Development of Green Mold in Degreened Oranges

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ABSTRACT

The incidence of green mold caused by *Penicillium digitatum* is reduced by degreening oranges at relative humidities of 90-96% at 30 C. Degreening for 2 or 3 days at these high relative humidities induced lignin formation in injured flavedo tissue. Lignification rendered the injuries less susceptible to infection by *P. digitatum*. No lignification occurred where injured fruit were degreened at relative humidities of 55-75% at 30 C, and consequently the fruit were much more readily invaded by this fungus. Degreening at 90-96% relative humidity at 27 C did not reduce infection by *P. digitatum* as effectively as degreening at 30 C. Injuries with severe peel oil damage were usually invaded by *P. digitatum* even when the injured fruit were degreened at 90-96% relative humidity. The oil killed many of the cells surrounding the injury and thereby prevented lignification. *P. digitatum* usually invaded deep injuries which exposed albedo cells that never synthesized lignin during degreening. Fruit with desiccated flavedo or albedo injuries were not invaded by *P. digitatum*. Infection did occur, however, when these fruit were packed in consumer packages, especially polyethylene bags, where a rapid buildup of relative humidity provided moisture for spore germination. However, less green mold developed where the fruit injuries had lignified.

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The most important postharvest decay of citrus fruit is green mold caused by the fungus *Penicillium digitatum* Sacc. This organism is able to gain entrance into the fruit only through injuries to the peel. Such injuries usually occur during harvesting and subsequent handling.

The incidence of green mold in Florida citrus fruit is reduced by degreening (10), which is a procedure to improve fruit color by reducing chlorophyll in the peel, thereby exposing the yellow and red pigments (7). This is accomplished by placing fruit in special degreening rooms held at ca. 30 C and 90-96% relative humidity which contain ca. 5 μliters ethylene per liter of air. Cultivars which mature in September through November usually require degreening for 2-4 days. The previously reported effect of degreening in reducing green mold is known to be associated with high relative humidity (10, 11, 12) and not with the presence of ethylene (10).

These studies were made to determine why degreening of Florida oranges at 30 C with high relative humidity reduces the incidence of green mold.

MATERIALS AND METHODS.—Oranges, *Citrus sinensis* (L.) Osbeck 'Hamlin', 'Pineapple', or 'Valencia', were washed and injured before degreening. Injuries to the flavedo were formed by rubbing the fruit a distance of 6 cm on No. 60 coarse sandpaper (3-M Company No. 9003) causing a circular injury ca. 14 mm in diam. Deep injuries of similar size were made into the albedo by removing the epidermis and flavedo with a scalpel. Fruit were degreened with 5 μliters ethylene per liter of air at temperatures of 27, 30, or 33 C and high (90-96%) or ambient (55-75%) relative humidities.

Spores of *P. digitatum* were collected (18) from sporulating fruit inoculated 7-12 days previously and incubated at 22 C. Spores were dried over silica gel at 25 C for 3 days and then stored at 40-50% relative humidity for use within the subsequent 30 days.

Injured fruit were placed on plastic rings in a plastic dishpan (30 X 28 X 13 cm). The fruit were then inoculated, by dusting dry spores of *P. digitatum* on the injury (ca. 500 spores/mm^2), and held at 25 C. An atmosphere of 100% relative humidity was attained by pouring 50 ml of water into the bottom of the dishpan and covering the pan with a 0.2-mm-thick sheet of polyethylene sheeting.

Fruit to be packed in cartons or consumer packages were waxed with a solvent wax after degreening and before inoculation. In these trials, fruit were not placed in dishpans but were packed in 0.03-m^3 ventilated fiberboard cartons with or without lids; or in 2.27-kg polyethylene film consumer bags having 72 holes, each 6.3 mm in diam; or in 2.27-kg mesh bags of polyethylene net (5). Eight "poly" or mesh bags were then packed in a 0.05-m^3 ventilated fiberboard master carton. After such packaging, all fruit were stored in a room maintained at 21 C and 60-70% relative humidity to simulate unventilated warehouse conditions.

Injuries to the fruit peel were examined microscopically by sectioning the tissue at a thickness of 24-36 μ with a Hooker Plant Microtome (Lab-Line Instruments, Inc., Melrose Park, Ill.). Unstained sections were examined as well as sections stained with aniline blue (13), phloroglucinol-HCl (13), or 1 M KOH (17) to detect the presence of callose, lignin, or chlorogenic acid and its related compounds, respectively.

RESULTS.—*Development of P. digitatum in oranges inoculated before degreening.*—Inoculated fruit were degreened for 3 days at 27, 30, or 33 C at either high or ambient relative humidities. After 24 hr of degreening at 90-96% relative humidity at 27 C, germ tubes from spores of *P. digitatum* had already

TABLE 1. Influence of temperature and relative humidity on the development of *Penicillium digitatum* in oranges during degreening and subsequent storage

Degreening temperature[a] C	% of fruit infected with green mold after degreening at relative humidities of	
	90-96%	55-75%
27	89 wx[b]	71 xy
30	63 y	93 w
33	21 z	97 w

[a] 24 fruit were degreened at each temperature at each range of relative humidity for 3 days and then stored at 25 C and 100% relative humidity for 4 days.

[b] Values followed by unlike letters are significantly different at the 5% level of probability.

TABLE 2. Percentage green mold after 7 days of storage at 25 C in oranges inoculated with *Penicillium digitatum* after injuring the fruit and degreening for 0, 1, 2, and 3 days, respectively, at high and ambient relative humidities

Days of degreening[a]	% of fruit infected with green mold after degreening fruit at relative humidities of	
	90-96%	55-75%
0	100 x[b]	100 x
1	88 x	100 x
2	50 y	96 x
3	21 z	88 x

[a] 24 fruit were treated at each degreening period at each level of relative humidity.

[b] Values followed by unlike letters are significantly different at the 5% level of probability.

penetrated living cells beneath the injured flavedo tissue. But, after the same period of degreening at 30 and 33 C, the germ tubes still had not grown beyond the injured cells. No spore germination occurred on the injured areas of fruit held at 55-75% relative humidity during degreening. After 3 days of degreening at 27, 30, and 33 C at high relative humidities, 44, 25, and 0% of the total number of fruit exhibited green mold, respectively. All of the fruit were then held at 25 C and 100% relative humidity for an additional 4 days (Table 1). During this period, spores of *P. digitatum* germinated and infected many of the fruit degreened at 55-75% relative humidity. Therefore, at the end of the 7 days, significantly less mold was present, except at 27 C, in fruit degreened at a high relative humidity than at ambient (Table 1).

Green mold in oranges inoculated after degreening.—Oranges with flavedo injuries were degreened at 30 C for 0, 1, 2, and 3 days at either high or ambient relative humidities. The fruit were then inoculated and held at 25 C and 100% relative humidity. Percentage of fruit with green mold 7 days after inoculation is shown in Table 2. All fruit inoculated immediately following injury decayed, as did the fruit degreened 1 day at ambient relative humidity. As the degreening time was increased to 2 or 3 days, significantly less green mold developed in fruit that were degreened at 90-96% relative humidity.

Lignin was discernible in two or three layers of living epidermal or flavedo cells adjacent to the injury (Fig. 1-A, B) after 2 or 3 days of degreening at high relative humidity. Accumulation of lignin was usually greater at 3 than at 2 days. Lignin was not detected in freshly made injuries or in injuries of fruit degreened at ambient relative humidities. Chlorogenic acid and its related compounds were also produced under conditions similar to those required for lignin production. No callose could be detected in any of the injuries. Spores of *P. digitatum* germinated on lignified injuries, but there was little hyphal growth beyond the lignin-containing cells.

Peel oil, released from the oil glands during injury of the flavedo, was usually absorbed by the physically damaged cells. Frequently, however, peel oil killed some cells adjacent to the injury, thereby preventing them from synthesizing lignin (Fig. 2). The incidence of green mold was increased after degreening at high relative humidity if extensive oil damage was induced

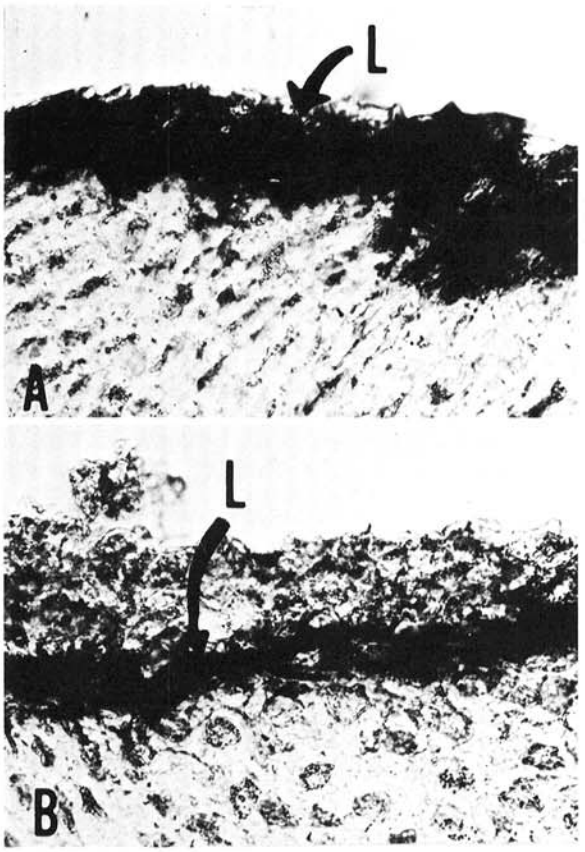

Fig. 1. Production of lignin (indicated by arrows) by living cells adjacent to injured cells in the **A)** epidermis and **B)** flavedo (× 160).

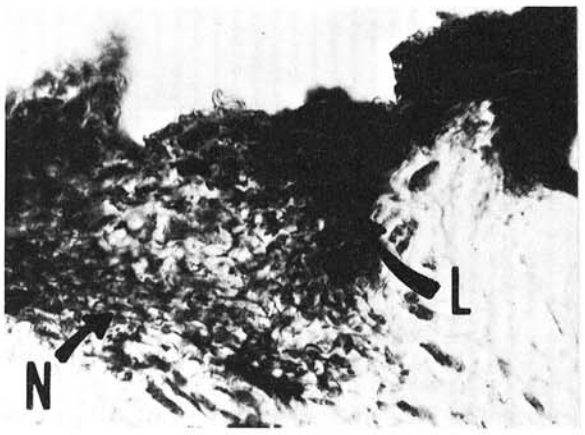

Fig. 2. Necrosis (N) of cells in the flavedo caused by peel oil released during injury. Lignin (L) formation in adjacent living cells (× 160).

by rubbing the fruit on sandpaper impregnated with peel oil which subsequently interfered with lignification. However, if the injured area of the fruit was washed in water or blotted on filter paper to remove part of the peel oil, lignification was improved and the amount of green mold decreased.

Deep injuries into the peel exposed albedo tissues which did not synthesize lignin. Such injuries to oranges degreened 3 days at 90-96% relative humidity were normally invaded by *P. digitatum* from inoculations after degreening, if moisture was available for spore germination.

Green mold during storage of oranges in fresh fruit containers.—Oranges with flavedo injuires were degreened for 3 days at 30 C at high or ambient relative humidities and then were inoculated and packed in fresh fruit containers. Humidity levels surrounding the fruit were influenced greatly by the type of container. Within 24 hr after packaging, water had condensed on the inner surface of the "poly" bags of fruit packed in master cartons. The incidence of green mold varied significantly in fruit packed in the various containers during a period of 10 days (Table 3). Fruit degreened at 90-96% relative humidity developed the least green mold, and its incidence was not significantly influenced by the storage container. The type of container did significantly affect development of green mold in fruit degreened at low relative humidities. By packaging these fruit in "poly" bags in master cartons, 35% of the fruit contained green mold after 10 days of storage (Table 3). Only 6% of this fruit developed green mold in open cartons where the fruit were exposed to the relative humidity of the storage room.

TABLE 3. Effect of different types of container storage on the incidence of green mold in injured fruit that were degreened at high or ambient relative humidities for 3 days and then inoculated with *Penicillium digitatum*

Type of container[a]	% of fruit infected with green mold[b] after degreening at relative humidities of	
	90-96%	55-75%
Open carton	1 z[c]	6 xyz
Closed carton	3 yz	13 x
Master carton–vexar	1 z	11 xy
Master carton–poly	2 z	35 w

[a] 160 fruit were treated and placed in each type of container at each range of relative humidity.

[b] Green mold after 10 days storage at 21 C.

[c] Values followed by unlike letters are significantly different at the 5% level of probability.

DISCUSSION.—Lignification during degreening at high relative humidities at a temperature unfavorable for optimal growth of *P. digitatum* apparently accounts for the reduced incidence of green mold in degreened Florida fruit. *P. digitatum* does not readily invade lignified injuires nor does it grow very rapidly at 30 C (4). Spore germination is delayed significantly during degreening, whereas lignification proceeds and effectively inhibits *P. digitatum* from penetrating many injuries. Temperatures of 21-27 C, used during degreening of fruit in other citrus-producing regions (2, 8), encompasses an optimal temperature range for the growth of *P. digitatum* (4). Degreening at these lower temperatures reduces green mold less than at 30 C, since penetration may occur before an effective lignin barrier is formed. Also, a slower rate of lignification may occur at temperatures below 30 C. In sweet potatoes, suberization and wound periderm formation (1), later reported as lignification (17), was most rapid between 27 and 34.5 C. If oranges respond similarly, lignification during degreening at less than 30 C may be inadequate to prevent penetration and decay.

Lignin is not produced in all injuries to oranges during degreening at 30 C and 90-96% relative humidity. Albedo tissues of the orange peel do not synthesize lignin and neither do dead cells of the flavedo killed by peel oil released during injury. Some oranges developed green mold (Table 2) when inoculated even after degreening at 30 C at high relative humidities for 3 days. Presumably, damage from peel oil prevented complete lignification of injuries to all fruit.

Desiccated injuries are not infected by *P. digitatum* because moisture is needed for spore germination (3) regardless of whether the injury is lignified, damaged by peel oil, or located in flavedo or albedo tissues. However, fruit are exposed to high relative humidities during packaging in consumer packages (6, 9, 14) which will provide adequate moisture for spore germination that may lead to infection and decay, except in oranges with lignified injuries.

Several factors may be involved in the failure of *P. digitatum* to penetrate lignified flavedo cells. Lignin may function as a physical barrier to penetration by hyphae of *P. digitatum*, and/or phenolics involved in the synthesis of lignin may inhibit fungal growth. It is

known that flavedo tissue of oranges injured by shallow wounds between oil glands without the release of peel oil is generally resistant to infection by *P. digitatum* (15). Resistance of these injuries to infection has been attributed to the lack of specific pectic substances in such injuries. Without these substances, *P. digitatum* may not be induced to secrete pectin enzymes required for infection (16). Secretion of these enzymes may not occur in lignified injuries either, since pectic substances are probably not readily available from cells with lignified walls.

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