Field induction of Fusariosis in pineapple fruit with *Fusarium moniliforme* SHELD. var. *subglutinans* WR.& RG.

J.A. VENTURA, L.A. MAFFIA and G.M. CHAVES*

INOCULATION DE *FUSARIUM MONILIFORME* SHELD. VAR. *SUBGLUTINANS* WR. ET RG. A DES ANANAS DANS LES CONDITIONS DU CHAMP

J.A. VENTURA, L.A. MAFFIA et G.M. CHAVES

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RESUME - Les auteurs ont étudié, dans les conditions du champ, l'effet de trois concentrations de spores (10^1, 10^4, 10^7 spores par ml) de *Fusarium moniliforme* var. *subglutinans*, appliquées en inoculation à des ananas de la variété Cayenne lisse, à différentes périodes après l'induction florale (2, 6, 10, 14 et 18 semaines).
Le plus grand nombre de fruits atteints a été obtenu avec des inoculations effectuées 6 semaines après l'induction florale et des dégâts importants ont été observés avec des inoculations survenues 2 semaines après l'induction florale. Dans les deux cas, la maladie était plus intense avec les concentrations de 10^4 et 10^7 spores par ml.
En fonction de ces résultats, il semble que l'inflorescence, avant et pendant l'anthèse, peut être considérée comme le lieu primaire de l'infection.

INTRODUCTION

The Fusarium disease of pineapple [*Ananas comosus* (L.) MERRIL.] was reported in Brasil by KIMATI and TOKESHI (1964) and its causal'agent was described as *Fusarium moniliforme* SHELD. var. *subglutinans* WR. and RG. It can infect all plant parts causing tissue rot and inducing very typical symptoms depending on the infection site (PISSARRA et al., 1979, and ROBBS et al., 1965). The disease first appears in the fruitlets indicating that infection takes place in the floral cavities. This was observed by BOLKAN et al. (1979), who concluded that flowers are important infection sites. CAMARGO and CAMARGO (1974) reported great inoculation efficiency when conidial suspensions of *F. moniliforme* var. *subglutinans* were sprayed on inflorescences with opened flowers. ROHRBACH and PFEIFFER (1976) inoculating pineapple fruits with *Penicillium funiculosum* under field conditions obtained very significant infection levels after floral induction. Higher infection was observed with inoculations made 6 weeks after induction.

Determination of the inflorescence stages most feasible to infection by *F. moniliforme* var. *subglutinans* should be very important to establish effective control measures. One approach of this subject was previously presented by VENTURA et al. (1979).

* - José Aires VENTURA - Empresa Capixaba de Pesquisa Agropecuária-EMCAPA, Setor de Fitopatologia, Cx. postal 391, Vitória (ES) 29 000 Brasil.
Luiz Antonio MAFFIA et Geraldo Martins CHAVES - Universidade Federal de Viçosa, Dept. de Fitopatologia, Viçosa-MG, 56 570 Brasil.

MATERIAL AND METHODS

The work was carried out in 1977 and 1978, under field conditions,in Serra County,Espirito Santo State, Brazil,with the cultivar Smooth Cayenne grown according to the recommended practices for that State (EMBRATER/EMBRAPA, 1976). The experimental design was randomized blocks, in a 4x5x3 factorial schem, with four inoculum levels, five inoculation times and three replications. In each plot 10 plants were arranged in lines separated by border plants. Flowering was chemically induced by calcium carbide (GIACOMELLI, 1975).

F. moniliforme var. *subglutinans* was isolated from naturally infected fruits, and grown on PDA (potato-dextrose-agar) for 12 to 15 days at 26-28°C in Roux flasks. Inoculum was prepared by adding sterile distilled water plus adesive Novapal (Bayer do Brasil S.A.) 0,1 % into each flask, scrapying the cultural surface with a spatula and filtering the suspensions through cheesecloth.

Conidial concentrations were adjusted to 10^7, 10^4 and 10^1 conidia/ml. About 15 ml of each conidial suspension or 15 ml of sterile distilled water were sprayed on foliar rosette 2, 6, 10, 14 and 18 weeks after artificial floral induction.

Harvest was done when about 50 % of the fruitlets presented indication of ripening. Infection was estimated by the number of infected fruitlets on fruits and the disease index determined by Meckinney's formula (CIRULI and ALEXANDER, 1966).

$$DI = \frac{\Sigma (f.v.).\ 100}{NX},$$ where DI = disease index;

Σ = summation ; f = number of fruits in each infection degree ; v = disease score (0 = none fruitlet showing symptoms ; 1 = one fruitlet with symptoms ; ... 5 = five fruitlets with symptoms) ; N = total number of fruits ; and x = maximal value of infection degree.

RESULTS AND DISCUSSION

Inoculations until 10 weeks after artificial flower induction were very effective and the disease severity reached high level after two and six weeks, mainly with the higher inoculum concentrations, as shown in figure 1.

Comparisons between the averages of DI at different inoculum levels and on different inoculation times are given in table 1. During the second week after forcing, all DI averages showed statistically significant differences, and the highest average of infected fruits was obtained with the higher inoculum concentration. The disease index in the control, represented natural infection. The inoculum concentrations, of 10^4 and 10^7 conidia/ml six weeks after floral induction, produced the highest infection indices. The results observed in the control and 10^1 conidia/ml treatments were similar. All inoculum concentrations showed similar averages at 14 and 18 weeks after induction. Within these periods, although the inoculum concentrations varied, the flowers were probably closed and no infection sites were available.

Control averages for all times were similar. The highest levels of diseases with 10^1, 10^4 and 10^7 conidia/ml were observed at six weeks, and the two weeks. These results differ from those obtained by BOLKAN et al. (1979), who reported infection only after the opening, of the flowers. In the climatic conditions of Espírito Santo State Fusarium infection was observed before anthesis. This can be explained by the appearance of microfissures during inflorescence formation, and by insect injuries, since after the 7th week the insecticide carbaryl was used for control of fruit borer (*Thecla basilides*). Probably few flowers remained open after 14 weeks, since the infection level remained the same.

Among the inoculum concentrations the infection indices were higher from sixth to second weeks after induction showing that the period between the second and sixth weeks seems to be critical for disease occurrence.

CONCLUSIONS

The developing stage of the pineapple inflorescence is very important for infection by *F. moniliforme* var. *subglutinans*. KERNS et al. (1936) verified that the flowers and fruitlets of the cv. Smooth Cayenne began developing since the first week after floral induction and that the inflorescence was completely formed by the fifth and sixth weeks, but only emerge from the foliar rosette after the sixth weeks. In this work the highest infection levels were obtained between the second and sixth weeks after flower induction. The inoculations at the 14th and 18th weeks after induction did not differ of the control suggesting that the flowers were closed. These data are similar to those obtained by ROHRBACH and PFEIFFER (1976) with fruitlet core rot disease caused by *Penicillium funiculosum*. Recently, MATOS (1978), evaluating inoculation methods of *F. moniliforme* var. *subglutinans* in fruits of cv. Pérola, obtained high infection levels before flower opening, although CAMARGO and CAMARGO (1974) made inoculations only when the flowers were opened. The present data showed that inoculations made before anthesis were very efficient. The results, characterized by a quicker disease incidence curve than that obtained by BOLKAN et al. (1979), could probably be attributed to insect interference, since we applied insecticides only after the seventh week, and throughout growth cracks, which occur naturally during inflorescence development.

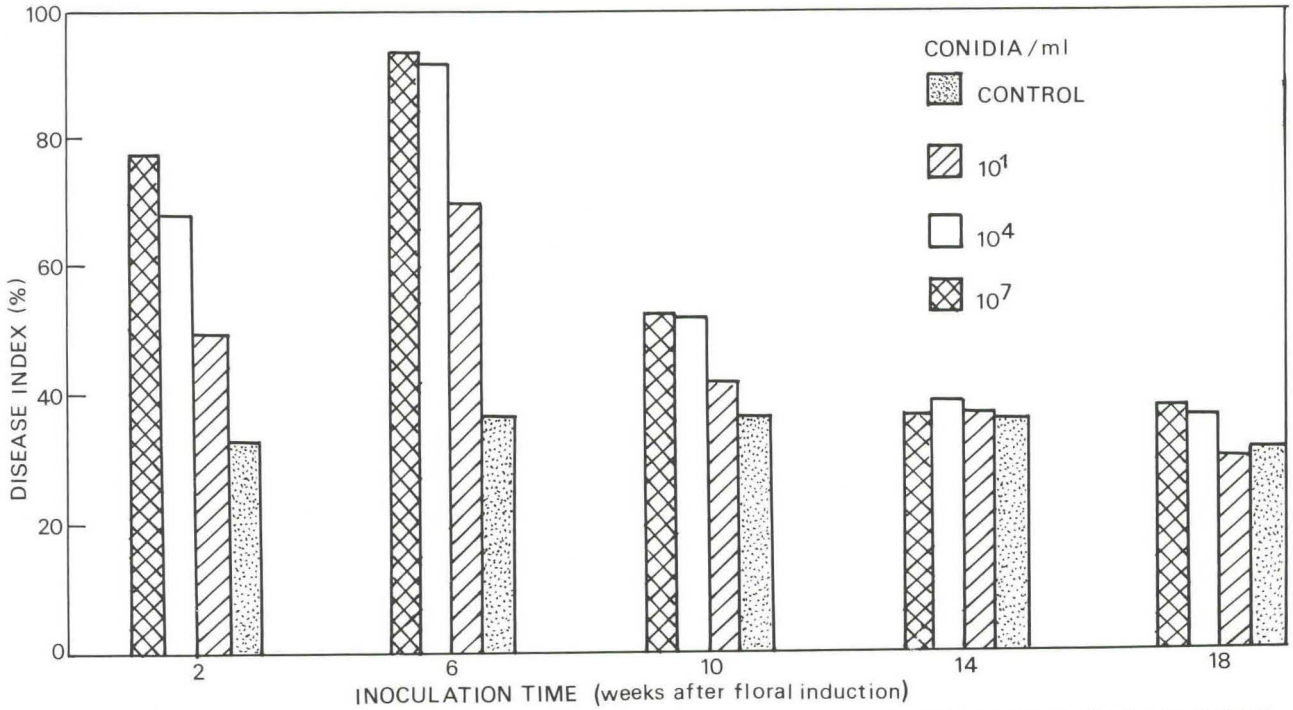

Figure 1 • FUSARIUM DISEASE INDICES IN PINEAPPLE FRUITS, CV. SMOOTH CAYENNE, UNDER FIELD CONDITIONS, OBTAINED WITH 3 INOCULUM CONCENTRATIONS AT FIVE DIFFERENT INOCULATION TIMES, IN THE STATE OF ESPIRITO SANTO, BRAZIL.

TABLE 1 - Effect of *F. moniliforme* var. *subglutinans* applied at five different times after floral induction and four different inoculum concentrations on cv. Smooth Cayenne, in Espírito Santo State conditions. Disease Index in % (w).

Inoculum concentration (Conidia/ml)	weeks after floral induction				
	2	6	10	14	18
0	35.26 d (x) A (y)	37.02. c A	37.32 b A	36.56 a A	36.96 a A
10^1	44.45 cB	56.62 b A	40.20 b C	37.92 a CD	35.71 a D
10^4	56.01 b B	73.10 a A	45.68 a C	38.21 a D	37.17 a D
10^7	61.54 a B	74.77 a A	46.06 a C	37.14 a D	37.64 a D

(w) - Data were transformed to arc $\sin\sqrt{\%}$, and each value is the mean of three replications.
(x) - Values in each column followed by the same small letter are not significantly different, according to Tukey's test (p = 0,01).
(y) - Values in each line followed by the same capital letter are not significantly different, according to Tukey's test (p = 0,01).

Knowledge of the critical stage of pineapple inflorescence infection by *F. moniliforme* var. *subglutinans* could aid the investigation of resistant cultivars and the knowledge of its etiology. It would also allow to develop a schedule for fungicide application. Further research is necessary to establish more accurately the infection time.

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