

# Gibberellic Acid Levels and Quality Effects of Gibberellic Acid in Treated Table Grapes

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Submitted for publication: March 1992

Accepted for publication: September 1992

Key words: Gibberellic acid, table grapes, residue, bunch quality, growth regulator

**Sultanina and Waltham Cross vineyards were treated with increasing dosages and late applications of gibberellic acid. Berries with sampled at different stages after treatment as well as after four weeks' cold storage. The gibberellic acid levels of treated and untreated berries were determined using a gibberellic acid-specific radio-immunoassay. Irrespective of treatment, late applications and high dosages increased gibberellic acid levels in berries at harvest but did not exceed the 200 ng/g fresh berry mass maximum limit. The effects of treatments on bunch quality, i.e. ripeness, berry size, berry crack, berry shatter, decay, SO<sub>2</sub> damage, colour, blemishes, even berry size, straggleness and dry stems were also determined. Berry ripening was delayed and blemishes were induced by high GA dosages and late GA applications.**

In South Africa Sultanina and Waltham Cross (Dattier de Beyrouth) are the only two table grape cultivars treated with gibberellic acid (GA) to improve quality (Van der Merwe, Geldenhuys & Botes, 1991; Wolf, Van der Merwe & Orth, 1991). Sultanina bunches, which are normally compact with small berries, are treated with GA at bloom and after berry set to thin out berries and to increase berry size (Weaver, 1954).

Premium-quality Sultanina bunches with a minimum berry mass of 3,6 g are then produced and marketed as Thompson Seedless. Waltham Cross, known for setting seedless berries which are smaller at harvest than seeded berries of the same bunch, is treated with GA after berry set to ensure an even berry size. This ensures a top-quality product without incurring additional labour expenses to remove undersized berries.

The timing of GA applications is critical with regard to the physiological development stages of bunches (Wolf, Van der Merwe & Orth, 1991). Table grape producers tend to increase GA dosages for acceptable berry size when applications extend beyond the optimal physiological GA berry-sizing stage (10 mm berry size). These GA applications are made to compensate when large differences in physiological development of bunches occur. This could cause GA residues in treated table grapes to exceed the internationally set maximum limit of 200 ng/g fresh berry mass. Although naturally occurring GA levels have been determined in various cultivars from anthesis up to harvest (Coombe, 1960; Hagiwara, Ryugo & Olmo, 1980; Scienza *et al.*, 1978), no work has been done on Sultanina and Waltham Cross berries treated with high GA dosages and late GA applications or on berries which have undergone cold storage. In this study the effect of late applications and high GA dosages on residue levels of berries

from 10 mm berry size up to four weeks after harvest was investigated. The effect of each treatment on bunch quality was also determined.

## MATERIALS AND METHODS

**Experimental vineyard:** The Sultanina and Waltham Cross vineyards used for this study were situated in the Paarl table grape-producing region of the Western Cape, South Africa. The Sultanina vines were five years old, trained on a roof trellis (Uys, 1976) with a planting distance of 3 m x 3 m. The Waltham Cross vines were six years old, trained on a slanting trellis (Uys, 1976) with a planting distance of 3,6 m x 1,8 m. Both vineyards were grafted on rootstock Ramsey and irrigated by micro-jets.

**Experimental design:** Various GA dosages and a control treatment were compared on Sultanina (Table 1) and Waltham Cross vines (Table 2). A randomized experimental design consisting of three single-vine replicates was used. All bunches on a vine were treated with GA using a dip application and timed according to Wolf, Van der Merwe & Orth (1991). Bunch samples of three bunches from each vine were collected at five stages, i.e. 10 mm berry size, véraison, three weeks before harvest, at harvest and after four weeks' cold storage to determine GA levels. Waltham Cross berry samples were separated into seed and flesh samples. Bunches were harvested at an average ripeness of 18°Balling (B) for Sultanina and 15°B for Waltham Cross, packed according to South African export standards (Anonymous, 1990) and placed in cold storage at -0,5°C for four weeks. After cold storage various grape-quality parameters (Table 3) were evaluated according to the standard methods of the Nietvoorbij Institute for Viticulture and Oenology (Nietvoorbij).

*Acknowledgements: J.A. Viljoen, Rentia Theron and Aubrey Gerber for their technical assistance. Dr. J. Cutting for his advice and assistance during the GA-determinations.*

TABLE 1  
Treatments applied to Sultanina bunches.

No.	Gibberellic acid treatments	*Dosage (ppm)
1	Control	0
2	Late application (3 weeks pre-harvest)	1 x 40
3	Normal dosage	2 x 10 plus 3 x 20
4	Normal dosage plus late application	2 x 10 plus 3 x 20 plus 1 x 40
5	High dosage A	2 x 10 plus 3 x 40
6	High dosage A plus late application	2 x 10 plus 3 x 40 plus 1 x 40
7	High dosage B	2 x 10 plus 3 x 66
8	High dosage B plus late application	2 x 10 plus 3 x 66 plus 1 x 40

\*Dosages applied according to Wolf, Van der Merwe & Orth (1991).

TABLE 2  
Treatments applied to Waltham Cross bunches.

No.	Gibberellic acid treatments	*Dosage (ppm)
1	Control	0
2	Late application (3 weeks pre-harvest)	1 x 15
3	Normal dosage	1 x 20
4	Normal dosage plus late application	1 x 20 plus 1 x 15
5	High dosage	1 x 40
6	High dosage plus late application	1 x 40 plus 1 x 15

\*Dosages applied according to Wolf, Van der Merwe & Orth (1991).

TABLE 3  
Quality parameters for export table grapes.

Parameter	Unit
Ripeness	°B
Berry size	g
Berry crack	%
Berry shatter	%
Decay	%
SO <sub>2</sub> damage	%
Colour	1 to 5 <sup>a</sup>
Blemishes	1 to 5 <sup>b</sup>
Even berry size	1 to 5 <sup>c</sup>
Straggly bunches	1 to 5 <sup>d</sup>
Dry stems	1 to 5 <sup>e</sup>

<sup>a</sup> 1 = Maximum quality.

5 = minimum quality.

<sup>b</sup> 1 = no blemishes.

5 = more than 75% blemishes.

<sup>c</sup> 1 = even berry size.

5 = uneven berry size.

<sup>d</sup> 1 = full bunches.

5 = straggly bunches.

<sup>e</sup> 1 = green stems.

5 = dry stems.

### Gibberellic acid determination

**Extraction:** Gibberellic acid was extracted from pulp and seed samples using a modified method of Milborrow & Mallaby (1975). Samples were homogenized in a 1:2 ratio (m/v) with a chilled 80% aqueous methanol solution containing glacial acetic acid (20 ml/l), 2,6-di-*t*-butyl-4-methyl-phenol (BHT) (50 mg/l) and ascorbic acid (50 mg/l). Samples were then shaken at low temperature (4-10°C) in the dark for 24 hours. To each sample 10 000 cpm <sup>3</sup>H-GA<sub>1</sub> (32.2 Ci/mmol from Amersham International) was added to determine the recovery of GA after extraction and purification. After incubation samples were centrifuged for 10 minutes at 15 000 X g and the pellet discarded.

**Purification:** The GA-containing supernatant was partitioned with 2 x 20 ml ethyl acetate (EtOAc) and the aqueous phase retained. After adjusting the pH to 2.5 with 2 N HCl this phase was partitioned with 1 x 20 ml and 1 x 10 ml EtOAc. The organic phase was retained and reduced to dryness. All samples were redissolved in 2 ml potassium phosphate 0.1 M, pH 8 buffer and passed through a 5 ml polyvinylpyrrolidone (PVP) column (Polyclar AT, BDH Chemicals Ltd.). Columns were washed with four volumes phosphate buffer and the pH of the eluent was adjusted to 2.5 with 5 N HCl prior to partitioning with 2 x 15 ml EtOAc. The organic phase was reduced to dryness, the residue was redissolved in HPLC grade methanol, again reduced to dryness and stored at -30°C.

**Radio-immunoassay:** Prior to determining GA levels with a radio-immunoassay (RIA) using antiserum which cross-reacts with GA<sub>1,3,20</sub>, the samples were methylated using diazomethane (Hofman, 1990). To each sample 100 µl <sup>3</sup>H-GA<sub>1</sub> (10 000 cpm of 37.7 Ci/mmol from Amersham International), 100 µl GA antiserum and 500 µl bovine serum albumin solution were added. The samples were then shaken and incubated at 37°C for 30 minutes. After addition of 850 µl 90% ammonium sulphate, samples were shaken and the protein allowed to precipitate during a 20-30 minute period. The samples were subsequently centrifuged for 10 minutes at 5 000 X g, the supernatant decanted and the pellet washed with 1.5 ml 50% ammonium sulphate. The samples were again centrifuged and the supernatant decanted. The pellet was dissolved in 250 µl distilled water and 2 ml Picofluor 40 scintillation cocktail was added before samples were passed through a Packard 1900CA liquid scintillation analyser.

All GA-RIA determinations were done in triplicate and raw data were analysed using a Securia data reduction RIA computer package (Packard Instrument Company,

1986 Publication No. 169-3016). Percentage recovery for each sample was calculated using the  $^3\text{H-GA}_1$  spiked samples as references. All other data were analysed using an analysis of variance procedure incorporated in a statistical computer package (Genstat 5, 1987, Statistics Department, Rothamsted Experimental Station).

## RESULTS AND DISCUSSION

Gibberellic acid<sub>1,3,20</sub> levels decreased in all treatments from just after application (10 mm berry size) up to four weeks post-harvest (Figs. 1 & 2).

None of the samples taken at harvest and post-harvest indicated GA<sub>1,3,20</sub> levels higher than 200 ng GA/g fresh

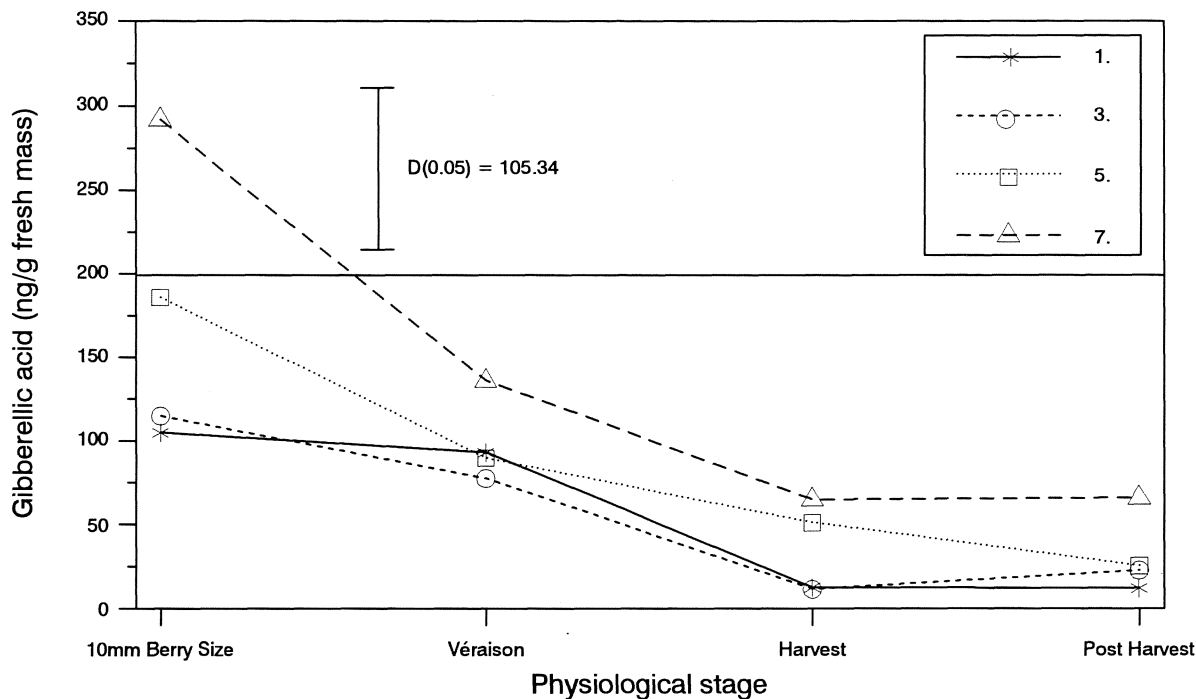


FIGURE 1

Gibberellic acid levels in Sultanina samples (berry flesh) taken at 10 mm berry size, véraison, harvest and post harvest. For a definition of treatments 1, 3, 5 & 7 see Table 1.

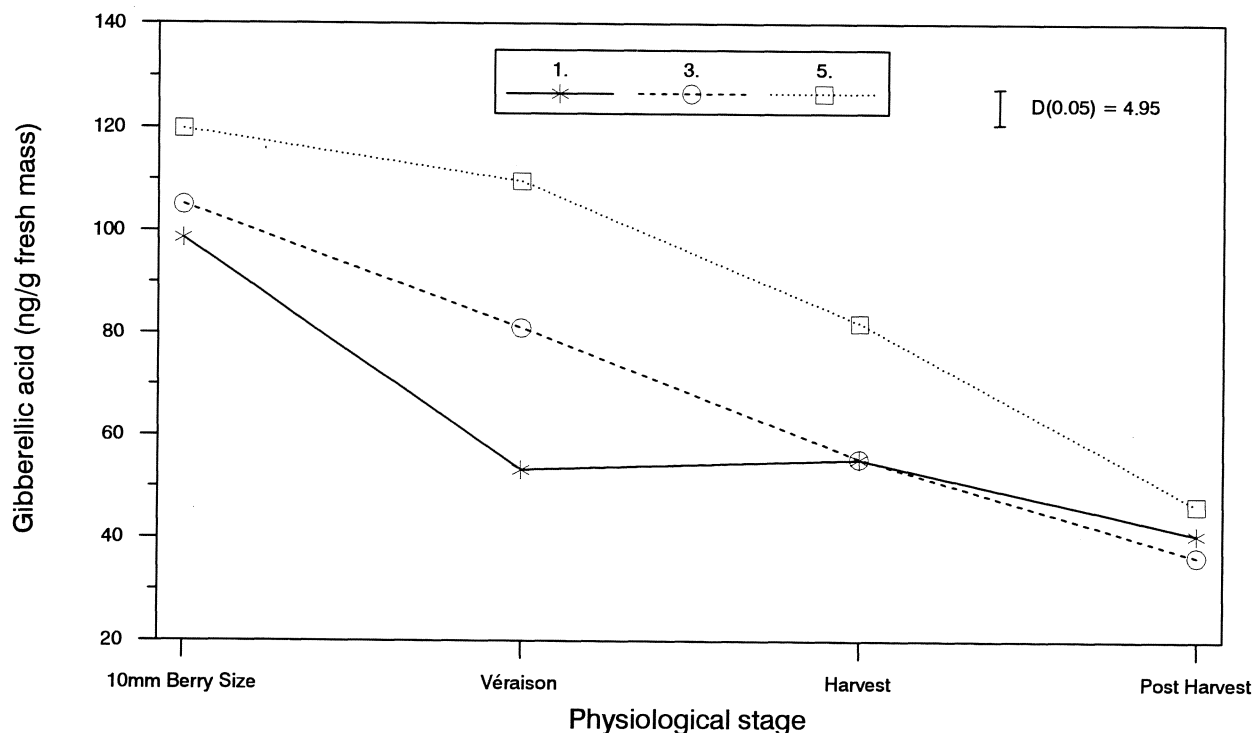


FIGURE 2

Gibberellic acid levels in Waltham Cross samples (berry flesh) taken at 10 mm berry size, véraison, harvest and post harvest. For a definition of treatments 1, 3 & 5 see Table 2.

berry mass. As the  $GA_{1,3,20}$  are the most prolific forms of GA in grapes it is probable but not conclusive that the recommended maximum allowable GA level was not exceeded. It is noteworthy that the untreated control showed comparable levels of  $GA_{1,3,20}$  during later sampling. In all samples the  $GA_{1,3,20}$  levels in Sultanina were higher than in

Waltham Cross, while no measurable levels were found in Waltham Cross seeds (data not shown). In both cultivars, irrespective of dosage, late GA applications showed higher levels just after application and at harvest than the normal dosage (Figs. 3 & 4). Generally  $GA_{1,3,20}$  levels in samples increased significantly as dosages increased.

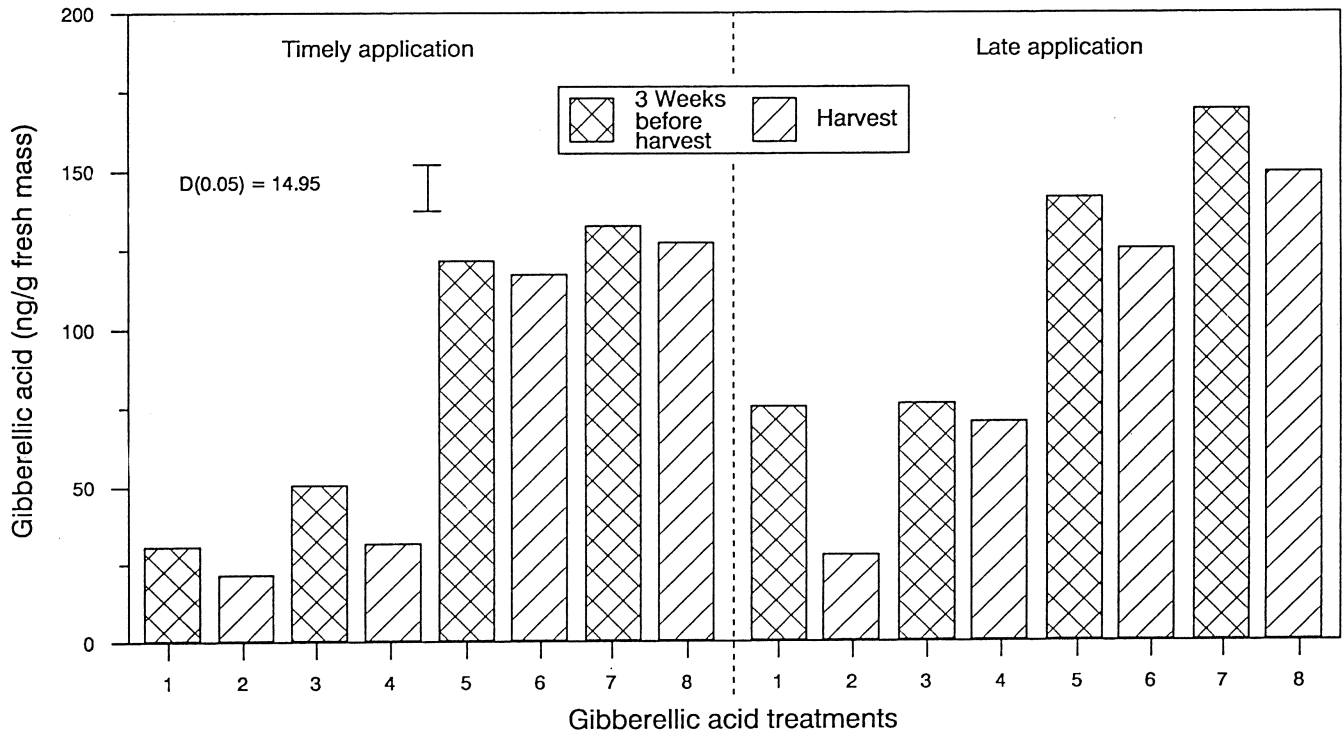


FIGURE 3

Gibberellic acid levels in Sultanina samples (berry flesh) treated with GA applications. For a definition of treatments 1, 3, 5 & 7 see Table 1.

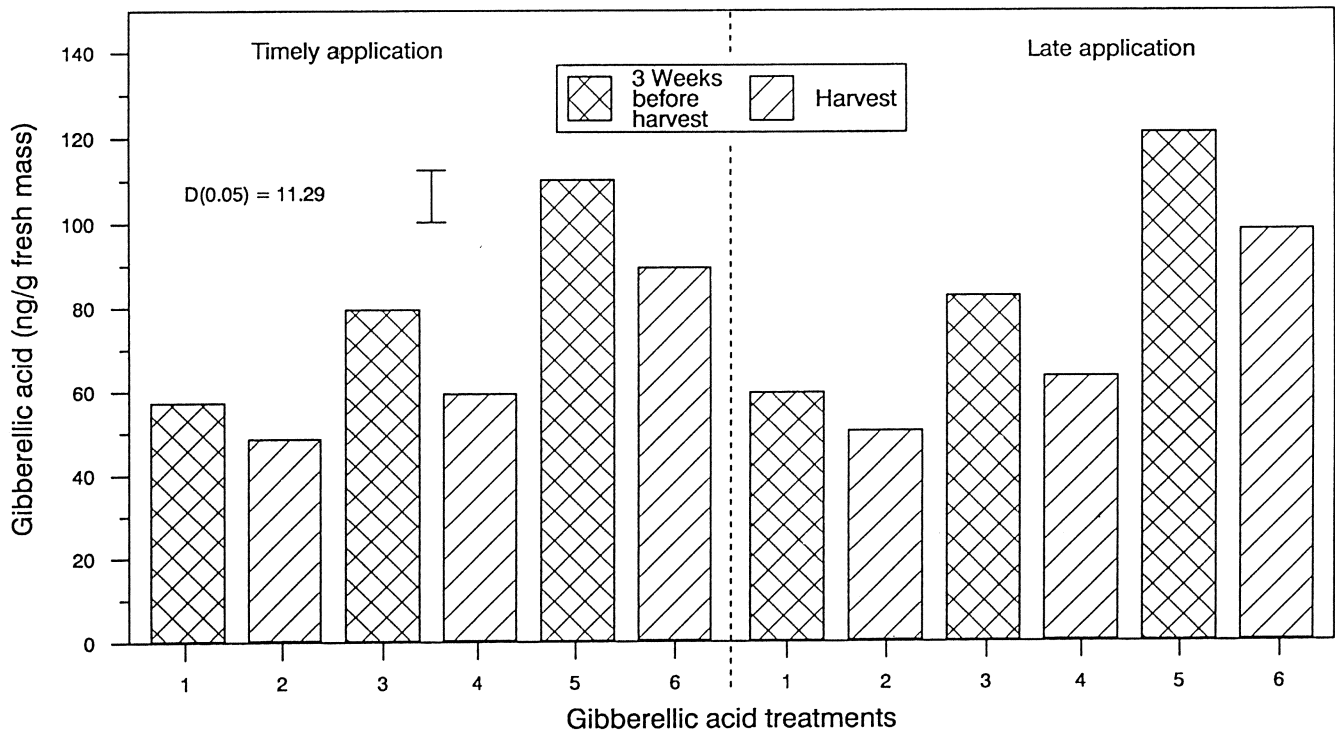


FIGURE 4

Gibberellic acid levels in Waltham Cross samples (berry flesh) treated with GA applications. For a definition of treatments 1 to 6 see Table 2.

The GA levels found in this study cannot be compared directly to those found by Coombe (1960), Hagiwara *et al* (1980) and Scienza *et al.* (1978) due to differences in GA-level expression as well as cultivar. A comparison can, however, be made with regard to the decrease in GA levels during similar physiological stages. The decrease in

GA levels found in both GA-treated and untreated Sultanina and Waltham Cross coincided with those found by Coombe (1960), Hagiwara *et al.* (1980) and Scienza *et al.* (1978).

Quality evaluations after four weeks' cold storage showed a tendency to decreasing ripeness with increasing

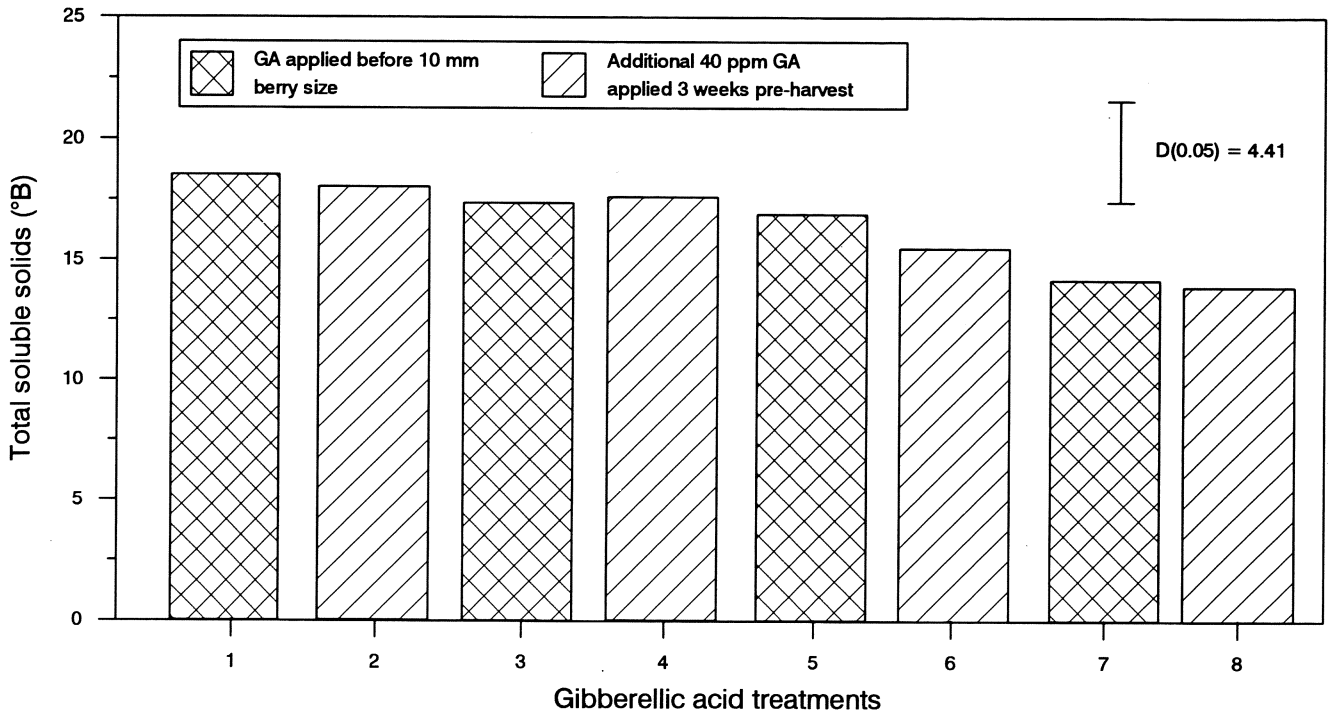


FIGURE 5

Total soluble solids (ripeness) of Sultanina samples (berry flesh) treated with timely and late GA applications. For a definition of treatments 1 to 8 see Table 1.

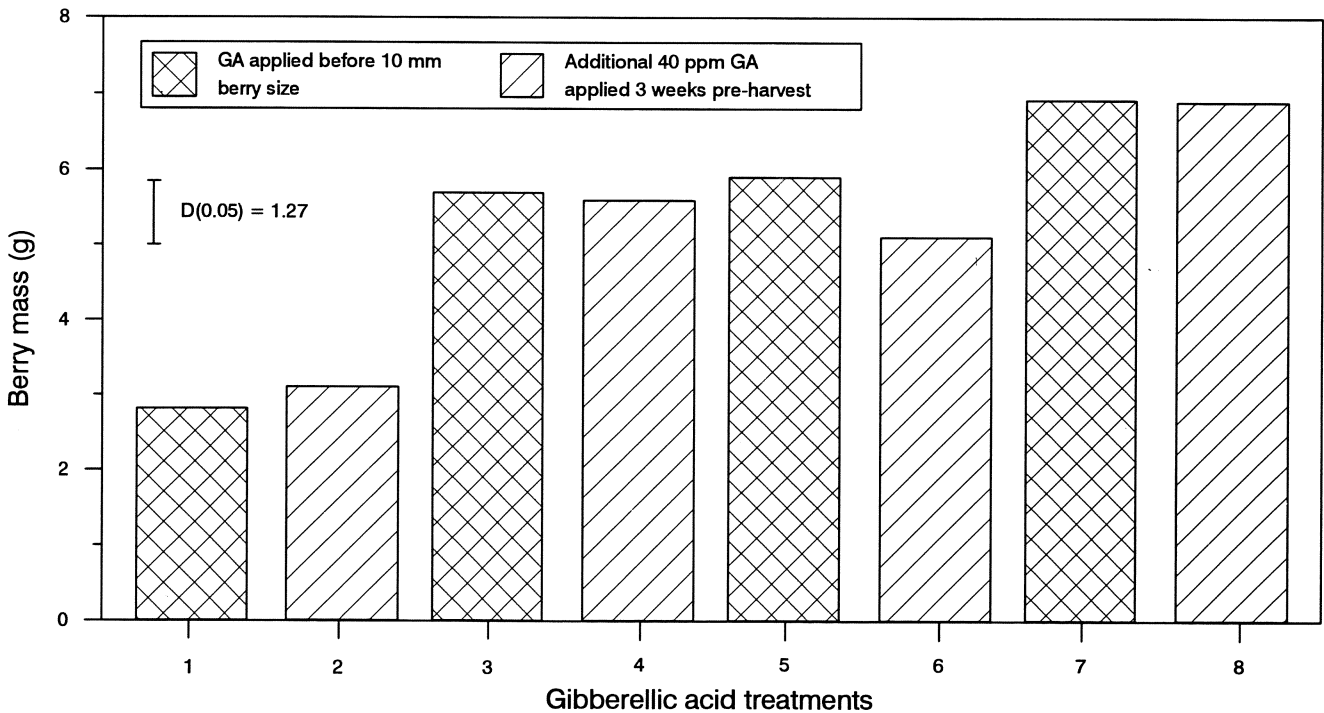


FIGURE 6

Berry mass of Sultanina grapes treated with increasing gibberellic acid dosages. For a definition of treatments 1 to 8 see Table 1.

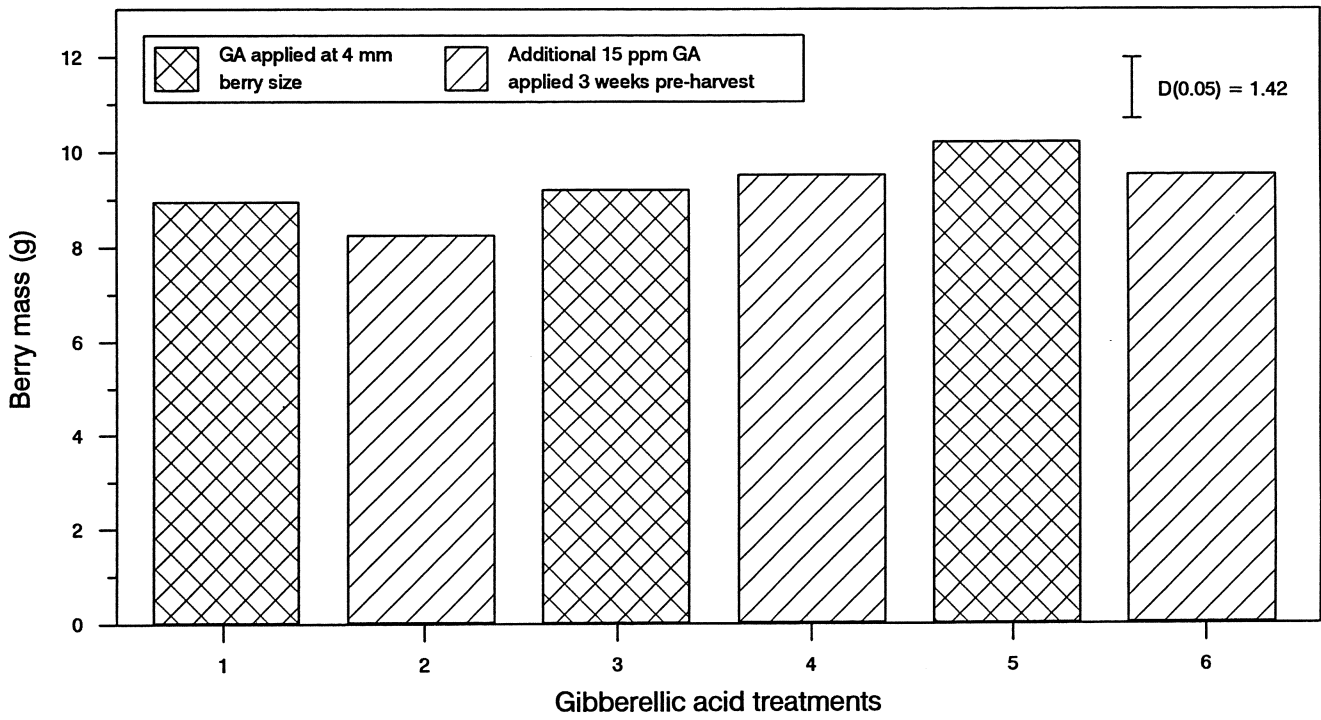


FIGURE 7

Berry mass of Waltham Cross grapes treated with increasing gibberellic acid dosages. For a definition of treatments 1 to 6 see Table 2.

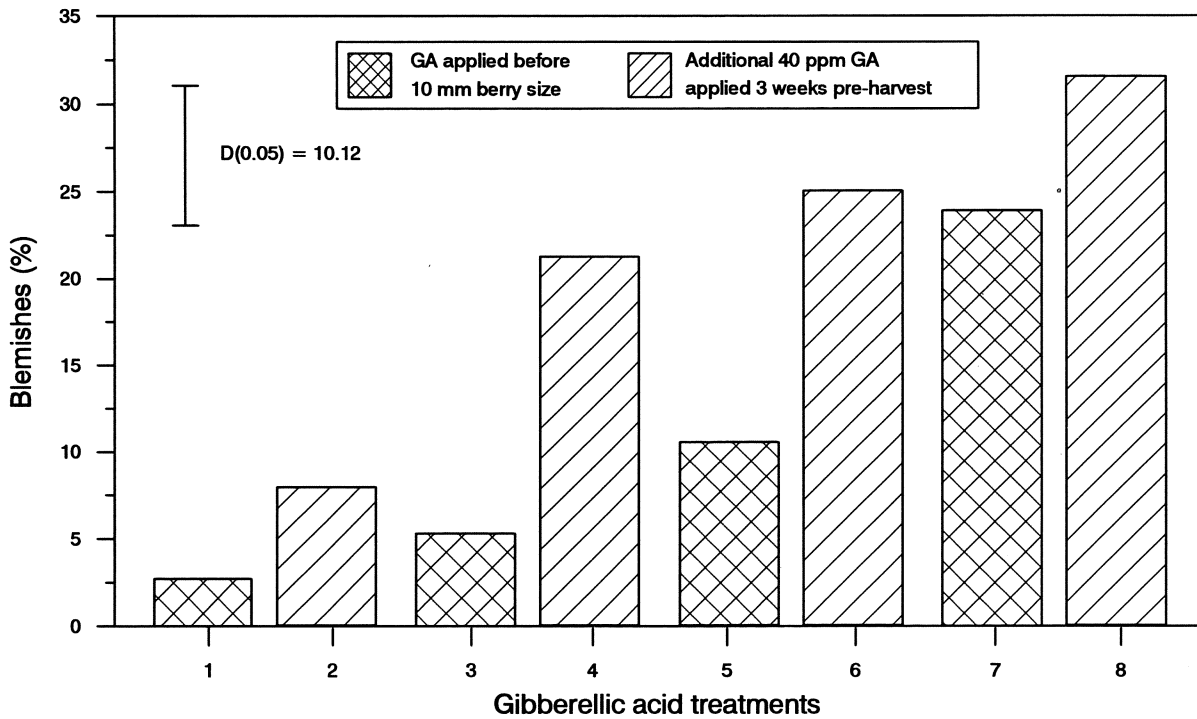


FIGURE 8

Occurrence of blemishes on Sultanina berries and stems as a result of high gibberellic acid dosages and late applications. For a definition of treatments 1 to 8 see Table 1.

GA dosages (Fig. 5). It was found that while higher GA dosages resulted in a significant increase in berry mass of Sultanina (Fig. 6), only a tendency to increased berry mass was found in Waltham Cross (Fig. 7). In both cases the late GA applications had no additional effect on berry mass. The higher GA dosages and late GA applications did, however, result in an increase in the occurrence of blemishes on Sultanina berries (Fig. 8). No other quality defects were found on GA-treated bunches.

#### CONCLUSION

The results of this study indicates that late applications (three weeks before harvest) and high GA dosages increased GA<sub>1,3,20</sub> levels in grape berries significantly. As the GA<sub>1,3,20</sub> are the most prolific forms of GA found in grapes, it is probable that the maximum allowable limit of 200 ng GA/g fresh berry mass at harvest was not exceeded. This is probably due to the systematic breakdown of GA after application.

The results also show that both high GA dosages and late GA applications cannot be recommended due to a delay in ripening of Sultanina and Waltham Cross. An increase in berry blemish (Sultanina) was also caused by these treatments.

To maximize bunch quality with minimal costs, GA dosages and application times for Sultanina and Waltham Cross as recommended by Van der Merwe, Geldenhuys & Botes (1991) and Wolf, Van der Merwe & Orth (1991) should be used.

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