Regreening of Valencia Orange as Influenced by Potassium Gibberellate 1, 2, 3

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In Southern California the Valencia orange (Citrus sinensis L.) attains maximum orange rind color during winter and regreenes somewhat by harvest time the following summer. The degree of regreening has been correlated with temperature patterns and with nitrogen nutrition (2, 5). It has also been reported (3) that applications of potassium gibberellate (KGA) to Valencia trees were associated with enhanced regreening of the fruit. The objective of this investigation was to obtain additional information about the influence of KGA on regreening of the Valencia orange fruit.

Materials & Methods

Valencia orange fruit, located on large trees at Riverside, Cal., were selected for uniformity of size and color. Fruit were predominantly orange with almost no green in the rind. The experimental design was a paired comparison with fruit of a pair in close proximity on the same tree. Color and size variations between fruit of a pair were much less than among pairs. One member of the pair served as the untreated control. The second fruit and a few adjacent leaves were submerged for 30 seconds in aqueous solutions of 500 ppm acid equivalent potassium gibberellate containing 0.013 (v/v) X-77 wetting agent. The principal functioning agents of X-77 are alkylarylpolyoxyethylene glycols, free fatty acids, and isopropanol.

During the interval of 17 weeks after treatment, fruit were harvested, and absorbance of rind extracts was determined at 433 and 666 μm on a Beckman DU spectrophotometer. Four 21-mm diameter discs of peel were removed from evenly spaced locations in the equatorial region of the fruit. The tissue was ground in acetone in a Lourdes grinder with the grinding beaker submerged in ice water. Grinding was done for 1 minute at low speed (ca. 2,000 rpm) and for 7 minutes at high speed (ca. 10,000 rpm). The resulting mixture was filtered through Whatman No. 1 filter paper, the residue was washed several times with acetone, and the filtrate was brought to volume in a 100 ml flask. The procedure was similar to that used by Erickson (4). Absorption at the longer wavelength was due almost exclusively to chlorophylls, while absorption at the shorter wavelength was due to chlorophylls and carotenoids (6).

During the interval of 16 to 19 weeks after treatment, analyses were made for chlorophyll pigments in rind removed from apical, equatorial, and basal (stem end) regions of the fruit. Procedures used are similar to those reported by Smith and Benitez (7). The grinding procedure was similar to that described above. Four discs were ground in 80 ml acetone, and the resulting mixture was flushed from the grinding apparatus with 40 ml diethyl ether into a 250 ml erlenmeyer flask. The mixture was stored overnight at 0 C, then filtered with Whatman No. 1 paper. The filter paper and residue were washed with 50 ml ether. A capillary stream of the ether-acetone filtrate was washed through 100 ml of water contained in a 250 ml separatory funnel. The water-acetone layer thus formed was discarded and the ether layer was washed an additional two times to complete the removal of acetone. The ether solution was concentrated with an air stream to a final volume of 15 ml and stored overnight at 0 C. The ether solutions were used for absorbance determinations at 624, 644, and 662 μm. Concentrations of chlorophyll a, chlorophyll b, and protochlorophyll were calculated by simultaneous equations and specific absorption coefficients reported by Smith and Benitez (7).

Results & Discussion

Acetone extracts from treated fruit had lower absorbance values at 433 μm as early as 59 days after application, and absorbancy was lower than untreated fruit for the duration of the experiment (table 1).

<table>
<thead>
<tr>
<th>Days After treatment</th>
<th>Average absorbancy</th>
<th>433 μm</th>
<th>666 μm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>22</td>
<td>1.20</td>
<td>1.19NS</td>
<td>0.002</td>
</tr>
<tr>
<td>59</td>
<td>1.39</td>
<td>1.15***</td>
<td>0.010</td>
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<tr>
<td>85</td>
<td>1.61</td>
<td>1.15***</td>
<td>0.013</td>
</tr>
<tr>
<td>100</td>
<td>1.42</td>
<td>1.16**</td>
<td>0.017</td>
</tr>
<tr>
<td>120</td>
<td>1.49</td>
<td>0.91***</td>
<td>0.019</td>
</tr>
</tbody>
</table>

* ** *** NS Statistical significance at the 0.05, 0.01, and 0.001 levels of probability, respectively. Non-significant at 0.05 level.

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2 This investigation was supported in part by grants from Merck & Company, Inc., Rahway, N. J., and Eli Lilly & Co., Indianapolis, Ind.
3 Paper number 1369.
Treated fruit gave higher than control absorbance for all harvest dates at 666 μm, indicating that treatment increased chlorophyll concentrations in the rind. Since absorbance readings at 433 μm were lower, even though chlorophylls contributed to the readings, it was concluded that KGA caused a reduction in concentration of carotenoids.

Data in Table II support the conclusion that treated fruit possessed elevated concentrations of chlorophyll pigments. In the basal region, treatment increased protochlorophyll, chlorophyll a, and chlorophyll b concentrations. Increases in chlorophylls a and b were associated with KGA treatments in equatorial and apical regions. Chlorophyll a accumulation was enhanced relatively more than chlorophyll b, at least in equatorial and apical areas, as shown by ratios of the two pigments.

The fact that chlorophyll a was influenced relatively more than chlorophyll b supports the view that protochlorophyll is converted directly to chlorophyll a (6), with subsequent conversion of chlorophyll a to chlorophyll b (1). If it is assumed that the mechanism for chlorophyll synthesis is accelerated in a system almost free of chlorophylls, it is easy to visualize the production of protochlorophyll, the conversion to and accumulation of chlorophyll a with a slower rate of accumulation of chlorophyll b.

Among the controls and among the treated fruit, data from apical and basal regions were compared statistically. For controls, chlorophyll a, chlorophyll b, and the ratios of chlorophyll a to b were higher in the basal areas. The same was true of treated fruit, with the additional difference that basal areas contained higher levels of protochlorophyll than apical regions.

It is a common observation that the rind of regreened fruit is greenest at the basal region and progressively less so toward the apical. The reason for greater accumulation in the basal region is not known. Perhaps chloroplasts in the basal region do not become as disorganized as in other areas when chlorophyll is lost during maturation. One may then speculate that when conditions are favorable for the synthesis of chlorophyll, it appears predominantly in the basal region. This is the only region where protochlorophyll accumulated in response to KGA, suggesting either a higher degree of organization of plastids or a more favorable position with respect to environmental conditions or to translocation of metabolites.

**Summary**

Potassium gibberellate (KGA) modified the accumulation of chlorophyll and carotenoid pigments in the Valencia orange. This was shown by applying KGA to fruit which were predominantly orange with almost no green in the rind. Analyses of rind pigments were made 19 weeks after treatment. The rind of treated fruit contained lower concentrations of carotenoid pigments and higher levels of chlorophyll pigments than untreated fruit.

Treated fruit contained higher levels of chlorophyll a and chlorophyll b in apical, equatorial, and basal (stem end) regions of the rind than did untreated fruit. Treatment increased protochlorophyll in the basal region. Chlorophyll a accumulation in treated fruit was enhanced proportionally more than chlorophyll b. In treated and untreated fruit, chlorophyll a and b were higher in basal than in apical regions.

It appears that KGA-treated fruit exhibit accelerated rates of synthesis of protochlorophyll which lead to enhanced regreening through accumulation of chlorophyll a and chlorophyll b. Regreening was more pronounced in basal than in equatorial and apical areas, a pattern similar to that exhibited by untreated fruit. The mechanism of regreening, already present in the fruit, appears to be favored by gibberellic acid.

**Acknowledgments**

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Literature Cited


Ribonucleic Acid-Polyphosphate From Algae
I. Isolation & Physiology

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An RNA-polyphosphate complex has been isolated from Mycobacteria by Winder and Dennen (31) and from Aspergillus by Belosersky and Kulaev (3). Both groups of investigators postulated that chemical bonds existed between the RNA and the polyphosphate. P232-phosphate was rapidly incorporated into acid-insoluble RNA-polyphosphates in Aspergillus (22) and the specific activity of the polyphosphate was shown to be higher than the specific activity of the RNA. In the case of Azotobacter, Zaetseva, and Belozersky (32) showed a similar pattern of incorporation of P232 into the acid-insoluble polyphosphates and the only phosphate fraction labeled more rapidly was ATP. These properties indicate that a metabolic importance should be associated with the RNA-polyphosphate fraction from microorganisms.

No careful purification or detailed characterization of an RNA-polyphosphate has been reported. Also no convincing evidence for a metabolic role for these complexes has been demonstrated. However, all reports indicate that the RNA-polyphosphates are major components of the phosphorus and nucleic acid fractions from microorganisms. These reports also agree that there is a rapid turnover of the polyphosphate in these complexes. Consequently, this paper presents results of a more detailed study of the isolation and properties of RNA-polyphosphate with some speculations about its possible metabolic role.

Many reports have established that polyphosphates are normal constituents of algae (1, 9, 13, 18, 25, 27, 28, 29). In Acetabularia the polyphosphates have been found in spheres in the cytoplasm (29), while in Zygnemataceae the polyphosphates were found in the chloroplasts along with a high concentration of RNA (18). Polyphosphates have been reported in nuclear equivalents which contain RNA and DNA, in addition, and which have also been referred to as pseudovacuoles (13, 21). In all these previous reports fractions containing only polyphosphates and those containing both the polyphosphate and RNA have not been differentiated.

Materials & Methods

▶ Anabaena Culture. An inoculum of the blue-green alga, Anabaena variabilis Kütz, was obtained from the Algal Culture Laboratory, Botany Department, University of Indiana. This alga was mass cultured in 6-liter flasks with medium C, as described by Kratz and Myers (20). The cultures at 30℃