Reduction of Green Mold on Grapefruit after Hot Forced-Air Quarantine Treatment

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ABSTRACT

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Lesions on grapefruit developing from wounds inoculated with a spore suspension of *Penicillium digitatum* just prior to heating for 300 min in 46°C moist, forced air developed less rapidly during 4 days of storage at 21°C than lesions formed from inoculations made after the fruit were heated, or lesions on non-heated fruit. Since the lesion size on fruit inoculated after heating was similar to that on fruit that were not heated, induced host resistance via lignification or accumulation of phytoalexins probably did not occur. Results from this research demonstrate that development of green mold on grapefruit caused by *P. digitatum* is inhibited by time and temperature regimes of moist, forced air that are known to provide quarantine security against Mexican fruit fly.

Additional keywords: citrus, disinfestation, high temperature forced air, marketing

Infection of grapefruit (*Citrus paradisi* Macf.) by *Penicillium digitatum* (Pers.:Fr.) Sacc. results in green mold, a predominant postharvest disease of citrus (5). Spores of this fungus are ubiquitous in citrus-growing areas and in the fruit-handling environment. Initial infection of the fruit occurs prior to or after harvest via wounds in the fruit peel (17). Decay progresses most rapidly at an ambient temperature of 24°C, and more slowly at temperatures above 30°C or below 10°C. Commercial decay control measures commonly include careful handling to minimize fruit injury, pre- and postharvest use of fungicides, postharvest disinfestants, and temperature management. Resistance of *P. digitatum* to fungicides has been reported, particularly to the benzimidazoles (5).

Quarantine against the importation of fresh grapefruit from areas where fruit flies (Diptera: Tephritidae) are endemic can restrict the movement of grapefruit in domestic and international markets. Adult fruit fly females oviposit in developing or mature fruit, and larvae feed and develop in the fruit pulp. Fruit grown in fruit fly-infested areas must be subjected to some type of disinfestation treatment prior to marketing to prevent inadvertently intro-

ducing a new fruit fly population into a virgin geographic area. Heating grapefruit in moist, forced air at 46°C for 300 min has been demonstrated to provide quarantine security against the Mexican fruit fly (*Anastrepha ludens* Loew) with no deleterious effect on fruit market quality (15).

Heat is an effective method for controlling postharvest decay of fresh fruits and vegetables (1). However, heat treatments targeted specifically for controlling fungal decay differ from those for disinfesting fruit, in that decay control treatments usually involve a shorter exposure period (30 s to 10 min for water immersion and 10 to 60 min for hot air) at higher temperatures (up to 60°C for water or 54°C for hot air).

The objective of this study was to determine whether exposure to moist, forced air at 46°C for the duration known to provide quarantine security against the Mexican fruit fly was also effective in reducing the growth of green mold on grapefruit during subsequent fruit storage.

MATERIALS AND METHODS

Spores of *P. digitatum* were isolated from Rio Red grapefruit and maintained on potato-dextrose agar. A suspension containing 10^6 spores ml^{-1} of distilled water was prepared by counting the spores under a light microscope with a hemacytometer and adjusting final volume. A drop of Tween-20 was added, and the suspension was agitated on a shaker for 3 h prior to inoculation.

Sixty Rio Red grapefruit were harvested at commercial maturity four times in 1995 and nine times in 1996 from an orchard at the United States Department of Agriculture, Agricultural Research Service (USDA-

ARS) Crop Quality and Fruit Insects Research Unit, Weslaco, Texas. The fruit were harvested in 1995 at the end of the citrus season, during March and April. The 1996 harvests were performed three times, during December 1995 and February and April 1996, to provide replicated harvests of early-, mid-, and late-season fruit. The fruit were harvested 1 day prior to treatment, hand-washed in soapy water, and left overnight at ambient temperature (21°C) to dry.

Twenty grapefruit from each harvest were randomly allocated into each of three treatment groups. The three treatments were: (i) inoculation before heating, (ii) inoculation after heating, and (iii) inoculation without heating. Each fruit was inoculated by making four equidistant, 6-mm-deep punctures into the flavedo around the fruit equator with a 1-mm-wide, sterile nail, and inserting 10 μl spore suspension into each wound. Fruit not exposed to the heat treatment were inoculated at the same time as fruit inoculated after heating.

Within 30 min after inoculation, the fruit to be heated were placed inside a forced-air chamber similar to that described by Gaffney (11), and heated for 300 min by rapidly circulating air at 46°C. The fruit were placed in a bin with a perforated bottom, and the bin was placed over the outlet vent inside the chamber. Air heated to 46°C was forced over the surface of the fruit. Throughout the duration of the heat treatment, the dewpoint temperature inside the chamber was maintained 2°C cooler than the coolest fruit surface to avoid condensation on the fruit surface. Air speed inside the treatment chamber was maintained at 2 m s^{-1} . The temperature, relative humidity (RH), and air speed inside the chamber, and the fruit surface and center temperatures, were recorded every 60 s. Temperatures were measured by 36-gauge (0.13-mm) Type T copper constantan thermocouples. After 300 min of exposure, the heated fruit were cooled to 28°C in relatively still, 21°C air. Fruit not exposed to heat were stored at 21°C for the duration of the heat treatment and cool-down period.

The twenty inoculated fruit from each treatment group were placed into one-half standard cardboard boxes and stored for 4 days at 21°C in 80% RH. Development of decay was evaluated after 4 days of storage, and the lesion diameter was measured at each wound inoculation site. The aver-

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age lesion diameter of the four wound sites was calculated for each fruit.

A balanced, randomized experimental design was used with harvests considered as replications (1995) or blocks (1996). The average lesion diameter of 20 observations per treatment group was analyzed. Data from 1995 and 1996 were subjected to an analysis of variance (ANOVA) using SAS (GLM procedure, SAS Institute, Inc., Cary, NC). Mean separation for significant main effects was accomplished using Duncan's new multiple range test. Data from 1995 were analyzed as an ANOVA, with treatment as the main effect and treatment $\times$ harvest as the error term. Data from 1996 were analyzed as a factorial ANOVA, with fruit maturity (early, mid, or late) and treatment as the main effects, and maturity $\times$ treatment $\times$ harvests as the error term.

RESULTS

The order in which the fruit were heated relative to inoculation significantly affected lesion diameter (Table 1). Lesions on fruit inoculated prior to heating were significantly smaller in 1995 and 1996 than those of fruit inoculated after heating or of inoculated fruit that were not heated. Fruit inoculated after heating in 1995 and 1996 developed a similar lesion diameter as inoculated fruit that were not heated. Fruit maturity per se had no influence on lesion diameter (Table 1); nor did fruit maturity interact significantly with any level of the treatment. The average lesion diameter of early-, mid-, and late-season fruit inoculated prior to heating in 1996 was smaller than that of fruit inoculated after heating or inoculated fruit that were not heated.

Nearly identical temperature and dewpoint inside the forced-air chamber during experiments conducted in 1995 and 1996 resulted in similar fruit heating profiles (Fig. 1). The forced air chamber heated at a rate of 2.2°C/min, and reached the set-point of 46°C within 10 min. The temperature at the surface of the fruit reached 41°C after only 10 min of exposure to moist, forced air at 46°C. The fruit surface temperature reached a maximum of 45°C after about 100 min of exposure to moist, forced air at 46°C. The dewpoint temperature inside the forced-air chamber also reached a maximum and stabilized at 43°C after 100 min of exposure. When the fruit surface and chamber dewpoint temperatures had reached their maximum (100 min), the temperature at the center of the fruit was 5°C cooler (40°C) than the fruit surface. The temperature at the center of the fruit reached a maximum temperature of 44.5°C after an additional 100 min of exposure (200 min exposure duration). After removal from the forced-air chamber, the center temperature of the grapefruit cooled to 28°C at a rate of 0.1°C/min. Fruit were inoculated no sooner than 2.5 h after termination of the heat treatment.

DISCUSSION

Results from this study suggest that the mechanism by which exposure to moist, forced air at 46°C reduced the progression of decay was not due to a thermal-induced resistance of the grapefruit. If heating had enhanced rind resistance due to phytoalexin (2,13) or lignin biosynthesis (4,6),

then fruit inoculated after heating should have had a smaller lesion diameter than fruit that were not heated. Since the lesion size of fruit inoculated after heating was similar to that of fruit that were not heated, induced host resistance via lignification or accumulation of phytoalexins probably did not occur. Exposure to 46°C reportedly

Table 1. Average lesion diameter of sites inoculated with *Penicillium digitatum* on Rio Red grapefruit harvested in 1995 or 1996 after 4 days of storage at 21°C

Treatment	Average lesion diameter (cm) ^x			
	1995		1996	
Pre-heat inoculation	2.5 a ^y		0.9 a	
Post-heat inoculation	6.1 b		5.3 b	
No heat inoculation	6.4 b		5.7 b	
Source	df	Mean square	df	Mean square
Treatment	2	743.19***	2	2,573.78**
Error	6	266.38	18	778.79
Maturity			2	191.16
Maturity $\times$ treatment			4	456.26

^x Each of 20 fruit per treatment group were inoculated in four wound sites with 10 μ l of 10⁶ spores ml⁻¹.

^y Means within a year column followed by different letters are significantly different according to Duncan's new multiple range test at $P = 0.05$.

^z Mean square significant at $P = 0.01$.

Fig. 1. (A) Temperature at the center ● and surface ▼ of grapefruit and (B) dewpoint ▼ and dry bulb ● temperature of the forced-air chamber during forced-air treatments of grapefruit in 1995 and 1996. Open symbols represent data from 1995 for the average of four treatment replications ($n = 20$ fruit). Solid symbols represent data from 1996 for the average of nine treatment replications ($n = 45$ fruit).

inhibits protein synthesis (3), suggesting that the mode of action was possibly due to a heat-induced inhibition of pathogen growth.

The fruit evaluated in this study were heated immediately after inoculation, and heating retarded but did not inactivate spore development. Brown et al. (7) reported spore inactivation when inoculated fruit were held at ambient temperature for 24 h prior to a 270-min exposure to 43.5°C forced, vapor heat, but much less consistent control when the fruit were heated immediately after inoculation. The efficacy of heat for controlling the growth of *P. digitatum* is known to be influenced by the moisture content and metabolic activity of the spores, by the heating medium, and by exposure temperature and duration (1). Dehydrated, dormant spores require higher exposure temperatures and longer exposures for inactivation than hydrated, germinating spores. Immersion in hot water (16) and exposure to vapor heat (7) most effectively control green mold when fruit are heated 24 h after harvest or after inoculation, permitting the spores to hydrate, germinate, or interact with the fruit environment prior to treatment. In addition to an incubation period, the heat treatment evaluated by Brown et al. (7) was 30 min shorter and 2.5°C cooler than the heat treatment evaluated in the present study. It is possible that the moist, forced-air treatment evaluated in the present study could have resulted in spore inactivation if we had incubated the inoculated fruit at room temperature for 24 h prior to heating.

Heat treatments developed for disinfesting fruit of fruit flies have been shown to control postharvest decay (7–9,14). Like the moist, forced-air treatment evaluated in

this study, most heat disinfestation treatments do not provide satisfactory levels of disease control, and have little if any residual activity for protection during postharvest marketing and storage. These heat treatments, however, may be commercially most useful when applied in tandem with an additional postharvest treatment for decay control. For example, biocontrol of green mold by *Pseudomonas glathei* is enhanced when heat is used to retard the spore germination of *Penicillium digitatum* while simultaneously stimulating the multiplication of the protective bacteria (12). Heat may also accelerate the growth of an avirulent culture of *Geotrichum candidum* that reduces the incidence of green mold on grapefruit (10). Our results suggest that moist, forced air can be used not only to disinfest grapefruit of the Mexican fruit fly, but also to assist in delaying green mold development.

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