Method for early quantification of quiescent infections of *Colletotrichum musae* on bananas

Luc de Lapeyre de Bellaire[1]*, Marc Chillet[2], Yolande Chilin-Charles[3]

[1] CIRAD, Persyst, UPR Systèmes, bananes et ananas, CARBAP, BP 832, Douala, Cameroon
luc.de_lapeyre@cirad.fr,

[2] CIRAD, Persyst, UMR Qualisud, Faculdade de Farmacia, Universidade de São Paulo, avenida Lineu Prestes, 580, Bloco 14 05508-900 São Paulo, SP, Brasil
marc.chillet@cirad.fr

[3] CIRAD, BIOS, UPR 75, Station de Neufchâteau, 97130 Capesterre belle eau, Guadeloupe, France
yolande.chilin-charles@cirad.fr

Method for early quantification of quiescent infections of *Colletotrichum musae* on bananas.

Abstract — Introduction. This protocol aims at detecting and quantifying quiescent infections of *Colletotrichum musae* on bananas. The principle, key advantages, starting plant material, time required and expected results are presented. **Materials and methods**. The materials required and details of the three steps of the protocol (fruit sampling, fruit ripening and anthracnose lesion quantification) are described. Possible troubleshooting is discussed. **Results**. The protocol results in the quantification of anthracnose lesions on the fruits, which makes it possible to predict postharvest losses due to anthracnose (peel rot), and also to propose a better management of postharvest fungicide applications.

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

Méthode pour une quantification précoce des infections quiescentes de *Colletotrichum musae* sur bananes.

Résumé — Introduction. Ce protocole vise à détecter et à mesurer les infections quiescentes de *Colletotrichum musae* sur les bananes. 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 trois étapes de réalisation du protocole (échantillonnage, maturation du fruit et quantification du nombre de lésions dues à l'anthracnose) sont décrits. Des problèmes potentiels sont évoqués. **Résultats**. Le protocole conduit à la quantification des lésions d'anthracnose à la surface des fruits; cela permet d'estimer les risques potentiels de dégâts après-récolte dus à l'anthracnose (chancre) et de proposer une meilleure gestion des applications de fongicide après la récolte.

France (Guadeloupe) / *Musa* sp. / *Colletotrichum musae* / contrôle de maladies / méthode / lutte après récolte

* *Correspondence and reprints*

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1. Introduction

Application

This protocol aims at detecting and quantifying quiescent infections of *Colletotrichum musae* on bananas. This information is essential for the prediction of postharvest losses due to anthracnose (peel rot) and also for a better management of postharvest fungicide applications.

Principle

Independently of the fruit age, the quantification of appressoria on the fruit surface is based on the breaking of appressorial dormancy through a fast ripening of fruit at 32 °C under a continuous contact with a high ethylene concentration [1]. In other words, since most contaminations occur during the 40 days following bunch emergence [2], the level of fruit contamination at harvest is foreseeable several weeks before the harvest.

Key advantages

– There is no development of senescent spots that can be confused with anthracnose lesions, since the high temperature favours fungal development and hastens fruit ripening without the development of senescent spots (fruits remain soft and green, which is called green ripe).

– This method can be applied to most genotypes of bananas, no matter the fruit origin, or fruit age (from 4 weeks after flowering until harvest).

– This method is well adapted for routine analysis: it is easy to carry out and does not require sophisticated equipment.

Starting material

The protocol is applied to young banana fruit.

Time required

The protocol needs 30 min for fruit sampling, 5 d for fruit ripening and 30 min for assessment of the number of anthracnose lesions.

Expected results

The method allows the assessment of the average number of anthracnose lesions per fruit.

Figure 1.
A banana bunch at the "horizontal finger stage". Age is indicated by a colour belt hanging on the bunch.

2. Materials and methods

Laboratory materials

The protocol requires:

– a ripening room regulated at 32 °C or an airtight ripening tank stored in a controlled temperature chamber regulated at 32 °C,
– ethylene, *e.g.*, a bottle of azethyl gas (95% nitrogen + 5% ethylene).

Protocol

• Step 1: Fruit sampling
– Sample 20 bananas on 20 banana trees of the same plot.
Note: harvest bananas at the same physiological stage. It is advised to harvest 3 weeks before the forecasted harvest date. The date of flowering is indicated by tying a colour belt on the bunch at the horizontal finger stage (*figure 1*).
– Collect the fruit on the third hand, on the centre of the outer row.
– Stock fruits in a plastic box.

• Step 2: Fruit ripening
– Introduce the plastic box into a ripening room regulated at 32 °C or an airtight ripening tank stored in a controlled temperature chamber regulated at 32 °C.
– Inject ethylene gas into the ripening room at a concentration of 1000 $\mu L \cdot L^{-1}$.
– Store the fruits for 5 d in the ripening room.

Note: Take care not to open the room or the tank to maintain ethylene concentration above 1000 $\mu L \cdot L^{-1}$. Humidity in the room or tank should not be inferior to 90%. During fruit conservation, CO_2 concentration should not exceed 0.5%: take care to introduce no more than 20 kg bananas$\cdot m^{-3}$.

• Step 3: Anthracnose lesion quantification
Five days after the ethylene treatment, count all anthracnose lesions on the fruits. Calculate the average number of lesions per fruit (NLF) and the percentage of necrotic fruit (PNF).

Note: it is difficult to consider a general threshold value of NLF in order to manage postharvest applications, since anthracnose (peel rot) development in commercial conditions will depend not only on fruit contamination, but also on fruit susceptibility, packing practices and logistics. Nevertheless, it can be considered that, when NLF is high (above 5 to 10 lesions per fruit), the risk of anthracnose at the commercial level is more significant.

Troubleshooting

Two main problems can occur:
(a) Bananas remain hard and do not ripen, no anthracnose lesions are observed, which can result from two reasons:
– The ethylene treatment has been disrupted during the 5 days of conservation.
Solution: take care with the airtightness of the ripening room or tank.

– CO_2 concentration is too high and prevents fruit ripening.

Solution: do not exceed 20 kg bananas·m^{-3} in the ripening room or tank; introduce KOH into the ripening room or tank to absorb CO_2.

(b) All bananas are constantly and abnormally heavily spotted, which means that new contamination did occur in the ripening room.

Solution: clean the ripening room with chlorinated water between tests.

3. Typical results obtained

Typical lesions are observed on fruits sampled (*figure 2*).

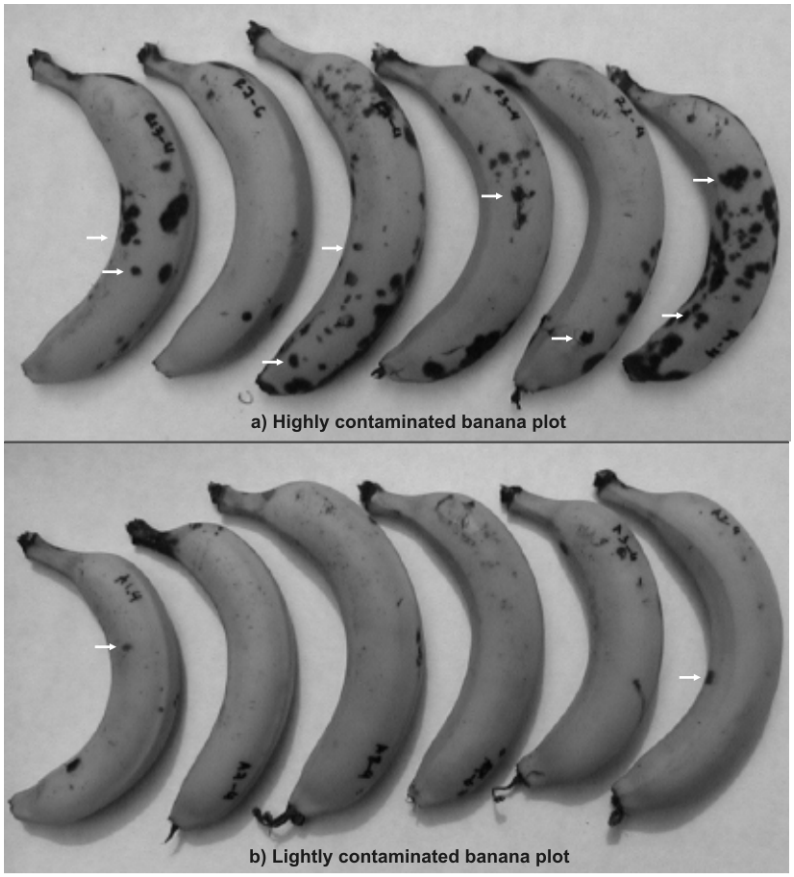

Figure 2.
Anthracnose lesions (arrows) observed on bananas and contamination levels observed in:
– heavily spotted fruits (a): in this case, 100% of fruits were spotted and an average of 21 lesions per fruit was counted; a postharvest fungicide application is then recommended;
– lightly spotted fruits (b): in this case, 33% of fruits were spotted and an average of 0.5 lesions per fruit was counted; a postharvest fungicide application is not recommended.

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