

Reaction of pineapple, cultivars Pérola and Smooth Cayenne, to inoculation with *Fusarium moniliforme* SHELDT. var. *subglutinans* WR. & RG.

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COMPORTEMENT DES CULTIVARS PEROLA ET CAYENNE LISSE A L'INOCULATION AVEC *FUSARIUM MONILIFORME* SHELDT. VAR. *SUBGLUTINANS* WR. & RG.

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RESUME - Deux cultivars d'ananas Pérola et Cayenne lisse ont été inoculés par *Fusarium moniliforme* var. *subglutinans*, à différents stades de développement de l'inflorescence.

Les pourcentages d'yeux infectés et la sévérité de l'attaque ont été plus importants lorsque l'inoculation a été réalisée 4, 7 et 10 semaines après l'induction florale.

Les inoculations effectuées 13 semaines après l'induction florale n'ont entraîné qu'un développement faible de la maladie sur les fruits des deux cultivars. Les témoins, non inoculés, présentaient les pourcentages d'infestation les plus faibles. Le cultivar Pérola s'est montré plus sensible que le cultivar Cayenne lisse, aussi bien après inoculations sur les différents stades du développement de l'inflorescence qu'après inoculation sur rejets de type «bulbille».

INTRODUCTION

The fusariosis, caused by *Fusarium moniliforme* SHELDT. var. *subglutinans* WR. & RG., is the most serious pineapple disease in Brazil, causing very high losses in all pineapple producing areas (MAFFIA, 1978 ; MATA, 1978 ; MATOS 1978a, 1979, 1981 ; PISSARRA, 1978 ; ROBBS, 1965). This disease was first reported by KIMATI & TOKESHI (1964), who isolated the pathogen from infected fruits, cultivar Smooth Cayenne. The pathogen is able to infect

all parts of the pineapple plant and the development of the disease results in a characteristic gum exsudation from the infected tissue.

Pineapple inflorescence, inoculated with *F. moniliforme* var. *subglutinans* at several stages of development, originate infected fruits, whether under greenhouse conditions (BOLKAN et al, 1979 ; MATOS, 1978b) or in field experiments (VENTURA et al, 1981).

This study was undertaken to determine the stage of inflorescence development of pineapple which is more susceptible to infection by *Fusarium moniliforme* var. *subglutinans* and also if the cultivars Perola and Smooth Cayenne, which are naturally very susceptible to fusariosis, would have differential susceptibility when artificially inoculated.

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MATERIALS AND METHODS

The pineapple cultivar Perola and Smooth Cayenne, used in this study, were grown according to standard procedures. The chemical treatment to initiate flowering was performed by adding, approximately, 50 ml of a water solution of calcium carbide (90 g of Ca Ce/20 l of cold water) directly into the growing point ; this practice was repeated 3 times, every other day.

A single spore culture of *F. moniliforme* var. *subglutinans*, isolated from basal sections of infected leaves, was maintained on potato-dextrose-agar (PDA) slants, under laboratory conditions, for 7 to 14 days. The conidial suspension was obtained by adding sterile distilled water to the culture slants, scraping the culture surface with a small brush to liberate the conidia, and filtering the conidial suspension through three layers of cheese cloth. The inoculum concentration was determined with a hemacytometer, Neubauer type, and the spore suspension was diluted to 1×10^3 , 1×10^4 and 1×10^5 conidia/ml. The inoculations were performed 1, 4, 7, 10 or 13 weeks after forcing (ROHRBACH & PFEIFFER, 1976) using a sprayer, 500 ml capacity. Approximately 10 ml of inoculum were applied into the differentiated growing point of the plant.

The test design was a split-split-plot with time of inoculation as the main plot, cultivars as the sub-plot and the inoculum concentration as the sub-sub-plot, with four replications. Each sub-sub-plot consisted of six test plants with two plants on each and as buffer plants.

At harvest time the fruits were evaluated for incidence of fusariosis by establishing the percentage of infected fruits and the percentage of infected fruitlet/fruit. For statistical analysis the data were transformed to $\arcsin \sqrt{\%}$.

Slips of the pineapple cultivars Perola and Smooth Cayenne were obtained from experimental areas at the National Research Center for Cassava and Fruit Crops located in Cruz das Almas, State of Bahia, Brazil. After selection for disease incidence, the slips were inoculated with *F. moliniiforme* var. *subglutinans*. The inoculation was performed according to the technique described by MATOS (1978b), which consists of the immersion of the base of the slips, with 3 wounds, into a conidial suspension (1×10^5 conidia/ml), for 3 minutes. Immediately after inoculation, the slips, 10 per each cultivar, were planted in plastic pots (16 cm diameter x 13 cm high), and kept under greenhouse conditions. The plants were observed weekly, in order to determine the period of time between inoculation and symptoms expression and death of plants.

RESULTS

Time of inoculation and cultivar had a significant effect on the percentage of infected fruits, whereas the percentage of infected fruitlet/fruit was significantly influenced by the time of inoculation, inoculum concentration and their interaction (Table 1). Fusariosis symptoms developed at significant levels (percentage of infected fruits and percentage of infected fruitlet/fruit) when inoculations were made, 4, 7 or 10 weeks after forcing ; inoculations 13 weeks after forcing induced the lowest levels of disease development (Table 2). Increasing the inoculum concentration had no influence on the percentage of infected fruit, but the percentage of infected fruitlet/fruit, in both cultivars, increased as the inoculum concentration was increased (Table 3). The pineapple cultivar Perola was significantly more susceptible to *F. moniliforme* var. *subglutinans* than 'Smooth Cayenne', in relation to percentage of infected fruit (Table 4).

Symptoms of fusariosis were detected on slips of both cultivars, 3 weeks after inoculation with *F. moliniiforme* var. *subglutinans*, when the cultivar Perola showed 80 % of infected slips, while the 'Smooth Cayenne' showed only 20 % of infection. The period of time between inoculation and death of the slips was 6 weeks for the cultivar Perola and 13 weeks for 'Smooth Cayenne' (Table 5), thus indicating that the pineapple cultivar Perola is more susceptible to the pathogen than the 'Smooth Cayenne', not only when the evaluation was on inoculated fruits but also on inoculated slips.

DISCUSSION

Highly significant levels of fusariosis were induced by inoculations with *F. moniliforme* var. *subglutinans* 4, 7 or 10 weeks after forcing. This is in accordance with the results obtained by MATOS (1978 b) and by VENTURA et al (1981). According to BOLKAN et al (1979), the open flowers are the principal infection sites for the pathogen. Since the flower opening occurs, approximately, 10 weeks after forcing, the high levels of infection induced by inoculations prior to the anthesis suggest : a - *F. moniliforme* var. *subglutinans* is able to survive in the growing point, and latter, on the inflorescence, until the anthesis, when the infection takes place ; b - the pathogen is able to infect the inflorescence before the anthesis ; results obtained by CUNHA (1980), who observed fusariose development in inflorescences which had their anthesis inhibited by spraying with ethyl-phosphonic acid, support this possibility ; c - as the flower primordia are completely developed 5 to 6 weeks after forcing (KERNES et al, 1936), it is possible that the infection occurs at early stages of inflorescence development, before the anthesis.

Inoculations with *F. moniliforme* var. *subglutinans* 13 weeks after forcing induced the lowest levels of fusa-

TABLE 1 - Summary of the statistical analysis of the reaction of the pineapple, cultivars Perola and Smooth Cayenne, inoculated with *Fusarium moniliforme* SHELDT. var. *subglutinans* WR. & RG., at several stages of the inflorescence development. CNPMF, Cruz das Almas, BA., Brazil, 1984.

Source of variation	Degree of freedom	Mean square infection	
		fruits	fruitlet/fruit
Time of inoculation (T)	4	3,830.48**	1,094.32**
Error (a)	12	224.33	42.89
Inoculum concentration (C)	2	197.24 n/s	798.54**
Interaction : T x C	8	38.01	130.93*
Error (b)	30	115.15	47.71
Cultivar (Cv)	1	891.73*	3.50
Interaction : T x Cv	4	324.34 n/s	75.78 n/s
C x Cv	2	55.79	11.15
T x C x Cv	8	115.07	41.56
Error (c)	45	160.59	41.90

ns : not significant ** : $P \leq 0.01$ * $P \leq 0.05$

TABLE 2 - Reaction of pineapple fruits, cultivars Perola and Smooth Cayenne, to inoculation with *Fusarium moniliforme* SHELDT var. *subglutinans* WR. & R.G. at several stages of inflorescence development. CNPMF, Cruz das Almas, BA, Brazil, 1984.

Weeks after forcing	Infection *	
	fruits (%)	fruitlet/fruit (%)
1	97.5 b	9.7 b
4	98.6 ab	15.9 ab
7	98.8 a	20.0 a
10	99.8 a	14.0 ab
13	70.4 c	2.3 c

* - values followed by the same letter, in the same column, have no difference by Tukey's test ($P \leq 0.05$).

TABLE 3 - Effect of the inoculum concentration on the fusariosis development in pineapple cultivars Perola and Smooth Cayenne, inoculated with *Fusarium moniliforme* SHELDT, var. *subglutinans* WR. & RG. at several stages of inflorescence development. CNPMF, Cruz das Almas, Bahia, Brazil, 1984.

Inoculum concentration (conidia/ml)	Infection (%) *	
	fruit	fruitlet/fruit
1×10^3	90.0 a	7.0 c
1×10^4	96.1 a	12.2 b
1×10^5	97.8 a	16.8 a

* - values followed by the same letter, in the same column, have no difference by Tukey's test ($P \leq 0.05$).

TABLE 4 - Reaction of the pineapple, cultivars Perola and Smooth Cayenne, inoculated with *Fusarium moniliforme* SHELDT. var. *subglutinans* WR. & RG. at several stages of inflorescence development. CNPMF, Cruz das Almas, Bahia, Brazil, 1984.

Cultivar	Infection (%) *	
	fruit	fruitlet/fruit
Perola	98.0 a	11.9 a
Smooth Cayenne	94.8 b	11.5 a

* - values followed by the same letter, in the same column, have no difference by the test F.

TABLE 5 - Infection percentage of slips on the pineapple, cultivars Perola and Smooth Cayenne, inoculated with *Fusarium moniliforme* SHELDT. var. *subglutinans* WR. & RG. CNPMF, Cruz das Almas, Bahia, Brazil. 1984 *

Weeks after inoculation	Cultivars **			
	Perola		Smooth Cayenne	
	IS	D	IS	D
3	80.0	0.0	20.0	0.0
4	100.0	0.0	40.0	0.0
6		100.0	60.0	0.0
7			100.0	0.0
9				40.0
10				60.0
12				80.0
13				100.0

* - inoculation by immersion of the base of the slips, with 3 wounds, in the inoculum 1×10^5 conidia/ml

** - IS - Infected slips showing fusariosis symptoms ; D - dead plant due to fusariosis development.

riosis in pineapple fruit. This is in accordance with previous reports (BOLKAN et al, 1979 ; MATOS, 1978 b ; VENTURA et al, 1981) and it is, probably, due to the stage of inflorescence development at the time of inoculation, when most of the flowers were already closed.

The inoculum concentration tested in this study had no effect on the percentage of infected fruit, but the percentage of infected fruitlet/fruit increased significantly as the inoculum concentration was increased. Similar results have been reported by BOLKAN et al (1979).

The pineapple cultivar Smooth Cayenne showed to be less susceptible to *F. moniliforme* var. *subglutinans* than the cultivar Perola, either when the inoculation was

performed on the developing inflorescence or in the slips. This suggests that there is a relationship between the reaction of the slips and fruits inoculated with *F. moniliforme* var. *subglutinans*, however, further studies, involving more cultivars, are necessary, in order to determine if this relationship is true or not.

Working under field conditions, MATA (1978) found no difference between the pineapple cultivars Perola and Smooth Cayenne, relative to fusariosis incidence in fruit, while the data presented in this paper show that 'Perola' is more susceptible to *F. moniliforme* var. *subglutinans* than 'Smooth Cayenne'. It is possible that, under field conditions, the inoculum potential was not the same for both cultivars, thus resulting in the same percentage of fusariosis incidence in 'Perola' and 'Smooth Cayenne'.

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