Promotion of Berry Set in Grapes by Growth Retardants

III. Effects of the Prebloom Application of SADH and CCC on Gibberellin and Cytokinin Activity in Florets of Grape Varieties, Kyoho and Muscat of Alexandria

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Summary

The prebloom application of SADH (succinic acid-2, 2-dimethyl hydrazide) to clusters of Kyoho (V × L) by spraying at 2500 ppm was as effective for increasing the set of seeded berries as foliar treatment at the same concentration or prebloom shoot pinching. The spraying on cluster had no inhibitory effect on shoot growth during bloom and set stages. Nearly the same trend as above was observed in Muscat of Alexandria (V) to which CCC (2-chloroethyl trimethylammonium chloride) was applied at 200 ppm. These results reconfirmed those of our earlier study. In both cultivars, the application of the growth retardants to either clusters or leaves distinctly reduced the gibberellin activity in florets at the beginning of bloom, whereas the shoot pinching raised the activity in Muscat or did not affect it in Kyoho. On the other hand, the cytokinin activity in florets was markedly enhanced by the retardant applications and the shoot pinching in both cultivars.

These results indicate that reduction of shoot growth is not essential to exert the promotive effect of growth retardants on the set of seeded berries and that the increased level of cytokinin activity in florets induced by the chemicals is causally associated with the promoted berry set.

Introduction

Our previous researches (13,14) showed that the growth retardants, SADH (succinic acid-2, 2-dimethyl hydrazide) and CCC (2-chloroethyl trimethylammonium chloride) consistently promoted the set of seeded berries of grapes without any depressive effect on shoot elongation when applied directly to clusters before anthesis by means of dipping or spraying. These results, however, are contradictory to the finding of Coombe (3) that wherever CCC is applied to a shoot even directly to clusters, its promotive effect on berry set is due to correlated inhibition of shoot growth as in the case of shoot pinching.

Thus, it is important for the interpretation of the effect of growth retardants on berry set to make clear whether or not the cluster treatments with them inhibit the shoot growth, and one of the aim of the present research was to obtain conclusive evidence for this matter.

It has been shown clearly that fertilization is one of the momentous factors to fruit set, but pollen germination (14) and fertilization (1,12) are not affected by the prebloom application of CCC to leaves and/or clusters. It is likely, therefore, that the CCC treatment is effective on preventing the abscission of fertilized berries at the critical stage for setting. For elucidating the action of growth retardants in this respect, it seems meaningful to know their effects on the activities of endogenous growth regulators in florets, since they may more or less participate in controlling berry set and development (4) and it has been illustrated that growth retardants affect the levels of endogenous gibberellins (GAs) (5,7,8,19,26,27) and cytokinins (20).

Materials and Methods

Adult vines of Kyoho (Tetraploid, Vitis
vinifera L. × Vitis labrusca L.) and Muscat of Alexandria (Vitis vinifera L.), both of which usually set poorly especially in the case of vigorous vines, were used in this research as same as in the previous study (14). Four treatments were applied to Kyoho vines grown in an orchard of Shimane University (A orchard) through two years’ experiments in 1973 and 1974, that is, shoot spraying and cluster spraying with SADH at 2500 ppm about 3 weeks before full bloom, and shoot pinching leaving 6 leaves per shoot 2 weeks before full bloom and untreated controls. The same treatments except for shoot pinching were also applied in 1973 to Kyoho vines grown in another orchard of the university (B orchard) with the sole object of sampling clusters for the analysis of growth regulators. In the same year, 4 treatments, shoot spraying and cluster dipping with CCC at 200 ppm, shoot pinching and untreated controls were applied to Muscat of Alexandria vines grown in a glass house of the university with timings almost identical to those mentioned above.

In every experiment, Atlox BI was added to the solutions as a wetting agent as a concentration of 0.1%. Prior to treatments, clusters were thinned to 2 per shoot, one of which was removed later for the analysis of GA and cytokinin. In the shoot spray treatment, clusters were covered with polyethylene film bags during treatment to avoid the contamination of applied retardants. In each cultivar and year, treatments were replicated at least 4 times with a randomized block design.

For extraction of GA and cytokinin, clusters were collected just before anthesis in 1973, when the first blooming was detected, and were frozen at \(-20^\circ\text{C}\) until use. Clusters collected and stored in the previous experiments (14) were also used for the extraction. After clusters were lyophilized, florets were separated from rachises, and ground to a powder. Five gram portions of the ground tissue were extracted with 80, 90 and 100% methanol in the order. The combined extracts were evaporated under reduced pressure below 40°C to remove methanol. The remainder was adjusted to pH 6.5 and centrifuged to remove insoluble substances. The supernatant was adjusted to pH 2.5 and partitioned 3 times with ethylacetate.

Assay of GA activity: After dehydration with anhydrous sodium sulfate, the ethylacetate phase was evaporated to dryness, and the residue was purified by column chromatography using silica-gel as solid phase and mixtures of ethylacetate and normal hexane as liquid phase (18). The eluate was evaporated to dryness and dissolved in a small volume of ethanol. A portion of the ethanol solution equivalent to 100 or 250 mg dry matter was chromatographed using Toyo No. 51 filter paper strip (2×40 cm) and a mixture of isopropanol, ammonium hydroxyde and water (10 : 1 : 1v/v) at 25°C, and the activity was assessed with a barley endosperm assay (2).

Assay of cytokinin activity: The residual water phase of the ethylacetate partition was purified using a Dowex 50 (H\(^+\)) 200-mesh ion exchange column according to the method reported by Gazit et al. (6). The aqueous concentrate was loaded on the column, which was eluted with water, 70% methanol, water, 1.5 N ammonium hydroxyde and water, in the order. Ammonium hydroxyde and subsequent water fractions were combined, evaporated to dryness, and dissolved in a small volume of 80% ethanol. A portion of the ethanol solution was tested for cytokinin activity by a soybean cotyledon callus assay (12) after paper chromatography with a mixture of normal butanol, acetic acid and water (4 : 1 : 1 v/v).

**Results**

Shoot elongation of Kyoho and Muscat of Alexandria around blooming time in 1973 as affected by each treatment is depicted in Fig. 1. Though shoot spraying with SADH or CCC significantly depressed the shoot elongation of both grape varieties, the direct application of each chemical to clusters by spraying or dipping had no effect throughout the period observed. These results are completely in agreement with those obtained in the previous year’s experiment (14). The lateral bud of the top node on the pinched shoot began to grow several days after treatment, but the length still remained much shorter than that of controls even after full bloom. As shown in Fig. 2, each treatment exhibited the same
effect on shoot elongation in the next year's experiments for Kyoho with the sole exception that the growth rate of pinched shoots was much lower.

Eventhough Kyoho is a seeded cultivar, it is endowed with parthenocarpic ability to some extent, but the size of seedless berries is at most one third of seeded ones, the mean weight of which is usually more than 10 g under adequate growing conditions. Thus, increased set of seeded berries is favorable for getting high grade bunches for table use of large berries even if thinning is necessary. The increased number of seedless berries, however, is even troublesome for growers because most of them have to be hand-thinned at the immature stage for the same purpose. As shown in Table 1, the cluster treatment with SADH for Kyoho significantly increased the number of seeded berries just the same as shoot spraying despite their different effects on shoot growth as mentioned above. Though shoot pinching was as effective as both the SADH treatments for increasing the set of seeded berries, the former tended to increase and the latter, by contrast, to reduce the number of seedless berries so that a significant difference was detected between them. In Muscat, only the total number of set berries were counted due to its less parthenocarpic tendency. As in the case of Kyoho, each treatment was equally effective for promoting the set of berries, most of which were probably seeded.

As far as the mechanism of the promotive action of growth retardants are concerned, the effect of each treatment on the activities of GA and cytokinin in florets just before anthesis is very interesting. Fig. 3. depicts
the GA activity in Kyoho as affected by each treatment. A dominant peak of GA appeared at Rf 0.5 to 0.6 under the solvent system, the level of which was distinctly lowered by the SADH treatment either with shoot spraying or cluster spraying (dipping). Shoot pinching, however, did not affect the GA level. Almost the same trend was observed in experiments of Muscat with CCC (Fig. 4) with the exception that the shoot pinching raised the GA activity. In order to make the comparison between treatments easier, the approximate amount of GA in a unit dry weight of florets was calculated using the standard curves of GAs. As shown in Table 2, the application of growth retardants either to leaves or to clusters reduced the amount of GA levels ranging from 54 to 10% of untreated controls depending on year, variety and orchard. By contrast, shoot pinching had no effect on the level in Kyoho or stimulated it up to 4-fold in Muscat.
The responses of soy bean callus to chromatographed extracts of florets affected by each treatment are shown in Fig. 5. Chromatographic separation of the components was not good in some cases so that the results are inadequate to imply qualitative changes in cytokinin activity which may have been caused by some of the treatments. However, it can be said at least that each of the treatments tended to raise the total cytokinin activity. This is more distinctive in Table 3 manifesting that the application of retardants in both ways as well as the shoot pinching increased the estimated amount of cytokinins in florets to levels ranging from 1.1- to 4.1-fold greater than for untreated controls.

Table 2. Estimated amount of endogenous gibberellins in florets just before anthesis in Kyoho and Muscat of Alexandria grapes as affected by the application of growth retardants and the pinching.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Year</th>
<th>Shoot spraying</th>
<th>Cluster spraying</th>
<th>Cluster dipping</th>
<th>Shoot pinching</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kyoho</td>
<td>1972</td>
<td>0.04 (10)</td>
<td>0.05 (13)</td>
<td>0.06 (16)</td>
<td>0.46 (102)</td>
<td>0.38 (100)c</td>
</tr>
<tr>
<td></td>
<td>1973</td>
<td>0.14 (32)</td>
<td>0.16 (35)</td>
<td>0.11 (18)</td>
<td>0.45 (100)</td>
<td>0.61 (100)</td>
</tr>
<tr>
<td>Muscat of Alexandria</td>
<td>1972</td>
<td>0.05 (21)</td>
<td>0.04 (17)</td>
<td>0.03 (23)</td>
<td>0.31 (129)</td>
<td>0.24 (100)</td>
</tr>
<tr>
<td></td>
<td>1973</td>
<td>0.07 (54)</td>
<td>0.00 (18)</td>
<td>0.03 (23)</td>
<td>0.31 (129)</td>
<td>0.13 (100)</td>
</tr>
</tbody>
</table>

a) SADH 2500 ppm.  b) CCC 200 ppm.  c) Percent of controls.

Table 3. Estimated amount of endogenous cytokinins in florets just before anthesis in Kyoho and Muscat of Alexandria grapes as affected by the application of growth retardants and the pinching.

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<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kyoho</td>
<td>1973</td>
<td>3.98 (138)</td>
<td>3.16 (110)</td>
<td>4.44 (154)</td>
<td>2.88 (100)c</td>
<td>1.05 (100)</td>
</tr>
<tr>
<td></td>
<td>1973</td>
<td>1.97 (188)</td>
<td>4.27 (407)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscat of Alexandria</td>
<td>1973</td>
<td>1.26 (203)</td>
<td>1.66 (268)</td>
<td>1.49 (240)</td>
<td>0.62 (100)</td>
<td></td>
</tr>
</tbody>
</table>

a) SADH 2500 ppm.  b) CCC 200 ppm.  c) Percent of controls.
Discussion

The results obtained in this research confirm our previous data (13, 14) providing conclusive evidence that the direct application of growth retardants to grape clusters before anthesis promotes the set of seeded berries without any inhibitory effect on shoot growth. It must be presumed, therefore, that growth retardants applied to clusters act directly to the retention of berries at the critical stage for setting. Furthermore, even when the retardants were applied to shoots but not to clusters, it seems questionable whether the improved set of seeded berries is only due to the depressed shoot growth whereby organic and inorganic nutrients may be diverted to developing ovaries as in the case of shoot pinching (3, 16, 17, 21). The assumption was proposed in our previous report (14) based on the enhanced set of Muscat by shoot spraying with CCC when shoot pinching had no obvious effects on the set even though the pinching was more depressive for shoot elongation than the shoot spraying. This may be further supported by the fact shown in this research that the SADH shoot spraying as well as the cluster treatment had somewhat reverse effects to those for shoot pinching with regard to the set of seedless berries in Kyoho. In the foliar treatment, a part of the chemicals absorbed by leaf tissues may be transported to clusters (9, 11), particularly from leaves basal to the clusters (22), playing a similar role with regard to set that cluster treatment does.

It is widely recognized that growth retardants reduce the level of endogenous GAs in the plant tissues through the inhibition of their biosynthesis (5, 7, 8, 19, 26, 27). This was true in our experiments in which the GA activity in florets just before anthesis was lowered by the cluster treatments. It is interesting that the shoot spraying depressed the GA activity as well whereas the pinching did not affect it or raised it. Thus, no direct relation was found between the GA level in florets at the

Fig. 5. Effect of SADH and CCC applied to shoots or clusters, and pinching on cytokinin activity in florets just before anthesis in Kyoho and Muscat of Alexandria grapes in 1973. Solvent: n-butanol: acetic acid: water (4:1:1). Bioassay: soy bean callus test.

a) 200 mg dry matter equivalent. b) 1000 mg dry matter equivalent.
stage and the set of seeded berries. However, the reciprocal changes in the number of seedless berries induced by these treatments seem to be a function of the GA level which may influence strongly the set and development of seedless berries (4, 23). In addition, these results also indicate the possible transport of the retardants from treated leaves to non-treated clusters as suggested above.

On the other hand, the cytokinin activity in florets at the stage was greatly raised by either the application of growth retardants or the shoot pinching. Skene (20) has demonstrated that the cytokinin concentration in the bleeding sap of grape vines is markedly increased by the application of CCC to the culture medium, and suggested that the increased level of cytokinin may be implicated in the stimulation of set by CCC. The tissues to which retardants were applied in our experiments are different from the tissue in Skene's. Further, the biochemical pathway through which the growth retardants increase the cytokinin level in florets is unexplainable at this time. It is possible to presume, however, that the shift of cytokinin level promotes the transport, accumulation and retention of metabolites in the organ (10) and results in the improved berry set. In fact, there have been some reports showing that the application of cytokinins by vine spraying or cluster dipping prior to or at anthesis promotes the set of seeded and seedless grape cultivars (15, 24). It is suggestive that the application of cytokinins to seeded cultivars in those experiments, in contrast to the SADH treatments in our research, causes a marked increase in the number of seedless berries (shot berries) in addition to that of seeded berries.

In conclusion, our results indicate that an increased cytokinin level in florets caused by the prebloom application of growth retardants to leaves or clusters is causally related to the increased set of seeded berries, although the retardation of shoot growth may not be eliminated as an influencing factor for set in the case of shoot spraying.

**Literature Cited**


14. **--- ---, and T. HAYASHI.** 1974. Promotion of berry set by growth retardants. II. Effects of SADH and CCC applied directly to clusters on berry set and shoot growth in Kyoho and Muscat of Alexandria grapes. *J.*


生長抑制剤によるブドウの花ふるい防止に関する研究（第3報）

SADHおよびCCCの開花前処理が花らしい中のジベレリンならびにサイトカイン活性に及ぼす影響

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摘要

巨峰に対するSADH2500ppmの開花前の花房散布は、新しよう散布および摘心と同様に有効果の着果を有意に促進した。その場合、新しよう散布および摘心は、開花着果期の新しようの生育を有意に抑制したが、花房散布の影響はまったく認められなかった。CCC200ppmを用いて行なったマスカル・オブ・アレキサンドリアについての実験でも、ほぼ同様の結果が得られた。これらの結果は前報と完全に一致した。

両品種におけるそれぞれの抑制剤の処理により、開花直前の花らしい中のジベレリン活性は顕著に減少したが、一方、摘心の影響は巨峰では現われず、マスカットでは著しく増加した。このようにこの時期の花らしい中のジベレリン活性と有効果の着果との間に対応関係は認められなかった。これに対し、サイトカイン活性は着果を促進した抑制剤の両処理、摘心のいずれによっても明らかに増加した。

これらの結果より、生長抑制剤のブドウ有効果の着果促進作用は、従来示されている新しようの生育抑制をとおして新しようと花（果）房との養分競合の回避という考え方ののみで説明出来ないことは明らかで、抑制剤処理による花らしい中のサイトカイン活性の増加が関係しているようにと思われる。