Vitis **10**, 292—297 (1972)

Department of Viticulture and Enology, University of California, Davis, USA

Effect of exogenous gibberellin on endogenous hormone content and development of 'Black Corinth' grapes

by

D. E. Bertrand and R. J. Weaver

Introduction

Auxin activity has been demonstrated in grape berries (2, 7, 9, 11), as well as in several other fruits (1, 3, 6, 8). However, there are few reports concerning changes in auxin concentration during berry development. Nitsch *et al.* (11) reported that auxin concentration in 'Concord' berries increased with the first burst of fruit growth, then decreased during the period of suspended growth, with no rise when the fruits entered their second period of accelerated growth. Coombe (2) reported a peak in the auxin level of 'Black Corinth' berries 11 days after anthesis, corresponding with the first period of rapid growth. The auxin concentration then decreased and remained at a low level until maturity. Ito *et al.* (7) found the concentration of auxin roughly parallelled the growth rate of 'Delaware' grapes from anthesis to 60 days after bloom, when fruit growth was renewed. There was no increase in auxin with this second burst of growth.

The purpose of the present study was to correlate changes in concentration of auxin with berry growth of both KGA_3-treated and untreated 'Black Corinth' grapes.

Materials and Methods

Mature, vigorous, and fruitful vines of 'Black Corinth' growing in an irrigated vineyard at the University of California, Davis, were used. The vines were usually pruned to four canes, each bearing 8 to 12 buds.

Potassium gibberellate (KGA_3) containing 80% active ingredient, with Triton B-1956 as a wetting agent, was applied with a 3-gallon hand sprayer to both clusters and foliage until run-off. The concentration used (25 ppm) is expressed on an acid-equivalent basis.

On May 21, 3 days after full bloom, 40 vines were sprayed with KGA_3. Full bloom was considered to be that stage at which 95% of the calyptras had fallen (capfall). A group of unsprayed vines served as control.

At sampling time, berries from each treatment were removed from the clusters and thoroughly mixed. Average berry size was determined either by weighing subsamples of 25 berries or by measuring berry width and length with calipers. Samples were collected for measurement of berry growth at 5-day intervals from the date of treatment until the fruits were ripe on July 31 (Fig. 1). Volume of the subsamples was measured by water displacement, and dry weight was determined after the berries were dried at 70° C. The remaining berries were crushed and per cent soluble solids in the juice was measured with a hand refractometer. Total acidity was determined by diluting 10 ml of the juice to 50 ml with distilled water, and titrating with 0.133 N NaOH, using phenolphthalein as an indicator.

Auxin activity

Twenty-one samples were collected on 15 sampling dates at intervals from 1 hour after treatment with KGA_3 until July 31 (Fig. 5). The samples were frozen shortly after collection and stored at —12° C until extractions were made. Samples containing 20 g frozen berries were ground and then filtered three times with 100 ml of methanol at 0° C. The combined extracts were evaporated to dryness in a Rinco evaporator and then dissolved in 20 ml of methanol.

A 0.1-ml sample of the dissolved extracts was streaked on Whatman No. 3 MM paper, and developed by ascending chromatography to 20 cm in isopropanol, ammonium hydroxide (38%), and water (80 : 0.1 : 19.9 v/v/v). The chromatograms were air dried, and developed to 24 cm in hexane, chloroform, and water (75 : 15 : 10 v/v/v). They were then air dried and cut into 12 strips each 2 cm wide.

The *Avena* first internode bioassay developed by NITSCH and NITSCH (10), and modified as follows, was used to determine auxin activity. The oat seeds were grown in vermiculite for 60 hr at 26° C. Then groups of 5 first internodal sections were each placed in tubes containing 1.0 ml of sucrose buffer solution and a chromatogram strip. Each treatment was replicated twice. The tubes were rotated at ⅓ rpm in a test tube roller apparatus. Solutions of IAA, at concentrations ranging from zero to 0.3 μg per ml were chromatographed as controls. After 20 hr, the sections were measured to the nearest tenth millimeter using a binocular microscope. The results were converted to growth promotion per mg weight, using the fresh weight-to-dry weight ratios to make the calculation for the various samplings. These were then converted to μg equivalents IAA per g dry weight, using the dosage response curve of IAA. The first internode test of NITSCH is also sensitive to gibberellins. Therefore, the "auxin" value obtained included the activity of any endogenous gibberellins present. The exogenous KGA_3 is not considered to have significantly affected the "auxin" values, because the results for control and treated berries were almost the same 18 and 24 hours after treatment.

Results

Berry growth

Changes in fresh and dry weights, length and diameter, soluble solids and total acidity are indicated in Figures 1—4. Berries from vines treated with KGA_3

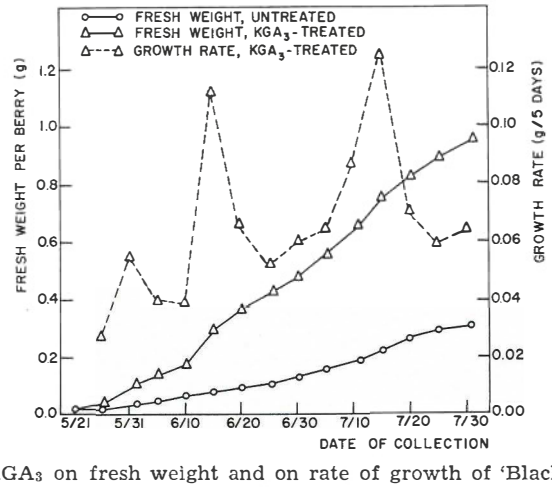

Fig. 1: Effect of KGA_3 on fresh weight and on rate of growth of 'Black Corinth' berries.

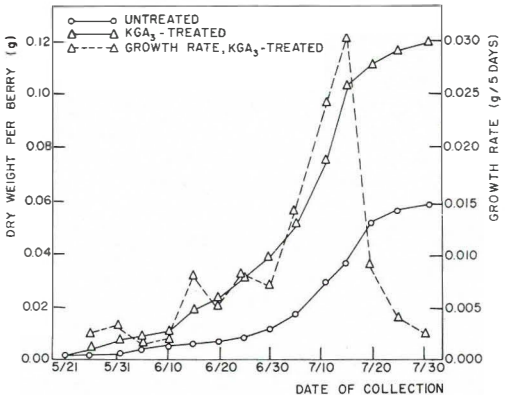

Fig. 2: Effect of KGA_3 on dry weight and rate of growth of 'Black Corinth' berries.

were larger than those from untreated vines within 10 days, and remained larger throughout the season. Although KGA_3 greatly stimulated growth, the general shape of the growth curves was not altered.

The growth curve of treated berries was slightly double sigmoid. The first period of rapid growth (stage I) lasted until June 15. Berry growth was then slow (stage II) until July 10, the start of the second period of rapid growth (stage III). Thereafter, growth was rapid until harvest. The growth rate curve for fresh weight had peaks corresponding to the end of stage I and to III (Fig. 1). There was also a small peak near the middle of stage I. The growth curves and rate of growth curves for volume (not shown) were almost identical to those for fresh weight. The growth rate curve for dry weight of KGA_3-treated berries showed a large peak in stage III, but only a small one in stage I (Fig. 2). The double sigmoid curve was evident in curves for length and diameter KGA_3-treated berries (Fig. 3).

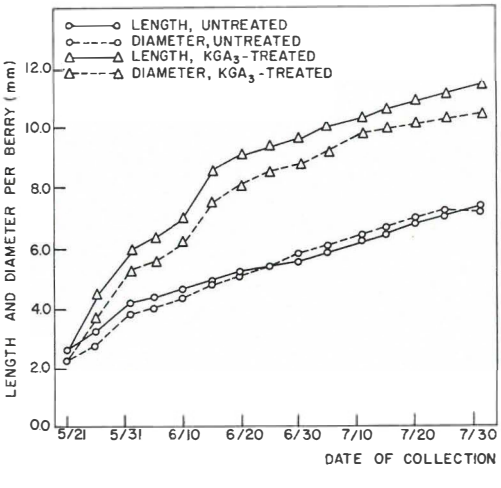

Fig. 3

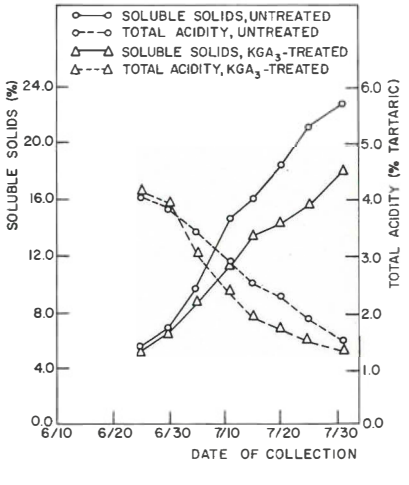

Fig. 4

Fig. 3: Effect of KGA_3 on berry length and diameter of 'Black Corinth' berries.
Fig. 4: Effect of KGA_3 on soluble solids and total acidity of 'Black Corinth' berries.

Total soluble solids increased rapidly near the end of stage II (Fig. 4), and were higher in control berries than in KGA_3-treated berries. Total acidity, however, was negatively correlated with total soluble solids, and decreased sharply near the end of stage II (Fig. 4). Acidity declined more rapidly in KGA_3-treated berries than in control berries, but at harvest there was little difference between values for treated and the control.

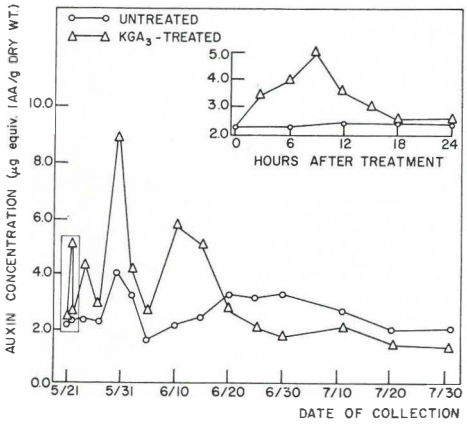

Fig. 5: Changes in auxin concentration in KGA_3-treated and untreated berries during development.

Auxin content during berry development

Application of KGA_3 increased auxin activity within 3 hours after treatment (Fig. 5). In both treated and untreated berries, auxin activity reached a peak about 12 days after anthesis. Berries from KGA_3-treated vines maintained a higher level of auxin activity during stage I and stage II than did those from untreated vines. There was no increase in auxin activity in either treated or untreated berries during the second period of rapid growth (stage III).

Discussion

The growth curves of 'Black Corinth' berries in this study were double sigmoid, as had been noted in previous studies by Coombe (1960) and Weaver and McCune (1959). These workers obtained a rather smooth curve when either berry diameter or berry volume were measured. The growth of seeded berries is similar, except that the period of arrested growth is longer and the suspension of growth is more pronounced (20).

The auxin concentration of KGA_3-treated berries remained high during stage I, but fell rapidly to a low level during stage II. However, the auxin concentration of untreated berries declined prior to the start of stage II, indicating that a decreased auxin level probably does not initiate the arrested growth of stage II, as might be indicated by results obtained with the KGA_3-treated berries. The results of this study confirm earlier reports (2, 7, 11) that there is no direct relation between berry growth and the concentration of auxin in the fruits.

In the KGA_3-treated berries, auxin activity rose rapidly within 24 hr in response to application of KGA_3 3 days after full bloom. Ito *et al.* (7) reported KGA_3 in-

creased both auxin and (gibberellin) GA activity in 'Delaware' grapes shortly after application. The increase in auxin activity may have been due to the effect of GA on the conversion of auxin precursors (15, 16). Although applications of GA increase auxin production, Galston and Davies (5) suggest this is undoubtedly only one of the consequences of GA application.

Gibberellin regulation of plant growth may involve nucleic acid metabolism. Soleimani, Kliewer, and Weaver (14) recently reported that protein, RNA, and DNA were markedly increased in 'Black Corinth' grapes during the first 24 hr after application of KGA_3. Sachs and Weaver (12) noted an increase in the number of cells in pericarp tissue of 'Black Corinth' berries within 48 hr after treatment with KGA_3. This increase in cell number would result in an increase in the amount of DNA, since the amount of DNA in any cell of a given species is the same (14), and subsequently in the amounts of RNA and protein. Gibberellin-induced cell elongation may also be occurring in the berries, accompanied by synthesis of RNA and protein (5, 17). It has been suggested by Soleimani (13) that GA is involved in stimulating the fundamental synthesis processes which cause plant tissues to be more active in production of DNA, and hence of RNA and protein.

There is increasing evidence to indicate that the role of hormones in fruit set and development may be to mobilize elaborated food materials (3, 4). Our results support the hypothesis that mobilization of metabolites into 'Black Corinth' clusters, due to the presence of endogenous hormones, may account for the first flush of rapid growth (stage I). It has been demonstrated that KGA_3 and 4-CPA increased both fresh and dry weights of 'Black Corinth' berries within 24 hr after application (12). This mobilization of solutes and accompanying water uptake may be related to increases in endogenous growth regulators (7) or to increases in proteins and nucleic acids (14).

Application of KGA_3 to the fruits of 'Black Corinth' has usually increased the rate and amount of movement of assimilates into the grape berries, resulting in a rapid increase in both fresh and dry weights (19). Zaerr and Mitchell (21) have found that once a center of new cell growth is induced with an exogenous regulator, plant constituents required for this new growth move to the growth center. Hence, the greater size of KGA_3-treated berries is probably due to their greater mobilization capability.

Summary

The relationship between growth and development of potassium gibberellate (KGA_3)-treated 'Black Corinth' berries and changes in the level of hormones was studied from anthesis to maturity. Growth rate curves for fresh weight and volume had peaks corresponding to stages I and III. The curve for dry weight showed only one large peak that occurred in stage III. Associated with the first period of rapid growth (stage I) was a rapid increase in concentration of hormone. Application of KGA_3 increased auxin activity within 3 hours after treatment, and berries from KGA_3-treated vines maintained a higher level of auxin activity during stage I and stage II than did berries from untreated vines. Hormone content decreased during the period of retarded growth (stage II), and continued at a low level until harvest.

Acknowledgement

The authors thank Merck and Co., Inc., Rahway, New Jersey for financial support of this investigation.

Literature Cited

1. Collins, W. B., Irving, K. H. and Barker, W. G., 1966: Growth substances in the flower bud and developing fruit of *Vaccinium angustifolium* Act. Proc. Amer. Soc. Hort. Sci. **89**, 243—247.
2. Coombe, B. G., 1960: Relationship of growth and development to changes in sugars, auxins, and gibberellins in fruit of seeded and seedless varieties of *Vitis vinifera*. Plant Physiol. **35**, 241—250.
3. Crane, J. C., 1964: Growth substances in fruit setting and development. Ann. Rev. Plant Physiol. **15**, 303—326.
4. — — , 1969: The role of hormones in fruit set and development. HortScience **4**, 108—111.
5. Galston, A. W. and Davies, P. J., 1969: Hormonal regulation in higher plants. Science **163**, 1288—1297.
6. Gustafson, F. G., 1939: The cause of natural parthenocarpy. Amer. J. Bot. **26**, 135—138
7. Ito, H., Motomura, Y., Konno, Y. and Hatayama, T., 1969: Exogenous gibberellin as responsible for the seedless berry development of grapes. I. Physiological studies on the development of Seedless Delaware grapes. Tohoku J. Agricult. Res. **20**, 1—18.
8. Nitsch, J. P., 1950: Growth and morphology of the strawberry as related to auxin. Amer. J. Bot. **37**, 211—215.
9. — — and Nitsch, C. S., 1955: The separation of natural plant growth substances by paper chromatography. Beitr. Biol. Pflanzen **31**, 387—408.
10. — — and — — , 1956: Studies on the growth of coleoptile and first internode sections. A new, sensitive, straight growth test for auxins. Plant Physiol. **31**, 94—111.
11. — — , Pratt, C., Nitsch, C. and Shaulis, N. J., 1960: Natural growth substances in Concord and Concord Seedless grapes in relation to berry development. Amer. J. Bot. **47**, 566—576.
12. Sachs, R. M. and Weaver, R. J., 1968: Gibberellin and auxin-induced berry enlargement in *Vitis vinifera* L. J. Hort. Sci. **43**, 185—195.
13. Soleimani, A., 1968: Effect of gibberellin A_3 on concentration of DNA, RNA, and protein in the pea plant (*Pisum sativum* L.). HortScience **3**, 276.
14. — — , Kliewer, W. M. and Weaver, R. J., 1970: Influence of growth regulators on concentration of protein and nucleic acids in 'Black Corinth' grapes. J. Amer. Soc. Hort. Sci. **95**, 143—146.
15. Valdovinos, J. G., Ernest, L. C. and Henry, E. W., 1967: Effect of ethylene and gibberellin on auxin synthesis in plant tissues. Plant. Physiol. **42**, 1803—1806.
16. — — and Sastry, K. S. S., 1968: The effect of gibberellin on tryptophan conversion and elongation of *Avena* coleoptile. Physiol. Plant. **21**, 1280—1286.
17. Van Overbeek, J., 1966: Plant hormones and regulators. Science **152**, 721—731.
18. Weaver, R. J. and McCune, S. B., 1959: Effect of gibberellin on seedless *Vitis vinifera*. Hilgardia **29**, 247—275.
19. — — , Shindy, W. and Kliewer, W. M., 1969:Growth regulator induced movement of photosynthetic products into fruits of 'Black Corinth' grapes. Plant Physiol. **44**, 183—188.
20. Winkler, A. J. and Williams, W. O., 1936: Effect of seed development on the growth of grapes. Proc. Amer. Soc. Hort. Sci. **33**, 430—434.
21. Zaerr, J. B. and Mitchell, J. W., 1967: Polar transport related to mobilization of plant constituents. Plant Physiol **42**, 863—874.

Eingegangen am 16. 9. 1971

Prof. Dr. R. J. Weaver
Dept. of Viticulture and Enology
Univ. of California
Davis, Calif.
USA