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Proc. Fla. State Hort. Soc. 93:305-307. 1980.

SOFT ROT OF CITRUS FRUIT CAUSED BY PENICILLIUM DIGITATUM AND P. ITALICUM[1]

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Additional index words. green mold, blue mold, postharvest decay.

Abstract. **Peel tissue of citrus fruit rotted by** Penicillium digitatum **Sacc. and** P. italicum **Wehmer was similar in degree of softness and relative water content. Pectolytic enzymes produced by these fungi degraded pectin of the cell wall middle lamella resulting in loss of cell coherence. This process is considered important in aiding hyphal penetration. Rate of development of the decay was not determined by the macerating ability of the pectolytic enzymes but apparently due to the growth rate of each organism.**

Penicillium digitatum Sacc. (green mold) and *P. italicum* Wehmer (blue mold) are 2 important postharvest fungal pathogens of citrus fruit. In Florida, green mold is by far the more important decay of the 2 types. Infection of citrus fruit by these 2 organisms requires an injury which is usually incurred at some time during harvesting and/or during postharvest preparation (4). Rot caused by the 2 organisms is similar in appearance and texture and may spread to contiguous healthy fruit in packed cartons.

The importance of pectolytic enzymes produced by various pathogens in the development of soft rots of other fruits is well documented (3). The enzymes degrade pectic substances of the cell wall middle lamella causing loss of tissue cohesiveness and death of cells.

The following discussion reports similarities in properties of the 2 decays and the identity of pectolytic enzymes produced by each pathogen.

Materials and Methods

Inoculations. Mature 'Valencia' oranges (*Citrus sinensis* (L.) Osbeck) were washed and inoculated through a puncture, 5 mm deep, into the albedo with spores (approx. 10^6 spores/ml) of either *P. digitatum* or *P. italicum* in water containing 0.01% Triton X100. The fruit were incubated at near 100% relative humidity and 25°C.

Pectolytic enzyme analysis. Methods used in the extraction and characterization of the pectolytic enzymes in the decayed tissues have been published (1, 2).

Organic acid analysis. The organic acids in the decayed tissue were extracted according to the procedure of Fernandez-Flores et al. (8) as modified by Ting (unpublished, Florida Dept. of Citrus, Lake Alfred). The decayed peel was homogenized in water, centrifuged and the supernatant was adjusted to 80% ethanol and centrifuged again. Saturated lead acetate solution was added to the supernatant, centrifuged and the pellet was serially washed with ethanol, acetone, diethyl ether and then vacuum dried. The organic acids in the pellet were silylated with Tri-Sil (Pierce Chemical Co., Rockford, IL 61105) and analyzed with a Hewlett-Packard gas chromatograph, Model 5736, equipped with a flame ionization detector and an SE 30 column. A temperature program of 70 to 210°C at a rate of 4°C per minute was used.

[1]Florida Agricultural Experiment Stations Journal Series No. 2708.

Puncture test. Peel strength of infected oranges was determined by measuring the amount of force in Newtons (kg m/sec^2) (N) required to puncture the peel with a 0.64 cm flat tipped rod. An Instron Model 1122 Universal Testing Instrument was used according to the procedure of Churchill et al. (5). Twenty fruit were tested each at 3 different points in the water-soaked area of the lesion. A set of noninfected fruit was similarly tested.

Relative water content. Peel discs, 8 mm in diameter, were taken from the water-soaked area of the lesion, weighed and placed in water. After 2 hr, they were removed and surface water removed by blotting and the peel was weighed again. Relative water content (RWC) was calculated according to the procedure of Weatherley (13). Three determinations, each using 5 discs, were made on infected and noninfected tissue.

Histology. Procedures for histological studies of infected and noninfected tissues have been published (1, 2).

Results and Discussion

Green mold develops at a rate twice that of blue mold on fruit incubated at 25°C. This difference in growth rate also exists when these 2 fungi are grown on agar plates (7). Softness of the 2 decayed tissues was similar; 4.2 ± 1.3 N were required to puncture green mold tissue whereas 3.0 ± 0.6 N were required for blue mold. Peel strength of noninfected fruit was 44.1 ± 7.1 N. The RWC of tissues decayed by either organism was 95% while that of noninfected tissue was 64%. These data indicate that the properties of peel strength and moisture content were similar for the 2 decays and suggest that the macerating systems are similar.

The sequence of events leading to the maceration of infected tissue is shown in Table 1. Both fungi produce a pectolytic enzyme during pathogenesis (1, 2). The pectolytic enzyme produced by *P. digitatum* is an exo-polygalacturonase (exo-PG) which hydrolyzes the terminal galacturonide of the pectin chain by hydrolytic action. The primary product of pectin degradation, both *in vitro* (1) and *in vivo*, is galacturonic acid (Table 2). In contrast, *P. italicum* produces an endo-PG which randomly cleaves the pectin chain (2). The primary end products of the reaction *in vitro* are short polymers of pectin. However, in decayed tissue galacturonic acid content is high because of extensive degradation periods and continual synthesis of the enzyme by *P. italicum* (Table 2).

Table 1. Process of tissue degradation of orange rind infected by either *Penicillium digitatum* or *P. italicum*.

1. Production of polygalacturonase by fungus
   a) *P. digitatum*—exo-Polygalacturonase
   b) *P. italicum*—endo-Polygalacturonase
2. Degradation of pectin and pectic substances of the cell wall to short polymers and galacturonic acid
3. Galacturonic acid diffusion into healthy tissue causing:
   a) Cell plasmolysis
   b) Cell death
   c) Leakage of endogenous pectinmethylestrase (PME) and nutrients into cell wall matrix
   d) Cell wall swelling
4. Demethylation of pectin by PME
5. Advanced degradation of pectin causing cell separation
6. Hyphal penetration

Based on *in vitro* studies, exo-PG is not considered to be an effective macerating enzyme because of its terminal hydrolytic action. In contrast, endo-PG will completely macerate tissue within several hours. Yet, the appearance of tissues from the lesion of both green and blue mold are comparable (1, 2). Both enzymes require demethylated sites on the pectin chain for hydrolytic action (10). A low degree of demethylation, which is often the case in *in vitro* studies, limits the extent of pectin degradation by exo-PG and to a lesser extent, by endo-PG.

Table 2. Organic acids of 'Valencia' orange rind as affected by infection by *Penicillium digitatum* and *P. italicum*.

Organic acid	Concentration (mg/g fresh weight)		
	Noninfected	*P. digitatum*	*P. italicum*
Malic	0.8	0.9	0.4
Citric	0.1	2.1	1.9
Quinic	0.7	0.0	0.0
Succinic	0	0.1	0.1
Galacturonic	0	12.5	8.6
Total	1.6	15.6	11.0

Pectin within the lesions of green or blue mold is almost completely demethylated to pectic acid as a result of endogenous PME. Thus, pectin degradation is not limited under this condition. Endogenous PME is released into the cell wall matrix following cell damage at the lesion front (1, 2), as a result of galacturonic acid and other solutes diffusing from the macerated area into the healthy tissue. The increase in solutes in conjunction with a low pH, 3.1, is sufficient to cause cell plasmolysis and cell death. Increased leakage of cell constituents is characteristic of these cells (11). In addition, nutrients leaked from these cells would also support growth of the pathogen.

The diffusion of galacturonic acid into the healthy tissue is also associated with swelling of the cell wall (1, 2). This is considered indicative of cell wall weakening (6). Increased levels of water extractable pectin are also found in this area (1). Further weakening is caused by PG action which culminates in a partial separation of the cells at the time of hyphal penetration. Maceration is generally observed first in the albedo tissue and then in the flavedo. The flavedo is composed of compact cells whereas cells of the albedo are less compact and contain numerous schizogenous air spaces (12). Such loosely bound tissues are apparently more easily degraded by both enzyme systems.

The difference in the rate of development between green and blue mold cannot be attributed to the type of pectolytic enzyme produced during pathogenesis. *Penicillium italicum* produces a more active macerating PG than does *P. digitatum*, but green mold is a more rapid developing decay than blue mold. Thus, factors other than the rate of tissue degradation must be involved in determining the rate of development and the most obvious factor is growth rate. The galacturonic acid produced as a product of pectin degradation has an active role in the decay process. It is the diffusion of this acid that contributes to increased pectin degradation and cell wall weakening. Green (9) also has shown that acids, e.g. citric and oxalic, will increase the infection of citrus rind by both *P. digitatum* and *P. italicum*.

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Proc. Fla. State Hort. Soc. 93:307-308. 1980.

INHIBITION OF ALTERNARIA ROT OF TOMATOES AND BELL PEPPERS BY POSTHARVEST TREATMENT WITH CGA-64251 OR IMAZALIL[1,2]

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Additional index words. Lycopersicon esculentum, Capsicum annuum.

Abstract. **Dipping tomatoes** (Lycopersicon esculentum **Mill.) and bell peppers** (Capsicum annuum **L.) for 10 seconds in an aqueous solution of 50 to 250 μg a.i./ml of CGA-64251 or imazalil inhibited development of rot by** Alternaria alternata **(Fr.) Keissler. Residues of imazalil were less than 1 μg/g in tomatoes analyzed after treatment in 100 or 250 μg/ml of imazalil.**

Development of alternaria rot during marketing of Florida produce in the Greater New York Market from 1974-77 caused 1.2 and 1.3% retail and 1.4 and 1.2% consumer losses in prepackaged and loose-packaged tomatoes, respectively (2). These figures represent 20-35% of the total retail and 18-24% of the total consumer losses determined in this survey. A similar study in the same area from 1971-73 showed the alternaria rot caused 0.26% wholesale and 0.26% retail losses in loose-packaged bell peppers (1). These figures represent 6.4% of the total wholesale and 8.2% of the total retail disease losses determined in this survey. However, losses in Florida peppers are often much higher in export markets (4) with 1.9% wholesale and 16.2% retail losses due to alternaria rot in peppers shipped to Europe (3).

Imazalil has been reported (5) to control alternaria rot of tomatoes. Our preliminary tests showed that the experimental chemical, CGA-64251, inhibited the *in vitro* growth of *A. alternata*. We compared imazalil and CGA-64251 for controlling alternaria rot of both tomatoes and peppers and determined residues for tomatoes treated with imazalil.

Materials and Methods

The following experimental fungicides were tested *in vitro* at 1, 10, and 100 μg a.i./ml of potato-dextrose agar (PDA): 1-[2-(2,4-dichlorophenyl)-4-ethyl-1,3-dioxolan-2-ylmethyl]-1H-1,2,4-triazole (CGA-64251) (Ciba-Geigy, Greensboro, NC 27409) and 1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole (imazalil) (Janssen Pharmaceutica, B-2340 Beerse, Belgium and Decco Pennwalt, Monrovia, CA 91016). A 7-mm disk with spore-mycelial growth of *A. alternata* from a 1- to 2-week-old culture on PDA was placed in the center of a petri plate containing PDA with or without fungicide. Four duplicate plates were run for each treatment, and the entire test was repeated at another time. The plates were incubated at 25°C for 7 days, and then the average diameter of growth was measured.

Large, commercially waxed, non-ethylene-treated, mature-green 'Flora-Dade' tomatoes and medium-sized, mature-green 'California Wonder' bell peppers were purchased at intervals from Dec. 1979 to Apr. 1980, from local sources in Dade County, Florida. Fruits were sorted into 8 lots of 20-25 fruit with similar size, appearance, and freedom from decay and injury. Fruit surfaces were then disinfested by washing them with 70% ethanol. Fruits were inoculated by inserting a small amount of sporulating mycelial growth from a PDA culture of *A. alternata* into a small pocket (about 2 mm wide) just beneath the skin. Each fruit was inoculated at two sites on opposite sides along the equator to double the data obtained per fruit.

Within 2 hr of inoculation, tomatoes and peppers were dipped for 10 seconds in an aqueous solution of 0-250 μg a.i./ml of CGA-64251 or imazalil, drained of excess liquid, placed in a plastic tray, and covered loosely with a polyethylene bag to maintain humidity. The fruits were stored for 2 weeks at 5°C to induce chilling injury and allow fungal growth and then transferred to 13°C for 1-2 weeks to allow lesions to develop. The roughly elliptically shaped lesions were measured as the area of an ellipse with the vertical measurement as the major axis and the horizontal as the minor axis. Data were then converted to show the percent inhibition of lesion development due to fungicidal action.

Imazalil residues on mature-green tomatoes treated for 10 seconds in 100 or 250 μg a.i./ml of imazalil were determined. Three tomatoes were selected at random from a treated lot and analyzed separately for imazalil residue. Analyses were run within 2 hr of treatment, after 3 days of simulated transit at 13°C, and after ripening to the red stage at 21°C. A fruit for analysis was homogenized, and a representative portion (20 g) of the homogenate was shaken for 1 hr with 20 ml of benzene. The sample was then centrifuged, and the benzene phase analyzed for imazalil by gas chromatography at 260°C. The instrument was equipped with a ^{63}Ni linearized electron capture detector and a glass column (4-mm I.D. x 25 cm) packed with 20% OV-17 on

[1]Mention of a fungicide, trademark, proprietary product, or vendor does not constitute a guarantee or warranty by the U.S. Department of Agriculture nor does it imply registration under FIFRA.

[2]The authors express their thanks and appreciation for the technical assistance of Messrs. James D. Teas and William G. H. Latham.