Induction and Chemical Control of Rot Caused by Ceratocystis paradoxa on Pineapples

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ABSTRACT

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High levels of butt rot of pineapple propagative materials and fruit rot, both incited by the fungus *Ceratocystis paradoxa*, were induced by artificial inoculation and the infected plant material was used to screen fungicides for efficiency of control. Rot of vegetative propagative materials was controlled by dipping in benomyl, carbendazim (acidified), and thiabendazole. Thiabendazole and carbendazim dips resulted in increased plant weights. Benomyl was superior to thiabendazole in reducing fruit rot, but it reduced plant weights. Sodium-*o*-phenylphenol was not effective for the control of fruit rot.

Additional key words: *Ananas comosus*.

The fungus, *Ceratocystis paradoxa* (de Seynes) Moreau, causes three distinct syndromes in pineapple, *Ananas comosus* (L.) Merr. (8): leaf spot, butt rot (basal rot of the asexual propagative part), and fruit rot. The leaf spot generally is not of economic importance in Hawaii, because disease levels are low in the commercial cultivar, Smooth Cayenne. The rotting of the asexual propagative parts, which may be crowns, slips, or quartered crowns has been termed butt rot (8, 16). Butt rot and fruit rot occur sporadically, but can result in severe damage. This sporadic occurrence has made fungicide testing and disease development studies difficult and inconclusive. The objectives of this study were to develop a technique to induce consistent disease under field conditions to facilitate the screening of fungicides for disease control, and to obtain information on disease development. Abstracts of portions of this study have been reported previously (21, 22).

MATERIALS AND METHODS

Inoculation procedure.—An isolate of *C. paradoxa* obtained from a naturally infected pineapple crown was maintained on slants of Hoyer's agar (5). Cultures for production of inoculum were grown on plates of Difco potato-dextrose agar (PDA) and incubated for 1 wk at 25 C. Spore suspensions were prepared by flooding the cultures with distilled water containing 0.005% Ortho X-77 Spreader (Chevron Chemical Company, San Francisco, CA 94104), scraping the culture surface with a rubber policeman, and filtering the spore suspension through one-ply tissue paper (Kimwipes, Kimberly Clark Corp., Neenah, WI 54956). Spore concentration was determined microscopically using a hemacytometer, and was adjusted to the desired inoculum level by dilution with distilled water. Inoculations were made by atomizing the spore suspension on the cut stem surfaces of freshly harvested whole crowns or the surface of the entire fruit.

Effect of inoculum level on butt rot and fruit rot.—The effects of inoculum levels on disease incidence were determined by applying 1 ml of spore suspension at various dilutions on crowns or 5 ml on each fruit. After inoculation, crowns were planted immediately and fruits were packaged in standard five-fruit cardboard boxes and incubated at ambient temperatures (18 to 26 C). The incidence and severity of butt rot were evaluated 2-6 mo after planting and fruit rot after 7-9 days.

Fungicidal treatments for control of butt and fruit rot.—Butt rot control treatments compared the following fungicides: benomyl (Benlate 50W) at 187.3 μg/ml, and 748 μg/ml, acidified carbendazim (pH 2.5) [methyl-2-benzimidazolecarbamate, the product from acid hydrolysis of Benlate 50W prepared according to McCain (17)] at 187 μg/ml, guazatine (SN-513) at 187 μg/ml, thiabendazole (Mertect 120F) at 187.3 μg/ml and 374 μg/ml, captafol (Difolatan 4F) at 14,300 μg/ml, and captan (Orthocide 50W) at 11,900 μg/ml. A water dip was used as a control in all tests. Each test comprised five replications containing 10 crowns per replication. Whole crowns were dipped in the various fungicide treatments, allowed to dry partially in air; each crown then was inoculated by spraying about 1 ml of a spore suspension containing 2×10^5 spores/ml. All propagative materials

were planted immediately after treatment and inoculation.

Fruit dip treatments for rot control compared the sodium salt of *o*-phenylphenol (Dowcide A) at 7,200 μg/ml with benomyl (Benlate 50W) at 500 and 1,200 μg/ml, and thiabendazole (Mertect 120F) at 500 and 1,000 μg/ml. A water dip was used as a control. Each test comprised five replications containing five fruits per replication. Mature fruits were dipped in the various fungicide treatments, allowed to dry partially in air, and 5 ml of a spore suspension containing 1×10^4 spores/ml was atomized over the entire fruit surface. Inoculated fruits were packaged as previously described and incubated at ambient temperatures (18 to 26 C) for 7-9 days.

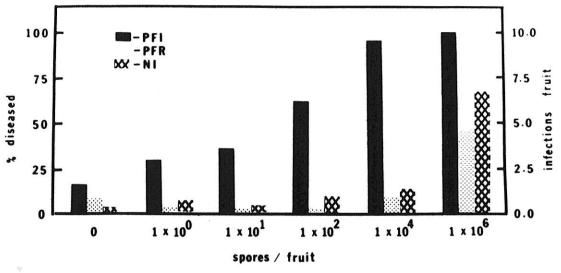

Fig. 1. Effects of various inoculum levels of *Ceratocystis paradoxa* on the percent of fruit infected (PFI), the percent of each fruit rotted (PFR), and the number of infections per fruit (NI), based on determinations made from fruits 7-9 days after inoculation. Fruits were inoculated by applying 5 ml of the desired spore dilution over the entire fruit surface.

TABLE 1. Effects of inoculum level on death of whole crowns due to butt rot caused by *Ceratocystis paradoxa* evaluated 2 mo after inoculation and planting

Inoculum level (Spores/ml)	Crowns killed[y] (%)
2×10^5	66 a
2×10^3	48 ab
2×10^1	44 ab
2	34 b
0	12 c

[y]Average of five replicates each comprising ten plants. Numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. The 12% killed at the 0 spores/ml inoculum level represents natural field infection.

Analysis of disease.—Data on the effect of treatment of propagative material (percent mortality and plant weight) were taken 6 mo after planting. Fruit rot was evaluated on fruit after it was cut into four longitudinal quarters. Records were kept on (i) percent of infected fruits, (ii) the percent of each fruit that was rotted, and (iii) the average number of infections per fruit. All data were analyzed by use of the analysis of variance and Duncan's Multiple Range Test for significance ($P = 0.05$) between means (11).

RESULTS

Effects of inoculum levels on butt rot and fruit rot.—All levels of inoculum tested on whole pineapple crowns resulted in a significant increase in the percent mortality as compared with the noninoculated controls (Table 1). Increasing the levels of inoculum from 0 to 2×10^5 spores/ml resulted in an increase in mortality from a low of 12% at the 0 spores/ml to a high of 66% at the 2×10^5 spores/ml. Plant mortality at the 0 spores/ml level probably represents natural field infection from inoculum which survived in the soil from the previous crop.

Pineapple fruit inoculated with 1×10^6 and 1×10^4 spores/fruit resulted in a significant increase in infected fruit, percentage of each fruit rotted, and the number of infections per fruit in comparison to lower inoculum levels (Fig. 1). Inoculation with 1×10^2 spores/fruit resulted in a significant increase only in the number of infected fruits, and the percentage of each fruit rotted. Increasing inoculum levels resulted in an increase in fruit infection, and inoculation with 1×10^6 spores/fruit induced 100% infection. Sixteen % infection observed on the noninoculated treatment probably represents infection from natural inoculum.

Inoculum levels that resulted in the highest mortality of plants due to butt rot and the maximum number of infected fruit for fruit rot were used in field-inoculation and fungicide testing trials.

Screening fungicides for butt rot control.—Several field trials were conducted to evaluate the effectiveness of

TABLE 2. Effect of preplant fungicidal dips on percent of dead plants and plant weight 6 mo after inoculation of whole pineapple crowns with *Ceratocystis paradoxa*

Fungicide	Conc. (μg/ml)	Percent killed[x]			Plant wts. (g)[x]		
		Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
Guazatine	187.3	2 b[y]	...	...	490.3 c	...	...
Thiabendazole	374.0	...[z]	1.0 c	...	...	658.3 e	...
Thiabendazole	187.3	...	28.3 b	...	...	640.1 d	...
Captafol	14300.0	...	...	1.6 c	...	...	513.0 b
Captan	11900.0	...	...	14.7 b	...	...	376.8 a
Carbendazim (acidified)	187.3	...	0 c	...	...	631.1 d	...
Benomyl	187.3	0 b	1.7 c	3.7 c	517.6 c	558.4 b	481.2 b
Inoculated Control		50 a	41.7 a	22.2 a	227.0 a	467.6 a	354.1 a
Noninoculated Control		42 a	43.3 a	19.5 ab	336.0 b	594.7 c	376.8 a

[x]Average of five replicates each comprising ten plants.
[y]Numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z]Fungicide not evaluated.

TABLE 3. Effect of postharvest fungicide dips on control of pineapple fruit rot caused by *Ceratocystis paradoxa* evaluated 7-9 days after inoculation

Fungicide	Conc. (μg/ml)	Disease Incidence (%)[x] Test 1 PFI	Test 1 PFR	Test 1 NI	Test 2 PFI	Test 2 PFR	Test 2 NI
Benomyl	500	...[y]	...	...	20 ab	1.40 a	0.20 a
Benomyl + Wax	500	...	...	...	72 b	6.00 b	0.72 b
Benomyl	1200	64 b[z]	3.84 a	0.64 b	...	...	...
Thiabendazole	500	...	...	...	92 c	9.15 c	1.04 c
Thiabendazole	1000	...	...	...	72 b	5.56 b	0.72 b
O-phenylphenol	7200	92 c	11.64 b	0.92 c	...	...	...
Inoculated Control		100 c	11.0 b	1.00 c	100 c	14.36 d	1.28 d
Noninoculated Control		20 a	3.8 a	0.20 a	16 a	1.70 a	0.16 a

[x]Average of five replicates each comprising five fruits. Rot was evaluated as percent of fruit infected (PFI), percent of each fruit rotted (PFR), and number of infections/fruit (NI).

[y]Fungicide not evaluated.

[z]Numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

various fungicides for control of butt rot of crown propagative material. All treatments significantly reduced percent mortality and increased plant weight as compared with the inoculated control (Table 2). In test 2, fungicide treatments with thiabendazole and acidified carbendazim were superior to benomyl with respect to increased plant weight. The plant weight from crowns treated with thiabendazole at 374 μg/ml was significantly higher than all other treatments. The percent mortality (28.3%) was significantly higher for the low rate of thiabendazole (187.3 μg/ml) than for the other treatments.

In test 3, treatments with captafol (14,300 μg/ml) and benomyl at two rates (749 μg/ml and 187 μg/ml) resulted in higher plant weights and lower mortality than treatment with captan (11,900 μg/ml). Of these treatments, captafol resulted in higher plant weights [513 g (1.13 lbs)/plant] than from the higher benomyl rate [445 g (0.98 lbs)/plant].

Evaluation of fungicidal control of fruit rot.—Two tests were conducted to evaluate the effectiveness of fungicides for control of postharvest fruit rots (Table 3). In test 1, the benomyl treatment at 1,200 μg/ml reduced all disease parameters compared with the inoculated control. However, fruits treated with benomyl (1,200 μg/ml) had more rot and more infection per fruit than the noninoculated control in which 20% of the fruit were rotted and the number of infections per fruit was 0.2. The *o*-phenylphenol treatment failed to control fruit infections by *C. paradoxa*.

In test 2, all treatments reduced the number of rotted fruits and the number of infections per fruit compared to the inoculated control. Also all treatments except thiabendazole at 500 μg/ml reduced the percentage of each fruit rotted. Treatment with benomyl at 500 μg/ml was superior to other treatments in reducing fruit rot. Addition of wax to the benomyl treatment resulted in a significant decrease in its effectiveness for rot control.

DISCUSSION

The incidence of plants killed by butt rot under natural field conditions was quite variable (12% to 43.3%). This variation in disease incidence may be attributed to the different levels in inoculum density. The number of *C. paradoxa* chlamydospores apparently depends upon the amount of plant residue left from the previous crop which could serve as a substrate for growth and reproduction of the fungus (19). The low inoculum levels encountered under natural conditions may explain previous failures encountered in fungicide control evaluations. In this respect, inoculation of propagative material and fruit overcame this difficulty.

Our results confirm previous reports for the control of *C. paradoxa* rot of pineapple fruit with benomyl and thiabendazole (12, 13, 18). Fungitoxicity of benomyl has been attributed to its breakdown products carbendazim and the volatile butyl isocyanate (14). Although butyl isocyanate has been shown to inhibit respiration in certain fungi (14), most of benomyl's toxicity has been attributed to carbendazim, which inhibits the mitotic process of fungi (7, 9, 14). In this respect, both benomyl and carbendazim equally reduced butt rot incidence; however, plant weights were greater for the carbendazim treatment. Although benomyl penetrates host tissues better than carbendazim (24), the hydrochloride salt form of carbendazim penetrates better than benomyl (2, 3, 4). Also the current rate of benomyl used to treat pineapple propagative materials (approximately 374 μg/ml) is reported to reduce plant growth rates and fruit yields more than lower rates of benomyl (D. D. F. Williams, *personal communication*). Phytotoxicity of benomyl on several crops has been reported (1, 15, 20, 23). The decreased weight of plants treated with benomyl may be due to phytotoxicity and its penetration properties.

Resistance to benomyl and related benzimidazole fungicides have been reported for a number of phytopathogenic fungi (6, 10). To reduce the development of resistance, fungicides with different mechanisms of fungitoxicity should be applied in alternation or combination (10). In this respect, guazatine and captafol were shown to reduce butt rot incidence.

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