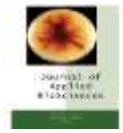

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Susceptibility of rubber genotypes [*Hevea brasiliensis* Muell. Arg. (Euphorbiaceae)] to Corynespora Leaf Fall disease in graft wood gardens in southwestern Côte d'Ivoire

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ABSTRACT
Objective: This work to determine the susceptibility of 20 rubber genotypes to leaf fall disease caused by *Corynespora cassiicola* (CLF) was conducted in the San Pedro Rubber Sector in southwestern Côte d'Ivoire.
Methods and Results: These rubber genotypes were selected in large-scale clone fields and tested for susceptibility to CLF disease under natural infection in the transplant garden. In a clonal plot, the rubber plants were planted in line, with a spacing of 1 m x 1 m between the lines. Overall, the results showed that the susceptibility of rubber genotypes fluctuated over the past five years. In addition, some rubber genotypes such as IRCA 430, IRCA 101, IRCA 733, IRCA 321, IRCA 523, IRCA 41, IRCA 230, IRCA 323 and IRCA 229, once resistant or tolerant, showed over time a susceptibility to CLF disease. On the other hand, three genotypes: PB 217, IRCA 101 and IRCA 538 expressed a good level of resistance to *C. cassiicola*, unlike the sensitive control IRCA 18 which showed a high susceptibility throughout the 5 years of observation.
Conclusion and Recommendation: Of the 20 rubber genotypes tested, only three (PB 217, IRCA 101 and IRCA 538) are good candidates for large-scale rubber crop referrals to minimize the impact of CLF disease in the South-West Côte d'Ivoire rubber orchard.
Keywords: susceptibility, *Corynespora cassiicola*, *Hevea brasiliensis*, graft wood garden, CLF disease, Côte d'Ivoire

INTRODUCTION

Hevea brasiliensis (Muell. Arg), a plant species of the Euphorbiaceae family, is native to the amazon basin. It is cultivated in several tropical countries. Its cultivation started in Côte d'Ivoire in the 1950s by agro-industrial companies (Canh, 1999) and is steadily increasing. Côte d'Ivoire is now the leading African producer and the fourth largest in the world, with a production of 1,100,000 tonnes of dry rubber representing 80% of African production (APROMAC, 2022). *Hevea brasiliensis* is currently the main source of tapped natural rubber (Rahman *et al.,* 2013) and also the only commercially planted and prized species in the world with better rubber (Dusotoit-Coucaud *et al.,* 2009). The demand for natural rubber is constantly increasing, especially due to the growing demand in recent years from emerging countries such as China, Brazil and India. Numerous research activities have been developed around the rubber tree, notably the selection of high latex-producing clones. However, the agronomic performance of the selected clones is still hampered by some diseases, particularly leaf diseases. Currently, one of the leaf diseases of greatest concern to rubber growers is Corynespora Leaf Fall (CLF) disease. The pathogen responsible for this disease is a fungus of the class Dothideomycetes called *Corynespora cassiicola* (Schoch *et al.,* 2009). First observed in Côte d'Ivoire in the 1990s on clones RRIC 103 and RRIC 110 (Wahounou *et al.,* 1996), CLF disease has been in epidemic phase since 2010. Indeed, heavy attacks of this disease observed in almost all plantations of clone IRCA 18 in Côte d'Ivoire, have contributed to increase the concern of farmers and the rubber industry. Today, the problem is managed by regularly treating trees with antifungal products. However, these chemical treatments are difficult to implement on a plantation scale, and are harmful to the environment. Therefore, the adverse effects of these chemicals lead to the consideration of alternative, more environmentally friendly control methods. As an alternative, rubber genotypes showing tolerance to *Corynespora cassiicola* and having good production dispositions could be used in rubber cultivation. This study specifically aims to evaluate the susceptibility of 20 rubber genotypes to CLF disease in the rubber growing area of San Pedro in southwestern Côte d'Ivoire in a graft wood garden.

MATERIALS AND METHODS

Study site: The study was carried out in the graft wood gardens (GWG) located in the rubber sector of San Pedro (Figure 1). The San Pedro site is located 368 km from Abidjan, in the southwest of Côte d'Ivoire, not far from the border with Liberia. The San Pedro region has a humid equatorial climate, characterised by abundant rainfall. The average annual temperature in the San Pedro area is 26.1°C (Climate-Data.org). The hottest months in this area are February, March and April (28.5-29°C) and the coolest months are August and September with an average of 25.6°C (Kéli *et al.,* 1992). The average annual rainfall is 1900 mm (fr.weatherspark.com). The choice of this site takes into account, on the one hand, the high representation of rubber cultivation in the southern zone representing the traditional area of rubber cultivation in Côte d'Ivoire, and on the other hand, the favourable climatic conditions conducive to the expression of the susceptibility of genotypes to cryptogamic diseases.

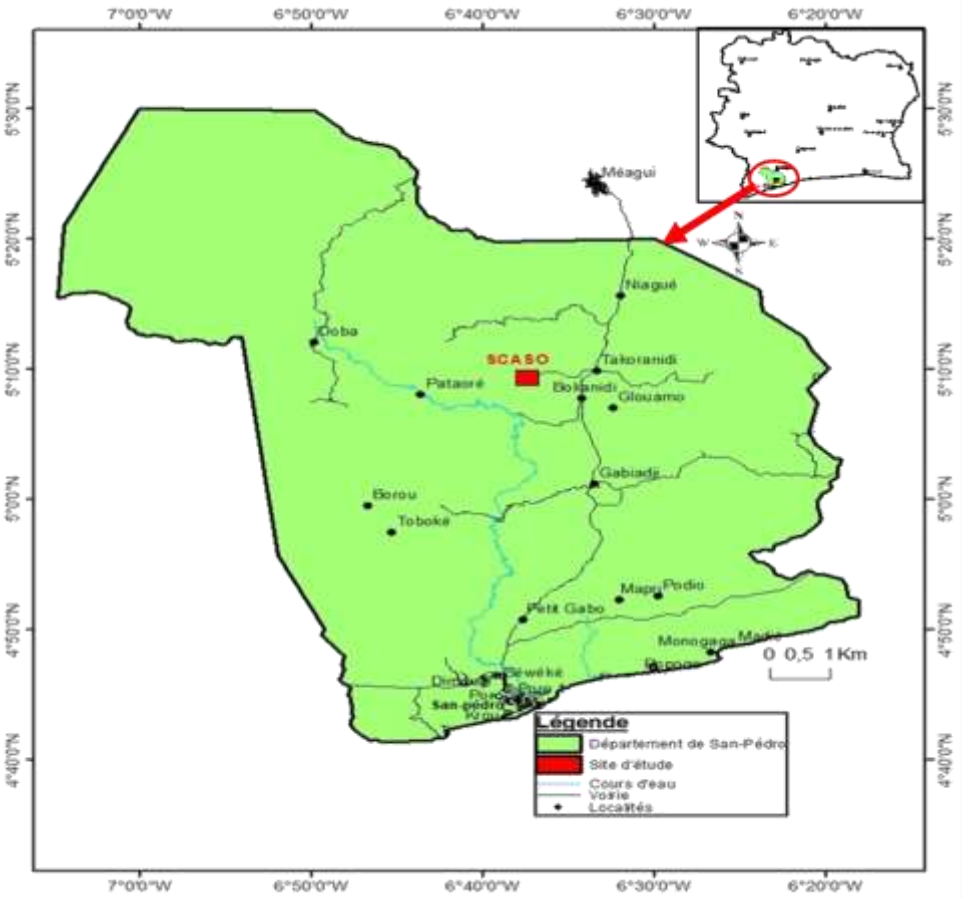

Figure 1: Location of study site (CNRA, 2019)

Plant material: The plant material consisted of 20 rubber genotypes selected in large-scale clone fields (LSCF) and a control genotype IRCA 18. The LSCF are located in the traditional rubber growing area of Côte d'Ivoire. The crosses of these rubber genotypes are distributed as shown in table 1.

Table 1: Descendants of rubber genotypes and their parents used in this study

Numbers	Descendants	Parents (Female x Male)
1	IRCA 18 (control genotype)	PB 5/51 x RRIM 605
2	IRCA 41	GT 1 x PB 5/51
3	IRCA 230	GT 1 x PB 5/51
4	PB 217	PB 5/51 x PB 6/9
5	IRCA 101	PB 5/51 x IR 22
6	IRCA 122	PB 5/51 x RRIM 600
7	IRCA 229	GT 1 x PB 5/51
8	IRCA 317	GT 1 x PB 5/51
9	IRCA 323	GT 1 x PB 5/51
10	IRCA 523	PB 5/51 x RRIM 703
11	IRCA 538	PB 5/51 x RRIM 703
12	IRCA 733	PB 5/51 x PR 228
13	RRIM 712	RRIM 605 x RRIM 71
14	IRCA 428	GT 1 x AF 261

15	IRCA 27	PB 5/51 x RRIM 623
16	IRCA 321	GT 1 x PB 5/51
17	IRCA 840	PB 5/51 x PB 252
18	IRCA 408	PB 5/51 x NAB17
19	BPM 24	GT 1 x AVROS 1734
20	RRIM 729	RRIM 623 x FX 25
21	IRCA 430	RRIM 605 x AF 261

Methods: The experiments involved 21 selected rubber genotypes including the susceptible control IRCA 18. These genotypes were selected in large-scale clone fields and tested for susceptibility to Corynespora Leaf Fall (CLF) disease under natural infection in graft wood gardens. Observations of CLF disease symptoms were made over five consecutive years (2018 - 2022) in graft wood gardens. In a clonal plot, rubber plants were planted in rows with a spacing of 1 m x 1 m between rows. Each plot was divided into five relatively equidistant observation points on the diagonals. In each clonal plot, 100 plants were scored. Leaf symptoms such as necrotic lesions with yellow halo and vein necrosis giving a typical "fishbone" pattern were used to identify plants attacked by CLF disease. The Manju *et al.* (2010) scale was used to assess the level of disease severity. 0 = no symptoms, 1 = very mild (five lesions), 2 = mild (6-10 lesions and 5% leaf drop), 3 = moderate (more than 10 lesions and 10.1-25% leaf drop), 4 = severe (large lesions and 25.1-50% leaf drop), 5 = very severe (large lesions and >50% leaf drop). The disease severity index (DSI) caused by *C. cassiicola* was determined by the following formula:

$$DSI = \frac{\sum (Ni \times Ii)}{Nt}$$

Ii: severity score (0 to 5); Ni: number of trees with score i; Nt: total number of trees examined. Clonal susceptibility was assessed on a scale of 0 to 5 as follows: 0 = immune (DSI = 0); 1 = resistant genotype ($0 < DSI \leq 1$); 2 = tolerant ($1 < DSI \leq 2$); 3 = moderate ($2 < DSI \leq 3$); 4 = susceptible ($3 < DSI \leq 4$) and 5 = highly susceptible ($4 < DSI \leq 5$).

Statistical analysis: Analysis of variance (ANOVA) was used to compare *C. cassiicola* disease severity indices across rubber genotypes. When a significant difference was observed between the means, the ANOVA was complemented by multiple comparisons using the least significant difference (LSD) test. The significance of the tests was determined at the α=0.05 threshold. These analyses were carried out with Statistica 10 software.

RESULTS

Susceptibility of selected rubber genotypes to *Corynespora cassiicola* across years in the San Pedro area: The analysis of variance (ANOVA) performed to compare the disease severity index of rubber genotypes over the five years (2018-2022) of observation revealed a significant difference (Table 2). This table shows that rubber genotypes such as IRCA 430, IRCA 321, IRCA 41, IRCA 230, IRCA 323 IRCA 229, IRCA 428, IRCA 317 and BPM 24 that showed resistance to *C. cassiicola* in the year 2018, subsequently showed low to high Corynespora disease severity indices. Although the susceptibility of rubber genotypes has fluctuated over the years, there is one batch of genotype with good level of resistance to *C. cassiicola* throughout the five years of observation. This is the case with PB 217, IRCA 101 and IRCA 538. The susceptible control IRCA 18 showed high susceptibility to *C. cassiicola* throughout the five years of observation.

Table 2: Susceptibility of selected rubber genotypes to *Corynespora cassiicola* over five years (2018-2022) of observation

	2018	2019	2020	2021	2022
Rubber genotypes	**Disease Severity Index (DSI)**				
IRCA 18	5.000 a	5.000 a	5.000 a	5.000 a	5.000 a
IRCA 430	0.000 c	2.400 c	1.000 d	2.500 f	3.200 de
RRIM 729	1.200 b	1.200 e	2.300 c	5.000 a	1.633 h
IRCA 101	0.000 c	0.000 g	1.500 d	0.000 h	0.000 i
IRCA 733	0.000 c	2.500 c	2.800 b	0.000 h	0.000 i
RRIM 712	4.000 a	5.000 a	5.000 a	0.000 h	2.633 g
IRCA 27	1.400 b	2.500 c	2.900 b	0.000 h	4.000 c
PB 217	0.000 c	1.000 e	1.000 d	0.600 g	0.000 i
IRCA 321	0.000 c	0.600 f	1.000 d	4.600 b	3.000 ef
IRCA 538	0.000 c	0.000 g	0.000 f	0.000 h	0.000 i
IRCA 523	0.200 c	0.600 f	0.700 e	5.000 a	2.800 fg
IRCA 41	0.000 c	0.800 ef	1.000 d	5.000 a	3.833 c
IRCA 230	0.000 c	3.200 b	1.000 d	5.000 a	4.000 c
IRCA 323	0.000 c	0.700 f	2.500 c	2.300 f	2.533 g
IRCA 428	0.000 c	2.700 c	2.000 c	3.767 d	4.000 c
IRCA 229	0.000 c	2.000 cd	2.800 b	4.367 c	3.333 d
IRCA 122	4.000 a	4.000 a	2.400 c	4.467 bc	4.467 b
IRCA 840	1.000 b	1.600 d	1.200 d	5.000 a	4.000 c
IRCA 317	0.000 c	0.700 f	2.800 b	3.200 e	3.800 c
IRCA 408	0.700 bc	1.000 e	2.400 c	3.067 e	4.000 c
BPM 24	0.000 c	3.500 b	1.300 d	5.000 a	3.400 d
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

In each column, values with the same letter are not significantly different (5% Newman Keuls test); 0 = no disease (DSI = 0); 1 = resistant genotype (0 < DSI ≤ 1); 2 = tolerant (1 < DSI ≤ 2); 3 = moderate (2 < DSI ≤ 3); 4 = susceptible (3 < DSI ≤ 4); and 5 = highly susceptible (4 < DSI ≤ 5)

Overall effect of the association of years and rubber genotypes on the severity index of CLF disease caused by *Corynespora cassiicola:* Multivariate analysis of variance (MANOVA) showed that there is very highly significant influence (P < 0.0001) of the observation years and rubber genotype on the severity index of CLF disease caused by *Corynespora cassiicola*. Multivariate analysis of variance (MANOVA) revealed an interaction (P < 0.05) of years of observation and rubber genotypes on the severity index of CLF disease caused by *C. cassiicola* (Table 3).

Table 3: MANOVA result on the effect of the association "years and rubber genotypes" on the severity index of the disease caused by *C. cassiicola*

	DL	***F***	***P***
Years	4	5850.618	< 0.0001
Rubber genotypes	20	1913.721	< 0.0001
Years x Rubber genotypes	80	344.078	< 0.0001

Influence of the year on the severity index of the disease caused by *C. cassiicola* in the San Pedro area: Figure 2 presents the results on the influence of the years on the severity index of the CLF disease caused by *C. cassiicola* in the San Pedro area. The results of the five years

of observation show that the year 2021 stood out from the others with an increase in the disease severity index (DSI = 3.14). On the contrary, the year 2018 was marked by very low disease severity indices (DSI = 0.93).

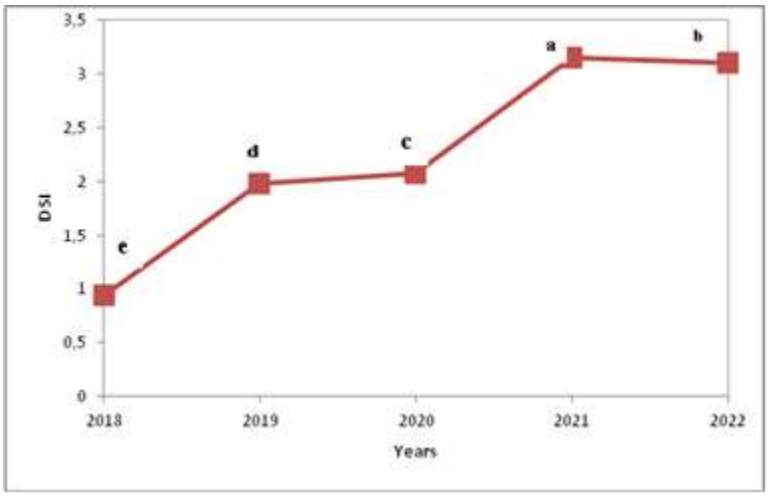

Figure 2: Graph of the dynamics of CLF disease caused by *C. cassiicola* over a period of 5 years in the San Pedro area

Some symptoms of CLF disease caused by *Corynespora cassiicola* in the graft wood garden:

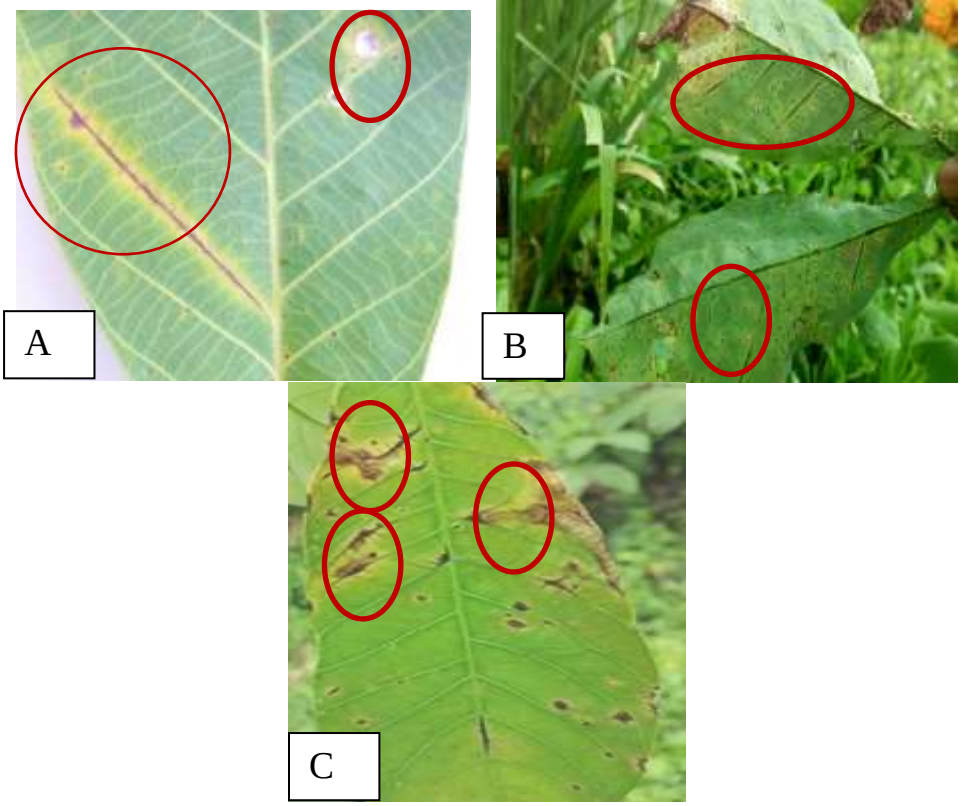

Figure 3: Symptoms of CLF disease due to *Corynespora cassiicola* in graft wood gardens (A: characteristic "fishbone" lesions on the leaf; B: burnt appearance of the leaf veins; C: lesions surrounded by a yellow halo on the leaf)

DISCUSSION

The results of the study of the susceptibility of rubber genotypes to *C. cassiicola* in graft wood garden showed that the formerly resistant rubber genotypes show over time a susceptibility to *C. cassiicola*. Indeed, the rubber genotypes in particular IRCA 430, IRCA 101, IRCA 733, IRCA 321, IRCA 523, IRCA 41, IRCA 230, IRCA 323 and IRCA 229 that showed resistance to *C. cassiicola* in 2018 have subsequently shown severe symptoms of *C. cassiicola* disease. This could be related to several factors such as climate change, introduction or development of a new pathotype of *C. cassiicola* in the rubber orchard. Similar observations were reported by Mathew (2006) on clonal susceptibility to the disease caused by *C. cassiicola* in various natural rubber producing countries. He pointed out that the tolerance level of widely grown clones is declining. The different ranges of susceptibility observed could be explained by the fact that rubber genotypes do not react in the same way after being attacked by the fungus (Tran, 2016). Similar observations were reported by Jayasinghe (2003) on rubber genotypes such as AVROS 2037, PB 260, RRIM 600, PB 235, PR 107, RRIC 110, IAN 873, RRII 105 and PR 261 to the disease caused by *C. cassiicola*. There is a group of rubber genotypes that did not show any symptoms of the disease (DSI=0). This observed phenomenon could be described as vertical or monogenic resistance, which appears only against certain races of the pathogen. A study by Breton *et al.* (1997) showed that this type of resistance can be explained first by the accumulation of a phytoalexin, scopoletin, and then by the lignification of cell walls, which forms a physical barrier to the spread of the fungus. According to d'Auzac (1995), this defence mechanism can be explained by the cross-linking of the parietal proteins responsible for blocking the mycelium in the necrotic tissues at the point of penetration. Our results also show the presence of a group of rubber tree genotypes tolerant to the disease ($1 < DSI \leq 2$). This tolerance response could be explained by the fact that the plant has adopted more sophisticated mechanisms to inhibit the reproduction of the pathogen after having bypassed its preformed defences (Thordal-Christensen, 2003). This type of defence mechanism is usually polygenic and is called horizontal resistance. This hypothesis is corroborated by the work of Othman *et al.* (1996). According to these authors, this phenomenon is due to the effects of polygenic characters that lead to resistance to *Corynespora cassiicola*. The high rate of susceptible rubber genotypes observed in the graft gardens of southwestern Côte d'Ivoire could be mainly related to climatic conditions, especially rainfall. Indeed, this rubber-growing region of southwestern Côte d'Ivoire receives heavy rainfall (about 2000 mm) every year, providing favourable conditions for the proliferation of *Corynespora cassiicola* spores. This result is consistent with what Jayasinghe (2003) reported. This author showed that moisture generated by rainfall favours sporulation, spore germination and increased pathogenicity. It was shown that the *C. cassiicola* susceptible control, particularly IRCA 18, displayed susceptibility to CLF disease throughout the observation period (2018-2022). This susceptibility could be due to the disease outbreak first reported in 2010 on this genotype, which is cultivated over large areas in the traditional rubber growing zone in Côte d'Ivoire; thus, increasing the pest pressure in this area. The high susceptibility to CLF diseases observed in the majority of genotypes in this study could be explained by the fact that these rubber genotypes are hosts of *C. cassiicola* breed confined in this area. This result may also be due to the high rainfall observed in this study area. Similar results were reported by Manju *et al.* (2010), in a study conducted in Karnataka district (India)

on the susceptibility of 62 *Hevea brasiliensis* genotypes to the disease caused by *C. cassiicola*. Ultimately, epidemiology is very important for designing and developing appropriate control methods. Diagnosis based on the observation of symptoms is a prerequisite for the development of control methods against the fungi.

CONCLUSION AND RECOMMENDATION

At the end of this study, it was found that the susceptibility of rubber genotypes to CLF disease has fluctuated over the years. In addition, some rubber genotypes such as IRCA 430, IRCA 101, IRCA 733, IRCA 321, IRCA 523, IRCA 41, IRCA 230, IRCA 323 and IRCA 229, formerly resistant or tolerant, show susceptibility to CLF diseases over time. However, three genotypes; PB 217, IRCA 101 and IRCA 538 expressed a good level of resistance to *C. cassiicola*, in contrast to the susceptible control IRCA 18 which showed a high susceptibility to *C. cassiicola* throughout the 5 years of observation. These three genotypes (PB 217, IRCA 101 and IRCA 538) are good candidates for large-scale rubber crop referrals to minimize the impact of CLF disease in the South-West Côte d'Ivoire rubber orchard.

BIBLIOGRAPHIC REFERENCES

APROMAC. 2022. Rubber culture is an opportunity for environmental preservation, APROMAC, 15th Conference of the parties (COP15). Côte d'Ivoire depressed agency of 20 May 2022, Abidjan (Côte d'Ivoire).

Breton F, Sanier C, d'Auzac J. 1997. Scopoletin production and degradation in relation to resistance of *Hevea brasiliensis* to *Corynespora cassiicola*. *Journal of Plant Physiology.,* 151: 595-602. https://doi.org/10.1016/S0176-1617(97)80236-2

Brou YT, Akindes F, Bigot S. 2005. Climate variability in Côte d'Ivoire: between social perceptions and agricultural responses, *Cahiers Agricultures*. 14 (6): 533-540. https://revues.cirad.fr/index.php/cahiers-agricultures/article/view/30548

Canh TV. 1999. Research for the rubber industry in Côte d'Ivoire. Plantation, Research, Development, 6 (2): 102–105.

CNRA. 2019. Promotion of new rubber clones, FIRCA projet. Progress Report 1, 32 p.

D'Auzac J, Prevot JC, Jacob C. 1995. What's new about lutoids? A vacuolar system model from Hevea latex. *Plant Physiol. Biochem.*, 33: 765–777. https://agritrop.cirad.fr/388097/

Dusotoit-Coucaud A, Brunel N, Kongsawadworakul P, Viboonjun U, Julien J-L, Chrestin H, Sakr S. 2009. Sugar importation into laticiferous cells of *Hevea brasiliensis*, in relation to ethylene stimulation of latex yield Annals of botany in press.

Jayasinghe CK. 2003. Corynespora Leaf Fall and future of the leading rubber clones in the world. Bulletin of the Rubber Research Institute of Sri Lanka, 44: 5-11.

Keli JZ, Obouayeba S, Zehi B. 1992. Influence of some food systems on the behaviour of young rubber trees in lower Côte d'Ivoire. Agricultural Systems in Africa, 2 (1): 41 - 48.

Manju MJ, Vinod KK, Idicula SP, Kuruvilla Jacob C, Nazeer MA, Benagi VI. 2010. Susceptibility of *Hevea brasiliensis* Clones to Corynespora Leaf Fall disease. *Hevea Breeding Sub-Station, Rubber Research Institute of India, J. Mycol Plant Pathol.,* 40 (4) 603 - 609.

Mathew J. 2006. Clonal resistance of *Hevea brasiliensis* to Corynespora leaf fall diseases. Corynespora leaf disease of *Hevea brasiliensis.* Strategies for Management (Jacob CK eds) Rubber Research Institute of India. 188 pp.

Othman R, Benong M, Ong SH, Hashim I. 1996. Strategies and development of resistant Hevea clone against Corynespora leaf fall. *Proc Workshop, Corynespora Leaf Fall Disease of Hevea Rubber*, Indonesian Rubber Research Institute, Medan, Indonesia, pp 177-193.

Rahman AYA, Usharraj AO, Misra BB, Thottathil GP, Jayasekaran K, Yun F, Hou S, Ong SY, Ng FL, Lee LS, Tan HS, Sakaff MK, Muhd L, The BS, Khoo BF, Badai SS, Aziz NA, Yuryev A, Knudsen B, Dionne-Laporte A, Mchunu NP, Yu Q, Langston BJ, Freitas TAK, Young AG, Chen R, Wang L, Najimudin N, Saito JA, Alam M. 2013. Draft genome sequence of the rubber tree *Hevea brasiliensis*; *BMC Genomics*, 14:75. doi : 10.1186/1471-2164-14-75.

Schoch CL, W CP, Z GJA, Bew IBT, de Gruyter JGS, de Hoog LJDM. 2009. A class-wide phylogenetic assessment of Dothideomycetes. *Studies in Mycology,* 64: 1-15.

Thordal-Christensen H. 2003. Fresh insights into processes of nonhost resistance. *Curr. Opin. Plant Biol.*, 6, 351–357. https://doi.org/10.1016/S1369-5266(03)00063-3

Tran DM. 2016. The toxin test: a phenotyping method for the study of the interaction *Hevea brasiliensis* x *Corynespora cassiicola* and the identification of genetic factors of susceptibility. Doctoral thesis, Montpellier University 2, France, 257 p. https://tel.archives-ouvertes.fr/tel-01689822

Wahounou PJ, Tran Van Canh C, Keli JZ, Eschbach JM. 1996. Development of *Corynespora cassiicola* and Colletotrichum gloesporioides leaf fall diseases in rubber plantation in Africa. In: Proceeding of the workshop on Corynespora Leaf Fall disease. Medan, Indonesia, pp 99–106. https://agritrop.cirad.fr/389621