The Effect of Ethephon on the Berry Colour of Flame Seedless and Bonheur Table Grapes

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Flame Seedless grafted onto Richter 99 situated in the Lower Orange River region and Bonheur grafted onto Ramsey in the Berg River Valley region of South Africa were used to test ethephon treatments for berry colour improvement. The trial was repeated over three seasons. Four ethephon dosages (100, 200, 300 and 400 mg/L) in combination with five application times (weekly intervals starting from acid maximum) were compared to an untreated control. As soon as general minimum maturity for export was reached, pack-out percentage was determined and representative bunch samples harvested and packed as for export. After storage for four weeks at -0.5°C and one week at 10°C, bunch quality was evaluated according to standard industry norms. As a preliminary determination anthocyanin and phenol absorbance levels were determined during one season to possibly complement visual evaluation of berry colour. The best overall result with regard to the timing of application for both Flame Seedless and Bonheur was obtained with an ethephon application two weeks after acid maximum (30 to 40% colour). Significant seasonal differences were observed regarding various quality variables of Bonheur. This timing was not very critical for improved export pack-out or meeting the berry colour quality norm. Treatments closer to harvest increase the risk of quality defects during cold storage, such as berry crack, SO_2 burn, as well as soft and loose berries. An ethephon dosage of 300 mg/L for Flame Seedless and 200 mg/L for Bonheur gave satisfactory results regarding export pack-out, export-quality berry colour at harvest and post-cold-storage quality. The preliminary evaluation of anthocyanin and total phenol absorbance levels did not correlate well with visual evaluation of berry colour, showing the difficulty of sensory determinations for quality evaluation.

Flame Seedless is a complex hybrid whose parents include Sultanina, Cardinal, Malaga and Muscat d'Alexandrie, and was developed in the early 1960s by John Weinberger in California (Van der Merwe *et al.,* 1991). It is a popular red seedless table grape, with a firm, crispy texture and sweet, neutral taste. Currently, it is the most widely planted red seedless table grape cultivar in the world. It is widely planted in the Lower Orange River region of South Africa, where it ripens before Sultanina. The Lower Orange River is a semi-desert region in the Northern Cape province, with mild winters and hot summers. Due to its earliness, Flame Seedless is highly priced on the export market. It is harvested from about mid-November (week 46) in the earliest areas. In the 2000/2001 season approximately 0.5 million 4.5 kg cartons (1.3% of total exports) Flame Seedless were exported.

Bonheur is a locally bred table grape cultivar resulting from a cross between Alphonse Lavallée and Barlinka. It was released by ARC Infruitec-Nietvoorbij during 1986 and is one of the most successful seeded black export cultivars in South Africa (Evans & Ellis, 1987). It has excellent eating and cold-storage quality, with a firm skin and sweet, neutral taste. In the 2000/2001 season approximately 1.2 million 4.5 kg cartons (3.4% of total exports) Bonheur were exported. The Berg River Valley region is one of the largest table grape regions in the Western Cape province of South Africa. It has a moderate Mediterranean climate resulting in grapes ripening from December (week 51) until the end of March (week 13).

In 1946 (2-chloroethyl) phosphonic acid was developed and named ethephon (De Wilde, 1971). In plants this compound releases ethylene, a natural plant growth regulator (PGR), which is responsible for the physiological action of the product. It has found many applications on various crops in the agricultural field, including promoting flowering, inhibiting vegetative growth, as a thinning agent, inducing disease and freezing resistance, improving latex production and improving ripening and maturity, inducing better fruit colour (De Wilde, 1971). Ethephon has been used since the 1970s to improve colour and hasten maturity of grapes (Weaver & Pool, 1971; Cirami *et al.*, 1992; Leão and De Assis, 1999), as well as in other fruits (De Wilde, 1971). If ethephon is

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used to enhance colouring and maturity of table grapes, there is the danger of negative quality effects being enhanced, for example soft berries, loose berries and lower post-harvest quality (Weaver & Pool, 1971). Although ethephon is used in South Africa to improve the colour of table grapes (Blommaert & Steenkamp, 1977), it is presently only registered for use on Barlinka.

The use of ethephon for improvement of colour and quality of Flame Seedless table grapes in the Lower Orange River region and Bonheur table grapes in the Berg River Valley region of South Africa was investigated over three seasons to determine optimal dosages and timing of application.

MATERIALS AND METHODS

Vineyard

Flame Seedless

Commercial Flame Seedless vines, grafted onto Richter 99 (*Vitis Berlandieri* x *Vitis rupestris*), were planted in 1990 at Kanoneiland in the Lower Orange River region of South Africa (28°38' S, 21°07' E). Vines were spaced 3.5 m x 2.0 m and trained onto a gable trellis system, where the vines were split into two sections that were trellised onto each half of the gable "V". A micro-jet irrigation system was used on the loamy alluvial soil. Crop load was regulated to five bunches per square metre canopy. In general, vine growth was vigorous. The bunches were treated by means of backpack sprays with 10 mg/L gibberellic acid (GA_3) at 50% and 80% bloom and with 30 mg/L GA_3 at 6 mm and 8 mm berry size.

Bonheur

Commercial Bonheur vines, grafted onto Ramsey *(Vitis champinii),* were planted in 1989 at Saron in the Berg River Valley region of South Africa (33°08' S, 18°59' E). Vines were spaced 3.5 m x 2.0 m and trained onto a gable trellis system, where the vines were split into two sections that were trellised onto each half of the gable "V". A drip irrigation system was used on the loamy alluvial soil. Vine growth was balanced, i.e. no excessive growth was observed.

Irrigation was scheduled according to class A pan evaporation readings and specific crop factors, as well as investigatory holes, which were dug to determine deeper soil water status. Vines were fertilised according to standard practices as defined for the particular region and cultivar as well as vine leaf petiole analyses during the growth season. Both cultivars were stem girdled (3 mm cut) at 6 mm berry size. All other viticultural treatments were as recommended for the production of export quality Flame Seedless and Bonheur table grapes (Van der Merwe *et al.*, 1991).

Treatments

Ethephon (active ingredient of Ethrel®, a registered product of Aventis, containing 480 mg/L ethephon) dosages of 100, 200, 300 and 400 mg/L were applied at a spray volume of 1000 L/ha to bunches using a backpack pressure sprayer with hand lances to ensure thorough wetting. A standard buffering wetting agent, Bladbuff® 5, was added at recommended rates, although final spray pH was not measured. Applications were made weekly for five weeks and commenced at the acid maximum (approximately one week before beginning of colour or véraison). The application times (AT) may be described as follows: AT 1 – 0 to 5% berry colour; AT 2 – 10 to 20% berry colour; AT 3 – 30 to 40% berry colour; AT 4 – 60 to 80% berry colour; and AT 5 – one week before harvest. Colour was determined visually in the vineyard.

Statistical procedures

The experiment was a completely randomised block design with 25 treatment combinations replicated in three blocks. The treatment design was a 5 x 5 factorial with the following factors: four ethephon dosages and a control (0 control, 100, 200, 300 and 400 mg/L) and five weekly application times (AT 1, 2, 3, 4 and 5). An experimental unit consisted of five consecutive vines in a row, of which the three inner vines were used to harvest representative bunch samples at export maturity. There were no buffer rows. Treatments were applied to the same plots for three consecutive seasons, giving repeated measurements over seasons. Seasons were considered as a sub-plot factor (Little & Hills, 1972). Standard split-plot analyses of variance were performed on the data, using SAS statistical software version 6.12 (SAS, 1999). The qualitative factors were broken down into single degree polynomials to determine the polynomial order to be fitted with the bivariate polynomial regression functions. Response surface plots and contour plots were created to examine the variable performance. The Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). Student's t-test of least significant difference was calculated at the 5% level to compare treatment means.

Measurements

Representative bunch samples (5 kg) of each treatment were harvested when buffer vines reached minimum export maturity (18°Brix for Flame Seedless and 16°Brix for Bonheur), from each treatment replicate. All samples were packed as for export and stored for four weeks at -0.5°C and one week at 10°C to simulate export conditions. Pack-out percentage was visually determined in the vineyard, while representative bunches were taken and total soluble solids (TSS in °Brix) and total titratable acids (TTA in g/L) determined in the laboratory. The following post-cold-storage quality norms were measured, namely bunch (berry) colour (reject, export medium and export dark colour), loose berries, decay, lesions, crack, bruising, sulphur-dioxide (SO_2) – damage, average berry mass and bunch straggliness. These measurements were done according to standard quality-control procedures by IHS Quality Control in Cape Town Harbour. Bunch (berry) colour was visually evaluated according to minimum export-quality colour charts, as used by the export table grape industry.

A preliminary study for one season of anthocyanin extraction of berry skins was done to possibly provide a simple support for visual determination of berry colour. The absorbancies of anthocyanins and total phenols were determined as follows: 24 berries were randomly selected from each sample after post-cold-storage quality evaluation and frozen at -25°C until extraction. Two 10 mm skin disks were removed from the top and bottom of each frozen berry using a cork borer and forceps, taking care not to include any pulp. The disks were placed in a glass bottle containing 20 mL acidified methanol (containing 1% hydrochloric acid v/v). These were extracted at room temperature in darkness for 24 hours, during which the bottles were gently shaken by hand every eight hours. After this period the contents were mixed for 5 seconds on a vortex mixer and allowed to settle for 30 minutes. The absorbance of a 5 mL aliquot was determined at 520 nm (total anthocyanins) and 280 nm (total phenols) using a spectrophotometer (LKB Biochrom Ultrospec II E Model 4057). As the study was done to determine relative differences between treatments, actual anthocyanin levels and efficacy of extraction were not determined and only the relative differences in absorbancies were compared.

Meteorological parameters of each region were measured to monitor possible climatic effects on the efficacy of treatments.

RESULTS

Meteorological summary

For the purpose of discussing the meteorological conditions in both regions, a growth season was described as the period from July (budbreak) up to the following June (dormancy). The summaries for both regions are discussed below.

Lower Orange River region (Flame Seedless)

Harvest of the trial vineyard was normally during December. Total rainfall in the 1994/1995 season (July 1994 to June 1995) was lower than normal, although higher levels than the long-term average were recorded during December, when harvest occurred. Temperatures were higher during spring and similar to long-term levels during October to December 1994 (harvest) (Figs. 1 a and b). The 1995/1996 season was characterised by high rainfall levels in December (harvest), January and February. Temperatures were cooler during the flowering period in October and also during harvest in December 1995. As expected, due to the high rainfall levels during harvest, bunch quality was lower in this season due to a higher occurrence of pre- and post-harvest decay and berry crack. The 1996/1997-season had no rainfall during the period September-December 1996. Temperatures were lower during budburst in spring and increased to similar levels than the long-term average during November and December 1996 (harvest).

Berg River Valley region (Bonheur)

Harvest of the trial vineyard normally took place during February. The 1994/1995 season had lower total rainfall than the long-term average, especially during November 1994 to February 1995 (harvest). Maximum temperatures were warmer than normal, especially during December 1994 to February 1995, which resulted in high levels of heat damage to berries (Figs. 2 a and b). The 1995/1996 season had a slightly higher level of total rainfall than the long-term average, importantly during winter and December 1995. Temperatures, although initially quite mild in spring, were higher that the long-term average during November 1995 to February 1996 (harvest). The 1996/1997 season was characterised by higher than average rainfall levels from spring (September 1996) to December 1996. Temperatures were lower than average and remained so until harvest in February 1997, resulting in a delayed harvest. In general, bunch quality was found to be lower due to a higher occurrence of pre- and post-harvest decay and berry crack.

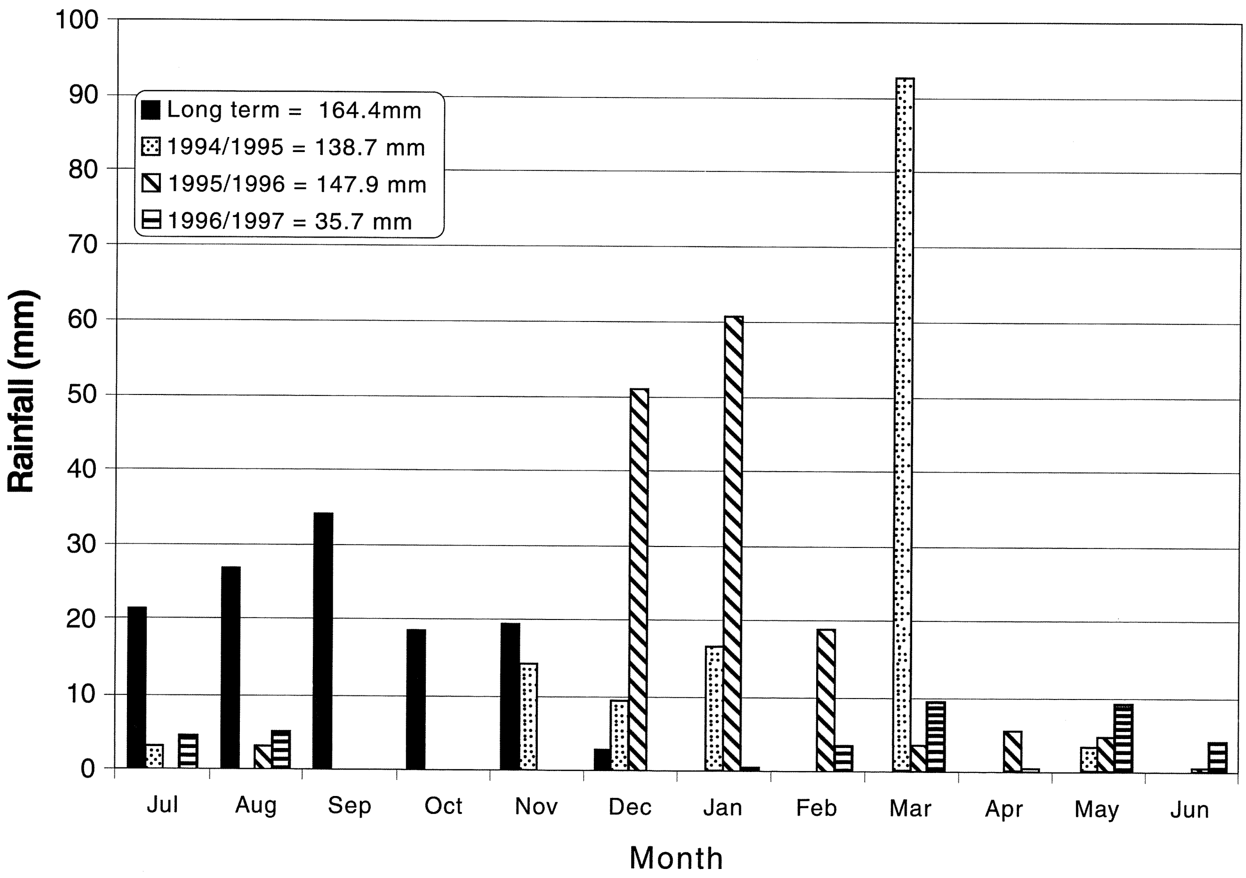

FIGURE 1a
Total monthly rainfall at Kanoneiland, Lower Orange River region, South Africa, 1994/1995 to 1996/1997.

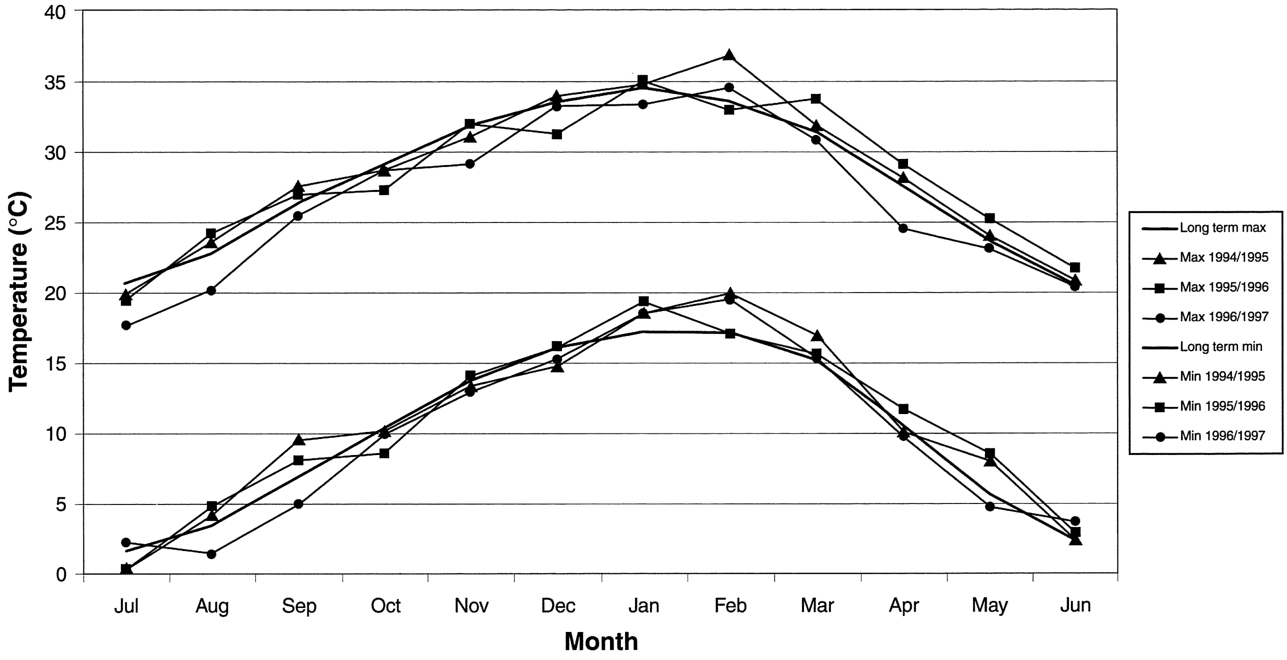

FIGURE 1b

Average monthly minimum and maximum ambient temperatures at Kanoneiland, Lower Orange River region, South Africa, 1994/1995 to 1996/1997.

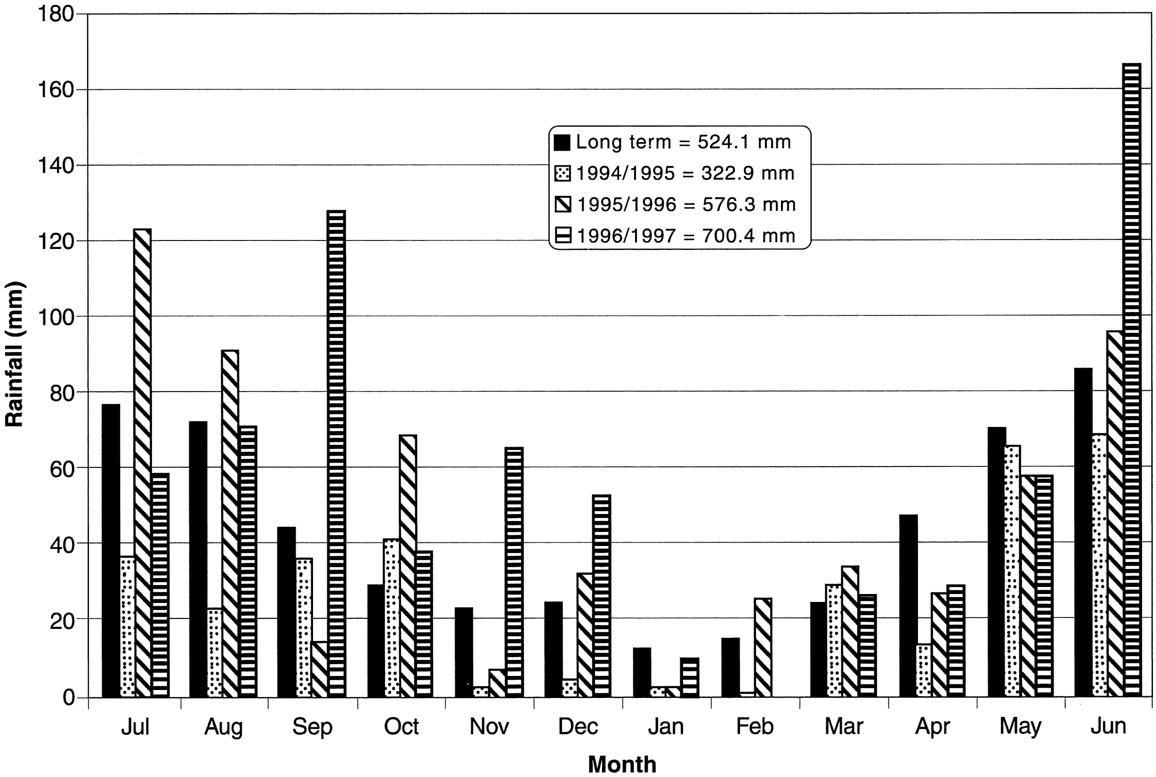

FIGURE 2a

Total monthly rainfall at Saron, Berg River Valley region, South Africa, 1994/1995 to 1996/1997.

Flame Seedless

For both Flame Seedless and Bonheur, the data regarding both ethephon dosage and application timing were deemed to be sufficiently similar to enable the three seasons' data to be combined. Although there were seasonal differences, there were no seasonal interactions regarding ethephon dosage. Interactions concerning application timing were nevertheless observed. The analysis of variance summary of Flame Seedless variables is presented in Table 1.

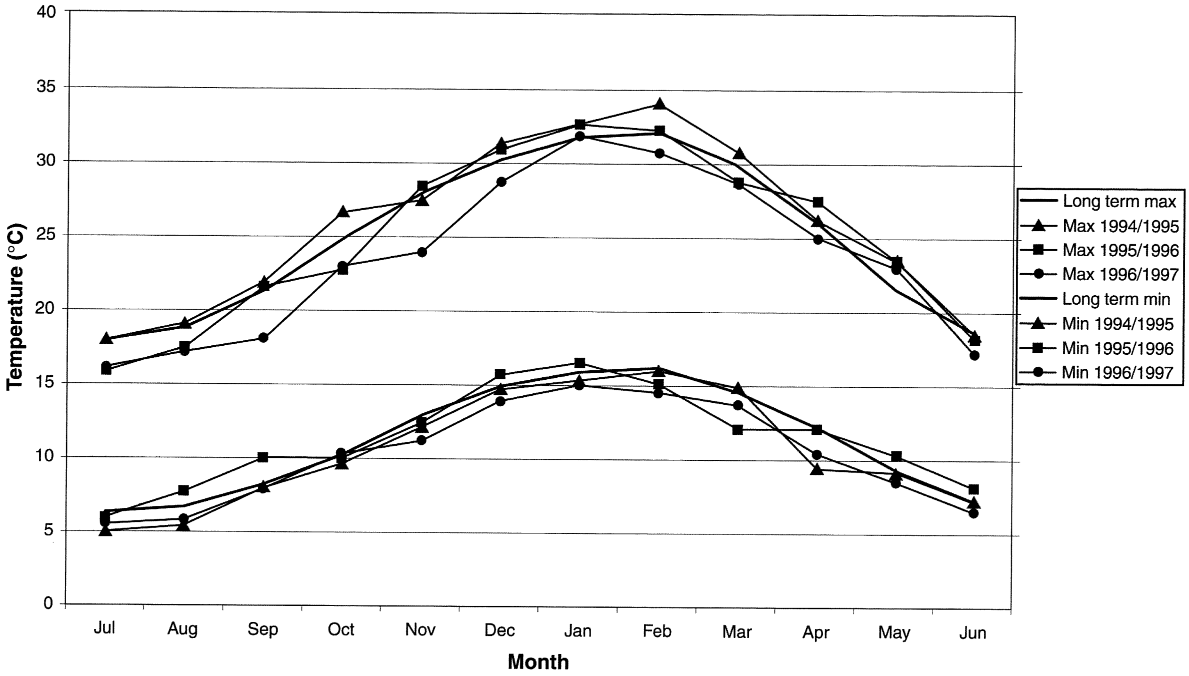

FIGURE 2b

Average monthly minimum and maximum ambient temperatures at Saron, Berg River Valley region, South Africa, 1994/1995 to 1996/1997.

Only those variables that showed significant differences in the various seasons are discussed. Average values of the measured quality variables were similar to average quality variables for the industry as determined by Quality Control at IHS in Cape Town Harbour in the different seasons. The Flame Seedless trial was harvested on 13 December 1994 (1994/1995 season), 20 December 1995 (1995/1996 season) and 23 December 1996 (1996/1997 season).

Dosage

The results regarding ethephon dosage over these three seasons were sufficiently similar to be presented as combined averages and are presented in Table 2. As there were no seasonal interactions regarding dosage, seasonal influence on dosage was not significant. All dosages produced a significantly higher export pack-out percentage than the untreated control. A slight (not always significant) increase in average berry mass was observed with increased dosages. Dosages of 200 to 400 mg/L increased berry mass significantly, with 400 mg/L the most effective. Increasing dosages tended to increase the occurrence of loose berries and berry crack, although not significantly. Reject light berry colour, determined from the number of reject bunches due to poor colour, was significantly higher for both the untreated control and the 100 mg/L dosage, compared to higher dosages. The dark export-quality and total export-quality colour of berries increased with dosage, and were highest at the 200, 300 and 400 mg/L dosages, which were significantly higher than the untreated control. Both TSS and TTA levels decreased or tended to decrease with increasing dosages, above 100 mg/L, the higher dosages having significantly lower TSS and TTA levels than the control. The ethephon dosages increased anthocyanin concentration, with the highest levels at 200 and 400 mg/L which were significantly higher than the untreated control.

Application timing

Season had an effect on time of application and thus the results are presented per season (Table 3). In 1994/1995, ethephon applications at AT 4 and 5 significantly decreased export pack-out percentage. Berry mass tended to decrease with later applications. The total percentage of berries with export-quality colour did not differ significantly between applications. Nevertheless, differences did occur in the percentage of export-quality medium-and dark-coloured berries, with later applications producing higher levels of medium-coloured export-quality berries. AT 5 had the most detrimental effect. Medium-coloured berries are sought after for Flame Seedless, as the colour is naturally red, rather than darker berries that appear very dark. No significant increase in TSS was found, while TTA increased significantly with applications during AT 4 and 5. In 1995/1996 increased export pack-out was mainly due to improved colouring of berries at harvest. Application times showed minimal differences. In 1996/1997, later applications increased export pack-out, though not significantly, but led to a significant increase in berry crack and decrease in export-quality berry colour in AT 5. Although not significant, the improvement in export-quality berry colour was highest at AT 4. Berry mass also improved with later application timings, peaking at AT 3. The last application (AT 5) produced significantly higher levels of reject light-coloured berries, while AT 4 produced the lowest levels of light-coloured reject berries.

Contour graphs

To enable visualisation of the optimum dosage and application timing of ethephon, a number of contour graphs were drawn for certain important variables (Figs. 3 a to d). These graphs show values as contours, with the lowest values in the bottom portion and the highest values at the top. In this way the optimum dosage and applica-

TABLE 1

Analysis of variance for different variables of Flame Seedless table grapes treated with ethephon for improvement of berry colour in the Lower Orange River region, South Africa, 1994/1995 to 1996/1997.

		Export pack-out		Average berry mass		Loose berries		Loose berries		Berry colour: Reject (light)		Berry colour: Export-quality medium		Berry colour: Export-quality dark		Berry colour: Export-quality total		TSS		TTA		Anthocyanin absorption*	
		(%)		(g)		(%)		(%)		(%)		(%)		(%)		(%)		(°B)		(g/L)		(A 520 nm)	
Source	**DF**	**MS**	**P**	**MS**	**P**	**MS**	**P**	**MS**	**P**	**MS**	**P**	**MS**	**P**	**MS**	**P**	**MS**	**P**	**MS**	**P**	**MS**	**P**	**MS**	**P**
Block	2	722.13	0.08	2.4058	<0.01	2.8048	0.01	10.8923	0.01	246.41	0.63	771.37	0.30	269.0	0.75	221.1	0.67	0.2790	0.67	0.228	0.17	0.0017	0.94
Ethephon	4	798.37	0.03	1.4225	<0.01	0.7735	0.21	2.2857	0.39	2938.49	<0.01	352.74	0.69	2093.4	0.08	2869.5	<0.01	1.6480	0.07	2.210	<0.01	0.0909	0.02
Eth-Linear	1	1631.35	0.02	5.3486	<0.01	0.3872	0.39	8.6528	0.05	9613.29	<0.01	388.65	0.44	6046.3	0.01	9498.1	<0.01	4.5804	<0.01	7.553	<0.01	0.2605	<0.01
Eth-Quadratic	1	1451.91	0.03	0.0019	0.93	1.6869	0.07	0.0016	0.98	1725.08	0.08	0.43	0.98	1551.6	0.20	1603.2	0.09	1.5650	0.14	0.362	0.09	0.0154	0.46
Eth-Cubic	1	83.55	0.58	0.2954	0.30	1.0177	0.16	0.4802	0.64	110.31	0.65	1021.22	0.21	514.1	0.46	86.9	0.69	0.0002	0.99	0.925	<0.01	0.0675	0.12
Eth-Quartic	1	26.68	0.76	0.0443	0.69	0.0021	0.95	0.0083	0.95	305.26	0.45	0.66	0.97	261.4	0.60	289.9	0.47	0.4464	0.43	0.000	0.95	0.0201	0.39
Time	4	294.74	0.37	0.0872	0.86	1.6184	0.02	4.7422	0.08	694.69	0.28	1226.76	0.12	2320.8	0.06	687.1	0.30	0.4346	0.65	1.044	<0.01	0.0298	0.37
Time-Linear	1	405.94	0.23	0.0207	0.78	3.8457	0.01	4.5000	0.16	632.01	0.28	3349.98	0.03	6607.1	<0.01	546.3	0.32	0.1352	0.66	3.363	<0.01	0.0686	0.12
Time-Quadratic	1	487.87	0.19	0.3260	0.28	1.9444	0.06	13.2603	0.02	1154.12	0.15	265.79	0.52	2580.0	0.10	1188.2	0.15	1.1231	0.21	0.443	0.06	0.0436	0.21
Time-Cubic	1	283.54	0.31	0.0016	0.94	0.0854	0.68	0.2689	0.73	874.73	0.21	890.70	0.24	0.1	0.99	909.9	0.20	0.0910	0.72	0.060	0.49	0.0069	0.62
Time-Quartic	1	1.62	0.94	0.0006	0.96	0.5980	0.28	0.9395	0.51	117.89	0.64	400.57	0.43	95.8	0.75	103.8	0.66	0.3889	0.46	0.309	0.12	0.0001	0.96
Ethephon*Time	16	139.78	0.93	0.3522	0.24	0.2677	0.92	3.8149	0.07	426.62	0.68	1086.32	0.07	1089.2	0.33	424.4	0.70	0.9610	0.20	0.295	<0.01	0.0264	0.50
Error a	**48**	**271.52**		**0.2725**		**0.5067**		**2.1570**		**535.66**		**630.83**		**932.2**		**545.6**		**0.7016**		**0.123**		**0.0272**	
Season	2	11433.84	<0.01	61.7731	<0.01	10.1059	<0.01	134.3592	<0.01	14691.94	<0.01	22239.26	<0.01	62457.3	<0.01	14582.7	<0.01	16.7303	<0.01	4.298	<0.01		
Season*Ethephon	8	174.72	0.62	0.1798	0.76	0.7105	0.24	1.6246	0.77	1235.92	<0.01	545.61	0.74	958.4	0.48	1240.7	<0.01	1.1895	0.15	0.463	<0.01		
Season*Time	8	836.09	<0.01	0.8456	0.01	0.8152	0.16	5.6530	0.04	615.26	0.07	801.78	0.49	1032.7	0.42	619.8	0.07	0.2034	0.98	0.646	<0.01		
Season*Ethephon*Time	32	100.69	0.99	0.3227	0.34	0.7205	0.14	3.0582	0.31	233.20	0.87	950.94	0.34	1151.1	0.30	239.3	0.84	0.6324	0.74	0.270	<0.01		
Error b	**100**	**224.85**		**0.2902**		**0.5375**		**2.6840**		**329.68**		**854.50**		**1005.6**		**327.5**		**0.7741**		**0.135**			
Corrected Total	**224**																						

DF = Degrees of freedom. MS = Mean Square. P = Probability of F-ratio test.

TABLE 2

The effect of increasing ethephon dosages on export pack-out and post-cold storage quality of Flame Seedless table grapes, Lower Orange River region, South Africa, 1994/1995 to 1996/1997.

Ethephon	Export pack-out	Average berry mass	Loose berries	Berry crack	Berry colour: Reject (light)	Berry colour: Export-quality medium	Berry colour: Export-quality dark	Berry colour: Export-quality total	TSS	TTA	Anthocyanin absorption*
(mg/L)	%	%	%	%	%	%	%	%	(°B)	(g/L)	(A 520 nm)
control	67.7b	4.21c	0.91a	0.84a	25.8a	29.4a	44.8b	74.2b	18.25a	6.69a	0.57b
100	75.9a	4.38bc	0.95a	1.08a	19.3a	25.8a	54.9ab	80.7b	18.06ab	6.34b	0.68ab
200	77.6a	4.47b	1.14a	1.17a	9.2b	29.9a	60.9a	90.8a	17.80b	6.27b	0.73a
300	78.0a	4.49ab	1.20a	1.22a	8.3b	33.7a	57.9a	91.7a	17.86b	6.26b	0.68ab
400	76.2a	4.70a	0.94a	1.46a	8.3b	30.1a	61.6a	91.7a	17.85b	6.06c	0.78a
LSD	6.9	0.22	0.30	0.62	9.8	10.6	12.9	9.9	0.36	0.15	0.12

Values with the same letters do not differ significantly from each other at the 5% significance level.
* Only in the 1995/1996 season.

TABLE 3

The effect of application timing of ethephon and season on export pack-out and post-cold-storage quality of Flame Seedless table grapes, Lower Orange River region, South Africa, 1994/1995 to 1996/1997.

Season	Weekly application times**	Export pack-out	Average berry mass	Loose berries	Berry crack	Berry colour: Reject (light)	Berry colour: Export-quality medium	Berry colour: Export-quality dark	Berry colour: Export-quality total	TSS	TTA	Anthocyanin absorption*
(AT)	(%)	(g)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(°B)	(g/L)	(A 520 nm)
1994/1995	AT 1	69.4bc	5.39ab	0.75e	0.47d	6.5efg	26.6bcd	66.9ab	93.5abc	18.39abc	6.34cd	
1994/1995	AT 2	65.2cd	5.62a	0.79de	0.57cd	14.1defg	31.3abc	54.6bc	85.9abcd	18.40abc	6.42cd	
1994/1995	AT 3	68.6bcd	5.22b	0.97cde	0.21d	3.0fg	43.1ab	53.9bc	97.0ab	18.59a	6.27cde	
1994/1995	AT 4	58.4d	5.25ab	0.94cde	0.78cd	11.2defg	46.9ab	41.8cd	88.8abcd	18.65a	6.80b	
1994/1995	AT 5	44.0e	5.06bc	0.69e	0.22d	14.8defg	49.6a	35.6cd	85.2bcd	18.50ab	7.16a	
1995/1996	AT 1	78.4ab	3.62f	0.72e	0.20d	5.1fg	9.3d	85.6a	94.9ab	17.65d	6.11de	0.71a
1995/1996	AT 2	77.4ab	3.36f	1.10cde	0.25d	3.3fg	9.9d	86.8a	96.7ab	17.71d	6.29cd	0.71a
1995/1996	AT 3	76.4ab	3.47f	0.88de	0.33d	2.7fg	8.0d	89.4a	97.3ab	17.93bcd	6.31cd	0.71a
1995/1996	AT 4	85.7a	3.52f	0.65e	0.38d	1.1g	9.7d	89.2a	98.9a	17.62d	6.16cde	0.69a
1995/1996	AT 5	81.1a	3.54f	0.65e	0.41d	2.1fg	12.6cd	85.3a	97.9ab	17.81cd	6.31cd	0.61a
1996/1997	AT 1	80.3a	4.15e	1.75ab	2.93b	28.9bc	44.1ab	27.0de	71.1ef	17.47d	6.11de	
1996/1997	AT 2	83.7a	4.40de	1.89a	2.27b	24.1bcd	33.8abc	42.1cd	75.9def	17.84cd	6.11de	
1996/1997	AT 3	85.2a	4.79cd	1.31bcd	1.74bc	31.9b	28.0bcd	40.1cd	68.1fg	17.83cd	6.06e	
1996/1997	AT 4	86.1a	4.68cd	1.46abc	2.23b	18.6cde	51.7a	29.7de	81.4cde	17.52d	6.26cde	
1996/1997	AT 5	86.8a	4.64d	0.85de	4.32a	45.3a	42.1ab	12.6e	54.7g	17.51d	6.19de	
	LSD	10.9	0.39	0.53	1.19	13.2	21.2	23.0	13.1	0.64	0.27	0.12

Values with the same letters do not differ significantly from each other at the 5% significance level.
* Only in the 1995/1996 season.
** AT 1: 0 to 5% berry colour; AT 2: 10 to 20% berry colour; AT 3: 30 to 40% berry colour; AT 4: 60 to 80% colour; AT 5: one week before harvest.

tion timing areas can be visualised. The specific minimum and maximum values and the corresponding dosage and AT are given in each figure. The dosage and timing graph for the variable export pack-out (Fig. 3 a) shows that maximum export pack-out was obtained between 200 to 300 mg/L and at a timing optimum between AT 2 and 3.5. The highest export pack-out was reached with a dosage of 256 mg/L applied at AT 2.6. Optimum berry mass was obtained at a dosage of between 300 and 400 mg/L applied between AT 1 and 4 (Fig. 3 b), with maximum berry mass at a dosage of 400 mg/L applied at AT 2.4. To evaluate berry colour variables, the light (reject) berry colour (Fig. 3 c) and total export-quality berry colour (Fig. 3 d) graphs are shown. The minimum level of light (reject) berries was reached with a dosage between 300 and 400 mg/L applied between AT 2 and 4, with the lowest levels at 350 mg/L applied at AT 3.1. Total export berry colour had an optimum peak between 250 and 400 mg/L applied between AT 2 and 4, with a maximum at 350 mg/L applied at AT 3.2.

Bonheur

The analysis of variance summary of Bonheur variables is presented in Table 4. The Bonheur trial was harvested on 07 February 1995 (1994/1995 season), 13 February 1996 (1995/1996 season) and 24 February 1997 (1996/1997 season).

Dosage

Increasing ethephon dosages did not show significantly higher export pack-out percentages or better berry colour than the untreated control (Table 5). Average berry mass was influenced by dosage and tended to reach a maximum at 200 mg/L, whereafter it decreased. Loose berries and berry decay tended to increase with dosage, while berry crack tended to decrease with increased dosages. When compared to the untreated control, an increase in dosage tended to increase both total export berry colour and dark-coloured export berries, peaking at 200 mg/L. Medium-coloured export berries decreased slightly compared to the control, with the 100 mg/L dosage significantly lower than both the control and the 200 mg/L dosage. It is important to remember that in the case of Bonheur, which is a black grape, dark-coloured berries are more sought after than medium-coloured berries. The levels of reject light-coloured berries tended to decrease with dosage and were lowest at a dosage of 200 mg/L. Berry maturity (TSS and TTA) was not affected by ethephon treatments. In the preliminary study in the 1995/1996 season, both anthocyanin and phenol absorbance levels increased significantly with the application of ethephon.

Application timing

Applications of ethephon after 10 – 20% colour (AT 2) significantly increased export pack-out percentages (Table 6). The number of loose berries decreased significantly with applications later than AT 2, reaching the lowest level at AT 3. The AT 4 and 5 applications tended to increase berry crack, AT 4 significantly so. Export-quality berry colour was positively influenced by later appli-

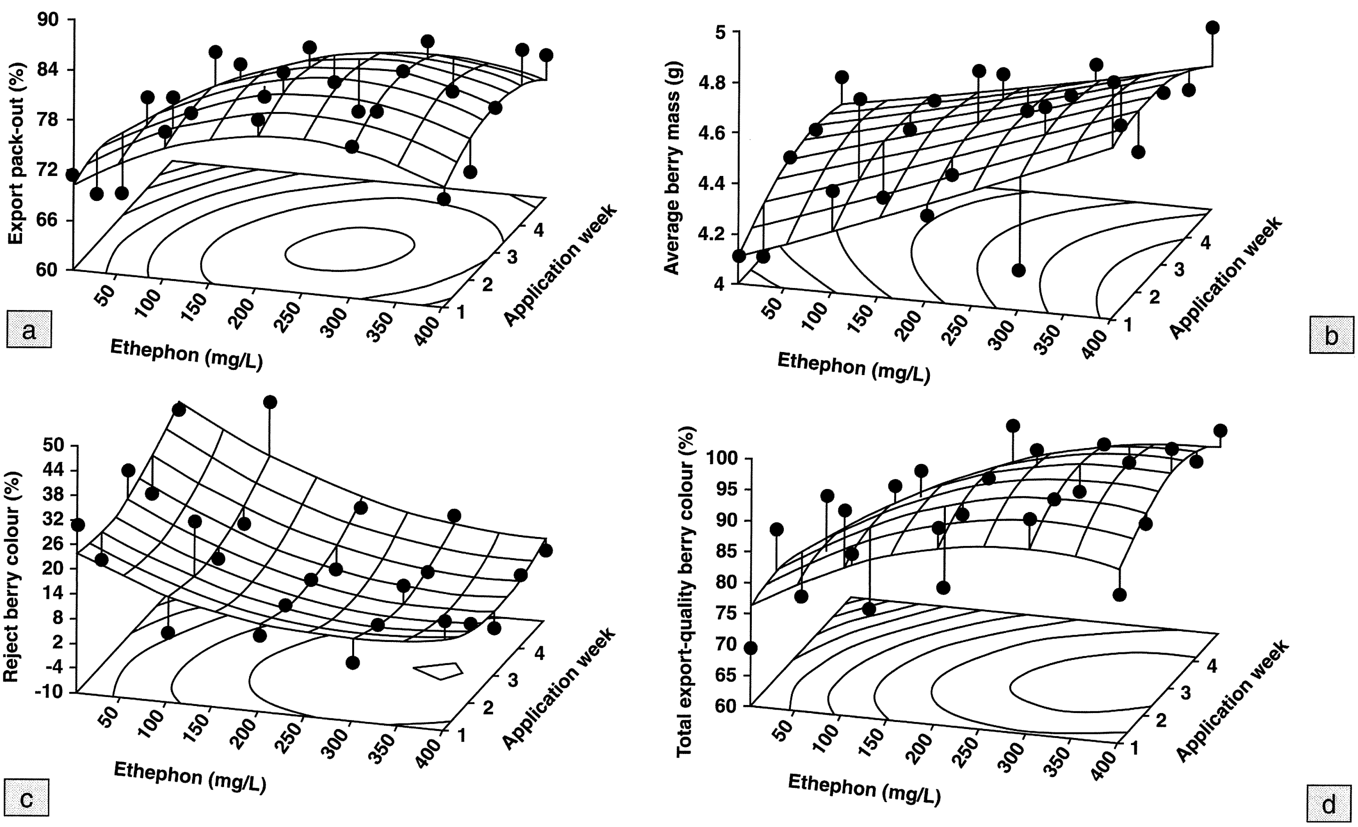

FIGURE 3

Bivariate second-order polynomial contour plots for different variables of Flame Seedless table grapes treated with ethephon for berry colour improvement in the Lower Orange River region, South Africa, 1994/1995 to 1996/1997.

(a) Export pack-out (%) $z=a+bx+cy+dx^2+ey^2+fxy$ r^2=62.9% a=67.6105 b=0.0665 c=3.4427 d=-0.00015 e=-0.8800 f=0.0044 Max=80.6% x=257 y=2.6 Min=62.8% x=0 y=5;
(b) Average berry mass (g) $z=a+bx+cy+dx^2+ey^2+fxy$ r^2=51.2% a=3.9504 b=0.0015 c=0.1783 d=1.71e-07 e=-0.0227 f=-0.00018 Max=4.7g x=400 y=2.4 Min=4.1g x=0 y=1;
(c) Reject berry colour (%) $z=a+bx+cy+dx^2+ey^2+fxy$ r^2=64.2% a=27.6986 b=-0.0882 c=-5.3209 d=0.00017 e=1.3535 f=-0.0081 Max=34.9% x=0 y=5 Min=5.4% x=400 y=3.2;
(d) Total export-quality berry colour (%) $z=a+bx+cy+dx^2+ey^2+fxy$ r^2=64.1% a=72.1427 b=0.0847 c=5.4677 d=-0.00016 e=-1.3733 f=0.0084 Max=94.6% x=400 y=3.2; Min=65.1% x=0 y=5.

cation timings. Medium-coloured export berries decreased slightly with later timings, while sought-after dark-coloured export berries increased significantly with later timings. Total export-colour berries also increased with later timings, significantly from AT 2, while light-coloured reject berries decreased significantly with the application of ethephon. Later applications tended to increase TSS levels, AT 3 and 4 treatments significantly more so than the earliest AT 1 treatment. There was no significant effect on TTA levels. In the 1995/1996 season both anthocyanin and phenol levels increased significantly with applications at AT 2 and 3.

Seasonal differences

Seasonal differences were observed (Table 7). The only seasonal interactions were for export pack-out (two-factor interaction) and medium export berry colour (three-factor interaction). All variables showed significant differences between seasons, except loose berries and medium export berry colour. A factor that may be responsible for these differences is the lower temperatures during the ripening period and harvest of the 1996/1997-season, contrary to higher temperatures experienced in the previous two seasons, especially 1994/1995, which resulted in heat damage to the berries. Anthocyanin and total phenol absorbancies gave some indication of the fluctuations of these compounds in the 1995/1996 season (Tables 5 and 6).

Contour graphs

As with the Flame Seedless data, contour graphs were drawn for certain important variables to enable a visualisation of the optimum dosage and application timing of ethephon (Figs. 4 a to d). The dosage and timing graph for the variable export pack-out (Fig. 4 a) shows that maximum export pack-out was reached between 200 to 400 mg/L and optimum timing between AT 4.5 and 5. The maximum export pack-out was achieved with 400 mg/L applied at AT 5. An optimum berry mass was obtained at a dosage between 100 and 250 mg/L applied between AT 1.5 and 4 (Fig. 4 b), with the maximum at 173.7 mg/L applied at AT 2.8. To evaluate the berry colour variables, the light (reject) berry colour (Fig. 4 c) and total export–quality berry colour (Fig. 4 d) graphs are shown. The minimum levels of reject, light berries were reached with a dosage between 200 and 400 mg/L applied between weeks 3.5 and 5, with the lowest levels at 321.8 mg/L applied at AT 4.4. The total export berry colour produced an optimum peak between 200 and 400 mg/L between AT 4 and 5, with the maximum level achieved at 315.2 mg/L applied at AT 4.7.

TABLE 4

Analysis of variance for different variables of Bonheur table grapes treated with ethephon for improvement of berry colour in the Berg River Valley region, South Africa, 1994/1995 to 1996/1997.

		Export pack-out (%)		Average berry mass (g)		Loose berries (%)		Berry decay (%)		Berry crack (%)		Berry colour: Reject (light) (%)		Berry colour: Export-quality medium (%)		Berry colour: Export-quality dark (%)		Berry colour: Export-quality total (%)		TSS (°B)		TTA (g/L)		Anthocyanin absorption* (A 520 nm)	
Source	DF	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Block	2	84.48	0.75	0.4692	0.60	0.4955	0.24	0.0928	0.51	0.6588	0.45	1918.4	0.07	475.15	0.44	581.8	0.57	1787.2	0.08	4.9666	0.02	0.107	0.56	0.0045	0.85
Ethephon	4	261.51	0.48	2.1328	0.07	0.4732	0.24	0.4048	0.03	1.3301	0.18	537.7	0.54	1468.03	0.05	1356.4	0.27	424.1	0.64	0.2257	0.94	0.020	0.98	0.1421	<0.01
Eth-Linear	1	393.12	0.13	0.9032	0.21	0.0192	0.77	1.0677	0.01	3.5520	0.08	482.4	0.41	249.91	0.48	840.6	0.31	173.9	0.63	0.0214	0.87	0.001	0.89	0.4862	<0.01
Eth-Quadratic	1	328.03	0.16	5.7333	<0.01	0.6867	0.08	0.1493	0.34	1.0048	0.34	233.1	0.57	512.30	0.31	1395.0	0.19	216.6	0.59	0.5200	0.41	0.057	0.38	0.0652	0.13
Eth-Cubic	1	201.60	0.27	0.1674	0.59	0.1447	0.42	0.0314	0.66	0.1280	0.73	70.0	0.75	2118.79	0.04	2819.1	0.06	49.9	0.80	0.1318	0.68	0.006	0.78	0.0154	0.46
Eth-Quartic	1	123.29	0.39	1.7272	0.08	1.0420	0.03	0.3709	0.13	0.6357	0.45	1365.3	0.17	2991.12	0.02	370.8	0.50	1256.0	0.20	0.2297	0.58	0.016	0.64	0.0014	0.82
Time	4	2010.46	<0.01	0.6686	0.57	1.7531	<0.01	0.1607	0.33	1.9789	0.06	2728.0	<0.01	618.08	0.38	4697.9	0.00	2998.2	0.00	2.2382	0.13	0.204	0.35	0.1187	<0.01
Time-Linear	1	7267.36	<0.01	0.0998	0.68	4.2593	<0.01	0.0374	0.63	4.5501	0.05	8716.4	<0.01	1190.36	0.13	17941.7	<0.01	9889.2	0.00	3.3627	0.04	0.053	0.40	0.0657	0.13
Time-Quadratic	1	305.35	0.18	1.3038	0.13	0.9189	0.04	0.2504	0.22	0.4680	0.52	1708.3	0.12	240.45	0.49	582.5	0.40	1571.5	0.15	5.3951	<0.01	0.343	0.03	0.2912	<0.01
Time-Cubic	1	436.70	0.11	0.0460	0.78	0.1187	0.46	0.2763	0.19	1.0903	0.32	477.9	0.41	886.57	0.19	46.8	0.81	526.2	0.41	0.1318	0.68	0.016	0.64	0.0549	0.17
Time-Quartic	1	32.45	0.66	1.2247	0.15	1.7155	0.01	0.0790	0.49	1.8072	0.20	9.4	0.91	154.92	0.58	220.4	0.60	5.8	0.93	0.0631	0.77	0.405	0.02	0.0629	0.14
Ethephon*Time	16	463.33	0.11	0.7169	0.68	0.1466	0.96	0.3735	<0.01	0.9954	0.29	553.3	0.67	998.53	0.07	1526.7	0.14	556.3	0.65	1.1482	0.50	0.158	0.60	0.0150	0.91
Error a	**48**	**293.87**		**0.8976**		**0.3330**		**0.1369**		**0.8137**		**684.4**		**574.83**		**1024.1**		**672.8**		**1.1840**		**0.180**		**0.0278**	
Season	2	33275.21	<0.01	46.2348	<0.01	0.1098	0.60	1.5945	<0.01	43.8354	<0.01	21400.1	<0.01	711.86	0.25	25881.3	<0.01	22047.9	<0.01	4.9752	<0.01	103.523	<0.01		
Season*Ethephon	8	398.66	0.02	0.1227	0.99	0.3420	0.14	0.5096	<0.01	1.7701	0.14	950.4	0.23	387.88	0.63	418.9	0.84	982.7	0.25	1.0584	0.21	0.058	0.62		
Season*Time	8	542.69	<0.01	0.3624	0.74	0.4288	0.06	0.2319	0.19	1.2452	0.35	782.0	0.37	1305.60	0.01	1062.3	0.24	748.4	0.45	1.4579	0.06	0.055	0.66		
Season*Ethephon*Time	32	221.52	0.15	0.5606	0.50	0.2986	0.12	0.2554	0.05	1.4054	0.19	486.5	0.89	894.88	0.02	858.0	0.39	506.8	0.90	0.8051	0.40	0.061	0.73		
Error b	**100**	**166.74**		**0.5682**		**0.2172**		**0.1616**		**1.1083**		**707.8**		**501.73**		**805.6**		**755.5**		**0.7576**		**0.074**			
Corrected Total	**224**																								

DF = Degrees of freedom. MS = Mean Square. P = Probability of F-ratio test.

TABLE 5

The effect of increasing ethephon dosages on export pack-out and post-cold-storage quality of Bonheur table grapes, Berg River Valley region, South Africa, 1994/1995 to 1996/1997.

Ethephon	Export pack-out	Average berry mass	Loose berries	Berry decay	Berry crack	Berry colour: Reject (light)	Berry colour: Export-quality medium	Berry colour: Export-quality dark	Berry colour: Export-quality total	TSS	TTA	Anthocyanin absorption*	Phenolics absorption*
(mg/L)	(%)	(g)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(°B)	(g/L)	(A 520 nm)	(A 280 nm)
control	73.5a	10.66ab	0.36b	0.16b	1.19a	31.2a	41.1a	27.7b	68.8a	16.60a	4.64a	0.41b	0.67b
100	75.7a	10.72ab	0.43ab	0.14b	1.00ab	31.0a	26.3b	42.7a	69.0a	16.52a	4.59a	0.56a	0.76ab
200	76.2a	11.05a	0.61a	0.26ab	0.77b	24.2a	38.7a	37.1ab	75.8a	16.53a	4.62a	0.60a	0.79a
300	71.2a	10.71ab	0.37ab	0.19b	0.89ab	31.1a	33.4ab	35.4ab	68.9a	16.44a	4.61a	0.63a	0.81a
400	71.1a	10.44b	0.42ab	0.37a	0.80b	28.1a	33.8ab	38.1ab	71.9a	16.61a	4.64a	0.66a	0.83a
LSD	7.3	0.40	0.24	0.16	0.38	11.1	10.2	13.6	11.0	0.46	0.18	0.12	0.09

Values with the same letters do not differ significantly from each other at the 5% significance level.
* only in the 1995/1996 season.

TABLE 6

The effect of application timing of ethephon on export pack-out and post-cold-storage quality of Bonheur table grapes, Berg River Valley region, South Africa, 1994/1995 to 1996/1997.

Weekly application times**	Export pack-out	Average berry mass	Loose berries	Berry decay	Berry crack	Berry colour				TSS	TTA	Anthocyanin absorption*	Phenolics absorption*
						Reject (light)	Export-quality						
							medium	dark	total				
(AT)	(%)	(g)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(°B)	(g/L)	(A 520 nm)	(A 280 nm)
AT 1	65.19b	10.66a	0.67a	0.22a	0.81b	42.7a	35.0a	22.3c	57.3b	16.16b	4.71a	0.44c	0.66c
AT 2	67.84b	10.72a	0.62a	0.27a	0.80b	30.2b	40.6a	29.2bc	69.8a	16.59ab	4.55a	0.59ab	0.79ab
AT 3	75.54a	10.92a	0.22b	0.16a	0.73b	25.7b	34.6a	39.7ab	74.3a	16.69a	4.64a	0.69a	0.87a
AT 4	79.82a	10.65a	0.36b	0.19a	1.19a	25.2b	31.7a	43.1a	74.8a	16.70a	4.56a	0.56bc	0.77b
AT 5	79.29a	10.62a	0.31b	0.30a	1.11ab	21.8b	31.3a	46.9a	78.2a	16.54ab	4.65a	0.57bc	0.76bc
LSD	7.27	0.40	0.24	0.16	0.38	11.1	10.2	13.6	11.0	0.46	0.18	0.12	0.09

Values with the same letters do not differ significantly from each other at the 5% significance level.
* only in the 1995/1996 season.
** AT 1: 0 to 5% berry colour; AT 2: 10 to 20% berry colour; AT 3: 30 to 40% berry colour; AT 4: 60 to 80% colour; AT 5: one week before harvest.

TABLE 7

The effect of season on export pack-out in vineyard and post-cold-storage bunch quality of Bonheur, Berg River Valley region, South Africa, 1994/1995 to 1996/1997.

Season	Export pack-out	Average berry mass	Loose berries	Berry decay	Berry crack	Berry colour				TSS	TTA
						Reject (light)	Export-quality				
							medium	dark	total		
	%	%	%	%	%	%	%	%	%	(°B)	(g/L)
1994/1995	49.51c	9.86c	0.45a	0.06b	0.28c	21.1b	37.13a	41.35b	78.47a	16.24b	3.44c
1995/1996	82.27b	11.39a	0.39a	0.29a	0.74b	18.1b	31.19a	51.69a	82.89a	16.66a	4.65b
1996/1997	88.83a	10.89b	0.47a	0.32a	1.77a	48.8a	35.62a	15.62c	51.23b	16.72a	5.79a
LSD	4.18	0.24	0.15	0.13	0.34	8.6	7.26	9.19	8.90	0.28	0.09

Values with the same letters do not differ significantly from each other at the 5% significance level.
* only in the 1995/1996 season.

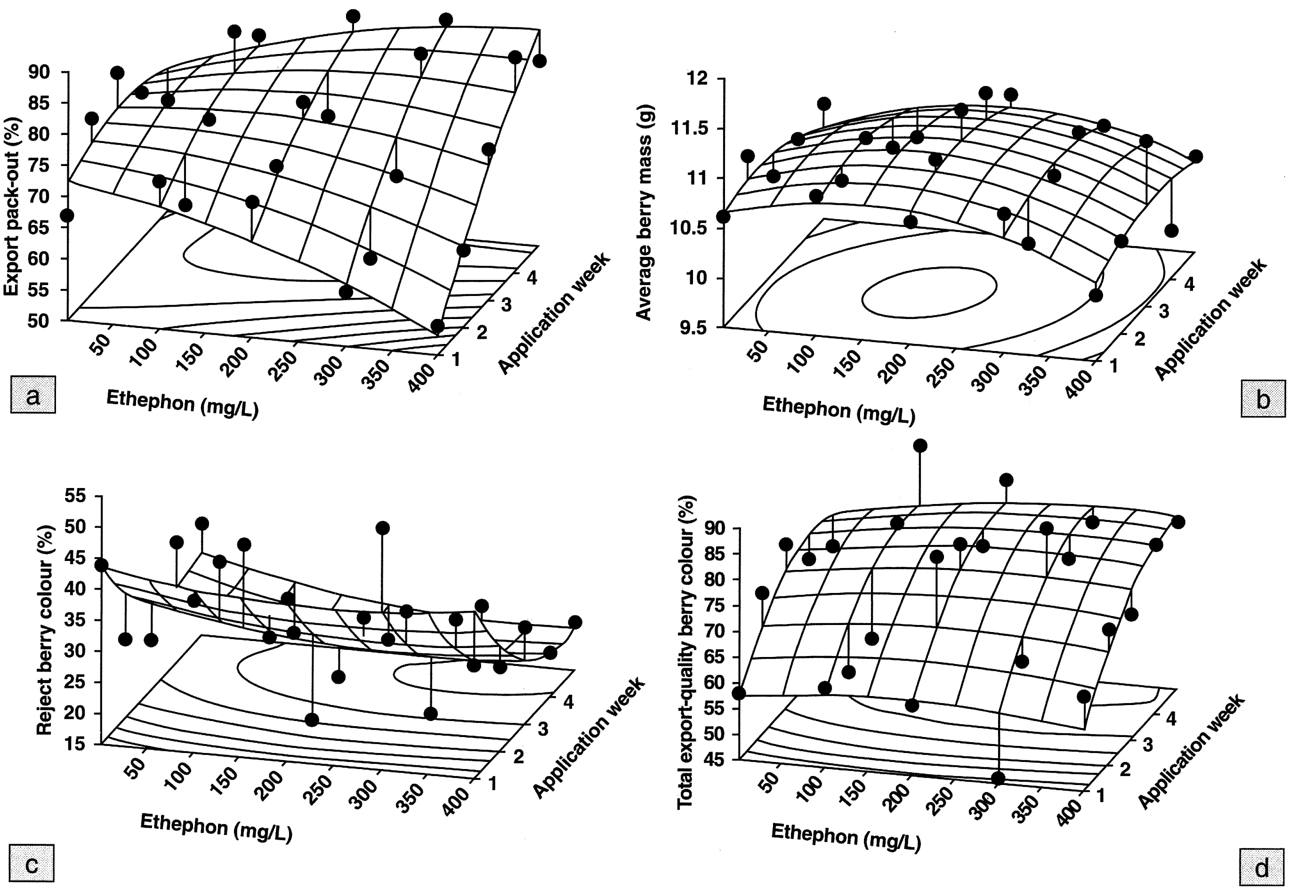

FIGURE 4

Bivariate second-order polynomial contour plots for different variables of Bonheur table grapes treated with ethephon for berry colour improvement in the Berg River Valley region, South Africa, 1994/1995 to 1996/1997.

(a) Export pack-out (%) $z=a+bx+cy+dx^2+ey^2+fxy$ $r^2=70.8\%$ a=68.6799 b=-0.0387 c=4.3136 d=-7.22e-05 e=-0.6962 f=0.0194 Max=84.6% x=400 y=1 Min=53.0% x=400 y=6;

(b) Average berry mass (g) $z=a+bx+cy+dx^2+ey^2+fxy$ $r^2=37.6\%$ a=10.4791 b=0.0027 c=0.2116 d=-9.54e-06 e=-0.0455 f=0.00023 Max=11.0g x=147 y=2.8 Min=10.4g x=400 y=6;

(c) Reject berry colour (%) $z=a+bx+cy+dx^2+ey^2+fxy$ $r^2=51.2\%$ a=55.5124 b=-0.0254 c=-13.6654 d=6.08e-05 e=1.6467 f=-0.0031 Max=41.8% x=400 y=1 Min=21.0% x=322 y=4.5;

(d) Total export-quality berry colour (%) $z=a+bx+cy+dx^2+ey^2+fxy$ $r^2=52.9\%$ a=45.5149 b=0.0187 c=13.4351 d=-5.86e-05 e=-1.5794 f=0.0036 Max=79.0% x=315 y=5 Min=56.9% x=400 y=1.

DISCUSSION

The results of the various ethephon application times and dosages showed variation between seasons, which complicated the determination of the optimum dosage and time of application. Few clear statistically significant differences were observed between the different dosages and application timings. However, certain tendencies were observed and, with the help of contour graphs, an indication of optimum dosage and application timing for these cultivars could be obtained.

Flame Seedless

The best overall results regarding the timing of application, based on export pack-out in the vineyard, export-quality berry colour and other post-cold-storage quality norms, were obtained with ethephon applied at two weeks after acid maximum (30 to 40% colour, AT 3) (Table 3, Figs. 3 a – d). This is later than results obtained by Jensen (1987) on Flame Seedless in the Coachella Valley in California, who obtained best results with an application one week after véraison, at the beginning of colour, with a dosage of 225 mg/L ethephon. Generally producers currently apply ethephon at this stage in the Lower Orange River region of South Africa. Timing of treatments closer to harvest did, however, increase the risk of quality defects such as berry crack, SO_2 burn, soft berries and loose berries. An ethephon dosage of 300 or 400 mg/L gave the best overall results regarding export pack-out in the vineyard, export-quality berry colour and other post-cold-storage quality norms (Table 2, Figs. 3 a – d). As one should always keep the dosage of PGR as low as possible, the lower dosage of 300 mg/L is recommended.

Bonheur

The best overall results over the three seasons regarding the timing of application, based on export-quality berry colour, export pack-out in the vineyard and post-cold-storage quality, were obtained with ethephon applied at two weeks after acid maximum (30 to 40% colour, AT 3) (Table 6, Figs. 4 a to d). In cooler seasons application could possibly be made a week later (60 to 80% colour). A later ethephon application (three weeks after acid maximum, 60 to 80% colour) in a cooler season (as in 1995/1996) and earlier (one week after acid maximum, 10 to 20% colour) in a warmer season (as in 1994/1995) could also be possible. Timing of treatments closer to harvest increases the risk of quality defects such as berry crack, loose berries, SO_2 burn and soft berries. Bonheur produced satisfactory berry colour and post-cold-storage quality at an ethephon dosage of 200 mg/L which increased export pack-out percentage, as well as average berry mass, and improved total export berry colour without negatively influencing other quality norms (Table 5, Figs. 4 a to d).

It is important to remember that different table grape cultivars will, however, differ in their response to ethephon due to their unique genetic, rootstock (which will affect growth vigour and thus response to ethephon) and cultivation differences. Crop load, climate and vigour also have an influence on the efficacy of the treatment. Therefore adjustments in dosage and timing may be necessary. The preliminary study to possibly use total anthocyanin and phenol absorption levels to complement visual berry colour evaluation would need to be repeated over a number of seasons with improved laboratory extraction techniques. However, due to the increased cost and time involved, it is doubtful whether it could be used as a practical tool for the producer. A speculative thought for seasonal variation could be that in warmer seasons (i.e. not a great variation between day and night temperatures), natural anthocyanin (colour pigments) production could be lower and a higher dosage of ethephon (applied a week earlier in the case of Flame Seedless) could be needed to ensure adequate colour. On the other hand, in a season where the day and night temperatures show greater variation, the natural anthocyanin production should be higher and a lower dosage of ethephon (applied a week later in the case of Flame Seedless), could be possible. What the precise temperature variation should be, and at what physiological stage for optimum natural colour development this variation should occur, was not determined, as it did not fall within the objectives of this study. The development of a model to accurately predict the natural development of anthocyanins in a specific season, and to prescribe the necessary ethephon dosage and timing would be of great benefit. The method used to determine the application timing, which was linked to acid maximum or bunch colour in this trial, can be improved. Recent attempts by local producers to rather use TSS (in °Brix) of bunches for timing show promise as a more accurate and quantifiable method to link application timing to a specific physiological maturity stage of the bunch. Multiple applications at different timings are a further method that could lessen the risk of not applying at the optimum timing and dosage for best colour development. This, however, needs to be investigated in further studies.

CONCLUSIONS

General recommendations are made of 300 mg/L ethephon for Flame Seedless and 200 mg/L ethephon for Bonheur table grapes, both applied at two weeks after acid maximum (30 to 40% colour). These recommendations apply to vines of normal vigour that are subjected to normal crop-load, i.e. vines with well-balanced growth. NOTE: Ethephon is presently not registered for colour improvement on Flame Seedless and Bonheur table grapes.

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