Etiology

Pathogenicity of Epiphytic *Fusarium moniliforme* var. *subglutinans* to Pineapple

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ABSTRACT

Dianese, J. C., Bolkan, H. A., da Silva, C. B., and Couto, F. A. A. 1981. Pathogenicity of epiphytic *Fusarium moniliforme* var. *sublgutinans* to pineapple. Phytopathology 71:1145-1149.

Two hundred fifteen isolates of *Fusarium* spp. were obtained from washings of 0.9^2 of symptomless leaf surface of pineapple cultivar Smooth Cayenne. Twenty-one (9.7%) of the isolates were *F. moniliforme* var. *subglutinans*. Nineteen of these isolates induced symptoms with variable degrees of disease severity on leaves, inflorescences, and/or lateral sprouts of susceptible pineapple cultivars. An epiphytic isolate of *F. oxysporum* and another of *F. solani* were both nonpathogenic. *Fusarium* spp. found in similar leaf washings from cultivar Perola included 26.9% of *F. moniliforme* var. *subglutinans*.

Additional key words: pineapple fruit and basal rot, pineapple gummosis.

Fruit rot caused in pineapple (*Ananas comosus* (L.) Merr.) by *Fusarium moniliforme* var. *subglutinans* Wr. & Reink. is a serious disease in Brazil that causes losses of up to 80% of the commercial fruits (9). The fungus survives in plant debris (2,8); however, other ecological aspects of the disease have not been considered except for a preliminary study on the microbial flora of the leaf surface of pineapple (5).

The purpose of this investigation was to study further the epiphytic population of *F. moniliforme* var. *subglutinans* present on symptomless leaves of pineapple and to determine its ability to induce disease on leaves, fruits, and sprouts of that plant.

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MATERIALS AND METHODS

Isolation of *F. moniliforme* var. *subglutinans* from the surfaces of symptomless pineapple leaves. Fifty-eight leaves collected at

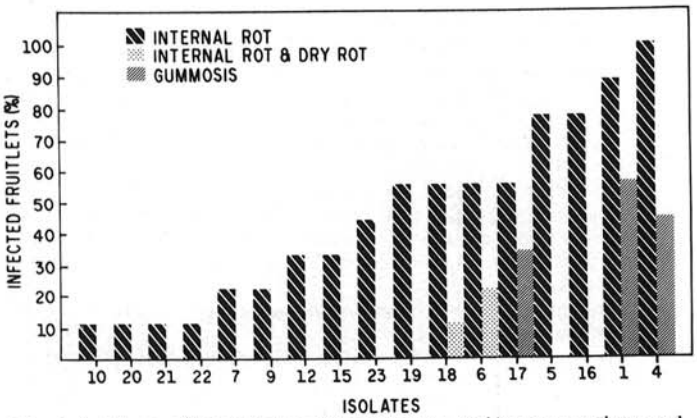

Fig. 1. Incidence of internal rot, external dry rot, and/or gummosis caused in fruitlets of pineapple cultivar Boituva by epiphytic isolates of *Fusarium moniliforme* var. *subglutinans* from symptomless leaves. Data were recorded 30 days after inoculation.

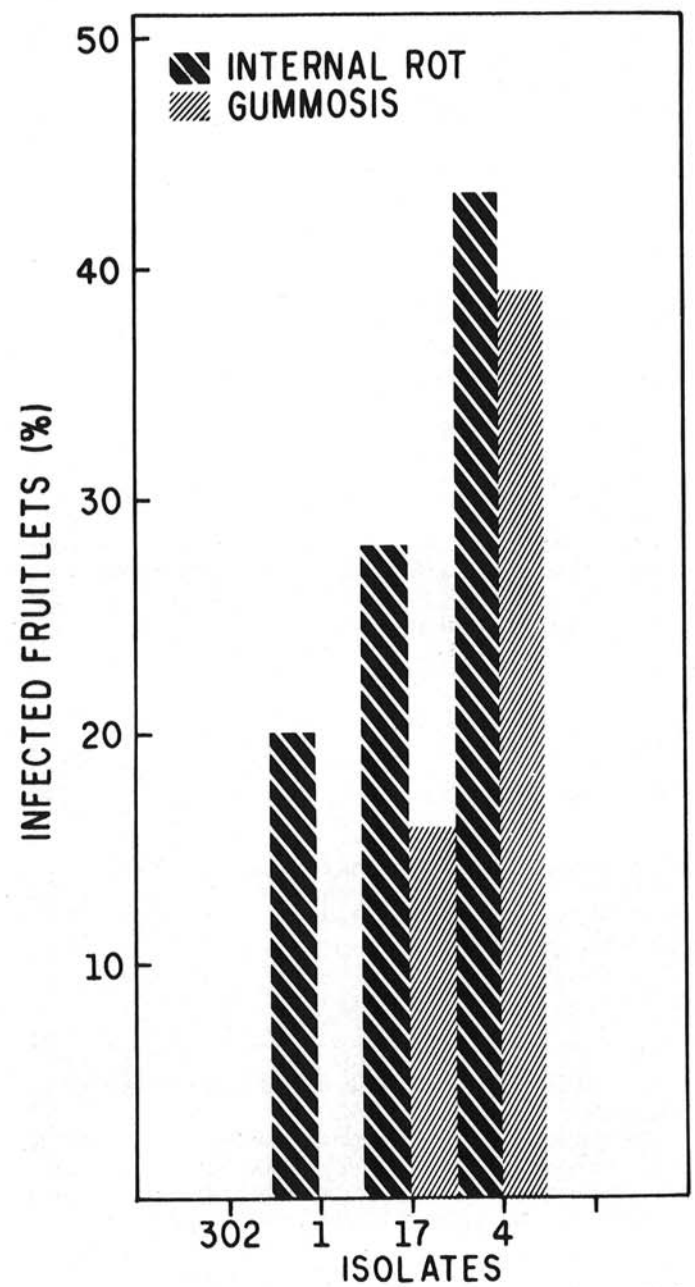

Fig. 2. Virulence of *Fusarium moniliforme* var. *subglutinans* isolates UnB-1, UnB-4, UnB-17, and UnB-302 to pineapple cultivar Smooth Cayenne 30 days after inoculation.

random from symptomless cultivar Smooth Cayenne pineapple plants growing in a 3-ha field in Brasília, Brazil, were taken to the laboratory for analysis. Each leaf was dipped into a sterile 1-L graduate cylinder containing 800 ml of sterile distilled water. The cylinder was capped and shaken by inverting it 10 times. The resulting liquid suspension was centrifuged for 20 min at 16,000 *g* in sterile bottles at 5 C. The pellet was suspended in 20 ml of sterile distilled water. Samples (1 ml) were pipetted into each of 20 sterile petri plates and 20 ml of Komada's medium (6) at 45 C with 2% sucrose added as the carbon source. Control treatments contained only sterile water and the medium. The same procedure was applied to a similar leaf sample from plants of cultivar Perola.

The plates were incubated at 24 ± 2 C. After 7 days, colonies of *Fusarium* spp. were transferred to potato-dextrose agar (PDA) for final identification. The isolates of *F. moniliforme* var. *subglutinans* were numbered from UnB-1 to UnB-23, except for UnB-11 and UnB-14, which were used to designate isolates of *Fusarium solani* and *F. oxysporum*, respectively. Isolate UnB-302 (ATCC-38067) from rotting pineapple fruit also was used to compare the virulence of the isolates from leaf samples.

Inflorescence infection. Individual pineapple inflorescences 20–25 cm long with a diameter of 15–18 cm were inoculated with a conidial suspension containing 10^7 spores of *F. moniliforme* var. *subglutinans* per milliliter. A sterile toothpick was dipped in inoculum and introduced into individual flowers of each inflorescence to a depth of 2.5 cm. Control flowers received the same treatment except the toothpicks were dipped in sterile distilled water. Three flowers on each of three inflorescences of pineapple cultivar Boituva were inoculated with each of 21 isolates in an experiment using cv. Boituva. For the test with cultivar Smooth Cayenne, four isolates were inoculated, each in 21 flowers (three per inflorescence). In all cases, inflorescences also received the control treatment. Results were read 30 days after inoculation of flowers when developing fruits were still in the green stage.

Leaf infection. Detached leaves of cultivar Boituva 75–80 cm long were selected for inoculation. Leaf surfaces were rubbed with cotton soaked in a 1% sodium hypochlorite solution washed with sterile distilled water before being inoculated. Mycelial plugs (4-mm diameter) taken from 8-day-old PDA cultures of *F. moniliforme* var. *subglutinans* isolates UnB-4, UnB-17, UnB-23, UnB-302, and *F. oxysporum* isolate UnB-14 were placed on puncture wounds made on the detached leaves with sterile toothpicks. The control treatments received only PDA plugs. The leaves were held vertically with their bases in 3 cm of water at 24/26 C on a laboratory bench, as described previously (3,4). The inoculations were replicated three times and made at 8, 30, and 45 cm from the base of the leaf and the results were recorded after 20 days. In addition, 8-cm-long foliar segments, cut either from the leaf base or from an area located between 15 and 23 cm from the leaf base, were inoculated with the same isolates and incubated for 10 days at 100% relative humidity at 26 C. Sterile toothpicks were used to wound the leaf pieces at their centers where 4-mm-diameter agar plugs containing the fungal growth were placed in contact with the host tissue. Each inoculation was replicated three times.

Infection of sprouts. Pineapple sprouts (20 cm long) were inoculated with each of the 21 isolates of *F. moniliforme* var. *subglutinans* obtained from leaf washings and the fruit isolate UnB-302. *F. oxysporum* isolate UnB-14 and *F. solani* isolate UnB-11, both isolated from pineapple leaf surfaces, were also included. The inoculation procedure consisted of immersion of sprouts (punctured with a 1-mm-diameter nail) into a suspension of microconidia and macroconidia containing a total of 10^7 spores per milliliter, as described by Souto and Matos (10). All inoculations were replicated five times.

RESULTS

Isolation of *F. moniliforme* var. *subglutinans* from symptomless pineapple leaves. Two hundred fifteen colonies of *Fusarium* spp. were recovered from the washings of 58 symtomless leaves of cultivar Smooth Cayenne with a total surface area of 0.9 m^2. Twenty-one (9.7%) of the colonies were identified as *F.*

moniliforme var. *subglutinans*. A sample taken from cultivar Perola contained *F. moniliforme* var. *subglutinans* in 26.9% of its total Fusarium population.

Inflorescence infection. The 21 epiphytic isolates of *F. moniliforme* var. *subglutinans* varied in degree of virulence to the resulting immature fruits of cultivar Boituva. Leaf isolates UnB-2, UnB-3, UnB-8, and UnB-13 were not virulent to cultivar Boituva; however, the other epiphytic isolates (Fig. 1) induced either internal rot, external dry rot, or gummosis of the fruitlets. Other Fusaria (UnB-11 and UnB-14) and the fruit isolate of *F. moniliforme* var. *subglutinans* (UnB-302) were avirulent to that cultivar.

When inoculated on cultivar Smooth Cayenne (Fig. 2), isolates UnB-4 and UnB-17 were virulent to field-grown plants producing gummosis in 33 and 44% of the fruitlets of cultivar Boituva, compared to 16 and 40% of those of cultivar Smooth Cayenne. Isolate UnB-1 caused 20% internal fruit rot and no gummosis on cultivar smooth Cayenne; however, on cultivar Boituva it produced 89% of internal rot and 55% of gummosis. Isolate UnB-

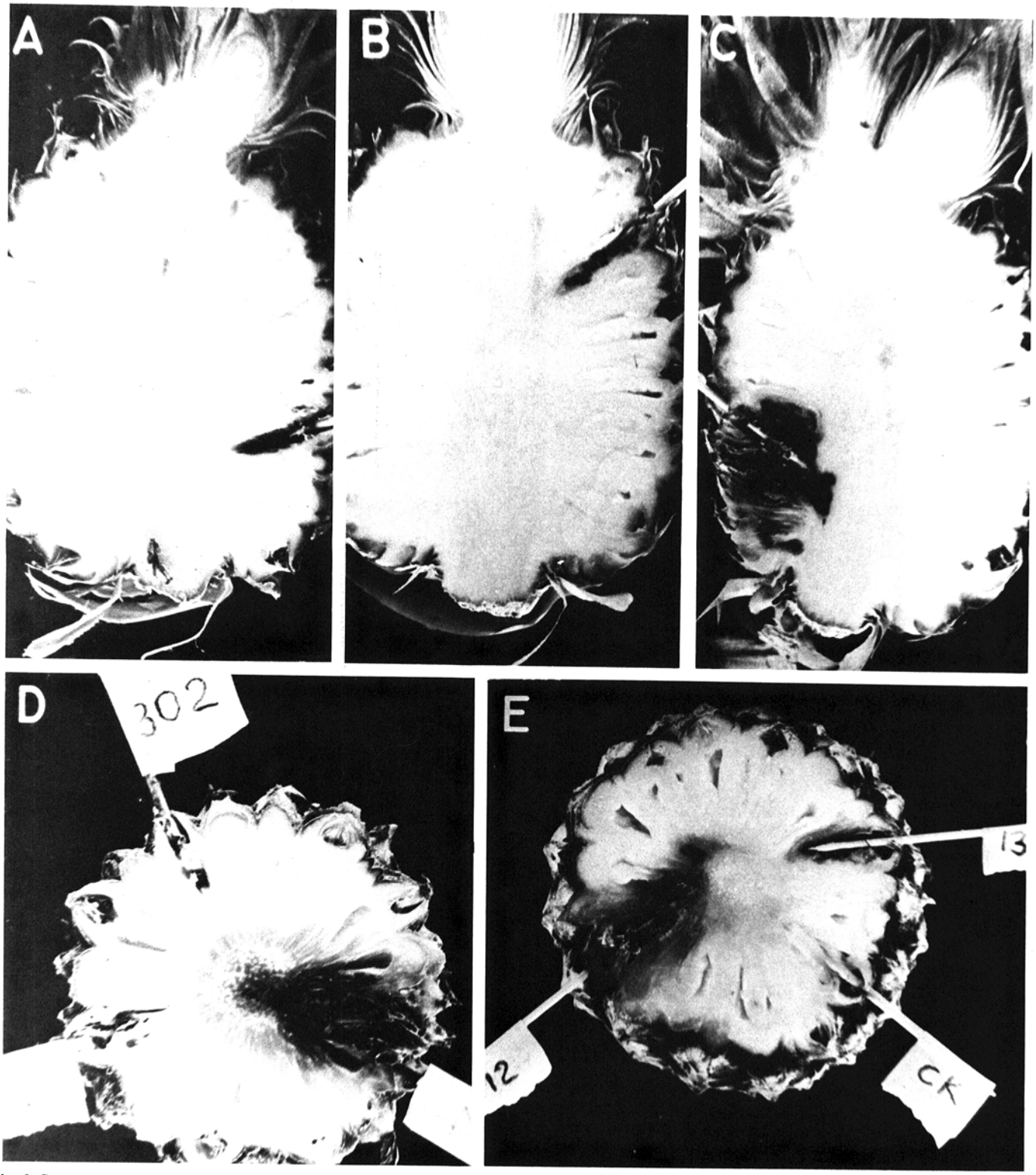

Fig. 3. Symptoms produced in the field 30 days after inoculation with *Fusarium moniliforme* var. *subglutinans* on fruits of pineapple cultivar Smooth Cayenne (A = control treatment, B = isolate UnB-302, C = isolate UnB-4) and cultivar Boituva (D = isolates UnB-302 and UnB-4, E = isolate UnB-12 and UnB-13).

302 was avirulent to both cultivars. Figure 3 illustrates the variability in virulence found among the isolates tested.

Leaf infection. Isolates UnB-4, UnB-17, and UnB-23 were pathogenic when inoculated at a distance of 8 cm from the leaf base; however, isolates UnB-14 and UnB-302 were not. When inoculations were done between 30 and 45 cm from the leaf base, where tissue was more differentiated, none of the isolates caused an infection.

Inoculations of 8-cm-long segments cut from leaves produced similar results, but all isolates, including the nonpathogenic *F. oxysporum* isolate UnB-14, infected segments cut from the leaf base (Fig. 4). Pathogenic isolates UnB-4, UnB-17, and UnB-23 could be separated from the nonpathogenic UnB-302 and UnB-14 isolates when inoculation was performed on leaf segments taken from between 15 and 23 cm from the leaf base (Fig. 5).

Infection of sprouts. Sprouts of cultivar Perola inoculated with isolates of *F. moniliforme* var. *subglutinans* epiphytic on cultivar Smooth Cayenne, showed variable responses which permitted grouping of the isolates according to their degree of virulence: highly virulent, able to rot and kill all five sprouts inoculated, and prevent root formation (six isolates); virulent, able to infect all five sprouts but allowing root formation on at least two sprouts (11 isolates); avirulent, unable to infect any sprout (two isolates). An isolate of *F. solani* (UnB-11) and another of *F. oxysporum* (UnB-14) were not pathogenic to sprouts of cultivar Perola.

DISCUSSION

The twenty-one isolates of *F. moniliforme* var. *subglutinans* obtained from 0.9 m^2 of leaf surface of Smooth Cayenne pineapple suggested that there could be 35 propagules of the fungus on a typical plant with 10 (2–5 cm basal width × 60 cm length) triangular leaves (representing an average plant size on a 12-mo-old plantation). The epidemiological meaning of these figures should be evaluated, because the pineapple plant, due to its morphological characteristics, functions as a natural spore trap, receiving propagules like an inverted umbrella. Thus, fungal populations could be monitored through periodic washings of a known leaf area to yield an estimate of the inoculum level at the plant surface. These data could then be correlated with infection levels to serve as an indicator of potential epidemics of pineapple fruit rot. The relationship between fungal propagules and leaf surface should be explored to determine whether *F. moniliforme* var. *subglutinans* is a true leaf resident (7).

Isolates of *F. moniliforme* var. *subglutinans* from pineapple leaves infected inflorescences which resulted in diseased fruits. Leaves and sprouts were infected also. Furthermore, those isolates varied in virulence to pineapple cultivars commonly grown in Brazil. Isolate (UnB-302) from pineapple fruit previously shown to be pathogenic (1) apparently lost its virulence after 4 yr of storage in culture.

Inoculations of pineapple leaves with isolates of *F. moniliforme* var. *subglutinans* showed that they varied in virulence (4). No variability in virulence to fruits and sprouts was observed. Most economic loss is experienced when these plant organs are infected (1,9).

Mature tissue was more resistant than juvenile leaf tissue. Thus, the same isolate may exhibit either high virulence or avirulence if inoculated at different heights on the same leaf. For results to be comparable, leaf segments should be taken from identical areas of similar leaves.

These results suggest that the role of the epiphytic stage of the fungus in the dissemination of the pathogen should be an important area of investigation in studies on pineapple fruit rot in Brazil.

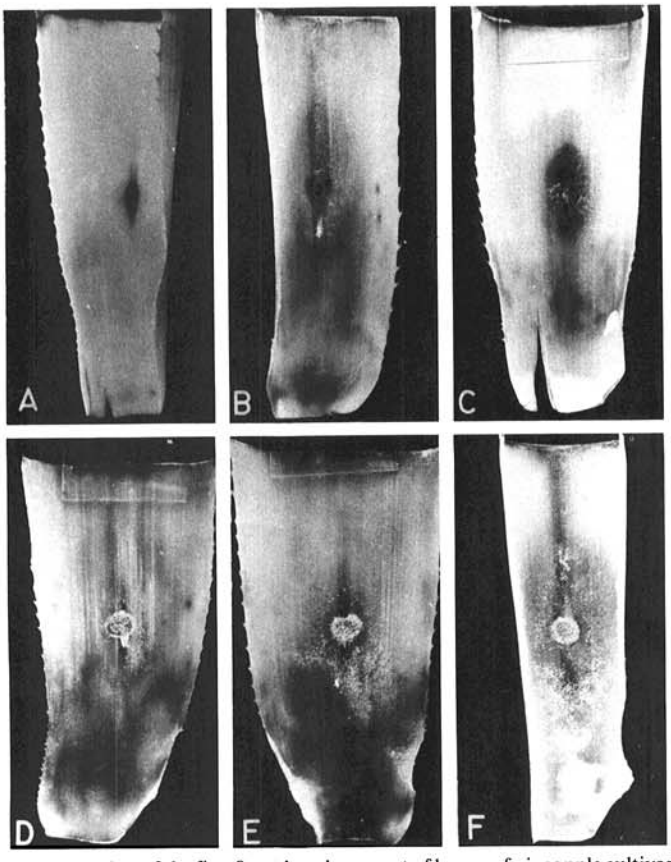

Fig. 4. Rotting of the first 8-cm basal segment of leaves of pineapple cultivar Boituva following puncture inoculation with *Fusarium moniliforme* var. *subglutinans* isolates (**B**, UnB-302; **D**, UnB-23; **E**, UnB-17; and **F**, UnB-4), and **C**, isolate UnB-14 of *Fusarium oxysporum* compared to **A**, the punctured but uninoculated control.

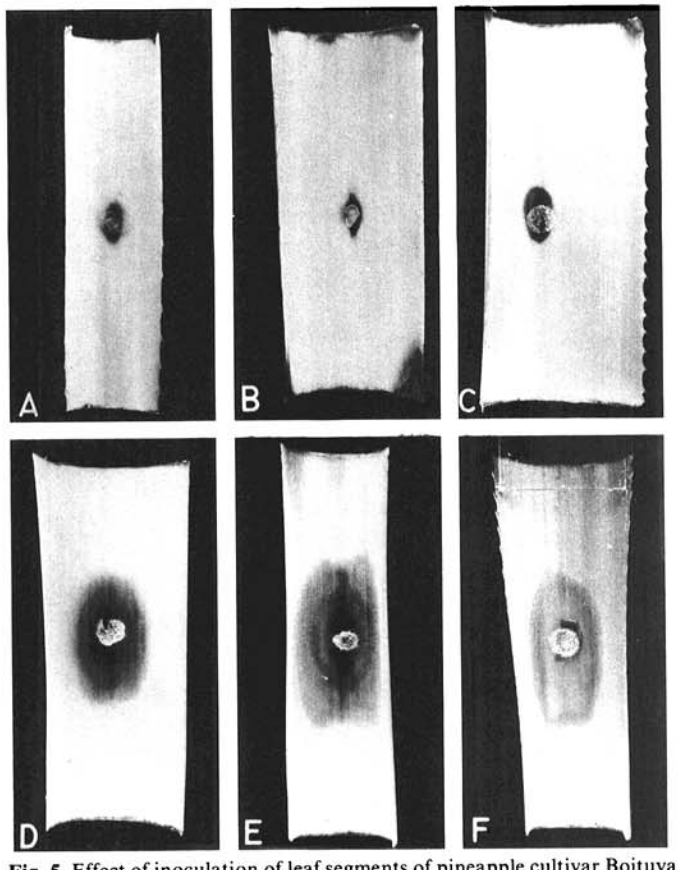

Fig. 5. Effect of inoculation of leaf segments of pineapple cultivar Boituva between 15 and 23 cm from the leaf base with *Fusarium moniliforme* var. *subglutinans* isolates (**B**, UnB-302; **D**, UnB-23; **E**, UnB-17; and **F**, UnB-4) and an isolate of *F. oxysporum* (**C**, UnB-14) compared to **A**, the punctured but uninoculated control.

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