Effects of a Growth Retardant, CCC, on Leaf Growth in *Phaseolus vulgaris*

G. M. Felippe* and J. E. Dale

Botany Department, University of Edinburgh

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Summary. 1. CCC applied at $10^{-2}$ M during early stages of germination of dark-grown plants led to increases in cell number and fresh and dry weight of the primary leaves. These effects could be reversed by application of 10 µg GA$_3$ per plant after emergence above the soil.

2. Irradiation with red light for short periods also increased cell number and fresh weight of leaves. This effect could be reversed by far-red radiation, but not by application of GA$_3$.

3. Responses to both CCC and light were dependent upon the continued presence of the cotyledons, which it is suggested supply a growth factor required by stem and leaves.

4. Treatment of plants grown in 12-hour days with CCC, reduced area and rate of expansion of primary and trifoliate leaves. For the latter, a change in leaflet shape was found. Cell number of primary leaves was not affected under these conditions, but that of trifoliate leaves was reduced. A smaller reduction in cell number was noted for the third trifoliate leaves on treated plants; this was shown to be due to the fact that this leaf was not shaded by the primary leaves as was the case for first and second trifoliate leaves.

5. The effects of GA$_3$ in counteracting the responses to CCC are described. It is suggested that applications of CCC and GA$_3$ may modify the pattern and polarity of cell expansion in leaves.

Introduction

Although the effects on stem growth of the quaternary ammonium compound 2-chloroethyl trimethyl ammonium chloride, CCC, are well known (Cathey, 1964), effects of this growth retardant on the development and growth of leaves are less well characterised. There are reports that total leaf area of treated plants is greater than that of untreated controls (Lindstrom and Tolbert, 1960; Humphries, 1963) and that treatment leads to an area reduction (Humphries and French, 1965). Similarly, there are reports that area of individual leaves on treated plants may be either increased or decreased according to the species (e.g. Dyson, 1965), while the number of leaves produced following treatment may also be affected (Humphries and French, 1965).

In an earlier paper (Dale and Felippe, 1968), it was shown that treatment of dark-grown seedlings of *Phaseolus vulgaris* with $10^{-2}$ M CCC...
led to a diversion of dry matter from the cotyledons to the primary leaves so that these were heavier than in control plants, whereas in contrast, dry weight of the stems was reduced for treated plants. It has been established that this diversion of material to the leaves is associated with enhanced leaf growth in plants grown in darkness, but not in plants grown in light conditions. This paper reports these results in detail.

It is well known that many effects of CCC on plant growth can be counteracted by application of gibberellic acid, GA₃. This is shown to be true also for effects of CCC on leaf growth in Phaseolus, and these results are discussed in the light of effects of CCC on the early development of young seedlings of French bean (DALE and FELIPE, 1968).

**Materials and Methods**

Plants of Phaseolus vulgaris cv. Suttons Selected Canadian Wonder were grown in sand under controlled conditions as already described (DALE, 1964a; DALE and FELIPE, 1968). For plants grown in complete darkness temperature was 25 ± 1°C; for plants grown in the light temperature was 22.5 ± 1°C, with 12 hour daylength and a light intensity from warm white fluorescent tubes of 9200 lux.

CCC was applied as a soil drench to plants, 100 ml of solution at 10⁻² M being given on the fourth or seventh day after planting in the case of the light-grown plants, and at various times up to day 8 after planting for material grown in darkness. It was found that CCC at lower concentrations (10⁻⁶ and 10⁻⁴ M) had no detectable effects on leaf development although slight effects on stem elongation were found in the case of plants grown in the light; dark-grown plants showed no response in stem or leaf characters to the lower levels of CCC. In some experiments gibberellic acid, GA₃, was applied as a 10 μl drop of solution containing 1000 ppm, to the stem apex on the seventh day from planting.

Where dark-grown plants were given a white, red or far-red light treatment for 10 minutes, this was done using a light bank holding three 5 ft. 80 w. cool white fluorescent tubes and twenty 25 w. tungsten bulbs which could be used separately or together. For white light, only the fluorescent tubes were used to give 0.8 J. cm⁻². Red light was obtained by passing radiation from the fluorescent tubes through cellophane sheets to give 0.15 J. cm⁻² of radiation in the range 600--700 nm. Far-red light was generated by passing light from the tungsten bulbs through red cellophane and a blue glass sheet and in 10 minutes energy supplied in the range 700--780 nm was 0.20 J. cm⁻².

Leaf area was measured using an EEL meter for which calibration curves were prepared using bean leaf tissue (HURD and REES, 1966). Length of the median leaflet of the trifoliate leaves of light-grown plants was measured daily. Since these leaves unfolded at different times in different treatments, the starting point for the observations was taken as the time when the leaflet reached 3 cm in length; hence in Fig. 5 the time scale represents time after reaching the minimal length of 3 cm.

Cell number was estimated for leaves macerated in 5 percent chromic acid. Counts for leaves of dark-grown plants were made using fresh material (DALE, 1964b). It was not always possible to count immediately, cells in the large number of samples collected in many runs on light-grown plants. Use was therefore made of the method of DALE (1964c) for counting cells in dried leaf material.