Technical note

Response of Dwarf Cavendish banana plantlets to inoculation with races 1 and 4 of *Fusarium oxysporum* f. sp. *cubense* at different levels of Zn nutrition

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Response of Dwarf Cavendish banana plantlets to inoculation with races 1 and 4 of *Fusarium oxysporum* f. sp. *cubense* at different levels of Zn nutrition.

Abstract — Introduction. The causal agent of Panama disease in banana is *Fusarium oxysporum* f. sp. *cubense* (FOC). The authors put forward a hypothesis to account for certain disturbances of the natural defence mechanisms of banana plants against Panama disease as a consequence of very low Zn levels which could alter the mechanism of tylose formation. **Materials and methods**. A long-term experiment was carried out with 2-month-old banana plants in a growth chamber at 23 °C using three different levels of Zn nutrition. The nutrient solutions were inoculated with FOC 1 and FOC 4. **Results and discussion**. None of the total plants growing in a nutrient solution inoculated with FOC 1 showed infected rhizomes at the end of the experiment, but 75% of the plants growing in pots inoculated with FOC 4 had infected rhizomes. On the other hand, rhizome infection by FOC 4 was influenced by the nutrient solutions. The data showed that the number of plants with damaged rhizomes was significantly higher in the Zn-deficient treatments (100% of plants) than in the normal Zn solution (25% of plants), these internal symptoms being seen to worsen as the presence of Zn decreased in the nutrient solution. **Conclusions**. The results obtained seem to confirm the role played by Zn nutrition of banana plants in the appearance of Panama disease and are in accordance with our hypothesis concerning the relationships among plant Zn nutrition, plant IAA level, tylose formation and the incidence of the disease. Likewise, under our experimental conditions, race 1 of FOC is confirmed to be non-pathogenic against this banana cultivar, while race 4 is indeed pathogenic.

Spain / *Musa* / fungal diseases / pathogens / *Fusarium oxysporum* / plant nutrition / zinc

Réponse de plantules de bananiers Dwarf Cavendish, soumises à différents niveaux de nutrition en Zn, vis-à-vis d'une inoculation par les races 1 et 4 du *Fusarium oxysporum* f. sp. *cubense*.

Résumé — Introduction. L'agent causal de la maladie de Panama du bananier est *Fusarium oxysporum* f. sp. *cubense* (FOC). Les auteurs ont proposé une hypothèse pour expliquer certaines perturbations des mécanismes normaux de la défense des plants de bananier contre la maladie de Panama ; selon cette hypothèse, des niveaux très bas en Zn pourraient affecter le mécanisme de la formation de tyloses. **Matériel et méthodes**. Une expérience à long terme a été effectuée avec des plants de bananiers âgés de 2 mois placés en chambre de culture à 23 °C et testant trois niveaux différents de nutrition en Zn. Les solutions nutritives ont été inoculées avec du FOC de races 1 et 4. **Résultats et discussion**. En fin d'expérience, aucun des plants développés sur solution nutritive inoculée avec FOC 1 n'a présenté de rhizomes infectés mais 75 % des plants mis en pots en présence de FOC 4 ont eu leurs rhizomes infectés. D'autre part, l'infection des rhizomes par FOC 4 a été influencée par les solutions nutritives testées. Nos résultats ont montré que le nombre de plants présentant des rhizomes endommagés était sensiblement plus élevé dans les traitements déficients en Zn (100 % des plants atteints) que dans la solution avec une concentration normale en Zn (25 % des plants atteints), ces symptômes internes empirant en même temps que diminuait la concentration du Zn dans la solution nutritive. **Conclusions**. Les résultats obtenus semblent confirmer le rôle joué par la nutrition en Zn apportée aux plants de bananier dans le contexte de la maladie de Panama ; ils sont conformes à notre hypothèse au sujet des relations existant entre la nutrition des plants en Zn, leur teneur en AIA, la formation de tyloses et l'incidence de la maladie. De même, dans nos conditions expérimentales, la race 1 du FOC est confirmée comme étant non pathogène pour le cultivar Dwarf Cavendish, alors que la race 4 est pathogène.

Espagne / *Musa* / maladie fongique / agent pathogène / *Fusarium oxysporum* / nutrition des plantes / zinc

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Fruits, 2004, vol. 59, p. 319–323

DOI: 10.1051/fruits:2004030

***Resumen Español,** p. 323*

1. Introduction

Panama disease is one of the most serious threats to banana crops worldwide. The causal agent of Panama disease in banana is *Fusarium oxysporum* f. sp. *cubense* (FOC).

Gutiérrez-Jerez *et al.* [1] published a statistical study on the physical and chemical properties of Canary Island banana soils, comparing FOC-infected banana soils with uninfected banana soils. These authors suggested that several parameters seemed to significantly affect the appearance of Panama disease. Borges-Pérez *et al.* [2] put forward a hypothesis to account for certain disturbances of the natural defence mechanisms of banana plants against Panama disease as a consequence of soil K-Mg and P-Zn imbalances. These same authors also suggested that very low plant Zn levels could alter the mechanism of tylose formation.

According to Mace [3], tylose formation in banana roots is stimulated by indoleacetic acid (IAA). Researchers such as Skoog [4], Tsui [5], Takaki and Kushizaki [6], and Salami and Kenefick [7] cited by Marschner [8] have pointed out the relationship between Zn and IAA levels in plants. If the timing of tylose formation in banana plants infected by FOC is not correct, the infection may progress to the rhizome, even in the case where resistant gels exist [9]. The disturbances of plant Zn nutrition can be conditioned in the banana soils of the Canary Islands by low values of Zn and organic matter aggravated by the poor physical conditions and high values of P of these soils. Interactions of P with Zn in the plant may be observed under certain conditions [10]. In the literature on the role played by Zn banana plant nutrition in relation to Panama disease, Primavesi [11] working on "banana maça" related low Zn absorption by the plant due to strong sun irradiation (Brazil) to an increase in disease incidence but made no reference to resistance mechanisms. Borges-Pérez *et al.* [12] published a work on the enhanced resistance of Dwarf Cavendish banana plants to *Fusarium oxysporum* f. sp. *cubense* by controlled Zn nutrition under field conditions. Later, Hecht-Buchholz *et al.* [13] described the influence of Zn nutrition, exogenous IAA and Zn nutrition–exogenous IAA interaction, on *Fusarium* wilt of banana in a growth chamber at 28 °C.

Stover and Waite [14] pointed out that the outbreak of Panama disease in the Cavendish cultivar was due to the action of nutritional factors that caused a decrease in the resistance of this cultivar to race 1 of *Fusarium oxysporum* f. sp. *cubense* (FOC 1).

In 1977, a new race of FOC named 'FOC 4' was described in the Dwarf Cavendish cultivar in Taiwan [15]. Sun and Su [16] reported on a rapid method for determining differential pathogenicity of *Fusarium oxysporum* f. sp. *cubense* using banana plantlets. Later, Su *et al.* [17] cited the existence of race 4 in isolates from diseased Dwarf Cavendish banana plants from the Canary Islands. This was confirmed the following year by Hernández-Hernández and Sala-Mayato [18], who reported the presence of FOC 4 in isolates from the island of Tenerife (Canary Islands).

We undertook a long-term experiment to evaluate the response of Dwarf Cavendish banana plantlets derived from meristem culture to inoculation with races FOC 1 and FOC 4 at different levels of Zn nutrition in a growth chamber.

2. Materials and methods

A long-term experiment (six treatments, four replicates per treatment) was carried out with 2-month-old banana plants in a growth chamber at 23 °C using different nutrient solutions (aerated): complete nutrient solution [Cns_{+Zn}] [19], low Zn level [$Cns_{+Zn}/5$] and very low Zn level [$Cns_{+Zn}/10$]. The nutrient solutions were inoculated (3×10^4 spores·mL^{-1} of nutrient solution) with FOC 4 (VCG-0120, Canary Islands isolate) and FOC 1 (VCG-0126, Central American isolate). The banana plants were Dwarf Cavendish plantlets derived from meristem culture from the firm Cultivos Vegetales *in vitro* de Tenerife, S.A., CULTESA. They were grown in pots (2000 mL nutrient solution per pot, one plant per pot). The relative humidity was kept around 70%, with light intensity (PAR) of 18 μE·m^{-2}·s^{-1}, and a 12-h photoperiod. Nutrient solutions were renewed every 10 days. Water losses due to evapotranspiration were compensated with

Table I.
Number of plants with damaged rhizome for Dwarf Cavendish banana plants inoculated with races 1 and 4 of *Fusarium oxysporum* f. sp. *cubense* (FOC) at 16 weeks post-inoculation, according to the nutrient solution used.

Nutrient solution	Counting of plants	FOC 4	FOC 1
Complete nutrient solution (Cns_{+Zn})	No. plants tested	1	1
	No. plants with damaged rhizome	0	0
[(Cns_{+Zn})/5]	No. plants tested	1	1
	No. plants with damaged rhizome	1	0
[(Cns_{+Zn})/10]	No. plants tested	1	1
	No. plants with damaged rhizome	1	0

distilled water. For the first month after inoculation, the nutrient solution was not renewed every 10 days to avoid loss of the inoculum. However, water losses due to evapotranspiration were compensated with distilled water. In order to detect FOC infection in plant rhizomes of the different treatments, the rhizomes of one plant from each treatment were examined at 16 weeks post-inoculation while those of the remaining plants were checked at the end of the assay (36 weeks post-inoculation).

Table II.
Percentage (%) of plants with damaged rhizome for Dwarf Cavendish banana plants inoculated with races 1 and 4 of *Fusarium oxysporum* f. sp. *cubense* (FOC) at 36 weeks post-inoculation, according to the nutrient solutions used.

Nutrient solution	FOC 4	FOC 1
Complete nutrient solution (Cns_{+Zn})	25 a	0
[(Cns_{+Zn})/5]	100 b	0
[(Cns_{+Zn})/10]	100 b	0

Values within the same column followed by different letters are significantly different at the $P = 0.05$ level.

3. Results and discussion

After 16 weeks post-inoculation, none of the three banana plants inoculated with FOC 1 showed infected rhizomes (*table I*). However, two of the three banana plants inoculated with FOC 4 showed infected rhizomes. At the end of the experiment (36 weeks post-inoculation), none of the total banana plants inoculated with FOC 1 showed infected rhizomes (*table II*). However, 75% of the plants inoculated with FOC 4 showed infected rhizomes.

The results obtained (16 and 36) weeks after inoculation confirmed that, under our experimental conditions, FOC 1 is not pathogenic against the Dwarf Cavendish banana plants. The results obtained with FOC 4 also confirmed that this race is pathogenic against this banana cultivar. Our data are in accordance with the results obtained by other authors using different experimental systems [16, 17].

In the treatments with the pathogenic race (FOC 4), rhizome infection was influenced by the nutrient solutions. Indeed, the data indicate that the number of plants with damaged rhizomes was significantly higher in the Zn-deficient treatments (Cns_{+Zn}/5 and Cns_{+Zn}/10), with 100% of affected plants, while those developed at optimum levels of Zn [Cns_{+Zn}] displayed only 25% of rhizomes infected by FOC 4 (*table II*). The internal symptoms were found to worsen as the Zn level decreased in the nutrient solution.

Hecht-Buchholz *et al.* [13] working in a growth chamber at 28 °C (system Dwarf Cavendish banana plants – FOC 4) did not observe statistically significant differences in the mean gravity of rhizome damage between treatments (optimum Zn + FOC 4) and (– Zn + FOC 4), whose results are in sharp contrast with those obtained by us in

the present work using the same experimental plant-pathogen system, but in a growth chamber at 23 °C. Apparently, FOC 4 causes greater damage to the plants at 28 °C than at 23 °C, confirming previous work carried out with the system Gros Michel banana plants-FOC 1.

Indeed, Beckman *et al*. [20] found that, at 27 °C, the host's (Gros Michel) responses failed consistently to check the advance of the pathogenic *Fusarium oxysporum* f. sp. *cubense* (FOC 1) spores and plants grown at this temperature were highly susceptible, reaching the conclusion that soil temperature is a significant factor in determining resistance and susceptibility.

4. Conclusion

The results obtained in this work seem to confirm the role played by Zn nutrition of banana plants in the appearance of Panama disease and are in accordance with our hypothesis concerning the relationships among plant Zn nutrition, plant IAA level, tylose formation and the incidence of the disease. Likewise, it was verified that, under our experimental conditions, race 1 of FOC is not pathogenic against Dwarf Cavendish banana cultivar, while the opposite conclusion was reached with race 4.

Acknowledgements

The authors would like to thank J.M. Hernández Hernández from the *Departamento de Patología Vegetal*, *Instituto Canario de Investigaciones Agrarias* (ICIA), for kindly providing FOC 1 and FOC 4 inocula.

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Respuesta de plántulas de bananas Dwarf Cavendish a la inoculación con las razas 1 y 4 de *Fusarium oxysporum* f. sp. *cubense* a diferentes niveles de nutrición en Zn.

Resumen — Introducción. El agente causal de la enfermedad de mal de Panamá en bananas es el *Fusarium oxysporum* f. sp. *cubense* (FOC). Los autores han apuntado una hipótesis atribuyendo a ciertos desórdenes en los mecanismos naturales de defensa de las plantas de banana frente al mal de Panamá como consecuencia de bajos niveles nutricionales de Zn que pudieran alterar el mecanismo de formación de tilosas. **Material y métodos**. Este experimento se realizó con plántulas de banana de 2 meses de edad en cámara de crecimiento a 23 °C usando tres diferentes niveles de nutrición en Zn. Las soluciones nutritivas fueron inoculadas con FOC 1 y FOC 4. **Resultados y discusión**. Al final de la experiencia, ninguna del total de plantas creciendo en la solución nutritiva con FOC-1 mostró sus rizomas infectados, pero el 75% de las plantas desarrolladas en las macetas inoculadas con FOC 4 vieron sus rizomas infectados por el hongo. Por otra parte, la infección del rizoma estuvo influenciada por la concentración de Zn de las soluciones nutritivas. Los datos mostraron que el número de plantas con rizoma dañado por el hongo fue significativamente mayor en los tratamientos deficitarios en Zn (100% de las plantas) que en aquellos con soluciones adecuadas en este micronutriente (25% de las plantas). Asimismo, se observó que estos síntomas internos fueron más pronunciados a medida que iba decreciendo la presencia de Zn en la solución nutritiva. **Conclusiones**. Los resultados obtenidos en este estudio parecen confirmar el papel jugado por la nutrición de Zn en las plantas de banana en la aparición del mal de Panamá y están en consonancia con nuestra hipótesis que relaciona la nutrición en Zn, nivel de IAA, formación de tilosas y la incidencia del mal. Asimismo, y bajo las condiciones en que esta experiencia fue llevada a cabo, se confirma que la raza 1 de FOC es no-patogénica a este cultivar mientras que la raza 4 si lo es.

España / *Musa* / enfermedades fungosas / organismos patógenos / *Fusarium oxysporum* / nutrición de las plantas / cinc