Reaction of pineapple accessions to inoculation with *Fusarium moniliforme* var. *subglutinans.*

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REACTION OF PINEAPPLE ACCESSIONS TO INOCULATION WITH *FUSARIUM MONILIFORME* VAR. *SUBGLUTINANS.*

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ABSTRACT - The reaction of pineapple accessions to *Fusarium moniliforme* var. *subglutinans*, the causal agent of the pineapple fusariose, the most serious disease of that crop in Brazil, was evaluated under greenhouse conditions. Plantlets obtained either from tissue culture, from stem section technique, or from lateral bud development (destruction of the apical meristem) were wounded at the base, immersed in an inoculum, 10^5 conidia/ml, for 3 minutes and transferred to pots, 7 x 7 x 6.5 cm, containing a mixture of 1/3 «terreau neuhaus» + 1/3 «terre de bruyère» + 1/3 sand as substrate. Disease incidence estimates, performed 2 months after inoculation, were based on a zero to 6 numerical rating system. Resistance to *F. moniliforme* var. *subglutinans* was detected in *Ananas comosus*, accessions Blanca, Samba, Angelita 1, Iris 1, BR 189 and Tapiricanga ; in *Ananas bracteatus*, accessions Ananas São Bento, Branco do Mato and BR 123 ; and in *Ananas parguazensis*, access VE 64. The accessions GU 101, Ignacio 1, Valera Amarilla, Cabello 1 and Roxo de Tefé showed tolerance to the pathogen.

COMPORTEMENT, APRES INOCULATION, DE DIFFERENTS ANANAS VIS-A-VIS DE *FUSARIUM MONILIFORME* VAR. *SUBGLUTINANS.*

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RESUME - La réaction de différentes accessions d'ananas vis-à-vis de *Fusarium moniliforme* var. *subglutinans* agent causal de la fusariose, maladie la plus grave sur cette production au Brésil, a été évaluée par inoculation expérimentale en conditions contrôlées. Le matériel obtenu soit par culture *in vitro*, soit après multiplication végétative est inoculé par trempage de la base des plantules blessées dans une suspension conidienne. Les plants sont ensuite transférés en serre et repiqués sur un substrat 1/3 «terreau» + 1/3 «terre de bruyère» + 1/3 sable. La maladie est évaluée 2 mois après l'inoculation. Un niveau de résistance élevé est mis en évidence chez *Ananas comosus* : Blanca, Samba, Angelita 1, Iris 1, BR 189 et Tapiricanga ; chez *Ananas bracteatus* : Ananas São Bento, Branco do Mato et BR 123 ; et chez *Ananas parguazensis* : VE 64. Les accessions GU 101, Ignacio 1, Valera Amarilla, Cabello 1 et Roxo de Tefé se sont révélées partiellement résistantes.

INTRODUCTION

The pineapple fusariose, caused by *Fusarium moniliforme* Sheld. var. *subglutinans* WR. and RG., was first reported in Brazil causing fruit rot on the cultivar Smooth Cayenne (Kimati and Tokeshi, 1964). Currently the disease is widespread all over the pineapple producing areas of the country (Matos, 1987) causing losses as high as 80% of marketable fruits (Robbs *et al.*, 1965). Besides causing high losses of marketable pineapple fruits, the pathogen infects approximately 40% of the asexual propagative material and about 20% of the pineapple plants prior to harvest (Aguilar, 1981).

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Control of the pineapple fusariose has been based mainly on the use of pathogen free propagating material (Reinhardt and Cunha, 1981), on the evasion of the pathogen by performing the flowering induction treatment in periods which allow the inflorescence development under environmental conditions unfavorable to disease development (Matos, 1987) and on the direct protection by chemical control (Choairy *et al.*, 1984 ; Matos and Caldas, 1978).

The potential for disease resistance as a control measure for the pineapple fusariose has been suggested in studies based on observations carried out either under field conditions (Giacomelli *et al.*, 1969 ; Giacomelli and Teófilo Sobrinho, 1984) or under artificial inoculation technique (Souto and Matos, 1978 ; Cabral *et al.*, 1985 ; Matos and Cabral, 1987). Considering that growing resistant cultivars constitute one of the most cheap and efficient control measure of plant diseases, the identification of sources of resistance to *F. moniliforme* var. *subglutinans* is a very important step to obtain pineapple varieties resistant to the pathogen.

MATERIAL AND METHODS

The current study was conducted in the Plant Pathology Laboratory and greenhouses of the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) - Institut de Recherches sur les Fruits et Agrumes (IRFA), located in Montpellier, France, from November, 1990 to September 1991.

Pineapple accessions. The 45 pineapple accessions evaluated for their reaction to inoculation with *F. moniliforme* var. *subglutinans* are listed in Table 1. Evaluation was performed either on tissue culture pineapple plantlets (Pannetier and Lanaud, 1976), on plantlets obtained by stem section technique (Collins, 1960), or by eliminating the apical meristem to force lateral bud development. The CIRAD-IRFA, Martinique, provided the propagative material used to produce plantlets by stem sectioning and by lateral bud development.

TABLE 1 - Accessions of the Pineapple Collection of the Centre de Coopération internationale en Recherche Agronomique pour le Développement (CIRAD) - Institut de Recherches sur les Fruits et Agrumes (IRFA) evaluated, by artificial inoculation, for resistance to *Fusarium moniliforme* var. *subglutinans*.

Species	Accessions	Geographic origin
Ananas ananassoides	VE 79	Venezuela
	VE 80	Venezuela
	BR 47 - Ananas dos Indios *	Brazil
Ananas bracteatus	BR 20 - Branco do Mato *	Brazil
	GU 54 - Branco var. typica *	Guinea
	BR 123 - FRF 19	Brazil
	BR 2 - Ananas São Bento	Brazil
Ananas comosus	AN 38 - Champaka *	Hawaii
	GU 71 - Red Spanish *	Puerto Rico
	SI 85 - Singapore Canning	Singapore
	BR 1 - Pérola	Brazil
	TA 39 - Pomare	Tahiti
	BR 16 - Roxo de Tefé	Brazil
	BR 77 - Barreiras 2	Brazil
	RE 43 - Victoria *	Reunion Island
	BR 189 - FRF 175	Brazil
	BR G01 - C BAU *	Brazil
	BR G03 - Boituva *	Brazil
	VE 29 - Angelita 1 *	Venezuela
	VE 69 - Iris 1	Venezuela
	VE 88 - Ignacio 1	Venezuela
	VE 145 - Valera Amarilla	Venezuela
	PE 56 - Roja Trujiliana	Peru
	PE 57 - Blanca	Peru
	PE 67 - Samba	Peru
	RE 83 - Burguat	Reunion Island
	FI 84 - Ripley Queen	Fidji Island
	VE 57A - Cabello 1	Venezuela
	YO - Smooth Cayenne **	
	HT - Queen **	
	A - Queen	
	B - Queen	
	KE 15 - Kenia *	Kenia
	BR G05 - Tapiricanga *	Brazil
	BR 58 - Inerme de Rondônia *	Brazil
Ananas lucidus	BR 67 - Selvagem 6 *	Brazil
Ananas parguazensis	VE 64	Venezuela
	VE 131 - Cucurital 2	Venezuela
	VE 78 - Pacairo	Venezuela
Undetermined	VE 129	Venezuela
	PH 79 - Phi 1	Philippines
	PH 80 - Phi 2	Philippines
	PH 86 - Phi 3	Philippines
	GU 101	Guinea
	BR 39 - BGA 5	Brazil

* - Accessions supplied by Dr. M-F Duval, CIRAD-IRFA, Martinique.
** - Accessions obtained from VITROPIC - France.

Pineapple plantlets, issued from tissue culture, were removed from tubes, washed in running tap water, transferred to pots, 7 x 7 x 6.5 cm, containing a mixture of 1/3 «Terreau neuhaus» + 1/3 «Terre de bruyère» + 1/3 sand as substrate, and kept under growth chamber conditions, 26 to 28°C and 80% relative humidity, for one month before inoculation. Plantlets obtained either by stem section technique or by lateral bud development (destruction of the apical meristem) were kept under greenhouse conditions prior to inoculation with the pathogen.

Pathogen. *Fusarium moniliforme* var. *subglutinans*, isolated from naturally infected pineapple fruit, cultivar Pérola, was maintained on potato-dextrose-agar (PDA) slants at about 4°C. Inoculum was prepared from 10 day old cultures, grown on PDA, by aseptically adding sterile distilled water to the petri dishes ; conidia were harvested by scraping the surface of the colony with a small brush. The obtained conidial suspension was filtered through three layers of cheesecloth ; concentration was determined with the aid of a hemacytometer and adjusted to 10^5 conidia/ml (Matos, 1978) ; the pathogen isolate Fms 40, selected as highly virulent to the cultivar Pérola, was used in this study.

Inoculation. Plantlets were wounded with an apparatus consisting of an 1 mm diameter stainless needle the blunt end of which protruded 3 mm from a rubber block. A puncture wound was produced by pressing the protruding needle against the surface of the pineapple plantlets, at the basal region. Inoculation was performed by dipping wounded plantlets in an inoculum, 10^5 conidia/ml, for 3 minutes (Matos, 1978). After inoculation the plantlets were kept under greenhouse conditions, 25 to 30°C, for 2 months, and then inspected for disease development. Evaluation was based on a zero to 6 numerical rating system in which 0 = no disease development in the stem ; 1 = 1 to 2% of the stem infected by the pathogen ; 2 = 3 to 5% ; 3 = 6 to 10% ; 4 = 11 to 20% ; 5 = 21 to 50% ; and 6 = 51 to 100% (James, 1971).

TABLE 2 - Comparative virulence of isolates of *Fusarium moniliforme* var. *subglutinans* inoculated in pineapple plantlets, cultivar Pérola.

Isolates	Disease index *
Fms 40	5.8 a
Fms 33	5.8 a
Fms 36R	5.7 a
Fms 31	5.5 ab
Fms 36B	5.4 ab
Fms 44	4.1 abc
Fms 32	3.7 bc
Fms 26	3.0 bc
Fms 28	2.9 c
Fms 42	0.5 d

* - values followed by the same letter are not significantly different (Tukey's test, P = 0.01)

For the purpose of this study, resistant reaction is defined as no disease development in inoculated plantlets, while accessions in which infection takes place but the colonization of the host tissue proceeds slowly, reaching a rate of 3 or less of disease index, two months after inoculation, and which shows significantly less disease severity than the susceptible control, are considered as tolerant to the pathogen.

The experiments were conducted in a completely randomized design, with 5 replicates. The pineapple cultivars Pérola, Smooth Cayenne or Queen were used as susceptible control.

During the conduction of the experiments watering was performed as needed.

RESULTS AND DISCUSSION

The study of comparative virulence (Table 2) showed

TABLE 3 - Comparison between the behavior of tissue culture pineapple plantlets and asexual propagative parts of pineapple accessions regarding to the inoculation with *Fusarium moniliforme* var. *subglutinans*.

Pineapple accessions	Reaction *	
	TCP	APP
BR 1 - Pérola	S	S
BR 2 - Ananas São Bento	R	R
BR 16 - Roxo de Tefé	T	T
BR 20 - Branco do Mato	R	R
BR 39 - BGA 5	S	S
BR 58 - Inerme de Rondônia	S	S
BR 67 - Selvagem 6	S	S
BR 189 - FRF 175	R	R
FI 84 - Ripley Queen	S	S
GU 71 - Red Spanish	S	S
YO - Smooth Cayenne	S	S

* - TCP = tissue culture plantlets (which were allowed to grow for one month, under greenhouse conditions, before inoculation) ;
APP = asexual propagative parts ;
R = resistant ; T = tolerant ; S = susceptible.

TABLES 4 TO 11 - Incidence of *Fusarium moniliforme* var. *subglutinans* on inoculated pineapple plantlets.

TABLE 4 -

Pineapple accessions	Disease index *
A - Queen	6.0 a
B - Queen	6.0 a
YO - Smooth Cayenne	6.0 a
HT - Queen	5.2 a
BR 123 - *Ananas bracteatus*	0.0 b
VE 64 - *Ananas parguazensis*	0.0 b
PE 67 - Samba	0.0 b
BR 2 - Ananas São Bento	0.0 b

* - values followed by the same letter are not significantly different (Tukey's test, P = 0.01).

TABLE 5 -

Pineapple accessions	Disease index *
FI 84 - Ripley Queen	6.0 a
PH 80 - Phi 2	6.0 a
RE 83 - Burguat	6.0 a
PH 79 - Phi 1	5.8 a
PH 86 - Phi 3	5.6 a
VE 145 - Valera Amarilla	3.0 b
VE 88 - Ignacio 1	2.6 b
PE 57 - Blanca	0.0 c
VE 64 - *Ananas parguazensis*	0.0 c

* - Means followed by the same letter are not significantly different (Tukey's test, P = 0.01)

TABLE 6 -

Pineapple accessions	Disease index *
HT - Queen	5.4 a
VE 129	4.6 a
VE 78 - Pacairo	4.4 a
VE 131 - Cucurital 2	3.8 a
VE 80 - *Ananas ananassoides*	3.6 a

* - Values followed by the same letter are not significantly different (Tukey's test, P = 0,01).

TABLE 7

Pineapple accessions	Disease index *
HT - Queen	6.0 a
VE 79 - *Ananas ananassoides*	4.0 b
VE 57A - Cabello 1	2.6 c
VE 69 - Iris 1	0.0 d
PE 57 - Blanca	0.0 d

* - Means followed by the same letter are not significantly different (Tukey's test, P = 0.01)

that the isolates Fms 40, Fms 33 and Fms 36R were highly virulent to pineapple plantlets, cultivar Pérola, even though no significant difference was observed between them and the isolates Fms 31, Fms 36B and Fms 44. On the other hand, Fms 42 expressed the lowest level of virulence, while

TABLE 8

Pineapple accessions	Disease index *
RE 43 - Victoria	5.8 a
BR 1 - Pérola	5.6 a
SI 85 - Singapore Canning	4.8 ab
GU 54 - Branco var. typica	4.4 ab
BR 47 - Ananas dos Indios	4.0 ab
GU 71 - Red Spanish	3.4 b

* - Numbers followed by the same letter are not significantly different (Tukey's test, P = 0.01).

TABLE 9

Pineapple accessions	Disease index *
BR 1 - Pérola	5.5 a
BR 67 - Selvagem 6	4.8 a
TA 39 - Pomare	4.7 a
BR 39 - BGA 5	4.3 a
PE 56 - Roja Trujiliana	4.3 a
BR 16 - Roxo de Tefé	2.0 b
GU 101	1.8 b

* - Means followed by the same letter are not significantly different (Tukey's test, P = 0.01)

TABLE 10

Pineapple accessions	Disease index *
FI 84 - Ripley Queen	6.0 a
AN 38 - Champaka	5.8 a
BR G01 - C BAU	5.8 a
VE 29 - Angelita 1	0.0 b
BR G05 - Tapiricanga	0.0 b

* Values followed by the same letter are not significantly different (Tukey's test, P = 0.01)

TABLE 11

Pineapple accessions	Disease index *
BR G03 - Boituva	6.0 a
BR 58 - Inerme de Rondônia	5.6 a
BR 77 - Barreiras 2	5.2 ab
BR 1 - Pérola	5.2 ab
KE 15 - Kenia	4.2 b
BR 189 - FRF 175	0.0 c
BR 20 - Branco do Mato	0.0 c

* - Numbers followed by the same letter are not significantly different (Tukey's test, P = 0.01).

Fms 32, Fms 26 and Fms 28 showed intermediate reaction. These results are in accordance with those reported by Perriot (1980) and by Matos and Cabral (1987) who observed virulence variation in isolates of *F. moniliforme* var. *subglutinans* obtained from several pineapple growing areas.

The data in Table 3 show that the reaction of the pineapple accessions to inoculation with *F. moniliforme* var.

subglutinans was not affected by the kind of propagating material (tissue culture plantlets or asexual propagative parts) undergoing evaluation. These results indicate that the inoculation of tissue culture pineapple plantlets, one month after being transferred to soil, for evaluation of resistance to fusariose, constitute a fairly reliable technique.

The data concerning to the reaction of pineapple accessions to inoculation with *F. moniliforme* var. *subglutinans* under greenhouse conditions are shown in Table 4 to 11. A summary of the reactions of all evaluated accessions is presented in Table 12.

Under the conditions in which the current study was conducted, resistance to the pineapple fusariose, as expressed by no disease development in inoculated plantlets, was detected in the species *Ananas comosus* (L.) Merr., accessions Samba (Table 4), Blanca (Table 5), Iris 1 (Table 7), Angelita 1, Tapiricanga (Table 10) and BR 189 (Table 11) ; in the species *Ananas bracteatus* (Lindl.) Schultes, accessions Ananas São Bento, BR 123 (Table 4), and Branco do Mato (Table 11) ; and in *Ananas parguazensis* Camargo, accession VE 64 (Table 4). These results confirm those previously reported by Souto and Matos (1978), Giacomelli and Teófilo Sobrinho (1984) and Matos and Cabral (1987) who observed resistance to *F. moniliforme* var. *subglutinans* in the accessions Ananas São Bento, Tapiricânga and Branco do Mato, respectively. On the other hand the accessions Blanca, Samba, Angelita 1, Iris 1, BR 123, BR 189, and VE 64 constitute new sources of resistance to the pathogen, that can be used in pineapple breeding programs aiming to obtain fusariose resistant cultivars.

Resistance to *F. moniliforme* var. *subglutinans* in species of Bromeliaceae other than *Ananas comosus*, as found in this study, is in accordance with the results reported by Matos and Cabral (1987), who detected resistance to the pathogen in accessions of *Ananas ananassoides* (Baker) L.B. Smith, *Ananas bracteatus, Pseudananas sagenarius* (Arr. Câmara) Camargo, *Bromelia balançae* Mez., *Bilbergia* sp. and *Tillandsia* sp.

The pineapple accessions Ignacio 1, Valera Amarilla (Table 5), Cabello 1 (Table 7), Roxo de Tefé and GU 101 (Table 9) showed significantly less disease severity ($P = 0.01$) than the susceptible control, thus expressing tolerant reaction to *F. moniliforme* var. *subglutinans*. The tolerant reaction of Roxo de Tefé, as observed in this study, confirm the results found by Matos and Cabral (1987) in which Roxo de Tefé expressed tolerant reaction to 6 isolates of the pathogen.

Susceptibility to *F. moniliforme* var. *subglutinans* was detected in 30 out of 45 evaluated accessions, most of them cultivated in several pineapple growing areas of the world. The susceptible reaction of the accessions Smooth Cayenne (Table 4), Red Spanish, Pérola (Table 8), Selvagem 6 (Table 9), C BAU (Table 10), Boituva and Inerme de Rondônia (Table 11) as observed in this study, has been already reported (Kimati and Tokeshi, 1964 ; Giacomelli *et al.*, 1969 ; Giacomelli and Teófilo Sobrinho, 1984 ; Cabral *et al.*, 1985 ; Matos and Souto, 1985 ; Matos and Cabral, 1987). The accessions Queen - A, B and HT (Table 4), Ripley Queen, Phi 1, Phi 2, Phi 3, Burguat (Table 5), Pacairo, Cucurital 2, VE 80, VE 129 (Table 6), VE 79 (Table 7), Branco var. typica, Singapore Canning, Ananas dos Indios, Victoria (Table 8), Roja Trujiliana, Pomare, BGA 5 (Table 9), Champaka (Table 10), Kenia and Barreiras 2 (Table 11), where also, identified as susceptible to the pathogen.

TABLE 12 - Reaction of pineapple accessions to inoculation with *Fusarium moniliforme* var. *subglutinans*.

Pineapple accessions	Reaction *
PE 57 - Blanca	R
PE 67 - Samba	R
VE 29 - Angelita 1	R
VE 64	R
VE 69 - Iris 1	R
BR 189 - FRF 175	R
BR 2 - Ananas São Bento	R
BR 20 - Branco do Mato	R
BR 123 - FRF 19	R
BR G05 - Tapiricanga	R
GU 101	T
VE 57A - Cabello 1	T
VE 88 - Ignacio 1	T
VE 145 - Valera Amarilla	T
BR 16 - Roxo de Tefé	T
HT - Queen	S
A - Queen	S
B - Queen	S
FI 84 - Ripley Queen	S
YO - Smooth Cayenne	S
AN 38 - Champaka	S
KE 15 - Kenia	S
PE 56 - Roja trujiliana	S
PH 79 - Phi 1	S
PH 80 - Phi 2	S
PH 86 - Phi 3	S
RE 43 - Victoria	S
RE 83 - Burguat	S
GU 54 - Branco var. typica	S
GU 71 - Red Spanish	S
SI 85 - Singapore canning	S
TA 39 - Pomare	S
VE 78 - Pacairo	S
VE 79	S
VE 80	S
VE 129	S
VE 131 - Cucurital 2	S
BR 1 - Pérola	S
BR 39 - BGA 5	S
BR 47 - Ananas dos Indios	S
BR 58 - Inerme de Rondônia	S
BR 67 - Selvagem 6	S
BR 77 - Barreiras 2	S
BR G01 - C BAU	S
BR G03 - Boituva	S

* - R : resistant ; T : tolerant ; S : susceptible

CONCLUSIONS

The accessions Blanca, Samba, Angelita 1, VE 64, Iris 1, Ananas São Bento, Branco do Mato, BR 123, BR 189 and Tapiricanga are resistant to *F. moniliforme* var. *subglutinans*.

Resistance to *F. moniliforme* var. *subglutinans* is found in accessions of species of Bromeliaceae other than *Ananas comosus*, such as *Ananas bracteatus* and *Ananas parguazensis*.

The accessions GU 101, Ignacio 1, Valera Amarilla, Cabello 1 and Roxo de Tefé express tolerant reaction to *F. moniliforme* var. *subglutinans*.

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RESUMEN - La reacción de diferéntes introducciones de piña a *Fusarium moniliforme* var. *subglutinans*, el agente causal de la fusariosis de la piña, la principal enfermedad de este cultivo en Brasil, fué evaluada bajo condiciones de invernadero. Las plantulas fueron obtenidas por cultivo de tejido, por la técnica de corte de tallo, ó por el desarrollo de yemas laterales (destrucción del meristemo apical) ; estas plantulas fueron heridas en su base, sumergidas en una suspensión de inoculo (10^5 conidios/ml) por 3 minutos y transferidas a macetas (7 x 7 x 6,5 cm) conteniendo una mezcla de 1/3 «terreau neuhaus» + 1/3 «terre de bruyère» + 1/3 de arena, como substrato. Estimaciones de la incidencia de la enfermedad, se hiciéron 2 meses despues de la inoculación, basandose en una escala numérica de cero a 6. La resistencia a *F. moniliforme* var. *subglutinans* se detectó en *Ananas comosus*, introducciones Blanca, Samba, Angelita 1, Iris 1, BR 189 y Tapiricanga. En *Ananas bracteatus*, introducciones Ananas São Bento, Branco do Mato y DR 123 ; y en *Ananas parguazensis*, introdución VE 64. Las introduciones GU 101, Ignacio 1, Valera Amarilla, Cabello 1 y Roxo de Tefé mostraron tolerancia al patogeno.