Effect of Postharvest Treatments on Stemphylium Rot of Papaya

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ABSTRACT

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The size of lesions caused by *Stemphylium lycopersici* on papaya (*Carica papaya*) fruits was affected by type of wounding, fruit maturity, duration of postharvest hot-water treatment, and duration of cold storage. A hot-water immersion treatment resulted in larger lesions than a hot-water spray. Susceptibility to infection increased with increasing maturity.

A storage disease occurred on fruit of papaya (*Carica papaya* L. 'Kapoho Solo') when refrigerated during transport to distant markets (1). Affected fruit had round, black, velvety lesions surrounded by a maroon ring. The causal fungus was identified as *Stemphylium lycopersici* Yamamoto (1).

Conditions permitting infection of papaya fruit by *S. lycopersici* are not well understood. Couey and Farias (5) found an increase in the incidence of *Stemphylium* sp. after hot-water immersion. In our study, hot-water treatments of fruit at various levels of maturity and lengths of refrigeration were compared for their influence on diameter of *S. lycopersici* lesions.

MATERIALS AND METHODS

S. lycopersici strain S003, isolated from natural infections on papaya fruit (1), was maintained on V-8 agar (10% V-8 juice, 0.02% $CaCO_3$, 1.5% agar) at room temperature under continuous light. Cultures 10 days to 3 wk old were used in the experiments.

Spore formation of the fungal cultures was induced by transferring 10- to 14-day-old cultures to continuous darkness for 1 wk. Heat inactivation of spores was studied by collecting spores in sterile water and transferring them to sterile capillary tubes (0.13 ml) that were sealed at one end. Three filled capillary tubes were used per treatment. The tubes were held in a hot-water bath at 46, 48, and 50 C (±0.2 C) for 10, 20 and 22 min and at 54 C (±0.2 C) for 1, 3, and 5 min. The hot-water-treated spore suspensions were transferred to V-8 agar plates and the developing colonies were counted after 2 days of incubation at 25 C. Controls received the same treatment with omission of a hot-water incubation. The experiment was replicated six times.

Papaya fruits were harvested in early morning and used in experiments the same or following day. Fruits were selected visually in the colorbreak, one-quarter ripe, and one-half ripe stages and measured individually at the inoculation site with a Hunter colorimeter standardized with the white master standard ($L_L = 92.50$; $a_L = -0.9$; $b_L = -0.1$). A heat treatment was given by either running the fruit through a hot-water spray (54 C) with equipment similar to that used by Hundtoft and Akamine (6) or immersing the fruit in a hot-water tank (49 C) for 20 min according to standard procedures (4).

On the inoculation site, a small block of agar with fungus (2 × 2 mm) was placed on 1) a slash wound made with a razor blade, 2) a pinpoint wound made with an insect needle, and 3) an unwounded area. In the experiments comparing spray and immersion treatments and effect of incubation time, two wounded and two unwounded sites were inoculated on each fruit. Fruits were incubated in moistened plastic bags for 48 hr, then the plastic bags were removed and the fruit stored in vented plastic bags in fiberboard boxes at 10 C for 0, 1, or 2 wk. Each sample consisted of 10 fruits. After refrigeration, the fruits were left at room temperature for another 5 days before measuring the lesions. All experiments were replicated five or six times. All experimental data were evaluated by analysis of variance. A split-plot technique was used where necessary (2). Frequency of infection of the unwounded fruit sites was analyzed by calculating the *G* statistic as described by Sokal and Rohlf (8).

RESULTS

Lesions did not develop on inoculated fruit that had been stored only at room

Table 1. Influence of heat treatment, length of cold storage, and type of wounding on size of *Stemphylium lycopersici* lesions on papaya fruit (10 fruits per sample)

Hot-water spray at 54 C (min)	Cold storage (wk)	Type of wounding		
		None (cm)	Pinpoint (cm)	Slash (cm)
0	1	0.08	0.71	0.89
	2	0.37	1.77	1.60
3	1	0.12	1.17	1.04
	2	0.52	2.22	2.05
5	1	0.31	1.42	1.17
	2	0.26	2.64	2.25

Source	df	*MS*[a]
Replicates	4	6.694**
Time in hot-water spray (A)	2	1.491*
Regression on time	1	2.979**
Deviations	1	0.007
Error A	8	0.190
Time in cold storage (B)	1	12.709**
Interaction (AB)	2	0.032
Error B	12	0.228
Type of wounding (C)	2	17.077**
Wounded vs. unwounded	1	33.800**
Between wounded	1	0.347
Interaction (AC)	4	0.334
Interaction (BC)	2	1.693**
Interaction (ABC)	4	0.109
Error C	48	0.173

[a]Split-plot analysis of variance. * = Significant *F* test ($P = 0.05$) and ** = significant *F* test ($P = 0.01$).

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temperature. During cold storage, water-soaked areas developed that increased in size with increasing length of cold storage. During subsequent storage at room temperature, these water-soaked areas developed into large, black, velvety lesions surrounded by a maroon ring. Lesion diameter increased with duration of hot-water spray and of cold storage (Table 1). Infection occurred at almost all wounded inoculation sites and the size of the lesion was a quantitative measure of degree of susceptibility. Inoculations at unwounded sites frequently did not result in infection, but when infection did occur, it usually was in the ripest fruit in the more severe treatments—those with the longest hot-water spray and cold storage exposures.

The hot-water spray at 54 C for 3 min had been used previously in our laboratory and had proven comparable in effectiveness to hot-water immersion treatments for control of stem-end rot and anthracnose (4). Most *S. lycopersici* spores were killed by exposure to 48 C for 18 min; although 1 min at 54 C resulted in 2.5% viable spores, 3 min at 54 C killed 99% of the spores (Table 2). Therefore, the 3-min hot-water spray would also be effective against *S. lycopersici* on papaya. Hot-water sprays for 3 min were compared with routine hot-water immersion. The mean diameters of *S. lycopersici* lesions were larger on immersed than on sprayed fruit (Table 3), and considering only the unwounded sites, more fruits became infected in the 20-min immersion treatment at 49 C than in the 3-min spray at 54 C (Table 4).

In fruit incubated after inoculation for 24 or 48 hr before refrigeration, the lesion diameter at wounded sites and the frequency of infection at unwounded sites increased with longer incubation time (Tables 5 and 6). Fruit inoculated and incubated for 48 hr before hot-water spray or immersion treatment did not become infected. Lesion diameters on both wounded and unwounded sites and frequency of infection at unwounded sites all increased with increasing fruit ripeness (Tables 3–6).

Hunter b values were significantly different for the three degrees of maturity established by visual evaluations (Tables 3 and 5). Among replicates, a significant difference in Hunter b values was present (Table 3), indicating that visual determination of fruit maturity differed in the experiments.

DISCUSSION

The size of *Stemphylium* lesions on papaya fruit was increased by wounding the fruit and by increasing the duration of hot-water treatments and of cold storage (Table 1).

Two weeks of cold storage (10 C) of inoculated fruit resulted in much larger lesions than storage for 1 wk. Chilling injury causes metabolic disturbances and permeability changes in the cells that increase susceptibility to otherwise nonpathogenic fungi (3,7).

Five-minute spray treatments with hot water at 54 C resulted in larger *Stemphylium* lesions on papaya fruit than 3-min spray treatments. Exposure to heat most likely caused some injury to the fruit, increasing its susceptiblity to the fungus. Because a 3-min hot-water treatment was sufficient to kill almost all *S. lycopersici* spores (Table 2), longer

Table 2. Heat inactivation of *Stemphylium lycopersici* spores

Temperature (C)	Length of heat treatment (min)	Total no. of colonies	Colonies (% of 25 C control)
25	0	744	100.0
46	18	239	31.6
	20	241	32.4
	22	195	28.7
48	18	10	1.9
	20	2	0.6
	22	10	1.5
50	18	2	0.6
	20	3	1.0
	22	0	0.0
54	1	14	2.5
	3	1	0.1
	5	4	1.2

Source	df	*MS*[a]
Replicates	5	0.0128
Treatments	11	0.1102*
Regression on temperature and time	2	0.3344*
Deviations	9	0.0603
Error	55	0.00638

[a] * = Significant *F* test ($P = 0.01$).

Table 3. Influence of hot-water spray (54 C, 3 min) vs. hot-water immersion (49 C, 20 min) treatments and fruit maturity on diameter of *Stemphylium lycopersici* lesions on papaya fruit

Fruit maturity	Heat treatment	Hunter b values[a]	Diameter of lesions (cm)	
			Unwounded	Wounded
Colorbreak	Immersion	13.60	0.22	1.52
	Spray	14.07	0.04	0.98
One-fourth ripe	Immersion	14.52	0.31	1.85
	Spray	14.37	0.22	1.64
One-half ripe	Immersion	19.25	0.83	2.31
	Spray	17.90	0.56	2.13

Source	df	*MS*[b]	*MS*	*MS*
Replicates	5	242.5088	1.09	3.53
Maturity (A)	2	799.46**	10.43*	28.11**
Regression on Hunter b	1	···	16.89**	46.90**
Deviations	1	···	3.96	2.54
Error A	10	33.41	0.95	3.67
Heat method (B)	1	10.82	2.95*	8.87**
Interaction (AB)	2	25.68*	0.25	1.20
Error B	15	4.72	0.37	0.77
Subsampling error	324	6.78	0.22	0.27

[a] Hunter b values were measured with a Hunter colorimeter at the time of inoculation.
[b] Split-plot analysis of variance. * = Significant *F* test ($P = 0.05$) and ** = significant *F* test ($P = 0.01$).

Table 4. Frequency of infection of unwounded papayas by *Stemphylium lycopersici* related to fruit maturity and method of heating[a]

Fruit maturity	Heat treatment	No. of fruit with indicated no. of lesions		
		0	1	2
Colorbreak	Immersion	24	17	18
	Spray	47	4	9
One-fourth ripe	Immersion	26	15	18
	Spray	29	10	21
One-half ripe	Immersion	11	16	33
	Spray	21	15	24
Total		158	77	123
Expected[b]		108	177	73

	df	*G*[c]
Maturity	4	28.36**
Heat method	2	14.84**
Interaction	4	10.47*
Total	10	53.67**
Goodness of fit	2	121.40**

[a] Two inoculations per fruit, immersion at 49 C for 20 min or spray at 54 C for 3 min.
[b] Expected values calculated from proportion of inoculations that were infected (I) and uninfected (N): $n(I + N)^2 = n(I^2 + 2IN + I^2)$ 358 $(0.45 + 0.55)^2 = 0.303(358) + 0.495(358) + 0.203(358)$.
[c] *G* values calculated as in Sokal and Rohlf (8). * = Significant chi-square test ($P = 0.05$) and ** = significant chi-square test ($P = 0.005$).

Table 5. Influence of incubation period at room temperature on lesion size of *Stemphylium lycopersici* lesions on papaya fruit[a]

Fruit maturity	Moist incubation (hr)	Hunter b values	Diameter of lesions (cm)[b]	
			Unwounded	Wounded
Colorbreak	24	13.20	0.03	0.73
	48	12.87	0.03	0.82
One-fourth ripe	24	14.99	0.06	1.18
	48	15.30	0.12	1.41
One-half ripe	24	18.21	0.33	1.82
	48	18.67	0.49	2.14
Source	**df**	***MS*[c]**	***MS***	***MS***
Replicates	5	241.80	1.00	5.15
Maturity (A)	2	889.73**	4.975**	43.56**
Regression on Hunter b	1	···	9.307**	86.84**
Deviations	1	···	0.643	0.29
Error A	10	35.21	0.26	2.71
Incubation time (B)	1	1.90	0.47	4.04*
Interaction (AB)	2	5.34	0.21	0.39
Error B	15	12.21	0.30	0.59
Subsampling error	324	6.85	0.109	0.273

[a] Three levels of fruit maturity were used on the basis of visual determination and these were correlated with Hunter colorimeter values measured at the time of inoculation.
[b] Average of two inoculations per fruit.
[c] Split-plot analysis of variance. * = Significant F test ($P = 0.05$) and ** = significant F test ($P = 0.01$).

Table 6. Frequency of infection of unwounded papayas by *Stemphylium lycopersici* related to fruit maturity and incubation period before refrigeration (two inoculations per fruit)

Fruit maturity	Incubation time	No. of fruit with indicated no. of lesions		
		0	1	2
Colorbreak	24	54	6	0
	48	53	4	3
One-fourth ripe	24	54	4	2
	48	42	11	7
One-half ripe	24	33	17	10
	48	22	16	22
Total	···	258	58	44
Expected[a]	···	229	116	15
	df	***G*[b]**		
Maturity	4	64.81*		
Incubation time	2	11.95*		
Interaction	4	7.32		
Total	10	84.08*		
Goodness of fit	2	77.05*		

[a] Expected values calculated from proportion of inoculations that were infected (I) and not infected (N): $n(I + N)^2 = n(I^2 + 2IN + I^2)$ 358 $(0.45 + 0.55)^2 = 0.303(358) + 0.495(358) + 0.203(358)$.
[b] G values calculated as in Sokal and Rohlf (8). * = Significant chi-square test ($P = 0.005$).

hot-water spray treatments are unnecessary. The hot-water immersion treatment resulted in significantly larger lesions than the 3-min hot-water spray (Table 3). The amount of damage caused by heat is therefore slightly greater in the immersion treatment.

Moist incubation of inoculated fruit for 24 or 48 hr before cold storage caused a small but significant difference in lesion diameter (Table 5). The extra day of incubation at room temperature may have increased the ripeness of the 48-hr series sufficiently to account for the significant increase in lesion diameter. The fungus did not penetrate tissue beyond the wounded area in inoculated fruit stored at room temperature. Thus, cold storage after incubation appeared to be essential for disease development.

It is clear that papaya fruits are initially highly resistant to infection by *S. lycopersici* and that the subtle damage inflicted by heating or cold storage is responsible for increasing susceptibility. Whatever these changes may be, they occur on a whole-fruit basis. If all the fruits were equally susceptible to infection and all were gradually becoming more susceptible to infection, infections would be distributed randomly among the inoculation sites and a binomial distribution of fruits into our three infection classes would be expected. However, the proportion of fruits with either no lesions or two lesions at the unwounded sites was much greater than predicted by binomial distribution (Tables 4 and 6).

The size of *S. lycopersici* lesions on papaya fruit was affected by wounding the fruit, the duration of hot-water treatments, the time in cold storage, and the ripeness of the fruit.

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