

Relation of plant regulators to bud rest in *Vitis vinifera* grapes

by

R. J. WEAVER, KANG YEOU-DER¹⁾, and R. M. POOL

Introduction

Only limited data are available as to the length of rest period of grape buds of various varieties. At Davis, California, the buds usually enter a rest state in autumn and do not emerge from it until about January (11). Low temperature after fruit ripening is necessary to terminate the rest. One objective of our research was to determine the effects of various growth regulators on bud rest. With this information, it might be possible to terminate bud rest at will. If so, rest could be terminated in autumn so that shoots would develop under greenhouse conditions and cluster counts could be made early and level of crop predicted (11). Also, the occurrence of endogenous promoters and inhibitors was followed in one variety during the dormant season.

Materials and Methods

To study length of rest period of nontreated buds, 30 cuttings, each with 14 to 16 buds, of each of 13 varieties were taken from the vineyard on March 21, 1966. The cuttings were placed in containers so that their bases were in 4 or 5 inches of water at 25° C. Counts of cuttings showing bud growth were made at weekly intervals. Rest was considered broken in this and subsequent experiments when buds on cuttings placed at 25° C began to show green color.

A second experiment was designed to study effect of various exogenous growth regulators on bud rest. Thirty cuttings of 'St. Emilion' were collected on November 14, 1966. These were soaked in a cytokinin, benzyladenine (BA), at 1000 ppm for 30 minutes. After being rinsed in tap water, the cuttings were placed with their bases in water and the termination of bud rest noted. Other cuttings were similarly treated with the plant growth retardants 2-chloroethyltrimethylammonium chloride (CCC) or N-dimethylamino succinamic acid (B-9), at 20, 200, or 2000 ppm. One group was treated with the auxin benzothiazole-2-oxyacetic acid (BOA) at 1000 ppm. Control cuttings were soaked in water.

To study effects of BA on endogenous inhibitors and promoters, cuttings of 'St. Emilion' were obtained from the vineyard on November 30, 1966. Seventyfive cuttings were soaked for 30 minutes in BA at 1000 ppm, rinsed and then stored with bases in water at 25° C. The control group was soaked in cold tap water. Five days after start of treatment, 25 buds each (about 1 g) from both untreated and treated cuttings were removed from canes and extracted. Growth regulating activity was measured by the mung bean test. Similar measurements were made 10, 15, 20, 25, and 30 days after start of treatment. Solutions of BA at 1 and 100 ppm were

¹⁾ Present address: Department of Horticulture, National University of Taiwan, Taipei, China.

prepared from stock solutions and bioassayed to determine whether there was any stimulatory or inhibitory activity in the BA itself.

In the fourth experiment the occurrence of endogenous promoters and inhibitors was followed during the dormant season. Twenty cuttings of 'St. Emilion' were collected from the vineyard on each of the following dates: December 5 and 19, 1966; January 3, 16, and 30; February 13 and 27; March 13 and 27; and April 10, 1967. At the last collection the buds were beginning to show green color. About 100 buds (4 g) were removed at each collection. The buds were extracted with methanol and extract equivalent to 2 g of buds was streaked on duplicate sheets of Whatman No. 1 filter paper.

To obtain endogenous inhibitors and promoters, buds were removed from the cuttings and extracted for 8 hours with 40 ml of 100% methanol in a Soxhlet-type extractor. The extract was stored at -10° C until use. At that time the liquid was evaporated to dryness in a Rinco evaporator, and then 50 ml of water were added. This mixture was extracted three times with an equal volume of ethyl acetate. Then the ethyl acetate was evaporated and the residue dissolved in 1 ml of methanol. The resultant solution was streaked on sheets of Whatman No. 1 paper (12 x 57 cm). A solution of isopropyl alcohol, ammonia, and water (10:1:1 V/V) was used in ascending chromatography. In about 8 hours the front reached the 20-cm mark from the origin. The paper was then dried and cut into 15 strips and the strips stored at -10° C until use.

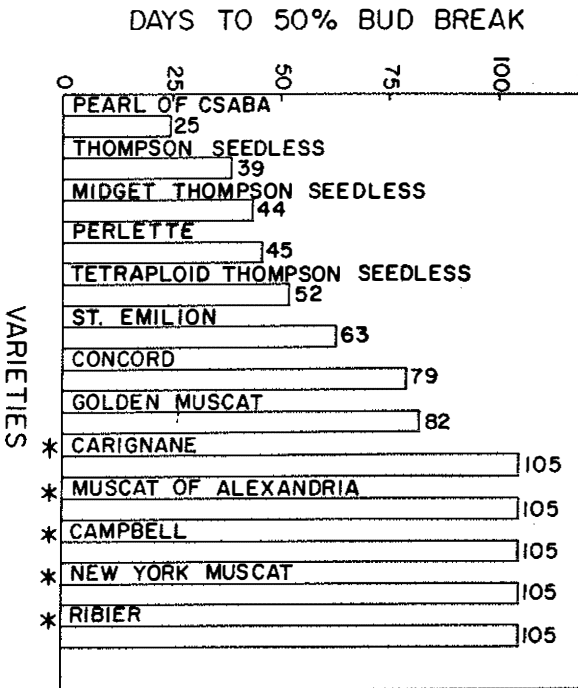


Fig. 1: Days required for various varieties of grapes to terminate rest when placed at 25° C. Experiment was terminated after 105 days. Varieties marked with asterisks (*) had no evidence of bud break.

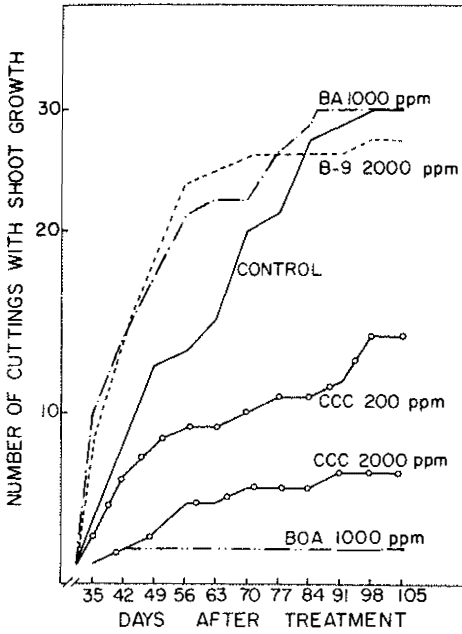


Fig. 2: Days required for bud break on cuttings of 'St. Emilion' treated with indicated growth-regulators and then stored at 25° C.

Compounds used were N-dimethylamino succinic acid (B-9), 2-chloroethyltrimethylammonium chloride (CCC), benzyladenine (BA), and benzothiazole-2-oxyacetic acid (BOA). B-9 at 20 and 200 ppm and CCC at 20 ppm gave results similar to those of the controls and are not shown.

In the experiment in which the occurrence of endogenous promoters and inhibitors was followed during the dormant season, each paper was divided after development into four parts in such a way that there was about 0.5 g of extract on each piece. Two pieces were used for the mung bean test, and one for the wheat coleoptile test.

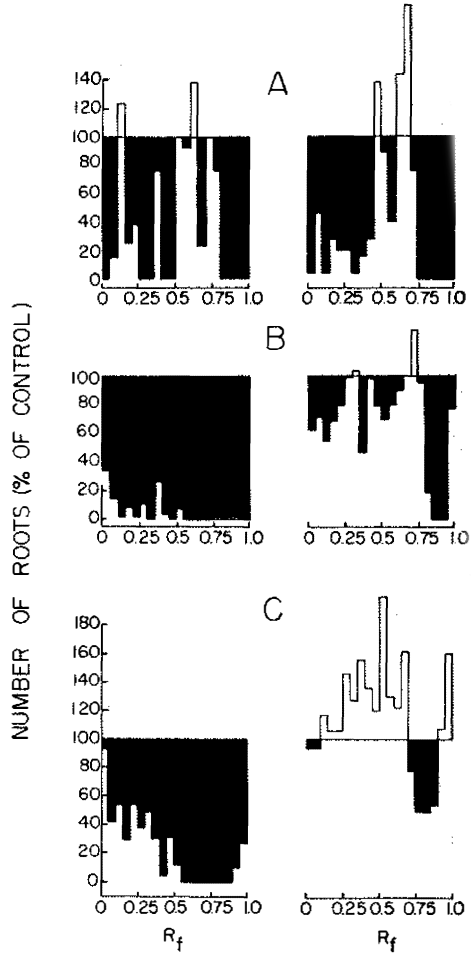


Fig. 3: Root-promoting or -inhibiting activity of bud diffusates of 'St. Emilion' from chromatogram strips tested by the mung bean bioassay.

Each chromatogram represents 1.0 gram (fresh weight) of buds, developed with isopropanol-ammonia-water (10:1:1 V/V). Buds collected 5 (A), 19 (B), and 30 (C) days after treatment with benzyladenine (BA). Left, control; right, treated with BA. Control is 100%.

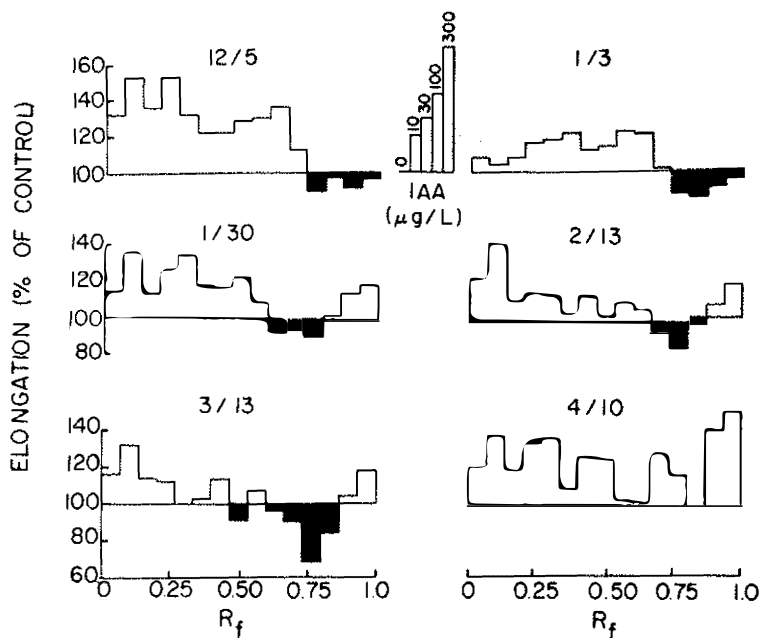


Fig. 4: Growth-regulating activity of bud diffusates of 'St. Emilion' from chromatogram strips tested by the wheat coleoptile test.

Each chromatogram represents 0.5 gram (fresh weight) of buds, developed with isopropanol-ammonia-water (10:1:1 V.V). Buds collected on December 5, January 3, 30, February 13, March 13, and April 10. Control is 100%.

The wheat coleoptile test (7) and a modification of the mung bean test of Hess (4) were used for bioassays. In the mung bean test, seeds were treated for 5 minutes in a clorox solution (1 part clorox: 16 parts water), rinsed, and then soaked in running water for 24 hours. They were then planted in moist vermiculite and germinated in a growth chamber at 29° C daytime and 16° C nighttime. There were 12 hours daylight, and the relative humidity was about 70%. After 10 days the bean cuttings were prepared by removing the seedling root system 3.5 cm below the cotyledonary node. The cuttings then consisted of 3.5 cm of hypocotyl, the epicotyl, the primary leaves, and the trifoliate buds. Five plants were placed in each of two 20 × 68-mm shell vials containing a chromatographic section in distilled water. A strip from below the origin was used for the control. Each treatment was duplicated once. Sufficient water was added to the vials each day to retain the original volume (10 ml) of solution. After 5 days the number of roots on each cutting was compared to the number on the control.

Results

Varietal differences in relation to termination of rest

The results indicate a great difference among varieties in the time required for termination of rest (Fig. 1). The most rapid growth and the highest percentage of cuttings showing growth occurred with 'Pearl of Csaba', 'Thompson Seedless', 'Midget Thompson Seedless', and 'Perlette'. 'Tetraploid Thompson Seedless' also overcame

rest relatively early, while 'St. Emilion' took somewhat longer. 'Concord' and 'Golden Muscat' emerged from the resting condition later than 'St. Emilion'. The buds of 'Carignane', 'Muscat of Alexandria', 'Campbell', 'New York Muscat', and 'Ribier' dried out before growth was evident. The experiment was terminated after 105 days. Effect of exogenous growth regulators

In these experiments BA at 1000 ppm and B-9 at 2000 ppm hastened termination of rest (Fig. 2). The most effective compound in delaying termination of rest was BOA, although CCC at 200 and 2000 ppm was also markedly effective. Nearly all buds on canes treated with BOA remained in a rest condition.

Effect of BA on endogenous inhibitors and promoters

Five days after start of treatment both the BA-treated buds and the control buds contained considerable inhibitor (Fig. 3). After 10 days much of the inhibitor had disappeared from the BA-treated buds, and that which did remain may have been due to an impurity in the BA stock solution. Controls contained much inhibitor. Chromatographs of extracts taken 15, 20, and 25 days after treatment were similar to those made 10 days after treatment. After 30 days, the only significant amount of inhibitor present was from R_f 0.7 to 0.9, where there was also inhibition in the BA stock solution. Also, there was stimulatory activity at most other R_f 's.

Changes in endogenous promoters and inhibitors in buds during the dormant season

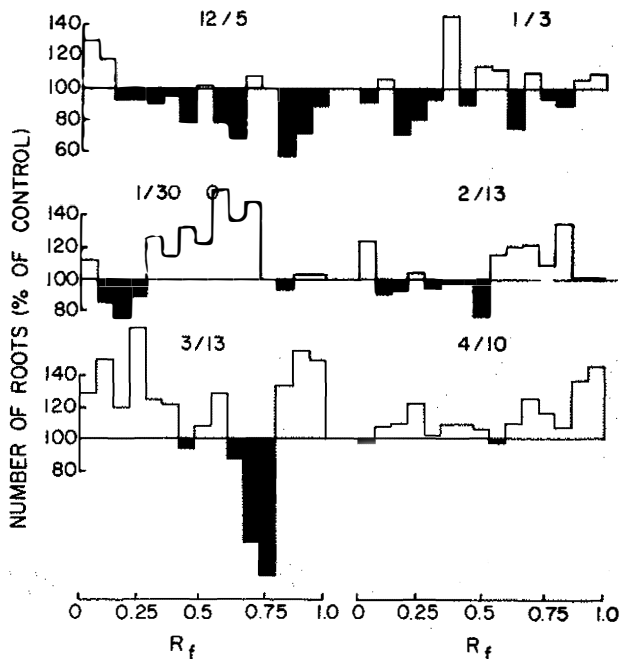


Fig. 5: Root-promoting or -inhibiting activity of bud diffusates of 'St. Emilion' from chromatogram strips tested by the mung bean bioassay.

Each chromatogram represents 0.5 gram (fresh weight) of buds, developed with isopropanol-ammonia-water (10:1:1 V/V). Buds collected on December 5, January 3, 30, February 13, March 13, and April 10. Control is 100%.

The wheat coleoptile test on December 5 revealed stimulatory activity from R_f 0 to 0.7 and inhibitory activity from R_f 0.7 to 1.0 (Fig. 4). Activity on December 12 was about the same as on the earlier date. By January 3 the stimulatory activity had decreased, but inhibitory activity was still present at the same R_f 's in slightly larger amounts than on the earlier dates. On January 30 promotive activity was evident from R_f 0 to 0.6 and 0.9 to 1.0. Activity was similar through the March 27 collection; but by April 10, when shoots began to grow, all inhibitory activity had disappeared.

Results with the mung bean test also indicated inhibitory substances were present up until the time the shoots began to grow (Fig. 5). However, the inhibitory substances were found at various R_f 's not indicated by the wheat coleoptile test. For example, on December 5 inhibitory substances were found from R_f 's 0.2 to 0.5, 0.6 to 0.7, and 0.8 to 1.0. On January 30 much stimulatory activity was present in the sample from R_f 0.3 to 0.6. On February 13 there was strong inhibition from R_f 0.4 to 0.5, as well as stimulation from R_f 0 to 0.1 and 0.5 to 0.8. By initiation of shoot growth on April 10 inhibitors had almost disappeared, although there was still considerable stimulatory activity.

Discussion

In the experiment with exogenous growth regulators, the early termination of rest in 'St. Emilion' by BA confirms previous results obtained with 'St. Emilion' 'Thompson Seedless', and 'Tokay' (10). The prolongation of rest by CCC and the hastening of termination of rest by B-9 indicate the mechanism of action of these two growth retardants evidently is different. A difference in effects on gibberellin production in *Fusarium* cultures was noted by NINNEMANN *et al.* (6). CCC strikingly inhibited gibberellin production, but B-9 caused no decrease. It is noteworthy that the auxin BOA, which strikingly delayed termination of rest, also caused a striking delay in maturation of berries (9).

It has been demonstrated in many species that when shoot growth begins in the spring, the inhibitors rapidly disappear. Our work indicates that BA may terminate rest by decreasing the concentration of inhibitors in the buds. However, the mechanism of action is not clear. In 'St. Emilion' inhibitors remained through the March 13 sampling; but after shoot growth commenced all inhibitors disappeared, and there was a marked increase in stimulatory materials. SPIEGEL (8) observed that inhibitors disappeared from grape cuttings just before bud break in the spring.

Rest in grapes is terminated in mid-winter at DAVIS (11), but inhibitors are present until shoot growth in the spring. Thus, in our experiment there was no correlation between termination of rest and level of inhibitors. DENNIS and EDGERTON (2) also found no correlation between inhibitory activity of bud extracts and the resting condition of peach buds (*Prunus persica*). In contrast, KAWASE (5) found in four woody plants other than grapes that inhibitors were at a maximum during the winter season and generally declined towards spring. Breaking of bud dormancy occurred along with the disappearance of inhibitors.

CORGAN (1) found in peach buds that the concentration of the inhibitor compound naringenin remained high more than 30 days after rest was terminated, and that there was an apparent dilution of the compound as buds expanded. FADL and HARTMANN (3) found high levels of inhibitors in 'Old Home' pear buds only during rest (November and December). In contrast, there were large amounts of inhibitors in 'Bartlett' pear buds most of the year.

An explanation as to why the inhibitor concentration of buds usually fails to decrease at termination of rest may lie in the finding of DENNIS and EDGERTON (2).

They reported that in peach the inhibitory compounds are confined to the bud scales. CORGAN (1) suggested that rest may be controlled by diffusion of naringenin from bud scales into flower primordia, and that any change which interferes with this diffusion could terminate rest. The distribution of inhibitors in the grape bud has not been determined.

It has been suggested that rest is caused by high levels of auxin or by a balance of inhibitors and promoters (2). However, our data indicate there was considerable activity of promoter and inhibitor both during and after rest.

There were considerable differences between results obtained with the wheat coleoptile test and those obtained with the mung bean test. Evidently rooting of the mung bean is more sensitive to certain inhibitory substances than is elongation of the wheat coleoptile.

Summary

A large varietal difference was noted in time required for grape buds to terminate rest. 'Pearl of Csaba' and seedless varieties terminated rest most rapidly.

Exogenous applications of benzyladenine (BA) at 1000 ppm or of N-dimethylamino succinamic acid (B-9) at 2000 ppm hastened termination of rest. Benzothiazole-2-oxyacetic acid (BOA) at 1000 ppm and 2-chloroethyltrimethylammonium chloride (CCC) at 200 or 2000 ppm markedly delayed it.

In cuttings of 'St. Emilion' treated with exogenous BA, much inhibitor disappeared from the treated buds within 10 days after treatment. We suggest that the effect of BA in terminating rest may be due to its destructive effect on inhibitor concentration in the bud.

Inhibitory substances were present in buds from 'St. Emilion' from December 5 through the dormant season. The compounds disappeared and stimulatory activity increased at the start of shoot growth.

Literature Cited

1. CORGAN, J. N.: Seasonal change in naringenin concentration in peach flowering buds. Proc. Amer. Soc. Hort. Sci. 35, 129-132 (1965).
2. DENNIS, F. G. and EDGERTON, L. J.: The relationship between an inhibitor and rest in peach flower buds. Proc. Amer. Soc. Hort. Sci. 77, 107-116 (1961).
3. FADL, M. S. and HARTMANN, H. T.: Relationship between seasonal changes in endogenous promoters and inhibitors in pear buds and cutting bases and the rooting of pear hardwood cuttings. Proc. Amer. Soc. Hort. Sci. 91, 96-112 (1967).
4. HESS, C. E.: Naturally occurring substances which stimulate root initiation. In NITSCH, J. P. (Ed.): *Regulateurs naturels de la croissance végétale*. C. N. R. S., Paris, 517-527 (1964).
5. KAWASE, M.: Growth inhibitory substance and bud dormancy in woody plants. Proc. Amer. Soc. Hort. Sci. 89, 752-757 (1966).
6. NINNEMANN, H., ZEEVAART, J. A. D., KENDE, H. and LANC, A.: The plant growth retardant CCC as inhibitor of gibberellin biosynthesis in *Fusarium moniliforme*. *Planta* 61, 229-235 (1964).
7. NITSCH, J. P. and NITSCH, C.: Studies on the growth of coleoptile and first internode sections. A new, sensitive straight-growth test for auxins. *Plant Physiol.* 31, 94-111 (1958).
8. SPIEGEL, P.: Auxin and inhibitors in canes of *Vitis*. *Israel Res. Council Bull.* 4, 176-183 (1954).
9. WEAVER, R. J.: The effect of benzothiazole-2-oxyacetic acid on maturation of seeded varieties of grapes. *Amer. J. Enol. Viticult.* 13, 141-149 (1962).
10. — — : Use of kinins in breaking rest in buds of *Vitis vinifera*. *Nature* 198, 207-208 (1963).
11. — — , McCUNE, S. B. and COOMBE, B. G.: Effects of various chemicals and treatments on rest period of grape buds. *Amer. J. Enol. Viticult.* 12, 131-142 (1961).

Eingegangen am 9. 4. 1968

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