# An early quantification method of quiescent infections of *Colletotrichum musae* on bananas using ethylene treatment

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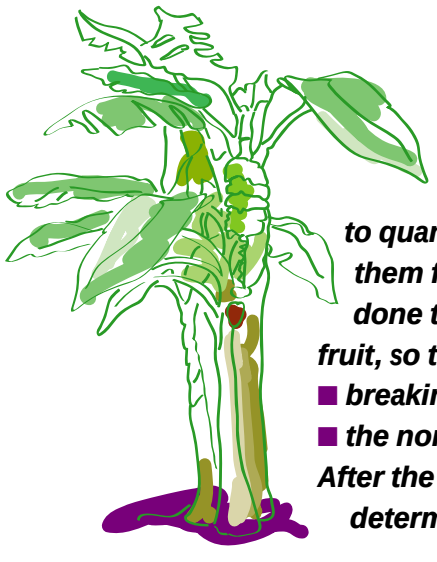

***Anthracnose is the main post-harvest disease of bananas grown in the French West Indies. Colletotrichum musae, the causal agent, forms quiescent infections on the fruit peel that lead to necrosis at maturity. The existence of quiescent infections makes it impossible to assess the inoculum level on the fruit peel before the ripening of the banana.The development of a technique to quantify the inoculum level on the fruit before harvest would be very interesting for banana growers; it can lead them for certain technical decision such as whether or not to apply fungicides after harvest. This work has been done to determine a practical method, usable in routine, to quantify the inoculum level on mature and immature fruit, so that it can be used as a method of early prediction. Two points have to be considered:***

- ***breaking the dormancy of the appressoria,***
- ***the non apparition of senescent spots.***

***After the elaboration of the method, we have considered the role of the ethylene treatment as the primary determinant of the breaking of the dormancy of the appressoria.***

## Material and methods

### Plant material and fruit sampling

All fruit belonged to the cultivar Grande Naine (Cavendish subgroup). Two stages of maturity were sampled: 5-6 weeks after flower emergence (immature fruit) and commercial harvest stage (mature fruit).

### Inoculation of fruit

Two method of inoculation were used:

- deposit of 20 10-µl droplets of a conidial suspension of *C. musae* ($10^3$ per ml) were applied on one side of the fruit,
- fruit dipped in a conidial suspension (5 x $10^2$ per ml) containing 0.25‰ Triton X-100.

After inoculation, fruit were placed at 25°C, 100% HR to optimise appressoria formation.

### Influence of storage temperature on lesion development

After the ethylene treatment (1 200 ppm during 24 h at 25°C), inoculated fruit were placed at 25, 29, 32 and 35°C.

### Influence of different storage condition on lesion development

After the ethylene treatment (1 200 ppm during 24 h at 25°C), inoculated fruit were placed in different storage condition: **1.** Air-32°C; **2.** Sealed plastic bag-25°C; **3.** Sealed plastic bag-32°C; **4.** 2% $O_2$, 15% $CO_2$ - 32°C; **5**. Ethylene 1 200 ppm-32°C.

### Role of ethylene in the development of *Colletotrichum*

Before inoculation, fruit were treated with 1-MCP to block fruit maturation (24 h, 0,5 µl.l$^{-1}$). After inoculation, fruit were treated with 1 000 ppm ethylene and stored at 25°C.

## Results

### Influence of storage temperature

Figure 1 and photo 1 show that at 29 and 32°C, the development of necroses was very good and similar. However, at 32°C, the senescent spot did not develop and the skin remained green.

### Influence of different storage condition

Figure 2 shows that the only method of storage that resulted in constant development of lesion was in which the fruit remained at 32°C in the maturation chamber for 6 days after the ethylene treatment.

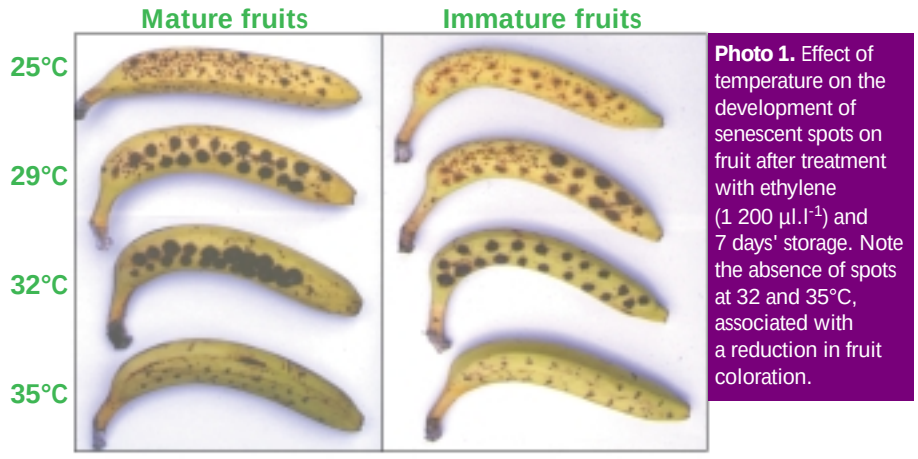


**Photo 1.** Effect of temperature on the development of senescent spots on fruit after treatment with ethylene (1 200 µl.l$^{-1}$) and 7 days' storage. Note the absence of spots at 32 and 35°C, associated with a reduction in fruit coloration.

**Table 1.** Effect of the 1-MCP treatment on the lesion development. Fruits were treated or not with 1-MCP, and then inoculated with a spore suspension and treated with 1 000 ppm ethylene.

| Treatment | Surface of necrosis (mm$^2$) | Homogene group (Newman-Keuls test) |
|---|---|---|
| Bananas without 1-MCP | 175 | A |
| Bananas with 1-MCP | 0 | B |

### Role of ethylene in the development of *Colletotrichum*

Table 1 shows that there are no lesion development when the fruit is pre-treated with 1-MCP. Ethylene seems not to be the primary determinant of breaking the dormancy of appressoria of *Colletotrichum*, but ripening of the fruit is an essential primary stage.

## Discussion

This study describes a method to reveal quiescent infections of *C. musae* on bananas based on treating the fruit for 6 days with high level of ethylene and storing at 32°C. At this temperature, the fruit remained green and there is no development of senescent spots. This temperature is optimal for fungal growth of *C. musae;* it is why there is higher disease expression. The duration of exposure to ethylene (6 days) is greater for the revelation of the inoculum on immature fruits. Ethylene has not a direct action on lesion development, but is essential for initiation of fruit ripening. This method should be used by bananas growers for predicting the levels of contamination by *C. musae*. If the level is very low, they can decide to export fruit without postharvest fungicide treatment.

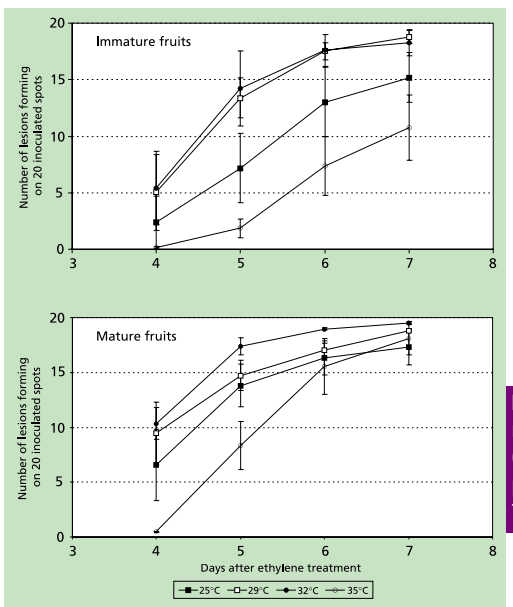


**Figure 1.** Effect of temperature on the development of necroses on immature (top) and mature (bottom) fruits inoculated by deposition of a suspension of spores calibrated at $10^3$ conidia.ml$^{-1}$. The bars represent the standard error.

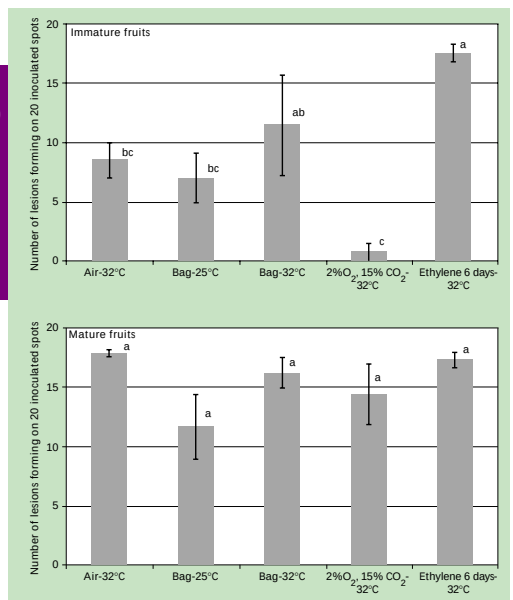


**Figure 2.** Effect of different storage methods on the development of necroses on immature (top) and mature (bottom) fruits inoculated by deposition of a spore suspension calibrated at 5 x $10^2$ conidia.ml$^{-1}$. The bars represent the standard error and treatments marked with the same letter do not differ according to the Newman-Keuls test at the 5% probability level.

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