# Ethylene-Induced Carotenoid Accumulation in Citrus Fruit Rinds[1]

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*Abstract.* Citrus trees were sprayed with ethephon when the fruit were mature but still green or partially degreened. Harvested fruit that were mature and partially or fully degreened were held in an atmosphere containing 10 ppm ethylene for 5 to 9 days. One preharvest ethephon spray hastened carotenoid accumulation in rinds of green 'Nova' tangerines, partially degreened 'Robinson' and 'Dancy' tangerines, and fully degreened 'Robinson' tangerines, but was not effective on green 'Bearss' lemons and partially degreened 'Hamlin' oranges. Effective concn varied between 50 and 500 ppm and with the cultivar evaluated. Two 500-ppm ethephon sprays, applied 2 weeks apart, hastened carotenoid accumulation in rinds of partially degreened 'Hamlin' oranges and 'Robinson' tangerines. Postharvest-ethylene treatment induced carotenoid accumulation in rinds of partially degreened 'Bearss' lemons and 'Lee' and 'Dancy' tangerines and degreened 'Robinson' and 'Dancy' tangerines. Tangerines showed greater ethylene-induced increases in rind carotenoids than did 'Hamlin' oranges and 'Bearss' lemons. Fruit which had higher rind carotenoid contents as a result of ethylene or ethephon application had better visible external color.

Carotenoids in the rinds of many citrus cultivars increase in the fall and winter as the fruit mature[3] (10, 18, 19). Early fall increases in carotenoids often occur during the same time that chlorophyll in the rind begins to decrease[3] (10, 19), although carotenoids continue to accumulate after chlorophyll destruction. These 2 processes are accelerated by cool temp (3, 5, 22), and chlorophyll destruction has been shown to be increased with exogenously applied ethylene or chemicals that release ethylene (1, 4, 11, 12, 14, 15, 23). Cool temp have also been shown to increase endogenous ethylene in citrus fruit (3, 5).

The effect of ethylene on carotenoid pigments has been controversial. Miller et al. (17, 19) found that the total extractable carotenoids of orange, grapefruit, lemon, and lime rinds were not affected by postharvest exposure to ethylene, while Eilati[3] found that they were in 'Shamouti' oranges. Other reports (1, 2, 4, 11), where visual color ratings were made, indicated that citrus fruit treated with ethylene or ethylene-producing chemicals had better external color. This may have reflected not only chlorophyll destruction, but increased carotenoids (2, 4). Several reports[3] (18, 20) have also indicated changes in levels of individual carotenoids associated with the development of better external color.

This paper presents evidence that exogenously applied ethylene accelerates the accumulation of total extractable carotenoids in citrus fruit rinds.

## Materials and Methods

*Plant material.* Trees in orchard plantings of the following were used for test purposes: 'Robinson', 'Nova', and 'Lee' tangerines [*Citrus reticulata* Blanco X (*C. paradisi* Macf. X *C reticulata* Blanco)]; 'Dancy' tangerine (*C. reticulata* Blanco); 'Hamlin' orange [*C. sinensis* (L.) Osbeck]; and 'Bearss' [*C. limon* (L.) Burm. f.]. 'Robinson' and 'Nova' trees were 4 to 5 years old, while 'Hamlin', 'Lee', 'Dancy', and 'Bearss' trees were 8 to 10 years old. 'Robinson', 'Nova', 'Hamlin', and 'Dancy' trees were located in the central ridge portion of the State; the 'Lee' trees were in the west-central area; and the 'Bearss' lemon planting was in south Florida.

*Preharvest spray applications.* Sprays of 0, 50, 100, 200, 300, 500, and 750 ppm (2-chloroethyl)phosphonic acid (ethephon)[4] were applied with a 50-gal sprayer at 200 psi. Each tree was sprayed with 4 gal of solution. Triton X-100 was added as a surfactant at a rate of 0.1%. Tests on 'Robinson', 'Lee', 'Dancy', 'Hamlin', and 'Bearss' were conducted on single trees with 2 replicates, and the 'Nova' tests on 4 replicates. All trees were

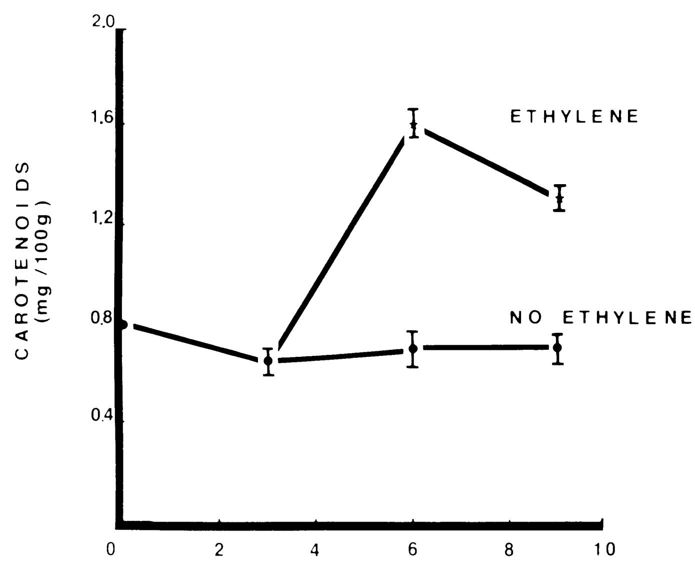


*Fig. 1.* Changes in carotenoid content (± standard deviation) on 'Bearss' lemon fruit rinds (△OD .500) during postharvest exposure to 10 ppm ethylene.

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[3]Eilati, S. 1970. The changes in color of the ripening orange fruit as affected by endogenous and exogenous factors. Ph.D. Thesis, The Hebrew University of Jerusalem.

[4]This is a report on the current status of research involving use of certain chemicals that require registration under the Federal Insecticide, Fungicide, and Rodenticide Act. It does not contain recommendations for the use of such chemicals, nor does it imply that the uses discussed have been registered. All uses of these chemicals must be registered by the appropriate State and Federal agencies before they can be recommended.

[5]Young, R., and O. Jahn (unpublished data).

*Table 1.* Effect of preharvest ethephon applications on carotenoid content (mg/100 g) of fruit rinds of 6 cultivars of citrus.

| Ethephon[a] | Bearss lemon | Hamlin orange | Lee tangerine | Nova tangerine | Robinson tangerine | | Dancy tangerine |
|---|---|---|---|---|---|---|---|
| concn (ppm) | (△OD.770)[b] 9/8 | (△OD.313) 11/18 | (△OD.453) 11/6 | (△OD.725) 10/8 | (△OD.481) 10/8 | (△OD.200) 11/10 | (△OD.438) 11/23 |
| 0 | 0.6 | 2.1 | 1.5 | 1.0 | 1.8 | 12.3 | 5.4 |
| 50 | - | - | 3.4 | 1.7 | 1.8 | 14.2 | - |
| 100 | 0.7 | 1.9 | 3.3 | 3.5 | 2.8 | 13.0 | 8.2 |
| 200 | - | 2.7 | 5.8 | 4.4 | 3.3 | - | 8.9 |
| 250 | 0.8 | - | - | - | - | - | - |
| 300 | - | 2.6 | 9.2 | 3.4 | 2.8 | 12.7 | 8.7 |
| 500 | 0.7 | 3.0 | 5.6 | 2.4 | 3.2 | 10.5 | - |
| 750 | 0.9 | - | - | - | - | - | - |
| L.S.D. .05 | N.S. | N.S. | 2.2 | 1.1 | 1.0 | 0.7 | 3.3 |

[a]Ethephon applied 6 to 7 days before harvest date indicated.

[b]△OD values are the chlorophyll readings for untreated fruit at harvest date.

sprayed once 6 or 7 days prior to harvest, except one test each with 'Hamlin' and 'Robinson', where 500 ppm sprays were repeated after 2 weeks.

*Postharvest ethylene treatment.* Two 100-fruit lots of 'Lee', 'Robinson' and 'Dancy' tangerines and 'Bearss' lemon were exposed to 10 ppm ethylene in a room maintained at 80°F and 80 to 90% R.H. 'Dancy' fruit were separated into 3 color classes according to chlorophyll content. Fruit were removed periodically for carotenoid analysis. Harvest dates for these cultivars were Nov. 9, Nov. 16, Dec. 7, 1970, and March 2, 1971, respectively.

*Carotenoid and chlorophyll analyses.* Carotenoids were extracted, analyzed, and calculated by the procedures described by Meredith and Young (16). Ten g rind from 20 fruit on each sprayed tree, or from 10 fruit at each sampling time in postharvest tests, was diced and blended in water (1:3 w/v) with an omnimixer. The water extract was diluted with an equal vol of methanol, 10 g supercell was added, and the slurry filtered on a Büchner funnel. The mat remaining on the funnel was extracted 3 times with hexane-acetone (1:1). The extracts were combined and washed with water in a separatory funnel to remove the acetone. Methanol saturated with KOH was added and, after 1 hr, the hexane extract was washed with water to remove saponified material. The hexane extract was read in a dual-beam spectrophotometer at 450 and 502 nm, and the results expressed as mg total carotenoids/100 g fruit wt of rind. This method is an approximation, since there are many individual carotenoids in citrus fruit rinds, but is satisfactory to give trends in carotenoid accumulation.

Chlorophyll (△OD) was measured on intact fruit with a light transmittance difference meter as previously described (15). Twenty fruit from each sprayed tree or from each 100-fruit lot were measured. Chlorophyll values (△OD) indicate the stage of natural degreening of the untreated fruit at the time of harvest. High △OD values (.600 to .800) indicate the fruit are quite green, while low △OD values (.100 to .250) indicate the fruit are degreened.

## Results

*Preharvest ethephon applications.* Our results indicate that single preharvest sprays of ethephon up to 500 ppm applied to partially degreened 'Hamlin' oranges (△OD .313) and up to 750 ppm on green 'Bearss' lemons (△OD .770) did not significantly affect total rind carotenoids. Carotenoid content of 'Hamlins' ranged from 1.9 to 3.0 mg/100 g and on 'Bearss' lemons from 0.6 to 0.9 mg/100 g (Table 1). Chlorophyll level in rinds of lemons sprayed with 750 ppm ethephon was similar to that in unsprayed fruit, although high ethylene evolution rates were measured from fruit 1 day after spray application. The 'Hamlin' oranges degreened with 500 ppm ethephon. Ethephon between 200 and 500 ppm increased carotenoid levels in rinds of 'Lee' (△OD .453), 'Nova' (△OD .735), and 'Robinson' (△OD .481, .200) tangerines. Ethephon at 100 ppm was also effective in increasing carotenoid levels in 'Nova' and 'Robinson' as were 200 and 300 ppm in 'Dancy' (△OD .438). In 1 test on 'Robinson', 50 and 100 ppm ethephon were effective, while 300 and 500 ppm were not. Changes in total carotenoids of tangerine rinds 1 week after ethephon application ranged from 1.0 to 7.7 mg/100 g. 'Lee', 'Nova', and 'Robinson' fruit were partially or totally degreened on the tree, depending on the initial rind chlorophyll level and ethephon concn[5]. In tests with these tangerines, 500 ppm ethephon appeared less effective than

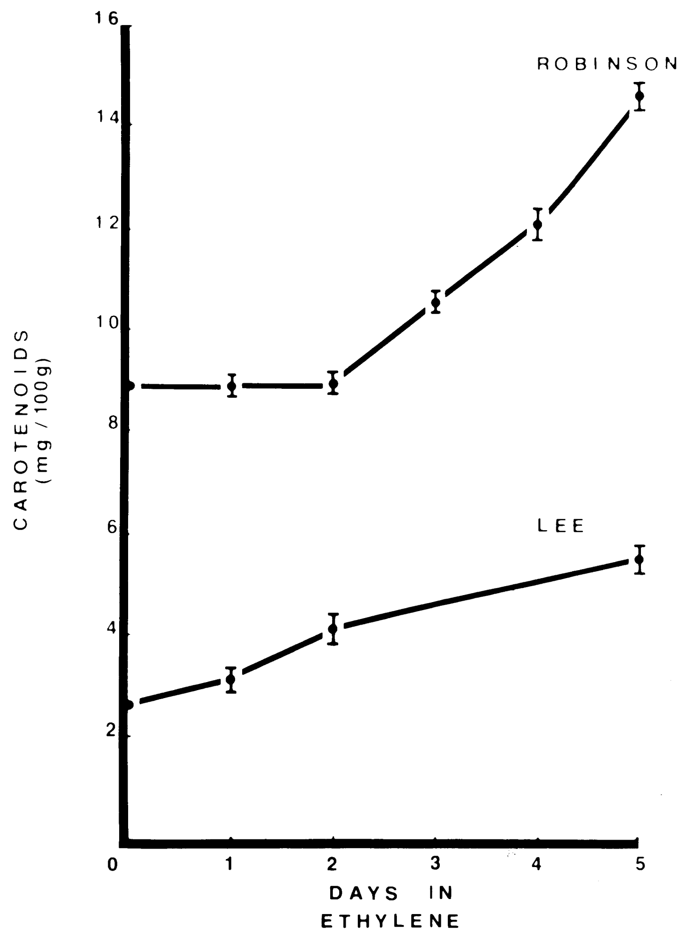


*Fig. 2.* Changes in carotenoid content (± standard deviation) of 'Robinson' (△OD .170) and 'Lee' (△OD .352) tangerine fruit rinds during postharvest exposure to 10 ppm ethylene.

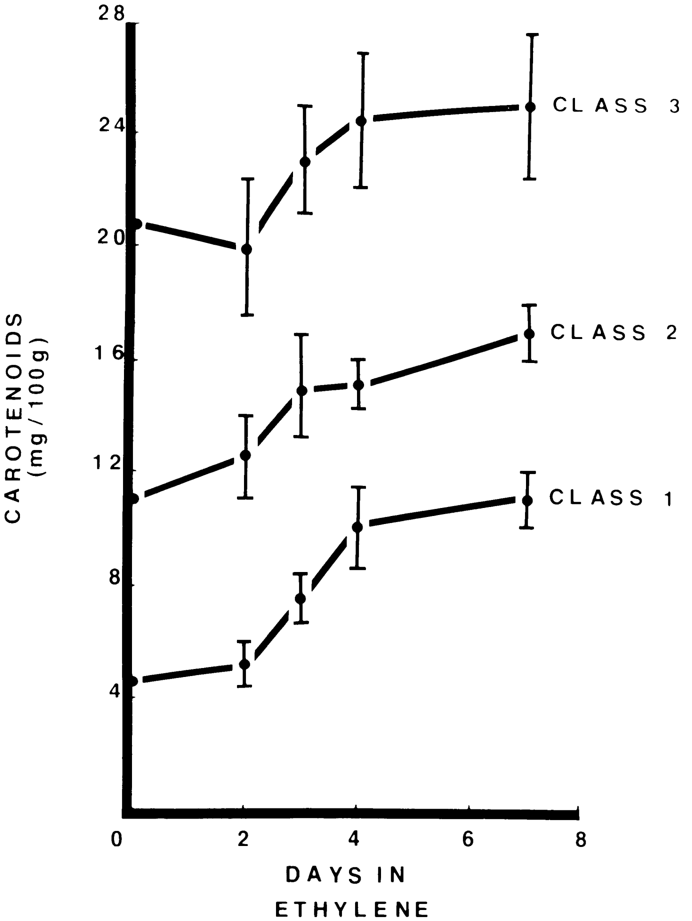


*Fig. 3.* Changes in rind carotenoid content (± standard deviation) of 3 color classes of 'Dancy' tangerine fruit (Class 3 - ΔOD .180; Class 2 - ΔOD .300; Class 1 - ΔOD .438) during postharvest exposure to 10 ppm ethylene.

300 ppm (Table 1).

Two 500-ppm sprays on 'Hamlins' increased the rind carotenoid content slightly (1.9 mg/100 g), while 2 500-ppm sprays greatly increased the rind carotenoid content of 'Robinson' 15.3 mg/100 g (Table 2). 'Hamlin' and 'Robinson' fruit were both degreened on the tree with 2 500-ppm sprays.

*Postharvest ethylene application.* Carotenoids increased slightly in 'Bearss' lemon rinds (ΔOD .500) during 9 days' exposure to 10 ppm ethylene, but did not in the absence of ethylene (Fig. 1). Total increase was 0.9 mg/100 g. Carotenoids increased in 'Robinson' (ΔOD .170) and 'Lee' (ΔOD .352) tangerine rinds during 5 days in ethylene, and the total increase was 5.7 and 3.2 mg/100 g, respectively (Fig. 2). 'Dancy' tangerine fruit, separated into 3 color classes after harvest (Class 1 - ΔOD .438; Class 2 - ΔOD .300; Class 3 - ΔOD .180), increased in carotenoids during 7 days in ethylene (Fig. 3). Total increases were 6.3, 6.0, and 4.2 mg/100 g for Classes 1, 2, and 3, respectively. All of the fruit exposed to postharvest ethylene for 5 to 9 days were degreened. Largest increases in carotenoids occurred in all cultivars tested after exposure to ethylene for at least 2 days.

## Discussion

These results are in agreement with the conclusions of Eilati[3] using 'Shamouti' oranges, and in contrast to those of Miller et al. (17, 18) using 'Valencia', 'Pineapple', and 'Parson Brown' oranges, 'Villa Franca' and 'Perrine' lemons, 'Duncan' grapefruit, and 'Persian' and 'Key' limes.

Extraction procedures in our studies and those used by Miller were quite similar, and not likely to the source of the discrepancies in the results, but sampling and spectrophotometric procedures did vary. Miller used a photometer with a filter which transmitted light at approx 458 mμ while we used a grating spectrophotometer at exactly 450 and 502 mμ. The cultivars studied by Miller all had moderate-to-low levels of carotenoids and to demonstrate small differences would require more precision than he had available.

In our tests, the largest changes in ethylene-induced carotenoids occurred in 'Robinson' and 'Dancy' tangerines which when fully colored have a natural high rind carotenoid content. Rinds of these fruit degreened naturally (Figs. 2 and 3, Table 1), increased in carotenoids following exposure to ethylene nearly as well as mature, green, or partially degreened fruit (Table 1, Fig. 3). Also, we were able to demonstrate small increases in ethylene-induced carotenoids in the less-colored 'Hamlin' orange and 'Bearss' lemon.

The largest percentage change in carotenoids occurred in fruit which exhibited natural color break (chlorophyll readings of .350 to .500), which confirms the findings of Eilati[3]. Total carotenoid levels were comparable to those reported for other lemon, orange, and tangerine cultivars (6, 8, 9, 17, 19, 20). Fruit which had higher rind carotenoid contents, as a result of ethylene or ethephon application, had better visible external color. It would appear that citrus cultivars respond to ethylene stimulation of carotenoid development in accordance with their inherent capacity to make carotenoids.

The absorption spectra of carotenoid extracts for ethylene-treated and ethephon-treated fruit were similar to untreated fruit, qualitatively. No attempt was made to separate individual pigments. In the case of ethephon-sprayed 'Hamlin' oranges, we did observe a deeper orange color to the rind, although there were only slight changes in total carotenoids. This suggested a possible shift in the levels of individual carotenoids as a result of ethylene treatment. A similar effect was noted with ethylene-treated 'Bearss' lemons, although the

*Table 2.* Effect of 1 and 2 preharvest ethephon applications on carotenoid content (mg/100 g) of 'Hamlin' orange and 'Robinson' tangerine fruit rinds.

| Ethephon concn (ppm) | Hamlin[a] Single spray | Hamlin[a] Double spray | Robinson[b] Single spray | Robinson[b] Double spray |
|---|---|---|---|---|
| 0 | 2.1 | 2.1 | 1.8 | 6.0 |
| 500 | 3.0 | 4.0 | 3.2 | 21.3 |
| L.S.D. 05 | N.S. | 1.0 | 1.0 | 2.1 |

[a]Single spray applied on 'Hamlin' 11/12/70, double sprays applied 10/30 and 11/12/70; all fruit harvested 11/18/70.

[b]Single spray applied on 'Robinson' 9/22/70 and harvested 10/8; double sprays applied 9/22 and 10/13/70, and all fruit harvested 10/19/70.

pigments involved were yellow. 'Lee', 'Nova', 'Robinson', and 'Dancy' tangerines all appeared to develop more orange and orange-red pigments, coincident with increases in total extractable carotenoids. Others (6, 7, 8, 9, 20, 21) have reported detailed studies on individual pigments of citrus fruit rinds, but not in relation to ethylene treatment.

The decreased carotenoids in 'Lee', 'Nova', and 'Robinson' tangerine fruit rinds sprayed with 500 ppm ethephon, as compared to 300 ppm, may represent an inhibition. Hield et al. (13) reported that ethephon inhibited carotenoid accumulation in navel oranges, and Fuchs and Cohen (11) reported that high ethephon concn inhibited chlorophyll destruction in 'Shamouti' oranges and 'Clementine' tangerines. Jahn et al. (14) reported that 20,000 ppm ethylene did not inhibit degreening of 'Hamlin' oranges; and our results indicate that 10 ppm ethylene was not inhibitory to carotenoid accumulation. This suggested that ethylene probably was not the inhibitory factor involved, but that 1 of the other breakdown products of ethephon or pH might have been.


## Summary

Ethylene applied as a gas to harvested fruit or as an ethephon spray to citrus fruit on the tree undergoing natural degreening and coloring increased the rind carotenoids. The acceleration of carotenoid development occurred within 2 to 9 days after treatment. 'Hamlin' oranges and 'Bearss' lemons did not respond to ethylene as well as 'Lee', 'Nova', 'Robinson', and 'Dancy' tangerines. The greatest potential for ethylene-color stimulation was in fruit undergoing natural coloring. Fruit which had higher rind carotenoid contents as a result of ethylene or ethephon application had better visible external color. Ethylene appeared to hasten the development of the carotenoids, rather than to change the inherent potential for color development.


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