Determination of banana fruit susceptibility to post-harvest diseases: wound anthracnose, quiescent anthracnose and crown rot

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Abstract — Introduction. This protocol aims at (a) evaluating the resistance to post-harvest diseases within different genotypes of bananas, and (b) comparing different origins of bananas (geographic origin, physiological stage, etc.) for their susceptibility to post-harvest diseases. The principle, key advantages, starting plant material, time required and expected results are presented. **Materials and methods**. Materials required and details of the twelve steps of the protocol (fruit sampling and inoculum preparation, wound anthracnose resistance study, quiescent anthracnose resistance study and crown-rot resistance study) are described. **Results**. Typical symptoms of the different diseases are obtained after artificial inoculation.

France (Guadeloupe) / *Musa* sp. / *Colletotrichum musae* / disease resistance/ methods

Détermination de la sensibilité des bananes aux maladies de conservation : anthracnose de blessure, anthracnose quiescente, pourriture de la couronne.

Résumé — Introduction. Ce protocole vise (a) à évaluer la résistance aux maladies de conservation pour différents génotypes de bananiers, (b) à comparer différentes origines de bananes (origine géographique, stade physiologique, etc.) vis-à-vis de leur sensibilité aux maladies de conservation. Le principe, les principaux avantages, le matériel végétal de départ, le temps nécessaire et les résultats attendus de la méthode sont présentés. **Matériel et méthodes**. Le matériel nécessaire et le détail des douze étapes de réalisation du protocole (prélèvement de fruits et préparation d'inoculum, étude de résistance à l'anthracnose de blessure, étude de la résistance à l'anthracnose quiescente et étude de résistance à la pourriture de couronne) sont décrits. **Résultats**. Des symptômes types des différentes maladies sont obtenus après inoculation artificielle.

France (Guadeloupe) / *Musa* sp. / *Colletotrichum musae* / résistance aux maladies / méthode

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1. Introduction

Application

This protocol aims at:

– evaluating the resistance to post-harvest diseases within different genotypes of bananas,
– comparing different origins of bananas (geographic origin, physiological stage, etc.) for their susceptibility to post-harvest diseases.

Principle

The principle of the method is to simulate the conditions of natural infections. The evaluation of the resistance is based on artificial inoculation with the pathogen involved in the various post-harvest diseases.

For wound anthracnose and quiescent anthracnose, that should be considered as two different post-harvest diseases, the pathogen is *Colletotrichum musae* [1]. For crown rot, the most common pathogens

implied are *Colletotrichum musae*, *Fusarium moniliforme*, *Fusarium pallidoroseum*, *Botryodiplodia theobromae* and *Cephalosporium* spp. [2–5]. However, *Colletotrichum musae* is the most pathogenic species and will be used for these studies.

Key advantages

While this method is time-consuming and more fastidious than the observation of natural infections, its key advantage lies in its accuracy and reliability. Under natural contamination, the development of post-harvest diseases is too erratic.

Starting material

The method requires mature, freshly harvested bananas and fungal cultures of the different pathogens involved in the post-harvest diseases.

Time estimation

For wound anthracnose, the time required is 15 min for fruit sampling; 20 min for fruit inoculation; 15 min for fruit wounding; 10 min for disease assessment.

For quiescent anthracnose: 15 min for fruit sampling; 20 min for fruit inoculation; 10 min for disease assessment.

For crown rot: 15 min for fruit sampling; 20 min for fruit inoculation; 15 min for disease assessment.

Expected results

We obtain (a) for wound anthracnose, the surface of lesion; (b) for quiescent anthracnose, the surface of lesion; (c) for crown rot, the internal surface of rot (ICR).

2. Materials and methods

Laboratory materials

The protocol requires:

– agar plates with Mathur's medium ($MgSO_4$ $7H_2O$ 2.5 g; peptone 1 g; yeast extract 1 g; saccharine 10 g; agar 15 g; water 1 L),

– a Malassez counting cell,

– sterile distilled water,

– a microscope,

– 50% alcohol,

– a computerized penetrometer with a rounded probe (1-cm diameter),

– a controlled environment cabinet regulated at 13 °C,

– a controlled environment cabinet regulated at 20 °C,

– a controlled environment cabinet regulated at 25 °C.

Protocol

Fruit sampling and inoculum preparation

• Step 1. Fruit sampling

– In order to minimize the effect of natural infections that might occur in the field, floral remnants should be removed early (when fruits are in a horizontal position), and bunches should be covered with a plastic sleeve just after [1].

– In order to minimize within-bunch variability, use bananas of the third hand (eventually also those of the second hand).

– Harvest bananas at the same physiological age corresponding to 75% of the thermal sum that this genotype would reach at the "first yellow-fruit stage". For Cavendish bananas, 900 °C-days is the thermal sum recommended. This thermal sum will differ according to the different genotypes of bananas studied.

– The optimum situation is to harvest 20 bunches per cultivar or treatment, each bunch being then considered as a replicate. When it is not possible to harvest 20 bunches at the same time (various cultivars), a minimum of 10 bunches should be harvested on different dates and compared in each experiment with the cultivar Grande Naine as reference.

• Step 2. Inoculum preparation

– Inoculate Mathur's medium plates with a small plug from a fungal colony of *Colletotricum musae*. Fungal cultures should be monosporic and should not be sub-cultured more than 5 times.

Note: initiate new cultures from frozen conidial suspensions conserved at – 80 °C in 30% glycerin.
– Store fungal cultures at 25 °C for 10 d.
– After 10 d of incubation, flow the fungal cultures with distilled sterile water.
– Filtrate the conidial suspension through a 35-µm sieve.
– Calibrate the conidial suspension to (10^6 and 10^4) conidia·mL^{-1} using the Malassez counting cell.

Wound anthracnose resistance study

• Step 3
Sample a fruit on the external row of the third hand of each bunch (ideally 20 per treatment or cultivar).

• Step 4
On one of the side faces of the fruit, deposit 25 µL of the *Colletotricum musae* conidial suspension calibrated to 10^6 conidia·mL^{-1} (locate inoculation area with a felt pen). Once the droplet is dry, cover the inoculated area with a humidified swab. Wrap with plastic to maintain humidity. Store the fruits at 25 °C for 48 h.

• Step 5
Bruise the fruit at the place where inoculum was deposited. Crushing is done with a computerized penetrometer equipped with a rounded probe. The speed of the probe is 5 mm·s^{-1} and a 5-mm compression is exerted on the fruit for 4 s. Store the fruits at 20 °C.

• Step 6
Ten days after inoculation, start measuring the surface of the lesions, assuming that the lesion is elliptical [length × width × (Π/4)], and repeat it every 2–3 d until fruits are ripe. For Cavendish bananas, another possibility is to store the fruits for 10 d at 13 °C, 10 d at 20 °C, and then measure the surface of the lesions.

Quiescent anthracnose resistance study

• Step 7
Sample as described in step 3 and inoculate the *Colletotricum musae* conidial suspension as described in step 4.

• Step 8
Store the fruits at 20 °C.

• Step 9
Ten days after inoculation, start measuring anthracnose lesions as in step 6.

Crown-rot resistance study

• Step 10
Sample a cluster of four fruits on the third hand of each bunch (ideally 20 per treatment or cultivar).

• Step 11
For each cluster, remove a thin slice of crown on all sections. Wait 30 min for latex flow and sterilize the crown by dipping in 50% ethanol. Wait at least 30 min for the alcohol to dry and deposit 50 µL of the conidial suspension, calibrated to 10^4 conidia·mL^{-1}, on the upper face of the crown. Cover this droplet with a 1-cm^2 paper filter and place the fruits at 25 °C for 3 h before storage at 13 °C.

• Step 12
Thirteen days after storage at 13 °C, split the cluster crown into two parts, and measure the internal crown rot surface (ICR).

Troubleshooting

• Step 13
Very few failures should happen; nevertheless, if the fungal cultures are sub-cultured for a long period (> 5 subcultures), the strains might lose their pathogenicity: regularly initiate new fungal cultures from frozen conidial suspensions.

3. Typical results obtained

Typical symptoms of wound anthracnose (*figure 1*) and crown rot (*figure 2*) are obtained after artificial inoculation.

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Figure 1.
Wound anthracnose assessed on Cavendish bananas. Results on bananas stored for 10 d at 13 °C and showing young brown lesions starting from the inoculated area.

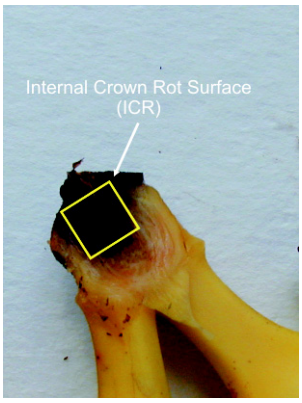

Figure 2.
Crown rot of bananas assessed 13 d after storage.

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