Postharvest Pathology and Mycotoxins

Prestorage Heat Treatment for Control of Decay of Pear Fruit

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This project is supported in part by Cooperative Agreement 58-0401-5-00036 between the USDA and Oregon State University for pome fruit disease research and by the Winter Pear Control Committee.

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Oregon Agricultural Experiment Station Technical Paper 8032.

Accepted for publication 24 June 1987 (submitted for electronic processing).

ABSTRACT

Spotts, R. A., and Chen, P. M. 1987. Prestorage heat treatment for control of decay of pear fruit. Phytopathology 77:1578-1582.

The effects of prestorage heat treatment and relative humidity during heating on fruit quality and incidence of decay of pear fruits caused by Botrytis cinerea, Mucor piriformis, Penicillium expansum, and Phialophora malorum were studied. Heating of fruit at temperatures from 21 to 38 C for 1–7 days significantly reduced decay caused by P. malorum, and large decreases in decay occurred after 1, 3, and 5 days heating at 32, 27, and 21 C, respectively. Prestorage heating was less effective for decay control in overmature than optimum mature fruits. Dessert quality of fruits heated to 32 and 38 C was not acceptable because proper ripening was inhibited. Less Mucor rot occurred when fruits were heated at 90 than at

100% relative humidity, but relative humidity from 80 to 100% during heating did not affect decay caused by *B. cinerea*, *P. expansum*, or *P. malorum*. Although heating reduced incidence of decay caused by *M. piriformis* and *P. malorum*, decay caused by *B. cinerea* was not affected and was increased in fruits inoculated with *P. expansum*. When fruits were both wounded and inoculated after heating, no control of decay occurred. However, heating wounded fruits reduced Mucor rot and Phialophora side rot even when fruits were inoculated after heating, indicating that apart from direct effects on the pathogen, heating may promote a wound healing response.

Important postharvest losses to pear (*Pyrus communis* L.) in the Pacific Northwest are caused by *Botrytis cinerea* Pers: Nocca & Balbis, *Mucor piriformis* Fischer, *Penicillium expansum* Lk: Thom., and *Phialophora malorum* (Kidd & Beaum.) McCulloch. (2,3,19). Survival of these fungi in the orchard is dependent on temperature and relative humidity (25), and growth of fungi and infection of fruit also are related to these environmental factors (4, 23).

Postharvest treatment with hot air has been used to reduce decay of several crops, including pumpkin (9), strawberry (6,22), raspberry (27), and apple (20). Treatment of apples immediately after harvest at 38 C for 4-6 days reduced decay caused by *Penicillium* and *Corticium* spp. and suppressed softening during subsequent storage at -1 C (20). Detached pear fruits inoculated with *P. malorum* and *Pezicula malicorticis* (Jacks.) Nannf. were not infected when incubated at 20 C, but severe decay occurred at -1 C (26). Problems encountered with heat treatment for decay control include development of adverse flavors, excessive moisture loss, and fruit injury (7,24).

The objectives of this study were to determine the effects of prestorage heat treatment of pear fruits with selected temperatures and relative humidities on decay caused by *B. cinerea*, *M. piriformis*, *P. expansum*, and *P. malorum*. Temperature effects were studied on fruits harvested at two stages of maturity. In addition, the effects of heat treatment on fruit quality were evaluated.

MATERIALS AND METHODS

Effects of temperature, duration of heating, and harvest maturity on side rot and fruit quality. Pear fruits (cultivar Bosc) were harvested at optimum maturity (62 Newtons flesh firmness) from mature trees at the Mid-Columbia Agricultural Research and Extension Center on 18 September 1984 and at an overmature stage on 2 October. At each harvest, fruits were disinfested in 0.525% sodium hypochlorite, rinsed with tap water, then puncture-wounded with a blunt metal instrument (6mm diameter and 3mm deep) on four locations per fruit. Conidia of *P. malorum* were washed from 2-3-wk-old cultures growing on potato-dextrose agar (Difco) acidified with 1.5 ml of 85% lactic acid per liter. Each wound was inoculated with $50 \mu l$ of a spore suspension containing 2.5×10^4 conidia per milliliter.

Heat treatment temperatures were based on results of a preliminary study in which Bosc pear fruits were inoculated with P. malorum and exposed to prestorage temperatures ranging from -1 to 21 C for 1-14 days. After exposure to temperatures of 16 and 21 C for 5-7 days, decay incidence was reduced approximately 50%. Based on these data, fruits were placed in wooden containers lined with polyethylene bags, and three replicate containers were placed in each of four growth chambers at 21, 27, 32, and 38 ± 1.5 C. Air temperature and relative humidity were monitored in the center of the fruit mass in one container per chamber with thermocouples and narrow-range lithium chloride humidity sensors (American Instrument Co., Silver Springs, MD). Temperature and relative humidity of air in the growth chambers were recorded with

calibrated hygrothermographs. Relative humidity in the containers of fruit was maintained at 97–100% with the chamber humidifier supplemented with sterile, wet paper towels inside the polyethylene bags. In each chamber, two sets of unwounded fruits also were heat treated. One set was wounded after heating and inoculated as described above. The other set was left unwounded and used for evaluation of quality as described later.

After 1, 3, 5, and 7 days of heating, 10 inoculated and 20 unwounded fruits were removed from each container, 10 of the unwounded fruits were puncture-inoculated, and all fruits stored in polyethylene-lined boxes at -1 C. At each harvest, a set of inoculated fruits was placed immediately at -1 C without a prestorage heat treatment. Three months after treatment, inoculated fruits were evaluated for incidence of side rot decay. Unwounded fruits were ripened for 9 days at 20 ± 1 C and $94\pm2\%$ relative humidity, and texture, flavor, and juiciness were evaluated organoleptically by the authors on a scale of 1–5, where 1 represented the lowest quality and 5 the highest.

The experiment was analyzed as a $2 \times 4 \times 4$ factorial with harvest date, temperature, and duration of heating as factors. Decay percentages were converted to arcsin $\sqrt{-\%}$ before statistical analysis.

Effects of relative humidity during heating on fruit quality and decay caused by four fungi. Bosc and Anjou pear fruits were harvested at commercial maturity (52 and 56 Newtons flesh firmness, respectively) on 16 September 1985. Fruits were surface disinfested with 95% ethanol and puncture-wounded as described above. Fungal cultures 1-2 wk old were flooded with sterile distilled water and spore suspensions were adjusted to obtain 2× 10^{3} (M. piriformis ATCC 60988 and P. expansum), 4×10^{3} (B. cinerea), or 2.5 × 104 (P. malorum) conidia per milliliter. Bosc fruits were inoculated with P. malorum and Anjou fruits with the other three fungi using 50 µl of conidial suspension per wound. Fifteen fruits were inoculated at four locations per fruit with each fungus and placed in a perforated plastic container. Three replicate containers were placed at each of the following relative humidities: $80\pm5,90\pm4$, and 100%. At all relative humidities, air temperature within the fruit mass was maintained at 27 \pm 1.5 C. Temperature and relative humidity were maintained within growth chambers as described above and monitored with thermocouples and relative

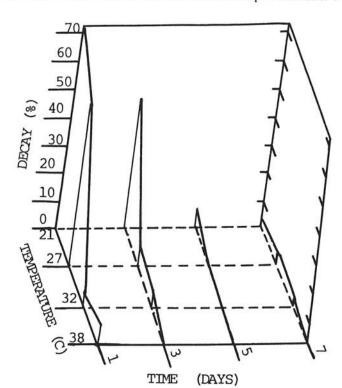


Fig. 1. Effect of prestorage heat temperature and duration of heating on incidence of side rot caused by *Phialophora malorum* of Bosc pear fruits harvested at optimum maturity.

humidity sensors at the center of one container per chamber. A set of 15 unwounded fruits was placed in each container for evaluation of quality. After 1, 2, and 3 days of treatment, five inoculated and five unwounded fruits were removed from each container and stored at -1 C as described above. In addition, a set of 21 fruits inoculated with each organism was placed immediately into -1 C without any heat treatment.

Decay incidence was evaluated monthly for 3 mo, and monthly decay values were summed to obtain total decay. Decayed fruits were removed monthly to prevent secondary spread. Quality of nonwounded fruits was evaluated after 3 mo as described above. The experiment was repeated once. Statistical analysis was done using a 3×3 factorial with relative humidity and duration of heating as factors. Decay percentages were converted to arcsin $\sqrt{-\%}$ before analysis.

Effect of time of inoculation relative to time of heat treatment and wounding. Three different sequences of inoculation, heating, and wounding were compared. Treatments included: 1) wound, heat, then inoculate; 2) wound, inoculate, then heat; and 3) heat, wound, then inoculate. Fruits were harvested and disinfested as described above in the relative humidity experiment. Bosc pear fruits were inoculated with *P. malorum* $(2.5 \times 10^4 \text{ conidia per milliliter})$ and Anjou fruits with *M. piriformis* $(2 \times 10^3 \text{ conidia per milliliter})$ as described above. Each of the three treatments consisted of three replicates per fungus with 15 fruits per replicate. Fruits were heated at $27 \pm 1.5 \text{ C}$ and 98-100% relative humidity for 3 days, then stored at -1 C as described above. Incidence of decay was evaluated monthly for 3 mo, and values were added to obtain total decay.

RESULTS

Effects of temperature, duration of heating, and harvest maturity on side rot and fruit quality. Incidence of side rot of inoculated, unheated fruits was 95%. Heating of inoculated fruits harvested at optimum maturity at 21 C for 1, 3, 5, and 7 days

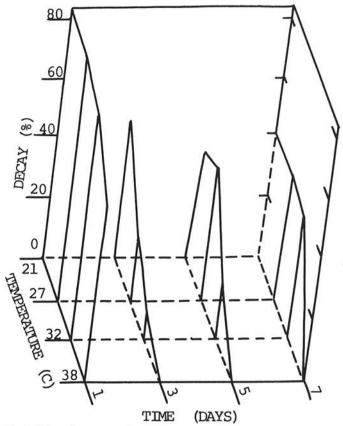


Fig. 2. Effect of prestorage heat temperature and duration of heating on incidence of side rot caused by *Phialophora malorum* of overmature Bosc pear fruits.

reduced decay to 70, 45, 6, and 2%, respectively (Fig. 1). Incidence of decay after heating at 38 C for 1, 3, 5, and 7 days was 8, 0, 0, and 0%, respectively (Fig. 1). Large decreases in decay occurred after 5, 3, and 1 days of heating at 21, 27, and 32 C, respectively. Effects of both duration of heating and temperature on decay were highly significant (P = 0.01).

The effects of heating on side rot of inoculated fruits harvested 2 wk after optimum maturity were different than for fruits harvested earlier. Overall incidence of decay of overmature fruits was 37 vs. 13% for mature fruits, and the difference was highly significant (P = 0.01). Although decay was significantly less (P = 0.01) after heating for 3, 5, and 7 days than it was after 1 day, regardless of temperature, decay appeared to increase after 5 days at 27 C or 7 days at 21 C (Fig. 2). However, only the increase between 5 and 7 days at 32 C was significant (P = 0.05). At 38 C, decay incidence decreased from 60% after 1 day of heating to 0% after 3, 5, and 7 days (Fig. 2).

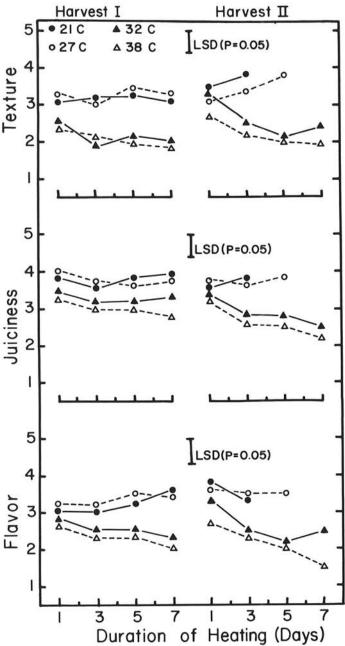


Fig. 3. Relationships between prestorage heat treatment temperature, duration of heating, and harvest maturity to fruit texture, juiciness, and flavor after 3 mo storage at -1 C and 9 days ripening at 20 C. Quality ratings are on a scale of 1-5 where 1 represents the lowest and 5 the highest quality.

When fruits harvested at either date were wounded and inoculated after heat treatment, no reduction of decay occurred with any of the time-temperature combinations. Overall incidence of side rot was 91 and 93% for overmature and mature fruits, respectively, compared with 95% for unheated fruits.

Unwounded fruits that were heated, then stored at -1 C for 3 mo were ripened and evaluated for texture, flavor, and juiciness. All three quality parameters were significantly lower (P = 0.05) for fruits heated at 32 and 38 C than for those at 21 and 27 C, regardless of harvest date (Fig. 3). Duration of heating did not affect quality of optimum mature fruits. However ripening of overmature fruits was initiated after 5 and 7 days at 21 and 27 C, respectively, and senescent breakdown occurred in storage. Quality of overmature fruits declined with increasing duration of heating at 32 and 38 C (Fig. 3).

Effects of relative humidity during heating on fruit quality and decay caused by four fungi. Prestorage heat treament at 27 C and various relative humidities differentially affected each of the four decay fungi. Incidence of decay of unheated fruits inoculated with B. cinerea was 91%. Average decay was 95% after 1, 2, or 3 days at 27 C, with all relative humidities combined, and 95, 93, and 95% at relative humidities of 80, 90, and 100%, with all heating durations combined, respectively. Thus, neither duration of heating nor relative humidity affected incidence of decay caused by B. cinerea.

Decay of nonheated fruits inoculated with *P. expansum* was 63% and increased to 83, 95, and 99% after heating for 1, 2, and 3 days, respectively. Decay after 2 and 3 days was significantly (*P* = 0.05) greater than decay of unheated fruits. Relative humidity had no effect on decay caused by *P. expansum*.

P. malorum caused 88% decay of unheated fruits, and decay decreased to an average of 79, 48, and 49% after heating for 1, 2, and 3 days, respectively. Decay after 2 and 3 days of heating was

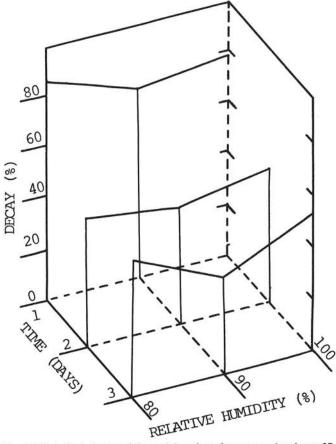


Fig. 4. Effect of relative humidity and duration of prestorage heating at 27 C on side rot incidence of Bosc pear fruit. Fruits wound-inoculated with 2.5×10^4 conidia per milliliter of *Phialophora malorum*, heated, then evaluated after a 3-mo storage at -1 C.

significantly (P = 0.01) less than after 1 day at all relative humidities (Fig. 4). Relative humidity had no effect on incidence of side rot.

M. piriformis caused 70% decay of unheated fruits, and heating for 1, 2, and 3 days significantly (P=0.05) reduced decay to 29, 11, and 10%, respectively. Reductions of decay after both 2 and 3 days of heating were significant compared with 1 day (Fig. 5). Decay at 80 and 90% relative humidity was not different, but decay at 100% relative humidity was significantly (P=0.01) greater than at 90% after 1 and 3 days of heating (Fig. 5).

Neither relative humidity nor duration of heating affected fruit quality (Fig. 6). The numerical quality rating of Anjou was significantly (P = 0.05) greater than Bosc (Fig. 6).

Effect of time of inoculation relative to time of heat treatment and wounding. When fruits were puncture-wounded before heat treatment, no difference was observed whether wounds were inoculated before or after heat treatment (Table 1). However, when fruits were wounded and inoculated after heating, incidence of side rot and Mucor rot was significantly greater (P = 0.01) than in fruits wounded before heating (Table 1).

DISCUSSION

Prestorage heating of pear fruits at 27 C for 2 days decreased side rot and Mucor rot and, in some cases, less decay occurred after 1 day at 21 C. As temperature increased to 38 C and duration of heating to 7 days, decay control increased. However, several problems were associated with higher temperatures and longer duration of heating. First, at 32 and 38 C, neither optimum mature nor overmature fruits ripened properly, thus resulting in a decline

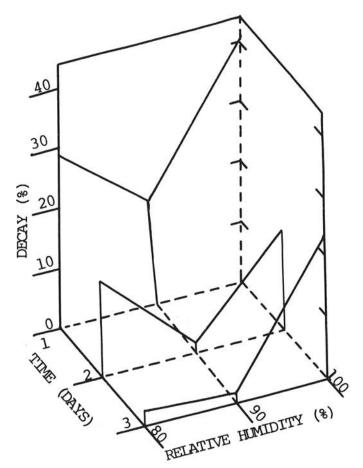


Fig. 5. Effect of relative humidity and duration of prestorage heating at 27 C on Mucor rot incidence of Anjou pear fruits. Fruits wound-inoculated with 2×10^3 sporangiospores per milliliter of *Mucor piriformis*, heated, then stored at -1 C and evaluated monthly for 3 mo. Monthly values summed and total decay shown.

in quality. Polygalacturonase, which is an important enzyme in the ripening process, was inactivated in solutions within 3 hr at 30 C (13), and denaturation of this enzyme could account for the lack of softening and low ratings for texture, flavor, and juiciness. Ripening, ethylene production, and sensitivity to ethylene were inhibited when Bartlett pear fruits were warmed to 40 C (10,16,18). Second, heat treatment was less effective for controlling decay in overmature than optimum mature fruits. Heating for up to 3 days decreased decay of overmature fruits, but after 5-7 days at 21-32 C, decay appeared to increase and was related to physiological breakdown of fruit during storage as a result of initiation of ripening during heating.

Relative humidity during heating did not affect decay control except with M. piriformis, which caused less decay at 90 than 100%

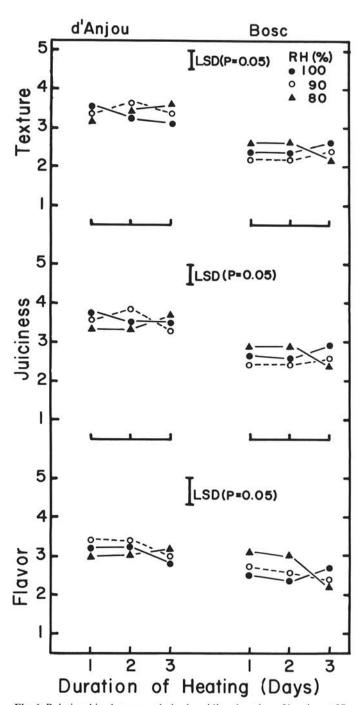


Fig. 6. Relationships between relative humidity, duration of heating at 27 C, and texture, juiciness, and flavor of Anjou and Bosc pear fruits. Fruits were heated, stored at -1 C for 3 mo, ripened at 20 C for 9 days, then evaluated. Quality ratings are on a scale of 1-5 where 1 represents the lowest and 5 the highest quality.

TABLE 1. Effect of time of inoculation relative to time of prestorage heat treatment and wounding on decay of pear fruits

| Treatment sequence ² | Percent decay caused by | |
|---------------------------------|-------------------------|------------------------|
| | Mucor piriformis | Phialophora malorum |
| Wound, heat, then inoculate | 11 a | 45 a |
| Wound, inoculate, then heat | 2 a | 53 a |
| Heat, wound, then inoculate | 88 b | 99 b |

Each value represents the average of three replications of 15 fruit per replicate, each fruit wounded four times. Numbers followed by the same letter within columns are not significantly different (P=0.01) according to Duncan's new multiple range test.

relative humidity. M. piriformis previously was shown to be adversely affected by small decreases in relative humidity (23).

Prestorage heat treatment decreased decay in the absence of inoculum, indicating that apart from direct effects on the pathogen, heating also may affect the host. However, the increased resistance was not observed when unwounded fruits were heated, then puncture-inoculated. Therefore, the effect of heating probably is on the wound site and may involve some type of healing response. Sweet potato wound periderm formation increased as temperature was increased from 12-32 C at 90% relative humidity (15). Wound responses in citrus, including an increase in free phenolics, deposition of lignin, and production of phellem and phellogen may be involved in disease resistance (5,11,12,14). Ligninlike substances deposited around inoculated tissue of lemon may represent a defense mechanism against infection (1). Eludication of the mechanisms involved in reduction of decay of pear fruits caused by M. piriformis and P. malorum requires further research.

Prestorage heat treatment controlled M. piriformis and P. malorum but not B. cinerea and P. expansum. P. expansum is considered as heat tolerant with an LD50 of 53 C for 4 min, and B. cinerea is somewhat less tolerant with an LD50 of 43-47 C (N. F. Sommer and R. J. Fortlage, unpublished). P. malorum has an optimum growth temperature of between 23 and 27 C but grew and sporulated readily at 30 C (8). Control of decay caused by P. malorum with heat probably is more a result of host responses than an effect of temperature on the pathogen. However, M. piriformis has a maximum growth temperature of 20 C (17) and may be adversely affected by heat conditions used herein. At 27 C, germination of M. piriformis was abnormal and growth was yeastlike (21).

For heat treatment to be of practical value for control of pear fruit decay caused by M. piriformis and P. malorum, fungi such as B. cinerea and P. expansum must be controlled by means other than heat. Current pear industry control of B. cinerea and P. expansum is with fungicides such as benomyl, thiobendazole, and captan, but no registered fungicides are effective for postharvest control of M. piriformis or P. malorum. Therefore, a combination of heat and fungicide application may be necessary to achieve control of these four main decay fungi. In addition, problems with early ripening and subsequent senescence during storage of overmature fruits preclude use of heating on fruits harvested past optimum maturity.

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Fruits heated at 27 C and 98-100% relative humidity for 3 days, then evaluated after storage at -1 C for 1-3 mo.