Diagnostic survey on the occurrence of pineapple fruitlet core rot and relationship with phenolic compounds in Réunion Island

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Abstract

Fruitlet core rot (FCR) is a postharvest disease that develops once the pineapple fruit reaches maturity. In the tropical island of Réunion, it mainly occurs during the winter season. The two fungi responsible *Talaromyces stollii* and *Fusarium ananatum* cause black spot in the flesh of the fruit. These internal damages make the FCR difficult to diagnose for the producers and consumers. To get a better idea of the extent of the disease, we conducted a survey of agricultural practices over 27 plots distributed throughout the island. Five hundred forty pineapples were sampled at the same time to determine the occurrence of the disease. During the winter season, almost all pineapples had the FCR symptoms. In order to assess the variability of fruit susceptibility to FCR, 80 pineapple fruits were inoculated with a solution of *Fusarium ananatum* spores (10^3 sp. mL^{-1}). Free and bound phenolic acids were monitored in the healthy and infected fruits. High level of phenolic compounds in healthy fruits provides resistance to FCR. All the infected fruitlets react to the fungal infection with a high accumulation in free and bound phenolic compounds. The links between certain mechanisms of resistance and cultural conditions are discussed. The problem of the fruitlet core rot is certainly underestimated in view of its strong presence.

Keywords: fruitlet core rot / *Fusarium ananatum* / Pineapple / Postharvest disease /Phenolic acids

INTRODUCTION

Pineapple is the first fruit production in Réunion Island and one of the most consumed tropical fruits in the world. Consumer demand increases every year and to meet this demand, producers expand their areas dedicated to pineapple. This intensification favors the development of diseases and notably the fruitlet core rot, a fungal disease.

Fusarium ananatum and *Talaromyces stollii* are the main causal agents. The process of infection start at the flowering stage, the fungus penetrate the plant through the stylar canals and nectary ducts (Rohrbach and Pfeiffer, 1976). When the fruit ripens, the pathogen emerges from its latency and affects the flesh causing black spot located in the fruitlets. The physiological changes during maturation favor the development of the disease.

Chemical control has been tested (Petty et al., 2005). Two fungicides in combination gave encouraging results. However, they are now submitted to restriction or ban in accordance with European legislation. As these diseases are hard to be controlled with chemical control nowadays, it is important to better understand what fosters its development. Fournier et al. (2015) showed that climatic conditions play a role in the occurrence of the fruitlet core rot. Cold temperatures and high humidity during the open heart to harvest stages promote the appearance of the disease.

If environmental conditions affect the presence of the fruitlet core rot, what about cultural practices?

A diagnostic survey was conducted on the various pineapple production areas of Reunion Island. Firstly, the occurrence of the FCR disease during the winter season was investigated, and the relationships between FCR and the agricultural practices was also explored. Finally, biochemical analyses were carried out on pineapple fruits and on crown.

MATERIALS AND METHODS

Survey

The survey was conducted during the winter season, between June and October 2016, in the main areas of pineapple production in Réunion Island (Figure 1). Quantitative and open discussion questions were formulated directly at the same time as the sampling. For the quantitative questions, growers were asked to provide average values based on their records for: planting density; amounts of nitrogen (N), phosphorus (P), and potassium (K) applied; total yield; fruit size …

Plot sampling

The 27 plots of pineapples (12 in the east, 12 in the south, 1 in the west and 2 in the north) chosen represent the distribution of pineapple producers. The location of the plots, which is directly related to the temperature and rainfall, and the level of intensification, notably fertilization, planting density and previous crop describe the entire climatic and agricultural practices over the island.

On each selected plot, twenty pineapples at the C3 stage (when the fruit color shell is three-quarter yellow) were harvested and preserved at 22°C for six days.

Assessment of the susceptibility of fruit to FCR

After these 6 days of storage, the number, length and breadth of the natural black spots were measured on the 20 pineapples by separating the upper and lower part of the fruit. The area of the black spots was estimated using the formula for the area of an ellipse. Total soluble solids (°Brix), pH, and titratable acidity of the fruit flesh were also measured.

From the 20 fruits harvested by plot, 4 fruits were inoculated with 25 µL of *Fusarium ananatum* at 1 x10^3 spores mL^{-1}, using a 50 µL Hamilton syringe. The fruitlets were inoculated on one side, at the day of harvest. The healthy part of the fruit and the infected fruitlets were sampled separately, after the 6 days of storage. The area of the black spots was also measured. Free phenolic compounds were extracted as previously described (Barral, 2017). For the bound phenolic acids, 10 mL of ethanol-water solution (80:20, vol:vol) was added to 100 mg of lyophilized pulp and sonicated at 40°C. After centrifugation, only the pellet was kept and 5 mL of NaOH 2N was added for alkaline hydrolysis. After 2 H agitation, pH was adjusted to 5 with HCl. Finally, samples were filtered with a 0.45 µm filter (Millipore) and injected into a high-pressure liquid chromatography (HPLC). Phenolic acids identification was resolved on the basis of ion molecular mass, MS^n, and UV-visible spectra using a LCQ ion trap mass spectrometer fitted with an electrospray interface (Thermo Finnigan, San Jose, CA, USA). Quantification of phenolic acids was performed using commercial standards of trans-*p*-coumaric acid, chlorogenic acid, trans-sinapic acid and gallic acid purchased from Sigma-Aldrich (France). HPLC

Diagnosis of mineral nutrition

A sampling of 20 pineapple crown per plot was collected for mineral analysis. The elements analyzed were N, P, K, Ca, Mg, Na, Mn, Fe using the Dumas combustion method and the inductively coupled plasma atomic emission spectroscopy (ICP-AES).

Statistical analysis

All statistical analyses were conducted in R (R Development Core Team, 2015). Due to the large number of quantitative variables from the diagnostic survey and the physicochemical analyses, a Pearson correlation matrix was employed to make a first selection. Then, the variable "number of black spots" has been transformed into qualitative variable and a canonical variate analysis (CVA) was performed.

RESULTS AND DISCUSSION

Occurrence of the fruitlet core rot

FCR symptoms were present at least once on every plot studied. Regardless of the location of the plots or the technical itinerary, winter conditions are very favorable for the presence of the disease. The disease severity was grouped into five classes ranging from "1-5" black spots to ">20" black spots. Figure 2A shows the distribution of the number of black spots per fruit over the 540 pineapples sampled. 74% of the fruits had at least one black spot. The largest portion of diseased fruits was ranged between one and five spots per fruit. It is noteworthy that 12% of the pineapples sampled had more than 20 black spots per fruit.

The lower part of the fruit has on average more black spots than the upper part as shown in figure 2B, with respectively 6.5 and 4 black spots. The pineapple is an infructescence; maturity begins at the base and then goes up to the crown (Dou et al., 2010). The lower part is ripe first/earlier with higher sugar levels and structural changes. These conditions favorable to the development of fungi could explain this preferential distribution in the lower part.

Potassium, Nitrogen fertilization and climatic conditions affect FCR occurrence

The canonical variate analysis allowed us to extract variables related to the occurrence of FCR. Pluviometry, N and K inputs affected FCR occurrence (Figure 3). There was a large variation in fertilization; One plot did not receive fertilizer while on others N and K application were up to 400 and 600 kg ha^{-1} respectively. These high levels correspond to the plots where growers continue to fertilize after flower induction. Moreover, Figure 3A shows that the amount of potassium applied is positively correlated with the resistance of the pineapple FCR up to 600 kg ha^{-1} and beyond that point the fruit becomes extremely susceptible. Nitrogen follow the same pattern (figure 3B). A reasoned application of nitrogen and potassium could help to regulate the FCR. Finally, the more the rainfall during flowering stage, the more the fruit is susceptible to FCR (Figure 3C), as shown by Fournier et al. (2015).

Table 1 shows the average mineral composition of the crown leaves of the 27 plots. Values of the minerals are similar to those found by Alvarez et al (1993) in the "D" leaves. No significant relation has been made between any of those elements and the susceptibility of the fruit to FCR.

Phenolic acids involved in fruit resistance to FCR

Free and bound phenolic acids were measured in the healthy part of the fruits and in the infected fruitlets. Figure 4 shows levels of phenolics acids measured in the healthy part of the fruits and their relation with fruit disease susceptibility. High levels of phenolic acids provide resistance to FCR. For example, in Figure 4A more than 70% of the fruits were healthy with levels in hydroxybenzoic acids above 200 µg g^{-1} of FW. The same pattern is observed for the other free phenolic acids. It is also true for the bound phenolic acids, levels above 160 and 1400 µg g^{-1} of FW of coumaric and ferulic acids respectively provide more than 60% of healthy fruits (Figure 4E and 4F). Phenolic acids are known for their implication in plant resistance after pathogen infection.

Conclusion

In favorable conditions, 74% of the fruits were affected by the fruitlet core rot. This is a disturbing figure for places where the 'Queen' cultivar is produce, like in Réunion island. We showed that potassium fertilization was positively correlated to pineapple resistance to FCR. However, very high level of Potassium fertilization which correspond to post floral induction application (survey data), had opposite effect. A reasoned application of nitrogen and potassium could help to regulate the FCR, and application of fertilizer after floral induction should be banned.

Finally, we showed that both free and bound phenolics were involved in the natural resistance of pineapple to fruitlet. It would be interesting to find a way to enhance these compounds in order to reduce the risk of infection by the fungus.

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Figures

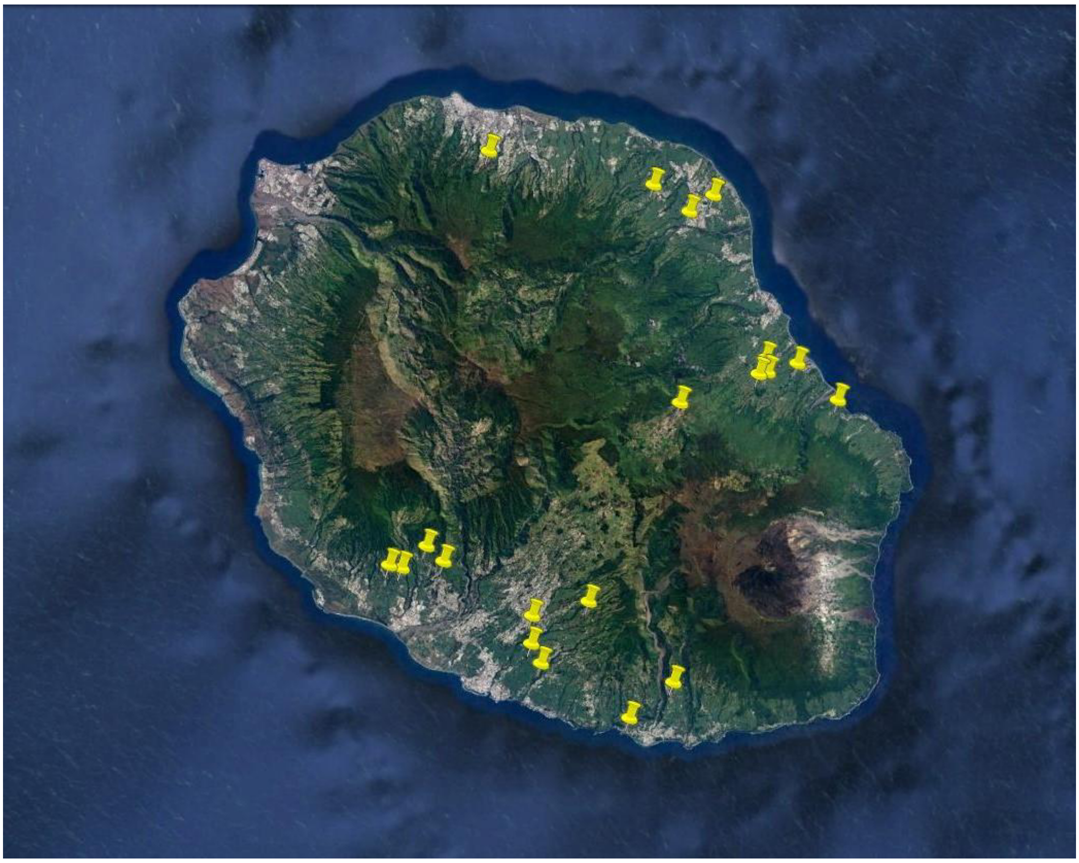

Fig. 1. Distribution of pineapple producers on Reunion Island.

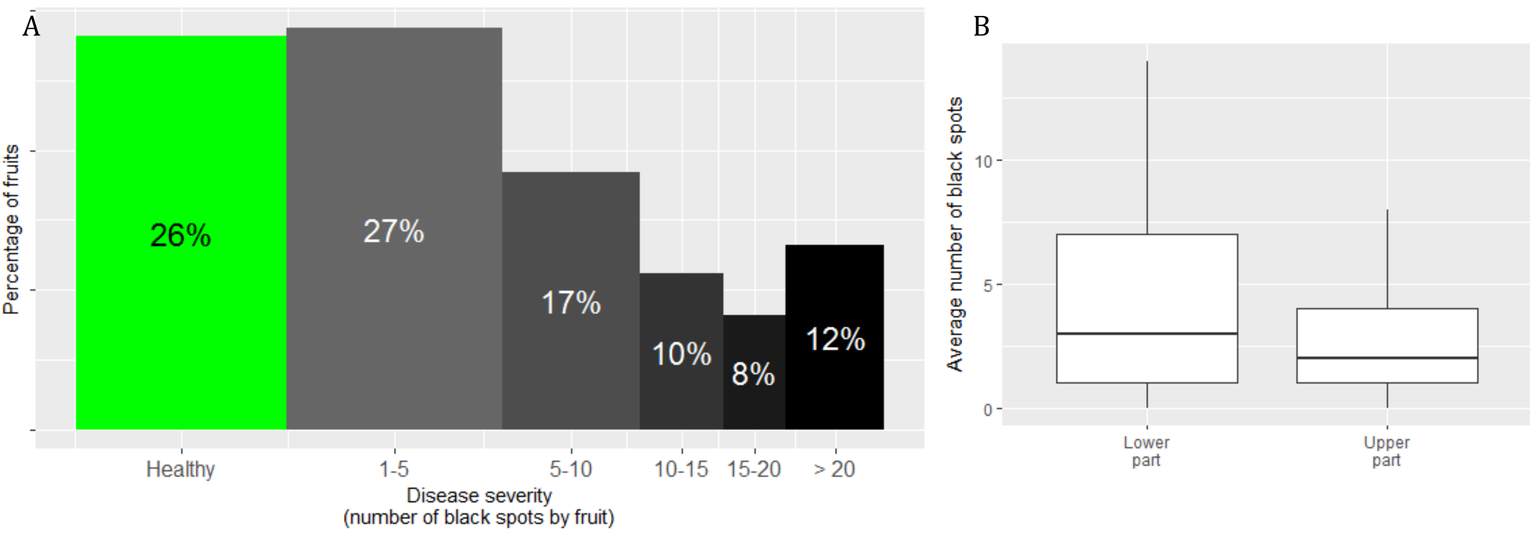

Fig. 2. Percentage of healthy fruits (green) and class distribution of the number of black spots (grayscale) over the 540 fruits sampled (A) and mean distribution of the fruitlet core rot symptoms inside the fruit (B).

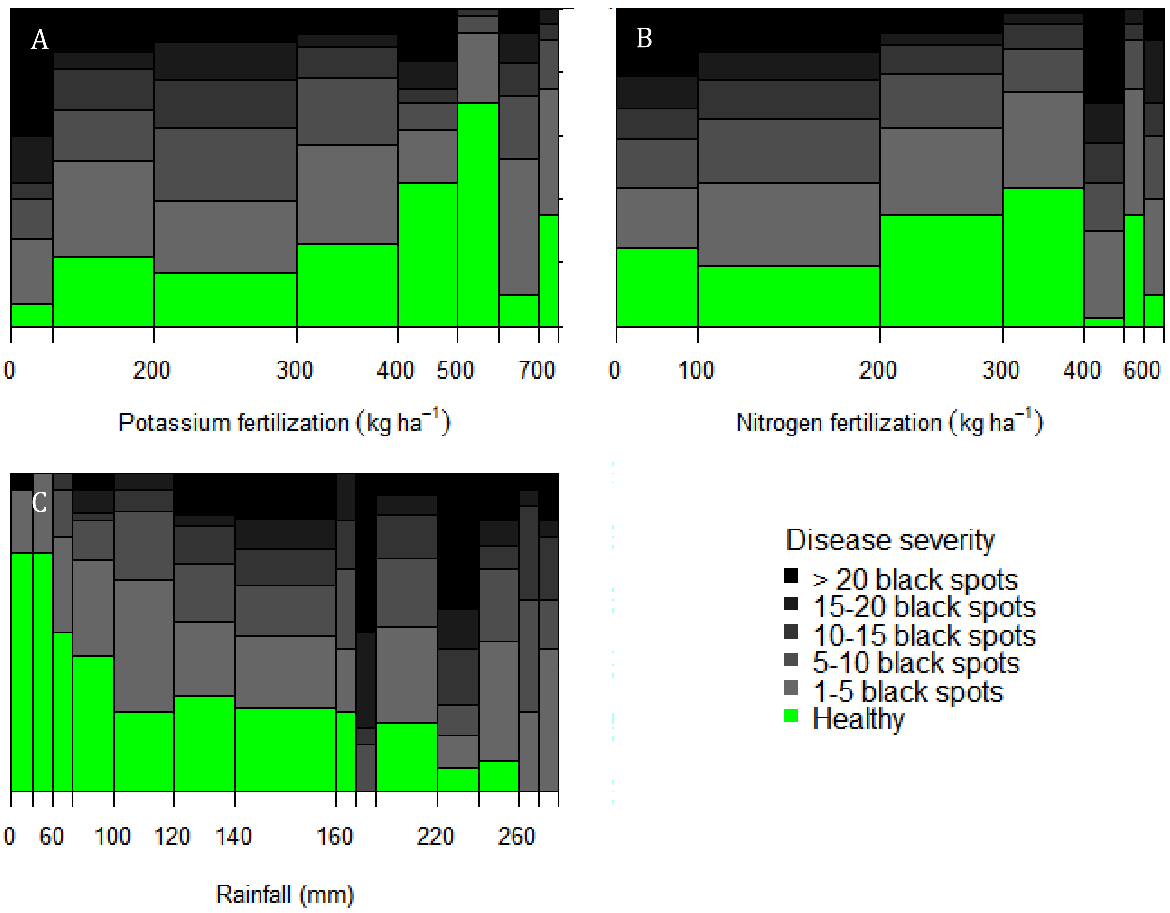

Fig. 3. Relation between Potassium fertilization (A), Nitrogen fertilization (B), pluviometry (C) and fruit susceptibility of the fruit to fruitlet core rot. The x-axis integrates the representativeness of each class of fertilizer level or rainfall of all the plots. The y-axis integrates the percentage of each disease severity class.

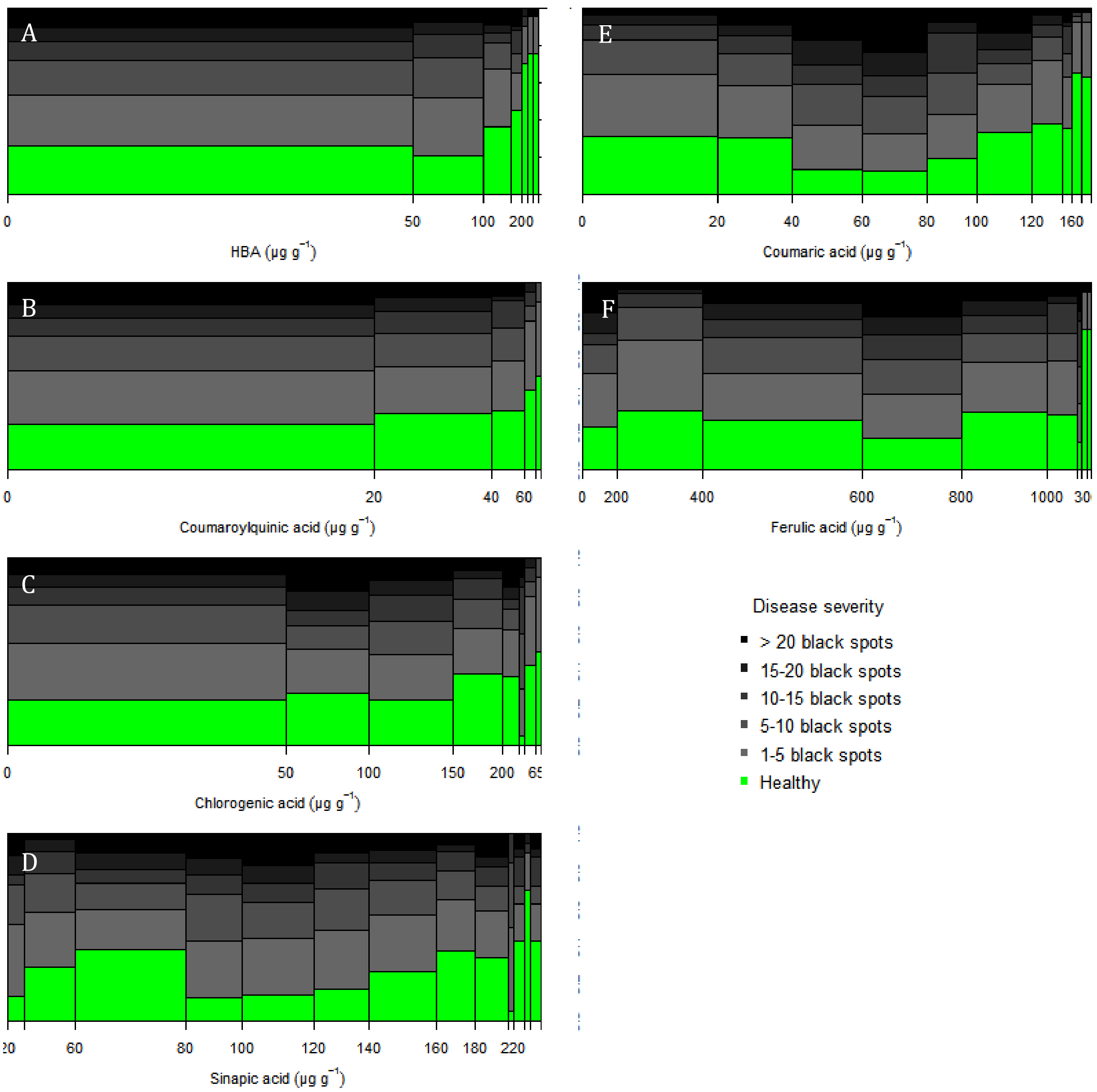

Fig. 4. Relation between levels in free phenolic acids (left column: Hydroxybenzoic acids (HBA) (A), coumaroylquinic acid (B), chlorogenic acid (C), sinapic acid (D)); bound cell wall phenolic acids (right column: coumaric acid (E), ferulic acid (F)) measured in healthy part of the fruit and fruit susceptibility of the fruit to fruitlet core rot. The x-axis integrates the representativeness of each class of phenolic compounds of all the plots. The y-axis integrates the percentage of each disease severity class.

Tables

Table 1. Mineral composition of the crown of pineapple (n = 27)

	Ca (% DW)	**Mg (% DW)**	**P (% DW)**	**N (% DW)**	**K (% DW)**	**C (% DW)**	**Mn (mg kg^{-1})**	**Na (mg kg^{-1})**
Mean	0.113	0.194	0.178	1.129	3.468	46.39	196.71	225.12
Se	0.044	0.051	0.037	0.221	0.74	1.329	150.45	74.14