Hand position on the bunch and source–sink ratio influence the banana fruit susceptibility to crown rot disease

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Abstract

The postharvest development of crown rot of bananas depends notably on the fruit susceptibility to this disease at harvest. It has been shown that fruit susceptibility to crown rot is variable and it was suggested that this depends on environmental preharvest factors. However, little is known about the preharvest factors influencing this susceptibility. The aim of this work was to evaluate the extent to which fruit filling characteristics during growth and the fruit development stage influence the banana susceptibility to crown rot. This involved evaluating the influence of (a) the fruit position at different levels of the banana bunch (hands) and (b) changing the source–sink ratio (So–Si ratio), on the fruit susceptibility to crown rot. The fruit susceptibility was determined by measuring the internal necrotic surface (INS) after artificial inoculation of Colletotrichum musae. A linear correlation \( r = -0.95 \) was found between the hand position on the bunch and the INS. The So–Si ratio was found to influence the pomological characteristics of the fruits and their susceptibility to crown rot. Fruits of bunches from which six hands were removed (two hands remaining on the bunch) proved to be significantly less susceptible (INS = 138.3 mm²) than those from bunches with eight hands (INS = 237.9 mm²). The banana susceptibility to crown rot is thus likely to be influenced by the fruit development stage and filling characteristics. The present results highlight the importance of standardising hand sampling on a bunch when testing fruit susceptibility to crown rot. They also show that hand removal in the field has advantages in the context of integrated pest management, making it possible to reduce fruit susceptibility to crown rot while increasing fruit size.

Introduction

Crown rot disease affects export bananas in all producing countries and is viewed as one of the main postharvest diseases of bananas. The disease develops during shipping, ripening and storage and has a negative impact on the market value of bananas (Slabaugh & Grove, 1982). It results from the development of several relatively nonspecific pathogens, but many authors agree on the high pathogenicity of Colletotrichum musae, which can trigger an infection from a very small inoculum (Finlay & Brown, 1993; Lassois et al., 2008).

Geographic and seasonal variations have been noted in the incidence of banana postharvest diseases (Lukezic et al., 1967; Shillingford, 1978; Chillet & de Lapeyre de Bellaire, 1996; Krauss & Johanson, 2000; Chillet et al., 2007; Lassois et al., 2008). It has been suggested that these spatiotemporal fluctuations may reflect variations
in the banana fruit quality potential that develops in the field and which determines the postharvest onset or absence of diseases (Chillet & de Lapeyre de Bellaire, 1996; Lassois et al., submitted). The quality potential comprises a physiological and parasitic component, which both depend on agro-technical factors and on soil and climate environment conditions (namely pedo-climatic factors).

The physiological component here refers to the fruit susceptibility to crown rot. In order to overcome the parasitic component, this susceptibility level of the fruit is measured by the size of lesions in standardised artificial inoculations (de Lapeyre de Bellaire et al., 2008).

In the case of anthracnose it has been shown that fruit susceptibility at harvest is a key factor in the development of this disease and its control (Chillet et al., 2006, 2007). Little is known, however, about the factors influencing the banana susceptibility to crown rot. Although pedo-climatic conditions and agro-technical factors are known to influence the development of this postharvest disease (Lukezic et al., 1967; Shillinglord, 1978; Krauss & Johanson, 2000), there are few studies linking such fluctuations to the field susceptibility (Lassois et al., 2008).

The aim of this work was to characterise the importance of some preharvest factors in determining fruit susceptibility to crown rot. In particular, we were interested in the influence of the fruit development stage and fruit filling characteristics. First, we examined whether the position of a hand in a banana bunch influences the susceptibility of its fruits to crown rot, taking advantage of the fact that the hands of a bunch differ as regards to both their development stage and filling status (Jullien et al., 2001a). Next, we evaluated how a change in the source–sink ratio (So–Si ratio) during growth of the bunch affects fruit susceptibility to crown rot (leaves being the source and fruit the sink) considering that So–Si ratio modification leads, among others, to changes in the rate of fruit filling (Jullien et al., 2001b). The source–sink terms are commonly used in plant characterisation. Leaves were considered as source tissues because they produce excess of assimilate, while fruits were sink organs. With respect to metabolism, plant organs are generally divided into source and sink tissues. Source tissues like mature leaves produce excess of assimilates which are transported via the phloem to the different sink tissues not able to produce themselves sufficient amounts of assimilates.

Materials and methods

A banana plant produces an inflorescence called a bunch. Fruits on a bunch are grouped into female and male hands arranged helicoidally around a central axis called the stalk. The hand at the top of the bunch is the first to be initiated and hands are traditionally numbered from this one downward. Each hand can be divided into clusters consisting of several banana fruits called fingers (Fig. 1).

Plant material

For each performed test, homogeneous sets of banana plants (Musa acuminata [AAA group, Cavendish subgroup] cv. Grand Nain) which have grown under the same agro-technical practices were randomly selected at the Dia-Dia commercial plantation (PHP, Njombe, Cameroon) (altitude: 80 m; annual mean temperature: 26.5 °C; annual mean rainfall: 3500 mm). Nonsystemic fungicide applications were used to control foliar diseases. The crown tissues have never been in contact with fungicides. The date of flowering was indicated by tying a coloured belt to each bunch at the horizontal finger stage in order to predict the time of harvest. Bunches were also covered with a plastic sleeve at this stage. Bunches were harvested at a constant physiological age (Jullien et al., 2008), that is when the mean daily temperature sum accumulated by the fruit at the 14 ºC threshold between flowering and harvest reached 900 degree days (dd). Temperatures were recorded at a weather station on the plantation. The daily average temperature (Td) was estimated from measurements of maximum temperature (Tmax) and minimum temperature (Tmin).

Evaluation of susceptibility to crown rot

Hands of bananas collected on the day of the experiment and transported to the laboratory were cut into clusters of four fingers without defects. The crown surfaces were refreshed with a knife. These cuttings were square, with regular, clean-cut sections in order to obtain similar crowns. Smoothly cut crowns were obtained with a sharp knife, leaving as much crown tissue as possible. Latex from crown tissues was dried with absorbent paper and the crowns were surface-sterilised by submerison in 50% ethanol. Fifty microlitres of C. musae conidial suspension containing 10^4 conidia mL^-1 was applied to the centre of the freshly exposed crown tissue and covered with a small paper filter. The C. musae strain was isolated in Njombe, Cameroon. It is sensitive to thiabendazole and was stored at −20 ºC in a glycerol solution (30%). C. musae cultures were grown at 25 ºC in Mathur medium (MgSO_4.7H_2O: 2.5 g L^-1; KH_2PO_4: 2.7 g L^-1; peptone: 1 g L^-1; yeast extract: 1 g L^-1; saccharose: 10 g L^-1; agar: 15 g L^-1) for 10 days. Conidia were removed by flooding the plates with sterile distilled water and filtration through a 45 μm sieve. Their concentrations were determined.
Figure 1  Organisation of a banana bunch.
with a Mallassez cell. Two hours after application of the conidial suspension, the clusters were packed in punched polyfilms normally used in the industry, placed in commercial boxes, and stored at 13°C for 10 days to simulate shipment. Artificial ripening was then initiated by dipping the bananas for 5 s in an ethrel solution (480 g L\(^{-1}\)), after which the clusters remained at 20°C for another 3 days before crown rot assessment. The internal progression of rot was determined by cutting the cluster crown longitudinally in two and measuring the surface of rot spread into the crown, from the original inoculation point. This ‘internal necrotic surface’ (INS), calculated by assuming a rectangular shape, was expressed in mm\(^2\). Its average value was taken as a measure of fruit susceptibility to crown rot.

**Intrabunch variation of fruit susceptibility to crown rot**

From March to May 2005 and during 10 weeks, 1 bunch per week having reached 900 dd was harvested (Fig. 2). Hands were separated from the bunch to evaluate the susceptibility to crown rot of each hand and numbered from 1 to 8 by order of appearance on the bunch, Hand 1 being the first to have appeared at the top of the bunch (Fig. 1). Each hand was then divided into three clusters of four fingers. The susceptibility of each cluster to crown rot was assessed as described in the section on *Evaluation of susceptibility to crown rot*. The average INS values calculated for the three clusters of a hand were subjected to two-way crossed-mixed ANOVA (Hand, Bunch) performed with Minitab software. Finally, results were submitted to a linear regression analysis.

**Effect of modifying the So–Si ratio on the crown rot susceptibility**

The So–Si ratio was modified as follows during fruit growth by removal of leaves (L) and hands (H) at the flowering stage (horizontal finger stage) so as to obtain the following treatments:

- 12L/2H: 12 leaves and 2 hands (the first two to have appeared on the bunch) remaining at the flowering stage;
- 12L/8H: 12 leaves and 8 hands (the first eight to have appeared on the bunch) remaining at the flowering stage;
- 5L/2H: five leaves (the last to have emerged) and two hands (the first two to have appeared on the bunch) remaining at the flowering stage;
- 5L/8H: five leaves (the last to have emerged) and eight hands (the first eight to have appeared on the bunch) remaining at the flowering stage;

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**Figure 2** Chronology and modalities of the various tests performed.
Intrabunch variation of fruit susceptibility to crown rot

Results

Intrabunch variation of fruit susceptibility to crown rot

The position of fruit on the bunch was found to influence fruit susceptibility to crown rot very highly significantly ($P < 0.001$) (Table 1). Although very highly significant differences were observed between bunches (weeks) ($P < 0.001$), the trend was the same whatever the bunch, as no effect of the interaction ‘hand × bunch’ was observed ($P = 0.218$). A gradient of susceptibility was observed between hand 8, with an INS average of 73.6 mm$^2$, and hand 1, with an INS average of 150.9 mm$^2$ (Fig. 3). Hand rank is an equidistant ordinal variable that can be compared to a quantitative value in the calculations. Hence, a linear correlation was found ($r = −0.95$) between the hand position on the bunch and the eight corresponding INS means.

Effect of the Source–Sink ratio modification on fruit grade and length

The change in the So–Si ratio imposed during flowering by removal of both leaves and hands from the bunch had a highly significant effect on the fruit susceptibility to crown rot ($P = 0.004$) (Table 2). Although there was also a highly significant effect of the year factor ($P = 0.004$), the trend was the same in both years because no interaction was observed between the treatments and the years ($P = 0.225$), leading to analyse the results of both years together. The fruit susceptibility to crown rot showed a very highly significant variation ($P < 0.001$) from week to week within a year without consequence on the preceding conclusions. The ranking of different treatments (Table 3) shows that the fruits of bunches from which six hands were removed (treatments 12L/2H and 5L/2H) were significantly less susceptible to crown rot (INS = 138.3 mm$^2$) than those of bunches with eight hands (12L/8H treatment and 5L/8H, INS = 237.9 mm$^2$). There appeared no susceptibility difference, however, between treatments 12L/8H and 5L/8H or between treatments 12L/2H and 5L/2H. Thus, the removal of 6 out of 8 hands had a significant effect on fruit susceptibility to crown rot but the removal of 7 out of 12 leaves did not. Table 3 also shows the classification of INS by order of decreasing empirical So–Si ratio. It is noteworthy that in trend terms, the crown susceptibility increased as the empirical So–Si ratio decreased.
of many hands thus has a significant effect on the morphometric characters of the fruit. No difference in fruit grade or length was observed, however, between treatments 12L/8H and 5L/8H or treatments 12L/2H and 5L/2H. Thus, leaf removal had no significant effect on these morphometric characteristics (Table 3). It is noteworthy that the fruit pomological characteristics and banana yield increased as the empirical So–Si ratio decreased (Table 3). The various changes in the So–Si ratio had an effect on fruit filling.

Discussion

We show here that fruit development stage and filling characteristics are parameters influencing postharvest susceptibility of bananas to crown rot. Within a bunch,

**Figure 3** Mean values and standard deviations of internal necrotic surface (INS mm²) as a function of the hand position on the bunch.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>F-value</th>
<th>P-value</th>
<th>F-value</th>
<th>P-value</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>3</td>
<td>57.87</td>
<td>0.004</td>
<td>140.46</td>
<td>&lt;0.001</td>
<td>103.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Year</td>
<td>1</td>
<td>13.29</td>
<td>0.004</td>
<td>10.85</td>
<td>0.001</td>
<td>102.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment × Year</td>
<td>3</td>
<td>1.52</td>
<td>0.225</td>
<td>4.80</td>
<td>0.003</td>
<td>2.44</td>
<td>0.065</td>
</tr>
<tr>
<td>Week (Year)</td>
<td>11</td>
<td>10.14</td>
<td>&lt;0.001</td>
<td>7.65</td>
<td>&lt;0.001</td>
<td>2.58</td>
<td>0.005</td>
</tr>
<tr>
<td>Treatment × Week (Year)</td>
<td>33</td>
<td>1.77</td>
<td>0.009</td>
<td>1.14</td>
<td>0.283</td>
<td>1.64</td>
<td>0.022</td>
</tr>
<tr>
<td>Bunch (Treatment Year Week)</td>
<td>191</td>
<td>2.32</td>
<td>&lt;0.001</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**Table 2** Results of a partially hierarchical mixed four-way ANOVA (Bunch, Treatment, Week, Year) on internal necrotic surface (mm²) and of two partially hierarchical mixed three-way ANOVAs (Treatment, Week, Year) on fruit grade and length.
there is a gradient of susceptibility to crown rot, the hands initiated first (the upper ones) being more susceptible than those initiated last (the lower ones). It has already been established that morphological differences between hands of a same bunch result from differential development associated with cell division and fruit filling characteristics (Jullien et al., 2001a). Here it appears that competition within a bunch affects not only the morphological characteristics of the fruits but also the fruit quality potential. Taking into account the observation of Jullien et al. (2001a) that the hands initiated first are approximately 70 dd ahead of those initiated last, the susceptibility gradient might be because of this physiological age gap between fruits in the same bunch. Effectively, in the case of anthracnose of banana, the fruit susceptibility has been shown to increase with the physiological age of the fruit expressed in dd (Chillet et al., 2006, 2007).

The position of a hand on the bunch has also been shown to influence fruit filling. This results in gradients of pulp dry weight, cell number per fruit, and starch grain number per cell (Jullien et al., 2001a) and also in differences in sap concentration and composition within the same bunch (Kurien et al., 2000). Furthermore, it is well known that partitioning of assimilates between various sink organs is complex and not equally distributed (Kozlowski, 1992). These differences in filling characteristics within a bunch may be involved in the observed susceptibility changes.

We confirm here that So–Si ratio changes have a significant effect on fruit morphology, as previously demonstrated in several studies (Daniells et al., 1987, 1994; Israeli et al., 1995; Johns 1996; Kurien et al., 2000; Jullien et al., 2001b; Mouen Bedimo et al., 2003; Chillet et al., 2006). We reveal, furthermore, a new effect of a So–Si ratio change on the fruit quality potential: when the sink is decreased by removal of many hands, the fruit susceptibility to crown rot decreases. Few studies on various plants have linked the importance of So–Si ratio, and thus photosynthetic assimilate distribution, in plant–pathogen interactions (Dood, 1980; Barrière et al., 1981; Barrière, 1985; Pegg, 1986; Seetharama et al., 1991). However, in the case of bananas, the present results contrast with the previously reported observation that modifying the So–Si ratio has no effect on fruit susceptibility to anthracnose or on the fruit conservation potential (Chillet et al., 2006). This suggests that different mechanisms govern the susceptibility of fruit to crown rot and anthracnose. Jullien et al. (2001b) have also highlighted the impact of this So–Si ratio on fruit filling characteristics and notably on the rate of cell filling. In our study, we consider that hand and leaf removal result in a modification of the empirical So–Si ratio. In keeping with the observation of Jullien et al. (2001b), we assume that an increased empirical So–Si ratio results in an increased fruit filling rate. One might hypothesise that this increased cell filling rate is involved in the observed reduction of the fruit susceptibility to crown rot. On the other hand, the impact of the So–Si ratio on nutrient availability, distribution, storage and assimilative transformation has been extensively documented in many models (Famiani et al. 2000; Noquet et al., 2004; Savin et al., 2006; Niu et al., 2008; Dordas, 2009). We suggest that a modification of the So–Si ratio leads to a change in the partitioning of assimilates between various sinks which influences the formation of secondary metabolites involved in plant–pathogen interaction. When hands are removed, the competition between sinks is reduced and the availability of mobile assimilates for remaining hands is more important.

It is noteworthy that in our study source reduction (leaf removal) had a lesser effect on fruit susceptibility to crown rot than sink reduction, because removal of about 60% of the leaves (7 out of 12) had little effect on fruit susceptibility, in contrast to removal of 75% of the fruits (6 out of 8). It is recognised that the impact of defoliation on the qualitative and quantitative development of bananas is variable and highly dependent on (a) when the defoliation is performed (Arcila et al., 1995), (b) the intensity of defoliation (Robinon & Anderson, 1990; Israeli et al., 1995; Rodriguez et al., 2005) and (c) how defoliation is performed: mechanically or through the action of pathogens (Robinon et al., 1992). Here, mechanical defoliation appears not to have been sufficiently early and/or severe to influence the processes determining fruit susceptibility to crown rot. On the other hand, a compensatory phenomenon reducing the impact of defoliation might also be involved, as the development of a bunch results from the distribution of dry matter.

### Table 3 Mean values and standard deviations of internal necrotic surfaces (INS) mm², fruit grade (mm), and length (cm) after treatments affecting the source–sink ratio

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Source–sink ratio</th>
<th>INS (mm²)</th>
<th>Grade (mm)</th>
<th>Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12L/2H</td>
<td>4</td>
<td>130.2 ± 65.4a</td>
<td>37.9 ± 0.3b</td>
<td>22.0 ± 0.2c</td>
</tr>
<tr>
<td>5L/2H</td>
<td>1.7</td>
<td>146.4 ± 60.2a</td>
<td>36.8 ± 0.3b</td>
<td>21.3 ± 0.2a</td>
</tr>
<tr>
<td>12L/8H</td>
<td>1.0</td>
<td>227.4 ± 66.7a</td>
<td>33.3 ± 0.2a</td>
<td>19.5 ± 0.2a</td>
</tr>
<tr>
<td>5L/8H</td>
<td>0.4</td>
<td>248.4 ± 72.0a</td>
<td>32.0 ± 0.2a</td>
<td>18.6 ± 0.2b</td>
</tr>
</tbody>
</table>

All fruits were obtained from hand 2. The letters a, b, c, d, e and f represent groups of statistically similar fruits based on contrast testing. 12L/2H: banana plant with 12 leaves and two hands; 5L/2H: banana plant with five leaves and two hands; 12L/8H: banana plant with 12 leaves and eight hands; 5L/8H: banana plant with five leaves and eight hands. The source–sink ratio was estimated according to the number of leaves and fruits removed in each treatment, as compared to unaltered banana plants (12L/8H).
not only from the leaves but also from other parts of the plant. In this way, the rachis and pseudostem may partially compensate for late-occurring defoliation (Eckstein et al., 1995). It has also been shown that an increased photosynthetic capacity of the remaining leaves may partially compensate for losses caused by defoliation (Robinson et al., 1992).

Conclusion
The susceptibility of bananas to crown rot is thus likely to be influenced by the stage of fruit development and by filling characteristics, these parameters being in close interaction and dependent on the soil–climate conditions and agro-technical factors of the production area. It is essential not to lose sight of the fact that the regulation of plant susceptibility is, in all cases, the result of nutritional balance established during plant growth. This balance is the consequence of all physiological relations of the whole plant and the environmental factors and might affect plant–pathogen interaction by two ways: first, by influencing the ability of the plant to establish defence mechanisms, notably through changes in secondary metabolism; and secondly, by altering the bioavailability of nutrients necessary for pathogen development.

However, the molecular underlying mechanisms implied in the susceptibility variations observed in this study are still unknown.

The fact that the fruit susceptibility depends on the hand position in the bunch shows the importance of standardising the sampling method when measuring the susceptibility of bananas to crown rot. The hands collected for an experiment should be collected systematically from the same position on each bunch. As the hands in the upper portion have more fruits and allow division into more four-fruit clusters, it has been recommended to use the third hand of the bunch, which is more stable from one bunch to another than the first two hands (Jannoyer, 1995). If more than one hand per bunch is needed to carry one bunch to another than the first two hands (Jannoyer, 1995). If more than one hand per bunch is needed to carry out the experiment, it is essential to work with successive hands as demonstrated in this paper.

Lastly, the main method used to control crown rot is a systematic chemical postharvest treatment. Apart from the environmental, social and legislative problems resulting from this chemical control strategy, growers also face problems of treatment efficiency which are notably related to the fruit susceptibility in some specific areas. Only a truly integrated pest management strategy applied to the whole chain can provide effective alternatives to chemical treatment (Lassois et al., submitted). We have shown that an increase in the empirical So–Si ratio makes it possible to reduce fruit susceptibility to crown rot while increasing fruit size at harvest. Thus, early hand removal in the field might be used as part of an integrated pest management scheme.

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