

Postharvest Fruit Rot of Papaya Caused by *Stemphylium lycopersici*

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ABSTRACT

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A previously undescribed storage disease of papaya fruit caused by *Stemphylium lycopersici* is characterized by dark brown sunken lesions with distinct reddish brown margins. The pathogen infects wounded fruits more readily than it does unwounded fruits, and symptoms usually appear after extended cold storage (7–21 days). Lesion size and disease incidence were reduced by decreasing the storage temperature from 10 C (normal shipping temperature) to 4 C, but cold injury was observed on fruits stored at 6 C or lower. The fungus was pathogenic to lettuce, alfalfa, bean, and tomato.

The extended periods of cold storage required for surface shipment of papaya from Hawaii to the U.S. mainland increase the incidence of postharvest fruit rot caused by various fungi (2). In spring 1978, unusual dark brown lesions were first observed on papaya fruit stored for 3 wk at 10 C in quality-control studies on the island of Hawaii. Similar symptoms occurred sporadically on refrigerated fruits during subsequent years. A *Stemphylium* sp. was consistently isolated from the lesions. The purpose of this study was to examine the role of the *Stemphylium* sp. in postharvest disease of papaya and to characterize the fungus.

MATERIALS AND METHODS

Isolation and production of inoculum.

A *Stemphylium* sp. was isolated from infected papaya by placing tissue samples on 1.6% water agar (WA). Single conidial cultures derived from 11 isolates were

maintained on 10% vegetable juice agar (VJA) (100 ml V-8 juice, 16 g Difco agar, 2 g CaCO₃, and 900 ml distilled water). Six- to 7-day-old cultures grown on VJA at 20–24 C with continuous fluorescent light were subjected to complete darkness for 2 days for conidial production. Because all isolates showed similar conidial morphology and cultural characteristics, only one isolate (S-006) was used in this study.

Pathogenicity tests. Fruits at color-break stage were surface-disinfested by immersion in hot water (48 C) for 20 min (1). Inoculum consisted of conidial suspensions in distilled water or agar blocks (VJA, about 3 × 3 mm) containing mycelium and conidia. Methods of inoculation were compared by 1) bruising the fruit surface with a glass rod with a rounded end, 2) slashing (2–4 mm) with a sterile scalpel, or 3) pricking with an insect pin. Bruised fruits had lower incidence of infection than fruits wounded by the other two methods. Suspensions containing 2,000–10,000 conidia per inoculated site or agar blocks resulted in 70–90% infection on prick-wounded fruits. Based on these results, the agar block method was selected for further studies. Inoculum was placed on wounded or unwounded papaya fruit surfaces. Fruit were also inoculated by placing mycelial blocks on severed fruit stems after harvest. Fruit were incubated

24 hr in moist chambers at room temperature (RT) (20–24 C), then stored for 10 days at 10 C. Disease incidence was recorded 5 days after removal of fruit from storage. The experiment was repeated three times with 100 fruits per treatment in each experiment. Distilled water (0.05 ml) was used on control fruit in place of inoculum.

Effect of storage temperature on disease development. Detached fruit at color-break stage were surface-disinfested and inoculated as described. After 1 day of incubation in moist chambers, the fruit were stored at 4, 6, 8, 10, 13, or 16 C for 9 days. Disease incidence and lesion diameters were recorded 5 days after removal of fruit from storage. The experiment was repeated three times, with 40 fruit per treatment in each experiment.

Pathogenicity of *Stemphylium* sp. on other plants. Alfalfa, lettuce, cucumber, and soybean seedlings were inoculated with a suspension containing fungal conidia and mycelium in distilled water. One hundred seedlings wounded with insect pins and 100 unwounded seedlings were used in each trial. Distilled water was used on wounded and unwounded control plants. Seedlings were incubated in moist chambers for 24 hr at RT and subsequently placed on the greenhouse bench. Leaves of 5-wk-old tomato plants were similarly inoculated and incubated. Disease incidence was recorded 2 and 14 days after incubation. Fifty ripe, detached tomato fruits were inoculated at two sites per fruit, one site wounded with an insect pin and the other without wounding, using the methods described; distilled water was placed on two additional sites (wounded and unwounded) per fruit as controls. Fruit were incubated 24 hr in moist chambers at RT and stored 10 days at 10 C. Disease incidence was recorded 5 days after removal of fruit from storage.

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Fungal characteristics. Cultures were grown for 6–7 days under continuous fluorescent light at RT, subjected to complete darkness, and examined at 2-hr intervals for 16 hr. The conidial germination rate was determined in double-distilled water, WA, VJA, and papaya latex. Sporulating cultures were flooded with sterile distilled water, the conidial suspension filtered through double layers of cheesecloth, and the filtrate centrifuged and readjusted to about 200 conidia per 10 μ l with sterile distilled water. Ten microliters of conidial suspension was pipetted onto a microscope slide coated with WA, VJA, or papaya latex. The slides were incubated in moist chambers and examined hourly with a light microscope

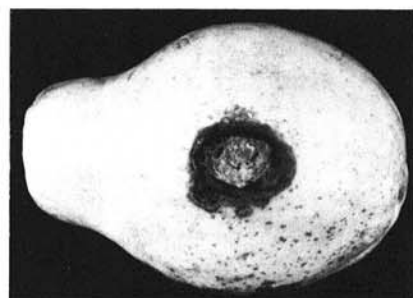


Fig. 1. Papaya fruit showing typical lesion caused by *Stemphylium lycopersici*.

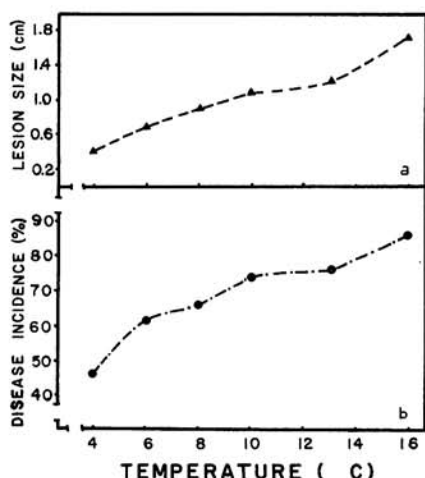


Fig. 2. Effect of temperature on (A) lesion development and (B) percentage of fruit infected after wound inoculation with *Stemphylium lycopersici*.

for 8 hr, then 2 days after incubation for conidial germination. A conidium was considered germinated when one of its germ tubes had reached at least half the length of the conidium. Six hundred conidia were counted for each treatment.

The effect of temperature on fungal growth was also determined. Mycelial disks (5 mm diam.) taken from edges of 6-day-old cultures were seeded at the edges of petri dishes (9.5 cm diam.) containing 12 ml VJA per plate. The plates were incubated at 6, 10, 12, 14, 18, 22, 26, 30, or 34 C with continuous light for 6 days, and the diameter of the fungal colonies was measured. The experiment was repeated three times with 10 plates per treatment in each experiment.

RESULTS

Small, round, dark brown lesions were observed on wounded inoculated papaya fruits when removed from storage. Five days later, lesion margins were reddish brown. Lesion centers were slightly sunken and covered with a dense dark green conidial mass. At an advanced stage, gray to white mycelium grew over the lesions (Fig. 1). Cross sections of infected areas examined under the light microscope showed darkened parenchyma and vascular tissue. The parenchyma cells at lesion centers collapsed. Cells at the

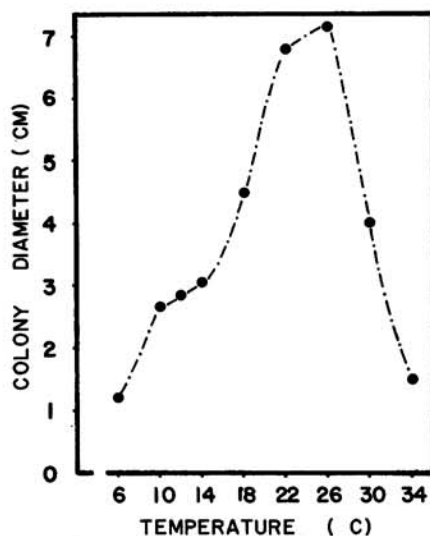


Fig. 3. Effect of temperature on radial extension of *Stemphylium lycopersici* grown on 10% V-8 agar.

newly infected area were usually filled with mycelium, forming a visible boundary beyond which cells appeared healthy. Percentages of infection on wounded and unwounded fruit surfaces were 89 and 5, respectively. All inoculations on severed fruit stems resulted in stem-end rots within 5 days of removal of fruit from storage.

Both disease incidence and lesion size were significantly reduced by decreasing the storage temperature from 10 C (normal shipping temperature) to 4 C (Fig. 2). Fruit stored at 4 and 6 C ripened unevenly, showing patches of dark green and light yellow tissue.

The wounded and unwounded seedlings of alfalfa and lettuce showed chlorosis 2 days after inoculation and were killed within 14 days. Chlorotic spots were visible on leaves of soybean seedlings 2 days after inoculation, but the lesions did not enlarge. Lesions on tomato leaves were circular and brown. The central areas of some old lesions cracked and later excised. Lesions on tomato fruit were water-soaked, brown, and soft. Results of the host-range test are summarized in Table 1.

Fungal characteristics. Conidia were light brown, minutely warty, and pointed with two or three transverse and several longitudinal septa. The medial transverse septum was most prominent. Conidial size range was $72.5\text{--}45 \times 27.5\text{--}15 \mu\text{m}$. The colony diameter of the papaya pathogen increased as temperature increased from 6 to 26 C, then abruptly decreased (Fig. 3). Sporulation was observed on cultures incubated under continuous fluorescent light at 10, 12, and 14 C. Cultures grown under continuous light at 18, 22, and 26 C formed numerous dark conidiophores and required an additional 8–10 hr of darkness to stimulate spore formation. At 30 and 34 C, fungal cultures did not form conidiophores, and pinkish red pigment diffused into the medium.

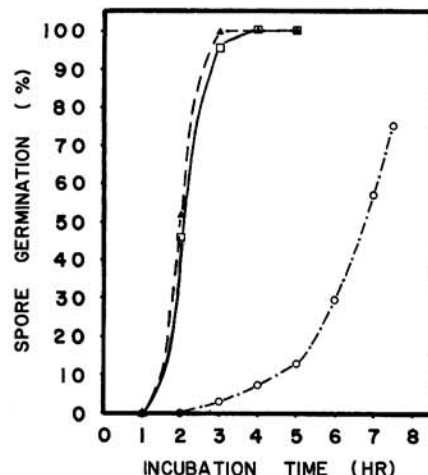


Fig. 4. Germination of *Stemphylium lycopersici* conidia incubated on 10% V-8 agar (\blacktriangle — \blacktriangle), water agar (\square — \square), and papaya latex (\circ — \circ).

Table 1. Pathogenicity of *Stemphylium lycopersici* on various plant species^a

Host	Inoculated plants that developed disease symptoms (%)	
	Wounded	Unwounded
Alfalfa (<i>Medicago sativa</i> L.)	100	82
Lettuce (<i>Lactuca sativa</i> L.)	100	100
Bean (<i>Phaseolus vulgaris</i> L.)	50	46
Onion (<i>Allium cepa</i> L.)	0	0
Cucumber (<i>Cucumis sativa</i> L.)	0	0
Tomato leaf (<i>Lycopersicon esculentum</i> Mill.)	78	2
Tomato fruit (<i>L. esculentum</i> Mill.)	93	63

^aOne hundred wounded and unwounded seedlings were used for each host. Fifty ripe tomato fruits were inoculated at two sites per fruit.

Percentage germination of conidia incubated in WA, VJA, and papaya latex is shown in Figure 4. Conidia in double-distilled water did not germinate within 1 day of incubation at RT. Mycelial growth of the fungus was observed on WA, VJA, and papaya latex 2 days after incubation. Based on conidiogenesis and conidial morphology described for the genus (6), the fungus isolated from papaya was identified as *Stemphylium*. The species was identified as *S. lycopersici* Yamamoto (= *S. floridanum* Hannon & Weber) by E. G. Simmons, Department of Botany, University of Massachusetts, Amherst 01003 (personal communication).

DISCUSSION

S. lycopersici has been reported to cause disease on tomato, chrysanthemum, gladiolus, onion, and other plants (4) but not on papaya fruit. The *S. lycopersici* isolated from papaya corresponds to the descriptions by Hannon (5) and Ellis (4), except the papaya fungus is pathogenic to tomato fruit and not pathogenic to onion.

Low disease incidence on unwounded papaya fruit indicated that *S. lycopersici*

is primarily a wound pathogen on papaya. Fungal conidia and mycelial fragments probably lodge on latex that exudes freely from papaya fruit surfaces as a result of minor wounds. Conidia may germinate in latex and cause lesions under favorable conditions.

Stemphylium rot is unusual on fruit stored at room temperature and ripened within 5–7 days after harvest. However, surface shipment of papaya fruit from Hawaii to mainland or foreign markets requires 7–18 days of storage at 10–15 C (2), and such conditions promote disease development on papaya fruit. Although lower storage temperatures would reduce disease incidence, papaya fruit stored at or below 6 C are sensitive to cold injury and do not ripen normally.

Stemphylium fruit rot occurs sporadically and disease incidence is not well documented. In postharvest trials spanning a period of 8 mo in 1980–1981, about 25% of 510 untreated fruits had Stemphylium rot. Incidence was not reduced by a hot-water immersion treatment (48 C, 20 min) (Couey et al, unpublished). In orchard spray trials,

Stemphylium rot incidence varied from 7 to 15% of 5,863 fruits harvested from plots sprayed with chlorothalonil, copper-sulfur, or mancozeb (3). In both cases, fruit were refrigerated 7 days before grading.

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