Field Induction of Pineapple Interfruitlet Corking, Leathery Pocket, and Fruitlet Core Rot with Penicillium funiculosum

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ABSTRACT

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Two pineapple cultivars were inoculated in the field with 1 $\times$ 10^7 spores/ml of *Penicillium funiculosum* at 1, 4, and 7 weeks following chemically forced flowering. Infection induced interfruitlet corking, leathery pocket, fruitlet core rot, and inhibition of fruitlet enlargement. The latter accounted for significant reductions in fruit size in cultivar A but not in cultivar Smooth Cayenne. Inoculation at 10 weeks induced disease symptoms only in Smooth Cayenne. No insect associations were noted.

Additional key words: Ananas comosus, inoculation techniques.

Interfruitlet corking, leathery pocket, and fruitlet core rot of pineapple, *Ananas comosus* (L.) Merr., fruit are described in the literature as separate diseases (1, 4, 6). Interfruitlet corking was described by Hepton and Anderson (4) as an epidermal corking between pineapple fruitlets. Lack of trichomes, "glossiness", retarded fruitlet development or eye inhibition, nonflowering eyes, and increased internal blemishes were associated with the disease. *Penicillium funiculosum* Thom was identified as the causal organism (4).

Leathery pocket is characterized by a corking of the surface of the locules (1). This disease has generally been attributed to tarsonemid mite invasion of the locules through the nectary ducts or stylar canal (1, 7), although Hepton and Anderson (4) noted its occurrence in fruit that exhibited symptoms of interfruitlet corking.

Fruitlet core rot, the most economically significant of these three diseases is characterized by a light- to dark-brown soft rot of the fruitlet core area (1, 6). *Penicillium funiculosum, Fusarium moniliforme,* and certain yeasts and bacteria were associated with the fruitlet core rot symptom (1, 4, 6, 7, 8, 9, 10, 11, 12). These organisms may enter through the stylar canals and nectary ducts (1) as well as insect injuries and natural growth cracks from the flowering stage to fruit maturity (10, 11).

Field inoculations with *P. funiculosum* have produced high levels of interfruitlet corking (4); however, the association of *P. funiculosum* with leathery pocket and fruitlet core rot is not well established. Pathogenicity studies on fruitlet core rot have always included some method of wounding to initiate infection (6, 10).

These studies were undertaken to develop a field technique to screen pineapple cultivars for resistance to *P. funiculosum* infection and to establish the nature of the association of these separately described diseases.

MATERIALS AND METHODS

Two pineapple cultivars (*Ananas comosus* 'Smooth Cayenne' and cultivar 'A') were grown according to standard cultural practices for growth of Smooth Cayenne, the commercial cultivar in Hawaii (1). The test design was a randomized complete block with four replicates for each forcing date with cultivars placed adjacent to each other. The plots comprised six plants and were separated by four border plants. Flowering in plants of both cultivars was chemically forced (2) on 18 August, 15 October, and 9 December 1973 so maturity would be reached in harvest periods of 27 February to 2 April, 15 May to 17 July, and 25 June to 6 August 1974, respectively.

Preparation and application of inoculum.—The isolate of *P. funiculosum* used was obtained from A. Hepton, Dole Co., Honolulu, Hawaii. The fungus was cultured on Difco potato-dextrose agar for 2-4 weeks at room temperature (20-22 C) in Roux culture flasks. A spore suspension was obtained by adding tap water [0.005% Ortho X-77 (Chevron Chemical Co., San Francisco, California) added to disperse spores] to the flask, scraping the culture surface with a spatula; and filtering the spore suspension through single-ply tissue (Kimwipes, Kimberly Clark Corporation, Neenah, Wisconsin) to remove mycelium. Spore concentrations were determined with a Howard mold counter and the spore suspension was diluted to 1 $\times$ 10^7, 1 $\times$ 10^4, and 10 spores/ml. Approximately 25 ml of each inoculum level and a tap water check (Ortho X-77, 0.005%) were applied into the differentiated growing point of each plant with a single-nozzle compressed air sprayer. A single application of each inoculum level was applied at 1, 4, 7, 10, 13, and 16 weeks after forcing. Two of these time periods represent

stages of inflorescence development: 7 weeks, the beginning of open heart, defined as the stage when the inflorescence begins to emerge from the immature leaves of the growing point; and 13 weeks, mid-flower, the stage in which anthesis is occurring in the central part of the inflorescence.

Evaluation of fruit.—Fruit were harvested when approximately 50-100% of the fruitlets were yellow. The percentage of interfruitlet corking and inhibition of fruitlet enlargement were recorded by examination of the fruit shell. Fruit size in pineapple terminology is determined by classifying the percent "2-1/2 fruit" size which is defined as fruit not passing through a 12.7-cm (5-inch) diameter ring. Following removal of the fruit shell, the percentage of leathery pocket and fruitlet core rot 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 diseased fruit and percent 2-1/2 fruit size were analyzed by an analysis of variance and Duncan's Bayesian Least Significant Difference (LSD) test for significance between means (P = 0.05) (3). Minimum sample size for any treatment was 24 fruits.

RESULTS

In general, the disease level varied among the three harvest periods. Fruit harvested during the 27 February to 2 April harvest period had only slight disease, that harvested in the 15 May to 17 June period had very severe disease, and that harvested in the 25 June to 6 August period had moderate disease levels. Figure 1 and 2 summarize the percent diseased fruit for each of the disease indices as an average of the three harvest periods. In cultivar Smooth Cayenne (Fig. 1), significant levels of interfruitlet corking were induced with inoculations of 1×10^7 *P. funiculosum* spores per milliliter at 1, 4, 7, and 10 weeks. Significant incidence of eye inhibition, leathery pocket, and fruitlet core rot also was induced; eye inhibition and fruitlet core rot were greatest at 7 weeks and leathery pocket at 4 weeks postflower induction. At 1×10^4 spores/ml, significant levels of eye inhibition were induced when plants were inoculated 1, 4, and 7 weeks after forcing, interfruitlet corking by inoculation at 4 and 7 weeks, and fruitlet core rot by inoculation at 7 and 10 weeks.

In cultivar A (Fig. 2), significant levels of interfruitlet corking, eye inhibition, and fruitlet core rot were induced

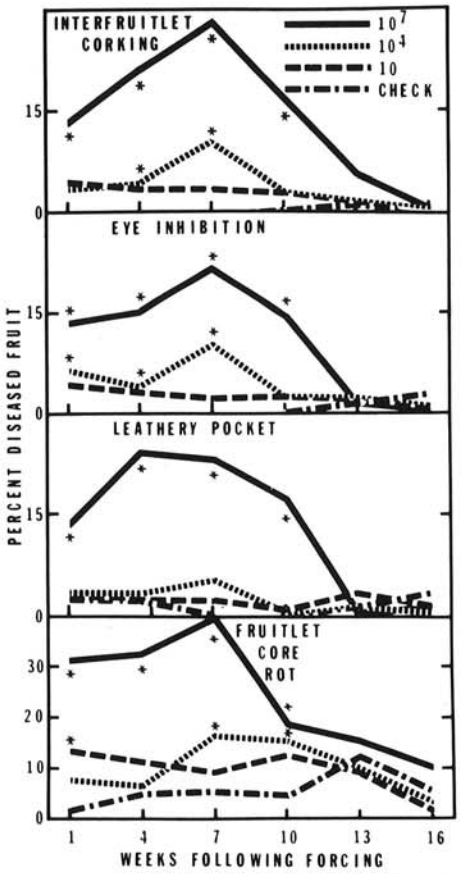

Fig. 1. The effects of inoculation of pineapple cultivar Smooth Cayenne with *Penicillium funiculosum* at inoculum levels of 10^7, 10^4, and 10 spores/ml at various times after forcing on the average percent diseased fruit of three harvest periods for interfruitlet corking, eye inhibition, leathery pocket, and fruitlet core rot [asterisk denotes significance (P = 0.05) from the check].

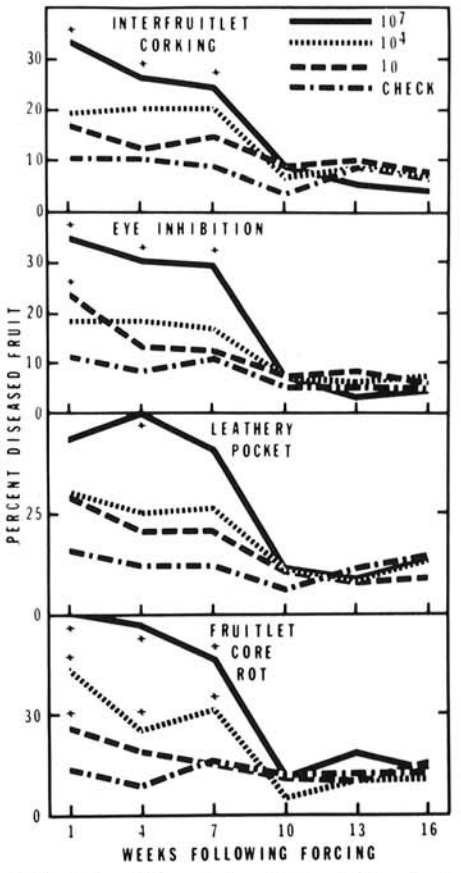

Fig. 2. The effects of inoculation with *Penicillium funiculosum* at inoculum levels of 10^7, 10^4 and 10 spores/ml at various times after forcing pineapple cultivar 'A' on the average percent diseased fruit for three harvest periods for interfruitlet corking, eye inhibition, leathery pocket, and fruitlet core rot [asterisk denotes significance (P = 0.05) from the check].

with 1×10^7 spores/ml at 1, 4, and 7 weeks postflower induction. The highest level of disease was at the 1-week inoculation. A significant level of leathery pocket was induced only at 4 weeks after forcing. Significant levels of fruitlet core rot were also induced at 1, 4, and 7 weeks with 1×10^4 spores/ml. At 10 spores/ml, significant levels of eye inhibition and fruitlet core rot were induced at 1 week after forcing.

Significant reductions in percent 2-1/2 fruit size were noted at 1, 4, and 7 weeks after forcing with 1×10^7 spores/ml; at 1 and 7 weeks with 1×10^4 spores/ml; and, at 1 and 4 weeks with 10 spores/ml in cultivar A (Table 1). No significant differences occurred in Smooth Cayenne.

Correlation values for the diseases described above were significant with the exception of fruitlet core rot and percent 2-1/2 fruit size in Smooth Cayenne (Table 2). The highest correlation was between interfruitlet corking and eye inhibition in both cultivars (r = 0.9349 for Smooth Cayenne and 0.9305 for cultivar A). Correlation values for leathery pocket and fruitlet core rot with the other diseases were higher in cultivar A than in Smooth Cayenne. Correlation values for percentage of 2-1/2 fruit size with other diseases were negatively correlated but high in cultivar A.

Isolations from leathery pocket and fruitlet core rot consistently yielded *P. funiculosum*.

DISCUSSION

Highly significant levels of interfruitlet corking were induced by inoculations with *P. funiculosum* shortly after forcing; the last effective inoculations were 3-5 weeks before anthesis. While Hepton and Anderson (4) only inoculated forced plants when the inflorescence was first visible or approximately 8 weeks following forcing and later, our studies show inoculations before 8 weeks to be much more effective in inducing interfruitlet corking.

Leathery pocket, although associated with interfruitlet corking by Hepton and Anderson (4), has generally been attributed to feeding of mites (1, 7). Our results indicate that leathery pocket is also a symptom of infection by *P. funiculosum*.

Collins (1) attributed fruitlet core rot to infection of unplugged stylar canals and nectary ducts following withering of the blossom. The high levels of fruitlet core rot induced by *P. funiculosum* in the present study indicates that infection of the pineapple flower occurs at least 3-5 weeks before anthesis and that *P. funiculosum* plays a major role in fruitlet core rot in Hawaii. Additionally, observations indicate that the first evidence of flower necrosis occurs on the anthers and style 2-3 weeks before normal anthesis.

The stage of inflorescence development in relation to inoculation and, therefore, infection is of particular interest. In a study by Kerns et al. (5) using cultivar Smooth Cayenne, no individual fruitlets or florets had formed at one week following forcing, in this case natural differentiation. Individual florets began forming during the first and second week following flower induction and all florets on the inflorescence were formed by 5-6 weeks. Also, the developing inflorescence did not emerge through the heart leaves to the external inoculation site until 7-9 weeks. In the present study, peak levels of disease were noted with inoculations at 4 and 7 weeks after forcing in Smooth Cayenne and 1 and 4 weeks after forcing in cultivar A. Therefore, the actual mode of infection in relation to floret initiation and development, and inflorescence emergence is not clear at this time.

TABLE 1. The effects of inoculation with *Penicillium funiculosum* at 1, 4, 7, 10, 13, and 16 weeks following forced flower induction with three inoculum levels on percent "2-1/2 pineapple fruit" size[a] in cultivar 'A' harvested 15 May to 17 July

Weeks following forced flower induction	Inoculum level (spores/ml)			
	1×10^7	1×10^4	10	0
1	13.7 a	53.3 cd	49.3 cd	87.1 fg
4	8.1 a	77.7 efg	62.5 cde	95.6 g
7	31.4 b	47.9 bc	77.1 efg	95.6 g
10	95.6 g	95.6 g	68.3 def	91.3 fg
13	87.1 fg	87.0 fg	87.0 fg	86.3 fg
16	91.3 fg	95.6 g	95.6 g	78.7 efg

[a]In pineapple grading, 2-1/2 fruit size refers to all fruit too large to pass through a 12.7-cm (5-inch) diameter ring. Means followed by the same letter are not significantly different ($P = 0.05$).

TABLE 2. Correlation values (r) for disease indices from pineapple fruit of two cultivars infected with *Penicillium funiculosum*

Cultivar and disease	Eye inhibition	Leathery pocket	Fruitlet core rot	Percent 2-1/2[a] fruit size
'Smooth Cayenne'				
Interfruitlet corking	.9349	.7513	.3962	−.3887
Eye inhibition		.7831	.3518	−.3861
Leathery pocket			.3835	−.3503
Cultivar 'A'				
Interfruitlet corking	.9305	.8313	.6801	−.6362
Eye inhibition		.8676	.6863	−.6211
Leathery pocket			.6909	−.5624
Fruitlet core rot				−.5395

[a]In pineapple grading, 2-1/2 fruit size refers to all fruit too large to pass through a 12.7-cm (5-inch) ring.

Significant levels of disease were induced by inoculation at 10 weeks following forcing in Smooth Cayenne but not in cultivar A. Observations at each of the inoculation times indicated that flower development in Smooth Cayenne was 10 days to 2 weeks behind cultivar A at the 10-week inoculation. Therefore, the 10-week Smooth Cayenne inoculation was actually at an 8-week stage of development in relation to cultivar A.

In conclusion, *P. funiculosum* was shown to be the causal organism of the previously described pineapple fruit diseases termed interfruitlet corking, leathery pocket, and fruitlet core rot. The inoculation techniques developed here will allow cultivar screening for disease resistance and studies on the etiology of these diseases.

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