

cedure produced no statistically significant differences in content between soft- and hard-finisher treatments of juice in either variety. Significant flavor correlation with limonin was found in the 'Valencia' variety, although overall limonin contents were lower in 'Valencia' juice than limonin contents found in juice from the 'Pineapple' orange variety.

The fact that optical density was the only variable studied which gave a significant flavor correlation common to both 'Pineapple' and 'Valencia' varieties, coupled with results that increased yields from hard finish did not produce significant corresponding flavor correlations, indicated that under the conditions of this study, the effects of finishing were determined to be not critical to overall juice quality.

Finally, a flavor prediction equation with an r^2 value of 0.770 was developed.

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STUDIES ON THE INHIBITION OF ALTERNARIA CITRI IN STORED CITRUS FRUITS¹

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Abstract. During extended storage of citrus fruits for 3-4 months, a type of disease known as "Black-Rot" caused by *Alternaria citri* becomes a problem. Post-harvest treatments proved ineffective to control this type of decay. Now it has been demonstrated that the application of an antifungal complex (Antibiotic F), derived from a local strain of *Bacillus subtilis* AECL 69, on the cut stem-ends (in a radius of 2 cm) prior to storage significantly controls this disease in 'Valencia' oranges and 'Kinnow' mandarins. No residual bioactivity was detected from the peel and juice of the treated fruits after a storage period of 3-4 months at 4-5°C.

In stored citrus fruits the major spoilage organisms were found to be *Penicillium digitatum*, *Penicillium italicum* and in 'Kinnow' mandarins, a type of soft rot caused due to *Erwinia citri* maculan was also observed (6). In extended storage of both oranges and mandarins, Black-Rot caused by *Alternaria citri* was also observed. In order to minimise postharvest losses in citrus fruits, several experiments were carried out in our laboratory using different postharvest treatments (1, 2, 4, 5).

None of these treatments and others reported from elsewhere (8) were effective in controlling Black-Rot in citrus fruits. The antifungal antibiotic F derived from a local strain of *Bacillus subtilis* AECL 69 was found quite effective against *Alternaria citri* in stored 'Valencia' oranges (7). Further in a detailed study, the effect of Antibiotic F on the inhibition of *Alternaria citri* in stored oranges and man-

darins was investigated during 1979-81, the results of which are reported in this paper.

Materials and Methods

Stored citrus fruits decaying with 'Black-Rot' were collected and the causative fungus, *Alternaria citri*, was identified by direct microscopic examination of blackened tissues. The infected tissues from oranges as well as mandarins were streaked on to malt extract agar (pH 6.5) and a dozen of pure culture isolates of *Alternaria citri* were maintained on malt extract agar slants and were tested against the killing effect of antibiotic F by cup plate technique.

To test the effectiveness of antibiotic F to control the postharvest decay of citrus by *Alternaria citri*, the mature fruits of *Citrus sinensis* Osbeck cv. 'Valencia' and *Citrus reticulata* Blanco cv. 'Kinnow' were procured from a private orchard, stem-ends cut to the shoulders, washed and dipped in aqueous suspension of thiabendazole (1000 ppm conc.) against *Penicillium* rots (5). The fruit skin portions around stem-ends were encircled (radius 2 cm) with a felt-tipped marker and the aqueous solution of antibiotic F (inhibition zone 25 mm against *A. citri*) was applied with the help of a sterilized pasteur pipette to the encircled skin area. This was repeated three times after an interval of five minutes. Plain water drops were applied to another lot of fruits that served as controls. After drying in normal air, the treated as well as nontreated oranges and mandarins were held in perforated cardboard boxes lined with newspaper (0.093 mm thick). The fruit containers were stored for 3 months (Kinnow), 3 and 4 months (Valencia) in a commercial cold storage (4-5°C) and then transferred to room temperature (25-30°C) for a week. The fruits were then cut across and the incidence of Black-Rot (also known as internal blackening) recorded.

For the residual effect of the antibiotic F, treated fruits 3 (3x3) were collected at random after a storage period of 4 months. The skin portion from 2 cm dia around the stem-ends were removed with a sharp sterilized blade and skin

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tissue meshed into small pieces in a blender. Since antibiotic F is soluble in water (3, 9, 10), each lot of washed tissues was taken in 10 ml of sterilized water and vigorously shaken to dissolve out any antibiotic F, if present in the treated skin. These supernatants were then passed through Seitz filters and completely dried over a rotary evaporator under vacuum. This dried material was dissolved in 0.5 ml of sterile water and tested against *Aspergillus niger* (the most sensitive test organism for antibiotic F) bioactivity. In another experiment, the skin from fruits 36 (12x3) were extracted with water as above and pooled together, dried over rotary evaporator and redissolved in 0.5 ml of sterilized water. The bioactivity of this material was also determined. In the third experiment, the juice of the treated fruits was passed through Seitz filter and the filtrate was completely dried over a rotary evaporator under vacuum and then redissolved in 0.5 ml of sterilized distilled water and tested for antibiotic F bioactivity against *Aspergillus niger* and *Alternaria citri*. In a preliminary study to find out the time scale recovery of the bioactivity of the treated skin portions of the fruits (0, 1, 3, 7, 15 days), the ground peel slurry from 5 fruits was prepared in water, passed through a Seitz filter followed by charcoal column and finally dried under vacuum and dissolved in 0.5 ml water and tested for the bioactivity.

Results and Discussion

The killing effect tests on 12 *Alternaria citri* culture isolates from decayed oranges and mandarins were carried out in petri plates while *Aspergillus niger* served as standard for the bioactivity of antibiotic F (Table 1). So far we have not found any isolate showing resistance to the antibiotic F killing effect.

The incidence of Black-Rot after a storage period of 3

Table 1. In vitro testing of antibiotic F against *Alternaria citri* isolates.

Culture No.	Inhibition zone (mm)
<i>Aspergillus niger</i> (40.0 standard test zone)	
<i>Alternaria citri</i> No. V1 ^z	25
No. V2	27
No. V3	25
No. V4	25
No. V5	26
No. V6	25
<i>Alternaria citri</i> No. K1 ^y	26
No. K2	25
No. K3	25
No. K4	26
No. K5	25
No. K6	25

^zNo. V1, No. V2 . . . No. V6 = Culture isolates from 'Valencia' oranges.
^yNo. K1, No. K2 . . . No. K6 = Culture isolates from 'Kinnow' mandarins.

Table 2. Time-scale recovery of antibiotic F from treated 'Valencia' oranges.

Time (days)	Antibiotic F recovered IZD/A. niger (mm) ^z
0	50
1	40
3	30
7	25
15	N.D. ^y

^zIZD/A. niger = Inhibition zone diameter against *A. niger*.

^yN.D. = Bioactivity not detected.

months showed a highly significant difference ($P < 0.001$) between the treated and untreated fruits (Fig. 1). In the

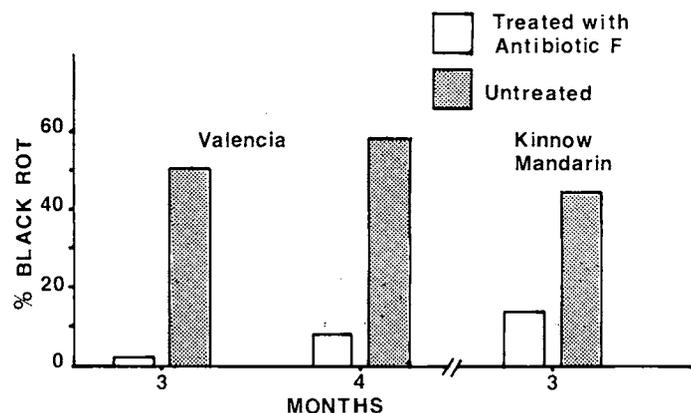


Fig. 1. Effect of antibiotic F on the control of black rot in citrus fruits during cold storage (4-5°C).

second experiment on 'Valencias', the trend of the control of decay remained the same, however, the percentage of the 'Black-Rot' incidence increased with increase in storage time. The reason for this may be due to the inactivity of the antibiotic. The effect of this antibiotic on the control of Black-Rot in 'Kinnow' cultivar of mandarin was similar as in 'Valencia' (Fig. 2). As regards residual bioactivity of the antibiotic in the peel and juice of the treated fruits, no such activity was detected after 3-4 months storage period (3 for Kinnow and 4 for Valencia, respectively). It is presumed that the antibiotic applied on the stem-ends and the surrounding areas (2 cm) sterilized this portion of the fruit in the first instance and possibly decomposed during subsequent storage period, hence, the bioactivity of the antibiotic could not be detected either from peel or juice of the treated fruits. Time scale recovery of the antibiotic from the treated portion of the skin of the fruits showed that the bioactivity was reduced below the level of inhibition zone detection against *Aspergillus niger* at an interval of 2 weeks. The metabolic fate of the antibiotic F in citrus fruits, its mutagenic effects using Ames *Salmonella*/microsome test, and toxicological studies on mice are being investigated.

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