THE EFFECT OF (2-CHLOROETHYL) TRIMETHYLAMMONIUM CHLORIDE (CCC) ON THE GROWTH PATTERN OF BARLEY

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1. INTRODUCTION

The extensive investigations that have followed the rather recent discovery of the action of plant growth substances, have extended the use of these substances for the improvement of crop species. Unlike the more recently developed organic herbicides, a number of these organic compounds act in such a way as to delay the growth processes of the plant rather than to promote its death. The practical value of such a chemical substance has already been demonstrated in the field of plant improvement, particularly in the field of ornamentals and vegetables. The desirability of chemically inducing a prolonged flowering period in floral species requires no explanation. Similarly, the ability of these same compounds to suppress the height of certain ornamental species, to induce dormancy in root crops, as well as to reduce the "bolting" tendency in some vegetable crops, has led to their increased use on a commercial scale.

(2-chloroethyl) trimethylammonium chloride (or commercially known as CCC) is one of a number of compounds which when applied to certain plant species, suppresses growth. Many of the plants tested with CCC and related compounds have been dicotyledonous species. Recently, however, workers have devoted some attention to the effect of this chemical on cereals with particular reference to its potential value in decreasing losses incurred from lodging.

Hordeum vulgare L., common barley, is a crop which inherently possesses relatively weak straw and is therefore susceptible to lodging. This problem becomes even more serious when the crop is grown under irrigation or abundant rainfall. The use of nitrogen fertilizers which are now being applied liberally, aggravates the problem by increasing the yields of grain and amount of vegetative growth produced by a plant.

The possibilities of improving lodging resistance through conventional plant breeding methods or by induced mutation have attracted much attention from barley breeders in the past. Because of the lack of superior sources of barley germ plasm possessing this highly desirable trait however, success in developing varieties with improved straw strength has been limited. In view of this, some consideration has been directed to the possibility of accomplishing the same objective by reducing straw length, thereby indirectly improving straw strength. Mann (1953) found that in barley height of culm is positively correlated with internode length rather than with internode number.

This association might suggest that the application of an appropriate growth retarding compound could reduce the rate of cell division at the intercalary meristematic regions of the culm of the cereal plant. Internode length, thus culm height would be reduced as a result. In addition to its property of retarding plant growth, recent reports have shown that plants treated with CCC also are able to tolerate more readily conditions of drought and high soil salinity.

The present study was initiated with the objective of studying the biological response of the barley plant to CCC when grown under a range of available moistures. Particular reference is to be made to the effect of this chemical on such plant characters as height, internode lengths and components of yield.

2. REVIEW OF LITERATURE

The growth retarding effect of certain ammonium compounds on plants has been reported by a number of workers (Wirwille and Mitchell, 1950; Krewson, 1959; Humphries and Wheeler, 1963). The biological activity compounds, which are structurally related to the quarternary ammonium cation $(CH_3)_3N + CH_2 - CH_2$ X, was examined by Tolbert (1960c). Three very active and closely related compounds were discovered in which "X" was a chloro, bromo or $= CH_2$ group. (2-chloroethyl) trimethylammonium chloride has been the most thoroughly investigated of the three compounds. It has the structural formula $ClCH_2CH_2N + (CH_3)_3Cl^-$, and is commercially known as "cycocel" or "CCC". These compounds are sometimes referred to as derivatives of choline, thus "CCC" could be called chlorocholine chloride.

Experiments on numerous plants have demonstrated that the effects of (2-chloroethyl) trimethylammonium chloride and related compounds are characterized by a suppression of vegetative development, the production of stems with short internodes, and by the bearing of foliage dark green in color (Tolbert, 1960b; Wittwer and Tolbert, 1960a; Lindstrom and Tolbert, 1960). The symptoms produced by these compounds are similar to those observed in plants grown under short photoperiods, low temperatures, and/or high light intensities (Bukovac 1962).

2.1 Effect of "CCC" on cereals

The reaction of wheat seedlings, *Iriticum aestivum* var. Thatcher to CCC was tested extensively under greenhouse conditions

(Tolbert, 1960b). Concentrations ranging from 10^{-2} to 10^{-6}M were found effective when either applied to the soil or used in nutrient culture. Less effective were treatments in which the chemical was either sprayed on the leaves or incorporated into the seed by soaking. Regardless of the method of application, a single treatment was found to be effective in reducing the total growth of the plant. The most characteristic growth alterations in treated plants in comparison with controls were shorter and thicker stems, broader and greener leaves, earlier and stronger tillering, and more uniform growth throughout the plant population. The shorter and thicker stems resulted in wheat plants which grew very erect with no tendency to lodge. Moreover, treatment enhanced tiller development with the newly-formed tillers being about equal to the main tiller in their rates of growth. Little or no tillering occurred during subsequent growth of the treated plants. The number of leaves was not altered by the chemical treatments, and time of appearance of the early leaves was not appreciably delayed. Tolbert (1960b) noted that the visual changes in the growth of wheat after one treatment developed slowly over a period of two weeks. A difference in stem length between controls and treated plants could first be detected within three to five days after treatment. Despite their reduced stature, treated plants were similar in their wet and dry weights to untreated plants. In addition, grain yields of treated and untreated plants remained comparable with some indication of an increase in yield induced by concentrations of the magnitude of 10^{-5} to 10^{-6} M.

The action of different doses of CCC (10^{-5}, 10^{-4} and 10^{-3}M) on spring wheat grown in the greenhouse was investigated by Linser <u>et.al.</u> (1961). An application of 79.0 milligrams per pot at two weeks after the three-leaf stage reduced the culm lengths 24 percent in comparison with controls.

Linser and Kühn (1962) found considerable differences in response of different wheat varieties to CCC chemical. They reported that although the total height of the plants grown on light and heavy soils were similarly affected at early stages of growth, those grown on heavy soil completely recovered at later stages of growth while those on light soil remained dwarfed.

An application of 35 pounds per acre of nitrogen fertilizer together with 20, 30 and 40 pounds of actual ingredient of CCC per acre did not affect the straw strength of either wheat, barley or oats (Cooper, 1964, personal communication). A 40-pound rate of CCC applied on wheat at the shot-blade stage resulted in a reduction in height of approximately 16 inches in overall height. On light soil, plant height was reduced by 28 percent by an application of CCC amounting to less than five percent by weight of the nitrogen fertilizer used (Linser and Kūhn, 1962). The best time of chemical application was found to be either at the time of tillering or just before heading. Although these workers found that root weight was increased by application of CCC to wheat grown on light soil, grain yield was not affected.

Another ammonium compound (2-bromoethyl) trimethylammonium bromide, was also tested by Tolbert (1960c)and was found to have similar effects to CCC. A variety of spring wheat, Russell, also the variety Genesee, a winter wheat, were used in this study.

Montcalm barley and Garry oats exhibited a similar growth pattern as wheat when treated with this compound but to a less marked degree.

Vegetative growth in dwarf as well as tall varieties of maize was reduced following the treatment with CCC (Wittwer and Tolbert, 1960b). In this experiment, dwarfism as conditioned by genetic factors was accentuated by chemical treatment.

2.2 Effect on other crops

Controlling vegetative growth by means of chemical application would be a great asset in the production and marketing of many horticultural crops. As a result of this possibility, extensive studies have been made in this field. Those growth retarding chemicals which have been most effective on wheat, have also induced a response on both early and late maturing varieties of tomato (Wittwer and Tolbert, 1960a). Within 5-7 days after treatment the foliage of plants receiving concentrations of 10^{-3} or 10^{-4}M of CCC became intensely dark green while the leaves assumed a wrinkled appearance and the newly formed internodes became shorter and thicker than those of untreated plants. Such plants subsequently developed a very stocky and compact type of growth. The flowering period of treated plants was noticeably earlier than that of controls. Thus, earliness and fruit development of market tomatoes, both under greenhouse conditions and in the field were promoted with the use of CCC.

Tissen (1962) studied the effect of temperature on the response of tomatoes and peppers to both CCC and trimethylammonium bromide. When applied to the foliage, CCC resulted in an increased pepper yield, a greater response occurring under cool temperature conditions (minimum night temperature 12 - 13°C.) than under higher temperatures (minimum night temperature 18 - 20°C.). In contrast, he found that a soil application of CCC reduced the yield of tomato plants in comparison with controls.

Cucumber plants treated with 10^{-5}M solutions of CCC developed very short internodes and the formation of tendrils was prevented (Krewson, 1959).

An application of CCC to mustard plants grown in a greenhouse increased the total leaf area per plant by way of an increased number of lateral branches as induced by the treatment (Humphries, 1963). Under conditions of controlled environment and high light intensity, formation of laterals was maintained at a minimum, nevertheless the total area of treated plants exceeded that of controls by virtue of large leaves produced on the main stem. The stem growth of treated plants however, was decreased.

Whether the increase in leaf size found by Humpheries to occur on treated mustard plants was the result of stimulation in the rate of cell division or whether it was due to greater cell enlargement is unknown. It is of interest in this regard that a study by Sachs and Lang (1961) revealed that cell division in the sub-apical meristem of treated plants was reduced by CCC treatment, thereby resulting in reduced internode length.

Bukovac (1962) found that shoot elongation of apple seedlings and one-year-old cherry trees was temporarily inhibited with the application of CCC and related compounds. The number of leaves per shoot, the length and width of leaf and the dry weight of tops were decreased with increasing concentrations of CCC ranging from 10^{-4} to 10^{-2}M.

In many floricultural crops shorter plants would be preferred over existing varieties. The response of a number of these horticultural species to ammonium compounds also has been studied. With chrysanthemum and poinsettias, one or two soil applications of 100 millilitres of 10^{-2}M CCC resulted in plants with a more desirable floral characteristic than the untreated plant (Lindstrom and Tolbert, 1960). Plants treated with 5 x 10^{-2}M solutions although short in stature, underwent a prolonged floral period displaying flowers of excellent quality. Growth of poinsettias could be inhibited most effectively when the chemicals were applied as a soil drench or when they were mixed directly into the soil before planting (Lindstrom, 1961). Only those plants which received three treatments of CCC had internodes which remained consistently short throughout the growth of the plant. For pot-plant marketing, the height of poinsettias was found to be effectively reduced by using soil application of CCC just before or immediately after transplanting from small to larger pots. CCC treatment resulted in well proportioned plants of high commercial quality (Kofranek et al., 1962a, and 1963).

In certain flower species, floral induction can also be affected by CCC and related compounds. CCC treatment of Gardenia jasminoides "Veitchii" was reported to result in a higher number of flower buds and flowers (Joiner and Poole, 1962).

Strong inhibition of stem growth in the long-short-day-plant of Bryophyllum daigremontianum by application of high doses of CCC to the roots of plants subjected to short-days was found to be associated with suppression of flower formation (Zeevaart and Lang, 1963). Flowering can be induced either by exposure of the plants to long days followed by short days, or by application of gibberellic acid to plants grown continuously under short-day conditions. Flower bud initiation was promoted with CCC application to azalea (Rhododendron spp.) plants of varying ages (Stuart, 1961). This response occurred under environmental conditions which normally prevented or limited flower bud initiation in untreated plants. The effect of CCC on floral initiation and growth of Pharbitis nil, a short-day plant was recently reported (Zeevaart, 1964). Although flowering was not inhibited by application of CCC to the plumules or cotyledons, root application proved successful. Anatomical observations showed that CCC strongly reduces cell number in the first internode which is just beginning to expand at the time of long-day photoperiod induction. This chemical was found to have a dual effect on flower formation in Pharbitis; it suppressed flowering when applied to seedlings just before photoperiodic induction, but promoted flower formation in plants grown under long-day. The shorter internodes which resulted from CCC treatment were attributed to inhibition of cell division (Zeevaart, 1964). As in a number of other species, treatment resulted in short, thick internodes and dark green leaves. Growth rates in the treated plants remained low for a considerable period after the last application, but ultimately reached the same level as in untreated plants.

2.3 Physiological effects of growth retarding compounds

The modifying influences of (2-chloroethyl)trimethylammonium and related compounds on the responses of the auxins and gibberellins have been studied (Wittwer and Tolbert, 1960b). Studies with CCC have demonstrated that it can suppress both gibberellin and light-induced germination of lettuce seed. It has been shown also to reverse GA-induced vegetative extension of genetically dwarf and normal plants, as well as the reduction of height of plants grown under either light or dark conditions. Elongation of Avena coleoptile sections was suppressed by CCC both in the presence and absence of 3-indole-acetic acid.

These workers have pointed out the mutually antagonistic effects of CCC and gibberellins in light of their ability to counteract the effects of one other under different regimes of light, temperature and photoperiod. As in the case of wheat plants treated with CCC, rapid elongation of the treated plants occurred at the time of heading, so that the final height of both treated and control plants was nearly identical. This apparent recovery of plants treated with CCC early in the seedling stage has led some workers to conclude that there is produced during the heading or flowering stage a natural Gibberellin-like material which counteracts the effect of CCC induced earlier (Tolbert, 1960d). Other evidences, (Kuraishi, et al., 1963) however, suggest that CCC-inhibited stem growth may result from the amount of auxin present in the meristematic tissue rather than by the level of gibberellin. The diffusible auxin content of apices of the stem sections cut from peas, was measured by these workers using the Avena curvature test. They found that untreated peas had about six times the amount of diffusible auxin than produced in peas treated with CCC.

Two conditions have now been reported in which CCC and related compounds have been found to stimulate growth (Wittwer and Tolbert, 1960b). Increased vegetative and dry matter accumulation has been obtained with the use of low concentrations of CCC (10^{-7}M), where presumably auxin and gibberellin were not limiting. The second condition is the synergistic response from a combination of IAA, GA and CCC on parthenocarpic fruit development. Concentrations of CCC in the order of 10^{-2}, 10^{-3} or 10^{-4} in combination with both 10^{-3}M IAA and 10^{-5} gibberellin, markedly accelerated ovary growth of tomatoes.

Kelley and Postlethwait (1962) reported that CCC treatment promotes growth of fern gametophytes by stimulating their rate of cell division. The transition from filamentous to biplanar and triplanar cell divisions was hastened, resulting in earlier developing and more numerous gametangia on treated prothalli.

Investigating the growth responses of chrysanthemum and poinsettia to CCC, Lindstrom and Tolbert (1960) observed that treated plants seemed to have greater tolerance to drought than control plants and as a result did not show signs of wilting when subjected to moisture stresses. High temperatures following periods of low light intensities caused wilting in control plants, while treated plants remained turgid. The drought responses of bean plants, *Phaseolus vulgaris* subjected to CCC and phosphon treatment was later studied by Halevy and Kessler (1963) in two different experiments. In the first experiment, treated plants survived a much longer time than untreated plants when grown in soil in which the moisture level was at or just below the wilting point. In the second experiment the soil moisture was determined by means of weighing each pot and its contents daily and maintaining a predetermined level of soil moisture for a particular treatment throughout the experiment. From the result, it was evident that the fresh and dry weights of treated plants was higher in comparison with those of controls. The authors concluded that plants treated with either CCC or with phosphon were able to withstand moisture deficits better than untreated plants.

Translocation of phosphorus P^{32} in barley seedlings was found to be inhibited when CCC was added to the nutrient solution (Gohlke and Tolbert, 1962). This inhibition was accompanied by a proportional decrease in water uptake by the seedlings. Many investigators agree that CCC induces a dark green coloration of the leaves and results from studies using tobacco, indicate that in CCC-treated plants the chlorophyll content per leaf as well as per gram fresh weight was increased (Humpheries, 1963). Such leaves were found to also contain a higher nitrate content in comparison with control plants, although the total nitrogen in the plant as a whole was little affected.

The ability of plants treated with CCC to tolerate abnormal levels of pH has also been reported (Miyamoto, 1962). Seed treatment with CCC increased the resistance of wheat seedlings to high concentration of soil salts. Similarly, soybean plants dwarfed by spray or soil application of either CCC or AMO-1618, were found by Marth and Frank (1961) to survive concentrations of salt in the soil which proved toxic to untreated plants.

3. MATERIALS AND METHODS

3.1 Preparation of plant material for treatment and analysis

Uniformly large seeds of the variety Parkland and Hannchen were used in these studies and were obtained from purified barley stocks produced by the Crop Science Department, Saskatoon. The seeds were usually germinated in the petri dishes prior to planting them in plastic containers in the growth room. By selecting for planting only those seeds which were at a comparable stage of germination, it was possible to obtain a very uniform population of plants for treatment. Measured amounts of fertilizer of the type 11-48-0 was applied to the pots at the time of planting, and again before and after the heading stage of plant growth. Pots were arranged in a randomized block design containing four replications.

All the experiments (except experiment no. 1 below) were conducted in a growth room with controlled light and temperature. Light was supplied from fluorescent bulbs with the intensities at bench level varying from 2200 foot candles measured below the centre of the light bank, to 1600 foot candles measured at its periphery. By a continuous rotation of the position of pots every three or four days, the effect on plant growth of variation in intensity below each light bank was minimized. The photoperiod was controlled at 16 hours, with day temperatures maintained at 20^{o}C. and night temperatures at 18^{o}C.

3.2 The chemical and its application

The compound (2-chloroethyl) trimethylammonium chloride used in these studies was either in the form of crystalized powder (Eastman Organic Chemicals, New York), or as a concentrated solution with 11.8 percent active ingredients as supplied by Cyanamid of Canada, Limited. Water solutions of the chemical with designated molar concentrations were prepared by dissolving the crystals or diluting the concentrated solution just before use.

As indicated previously, (2-chloroethyl) trimethylammonium chloride or according to other designations, chlorocholine chloride, cycocel or "CCC", is a stable white solid with a molecular weight of 158.03 and which is very hygroscopic. The empirical formula of CCC is $C_5H_{13}NCl_2$ while its structural formula is $ClCH_2CH_2N^+(CH_3)_3Cl^-$. It is soluble in water and the lower alcohols. The pH of a 50 percent aqueous solution of CCC by weight is 3.8 while the freezing point of a 10 percent solution by weight is -4°C. CCC appears to be non-toxic and no gross pathology has been observed in the test animals of male albino rats. No sign of skin irritation has been observed at any dosage level (Cyanamid Technical Bulletin).

The two main methods of application of the chemical to the plants was by spray and by soil drench. Preliminary experiments were also carried out in an attempt to incorporate the chemical into the seed by soaking. For the soil treatment 200 millilitres of a designated molarity of chemical was poured onto the soil of each pot containing a single plant. For spray treatments, solutions containing about 0.01 percent "Tween 20" as a wetting agent were sprayed onto the leaves of the seedlings until the leaves were wet. In order to prevent rapid evaporation, sprayed plants were placed overnight in a large box covered with moist canvas. In certain experiments in which a supplement to the soil drench was employed, a "wick method" of feeding was used. In this method a twisted strand of cotton was threaded through the lower part of plant stem by a needle both ends of the thread being inserted into a 20 ml. stoppered vial containing the chemical solutions. The solution passed into the plant through capillary action along the "wick".

3.3 Maintenance of soil moisture regimes

Soil used in these studies was classified as sandy loam. All soil batches were thoroughly air dried prior to drawing samples for the purpose of determination of field capacity and wilting point percentages. An approximation of field capacity determinations was made using a pressure plate with 3/10 Bars tension, while the wilting point levels were measured under 15 Bar tension by means of a pressure membrane. With the exception of experiments 1 and 5 described below, soil moistures were controlled on the basis of water regimes indicated in each experiment in terms of percentage of available water. Plastic containers of approximately equal weight and with a capacity of about four pounds of soil were used as plant containers. Equal amounts of dry soil were used in all the pots for which the amount of water per pot required to establish a given water regime was calculated. Soil moisture control was started about four weeks after seeding, at which time the seedlings were well established. The predetermined soil moisture level for each regime was maintained thereafter by weighing daily each plant pot and its contents and adding sufficient water to maintain the desired soil moisture level.

3.4 Measurement of root weights

For measuring root weights, it was essential to clean the roots of any soil or foreign materials, and also to avoid losing the delicate root hairs during the washing and handling process. To facilitate the root washing procedure, soil was first saturated with a three percent hydrogen peroxide solution which was added to each plant container after the aerial parts of the plant were harvested. After a soaking period of approximately four hours, the roots were then placed on a fine mesh screen and were gently washed with a spray of tap water. After being allowed to reach air-dried weight, roots of each plant were then weighed.

3.5 Series of experiments

3.5.1 Experiment Number 1

Seeds from *Hordeum vulgare* L. var Parkland and *H. distichum* L. var. Hannchen were first germinated in petri dishes and then transferred to 5-inch clay pots in the greenhouse. As the experiment was conducted during the summer months, no supplementary light was needed to provide adequate photoperiod. Maximum day temperature was controlled at about 21°C. Two types of treatment were used; a soil drench and a leaf spray. For the soil drench, 200 millilitres of the following concentrations were applied, at the 2-leaf stage of growth.

(1) Control

(2) 50 p.p.m. (3.15×10^{-4}M)

(3) 200 p.p.m. (1.26×10^{-3}M)

(4) 500 p.p.m. (3.15×10^{-3}M)

(5) 1000 p.p.m. (6.30×10^{-3}M)

(6) 2000 p.p.m. (1.26×10^{-2}M)

For the leaf spray treatment, concentrations of 0, 50 and 2000 p.p.m. were used.

As a measure of the degree of growth retardation resulting from treatment, the distance between the bases of the first and second leaves was measured two weeks after application of the chemical. The general appearance and height of treated plants, was recorded at weekly intervals from the time of treatment to the time of maturity.

3.5.2 Experiment Number 2

The two barley varieties, Parkland and Hannchen, were used again in this experiment. Germinated seeds were transferred to the plastic pots each containing 1800 grams of soil with a field capacity and a wilting point of 26.84 and 11.67 percent moisture respectively. When seedlings were at a two-leaf stage, CCC was added in the form of a soil drench at the following concentrations:

(1) Control

(2) 250 p.p.m (1.57×10^{-3}M)

(3) 1000 p.p.m (6.30×10^{-3}M)

Following treatment, the following three different soil moisture regimes were maintained within each treated population:

(1) near wilting point

(2) 20 percent available water

(3) 80 percent available water

In addition to carrying out the same measurements as outlined above under Experiment 1, the air-dry weight of the plants, root weight, and date of maturity were also recorded for this experiment.

3.5.3 Experiment Number 3

In this experiment only variety Parkland was used, because of its better response to the chemical, and also due to the limited space available in the growth room. The following concentrations of CCC were used as soil drench:

(1) Control

(2) 200 p.p.m. (1.26×10^{-3}M)

(3) 2000 p.p.m. (1.26×10^{-2}M)

Under the water regimes maintained in the previous experiment, a true picture of the plant growth under high and low moisture conditions of the soil was not obtained, 80 percent available water being too high and wilting point being too low. Therefore, in this experiment, two compromised levels of soil moisture were chosen, 50 percent and 10 percent available water for the normal and the dry conditions of moisture respectively. The amount of water which had to be added each day to maintain the soil moisture at the above levels was recorded in millilitres. Field capacity of the soil used in this experiment was 26.25 percent, and the wilting point, 9.03 percent.

Plants receiving the soil drench treatment early in their development, usually regain their normal height by the time they are nearing maturity. In order to perpetuate the initial effect of the chemical plants treated initially through the soil, received additional amounts of CCC through the stem by means of the "wick method". Plants treated in this manner were divided into three groups: one group was wick-treated for a period of 48 hours two weeks following soil treatment; while the second and third groups received the same treatment three and four weeks respectively following the initial soil drench. In order to provide optimum conditions for cleaning and measuring the root weight, the soil used in this experiment was finely sieved and no peatmoss was added. Unfortunately the deletion of both sand and peatmoss from the soil resulted in poor aeration and consequent poor plant growth. Yield data were not obtained from this experiment, although during the early stages of plant growth it was possible to record data pertaining to plant height, number of tillers, date of heading and distance between first and second leaf base of all treated plants.

3.5.4 Experiment Number 4

This experiment was conducted much in the same way as described for the previous one, except that peatmoss and sand were mixed with the soil to improve its physical properties. Field capacity of this soil was 20.90 percent, and the wilting point 9.92 percent. All the measurements described in the previous experiments were made, in addition to measurement of grain yield and its components (number of heads per plant, number of kernels per head and kernel weight).

3.5.5 Experiment Number 5

This experiment was conducted in much the same way as those previously described, except that plants receiving the spray treatments were divided into two groups; one group being treated at the two-leaf stage as previously described, the other group being sprayed two weeks following the first spray when the plants were approximately close to flag leaf stage. Only one variety, Parkland, was spray treated and the following concentrations were used:

0 (control), 10^{-1}M, 10^{-2}M, and 10^{-3}M

Both varieties, Parkland and Hannchen, were used in the soil drench phase of this treatment, each receiving the following concentrations:

0 (control), 10^{-1}M, 10^{-2}M, 10^{-3}M, and 10^{-4}M

Data recorded from this experiment included measurements of the distance from the base of first to second leaf blade two weeks after treatment, the general appearance of treated plants and their height as measured weekly, the number of leaves per plant at near maturity, the period required for treated plants to reach maturity, total grain yield per plant and finally, the components of yield.

4. RESULTS

4.1 General effect of CCC on the plant

The general effect of (2-chloroethyl) trimethylammonium chloride on barley plants was found to be expressed as a reduction of growth results in plants with short, thick stems and with broad dark green leaves. Such plants, because of their short stature grew erect with no tendency to lodge as they neared the final stages of their vegetative growth. The visual change in the growth of barley plants as a result of treatment developed gradually over a period of about two weeks after application of the chemical. A difference in stem length between controls and treated plants could first be detected after three to five days on the basis of the reduced distance between successive leaf blade bases. Tillering was usually initiated earlier in treated plants than in controls, and was first noticeable within a few days to two weeks after treatment. Treated plants exhibited uniform height as a result of tillers having a rate of growth comparable to that of the retarded main tiller. Maturity was generally delayed for one or two weeks depending upon the concentration of the chemical used.

4.2 Experiment I

Table 4.1 shows the results of the first of a series of experiments in which the varieties Hannchen, a two-rowed barley, and Parkland, a six-rowed barley were treated with CCC applied as a soil drench and as a spray. Distinct varietal differences in response to treatments occurred (Fig. 4.1). With the exception of the

Table 4.1 Effect of CCC on height (in cm.) of two barley varieties treated with CCC.

Weeks after treatment	Parkland							
	Soil drench						Spray	
	2000 p.p.m.	1000 p.p.m.	500 p.p.m.	200 p.p.m.	50 p.p.m.	0 p.p.m.	50 p.p.m.	2000 p.p.m.
2	5.8	6.7	8.5	10.3	10.2	11.3	8.9	9.5
3	9.3	9.2	11.6	14.7	14.5	15.0	14.1	14.8
4	12.6	14.0	17.5	22.2	22.2	24.5	23.7	22.2
5	15.4	18.6	25.5	31.9	33.0	34.0	34.2	44.7
6	25.5	32.0	37.2	53.1	40.0	53.6	54.7	61.0
7	47.6	47.0	45.5	55.4	52.2	62.0	64.7	66.0
8	47.6	50.0	48.5	55.8	52.6	62.1	65.0	66.5

Weeks after treatment	Hannchen							
	Soil drench						Spray	
	2000 p.p.m.	1000 p.p.m.	500 p.p.m.	200 p.p.m.	50 p.p.m.	0 p.p.m.	50 p.p.m.	2000 p.p.m.
2	7.1	6.8	7.7	6.9	8.7	9.6	8.7	8.5
3	9.7	9.2	10.0	7.6	12.3	11.7	11.1	13.3
4	15.6	14.5	14.9	12.9	20.6	17.7	17.5	17.7
5	19.0	19.1	20.0	17.2	27.0	21.7	21.4	19.6
6	26.7	27.9	29.6	24.7	39.0	31.0	30.5	32.5
7	51.0	56.1	51.1	45.3	62.7	60.0	58.9	62.9
8	61.0	64.0	63.9	59.1	62.7	61.4	67.4	72.0

For comparison between heights at eight weeks after treatment

L.S.D.(0.05) = 5.88

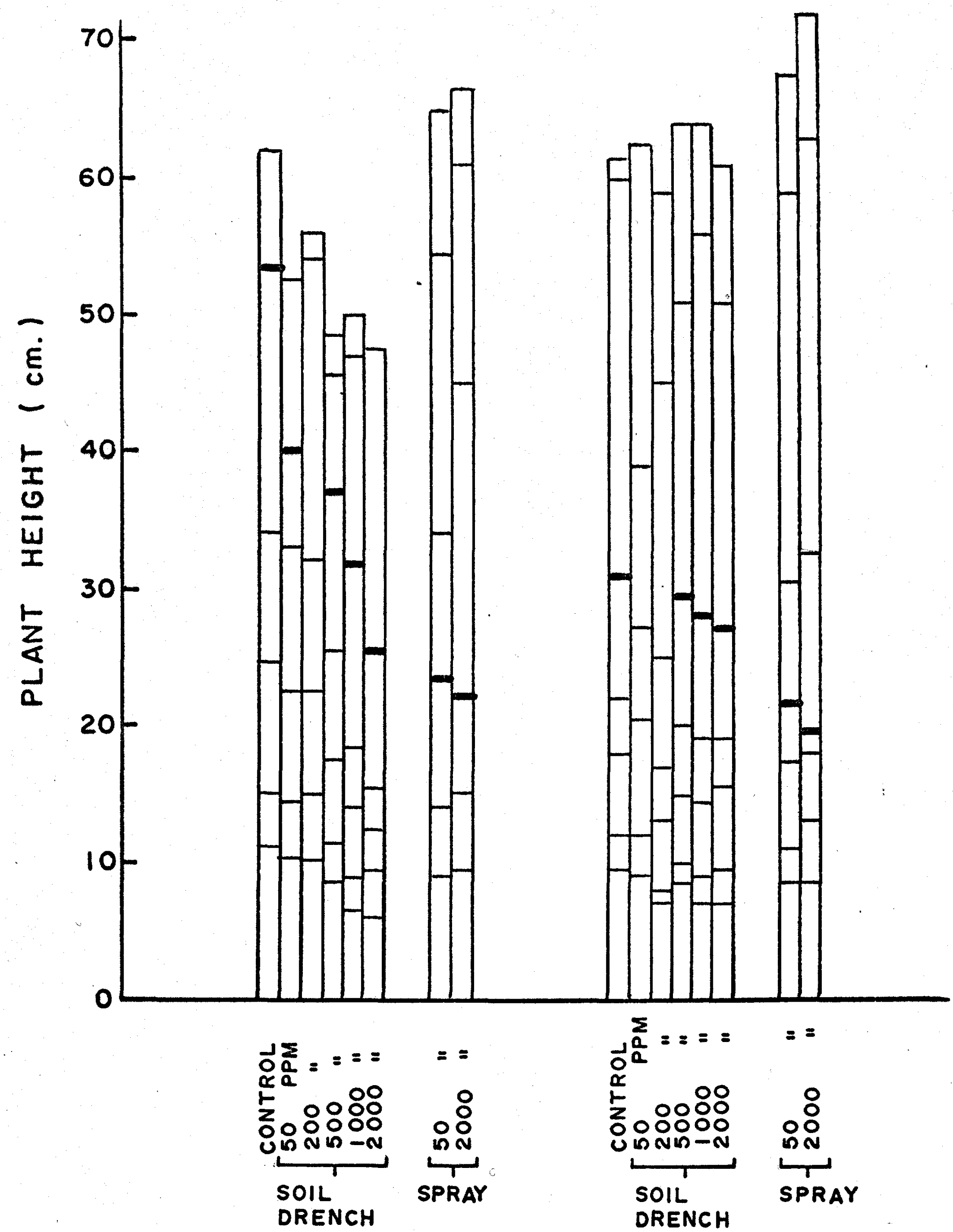

FIG. 4.1: AVERAGE HEIGHT OF TWO BARLEY VARIETIES FOLLOWING TREATMENT WITH " CCC " APPLIED AS A SOIL DRENCH AND AS A SPRAY. MEASUREMENTS WERE MADE AT WEEKLY INTERVALS, FROM TWO WEEKS AFTER TREATMENT TO NEAR-MATURITY.

Note -In this and subsequent similar Figures the heavy cross-bars in the columns designate the time and height at which the chemical had no further observable effect.

200 p.p.m. treatment on Parkland and also the 50 and 200 p.p.m. treatments on Hannchen, the most obvious effect of the chemical as applied to the soil was to initially reduce the height of both varieties in accordance with concentration used. In Hannchen, this condition persisted up to about the sixth week after treatment. After this time, an accelerated growth rate occurred in treated plants of this variety which resulted in almost a complete recovery of height by the time of near-maturity. In contrast, Parkland exhibited a permanently retarded growth right up to the time of ripening.

Apart from the initial "dwarfing" effect of the chemical on the plant, even the highest concentration used (2000 p.p.m.) when applied to the soil produced no visibly harmful symptoms. Spray treatment with this same concentration caused some necrosis of the leaves but as the new unaffected leaves were undergoing rapid growth following treatment, this temporary damage had no obvious effect on the general vigor of the plants. Results of this experiment indicated that spray applications of the chemical at 50 and 2000 p.p.m. had a slight inhibitory effect up to fourth week after treatment after which time it stimulated plant growth. As a result, both spray-treated and control plants were of about the same height at the time of maturity (Fig. 4.1).

It is noteworthy that despite the fact that the two varieties tended to completely recover their normal height, the average distance between the bases of first and second leaf blades were permanently reduced (Table 4.2). As will be seen later, this condition is reflected in reduced internode lengths at the base of the plant which may have some significance from the standpoint of increased lodging resistance.

Table 4.2 Distance between first and second leaf blade bases (mm.) of two barley varieties two weeks after treatment with CCC (average of four replicates).

Parkland							
Soil drench						Spray	
2000 p.p.m.	1000 p.p.m.	500 p.p.m.	200 p.p.m.	50 p.p.m.	Control	50 p.p.m.	2000 p.p.m.
13	18	25	30	27	36	21	21

Hannchen							
Soil drench						Spray	
2000 p.p.m.	1000 p.p.m.	500 p.p.m.	200 p.p.m.	50 p.p.m.	Control	50 p.p.m.	2000 p.p.m.
21	23	24	29	34	32	28	26

L.S.D. (0.05) = 5.13 mm.

4.3 Experiment 2

In this experiment three concentrations of CCC (0,250 and 1000 p.p.m.) each were applied to the varieties Parkland and Hannchen which were grown under three soil moisture regimes; wilting point (11.67 percent moisture), 20 percent, and 80 percent available water. Effects of the chemical on the growth pattern of plants grown in this manner were measured in terms of number of tillers per plant, total height (all tillers per plant), height of main tiller only, and distances between the bases of successive leaf blades. These data are shown in Table 4.2.

The general appearance of treated plants grown under both 20% available water and at the wilting point was superior to that of untreated plants reared under comparable conditions. Treated plants remained more turgid under the low water regime than did untreated ones and a yellowish leaf color which was general throughout the untreated population grown under this regime was absent in plants of the treated population.

Reference to Table 4.3 shows that the number of tillers produced on treated plants of this experiment grown within any one moisture regime, generally was no greater than the number formed on control plants grown at the same moisture level. When the combined height of all tillers is considered, however, it can be seen that particularly in the case of the 1000 p.p.m. treatment, a marked increase in this character at all moisture levels occurred (Table 4.3). This same trend was also apparent in response to the 250 p.p.m. treatment, but was limited however, to only those plants grown under conditions of wilting. For reasons that are not too

Table 4.3 Effect of CCC on certain plant characters as influenced by availability of soil moisture (average of four replicates).

		Parkland				Hannchen				
	CCC p.p.m.	W. pt.1/	20% av. w.2/	80% av. w.	% of control	W. pt.	20% av. w.	80% av. w.	% of control	L.S.D. (0.05)
Distance between 1st and 2nd leaf blade (mm.)	0	4.9	4.6	5.8	100.0	4.8	5.5	7.4	100.0	1.08 mm.
	250	4.6	4.7	4.1	87.6	4.3	5.0	5.8	78.4	
	1000	5.8	5.1	5.4	106.5	4.2	4.7	5.2	70.3	
Height of main tiller (cm.)	0	41.7	47.0	61.5	100.0	22.7	30.2	46.9	100.0	
	250	39.9	44.1	55.2	87.5	21.7	29.7	43.8	93.4	4.57 cm.
	1000	35.5	42.2	54.9	86.6	20.4	27.2	39.5	84.3	
Total height of all tillers (cm.)	0	84.6	158.0	170.5	100.0	118.1	162.9	222.0	100.0	
	250	99.8	149.0	158.1	98.5	119.9	154.2	210.4	96.4	23.73 cm.
	1000	90.5	201.6	219.0	123.7	133.3	164.0	232.2	104.6	
Number of tillers	0	4	7	6	100.0	10	14	17	100.0	
	250	5	7	7	111.7	9	12	16	94.1	
	1000	5	7	7	111.7	11	14	18	105.9	

1/ av. w. = available water
2/ w. pt. = wilting point

clearly defined, this same treatment caused an actual reduction of overall tiller height of both varieties grown at both the 20% and 80% available moistures.

The influence of treatment on the height of the main tiller alone can also be seen from data in Table 4.3. Expectedly, as the availability of soil moisture increased, so did the length of the main tiller. In response to treatment, however, the main tiller height of both varieties was decreased without exception at all moisture regimes in a direct relationship to the concentration of chemical used as treatment. For example, in comparison with untreated plants, the average height reduction for the 250 p.p.m. treatment at all moisture levels was 12.5 and 6.6 percent for Parkland and Hannchen respectively. Similarly, the main tiller of these same two varieties treated with 1000 p.p.m. was shortened 13.4 and 15.7 percent of the height of control plants. It would appear that the growth retarding action of CCC acts solely on the main tiller. Its dominant growth habit is suppressed. In turn however, other tillers of the treated plant appear to be stimulated to undergo an accelerated growth rate. The result is a plant in which all culms are of about equal length and with exception of the main tiller, are somewhat longer than they would be under normal conditions.

Data pertaining to plant weight as influenced by CCC treatment are shown in Table 4.4. As expected, the average root weight per plant increased with successively higher soil moisture levels. Treatment effect within any one moisture regime, however, appeared to have little effect on root weight. Comparing the total

Table 4.4 Average dry weight in grams of roots and above ground parts of mature plants of Parkland and Hannchen barley treated with CCC (average of four replicates).

		Parkland			Hannchen		
		Control	250 p.p.m.	1000 p.p.m.	Control	250 p.p.m.	1000 p.p.m.
Wilting point	Roots	2.1	1.8	2.2	1.6	1.4	1.7
	Total	8.6	8.1	8.9	10.4	10.1	10.8
20% available water	Roots	2.6	2.5	2.8	2.1	1.9	2.2
	Total	15.9	13.9	14.8	17.6	16.5	16.7
80% available water	Roots	3.7	3.6	3.9	4.1	3.9	4.3
	Total	28.0	26.9	26.2	29.1	26.3	26.4

L.S.D. (0.05) Roots = 0.62

Total = 0.62

weight per plant (root + tops), it is seen that at the low moisture regime, CCC has little, if any influence on this character. At both the 20 percent and 80 percent available moisture levels, both varieties showed a decrease in total plant weight in comparison with controls when treated with either the 250 or 1000 p.p.m. of chemical. This decrease may be attributable to the corresponding decrease in overall tiller height obtained in treated plants grown under these sametwo water regimes.

4.4 Experiment 3

As mentioned above in describing the results of experiment 1, there was a strong tendency for plants treated with CCC early in their growing period to overcome the effects of the chemical and to regain their normal height by the time of maturity. An attempt was made to perpetuate the inhibitory effect of the chemical on the growth of the plant by supplementing the initial soil treatment with subsequent applications supplied by the "wick method". Moreover, since the potential of this chemical to induce drought tolerance can best be measured in terms of the actual water consumption of treated plants, this factor was carefully measured in this experiment as well as in the one to follow. Although the general conditions of plant growth were adversely affected by the heavy soil used in this particular experiment, the characteristic growth patterns following CCC application were produced, as may be seen from the results in Table 4.5 and Fig. 4.2. It is seen that the magnitude of the height differences attributable to the various levels of treatment used, was very marked. Although the plants were reduced in height as a result of the sub-optimal

Table 4.5 Average height (cm.) of four replicates of Parkland barley grown on heavy soil at 10 percent and 50 percent available water and treated with two concentrations of CCC.

Week	10% available water									50% available water								
	0	200 p.p.m.				2000 p.p.m.				0	200 p.p.m.				2000 p.p.m.			
		Soil 1/	Soil +2	Soil +3	Soil +4	Soil	Soil +2	Soil +3	Soil +4		Soil	Soil +2	Soil +3	Soil +4	Soil	Soil +2	Soil +3	Soil +4
2	9.5	5.8	6.5	6.7	5.6	5.1	5.5	5.0	5.4	7.5	5.9	6.5	6.4	6.3	5.0	5.3	5.1	5.0
3	12.1	8.0	8.1	8.4	8.2	6.9	7.3	7.2	7.6	10.6	8.7	8.4	9.3	8.9	7.1	7.9	7.2	7.3
4	13.6	8.6	8.4	8.7	8.4	8.0	8.9	8.2	9.1	11.9	10.5	9.6	10.3	10.1	8.3	8.5	8.0	8.4
5	19.4	11.1	10.2	11.7	11.2	10.7	11.1	10.0	19.3	19.4	14.7	12.4	15.4	14.3	14.0	12.9	11.8	11.1
6	30.3	20.6	20.7	21.6	22.5	17.0	17.8	16.7	15.9	30.9	26.9	27.5	29.0	28.2	23.3	21.5	19.2	19.5
7	32.5	25.4	24.0	25.9	27.9	23.3	23.0	22.1	21.5	35.0	29.1	30.5	31.5	32.0	27.2	26.4	25.3	26.0

1/ Soil = soil drench
Soil +2 = soil drench followed 2 weeks later with "wick treatment"
Soil +3 = soil drench followed 3 weeks later with "wick treatment"
Soil +4 = soil drench followed 4 weeks later with "wick treatment"

L.S.D. (0.05) = 2.94

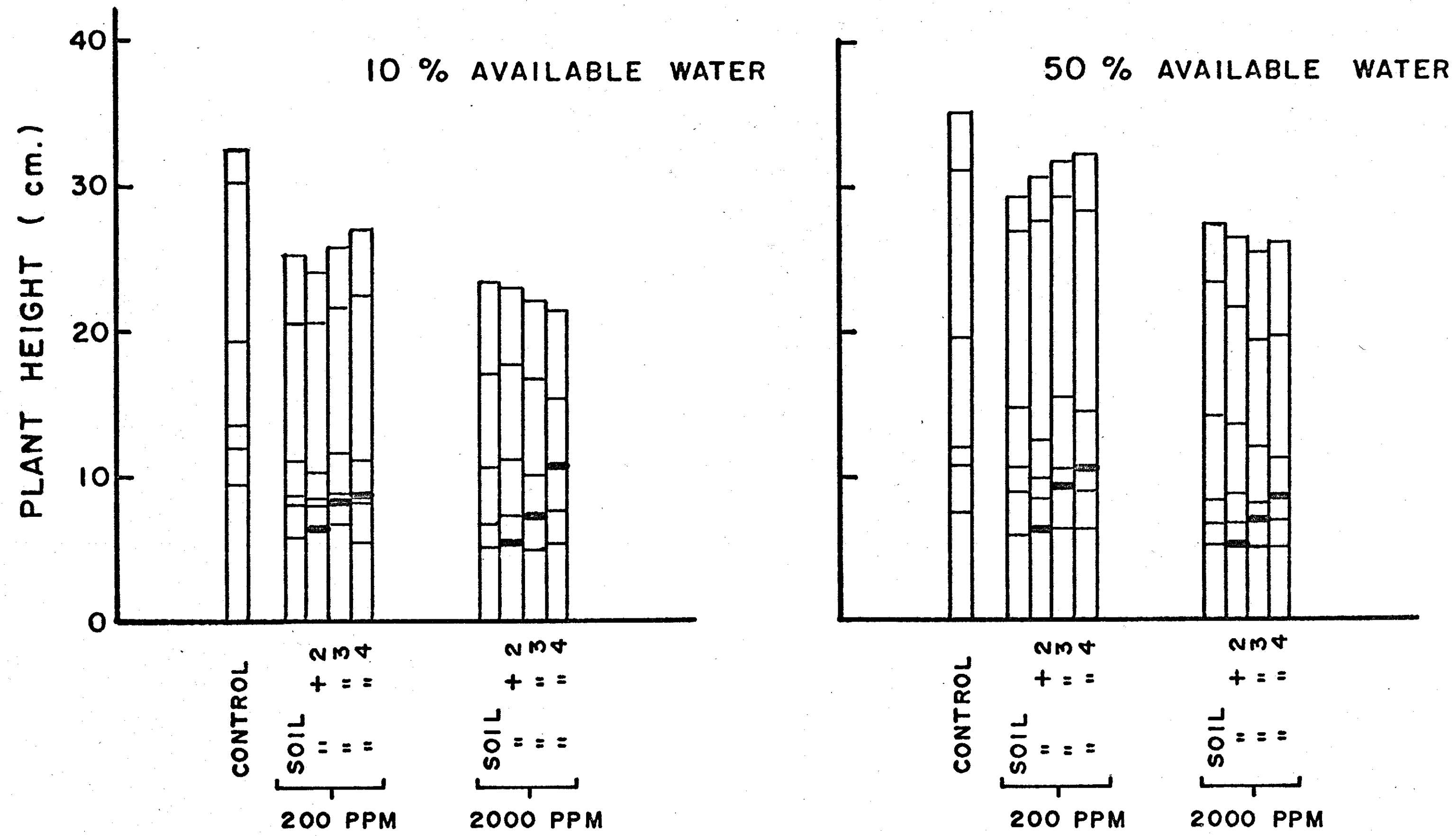

FIG. 4. 2: AVERAGE HEIGHT OF PARKLAND BARLEY GROWN ON HEAVY SOIL UNDER TWO WATER REGIMES AND TREATED WITH TWO CONCENTRATIONS OF "CCC".

SOIL DRENCH — 2 WEEKS AFTER SEEDING
SOIL + 2 — SOIL DRENCH FOLLOWED 2 WEEKS LATER WITH "WICK TREATMENT"
SOIL + 3 — " " " 3 " " " " "
SOIL + 4 — " " " 4 " " " " "

conditions of soil texture, the 200 p.p.m. and particularly the 2000 p.p.m. applications of CCC further reduced plant height under both moisture regimes. This reduction was particularly noticeable during the period between the third and fourth week after soil treatment in those plants grown at a low water level.

During this period, the plant growth was retarded by 200 p.p.m., and completely inhibited by 2000 p.p.m. of CCC (Fig. 4.2). Under high water level, however, such drastic growth alterations did not occur, although differences in height between treated and control plants, and also between the two treated populations themselves were apparent.

It is interesting to note that supplementary application of the chemical following the initial soil treatment did not completely suppress the tendency for the occurrence of an accelerated growth rate of treated plants following a period of temporary arrestment. Although a slight height reduction occurred during a period immediately following each treatment, an hastened rate of growth thereafter largely compensated for this initial period during which growth was retarded (Fig. 4.2).

The actual amounts of water used by treated and control plants from a period two weeks after treatment to flowering time and also from this same period to near maturity are given in Table 4.6. It may be seen that in comparison with untreated material, the 2000 p.p.m. treatment of CCC reduced the water use by plants growing under both the 10% and the 50% moisture regimes. Expressing the water consumption of control plants as 100%, the total consumption from a period two weeks after treatment to near-maturity was decreased

Table 4.6 Water consumption (ml.) of barley plants (var. Parkland) grown on heavy soil under two water regimes following treatment with CCC (average of four replicates).

Time	% available water	Control		200 p.p.m.		2000 p.p.m.		L.S.D. (0.05)
		ml.	% of control	ml.	% of control	ml.	% of control	
2 weeks after treatment to flowering	10	921.0	100	765.0	83.1	685.0	74.4	
	50	1094.0	100	1214.0	111.0	864.0	78.8	
2 weeks after treatment to near-maturity	10	1066.0	100	824.4	77.3	715.0	67.1	155.08
	50	1325.0	100	1414.5	106.7	1073.0	81.0	210.37

32.9% for plants maintained at a 10% soil moisture and by 19% for those grown at a 50% level of moisture.

The effect of the 200 p.p.m. treatment on water utilization by the plant however, was not consistent. It is interesting to note that only those plants grown under the 10% moisture regime were found to consume less water in response to this treatment. For the full period of their development, plants growing at this moisture level reduced their water consumption by 22.7%. In contrast, this same treatment applied to plants grown at 50% soil moisture, tended to increase their water use. This increase occurred during both the pre-flowering stage of development (11%), while the overall increment from the seedling to the mature plant stage was only 6 percent.

4.5 Experiment 4

An attempt was made to study the growth patterns of CCC treated barley plants under controlled moisture levels on a soil lighter in texture than that previously described (Section 3). Improved physical condition of the soil resulted in a more favorable growth of plants under both low and high water regimes as may be seen from the height measurements shown in Table 4.7 and Fig. 4.3. In general, all plants grown in this experiment were taller than those studied in the previous experiment in which soil conditions were sub-optimal. In spite of this, height differences between control plants and treated plants, particularly those that received the 2000 p.p.m. treatment, were pronounced.

It was apparent that the main difference in height between treated and control plants may be attributable to an

Table 4.7 Effect of CCC on height of Parkland barley grown on light soil maintained at 10 and 50 percent available water.

	10% available water									50% available water								
0	200 p.p.m.				2000 p.p.m.				0	200 p.p.m.				2000 p.p.m.				
	Soil[1]	Soil +2	Soil +3	Soil +4	Soil	Soil +2	Soil +3	Soil +4		Soil	Soil +2	Soil +3	Soil +4	Soil	Soil +2	Soil +3	Soil +4	
4.5	3.9	3.8	3.2	3.2	2.7	3.1	2.9	3.6	4.5	3.1	3.2	3.4	3.1	2.8	2.7	2.9	2.6	
16.9	10.6	11.2	11.4	11.4	8.7	10.3	11.3	10.7	17.6	13.2	12.7	13.5	13.2	10.9	10.5	11.5	10.6	
24.3	16.1	16.0	17.1	16.9	13.7	13.2	14.1	14.6	30.0	22.1	20.8	22.5	21.6	17.1	16.0	17.9	18.4	
40.0	26.8	23.8	24.7	27.3	23.2	22.1	22.5	26.2	46.1	37.8	35.0	35.3	37.5	31.9	28.8	29.3	33.0	
43.9	39.4	37.2	37.3	38.4	35.4	35.6	33.7	32.5	50.0	46.9	46.5	46.8	47.9	40.8	39.0	37.9	39.8	

1/ Soil = soil drench
Soil +2 = soil drench followed 2 weeks later with "wick treatment"
Soil +3 = soil drench followed 3 weeks later with "wick treatment"
Soil +4 = soil drench followed 4 weeks later with "wick treatment"

L.S.D. (0.05) for the final height = 4.001

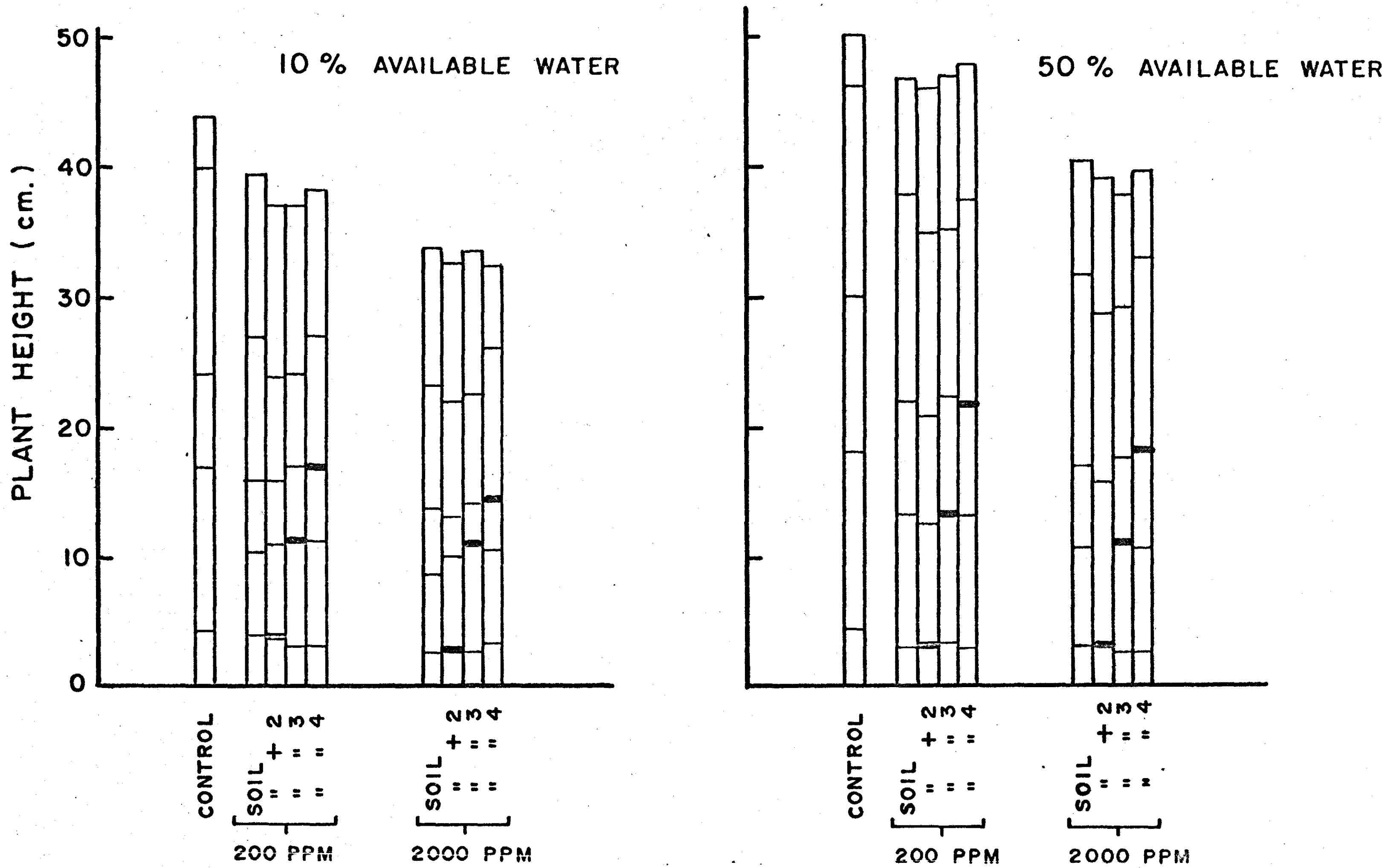

FIG. 4.3: AVERAGE HEIGHT OF PARKLAND BARLEY GROWN ON LIGHT SOIL UNDER TWO WATER REGIMES, AND TREATED WITH TWO CONCENTRATIONS OF "CCC"

SOIL DRENCH — 2 WEEKS AFTER SEEDING.
SOIL + 2 — SOIL DRENCH FOLLOWED 2 WEEKS LATER WITH "WICK TREATMENT"
SOIL + 3 — " " " 3 " " " " "
SOIL + 4 — " " " 4 " " " " "

inhibition of growth during the period of second to fifth week after treatment under low soil moisture, and between the second and fourth week under the high water level. Comparison of the heights of plants receiving different treatments during their latter stages of vegetative growth (Fig. 4.3) show that CCC treated plants eventually regain their normal height during this period.

The effects of supplementary treatment by the "wick method" was less distinct in so far as the final height difference of the plants is concerned. As in the previous experiment this treatment seems to have a slight effect on reducing the plant's growth for only one or two weeks following treatment. Measurements of this response may best be seen from Fig. 4.3 in which the effect of application of the 48-hour wick treatment is depicted on each vertical bar by a heavy line.

In spite of a general reduction in plant height resulting from chemical treatment, particularly with the 2000 p.p.m. concentration, the overall capacity of these plants to produce a normal amount of top growth was not appreciably changed (Tables 4.7 and 4.8). Particularly under the low water regime, both control plants and those which received a 200 p.p.m. treatment were about equal in average total tiller height. The 2000 p.p.m. treated plants did not differ greatly from the ones which were treated with 200 p.p.m. although a decreased trend in top growth as produced with this treatment was apparent. Exactly this same relationship between total length of tillers and concentration of treatment existed at the 50% moisture regime, with even a greater reduction in total tiller height attributable to the 2000 p.p.m. treatment

Table 4.8 Effect on total tiller height (cm.) of 200 and 2000 p.p.m. of CCC applied to Parkland barley grown under 10 and 50 percent available water.

Water regime	Control	200 p.p.m.	2000 p.p.m.
10%	177.0	175.0	160.0
50%	361.0	369.6	273.0

L.S.D. (5%) = 50.10

occurring at this moisture level than at the 10% one.

As was found in Experiment 2, the 2000 p.p.m. treatment caused an increase in total dry plant weight. In comparison with controls, an increase of 0.5 and 1.3 grams per plant was obtained under soil moistures of 10% and 50% respectively (Table 4.9).

Table 4.9 Effect of CCC on average dry weight per plant (in grams) of Parkland barley grown under two water regimes.

Water regime	Control	200 p.p.m.	2000 p.p.m.
10%	13.3	12.6	13.8
50%	21.0	19.6	22.3

L.S.D. (5%) = 2.39

The 200 p.p.m. treatment, also showed similar patterns in both experiments by reducing dry weight of the plants in comparison with controls.

The results of yield measurements in this experiment are given in Tables 4.10 and 4.11. Grain yield of plants receiving a soil treatment of 2000 p.p.m. of CCC was significantly increased over that of controls (P = .05). One treatment, i.e. the 200 p.p.m. soil application supplemented by a 48-hour continuous treatment two weeks later, resulted in a significant yield reduction.

The average number of fertile tillers per plant (Table 4.12) did not show a great variation between treated and control plants. The number of seeds harvested per plant however, was decreased by the 2000 p.p.m. treatment applied at the 10% moisture regime. In contrast, plants grown under the 50% water regime, produced an increased number of seeds in response to this same treatment.

The 1000-kernel weight of the 2000 p.p.m. treated plants' was comparable to that of controls under both water regimes. Plants from the 200 p.p.m. treatment, however, exhibited an increased kernel weight under low water regime but a decreased weight under the high water regime (Table 4.13). In spite of the fact that overall plant height was decreased and that dry weight, including grain yield, was increased by treatments of high concentration, it is interesting to note that water consumption of treated plants grown under light soil conditions was lowered in comparison with untreated plants. Table 4.14 shows that under a low water regime, a reduction of 12.7 percent and a 19.1 percent reduction of water consumption occurred up to the time of flowering in plants

Table 4.10 Effect on yield of barley of soil and spray treatments of CCC followed by a supplementary application by wick (average of four replicates grown under two water regimes). Yields in grams per plant.

Water regime	Control	200 p.p.m.				2000 p.p.m.			
		Soil[1]	Soil +2	Soil +3	Soil +4	Soil	Soil +2	Soil +3	Soil +4
10%	3.21	2.50	2.07	3.16	2.62	3.30	1.97	3.36	2.50
50%	4.51	3.77	2.65	4.47	3.76	6.31	4.18	3.49	4.51
$\bar{X}$	3.86	3.13	2.36*	3.82	3.19	4.80*	3.08	3.43	3.51

1/Soil = soil drench
Soil +2 = soil drench followed 2 weeks later with "wick treatment"
Soil +3 = soil drench followed 3 weeks later with "wick treatment"
Soil +4 = soil drench followed 4 weeks later with "wick treatment"

* = Significant at 0.05

Table 4.11 Analysis of variance of effect of CCC on yield.

Source	d.f.	F value	
		Calculated	Table (.010)
Water regimes	1	95.02**	7.09
Chemical treatments	8	9.29**	2.83
Replications	3	40.72**	4.14
Error	59		
Total	71		

L.S.D. (5%)= .886
C.V. = 18.09

Table 4.12 Effect of CCC on numbers of fertile spikes and seeds per plant of Parkland barley grown under two water regimes (average of four replicates).

Water regime	Control		200 p.p.m.		2000 p.p.m.	
	No. of heads/ plant	No. of seeds/ plant	No. of heads/ plant	No. of seeds/ plant	No. of heads/ plant	No. of seeds/ plant
10%	5.5	109.0	6.0	98.0	5.0	81.0
50%	11.0	117.0	11.2	98.0	11.0	161.5

L.S.D (0.05) for No. of seeds/plant = 32.98

Table 4.13 Average 1000-kernel weight of barley plants (var. Parkland) grown on light soil and treated with CCC (average of four replicates).

Available water	Control	200 p.p.m.	2000 p.p.m.
10%	33.15	36.13	33.09
50%	39.55	37.61	39.31

L.S.D. (0.05) = 2.59

Table 4.14 Effect of CCC on water consumption of Parkland barley grown on light soil maintained at 10 and 50 percent available water (average of four replicates).

Time	% available water	Control		200 p.p.m.		2000 p.p.m.	
		ml.	% of control	ml.	% of control	ml.	% of control
2 weeks after treatment to flowering	10	1745	100	1523	87.3	1141	80.9
	50	2661	100	2590	97.3	2527	95.0
2 weeks after treatment to near-maturity	10	2787	100	2422	97.4	2283	91.8
	50	4253	100	4180	98.3	4119	96.8

L.S.D. (0.05)for 2weeks after treatment to near-maturity:

10 81.36 ml.

50 Nil

treated with 200 and 2000 p.p.m. respectively. The magnitude of this reduction was not as great for this same treatment when applied under conditions of more favorable moisture supply.

4.6 Experiment 5

The concentrations of CCC used in studies reported above, approximated those used by other workers investigating the effects of the chemical on wheat and other cereals (Tolbert, 1960). The present studies indicate that a differential response of the two barley varieties to treatment occurred, with the two-rowed variety Hannchen being effected less than Parkland. If this chemical has any potential value from the standpoint of reducing the plant height, thereby improving the tolerance of treated plants to conditions of lodging, it would be desirable to test the effect of higher concentrations than those used previously. Accordingly, in this experiment, concentrations of 10^{-1}M (15800 p.p.m.), 10^{-2}M (1580 p.p.m.), 10^{-3}M (158p.p.m) and 10^{-4}M(15.8p.p.m.) were used as soil drenches on both varieties Hannchen and Parkland, and as a spray treatment on Parkland.

In regard to the general appearance of treated plants, even the highest concentration caused no discernable harmful effects. Applications of 10^{-1} and 10^{-2}M solutions as a spray caused some necrosis and discoloration on the leaves, however, these symptoms were temporary and the plants soon regained their normal appearance. As noted in previous experiments, all of the characteristic growth effects induced by this chemical (e.g. the production of deep green coloration of foliage, increased tillering, and depressed internode growth) were apparent within two to three weeks after treatment.

Weekly measurements of height of plants from two weeks following both the soil and first spray treatments to the time at which a constant height was reached are shown in Table 4.15 and Fig. 4.4. It may be seen that as concentration of chemical used in both treatments increased, height of both varieties was correspondingly decreased. Moreover, the reduction in height induced by the various soil treatments persisted to the final stages of plant growth, a condition which was particularly marked in Parkland. The effect of spray treatment applied at the same time as the soil drench (two-leaf stage) also induced a more permanent response than obtained in previous experiments. This effect persisted up to the fourth week after treatment after which time the characteristic accelerated growth rate obtained in previous experiments again became apparent. The result was that although sprayed plants were of shorter stature than control plants at the time of maturity, this difference was not as great as that obtained with soil treatments.

It should be noted that these same chemical applications applied as a spray at a late stage of growth (two weeks after two-leaf stage) affected height in much the same manner as the early application with exception of the lower concentration (10^{-3}M). Whereas both the 10^{-1}M and 10^{-2}M solution applied during the late stage resulted in a height reduction equal to that obtained with a seedling treatment, the 10^{-3}M concentration was ineffective in inhibiting growth of tillers at this late stage of plant growth.

From the Table 4.16 one can see a definite pattern for CCC treatments to increase the number of fertile spikes per plant. In both varieties the highest concentration used (10^{-1}M) resulted in a maximum increase of this component which was presumably reflected in increased yields per plant.

The results of yield measurements in this experiment are given in Table 4.17. Soil treatment of both barley varieties with CCC resulted in a yield increase in comparison with controls. These results show that in the variety Parkland, increased chemical concentrations of 10^{-4}, 10^{-3} and 10^{-1}M all resulted in yield increases closely corresponding to the concentration of chemical used.

Yields of spray-treated plants varied depending upon the stage of treatment. Those sprayed with 10^{-3}, 10^{-2} and 10^{-1}M concentrations at the two-leaf stage (Time 1) yielded about equal to control plants, while spray treatments with 10^{-1} and 10^{-2}M concentrations two weeks later, (Time 2) resulted in an increased yield.

Table 4.15 Average weekly heights (cm.) of Parkland and Hannchen barley following treatment with CCC applied as a soil drench and as a spray at the seedling and flag leaf stages of growth.

Week	Parkland											Hannchen				
	Soil drench					Spray						Soil drench				
						Time 1			Time 2							
	10^{-4} M	10^{-3} M	10^{-2} M	10^{-1} M	0	10^{-3} M	10^{-2} M	10^{-1} M	10^{-3} M	10^{-2} M	10^{-1} M	10^{-4} M	10^{-3} M	10^{-2} M	10^{-1} M	0
2	9.7	9.4	7.0	6.3	14.6	8.1	10.4	6.3	16.4	16.2	14.4	9.1	7.5	6.7	5.2	11.0
3	19.1	19.7	18.1	17.5	30.1	20.2	20.1	13.0	41.4	28.4	27.7	17.1	13.2	13.2	11.7	21.0
4	55.5	53.4	50.4	46.0	72.0	61.1	55.4	37.0	79.6	64.0	55.6	40.6	35.0	30.2	23.4	43.1
5	75.7	69.0	66.0	64.5	86.0	70.0	70.5	59.6	86.0	77.0	67.1	68.9	52.2	55.7	35.7	64.4
6	76.3	69.5	66.0	65.0	86.7	70.7	76.5	68.5	86.0	77.9	68.5	71.2	70.0	65.5	59.0	70.1

L.S.D. (0.05) for the final height = 10.03

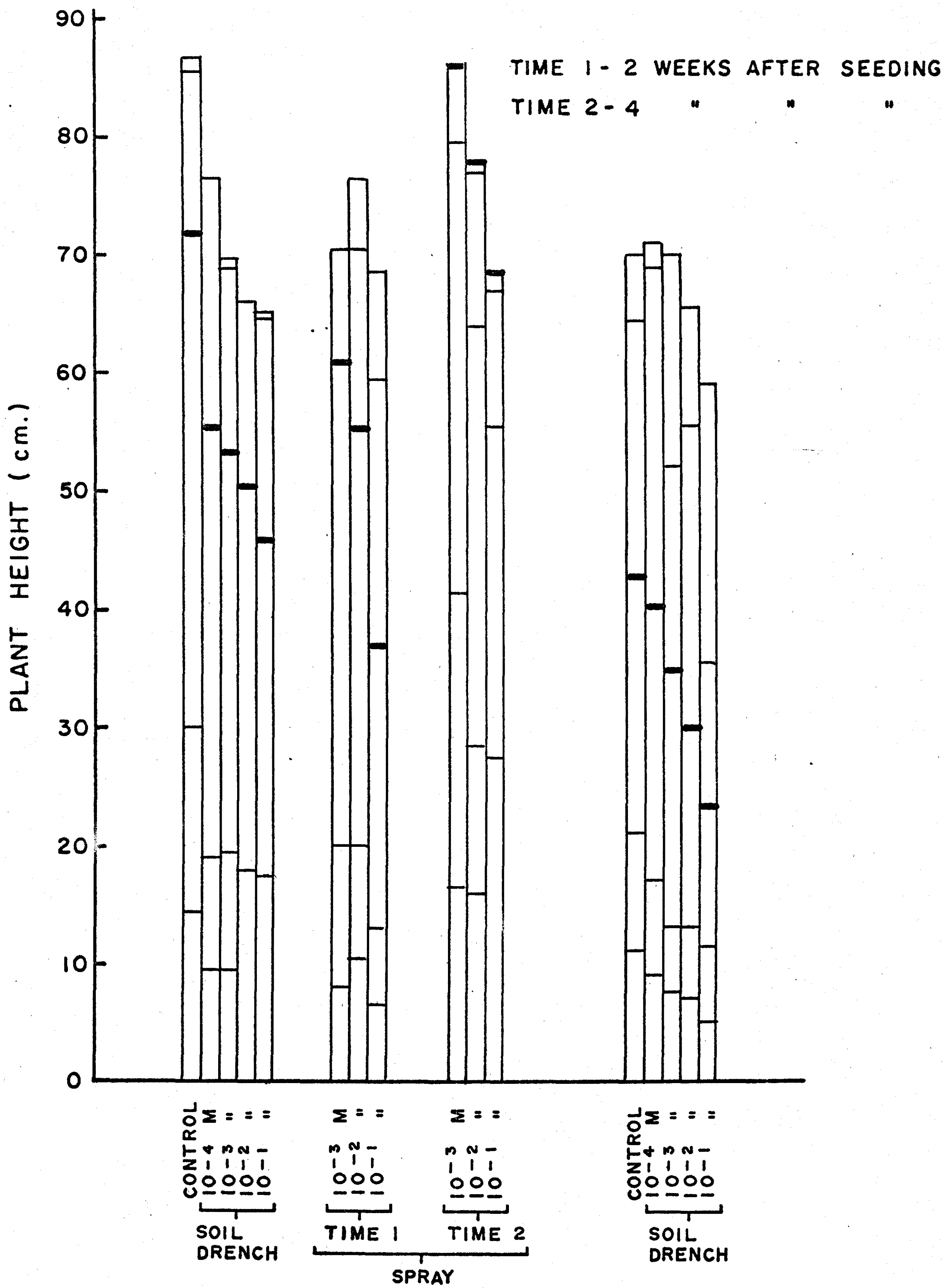

FIG. 4.4: AVERAGE HEIGHT OF TWO BARLEY VARIETIES MEASURED WEEKLY FOLLOWING TREATMENT WITH "CCC" APPLIED AS A SOIL DRENCH AND AS A SPRAY AT TWO STAGES OF PLANT GROWTH.

Table 4.16 Number of fertile heads in two barley varieties following soil and spray treatment with CCC, (average of four replicates).

Reps.	Parkland											Hannchen				
	Soil drench					Spray							Soil drench			
						Time 1			Time 2							
	10^{-1} M	10^{-2} M	10^{-3} M	10^{-4} M	0	10^{-1} M	10^{-2} M	10^{-3} M	10^{-1} M	10^{-2} M	10^{-3} M	0	10^{-1} M	10^{-2} M	10^{-3} M	10^{-4} M
$\bar{X}$	2.7	2.0	1.7	1.5	1.2	2.5	2.0	1.5	2.7	2.2	1.2	3.2	6.7	4.5	4.2	3.5

L.S.D. (0.05) = 0.7

Table 4.17 Grain yield of two barley varieties following soil and spray treatment with CCC, (average of four replicates). Yield in grams per plant.

Reps.	Parkland												Hannchen			
	Soil drench					Spray							Soil drench			
						Time 1			Time 2							
	10^{-1} M	10^{-2} M	10^{-3} M	10^{-4} M	0	10^{-1} M	10^{-2} M	10^{-3} M	10^{-1} M	10^{-2} M	10^{-3} M	0	10^{-1} M	10^{-2} M	10^{-3} M	10^{-4} M
$\bar{X}$	3.36	2.84	2.47	2.27	1.95	1.81	1.98	2.02	3.00	2.88	1.97	2.39	3.81	2.73	2.86	2.71
% of control	172.3	145.6	126.7	116.4	100	92.8	101.5	103.6	153.8	147.7	101.0	100	159.4	114.2	119.7	113.4

L.S.D. (0.05) for the soil drench treatments = 0.35

5. DISCUSSION

5.1 General effects of CCC on growth pattern of plants

It generally appears that the growth patterns produced in barley after treatment with (2-chloroethyl) trimethylammonium chloride are similar to the effects previously reviewed for other plant materials. Changes in the growth behaviour of stems and particularly internode length as induced by CCC treatment are of primary interest from the standpoint of increasing the resistance of barley plants to lodging. Tolbert (1960 a,b,d) reported that when wheat seedlings were treated once with CCC, the major growth difference observed was the development of plants with shorter and thicker stems compared with those of untreated plants and also the production of leaves that were dark green in color. The distance between the base of all leaf blades was substantially reduced during their early vegetative growth cycle, an effect, however, from which treated plants recovered by the time of heading. Corresponding to the decrease in stem length, the stem diameter of treated plants was greater than stems of control plants. The relatively short, thick stems resulted in wheat plants which grew very erect with no tendency to lodge.

From the results obtained in the present studies, a marked reduction in the height of treated plants in comparison with controls was evident. This difference, as shown by the weekly height measurements in Figures 4.1 to 4.4, is more pronounced during the periods immediately following treatments. In wheat plants, the decreased distance between first and second leaf blade bases has been reported (Tolbert, 1960b) to correspond with the concentration of chemical and therefore can be considered as a reliable criterion for measuring the effectiveness of treatment. In most cases the same thing appeared to be true in the present studies. It may be seen from the results presented in Table 4.3, however, that some discrepancy in the relationship between concentration and basal internode length occurred, thereby casting some doubt on the reliability of this character as a criterion for evaluating at an early stage of the plant's development the effectiveness of any given treatment.

Mann (1953) found plant height positively correlated with culm-internode length rather than culm-internode number. It is also found (Abdallah and Omar 1957) that lodging resistance in barley is associated with (1) high number of vascular bundles, (2) small bundles round in shape with numerous fibres, (3) large amounts of schlerenchyma, and (4) narrow or thick culms. Further, it is reasonable to assume that internode distances at the lower portion of the culm may have some effect on determining the lodging resistance of cereal plants. A comparison of internode distance on the culms of CCC-treated and control plants was made for the two barley varieties used in experiment 5. The general trend in treated plants grown under the conditions of this study, was for the first four internodes to be reduced in length in comparison with normals. Sachs and Lang (1961) found that cell division in the sub-apical meristem was reduced after CCC treatment resulting in decreased internode elongation.

In addition to being short in stature, treated plants in these studies appeared to have thicker stems than control plants. Thus, the assumption could be made that if under proper conditions, plants treated with CCC showed less tendency to lodge, this effect could be attributed to, (1) a decreased total plant height, (2) an increased stem diameter, and (3) short distances between lower internodes. It is realized that the growing conditions under which these studies were made i.e. the small volumes of soil used and the growing of the single plant per pot with no physical limitation or competition among the plants are not strictly applicable to field conditions and the results may not reflect the true response that would be obtained under field conditions.

Linser and Kuhn (1962) reported that different varieties of wheat exhibited differential response to application of CCC. Similarly, the results obtained from the two barley varieties used in this study, Parkland and Hannchen, reacted differently to treatment (Table 4.1 Fig. 4.1). Hannchen appeared to require higher concentrations of chemical than Parkland to produce comparable growth patterns. Moreover, Hannchen remained responsive to the effects of the chemical for considerably shorter periods of time than did Parkland.

Early maturity is an important agronomic factor in the adaptation of barley to certain areas of production. Earliness is often important in enabling a plant to escape hot, dry seasonal periods also the ravages of disease or insects, as well as in promoting the growth of companion crops of legumes or grasses. Any delay of maturity as induced by CCC therefore, might be considered as an undesirable characteristic under those conditions where earliness is of primary significance.

5.2 Effect of time and methods of application

Tolbert (1960) found that CCC concentrations ranging from 10^{-2} to 10^{-6}M were effective on wheat when either applied to the soil or used in nutrient culture. Application of 10^{-3}M or stronger solutions reduced the first interleaf base distance essentially to zero. Treatments in which the chemical was either sprayed on the leaves or incorporated into the seed by soaking was found to be less effective than soil treatments. According to Cooper (1964, personal communication) applications of CCC to the seed of spring wheat prior to planting also produced the typical retarded growth patterns as observed shortly after emergence, however, complete recovery appeared to have occurred approximately six weeks later.

Preliminary experiments with two varieties of barley used in the current study, showed that soaking the seeds in concentrations of 1.5×10^{-3}M and 6.30×10^{-3}M for 24 hours prior to planting had no apparent effect on plant growth. Moreover, soil applications of less than 10^{-4}M CCC produced no observable effect on the barley plant. From the results in Table 4.15 and Fig 4.4 it may be concluded that concentrations of 10^{-3} and 10^{-2} applied as a soil drench initially resulted in a reduction of growth of both varieties.
In Hannchen, the effect was only a temporary one and as a result this variety had almost regained its normal height by the time of maturity. Only by increasing the chemical concentration to 10^{-1}M was a marked height reduction realized which persisted to stage of harvest. With

this treatment, Parkland and Hannchen were reduced in height by 18.5 and 15.7 percent respectively. Accompanying this effect was a reduction of the distance between first and second leaf blades bases by an average of 2.5 mm. in Parkland and 2.3 mm. in Hannchen.

Wittwer and Tolbert (1960b)reported two instances in which CCC had a stimulatory effect on the plant growth. Low concentrations of CCC (10^{-7}M) increased vegetative growth as well as dry matter content of tomato plants. Synergistic response from a combination of IAA, GA and CCC (10^{-4} to 10^{-2}M) on parthenocarpic fruit development also markedly accelerated ovary growth of tomatoes. In the present study, spray treatments of CCC (3.15×10^{-4}M and 1.26×10^{-2}M) at the two-leaf stage also resulted in growth stimulation of both barley varieties under greenhouse conditions (Table 4.1 Fig. 4.1). Under controlled conditions in the growth room, however, spray treatments with concentrations ranging from 10^{-3} to 10^{-1}M at a comparable stage resulted in typical growth inhibiting effects. This highly variable growth response of barley plants to CCC as observed under the two different environments might be due either to differences in concentration *per se* or to differences due to an interaction between chemical effect and growing conditions (photoperiod, temperature, etc.). It is possible to conclude therefore that only under a rather narrow range of varying climatic conditions is the full effectiveness of this chemical expressed by the plant. Similar results have been reported by Tolbert (1961b)and also by Cathey and Stuart (1961) in which it was found that under greenhouse conditions, CCC was found to be more effective in winter than summer. Assuming therefore, that a plant's response to CCC can be reversed as a result of environmental conditions subsequent to treatment and/or differences in concentration beyond a certain threshold, the value of this chemical from a practical standpoint deserves consideration. Factors such as partial exposure of the plants to the spray treatment, or dilution of the chemical on the leaf surface by rain could conceivably result in a stimulation rather than a suppression of plant growth.

The time of CCC application is also an important factor which determines the degree of effectiveness of treatment. In the experiments on cereal crops so far reported, Tolbert (1960) treated winter and spring wheats, oats, barley and maize all at the two-leaf stage of development. Linser et. al. (1961) examined the effect of CCC on spring wheat treated at three weeks after the three leaf-stage and found that the plant height was reduced up to 24 percent compared with control. Linser and Kühn (1962) found that the best time for chemical application on wheat was either at the time of tillering or just before heading. Cooper (1963, personal communication) indicated that in Europe, usually two sprays of CCC have been used on cereals, but reducing it to one spray just prior to boot stage has been under consideration.

Experiments with the two barley varieties currently studied showed that both soil and spray applications were almost equally effective in reducing plant height when applied at two-leaf stage. Spray treatments delayed for a period of two weeks following the two-leaf stage of growth produced similar results to the early spray treatments, particularly at the higher concentration (10^{-1} and 10^{-2}M). The low concentration (10^{-3}M) however, had no effect when applied late (Fig. 4.4).

5.3 Effect of chemical on increasing drought tolerance

The possible value of CCC in improving the tolerance of plants to drought was pointed out by Lindstrom and Tolbert (1960). They observed that, when poinsettia and chrysanthemum plants growing under moisture stress were treated with CCC, they showed less tendency to wilt than untreated plants.

Halevy and Kessler (1963) reported that CCC-treated bean plants survived a much longer period of time than did controls on the soil with moisture level at or just below the wilting point. They also measured both fresh and dry weight of plants grown under predetermined moisture conditions (wilting point, 20 and 95 percent available water) and found that treated plants had a greater weight in comparison with untreated plants.

The system of controlling soil moisture on the basis of water regime has been described (Hudson, 1957). As he pointed out, this system is a satisfactory method of studying the relationship between plants and their water requirements, provided the roots are allowed to become well established and are uniformly distributed throughout the plant container prior to commencement of studies. Vaadia et al. (1961) pointed out that although "available moisture" has been defined as the difference between field capacity and wilting point, plants may use considerable amounts of water from soil having moisture contents above field capacity or possibly even some water below the wilting point.

In the present studies, treated barley plants under the low water regimes remained turgid and were more vigorous than control plants. The dry weight of CCC-treated plants was shown to be less than that of controls under high soil moisture regimes, while under wilting point average weights of the two populations were comparable (Table 4.4). Furthermore, from the measurements of actual water consumption, it was evident that the heaviest concentration of CCC used in this study (2000 p.p.m.),induced a reduction in the water consumption of treated plants grown on both a heavy and a light-textured soil. This reduced water usage moreover, was apparent at both soil moistures maintained throughout the course of study (10% and 50% available water). If the practical value of treatments with CCC for the induction of drought tolerance, is to be realized, present results pertaining to the experiment conducted on light soil is of particular interest. Such results indicate that the greatest reduction in water consumption occurred during a period from the seedling stage of the plant to flowering. After this time water consumption per plant approximated that of controls, a condition which might be explained on the basis of the tendency for treated plants to undergo an accelerated growth rate in relation to normal plants.

It is interesting to note that similar results were obtained by at least one investigation carried out with plants cultivated <u>in vitro</u>. Gohlke and Tolbert (1962) reported that CCC decreased the water uptake in young barley seedlings grown in nutrient solution. Water uptake as measured by potometer studies was reduced to 60 to 80 percent of that of control plants with 10^{-4} to 10^{-3}M concentrations of CCC. Water translocation, as measured by the quantity of xylem exudate which could be collected after decapitation, similarly was decreased.

5.4 Effect of the chemical on yield

Humphries (1963) found that in addition to its characteristic dwarfing effect , CCC increased the leaf area of treated plants. He also suggested that decrease in internode growth possibly increased the meristematic activity in leaf primordia, producing more cells per leaf.

Decreased internode length and plant height in barley resulting from these same treatments, also possibly affects leaf area. On the basis of observation, this assumption appeared valid although no actual leaf area measurements were made. All investigators agree that CCC results in plants developing dark green leaves. Furthermore, some findings from work carried out with tobacco (Humphries 1963) suggest that the chlorophyll content per leaf as well as per gram fresh weight was increased by treatment. With the important role which leaf size, particularly flag leaf size is believed to play in governing yield in cereals, at least part of the yield increase observed in the present study possibly may be explained on the basis of an increased leaf area resulting from treatment.

In the present study, the effect of CCC on the component parts of yield was examined. As explained by Grafius et.al.(1952) total grain yield of a cereal plant may be considered as representing the volume of a box, the three dimensions of which being (1) number of tillers per plant, (2) number of seeds per spike, and (3) weight per 1000 kernels. Increasing any one dimension while holding the other two constant, theoretically should result in an increased volume (yield). Examining the results of the present study, it is found that in the first of two experiments in which yield data were obtained, CCC-treatment significantly increased yields per plant. This effect was most consistently marked with the highest concentration (2000 p.p.m.), applied to plants growing under a 50% level of available water. Analysing in turn each of the three "components" of yield, it was evident that the one factor contributing to the improved yield returns was the increase in the number of seeds obtained from treated plants. Neither the number of fertile tillers nor seed weight appeared to be positively affected.

Unfortunately, some sterility occurred in all plants grown in this experiment. The exact cause of this abnormality cannot be ascertained although it is known barley is hypersensitive to temperature during the time of flowering. If during this critical period the movable bank of fluorescent tubes under which the entire experiment was conducted was inadvertently brought too close to the flowering spikes, partial sterility could be induced. Furthermore, despite the fact that the position of both treated and control plants were rotated weekly under the light bank, a differential effect of temperature on the treated populations was possible. Plants which received the high concentration (2000 p.p.m.) were reduced in height compared with other treatments and therefore if temperature is the factor responsible for the partially sterile condition, the shortened plants would receive a reduced amount of heat energy, and would not be damaged to the same degree.

A second attempt (Experiment No. 5) to obtain a more reliable estimate of CCC on yield under controlled condition produced results which it is thought are more reliable. Unlike the previous investigation, the level of fertility in this experiment was high. From the results (Table 4.17) similar trends to those observed in the previous experiment, were obtained. The highest concentrations (particularly 10^{-1} and 10^{-2}M) applied either as a soil drench or as a late spray (early boot stage) increased the yields of both Parkland and Hannchen.

A field scale experiment in which CCC was applied in various concentrations to both Parkland and Hannchen barley is also presently underway. It is hoped that with the data obtained under natural field conditions, the investigations carried out under controlled conditions and as reported herein will be corroborated. In addition, the field experiment will provide an adequate supply of seed for a complete analysis of the effect of CCC on the components of quality (grain nitrogen, wort nitrogen, malt extract, and saccharifying activity).

6. SUMMARY

1. The effects of (2-chloroethyl) trimethylammonium chloride on the growth patterns of two varieties of barley, including height, maturity, drought tolerance and yield, were studied.

2. Soil application of the chemical was found to be more effective than seed and spray treatments.

3. Treated plants appeared to have thicker stems, shorter and broader leaves with darker green color. These same plants were later and had higher number of tillers in comparison with control plants.

4. The chemical considerably reduced the plant height for some period of time, after which treated plants tended to recover their normal height. Reduced height appeared to have resulted from decreased length of lower internodes in the plants.

5. Differential response was found in height alteration by the chemical between two varieties of barley, Hannchen and Parkland. Hannchen being less responsive.

6. Spray application of high concentration (10^{-1} and 10^{-2}M) at both two-leaf stage and at two weeks later produced similar effects on decreasing the plant height, while lower concentration (10^{-3}M) was slightly effective only when applied at an early stage (two-leaf).

7. Water consumption of treated plants, particularly with high concentration (1.26×10^{-2}M) was decreased considerably when grown on a heavy soil, but on a lighter soil the reduction was negligible.

8. Concentrations of ($1.26 \times 10^{-2}M$) and higher increased the yield of barley plants. Increased number of seeds per head in one experiment was found to be responsible for yield increase.

7. LIST OF REFERENCES

Abdallah, A.K., and A.A.M. Omar, 1957. A study of the relation of some morphological character to lodging barley. Ann. Agr. Sci. Cairo, 2: 243-247.

Audus, L.J., 1959. Plant growth substances. Leonard Hill (Books) Limited, London.

Bukovac, M.J., 1962. Modification of the vegetative development of apple seedlings and one-year-old cherry trees with Gibberellin A_3 and (2-chloroethyl) trimethylammonium chloride and related compounds. Advancing Frontiers of Plant Sciences 1: 7-22.

Cathey, H.M. and N.W. Stuart, 1961. Comparative plant growth-retarding activity of AMO-1618, PHOSPHON and CCC. Bot. Gaz. 123(1): 51-57.

__________ and R.L. Taylor, 1963. Growth control of poinsettias by use of cyclic lighting and (2-chloroethyl) trimethylammonium chloride. Amer. Soc. Hort. Sci. Proc. 82: 532-540.

Gohlke, A.F. and N.E. Tolbert, 1962. Effect of (2-chloroethyl) trimethylammonium chloride on phosphate absorption and translation. Proceedings of the plant physiology meetings. Plant Physiol. 37 (Suppl.) xii.

Grafius, J.E., W.L. Nelson and V.A. Dirks, 1952. The heritability of yield in barley as measured by early generation bulked progenies. Agron. J. 44: 253-257.

Halevy, A.H. 1962. Inverse effect of Gibberellin and AMO-1618 on growth, catalase and peroxidase activity in cucumber seedlings. Experimentia 18: 74-76.

__________ and B. Kessler, 1963. Increased tolerance of plants to soil drought by means of growth-retarding substances. Nature 197 (4864): 310-311.

Hudson, J.P., 1957. Control of the plant environment. Butterworths Scientific Publications (London).

Humphries, E.C. 1963. Effects of (2-chloroethyl) trimethylammonium chloride on plant growth, leaf area, and net assimilation rate. Annals of Botany, new series 27(107): 517-531.

______________ and A. W. Wheeler, 1963. Quarternary ammonium compounds. Ann. Rev. of Plant Physiol. 14: 404.

Joiner, J.N. and R.T. Poole, 1962. Variable photoperiod and CCC effects on growth and flowering of Gardenia jasminoides "Veitchii" Proc. Florida State Hort. Soc. 75: 449-451.

Kelley, A.G. and S.N. Postlethwait, 1962. Effects of (2-chloroethyl) trimethylammonium chloride on fern gametophytes. Amer. J. Bot. 49(7): 778-786.

Kofranek, A.M., R.H. Sciaroni, and T.G. Byrne, 1962(a). Shortening poinsettias with CCC for proportioned potted plants. Calif. Agric. 16(12): 4-5.

____________, ____________, and Y.J. Kubota, 1962(b) Dwarfing carnations with CCC for pot plant sales. Calif. Agric. 16(9): 12-13.

____________, ____________, and __________, 1963. The dwarfing effect of CCC on carnation plants. Carnation Craft 60: 2-4.

Krewson, C.F., 1959. Synthesis and biological activity of some quarternary ammonium and related compounds that suppress plant growth. J. Agr. Food Chemistry 7: 264-268.

Kuraishi, S. and R.M. Muir, 1963. Mode of action of growth retarding chemicals. Plant Physiol. 38(1): 19-24.

Lindstrom, R.S., 1961. The response of floricultural crops to various growth regulators. I. Effect of (2-chloroethyl) trimethylammonium chloride on poinsettias, (Euphorbia pulcherima, Wild). Michigan Quarterly Bulletin 43(4): 839-847.

____________ and N E. Tolbert, 1960. (2-chloroethyl) trimethylammonium chloride and related compounds as plant growth substances. IV. Effect on chrysanthemums and poinsettias. Michigan Quarterly Bulletin 42(4) 917-928.

Linser, H. and Kühn, H, 1963. The rate and timing of (2-chloroethyl) trimethylammonium applications as a means of reducing straw length. Z.F. Pflanzenernahr, Dungung, Bodenk, 101(3): 206-210.

________, H.H. Mayer and G. Bodo. 1961. The effects of CCC on summer wheat; preliminary report. Bodenkulture 12(4): 279-280.

Lockhart, J.A., 1961. Physiological mechanism of action of stem growth inhibitors. Plant Physiol. 36: (Suppl.)38.

Mann, H.O., 1953. Height inheritance in barley. M.Sc. Thesis, Colorado State Univ.

Marth, P.C. and Frank J.R., 1961. Dwarfed soybeans gain salt tolerance. Agric. Res. Service U.S.D.A. 10(4): 5.

Miyamoto, T. 1962. Increasing the resistance of wheat to high concentrations of salt by treating the seed with (2-chloroethyl) trimethylammonium chloride. Naturwissenschaften 49(9): 213.

__________., 1962. Effects of the seed treatment with (2-chloroethyl) trimethylammonium chloride on the resistance to high and low pH values of soil in wheat seedlings. Naturwissenschaften 49(16): 377.

Nilan, R.A., 1964. The cytology and genetics of barley 1951-1962. Washington State Univ. 32(1).

Sachs, R.M., and Lang, A., 1961: Shoot Histogenesis and the Sub-Apical Meristem: The Action of Gibberellic Acid, AMO-1618 and Maleic Hydrazide. In Plant Growth Regulation. Iowa, 567.

Stuart, N.W., 1961. Initiation and development of flower buds in Rhododendron following application of growth retardants. Plant Physiol. 36: (Suppl.) liv.

__________ and H.M. Cathey, 1961. Learning about growth regulators. Agr. Res. Ser. U.S.D.A. 9(10): 10-11.

Tissen, H., 1962. The influence of various temperatures and (2-chloroethyl) trimethylammonium and (allyl) trimethylammonium bromide on peppers and tomatoes. Can. J. Plant Sci. 42: 142-149.

Tolbert, N.E., 1960(a). Effect of (2-chloroethyl) trimethylammonium chloride and related compounds upon plant growth. Federation Proceedings 19: 328.

__________, 1960(b). (2-chloroethyl) trimethylammonium chloride and related compounds as plant growth substances. II. Effect on growth of wheat. Plant Physiol. 35: 380-385.

__________, 1960(c). (2-chloroethyl) trimethylammonium chloride and related compounds as plant growth substances. I. Chemical structure and bioassay. J. Biol. Chemistry, 235(2): 475-479.

__________ , 1961(d). (2-chloroethyl) trimethylammonium chloride and related compounds as plant growth substances. In Plant Growth Regulation. Iowa State Univ. Press.

Vaadia, Y., F.C. Raney, and R.M. Hagan, 1961. Plant water deficits and physiological processes. Ann. Rev. of Plant Physiol. 12: 245-292.

Wirwille, J.W. and J.W. Mitchell, 1950. Six new plant growth inhibiting compounds. Bot. Gaz. 111: 491-494.

Wittwer, S.H. and N.E. Tolbert, 1960(a). (2-chloroethyl) trimethylammonium chloride and related compounds as plant growth substances. III. Effect and growth and flowering of the tomato. Amer. J. Bot. 47(7): 560-565.

____________ and ____________, 1960(b). (2-chloroethyl) trimethylammonium chloride and related compounds as plant growth substances. V. Growth, flowering and fruiting responses as related to those induced by auxin and Gibberellin. Plant Physiol. 35: 871-877.

____________ and ____________, 1960(c). Comperative effects of CCC with Gibberellin. Amer. J. Bot. 47: 560-563.

Zeevaart, J.A.D., 1964. Effects of the growth retardant CCC on floral initiation and growth in <u>Pharbitis nil</u>. Plant Physiol. 39(3): 402-408.

____________ and A. Lang, 1963. Suppression of floral induction in <u>Bryophyllum daigremontianum</u> by a growth retardant. Plants 59: 509-517.