Biochemical Changes in Berry Development in the Cardinal Grape, *Vitis vinifera*, L.[1]

Justo López García[2, 3]

ABSTRACT

The Cardinal grape cultivar is widely cultivated, especially in California and Arizona. This cultivar sets both seeded and seedless (shot) berries on the same cluster, which poses a problem in grading and packing for marketing. Investigations were conducted in an attempt to solve it.

Maleic hidrazide, indoleacetic acid, gibberellic acid, and the removal of 0.64 cm from the floral apex did not reduce the number of shot berries, when applied at prebloom or at 50% bloom. There were highly significant differences in nucleic acid content between the prebloom, full bloom, 2 weeks after full bloom, and ripe stages. There were no significant differences between the sections of the cluster at the same growth stages. There were significant differences on protein content between the four growth stages. There were no significant differences between the sections of the clusters.

INTRODUCTION

Grapes are considered to be one of the most important fruits from the economic standpoint. They are used fresh as table grapes, processed as raisins, or for the production of wine. The economy of several countries depends to a major extent on grape production.

World consumption of table grapes is constantly increasing. Puerto Rico imports table grapes from the United States mainland and from other countries. According to the Puerto Rico Department of Agriculture, imports from the United States are increasing yearly. During fiscal year 1975–76, Puerto Rico imported almost 3,000 tons of grapes valued at about $2 million (2).

Cultivar Cardinal is one of the leading grapes in California and Arizona. It was obtained from a cross of the Flame Tokay × Ribier (Alphonse Lavalée) cultivars (19). The Cardinal, as grown in Arizona, has mainly large, purple-colored, seeded berries. It also has the characteristic of setting a variable number of seedless berries, which fail to abscise and remain on the clusters through the ripening period as small, usually green, shot berries. The presence of large numbers of shot berries reduces the grade of the clusters and increases costs because of the time and labor required to remove them during packing (17).

Several studies have been conducted in an effort to clarify the correlations between berry development in seeded grape cultivars and the

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[2] Associate Horticulturist and Administrator, Corozal Substation, Agricultural Experiment Station, Mayagüez Campus, University of Puerto Rico, Río Piedras, P.R.

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ultimate quality of the clusters. Olmo (16) reported that shot (tiny seedless) berries might be due to lack of pollination. He concluded that pollination was necessary to obtain both seeded and seedless berries. Meanwhile, Sharpless et al. (18), studied the effect of insect pollinators on cultivar Cardinal, and concluded that caging vines with honey bees did not increase the set of normal seeded berries or reduce the number of shot berries.

It appears that during the development of berries, biochemical changes occur which might be responsible for the setting of both types of berries. In both the pollination and the fertilization processes, presumably endogenous growth hormones are involved. As the flower develops, the activity of these substances is expected to increase, reaching a peak at or near the opening stage. Nistch et al. (15), found that the seedless strain of Concord grape had a higher growth rate and higher auxin content than the seeded strain of the same cultivar, in a period between bloom and about 7 days after bloom. However, between 20 to 26 days after bloom, the endosperm developed rapidly in the seeds and higher growth rate of the berries and higher auxin content were observed during this period. In the Concord seedless strain, on the contrary, the endosperm started to degenerate at this time, and both growth rate and the auxin content level decreased.

Some growth regulators have been used in an effort to reduce the number of shot berries to improve the quality of clusters of the Cardinal grape.[4] This paper reports on a study of the effect of growth regulators and floral apex removal on the development of shot berries in the Cardinal grape cultivar, and on the content of nucleic acid and protein in the clusters of this cultivar during four stages of development.

MATERIALS AND METHODS

The study was performed at the University of Arizona at Tucson. The vines were about 15 years old, looked healthy and free of insects. They had been pruned the last week of December of the previous year, following the cordon pruning method (21).

For the different treatments, 165 clusters were randomly selected and tagged. The treatments given, either at prebloom (about 1 week before bloom) or at 50% bloom, were as follows: a) Maleic hydrazide (MH) at 10, 50, and 100 p/m; b) indoleacetic acid (IIA) at 10, 50, and 100 p/m; c) floral apex removal (0.64 cm); d) gibberellic acid (GA) at 20 p/m; and e) control.

Ten clusters were used per treatment, except for GA, for which 15 clusters were used.

MH, IIA, and GA solutions were freshly prepared and applied using the dipping method. The floral apex removal consisted of the pruning of

[4] Kuykendall, J. R., personal communication.

about 0.64 cm from the apex of the clusters. This operation was performed with standard grape-thinning shears.

Data were taken on the total number of normal seeded berries, and the number of shot berries. The number of normal seeded berries was also recorded for the GA treatment. The berries of this treatment were cut across with a razor blade in order to determine the number of seeds, if any.

Preliminary observations revealed that there was an apparent tendency for the first flowers to open on the middle section of the clusters, the next on the top, and the last on the apex. There was also a tendency for the clusters to set more shot berries on the top and apex than on the middle. In order to try to confirm this preliminary observation an experiment was designed to study the flower-opening pattern of this cultivar.

Fifteen clusters were randomly selected and tagged during the spring of the second year. The number of flowers on each shoulder, as well as the number of shoulders of each cluster, were counted and recorded. To facilitate the recording of the data, a diagram of each cluster was prepared. Each shoulder was numbered according to its position on the cluster. The flowers were counted when they were large enough to facilitate the operation, but well before they started to open.

To record the number of flowers as soon as they opened, the orchard was visited twice a day, in the early morning and in the late afternoon. As soon as the flowers opened, they were counted and recorded on the diagram. In general, all the flowers of a determined cluster opened in 3 days.

Although the main objective of this part of the study was to determine the flower opening pattern of this cultivar, observations were continued until harvest. The number of normal seeded and shot berries was counted for each section of the clusters. For this purpose the clusters were divided into three sections: top (shoulders 1 through 5), middle (shoulders 6 through 11), and apex (shoulders 12 through the tip).

During the second year, another experiment was designed to study the NA content of the sections of the clusters at different growth stages and the possible correlation with the set of shot berries.

About 100 clusters were randomly selected and tagged. The material was harvested at the following growth stages: prebloom (about 1 week before the first flower opened); first flower opened; 50% bloom; full bloom, shattering; 2 weeks after full bloom; green stage; color break; and ripe. The clusters were divided into three sections: top, middle, and apex. The harvested material was immediately frozen with dry ice. The frozen material was kept in a freezer until the biochemical assays were performed.

Several methods have been used for the NA determinations of plants and fruits (8, 20), but for this study the Cherry II method, slightly

modified was followed (3). The modification consisted of the following: in step No. 2 the insoluble pellet was washed four times, instead of three, with methanol and in step No. 3 the residue was extracted twice, instead of once, with perchloric acid.

Centrifugations were performed in a Sorvall SS - Automatic Superspeed[5] centrifuge. Absorbancy at 260 and 290 nan (UV) was read in a Beckman Reading Quartz Spectrophotometer. The difference between the readings at 260 and 290 nan was multiplied by a factor of 57 in order to determine the total NA. The DNA determinations were made following the method of Burton (1).

During the third year, an experiment was designed to determine the protein content of the sections of the clusters in an attempt to find the degree of correlation, if any, between this and the set of shot berries. Sixty-five clusters were randomly selected and tagged. The material was harvested at prebloom, full bloom, 2 weeks after full bloom, and ripe stages. Material was also harvested at the first flower open stage, 50% bloom, green, and color break stages. The harvested material was immediately frozen with dry ice. The frozen material was kept in a freezer until the protein determinations were made. These were performed following the method of Lowry et al. (14).

All data were tabulated and statistically analyzed.

RESULTS AND DISCUSSION

The data indicated that MH at 10 p/m, applied at prebloom, tended to reduce the number of shot berries. However, MH at 50 and 100 p/m, IIA at 10, 50, and 100 p/m, and floral apex removal greatly increased the number of shot berries.

When the same treatments were applied at 50% bloom, MH at 10 p/m and floral apex removal tended to reduce the number of shot berries. The other treatments significantly increased the number of shot berries. Floral apex removal, at either growth stage, considerably improved the shape and quality of clusters. Similar findings have been reported by Sharpless et al. (17).

When GA at 20 p/m was applied at prebloom and at 50% bloom, the whole clusters were notably affected. Generally, epinasty was noted on almost all treated clusters. Both the rachis and berries were greatly affected. The ripe berries abscised when the clusters were harvested. About 84% of the normal berries were seedless. The number of shot berries was markedly increased (69% GA, 31% control).

[5] Trade names are used in this publication solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico or an endorsement over other equipment or materials not mentioned.

It seems likely that GA applied at these growth stages, at this concentration, sufficed for both the pollination and fertilization processes resulting in seedless berries. It also appears that either the concentration used or the stages of development at which the treatment was given were not the appropriate ones to reduce the number of shot berries or to produce 100% normal seedless berries. GA has been used on seeded cultivars to produce almost 100% seedless berries (4, 11). Several investigators have reported that the GA activity was always more marked on the seedless cultivars than on the seeded ones (5, 9, 13, 22).

Although the growth substances and floral apex removal, as used in the present study, tended to affect the setting of shot berries on the Cardinal grape cultivar, the results suggest that additional studies should be performed in an effort to obtain more precise information on the problem.

The data on flower opening pattern recorded the second year tend to confirm the information gathered during the previous year. Thirteen out of 15 clusters studied behaved similarly. The flowers started to open at the middle section of the cluster, then at the top, and finally at the apex.

The total population comprised 10,336 flowers on 15 clusters. Usually, the top sections had a larger number of flowers than each one of the other sections. The tops had a total of 5,573 flowers; the middles 2,678; and the apices 2,085.

The 15 clusters produced a total of 1,510 berries. Out of these, 1,086 were normal seeded and 424 shot berries. The top sections produced 478 seeded and 186 shot berries. The middles produced 297 seeded and 109 shot berries. Finally, the apices produced 311 seeded and 137 shot berries.

The largest number of shot berries was produced by tops and the smallest by the middles. It appears that the first flowers to open on the middle sections exerted an important action on the setting of berries on the other sections. If endogenous growth substances are synthesized as the flower develops, reaching a peak when it opens, it seems likely that these hormones are translocated to the tops and apices where they partially suffice for the pollination and fertilization processes. Coombe (6), when working with the grape cultivars Corinth and Sultanina, suggested that organic nutrients move from elsewhere in the plant into a defoliated shoot and permit normal set within its clusters. It is probable that growth substances might move within the plant similarly. It appears that some kind of stimulus, probably produced by endogenous hormones, is not strong enough for the setting of normal seedless berries and as a result the shot berries are set. Also, it might be that the flower, as it opens, acts as a source of endogenous growth substances.

Table 1 shows the total nucleic acid (NA) content of sections of the clusters at prebloom, full bloom, 2 weeks after full bloom, and ripe stages

of growth. Analyses of variance for the NA content at these stages of development indicated that there were highly significant differences between the stages of development, but not between the sections of the clusters at the same growth stages. There were no significant differences in RNA content between either the 2 weeks after full bloom and the ripe stage or between the prebloom and full-bloom stages (table 2). Similarly, there were no significant differences between sections of the clusters at the four stages of growth. Concerning the DNA content, there were significant differences between ripe, 2 weeks after full bloom, full bloom, and prebloom stages (table 3). Again, there were no significant differences between the sections of the cluster at these growth stages.

The nucleic acid (NA) pattern at the different stages of development of the clusters is noteworthy. At the prebloom stage, when there was an

TABLE 1.—*NA concentration in Cardinal grape clusters at four stages of development*

Stage of development	Tops	Middles	Apices
	(mg NA/g DW, means of 4 replicates)		
Prebloom	199.7 a[1]	200.4 a	229.5 a
Full bloom	185.9 a	181.9 a	188.2 a
Two weeks after full bloom	15.4 b	14.5 b	14.1 b
Ripe	31.5 b	32.9 b	34.5 b

[1] Values in columns followed by the same letter do not differ significantly at the 5% level.

TABLE 2.—*RNA concentration in the sections of Cardinal grape clusters at four stages of development*

Stage of development	Tops	Middles	Apices
	(mg RNA/g DW, means of 4 replicates)		
Prebloom	195.0 a[1]	194.9 a	223.4 a
Full bloom	181.5 a	177.0 a	183.2 a
Two weeks after full bloom	14.4 b	12.4 b	13.2 b
Ripe	31.2 b	28.7 b	30.1 b

[1] Values in columns followed by the same letter do not differ significantly at the 5% level.

active enzymatic activity in the flower, the NA, content was higher than in the other stages. At 2 weeks after full bloom, the NA content reached the lowest level, probably, because at this stage the enzymatic activity was very slow. After this stage was over, the physiological processes rose again and the NA content consequently increased, but not to the same levels as in the first two stages.

The NA content per flower indicated that there were slight differences both between stages of development and between sections of the clusters. At the first-flower-opened stage, the NA content was higher than at the 50% bloom, full bloom, or shattering stages. At the 50% bloom, the NA content was at the lowest levels.

On a per berry basis, the NA content showed that the middle sections at the green and ripe stages were markedly lower than the other sections of the clusters at these stages. At color-break stage, the NA content was always higher than at the other stages. The DNA content per berry was lower in the middles at the color-break and ripe stages.

The seeds are active organs of the plant. These are supposed to be rich both in protein and NA. The NA content per seed was rather low at the green stage, but increased at color break and then slowed down slightly when ripe. At the green stage, the seed is still growing, attaining maturity at color break. A similar pattern was followed by RNA. DNA increased all the way from the green to the ripe stage.

The study of the NA of the three sections of the clusters at different stages of growth did not show any direct relationship between these and the setting of shot berries.

Data on the protein content of the three sections of the clusters at four stages of development are presented in table 4. There were significant differences between stages of growth. The highest values were obtained

TABLE 3.—*DNA concentration in the sections of Cardinal grape clusters at four stages of development*

Stage of development	Tops	Middles	Apices
	(mg DNA/g DW, means of 4 replicates)		
Prebloom	4.7 a[1]	5.5 a	6.1 a
Full bloom	4.4 a	4.9 b	5.0 b
Two weeks after full bloom	1.0 c	2.1 d	0.9 d
Ripe	4.3 b	4.2 c	4.4 c

[1] Values in columns followed by the same letter do not differ significantly at the 5% level.

at the prebloom and the least at the 2-weeks-after-full-bloom stage. At prebloom, presumably, a high degree of enzymatic activity was taking place. At 2 weeks after full bloom, on the contrary, the smallest concentration of protein might be due to the fact that, at this stage, the tiny berries started to grow and active cell division was taking place. This is in accordance with the finding of Hulme (10), when working with apples.

There were no significant differences between the protein content of the sections of clusters at a given stage.

The data did not confirm the assumption that the setting of shot berries was related to the protein concentration in a given section of the cluster. It could be possible that the setting of shot berries is due to the kind of protein present rather than to the total protein content. Different attempts were made in an effort to separate the protein but, due to the high amount of tannins present in the grape tissue, the author was unable to accomplish it.

At the green stage, the apices had more protein than either the tops or the middles. When ripe, the middles had more protein than either the

tops or the apices. Protein content increased as the berries approached maturity. The ripening process requires enzymes, but as the fruit matures the reserve of protein in the seed increases (7).

At color break, the pulp had more protein than at the green or ripe stages. It seems likely that at this stage the berry synthesized the majority of the enzymes involved in the ripening process. The seeds are usually the richest organs in protein content. As the seed matured, the protein concentration increased steadily, reaching the highest values when ripe.

The highest values in protein content per flower (0.0136, 0.0135, and 0.0127 mg/g FW for the tops, middles, and apices, respectively) were obtained when the first flower opened, and the least values (0.0029, 0.0032, and 0.0045 mg/g FW) at full bloom. Probably, the physiological activities were at a maximum at the prebloom or near the stage when the first flower opened. These activities are controlled by enzymes. Therefore, more proteins were expected to be synthesized at this stage than either at 50% bloom or at full bloom.

TABLE 4.—*Protein content in the sections of Cardinal grape clusters at four stages of development*

Stage of development	Tops	Middles	Apices
	(mg/g DW, means of four replicates)		
Prebloom	0.3916 a[1]	0.3931 a	0.4882 a
Full bloom	.3011 b	.3493 b	.3715 b
Two weeks after full bloom	.1158 d	.0999 d	.0891 d
Ripe	.2534 c	.2533 c	.2083 c

[1] Values in columns followed by the same letter do not differ significantly at the 5% level.

The results herein reported suggest that additional investigations should be conducted in an effort to elucidate the problem of setting of both seeded and seedless (shot) berries on the same cluster in the Cardinal grape cultivar.

RESUMEN

Se realizaron varios experimentos con la uva de mesa del cultivar Cardinal. El propósito de estos trabajos era determinar la causa de la producción de frutas normales con semillas y frutas pequeñas sin semillas en el mismo racimo, ya que esto causa muchos problemas al momento de empacar las frutas.

Se usaron hidracida maleica y ácido indoleacético a razón de 10, 50 y 100 ppm. Otro tratamiento fue la poda de 0.64 cm del ápice del racimo. Estos tratamientos se dieron como una semana antes de florecer o cuando aproximadamente el 50% de las flores habían abierto. El ácido giberélico a razón de 20 ppm se usó dos veces: antes de florecer y cuando el 50% de las flores habían abierto.

Solamente la hidracida maleica a razón de 10 ppm aplicada antes de florecer tendió a reducir las frutas pequeñas sin simillas (shot berries). Los demás tratamientos no disminuyeron substancialmente el número de estas pequeñas frutas. El ácido giberélico produjo cerca de 84% de frutas normales sin semillas.

Se estudió el patrón de floración de los racimos. Se encontró que las flores abren primero en la sección del medio, luego en la parte superior y finalmente en el ápice del racimo. Se encontró que había más frutas pequeñas sin semillas en la parte superior y en el ápice que en el medio.

Se determinó el contenido en ácidos nucleicos en cuatro estadios de crecimiento: antes

de florecer, cuando todas las flores habían abierto, 2 semanas después de florecer y al madurar. Hubo diferencias significativas entre estos estadios de crecimiento, pero no entre las secciones de los racimos.

Igualmente se determinó el contenido en proteína para los mismos estadios de crecimiento. Hubo diferencias significativas entre éstos, pero no entre las secciones de los racimos.

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