and organoleptic evaluation was collected. In this experiment, none of the judges detected off-flavour in unwaxed 'Feutrell's Early' packed with different lining materials up to 5-weeks, however, off-flavour in waxed fruit was detectable after 2 weeks storage at 12-22° C. In waxed 'Kinnows' stored at 23-36° C off-flavour developed after one week but in unwaxed 'Kinnows' packed with different materials as container liners, no such problem was noticed. It is interesting to note that no off-flavour was noticed in waxed fruits of 'Pineapples' stored at 12.5-20° C for 5 weeks indicating that the 'Pineapple' orange is more resistent to development of off-flavour than is 'Kinnow' mandarin. Further research in this direction is in progress in this laboratory.

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LIGNIFICATION OF INJURIES TO CITRUS FRUIT AND SUSCEPTIBILITY TO GREEN MOLD[1]

G. Eldon Brown and M. A. Ismail
Florida Department of Citrus

C. R. Barmore
Agricultural Research and Education Center
University of Florida, IFAS
P. O. Box 1088, Lake Alfred, FL 33850

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Abstract. **Green mold caused by** Penicillium digitatum **is one of the major fungal decays of Florida fresh citrus. Injuries to the fruit peel are required for penetration of the fugus. Penetartion by** P. digitatum **was inhibited in injuries to oranges and grapefruit where cells at the injured surface produced lignin before fungal entry. Accumulation of lignin occurred most rapidly at 30C and at relative humidities above 90%. Under these conditions, lignin developed within 12 hours following injury. Lignification was delayed or inhibited by peel oil or desiccation which caused damage to cells near the injury. These injuries were easily penetrated by** P. digitatum **as were injuries into the albedo where cells were incapable of producing lignin.**

Green mold caused by *Penicillium digitatum* Sacc. is one of the major decays of fresh citrus. Infection by the causal organism occurs by germination of spores within injuries formed in the peel during handling at harvest and packing. Success of infection is influenced by location and depth of the injury. Shallow injuries between oil glands are generally more resistant to infection than deeper injuries into the albedo and injuries involving oil glands (10).

The purpose of this paper is to report the association of lignin with injuries that are resistant to penetration by *P. digitatum* and observations of factors that curtail or promote the lignification process.

Materials and Methods

Mature fruit of *Citrus sinensis* (L) Osbeck ('Valencia' oranges) or *C. paradisi* Macf. ('Ruby Red' grapefruit) were washed and injured at the equator by rubbing the fruit a distance of 10 cm on 400 and 220 grit waterproof silicon carbide paper, respectively. By submerging the paper under 15 mm of flowing water during injury, the released peel oil was removed rapidly and oil damage was minimized. The injury was also immediately flushed with additional fresh water to remove remaining oil. For inoculation, the injured area, approximately 2 cm in diameter, was covered with spores (2×10^5/ml) suspended in distilled water containing a trace amount of Triton X 100 as a surfactant. Injured fruit held at relative humidities of 95 to 100% (high) were supported on plastic rings within a plastic pan containing a thin film of water on the bottom surface. Fruit were enclosed by covering the pan with a 0.2 mm-thick sheet of polyethylene film and the pans were held at temperatures of 25, 27, 30 or 33C. Low relative humidities (55 to 70%) were obtained by holding fruit in pans without water or plastic covering.

Injuries to the fruit peel were examined microscopically by sectioning the tissue at a thickness of 24-36 μm with a Hooker Plant Microtome (5). Lignin was detected using phloroglucinol–HCL (9).

Procedures for extraction of phenolic compounds from injured peel have been published previously (7). Lignin-like material associated with injured tissue was measured using the difference spectrum method of Stafford (11).

Results

Lignification. Lignin was detected in oranges with the phloroglucinol–HCL stain within 12 hours after injury and storage at 30C (Fig. 1.). Heavier accumulations were evident

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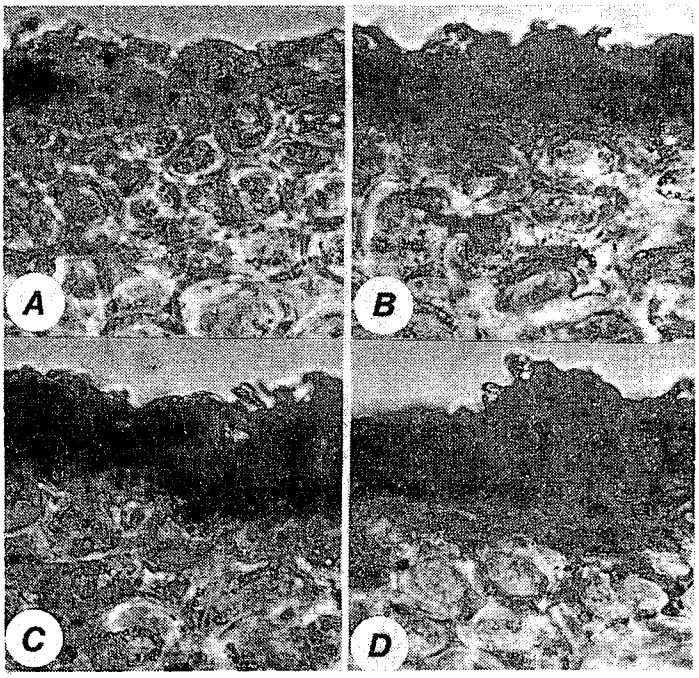

Fig. 1. Darkening of 'Valencia' orange tissue from the phloroglucinol-HCL stain for lignin at A) 12 hours B) 24 hours C) 48 hours and D) 60 hours after injury at 30C and high relative humidity. (X960).

by 24 hours, with relatively little additional increase after 48 hours. In additional studies, lignin formation was optimum at 30C. More rapid accumulation was not evident at 33C, while slower development occurred at 25 C.

Accumulation of lignin-like material measured by spectral analysis (Table 1) correlated well with the histochemical tests except that additional lignin-like material accumulated between 48 and 72 hours. Maintenance of high relative humidity following injury was critical to the formation of lignin. Much less lignin-like material was associated with injured flavedo from oranges held at low relative humidity because cells surrounding the injury were damaged from desiccation. This was also confirmed using the histochemical test for lignin.

Table 1. Accumulation of lignin-like material in injuries to 'Valencia' orange flavedo at 30C and low and high relative humidity.

Hours after injury (30C)	Total absorbance at 359 nm/gm dwt at low and high relative humidities	
	Low	High
0	0.0	0.0
24	3.9	11.5
48	7.2	13.1
72	5.9	21.8

Injuries of sufficient depth to involve albedo cells did not accumulate lignin even at 30C and high relative humidity. These cells apparently lacked the capacity to synthesize lignin. Peel oil, released when oil glands were injured, damaged cells near the injury and interfered with lignin formation.

Phenolics. Phenolics required for lignin synthesis were formed during lignification of injured orange flavedo (Table 2). Free phenolic constituents more than doubled after 48 hours at 30C if fruit were held at a high relative humidity. Formation of free phenolics was also temperature dependent (Table 3). In grapefruit, the rate of accumulation was most rapid at 30C, less at 24C, and no accumulation occurred at 15 or 5C.

Table 2. Accumulation of free phenolics in injuries to 'Valencia' orange flavedo at 30C and low and high relative humidity.

Hours after injury (30C)	Free phenolics mg/gm dwt	
	Low	High
0	1.47	1.47
24	1.40	1.59
48	1.98	4.06
72	2.48	5.13

Table 3. Accumulation of free phenolics in injuries to 'Ruby Red' grapefruit flavedo as influenced by temperature.

Hours after injury	Free phenolics (mg/gm dwt) at temperatures (C) of			
	5	15	24	30
0	1.30	1.30	1.30	1.30
24	1.21	1.25	1.31	1.32
48	1.00	0.95	1.33	1.52
72	0.85	1.05	1.62	2.00

Green mold. Inoculations with *P. digitatum* to fruit injured and held at high relative humidities caused less green mold as time between injury and inoculation increased from 0 to 72 hours (Table 4). The incidence of mold did not decrease with time between injury and inoculation if fruit were stored at low relative humidity. Spores of *P. digitatum* germinated when they were placed on all injured fruit surfaces. However, germ tubes did not penetrate cells with lignified walls, while in the absence of lignin, penetration and development of decay proceeded.

Table 4. Percentage green mold of 'Valencia' oranges injured and held at 25C at two relative humidities for 0, 24, 48, or 72 hours before inoculation.

Relative humidity after injury	Hours between injury and inoculation			
	0	24	48	72
High	92[z]	44	25	0
Low	—	100	94	100

[z]Each value represents decay of 36 fruit. All fruit were held near 100% relative humidity following inoculation.

Development of green mold was also influenced by the temperature at which fruit were held following injury and immediate inoculation (Table 5). At 27C, 88% of the fruit developed mold, while at 30 and 33C, the incidence of mold decreased to 63 and 21%, respectively. Growth of germ tubes was much slower at the higher temperatures, to the extent that penetration often failed to precede lignification.

Table 5. Influence of temperature on development of green mold in injured and inoculated 'Valencia' oranges held at high relative humidity.

Temperature after injury and inoculation[y]	% green mold
27	88[z]
30	63
33	21

[z]Each value represents decay of 24 fruit.
[y]Fruit were held at each temperature for 3 days and then stored at 25C for 4 days.

Discussion

Lignin has been associated with resistance of plants to infection by plant pathogenic microorganisms (3). Since most pathogens can not degrade lignin, lignified cell walls represent a barrier to infection by the pathogen. In some cases, lignin can be formed in response to infection or in other instances it can be produced in response to injury (3). Such apparently is the case with injured citrus flavedo. *P. digitatum* is unable to degrade cell walls of the flavedo once they become impregnated with lignin. Phenolics formed in the synthesis of lignin may also possess fungitoxic properties and may play a role in preventing penetration (8).

The occurrence of green mold is reduced during the degreening season (6). This reduction has been associated with increased resistance of injuries due to lignification during the degreening process (1, 7). The importance of maintaining proper temperature and relative humidity during degreening to insure rapid lignification is apparent. The temperature of 30C, used for degreening because of optimum degradation of chlorophyll (4), seems to be also optimum for phenolic and lignin systhesis. Growth of *P. digitatum* is also significantly reduced at 30C (2). Development of the fungus in injuries during degreening at 30C is slow, and in many instances lignification is probably initiated before penetration can occur. The fact that injuries into the albedo or those involving substantial release of peel oil are more susceptible to infection by *P. digitatum* (10) is probably due to the lack of lignin formation in such instances.

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SEPARATION OF FROZEN GRAPEFRUIT BY USING EMULSIONS OF DIFFERING SPECIFIC GRAVITIES

T. T. Hatton and R. H. Cubbedge
Agricultural Research,
Science and Education Administration,
U.S. Department of Agriculture,
Horticultural Research Laboratory,
Orlando, FL 32803

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Abstract. **Grapefruit (Citrus paradisi Macf.) damaged by the January 1977 freeze were separated from sound ones by using emulsions representing three specific gravities. Fruit either sank or floated in the emulsions, then fruit were cut and rated as U.S. No. 1, U. S. No. 2, or below, depending on the extent of internal dryness or freeze damage. Emulsions with a specific gravity of 0.78 and 0.80 were significantly better in yielding U.S. No. 1 and U.S. No. 2 fruit than the emulsion with a specific gravity of 0.82. However, no differences were observed between emulsions with specific gravities of 0.78 and 0.80.**

After the January 1977 freeze, a 10-day embargo was imposed to prevent the shipment of damaged fruit, as prescribed by the Florida Citrus Code (2). During the embargo, much of the seriously frozen fruit dropped from the trees and some internal drying, especially at the stem end, affected fruit that remained on the trees. When the embargo was lifted, the inspectors had the task of examining and grading fruit according to U.S. standards for Florida grapefruit (*Citrus paradisi* Macf.) (5). The inspectors cut and examined the fruit by the official procedure prescribed for grading Florida citrus for internal dryness or freeze damage (6). The industry has long known that an improved method was needed to separate frozen from sound grapefruit.

Dried fruit can be separated in the packinghouse by using either a water- or emulsion-type separator (3, 7). Both methods of separation are based on differences in specific gravity between freeze-damaged and undamaged fruit. Water separators depend on the greater buoyancy of frozen fruit when dropped into or released under a moving stream of water. Emulsion separators use a mixture of mineral oil and water. Sound fruit, which sink, and frozen fruit, which float, are carried out of the emulsion on separate conveyors. Manufacturers of fruit separators recommend the use of emulsions with a range of specific gravities, depending on the extent of dryness and the peel thickness of the fruit. Specifications proposed by manufacturers do not differentiate between oranges and grapefruit. One manufacturer is currently attempting to develop a device to separate grapefruit according to peel thickness (J. T. Liles, *personal communication,* 1978). The purpose of this study was to determine the accuracy of separation of sound and frozen grapefruit by use of oil-water emulsions of optimum specific gravity. Water-type separation was not considered in the study.