Production of Clean Planting Material for Managing Plantain Diseases

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This manual presents the results of a collaborative work with the Regional Fund for Agricultural Technology (FONTAGRO) and the Colombian Federation of Plantain Producers (FEDEPLÁTANO), with support from the Food and Agriculture Organization of the United Nations (FAO) and Agricultural Innovation Marketplace.

Contents

Importance of plantains and bananas

Plantain (*Musa balbisiana* ABB) and banana (*M. acuminata* AAB) are economically valuable crops that also contribute significantly to the food security of many countries throughout the tropical world. These crops are also important sources of employment and income in these countries.

World production of plantain reached about 38.9 million tons in 2011. Uganda, Ghana, Cameroon, Rwanda, Colombia, and Nigeria are the main producers. Banana production reached 106.5 million tons, with the main producers being India, China, the Philippines, Ecuador, and Indonesia (FAOSTAT, 2011).

Latin America and the Caribbean supplied 63.7% of the international plantain trade in 2010, principally consisting of production from Ecuador (33.2%), Colombia (10.2%), Costa Rica (10%), and Guatemala (8.6%). The world's five leading export countries (Ecuador, Colombia, the Philippines, Costa Rica, and Guatemala) supplied 73.6% of the world trade, with the remaining exporting countries contributing 26.4% (Fundación Produce de Guerrero, A.C., 2012).

The plantain varieties that are most widely cultivated do not produce seeds, their fruits being botanically known as parthenocarpic (*lit*. virgin fruit). Consequently, the plants' corms or suckers[1] are used as planting material (Canchignia and Ramos, 2004).

1. Corms or suckers are typical vegetative propagation units of the Musaceae (plantains and bananas). Their structure is differentiated into roots, bulked-up base, and pseudostem. They connect with the mother plant through the vascular system.

Major plantain and banana diseases

The diseases affecting bananas and plantains represent significant problems throughout the world. These diseases, caused by fungi, bacteria, and viruses, lead to the deterioration of all plant parts. Fungal diseases include black sigatoka (or black leaf streak disease), caused by *Mycosphaerella fijiensis*; and Panama disease (or fusarium wilt, caused by *Fusarium oxysporum f*. sp. *cubense* Foc race 1 and, recently, Foc race 4). The most important bacterial diseases are Moko disease (a vascular wilt caused by *Ralstonia solanacearum* race 2); pseudostem rot caused by *Dickeya (Erwinia) chrysanthemi*; and bacterial wilt caused by *Xanthomonas campestris* pv. *musacearum*. Viral diseases are distributed throughout most areas producing plantain and banana. They include banana streak (BSV) and banana mosaic (caused by cucumber mosaic virus [CMV]) (Ploetz, 2004).

Diseases transmitted by planting material

Moko disease, bacterial wilt, vascular wilt, maduraviche, or ereke

Causal agent of the disease:
Ralstonia solanacearum race 2

Caused by the bacterium *Ralstonia solanacearum*, this significant disease affects plantain, banana, and other crops across broad geographical areas. The extensive genetic diversity of the bacterial strains causing this wilt led to the concept of a *R. solanacearum* species complex (Genin and Denny, 2012), whereby strains are grouped into races and biovars (Denny, 2006). Races are based on the range of hosts that they infect (Álvarez, 2005); in this case, race 2 affects plantain and banana.

Symptoms. The disease is primarily a wilt of vascular origin, from which the infected plants eventually die (Figure1).

The bacterium moves throughout the plant's vascular bundles, blocking the passage of water, nutrients, and photo-assimilates (Gómez et al., 2005) (Figure 2). The blockage produces symptoms of chlorosis and wilt in the leaves, which then droop, rot, and fall off. Offshoots or suckers of infected plants may also show similar symptoms but, often, they remain asymptomatic, allowing the phytopathogen to disseminate through planting material.

Figure 1. Plantain plant with symptoms of Moko disease.

Figure 2. Vascular bundles in the plantain rachis are blocked by the pathogen causing Moko disease.

Dissemination. The bacterium *R. solanacearum* naturally inhabits soil. However, successive cropping of plantain varieties highly susceptible to Moko disease such as Dominico Hartón, the use of infected planting material, and a poverty of beneficial soil microorganisms all favor the phytopathogen's spread. Dissemination is further accelerated by increased contamination of both soil and runoff in crops (Figure 3).

Figure 3. Runoff from prolonged rains in a dissemination focus of Moko disease.

Other means of dissemination include infested surfaces of tools, equipment, and working clothes; and vector insects and animals (Moorman, 2013). This situation is currently reflected in the extensiveness of devastated cropping areas.

Management. The best strategy is preventive management, that is, the use of certified seed and planting in exclusion zones where the disease is not present.

In affected areas, to reduce risks of propagating this disease, management includes eradicating infected plants and constantly disinfecting all tools and machinery used in cultivation (Eyres et al., 2001).

Healthy racemes (i.e., bunches of fruit) can be protected by covering them with translucent plastic bags to help prevent dissemination by aerial vectors. Rotating with crops that do not host the bacterium such as cassava, maize, or beans will also help (Rodríguez and Avelares, 2012).

Bacterial soft rot or plantain corm necrosis

Causal agent of the disease:
Dickeya (Erwinia) chrysanthemi

Recent taxonomic revisions have reclassified the *Erwinia* genus as *Dickeya* (Genome Evolution Laboratory, 2007). This bacterium belongs to the Enterobacteriaceae family, most of which are phytopathogens that characteristically cause soft rots. That is, tissues are degraded or macerated through enzymes such as pectinases that break down plant cells, causing the exposed plant parts to release nutrients that then facilitate bacterial growth (Van Vaerenbergh et al., 2012).

Dickeya sp. can survive on those decomposing pseudopetioles that remain adhered to the pseudostem after leaf removal (Martínez-Garnica, 1998).

Incidence of this disease has increased recently, mostly in plantain, with crop losses of 30% to 100%. The presence of the disease is usually associated with bad drainage, poor phytosanitary conditions of the crop, high rainfall, and inadequate irrigation practices (Dita et al., 2012).

The crops that are most susceptible to the disease are those that have imbalanced nutrition, especially of potassium and boron (Belalcázar, 1991; Palencia et al., 2006). Such imbalances accentuate symptom severity.

Symptoms. The disease is characterized by chlorosis of the lower leaves, which then droop. This is followed by a general wilting of the plant as the disease progresses upwards, totally affecting all leaves (Gómez-Caicedo et al., 2001).

Aqueous yellow lesions also appear inside the pseudostem. These lesions eventually become dark brown and give off a fetid smell (Figure 4).

Dispersal. The bacterium colonizes xylem vessels, through which it can systemically infect the plant, causing it to wilt overall. This systemic condition is a cause for alarm in the vegetative propagation of plantain (and other crops), as the pathogen can remain latent within planting material and later propagate itself throughout the crop. The bacterium can also survive in crop residues, thus creating a risk of latent infection for the next crop (EPPO/CABI, 2011).

The bacterium can be transmitted on tools used to remove leaves and other dried aerial parts,[2] as well as by insects such as the silky cane banana weevils (*Metamasius* spp.), and by contaminated planting material.

2. Known in Spanish as *desgüasque*, trimming involves the removal of dried or decomposing sheaths of the pseudostem. If such material remains on the plant, the crop becomes potentially predisposed to serious phytosanitary problems such as infestations by pests, including the silky cane weevil, banana root borer, and screwworm fly larvae, as well as bacterial soft rot (Morales 2010).

Figure 4. Soft rot in sheaths of a plantain pseudostem.

Management. Constant disinfection of tools during routine work on the crop will reduce the spread of this plant pathogen. Controlling weevils and correctly applying fertilizers will also help in the timely management of the disease.

The frequent use of sodium hypochlorite, in its commercial presentation (at 5.25%) and dissolved in water at 1:1, is suitable for disinfecting tools (Gómez-Caicedo et al. 2001).

Mass multiplication of clean planting material

This handbook proposes a system of producing healthy plantain planting material (seed). This system combines two processes: *in vitro* propagation (Figure 5), and low-cost thermotherapy, using a thermal chamber (Figure 6).

Figure 5. *In vitro* plantain seed.

Figure 6. A CIAT prototype of a thermal chamber.

Thermal chamber. Corms and their induced buds are placed in this chamber and subjected to a cleaning system involving thermotherapy. Temperatures are set between 50° and 70°C, relative humidity from 30% to 100%, and the photoperiod may be as long as 24 hours (complemented with artificial light at night). These three parameters, together with frequency of fertigation (i.e., irrigation with a nutritional solution) are automated by means of a programmable logic controller (PLC) (Figure 7) and Software Alpha Programming Mitsubishi® 2001) (FONTAGRO, 2010).

Figure 7. Programmable logic controller (PLC).

Planting material. Corms (sword-type suckers), weighing between 1 and 2 kg, are used as seed. The corms are produced in plots set aside for elite plants obtained from seed developed *in vitro*. The corms are first disinfected in a solution of insecticide + fungicide, and then subjected to the technique of accelerated reproduction of seed or planting material (TRAS)[3] (Aguilar et al., 2004). This technique inhibits the growth of apical buds or meristems and induces lateral buds to sprout (Figure 8).

3. TRAS is the acronym for *Técnica de reproducción acelerada de semilla*, that is, accelerated seed reproduction technique. Buds are not separated from the corms; instead, whole corms are planted in small seedbeds previously prepared to facilitate the sprouting of axillary buds. The apical bud is extracted to a depth of 1 cm below the crown (which joins the corm's pseudostem). This prevents apical dominance and induces axillary buds to sprout (Aguilar et al., 2004).

Figure 8. Cuts made in a plantain corm to inhibit apical dominance.

The high temperatures inside the thermal chamber shorten the time vegetative buds take to sprout and develop. Within a relatively short time (18 days), larger numbers of buds sprout at a higher emergence rate under the higher temperatures of a thermal chamber than would have sprouted under outdoor conditions (29 days), using the same technique (Figure 9).

Figure 9. High shoot production from one corm, using the TRAS technique in a thermal chamber.

For plantain variety Dominico Hartón (*Musa* AAB), the best yield of planting material is obtained by applying the TRAS technique to corms of 1 to 2 kg placed in a thermal chamber and using previously sterilized sawdust as the planting substrate. Studies show that production in a thermal chamber can be as high as 90 shoots/m^2 per month, compared with 35 shoots/m^2 per month under outdoor conditions (Figure 10).

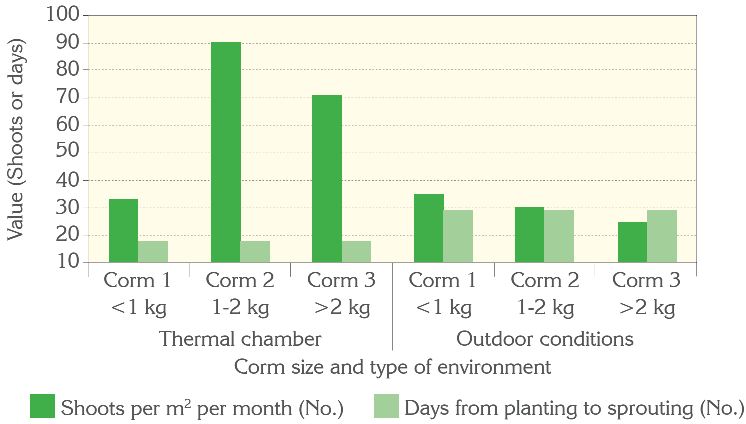

Figure 10. Effect of environment (i.e., thermal chamber or outdoor conditions) and corm size on the mass propagation of plantain variety Dominico Hartón.

Rooting and growth in the greenhouse

When shoots in the thermal chamber are 18 days old (Figure 11), they are extracted from the substrate and washed in a solution of 1% sodium hypochlorite. They are then immediately planted in black plastic bags containing sterilized substrate rich in organic matter such as rice husks + white-wood sawdust + fertile soil at a rate of 2:1:1.

Figure 11. Plantain shoot ready for transplanting to a seedbed.

Seedbed. Space should be made for establishing a seedbed or nursery (Figure 12), where the bags containing shoots will be placed. The area must be under natural shade (trees) or artificial shade constructed from saran with a 30% to 50% mesh.

Figure 12. Seedbed with healthy plantain shoots.

Beginning in the second week after transplanting, the plants, still in their bags, are given fertilizers weekly. The fertilizer used is a mixture of urea[4] (24.5%), MAP or DAP (37.7%), and KCl (37.7%) (Lardizábal and Gutiérrez, 2006; Coto, 2009). After 90 days, the seed will be ready for transplanting to the field (Figure 13).

Figure 13. Optimal plantain seed for transplanting to the field.

During this stage of growth and development, beneficial microorganisms should be applied, for example, bacteria that promote root growth (e.g., plant-growth-promoting rhizobacteria or PGPR), and the fungi *Trichoderma viride, T. harzianum*, and arbuscular mycorrhiza, which favor development of the root system and plant growth before transplantation to the field.

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4. Urea is a nitrogen-rich fertilizer (60% N); MAP or DAP is monoammonium or diammonium phosphate, rich in assimilable phosphorus (50% and 48% of P_2O_5, respectively); and KCl is potassium chloride (60% of K_2O).

Comparing propagation in the thermal chamber with the conventional method

Thermal chamber	Conventional method
• Automated and technified production system. • Seed size and weight are uniform, facilitating linkage with seed certification programs, and reducing transportation costs and seed price. • When elite material from *in vitro* propagation is used, phytopathogenic microorganisms and pests are excluded. • The first cropping cycle can be reduced by up to 2 months, depending on temperature ranges. • Planting material is consistently available all year round. • The root system develops and is protected by beneficial microorganisms. • From one corm, as many as 15 shoots can be produced and used as planting material.	• Production system is neither automated nor technified. • Seed size and weight are variable, complicating the certification of planting material, and increasing transportation costs and seed price. • Greater probability of spreading phytopathogenic microorganisms and pests. • Cropping cycle is normal, depending on temperature ranges. • Availability of corms may be limited. • The corms used do not have root systems. • Larger volumes of seed are required. • Conventional propagation of Musaceae (plantain and banana) allows separation of corms from their mother plants through desuckering (Martínez et al., 2004).

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