CONTROL OF GREEN MOLD IN MARSH GRAPEFRUIT WITH VAPOR HEAT QUARANTINE TREATMENT

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Abstract. Marsh grapefruit were inoculated in 3 separate tests with spores of Penicillium digitatum and subjected either immediately or 24 hours after inoculation to a vapor heat quarantine treatment of 43.5°C and 100% relative humidity for approximately 4.5 hours. Levels of green mold were associated with spore concentration except in one test of fruit treated immediately with vapor heat, where infection levels did not differ at concentrations of 500, 5000 or 50,000 spores per inoculation site. Best control was achieved with the delayed vapor heat treatment where spore concentration did not affect disease and the incidence was less than 2 percent.

Heat treatments for the control of fungal diseases and insect infestations were investigated rather extensively several years ago and periodically since that time (Couey, 1989). With the advent in more recent years of effective fungicides and fumigants, interest declined in the use of heat for pest and disease control. However, interest in the use of heat for postharvest treatments has experienced a revival recently because of several factors. Perceived worker and food safety concerns have caused the loss of several effective materials, one example being ethylene dibromide. More stringent regulatory requirements have caused deregistration of old materials and registration of new compounds to be expensive, less profitable, and a risky investment for pesticide manufacturers. Low volume requirements of chemicals for postharvest uses on a minor crop such as citrus also contribute to the lack of profitability.

Heat may be applied in the form of hot dry air, water-saturated hot air (vapor heat), or as hot water (Couey, 1989). In the past, aqueous treatments have been applied to citrus fruit for the control of brown rot (Klotz and DeWolfe, 1961), green mold, and stem-end rot (Smoot and Melvin, 1963; Smoot and Melvin, 1965). None of these treatments are in use today on a commercial level. With the loss of ethylene dibromide as a quarantine treatment for the eradication of fruit flies, heat treatments have been investigated as a replacement treatment (Gaffney and Armstrong, 1990; Gaffney et al., 1990; McGuire, 1991; Miller and McDonald, 1991; Miller et al., 1991; Miller et al., 1988; Miller et al., 1991; Sharp, 1989). During the course of these studies, treatments with heated air have caused some reduction in postharvest diseases (McGuire, 1991; Miller and McDonald, 1991; Miller et al., 1991).

The study reported herein was undertaken to evaluate the effect of a vapor heat treatment for the eradication of the Caribbean fruit fly (Anastrepha suspensa) on the control of green mold caused by Penicillium digitatum.

Materials and Methods

Fruit. 'Marsh' white grapefruit were obtained on 3 separate dates of January 10, 17, and 31, 1991 from a packinghouse in Vero Beach, Florida. All fruit were from groves located in Indian River or St. Lucie counties. On each occasion, fruit were taken from field bins before they were subjected to any packinghouse handling. This was generally within 24 hr of harvest. Fruit were transported in crates by truck to the U.S. Dept. of Agriculture (USDA) Horticultural Laboratory in Orlando where they were
washed and graded. Fruit were randomized into lots before receiving 1 of 4 levels of inoculum followed by 1 of 3 vapor heat treatments.

Inoculation. Spore concentrations were determined with a spectrophotometer at 700 nm, slit width 0.5 nm (Morris and Nicholls, 1978). Fruit were inoculated at 4 equidistant sites around the fruit equator. Spores were injected at concentrations of 500, 5000, or 50,000 per site in 5 µL of water using a Hamilton repeating dispenser attached to a 250 µL syringe fitted with a no. 19 Stylex hypodermic needle (Fig. 1). The needle was adapted to form a puncture 2 mm in depth. Control fruit were not injected.

Heat treatments. Fruit were held at 21°C and 92% relative humidity in wooden crates after inoculation and before treatment, and fiberboard 4/5 bushel cartons after treatment. For treatment immediately (0-hr) or 24 hr (24-hr) after inoculation, fruit were placed in plastic bins (Miller and McDonald, 1991) and stacked in the vapor heat chamber (Gaffney et al., 1990; Miller and McDonald, 1991). Fruit were treated for 4.5 hours with humid (95 ± 5% relative humidity) air heated to 43.5°C and applied at an air flow rate of 0.4 M³ sec⁻¹. Fruit were not waxed after treatment and were inspected for infection that developed at the inoculation site at 3 and 7 days following inoculation. Data are reported only for observations made at 7 days. Each treatment consisted of 3 replications with each replication containing 15 fruit and a total of 60 inoculation sites.

Analysis of data. Data were transformed using arcsine transformations before analysis as a factorial experiment with 4 spore concentrations, 3 vapor heat treatments, and 3 tests using the analysis of variance procedure (SAS Institute, 1988). Orthogonal comparisons were used to contrast individual treatments.

Results

The analysis showed a highly significant (P = 0.01) effect of vapor heat, spore concentration, and test on the incidence of green mold. Since differences among tests were significant, data from the 3 tests were analyzed separately and are shown in Table 1. Less decay (15.1%) developed in fruit of test 1 than in fruit of test 2 (20.2%) or 3 (20.3%).

A significant interaction (P = 0.01) between heat treatment and spore concentration for inoculation was observed in all 3 tests. In control fruit, infection increased as spore concentrations were increased. This also occurred with the 0-hr heat treatment except in test 2 where no differences occurred except in non-inoculated fruit. In this same test, the concentration of 500 spores caused more infection in the 0-hr vapor heat treatment than in the control. The 24-hr vapor heat treatment effectively prevented infection (< 2%) irrespective of the spore concentration (Table 1).

Slight peel damage was observed on a few fruit from the heat treatments. The lesions were observed at 3 days and were slightly darker by 7 days following heat treatment.

Discussion

Application of the vapor heat treatment at conditions necessary for eradication of larvae of the Caribbean fruit fly reduced green mold in these experiments where spore concentration and germination time were accurately controlled. Unlike these results, control of green mold in naturally infected grapefruit with vapor heat was not observed in a previous experiment (Miller et al., 1991). Lack of control in naturally infected fruit may be attributable to the various stages of spore germination that exist at the time of vapor heat treatment. Lack of any residual activity from the heat treatment also allows reinfection.

Ungerminated spores of Penicillium digitatum are less susceptible to heat than germinated ones. This was observed previously (Smoot and Melvin, 1963; Smoot and Melvin, 1965), where control of green mold in naturally colored oranges with hot water was best if the treatment was delayed 1 day following harvest. However, decay control was less effective if delays were for as long as 2-3 days, probably because of

![Fig. 1. Apparatus used to inoculate Marsh grapefruit with Penicillium digitatum.](image)

Table 1. Effect of vapor heat treatment on the incidence of green mold in Marsh grapefruit inoculated with Penicillium digitatum.

<table>
<thead>
<tr>
<th>Treatments*</th>
<th>A Spore Concentration x Sites infected (%)</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Contrasts</th>
<th>P &gt; F*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test 1*</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1. Control</td>
<td>0.9 3.3 47.6 89.5</td>
<td>1.B vs 1.CD</td>
<td>0.0001</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2. VH 0-hr</td>
<td>0.0 0.0 9.5 30.6</td>
<td>1.C vs 2.C</td>
<td>0.0001</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>3. VH 24-hr</td>
<td>0.0 0.0 0.0 0.0</td>
<td>1.D vs 2.D</td>
<td>0.0001</td>
<td></td>
<td></td>
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<tr>
<td>Test 2</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1. Control</td>
<td>1.1 8.9 66.1 95.0</td>
<td>1.B vs 2.B</td>
<td>0.0046</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. VH 0-hr</td>
<td>0.0 20.0 23.4 24.5</td>
<td>1.B vs 2.C</td>
<td>0.5430</td>
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</tr>
<tr>
<td>3. VH 24-hr</td>
<td>0.0 1.1 1.7 0.6</td>
<td>1.C vs 2.D</td>
<td>0.6984</td>
<td></td>
<td></td>
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<tr>
<td>Test 3</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1. Control</td>
<td>1.7 25.6 93.9 99.4</td>
<td>1.B vs 1.CD</td>
<td>0.0001</td>
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<tr>
<td>2. VH 0-hr</td>
<td>0.6 0.6 1.7 18.9</td>
<td>1.C vs 2.C</td>
<td>0.0001</td>
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<tr>
<td>3. VH 24-hr</td>
<td>0.0 0.0 0.0 0.0</td>
<td>1.D vs 2.D</td>
<td>0.0001</td>
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</tbody>
</table>

*Spores site:* A = 0; B = 500; C = 5,000; D = 50,000.
*Test dates: Test 1 = 1/10/91; Test 2 = 1/17/91; Test 3 = 1/31/91.
*Probability, using orthogonal contrasts, that values are different due to chance.
more advanced stages of infection. Physiological rind breakdown was also enhanced by delaying the treatment (Smoot and Melvin, 1963).

Stress from the heat treatment may predispose injuries to infection by *P. digitatum* if spore survival is sufficient or if reinfection occurs. More infection in the 0-hr heat treatment than in the control of test 2 at comparable low-level inoculum would also indicate that natural healing and resistance (Brown et al., 1978) of that injured tissue may be suppressed. Waxing has been shown to reduce phytotoxicity of the vapor heat treatment (Miller et al., 1991).

Control of pathogens of citrus other than green mold has been observed with heat treatments. Hot water treatments provided control of stem-end rot caused by *Phomopsis citri* (Smoot and Melvin, 1963), but were less effective against that caused by *Diplodia natalensis*. When heat treatments were applied to mangoes for control of pathogens also found on citrus, hot water significantly reduced anthracnose caused by *Colletotrichum gloeosporioides* and stem-end rot caused by *D. natalensis* (McGuire, 1991). The same diseases were also significantly reduced with heated forced air treatments (Miller et al., 1991).

Vapor heat temperatures and durations used for this study were those developed for fruit fly eradication. Some modifications may provide even more effective control of green mold and other postharvest diseases. Additional studies with heat may be justified if indeed fungicides become unavailable in the future. Though effective control with heat was achieved in these studies, additional evaluations would require tests with naturally infected fruit where various stages of infection may exist at the time of treatment.

Adaptation of heat for commercial usage would certainly not be without its challenges. Rather sophisticated instrumentation and large scale equipment would be required to treat large volumes of fruit at accurate temperatures. The process would certainly be more expensive, complicated, and inconvenient as compared to our present methods of fungicide treatment. Efficacy of heat in all probability would be less than present day fungicides, such as thiabendazole and imazalil, and risk of phytotoxicity would be greater. Sensitivity of citrus cultivars to heat treatments applied for decay control has been shown to vary, with mandarin types being more susceptible than oranges or grapefruit (Smoot and Melvin, 1965).

**Literature Cited**