THE EFFECTS OF CYCOCEL (CCC) ON TOMATO UNDER WATER STRESS

by

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STATEMENT

This thesis has not been previously submitted for a degree at this or any other University, and is the original work of the writer except where due reference is made in the text.

(SOLOMON AMOABIN)

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SUMMARY

CCC (2-chloroethyltrimethyl ammonium chloride) a synthetic plant growth retardant, has been previously found to increase the resistance of various plants to drought. Work done on this subject mostly attributes the CCC effect to reduced leaf area and increased root/shoot ratios due to the retardation of shoot growth though there are a few cases where CCC did not retard shoot growth but still induced drought resistance. A few workers have mentioned the involvement of CCC-induced stomatal closure in the drought resistance of CCC-treated plants. Some workers have associated the CCC-induced drought resistance with metabolic changes of CCC per se, and CCC-mediated metabolic changes in plants under water stress, without mentioning how these changes are related to the CCC-induced drought resistance.

This study was conducted to determine whether CCC affected the drought resistance of tomato, which is relatively sensitive to water stress, and, if so, to explore some of the possible mechanisms underlying the effect. Attention was directed towards the effects of CCC on the tomato plants under stress independent of its retardation effect on growth.

At a concentration of 1000ppm, CCC retarded the growth of the tomato plants 6 days after its application as soil drench. CCC reduced height, leaf area, fresh and dry weights of leaves and stem, without any retardation effect on root growth, resulting in an increased root/shoot ratio. Whether CCC retarded the growth of the plant or not before inducing water stress, the CCC-treated plants maintained higher water potential especially within the first few days after with-holding water, such that they did not wilt as quickly as the non-CCC-treated plants. Despite this prolonged survival under water stress due to CCC treatment, growth was not sustained. When PEG was used to induce stress, CCC did not have any effect on water potential and it could not reduce the toxic effect of PEG, in the form of leaflet margin chlorosis.

Under soil water depletion induced by with-holding water from the plants, RWC was higher in the CCC-treated plants but osmotic potential did not decrease as much in the CCC-treated plants as the non-CCC-treated plants. The relationships between water potential and RWC, osmotic potential and turgor potential were not altered by CCC which indicated that CCC did not enhance the treated plants' ability to adjust osmotically. This was supported by the apparent lack of effect of CCC treatment in promoting solute accumulation.

In normal well-watered plants, CCC caused a rapid differential increase in adaxial leaf diffusive resistance but not in abaxial resistance indicating a CCC-induced closure of the adaxial stomata, independent of its effects on growth.

This was consistent with a marked decrease in transpirational water loss from the adaxial leaf surface of the CCC-treated plants. Water stress *per se* (induced by with-holding water from the plants) also caused stomatal closure but this was quicker in the non-CCC-treated plants than in the CCC-treated plants. The same water potential threshold was found for stomatal closure and effective control of further water loss under stress, and this was unaffected by CCC. This indicated that, because of the initial CCC-induced adaxial stomatal closure and the concommitant reduced transpiration, the water potential of the CCC-treated plants declined less rapidly as the stress progressed, and that the time required to reach the water potential threshold for stomatal closure and effective control of water loss was prolonged by the CCC treatment.

It was concluded that, independent of its growth retardation effect, CCC enabled plants to delay the onset of severe internal water deficit and, therefore, prolonged survival through CCC-induced adaxial stomatal closure and the attendant decreased transpirational water loss. This did not seem to involve any CCC-induced osmotic adjustment. When CCC retarded growth, however, the increased root/shoot ratio and the reduced leaf area could be additional factors contributing to the CCC-induced resistance to water stress.

ABBREVIATIONS AND SYMBOLS

ABA	abscicic acid
ATP	adenosine triphosphate
^{14}C	radioactive carbon
Ca	calcium
^{o}C	degrees centigrade
CCC	cycocel
cm	centimetre(s)
cm^2	square centimetre(s)
CO_2	carbon dioxide
D_2O	deuterium oxide
dm^2	square decimetre(s)
F.C.	field capacity
Fig.	figure
g or gm	gram(s)
H^+	hydrogen ion
HCl	hydrochloric acid
H_2O	water
hr(s)	hours
i.e.	that is
ins.	inches
K	potassium
K^+	potassium ion
KCl	potassium chloride
kg	kilogram(s)
l	litre(s)
MeOH	methanol
Mg	magnesium
M.W.	molecular weight
MWC	methanol : chloroform : water
$\mu E.m^{-2}s^{-1}$	microeinstein per square metre per second
µg	microgram(s)
µl	microlitre(s)
mg	milligram(s)
ml	millilitre(s)
mm	millimetre(s)

N_2	nitrogen
N	normal
NMR	nuclear magnetic resonance
No.	number
P	phosphorus
^{32}P	radioactive phosphorus
PEG	polyethylene glycol
ppm	parts per million
^{86}Rb	radioactive rubidium
rpm	revolutions per minute
RWC	relative water content
Sec.	second(s)
Si	silicone
t	time
t-buOH (tert-BuOH)	tertiary butanol
Δt	change in time
>	greater than
%	percent
ψ	water potential
ψ_s	osmotic (solute) potential
ψ_p	turgor potential
ψ_m	matric potential

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CHAPTER I

INTRODUCTION

I.1 CCC AND PLANT GROWTH

I.1.1 The use of cycocel in general agriculture

The ultimate goal of Plant Breeders is to incorporate as many desirable characteristics as possible into a given plant. While this feat is really difficult to achieve, some of the desired characteristics can be produced chemically, through the application of plant growth regulators.

Cycocel (2-chloroethyltrimethyl ammonium chloride) is a synthetic plant growth regulator and, more specifically, a plant growth retardant. So far, knowledge available indicates that it is used commercially on ornamental crops such as poinsettias and azaleas to produce desirable plants, on cereals especially wheat to prevent lodging in many cases and increase yield as well in some cases and on some varieties of grape to enhance fruit set.

From the literature, there are potential uses of cycocel on a number of fruit, vegetable, field and other miscellaneous crops which include the following:

(i) Ultimate increase in yield
(ii) Compact plants
(iii) Promotion of flowering
(iv) Resistance to certain insects and plant diseases

These potential uses of cycocel will be outlined in the literature.

Furthermore, owing to the versatility of the chemical in the sense of species and varietal responsiveness, time of application, dosage, method of application and interaction with environmental conditions, it opens a broad spectrum for research work. Though quite a substantial amount of work has already been done, there is still the need for a lot more to properly explain some of the morphological, physiological, anatomical and biochemical changes in plants attributed to the chemical and possibly open new areas of usage.

I.1.2 Physical, chemical and physiological properties of cycocel

The physical and chemical properties of CCC are presented in Table I.1. However, attention must be drawn to the naming of the compound. It has a trade name of *Cycocel* and a generic name of *chlormequat*; the chemical names being 2-chloroethyltrimethyl ammonium chloride or chlorocohline chloride - commonly abbreviated as CCC. For convenience, the chemical will be referred to as CCC throughout this thesis.

The first observable effect due to CCC treatment on plants is intense greening of leaves. This precedesthe shortening of stems and overall reduction in plant size as reported by Tolbert (1960b). General responses of plants as a result of treatment with CCC include the following:

(i) Sturdier and more compact plants (Wittwer and Tolbert, 1960; Tolbert, 1960b).

(ii) Shortened and thickened internodes and in some plants general reduction in plant stature (Cathey, 1964; Tolbert, 1960b).

(iii) Intense green leaves (Wittwer and Tolbert, 1960; Tolbert, 1960b).

(iv) Lodging resistance and increase in yield in some cereals grains (Humphries, 1968)

(v) Resistance to environmental stresses, e.g. drought and salinity (Halevy and Kessler, 1963; El Damaty *et al.*, 1964)

(vi) Promotion of flower bud initiation and development (Cathey, 1964)

(vii) Resistance to certain insects and diseases (Tahori *et al.*, 1965a & 1965b).

I.1.3 CCC (and other compounds) as plant growth retardants

CCC falls within the category of chemicals designated *"Plant Growth Retardants"* (Cathey, 1964; Tolbert, 1960a). These are synthetic organic compounds which do not occur naturally in plants but when applied exogenously slow cell division and elongation in the shoot tissues, and regulate plant height physiologically, and indirectly affect flowering without malformations of the plants (Cathey, 1964). Thus growth inhibitors (e.g. maleic hydrazide) auxins, herbicides and germination inhibitors which ultimately stunt or completely suppress the growth of plants and/or cause visible malformations of the leaves, stems and flowers of the treated plants are excluded from the growth retardant group.

TABLE I.1: Physical and Chemical Properties of CCC

Characteristic	Effect
Chemical names	(i) 2-chloroethyltrimethyl ammonium chloride (ii) Chlorocholine chloride (CCC)
Generic name	Chlormequat
Empirical formula	$C_5H_{13}Cl_2N$
Molecular weight	158.1
Solubility	
(i) Water	Complete
(ii) Methanol	Complete
(iii) Ether and other hydrocarbons	Insoluble
Hygroscopicity	High
Melting point	245°C
Odor	Fish-like (typically amine)
Physical form	White crystalline solid
Persistence in soil	3-4 weeks
Time to noticeable response after treatment	1 week or less
Spray application	Relatively effective
Soil application	Effective
Plant spectrum	Many plants
Toxicity	Yellowing around veins at high concentration

The chemicals referred to as growth retardants can be grouped under different families with specific structural requirements for activity. As summerized by Cathey (1964), these include:

(i) *Nicotiniums* - e.g. 2,4-DNC (Fig. I.1, compound 1) which require chloride substitution for activity (Mitchell *et al.*, 1949)

(ii) *Quaternary ammonium carbamates* - e.g. AMO-1618 (Fig. I.1, compound 2) in which the reduction of any of the basic moieties, namely the carbamate nitrogen, the terpene ring, the quaternary nitrogen and the halide salt, removes activity (Wirwille and Mitchell, 1950)

(iii) *Hydrazines* - e.g. BOH (Fig. I.1, compound 3), in which the only requirement for activity is the C-C-N-N chain (Gowing and Leeper, 1955)

(iv) *Phosphoniums* - e.g. Phosphon D and Phosphon S (Fig. I.1, compounds 4a and 4b); the tributyl quaternary phosphonium cation of this group is indispensable for activity and the benzene ring requires a small nucleophilic and non-ionizable substituent in the 4-position (Pretson and Link, 1958).

(v) *Substituted cholines* - e.g. CCC (Fig. I.1, compound 5), the trimethyl quaternary ammonium cation is necessary for activity in this group. These compounds are analogs of choline with the general formula $CH_2X\text{-}CH_2\text{-}N\text{-}(CH_3)_3$ and for activity, a small nucleophilic and non-ionizable substituent at X is required. CCC is the chloride salt, however the bromide salt is also active (Tolbert, 1960[a]).

(vi) *Substituted maleamic and succinamic acid* - e.g. CO11 and B995 (Fig. I.1, compound 6). This group differs from the others in that it does not contain a benzene ring, quaternary ammonium or phosphonium cation or small nucleophilic and non-ionizable substituents (Cathey, 1964).

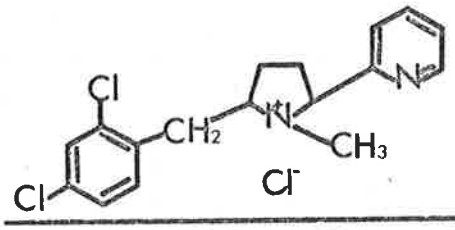
I.1.4 Effects of CCC on plant growth

I.1.4.1 *Specificity*

Responsiveness of plants to plant growth retardants is unrelated to taxonomic classification. Species vary in their response to various growth retardants (Cathey and Stuart, 1961), and even different cultivars of the same species vary in their responsiveness to the applied chemical (Cathey, 1964). As compared to AMO-1618 and phosphon, many plant species

FIG. I.1: Structural representation of some plant growth retardants (Cathey, 1964).

1)	2,4 DNC	[1-(2,4-dichlorobenzyl)-1-methyl-2-3-pyridyl, pyrrolidinium chloride]
2)	Amo-1618	[4-Hydroxyl-5-isopropyl-2-methylphenyl trimethylammonium chloride, 1-piperidine carboxylate]
3)	BOH	[β-hydroxyethylhydrazine]
4a)	Phosphon-D	[2,4-dichlorobenzyl-tributylphosphonium chloride]
4b)	Phosphon-S	[2,4-dichlorobenzyl-tributylammonium chloride]
5)	CCC	[(2-chloroethyl) trimethylammonium chloride]
6)	B995-(B-nine)	[N-dimethylamino maleamic acid]



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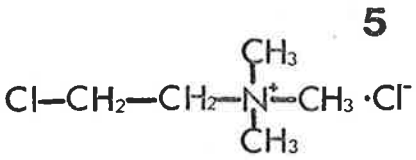

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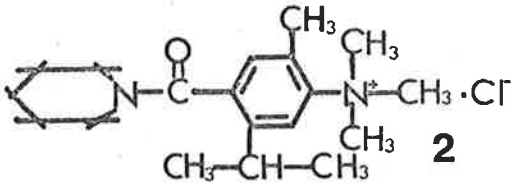

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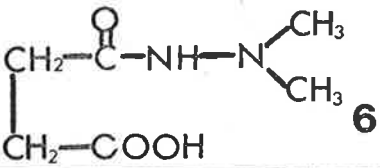

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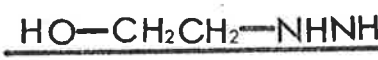

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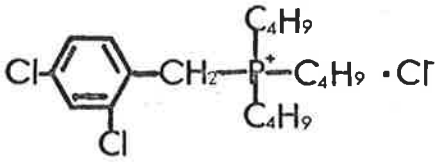

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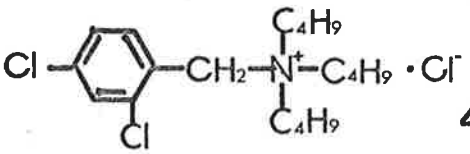


4b

are responsive to CCC. Out of 55 species tested by Cathey and Stuart (1961), only 6 were responsive to AMO-1618, 19 responsive to phosphon and 44 responsive to CCC. Cathey (1975) reported that out of 88 species tested, 5 responded to AMO-1618, 12 responded to phosphon and 21 responded to CCC. One thing peculiar with these growth retardants is that though CCC is effective on a broader spectrum of plants it does not necessarily follow that a plant responsive to AMO-1618 and/or phosphon will be responsive to CCC and *vice versa*.

Furthermore, the method of application of the retardant affects the responsiveness. CCC applied to dahlia as foliar spray was less effective than as soil drench (Bhattacharjee *et al.*, 1971). On wheat, soil application of CCC was more effective on light soil but on clay only spraying shortened the straw (Humphries, 1968).

Growth retardants are generally more effective on dicotyledoneous plants than monocotyledoneous plants (Cathey, 1964). Among cereal crops wheat has been very responsive especially to CCC; however, there is differential sensitivity among the varieties that are retarded by CCC.

I.1.4.2 *Effect of CCC on vegetative and reproductive growth*

As mentioned earlier the first, most easily observable response to CCC (as well as to other growth retardants) is intense greening of leaves and this is either attended with or followed by shortening and thickening of stems. Retardation of stem growth by CCC might be attributed to an inhibition of cell division in the subapical meristem as reported by Sachs *et al.* (1960). In wheat, Russel and Kimmins (1972) observed that CCC treatment decreased the total number of cells but the number of cells per unit fresh weight and per unit dry weight increased which suggested a decrease in cell size. They inferred, therefore, that CCC inhibited meristematic activity and cell elongation. Retardation of stem growth by CCC may be accompanied by an increased transverse growth. Sachs and Kofranek (1963) observed that CCC stimulated stem transverse growth in *Chrysanthemum* and this lateral expansion was attributed to the enlargement of cells. Zeevart (1965) reported that the diameter of pith parenchyma cells in *Pharbitis* seedlings were larger in CCC-treated plants than in untreated plants.

The effect of CCC on leaf growth is variable. A common visible effect is the intense green colour of leaves of CCC-treated plants. CCC reduced leaf area of sugar beet (Humphries and French, 1965), and tomato (Pisarczyk and Splittstoesser, 1979). Dyson (1965) showed that CCC

decreased the leaf area of potato and the higher the concentration of CCC the more the reduction in leaf area. CCC applied to *Phaseolus vulgaris* resulted in a reduction in leaf area which most probably was due to the reduction in the rate of expansion of leaves (Felippe and Dale, 1968). On the contrary, mustard plants treated with CCC responded by an increase in total leaf area which resulted from production of more lateral leaves or enlargement of stem leaves (Humphries, 1963). CCC did not have any significant effect on leaf lamina area in wheat (Humphries *et al.*, 1965).

Several reports indicate that CCC either retards root growth less than shoot growth or stimulates root growth. In wheat, CCC enlarges the root system to an extent which depends on the variety, as reported by Humphries (1968). According to Humphries, CCC increased root weight, area of absorbing surface and the amount of roots at all soil depths when applied to wheat. As usual with CCC, this proposed stimulation of root growth does not apply to all species. Not surprisingly, therefore, CCC retarded root growth at higher concentrations when applied to Norway Spruce (Dunberg and Eliason, 1972).

Generally, CCC retards the growth of various plants; nonetheless, this effect is not universal. In a few cases CCC, especially at low concentrations, stimulates growth, for example, in peas as reported by Adedipe *et al.* (1968). In snapdragon, Halevy and Wittwer (1965a) reported that a foliar spray with CCC increased stem height and dry weight of leaves and stem but soil application had no effect. Wünsche (1969) found that while a foliar spray of CCC stimulated stem elongation in snapdragon, soil application retarded growth even at low concentrations. However, in gladiolus, CCC applied as soil drench stimulated growth (Halevy and Shilo, 1970). CCC stimulated growth in lemons (Monselise *et al.*, 1966) and begonia (Heide, 1969), and, in tomato, an initial growth retardation due to CCC was followed by growth promotion (Van Bragt, 1969).

Application of CCC in certain cases caused suppression of vegetative growth and prompted flower initiation (Cathey, 1964). Wittwer and Tolbert (1960) reported that CCC applied to the roots of tomato promoted earlier flowering. Abdul *et al.* (1978) observed that CCC increased the number of flowers formed in the first inflorescence and also reduced flower abortion in tomato plants grown at high temperature with low light. Abdalla and Verkerk (1970) observed a reduction in flower drop and increased fruit-set and development in tomato plants treated with CCC through soil application. CCC drenches or sprays induced flower buds to form

earlier on azaleas than on untreated plants (Cathey and Stuart, 1961). Juntilla (1980) reported that CCC induced flower bud formation on immature plants of *Salix pentandra* as well as on cuttings from seedlings. Coombe (1965) showed that in some varieties of grapes, CCC increased the fruit set and cluster weight. In some species, for example in *Pharbitis nil*, CCC inhibits flowering as reported by Zeevart (1964).

I.1.5 CCC and nutrient content and uptake

Evidence available suggests that CCC treatment results in an increased concentration of macronutrients (N, P, Ca and Mg) but not K which is reduced (Robinson, 1975). Knavel (1969) also reported that tomato plants treated with CCC as foliar spray contained more N, P, Ca and Mg but less K than untreated plants. In a similar manner, young *Poinsettia* plants treated with CCC had more N and P but lacked K and Si (Crittendon and Kiplinger, 1969) and in pea plants, CCC treatment increased N, P and Mg in the vine but decreased K (Adedipe *et al.*, 1969).

El-Fouly *et al.* (1970) reported that CCC inhibited total ^{32}P uptake in cotton seedlings, and both foliar spray and addition to nutrient culture of CCC resulted in accumulation of ^{32}P in stem but decreased levels in the root. However, foliar spray increased, while addition of CCC to nutrient medium decreased, the ^{32}P content in the leaves. Contrary, Gholke and Tolbert (1962) found that the addition of CCC to the nutrient medium of barley seedlings resulted in a 3-4 fold increase in total ^{32}P uptake. This might have arisen from the several-fold increase in ^{32}P in the roots, but ^{32}P content in the leaves of CCC-treated plants was less than in untreated plants. Adedipe and Ormod (1972) reported that at high P rate, CCC did not have any effect on the total P uptake or on relative distribution in the leaf, stem and root; but at low P rate the root, relative to the leaves and stem, retained more P at 100mg/l CCC. Halevy and Wittwer (1965b) showed that CCC applied to the solution culture of bean plants resulted in a reduced mobility of ^{86}Rb to the upper stem but increased it to the roots; however, there was no effect on initial uptake when ^{86}Rb was applied to one of the expanded primary leaves. It stands to reason, therefore, that CCC affects the uptake, translocation and distribution of nutrients in different species differently though there is the general tendency for CCC to reduce the uptake of some nutrients.

I.1.6 Uptake and metabolism of CCC

It has been suggested that CCC could be taken up by plants either

through the leaves or roots (Dekhuijzen and Vonk, 1974; Lord and Wheeler, 1981; Birecka, 1967) though Blinn (1967) reported that it is absorbed slowly from foliar deposits on wheat. ^{14}C-labelled CCC applied to the leaf showed greater movement to the leaf tip, younger leaves, main stem and ear but less to the root in wheat than in barley and there was greater loss of labelled CCC in wheat than in barley.

According to Blinn (1967) ^{14}C-labelled CCC in wheat was not metabolized in that the labelled CCC was the only radioactive-labelled substance found in the wheat foliage, roots and grain. In support of this, Birecka (1967) found that CCC was not metabolized in wheat plants and that the increased amount of choline he observed in his work did not show any label from the ^{14}C-labelled CCC. This led him to infer that CCC might have blocked some enzymes involved in the metabolism of choline. On the other hand, Schneider (1967) showed that labelled choline and other labelled unknown metabolites resulted from labelled CCC-treated barley and *Chrysanthemum* shoots. Dekhuijzen and Vonk (1974) found that CCC was converted to choline which was further metabolized to betaine which, upon demethylation yields finally glycine and serine, they proposed that serine might have been formed from glycine with the evolution of $^{14}CO_2$ during photorespiration. The degradation of CCC in wheat to choline is also supported by evidence from El-Fouly and Jung (1969).

I.1.7 Mode of Action

Because CCC affects different species and even different cultivars of the same species differently, it has been difficult to propose a single well-defined mode of action for the chemical. Though it is quite logical for one to argue that CCC may act differently in different plants and under different conditions, the most popularly held view on the mode of action of the retardant involves its interaction with gibberellin.

Tolbert (1960b) and Wittwer and Tolbert (1960) observed that the effects of CCC on plants were opposite to those of gibberellin and these effects of CCC were reversed by gibberellin. It has also been found that CCC treatment resulted in the decrease of endogenous gibberellin in some plants (Jones and Phillips, 1967; Zeevart, 1966). Paleg *et al*. (1965) reported that the interference of CCC with the gibberellin might arise in five general ways, *viz:*

(i) Inhibition of the biosynthesis of endogenous gibberellin

(ii) Decrease in the level of compound or class of compounds on or with which gibberellin acts or reacts

(iii) Destruction or inactivation of gibberellin

(iv) An action preventing gibberellin from fulfilling it's primary or hormonal role

(v) Blocking of the physiological response of plants to gibberellins

The above authors favoured the first possibility - the inhibition of gibberellin biosynthesis - as the most suitable mode of action of CCC. Several studies lend support to the CCC inhibition of gibberellin biosynthesis. Lockhart (1962) reported that CCC partially blocks the system providing active gibberellins in bean (*Phaseolus vulgaris*) plants. Kende *et al.* (1963) reported that CCC completely inhibited formation of gibberellins in *Fusarium moniliforme* and this was extended by Ninnemann *et al.* (1964) who found that 10 ppm of CCC completely suppressed gibberellin production by the fungus and that the destruction of gibberellin by CCC was not observed. Further evidence came from Harada and Lang (1965), Cross and Meyers (1969) and Barnes *et al.* (1969) who located the CCC-inhibited steps in the gibberellin biosynthetic pathway.

Although the CCC-inhibition of gibberellin biosynthesis has been the most frequently reported mode of action of the chemical, there is evidence to show that it is not the only possible mechanism. In tomato plants, Van Bragt (1969) showed that CCC-treated plants contained slightly more gibberellin-like substances compared with control plants and, therefore, suggested that CCC did not inhibit gibberellin synthesis. Similarly, Halevy and Shilo (1970) reported of an increase in endogenous gibberellin in gladioli plants treated with CCC. Reid and Crozier (1970) observed, in pea seedlings, that CCC treatment resulted in an increase in gibberellin without there being any parallel stimulation of growth, which indicated that, in peas, the predominant factor in CCC-induced inhibition of stem growth may not be related to an effect of CCC on gibberellin biosynthesis. Devay *et al.* (1970) were of the opinion that, in *Phaseolus vulgaris*, the inhibition of the synthesis of giberellin-like substances was not the source of the physiological effect of CCC but, rather, the source could be an activation of a system which regulates the phytokinin level.

There are other modes by which the effects of CCC could be accounted for aside from the involvement of hormones. Heatherbell *et al.* (1966) suggested that in etiolated pea seedlings, CCC might act as an uncoupling agent, thus resulting in a block of the flow of energy into growth processes due to reduced mitrochondrial ATP production. Adedipe and Ormrod (1970) showed a decreased ATP level in pea tissue at low CCC concentration suggesting phosphate utilization as a regulatory site in the growth retarding effect of CCC.

Wittwer and Tolbert (1960) noted that the structural similarity of CCC to choline suggested a possible function in lipid metabolism and the

specificity of methyl groups in the ammonium cation implied that ^{14}C-labelled CCC was partially incorporated into phosphatidylcholine and lysophosphatidylcholine. Douglas (1974) found that CCC inhibited sterol biosynthesis in tobacco seedling at the squalene -2,3-epixode cyclase step.

Stoddart (1964) reported that CCC uncoupled assimilation and growth in *Lolium temulentum* plants. His evidence confirmed that there was a preferential conversion of carbohydrate to amino acids while nitrogen was adequate but fructosan was formed while nitrogen was inadequate. He therefore inferred that polymerization of free sugars, which should have been utilized to sustain growth, to storage polysaccharides occurred in the presence of CCC. From his work, he visualized a metabolic scheme (Fig. I.2) showing sites at which CCC might block plant metabolism.

I.1.8 CCC-induced resistance to pests and diseases

In some plants, CCC treatment results in their resistance to some insect pests and diseases. Tahori *et al.* (1965a) reported that cotton leaf-worm larvae did not cause as much destruction on bean plants sprayed with CCC as compared to the untreated plants. The same authors (1965) found that stem bases of oleander held in CCC solution were less infested with aphids as compared with the control. Van Emden (1964) observed that the populatin of cabbage aphid on brussel sprout was reduced on the plants treated with CCC as a soil drench.

CCC showed significant control of bacterial spot due to *Xanthomonas vesicatoria* on pepper plants (Crossan and Fieldhouse, 1964). Rawlins (1962) reported that CCC inhibited the multiplication of tobacco mosaic virus (TMV). According to Sinha and Wood (1964) tomato plants treated with CCC showed a decreased level of verticillium wilt infection. Bean seedlings treated with CCC and infected with stem rot fungus (*Sclerotium roffsii*) were less diseased than the untreated plants (Tahori *et al.*, 1965b). Diercks (1965) reported that treatment of winter wheat with CCC prevented lodging and reduced the incidence of stalks infected with eye-spot disease.

I.2 PHYSIOLOGY OF PLANT WATER STRESS RESISTANCE

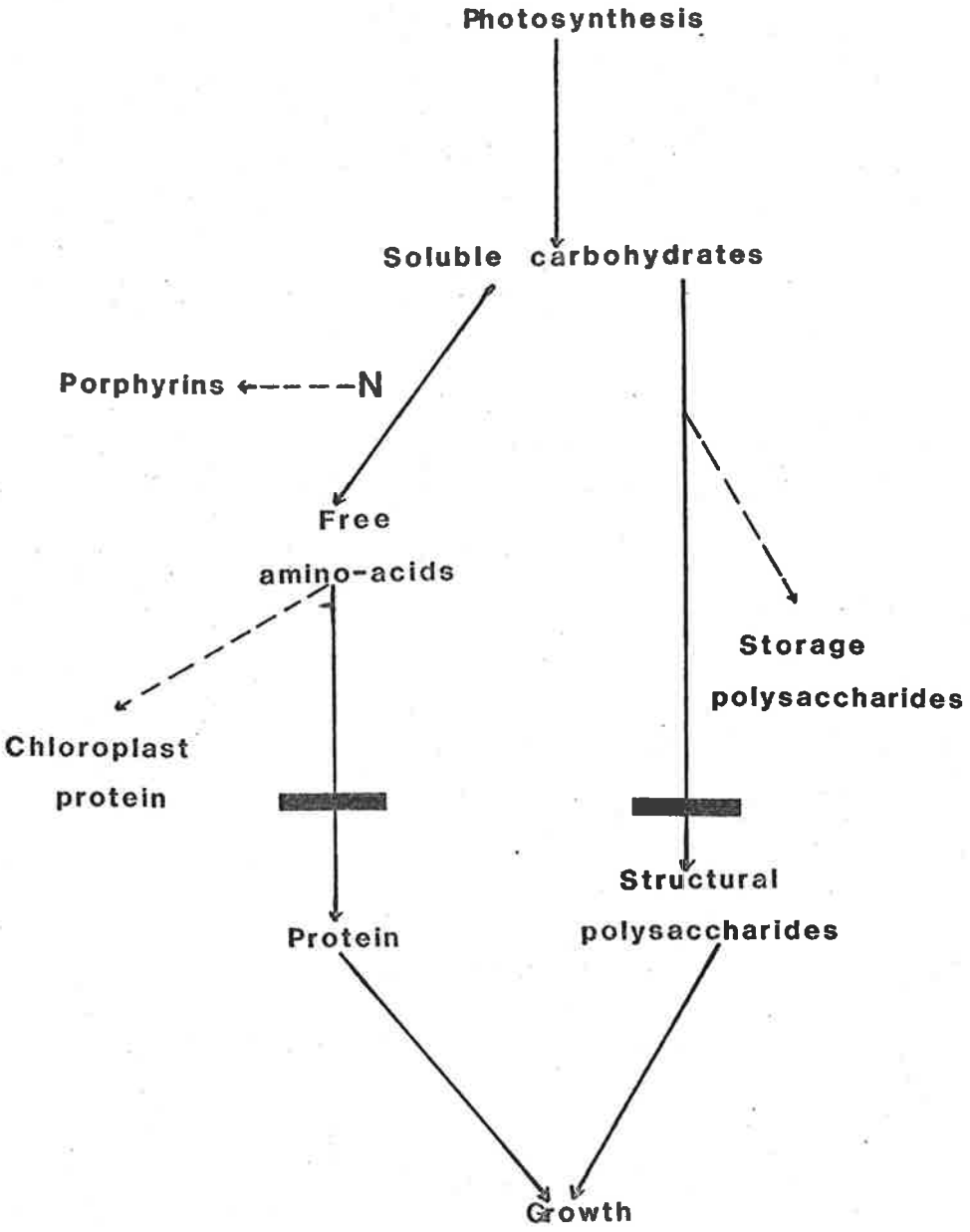
I.2.1 Development of water stress

The development of water stress in plants is dependent on the balance between the rate of water loss from the plant to the atmosphere and

FIG. I.2: Metabolic scheme for the mode of action of CCC by Stoddart (1964).

Possible position of CCC-induced metabolic block

---▸ Diversion pathway


Photosynthesis
Soluble carbohydrates
Porphyrins ←----N
Free
amino-acids
Chloroplast
protein
Storage
polysaccharides
Protein
Structural
polysaccharides
Growth

the rate of absorption of water from the soil and its subsequent transport to the evaporating surface. The movement of water through the soil-plant-atmosphere continuum is in response to gradients in water potential, and to soil, plant and atmospheric factors which influence the development of water deficits in plants. Water reaching the root system of a plant depends on the root absorbing area, soil water potential gradients and soil capillary conductivity. Similarly, the absorption and transport of water is dependent on plant water potential gradients and root and stem conductivities. Thus any condition creating an imbalance in the soil-plant-atmosphere continuum is likely to cause the development of water stress (Kozlowski, 1968; Begg and Turner, 1976; Augustin *et al.* 1968). For example a high evaporative demand usually results in the plant undergoing stress, likewise an excessive flood. Also the application of PEG to the root zone usually results in a plant water stress condition.

Although water stress occurs when water loss exceeds absorption, this is not the only condition under which water stress occurs. Rather, water stress is an inevitable consequence of the flow of water along a pathway in which frictional resistance and gravitational potential have to be overcome (Jarvis, 1975). Thus, all actively growing, transpiring plants experience some degree of water stress but the degree to which the stress affects growth and development processes will depend largely on the degree and duration of the stress and the extent to which the plants can adapt to the stress (Hsaio, 1973; Begg and Turner, 1976). Even in well-watered soils the development of regular diurnal internal water stress in plants is shown by the mid-day depression of water potential and the concomittant decrease in transpiration due to stomatal closure (Kozlowski, 1968).

As water stress develops, many physiological changes take place in the plant to reduce further loss of water, or to cope with the increased water loss. Some of these changes will be highlighted below.

I.2.2 Indicators of plant water status

There are a number of methods, direct and indirect, to evaluate the plant water content. The most widely accepted and probably the most meaningful criterion for measuring plant water status is the water potential designated Ψ (Hsaio, 1973; Knipling, 1967; Barrs, 1968). It depicts the chemical potential energy of the water in the tissue and in any situation the net water potential is the sum of the hydrostatic pressure or turgor pressure, the osmotic or solute potential and the matric potential which is normally

negligible, as in the following equation $\Psi = \Psi_p + \Psi_s + \Psi_m$ where Ψ_p is the turgor potential, Ψ_s the osmotic potential and Ψ_m the matric potential (Salisbury and Ross, 1969; Milburn, 1979; Gardner and Ehlig, 1965). Ψ and Ψ_s normally are negative values and Ψ_p is generally positive; however, the existence of negative turgor has been challenged by Tyree (1976). In addition to Ψ, measurements of Ψ_s and Ψ_p might be useful in assessing plant water status.

Another method for determining plant water status is the Relative Water Content (RWC) (Weatherly, 1950; Barrs and Weatherly, 1962; Barrs, 1968). This parameter is the ratio of the actual water content of the tissue to the water content at full turgor. RWC is related to Ψ of the same tissue and the relationship differs according to species (Connor and Tunstall, 1968; Sanchez-Diaz and Kramer, 1971; Shepherd, 1976), stages of growth and also environmental conditions (Knipling, 1967; Augustin *et al.*, 1968).

Other methods which may be useful include the measurements of stomatal aperture and resistance and visual symptoms such as wilting.

I.2.3 Mechanism of water stress resistance

Plants do adapt to withstand periods of limited soil water or drought by several means. The ephemerals which produce seeds that can survive periods of dry weather and also complete their life cycle before a period of serious plant water deficit develops are referred to as drought escapers (May and Milthorpe, 1962; Turner, 1979). The drought escapers normally do not possess any special physiological, biochemical or morphological mechanism to withstand water deficit but survive during periods of water deficit by rapid phenological development or developmental plasticity (Jones *et al.*, 1981). Another category of plants endure drought by the loss of their leaves. This group includes semi-desert shrubs (Shantz, 1927). These two mechanisms are the means by which many plants which cannot conserve water, but still have to experience periods of dry weather, have adapted to survive (Parker, 1968).

The other mechanisms by which plants can withstand drought involve physiological, morphological or biochemical responses which enable them to either avoid or tolerate the drought. Current thinking distinguishes drought tolerance from drought avoidance (Fischer and Sanchez, 1979). Drought avoidance depicts the situation in which high plant tissue water potentials are maintained in the presence of environmental drought (Hall and Schulze, 1980;

Begg and Turner, 1976) and this may be due to morphological and physiological adaptations to maintain water uptake (increased rooting and increased hydraulic conductance) and to reduce water loss (reduction in epidermal conductance, absorbed radiation and evaporative surface). Drought tolerance on the other hand involves the ability of plants to perform at low tissue water potentials under water deficit. This incorporates the maintenance of turgor by either solute accumulation or an increase in cell wall elasticity (Jones *et al.*, 1981).

As far as this work is concerned, the mechanisms of drought avoidance and drought tolerance are of much interest and will be discussed further. However, it must be pointed out that whether drought tolerance or avoidance is the most appropriate mechanism for a given crop is subject to the degree and duration of water deficit and the crop under consideration.

I.2.4 Drought avoidance

Drought avoidance, as mentioned earlier implies drought resistance at high internal plant water status (Turner, 1979; Jones *et al.*, 1981).

One of the most important physiological mechanisms by which water loss is reduced in plants is through stomatal control. Various studies show that stomata tend to close or do not display maximum opening when plants are experiencing water deficit. Moreover, there appears to be a threshold water potential at which the stomata close but this varies with the type of plant in question (Hsaio, 1973). Since the stomata are the entry points of CO_2 into the leaf, the closure of the stomata would imply ultimate reduction in assimilation and consequently reduced productivity. Due to the importance of this discussion to the present work, the response of stomata to water stress will be elaborated later in the text. In addition stomatal resistance, cuticular resistance to water loss also contributes, in some cases very substantially, to the control of transpirational water loss. Blum (1975) reported that, as a result of increased epidermal waxes on an isogenic line of sorghum, there was a reduction in cuticular transpiration which led to better water status of that line. In many cases, though cuticular resistance can play an important part in the control of transpirational water loss, it may be deemed insignificant as compared to stomatal control (Parker, 1968). In addition, nonstomatal factors in the leaf referred to as "mesophyll" and "wall" resistances may contribute to the reduction in transpirational water loss.

Another strategy for reducing water loss is the reduction in the radiation absorbed. This normally can be achieved by active and passive

leaf movements or changes in the reflectance of the leaves. Leaves of some stressed plants may be orientated parallel to the incident radiation.

Alternatively the control of water loss may be in the form of reduced evaporative area. This may be the direct effect of the water stress on inhibiting leaf expansion, as suggested by Hsaio *et al*. (1976), Boyer (1970) and Acevedo *et al*. (1971). In many plants water stress induces rapid senescence and dying of older leaves which results in the reduction of the total leaf area and reduced water loss; in natural communities shedding of leaves and the attendant reduction in leaf area serves as an important way of adapting to water stress (Kozlowski, 1976; Turner, 1979; Jones *et al*., 1981).

Drought avoidance does not involve only the control of transpirational water loss but also the maintenance of water uptake. An increase in root density or root depth is a great asset to plants under water stress since this may mean that more water can be reached and extracted. Therefore, the root/shoot ratio may increase in plants under water stress. Turner (1979) provided evidence for an absolute increase in root dry weight for a variety of species and an increase in root weight may involve increased root density or increased root depth or both.

For the maintenance of better water status in the leaves under water stress condition, there is the need for low resistance to water flow from the roots to the leaves. Kramer (1969) and Boyer (1971) have observed that the main resistance to water flow occurs in the roots; however, the resistance to flow in the stems and leaves should not be overlooked (Dimond, 1966; Begg and Turner, 1970; Boyer, 1974). On the contrary, Passioura (1972) reported that under water stress an increase in the resistance to water flow in wheat was advantageous since it served as a means of metering the limited amount of water.

1.2.5 Stomatal response to water stress

Stomatal control of transpiration in plants provides the principal mechanism for reducing evaporative water loss from the leaves. For example, in the wilty tomato mutant flacca, the lack of the capacity of the stomata to close results in excessive transpiration and resultant wilting (Imber and Tal, 1970; Tal and Imber, 1970). Although the closure of stomata to counteract excessive or further loss of water is a useful mechanism of conserving water, it in-

evitably increases the diffusion resistance to CO_2 entering the leaf and may, therefore, reduce photosynthesis. Therefore, a plant experiencing water deficit can conserve water by stomatal closure and maintain high tissue water potential at the expense of photosynthesis, or maintain a low tissue water potential but adjust osmotically which may result in partial opening of the stomata and at least partially sustain photosynthesis.

The distribution of stomata may differ on the upper and lower epidermis of the leaf depending on the species and sometimes environmental factors. Leaves with stomata in both epidermes are termed amphistomatous whereas those with stomata in the lower epidermis only are termed hypostomatous (Meidner and Mansfield, 1968). For example, tomato leaves are considered amphistomatous (Hurd, 1969); however, there are normally more stomata on the abaxial (lower) surface than on the adaxial (upper) surface. Under high light intensity the percent increase in the number of adaxial stomata was higher than the abaxial though the abaxial surface still had a greater number of stomata per unit area (Gay and Hurd, 1975).

The opening and closing of stomata result from turgor differences between guard cells and the surrounding subsidiary or epidermal cells (Hsaio, 1973; Zelitch, 1969). Potassium has been found to be the major solute which accumulates in guard cells in light and effects the turgor required for the opening; the loss of potassium from the guard cells in the dark results in stomatal closure (Raschke, 1975; Ehret and Boyer, 1979; Hsaio, 1973).

Under water stress conditions, the regulation of stomatal aperture is the key determinant of water and CO_2 exchange. Even in well-watered plants it has been established that there are diurnal trends in stomatal conductance or diffusive resistance depending on the evaporative demand. The diffusive resistance tends to be high at dawn and decreases to a minimum at sunrise; it may be followed by a slightly higher resistance at mid-day in some cases or the resistance may remain low till sunset and then increase (Jordan and Ritchie, 1971; Meyer and Green, 1981). Data available suggest that under conditions in which water potential is declining, there is a threshold water potential below which leaf resistance increases sharply indicating stomatal closure. This threshold differs in different plants, it is about -7 to -9 bars in tomato (Duniway, 1971), -16 to -18 in cotton (Jordon and Ritchie, 1971; Brown *et al.*, 1976) and -11 to -12 bars in beans (Kanemasu and Tanner, 1969). It has also been reported that stress preconditioning displaced this water potential threshold for stomatal closure such that stomata closed at a lower water potential, and this was more pronounced in the abaxial stomata (Brown *et al.*,

1976; McCree, 1974). In addition, it has been found that stomatal sensitivity to decreasing leaf water potential varies between field conditions and controlled environments. Davies (1977) observed that in both cotton and soybeans the stomatal sensitivity to declining water potential was in the order of chamber-grown > greenhouse-grown > field-grown plants.

Adaxial and abaxial stomata differ in their response to water stress in some cases. In such cases, the abaxial stomatal diffusive resistance was lower at the water potential threshold than the adaxial stomatal diffusive resistance (Sanchez-Diaz and Kramer, 1971; Kanemasu and Tanner, 1969; Brown *et al.*, 1976). The behaviour of abaxial and adaxial stomata is also affected by other external stimuli like light, darkness and some chemicals. Kanemasu and Tanner (1969) showed that in snap beans, high light intensity resulted in higher adaxial resistance than abaxial. Nagarajah (1978) provided evidence that in darkness the adaxial stomata of cotton leaves were more or less effectively closed while the lower stomata were partially open and, in addition, the reduction in stomatal aperture which occurs with the increase in age of leaves commenced earlier in the adaxial stomata and proceeded at a faster rate than the abaxial stomata. Moreshet (1975) reported that phenyl-mercuric acetate applied to both abaxial and adaxial surfaces of sunflower plants caused greater closure of the stomata on the adaxial than on the abaxial surfaces. Pemadasa (1981) reported that the responsiveness of stomata to light, CO_2, KCl and ABA was substantially greater in abaxial than in the adaxial stomatal cells of *Commelina communis* and that fusicoccin was remarkably effective in stimulating adaxial stomata to open more than adaxial. Under normal conditions the abaxial stomata usually have lower resistance than the adaxial, which may be explained both in terms of stomatal density and stomatal opening.

Since stomatal movement is turgor-dependent the differences in abaxial and adaxial stomatal response may be explained in these terms. Brown *et al.* (1976) attributed such differences in preconditioned cotton plants to the fact that the guard cells of the abaxial stomata could lower their osmotic potential more than those of the adaxial stomata. This is supported by Pemadasa (1979) who found from histochemical tests that more K^+ accumulated in the abaxial guard cells than the adaxial ones. One can therefore infer that, in certain cases, the adaxial and abaxial stomata behave differently and independently.

It has been suggested that water stress affects stomata *via* ABA levels. In the tomato mutant, flacca, which wilts rapidly under water deficit

due to the inability of the stomata to close, application of exogenous ABA to intact plants reversed this wilting symptom (Imber and Tal, 1970). In addition, Tal and Imber (1970) observed that the concentration of endogenous ABA-like substances in flacca was about 10 times lower than in the normal plant. An increase in ABA levels in plants under water stress has already been established (Hsaio, 1973; Vaadia, 1976; Rasmussen, 1976; Hiron and Wright, 1973; Mizrahi *et al.*, 1970. Apart from triggering stomatal closure under water stress, ABA may induce the accumulation of proline (Stewart, 1980; Rajagopal and Andersen, 1978) which is important for plant water stress resistance.

Mention is also made of kinetin as affecting stomatal movement. Interacting with ABA, reduced levels of kinetin affected stomatal closure (Bengston *et al.*, 1978) and Tal and Imber (1970) found that kinetin might have a role to play in the excessive opening of the stomata of flacca tomato,

I.2.6 Chemical induction of stomatal closure

In addition to ABA there are other compounds which, when applied exogenously to plants, result in stomatal closure and reduced transpiration. Zelitch (1961) found that α-hydrosulfonates induced stomatal closure and reduced transpirational water loss at high light intensities without diminishing photosynthetic CO_2 assimilation in tobacco. Slatyer and Birhuizen (1964) reported that phenyl mercuric acetate caused a decrease in transpiration in cotton leaves which they associated with the increase in diffusive resistance of the leaf to water vapour transfer due primarily to stomatal closure. In addition, phenyl mercuric acetate caused a proportionately greater reduction in transpiration than photosynthesis. Zelitch and Waggoner (1962) reported that phenyl mercuric acetate sprayed on tobacco and maize leaves induced stomatal closure only on the surface sprayed. They inferred that the substance was not translocated from one side of the leaf to the other; however, the adaxial stomata closed more readily than the abaxial. Also, the chemical reduced transpiration relatively more than CO_2 assimilation. Mishra and Pradhan (1968 and 1972) showed that phenyl mercuric acetate, CCC, B-9 and 8-hydroxyquinoline induced stomatal closure in tomato and thereby reduced transpiration. Moreshet (1975) reported a greater closure of adaxial sunflower stomata than abaxial when phenyl mercuric acetate was applied to both sides. According to Milborrow (1979), phenyl mercuric acetate and farnesol induced stomatal closure in spinach leaves and this was accompanied by an increased accumulation of ABA. However Sabine and Dörffling (1981) found that ABA, phenyl mercuric acetate and farnesol all decreased stomatal aperture but the

ABA level after stomatal closure in the phenyl mercuric acetate treated leaves was not significantly greater in *Spinacia* and *Commelina*. They challenged Milborrow's (1979) proposition that phenyl mercuric acetate-induced closure of stomata was mediated through ABA. Squire and Jones (1971) reported that phenyl mercuric acetate also reduced CO_2 fixation in the mesophyll cells, and this inhibition of mesophyll photosynthesis jeopardises the use of phenyl mercuric acetate as a commercial antitranspirant.

I.2.7 Drought tolerance

The importance of maintenance of turgor pressure for the growth of plants has been emphasized by Hsaio (1973) and Hsaio *et al.* (1976). Turgor pressure influences many of the physiological, biochemical and morphological processes in the plant. Therefore, for a plant under water stress to be able to perform well, turgor pressure must be maintained as water potential declines. There are two mechanisms by which this can be accomplished; a lowering of osmotic potential (normally referred to as osmotic adjustment or osmoregulation) or a high tissue elasticity.

I.2.7.1 *Osmoregulation*

Under conditions of water stress where the plant water potential (Ψ) is declining, the plant can maintain its turgor potential (Ψ_p) if the other components - osmotic potential (Ψ_s) and matric potential (Ψ_m) also decrease ($\Psi = \Psi_p + \Psi_s + \Psi_m$). Since, as already mentioned, matric potential is normally negligible turgor can be maintained under declining water potential if the osmotic potential is lowered. The lowering of tissue osmotic potential in plants may result from the concentration of existing solutes during dehydration or by the uptake or internal production of solutes in the cell. The former does not maintain turgor but the latter - lowering of osmotic potential as a result of accumulation of solutes does (Hsaio *et al.*, 1976; Jones *et al.*, 1981; Turner, 1979). The capacity to adjust osmotically is different in different plants. Turner (1974) found that sorghum adapts better than maize and tobacco; it has lower osmotic potential at full turgor and this is supported by Sanchez-Diaz and Kramer (1973) who observed larger changes in turgor pressure in sorghum than maize during the development of water stress and after re-watering. Stress preconditioning results in better osmotic adjustment as reported by Jones and Turner (1978). Though sorghum can adjust osmotically better than other crops, mention has been made of osmotic adjustment

in cotton (Cutler and Rains, 1978), and sunflower (Turner *et al.*, 1978; Jones and Turner, 1980).

The diurnal pattern of osmotic potential with respect to the diurnal changes in water potential, has been discussed by Hsiao *et al.* (1976). Working with maize, these authors reported that the lowering of water potential at noon due to high evaporative demand was accompanied by a lowering of osmotic potential, and the diurnal variation of osmotic potential lagged behind that of water potential. This diurnal variation in osmotic potential maintained turgor despite the rapid decline in water potential at mid-day and thereby sustained growth.

Several major solutes such as free amino acids, soluble sugars, carboxylic acids, inorganic cations and anions together are responsible for the osmotic adjustment in plants. In wheat apices and enclosed leaves, osmotic potential in the first few days of stress was mainly due to an increase in the content of ethanol-soluble carbohydrate but later increases in carbohydrate concentrations and amino acids, mainly asparagine and proline, made the major contributions. In cotton, Cutler and Rains (1978) reported that sugars made only a small contribution to the osmotic potential but potassium, nitrate and malate contributed substantially. According to Jones *et al.* (1980) the decrease in osmotic potential at full turgor in fully expanded sorghum leaves at a moderate level of stress was accounted for by an increase in the concentration of sugars (glucose and sucrose), potassium and chloride. In sunflower, however, half of the decrease in osmotic potential at full turgor was accounted for by increases in the concentrations of the inorganic ions, potassium, magnesium, calcium, nitrate and free amino acids. In partly expanded sunflower leaves exposed to severe stress treatment, the inorganic anions, chloride and nitrate and to a lesser extent carboxylic acids (principally aconitate) and free amino acids made a significant contribution to the decrease in leaf osmotic potential at full turgor. In four tropical pasture plants, as reported by Ford and Wilson (1981), the inorganic ions, sodium, potassium and chloride were the most important solutes involved in the alteration of bulk tissue osmotic potential in the stressed leaves though proline and betaine accumulated differently in different species.

Osmotic adjustment has been found to influence stomatal closure under water stress conditions. According to Brown *et al.* (1976) the abaxial stomata of cotton leaves showed a lower water potential threshold for closure than the adaxial stomata and this was attributed to the guard

cells of the abaxial having lower osmotic potential than the adaxial suggesting that the abaxial guard cells adjusted osmotically better than the adaxial and therefore stayed open at reduced water potential.

I.2.7.2 *Tissue elasticity*

Tissues with high elasticity tend to maintain higher turgor pressure as the water potential declines than tissues with low elasticity. Increased elasticity results in a smaller decrease in water potential with relative water content and also lowers the relative water content at which zero turgor is reached (Turner, 1979). This relationship is exemplified by maize (Weatherly, 1970). Even if the increased elasticity does not maintain turgor it may be important in the survival of the tissues, particularly under negative turgor pressure (Jones *et al.*, 1981).

I.3.1 CCC (and other retardants) and drought resistance

Generally, plant growth retardants have been shown to enhance the ability of plants to withstand some environmental stress conditions - water stress, salinity, cold temperatures etc. Earlier work by Halevy and Kessler (1963) suggested that CCC and Phosphon increased the drought tolerance of bean plant. Martin and Lopushincky (1966) observed a better water status in the leaves and fruits of apples treated with B-995: sunflower plants treated with B-995 also had better ability to recover after a wilting period. In slash pine seedlings, Asher (1963) found that CCC application resulted in reduced water loss. Larter *et al.* (1965) found that CCC treated barley used less water for the production of a unit of dry matter. El Damaty *et al.* (1965) reported that CCC-treated wheat plants under drought conditions used water at their disposal more economically especially for the production of kernels. Plaut and Halevy (1966) found that CCC and B-995 enhanced the regeneration of tillers in wheat plants subjected to drought conditions. Evapotranspiration and its ratio to the yield of grain of wheat were significantly decreased by CCC under moisture stress (Farah, 1969). Plaut *et al.* (1964) found a decrease in the transpiration rate of CCC-treated bean plants under drought. Kharanyan (1967) reported a better water status in CCC-treated bean and kidney bean plants. Wheat seeds treated with CCC produced seedlings which withstood high and low pH of the soil in which they were growing (Miyamoto, 1962). Michniewicz and Kentzer (1965) showed that CCC application induced frost tolerance in tomatoes and this is supported by the work of Birecka and

Zebrowski (1966) who found that CCC-treated tomato plants survived cold temperatures which killed the untreated plants. CCC, Phosphon and AMO-1618 induced salt tolerance in soybean and enabled the treated plants to withstand, without visible injuries, amounts of commercial fertilizer, 5-10-5, that killed the untreated plants (Marth and Frank, 1961). El Damaty *et al.* (1964) showed an increase in tolerance to salinity and drought by CCC treated wheat plants.

The resistance of plants to drought and salinity, as a result of treatment with growth retardants, especially CCC, has been quite well documented; however, the mode by which the growth retardants effect this resistance is still not clearly known. It would not be surprising if, in different plants, the mechanism by which CCC confers drought resistance was different since CCC *per se*, affects different species and even different varieties of the same species, differently. Plaut *et al.* (1964) found a decrease in transpiration rate in bean plants treated with CCC and a decrease in top/root ratio (i.e. an increase in root/shoot ratio) which might have contributed to the survival of the CCC-treated plants under conditions of water stress. In wheat, CCC promoted root growth as contained in Humphries' report (1968), and this involved an increase in the amount of roots at all soil depths which would help to sustain the uptake of water from the soil under limited soil water condition.

CCC has been reported to induce stomatal closure or inhibit stomatal opening. Mishra and Pradhan (1967 and 1972) reported that foliar spray of CCC resulted in a reduction of stomatal width and reduced transpirational water loss, and delayed wilting of tomato under soil moisture stress. The reduction of stomatal aperture and an increase in stomata density due to CCC treatment in cowpea has been found by Imbamba (1972). Lovett and Campbell (1973) also reported an increased number of stomata per unit area and increased diffusive resistance in the leaves of sunflower treated with CCC. Pill *et al.* (1979) reported that CCC applied foliarly and as soil drench to plants under NH_4^+ nutrition increased the leaf diffusive resistance and reduced the water deficit caused by the ammonium fertilizer.

El-Fouly *et al.* (1971) found that CCC applied to cotton under water stress significantly increased protein nitrogen and chlorophyll content of leaves and also decreased the loss of yield due to water stress but the changes in cotton yield were independent of those which occurred in chlorophyll and protein nitrogen content. The protection of protein under drought conditions is also supported by CCC-pretreated bean plant having more protein

nitrogen in the leaves than the untreated plants, under drought (Kharanyan, 1969). Marth and Frank (1961) observed that the salt tolerance induced by CCC, AMO-1618 and Phosphon in soybean was not entirely due to reduction in leaf area. They observed that mites, which are sucking insects, multiplied more rapidly on leaves of the untreated plants suggesting a development of chemical and physical changes. Singh *et al.* (1973) reported that CCC enhanced the accumulation of free proline in the organs of wheat under osmotic shock though CCC did not have any effect on the decrease in water potential. The accumulated proline disappeared more rapidly from the CCC-treated plants than the controls when the stress was relieved. They therefore suggested that the elevated concentrations of proline generated in the CCC-treated plants may play a direct role in supporting renewed growth once plant water potential increased.

Robertson and Greenway (1973) reported that application of CCC to young wheat and maize seedlings reduced transpiration as a result of reduced leaf area, and increased root/shoot ratios. The CCC-treated plants did not show any increase in tolerance to internal water deficits induced by osmotic shocks or desiccation, and CCC did not affect the uptake or metabolism of labelled glucose. It was therefore inferred that CCC increased the drought resistance of young maize and wheat plants only by delaying the onset of severe internal water deficit, i.e. by drought avoidance, rather than increasing the plant's tolerance to internal water deficits. Plaut *et al.* (1964) drew a similar conclusion that CCC and B-995 might increase drought avoidance in bean plants. Halevy (1967) did an extensive study with bean, tomato, cotton and gladiolus. He applied different growth retardants at a wide range of concentrations as foliar spray, soil drench or into nutrient culture medium with various osmotic values or under irrigation regimes. Studying transpiration, stomatal opening, osmotic potential, water saturation deficit and anatomical and morphological features, he found that none of these factors showed a consistent correlation with the effect of the chemicals on drought resistance. He was of the opinion that the effect of the growth retardants on increasing drought resistance might be related to their effect on delaying senescence; the basic influence of these chemicals being to slow the breakdown, under water stress, of nucleic acids and proteins.

CHAPTER II

MATERIALS AND METHODS

II.1 PLANT MATERIAL

Throughout this work one variety of tomato, namely 'Grosse Lisse' was used. This variety was supplied by Arthur Yates and Co. Pty. Limited, and its selection was made solely on its availability.

II.2 PLANT GROWTH ENVIRONMENT

Initial experiments were carried out in a glasshouse but the majority of experiments were in a controlled-environment growth room, and mention will be made in the text as to whether the plants were grown in the glasshouse or in the controlled-environment growth room.

The experiments in the glasshouse mostly took place in the summer when day temperatures were continually high; however, an automatic fan controlled the temperature to about 27°C ± 2°C during the day and temperature was about 17°C ± 2°C at night. There was no supplementation to natural light except that at that time of the year the glass was whitewashed to limit the excessive solar radiation.

The controlled-environment growth room had high pressure sodium lamps (8/400 watt), fluorescent tubes (10/60 watt) and incandescent bulbs (12/60 watts). The total light intensity was 800 $\mu E.m^{-2}S^{-1}$ and the sodium lamps, fluorescent tubes and incandescent bulbs contributed 85%, 10% and 5% respectively to the total light flux. Day temperature was 24°C ± 1°C and night temperature was 18°C ± 1°C. Daylength was maintained at 16 hours light, and 8 hours darkness.

II.3 CULTIVATION OF PLANT

Seeds were pre-germinated in Petri dishes lined with moistened Whatman No.1 filter paper (15 cm) overlayed with moistened "Kimwipes" fine grade wipers and the Petri dishes were kept in darkness by wrapping with aluminium foil. The filter paper and the Kimwipes' wiper were kept moist with distilled water whenever it was necessary. In 9-10 days later the germinated seeds with the radicle and plumule separated but the seed coat still attached, and the two cotyledons separated, were transplanted; normally 4-5 to

a pot containing the rooting medium. The plants were later thinned to one per pot for uniformity in size before the CCC application.

Plastic pots ranging from 102 mm to 152 mm (4 ins to 6 ins) in diameter were used. The rooting media used were as follows:

(i) Compost based recycled soil (Waite Institute) with nutrient level up to John Innes'.

(ii) International grade concrete sand - coarse mined sand washed to remove clay (clay 5%).

(iii) 1:1:1 Peat/Perlite/Vermiculite mixture.

In all cases, a known weight of the rooting medium underlied by a known weight of tree bark to facilitate drainage, was used.

In the experiments where plants were grown in sand or peat/perlite/vermiculite mixture, mineral nutrients were supplied by watering with Hoagland's solution (Hoagland and Arnon, 1938) daily. With the recycled soil, Hoagland's solution was supplied as a supplementation - twice a week.

II.4 PREPARATION AND APPLICATION OF CCC

Cycocel 100A, obtained from Cyanamid Australia Pty. Ltd., with an active ingredient of 100g/l was used. In all the experiments, CCC was prepared in Hoagland's solution and the quantity required for each experiment was prepared the same day that it was applied.

As mentioned earlier, the method of application of CCC affects the responsiveness of plants (Bhattacharjee *et al.*, 1971; Humphries, 1968). In tomato, CCC applied as a foliar spray has been effective (Pisarczyk and Splittstoesser, 1979) as well as when applied to the rooting medium (Wittwer and Tolbert, 1960; Abdalla and Verkerk, 1970; Abdul *et al.*, 1978). However, Wittwer and Tolbert (1960) observed that foliar spray, though effective, was not long lasting as compared to soil treatment. Moreover, in a limited space, drops of the CCC solution spreading onto plants not meant to be treated is not impossible. Therefore, in this work, it was thought appropriate to apply the CCC only to the rooting medium. The volume applied in each case is specified in the text.

II.5 METHODS OF STRESS IMPOSITION

Stress was imposed by with-holding water and allowing the available

soil moisture to be depleted. Alternatively polyethylene glycol (PEG M.W. 4000) was used to induce water stress.

Where water stress was induced by with-holding water to the rooting medium, the pots were embedded in polythene bags tied at the end to the lower portion of the stem to prevent direct evaporation from the rooting medium. Naturally, plant water deficit results when the water absorbed and transpired by plants is not replenished. This, in fact, sets the soil-plant-atmosphere continuum in an inbalance (Kozlowski, 1968) since obviously, the rate of water loss will override the rate of water absorption as the soil water is depleted.

PEG is a well known osmoticum. Husain and Aspinall (1970) and Singh *et al.* (1973) have used PEG (M.W.4000) to induce stress without any specific toxic effects. Lesham (1966) and Lawlor (1970) have reported toxic effects due to PEG.

In this work PEG (M.W. 4000), used to induce stress, was applied in Hoagland's solution to effect the required plant water potential. In the two experiments where PEG was used, abnormal symptoms developed especially in the leaves and this will be discussed below.

II.6 MEASUREMENT OF PLANT WATER STATUS

II.6.1 Leaf water potential

Leaf water potential was measured with the Spanner-type thermocouple psychrometer and also with the pressure bomb (Boyer, 1969; Barrs, 1968). With the pressure bomb technique, the petiolus of the leaflet was sealed in the chamber such that it protruded about 5 mm above the seal. The blade of the leaflet was subjected to pressure inside the chamber and at the point that the xylem sap appeared at the cut surface the pressure build-up was stopped and the water potential read off the pressure gauge. In order to minimize error in this operation the increase in pressure was so controlled that the pointer of the pressure gauge travelled at a reasonably moderate speed. In addition, the chamber was lined with moist filter paper to reduce the dehydration of the leaflet due to the pressure.

In the psychrometry, half of the leaflet blade was placed against the inner wall of the chamber and sealed with the thermocouple plug and then placed in a water bath at 25°C ± 0.001°C. An equilibration time of 2-3 hours

was allowed. The deflections from a moving chart recorder were used to compute the water potential after comparison with standard calibration values using known molar solutions of sodium chloride. Since the psychrometer used had 24 chambers the water potential of the sampled leaflet was measured twice with each half of the blade in two different chambers and the average value taken.

II.6.2 Leaf osmotic potential

Osmotic potential was determined with Spanner-type thermocouple psychrometer (Barrs, 1968). Throughout the experiments, the same tissue used for the determination of water potential was frozen between sheets of filter paper and thawed for about 30 minutes and then reloaded into the chamber and the same procedure for the psychrometric measurement of water potential (Section II.6.1) followed. Though many workers freeze their tissue in the chamber, this method can ruin the chamber due to repeated freezing and thawing. On the other hand, freezing the tissue in the open could lead to precipitation of atmospheric water on the tissue. In order to avoid these, the tissues were frozen and thawed in between sheets of filter papers and before reloading, all the chambers were wiped clean of all drops of water.

II.6.3 Relative water content

The relative water content (RWC) was determined using leaf discs 1.5 cm in diameter. The fresh weight of the leaf discs were taken immediately after punching them out and then the leaf discs were floated on distilled water under diffused light for 4 hours (Barrs and Weatherly, 1962). The discs were then blotted dry with filter paper and the turgid weight taken prior to oven drying at 80°C for 24 hours and measuring dry weight. The RWC was then calculated from the formula (Barrs, 1968):

$$RWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fully turgid weight} - \text{Dry weight}} \times 100$$

II.7 MEASUREMENT OF GROWTH PARAMETERS

II.7.1 Plant Height

The height of plant was determined by measuring the height of the stem from the soil surface to the apex. In intact plants, this was done by means of a piece of string trailed along the stem from the soil level to the

apex and the corresponding length of the string measured on a rule. In excised plant the stem height was measured directly on a rule after excision of the leaves.

II.7.2 Leaf Area

Leaf area was measured on a Paton electronic planimeter. The area of the leaflets was actually measured without the petiole since the device used could not handle the petiole. The leaflets were put one after the other on a transparent conveyor and passed between a line of photocells and a light source at a constant speed and total area read on a digital readout. The planimeter was equilibrated for at least 30 minutes and calibrated before use.

II.7.3 Fresh weight

The fresh weight of the leaves, stem and root were determined by weighing these organs on a Mettler (P1210) balance. Leaves including the petiole were excised, and weighed immediately. The stem was cut at the soil level and immediately weighed. Roots were extracted by carefully washing away the soil or sand and blotting dry prior to weighing.

II.7.4 Dry weight

Leaves, stem and roots were separately put into paper bags and oven-dried at 80°C for 48 hours and then weighed.

II.8 LEAF DIFFUSIVE RESISTANCE, STOMATAL COUNTS AND TRANSPIRATION

II.8.1 Leaf diffusive resistance

The leaf diffusive resistance was measured with a Li-Cor Diffusive Resistance Meter, model LI-60 (Lambda Instruments Corporation) with a horizontal Lambda model sensor (Kanemasu *et al.*, 1969). The whole assembly is normally referred to as a leaf diffusive resistance porometer. The diffusive resistance was measured by inserting the leaflet in the sensor such that the sensor cup was on the surface to be measured. The time required for a given amount of water vapour to diffuse into the sensor cup and be absorbed by the humidity sensing element was recorded. The resistance was computed by comparison with calibration values.

II.8.1.1 *Calibration of diffusive resistance Porometer*

Calibrations of the porometer were done in a constant temperature

room at 20°C and in the controlled-environment growth room with a temperature of 24°C ± 1°C, where the majority of the experiments were carried out. The calibration procedure developed by Kanemasu *et al.* (1969) was followed.

The resistance for the calibration were derived from a set of holes (of the same size as the aperture of the sensor of the diffusive resistance porometer) on the upper plate of a pair of acrylic plates. Strips of Whatman No.1 filter paper (24.0 cm), 1 mm thick, were moistened and placed on the acrylic base plate such that the ends dipped into a distilled water reservoir on both sides. These strips thus served as water wicks. A coarse chromatographic paper cut to the size of the plate was moistened and placed on the filter paper wicks. A Whatman No.1 (24.0 cm) filter paper also cut to the size of the plate, was moistened and placed over the moist coarse paper and run over with a glass rod to remove excess water. The upper acrylic plate which is the resistance plate was then fastened over the upper filter paper. The sensor cup of the porometer, always kept dry by storage in a dessicant, was attached to the resistance plate. The whole assembly was then allowed to equilibrate for about 1 hour and during this time dry air was intermittently pumped through the cup. With the sensor cup over a blank space on the resistance plate, the meter of the porometer was adjusted. The instrument was then calibrated for transit times between two meter readings, 30-70 using the "Humidity-2" level of the meter with a stop watch. Starting with the lowest resistance (i.e. the open space), several readings were taken for each set of resistance holes-open (Lo/α), 60, 30, 15 and 8 (Table II.1) in order of increasing resistance. The relationship between the time lapse Δt and resistance (sec. cm^{-1}) are presented in Fig.II.1 for the constant 20°C temperature room and the 24°C ± 1°C controlled environment growth room.

II.8.1.2 *Measurement of leaf diffusive resistance*

Before taking any set of readings, the meter of the porometer was switched on, adjusted to full scale (100) and allowed to equilibrate for 30 minutes during which time dry air was pumped through the sensor cup from time to time. Prior to a measurement dry air was pumped through the sensor cup such that the meter read 20 or less. The sample leaflet (usually the terminal leaflet) was inserted into the sensor carefully making sure that the leaflet was not crumpled in the sensor. The time lapse for the pointer of the meter to travel from 30-70 was recorded using a stop watch. The temperature of the leaflet was also recorded from the meter and later con-

TABLE II.1 Calibration values of the diffusive resistance porometer at 20°C and 24°C

A). Calibration at 20°C

t (secs)	Number of resistance holes				
	Open (L/α)	60	30	15	8
	35.2	43.7	64.3	111.2	181.8
	26.7	41.5	56.9	110.0	178.1
	27.1	43.8	59.2	107.6	180.5
	28.5	43.0	59.3	110.8	179.2
	29.3	42.4	56.8	109.3	181.0
	28.7	42.8	58.2	108.7	177.3
	27.2	43.2	57.9	110.5	178.9
	28.2	42.9	59.1	110.7	180.7
	27.5	41.8	58.7	108.9	178.5
	27.9	43.2	57.1	109.6	179.1
Mean t (secs)	28.63	42.83	58.75	109.73	179.51
Mean resistance (sec cm^{-1})	0.61	3.27	6.53	13.07	24.50

B). Calibration at 24°C

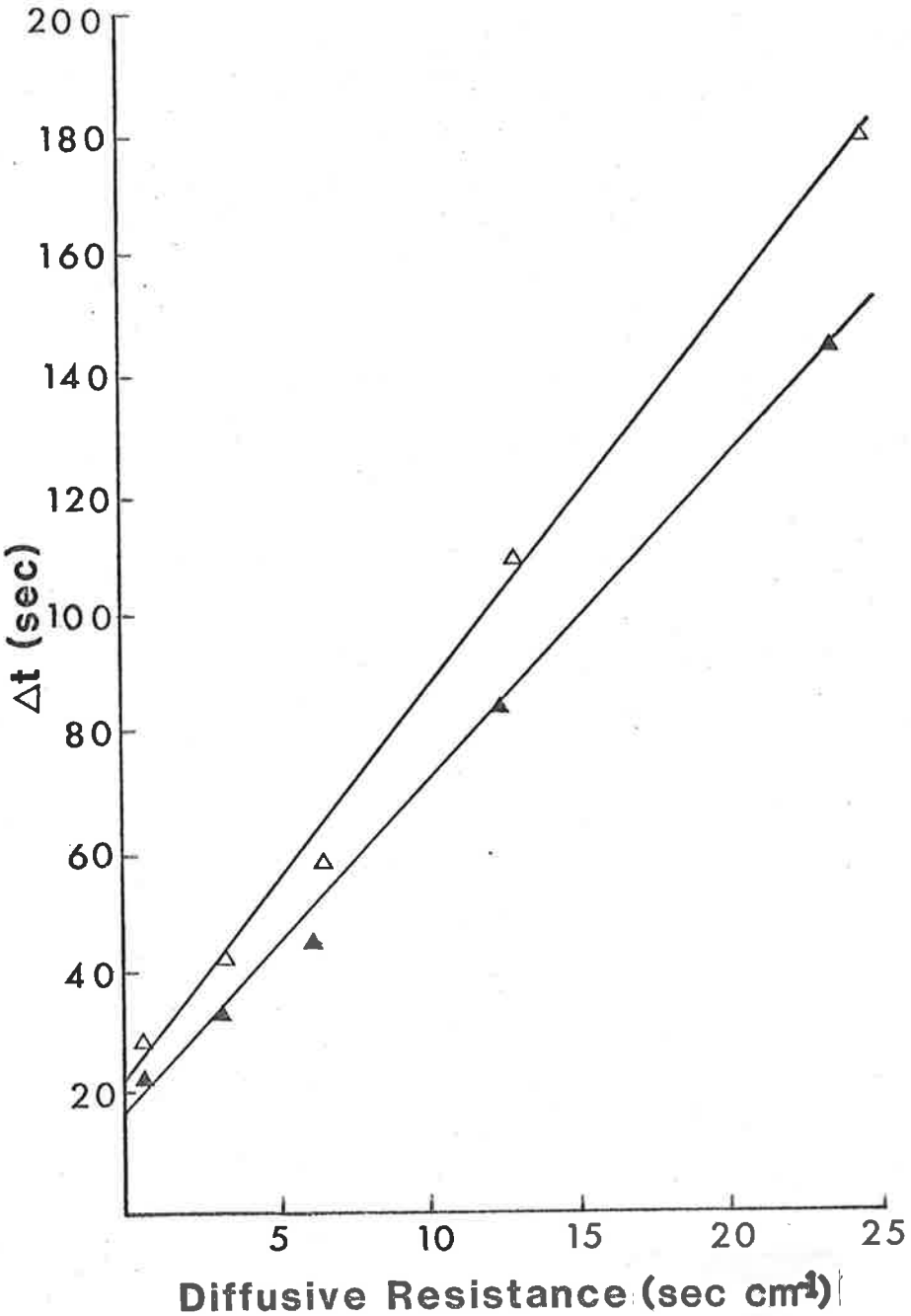
t (secs)	Number of resistance holes				
	Open (L/α)	60	30	15	8
	23.4	34.9	45.5	76.8	128.5
	23.0	33.3	45.0	88.2	149.3
	23.4	32.0	44.2	86.3	139.4
	22.3	30.4	43.0	86.4	152.5
	22.5	32.6	45.3	80.9	131.0
	22.0	35.0	45.3	81.0	146.8
	21.2	33.5	47.7	90.7	150.1
	24.1	34.6	43.5	86.5	151.2
	23.6	34.0	48.9	83.2	149.7
	23.5	35.2	45.8	87.1	148.5
Mean t (secs)	22.80	33.55	45.42	84.71	144.76
Mean resistance (sec cm^{-1})	0.80	3.13	6.27	12.54	23.51

FIG. II.1: Relationship between the time lapse (t) and diffusive resistance in the calibration of the porometer at 20°C and 24°C

(▲) 24°C (△) 20°C

The straight lines were fitted by linear regression analysis.

20°C	24°C
Y=6.44x + 22.05	Y=5.42x + 16.33
R=0.998	R=0.998


200
180
160
140
120
100
80
60
40
20
Δt (sec)
5
10
15
20
25
Diffusive Resistance (sec cm-1)

verted to °C. Initial experience with the porometer indicated that after the first measurement, and especially on the adaxial surface, a second measurement on the same surface of the same leaflet gave a higher diffusive resistance. Therefore, on a given leaflet the resistance of the adaxial surface was first measured before the abaxial surface. Care was also taken not to breathe on the leaflet at the time of measurement.

II.8.2.1 *Stomatal counts*

The initial attempts to peel off the epidermal layer were very frustrating since the layer was delicate and would not peel off easily. Therefore the method of Sampson (1961) was used for making imprints of the leaf surface. Leaf discs were punched from the blade of the leaflet and floated on distilled water to prevent wilting. Silicone rubber was spread on the surface after blotting the disc dry with filter paper and this hardened within 15 minutes. The hardened plastic was gently lifted away with forceps, washed in detergent and then rinsed in distilled water. This was thoroughly dried with filter paper and then placed flat with the impressed surface upwards in a dessicator containing silica gel for 15 minutes. Clear varnish (transparent nail polish) was spread over the dry and undisturbed impressed surface of the silicone rubber and immediately replaced into the dessicator for about 15 minutes. The varnish replica was separated from the silicone rubber and the number of stomates/unit area counted under a light microscope.

II.8.3 Transpiration

Total transpiration in intact plants was determined gravimetrically. Pots were embedded in polythene bags, the ends of which were tied around the lower portion of the stem. The whole system - pot (polythene bag) with plant - was weighed initially and later after a given time period. The transpiration rate was calculated from water loss from the intact plant for a known period of time per unit leaf area.

Transpiration rate in the excised leaf was determined by excising the leaf and cutting the petiole under water and then sealing it in a plastic bottle (as shown in Appendix I). The water loss in a known time interval was expressed on a unit leaf area basis.

II.9 CHEMICAL PROCEDURES

II.9.1 Determination of proline

The determination of free proline in the leaves was done following the method of Singh *et al.* (1973). Prior to the extraction and measurement of free proline, the leaf tissue was frozen in liquid nitrogen and freeze-dried. A known weight, normally about 200 mg, of the freeze-dried leaf tissue together with 1.5g of Decalso-F resin was placed in a Dual glass homogenizer and homogenised in 5 ml of methanol : chloroform : water (MWC 12:5:3) at room temperature. The homogenate was decanted into a centrifuge tube and 5 ml distilled water and 3 ml chloroform added to break the emulsion. The mixture was then shaken thoroughly on a centrifugal shaker and centrifuged at 2000 rpm for 5 minutes. The upper aqueous layer was then transferred to a boiling tube containing 5 ml glacial acetic acid and 5 ml of freshly prepared ninhydrin reagent (125 mg ninhydrin : 3 ml glacial acetic acid : 2 ml 6M orthophosphoric acid), and then placed in a boiling water bath for 45 minutes with a glass marble covering the boiling tube to prevent excessive evaporation. After cooling to room temperature, 5 ml toluene was added and then shaken vigorously. The two layers were allowed to separate for 30 minutes and the optical density of the toluene layer read at 520 nm. The concentration of proline was estimated from a standard curve (0 to 100 ug proline).

II.9.2 Extraction and assay of quaternary ammonium compounds using the N.M.R. technique

Fresh tomato leaf tissue (2-5 mg) was homogenized in 10 ml methanol : chloroform : water (MCW 12:5:3) in a glass centrifuge tube which sat in an ice bath during the homogenization. The grinding head of the homogenizer was washed with 5 ml of distilled water into the original homogenate and centrifuged at 3000 rpm. The supernatant (MeOH/water phase) was removed and the pH adjusted to a range of 6-7 and then loaded on to a Dowex column, followed by distilled water until 100 ml of eluent had been collected and this was discarded. The Dowex column consisted of 5g of Dowex 50W/H^+/50-100 mesh/2% cross-linkage. Before each use, it was washed with about 25 ml of 8N HCl and then flushed with distilled water until the pH of the eluent was 5. After the first elution with distilled water, the second elution of the Dowex column was 4N HCl and 100 ml was collected. This acid eluent was dried under vacuum using a rotary evaporator and a water bath at 50°C - 60°C. The dry residue was washed with 5 ml of distilled water and redried. The last traces of H_2O were removed by drying under a steady stream of N_2 for about

5 minutes. The sample was then dissolved in 0.6 ml D_2O. Care was taken for this dissolution since the dry sample had spread all over the evaporator flask but still had to be dissolved in the 0.6 ml D_2O. 0.4 ml was removed and transferred to a 5 mm NMR tube. The result from the NMR was thus multiplied by 1.5 as a volume correction factor. A reference standard, 20 ul of tert.-butanol was added to the NMR tube. The spectra was then run and the integrated areas obtained. The areas of the compounds of interest were expressed as percentages of the t-BuOH peak area and the amount of each compound was determined from a standard curve.

II.10 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

A randomized complete block design was used in all the experiments and the number of replicates used in each experiment is specified in the text. The pots, in which the plants were growing, were rotated intermittently to minimize positional effect.

Data were analysed by the analysis of variance method and depending on the factors involved either the randomized complete block analysis or the factorial analysis was used.

CHAPTER III

RESULTS AND DISCUSSION

III.1 CCC AND WATER STRESS EFFECTS ON WATER STATUS AND GROWTH OF TOMATO

III.1.1 Introduction

It has often been reported that CCC enhanced the ability of some plant species to withstand drought. The very early work by Halevy and Kessler (1963) showed that CCC increased the drought tolerance of bean plants and since then a substantial amount of work has been done relating CCC and drought resistance.

Tomato, as compared to other plants (brigalow and mulga) is very sensitive to drought (Connor and Tunstall, 1968) and it was, therefore, worth testing CCC on it under drought conditions. As mentioned earlier, CCC affects different species and even different cultivars of the species differently (Cathey, 1964). It was therefore necessary to carry out some initial studies on the growth and water status of the tomato cultivar used and how it was affected by CCC under normal conditions and under water stress conditions. This, as reported here, took the form of the:

(i) Establishment of an effective concentration of CCC
(ii) Effects of this concentration on the growth of tomato
(iii) Age or stage of growth of the plant at which this concentration could be applied.

III.1.2 Effects of different concentrations of CCC and water stress on water potential and growth

III.1.2.1 *Introduction*

Plaut *et al.* (1964) reported that different concentrations of CCC produced varied results on bean plants under various irrigation regimes. In potatoes, Dyson (1965) found that higher concentrations of CCC reduced stem growth more than lower concentration, and similar results were obtained by Abdul *et al.* (1978) in tomato. However, high concentrations could be toxic to the plant. This has been found in Norway Spruce *(Picea abies)* on which CCC at 300 mg/l was toxic (Dunberg and Eliason, 1972). It therefore became necessary for a satisfactory level of CCC

to be determined for this work since, under a given set of experimental conditions, too high a concentration could be toxic while too low a concentration could be ineffective.

Initial observations indicated that concentrations below 500 ppm were relatively ineffective but concentrations between 500 ppm and 1000 ppm were most effective. Those above 1500 ppm produced toxic effects mainly in the form of yellowing of the leaves starting from the mid-vein and spreading towards the margin of the leaf blade. Plants which received 1500 ppm could recover and grow normally but at 5000 ppm the plants did not recover. Thus, in this experiment, concentrations of 0, 750 and 1000 ppm were tested.

The tomato seeds were pregerminated as described in Section II.3 and transferred to 152 mm (6 ins) pots containing 2 kg of sand each with 100 gm of tree bark pieces to facilitate drainage. The plants were initially watered with half strength Hoagland's solution and later with full strength as they grew bigger. CCC (750 and 1000 ppm) was applied in Hoagland's solution at 200 ml/pot to 3-4 weeks old plants and the untreated plants received an equal volume of Hoagland's solution. Water stress was imposed by with-holding water 2 days after the CCC treatment and the pots were embedded in plastic bags to reduce evaporation.

Water potential of the uppermost fully developed leaf (3rd or 4th leaf) was measured with the pressure bomb. Four days after with-holding water the plants were harvested and the growth parameters measured. The experiment was carried out in the controlled-environment growth room with conditions specified in Section II.2, and the treatments were replicated 4 times.

III.1.2.2 *Results*

There were no significant differences between the water potential of the plants under field capacity but, with time water stress resulted in a decline of water potential in all the treatments. However, both the 750 ppm and 1000 ppm CCC treatments maintained significantly higher water potential (i.e. less negative) than the untreated plants under water stress (Fig. III.1.1). The 1000 ppm CCC treatment maintained the highest water potential in the course of the water stress development. By the fourth day after with-holding water, the water potential of the untreated plants

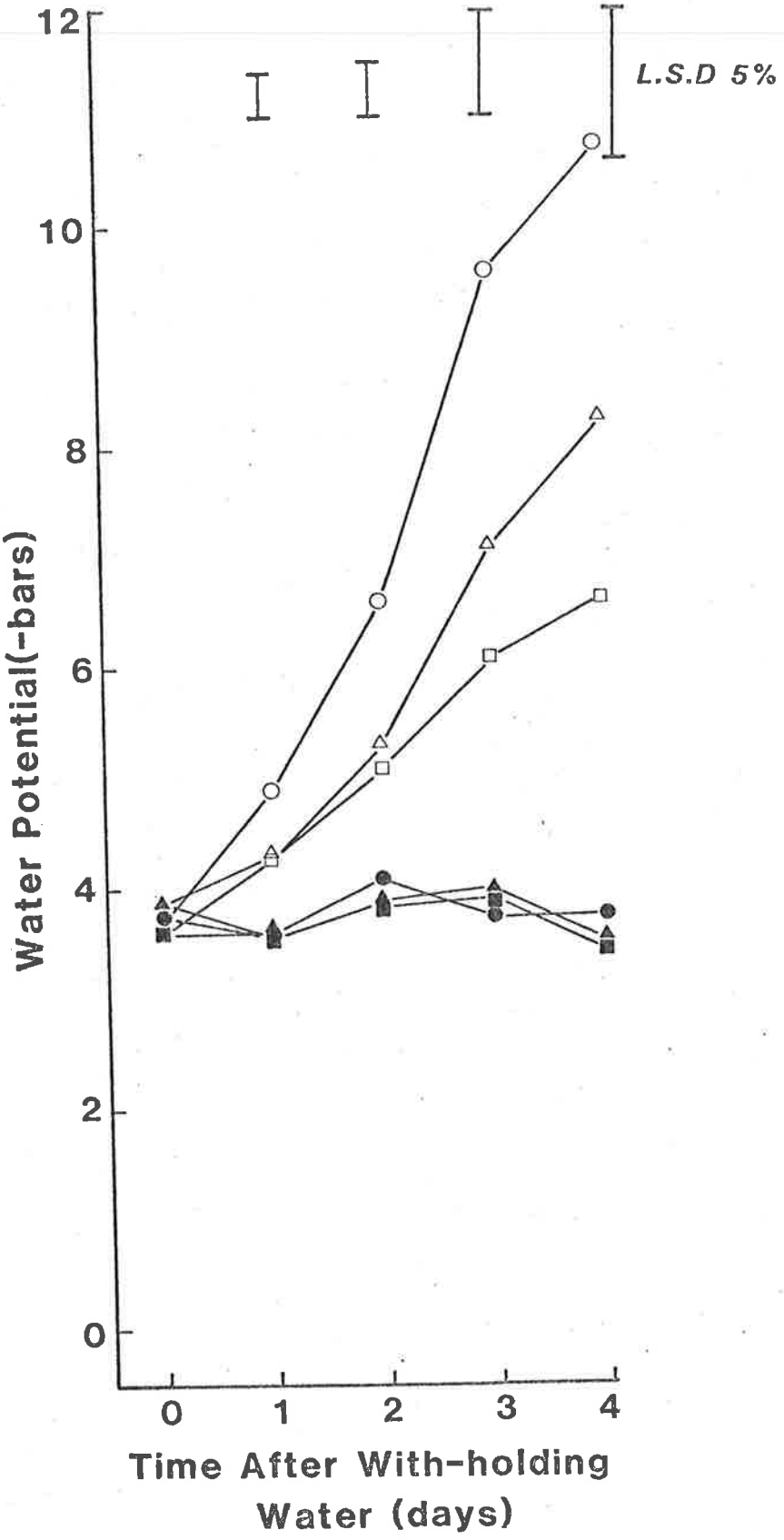
FIG. III.1.1: Effects of different levels of CCC pretreatment on water potential during 4 days of withholding water.

Control (0 ppm) - (●) F.C.
(○) Stress

750 ppm - (▲) F.C.
(△) Stress

1000 ppm - (■) F.C.
(□) Stress

(F.C. = Field Capacity)


L.S.D 5%
12
10
8
6
4
2
0
Water Potential(-bars)
0
1
2
3
4
Time After With-holding
Water (days)

under water stress had reached -10.75 bars while the 750 ppm and 1000 ppm CCC-treated plants had attained water potentials of -8.5 bars and -6.65 bars respectively. At this point the untreated plants showed symptoms of wilting - the leaves had collapsed and the leaflets were rolling up butthe 750 ppm CCC-treated plants had their leaves beginning to collapse with no rolling up of leaflets while the 1000 ppm CCC-treated plants did not show any of these symptoms as shown in Fig. III.1.2. The behaviour of tomato leaves under wilting conditions must be commented on at this juncture. Under water deficit, the first visible symptom is the collapse of the leaf petiole and this normally occurs at a water potential of between -8 bars and -9 bars. This is then followed by the rolling up of the leaflets as the water stress increases in severity and all these symptoms were delayed by the CCC treatments especially at 1000 ppm.

Both levels of CCC significantly reduced the height of the plants 6 days after the treatment and water stress also reduced the height of the untreated plants but not the CCC-treated plants (Table III.1.1). The total leaf area and the number of leaves per plant were unaffected by CCC alone but were decreased by water stress though the CCC-treated plants maintained higher leaf area than the untreated plants under water stress. The dry weight of the whole plant was only significantly reduced by water stress and this reduction could largely be accounted for by the reduction in the leaf dry weight since the stem and root dry weights were unaffected by water stress.

III.1.2.3 *Discussion*

The maintenance of higher water potential under water stress due to the CCC treatments agrees with the work of De *et al*. (1982) who found that in two varieties of wheat both seed treatment with CCC and CCC applied to 45 days old plants resulted in the treated plants maintaining higher water potential than the untreated plants under drought conditions in the field.

The height of the plant was retarded by the CCC treatment and similar results were obtained with tomato by Abdalla and Verkerk (1970). There was no further retardation in the height due to water stress in the CCC treatments but water stress reduced the height of the untreated plants. Leaf area and number of leaves were not affected by CCC alone

FIG. III.1.2: Photographs showing CCC-treated and control plants under well-watered condition and under stress induced by with-holding water. The CCC-treated plants were supplied with 1000 ppm CCC.

From right to left:
CCC F.C.
Control F.C.
CCC stressed
Control stressed

The Control stressed plant at the extreme left shows symptoms of wilting as described in Section III.1.2.3, whereas the CCC-treated and stress plant next to it does not show the wilting symptoms.

TABLE III.1.1: Effects of different levels of CCC and water stress on growth characteristics after 4 days of with-holding water.

	Height of plant (cm)		Leaf area (dm^2)		Number of leaves		Dry weight (g)							
							Leaves		Stem		Root		Whole plant	
	F.C.	Stress	F.C.	Stress	F.C.	Stress	F.C.	Stress	F.C.	Stress	F.C.	Stress	F.C.	Stress
0	19.76	17.52	1.36	0.48	6.42	5.67	0.60	0.46	0.18	0.20	0.09	0.11	0.87	0.76
750	17.15	16.78	1.38	0.84	6.50	5.33	0.73	0.49	0.24	0.24	0.13	0.12	1.10	0.84
1000	17.47	17.24	1.40	0.93	6.75	5.84	0.70	0.48	0.20	0.21	0.11	0.11	1.00	0.77
L.S.D. 5%														
CCC	1.27		-		-		-		0.03		-		-	
Stress	1.04		-		0.36		0.07		-		-		0.11	
CCC*Stress	-		0.28		-		-		-		-		-	

F.C. = Field capacity.

but were by water stress. The drastic reduction in the leaf area of the untreated plants by water stress could be attributed to the rolling up of the leaflets. The reduction in the number of leaves per plant in all the treatments, due to water stress, could have resulted from the senescence and subsequent dying of the lower older leaves, especially the first leaf. The dry weight of the leaves but not the stem or root was reduced in all the treatments by water stress despite the fact that the CCC treatments maintained higher leaf water potential. Similar results were found by Gates (1955) with tomato under water stress where the stress affected the dry weight of the leaves more than the other parts and both moderate and severe stress reduced the dry weight of the leaves to the same extent. This possibly suggests that, in tomato, leaf growth is more sensitive to water stress than the other parts and once water begins to become limited leaf growth is drastically affected.

III.1.3 The effects of 1000 ppm CCC on leaf, stem and root growth

III.1.3.1 *Introduction*

From the previous results (Section III.1.2.2) the 1000 ppm CCC treatment maintained high water potential under water stress and, on that basis, was chosen for all subsequent experiments.

Generally, CCC retards the stem growth of plants and in some cases the leaf and root growth as well. This CCC-induced inhibition of growth has been observed in tomato (Pisarczyk and Splittstoesser, 1979; Abdalla and Verkerk, 1970). This, therefore, necessitated a study of the growth of the tomato cultivar used for this work when treated with 1000 ppm CCC.

Pregerminated seeds were grown in 1 kg recycled soil (Waite Institute) in 127 mm (5 ins) pots and half strength Hoagland's solution was supplied to the plants twice a week. 1000 ppm CCC was applied as 150 ml/pot soil drench when the plants were 3 weeks old. Water stress was imposed in some of the plants by with-holding water 10 days after the CCC treatment. The height of the plants was measured in both the well-watered plants as well as the stressed plants with time. To another set of plants the 1000 ppm CCC was applied when they were 4 weeks old and

leaf growth and the fresh and dry weights of the various organs were studied in well-watered plants 5 days and 16 days after treatment with CCC. The experiments were carried out in the controlled-environment growth room with the same conditions as stated in Section II.2. The various treatments were replicated 3 times.

III.1.3.2 *Results*

The height of the plant was not significantly reduced by CCC in the well-watered plants until the 6th day after the CCC treatment (Fig. III.1.3) and thereafter the CCC-induced retardation in plant height became more and more significant. By the 16th day, the height of the CCC-treated plants under well-watered conditions had reached only 69% of their untreated counterparts. Water stress significantly reduced the height of the untreated plants but there was no further significant reduction in the CCC-treated plants due to water stress.

In the second batch of plants, 5 days after the CCC treatment none of the parameters measured had been affected by CCC (Table III.1.2). Sixteen days after the CCC treatment, the height of the plant, total leaf area, the fresh and dry weights of leaves and stem had been significantly reduced by CCC but not the fresh and dry weights of the root. Thus, the reduction in the shoot fresh and dry weights accounted for the reduction in the whole fresh and dry weights in the CCC-treated plants. The root/shoot ratio on fresh weight basis but not on dry weight basis was significantly higher in the CCC-treated plants.

A study of the leaf area and leaf length of the individual leaves with respect to their position on the stem showed that 5 days after the CCC treatment, there were no differences in either the leaf area (Fig. III.1.4a) or the leaf length (Fig. III.1.4b) at any position between the CCC-treated plants and the untreated controls. By the 16th day after the CCC application, the leaf area of the 3rd leaf and all the leaves higher than the 3rd had been reduced by CCC whereas the length of the 5th leaf and all the leaves higher than the 5th had been reduced by CCC.

FIG. III.1.3: Retardation effects of 1000 ppm CCC and water stress.

Control (0 ppm) - (●) F.C.
(○) Stress

CCC (1000 ppm) - (▲) F.C.
(△) Stress

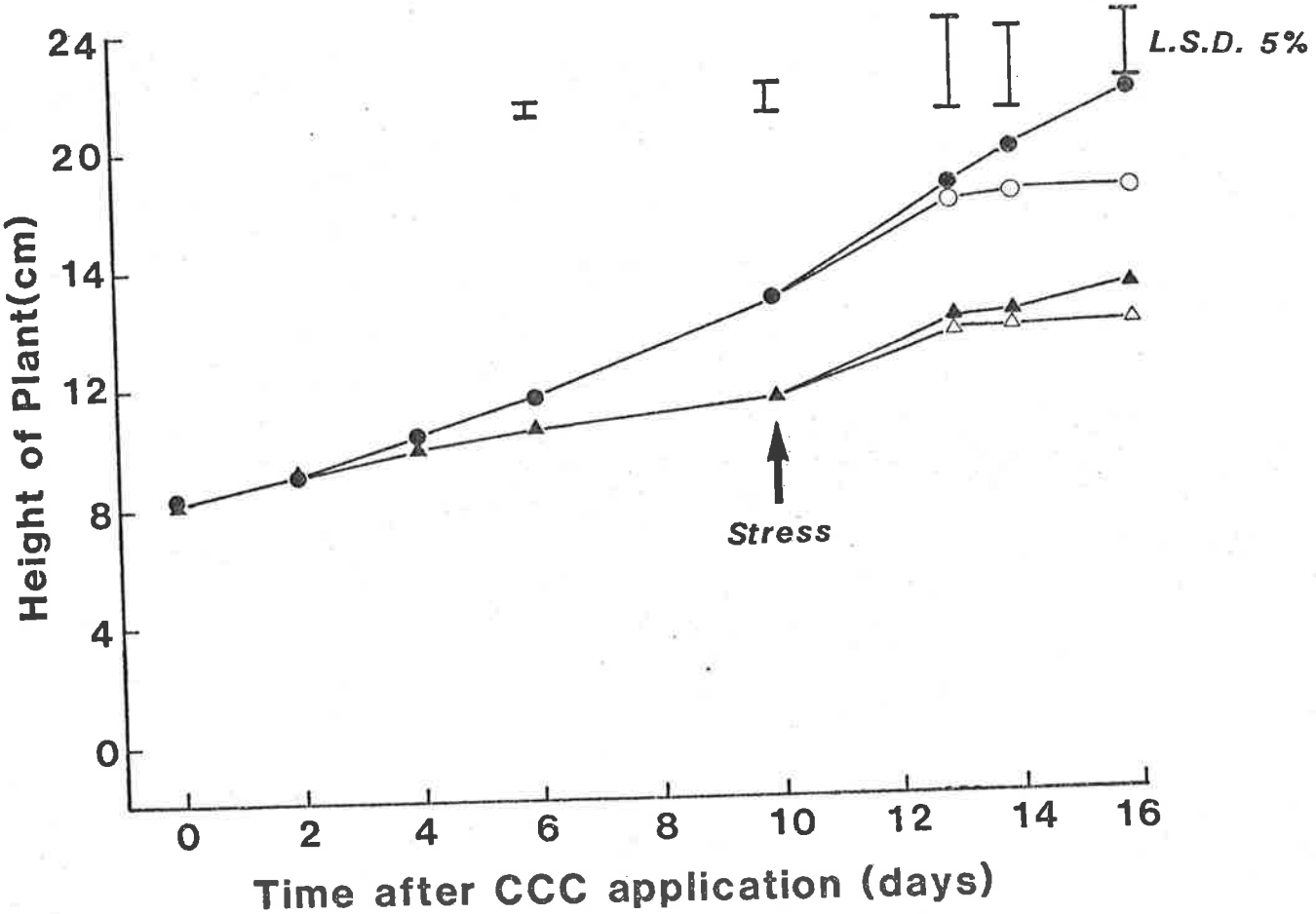

L.S.D. 5%
Stress
Height of Plant(cm)
Time after CCC application (days)
24
20
14
12
8
4
0
0
2
4
6
8
10
12
14
16

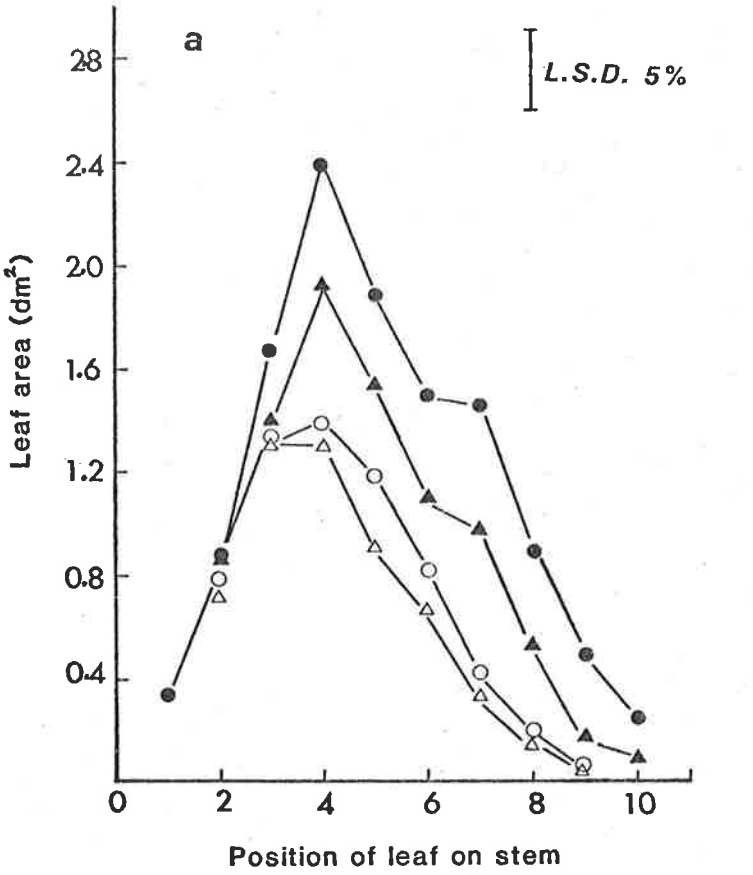
TABLE III.1.2: The effects of 1000 ppm CCC on various growth characteristics of well-watered tomato plants.

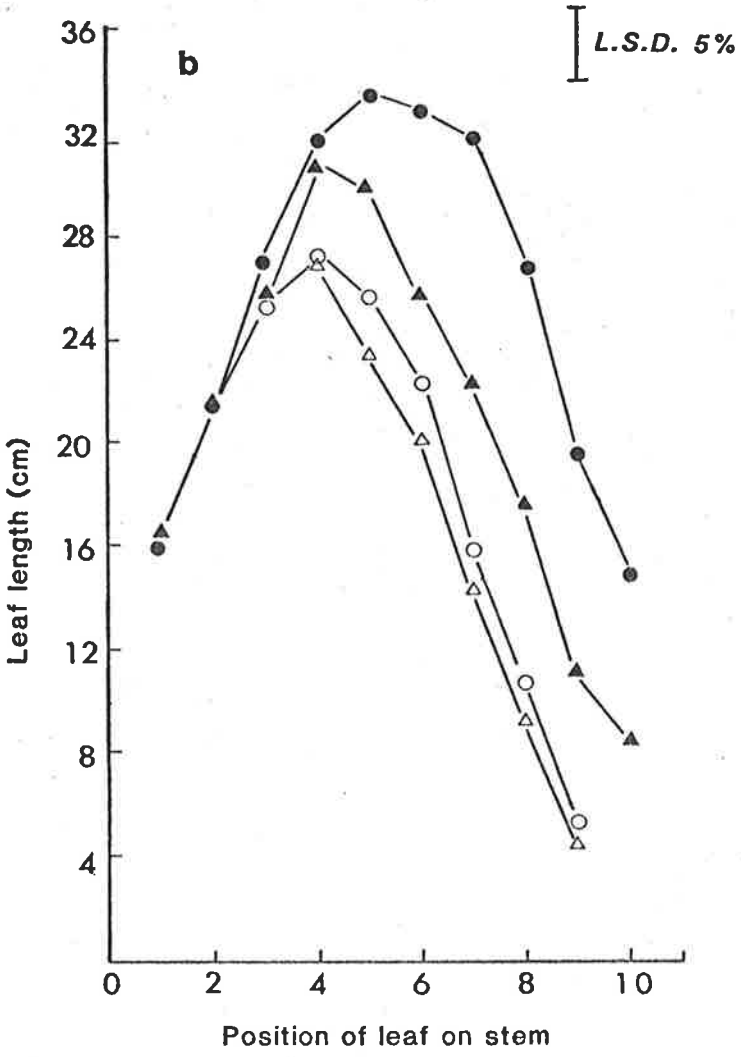
Characteristic	5 days after CCC treatment			16 days after CCC treatment		
	Control	CCC	L.S.D. (5%)	Control	CCC	L.S.D (5%)
Height of Plant (cm)	21.87	19.60	3.04	40.90	22.17	3.74
Leaf Area (dm^2)	6.37	6.32	0.57	12.13	8.95	1.75
Fresh weights (g)						
Leaves	26.96	26.41	4.12	59.33	49.96	5.38
Stem	10.17	9.92	0.97	28.65	17.64	3.42
Root	9.06	8.31	1.60	16.81	17.20	2.02
Whole plant	46.20	44.64	5.50	104.20	84.70	10.63
Dry weights (g)						
Leaves	3.96	3.75	0.88	8.60	7.45	1.00
Stem	0.47	0.43	0.24	2.39	1.53	0.43
Root	0.28	0.26	0.04	0.91	0.88	0.11
Whole plant	4.71	4.45	1.13	11.90	9.81	1.44
Root/Shoot ratio:						
Fresh weight basis	0.25	0.24	0.01	0.19	0.26	0.03
Dry weight basis	0.06	0.06	0.006	0.08	0.09	0.02

FIG. III.1.4: Effect of 1000 ppm CCC on (a) leaf area and (b) leaf length, with respect to leaf position, 5 days and 16 days after the CCC treatment.

5 days after CCC treatment - (○) Control (△) CCC

16 days after CCC treatment - (●) Control (▲) CCC


a
L.S.D. 5%
Leaf area (dm²)
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0
2
4
6
8
10
Position of leaf on stem


b
L.S.D. 5%
Leaf length (cm)
36
32
28
24
20
16
12
8
4
0
2
4
6
8
10
Position of leaf on stem

III.1.3.3 *Discussion*

The height of plants was not significantly reduced by CCC in the well-watered plants until after about 6 days (Fig. III.1.3). This agrees with findings of Abdalla and Verkerk (1970) in whose work CCC retarded the height of tomato plants after 7 days. In addition Wittwer and Tolbert (1960) observed that the shortening of internode length in CCC-treated tomato plants occurred within 5-7 days after treatment and van Bragt (1969) reported that CCC applied to the roots of tomato inhibited the increase in height of plants 5 days after the application of the CCC. Generally, work done with CCC shows that the shortening of the height of the plants can be observed about a week after the CCC application. For example, in wheat, Singh *et al.* (1973) observed a retardation in plant height by CCC 10 days after the treatment.

From Table III.1.2, leaf area and fresh and dry weights of the plant organs were unaffected when the height of the plant had not been substantially retarded by the CCC treatment (i.e. 5 days after CCC treatment). However, once the height had been retarded, as was the case 16 days after CCC treatment, the total leaf area, the fresh and dry weights of the leaves, stem and the plant as a whole were reduced by the CCC treatment. In tomato, Pisarczyk and Splittstoesser (1979) reported that 2 weeks after the application of CCC, the leaf area and the dry weight of the treated plants were less than the untreated. Contrary to the shoot, CCC treatment did not have any inhibitory effect on the root growth and this resulted in a higher root / shoot ratio, especially on fresh weight basis, in the CCC-treated plants. Several workers have observed an increase in the root / shoot ratio due to CCC treatment. As reported by Humphries (1968), CCC stimulated root growth in wheat. In the present work the increased root / shoot ratio found with the tomato plants was not due to increased root growth but rather to the inhibition of shoot growth without any inhibition of root growth.

By the 16th day after CCC application the leaf area and length of the 4th and 5th leaves respectively, and all the higher leaves had been significantly reduced (Fig. III.1.4). This suggests that CCC exhibited an inhibitory effect on the growth of the leaves which were still developing at the time of the CCC application and the leaves formed after

the CCC application, and this may account for the overall reduction in the total leaf area of the CCC-treated plants.

III.1.4 The effects of 1000 ppm CCC applied at different stages of growth and water stress on water potential and growth

III.1.4.1 *Introduction*

Generally, work done to study CCC-induced resistance to drought has been carried out such that drought conditions are imposed after CCC has effectively retarded growth. This poses the question as to whether the CCC-induced drought resistance is not merely due to the reduced evaporative surface resulting from growth retardation, which in turn, controls water loss and thereby maintains better water status. This experiment therefore was designed to study CCC and water stress interactions when CCC had retarded growth and before it had affected growth. From the previous experiments (Section III.1.3.2), CCC retarded the growth of the shoot by about 6 days after its application. This suggested that differences in the length of time of CCC application before stress imposition might affect the treated plants differently. Thus 1000 ppm CCC was applied at an early and at later stages of the vegetative growth so as to achieve a retardation of growth and non-retardation of growth respectively, by the time stress was imposed. This was to ensure that both the CCC-retarded and CCC-non-retarded plants were of the same physiological age by the time of stress imposition notwithstanding the complications of the plants being treated with CCC at different stages of growth and for different lengths of time before stress.

Pregerminated tomato seeds were grown in 1.5 kg recycled soil in 152 mm (6 ins) pots in the controlled environment growth room with conditions as stated in Section II.2. Half strength Hoagland's solution was supplied twice a week as a supplementation. 1000 ppm CCC was applied as 200 ml/pot to one batch of plants when they were 13 days old; they were designated "CCC Early" treatment. The "CCC Late" treatment comprised plants treated with CCC when they were 25 days old. Water stress was imposed by with-holding water from the plants 3 days after the "CCC Late" treatment, (i.e. 15 days after the "CCC Early" treatment). Just before imposing the stress a harvest was made from well-watered CCC-treated and

untreated plants and a second harvest was made at the end of the stress episode (i.e. 9 days after with-holding water). The treatments were replicated 3 times.

III.1.4.2 *Results*

Before the imposition of water stress (i.e. 3 days and 15 days after CCC treatment in the "CCC Late" and "CCC Early" respectively), the "CCC Early" treatment had retarded growth of both shoot and root, but increased root/shoot ratio, whereas the "CCC Late" treatment had not affected growth (Table III.1.3a).

Under normal well-watered conditions CCC did not affect the water potential (Fig. III.1.5). However, in the water-stressed plants, the non-CCC-treated plants showed a rapid decline in their water potentials after the 3rd day of with-holding water. This rapid decline was deferred by both CCC treatments especially the "CCC Early" treatment. Moreover in the course of the stress, both the "CCC Early" and "CCC Late" treatments maintained a significantly higher potential than the non-CCC-treatment, but water potential was still higher in the "CCC Early" treatment than in the "CCC Late" treatment. At the end of the stress episode the water potential of the "CCC Early","CCC Late" and the non-CCC-treatments were -15 bars, -15.8 bars and -18.8 bars respectively. The "CCC Late" treatment retarded growth of the shoot but not the root thereby increasing the root to shoot ratio 12 days after the CCC treatment (i.e. 9th day of stress) as shown by the "CCC Late" F.C. treatment in Table III.1.3b. Also, water stress inhibited the growth of plants in all the treatments and increased the root/shoot ratio.

III.1.4.3 *Discussion*

The lack of differences between the water potentials of the CCC treatments and the non-CCC treatment under well-watered conditions, but the maintenance of higher water potential in the CCC treatments than the non-CCC treatment of stressed plants agrees with the results in Section III.1.2.2 (Fig. III.1.1).

The retardation of root growth in the "CCC Early" treatment before the imposition of water stress (i.e. 15 days after CCC treatment),

FIG. III.1.5: Effects of 1000 ppm CCC applied at different stages of growth and water stress on water potential.

"CCC Early" ——— CCC was applied to 13 days old plants

"CCC Late" ——— CCC was applied to 25 days old plants

Control - (●) F.C.
(○) Stress

"CCC Early" - (▲) F.C.
(△) Stress

"CCC Late" - (◆) F.C.
(◇) Stress

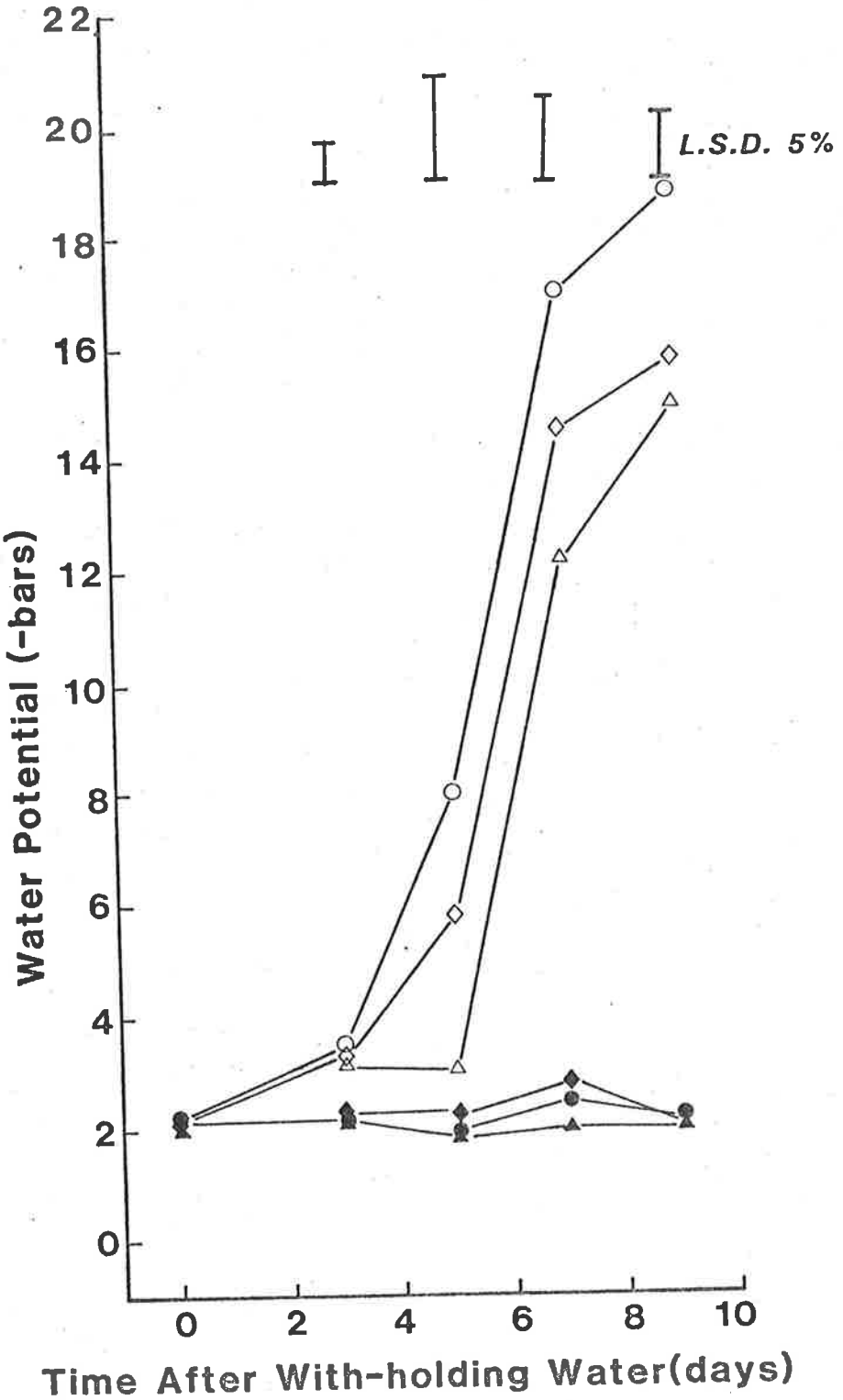

L.S.D. 5%
Water Potential (-bars)
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
10
Time After With-holding Water(days)

TABLE III.1.3: Effects of 1000 ppm CCC applied at different times (Early and Late) on growth characteristics before and after stress.

(a) - Before stress imposition

	Height of plant (cm)	Leaf area (dm^2)	Dry weight (g)			Root/Shoot ratio
			Leaves	Stem	Root	
Control	21.56	7.85	3.14	0.77	0.59	0.15
CCC Early	14.54	4.64	1.95	0.45	0.41	0.17
CCC Late	20.74	8.80	3.23	0.74	0.60	0.15
L.S.D. 5%	2.58	2.64	1.01	-	0.17	

(b) - After 9 days of stress

	Height of Plant (cm)		Dry weight (g)						Root/Shoot ratio	
			Leaves		Stem		Root			
	F.C.	Stress	F.C.	Stress	F.C.	Stress	F.C.	Stress	F.C.	Stress
Control	34.67	26.27	4.44	2.06	2.13	1.42	1.24	0.90	0.19	0.2
CCC Early	23.60	18.93	2.48	1.72	1.14	0.90	0.83	0.66	0.23	0.25
CCC Late	28.00	21.27	3.46	1.89	1.95	1.41	1.24	0.94	0.23	0.28
L.S.D. 5%										
CCC	2.25		0.85		0.42		0.21		0.02	
Stress	1.84		0.68		0.34		0.26		0.02	
CCC*Stress	-		-		-		-		-	

is not consistent with the result in Section III.1.3.2 (Table III.1.2) where CCC did not retard root growth 16 days after its application. This discrepancy could possibly be accounted for by the differences in the ages of the plants at which CCC was applied. In Section III.1.3.2, CCC was applied to older plants (4 weeks old) whereas in the present case the plants in the "CCC Early" treatment were treated with CCC at a relatively younger stage (13 days old). The increase in root/shoot ratio in the "CCC Early" treatment before stress imposition could possibly be due to shoot growth being retarded more than root growth. The "CCC Late" treatment did not have any effect on growth before stress imposition (i.e. 3 days after CCC application) but retarded the shoot growth without affecting root growth 12 days after the treatment and, thereby, increasing root/shoot ratio. Similar results were found in Section III.1.3.2 (Table III.1.2) where CCC did not affect growth 5 days after its application but retarded shoot growth without affecting root growth, 16 days after its application.

Despite the maintenance of higher water potential under water stress growth could not be sustained and similar results were found in Section III.1.2.2 (Table III.1.1). It is also evident that the CCC-induced maintenance of higher water potential under stress is independent of its growth retardation effect.

III.2 A COMPARISON OF PEG-INDUCED WATER STRESS AND SOIL WATER DEPLETION (BY WITH-HOLDING WATER) COMBINED WITH CCC TREATMENT

III.2.1 Introduction

In all the previous experiments, water stress was induced by with-holding water from the plants and there has been a repeatable effect of CCC treatment on the treated plants' ability to maintain better water status under stress. Robertson and Greenway (1973) reported that the growth of CCC-treated maize and wheat seedlings was affected to the same extent as the untreated plants when subjected to mannitol-induced water stress. Data from the work of Singh *et al.* (1973) showed that the water potential of CCC-treated wheat plants declined to the same extent as that of the untreated plants under PEG-induced water stress.

It was therefore found appropriate to compare the effects of CCC on tomato plants under water stress conditions induced by soil water depletion (i.e. by with-holding water) and an osmoticum. In this section, the effect of CCC on the water status of tomato under either PEG-induced stress or soil water depletion, and the growth of CCC-treated and untreated tomato plants under PEG stress were studied.

The tomato plants were grown from pregerminated seeds in 152 mm (6 ins) pots containing 2 kg sand. The plants were watered with half strength Hoaglands solution initially and later with full strength. CCC at 1000 ppm was applied (200 ml/pot) to the treated plants when they were $3\frac{1}{2}$ weeks old. Water stress was imposed 4 days after the CCC treatment. PEG (M.W. 4000) at two levels, -8 bars and -12 bars osmotic potential, was applied (300 ml/pot) in Hoagland's solution daily to the sand medium, to one batch of plants. In another batch of plants water stress was imposed by with-holding water from the plants; the pots were embedded in polythene bags to minimize evaporation of water from the surface of the sand. The non-stressed plants were watered daily with full strength Hoagland's solution. Before the imposition of stress (i.e. 4 days after CCC treatment), some plants were harvested and some growth parameters - height of plant, leaf area, dry weight of leaves, stem and roots were measured. Leaf water potential of the uppermost fully developed leaf was monitored with the pressure bomb during the stress period. At the end of the stress episode (i.e. 8 days of stress), a second harvest was made to evaluate the effect of PEG at both levels on the growth parameters mentioned above. The experiment, which was carried out in the controlled-environment growth room, with conditions specified in Section II.2, was replicated three times.

III.2.2 Results

Before the imposition of stress, CCC had not had any effect on the growth of the treated plants, as shown in Table III.2.1; the height of plant, leaf area and dry weight of leaves, stem and roots had not been affected by the CCC treatment.

As can be seen from Fig. III.2.1, -8 bars PEG and -12 bars PEG decreased the water potential, and there was no difference in effect on either the CCC-treated or untreated plants. Soil water depletion also in-

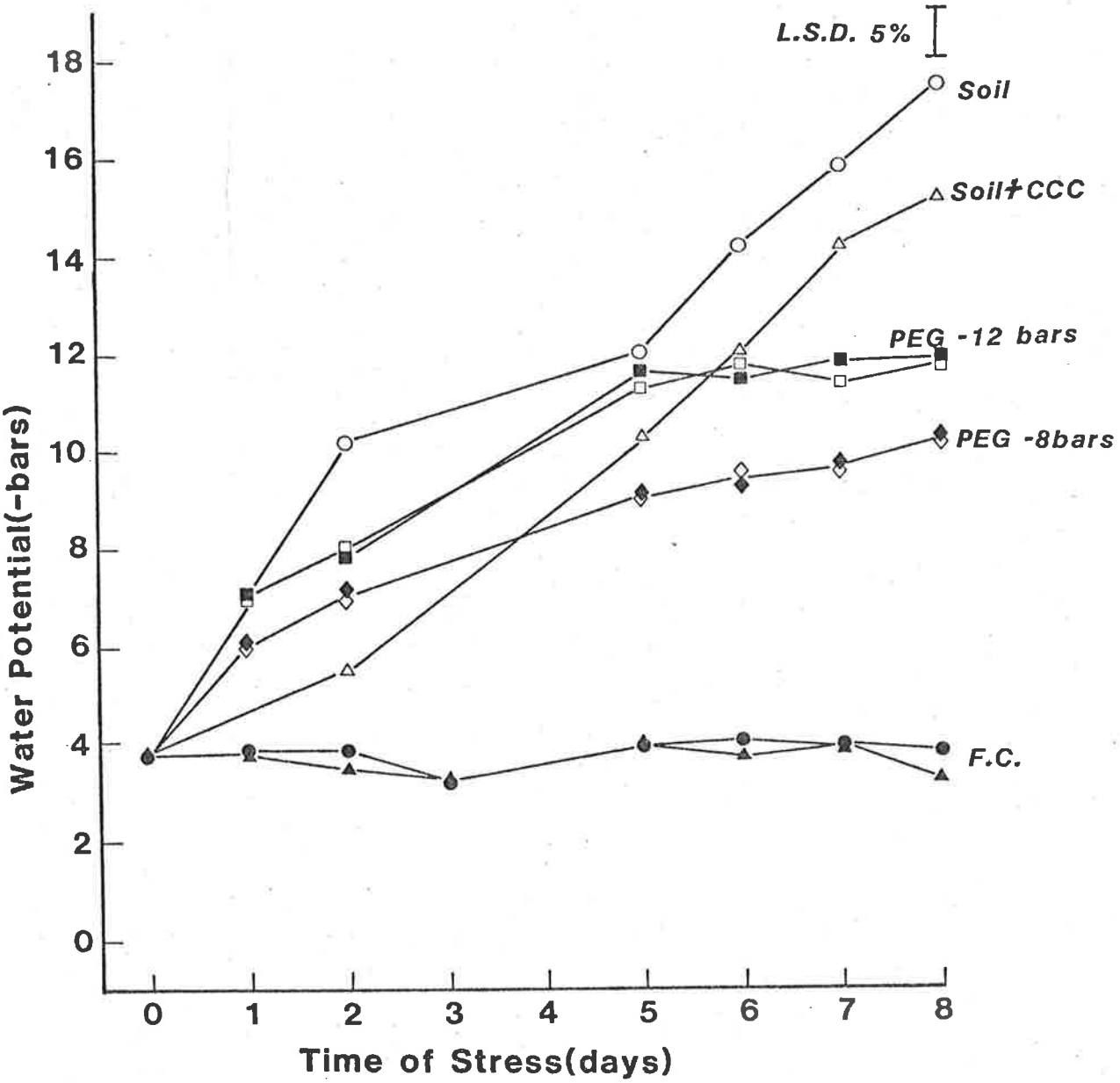
TABLE III.2.1: Effects of CCC on various growth characteristics before the imposition of stress.

	Height of plant (cm)	Leaf area (dm^2)	Dry weight (g)		
			Leaves	Stem	Root
Control	22.60	5.82	3.37	0.81	0.68
CCC	21.20	5.73	3.24	0.84	0.72
L.S.D. 5%	2.30	1.26	0.90	0.41	0.38

FIG. III.2.1: Effect of CCC on water potential under PEG-induced stress and soil moisture depletion induced by with-holding water.

Control -
(●) F.C.
(○) Soil water stress
(◆) -8 bars PEG stress
(■) -12 bars PEG stress

CCC -
(▲) F.C.
(△) Soil water stress
(◇) -8 bars PEG stress
(□) -12 bars PEG stress


L.S.D. 5%
Soil
Soil+CCC
PEG -12 bars
PEG -8bars
F.C.
Water Potential(-bars)
Time of Stress(days)
0
2
4
6
8
10
12
14
16
18
0
1
2
3
4
5
6
7
8

duced a decrease in water potential but, contrary to the PEG stress, the CCC-treated plants maintained higher water potentials than the untreated plants.

By the third day of stress, the PEG-treated plants showed symptoms of toxicity, these symptoms were more severe in the -12 bars PEG treatment. The margins of the leaflets were chlorotic and wilty and the symptoms spread slowly towards the mid-vein. The central portions of the leaflet lamina, however, remained non-chlorotic but were abnormally dark and shiny green and appeared water-logged. The petiole and stem, on the other hand, appeared turgid and even by the end of the stress they appeared more turgid than those of the plants subjected to soil water depletion. This PEG-induced toxicity syndrome affected the CCC-treated and non-CCC-treated plants to the same extent.

Data from Table III.2.2 indicates that at the end of the stress episode PEG, at both levels, had significantly decreased growth; height of plant, dry weight of leaves, stem and roots had been decreased in the CCC-treated and untreated plants alike. Under field capacity, however, CCC slightly decreased shoot dry weight but increased the root dry weight thereby increasing root/shoot ratios (Table III.2.2).

III.2.3 Discussion

CCC did not have any inhibitory effect on the growth of plants 4 days after its application (Table III.2.1). As discussed in Section III.1.3.3, CCC does not retard the growth of tomato until about a week after its application.

When tomato plants are subjected to soil water depletion, CCC enhances their ability to retain higher water potential, and this has been the case in all previous experiments. On the other hand, when subjected to PEG stress, water potential decreased to the same extent in the CCC-treated and non-CCC-treated plants. Singh *et al.* (1973) reported a similar effect of PEG in that the water potential of CCC-treated and untreated wheat plants were decreased to the same extent. It is not clear why CCC-treated plants behave differently when subjected to the two different methods of stress, but secondary effects of PEG may be involved.

TABLE III.2.2: Effects of CCC and PEG-induced stress on various growth characteristics.

	Height of plant (cm)	Leaf area (dm^2)	Dry weight (g)			Root/Shoot ratio (dry wt. basis)
			Leaves	Stem	Root	
Control F.C.	33.20	11.22	8.00	1.97	2.16	0.22
CCC F.C.	25.60	8.76	7.06	1.62	3.19	0.37
Control stress 1 (-8 bars PEG)	23.33		3.50	1.31	1.36	0.28
CCC stress 1 (-8 bars PEG)	22.77		3.41	1.52	1.56	0.31
Control stress 2 (-12 bars PEG)	23.07		2.43	1.20	1.10	0.30
Control stress 2 (-12 bars PEG)	22.57		2.56	1.29	1.20	0.31
L.S.D. 5%						
CCC	1.93	1.53	0.90	-	0.31	-
Stress	2.36	-	1.78	0.54	0.43	-
CCC*Stress	-	-	-	-	-	0.14

Toxic effects due to PEG have been found in certain situations. Lesham (1966) reported that PEG was toxic to *Pinus halepensis* seedlings. According to Lawlor (1970), the presence of PEG in leaves causes necrosis which may ultimately kill the leaves. Hodgson *et al.* (1949) reported that PEG treatment of tomatoes caused marginal wilting of leaflets while the leaflet centres, petioles and stems still had high water content comparable to the controls. They also found that a large portion of the PEG taken up by the plants was in the leaflet margins which wilted. Joyce (1980) observed that in radish, PEG-induced leaf margin necrosis led to unrealistically high water potential measurements in the pressure bomb; this he attributed to the ingress of air into the leaf through xylem exposed to the atmosphere due to the necrosis. Thus, the PEG-induced toxicity syndrome reported in this work is not unusual and the symptoms observed were similar to those reported by Hodgson *et al.* (1949). Nonetheless, the fact that CCC treatment could not maintain better water status under PEG stress and that toxic symptoms showed in the CCC-treated plants, as well as the untreated ones, suggests that the devastating effects of PEG on the tomato plants could not be ameliorated by the CCC treatment.

PEG decreased the growth of both CCC-treated and untreated plants. Results from Section III.1.4.2 indicate that CCC could not sustain growth under soil water depletion. This, therefore, suggests that irrespective of the source of water stress, growth under water stress is not sustained by CCC.

For all subsequent experiments, water stress was induced by withholding water. This method was adopted due to PEG toxicity and the lack of effect of CCC on water potential in the PEG-stressed plants.

III.3 CCC AND WATER RELATIONS OF TOMATO

III.3.1 Introduction

Previous results revealed that CCC-treated plants, when under water stress imposed by with-holding water, maintained higher water potential than the untreated plants. This, therefore, called for a further study into the water relations. In this section, the effects of CCC on water potential, relative water content (RWC), osmotic potential, turgor potential and their inter-relationships, particularly when growth had not been retarded by CCC before stress imposition, will be discussed.

III.3.2 Relative water content

III.3.2.1 *Method*

Pregerminated tomato seeds were grown in 127 mm (5 ins) pots containing 1 kg recycled soil and half strength Hoagland's solution supplied twice a week. 1000 ppm CCC (150 ml/pot) was applied to the soil when the plants were $3\frac{1}{2}$ weeks old. Water stress was imposed by with-holding water from the plants 3 days after CCC application. Water potential and RWC of the 5th leaf (the uppermost fully developed leaf) were measured as outlined in Sections II.6.1 and II.6.3 respectively. The experiment was carried out in the controlled-environment growth room with conditions specified in Section II.2, and was replicated three times.

III.3.2.2 *Results*

Water potential was higher (i.e. less negative) in the CCC-treated plants than the untreated plants under water stress but there were no differences in the well-watered plants (Fig. III.3.1).

Similarly, RWC was higher in the CCC-treated plants than the untreated plants under water stress and this difference was more pronounced after the 3rd day of stress (Fig. III.3.2). By the 8th day of the stress, RWC had dropped to 57% and 53% in the CCC-treated and untreated plants respectively. There were no significant differences in the RWC under well-watered conditions between the CCC treatment and non-CCC treatment.

The relationship between RWC and water potential (Fig. III.3.3) showed a pattern which fitted a linear regression analysis. However, the regression coefficients of the CCC and the non-CCC treatment were not significantly different (0.939 and 0.935 respectively), neither were these different from the regression coefficient of the pooled data (0.934). This indicated that data from both CCC treatment and non-CCC treated fitted the same linear regression. Thus, at a given water potential RWC did not differ in the CCC and non-CCC treatment or in other words, a unit fall in RWC decreased water potential to the same extent in treated and non-treated plants.

FIG. III.3.1: Effect of CCC and water stress on water potential.

Control - (●) F.C.
(○) Stress

CCC - (▲) F.C.
(△) Stress

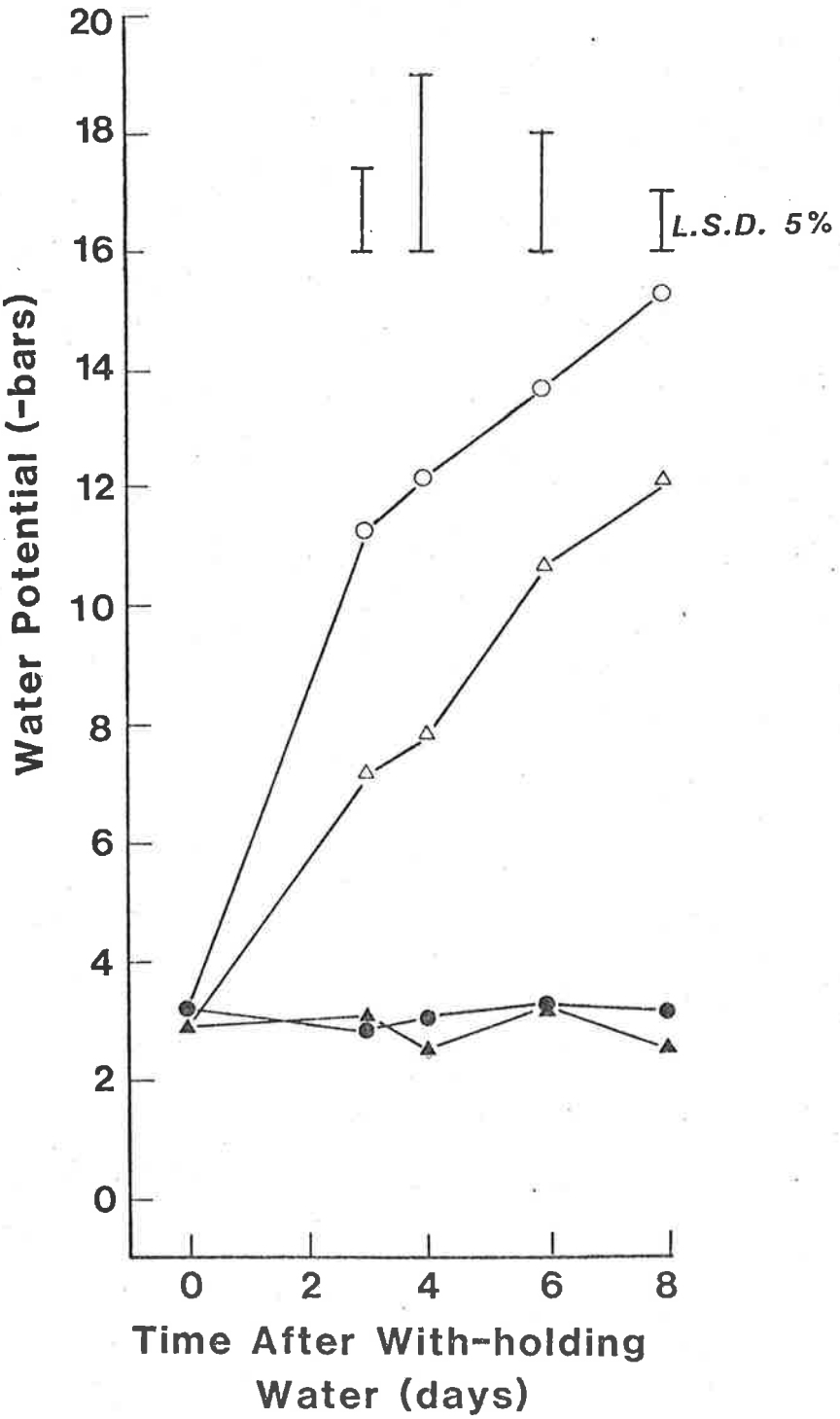

20
18
16
14
12
10
8
6
4
2
0
L.S.D. 5%
Water Potential (-bars)
0
2
4
6
8
Time After With-holding
Water (days)

FIG. III.3.2: Effects of CCC and water stress on RWC.

Control – (●) F.C.
(○) Stress

CCC – (▲) F.C.
(△) Stress

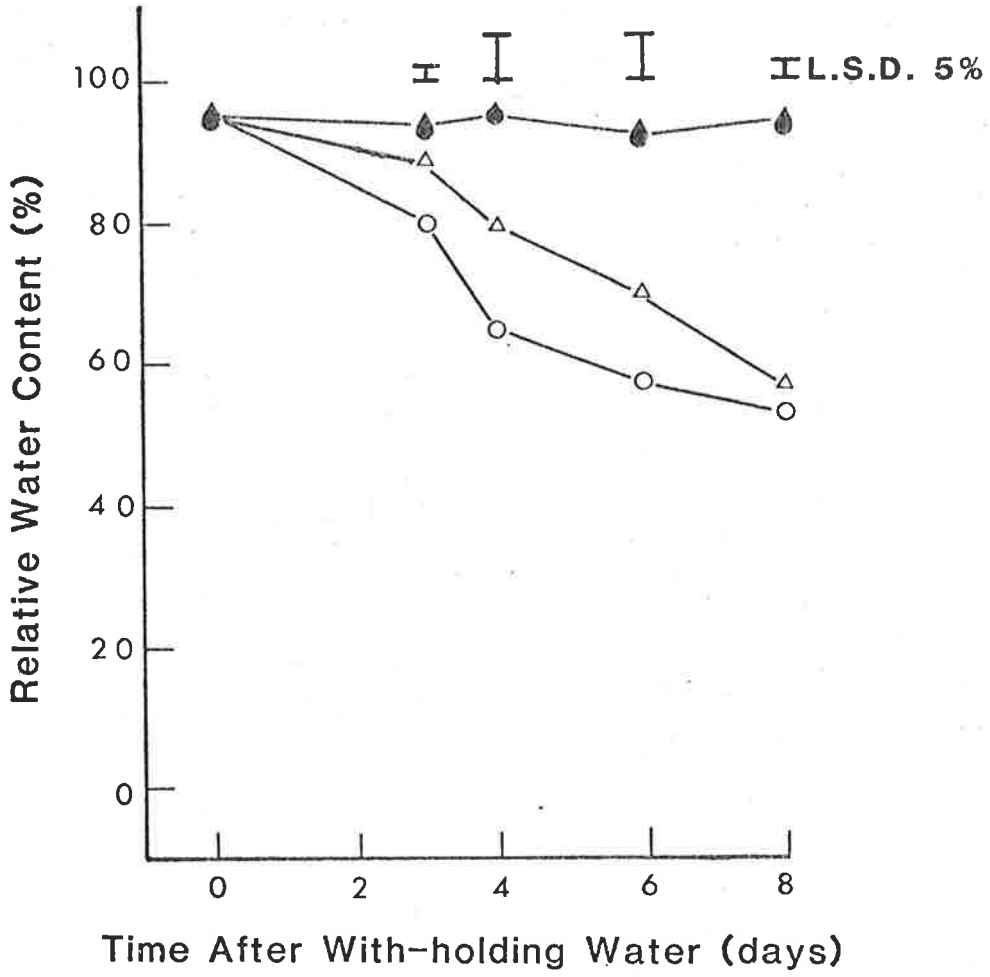

L.S.D. 5%
100
80
60
40
20
0
Relative Water Content (%)
0
2
4
6
8
Time After With-holding Water (days)

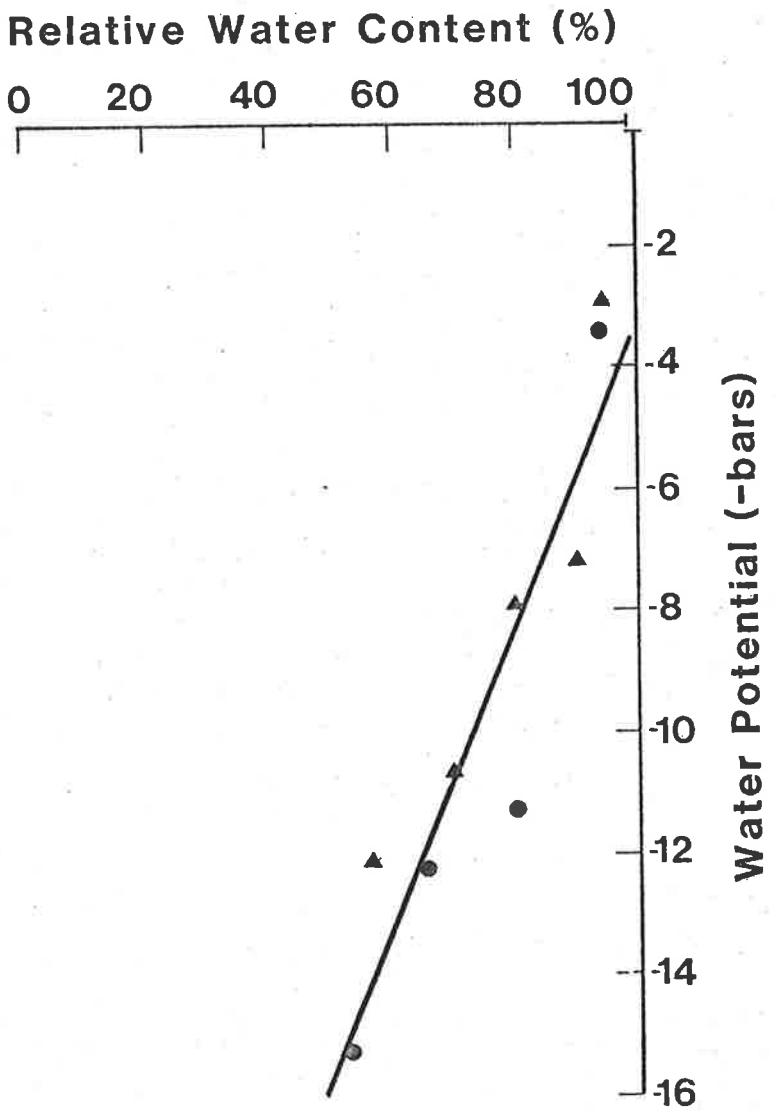
FIG. III.3.3: Relationship between RWC and water potential (moisture release curve).

(●) Control
(▲) CCC

The straight line was fitted by a linear regression analysis.

$$Y = 0.25X - 28.17$$

$$R = 0.934$$


Relative Water Content (%)
0
20
40
60
80
100
-2
-4
-6
-8
-10
-12
-14
-16
Water Potential (-bars)

III.3.3 Osmotic potential and turgor potential

III.3.3.1 *Method*

Tomato plants were grown from pregerminated seeds planted in 127 mm (5 ins) pots containing 1 kg 1:1:1 peat/perlite/vermiculite mixture. The plants were continuously supplied with half strength Hoagland's solution initially and then later with full strength as they grew bigger. 1000 ppm CCC was applied as 150 ml/pot when the plants were about 4 weeks old. Imposition of stress was by with-holding water 3 days after the CCC treatment. Some of the plants were rewatered after 6 days of water stress. Leaf water potential and osmotic potential were measured by the psychrometric method as the stress progressed and also during the recovery period. Turgor potential was deduced from the difference between osmotic potential and water potential. The experiment was carried out in the controlled-environment growth room with conditions as outlined in Section II.2 and was replicated three times.

III.3.3.2 *Results*

Similar to the previous experiments CCC treatment resulted in the maintenance of higher water potential under water stress, but after re-watering both the CCC-treated and untreated plants recovered at the same rate (Fig. III.3.4). Within 12 hours the water potential of all the treatments had reached normal values.

During the period of stress, osmotic potential of the non-CCC treatment was lower (i.e. more negative) than the CCC treatment (Fig. III.3.5) and there was no difference between the two treatments under well-watered conditions. Upon stress alleviation, the osmotic potential of the CCC-treated and untreated plants increased at the same rate but did not reach normal values as quickly as the water potential. The relationship between water potential and osmotic potential, as illustrated in Fig. III.3.6, shows that at a given water potential, the osmotic potential was the same for both treatments; however, stress alleviation altered the relationship at lower osmotic potentials with no effect of CCC.

FIG. III.3.4: Effect of CCC on water potential under stress and recovery.

Control - (●) F.C.
(○) Stress

CCC - (▲) F.C.
(△) Stress

Dotted line —— water potential under recovery.

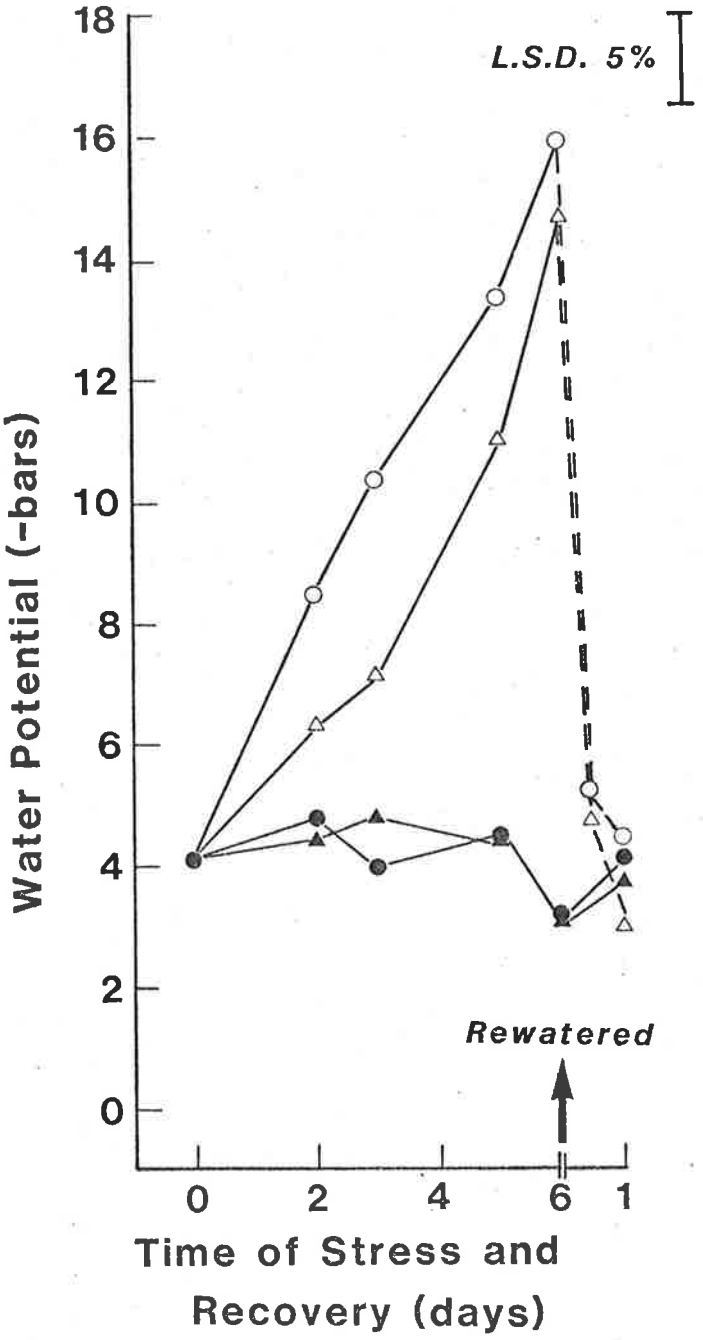

L.S.D. 5%
Water Potential (-bars)
18
16
14
12
10
8
6
4
2
0
Rewatered
0
2
4
6
1
Time of Stress and
Recovery (days)

FIG. III.3.5: Effect of CCC on osmotic potential under stress and recovery.

Control - (●) F.C.
(○) Stress

CCC - (▲) F.C.
(△) Stress

Dotted line —— osmotic potential under recovery.

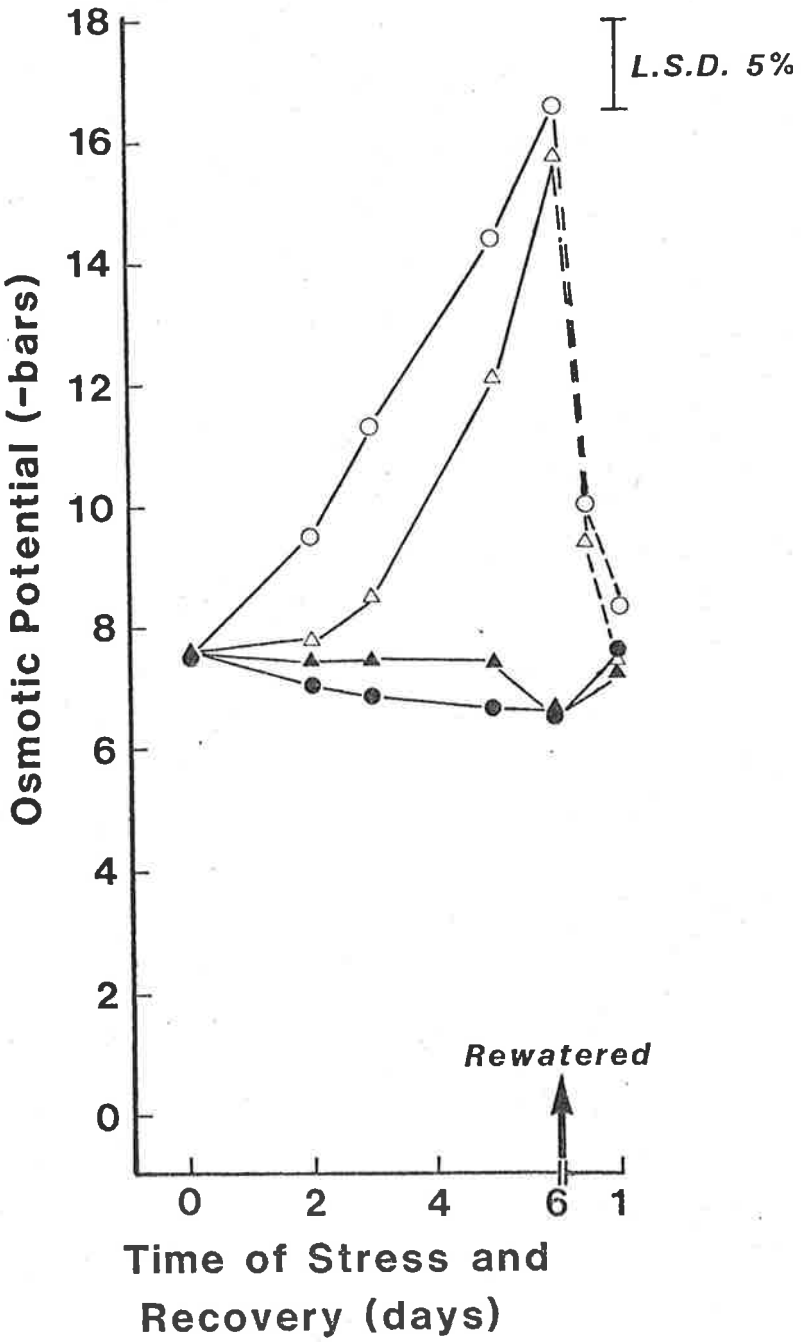
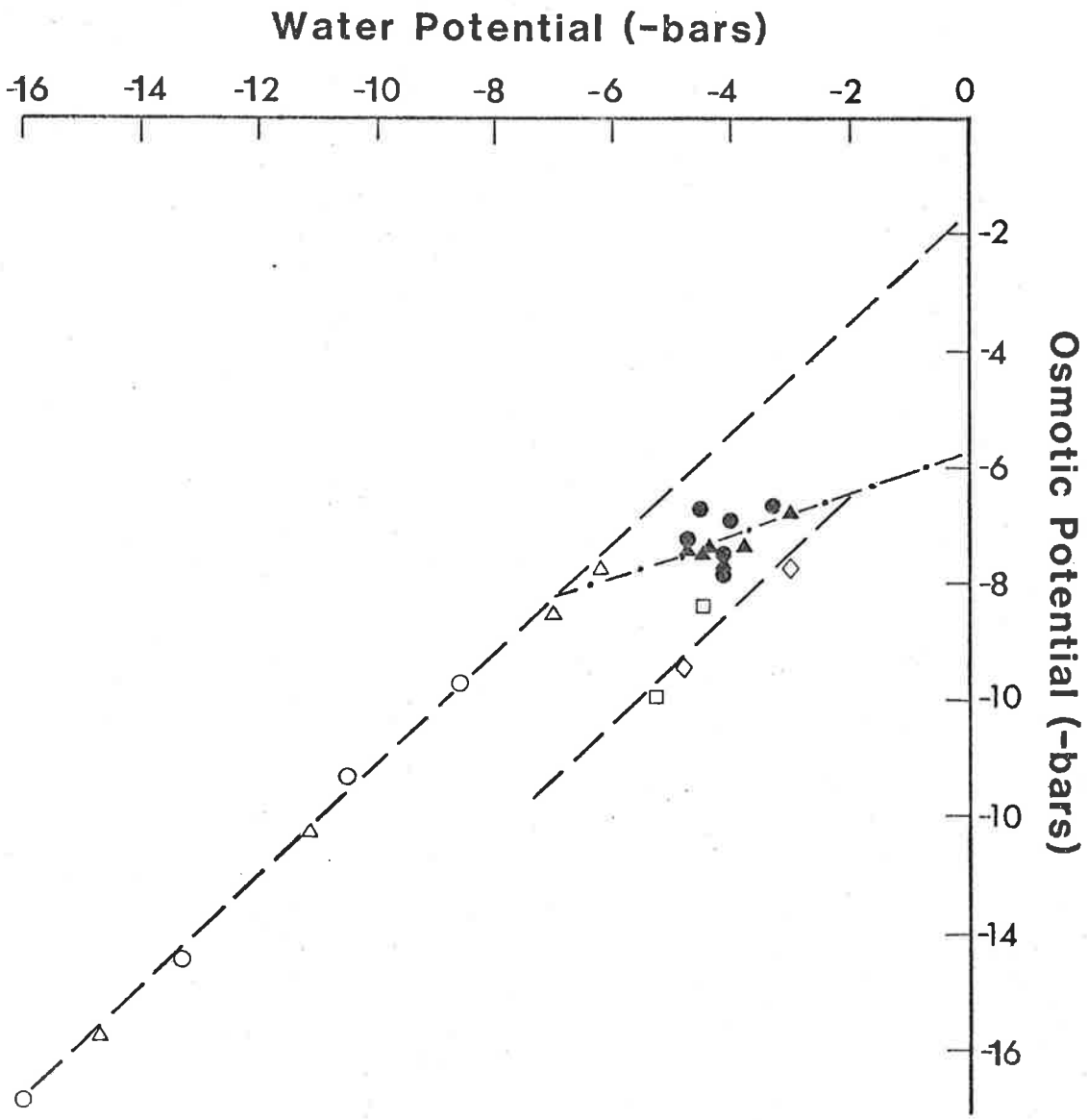

L.S.D. 5%
Osmotic Potential (-bars)
18
16
14
12
10
8
6
4
2
0
Rewatered
0
2
4
6
1
Time of Stress and
Recovery (days)

FIG. III.3.6: Relationship between water potential and osmotic potential.

Control - (●) F.C.
(○) Stress
(□) Recovery

CCC - (▲) F.C.
(△) Stress
(◇) Recovery

Lines fitted after Morgan (1977a).


Water Potential (-bars)
-16
-14
-12
-10
-8
-6
-4
-2
0
Osmotic Potential (-bars)
-2
-4
-6
-8
-10
-10
-14
-16

Turgor potential, deduced from the difference between osmotic potential and water potential, did not differ significantly between the two treatments under water stress or under well-watered conditions (Fig. III.3.7). Turgor was recovered very rapidly in the stressed plants after rewatering, though the rate of recovery was the same for the two treatments. At a given water potential turgor pressure was the same in the two treatments (Fig. III.3.8).

III.3.3.3 *Discussion*

The maintenance of higher water potential due to CCC treatment under water stress (Figs. III.3.1 and III.3.4) supports earlier findings; however, upon rewatering, CCC did not have any effect on rate of recovery of the water potential which could possibly be due to the rapidity with which the plants recovered.

The lower osmotic potential in the non-CCC-treated plants under stress (Fig. III.3.5) may be attributed to the fact that during the stress episode, the non-CCC-treated plants suffered a more severe internal water deficit than the CCC-treated plants as manifested in the water potential data (Fig. III.3.4). This less lowering of osmotic potential in the CCC-treated plants is reflected in the CCC treatment not significantly affecting turgor potential under stress (Fig. III.3.7). Upon stress alleviation osmotic potential increased at the same rate in both treatments but because osmotic potential did not reach normal values as rapidly as water potential (Figs. III.3.4 and III.3.5) there was a dramatic increase in turgor potential which did not differ between the two treatments during recovery period (Fig. III.3.7). Sanchez-Diaz and Kramer (1973) also found that, in maize and sorghum, turgor potential returned to values higher than normal after rewatering.

CCC did not affect the relationship between water potential and osmotic potential suggesting that both the CCC-treated and non-CCC-treated plants reached zero turgor at the same water potential (Fig. III.3.6); however, stress alleviation altered the relationship without any effect of CCC such that zero turgor might be reached at a lower water potential. In plants which can adjust osmotically to resist water stress, osmotic potential is so lowered that zero turgor is reached at a lower

FIG. III.3.7: Effect of CCC on turgor potential under stress and recovery.

Control - (●) F.C.
(○) Stress

CCC - (▲) F.C.
(△) Stress

Dotted line —— turgor potential under recovery.

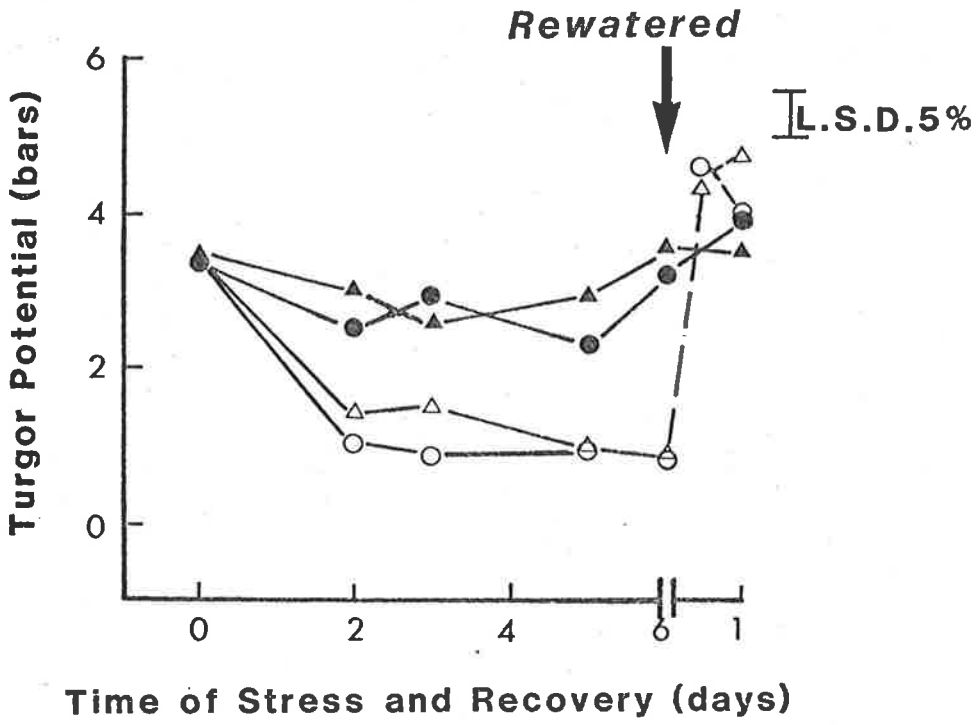
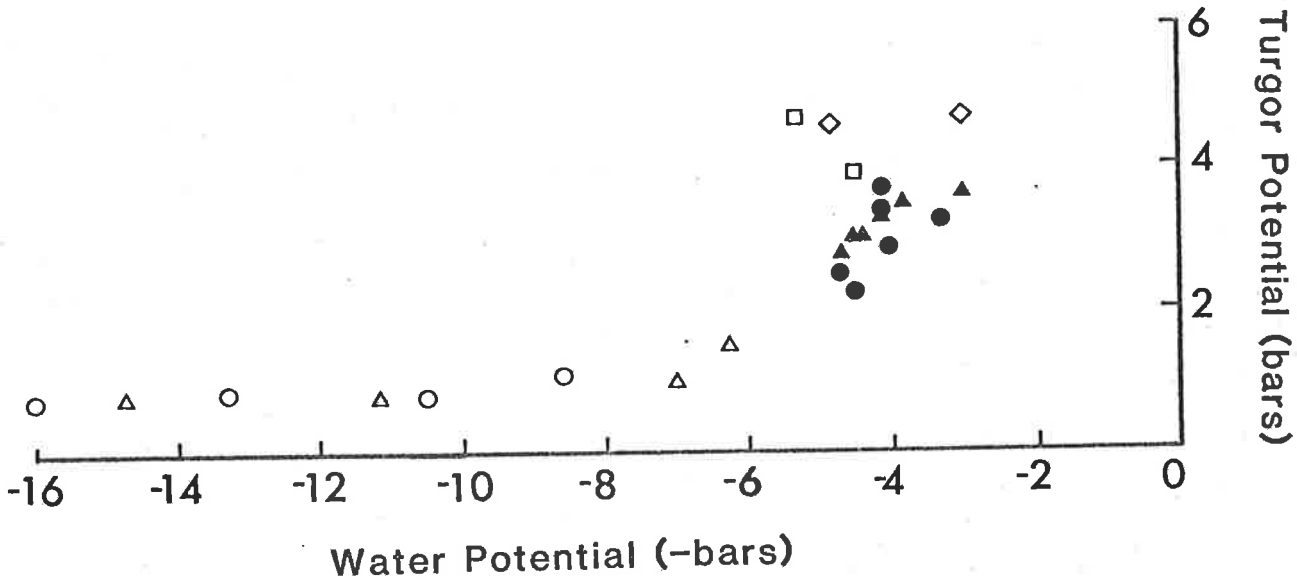

Rewatered
L.S.D.5%
Turgor Potential (bars)
6
4
2
0
0
2
4
1
Time of Stress and Recovery (days)

FIG. III.3.8: Relationship between water potential and turgor potential.

Control - (●) F.C.
(○) Stress
(□) Recovery

CCC - (▲) F.C.
(△) Stress
(◇) Recovery


Turgor Potential (bars)
6
4
2
0
-16
-14
-12
-10
-8
-6
-4
-2
Water Potential (-bars)

water potential (Morgan, 1977a; 1977b). Thus, sorghum can adjust osmotically better than maize and tobacco because its osmotic potential is lowered more (Turner, 1974). CCC, therefore, did not enhance the plants ability to adjust osmotically but stress alleviation did. This may explain the lack of difference in turgor potential at a given water potential (Fig. III.3.8) due to CCC treatment and the high turgor potential values during recovery. The lack of CCC-induced osmotic adjustment is also supported by the fact that CCC did not alter the relationship between water potential and RWC, i.e. the moisture release curve (Fig. III.3.3). According to Jones and Turner (1978) osmotic adjustment and the decrease in elasticity in sorghum under stress caused a shift in the moisture release curve such that there was a small decrease in RWC per unit decrease of water potential.

III.4 CCC AND ACCUMULATION OF PROLINE AND OTHER QUATERNARY AMMONIUM COMPOUNDS

III.4.1 Introduction

The previous results indicated that the prolonged survival of the CCC-treated plants did not involve CCC-induced osmotic adjustment. Singh *et al.* (1973), however, observed that CCC treatment promoted proline accumulation under water stress. Since proline accumulation has been suggested as a possible indicator of stress or drought resistance (Singh *et al.*, 1972; Palfi and Juhasz, 1971), the effect of CCC on proline accumulation in tomato under water stress was explored and, in addition, an attempt was made to ascertain whether quaternary ammonium compounds were accumulated that may be related to plant stress resistance.

Proline was extracted by the method outlined in Section II.9.1 in two separate experiments. In both experiments, the plants were grown in 127 mm (5 ins) pots containing 1 kg recycled soil and were supplied with half strength Hoagland's solution twice a week. Water stress was induced by with-holding water from the plants 3 days after CCC application. In both experiments, however, proline was extracted from the uppermost fully developed leaf of CCC-treated and untreated plants under water stress and

well-watered conditions. Water potential of the sample leaves was recorded. In yet another experiment, NMR techniques, outlined in Section II.9.2, were used to study the quaternary ammonium compounds which accumulated in the leaves of both the CCC-treated and untreated plants; leaf water potential was again measured and the plants were grown under similar conditions as described above.

III.4.2 Results

The data from the first experiment in which proline was extracted are presented in Fig. III.4.1 and Fig. III.4.2 for water potential and proline accumulation respectively. CCC-treated plants maintained higher water potential than the untreated plants under water stress induced by with-holding water (Fig. III.4.1). The untreated plants under water stress accumulated more proline than the CCC-treated plants until the 6th day after with-holding water when the CCC treatment overtook the non-CCC treatment, though CCC did not significantly increase the amount of proline accumulated by the 8th day of stress. The proline content remained the same in the non-stressed plants over the stress period and did not differ between the CCC-treated and non-CCC-treated plants (Fig. III.4.2). The results from the second experiment showed that CCC treatment maintained better water status under stress than the non-CCC treatment (Fig. III.4.3) and this agrees with the data from the first experiment (Fig. III.4.1). The trend of proline accumulation in the second experiment (Fig. III.4.4) was similar to that of the first experiment (Fig. III.4.2) except that the amounts of proline were less in the second experiment.

The NMR spectra (Figs. III.4.5 and III.4.6) indicated that CCC and choline were the major quaternary ammonium compounds detected as was proline in the leaves of the CCC-treated plants (Fig. III.4.5) while choline and proline alone were detected in the untreated plants (Fig. III.4.6). From Table III.4.1, the proline content in the leaves of the untreated plants under stress was higher than in the leaves of the CCC treatment but water potential was lower in the untreated plants (-11.20 bars) than the CCC-treated plants (-9.80 bars). The amount of choline was affected neither by CCC nor stress. The CCC content was higher in the well-watered than the stressed plants.

FIG. III.4.1: Effect of CCC and water stress on water potential.

Control - (●) F.C.
(○) Stress

CCC - (▲) F.C.
(△) Stress

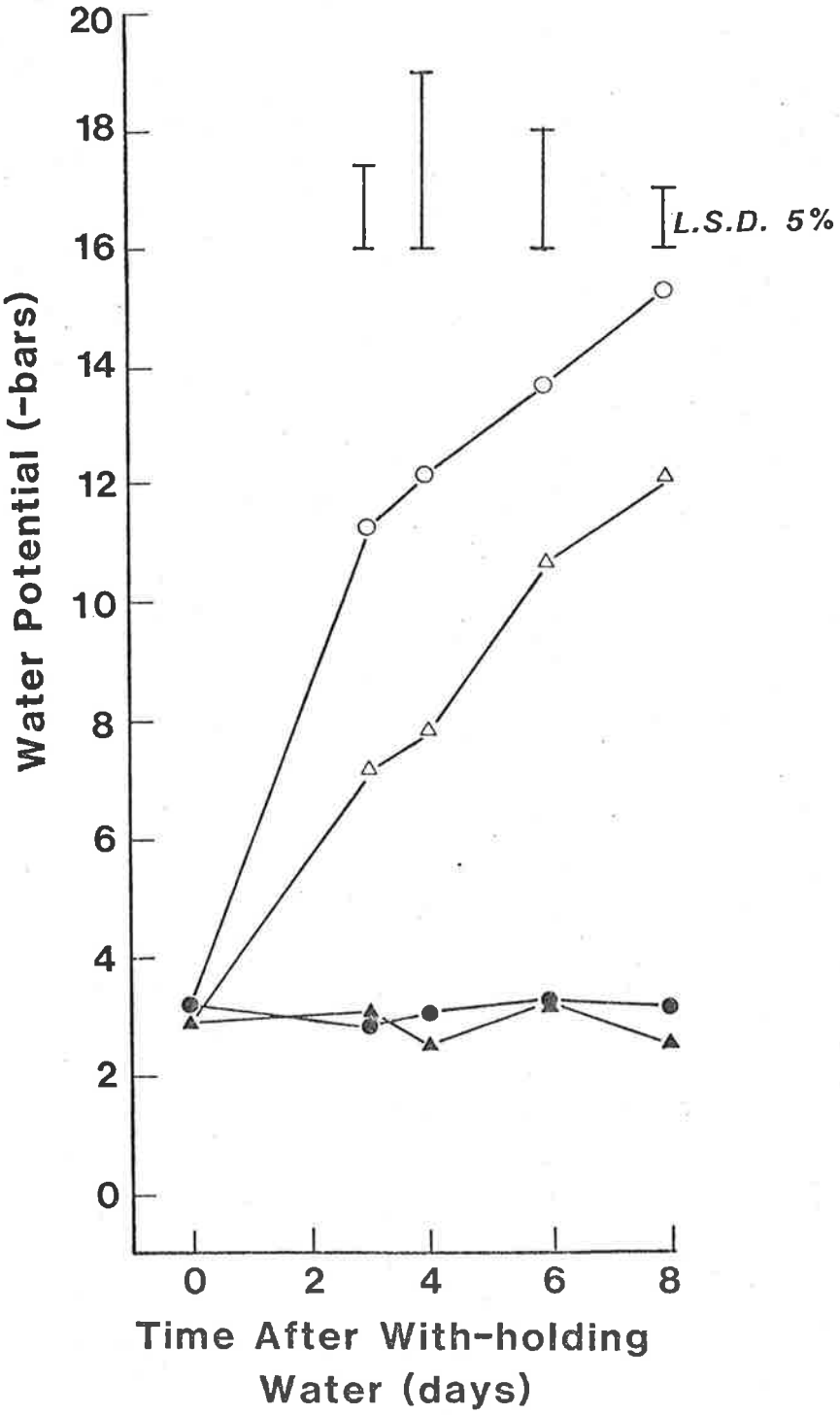

20
18
16
14
12
10
8
6
4
2
0
Water Potential (-bars)
L.S.D. 5%
0
2
4
6
8
Time After With-holding
Water (days)

FIG. III.4.2: Effects of CCC and water stress on proline accumulation.

Control - (●) F.C.
(○) Stress

CCC - (▲) F.C.
(△) Stress

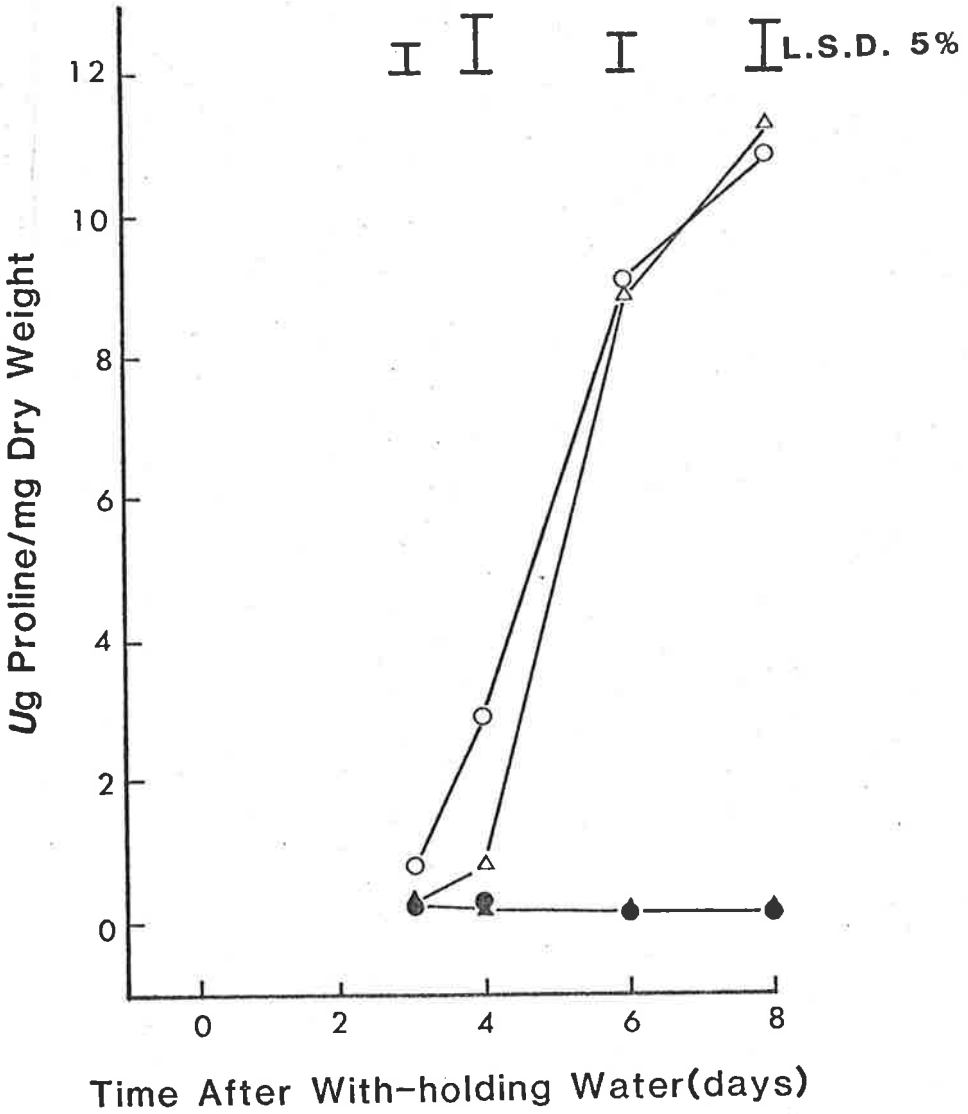

L.S.D. 5%
12
10
8
6
4
2
0
0
2
4
6
8
Ug Proline/mg Dry Weight
Time After With-holding Water(days)

FIG. III.4.3: Effects of CCC and water stress on water potential.

Control - (●) F.C.
(○) Stress

CCC - (▲) F.C.
(△) Stress

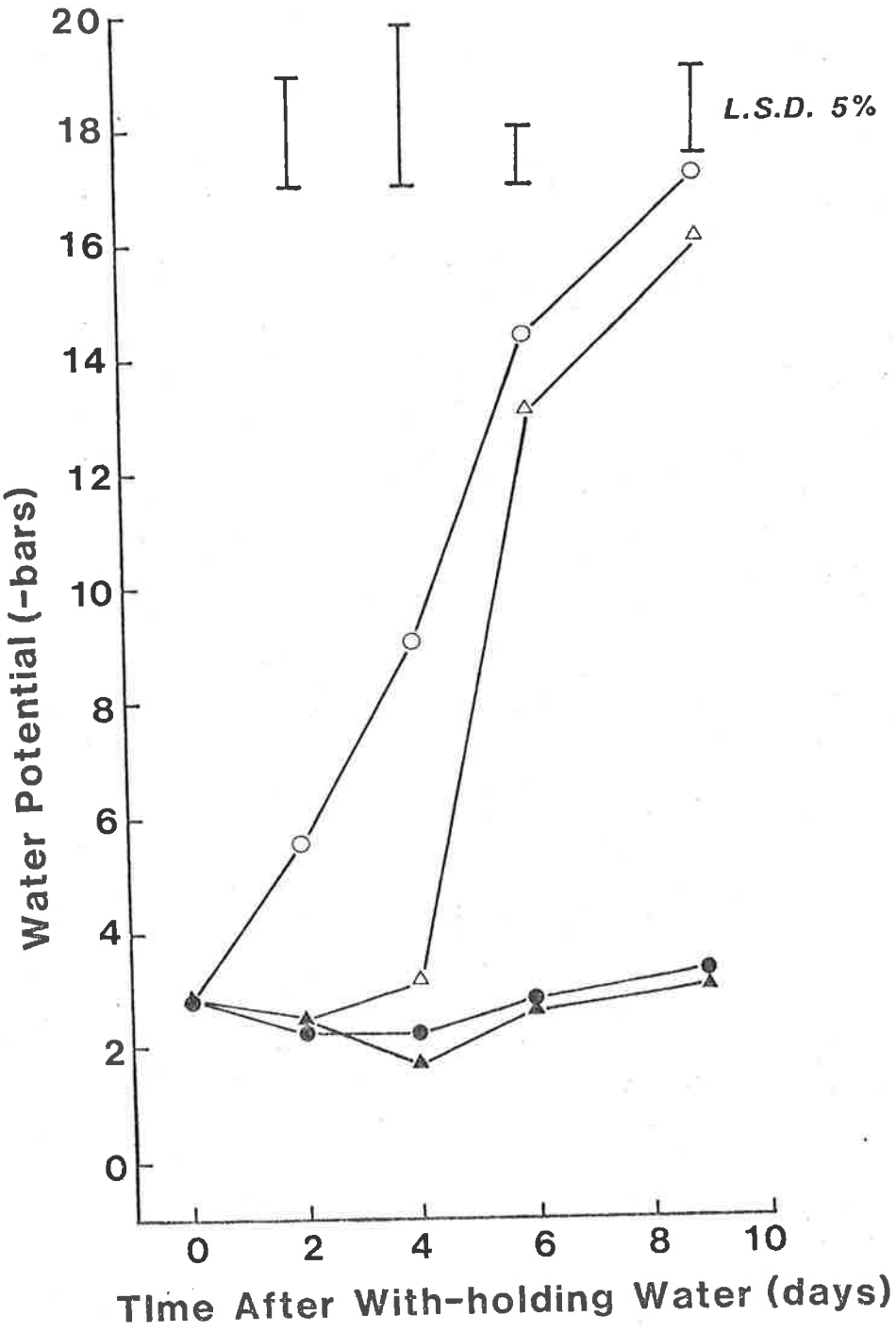

20
18
16
14
12
10
8
6
4
2
0
L.S.D. 5%
Water Potential (-bars)
0
2
4
6
8
10
Time After With-holding Water (days)

FIG. III.4.4: Effects of CCC and water stress on proline accumulation.

Control - (●) F.C.
(○) Stress

CCC - (▲) F.C.
(△) Stress

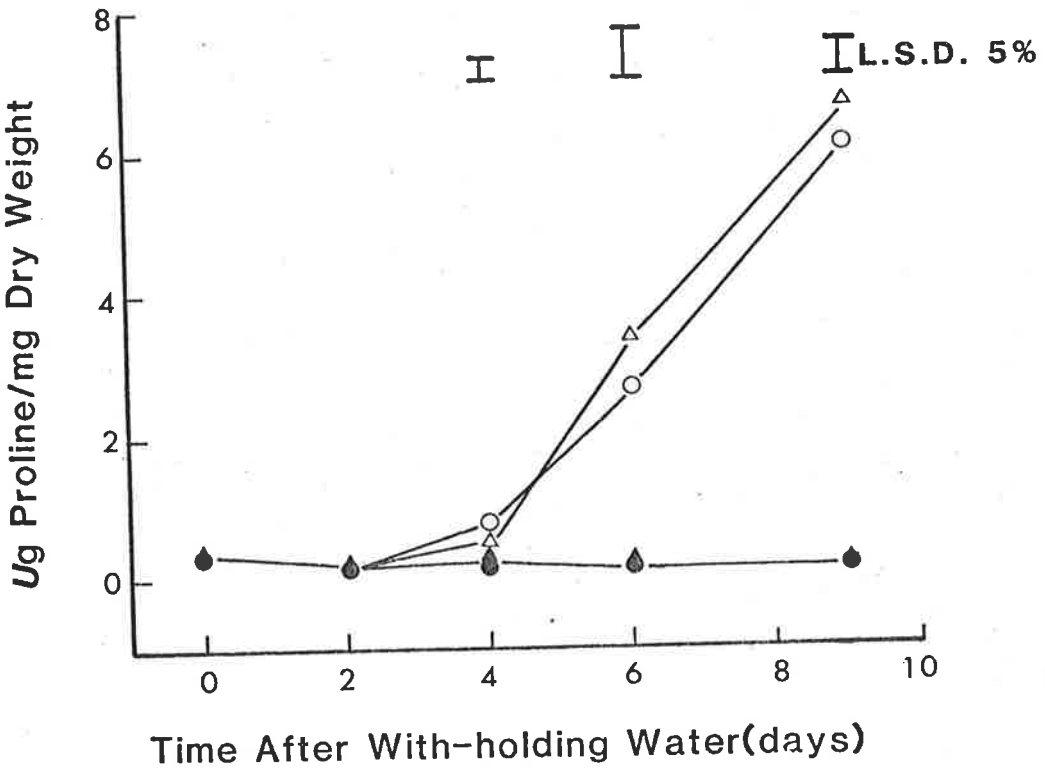

L.S.D. 5%
Ug Proline/mg Dry Weight
8
6
4
2
0
0
2
4
6
8
10
Time After With-holding Water(days)

FIG. III.4.5: NMR spectrum of an extract of a CCC-treated plant's leaf, showing choline, CCC and proline peaks.

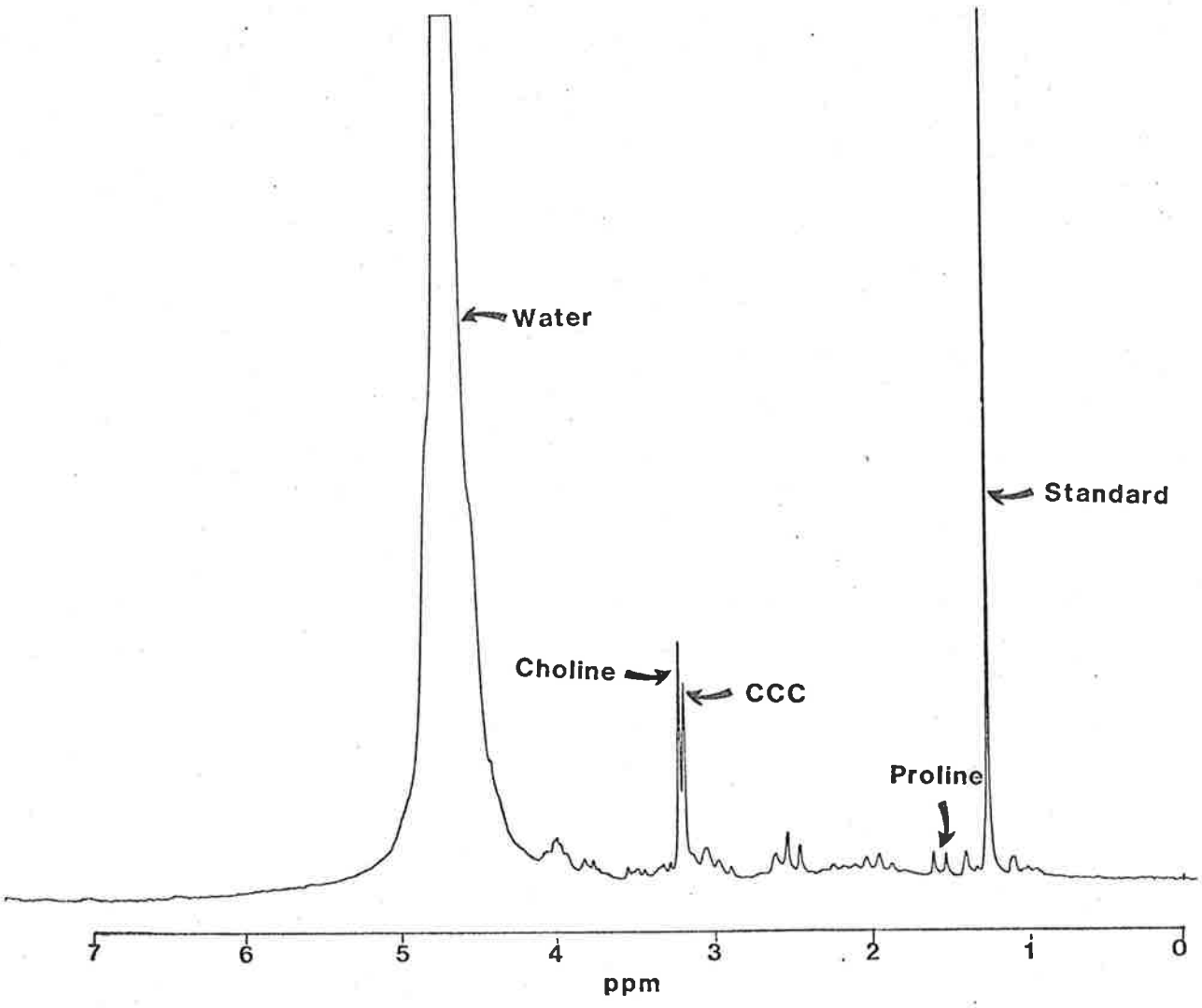

Water
Standard
Choline
CCC
Proline
7
6
5
4
3
2
1
0
ppm

FIG. III.4.6: NMR spectrum of an extract of a non-CCC-treated plant's leaf, showing choline and proline peaks.

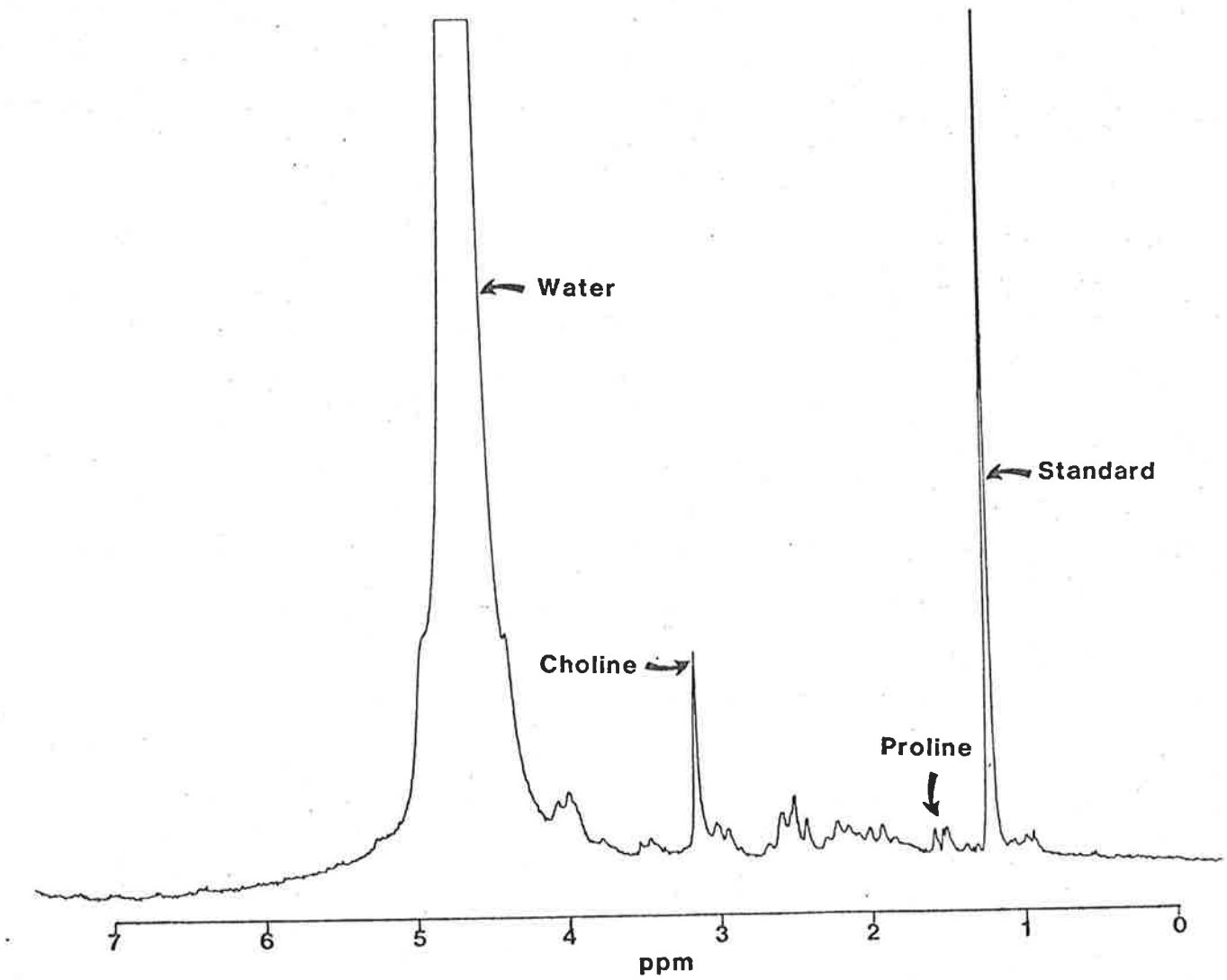

Water
Standard
Choline
Proline
7
6
5
4
3
2
1
0
ppm

TABLE III.4.1: Proline, choline and CCC content of leaf estimated by the NMR technique.

	Water potential (-bars)	µg/mg dry weight		
		Proline	Choline	CCC
Control F.C.	4.00	4.15	0.63	
Control stress	11.20	6.78	0.69	
CCC F.C.	4.20	-	0.78	3.40
CCC stress	9.80	5.24	0.62	1.07
L.S.D. 5%	2.05	1.00	0.22	1.23

III.4.3 Discussion

The maintenance of higher water potential, under water stress conditions, due to CCC treatment (Fig. III.4.1 and Fig. III.4.3), supports the repeatable effect of CCC-induced enhancement of water status under water stress induced by with-holding water from the plants, as found in the previous experiments.

Overall, CCC did not increase the plants' ability to accumulate proline in the leaves under water stress. The initial lag of proline accumulation in the CCC-treated plants may be attributed to the delay in the onset of severe internal water deficit, as manifested by the water potential data (Figs. III.4.1 and III.4.3). This suggests that the accumulation of proline is dependent on the water potential, as has been suggested by Aspinall and Paleg (1981). Though Singh *et al.* (1973) found that CCC promoted proline accumulation in wheat, the water potential declined to the same extent in both the CCC-treated and non-CCC-treated plants as a result of the PEG-induced stress. However, in addition to using different tissue, Singh *et al.* (1973) imposed stress by a different method.

Choline levels were high in the tomato leaves but neither CCC nor stress promoted its accumulation (Table III.3.1). This supports the finding of Mayr and Paxton (1962) who observed that the main quaternary ammonium base extractable from untreated tomato plants was an unidentifiable substance which was readily converted to choline. The occurrence of CCC in the leaves of the CCC-treated plants (Table III.3.1) suggests that CCC was translocated from the root zone, where it was applied, to the leaves. According to Birecka (1967), a majority of radioactive CCC supplied to the root of wheat was translocated to the leaves and a similar observation was made by Dekhuijzen and Vonk (1974). However, the marked decrease in the level of CCC under water stress, as in Table III.3.1, is hard to explain though the possibility that CCC might have been degraded faster under water stress is not over-ruled.

The apparent lack of CCC in promoting the accumulation of solutes under water stress may explain the lack of osmotic adjustment as discussed in Section III.3.4.

III.5 EFFECT OF CCC ON LEAF DIFFUSIVE RESISTANCE

III.5.1 Introduction

It has been reported that CCC induces stomatal closure and, thereby, increases leaf diffusive resistance. Mishra and Pradhan (1968 and 1972) reported that CCC treatment caused stomatal closure in tomato. Pill *et al.* (1979) observed an increased leaf diffusive resistance in tomato due to CCC treatment and similar observations have been made with sunflower by Lovett and Campbell (1973) and wheat by De *et al.* (1982).

Since CCC-induced stomatal closure would imply reduced transpirational water loss, it became imperative to study some of the effects of CCC on tomato leaf diffusive resistance. In this section the effects of CCC on the diffusive resistance in well-watered tomato plants is examined.

Firstly, the effect of CCC on the diurnal pattern of leaf diffusive resistance was studied in the glasshouse as well as in the controlled-environment growth room. Three and a half week old tomato plants grown in the glasshouse, were treated with 1000 ppm CCC as a soil drench and the control plants were watered as normal. On the 4th day after CCC treatment, leaf diffusive resistances were measured with a Li-Cor diffusive resistance porometer, as outlined in Section II.8.1.2, on both the adaxial (upper) and abaxial (lower) surfaces of the uppermost fully developed leaf (4th leaf). The measurements were done over a day-length period of 10 hours - from 8 a.m. to 6 p.m. In the controlled-environment growth room experiment, however, the adaxial diffusive resistance was also monitored daily at about 9.30 a.m. over a period of seven days, starting from the first day after CCC treatment. Diffusive resistance measurements were replicated 3 times. In another experiment, about 4 weeks old plants were treated with 1000 ppm CCC. Five days after CCC treatment, the adaxial and abaxial diffusive resistances of the 4th leaf, leaf water potential, transpiration rate - in intact plant and excised leaves, and leaf area were measured. At the same time, in another batch of plants, after measuring the adaxial and abaxial leaf diffusive resistances of the 4th leaves, they were excised and the transpiration rates of either the adaxial or abaxial surface measured by covering one surface with vacuum grease to block transpirational water loss from that surface. Fifteen days after CCC treatment, the adaxial and abaxial diffusive resistances, leaf water potential, transpiration rate and leaf area were measured on the last

batch of plants. This experiment was carried out in the growth room and replicated three times.

III.5.2 Results

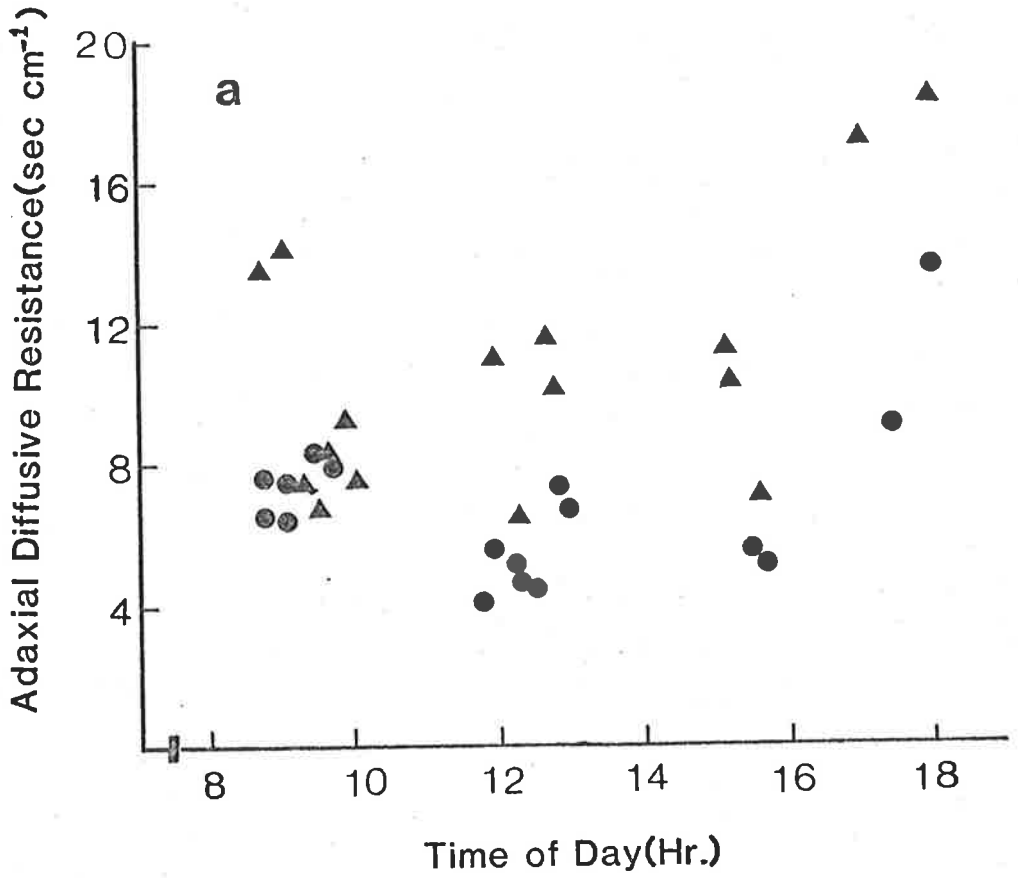
The results of a comparative study of the diurnal pattern of leaf diffusive resistance in CCC-treated and untreated plants in the glasshouse and controlled-environment growth room are presented in Figs. III.5.1 and III.5.2. In the glasshouse the adaxial resistances of both the CCC-treated and untreated plants showed a slight decrease in the afternoon (i.e. 12-16 hr) and then increased (Fig. III.5.1a). In the growth room, these adaxial resistances showed an increasing diurnal pattern (Fig. III.5.2a). Abaxial resistances (both CCC and non-CCC) did not show any marked diurnal variation (Figs. III.5.1b and III.5.2b) under the two growth environments. CCC markedly increased adaxial diffusive resistance irrespective of the growth environment but did not influence the abaxial resistance (Figs. III.5.1 and III.5.2). This CCC-induced increase in adaxial resistance commenced a day after the CCC treatment and persisted for 7 days, though after the 4th day the increase slightly diminished (Fig. III.5.3).

A further aim was to explore the growth retardation effect of CCC and its relationship with the CCC-induced increase in leaf diffusive resistance. Table III.5.1 shows that 5 days after CCC treatment, when the growth of plants has not been retarded (plant height and leaf area are not reduced), it caused an increase in the adaxial diffusive resistance, but not the abaxial, which was attended with a decrease in transpiration rate. Fifteen days after CCC application, the height of the plant and the leaf area were significantly reduced by CCC which did not cause a significant increase in adaxial resistance nor a significant reduction in transpiration rate. This suggests that the CCC-induced increase in adaxial resistance and, hence, decreased transpiration may diminish when CCC treatment causes a reduction in leaf area (or growth). CCC may also be metabolised by this time, or be translocated elsewhere in the plant.

The relationship between diffusive resistance and transpiration by adaxial and abaxial surfaces in light and in darkness is presented in Table III.5.2. CCC increased the adaxial diffusive resistance in light and hence decreased the transpirational water loss from the adaxial surface with-

FIG. III.5.1: Diurnal pattern of (a) adaxial and (b) abaxial diffusive resistances in CCC-treated and control plants, in the glasshouse.

(●) Control
(▲) CCC


a
Adaxial Diffusive Resistance(sec cm⁻¹)
20
16
12
8
4
8
10
12
14
16
18
Time of Day(Hr.)

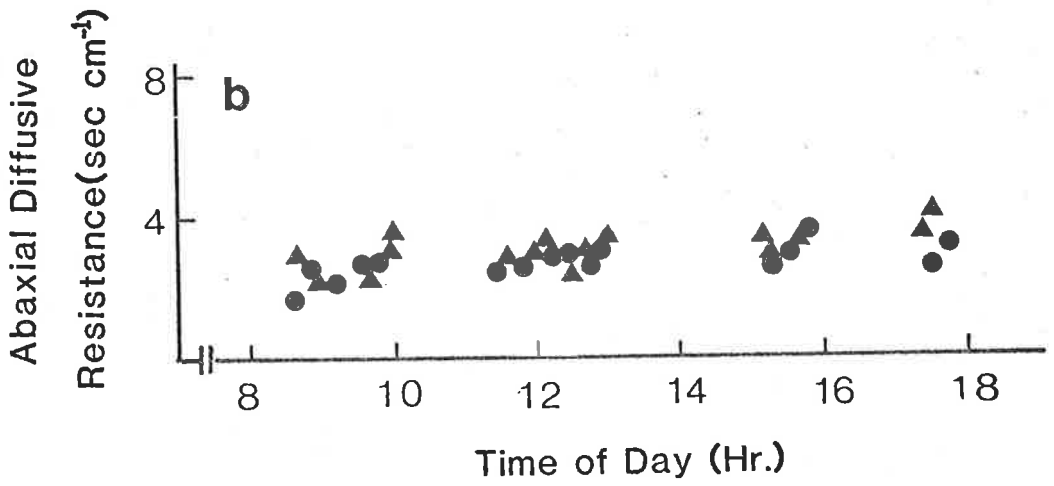

b
Abaxial Diffusive Resistance(sec cm⁻¹)
8
4
8
10
12
14
16
18
Time of Day (Hr.)

FIG. III.5.2: Diurnal pattern of (a) adaxial and (b) abaxial diffusive resistances in CCC-treated and control plants, in the growth room.

(●) Control
(▲) CCC

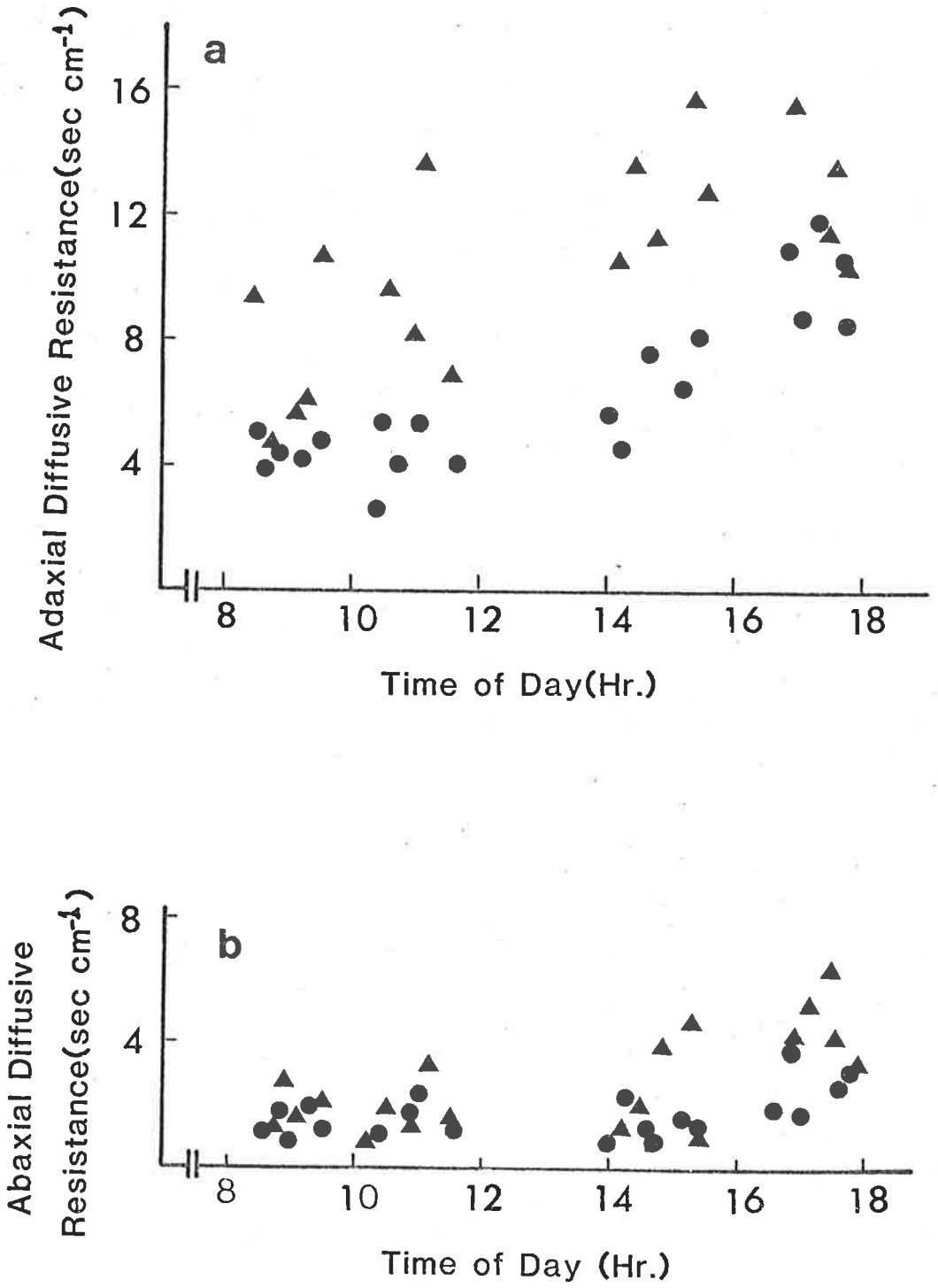

a
Adaxial Diffusive Resistance(sec cm-1)
16
12
8
4
8
10
12
14
16
18
Time of Day(Hr.)
b
Abaxial Diffusive Resistance(sec cm-1)
8
4
8
10
12
14
16
18
Time of Day (Hr.)

FIG. III.5.3: Effect of CCC on adaxial diffusive resistance within a period of 7 days.

(●) Control
(▲) CCC

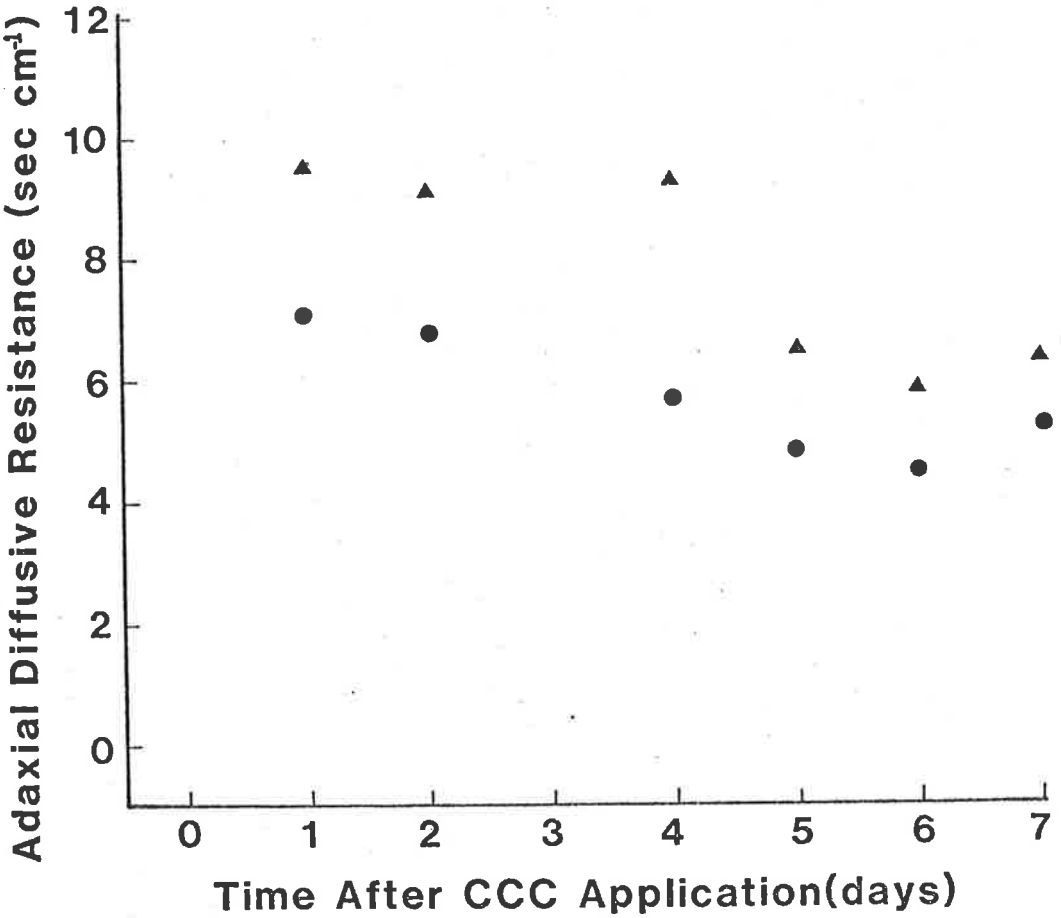

Adaxial Diffusive Resistance (sec cm-1)
12
10
8
6
4
2
0
0
1
2
3
4
5
6
7
Time After CCC Application(days)

TABLE III.5.1: Relationship between plant size, diffusive resistance and transpiration.

(a) - 5 days after CCC treatment

	Water potential (-bars)	Plant height (cm)	Leaf area (dm^2)	Diffusive resistance (sec cm^{-1}) Adaxial	Abaxial	Transpiration rate ($g/dm^2/hr.$) Intact plant	Excised leaf
Control	3.67	21.87	6.37	7.66	2.66	0.64	0.57
CCC	3.67	19.60	6.32	10.68	3.53	0.56	0.49
L.S.D. 5%	-	-	-	2.46	-	0.08	0.06

(b) - 15 days after CCC treatment

Control	3.91	40.90	12.13	11.08	3.06	0.45	0.35
CCC	3.83	22.17	8.95	12.04	3.16	0.42	0.39
L.S.D. 5%	-	5.80	2.80	-	-	-	-

TABLE III.5.2: Diffusive resistance of intact plant and transpiration rate of the Adaxial and Abaxial surfaces of excised leaves. Measurements were taken 5 days after CCC treatment.

	Diffusive resistance (Sec cm^{-1})				Transpiration rate ($g/dm^2/hr.$)			
	Light		Darkness		Light		Darkness	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Control	7.76	3.96	19.80	9.74	0.30	0.45	0.19	0.31
CCC	10.89	3.98	19.29	9.34	0.23	0.45	0.19	0.30
L.S.D. 5%	2.69		2.69		0.04		-	

out any effect on the abaxial surface. In darkness, however, CCC did not have any effect on adaxial and abaxial diffusive resistances or their transpiration rates.

III.5.3 Discussion

The slight decrease in adaxial diffusive resistances in both the CCC-treated and non-CCC-treated plants in the afternoon, as happened in the glasshouse (Fig. III.5.1a), portrays the normal diurnal trend under natural light (Jordan and Ritchie, 1971; Meyer and Green, 1981), and this is usually attributed to fluctuations in the intensity of sunlight. In the growth room, the increasing diurnal pattern of adaxial resistance may be attributed to the effect of the constant light intensity (Section II.2).

The glasshouse measurements of leaf diffusive resistances were consistent with the growth room measurements in that CCC induced an increase in the adaxial resistance but not the abaxial resistance under both environments. This differential effect of CCC in increasing the adaxial resistance but not the abaxial can be explained in terms of the different and independent behaviour of the stomata on the two surfaces. Kanemasu and Tanner (1969b) reported that the adaxial and abaxial stomata reacted differently to light. They observed that the abaxial stomata were fully open leading to a low abaxial diffusive resistance at a light intensity which was low enough to close the adaxial stomata and increase adaxial diffusive resistance. The same authors (1969a) also found a differential effect of water deficit on the adaxial and abaxial surface resistances in that the adaxial diffusive resistance increased sharply at a lower water potential than the adaxial. Distinct differential behaviour of adaxial and abaxial stomatal diffusive resistances at the same light level has also been found in sorghum and tobacco (Turner, 1968), and in cotton, snap bean, rough lemon, and corn (Erhler and van Bavel, 1968). Differences between the responses of adaxial and abaxial stomatal aperture to light in *Stachytarpheta indica*, *Coreopsis grandiflora* and *Crotolaria retusa* have been reported by Pemadasa (1979). Apparently, therefore, this differential behaviour of the stomata in the adaxial and abaxial leaf surfaces is a widespread phenomenon. The effect of CCC in differentially increasing adaxial but not abaxial resistance as reported here, is supported by the work of Moreshet (1975), who observed that phenyl-mercuric acetate (PMA), applied to both the adaxial and abaxial surfaces of sunflower

leaves caused a greater closure of the adaxial stomata than the abaxial.

The CCC-induced increase in adaxial diffusive resistance results in a decrease in transpiration from the adaxial surface, as evident from Table III.5.2, and contributes significantly to the reduced water loss from the treated plants (Table III.5.1a). Though the increased adaxial resistance due to CCC persists for at least a week (Fig. III.5.3), the effect decreases in about 2 weeks (Table III.5.1b). This agrees with evidence from Mishra and Pradhan (1972), who observed a decrease in the magnitude of CCC-induced stomatal closure in tomato with time.

III.6 EFFECTS OF CCC AND WATER STRESS ON LEAF DIFFUSIVE RESISTANCE

III.6.1 Introduction

The effect of CCC on leaf diffusive resistance, as discussed in Section III.5, indicated that CCC treatment caused a differential increase in adaxial diffusive resistance but not in abaxial, in well-watered tomato plants. Since CCC treatment induces the maintenance of higher water potential in the treated plants, as has been mentioned previously in this thesis, it became necessary to investigate the effects of CCC and water stress on leaf diffusive resistance.

The materials and methods used were the same as described in Section III.3.3.1 and data presented below involving leaf diffusive resistance were obtained by measurements on the same plants as in Section III.3.3.1. Leaf diffusive resistance was measured with a Li-Cor diffusive resistance porometer and measurements were made between 8.30 a.m. and 10.00 a.m.

III.6.2 Results

The data for water potential has already been presented in Fig. III.3.4 (Section III.3.3.2). The relationships between adaxial and abaxial diffusive resistances, and the time of stress and recovery from stress, are presented in Figs. III.6.1 and III.6.2, respectively. CCC delayed the sharp increase in both the adaxial and abaxial diffusive resistances and the diffusive resistances of the CCC-treated plants remained significantly lower

FIG. III.6.1: Effect of CCC on adaxial diffusive resistance under stress and recovery.

Control - (●) F.C.
(○) Stress

CCC - (▲) F.C.
(△) Stress

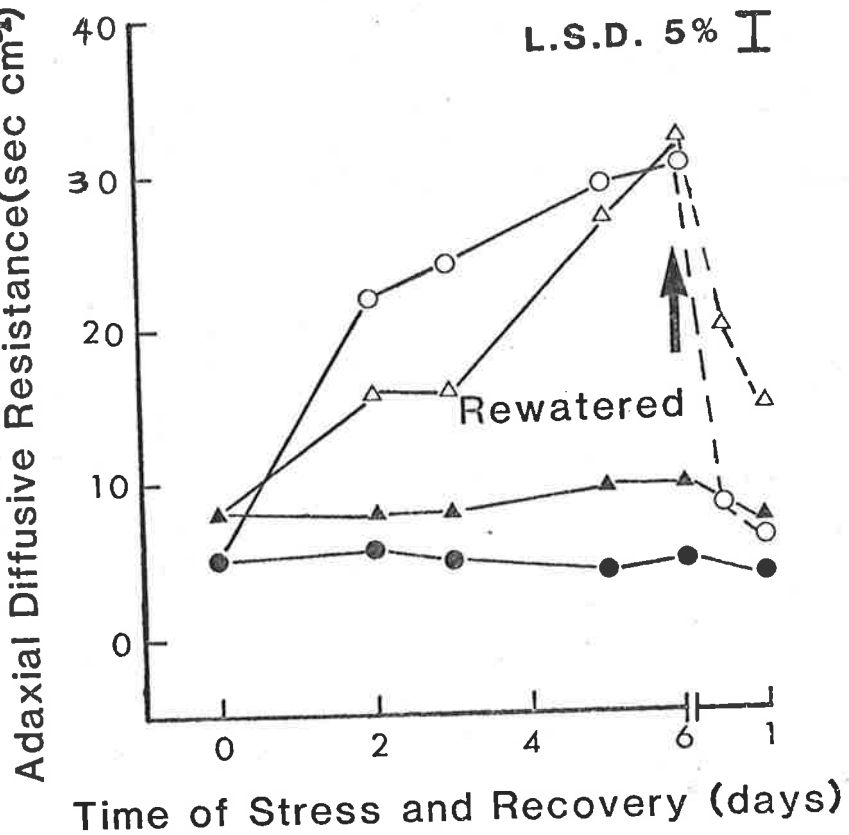
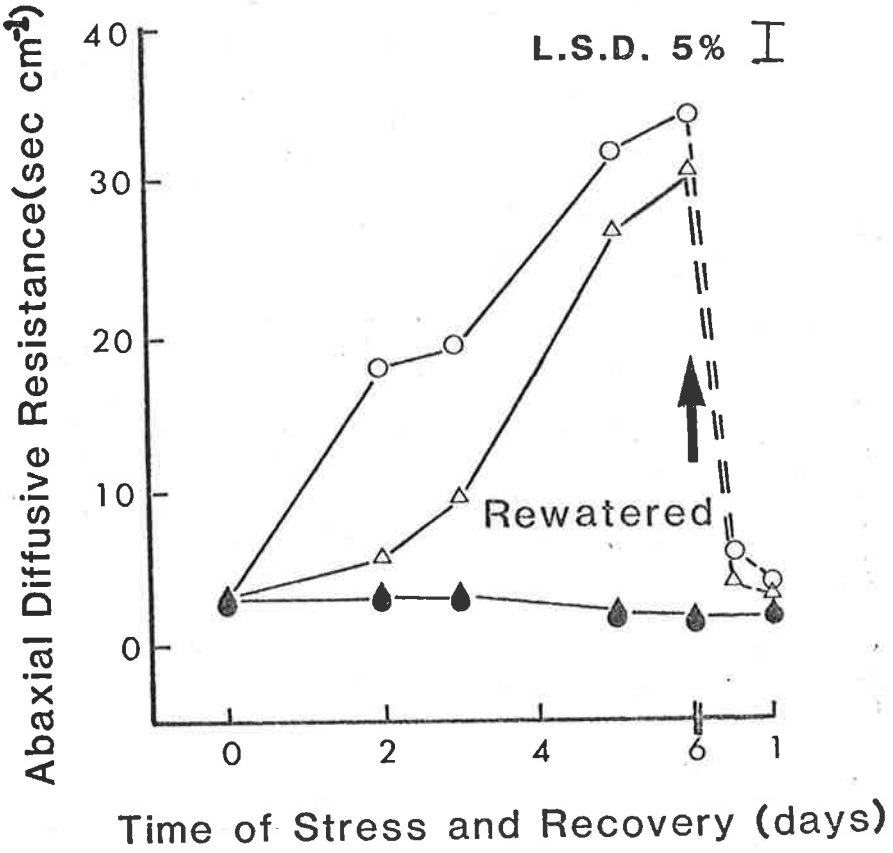

L.S.D. 5%
Adaxial Diffusive Resistance(sec cm⁻¹)
40
30
20
10
0
Rewatered
0
2
4
6
1
Time of Stress and Recovery (days)

FIG. III.6.2: Effect of CCC on abaxial diffusive resistance under stress and recovery.

Control - (●) F.C.
(○) Stress

CCC - (▲) F.C.
(△) Stress


L.S.D. 5%
Abaxial Diffusive Resistance(sec cm-1)
40
30
20
10
0
Rewatered
0
2
4
6
1
Time of Stress and Recovery (days)

than those of the untreated plants till the 5th day of stress on the adaxial surface, and even longer on the abaxial surface. When plants under stress were rewatered, adaxial resistances did not recover to normal values after a day and was higher in the CCC treatment; abaxial resistances recovered to normal values and did not show any CCC effect. Again, in the well-watered plants, CCC induced an increase in adaxial resistance (Fig. III.6.1) but not abaxial.

The relationships between adaxial and abaxial resistances and water potential showed a linear scatter but did not show any CCC-mediated effect on either the adaxial nor abaxial resistances (Figs. III.6.3 and III.6.4). When diffusive resistance values were transformed into their reciprocals, which are normally referred to as conductances, the scatters depicted curves, shown in Figs. III.6.5 and III.6.6 for adaxial and abaxial, respectively, and there was no effect of CCC. Both the adaxial and abaxial conductances decreased as water potential declined but became fairly constant below -7 bars and -9 bars in the adaxial and abaxial, respectively.

III.6.3 Discussion

The finding that adaxial and abaxial diffusive resistances are initally lower in the CCC-treated plants under water stress may imply that the stomata on both surfaces do not close as quickly as in the untreated plants under stress. This effect may be explained in terms of the CCC treatment delaying the onset of severe plant water deficit in the treated plants, as shown in Fig. III.3.4, in response to soil water deficit. As evident from Figs. III.6.5 and III.6.6, CCC has no effect on the decline of leaf conductance with declining water potential; however, there appears to be a threshold water potential of about -7 bars and -9 bars for adaxial and abaxial stomatal closure, respectively. Hsiao (1973) has discussed the existence of a threshold water potential, below which leaf diffusive resistance and, therefore, stomatal opening, remains constant. The present results agree with the finding of Duniway (1971) in that the water potential threshold for stomatal closure in tomato is about -7 and -9 bars. Moreover, the fact that abaxial stomata close at a lower water potential than adaxial stomata has already been found in snap bean by Kanemasu and Tanner (1969) and in cotton by Brown *et al.* (1976).

FIG. III.6.3: Relationship between water potential and adaxial diffusive resistance.

Control - (●) F.C.
(○) Stress

CCC - (▲) F.C.
(△) Stress

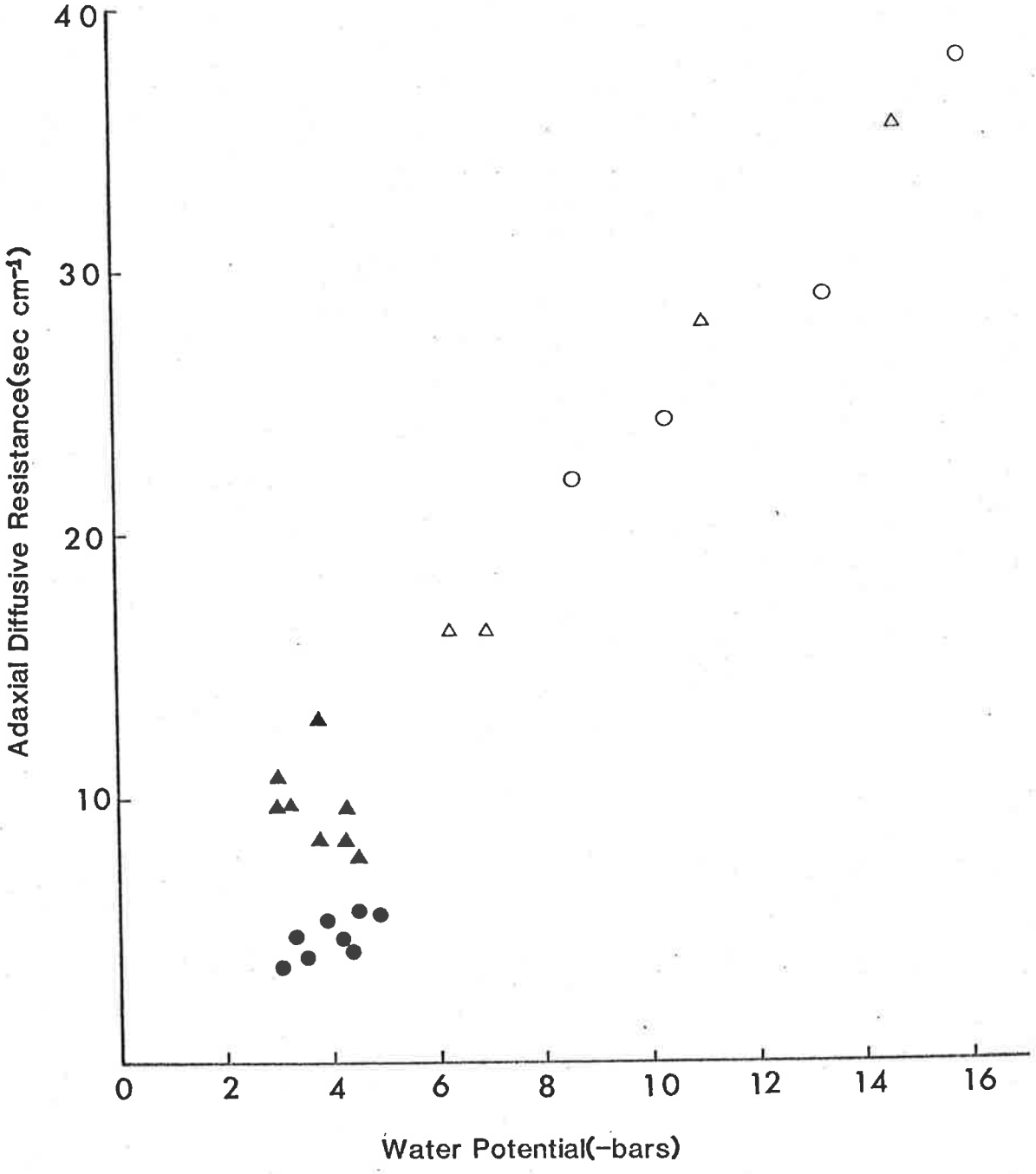

40
30
20
10
0
2
4
6
8
10
12
14
16
Adaxial Diffusive Resistance(sec cm⁻¹)
Water Potential(-bars)

FIG. III.6.4: Relationship between water potential and abaxial diffusive resistance.

Control - (●) F.C.
(○) Stress

CCC - (▲) F.C.
(△) Stress

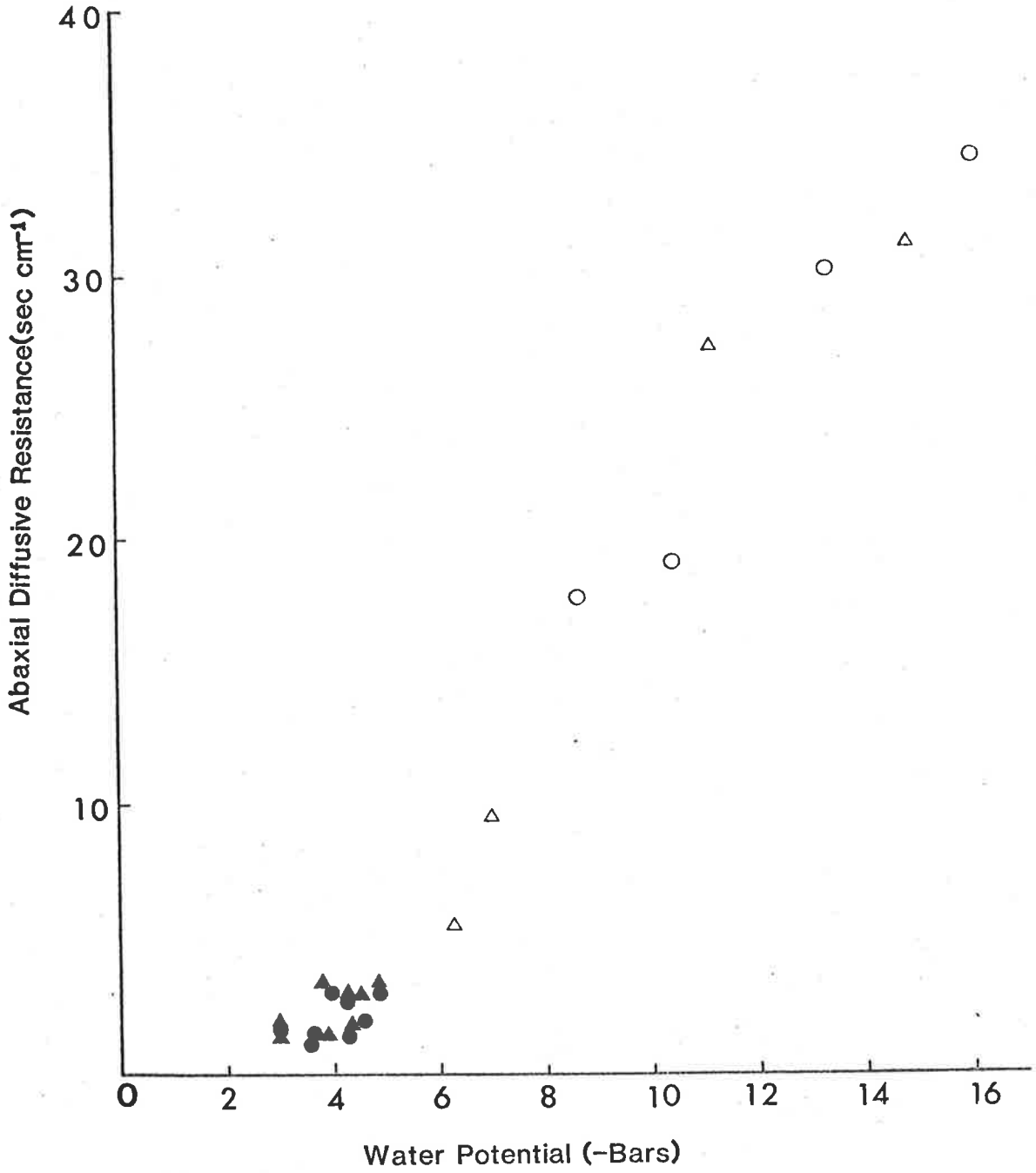

40
30
20
10
0
2
4
6
8
10
12
14
16
Abaxial Diffusive Resistance(sec cm-1)
Water Potential (-Bars)

FIG. III.6.5: Relationship between water potential and adaxial conductance.

Control - (●) F.C.
(○) Stress

CCC - (▲) F.C.
(△) Stress

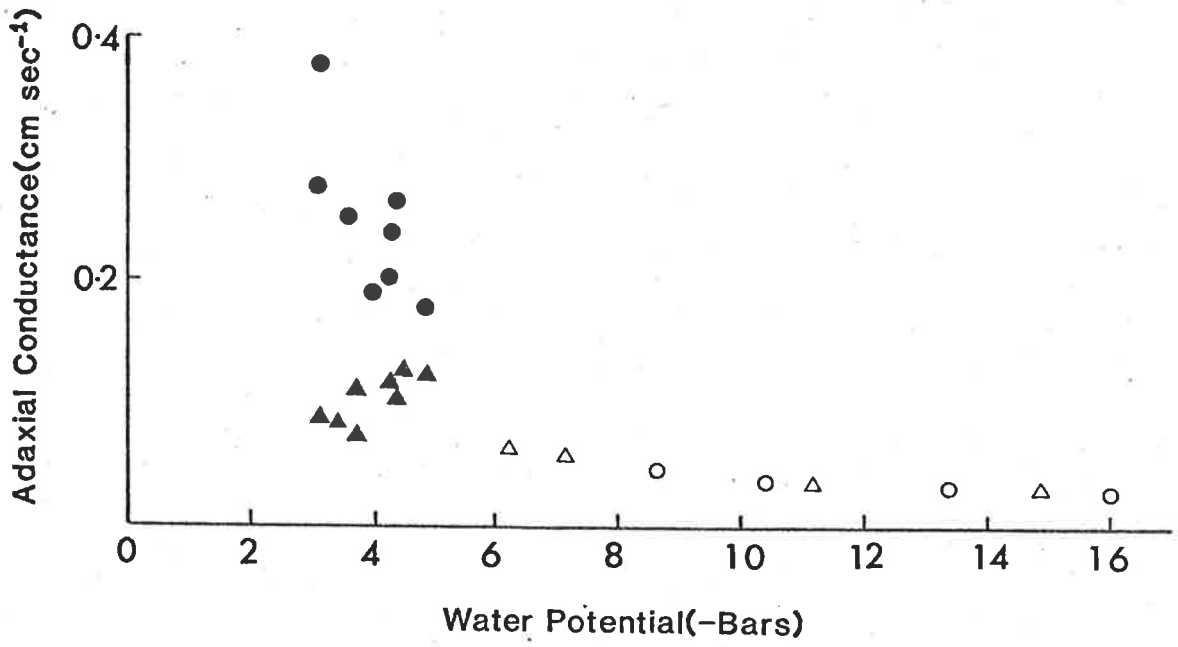
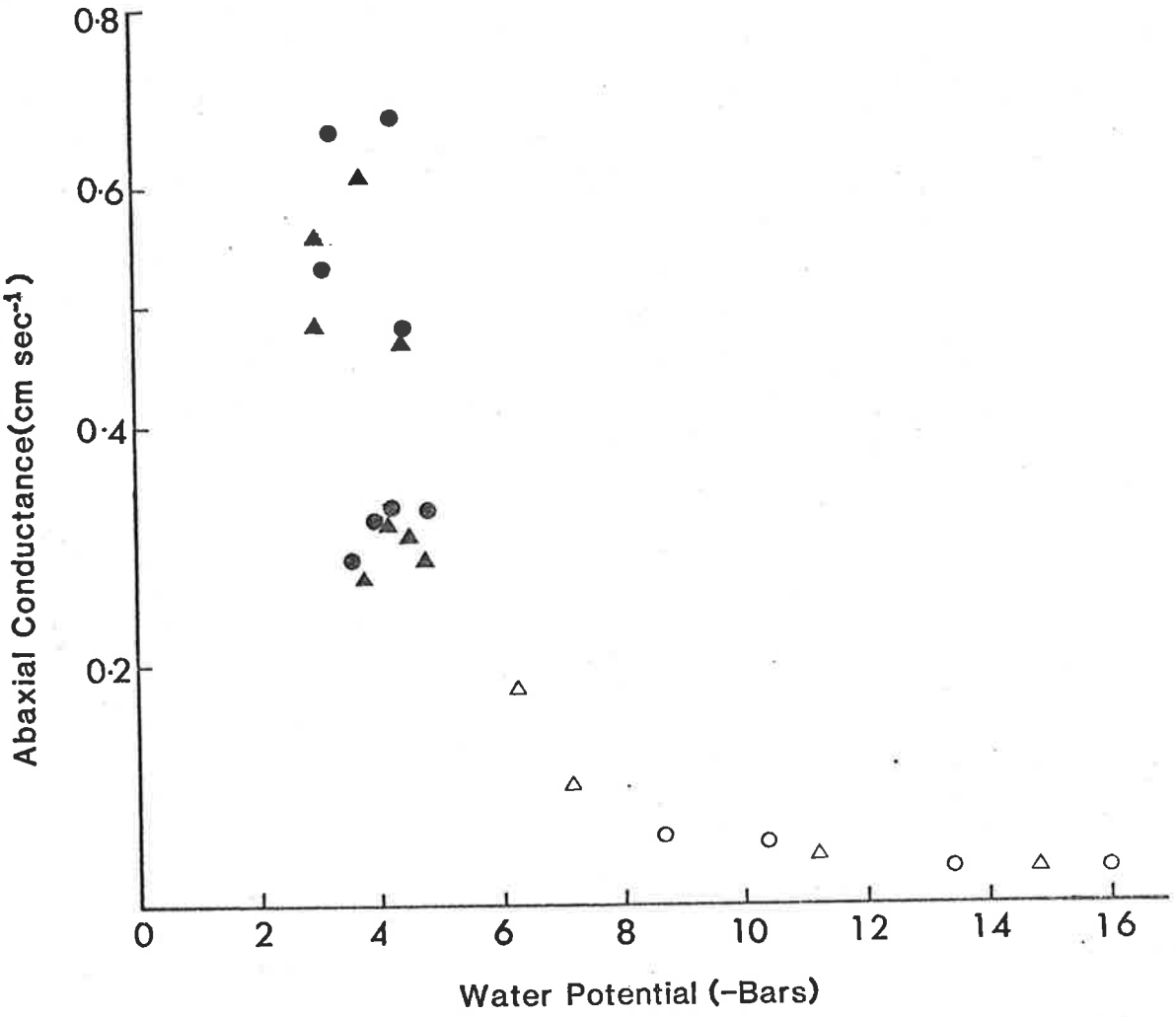

Adaxial Conductance(cm sec⁻¹)
0·4
0·2
0
2
4
6
8
10
12
14
16
Water Potential(−Bars)

FIG. III.6.6: Relationship between water potential and abaxial conductance.

Control - (●) F.C.
(○) Stress

CCC - (▲) F.C.
(△) Stress


0·8
0·6
0·4
0·2
Abaxial Conductance(cm sec⁻¹)
0
2
4
6
8
10
12
14
16
Water Potential (-Bars)

III.7 EFFECTS OF CCC AND WATER STRESS ON TOTAL WATER LOSS

III.7.1 Introduction

Results presented so far indicate that the CCC-induced maintenance of water status under stress may be explained in terms of the initial reduced transpirational water loss in CCC-treated plants, which leads to a delay in the onset of internal water stress in the treated plants. It has already been found that CCC-treated plants use water more economically under drought (El Damaty *et al.*, 1965; Farah, 1969; Plaut *et al.*, 1964). A study was therefore conducted to explore the effects of CCC on total water loss.

The tomato plants were grown in 1 kg recycled soil (Waite Institute) in 127 mm (5 ins) pots. Hoagland's solution was supplied as a supplementation whenever required. CCC 1000 ppm was applied as soil drench at 150 ml/pot to $3\frac{1}{2}$ weeks old plants. Water stress was imposed by withholding water from the plants, 4 days after CCC treatment. All the pots were embedded in polythene bags to minimize water loss from the soil. Pot weights were recorded daily at the start of the light period in the growth room (i.e. 6.00 a.m.) and at the end of the light period (10.00 p.m.). The non-stressed plants were returned daily to field capacity after weighing the pots in the morning. Leaf water potential was monitored with the pressure bomb during the course of the stress and the experiment was replicated three times.

III.7.2 Results

The water loss per day in light and in darkness is presented in Fig. III.7.1. Under well-watered conditions, the water loss during the light period was significantly less in the CCC-treated plants than the untreated plants, but there was no such difference in the dark period.
By the 3rd day of stress the water loss per day during the light period, in the non-CCC-treated plants, had decreased dramatically such that it was less than in the CCC-treated plants till the 5th day of stress.

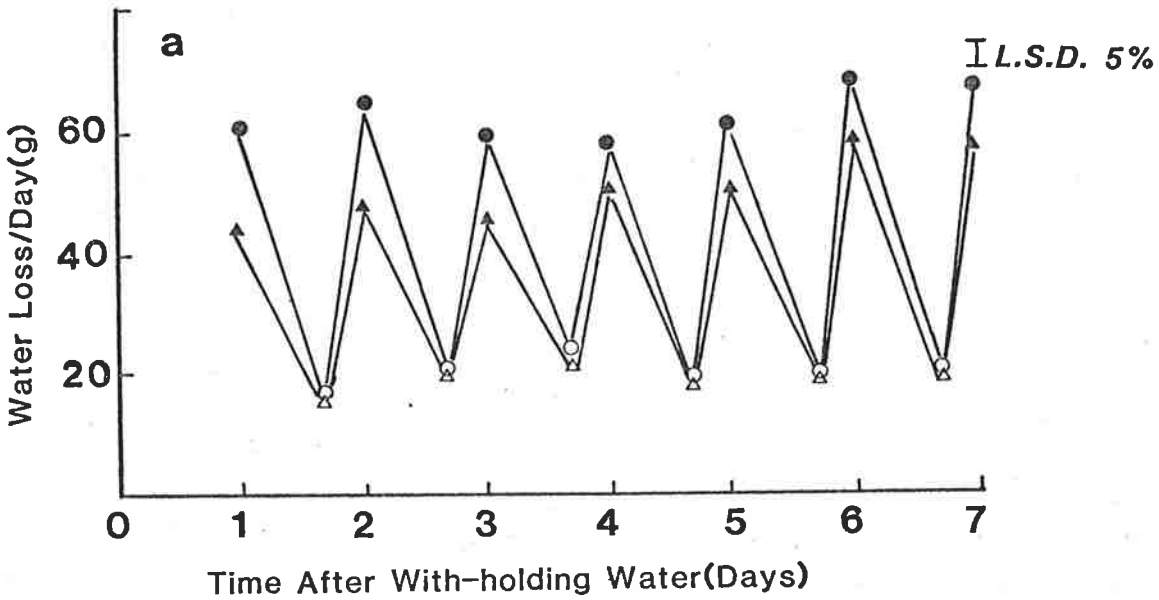
CCC significantly reduced the cumulative water loss in the well-watered plants. Under stress, however, the cumulative water loss was significantly decreased on the 3rd and 4th days of stress in the non-CCC-treated and CCC-treated plants respectively (Fig. III.7.2).

FIG. III.7.1: Effects of CCC and water stress on water loss/day.

(a) Well-watered plants
(b) Stressed plants

Water loss during the light period – (●) Control, (▲) CCC

Water loss during the dark period – (○) Control, (△) CCC


a
I L.S.D. 5%
Water Loss/Day(g)
60
40
20
0
1
2
3
4
5
6
7
Time After With-holding Water(Days)

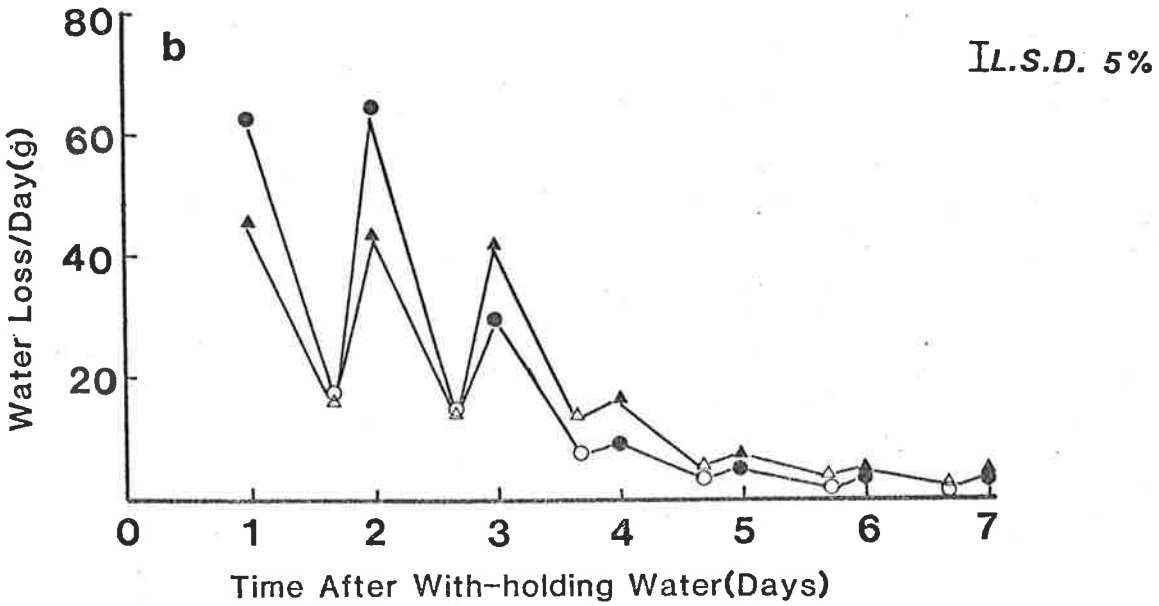
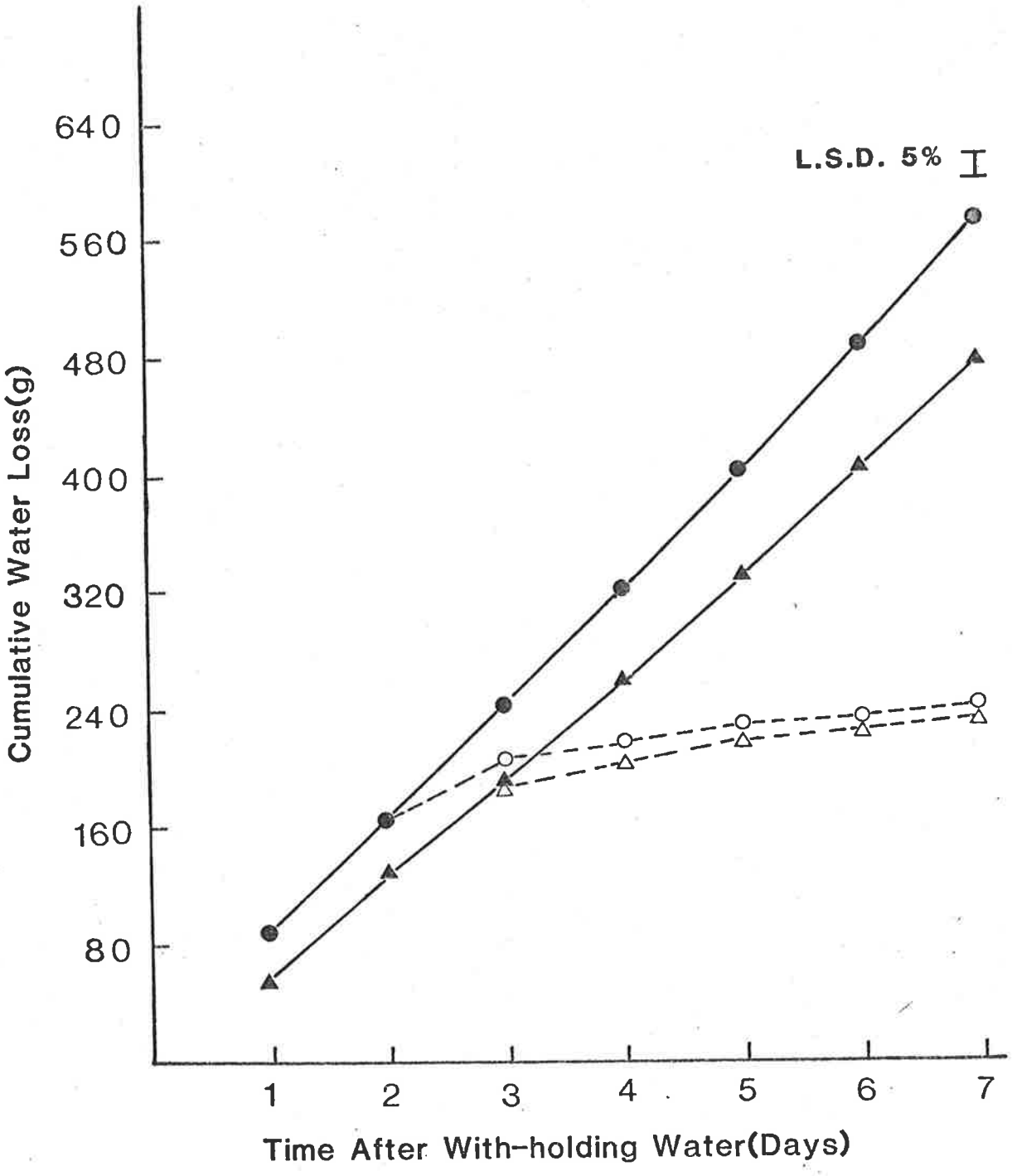

b
I L.S.D. 5%
Water Loss/Day(g)
80
60
40
20
0
1
2
3
4
5
6
7
Time After With-holding Water(Days)

FIG. III.7.2: Effects of CCC and water stress on cumulative water loss.

Control - (●) F.C.
(○) Stress

CCC - (▲) F.C.
(△) Stress


640
560
480
400
320
240
160
80
Cumulative Water Loss(g)
L.S.D. 5%
1
2
3
4
5
6
7
Time After With-holding Water(Days)

The relationship between water loss/day and water potential presented in Fig. III.7.3 shows that the water loss/day decreased as water potential declined in response to soil water deficit, till about -8 bars when the rate slowed down. Cumulative water loss, however, increased with declining water potential until about -8 bars when the rate slowed down, as shown in Fig. III.7.4.

III.7.3 Discussion

The decrease in the water loss due to CCC treatment under normal well-watered conditions may be attributable to the CCC-induced increase in leaf diffusive resistance, largely on the adaxial surface, as discussed in Section III.5. When the plants were subjected to soil water depletion, the CCC-treated plants, by virtue of their reduced water loss initially, delayed the onset of severe plant water deficit and maintained better water status and, therefore, their stomata remained more open than in the untreated plants, as in Figs. III.6.1 and III.6.2. This is reflected in the non-CCC-treated plants controlling further loss of water earlier (3rd day of stress) than the CCC-treated plants (4th day of stress) as shown in Fig. III.7.1.

The amount of water required to be transpired for the water potential to drop to a given level was unaffected by CCC. A threshold water potential of -8 bars was found for both CCC-treated and untreated plants under stress and this is consistent with the threshold water potential of -7 bars to -9 bars for stomatal closure as discussed in Section III.6.3.

FIG. III.7.3: Relationship between water potential and water loss/day.

(●) Control
(▲) CCC

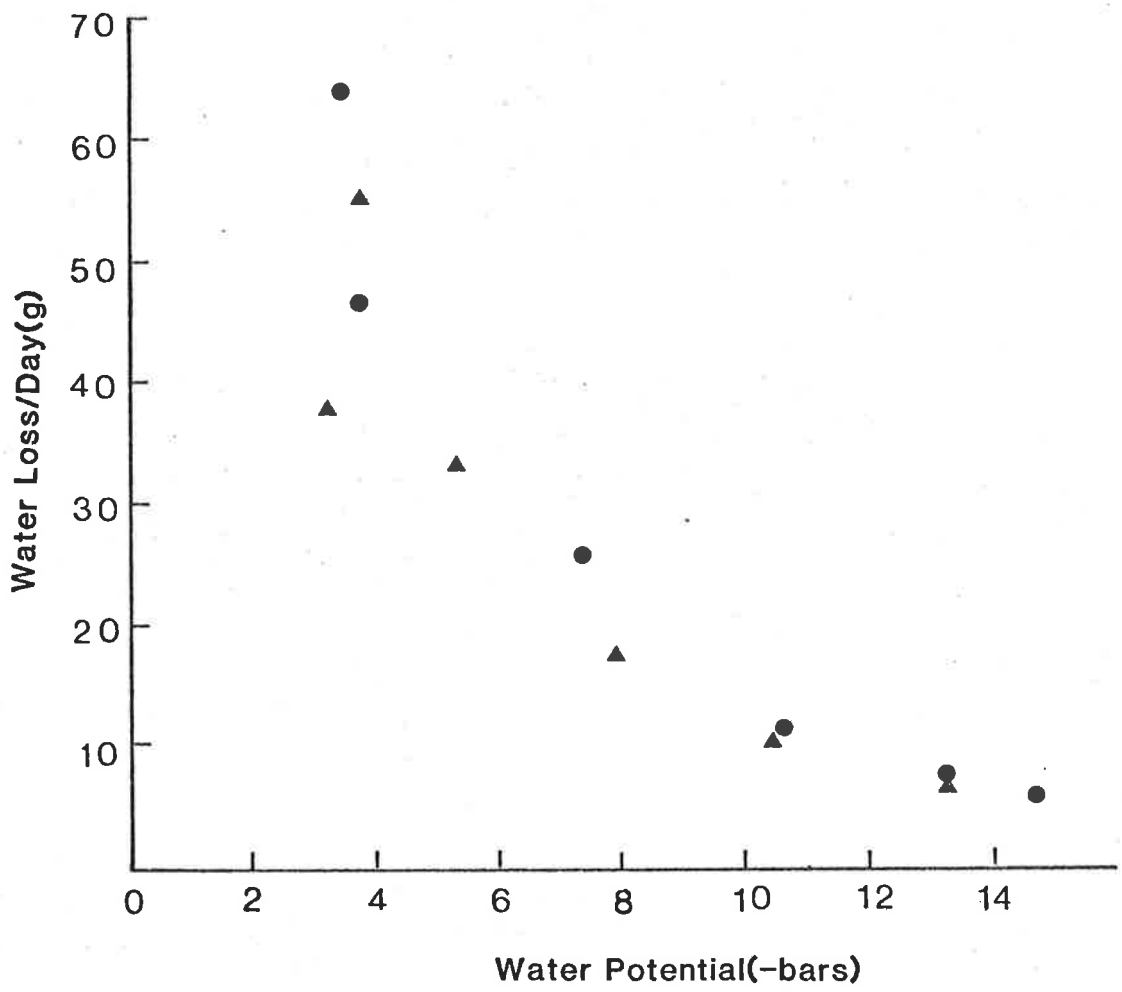
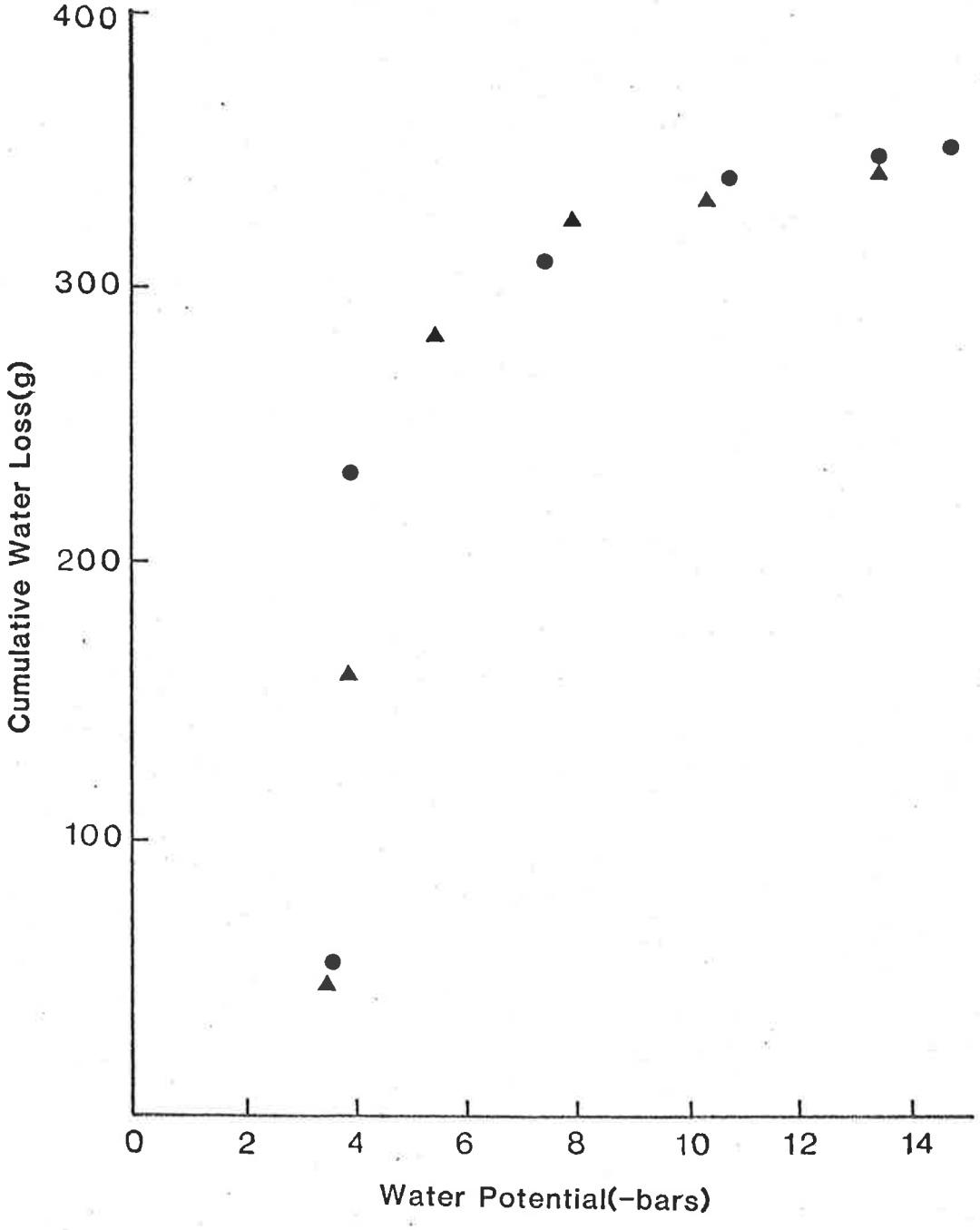

70
60
50
40
30
20
10
Water Loss/Day(g)
0
2
4
6
8
10
12
14
Water Potential(-bars)

FIG. III.7.4: Relationship between water potential and cumulative water loss.

(●) Control
(▲) CCC


400
300
200
100
Cumulative Water Loss(g)
0
2
4
6
8
10
12
14
Water Potential(-bars)

CHAPTER IV

GENERAL DISCUSSION

It is quite a widespread phenomenon that CCC increases the ability of some plants to resist drought, and various mechanisms have been proposed to explain this CCC-induced drought resistance.

Some workers claim that CCC increases the ability of plants to endure or tolerate stress through CCC-mediated metabolic changes such that even at low tissue water potential, CCC-treated plants may retain their turgor possibly through CCC-mediated accumulation of solutes. Singh *et al.* (1973), reported a promotion of proline accumulation in CCC-treated wheat plants subjected to an osmotic shock and suggested that the CCC-induced accumulated proline may enhance recovery from water stress. Stoddart (1964) observed that osmotic substances like polysaccharides and amino acids accumulate in the presence of CCC and proposed the possible involvement of these solutes in the CCC-induced resistance to drought. Marth and Frank (1961) speculated that the increased tolerance of CCC-treated soybean plants to soluble salts may involve some CCC-induced metabolic change since the population of mites feeding on the CCC-treated plants was reduced.

Other workers explain the CCC-induced resistance to drought in terms of reduced transpiration and increased root/shoot ratio, such that under water stress the CCC-treated plants have favourable water status. Robertson and Greenway (1973) reported that CCC-treated maize and wheat plants avoided drought by delaying the onset of severe internal water deficit, by virtue of reduced leaf area and increased root/shoot ratio. Plaut *et al.* (1964), claimed that a decrease in transpiration rate and top/root ratio in CCC-treated bean plants contributed to their prolonged survival under water stress. Moreover CCC-induced closure of stomata has been reported in tomato (Mishra and Pradhan, 1967; 1972; Pill *et al.* (1973), cowpea (Imbamba, 1972), sunflower (Lovett and Cambell, 1973), and wheat (De *et al.*, 1982) and this has been associated with reduced transpirational water loss and, hence, better water status under water stress.

Thus, CCC-induced drought resistance involves metabolic, morphological and physiological changes due to the CCC treatment; however, there has not been any detailed study of how these changes affect the water relations under stress. In the present work much emphasis was placed on mechanisms involved in the CCC-induced drought resistance of tomato, independent of the CCC effect on growth.

Results from this work revealed that CCC, at a concentration of 1000 ppm, enabled the treated tomato plants to delay wilting and survive longer under water stress even when growth was not retarded (Fig. III.1.2). At this concentration, however, CCC exterted an inhibitory effect on the height of the plants 6 days after its application (Fig. III.1.3). Wittwer and Tolbert (1960) and van Bragt (1969) also found that CCC retarded the height of tomato 5-7 days after its application. The reduction in height was followed by a reduction in total leaf area resulting from the reduction in the area of the leaves formed or still developing after CCC application (Fig. III.1.4). This CCC-inhibition of the growth of developing leaf tissues can be explained by the findings of Sachs *et al.* (1960) and Russel and Kimmins (1972), that the retardation of growth by CCC might be attributed to the inhibition of meristematic activity and cell elongation. Once the stature of the plant was reduced, the dry weight of the shoot was also reduced, but not the root, which resulted in an increase in the root/shoot ratio (Tables III.1.2 and III.1.3).

Whether CCC retarded growth or not, under water stress induced by with-holding water, CCC-treated plants maintained higher water potential and this better plant water status might have accounted for the prolonged survival under water stress, though CCC did not sustain growth.

When CCC has not affected growth the CCC-induced resistance to water stress could not be explained in terms of CCC-induced osmotic adjustment since CCC-treated plants did not lower their osmotic potential enough (Figs. III.3.5 and III.3.6) to maintain turgor at low tissue water potential. According to Morgan (1977b) and Turner (1974), in plants than can adjust osmotically to water stress, a unit decrease in water potential results in such great lowering of osmotic potential that turgor potential is maintained at lower tissue water potential. In addition, CCC did not alter

the moisture release curve (Fig. III.3.3) and, according to Jones and Turner (1978), osmotic adjustment may involve shifting the moisture release curve such that the decrease in RWC is less with a unit decrease in water potential. Furthermore, the apparent lack of CCC to significantly increase the accumulation of proline (Figs. III.4.2 and III.4.4) or any other quaternary ammonium compound (Figs. III.4.5 and III.4.6 and Table III.4.1) may suggest their non-involvement in the maintenance of better water status under stress by CCC.

The other evidence from this thesis showing a differential increase in adaxial diffusive resistance but not abaxial (Figs. III.5.1 and III.5.2), and the rapid increase in adaxial diffusive resistance due to CCC without an effect on growth (Fig. III.5.3) could be interpreted as CCC inducing stomatal closure in the adaxial leaf surface. The possibility of CCC inducing stomatal closure of the adaxial surface but not the abaxial has been discussed in Section III.5.3. This CCC-induced closure of adaxial stomata in normal well-watered tomato plants was consistent with the reduced transpirational water loss from the adaxial surface (Table III.5.2) and this, possibly, accounted for the reduced water loss in the CCC-treated plants (Fig. III.7.1a) under field capacity. Water stress also caused stomatal closure on both the adaxial and abaxial surfaces but this response was quicker in the non-CCC-treated plants (Figs. III.6.1 and III.6.2). Since the water potential thresholds for stomatal closure (on both adaxial and abaxial surfaces) were consistent with the water potential thresholds for controlling excessive water loss under stress (Figs. III.6.5, III.6.6, III.7.3 and III.7.4), and were unaffected by CCC, it may be argued that CCC delayed the time at which the water potential threshold for stomatal closure was reached; this agrees with the initial less rapid decline in water potential in the CCC-treated plants under water stress.

When CCC has affected growth the CCC-induced adaxial diffusive resistance may diminish (Table III.5.1b) but the reduced leaf area will inevitably reduce water loss and maintain higher water potential.

In conclusion, when growth is unaffected by CCC treatment, the closure of the adaxial stomata results in reduced water loss under normal well-watered conditions. This initial control of water loss enables the CCC-treated plants to maintain higher tissue water potential, thus delaying

the onset of severe water deficit in the plants and prolonging their survival under water stress. This CCC-induced prolonged survival under water stress did not involve sufficient osmotic adjustment to permit the CCC-treated plants to sustain growth under low tissue water potential. This could be due to the lack of CCC-induced metabolic changes under water stress. However, when CCC has retarded growth, the increased root/shoot ratio and reduced leaf area will inevitably play a very significant role in the CCC-induced resistance to water stress.

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APPENDIX I

FIG.1: Photograph showing the method of determination of transpiration rate in excised leaves as described in Section II.8.3.

APPENDIX II

Determination of stomatal density

The number of stomata per unit leaf area was determined from leaf surface imprints following the method described in Section II.8.2.

Results from Table 1 reveal that at 3 days after CCC treatment the number of stomata/unit area of leaf in the more matured 4th leaf and the younger 6th leaf were unaffected by CCC but inherently there were more stomata on the abaxial surface than the adaxial. However, after 10 days, the stomatal density increases markedly in the CCC-treated plants, especially in the younger leaves, which could be an effect resulting from the reduction in leaf area due to CCC treatment. As discussed in Section III.3.3, CCC retards the growth of developing or newly formed leaves. With a decrease in leaf area but unchanged total number of stomata, the number of stomata/unit leaf area subsequently increases.

An attempt to measure stomatal apperture was not successful since the stomata appeared closed as shown in Figures 2 and 3. The closure of stomata might have been caused by the silicone rubber used to make the epidermal imprints, as it was deleterious to the leaves. Figures 2 and 3 also show the increase in abaxial stomatal density as compared to adaxial and the lack of effect of CCC on stomatal density of the 4th leaf at 10 days after CCC application.

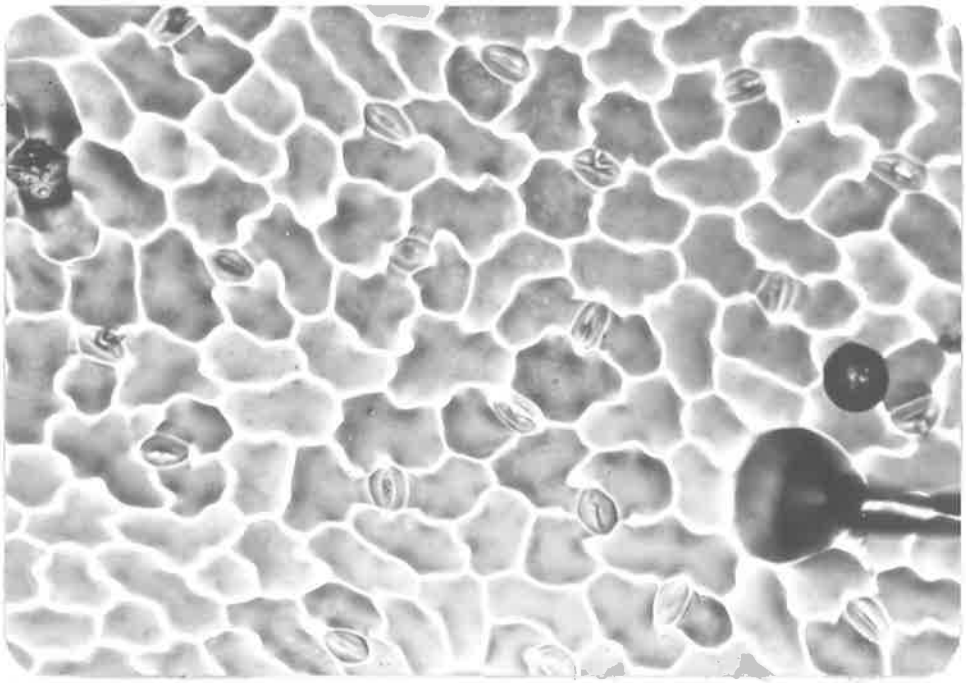
TABLE 1: Number of stomata/mm^2.

	3 days after CCC treatment				10 days after CCC treatment			
	4th leaf		6th leaf		4th leaf		6th leaf	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Control F.C.	75.70	186.00	136.00	319.00	93.00	216.00	122.70	363.00
Control stress					119.30	257.00	149.30	398.00
CCC F.C.	80.30	188.00	135.00	316.00	98.30	233.00	176.70	420.00
CCC stress					106.30	250.00	152.70	398.00
L.S.D. 5%								
CCC	-	-	-	-	-	-	29.49	51.63
Water stress	-	-	-	-	-	-	-	-
CCC*water stress	-	-	-	-	-	-	-	-

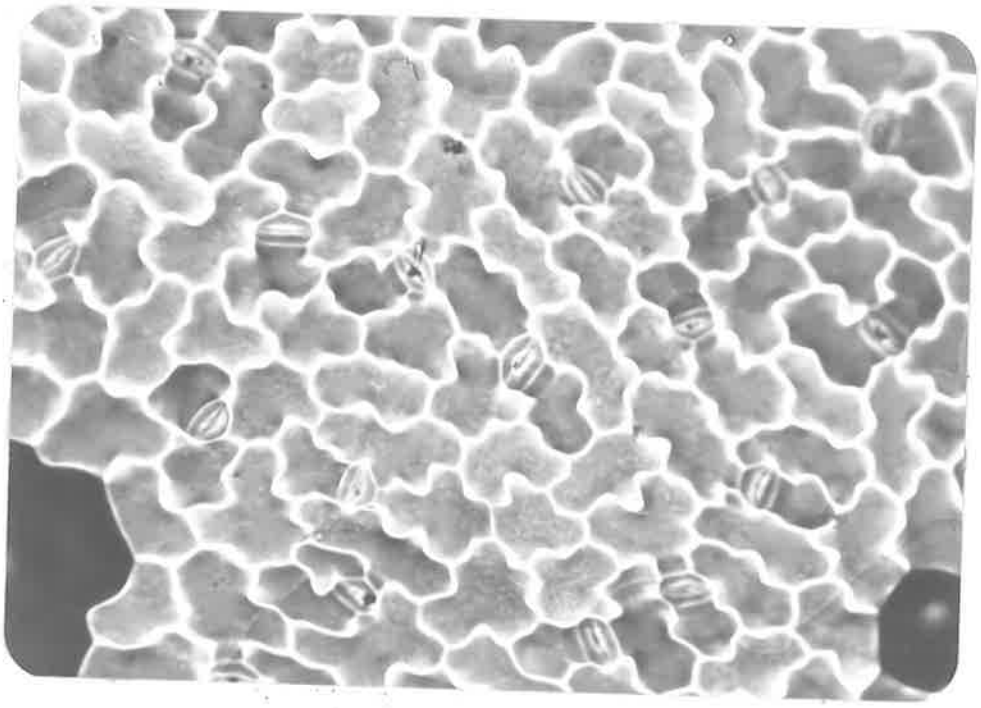
FIG.2: Photographs showing stomatal distribution on the adaxial surface.

Imprints of the 4th leaves were made 10 days after CCC treatment and the leaf tissues were from well-watered plants.

The stomata appeared close and, therefore, it was impossible to measure stomatal aperture.



CONTROL

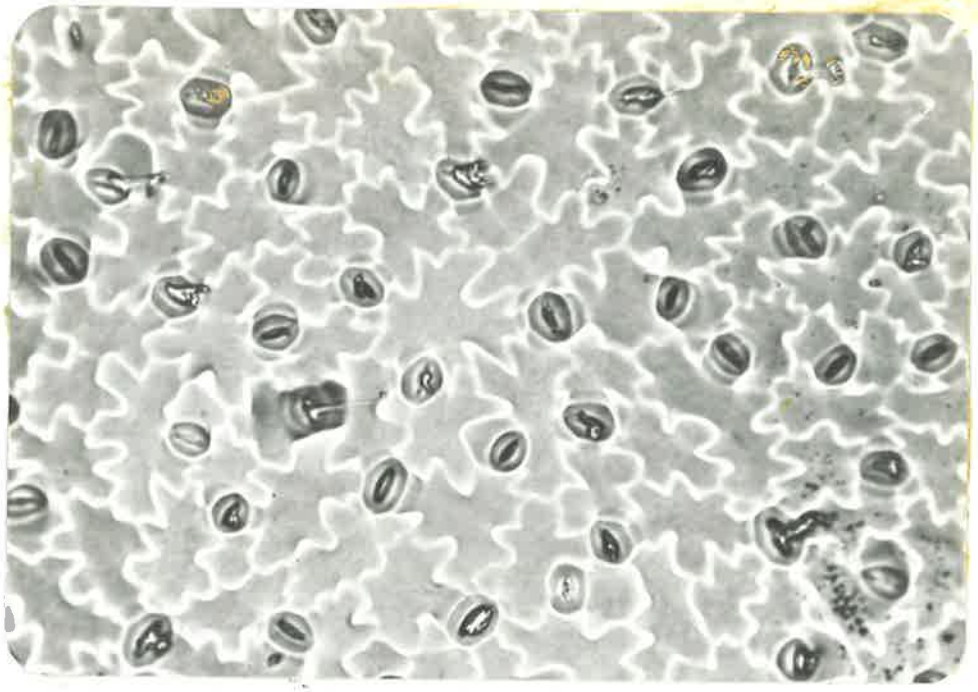


CCC

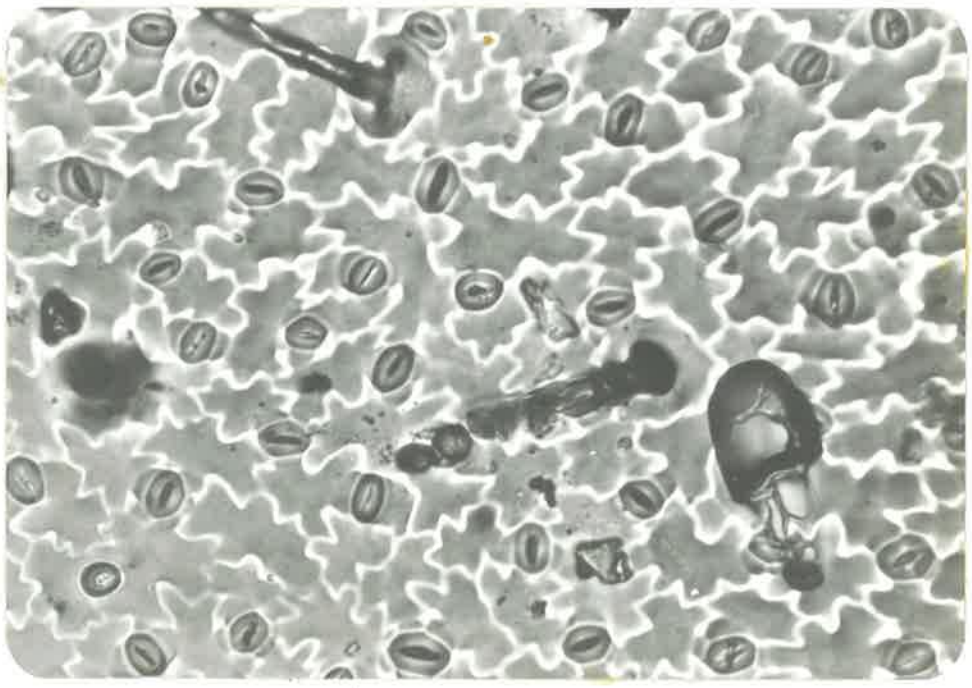
FIG.3: Photographs showing stomatal distribution on the abaxial surface.

Imprints of the 4th leaves were made 10 days after CCC treatment and the leaf tissues were from well-watered plants.

Stomatal density of the abaxial surface appears higher than the adaxial. However, stomatal aperture could not be measured as the stomata appeared close.



CONTROL



CCC