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Effects of Maturity, Storage, and Ethylene on the Induction of Carotenoid Synthesis in Citrus Fruits by 2-(4-chlorophenylthio)-triethylamine (CPTA)[1, 2]

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Abstract. Early in the season, postharvest applications of 2-(4-chlorophenylthio)-triethylamine (CPTA) had little effect on carotenoid synthesis in 'Bearss' lemon (*Citrus limon* Burm. f.), 'Robinson' tangerine (*C. reticulata* Blanco X (*C. paradisi* Macf. X *C. reticulata*)), 'Marsh' grapefruit (*C. paradisi* Macf.), and 'Hamlin' orange [*C. sinensis* (L.) Osbeck]. The responses increased as the fruit matured, but greater CPTA responses were induced by storage of the fruit at 16°C before treatment or by exposing treated fruit to ethylene. Observations suggested that cultivars with low natural carotenoid levels (lemon and grapefruit) are more responsive to CPTA applications than are those with higher levels (tangerine).Improved color of 'Hamlin' orange was obtained with CPTA applications made before or after a 3-day degreening treatment. This response did not appear to be prevented by waxing. However, the practical use of CPTA to improve the color of oranges appears limited, although it may be useful in research on carotenoid synthesis.

Satisfactory color, appropriate for the type of fruit, is important for citrus fruits. Although fruit color changes through the season, the consumer expects a relatively constant fruit color in the market. This has led to the use of dyes on early season FL oranges to produce a more typical orange color. The effect of 2-(4-chlorophenylthio)-triethylamine (CPTA) on carotenoid synthesis has suggested an alternative method of improving fruit color. This chemical has been shown to induce lycopene synthesis in many plant tissues, including those of citrus fruits (1). Other carotenoids also may be increased, and in fruit treated before maturity, decreases in some normally occurring carotenoids have been noted (3, 4). The tests reported here provide information on the effects of several factors on the response of citrus fruits to CPTA.

Materials and Methods

The response of 'Bearss' lemon rind to CPTA was studied in tests initiated in August and September, 1972. Fruit of 'Hamlin' orange, 'Robinson' tangerine, and 'Marsh' grapefruit were treated at biweekly intervals from September 19 to December 11, 1972. These periods represent the season when fruit requires degreening and when color is often unsatisfactory.

Fruit were treated with 0 or 500 ppm CPTA (adjusted to pH 9 with NH_4OH and with 0.1% Triton X 100 wetting agent) at harvest or after varying periods at 16°C. Samples were then held at 21°C for color development. Comparable samples of orange, tangerine and grapefruit were also treated at harvest and held in 10 ppm ethylene at 29°C. Some lemons stored at 16°C were treated with a gibberellic acid (GA) dip (20 ppm) prior to treatment with CPTA.

The effects of CPTA concn, pH, and time of application on color development in 'Hamlin' oranges were studied in a single test begun December 1, 1972. Oranges were treated with CPTA at 0, 50, 200, and 500 ppm either before or after a 3-day degreening period (10 ppm ethylene at 29°C). CPTA was applied at normal (±7.3) pH or adjusted to pH 9. Limited comparisons of applications at pH 8 and of drying methods were also included. All samples were washed and waxed at the end of the degreening period and then held at 21°C.

Carotenoid changes of fruit in selected tests were measured with an experimental reflectance photometer using wavelengths of 527 and 730 nm. Observations on color development were recorded and in each test rind samples for carotenoid analysis (6) were taken 0, 3, and 7 days from treatment.

Results and Discussion

Instrument measurements of lemons treated after 6 weeks' storage (Fig. 1) show rapid changes in the reflectance patterns associated with

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[2] This is a report of research on chemicals that require registration under the Federal Insecticide, Fungicide and Rodenticide Act. Any use of these chemicals must be registered by the appropriate agencies before recommendations can be made.

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the development of red color. Untreated fruit showed no change under these conditions. Analysis of rind samples from lemons in this test showed rapid increases in carotenoid content (Fig. 1). Although the red color resulting from CPTA applications is due largely to lycopene formation, other complex alterations in the carotenoid composition also occur (1, 3, 4). These data show that in stored and degreened lemons carotenoid levels in the fruit rind change rapidly in response to CPTA application.

'Bearss' lemons treated with CPTA at harvest during August and September did not show any visible color response in the fruit rind. Lemons stored 1 week at 16°C developed some pink spots within a few days after treatment. The intensity and uniformity of this color development increased with each additional week's storage, and after about 4 weeks' storage, the fruit attained a max red color within 4 to 7 days of treatment. This increased response paralleled the degreening changes in the fruit. Lemons treated with GA and stored at 21°C showed less subsequent response to CPTA. No reduction in carotenoid synthesis by GA was observed at 16°C, possibly because the fruit changes were too far advanced before the GA was applied.

'Marsh' grapefruit, 'Robinson' tangerine, and 'Hamlin' orange also showed no response to CPTA when treated at harvest during September and October and held at 21°C. As the fruit matured and natural color development proceeded, some increases in response occurred, but color development was still incomplete in fruit harvested December 11. Comparable fruit held at 29°C with 10 ppm ethylene also showed little response to CPTA early in the season, although the fruit did fully degreen during the 7-day period. However, with increasing maturity, the response of fruit treated with both CPTA and ethylene increased more rapidly than that of fruit receiving only CPTA. The grapefruit, tangerine, and orange samples stored for 2 to 6 weeks at 16°C responded rapidly when treated and held at 21°C. The response in these stored samples increased with storage time, much as the response increased as fruit maturity increased.

Instrument measurements of carotenoid levels in the December 11 test (Fig. 2) showed no response to CPTA in 'Marsh' grapefruit held at 21°C for 4 days. Fruit treated with ethylene at 29°C showed an increased carotenoid level associated with degreening, and this was increased further by CPTA. Fruit which had been stored at 16°C for 6 weeks had a much higher carotenoid level than fruit obtained from the tree at the start of the test. The carotenoid level was further increased by CPTA applications. Fruit left on the tree for this period received approximately 300 hr of exposure at temp below 16°C and 400 hr between 16 and 21°C compared with the 1008 hr at 16°C for stored fruit. Comparable data on 'Robinson' tangerine (Fig. 2) showed little evidence of a response to CPTA and only slight changes in the ethylene-treated fruit. The stored tangerines had consistently higher carotenoid levels than fruit left on the tree. Observations from other

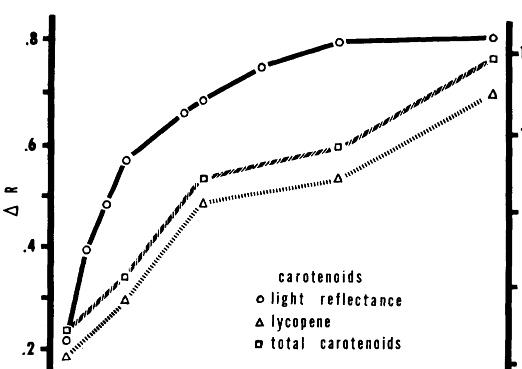

Fig. 1. Changes in carotenoid levels in 'Bearss' lemon treated with 500 ppm CPTA (pH 9) at 21°C, as shown by light reflectance measurements (ΔR) and lycopene and total carotenoid content of the flavedo. Fruit harvested on September 11, 1972 and stored at 16°C until treated on October 24. Data from single 10-fruit measurement samples and 5-fruit composited analysis samples.

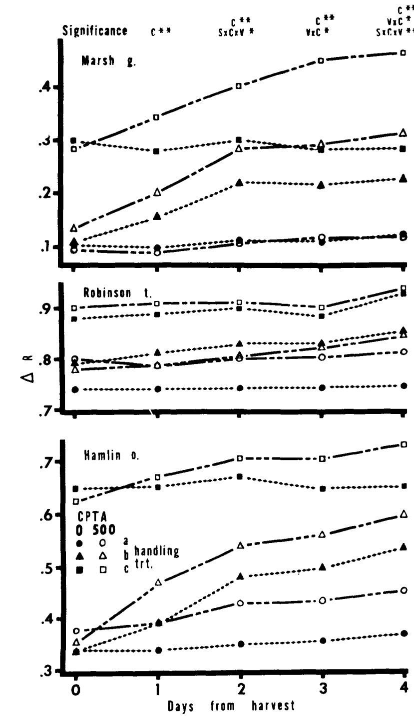

Fig. 2. Changes in carotenoid levels (ΔR) in 'Marsh' grapefruit 'Robinson' tangerine, and 'Hamlin' orange treated with 0 and 500 ppm CPTA (pH 9) at harvest on December 11, 1972 and held in air at 21°C (a) or in 10 ppm ethylene at 29°C (b), and changes in fruit stored 6 weeks at 16°C prior to treatment on December 11 (c). Data from single 10-fruit samples. Statistical significance is shown for: C-CPTA concn, S-storage, and V-cultivar and certain interactions. In addition, effects of storage, cultivar, and the storage X cultivar interaction were highly significant on all test dates.

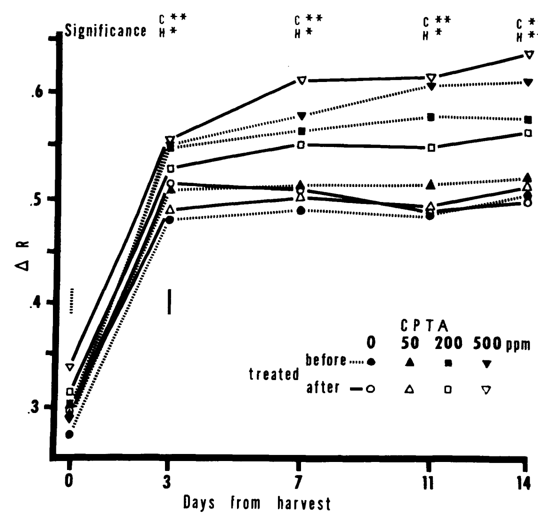

Fig. 3. Changes in carotenoid levels (ΔR) in 'Hamlin' orange treated with CPTA (pH 9) before or after a 3-day degreening period. Fruit was harvested on December 1, 1972 and all fruit was washed and waxed after removal from ethylene. Data from single 20-fruit samples. Statistical significance is shown for: C-CPTA concn, and H-pH of application. Data for pH 7.3 not shown.

tests in the series also suggested that 'Robinson' tangerine was less responsive to CPTA than were the other types of citrus studied.

Changes in carotenoid levels in 'Hamlin' orange (Fig. 2) showed responses similar to those in 'Marsh' grapefruit. Both the initial carotenoid levels and the responses to CPTA in oranges were intermediate between those for tangerines and grapefruit. The response to ethylene in the orange, as in the grapefruit, was associated with degreening and carotenoid changes in the maturing fruit. With greater maturity and higher natural carotenoid levels, these fruits, like the tangerines, showed less response to ethylene.

Applications of 200 and 500 ppm CPTA at pH 9 to 'Hamlin' orange induced significant increases in carotenoid levels (Fig. 3), but there was no response to 50 ppm. No consistent difference was shown between applications made before or after the 3-day degreening period. Since all samples were washed and waxed on December 4, color development after that date showed that changes were not inhibited by the wax coating. Application of CPTA at pH 7.3 (data not shown) gave significantly lower carotenoid levels and less uniform color. Applications at pH 8 were intermediate.

Observed responses to CPTA support reports of altered carotenoid synthesis patterns in other citrus cultivars (1, 3, 4). However, the effects of GA, ethylene and storage suggest that induced carotenoid changes are limited by the same factors affecting natural synthesis patterns. Although GA has been reported to have no effect on the CPTA response (1), our tests suggest that endogenous growth regulators, including GA, may have a controlling influence on the response, particularly at the start of the season. These responses indicate that CPTA may provide a useful tool to study carotenoid synthesis control patterns.

Some increase in response to CPTA with ethylene is to be expected, since ethylene increases carotenoid synthesis (2, 6). A similar effect of 2-chloroethylphosphonic acid on the response to CPTA has been reported (1). In our tests, the ethylene- and non-ethylene-treated samples could not be run at the same temp. Since carotenoid synthesis is favored by lower temp (5), and since observed color changes were rapid at both 21 and 29°C, this temp difference would not seriously affect the results.

Emphasis in our tests was on the nature and magnitude of the response of citrus fruits to CPTA. The increased carotenoid levels shown have no practical value except in 'Hamlin' oranges. The other types of citrus either are preferred with a yellow color (grapefruit and lemon), or normally have an adequate carotenoid level (tangerine). Although improved color of oranges was obtained even in the presence of a wax coating, 2 problems are evident. First, the inability of CPTA to override the natural controlling factors in carotenoid synthesis, limits its effectiveness during the early part of the season when improved color is most desirable. Second, only moderate increases in pigment levels will improve color, and these are difficult to control. The distinctive red color induced by CPTA is not normal to citrus, and therefore, more complete responses are undesirable. Although its practical value seems to be limited, the use of CPTA for studies on the mechanism of control of carotenoid synthesis appears promising.

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Incidence of Blackline in *Juglans regia* L. Propagated on Various Rootstock Species[1]

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Abstract. In a 16-year field study, all graft combinations of Persian walnut, *Juglans regia* scions with other rootstock species were susceptible to blackline. 'Sinensis #5', a selection of *J. regia*, did not exhibit symptoms of blackline when used as an interstock. In no case was blackline observed in graft combinations between *J. regia*.

Blackline is a graft union disorder which occurs between scions of *Juglans regia* and the commonly used rootstocks *J. hindsii* (northern California black walnut) and 'Paradox' (*J. hindsii* x *J. regia*). The disorder usually occurs in mature trees which are more than 20 yr old. Blackline is expressed as a thin layer of dead cambial cells at the graft union. Beginning at one site in the union, blackline progresses around the trunk at the rate of about 3 inches per year (1). With time, it also moves downward into the rootstock. Sprouts from the rootstock commonly develop a few years after the start of blackline.

The first reported case of blackline in the United States was in Oregon in 1924 (2). A few years later, this same disorder was found in the Central Coast County of Contra Costa in California (3). Since then its occurrence has become more widespread, but it is not clear whether the incidence of blackline has actually increased or techniques for positive identification have been improved, or both.

Even though blackline has decimated orchards in some districts, it has been given little attention by researchers, partly because numerous affected orchards were destroyed and the land used for dwellings and industry. As a result, the economic effect of this disorder was greatly mitigated. In 1959, Serr and Forde (1) described the nature of blackline at the union, its incidence in commercial orchards, age of the trees affected, rate of its advance in the graft union, and some possible factors favorable to its occurrence, i.e., rootstock, cultivars, soil, and climate. Their report was summation of work in grower orchards where blackline was prominent. Within the parameters measured by Serr and Forde, it was evident that the effect of blackline was devastating, and the cause unknown.

We examined numerous trees of selected species and cultivars for blackline during a 16-year period in an attempt to find resistance to this disorder.

Materials and Methods

Previous studies indicated that the greatest incidence of blackline in California was in the Central Coast area. For that reason, the trees used in this study were planted at the San Jose field station in 1952, well within the area of greatest incidence. Further information indicated that trees which were planted close together matured earlier and had earlier symptoms of blackline. Therefore, the planting was designed in a 1.2 × 4.5M pattern. Materials used in the various graft combinations are indicated in each table.

Graft unions were inspected annually by making V-shaped cuts through the bark down to the cambium. Any blackline discovered was marked with a nail on each end of its development. Wherever

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