Seedling responses to growth regulators and drought stress

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SEEDLING RESPONSES TO GROWTH REGULATORS AND DROUGHT STRESS

By

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TABLE OF CONTENTS

INTRODUCTION

Growth of plants is dependent on a delicate interaction between the plant itself and many environmental factors. Within limits plants are capable of adjusting to fluctuation in environmental factors but compounding effects can has serious consequences. Use of plant growth regulators have increased in recent years and these can influence the response of the plant to environmental factors. Therefore it is important to investigate combined environmental factors and plant growth regulator applications.

Plant growth regulators are usually defined as organic compounds, that, in small concentration, affect the physiological processes of plants (Nickell, 1979). Plant growth regulators can be divided into two types according to their source; natural or synthetic compounds, and according to their function, again they can be divided into two types; growth promoting and growth retarding compounds. Growth-promoting compounds are those when applied to the plant, enhance the growth e.g. compounds promote rooting and flowering, increase fruit set and increase the uptake of nitrogen and phosphorus. Growth-retarding compounds are chemicals which slow cell division and cell elongation in shoot tissues and regulate plant height physiologically without formative effects (Cathey, 1964). Growth-promoting and growth-retarding compounds are applied to the plant to alter their physiological processes in desired directions. Herbicides, however, can also be considered as plant growth regulators if they have growth promoting properties.

2,4-dichlorophenoxy acetic acid (2,4-D) and 2-chloro-ethyl-trimethyl-ammonium chloride (Cycocel; CCC), have been considered very important compounds in the world of agriculture. Their importance is attributed to their function as chemicals which can be used in some beneficial ways to increase the yield, improve the quality of crop plants and to reduce competition from weeds.

2,4-D is a selective herbicide with auxin activity. This herbicide is especially designed to control broad leaf weeds (dicotyledons) in cereal crop fields. It is generally accepted that 2,4-D is an auxin-like herbicide, because at low concentration it has growth promoting properties. The first herbicide reported to improve growth and yield of crops at sub-toxic level was 2,4-D (Ries, 1976). It has often been reported to increase yield of some crops such as bean, sugar beet, and potatoes. Also 2,4-D has been reported to increase protein content of wheat (Huffaker et al. 1967), potato (Payne et al. 1953), and kidney beans (Sell et al. 1949). Carbohydrate and moisture content also have been reported as increasing at sub-toxic level of 2,4-D (Payne et al. 1953). Moreover 2,4-D has been known as fruit drop controller, latex stimulator, disease controller, insect controller, senescence delayer, root inducer, flowering inducer, fruit set enhancer and fruit ripener (Nickell, 1982). The mode of action of such herbicides is not clearly known. For example it seems probable that sub-toxic application of phenoxyacids initially stimulate nucleic-acid metabolism and protein synthesis which results in providing some factor partially limiting growth (Ries, 1976).

In contrast to sub-toxic effects of 2,4-D, at high concentrations it can be toxic. The selectivity of 2,4-D or its toxicity, depends on: inherent resistance or susceptibility of particular species, the stage of growth of the plant, the environment under which the plant has grown, and the dosage levels. Graminaceous plants,are generally resistant to 2,4-D whilst most of the dicotyledonous cultivated plants are sensitive, but there is a high degree of variation in susceptibility with the stage of development. This is shown by the fact that sensitivity decreases as the plant gets older. The environmental conditions under which the plant has been grown also are very important, e.g. water stress following 2,4-D application can cause serious damage to the plant, whilst water stress before 2,4-D application may increase plant resistance (Muzik, 1976). The interaction between plant, chemicals and environment is very complex and not easy to understand. However, 2,4-D at high concentrations has often been reported to damage some plants which would normally be regarded as resistant to this herbicide. Abnormalities in the spikes and roots of wheat were reported by (Johanson and Muzik,1961). Hamner et al, 1946 found soil previously treated with 2,4-D affect the germination and growth of many seeds. Hoshaw et al. 1951, found that both pre-emergence and post-emergence spray application of 2,4-D resulted in morphological and anatomical response in young corn plants.

From this brief review it is clear that 2,4-D can be growth regulator used to enhance the growth, increase the yield and improve the quality of crop plants, or can be used as a selective herbicide to kill dicotyledon weeds in cereal crop fields, and in this case it may cause great damage to non-target plants (cereals). Moreover, the damage may extend to dicotyledon crops which subsequently grow in the cereal fields as a result of 2,4-D persistence in the soil.

Cycocel (CCC), is known as sugar cane ripener, growth retardant, lodging reducer, germination inhibitor, flower bud stimulator, pigment former, sex changer, senescence delayer, fruit set enhancer, insect controller and anti-transpirant (Nickell, 1982).

Cycocel has been shown to act by inhibiting gibberellin biosynthesis in the plants to which they are applied. Cycocel and its related compounds were effective from 10^{-2} to 10^{-6}M, when poured onto the soil or used in nutrient culture (Tolbert, 1960). Tolbert also found that the chemical was most effective when applied to the soil. They could also be sprayed on the leaves, or added by soaking of seeds before planting. Many investigators (e.g. Tolbert, 1960, Cathey, 1961; Humphries, 1963; Emden, 1967; Cockshull, 1969; Farah, 1969; Humphries, 1977; Bode, 1984), found that total height of the plant and the internode length were reduced by Cycocel-treatment. Leaves in most treated plants were much darker green than those untreated (Cathey et al. 1961; Tolbert, 1960; Humphries, 1963). Reduction in fresh and dry weights in CCC-treated plants also reported by (Cathey et al. 1961; Emden et al. 1951). Drought tolerance in Cycocel-treated plant has been reported by (Halevy et al. 1963; Emden et al. 1967).

In the response of a plant to aherbicides or growth regulators, there are always at least three components : a genetic component, stage of growth component and environmental component (Muzik, 1976). All these components have been reviewed recently (Audus, 1976), and are outside the scope of this project. Water-stress as environmental factor is the only one point we intend to discuss here in relation to 2,4-D and CCC application.

Plants are said to experience water stress, or water deficit, when their cells and tissues are less than fully turgid (Wareing and Phillips, 1981). Plant responses to water stress and physiology of plants under drought have been reviewed by many workers (e.g. Henckel, 1964; Hsiao, 1973; Bewley, 1979). The first change is most likely a slowing down of shoot and leaf growth, as a result $\Psi\rho$ reduction, followed by a reduction in cell wall and protein synthesis. As tissue Ψ decreases further cell division may slow and levels of some enzymes, such as nitrate reductase start to decline. Stomata may begin to close, with a consequent reduction in transpiration and Co_2 assimilation, and ABA probably begin to accumulate. As a stress continues and tissue Ψ decreases still further, declines in respiration, translocation of photosynthate, and cytokinins may become substantial. Levels of some hydrolytic enzymes are likely to increase and ion transport can be slow. Finally water deficits become severe enough to cause marked proline accumulation, Co_2 assimilation becomes very low. Senescence induced by stress may become clear in older leaves.

Reduction in the yield as a result of the reduction in stomatal opening and cell growth is possible. Reduction of stomatal opening may reduce Co_2 assimilation and the latter may reduce dry matter production by reducing photosynthesis. Reduction of cell growth may also reduce the development of leaf surface area which subsequently reduce the production of total dry matter.

Many of these effects are similar to those caused by herbicides, as a result of their interference with water uptake and translocation. Herbicides such as 2,4-D interfere with phloem, xylem production (Ries, 1976), thus the plant becomes water-stressed even in the presence of water.

Severe drought may make plants sensitive to herbicides where they are normally resistant. Water stress following 2,4-D application to young wheat for example resulted in more damage whereas plants moist for 14 days after application, recovered and grew normally (Muzik et al. 1964). The effect of the drought may be increased as a result of the stress caused by herbicide thus making the plant more sensitive to drought stress. Muzik explained that increased damage from drought following application of auxin-type herbicides is water stress in the tissues caused by damage to the roots from the herbicides accentuated by the drying effect.

Some growth regulators have been found to be involved in the control of water balance of plants (e.g. ABA, Cytokinins and Ethylene). Effects of external hormone application in relation to water stress is not fully understood. Cycocel for example reported to increase water tolerances in plants, it may have an effect on the stomatal opening.

In this project the selectivity of 2,4-D and CCC will be investigated on wheat and radish in relation to ecological factors, particularly drought stress. Growth inhibition and changed protein contents will be used as parameters of effect. The persistence of both 2,4-D and CCC and effects on seeding growth and establishment will also be tested.

MATERIALS AND METHODS

The experiments were conducted under unheated glass house conditions (the main temperatures were 20 °C) at the Department of Botany, University of Durham between May and July 1985.

1. PLANT MATERIALS:

Pre-soaked (for 24 hours) Caryopses of wheat (*Triticum aestivum* L.) and seeds of radish (*Raphanus sativus* L.) cv. French Breakfast, were planted in John Innes No.2 Compost contained in trays 5 cm deep, 32 cm long and 19 cm wide. The seedlings were watered every two days by bringing to field capacity.

2. APPLICATION OF GROWTH REGULATORS:

The growth regulators used were 2,4-dichlorophenoxy acetic acid (2,4-D) and 2, chloroethyl-trimethyl-ammonium chloride (Cycocel,CCC). Aqueous sprays were prepared from the commercial 2,4-D salt, supplied by B.D.H Laboratory, and CCC solution containing 40% weight/volume of chlormequat, supplied as (Cycocel) by Argro Ltd. Each compound was used at one standard concentration (100 ppm), which represents a functional level for most plants. The application of both compounds was carried out using a small hand spray directly to the leaves. The volume of spray used was equal in all treatments (2 ml. per tray) for each compound. Plants were sprayed once two weeks after planting. The amounts were about 247 μℓ per plant (0.025 mg) for radish and about 333 μℓ per plant (0.033 mg) for wheat.

3. GROWTH EXPERIMENTS:

Three growth experiments were carried out using distinct treatments as follows:

EXPERIMENT I : The growth responses of wheat and radish seedlings to a foliar spray of 2,4-D and CCC followed by drought stress.

In this treatment caryopses of wheat, seeds of radish, and a mixture of wheat caryopses and radish seeds in equal amounts were planted in 18 trays; 6 trays of wheat, 6 trays of radish and 6 trays of the mixture of wheat and radish. Two weeks after planting 6 of the trays were sprayed with CCC, 6 with 2,4-D and 6 remained untreated as a control. Each treatment group comprised of 2 trays of wheat, 2 of radish and 2 of mixed radish and wheat as in Table I. After spraying seedlings were subjected to drought stress by withholding further water.

Plants	No. of trays	Treatment		
		CCC	2,4-D	Control
Wheat	2	+		
	2		+	
	2			+
Radish	2	+		
	2		+	
	2			+
Mixed	2	+		
	2		+	
	2			+

Table I : Treatments given in experiment I.

EXPERIMENT II : The growth responses of radish seedlings to residual soil 2,4-D and CCC under a normal watering regime.

In this experiment, seeds of radish were planted into 12 of the same trays, still containing the same soil as was used in Experiment I. These treated trays were chosen at random from those available in Experiment I. The density was 20 equally-spaced seeds per tray. Four of the trays were used to test the residual effect of 2,4-D, four to test the residual effect of CCC, and four were controls as in Table II. The seedlings were watered normally every two days.

Plant	No.of trays	Treatment 2.4-D	CCC	Control
Radish	4	+		
	4		+	
	4			+

Table II : Treatments given in experiment II.

EXPERIMENT III : The differential responses of wheat and radish seedlings to a foliar spray of 2,4-D and CCC when grown under the normal watering regime and with drought stress (withholding water).

In this experiment the procedure described for Experiment I was adopted, but 9 of the trays were kept under drought stress (withholding water) and 9 were kept under the normal watering regime as in Table III. This experiment was set up specifically for protein estimation.

Plants	No.of trays W.r	D.s	2,4-D	Treatment CCC	Control
	1	1	+		
Wheat	1	1		+	
	1	1			+
	1	1	+		
Radish	1	1		+	
	1	1			+
	1	1	+		
Mixed	1	1		+	
	1	1			+

Table III : Treatments given in experiment III.

4. MEASUREMENTS OF DEVELOPMENT AND GROWTH RATE

A variety of parameters were used to assess the effects of 2,4-D and CCC on the development and growth rates of wheat and radish seedlings. These parameters included:

I. LEAF AREA : The radish cotyledon can be approximated in shape to a disc; the area of which was thus calculated from the breadth i.e. diameters ($A \simeq \pi (\frac{d}{2})^2$). The wheat leaf blade similarly can be approximated to a rectangle; the area of the leaf blade was thus calculated from the length and breadth (i.e. $A = l \times b$). The determinations of leaf area by these means could be carried out whilst the plants were growing in order to assess the impact of the growth regulators upon leaf development particularly leaf expansion.

II. NUMBER OF LEAVES PER PLANT : The plants were harvested at the end of the experiment and the number of leaves per plant counted.

III. NUMBER OF TILLERS IN WHEAT : The plants were harvested at the end of the experiment and the number of expended tillers per plant counted.

IV. FRESH WEIGHT : The fresh weight of the whole plant, its shoot, and its root (radish), were recorded by weighing the freshly harvested plants.

V. DRY WEIGHT : Harvested plants were dried in an oven at 110°C for 24 hours and then weighed to determine dry weight.

VI. LENGTH AND DIAMETER OF RADISH TUBER : The swollen parts (hypocotyl and root) were measured using calipers.

VII. ESTIMATION OF PROTEIN:

Changes in protein content were used as a parameter for growth regulators effects. The content of soluble and insoluble proteins in sprayed seedlings was estimated under the normal watering regime and under drought stress using the following procedure:

0.2 gm. of leaf tissue was ground in a pestle and mortar using 4 cm^3 of buffer [tris-hydroxy-methylaminomethane, (TRIS/Hcl) 0.04 M pH 7.5; Magnesium sulphate ($MgSo_4$) 0.1 M; and ethyl-endinitrilotetraacetic acid disodium salt, (EDTA 0.025 M]. The resulting syspension was centrifuged for 10 min. in a

Piccolo bench centrifuge at full speed in order to sediment insoluble proteins. The resulting pellet contained the insoluble proteins whilst the supernatant contained the soluble proteins.

Extraction of soluble proteins:

2 cm^3 of 10% trichloracetic acid (TCA) were added to 2 cm^3 of the protein supernatant in a test tube and kept on ice for 30 mins before centrifugation at full speed. The supernatant was discarded and the pellet was resuspended in 5% TCA in order to wash it. Following centrifugation the protein pellet was suspended in 1 cm^3 of IN Sodium hydroxide (NaoH) and dissolved by heating to 80°C.

Extraction of insoluble proteins:

The initial protein pellet was taken and chlorophyll removed by washing three times in 2 cm^3 1:1 a chloroform/methanol mixture, centrifugation being repeated at each washing. The resulting pellet was suspended in 1 cm^3 IN NaoH and heated in a water bath to 80°C.

SUMMARY FOR PROTEIN EXTRACTION PROCEDURE

0.2 gm. of leaf tissue

↓

ground in a pestle and mortar

using 4 cm^3 of buffer solution

↓

the resulting suspension was centrifuged

for 10 min.

↓

Left branch:

pellet containing sol.proteins

↓

washed in 2 cm^3 of 1 : 1

chloroform/methanol

↓

centrifuged for 10 min.

↓

pellet — supernatant discarded

↓

washed in 2 cm^3 of 1:1 chloroform/methanol

↓

centrifuged for 10 min.

↓

pellet — supernatant discarded

↓

washed in 2 cm^3 of 1:1 chloroform/methanol

↓

centrifuged for 10 min.

↓

pellet — supernatant discarded

↓

was suspended in 1 cm^3 of 1N NaoH

↓

heated in water bath to 80 C

Right branch:

supernatant containing sol.proteins

↓

2 cm of 10% TCA were added to 2 cm^3 of protein

supernatant

↓

kept in ice for 30 min.

↓

centrifuged

↓

pellet — supernatant was discarded

resuspended in 5% TCA in order to wash it

↓

centrifuged

↓

pellet — supernatant was discarded

↓

was suspended in 1 cm^3 of 1N NaoH

↓

dissolved by heating to 80 C

Both branches:

↓

5 cm^3 copper sulphate reagent was added

↓

left for 10 min.

↓

0.5 cm^3 Folin-Ciocalteu's was added

↓

the mixture allowed to stand for 30 min.

↓

the optical density of resulting blue-coloured

solution was measured colorimeterically

Estimation of proteins : (Lowery's method)

To both extracted proteins (soluble and insoluble) 5 cm^3 copper sulphate reagent was added, this being made up of [0.5 cm^3 copper sulphate ($CuSo_4$) + 0.5 cm^3 sodium/potassium tartrate, and + 50 cm^3 sodium carbonate (Na HCo_3)] . After 10 mins. 0.5 cm^3 Folin-Ciocalteu's reagent at 5% dilution of Sigma supplied stock, was added and the mixture allowed to stand for 30 mins. The optical density of the resulting blue-coloured solution was measured colorimeterically, using a Cornin Colorimeter, with green filter No.520.

In both cases the Colorimeter readings were quantified using a plot of the readings obtained from standard protein solutions of known concentrations.

Colorimeter calibration :

A stock solution at 10 mg $m\ell^{-1}$ was prepared using Bovin Serum Album en (BSA). From this stock solution the following concentrations were prepared by serial dilutions:

Protein conc. mg. $m\ell^{-1}$	Protein solution $m\ell$	1N NaoH $m\ell$
0.0	0.00	1.00
0.5	0.05	0.95
1.0	0.10	0.90
1.5	0.15	0.85
2.0	0.20	0.80
2.5	0.25	0.75
3.0	0.30	0.70
10.0	1.00	0.00

Table IV : Colorimeter Calibration.

5 cm^3 of $CuSo_4$ reagent and 0.5 cm^3 of Folin-Ciocalteu's were added to each. After 30 min. the optical density of each was measured in the colorimeter. The resulting values were plotted against protein concentration.

RESULTS

MORPHOLOGICAL EFFECTS OF 2,4-D ON RADISH SEEDLINGS UNDER DROUGHT STRESS

SHOOTS - Two hours after treatment extreme bending and twisting of the whole seedlings was evident particularly the petioles (Fig.1). Some burning spots were also noted in the middle of a few cotyledons. By the second day all seedlings were completely twisted. A few seedlings, however, began to recover after five days, but most seedlings remained twisted. An application of 2,4-D to radish seedlings followed by drought stress showed a remarkable influence on the further growth of the third leaf. The third leaf of treated seedlings was curled and grey in colour. At the same time, the third leaf of untreated seedlings was growing normally. Third leaf area growth in treated seedlings showed significant reduction with respect to the controls (Fig.2). As a result of this influence on the further growth and development of the new leaves, reduction in fresh and dry weight of shoots was significant (Table V).

TUBERS - An inhibition of tuber formation and tuber growth after 2,4-D treatment was noted. Hypocotyl and root malformation were very clear. The hypocotyl was elongated and extended some distance above the soil level, and the roots were swollen behind the tips. As a result of this bending and growth retardation, the fresh and dry weight of the tubers were significantly decreased (Table V).

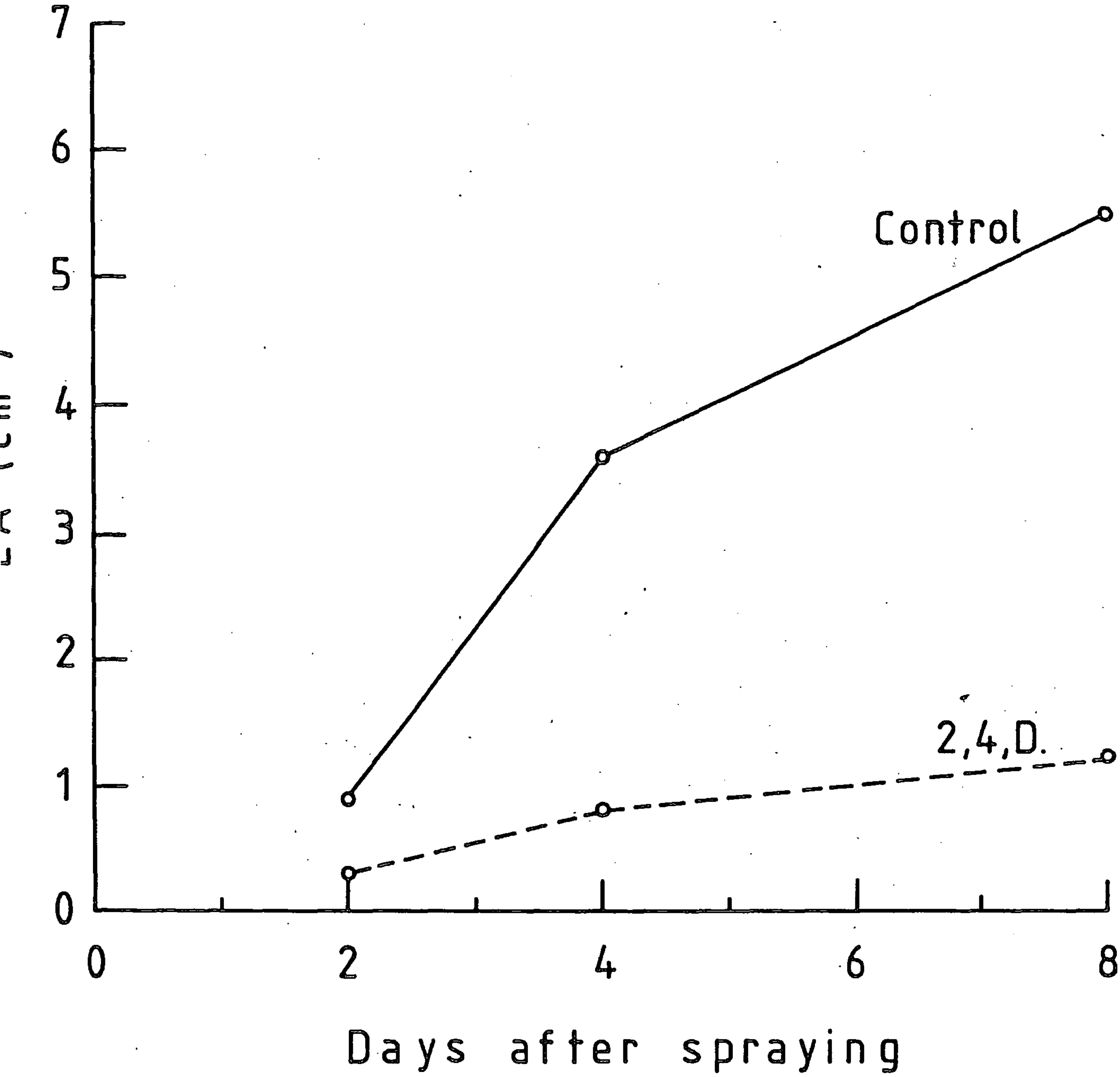
Treatment of radish seedlings with 2,4-D followed by drought stress showed a marked inhibitory effect on the growth and development of shoots and tubers. Treated seedlings, however, started wilting five days after treatment whilst the controls started wilting about 10 days after treatment. After 30 days all the treated seedlings had died, however, the controls remained a few days after that.

Parameters	2,4-D treated seedlings/gm.	Control seedlings/ gm.	p.t	p	D.f
Means of Shoot F.W	0.30	1.1	15.71	0.05	19
Shoot D.W	0.10	0.2	10.66	0.05	19
Tuber F.W	0.20	0.4	5.37	0.05	19
Tuber D.W	0.03	0.1	3.98	0.05	19

Table V : Effect of 2,4-D on growth and development of radish seedlings growing under drought stress. (3 weeks after treatment).

Fig. 1 Effect of 2,4-D on radish seedlings under drought stress, 5 days after treatment (Comparison between treated and untreated seedlings).

Fig. 2



The effect of 2,4,D. on the development of the third leaf in radish seedlings under drought stress.

MORPHOLOGICAL EFFECTS OF 2,4-D ON RADISH SEEDLINGS UNDER NORMAL WATERING REGIME

SHOOTS - The same symptoms described in experiment I developed within a few hours of treatment. When the seedlings were watered two days after treatment, twisting and bending were mostly overcome. By the end of one week, however, the treated seedlings had recovered, and the seedlings continued the growth normally as the controls. Leaves of treated seedlings showed no sign of injury. Even so, the reduction in fresh and dry weight of shoots suggested that 2,4-D had remarkable effect on radish seedlings under watering regime as well as under drought stress, but the effect was greater under drought stress.

TUBERS - The effect of 2,4-D on hypocotyl and root was more severe than on the shoots. An inhibition of tuber formation and tuber growth after 2,4-D treatment was evident. Root malformation was marked (Fig.3). Reduction of fresh and dry weights of tubers as well as in the diameter and the length were significant (Table VI).

Parameters	2,4-D treated seedlings	Control seedlings	p.t	p	D.F
Means of					
Plant F.W	6.2 gm.	11.9	7.73	0.05	19
Plant D.W	0.7	1.3	4.53	0.05	19
Shoot F.W.	3.5	4.8	2.61	0.05	19
Shoot D.W	0.4	0.6	2.86	0.05	19
Tuber F.W	2.7	7.2	9.28	0.05	19
Tuber D.W	0.4	0.7	4.92	0.05	19
Tuber diam.	1.3 cm.	2.0	6.75	0.05	19
Tuber length	3.1 cm	4.4	5.13	0.05	19

Table VI : Effect of 2,4-D on growth and development of radish seedlings under normal watering regime. (30 days after treatment)

Fig,3 Effect of 2,4-D on radish seedlings under normal watering regime, 30 days after treatment
(Comparison between treated and untreated seedlings).

EFFECT OF 2,4-D ON PROTEIN LEVEL IN RADISH SEEDLINGS

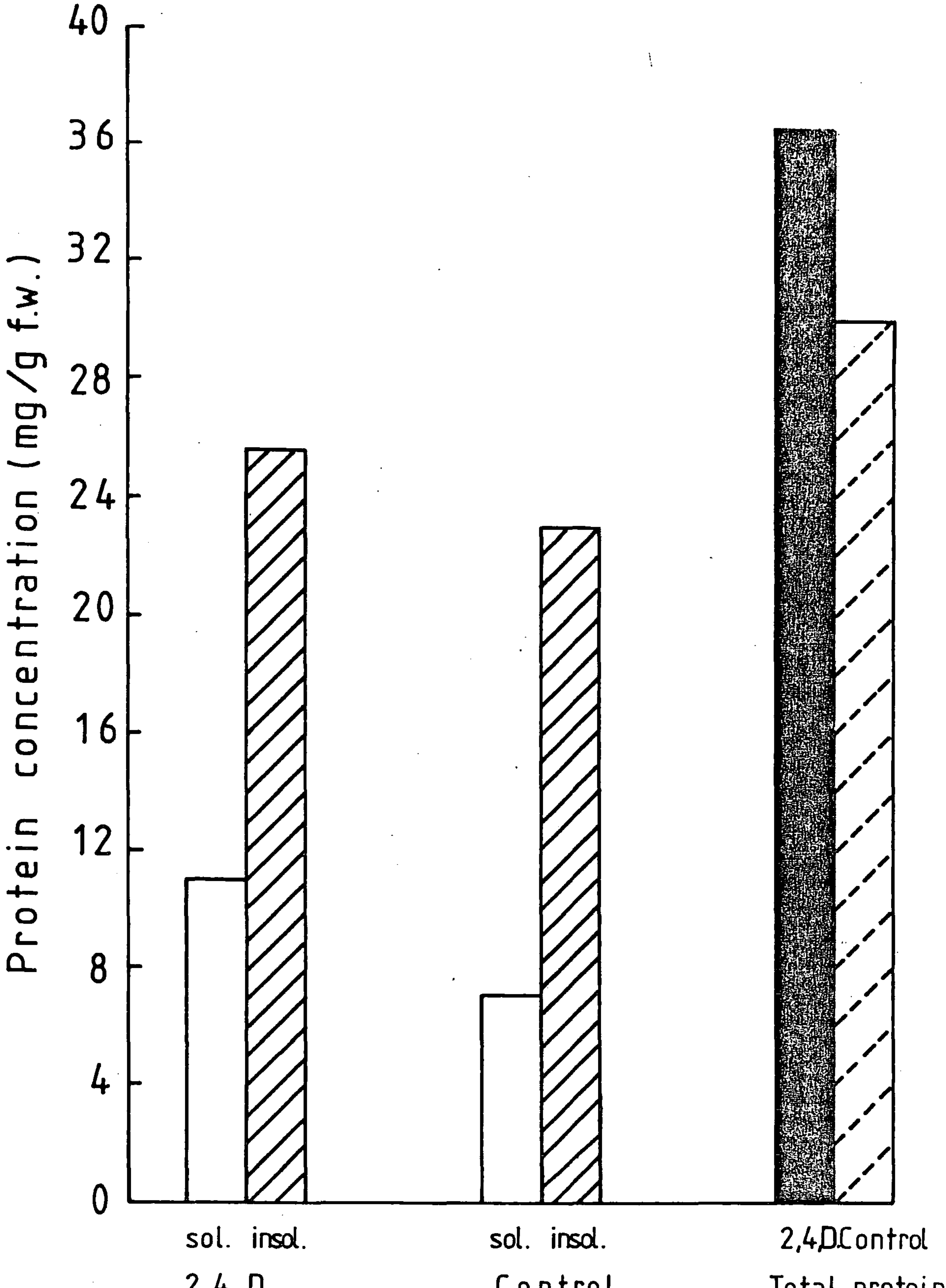
A. Under normal watering regime:

In general, leaves of both treated and untreated seedlings, showed higher levels of insoluble proteins than soluble proteins, but the total protein content was higher in 2,4-D treated seedlings (Fig.4), 14 days after treatment soluble protein was 9.0 mg/gf,wt, whilst in the control seedlings 7.0 mg/gfr.wt., and insoluble protein was 27.5 mg/gfr.wt. in 2,4-D treated seedlings whilst in untreated seedlings 23.0 mg/gfr.wt. only.

B. Under drought stress:

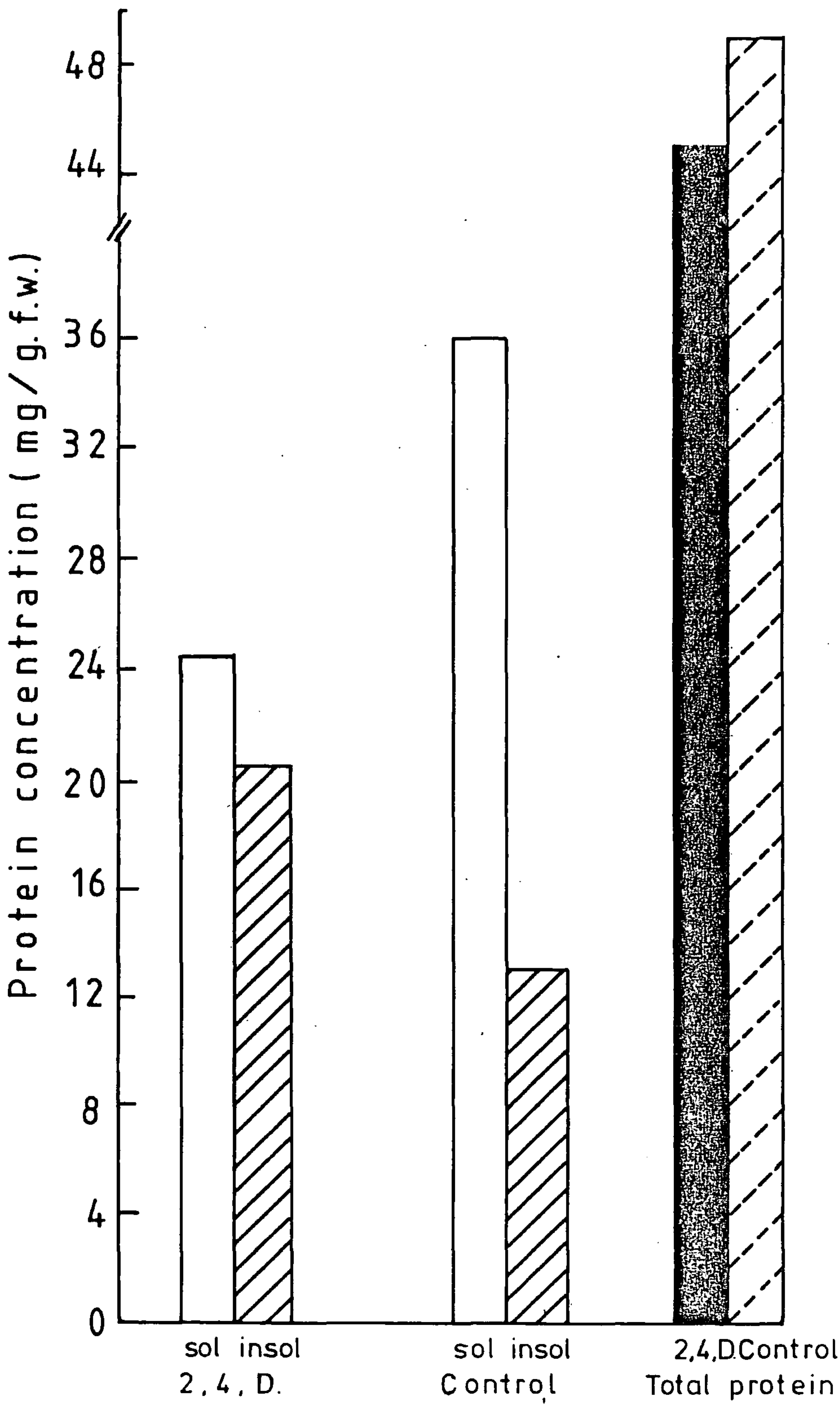
In contrast here the total protein contents were less in 2,4-D treated seedlings. The soluble proteins, however, were higher than the insoluble proteins in both treated and untreated seedlings. The soluble proteins were much higher in control than in 2,4-D treated seedlings, (36.0 mg/gfr.wt) in controls and (24.5 mg/gfr.wt.) in 2,4-D treated seedlings, whilst the insoluble proteins were higher in 2,4-D treated seedlings (20.5 mg/gfr.wt) and 13.0 mg/gfr.wt) in the controls (Fig.5).

Fig. 4



Protein content in 2,4,D. treatmed and untreated radish leaves under normal watering regime.(14 days after treatment).

Fig. 5



Protein content in 2,4,D. treated and untreated radish leaves under drought stress. (14 days after treatment.)

RESIDUAL EFFECT OF 2,4-D ON GERMINATION OF SEEDS AND DEVELOPMENT OF RADISH SEEDLINGS

Radish seeds planted in the soil 5 weeks after the time of spraying germinated normally indicating that the residual of 2,4-D had no inhibitory effect on germination of radish seeds at this concentration. However, most of the seedlings developed root malformation and marginal burning of the leaves, indicating that the 2,4-D was still active in the soil. The leaves of seedlings were shorter in length and in width, and the plants were stunted in appearance (Fig.6). The tubers appeared to be smaller than the tubers of the controls. Moreover, reduction in the growth of tubers were significant as measured by diamter, fresh and dry weight (Table VII). Depression in the rate of leaf growth was evident as measured by fresh and dry weight of shoots and development of leaves.

Parameters	2,4-D treated seedlings	Control seedlings	P.t	p	D.F
Means of					
Tuber diam.	1.5 cm	1.7	2.70	0.05	14
No. of leaves	4.8	6.4	5.52	0.05	14
Shoot F.W	1.5 gm.	2.2	3.19	0.05	14
Tuber F.W	4.8 gm.	7.1	3.44	0.05	14
Shoot D.W	0.2 gm.	0.2	2.57	0.05	14
Tuber D.W	0.4 gm.	0.5	2.95	0.05	14

Table VII : Residual effect of 2,4-D on the growth and development of radish seedlings under normal watering regime (30 days after planting).

Fig. 6 Residual effect of 2,4-D on radish seedlings under normal watering regime, 30 days after treatment. (Comparison between treated and untreated seedlings).

MORPHOLOGICAL EFFECTS OF CCC ON RADISH SEEDLINGS UNDER DROUGHT STRESS

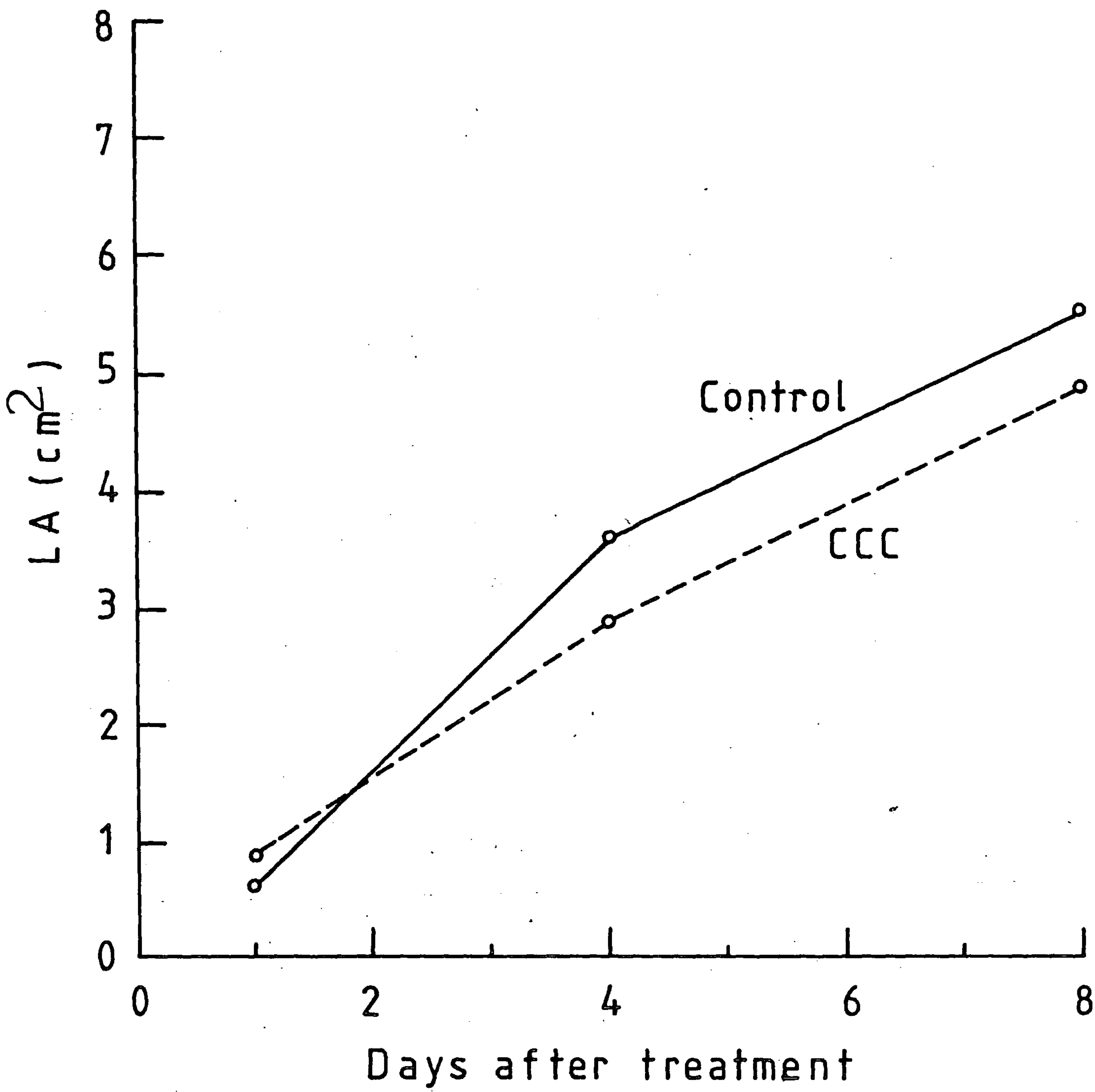
SHOOTS - Radish seedlings grew without apparent distortion response to CCC, and treated shoots showed no apparent signs of injury. Measurements of third leaf development, however, showed slight reduction in leaf area. In addition, treated seedlings were, by visual inspection, darker green in colour than untreated seedlings. Also reduction in fresh and dry weight of shoots indicated that the growth of treated seedlings was significantly less than that of untreated seedlings (Table VIII).

TUBERS-No apparent distortion effect was noted on the formation or growth of tubers (hypocotyl + root). The effect of CCC on the total growth of tubers as measured by weight was reduced, but there were no other toxicity symptoms. In general the treatment caused an initial depression in the rate of growth of shoot and tubers, but leaf or root abnormalities were not found (Table VIII).

Parameters	CCC-treated seedlings	Control seedlings	P.t	p	D.F
Means of					
Shoot F.W	0.50 gm.	1.1	12.16	0.05	19
Shoot D.W	0.10 gm.	0.2	4.19	0.05	19
Tuber F.W	0.20 gm.	0.4	5.16	0.05	19
Tuber D.W	0.03 gm.	0.1	3.64	0.05	19

Table VIII : Effect of CCC on growth and development of radish seedlings growing under drought stress (3 weeks after treatmenr).

Fig. 7



The effect of CCC on the development of the third leaf in radish seedlings under drought stress.

MORPHOLOGICAL EFFECTS OF CCC ON RADISH SEEDLINGS UNDER NORMAL WATERING REGIMES

Cycocel showed no clear signs of distortion effects on the treated seedlings such as leaf injury or root malformation, but did show clear effect on the growth habit; the seedlings were more uniform and the leaves were dark green. Inhibition of growth was significant as measured by fresh and dry weight and length and diameter of tuber (Table IX). These results suggested significant depression in the rate of growth.

Parameters	CCC-treated seedlings	Control seedlings	P.t	p	D.F
Means of					
Plant F.W	8.8 gm.	11.9	3.02	0.05	19
Plant D.W	0.9 gm.	1.3	4.24	0.05	19
Tuber F.W	4.5 gm.	7.2	4.28	0.05	19
Tuber D.W	0.4 gm.	0.7	5.72	0.05	19
Tuber diam.	1.6 cm.	2.0	5.16	0.05	19
Tuber length	3.5 cm.	4.4	3.12	0.05	19

Table IX : Effect of CCC on growth and development of radish seedling growing under normal watering regime (3 weeks after treatment).

EFFECT OF CCC ON PROTEIN LEVEL IN RADISH SEEDLINGS

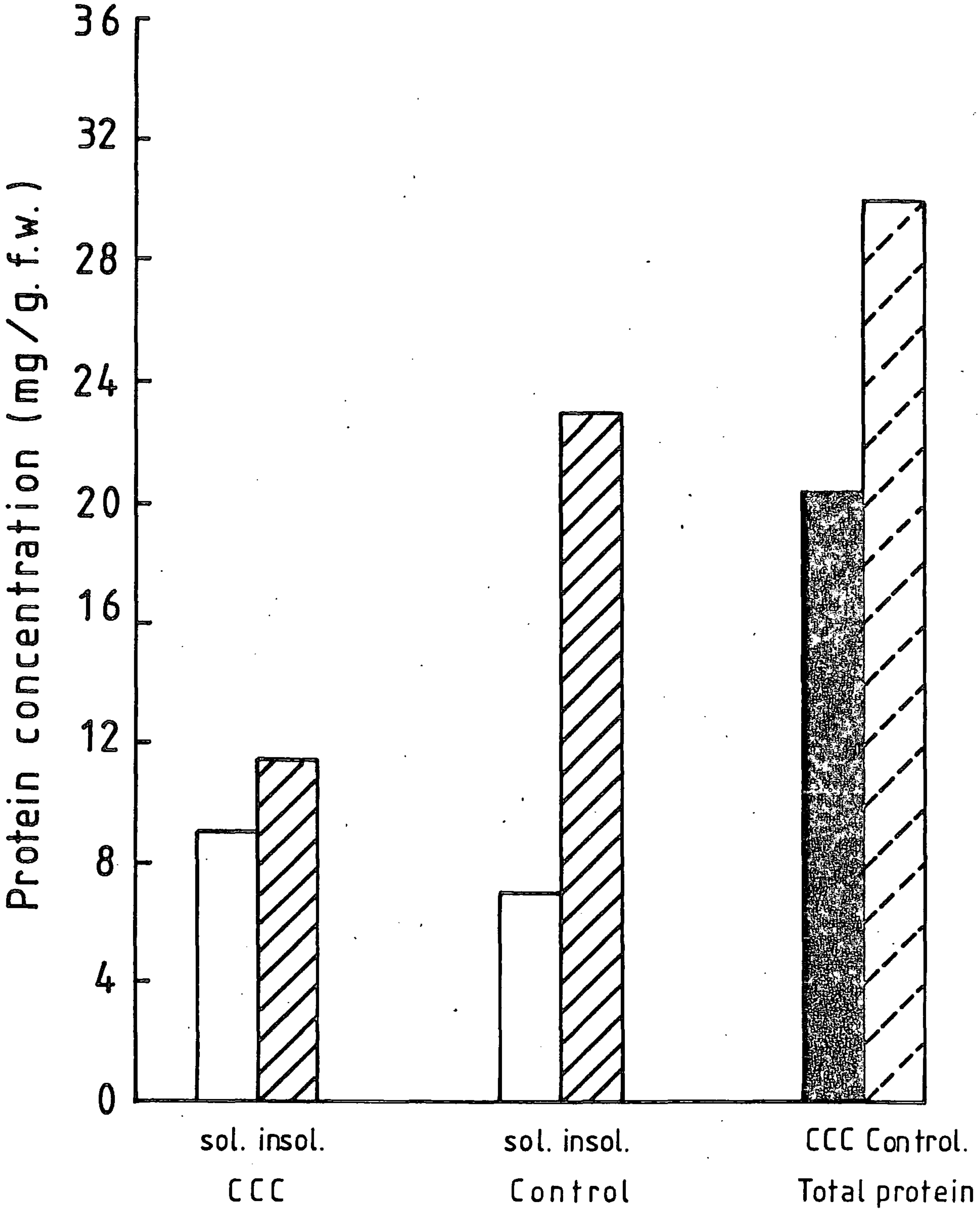
A. Under normal watering regime :

CCC- treated seedlings showed a large decline in total protein content in comparison to the controls. The soluble protein in both treated and untreated seedlings were less than the insoluble proteins, and the soluble proteins were higher in CCC-treated seedlings (Fig. 8). The soluble protein was 9.0 mg/g fr.wt. in CCC-treated seedlings and 7.0 mg/g fr.wt. in untreated seedlings, whilst the insoluble protein was 11.5 mg/g fr.wt in CCC.treated seedlings and 23.0 mg/g fr.wt in the controls.

B. Under drought stress

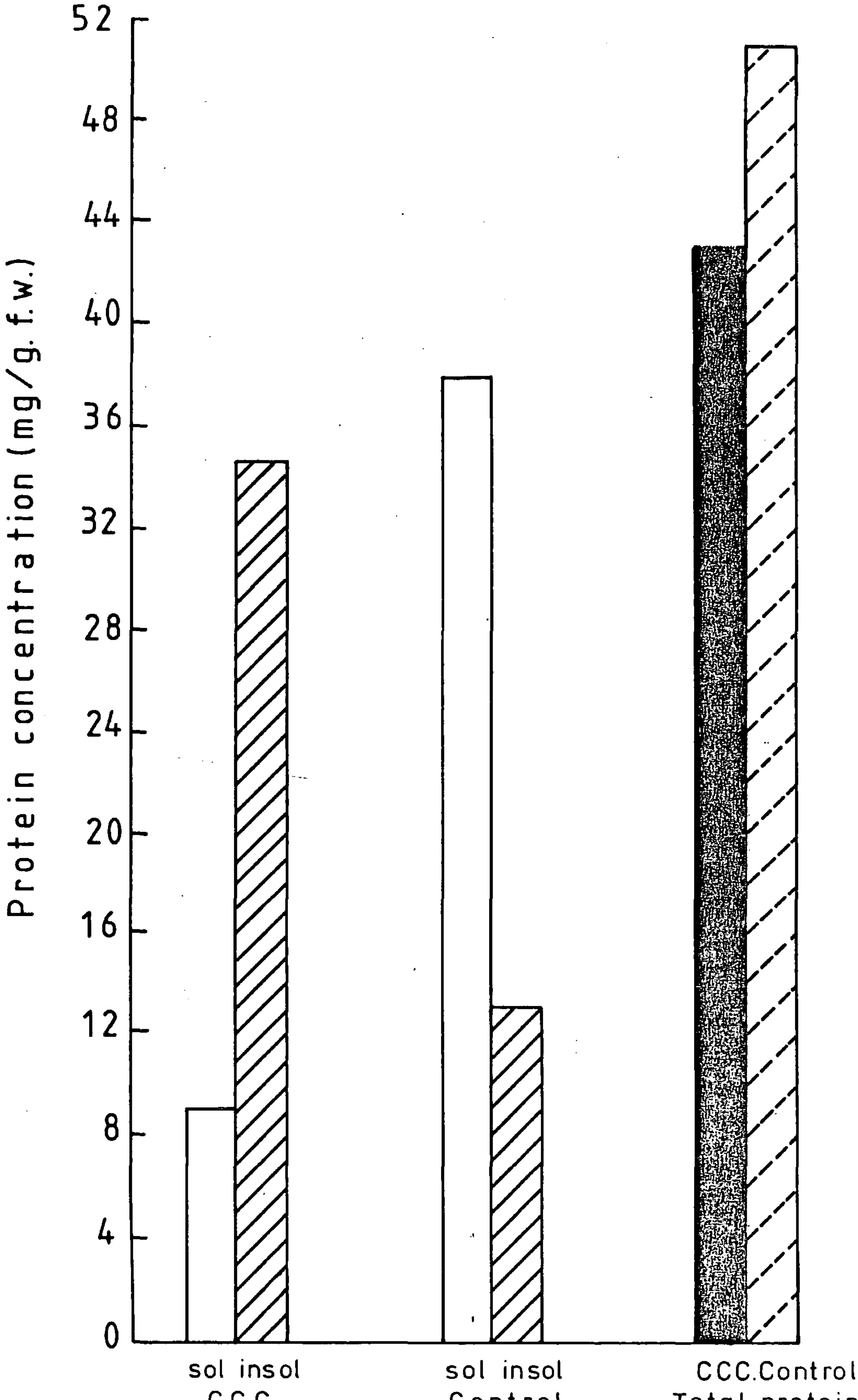
CCC-treated seedlings again showed a large decline in total protein content in comparison with the controls (Fig.9). Although the concentration ofinsoluble proteins were much higher in CCC-treated seedlings (34.5 mg/g fr.wt.) whilst in controls (13.0 mg/g fr.wt.) only. In contrast to insoluble proteins, the soluble proteins were higher in untreated seedlings (30.0 mg/g fr.wt.) whilst in CCC-treated seedlings (9.0 mg/g fr.wt.) only.

Fig. 8.



Protein content in CCC treated and untreated radish leaves under normal watering regime.

Fig. 9



Protein content of CCC treated and untreated radish leaves.under drought stress. (14 days after treatment)

RESIDUAL EFFECT OF CCC ON GERMINATION OF SEEDS AND DEVELOPMENT OF RADISH SEEDLINGS

Radish seeds planted in the soil treated 5 weeks previously with CCC showed no signs of effect. They germinated normally indicating that any residual CCC was not inhibited the germination of radish seeds. Leaves and tubers of the seedlings growing in treated soil showed no visible abnormalities or injury. However, the growth and rate of development as measured by fresh and dry weight of shoots and tubers, length and diameter of tuber and the number of leaves per plant suggested that the growth of seedlings growing in treated soil was significantly less than that of seedlings growing in untreated soil. Also seedlings growing in CCC-treated soil were by visual inspection darker green in colour and more uniform in comparison to controls (Table X).

Parameters	CCC-treated seedlings	Control seedlings	Pt	p	D.F
Means of					
Plant F.W	4.5 gm.	6.7	4.403	0.05	14
Tuber F.W	2.8 gm.	5.1	3.248	0.05	14
Tuber D.W	0.3 gm.	0.4	2.542	0.05	14
Tuber diam.	1.2 cm.	1.6	2.995	0.05	14

Table X : Residual effect of CCC on the growth and development of radish seedlings under normal watering regime (30 days after planting).

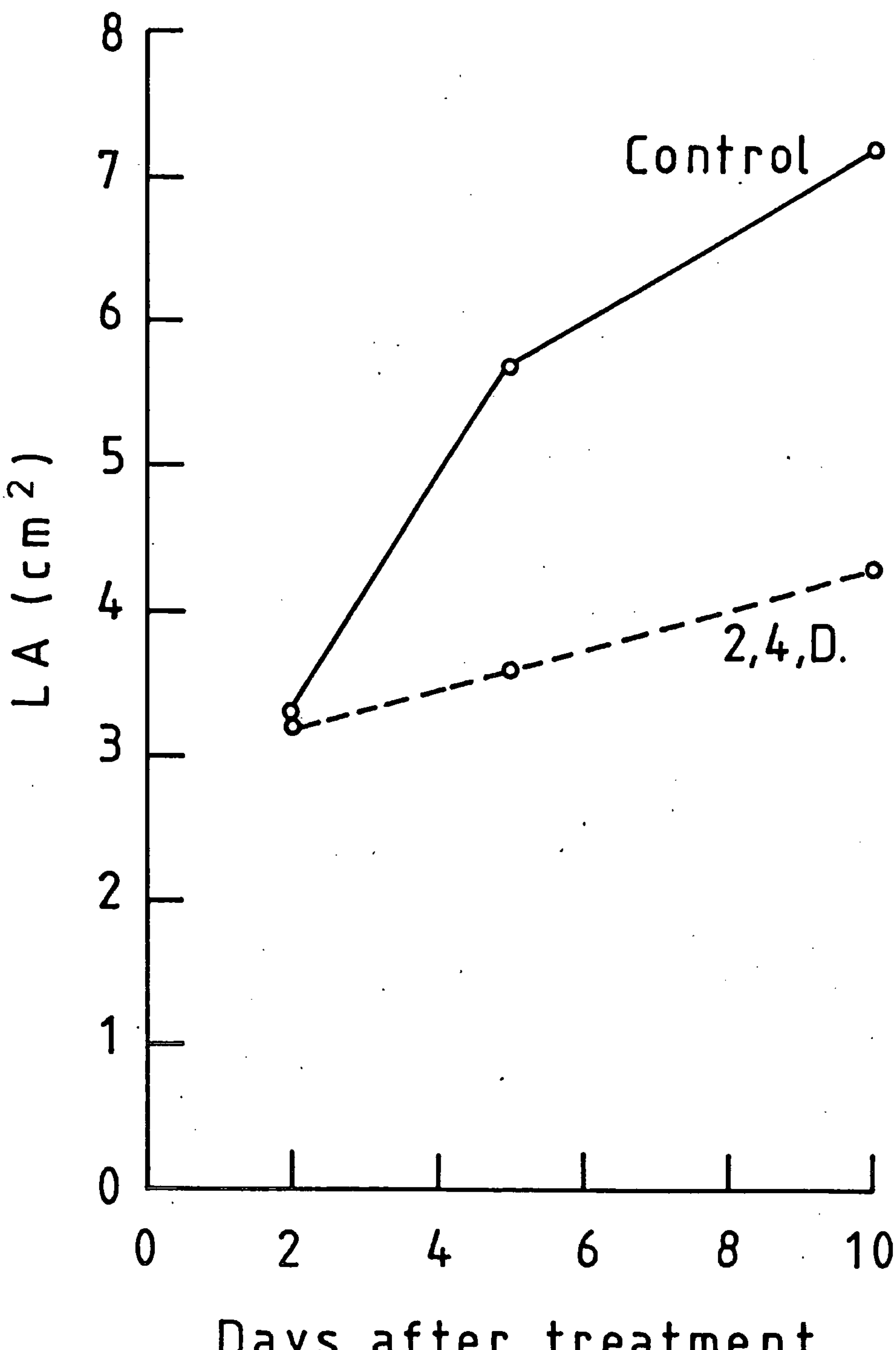
MORPHOLOGICAL EFFECTS OF 2,4-D ON WHEAT SEEDLINGS UNDER DROUGHT STRESS

The treated seedlings showed no apparent signs of injury on leaves or tubers. Wheat seedlings grew without clear response to 2,4-D, but the first two leaves in treated seedlings began to senesce earlier than in untreated seedlings. Also the treated seedlings started wilting earlier than the untreated seedlings, and died 30 days after treatment, whilst the control seedlings continued for several days after that. Moreover the fresh and dry weights decreased significantly, and the number of leaves per plant were reduced. Furthermore there were reductions in the third leaf development (Fig. 10), (Table XI).

Parameters	2,4-treated seedlings	Control seedlings	P.t	p	D.F
Means of					
Plant F.W	0.4 gm.	0.8	8.28	0.05	19
Plant D.W	0.1 gm.	0.2	4.64	0.05	19
No.of leaves	4.8	5.3	4.81	0.05	19

Table XI : Effect of 2,4-D on growth and development of wheat seedlings under drought stress. (3 weeks after treatment)

Fig. 10



The effect of 2,4,D. on the development of the third leaf in wheat seedlings under drought stress.

MORPHOLOGICAL EFFECTS OF 2,4-D ON WHEAT SEEDLINGS UNDER NORMAL WATERING REGIME

Under a normal watering regime there was no clear effect on the early growth or development of wheat seedlings. A recorded effect, however, was that the treated plants showed an earlier senescence of some of the leaves. As a result the number of leaves per plant was significantly reduced in comparison with that of the controls (Fig. 11).

EFFECT OF 2,4-D ON PROTEIN LEVEL IN WHEAT SEEDLINGS

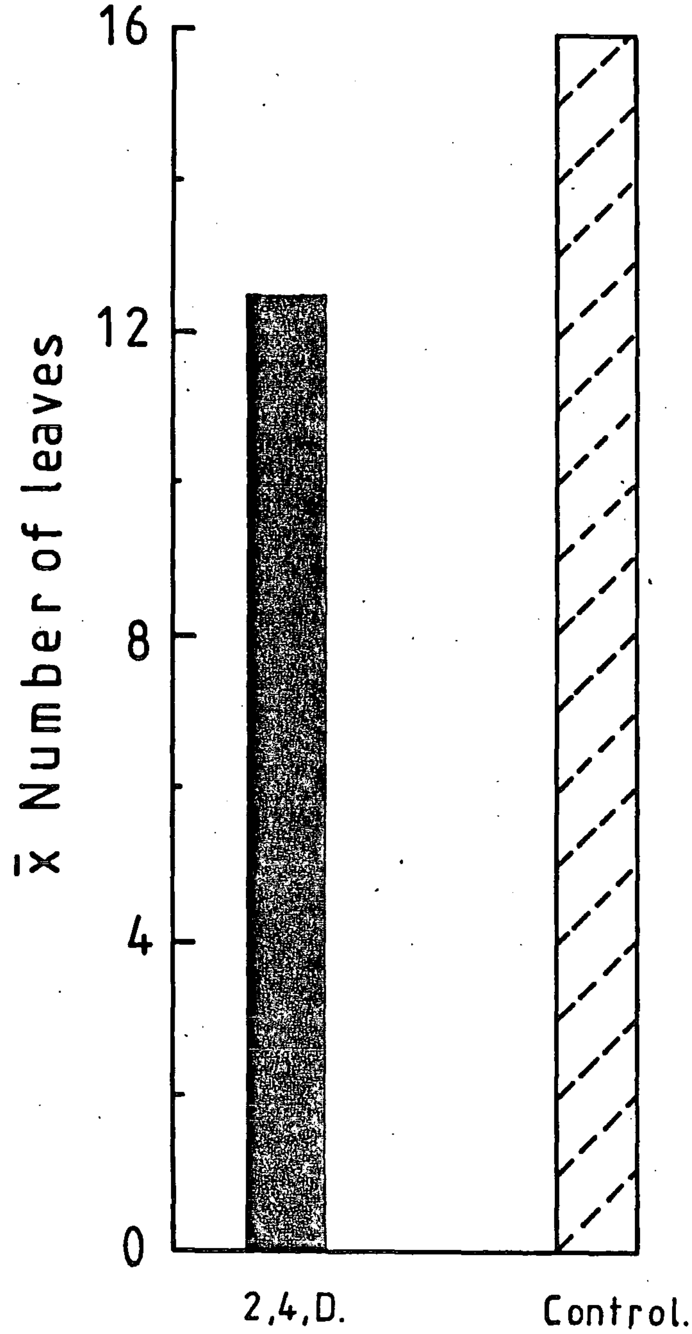
A. Under normal watering regime :

2,4-D treated seedlings showed an increased total protein content over that of the controls, although the concentration of insoluble proteins were higher than the soluble proteins in both treated and untreated seedlings. The insoluble proteins were 49.0 mg/g fr.wt in 2,4-D treated seedlings and 43.0 mg/g fr.wt in the controls. The soluble proteins were 26.0 mg/g fr.wt. in 2,4-D treated seedlings and 16.5 mg/g fr.wt in the controls (Fig.12).

B. Under drought stress :

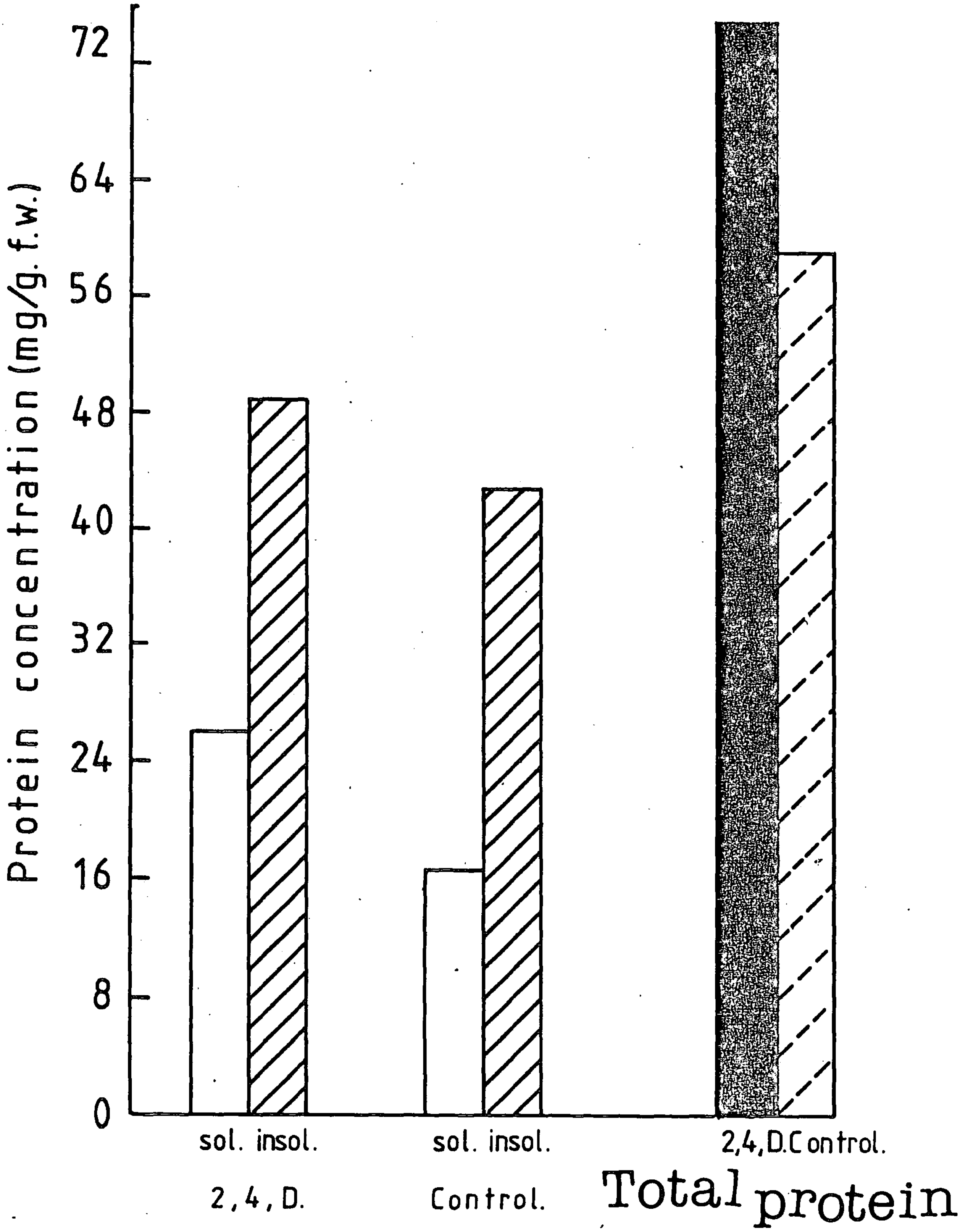
In contrast to the seedlings growing under watering regime, the total protein content in 2,4-D treated seedlings growing under drought stress, was less than that of the controls (Fig.13). However, the soluble proteins were higher than insoluble in both treated and untreated seedlings. The soluble proteins were 25.0 mg/g fr.wt. in the controls and 20.5 mg/g fr.wt. in 2.4-D treated seedlings, whilst the insoluble proteins were 21.5 mg/g fr.wt. in untreated seedlings and 19.0 mg/g fr.wt. in 2,4-D treated seedlings.

Fig.11



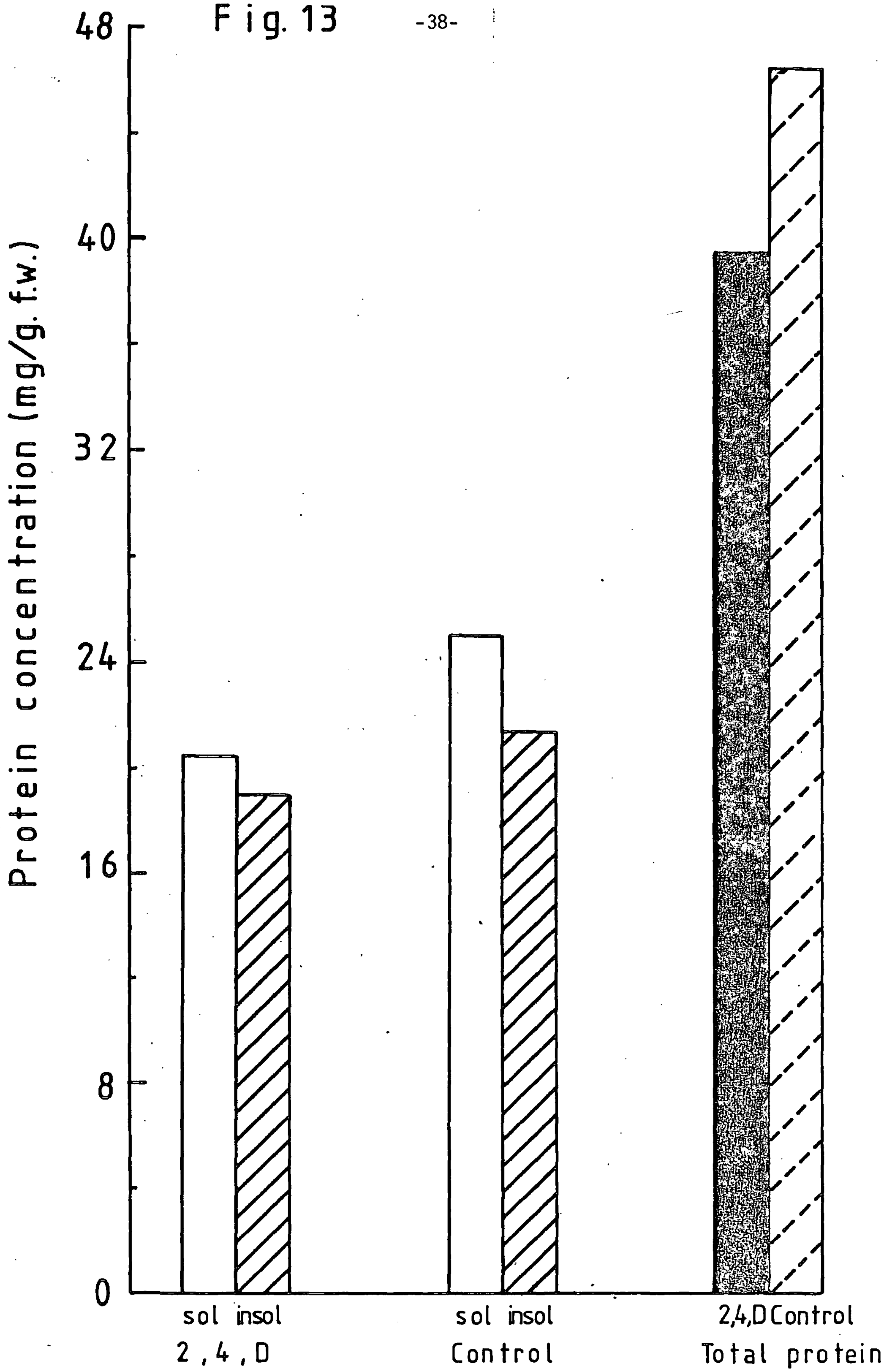
The effect of 2,4,D. on the number of leaves in wheat under normal watering regime.

Fig. 12



Protein content in 2,4,D. treated and untreated wheat leaves under normal watering regime. (14days after treatment.)

Fig. 13



Protein content in 2,4,D. treated and untreated wheat seedlings under drought stress (14 days after treatment)

MORPHOLOGICAL EFFECTS OF CCC ON WHEAT SEEDLINGS UNDER DROUGHT STRESS

Wheat seedlings treated with CCC, were shortened,thickened and darker green. Although the reduction in the fresh and dry weights and number of leaves (Table XII) indicated that the growth of seedlings was inhibited by CCC treatment, the CCC had a marked positive effect on the seedling. Wheat seedlings treated with CCC were the last to wilt and die in comparison with the controls; CCC-treated seedlings died 40 days after treatment without any change in the colour of the leaves

Parameters	CCC treated seedlings	Control seedlings	p.t	p	D.F
Means of					
Plant F.W	0.6 gm.	0.8	4.36	0.05	19
Plant D.W	0.1 gm.	0.2	4.78	0.05	19
No. of leaves	4.8	5.3	3.26	0.05	19

Table XII : Effect of CCC on growth and development of wheat seedlings under drought stress (3 weeks after treatment)

MORPHOLOGICAL EFFECTS OF CCC ON WHEAT SEEDLINGS UNDER NORMAL WATERING REGIME

Reduction in the number of tillers and number of leaves per plant (Fig. 14); indicating that the growth of seedlings treated with CCC was significantly less than that of the untreated seedlings. In addition to that treated seedlings were by visual inspection shorter, darker green in colour and thicker than the controls.

EFFECT OF CCC ON PROTEIN LEVEL IN WHEAT SEEDLINGS

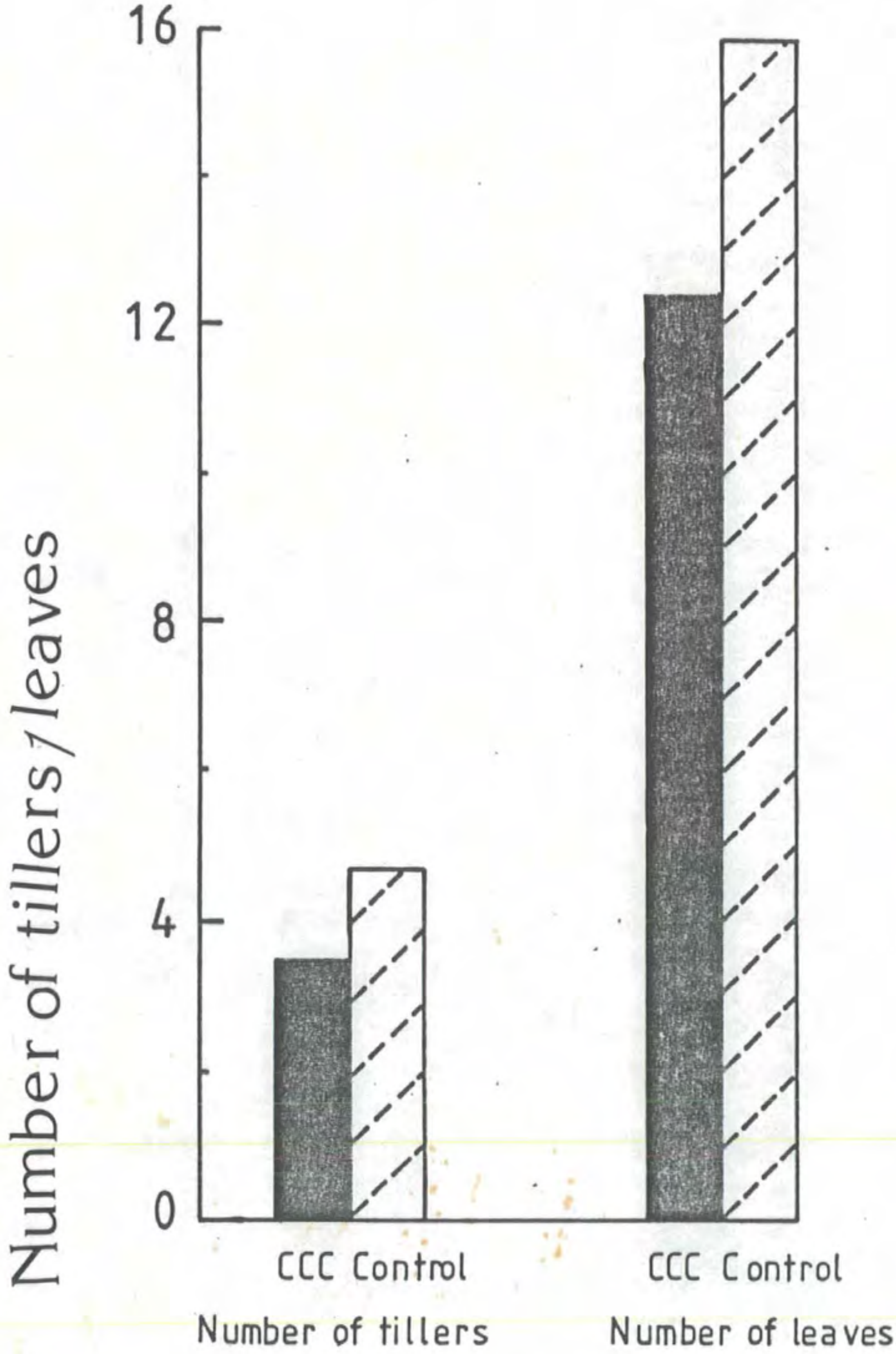
A. Under normal watering regime

The total protein content was higher in CCC-treated seedlings than that of the controls (Fig. 15). The soluble proteins were also much higher in CCC-treated seedlings than that of the controls. The insoluble proteins, however, showed no great differences between CCC-treated and untreated seedlings. The soluble proteins were 35.0 mg/g. fr.wt. in CCC-treated seedlings and 16.5 mg/g.fr.wt. only in the controls. In the meantime the insoluble proteins were 42.0 mg/g fr.wt. in CCC-treated seedlings and 43.0 mg/g fr.wt. in the controls which were higher.

B. Under drought stress

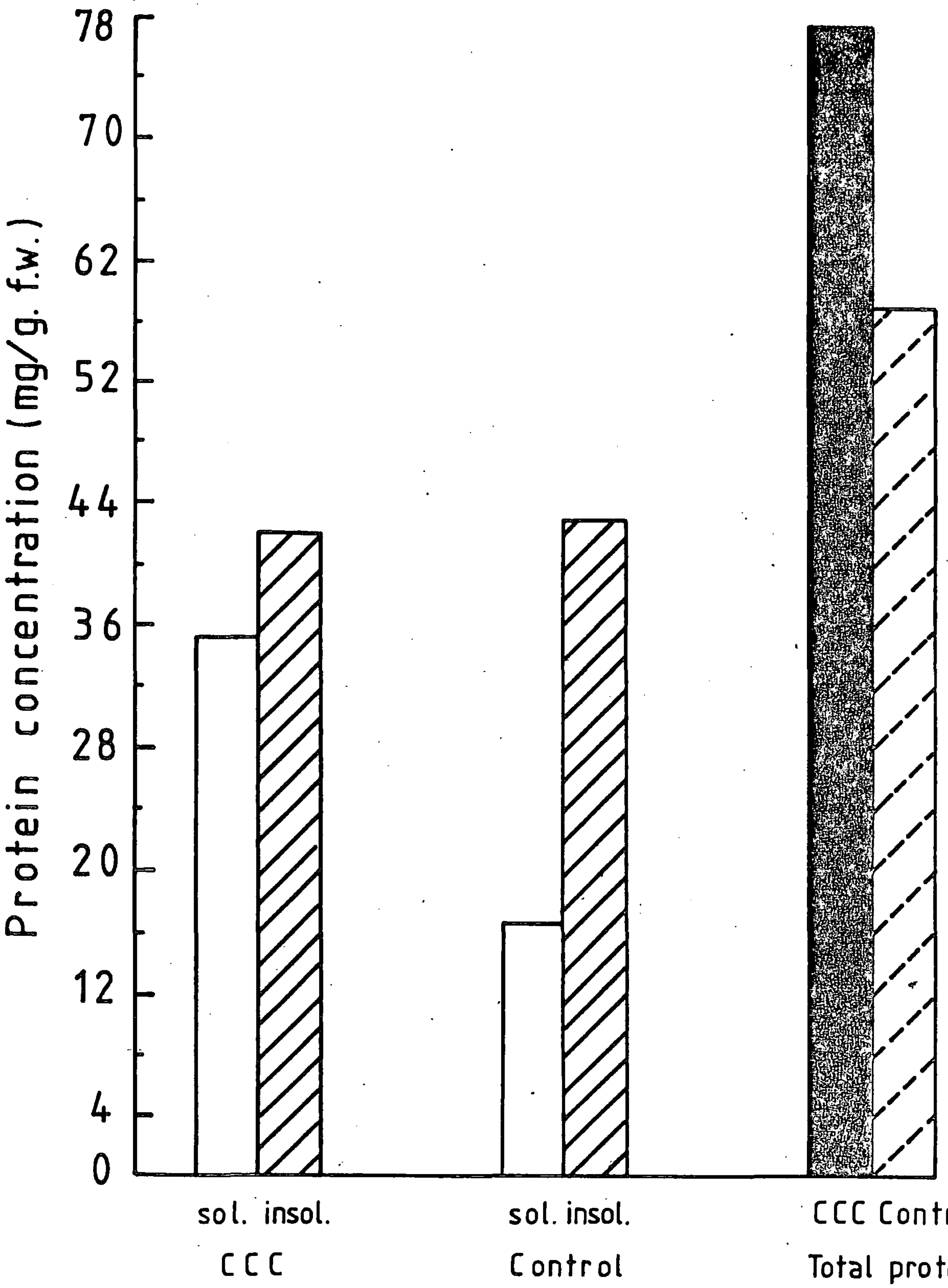
An increase in the total protein content was evident in CCC-treated seedlings. In contrast to the controls, the insoluble proteins were higher than the soluble (35.0 mg/g.fr.wt.) whilst the soluble proteins were (22.5 mg/g.fr.wt. only). In untreated seedlings the soluble proteins were higher 25.0 mg/g.fr.wt. whilst the insoluble proteins were (26.5 mg/g.fr.wt) only. (Fig.16).

Fig. 14



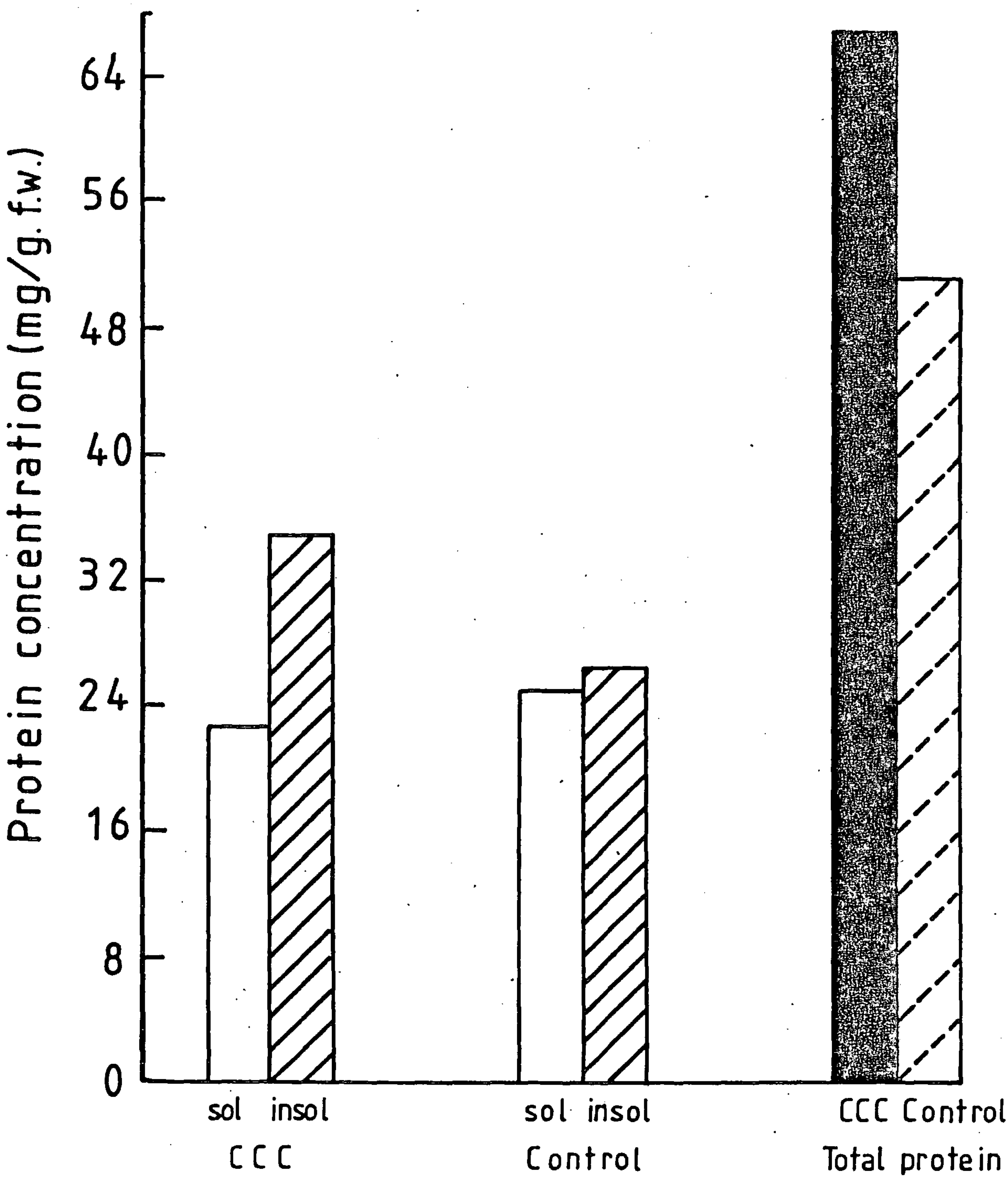
The effect of CCC on the number of leaves and tillers in wheat seedlings under normal watering regime.

Fig. 15



Protein content in CCC treated and untreated wheat leaves under normal watering regime. (14 days after treatment.)

Fig. 16



Protein content in CCC treated and untreated wheat leaves under drought stress. (14 days after treatment.)

DISCUSSION

A. EFFECT OF 2,4-D ON RADISH SEEDLINGS :

The symptoms which resulted from 2,4-D application to radish seedlings were as expected. Since radish is a dicotyledon plant, it is very sensitive to auxin-herbicides. Morphological bending, twisting and swelling of plant organs and plant tissue are commonly seen during the action of auxin-herbicides (Fedtke , 1982). These symptoms are attributed to increase in the production of nucleic acids in the treated tissue. As a result many chemical processes are stimulated, the stimulation of which promotes cell division and creates new cambial promordia. Consequently cell growth and tissue swelling developed (Fedtke, 1982). The final result is plant break down at a cellular level. According to that root and hypocotyle malformation may be explained by increased volume of cortical cells above the root apex and abnormal cell division in the pericycle and endodermis resulting in the extension of the hypocotyle and root swelling. This suggested that 2,4-D stimulates cell division in the roots but inhibited the elongation of those roots.

In addition to the morphological effects of 2,4-D on radish seedlings, reduction in fresh and dry weight of shoots and tubers was significant, both for seedlings growing under drought stress and a normal watering regime following the application of 2,4-D. These results suggested that 2,4-D has an inhibitory effect on the basic metabolic pathway of photosynthesis, respiration, protein synthesis and nucleic acid synthesis (Fedtke, 1982), under a normal watering regime and under drought stress, but under drought stress the problem becomes worse, since drought stress has a similar inhibitory effect in the metabolic pathways (Hsiao, 1973). Because of those combined effects of drought stress and 2,4-D, the plants had no chance to recover or to overcome the bending effect of 2,4-D. Such effects argue that the herbicides place a stress on the treated plants and the stress makes them more susceptible to drought stress (Muzik, 1976). The opposite was true for the seedlings kept under normal watering regime. The seedlings had recovered particularly the shoots where twisting and curling of cotyledons and petioles was no longer seen and developed normally. These are very interesting and unexpected results, since the radish seedlings are very sensitive to auxin-herbicides particularly in this stage of development, which suggested that excess of water following 2,4-D treatment may remove the expression of symptoms and decreases phototoxicity. Even so, root malformation was evident in the seedlings growing under watering regime, which may result from 2,4-D persistence in the soil or accumulation of 2,4-D in the roots from the shoots through the transport system.

Although many reports indicated that sub-toxic application of phenoxy herbicides, particularly 2,4-D may increase the total protein yield (Ries,1976), the data indicated that application of 2,4-D, at toxic level, followed by drought stress, decreased the total protein level in radish leaves in comparison to the controls. The results suggested that reduction in total protein level in treated seedlings resulted, because of the combined effect of 2,4-D and drought stress. This suggestion is supported by the data obtained from the radish seedlings, which were kept under a normal watering regime. The data show that total protein content in 2.4-D-treated seedlings may be explained by decreasing the phytotoxicity of 2,4-D to the plant as a result of application of water which may increase the stimulatory effect of 2,4-D and subsequently the total protein level as indicated at sub-toxic level. In the meantime increased level of soluble proteins in treated and untreated seedlings under drought stress indicated that drought stress had pronounced effect on protein synthesis and metabolism. Unlike the seedlings growing under drought stress, seedlings growing under a normal watering regime showed increased insoluble proteins in both treated and untreated seedlings. These results indicated that those seedlings were less stressed.

Radish seeds planted in the soil 5 weeks after the time of spraying germinated normally indicating that residual 2,4-D had no inhibitory effect on germination of radish seeds. At the same time most of the seedlings developed root malformation and marginal burning of leaves, indicating that the 2,4-D was still active in the soil. These results are in agreement to a certain extent with what have been found by (Hamner et al. 1946) that bean seeds planted in 'muck soil' 3 weeks after it had been treated with 2,4-D germinated and grew with only slight symptoms of the presence of 2,4-D. Primary leaves of a few plants developed virus-like symptoms indicating that some of the chemical was still active in the 'muck'. In the same experiment (Hamner et al. 1946) found that bean and pea seeds planted in muck 4 weeks after the time of treatment germinated and grew normally, indicating that the toxic effect of 2,4-D had been dissipated. In the meantime they found that peas and beans planted in muck 1 week after it had been treated with 2,4-D were severely affected; the peas failed to germinate and the beans exhibited pronounced formative defects. They concluded that the toxic effect of 2,4-D in the muck disappeared to a great extent after 3 weeks. Our data suggested that in compost soil the toxic effect may extend up to 5-6 weeks. This result is in great contrast with that found by (Mitchell et al. 1945). They found that 2,4-D was not persistent under greenhouse conditions in the compost, however, since seeds of three species of grass germinated readily and became established when planted in the soil 5 weeks after its surface had been sprayed. The germination of grass seeds in Mitchell's experiment does not necessarily mean 2,4-D was not persistent under greenhouse conditions because grass seeds and seedlings are not good indicators for 2,4-D persistence but the opposite may be true since a small quantity of 2,4-D may stimulate the germination and growth of such plants. It could be concluded from these facts that species of plant, soil type, and environmental condition in relation to mode of action, phylotoxicity, mobility and degradability of such herbicides should be considered in this context.

B. EFFECT OF CCC ON RADISH SEEDLINGS :

Seedlings treated with Cycocel showed no symptoms of toxicity such as bending, twisting or swelling, under both drought stress and normal watering regime. The effect here is different to that of a toxic herbicide since it acts as a positive regulator of plant growth. Such compounds do regulate the growth of plants without formative effect. Even so, CCC-treated seedlings showed significant reduction in fresh and dry weight of shoots and tubers under drought stress and under normal watering regime as well. The same results have been reported by (Cathey et al. 1961; and Emden et al. 1967). The reduction of fresh and dry weight may be due to the inhibition of cell division and cell elongation in treated seedlings (Nickell, 1982), or due to the reduction of the number of stomata per unit area of leaf surface and this response could have affected the rate of diffusion, carbon dioxide into the leaf and the rate of water loss from the leaf, thus reducing total dry and fresh weight (Emden, 1967). The other possibility is that there was a reduction of the leaf surface resulting from shorter, and narrower leaves which could be the cause of weight reduction (Bode et al. 1984). Cycocel treated seedlings also showed slight reduction in the rate of development of third leaf as measured by leaf area. Reduction of leaf area in radish was reported by (Humphries, 1963) with all dosages of CCC. By visual inspection treated leaves were thicker and darker green in colour. The thickness of the leaves could be due to the inhibitory influence of CCC on all enlargement (Bode et al. 1984).

Although CCC has been reported to increase nitrogen and some other minerals, our data indicated that CCC retarded protein accumulation in radish leaves. Under drought stress and normal watering regime the total protein contents in CCC-treated seedlings were decreased in comparison to the controls. However, under normal watering regime soluble proteins were less than insoluble in both treated and untreated seedlings in comparison to the controls, which indicated that radish seedlings were not stressed as a result of CCC-treatment. The point which is of interest was that under drought stress insoluble proteins were much higher than soluble proteins in CCC-treated seedlings which again indicated that CCC-treated seedlings were not stressed or disturbed as a result of CCC. Whilst the opposite was true in the controls. However, retardation of protein accumulation has been reported by Russian workers on winter wheat (Nickell, 1982). CCC has been shown to act as inhibitor of protein synthesis.

The nature of the interaction between environmental factors and CCC is not clear, but such an interaction does exist. Cathey et al. 1961, for example reported that growth retardation following application of CCC was much less in summer than in winter. Many other workers (e.g. Farah, 1969; Emden et al. 1967 and Cockshull et al. 1969) have reported that CCC increased drought tolerance in treated plants. Our data also indicated that radish seedlings treated with CCC showed less susceptibility to drought stress.

Cycocel has been reported to delay the germination of seeds (Cathey et al. 1961). In this experiment, radish seeds planted in the soil treated 5 weeks previously with CCC, showed no signs of effect. They germinated at the same time as the controls. This result indicated that persistent CCC did not inhibitit germination. However, the same symptoms described for sprayed seedings appeared on the seedlings growing in the soil contained persistent Cycocel. Reduction of plant fresh weight and tuber fresh and dry weight was significant, although the reduction of plant dry weight was not significant.

C. EFFECT OF 2,4-D ON WHEAT SEEDLINGS :

The application of 2,4-D to the foliage of wheat seedlings appeared to have no distortion effect on the leaves or tillers, under both drought stress and normal watering regime. That was because of the genetic nature of this plant as auxin-herbicide resistant, Even so, the reduction of fresh and dry weight of seedlings and reduction of third leaf area under drought stress and reduction of the number of leaves per plant under normal watering regime indicated that 2,4-D may have had an indirect effect on the growth and development of wheat seedlings. These results suggest that decreased fresh and dry weight of the seedlings resulted from foliage application of 2,4-D may be due to the damage of roots, even though the foliage may appear undamaged (Johanson et al. 1961). The effect of damaged roots may be increased under drought stress, since the reduction of fresh and dry weight was under drought stress only. Reduction in the number of leaves per plant under both drought stress and normal watering regime may be due to increase of ethylene production in treated leaves, which makes leaves senesce early (Nickell, 1982). Wilting of 2,4-D treated seedlings early in comparison to the controls suggested that damaged roots were unable to provide enough water for the whole plant.

In 2,4-D-treated leaves a reduction in total protein contents was marked under drought stress in comparison to the controls and the opposite was true under normal watering regime. It seems to be that reduction in total proteins in 2,4-D treated seedlings under drought stress more likely resulted from drought stress than 2,4-D, since total proteins in treated seedlings increased under normal watering regimes. Moreover soluble proteins were higher than insoluble proteins in both treated and untreated seedlings growing under drought stress, and the opposite was true under normal watering regime. These changes in protein from insoluble to soluble were only under drought stress, which suggested that drought stress had pronounced effect on protein synthesis and metabolism in wheat seedlings, rather than 2,4-D.

The herbicide 2,4-D has often been reported to damage wheat plants (Johanson et al, 1961). Most investigators agree that wheat is most sensitive at the spikelet initiation and formation of flower parts stages. In this experiment 2,4-D application to wheat seedlings showed no great damage under normal watering regime, whilst under drought stress the damage was marked. These results indicated that combined effect of 2,4-D and environmental condition such as drought stress may increase the possibility of damage since drought stress makes plants more sensitive to herbicides (Muzik et al. 1964).

D. EFFECT OF CCC ON WHEAT SEEDLINGS:

Cycocel application to wheat to reduce the size of overall plant has become normal practice in modern agriculture, especially in the growing of wheat in Europe (Nickell, 1982). The reaction of plants to treatment by CCC under drought stress is of special interest. These results showed that the application of CCC to wheat seedlings under drought stress resulted in reduction of fresh and dry weight of wheat seedlings and the number of leaves per plant in comparison to the controls. The same results have been reported by (Cathey et al. 1961, and Emden et al. 1967). The reduction of fresh and dry weight may be due to the inhibition of cell division and cell elongation in treated seedlings (Nickell, 1982), or due to the reduction in the number of stomata per unit area of leaf surface and this response could have affected the rate of diffusion carbon dioxide into the leaf and the rate of water loss from the leaf, thus reducing total dry and fresh weights (Emden, 1967). The reduction of fresh and dry weights was under drought stress only. Reduction of the number of leaves was under both drought stress and normal watering regime. This may be the result of the action of CCC decreasing the rate of development. By visual inspection the leaves of treated seedlings were thicker and darker green which could be due to the inhibitory influence of CCC on cell enlargement (Bode et al.1984). Also in this experiment the progress of wilting was observed in untreated seedlings and seedlings treated with CCC. The same results obtained by (Cockshull et al. 1969; and Emden et al. 1967), suggested that leaves of treated seedlings retain more water than the controls.

Total protein content in the leaves of treated seedlings was higher under both drought stress and normal watering regime. It seems that CCC increases protein content in wheat, since this increase was under both drought stress and watering regime. Elsewhere Russian studies on winter wheat in the field showed the opposite (Nickell, 1982). However, increased insoluble protein in treated seedlings in comparison to the controls indicated that treated seedlings were at less stress which suggested that Cycocel increased the tolerance of plants to drought stress as has been suggested by some investigators. It is, however, interesting that soluble protein remained higher than insoluble in leaves of CCC-treated seedlings growing under normal watering regime.

Finally, one may conclude that :

1. Drought stress does have an effect on protein level.
2. Application of herbicides/growth regulators must be considered in relation to other environmental factors.
3. Persistence, whilst not apparent at an early stage may become a problem in long term growth.
4. Radish is a good assay for persistent 2,4-D.

SUMMARY

Under glasshouse conditions, Caryopses of wheat (Triticam aestivum L.) and seeds of radish (Raphanus sativus L.) cv French Breakfast were planted in John Innes No.2 compost contained in trays. Two weeks after planting, seedlings of wheat and radish were sprayed with an equal volume of 2,4-D and CCC at one standard concentration (100 ppm). Three growth experiments were carried out using distinct treatments. A variety of parameters were used to assess the effects of 2,4-D and CCC on the development and growth rates of wheat and radish seedlings. The effects of 2,4-D and CCC on both radish and wheat seedlings can be summarized as follows:

1. EFFECT OF 2,4-D ON RADISH SEEDLINGS :

Two hours after treatment extreme bending and twisting of the whole seedlings was evident particularly the petioles. An application of 2,4-D to radish seedlings followed by drought stress showed a remarkable influence on the further growth of the third leaf. As a result, reduction in fresh and dry weight of shoots was significant. In seedlings watered normally two days after treatment, twisting and bending were mostly overcome and the seedlings continued to grow normally, as the controls. Even so, the reduction in fresh and dry weight of shoots suggested that 2,4-D had marked effects on radish seedlings under watering regime as well as under drought stress, but the effect was greater under drought stress.

An inhibition of tuber formation and tuber growth after 2,4-D treatment was noted under both drought stress and normal watering regime. Hypocotyl and root malformation were very clear. As a

result of this bending and growth retardation the fresh and dry weight of tubers under both drought and normal watering regime were significantly decreased. Also, reduction of the diameter and length of tuber under normal watering regime was significant.

Additional to that, the data indicated that application of 2,4-D, at toxic level, followed by drought stress decreased total protein level in radish leaves, whilst this increased under normal watering regime. In the meantime increased levels of soluble proteins in treated and untreated seedlings under drought stress indicated that drought stress had a pronounced effect on protein synthesis and metabolism. Unlike the seedlings growing under drought stress, seedlings growing under normal watering regime showed increase in insoluble proteins, in both treated and untreated seedlings with 2,4-D.

Radish seeds planted in the soil 5 weeks after the time of spraying germinated normally indicating that the residual of 2,4-D had no inhibitory effect on the germination of radish seeds. However, most of the seedlings developed root malformation and marginal burning of the leaves, indicating that the 2,4-D was still active in the soil. Reduction in the growth of tubers was significant as measured by diameter in fresh and dry weight.

2. EFFECT OF CCC ON RADISH SEEDLINGS :

Radish seedlings grew without apparent distortion in response to CCC, under both drought stress and normal watering regimes. Treated seedlings were, by visual inspection, darker green in colour than untreated seedlings. However, reduction in fresh and dry weight of shoots and tubers under drought stress and normal watering regimes was significant, and the reduction in diameter and length of tuber was significant under normal watering regime.

The results indicated that CCC retarded protein accumulation in radish leaves under both drought stress and normal watering regimes. However, under normal watering regime soluble proteins were less than insoluble in treated and untreated seedlings in comparison to the controls, and under drought stress insoluble proteins were much higher than soluble proteins in CCC-treated seedlings. These results suggested that CCC-treated seedlings were less susceptible to drought stress than the controls.

These results also indicated that persistent CCC was not inhibiting germination of radish seeds; however, reduction of plant fresh weight and tuber fresh and dry weight was significant and indicating that CCC was still active in the soil.

3. EFFECT OF 2,4-D ON WHEAT SEEDLINGS :

The application of 2,4-D to the foliage of wheat seedlings appeared to have no distortion effect on the leaves or tillers, under both drought stress and normal watering regimes. Even so, the reduction of fresh and dry weight of seedlings and reduction of third leaf area under drought stress and reduction of the number of leaves per plant under normal watering regime indicated that 2,4-D had indirect effects on the growth and development of wheat seedlings.

In 2,4-D treated leaves reduction in total protein content was marked under drought stress in comparison to the controls, and the opposite was true under normal watering regime. Moreover, soluble proteins were higher than insoluble proteins in both treated and untreated seedlings growing under drought stress and the opposite was true under normal watering regime.

4. EFFECT OF CCC ON WHEAT SEEDLINGS :

An application of CCC to wheat seedlings under drought stress resulted in reduction of fresh and dry weight of the plant and the number of leaves per plant in comparison to the controls. By visual inspection the leaves of treated seedlings were thicker and darker green.

Total protein content in the leaves of treated seedlings was higher under both drought and normal watering regimes than the controls. It seems that CCC increased protein content in wheat seedlings since this increase was under both drought stress and watering regime. However increased insoluble protein in treated seedlings in comparison to the controls indicated that seedlings were less stressed, even though they were growing under drought stress, which suggested that Cycocel increased the tolerance of wheat seedlings to drought stress.

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