Plant Physiol. (1975) 56, 765–767

Cellulase Activity, Endogenous Abscisic Acid, and Ethylene in Four Citrus Cultivars during Maturation

Received for publication April 22, 1975 and in revised form July 31, 1975

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ABSTRACT

At maturity, the fruit of two early maturing orange cultivars, Hamlin and Pineapple (*Citrus sinensis* [L.] Osbeck), contained more ethylene and abscisic acid than the late maturing Valencia and Lamb Summer (*C. sinensis* [L.] Osbeck) cultivars. Ethylene (up to 95 nl/l in internal atmosphere) and abscisic (50 μg/kg dry weight flavedo) increased most rapidly in Pineapple, leading to increased cellulase activity and loosening of the fruit. Fruit of the two late maturing cultivars contained less than 25 nl/l ethylene and 40 μg abscisic acid/kg dry weight of flavedo at peak maturity. Cellulase activity and loosening of the fruit of these late maturing cultivars was slight.

The fruit removal force of early and midseason oranges is reduced readily by ethylene-generating chemicals, whereas late season oranges in Florida do not respond consistently during their peak maturity (2). Several reasons for this difference in response have been hypothesized, including increased GA_3 synthesis or translocation from young leaves of Valencia oranges associated with weather causing tissue growth (9), hormone level difference between varieties (2), and regeneration of tissues in the separation zones of Valencia oranges caused by auxins translocated from young leaves to these zones (3).

Cellulase activity in separation zones causes loosening of citrus fruit (7, 8). Abscisic acid, when applied properly (10), and ethylene (6, 11) stimulate cellulase activity and loosening of fruit. In some cultivars, such as the navel, ethylene increases during maturation to several times that found in Valencia (1). Although no FRF[1] values were reported for navels, the ethylene level is such that cellulase is probably stimulated. Also, ABA increases during maturation of Valencia; however, the FRF is not lowered during regreening time, possibly because of the overriding effect of GA_3 (2, 9). The FRF of Pineapple oranges decreases gradually during maturation, leading to fruit drop, whereas Valencia maintains an average FRF above 6.8 kg, with little fruit drop (4).

This paper presents the results of investigations on the changes in endogenous ABA, ethylene, and cellulase in four citrus cultivars during maturation. The loosening of the fruit and effectiveness of abscission-inducing chemicals are closely related to the levels of these three factors.

MATERIALS AND METHODS

Fruit of Hamlin, Pineapple, Valencia, and Lamb Summer (*Citrus sinensis* [L] Osbeck) were selected as representative early, midseason, and two late maturing orange cultivars, respectively. Samples were collected at monthly intervals beginning in December and ending after peak fruit maturity, for two seasons. The data presented are averages for the 2 years. Ten fruit per sample were used for ABA, ethylene, and cellulase assays. Ten other fruit were used for FRF measurements (11). Methods for ethylene in the internal atm by gas chromatography (11), cellulase in separation zone tissue by viscosometry (8), and ABA in the flavedo (exocarp) by bioassay (9) have been published. ABA was identified qualitatively, as well, by identification of its methyl ester by GLC with a ^{63}Ni EC detector.

RESULTS

ABA in Flavedo. The flavedo of Hamlin and Pineapple contained about 30 μg ABA/kg dry weight in December when sampling started (Fig. 1A). ABA increased faster and remained higher in Pineapple than in the other cultivars under final harvest. Mature Pineapple flavedo contained 48 ± 4 μg ABA/kg dry weight compared to 42 ± 4 μg/kg dry weight in Hamlin.

Valencia and Lamb Summer flavedo contained about equal amounts of ABA during maturation. ABA ranged from about 20 ± 2 μg/kg dry weight in December to 40 ± 3 in Valencia and 38 ± 2 in Lamb Summer in May. ABA was higher during April in Valencia than in Lamb Summer. This difference may be due to dry weather and a cultivar difference, because Lamb Summer is a Valencia selection that is supposed to remain on the tree longer than Valencia. Neither Valencia nor Lamb Summer contained as much ABA as Pineapple or Hamlin at their respective peak maturities.

Ethylene in Internal Atmosphere. Ethylene in the internal atm of the fruit followed nearly the same pattern as ABA in the flavedo (Fig. 1B). Hamlin and Pineapple contained at least two times as much ethylene during the sampling period as did Valencia and Lamb Summer. Ethylene in Hamlin and Pineapple ranged from 46 to 64 nl/l in December, increased slightly to 50 to 60 by February, and in Pineapple, increased to nearly 100 in April. Pineapple was past peak maturity in April, and the high ethylene content may have indicated senescence and dry weather. Ethylene in Hamlin increased only to about 75 nl/l.

Late maturing Valencia and Lamb Summer increased only slightly in ethylene during the sampling period (Fig. 1B). The level ranged from 10 to 15 nl/l in December to 35 ± 7 nl/l in Valencia and 25 ± 8 nl/l in Lamb Summer in July.

Separation-Zone Cellulase. Cellulase activity increased in the separation-zone tissue as maturity of the fruit increased (Fig. 1C). The most rapid increase was in Pineapple, from 10 ± 5 Δn/hr in December to 38 ± 9 Δn/hr in April. Cellulase activity in the other cultivars increased slowly during maturation. Since Hamlins were legally mature when sampling started, cellulase activity was higher in their separation zones than in those of Valencia or Lamb Summer oranges. Cellulase in Valencia peaked in May and then decreased slightly.

[1] Abbreviation: FRF: fruit removal forces.

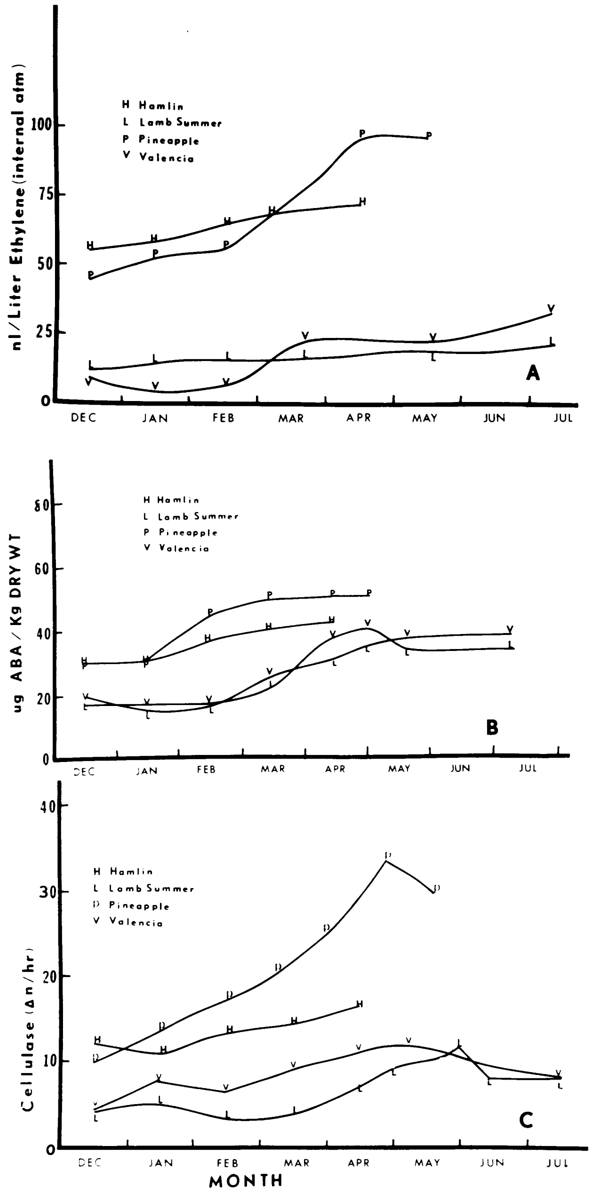

FIG. 1. A: Abscisic acid in flavedo of fruit of four citrus cultivars during maturation. (Each point is the average of two samples of 10 fruit collected each month.) B: Internal atmosphere ethylene in fruit of four citrus cultivars during maturation. (Each point is the average of 10 fruit per month during 2 seasons.) C: Cellulase in the separation zone of fruit of four citrus cultivars during maturation. (Each point is the average of two samples of 10 fruit collected each month. Δn/hr: change in viscosity/hr.)

Fruit Removal Force. The FRF decreased as the season advanced (Table I). The FRF of Hamlin orange decreased from about 7.7 to 6 kg, and the FRF of Pineapple orange decreased from 7.8 to 4.2 kg during 5 months of maturation. The FRF of Valencia and Lamb Summer changed very slowly, and the Valencia fruit exhibited tightening to their stems during May and June, which was not evident in the Lamb Summer. The FRF of Lamb Summer, however, was higher during April and May than that of Valencia.

DISCUSSION

Ethylene increased in the internal atm of the four citrus cultivars as maturity increased. Ethylene in the internal atm of navel and Valencia oranges in California follows trends similar to those shown in these tests (1). Ethylene increased most in Pineapple, in which cellulase activity and fruit loosening was the greatest. Ethylene is not the only factor which stimulates cellulase activity When ethylene levels are lowered by hypobaric conditions, ABA stimulates cellulase in the separation zones of citrus fruit (10). In some instances, ABA induces senescence, which brings about more ethylene synthesis by the fruit. Senescent fruit are more susceptible to wounding by abscission-inducing chemicals. More ethylene is produced and the fruit abscise rapidly. Applied auxins may retard citrus leaf and fruit abscission even in the presence of ethylene (5). That endogenous ABA may be counteracting the auxin effect in these tissues is unlikely because the auxin level in mature citrus fruit in most instances is too low to measure. Reduced auxin translocation from leaves probably is not a factor in mature citrus fruit abscission. The possibility exists that ethylene and ABA influence some metabolic processes which influence the early stages of abscission. The possibility that other enzymes, such as polygalacturonase (12), membrane permeability (8), and a senescence-inducing factor (6) may be affected by endogenous ABA or ethylene should not be overlooked.

The data presented in this report suggest that ABA and ethylene in Hamlin and Pineapple are high enough, so that abscission-inducing chemicals applied to them are more efficient than those applied to Valencia and Lamb Summer, in which ABA and ethylene are much lower. The late maturing cultivars are also susceptible to regreening (9) and have higher GA levels. The GA probably counteracts the effect of the endogenous ABA, which results in more "juvenile" tissues that are more resistant to wounding by abscission-inducing chemicals.

Table I. *Fruit Removal Force of Four Citrus Cultivars from December through Fruit Maturation*

Month	Fruit Removal Force			
	Hamlin	Pineapple	Valencia	Lamb Summer
		kg		
Dec	7.7 ± 0.5	7.8 ± 0.5	8.6 ± 0.3	8.4 ± 0.4
Jan	7.3 ± 0.4	7.3 ± 0.6	8.8 ± 0.5	8.2 ± 0.3
Feb	6.7 ± 0.4	6.5 ± 0.5	8.6 ± 0.4	8.5 ± 0.4
March	6.0 ± 0.6	4.8 ± 0.2	7.7 ± 0.3	8.7 ± 0.2
April		4.2 ± 0.8	6.5 ± 0.8	7.8 ± 0.4
May			7.1 ± 0.2	7.4 ± 0.3
June			7.5 ± 0.6	6.9 ± 0.5
July			6.8 ± 0.2	7.0 ± 0.4

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