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# EFFECTS OF (2-CHLOROETHYL) TRIMETHYL-AMMONIUM CHLORIDE (CCC) ON GROWTH AND OF GIBBERELLIC ACID ($GA_3$) AND CCC ON CHLOROPHYLL AND NPK CONTENT OF LEAVES OF YOUNG TEA PLANTS

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The effects of foliar sprays (2,000 to 16,000 p.p.m.) and soil application (20,000 to 50,000 p.p.m.) of CCC on the growth of young tea plants were studied in three experiments. In a fourth experiment the effects of foliar sprays of CCC (6,000 p.p.m.) and of $GA_3$ (200 p.p.m.) on the chlorophyll and NPK content of leaves were determined.

Foliar sprays of CCC at concentrations greater than 8,000 p.p.m. reduced plant height and dry weight but soil application reduced these attributes at 50,000 p.p.m. and when the terminal buds of plants were in the mature dormant condition. Foliar sprays of CCC applied at different intervals from the planting of cuttings were effective only on rooted cuttings. $GA_3$ reduced the concentration of chlorophyll a and b while CCC increased them. In general, $GA_3$ reduced N and P and increased K, while CCC increased N, P and K.

## INTRODUCTION

It is common practice in tea nurseries artificially to remove the apex of plants by operations such as disbudding, removal of the terminal bud with two adjoining leaves and the intervening portion of stem (referred to as 'pinching' or 'thumb-nailing'), or even by making a cut lower down the stem, all in order to encourage the lateral spread of the plant at an early stage. The latter two operations are more drastic when done early, as they remove a fair proportion of growth and photosynthetic area already produced, causing a setback to the growth of the plant. For practical purposes it is advantageous to obtain a low spreading frame on which, in time, secondary and higher order shoots would be borne from which the tea of commerce is made by harvesting the bud and first two leaves and the intervening portion of stem. It was envisaged that the same effects of encouraging the lateral growth of buds could be achieved by the use of a growth retardant without the attendant disadvantage of any of these forms of pruning.

Several chemicals have been reported which possess the property of inhibiting plant growth by suppressing subapical meristematic activity. Among the most active of these dwarfing agents is a choline derivative, (2-chloroethyl) trimethyl-ammonium chloride or CCC (Cycocel), first reported by Tolbert (1960).

In order to study in some detail the effect of growth retardants on apical dominance in young tea, it was necessary first to carry out preliminary experiments to obtain general information on the response shown by tea plants to these substances. These experiments were conducted on plants at a relatively early age, in their growth as a prelude to a study of the responses of growth substances on older plants.

This paper reports experiments on the optimal concentrations of CCC to use in soil drenches and foliar sprays applied to tea plants of varying age. An experiment on the effects of $GA_3$ and CCC on the chlorophyll and NPK contents of leaves of young tea plants is also reported.

## MATERIALS AND METHODS

In the following experiments all foliar sprays were applied using a 0.35 l hand atomiser. All treatments including the control had a non-ionic wetting agent (Lissapol NX) included at a concentration of 0.05%.

### Determination of the optimal concentration of CCC for foliar sprays

The following concentrations of CCC were used:

1. Control — distilled water
2. 2,000 p.p.m.
3. 4,000 p.p.m.
4. 6,000 p.p.m.
5. 8,000 p.p.m.

Plants of clones TRI 2025 and DT 1 were used when they were 4 months old, at which stage they had about four fully expanded leaves. The layout was a randomized block design with 5 replicates, involving a total of 50 plants per treatment per clone. Five weekly applications of each spray solution were given.

About 150 ml of solution of each treatment was used for applications 1 to 3. Since the sprays were given over a period of 5 weeks, this amount was increased to 300 ml of each treatment for the fourth and fifth spray to make allowance for the new growth over this period. An assessment of effects was made 12 weeks after the first spray.

### The effect of plant age on the response to different concentrations of CCC applied as foliar sprays

In this experiment plants of clones TRI 2025 and DT 1 were raised from cuttings planted on the same date. CCC was applied to the plants at 8,000 and 16,000 p.p.m. as follows: One group of 120 plants received the sprays when they were 4 weeks old from the date of propagation; of this, 60 plants received the 8,000 p.p.m. concentration while the other 60 received the 16,000 p.p.m. concentration; another group was first sprayed when 8 weeks old; and a third group first received CCC when 12 weeks old. Each group received 4 applications at 2-3 day intervals, the 4 sprays being completed within 10 days. The 4- and 8-week-sprayed plants received 3 ml of solution per plant per application, but as the 12-week plants had unfolded more leaves, each of them received 5 ml of solution in order to obtain better foliar coverage. The layout was a randomized block design with 3 replicates. Measurement of the height of the plants commenced when they were 4 weeks old and continued at fortnightly intervals until the 20th week, when the dry weight of plant parts were determined.

**Effect of a soil drench of CCC on the growth of young tea plants grouped according to the condition of terminal bud on the main shoot**

In this experiment 4-month-old plants of clone TRI 2026 were grouped as follows:

1. Plants with terminal buds just gone dormant (soft banji)
2. Plants with terminal buds dormant (hard banji)
3. Plants with terminal buds resuming growth
4. Plants with terminal buds active.

The growth of the tea plant is characterised by a rhythmic alternation of periods of active and dormant growth. However, as these phases are not synchronised even in all plants of the same age, it is always possible to obtain plants in various conditions of terminal bud growth simultaneously.

The plants were raised in soil filled polythene sleeves, 10 cm diameter and 23 cm in length and open at both ends. Plants of each group were arranged in a block of 5 rows of 4 plants each and soil drenches were applied at random in such a way that one row of each block received one of the following CCC treatments:

(1) Control (water only); (2) 20,000 p.p.m.; (3) 30,000 p.p.m.; (4) 40,000 p.p.m.; (5) 50,000 p.p.m.

Each plant received 25 ml of solution at the appropriate concentration per application, the control plants receiving an equivalent amount of water. At the time of the soil drench the plants had a mean height of 18 cm. Three applications were made at weekly intervals and an assessment was made 18 weeks after first application.

**Effect of foliar application of $GA_3$ and CCC on NPK and chlorophyll content**

Five-month-old plants of clones DT 1 and TRI 2025 were used to obtain leaf samples for NPK and chlorophyll analyses. The treatments previously applied were: (1) Control (water only); (2) $GA_3$ (200 p.p.m.); (3) CCC (6,000 p.p.m.).

Each treatment was applied by spraying 10 rows of each clone at weekly intervals on five occasions. There were 10 plants per row and thus 100 plants of each clone per treatment. Leaf samples for the NPK and chlorophyll analyses were taken 10, 20 and 30 days after the final spray.

For the NPK analysis the third fully expanded leaf sample from the apex was chosen (Sivasubramaniam, 1970). NPK analyses were carried out using standard methods of chemical analysis. Analysis of the chlorophyll content were also done on third leaf samples by the method described by Arnon (1949). Sufficient leaf samples were removed from the plants and 25 leaf discs punched out immediately with a cork borer (diam. 1 cm). The leaf discs were left in the dark overnight in 15 ml 80% acetone in conical flasks. The contents of the flasks were shaken from time to time to ensure as complete extraction of the chlorophyll as possible. The absorbancy of each extract was measured at 645 nm and 663 nm in a spectrophotometer. The chlorophyll concentrations were worked out on a weight per $dm^2$ of leaf area.

Both the NPK and chlorophyll contents have been expressed as the mean of four determinations on each occasion.

## RESULTS

### Determination of the optimal concentration of CCC

**Effect on height.** The highest concentration of CCC reduced plant height 6 weeks after the first spray, the effect lasting for 4 weeks (Fig. 1). Further, the 4,000 and 6,000 p.p.m. concentrations reduced plant height 8 weeks after the first spray, the effect lasting for 2 weeks. At 12 weeks after the first spray the 2,000 and 4,000 p.p.m. concentrations increased plant height.

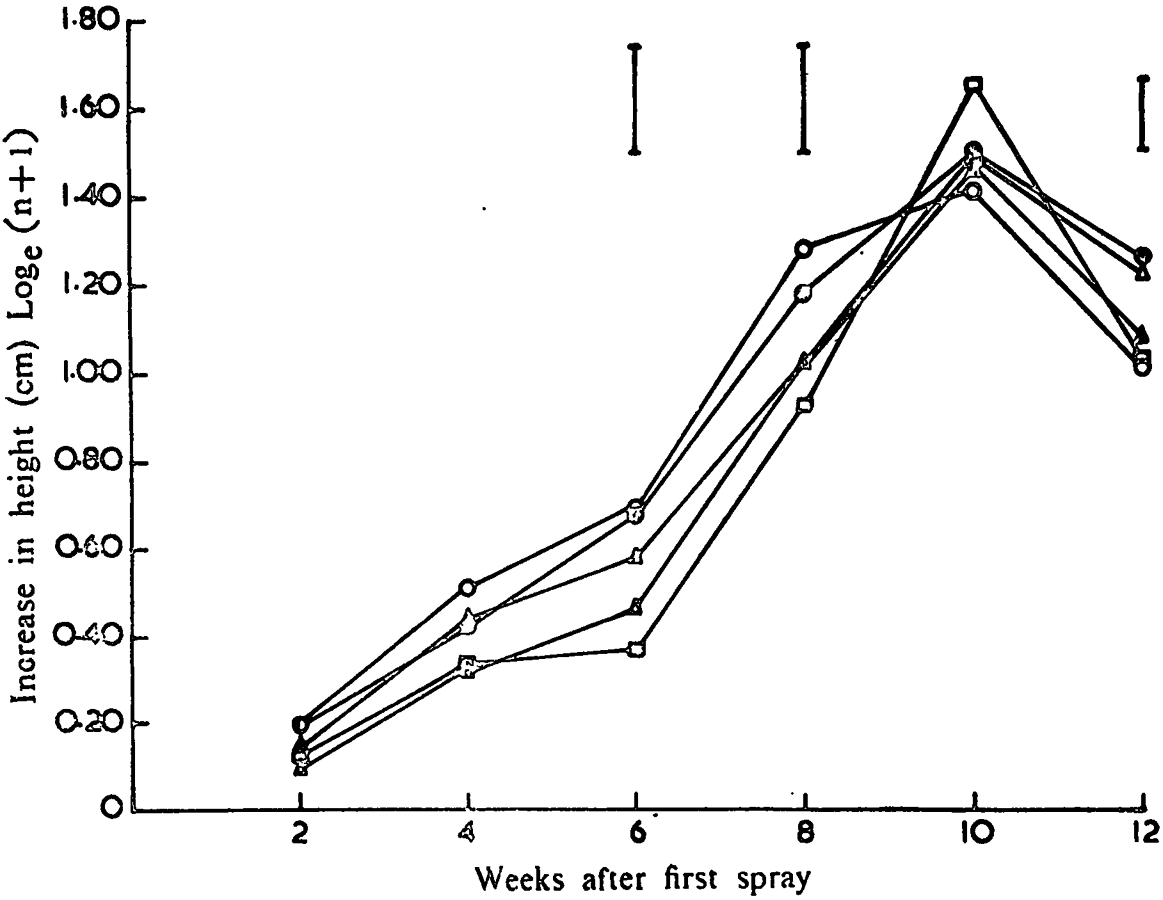


*Fig. 1. Effect of different concentrations of CCC on increase in height averaged over two clones (means of 100 plants). ○=control; ◎=2,000 p.p.m.; ◻=4,000 p.p.m.; △=6,000 p.p.m.; □=8,000 p.p.m.; Vertical bars show LSD (P=0.05)*

**Effects on dry matter production.** The CCC treatments showed less dry weight of stems (6,000 and 8,000 p.p.m.) and of plant (8,000 p.p.m.) but more dry weight of leaves (6,000 and 8,000 p.p.m.) (Table 1).

TABLE 1 — *Effect of different concentrations of CCC on dry matter production (means of 100 plants)*

| *Treatments* | *Dry weight (g)* | | | |
|---|---|---|---|---|
| | *Leaves* | *Stems* | *Roots* | *Plant* |
| Control | 1.33 | 1.05 | 0.78 | 3.16 |
| 2000 p.p.m. | 1.29 | 0.97 | 0.86 | 3.12 |
| 4000 p.p.m. | 1.35 | 0.99 | 0.72 | 3.06 |
| 6000 p.p.m. | 1.49 | 0.93 | 0.68 | 3.10 |
| 8000 p.p.m. | 1.52 | 0.88 | 0.64 | 3.04 |
| LSD (P=0.05) | 0.11 | 0.12 | NS | 0.12 |

**The effect of age of plants on response to different concentrations of CCC**

**Effect on height.** Both concentrations of CCC produced a reduction in plant height when applied at the 4-week stage. The reduction was observed 2 weeks after the first spray and the effect lasted for 4 weeks (Table 2). When the plants were sprayed at the 8-week stage only the higher concentration reduced plant height. This occurred 8 weeks after the first spray on 8-week-old plants and the effect lasted for 6 weeks. When the plants were sprayed at the 12-week stage both concentrations reduced plant height. This occurred 4 weeks after first application on 12-week-old plants and the effect lasted for 6 weeks.

TABLE 2 — *Effect of age of plants on response to different concentrations of CCC on increase in height averaged over two clones*

| *Treatments* | *Fortnightly increase in mean height per plant (cm) $log_e (n+1)$ from first spray on 4-week-old plants* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *2* | *4* | *6* | *8* | *10* | *12* | *14* | *16* |
| Control | 1.07 | 0.84 | 0.23 | 0.15 | 0.13 | 0.30 | 0.44 | 0.59 |
| 4 weeks — 8000 p.p.m. | 0.80 | 0.63 | 0.29 | 0.15 | 0.17 | 0.29 | 0.39 | 0.52 |
| — 16,000 p.p.m. | 0.84 | 0.47 | 0.34 | 0.15 | 0.20 | 0.23 | 0.36 | 0.50 |
| 8 weeks — 8,000 p.p.m. | 1.12 | 0.84 | 0.21 | 0.12 | 0.13 | 0.18 | 0.34 | 0.45 |
| — 16,000 p.p.m. | 1.08 | 0.87 | 0.23 | 0.08 | 0.06 | 0.10 | 0.28 | 0.38 |
| 12 weeks — 8,000 p.p.m. | 1.06 | 0.82 | 0.30 | 0.14 | 0.08 | 0.09 | 0.25 | 0.35 |
| — 16,000 p.p.m. | 1.05 | 0.80 | 0.31 | 0.07 | 0.08 | 0.07 | 0.23 | 0.31 |
| LSD (P=0.05) | 0.13 | 0.12 | NS | NS | NS | 0.15 | 0.14 | 0.17 |

**Effects on dry matter production.** The higher concentration reduced the dry weight of stems of 8-week-old plants while both concentrations reduced the dry weight of the whole plant (Table 3). When CCC was sprayed at the 12-week-stage both concentrations showed less dry weight of stems and plant. The higher concentration also showed a less dry weight of leaves.

TABLE 3 — *Effect of age of plants on response to different concentrations of CCC on dry matter production*

| *Treatments* | *Leaves* | *Stems* | *Roots* | *Plant* |
|---|---|---|---|---|
| | *Dry weight (g)* | | | |
| Control | 0.85 | 0.68 | 0.56 | 2.09 |
| 4 weeks — 8,000 p.p.m. | 0.78 | 0.65 | 0.54 | 1.97 |
| — 16,000 p.p.m. | 0.81 | 0.61 | 0.50 | 1.92 |
| 8 weeks — 8,000 p.p.m. | 0.74 | 0.58 | 0.48 | 1 80 |
| — 16,000 p.p.m. | 0.82 | 0.49 | 0.51 | 1.82 |
| 12 weeks — 8,000 p.p.m. | 0.74 | 0.51 | 0.49 | 1.74 |
| — 16,000 p.p.m. | 0.68 | 0.42 | 0.47 | 1.57 |
| LSD (P=0.05) | 0.14 | 0.15 | NS | 0.27 |

**Effects of a soil drench of CCC on the growth of plants grouped according to the condition of the terminal bud**

**Effect on height.** The height of plants with terminal buds just gone dormant as well as those with dormant terminal buds was reduced by the highest concentration of CCC applied (Fig. 2). No differences in height were noted in plants with active terminal buds and those with dormant buds resuming growth due to any of the CCC treatments compared to the control.

**Effects on dry matter production.** Plants with terminal buds just gone dormant showed less dry weight of leaves, stems and whole plant with the highest concentration of CCC (Table 4). When CCC was applied at 40,000 p.p.m. to plants with dormant terminal buds it reduced the dry weight of leaves, roots and of whole plant. The 50,000 p.p.m. concentration was effective in reducing the dry weight of stems in addition to reducing those of leaves, roots and whole plant. CCC at 30,000 p.p.m. concentration applied to plants with dormant buds resuming growth reduced the dry weight of stems. No reduction in dry matter of plant components due to any of the CCC treatments were noted when applied to plants with active terminal buds.

TABLE 4 — *Effect of soil application of CCC on dry matter production*

| | *Treatments* | *Leaves* | *Stems* | *Roots* | *Plant* |
|---|---|---|---|---|---|
| | | *Dry weight (g)* | | | |
| **Terminal bud just gone dormant (soft banji)** | Control | 1.79 | 1.31 | 1.20 | 4.30 |
| | 20,000 p.p.m. | 2.12 | 1.64 | 1.97 | 5.73 |
| | 30,000 | 2.23 | 1.54 | 2.04 | 5.81 |
| | 40,000 | 1.90 | 1.18 | 1.29 | 4.37 |
| | 50,000 | 1.45 | 1.03 | 1.42 | 3.90 |
| **Terminal bud dormant (hard banji)** | Control | 1.72 | 1.70 | 1.90 | 5.32 |
| | 20,000 | 2.86 | 2.10 | 2.56 | 7.52 |
| | 30,000 | 2.03 | 1.69 | 1.09 | 4.81 |
| | 40,000 | 1.30 | 1.24 | 1.07 | 3.61 |
| | 50,000 | 1.32 | 0.90 | 1.45 | 3.67 |
| **Dormant bud resuming growth** | Control | 2.05 | 1.65 | 1.04 | 4.74 |
| | 20,000 | 2.42 | 2.15 | 1.93 | 6.50 |
| | 30,000 | 2.02 | 1.37 | 1.34 | 4.73 |
| | 40,000 | 2.25 | 1.61 | 1.49 | 5.35 |
| | 50,000 | 2.36 | 1.84 | 2.19 | 6.39 |
| **Terminal bud fully active** | Control | 1.88 | 1.36 | 1.45 | 4.69 |
| | 20,000 | 3.29 | 2.65 | 2.08 | 8.02 |
| | 30.000 | 3.06 | 2.20 | 1.90 | 7.16 |
| | 40,000 | 2.99 | 2.08 | 2.00 | 7.07 |
| | 50,000 | 2.61 | 1.77 | 1.67 | 6.05 |

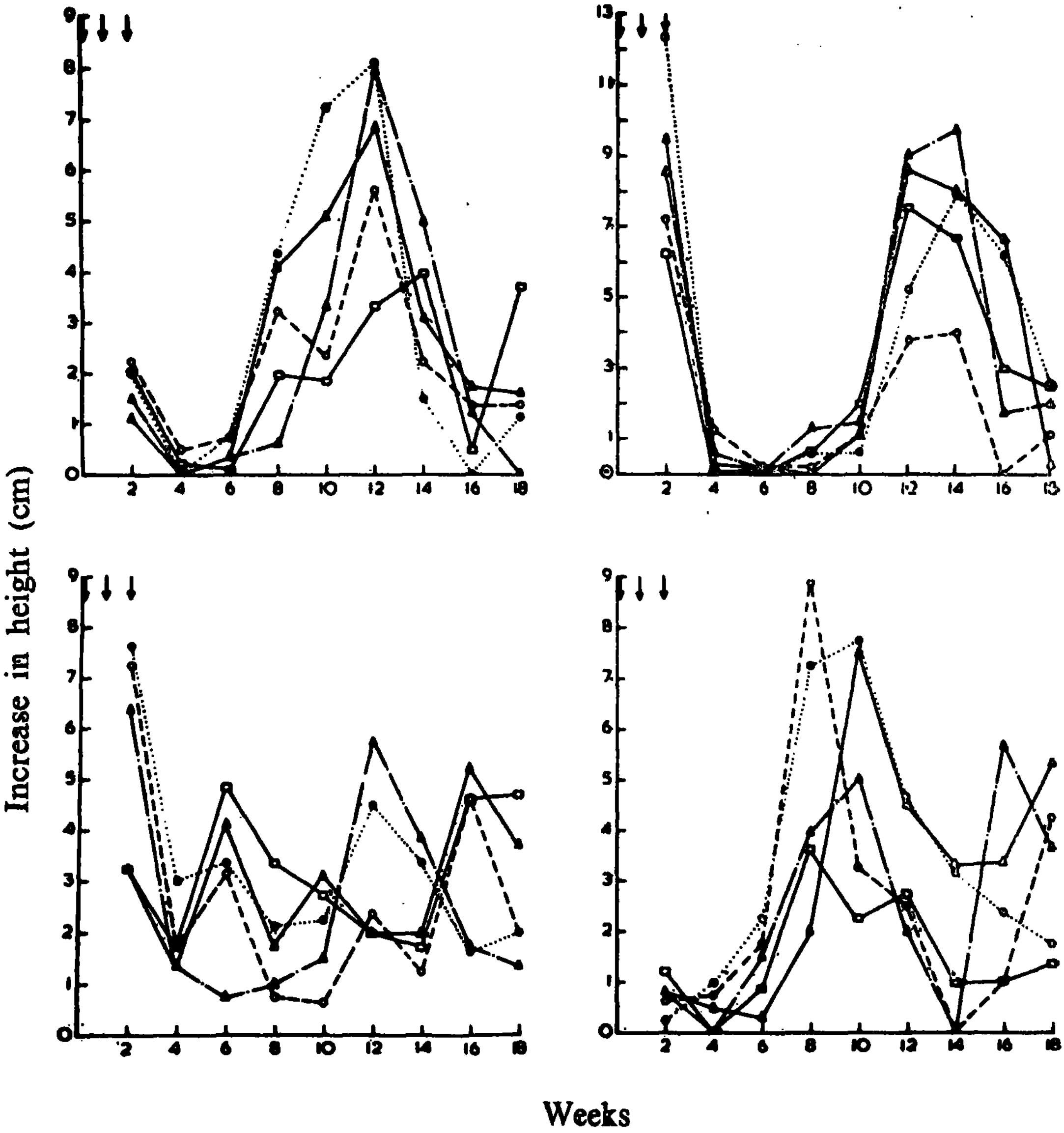


*Fig. 2. Effect of soil application of CCC on increase of height of plants of clone TRI 2026 grouped according to condition of terminal bud. Arrows indicate time of application of treatments. Top left: Terminal buds just gone dormant; Top right: terminal buds fully active; Bottom left: Dormant buds resuming growth; Bottom right: Terminal buds dormant. ○=control; ●=20,000 p.p.m.; ▲=30,000 p.p.m.; ▲=40,000 p.p.m.; ■=50,000 p.p.m.*

TABLE 5 — *Effect of foliar application of $GA_3$ and CCC on chlorophyll ($mg/dm^2$) and NPK (% dry weight) content of leaves of clones DT 1 and TRI 2025*

| *Treatments* | *Clone DT 1* | | | | | | *Clone TRI 2025* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Chlorophyll a* | *Chlorophyll b* | *Ratio chlorophylls (a : b)* | *N* | *P* | *K* | Chlorophyll *a* | Chlorophyll *b* | *Ratio chlorophylls (a : b)* | *N* | *P* | *K* |
| **1st sampling 10 days after last spray** | | | | | | | | | | | | |
| Control | 2.33 | 1.24 | 1.88 | 3.62 | 0.15 | 1.07 | 2.75 | 1.36 | 2.02 | 3.50 | 0.12 | 0.88 |
| $GA_3$ | 1.82 | 1.14 | 1.60 | 2.60 | 0.12 | 1.67 | 2.12 | 1.20 | 1.77 | 2.87 | 0.04 | 1.23 |
| CCC | 2.74 | 1.38 | 1.99 | 3.40 | 0.18 | 1.38 | 4.03 | 1.89 | 2.13 | 3.35 | 0.15 | 1.08 |
| **2nd sampling 20 days after last spray** | | | | | | | | | | | | |
| Control | 2.53 | 1.41 | 1.79 | 3.12 | 0.15 | 1.26 | 2.82 | 1.53 | 1.84 | 3.46 | 0.13 | 0.32 |
| $GA_3$ | 1.91 | 1.18 | 1.62 | 2.98 | 0.13 | 1.78 | 2.23 | 1.31 | 1.70 | 3.25 | 0.10 | 0.81 |
| CCC | 2.96 | 1.56 | 1.90 | 4.20 | 0.25 | 1.18 | 3.65 | 1.91 | 1.91 | 3.81 | 0.15 | 0.32 |
| **3rd sampling 30 days after last spray** | | | | | | | | | | | | |
| Control | 2.45 | 1.33 | 1.84 | 3.36 | 0.14 | 1.56 | 2.76 | 1.48 | 1.86 | 3.57 | 0.13 | 1.26 |
| $GA_3$ | 2.26 | 1.26 | 1.79 | 3.50 | 0.09 | 2.05 | 2.50 | 1.42 | 1.76 | 3.61 | 0.08 | 0.94 |
| CCC | 2.60 | 1.33 | 1.95 | 3.22 | 0.20 | 1.89 | 2.95 | 1.53 | 1.93 | 3.36 | 0.11 | 1.14 |

**Effect of $GA_3$ and CCC on chlorophyll and NPK content of leaves**

**Chlorophyll content of leaves.** In both clones the concentration of chlorophyll a and b and the ratio of chlorophyll a:b was reduced by $GA_3$, while CCC produced the opposite effect (Table 5). Though differences between the treatments were still seen at the 3rd sampling, they were less marked than in the previous samplings. The young leaves of $GA_3$-treated plants were paler while those of CCC treated plants were darker green compared with the controls.

**NPK content of leaves.** In both clones, the N content of leaves was reduced by $GA_3$ at the first two samplings and increased by CCC at the second sampling (Table 5). In general, $GA_3$ reduced P and increased K, while CCC increased both P and K.

## DISCUSSION

The experiments on the different concentrations of CCC that would elicit a response in young tea plants showed that the higher concentration produced smaller sized plants (Fig. 1, Table 1). Marcelle (1966) using 15 sprays of CCC at 1,000 and 2,000 p.p.m. on young apple trees noted that the growth of the main stem was retarded, the effect being very significant and increasing linearly with dose. Again, CCC was also effective in reducing plant size when applied to 12-week-old plants but not the 4- or 8-week-old plants (Tables 2 and 3). Depending on the elevation, tea cuttings establish as rooted plants around 10-12 weeks and it is at this stage that fertilizer application is commenced. It would thus appear that roots are necessary for effective reduction of plant size by CCC. In the culture of the tea plant it is advantageous to put out plants with a low frame in the field in order to enhance the lateral spread of the tea bush, which would result in a larger number of harvesting units. Further, the height of the tea plants must be kept within conformable limits to facilitate easy harvest.

In this study CCC sprayed to 12-week-old plants did not reduce apical dominance and produce more side shoots. When a tea cutting grows it produces 4 to 6 expanded leaves before becoming dormant at about 3 to 4 months of age. During this phase of growth apical dominance is strong and no side shoots are produced. However, when it re-commences to unfold leaves about 4 to 6 weeks later, some side shoots are produced below when apical dominance is reduced. In another study with relatively older plants commencing growth following dormancy, when apical dominance is naturally reduced, CCC was found to enhance the production of side shoots.

Most studies of the effects of CCC have been carried out using sprays. In the present work CCC was also used as a soil drench in order to determine whether it would be as effective as foliar sprays. The soil irrigations were effective in reducing plant size only at the higher concentrations and when applied to dormant plants, but not the actively growing plants (Fig. 2, Table 4). It has been shown that actively growing tea shoots which continue to unfold leaves have greater amounts of gibberellins compared to dormant shoots (Kulasegaram and Kathiravetpillai, 1979). It is known that CCC inhibits gibberellin biosynthesis (Cathey, 1964: Harada and Lang, 1965). Since biosynthesis of gibberellins commences with bud break it appears that CCC was effective only in dormant plants by preventing their biosynthesis but not in active plants where their levels would already have been high.

When $GA_3$ was applied as a spray the chlorophyll content per unit leaf tended to decrease (Table 5) and the young leaves appeared to be paler in colour compared with the leaves of control plants. Wolf and Haber (1960) attribute such decreases to a dilution effect of the same amount of chlorophyll over the area of a larger leaf which results from a failure of chlorophyll synthesis to keep pace with increased cell expansion while Wittwer and Bukovac (1958) consider the paling of leaves to be associated with moisture stress and temporary mineral nutrient deficiencies, the concomitants of an unfavourable shoot/root ratio, particularly when soil moisture and fertility levels are marginal.

The leaves of CCC - treated plants were much greener and had a greater chlorophyll content with an increased proportion of chlorophyll a. Marcelle (1966) found that not only was the chlorophyll content greater in CCC - treated apple plants but also chlorophyll a accumulated more rapidly in them than chlorophyll b.

The results also showed that, in general, $GA_3$ decreased the content of N and P in the leaves and almost consistently increased K while CCC increased N and P (Table 5). Humphries (1963) observed that CCC increased N in leaves and stems of tobacco plants. The increase in N caused by CCC was attributed by him to the fact that CCC contains N and it is probable that some of the N could have come from this source.

In the nursery several methods are adopted on young tea plants in order to prepare them for bearing shoots of several orders when they are planted in the field. If a tea plant is allowed to grow freely, the main shoot shows extension growth and will later bear lateral shoots at varying distances above the ground level and the plant soon assumes the form of a pyramid. The various methods adopted to maintain the bush in a shape and size conformable to economic demands act by preventing the centre of the bush dominating the outer branches. In the nursery artificial practices such as disbudding, removal of the terminal bud with two adjoining leaves with included stem or a cut lower down the stem are devices done to ensure a more equitable distribution of growth over the frame. However, it is to be noted that these operations, particularly the latter two, are more drastic when done on young plants as the removal of a fair proportion of growth could be expected to curtail photosynthetic activity and reduces root growth (Kathiravetpillai and Kulasegaram, 1982), causing a setback to the growth of the plant. The results of this study indicate that it is possible to obtain smaller sized plants and from a subsequent study plants of a low spreading habit (Kathiravetpillai and Kulasegaram, 1981) by the use of a growth retardant like CCC without reduction of roots as a consequence of manual pruning. A further advantage is the reduction in labour requirement which would be quite considerable in commercial nurseries.

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