Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Botany

Appl. Botany 161 (2021) 55881-55888

ISSN: 2229-712X

Screening of Cocoa Hybrids of Yangambi Breeding for Resistance to Black Rot Disease of Cocoa Pods Caused by Lasiodiplodia Theobromae in ihe Democratic Republic of Congo (DRC)

Limba, KG mukwa Kibuka [1], Onautshu O. Didy[2], Mukandama J. P[3], Mubenga Onesime[3] and, Monde Godefroid [4]

[1]National Cocoa Research Program at the National Institute for Agronomic Study and Research of Yangambi (INERA-Yangambi)

[2]Laboratory of Microbiology and Phytopathology, Department of Biotechnological Sciences, Faculty of Sciences, University of Kisangani

[3]Faculty of Renewable Natural Resources Management, University of Kisangani

[4]Plant Pathology and Biotechnology Laboratory, Yangambi Faculty of Agricultural Sciences Institute (IFA-Yangambi)

ARTICLE INFO

Article history:
Received: 20 October 2021;
Received in revised form:
15 December 2021;
Accepted: 25 December 2021;

Keywords
Lasiodiplodia Theobromae,
Black rot,
Pod,
Resistance,
DRC.

ABSTRACT

Black rot of cocoa pods, a disease caused by *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., causes significant production losses all over the world. To reduce the impact of this pest, genetic control using resistant varieties is favored.To do this, inoculations having shown the interest of the INERA Yangambi collection, consisting in the evaluation of the resistance of 14 "CRY" clones, vis-à-vis inoculations of a strain of L. theobromae were operated. This study, carried out in Yangambi in the DRC, using inoculation on detached pods with series of tests and a statistical test adapted to the ordinal nature of the data confirms that two clones representing 14.28% of the subjects tested do indeed constitute resistant materials. L. theobromae. In addition, 5 clones or 35.71% are considered moderately resistant. However, the other remaining materials could be integrated into many cocoa genetic improvement programs, especially those with other notable qualities. The Yangambi collection with 62 accessions.

Introduction

New so-called emerging diseases are constantly appearing. Indeed, it is and remains indisputable that in recent years new parasitic diseases have arisen and are often gaining extension in all countries of the world. This is how Robinson (1996) will describe emergence as being the result of the unprecedented encounter of a parasite and a plant which until then was not or no longer its host. The phenomenon follows the occurrence of conditions favorable to the development and dissemination of a pathogen already present (indigenous) or introduced (exotic). Thus, the 19th century was marked by large parasitic invasions, in particular the late blight of the potato caused by Phytophthora infestans, at the origin of the great famine which decimated 20% of the Irish population between 1845 and 1849 (Ristaino, 2002).

In this work, we were mainly interested in a pathogenic fungus, Lasiodiplodia theobromae. Its resurgence is closely linked to the globalization of trade and the subsequent introduction of pathogens into new ecosystems (Brown et al., 2001). Anderson et al., 2004 propose that an emerging disease is caused by a pathogen (i) whose incidence, geographic distribution or host range has increased; (ii) whose pathogenicity has increased significantly; (iii) which has recently evolved genetically or (iv) whose discovery is recent. Environmental changes, the domestication of wild species, increased trade and the intensification of production systems are all factors that may explain the emergence or re-emergence of plant diseases.

Like all other crops around the world, the cocoa tree is subject to attack from various diseases and insects. This is the case of black rot of the cocoa tree caused by the fungi of the genus Botryodiplodia (synonym Lasiodiplodia) and in particular, by the species Lasiodiplodia theobromae, which causes significant production losses in all cocoa growing areas, which can vary from 30 to 90% depending on the conditions (Bowers et al., 2001).

The emergence of this disease in the cocoa growing area of Bengamisa had arrested more than one person and, following the report of the experts in 2013 (Limba 2013), the central government had released a sum of the order of USD 223,500, to buy phytosanitary products to fight against this disease in this cocoa growing area. Let us recall here that the cocoa zone of Bengamisa (CABEN) consists of two blocks of cocoa trees: the industrial block with an area of 443 hectares and the family block of 628 hectares of cocoa trees. This spontaneous intervention by the central government, not only was insignificant for the industrial bloc managed by the government because of the social charges of the workers, but also did not concern the family bloc managed by the peasants, which is nevertheless the most important (628 Ha). The disease is therefore far from being eradicated in this cocoa growing area, with the risk of contamination in other neighboring areas such as the hinterland of the city of

Tele:
E-mail address: gastonlimba49@gmail.com

Kisangani, why not in all the cocoa growing areas of the country if no adequate security arrangements are made. is taken quickly. We should also point out that, out of ignorance, most of the new cocoa tree planters obtain their seeds from CABEN and even from the farmers supervised by it. This creates a permanent danger in the area. In fact, lacking sufficient financial means, these farmers do not have access to phytosanitary products which are initially unavailable on local markets. This situation calls in the foreground, the research supposed to be able to find the durable solution awaited by the planters. Thus, a study which had been carried out in this area had shown that there was a need to consider an intervention in order to eradicate this disease because the rate of pods found diseased was very high. And the resulting predictions have shown that if we did not intervene within a few years, the disease would worsen (LIMBA, 2020). This same study had identified the fungus L. theobromae as being responsible for the disease in this cocoa growing area. Among the solutions which had been envisaged, the use of resistant clones was favored in view of its numerous advantages. And the resulting predictions have shown that if we did not intervene within a few years, the disease would worsen (LIMBA, 2020). This same study had identified the fungus L. theobromae as being responsible for the disease in this cocoa growing area. Among the solutions which had been envisaged, the use of resistant clones was favored in view of its numerous advantages. And the resulting predictions have shown that if we did not intervene within a few years, the disease would worsen (LIMBA, 2020). This same study had identified the fungus L. theobromae as being responsible for the disease in this cocoa growing area. Among the solutions which had been envisaged, the use of resistant clones was favored in view of its numerous advantages.

Indeed, today, resistance to parasitic plagues is a major objective in cocoa tree breeding. This is because chemical control is not always effective and represents a high cost for the planter. The use of varieties resistant to this plague is an essential ecological and economic solution in an integrated and sustainable control. Thus, as for the brown rot of cocoa pods caused by Phytophtora (Nyassé et al., 2007; Pokou et al., 2008), great hopes must be placed in the selection and multiplication of cocoa varieties resistant to black rot of cocoa trees. pods at Lasiodiplodia. For this reason,

Research questions

Are there materials resistant or tolerant to the black rot disease of cocoa pods caused by the fungus of L. theobromae in the INERA Yangambi collection?

To this main question is added a second: do the climatic conditions as well as the morphological characteristics of the varieties thus play a role in the resistance of the clones to infection?

To answer these questions, the following hypotheses have been formulated

Hypotheses

- Among the genetic materials of INERA, there are those that may be "resistant" to black rot disease in cocoa growing areas of the DRC.
- The morphological characteristics of the pods as well as the climatic conditions would play a role in the resistance and the infection of the clones to the disease.

Main objective of the Study

Select cocoa clones resistant to black pod rot in the Democratic Republic of Congo (DRC).

Specific objectives

- Testing the genetic materials of the Yangambi cocoa tree for their resistance to pod rot;
- Select those that would behave better in the face of inoculation for resistance to black pod rot disease for dissemination in all cocoa growing areas of the country;
- Make a connection between the morphological characteristics of the pod, climatic conditions and resistance to infection.

Material and methods

➢ Location

The study concerned two cocoa growing areas namely, the cocoa growing area of Bengamisa located 36 km from the city of Kisangani and the cocoa growing area of Yangambi, 100 km from Kisangani. The two cocoa growing areas are 150 km apart.

➢ Rainfall during the study periods

The rainfall records for 2018 and 2019 are shown on the graph in Figure 1 below. It should be noted that the observation periods were from 06 to 18/5/2018 and from 07 to 19 Nov./2019. Note also that the pods were harvested at the maximum age of 4 months. That said, the pods inoculated in May, received during their development 557.8 mm of the rains that fell from January to April and, those inoculated in September, the rains from May to September are, 755mm of the rains before their entry into the laboratory.

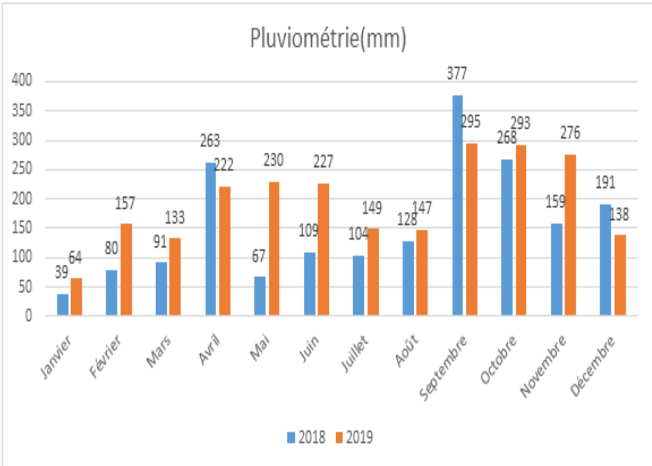

Figure 1. Rainfall readings for the years 2018 and 2019.

➢ Relative Humidity

During this same period, the relative humidity readings were recorded and illustrated in Figure 2 below:

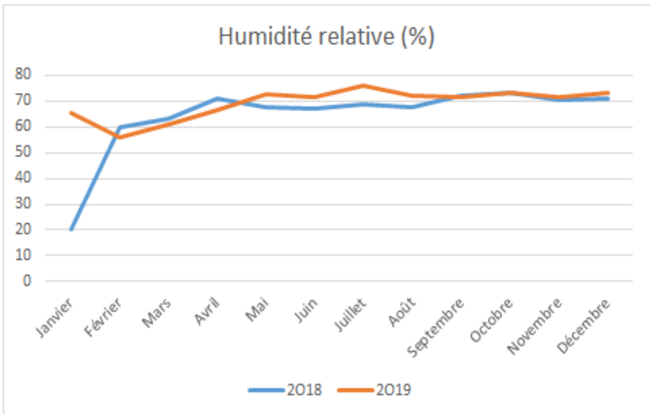

Figure 2. Reading the relative humidity of the air during the study period.

Note that the humidity inside the room was kept almost the same during both periods of the study.

➢ **Plant Material**

The choice of material was dictated by the previous genetic study which showed great genetic diversity in the collection of hybrids selected by INERA Yangambi (Hayley et al., 2018). The idea was therefore to consider genetically distant individuals in order to observe their reactions to inoculations of the disease. Thus the screening was carried out on well identified and different material, obtained by self-pollination. This made it possible to obtain material of relatively the same age (Figure 3).

(A) Emasculation of the flower; (B) Insulation of the flower

Figure 3. Process of artificial pollination

The plant material consisted of 14 clones of the new selection from INERA Yangambi, listed with their morphological characteristics in Table 1 below.

➢ **Fungal Material.** The material that served as the inoculum was strains of L. theobromae isolated in the Microbiology and Phytopathology laboratory of the Faculty of Sciences of the University of Kisangani. These materials had been collected in the fields of CABEN. It should be noted that the CABEN cocoa trees had manifested black pod rot disease only three years after their entry into production in plots I and V. The study which has just been carried out in this cocoa growing area shows that the rate of black pod disease infection is around 71% (Limba 2020). Let us recall that the basic seeds of the fields of CABEN and its population had been imported from Côte d'Ivoire.

➢ **Methods**

For this study, the screening method was adopted by the test on detached pods. This test is considered to be the most economical and efficient screening method compared to the selection of resistant genotypes in the field (Ling A., 2017).

▪ *Experimental apparatus.* This was completely randomized and installed in the Phytopathology laboratory of INERA

▪ *Collection and preparation of pods for inoculation.* Unripe, same-stage, unripe pods of similar dimensions (3-4 months old) were carefully collected in the morning between 7 a.m. and 10 a.m. and kept in clear, labeled plastic bags. No more than two pods were kept together in contact, locked in a bag with the others.The harvested pods were brought to the INERA laboratory Yangambi where they have been rinsed with distilled water and labeled. They were thenscarified by simulating the bites of mirids in order to allow the penetration of the inoculum, finally, they were placed on the bench in the INERA laboratory (figure 4).

(A) Arrangement of pods; B) Maintain humidity on pods

Figure 4. Location of pods in the laboratory.

▪ The room temperature was at 25 ° C for a minimum of 12 hours in order to obtain a uniform condition before inoculation was accomplished.

▪ *Sample size*

• 5 pods per candidate or 70 per repetition, 560 for the two repetitions.

• Two rehearsals were performed, one in season A in May and the other in September respectively in 2018 and 2019.

▪ Isolates of the fungus were taken from pods clearly showing symptoms of black rot from the cocoa area of Bengamisa. These isolates were cultured in the juice of the V8-calcium carbonate culture medium aged 10 days (4 days in the dark and 6 days alternated with light in the microbiology and phytopathology laboratory of the University of Kisangani. zoospores were obtained by adding 10 ml of distilled water (heated to 10 ° C) and incubated at 26 ± 2 ° C. The release of zoospores was induced by the addition of sterile distilled water cooled for 25 minutes (5 ° C) and incubate in the dark at 25 ° C for 30 minutes

▪ Inoculation

The inoculation was made by sprinkling half of the pod 30 cm away using a squeeze bottle. An average of 1ml of the zoospore suspension should be deposited over 150cm² of the pod surface. The inoculated pods were covered with damp paper to retain moisture.

Table 1. Morphological characteristics of the clones

Variety	Reference group	Pod color	Pod surface	Shell thickness (mm)
CRY12	National	Green	Warty	14
CRY1323	Criollo-Trinitario	Green	Warty	13
CRY14	National	Green	Warty	28
CRY211	CRY	Red	Warty	22
CRY115	Nanay	Red	Smooth	18
CRY3	Trinitario	Green	Little warty	20
CRY10	Marannn-Nanay	Red	Warty	17
CRY711	CRY	Red	Warty	15
CRY124	Nanay	Green	Little smooth	24
CRY4	Trinitario	Green	Little warty	18
CRY235	CRY	Green	Warty	13
CRY13	Marannn-Nanay	Green	Little warty	23
CRY1111	CRY	Green	Little warty	15
CRY633	CRY	Green	Little warty	13

Source: National Cocoa Research Program INERA Yangambi (2008)

Observed parameters

➢ The incubation time.

This is the time that elapses between incubation and the manifestation of the first symptom of the disease. It gives an idea of the time taken for the symptoms of the disease to appear on the surface after inoculation. In other words, this duration gives us an indication of the susceptibility of the variety to black pod rot disease. Figure 5 illustrates the onset of symptoms of pod disease

➢ The dimensions of the lesions formed (the mean diameter of the lesions on the surface and the depth in the vessels of the bark) were measured for 2 weeks from the manifestation of the first symptoms.

- The mean diameter of the lesion was obtained using a graduated slat and was calculated using the following formula:

Ø medium= Ø maximum + Ø minimum / 2

This dimension tells us how the disease stain is spreading on the pod.

- The depth of the lesion was taken on the last day of observations (14th day). This measurement shows how the disease progresses inside the pod to reach the beans.

➢ The frequency and development of translucent spots appearing on an inoculated pod were scored at the two-day interval through day 14 according to the international rating scale (Table 2). This scale was used to assess the importance of the disease on the pod.After two days of incubation, the inoculated pods were returned for their reaction to inoculation. The estimate was based on the frequency and size lesion formed.

➢ The severity of infection was estimated on an eight-point scale (Table 2)

➢ ci were compared with the dimensions of the scale in order

❖The lesion distribution was calculated from the results obtained from the disease rating scale. From the observations of the spots, the IMPs were calculated using the indices recorded for each variety. This index is essentially an average of the indices of pod diseases from plants of the same clone, reported on a scale of 100. In our case, this was done for 5 pods of the same variety.

$$IMP = \left(\frac{(indice\ 1 + indlce\ 2 + indice\ 3 + indice\ 4 + indice\ 5)}{5}\right) /8) * 100$$

➢ Statistical analyzes.

The differences between the disease indices of plants belonging to different varieties were analyzed using a Wilcoxon test by pair of samples. For the comparison of means, we used Tukey's test.

Results and Discussion

1. Incubation time

The results relating to the incubation period and the depth of the infection in the shell are given in Table 3 below.

The incubation period is the number of days it takes for the first symptom of the disease to appear outside. It varies from pod to pod and variety to variety.

The average number of incubation days for infection was 3.38 days for the year 2018. In 2018, 64.28% of inoculated varieties showed symptoms of infection well before reaching their mean. . While in 2019 the average incubation period was 2.78 days. For this year, 50% of varieties had exterminated the symptoms of the disease before their average.

In general, for both replicates, the CRY materials exhibited symptoms of black pod rot disease but at different intervals. However, 57.14% manifested disease symptoms within 4 days of coming into contact with the inoculum, while 42.86% manifested disease symptoms from day 4.

Table 2. Pod disease estimation scale

Degree of disease	Level of infections	Classification of sensitivity
1	No symptoms	highly resistant to penetration
2	1-5 localized lesions	Resistant
3	6-15 localized lesions	Moderately resistant
4	> 15 localized lesions	Partially resistant / resistant to the spread of the lesion
5	1-5 lesions developed	Partially resistant / resistant to a single penetration
6	6-15 lesions developed	Moderately susceptible
7	> 15 lesions developed	Susceptible
8	Merged lesions	Highly susceptible

Source: Working procedures for cocoa germplasm evaluation and selection (1998).

Table 3. Results on the duration and depth of the lesion Incubation time and lesion depth in the shell

Variety	Thickness of the hull (mm)	2018		2019	
		Duration incubation (jr)	Depth of the lesion (mm)	Duration incubation (jr)	Depth of the lesion (mm)
CRY14	27	4	26	3	27
Cry10	17	2	17	1	17
CRY12	14	2	14	1	17.8
CRY1111	15	2	15	1	13.8
CRY4	24	2	11	1	12
CRY211	22	9	0	5	0.4
CRY235	13	3	8	2	4.2
CRY13	23	4	5	4	16.8
CRY1323	13	3	10	3	8.2
CRY711	15	3	6	3	6
CRY633	13	4	4	2	2.4
CRY115	18	5	0	5	1.4
CRY3	20	8	0	7	1
CRY124	24	2	12	1	13
% average	16.46	3.38	10.38	2.78	10.22
CRY1424 (T)		14	0	14	0

Varieties CRY3 and CRY211 manifested disease symptoms 8 and 9 days, respectively, after inoculation. They were therefore less sensitive to inoculation than the other varieties inoculated on the same day.

This situation indicates that a good part of the CRY material is susceptible to black pod rot disease but to different degrees (Figure 5).

(A) Surface manifestation of the lesion; (B) In-depth manifestation of the lesion (day).

Figure 5. Manifestation of the lesion after inoculation.

- **Average diameter of the lesion on the surface**

This dimension gives us overall the space that the lesion occupied on the pod from the appearance of the first symptom until the end of the observations (14th day). The results of the statistical calculations are illustrated by Figures 2 and 3 below:

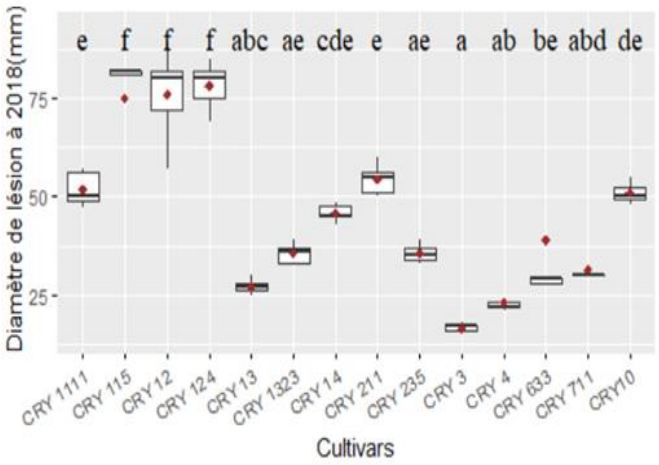

Figure 6. Lesion diameter in 2018.

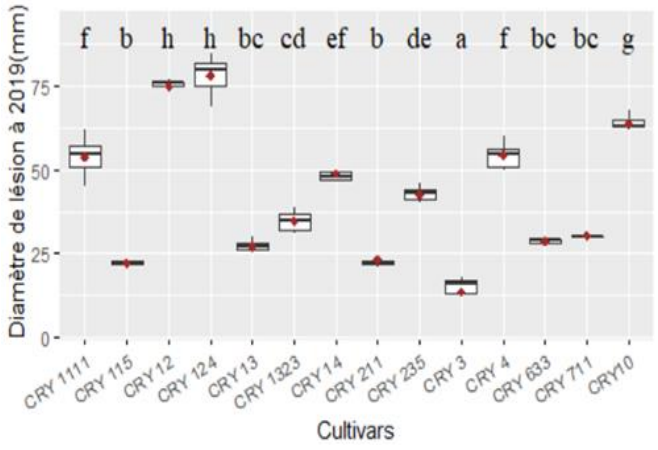

Figure 7. Lesion diameter in 2019.

In 2018, relative to the lesion diameter parameter, CRY3 and CRY4, developed a lesion diameter comparable to each other and small, while, on CRY 115, 12 and 124 the symptoms of the disease had spread over large surfaces (Figure 6).

In 2019 CRY 3, CRY115 and 211 developed a small lesion diameter (less than 23mm) (figure 7), while CRY12 and 124 developed a larger lesion diameter.

By comparing the two years of observations with respect to this parameter, CRY 3 kept its position during the two years while CRY4 did not keep its sensitivity level of 2018. This variety will have to be integrated in future tests in view. to confirm its real position.

- **Depth of lesion in the inoculated pod**

The depth of the lesion was taken on the last day of observations (14th day). This measurement shows how the disease has progressed inside the pod to reach the beans. The results of these observations are given in Figures 8 and 9 below:

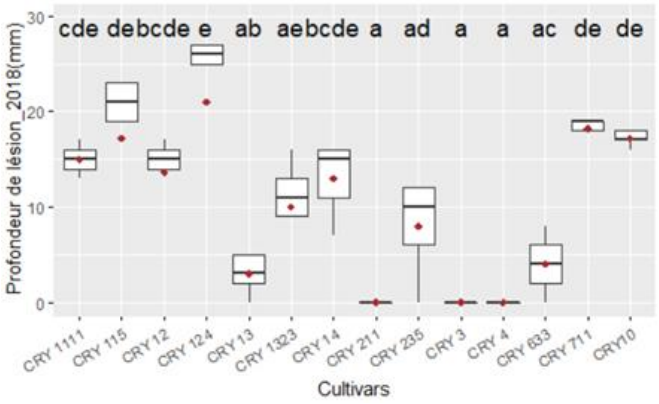

Figure 8. Depth of lesion in the pod in 2018.

It emerges from these observations that in 2018, three clones: CRY211, CRY3 and CRY4 did not develop the symptoms of infection visible in the pod shell. While on the CRY124, the symptoms were found deep in the hull. This variety can be considered highly sensitive with respect to this parameter.

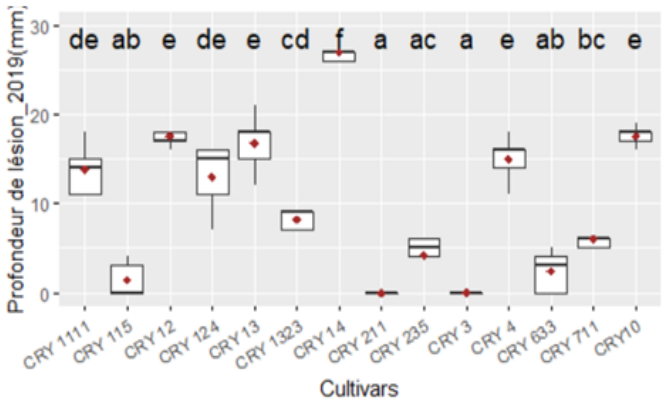

Figure 9. Depth of lesion in the pod in 2019

In 2019, CRY 211 and CRY3 did not allow the lesion to progress inside the pod. While CRY12, 13 and 10 did not resist the attack until the symptoms penetrate inside the hull.

The results of two years of observations (2018 and 2019) confirmed that the cuticles of CRY 3 and CRY 211, resisted the penetration of disease infection.

If we observe Table 4, it emerges that, although the disease was found on the surface, it did not penetrate into the pod, the beans remained intact. We can, conclude that these varieties have resisted the penetration of the fungus are therefore resistant

- **Distribution of infection**

Since the infection increases over time, it was interesting to analyze the sensitivity of different clones to a specific time period between different varieties. Based on the sensitivity of each pod, the result was reported on stacked bars. Light colors (white) indicating low infections and dark colors (black) indicating high infections. This has been visualized in FIGS. 10 and 11 respectively for the years 2018 and 2019. These indices make it possible to identify "possible first" resistant "clones.

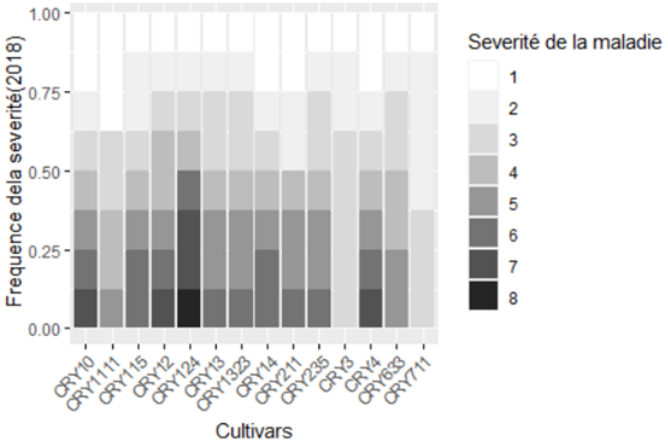

Figure 10. Distribution of disease severity indices on different clones in 2018.

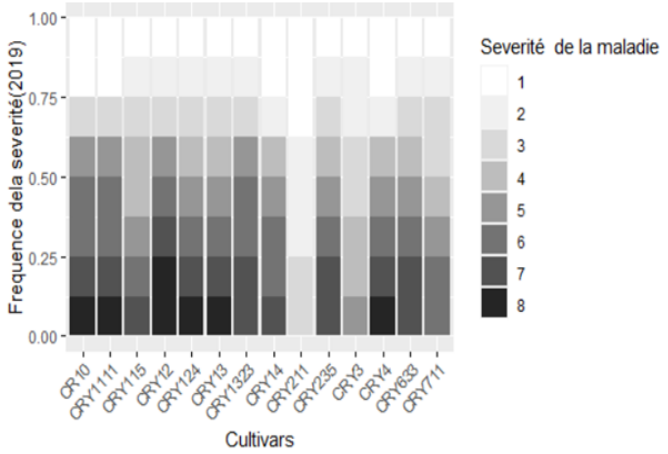

Figure 11. Distribution of disease severity indices by pod in 2019.

In 2018, the infection rate was low for the CRY 3, CRY711 CRY 1111 and CRY633 their index varying between 1 and 3, while the infection was greater and elevated for the clones CRY 12 and CRY4, which have developed a very high disease index between 5 and 8. which suggests that they are more susceptible to disease than previous clones.

In 2019, the CRY211 and CRY3 clones have a very low disease index around 1 according to the disease scale. While CRYs 124,12 and 10 have developed a very high susceptibility to infection.

The two years of observation compared retain CRY3 as a resistant clone in the INERA collection with respect to this parameter.

By observing all the parameters observed, we notice that CRY3 and CRY211 have well resisted infection by L. theobromae. The hypothesis that there is at least one clone resistant to the disease is confirmed.

From these parameters we can already conclude that CRY 3 and CRY211 can be considered as resistant clones of the INERA Yangambi collection.

Evolution of the disease over time

In order to follow the daily evolution of the infection on the varieties we had considered the average sensitivity indices of each variety reported on a curve. The results are shown in Figures 12 and 13.

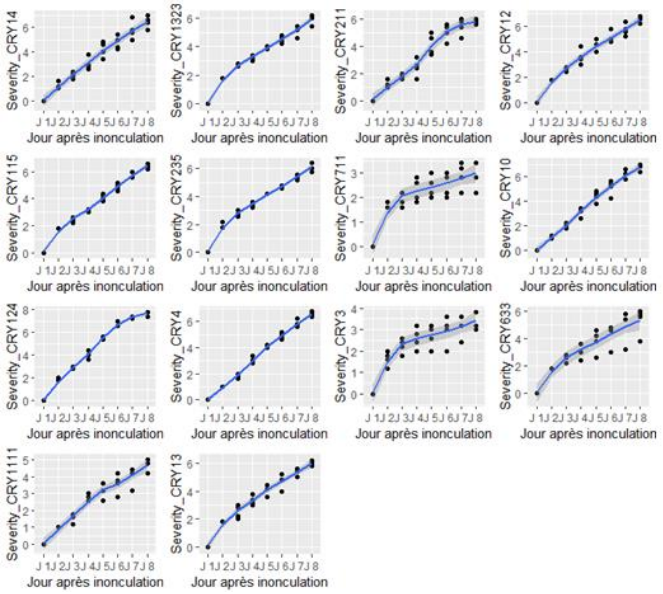

Figure 12. Daily evolution of the severity of the disease by cultivars in 2018

It emerges from this figure that in 2018, the daily evolution of the disease was very low for CRY3 and higher for CRY 124. The lesion therefore had difficulty developing on pods of the CRY3 variety than on those of the CRY124 for this year of observations.

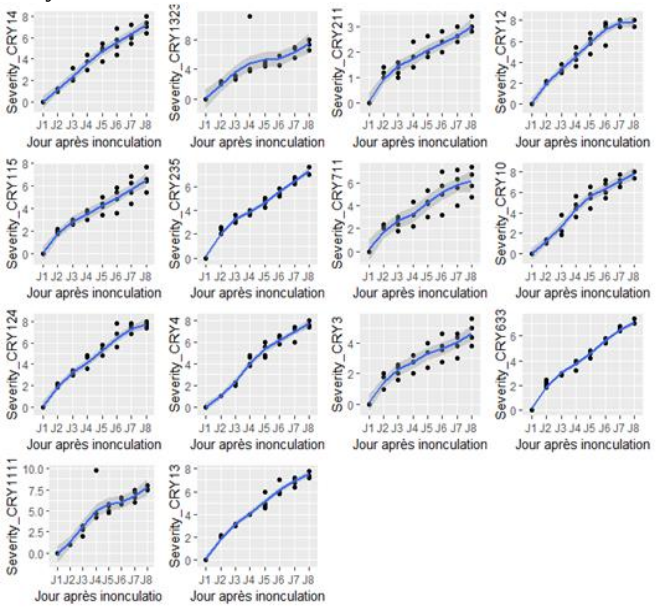

Figure 13. Daily evolution of the severity of the disease by cultivars in 2019.

In 2019, this evolution was again lower for CRY3 and higher for CRY 124 and CRY 4 which, at the end of the 7th day, had already reached the highest index. It was found that the evolution of the severity of the disease was a function of time. Indeed, when the fruits are infected, the pathogen can sometimes remain latent until the time when the fruit is ripe, the cuticle softens to act (Ventura et al., 2004). In addition, to penetrate the fungus emits enzymes that soften the cortex easily when it becomes less hard. For resistant varieties, this softening is difficult or late compared to sensitive varieties.

Although CRY211 and CRY 3 were identified as resistant, however they showed symptoms of the disease because they had previously been mechanically injured. This proves that theMeans of entry of L. theobromae into hosts is through injury from work tools, insects, or natural causes. This had also been reported byPloetz (2003).

The results given by the observations on the mean depth of the lesion show that apart from these two clones, other clones such as: CRY 235; CRY13, CRY1323, CRY4 and CRY633 did not allow the lesion to reach the beans. This does not necessarily mean that the fungus had not reached the beans. Because, it must be kept in mind that theThe fungus, colonizing the vascular system, advances before visible symptoms. This had also been reported by Burgess et al., 2006; Shahbaz et al., 2009. It is therefore quite possible for these clones that the fungus had already reached the beans without there being any visible manifestation of symptoms. However, if harvested on time, these pods can produce good quality beans. However, we can recommend other inoculations in which these varieties will be integrated in order to further test their resistance.

❖Comparison of severity levels between 2018 and 2019

The severity levels were compared between the two years of observations in order to identify which of the two years was most affected by black pod rot disease. The results of these observations are given in Figure 14 below:

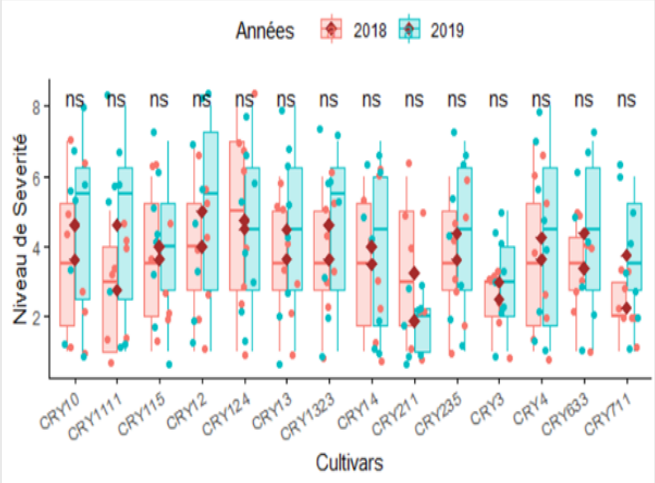

Figure 14. Comparison of severity level in 2018 and 2019

The observation of Figure 8 shows that the level of severity was apparently low in 2018 than in 2019. This can be explained by the fact that, the amount of rainwater that fell until the pod harvest in May was 640 mm against 1048 mm of water dropped during the development of the pods harvested in September 2019. The amount of water could influence the predisposition of the pod cells to the development of infection. He is well knownthat during periods of rain there is a greater production of spores, which can be dispersed by raindrops and wind (Ventura et al., 2009). In addition, it is known that when there is not enough water, the cells become less turgid, the pods mummify and the fungus persists in the cells of the tissues. dead trees or in the ground (Pegg et al., 2003) and especially on mummified fruits (Ploetz, 2003). The spread of the fungus becomes weak.

Although there is apparently a difference in severity between the two years, it is not, however, statistically significant according to Tukey's test. The disease having developed with the same intensity during the two years of observations.

Behavioral modeling of disease severity on pods

In order to predict the severity of the disease over time, it was necessary to model the severity by adopting the logarithmic model and the results are presented in FIG. 15 below.

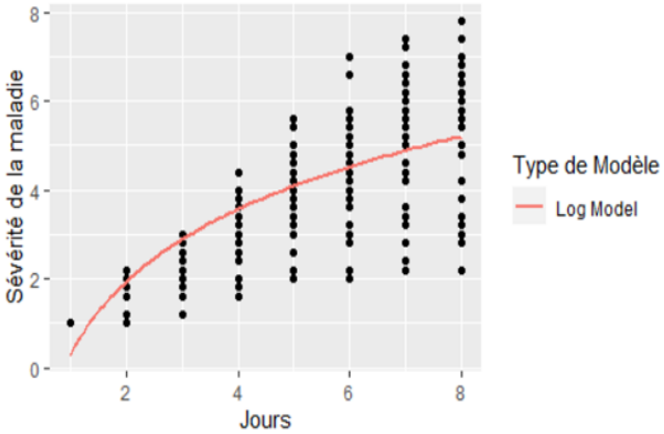

Figure 15. Modeling of disease severity as a function of days

Figure 15 above demonstrates that, the course of the disease severity was a function of time. Details on the models describing the severity of the disease on each cultivars in 2018 and 2019 can be found in the model tables in the appendix (Appendix 1 and 2).

Morphological characteristics of varieties and disease infection

The morphological characteristic of the varieties which resisted inoculation is as follows: CRY211 of the reference group CRY is red in color and the surface of its shell is viscous; the CRY3 clone of the national reference group, on the other hand, is green and viscous. Although the surface of their hulls is similar, viscous, the color of the hull differs as well as their genetic origin is different. In addition, CRY12 (Nacional) and 1323 (Criolo-trinitario), which had been shown to be sensitive to inoculation, have the same characteristics as CRY3, without having the reaction similar to the latter. We can deduce that the resistance is not a function of the morphology of the pod so our second hypothesis is rejected.

Conclusion

1. Both the analysis of the depth of the lesion, the width of the lesion, the frequency of the disease and the course of the disease over time, CRY211 and CRY3 were shown to be resistant to black rot in L. theobromae.
2. This resistance is genetic for CRY3 since the results of molecular analyzes carried out in 2018 classified this clone in the same group as SCA6 recognized as resistant to several cocoa diseases.
3. While rainfall favors the spread of the disease, morphological characteristics play no role in the resistance to infection of pods
4. The prediction as found by the logarithmic model shows that the disease progresses over time. Thus, the pods must be harvested as soon as they ripen.
5. Sanitary harvests and regular maintenance of plantations are preventive methods to be applied

Bibliographical references

Anderson, PK, Cunningham, AA, Patel, NG, Morales, FJ, EPSTEIN, P. 2004

Barnouin, J.& Sache,I. 2010.Emerging diseases. Epidemiology in plants, Bareto et al 2015

Bowers, JH, Bailey, BA, Hebbar, PK, Sanogo, S., Lumsden, RD (2001). The 7 impact of plant diseases on world chocolate production

Burgess TI, Barber PA, Mohali S, Pegg G, de Beer W and. Wingfield MJ 2006. Three new *Lasiodiplodia* spp. from the tropics, recognized based on DNA sequencecomparisons and morphology. Mycologia 98: 423-435

Desprez-Losustau, M.-L., Robin, C., Buee, M., Courtecuisse, R., Garbaye, Suffert, F., Sache, I. & Rizz, DM 2007. The fungal dimension of biological invasions. Trends In Ecology & Evolution, 22, 472-480.

Hayley Rottiers1,2 & Helena Everaert1,2 & Pascal Boeckx3 & Gaston Limba4 & GeertBaert5 & Jocelyn De Wever1 & Kevin Maebe6 & Guy Smagghe6 & Koen Dewettinck2 & Kathy Messens1. Unraveling the genetic background of the Yangambi Research Center cacao germplasm collection, DR Congo. https://link.springer.com/article/10.1007/s11295-018-1285-6. 2018.

Limba, G. 2008. Annual report of the PNR Cacaoyer (unpublished).

Limba, L. 2013. Mission report to CABEN. Unpublished.

Limba K. mukwa Kibuka Gaston, Mukandama Jean-Pierre, Kikukama Kezile Laurent, Bahati David and Wembonyama Shako François. Monitoring of Black pod Rot Disease and Identification of the Causal Agent in the Bengamisa Cocoa zone in the Democratic Republic of Congo (DRC) inElixir Agriculture. 2020.

Nyassé, S. Bruno Mousseni Efombagn. BI Kébé. Mr. Tahi. Integrated disease management Phytophthora on cocoa (Theobroma cacao L): impact of plant breeding on the incidence of pod rot.Crop protection (CROPPROT 26 (1): 40-45. January 2007.

Ploetz RC. 2003. Diseases of Tropical Fruit Crops. CABI Pu blishing. Wallingford, UK. pp76-77.

Pegg KG, Coates LM, Korsten L and Harding RM. 2003. Foliar, fruits and soilborne diseases. 299-337. AW Whiley AW, Schaffer B and Wolstenholme BN (eds.). Tea Avocado:Botany, Production, and Uses. CABI Publishing, UK. 560 p

Pokou, ND, JAK N'goran, I. Kébé, A. Eskes, M. Tahi, A. Sangaré (2008). Levels of 1resistance to Phytophthora pod rot in cocoa accessions selected on-farm in Côte ivory. Crop Protection 27: 302-309. 3

Ventura JA, Costa H and Tatagiba J. 2004. Papaya diseases and integrated control. p.201-268.: Naqvi SAMH (ed.). Diseases of Fruits and Vegetables: Diagnosis and Management. Volume II. Kluwer Academic Publishers Dordrecht, United States of America. 704 p

Ristaino, J. B, Anderson, P., Brauman, KA, Cunniffe, NJ, Federoff, N., Garrett, KA, Gilligan, C., Holmes, T., Martin, M., MacDonald, GK, Neenan, P., Records, A., Schmale, D., Tateosian, L. 2020. The Persistent Threat of Emerging Plant Diseases to Global Food Security. Proc. National Academy Sciences, USA

Ristaino, JB; 2002. Tracking historic migrations of the Irish potato famine pathogen, Phytophthora infestans.Microbes and Infection.4: 1369-1377

Shahbaz M, Iqbal Z, Sallem A and Anjum MA. 2009. Association of Lasiodiplodia theobromae with different decline disorders in mango (Mangifera indica L.). Pakistan Journal of Botany 41: 359-368.