Influence of Temperature and Humidity on Survival of *Penicillium digitatum* and *Geotrichum citri-aurantii*

J. L. Smilanick and M. F. Mansour, USDA ARS, San Joaquin Agricultural Sciences Center, 9611 South Riverbend Avenue, Parlier, CA 93648

ABSTRACT

Smilanick, J. L., and Mansour, M. F. 2007. Influence of temperature and humidity on survival of *Penicillium digitatum* and *Geotrichum citri-aurantii*. Plant Dis. 91:990-996.

Longevity of conidia of *Penicillium digitatum* (cause of citrus green mold) and arthroconidia of *Geotrichum citri-aurantii* (cause of sour rot of citrus) was determined under controlled temperature and relative humidity (RH) or ambient summer conditions in central California. Longevity at low RH was longer than at high RH. Hours to kill 99% of the conidia (LT₉₉) of nine *P. digitatum* isolates were determined at 50°C and 75 or 95% RH. At 75 and 95% RH, the LT₉₉ was 24.9 and 4.9 h, respectively. The LT₉₉ was 30 and 42 days, respectively, for conidia of *P. digitatum* under ambient conditions at two outdoor locations. The LT₉₉ of arthroconidia of *G. citri-aurantii* from colonies cultured on potato dextrose agar was briefer than that of conidia of *P. digitatum*. At 45°C and 75 or 95% RH, the LT₉₉ was about 4 and 2 h, respectively, whereas at 50°C, none was viable after 1 h at either humidity. Sanitation is an important practice for managing these diseases. Since there was little or no survival of either fungus after 1 day at 50°C and 75% RH or higher, we conclude that commercial sanitation could employ a similar regime.

Additional keywords: *Geotrichum candidum*, heat treatment, postharvest

Sanitation is an important component in citrus packinghouse management, because various airborne pathogens usually bridge their survival between seasons by means of resting structures that contaminate these buildings. Sanitation aims to reduce or eliminate the fungal population from all sources, thus breaking the continuity of pathogen survival in time and space between seasons. It is particularly important in the management of fungicide-resistant isolates of *Penicillium digitatum* (Pers.:Fr.) Sacc. (cause of citrus green mold), since fungicide-resistant isolates of this pathogen occur primarily within packinghouses in California, where fungicides are used, and not in groves, where fungicides are not used (11). Therefore, effective sanitation within packinghouses can substantially reduce fungicide resistance problems. Sanitation is also an important method to control *Geotrichum citri-aurantii* (Ferraris) Butler, cause of citrus sour rot, because postharvest fungicides to effectively control this disease are not registered in the United States. Both of these diseases can occur during ethylene degreening, long-

term storage of the fruit, or delayed sales of fruit in cartons, all of which can result in severe contamination of storage rooms and boxes with conidia or arthroconidia. Currently, formaldehyde fumigation or spray applications of alcohol or quaternary ammonium compounds are used for sanitation in citrus packing and storage facilities.

Hong et al. (9) summarized information on the longevity of fungal spores, and noted that what is known about spore longevity is limited and somewhat ambiguous. In general, temperature, conidial moisture content, and the humidity of the storage atmosphere are the major factors that influence spore longevity (9). For most fungi, the length of life of spores, as measured by their ability to germinate after various periods, decreases as temperature, conidial moisture content, or equilibrium relative humidity (RH) increase. At a fixed RH, increasing temperature (but below that which kills spores) generally decreases the longevity of fungal spores, whereas lower temperatures (above freezing) increase longevity. The relationship between RH and viability of fungal spores does not appear to be as simple; most persist longer at lower humidity, conversely, some species die more rapidly at lower humidity; and an isolate of *Aspergillus flavus* was reported to lose viability rapidly at 75% RH while persisting much longer at lower or higher RH (27). The survival of postharvest pathogenic fungi of fresh pome fruit has been characterized (26), but little has been published about those of citrus fruit.

The objectives of this study were to determine the optimum combinations of temperature and RH that can kill conidia of *P. digitatum* and arthroconidia of *G. citri-aurantii* within a reasonable period of time, which could be applied in packinghouses and storage rooms for sanitation purposes in place of chemical sanitizers. We also determined the survival of conidia of *P. digitatum* at two outdoor locations under ambient conditions.

MATERIALS AND METHODS

Fungi. *P. digitatum* isolates used in this study were collected from citrus groves and packinghouses in California during 2004. Many aspects of their growth, virulence, and fungicide sensitivity have been studied (11). Isolates included the fungicide-sensitive isolates PD90, PD172, PD180, and PD182, imazalil-resistant isolates PD136 and PD149, imazalil- and thiabendazole-resistant isolates PD120 and PD130, and imazalil-, thiabendazole-, and sodium ortho-phenyl phenol-resistant isolates PD145 and PD155. The conidia employed in all experiments were collected from sporulating lesions on orange fruit. Oranges were inoculated by injection of 0.2 ml of a suspension that contained about 1×10^6 spores-ml⁻¹ about 1 cm beneath the rind surface (2) followed by incubation of the fruit for 7 days at 20°C before use. Conidia of *P. digitatum* were collected from decay lesions on oranges by brushing the surface of the lesions with a camel hair brush, and collecting conidia in a glass vial. The conidia were used immediately. The isolate of *G. citri-aurantii* used was from an infected lemon from a packinghouse in Ventura County, CA. It was grown on potato dextrose agar (PDA; Difco Laboratories, Detroit MI) plates for 2 weeks at 24°C before use.

In some experiments, about 2 g of conidia of *P. digitatum* were placed in glass vials with porous fiber cloth cover (Miracloth, Calbiochem, La Jolla, CA). The cylindrical vials were 5.5 cm in length, 2.5 cm in diameter, and had an opening 2 cm in diameter. The vials were placed inside rooms of known and stable temperature and RH, or they were placed in outdoor locations where temperature and RH were recorded hourly. In subsequent experiments, about 1 g of conidia was dispersed in a 2 by 3 cm plastic weigh boat, and then placed inside humidity controlled containers at various temperatures. In both cases,

Corresponding author: J. L. Smilanick
E-mail: Jsmilanick@fresno.ars.usda.gov

Accepted for publication 19 March 2007.

doi:10.1094/PDIS-91-8-0990

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2007.

small samples of conidia were withdrawn at intervals to determine their germinability.

Arthroconidia of *G. citri-aurantii* were collected from PDA cultures by flooding the colonies with sterile water containing 0.01% (wt/vol) Triton X-100 (Sigma Chemical Co., St Louis, MO). A dense suspension was prepared that contained about 2×10^6 arthroconidia-ml⁻¹, as determined with a spectrophotometer (2). Sterile wood craft sticks, 11.3 cm in length, 1 cm wide, and 2 mm thick, were placed in the suspension for 2 min, dried in air under a sterile laminar-air flow hood for several minutes, then placed inside humidity controlled containers at various temperatures. A single stick sample containing the arthroconidia was withdrawn at intervals to determine their germinability. To resuspend the arthroconidia, the sticks were suspended in sterile water and vortexed at high speed for 1 min.

The germinability of conidia of *P. digitatum* was determined by the preparation of dense initial suspensions that contained about 1×10^6 conidia, as determined with a spectrophotometer (2). Then a series of dilutions was prepared, 10 µl of each dilution was plated on PDA, and each was incubated at 25°C. Arthroconidial suspensions were diluted but not adjusted before dilution, because the density of suspensions recovered from the sticks was not high. After incubation for 18 h, the conidia or arthroconidia were stained with acid-fuchsin and examined by light microscopy ($\times 100$). The acid-fuchsin (0.1 g acid fuchsin, 100 ml lactic acid, and 100 ml deionized water) stopped germination and improved contrast of the conidia or arthroconidia (25). They were considered to have germinated when the length of the germ tube was equal to one-half of their diameter (19). In some experiments, unstained conidia or arthroconidia were incubated on PDA for periods as long as 3 to 5 days, to determine if germination had been delayed rather than stopped by thermal treatments.

Thermal and humidity treatments. RH and temperature were measured hourly during experiments with a temperature-RH logger (HOBO Model U12, Onset Computer Corp., Bourne, MA). The accuracy of these loggers was compared to measurements of temperature by a precision mercury thermometer (Miller and Weber, Inc., New York) and to measurements of RH by a chilled-mirror psychrometer (Model M2-Plus, General Eastern Instruments, Woburn, MA). As indicated by the manufacturer of the temperature-RH loggers, the temperature accuracy was high throughout the ranges used in our studies, while RH measurement accuracy was excellent at intermediate RH and less so above 95%.

In a preliminary study to evaluate the longevity of conidia of *P. digitatum* isolate

PD90, about 2 g of conidia were placed inside glass vials with a porous fiber cloth cover at the top to allow for gas exchange. Vials were placed in incubators at 1°C with 99% RH, 5°C with 86% RH, 10°C with 85% RH, 20°C with 54% RH, 25°C with 37% RH, 30°C with 47% RH, or 40°C with 19% RH. Three replicate vials of conidia were placed in each environment. A representative sample was collected weekly, and germination was assessed to determine the viability of the conidia. Samples were collected for 10 weeks. Temperature-RH loggers were placed in each. Temperature changed no more than 0.5°C and RH no more than 5% during this experiment.

In subsequent experiments, the longevity of *P. digitatum* conidia or *G. citri-aurantii* arthroconidia was determined in a sealed plastic container with two asymmetrical compartments that shared a common head space (1.13-liter capacity; Rubbermaid Inc., Wooster, OH). The larger compartment was 12 cm long, 12 cm wide, and 3 cm deep. The smaller compartment was 12 cm long, 8 cm wide, and 3 cm deep. In the larger compartment, 200 ml of saturated salt solution were present. The conidia or arthroconidia, and in some tests, a RH and temperature logger, resided in the smaller compartment. RH of 95, 75, and 32% were maintained within the compartments using oversaturated solutions of K₂SO₄, NaCl, or MgCl₂ (Sigma), respectively.

Five similar experiments that assessed survival of conidia of *P. digitatum* under conditions of controlled temperature and RH were conducted. Each experiment was done once. In a first experiment, three replicate vials containing conidia of *P. digitatum* isolate PD90 were kept under a constant temperature at 40 or 45°C at an RH of 95, 75, or 32%. Germination of conidia was determined weekly for 3 weeks. In a second experiment, conidia of *P. digitatum* isolate D201 were incubated within three replicate vials at 35, 40, 45, or 50°C at a RH of 95, 75, or 32%. Germination of conidia was determined after 4, 7, and 12 days. In a third experiment, conidia of *P. digitatum* isolate PD90 were incubated within three replicate vials at 40, 45, or 50°C at a RH of 95, 75, or 32%. Germination of conidia was determined after 1, 2, 3, 7, and 15 days. In a fourth experiment, conidia of *P. digitatum* isolate PD90 were incubated within three replicate vials at 45 or 50°C at a RH of 95 or 75%. Germination of conidia was determined after 1, 2, 24, 36, 48, and 60 h. In the fifth experiment with controlled temperature and RH, conidia of nine isolates of *P. digitatum* (fungicide-sensitive isolates PD172, PD180, and PD182, imazalil-resistant isolates PD136 and PD149, imazalil- and thiabendazole-resistant isolates PD120 and PD130, and imazalil-, thiabendazole-, and sodium ortho-phenyl phenol-resistant

isolates PD145 and PD155), each in a single vial for each combination of RH and temperature, were placed at 50°C at 75 or 95% RH. Germination of conidia was determined every 2 h for 24 h. The fourth and fifth experiments were conducted specifically to determine the LT₉₉ values (the duration in days or hours required to inhibit germination of 99% of spores) for the combinations of RH and temperature tested.

An experiment to assess survival of arthroconidia of *G. citri-aurantii* under conditions of controlled temperature and RH was conducted. Arthroconidia on craft wood sticks were placed at 40, 45, or 50°C at 75 or 95% RH. Germination of arthroconidia was determined from two replicate craft wood sticks collected hourly for a period of 12 h. The experiment was repeated.

Two experiments were conducted to determine the longevity of conidia of *P. digitatum* isolate PD90 under natural ambient summer conditions in the San Joaquin Valley of California. Two locations were selected, and the conidia were placed to avoid direct sunlight. The first location (Parlier) was within a tree at a height of 1.5 m in Fresno County (longitude N 36°35.789', latitude W 119°30.803'), and the second location (Lindcove) was within a citrus grove in Tulare County (longitude N 36°21.607', latitude W 119°03.595') at a height of 1 m. About 2 g of conidia were placed inside glass vials as previously described with a porous fiber cloth cover at the top to allow for gas exchange. At each location, temperature and RH were recorded hourly (HOBO Model U12). Germination of conidia was determined weekly for a period of 16 weeks.

Statistical analyses. The percentage of germination was calculated, and mean values and their standard deviations are reported. Probit analysis (SPSS 14.5, Chicago, IL) was applied to estimate the time in hours or days, with 95% confidence intervals, when germination decreased by 99% (LT₉₉) compared to that of the initial germinability of the conidia or arthroconidia (5). Before the LT₉₉ was calculated from germination counts, tests for normality and homogeneity of variance were applied.

RESULTS

In a preliminary test that assessed germination after storage in wide range of static conditions, temperatures of 1, 5, or 10°C (with RH 99, 86, or 85%, respectively) had little or no effect on germination of *P. digitatum* conidia during the 10-week period of the experiment (Fig. 1). At 20 or 25°C (with RH 54 and 37%, respectively), the decline in germination percentage was moderate at the end of this period. At 30°C with RH 47%, there was a sharp decline in germinability of conidia after 8 weeks of exposure, although at the end of

the 10-week storage period, 8% germinated. However, at 40°C with 19% RH, germination declined relatively rapidly, and none was observed after 9 weeks.

In the first experiment that assessed survival of conidia of *P. digitatum* at 40 or 45°C and RH of 32, 75, or 95%, increasing temperature and RH both reduced the percentage of germinable conidia (Fig. 2). At either temperature with 95% RH, very few spores were viable after 1 week. The germination percentage observed was similar if the germination was counted after 18 h

Fig. 1. Effect of different combinations of temperature and relative humidity on the longevity (as determined by % germination) of conidia of *Penicillium digitatum* isolate PD90. Germination was assessed from conidia removed weekly for 10 weeks.

Fig. 2. Longevity of conidia of *Penicillium digitatum* isolate PD90 stored at a constant temperature of 40 or 45°C at a relative humidity of 32, 75, or 95%. Germination percentage was assessed from conidia removed weekly for 3 weeks.

or up to 5 days on PDA after exposure of the conidia to controlled temperature and RH conditions. This indicates that the ability of conidia to germinate was not delayed but stopped.

In the second experiment that assessed survival of conidia of *P. digitatum* at 35, 40, 45, or 50°C and RH of 32, 75, or 95%, increasing temperature and RH both reduced the percentage of germinable conidia (Fig. 3). At 40, 45, or 50°C with 95% RH, very few spores were viable after 4 days, while at 35°C or lower RH, germination was higher. At 50°C, regardless of RH, none of the conidia germinated after 4 days exposure, which was the briefest exposure period evaluated. As in the prior experiment, the germination percentage observed was similar if the germination was counted after 18 h or up to 5 days on PDA after exposure of the conidia to the controlled temperature and RH conditions.

In the third and fourth experiments that assessed survival of conidia of *P. digitatum* at 40, 45, or 50°C and RH of 32, 75, or 95%, increasing temperature and RH both reduced the percentage of germinable conidia as in prior experiments (Fig. 4). Data from both tests were combined to determine LT_{99} values over a wide range of exposure periods. At these temperatures, mortality of conidia at 32% RH occurred very slowly compared to that at higher RH. At 45 and 50°C with 95% RH, the LT_{99} predicted values were very brief, only 7.8 and 4.9 h, respectively.

In the fifth experiment, testing the effect of 50°C at 75 or 95% RH on fungicide-

sensitive and -resistant isolates of *P. digitatum*, all isolates responded similarly at 95% RH with LT_{99} of about 5 h (Fig. 5). The LT_{99} values at 75% RH indicated that there was some variation among the isolates; the LT_{99} of isolate PD155 was lower at 11.5 h, and that of isolate PD120 was higher at 33.5 h, than most of the other isolates, which had LT_{99} values between 20 and 30 h.

The ability of conidia to germinate after exposure to summer conditions at both locations in the San Joaquin Valley declined by one-half approximately every 2 weeks (Fig. 6). Environmental conditions at both locations were similar, although occasionally direct sunlight was present briefly on the sample site at times at the Parlier location that slightly increased the mean temperatures and greatly increased the daily peak temperatures. This period (8 July to 30 August 2005) was exceptionally hot. Estimates of LT_{99} (95% confidence interval) at the Lindcove and Parlier locations were 30.2 (27.2, 34.3) and 41.6 (34.2, 55.1) days, respectively.

The ability of arthroconidia of *G. citri-aurantii* to germinate declined more rapidly than that of conidia of *P. digitatum* (Fig. 7). However, unlike *P. digitatum*, after exposure of the arthroconidia to the controlled temperature and RH conditions, the percentage of germination observed increased when germination counts made after 18 h were compared to those made after 5 days incubation on PDA. This indicates the ability of some arthroconidia to germinate was delayed by the environ-

Fig. 3. Longevity of conidia of *Penicillium digitatum* isolate D201 stored at a constant temperature of 35, 40, 45, or 50°C at a relative humidity of 32, 75, or 95%. Germination was assessed from conidia removed after 4, 7, and 12 days of storage.

mental treatments before the ability to germinate was stopped. At 50°C with both 75 and 95% RH, germination of all arthroconidia was inhibited completely after 2 h of exposure. At 45°C with 95% RH, germination of all arthroconidia was inhibited completely after 3 h of exposure.

DISCUSSION

Survival of conidia of other postharvest pathogens is similar in many respects to that of *P. digitatum*. Spotts (26) characterized the longevity of several fungi that cause postharvest decay of pome fruit. He reported that conidia of *P. expansum*, the cause of postharvest blue mold of pome fruit, and *Botrytis cinerea*, the cause of postharvest gray mold, persisted about 40 days and more than 63 days, respectively, on the surface of pear fruit in orchards. Under controlled conditions, increasing RH and temperature decreased their persistence. Moyano and Melgarejo (17) reported significant differences in survival of conidia of *B. cinerea* associated with temperature, RH, and their interaction. High temperatures caused a decrease in survival of conidia; at 40°C, conidia survived for only 7 days at 80% RH, whereas at 22°C, they survived for 30 to 90 days depending on the RH.

Prior research on the influence of RH on the survival of conidia of other fungi can provide an interpretation of our findings with conidia of *P. digitatum* and arthroconidia of *G. citri-aurantii*. The moisture content of conidia is a function of RH, and the moisture content of fungal conidia spans a wide range, from 8 to 88% of fresh weight, depending upon species and conditions of growth (6). Fungal conidia, and spores of bacteria and seeds, invariably exhibit an S-shaped plot of water sorption as a function of RH. Between 0 and 10% RH, most biological materials exhibit a sharp increase to as much as 5% water content. A more gradual increase is observed between 10 and about 80% RH, to a water content of about 10 to 25%. This water is considered to be "bound" at charged or polar sites with varying degrees of tightness (12). Above 80% RH, there is a sharp increase in the water content to a value usually in excess of 35% at 100% RH. This is considered to represent "bulk" water accumulating in the regions between hydrated molecules (12). Fahey et al. (4) reported that damp conidia of *Neurospora* (about 65% water content) exhibited significant metabolic activity, as evidenced by a reduction of oxidized to reduced glutathione (GSH), degradation of GSH, and changes in the pool sizes of certain amino acids, whereas dry conidia had little metabolic activity. Dry conidia had greater longevity than wet conidia. Furthermore, dry and quasi-dry conidia had substantial resistance to heating at 100°C, whereas wet conidia had no resistance. They stated that dehydration appears to be essential to

thermal resistance in conidia, probably via a direct effect upon the stability of macromolecules. Dry proteins survive heating more readily than wet proteins, and lyophilized enzymes have withstood extended heating under vacuum at temperatures in excess of 200°C (12). Rotem (20) found longevity of mycelium of *Alternaria porri* f. sp. *solani* at all humidities decreased with increasing temperature. The optimal relative humidities for longevity were from 7 to 38%, depending upon the temperature. An increase in humidity was associated with a sharp decrease in longevity, and a decrease in RH with a slow decline. Similar effects were found in relation to the longevity of spores. Mycelium and spores died within 2 h at 90 and 60°C, respectively. Although the survival rate of this fungus is much greater at low temperature, any increase of atmospheric humidity depresses it markedly. Teitell (27) found a narrow band of RH which had a pronounced lethal effect on conidia of several

isolates of *Aspergillus flavus*. At 29°C, this lethal RH was 75%; conidial survival was not affected at relative humidities of 73% or lower and 77% or higher (27).

The longevity of conidia of *P. digitatum* and arthroconidia of *G. citri-aurantii* that we report, and the observation that it was inversely proportional to both temperature and RH, is similar to reports about the longevity of many other fungi. The longevity of mycelium and spores of several fungi, including *Penicillium italicum*, was diminished in 1 day at 53°C when the RH was 74% or higher (20). Schein and Rotem (22) stored uredospores of the bean rust fungus, *Uromyces phaseoli*, under different temperatures and RH, and reported that as the temperature and humidity of the storage atmosphere increased, the longevity of spores, as determined by their germinability, declined, and this decrease was inversely proportional to both temperature and humidity of the atmosphere. The RH during storage of the conidia of *Helmin-*

Fig. 4. Longevity of conidia of *Penicillium digitatum* isolate PD90 stored at a constant temperature of 40, 45, or 50°C at a relative humidity of 95, 75, or 32%. Values are the times in hours when the percentage of conidia that could germinate had declined by 99% (LT₉₉), as estimated by probit analysis. Germination percentage was assessed from conidia removed hourly when mortality was rapid, at 45 or 50°C at 75 or 95% RH, or daily when mortality was slow, at 40°C or at 32% RH at all temperatures. Data from two experiments were combined.

thosporium oryzae was a more important factor influencing longevity than temperature alone (18). For example, at a RH of 20%, the conidia survived for 6 months with little loss of viability even at 31°C, whereas at a RH of 95%, they failed to germinate after 1 month at this temperature. Viability of conidia of *Metarhizium flavoviride* declined relatively rapidly at high temperatures (up to 55°C) when dried to a moisture content of 19%, whereas the viability of those dried to 4 to 5% moisture content declined slowly (8). Hong et al. (9), Sandhu et al. (21), and Spotts (26) have characterized the longevity of many fungi, including *A. porri*, *Beauveria bassiana*, *Beauveria brongniartii*, *Botrytis cinerea*, *Helminthosporium oryzae*, *Metarhizium anisopliae*, *Mucor pyriformis*, *Paecilomyces farinosus*, *Penicillium ex-*

pansum, *Sclerotinia sclerotiorum*, and *Uromyces appendiculatus*, and showed declining longevity with increasing moisture content or RH and increasing temperature. Hong et al. (10) developed models driven by conidial moisture content and temperature that predicted longevity of isolates of *Beauveria bassiana*. They were concerned with the shelf life of this formulated entomopathogenic fungus, and increasing temperature and humidity shortened it. Not all fungi respond in this manner, however. Temperature, and not RH, was the main climatic factor affecting longevity of urediniospores of *Tranzschelia discolor* (3). A model based on temperature showed that urediniospores of *T. discolor* were critically affected by temperatures in the range 25 to 30°C; exposure to higher temperatures resulted in a

34-fold faster rate of decline. Temperatures above the optimum for growth generally represent a stress for fungi that reduces the longevity of their spores. For example, optimal growth of *Aspergillus carbonarius* occurs from 25 to 35°C (1); however, Leong et al. (13) reported that 37°C severely retarded growth and 42°C killed the conidia. Heat stress at 37°C contributed to the relatively rapid death of *A. carbonarius* spores (14). In our work and that of most others, constant and controlled conditions of temperature and humidity were applied; however, it is conceivable that prior activation of conidia or arthroconidia could influence their subsequent longevity. Lingappa and Sussman (15) reported that heat resistance declined after activation of ascospores of *Neurospora tetrasperma*. Activation, which consisted of 5-min incubation at 27°C, rendered spores susceptible to substantial mortality after a 5-min "heat flash" of 65°C. When the heat flash was applied 25 min after activation, none of the ascospores survived, whereas more than half of unactivated ascospores survived exposure for 30 min to 65°C. Dry heat was less effective than wet heat in activating ascospores.

The longevity of arthroconidia of *G. citri-aurantii* during incubation at warm temperatures was less than that of conidia of *P. digitatum*. For example, at 50°C with 95% RH, the LT₉₉ of conidia of *P. digitatum* was about 5 h, whereas no arthroconidia of *G. citri-aurantii* germinated after 2 h incubation under these conditions. At 45°C with 95% RH, the LT₉₉ of conidia of *P. digitatum* was about 8 h, whereas no arthroconidia of *G. citri-aurantii* germinated after 3 h incubation under these conditions. In prior work to determine the influence of hot water on the survival of these fungi, conidia of *P. digitatum* were also more resistant to heat than arthroconidia of *G. citri-aurantii*. To inactivate 99% of the conidia of *P. digitatum* in 15 s, a water temperature of 63.9°C was needed, whereas only 58.8°C was needed to inactivate 99% of the arthroconidia of *G. citri-aurantii* (25).

The temperatures that inactivate conidia of *P. digitatum* and arthroconidia of *G. citri-aurantii* are relatively low, and it is probably feasible to develop a 1- or 2-day thermal and humidity regime that would reliably disinfest packinghouses, contaminated harvest bins, or storage rooms. Unlike chemical sanitation, the use of heat and moisture avoids many issues including worker hazard, chemical storage, fumigant containment, corrosion, or residues. The approach that we propose is similar to that recently reported for solar disinfestation of greenhouses (24). Shlevin et al. (24) found dry heating to 60°C and higher with a consequent low relative humidity (RH = 15%) required an extended period for thermal inactivation of *Fusarium oxysporum* f. sp. *melonis* and *F. oxysporum* f.

Fig. 5. Longevity of conidia of nine *Penicillium digitatum* isolates stored at a constant temperature of 50°C at a relative humidity of 75 or 95%. Isolates were resistant (R) to the fungicides imazalil (IMZ), thiabendazole (TBZ), and sodium ortho-phenylphenate (SOPP) or sensitive (S) to all of them. Values are the times in hours when the percentage of conidia that could germinate had declined by 99% (LT₉₉), as estimated by probit analysis. Germination percentage was assessed from conidia removed hourly.

sp. *radicis-lycopersici*. They enhanced pathogen control by increasing moisture during the hot hours of the day; the ED₅₀ value of thermal inactivation was reduced from 15 days to 1 day. Their system provided both high temperature (about 60°C) and high RH (about 100%). Grain mills are also disinfested by heat, although the objective is primarily to control insects. Regimes of approximately 50°C for 24 to 36 h killed all life stages of *Tribolium castaneum*, the red flour beetle (16), and this approach cost less than one-third that of chemical fumigation (7).

There are locations within citrus packinghouses where thermal disinfestation is particularly feasible. Treatments of sufficient temperature and humidity would require the acquisition of heaters and humidifiers and the energy to operate them. From our work, it is likely good disinfestation of both pathogens should result after a 1- or 2-day treatment of 50°C or more with

a RH exceeding 75%. It is likely the surfaces of flooring and walls may require some period to reach effective temperatures, and the length of this period is likely to vary among facilities. Disinfestation of spaces that do not contain packing equipment is more practical, because damage to the electronic components of sizing and grading equipment would be avoided. Within citrus packinghouses, chambers (degreening or "sweat" rooms) to apply ethylene gas treatments are common. In California, they incorporate heating, air-circulation, and humidity regulation because the treatment is typically conducted at 20°C with high humidity for several days before the fruit are packed (29). Often fruit will decay during degreening and these rooms can become severely contaminated with conidia of *P. digitatum*. Storage rooms, usually operated at 10°C or cooler, and containing fruit in cartons or storage boxes for periods up to months, can also

become severely contaminated with both *P. digitatum* and *G. citri-aurantii*. Fruit field bins and other equipment contaminated with these pathogens could also be disinfested by thermal treatment within these rooms.

The temperatures that inactivate conidia, however, are too high to be tolerated by fresh citrus fruit without risk of harm to their appearance and internal quality. Curing treatments for citrus fruit, applied to retard decay and increase chilling tolerance, are usually of 24 to 48 h in duration at about 35°C; it is unlikely fruit would tolerate temperatures 10 to 15°C higher than this without alterations such as increases in ethanol and acetaldehyde, poor color development, decreased flavor scores, and increased weight loss (23,28).

From the results presented here, it is likely conidia could survive in large numbers within California packinghouses from one harvest season to another, while those in the groves decline markedly. In our two outdoor locations, the ability of conidia to germinate was estimated to have declined by 99% by 30 or 42 days. Conversely, conidia in the protected environment of a citrus packinghouse persisted much longer. We determined the viability of conidia of *P. digitatum* weekly for 16 weeks that were

Fig. 6. Relative humidity and temperature of two outdoor locations (Lindcove and Parlier), from 8 July to 30 August 2005, where conidia of *Penicillium digitatum* isolate PD90 were placed. Germination (% $\pm$ standard deviation of four samples) was determined from conidia collected weekly. Estimates of the time in days when the percentage of conidia that could germinate had declined by 99% (LT₉₉ with lower and upper 95% confidence intervals) by probit analysis were 30.2 (27.2, 34.3) and 41.6 (34.2, 55.1) days, respectively, at the Lindcove and Parlier locations.

Fig. 7. Longevity of arthroconidia of *Geotrichum citri-aurantii* stored at a constant temperature of 45 or 50°C at a relative humidity of 75 or 95%. Germination was assessed from arthroconidia removed hourly. Two examinations of arthroconidia germination were made, the first (exam 1) after 18 h, and a second (exam 2) after 5 days incubation on potato dextrose agar at 24°C.

placed in a citrus packinghouse where daily temperatures were 20 to 30°C and the RH was 30 to 60% (J. L. Smilanick and M. F. Mansour, *unpublished*). Initially and after 16 weeks, germination was 97 and 25%, respectively. Knowledgeable industry technicians stated that sanitation in Florida packinghouses is of less concern than those in California, because populations of conidia of *P. digitatum* in the humid Florida packinghouses decline rapidly, whereas those in drier California packinghouses persist for many months. Our results support their observations, and emphasize the need for packinghouse sanitation in California, particularly between harvest seasons.

ACKNOWLEDGMENTS

We thank statistician Bruce Mackey of the Biometrics Unit of USDA ARS Western Regional Research Center for advice and assistance, the California Citrus Research Board for financial assistance, Walter Stutzman of the University of California Lindcove Research and Extension Center, Exeter, CA, and David Sorenson of Fruit Growers Supply, Exeter, CA.

LITERATURE CITED

1. Belli, N., Marin, S., Sanchis, V., and Ramos, A. J. 2004. Influence of water activity and temperature on growth of isolates of *Aspergillus* section *Nigri* obtained from grapes. *Int. J. Food Microbiol.* 96:19-27.
2. Eckert, J. W., and Brown, G. E. 1986. Evaluation of postharvest treatments for citrus fruits. Pages 92-97 in: *Methods for Evaluating Pesticides for the Control of Plant Diseases*. K. D. Hickey, ed. American Phytopathological Society, St. Paul, MN.
3. Ellison, P. J., McFadyen, L. M., Cullis, B. R., and Kable, P. F. 1995. Survival of dispersed urediniospores of *Tranzschelia discolor* Fckl. (Tranz. Litv.) on leaves of *Prunus domestica* L. cv. 'd'Agen' in spring and summer in the Murrumbidgee irrigation areas. *Aust. J. Agric. Res.* 39:847-856.
4. Fahy, R. C., Mikolajczyk, S. T., and Brody, S. 1978. Correlation of enzymatic activity and thermal resistance with hydration state in ungerminated *Neurospora* conidia. *J. Bacteriol.* 135:868-875.
5. Finney, D. J. 1971. *Probit Analysis*. Cambridge University Press, New York.
6. Hawker, L. E., and Madelin, M. F. 1976. The dormant spore. Pages 1-72 in: *The Fungal Spore: Form and Function*. D. J. Weber and W. M. Hess, eds. John Wiley & Sons, New York.
7. Heaps, J. W., and Black, T. 1994. Using portable rented electric heaters to generate heat and control stored product insects. *Assoc. Operative Millers Bull.*, July 1994, pp 6408-6411.
8. Hedgecock, S. 1995. Influence of moisture content on temperature tolerance and storage of *Metarhizium flavoviride* conidia in an oil formulation. *Biocontrol Sci. Technol.* 5:371-378.
9. Hong, T. D., Ellis, R. H., and Moore, D. 1997. Development of a model to predict the effect of temperature and moisture on fungal spore longevity. *Ann. Bot.* 79:121-128.
10. Hong, T. D., Gunn, J., Ellis, R. H., Jenkins, N. E., and Moore, D. 2001. The effect of storage environment on the longevity of conidia of *Beauveria bassiana*. *Mycol. Res.* 105:597-602.
11. Kinay, P., Mansour, M. F., Gabler, F. M., Margosan, D. A., and Smilanick, J. L. 2007. Characterization of fungicide-resistant isolates of *Penicillium digitatum* collected in California. *Crop Prot.* 26:647-656.
12. Kuntz, I. D., Jr., and Kauzmann, W. 1974. Hydration of proteins and polypeptides. *Adv. Protein Chem.* 28:239-345.
13. Leong, S. L., Hocking, A. D., and Pitt, J. I. 2004. Occurrence of fruit rot fungi (*Aspergillus* section *Nigri*) on some drying varieties of irrigated grapes. *Aust. J. Grape Wine Res.* 10:83-88.
14. Leong, S. L., Hocking, A. D., and Scott, E. S. 2006. Effects of water activity and temperature on the survival of *Aspergillus carbonarius* spores *in vitro*. *Lett. Appl. Microbiol.* 42:326-330.
15. Lingappa, Y., and Sussman, A. S. 1959. Changes in the heat-resistance of ascospores of *Neurospora* upon germination. *Am. J. Bot.* 46:671-678.
16. Mahroof, R., Subramanyamb, B., and Eustace, D. 2003. Temperature and relative humidity profiles during heat treatment of mills and its efficacy against *Tribolium castaneum* (Herbst) life stages. *J. Stored Products Res.* 39:555-569.
17. Moyano, C., and Melgarejo, P. 2002. Survival of *Botrytis cinerea* in soil in South-Eastern Spain. *J. Phytopathol.* 150:536-540.
18. Page, R. M., Sherf, A. F., and Morgan, T. L. 1947. The effect of temperature and relative humidity on the longevity of the conidia of *Helminthosporium oryzae*. *Mycologia* 39:158-164.
19. Paul, G. C., Kent, C., and Thomas, C. R. 1992. Viability testing and characterization of germination of fungal spores by automatic image analysis. *Biotechnol. Bioengineering* 42:11-23.
20. Rotem, J. 1968. Thermoxerophytic properties of *Alternaria porri* f. sp. *Solani*. *Phytopathology* 58:1284-1287.
21. Sandhu, S. S., Rajak, R. C., and Agrawal, G. P. 1993. Studies on prolonged storage of *Beauveria bassiana* conidia: Effects of temperature and relative humidity on conidial viability and virulence against chickpea borer, *Helicoverpa armigera*. *Biocontrol Sci. Technol.* 3:47-53.
22. Schein, R. D., and Rotem, J. 1965. Temperature and humidity effects on uredospore viability. *Mycologia* 57:397-403.
23. Schirra, M., Mulas, M., Fadda, A., and Cauli, E. 2004. Cold quarantine responses of blood oranges to postharvest hot water and hot air treatments. *Postharvest Biol. Technol.* 31:191-200.
24. Shlevin, E., Mahrer, Y., and Katan, J. 2004. Effect of moisture on thermal inactivation of soilborne pathogens under structural solarization. *Phytopathology* 94:132-137.
25. Smilanick, J. L., Sorenson, D., Mansour, M. F., Aiyebe, J., and Plaza, P. 2003. Impact of a brief postharvest hot water drench treatment on decay, fruit appearance, and microbe populations of California lemons and oranges. *Hort-Technology* 13:333-338.
26. Spotts, R. A. 1985. Environmental factors affecting conidial survival of five pear decay fungi. *Plant Dis.* 69:391-392.
27. Teittel, L. 1958. Effects of relative humidity on viability of conidia of *Aspergilli*. *Am. J. Bot.* 45:748-753.
28. Vázquez, D. E., Meier, G. E., and Ponte, M. 2005. Influence of postharvest curing on 'Marsh' grapefruit quality during long-term storage. *Acta Hort.* 682:1257-1264.
29. Wardowski, W. L., Miller, W. M., and Grierson, W. 2006. Degreening. Pages 277-298 in: *Fresh Citrus Fruits*. 2nd ed. W. F. Wardowski, W. M. Miller, D. J. Hall, and W. Grierson, eds. Florida Science Source, Inc., Longboat Key, FL.