Growth Regulator Induced Movement of Photosynthetic Products Into Fruits of 'Black Corinth' Grapes

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Abstract. The effect of exogenous growth regulators on movement of assimilates into flowers and young fruits of 'Black Corinth' grapes was studied. Clusters were treated with growth regulator and after 0.5 hr to 5 days the leaves above the clusters were exposed to \( ^{14}\text{CO}_2 \). Control shoots received \( ^{14}\text{CO}_2 \) but no growth regulator. At harvest, counting and radioautographic techniques were used to ascertain amount and distribution of activity in clusters. Clusters were dipped in 4-CPA (4-chlorophenoxyacetic acid), \( \text{GA}_3 \) (gibberelllic acid), or BA (benzyladenine). All berries were heavier than controls within 3 days. Total counts in the fruits were increased by 4-CPA, and the distribution of radioactivity among the sugar, organic acid, and amino acid fractions was usually altered by all treatments. In a time series experiment, within 6 hr after treatment of fruits with \( \text{GA}_3 \) there was almost an 8-fold increase in total counts relative to the control. After 12 hr there was about a 9-fold and 6-fold increase in counts in tartaric and malic acids, respectively, and in \( \gamma \)-aminobutyric acid, pipecolic acid, and valine increases of 56, 150, and 330 %. Radioactivity in fructose was increased 70 % in gibberellin-treated clusters over the controls. After 96 hr there were only about 1000 cpm per g fr wt in controls, but there were about 31,000 cpm counts in treated clusters. Treatment of clusters with gibberelin attracted less assimilates into the fruits when shoots had also been sprayed with gibberelin. Dipping portions of clusters in gibberellin increased the movement of \( ^{14}\text{C} \) assimilates into the treated portions. Hormonal control of mobilization is discussed.

It is well known that cytokinins are strong mobilizing agents, and that a drop of cytokinin on a leaf can attract such substances as amino acids from adjacent, untreated tissue (10). Shindy and Weaver (15) recently have shown that auxin and gibberellin also induce mobilization and concomitant alteration of translocation patterns in shoots of grapevines. Little work has been done concerning the effect of exogenous growth regulating agents on movement of elaborated food materials into fruits. However, it is well known that gibberellins (18), the auxin 4-chlorophenoxyacetic acid (16) and to a lesser extent the cytokinins (19) increase rate of fruit enlargement and final size of fruit in 'Black Corinth' as well as other seedless varieties of grapes. This would suggest that these growth regulators enhance the mobilizing power of flowers and fruits, and stimulated initiation of the research presented in this paper.

Materials and Methods

The plants were growing in a vineyard at the University of California at Davis. Shoots of mature 'Black Corinth' plants (\( Vitis \) \( \text{vinifera} \) L.) with clusters at the late bloom or early fruit-set stage were used. One cluster per shoot was retained and all clusters were trimmed to about the same size. Clusters or portions of clusters were dipped momentarily in solutions of \( 6 \times 10^{-4} \) M gibberellic acid (\( \text{GA}_3 \)), \( 1.3 \times 10^{-4} \) M of the auxin 4-chlorophenoxyacetic acid (4-CPA), or \( 8.9 \times 10^{-3} \) M of the cytokinin benzyladenine (BA), using B-1956 at 0.05 % as a wetting agent. These concentrations of growth regulators were utilized because they had been previously shown to cause a large increase in size of 'Black Corinth' flowers and fruits (16, 18, 19). Two shoots were utilized per vine, and in all instances the same treatment was made to these 2 shoots on a given vine. Previous research revealed there is little or no movement of growth regulator out of a cluster, as they are importing photosynthetic and other compounds (17, 18). Treatments were made between 8:00 and 9:00 AM. After a time interval ranging from 0.5 hr to 5 days the leaves immediately above and on the same side of the stem as the cluster was treated with \( ^{14}\text{CO}_2 \) for 30 min. A plastic bag was placed around the leaf to be treated, and a measured volume of air (10-20 ml) containing \( ^{14}\text{CO}_2 \) was injected into the bag with a 100-ml hypodermic syringe.

The source of the \( ^{14}\text{CO}_2 \) was a plastic bag in which was a capped vial containing lactic acid and radioactive barium carbonate (4). Carbon-14-labeled barium carbonate with an activity of 0.120

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me/mg was used at a rate of 0.4 mg per leaf. The open part of the plastic bag was wrapped tightly around a rubber cork through which a glass tube, with a soft rubber cap on the outer end, was inserted (Leonard, private communication). Air was evacuated from the bag with a 100-ml hypodermic syringe. A given volume of air (usually 200 ml) then was injected into the bag with the syringe, after which the cap was removed from the vial to release the $^{14}$CO$_2$.

With this method a large number of leaves could be rapidly treated. After injection of the $^{14}$CO$_2$, the hole in the bag was covered with masking tape.

Clusters were harvested and weighed 6 hr after the beginning of exposure to $^{14}$CO$_2$. Then 25 berries from each cluster were weighed and the fresh and dry weights usually obtained. The remainder of the cluster was cut up and placed in hot 80% ethanol for 30 min (6). The procedures for separating the amino acid, organic acid, and sugar fractions, and for determination of radioactivity, have been previously described (7). Some shoots were mounted for radioautography according to the method of Yamaguchi and Crafts (21).

### Experimentation and Results

**Experiment 1.** Effects of various growth-regulators on mobilization. In the latter part of bloom on June 2, 1967, clusters were dipped in solutions of $6 \times 10^{-5}$ M GA$_3$, $1.3 \times 10^{-4}$ M 4-CPA, or $8.9 \times 10^{-3}$ M BA. One series of clusters was not treated with growth-regulator. Shoots were 3 to 4 feet long, and the clusters were about 6 inches long. There were 6 clusters per treatment, 4 for analyses and 2 for radioautography.

Leaves were treated with $^{14}$CO$_2$ 3 days after the clusters were dipped. The leaves were fully expanded and about 6 inches in width. Laterals 4 to 12 inches long grew from the leaf axils. Clusters were harvested 6 hr after exposure to $^{14}$CO$_2$.

Berries treated with growth-regulators were heavier than control berries (table I). Total counts were strikingly increased only by the 4-CPA treatment, which resulted in over twice as many counts as in the controls. The 4-CPA resulted in an increase in the sugar, organic acid, and amino acid fractions, but the distribution of the radioactivity among the fractions was usually altered from that of the control. Treatments with GA$_3$ or BA resulted in a marked increase in the sugar fraction. Gibberellin decreased activity in the organic acid fraction, while both GA$_3$ and BA markedly decreased activity in the amino acid fraction (table I).

**Experiment 2. Time Series.** The objective was to compare the movement of $^{14}$C-assimilates from leaves into undipped clusters with the movement into clusters dipped in $6 \times 10^{-5}$ M GA$_3$. Clusters were dipped at anthesis, when about 95% of the calypters had fallen. Then the $^{14}$CO$_2$ was applied to the leaf immediately above each cluster as rapidly as possible. Thirty min after dipping the clusters of 1 series were harvested. Other series were harvested 6, 12, 24, 48, or 96 hr after dipping. There were 4 clusters per treatment. Clusters which were undipped, but which had been treated with $^{14}$CO$_2$, were used as controls. Three clusters to be used for background counts received neither GA$_3$ nor $^{14}$CO$_2$.

Average dry weight per berry 0.5, 6, 12, 24, 48, and 96 hr after treatment with GA$_3$ was 0.73, 0.80, 0.86, 0.94, 1.46, and 1.71 mg, respectively. The

### Table I. Weight of 'Black Corinth' Berries and Relative Amount of $^{14}$C Incorporated Into the Sugar, Organic Acid, and Amino Acid Fractions of Berries as Influenced by Growth Regulators

<table>
<thead>
<tr>
<th>Growth regulator</th>
<th>Fresh wt per berry</th>
<th>Dry wt per berry</th>
<th>Total $^{14}$C in ethanol extract</th>
<th>Radioactivity of fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.99</td>
<td>0.90</td>
<td>1395</td>
<td>Sugar</td>
</tr>
<tr>
<td>GA$_3$, $6 \times 10^{-5}$ M</td>
<td>6.51</td>
<td>1.02</td>
<td>1120</td>
<td>63</td>
</tr>
<tr>
<td>4-CPA, $1.3 \times 10^{-4}$ M</td>
<td>8.11</td>
<td>1.26</td>
<td>3480</td>
<td>39</td>
</tr>
<tr>
<td>BA, $8.9 \times 10^{-3}$ M</td>
<td>9.23</td>
<td>1.24</td>
<td>1500</td>
<td>53</td>
</tr>
</tbody>
</table>

1 Values with different superscripts are significantly different at the 5% level.
values in the same order for untreated clusters were 0.73, 0.82, 0.79, 0.80, 1.04, and 1.27, respectively.

Within 6 hr after treatment there was an almost 8-fold increase in total counts in GA_3-treated berries relative to the control (fig 1). After 12 hr there was about 4 times as many counts. At 24 and 48 hr after treatment there were fewer counts in the treated clusters than in the control, but at the fruit sampling after 96 hr there were few counts in the controls while counts in treated clusters remained high.

In the control the highest counts were usually in the sugar fraction and the lowest in the amino acid fraction (fig 2a). In the gibberellin-treated clusters, there were sharp peaks 12 hr after treatment in sugar and organic acid fractions, although the amino acid was low throughout the course of the experiment as it was for the control (fig 2b).

Twelve hr after treatment 100 μl of each extract were spotted and chromatographed on paper to determine the activity in the various constituents. Radioactivity in fructose was increased in GA_3-treated clusters, but the counts in the sucrose and glucose were not significantly increased (fig 3). Gibberellin resulted in about a 9- and 6-fold increase in activity tartaric and malic acids, respectively, over that of the controls (fig 4). Fifteen amino acids were isolated for counting (fig 5). Except

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**Table II. Influence of GA_3 on Weight Increase of ‘Black Corinth’ Berries and on Relative Amount of ^14C Incorporated Into the Sugar, Organic Acid, and Amino Acid Fractions After Treatment of Clusters or Clusters and Shoots With Growth-Regulator and ^14CO_2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry wt per berry (mg)</th>
<th>Total ^14C in ethanol extract (cpm/g fr wt)</th>
<th>Radioactivity of fractions (% of total radioactivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, no GA_3</td>
<td>2.20</td>
<td>20,110</td>
<td>Sugar 74 / Organic acid 16 / Amino acid 10</td>
</tr>
<tr>
<td>Clusters only treated with GA_3</td>
<td>3.80</td>
<td>51,770</td>
<td>Sugar 72 / Organic acid 20 / Amino acid 8</td>
</tr>
<tr>
<td>Clusters and shoots treated with GA_3</td>
<td>3.10</td>
<td>32,310</td>
<td>Sugar 72 / Organic acid 20 / Amino acid 8</td>
</tr>
</tbody>
</table>

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**Fig. 2a.** (Upper) Radioactivity in sugar, organic acid, and amino acid fractions in untreated grape berries at various time intervals after beginning of exposure of a leaf above cluster to ^14CO_2 for 30 min.

**Fig. 2b.** (Lower) Radioactivity in sugar, organic acid, and amino acid fractions in grape berries at various time intervals after gibberellin treatment. Leaf above cluster was exposed to ^14CO_2 for 30 min immediately after growth regulator treatment of cluster.

**Fig. 3.** Radioactivity in various grape sugars 12 hr after flowers and berries were treated with gibberelin. Leaf above cluster was exposed to ^14CO_2 for 30 min immediately after growth regulator treatment of cluster. Note that gibberellin increased amount of ^14CO in fructose.
for cysteine, cystine, glutamic acid, glutamine, and hydroxyproline, there was more activity in amino acids from GA$_3$-treated berries. The increases were greatest in $\gamma$-aminobutyric acid, piperolic acid, and valine.

Experiment 3. Effects of dipping clusters in GA$_3$ versus dipping clusters in GA$_3$ plus spraying the whole shoot. The objective was to see whether spraying the shoot and foliage altered the amount of photosynthetic that entered the cluster.

About 3 days past anthesis, shoots 16 to 36 inches long were thinned to 1 cluster each on June 9, and the retained clusters on 5 shoots were dipped in $6 \times 10^{-5} \text{m} \text{GA}_3$. On 5 other shoots the clusters were dipped in $6 \times 10^{-5} \text{m} \text{GA}_3$ and then the whole shoot including foliage was sprayed with the solution. Five shoots that were not treated served as controls.

![Graph showing radioactivity in various amino acids after dipping and spraying clusters.]

In each group 3 clusters were harvested to obtain counts, and 2 were used for radioautographs. Radioactive $^{14}\text{CO}_2$ was applied to the leaf above the cluster for 30 min on June 14, and after 6 hr the clusters were harvested.

At harvest there was less increase in berry size in dipped clusters from sprayed shoots, but the control berries were the smallest (Table III). Gibberellin had resulted in elongation of pedicels and berries.

Dipping of the cluster in gibberellin, with no spraying resulted in the highest number of counts (Table III). Counts in clusters that were dipped and the shoots sprayed with GA$_3$ were increased, but to a lesser degree. The largest increases were in the organic acid and sugar fractions, but the percentages of radioactivity in the fractions were not markedly altered by application of hormone.

Experiment 4. Movement of photosynthetic products into the berries after apical third of cluster had been dipped in GA$_3$. It has been demonstrated in cluster treatments that only the berries treated with a growth-regulator enlarge (18). The purpose of this experiment was to correlate the increase in

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Table III. Influence of GA$_3$ on Weight of 'Black Corinth' Berries and on Relative Amount of $^{14}\text{C}$ Incorporated Into the Sugar, Organic Acid, and Amino Acid Fractions After Treatment of Apical Third of Cluster With Growth-Regulator and $^{14}\text{CO}_2$

<table>
<thead>
<tr>
<th>Cluster part and treatment</th>
<th>Dry wt per berry</th>
<th>Total $^{14}\text{C}$ in ethanol extract</th>
<th>Radioactivity of fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CPU/g fract</td>
<td>g of total radioactivity</td>
</tr>
<tr>
<td>Apical, no GA</td>
<td>2.27</td>
<td>25,316</td>
<td>68</td>
</tr>
<tr>
<td>Basal, no GA</td>
<td>2.23</td>
<td>24,863</td>
<td>68</td>
</tr>
<tr>
<td>Apical, GA$_3$</td>
<td>2.70</td>
<td>163,470</td>
<td>57</td>
</tr>
<tr>
<td>Basal, GA$_3$ on apical</td>
<td>1.50</td>
<td>128,175</td>
<td>72</td>
</tr>
</tbody>
</table>

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Fig. 4. Radioactivity in various organic acids 12 hr after flowers and berries were treated with gibberellin. Leaf above cluster was exposed to $^{14}\text{CO}_2$, for 30 min immediately after growth regulator treatment of cluster. Note that gibberellin resulted in marked increases of $^{14}\text{C}$ in tartaric, glyceric, citric, malic, and ascorbic acids.

Fig. 5. Radioactivity in various amino acids 12 hr after flowers and berries were treated with gibberellin. Leaf above cluster was exposed to $^{14}\text{CO}_2$, for 30 min immediately after growth regulator treatment of cluster. Note that gibberellin markedly increased the amount of $^{14}\text{C}$ in certain amino acids (arginine, aspartic acid, lysine, piperolic acid, proline, valine, serine, and $\gamma$-aminobutyric acid), and decreased it in others (cysteine, glutamine).

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size of treated berries with mobilization of elaborated food material.

On June 9, 1967, shoots 18 to 36 inches long, 3 days past anthesis, were thinned to 1 cluster per shoot. The apical one-third of the cluster on each of 5 shoots was dipped in $6 \times 10^{-5} \text{ m GA}_3$. Five undipped clusters were exposed to $^{14}\text{CO}_2$, and used as controls. Three of the clusters in each group were used for counting, and 2 were radioautographed.

Five days after the clusters were dipped, the leaf immediately above each cluster was treated with $^{14}\text{CO}_2$. At harvest 6 hr after exposure of leaf to $^{14}\text{CO}_2$, the berries on the apical one-third and those on the basal one-third of each cluster were weighed as separate groups. Dry weight of the dipped berries was greater than that of the undipped (table III).

There was a very significant increase in counts in the dipped portion of the cluster as compared to counts in the undipped part (table III). However, the undipped basal portion of the cluster also had a great increase in counts relative to the control. In the apical portion the greatest increase was in the amino acid fraction.

In all instances where radioautographs were made the results were in agreement with those obtained by counting.

**Discussion**

Hormonal control of translocation of photosynthetically assimilated $^{14}\text{C}$ has been demonstrated in soybean (5) and in grape shoots (15). Similar results have been obtained with detached corn (Zea mays L.) leaves (11, 12) and detached Victory oat (Avena sativa) leaves (3), by the use of radioactive glycine and $^{32}\text{P}$, respectively. Little such work has been done with flowers or fruits. However, Kriedemann (9) recently has shown that the application of kinetin to a young fruit of orange cv. Washington Navel enhances its ability to import photosynthetic assimilates.

Application of growth regulator to flowers and fruits of 'Black Corinth' grapes usually resulted in a rapid increase in fresh and dry weight of berries. This result is in agreement with that obtained by Sachs and Weaver (13), who demonstrated that gibberellic and 4-chlorophenoxyacetic acid increased both fresh and dry weights of 'Black Corinth' berries within 24 hr after treatment.

In our study, application of GA$_3$ to fruits usually rapidly increased the rate and amount of movement of assimilates into the grape berries. Our data support the suggestion that the role of hormones in fruit development may be to mobilize elaborated food materials (2). However this suggestion does not explain why various growth regulators produce grape berries of different shapes (13). While our data indicate that movement of assimilates into the fruit is strongly enhanced by growth regulators, it also indicates that different growth regulators have different quantitative attraction for various sugars, amino acids, and organic acids. Could this explain, in part at least, differences in berry shape resulting from different growth regulators?

In experiment 1 GA$_3$ caused no increase in total counts in the clusters, while in the other experiments marked increases occurred. The explanation for this probably lies in the fact that clusters were at a younger developmental stage in experiment 1 than in the others. It has been demonstrated that grape clusters become much stronger sinks when they develop from the flowering into the fruit-set stage (4).

The highest activity for the organic acid and sugar fractions for berries treated with GA$_3$ was 12 hr after treatment, but for controls it was 24 hr. Thereafter activity decreased, especially in controls, where it was very low after 96 hr. No doubt much $^{14}\text{C}$ was lost in respiration. Some of the decrease undoubtedly was due to synthesis of ethanol-insoluble materials, such as proteins and hemicellulose, from the ethanol-soluble acid and sugar fractions. The increased amount of $^{14}\text{C}$ retained in GA$_3$-treated berries relative to the control may have been due to incorporation of $^{14}\text{C}$ into compounds that were not as easily respired or into compounds, such as tartaric acid, that were not used as much for synthesis of higher molecular weight insoluble materials.

Amino acids remained at a low level in both untreated and treated fruits, and there were low peaks in both 24 hr after the start of the experiment. Kliewer (8) found that amino acids are translocated in large amounts mainly as fruits approach ripeness, and that movement is low at earlier stages. Kliewer and Nassar (unpublished data) investigated the relative amount of activity in various free amino acids that were translocated to fruits from leaves treated with $^{14}\text{CO}_2$. They found that $\alpha$-alanine, serine, and $\gamma$-aminobutyric acid were the most prominently labeled amino acids in immature fruit clusters, an indication that these compounds may be the principal amino acids translocated to immature berries. The data in figure 5 indicate that these amino acids plus pipecolic acid and valine from GA$_3$-treated fruits contained significantly greater amounts of label than did the control fruits.

The sum of the counts of the individual organic acids exceeded the total count of the organic acid fraction. This can be explained by the fact that there is less quenching with the individual acid samples separated by chromatography than with the whole fraction containing all the acids.

The mechanism of mobilization is not known. Zaerr and Mitchell (22) have obtained data with hypocotyl segments of bean (Phaseolus vulgaris 'Pinto') to support their hypothesis that polar movement of indoleacetic acid and other endogenous and exogenous substances is controlled by mobilization and utilization of plant constituents at the growth centers. Rapid changes occur when 'Black Corinth' flowers or fruits are treated with growth regulator. Soleimani, Kliewer, and Weaver (unpublished data)
showed that there were detectable increases in the concentrations of RNA and DNA within 1 hr after treatment of grape flowers or berries with GA₃ or 4-CPA. The time curves for changes in the concentrations were about the same as we found for ¹⁴C entering the berry after treatment with GA₃. Large increases in the levels of RNA and DNA generally are followed by increases in cell number. The process of cell multiplication may account for the increases of ¹⁴C entering the GA₃-treated fruits due to greater demand for metabolites.

Went (20) suggested that the role of auxin in the correlative inhibition of lateral buds may be to stimulate the translocation of nutrients to meristematic regions. Strong mobilizing regions in grapes are shoot tips and young, rapidly growing fruits. Weaker ones are cambium, lateral buds, and tendrils (4). These regions are also generally high in growth hormones. This lends support to the idea that hormones are intimately involved in mobilization phenomena. Booth et al. (1) decapitated plants of Solanum andigena in the first fully extended internode and treated the cut surface with lanolin or with lanolin containing indole-3-acetic acid. When radioactive ¹⁴C-sucrose was applied to the third or fourth nature leaves, there was a much greater accumulation of radioactivity in the stumps of the uppermost internodes of decapitated plants treated with indole-3-acetic acid than in those supplied only with lanolin. Subsequently Seth and Wareing (14), using the same system but with decapitated peas and beans, showed that kinetin and gibberellic acid alone have little effect on phloem transport, but when applied in conjunction with indoleacetic acid, they markedly enhance the effect of the latter.

Dipping 'Black Corinth' clusters in gibberellin attracted more assimilates to berries on shoots unsprayed than to berries on shoots sprayed with gibberellin. This can be explained by the fact that gibberellin enhances the mobilizing power of shoots, so that much ¹⁴C from the treated leaf was diverted away from the cluster (15).

In the experiment with 3 growth-regulators, only the 4-CPA markedly increased radioactivity in the ethanol fruit extracts 3 days after treatment. Soleiman, Kliewer, and Weaver (unpublished data) showed that the DNA, RNA, and protein concentrations of 'Black Corinth' fruits increased considerably during the first 24 hr after treatment with GA₃ or 4-CPA, but that there was little difference after 48 hr. However, the 4-CPA, GA₃, and benzyladenine all altered the percentage of ¹⁴C in the different fractions. Perhaps each growth-regulator affects differently the phloem translocation of various sugars, organic acids, and amino acids.

**Literature Cited**