POSTHARVEST BROWN ROT OF PEACHES AND INOCULUM DENSITY OF *MONILINIA FRUCTICOLA* (Wint.)

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INTRODUCTION

Brown rot of stone fruit in California is caused by *Monilinia fructicola* (Wint.) and *M. laxa* (Alderh. and Ruhl.) Honey (*4*, *6*).[2] Large numbers of conidia (170,000 spores per fruit) of *M. fructicola* have been found on sound stone fruit at or near harvest (*9*), and may function as inoculum for postharvest rot. The occurrence of a fungal disease generally increased proportionately with an increase in infective propagules (*1*, *2*, *15*).[3] At low inoculum densities, infection may increase linearly with increasing inoculum (*15*); but at higher densities, the disease incidence per unit of inoculum normally decreases and departs radically from linearity (*1*, *2*, *15*). The amount of postharvest rot of stone fruit depends not only on inoculum density but also on handling and on postharvest application of chemicals. Handling may cause injury, such as broken trichomes and punctures, which allows the fungus to penetrate fruit (*14*). Postharvest application of a fungicide destroys or inactivates inoculum, thus protecting the fruit from infection. Common fungicide treatments include chlorine, DCNA (2,6-dichloro-4-nitroaniline), and a combination of DCNA and benomyl (methyl 1-(butacarbamoyl)-2-benzimidazolecarbamate) (*9*, *10*, *11*, *17*, *18*).

Chlorine, as ClO^-, is usually applied when the fruit is hydrocooled or unloaded into water. At 10 parts per million or above in water, ClO^- will kill conidia on the surface of inoculated fruit and reduce brown rot (*17*, *9*). A wide range of DCNA concentrations will reduce brown rot (*17*, *18*); 10 to 12 µg/L, based on the fresh weight of the fruit, controlled 80 percent of the brown rot on plums (*11*). The application of DCNA with benomyl reduces brown rot (*8*, *14*, *17*); combinations of 2 to 4 µg DCNA and 0.5 to 1.5 g benomyl per gram of fruit control Rhizopus rot and brown rot (*17*).

Thermotherapy, that is, hot-water (50°C) treatment of peaches for 1.5 to 2.5 min, also reduces brown rot effectively; but recontamination of the heat-treated fruit may result in substantial brown rot (*13*, *14*).

The incubation period for brown rot is reportedly decreased by high inocu-

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[2]Italic numbers in parentheses refer to Literature Cited, p. 7.

[3]A propagule is a part of a plant that is able to propagate or grow.

lum densities (10^5 conidia per fruit) (*3*), but the influence of inoculum density on the effectiveness of postharvest control measures apparently has not been studied. We report the postharvest occurrence of brown rot on fruit inoculated with various numbers of conidia per fruit. Some test fruit were heat treated before inoculation, and others not heat treated were treated with chlorine, DCNA, or benomyl plus DCNA after inoculation. Part of this work has been reported in an abstract (*7*).

MATERIALS AND METHODS

General Methods

Cultivars of freshly harvested peaches were obtained from orchards that had not been sprayed with fungicides. One cultivar was used per test. Field inoculum on each cultivar was estimated by plating on Monilinia selective medium (*10*). In all tests in all years, this estimate was 100 spores per fruit or less, often zero. In 1971, the cultivars 'Early Coronet' and 'Regular Coronet' were picked June 21 and June 29, respectively. Heat-treated and unheated fruit were inoculated at 0, 100, 1,000 or 10,000 conidia per fruit. Thus, each cultivar received eight treatments, and 100 fruit were used per treatment.

In 1973, the cultivars 'Regina', Suncrest', 'Rio Oso', and '49er' were tested. They were picked July 2, July 17, July 21, and August 15, respectively. Heated fruit were inoculated with 0, 10, 100, 1,000, and 10,000 conidia per fruit, and unheated fruit were inoculated at 0, 100, 1,000, 10,000, and 100,000 conidia per fruit. Unheated, inoculated fruit from each inoculum level were held for 18 h at 22°C and then treated with chlorine, DCNA, or benomyl and DCNA. Fruit not chemically treated were held after inoculation at 22°C until the fungicide treatment of the other fruit was completed. (Each cultivar received 25 treatments, and 125 fruit were used per treatment.) Twenty-five fruit per treatment were used for fungicide assay.

In 1976, the cultivars, 'Regina', 'Red Top', 'Pruess Suncrest', and 'Fayette', were tested. Two hundred fruit of each cultivar were picked before commercial harvest, and after commercial harvest (June 28, July 7, and July 20 for 'Regina' and 'Red Top', and July 21, August 3, and August 12 for 'Suncrest' and 'Fayette'). At each picking, fruit were inoculated with 100 and 500 conidia per fruit (100 fruit per level of inoculum).

Inoculation

In 1971 and 1976, inoculum for the tests was collected from 1- to 2-week-old cultures of *M. fructicola* (ATCC[4] 32670) grown on potato dextrose agar (PDA) and, in 1973, from *M. fructicola* sporulating on freshly harvested peaches. Conidia were washed from agar plates or fruit, after fruit passed through cheesecloth to remove hyphae, and diluted in water to the desired concentration, as determined by counting with a hemocytometer. Each milliliter of the wash water contained 0.003 ml Tween-20 wetting agent (Atlas Chemical Industries).

Fruit were inoculated by (1) a 30-s dip into the desired spore concentration

[4]American Type Culture Collection.

(1971) or (2) a spray of inoculum (1973 and 1976). The droplet spray was applied with an atomizer until the inoculum dripped from the fruit. The number of spores remaining in the fruit was estimated from the average weight increase of the fruit after the dipping or spraying. Each fruit held 1 to 3 g of spore suspension, depending on its size and texture.

Heat Treatment

Fruit were placed in wire baskets and immersed 90 s in an insulated stainless steel tank containing about 300 L of 50°C water. Water was circulated constantly during the treatment, and the temperature was maintained by a controller equipped with a thermistor probe.

Chlorine Treatment

A hundred liters of 150 µg/ml ClO^- was prepared from sodium hypochloride in a plastic lined tank. The solution was adjusted to pH 6.8, and the concentration of ClO^- verified with standard thiosulfate. Fruit samples were dipped for 5 min ('Regina' and 'Suncrest' cultivars) or 10 min ('Rio Oso' and '49er' cultivars) in the 1973 test. The treatment solution was maintained at 24° to 28°C, and fruit were agitated by hand during treatment. During the last two 1973 tests, the fruit were held 18 h at 4°C instead of 22°C, during the interval between inoculation and chemical treatment. Changes in dip time and holding temperature were made to improve the effectiveness of the chlorine treatment and to facilitate observation of rot response to increasing inoculum.

DCNA and Benomyl-DCNA Treatments

Samples of fruit were dipped for 30 s into 42 L of one of two 10:1 (v/v) mixtures of water and Decco Peach Wax WT 52 (Pennwalt Corp.). One mixture contained 1200 µg active DCNA (75 percent wettable powder (WP)) per milliliter; the other, 100 µg active benomyl (50 percent WP) and 300 µg DCNA per milliliter.

Fruit dipped into 1200 or 300 µg DCNA/ml retained, on the average, 10 µg or 3 µg of DCNA, respectively, per gram of fruit. Residues of DCNA were determined by gas chromatography (M. Uota, unpublished data). Retention of benomyl was 1.5 µg/g fresh fruit, as estimated by bioassay (*8*).

Handling and Evaluation of Treatments

After inoculation in 1971 and 1976 or after the 18-h holding period plus treatment period in 1973, the fruit were packed into boxes (25 per box) fitted with plastic trays. The boxes were held in random blocks for 2 days at 4°C and 4 days at 26°C; then the fruit were evaluated for decay.

Analysis of Data

The percentage of fruit with brown rot in each box was recorded. Arcsine transformations of the percentages were subjected to standard analysis of variance and were used for comparison of treatment or cultivar differences. Inoculum density response curves using the number of spores added by inoculation were plotted as log X versus probit y, log X versus log y, and X versus $\log_e 1/(1-y)$,

where x is an estimate of the number of conidia per fruit and y the proportion of infected fruit. Although all plots showed significant linear correlations, only the last type is reported because it seemed to clearly represent our data and because the term $\log_e 1/(1-y)$ may be interpreted as an estimate of the number of infections per fruit (*15*).

RESULTS

Heat Injury Before Inoculation

In 1971, heat-treated peaches had less rot than nontreated fruit when both were noninoculated. Of the inoculated fruit, those that were heat treated (heat-injured) had a higher incidence of rot than unheated fruit (table 1, fig. 1).[5] In 1973, the heat treatment again increased rot of the inoculated fruit, although of the four cultivars, the increase was significant from the unheated control only in the Suncrest peaches (tables 2 and 8).

Chemical Treatments After Inoculation

Chlorine was effective only in those peaches held at 4°C during the interval between inoculation and chemical treatment. When the inoculated fruit were held at 4°C, chlorine reduced rot to about a 10 percent loss at 100 and 1,000 spores per fruit, whereas at 10,000 and 100,000 spores per fruit losses were 40 to 70 percent (table 3). Like chlorine, DCNA adequately controlled brown rot only at inoculum densities or at less than 10,000 spores per fruit (table 4). Benomyl-DCNA controlled brown rot at all inoculum densities (tables 5 and 8).

Fruit not Heated or Chemically Treated

In fruit not heated or chemically treated, the number of fruit with rot increased rapidly to 80 percent at 10,000 spores per fruit. Further increases in the number of spores did not markedly increase the incidence of rot as it approached 100 percent loss (table 6).

Incidence of disease was similar in both cultivars in 1971; each was picked within a week of the other (table 1). Losses in 1973 were significantly greater for the two early cultivars than for the two later cultivars (table 6).

Following the 1973 observation of cultivar difference, the results of the 1976 test, designed to compare cultivars, indicated rot incidence was again greater in two early cultivars than in two later cultivars (table 7). In this test, fruit picked before the normal harvesting time had the least rot, especially in the late-ripening cultivars (table 7).

The Effect of Inoculum

In treated or untreated fruit, brown rot increased with the number of conidia added per fruit (figures 1 and 3). Field inoculum was low and was ignored in the analysis of inoculum response. The estimate of infection, $\log_e 1(1-y)$, was often significantly and linearly associated with the number of conidia added per fruit; however, the relation between the two variables could better be expressed by a curved rather than a linear line. The form of the curve is similar to that

[5]Tables follow the Literature Cited section.

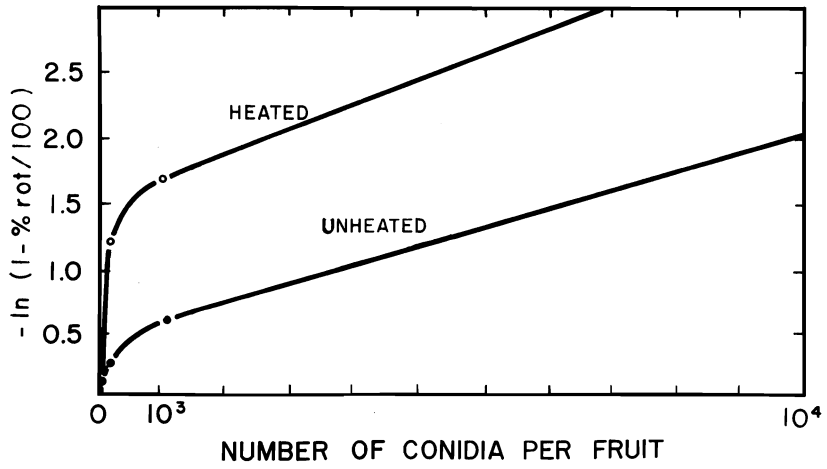

Figure 1.--The effect of heat-treatment injury on the occurrence of brown rot on 'Early Coronet' and 'Regular Coronet' peaches inoculated after the heat-treatment with 0, 10, 100, and 1,000 conidia of *Monilinia fructicola* per fruit.

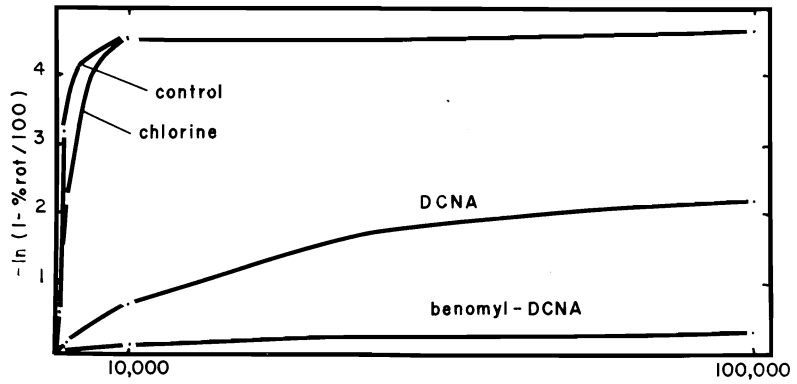

Figure 2.--The effect of chlorine, DCNA, or benomyl-DCNA on brown rot on 'Regina' peach inoculated in 1973 with 0, 100, 1,000, 10,000, or 100,000 conidia of *Monilinia fructicola* per fruit.

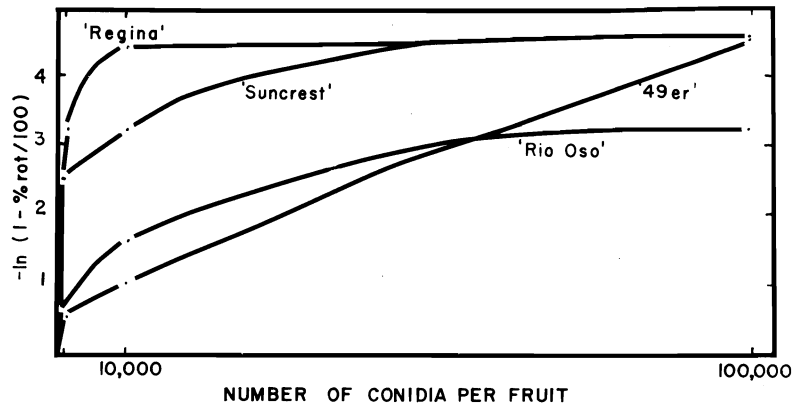

Figure 3.--The brown rot on four cultivars of untreated peaches inoculated in 1973 with 0, 100, 1,000, 10,000, or 100,000 conidia of *Monilinia fructicola* per fruit.

of the single or multiple Poisson equation described by van der Plank (*15*): $Y = N\ (1-e^{-ax})$ or $Y = N_1\ (1-e^{-a_1 x}) + N_2\ (1-e^{-a_2 x})$, where Y is the number of infections, x the number of spores, and N and a are parameters.

DISCUSSION

Our finding may clarify the often confusing relation between inoculum density and postharvest development of brown rot. The postharvest occurrence of brown rot on inoculated peaches depended on the amount of inoculum on the fruit, the fruit cultivar, and the type of postharvest treatment. Heat injury of the fruit, as shown by some but not all the heat-treated fruit, increased the occurrence of brown rot. Fungicides reduced losses at all inoculum densities but often gave commercially acceptable rot control (<10 percent) only at densities below 1,000 spores per fruit. This lack of effective control may result from that penetration into the untreated flesh of the fruit by *M. fructicola* at high inoculum densities. Poor control also may result from incomplete coverage with fungicide; such a coverage would be less important with the systemic fungicide benomyl.

In general, the inoculum density response curve for untreated fruit rose steeply near the origin and then less so as inoculum density increased (figs. 1, 2, 3). This shows a marked interaction between sites or marked differences in the susceptibility of sites (*15*). These sites may include (1) handling or picking wounds, (2) less susceptible sites such as stomata (*12*), and (3) sites of latent infection (*5*, *16*).

We have no explanation for the differences in disease incidence between early and late maturing cultivars of peaches in 1973 and 1976. Some undefined characteristics of the fruit appear to be important in reducing postharvest losses due to brown rot; however, even the most resistant cultivars would have required fungicide treatment to be commercially acceptable.

CONCLUSIONS

Ungerminated conidia of *M. fructicola*, found on the surface of freshly harvested California peaches (*7*), can cause brown rot after packing. Washing and brushing the peaches before packing provides the water and injury on the surface of the fruit, which stimulate germination of the spores.

Treatment with postharvest fungicide is usually necessary to reduce decay losses to below 10 percent of the packed fruit. When large amounts of inoculum are present, treatment with a postharvest fungicide must be prompt and thorough to be effective. Prompt treatment is especially needed for chlorine treatments, which leave no active residue. Even treatments that leave an active residue may allow some areas on the fruit to escape the treatment, permitting spores in these untreated areas to germinate and infect the fruit. A systemic residue on the fruit, such as the residue left by benomyl, reduces the growth from untreated areas and, therefore, reduces the number of sites that can be infected. If the residue can move systemically into the fruit, as does benomyl, both surface and most subsurface fungal growth are controlled, but even with the added benefit of systemic action, the fungicide treatment needs to be prompt and thorough to achieve the best possible control.

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APPENDIX

Table 1.--*Percentage of postharvest brown rot in heat-treated and untreated peaches inoculated with* Monilinia fructicola *in 1971*

Cultivar and treatment	Number of conidia added per fruit[1] 0	10^2	10^3	10^4	Cv. means
	Percent rot				
'Early Coronet' peach:					
Unheated	25	139	41	83	
Heated	5	61	72	91	52.1a
'Regular Coronet' peach:					
Unheated	0	21	48	90	52.8a
Heated	0	77	88	99	
Inoculum means	7.5a	49.5b	62.3c	90.8d	
Treatment means:					
Heated	43.4a				
Unheated	61.6b				

[1]Each datum based on four 25-fruit samples. Overall means within a row or column without a letter in common differ at the 95-percent confidence level.

Table 2.--*Percentage of postharvest brown rot in 3 cultivars*[1] *of peaches heat treated before inoculum with 0, 10, 100, 1,000, and 10,000* Monilinia fructicola *conidia per fruit*

Cultivar	Number of conidia added per fruit[2] 0	10^1	10^2	10^3	10^4	Cv.[3] means
	Percent rot					
'Suncrest'	0	23	67	99	100	57.8a
'49er'	5	8	13	44	71	28.2b
'Rio Oso'	2	11	25	56	92	37.2c
Inoculum means[3]	2.3a	13.6b	38.1c	66.0d	87.4e	

[1]'Regina' peaches were (inadvertantly) not heat treated.
[2]Each datum represents 100 fruit (four 25-fruit replicates).
[3]Inoculum or cultivar means within a row or column without a letter in common differ at 95-percent confidence level.

Table 3.--*Percentage of postharvest brown rot after chlorine treatment of 4 cultivars of peaches inoculated with 0, 100, 1,000, 10,000, and 100,000* Monilinia fructicola *conidia per fruit. 'Regina' and 'Suncrest' cultivars were held 18 h at 22°C, '49er' and 'Rio Oso' were held 18 h at 4°C, after inoculation but before treatment*

Cultivar	Number of conidia added per fruit[1] 0	10^2	10^3	10^4	10^5	Cv.[2] means
	Percent rot					
'Regina'	2	26	89	100	100	63.4a
'Suncrest'	21	79	99	100	100	79.8b
Inoculum means[2]	11.5a	52.5b	94c	100c	100c	
'49er'	7	12	8	27	56	22.1a
'Rio Oso'	13	4	8	40	79	29.0b
Inoculum means[2]	10a	8a	8a	38.7b	67.5c	

[1]Each datum represents 100 fruit (four 25-fruit replicates).
[2]Inoculum or cultivar means within a row or column without a letter in common differ at 95-percent confidence level.

Table 4.--*Percentage of postharvest brown rot in 4 cultivars of peaches treated with DCNA (2,6-dichloro-4-nitroaniline) 18 h after inoculation with 0, 100, 1,000, 10,000, and 100,000* Monilinia fructicola *conidia per fruit*

Cultivar	Number of conidia added per fruit[1]					Cv.[2] means
	0	10^2	10^3	10^4	10^5	
	Percent rot					
'Regina'	2	2	11	48	89	30.4a
'Suncrest'	1	11	28	29	60	23.8a
'49er'	2	4	9	28	36	15.8a
'Rio Oso'	3	8	5	2	36	10.8b
Inoculum means	2a	6.3b	10.8b	26.8c	55.25d	

[1]Each datum represents 100 fruit (four 25-fruit replicates).
[2]Inoculum or cultivar means within a row or column without a letter in common differ at 95-percent confidence level.

Table 5.--*Percentage of postharvest brown rot in 4 cultivars of peaches treated with a combination of DCNA (2,6-dichloro-4-nitroaniline) and benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate 18 h after inoculating with 0, 100, 1,000, 10,000, and 100,000* Monilinia fructicola *conidia per fruit*

Cultivar	Number of conidia added per fruit[1]					Cv.[2] means
	0	10^2	10^3	10^4	10^5	
	Percent rot					
'Regina'	0	1	7	10	29	9.4a
"Suncrest'	2	0	0	0	1	.6b
'49er'	2	1	1	2	3	1.8b
'Rio Oso'	11	8	6	4	11	8.0a
Inoculum means[2]	3.8a	2.5a	3.5a	4.0a	11.0b	

[1]Each datum represents 100 fruit (four 25-fruit replicates).
[2]Inoculum or cultivar means within a row or column without a letter in common differ at 95-percent confidence level.

Table 6.--*Percentage of postharvest brown rot in 4 cultivars of peaches. The untreated peaches were inoculated with 0, 100, 1,000, 10,000, and 100,000* Monilinia fructicola *conidia per fruit*

Cultivar	Number of conidia added per fruit[1]					Cv.[2] means
	0	10^2	10^3	10^4	10^5	
	Percent rot					
'Regina'	16	42	96	100	100	70.8a
'Suncrest'	54	49	93	96	100	78.4a
'49er'	17	17	49	80	96	51.8b
'Rio Oso'	21	21	40	62	99	50.7b
Inoculum means[2]	27.1a	32.2a	71.9b	84.4c	99d	

[1]Each datum represents 100 fruit (four 25-fruit replicates).
[2]Inoculum or cultivar means within a row or column without a letter in common differ at 95-percent confidence level.

Table 7.--*Percentage of brown rot in 4 peach cultivars inoculated with* Monilinia fructicola *picked at 3 stages of maturity in 1976*

Picking stage[1]	100 conidia added per fruit of the following cultivars[2 3 4]				500 conidia added per fruit of the following cultivars[2 3 4]				Row means
	R	RT	PS	F	R	RT	PS	F	
	Percent rot[5]								
Before harvest	11	5	2	4	44	27	6	7	13a
At harvest	8	13	13	19	15	38	34	34	22b
After harvest	26	31	10	13	29	54	38	25	28b
Column means	15ab	16ab	8a	12a	29cd	40d	26cb	22cb	

[1]In relation to normal commercial practice.
[2]Noninoculated control fruit had no brown rot.
[3]R = 'Regina'; RT = 'Redtop'; PS = 'Pruess Suncrest'; F = 'Fayette'.
[4]'Redtop' and 'Regina' picked June 28, July 7 and 20; 'Pruess Suncrest' and 'Fayette' picked July 21, Aug. 3 and 12.
[5]Each datum based on four 25-fruit samples. Overall means within a row or column without a letter in common differ at the 95-percent confidence level.

Note: Cultivar means are summarized as follows: R = 22ab, RT = 28c, PS = 17a, and F = 17a. A group comparison of early versus late cultivars was 25a and 17b, respectively.

Table 8.--*The effect of chlorine, DCNA,*[1] *benomyl*[2]*-DCNA, or heat treatment on the occurrence of brown rot on '49er' peaches inoculated with 0, 100, 1,000, and 10,000 conidia of* Monilinia fructicola *per fruit in 1973*

Fruit treatment	Number of conidia added per fruit[3]				Overall means for treatment
	0	10^2	10^3	10^4	
	Percent rot				
Unheated	21bcde	21bcde	40ef	62f	37.4c
Heated	2a	25bcde	56f	91f	40.5c
Chlorine	13abcd	4ab	9abc	40def	21.6b
DCNA	3a	8abc	5a	2a	8.6a
Benomyl-DCNA	11abc	7ab	6ab	4a	11.3a
Overall means for inoculation	15.1A	18.3A	25.6B	36.5C	

[1]2,6-dichloro-4-nitroaniline.

[2]Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate.

[3]Overall means or means within treatments without a letter in common differ at the 95-percent confidence level. Each datum represents four samples of 25 fruits.

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