Role of *Penicillium funiculosum* Strains in the Development of Pineapple Fruit Diseases

Tong-Kwee Lim and K. G. Rohrbach

Graduate student and plant pathologist, respectively, Department of Plant Pathology, University of Hawaii, Honolulu 96822. Present address of senior author, Department of Plant Protection, University of Agriculture Malaysia, Selangor, Malaysia.
Technical assistance by Yukio Nakahira and Robert Bray and statistical assistance by J. A. Silva are gratefully acknowledged.
Journal Series Paper 2385 of the Hawaii Agricultural Experiment Station at Manoa.
Accepted for publication 11 December 1979.

ABSTRACT

LIM, T-K., and K. G. ROHRBACH. 1980. Role of *Penicillium funiculosum* strains in the development of pineapple fruit diseases. Phytopathology 70: 70:663-665.

Three strains of *Penicillium funiculosum*, viz a nonpigmented reverse strain P1, and two red-pigmented strains P2 and P3, were isolated from diseased pineapple fruits, withered pineapple flowers, debris in the plant heart, pineapple trash, and insects found on or around the plant. Comparative pathogenicity tests revealed that all isolates of the P1 strain were virulent and induced significantly high levels of interfruitlet corking, leathery pocket, and fruitlet core rot. Isolates of the red-pigmented strains and strains of *P. funiculosum* from other sources were not pathogenic. Inoculations at 1 and 4 wk after chemical flower induction with isolates of the red-pigmented strains resulted in disease levels significantly lower than in uninoculated check plants.

Additional key words: biological control, *Ananas comosus*.

Two strains of *Penicillium funiculosum* Thom, viz a nonpigmented reverse and another with a red-pigmented reverse on potato dextrose agar (PDA), have been associated with interfruitlet corking (IFC), leathery pocket (LP), and fruitlet core rot (FCR) of pineapple (*Ananas comosus*) (6,11–13,15–17). Recent studies (15–17) established that the nonpigmented reverse strain of *P. funiculosum* is the causal agent of IFC, LP, and FCR. The role of the red-pigmented strain in the development of these diseases is unknown.

In recent studies a second red-pigmented strain of *P. funiculosum* was found associated with diseased pineapple fruits (9). These strains, herein designated P1, P2, and P3 (See footnote Table 2), also were isolated from withered pineapple flowers, debris in the plant heart, pineapple trash, and insects found on or around the pineapple plant (9).

Studies were undertaken to determine the role of the three strains in the development of the three described diseases of pineapple and to determine the pathogenicity of strains of *P. funiculosum* from other sources. Cultural characteristics of the P1, P2, and P3 strains will be the subject of a second paper.

MATERIALS AND METHODS

Two pineapple cultivars (*Ananas comosus*, 'Smooth Cayenne' and 'A') were grown according to standard cultural practices for Smooth Cayenne, the commercial cultivar in Hawaii (3). Three pathogenicity tests were conducted. Plots consisted of eight or 10 plants with two buffer plants at each end.

Test 1. The design of the first test was a factorial split-split plot replicated four times. Main plot treatments of harvest period were chemically forced (4) on 24 September 1975 and 15 October 1975 which resulted in harvest periods of 18 May 1976–22 June 1976 and 8 June 1976–6 July 1976, respectively. Sub-plot treatments consisted of the above two cultivars. Sub-subplots consisted of an uninoculated check and nine inoculation treatments. The nine treatments included inoculations with isolates of *P. funiculosum* from diseased pineapple fruit tissues (P1-1,P2-1), decaying pineapple trash (P1-3), and rotting gladiolus corms (G 100, G 200); two isolates (S 100, S 200) of the closely related species, *P. verruculosum*, in the same *P. funiculosum* series; a pineapple trash-washing, and a pineapple soil suspension. Identification of fungus species were confirmed by K. B. Raper, Dept. of Bacteriology, University of Wisconsin, Madison.

Test 2. The second test was a factorial randomized complete block design consisting of three forcing dates, two cultivars, and four inoculation treatments. Forcing dates were 23 September, 14 October, and 4 November 1976 which resulted in 1977 harvest periods of 23 May–27 June, 6 June–11 July, and 27 June–25 July, respectively. Inoculation treatments consisted of an inoculated check and three isolates of *P. funiculosum* that were reisolated from diseased tissues from test 1 and compared with original isolates (isolate P1-1, isolate P2-1, and isolate P3-1).

Test 3. The third test was a randomized complete block design with eight treatments. The forcing date was 4 November which resulted in a harvest period of 27 June–25 July 1977. Eight treatments consisted of an uninoculated check and inoculations with seven isolates of *P. funiculosum*: P1-1, P2-1, P3-1 reisolated from diseased pineapple fruit, P2-4 from withered pineapple flowers and debris in the plant heart, P1-7 and P2-6 from the exoskeleton of insects, and P1-4, a sparsely sporulating mutant derived from an old culture of P1-1.

Preparation and application of inoculum. The isolates were cultured on acidified potato dextrose agar (APDA [pH 4.0–4.5]) for 2–3 wk at temperatures of 27–28 C in Roux culture flasks. A spore suspension of 10^7 spores per milliliter was prepared as described previously (15). The soil suspension was prepared by suspending the equivalent of 100 g of oven-dry soil in 1 L of tap water. The soil suspension was shaken for 30 min in a gyratory shaker, decanted after settling for 5 min, and the supernatant was filtered through double ply tissue (Kimwipes, Kimberly Clark Corporation, Neenah, WI 54956) to remove organic debris. Pineapple trash washing was prepared in a similar manner by suspending 5 g of pineapple trash collected from a freshly "knocked-down" field in 1 L of water. The inoculum level of the P1 strain in both soil suspension and trash washing was determined by the soil dilution technique (7) to be 64 and 2.4×10^4 spores per milliliter, respectively. The heart of each plant was inoculated with 25 ml of each suspension applied with a single-nozzle compressed air sprayer at 1 and 4 wk after chemical forcing.

Evaluation of fruit. Fruits were harvested when 50–100% of the fruitlets were yellow. The percentage of IFC and cracked fruits was recorded by examination of the fruit shell. The percentage of 2-1/2 fruit size (defined in pineapple terminology as fruit not passing through a 12.7 cm [5-inches] diameter ring) was recorded. The percentage of LP and FCR was recorded after removal of the fruit

0031-949X/80/07066303/$03.00/0

shell. Data on percent diseased fruit, cracked fruit, and percentage of 2-1/2 fruit size were analyzed by analysis of variance and Duncan's Bayesian LSD test for significance between means at $P = 0.05$ (5).

Reisolations. Isolations were made from diseased fruits after disease evaluation. Tissues were surface sterilized in 1.05% sodium hypochlorite solution for 4–5 min, rinsed in distilled water, and plated on a semi-selective media developed in preliminary studies. The medium, a modified Tergitol NPX-acidified potato dextrose agar (NPX-APDA), consisted of 1 μl/ml of Tergitol NPX (Union Carbide Corporation, New York, NY 10017), 100 μg/ml each of tetracycline hydrochloride and streptomycin sulfate (Calbiochem, San Diego, CA 92112). The NPX-APDA medium was acidified to pH 4 with 25% lactic acid. Isolations were made in proportion to the percentage diseased fruit of each treatment. The reisolated fungi were compared with standard isolates.

RESULTS

Test 1. The P1-1 and P1-3 isolates of *P. funiculosum* induced significantly higher levels of IFC and FCR than any other treatment. The G 100 and G 200 isolates, trash-washings, and uninoculated check treatments had higher levels of disease than did the soil-washing and the S 100 and S 200 isolate treatments. The P2-1 isolate resulted in the lowest level of IFC and FCR, significantly lower than the uninoculated check. Similar trends were observed for leathery pocket (Table 1).

Significant reductions in percentage of 2-1/2 fruit size were observed for plants inoculated with P1-1 and P1-3 isolates. The highest yield of 2-1/2 fruit size was obtained with the P2-1 inoculation (Table 1).

Percent reisolations of strains. Isolations from LP and FCR (Table 2) lesions consistently yielded the P1 cultural type strain of *P. funiculosum*. Isolations from IFC lesions also yielded largely the P1 strain. None of the G 100 and G 200 cultural type strains of *P. funiculosum* or S 100 and S 200 strains of *P. verruculosum* were reisolated from diseased tissues. Two other strains of *P. funiculosum* viz P2 and P3 and a *Fusarium* sp. were isolated from diseased fruits of some of the treatments. The percentage of isolations of the P2 and P3 strains were low, varying from 1 to 29%. The *Fusarium* sp. was isolated less frequently (less than 20%) and only from the S 100, G 100, soil-washing and uninoculated check treatments. LP fruit yielded 1% P2 and P3 strains and FCR lesions yielded 5% P2 and P3 strains from plants inoculated with P2-1.

Test 2 and 3. Disease incidence due to the mutant P1-4 was lower than that caused by P1-1 or P1-3, but higher than that in the uninoculated check or in plants inoculated with isolates of P2 (P2-1, P2-4, P2-6) and P3 (P3-1). Inoculations with isolates of P2 and P3 produced the lowest disease incidence (Table 3).

Irrespective of the strain used for inoculation, over 83% of the isolations from LP and FCR lesions yielded P1 strain (Table 4). Less than 25% of the isolations yielded strains P2 and P3. A *Fusarium* sp. was isolated from some FCR lesions. The mutant isolate P1-4, which could be distinguished easily by its deep yellow, sparsely sporulating, and strongly mycelial colonies, was not reisolated. Results of Test 2 were similar to those for Test 3.

TABLE 1. Effect of some *Penicillium funiculosum* isolates and a closely related species *Penicillium verruculosum* on percentage of pineapple fruit having interfruitlet corking (IFC), leathery pocket (LP), and fruitlet core rot (FCR) in test 1

Isolate inoculated[a]	Percentage 2-1/2 fruit size[b]	Percentage diseased fruit[c] IFC	LP	FCR
P1-1	7 e	95 a	77 a	55 a
P1-3	2 e	97 a	81 a	56 a
P2-1	52 a	4 e	6 e	8 e
S 100	45 abc	22 d	18 d	14 de
S 200	36 cd	19 d	22 cd	16 cd
G 100	41 bcd	26 cd	23 cd	23 bc
G 200	31 d	39 b	43 b	25 b
Trash wash	42 abc	29 bc	29 c	19 bcd
Soil wash	49 ab	21 d	16 d	16 bcd
Check	40 bcd	28 bc	26 c	18 bcd

[a] *Penicillium funiculosum* isolates P1-1 and P2-1 are from diseased pineapple fruit, isolate P1-3 is from decaying pineapple trash, G 100 and G 200 are from rotting gladiolus corms; isolates S 100 and S 200 are *P. verruculosum*; the trash wash and soil wash contained spores of *P. funiculosum* strain P1 at 64 and 2.4×10^4 spores per gram, respectively; pure culture spore suspensions contained 10^7 spores per milliliter.

[b] 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.

[c] Means in columns followed by the same letters are not significantly different, $P = 0.05$, according to Duncan's Bayesian LSD test.

TABLE 2. Percentage isolation of *Penicillium funiculosum* strains P1, P2, and P3 from leathery pocket (LP) and strains P1, P2, P3, and a *Fusarium* sp. (Fus) from fruitlet core rot (FCR) diseased fruit in test 1

Isolate inoculated	Isolation (%) LP fruit: Total diseased fruit	Strain isolated[a] P1	P2	P3	FCR fruit: Total diseased fruit	Strain isolated[a] P1	P2	P3	Fus
P1-1	200	90	0	0	200	93	2	10	0
P1-3	210	98	0	0	198	89	0	3	0
P2-1	38	83	1	1	40	73	5	5	0
S 100	90	85	19	17	80	82	19	6	3
S 200	130	88	7	7	67	86	12	13	0
G 100	110	80	8	6	100	95	4	10	15
G 200	160	85	12	27	90	84	3	14	0
Trash wash	144	87	7	29	80	88	9	19	0
Soil wash	100	80	17	14	80	78	8	5	11
Check	104	90	7	9	80	86	13	12	11

[a] Strain P1 is nonpigmented reverse on acidified potato dextrose agar and yellow-pigmented reverse on Czapek's agar; Strains P2 and P3 are red-pigmented reverse on acidified potato dextrose agar, P3 being deeper red than P2; on Czapek's agar, P2 is reddish brown reverse while P3 is orangish brown (9).

DISCUSSION

The high IFC and FCR levels and high reisolation frequency of the nonpigmented reverse P1 strain of *P. funiculosum* confirms its role in the occurrence of IFC, LP, and FCR in Hawaii previously reported by Hepton et al (6) and Rohrbach et al (15,16). None of the other isolates were reisolated consistently or in high frequency from their respective inoculation treatments, demonstrating that they have limited ability to invade unwounded tissue. The low isolation frequency of the red pigmented P2 and P3 strains of *P. funiculosum* and the *Fusarium* sp. at low frequencies from some treatments, suggests that these three organisms are secondary invaders. Oxenham (11–13) also reported the isolation of yellow- and red-pigmented strains of *P. funiculosum*, and a *Fusarium* sp. from FCR-diseased fruits. All three organisms were found to be pathogenic by wound inoculation, indicating that they can invade damaged fruits. However, our findings support the proposition

TABLE 3. Effect of *Penicillium funiculosum* isolates on percentage of pineapple fruit having interfruitlet corking (IFC), leathery pocket (LP), and fruitlet core rot (FCR) in test 3

Isolate inoculated	Percentage diseased fruit[a] IFC	LP	FCR
P1-1	56.0 a	53.0 a	23.0 a
P1-4	27.0 b	27.5 b	12.0 b
P1-7	62.5 a	54.0 a	26.5 a
P2-1	1.5 d	4.0 cd	0.0 d
P3-1	1.5 d	5.0 cd	2.5 d
P2-4	1.5 d	1.5 d	1.5 d
P2-6	1.5 d	3.0 cd	4.0 cd
Check	11.5 c	13.0 bc	7.5 bc

[a] Means in column followed by same letters are not significantly different as determined by the Duncan's Bayesian LSD test, $P = 0.05$.

TABLE 4. Percentage isolation of *Penicillium funiculosum* strains P1, P2, P3, and a *Fusarium* sp. from leathery pocket (LP) and fruitlet core rot (FCR) diseased pineapple fruits in test 3

Isolate inoculated	Isolations from LP fruit (%)					Isolations from FCR fruit (%)				
	Total diseased fruit	Organism isolated				Total diseased fruit	Organism isolated			
		P1	P2	P3	Fus		P1	P2	P3	Fus
P1-1	42	95	5	10	0	18	94	11	11	0
P1-4	21	95	10	0	0	9	100	11	0	0
P1-7	31	95	8	13	0	22	86	5	9	10
P2-1	3	100	0	0	0	0	...	...	...	...
P3-1	4	100	25	0	0	2	100	0	0	0
P2-4	2	100	0	0	0	1	100	0	0	0
P2-6	2	100	0	0	0	3	100	0	0	33
Check	10	90	10	10	0	22	86	5	9	18

that only the P1 strain of *P. funiculosum* is a primary pathogen of unwounded fruit although *Fusarium moniliforme* can be important in the incidence of FCR (17).

The intermediate disease levels obtained with the mutant isolate of the P1 strain indicate that under continuous laboratory cultivation *P. funiculosum* strains can undergo mutation which results in a reduction in virulence and an inability to develop normal conidial structures.

The lower disease levels in treatments inoculated with suspensions washed from pineapple trash or from soil may be explained by the low inoculum level of P1 in the washings (previously determined by soil dilution technique to be 2.4×10^4 spores per gram of trash and 64 spores per gram of oven-dry soil) in comparison to the high inoculum density of 1×10^7 spores per milliliter of the P1-1 and P1-3 inoculations or the lower disease levels may have resulted from fungistatis.

Both P2 and P3 strains reduced infection of the pineapple inflorescence by the P1 strain, since the disease levels of plants inoculated with the P2 and P3 strains were significantly lower than those of the uninoculated check plants. This reduction of disease suggests the possibility of biological control.

Two possible explanations may be given for the disease reduction. First, the P2 and P3 strains may induce the production of phytoalexins in the pineapple plant. Last and Warren (8) reported phytoalexin stimulation by leaf surface saprophytes. Weber et al (19) showed that avirulent isolates of *Ceratocystis fimbriata* increased resistance of sweet potato against subsequent challenge inoculations. Second, the P2 and P3 strains may successfully compete with P1 on the developing inflorescence. Competition for nutrients on plant surfaces is an important factor affecting the establishment of disease (1). Many workers have demonstrated that prior colonization of substrata by saprophytes or nonpathogens can prevent infection by pathogens (2,10,14,18,19).

In conclusion, although it is generally admitted that biological control using antagonistic microorganisms against fungal pathogens is rather impractical for widespread use on field crops (18), our evidence indicates a distinct possibility of using strains P2 and P3 as biological agents for the control of interfruitlet corking, leathery pocket, and fruitlet core rot of pineapple.

LITERATURE CITED

1. BAKER, K. F., and R. J. COOK. 1974. Biological control of plant pathogens. W. H. Freeman, San Francisco, CA. 433 pp.
2. BHATT, D. D., and E. K. VAUGHAN. 1962. Preliminary investigations on biological control of gray mold (*Botrytis cinerea*) of strawberries. Plant Dis. Rep. 46:342-345.
3. COLLINS, J. L. 1960. The Pineapple. Interscience, New York, NY. 294 pp.
4. COOKE, A. R., and K. I. RANDALL. 1968. 2-Haloethane-phosphonic acids as ethylene releasing agents for the induction of flowering in pineapple. Nature (Lond.) 218:974.
5. DUNCAN, D. B. 1965. A Bayesian approach to multiple comparisons Technometrics. 7:171-222.
6. HEPTON, A., and E. J. ANDERSON. 1968. Interfruitlet corking of pineapple fruit, a new disease in Hawaii. Phytopathology 58:74-78.
7. JOHNSON, L. F., and E. A. CURL. 1972. Methods for research on the ecology of soil borne plant pathogens. Burgess, Minneapolis, MN. 47 pp.
8. LAST, F. T., and R. C. WARREN. 1972. Non-parasitic microbes colonizing green leaves: their form and functions. Endeavour 31:143-150.
9. LIM, T-K. 1977. Etiology of three pineapple fruit diseases caused by *Penicillium funiculosum*. Ph.D. Dissertation, University of Hawaii.
10. NEWHOOK, F. J. 1951. Microbiological control of Botrytis cinerea Pers. I. The role of pH changes and bacterial antagonism. II. Antagonism by fungi and actimomycetes. Ann. Appl. Biol. 38:169-184, 185-202.
11. OXENHAM, B. L. 1957. Diseases of pineapple. Queensl. Agric. J. 83:13-26.
12. OXENHAM, B. L. 1958. Notes on two pineapple diseases in Queensland. Queensl. J. Agric. Sci. 10:237-245.
13. OXENHAM, B. L. 1962. Etiology of fruitlet core rot of pineapple disease in Queensland. Queensl. J. Agric. Sci. 19:27-31.
14. RISHBETH, J. 1975. Stump inoculation: a biological control of *Fomes annosus*. Pages 158–162 in: C. W. Bruehl, ed. Biology and Control of Soil-Borne Plant Pathogens. Am. Phytopathol. Soc., St. Paul, MN.
15. ROHRBACH, K. G., and J. B. PFEIFFER. 1976. Field induction and etiology of pineapple interfruitlet corking, leathery pocket and fruitlet core rot with *Penicillium funiculosum*. Phytopathology 66:392-395.
16. ROHRBACH, K. G., and J. B. PFEIFFER. 1976. Infection of pineapple flowers by *Penicillium funiculosum* in relation to inflorescence development. (Abstr.) Proc. Am. Phytopathol. Soc. 3:231.
17. ROHRBACH, K. G., and J. B. PFEIFFER. 1976. Susceptibility of pineapple cultivars to fruit diseases incited by *Penicillium funiculosum* and *Fusarium moniliforme*. Phytopathology 66:1386-1390.
18. SKIDMORE, A. M. 1976. Interactions in relation to biological control of plant pathogens. Pages 507–528 in: C. H. Dickinson and T. F. Preece, eds. Microbiology of Aerial Plant Surfaces. Academic Press, New York, NY.
19. WEBER, D. J., and M. A. STAKMAN. 1964. *Cerotocycstis* infection in sweet potato: its effect on proteins, isoenzymes, and acquired immunity. Science 148:929-931.