure uploaded by Elke Plessers) of bracken fern (*Pteridium aquilinum*), available at web in 20.10.2019.

Table 1S. Cholinesterase determination (in triplicate).

Treatment	Time (min)				ChE (U/L)	Mean ± S.E.M
	A0	A1	A2	A3		
BioControl N (Range: 2450 – 4000 U/L)	1778	1742	1706	1669	3166	3283.1 ± 100.6
	1761	1719	1684	1646	3341	
	1753	1716	1676	1638	3341	
PEG 400	1708	1687	1653	1618	2614	2837.6 ± 197.7
	1625	1594	1561	1525	2905	
	1730	1699	1663	1627	2992	
Neostigmine 1 mg/ml	1815	1816	1813	1810	145.2	87.1 ± 58.1 (*)
	1813	1817	1815	1812	29	
	1843	1846	1843	1840	87.1	
N. exaltata (1 mg/ml)	1766	1737	1704	1671	2687	2866.6 ± 200.7
	1700	1676	1638	1600	2828	
	1753	1718	1681	1644	3083	
N. exaltata (3 mg/ml)	1783	1748	1713	1665	3338	3092.9 ± 214.2
	1783	1748	1713	1679	2942	
	1784	1748	1713	1678	2998	

S.E.M., Standard Error Mean. *, p<0.05 compared to BioControl N, PEG 400 and *N. exaltata*.

Table 2S. Preliminary toxicity of *N. exaltata* extract on TA100 strain (-S9) Salmonella/Microsome assay.

TA 100		Nº of revertant/plate		
		µg/placa	Nº revertant	±SD
N. exaltata (50 mg/ml)	C (+)	-	1310	197
	C (-)	-	130	19
	100 µl	5000	140	19
	75 µl	3750	182	36
	50 µl	2500	179	101
	25 µl	1250	188	22
	12.5 µl	625	149	9

Table 3S. Average root growth after exposure to different treatments.

Group	Average root growth (n=6) (cm)	Treatment	% of inhibition
Control -	1.4 ± 0.18	Potable tap water	0
Control +	0.7 ± 0.13	Paracetamol 750 mg/L	50
N. exaltata extract	0	2 g	100
	0.01 ± 0.03	1 g	93
	0.2 ± 0.18	0.5 g	85

Table 4S. Altered cells count.

Group	Total number of cells	Treatment	Altered cells
Control -	79	Potable tap Water	0
Control +	85	Paracetamol 750 mg/L	0
N. exaltata extract	None root growth	2 g	Not counted
	None root growth	1 g	Not counted
	57	0.5 g	0

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Distribution and ecological drivers of family celastraceae in Côte D'ivoire

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Most studies on drivers of plant diversity and distribution have focused on trees and combine several plant families. Climbers which are part of the particular characteristics of tropical rainforests due to their richness and abundance have been rarely related to ecological factors. This study evaluates the importance of vegetation type and total annual rainfall on the distribution of the Celastraceae plant family which are mostly climbers in Côte d'Ivoire using a herbarium database. A total of 1520 samples, encompassing16 genera, 60 species and 12 varieties of Celastraceae from over 363 localities in Côte d'Ivoire, were extracted from a database on Ivorian flora. Species' occurrences in localities were related to vegetation type and annual rainfall through a principal component analysis. A strong positive correlation (r = 0.81, P < 0.001) was found between the Celastraceae distribution and both the vegetation types and the rainfall. The Coastal evergreen and Western evergreen forests showed higher richness of Celastraceae climbers while the Sub-sudanian and Sudanian Savannas experienced lower richness than other vegetation types in Côte d'Ivoire.

Key words: Celastraceae, Tropical forest and savanna, climbing plants, species richness and spreading, rainfall.

INTRODUCTION

Among the West African vascular plant families, the Celastraceae *sensu* APG (1998, 2003, 2009), including Hippocrateaceae, is known at a regional scale only from Hallé (1958, 1962) and Hedin (1999). Taxa of African Tropical Celastraceae are mostly medium and tall climbers overlapping the high forest canopy. In addition, individuals available in the forest understory are mostly sterile and do not lead to full systematic identification. Moreover, it makes it difficult to link the diversity of such plants to its possible environmental drivers. This study aims to fill up for this gap by using large-scale digitized data of herbarium specimens and analysing the impacts of annual rainfall and vegetation types on Celastraceae species distribution in Côte d'Ivoire where this family has been intensively collected by several Botanists (Hallé, 1958, 1962; Hutchinson and Dalziel, 1958; Aké Assi,

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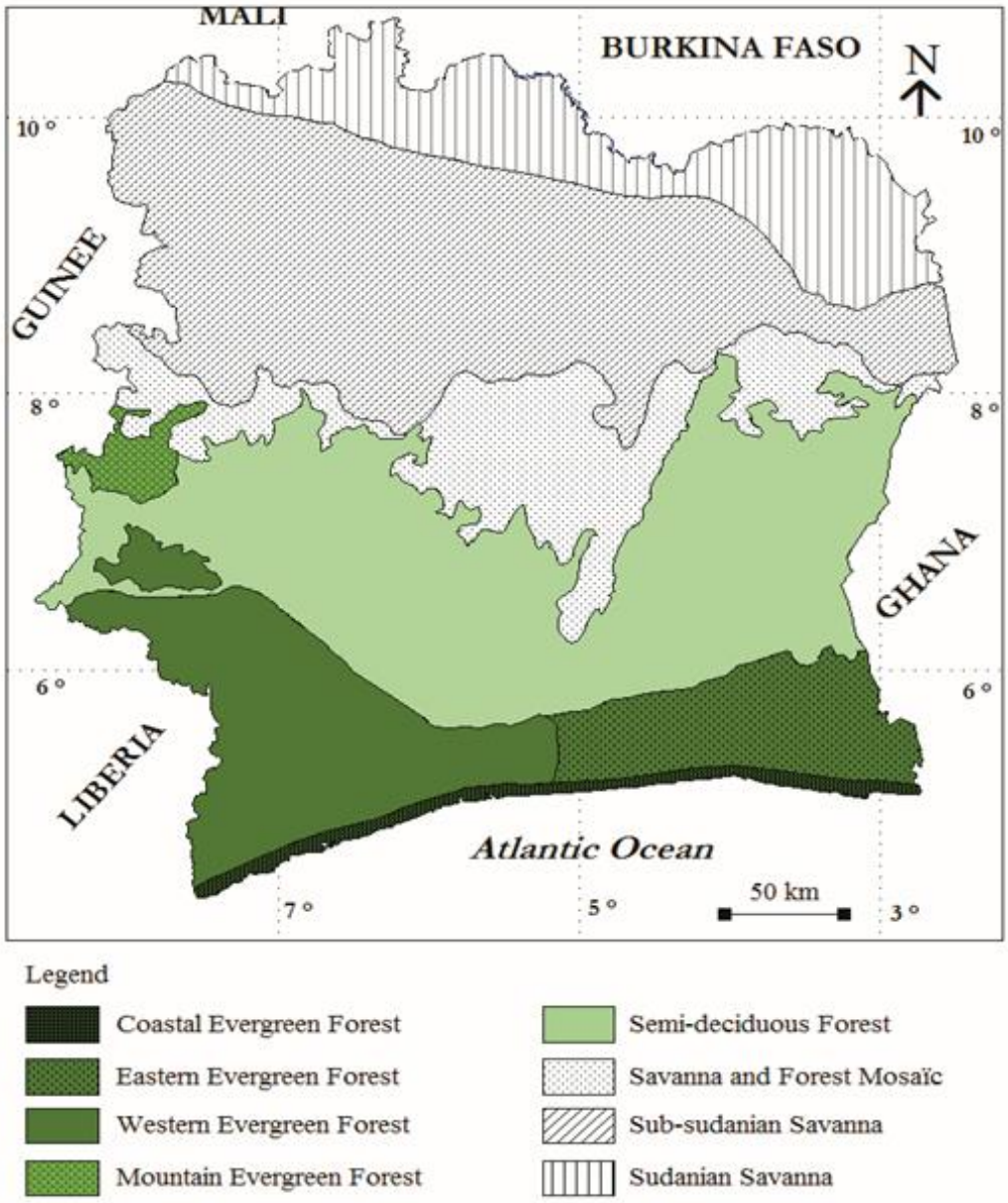

Figure 1. Map of main vegetation types of Côte d'Ivoire. This map is a result of combination of those of Monnier (1983) and Kouamé and Zoro Bi (2010)

2001; Jongkind, 2005).

MATERIALS AND METHODS

The materials source is the Ivoire-database compiled since 1997 by the Geneva Conservatory and Botanical Garden, which consists of botanical information-based on ca. 60'000 plant collections were deposited in various herbaria (Geneva, Abidjan, Wageningen and Paris) with geographic and ecological information (Gautier et al., 1999). From the Ivoire-database, the Celastraceae family consisting of about 1520 samples from 16 genera, 72 taxa made up of 60 species and 12 varieties collected over 363 localities were extracted. The occurrences and samples numbers of taxa were calculated and related to the eight main vegetation types (Figure 1) in Côte d'Ivoire. The similarity index of Sørensen (1948) was used to perform the comparison of the vegetation types according to their richness in Celastraceae taxa. The samples numbers and richness of Celastraceae were related to the total annual rainfall from the general meteorological data in localities through a linear regression. The distribution of taxa in the vegetation types and main ecosystems was performed using Principal Component Analysis with R Software and Venn diagram (Venn, 1880) respectively.

RESULTS

The Celastraceae plants recorded in Côte d'Ivoire are

essentially (86%) climbers and belong mostly to the genus *Salacia* L. which represents 51.4% of the flora (Table 1 and Figure 2). The climbing plants are medium size (2-8 long) for 64% and tall size (> 8 m long) for 22%; the non-climbing plants are medium size (2-8 m high) for 13% and small size (< 2m high) for 1% (Table 1 and Figure 2). Highest sample numbers and richness of Celastraceae were found in both coastal and western evergreen forests; whereas, their lowest values were obtained in sub-sudanian and Sudanian savannas areas (Table 1 and Figure 3). A strong positive correlation was found between the annual total rainfall and the Celastraceae richness (Figure 3).

No Celastraceae taxa were common to all the vegetation types (Table 1 and Figure 3). The commonest taxa were *Apodostigma pallens* (Planch.) R.Wilczek, *Helictonema velutinum* (Afz.) Pierre ex N.Hallé, *Salacia erecta* (G.Don) Walp. and *Salacia owabiensis* Hoyle sampled in 87.5% of the vegetation types (Table 1 and Figure 3). The taxa occurring in 37.5% of the vegetation types were the most abundant and expressed by 30.6 % of the flora (Figure 3). The ten rarest taxa as *Campylostemon laurentii* De Wild., *Maytenus buchananii* Loes., *Maytenus ovatus* Loes. var. *ovatus*, *Maytenus senegalensis* (Lam.) Exell, *Maytenus undata* (Thumb.) Blakel., *Reissantia parvifolia* (Oliv.) N.Hallé, *Salacia lehmbachii* Loes. var. *cucumerella* N.Hallé, *Salacia leptoclada* Tul., *Salacia longipes* (Oliv.) N.Hallé and *Simirestis atractaspis* N.Hallé were found in a single (12.5%) but variable vegetation type (Table 1 and Figure 3).

In terms of samples number, the five most collected Celastraceae taxa in Côte d'Ivoire (6.9%) were *Salacia nitida* (Benth.) N.E.Br., *Salacia owabiensis* Hoyle, *Apodostigma pallens* (Planch.) R.Wilczek, *Salacia erecta* (G.Don) Walp. and *Simicratea welwitschii* (Oliv.) N.Hallé with record number varying from 57 to 92 respectively (Table 1). The six (8.3%) less-collected Celastraceae taxa in Côte d'Ivoire are *Campylostemon laurentii* De Wild., *Maytenus undata* (Thumb.) Blakel., *Salacia lehmbachii* var. *cucumerella* N.Hallé, *Salacia leptoclada* Tul., *Salacia longipes* (Oliv.) N.Hallé and *Simirestis atractaspis* N.Hallé represented by only one sample each (Table 1).

In terms of occurrences of Celastraceae taxa, all the rainforests of Côte d'Ivoire, consisting of the evergreen forests except the montane evergreen forest and the semi-deciduous forest, are much more similar due to their high values of the similarity index (Table 2). The semi-deciduous forest and the savanna and forest mosaic are also much more similar. The Sub-sudanian savanna and the Sudanian savanna are more similar but different to all the other vegetation types (Table 2).

In terms of richness, there was a very strong influence ($r = 0.81$, $P < 0.001$) of both vegetation type and annual total rainfall on the Celastraceae plants distribution in Côte d'Ivoire (Figure 4). This influence of rainfall is positive. Among the vegetation types, coastal evergreen forests expressed the highest richness in Celastraceae while the Sudanian savanna was the lowest (Figure 4) in Côte d'Ivoire. The lowland rainforests showed higher richness in Celastraceae plants than the montane rainforests (Figure 4).

Based on the distribution of Celastraceae plants in Côte d'Ivoire, using PCA, three main groups of vegetation have been demonstrated (Figure 5). Eastern, coastal and montane evergreen forests are closer and constitute the Group 1. Western evergreen and semi-deciduous forest are closer to each other and designated as the Group 2. The third group is composed of the savanna vegetation types including the forest and savanna mosaic (Figure 5). Each vegetation group hosts some endemic taxa and some other taxa that are shared with one or two other groups (Figure 6 and Table 3). Group 1 showed the highest value of group endemic taxa with 11 taxa (15.3%). Group 2 with one taxa (1.4%) showed lowest value of group endemic taxa; while group 3 expressed intermediate value of group endemics with four taxa (5.6%). Nine-teen (19) taxa (26.4%) are common to the three groups of vegetation. Group 1 and 2 shared the highest value of common taxa between pairs of groups with 35 taxa (48.6%); while Group 1 and 3 had no taxa in common (Table 3).

The spatial distribution of Celastraceae plant taxa recorded in Côte d'Ivoire, using PCA, showed four groups of taxa.There was a large group around the center of the axes and three small groups of two-three taxa each above and below axis 1 (Figure 7). Above axis 1, there were two small groups of taxa on the right and the left of axis 2. The group at the right to axis 2 was constituted by *Salacia nitida* (Benth.) N.E.Br., *Salacia owabiensis* Hoyle and *Salacia whytei* Loes. (Figure 7). The latter taxa (*Salacia whytei* Loes.) is endemic to evergreen forests except montane forests; while *Salacia nitida* (Benth.) N.E.Br. is endemic to all evergreen and semi-deciduous forests. *Salacia owabiensis* showed larger distribution area including 87.5% of all the vegetation types. The group at the left of axis 2 encompases *Loeseneriella africana* R.Wilczek and *Salacia baumannii* Loes., which are common to both rainforests and savannas. The small group below axis 1 includes *Apodostigma pallens* (Planch.) R.Wilczek, *Salacia erecta* (G.Don) Walp. and *Salacia stuhlmaniana* Loes., which are also common to both rainforests and savannas. The largest group of taxa around the center of axes (Figure 7) is made of all the 64 remnant taxa. Therefore, the total annual rainfall and the 8 main vegetation types included in this manuscript cannot provide adequate separation for most of the Celastraceae plant taxa recorded in Côte d'Ivoire.

DISCUSSION

Family Celastraceae gathers 60 genera and nearly 850 worldwide mainly tropical but some representatives reach

Table 1. Samples numbers and richness of Celastraceae plants in the main vegetation types of Côte d'Ivoire.

Biology	Taxa	Coastal Everg. Forest	Eastern Everg. Forest	Western Everg. Forest	Montane Everg. Forest	Semi-decid. Forest	Savanna-Forest mosaïc	Sub-Sudanian Savanna	Sudanian Savanna	Total sample
MC	*Apodostigma pallens* (Planch.) R.Wilczek	4	10	22		10	10	8	4	**68**
MC	*Apodostigma pallens* var. *buchholzii* N.Hallé	1	2							**3**
TC	*Bequaertia mucronata* (Exell) R.Wilczek	2	5	3	16	2				**27**
TC	*Campylostemon angolense* Welw. ex Oliv.					2	6	6		**14**
MC	*Campylostemon laurentii* De Wild.			1						**1**
TC	*Campylostemon warneckeanum* Loes. ex Fritsch	3	3	8	1	8	7			**29**
TC	*Cuervea macrophylla* R.Wilczek ex N.Hallé	16	5	9		2				**31**
MC	*Helictonema velutinum* (Afz.) Pierre ex N.Hallé	5	5	1	1	2	7	2		**24**
TC	*Hippocratea myriantha* Oliv.	23	3	18						**44**
TC	*Hippocratea vignei* Hoyle	4		3		17				**24**
MC	*Loeseneriella africana* R.Wilczek var. *africana*	2	2		5	7				**15**
MC	*Loeseneriella africana* var. *schweinfurthiana* Loes.	1		3		3	3	3	1	**14**
TC	*Loeseneriella apocynoides* N.Hallé ex Raynal	7	3	3		1				**14**
TC	*Loeseneriella apocynoides* var. *guineensis* N.Hallé	4	4	5		2				**14**
TC	*Loeseneriella clematoides* (Loes.) R.Wilczek ex N.Hallé	8	3	2	6		2			**21**
TC	*Loeseneriella ectypetala* N.Hallé	6		6		12	2			**25**
TC	*Loeseneriella iotricha* (Loes.) N.Hallé	7	4	3	3	6	4			**27**
MC	*Loeseneriella rowlandii* (Loes.) N.Hallé	4	3	6	4	16	7			**40**
MT	*Maytenus buchananii* Loes.	5								**5**
MT	*Maytenus ovatus* Loes. var. *ovatus*	2								**2**
MT	*Maytenus senegalensis* (Lam.) Exell							14	3	**17**
MT	*Maytenus serrata* (Hochst. ex A.Rich.) Wilczek	8			1					**9**
MT	*Maytenus undata* (Thumb.) Blakel.				1					**1**
TC	*Prionostemma unguiculata* (Loes.) N.Hallé	16	4	2		14				**36**
MC	*Pristimera panicualata* (Vahl) N.Hallé	10		2		4	2			**17**
TC	*Pristimera plumbea* (Blak. & Wilczek) N.Hallé	1		1		3				**5**
TC	*Reissanthia indica* var. *astericantha* N.Hallé	4		1	1					**7**
MC	*Reissantia indica* N.Hallé var. *loeseneriana*	8	2		4	17	5	5		**41**
MC	*Reissantia parvifolia* (Oliv.) N.Hallé						2			**2**
MC	*Salacia adolfifriderici* Loes. ex Harms	2		2						**3**
MC	*Salacia baumannii* Loes.	5	3		5	5		2	2	**20**
MC	*Salacia cerasifera* Welw. ex Oliv.	3	3	3						**9**
MC	*Salacia chlorantha* Oliv.	1				1	2			**3**
MC	*Salacia columna* N.Hallé var. a*keassii* N.Hallé		2	5						**7**
MC	*Salacia columna* N.Hallé var. *columna*	9	4	4	6					**22**

Table 1. Contd.

MC	*Salacia columna* N.Hallé	11			11					**22**
MC	*Salacia cornifolia* Hook.f.	7		11		4				**22**
MC	*Salacia debilis* (G.Don) Walp.	3	3	7	7	18				**38**
MC	*Salacia elegans* Welw. ex Oliv.	4		6	7	4	2			**22**
MC	*Salacia erecta* (G.Don) Walp.	12	3	19	7	12	9	3		**64**
MC	*Salacia howesii* Hutch. & Moss	3		1		1				**5**
MC	*Salacia ituriensis* LoeSalacia	5				2				**6**
MC	*Salacia lateritia* N.Hallé	8	10	23	2					**42**
MT	*Salacia lehmbachii* Loes.	18	2	6	2	7				**35**
MT	*Salacia lehmbachii* Loes. var. *aurantiaca* N.Hallé	10	2	6		6				**24**
MT	*Salacia lehmbachii* Loes. var. *leonensis* N.Hallé	1	1	17	8	6				**33**
MT	*Salacia lehmbachii* var. *cucumerella* N.Hallé				1					**1**
MC	*Salacia leptoclada* Tul.							1		**1**
MC	*Salacia letestui* Pellegr.	1		1		1				**3**
MC	*Salacia longipes* (Oliv.) N.Hallé	1								**1**
MC	*Salacia longipes* var. *camerunenis* N.Hallé	5			2					**6**
MC	*Salacia miegei* N.Hallé	6	2	12						**20**
MC	*Salacia nitida* (Benth.) N.E.Br.	12	48	24	4	4				**92**
MC	*Salacia nitida* N.E.Br.var. *bipindensis* Loes.	2		1			1			**4**
MC	*Salacia oliveriana* Loes.	6	6	3						**14**
MC	*Salacia oliveriana* Loes. var. *adiopodoumella* N.Hallé	11	7			2				**20**
MC	*Salacia owabiensis* Hoyle	18	16	8	14	15	2		3	**75**
ST	*Salacia pallens* Oliv.	7		2	2	9		2		**23**
MC	*Salacia pyriformis* (Sabine) Steud.		2	2	3					**7**
MC	*Salacia staudtiana* Loes.	13	3		7	7				**30**
MC	*Salacia staudtiana* Loes. var. *leonensis* Loes.	8	7		4					**19**
MC	*Salacia staudtiana* var. *tshopoensis* De Wild.	1	1	1						**2**
MC	*Salacia stuhlmaniana* Loes.			10		5	5	6	3	**29**
MC	*Salacia togoica* Loes.	3		4	3	9	5	3		**26**
MC	*Salacia whytei* Loes.	18	9	14						**41**
MC	*Salacia zenkeri* Loes.	6	6	15						**27**
TC	*Salacighia letestuana* (Pellegr.) Blak.	17	5	14	3	3				**41**
MC	*Simicratea welwitschii* (Oliv.) N.Hallé	29	5	5	2	13	5			**57**
MC	*Simirestis atractaspis* N.Hallé							1		**1**
MC	*Simirestis dewildemaniana* N.Hallé	3		3	3					**9**
MC	*Simirestis tisserantii* N.Hallé	2	1		1					**4**
TC	*Tristemonathus nigrisilvae* N.Hallé	7	4	1						**12**

Table 1. Contd.

Samples	**424**	**207**	**325**	**147**	**258**	**86**	**56**	**16**	**1520**
Richness	**61**	**40**	**49**	**33**	**39**	**21**	**13**	**6**	

MC, Medium climber (2-8 m long); TCT, all climber (> 8 m long); MT, Medium tree (2-8 m high); ST, Small tree (< 2 m high). Everg., evergreen; decid., deciduous.

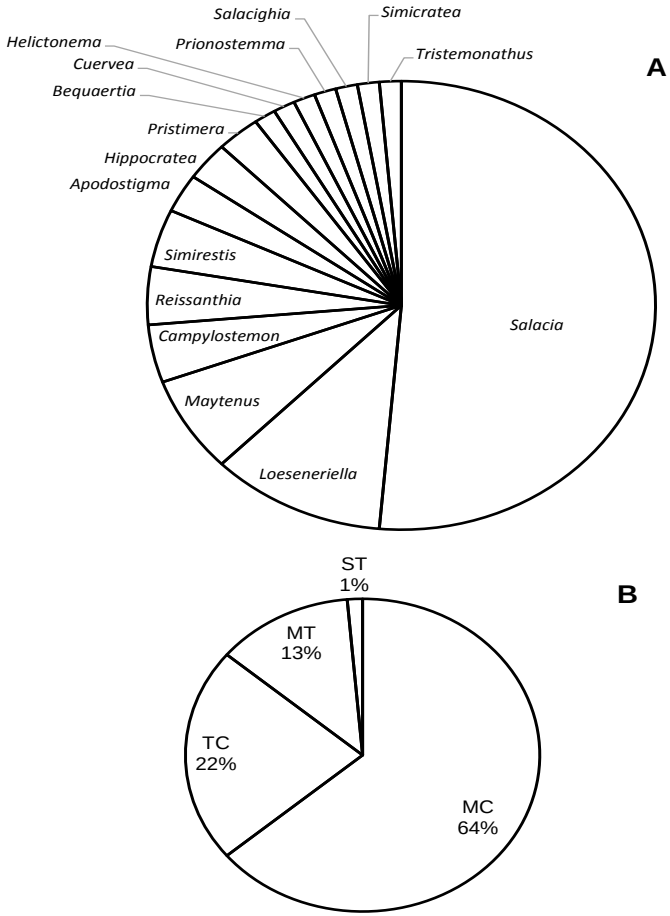

Figure 2. Diagrams of Celastraceae genera (A) and plant categories (B) assessed in Côte d'Ivoire. MC = Medium climber (2-8 m long), TC = Tall climber (> 8 m long), MT = Medium tree (2-8 m high) and ST = Small tree (< 2 m high).

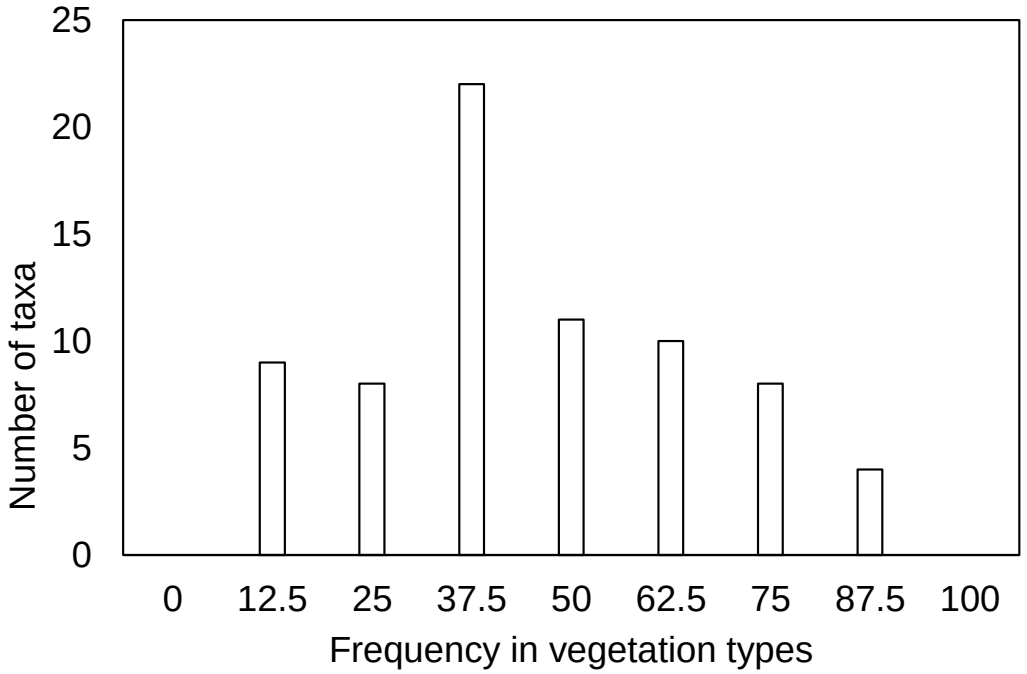

Figure 3. Diagram of the Celastraceae plant distribution frequency in the main vegetation types of Côte d'Ivoire.

Table 2. Sørensen's similarity coefficient between couples of vegetation types.

	Coastal Everg. Forest	Eastern Everg. Forest	Western Everg. Forest	Montane Everg. Forest	Semi-decid. Forest	Savanna and Forest mosaïc	Sub-Sudanian	Sudanian Savanna
Coastal Everg. Forest		71	77	58	71	43	24	13
Eastern Everg. Forest			72	58	62	35	20	14
Western Everg. Forest				54	77	55	22	14
Montane Everg. Forest					57	50	27	11
Semi-decid. Forest						68	36	20
Savanna and Forest mosaïc							47	26
Sub-Sudanian								53
Sudanian Savanna								

Coefficient values are in percentage and expressed above the diagonal. Higher coefficient between couple of vegetation types means strong similarity between these vegetation types. Below the diagonal, these valeus have been replaced by colors to illustrate the blocs of vegetation types according to Celastraceae richness and diversity. Everg. means evergreen; decid. means deciduous.

the temperate regions (Spichiger et representatives reach the temperate regions (Spichiger et al., 2000; Botineau, 2010). The most important genera are Maytenus Molina with 200 species form the hot tropical regions, Salacia L. Celastraceae flora in Côte d'Ivoire follows the characteristics of several large families of vascular plants occurring in the tropics. Indeed, large tropical plant families such as Orchidaceae, Poaceae and Rubiaceae have usually some large genera such as *Bulbophyllum* Thouars, *Panicum* L. and *Psychotria* L. respectively coexisting with several small genera (Hutchinson and Dalziel, 1954, 1958; 1963, 1968, 1972; Lebrun and Stork, with 200 species form the tropics, Euonymus L. with 180 species from the temperate regions and Hippocratea L. with 100 species from the tropics (Botineau, 2010).The predominance of the genus *Salacia* L. in the 1991, 1992, 1995, 1997; Hawthorne and Jongkind, 2006). But the substantial occurrence (51.4%) of taxa represented by *Salacia* L. among Celastraceae plants in Côte d'Ivoire (Table 1 and Figure 2) was exceptional. *Salacia* L. is about 200 species of lianas, shrubs, and small trees found throughout the tropics (Simmons, 2004; Botineau, 2010). Around 100 species are found in tropical Africa (Jongkind, 2006) and around 80 of these in the

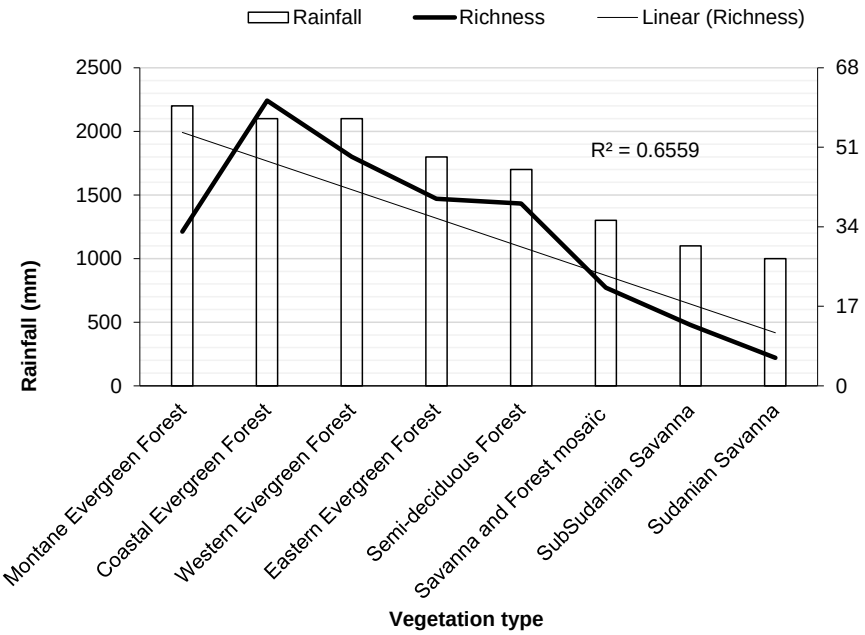

Figure 4. Ordination of Celastraceae plants' richness according to both the vegetation types and annual total rainfall **.**

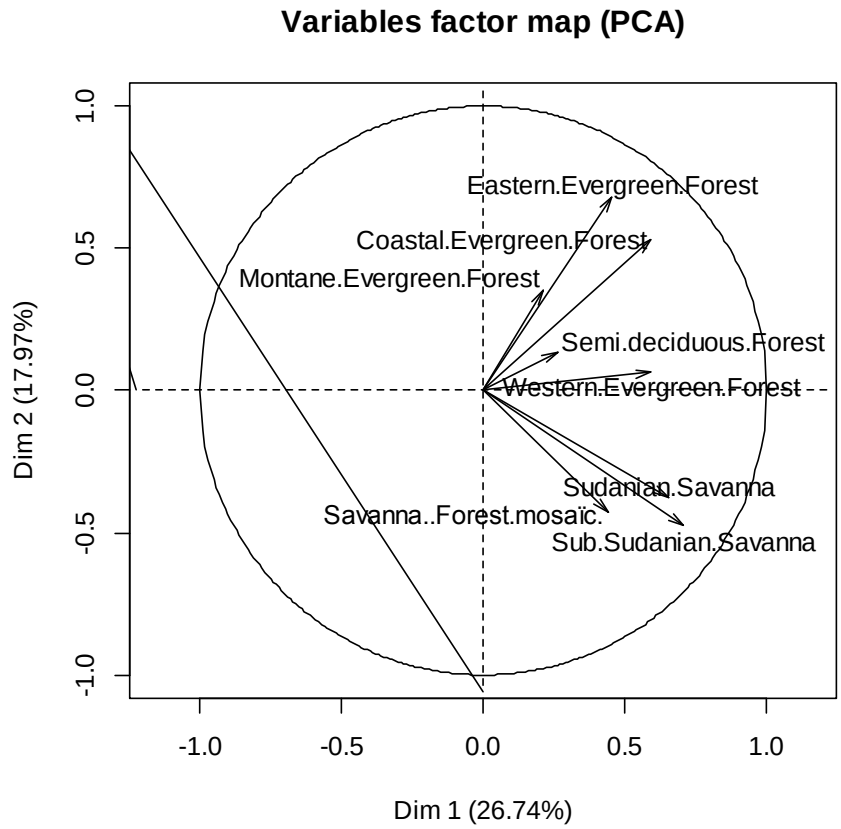

Figure 5. Groups of vegetation according to Celastraceae plants distribution in Côte d'Ivoire. Group 1 rich of 65 taxa consists of evergreen forests except Western evergreen forest; Group 2 with 57 taxa gathers Western evergreen and semi-deciduous forest; Group 3 about 25 taxa unifies all vegetation types in savanna area including the savanna and forest mosaic.

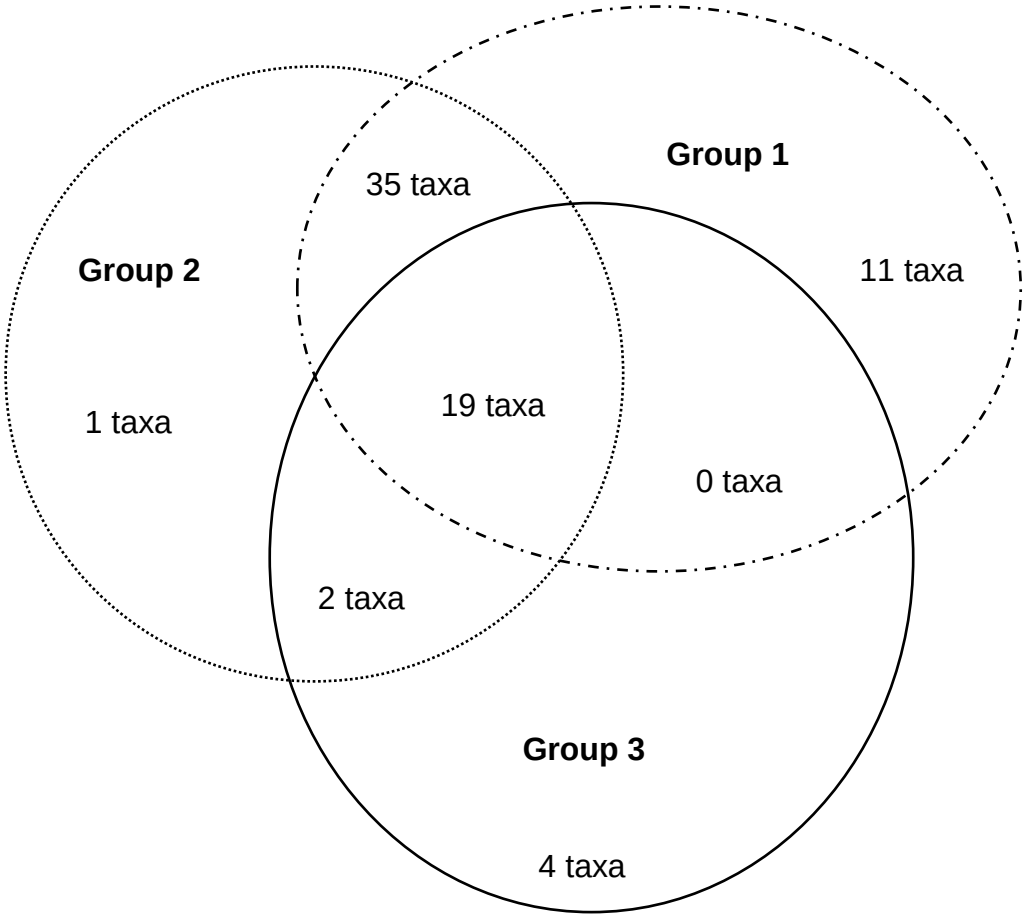

Figure 6. Venn diagram of the vegetation groups of Côte d'Ivoire according to Celastraceae distribution. Taxa endemic to these forest groups are as follow: **Group 1**. *Apodostigma pallens* var. *buchholzii* N.Hallé, *Maytenus buchananii* Loes., *Maytenus ovatus* Loes. var. *ovatus*, *Maytenus serrata* (Hochst. ex A.Rich.) Wilczek, *Maytenus undata* (Thumb.) Blakel.,*Salacia columna* N.Hallé, Salacia lehmbachii var. *cucumerella* N.Hallé, *Salacia longipes* (Oliv.) N.Hallé, *Salacia longipes* var. *camerunenis* N.Hallé, *Salacia staudtiana* Loes. var*. leonensis* Loes., *Simirestis tisserantii* N.Hallé. **Group 2.** *Campylostemon laurentii* De Wild. **Group 3.** *Maytenus senegalensis* (Lam.) Exell, *Reissantia parvifolia* (Oliv.) N.Hallé, *Salacia leptoclada* Tul., *Simirestis atractaspis* N.Hallé; Taxa common to all the three groups are *Apodostigma pallens* (Planch.) R.Wilczek, *Campylostemon warneckeanum* Loes. ex Fritsch, *Helictonema velutinum* (Afz.) Pierre ex N.Hallé, *Loeseneriella africana* var. *schweinfurthiana* Loes., *Loeseneriella clematoides* (Loes.) R.Wilczek ex N.Hallé, *Loeseneriella ectypetala* N.Hallé, *Loeseneriella iotricha* (Loes.) N.Hallé, *Loeseneriella rowlandii* (Loes.) N.Hallé, *Pristimera panicualata* (Vahl) N.Hallé, *Reissantia indica* N.Hallé var. *loeseneriana, Salacia baumannii* Loes., *Salacia chlorantha* Oliv., *Salacia elegans* Welw. ex Oliv., *Salacia erecta* (G.Don) Walp., *Salacia nitida* N.E.Br.var. *bipindensis* Loes., *Salacia owabiensis* Hoyle, *Salacia pallens* Oliv., *Salacia togoica* Loes., *Simicratea welwitschii* (Oliv.) N.Hallé. Refer to Table 3 for taxa common to couples of vegetation types.

Cameroon-Gabon centre of biodiversity (Gosline and Cheek, 2014). The most recent review of the entire genus is an unpublished thesis by Hedin (1999). Recent species description has been confined to South America (Lombardi, 2007, 2009, 2010) and India (Udayan et al., 2012, 2013).

A proportion of about 86% of the Celastraceae flora in Côte d'Ivoire, represented by climbing plants confirmed the character of tropical plant family attributed to Celastraceae (Hallé, 1958, 1962; Hutchinson and Dalziel, 1954; Lebrun and Stork, 1991, 1992; Hawthorne and Jongkind, 2006) which is among the vascular plants medium occurring families usually common to African tropical forests understory and canopy.

The decreasing of both Celastraceae samples number and richness (Table 1 and Figure 4) from the rainforest to the savanna types follows the natural distribution rules of climber plants in tropical vegetation types (ORSTOM and

Table 3. Celastraceae plant taxa common to couples of vegetation groups.

	Group 1	Group 3
Group 2	*Bequaertia mucronata* (Exell) R.Wilczek *Cuervea macrophylla* R.Wilczek ex N.Hallé *Hippocratea myriantha* Oliv. *Hippocratea vignei* Hoyle *Loeseneriella africana* R.Wilczek var. africana *Loeseneriella apocynoides* N.Hallé ex Raynal *Loeseneriella apocynoides* var. *guineensis* N.Hallé *Prionostemma unguiculata* (Loes.) N.Hallé *Pristimera plumbea* (Blak. & Wilczek) N.Hallé *Reissanthia indica* var. *astericantha* N.Hallé *Salacia adolfifriderici* Loes. ex Harms *Salacia cerasifera* Welw. ex Oliv. *Salacia columna* N. Hallé var. *akeassii* N. Hallé *Salacia columna* N. Hallé var. *columna* *Salacia cornifolia* Hook.f. *Salacia debilis* (G.Don) Walp. *Salacia howesii* Hutch. & Moss *Salacia ituriensis* Loes. *Salacia lateritia* N. Hallé *Salacia lehmbachii* Loes. *Salacia lehmbachii* Loes. var. *aurantiaca* N.Hallé *Salacia lehmbachii* Loes. var. *leonensis* N.Hallé *Salacia letestui* Pellegr. *Salacia miegei* N. Hallé *Salacia nitida* (Benth.) N.E.Br. *Salacia oliveriana* Loes. *Salacia oliveriana* Loes. var. *adiopodoumella* N.Hallé *Salacia pyriformis* (Sabine) Steud. *Salacia staudtiana* Loes. *Salacia staudtiana* var. *tshopoensis* De Wild. *Salacia whytei* Loes. *Salacia zenkeri* Loes. *Salacighia letestuana* (Pellegr.) Blak. *Simirestis dewildemaniana* N.Hallé *Tristemonathus nigrisilvae* N.Hallé	*Salacia stuhlmaniana* Loes. *Campylostemon angolense* Welw. ex Oliv.
Group 3	No common taxa	

UNESCO, 1983; Blanc, 2002). Indeed, tropical rainforests have this characteristic to harbour many medium and tall climbers (Schimper, 1903; Richards, 1996) among which Celastraceae is one of the most abundant families in Côte d'Ivoire (Aké Assi, 2001, 2002; Kouamé et al., 2007). Moreover, Celastraceae is almost exclusively a forest plants family (Hallé, 1958, 1962; Hutchinson and Dalziel, 1958; Hawthorne and Jongkind, 2006); even in savanna and montane areas where these taxa occur in forest patches on plateaus and along rivers. The genus *Maytenus* Molina possesses exclusively medium size trees (Table 1) and is better spread in coastal evergreen forests, montane evergreen forests and savanna area in Côte d'Ivoire. The different groups of taxa according to their distribution in the vegetation types (Tables 1 to 3 and Figures 5 to 7) express the capacity of these taxa to live or adapt as a response to local ecological conditions. Hence, ubiquitous taxa such as *Apodostigma pallens* (Planch.) R.Wilczek, *Helictonema velutinum* (Afz.) Pierre ex N.Hallé, *Salacia erecta* (G.Don) Walp., *Salacia owabiensis* Hoyle have been assessed in 87.5% of the vegetation types (Table 1) due to their large tolerance to local ecological conditions. Higher samples numbers of these taxa (Table 1) confirmed the overall local abundance of such Celastraceae in Côte d'Ivoire. Consequently, Celastraceae

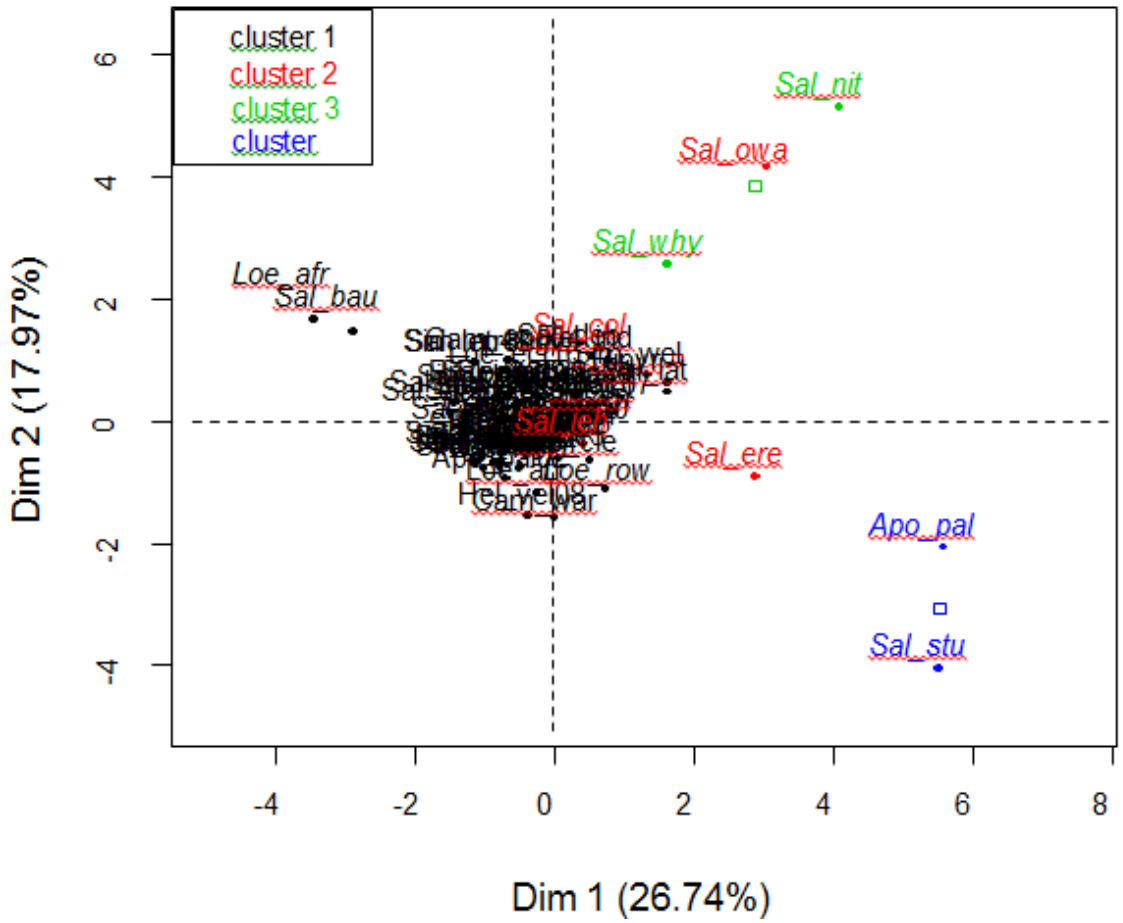

Figure 7. Spatial distribution of Celastraceae plants assessed in Côte d'Ivoire through a PCA. The taxa names are abbreviated using the three first letters of the genera and those of the specific epithets.

species has been able to live and develop in all the 8 vegetation types in Côte d'Ivoire (Table 1 and Figure 3) revealing that ecological conditions are quite different in these main vegetation types.

Among the rainforest types, the highest samples number and richness of Celastraceae found in both coastal and western evergreen forests (Table 1 and Figure 3) could be explained by the higher intensity of plants assessments in both areas in Côte d'Ivoire. Banco forest and Taï forest which do two national parks exist respectively in these forest zones are also the most studied forests in Côte d'Ivoire (de Koning, 1983; Riezebos et al., 1994). But this difference of sampling effort cannot explain the lowest values of both sample number and richness of Celastraceae, in comparison to the rainforest types (Figure 3).

The strong and positive correlation between the annual rainfall and the Celastraceae richness (Figure 4) reveals that local annual total rainfall is among the factors that lead to establishment and development of Celastraceae in an area as reported by Parmentier et al. (2007, 2011) and Fayolle et al. (2014) for tropical trees. Therefore, Celastraceae taxa endemic to one or other vegetation type (Table 1) such as *Maytenus buchananii* Loes., *Salacia lehmbachii* Loes var. *cucumerella* N.Hallé, *Reissantia parvifolia* (Oliv.) N.Hallé can establish and develop only in the ecological conditions prevailing in these vegetation types.

Conclusion

The Celastraceae plant family consists of almost exclusively of climbing life habits that live in all vegetation types of Côte d'Ivoire, even though there are different levels of abundance and richness across these vegetation types. The total annual rainfall was to positively influence the richness of Celastraceae as well. Based on spatial distribution of these plants, the natural main vegetation types of Côte d'Ivoire could be classified into three groups of two to three vegetation types each. Despite the difficulties to assess fertile materials of most climbing plants such as those in the family Celastraceae, a database such as the one used here holds promise to

improve our understanding of some major biogeographic and ecological patterns of these climbers across broad geographic localities.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Appendix

Celastraceae distribution frequency in the main vegetation types.

REFERENCES

Aké Assi L (2001). Flore de la Côte d'Ivoire : catalogue systématique, biogéographique et écologie. I. Biossiera 57:396.
Aké Assi L (2002). Flore de la Côte d'Ivoire: catalogue systématique, biogéographie et écologie. II. Boissiera 58:401.
APG (1998). An ordinal classification for the families of flowering plants. Annals of the Missouri Botanical Garden 85(4):531-553.
APG (2003). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Botanical Journal of the Linnean Society 141:399-436.
APG (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Botanical Journal of the Linnean Society 16:105-121.
Blanc P (2002). Être plante à l'ombre des forêts tropicales, Nathan/VUEF, Paris. 432p.
Botineau M (2010). Botanique systématique appliquée des plantes à fleurs. Tec & Doc., Paris. 1006p.
De Koning J (1983). La forêt du Banco. I. La forêt. II. La flore, Wageningen Agricultural University, tome I, 156pp & tome II, 921pp.
Fayolle A, Swaine MD, Bastin J-F, Bourland N, Comiskey JA, Dauby G, Doucet J-L, Gillet J-F, Gourlet-Fleury S, Hardy OJ, Kirunda B, Kouamé FN, Plumptre AJ (2014). Patterns of tree species composition across tropical African forests. Journal of Biogeography 41(12):2320-2331.
Gautier L, Aké Assi L, Chatelain C, Spichiger RE (1999). Ivoire: A geographic information system for biodiversity management in Ivory Coast. In Timberlake J, Kativu S (eds), African plants: biodiversity, taxonomy and uses. Royal Botanic Gardens, Kew. pp. 183-194.
Gosline G, Cheek M (2014). Two new African species of *Salacia* (Salacioideae, Celastraceae). Blumea 59(1): 26-32.
Hallé N (1958). Hippocrateacées nouvelles d'Afrique Occidentale. Bulletin du Muséum National d'Histoire Naturelle. Paris 2(30):464-474.
Hallé N (1962). Monographie des Celastraceae d'Afrique Occidentale, IFAN, n° 64.245pp.
Hawthorne WD, Jongkind CCH (2006). Woody plants of western African forests. A guide to the forest trees, shrubs and lianes from Senegal to Ghana. Kew Publishing, UK. 1023pp.
Hedin JPT (1999). Systematic studies of the neotropical species of Salacia L. (Hippocrateaceae) and its relatives. PhD thesis, Unpubl. Washington University, St. Louis.
Hutchinson J, Dalziel JM (1954). Flora of West Tropical Africa. 2e ed., Vol. 1, part 1, Millbank, London 295p.
Hutchinson J, Dalziel JM (1958). Flora of West Tropical Africa, 2e ed., vol. 1, part 2, Millbank, London pp. 296-828.
Hutchinson J, Dalziel JM (1963). Flora of West Tropical Africa. 2e ed., vol. 2, Millbank, London 544p.
Hutchinson J, Dalziel JM (1968). Flora of West Tropical Africa. 2e ed., vol. 3, part 1, Millbank, London 276p.
Hutchinson J, Dalziel JM (1972). Flora of West Tropical Africa. 2e ed., vol. 3, part 2, Millbank, London pp. 277-574.
Jongkind CCH (2005). Checklist of climber species in Upper Guinea. In Bongers F, Parren MPE, Traoré D (eds), Forest climbing plants of West Africa. Diversity, Ecology and Management, CABI Publishing, UK pp. 231-264.
Jongkind CCH (2006). Novitates Gabonenses 60. *Salacia hallei*, a name for a rare Celastraceae species from Western Central Africa. Adansonia, sér 3,28(1):101-104.
Kouamé FN, Bakayoko A, Traoré D, Bongers F (2007). Checklist of the Upper Guinea forest area climbers of Côte d'Ivoire. Sciences et Nature 4(2):149-170.
Kouamé FN, Zoro Bi IA (2010). Nouveau découpage de la zone de forêt dense humide de la Côte d'Ivoire. Sciences et Nature 7(2):177-194.
Lebrun J-P, Stork AL (1991). Enumération des plantes à fleurs d'Afrique Tropicale: Généraités et Annonaceae à Pandaceae. Vol. I, CJB, Genève 249p.
Lebrun J-P, Stork AL (1992). Enumération des plantes à fleurs d'Afrique Tropicale: Chrysobalanaceae à Apiaceae. Vol. II, CJB, Genève 257pp.
Lebrun J-P, Stork AL (1995). Enumération des plantes à fleurs d'Afrique Tropicale. Monocotylédones : Limnocharitaceae à Poaceae. Vol. III, CJB, Genève 341pp.
Lebrun J-P, Stork AL (1997). Enumération des plantes à fleurs d'Afrique Tropicale. Gamopétales: Ericaceae à Lamiaceae. Vol. IV, CJB, Genève 712 pp.
Lombardi JA (2007). Three new South American species of *Salacia* with fasciculate inflorescences (Celastraceae, Hippocrateoideae). Novon 17:33-39.
Lombardi JA (2009). Three new species of *Salacia* from Mesoamerica (Celastraceae, Salacioideae). Novon 19:372-379.
Lombardi JA (2010). Three new species of Salacioideae (Celastraceae). Nordic Journal of Botany 28(3):316-320.
Monnier Y (1983). Carte de la végétation de la Côte d'Ivoire. In Vennetier P, Laclavère G (eds), Atlas de Côte d'Ivoire. 2e éd., Jeune Afrique, Paris 72p.
ORSTOM, UNESCO (1983). Écosystèmes forestiers tropicaux d'Afrique. Collection Recherches sur les Ressources Naturelles, n° 19, Paris 473pp.
Parmentier I, Harrigan RJ, Buermann W, Mitchard ETA, Saatchi S, Malhi Y, Bongers F, Hawthorne WD, Leal ME, Lewis SL, Nusbaumer L, Sheil D, Sosef MSM, Affum-Baffoue K, Bakayoko A, Chuyong GB, Chatelain CV, Comiskey JA, Dauby G, Doucet J-L, Fauset S, Gautier L, Gillet J-F, Kenfack D, Kouamé FN, Kouassi EK, Kouka LA, Parren MPE, Peh KS-H, Reitsma JM, Senterre B, Sonké B, Sunderland TCH, Swaine MD, Tchouto MGP, Thomas D, van Valkenburg JLCH, Hardy OJ (2011). Predicting alpha diversity of African rain forests: Models based on climate and satellite-derived data do not perform better than a purely spatial model. Journal of Biogeography 38(6):1164-1176.
Parmentier I, Malhi A, Senterre B, Whittaker RJ, ATDN, Alonso A, Balinga MPB, Bakayoko A, Bongers F, Chatelain CV, Comiskey JA, Corthay R, Djuikouo-Kamdem M-N, Doucet J-L, Gautier L, Hawthorne W D, Issembe YA, Kouamé FN, Kouka LA, Leal ME, Lejoly J, Lewis SL, Nusbaumer L, Parren MPE, Peh KS-H, Phillips OL, Poorter L, Sheil D, Sonké B, Sosef MSM, Sunderland TCH, Stropp J, ter Steege H, Swaine MD, Tchouto MGP, van Gemerden BS, van Valkenburg JLCH, Wöll H (2007). The odd man out? Might climate explain the lower tree α-diversity of African rain forests relative to Amazonian rain forests? Journal of Ecology 95:1058-1071.
Richards PW (1996). The tropical rain forest. Cambridge University Press, 2e ed. 575p.
Riezebos EP, Vooren AP, Guillaumet J-L, Sloot PHM, Hazeu GW

(1994). Le Parc National de Taï, Côte d'Ivoire. I. Synthèse des connaissances. II. Bibliographie. Tropenbos Series, 8, Wageningen. 323 pp. + 1 disc.
Schimper AFW (1903). Plant-geography upon a physiological basis. Oxford University Press. 1028pp.
Simmons MP (2004). Celastraceae. In Kubitzki K (eds), The families and genera of vascular plants 6. Springer pp. 29-64.
Sørensen T (1948). A method of establishing groups of equal amplitude in plant sociology based on similarity of species content. Det Kongelige Danske Videnskabernes Selskab. Biologiske Skrifter 5(4):1-34.
Spichiger RE, Savolainen VV, Figeat M (2000). Botanique systématique des plantes à fleurs. Une approche phylogénétique nouvelle des Angiospermes des régions tempérées et tropicales. Presses polytechniques et universitaires romandes, Lausanne 372p.
Udayan P, Yohannan R, Devipriya M, Devipriya V, Pradeep A (2012). A new species of *Salacia* (Hippocrateaceae) from South India. Edinburgh Journal of Botany 69(2):255-258.
Udayan P, Yohannan R, Devipriya M, Devipriya V, Pradeep A (2013). *Salacia vellaniana* Udayan, Yohannan, Pradeep (Celastraceae), a new species from India. Candollea 68(1):147-149.
Venn J (1880). On the diagrammatic and mechanical representation of propositions and reasonings. Philosophical Magazine and Journal of Science 9(59):1-18.

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