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MORPHOLOGICAL AND ANATOMICAL CHANGES INDUCED IN SUNFLOWER BY CHLORMEQUAT AND THEIR POSSIBLE SIGNIFICANCE

The plant growth retardant Chlormequat (CCC, 2-Chloroethyltrimethylammoniumchloride) may have considerable commercial potential in Australia both in controlling the height of the present tall cultivars and in the alleviation of moisture stress which many crops experience during their growth cycle.

Yet the underlying mechanisms of CCC effect are not clearly understood, or adequately documented. The authors suggested that since reductions in transpiration following treatment are immediate, CCC may cause stomatal closure initially. However, they regarded this as a short term effect and did not exclude the possibility that morphological and anatomical changes may also be induced which would favourably influence plant water relations in the longer term.

Individual plants of the sunflower cultivar Peredovik were grown in 23 cm plots containing a mixture of loam and peat (ratio 3:1) in the glasshouse. Environmental conditions during the growing period were: - mean day temperature 26°C, mean night temperature 15°C and daylength 12 hours. A complete commercial nutrient solution was applied at weekly intervals throughout the experiment.

At the ten-leaf stage CCC was applied as a foliar spray of a 4000 ppm aqueous solution to half the plants.

Water use was measured daily be weighing, after which pots were rewatered to the predetermined 90% field capacity. Polystyrene balls were placed on the surface to minimize evaporation.

Leaf area was measured on the same plants throughout the growing period using the relationship: leaf area = max. length x max. width x 0.7. Stomatal resistance was recorded by means of a Diffusion Porometer on leaves which were expanded at the time of treatment and on newly expanding leaves. Leaf impressions were taken on similar leaves to determine stomatal size and frequency. Material for anatomical analysis was sampled on the day of spraying and at seven and fourteen days after spraying. Leaves which were expanded at the time of treatment and newly expanding leaves were harvested, together with the respective petioles and a portion of the stem from below the node. Stem apices were also sampled. Dehydration and infiltration techniques were similar to those described by Johansen and sections were stained with Safranin and Fast Green for subsequent microscopic examination.

The pattern of water use revealed that to application of CCC resulted in an immediate reduction in transpiration, and water use of treated plants was significantly (5%) lower for three weeks after treatment than in the untreated plants by 12.7, 16.1 and 9.3% respectively. In the fourth week transpiration rose to the level of untreated plants and did not significantly differ for the remainder of the experiment.

Expanding leaves are much deformed and their diffusion stomatal resistance was therefore measured in four days after treatment. The stomatal resistance of these leaves was significantly increased, and the tendency suggests that it may have been increased prior to this, a circumstance which would explain the rapid effect of CCC on transpiration (Table 1). CCC did not affect the stomatal resistance of leaves which were expanded at the time of treatment. This is confirmed by microscopic observations of leaf impressions taken five days after treatment.

Table 1

Effect of CCC on Stomatal Resistance (s cm^{-1}) of Newly Expanding Leaves

Days from treatment	4	5	6	7	8	9	10	11
CCC	3.50	2.18	2.95	2.80	2.70	2.58	1.63	1.43
Control	1.68	1.58	2.18	1.98	1.83	1.25	1.05	1.15
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	N.S.	N.S.

CCC had no apparent affect on stomatal number or width of the lower leaves. In the newly expanding leaves, however, stomata were partially closed on CCC-treated plants. Stomata per unit area were also slightly increased in these leaves, though not significantly, which may have resulted from reduced leaf expansion.

The effects of CCC on stomatal resistance diminished after ten days while water use was significantly reduced for three weeks after treatment. Measurements of leaf area showed that control plants developed a greater leaf area during the four weeks after spraying although differences between treated and untreated plants were not statistically significant.

The mechanism by which CCC reduces water use would therefore appear to be an initial increase in stomatal resistance loading to an immediate but short term decrease in transpiration, followed by a decrease in leaf area. The latter effect would reduce the amount of radiation intercepted by the plant and, hence, transpiration.

CCC-induced stomatal closure has previously been related to decreased pyrophosphatase activity. In this experiment such an affect may have been prolonged by leaf chlorosis following treatment which would tend to reduce the photosynthetic rate of affected leaves and cause stomatal closure through a build up of intercellular carbon dioxide.

If photosynthesis is reduced by CCC it may results in less assimilate being available for leaf expansion. However, the situation is complicated by the fact that in treated plants less demand is made on the leaves for assimilates since CCC treatment reduces the growth of a powerful sink i.e. the stem.

Preliminary studies have been undertaken to determine the effects of CCC on both photosynthesis and translation, using $^{14}CO_2$ techniques similar to those described by McWilliam et al. Initial results indicate that the photo-

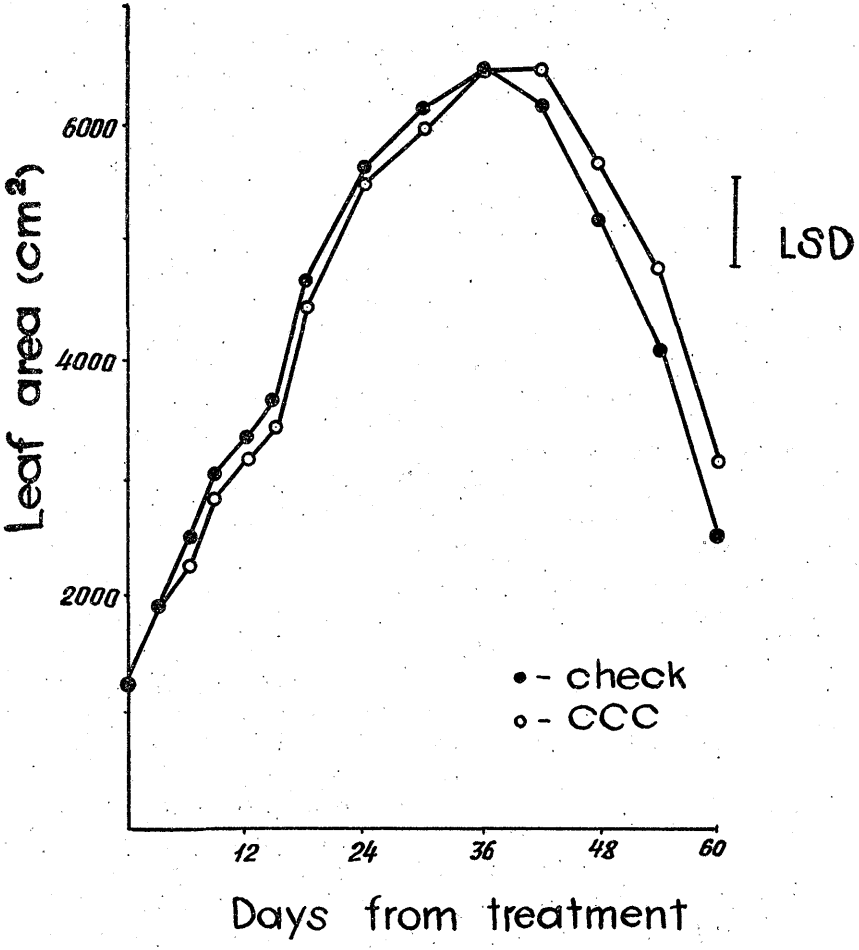

Fig. 1. Effect of CCC on leaf area

synthetic rate of the upper leaves is considerably reduced by CCC treatment but this is compensated for, in part, by a greater retention of assimilates (Table 2). In both treated and untreated plants the major sinks remain the same but differences in their relative importance are evident. It is interesting to note the increased percentage of photosynthate which is translocated to the roots in treated plants.

During the phase of leaf senescence CCC-treated plants possessed a greater leaf area than controls, and although the former had a slightly higher transpiration rate this was not proportional to the increased leaf area. It would appear, therefore, that a more permanent effect on the plant may have been induced by treatment. Anatomical studies, however, were able to detect only small differences between treated and untreated plants, although this was partly due to the inherent variability present in Peredovik. For example, there was no consistent effect on leaf thickness while vascular differentiation in leaves, petioles and stems appeared unaffected by treatment. At the stem apex little effect could be seen in the region of active cell division but cell extension was reduced in treated plants. This reduction persists until maturity and results in reduced internode length of the treated plants. As a consequence of this, water use of treated plants may be reduced through increased mutual shading of the leaves.

Thus, CCC is able to cause a short-term increase in stomatal resistance followed by a delay in leaf expansion. Moreover, the apex's cell division is inhibited and the plant height is reduced. A similar reaction can be observed in sunflowers which experience moisture stress, and thus the action of CCC can be seen as that of pre-adapting the plant to stress conditions. This CCC effect may be of importance in arid areas.

Table 2

The Effect of CCC on Translocation. (Plants were labelled in the leaf 11 position 5 days after treatment and harvested 48 hours later)

Plant fraction	Distribution of $^{14}CO_2$ (as a % of plant total)	
	control	CCC
Leaf 11 (fed leaf)	25	33
Petiole 11	5	6
Lower leaves	0	0
Upper leaves	2	5
Lower petioles	0	0
Upper petioles	1	2
Lower stem	42	33
Upper stem (including apex)	20	12
Roots	5	9

It would seem that CCC may be able to induce drought avoidance at any stage of growth at which leaf expansion occurs. Further studies are needed to clarify. These studies may also encompass the effects of treatment on other metabolic processes so that a fuller understanding of the action of CCC may be obtained.

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