

## Effects of Growth Retardants on *Vitis vinifera* L.

by

B. G. COOMBE

### Introduction

The group of chemicals known as plant growth retardants have only become widely known during the last decade but during that time have attracted intense interest. CATHEY (1964) defines them as "chemicals that slow cell division and cell elongation in shoot tissues and regulate plant height physiologically without formative effects". They have many other effects all of which are modifications of normal growth; for example, thickened stems, smaller and greener leaves, promotion of flower initiation in some species, and increased ability to withstand adverse water, temperature and salinity conditions (CATHEY 1964). Since 1964, two additional effects have been described: retarded senescence of harvested produce (HALEVY and WITTEWEN 1966) and increased fruit set (COOMBE 1965, EGERTON and HOFFMAN 1965).

This paper describes some of the effects of growth retardants on *Vitis vinifera* grapes in South Australia, a preliminary account of which was reported earlier (COOMBE 1965).

### Materials and Methods

These experiments were carried out on own-rooted *V. vinifera* vineyards in all of the major grape areas of South Australia. Aqueous solutions of the growth retardants (to which were added 0.05% Tween 20) were applied in two ways: as a cluster dip where single inflorescences were immersed momentarily in a solution, or as a vine spray where the whole vine was sprayed to run-off. At the rate of spraying used, 1000 ppm would represent an application rate of 200 to 250 g active ingredient per acre.

Four growth retarding chemicals were tested: CCC or Cycocel [(2 chloroethyl) trimethylammonium chloride, supplied by American Cyanamid Co.], Phosfon-D (tributyl-2,4-dichlorobenzylphosphonium chloride, supplied by Virginia-Carolina Chemical Corp.), Alar or B 995 (N,N-dimethylaminosuccinamic acid, supplied by Naugatuck Chemicals, U. S. Rubber Co.) and CO 11 (N,N-dimethylaminomaleamic acid, supplied by Naugatuck). CCC was supplied as a 50% aqueous solution and the others as white crystalline solids.

All experiments were designed as randomised complete blocks. Cluster dips were, wherever possible, applied to a single vine which served as a block and these were replicated ten times. The vine spray experiments were replicated five to eight times with three to five vines per plot.

Records were taken as indicated in the results. The dipped clusters were harvested when mature, their weight and length measured, and the numbers of berries per cluster counted after classification into seeded and seedless. A sample of twenty typical berries (seeded or seedless depending on the cultivar) was taken and measurements were made of their fresh weight and of their cumulative length

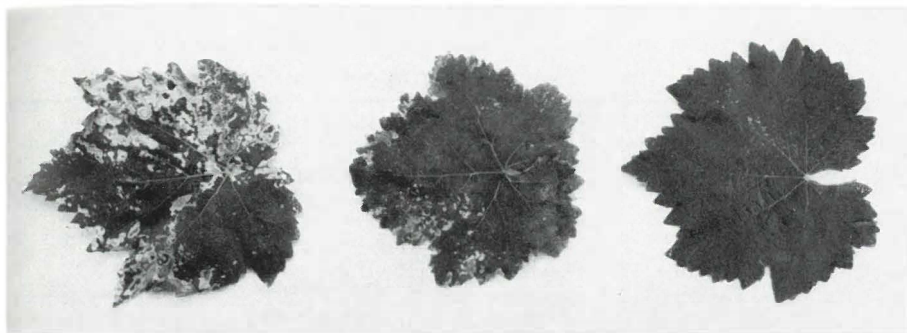


Fig. 1: Effect of 100 ppm Phosfon-D on leaves of Corinth (left), Muscat of Alexandria (middle) and Sultanina (right).

and breadth. After crushing, the juice was used to determine refraction (expressed as % sucrose) and titratable acidity. The same parameters were assessed in the vine spray experiments by weighing and measuring length of twenty clusters selected as typical of the clusters on each vine. Berries in a weighed subsample of about one tenth of each cluster were then counted. Finally a 50- or 100-berry sample was taken to measure weight, length, breadth, juice refraction and acidity.

## Results

### Effects on leaves

In fourteen experiments involving 8 cultivars in which CCC had been sprayed onto shoots or whole vines at concentrations between 100 and 1000 ppm, no leaf chlorosis was observed. Alar and CO 11 were also non-toxic but Phosfon-D at 100 ppm caused extensive patchy chlorosis and necrosis (Fig. 1).

Mature leaves treated with CCC were often darker green than untreated leaves. Discs were taken from such leaves at comparable node positions and extracted with 80% aqueous methanol. The optical density at 665 m $\mu$  of the filtered extract was from 10 to 11 per cent greater in discs from treated than from untreated leaves showing that the darker green colour was due to an increased chlorophyll content per unit area of leaf. Treated leaves appear to be thicker and of coarser texture but no sections or measurements have been made to test these observations. Leaves which were produced after CCC treatment were usually smaller in area than those on untreated vines. This is illustrated by Table 1 which shows the dimensions of parts of typical shoots from untreated vines and vines treated with CCC (100 ppm) 15 days pre-anthesis when the shoots had 9 visible nodes.

### Effects on stems and tendrils

The concentrations of CCC applied (<1000 ppm) affected stem growth in many, though by no means all, experiments. The effect was a reduction in internode length in the new growth after treatment and an earlier cessation of node formation by the apex (Table 1). A more consistent effect of CCC was on tendril growth. New growth frequently had greatly inhibited tendrils which were slow to lignify or abscised readily (Table 1, Fig. 2). This effect was greater and more

Table 1  
Dimensions of parts of typical shoots from untreated and CCC sprayed Muscat of Alexandria vines<sup>1)</sup>

Dimension (cm)	Node Number <sup>2)</sup>												
	6	7	8	9	10	11	12	13	14	15	16	17	18
Untreated													
Petiole length	9	9.5	8.5	7.5	5.5	4.5	3.5	3	2.5	2.3	2	1	0.5
Leaf length	12	14	14	12	12	10	9	8	7	5	4	2	1
Leaf width	12	14	13	12	12	10	9	9	8	5	4	2	1
Internode length <sup>3)</sup>	4	5	6	5	4.5	6	4.5	3	3	3	2	2	1
Tendrils length	—	12	15	—	13	13	—	11	8	—	4	2	—
CCC treated													
Petiole length	8.5	8	8	5	4	3	2	1.5	1	0.5	—	—	—
Leaf length	14	15	13	10	9	7	6	5	3	1	—	—	—
Leaf width	12	13	12	10	9	7	5.5	5	3	1	—	—	—
Internode length <sup>3)</sup>	5	4	5	4	3	3	2.5	1.5	2	1	—	—	—
Tendrils length	—	9	10	—	7	6	—	1.5	1	—	—	—	—

<sup>1)</sup> Treated 15 days before anthesis, measured 21 days after anthesis.

<sup>2)</sup> These shoots bore inflorescences at nodes 4 and 5. There were no differences in dimensions at the first 5 nodes.

<sup>3)</sup> The internode which is proximal to the node.

frequent than the inhibition of shoot growth with the result that some experiments showed shoots unaffected in length but with inhibited tendrils: Figure 2 is an example. Similarly, Table 1 shows that in this experiment all organs were affected by CCC but the reduced dimensions of internodes, petioles and leaf length and breadth were not evident until node No. 9 whereas tendrils were inhibited at nodes 7 and 8.

#### Effects on inflorescence initiation

The frequency of occurrence of secondary inflorescences on lateral (axillary) shoots is often greater on CCC treated vines, especially in those cultivars prone to form them. The increase results from a greater number of laterals bearing inflorescences and, most interestingly, a greater number of inflorescences per lateral. Fig. 3 illustrates a lateral from Muscat of Alexandria cultivar treated 2 weeks before anthesis with 300 ppm CCC; all of its 8 „tendrils“ are inflorescences.

The same transformation of tendrils into inflorescences was also seen at nodes 10, 12 and 13 of one primary



Fig. 2: Comparison of Muscat of Alexandria shoots, with leaf blades removed, from untreated vines (left) and vines treated just before anthesis (on 20. 10. 62) with 100 ppm CCC (right). Photographed 4. 2. 63.

shoot on a treated vine. In this instance the tendrils apparently did not abscise but the course of their development was changed so that inflorescences formed.

#### Next season's growth

There was no indication of residual effects on vegetative or reproductive growth in the years following spraying with CCC, even on vines which were treated for three successive years.

#### Effects on berry set and growth

Between 1962 and 1965 the effect of CCC at 100 or 300 ppm, as a vine spray or a bunch dip, was tested along with other treatments in forty-six experiments involving eight cultivars and four districts. The data from CCC-treated plots are expressed as a percentage of the untreated control in each experiment and have been plotted as frequency distribution histograms for parameter (Figs. 4 and 5). By this method a nil result would show as a normal distribution centred about zero percentage-difference-from-control, and a positive or negative effect would shift the curve to the right or left respectively. Those histograms farthest removed from the centre contain the greatest proportion of results in which the effect was significant but this method of presentation does not permit the display of individual significances.

Clearly, CCC has increased the number of berries per bunch due to an increase in fruit-set. The mode is 15 per cent increase over control but over half of the experiments show more than 20% increase. This increase did not involve any consistent change in the proportion of seeded and seedless berries, although a few experiments showed a small significant decrease in the per cent seedless berries. Another consistent effect of CCC was the reduction of berry size as shown by the histograms of berry weight, width and length. The mode for weight is 13 per cent decrease. This reduction counterbalances some of the berry set increases with the result that the product, cluster weight, shows a shift back towards the centre. The histograms for cluster weight, in fact, appear to be bimodal with one group

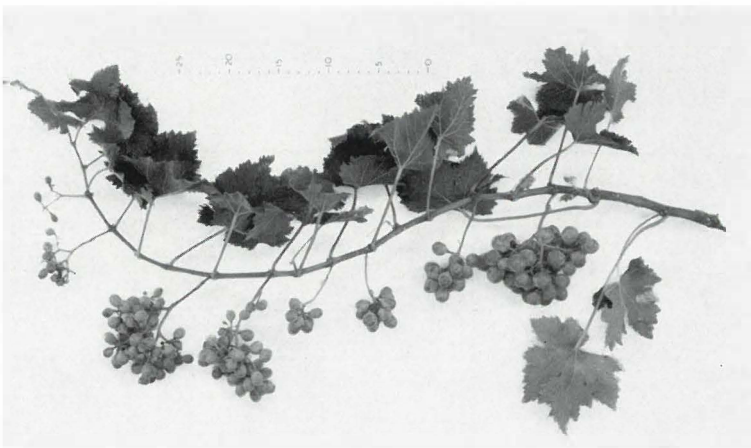
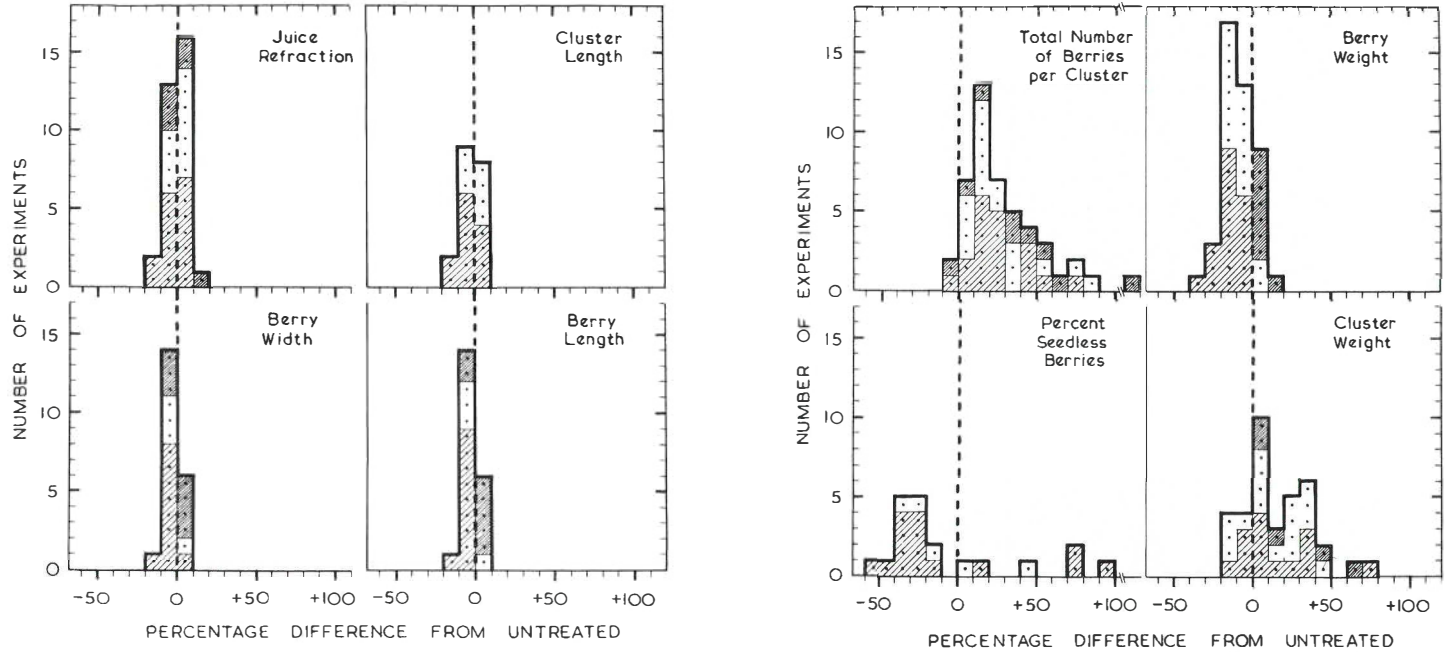


Fig. 3: Lateral shoot from a Muscat of Alexandria vine treated just before anthesis (on 10. 11. 66) with 100 ppm CCC. Photographed 23. 3. 67.



Figs. 4 and 5: Frequency distribution histograms showing the number of experiments giving certain percentage differences between CCC treatment (100—300 ppm) and untreated for eight parameters as shown. Each dot represents one experiment. Dark hatching represents Corinth, light hatching represents Muscat of Alexandria and the rest, unhatched, represents six other cultivars.

showing no response and the remainder, comprising 40%, showing an increase of 20% or more over untreated. The similarity between the berry width and length histograms reflects the lack of effect of CCC in altering berry shape, although in a small number of experiments there has been a minor but significant decrease in the length/width ratio of berries. Juice refraction, a measure of total soluble solids, was unaltered by the treatment. Cluster length was largely unaltered; though slightly more experiments occur on the negative side none of them were significant. There were no differences noted in other factors examined such as titratable acidity of juice, berry taste, and numbers of seed per berry. In summary, treatment with CCC increased set and decreased size but had no other notable effects on berry growth.

The histograms are hatched according to the cultivars used: dense hatching represents Corinth, light hatching represents Muscat of Alexandria and unhatched comprises the remaining six cultivars (Sultanina, Palomino, Doradillo, Grenache, Shiraz, Cabernet sauvignon). It can be seen that there are no noteworthy differences in the response of the cultivars apart from those parameters associated with berry size (weight, width and length). With these, Corinth does not show the usual reduction after CCC treatment (probably because they normally have small berries).

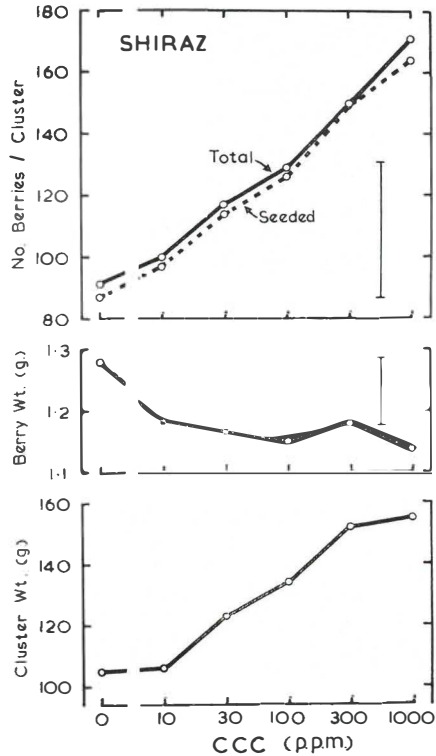
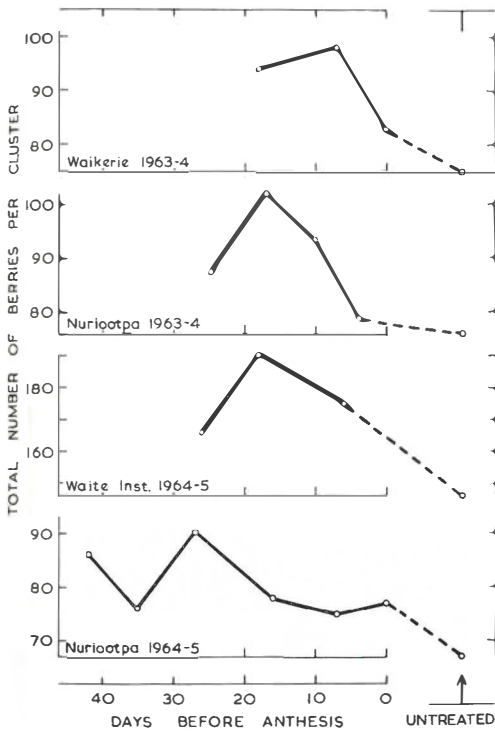


Fig. 6: Effect of time of dipping with 100 ppm CCC on setting in four experiments with Muscat of Alexandria clusters. In all of these experiments the high values were significantly greater than untreated control ( $p < 0.05$ ).

Fig. 7: Effects of five concentrations of CCC solutions as pre-anthesis cluster dips on setting, berry weight and cluster weight of Shiraz.



Twelve of the 46 experiments were treated by spraying and the rest cluster dipped. A comparison of the histograms of setting showed no differences between the two methods of treatment.

#### Time of application of CCC

When this response was first reported (COOMBE 1965) it was shown that a treatment 2 to 3 weeks before anthesis was effective but that treatments at or after anthesis had no effect. Subsequent trials comparing a greater array of timings have largely confirmed this result. The results of four experiments on Muscat of Alexandria using bunch dips of 100 or 300 ppm CCC are shown in Fig. 6. Applications just before or at anthesis give only slight increases in set. The largest increases were obtained at one to three weeks before anthesis during which the timing does not appear to be very critical.

#### Concentration of growth retardants

In most experiments CCC has been used at 100 ppm as a spray or dip. In 1964—65, cluster dips of 0, 10, 30, 100, 300 and 1000 ppm were compared on six cultivars and vine sprays of 0, 30 and 300 ppm compared on four, all treatments being applied about ten days before flowering. The results obtained with Shiraz are presented in Fig. 7. Set was increased linearly with increasing concentration and the results suggest that as little as 30 ppm gives some response. This increase is due entirely to seeded berries since there is no change in the number of seedless berries per cluster. Berry weight was reduced by about 10% at all CCC levels with only a slightly greater reduction at high concentrations. Cluster weight responded in a similar fashion as did set.

The cultivars Cabernet sauvignon, Muscat of Alexandria and Grenache, responded to bunch dips similarly to Shiraz, but Palomino and Doradillo responded erratically. However, all four cultivars in the vine spray experiments (Shiraz, Muscat of Alexandria, Palomino and Doradillo) gave similar results (Table 2).

Alar was tested with CCC at the same array of concentrations in these cluster dips and vine spray experiments but it had practically no significant effects on any parameter. Only three treatments of the 38 tested (all concentrations in 10 experiments) gave significant increases in set and these were only just significant at  $p < 0.05$ . Similarly only one of the treatments gave a significant change (reduction) in berry size.

CO 11 was tested in a more limited way in earlier experiments but was without effect. Phosfon-D, like CCC, caused an increase in set (see COOMBE 1965) but was phytotoxic to leaves.

### Discussion

Several separate aspects of vine growth have been altered by treatment with CCC but all of them are essentially "normal". Leaves were darker green, shoots were shortened, tendrils retarded, laterals differentiated more inflorescences, more berries set and berry size was decreased. None of them represent abnormal growth so that CATHEY's generalisation for the effect of growth retardants on other plants (CATHEY 1964) has been confirmed for *Vitis vinifera*. The effects, however, could be regarded as unusual in that no other exogenous chemicals have brought about these

Table 2

Comparison of the effects of two CCC concentrations (ppm) as pre-anthesis sprays on four cultivars

Parameter	Shiraz			Muscat of Alexandria			
	0	30	300	0	30	300	
No. berries per cluster	Seeded	122	133	159*	63	68	81
	Seedless	13	4	1	30	25	29
	Total	135	137	160	93	93	110
Berry weight (mg)	1276	1181*	1148*	4818	4258*	4155*	
Cluster weight (g)	156	147	169	331	298	343	
Refractometer (%)	22.2	22.2	22.4	19.0	18.7	18.5	

Parameter	Palomino			Doradillo			
	0	30	300	0	30	300	
No. berries per cluster	Seeded	136	157	181*	197	210	252*
	Seedless	13	15	18	30	21	13
	Total	149	172	199*	227	231	265*
Berry weight (mg)	2536	2305*	2378*	3370	3307	2983*	
Cluster weight (g)	352	370	438*	719	703	741	
Refractometer (%)	20.4	20.4	20.0	16.7	15.7	15.2*	

\*) Significantly different from 0 (control) at  $p < 0.05$ .

effects on grapes, especially increased setting of seeded berries and the marked alteration in tendril/inflorescence morphogenesis. The wide spectrum of effects suggests some far-reaching alteration in cell metabolism.

Gibberellic acid applied to grapes has effects which are generally the reverse of those induced by CCC: leaves are paler green, shoots are elongated, tendril growth promoted, inflorescence initiation reduced, set is decreased, and berries are elongated and increased in size. These effects are not invariable and require some qualification; reduced inflorescence numbers were noted in the year following  $GA_3$  treatment (JULLIARD and BALTHAZARD 1965); the decrease in setting caused by  $GA_3$  can be confused by the relative numbers of seeded and seedless berries on which  $GA_3$  has a potent effect (COOMBE 1965); the increase in berry size refers mainly to seedless berries (WEAVER and McCUNE 1959). Nevertheless the contrast between the effects of CCC and  $GA_3$  is striking and suggests that, in an investigation of the mode of action of CCC on grapes, the gibberellin metabolism should be included.

Effects on berry set and berry growth were obtained with both cluster dip treatment and vine spraying but in the former method no effects on shoot growth were observed. This indicates that the changes leading to alterations in berry set and size were local, within the inflorescence. Further, the inverse correlation between berry size and berry setting is poor (see Fig. 7 and Table 2 for example) implying that CCC influences these growth processes separately.

The lack of evidence for any alteration in vine behaviour during the year following treatment, either vegetatively or reproductively, suggests a lack of carry-over from year to year. This is not surprising in view of the fact that only about 100 mg is applied early in the growing season and that a major part of the current season's growth is removed by pruning.



Two other reports have described the effects of growth retardants on grapes. CLAUS (1965) tested CCC on *V. vinifera* grapes in Germany (cvs. Riesling, Traminer, Müller-Thurgau and Silvaner). He found differences in response of cultivars but in general his results agree with those described here. One noteworthy exception was his description of CCC's effect in shortening clusters; this, together with an apparent increase in set, led to compact clusters. TUKEY and FLEMING (1967) have tested Alar on both *V. labrusca* and *V. vinifera* grapes. They describe an increase in berry setting but they found no reduction in berry size on *vinifera*'s. Also, their most effective time of treatment was closer to anthesis than I have found for CCC. This suggests that the lack of effect of Alar in the present experiments may have been due to too-early treatment.

While the effects of CCC on berry set and size described here are considerable, they should not be regarded as a definitive study of its effects under commercial conditions. However, the results suggest that this retardant could be useful on winegrapes which set poorly or irregularly. Even if the treatment had no effect on yield it may be useful on red winegrapes in increasing the proportion of skins through an increase in number but a decrease in size of berries. No use is foreseen for it on table grapes and its use on raisin grapes remains uncertain.

### Summary

Four growth retardants, CCC, Phosfon-D, Alar and CO 11, have been tested on *Vitis vinifera* over five seasons. Applications have been made by vine spraying or cluster dipping.

Spraying with CCC resulted in darker green leaves, shortened internodes, retarded tendrils, increased numbers of inflorescences differentiated on lateral shoots, greater berry set and smaller berry size. Not all of these effects occurred together, the most frequent effects being on tendrils and berry set and size. Cluster dipping affected berry set and size in the same way as vine spraying but had no effects on other organs.

Set was increased more than 20 per cent in about half of the 46 experiments. At the same time, berry size was reduced by about 10 per cent so that cluster weight or yield was only increased by more than 20 per cent in two-fifths of these experiments.

Comparison of concentrations of CCC between 10 and 1000 ppm showed, in general, an increasing effect on set with increasing concentration. The most effective time of treatment was one to three weeks before anthesis; timing does not appear to be critical.

Phosfon-D, like CCC, increased setting but caused a severe patchy chlorosis of leaves. Alar and CO 11 were, in general, without effect in these experiments.

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B. G. COOMBE  
Department of Plant Physiology  
Waite Agricultural Research Institute  
University of Adelaide  
Glen Osmond  
South Australia.