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Effects of Ethylene and 2-Chloroethylphosphonic Acid on the Ripening of Grapes

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ABSTRACT

The effects of ethylene gas, 2-chloroethylphosphonic acid, and the auxin, benzothiazole-2-oxyacetic acid, on the ripening of grapes (*Vitis vinifera* L.) was investigated. Ethylene hastened the start of ripening of Doradillo grapes when it was aplied for 10 days starting midway through the slow growth phase. 2-Chloroethylphosphonic acid applied to Shiraz grapes showed the same effect, but when it was applied earlier, during the second half of the first rapid growth phase or at the start of the slow growth phase of berry development, it delayed ripening. 2-Chloroethylphosphonic acid and benzothiazole-2-oxyacetic acid delayed the ripening of Doradillo grapes, and ethylene partially reversed the effect of benzothiazole-2-oxyacetic acid. The results demonstrate the importance of the slow growth stage in grape berry development and suggest that an auxin-ethylene relationship may be involved in the regulation of grape ripening.

The ripening of fleshy fruits is preceded by a shift in metabolism which leads to characteristic changes in composition, texture, and color of the fruit (3). In grapes, which have a double sigmoid growth curve (9), this shift occurs at the end of the slow growth phase of berry development; the changes include berry softening, an increase in reducing sugar, a fall in acidity, a loss of green color and, in some cultivars, the appearance of anthocyanin pigments. Changes in enzyme complement also occur (15, 16).

Ethylene accelerates the ripening of all fruits on which it has been tested (5, 6), and there is strong evidence that it is a fruit-ripening hormone (6, 13, 21). The ripening of grapes is delayed by auxins (4, 12, 22, 24), but there is no information on the effects of ethylene. This paper describes the effects of ethylene and 2-chloroethylphosphonic acid on the development of the grape berry. Their interaction with the auxin, benzothiazole-2-oxyacetic acid, was also explored.

MATERIALS AND METHODS

Ethrel on cv. Shiraz. Mature vines of *Vitis vinifera* L. cv. Shiraz, a black wine grape growing in a vineyard near Adelaide, were used. Bunches of grapes, selected for uniformity of development, were dipped in 500 or 1200 mg/l aqueous solutions of Ethrel[1] containing 0.05% (v/v) of a nonionic wetting agent. Bunches were dipped at 4, 5, 6, 7, 8, or 9 weeks after anthesis or six times at weekly intervals starting at 4 weeks after anthesis. In addition there were two untreated control treatments. There were 10 bunches of 150 to 200 berries in each treatment in a randomized block design.

The start of ripening was assessed visually by noting the first appearance of anthocyanin pigments, and the progress of ripening was followed by counting the number of colored berries on the bunches at intervals during the early stages of ripening. The fruit of a particular treatment was harvested 2 weeks after the start of ripening, and the reducing sugar and titratable acid contents were determined. Reducing sugar was determined by the 3,5-dinitrosalicylic acid reagent (1) and titratable acid by titration with 0.05 N NaOH to the phenolphthalein end point. Titratable acid is expressed as grams of tartaric acid.

Ethylene, Ethrel, and BTOA on cv. Doradillo. Mature vines of *V. vinifera* L. cv. Doradillo growing in the Waite Agricultural Research Institute orchard near Adelaide were used. This cultivar has large berries with a prolonged slow growth stage.

Twelve bunches were selected in four replicate groups of three on two adjacent vines. Each bunch was trimmed to two laterals each of about 15 berries from which 5 berries of 14.0 ± 0.4 mm diameter were selected and labeled. One lateral on each bunch was dipped in 20 mg/l BTOA, and the other was left untreated.

Three treatments were allotted at random to each group of three bunches: (*a*) untreated control; (*b*) dip in 400 mg/l Ethrel solution; (*c*) ethylene gas. All bunches were enclosed in polythene bags and subjected to an air flow of 100 ml/min. The bags were fitted with appropriate sealed inlets. A hypodermic needle outlet provided back pressure. Ethylene was injected from a cylinder to make a concentration of 20 mg/l in the air leading to group *c* bunches. Aluminum foil was wrapped around the bags to avoid heat injury to the bunches. These treatments were applied 10 weeks after anthesis, *i.e.*, midway through the slow growth stage of berry development, and the gas treatments were continued for 10 days, after which the polythene bags were removed.

Berry diameters were measured twice weekly between 9 and 11 AM with constant tension calipers at two spring tensions giving 60*g* and 240*g* force, respectively, at the measuring surface. The differences between the two diameters gave a measure of the compressibility of the berries. An increase in compressibility occurs several days before any increase in diameter can be detected (Coombe, unpublished data) and is the first indication of the start of ripening. These methods were developed in a preliminary experiment during the preceding growing season which gave similar results.

RESULTS

Shiraz Experiment. The effect of Ethrel on the appearance of anthocyanin pigments in berries is shown in Figure 1. Treatment

[1] Abbreviations: Ethrel: 2-chloroethylphosphonic acid; BTOA: benzothiazole-2-oxyacetic acid.

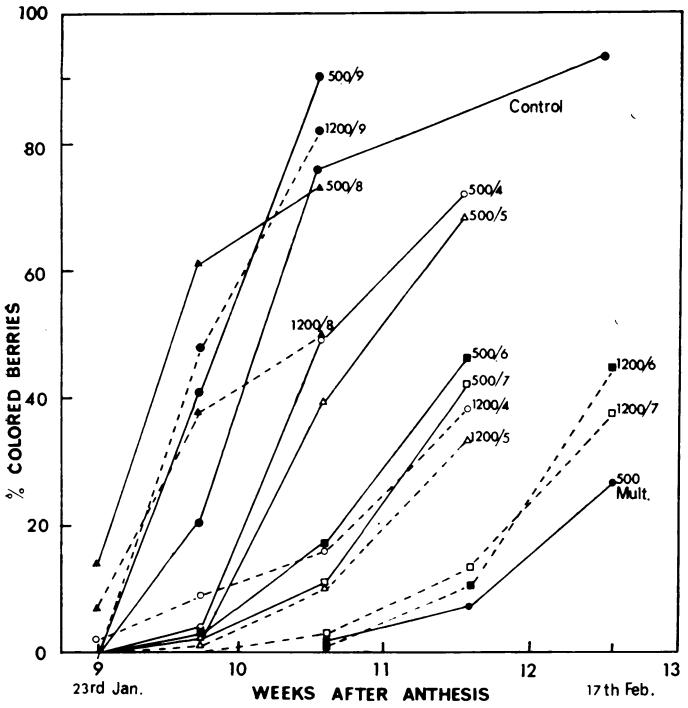

FIG. 1. Effect of Ethrel on the ripening of grape berries. Shiraz grapes were dipped in 500 or 1200 mg/l Ethrel solutions at 4, 5, 6, 7, 8, or 9 weeks (23rd January) after anthesis or on all treatment dates (Mult.). The points represent the mean of the number of berries showing first signs of color expressed as a percentage of the total number of berries per bunch. Individual curves are designated by concentration and age at application; *e.g.*, 500/4 means 500 mg/l applied 4 weeks after anthesis.

at 8 or 9 weeks after anthesis hastened the start of coloring while treatment at 4, 5, 6, or 7 weeks and the multiple treatment caused progressively greater delays. There were significantly more colored berries at the first count in the treatment at 8 weeks than any other treatment. At the second count, treatments at 8 or 9 weeks had significantly higher proportions of colored berries than treatments at 4, 5, 6, and 7 weeks and the multiple treatment, but only treatments with 500 mg/l at 8 weeks and 1200 mg/l at 9 weeks were higher than the control. At the second and third counts, treatments at 4, 5, 6, or 7 weeks and the multiple treatment had a significantly lower proportion of colored berries than the control treatment. The multiple treatments were toxic, and many berries shriveled and abscised. This effect was most severe in the 1200 mg/l treatment, where whole bunches were killed. Berries dipped in 500 mg/l of Ethrel colored sooner than berries dipped in 1200 mg/l, irrespective of whether the treatment advanced or delayed coloring.

The major compositional changes in ripening grapes are an increase in reducing sugar and a fall in acid content. The sugar to acid ratio integrates these changes and is used here as a measure of ripeness. Table I shows the sugar to acid ratios of fruit from treatments which hastened coloring. The colored berries which had been treated 8 weeks after anthesis had a significantly higher sugar to acid ratio, *i.e.*, were riper, than untreated colored berries, and green berries which had been treated with 1200 mg/l at 8 weeks were less ripe than untreated green berries. Treatment at 9 weeks had no significant effect on the ripeness of colored berries, but treated green berries were less ripe than untreated green berries. Thus Ethrel treatment at 8 or 9 weeks hastened ripening of the berries which were colored at the time of harvest but delayed ripening of the berries which were green. A differential ripening response by berries treated with Ethrel at the same time is also shown by the inflection in the curve for

Table I. *Effect of Ethrel on the Sugar to Acid Ratio of Shiraz Grapes Harvested 10.5 Weeks after Anthesis*

Ethrel Concn	Time of Treatment	Sugar to Acid Ratio	
		Colored berries	Green berries
mg/l	*weeks after anthesis*		
Control	...	5.5	1.1
500	8	8.9	0.8
1200	8	8.9	0.6
500	9	6.5	0.5
1200	9	6.1	0.5
LSD (P = 0.05)		1.5	0.35

Table II. *Effect of Ethrel on the Sugar to Acid Ratio of Shiraz Grapes*

Time of Harvest	Ethrel Concn	Time of Treatment	Sugar to Acid Ratio
weeks after anthesis	*mg/l*	*weeks after anthesis*	
11.5	500	4	6.8
11.5	500	5	6.7
11.5	500	6	4.4
11.5	500	7	3.7
11.5	1200	4	4.9
11.5	1200	5	3.9
LSD (P = 0.05)			1.85
12.5	Control	...	14.1
12.5	1200	6	4.3
12.5	1200	7	4.4
12.5	500	4, 5, 6, 7, 8, 9	3.6

percentage of colored berries for the treatments made at the 8th week after anthesis (Fig. 1). Table II shows that ripening was delayed by treatment with Ethrel at 4, 5, 6, and 7 weeks after anthesis (the later the treatment the greater the delay) and that 1200 mg/l delayed ripening more than 500 mg/l.

Doradillo Experiment. The compressibility of grape berries during the slow growth stage was 0.40 ± 0.06 mm, and once softening started the values rose steadily to over 1.0 mm. The averages of compressibility readings for all treatments are shown for each date in Figure 2. The proportions of berries which had softened at each date were calculated by taking a compressibility value of 0.55 mm as the turning point. These proportions are shown in Figure 3. The results show that ethylene advanced the start of softening by about 6 days and that softening occurred more rapidly than in the control berries. Both Ethrel and BTOA delayed softening, and Ethrel plus BTOA delayed softening more than Ethrel or BTOA alone. Ethylene partially reversed the delaying effect of BTOA, the combination of promotion and inhibition leading to discontinuity in the curves for compressibility and proportion of berries softening. In other treatments, however, both these parameters of ripening progressed uniformly once started.

DISCUSSION

It is likely that ethylene released by the decomposition of Ethrel was the cause of the response to the Ethrel treatment, (8, 10, 11, 18, 25). In the experiments reported here, both ethylene and Ethrel hastened the onset of ripening of grape berries but only if applied at a restricted period immediately before the normal time of the start of ripening. In the cultivar Doradillo, with its prolonged slow growth stage, the onset of ripening was

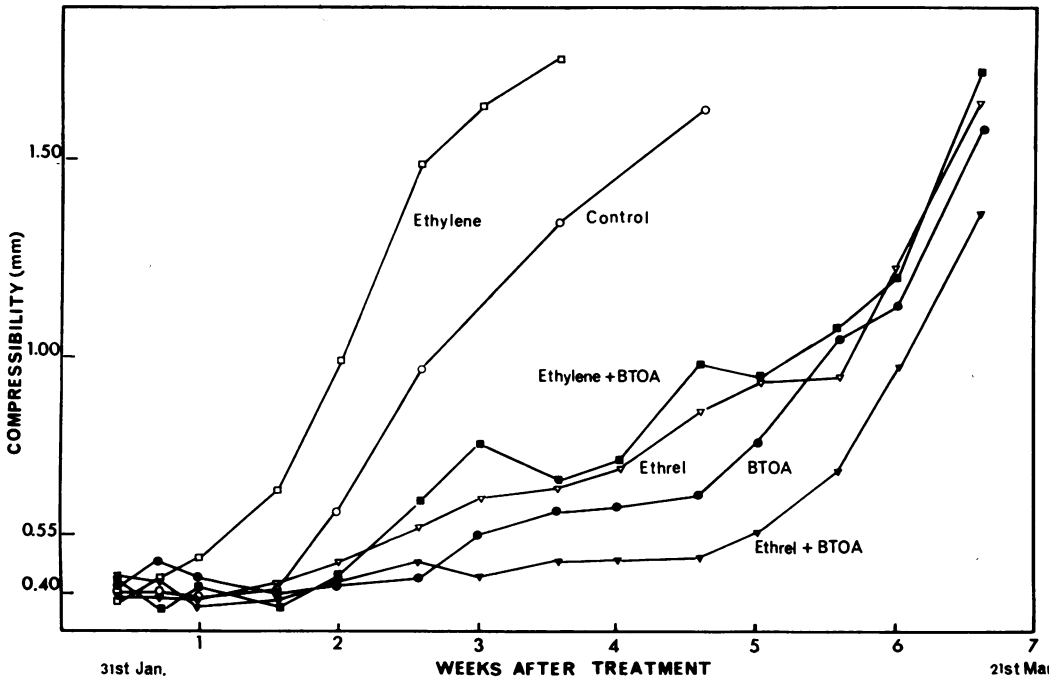

FIG. 2. Effect of ethylene, Ethrel, and BTOA on the average compressibility of Doradillo berries. The concentrations were ethylene: 20 μl/l; Ethrel: 400 mg/l; BTOA: 20 mg/l. The treatments were applied on 31st January (10 weeks after anthesis), and the ethylene treatments were continued for 10 days.

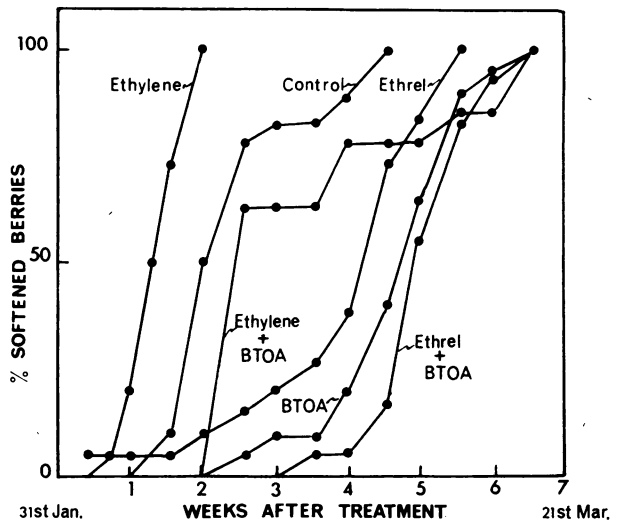

FIG. 3. Effect of ethylene, Ethrel, and BTOA on the proportion of softened Doradillo berries. Treatment details as in Figure 2 legend.

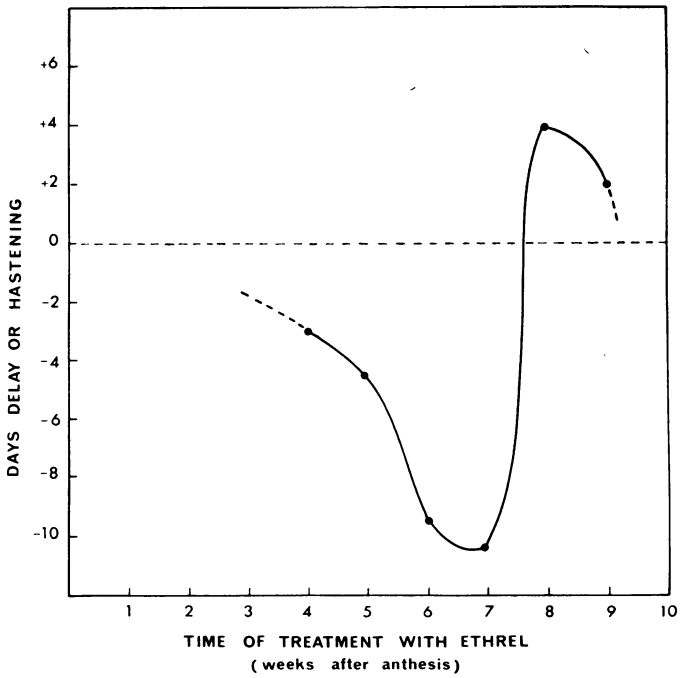

FIG. 4. Effect of time of treatment with Ethrel on days delay (−) or hastening (+), relative to untreated berries, to reach 40% colored berries, calculated from Figure 1.

advanced by 6 days (Figs. 2 and 3). With Shiraz, which has a shorter slow growth stage, the advance achieved was only 4 days (Fig. 1). It is probable that the effect would be even smaller and the responsive stage briefer in early maturing cultivars such as Zante Currant and Sultanina, which have brief slow growth stages (9).

When Ethrel was applied earlier to Shiraz, the onset of ripening was delayed, not advanced. The extent of the delay was considerable, up to 3 weeks, and was greater the later the application.

This difference between ripening response to Ethrel, *i.e.*, treatment in stage I inhibiting and treatment in stage II hastening, is similar to the situation when figs are treated with ethylene (17). The growth of the fig, however, differs from the grape in that growth during stage I is primarily by cell division (17), whereas in the grape cell division ceases about 4 weeks after anthesis (9, 14) and subsequent growth is by cell enlargement, so there would not have been any meristematic activity at 7 weeks after anthesis, when Ethrel caused the greatest delay in ripening.

The sudden reversal in response to Ethrel as the berry ages is

illustrated in Figure 4, which has been drawn from Figure 1. It shows that Ethrel treatment delayed ripening more the later it was applied and then suddenly reversed its effect from delay to promotion; the intensity of the promotive effect then declined. The reversal occurred in Shiraz berries in the 8th week after anthesis, and stage III commenced a week later. It is suggested that the same phenomenon occurred with Doradillo grapes, thus explaining the apparent anomaly of Ethrel delaying and ethylene hastening ripening. The reversal from delay to hastening by ethylene occurred in the 11th week after anthesis. As Ethrel was applied at 10 weeks it inhibited ripening. Ethylene treatment, however, was continued for 10 days into the 12th week after anthesis and hastened ripening, the inhibitory effect of the initial period being overcome by the continued application of ethylene. This hypothesis is supported by the partial reversal of the BTOA-induced delay by ethylene (Fig. 2).

At no stage of development have auxins been observed to accelerate the ripening of grapes (4, 12, 22, 24) as they do other fruits (2), where the hastened ripening is attributed to auxin-induced ethylene production (10, 17). Therefore, it seems that any increased ethylene production resulting from auxin treatment of grapes is insufficient to overcome the inhibitory effect of the auxin on their ripening. The similarity in the auxin- (12) and Ethrel-induced (Fig. 1) delays in ripening and the reversal of the auxin-induced delay by ethylene (Fig. 2) suggest that an auxin-ethylene relationship is involved in the regulation of grape ripening.

A second minor peak in auxin activity occurs in grape berries during the slow growth stage, and auxin begins to decrease at the time of the start of the second rapid growth stage (9). This, together with the auxin-induced prolongation of the slow-growth stage (12), indicates that a certain level of auxin is necessary to maintain the homeostasis of the berry at its slow growth stage of development. A higher auxin level at this stage of development may protect against the promotion of senescence by ethylene (20, 23). It is possible that applied ethylene delays the start of ripening by inhibiting auxin transport (7, 19) out of the berry or to a site of auxin destruction, thereby keeping the auxin level above a critical value. Exogenous auxins would have a similar effect on the auxin level.

The results presented in this paper show the physiological importance of the slow growth stage of berry development. During this stage the berry ages and reverses its response to ethylene and develops its potential to undergo the ripening changes. The role of auxin and possibly other hormones should be considered as well as ethylene in the regulation of fruit ripening.

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