Plant Physiol. (1986) 82, 324-326
0032-0889/86/82/0324/03/$01.00/0

Communication

Accumulation of Putrescine during Chilling Injury of Fruits

Received for publication April 15, 1986

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ABSTRACT

Putrescine (Put) increased 68% in lemon (*Citrus limon* (L.) Burm. f. cv Bearss) flavedo, 39% in grapefruit (*C. paradisi* Macf. cv Marsh) flavedo, 49% in grapefruit juice, and 149% in pepper (*Capsicum annuum* L. cv Early Calwonder) pericarp when fruits were stored at chilling temperatures. In lemon flavedo, the coefficient of correlation (r^2) between Put concentration with severity of chilling was 0.90 and Put levels almost doubled; the injury index going from 1 to 2 units. Pepper pericarp, which was the most chilling-sensitive tissue tested (injury index going from 1 to 3.8 units), showed the greatest difference in Put accumulation (166 to 413 nanomoles per gram fresh weight) between storage temperatures of 7.2 and 1°C. The least difference (338 to 470 nanomoles per gram fresh weight) was found in grapefruit flavedo between storage temperatures of 15.5 and 4.4°C; the injury index going from 1 to 1.3 units.

Chilling injury is induced in a wide variety of horticultural commodities when they are exposed to low but nonfreezing temperatures either before or after harvest. As a result, chill-damaged commodities are more susceptible to fungal and bacterial decays. Although several hypotheses relating to the causes of CI[2] have been postulated, the primary mechanism(s) of CI remain unclear. As biochemical differences probably exist between fruits sensitive and resistant to CI, elucidation of these differences could lead to a means for CI control.

The diamine Put has been linked to various plant stress conditions. Put has been found to accumulate in potassium-deficient barley seedlings (6) in magnesium-deficient barley, pea, bean, and radish leaves (1), and in plants supplied with excess ammonium (8) and sodium (9). Sulfur dioxide fumigation of pea seedlings has induced accumulation of Put (5). In peeled cereal leaf segments, Put concentration increased when tissues were exposed to osmotic shock (3) or subjected to acid stress (10). In a recent review (7), it was suggested that Put accumulation could be a cause of abiotic stress-induced injuries. However, to our knowledge, data correlating Put with CI have never been published. The objective of this study was to examine possible changes in Put concentrations with CI.

MATERIALS AND METHODS

Plant Materials. Lemons (*Citrus limon* (L.) Burm. f. cv Bearss) were harvested August 19, 1985, and dipped for 30 s in 600 μg/ml benomyl to prevent fungal growth. The fruit were cured (degreened) without added ethylene at 15.5°C and at 80 to 92% RH for 28 d, at which time they were sufficiently yellow in color for commercial acceptability. They were then washed, drenched with 600 μg/ml benomyl, waxed, and stored for 21 d at either 1° or 10°C under RH conditions of 80 to 92%. Ten fruit were removed at random from each storage regime and rated for CI as follows: 1 = none, 2 = slight, 3 = moderate, and 4 = severe.

Grapefruit (*C. paradisi* Macf. cv Marsh) were harvested November 18, 1985, and degreened with 5 μ/L ethylene for 3 d at 29.4°C and 80 to 90% RH. They were then washed, drenched with 600 μg/ml benomyl, waxed, and stored for 60 d at either 4.4 or 15.5°C at 80 to 92% RH. At the end of the storage period, 10 fruit were removed at random from each storage regime and rated for CI in the same manner as the lemons.

Peppers (*Capsicum annuum* L. cv Early Calwonder) were harvested January 27, 1985, spray-washed with 50 μg/ml chlorine, and stored for 21 d at either 1 or 7.2°C at 80 to 92% RH. At the end of the storage period, 10 fruit were removed at random and rated for CI in the same manner as the lemons and grapefruit.

Chemicals. Benomyl[3] was obtained from Dupont and the wax used was Fresh Mark 3202 (Fresh Mark Chemical Corp., Orlando, FL). Put was obtained from Calbiochem; reagent grade benzoyl chloride and HPLC grade acetonitrile from Baker.

Putrescine Analysis. A total of 5 g fresh weight of lemon or grapefruit flavedo, pepper pericarp, or 5 ml of grapefruit juice from 10-fruit samples were blended for 20 s at 4°C in 50 ml of 5% (v/v) $HClO_4$ to give a final concentration of 0.1 g/ml using a Polytron homogenizer (Brinkman Instruments, Div. of Sybron Corp., Westbury, NJ). The homogenate was centrifuged at 20,000*g* for 20 min at 4°C and the supernatant was used for the analysis of Put according to a previously described procedure (2). A total of 0.5 ml of the tissue extract was added to 2 ml of 4 N NaOH and 5 μl benzoyl chloride. The reaction mixture was vortexed for 15 s and incubated for 20 min at 35°C in a water bath. The reaction was terminated by the addition of 4 ml saturated NaCl and the benzoylated Put was extracted with 4 ml chilled ethyl ether. The ether fraction was collected and centrifuged at 1,500*g* for 10 min and a 2-ml aliquot was vacuum dried at room temperature and then resuspended in 200 μl acetonitrile. Put analysis was carried out on a Waters HPLC System (Waters Associates, Inc., Milford, MA) equipped with a UV detector and C-18 reverse phase column (30 cm × 4 mm). Acetonitrile/water (1:1) was used as the mobile phase in an isocratic elution.

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[2] Abbreviations: CI, chilling injury; Put, putrescine.

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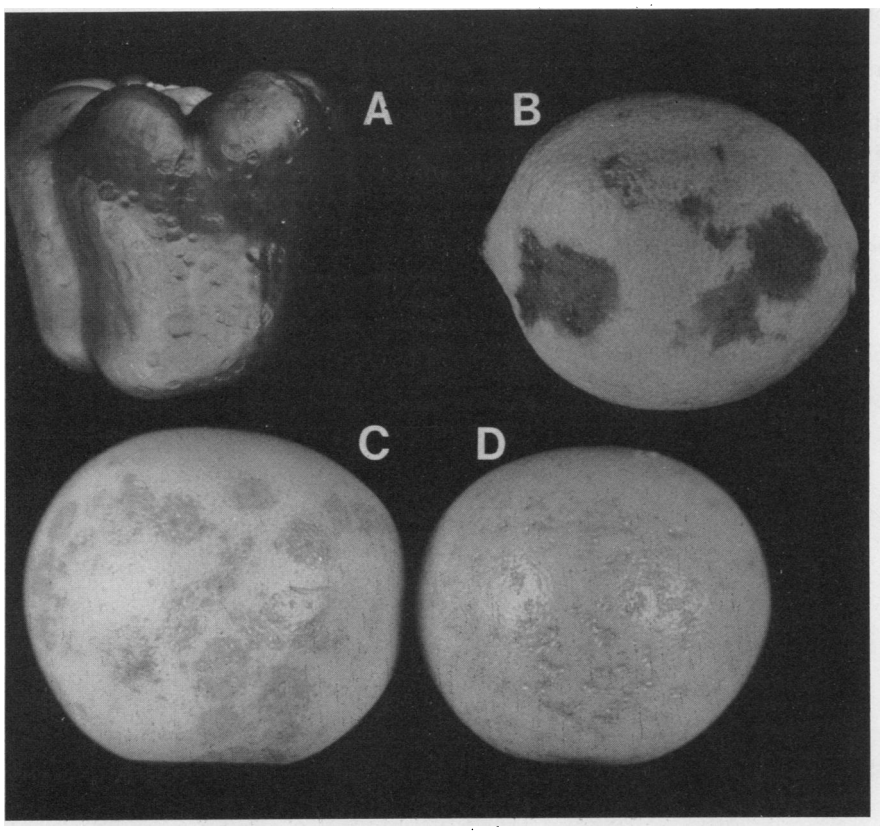

FIG. 1. CI of pepper (A), lemon (B), grapefruit (brown staining) (C), and grapefruit (pitting) (D).

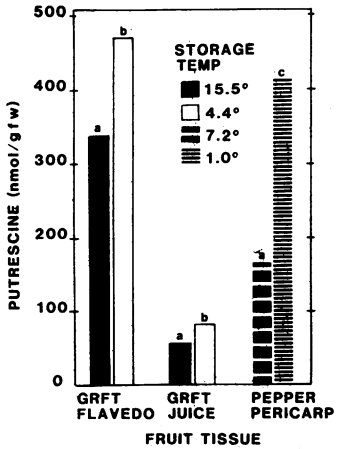

FIG. 2. The concentrations and amounts of putrescine in grapefruit flavedo and juice after 60 d and in pepper pericarp after 21 d at chilling and optimum storage temperatures. Numbers above bars indicate CI index: a = 1.0; b = 1.3; and c = 3.8.

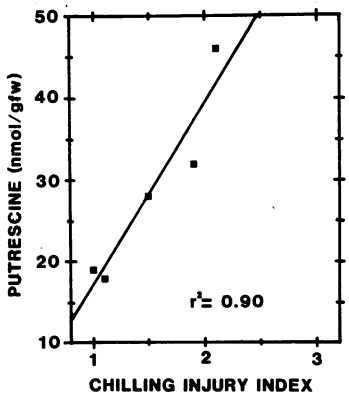

FIG. 3. Relationship between CI rating and putrescine concentration in lemon flavedo after 21 d at a chilling temperature (1°C).

RESULTS AND DISCUSSION

Figure 1 shows CI symptoms on the three types of fruits used in this study. Pepper fruits demonstrate CI with sheet pitting, and individual spots which are flat or slightly sunken, and large affected areas which are dappled in appearance. Symptoms of CI on citrus fruits are varied. 'Brown staining' is a diffuse, irregular, tan-to-brown superficial discoloration of the peel while 'pitting' is characterized by depressed spots which turn tan to brown in color.

The Put concentrations in chilled and nonchilled grapefruit and pepper fruits are shown in Figure 2. Put accumulation was greater in chilled compared to that in nonchilled tissue. The greatest increase in Put concentration (approximately 2.5-fold) occurred in pepper pericarp tissue stored at 1°C compared to fruit stored at 7.2°C. A storage temperature of 4.4°C resulted in 39 and 49% Put increases in grapefruit flavedo and juice, respectively, over fruit stored at 15.5°C. There was nearly a linear relationship between the severity of CI and Put level in lemon flavedo (Fig. 3). A coefficient of correlation of 0.90 would indicate that CI is highly related to Put accumulation.

Spermidine and spermine levels were determined in all chilled and nonchilled fruits. However, no significant relationships were found between these polyamines and CI, therefore, data are not presented.

The greater differences in Put levels observed in peppers may be due to the severity of CI in these fruits. Most of the peppers had severe CI symptoms, with a composite rating of 3.8. Grapefruit had a composite rating of 1.3, indicating that CI was not as acute as in the peppers. Therefore, one would not expect such proportionally large differences in grapefruit tissue Put levels. Tissue sampled from fruit without visible CI or portions of fruit surfaces without visible CI may not contain elevated

levels of Put and thus tends to dilute the effect of tissue with CI on Put levels.

It has been observed in cereal leaves that temperature alone did not affect the Put level (4). Therefore it may be that chilling stress affected the Put levels in the chilling sensitive fruit tissues in this study and induced CI. Moreover, as it has been suggested (7), Put accumulation may be a cause of stress-induced injury, and in this case, the stress of chilling.

Acknowledgment—We wish to thank Dr. M. Bausher for the use of his research facilities.

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