

Susceptibility of Pineapple Cultivars to Fruit Diseases Incited by *Penicillium funiculosum* and *Fusarium moniliforme*

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ABSTRACT

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Several unnamed pineapple cultivars were inoculated in 2 successive years with *Penicillium funiculosum* and *Fusarium moniliforme* 1, 4, and 7 weeks following chemically-induced flowering. Unnamed cultivars A, D, and a commercial cultivar, Smooth Cayenne (cultivar X) were highly susceptible to fruitlet core rot (FCR) incited by *P. funiculosum*. Cultivar C was highly susceptible to FCR incited by *F. moniliforme*. Cultivar B was susceptible to FCR incited by *F. moniliforme* in fruit harvested in 1974 but not susceptible in 1975. In 1975, all cultivars were susceptible to FCR incited by *P. funiculosum*. Symptoms of FCR incited

by *F. moniliforme* were greatest in fruit harvested in February-March of both years and those incited by *P. funiculosum* were greatest from April through July. Cultivars A and X were significantly more susceptible to interfruitlet corking (IFC) than cultivars B and C. Cultivar A was significantly more susceptible to leathery pocket (LP) than cultivars B and X. Interfruitlet corking and LP were induced only by *P. funiculosum* and were most severe in harvests from April through June in 1974 and April through July in 1975.

Additional key words: *Ananas comosus*, inoculation techniques.

Inoculation of the developing flower of the pineapple, *Ananas comosus* (L.) Merr., with a cultural type of *Penicillium funiculosum* Thom having little 'reverse' pigmentation has been shown to induce the pineapple fruit diseases termed 'fruitlet core rot' (FCR), 'interfruitlet corking' (IFC), and 'leathery pocket' (LP) (5, 11). Other organisms such as a second cultural type of *P. funiculosum* having a red-pigmented 'reverse' (underside of the mycelial mat viewed through the bottom of the petri plate) on potato-dextrose agar, *Fusarium moniliforme* Sheldon, certain yeasts, and bacteria also have been associated with the FCR symptom (1, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13). It has been suggested that these organisms enter the flower through styler canals and nectary ducts during anthesis (1). Insect injuries and natural growth cracks from the period of anthesis to fruit maturity also have been suggested as infection sites for FCR organisms (4, 10, 12).

In recent studies on the field induction of FCR, inoculations with *P. funiculosum* at 1, 4, and 7 weeks following chemical flower induction incited high levels of FCR without wounding. One of the two cultivars used in these studies appeared more susceptible than the other although the test was not designed to demonstrate this point (1). Previous studies with *F. moniliforme* have suggested that wounding is needed for infection (6, 9, 10). Effective field inoculation techniques with *F. moniliforme* have not been reported. Also, the

susceptibility of various pineapple cultivars to *F. moniliforme* is unknown.

This study was undertaken to determine if pineapple cultivars having high natural levels of FCR would have differential susceptibility to *P. funiculosum* and *F. moniliforme* when inoculated by the technique reported for *P. funiculosum* (11), and if identified cultivars having high susceptibility to FCR, IFC, and LP could be used in further elucidating the etiology of these diseases.

MATERIALS AND METHODS

Cultivar culture and test design.—Four pineapple cultivars *Ananas comosus* (L.) Merr. 'A', 'B', 'C', 'D' (from the breeding program of the Pineapple Research Institute of Hawaii), and 'X' (= 'Smooth Cayenne', a commercial cultivar), were selected for various levels of natural or induced (11) susceptibility to FCR. All cultivars were grown according to standard cultural practices for growth of cultivar Smooth Cayenne. The tests were designed for factorial split-split-plot analysis with variables of harvest period, pineapple cultivar, and species of fungal organism introduced. Test design was a split-split-plot with harvest period as the main plot, cultivar as the sub-plot, and organism introduced as the sub-sub-plot with four replications. Each plot consisted of eight test plants with two plants on each end as buffer plants. For the first test, plants of cultivars B, C, D, and X were chemically treated (2) on 23 July, 3 September, 15 October, and 26 November, 1973 to initiate flowering that resulted in harvest periods of 6 February to 20 March, 2

April to 7 May, 15 May to 25 June, and 25 June to 30 July, 1974. In the second test, plants of cultivars A, B, C, and X were similarly treated on 12 August, 16 September, 15 October, and 11 November, 1974 resulting in harvest periods of 25 February to 31 March, 15 April to 27 May, 20 May to 25 June, and 16 June to 28 July, 1975.

Preparation and application of inoculum.—The isolate of *Penicillium funiculosum* [a cultural type with little 'reverse' pigmentation (10) and a yellow-pigmented mycelium] was cultured as previously described (11). *Fusarium moniliforme* was obtained from an FCR-infected fruit from the Pineapple Research Institute of Hawaii and cultured on Difco potato-dextrose agar for 1-2 weeks. Spore suspensions were prepared as described previously for *P. funiculosum* (11) and applied at 1×10^7 spores/ml (*P. funiculosum*) and 1×10^5 spores/ml (*F. moniliforme*). Treatments in 1974 and 1975 were *P. funiculosum* and *F. moniliforme* applied separately and, in 1975, a combination of both fungi was included. Approximately 25 ml of the spore suspension was applied into the differentiated growing point of each plant with a single-nozzle, compressed air sprayer at 1, 4, and 7 weeks following forcing.

Evaluation of fruit.—Fruits were harvested when approximately 50 to 100% of the fruitlets were yellow. Incidence of IFC was recorded by examination of the fruit shell. Following removal of the fruit shell, LP and FCR were recorded. The severity of each symptom was scored according to the following scale: 0 = no fruitlets showing symptoms, 1 = 1-2% of the fruitlets with symptoms, 2 = 3-5%, 3 = 6-10%, 4 = 11-25%, 5 = 26-50%, and 6 = 51-100%. Data on percent FCR, IFC, and LP were transformed ($\sqrt{x + 1}$) and analyzed using an

analysis of variance and Duncan's Bayesian LSD test for significance between means ($P = 0.05$) (3).

Reisolation of inoculated organisms from fruitlet core rot.—A 2-mm portion of an individual fruitlet showing FCR was removed aseptically from the margin of the rot and plated on Difco potato-dextrose agar. Reisolates were identified by comparison with original cultures. Since two cultural types of *P. funiculosum* have been reported, one type with slight 'reverse' (as seen from beneath the petri plate) pigmentation and yellowish mycelium, and, the other type with a red 'reverse' (10), reisolation of both types was noted.

RESULTS

Fruitlet core rot susceptibility interactions.—*Penicillium funiculosum* induced FCR in cultivars D and X in 1974 and A, B, and X in 1975. Cultivar A, tested only in 1975, was significantly more susceptible to FCR than B and X. *Fusarium moniliforme* induced FCR in cultivars B, C, and X in 1974 and only in C in 1975. Cultivar C was significantly more susceptible to FCR than cultivars B and X. Significantly less FCR was induced by *F. moniliforme* than *P. funiculosum* in cultivar X (Table 1).

The percent FCR was highest in the February-March harvest period for cultivars B and D, whereas for cultivar X it was highest in the April-May and May-June harvest periods. For cultivar A it was highest in the April-May, May-June, and June-July harvest periods. For cultivar C it was highest in the February-March and May-June harvest periods (Table 2).

Severity of FCR ranged from 1.0 to 1.5 with the

TABLE 1. Percent fruitlet core rot of pineapple fruit in five cultivars inoculated with *Penicillium funiculosum* and *Fusarium moniliforme* harvested from February through July in 1974 and 1975

Organism	Cultivar ^y							
	A		B		C		X	
	1975 (%)	1974 (%)	1975 (%)	1974 (%)	1975 (%)	1974 (%)	1974 (%)	1975 (%)
<i>Penicillium funiculosum</i>	58 a ^z	25 c	33 cd	40 b	43 abc	44 b	42 b	34 cd
<i>Fusarium moniliforme</i>	11 fgh	40 b	12 efgh	70 a	52 ab	22 c	18 c	15 efg
Noninoculated	5 h	19 c	19 ef	46 b	34 cd	17 cd	9 d	8 gh

^yCultivars A, B, C, and D are unnamed cultivars from the breeding program of the Pineapple Research Institute of Hawaii and cultivar X is the commercial cultivar Smooth Cayenne.

^zMeans followed by the same letter are not significantly different ($P = 0.05$) within years.

TABLE 2. Percent fruitlet core rot of pineapple fruit in five cultivars harvested during four different periods in 1974 and 1975

Harvest periods	Cultivar ^y							
	A		B		C		X	
	1975 (%)	1974 (%)	1975 (%)	1974 (%)	1975 (%)	1974 (%)	1974 (%)	1975 (%)
Feb. - March	16 fgh ^z	63 a	34 bcd	69 a	62 a	47 b	16 e	21 efg
April - May	31 bcde	26 d	25 def	41 bc	43 b	32 cd	32 cd	33 bcd
May - June	33 bcd	15 e	23 def	69 a	28 cde	24 d	36 c	32 bcde
June - July	41 bc	16 e	11 h	32 cd	34 bcd	11 ef	8 f	13 gh

^yCultivars A, B, C, and D are unnamed cultivars from the breeding program of the Pineapple Research Institute of Hawaii and cultivar X is the commercial cultivar Smooth Cayenne.

^zMeans followed by the same letter are not significantly different ($P = 0.05$) within years.

exception of cultivar C which had 1.7 with *F. moniliforme* in the February-March harvest periods.

Interfruitlet corking susceptibility interactions.—Cultivars A and C were significantly more susceptible to IFC incited by *P. funiculosum* in 1975 than were cultivars B and X. *Fusarium moniliforme* did not induce IFC (Table 3). Results from 1974 harvest were similar.

The highest levels of IFC occurred in the April-May and May-June harvest periods in both years in all cultivars. Additionally, cultivars A and C also were significantly more susceptible in the June-July harvest period in 1975 (Table 4).

Severity of IFC generally is correlated with disease incidence. Severity of IFC ranged from 2.5 to 5.1 with cultivar A being the most seriously affected.

Leathery pocket susceptibility interactions.—The symptoms of LP were induced in all cultivars by inoculation with *P. funiculosum* but not *F. moniliforme*. Severity of LP generally approximated IFC. Cultivars A and C were significantly more susceptible than B and X in 1975 (Table 5). No significant interactions occurred in the 1974 harvest periods.

Incidence of LP in 1974 was highest in the May-June harvest period for all cultivars. In 1975, highest incidence of LP for cultivars A, C, and X occurred in the April-May and May-June harvest periods. Cultivar A also had significant LP in the June-July harvest period, but in cultivar B it was high only in the May-June harvest period (Table 6).

Incidence of LP was similar to IFC for both years when

inoculated with *P. funiculosum* (Table 4).

Severity of LP ranged from 1.0 to 2.2 in 1974 and from 1.0 to 3.8 in 1975 following inoculation with *P. funiculosum*. Cultivar C had the most severe LP in 1974, but cultivar A was most severely affected in 1975.

Reisolations from fruitlet core rot lesions.—Reisolations of *P. funiculosum* and *F. moniliforme* from FCR tissue varied with the cultivar (Table 7). In cultivar A, FCR was caused predominantly by the nonpigmented 'reverse' yellow-mycelium cultural type of *P. funiculosum* even when inoculated with *F. moniliforme*. In cultivars B and C, FCR was caused predominantly by the nonpigmented 'reverse' yellow-mycelium cultural type of *P. funiculosum* when inoculated with the same type and by *F. moniliforme* when inoculated with *F. moniliforme*. Cultivars D and X were similar to cultivars B and C except that both cultural types of *P. funiculosum* and *F. moniliforme* were isolated when inoculated with *F. moniliforme*. When inocula of both the nonpigmented 'reverse' yellow-mycelium cultural type of *P. funiculosum* and *F. moniliforme* were combined, only the same cultural type of *P. funiculosum* was isolated from FCR.

DISCUSSION

This study confirms the role of the nonpigmented 'reverse' yellow mycelial cultural type of *P. funiculosum* in pineapple FCR, IFC, and LP. The role of the other cultural type of *P. funiculosum* is unknown. In addition,

TABLE 3. Percent interfruitlet corking of pineapple fruit in four cultivars inoculated with *Penicillium funiculosum* and *Fusarium moniliforme* harvested from February through July 1975

Organism	Cultivar ^y			
	A	B	C	X
<i>Penicillium funiculosum</i>	54.0 a ^z	33.0 b	46.0 a	31.0 b
<i>Fusarium moniliforme</i>	8.1 c	2.2 def	1.6 f	5.6 cd
Noninoculated	6.7 c	1.4 f	1.9 ef	5.3 cde

^yCultivars A, B, and C are unnamed cultivars from the breeding program of the Pineapple Research Institute of Hawaii and cultivar X is the commercial cultivar Smooth Cayenne.

^zMeans followed by the same letter are not significantly different ($P = 0.05$).

TABLE 5. Percent leathery pocket of pineapple fruit in four cultivars inoculated with *Penicillium funiculosum* and *Fusarium moniliforme* harvested from February through July 1975

Organism	Cultivar ^y			
	A	B	C	X
<i>Penicillium funiculosum</i>	58 a ^z	16 d	53 a	28 bc
<i>Fusarium moniliforme</i>	14 d	3 e	5 e	4 e
<i>Penicillium funiculosum</i> + <i>Fusarium moniliforme</i>	60 a	20 cd	50 a	32 b
Noninoculated	16 d	2 e	4 e	6 e

^yCultivars A, B, and C are unnamed cultivars from the breeding program of the Pineapple Research Institute of Hawaii and cultivar X is the commercial cultivar Smooth Cayenne.

^zMeans followed by the same letter are not significantly different ($P = 0.05$).

TABLE 4. Percent interfruitlet corking of pineapple fruit in five cultivars harvested during four different harvest periods in 1974 and 1975

Harvest periods	Cultivar ^y							
	A		B		C		X	
	1975 (%)	1974 (%)	1975 (%)	1974 (%)	1975 (%)	1974 (%)	1975 (%)	1975 (%)
Feb. - March	0 i ^z	0 f	1 i	0 f	0 i	0 f	0 f	1 i
April - May	48 cde	18 e	31 fg	19 de	29 g	30 bed	26 cde	41 de
May - June	69 a	45 a	49 cd	39 abc	55 bc	37 abc	41 ab	63 ab
June - July	39 ef	2 f	8 h	1 f	24 g	2 f	1 f	7 h

^yCultivars A, B, C, and D are unnamed cultivars from the breeding program of the Pineapple Research Institute of Hawaii and cultivar X is the commercial cultivar Smooth Cayenne.

^zMeans followed by the same letter are not significantly different ($P = 0.05$) within years.

inoculation with *F. moniliforme* prior to anthesis induced significant levels of FCR in susceptible cultivars, but not IFC or LP. Natural or insect wounding was not necessary as hypothesized previously (4, 10, 12). The actual stage of flower development in which *F. moniliforme* infection occurs was not established in this study. However, since early inoculations induced high levels of fruitlet core rot, infection probably occurred in the same time sequence as reported for *P. funiculosum* (11).

Cultivars varied in their susceptibility to FCR caused by *P. funiculosum* and *F. moniliforme*. Cultivar A was

most susceptible to FCR induced by *P. funiculosum* with corresponding susceptibility to IFC and LP. Reisolations indicate an exclusive susceptibility to *P. funiculosum* since *F. moniliforme* could not be isolated when it was inoculated. On the other hand, cultivar C was more susceptible to FCR induced by *F. moniliforme*, although *P. funiculosum* also induced significant disease. Reisolations confirmed the high susceptibility to *F. moniliforme*. The cultivar Smooth Cayenne (X) and cultivar B were seasonally susceptible to *P. funiculosum* and *F. moniliforme*. This also was confirmed by reisolations. All FCR that occurred in cultivar C could

TABLE 6. Percent leathery pocket of pineapple fruit in five cultivars harvested during four different harvest periods in 1974 and 1975

Harvest periods	Cultivar ^y							
	A		B		C		X	
	1975 (%)	1974 (%)	1975 (%)	1974 (%)	1975 (%)	1974 (%)	1974 (%)	1975 (%)
Feb. - March	1 gh ^z	5 fg	1 gh	2 g	2 h	2 g	3 g	0 h
April - May	58 ab	8 def	13 e	6 efg	40 c	11 cde	16 bcd	37 c
May - June	69 a	20 bc	33 cd	34 a	53 b	22 b	38 a	54 b
June - July	52 b	2 g	4 fg	3 g	27 d	3 g	2 g	7 f

^yCultivars A, B, C, and D are unnamed cultivars from the breeding program of the Pineapple Research Institute of Hawaii and cultivar X is the commercial cultivar Smooth Cayenne.

^zMeans followed by the same letter are not significantly different ($P = 0.05$) within years.

TABLE 7. Percent isolation of organisms from fruitlet core rot of pineapple fruit of five pineapple cultivars harvested from February through July in 1974 and 1975

Cultivar ^x	Organism inoculated	Total no. of platings	Organism isolated ^y			
			P.f.y	P.f.r	P.x	F.m
A	<i>Penicillium funiculosum</i>	7	100 ab ^z	29 ab
	<i>Fusarium moniliforme</i>	5	100 a	20 a
	<i>P. funiculosum</i> + <i>F. moniliforme</i>	13	85 abcd	38 abcd	8	...
	Noninoculated	2	...	100
B	<i>P. funiculosum</i>	14	71 a	7 a	7 b	29 b
	<i>F. moniliforme</i>	21	10	5 a	14 ab	81 b
	<i>P. funiculosum</i> + <i>F. moniliforme</i>	11	91 a	18 a
	Noninoculated	8	38 a	13	25 b	50 ab
C	<i>P. funiculosum</i>	12	67	8 a	8	25 a
	<i>F. moniliforme</i>	54	4 a	9 ab	4 c	91 bc
	<i>P. funiculosum</i> + <i>F. moniliforme</i>	6	83 a	17 a	17	...
	Noninoculated	22	10	5	14 a	77 a
D	<i>P. funiculosum</i>	17	100 ab	12 ab	6 a	...
	<i>F. moniliforme</i>	13	38 ab	46 abc	15	23 c
	Noninoculated	11	18 a	27	27 a	36
X	<i>P. funiculosum</i>	24	96 ab	13 ab
	<i>F. moniliforme</i>	9	56 a	22 a	...	33
	<i>P. funiculosum</i> + <i>F. moniliforme</i>	15	87 abcde	47 abcde
	Noninoculated	3	100 a	50 a

^xCultivars A, B, C, and D are unnamed cultivars from the breeding program of the Pineapple Research Institute of Hawaii and cultivar X is the commercial cultivar Smooth Cayenne.

^yP.f.y = *Penicillium funiculosum*, nonpigmented 'reverse' yellow mycelial cultural type; P.f.r = *Penicillium funiculosum*, red-

not be accounted for by inoculation with *P. funiculosum* or *F. moniliforme* and it is possible that additional unidentified factors are involved. We cannot explain the failure to reisolate *F. moniliforme* following inoculation with *P. funiculosum*.

In general, levels of fruitlet core rot induced by *F. moniliforme* in susceptible cultivars were highest in the February-March harvest period and *P. funiculosum* fruitlet core rot was highest in the later harvest periods. This may result from greater *P. funiculosum* infection during the cooler part of the year (5).

Susceptibility to interfruitlet corking and leathery pocket varied, with cultivars A and C being more susceptible than B and X.

In conclusion, the results of the present study demonstrate that early inoculations of developing pineapple flowers prior to anthesis with *P. funiculosum* and *F. moniliforme* can be used to identify cultivars susceptible or resistant to fruitlet core rot, interfruitlet corking, and leathery pocket. Cultivars shown to be more susceptible than the cultivar Smooth Cayenne can be used to elucidate the etiology of these diseases.

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