Predisposition of Citrus Fruits to Sour Rot When Submerged in Water

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ABSTRACT

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Sour rot developed at more than 90% of the wounded sites on lemons, oranges, and grapefruit inoculated with 5 μl of an aqueous suspension of *Geotrichum candidum* conidia (10^4–10^6/ml) when inoculated fruit were submerged in water at room temperature for 24–36 hr before storage. The frequency of infection was similar in yellow fruit exposed to a water-saturated atmosphere, but this treatment was much less effective than prolonged submersion in water for stimulating sour rot in silver lemons and in mature and immature green fruit. The maximum effect of the water treatment was induced by inoculation with a minimum of 10^5 conidia per milliliter, followed by immersion in water at room temperature for at least 24 hr. This simple procedure for predisposition of citrus fruits to sour rot can be scaled up for fungicide tests and research projects that require a dependable, high level of infection.

Sour rot, caused by the fungus *Geotrichum candidum* Link, is a major postharvest disease of citrus fruit in most areas of the world (1,11,16). The disease develops rapidly on tree-ripe fruit (4,10,14), especially if harvested when the rind tissue is highly turgid (4,5). Healthy fruits are infected by the fungus only through wounds or by contact with decaying fruits. Inoculation is followed by a rapidly advancing, soft-slimy rot or by arrested, dry lesions, which rarely lead to decay (4,10). Inoculum additives such as cycloheximide, pectinase, and sodium polypectate have been reported to ensure a high level of infection and thereby facilitate fungicide tests (5,6,13,15). Nutrition and pH of the tissue have been suggested as important factors in the establishment of the fungus in oranges (18), but attempts to increase susceptibility of lemons by modification of these factors have not been successful (6,13). Inoculum containing a mixture of decayed tissue and water has been proposed as a method for increasing infection rates (17). Preinoculation fruit treatments that weaken the rind or cause injuries, such as a hot water dip, steam, or brief exposure to microwaves, increase sour rot infection (E. Cohen, *unpublished data*). However, wound-inoculation of the peel of ripe citrus fruit, without any of the predisposing treatments, results in a variable and often low incidence of decay (4). Mature green fruit are resistant to infection. In this paper, we describe a simple, reliable method for predisposing citrus fruits to sour rot. Factors found to be related to disease initiation in earlier studies, including maturity, size, age, and inoculum concentration, were also evaluated.

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MATERIALS AND METHODS

Citrus fruit. Fruits of lemon (*Citrus limon* (L.) Burm. f.) cultivars Lisbon and Eureka were hand-picked with clippers in southern California groves (Coachella Valley, Corona, South Coast Field Station, and Ventura County). The uniform-sized (about 60 mm diameter) fruit were sorted into color categories (dark green, light green, silver, yellow, and deep yellow). For some experiments, immature dark green lemons (40–50 and 30–40 mm diameter) were used. For other experiments, oranges (*C. sinensis* (L.) Osbeck)(cultivars Washington navel and Valencia) and grapefruit (*C. paradisi* Macf.) (Marsh seedless) harvested in southern California were used. Fruit were kept in polyethylene bags for 1 or 2 days at 25 C or for longer periods at 10 C until inoculated.

Pathogen. Isolate GW 20 of *G. candidium* (originally isolated from a decayed lemon) was maintained on potato-dextrose agar. Five- to ten-day-old slant cultures were flooded with sterile 0.05 M potassium phosphate buffer, pH 6.0, containing 0.01% Triton X-100. The culture surface was gently rubbed with a scalpel, and the resulting suspension was filtered through sterile cheesecloth to remove hyphal fragments. The concentration of conidia was estimated with a hemacytometer and the concentration was adjusted with buffer solution to provide suspensions of approximately 10^2–10^8 conidia per milliliter.

Fruit inoculation. Fruit were dipped in 50% aqueous ethanol for 1 min, were allowed to dry, and were inoculated by injecting 5 μl of an aqueous conidial suspension into the peel with a 250-μl syringe equipped with a repeating dispenser (Hamilton Company, Reno, NE). The syringe was fitted with a 19-gauge needle (1.07 mm o.d.) with a 45° tip, which protruded 2.5 mm from a Teflon sleeve. Each lemon was inoculated at 10 sites distributed around the fruit and equidistant from the equator (five sites on either side of the equator). The inoculation wound was approximately 1 × 2.5 mm and perpendicular to the fruit surface. The exact volume of inoculum deposited in each wound was evaluated in an earlier study (4).

Sour rot activation. In experiments comparing liquid water and water-saturated air pretreatments, lemons with five inoculation sites on each hemisphere were half-submerged in water in a vertical position so that one-half of the inoculation sites were covered with water; the other five inoculation sites on the

same fruit were exposed to a water-saturated atmosphere, created by covering the container with a polyethylene film. The fruit were exposed to the two moisture environments for 0–48 hr and then stored at 25 C and high humidity up to 6 days. In experiments evaluating the effects of *Geotrichum* inoculum concentration (Fig. 1) and water submersion time (Fig. 2) on infection, lemons were completely submerged in water. Each experiment was repeated two to four times.

RESULTS

Effect of fruit color, maturity, cultivar, and postharvest age on sour rot development. Lemon fruit at the yellow stage of ripeness were highly susceptible to sour rot (Table 1). Lesions developed at each of the wound sites. However, the immersion treatment appeared to shorten the incubation period; lesions developing at wounds that had been submerged in water were significantly larger than those developing at wounds that were exposed to the high-humidity atmosphere above the water. In contrast, with silver and green lemons, few lesions developed at wounds that had not been submerged, whereas lesions developed at most sites that had been submerged in water. However, decay advanced more rapidly in the yellow, compared with silver and green, fruit.

The water submersion treatment increased the susceptibility of green lemons at several stages of maturity, from almost complete resistance to highly susceptible (Table 2). The lesions expanded more rapidly in the mature than in the immature green fruit. The water treatment also resulted in a high frequency of lesion development in immature green grapefruit, mature Washington navel oranges, and immature dark-green Valencia oranges. After these fruits had been incubated for 48 hr at 25 C, the average lesion diameter was 19.7 ± 2.2, 20.4 ± 3.2, and 12.4 ± 2.2 mm, respectively. In contrast, lesions did not develop at inoculation sites that had been exposed only to a high-humidity atmosphere. The disease symptoms were typical of sour rot in all cases, and *G. candidum* was the only fungus isolated from diseased tissue.

The postharvest age of the fruit affected the susceptibility of lemons to sour rot when they were subjected to prolonged submersion in water. Treatment of lemons, stored for 30 days after harvest, resulted in the development of active lesions at each inoculation site. Among fruit stored for 50 days, the incidence fell to 80%, and lesion diameter was reduced by 40%.

Effect of inoculum concentration and water immersion time on sour rot development. When sour rot lesions were evaluated after 3 days of incubation at 25 C after fruit were immersed in water for 24 hr, there was a close curvilinear relation between the number of sour rot lesions/fruit and the log of the number of conidia per inoculation (Fig. 1). However, the relation between lesion diameter and number of conidia per inoculation was linear throughout the range studied (Fig. 1). In fruit inoculated with 5×10^3 conidia per site, there was a close curvilinear relation between the number of lesions and the hours of postinoculation submersion in water (Fig. 2). No significant difference was found in response

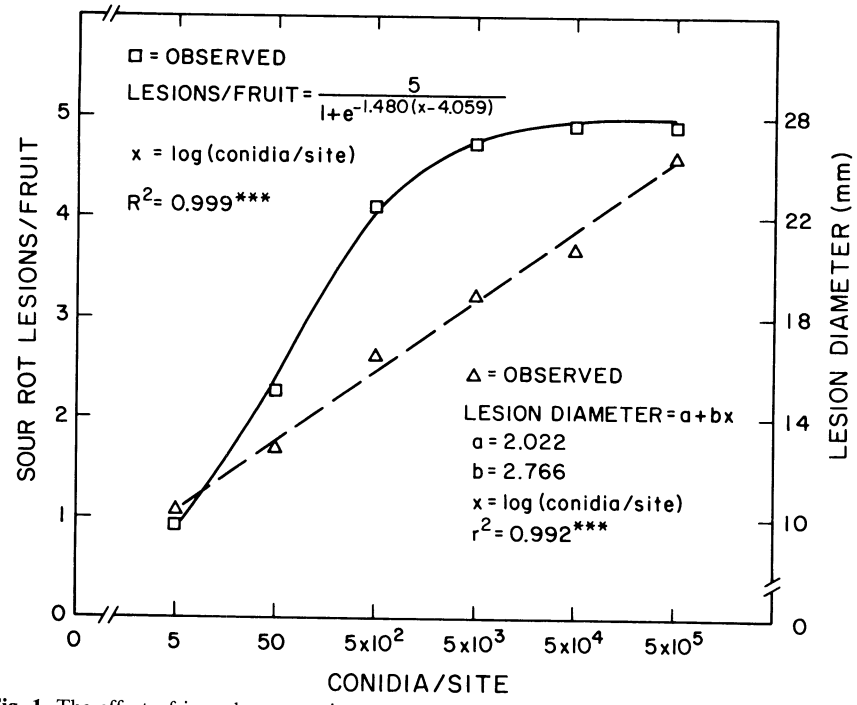

Fig. 1. The effect of inoculum quantity on sour rot incidence and lesion expansion in mature green lemon fruit immersed in water for 24 hr after inoculation. Disease was evaluated after 3 days of storage at 25 C and approximately 90% relative humidity. Means from 15 lemons, five inoculation sites each. *** indicates 0.001 probability.

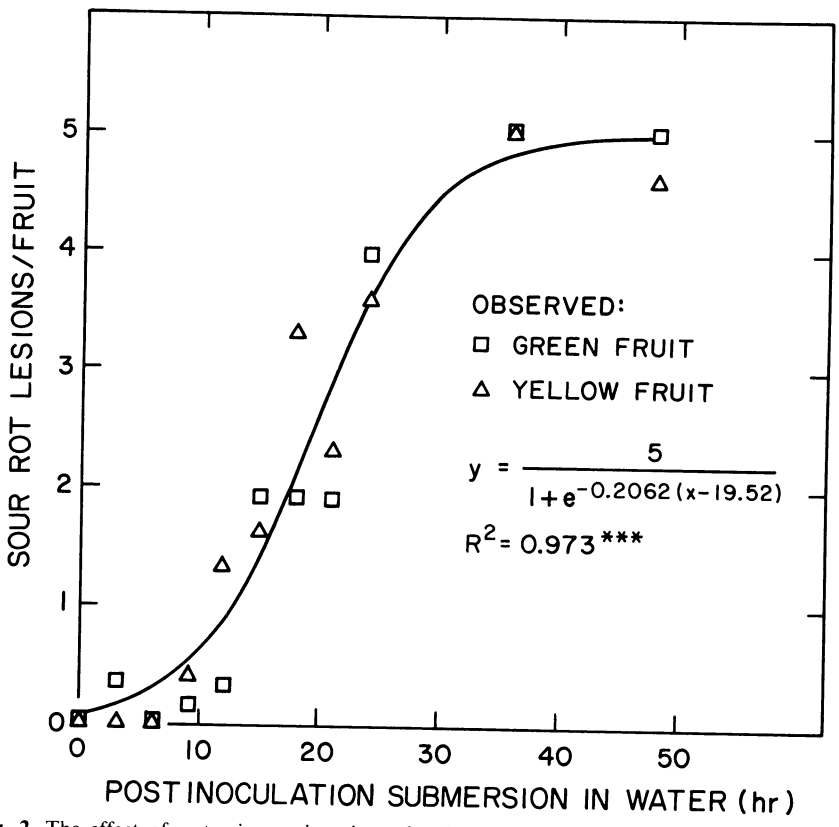

Fig. 2. The effect of water immersion time after inoculation (5×10^3 conidia/site) on sour rot incidence in green and yellow lemon fruit. Disease was evaluated after 3 days' storage at 25 C and approximately 90% relative humidity. Means from 10 to 40 green and 5 to 10 yellow lemons, five inoculation sites each. *** indicates 0.001 probability.

Table 1. Influence of a high-humidity atmosphere or water immersion on predisposition of lemon fruit to sour rot[y]

Fruit color	Active lesions/fruit[z] (no.)		Diameter of rot lesion[z] (mm)	
	High humidity	Water immersed	High humidity	Water immersed
Yellow	4.6 a	5.0 a	10.8 b	14.4 a
Silver	0.5 b	4.8 a	7.1 c	8.1 ab
Light to dark green	0.7 b	5.0 a	6.6 c	7.6 c

[y] Each inoculation site was injected with 5 μl of 1×10^6 conidia per milliliter. The sites were immersed in water or kept in high humidity for 24 hr, followed by storage at 25 C and approximately 90% relative humidity for 6 days.
[z] Mean of 10 lemons, five inoculation sites each. Duncan's multiple range test at 5% level.

Table 2. Influence of a high-humidity atmosphere or water immersion on predisposition of mature and immature green lemon fruit to sour rot[y]

Fruit maturity and diameter	Active lesions/fruit[z] (no.)		Diameter of rot lesion[z] (mm)	
	High humidity	Water immersed	High humidity	Water immersed
Mature, 60 mm	0.3 b	5.0 a	5.3 c	19.8 a
Immature, 40–50 mm	0.0	5.0 a	0.0	15.8 ab
Immature, 30–40 mm	0.0	4.9 a	0.0	13.0 b

[y] Each inoculation site was injected with 5 μl of 1×10^6 conidia per milliliter. The sites were immersed in water or kept in high humidity for 24 hr, followed by storage at 25 C and approximately 90% relative humidity for 6 days.
[z] Mean of 10 lemons, five inoculation sites each. Duncan's multiple range test at 1% level.

of green and yellow lemons to immersion duration.

DISCUSSION

Disease incidence in citrus fruits that have been wound-inoculated with approximately 5×10^3 conidia of *G. candidum* in buffer is often low and variable compared with that produced by similar inoculum of *Penicillin digitatum* (Pers.: Fr.) Sacc. or *P. italicum* Wehmer (4,17, 19). Several factors are recognized to favor infection by *G. candidum*: Fruit ripeness and a high water content in the peel (4,5,6,14); fungal isolate, conidial concentration, and endopolygalacturonase production (2,4,9,19); and a warm (25–30 C) humid environment (8,14,16). The infectivity of inoculum is further enhanced by certain extraneous amendments: cycloheximide to block the development of host defenses (5,7,13,15); pectolytic enzymes and polygalacturonic acid, but not simple nutrients (6,13); and synergistic fungi (13,19). Gutter (17) added homogenized diseased orange peel to the inoculum to obtain a high level of infection.

The water-immersion procedure described here evolved from earlier observations (4,5) that the susceptibility of lemons to sour rot was increased if 1 ml of water was injected into the albedo tissue 1 hr before inoculation at that site or if the water content of the peel was increased by immersing the fruit stem in water. Covering the inoculation site with a drop of water virtually assured infection, whereas soaking the uninjured fruit in water for 20 hr was less effective, apparently because the peel did not absorb much water under these conditions. Our procedure involves immersion of the wounded-inoculated fruit for at least 24 hr, which may bring about a localized imbibition of water by the wounded peel (4). An increased water level of the inoculation sites could increase the frequency of infection in several ways: A low O_2, high CO_2 environment could stimulate the development of *G. candidum* (20); free water might stimulate the induction of endopolygalacturonase by *G. candidum*, a process known to be osmotically sensitive (12), dilute an endogenous endopolygalacturonase inhibitor (3), or transport fungal enzymes into the intercellular spaces of the albedo tissue.

The simple water-immersion procedure described here can be readily scaled up for tests of various control strategies, and comparison of the pathogenicity of isolates under circumstances that discourage the use of cycloheximide (15). The method may also be useful for further determination of the well-known resistance of green and immature fruit to sour rot (5,6,14).

ACKNOWLEDGMENT

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