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## **Exogenous Gibberellin as Responsible for the Seedless Berry Development of Grapes.**

### **IV. Effects of Temperature on the Activity of Applied Gibberellin, on the Seedlessness and the Seedless Berry Development in Delaware and Campbell Early Grapes.**

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#### **Summary**

The effectiveness of exogenous GA in the formation of the seedless fleshy berries affected by temperature was studied in Delaware and Campbell Early grapes. Vines were kept at different temperatures immediately after GA application till the completion of flowering and then removed to a fixed temperature.

At low temperatures, corresponding to the considerably high level of GA activity maintained in the flowers, flower weight was heavier, sugar concentrations were higher, and seedless and seedless fleshy berries were obtained in high ratio, although their subsequent growth was not sufficient, compared with those at high temperatures.

It seems that to induce seedlessness, a sufficient level of GA activity must be maintained for a comparatively short term at the suitable stage of blossom bud growth. In addition, for the induction of fleshy growth of seedless berries, GA activity must be maintained for the entire period till flowering. In this connection, some arguments were made on the difference of the effectiveness of exogenous GA in Delaware and Campbell Early.

The effects of temperature on the development of grape berries were studied by several investigators (1-10), but not in relation to gibberellin (GA) application now effectively used for seedless berry production in some cultivars.

In the previous papers (11 and 12), the authors reported that the change of the activity of GA from application to flowering had an influence on the blossom bud growth and flower size at anthesis, and moreover it seemed to be related to the seedlessness and the seedless berry development in the long run.

Now, it seemed probable that GA activity would be affected by temperature, thus, the effectiveness of GA on the seedless berry development might also be affected by temperature after GA application. To ascertain this assumption was the purpose of this study.

Two cultivars, Delaware and Campbell Early, were used in comparison. The potted vines were kept under different temperatures immediately after GA application till the end of flowering, then transferred to a fixed temperature and grown uniformly.

### Materials and Methods

Delaware and Campbell Early grapes were used. The vines were, in both cultivars, 3 years old on the first year of the experiments, which were carried out over 7 years from 1970 on Delaware and over 3 years from 1974 on Campbell Early. They were grown in 30 cm pots and 4 vines in 4 pots were used as one set for each temperature treatment. Inflorescences were thinned out before GA application, so that one inflorescence was borne on a current shoot. Potted vines were kept outdoors until GA application.

GA solution (100 ppm GA with 100 ppm Aerol OP) was prepared as described in the previous papers (11 and 12). GA was applied to the inflorescences by dipping at tetrad stage of pollen, and then moved into glass rooms of controlled temperatures under natural daylight. Temperature treatments were 30°, 25°, 20° and 15°C for day temperatures (from 6 AM to 6 PM) combined with night temperatures 5°C lower respectively, represented hereinafter as 30°-25°C, 25°-20°C etc. In each room vines without GA application were added as the control. On the completion of flowering in each temperature treatment, all vines were moved into a room kept at 25°C in the day and 20°C in the night and grown uniformly.

Blossom buds were sampled and weighed every 3 days from the day of GA application, while flowers were sampled everyday on their anthesis throughout the flowering period and their number and weight were recorded. The weights were for 100 blossom buds or flowers. Here, anthesis means the separation of the calyptra from the receptacle in individual flowers.

The bioassay of GA activity was carried out with 100 blossom buds or flowers using the same method described in the previous paper (12), and GA activity was represented as the length of the second leaf sheath of the rice seedling.

For sugar analyses, daily flower samples were extracted with 80% ethanol. On the extracts, total invert sugars and reducing sugars were determined by the Nelson method; for total invert sugars the extracts were previously hydrolysed by heating with 0.1% HCl on a boiling water bath for 30 minutes and then neutralized with NaOH. Nonreducing sugars were calculated as the difference between total invert sugars and reducing sugars.

Fruit clusters were harvested when the seedless fleshy berries ripened and the seeded berries were still green. Percentage distributions of seeded berries, seedless fleshy berries and shot berries were calculated as described in the previous paper (11).

## Results

### 1. Blossom Bud Growth, Flowering and Flowers.

The increase of blossom bud weight to anthesis in relation to temperature and GA application is shown in Fig. 1 (a and b). Blossom bud weight, in this paper, means the total weight of calyptra, stamens, pistil and receptacle, while flower weight means the total of calyptra, stamens and receptacle, calyptra being excluded because of its behaviour of falling off when flowering. It appears in Fig. 1 (a and b) that both high temperatures and GA application accelerated the growth of blossom buds, which resulted in earlier flowering. In Fig. 2 the flowering period and the day of full bloom are shown. The day of full bloom means the day when the calyptras separated from the receptacle in about 50–70% of the flowers in each inflorescence. As is evident in Fig. 1 and 2, the higher the temperature, the earlier the beginning of flowering as well as the day of full bloom. The flowering period, however, was longer at low temperatures, and especially so at 15°–10°C. GA application not only hastened somewhat the flowering, especially at low temperatures, but also prolonged the flowering period, though only a little, regardless of temperature. The circumstances were the same for the two cultivars tested.

Change of daily flower weights and their total average throughout the flowering period are shown in Fig. 3 and 4. It is evident from Fig. 3 that daily flower

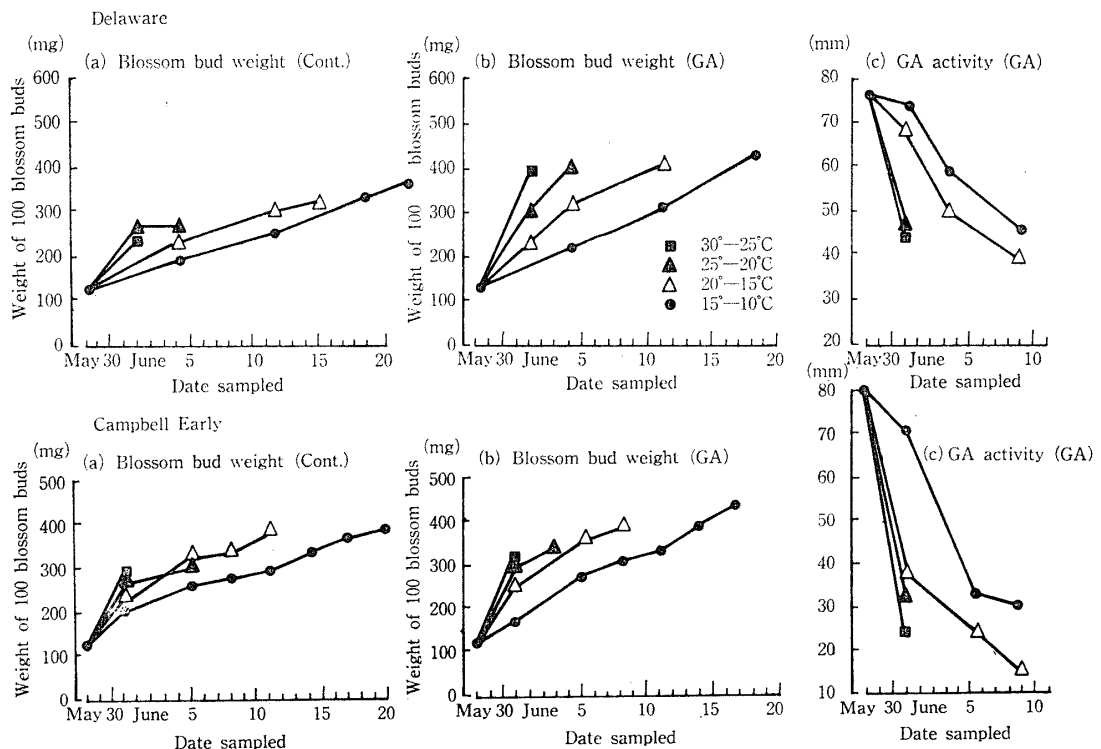
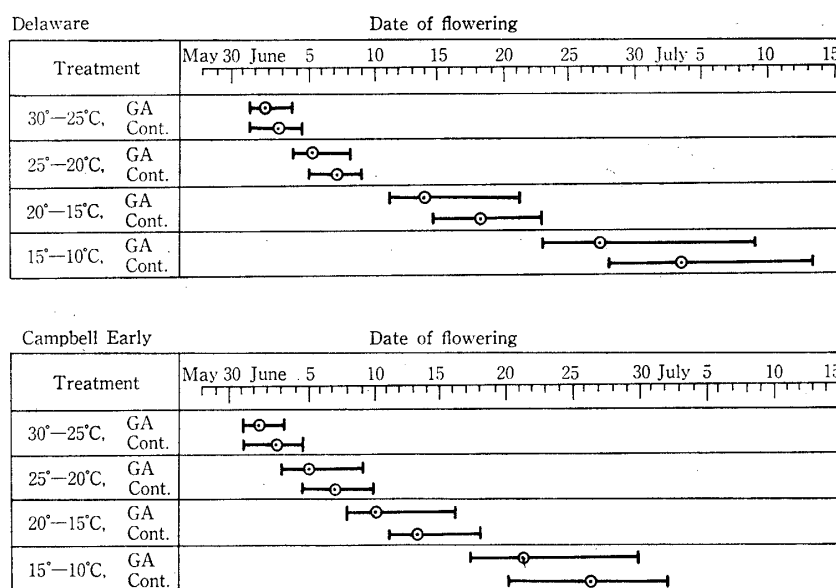


FIG. 1. Effects of temperature on the blossom bud growth and biological activity of applied GA



On May 25 applied with GA and moved into 4 different temperatures.  
 ⊙ : Day of full bloom

FIG. 2. Effects of temperature on the flowering period.

weights had a maximum on the beginning of flowering, then decreased successively with the day of flowering, regardless of temperature or GA application except at 15°-10°C, where the maximum value was obtained at the middle of the flowering period. Furthermore, Fig. 3 and 4 show that GA application increased both the daily flower weights and their total average in both cultivars. Also low temperatures caused an increase especially in Delaware applied with GA. In general, the variation of flower weight due to temperature or GA application was smaller in Campbell Early than in Delaware as is shown in Fig. 4.

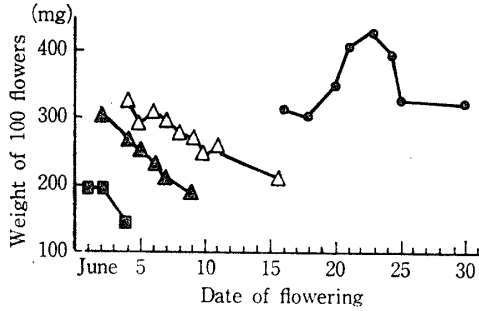
2. *Activity of Applied GA in Blossom Buds and Flowers.*

As shown in Fig. 1 (c), the biological activity of applied GA in blossom buds decreased rapidly at high temperatures, while slowly at low temperatures, in both cultivars immediately after application. In comparing the two cultivars, however, the decrease was more rapid in Campbell Early than in Delaware.

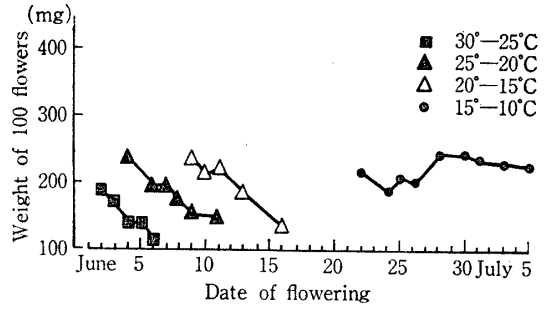
Fig. 3 (a' and b') show the biological activity of GA in the flowers in relation to the day of their anthesis in both cultivars. It appears from these figures that in GA applied flowers there was a clear trend toward a decrease in GA activity with the day of flowering regardless of cultivars. This corresponded well with the decrease in daily flower weights. The maximum activity was obtained in most cases on the beginning of flowering and it was higher or, at least, not lower at low temperatures than at high temperatures, irrespective of the fact that more days had been required at low temperatures from GA application to flowering. In the

Delaware

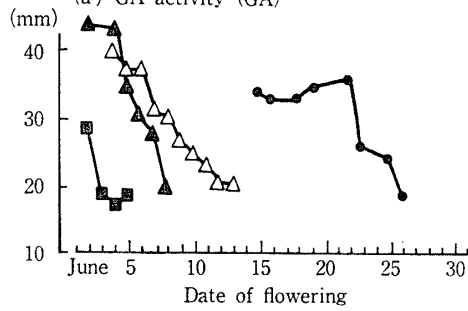
(a) Flower weight (GA)



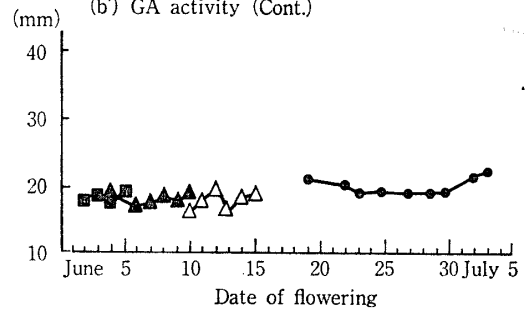
(b) Flower weight (Cont.)



(a') GA activity (GA)

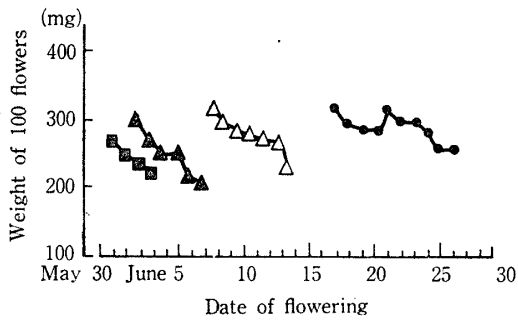


(b') GA activity (Cont.)

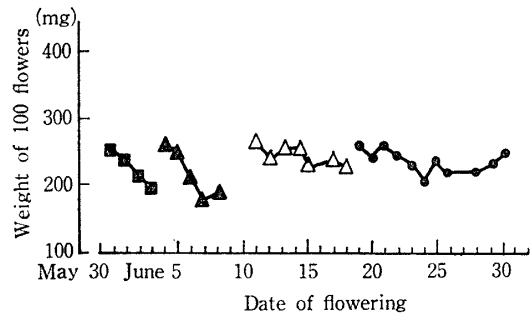


Campbell Early

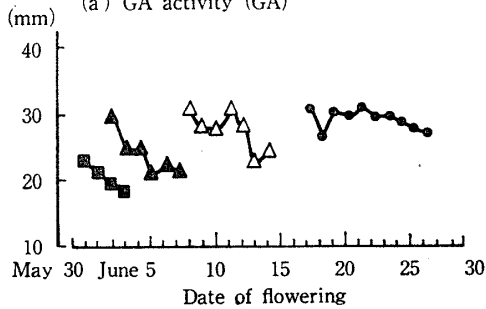
(a) Flower weight (GA)



(b) Flower weight (Cont.)



(a') GA activity (GA)



(b') GA activity (Cont.)

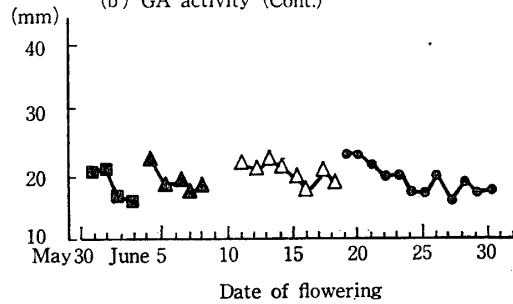


FIG. 3. Effects of temperature on the daily flower weight and biological activity of flowers.

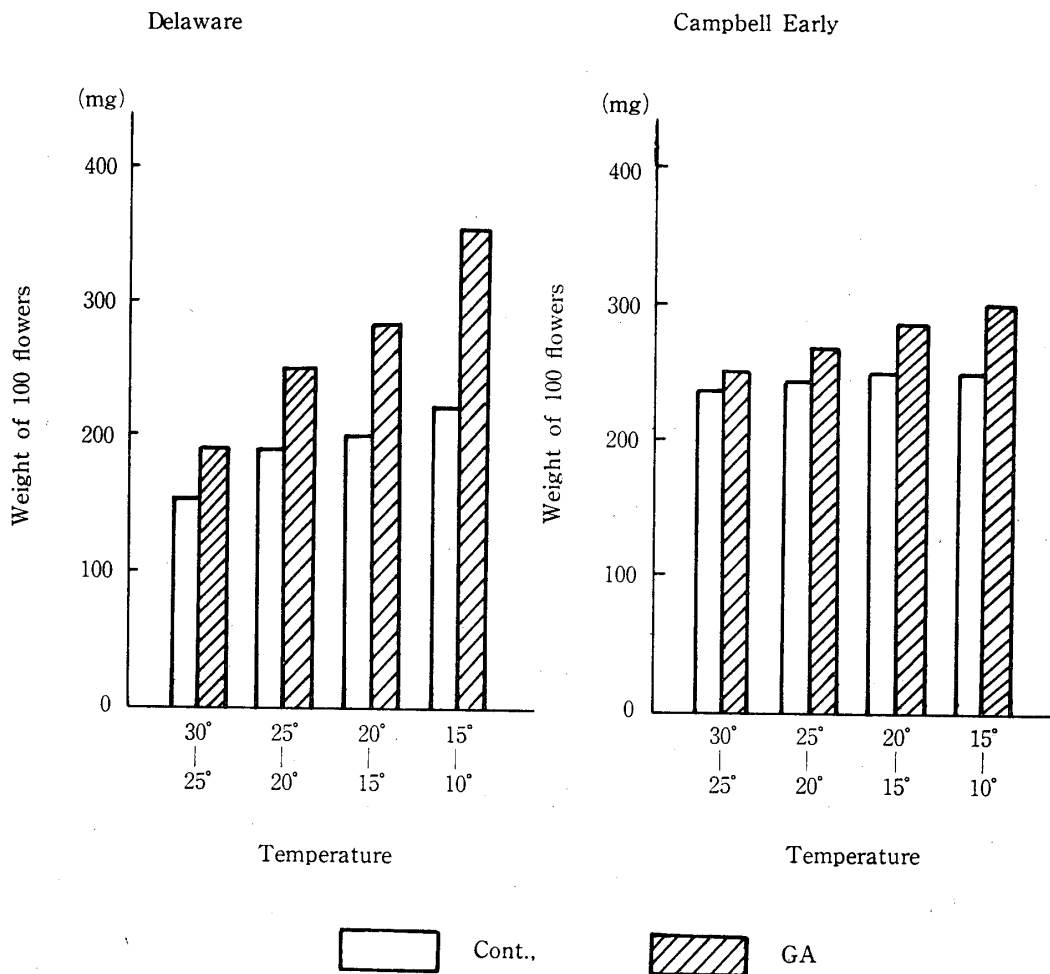


FIG. 4. Effects of temperature on the total average of flower weight.

flowers, without GA application, however, the biological activity of GA was low and almost constant in Delaware throughout the flowering period and showed no correspondence with the change of daily flower weight. While, in Campbell Early, a slight decrease was observed in GA activity together with daily flower weight.

### 3. Sugar Contents of Flowers.

As shown in Fig. 5, the concentrations of total invert sugars and reducing sugars in Delaware flowers were higher at low temperatures than at high temperatures regardless of GA application. By GA application, however, the reducing sugar concentration was decreased at high temperatures and increased at low temperatures. Thus, the reducing sugar concentration was lower in GA-applied flowers, but their size was so large that the amount of reducing sugars contained in them was greater than that in the flowers without GA application. In the concentration of nonreducing sugars there was found no consistency, but it is notable that at low temperatures it was conspicuously increased by GA application.

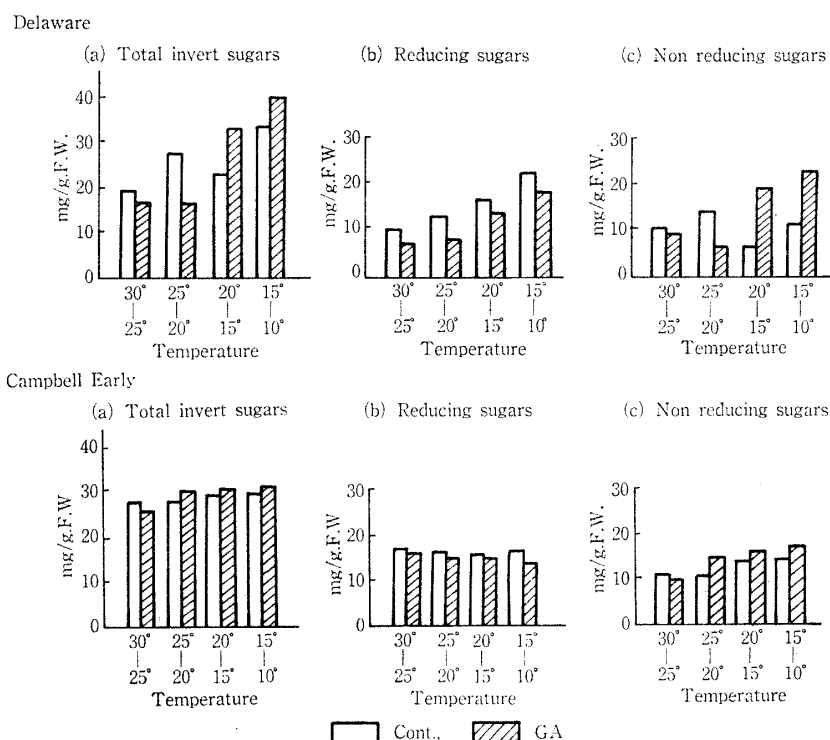


FIG. 5. Effects of temperature on the sugar concentrations in flower.

In Campbell Early, sugar concentrations were not so much affected as in Delaware by temperature or by GA application. The concentration of total invert sugars and that of nonreducing sugars were a little higher, while that of reducing sugars was slightly lower, at low temperatures.

#### 4. Berry Development.

In Delaware kept at low temperatures seedless berries (seedless fleshy berries + shot berries) were harvested in high ratio and at 15°–10°C all of the berries were seedless. In addition, the ratio of seedless fleshy berries was higher at low temperatures although their growth was inferior. While at 30°–25°C, however, many berries had dropped soon after flowering, and all of the berries harvested were fleshy and seeded.

In Campbell Early, on the contrary, the ratio of seeded fleshy berries was low in general and not more than 25% even at 30°–25°C, and the others were all shot berries except that at 15°–10°C a few seedless fleshy berries were obtained.

### Discussion

In Delaware grapes, blossom bud growth as well as flowering were delayed and the flowering period was prolonged at low temperatures. These results may be well explained considering that in most fruit trees flowering time is influenced



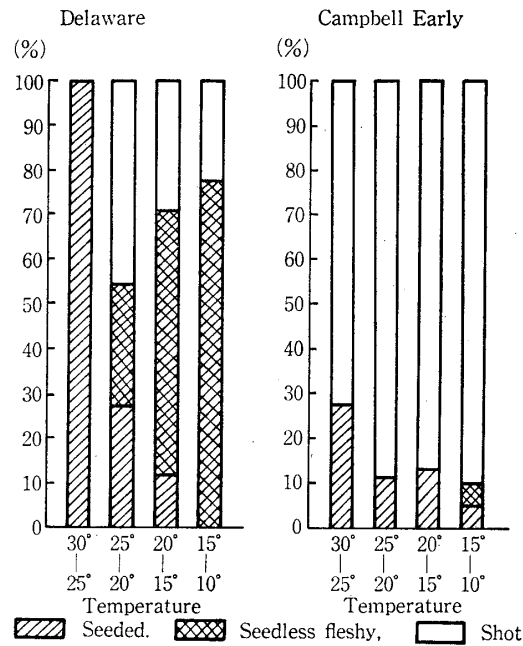


FIG. 6. Effects of temperature on the percentage distribution of seeded, seedless fleshy and shot berries in GA treated inflorescences.

mainly by the cumulative temperature during a certain period before flowering. In addition, the blossom buds applied with GA and kept at low temperatures were delayed in their flowering, but their GA activity was maintained higher, though gradually decreased, throughout from the blossom bud stage towards flowering. At temperature as high as 30°–25°C, however, GA activity decreased rapidly throughout the blossom bud stage and flowering period. These activities appear to be due to GA application, because in flowers without GA application GA activity was low and constant throughout the blossom bud stage to flowering.

It is interesting that at low temperatures, corresponding to the considerably high level of GA activity maintained in the flowers, the flower weight was heavier, the sugar concentrations were higher, and seedless and seedless fleshy berries were obtained in higher ratio compared with those at high temperatures.

Kobayashi *et al.* (13) reported that the weight of pistil at the full bloom was heavier at low temperatures in Delaware grapes. Also, Harris and Scott (14) showed in carnation that relatively large flowers were produced at low temperatures, though their flowering was delayed markedly, because the increase in the duration of growth more than compensated for the decreases in rate of growth. In relation to their results, their viewpoint that the partition of assimilates between flower and remainder of the shoot system is under the direct control of temperature seems to be very promising.

For the production of seedless fleshy berries in Delaware, two applications of GA are practiced. By the prebloom application seedlessness and seedless berry

development are induced, while the postbloom application is required to get berries grown to enough size for market. In the previous paper (11), we demonstrated the importance of the time factor for the effectiveness of the prebloom application. GA must be applied about two weeks before the anticipated day of full bloom, but this criterion fails occasionally. From the preceding results, it appears probable that these failures occur due to the temperature conditions after the prebloom application.

To induce seedlessness, a sufficient level of GA activity kept for a comparatively short term at the suitable stage of blossom bud growth seems to be necessary. In addition the fleshy growth of the seedless berries requires GA activity being maintained for a long period till flowering or thereafter. Thus, at temperatures as high as 30°–25°C, GA activity decreased so rapidly that all the berries harvested were seeded, though many berries had dropped soon after flowering, while at temperature as low as 15°–10°C, seedless fleshy berries were obtained in high ratio, although their subsequent growth was not sufficient.

Hale and Weaver (15) showed in grapes that inflorescences were strong sinks for assimilates while increasing their size rapidly before flowering, but their sink activity decreased as they approached the stage of flowering. It appears probable that postbloom application of GA may increase the sink activity and promote the subsequent growth of seedless and fated-to-fleshy berries induced by the prebloom GA application. In addition, large sized flowers with high sugar contents due to prebloom GA application are considered to have a high ability for the subsequent growth.

Campbell Early is one of cultivars in which GA has little effect on the production of seedless fleshy berries. In this cultivar, compared with Delaware, the activity of applied GA decreased more rapidly immediately after its application. Nevertheless, and even at 30°–25°C, seedlessness could be induced at a high ratio. The subsequent growth, however, did not proceed at all and all the berries remained shot. In addition, in this cultivar, it is difficult to promote the subsequent growth of berries by the postbloom application. Thus, in Campbell Early, it is inferred, aside from the rapid inactivation of GA, that there might be different kinds of growth substances other than GA which are involved in the berry development.

To explain the decrease of GA activity, we will only indicate here two possibilities, that is, 1) the translocation of GA to the other parts out of inflorescences and 2) the inactivation in the inflorescences in situ, about which we will discuss at another time. Also, in a later paper will be discussed the participation of GA in the activity of inflorescences as a sink for the assimilates.

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