Crown Rot Disease of Bananas

Ludivine Lassois[1] and Luc de Lapeyre de Bellaire[2]

[1]Forest, Nature and Lanscape Department, Liège University, Gembloux Agro-Bio Tech, Gembloux, Belgium;
[2]CIRAD, Research Unit Banana, Plantain and Pineapple Cropping Systems, Montpellier, France

Contents

OVERALL IMPORTANCE OF THE DISEASE

Crown rot disease is considered to be the main export banana postharvest disease (Reyes et al., 1998; Krauss and Johanson, 2000) and it affects export bananas in all banana-producing countries. Crown rot affects tissues of the so-called 'crown', which unites the peduncles (Fig. 3.1A). Since crowns are mainly infected at harvest time, future infection is unpredictable. The symptoms develop rapidly during shipping and ripening when the physiology of the fruit undergoes modifications that facilitate fungal development (Jones, 2000). The rot begins with mycelial development on the surface of the crown (Fig. 3.1B), followed by the peduncles and fruit (Fig. 3.1C–F). The bananas may detach from the peduncle in cases of severe infection. Crown rot results from the development in the crown of several common fungi, which constitute a fungal complex, and leads to softening and blackening of tissues at the site of the wound left when the cluster was cut from the bunch.

Crown rot affects the fruit quality because of the development of necrosis on the fruit, and it can also trigger early ripening of bananas during shipping (Peacock, 1973). Ripening may be caused by ethylene released by stressed and necrotic tissues (Simmonds, 1963; MacCracken and Swinburne, 1980; Dominguez and Vendrell, 1993) but also by ethylene produced by mycelia of fungi such as *Colletotrichum musae* (Peacock and Muirhead, 1974; Daundasekera et al., 2003). The result is a green life reduction and an accelerated maturation of the fruits. Overall, crown rot incidence involves a depreciation of the product inducing significant economic losses. Onset of the disease cannot be predicted and it spreads in an irregular pattern on all clusters in a shipping box. The same box may contain both healthy and infected clusters. The incidence of crown rot periodically increases during the rainy season, and losses of over 10% have been recorded in the UK in bananas coming from the Windward Islands that were harvested during this period (Krauss and Johanson, 2000). Losses of up to 86% have also been reported in non-chemically treated bananas from the Philippines (Alvindia et al., 2000).

Crown rot disease became a major problem in the banana industry during varietal reconversion initiatives in the 1960s. Up until 1960, only Gros-Michel subgroup cultivars were cropped for export, and for economic and practical reasons bananas were shipped in complete bunches consisting of double layers of fruits, called hands, which are arranged helicoidally around a central axis called the stalk. These cultivars were, however, susceptible to Panama disease (*Fusarium oxysporum* f.sp. *cubense*) and
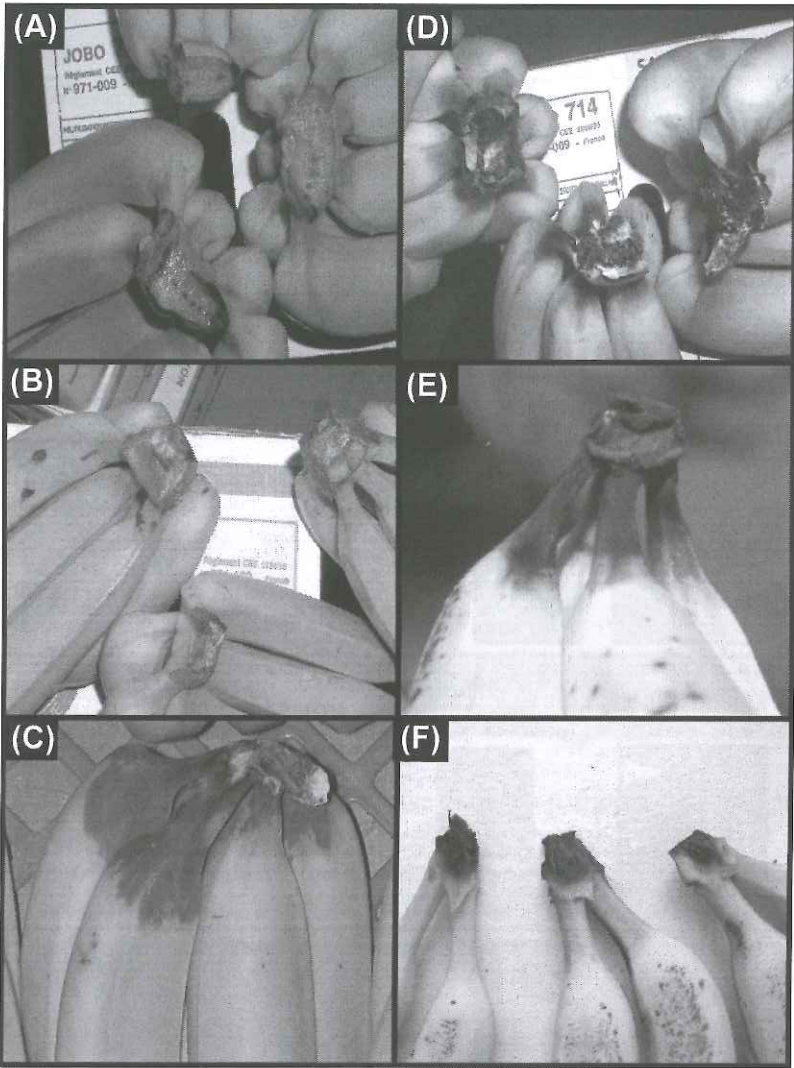
Figure 3.1 Crown rot symptoms. (A) Healthy crowns after maritime shipping before being placed in ripening rooms. (B) Crowns diseased with a superficial mycelium after maritime shipping. (C) Rot noted on bananas after maritime shipping. (D) Rot on the peduncles inducing banana ripening upon their arrival after maritime shipping. (E) External crown rot symptoms after fruit ripening. (F) Internal crown rot symptoms after fruit ripening (Lassois et al., 2010b).

thus were replaced by Cavendish subgroup cultivars because of their resistance to Panama disease (Stover, 1972). However, Cavendish bananas are more fragile during shipping and this triggered a revolution in the banana industry as bunch shipping was discontinued. Bananas were instead cut
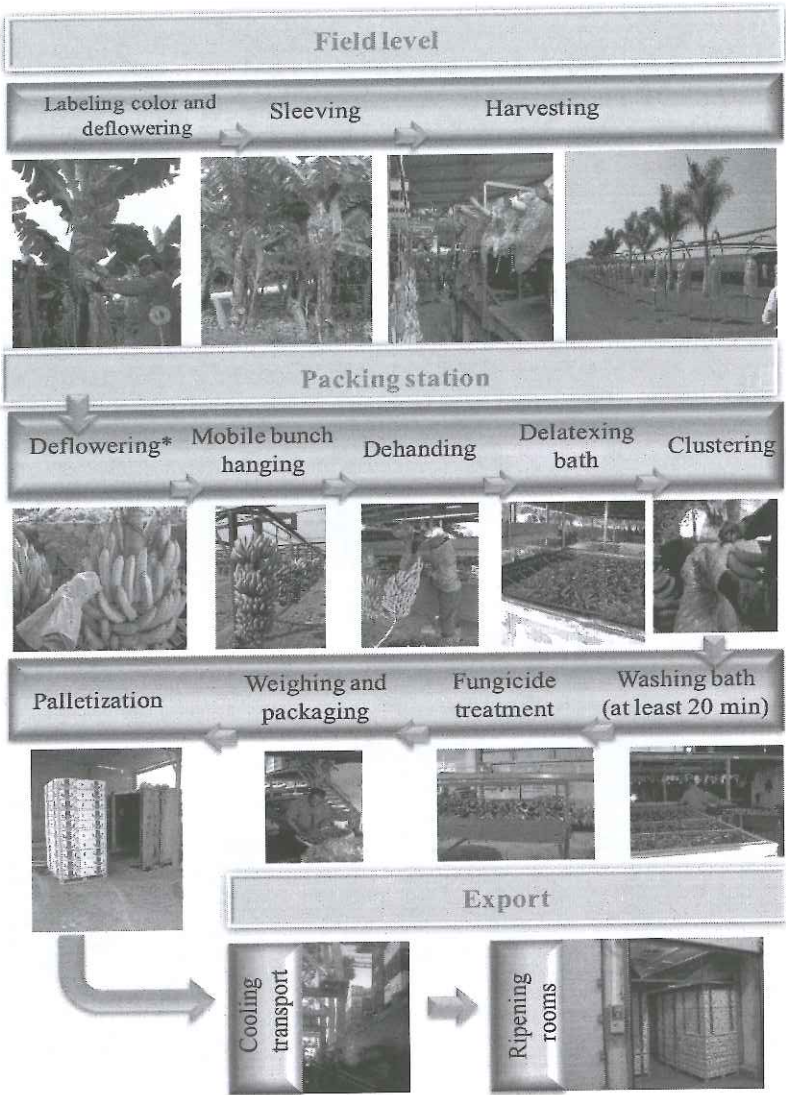
Figure 3.2 Successive operations carried out from the field to the banana marketing stage. *Deflowering is done in the packing station when it has not been done in the field (Lassois et al., 2010b).

into clusters consisting of several banana fruits joined by the crown tissues and boxed for shipping (Eckert and Ogawa, 1985). The crown became a prime site for infection by different pathogens (Lukezic et al., 1967; Griffee and Burden, 1976; Jones, 2000).

The successive operations carried out from the field to the banana marketing stage are presented in Figure 3.2 and will help to a better understanding of the contamination process and the control methods.

TAXONOMY AND MORPHOLOGY

Crown rot is the result of the activity of a fungal complex. The role of bacteria in the complex has not been confirmed by detailed research (Lukezic et al., 1967). The microorganisms most commonly isolated in crown rot are: *Musicillium theobromae, Colletotrichum musae, Ceratocystis paradoxa, Lasiodoplodia theobromae, Nigrospora sphaerica, Cladosporium* spp., *Acremonium* spp., *Penicillium* spp. and *Aspergillus* spp., as well as many *Fusarium* species including *F. semitectum, F. verticillioides, F. sporotrichoides, F. oxysporum* and *F. solani* (Greene and Goos, 1963; Lukezic et al., 1967; Wallbridge and Pinegar, 1975; Griffee and Burden, 1976; Shillingford, 1976; Wallbridge, 1981; Ogundero, 1987; Mesturino and Ragazzi, 1988; Johanson and Blasquez, 1992; Marin et al., 1996; Anthony et al., 2004). Several organisms may be involved in disease development, however, disease severity and the nature of the complex may vary substantially, depending on the production area and season (Table 3.1). Lukezic and Kaiser (1966) showed that fungal populations may differ between banana plants and even between clusters originating from the same plant.

Fungi of the complex do not all have the same pathogenicity and variations have been reported between regions. Knight (1982) considered that *F. oxysporum, F. verticillioides* and *F. graminearum*, which have been isolated frequently from crowns of Windward Island banana production, are primary pathogens, whereas *L. theobromae, M. theobromae*, and *N. sphaerica* are considered to be relatively non-pathogenic species. According to the findings of Marin et al. (1996) in Costa-Rica, *F. verticillioides* and *F. semitectum* are the most pathogenic species, while Griffee (1976) considered *C. musae, L. theobromae, C. paradoxa, F. semitectum* and *F. graminearum* as major pathogens involved in this disease in the Caribbean. However, many authors agree on the strong pathogenicity of *C. musae*, which may trigger an infection from a very small amount of inoculum since other pathogens require larger amounts of inoculum to induce crown rot symptoms (Greene and Goos, 1963; Lukezic et al., 1967; Griffee, 1976; Shillingford, 1976; Finlay and Brown, 1993; Krauss, 1996; Krauss et al., 1998; Lassois et al., 2008).

The nature of the complex, the specific pathogenicity of the different microorganisms involved as well as interactions among them may alter the

Table 3.1 Bibliographic Data on the Fungal Species Isolated from Crown Rot in Different Banana-producing Regions.

Countries	References	Colletotrichum musae	Fusarium semitectum	Fusarium verticillioides	Fusarium sporotrichoides	Fusarium oxysporum	Fusarium solani	Lasiodiplodia theobromae	Musicillium theobromae	Gliocladium roseum	Nigrospora sphaerica	Acremonium sp.	Penicillium sp.
Honduras	1	3%	80%	14%					12%	81%			93%
Central and South America	2	F	MF	X					R	F		R	
	3	0-10%	4-50%	0-28%							0-30%	0-8%	0-33%
Windward Islands (WI)	4	23-33%								5-7%	5%		
	5	36%	27%	6%					2%	8%		<1%	
	6	24%	18%	3%			3%	2%	2%	3%	2%	<1%	<1%
	7	26-44%	7-23%	6-21%					3-9%	4-18%	0-10%	24-26%	0-10%
	8	I	I	I									
	9	X	X	X					X				
	10		I										
	11	I	I	I		I	I		I	I		I	
	12	I	I	I		I	I		I	I		I	
Jamaica	13	11%	F	X			X		3%	13%		1%	3%
Sri Lanka	14	X		X					X				
Nigeria	15	27%	3%	10%		4%		6%	26%	2%			
Somalia WI, Guatemala	16	38%	X	X		20%				2%			4%

Percentage values correspond to isolation frequencies when given by the authors. "X" indicates that the pathogen was identified on the crown by the author(s), but without providing any isolation frequency data. F: frequently isolated; MF: most frequently isolated; R: rarely isolated. "I" indicates that authors evaluated fungal pathogenicity without information about the isolation frequencies. Information about the pathogenicity are shown when given by authors. ▮: Highly pathogenic; ▮: Medium pathogenicity; ▮: Slightly or non pathogenic. 1: Lukezic et al., 1967; 2: Green and Goos, 1963; 3: Marin et al., 1996; 4: Wallbridge and Pinegar, 1975; 5: Griffee and Burden, 1976; 6: Wallbridge, 1981; 7: Johanson and Blasquez, 1992; 8: Lassois et al., 2008; 9: Finlay and Brown, 1993; 10: Knight et al., 1977; 11: Knight, 1982; 12: Griffee, 1976; 13: Shilingford, 1976; 14: Anthony et al., 2004; 15: Ogundero, 1987; 16: Mesturino and Rgazzi, 1988.

pathogenicity of the complex. *Colletotrichum musae* was more pathogenic when it was inoculated alone than when it was co-inoculated with other species (Lassois et al., 2008), however, Anthony et al. (2004) found that *L. theobromae, F. verticillioides*, and *C. musae* were more highly pathogenic when co-inoculated than when they were inoculated separately.

The respective roles of the different species that could belong to the fungal complex have been thoroughly studied, whereas little information is available on the antagonistic or synergistic relationships among these different species.

More specifically, *C. musae*, the most pathogenic fungi associated with crown rot disease is a specific pathogen of banana. The genus *Colletotrichum* comprises imperfect fungi (Deuteromycetes) belonging to the order of Melanconiales. *Colletotrichum musae* is a distinct lineage in the 'gloeosporiodes' species complex (Su et al., 2011). The mycelium of *C. musae* is aerial, white turning an orange color with age (de Lapeyre de Bellaire, 1999; Abd-Elsalam et al., 2010). Conidia of *C. musae* are hyaline, non-septate, and mostly ellipsoid, ranging from 12–17 μm × 4–8 μm in size (Lim et al., 2002; Abd-Elsalam et al., 2010) and are produced on septate conidiophores that are up to 30 μm long and 3.5 μm wide. Appressoria are highly melanized, dark brown and irregularly shaped ranging from 6–11 × 5–10 μm in size (de Lapeyre de Bellaire, 1999; Lim et al., 2002). Conidiophores are gathered within acervuli that are up to 400 μm (Jones and Slabaugh, 1994). *Colletotrichum musae* produces its conidia in a mucilage which protects them and makes it possible to ensure their survival within acervuli (Mondal and Parbery, 2005).

BIOLOGY OF CROWN ROT INFECTION PROCESS

The large number of pathogens involved in the complex and the fact that the characteristics of each species differ complicates studies on the epidemic cycle and very little information is available on the epidemiology of the crown rot complex. Fruit contamination might occur in the field on the bunch or in the washing tanks in the packing station but, although field infections cannot be excluded, infections mainly occur during harvest when clusters are trimmed from bunches.

Field Contamination
Conidial Dispersion
Spores of some species, such as *C. musae*, are mainly disseminated by rainwater (Jeffries et al., 1990; de Lapeyre de Bellaire and Mourichon, 1997; de Lapeyre de Bellaire et al., 2000). Particularly, de Lapeyre de Bellaire et al.

(2000) have shown that the streaming of rainwater is an essential factor for the transport of the conidia of *C. musae* on the surface of the fruit. Other banana pathogens are airborne and most species involved in the fungal complex are found in the air spora of a banana plantation (Meredith, 1962a), and it has also been shown that some *Fusarium* species can be wind disseminated (Lukezic and Kaiser, 1966).

Fruit Contamination in the Field

Fruit pollution and fruit contamination must be distinguished. Fruit pollution, which occurs in the field, concerns the quantity of conidia that reach the fruit surface. Fruit contamination is related to the quantity of conidia that infect the crown and leads to disease development. In banana plantations, fungal species sporulate abundantly on all putrescent organs such as leaves (Simmonds, 1941; Meredith, 1962a), floral parts (Agati, 1992; de Lapeyre de Bellaire and Mourichon, 1997), and bracts (Lukezic and Kaiser, 1966; de Lapeyre de Bellaire et al., 2000). *Colletotrichum musae* and some *Fusarium* species are primary colonizers of decomposing leaves (Meredith, 1962b) and floral parts (de Lapeyre de Bellaire et al., 2000). This fungus does not sporulate on the still green parts of the banana tree but only on the senescent organs. It develops as a saprophyte on all decomposition matter of the banana plantation. According to Meredith (1962b), *C. musae* is a primary colonizer of the decomposing leaves. This author explains that the infectious structures are formed on the leaves when they were still green, which confers a decisive advantage for the colonization of senescent organs. de Lapeyre de Bellaire and Mourichon (1997) have shown in a study carried out in Guadeloupe that the pollution of bananas by *C. musae* and various *Fusarium* species usually occurs during the first 40 days following bunch emergence and sharply decreases thereafter. For these authors, the floral parts and the last bunch bract are the two main effective sources of inoculum for the contamination of the fruits but, because they are closest to the fruit, the floral parts are the most effective inoculum source for fruit pollution.

Fruit Contamination in the Packing Station

The fungus *C. musae*, which is also the causal agent of anthracnose, establishes quiescent infections in the field during the first month following banana flowering (de Lapeyre de Bellaire et al., 2000). These quiescent infections could also contribute to the onset of crown rot if the pathogen has an opportunity to colonize the crown region (Griffee and Pinegar,

1974). However, although field infections cannot be excluded, infections of the crown mainly occur during harvest and when clusters are trimmed from bunches (Meredith, 1971). Contamination generally takes place when hands are cut with a contaminated knife (Greene and Goos, 1963; Stover, 1972; Wallbridge, 1981; Finlay et al., 1992) or when clusters are cleaned with contaminated washing water (Shillingford, 1977) (see Fig. 3.2). At harvest, the fruit and senescent floral organs bear high quantities of spores that could potentially contaminate the crowns. Some of these spores could be removed by washing and then accumulate in the washing water (Slabaugh and Grove, 1982). The washing tanks are the main source of inocula according to Shillingford (1977). After the banana clusters are dipped in the washing water, the spores can passively penetrate a few millimeters into the vascular vessels of the crown, and disease is then hard to control with a fungicide spray treatment (Eckert and Ogawa, 1985). Greene and Goos (1963) have shown that a suspension of *C. musae* spores could penetrate 5–7 mm into the crown tissues within only 3 min. Airborne spores may also contaminate the crowns before the fruit are placed in boxes in the packing station. The risk is especially high when the facilities are dirty or if debris (pistils, stalks, and fruits) is piled up nearby.

PREHARVEST FACTORS FAVORING CROWN ROT DISEASE

Most of the studies on crown rot focused on the etiology of the disease, especially on the identification of the most pathogenic fungal species involved, and on postharvest control methods. Unfortunately, these studies provided neither a good understanding of the conditions most conducive for disease expression nor adequate control of the disease at all spatio-temporal scales. There is now some evidence that fruit physiology at harvest influences crown rot development and that all interactions among the microorganisms implicated should be considered. Taking these into account, a new concept of 'banana quality potential at harvest' and a model of elaboration of this quality potential were proposed by Lassois et al. (2010b) (Fig. 3.3). In this model, the fruit quality potential at harvest is presented as a key factor in crown rot development. The quality potential develops during banana growth in the field and depends on two components: (1) a fruit physiological component, which determines the fruit susceptibility to the disease; and (2) a parasitic component that reflects a level of crown contamination by the fungal complex, as well as the pathogenicity of this complex. Both these components are major in
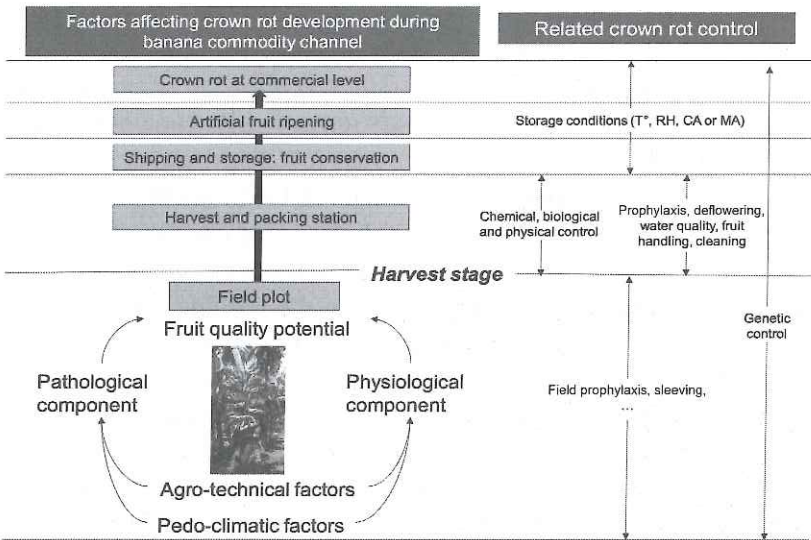
Figure 3.3 Diagram representing different key factors that arise throughout the commodity channel concerning crown rot development at commercial stage, and related control methods. T° = temperature; RH = relative humidity; CA = controlled atmosphere; MA = modified atmosphere (Lassois et al., 2010b).

the postharvest development of the crown rot disease and are greatly influenced by all environmental and agrotechnical preharvest factors. Only a few studies link them to either fruit susceptibility or to fruit contamination, and these are presented hereafter.

Influence of Environmental Factors on the Potential of Quality

Geographical and seasonal variations have been observed in the incidence of crown rot disease of bananas (Lukezic et al., 1967; Shillingford, 1978; Krauss and Johanson, 2000). Lukezic et al. (1967) have shown that the incidence of this disease varied throughout the year in Honduras. It is generally higher during the summer (March to September) and declines during the coldest period (October to February). Moreover, in Jamaica, a high disease incidence was found to be correlated with periods during the year when temperatures were highest (Shillingford, 1978), whereas in the Windward Islands, the incidence was reported to be highest during the rainy period (Krauss and Johanson, 2000). Therefore, it was important to disentangle whether these spatio-temporal fluctuations affected the parasitic and/or the physiological component of the fruit quality potential. Some experiments were carried out through controlled inoculations and

demonstrated that the spatio-temporal factors have an impact on the physiological component of the fruit quality potential. A multilocal study carried out in Cameroon over a 1-year period showed less crown rot development at higher altitude (500 m) than at lower altitude (80 m) (Ewanéet al., 2013b). This study also suggested that fruits harvested in a low-altitude plantation during the rainy season might be more susceptible to crown rot than those harvested during the dry season. On the other hand, continuous variations of fruit susceptibility have been shown in the same banana plot through studies realized in Cameroon (Lassois, et al., 2010a) and Guadeloupe (Lassois et al., 2008).

Influence of Harvest Stage on Fruit Susceptibility

Harvest stage is expressed in accumulated degree day (dd) (Jullien et al., 2008) from flowering to harvest. It has been shown that harvest stage affects fruit susceptibility to crown rot: (1) an intra-bunch susceptibility gradient has been documented with a linear correlation ($r = 0.95$) between the hand position on the bunch and crown rot development (Lassois, et al., 2010a). Clusters growing on the first hands of a bunch are, on average, 70 dd more advanced than the hands initiated last (Jullien et al., 2001); (2) a linear relationship between fruit age (in dd) at harvest and the susceptibility of the fruit to crown rot was reported, with the oldest fruit being most susceptible to this disease (Forret, 2008).

Influence of Source/Sink Ratio on Fruit Susceptibility

Leaves are considered as 'source' tissues because they produce an excess of assimilates, while fruit are considered as 'sink' organs because they are not able to produce sufficient amounts of assimilate. It was demonstrated that the source/sink ratio during bunch growth affects the pomological characteristics of the fruits but also their susceptibility to crown rot (Lassois et al., 2010a). Fruits of bunches from which six hands were removed (high source/sink ratio, two hands remaining on the bunch) proved to be significantly less susceptible to crown rot than those from bunches with eight hands remaining on the bunch. It has also been shown that in the case of severe defoliations (low source-sink ratio), with only one or two leaves remaining on the tree, fruit susceptibility to crown rot disease was increased (Ewané, 2012). The authors considered that source/sink ratio modification leads, among other factors, to changes in the rate and characteristics of fruit filling which influences the formation of secondary metabolites involved in plant–pathogen interaction. A study was conducted to highlight underlying

molecular mechanisms implied in the susceptibility variations observed when the source/sink ratio was modified (Lassois, et al., 2011a). Some genes involved in the susceptibility variation of bananas to crown rot disease were identified and results suggest that the catecholamine pathway could be implied in this phenomenon (Lassoiset al., 2011b). In the past, it had been suggested that preformed fungitoxic polyphenolic compounds could be involved in banana resistance to postharvest diseases (Mulvena et al., 1969; Muirhead and Deverall, 1984; Abdel-Saatar and Nawwar, 1986; Alam et al., 1993; Chillet et al., 2007), and it has also been shown that the modification of the source/sink ratio might induce modifications in the content of polyphenolics that might be implied in the modification of fruit susceptibility (Ewané, 2012).

Effect of Some Biotic Factors on Fruit Susceptibility

A study was carried out to evaluate the influence of some preharvest biotic factors on the fruits' susceptibility to postharvest crown rot development (Ewané et al., 2013a). It has been proven that the black leaf streak disease (*Mycosphaerella fijiensis*) severity at flowering and at harvest is positively correlated with the postharvest development of crown rot (Ewané et al., 2013a). This must be linked to the fact that black leaf streak disease reduces the source/sink ratio.

Influence of Sanitation Practices on Fruit Contamination

It is well known that most species involved in the fungal complex are saprophytes that occur on senescent banana organs, especially on decomposing leaves (Meredith, 1962b). Old leaves and other senescent organs present in the banana plantation may harbor inocula that could be responsible for severe contamination of bananas by this fungal complex (Simmonds and Mitchell, 1940).

POSTHARVEST FACTORS FAVORING CROWN ROT DISEASE

As can be seen in Figure 3.3, agronomic and environmental factors influence the fruit quality potential in the field and its propensity to develop crown rot. However, this figure also shows the importance of postharvest stages, such as harvesting, handling, transport and storage conditions in the development of crown rot. Fruit injuries enhance fruit ethylene production which has an impact on crown rot development. Furthermore, wounds on the fruits favor infection by pathogens. Fruit injuries must thus be avoided

at all steps of the banana commodity channel. The inoculum pressure must be limited in all steps of the postharvest chain production to control efficiently crown rot development.

Organization of the Banana Commodity Channel

Due to the highly perishable nature of banana fruit, the commodity channel has to be well organized at all levels (Krauss and Johanson, 2000). This includes timely harvesting, a rapid cooling of fruit which is a prerequisite for effective crown rot control, an effective 'in' and 'out' quality control, an integrated efficient postharvest crown rot control, rapid loading into the boats and a continuous cooling chain from the beginning of the voyage to ripening centers and retailers in the importation countries. Shortcomings here can negate other efforts made to maintain healthy fruits.

It is difficult to control most of the preharvest factors in order to reduce crown rot impact. Inversely, most of the postharvest factors have to be controlled to reduce crown rot disease development. The impact of these different stages on disease development, as well as the methods applied to limit them, will be described in the following section concerning postharvest control methods.

CONTROL METHODS AND ALTERNATIVES TO CHEMICAL CONTROL

Conventional Chemical Control

Banana crown rot, like other storage diseases, has an especially detrimental impact on export produce. Routine postharvest fungicide treatment is still the most efficient and used crown rot control method (Table 3.2). This strategy was introduced in the late 1960s with the discovery of systemic benzimidazole derivative fungicides (thiabendazole and benomyl). They are classified as antimitotic compounds. Gradually, other fungicides that inhibit ergosterol biosynthesis, such as imazalil and bitertanol, were also introduced (Frossard et al., 1977; de Lapeyre de Bellaire and Nolin, 1994). The recent exclusion of bitertanol led to the use of QoI fungicides like azoxystrobin. The application methods vary markedly, including dipping, spraying and cascade treatment (see Fig. 3.2) but, in every case, the bananas must be thoroughly wetted to ensure fungicide treatment efficacy (de Lapeyre de Bellaire and Nolin, 1994). Alum is often combined with fungicide slurries to neutralize latex residue remaining on crowns when the bananas are removed

Table 3.2 Summary of Different Fungicides Used for Controlling Postharvest Diseases of Bananas in Commercial Banana Plantations.

Active Ingredient	Mode of Action	Recommended Rate (mg L^{-1})	Commercial Name	Observations
Benomyl	antimicotic	250	Benlate®	Banned
Thiabendazole	antimicotic	450	Tecto®, Metect®, Decco®	Efficient on crown rot and anthracnose
Imazalil	ergosterol biosynthesis inhibitor (DMI)	375	Fungaflor®, Sulima®, Neozil®	Effective on crown rot, not on anthranose
Bitertanol	ergosterol biosynthesis inhibitor (DMI)	200	Baycor®	Efficient on crown rot and anthracnose. Recently banned
Azoxystrobin	Quinone oustside inhibitor (QoI)	60	Ortiva®, Bankit®	Efficient on crown rot and anthracnose

from the washing bath (Muirhead and Jones, 2000), however, this mixture seems to have a negative impact on the performance of some active ingredients such as thiabendazole (Joas and Malisart, 2001). The time between crown trimming and fungicide application is critical. Crown rot severity seems to increase when fungicide application is delayed (Greene and Goos, 1963). Bananas are, nevertheless, usually treated in time in packing stations, i.e. just after they are removed from the washing bath. Problems concerning the development of resistant strains may arise with this practice. Postharvest fungicides generally have the same mode of action as those sprayed in the field to control Sigatoka disease. *Colletotrichum musae* strains resistant to thiabendazole, and generally to all antimitotic compounds, have been detected in many banana-producing countries (Hostachy et al., 1990; Johanson and Blasquez, 1992; de Lapeyre de Bellaire and Dubois, 1997; Krauss and Johanson, 2000). In Guadeloupe, it has been shown that thiabendazole-resistant *C. musae* strains developed after exclusive foliar applications of benomyl over a decade (1972–1982) for Sigatoka disease control (de Lapeyre de Bellaire and Dubois, 1997). Postharvest use of chemicals could ultimately be prohibited because the number of active substances registered for postharvest treatment has been reduced by current regulations, and legislation is

becoming increasingly restrictive. Moreover, the efficacy of intensive fungicide treatments is not always adequate. In some areas where very few resistant strains are present, the efficacy of fungicides for postharvest disease control was found to vary substantially depending on season and production area (Chillet and de Lapeyre de Bellaire, 1996). Finally, consumers are now highly selective with respect to food safety concerns, and demand is increasing for produce that has not undergone any postharvest chemical treatments. Alternative non-fungicide solutions are therefore being sought.

Non-Conventional Control

Because the systematic fungicide control approach is not satisfactory and is increasingly criticized, several biological control approaches have been developed in recent years to find alternatives. We present here a set of methods that should be implemented in an integrated way to achieve efficient crown rot control (see Fig. 3.3) as an alternative to chemical control.

Field Control Methods
Bunch Sanitation

- Leaf removal: since senescent leaves hanging on the bunch might enhance fruit contamination (Meredith, 1962b; de Lapeyre de Bellaire et al., 2000), these leaves must be removed to reduce fruit contamination.
- Bunch sleeving (see Fig. 3.2): no accurate studies have been carried out to assess the impact of sleeving on crown rot development rate. It is, nevertheless, known that bunch sleeving with perforated plastic film protects bunches from fungal contamination and can reduce contamination by *C. musae* by over 80% (de Lapeyre de Bellaire et al., 2000). These results suggest that sleeving directly reduces crown contamination in the field, or that sleeved fruits release fewer spores in washing water during packing operations. Furthermore, this practice protects fruits from injuries.
- Deflowering (see Fig. 3.2): the main inoculum sources for *C. musae* and several *Fusarium* species are the floral remnants of the fruits and their early removal, generally when the young hands assume a horizontal position, is an important step further to limit bunch contamination (de Lapeyre de Bellaire et al., 2000).

Controlling the Banana Harvest Stage
Bananas are climacteric fruits, and the period between harvest and climacteric, the preclimacteric life, is commonly called the green life.

Bananas should be harvested at an age that will ensure a sufficient preclimacteric life during husbandry, because they should reach the ripening rooms still unripe (green). Furthermore, bananas are exported to Europe when they have grown to a commercial grade specified by European regulations (Commission Regulation (EC) No 2257/94 of 16 September 1994, setting quality standards for bananas) and market requirements. Thus, both age and grade of bananas are closely monitored during bunch development to determine the optimum date for harvest. Generally, once a week, newly emerged bunches are tagged with a colored plastic ribbon; different colors correspond to different weeks. Based on this colored system, the age of the banana is either estimated (1) in days or (2) in physiological age of the fruits. This age is then expressed as a sum of accumulated temperatures from flowering to harvest rather than in days (Jullien et al., 2008). It has been shown that physiological age is the best estimator of the green life. It has also been shown that younger fruits are less susceptible to crown rot than older fruits of the same grade (Forret, 2008), so good harvest stage management is an essential step to reduce harvesting of very susceptible fruits.

Black Leaf Streak Control
Good control of black leaf streak (Ewané et al., 2013a) or other foliar disease is important, since these disease promote fruit crown rot susceptibility by reducing the source/sink ratio. This requires proper chemical strategies as well as other integrated practices (de Lapeyre de Bellaire et al., 2010).

Genetic Control
The range of varieties grown for dessert banana export is very narrow as all clones belong to the Cavendish subgroup (Bakry et al., 1997). Moreover, breeding is complicated because triploid banana varieties are generally sterile. In the past, banana genetic improvement programs were mainly focused on obtaining varieties resistant to Sigatoka and Panama diseases. Resistance to crown rot of improved varieties has not been considered in any breeding programs to date. According to Marin et al. (1996), FHIA-01 and FHIA-02, two hybrids produced by the FHIA breeding program, are partially resistant to crown rot. Conversely, Perez-Vicente and Hernandez (2002) consider that these two varieties are more susceptible to crown rot caused by *F. semitectum* and *C. musae* compared with Cavendish varieties. According to these authors, only FHIA-23 is more resistant to crown rot than Grande-Naine. Finally, the first FHIA hybrids were introduced in the late 1980s, but their characteristics

differ from those of Cavendish bananas and consumers have shown little interest in them, thus limiting their distribution.

Postharvest Control Methods
Deflowering and Sanitization
Deflowering involves the removal of all floral parts that could potentially harbor inocula before the dehanding operation (see Fig. 3.2). When the floral parts have not been removed in the field, deflowering is done in the packing station before bunch trimming, thus reducing the risk of contamination of the washing baths (Shillingford, 1976; Krauss and Johanson, 2000).

Packing Station Water Quality
Contamination of crowns when the bananas are processed in the dehanding and washing tanks is a key step in crown rot development because the wounds resulting from the clustering of banana hands are the main portal for infection by crown rot pathogens. Spore accumulation in the water can be reduced by regularly changing the washing water in the washing and delatexing baths (Slabaugh and Grove, 1982). These baths can contain a high quantity of spores, mainly *Fusarium* spp. and *Verticillum* spp., that detach from the peel surface (Shillingford, 1977), whereas *C. musae* conidia are less often found (Shillingford, 1976). Care should be taken to avoid contaminating the baths with plant debris (pistils, leaves, trimming waste, etc.) and the water should be regularly refreshed.

It is also recommended that the bath water be treated with active chlorine at the rate of $1-2\,\text{mg L}^{-1}$ (Arneson, 1971; Slabaugh and Grove, 1982; Eckert and Ogawa, 1985) or quaternary ammonium disinfectants in order to hamper contamination (Shillingford, 1977), but the efficacy of this disinfection procedure is controversial (Slabaugh and Grove, 1982; Eckert and Ogawa, 1985). The chlorine concentration should be regularly adjusted to offset the high observed losses by volatilization or through redox reactions with latex or other organic matter in the washing baths (Shillingford, 1977).

Packing Station Sanitation
Protective measures implemented in the packing station are aimed at keeping the crowns of freshly trimmed bananas away from all inoculum sources. To ensure efficient crown rot control, it is thus essential to keep the packing station and the adjoining facilities clean. Waste (stalks, low-grade fruit, etc.) located in the vicinity of the banana packing area, which could contaminate the air in the station, must be eliminated (Finlay et al., 1992; Krauss and

Johanson, 2000). It has been shown that trimming clusters in a clean environment rather than in the field can reduce crown rot incidence by 50%.

Banana and Crown Trimming

The fruits should also be cleaned before being trimmed from the bunch. A contaminated trimming knife could spread inocula from the peel into the crown tissues (Lukezic et al., 1967). This may be avoided by washing the bunches in chlorinated water before they are trimmed into clusters (Krauss and Johanson, 2000). Bananas should be trimmed with a clean stainless steel blade. It was shown that roughly trimming the crowns, or ripping them off the hands, significantly increased the level of crown rot disease. Tissue fragments on the surface of the crowns dry out and quickly become senescent, thus providing an ideal site for rot development. Moreover, banana trimming knife tips are rounded to avoid banana fruit wounds. It is also important to cut wide crown sections containing as much crown tissue as possible, a technique that seems to enhance crown resistance to rot and seldom leads to the spread of rot into the fruit pedicels (Muirhead and Jones, 2000).

Packing

Although most pathogens require a high relative humidity for their *in vitro* development (Badger, 1965), bananas are less susceptible to crown rot under these conditions. A high relative humidity seems to hinder transpiratory water loss from the fruit, which is essential to ensure a long green life. Indeed, this ripening stage is markedly reduced under low relative humidity atmospheric conditions (30–40%) as a result of ethylene production from the fruit peel (Peacock, 1973). The senescence of banana crown tissues can then be hampered by maintaining their turgidity in plastic bag packaging (Muirhead and Jones, 2000).

Shipping

Bananas must be cooled as soon as possible to 13°C after packing and delivered promptly to a ripening facility (see Fig. 3.2). One way to slow down banana metabolism, and thus to delay crown rot development, is to refrigerate the fruit in boxes at the lowest possible temperature without inducing physiological disorders. Cooling should be continuous throughout the shipping phase which, in turn, should be as short as possible. Containers designed for maritime shipping of bananas are climate controlled at 13–14°C, because temperatures below 12°C provoke peel browning due to oxidization (Muirhead and Jones, 2000). Fungal growth is slowed down at 13°C (Finlay

and Brown, 1993) which is a good trade-off with respect to preserving the bananas and hampering fungal colonization of the crowns. However, this is much lower than the optimal temperatures for germination and growth of fungi responsible for crown rot, but it is not low enough to slow down the activity of these fungi, which can take place within a broad temperature range, i.e. 8–36°C (Simmonds and Mitchell, 1940). As a result, crown rot incidence and severity are higher in bananas exposed to temperatures over 16°C (Slabaugh and Grove, 1982).

Controlled and Modified Atmospheres

The composition of the atmosphere around bananas during shipping can be manipulated to slow down metabolic activity. Moreover, high CO_2 (>15%) and low O_2 (<1%) levels are toxic to many fungi (Goos and Tschirsch, 1962; Al Zaemey et al., 1994). Unfortunately, bananas cannot be stored under these conditions because major physiologic alterations generally occur during ripening when the atmospheric CO_2 level is above 7–12% or when the O_2 level is below 1–2% (Daun et al., 1973; Wilson, 1976; Thompson, 1998). Then, ideally, bananas should be stored in an atmosphere of 5–7 % CO_2 and 2% O_2 (Daun et al., 1973; Thompson, 1998). Modified atmospheres (MA) can be achieved by packing bananas in sealed plastic bags (polybags), and controlled atmospheres (CA) are obtained by injecting pure nitrogen into the storage rooms. It was shown that crown rot can be partially controlled (20%) by packing bananas in MA (1–2% O_2 and 12–14% CO_2) (Bastiaanse et al., 2010). For MA, the balance of O_2 and CO_2 contents depends on the extent of fruit respiration, bag permeability, and the composition of the air outside the bag (Thompson, 1998). The O_2 and CO_2 contents generally range from 1 to 10% and 2 to 14%, respectively, depending on the quality and thickness of the plastic packaging (Marriott and Palmer, 1980; Thompson, 1998). These plastic bags must also have a high mechanical strength because even small punctures will upset the MA (Chillet and de Lapeyre de Bellaire, 1996).

Hot Water Treatments (HWT)

To date, there has been no commercial adaptation of this technique to bananas grown for exportation (Cavendish), like in other tropical fruits such as papaya (Couey et al., 1984; Nishijima et al., 1992) and mango (Spalding and Reeder, 1978; Coates et al., 1993). However, some recent work has been done on local cultivars grown in Asian countries. Hot water treatment (20 min at 50°C) is ineffective in killing dormant spores but causes a delay

in mycelium growth and conidial germination on cv 'Bungulan'(AAA) grown in the Philippines (Alvindia, 2012). De Costa and Erabadupitiya (2005) have shown that the optimal temperature and exposure time for controlling crown rot on 'Embon' (AAA) and 'Kolikuttu' (AAB) varieties from Sri Lanka was 50°C for 3 min. Higher temperatures lead to pale fruit, and exposure times of over 5 min reduce Brix values (De Costa and Erabadupitiya, 2005) and caused unacceptable fruit browning on cv 'Kluai-Hom Thong' (AAA) from Thailand (Win et al., 2007). In Brazil, a 20 min longer treatment at a temperature under 45°C was effective for crown rot control in cv 'Santa CatarinaPrata' (AAB) and 'Williams' (Cavendish, AAA) with the percentage of infected bananas decreasing from 100% to less than 15% (Reyes et al., 1998). However, commercial tests on naturally infected fruit have not achieved crown rot control, and ripening delays have also been noted.

UV and Gamma Radiation Treatment

Ultraviolet light (UV) treatments have considerable potential for fruit and vegetable postharvest disease control. Stevens et al. (2005) discussed the possibility of inducing apple resistance to *C. gloeosporioides* through UV-C treatments. In bananas, however, the peel seems to be too sensitive to UV for the use of this technique for postharvest control of some pathogens of the complex of *C. musae* (Joas, 1997).

Kanapathipillai et al. (1987) pointed out that gamma ray treatment (38 min at 4 kGy) inhibited spore germination, the formation of *C. musae* appressoria, and all fungal development on the surface of fruit pieces. However, these authors did not investigate the effects of gamma rays on whole fruit, or on their complex components. Although dosages of around 0.5 kGy can extend banana green life (Marriott and Palmer, 1980; Wills et al., 1998), Marriott and Palmer (1980) noted that irradiation can alter the banana peel; the maximum dose tolerated by the fruits is likely around 0.5 kGy (Wills et al., 1998). Finally, the development of postharvest fruit irradiation has been hampered especially by the high cost of such treatments and their low consumer acceptance.

Biological Control

Currently, there is a continuation of a study on developing a biocontrol formulation to treat crown rot of banana and to replace the systematic fungicide treatment. The following examples are far from exhaustive. Postharvest biological control is very promising because the crown rot infection site

on the fruit is limited, the environmental conditions during storage are clearly defined and stable, and bananas have a high added value (Jijakli and Lepoivre, 1999). The results of many studies have suggested that the use of microorganisms such as fungi, bacteria, and yeasts could provide partial crown rot control (Postmaster et al., 1997; De Costa and Subasinghe, 1998; East and Kenyon, 1998; Krauss et al., 1998; Gunasinghe et al., 2004; De Costa and Erabadupitiya, 2005; Alvindia and Natsuaki, 2008; Lassois et al., 2008; Williamson et al., 2008; Sangeetha et al., 2010a). It has also been shown that biological control efficacy increases with the antagonistic agent concentration and with the incubation time between the application of the antagonist and crown contamination by the fungal complex (Postmaster et al., 1997; Lassois et al., 2008). Antagonists can be used to reduce significantly lesions induced by the fungal complex that causes crown rot, but the control efficacy is limited and variable (Lassois et al., 2008). This type of control, when used alone, cannot provide total crown rot control, and so should be combined with other control tactics such as inorganic salts and MA packaging to improve the effectiveness (Bastiaanse et al., 2010; De Costa and Gunawardhana, 2012; Alvindia, 2013).

Natural Compounds

The impact of several natural substances or non-synthetic fungicides, such as preparations of calcium, plant extracts or organic acids, on crown rot development has also been evaluated. Garlic (*Allium sativum*) extracts (Krauss and Johanson, 2000) and essential oils of cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*) (Ranasinghe et al., 2002, 2005), citronella (*Cymbopogon nardus*), and basil (*Ocimum basilicum*) (Anthony et al., 2004; Sangeetha et al., 2010b) have also been found to have fungicidal activity. Win et al. (2007) showed that cinnamon extracts ($5 \, g \, L^{-1}$) partially reduced crown rot severity (25%), increased green life, and had no negative effects on postharvest banana quality. These plant extracts have fungistatic and fungitoxic activity and can inhibit completely conidial germination and mycelial growth of *C. musae*, *Fusarium* spp., and *L. theobromae*. However, cases of phytotoxicity have been reported and the level of control provided by these natural substances is not sufficient to meet market requirements. The use of antioxidants (Khan et al., 2001) and organic salts (Alvindia et al., 2004), sometimes combined with surfactants (Alvindia and Natsuaki, 2007), can also enable partial crown rot control (up to 60%). Finally, experiments showed that some treatments were found to provide the same level of crown rot control as fungicide treatment (Alvindia, 2013; Sangeetha et al., 2013).

This is the case with Biocto 6® (seed extract from citrus) (Productos Bio-genicos S.A., San Jose, Costa Rica) combined with a wax-based additive (Verdiol, Productos Biogenicos S.A., San Jose, Costa Rica) (Demerutis et al., 2008). In the same way, Sangeetha et al. (2013) showed that the dipping of banana in zimmu leaf extract at 25% concentration exhibited 100% inhibition of crown rot disease in cold storage (14°C) up to 35 days and increased the shelf-life to 64 days without altering organoleptic properties. These seem to be a promising potential ecofriendly technology substituting fungicides in the control of the disease but further studies would be needed prior to recommending them in commercial packing houses, including validation of the results in real full-scale commercial exportation conditions, identification of the principle compounds responsible for the control and also the performance of safety tests.

CONCLUDING REMARKS

Crown rot studies and control are especially complex because of the observed diversity and variability in the composition and pathogenicity of the complex involved in the development of this disease. The broad range of possible situations complicates studies on both the parasitic and physiologic components of fruit quality. Further studies are thus required to gain better insight into this disease, especially because little documentation is available on certain aspects such as the epidemiology of crown rot. It is also very important to enhance the overall understanding of banana physiological mechanisms involved in the induction of fruit resistance to crown rot pathogens with the aim of improving control.

There is considerable growing interest in alternative methods to reduce or even completely eliminate fungicide treatments. To be efficient, these alternative control methods should not be too specific considering the broad spectrum of pathogens involved in the complex. Crown rot research should enhance the overall understanding of this disease and thus lead to the development of an effective integrated control strategy.

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