

## Optimizing Chemical Control of Storage Diseases Cavendish Banana (Musa Spp. AAA Group) Cultivar Grand Nain in Côte d'Ivoire

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### Abstract

Côte d'Ivoire is the leading dessert banana producing country in Africa. It exports 80% of its production to the European Union markets. Growing large dwarf dessert bananas is profitable for both operators and the country. However, storage diseases threaten the quality of production. With the aim of contributing to the fight against the fungi responsible for these diseases, samples of banana bunches treated without the apex with Azoxystrobin and Boscalid were collected for three years in the production zones. These samples were stored for 21 days in the laboratory. The symptoms developed by the banana bunches and fingers have been observed and described. From infected banana explants, the fungi associated with the symptoms observed were identified. A pathogenicity test was used to identify the fungi responsible for storage diseases. Fungi present at the apex of fingers treated with antifungals were identified. Bunches of bananas were then treated with the antifungals mentioned, targeting the apex. Infection rates for storage diseases in treated and untreated apex fingers were compared. The banana bunches developed crown and apex rot and pericarp necrosis during storage. The fungi responsible for these infections were of the genera *Botryodiplodia*, *Fusarium*, *Colletotrichum* and *Musicillium*. The same isolates responsible for storage diseases identified on bananas without the apex and infected after 21 days of storage were identified at the apex of the fingers after 48 hours of storage. Treatment with Azoxystrobin and Boscalid targeted at the apex of the fingers reduced storage diseases in dessert bananas by more than 80 to 90%.

**Keywords:** Banana; Cavendish; Fungi; Diseases; Storage; Côte d'Ivoire

### Introduction

Once extensive agriculture is facing climate change. Fertile land is scarce, sometimes leading to recurring social conflicts in Africa. In Côte d'Ivoire, especially industrialists then grow crops on plots of land intensively. Industrialists who have the financial means to meet the costs most often practice intensive agriculture. Intensive monoculture of bananas, the Grand Nain variety from the Cavendish sub-group in Côte d'Ivoire, grown on more than 5,897 ha at 32 production sites. Selling dessert bananas is a profitable business. After a drop in production due to the 2014 floods, Côte d'Ivoire has been Africa's leading dessert banana producer since 2019, with more than 450,000 tons. Dessert banana companies have a turnover of over 145 billion CFA francs, representing 7% of agricultural gross domestic product (GDP) and 3% of national GDP. In recent years, Côte d'Ivoire has seen the arrival of groups from Martinique and the West Indies, producing over 75,000 tons of bananas a year [1,2].

However, Côte d'Ivoire exports 80% of dessert banana production to European Union markets. From then on, production was subject to the quality requirements of the international market. The use of alternative fungicides that have little impact on the environment, and strict compliance with the MRL values of the fungicides applied, are all measures that regulate the international marketing of dessert bananas, to which ivoirien producers must adhere. An upsurge in storage diseases have compounded all these constraints in recent years, which are causing producers considerable financial losses. Several factors could contribute to the development of storage diseases in bananas. To control these diseases, a number of fungicides are used and results are not always satisfactory [3].

Several factors could contribute to the development of banana conservation diseases. The conditions of high humidity and hypoxia observed into bananas boxes could favor the lifting of quiescence and lead to the development of viable structures represented by the

observed fungal colonies. Effective control of post-harvest banana diseases should take into account the quantity and the diversity of fungi that evolve in banana plantations. Once out of the plantations, the diets carry inoculum that is always present and viable on the banana fingers. In order to make chemical control of fungi responsible for conservation diseases more effective, it is necessary to reach the points of existence and conservation of these pathogenic fungi on bananas [4].

### More specifically, it involved to:

- do an inventory of banana conservation diseases in Côte d'Ivoire identify the fungi responsible for banana storage diseases.
- Study the effect of treatment of banana finger apices on the development of banana storage diseases.

### Material and Methods

#### Phytosanitary survey and sampling of bananas in production and packaging zone in Côte d'Ivoire

Phytosanitary surveys were carried out from 2021 to 2023 in seven production areas in the dessert banana production areas in Côte d'Ivoire for three years. Every year, two collections of banana samples were made every six months. However, the first survey was carried out for an inventory of the production and packaging of bananas in the

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plants without collecting samples of bananas. One to three stations were visited per production zone depending on availability in Dabou, Abengourou, Agboville, Azaguié, Motobé and Ndouci. Five collections have been completed. At each collection, three box of Azoxystrobin and Boscalid-treated bananas ready for export were collected per zone. 105 banana bunches box were collected for the study [5].

### **Inventory of banana storage diseases in Côte d'Ivoire**

The batch of 14 box was also stored for 21 days under the same conditions as previously.

After these storage periods, the boxes were stripped. In banana fingers preserved for 48 hours, the apex was observed and described. The symptoms developed on banana bunches preserved for 21 days were observed and described. Banana bunches infected with each type of storage disease were counted. The number of bouquets and fingers were also counted. For each type of disease developed by banana bunches or fingers, the infection rate was calculated to identify the predominant storage disease regardless of the area from which the bouquets originated. The ratio between the number of infected bunches and the total number of bunches or the ratio between the number of infected fingers and the total number of fingers was calculated [6,7].

### **Isolation and identification of fungi responsible for preservation diseases of dessert banana bunches**

Isolations of fungi associated with symptoms of banana preservation diseases were made from explants taken from infected bananas after 24 hours and 21 days of storage. To do this, the infected banana explants were inoculated on PDA (potato dextrose agar) medium for the isolation of the fungi present according to the Davet and Roux method. Successive sub culturing until homogeneous individualized colonies were obtained purified the colonies of fungi developed. These colonies were then observed and described after one week of culture on the PDA medium in Petri dishes [8].

### **On the basis of macroscopic and microscopic morphological characteristics, the genus of the fungus isolates was identified.**

Following the production of the fungi associated with the various symptoms of the storage diseases developed, the fungi responsible for these infections were identified by a pathogenicity test. The pathogenicity test was carried out by gentle inoculation of bananas with isolated fungi to identify those responsible for banana infections in storage. To do this, an inoculum with a concentration of  $\geq 10^6$  conidia/ml of each fungus was prepared. Soft inoculation of the bananas was carried out by depositing a 50 $\mu$ l droplet of the conidial suspension and covering it with a Whatman paper pellet at three points:

- on the crown, refreshed with a sterile knife from the individualized crown of the banana, at a rate of thirty bananas per fungi tested;
- on the pericarp, disinfected with 10% diluted hypochlorite at a rate of 30 bananas per fungi tested;
- at the apex, refreshed with a sterile knife at a rate of 30 bananas per fungi tested;
- 30 control bananas were only inoculated with the simple agar according to the three types of inoculation.

All inoculated bananas were incubated under controlled conditions in sterile conditioning tanks and observed daily. After symptoms developed on bananas, these were described.

The Koch's Postulate test was used to identify the fungi responsible for the various infections of bananas during storage. Responsible fungi were identified base from identify keys of confirmed by researches. Three repetitions of this test were carried out [9]

### **Apex antifungal treatment Effect of dessert banana fingers on the development of storage diseases**

The banana bunches selected were treated with Azoxystrobin and Boscalid at the plant in accordance with the manufacturer's instructions. However, the sprayer nozzle was directed only at the apex of the banana bunch fingers during treatment. The treated bananas were packaged in transparent polyethylene and then placed in cardboard boxes for transport to the laboratory. The treated bananas were packaged in transparent polyethylene and then placed in cardboard boxes for transport to the laboratory. Once in the laboratory, the treated bouquets were stored for 21 days. After this time, the boxes were unpacked and the various symptoms were observed and described. The numbers of infected and non-infected fingers and crowns were counted. Average infection rates were calculated to determine the health status of the bananas with regard to storage diseases [10,11].

### **Statistical analysis**

Statistical analyses were performed using STATISTICA 7.1 software. The mean prevalence rates of the diseases were analyzed using a series of ANOVA 1. In the event of a difference at the 5% threshold, The Newman-Keuls test was used to compare the mean values of the treatments and determine the homogeneity groups [12].

### **Résult**

#### **Diversity of storage diseases symptoms on bananas after 21 days**

Boxes of banana bunches treated with antifungals and protected in polyethylene; collected in the various production areas were stripped after 21 days of storage under aseptic conditions in the laboratory. The banana fingers on bunches developed a number of symptoms.

The control bananas bunches uninfected showed fingers that were green, firm, with an intact crown well attached to the fingers. On the other hand, bunches of bananas infected in the same box showed three characteristic symptoms, irrespective of their area of origin: necrotic crowns, sometimes black on the surface, but also deep down to the stalk of infected fingers when small. The fingers of ripe or unripe bananas are detached by lifting the bunch by the crown. Bunches of preserved bananas have developed crown rot [13].

The apex of infected fingers showed a brownish discoloration, often covered with white mycelial colonies. To the touch, the finger is soft with an pericarp that detaches easily. This symptom is always localized to the apex of infected banana fingers. This is apex rot.

The pericarp of infected banana fingers showed black, spots of highly variable diameters on average, 2 to 3 cm in radius and randomly distributed. The spots are sometimes covered with a fine orange powder in the center. Other spots, on the other hand, have a depression in the pericarp. Infected fingers showed characteristic symptoms of pericarp necrosis (Figure 1A, Figure 1B, Figure 1C and Figure 1D)

### **High prevalence of diseases from bananas after 21 days of storage**

Bunches of bananas developed symptoms of storage diseases with different proportions. However, the average prevalence rates of these developed storage diseases varied from one production zone



**Figure 1:** Symptoms of storage diseases of dessert bananas observed after 21 days of storage, A: bunches of uninfected bananas, B: bunches of bananas with crown rot, C: fingers of bananas with rotted apex, D: fingers of bananas with necrotic pericarp.

**Table 1:** Average prevalence of storage diseases in dessert bananas according to production zone.

Zones of production	Average prevalence of storage diseases in dessert bananas		
	Crown rot	Pericarp necrosis	Apex rot
Abengourou	68,26 <sup>a</sup> ± 7,2	36,06 <sup>a</sup> ± 6,8	23,28 <sup>ab</sup> ± 8,9
Aboisso	36,76 <sup>b</sup> ± 11,2	19,4 <sup>ab</sup> ± 10,5	8,9 <sup>b</sup> ± 3,7
Agboville	38,83 <sup>b</sup> ± 8,27	0,3 <sup>b</sup> ± 0,3	11 <sup>b</sup> ± 7,6
Azaguié	45,56 <sup>ab</sup> ± 8,6	18,6 <sup>ab</sup> ± 10,9	8 <sup>b</sup> ± 3,8
Dabou	58,06 <sup>ab</sup> ± 4,05	31,68 <sup>a</sup> ± 5,16	28,42 <sup>ab</sup> ± 11,06
Tiassalé	32,50 <sup>b</sup> ± 11,06	26,97 <sup>a</sup> ± 8,4	37,09 <sup>a</sup> ± 10,3
Motobé	58,3 <sup>ab</sup> ± 11,02	33,24 <sup>a</sup> ± 8,8	17,07 <sup>ab</sup> ± 6,1
P	0,06	0,03	0,11
F	2,2	2,7	1,9

Means followed by the same letter are not significantly different at the 5% threshold (Newman-Keuls test)

to another. Crown rot was more developed on bunches of bananas from Abengourou at 68.26% and less so on those from Aboisso and Agboville at 36.76, 38.83 and 32.50% respectively. Crown rot was more developed on bunches of bananas from Abengourou at 68.26% and less so on those from Aboisso and Agboville at 36.76, 38.83 and 32.50% respectively. Pericarp necrosis was less developed on bunches of bananas from the Agboville zone at 0.3%, in contrast to bunches from other collection areas where the highest infection rates were observed. On the other hand, apex rot rates were lowest in Aboisso, Agboville and Azaguié (Table 1) [14].

**Diversity of post-harvest fungi associated with symptoms of banana storage diseases**

A diversity of fungi from four genera was isolated and identified on bananas infected with banana storage diseases. Macroscopic and microscopic characteristics were observed in colonies grown for one week on PDA medium. *Musicillium* showed a very cottony yellow downy colony. A branched septate mycelium surmounted by phialides bearing small spherical first-formed conidia 4-15 × 2-4µm, and small conidia 2-4 × 1-2µm were observed under the microscope. Isolates of the *Colletotrichum* genus showed a lightly lined white colony which discolored the culture medium pink. Under the microscope, oval conidia, sometimes slightly curved without a septum, attached to a branched septate mycelium were observed.. The macroscopic morphological characteristics of isolates of the *Botryodiplodia* genus are black, spiky colonies that stain the culture medium black [15]. The mycelium is black, hyaline, septate and branched under the microscope. Oval conidia with an equatorial septum measuring approximately 10-8 × 17-43µm were also observed. The conidia were hyaline, septate, mostly ellipsoid, and 14 × 7µm in size under the microscope. The characteristics of the mycelial colonies were white in color, not very flaky and a little bristly [16].

Isolations of fungi from the crown, pericarp and distal tip

showed a diversity of fungi belonging to four fungal genera. These were *Musicillium*, *Colletotrichum*, *Fusarium* and *Botryodiplodia*. The fungi were isolated according to the infected parts of the banana. *Botryodiplodia*, *Fusarium* and sometimes *Colletotrichum* fungi were isolated from the crown.

However, the *Musicillium* fungus was mainly isolated from the distal end of infected bananas. In addition, it should be noted that several morphotypes were isolated per fungal genus. For the genera *Colletotrichum*, *Botryodiplodia* and *Fusarium*, three morphotypes of each were isolated. Isolates of *Botryodiplodia* sp., *Fusarium* sp. and *Colletotrichum* sp. were associated with crown rot. Isolates of *Colletotrichum* sp. were associated with pericarp necrosis. *Musicillium* sp. was associated with distal tip rot (Figure 2).

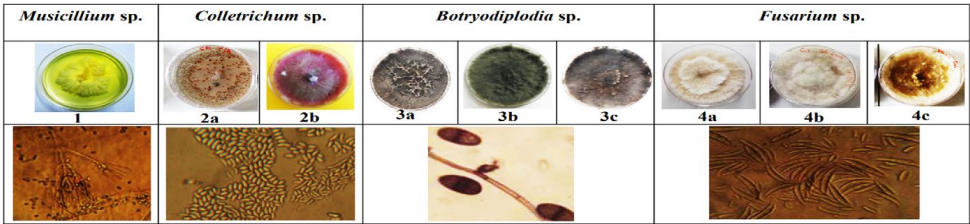
**Post-harvest fungi responsible for storage diseases of dessert bananas**

In the presence of the fungi *Botryodiplodia* sp., *Colletotrichum* sp., *Fusarium* sp. and *Musicillium* sp., bananas developed post-harvest diseases in varying proportions. Post-harvest diseases were more developed in the presence of fungi of the *Botryodiplodia* sp. and *Colletotrichum* sp. genera. The most common symptoms were crown rot and pericarp necrosis, with crown rot dominating between 79.1% and 100%. From specific morphological characters of fungi identification keys, isolates responsible of banana storage diseases seemed to be *Botryodiplodia theobromae*, *Colletotrichum musae*, *Fusarium musae*, *Fusarium verticillioides* and *Musicillium theobromae* (Table 2) (Figure 3).

**Fungi isolated on the apex of dessert banana fingers**

Isolations of fungi made after 48 h of storage from the apex of bananas treated with the antifungals Azoxystrobin and Boscalid showed a diversity of white fungal colonies visible at the apex of the fingers whatever their origin. Morphological identification of these





**Figure 2:** Macroscopic and microscopic characteristics of fungi associated with symptoms of storage diseases of dessert banana, 2.1: *Musicillium* sp.; 2.2: two morphotypes of *Colletotrichum* sp.; 2.3: three morphotypes of *Botryodiplodia* sp.; 2.4: three morphotypes of *Fusarium* sp.

**Table 2:** Percentage development of storage diseases according to the fungi responsible.

Fungi inoculated to banana	Percentage of diseases developed		
	Crown rot	Pericarp necrosis	Apex rot
<i>Musicillium</i> sp.	79,17	0	4,17
<i>Colletotrichum</i> sp.	100	100	4,1
<i>Botryodiplodia</i> sp.	100	100	8,3
<i>Fusarium</i> sp.	79,1	0	4,1



**Figure 3:** Symptoms developed.

colonies after culture on PDA medium revealed *Botryodiplodia* sp., *Colletotrichum* sp., *Fusarium* sp. and *Musicillium* sp. isolates as on infected crowns, pericarps and apices after 21 days of storage above (Figure 4) [17].

**Reducing the prevalence of storage diseases in dessert bananas by apex treatment**

Bunches of bananas treated by targeting the apex of the fingers significantly reduced banana storage diseases. In banana bunches treated with Azoxystrobin and Boscalid, a significant difference was noted in the average rates of development of storage diseases on bunches in storage ( $p<0.05$ ) [18].

Crown and pericarp without apex, from the seven production zones, crown rot was more developed at 48.06%, followed by pericarp necrosis at 23.11%. Crown rot was least developed at 18.88% by the banana bunches. A significant difference was also observed between the average rates of development of storage diseases on bunches of bananas treated as previously, targeting the apex of the fingers ( $p<0.05$ ). Crown rot was least developed at 18.88% by the banana bunches. A significant difference was also observed between the average rates of development of storage diseases on bunches of bananas treated as previously, targeting the apex of the fingers ( $p<0.05$ ). Crown rot was also the most developed at 7.7% by bunches in storage. On the other hand, pericarp rot was the least developed at 0.15% in the banana bunches [19].

By comparing the average infection rates for the development of conservation diseases observed between bunches treated at the crown and on the pericarp with antifungals and those treated by targeting the apex, the disease reduction rates were very high. Crown rot was reduced by 83.97%, pericarp necrosis by 83.12% and apex rot by 99.20%.



**Figure 4:** Apex fungal colonies development on PDA medium from banana crown and pericarp treated with Azoxystrobin and Boscalid.

Targeted treatment of the apex of banana fingers with Azoxystrobin and Boscalid reduced storage diseases of dessert bananas by an average of 88.76% (Table 3) [20].

**Discussion**

Bunches of dessert bananas treated with Azoxystrobin and Boscalid; collected in the production areas developed crown and apex rot in addition to necrosis of the pericarp during storage. The infected bunches were found to be in favourable conditions for the development of storage diseases, revealing the presence of fungal structures that were still viable on the bananas after the antifungal treatments.

In fact, from the field, bananas could be contaminated by fungal structures maintained right through to packaging. On the plantations, in addition to mineral fertilization, out-of-class bunches and banana waste from the stations are used as organic fertilizers. This rotting organic matter is a source of various fungi capable of colonizing the air and the bunches in the plantations with their spores. Failure to act against these spores continually increases fungal parasite pressure in plantations and on harvested bunches. Once on the bunches, these spores could start infections. The fungi responsible for pericarp necrosis induce two types of necrosis. Latent pericarp necrosis can start at the beginning of the season, before the bunch is harvested. The pathogen lives a slow life in the form of subcuticular hyphae until the fruit approaches maturity. It resumes its infection at ripening, causing characteristic brown necrotic spots on infected fingers.

However, the agent can also develop on fingers that are still green, even when kept cold at 12-14°C. Contact antifungal treatments are ineffective against these early infections. In addition to latent infection, there is also non-latent infection, according to the same authors cited above. Non-latent infection appears on small wounds from harvest onwards and continues to develop without a dormant period. On green fruit, it causes lesions that are generally dark brown or black in color, surrounded by a pale halo, lenticular and slightly depressed. On green fruit, it causes lesions that are generally dark brown or

Table 3: Reduction rate of storage diseases of banana according to the parts of the finger treated with the antifungals Azoxystrobin and Boscalid.

Storage diseases	Average prevalences according to the parts of the banana treated		Rate of storage disease reduction rate (%)
	Crown and pericarp	Apex	
Crown rot	48,06 <sup>a</sup> ± 3,7	7,7 <sup>a</sup> ± 2,4	83,97
Pericarp necrosis	23,11 <sup>b</sup> ± 3,3	3,9 <sup>ab</sup> ± 2,4	83,12
Apex rot	18,88 <sup>b</sup> ± 3,2	0,15 <sup>b</sup> ± 0,1	99,20
P	0	0,03	
F	2,1	3,7	

Means followed by the same letter are not significantly different at the 5% threshold (Newman-Keuls test).

black, surrounded by a pale halo, lenticular and slightly depressed, as observed on bananas collected and stored. This non-latent necrosis was observed with depressions on the pericarp. According to, in the case of anthracnose, non-latent infection manifests itself on ripe fruit as numerous small brown or black circular spots. These spots enlarge and become coalescent, forming large necrotic patches on the pericarp. As the disease progresses, the necrotic areas become depressed and are covered at the center with clusters of salmon-pink spores. Infected fingers quickly ripen and rot.

The banana bunch is cutting in water baths to select the banana bunches. These waters are highly concentrated in spores. Crown rot results both from the opening of the stem during bunch selection and from contamination of the crown from the spore solution in bunches cutting water, from rinsing in the tanks and from infection under conditions favourable to the contaminating fungi. Any contact of the open crown with these waters constitutes forms of inoculation with spores of the fungi responsible for rotting.

To combat these infections, growers use azoxystrobin and Boscalid after harvesting the banana bunches. Azoxystrobin is a systemic, active, preventive, curative and translaminar fungicide with slow foliar absorption and not in the fruit. It moves only in the xylem and not in the reserve organs. It is effective in the early stages when spores germinate, not afterwards. This could justify its ineffectiveness against the early infections that lead to these different forms of necrosis of the banana pericarp. As for Boscalid, it must be applied strictly as a preventive measure and is therefore ineffective against early infections in banana plantations. The fungi identified as being associated with and responsible for epicarp necrosis, crown rot and apex rot were isolates of *Botryodiplodia* sp., *Colletotrichum* sp., *Fusarium* sp. and *Musicillium* sp.

In fact, the fungi most commonly identified as associated with crown rot of dessert banana are *Colletotrichum musae*, *Fusarium roseum*, *Fusarium semitectum*, *Botryodiplodia theobromae* and *Musicillium theobromae*. *Colletotrichum musae* is often considered to be the primary pathogen of banana crown rot, as it is the most virulent and is capable of causing severe symptoms from minute quantities of inoculum. Pericarp necrosis, also known as anthracnose, caused by *Colletotrichum musae* (Berck; Curt.) Von Arx is an important post-harvest infection of bananas. The fungi responsible for the storage diseases identified were also present at the apex of banana fingers not treated with fungicides by producers before packing and palletizing. The apex, where old floral parts are inserted, is thought to be a reservoir and a high concentration point for the spores of the fungi responsible for storage diseases. Indeed, Meredith and Shillingford have already identified senescent organs (floral parts and leaves) as a potential source of inoculum. According to Lapeyre, removal of the inflorescence bract and floral parts significantly reduces the quantity of

spores contaminating banana fingers in plantations. As a result, these organs and the apex are the main sources of inoculum for the fungi involved in the development of banana storage diseases.

The antifungal treatment targeted at the apex significantly reduced the fungus populations present on this part of the banana finger. This confirms the low rates of disease development observed after treatment of the banana finger apex. Control of storage diseases of banana cannot be achieved by the control method alone, but targeted application of antifungal products could be quite effective against the fungi responsible for storage diseases. The present study shows that chemical treatment directed against the reservoir parts of fungal inoculum could considerably reduce banana storage diseases and, consequently, the quantity of antifungal product used by growers to control the fungi responsible.

Conclusion

The fight against the fungal diseases responsible for the destruction of the banana requires a combination of several actions, taking into account the diversity of the factors involved in the development of these diseases on the banana. In the context of this study, it should be noted that the main banana conservation diseases observed in Côte d'Ivoire are crown and apex pests and pericarp necrosis. The fungi responsible for these infections belong to the *Botryodiplodia theobromae*, *Fusarium* sp., *Colletotrichum musae* and *Musicillium theobromae* genera. All or most of these fungi are found in the apex of the fingers of banana bunches. To control the fungi responsible for banana storage diseases, the application of a contact fungicide would be more effective if it applied before the fungi come into contact with the banana in farm or, precisely targeted at the apex of the banana fingers during the post-harvest antifungal treatment at the packing station, to ensure successful storage during export.

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