<Original>Differences in Polyamine Levels between 'Kyoho,' 'Campbell Early' and 'Muscat Bailey A' Grapes with Differing Features of Berry Drop

Differences in Polyamine Levels
between 'Kyoho,' 'Campbell Early' and 'Muscat Bailey A'
Grapes with Differing Features of Berry Drop

Shuji Shiozaki, Tsuneo Ogata, and Shosaku Horiuchi

(Laboratory of Pomology, Graduate School of Agriculture and Biological
Science, Osaka Prefecture University)

Abstract

A comparative study was conducted of berry dropping and changes in polyamine levels in 'Kyoho,' 'Campbell Early' and 'Muscat Bailey A' grapes. Berry dropping occurred first in 'Muscat Bailey A' grapes, peaking 6 days after full bloom. In 'Kyoho' grapes, dramatic berry dropping began 8 days after full bloom and reached a peak two days later. In these two cultivars, berry dropping had almost ceased by 18 days after full bloom. On the other hand, although berry dropping took place in 'Campbell Early' from 4 days after full bloom, fairly constant dropping occurred for the duration of the experiment. The berry setting rate after cessation of berry dropping was highest in 'Campbell Early,' and lowest in 'Kyoho' grapes. Putrescine (Put) predominated in free, in soluble-conjugated and in insoluble bound fractions within each cultivar. In 'Muscat Bailey A' grapes, changes in free PA levels were not found to be associated with berry dropping, but in 'Kyoho' and 'Campbell Early' berry drop was preceded by a reduction in free Put. The decline in free Put level was more pronounced in 'Kyoho' grapes. In 'Kyoho' grapes, changes in the levels of soluble conjugated and insoluble bound PA were anticorrelated to changes in free PA.

Key Words: polyamine, grape, abscission

Introduction

Fruit abscission occurs naturally just after full bloom in grapes, as in other fruits. In grapes, berry abscission inducing a very poor berry set is described as "Coulure" (Winkler et al., 1962) or "Hana-burui" in Japanese. Coulure is a serious problem that causes deterioration of table grapes, and is most liable to occur in grapes which grow vigorously such as the tetraploid grape 'Kyoho' (*Vitis labruscana* Bailey × *V. vinifera* L.). Suggested causes of coulure include abnormal morphology of pollen (Ahmedullah, 1983; Cargnello et al., 1980), of stigma (Carraro et al., 1979) or of the embryo sac and ovule (Pratt, 1973; Carraro et al., 1979), lack of fertilization (Winkler et al., 1962), degeneration of the ovule after fertilization (Okamoto and Imai, 1982; Pratt, 1973), disproportionate distribution of nutrient between berries and bearing shoots (Coombe,

1962), low availability of carbohydrates (Caspari et al., 1998), and inhibition of pollen tube growth in the pistil (Naito and Kawashima, 1980; Naito et al., 1983; Okamoto et al., 1984). The shedding of berries in coulure is induced by dissolution of the cell wall between adjacent living cells at the abscission zone of the pedicel, and like many plant physiological processes this is most likely to be controlled directly by endogenous plant growth regulators. There is good evidence in many plant systems for the participation of endogenous plant growth regulators, in particular ethylene and auxin, in the abscission of plant organs including fruit (Brown, 1997).

Polyamine (PA) is likely to be another potent candidate in regulation of fruit abscission. A substantial inhibitory effect of exogenous polyamine on fruit abscission has been found in many fruit species, including the

apple (Costa and Bagni, 1983), lychee (Mitra and Sanyal, 1990), olive (Rugini and Mencuccini, 1985), pear (Crisosto et al., 1988) and mango (Singh and Singh, 1995). The observation that fruit sets when the endogenous level and the biosynthesis activity of polyamines are high (Biasi et al., 1991) implies a role for polyamine in fruit abscission and fruit setting. However, little is known about the role of endogenous polyamines in berry abscission and berry setting in grapes.

The present study analyzed polyamine levels in the berries of 'Kyoho,' 'Campbell Early' (*Vitis labruscana* Bailey; ×2) and 'Muscat Bailey A' (*Vitis labrusca* L. ×*V. vinifera* L.; ×2) during the berry abscission period just after bloom. In the light of these results the correlation is studied between changes in the levels of polyamines and berry abscission and the possible role of endogenous polyamines in coulure.

Materials and Methods

Plant materials and berry abscission. X-shape-trellised field-grown 'Kyoho,' 'Campbell Early' and 'Muscat Bailey A' mature vines were used for this experiment. These were grown at experimental farm in Osaka Prefecture University. In each cultivar, 21 bearing shoots of similar development were chosen, and clusters were pruned to one per shoot. The shoulder of cluster was eliminated 4 days before full bloom. The average flower number per cluster after elimination of the shoulder was 310 for 'Kyoho,' 230 for 'Campbell Early,' and 450 for 'Muscat Bailey A' grapes. A random set of 6 of the 21 clusters for each cultivar was bagged using a gauze bag, in order to determine changes in the rate of berry abscission. The remaining clusters were used to determine the PA levels in each cultivar. The number of fallen berries in the bag was counted at two-day intervals, beginning two days before full bloom and continuing to 18 days after full bloom (DAB). Between days 20 to 27, the numbers of berries on the clusters and the number of seeds per berry were determined. Flowers and berries from each cultivar were randomly sampled at 0, 3, 6, 9, 15 DAB; each sample was drawn from three clusters. Replicate samples (500 to 750 mg) of flowers and berries were frozen in liquid nitrogen and stored at -30℃ until PAs extraction.

Polyamine extraction. PAs were extracted as specified by Smith (1991). Samples were homogenized in cold 10% perchloric acid (PCA) (0.1 g tissue/ml PCA) using a glass homogenizer, and the homogenate was maintained at 4℃ for 30 min. The extracts were centrifuged for 20 min at 12,500g, and the supernatant fraction was used to determine levels of free PAs and of PCA-soluble conjugated PAs. For the determination of PCA-insoluble bound PAs, the pellet was used. The pellet was washed in 5 ml of PCA, centrifuged for 20 min at 12,500g, and then resuspended in the original volume of PCA by vortexing. The pellet suspension and the original supernatant (0.2 ml each) were hydrolyzed for 18 hr with 0.2 ml of 12N HCl at 110℃ in a reaction vial. The hydrolysate was centrifuged and 0.1 ml aliquot of the supernatant was dried in vacuo at 60℃, then dissolved in 0.1 ml PCA. The soluble conjugated PAs were estimated as being the concentration of PAs in the hydrolysate of the original supernatant less the concentration of free PAs.

Dansylation of polyamines and HPLC analysis. The PCA extracts were dansylated according to the methodology of Flores and Galston (1982). An aliquot (0.1 ml) of extract was added to 0.2 ml saturated sodium carbonate and 0.4 ml dansyl chloride in acetone (7.5 mg/ml). The mixture was incubated at 60℃ for 30 min in the dark. To eliminate excess dansyl chloride, 0.1 ml of proline (0.1 g/ml) was added to the mixture, which was then incubated at room temperature for 15 min in the dark. Dansylated PAs were extracted by vortexing for 1 min with 0.5 ml toluene, and a 0.2 ml aliquot was dried. The resulting derivatives were dissolved in methanol and analyzed by reverse-phase HPLC with a fluorescence detector. Excision and emission wavelengths were respectively 365 and 510 nm. Samples were eluted from the reversed-phase HPLC column (4.6 × 250 mm) using a linear solvent gradient, from 60% methanol in pH 3.5 acetate buffer to 95%

methanol in 25 min; the 95% methanol runs for 10 min. Flow rate of the solvent was 1 ml/min in all gradient steps. Extraction and analysis of PA for each sample was performed in triplicate.

Results

Berry drop and seed content. In all the cultivars tested here, berry dropping occurred with flowering, and had almost ceased by 18 days after full bloom (DAB). Berry dropping of 'Muscat Bailey A' grapes increased gradually after full bloom, and peaked at 6 DAB; 26.5% of berries fell at the peak (Fig. 1). After the peak the berry drop rate decreased, with no clear dropping on days 16 and 18. In 'Kyoho' grapes, berry dropping had hardly begun by day 6, but obvious dropping was found at 8 and 10 DAB. About 21% and 25% of the berries in one cluster respectively fell at 8 and 10 DAB. From 10 DAB, the rate of berry dropping of 'Kyoho' grapes decreased as in 'Muscat Bailey A.' In 'Campbell Early,' discernible peaks in the berry dropping rate were found at days 6 and 16. However, the dropping rates at these peaks were not as high as at the peaks for 'Muscat Bailey A' and 'Kyoho' grapes; the rates of berry dropping of 'Campbell Early' grapes at 6 and 16 DAB were
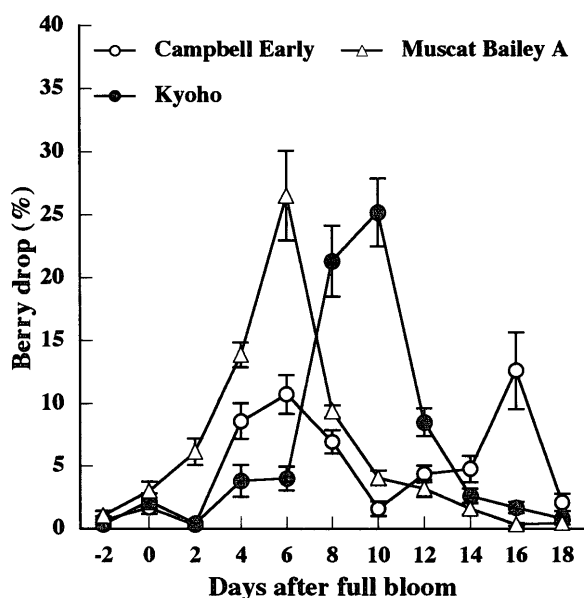
10.7 and 12.6% respectively. The berry setting rate was significantly higher in 'Campbell Early' than in the other two cultivars: 43.5% of berry setting by that stage in 'Campbell Early,' 26.8% in 'Kyoho' and 29.8% in 'Muscat Bailey A' grape berries (Fig. 2). In this experiment there were no seedless berries or berries containing poorly developed seeds. The average seed number per berry was greatest in 'Campbell Early' grapes, and least in 'Kyoho' grapes.

Changes in polyamine levels during berry drop. In all fractions (free, PCA-soluble conjugated, and PCA-insoluble bound), putrescine (Put), spermidin (Spd) and spermine (Spm) were detected in extracts from flowers and berries of 'Kyoho,' 'Campbell Early' and 'Muscat Bailey A' grapes (Fig. 3, 4, 5). Put levels were the highest in all cultivars, followed by Spd and Spm, in all fractions and throughout the experiment. Also, the PCA-insoluble bound PA fraction was the largest in each cultivar. The Spm extracted from each

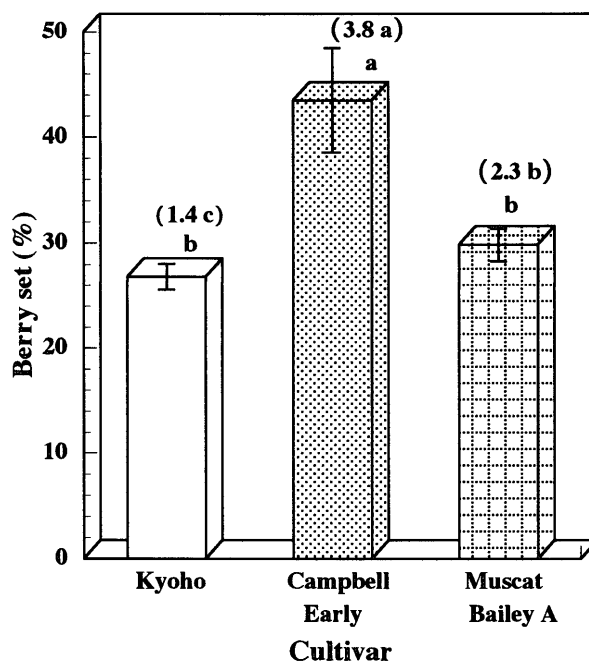Fig.1. Changes in the berry dropping rate of 'Kyoho,' 'Campbell Early' and 'Muscat Bailey A' grapes.

Fig.2. The rate of berry setting and seed number per berry after cessation of berry dropping in 'Kyoho,' 'Campbell Early' and 'Muscat Bailey A' grapes. The number in the parenthesis is the seed number per berry. Data with the same letters are insignificantly different by Sheffe's F test, $P<0.05$.

cultivar showed little change in concentration across fractions. Free Put levels were greatest at full bloom in each cultivar, and for 'Kyoho,' 'Campbell Early' and 'Muscat Bailey A' grapes respectively were 76.3, 73.7 and 44.9 nmol/gfw

(Fig. 3). In 'Kyoho' grapes, the free Put concentration decreased markedly from 75.6 to 30.9 nmol/gfw from day 3 to day 6. After day 6, levels of free Put scarcely changed. Free Put in 'Campbell Early' grapes decreased
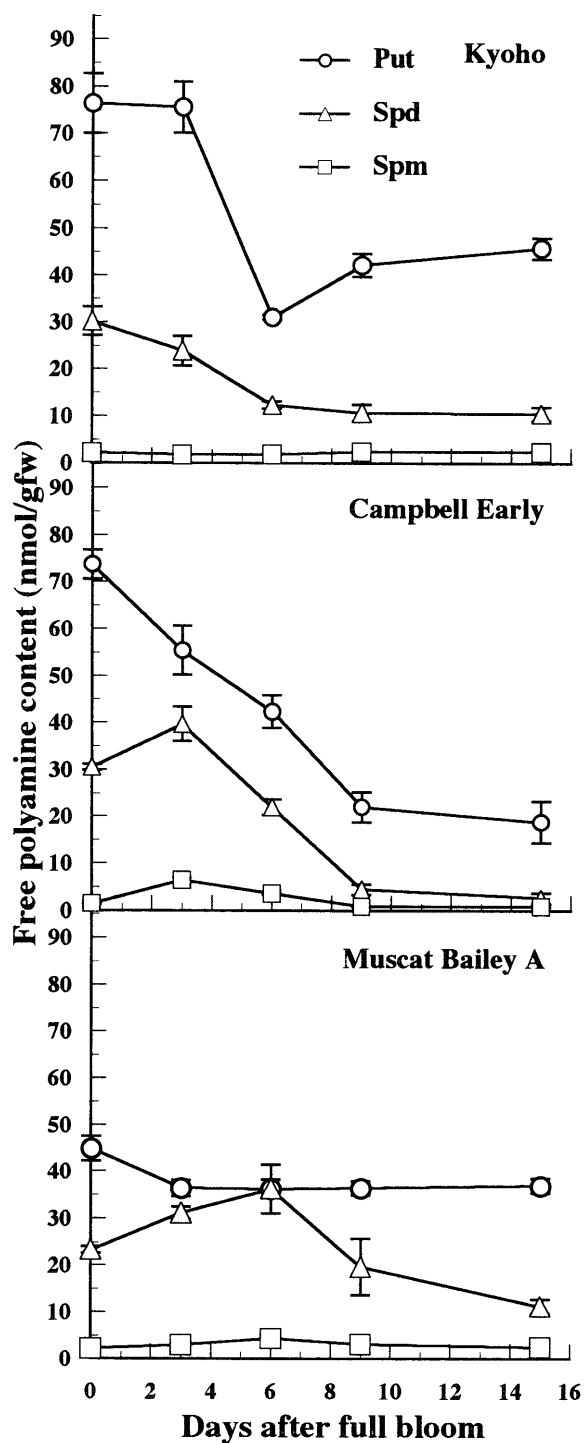

Fig.3. Changes in free polyamine levels during berry setting of 'Kyoho,' 'Campbell Early' and 'Muscat Bailey A' grapes.
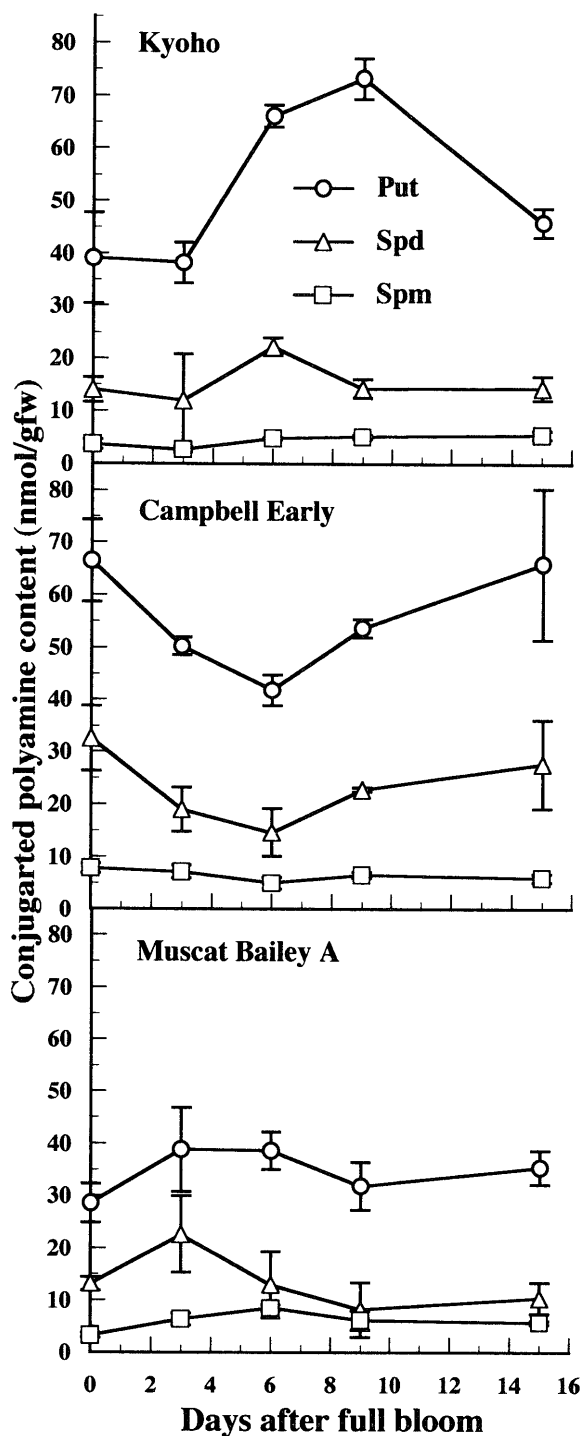
Fig.4. Changes in conjugated polyamine levels during berry setting of 'Kyoho,' 'Campbell Early' and 'Muscat Bailey A' grapes.

gradually by 30% from full bloom to 9 DAB. In 'Muscat Bailey A' grapes, free Put decreased slightly from full bloom to 3 DAB, and its levels then changed little up to 15 DAB. The levels of free Spd at full bloom in all cultivars w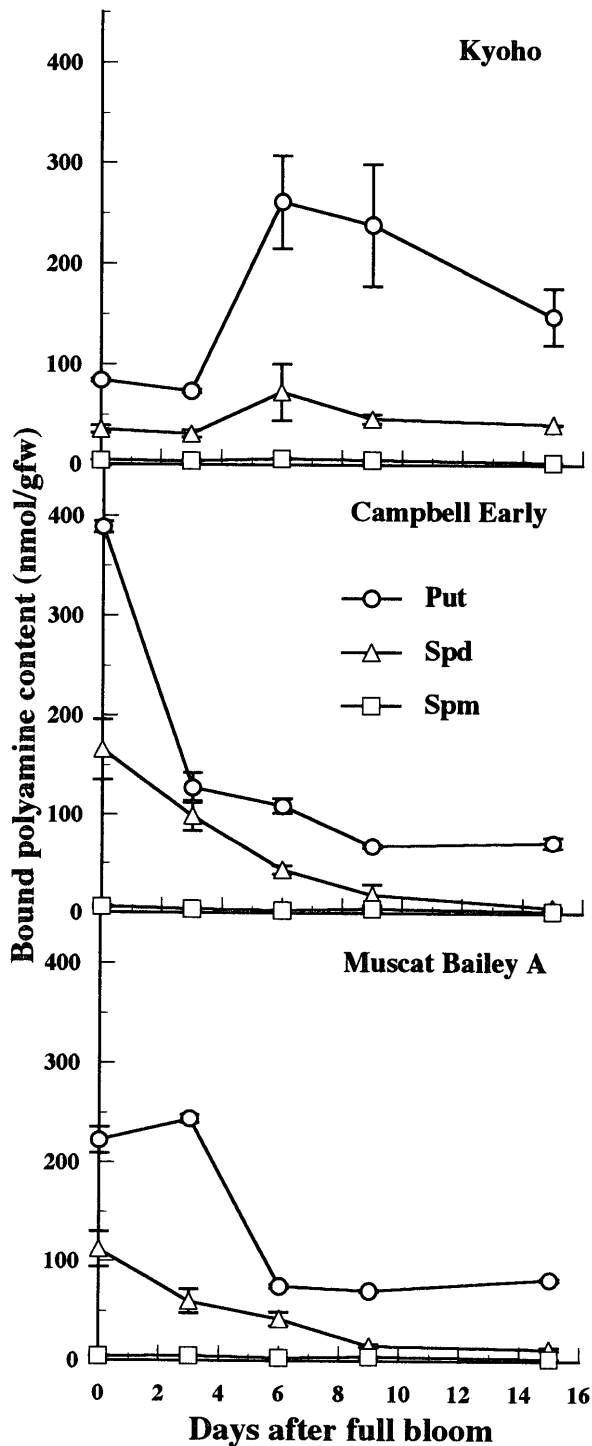ere about 40-50% of those of free Put. In 'Kyoho' grapes, free Spd concentration decreased between day 0 and 6, leveling off by 15 DAB. In 'Campbell Early' and 'Muscat Bailey A' grapes, the levels of free Spd increased slightly after full bloom, decreasing thereafter with berry development.

Mean levels of PCA-soluble conjugated PAs were least in 'Muscat Bailey A' grapes throughout the experiment. Changes in the levels of PA conjugates depended on cultivar (Fig. 4). However, the levels of Put and Spd conjugates changed in similar fashion in each cultivar, in contrast to free Put and Spd. In 'Kyoho' grapes, Put conjugate increased by 27.9 nmol/gfw between 3 and 6 DAB, and Spd conjugate increased about 87% at 6 DAB. This is the interval in which levels of free Put decreased. After peaking, Put and Spd conjugates decreased nearly to the level found on day 3. Put and Spd conjugates in 'Campbell Early' grapes decreased from full bloom to 9 DAB, then increased to approximately the full bloom level by 15 DAB. In 'Muscat Bailey A' grapes, Put conjugate levels increased to 3 DAB and remained constant to 6 DAB, then decreased to the full bloom level. An increase in the level of Spd conjugate was also found at 3 DAB.

Changes in the levels of PCA-insoluble bound PAs, especially Put and Spd, were substantially different in 'Kyoho' grapes from the other two cultivars (Fig. 5). Moreover, bound Put and Spd showed changes similar to their conjugates in the 'Kyoho' grape. At full bloom, the levels of bound Put and Spd in 'Campbell Early' and 'Muscat Bailey A' grapes were about 5.4 and 4.8 times greater than the levels of free Put and Spd, but in the 'Kyoho' grape their levels were nearly the same as levels of free Put and Spd. In 'Campbell Early' and 'Muscat Bailey A' grapes, the levels of bound Put and Spd decreased with berry development, while in the 'Kyoho' grape, bound Put increased by 187.8 nmol/gfw and Spd by 43 nmol/gfw to 6 DAB (when free Put decreased), decreasing thereafter with berry development. Bound Spd in the 'Kyoho' grape, like Spd conjugates, increased by about 143% to 6 DAB.

Fig.5. Changes in bound polyamine levels during berry setting of 'Kyoho,' 'Campbell Early' and 'Muscat Bailey A' grapes.

Discussion

The berry abscission profile of some grape cultivars has been hitherto showed in a previous paper (Komatsu and Nakagawa,1989), in which the lowest berry setting rate was observed in the tetraploid grapes 'Kyoho'. The number of seeds per berry depends on cultivars (Olmo, 1946) and was less in 'Kyoho' grapes than 'Campbell Early' or 'Muscat Bailey A' (Komatsu and Nakagawa,1989). Present observations confirm these results.

In free PAs, especially in the dominant Put, the highest level was found at full bloom in all three cultivars, as in other plants (Biasi et al., 1988; Nathan et al., 1984; Palavan and Galston, 1982). This could distinguish PAs from other plant growth regulators (PGRs) such as auxin or gibberellin, which are found at very low levels at full bloom, and increase along with the rapid development of berries in some grape cultivars (Inaba et al., 1976; Nitsch, et al., 1960). This also implies that PAs might be more directly involved in the berry setting process starting just after full bloom than other PGRs.

Comparison of changes in the berry dropping rate and PA levels reveals that the relation between berry abscission and PA levels varies with the cultivar. In three cultivars, berry dropping was observed earliest in 'Muscat Bailey A.' This cultivar had the lowest PA levels in free and conjugated fractions at full bloom. Although the total free PA level was almost the same in 'Kyoho' and 'Campbell Early' grapes at full bloom, the level in 'Muscat Bailey A' grapes was about 30% lower. It has been reported that male and imperfect hermaphroditic flowers in which the pistil does not develop properly comprise about 40 to 60% of flowers per single cluster in 'Muscat Bailey A'; moreover the berry setting rate of imperfect hermaphroditic flowers is inferior to that of perfect hermaphroditic flowers (Iizuka et al., 1968; Takagi et al., 1977). There is abundant evidence that PAs are involved in flower morphogenesis in grapes and some other plants (Applewhite et al., 2000; Geny et al., 1997; Geny et al., 1999; Tarenghi and Martin-Tanguy 1995). The lower levels of PA in 'Muscat Bailey A' could therefore be closely correlated with inferior development of the pistil. In 'Muscat Bailey A', although berry dropping was dramatic with a distinct peak 6 days after full bloom, no obvious change in the levels of PA was found that correlated with it. This suggests that, in 'Muscat Bailey A' grapes, imperfect flower morphology, due probably to low levels of PA, is the primary cause of berry dropping, rather than changes in endogenous PA levels after full bloom.

In 'Kyoho' and 'Campbell Early,' however, changes in the levels of PA, and in particular free PA, are more influential in berry setting. A reduction in free PA related to berry dropping was commonly found in these two cultivars. The decrease in free PA, especially Put, just before the peak of berry drop was more pronounced in 'Kyoho,' which had a clearer peak of dropping than 'Campbell Early'. It is proposed that free PA is involved in regulating berry setting, and the involvement of free PA seems to be more significant in 'Kyoho' grapes in which the berry set is the poorest. Okamoto (1982) demonstrated that in 'Muscat of Alexandria' grapes, which have a poor berry set in cultivation, degeneration of ovules occurred just before berry dropping following full bloom; only about 20% of all the dropped berries had normal ovules. Degeneration of ovules after full bloom was likewise observed in 'Kyoho' grapes (Komatsu, 1996). It seems that a decrease in the levels of free PA is closely correlated with ovule development.

As regards the roles of conjugated and bound PA, their participation in berry setting may not be as direct as that of free PA. In general, conjugated PAs are linked to compounds of low molecular weight. Bound PAs are found in covalent linkage with high molecular weight compounds, including protein (Dinnella et al., 1992; Mizrahi et al., 1989) and cell wall components such as uronic acid (Goldberg and Perdrizet, 1984) and lignin (Valee et al., 1983). Although a role of conjugated and bound PAs has been claimed on floral initiation and flowering, there is no information available on their precise role in other physiological phenomena such as fruit

set and development. It has been reported that conjugated and bound PAs decreased rapidly to low levels after anthesis in *Vitis vinifera* 'Cabernet Sauvignon' and 'Merlot' (Geny et al., 1997; Geny et al., 1999). In our experiment, however, although bound PA levels decreased dramatically after full bloom in 'Campbell Early' and 'Muscat Bailey A', changes in the levels of conjugated PA differed greatly between cultivars, and the pattern for bound PA in the 'Kyoho' grape was unique. Changes in conjugated and bound PA levels in 'Kyoho' grapes showed trends opposite to that of free PA. Translocation of PA must be significant in regulating PA levels in plant tissues and organs, including grapes (Friedman et al., 1986). However, there is no direct evidence that PA, except in free form, can be translocated through the xylem and phloem (Antognoni et al., 1998). Availability of free PA therefore seems to determine the levels of conjugated or bound PA in the corresponding tissue or organ. The elevated levels of conjugated and bound PA from day 3 to 6 were respectively about 28 and 180 nmol, and the sum of these was about 5 times the decreased level of free PA in the same period in 'Kyoho' grapes. This finding indicates that free PA existed already, and also that free PA was *de novo* synthesized or translocated from other organs to be rapidly conjugated during that period. It follows that an activated conjugation process of PA is responsible for the depletion of free PA, causing the heavy drop of berries in 'Kyoho'.

In conclusion, there might be no simple correlation between PA levels *per se* and berry set. Based on our results, however, it is clear that abscission potential and berry set depends on the free PA level of the berries, especially free Put. This is remarkable in a cultivar susceptible to "Coulure" such as the 'Kyoho' grape.

References

Ahmedullah, M. 1983. Pollen morphology of selected Vitis cultivars. J. Amer. Soc.Hort. Sci., **108**, 155-160.

Antognoni, F., Fornale, S., Grimmer, C., Komor, E., and Bagni., N. 1998. Long-distance translocation of polyamines in phloem and xylem of *Ricinus communis* L. plants. Planta, **204**, 520-527.

Applewhite, P. B., Kaur-Sawhney, R., and Galston, A. W. 2000. A role for spermidine in the bolting and flowering of Arabidopsis. Physiol. Plant., **108**, 314-320.

Biasi, R., Bagni, N., and Costa, G. 1988. Endogenous polyamines in apple and their relationship to fruit set and fruit growth. Physiol. Plant, **73**, 201-205.

Biasi, R., Costa, G., and Bagni, N. 1991. Polyamine metabolism as related to fruit set and growth. Plant Physiol. Biochem., **29**, 497-506.

Brown, K. M. 1997. Ethylene and abscission. Physiol. Plant., **100**, 567-576.

Carraro, L., Lomnardo, G., Cargnello, G., and Gerola, F. M. 1979. Studies on the embryo sac and on the stigmatic receptivity of Vitis cultivars with different productivity (Picolit giallo and Verduzzo friulano). Vitis, **18**, 285-290.

Cargnello, G., Carraro, L., Lombardo, G., and Gerola, F. M. 1980. Pollen morphology of Picolit grown in different Italian regions. Vitis, **19**, 201-206.

Caspari H. W., Lang, A., and Alspach, P. 1998. Effects of girdling and leaf removal on fruit set and vegetative growth in grape. Amer. J. Enol. Vitic., **49**, 359-366.

Costa, G. and Bagni, N. 1983. Effects of polyamines on fruit-set of apple. HortSci., **18**, 59-61.

Coombe, B. G. 1962. The effect of removing leaves, flowers and shoot tips on fruit-set in *Vitis vinifera* L.. J. Hort . Sci., **37**, 1-15.

Crisosto, C. H., Lombard, P. B., Sugar, D., and Polito, V. S. 1988. Putrescine influences ovule senescence, fertilization time, and fruit set in Comice pear. J. Amer. Soc. Hort. Sci., **113**, 708-712.

Dinnella, C., Serafini-Fracassini, D., Grandi, B., and Duca, S. D. 1992. The cell cyclein Helianthus tuberosus: analysis ofpolyamine-endogenous protein conjugates by transglutaminase-like activity. Plant Physiol. Biochem., **30**, 531-539.

Flores, H. E. and Galston, A. 1982. Analysis of polyamines in higher plants by high perform-

ance liquid chromatography. Plant Physiol., **69**, 701-706.

Friedman, R., Levin, N., and Altman, A. 1986. Presence and identification of polyamines in xylem and phloem exudates of plants. Plant. Physiol., **82**, 1154-1157.

Geny, L., Broquedis, M., Martin-Tanguy, J., and Bouard, J. 1997. Free, conjugated and wall-bound polyamines in various organs of fruiting cutting of *Vitis vinifera* L. cv. Cabernet Sauvignon. Am. J. Enol. Vitc., **48**, 80-84.

Geny, L., Colin, L., Brezillon, I., and Broquedis, M. 1999. Analysse des polyamines conjugates aux acides hydroxycinnamiques dans les fleurs de vigne et les baies de raisin. Vitis, **38**, 157-160.

Goldberg, R. and Perdrizet, E. 1984. Ratio of free to bound polyamines during maturation in mung bean hypocotyls cells. Planta, **161**, 531-535.

Iizuka, M., Kim, K. R., Rao, L. M., and Kozaki, I. 1968. Unusual flower types in 'Muscat Bailey A' grapes. Japan. J. Breeding, **18**, 217-220.

Inaba, A., Ishida, M., and Sobajima, Y. 1976. Changes in endogenous hormone concentrations during berry development in relation to the ripening of Delaware grapes. J. Japan. Soc. Hort. Sci., **45**, 245-252.

Komatsu, H. 1996. Berry shatter. *In* "Nippon Budou Gaku" (eds. by Nakagawa, S., Horiuchi, S., and Matsui, H). Yokendo Ltd. Tokyo, 440-453.

Komatsu, H. and Nakagawa, S.1989. Characteristics of berry setting in 'Kyoho' grape. Proc. Fac. Agri., Kyushu Tokai Univ., **8**, 1-8.

Mitra S. K. and Sanyal, D. 1990. Effect of putrescine on fruit set and fruit quality of Litchi. Gartenbauwissenshaft, **55**, 83-84.

Mizrahi, Y., Applewhite, P. B., and Galston, A. W. 1989. Polyamine binding to proteins in oat and petunia protoplasts. Plant Physiol., **91**, 738-743.

Naito, R., Fujimoto, J., and Yamamura, Y. 1983. Effects of shoot vigor and SADH application to clusters before anthesis on berry set in Kyoho grapes. J. Amer. Enol. Vitc., **34**, 142-147.

Naito, R. and Kawashima, T. 1980. Promotion of berry set in grapes by growth retardants. IV. Comparison of SADH cluster dipping, shoot pinching and flower thinning with regards to their effect on berry set in Kyoho grapes. J. Japan. Soc. Hort. Sci., **49**, 297-310.

Nathan, R., Altman, A., and Monselise, S. P. 1984. Changes in activity of polyamine biosynthetic enzymes and in polyamine contents in developing fruit tissue of 'Murcott' mandarin. Sci. Hort., **22**, 359-364.

Nitsch J. P., Pratt, C., Nitsch, C., and Shaulis, J. 1960. Natural growth substances in Concord and Concord seedless grapes in relation to berry development. Amer. J. Bot., **47**, 566-576.

Okamoto, G. and Imai, S. 1982. A histological study of berry setting in 'Muscat of Alexandria' grapes. J. Japan. Soc. Hort. Sci., **50,** 436-444.

Okamoto, G., Yamamoto, K., and Shimamura, K. 1984. A study on the difference in the occurrence of seedless berries among the tetraploid grapes related to Kyoho variety. J. Japan. Soc. Hort. Sci., **53**, 251-258.

Olmo, H. P. 1946. Correlations between seed and berry development in some seeded varieties of *Vitis vinifera*. Proc. Amer. Soc. Hort. Sci., **48**, 291-297.

Palavan, N. and Galston, A.W. 1982. Polyamine biosynthesis and titer during various developmental stages of *Phaseolus vulugaris*. Physiol. Plant., **55**, 438-444.

Pratt, C. 1973. Reproductive system of 'Concord' and two sports (*Vitis labruscana* Bailey). J. Amer. Soc. Hort. Sci., **98**, 489-496.

Rugini, E. and Mencuccini, M. 1985. Increase yield in the olive with putrescine treatment. Hort Sci., **20**, 102-103.

Singh, Z. and Singh, L. 1995. Increase fruit set and retention in mango with exogenous application of polyamines. J. Hort. Sci., **70**, 271-277.

Smith T. A. 1991. Chromatographic methods for the identification and quantitation of polyamines. *In* "Biochemistry and Physiology of Polyamines in Plants" (eds. by Slocum, R.D. and Flores, H.E.). CRC Press. Boca Raton Ann Aorbor London, 229-242.

Takagi, T., Furukawa, Y., Soejima, J., and Tomana, T. 1977. Studies on the stabiliza-

tion of GA-induced seedless berry production on Muscat Bailey A grapes. I. Variation in flower types and its effect on seedlessness. J. Japan. Soc. Hort. Sci., **40**, 173-180.

Tarenghi, E. and Martin-Tanguy, J. 1995. Polyamines, floral induction and floral development of strawberry (*Fragaria ananassa* Duch.) Plant Growth Regul., **17**, 157-165.

Vallee J.C., Vansuty, G., JNegrei, J., Pedrizet,

E., and Prevost, J. 1983. Misen en evidence d'amines liees a des structures cellulaires chez Nicotiana tabacum et Lycopersicum esculentum. Physiol. Plant., **57**, 143-148.

Winkler, A. J., Cook, J. A., Kliewer, W. K., and Lider, L. A. 1962. General Viticulture, Univ. Calf. Press, Berkley, 710pp.