Seedlings' growth in response to drought stress and 2,4-dichlorophenoxyacetic acid (2,4-D)

Alaib, M. A.

SEEDLINGS' GROWTH IN RESPONSE TO DROUGHT STRESS AND 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

By

M. A. Alaib

B.Sc. University of Garyounis, Libya.

M.Sc. University of Durham, England.

A thesis submitted for the degree of Doctor of Philosophy of the University of Durham, England.

Department of Biological Sciences, February, 1989.

DECLARATION

The results in this thesis are entirely my own work and no part of this thesis has been submitted for any degree in this or any other University.

DEDICATION

To the soul of my beloved mother

ABSTRACT

SEEDLINGS GROWTH IN RESPONSE TO DROUGHT STRESS AND 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

The aim of this work was to study the effect of the herbicide, 2,4-D on a monocot (*Lolium temulentum* Linn.) and a dicot (*Raphanus sativus* Linn.) in relation to drought stress, in order to elucidate if the combined treatments altered the survival of the plants. Herbicide effects were investigated on a number of plant developmental stages; germination, seedling growth, mature leaves and root function, and were combined with various water stress regimes.

2,4-D did not alter the germination percentage in either species when applied singly or with polyethylene glycol (PEG) induce water stress. However, rate of seedling emergence and accumulation of chlorophyll, protein and proline were inhibited. Foliar application of 2,4-D at selective concentrations showed that in addition to induced growth distortion the herbicide reduced the survivial capacity of radish but enhanced that of rye grass to later drought stress. Analysis of the content of proline (a stress metabolite) in both species indicated that the accumulation of this compound was reduced in radish but enhanced in rye grass. In contrast, when 2,4-D applied via the roots, from water culture, the selectivity of effect was lost since proline accumulation was reduced in both species. Use of ^{14}C-2,4-D showed that the herbicide remained in the roots when applied in water culture and that since a major response was seen in the roots this implied that some signalling was occuring between the two organs.

From the results it would appear that the use of low doses of herbicides such as 2,4-D may be valuable in protecting certain plants from drought stress, whilst the susceptability of other plants could be increased hence making the herbicide more effective at low concentrations.

ACKNOWLEDGEMENT

My profuse thanks are due to my supervisor, Dr. J. A. Pearson for his close supervision, invaluable criticism, kindness and helpful comments during the course of this study.

Thanks are also due to Professor D. Boulter Head of Biological sciences Department for providing the facilities in the department, and also due to other staff members in the department, especially Mrs. G. A. Walker and Mr. P. Sidney for their help.

I am also greateful to the library staff for their excellent services, kindness and help, and to members of the staff of the computer center for their valuable advice and help in producing this thesis.

I am inbebted to Dr. P. Johns and to the staff of Ward 8 in the RVI for their tremendous care and invaluable services in helping my son in days of difficulty which I will never forget.

I am indebted to the Secreatariate of Education and University of Garyounis for their financial support throughout the course of this study.

On a more personal level, I wish to express my great appreciation to Saleh Shueb for his help, support and engargement in days of difficulty and pressure. I also wish to thank A. Abdullah, S. Yaish and his wife, I. El-Rawas and his wife, R. Sabbagh and his wife and A. Al-Shehri for their support, encouragement and sympathy.

Finally I am sincerely greateful to my wife Zakia and my children Iman, Aimen, Amin and Aya for their outstanding patient and moral support during this study.

ABBREVIATIONS

A	Absorbance
ABA	Abscisic acid
ANOVA	Analysis of variance
B	Boron
BDH	British Drug House
°C	Degrees Celsius
$Ca(NO_3)_2$	Calcium nitrate
CCC	Cycocel
Chl	Chlorophyll
Chl a	Chlorophyll a
Chl b	Chlorophyll b
$(CHOH.COOH)_2$	Tartaric acid
Cl	Chlorine
cm	centimetre
cm^2	Square centimetre
cm^3	Cubic centimetre
CMPP	(±)-2-(4-chloro-2-methylphenoxy)propionic acid
4CPA	4-chlorophenoxyacetic acid
CPM	Counts per minute
Cu	Copper
$CuSO_4.5H_2O$	Cupric sulphate
D.F	Degree of Freedom
2,4-DB	γ-(2,4-dichlorophenoxy)butyric acid
2,4-DP	α-(2,4-dichlorophenoxy)propionic acid
DW	Dry weight
EDTA	Ethylene diamine tetra-acetic acid
F	Variance ratio
$FeSO_4$	Ferrous sulphate

Fig.	Figure
FW	Fresh weight
g	gram
H_2O	Water
H_3BO_3	Boric acid
HCl	Hydrochloric acid
IAA	Indole acetic acid
Kg	Kilogramme
KNO_3	Potassium nitrate
l	litre
Linn	Linnaeus
LSD	Least significant difference
M	Molar
m	metre
MCPA	4-chloro-2-methylphenoxyacetic acid
MCPB	γ-(4-chloro-2-methylphenoxy)butyric acid
mCi	millicurie
mg	milligram
$MgSO_4.7H_2O$	Magnesium sulphate
min	minute
ml	millilitre
mmol	mole
Mn	Manganese
$MnCl_2.4H_2O$	Manganous chloride
Mo	Molybdenum
N	Normal
NaOH	Sodium hydroxide
ng	nanogram
$NH_4.H_2PO_4$	Ammonium hydrogen orthophosphate
$(NH_4)_6Mo_7O_24.4H_2O$	Ammonium molybdate

nm	nanometer
PROB	Probability
r	Correlation coefficient
sec.	second
SE	Standard error
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
2,4,5-TB	γ-(2,4,5-trichlorophenoxy)butyric acid
TCA	Trichloroacetic acid
2,4,5-TB	2-(2,4,5-trichlorophenoxy)propionic acid
Tris	Tris(hydroxymethyle)-aminomethane
UK	United Kingdom
V/V	Volume per volume
W	watt
μCi	microcurie
μg	microgram
μl	microlitre
μmole	micromole
Zn	Zinc
$ZnSO_4.7H_2O$	Zinc sulphate

SIGNIFICANCE SYMBOLS

N.S	Not-significant; P>0.05
*	Significant at P<0.05
**	Significant at P<0.01
***	Significant at P<0.001

CONTENTS

LIST OF FIGURES

LIST OF PLATES

CHAPTER 1

INTRODUCTION

1.1. General Introduction

The 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 fluctuations in environmental factors but compounding effects can have serious consequences. The use of herbicides has increased in recent years and this can influence the response of a plant to environmental factors. Therefore it is important to investigate combined environmental factors and herbicides.

2,4-dichlorophenoxyacetic acid (2,4-D) is a herbicide which is highly selective to broad-leaf weeds and is translocated throughout the plant. It belongs to a very large class known as "hormone-type herbicides" or phenoxyalkane carboxylic acids which are comprised of three major groups and several derivatives. These groups include; phenoxyacetic acids (4CPA, 2,4-D, MCPA, 2,4,5-T); α-phenoxypropionic acids (2,4-DP, CMPP, 2,4,5-TP); and γ-phenoxybutyric acids (2,4-DB, MCPB, 2,4,5-TB). 2,4-D has been used since the nineteen forties and has the greatest significance of all known herbicides. More than 32 million Kg in the United States (Ware, 1983), and about 10^5 tons world-wide (Klopffer *et al.*, 1982, cf. Ware, 1983) are produced annually for agricultural purposes in many formulated products and in different forms.

2,4-D is chiefly used to control broad-leaf weeds (dicotyledons) in cereal crop fields (Nutman, Thornton & Quastel, 1945; Slade, Templeman & Sexton, 1945, cf. Hathway, 1986; Ashton & Crafts, 1981; Gile, 1983; Ware, 1983), grazing land (Griffen *et al.*, 1984; Hamann & Kettrup, 1987), turf and lawns (Gile, 1983; Ware, 1983), roadsides and in forest conservation programs (Ware, 1983), and has further-

more been used as a defoliating agent (Hamann & Kettrup, 1987) and growth regulator (Riederer & Schonherr, 1984) as well as for the control of water weeds (Hamann & Kettrup, 1987). Combinations of 2,4-D with other herbicides are used, such as 2,4,5-T for brush wood control (Smith, 1979) and dicamba to control grassy and broad-leaf weeds in wheat (Malik *et al.*, 1986), pastures, rangelands and roadsides (Lyon & Wilson, 1986). Several other herbicides like dichlorprop, difenzoquat, and TCA have also been used with 2,4-D for control of different weed species (Smith, 1979).

It is generally accepted that 2,4-D is an auxin-like herbicide (Zsoldos *el al.*, 1986; Wernicke & Milkovits, 1987; Shimabukuro *et al.*, 1986; Wernicke *et al.*, 1986), because at low concentration it has the properties of stimulating growth similar to the natural auxin IAA (Ries, 1976). 2,4-D was the first herbicide reported to improve growth and yield of crops at sub-toxic levels. It has often been reported to increase the yield of some crops such as bean, sugar beet and potatoes (Ries, 1976) and to increase the protein content of wheat (Huffaker *et al.*, 1967, cf. Ries, 1976) potato (Payne *et al.*, 1953, cf. Ries 1976), and kidney bean (Sell *et al.*, 1949, cf. Ries, 1976). Carbohydrate and moisture content also have been reported to increase by treatment with sub-toxic levels of 2,4-D (Payne *et al.*, 1953, cf. Ries). Moreover 2,4-D has been known as a fruit drop controller, senescence delayer, root inducer, flowering inducer, fruit set enhancer, and fruit ripener (Nickell, 1979, 1982).

In contrast to sub-toxic effects of 2,4-D, at high concentration it can be toxic. The selectivity of 2,4-D, or its toxicity, depend on; inherent resistance or susceptibility of particular species (Muzik, 1976), the stage of the growth of the plant (Muzik & Mauldin, 1964; Muzik, 1976; Van Andel *et al.*, 1976; Åberg & Stecko, 1976; Cartwright, 1976), the environment under which the plant has grown (Muzik & Mauldin, 1964; Muzik, 1976), and the dosage levels (Cartwright, 1976). Gramineaseous plants are generally resistant to 2,4-D whilst most of the dicotyledonous plants are sensitive (Taylor & Maj, 1946; Hagin, 1970; Davidonis *et al.*, 1982;

Shimabukuro *et al.*, 1986).

The basis of 2,4-D selectivity in plants has been discussed by many investigators (e.g. Bovey, 1980; Ashton & Crafts, 1981; Shimabukuro, 1985; Gressel, 1985). They concluded that plants are resistant to 2,4-D because of their ability to detoxify the herbicide by conjugation with plant constituents (Davidonis *et al.*, 1982; Chkanikov *et al.*, 1982; Davis & Linscott, 1986) or by metabolism (Hagin *et. al.*, 1970) or possess morphological characteristics that are barriers to herbicide absorption and translocation (Bovey, 1980; Zemskaya *et al.*, 1984).

According to Ashton and Crafts (1981) 2,4-D and chlorophenoxy acids in general have profound effects on the growth and structure of plants. These herbicides produce epinastic bending, cessation of growth, tumour formation and secondary root induction. Moreover meristematic cells of treated plants also cease to divide and cells which normally would elongate expand only radially. In mature plants, parenchyma cells swell, divide and produce callus tissue and expanding root primordia. Furthermore, root elongation stops, root tips swell and young leaves stop expanding. In addition these herbicides are known to modify nucleic acid metabolism in plants (Hanson & Slife, 1969) and interact with numerous enzyme systems (Woodford *et al.*, 1958). Enhanced ethylene production by 2,4-D treated plants has also been reported (Hanson & Slife, 1969; Holm & Abeles, 1968; Pinfield et al., 1984; Zemskaya *et al.*, 1985; Tittle, 1987).

At high concentrations sensitive plants showed varying responses to 2,4-D. According to Hamner & Tukey (1944) these responses including epinastic curvatures, splitting of hypocotyls or stems, swelling of hypocotyls or stems and roots, browning of leaves, stem and roots, chlorosis of leaves and stems, chlorosis and enlargement of petioles, swelling of root tips, swelling of some shoot meristems, severe twisting and curling of leaves, root shortening and thickening.

2,4-D at high concentrations has often been reported to damage some plants which would normally be regarded as resistant to this herbicide, inhibiting growth and causing distinct morphological changes in these plants. Abnormalities in the spikes and roots of wheat were reported by Johanson & Muzik, 1961 . Hamner *et al.* (1946) found that soil previously treated with 2,4-D affected the germination and growth of many grass seeds. Hoshaw & Guard (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.

The mechanism of sub-toxic and toxic action of phenoxy herbicides in general and 2,4-D in particular have been reviewed appreciably by many investigators (*e.g.* Ries, 1976; Bovey, 1980; Ashton & Crafts, 1981). They concluded that the initial action of 2,4-D involves absorption and penetration of plant surfaces, absorption into symplast (the living parts of plant, i.e. the cells containing cytoplasm.), migration across parenchyma tissue to the vascular system and translocation from leaves to stems and roots with manufactured foodstuffs. At the same time 2,4-D can be absorbed by roots with water and moves throughout the plant in the apoplast (the non-living parts of plant, i.e. the xylem, the cellulose, the cell walls and the intercellular spaces). All these processes in turn depend on the, environmental factors, stage of growth of plant, and herbicide formulation (Kasasian, 1971). During and after distribution of the herbicide within the plant, many biochemical responses are triggered which may lead to abnormal growth resulting in epinastic manifestations of plant parts and plugging of vascular channels, ultimately starving the plant.

From this brief review it is clear that 2,4-D can be a 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 dicotyledonous 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 dicotyledonous crops which subsequently grow

in the cereal fields as a result of 2,4-D persistence in the soil. Furthermore spray droplet and vapour drift from this compound can also cause damage to susceptible crops in nearby fields (Ahmedullah *et al.*, 1985; Lyon & Wilson, 1986).

In the response of a plant to herbicides, 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. Drought stress as an environmental factor is the only one point intended for discuss here in relation to 2,4-D application.

Plants are said to experience water deficit when their cells and tissues are less than fully turgid (Wareing & Phillips, 1981). Plant responses to drought stress and physiology of plants under drought stress have been reviewed by many workers (*e.g.* Henckel, 1964; Hsiao, 1973; Bewley, 1979; Hanson & Hitz, 1982; Morgan,1984; Schulze, 1986). The first change is most likely a slowing down of shoot and leaf growth, as a result of turgor pressure (ψ_p) reduction, followed by a reduction in cell wall and protein synthesis. As tissue water potential (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 carbon dioxide (CO_2) assimilation whilst abscisic acid (ABA) probably begins to accumulate. As stress continues and tissue ψ decreases still further, decline in respiration, translocation of photosynthates, and cytokinins may become substantial. Levels of some hydrolytic enzymes are likely to increase and ion transport can be slow. Finally, as water deficits become severe enough to cause marked proline accumulation, CO_2 assimilation becomes very low. Senescence induced by stress may become apparent 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 will reduce CO_2 assimilation and the latter will reduce dry matter production by reducing photosynthesis. Reduction of cell growth may also reduce the development of leaf surface area which subsequently reduces 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 and xylem production (Ries, 1976). Thus the plant becomes water-stressed even in the presence of water.

Responses of plant to a herbicide depend upon the environmental factors before treatment, during treatment, and following treatment (Muzik, 1976). A major factor in this respect is drought stress. During recent years a considerable body of literature has been accumulated relative to the interaction between 2,4-D and environmental factors (Marth & Davis, 1945; Kelly, 1949; Pallas, 1959 ; Basler *et al.*, 1961; Pallas & Jr, 1960; Muzik & Mauldin,1964; Muzik,1976; Richardson, 1977; Fowler *et al.*, 1986). However, few studies report on the interaction between 2,4-D and drought stress.

Plants which have been grown under drought stress condition can be more resistant to herbicide than plants which have been grown under sufficient amounts of water. Such increases in resistance may be due to the morphological and physiological changes imposed by drought stress upon the plant. Drought stress may make the cuticle less permeable to water solutions (Levitt, 1972) and increases the contact angle of spray droplets with consequent decreased wettability (Fogg, 1947, cf. Muzik, 1976). Drought stress over a prolonged period may lead to an increased thickness and density of cuticle, increased pubescence and therefore prevent the entry and transport of herbicide (Muzik, 1976). Moreover, drought stress reduces translocation (Basler *et al.*, 1961; Pallas, 1959) with a consequent slowing of the absorption of herbicide

(Hauser, 1955). As a result of these morphological and physiological modifications plant becomes more resistant to herbicide. This, however, may be the the case for susceptible species but not a general rule. In contrast, plants growing under drought stress conditions can be very susceptible to herbicide, particularly resistant species (Muzik, 1976). He argued that adverse weather conditions may accentuate the injury caused by herbicides and therefore make plants susceptible to herbicides which they normally resist.

On the other hand response of plants to drought stress, following herbicide treatment is very important. Herbicide may cause damage to roots and makes them unable to compete for moisture and subsequently reduce the capacity of plant to resist drought. Muzik & Mauldin (1964) found that water stress following 2,4-D application to young wheat resulted in more damage; whereas plants kept moist for 14 days after application, recovered and grew normally. They explained that the damaged roots of the treated plants are unable to compete for moisture as well as uninjured roots. With adequate moisture the plants recover, but under dry conditions, growth and development of the sprayed plants may be severely affected.

1.2. Aims

From the above literature data it is clear that the effects of 2,4-D are markedly influenced by several environmental factors. Information on the response of plants to drought stress and 2,4-D is required for a better understanding of the interaction between 2,4-D and drought stress and the response of plants to both. In this thesis an account is given of the results of serial experiments, in which the combined effects of 2,4-D and drought stress were investigated in monocot and dicot seedlings. Although 2,4-D has been extensively used as a herbicide since the forties and its effects, translocation and mechanism of action have been reviewed (Bovey,

1980; Ashton & Crafts, 1981), very little data is available on the combined effects of 2,4-D and drought stress on the growth and development of seedlings.

In the course of these investigations attention was concentrated on the morphological, and biochemical responses. In addition to that absorption and translocation of 2,4-D in both monocot and dicot seedlings were also investigated under normal watering regime and drought stress condition.

Preliminary investigation into the effects of 2,4-D on seedling growth have already been recorded (Alaib, 1985).

The first Chapter reviews the literature relating to the effect of 2,4-D and drought stress on plants and the general principles underlying the processes governing the fate of foliar-applied 2,4-D. It also describes the general materials and methods used throughout this work. In Chapter 2, 3 and 4 the experimental work is reported of the separate effects of the herbicide and its combination effect with drought (water withhelded) or water stress (PEG treatment to roots). Throughout this work two model plants system were used; *Lolium temulentum* (a monocot) and *Raphanus sativus* (a dicot). Chapter 3 describes the results obtained from an investigation in to the changes in specific cellular components which were thought to be important in explaining the responses to the herbicide and drought. These components were; chlorophyll, protein and proline. Chapter 4 describes data relating to uptake of radioactive 2,4-D into roots in order to elucidate if the effects seen in the roots and shoots were due to the herbicide being translocated to these sites. In chapter 5 a summary and overall disscution of the results and conclusions is given.

1.3. General Materials and Methods

The experiments were conducted either under glasshouse condition (heated in Winter only) or under semi-controlled conditions of temperature and controlled light in laboratory at the Department of Botany University of Durham, as stated for individual experiments.

1.3.1. Plant Materials

Seeds of radish (*Raphanus sativus* Linn.) cv. French Breakfast, were obtained from the local market (Durham), supplied by Suttons Seeds Ltd. England, (EEC Rules & Standards). Caryopses of rye grass (*Lolium temulentum* Linn.) and seeds of maize (*Zea mays* Linn.) cv. Cistron, were provided by the Welsh Plant Breeding and the Botany Department at the University of Durham. Radish seeds were kept in bags in which they were supplied, rye grass and maize seeds were sealed in plastic containers and stored in the laboratory at room temperature until required for sowing.

1.3.2. Preparation of the Herbicide Solutions

Stock solutions of 2,4-D were prepared from 2,4-D salt, supplied by BDH. The required amount of the compound was first dissolved in 0.5 ml of ethanol and made up to desired volume with distilled water to give the final concentration (ppm). Storage was at 4 °C until required.

1.3.3. Application Methods

For 2,4-D application the following methods were used:

1.3.3.1. Foliar Application

I. Direct spray to the foliage of seedlings, using small hand sprayer, to give a known dose as detailed for experiments.

II. Addition of microdrops of the solution to the cotyledons or the leaves of seedling, using a 1 ml syringe.

1.3.3.2. Soil Application

Soil treatment was carried out by spraying and mixing the soil thoroughly with aqueous solutions of the herbicide, to give a known dose as detailed for the experiments.

1.3.3.3. Root Application

Roots were treated by adding certain amounts of the herbicide to nutrient solutions and by feeding to seedlings by growing them in plastic containers with their roots immersed in this solution.

1.3.3.4. Seed Application

Seeds were treated by sowing them in 9-cm Petri dishes on filter paper moistened with an aqueous solution of 2,4-D, or by wrapping in filter paper and soaking them for a period of time in a known volume of the herbicide as stated for individual experiments.

1.3.4. Cultural Methods

Seeds were planted in Levington's Compost contained either in plastic trays 4.5 cm deep, 21 cm long and 15.5 cm wide or in 4.5 cm plastic pots.

For nutrient solution culture seeds were germinated in 9-cm Petri dishes on filter paper moistened with distilled water. When the seedlings had attained the desired size (as stated for individual experiments), they were transferred to small plastic nutrient-solution containers with a removable cover. The roots of the seedlings were inserted through holes on the cover to the nutrient solution, and the shoots were kept above surface, held in place by cotton wool. All sides of the containers were covered with aluminum foil to exclude light (and thus algae). An air pump was connected to each container to provide aeration to the seedling roots.

1.3.5. Drought Stress Treatment

The seedlings were exposed to a drought stress after a period of time growth as follows:

1.3.5.1 Seedling Grown in Soil

Seedling grown in soil were subjected to a drought stress by withholding further water supply. The non-stressed seedlings were irrigated every other day throughout the experiment.

1.3.5.2. Nutrient Solution Grown Seedlings

Polyethylene glycol (PEG) has been used successfully as an osmoticum to induce drought stress in hydroponically grown plants (e.g Talouizite & Champigny, 1988, Heath *et al.*, 1985; Vavrina, 1983; Matssuda & Riazi, 1981; Rajagopal & Andersen, 1978; Kaufman & Eckard, 1971; Resnik, 1970). Accordingly seedlings grown in water solutions were subjected to a drought stress by flooding the rooting medium with PEG solution. Aqueous solutions of PEG 6000 with varying water potential were prepared according to Resnik (1970). 125 g/l for -5 bars, 200 g/l for -10 bars and 230 g/l for -15 bars.

1.3.6. Nutrient Solution

Hoagland's nutrient solution at half strength and pH 6, was used as nutrient media for nutrient solution culture with little modification. The solution was prepared from nutrient elements with specific concentrations described by Hoagland & Arnon (1938). The following table shows the proper concentrations of the elements.

Table 1.1. Hoagland's nutrient solution.

Salts	mg/l
KNO_3	606.60
$Ca(NO_3)_2$	656.36
$NH_4H_2PO_4$	115.03
$MgSO_4.7H_2O$	492.94
Mixture of 0.5 % $FeSO_4$ & 0.4 % $(CHOH.COOH)_2$ 0.6 ml/l added 3 times /wk	
$MnCl_2.4H_2O$	0.5 Mn,6.5 Cl
H_3BO_3	0.5 B
$ZnSO_4.7H_2O$	0.05 Zn
$CuSO_4.5H_2O$	0.02 Cu
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.05 Mo

1.3.7. Sources of Chemicals

Unless stated otherwise, all chemicals used were obtained either from BDH, Poole or Sigma, Poole, UK. They were of analytical grade.

1.3.8. Statistical Treatment of Data

The data were subjected to the analysis of variance (ANOVA) in most

cases, followed by multiple range test (LSD), presented in the form in which it was analysed (Parker, 1983). In some cases linear regression and correlation were used. Statistical Package for the Social Sciences (SPSS-X) program was used for the ANOVA test and Statistics for Biologists Version 28. 11.86 was used for correlation test, at the computer center, University of Durham.

Analysis of variance is a technique used in comparing more than two samples. It combines with one procedure all combinations of t-tests which would otherwise have to be performed and reduces the chances of rejecting the null hypothesis when it is really true.

Analysis of variance compares the variation between each treatment represented by the samples (between treatment variance) to the amount of variation within each treatment represented by the samples (within treatment variance).

The results of analysis of variance are presented in an analysis of variance table which, in the case of one-way classification (used in this study), is composed of the error mean square, the treatment mean squaqre, and the result of a variance ratio or F-test by which the significance of the treatments can be tested. The calculations are preformed on the sums of squares of the deviations from the means, which become mean squares or variances when divided by the appropriate number of degrees of freedom. The degrees of freedom are closely related to the number of observations which contribute to the sums of deviations.

1.3.9. Text Processing and Graphics

This thesis was prepared on the mainframe computer (Dur.MTS). TEX program (Knuth, 1986) was used for text processing and GIMMS programme (Waugh & McCalden, 1983) was used for plotting graphs. Text and Graphics were printed on a QMS Lasergrafix 800 laser printer at the computer center, University of Durham.

CHAPTER 2

EFFECTS OF 2,4-D AND DROUGHT STRESS ON SEED GERMINATION AND SEEDLING DEVELOPMENT

2.1. Introduction

A very large number of herbicides can inhibit germination. All those herbicides which are toxic to plants will also, at toxic concentration, inhibit germination. Many of the commonly used herbicides such as 2,4-D affect germination at comparatively low concentrations (Mayer *et al.*, 1975; Parker, 1976). Hamner *et al.* (1946) found that soil previously treated with 2,4-D affected the germination of many seeds, at concentrations of the acid as low as 1 ppm. Reduction in the rate of germination of barley and mustard as a result of soil treatment with 2,4-D were found to be 80-90 % (Mitchell & Marth, 1945). In studies in moist chambers 2,4-D was found to delay germination and to cause abnormalities in the seedlings of twenty-two broadleaf and cereal species (Allard *et al.*, 1946). Sasaki *et al.* (1968) found that 2,4-D suppressed early germination of *Pinus resinosa* Linn. seeds only at concentrations higher than 100 ppm and checked final germination at comparatively high concentrations. Audus & Quastel (1947) however, found no significant effect on germination of cress, radish, mustard, carrot, onion, cabbage, beet root and mixed lawn grass seeds when treated with 2,4-D at low concentrations up to 10 ppm.

Despite the inhibitory effect of 2,4-D, there were some exceptions, where it has been shown to have stimulatory action on seed germination. By soaking the seeds of *Amaranthus retroflexus* Linn. in 2,4-D solution (20 mg/l) for 20 hours, Rojas-Garciduenas & Kommedahl (1960, cf. Parker, 1976) were able to increase the percentage of germination from (66 % to 82 %). In another experiment Rojas-Garciduenas *et*

al. (1962, cf. Parker, 1976) again increased germination of *A retroflexus* Linn. from (43 % to 85 %) this time by exposing the seeds continuously to a solution of 2,4-D at 1 mg/l. Milyi (1972, cf. Parker, 1976) succeeded in accelerating germination of *A retroflexus* Linn. and *Setaria lulescens* Linn. by mixing soil with a 800 mg/l of solution of 2,4-D. Åberg *et al.* (1948, cf. Parker, 1976) reported that spraying *Galium aparine* Linn. with 2,4-D resulted in the production of seeds which would germinate on the soil soon after harvest. Aomisepp (1959, cf. Parker, 1976) showed that the seeds from plants of *A fatua* Linn. which had been sprayed with 2,4-D give increased germination compared with seeds from untreated plants.

In addition to its effect on seed germination, 2,4-D is known for its strong inhibitory effect on seedling establishment (Cartwright, 1976); dicotyledons are more severely affected but many monocotyledons are also sensitive, e.g. a corn germination test was shown to be very sensitive to 2,4-D (Thompson *et al.*, 1946). Elongation of the radicle, however, is more sensitive than shoot growth. Inhibition of root growth and development of cotyledon of red pine seedlings shortly after seed germinated have been reported by Sasaki *et al.* (1968). Inhibition of root production and top growth was also reported by Hamner *et al.* (1946), when they treated sudan grass by soaking in a solution of the acid at 100 ppm for 4 hours. In the same experiment they treated bean (*Phaseolus vulgaris* Linn.) and pea (*Pisum sativum* Linn.) seeds also by soaking them in 10 and 100 ppm solutions; seedlings of both plants were severely checked by the acid at 10 ppm. The roots as well as the tops were checked. In Seeds treated with the compound at 100 ppm, growth of the seedlings was almost completely checked. No top or root growth occurred in the pea, while only 1 % of the beans grew, they did so with feeble growth. Johanson & Muzik (1961) treated two different varieties of winter and spring wheat seeds by immersing in various 2,4-D solutions producing seedlings with thickened and fasciated roots. They concluded that stems appeared to be less affected than the roots and the longer period

of immersion generally produced more abnormalities, but morphological abnormalities were pronounced for both varieties used. Hoshaw & Guard (1951) also reported that pre-emergence treatment of corn resulted in extreme morphological responses, such as seedlings with much elongated coleoptiles and first internodes; most seedlings also shown severe bending and twisting. Complete inhibition of roots in red clover at 10 and 100 ppm again has been reported by Nutman & Thornton (1945).

2,4-D also has profound effects on the growth and development of growing plants. Its effect depends to great extent on the species of plant (susceptible or resistant) (Cartwright, 1976; Muzik, 1976). It is clear that the growth and development of susceptible species are inhibited by 2,4-D. The degree of inhibition varies with; herbicide dose, stage of growth , and other factors (Cartwright, 1976; Muzik & Mauldin, 1964; Muzik,1976). As described by Hanson & Slife (1961), when susceptible seedlings are treated with 2,4-D normal growth patterns change rapidly. These changes in the growth processes result in physiological malfunctioning and therefore death of the whole plant (Cartwright, 1976).

The production of varieties of developmental abnormalities following treatment of young seedlings with 2,4-D has been reported for a number of susceptible species. Beal (1944) treated kidney bean at the seedling stage with a 1 % mixture of 2,4-D, the application of the mixture to the leaves resulted in epinasty and stem curvature within 6 hours following treatment. At 24 hours the curvature was so great as to change the growing points and heart-shaped leaves of nearly all plants. The petioles of a few leaves begun to swell, and after 48 hours petioles and second internodes of most plants were distinctly enlarged. Nine days after treatment seedlings began to die. In experiments on a number of dicotyledonous plants Audus & Quastel (1947), reported that 2,4-D at concentrations of 1-10 ppm gave rise to characteristic formative effects on the base of the hypocotyl and root growth was reduced to very low values in the highest concentrations of 10 ppm. Audus (1949) treated cress, radish, and gar-

den pea with different concentrations of 2,4-D in water culture solutions, he reported inhibition of root growth for all concentrations. Taylor & Maj (1946) found that treating soil and solution cultures with 2,4-D resulted in swelling of the hypocotyl region and stimulated growth of adventitious roots of kidney bean. The root systems of seedlings were much stunted in all solution-culture treatments. Application of 8 ppm to solution cultures resulted in the death of kidney bean and soybeans by the 6th day. Dry-weights of seedlings grown in soil or solution cultures were also decreased. Treatment of marrow seedlings with 2,4-D, immediately following germination, has been shown to be markedly affected by the presence of comparatively low concentrations (Yousif *et al.*, 1979; Pinfield & Yousif, 1980; Pinfield *et al.*, 1984). Among the morphological abnormalities observed were swelling of root/shoot axis at the hypocotyl base, which resulted largely from cell expansion, and severe inhibition of extension growth in both hypocotyl and radicle. Exposure of tomato seedlings to a range of concentrations (5-50 ng/l) of vapour of 2,4-D resulted in epinasty on the youngest leaves and petioles and within 140 minutes, all seedlings had begun to bend. Dry-weight and dry matter were 8 % and 9 % respectively after 7 weeks of treatment, compared with control seedlings (Breeze & West, 1987).

Resistant species show varying degrees of susceptibility to 2,4-D depending on the development stage of the plant, the dose, and the formulation of the herbicide (Cartwright, 1976). Stage of growth, however, appeared to be an important factor in the response of resistant species. According to Johanson & Muzik (1961), the seedling and early flowering shown to be the most susceptible stages. They noted that abnormalities in the spikes which occurred when wheat was sprayed in either the seedling or boot stage included a pronounced curvature and doubling at the rachis joint. The wheat treated at other stages looked normal, and there were no abnormalities in the untreated plants. Johanson & Muzik also noted that wheat seedlings responded differently to various formulation of 2,4-D. Foliar treatment with 2,4-D as

solubilized acid at 2000 ppm stimulated elongation of the primary root of wheat, but inhibited lateral root growth. Treatment with isopropyl ester at concentrations of 1000, 2000, and 4000 ppm stopped the root growth within few hours; plants withered and died within 24 hours. The triethanolamine salt was tested at concentrations of 500, 1000, 2000, and 4000 ppm, primary root elongation was stimulated by 500 ppm, but the other treatments reduced elongation. All treatments caused a shortening of the lateral roots and swelling of both the lateral root and base of the culm. Taylor & Maj (1946) treated wheat seedlings during the 4th and 5th weeks of growth with 2,4-D at concentrations of 1.5-3.0 ppm in nutrient solutions, they reported inhibition of shoot growth 4 days after treatment. On the 6th day the shoots of the seedlings were chlorotic and most seedlings growing in 3 ppm were dead. Fresh-weight for these treatments indicated that, in proportion to the concentration supplied, 2,4-D significantly reduced the increases in fresh weight of shoots. Elongation of fibrous roots also reduced at 0.05 ppm and completely inhibited at 1 ppm, 0.1 ppm reduced lateral roots and at 2 ppm no new branches appeared. Concentrations from 0.1-3.0 or 4 ppm, caused a development of localized swellings on all root tips which was at a maximum at approximately 1.5 ppm and was progressively less in higher concentrations until little swelling occurred in 4 ppm or more. Concentration of 2,4-D between 0.75-4 ppm stimulated adventitious root growth from the crown regions of plants, this reaction reached a maximum at 2-3 ppm and again was lacking at 4-5 ppm. Appreciable crown swelling was caused by 1.0 ppm before plants were killed.

Other resistant species have also been reported to have such morphological and physiological malformation as a result of 2,4-D treatment. Post-emergence treatment of corn seedlings with 3000 and 5000 ppm of 2,4-D caused bending and twisting at the lower node within 24 hours after application (Hoshaw & Guard, 1951). This bending intensified and at the end of 12 days after application most of the shoots were in a horizontal position. By the end of 5 weeks, however, the treated plants

had recovered or partially recovered from the horizontal position but were markedly stunted. Their average height was considerably less, and their leaf blades were much narrower than those of untreated plants. Large masses of root tissue occurred just above the soil surface, and some of the fasciated roots failed to penetrate the soil. Treating corn with 2,4-D in nutrient solutions at 0.5 and 8 ppm did not kill them, though their dry weight was significantly decreased by the lowest concentration (Taylor & Maj, 1946). They also reported wilting and drying of leaves and swelling of the crown region, from which the growth of adventitious roots was stimulated. Jacobson *et al.* (1985) reported a 30 % reduction of primary oat roots when compared with the control as a result of 2,4-D application to the roots. 2,4-D has also been reported to cause root shortening and thickening, and caused callus growth in corn in root tissue cultures at 1-10 micromolar (Shimabukuro *et al.*, 1986) .

The growth pattern of plants is determined by the activities of the meristematic tissues (Van Andel *et al.*, 1976). Many herbicides can affect these tissues, thus causing abnormalities in the development and subsequently in the shape of plant.

Herbicidal action might result from a direct interference with cell division, enlargement, or differentiation of meristematic tissues(Cartwright, 1976), causing different types of morphological disorders. In order to identify the origin of these morphological abnormalities in different organs of plant, many investigators have studied the anatomy of 2,4-D-treated plants, both monocots and dicots (e.g. Tukey *et al.*, 1945; Swanson, 1946; Allard *et al.*, 1946; Eames, 1949, 1950; Wilde, 1951; Wu *et al.*, 1971; Robnett & Morey, 1973; Nadakavukaren & McCracken, 1977; Herdi, 1983; Hariharan & Unnikrishman, 1985). They all agreed that an application of 2,4-D resulted in a loss of control of the balance between cell division, enlargement, differentiation and disturbance in the functioning organs of susceptible plants. Resistant plants, however,

may be susceptible at some developmental stages and when the susceptibility does occur the responses are essentially of the same type as those in susceptible species.

Swanson (1946) reported histological effects on young bean seedlings given spray treatment with 2,4-D. In a study of bean seedlings growing in soil treated with 2,4-D , Allard *et al.* (1946) also reported that the hypocotyl of treated seedlings differed from the normal in having a solid pith, slower maturation of primary conducting tissues, an enlargement of cortical and pith cells, and in a weak stimulation of proliferation in primary phloem and rays. With application of higher concentrations fasciated roots developed. They also reported proliferation of cells in the region of inner cortex and endodermis and that the proliferation made identification of the specific region involved difficult and uncertain.

Eames (1950) investigated the origin and development of the sheath of proliferating tissue formed in the hypocotyl of bean seedlings after treatment with 2,4-D, with attention especially on the effect upon the phloem. He reported that all tissues between the cortex and primary xylem were involved. The first divisions were in the endodermis; these were immediately followed by those in the inner pericycle, primary phloem, and all immature cambium derivatives. In all tissues the first divisions were periclinal, these were soon followed by proliferating tissue which built up. In the primary phloem, which was mature at time of treatment, the parenchyma cells proliferated freely, disrupting the phloem strands, within which they lie, and soon crushing the companion cells and smaller sieve tubes. The remnants of these cells were absorbed, and later the larger sieve tubes became empty and were transversely ruptured. After 8-13 days no phloem, as such, was present in the hypocotyl, the region between the photosynthetic organs and the root system. The scattered remnants of the primary phloem were moved farther apart and outward by continuing proliferation throughout the sheath. After 13 days the the only vestiges of the phloem present at time after treatment were a few crushed, broken, and empty sieve tubes. No secondary

phloem formed because only initials of these tissue were present at time of treatment and these became a part of the proliferating tissue. By the 16th day even the last broken empty seive tubes had disappeared, their former position was indicated only by remnants of their collapsed cell walls. He concluded that destruction of the phloem was doubtless a contributing factor in the killing of bean seedlings with 2,4-D.

Wilde (1951) noticed distinct abnormalities in the growth and anatomy of roots of bean seedlings growing in 2,4-D-treated soil. The growth in length of the roots was checked and an abnormal number of lateral roots was produced on immature, and also to some extent on mature, parts of root. Enlargement back from the tip in all roots was visible within 3 days after treatment. By the 6th day these elargements had developed into two or four conspicuous shoulders of tissue, merging upward in large roots into numerous closely placed stubly lateral roots. Four shoulders of tissue were formed on primary and large secondary root tips; on small lateral root tips two opposite shoulders were formed giving the root tips the shape of arrow-heads. An anatomical study of these structural abnormalities showed that the shoulders, which were opposite the protoxylem points, were composed chiefly of proliferated pericycle. Proliferation, beginning just outside the provascular core, established a meristematic zone which, by tangential divisions, added new cells mainly towards the inside with fewer towards the outside, thereby the meristematic zone was continuosly pushed outward. He explained that such swellings were produced by abnormal enlargement of cortical cells as well as by proliferation of pericycle, and sometimes endodermis, as a result of 2,4-D stimulatory effect on the activity of meristematic cells of root tips. Moreover cells in the region of cell division may divide abnormally; those in the region of normal elongation may be stimulated to abnormal enlargement. When the effect of the stimulus was greater, a meristematic zone outside the provascular core developed producing masses of proliferating tissue, mainly pericyclic. Wilde (1951) concluded that root tip enlargement, whether the result of cell enlargement or cell proliferation,

was caused by the action of 2,4-D on the normal activity of meristematic cells. The severity of the effect varies with kind of plant and its stage of development.

Watson (1948, cf. Linck, 1976) followed the morphogenetic changes in bean leaves in intact plants treated with 2,4-D. He reported that marked leaf distortions were caused by the failure of normal lateral leaf expansion and the development of replacement tissue which was described as thick-walled turgid, parenchyma-like cells. Hallam (1970) studied the effect of 2,4-D on the ultrastructure of primary leaves of *Phaseolus vulgaris* Linn. and noted changes in the morphological and internal structure of the chloroplasts occurring primarily in the light-treated leaves. Just 4 hours after application, 2,4-D caused a breakdown in the membranes of cells of the epidermis, palisade and mesophyll. Hallam (1970) pointed out that after 8 hours, the chloroplasts were distorted, the granularity of the stroma was more marked, vesicles appeared in the stroma, outer chloroplast membranes were broken in many places and invaginations of the inner membrane into the stroma appeared. At that time the plasmalemma moved away from the cell wall and the cytoplasm. The effects of the 2,4-D were even more pronounced 24 hours after treatment. At this time the cytoplasm was even more condensed giving the appearance of a plasmolysed cell and the cytoplasm was densely packed with ribosomes. By this time the extreme distortion of the chloroplasts made the identification of internal structure very difficult and membranes appeared to bound the vesicles enlarging within the stroma and the osmiophilic granules had aggregated.

Further investigation on the structural abnormalities in susceptible species was carried out by Wu *et al.* (1971). They studied the effect of 2,4-D on early development of *Pinus resinosa* Linn. seedlings and reported proliferation and expansion of parenchyma cells in the stem and cotyledons, causing abnormal thickening of seedlings. Disorganization and collapse of parenchyma cells in the upper stem were followed by callus formation. Formation of vascular strands was retarded. Division

and expansion of mesophyll cells were stimulated causing a decrease in intercellular species. Numbers of stomata and chloroplasts were less in cotyledons of treated plants than in controls.

2,4-D has also been shown to effect the ultrastructure of many resistant species (grasses). Hoshaw & Guard (1951) studied the effects of 2,4-D at concentrations of 2000 & 5000 ppm on the stem internodes of corn seedlings 21 days after pre-emergence or post-emergence treatments. They reported proliferation in the meristematic zone, and pointed out that there were three regions visible in the proliferated area, and these corresponded to the three histogens found in corn root tip. Root fasciation was extreme, particularly in plants examined 21 days after post-emergence treatment. Large masses of tissue were externally visible. This tissue consisted chiefly of small isodiametric or slightly elongated cells. Anatomical examination indicated that tissues other than the pericycle and meristematic zone were unresponsive to 2,4-D treatment used. Treating wheat seedlings with comparatively high concentration of 2,4-D (up to 2000 ppm) caused marked histological effects on roots. The volume of cortical cells above the root apex was also increased and abnormal cell divisions in the pericycle and endodermis resulted in the formation of numerous lateral root primordia. Johanson & Muzik (1961) found that a major portion of the root swelling was the result of enlargement of cortical cells. Therefore, it appears that 2,4-D stimulated cell division and the differentiation of the lateral roots but inhibited elongation of these roots. Friesen & Olsn (1953, cf. Linck, 1976) followed the morphogenetic changes in barley treated with 2,4-D, they found two critical stages of injury following application of 2,4-D. The first was at the seedling stage and the second was at the advanced boot stage just before spike emergence. Deformity of leaf initials in seedling stage and sterility was also induced at the time when the anther and stigma were differentiating.

Like 2,4-D, drought stress can have very marked effects on seed germination and seedling development of many plant species. That is because water is essential for the rehydration of seed as the initial step toward germination and also substantial amount of water is needed for establishment and subsequent growth of the seedling (Bewley & Black, 1978). Therefore it is very important for the seed to imbibe water in order to carry out the process of germination. The extent to which imbibition occurs is determined by three factors (Mayer & Poljakof-Mayber, 1982), the composition of the seed, the permeability of seed coat to water and the availability of water in the environment. The availability of water is very much dependent on the water potential of both seed and its surroundings. In general, water potential in air-dried seeds is lower than that in the moist medium (Shaykewich & Williams, 1971), so water moves in the direction of decreasing water potential, i.e. from the medium into the seed. Any decrease in water potential around the seed therefore can inhibit water uptake (Shaykewich & Williams, 1971), and subsequently delays the completion of germination (Bewley & Black, 1978) and slows down the rate at which seeds completes germination (Etherington & Armstrong, 1975; Bewley & Black, 1978), or reduces total germination (Bonner, 1968; Etherington & Armstrong, 1975). The specific effects, however, vary with species or variety, environmental factors (Bewley & Black, 1978), and the degree of contact between the seed and its surroundings (Etherington & Armstrong, 1975).

On the other hand exposure of the seed to water deficit after rehydration may result in water loss from the seed to its surroundings. However, desiccation of seeds between early stages of imbibition and the time of cell division and vacuolation of the developing seedling usually has no permanent deleterious effects on subsequent germination and growth (Bewley, 1979). As seedlings develop they become sensitive to water deficit (Bewley, 1979). Their sensitivity depends on the severity of water deficit (Hsiao, 1973), its duration and on other environmental factors (Hanson & Hitz,

1982) under which the plants are growing. As a whole, water deficit has consequences which involve all physiological functions ranging from primary biochemical processes to overall reduction of growth and development (Etherington & Armstrong, 1975). Water deficit is likely to reduce water uptake, the corresponding fall in plant water potential and the consequent stomatal closure will limit carbon dioxide supplies to mesophyll cells (Fitter & Hay, 1983; Kaiser, 1987). Therefore it is possible that the rate of photosynthesis in these cells may be reduced (Fitter & Hay, 1983; Schulze, 1986) with subsequent decrease in accumulation of dry matter, decreased extension growth, and changes in morphology (Brix 1962). Growth responses to water deficit varies with the stage of life-cycle and also with physiological mechanism through which it is mediated (Etherington & Armstrong, 1975). In general the rate of growth of plant cells and the efficiency of their physiological process are highest when the cells are at maximum turgor (Fitter & Hay, 1983). Thus cell growth is highly sensitive to water stress because cell expansion is caused by the action of turgor pressure upon softened cell walls (Greacen & Oh, 1972; Ordin, 1960). Therefore even under mild stress when turgor pressure is reduced by only a few bars, there is a significant decrease in growth. Since turgor is essential for cell enlargement cell growth ceases at zero turgor pressure or more likely, at a threshold turgor before leaf wilting (Hsiao, 1973). Hsiao *et al.* (1976) demonstrated that very mild stress can reduce growth rate of corn and sorghum leaves. Boyer (1970) showed that stress levels required to reduce leaf elongation in sunflower, sugarbeet, and corn were substantially less than those needed to effect photosynthesis. Acevedo *et al.* (1971) also showed that growth rates of corn leaves could be changed within seconds following alterations of the water status of the root environment. Singh *et al.* (1973) found that induced water deficit in 10-day old barley seedlings resulted in a decrease in leaf dry weight and inhibition of primordium formation and apex elongation of the main shoot.

Water deficit is also known to effect respiration (Etherington & Armstrong,

1975; Hsiao, 1973), reduce translocation rate of phloem (Etherington & Armstrong, 1975), reduce transpiration (Hsiao, 1973) and several aspects of metabolism (Hanson & Hitz, 1982).

Many of these effects are similar to those ascribed to 2,4-D, suggesting that part of its effect is due to interference with water uptake and translocation (Muzik; 1976). 2,4-D is known to interfer with phloem translocation (Ashton & Bayer, 1976) and xylem production (Cartwright, 1976), therefore plant tissue may in effect become water stressed even though the plant is in a moist environment (Muzik, 1976). Along with the damage to the roots (Johanson & Muzik, 1961), the inhibiting effect of 2,4-D on xylem formation can contribute to a reduction in capacity for herbicide translocation.

Therefore 2,4-D can alter germination and its rate, inhibit seedling establishement and effect the growth and development of growing plants by causing a variety of developmental abnormalities and changes in the anatomical structure of plants. The effectiveness of 2,4-D depends upon the species of plant, dosage, stage of growth, methods of application and other factors.

Since many of the effects of the herbicide resemble those of stress on plants it is suggested that a combination of 2,4-D treatment with altered environmental factors may induce more sever responses by the plants than to each factor applied individually. Preliminary evidence for this has already been reported (Alaib 1985). Water stress in particular would appear to be important since it influences cell growth and 2,4-D is shown also to inhibit cell growth.

The major aim of the investigation reported in this chapter was to establish the effect of combined treatments with 2,4-D and drought stress on the germination of seeds, development and growth of monocot and dicot seedlings.

2.2. Materials and Methods

2.2.1. Effect of 2,4-D at different concentrations on germination of seeds and development of *Raphanus sativus* Linn. seedlings.

The seeds were placed in 9-cm Petri dishes on filter paper moistened with 15 ml of solutions containing different concentrations of 2,4-D (1-100 ppm). 20 seeds were placed in each dish, 5 dishes were used with each concentration plus 5 controls (distilled water). The seeds were germinated at temperatures between 21-25 °C, a light intensity of 45 $\mu mol.M^{-2}.S^{-1}$ provided by panels consisting of 6 fluorescent OSRAM 65/80-W tubes on a 16-hour photoperiod.

For each, percentage germination, and radicle and cotyledon expansion were calculated. After 7 days of growth the total percentages of seed germination for each concentration were calculated and on the 12th day the growth inhibition of primary roots was determined. Inhibition of growth caused by the application of 2,4-D at 100 ppm was designated as 100 % and inhibition of root growth resulting from other concentrations was expressed in terms of this percentage.

2.2.2. Effects of treating seeds with 2,4-D on germination of seeds and emergence of *Raphanus sativus* Linn. seedlings.

Seeds were soaked in three different concentrations of aqueous solutions of 2,4-D. The concentrations were 1, 10, and 100 ppm. In each 50 or 45 seeds were soaked in 30 ml of 2,4-D solution and distilled water (for controls). The soaked seeds were kept under laboratory temperature (about 21 °C for 24 hours in the dark after which time they were washed with distilled water and in equal amounts either planted in Petri dishes on filter paper, moistened with 10 ml of distilled water, or in Levington's compost contained in plastic pots.

For seeds planted in Petri dishes, 10 seeds were placed in each dish, 5 dishes were used with each concentration plus 5 controls. The experiment was conducted under growth chamber conditions. The mean temperature was 24 °C with a 24-hour photoperiod.

For seeds planted in Levington's compost, 5 seeds were planted in each pot. There were three treatments which were composed of three standard concentrations of 2,4-D, plus the controls. Each had nine replicates (one pot=one replicate). The experiment was conducted under unheated glasshouse conditions. Mean temperature was about 15 °C.

Percentages of germination were calculated daily for each concentration and the emerging seedlin-gs were counted every day for seed planted in the compost.

2.2.3. Effects of treating soil with 2,4-D on emergence and development of *Raphanus sativus* Linn. seedlings.

Equal amount of Levington compost contained in trays, were sprayed with aqueous solution of 2,4-D. The compound was used at one standard concentration (100 ppm). The volume of spray was equal in all treatments (4 ml per tray). After spraying the trays, the soil was thoroughly mixed to ensure the distribution of 2,4-D. Three treatments were given which comprised of one standard concentration of the herbicide, plus the controls. Each treatment had three replicates (one tray=one replicate), and three trays were used as a control. All trays were planted at the same time, whilst the spraying was carried out at intervals. Three trays were sprayed 2 weeks prior to planting; three were sprayed one week prior to planting and the final three at time of planting. The experiment was conducted under the same conditions which have been described in 2.2.2.

The number of emerged seedlings was recorded daily for 21 days and sub-

sequent development of seedlings was also recorded 30 days after planting.

2.2.4. Effects of 2,4-D and drought stress on seed germination and seedling development of *Raphanus sativus* Linn.

Seeds were placed in 9-cm Petri dishes on filter paper moistened with 15 ml of nutrient solution containing different concentrations of 2,4-D (1, 50 and 100 ppm or 125, 250, 500 and 1000 ppm), and the same concentrations in nutrient solution containing 200 g/l PEG (-10 bars), as water stress agent. 10 seeds were placed in each dish, 10 dishes were used with each concentration (5 containing 2,4-D only and 5 containing 2,4-D+PEG), plus 5 treated with PEG without 2,4-D and 5 controls (distilled water). The experiment was conducted under the same conditions which have been described in 2.2.1.

The number of germinated seeds was taken daily for five days. On the fifth day total percentages of germination was calculated, length of primary roots and hypocotyl was measured and fresh and dry weight of seedlings their seeds treated at high concentrations (more than 100 ppm) were also recorded.

2.2.5. Response of *Raphanus sativus* Linn. seedlings to drought stress following 2,4-D application to the roots in nutrient solution.

Seeds were germinated in 9-cm Petri dishes on filter paper moistened with 10 ml of distilled water, and germinated at a temperature between 20-24 °C, under condition of light described in 2.2.1. When the seedlings had attained the desired size (cotyledons were fully expanded), they were transferred to nutrient solution. 1 week after the seedlings had been transferred, they were treated with 10 ppm of 2,4-D. 5 ml of the compound was added to 200 ml of nutrient solution in each container. 10 containers out of 20 were treated and 24 hours after treatment, 5 of the treated

containers were exposed to drought stress by changing the nutrient solution and adding new nutrient solution contained PEG at 200 g/l (-10 bars) and 2,4-D. For the other 10 containers, 5 were treated with PEG only and 5 were used as controls.

One week after treatment the seedlings were harvested and their fresh and dry weights were recorded. Each replicate (4 seedlings) was weighed separately, so that every treatment had 5 replicates.

2.2.6. Response of *Raphanus sativus* Linn. seedlings to drought stress following foliar application of 2,4-D.

Equal amount of seeds were planted in Levington's compost contained in 24 trays. The seedlings watered every other day by bringing to field capacity. Two weeks after planting (the cotyledons were fully expanded), the trays were sprayed with three concentrations of the herbicide, 6 trays with 1 ppm, 6 trays with 10 ppm, 6 trays with 100 ppm and the other 6 remained untreated as a controls. Seedlings were sprayed once, with equal volume of the herbicide (4 ml per tray). After spraying, the number of trays were divided to two parts; half of the trays (3 for each treatment) were subjected to drought stress (by not watering) and the other half were kept under normal watering regime. The seedlings were kept under glass house (summer) condition .

Two weeks after treatment, the fresh and dry weight of shoots and hypocotyls were recorded and chlorophyll, protein, and proline content in the leaves of treated and untreated seedlings were also determined.

2.2.7. Response of *Raphanus sativus* Linn. seedlings to drought stress following 2,4-D treatment.

In this experiment the procedure in 2.6. was repeated, but this time all 24 trays were subjected to drought stress (treated and untreated seedlings), the method of herbicide application and the volume used were also changed. The application of the compound was carried out using 1 ml syringe. The volume of spray used was equal in all treatment (0.05 ml per seedling), added directly to the cotyledon. Throughout the test period the seedlings were kept in the laboratory under conditions of light and temperature described in 2.2.1.

To measure the response of seedlings to drought stress following 2,4-D treatment, water was withheld from the treated and untreated seedlings and the percentage survival after 2 weeks in the unwatered condition was taken for both treated and untreated seedlings for comparison.

2.2.8. Effects of 2,4-D at different concentrations on caryopsis germination and seedling development of *Lolium temulentum* Linn.

The procedure described in 2.2.1. was repeated here, the number of caryopses per dish was increased to 25. The total percentages of germination, root and coleoptile expansion were calculated after 7 days with length of primary root and coleoptile.

2.2.9. Effects of 2,4-D and drought stress on caryopsis germination and seedling development of *Lolium temulentum* Linn.

Caryopses were placed in 9-cm Petri dishes on filter paper moistened with 10 ml of nutrient solution containing one concentration of 2,4-D (100 ppm) or with 10 ml of nutrient solution containing 200 g/l PEG plus 2,4-D. 10 caryopses were placed in

each dish, 5 dishes were used with each treatment (5 dishes containing 2,4-D only and 5 dishes containing 2,4-D+PEG), plus 5 treated with PEG and 5 controls (distilled water). The plants were grown in the laboratory under the same condition described in 2.2.1.

The number of germinated caryopses, root and coleoptile expansion were recorded daily. On the 11th day total percentages of germination, root and coleoptile expansion were calculated and length of primary root and coleoptile were also measured.

2.2.10. Response of *Lolium temulentum* Linn. seedlings to drought stress following 2,4-D application to the roots in nutrient solution.

Caryopses were germinated in vermiculite in the laboratory, under conditions of temperature and light similar to those described in 2.2.1. When the the seedlings had attained the desired size (two-leaf stage), they were removed from the vermiculite, their roots were washed with running water and were transferred to nutrient solution. One week after the seedlings had been transferred to the nutrient solution, they were treated with aqueous solution of 2,4-D at 100 ppm. 5 ml of the herbicide solution was added to 200 ml of nutrient solution in each container. 12 containers out of 24 were treated and 24 hours later 6 of the treated containers were exposed to drought stress by changing the nutrient solution and adding new nutrient solution containing PEG at 200 g/l (-10 bars) and 2,4-D. For the other 12 containers, 5 were treated with PEG only and the remained 5 were used as controls. The seedlings were kept under temperature and light conditions similar to those described for caryopsis germination.

After one week of growth the seedlings were harvested and fresh and dry weight of their roots and shoots were recorded. Each replicate (4 seedlings) was

weighed separately, so that every treatment had 6 replicates.

2.2.11. Response of *Lolium temulentum* Linn. seedlings to drought stress following foliar treatment with 2,4-D.

Caryopses were soaked in distilled water for 24 hours and planted in Levington's compost contained in trays. The trays were watered every two days by bringing to field capacity. There were four treatments, each treatment comprised of 4 trays (replicates). 4 trays were sprayed with 2,4-D and kept under normal watering regime, 4 were sprayed with 2,4-D and subjected to drought stress, 4 were subjected to drought stress only and 4 were used as a controls. The herbicide was used at one standard concentration (100 ppm), which represents a functional level for most plants. The application of 5 ml per tray of the compound was carried out using a small hand spray directly to the leaves. Seedlings were sprayed once two weeks after planting, and kept under conditions of temperature and light similar to those described in 2.2.1.

Samples of seedlings were taken 1, 2, and 3 weeks after treatment and shoot fresh and dry weight was recorded and chlorophyll content was estimated. On the 3rd week proline content in the leaves was determined . The remained seedlings were transferred to the glasshouse to continue their growth to study the effects of the herbicide on later stages. During this period the morphological changes were recorded and the effect of herbicide on tillering, flowering and caryopsis set were also recorded.

2.2.12. Response of *Zea mays* Linn. seedlings to drought stress following 2,4-D application to the roots in nutrient solution.

Seeds of maize were soaked for 24 hours in distilled water and germinated in vermiculite contained in trays in the laboratory. When the seedlings had attained the desired size (two-leaf stage), their roots were washed using running water and

transferred to nutrient solution. There were three treatments and controls, each treatment had seven replications, one container with four seedlings being considered a replicate. 24 hours after the seedlings had transferred to nutrient solution, they were treated with 2,4-D at 100 ppm. 5 ml of the compound were added to 200 ml of the nutrient solution in each container. 14 container out of 28 were treated with the herbicide and 24 hours after treatment 7 of the treated containers were exposed to drought stress by changing the nutrient solution and adding new nutrient solution contained PEG at 200 g/l (-10 bars) and 2,4-D. For the other 14 containers 7 were treated with PEG only and the remained 7 were used as a control. Throughout the experimental period the seedlings were kept in the laboratory, under conditions of temperature and light described in 2.2.1.

After one week of growth the seedlings were harvested and fresh and dry weight of shoots and roots were recorded. Each replicate (4 seedlings) was weighed separately, so that every treatment had 7 replications.

2.2.13. Response of *Zea mays* Linn. seedlings to 2,4-D and drought stress.

Seeds were soaked for 24 hours in distilled water and germinated in 9-cm Petri dishes in dark. After germination seedlings of similar size were taken from the dishes and transferred to nutrient solution contained 2,4-D at 100 ppm or 2,4-D+PEG at 125 g/l (-5 bar) or PEG or nutrient solution only (control). There were three treatments plus control, each treatment comprised 5 containers (replicates) and each container contained 4 seedlings. 150 ml of nutrient solution were used with each treatment and 5 ml of 2,4-D. Seedlings were kept under conditions of temperature and light similar to those described in 2.2.1.

After one week of growth in nutrient solution seedlings were harvested and the number of expanded leaves was recorded, leaf area, length of primary root, first

internode and mesocotyl were also measured.

2.2.14. Measurements of Development and Growth Rate

A variety of parameters were used in this Chapter to asses the effects of 2,4-D, drought stress and combined 2,4-D and drought stress on seed germination and seedlings development. These parameters included:

1. % germination (radicle emergence), emergence (penetration of seedlings through the soil), root expansion, coleoptile expansion, cotyledon expansion, abnormal seedlings, and survival.

2. Fresh Weight

The fresh weight of the whole plant or its shoot or root, were recorded by weighing small tins empty after drying for a few minutes at 80 °C in an oven and then with the amount of fresh sample.

3. Dry Weight

Samples were dried for 24 hours in an oven at 80 °C The tins were removed from the oven, closed, allowed to cool, weighed and put back in the oven for further 24 hours periods until constant weight was reached.

4. Leaf Area

The leaves were outlined on squared paper, and the squares were counted.

5. Length of Parts

Length of root, hypocotyl, mesocotyl, and coleoptile were measured in cm using a ruler.

6. Number of Leaves

The plants were harvested at the end of the experiment and the number of expanded leaves per plant counted.

7. Number of Tillers

The plants were harvested at the end of the experiment and the number of tillers per plant counted.

8. Inflorescence Development

The plants were harvested at the end of the experiment and the number of inflorescences per plant, number of florets per inflorescence, and number of caryopses per floret counted.

2.3. Results

2.3.1. Effects of 2,4-D on seed germination and seedling development of *Raphanus sativus* Linn. when placed in direct contact with the seeds in Petri dishes.

Although the correlation between herbicide concentrations and percentages of germination was not very strong, it was still significant (Fig. 2.1). Whereas 2,4-D at low concentration did not affect germination significantly, at higher concentrations it was shown to reduce the percentage of germination 7 days after treatment. To distinguish between inhibition and slowing of germination, the seeds were also left in the Petri dishes for longer periods to complete their germination. 12 days after treatment 2,4-D was shown to reduce the percentage of germination and to slow the germination even at low concentrations (Fig. 2.2).

Radicle emergence and development in treated seeds were very restricted. 2,4-D was found to slow the emergence of the radicle and to cease its development immediately after germination. At high concentration 2,4-D inhibited the development completely and checked the final percentage of radicle emergence (Fig. 2.3).

Cotyledon expansion was closely related to the herbicide concentration in treated seeds, and was significantly inhibited by 2,4-D. The inhibition was increased by increasing concentration of the herbicide (Fig. 2.4). However, when the germinated seeds were allowed to develop in Petri dishes, the number of seedlings with expanded cotyledons increased with time, but cotyledon emergence in all 2,4-D treated seeds after 10 days was slower than that in the controls (Fig. 2.5). In seedlings which their seeds were treated with 2,4-D at lower concentration, cotyledons emerged in amounts which varied with herbicide concentration.

2,4-D also inhibited the elongation of primary roots. The degree of inhibition was closely related to the herbicide concentration. However, marked limitation in

Figure 2.1. Effects of 2,4-D at different concentrations on seed germination of *Raphanus sativus* Linn. when placed in direct contact with the seeds in Petri dishes for 7 days.

Figure 2.2. Effects of 2,4-D at concentrations of 1, 10 and 100 ppm on seed germination of *Raphanus sativus* Linn. when placed in direct contact with the seeds in Petri dishes for 12 days.

Figure 2.3. Effects of 2,4-D at concentrations of 1, 10 and 100 ppm on the expansion of radicles in *Raphanus sativus* Linn. seedlings their seeds treated by placing in direct contact with the herbicide solution for 10 days

Fig. 2.3

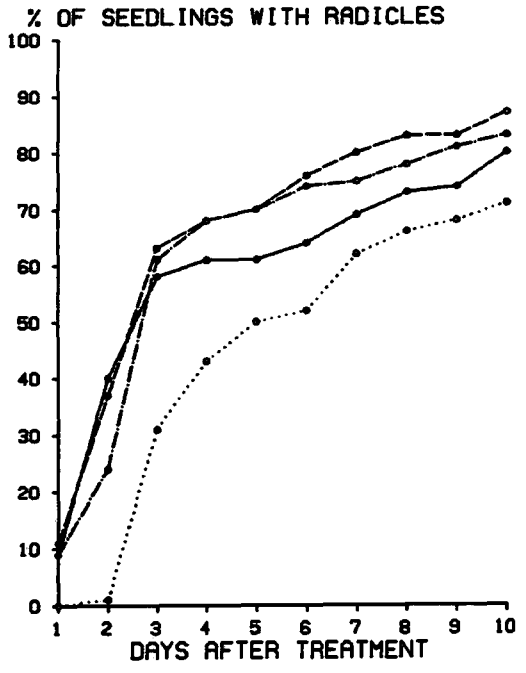


Fig. 2.2

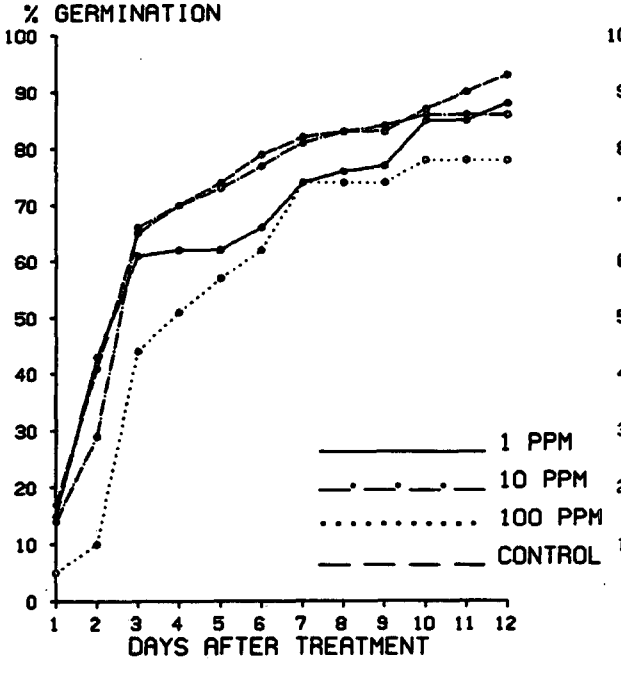


Fig. 2.1

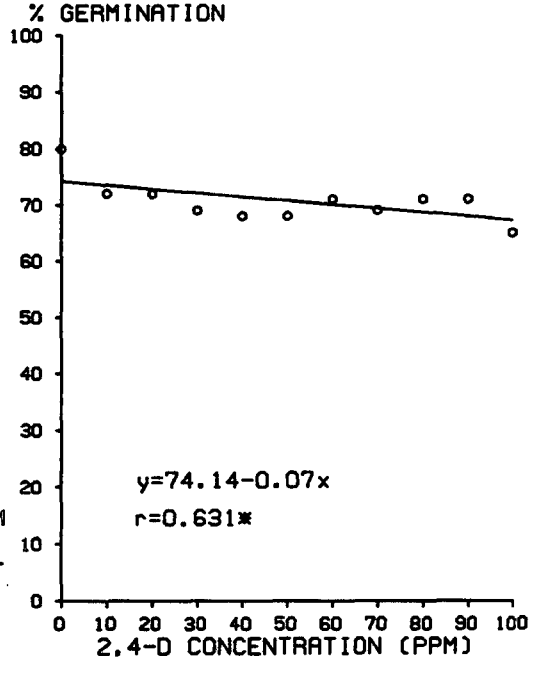
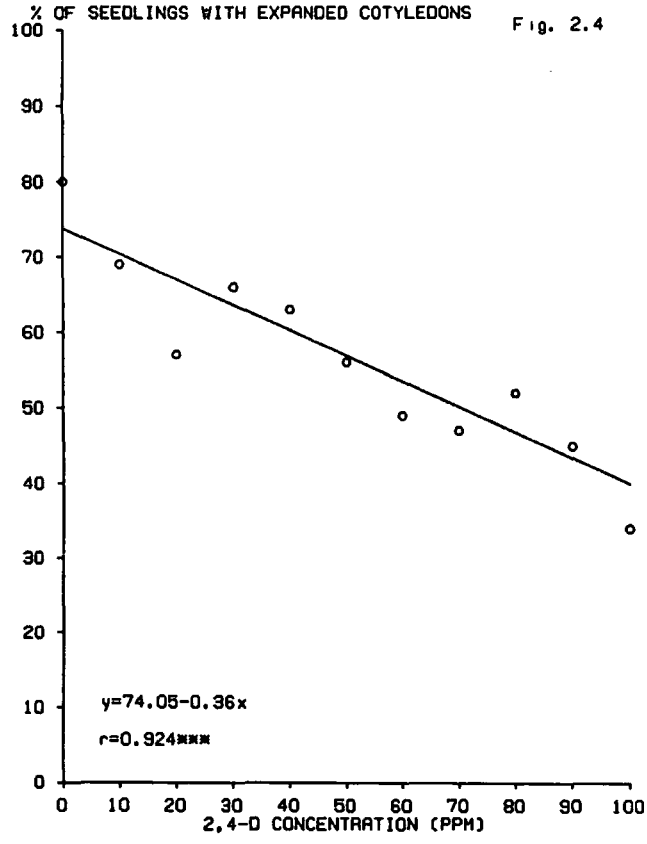


Figure 2.4. Effects of 2,4-D at different concentrations on the expansion of cotyledons in seedlings of *Raphanus sativus* Linn. Seeds were treated by placing in direct contact with the herbicide solution for 7-days.

Figure 2.5. Effects of 2,4-D at concentrations of 1, 10 and 100 ppm on the expansion of cotyledons in *Raphanus sativus* Linn. seedlings. Seeds were treated by placing in direct contact with the herbicide solutions for 10-days.


% OF SEEDLINGS WITH EXPANDED COTYLEDONS
Fig. 2.4
100
90
80
70
60
50
40
30
20
10
0
y=74.05-0.36x
r=0.924***
0 10 20 30 40 50 60 70 80 90 100
2,4-D CONCENTRATION (PPM)

EXPANDED

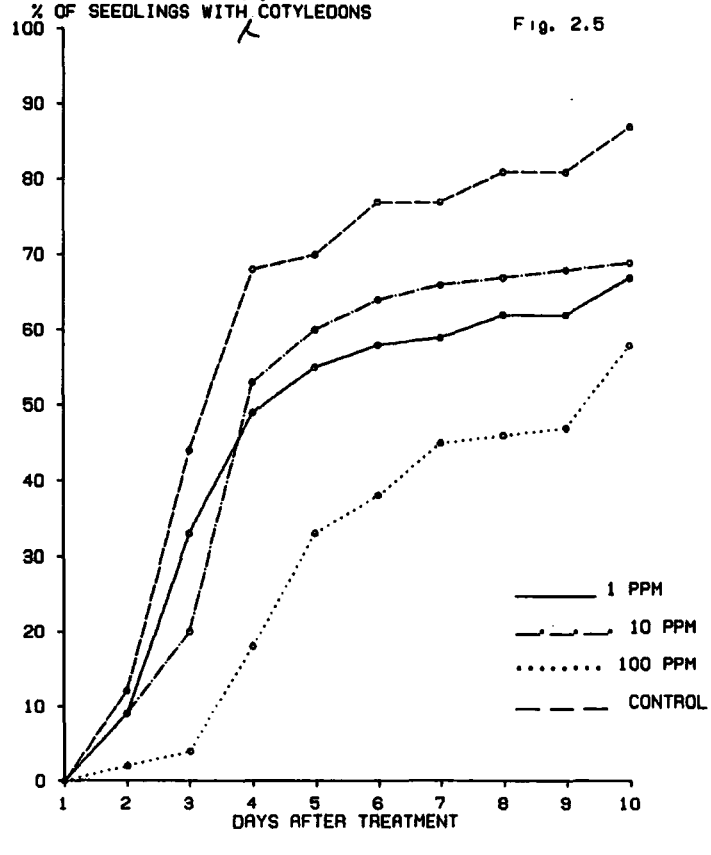
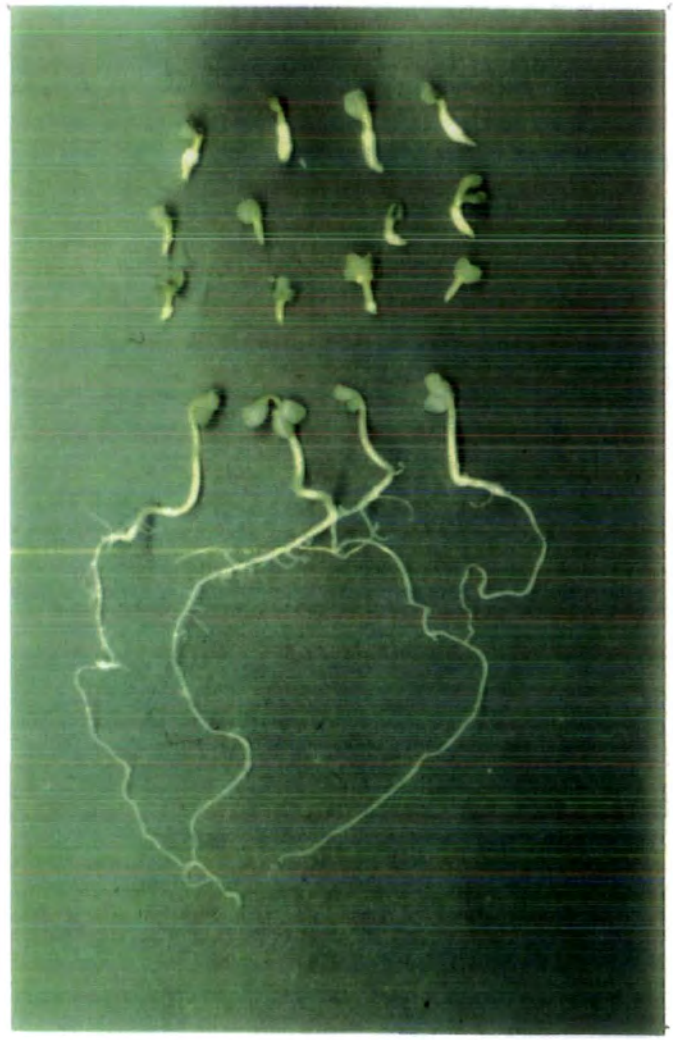

% OF SEEDLINGS WITH COTYLEDONS
Fig. 2.5
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10
DAYS AFTER TREATMENT
1 PPM
10 PPM
100 PPM
CONTROL

Plate 2.1 Effects of 2,4-D on the roots of *Raphanus sativus* Linn. seedlings.Starting with the top row, treatmentss were 1, 10, 100 ppm and the controls.



root length was observed at 10 days even at lowest concentration (Table 2.1). Inhibition of elongation and stunting of primary roots were associated with the production of large numbers of lateral hair-like roots. These laterals were situated so close together that they formed one single fused sheet and root-hypocotyl junction was also swollen (Plate 2.1).

Table 2.1. Effect of 2,4-D on the growth of primary root of *Raphanus sativus* Linn. seedlings.

2,4-D (ppm)	The average length of the primary root	Growth inhibition	% of growth inhibition
0	3.602	0.000	0.00
1	0.398	3.204	88.95
10	0.184	3.418	94.89
100	0.000	3.602	100

2.3.2. Effect of pre-treatment with 2,4-D on the germination of seeds and emergence of *Raphanus sativus* Linn. seedlings.

Treating seeds with 2,4-D by soaking for 24 hours had no effect on the germination at the concentrations used (1, 10, and 100 ppm) (Fig. 2.6). However,treatment of seeds with 2,4-D before planting them in the soil had marked effects on the emergence of seedlings. Despite the fact that 2,4-D had no effect at final percentage of emergence of seeds treated at low concentrations (1 and 10 ppm), it was shown to slow the emergence of the seedlings being more marked at high con-

centrations. In addition to the slowing of emergence, final percentage of emergence were dramatically reduced (Fig. 2.7).

2.3.3. Effects of treating soil with 2,4-D on emergence and development of *Raphanus sativus* Linn. seedlings.

The final percentages of emergence for seeds which were planted in compost contained in trays sprayed just before planting, one week before planting and 2 weeks before planting, with 100 ppm were similar to that of the controls. However, the seedling emergence from seeds planted in treated compost was to a certain extent slower than the controls. The effect, however, was not like that noted in seeds pre-treated with this concentration (Fig. 2.8).

After the emergence of seedlings, the most noticeable effect of 2,4-D on seedling development was stunting, slowing of leaf expansion and development of characteristic malformations on different organs. Leaves of seedlings growing in soil treated with 2,4-D, were drastically changed. The leaves were smaller and narrower than normal; their shape changed completely, with some leaves changed from a simple leaf to two leaves or semi-doubled leaves arising from one primordium. An increase in leaf thickness was also noted (Plate 2.2). These seedlings also showed characteristic formative effects on the base of the hypocotyl. where the lower half of the structure swelled to two or three times its normal diameter and became translucent in appearance particularly in the region of root-hypocotyl junction. Longitudinal ruptures of the surface frequently occurred in that region.

2.3.4. Effects of 2,4-D and drought stress on seed germination and seedling development of *Raphanus sativus* Linn. in Petri dishes.

Over the ranges of concentrations used (1, 50, and 100 ppm), no effect was

Figure 2.6. Effects on germination of pre-treating seeds of *Raphanus sativus* Linn. with 1, 10 and 100 ppm of 2,4-D by soaking in the herbicide solution for 24 hours in Petri dishes.

Figure 2.7. Effects on emergence of seedlings growing in compost of pre-treating seeds of *Raphanus sativus* Linn. with 1, 10 and 100 ppm of 2,4-D by soaking in the herbicide solutions for 24 hours on emergence of seedlings growing in compost.

Figure 2.8. Effect on the emergence of *Raphanus sativus* Linn. seedlings of treating compost soil with 100 ppm of 2,4-D.

- B.P. before planting of seeds.

Fig. 2.8

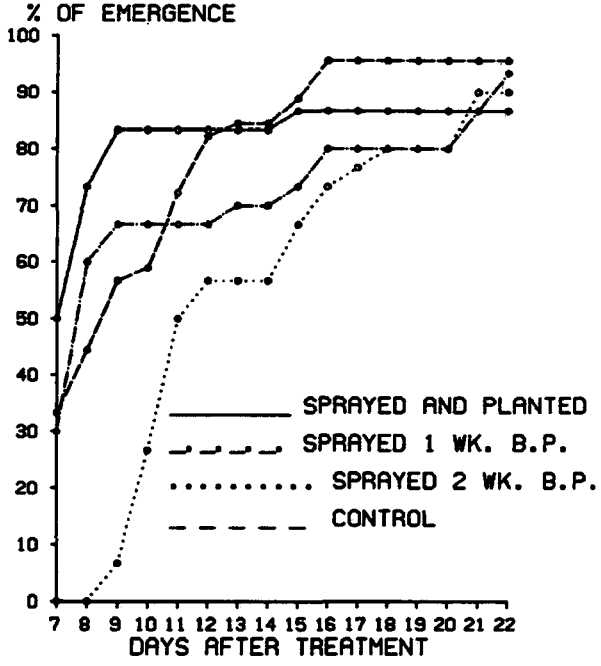


Fig. 2.7

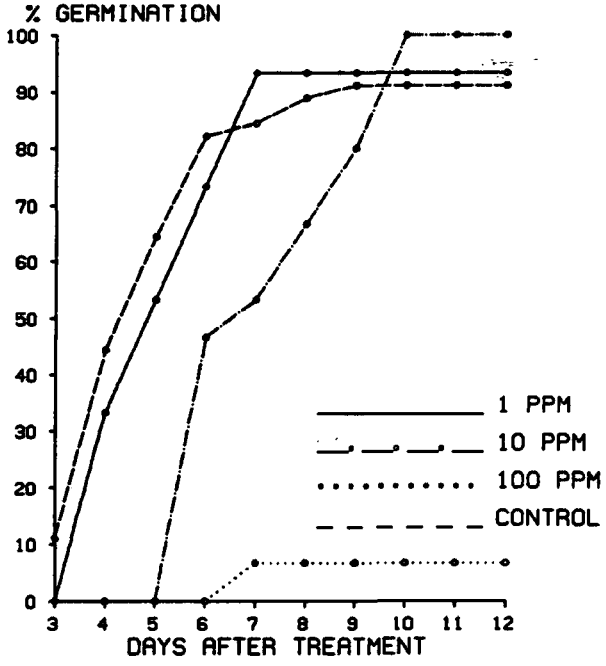


Fig. 2.6

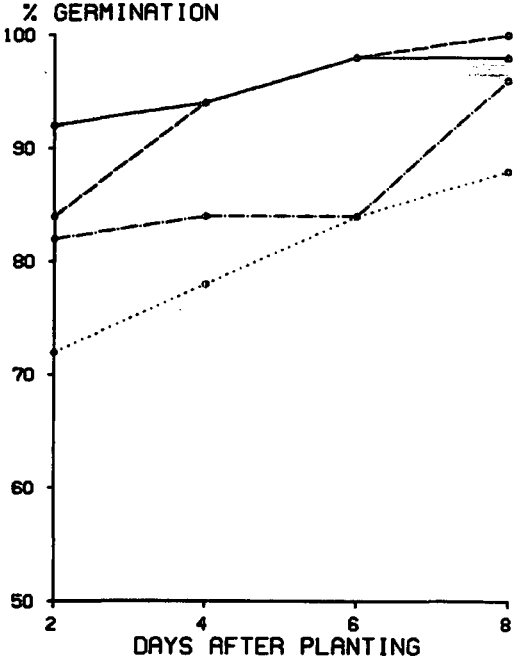


Plate 2.2 Effect of 2,4-D (100 ppm) on the growth and development of leaves of *Raphanus sativus* Linn. seedlings. The herbicide was added to the soil and the plants were grown in this for 20 days.

recorded on germination of seeds with or without PEG. All treated seeds germinated normally in a very short time (3 days) in comparison to the untreated seeds. It was noted, however, that for the seeds which were germinated in distilled water or solutions of 2,4-D without nutrient elements the germination in nutrient solution was faster and more uniform (Fig. 2.9). Seeds treated at higher concentrations (125, 250, 500, and 1000 ppm) with or without PEG, however, responded differently. Seeds treated with these concentrations without PEG germinated normally more or less similar to the controls. Whilst those treated with 2,4-D and PEG, showed a reduced germination rate. The reduction was closely related to the concentration of the herbicide (Fig. 2.10).

Elongation of the primary roots was completely inhibited by 50 ppm of 2,4-D or over with or without PEG. Therefore no measurements were made of primary root growth of these seedlings. The only possible measurement was for seedlings from seeds treated with 1 ppm and the control seedlings. Even at the low concentration the inhibition of primary roots was marked for both 2,4-D and combined 2,4-D and PEG without any significant different between them. Seeds germinated in PEG alone gave seedlings with the longest roots. The difference between control and PEG treated seedlings was also significant (Fig. 2.11).

The response of the hypocotyls was different from that of the primary roots. Although all treatments showed significant reduction in length of the hypocotyl, the most affected seedlings were those treated with 2,4-D+PEG, and the least affected those treated with PEG only (Fig. 2.12).

At high concentrations fresh and dry weight of seedlings were recorded and although it was very difficult to draw decisive conclusion, because there were many treatments, the general pattern was that 2,4-D reduced fresh weight significantly with or without PEG, but the reduction was higher for seedlings treated with 2,4-D plus

Figure 2.9. Effects of 2,4-D and drought stress (200 g/l of PEG) on seed germination of *Raphanus sativus* Linn. in Petri dishes.

Figure 2.10. Effects of 2,4-D at high concentrations and drought stress (200 g/l of PEG) on seed germination of *Raphanus sativus* Linn. in Petri dishes.

Figure 2.11. Effects of 2,4-D (1 ppm) and drought stress (200 g/l of PEG) on the expansion of primary roots in *Raphanus sativus* Linn. seedlings. Seeds were treated with the herbicide solution in Petri dishes.

Figure 2.12. Effects of 2,4-D (1 ppm) and drought stress (200 g/l of PEG) on the expansion of hypocotyl in *Raphanus sativus* Linn. seedlings. Seeds were treated with the herbicide solution in Petri dishes.

- Vertical bars represent standard error for the mean.

Fig. 2.9

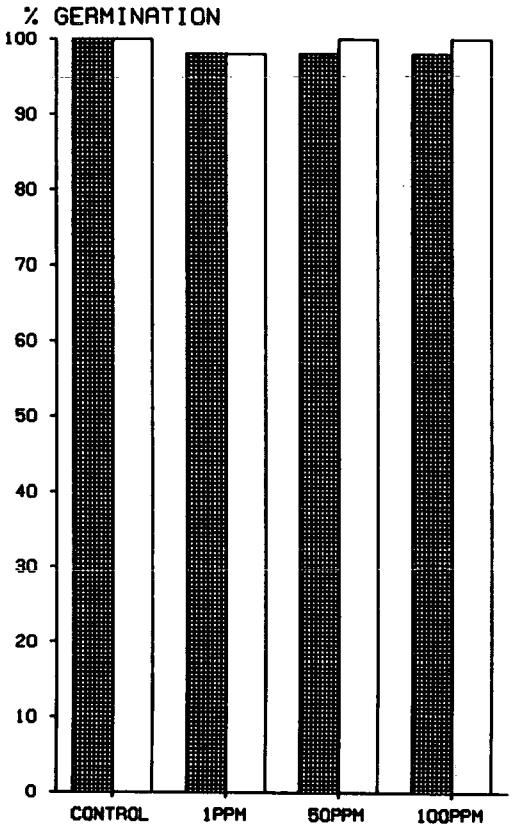


Fig. 2.11

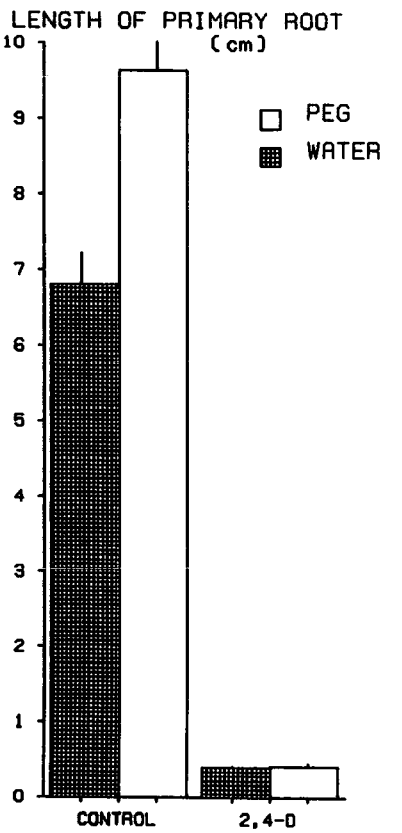


Fig. 2.10

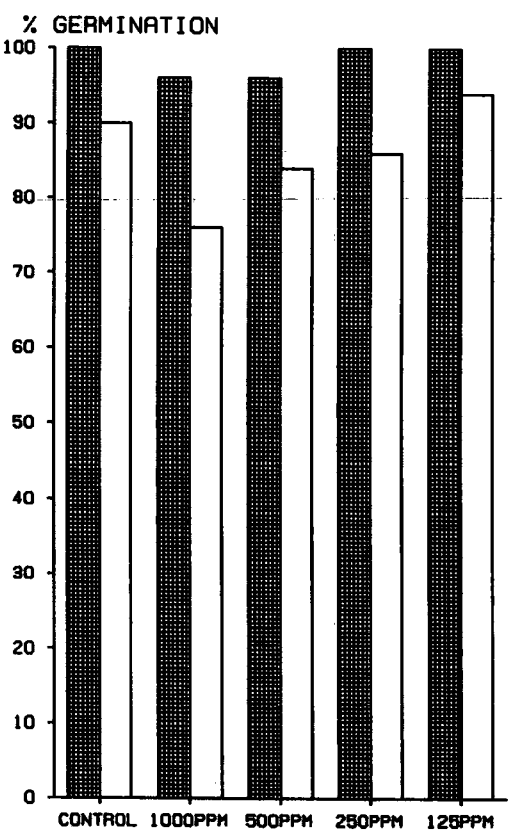


Fig. 2.12

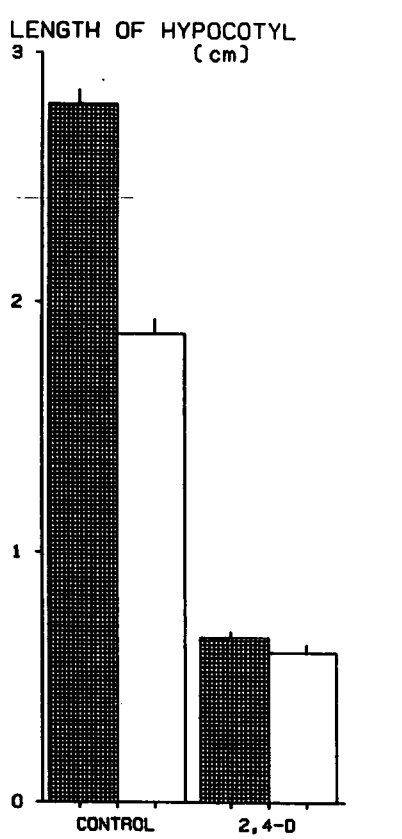


Figure 2.13. Effects of 2,4-D and drought stress (200 g/l of PEG) on fresh weight of *Raphanus sativus* Linn. seedlings. Seeds were treated with the herbicide solutions in Petri dishes.

Figure 2.14. Effects of 2,4-D and drought stress (200 g/l of PEG) on dry weight of *Raphanus sativus* Linn. seedlings. Seeds were treated with the herbicide solutions in Petri dishes.

- Vertical bars represent standard error for the mean.

Fig. 2.13

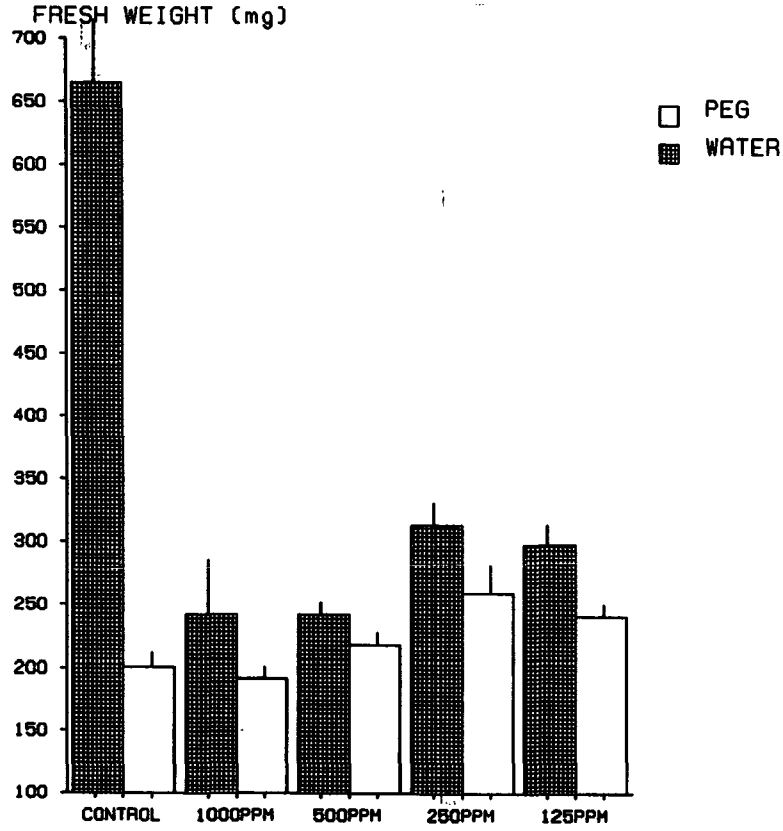
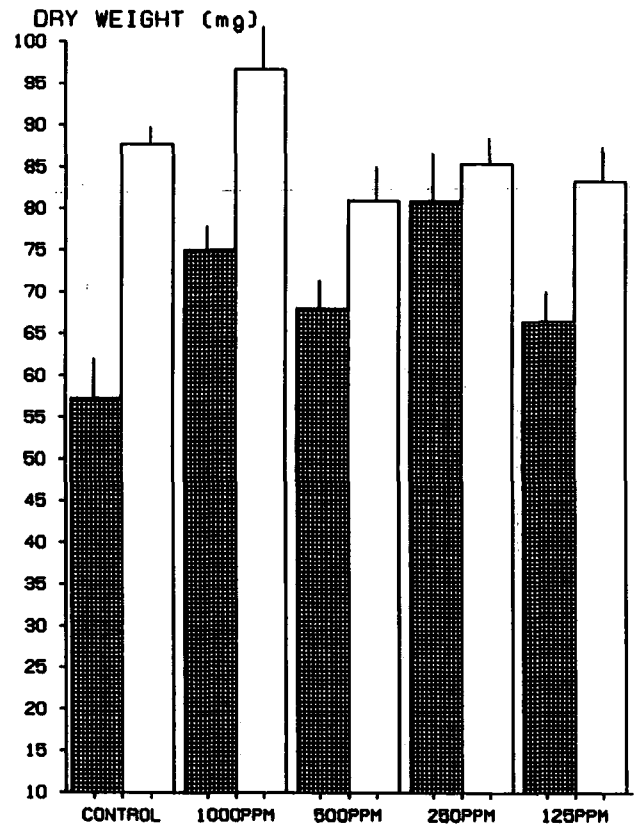


Fig. 2.14



PEG. In contrast dry weight of all treated seedlings was significantly higher than that of the controls and that of 2,4-D+PEG-treated seedlings was higher than that of seedlings treated with 2,4-D only (Fig. 2.13 and 2.14).

2.3.5. Response of *Raphanus sativus* Linn. seedlings to drought stress following 2,4-D application to the roots in nutrient solution.

The most consistent response of seedlings was extreme bending and twisting of the shoots, a few hours after treatment. 24 hours after treatment seedlings were completely twisted, especially the petioles, in addition the cotyledons were rolled. At this stage the seedlings were exposed to drought stress and after a few hours they began to show signs of wilting. 24 hours after PEG treatment complete cessation of growth was soon followed by wilting and drying of shoots of seedlings treated with 2,4-D followed by drought stress and seedlings under drought stress only. Very marked effects were also noticed on the expansion of the first leaf for both 2,4-D and 2,4-D+PEG-treated seedlings. The first leaf of treated seedlings was inhibited completely in some cases and for those where the leaves began expansion they were curled and gray in colour. By the end of the test period the leaves of 2,4-D treated seedlings were distinctly chlorotic and unhealthy. Meanwhile seedlings treated with 2,4-D and exposed to drought stress and seedlings under drought stress began to wilt permanently and their shoots began to dry out.

Consistently, the earliest and most outstanding symptoms which appeared on treated seedlings were swelling of the lower hypocotyl and parts of roots. The surface of the hypocotyls were corky and split by longitudinal fissures in all 2,4-D-treated seedlings with or without PEG.

Root growth was also completely arrested by the concentration used. Distinct suppression of root elongation, accompanied by thickening especially in the

region behind root tips, was evident in all 2,4-D-treated seedlings. The inhibition of elongation and stunting of primary roots were also accompanied by the production of a large number of lateral roots which remained stunted and did not develop beyond the primordial stage in 2,4-D-treated seedlings. In seedlings treated with 2,4-D+PEG, however, the effect was milder.

At the end of the test period fresh and dry weight of seedlings were recorded. All treatments including PEG reduced fresh weight significantly in respect to the controls. Seedlings treated with 2,4-D or PEG were the most affected ones with their fresh weight reduced significantly compared to all other treatments. 2,4-D+PEG-treated seedlings were the least affected in comparison to other treatments. In contrast PEG-treated seedlings had the highest dry weight, but this increase in dry weight was not significant compared with all other treatments except with that of 2,4-D-treated seedlings. There was no significant difference in dry weight between 2,4-D and 2,4-D+PEG-treated seedlings and between 2,4-D+PEG and control seedlings. Thus the only seedlings which showed significant reduction in dry weight were those treated with 2,4-D alone (Fig. 2.15 and 2.16).

2.3.6. Response of *Raphanus sativus* Linn. seedlings to drought stress following foliar application of 2,4-D.

Foliar application of 2,4-D at 1, 10 and 100 ppm had marked effects on growth and development of radish seedlings. Two hours after treatment extreme bending and twisting of the whole seedlings was evident particularly the petioles. Some discoloured spots were also noted in the middle of a few cotyledons treated at 100 ppm. By the second day all seedlings were completely twisted at all concentrations used. When the seedlings were watered two days after treatment, they began to recover and after

Figure 2.15. Effects on fresh weight of *Raphanus sativus* Linn. seedlings of drought stress (200 g/l of PEG) following 2,4-D (10 ppm) application to the roots in nutrient solution.

Figure 2.16. Effects on dry weight of *Raphanus sativus* Linn. seedlings of drought stress (200 g/l of PEG) following 2,4-D (10 ppm) application to the roots in nutrient solution.

- Vertical bars represent standard error for the mean.

Fig. 2.15

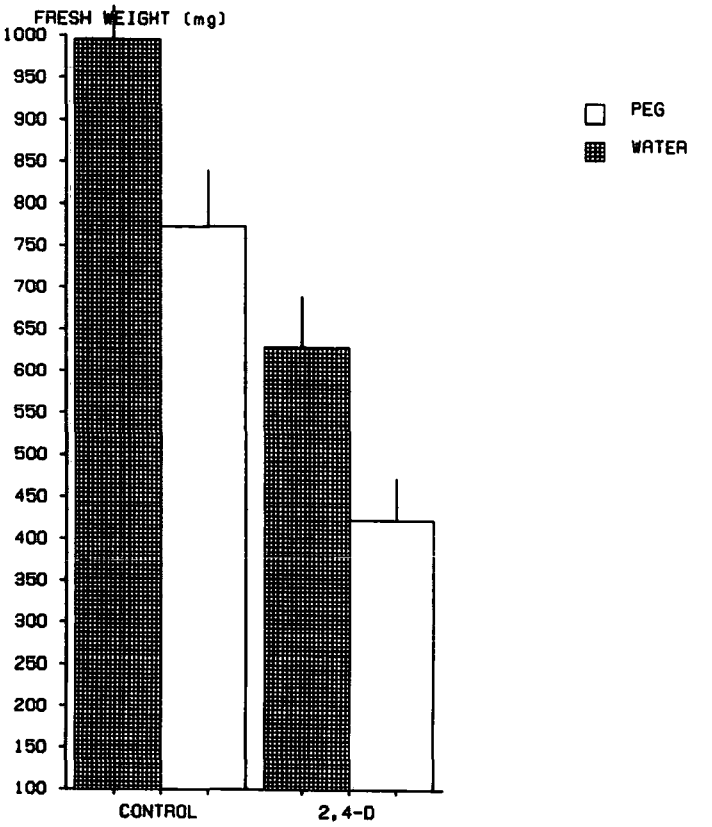
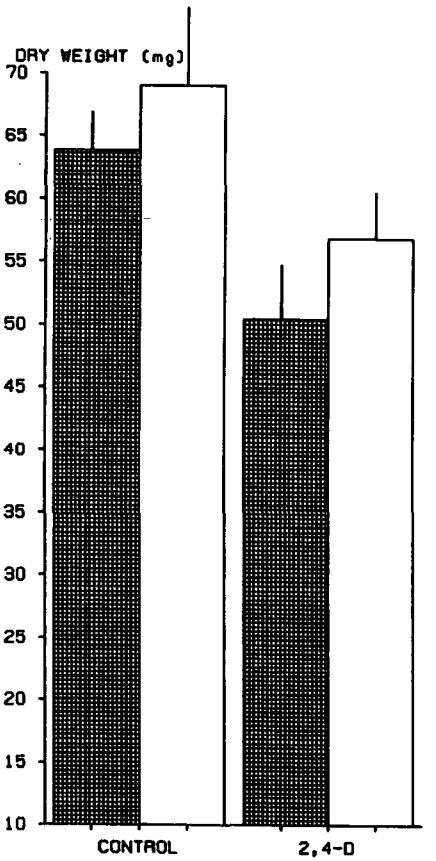


Fig. 2.16



5 days the twisting and bending were mostly overcome in seedlings treated with 1 and 10 ppm but not for those treated with 100 ppm or other seedlings which were kept under drought stress .

As water deficit developed seedlings under drought stress began to wilt and by the 7th day all seedlings were wilted. Normal development was seen in the control seedlings and in those kept under stress without 2,4-D treatment, although in the latter case it was clear that the rate of development was very much less than that of the controls. In contrast the growth of all treated seedlings was arrested, particularly those which were treated and kept under drought stress. The expansion of new leaves was very slow and expanded leaves were curled, thick and gray in colour. The hypocotyl was elongated, and extended some distance above the soil level, split longtudinally and abnormal callus tissue grew on the surface. As a result of these abnormalities the hypocotyls were very thin and long without any increase in diameter (tuber formation was inhibited). Although data suggested that seedlings under drought stress were more affected, the differences in the fresh and dry weights between these and the watered plants were not significant (Fig. 2.17, 2.18, 2.19, 2.21, 2.22 and 2.23). A significant increase in root to shoot ratio was found for all seedling treatments when compared with the controls (Fig. 2.20 and 2.24).

Some of the treated and untreated seedlings were left to develop in order to study the subsequent morphological effects of herbicide and drought stress on different parts of them. The following observations were recorded as shown in Plate 2.3 and 2.4.

Leaves–newly developed leaves (after treatment) showed some distortions in all concentrations used and for both seedlings growing under drought stress or under watering regime, but the abnormalities differed from those in the already developed leaves. The severity of the distortions and their type were related to the

Figure 2.17. Effects on root fresh weight of drought stress following foliar application of 2,4-D to the seedlings of *Raphanus sativus* Linn.

Figure 2.18. Effects on total fresh weight of drought stress following foliar application of 2,4-D to the seedlings of *Raphanus sativus* Linn.

Figure 2.19. Effects of drought stress following foliar application of 2,4-D to the seedlings of *Raphanus sativus* Linn. on total fresh weight.

Figure 2.20. Effects on root to shoot ratio of drought stress following foliar application of 2,4-D to the seedlings of *Raphanus sativus* Linn.

-Vertical bars represent standard error for the mean.

Fig. 2.17

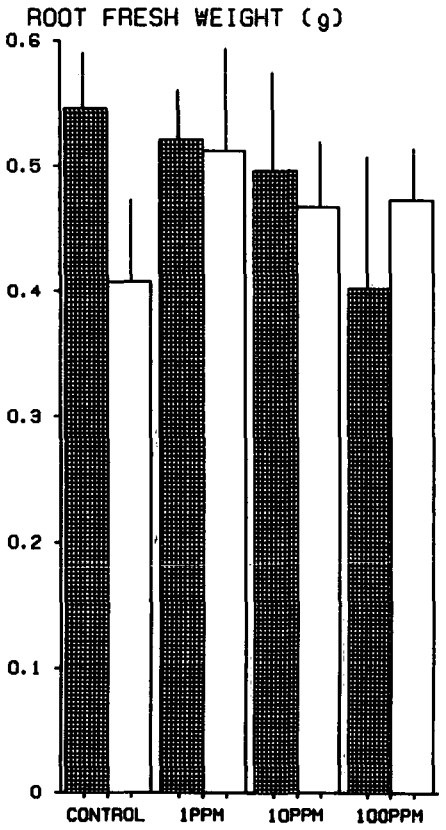


Fig. 2.18

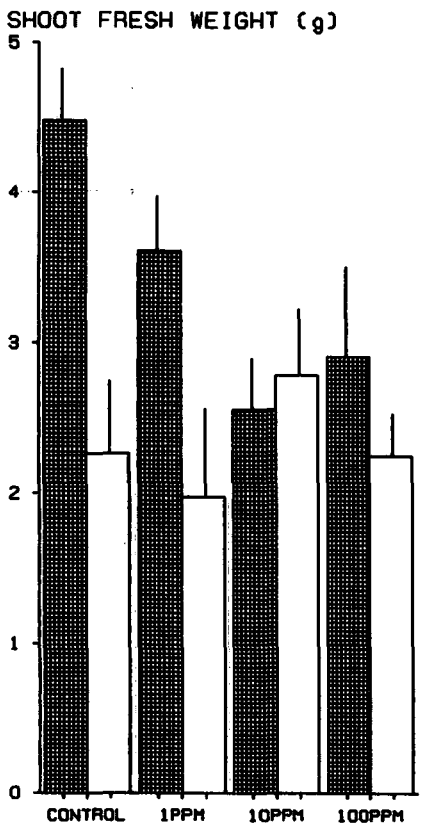


Fig. 2.19

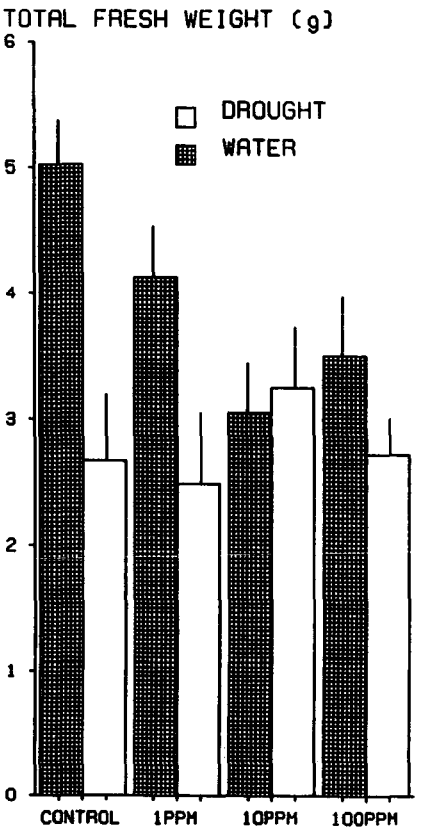


Fig. 2.20

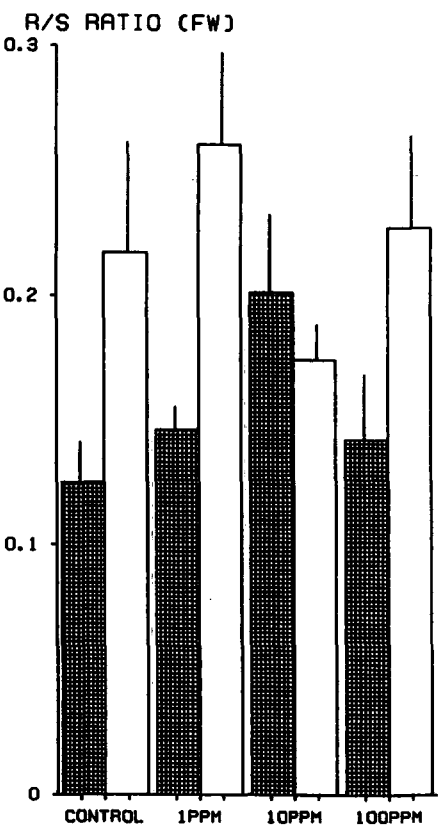


Figure 2.21. Effects on root dry weight of drought stress following foliar application of 2,4-D to the seedlings of *Raphanus sativus* Linn.

Figure 2.22. Effects on shoot dry weight of drought stress following foliar application of 2,4-D to the seedlings of *Raphanus sativus* Linn.

Figure 2.23. Effects on total dry weight of drought stress following foliar application of 2,4-D to the seedlings of *Raphanus sativus* Linn.

Figure 2.24. Effects on root to shoot ratio of drought stress following foliar application to the seedlings of *Raphanus sativus* Linn.

-Vertical bars represent standard error for the mean.

Fig. 2.21

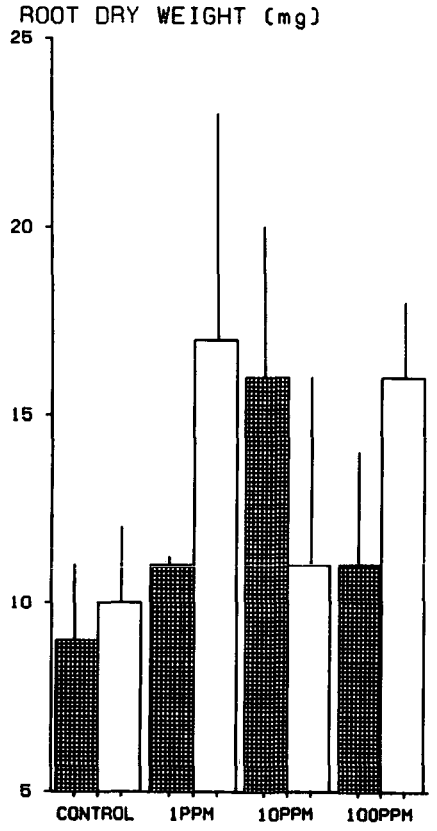


Fig. 2.22

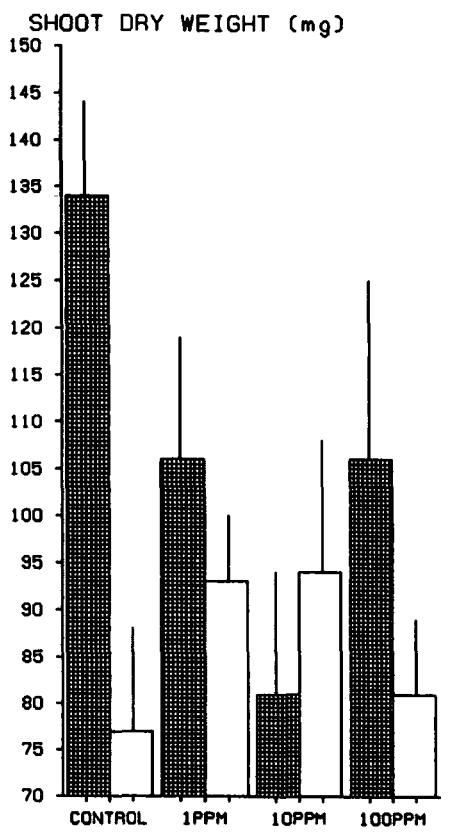


Fig. 2.23

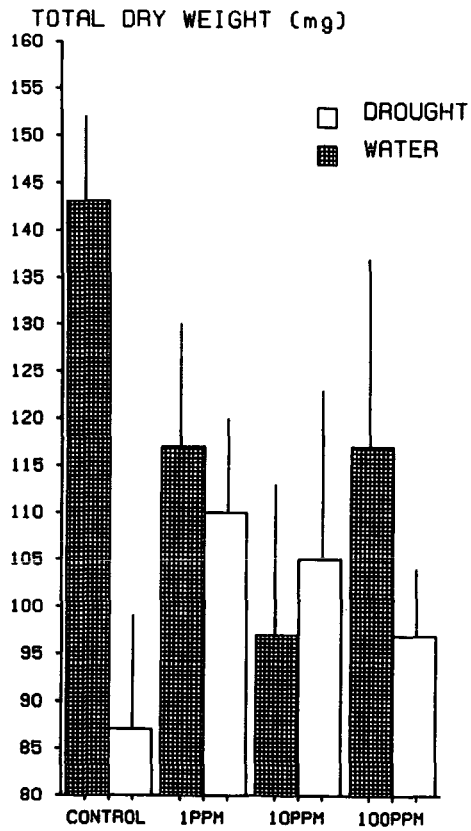
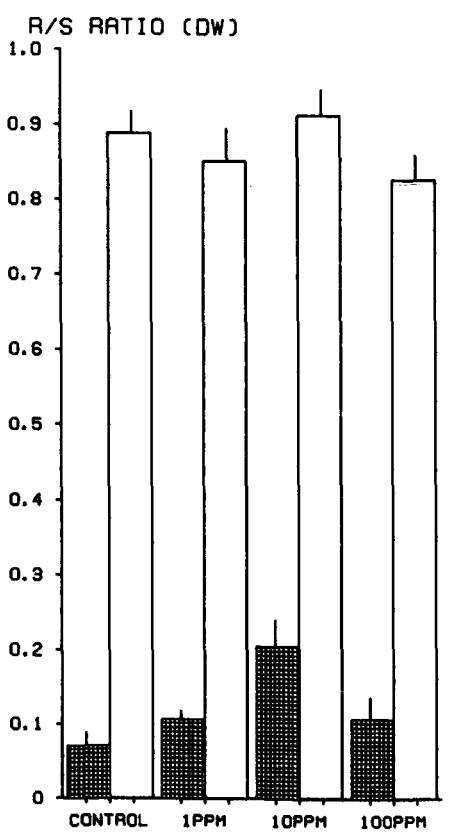


Fig. 2.24



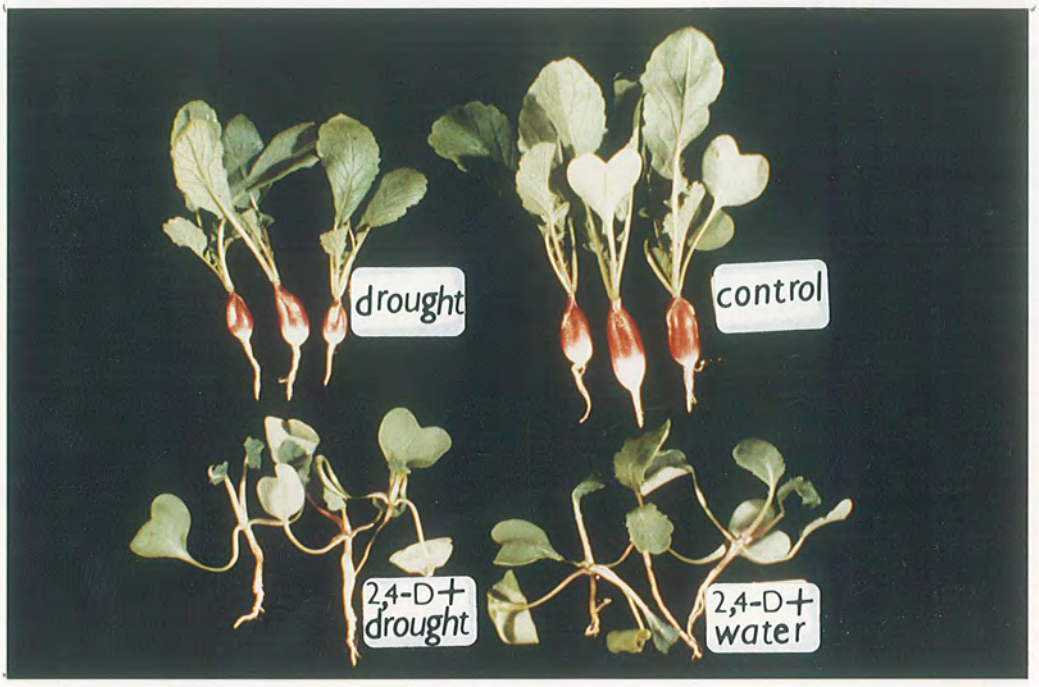
herbicide concentration and water status of seedlings i.e. seedlings treated with high concentrations produced more severely distorted leaves than seedlings treated with low concentrations. Seedlings growing under drought stress produced less severe and distorted leaves. Leaf malformations observed included; changes in width and length, and change in the thickness of the leaves. As a result of these changes leaf shape was totally changed giving rise to leaves with reduced mesophyll bulged out between the veins. Some leaves appeared as semi-double leaves on the same petiole and arising from one primordium.

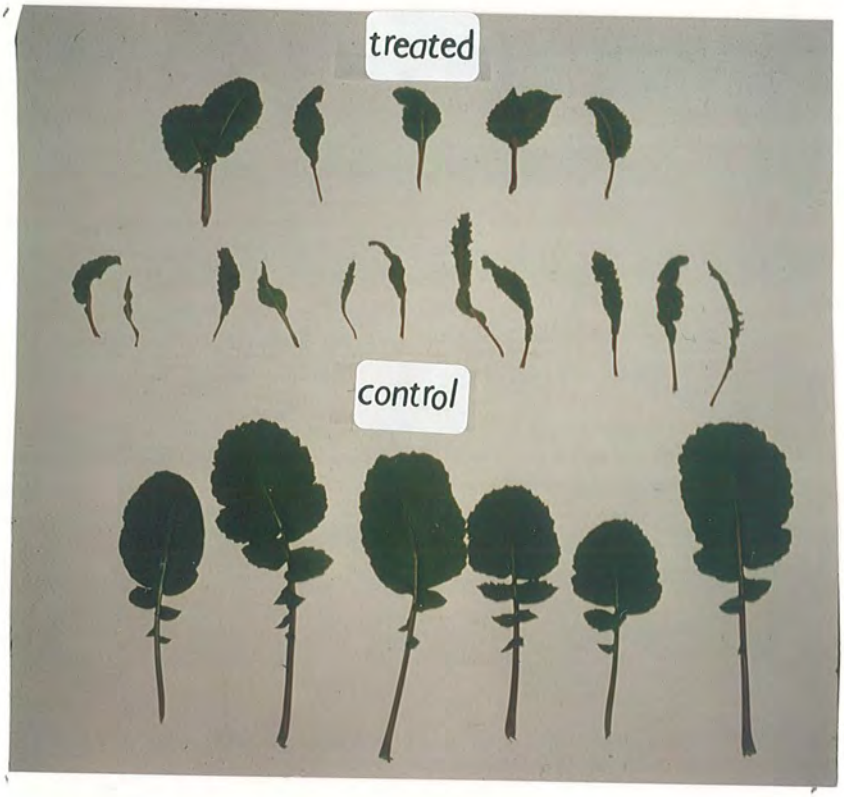
Hypocotyls–the hypocotyl was shown to be very sensitive to 2,4-D as it responded very early to herbicide treatment. Longitudinal ruptures of the surface and exposure of inner tissues occurred during the first week of treatment. Normal thickening also stopped totally and abnormal growth of tissues on the surface of the hypocotyl occurred frequently. As a result hypocotyls became pale in colour and very thin with no flesh at all and failed to penetrate the soil. By visual inspection it was noted that the most sensitive part appeared to be the root-hypocotyl junction, it was found that as abnormal growth began in the upper hypocotyl and down in the root the root-hypocotyl junction became weaker, and changed to a brown colour with subsequent separation of hypocotyl from root. This was probably the cause of death in the whole plant.

Roots–foliar treatment with 2,4-D had marked effects on the growth and morphology of radish roots. Growth in length was suppressed in all treated seedlings, resulting in a total reduction of root system with thick, non elongated primary root. Large number of lateral roots were produced on the main root, these roots were stunted and thick, and remained like that for a long time. In addition to these distortions, abnormal growth on the surface of the main root appeared as masses of proliferated cells in the region of elongation. These types of modifications were observed for all treated seedlings regardless of their water condition.

Plate 2.3 Effect of foliar applied 2,4-D (100 ppm) and drought stress on the growth and development of *Raphanus sativus* Linn. seedlings.

Plate 2.4 Effect of foliar applied 2,4-D (1-100 ppm) on the growth and development of leaves of *Raphanus sativus* Linn. seedlings.


drought
control
2,4-D+
drought
2,4-D+
water


treated
control

2.3.7. Effect of drought stress on survival rate of *Raphanus sativus* Linn. seedlings previously treated with 2,4-D.

Seedlings treated with three concentrations of 2,4-D (1, 10 and 100 ppm) and subjected to drought stress immediately after treatment showed the same kind of responses described before for radish seedlings treated with these concentrations. By the 5th day the first leaf had expanded in all untreated seedlings. However, the first leaf in treated seedlings was very small and thick and some did not expand at all after 5 days. At the same time all treated seedlings began to wilt permanently, particularly those which were treated with 100 ppm. By the end of second week most of the treated seedlings died and dessicated, and some of the untreated seedlings also died and some wilted.

At the end of 2 weeks period all seedlings were watered and allowed grow on; those which did not grow were classed as non-surviving. Survival rates are shown in Fig. 2.25.

2.3.8. Effects of 2,4-D at different concentrations on caryopsis germination and seedling development of *Lolium temulentum* Linn. in Petri dishes.

No significant effects were seen on germination of rye grass caryopses at concentrations of 10-100 ppm 2,4-D. 7 days after treatment treated caryopses showed almost complete germination for all concentrations in respect to the control caryopses (Fig. 2.26).

Root expansion and development in treated seedlings, however, were very restricted. 2,4-D slowed the expansion of roots and development ceased immediately after germination. At high concentrations 2,4-D inhibited the expansion and development completely (Fig. 2.27).

Figure 2.25. Effects on survival rate of *Raphanus sativus* Linn. seedlings of drought stress following 2,4-D treatment.

Fig. 2.25

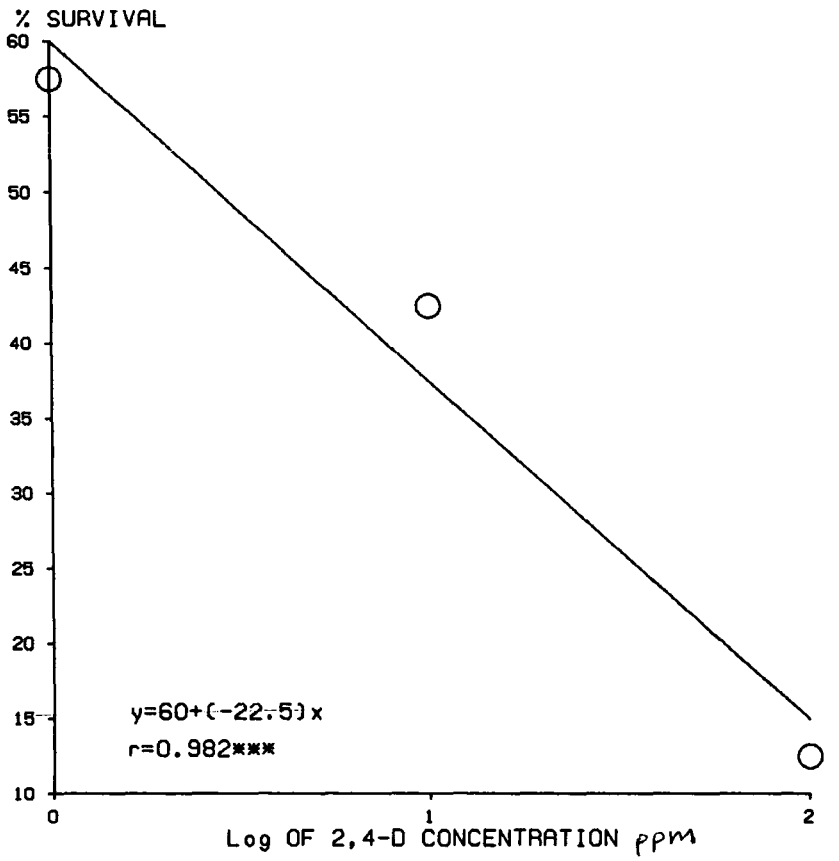

% SURVIVAL
60
55
50
45
40
35
30
25
20
15
10
y=60+(-22.5)x
r=0.982***
0
1
2
Log OF 2,4-D CONCENTRATION ppm

Figure 2.26. Effects on caryopsis germination of *Lolium temulentum* Linn. in Petri dishes of 2,4-D at different concentrations.

Figure 2.27. Effects on expansion of roots in *Lolium temulentum* Linn. in Petri dishes of 2,4-D at different concentrations.

Figure 2.28. Effects on expansion of coleoptiles in *Lolium temulentum* Linn. in Petri dishes of 2,4-D at different concentrations.

Figure 2.29. Effects on seedling development of *Lolium temulentum* Linn. of 2,4-D at different concentrations.

Fig. 2.26

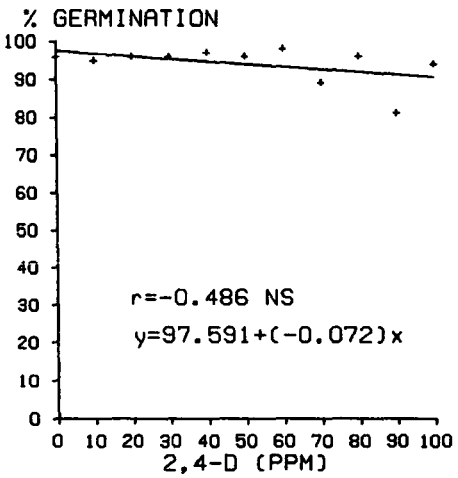


Fig. 2.27

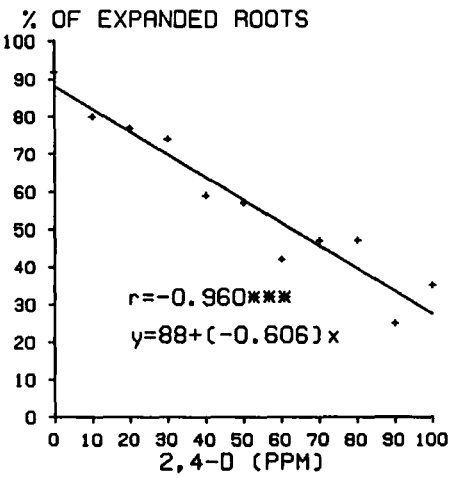


Fig. 2.28

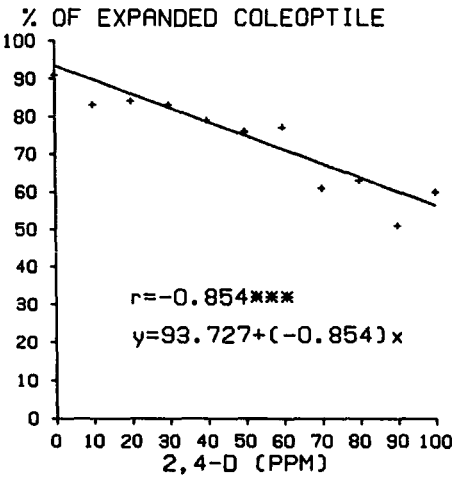
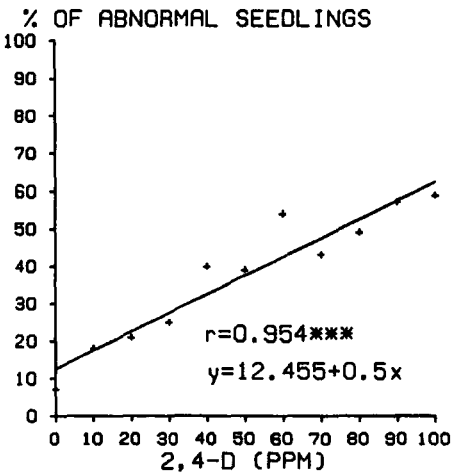


Fig. 2.29



2,4-D also inhibited the elongation of primary roots in treated seeds. The degree of inhibition was closely related to the herbicide concentration. However , root growth was checked by 2,4-D at all concentrations used. Marked limitations in root length were observed at 7 days even at lowest herbicide concentration.

In addition to root inhibition 2,4-D inhibited the elongation of the coleoptile over 7 day period (Fig. 2.28). As a result of the inhibition in root and coleoptile elongation a significant increase in the number of abnormal seedlings were noticeable in treated seedlings in comparison to the controls. It was found that the number of abnormal seedlings was decreased by decreasing 2,4-D concentration (Fig. 2.29).

2.3.9. Effects of 2,4-D and drought stress on caryopsis germination and seedling development of *Lolium temulentum* Linn. in Petri dishes.

Caryopses of rye grass germinated in 2,4-D solution at 100 ppm or in 2,4-D solution which contained PEG (200 g/l), germinated normally indicating that neither 2,4-D nor combined 2,4-D and PEG had any effect on germination. 11 days after treatment the rate of germination was almost the same for treated and untreated seeds (Fig. 2.30). 2,4-D and combined 2,4-D and PEG affected the growth and development of primary roots and coleoptile significantly. Reduction in the root expansion and in the elongation of primary root was significant for all treatments in respect to the control. The highest reduction was caused by 2,4-D alone followed by 2,4-D+PEG and the least reduction caused by PEG (Fig. 2.31 and 2.32).

In addition to the inhibition to root elongation and expansion, 2,4-D was shown to suppress the expansion and elongation of coleoptiles. The data suggested that coleoptiles were less responsive to the herbicide than the roots. The coleoptiles were more sensitive to PEG than 2,4-D. However, all PEG, 2,4-D and 2,4-D+PEG treatments suppressed the growth and development of coleoptiles in comparison to the

Figure 2.30. Effects on the germination of *Lolium temulentum* Linn. caryopses in Petri dishes of 2,4-D (100 ppm) and drought stress (200 g/l of PEG).

Figure 2.31. Effects on the expansion of roots in *Lolium temulentum* Linn. of 2,4-D (100 ppm) and drought stress (200 g/l of PEG).

Figure 2.32. Effects on the elongation of primary root in *Lolium temulentum* Linn. of 2,4-D (100 ppm) and drought stress (200 g/l of PEG).

-Vertical bars represent standard error for the mean.

Fig. 2.32

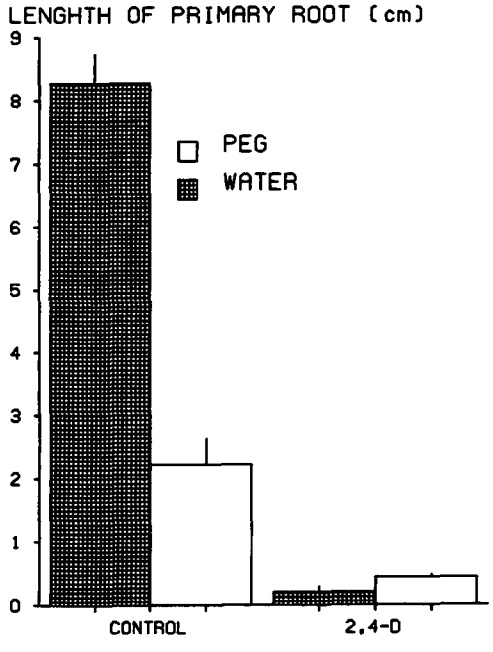


Fig. 2.31

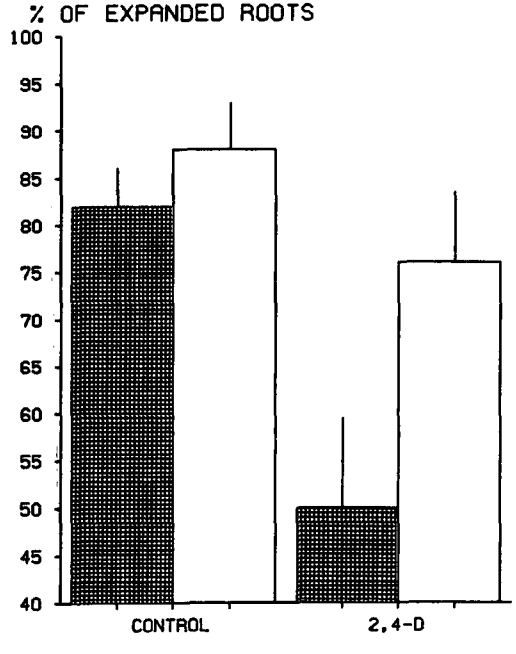


Fig. 2.30

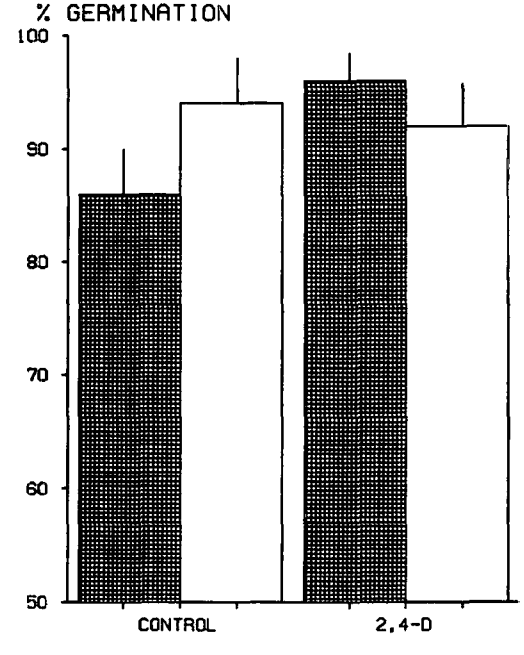


Figure 2.33. Effects on the expansion of coleoptiles in *Lolium temulentum* Linn. of 2,4-D (100 ppm) and drought stress (200 g/l of PEG).

Figure 2.34. Effects on the elongation of coleoptiles in *Lolium temulentum* Linn. of 2,4-D (100 ppm) and drought stress (200 g/l of PEG).

- Vertical bars represent standard error for the mean.

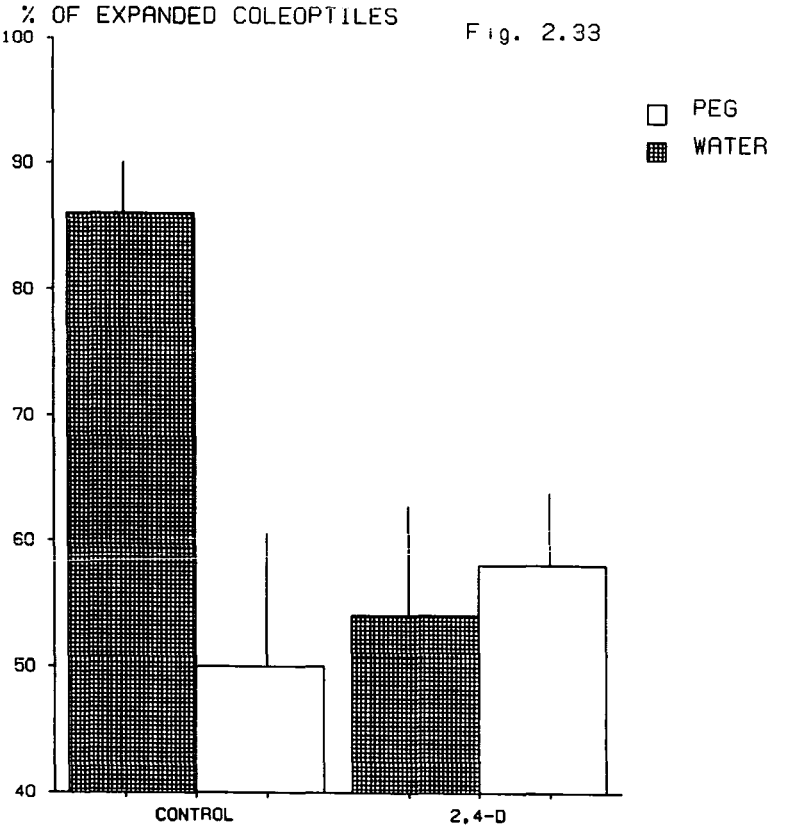


Fig. 2.33

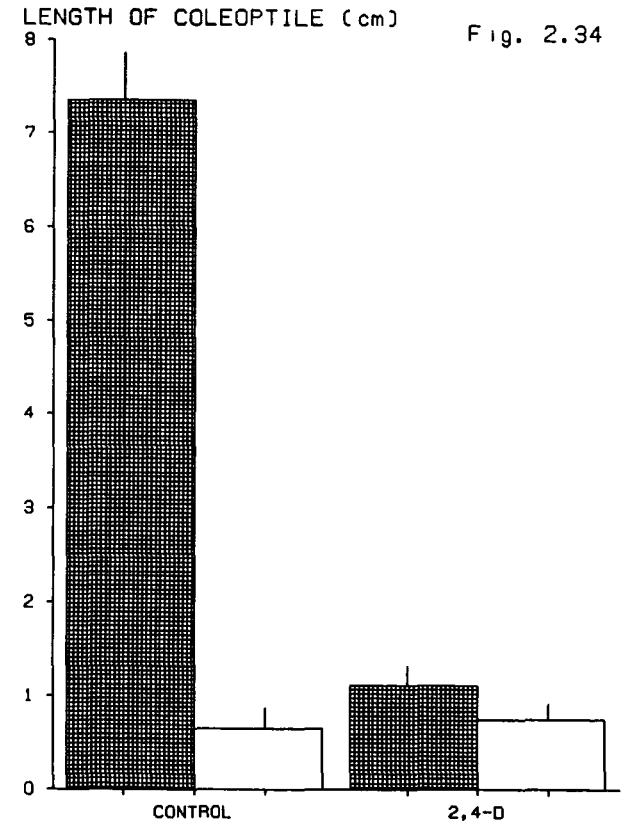


Fig. 2.34

controls. The response of the coleoptile to the treatments used was exactly opposite to that in the roots, the highest reduction in coleoptile length was caused by PEG, followed by 2,4-D+PEG and the lowest was caused by 2,4-D. There were no significant differences between treatments however, but the difference between all treatments and the controls were highly significant (Fig. 2.34). Similar responses were also observed for coleoptile expansion (Fig. 2.33).

2.3.10. Response of *Lolium temulentum* Linn. seedlings to drought stress following 2,4-D application to the roots in nutrient solution.

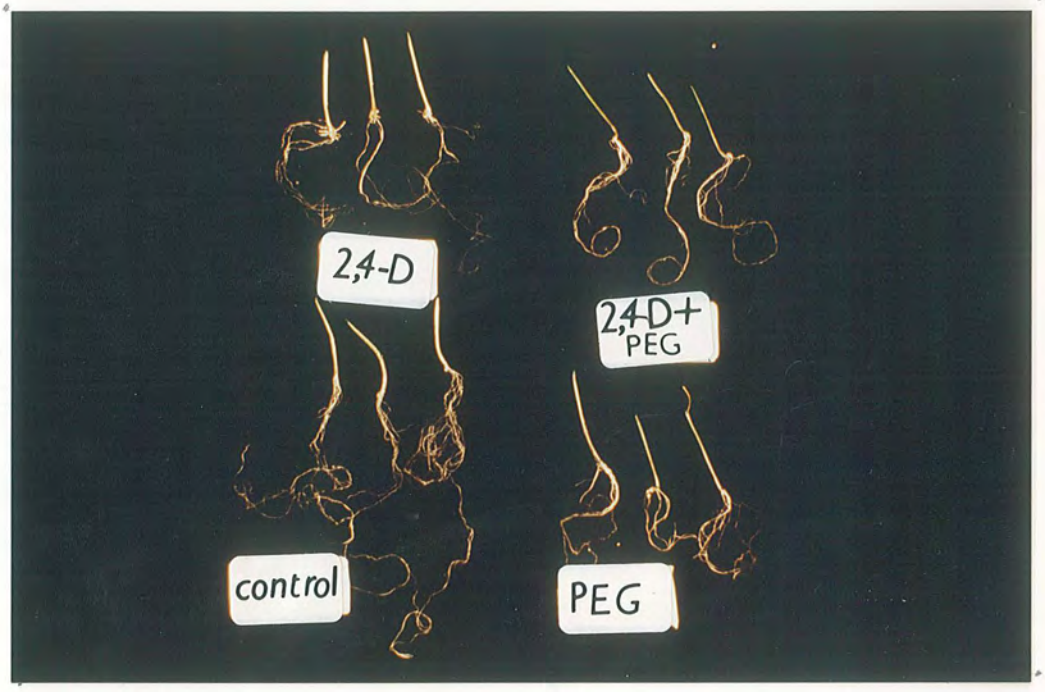
Rye grass seedlings treated for one week with 2,4-D or 2,4-D+PEG in nutrient solution developed no uniform or distinct symptoms of twisting or epinasty such as those observed on radish seedlings. However, the following effects on the seedlings were noted;

Leaves–growth of the first leaf was completely inhibited for all treatments used. In 2,4-D and in PEG-treated seedlings the second leaf expanded but its growth completely ceased immediately after expansion. In 2,4-D+PEG-treated seedlings, however, the expansion of new leaves was completely stopped. On the other hand leaves of 2,4-D+PEG and PEG-treated seedlings began to dry and die prematurely from the tip toward the base. At the same time leaves of 2,4-D treated seedlings were distinctly chlorotic and unhealthy in comparison to the controls.

Roots–normal growth and development of roots were almost completely inhibited by all treatments. The symptoms which developed on roots of rye grass seedlings were more or less similar to those observed on radish seedlings with regard to type and time of expression. Lateral roots were completely inhibited by 2,4-D, but in 2,4-D+PEG-treated seedlings lateral roots were expanded, although their growth ceased immediately after expansion and remained stunted. At the same time

Plate 2.5 Effect of 2,4-D (100 ppm) and drought stress (200 g/l of PEG) applied to the roots of *Lolium temulentum* Linn. in nutrient solution.

Plate 2.6 Effect of 2,4-D (100 ppm) and applied to the roots of *Lolium temulentum* Linn. in nutrient solution.


2,4-D
2,4-D+
PEG
control
PEG

lateral roots continued normal growth in PEG-treated seedlings without any visible symptoms of fasciation. Moreover growth of adventitious roots was inhibited by 2,4-D and 2,4-D+PEG, the inhibition was less noticeable, however, in 2,4-D+PEG-treated seedlings. Crowns of 2,4-D and 2,4-D+PEG-treated seedlings were also distinctly swollen. Meanwhile no signs of crown swelling or adventitious root inhibition were observed on seedlings treated with PEG (Plate 2.5 and 2.6).

7-days after treatment fresh and dry weight of shoots and roots were recorded. It was found that rye grass shoots responded differently to the treatment from the roots. The highest reduction in shoot fresh and dry weight was found to be by PEG, and the lowest was caused by 2,4-D. Whilst the effect of 2,4-D+PEG was intermediate (Fig. 2.35 and 2.38), however, all treatments showed a significant reduction in fresh and dry weights of seedlings in respect to the controls (Fig. 2.37 and 2.40). Roots were more responsive to combined 2,4-D+PEG, although there were no significant differences in root dry weight between treated and untreated seedlings (Fig. 2.39), all treatments reduced root fresh weight significantly. The highest reduction this time was caused by 2,4-D+PEG (Fig. 2.36).

2.3.11. Response of *Lolium temulentum* Linn. seedlings to drought stress following foliar treatment with 2,4-D.

Foliar application of 2,4-D to rye grass at the seedling stage (two weeks after germination) had no morphological effects on shoots during the early stages of development. Three weeks after 2,4-D application, no indication of any shoot curvature or abnormal growth were observed on the seedlings. As can be seen from Fig. 2.41, seedlings growing in drought or drought with 2,4-D showed reduced shoot fresh weight. However, no significant difference was found between controls and plants treated with 2,4-D only. Dry weight did not show any differences between

Figure 2.35. Effect of drought stress (200 g/l of PEG) on shoot fresh weight of *Lolium temulentum* Linn. seedlings following 2,4-D (100 ppm) application to the roots in nutrient solution.

Figure 2.36. Effect of drought stress (200 g/l of PEG) on root fresh weight of *Lolium temulentum* Linn. seedlings following 2,4-D (100 ppm) application to the roots in nutrient solution.

Figure 2.37. Effect of drought stress (200 g/l of PEG) on total fresh weight of *Lolium temulentum* Linn. seedlings following 2,4-D (100 ppm) application to the roots in nutrient solution.

-Vertical bars represent standard error for the mean.

Fig. 2.37

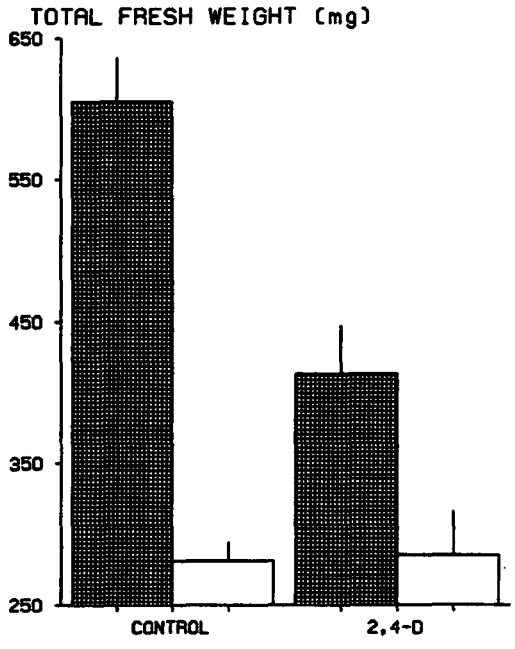


Fig. 2.36

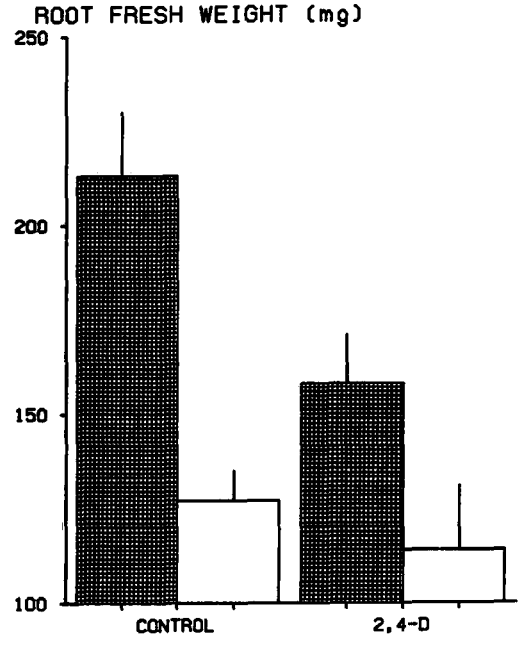


Fig. 2.35

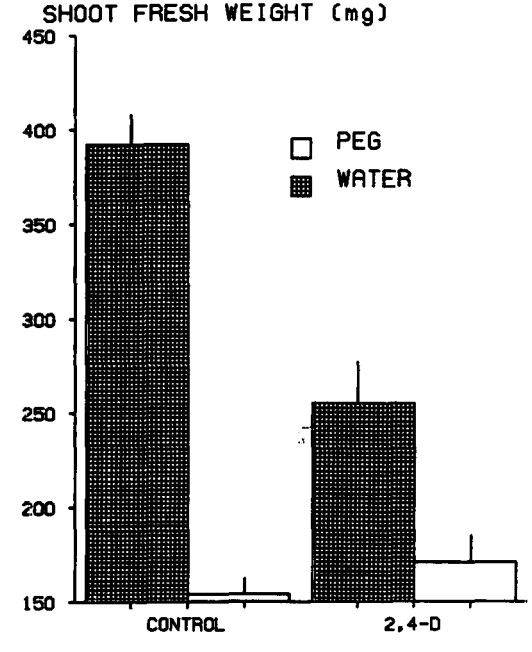


Figure 2.38 Effect of drought stress (200 g/l of PEG) on shoot dry weight of *Lolium temulentum* Linn. seedlings following 2,4-D (100 ppm) application to the roots in nutrient solution.

Figure 2.39. Effect of drought stress (200 g/l of PEG) on root dry weight of *Lolium temulentum* Linn. seedlings following 2,4-D (100 ppm) application to the roots in nutrient solution.

Figure 2.40. Effect of drought stress (200 g/l of PEG) on total dry weight of *Lolium temulentum* Linn. seedlings following 2,4-D (100 ppm) application to the roots in nutrient solution.

-Vertical bars represent standard error for the mean.

Fig. 2.40

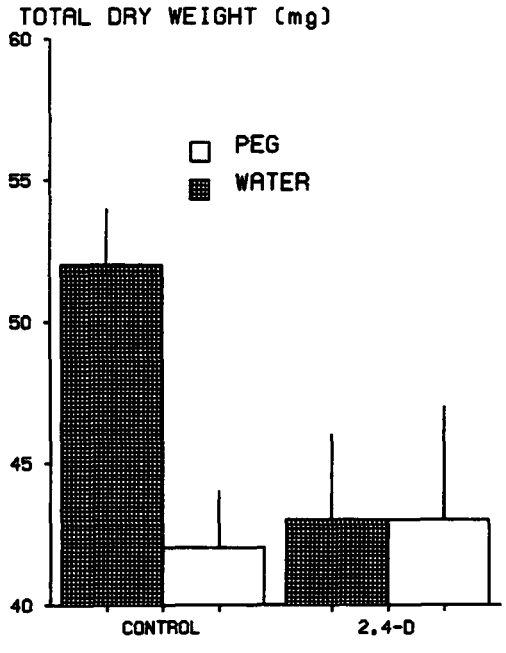


Fig. 2.39

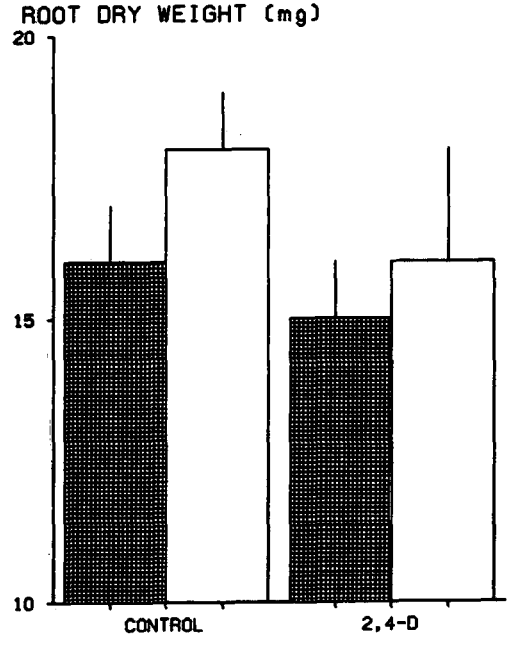
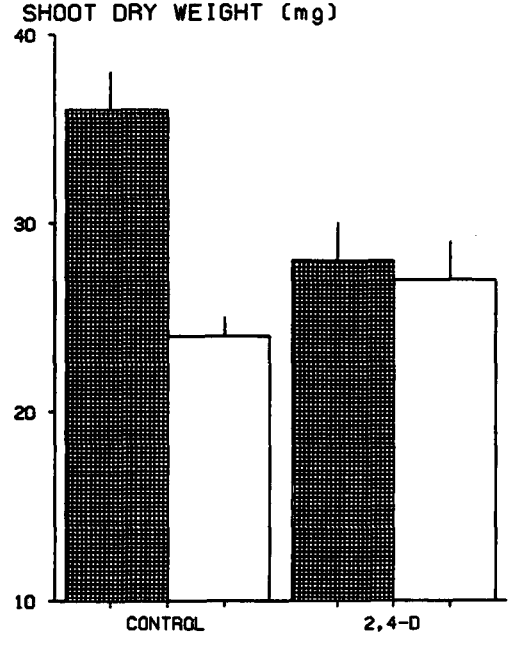


Fig. 2.38



treatments (Fig. 2.42).

Treated seedlings were allowed to grow on for several weeks after which no sign of morphological disorders were observed on 2,4-D-treated seedlings under both drought stress and watering regime. By visual inspection, however, seedlings growing under drought stress were growing slower than controls or 2,4-D treated seedlings. They were stunted, and had fewer leaves. When the plants reached the heading stage distortions began to appear; in all treated plants incomplete heading was very common. The infloresence remained partly or completely enclosed in the leaf, because the collar of the sheath was so constricted that only part of the infloresence emerged (Plate 2.7) As a result the production of bunchhead and bent infloresences was common (Plate 2.8). As a result distortion of infloresences was marked (Plate 2.9).

As a result of increased tillering in plants growing under drought stress (2,4-D-treated and untreated), numbers of inflorescences per plant were increased significantly. There were no significant differences found between 2,4-D-treated and control seedlings in the number of inflorescences per plant (Fig. 2.43). In contrast the number of florets per inflorescence was significantly higher in plants under watering regime (treated and untreated), and there were no significant differences found between treated and untreated plants growing under drought stress or under watering regime (Fig. 2.44). Numbers of caryopses per floret and per inflorescence were reduced significantly however, for all treatments in comparison to the controls. There were also significant differences in the number of caryopses between different treatments, with the highest reduction being caused by combined 2,4-D and drought stress, and the lowest reduction resulted from 2,4-D treatment (fig. 2.45 and 2.46). At the same time there were no significant differences in the weight of caryopses between treated and untreated plants as indicated by the weight of 100 caryopses (Fig 2.47).

Figure 2.41. Effect of drought stress on shoot fresh weight of *Lolium temulentum* Linn. seedlings following foliar treatment with 100 ppm of 2,4-D.

Figure 2.42. Effect of drought stress on shoot dry weight of *Lolium temulentum* Linn. seedlings following foliar treatment with 100 ppm of 2,4-D.

-Vertical bars represent standard error for the mean.

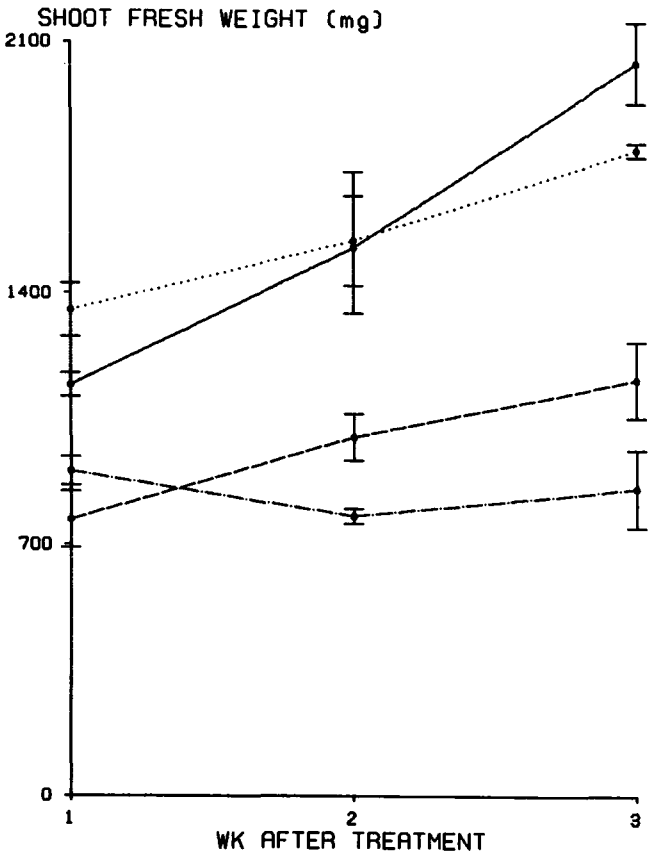


Fig. 2.41

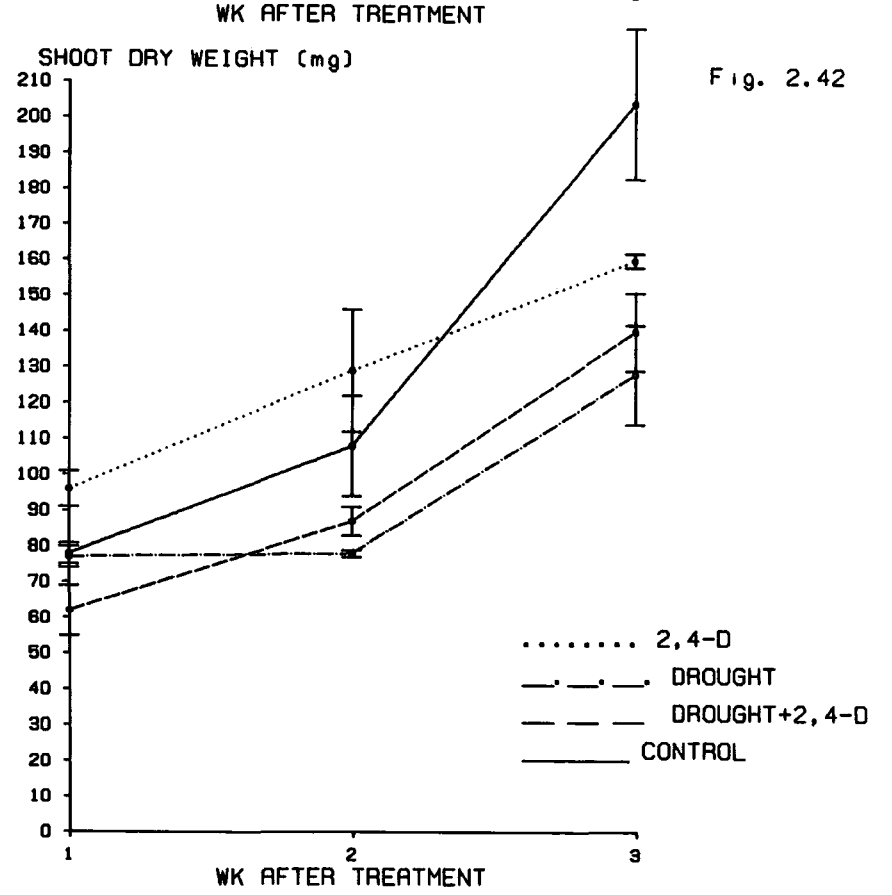


Fig. 2.42

Effect of foliar applied 2,4-D (100 ppm) on the growth and development of inflorescences of *Lolium temulentum* Linn. plants treated at the seedling stage.

Plate 2.7. Treated seedlings.

Plate 2.8. Treated seedlings (Inflorescences).

Figure 2.9. Control seedlings (Inflorescences).

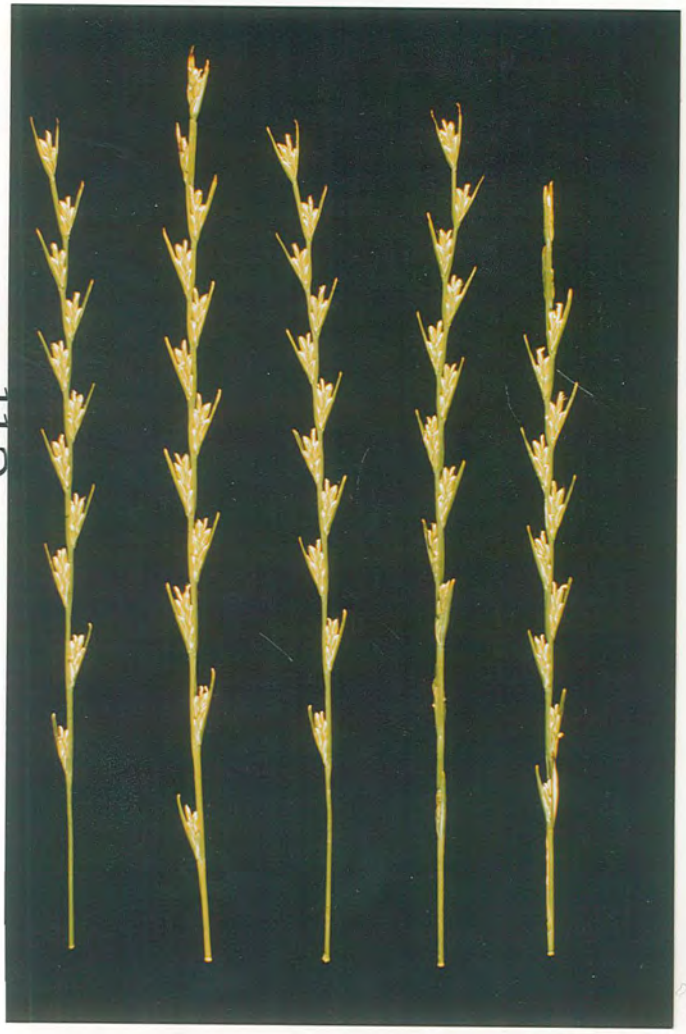


Figure 2.43. Effect of drought stress on number of inflorescences per plant in *Lolium temulentum* Linn. following foliar treatment with 100 ppm of 2,4-D.

Figure 2.44. Effect of drought stress on number of florets per inflorescence in *Lolium temulentum* Linn. following foliar treatment with 100 ppm of 2,4-D.

Figure 2.45. Effect of drought stress on number of caryopses per floret in *Lolium temulentum* Linn. following foliar treatment with 100 ppm of 2,4-D.

Figure 2.46. Effect of drought stress on number of caryopses per inflorescence in *Lolium temulentum* Linn. following foliar treatment with 100 ppm of 2,4-D.

Figure 2.47. Effect of drought stress on 100 caryopses weight in *Lolium temulentum* Linn. following foliar treatment with 100 ppm of 2,4-D.

-Vertical bars represent standard error for the mean.

Fig. 2.47

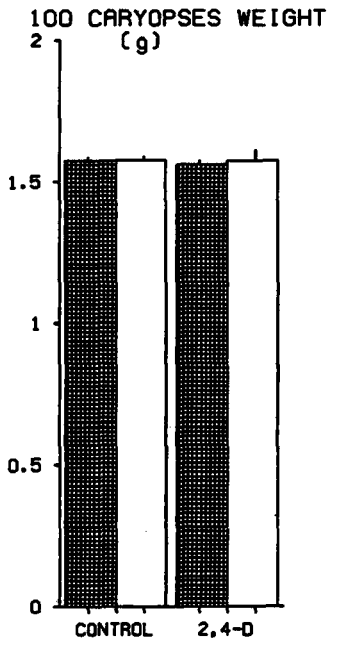


Fig. 2.46

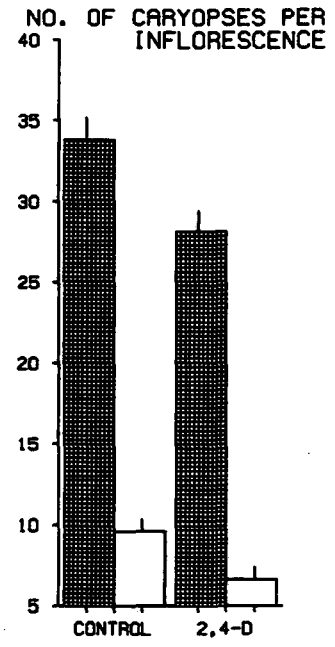


Fig. 2.45

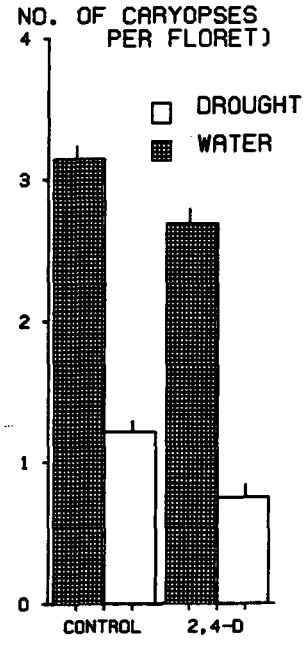


Fig. 2.44

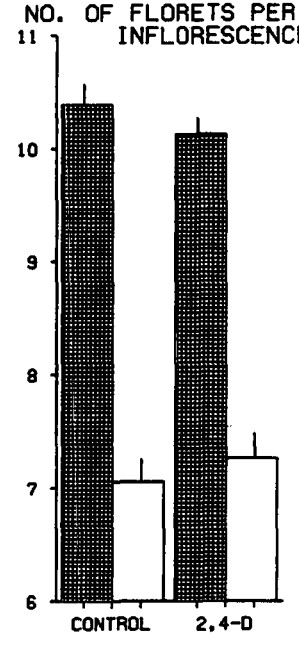
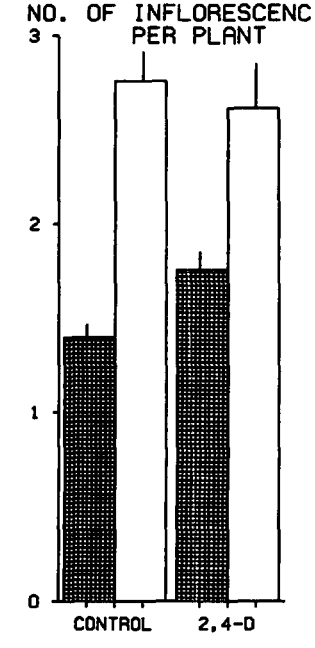


Fig. 2.43



2.3.12. Response of *Zea mays* Linn. seedlings to drought stress following 2,4-D application to the roots in nutrient solution.

Exposure of maize seedlings to drought stress by PEG (-10 bars), following 2,4-D (100 ppm) application to the roots had marked effects on the growth and development of shoots and roots of seedlings. One day after drought stress treatment (two days after 2,4-D treatment), seedlings growing in nutrient solution which contained 2,4-D+PEG and seedlings growing in nutrient solution which contained PEG, began to wilt and their leaf tips began to dry out, whilst seedlings growing in nutrient solution which contained 2,4-D only and control seedlings appeared normal. Three days later premature death of leaf tissues continued from leaf tips toward the base in seedlings under drought stress (2,4-D-treated and untreated seedlings). Some seedlings began to die after this period, but visual inspection showed that 2,4-D-treated seedlings growing under drought stress appeared more affected than those growing under drought stress alone. Expansion of the third leaf in these seedlings was also completely inhibited. At the same time seedlings growing in nutrient solution which contained 2,4-D and control seedlings grew normally and their third leaf began to expand. By the fifth day the leaves of seedlings growing in nutrient solution which contained 2,4-D began to show some signs of wilt and the tips of the leaves began to change to a yellow colour. This was unlike seedlings growing under drought stress where the leaf tips changed to dark green and then dried out indicating premature death of leaves.

Marked inhibition of root growth was caused by 100 ppm of 2,4-D in nutrient solution, root tips were swollen, and there was a general lack of development of stimulation of adventitious roots. At the end of the test period, normal development of roots had been almost completely inhibited by 2,4-D in seedlings growing under drought stress and seedlings under normal watering regime. The symptoms which developed on roots of maize seedlings were similar to those developed on roots of rye

grass seedlings (Plate 2.10)

As a result of the growth inhibition of the shoots, reduction in shoot fresh weight was significantly higher in all treatments compared to untreated seedlings. On the other hand shoot fresh weight was significantly less for seedlings growing in nutrient solution which contained 2,4-D+PEG compared to seedlings growing in nutrient solution which contained 2,4-D only, shoot fresh weight of which was significantly higher than seedlings growing in nutrient solution which contained PEG. Whilst there were no significant difference in shoot fresh weight between seedlings growing in nutrient solution which contained PEG and those growing in 2,4-D+PEG (Fig. 2.48). Although the same patterns were found for shoot dry weight, the reduction in shoot dry weight was significant only for seedlings growing under drought stress (2,4-D- treated and untreated) in respect to the controls (Fig. 2.52). Root dry weight data, however, indicated that treatment with PEG or combined 2,4-D+PEG had no effect on root dry weight, but 2,4-D reduced root dry weight significantly in respect to the controls and PEG treated seedlings (Fig. 2.53).

2.3.13. Response of *Zea mays* Linn. seedlings to 2,4-D and drought stress.

To investigate the effects of combined 2,4-D and PEG on early development of maize seedlings, they were treated at an early stage (immediately after germination and before leaf expansion), by immersion of roots in nutrient solutions which contained 2,4-D at 100 ppm or combined 2,4-D+PEG at mild water potential of -5 bars and allowed to grow in the solutions. The seedling growth data after one week indicated that PEG at the concentration used had no effect on the expansion of leaves compared to the controls. Whilst 2,4-D and combined 2,4-D+PEG were significantly reduced the number of leaves in respect to the controls. There was no significant difference in number of leaves between 2,4-D and 2,4-D+PEG treated seedlings (Fig.

Plate 2.10 Effect of root applied 2,4-D (100 ppm) and drought stress (200 g/l of PEG) on the growth and development of *Zea mays* Linn. seedlings.


2,4-D+PEG
2,4-D
CONTROL

Figure 2.48. Effect of drought stress (200 g/l of PEG) on shoot fresh weight of *Zea mays* Linn. seedlings following 2,4-D (100 ppm) application to the roots in nutrient solution.

Figure 2.49. Effect of drought stress (200 g/l of PEG) on root fresh weight of *Zea mays* Linn. seedlings following 2,4-D (100 ppm) application to the roots in nutrient solution.

Figure 2.50. Effect of drought stress (200 g/l of PEG) on total fresh weight of *Zea mays* Linn. seedlings following 2,4-D (100 ppm) application to the roots in nutrient solution.

Figure 2.51. Effect of drought stress (200 g/l of PEG) on root to shoot ratio of *Zea mays* Linn. seedlings following 2,4-D (100 ppm) application to the roots in nutrient solution.

-Vertical bars represent standard error for the mean.

Fig. 2.48

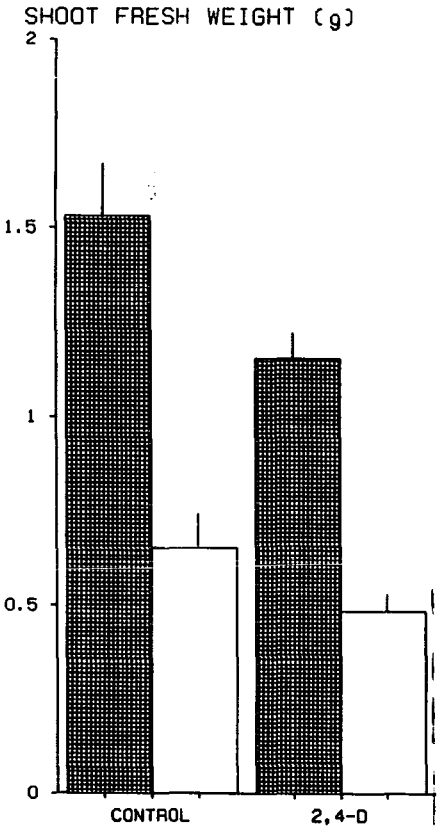


Fig. 2.49

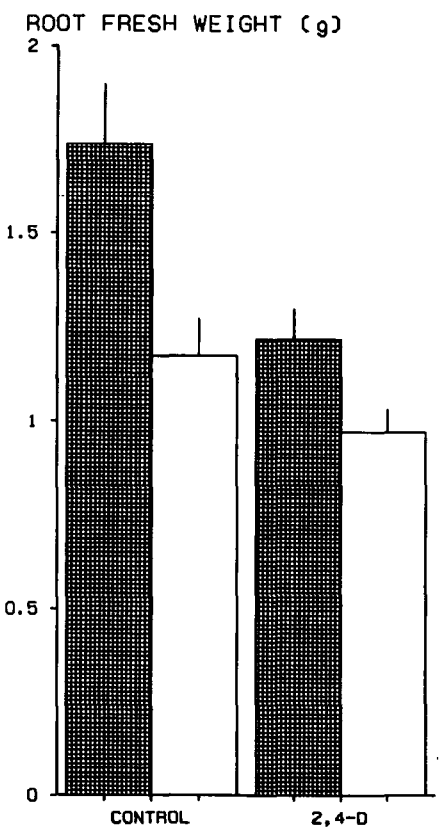


Fig. 2.50

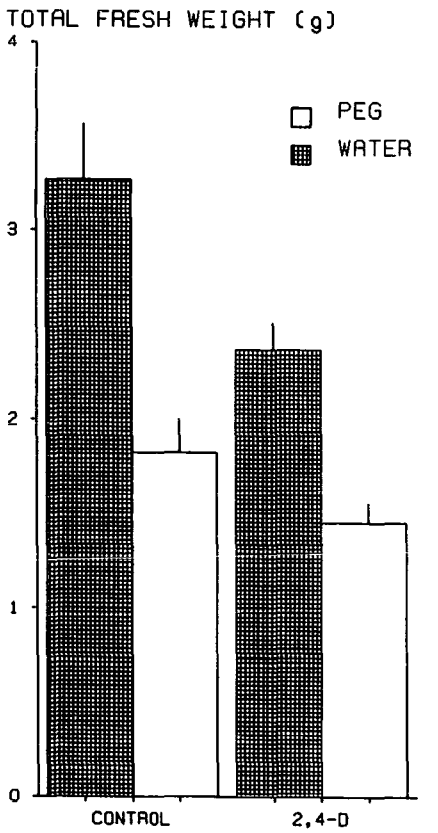


Fig. 2.51

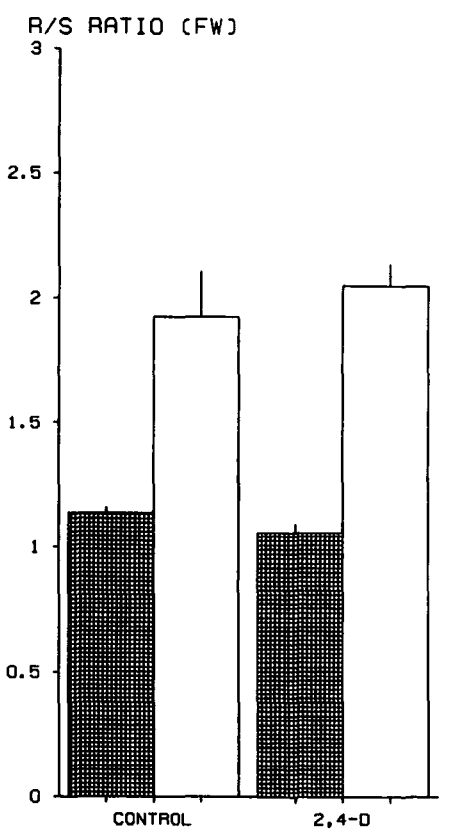


Figure 2.52. Effect of drought stress (200 g/l of PEG) on shoot dry weight of *Zea mays* Linn. seedlings following 2,4-D (100 ppm) application to the roots in nutrient solution.

Figure 2.53. Effect of drought stress (200 g/l of PEG) on root dry weight of *Zea mays* Linn. seedlings following 2,4-D (100 ppm) application to the roots in nutrient solution.

Figure 2.54. Effect of drought stress (200 g/l of PEG) on total dry weight of *Zea mays* Linn. seedlings following 2,4-D (100 ppm) application to the roots in nutrient solution.

Figure 2.55. Effect of drought stress (200 g/l of PEG) on root to shoot ratio of *Zea mays* Linn. seedlings following 2,4-D (100 ppm) application to the roots in nutrient solution.

-Vertical bars represent standard error for the mean.

Fig. 2.52

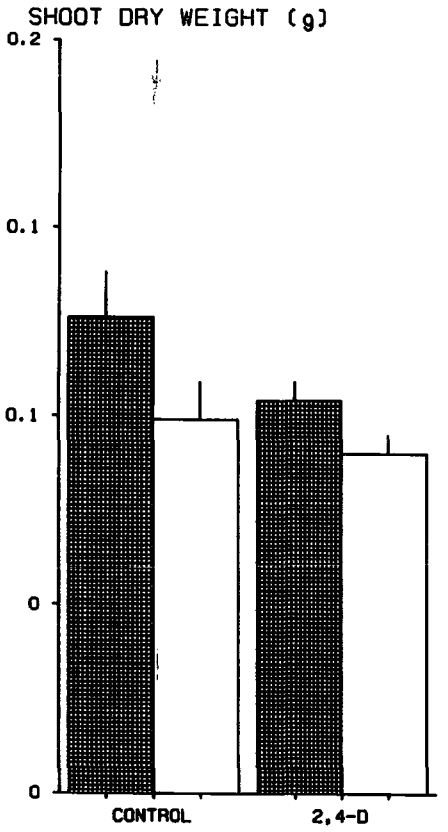


Fig. 2.53

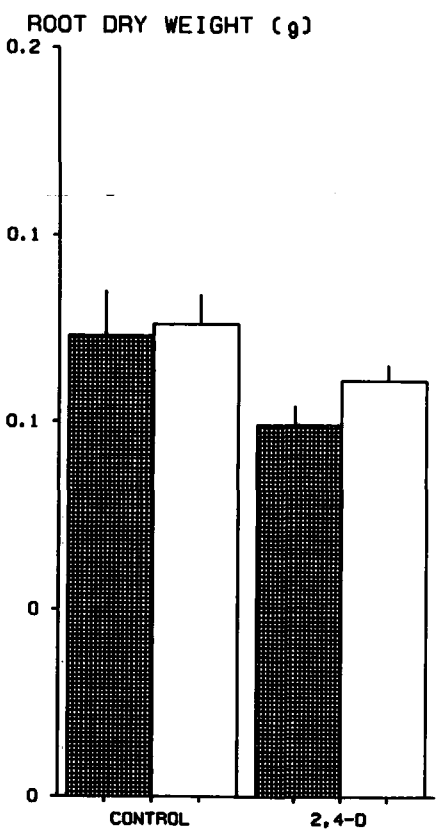


Fig. 2.54

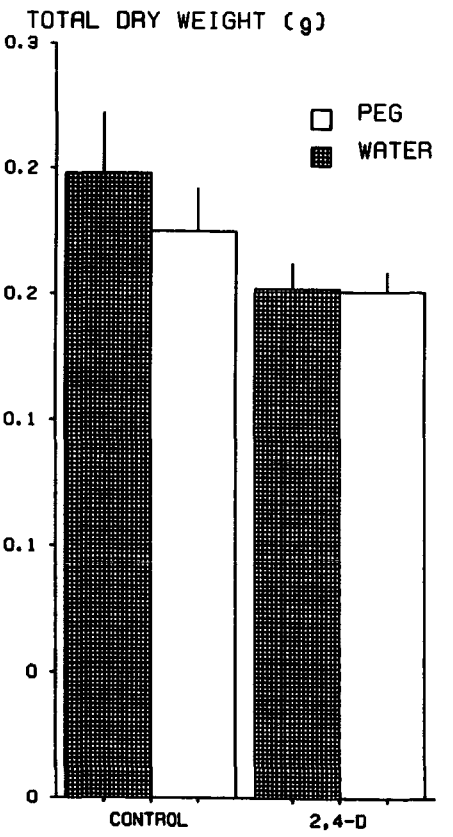


Fig. 2.55

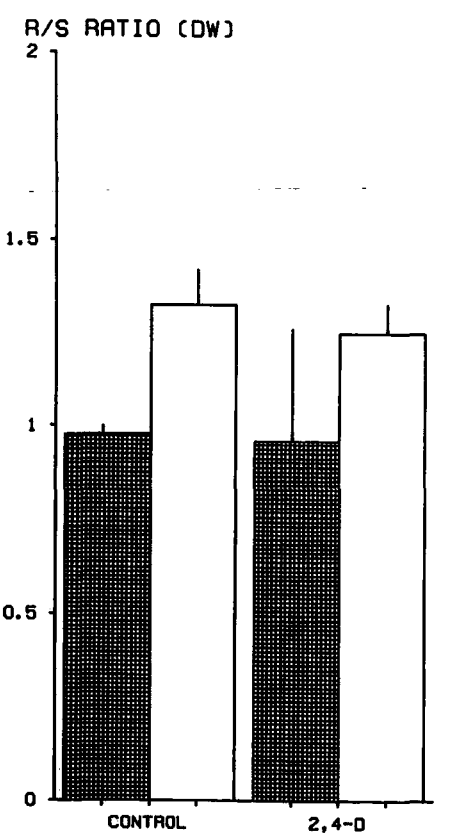


Figure 2.56. Effect of 2,4-D (100 ppm) and drought stress (125 g/l of PEG) on the number of leaves in *Zea may* Linn. seedlings treated through the roots in nutrient solution.

Figure 2.57. Effect of 2,4-D (100 ppm) and drought stress (125 g/l of PEG) on total leaf area in *Zea mays* Linn. seedlings treated through the roots in nutrient solution.

Figure 2.58. Effect of 2,4-D (100 ppm) and drought stress (125 g/l of PEG) on the elongation of second internode in *Zea mays* Linn. seedlings treated through the roots in nutrient solution.

Figure 2.59. Effect of 2,4-D (100 ppm) an drought stress (125 g/l of PEG) on the elongation of primary root in *Zea mays* Linn. seedlings treated through the roots in nutrient solution.

Figure 2.60. Effects of 2,4-D (100 ppm) and drought stress (125 g/l of PEG) on the elongation of mesocotyl in *Zea mays* Linn. seedlings treated through the roots in nutrient solution.

-Vertical bars represent standard error for the mean.

Fig. 2.60

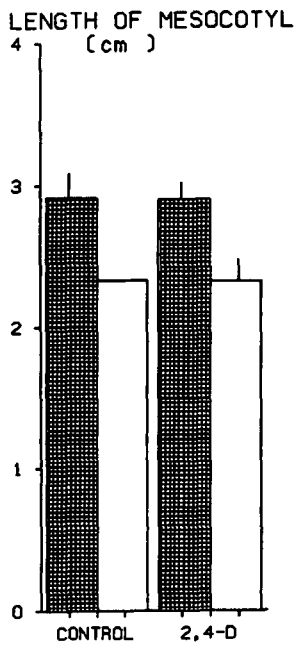


Fig. 2.59

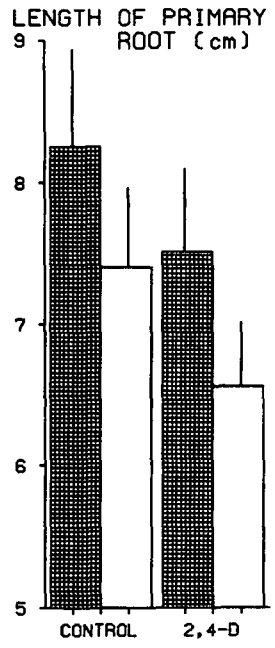


Fig. 2.58

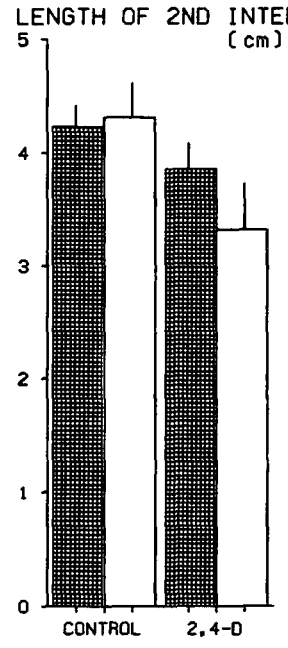


Fig. 2.57

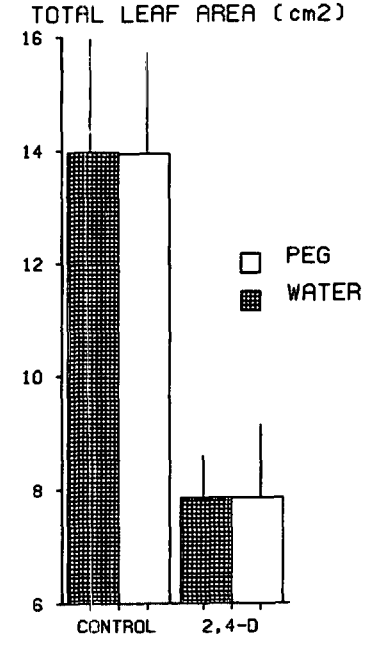
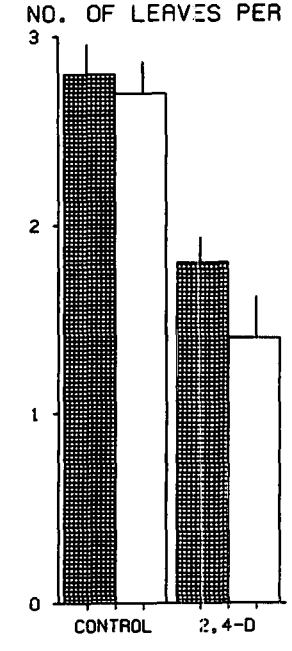


Fig. 2.56



2.56).

As a result of this reduction in number of leaves, total leaf area was significantly decreased by 2,4-D (Fig. 2.57). Meanwhile combined 2,4-D+PEG reduced the length of second internode significantly in respect to the controls and PEG treated seedlings, and there were no significant differences in the length of second internode between 2,4-D or PEG treated seedlings and the controls (fig. 2.58). On the other hand significant reduction in the length of primary root was caused by 2,4-D+PEG only in respect to the controls (Fig. 2.59). At the same time a significant reduction in the length of mesocotyl was caused by 2,4-D+PEG and PEG compared to 2,4-D and the controls (Fig. 2.60)

2.4. Discussion

2.4.1. Effects of 2,4-D and drought stress on seed germination.

At concentrations of 10-100 ppm 2,4-D maintained in direct contact with seeds of radish for periods of time up to 12 days, reduced the final percentages of germination at high concentration (more than 10 ppm) and increased the time needed to complete germination. In this respect the data represented here are in accord with that of Mitchell & Marth, 1945; Allard *et al.*, 1946; Audus & Quastel 1947; Sasaki *et al.*, 1968 , who noted that germination of many species was delayed at comparatively low concentrations and checked at high concentration by 2,4-D. In contrast treatment of radish seeds by soaking in solutions of 2,4-D at concentrations 1, 10, and 100 ppm for 24 hours (in Petri dishes) allowed normal germination without any delay in respect to the controls. Meanwhile when seeds were soaked in the same solutions for similar periods of time (24 hours), and planted in compost, the emergence of seedlings was delayed but there was no effect on the final percentages of seedling emergence for seeds treated at 1 and 10 ppm. However, emergence of seedlings from seeds treated at 100 ppm was dramatically reduced. When the seeds were investigated it was found that the inhibition of seedling emergence was not because of inhibition of germination, but because of the severe inhibition of roots which prevented the seedlings from penetrating the soil. Therefore, treating seeds for a period of time had no effect directly on seed germination, even though the germination was delayed and emergence of seedlings was also delayed or inhibited at high concentration. Treating soil at high concentrations (100 ppm), however, had no effect on seed germination or seedling emergence. This result is in great contrast with that found by Hamner *et al.*, 1946 who reported that soil previously treated with 2,4-D affected the germination of many seeds, at concentrations of the acid as low as 1 ppm.

When radish seeds were germinated in nutrient solutions containing 2,4-D at concentrations of 1, 50 and 100 ppm with or without PEG (-10 bars) as drought stress agent, the response of seeds was completely changed. 2,4-D, 2,4-D+PEG, PEG and control seeds germinated normally and in a very short period of time compared to seeds treated with solutions of 2,4-D without nutrient elements. It seemed that nutrient solutions somehow played an important role in increasing the final percentages of germination and reducing time needed for seeds to complete their germination therefore it appear that nutrient supply overrides the effect of 2,4-D to some extent. At the same time PEG showed no effect on germination of radish seeds at the concentrations used. When seeds were treated with 2,4-D at concentrations higher than 100 ppm (125, 250, 500, and 1000 ppm) with or without PEG, no effect was noted for these concentrations without PEG on germination, however, appreciable decreases in the final percentage of germination were caused by these concentrations with PEG. The decrease was closely related to the concentration of the herbicide. Since no reduction in the final percentages of germination were resulted because of 2,4-D or PEG alone, synergistic action of these two compounds was noted.

Unlike radish seeds, rye grass caryopses treated with different concentrations of 2,4-D (10-100 ppm), showed no altered germination with all concentrations used. Moreover, caryopses treated with 100 ppm of the herbicide in nutrient solution with or without PEG responded similarly indicating that neither 2,4-D nor PEG had any effect on the rate of caryopsis germination at the concentrations used.

In the light of these findings, what can be said here is that 2,4-D has very little effect on germination of seeds, despite the numerous reports about the inhibitory effect of it to the process (e.g. Mayer et al, 1975; Parker, 1976; Hamner et al, 1946). However 2,4-D may delay the process of germination or inhibit seedling emergence as a result of its deleterious effects on root expansion. Addition of PEG at a concentration of 200 g/l to the herbicide solution failed to modify the effects of 2,4-

D on caryopses of rye grass and to certain extend seeds of radish. Furthermore, PEG alone has no effect on germination of radish seeds or rye grass caryopses despite reports by many investigators about the effect of PEG as a drought stress inducer on seed germination, indicating delay of germination (Bewley & Black, 1978), slowing down the rate at which seeds complete germination (Etherington & Armstrong, 1975) or reducing total germination (Bonner, 1968). It could, however, be argued that the amount of PEG used was not enough to exert an effective water deficit under the environmental conditions given in the experiment.

2.4.2. Effects of treating seeds with 2,4-D and drought stress on subsequent development of seedlings.

In all seeds treated with 2,4-D at different concentrations and for different types of treatments, the growth and development of seedlings was reduced immediately after germination in both radish and rye grass species. The degree of inhibition was closely related to the concentration of the herbicide and to the sensitivity of the species. However, when the herbicide was applied directly to the seeds its selectivity decreased dramatically, and both monocotyledons and dicotyledons become sensitive.

In germinated radish seeds 2,4-D inhibited the expansion of the radicle and cotyledons and completely or partially inhibited the elongation of primary roots in both seeds treated with 2,4-D or 2,4-D+PEG. Addition of PEG to 2,4-D appeared to reduce the effect of the herbicide on the root and increase its effect on the hypocotyl.

Similar responses were found in germinated caryopses of rye grass. Inhibition of roots expansion and elongation of coleoptiles was evident at all concentrations used with or without PEG. In relation to root and shoot development, however, rye grass roots were more sensitive to 2,4-D alone than to the combined 2,4-D+PEG, whilst the shoots were more sensitive to PEG treatment.

The data obtained regarding the development of seedlings immediately after germination in 2,4-D treated seeds is in agreement with the data of Hamner *et al.* (1946), where they treated seeds of sudan grass, pea and bean by soaking them in solutions of the herbicide for 4 hours. Here they noted that 10 and 100 ppm of the herbicide inhibited the growth of roots in sudan grass and totally checked the growth and development of roots and shoots of bean and pea. The data are also in accord with that reported by Sasaki *et al.* (1968), who found that 2,4-D inhibited the elongation of the radicle and expansion of the cotyledons of red pine shortly after germination as a result of seed treatment. Thompson *et al.* (1946) have shown that the elongation of the radicle and coleoptile of corn were inhibited by 2,4-D, although the elongation of radicle was more sensitive than shoot growth. On the other hand, the increased effect of combined 2,4-D and PEG on shoots of both species can be attributed to PEG, since the same effect was noted on seeds treated with PEG without 2,4-D. Growth of cells are highly sensitive to water deficit and cell expansion very much depends on the water status of the tissue which acts as turgor pressure upon the softened cell walls. Therefore, even under mild stress when turgor pressure is reduced by only few bars there is a significant decrease in growth (Hsiao, 1973).

2.4.3. Response of seedlings to drought stress following 2,4-D application to the roots in nutrient solution.

Application of 2,4-D to the root system of radish, rye grass and maize seedlings growing in nutrient solutions resulted in marked reduction or inhibition of growth and caused distinct morphological distortions in these seedlings. Rye grass and maize were more resistant to the herbicide than radish, however.

Sudden exposure of seedlings to drought stress following herbicide treatment, also had very devastating effects on the growth and development of these

seedlings. All seedlings were shown to be more sensitive to drought stress than to the herbicide and their response to drought stress was also much more faster than to the herbicide. The sensitivity of treated seedlings to drought stress, however, appeared to have no connection with pre-drought stress treatment with 2,4-D i.e. 2,4-D did not increase the seedling sensitivity to drought stress. Although drought stress seemed to accentuate the effects of 2,4-D on the shoots and in particular on the expansion of leaves, at the same time, it appeared to lessen the effects on the roots, especially the inhibition of lateral and adventitious roots.

Bending and twisting of radish seedlings few hours after 2,4-D treatment are very common symptoms and have been reported by many investigators working on dicotyledonous plants (e.g. Beal, 1944; Audus & Quastel, 1947; Audus, 1949; Taylor & Maj, 1946). Exposure of radish seedlings to drought stress 24 hours later put the seedlings under a different type of stress. All seedlings which had been treated with PEG wilted and their shoots began to dry out (2,4-D treated and untreated) apparently because of water deficit. Inhibition of shoot growth was noted for seedlings treated with 2,4-D, PEG and combined 2,4-D+PEG. The inhibition of growth by 2,4-D may be the resulted of a subsequent action of its effects on cell division and cell elongation (Hanson & Slife, 1961). Whilst the effects of PEG resulted because of its inhibition of cell expansion and enlargement (Fitter & Hay, 1983). Therefore the effects of both compounds on plant cells can be considered as complementary to each other. This suggestion is supported by the observation made on seedlings treated with 2,4-D and later exposed to drought stress, which indicated that shoots of these seedlings were the most affected in respect to other treatments. In contrast drought stress was shown to relieve some of the stress put on the roots as a result of 2,4-D treatment. It was clear from the data and visual inspection that PEG increased the inhibition of elongation and minimized the morphological malformation in the roots. This may be because of the reduction in absorption of the herbicide by drought stress.

In relation to the hypocotyl PEG had no effect in modifying the mode of action of 2,4-D. Morphological malformation in the hypocotyl such as swelling, longitudinal splitting and inhibition of thickening were evident in all 2,4-D treated seedlings.

Unlike radish, rye grass seedlings treated with 2,4-D in nutrient solutions developed no uniform or distinct symptoms of twisting or epinasty such as those observed on radish seedlings, during the first 24 hours before drought stress treatment. This kind of response was expected because this plant is resistant to 2,4-D (Taylor & Maj, 1946; Hagin, 1970; Davidonis *et al.*, 1982). When the seedlings were exposed to drought stress, symptoms similar to those observed on radish seedlings developed, wilting of leaves of seedlings treated with PEG and inhibition of their growth were evident particularly in those treated with 2,4-D+PEG. The inhibition was less severe in seedlings treated with PEG or 2,4-D alone. Growth and development of roots was also suppressed by all treatments; 2,4-D, however, caused more damage to the roots than combined 2,4-D and PEG. Lateral and adventitious roots were completely inhibited by 2,4-D, the root crowns were also swollen. The severity of 2,4-D on roots was reduced by PEG in seedlings treated with combined 2,4-D and PEG. The lateral and adventitious roots were expanded and swelling of crowns of roots less visible although the expanded roots were stunted and unhealthy.

Maize seedlings treated with 2,4-D at 100 ppm and exposed to drought stress 24 hours later, were similar to rye grass seedlings in their response. However, when seedlings were treated at an early stage with the same concentration of 2,4-D and lower amount of PEG (125 g/l), drought stress seemed to have no effect on the shoots of seedlings. Seedlings treated with PEG showed normal leaf expansion. In contrast, seedlings treated with 2,4-D or 2,4-D+PEG exhibited reduced leaf number. It is clear from the data that 2,4-D alone is responsible for this reduction not PEG, since PEG was found to have no effect on leaf expansion, and there were no significant differences in the numbers of expanded leaves between 2,4-D and combined 2,4-D and

PEG-treated seedlings. Moreover, reduction in the total leaf area was found only in seedlings treated with 2,4-D. Combined 2,4-D and PEG, however, was shown to have marked effects on elongation of the second internode, primary root and mesocotyl. This is suggested as some interaction between the two compounds, since no effect was seen on these organs by either of the compounds alone except for PEG which reduced the length of mesocotyl.

2.4.4. Response of seedlings to drought stress following foliar application of 2,4-D.

Foliar application of 2,4-D to the seedlings of radish and rye grass resulted in marked inhibition of growth and development accompanied by morphological distortion of plant organs. Rye grass seedlings, however, showed more resistance to the herbicide than radish seedlings. As a result of 2,4-D treatment, response of the seedlings to drought stress was modified and subsequently their growth and development were dramatically changed. The symptoms which resulted from 2,4-D application to radish seedlings were as reported earlier. Morphological bending, twisting and swelling of plant organs and plant tissue are commonly seen during the action of auxin-herbicides (Fedtke, 1982). As a result of treatment many chemical processes are stimulated, the stimulation of which promotes cell proliferation and creation of new cambial promordia. Consequently cell growth and tissue swelling developed (Fedtke, 1982). The final result is plant breakdown at a cellular level in accord with root and hypocotyl swelling. This suggested that 2,4-D stimulated cell division in roots but inhibited the elongation of those roots.

In addition to morphological effects of 2,4-D on radish seedlings, reductions in total fresh and dry weight were significant, both for seedlings growing under drought stress and normal watering regime following the application of 2,4-D. These

results suggested that 2,4-D has an inhibitory effects 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 herbicide places a stress on the treated plants and this stress makes them more susceptible to drought stress (Muzik, 1976). This hypothesis was supported by the data represented in this work, where in radish seedlings treated with 2,4-D at 1, 10 and 100 ppm and exposed to drought stress, the survival rate of these seedlings was reduced significantly in respect to the controls.

The opposite was true for seedlings kept under normal watering regime. The seedlings recovered; particularly the shoots of seedlings treated at low concentrations where twisting and curling of cotyledons and petioles was lost. These result suggested that excess of water following 2,4-D treatment may remove the expression of symptoms and decreases phytotoxicity. Even so, root and hypocotyl 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.

Long term effects of 2,4-D on growth and development of radish seedlings were very severe. The most striking effect of 2,4-D was on the growing point of leaf. Leaf expansion was inhibited and subsequently the number of leaves was reduced. The morphology of leaves of radish treated with 2,4-D changed; some leaves changed from a simple leaf to semi-double leaves on the same petiole and arising from one primorda. 2,4-D-treated seedlings showed many different shapes of leaves. Some leaves were smaller and narrower than normal. This may be because of 2,4-D effect on leaf

expansion (Van Andel *et al.*, 1976). Van Andel stated that "the shape of the leaf is largely determined at early stage of development, and the growth in later phases consists mainly of cell enlargement and differentiation. Compounds inhibiting cell expansion may thus reduce the dimensions of the leaves". Additional to its effect on leaves 2,4-D was shown to effect root and hypocotyl development. Stunting and thickening of roots with longitudinal rupture, inhibition of thickening and proliferation of cells of the hypocotyl were marked in treated seedlings. These types of distortions may be produced as a result of 2,4-D interference with cell elongation and expansion or cell division (Van Andel *et al.*, 1976).

The application of 2,4-D to the foliage of rye grass seedlings appeared to cause no distortion effects on the shoots, under both drought stress and normal watering regime. That was because of the genetic nature of this plant as auxin herbicide resistant. However, in the long-term, and at later stages, 2,4-D proved to be toxic to these resistant seedlings. These effects indicated that the herbicide had in fact penetrated the tissue. The most striking effect was the development of abnormal inflorescences. All treated seedlings growing under normal watering regime or under drought stress developed abnormalities known as "incomplete heading" (Audus, 1959, cf. Van Andel *et al.*, 1976). Moreover reduction of the number of caryopses in 2,4-D-treated seedlings was also noted, this reduction seemed to increase in treated seeds growing under drought stress. The reduction of seeds is reported to be caused by the failure of ovules to develop (Kiermayer, 1956, cf. Van Andel *et al.*, 1976). Reduction in the number of florets was also observed in seedlings growing under drought stress (2,4-D-treated and untreated). In contrast the number of inflorescences was increased in these seedlings perhaps as a result of an increasing volumes of root system to overcome the drought stress which in turn resulted in more tillers and subsequently more inflorescences.

Finally, one may conclude that;

1. Application of 2,4-D to the seeds of monocots and dicots completely inhibited the growth and development of roots in both species at concentration from 1 ppm onward.

2. Root-applied 2,4-D reduced the growth of roots and inhibited completely the growth and development of adventious roots and lateral roots in both species.

3. Although seedlings of monocots and dicots responded differently to the foliar application of 2,4-D roots responded similarly to this herbicide.

4. Toxicity symptoms on monocots, whilst not appearent at early stages may become a problem in long term growth.

5. 2,4-D does influence the response of seedlings to drought stress and application of 2,4-D must be considered in relation to other environmental factors.

6. 2,4-D has effect in root cells by inhibiting growth therefore uptake of nutrients could be impared. It is possible that high levels of nutrient overcome the uptake problem by saturating the root system.

CHAPTER 3

BIOCHEMICAL RESPONSES OF SEEDLINGS TO 2,4-D AND DROUGHT STRESS

3.1. Introduction

The physiological, biochemical and metabolic responses of higher plants to phenoxy herbicides in general, and to 2,4-D in particular, have been extensively studied. Many reviews have been published that contain information on this subject, including Skoog (1951), Wort (1954), Woodford *et al.* (1958), Van Overbeek (1961), Brian (1964), Wort (1964), Penner & Ashton (1966), Robertson & Kirkwood (1970), Loos (1975), Ashton & Bayer (1976), Cherry (1976), Bovey (1980), Ashton & Crafts (1981), Fedtke (1982). All these investigators agreed that when 2,4-D has penetrated the apoplast and comes into contact with the living protoplasm a great variety of biochemical reactions may be altered. The specific reaction altered depend on plant species involved and the concentration of the herbicide (Ashton & Crafts, 1981).

The cellular processes which are likely to be changed by 2,4-D treatments include; protein, and nucleic acid metabolism (Key & Hanson, 1961; Hanson & Slife, 1969; Robertson & Kirkwood, 1970; Nakamura *et al.*, 1986; Sairam *et al.*, 1986; Golebski *et al.*, 1988), respiration and photosynthesis (Robertson & Kirkwood, 1970), starch content (Mangat *et al.*, 1985), chlorophyll content (Wolf, 1977; Nadakavukaren & McCracken, 1977; Sikka & Dubey, 1985; Golebski *et al.*, 1988) and lipid metabolism (Bovey, 1980; Ashton & Crafts, 1981).

The effects of 2,4-D on the nucleic acid and protein metabolism have been the most extensively studied and have been reviewed by Cherry (1976) for the period until 1976. Cherry concluded that treating a sensitive plant with 2,4-D gives an

enhancement of RNA polymerase activity with increased RNA and protein synthesis accompanied by massive cell proliferation in the tissues of certain organs. However, at high concentrations of the herbicide these processes can be inhibited. Since this review many other reviews have been published that contain information on the effect of 2,4-D on the nucleic acid and protein metabolism (e.g. Bovey, 1980; Ashton & Crafts, 1981; Fedtke, 1982). These later reviews also emphasized the same aspects reviewed by Cherry. Recently, Sikka & Dubey (1985) reported that treatment with up to 50 ppm of 2,4-D has no effect on RNase activity but this enzyme was inhibited at higher doses resulting in a many fold increase in RNA content. Sairam *et al.* (1986) treated oat plants with sub-herbicidal levels of 2,4-D reported enhancement of nitrate reductase activity and protein content .

2,4-D has also been reported to effect chlorophyll content of many plant species (Wolf, 1977; Nadakavukaren & McCracken, 1977). Golebski *et al.* (1988), reported significant reduction in chlorophyll content in the leaves of tobacco (*Nicotiana tabacum* cv. Samsum) plants 7 days after 2,4-D treatment at concentration of 1.5 l of commercial product (500 g/l) in 300 l water/ha. Reduction in chlorophyll content in maize leaves has also been reported by Sikka & Dubey (1985) at 2,4-D concentrations from 10 ppm onward. In contrast at sub-herbicidal levels 2,4-D has been shown to increase chlorophyll content in oats (*Avena sativa* Linn.). Manitasevic *et al.* (1984), however, found that 2,4-D at sub-herbicidal levels had no effect on chlorophyll content in the leaves of wheat (*Triticum aestivum*).

As with 2,4-D various metabolic changes are induced in plants subjected to drought stress including inhibition of protein synthesis and changes in amino acid metabolism (Aspinall *et al.*, 1973; Brady *et al.*, 1974; Cooke *et al.*, 1980; De Luca d'Oro & Trippi, 1987), reduction of photosynthesis capacity (Kaiser, 1987; De Luca d'Oro & Trippi, 1987) modification of chlorophyll content (De Luca d'Oro & Trippi, 1987), marked accumulation in free proline (Levy, 1983; Singh, 1973; Wample &

Bewley, 1975; Handa *et al.*, 1986), increase in abscisic acid (Pierce & Raschke, 1980; Henson & Quarrie, 1981; Ilahi & Dorffling 1982; Henson, 1982; Pekic & Quarrie, 1987), and substantial increase in ethylene production (Apelbaum & Fa Yang, 1981).

Although biochemical responses of many plant species to 2,4-D and to drought stress alone have been extensively studied, no data was found on the combined effects of 2,4-D and drought stress. The aim of the investigation reported in this chapter was to examine the combined effects of 2,4-D and drought stress on chlorophyll, protein and proline content in *Raphanus sativus* Linn. and *Lolium temulentum* Linn. seedlings in view of the combined effects reported in Chapter 2. In the course of these investigation attention was concentrated on the effect of 2,4-D on the accumulation of proline in seedlings growing under drought stress condition.

The specific aim of this part of the work was to investigate the relationships between the production of the stress metabolite, proline, and herbicide effects. Since it is known that drought enhances the levels of proline in many plants which resist drought, it was thought possible that the effect of 2,4-D in modifying water stress responses could be because the herbicide was modifying proline accumulation. The accumulation of proline therefore was followed in both the monocot and dicot in relation to drought stress, which was imposed either by withholding water or by PEG treatment, when the plants were co-treated with herbicide.

3.2. Materials and Methods

3.2.1. Plant Materials

Seedlings growth

Seeds of radish and caryopses of rye grass were germinated in trays on filter paper moistened with distilled water in the dark at temperatures between 21-25 °C. Seedlings which were used for studying the effect of 2.4-D and drought stress on the greening (chlorophyll biosynthesis) were treated and transferred to conditions of light and temperature described in 2.2.1. Seedlings which were used for proline treatment were left to grow under similar conditions for 5-days before treatment.

Seedlings treatment

For chlorophyll biosynthesis experiments seedlings were transferred to nutrient solution which contained 2,4-D at 100 ppm or 2,4-D+PEG at 230 g/l (-15 bars) or PEG or nutrient solution only (control).

For proline experiments seedlings were transferred to nutrient solution containing 2,4-D at 100 ppm or 2,4-D+PEG at 230 g/l (-15 bars) or PEG or nutrient solution only (control). Seedlings treated with combined 2,4-D+PEG were treated as follows:

1. one group was treated first with PEG only and 2,4-D was added to the solution at different intervals after PEG treatment (24, 48, 72, 96, 120 and 144 hours).
2. the second group was treated with 2,4-D first and then exposed to drought stress by PEG addition at 24, 48, 72, 96, 120 and 144 hours after 2,4-D treatment.

Seedlings were harvested at different intervals and extracted as appropriate.

3.2.2. Estimation of Chlorophyll

Plant materials (leaves or cotyledons) were ground in mortar and pestle with sand in 80 % acetone. The extracts from each sample were centrifuged at c. 2000×g for 10 min. at room temperature, made to a known volume, and the chlorophyll was determinated by measuring absorbance (A) at 645 and 663 nm in the spectrophotometer. Chlorophyll concentration was determined using the absorption coefficients of used by Arnon (1949) and checked by Bruinsma (1961) as follows:

Chl. a=12.7 A_{663}-2.69 A_{645}.

Chl. b=22.9 A_{645}-4.68 A_{663}.

Chl. a+b=8.02 A_{663}+20.20 A_{645}.

3.2.3. Determination of Proline

Proline content in the plant materials was determined after treatment following the method described by Bates *et al.* (1973).

3.2.3.1. Reagents

Acid ninhydrin was prepared by dissolving 1.25 g of ninhydrin in 30 ml glacial acetic acid and 30 ml 6M phosphoric acid. The mixture was warmed to 70 °C in water bath to ensure that the ninhydrin was completely dissolved. The reagent was kept cool at 4 °C . According to Troll & Lindsley (1955), it remained stable for 24 hours.

3.2.3.2. Procedure

1. 0.2 g of plant material (fresh weight) was homogenized in 25 ml of 3

% aqueous 5-sulphosalicylic acid with purified acid-washed sand to assure thorough grinding, using a pestle and mortar. The homogenate filtered through Whatman # 1 filter paper.

2. 2 ml of the filtrate was added to 0.15 g acid permutit in a test tube and shaken vigorously to remove interfering basic amino acids (Troll & Lindsley, 1955).

3. 2 ml of glacial acetic acid and 2 ml of acid ninhydrin were added to the 2 ml of the filtrate, heated in a water bath at 80 °C for 1 hour, and the reaction terminated in an ice bath. A pink colour was formed when the proline reacted with ninhydrin.

4. 4 ml of toluene was added to 4 ml of the reaction mixture in a separate test tube and shaken for 15-20 sec. The pink layer containing toluene separated out, was allowed to stand and the test tube was centrifuged at c. 2000 × g for 10 min. at room temperature in a bench top centrifuge.

5. The upper toluene layer was removed and its absorbance read at 520 nm in the spectrophotometer using toluene for a blank.

6. The proline concentration was determined from a standard curve prepared using Sigma proline and calculated on a fresh weight basis as follows: [(μg proline/ml × ml toluene)/115.5 μg/μmole]/ [(g sample)/2]=μmoles proline/g of fresh weight material.

3.2.4. Determination of Protein

The content of soluble and insoluble proteins in treated and untreated seedlings was determined under the normal watering regime and under drought stress using the following procedure:

0.2 g of leaf tissue (fresh weight) was ground in a pestle and mortar

using 4 ml buffer [tris (hydroxymethyl) aminomethane, (Tris/HCl) 0.04 M pH 7.5; magnesium sulphate ($MgSO_4$) 0.1 M; and ethylene-diaminetetra-acetic acid disodium salt, (EDTA) 0.025 M].

The resulting suspension was centrifuged at c. 2000 × g for 10 min. at room temperature in a bench top centrifuge in order to sediment insoluble proteins. The resulting pellet contained the insoluble proteins whilst the supernatant contained the soluble proteins.

3.2.4.1. Extraction of Soluble Proteins

2 ml of 10 % aqueous trichloroacetic acid (TCA) were added to 2 ml of the protein supernatant in a test tube and kept on ice for 30 mins. before centrifugation. The supernatant was discarded and the pellet was resuspended in 5 % aqueous TCA in order to wash it.

Following centrifugation the protein pellet was suspended in 1 ml of 1 N sodium hydroxide (NaOH) and dissolved by heating in a water bath to 80 °C.

3.2.4.2. Extraction of Insoluble Proteins

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

3.2.4.3. Measurement of Proteins

Proteins were determined following the method described by Lowry *et al.* (1951).

To both extracted proteins (soluble and insoluble) 5 ml of cupric sulphate reagents were added, this being made up of [0.5 ml 1 % cupric sulphate ($CuSO_4$) + 0.5 ml 2 % sodium/potassium tartrate, and + 50 ml 2 % sodium carbonate (Na_2HCO_3)]. After 10 mins. 0.5 ml of Folin & Ciocalteu's Phenol reagent at 1 N 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 at 520 nm in the spectrophotometer against a blank consisting of 1.0 ml NaOH treated as the sample.

In both cases the spectrophotometer readings were quantified using a plot of the readings obtained from standard protein solution of known concentrations. A standard curve was prepared using bovine serum albumin (BSA).

3.3. Results

3.3.1. Effects of 2,4-D and drought stress on the greening of *Raphanus sativus* Linn. cotyledons.

The chlorophyll content of cotyledons from all treatments increased linearly during the first 48 hours. However, during this period control seedlings synthesized chlorophyll approximately 10, 6 and 4 times faster than 2,4-D, 2,4-D+PEG and PEG-treated seedlings respectively. After 48 hours the total chlorophyll content in seedlings treated 2,4-D+PEG and PEG began to decline. At the end of 96 hours the control seedlings had more chlorophyll than treated seedlings.

24 hours after treatment all treatments showed a significant reduced chlorophyll a, b, a+b and a/b ratio in respect to the controls. The highest reduction in chlorophyll a, b, and a+b was caused by 2,4-D, followed by combined 2,4-D+PEG and the lowest was caused by PEG alone and there were no significant differences between these treatments. The highest reduction in a/b ratio, however, was caused by PEG alone which was significant in comparison to 2,4-D and combined 2,4-D+PEG, followed by 2,4-D+PEG which reduced a/b ratio significantly in respect to 2,4-D. Whilst the lowest reduction was caused by 2,4-D.

48 hours after treatment the chlorophyll content of cotyledons from all treatments continued to increase linearly, however, control seedlings synthesized chlorophyll many time faster than treated seedlings. Significant increases in chlorophyll a, b and a+b in control seedlings over all treatments used were seen with similar patterns of reduction found 24 hours after treatment. Significant reductions in the amount of chlorophyll a, b and a+b were caused by 2,4-D in respect to PEG. Combined 2,4-D+PEG also reduced total amount of chlorophyll in respect to PEG and there were no significant differences between 2,4-D and 2,4-D+PEG or between 2,4-D+PEG and PEG. On the other hand the highest increase in a/b ratio was caused

Effect of 2,4-D (100 ppm) and drought stress (230 g/l of PEG) on chlorophyll biosynthesis in the cotyledons of *Raphanus sativus* Linn. seedlings treated through the roots in nutrient solutions.

Figure 3.1. Chlorophyll a.

Figure 3.2. Chlorophyll b.

Figure 3.3. Chlorophyll a+b.

Figure 3.4. Chlorophyll a/b ratio.

-Vertical bars represent standard error for the mean.

Fig. 3.1

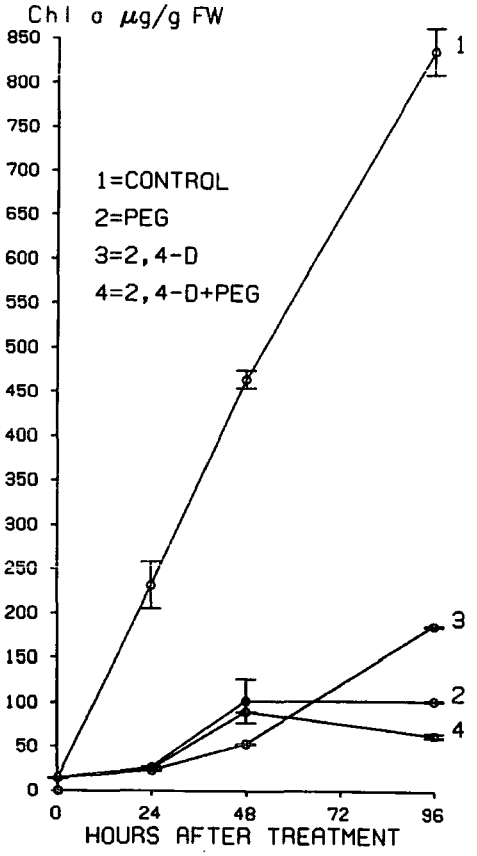


Fig 3.3

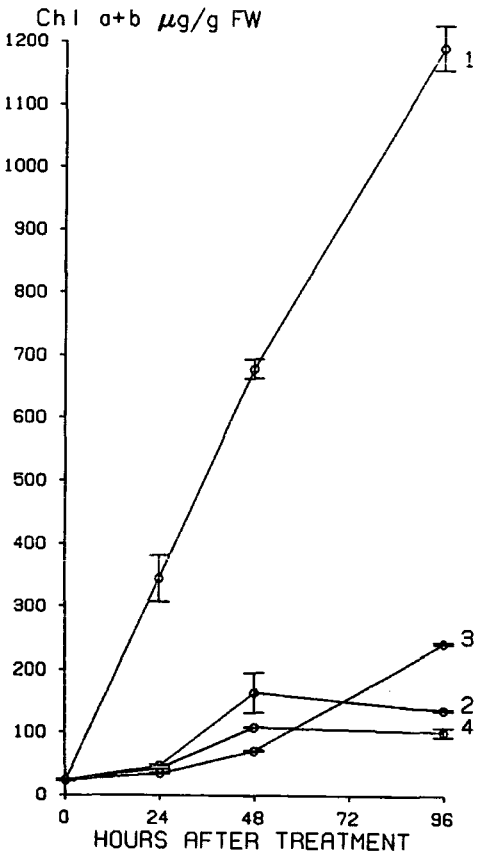


Fig 3.2

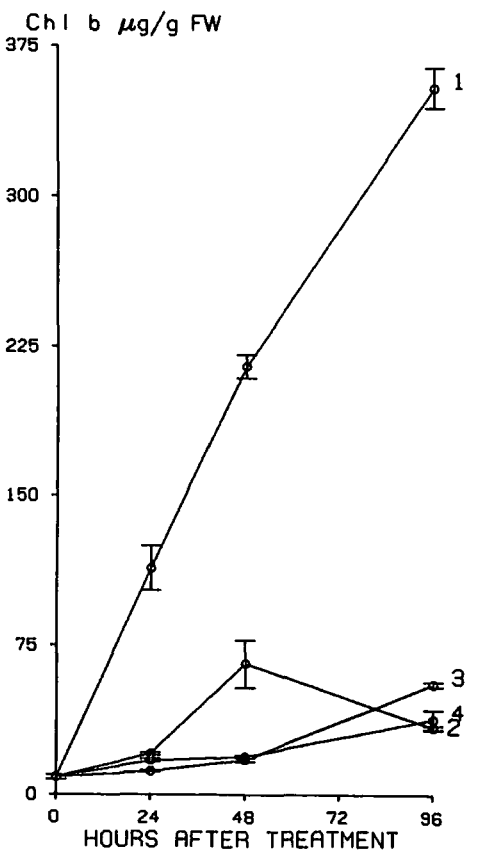
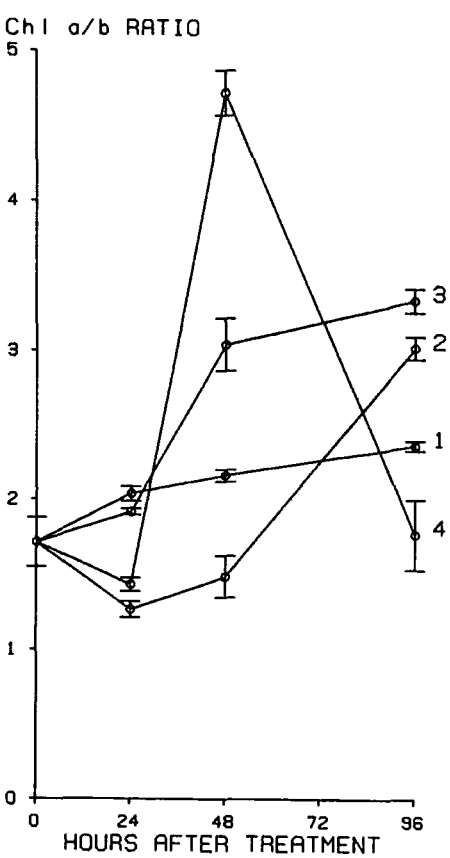


Fig. 3.4



by 2,4-D+PEG which was significant compared with all other treatments including the control, followed by 2,4-D which increased a/b ratio significantly in respect to PEG and control treatments. In contrast the a/b ratio was significantly decreased by PEG compared to all treatments including the control.

96 hours later all treatments showed a significant reduction in chlorophyll content compared to the controls. At this time the highest reduction in chlorophyll a and a+b was caused by 2,4-D+PEG and in chlorophyll b was caused by PEG. Whilst the lowest reduction was caused by 2,4-D. Differences in amount of chlorophyll a, b and a+b between 2,4-D alone and other treatments were significant and there was no significant difference between 2,4-D+PEG and PEG alone. Meantime the a/b ratio was significantly increased by 2,4-D in respect to 2,4-D+PEG and the control and there was no significant difference between 2,4-D and PEG. Whilst 2,4-D+PEG reduced this ratio significantly compared to all treatments. PEG also increased a/b ratio significantly in respect to controls and 2,4-D+PEG (Fig. 3.1, 3.2, 3.3 and 3.4)

3.3.2. Effects of 2,4-D and drought stress on the greening of *Lolium temulentum* Linn. first leaf.

24 hours after treatment significant increase in a, b and total amount of chlorophyll content of first leaf was caused by PEG alone compared to all other treatments including the control. In contrast a significant reduction was caused by 2,4-D in respect to other treatments, and no effect was found on a/b ratio .

48 hours after treatment all treatments showed a significant increase in chlorophyll content over the control. The highest increase was caused by PEG which was significant in respect to all treatments, followed by 2,4-D+PEG which increased chlorophyll content significantly compared to 2,4-D and control. Meanwhile 2,4-D increased the a/b ratio significantly in respect to all other treatments. However,

Effects of 2,4-D (100 ppm) and drought stress (230 g/l of PEG) on chlorophyll biosynthesis in the first leaf of *Lolium temulentum* seedlings treated through the roots in nutrient solution.

Figure 3.5. Chlorophyll a.

Figure 3.6. Chlorophyll b.

Figure 3.7. Chlorophyll a+b.

Figure 3.8. Chlorophyll a/b ratio

-Vertical bars represent standard error for the mean.

Fig. 3.5

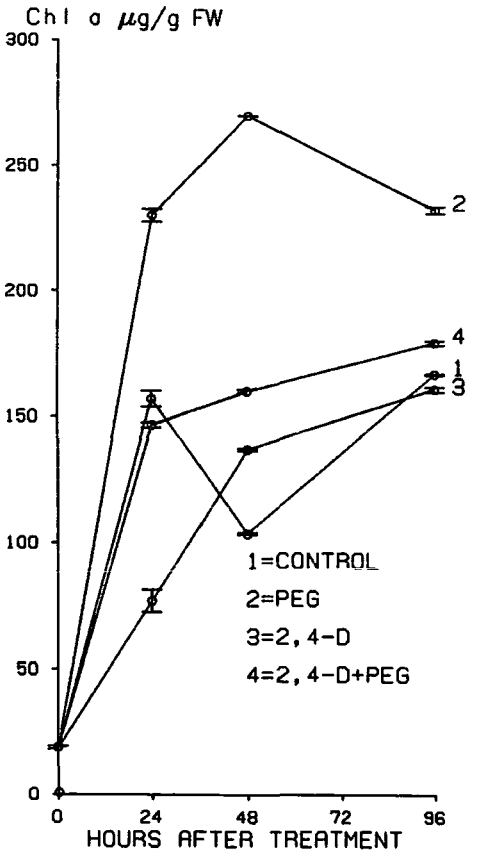


Fig 3.7

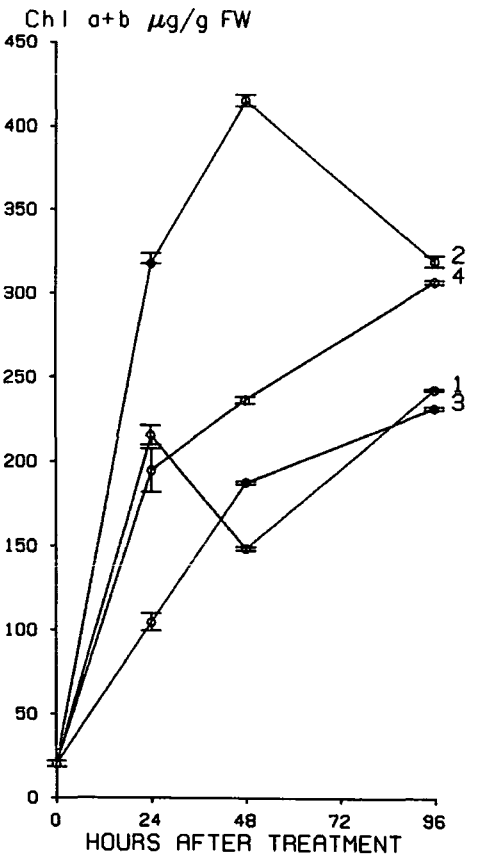


Fig 3.6

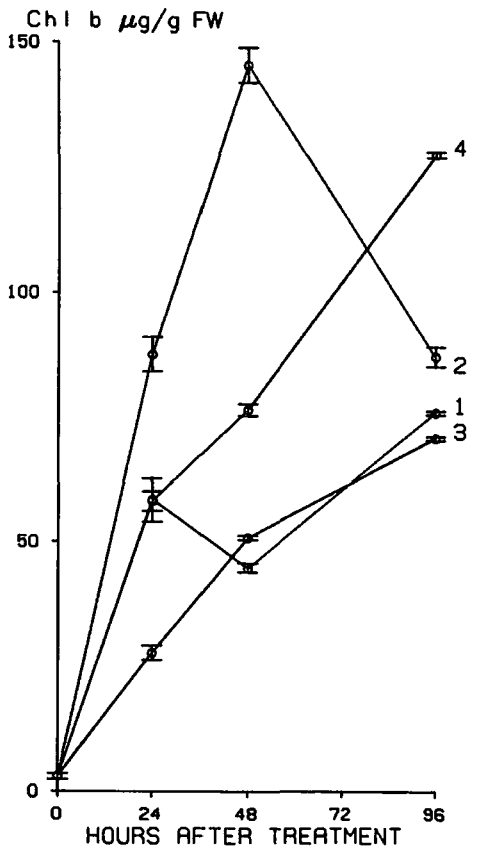
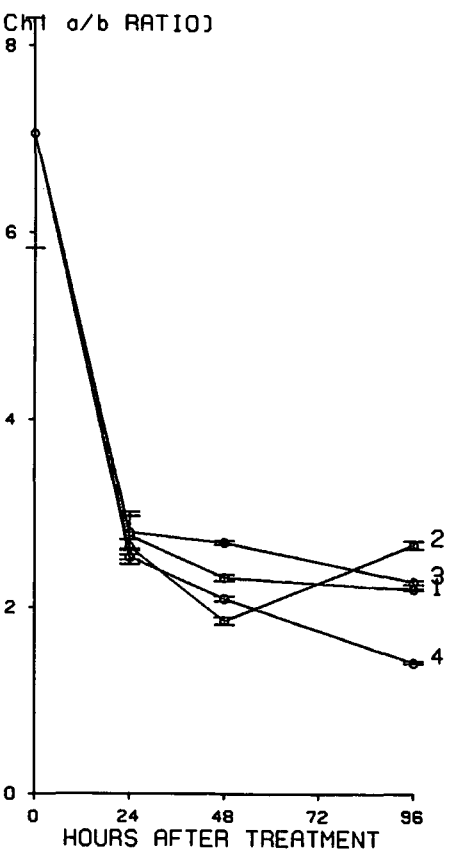


Fig. 3.8



PEG alone reduced it significantly.

96 hours after treatment PEG again caused significant increases in chlorophyll a, a+b and a/b ratio over all treatments including the control. In contrast 2,4-D reduced chlorophyll content significantly compared to all other treatments and there were significant differences between 2,4-D alone and 2,4-D+PEG and between PEG alone and 2,4-D+PEG (Fig. 3.5, 3.6, 3.7 and 3.8).

3.3.3. Effect on chlorophyll content of drought stress following 2,4-D application to the foliage of *Raphanus sativus* Linn. seedlings.

In the experiment described in 2.2.6, three days after treatment 2,4-D (10 ppm) had no effect on chlorophyll content. By the 6th day a significant increase on chlorophyll a, b and a+b was caused by drought stress in respect to 2,4-D and 2,4-D+drought stress. No significant differences, however, were found between drought stress and the controls or between controls and other treatments. Nine days later there were no significant differences between 2,4-D and 2,4-D+drought stress except in chlorophyll a and a/b ratio and there was significant in total chlorophyll in seedlings treated with 2,4-D+drought stress compared to the controls (Fig. 3.9, 3.10, 3.11 and 3.12).

In another treatment (2.2.6) when seedlings were sprayed with three different concentrations of 2,4-D (1, 10 and 100 ppm) all treatments showed significantly reduced total chlorophyll content 2 weeks after treatment in respect to the control except drought stress alone and 2,4-D at 1 ppm+drought stress. The highest reduction over the range of concentrations used was caused by 100 ppm of 2,4-D alone (Fig. 3.13, 3.14, 3.15 and 3.16).

Effects of foliar application of 2,4-D (10 ppm) and drought stress on the chlorophyll content in the leaves of *Raphanus sativus* Linn. seedlings.

Figure 3.9. Chlorophyll a.

Figure 3.10. Chlorophyll b.

Figure 3.11. Chlorophyll a+b.

Figure 3.12. Chlorophyll a/b ratio.

-Vertical bars represent standard error for the mean.

Fig. 3.9

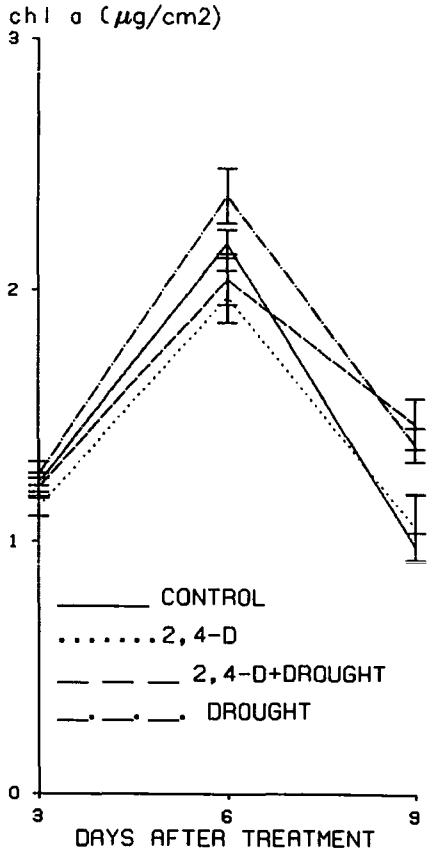


Fig 3.11

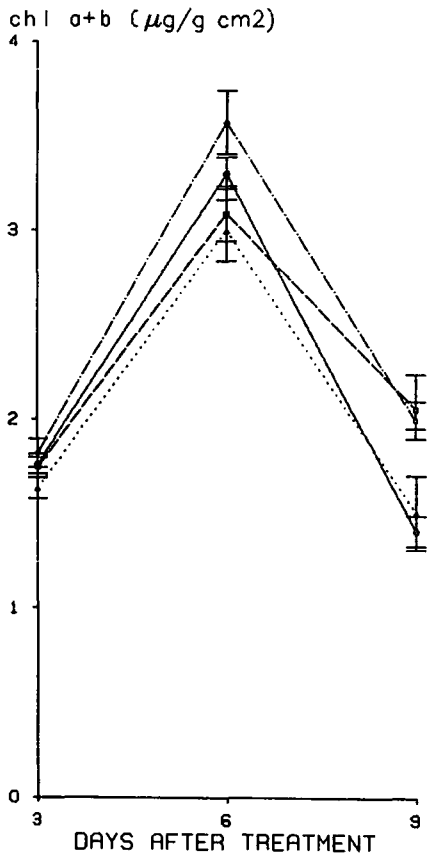


Fig 3.10

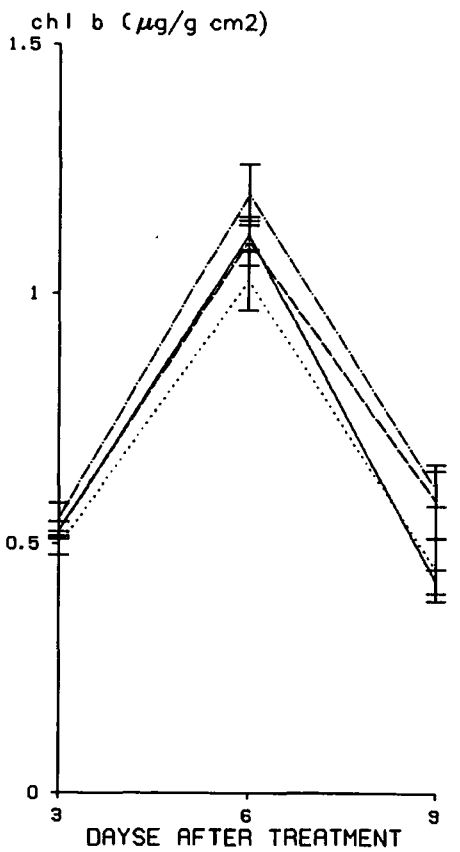
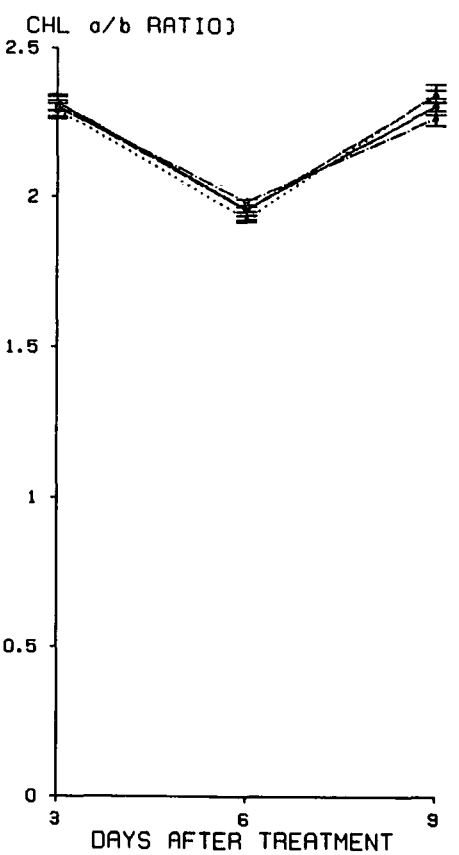


Fig. 3.12



Effects of foliar application of 2,4-D and drought stress on chlorophyll content in the leaves of *Raphanus sativus* Linn. seedlings.

Figure 3.13. Chlorophyll a.

Figure 3.14. Chlorophyll b.

Figure 3.15. Chlorophyll a+b.

Figure 3.16. Chlorophyll a/b ratio.

-Vertical bars represent standard error for the mean.

Fig. 3.13

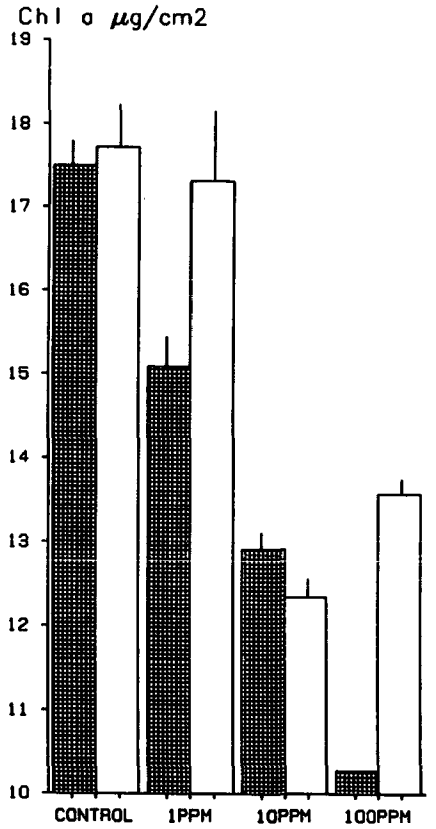


Fig. 3.14

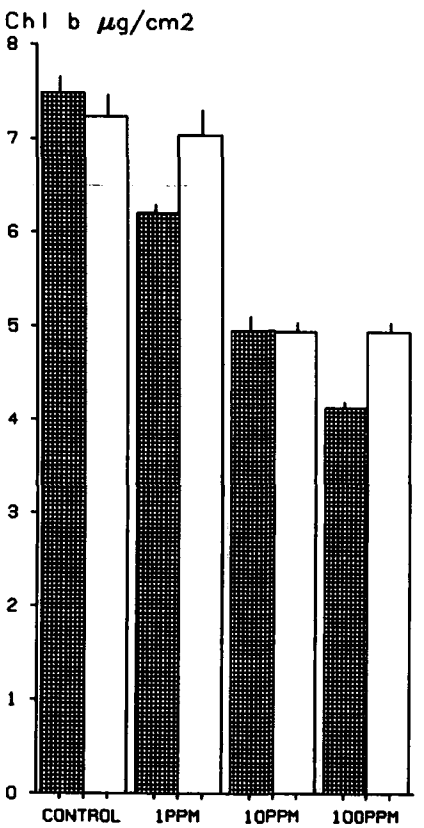


Fig. 3.15

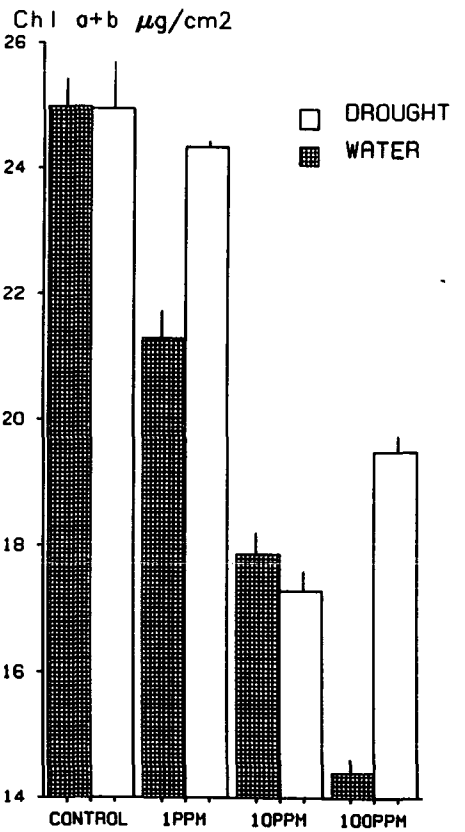


Fig. 3.16

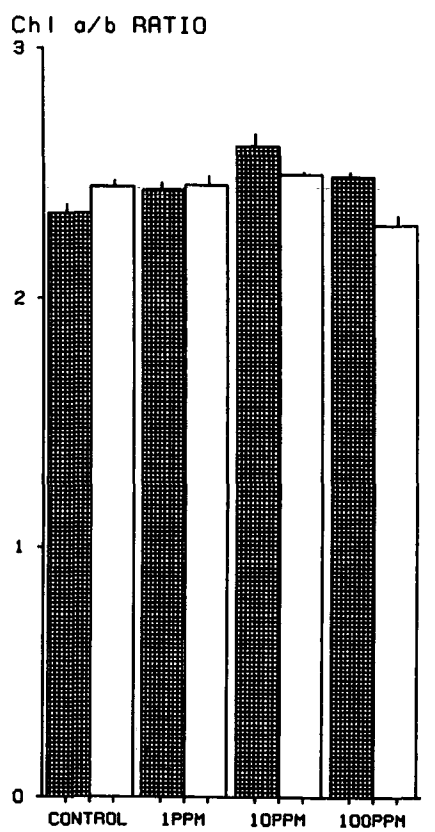
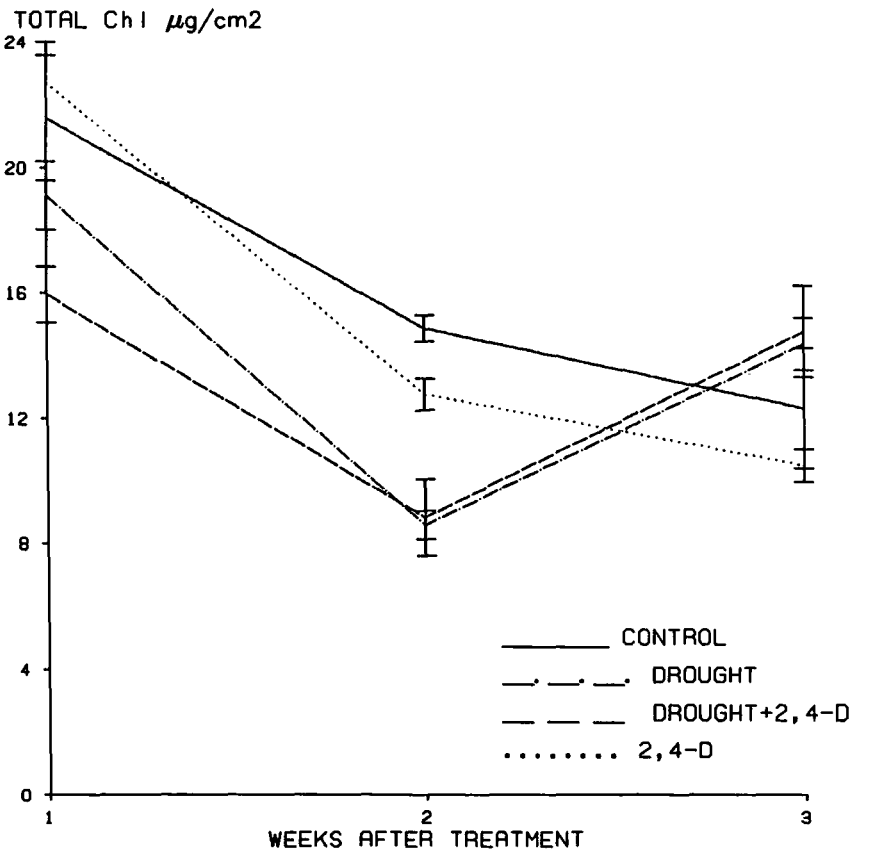


Figure 3.17. Effect of foliar application of 2,4-D (100 ppm) on the total amount of chlorophyll content in the leaves of *Lolium temulentum* Linn. seedlings.

-Vertical bars represent standard error for the mean.

Fig. 3.17


TOTAL Chl μg/cm2
24
20
16
12
8
4
0
1
2
3
WEEKS AFTER TREATMENT
CONTROL
DROUGHT
DROUGHT+2,4-D
2,4-D

3.3.4. Effect on chlorophyll content of drought stress following 2,4-D application to the foliage of *Lolium temulentum* Linn. seedlings.

In the experiment described in 2.2.11, one week after treatment combined 2,4-D+drought stress reduced the amount of total chlorophyll in the leaves of rye grass seedlings significantly in respect to control and 2,4-D alone, and there was no significant difference between drought stress and and 2,4-D+drought stress treatment. After 2 weeks a drop in the amount of chlorophyll content in seedlings growing under drought stress and under drought stress+2,4-D were noted. The drop was significant in respect to the control and to seedlings treated with 2,4-D and kept under watering regime. By the 3rd week the chlorophyll content in these seedlings recovered and showed a significant increase over seedlings treated with 2,4-D and kept under watering regime. Meanwhile there were no differences between seedlings treated with 2,4-D+drought stress and controls or seedlings under drought stress (Fig. 3.17).

3.3.5. Effect of drought stress following 2,4-D application to the foliage of *Raphanus sativus* Linn. seedlings on protein content.

In general, leaves of seedlings treated with drought stress alone, 2,4-D at 1 ppm+drought stress, control and 2,4-D at 1 ppm alone (2.2.6), showed significant increases in the level of soluble proteins in comparison to seedlings treated with 10 or 100 ppm growing under both drought stress and normal watering regime. There were no significant differences in the levels of soluble proteins between seedlings treated with drought, 2,4-D at 1 ppm+drought stress, controls and 2,4-D at 1 ppm alone, and between seedlings treated with 10 or 100 ppm growing under drought stress and seedlings treated 10 or 100 ppm growing under normal watering regime (Fig. 3.18).

Levels of insoluble proteins, however, were reduced significantly by all treatments used in respect to the controls. The highest reduction was caused by 1

Effects of foliar application of 2,4-D and drought stress on the levels of protein content in the leaves of *Raphanus sativus* Linn. seedlings.

Figure 3.18. Soluble protein.

Figure 3.19. Insoluble protein.

Figure 3.20. Total protein.

-Vertical bars represent standard error for the mean.

Fig. 3.20

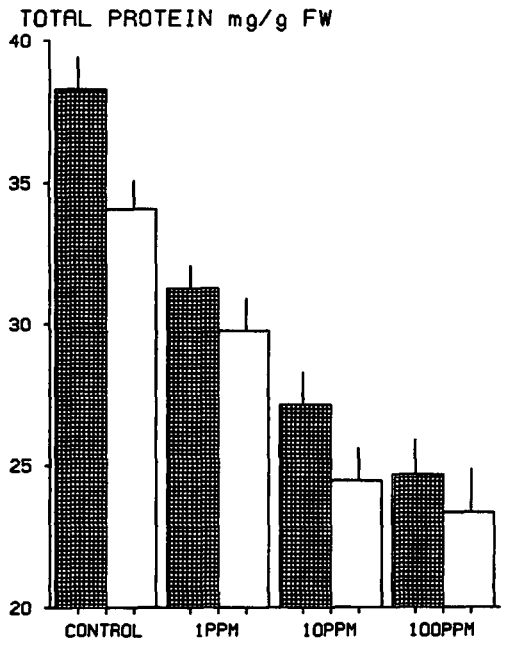


Fig. 3.19

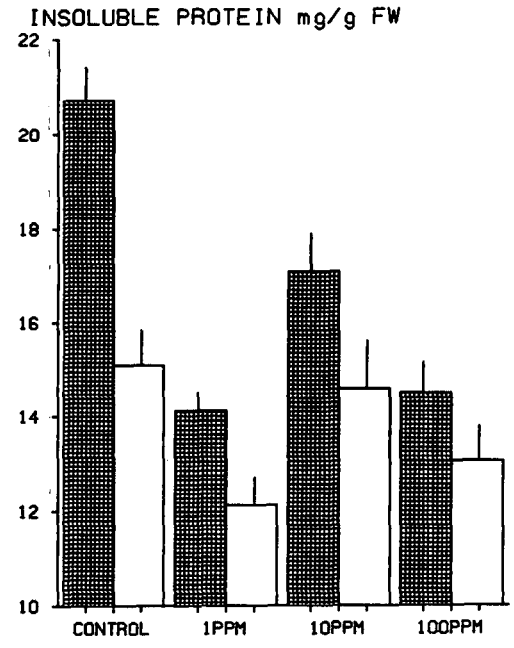
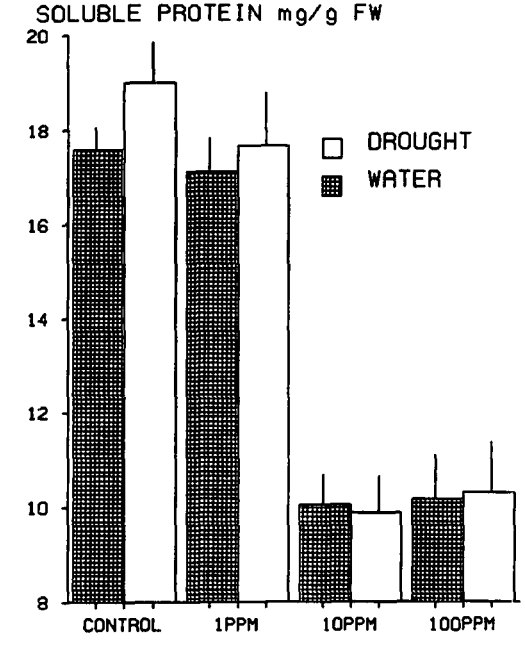


Fig. 3.18



ppm of 2,4-D+drought stress which reduced insoluble proteins significantly compared to 100 ppm of 2,4-D alone. A significant decline in insoluble proteins was also caused by 100 ppm of 2,4-D+drought stress in respect to drought stress alone and 10 ppm of 2,4-D alone. In addition 1, 100 ppm of 2,4-D and 10 ppm+drought significantly reduced insoluble proteins in respect to 10 ppm of 2,4-D alone(Fig. 3.19).

Total protein content was significantly reduced by all treatments used in respect to the controls. The highest reduction was caused by combined 100 ppm of 2,4-D+drought stress and the lowest was caused by drought stress alone. The reduction which was caused by 100 and 10 ppm of 2,4-D+drought stress was significant compared to 10 ppm of 2,4-D alone. Seedlings treated with 100 ppm of 2,4-D+drought stress showed significant reduction in total protein content in respect to those treated with 1ppm+drought, 1ppm of 2,4-D alone and drought stress alone. whilst seedlings treated with 10 ppm of 2,4-D alone their total protein was reduced significantly in comparison to those treated with 1 ppm of 2,4-D alone and drought stress alone. Total protein content in seedlings treated with 1 ppm of 2,4-D+drought stress was reduced significantly in respect to those treated with drought stress alone (Fig. 3.20).

3.3.6. Effects of 2,4-D and drought stress on proline accumulation in the cotyledons and hypocotyls of *Raphanus sativus* Linn. seedlings.

Treatment of radish seedlings with 2,4-D and exposing them to drought stress at intervals (24, 48, 72, 96 and 120) hours after treatment, resulted in a substantial reduction in the amount of proline produced by both cotyledons (Fig. 3.22) and hypocotyls (Fig. 3.24). The highest amount of proline was produced when both 2,4-D and PEG were added together at the same time, and the lowest when PEG was added 120 hours after 2,4-D treatment. However, proline accumulation by seedlings treated with 2,4-D followed by PEG was significantly higher in respect to those treated with

Figure 3.21. Effects of drought stress (230 g/l of PEG) followed by 2,4-D (100 ppm) in nutrient solution on proline accumulation in the cotyledons of *Raphanus sativus* Linn. seedlings.

Figure 3.22. Effects of 2,4-D (100 ppm) followed by drought stress (230 g/l of PEG) in nutrient solution on proline accumulation in the cotyledons of *Raphanus sativus* Linn. seedlings.

-Vertical bars represent standard error for the mean.

Fig. 3.22

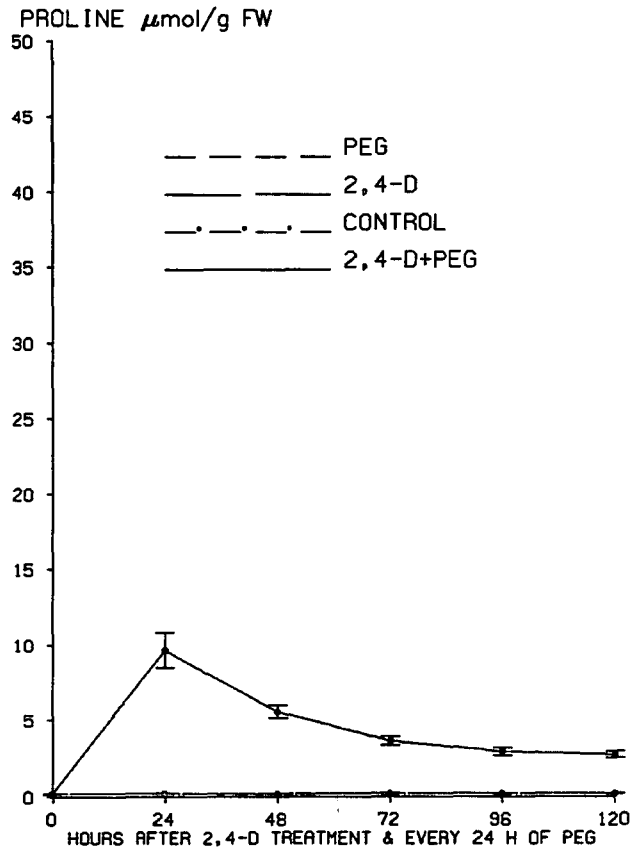


Fig. 3.21

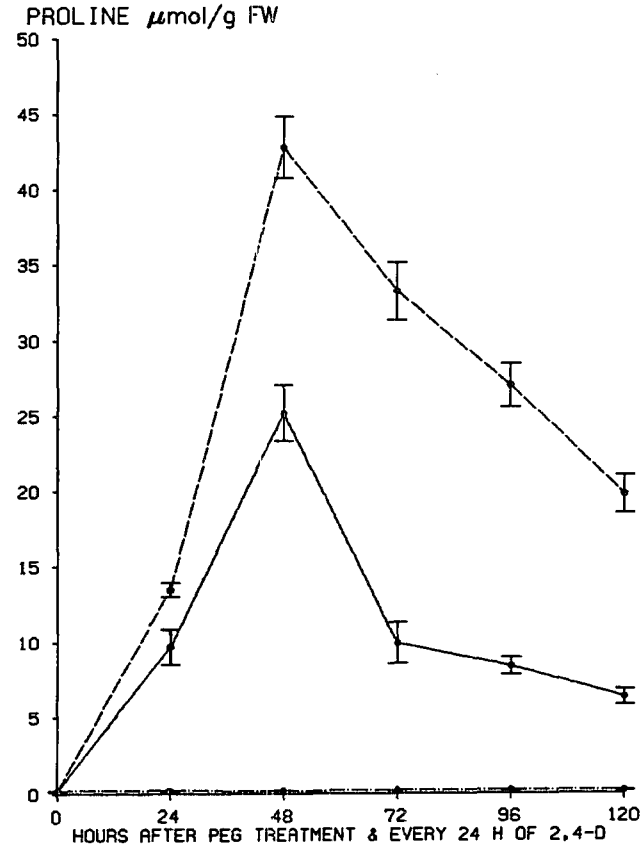


Figure 3.23. Effects of drought stress (230 g/l of PEG) followed by 2,4-D (100 ppm) in nutrient solution on proline accumulation in the hypocotyls of *Raphanus sativus* Linn. seedlings

Figure 3.24. Effects of 2,4-D (100 ppm) followed by drought stress (230 g/l of PEG) on proline accumulation in the hypocotyls of *Raphanus sativus* Linn. seedlings.

-Vertical bars represent standard error for the mean.

Fig. 3.24

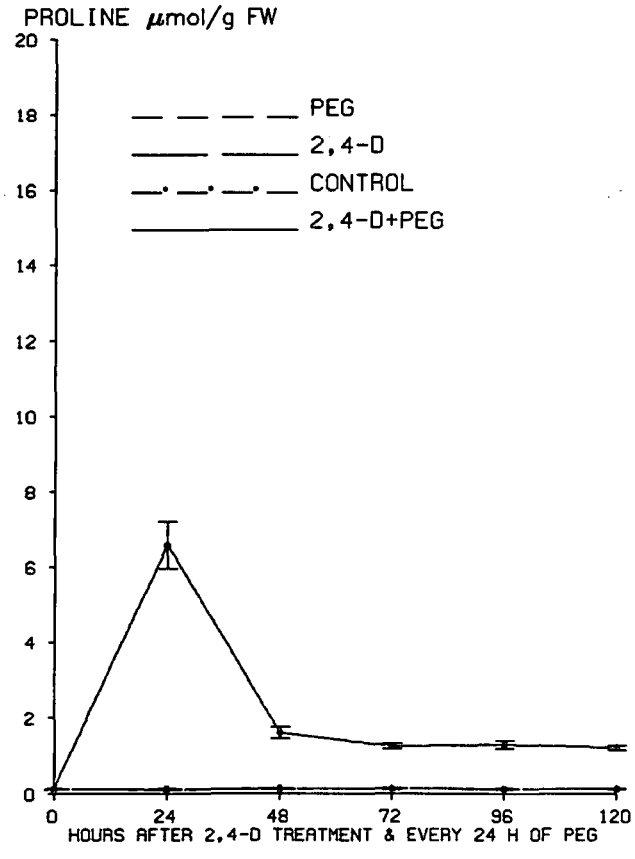
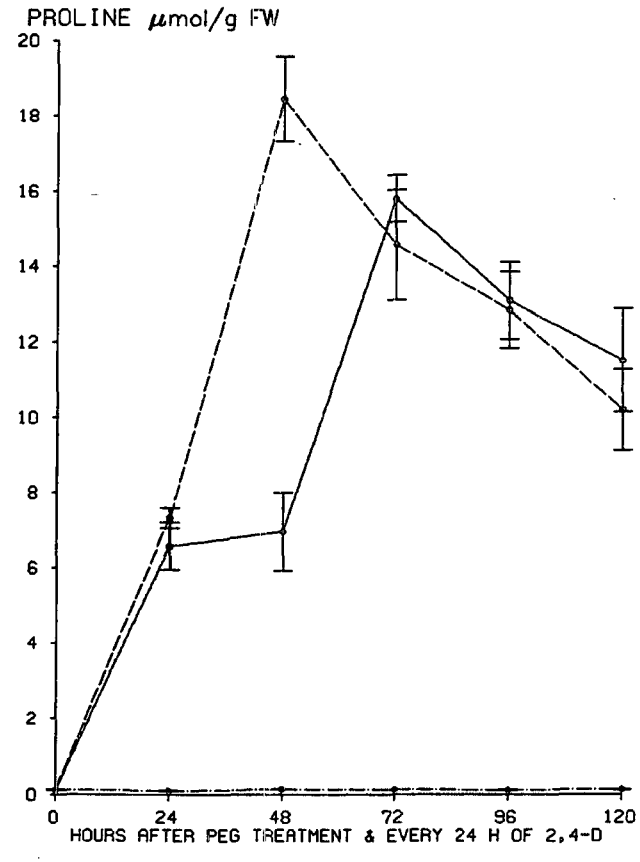


Fig. 3.23



2,4-D alone and controls. There was no significant difference between 2,4-D-treated and control seedlings in proline accumulation.

When the seedlings were subjected to drought stress first and then treated with 2,4-D, they accumulated more proline than those treated with 2,4-D and then subjected to drought stress (Fig. 3.21, 3.22, 3.23 and 3.24). In comparison to the seedlings treated with PEG alone, however, these seedlings accumulated significantly less proline. Proline in seedlings treated with PEG followed by 2,4-D reached the peak value 48 hours (in the cotyledons) and 72 hours (in the hypocotyls) after PEG treatment and then began to decline until reached the lowest level 120 hours after PEG treatment. Whilst in seedlings treated with PEG alone proline reached the peak just 48 hours after treatment, after which time it began to decline. Compared to the controls both treatments increased proline content significantly.

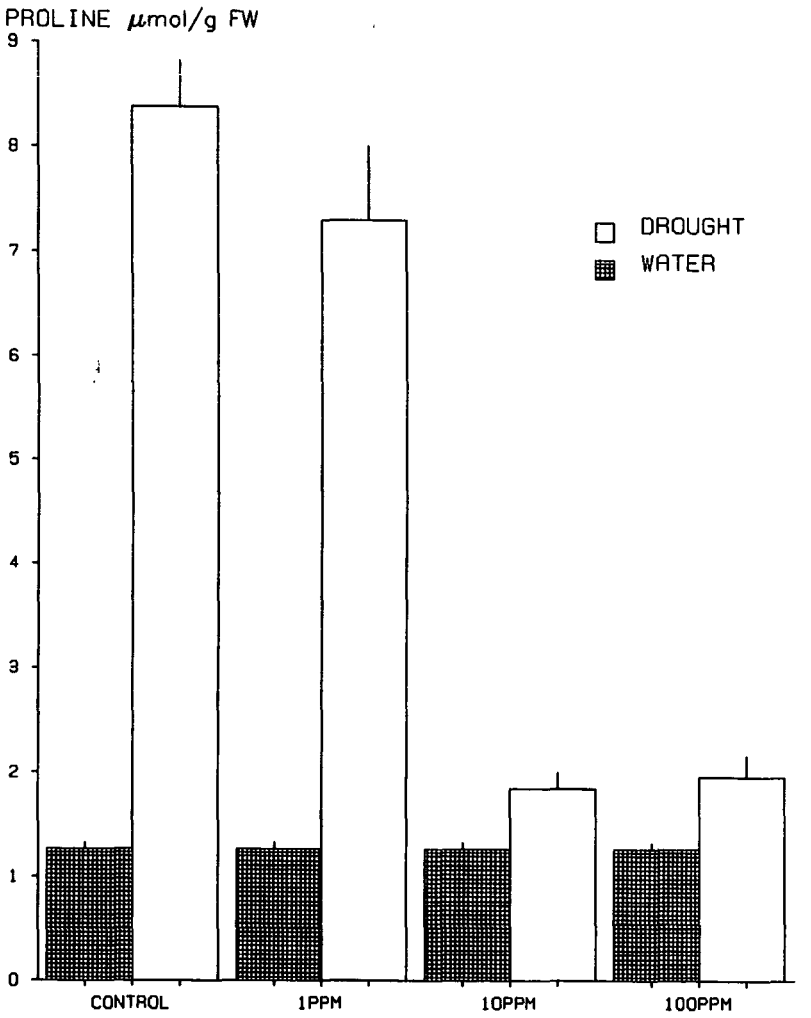
3.3.7. Effect of drought stress on proline accumulation in *Raphanus sativus* Linn. leaves following foliar treatment with 2,4-D.

Treatment of radish seedlings with 2,4-D (2.2.6) inhibited the accumulation of proline under drought stress condition at high concentrations (10 ppm onward) or significantly reduced the ability of seedlings to accumulate proline at low concentration (1 ppm). Seedlings treated with 10 and 100 ppm of 2,4-D and subjected to drought stress or kept under a normal watering regime did not accumulate proline as well as seedlings treated with 2,4-D at 1 ppm and kept under normal watering regime. Seedlings treated with 1 ppm of 2,4-D and subjected to drought stress, however, accumulated proline as untreated seedlings but proline levels in these seedlings were significantly less (Fig 3.25).

Figure 3.25. Effect of drought stress (water withheld) on proline accumulation in the leaves of *Raphanus sativus* Linn. seedlings following foliar treatment with 2,4-D.

-Vertical bars represent standard error for the mean.

Fig. 3.25


PROLINE μmol/g FW
9
8
7
6
5
4
3
2
1
0
DROUGHT
WATER
CONTROL
1PPM
10PPM
100PPM

3.3.8. Effects of 2,4-D and drought stress on proline accumulation in the leaves of *Lolium temulentum* Linn. seedlings.

Similar to radish, rye grass seedlings treated through the roots with 2,4-D followed by drought stress showed an overall reduction in proline accumulation in respect to those stressed first and then treated with 2,4-D. Addition of 2,4-D and PEG together at the same time reduced proline to the lowest level. Proline levels in these seedlings began to increase with time until they reached a peak at 120 hours after 2,4-D treatment and then began to decline again. Proline content, however, in the seedlings treated with 2,4-D followed by PEG increased significantly in respect to the controls and to those treated with 2,4-D alone 48, 72 and 96 hours after 2,4-D treatment. The levels of proline in rye grass seedlings treated with 2,4-D alone was shown to increase with time. The highest increase was 144 hours after treatment. This increase was significant 72, 96, 120, and 144 hours after treatment compared to the control and 144 hours in respect to 2,4-D+PEG (Fig. 3.27).

Stressed seedlings treated with 2,4-D contained much less proline than the untreated stressed seedlings. Proline levels continued increasing in these seedlings 48 hours after PEG treatment, reaching maximal values at 96 hours after PEG treatment. After reaching the maximum a gradual decline in proline content began. Meanwhile in untreated stressed seedlings proline levels began increasing 24 hours after treatment, reaching the peak values at 96 hours after treatment followed by sharp decrease. In both stressed seedlings that had been treated with 2,4-D and untreated stressed seedlings there were significant increase in proline content compared to the controls. The reduction in proline levels in stressed seedlings treated with 2,4-D was significant 24, 48, 72 and 96 hours after PEG treatment. After 96 hours proline levels in untreated stressed seedlings decreased significantly in respect to stressed 2,4-D-treated seedlings (Fig. 3.26).

Figure 3.26. Effects of drought stress (230 g/l of PEG) followed by 2,4-D (100 ppm) in nutrient solution on proline accumulation in the leaves of *Lolium temulentum* Linn. seedlings.

Figure 3.27. Effects of 2,4-D (100 ppm) followed by drought stress (230 g/l of PEG) in nutrient solution on proline accumulation in the leaves of *Lolium temulentum* Linn. seedlings.

-Vertical bars represent standard error for the mean.

Fig. 3.27

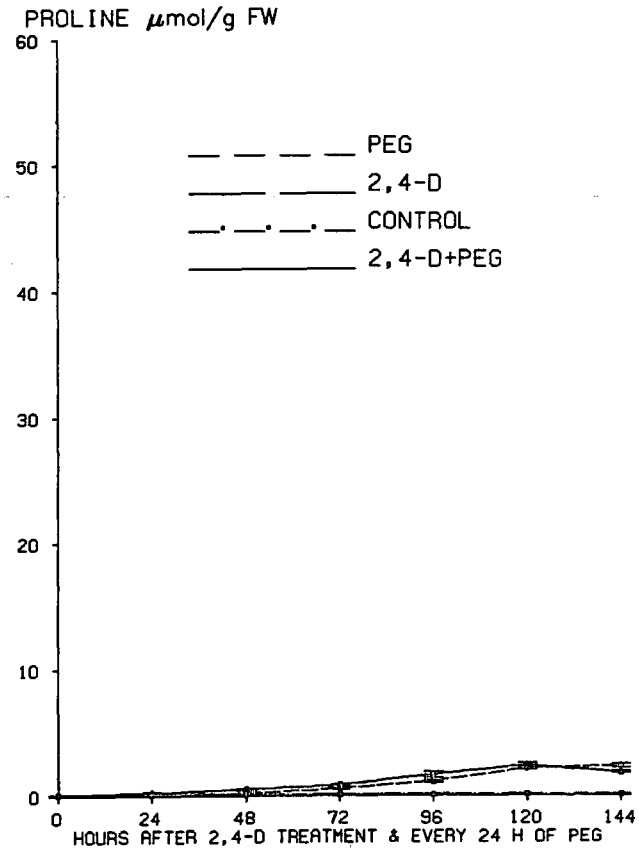
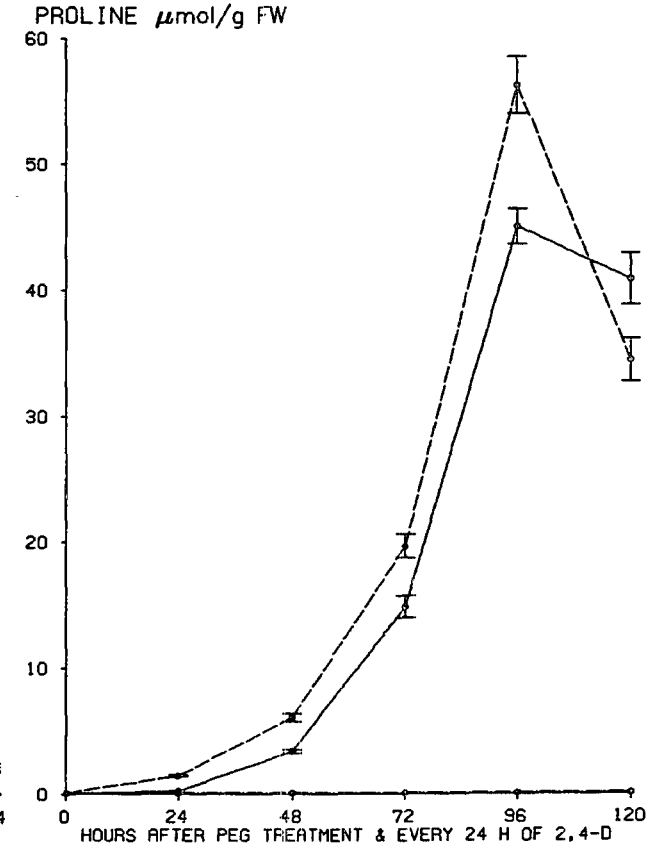


Fig. 3.26

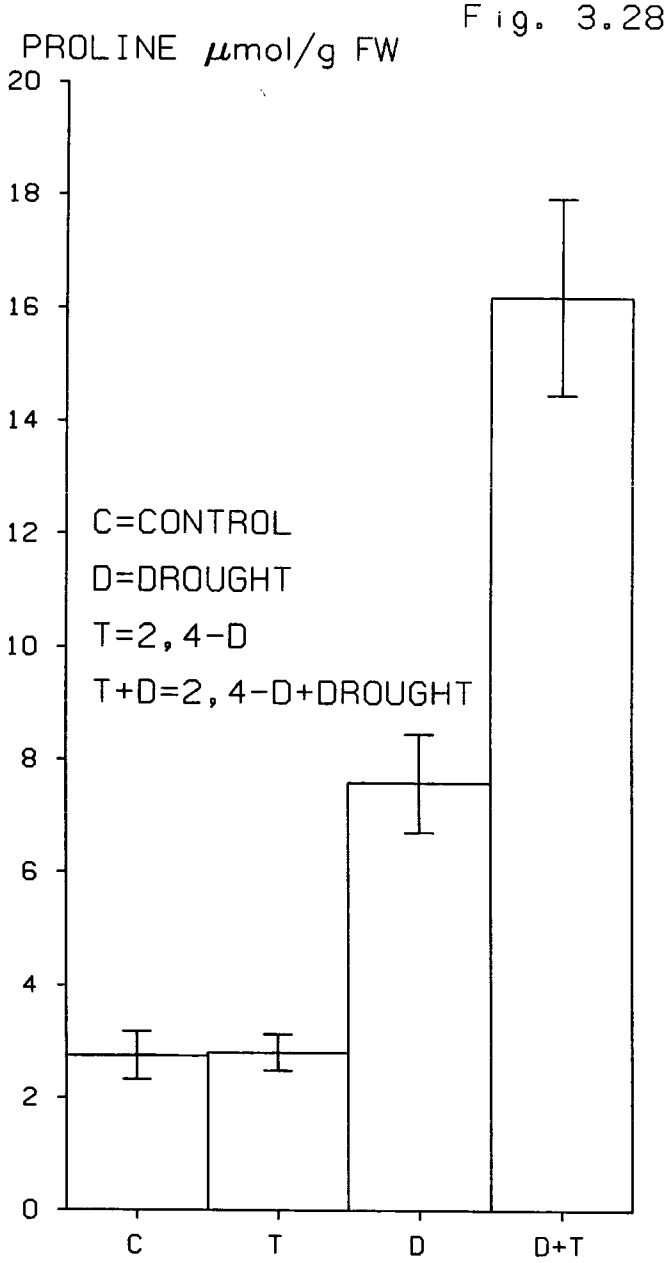


(water withheld)

Figure 3.28. Effects of drought stress on proline accumulation in the leaves of *Lolium temulentum* Linn. seedlings following foliar treatment with 2,4-D (100 ppm).

-Vertical bars represent standard error for the mean.

Fig. 3.28


PROLINE μmol/g FW
20
18
16
14
12
10
8
6
4
2
0
C=CONTROL
D=DROUGHT
T=2,4-D
T+D=2,4-D+DROUGHT
C
T
D
D+T

3.3.9. Effect of drought stress on proline accumulation in the leaves of *Lolium temulentum* Linn. seedlings following foliar treatment with 2,4-D.

In contrast to root treatment, foliar treatment of rye grass seedlings with 2,4-D (2.2.11) increased proline levels in stressed seedlings significantly compared to stressed untreated seedlings. Two weeks after 2,4-D treatment stressed seedlings which had been sprayed with 2,4-D showed about a two-fold increase in proline contents over untreated stressed seedlings. However, treated and untreated stressed seedlings contained significantly higher proline than those 2,4-D-treated and stressed and control seedlings, and there was no significant difference in proline levels between 2,4-D-treated (alone) and control seedlings (Fig. 3.28).

3.4. Discussion

The effects of 2,4-D, 2,4-D+PEG and PEG on chlorophyll biosynthesis in radish cotyledons were considerable. During the period of 96 hours control seedlings synthesized chlorophyll at a greater rate than did treated seedlings. 2,4-D-treated seedlings were the most affected ones and this is may have resulted from 2,4-D effects on the morphology and internal structure of chloroplast (Hallam, 1970; Nadakavukaren & McCracken, 1977). Whilst PEG was shown to lessen the effect of 2,4-D over the period of 72 hours after treatment, after this period it appeared to have more effect on the reduction in chlorophyll content than 2,4-D or PEG alone. This results suggest that the combined 2,4-D+PEG may show a synergistic effect. The chlorophyll a/b ratio in treated seedlings changed at different stages depend on the treatment which suggested that these treatments may had influenced both chlorophyll a and chlorophyll b.

Biosynthesis of chlorophyll in the first leaf of rye grass was different from that of radish cotyledons. Chlorophyll content of the first leaf in control seedlings increased linearly during the first 24 hours, followed by a decline at 48 hours and at 96 hours recovered more than it was at 24 hours after treatment. In PEG-treated seedlings, however, the chlorophyll content continued increasing until 96 hours and then began to decline. In the mean time chlorophyll content in 2,4-D and 2,4-D + PEG-treated seedlings increased linearly during the test period of 96 hours. At the end of 96 hours period, PEG-treated seedlings accumulated more chlorophyll than all other treatments and the controls, followed by 2,4-D+PEG and control seedlings. 2,4-D-treated treated seedlings accumulated the lowest chlorophyll in respect to other treatments. These data suggested that PEG may have altered the water status of the cells, therefore more tissues were used to give weight similar to 2,4-D-treated unstressed tissues.

Breakdown of chlorophyll in green seedlings treated with 2,4-D, 2,4-D+drought stress and drought stress alone was looked at in the light of the data obtained and it was found that chlorophyll is highly unstable. Therefore it is extremely difficult to draw a clear cut conclusion regarding the effect of each treatment, even though different treatments were shown to effect the processes leading to chlorophyll destruction in radish and rye grass leaves.

Drought stress alone was shown to reduce total proteins and to increase soluble proteins in the leaves of radish seedlings. These findings are in agreement with that found by Hsiao (1973), Brady *et al.*(1974), Dhindsa & Bewley (1976), Cooke *et al.* (1980) and Hanson & Hitz (1982), who demonstrated that protein was decreased by drought stress and with that found by Singh *et al.* (1973) who noted a significant increase in soluble protein content after drought stress was imposed. 2,4-D alone was also shown to reduce total protein levels. The reduction was closely related to 2,4-D concentration. These data are in accord with those reported by Key *et al.* (1966). Soluble protein was increased in preference insoluble protein only under the lowest concentration of 2,4-D (1 ppm). At high concentrations, however, 2,4-D alone decreased the levels of soluble protein. These results suggested that 2,4-D at low concentration may stimulated more enzyme synthesis, whilst at high concentration inhibited the function of soluble protein.

At the same time combined 2,4-D+drought stress reduced the levels of total proteins more than drought stress or 2,4-D alone did, which may suggest that both 2,4-D and drought stress have synergistic action. Whilst seedlings treated with 1 ppm of 2,4-D+drought stress showed an increase in soluble protein, at higher concentrations of herbicide, soluble protein was decreased. These results suggested that high levels of 2,4-D may have overcome the effect of drought so reducing the ability of seedlings

to respond to drought stress by increasing soluble protein.

Proline accumulation as a result of drought stress appears to be a general phenomenon in higher plants. The ability for proline accumulation, however, differs between different tissues of plants (Singh *et al.*, 1973) and is strongly influenced by previous exposure to drought stress and genotype (Singh *et al.*, 1973).

It has been suggested that proline accumulation in the drought-stressed plants resulted from an inhibition of protein and polysaccharide synthesis and a consequent channeling of amino acid and carbohydrate metabolism into the synthesis of proline (Stewart *et al.*, 1966). It is also possible that proline catabolism is inhibited during stress(Barnett & Naylor, 1966). The extremely high concentration of proline which developed in stressed tissues would also suggest lack of the usual control mechanisms of proline synthesis under these conditions (Singh *et al.*, 1973). Moreover high concentrations of proline have been attributed to the inhibition of its oxidation under drought stress (Stewart *et al.*, 1977), to inhibition of proline incorporation into proline-rich protein (Shiralipour & West, 1984, cf. Miranda-Ham & Loyola-Vargas, 1987), and to the continuous synthesis of this amino acid under stress condition (Miranda-Ham & Loyola-Vargas, 1987).

On the other hand, proline is considered to be involved in adaptation mechanisms in drought stress. Due to its high solubility, proline can remain in high concentration in cells. An osmoregulatory role (Stewart & Lee, 1974) has been suggested as well as protective function for enzymes and thus maintain their hydration (Schobert, 1979, cf. Hanson & Hitz, 1982). Hanson *et al.* (1979, 1980), however, suggested that accumulation of proline is an incidental response to severe drought stress and does not have any adaptive value.

It has been shown that proline induced by drought stress can be modified

by exogenous treatment with growth regulators. Singh (1973) reported that treatment with the growth retardant CCC and with gibberellic acid was modified the extent of proline accumulation. Rajagopal & Andersen (1978) demonstrated that root treatment of barley seedlings with solution containing PEG+ABA resulted in a significant increase in proline accumulation in respect to the PEG or ABA alone, this indicated an additive effect of the two compounds on proline. They suggested that PEG-enhanced proline content is through endogenous ABA level. Wample & Bewley (1975) showed that exposure of sunflower plants to drought stress following foliar treatment with BA caused a reduction in proline content compared to the untreated wilted plants. They proposed that a reduction in endogenous cytokinins as a result of wilting could in some way affect amino acid or protein metabolism and allow for an increase in free proline.

The data reported here showed that drought stress induced by withholding water or by PEG treatment resulted in proline accumulation in radish and rye grass shoots. These result s are in accord with those found by many investigators (e.g. Singh, 1973; Wample & Bewley, 1975; Rajagopal & Andersen, 1978; Levy, 1983). Pre-treatment of leaves or cotyledons with 2,4-D modified the effect of drought stress in terms of proline accumulation. Pre- or post-treatment of radish and rye grass seedlings with 2,4-D through the roots in combination with PEG treatments also resulted in changes in proline accumulation patterns.

Foliar treatment of radish seedlings growing in compost with 2,4-D followed by drought stress, resulted in the inhibition of proline accumulation in the leaves at high concentrations (10 ppm onward) or significant a reduction in the ability of seedlings to accumulate proline at low concentration (1ppm) compared to untreated stressed seedlings.

On the other hand, pre-treatment of radish seedlings through the roots in

nutrient solution with 100 ppm of 2,4-D followed by drought stress (PEG) at intervals, or exposure of these seedlings to drought stress (PEG) followed by 2,4-D treatments at intervals, showed a reduction in the accumulation of proline in the cotyledons and hypocotyls of these seedlings compared to untreated stressed seedlings. Pre-treatment of radish seedlings with 2,4-D before they were exposed to drought stress, however, was shown to have more effect in reducing the levels of proline.

These results suggest that 2,4-D has more effect on proline accumulation at the site of application where the bulk of this herbicide remained as suggested by many investigators (e.g. Hay, 1976, Zemskaya, 1984). This is supported by the data obtained from foliar-treated seedlings where the proline accumulation was inhibited completely at high concentrations and from root-treated seedlings (with the same concentration) where the effect was less severe. Moreover these data suggested that 2,4-D concentration was important since in foliar-treated seedlings, whereas 10 and 100 ppm inhibited proline accumulation completely, 1 ppm only reduced the ability of seedlings to produce proline.

In contrast to radish, foliar-treatment of rye grass seedlings with 2,4-D increased proline levels in stressed seedlings significantly in respect to those stressed but untreated with 2,4-D. Poor entry of the herbicide through the leaves was probably the main cause for proline increase in rye grass leaves. As small amount of 2,4-D may have stimulated proline accumulation, which suggest that morphological characteristics of rye grass leaves may play an important role in the resistance of this plant to 2,4-D herbicide.

Meanwhile root treatment of rye grass seedlings with 2,4-D before or after drought stress was imposed reduced proline accumulation in leaves of these seedlings in respect to stressed untreated seedlings. As in radish seedlings, pre-treatment of rye

grass seedlings with 2,4-D before they were exposed to drought stress was more effective in reducing the levels of proline than post-drought treatment. These findings suggested that the selectivity of the herbicide may be diminished if applied to the roots and similar effect on monocots and dicots species.

Finally one may conclude that shoot and root treatment with 2,4-D may effect the responses of susceptible plants to drought stress by reducing their ability to resist drought through accumulation of proline or soluble protein under drought stress condition. At the same time foliar-treatment with 2,4-D may improve the response of resistant plants to drought stress by enhancement of proline accumulation in these species. These suggested that 2,4-D may have an effect on overall metabolism of plant. The effect of 2,4-D, however, was dependent on the concentration, method of application and species of plants.

grass seedlings with 2,4-D before they were exposed to drought stress was more effective in reducing the levels of proline than post-drought treatment. These findings suggested that the selectivity of the herbicide may be diminished if applied to the roots and similar effect on monocots and dicots species.

Finally one may conclude that shoot and root treatment with 2,4-D may effect the responses of susceptible plants to drought stress by reducing their ability to resist drought through accumulation of proline or soluble protein under drought stress condition. At the same time foliar-treatment with 2,4-D may improve the response of resistant plants to drought stress by enhancement of proline accumulation in these species. These suggested that 2,4-D may have an effect on overall metabolism of plant. The effect of 2,4-D, however, was dependent on the concentration, method of application and species of plants.

CHAPTER 4

EFFECT OF DROUGHT STRESS ON THE UPTAKE AND DISTRIBUTION OF 2,4-D IN DIFFERENT ORGANS OF SEEDLINGS

4.1. Introduction

According to Muzik & Mauldin (1964), a systemic herbicide such as 2,4-D must, to be effective, do three things; it must enter the plant, it must be translocated throughout the plant, and it must exert a phytotoxic effect after it is translocated.

Research on the absorption and translocation of the phenoxy herbicides in general and 2,4-D in particular has been wide and has included several variables such as cuticle, species, stage of growth, intact plants, excised leaves, tissue segments, formulation, and environmental factors. The objective of many of these studies was to determine the basis of selectivity or to obtain more effective weed control (Ashton & Crafts, 1981). However, most research has been done regarding absorption and translocation of 2,4-D was concentrated on foliar-applied herbicide.

It is generally accepted that 2,4-D can be absorbed by both shoots and roots (Bovey, 1980; Ashton & Crafts, 1981). Foliar-applied 2,4-D involves the absorption and penetration of plant surfaces, absorption into the symplast, migration across parenchyma tissue to the vascular system, translocation from leaves to the sinks (roots) through the assimilate stream (Robertson & Kirkwood, 1970; Bovey, 1980; Ashton & Crafts, 1981). In root-applied 2,4-D the uptake can be by both passive and active mechanisms (Anonymous, 1968, cf. Bovey, 1980). Absorption occurs by the root hairs and cortical cells behind the root tip, migration via the symplast into the stele, and there a leakage from the symplast to the apoplast and transloca-

tion in the transpiration stream into the tops (Crafts, 1961). The passive entrance of 2,4-D into roots is primarly with absorbed water and it moves throughout the plant in the apoplast system, including the xylem. Active uptake involves entrance into the protoplasm and movement by the symplast system (Bovey, 1980).

Absorption and translocation of foliar-applied 2,4-D are known to be affected by environmental factors e.g. temperature, humidity, moisture stress and light; leaf factors e.g. leaf structure, and cuticle thickness; and chemical factors e.g. formulation, pH, surfactants, additives, concentration of the herbicide and molecular configuration. All these factors have been reviewed by Richardson (1977). Bukova (1976), however, emphasized that the uptake of the herbicide by roots is more affected by time-course of uptake, temperature, pH and concentration of the herbicide.

In this chapter drought stress as an environmental factor is the only point intended for discussion in relation to 2,4-D absorption and translocation. The available data regarding the absorption and translocation of 2,4-D in relation to moisture stress are very limited and all research has appeared to have been done in relation to foliar-applied 2,4-D.

Basler *et al.* (1961) demonstrated that moisture stress, measured as the relative turgidity of leaf tissue had no effect on absorption of 2,4-D acid by beans (*Phaseolus vulgaris* Linn). Similar results were obtained by Pallas & Williams (1962) in experiments on red kidney beans with 2,4-D. Translocation, however, is severely reduced by moisture stress. Hauser (1955), Basler *et al.* (1961) and Pallas & Williams (1962) have described 2,4-D reduced translocation under moisture stress.

In the light of the results obtained (as reported in Chapter 3) regarding the effect of 2,4-D and drought stress on proline accumulation and in particular the difference in response between foliar-applied and root-applied 2,4-D, it become appearent that it was important to investigate the uptake and distribution of 2,4-D

under drought stress and normal watering regimes. The intention was to investigate if the amount of the herbicide in different parts of the plant had an influence on the response of plant to this herbicide.

Herbicide movement within a plant appears to be important for its effectiveness. Given that root-applied 2,4-D has effect on the shoots of plants it is possible that this herbicide must move within the plant or alternatively elicit secondary responses in the roots which gives the effect in the shoots. The aime of the work reported here was to see if 2,4-D moved effectively from roots to shoots and if water stress treatment could modify this movement. Any change in 2,4-D movement could be related to modified physiological responses to the herbicide and drought stress.

4.2. Materials and Methods

4.2.1. Plant Materials

Seeds of radish and caryopses of rye grass were germinated in 9-cm Petri dishes on filter paper moistened with distilled water and kept at temperature between 21-24 °C, in the dark. When the seedlings had germinated, they were transferred to nutrient solution and left to grow for one-week before the treatment.

4.2.2. Preparation of Herbicide

2,4-dichlorophenoxy[2-^{14}C]acetic acid obtained from Amersham International plc England, had a specific activity of 56 mCi/mmol, radioactive concentration 50 μCi/ml and a radiochemical purity of 99 %. 200 μl of radioactive 2,4-D were added to 400 ml of distilled water or 400 ml of PEG solution (-10 bars) and were used as stock solutions.

4.2.3. Experimental Procedure

The seedlings were taken from the nutrient solution and their roots were immersed in 10 ml of either solutions (2,4-D[2-^{14}C]+PEG or 2,4-D[2-^{14}C]+H_2O), contained in glass vials. Two seedlings were put in each vial replicated five times at least for each treatment. Treated seedlings were kept under conditions of light and temperature similar to those described in 2.2.1. Six hours later radish and rye grass seedlings were removed and extracted for assay of radioactivity.

In order to study the process of translocation of radioactive herbicide in radish seedlings after uptake with or without water stress treatment, ten 2,4-D[2-^{14}C]+PEG-treated seedlings were removed and 5 were transferred to a PEG solution (-10 bars) and 5 were transferred to H_2O. Ten 2,4-D[2-^{14}C]+H_2O-treated seedlings

were also removed, and 5 were transferred to H_2O and 5 were transferred to a PEG solution. The seedlings were kept under conditions of light and temperature described above for another 6 hours, after which time they were removed and extracted for the assay of radioactivity.

Soil Treatment

To investigate the uptake of 2,4-D[2-^{14}C] by seedlings from the soil, compost was dried for 24 hours in an oven at 80 °C, weighed and put into 4 plastic trays. The trays were watered by bringing to field capacity and left for 24 hours to drain, after which time, they were weighed again. The compost, at 85 % of its field capacity, was treated with a 2,4-D[2-^{14}C] solution by spraying with 5 ml of a solution of 200 μl of radioactive 2,4-D in 20 ml of distilled water. The soil was thoroughly mixed to distribute the chemical.

Seeds of radish and maize were planted in the treated trays (two trays each) and left in a closed cabinet under conditions of light and temperature as described above. Thirteen days and 20 days after planting radish and maize seedlings were harvested and extracted for assay of radioactivity.

4.2.4. Liquid Scintillation Counting

Seedlings which had been treated with radioactive 2,4-D were washed several times with distilled water and homogenized in 2 ml of 5 % aqueous trichloroacetic acid (TCA), using a pestle and mortar. The homogenate was placed in scintillation vials, and 5 ml of scintillation fluid was added to the vials, this being made up of [4 g of 2,4-diphenyloxazole (PPO); + 1 l of toluene; + 500 ml of Triton-X-100], as described by Badenoch-Jones *et al.* (1983). The radioactivity was determined on a Beckman Ls8000 scintillation counter.

4.3. Results

4.3.1. Effect of drought stress on the uptake and distribution of 2,4-D[2-^{14}C] in *Raphanus sativus* Linn. seedlings.

Fig. 4.1 and 4.2 present results of experiments on the uptake of 2,4-D[2-^{14}C] by roots of radish and its distribution throughout the plant. It can be seen that radioactive 2,4-D in an aqueous solution without PEG was taken up by roots more than in solution which contained PEG.

With regard to the distribution of 2,4-D[2-^{14}C] which was taken up by the roots, it can be seen that the bulk remained in the treated roots. Hypocotyls were found to contain substantially less amount of the radioactive herbicide compared to the roots. The lowest amount was detected in the cotyledons of both seedlings treated with the radioactive 2,4-D with or without PEG. In seedlings fed 2,4-D without PEG, however, the amount of radioactive herbicide found in different parts was significantly higher than that found in those treated in PEG solution.

On the other hand the amount of radioactive 2,4-D in different organs of seedlings, calculated as a percentage of total amount of radioactive herbicide taken up by these seedlings, showed that about 73 % of the absorbed herbicide remained in the roots of seedlings treated with 2,4-D[2-^{14}C] in aqueous solution without PEG and 81 % in the roots of seedlings treated with the herbicide in PEG solution. Under normal watering condition higher amount of radioactive herbicide moved to the hypocotyls (24 %) compared to drought stress condition (13 %). In the cotyledons, however, about 4 % of the total amount of radioactive herbicide was found in seedlings treated in PEG, whilst only 1 % in seedlings treated in aqueous solution without PEG.

Total cpm from radish seedlings treated for 6 hours with 2,4-D[2-^{14}C] in PEG solution or H_2O and transferred for further 6 hours to PEG solution or H_2O were less than the cpm taken at 6 hours after treatment. The loss of radioactive mate-

Figure 4.1. Distribution of ^{14}C as a percentage of total radioactivity translocated and recovered 6 hours after the application of 2,4-D[2-^{14}C] to the roots of *Raphanus sativus* Linn. seedlings in water solution with or without PEG.

Figure 4.2. Distribution of ^{14}C as CPM of total radioactivity translocated and recovered 6 hours after the application of 2,4-D[2-^{14}C] to the roots of *Raphanus sativus* Linn. seedlings in water solution with or without PEG.

-vertical bars represent standard error for the mean.

R=roots, H=hypocotyls, C=cotyledons, T=total.

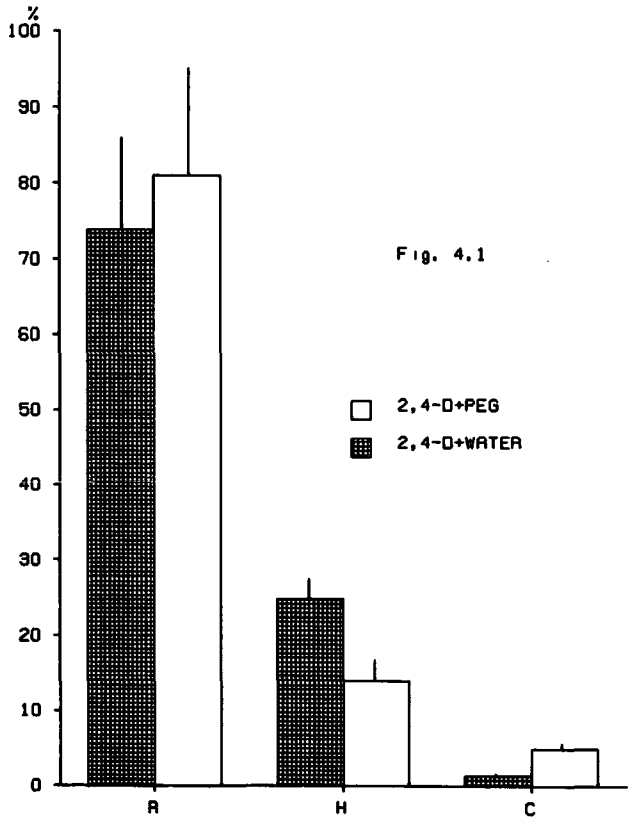

%
100
90
80
70
60
50
40
30
20
10
0
2,4-D+PEG
2,4-D+WATER
R
H
C

Fig. 4.1

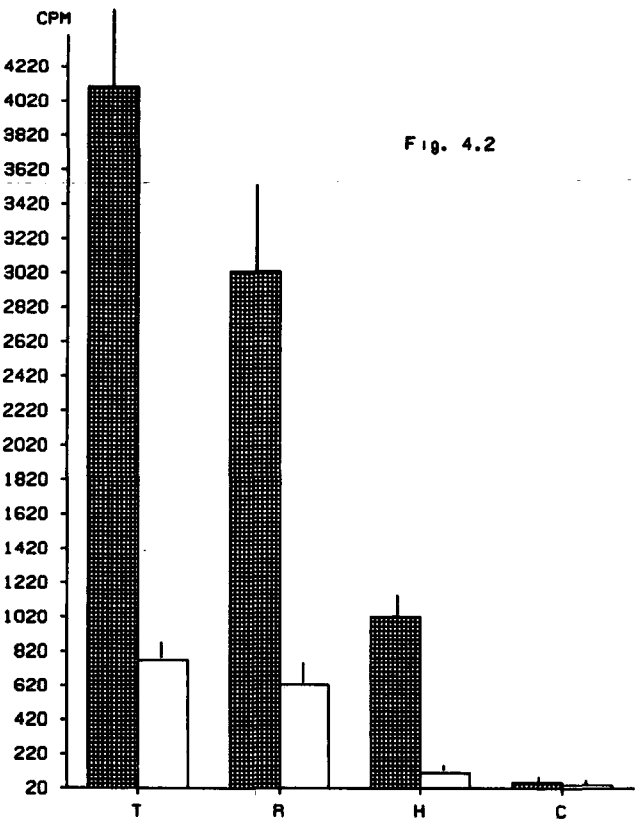

CPM
4220
4020
3820
3620
3420
3220
3020
2820
2620
2420
2220
2020
1820
1620
1420
1220
1020
820
620
420
220
20
T
R
H
C

Fig. 4.2

Figure 4.3. Distribution of ^{14}C as a percentage of total radioactivity translocated and recovered 12 hours after the application of 2,4-D[2-^{14}C] to the roots of *Raphanus sativus* Linn. seedlings in water solution with or without PEG.

Figure 4.4. Distribution of ^{14}C as CPM of total radioactivity translocated and recovered 12 hours after the application of 2,4-D[2-^{14}C] to the roots of *Raphanus sativus* Linn. seedlings in water solution with or without PEG.

-vertical bars represent standard error for the mean.

R=roots, H=hypocotyls, C=cotyledons, T=total.

Fig. 4.3

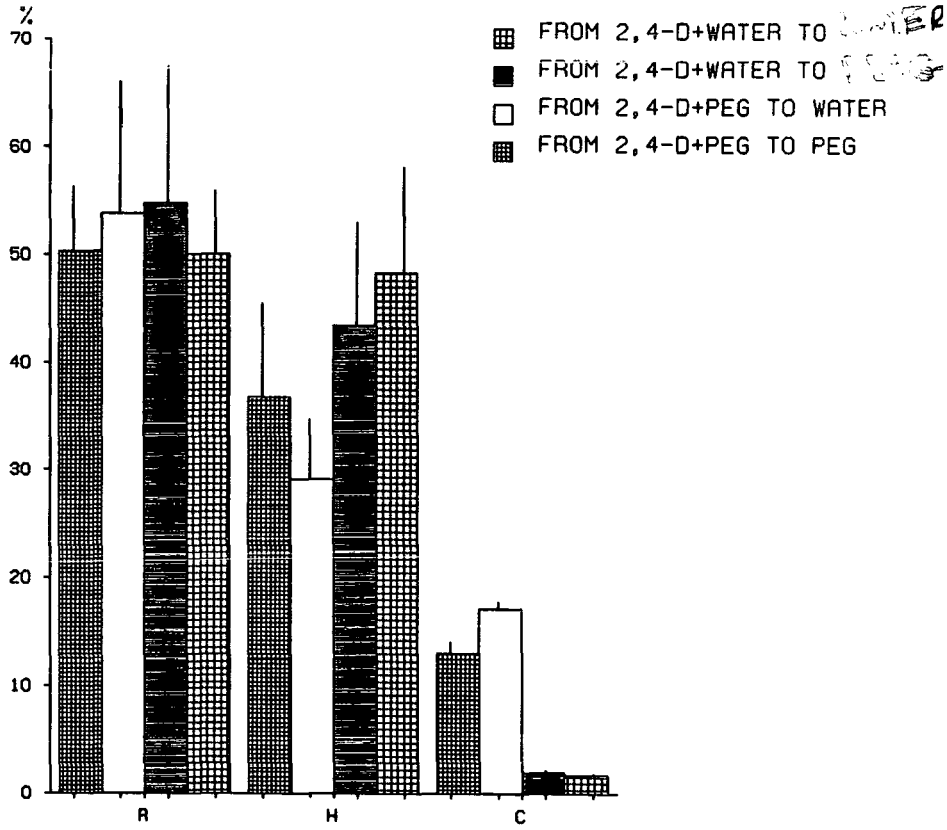
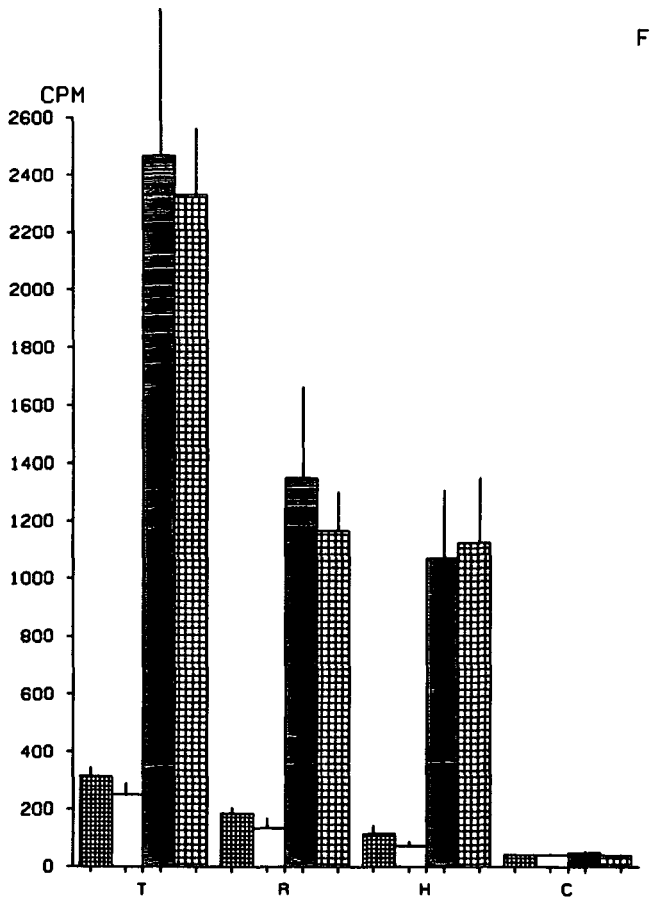

%
70
60
50
40
30
20
10
0
R
H
C
FROM 2,4-D+WATER TO WATER
FROM 2,4-D+WATER TO PEG
FROM 2,4-D+PEG TO WATER
FROM 2,4-D+PEG TO PEG

Fig. 4.4


CPM
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
T
R
H
C

rials from 2,4-D[2-^{14}C]+PEG-treated seedlings was greater than 2,4-D[2-^{14}C]+H_2O-treated seedlings and the loss from seedlings transferred to PEG was greater than those transferred to H_2O.

The greatest amount of radioactive materials lost was from the roots, followed by the hypocotyls and the lowest from the cotyledons. However, the loss of radioactive materials from the hypocotyls was only from seedlings treated with 2,4-D[2-^{14}C]+PEG and transferred to PEG, whilst in seedlings treated with 2,4-D[2-^{14}C]+PEG and transferred to H_2O or seedlings treated with 2,4-D[2-^{14}C]+H_2O and transferred to H_2O or PEG, a slight increase in radioactive materials was recorded. In the cotyledons, a slight increase in amount of radioactive materials was also noted in seedlings treated with 2,4-D[2-^{14}C]+PEG and transferred to PEG or H_2O. In contrast seedlings treated with 2,4-D[2-^{14}C]+H_2O and transferred to H_2O or PEG showed less counts at 12 hours than at 6 hours (Fig. 4.4).

With regard to distribution of 2,4-D[2-^{14}C], as shown in fig 4.3 the release from stress appeared to have no effect on the pattern of distribution of the radioactive materials in different parts of seedlings.

4.3.2. Effect of drought stress on the uptake and distribution of 2,4-D[2-^{14}C] in *Lolium temulentum* Linn. seedlings.

As in radish the amount of radioactive 2,4-D taken up by the roots of rye grass seedlings was decreased by the addition of PEG, with the bulk of the radioactive herbicide remaining in the roots with a very small amount being detected in the shoots of both seedlings fed with or without PEG. The percentages of total radioactive herbicide taken up by seedlings showed that there was no difference between with and without PEG treatment in both roots and shoots (Fig. 4.5 and 4.6).

Figure 4.5. Distribution of ^{14}C as a percentage of total radioactivity translocated and recovered 6 hours after the application of 2,4-D[2-^{14}C] to the roots of *Lolium temulentum* Linn. seedlings in water solution with or without PEG.

Figure 4.6. Distribution of ^{14}C as CPM of total radioactivity translocated and recovered 6 hours after the application of 2,4-D[2-^{14}C] to the roots of *Lolium temulentum* Linn. seedlings in water solution with or without PEG.

-Vertical bars represent standard error for the mean.

R=roots, S=shoots, T=total.

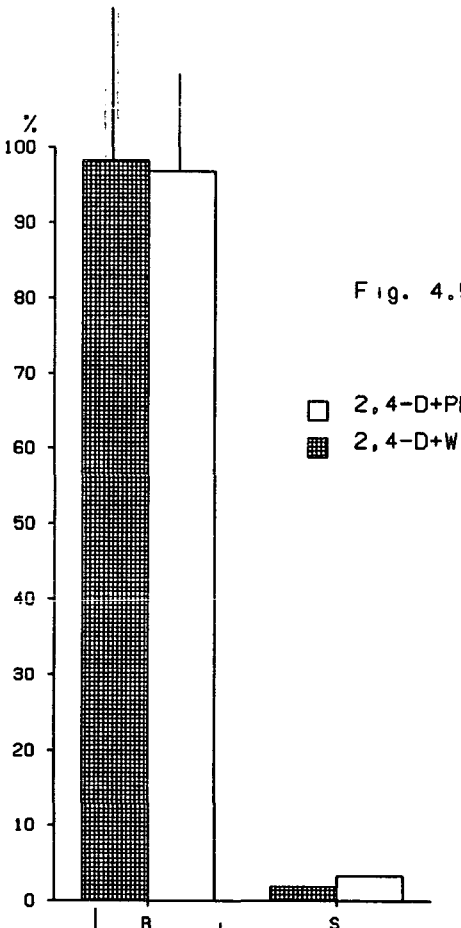

%
100
90
80
70
60
50
40
30
20
10
0
R
S
2,4-D+PEG
2,4-D+Water

Fig. 4.5

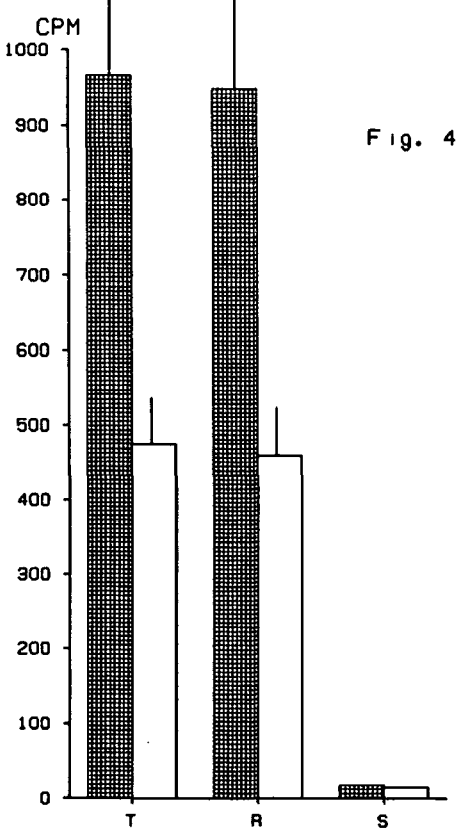

CPM
1000
900
800
700
600
500
400
300
200
100
0
T
R
S

Fig. 4.6

Figure 4.7. Distribution of ^{14}C CPM and a percentage of total radioactivity translocated in *Zea mays* Linn. seedlings and recovered 20 days after the application of 2,4-D[2-^{14}C] to the soil.

Figure 4.8. Distribution of ^{14}C CPM and a percentage of total radioactivity translocated in *Raphanus sativus* Linn. seedlings and recovered 13 days after the application of 2,4-D[2-^{14}C] to the soil.

-vertical bars represent standard error for the mean.

R=roots, H=hypocotyls, C=cotyledons and coleoptils, M=mesocotyls, L=leaves, T=total.

Fig. 4.7

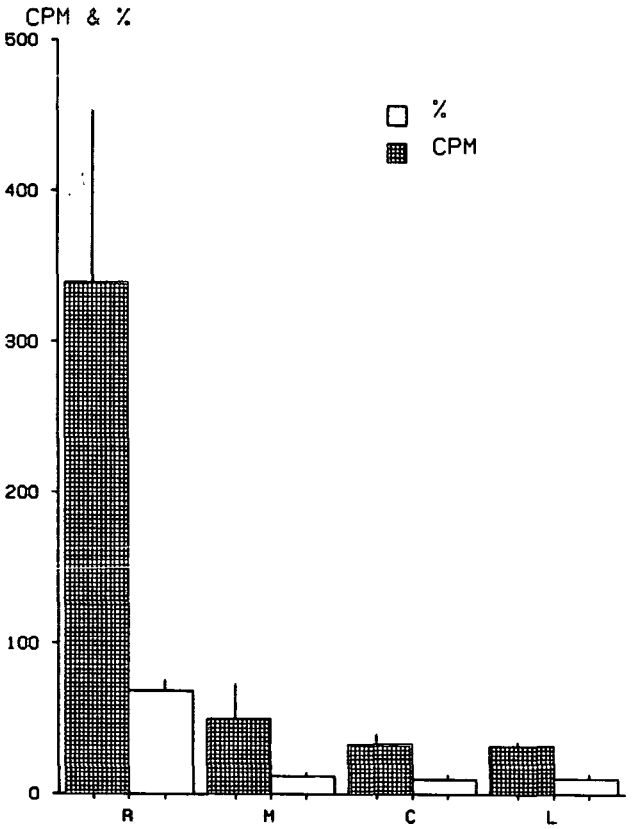
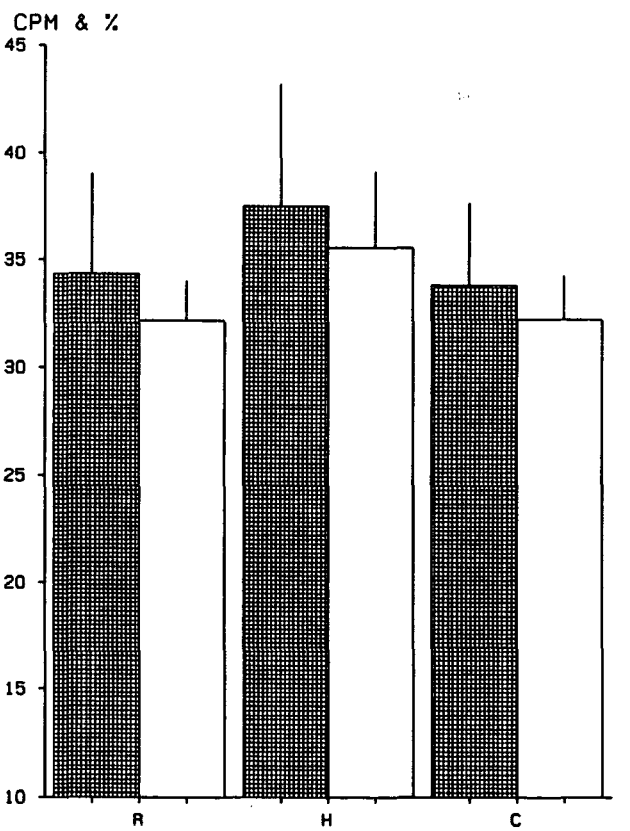


Fig. 4.8



4.3.3. The uptake of 2,4-D[2-^{14}C] by seedlings from soil.

In general the uptake of 2,4-D[2-^{14}C] from the compost by both radish and maize seedlings was very poor. The total amount of radioactivity detected being only slightly above background (Fig. 4.7 and 4.8).

With regard to the distribution of 2,4-D[2-^{14}C] in different parts of seedling, the data showed that there were no significant differences in the amount of radioactive materials in different parts of radish seedlings (Fig. 4.8). In maize seedlings, however the bulk of radioactive materials which were taken up remained in the roots. The amounts here were significantly higher in comparison to other parts of seedling. There were no significant differences in the amount of radioactive material between leaves, coleoptile and mesocotyl. Low uptake may have been due to imioblization loss or decomposition of the 2,4-D in the soil.

Since the uptake of radioactive 2,4-D in the soil was very low the use of soil for uptake experiments was not continued.

4.4. Discussion

It was evident from the obtained data that when seedlings of both radish (dicot) and rye grass (monocot) were fed through roots in aqueous solution with radioactive 2,4-D the bulk of it remained in these organs. These results are in agreement with those reported by many investigators (e.g. Crafts & Yamaguchi, 1958, cf. Scott & Morris, 1970; Hay, 1976; Hall *et al.*, 1982; Zemskaya *et al.*, 1984; Lingle & Suttle, 1985; Davis & Linscott, 1986), who used different techniques for the application of radioactive 2,4-D to different parts of plant and from autoradiographs, counting and bioassay analyses they found that the bulk of the material remained in treated tissues.

Addition of PEG to the aqueous solution reduced the total amount of radioactive materials taken up by seedlings. Regarding the distribution pattern of radioactive material this was eventually the same as for seedlings grown without PEG the bulk of this material remained in the treated roots and very little moved upward. The amount of radioactive herbicide was higher in different tissues of seedlings treated with the herbicide without PEG than in those treated in the presence of PEG. The reduction of 2,4-D up take in the presence of PEG was probably related to the plasmolysis of the cells and therefore reduced protoplast contact with the cell walls. These results suggested that drought stress may have an effect on the uptake and distribution of root-applied 2,4-D in both monocots and dicots plants, despite the data reported by Basler *et al.*, (1961) and Pallas & Williams (1962) which showed that moisture stress had no effect on the absorption of radioactive 2,4-D applied to the leaves of beans.

These data showed clearly that PEG reducing the uptake of radioactive 2,4-D, however, distribution patterns were the same for H_2O or PEG-treated seedlings at 6 hours. Since PEG reduced the uptake of 2,4-D then the effective concentration of this compound in these plants would be lower than for those taking the herbicide

from solution without PEG-tretment. This implies that the efficiency of 2,4-D in affecting plant function is increased by application of water stress condition through the use of PEG.

On the other hand after seedlings had been transferred from 2,4-D+PEG to H_2O or PEG and from 2,4-D+H_2O to H_2O or PEG, the patterns of distribution did not change. Therefore the release of stress appeared to have no effect on translocation patterns. Basler *et al.*, (1961) and Pallas & Williams (1962), however, indicated that moisture stress had some effect on 2,4-D translocation, but that was with leaf applied 2,4-D.

The loss of radioactive materials from seedlings transferred between solutions could be due to leakage from the seedlings or metabolism of the herbicide in plant tissue. This effect could be the result of the loss of extra cellular 2,4-D which would be removed most easily. With regard to the uptake of 2,4-D from the compost by both radish and maize seedlings, it was evident from the data that the amount taken up by these seedlings was very small. This was probably because 2,4-D was locked up in the soil and therefore it became unavailable for the seedlings. 2,4-D metabolism in the soil by microorganism or its degradation are another possibility.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Numerous reports have shown that 2,4-D can affect the process of germination; by reducing the rate of seed germination (Hamner *et al.*, 1946; Sasaki *et al.*, 1968) or delaying the germination of seeds for many species (Allard *et al.*, 1946; Sasaki *et al.*, 1968). However, most of the investigators stressed that the inhibition of seed germination only occurred when the seeds were treated with comparatively high concentrations (more than 100 ppm) (Sasaki *et al.*, 1968). At low concentrations of 2,4-D (up to 10 ppm) no significant effect was found on the germination of seeds (Audus & Quastel, 1947). 2,4-D has also been used as pre-emergence herbicide (Kasasian, 1971; Bovey, 1980) to prevent the germination of weed seeds in field crops.

In this respect a number of experiments were conducted to investigate the effect of different concentrations of 2,4-D on the germination of radish and rye grass seeds using different methods for the herbicide application. The results of these experiments showed that at concentrations of 10-100 ppm where 2,4-D was maintained in direct contact with seeds of radish for periods of time up to 12 days; it reduced the final percentages of germination at high concentration and increased the time needed to complete germination. In contrast treatment of radish seeds by soaking in solutions of 2,4-D at concentrations of 1, 10 and 100 ppm for 24 hours, allowed normal germination without any delay in respect to the control. Meanwhile when seeds were soaked in the same solution for similar periods of time, and planted in compost the emergence of seedlings was delayed but there was no effect on the final percentages of seedling emergence for seeds treated at 1 and 10 ppm. However, emergence of seedlings from seeds treated at 100 ppm was dramatically reduced. On

the other hand treating soil with 100 ppm of 2,4-D had no effect on seed germination or seedling emergence.

Unlike radish seeds, rye grass caryopses treated with 2,4-D at concentrations of 10-100 ppm, where 2,4-D was maintained in direct contact with the caryopses no altered caryopses germination was seen. Seven days after treatment, treated caryopses showed almost complete germination for all concentrations in respect to the control caryopses.

In the light of these results, it appeared that 2,4-D has very little effect on germination of seeds despite many reports about the inhibitory effect of 2,4-D to seed germination (e.g. Mayer *et al.*, 1975; Parker, 1976; Hamner *et al.*, 1946) but in agreement with Audus & Quastel (1947). However, 2,4-D was shown to delay the process of germination in radish seeds at comparatively low concentration and to reduce the percentages of seed germination only at high concentration when the herbicide was maintained in direct contact with the seeds. On the other hand 2,4-D had no effect on the germination of rye grass caryopses at the concentrations used.

When the radish seeds were germinated in nutrient solutions containing 2,4-D at concentrations of 1, 50 and 100 ppm with PEG, the response of seeds was changed. 2,4-D, 2,4-D+PEG, PEG-treated and control seeds germinated normally and in a very short period of time compared with seeds treated with solutions of 2,4-D without nutrient elements. It appears that nutrient supply overrides the effect of 2,4-D to some extent. At the same time PEG showed no effect on germination of radish seeds at the water potentials used. When the seeds were treated with 2,4-D at concentrations higher than 100 ppm (125, 250, 500 and 1000 ppm) with or without PEG, no effect was noted for these concentrations without PEG on germination, however, an appreciable decrease in the final percentages of germination was caused by these concentrations with PEG. The decrease was closely related to the herbicide

concentration. Since no effect on the final percentages of germination was recorded because of 2,4-D or PEG alone, synergistic action of these two compounds was noted.

Caryopses of rye grass germinated in 2,4-D solution, or in 2,4-D solution which contained PEG, germinated normally indicating that neither 2,4-D nor PEG had any effect on germination. From these results it can be seen that addition of PEG as a drought stress factor at a concentration of 200 g/l to the herbicide solution failed to modify the effects of 2,4-D on caryopses of rye grass and seeds of radish (at concentrations up to 100 ppm). In contrast, addition of PEG to solutions of 2,4-D at high concentrations (more than 100 ppm), however, was shown to act together to reduce the percentages of germination of radish seeds.

Whilst a very little (radish) or no effect (rye grass) was recorded for 2,4-D on seed germination with or without PEG, in all treated seed at all concentrations and in all different types of treatments, the growth and development of seedlings was reduced immediately after germination in both radish and rye grass species. The degree of inhibition was closely related to the concentration of the herbicide and to the sensitivity of the species.

In germinated radish seeds 2,4-D inhibited the expansion of the radicle and cotyledons and completely or partially inhibited the elongation of primary roots in both seeds treated with 2,4-D or 2,4-D+PEG. Addition of PEG appeared to reduce the effect of the herbicide on the root and to increase its effect on the hypocotyl .

Similar responses were noted in germinated caryopses of rye grass. Inhibition of root expansion and elongation of coleoptiles was evident at all concentrations used with or without PEG. In relation to root and shoot development, however, rye grass roots were more sensitive to 2,4-D alone than to the combined 2,4-D+PEG, whilst the shoots were more sensitive to PEG treatment.

These results suggested that 2,4-D is strongly inhibitory to seedling es-

tablishment as suggested by Cartwright (1976), and this makes 2,4-D successful as post-emergence herbicide. This suggestion is supported by the data obtained when seeds of radish were treated with the herbicide and planted in the soil; it was shown that 2,4-D inhibited seedling emergence. When the seeds were investigated it was found that the inhibition was not because of 2,4-D inhibition of germination, but because of severe inhibition of roots and hypocotyls which prevented the seedlings from penetrating the soil. Addition of PEG to 2,4-D solution in which seeds of radish and caryopses of rye grass were germinated was shown to modify its effect on seedling establishment.

Treatment of the root system of radish, rye grass and maize seedlings with 2,4-D in nutrient solutions resulted in a marked reduction or inhibition of growth and caused distinct morphological distortions in these seedlings. Rye grass and maize were more resistant to the herbicide than radish, however. These are very common symptoms for 2,4-D and have been reported by many investigators (e.g. Beal, 1944; Audus & Quastel, 1947; Audus, 1949; Taylor & Maj, 1946).

Sudden exposure of seedlings to drought stress following herbicide treatment by addition of PEG, also had very devastating effects on the growth and development of these seedlings. Seedlings of all three species were shown to be more sensitive to drought stress than the herbicide and their response to drought stress was also much faster than to the herbicide. These severe and fast responses to drought stress are possibly because of the sudden exposure of seedlings to drought stress which does not favour osmotic adjustment (Conroy *et al.*, 1988).

Bending and twisting of radish seedlings a few hours after 2,4-D treatment are very common symptoms. Exposure of the seedlings to drought stress 24 hours later was shown to put the seedlings under stress of a different type. 2,4-D treated and untreated seedlings which had been treated with PEG wilted and their shoots began

to dry out apparently because of water deficit. Inhibition of shoot growth was noted for seedlings treated with 2,4-D, PEG and combined 2,4-D+PEG. Inhibition of cell division and cell enlargement by 2,4-D (Hanson & Slife, 1961) and cell expansion and cell enlargement by drought stress (Fitter & Hay, 1983) could be the prime cause for the inhibition of growth by these compounds. PEG, however, was shown to decrease the inhibition and minimize the morphological malformation in the roots. This may be because of the reduction in absorption of the herbicide by drought stress. On the other hand PEG had no effect in modifying the mode of action of 2,4-D. Morphological malformation in the hypocotyl such as swelling, longitudinal splitting and inhibition of thickening were evident in all 2,4-D-treated seedlings.

Unlike radish, rye grass seedlings treated with 2,4-D in nutrient solutions developed no uniform or distinct symptoms of twisting or epinasty such as those observed on radish seedlings during the first 24 hours before drought stress treatment. This kind of responses was as expected, because this plant is resistant (monocot) to 2,4-D. When the seedlings were exposed to drought stress, symptoms similar to those observed on radish seedlings developed; wilting of leaves of seedlings treated with PEG and inhibition of their growth were evident particularly in those treated with 2,4-D+PEG. The inhibition was less severe in seedlings treated with PEG or 2,4-D alone. Growth and development of roots was also suppressed by all treatments. 2,4-D, however, caused more damage to the roots than combined 2,4-D+PEG. The severity of reduction of 2,4-D on roots was reduced by PEG in seedlings treated with 2,4-D+PEG.

Maize seedlings treated with 2,4-D at 100 ppm and exposed to drought stress (-10 bars) 24 hours later, were similar to rye grass seedlings in their response. One day after drought stress treatment, seedlings growing in nutrient solution which which contained 2,4-D+PEG and seedlings growing in nutrient solution which contained PEG, began to wilt and their leaf tips began to dry out. Three days latter pre-

mature death of leaf tissues continued from leaf tips towards the base in seedlings under drought stress (2,4-D-treated and untreated seedlings). Some seedlings began to die after this period, but visual inspection showed that 2,4-D-treated seedlings growing under drought stress appeared more affected than those growing under drought stress alone.

Inhibition of root growth, swelling of root tips and general lack of development of adventitious roots were also evident. As a result the growth inhibition of the shoots, reduction in shoot fresh and dry weight was significantly high in all treatments compared to untreated seedlings.

When maize seedlings were treated at an early stage (before the expansion of leaves) with the same concentration of 2,4-D (100 ppm) and lower amount of PEG (125 g/l), drought stress appeared to have no effect on the shoots of seedlings. PEG-treated seedlings showed normal leaf expansion. In contrast, seedlings treated with 2,4-D or 2,4-D+PEG exhibited reduced leaf number. It is apparent from the data that 2,4-D alone was responsible for this reduction not PEG, since PEG-treated seedlings showed no reduction in leaf number. Moreover reduction in the total leaf area was found only in seedlings treated with 2,4-D. Combined 2,4-D+PEG, however, was shown to have marked effects on elongation of the second internode, primary root and mesocotyl. This is suggested as some interaction between the two compounds, since no effect was seen on these organs by either of these compounds alone.

Foliar treatment of radish and rye grass seedlings with 2,4-D resulted in marked inhibition of growth and development accompanied by morphological distortion of plant organs. Rye grass seedlings, however, showed more resistance to the herbicide than radish seedlings. As a result of 2,4-D treatment, response of the seedlings to drought stress was modified and subsequently their growth and development were also affected.

Radish seedlings treated with different concentrations of 2,4-D developed morphological bending, twisting and swelling of plant organs. For seedlings which were kept under normal watering regime, the twisting of petioles, hypocotyls and curling of cotyledons disappeared and seedlings recovered. However, under drought stress 2,4-D-treated seedlings remained twisted. Moreover, reduction in total fresh and dry weight was significant both for seedlings growing under drought stress and normal watering regime following the application of 2,4-D. Further more the survival rate of radish seedlings treated with 2,4-D and exposed to drought stress was significantly reduced in respect to stressed untreated seedlings. These results suggested that 2,4-D may have an inhibitory effect on the basic metabolic pathway (Fedtke, 1982), under drought stress and under normal watering regime, but under drought stress the problem becomes worse, since drought stress has a similar inhibitory effect on the metabolic pathways (Hsiao, 1973). Because of this combined effect of 2,4-D and drought stress the seedlings had no chance to recover or to overcome the bending effect of 2,4-D. Such effects argue that the herbicide places a stress on the treated plants and that this stress makes them more susceptible to drought stress(Muzik, 1976).

Long-term effects of 2,4-D on growth and development of radish seedlings were very severe. The most striking effect was on the growing point of the leaf. Leaf expansion was inhibited and subsequently the number of leaves was reduced. The morphology of leaves was completely changed; some leaves changed from a simple leaf to semi-double leaves on the same petiole and arising from one primordia and some leaves were smaller and narrower than normal. In addition to its effect on leaves, 2,4-D was shown to effect root and hypocotyl development. Stunting and thickening of roots with longitudinal rupture, inhibition of thickening and proliferation of cells of the hypocotyls were marked in treated seedlings. These kinds of distortions showed that 2,4-D may have the ability to interfere in cell division, expansion and elongation

and have been noted by Van Andel *et al.* (1976).

Foliar treatment of rye grass seedlings with 2,4-D appeared to leave no distortion of the shoots under both drought stress and normal watering regimes. However, in the long-term and at later stages, 2,4-D proved to be toxic to these seedlings. All treated seedlings growing under normal watering regime or under drought stress condition developed abnormalities known as "incomplete heading" (Audus, 1959, cf. Van Andel *et al.*, 1976). Moreover, reduction of the number of caryopses in 2,4-D-treated seedlings was also noted. Number of inflorescences, however, was increased in these seedlings. The reduction in the number of caryopses has been reported to be caused by the failure of ovules to develop (Kiermayer, 1956, cf. Van Andel *et al.*, 1976). Whilst the increase in the number of inflorescences may be a result of an increase in the volumes of root system to overcome the drought stress condition which in turn results in an increase in the number of tillers and subsequently inflorescences.

According to Cherry (1976) and Fedtke (1982) cell proliferation in the tissues of 2,4-D-treated plants is accompanied by changes in nucleic acid and protein metabolism which in turn is likely to cause changes in other cellular processes. Exposure of treated seedlings to drought stress was shown to modify the pattern of changes in these processes.

The effect of root-applied 2,4-D, 2,4-D+PEG and PEG on chlorophyll biosynthesis in radish cotyledons were considerable. During the first 48 hours 2,4-D had more effect in reducing biosynthesis of chlorophyll a, b and a+b than PEG alone or 2,4-D+PEG. However, this effect appeared to be transient, since the biosynthesis of chlorophyll recovered 96 hours after treatment. Meanwhile the effect of PEG was shown to increase with time and was also shown to lessen the effect of 2,4-D in seedlings treated with 2,4-D+PEG over the period of 48 hours after treatment. After this period it appeared to have more effect on the reduction of chlorophyll biosyn-

thesis than 2,4-D or PEG alone. This result suggests that the combined 2,4-D+PEG may have synergistic effect. The chlorophyll a/b ratio in 2,4-D-treated seedlings was shown to increase with time until it reached its peak 96 hours after treatment with similar patterns of increase in both chlorophyll a and b. PEG-treated seedlings also showed similar patterns of increase but chlorophyll b decline sharply 48 hours after treatment. However, by the end of the test period a/b ratio was significantly higher in seedlings treated with 2,4-D and PEG alone than in the controls. At the same time 2,4-D+PEG increased the a/b ratio until it reached its peak 48 hours after treatment and then declined to the lowest level 96 hours after treatment.

Biosynthesis of chlorophyll in the first leaf of rye grass seedlings treated through the roots was different from that in radish cotyledons. Chlorophyll biosynthesis in control seedlings increased linearly during the first 24 hours, followed by a decline at 48 hours and at 96 hours recovered more than it was at 24 hours after treatment. 2,4-D was shown to have more effect on chlorophyll biosynthesis through out the test period. However, it was similar to radish cotyledons, the effect appeared to be transient. In PEG-treated seedlings chlorophyll biosynthesis continued increasing until 96 hours, at which time began to decline. In the mean time chlorophyll biosynthesis in 2,4-D+PEG-treated seedlings increased linearly during the test period of 96 hours. At the end of the experiment PEG-treated seedlings synthesized more chlorophyll than all other seedlings including the controls, followed by 2,4-D+PEG-treated seedlings and the controls. 2,4-D-treated seedlings synthesized the lowest chlorophyll in respect to all other seedlings. These data suggested that PEG may have altered the water status of the cells, therefore more tissues were used to give weight similar to 2,4-D-treated and untreated tissues. Moreover, these data showed that 2,4-D may have an effect on chlorophyll biosynthesis during the first 24 hours after treatment and exposure to light, after which chlorophyll biosynthesis appear to recover.

It is apparent from these results that 2,4-D is unlikely to have any direct

inhibition of chlorophyll synthesis. It is possible, however, that through its effect on proteins which include both enzymes directly involved in chlorophyll synthesis and structural proteins of the chloroplast, it could have affected chlorophyll biosynthesis. Exposure of treated seedlings to drought stress may have increased the effect.

Additional to its effect on chlorophyll biosynthesis 2,4-D was also shown to reduce total protein levels in the leaves of treated radish seedlings. The reduction was closely related to 2,4-D concentration. Soluble protein was increased in preference of insoluble protein only under the lowest concentration (1 ppm). At high concentrations, however, 2,4-D decreased the levels of soluble protein. Exposure of 2,4-D-treated seedlings to drought stress was shown to reduce the levels of total protein more than 2,4-D or drought stress alone. Whilst seedlings treated with 1 ppm of 2,4-D+drought stress showed an increase in soluble protein at higher concentrations of herbicide soluble protein was also decreased.

The results reported here regarding the effect of 2,4-D and drought stress on protein levels in leaves of radish seedlings, confirm the results reported by Hsiao (1973), Brady *et al.* (1974), Dhindsa & Bewley (1976), Cooke *et al.* (1980) and Hanson (1982) who demonstrated that protein was decreased by drought stress also the results of Singh *et al.*, (1973) who found a significant increase in soluble protein content after drought stress was imposed.

The obtained results also confirm these found by Key *et al.* (1966) who showed that 2,4-D can reduce total protein content. However, the increase in soluble protein at low concentration of 2,4-D and its decrease at high concentration, suggest that 2,4-D at low concentration may stimulate more enzyme synthesis, whilst at high concentration it inhibited the function of soluble protein.

On the other hand the reduction in protein by combined 2,4-D+drought stress suggested that 2,4-D and drought stress may have synergistic action. Moreover

the decrease in soluble protein at high concentration of the herbicide in preference of insoluble protein, suggest that high concentration of 2,4-D may have overcome the effect of drought stress so reducing the ability of seedlings to respond to drought stress.

Despite its effect on chlorophyll and protein biosynthesis 2,4-D alone appeared to have no effect on proline accumulation, at least in the short-term. However, it was shown to effect the production of proline in drought stressed seedlings. In contrast, the data presented here showed that drought stress alone, induced by withholding water or by PEG treatment in nutrient solution, resulted in the accumulation of considerable amounts of proline in radish and rye grass seedlings. These results are in agreement with the results reported by Singh (1973), Wample & Bewley (1975), Rajagopal & Andersen (1978) and Levy (1983). The present results also showed that the accumulation of proline by stressed radish and rye grass seedlings was modified by 2,4-D treatment.

Foliar treatment of radish seedlings with 2,4-D followed by drought stress resulted in the inhibition of proline accumulation in the leaves at high concentration or significant reduction at low concentration compared to untreated stressed seedlings.

Pre-treatment of radish seedlings through the roots in nutrient solution with 2,4-D followed by drought stress at intervals or exposure of these seedlings to drought stress followed by 2,4-D treatment at intervals, showed a significant reduction in the accumulation of proline in the cotyledons and hypocotyls of these seedlings compared to untreated stressed seedlings. Pre-treatment of radish seedlings with 2,4-D before they were exposed to drought stress, however, was shown to have more effect in reducing the levels of proline.

These results suggested that 2,4-D has more effect on proline accumulation at the site of application, where the bulk of it remained as found by Hay

(1976) and Zemskaya (1984). This suggestion is supported by the data obtained from foliar-treated seedlings where the proline accumulation was inhibited completely at high concentrations and from root-treated seedlings where the effect was less severe. Moreover these data showed that 2,4-D concentration was important, since in foliar-treated seedlings whereas 10 and 100 ppm of the herbicide inhibited proline accumulation completely, at 1 ppm only reduced the amount of proline accumulated by these seedlings.

Under foliar-treatment rye grass responded differently to the herbicide. Unlike radish, proline levels increased significantly in stressed seedlings in respect to those stressed but untreated with 2,4-D. At the same time root treatment of rye grass seedlings with 2,4-D before or after drought stress was applied, reduced proline accumulation in the leaves of these seedlings in respect to stressed untreated seedlings. Similar to radish, pre-treatment of rye grass seedlings with 2,4-D before they were exposed to drought stress was more effective in reducing the levels of proline than post-drought treatment.

These results suggested that poor entry of the herbicide through the leaves probably was the main cause of proline increase in foliar treated seedlings. As small amount of 2,4-D may have stimulated proline accumulation. In this respect the morphology of rye grass leaves can have great effect in the prevention of 2,4-D entry.

It is apparent from these results that responses of radish and rye grass seedlings to foliar-applied and root-applied 2,4-D are different in terms of proline accumulation. This led to the belief that the uptake and movement of 2,4-D may have affected the response of these seedlings.

It was evident from the data reported here that both radish and rye grass seedlings fed with aqueous solutions of radioactive 2,4-D through the roots show little transport of the herbicide out of these organs. These are results in accord

with those found by Hay (1976), Hall *et al.* (1982), Zemskaya *et al.* (1984), Lingle & Suttle (1985) and Davis & Linscott (1986). Addition of PEG to the solution reduced the total amount of radioactive materials taken up by these seedlings and decreased the amount present in different organs of seedlings particularly the leaves and the cotyledons. The reduction of 2,4-D uptake by PEG was probably a result of plasmolysis of the cells and therefore reduced protopast contact with the cell walls.

From these result it is apparent that very little amount of 2,4-D can get to the leaves of root-treated seedlings growing under drought stress, yet dramatic changes in proline levels in the leaves of these seedlings can be seen. This suggests the involvement of another factor(s), probably some signalling between the roots and the leaves. Whatever the signal is, it is not of necessity 2,4-D which elicits the response in the leaves. Since response to foliar spray is not the same as root application, this implies that some intermedia ry compound may be initiated from the roots. Such a factor could be abscisic acid since Pinfield & Tillberg (1987) have indicated that this compound increase in tissue which has been treated with 2,4-D.

Finally one may conclude that;

1. Application of 2,4-D to the seeds of monocots and dicots, whilst having no effect on germination at low concentration, at high concentration and in combination with drought stress may influence the process of germination. Moreover seed treatment was shown to inhibit completely or partially (depending on the concentration) the growth and development of roots and shoots in both species at concentration from 1 ppm onward.

2. Nutrient supply can overcome the effect the herbicide on seed germination.

3. Root-applied 2,4-D to the seedlings in nutrient solutions reduced the growth of roots and inhibited completely the growth and development of adventitious and lateral roots in both species. Addition of PEG to the herbicide solution was shown to reduce its effect on the roots and increase the effect on the shoots.

4. Although seedlings of monocots and dicots responded differently to the foliar application of 2,4-D, roots responded similarly to this herbicide.

5. Toxicity symptoms on monocots, whilst not appearent at early stages may become a problem in long-term growth.

6. 2,4-D does influence the response of seedlings to drought stress and application of 2,4-D must be considered in relation to other environmental factors.

7. Combined 2,4-D and drought stress appeared to show a synergistic effect.

8. Shoot and root treatment with 2,4-D may affect the responses of susceptible species to drought stress by reducing their ability to resist drought through an effect on the accumulation of proline and soluble proteins under drought stress condition. At the same time foliar treatment with 2,4-D may improve the response of resistant species to drought stress by enhancement of proline accumulation in these species which makes them more resistant to drought injury.

9. Drought stress can reduce the uptake and movement of root-applied 2,4-D in both monocots and dicots species.

REFERENCES

ÅBERG, E. & STECKO, V. (1976). Internal factors affecting toxicity. In *Herbicides: Physiology, Biochemistry, Ecology*, 2nd edn, vol.2 (ed. L.J. Audus), pp. 175-201, Academic Press, London, New York and San Francisco.

ACEVEDO, E., HSIAO, T. C. & HENDERSON, D. W. (1971). Immediate and subsequent growth responses of maize leaves to changes in water status. *Plant Physiology* **48**, 631-636.

AHMEDULLAH, M., MARQUIS, L., YANG, C. & KAWAKAMI, A. (1985). Metabolism of 2,4-D in grape. *Journal of American Society of Horticultural Science* **110**(4), 480-484.

ALAIB, M. A. (1985). Seedling Responses to Growth Regulators and Drought Stress. *M. SC. Dissertation*, University of Durham.

ALLARD, R. W., DEROSE, H. R. & SWANSON, C. P. (1946). Some effects of plant growth-regulators on seed germination and seedling development. *Botanical Gazette* **107**, 575-583.

APELBAUM, A. K. & YANG, S. F. A. (1981). Biosynthesis of stress ethylene induced by water deficit. *Plant Physiology* **68**, 594-596.

ARNON, D. I. (1949). Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*. *Plant physiology* **24**, 1-15.

ASHTON, F. M. & BAYER, D. E. (1976). Effects on solute transport and plant coonstituents. In *Herbicides: Physiology, Biochemistry, Ecology*, 2nd edn, vol.2 (ed. L. J. Audus), pp. 219-253, Academic Press, London, New York and San Francisco.

ASHTON, F. M. & CRAFTS, A. S. (1981). Absorption and translocation of herbicides. In *Mode of Action of Herbicides*, 2nd edn, pp. 20-39, A Wiley-Interscience Publication, John Wiley & Sons, New York, Chichester, Brisbane and Toronto.

ASHTON, F. M. & CRAFTS, A. S. (1981). Biochemical responses to herbicides.

In *Mode of Action of Herbicides*, 2nd edn, pp. 49-64, A Wiley-Interscience Publication, John Wiley & Sons, New York, Chichester, Brisbane and Toronto.

ASHTON, F. M. & CRAFTS, A. S. (1981). Phenoxys. In *Mode of Action of Herbicides*, 2nd edn, pp. 272-302, A Wiley-Interscience Publication, John Wiley & Sons, New York, Chichester, Brisbane and Toronto.

ASPINALL, D., SINGH, T. N. & PALEG, L. G. (1973). Stress metabolism. V. Abscisic acid and nitrogen metabolism in barley and *Lolium temulentum* L. *Australian Journal of Biological Science* **26**, 319-327.

AUDUS, L. J. (1949). The pH-relationships of root growth and its inhibition by 2,4-dichlorophenoxyacetic acid and coumarin. *New Phytologist* **48**, 97 -112.

AUDUS, L. J. & QUASTEL, J. H. (1947). Coumarin as a selective phytocidal agent. *Nature* **159**, 320-324.

BARNETT, N. M. & NAYLOR, A. W. (1966). Amino acid and protein metabolism in bermuda grass during water stress. *Plant Physiology* **41**, 1222-1230.

BARRIER, G. D. & LOOMIS, W. E. (1957). Absorption and translocation of 2,4-dichlorophenoxyacetic acid and P^{32} by leaves. *Plant Physiology* **32**, 225-231.

BASLER, E., TODD, G. W. & MEYER, R. E. (1961). Effects of moisture stress on absorption, translocation, and distribution of 2,4-dichlorophenoxyacetic acid in bean plants. *Plant Physiology* **36**, 573-576.

BATES, L. S., WALDREN, R. P. & TEARE, I. D. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil* **39**, 205-207.

BEAL, J. M. (1944). Further observations on the telemorphic effects of certain growth-regulating substances. *Botanical Gazette* **106**, 165-178.

BEAL, J. M. (1944). Some telemorphic effects induced in sweet pea by application of 4-chlorophenoxyacetic acid. *Botanical Gazette* **105**, 471-474.

BEWLEY, J. D. (1979). Phsiological aspects of desiccation tolerance. *Annual Review of Plant Physiology* **30**, 195-238.

BEWLEY, J. D. & BLACK, M. (1978). *Physiology and Biochemistry of Seeds.* vol.2,

Springer-Verlag, Brlin, Hedelberg and New York.

BONNER, F. T.(1968). Water uptake and germination of red oak acorns. *Botanical Gazette* **129**(1), 83-85.

BOVEY, R. W. (1980). Physiological effects of phenoxy herbicides in higher plants. In *The Science of 2,4,5-T and Associated Phenoxy Herbicides* (ed. R.W.Bovey & A.L.Young), pp. 217-238. John Wiley, New York.

BOYER, J. S. (1970). Leaf enlargement and metabolic rates in corn, soybean, and sunflower at various leaf water potentials. *Plant Physiology* **46**, 233-235.

BRADY, C. J., SCOTT, N. S. & MUNNS, R. (1974). The interaction of water stress with the senescence pattern of leaves. In *Mechanism of Regulation of Plant Growth.* (ed. R. L. Bieleski, A. R. Ferguson, M. M. Cresswell) Bulletin **12**, The Royal Society of New Zealand, Wellington.

BREEZE, V. G. & WEST, C. J. (1987). Long-and short-term effects of vapour of the herbicide 2,4-D butyl on the growth of tomato plants. *Weed Research* **27**, 13-21.

BREEZE, V. G. & WEST, C. J. (1987). Effects of 2,4-D butyl vapour on the growth of six crop species. *Annals of Applied Biology* **111**, 185-191.

BRIAN, R. C. (1964). The metabolism of herbicides. *Weed Research* **4**, 105-117.

BRIAN, R. C.(1976). The history and classification of herbicides. In *Herbicides: Physiology, Biochemistry, Ecology*, 2nd edn, vol.1 (ed. L. J. Audus), pp. 1-50, Academic Press, London, New York and San Francisco.

BRIX, H. (1962). The effect of water stress on the rates of photosynthesis and respiration in tomato plant and loblolly pine seedlings. *Physiologia Plantarum* **15**, 10-20.

BUKOVAC, M. J. (1976). Herbicide entry into plants. In *Herbicides: Physiology, Biochemistry, Ecology*, 2nd edn, vol.1 (ed. L. J. Audus), pp. 335-364, Academic Press, London, New York and San Francisco.

CARTWRIGHT, P. M. (1976). General growth responses of plants. In *Herbicides: Physiology, Biochemistry, Ecology,* 2nd edn, vol.1 (ed. L. J. Audus), pp. 125-

190, Academic Press, London, New York and San Francisco.

CHERRY, J. H. (1976). Action on nucleic acid and protein metabolism. In *Herbicides: Physiology, Biochemistry, Ecology*, 2nd edn, (ed. L. J. Audus), pp. 525-546, Academic Press, London, new York and San Francisco.

CHKANIKOV, D. I., MAKEEV, A. M., PAVLOVA, N. N. & NAZAROVA, T.A. (1982). Metabolism of 2,4-D in cultivated cereals. *Soviet Plant Physiology* English Translation **29** (32), 425-431.

CONROY, J. P., VIRGONA, J. M., SMILLIE, R. M. & BARLOW, E. W. (1988). Influence of drought acclimation and CO_2 enrichment on osmotic adjustment and chlorophyll a fluorescences of sunflower during drought. *Plant Physiology* **86**, 1108-1115.

COOKE, R. J., ROBERTS, K. & DAVIES, D. D. (1980). Model for stress-induced protein degradation in *Lemna minor*. *Plant Physiology* **66**, 1119 -1122.

CRAFTS, A. S. (1961). *The Chemistry and Mode of Action of Herbicides.* Interscience Publication, John Wiley & Sons, New York.

DAVIDONIS, G. H., HAMILTON, R. H. & MUMMA, R. O. (1982). Metabolism of 2,4-dichlorophenoxyacetic acid in 2,4-dichlorophenoxyacetic acid-resistant soybean cullus tissue. *Plant Physiology* **70**, 104-107.

DAVIDONIS, G. H., HAMILTON, R. H., VALLEJO, R. P., BULY, R. & MUMM-A, R. O. (1982). Biological properties of D-amino acid conjugates of 2,4-D. *Plant Physiology* **70**, 357-360.

DAVIS, C. & LINSCOTT, D. L. (1986). Tolerance of birdsfoot trefoil (*Lotus corniculatus*) to 2,4-D. *Weed Science* **34**, 373-376.

De LUCA d'ORO, G. M. & TRIPPI, V. S. (1987). Effect of stress conditions induced by temperature, water and rain on senescence development. *Plant Cell Physiology* **28**(8), 1389-1396.

DHINDSA, R. S, & BEWLEY, J. D. (1976). Water stress and protein synthesis. IV. Responses of drought-tolerant plant. *Journal of Experimental Botany* **27**(98), 513-523.

EAMES, A. J. (1949). Comparative effect of spray treatments with growth-regulating substances on the nut grass (*Cyperus rotundus L.*), and anatomical modifications following treatment with butyl 2,4-dichlorophenoxyacetic acid. *American Journal of Botany* **36**, 571-584.

EAMES, A. J. (1950). Destruction of phloem in young bean plants after treatment with 2,4-D. *American Journal of Botany* **37**, 840-847.

ETHERINGTON, J. R. & ARMSTRONG, W. (1975). Plants and water deficit: Physiological aspects. In *Enviromnment and Plant Ecology*, pp. 130-153, John Wiley & Sons, London, New York, Sydney and Toronto.

FEDTKE, C. (1982). *Biochemistry and Physiology of Herbicide Action.* Springer-Verlag, Berlin, Heidelberg and New York.

FITTER, A. H. & HAY, R. K. M. (1983). *Environmental Physiology of Plants*, Academic Press, London.

FOWLER, D. B., GRAHAM, M. L. & ASHFORD, R. (1986). Influence of fall applied 2,4-D amine and MCPA on cold hardiness and other agronomic characters of winter wheat and rye. *Canadian Journal of Plant Science* **66**, 837-843.

GILE, J. D. (1983). 2,4-D Its distribution and effects in a ryegrass ecosystem. *Journal of Environmental Quality* **12**(3), 406-412.

GOLEBSKI, W., MEYER, R. & WAGNER, R. G. (1988). Changes in the nucleotide pools in leaves and buds of tobacco plants upon treatment with 2,4-dichlorophen-oxyacetic acid. *Physiologia Plantarum* **72**, 29-35.

GREACEN, E. L. & OH, J. S. (1972). Physics of root growth. *Nature* **235**, 24-25.

GRESSEL, J. (1985). Herbicide tolerance and resistance: Alteration of site of activity. In *Weed Physiology*, vol.II, (ed. S. O. Duke), pp. 160-184, CRC Press, Boca Raton, Florida.

GRIFFIN, J. L., WATSON, V. H., KNIGHT, W. E. & COLE, A. W. (1984). Forage legume responses to dicamba and 2,4-D application. *Agronomy Journal* **79**, 487-490.

HAGIN, R. D., LINSCOTT, D. L. & DAWSON, J. E. (1970). 2,4-D Metabolism in

resistant grasses. *Journal of Agricultural and Food Chemistry* **18**, 848-850.

HALL, C., EDGINGTON, L. V. & SWITZER, C. M. (1982). Translocation of different 2,4-D, bentazon, diclofop, or diclofop-methyl combinations in oat (*Avena sativa*) and soybean (*Glycine max*). *Weed Science* **30**, 676-682.

HALLAM, N. D. (1970). The effect of 2,4-dichlorophenoxyacetic acid and related compounds on the fine structure of the primary leaves of *Phaseolus vulgaris* . *Journal of Experimental Botany* **21**(69), 1031-1038.

HAMANN, R. & KETTRUP, A. (1987). Determination of phenoxy acid herbicides in water samples. *Chemosphere* **16**(2/3), 527-536.

HAMNER, C. L., MOULTON, J. E. & TUKEY, H. B. (1946). Effect of treating soil and seeds with 2,4-dichlorophenoxyacetic acid on germination and development of seedlings. *Botanical Gazette* **107**, 352-361.

HAMNER, C. L. & TUKEY, H. B. (1944). Selective herbicidal action of midsummer and fall applications of 2,4-dichlorophenoxyacetic acid. *Botanical Gazette* **106**, 232-245.

HANDA, S., HANDA, A. K., HASEGAWA, P. M. & BRESSAN, R. A. (1986). Proline accumulation and the adaptation of cultured plant cells to water stress. *Plant Physiology* **80**, 938-945.

HANSON, A. D. & HITZ, W. D. (1982). Metabolic responses of mesophytes to plant water deficits. *Annual Review of Plant Physiology* **33**, 163-203.

HANSON, A. D., NELSON, C. E. (1980). Water: Adaptation of crops in drought-prone environments. In *The Biology of Crop Productivity*, (ed. P. S. Carlson), pp. 77-152, New York, Academic Press.

HANSON, A. D., NELSON, C. E., PEDERSEN, A. R. & EVERSON, E. H. (1979). Cpacity for proline accumulation during water stress in barley and its implications for breeding for drought resistance. *Crop Science* **19**, 489-493.

HANSON, J. B. & SLIFE, F. W. (1961). How does 2,4-D kill a plant ?. *Illinois Research* **3**(2).

HANSON, J. B. & SLIFE, F. W. (1969). Role of RNA metabolism in the action of

auxin-herbicides. *Residue Reviews* **25**, 59-67.

HARIHARAN, M. & UNNIKRISHMAN, K. (1985). Application of a low dose of 2,4-D to promote fruit development in *Piper nigrum*. *Seed Science & Technology* **13**, 257-264.

HATHWAY, D. E. (1986). Herbicide selectivity. *Biological Review* **61**, 435-486.

HAUSER, E. W. (1955). Absorption of 2,4-dichlorophenoxyacetic acid by soybean and corn plants. *Agronomy Journal* **47**, 32-36.

HAY, J. R. (1976). Herbicide transport in plants. In *Herbicides: Phsiology, Biochemistry, Ecology*, 2nd edn, vol.1 (ed. L. J. Audus), pp. 363-396, Academic Press, London, New York and San Francisco.

HEATH, R. L., FURBANK, R. T. & WALKER, B. A. (1985). Effects of polyethylene glycol-induced osmotic stress on transpiration and photosynthesis in pinto bean leaf discs. *Plant Physiology* **78**, 627-629.

HENCKEL, P. A. (1964). Physiology of plants under drought. *Annual Review of Plant Physiology* **15**, 363-386.

HENSON, I. E. (1982). Abscisic acid and water relations of rice (Oryza sativa L.): Sequential responses to water stress in the leaf. *Annals of Botany* **50**, 9-24.

HENSON, I. E. & QUARRIE, S. A. (1981). Abscisic acid accumulation in detached cereal leaves in response to water stress. I. Effects of incubation time and severity of stree. *Sonderdruck aus Zeitschrift Fur Pflanzenphysiologie, Band 101, Heft 5, Seite* 431-438.

HERDI, F. (1983). Histological changes in the leaf of sunflower (*Helianthus annuus* L.) in response to 2,4-D treatments. *Acta Agronomica* Palmira **32**(3/4), 288-297.

HOAGLAND. D. R. & ARNON, D. I. (1938). The water culture method for growing plants without soil. *CA, Circular* **347**, University of California College of Agriculture Experiment Station, Berkeley.

HOLM, R. E. & ABELES, F. B. (1968). The role of ethylene in 2,4-D-induced growth inhibition. *Planta* Berlin **78**, 293-304.

HOSHAW, R. W. & GUARD, A. T. (1951). Morphological and anatomical effects of 2,4-D on young corn plants. *Botanical Gazette* **113**, 65-74.

HSIAO, T. C. (1973). Plant responses to water stress. *Annual Review of Plant Physiology* **24**, 519-570.

HSIAO, T. C., ACEVEDO, E., FERERES, E. & HENDERSON, D. W. (1976). Water stress, growth, and osmotic adjustment. *Philosophical Transactions of the Royal Society Series B* **273**, 479-500.

ILAHI, I. & DORFFLING, K. (1982). Changes in abscisic acid and proline levels in maize varieties of different drought resistance. *Physiologia Plantarum* **55**, 129-135.

JACOBSON, A., SHIMABUKURO, R. H., MCMICHAELS, C. (1985). Response of wheat and oat seedlings to root-applied diclofop-methyl and 2,4-dichlorophenoxyacetic acid. *Pesticide Biochemistry and Physiology* **24**, 61-67.

JOHANSON, N. G. & MUZIK, T. J. (1961). Some effects of 2,4-D on wheat yield and root growth. *Botanical Gazette* **122**, 188-194.

KAISER, W. M. (1987). Effects of water deficit on photosynthetic capacity. *Physiologia Plantarum* **71**, 142-149.

KASASIAN, L. (1971). *Weed Control in the Tropics.* Leonard Hill, London.

KAUFMAN, M. R. & ECKARD, (1971). Evaluation of water stress control with polyethylene glycols by analysis of guttation. *Plant Physiology* **47**, 453-456.

KELLY, S. (1949). The effect of temperature on the susceptibility of plants to 2,4-D. *Plant Physiology* **24**, 534-536.

KEY, J. L. & HANSON, J. B. (1961). Some effects of 2,4-dichlorophenoxyacetic acid on soluble nucleotides and nucleic acid of soybean seedlings. *Plant Physiology* **31**, 145-152.

KEY, J. L., LIN, C. Y. GIFFORD, E. M. & DENGLER, R. (1966). Relation of 2,4-D-induced growth abberations to changes in nucleic acid metabolism in soybean seedlings. *Botanical Gazette* **127**, 87-94.

KNUTH, D. E. (1986). *The T_EX Book.* Wesley Publishing Company.

LEVITT, J. (1972). *Responses of Plants to Environmental Stress*, Academic Press, New York and London.

LEVY, D. (1983). Water deficcit enhancement of proline and α-amino nitrogen accumulation in potato plants and its association with susceptibility to drought. *Physiologia Plantarum* **57**, 169-173.

LINCK, A. J. (1976). Effects on the cytology and fine structure of plant cells. In *Herbicides: Physiology, Biochemistry, Ecology*, 2nd edn, vol.1 (ed. L. J. Audus), pp. 81-125, Academic Press, London, New York and San Francisco.

LINGLE, S. E. & SUTTLE, J. C. (1985). Amodel system for the study of 2,4-D translocation in leafy spurge. *Canadian Journal of Plant Science* **65**, 369-377.

LOOS, M. A. (1975). Phenoxyalkanoic acids. In *Herbicides*, vol.1 (eds. P. C. Kearney and D. D. Kaufman), pp. 1-28, Marcel Dekker, New York.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the folin phenol reagent . *Journal of Biological Chemistry* **193**, 265-275.

LYON, D. J. & WILSON, R. G. (1986). Sensitivity of fieldbeans (*Phaseolus vulgaris*) to reduced rates of 2,4-D and dicamba. *Weed Science* **34**, 953-956.

MALIK, R. K., BAYLAN, R. S. & BHAN, V. M. (1986). Sensitivity of wheat cultivars to 2,4-D + dicamba. *Pesticides Bomby* **20**(10), 26-28.

MANGAT, B. S., PASQUINI, F. & KAPOOR, N. N. (1985). Influence of 2,4-dichlorophenoxyacetic acid on the starch content of the biflagellate heterotroph *Polytoma uvella*: biochemical and scanning electron microscopic observations. *Canadian Journal of Botany* **63**(3), 527-532.

MANITASEVIC, J., DUMIJA, L. & HORGAS, D. (1984). Changes of pigments content in wheat cv. Sonja (*Triticum aestivum ssp. vulgare*) induced with herbicides in vivo and vitro. *Fragmenta Herbologica Jugoslavica* **13**(2), 13-18.

MARTH, P. C. & DAVIS, F. F. (1945). Relation of temperature to the selective herbicidal effects of 2,4-dichlorophenoxyacetic acid. *Botanical Gazette* **106**,

463-472.

MATSSUDA, K. & RIAZI, A. (1981). Stress-induced osmotic adjustment in growing regions of barley leaves. *Plant Physiology* **68**, 571-576.

MAYER, A. M. & POLJAKOFF-MAYBER. (1975). *The Germination of Seeds*, 2nd edn, Academic Press, New York and London.

MAYER, A. M. & POLJAKOFF-MAYBER. (1982). *The Germination of Seeds*, 3rd edn, Academic Press, New York and London.

MIRANDA-HAM, M. D. & LOYOLA-VARGAS, V. M. (1987). In vitro effect of PEG and proline on ammonium assimilation enzymes of maize. *Journal of Plant Physiology* **129**, 169-174

MITCHELL, J. W. & MARTH, P. C. (1945). Germination of seeds in soil containing 2,4-dichlorophenoxyacetic acid. *Botanical Gazette* **107**, 408-416.

MORGAN, J. M. (1984). Osmoregulation and water stress in higher plants. *Annual Review of Plant Physiology* **35**, 299-319.

MUZIK, T. J. (1976). Influence of environmental factors on toxicity to plants. In *Herbicides: Physiology, Biochemistry, Ecology,* 2nd edn, vol.2 (ed. L. J. Audus), pp. 203-247, Academic Press, London, New York and San Francisco.

MUZIK, T. J. & MAULDIN, W. G. (1964). Influence of environment on the response of plants to herbicides. *Weeds* **12**, 142-145.

NADAKAVUKAREN, M. J. & McCRACKEN, D. A. (1977). Effect of 2,4-dichlrophenoxy acetic acid on the structure and function of developing chloroplasts. *Planta* **137**, 65-69.

NAKAMURA, C., MORI, N., NAKATA, M. & ONO, H. (1986). 2,4-D resistance in a tobacco cell culture variant. II. Effects of 2,4-D on nucleic acid and protein synthesis and cell respiration. *Plant and Cell Physiology* **27**(2) , 243-251.

NICKELL, L. G. (1979). Controling biological behavior of plants with synthetic plant growth regulating chemicals. In *Plant Growth Substances* (ed. N. B. Mandava), pp. 263-279, *American Chemical Society*, Washington D.C.

NICKELL, L. G. (1982). *Plant Growth Regulators.* Springer-Verlag, Berlin, Heidelberg and New York.

NUTMAN, P. S. & THORNTON, H. G. (1945). Inhibition of plant growth by 2,4-dichlorophenoxyacetic acid and other plant-growth substances. *Nature* **155**, 498-500.

ORDIN, L. (1960). Effect of water stress on cell wall metabolism of *Avena* coleoptile tissue. *Plant Physiology* **35**, 443-450.

PALLAS, J. E. (1959). The effect of soil moisture on the absorption, transloction of 2,4-D. *Plant Physiology* Supplement **34**, xxi.

PALLAS, J. E. & JR. (1960). Effect of temperature & humidity on foliar absorption & translocation of 2,4-dichlorophenoxyacetic acid & benzoic acid. *Plant Physiology* **35**, 575-580.

PALLAS, J. E. & WILLIAMS, G. G. (1962). Foliar absorption and translocation of ^{32}P and 2,4-dichlorophenoxyacetic acid as affected by soil moisture tension. *Botanical Gazette* **123**, 175-180.

PARKER, C. (1976). Effects on dormancy of plant organs. In *Herbicides: Physiology, Biochemistry, Ecology*, 2nd edn, vol.1 (ed. L. J. Audus), pp. 165- 187, Academic Press, London, New York and San Francisco.

PARKER, R. E. (1983). *Introductory Statistics for Biology.* 2nd edn, Edward Arnold, London.

PEKIC, S. & QUARRIE, S. A. (1987). Abscisic acid accumulation in lines of maize differing in drought resistance: A comparison of intact and detached leaves. *Journal of Plant Physiology* **127**, 203-217.

PENNER, D. & ASHTON, F. M. (1966). Biochemical and metabolic changes in plants induced by chlorophenoxy herbicides. *Residue reviews* **14**, 39-113.

PIERCE, M. & RASCHKE, K. (1980). Correlation between loss of turgor and accumulation of abscisic acid in detached leaves. *Planta* **148**, 174- 182.

PINFIELD, N. J., SANCHEZ-TORRES, J. O. & McDERMOTT, C. N. (1984). The modifying effect of gibberellic acid and kinetin on the growth abnormalities

induced by 2,4-D in marrow seedlings and the possible involvement of endogenous ethylene . *Plant Growth Regulation* **2**, 99-109.

PINFIELD, N. J. & TILLBERG, E. (1987). The regulation by 2,4-dichlorophenoxyacetic acid of endogenous abscisic acid levels in seedlings of *Cucurbita pepo* L. *Journal of Plant Physiology* **129**, 211-217.

PINFIELD, N. J. & YOUSIF, O. A. F. (1980). The effects of 2,4-D on lipid mobilisation in germinating seeds of *Cucurbita peop* L. *L. Z. Pflanzenphysiol.*, **99**, 215-224.

RAJAGOPAL, V. & ANDERSEN, A. S. (1978). Does abscisic acid influence proline accumulation in stressed leaves ?. *Planta* **143**, 85-88.

RESNIK, M. E. (1970). Effect of mannitol and polyethylene glycol on phosphorus uptake by maize plants. *Annals of Botany* **34**, 497-504.

RICHARDSON, R. G. (1977). A review of foliar absorption and translocation of 2,4-D and 2,4,5-T. *Weed Research* **17**, 259-272.

RIEDERER, M. & SCHONHERR, J. (1984). Accumulation and transport of 2,4-dichlorophenoxyacetic acid in plant cuticles: I. Sorption in cuticular membrane and its components. *Ecotoxicology and Environmental Safety* **8**, 236-247.

RIES, S. K. (1976). Subtoxic effects on plants. In *Herbicides: Physiology, Biochemistry, Ecology*, 2nd edn, vol.2 (ed. L. J. Audus), pp. 313-344, Academic Press, London, New York and San Francisco.

ROBERTSON, M. M. & KIRKWOOD, R. C. (1970). The mode of action of foliage-applied translocated herbicides with particular reference to the phenoxy acid compounds. II. The mechanism and factors influencing translocation, metabolism and biochemical inhibition. *Weed Research* **10**, 94-120.

ROBNETT, W. E. & MOREY, P. R. (1973). Wood formation in Prosopis: Effect of 2,4-D, 2,4,5-T and TIBA. *American Journal of Botany* **60**(8), 745-754 .

SAIRAM, R. K. TOMER, P. S. & HARIKA, A. S. (1986). Effect of sub-herbicidal levels of 2,4-D, isoproturan and atrazine on the nitrogen metabolism and growth of oats (*Avena sativa* Linn.). *Indian Journal of plant Physiology* **XXIX**(3),

237-242.

SASAKI, S. & KOZLOWSKI, T. T. (1968). Effect of herbicides on seed germination and early seedling development of *Pinus redinosa*. *Botanical Gazette* **129**, 238-246.

SCHULZE, E. D. (1986). Carbon dioxide and water vapor exchange in response to drought in the atmosphere and in the soil. *Annual Review of Plant Physiology* **37**, 247-274.

SCOTT, P. C. & MORRIS, R. O. (1970). Quantitative distribution and metabolism of auxin herbicides in roots. *Plant Physiology* **46**, 680-684.

SHAYKEWICH, C. F. & WILLIAMS, J. (1971). Resistance to water absorption in germinating rapeseeds *Brassica napus Linn.* *Journal of Experimental Botany* **22**(70), 19-24.

SHIMABUKURO, R. H. (1985). Detoxication of herbicides. In *Weed Physiology*, vol.II, (ed. S. O. Duke), pp. 216-236, CRC Press, Boca Raton, Florida.

SHIMABUKURO, R. H., WALSH, W. C. & HOERAUF, R. A. (1986). Reciprocal antagonism between the herbicides, diclofop-methyl and 2,4-D, in corn and soybean tissue culture. *Plant Physiology* **80**, 612-617.

SIKKA, J. & DUBEY, P. S. (1985). Agroecosystem studies. III. Biochemical responses of a maize variety against 2,4-D. *Journal of Environmental Biology* **6**(1), 57-62.

SINGH, T. N., ASPINALL, D., PALEG, L. G. & BOGGESS, S. F. (1973). Stress metabolism. II. Changes in prolin concentration in excised plant tissues. *Australian Journal of Biological Science* **26**, 57-63.

SINGH, T. N., PALEG, L. G. & ASPINALL, D. (1973). Stress metabolism. I. Nitrogen mtabolism and growth in the barley plant during water stress. *Australian Journal of Biological Science* **26**, 45-56.

SINGH, T. N., PALEG, L. G. & ASPINALL, D. 91973). Stress metabolism. III. Variations in response to water deficit in the barley plant. *Australian Journal of Biological Science* **26**, 65-76.

SKOOG, F. (1951). *Plant Growth Substances.* University of Wisconsin Press, Madison.

SMITH, A. E. (1979). Soil persistence experiments with ^{14}C 2,4-D in herbicidal mixture, and field persistence studies with tri-allate and trifluralin both singly and combined. *Weed Research* **19**, 165-170.

STEWART, C. R., BOGGESS, S. F., ASPINALL, D. & PALEG, L. G. (1977). Inhibition of proline oxidation by water stress. *Plant Physiology* **59**, 930-932.

STEWART, G. R. & LEE, J. A. (1974). The role of proline accumulation in halophytes. *Planta* **120**, 279-289.

STEWART, C. R., MORRIS, C. J. & THOMPSON, J. F. (1966). Changes in amino acid content of excised leaves during incubation. II. Role of sugar in the accumulation of proline in wilted leaves. *Plant Physiology* **41**, 1585-1590.

SWANSON, C. P. (1946). Histological responses of the kidney bean to aqueous sprays of 2,4-dichlorophenoxyacetic acid. *Botanical Gazette* **107**, 522-531.

TALOUIZITE, A. & CHAMPIGNY, M. L. (1988). Response of wheat seedlings to short-term drought stress with particular respect to nitrate utilization. *Plant, Cell and Environment* **11**, 149-155.

TAYLOR, D. L. & MAJ, A. U. S. (1946). Observations on the growth of certain plants in nutrient solutions containing synthetic growth-regulating substances. I. Some effects of 2,4-dichlorophenoxyacetic acid. *Botanical Gazette* **107**, 597-611.

TAYLOR, D. L. & MAJ, A. U. S. (1946). Observations on the growth of certain plants in nutrient solutions containing synthetic growth-regulating substances. II. The influence of presentation time. *Botanical Gazette* **107**, 611-619.

TAYLOR, D. L. & MAJ, A. U. S. (1946). Observations on the growth of certain plants in nutrient solutions containing synthetic growth-regulating substances. III. The relative toxicity of isopropylphenylcarbamate and some phenoxyacetic acid derivatives to some cereals. *Botanical Gazette* **107**, 620-629.

TAYLOR, D. L. & MAJ, A. U. S. (1946). Observations on the growth of certain plants

in nutrient solutions containing synthetic growth-regulating substances. IV. The amount of growth in soil and solution cultures treated with equal weights of ammonium 2,4-dichlorophenoxyacetate. *Botanical Gazette* **107**, 630-632.

THOMPSON, H. E., SWANSON, C. P. & NORMAN, A. G. (1946). New growth-regulating compounds. I. Summary of growth inhibitory activities of some organic compounds as determined by three tests. *Botanical Gazette* **107**, 476-507.

TITTLE, F. L. (1987). Auxin-stimulated ethylene production in fern gametophytes and sporophytes. *Physiologia Plantarum* **70**, 499-502.

TROLL, W. & LINDSLEY, J. (1955). A photometric method for the determination of proline. *Journal of Biological Chemistry* **215**, 655-660.

TUKEY, H. B., HAMNER, C. L. & IMHOFE, B. (1945). Histological changes in bindweed and sowthistle following application of 2,4-dichlorophenoxyacetic acid in herbicidal concentrations. *Botanical Gazette* **107**, 393-407.

VAN ANDEL, O. M., ZWEEP, W. V. & GORTER, C. J. (1976). Morphogenetic responses of plants. In *Herbicides: Physiology, Biochemistry, Ecology*, 2nd edn, vol. 1 (ed. L.J.Audus), pp. 127-163, Academic Press, London, New York and San Francisco.

VAN OVERBEEK, J. (1961). Application of auxins in agriculture and their physiological bases. VIII. Physiology and biochemistry of 2,4-D. In *Handbuch der Pflanzenphysiologie*, vol.14 (ed. W. Ruhland), pp. 1149- 1155, Springer-Verlag, Brlin.

VAVRINA, C. S. & ASHLEY, R. A. (1983). Effect of alachlor on PEG6000 uptake, root osmotic potential, and root leakage. *Weed Science* **31**, 600- 603 .

WAMPLE, R. L. & BEWLEY, J. D. (1975). Proline accumulation in flooded and wilted sunflower, and the effects of benzyladnine and abscisic acid. *Canadian Journal of Botany* **53**, 2893-2896.

WARE, G. W. (1983). *Pesticides: Theory and Application.* W. H. Freeman and Company, San francisco.

WAREING, P. F. & PHILLIPS, I. D. J. (1981). *Growth and Differentiation in Plants.* Pergamon Press, London.

WAUGH, T. C. & MCCLDEN, J. (1983). *GIMMS Reference Manual*, edn, 4.5, GIMMS Ltd, Edinburgh.

WERNICKE, W., GORST, J. & MILKOVITS, L. (1986). The ambiguous role of 2,4-dichlorophenoxyacetic acid in wheat tissue culture. *Physiologia Plantarum* **68**, 597-602.

WERNICKE, W. & MILKOVITS, L. (1987). Rates of uptake and metabolism of indole-3-acetic acid and 2,4-dichlorophenoxyacetic acid by cultured leaf segments at different stages of development in wheat. *Physiolgia Plantarum* **69**, 23-28.

WILDE, M. H. (1951). Anatomical modifications of bean roots following treatment with 2,4-D. *American Journal of Botany* **38**, 79-91.

WOLF. F. T. (1977). Effects of chemical agents in inhibition of chlorophyll synthesis and chloroplast development in higher plants. *The Botanical Review* **43**, 395-425.

WOODFORD, E. K., HOLLY, K. & McCREADY, C. C. (1958). Herbicides. *Annual Review of Plant Physiology* **9**, 311-358.

WORT, D. J. (1954). Influence of 2,4-D on enzyme systems. *Weeds* **3**, 131-135.

WORT, D. J. (1964). Effects of herbicides on plant composition and metabolism. In *The Physiology and Chemistry of Herbicides*, (ed. L. J. Audus), pp. 291-330, Academic Press, New York.

WU, C. C., KOZLOWSKI, T. T., EVERT, R. F. & SASAKI, S. (1971). Effect of direct contact of *Pinus resinosa* seeds and young seedlings with 2,4-D or picloram on seedling development. *Canadian Journal of Botany* **49**, 1737-1741.

YOUSIF, O. A. F., FIELDER, N. A. & PINFIELD, N. J. (1979). Hypocotyl swelling in marrow seedlings in response to 2,4-D treatment. *New Phytologist* **82**, 37-40.

ZEMSKAYA, V. A., KHILIK, L. A., KARPOVA, G. YA., KALIBERNAYA, Z. V. & CHERNIKOVA, L. M. (1984). Penetration, transport, and conversion of 2,4-D in the lavender plant. *Soviet Plant Physiology* English Translation **30**(4), 549-556.

ZEMSKAYA, V. A., RAKITIN, V. YU., KALIBERNAYA, Z. B. & CHERNIKOVA, L. M. (1985). Effect of 2,4-D on growth of corn coleoptile segments and ethylene production in them. *Soviet Plant Physiology* English Translation **32**(52), 734- 738.

ZSOLDOS, F., HAUNOLD, E. & VASHEGYI, A. (1986). The effects of phosphate on uptake of potassium ions, 2,4-D and atrazine by wheat and maize. *Physiologia Plantarum* **68**, 154-158.

APPENDIX

Summary of Statistical Analysis of Data Included in Tables for Chapter; 2, 3 and 4.

Chapter 2

Table 2.1
Analysis of Variance for Fig. 2.11

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	325.1036	108.3679	224.4350	0.0000***
Within treatments	16	7.7256	0.4828		
Total	19	332.8292			

LSD Procedure

TREATMENTS	2,4-D	2,4-D+PEG	CONTROL	PEG
2,4-D				
2,4-D+PEG				
CONTROL	***	***		
PEG	***	***	***	

Table 2.2
Analysis of Variance for Fig. 2.12.

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	16.6693	5.5564	532.8416	0.0000***
Within treatments	16	0.1670	0.0104		
Total	19	16.8363			

LSD Procedure

TREATMENTS	2,4-D+PEG	2,4-D	PEG	CONTROL
2,4-D+PEG				
2,4-D				
PEG	***	***		
CONTROL	***	***	***	

Table 2.3
Analysis of Variance for Fig. 2.13.

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	9	3512.7250	390.3028	6.2241	0.0001***
Within treatments	30	1881.2500	62.7083		
Total	39	5395.9750			

LSD Procedure

TREATMENTS	1	2	3	4	5	6	7	8	9	10
CONTROL 1										
125 PPM 2										
500 PPM 3										
1000 PPM+PEG 4	*									
1000 PPM 5	**									
500 PPM+PEG 6	***	*	*							
250 PPM 7	***	*	*							
125 PPM+PEG 8	***	**	**	*						
250 PPM+PEG 9	***	**	**	**						
PEG 10	***	***	***	**	**	*				

Table 2.4
Analysis of Variance for Fig. 2.14.

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	9	686446.1000	76271.7889	45.1660	0.0000***
Within treatments	30	50661.0000	1688.7000		
Total	39	737107.1000			

LSD Procedure

TREATMENTS	1	2	3	4	5	6	7	8	9	10
1000 PPM+PEG 1										
PEG 2										
500 PPM+PEG 3										
125 PPM+PEG 4										
500 PPM 5										
1000 PPM 6										
250 PPM+PEG 7	*									
125 PPM 8	***	**	*							
250 PPM 9	***	***	**	*	*	*				
CONTROL 10	***	***	***	***	***	***	***	***	***	

Table 2.5
Analysis of Variance for Fig. 2.15.

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.8687	0.2896	19.2423	0.0000***
Within treatments	16	0.2408	0.0150		
Total	19	1.1095			

LSD Procedure

TREATMENTS	CONTROL	PEG	2,4-D	2,4-D+PEG
CONTROL				
PEG	*			
2,4-D	***			
2,4-D+PEG	***	***	*	

Table 2.6
Analysis of Variance for Fig. 2.16

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	999.6040	333.2013	3.2887	0.0479*
Within treatments	16	1621.0680	101.3167		
Total	19	2620.6720			

LSD Procedure

TREATMENTS	2,4-D	2,4-D+PEG	CONTROL	PEG
2,4-D				
2,4-D+PEG				
CONTROL	*			
PEG	**			

Table 2.7
Analysis of Variance for Fig. 2.17

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	7	0.0937	0.0134	0.5981	0.7526N.S
Within treatments	32	0.7163	0.0224		
Total	39	0.8100			

Table 2.8
Analysis of Variance for Fig. 2.18

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	7	23.9658	3.4237	3.5045	0.0067**
Within treatments	32	31.2618	0.9769		
Total	39	55.2276			

LSD Procedure

TREATMENTS	1	2	3	4	5	6	7	8
1 PPM+DROUGHT 1								
100 PPM+DROUGHT 2								
DROUGHT 3								
10 PPM+WATER 4								
10 PPM+DROUGHT 5								
100 PPM+WATER 6								
1 PPM+WATER 7	*	*	*					
CONTROL 8	***	**	**	**	*	*		

Table 2.9
Analysis of Variance for Fig. 2.19

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	7	25.5453	3.6493	3.2760	0.0097**
Within treatments	32	35.6470	1.1140		
Total	39	61.1922			

LSD Procedure

TREATMENTS	1	2	3	4	5	6	7	8
1 PPM+DROUGHT 1								
DROUGHT 2								
100 PPM+DROUGHT 3								
10 PPM+WATER 4								
10 PPM+DROUGHT 5								
100 PPM+WATER 6								
1 PPM+WATER 7	*	*	*					
CONTROL 8	***	**	**	**	*	*		

Table 2.10
Analysis of Variance for Fig. 2.20

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	7	3.2668	0.4667	1.0528	0.4155*
Within treatments	32	14.1849	0.4433		
Total	39	17.4516			

LSD Procedure

TREATMENTS	1	2	3	4	5	6	7	8
CONTROL 1								
100 PPM+WATER 2								
1 PPM+WATER 3								
10 PPM+DROUGHT 4								
10 PPM+WATER 5								
DROUGHT 6								
100 PPM+DROUGHT 7								
1 PPM+DROUGHT 8	*	*	*	*				

Table 2.11
Analysis of Variance for Fig. 2.21

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	7	0.0004	0.0001	0.9503	0.4830N.S
Within treatments	32	0.0020	0.0001		
Total	39	0.0024			

Table 2.12
Analysis of Variance for Fig. 2.22

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	7	0.0126	0.0018	2.3933	0.0435*
Within treatments	32	0.0241	0.0008		
Total	39	0.0367			

LSD Procedure

TREATMENTS	1	2	3	4	5	6	7	8
DROUGHT 1								
10 PPM+WATER 2								
100 PPM+DROUGHT 3								
1 PPM+DROUGHT 4								
10 PPM+DROUGHT 5								
1 PPM+WATER 6								
100 PPM+WATER 7								
CONTROL 8	**	**	**	*	*			

Table 2.13
Analysis of Variance for Fig. 2.23

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	7	0.0105	0.0015	1.5880	0.1747*
Within treatments	32	0.0302	0.0009		
Total	39	0.0408			

LSD Procedure

TREATMENTS	1	2	3	4	5	6	7	8
DROUGHT 1								
10 PPM+WATER 2								
100 PPM+DROUGHT 3								
10 PPM+DROUGHT 4								
1 PPM+DROUGHT 5								
1 PPM+WATER 6								
100 PPM+WATER 7								
CONTROL 8	**	*	*					

Table 2.14
Analysis of Variance for Fig. 2.24

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	7	5.6562	0.8080	169.8431	0.0000***
Within treatments	32	0.1522	0.0048		
Total	39	5.8085			

LSD Procedure

TREATMENTS	1	2	3	4	5	6	7	8
CONTROL 1								
1 PPM+WATER 2								
100 PPM+WATER 3								
10 PPM+WATER 4	**	*	*					
100 PPM+DROUGHT 5	***	***	***	***				
1 PPM+DROUGHT 6	***	***	***	***				
DROUGHT 7	***	***	***	***				
10 PPM+DROUGHT 8	***	***	***	***				

Table 2.15
Analysis of Variance for Fig. 2.30

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	280.0000	93.3333	1.4359	0.2693N.S
Within treatments	32	0.7163	0.0224		
Total	39	0.8100			

Table 2.16
Analysis of Variance for Fig. 2.31

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	42.0000	14.0000	5.7732	0.0071**
Within treatments	16	38.8000	2.4250		
Total	19	80.8000			

LSD Procedure

TREATMENTS	2,4-D	2,4-D+PEG	CONTROL	PEG
2,4-D				
2,4-D+PEG	*			
CONTROL	**			
PEG	**			

Table 2.17
Analysis of Variance for Fig. 2.32

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	213.4801	71.1600	144.9783	0.0000***
Within treatments	16	7.8533	0.4908		
Total	19	221.3334			

LSD Procedure

TREATMENTS	2,4-D	2,4-D+PEG	PEG	CONTROL
2,4-D				
2,4-D+PEG				
PEG	***	*		
CONTROL	***	***	***	

Table 2.18
Analysis of Variance for Fig. 2.33

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	40.0000	13.3333	4.5198	0.0177**
Within treatments	16	47.2000	2.9500		
Total	19	87.2000			

LSD Procedure

TREATMENTS	PEG	2,4-D	2,4-D+PEG	CONTROL
PEG				
2,4-D				
2,4-D+PEG				
CONTROL	**	**	*	

Table 2.19
Analysis of Variance for Fig. 2.34

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	159.5869	53.1956	113.4358	0.0000***
Within treatments	16	7.5032	0.4589		
Total	19	167.0900			

LSD Procedure

TREATMENTS	PEG	2,4-D+PEG	2,4-D	CONTROL
PEG				
2,4-D+PEG				
2,4-D				
CONTROL	***	***	***	

Table 2.20
Analysis of Variance for Fig. 2.35

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.2118	0.0700	45.9984	0.0000***
Within treatments	20	0.0307	0.0015		
Total	23	0.2425			

LSD Procedure

TREATMENTS	PEG	2,4-D+PEG	2,4-D	CONTROL
PEG				
2,4-D+PEG				
2,4-D	***	**		
CONTROL	***	***	***	

Table 2.21
Analysis of Variance for Fig. 2.36

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0353	0.0118	9.4692	0.0004***
Within treatments	20	0.0248	0.0012		
Total	23	0.0601			

LSD Procedure

TREATMENTS	2,4-D+PEG	PEG	2,4-D	CONTROL
2,4-D+PEG				
PEG				
2,4-D	*			
CONTROL	***	***	*	

Table 2.22
Analysis of Variance for Fig. 2.37

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.44171	0.1390	28.6899	0.0000***
Within treatments	20	0.0969	0.0048		
Total	23	0.5141			

LSD Procedure

TREATMENTS	PEG	2,4-D+PEG	2,4-D	CONTROL
PEG				
2,4-D+PEG				
2,4-D	**	**		
CONTROL	***	***	***	

Table 2.23
Analysis of Variance for Fig. 2.38

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0005	0.0002	8.6246	0.0007***
Within treatments	20	0.0004	0.0000		
Total	23	0.0009			

LSD Procedure

TREATMENTS	PEG	2,4-D+PEG	2,4-D	CONTROL
PEG				
2,4-D+PEG				
2,4-D				
CONTROL	***	**	**	

Table 2.24
Analysis of Variance for Fig. 2.39

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0000	0.0000	0.45729	0.7152N.S
Within treatments	20	0.0003	0.0000		
Total	23	0.0003			

Table 2.25
Analysis of Variance for Fig. 2.40

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0005	0.0002	2.7528	0.0695*
Within treatments	20	0.0011	0.0001		
Total	23	0.0016			

LSD Procedure

TREATMENTS	PEG	2,4-D+PEG	2,4-D	CONTROL
PEG				
2,4-D+PEG				
2,4-D				
CONTROL	*	*	*	

Table 2.26.1
Analysis of Variance for Fig. 2.41

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	1.0019	0.3340	18.5337	0.0000***
Within treatments	16	0.2883	0.0180		
Total	19	1.2902			

LSD Procedure

TREATMENTS	2,4-D+DROUGHT	DROUGHT	CONTROL	2,4-D+WATER
2,4-D+DROUGHT				
DROUGHT				
CONTROL	***	*		
2,4-D+WATER	***	***	*	

Table 2.26.2
Analysis of Variance for Fig. 2.41

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	2.2176	0.7392	10.9706	0.0004***
Within treatments	16	1.0781	0.0674		
Total	19	3.2757			

LSD Procedure

TREATMENTS	DROUGHT	2,4-D+DROUGHT	CONTROL	2,4-D+WATER
DROUGHT				
2,4-D+DROUGHT				
CONTROL	***	**		
2,4-D+WATER	***	**		

Table 2.26.3
Analysis of Variance for Fig. 2.41

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	4.5419	1.5140	33.9247	0.0000***
Within treatments	16	0.7140	0.0446		
Total	19	5.2560			

LSD Procedure

TREATMENTS	DROUGHT	2,4-D+DROUGHT	2,4-D+WATER	CONTROL
DROUGHT				
2,4-D+DROUGHT	*			
2,4-D+WATER	***	***		
CONTROL	***	***		

Table 2.27.1
Analysis of Variance for Fig. 2.42

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0028	0.0009	8.3821	0.0014**
Within treatments	16	0.0018	0.0001		
Total	19	0.0046			

LSD Procedure

TREATMENTS	DROUGHT	2,4-D+DROUGHT	CONTROL	2,4-D+WATER
2,4-D+DROUGHT				
DROUGHT	*			
CONTROL	*	***		
WATER+2,4-D	***	*	*	

Table 2.27.2
Analysis of Variance for Fig. 2.42

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0080	0.0027	4.1649	0.0233*
Within treatments	16	0.0102	0.0006		
Total	19	0.0181			

LSD Procedure

TREATMENTS	DROUGHT	2,4-D+DROUGHT	CONTROL	2,4-D+WATER
DROUGHT				
2,4-D+DROUGHT				
CONTROL				
2,4-D+WATER	**	*		

Table 2.27.3
Analysis of Variance for Fig. 2.42

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0166	0.0055	5.7612	0.0027**
Within treatments	16	0.0153	0.0010		
Total	19	0.0319			

LSD Procedure

TREATMENTS	DROUGHT	2,4-D+DROUGHT	2,4-D+WATER	CONTROL
DROUGHT				
2,4-D+DROUGHT				
2,4-D+WATER				
CONTROL	**	**	*	

Table 2.28
Analysis of Variance for Fig. 2.43

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	83.135	27.7712	25.1173	0.0000***
Within treatments	240	265.3586	1.1057		
Total	243	348.6721			

LSD Procedure

TREATMENTS	CONTROL	2,4-D	2,4-D+DROUGHT	DROUGHT
CONTROL				
2,4-D				
2,4-D+DROUGHT	***	***		
DROUGHT	***	***		

Table 2.29
Analysis of Variance for Fig. 2.44

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	1144.5425	381.5142	89.4954	0.0000***
Within treatments	469	1999.3222	4.2629		
Total	472	3143.8647			

LSD Procedure

TREATMENTS	DROUGHT	2,4-D+DROUGHT	2,4-D+WATER	CONTROL
DROUGHT				
2,4-D+DROUGHT				
2,4-D+WATER	***	***		
CONTROL	***	***		

Table 2.30
Analysis of Variance for Fig. 2.45

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	449.3568	149.7856	152.4782	0.0000***
Within treatments	492	483.3120	0.9823		
Total	495	932.6688			

LSD Procedure

TREATMENTS	2,4-D+DROUGHT	DROUGHT	2,4-D	CONTROL
2,4-D+DROUGHT				
DROUGHT	***			
2,4-D	***	***		
CONTROL	***	***	***	

Table 2.31
Analysis of Variance for Fig. 2.46

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	63058.5713	21019.5238	176.6459	0.0000***
Within treatments	486	57830.3267	118.9924		
Total	489	120888.8980			

LSD Procedure

TREATMENTS	2,4-D+DROUGHT	DROUGHT	2,4-D+WATER	CONTROL
2,4-D+DROUGHT				
DROUGHT	*			
2,4-D+WATER	***	***		
CONTROL	***	***	***	

Table 2.32
Analysis of Variance for Fig. 2.47

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0019	0.0006	0.1267	0.9437N.S
Within treatments	36	0.1781	0.0049		
Total	39	0.1800			

Table 2.33
Analysis of Variance for Fig. 2.48

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	4.7849	1.5950	26.8025	0.0000***
Within treatments	24	1.4282	0.0595		
Total	27	6.2131			

LSD Procedure

TREATMENTS	2,4-D+PEG	PEG	2,4-D	CONTROL
2,4-D+PEG				
PEG				
2,4-D	***	***		
CONTROL	***	***	**	

Table 2.34
Analysis of Variance for Fig. 2.49

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	2.2446	0.7482	9.5467	0.0002***
Within treatments	24	1.8810	0.0784		
Total	27	4.1256			

LSD Procedure

TREATMENTS	2,4-D+PEG	PEG	2,4-D	CONTROL
2,4-D+PEG				
PEG				
2,4-D	***	***		
CONTROL	***	***	**	

Table 2.35
Analysis of Variance for Fig. 2.50

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	13.0348	4.3449	16.6464	0.0040**
Within treatments	24	6.2643	0.2610		
Total	27	19.2992			

LSD Procedure

TREATMENTS	2,4-D+PEG	PEG	2,4-D	CONTROL
2,4-D+PEG				
PEG				
2,4-D	**			
CONTROL	***	***	**	

Table 2.36
Analysis of Variance for Fig. 2.51

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	5.6209	1.8736	25.8245	0.0000***
Within treatments	24	1.7412	0.0726		
Total	27	7.3621			

LSD Procedure

TREATMENTS	2,4-D	CONTROL	PEG	2,4-D+PEG
2,4-D				
CONTROL				
PEG	***	***		
2,4-D+PEG	***	***		

Table 2.37
Analysis of Variance for Fig. 2.52

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0048	0.0016	3.0334	0.0488*
Within treatments	24	0.0127	0.0005		
Total	27	0.0175			

LSD Procedure

TREATMENTS	2,4-D+PEG	PEG	2,4-D	CONTROL
2,4-D+PEG				
PEG				
2,4-D				
CONTROL	***	***	**	

Table 2.38
Analysis of Variance for Fig. 2.53

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0032	0.0011	2.4866	0.0848*
Within treatments	24	0.0103	0.0004		
Total	27	0.0135			

LSD Procedure

TREATMENTS	2,4-D	2,4-D+PEG	PEG	CONTROL
2,4-D				
2,4-D+PEG				
PEG	*			
CONTROL	*			

Table 2.39
Analysis of Variance for Fig. 2.54

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0105	0.0035	2.0005	0.1408N.S
Within treatments	24	0.0420	0.0018		
Total	27	0.0525			

LSD Procedure

TREATMENTS	2,4-D+PEG	2,4-D	PEG	CONTROL
2,4-D+PEG				
2,4-D				
PEG				
CONTROL	*			

Table 2.40
Analysis of Variance for Fig. 2.55

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.7290	0.2430	8.3728	0.0006***
Within treatments	24	0.6965	0.0290		
Total	27	1.4256			

LSD Procedure

TREATMENTS	2,4-D	CONTROL	2,4-D+PEG	PEG
2,4-D				
CONTROL				
2,4-D+PEG	**	**		
PEG	***	***		

Table 2.41
Analysis of Variance for Fig. 2.56

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	28.1500	9.3833	15.7078	0.0000***
Within treatments	76	45.4000	0.5974		
Total	79	73.5500			

LSD Procedure

TREATMENTS	2,4-D+PEG	2,4-D	PEG	CONTROL
2,4-D+PEG				
2,4-D				
PEG	*	*		
CONTROL	*	*		

Table 2.42
Analysis of Variance for Fig. 2.57

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	372.1020	124.0340	5.3483	0.0038**
Within treatments	36	834.8940	23.1915		
Total	39	1206.9960			

LSD Procedure

TREATMENTS	2,4-D	2,4-D+PEG	PEG	CONTROL
2,4-D				
2,4-D+PEG				
PEG	*	*		
CONTROL	*	*		

Table 2.43
Analysis of Variance for Fig. 2.58

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	12.2280	4.0760	2.3404	0.0800*
Within treatments	76	132.3600	1.7416		
Total	79	144.5880			

LSD Procedure

TREATMENTS	2,4-D+PEG	2,4-D	CONTROL	2,4-D
2,4-D+PEG				
2,4-D				
CONTROL	*			
PEG	*			

Table 2.44
Analysis of Variance for Fig. 2.59

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	29.0710	9.6903	1.4505	0.2348N.S
Within treatments	76	507.7370	6.6807		
Total	79	536.8080			

Table 2.45
Analysis of Variance for Fig. 2.60

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	6.7894	2.2631	4.4102	0.0065**
Within treatments	76	38.9995	0.5132		
Total	79	45.7889			

LSD Procedure

TREATMENTS	2,4-D+PEG	PEG	2,4-D	CONTROL
2,4-D+PEG				
PEG				
2,4-D	*	*		
CONTROL	*	*		

Chapter 3

Table 3.1.1
Analysis of Variance for Fig. 3.1

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	160260.5680	53420.1893	60.5646	0.0000***
Within treatments	16	14112.5861	882.0366		
Total	19	174373.1541			

LSD Procedure

TREATMENTS	2,4-D	2,4-D+PEG	PEG	CONTROL
2,4-D				
2,4-D+PEG				
PEG				
CONTROL	***	***	***	

Table 3.1.2
Analysis of Variance for Fig. 3.1

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	554194.0048	184731.3349	206.4882	0.0000***
Within treatments	16	14314.1433	894.6340		
Total	19	568508.1481			

LSD Procedure

TREATMENTS	2,4-D	2,4-D+PEG	PEG	CONTROL
2,4-D				
2,4-D+PEG				
PEG	*			
CONTROL	***	***	***	

Table 3.1.3
Analysis of Variance for Fig. 3.1

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	1976913.027	658971.0091	746.4269	0.0000***
Within treatments	16	14125.3423	882.8339		
Total	19	1991038.369			

LSD Procedure

TREATMENTS	2,4-D+PEG	PEG	2,4-D	CONTROL
2,4-D+PEG				
PEG				
2,4-D	***	***		
CONTROL	***	***	***	

Table 3.2.1
Analysis of Variance for Fig. 3.2

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	35473.7607	11824.5869	76.9504	0.0000***
Within treatments	16	2458.6413	153.6651		
Total	19	37932.4020			

LSD Procedure

TREATMENTS	2,4-D	2,4-D+PEG	PEG	CONTROL
2,4-D				
2,4-D+PEG				
PEG				
CONTROL	***	***	***	

Table 3.2.2
Analysis of Variance for Fig. 3.2

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	130339.4179	43446.4726	198.4289	0.0000***
Within treatments	16	3503.2373	218.9523		
Total	19	133842.6552			

LSD Procedure

TREATMENTS	2,4-D	2,4-D+PEG	PEG	CONTROL
2,4-D				
2,4-D+PEG				
PEG	***	***		
CONTROL	***	***	***	

Table 3.2.3
Analysis of Variance for Fig. 3.2

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	366084.3211	122028.1070	769.4943	0.0000***
Within treatments	16	2537.3155	158.5822		
Total	19	368621.6364			

LSD Procedure

TREATMENTS	PEG	2,4-D+PEG	2,4-D	CONTROL
PEG				
2,4-D+PEG	*			
2,4-D	*	*		
CONTROL	***	***	***	

Table 3.3.1
Analysis of Variance for Fig. 3.3

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	346268.1043	115422.7014	65.5810	0.0000***
Within treatments	16	28160.0301	1760.0019		
Total	19	374428.1344			

LSD Procedure

TREATMENTS	2,4-D	2,4-D+PEG	PEG	CONTROL
2,4-D				
2,4-D+PEG				
PEG				
CONTROL	***	***	***	

Table 3.3.2
Analysis of Variance for Fig. 3.3

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	1216558.432	405519.4772	252.2785	0.0000***
Within treatments	16	25718.8430	1607.4277		
Total	19	1242277.275			

LSD Procedure

TREATMENTS	2,4-D	2,4-D+PEG	PEG	CONTROL
2,4-D				
2,4-D+PEG				
PEG	**	*		
CONTROL	***	***	***	

Table 3.3.3
Analysis of Variance for Fig. 3.3

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	4036909.358	1345636.453	802.4328	0.0000***
Within treatments	16	26831.1362	1676.9460		
Total	19	4063740.495			

LSD Procedure

TREATMENTS	2,4-D+PEG	PEG	2,4-D	CONTROL
2,4-D+PEG				
PEG				
2,4-D	***	***		
CONTROL	***	***	***	

Table 3.4.1
Analysis of Variance for Fig. 3.4

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	2.0178	0.6726	70.5270	0.0000***
Within treatments	16	0.1526	0.0095		
Total	19	2.1704			

LSD Procedure

TREATMENTS	PEG	2,4-D+PEG	2,4-D	CONTROL
PEG				
2,4-D+PEG	*			
2,4-D	***	***		
CONTROL	***	***		

Table 3.4.2
Analysis of Variance for Fig. 3.4

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	29.2977	9.7659	107.1877	0.0000***
Within treatments	16	1.4578	0.0911		
Total	19	30.7555			

LSD Procedure

TREATMENTS	PEG	CONTROL	2,4-D	2,4-D+PEG
PEG				
CONTROL	**			
2,4-D	***	***		
2,4-D+PEG	***	***	***	

Table 3.4.3
Analysis of Variance for Fig. 3.4

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	7.3797	2.4599	29.1399	0.0000***
Within treatments	16	1.3507	0.0844		
Total	19	8.7304			

LSD Procedure

TREATMENTS	2,4-D+PEG	CONTROL	PEG	2,4-D
2,4-D+PEG				
CONTROL	**			
PEG	***	**		
2,4-D	***	***		

Table 3.5.1
Analysis of Variance for Fig. 3.5

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	58888.0617	19629.3539	415.4309	0.0000***
Within treatments	16	756.0094	47.2506		
Total	19	59644.07111			

LSD Procedure

TREATMENTS	2,4-D	2,4-D+PEG	CONTROL	PEG
2,4-D				
2,4-D+PEG	***			
CONTROL	***	*		
PEG	***	***	***	

Table 3.5.2
Analysis of Variance for Fig. 3.5

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	77705.0030	25901.6677	17307.0103	0.0000***
Within treatments	16	23.9456	1.4966		
Total	19	77728.9486			

LSD Procedure

TREATMENTS	CONTROL	2,4-D	2,4-D+PEG	PEG
CONTROL				
2,4-D	***			
2,4-D+PEG	***	***		
PEG	***	***	***	

Table 3.5.3
Analysis of Variance for Fig. 3.5

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	15877.8240	5292.6080	1153.3843	0.0000***
Within treatments	16	73.4202	4.5888		
Total	19	15951.2442			

LSD Procedure

TREATMENTS	2,4-D	CONTROL	2,4-D+PEG	PEG
2,4-D				
CONTROL	***			
2,4-D+PEG	***	***		
PEG	***	***	***	

Table 3.6.1
Analysis of Variance for Fig. 3.6

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	8957.1586	2985.7195	64.2645	0.0000***
Within treatments	16	743.3580	46.4599		
Total	19	9700.5166			

LSD Procedure

TREATMENTS	2,4-D	2,4-D+PEG	CONTROL	PEG
2,4-D				
2,4-D+PEG	***			
CONTROL	***			
PEG	***	***	***	

Table 3.6.2
Analysis of Variance for Fig. 3.6

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	31935.7042	10645.2347	575.4078	0.0000***
Within treatments	16	296.0053	18.5003		
Total	19	32231.7095			

LSD Procedure

TREATMENTS	CONTROL	2,4-D	2,4-D+PEG	PEG
CONTROL				
2,4-D	*			
2,4-D+PEG	***	***		
PEG	***	***	***	

Table 3.6.3
Analysis of Variance for Fig. 3.6

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	9944.6774	3314.8925	644.7693	0.0000***
Within treatments	16	82.2593	5.1412		
Total	19	10026.9367			

LSD Procedure

TREATMENTS	2,4-D	CONTROL	PEG	2,4-D+PEG
2,4-D				
CONTROL	**			
PEG	***	***		
2,4-D+PEG	***	***	***	

Table 3.7.1
Analysis of Variance for Fig. 3.7

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	114586.9371	38195.6457	117.9499	0.0000***
Within treatments	16	5181.2719	323.8295		
Total	19	119768.2091			

LSD Procedure

TREATMENTS	2,4-D	2,4-D+PEG	CONTROL	PEG
2,4-D				
2,4-D+PEG	***			
CONTROL	***			
PEG	***	***	***	

Table 3.7.2
Analysis of Variance for Fig. 3.7

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	208314.4808	69438.1603	2958.4709	0.0000***
Within treatments	16	375.5354	23.4710		
Total	19	208690.0162			

LSD Procedure

TREATMENTS	CONTROL	2,4-D	2,4-D+PEG	PEG
CONTROL				
2,4-D	***			
2,4-D+PEG	***	***		
PEG	***	***	***	

Table 3.7.3
Analysis of Variance for Fig. 3.7

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	29596.3555	9865.4518	604.9209	0.0000***
Within treatments	16	260.9386	16.3087		
Total	19	29857.2941			

LSD Procedure

TREATMENTS	2,4-D	CONTROL	2,4-D+PEG	PEG
2,4-D				
CONTROL				
2,4-D+PEG	***	***		
PEG	***	***	***	

Table 3.8.1
Analysis of Variance for Fig. 3.8

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.2147	0.0716	0.5340	0.6656N.S
Within treatments	16	2.1446	0.1340		
Total	19	2.3593			

Table 3.8.2
Analysis of Variance for Fig. 3.8

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	1.8832	0.6277	127.1231	0.0000***
Within treatments	16	0.0790	0.0049		
Total	19	1.9622			

LSD Procedure

TREATMENTS	PEG	2,4-D+PEG	CONTROL	2,4-D
PEG				
2,4-D+PEG	***			
CONTROL	***	***		
2,4-D	***	***	***	

Table 3.8.3
Analysis of Variance for Fig. 3.8

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	4.1904	1.3968	496.5177	0.0000***
Within treatments	16	0.459	0.0029		
Total	19	4.2363			

LSD Procedure

TREATMENTS	2,4-D+PEG	CONTROL	2,4-D	PEG
2,4-D+PEG				
CONTROL	***			
2,4-D	***	*		
PEG	***	***	***	

Table 3.9.1
Analysis of Variance for Fig. 3.9

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0447	0.0149	1.7959	0.1885N.S
Within treatments	16	0.1327	0.00838		
Total	19	0.1773			

Table 3.9.2
Analysis of Variance for Fig. 3.9

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.4715	0.1572	3.5558	0.0382*
Within treatments	16	0.7071	0.0442		
Total	19	1.1786			

LSD Procedure

TREATMENTS	2,4-D+WATER	2,4-D+DROUGHT	CONTROL	DROUGHT
2,4-D+WATER				
2,4-D+DROUGHT				
CONTROL				
DROUGHT	**	*		

Table 3.9.3
Analysis of Variance for Fig. 3.9

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.8630	0.2877	6.4969	0.0044**
Within treatments	16	0.7085	0.0443		
Total	19	1.5715			

LSD Procedure

TREATMENTS	CONTROL	2,4-D+WATER	DROUGHT	2,4-D+DROUGHT
CONTROL				
2,4-D+WATER	*			
DROUGHT	**	*		
2,4-D+DROUGHT	**	**		

Table 3.10.1
Analysis of Variance for Fig. 3.10

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0079	0.0026	1.2126	0.3372N.S
Within treatments	16	0.0347	0.0022		
Total	19	0.0426			

Table 3.10.2
Analysis of Variance for Fig. 3.10

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0926	0.0309	2.4220	0.1038N.S
Within treatments	16	0.2039	0.0127		
Total	19	0.2965			

Table 3.10.3
Analysis of Variance for Fig. 3.10

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.1344	0.0448	3.2619	0.0490*
Within treatments	16	0.2197	0.0137		
Total	19	0.3541			

bf LSD Procedure

TREATMENTS	CONTROL	2,4-D+WATER	2,4-D+DROUGHT	DROUGHT
CONTROL				
2,4-D+WATER				
2,4-D+DROUGHT	*			
DROUGHT	*	*		

Table 3.11.1
Analysis of Variance for Fig. 3.11

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0899	0.0300	1.6101	0.2264N.S
Within treatments	16	1.4578	0.0911		
Total	19	30.7555			

Table 3.11.2
Analysis of Variance for Fig. 3.11

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.9810	0.3270	3.1616	0.0534*
Within treatments	16	1.6549	0.1034		
Total	19	2.6360			

LSD Procedure

TREATMENTS	2,4-D+WATER	2,4-D+DROUGHT	CONTROL	DROUGHT
2,4-D+WATER				
2,4-D+DROUGHT				
CONTROL				
DROUGHT	*	*		

Table 3.11.3
Analysis of Variance for Fig. 3.11

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0286	0.0095	2.7928	0.0740N.S
Within treatments	16	0.0546	0.0034		
Total	19	0.0832			

Table 3.12.1
Analysis of Variance for Fig. 3.12

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0017	0.0006	0.1572	0.9235N.S
Within treatments	16	0.0570	0.0036		
Total	19	0.0587			

Table 3.12.2
Analysis of Variance for Fig. 3.12

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	0.0083	0.0028	1.4810	0.2574N.S
Within treatments	16	0.0301	0.0019		
Total	19	0.0384			

Table 3.12.3
Analysis of Variance for Fig. 3.12

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	1.7908	0.5969	6.3235	0.0049**
Within treatments	16	1.5104	0.0944		
Total	19	3.3012			

LSD Procedure

TREATMENTS	drought	CONTROL	2,4-D+WATER	2,4-D+DROUGHT
DROUGHT				
CONTROL				
2,4-D+WATER	**	*		
2,4-D+DROUGHT	**	**		

Table 3.13
Analysis of Variance for Fig. 3.13

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	7	267.1951	38.1707	47.1104	0.0000***
Within treatments	32	25.9277	0.8102		
Total	39	293.1228			

LSD Procedure

TREATMENTS	1	2	3	4	5	6	7	8
100 PPM+WATER 1								
10 PPM+DROUGHT 2	***							
10 PPM+WATER 3	***							
100 PPM+DROUGHT 4	***	*						
1 PPM+WATER 5	***	***	***	*				
1 PPM+DROUGHT 6	***	***	***	***	***			
CONTROL 7	***	***	***	***	***			
DROUGHT 8	***	***	***	***	***			

Table 3.14
Analysis of Variance for Fig. 3.14

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	7	53.0202	7.5743	58.4666	0.0000***
Within treatments	32	4.1456	0.1295		
Total	39	57.1658			

LSD Procedure

TREATMENTS	1	2	3	4	5	6	7	8
100 PPM+WATER 1								
10 PPM+DROUGHT 2	**							
10 PPM+WATER 3	***							
100 PPM+DROUGHT 4	***	***	***					
1 PPM+WATER 5	***	***	***					
1 PPM+DROUGHT 6	***	***	***	***	***			
DROUGHT 7	***	***	***	***	***			
CONTROL 8	***	***	***	***	***			

Table 3.15
Analysis of Variance for Fig. 3.15

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	7	554.5732	79.2247	52.6199	0.0000***
Within treatments	32	48.1793	1.5056		
Total	39	602.7525			

LSD Procedure

TREATMENTS	1	2	3	4	5	6	7	8
100 PPM+WATER 1								
10 PPM+DROUGHT 2	***							
10 PPM+WATER 3	***							
100 PPM+DROUGHT 4	***	**	*					
1 PPM+WATER 5	***	***	***	*				
1 PPM+DROUGHT 6	***	***	***	***	***			
DROUGHT 7	***	***	***	***	***			
CONTROL 8	***	***	***	***	***			

Table 3.16
Analysis of Variance for Fig. 3.16

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	7	0.3362	0.0480	9.6405	0.0000***
Within treatments	32	0.1594	0.0050		
Total	39	0.4956			

LSD Procedure

TREATMENTS	1	2	3	4	5	6	7	8
100 PPM+DROUGHT 1								
10 CONTROL 2								
1 PPM+WATER 3	**	*						
DROUGHT 4	**	*						
1 PPM+DROUGHT 5	**	*						
100 PPM+WATER 6	***	**						
10 PPM+DROUGHT 7	***	**						
10 PPM+WATER 8	***	***	***	**	**	*	*	

Table 3.17.1
Analysis of Variance for Fig. 3.17

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	135.0604	45.0201	4.7511	0.0149*
Within treatments	16	151.6133	9.4758		
Total	19	286.6737			

LSD Procedure

TREATMENTS	2,4-D+DROUGHT	DROUGHT	CONTROL	2,4-D+WATER
2,4-D+DROUGHT				
DROUGHT				
CONTROL	*			
2,4-D+WATER	*			

Table 3.17.2
Analysis of Variance for Fig. 3.17

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	142.2273	47.4091	17.5657	0.0000***
Within treatments	16	43.1834	2.6990		
Total	19	185.4107			

LSD Procedure

TREATMENTS	DROUGHT	2,4-D+DROUGHT	2,4-D+WATER	CONTROL
DROUGHT				
2,4-D+DROUGHT				
2,4-D+WATER	*	*		
CONTROL	*	*		

Table 3.17.3
Analysis of Variance for Fig. 3.17

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	58.8965	19.6322	4.1428	0.0237*
Within treatments	16	75.8210	4.7388		
Total	19	134.7175			

LSD Procedure

TREATMENTS	2,4-D+WATER	CONTROL	DROUGHT	2,4-D+DROUGHT
2,4-D+WATER				
CONTROL				
DROUGHT	*			
2,4-D+DROUGHT	*			

Table 3.18
Analysis of Variance for Fig. 3.18

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	7	1466.0727	209.4390	24.6454	0.0000***
Within treatments	88	747.8333	8.4981		
Total	95	2213.9062			

LSD Procedure

TREATMENTS	1	2	3	4	5	6	7	8
10 PPM+DROUGHT 1								
10 PPM+WATER 2								
100 PPM+WATER 3								
100 PPM+DROUGHT 4								
1 PPM+WATER 5	***	***	***	***				
CONTROL 6	***	***	***	***				
1 PPM+DROUGHT 7	***	***	***	***				
DROUGHT 8	***	***	***	***				

Table 3.19
Analysis of Variance for Fig. 3.19

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	7	600.3229	85.7604	13.5655	0.0000***
Within treatments	88	556.3333	6.3220		
Total	95	1156.6562			

LSD Procedure

TREATMENTS	1	2	3	4	5	6	7	8
1 PPM+DROUGHT 1								
100+DROUGHT 2								
1 PPM+WATER 3								
100 PPM+WATER 4	*							
10 PPM+DROUGHT 5	*							
DROUGHT 6	**	*						
10 PPM+WATER 7	***	***	**	*	*			
CONTROL 8	***	***	***	***	***	***	***	

Table 3.20
Analysis of Variance for Fig. 3.20

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	7	2312.6016	330.3717	20.6736	0.0000***
Within treatments	88	1406.2708	15.9804		
Total	95	3718.8724			

LSD Procedure

TREATMENTS	1	2	3	4	5	6	7	8
100 PPM+DROUGHT 1								
10+DROUGHT 2								
100 PPM+WATER 3								
10 PPM+WATER 4	*	*						
1 PPM+DROUGHT 5	***	**	**					
1 PPM+WATER 6	***	***	***	*				
DROUGHT 7	***	***	***	***	**			
CONTROL 8	***	***	***	***	***	***	*	

Table 3.21.1
Analysis of Variance for Fig. 3.21

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	465.5494	232.7747	88.8483	0.0000***
Within treatments	12	31.4389	2.6199		
Total	14	496.9883			

LSD Procedure

TREATMENTS	CONTROL	2,4-D+PEG	PEG
CONTROL			
2,4-D+PEG	***		
PEG	***	***	

Table 3.21.2
Analysis of Variance for Fig. 3.21

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	4577.6029	2288.8015	179.9140	0.0000***
Within treatments	12	152.6597	12.7216		
Total	14	4730.2626			

LSD Procedure

TREATMENTS	CONTROL	2,4-D+PEG	PEG
CONTROL			
2,4-D+PEG	***		
PEG	***	***	

Table 3.21.3
Analysis of Variance for Fig. 3.21

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	2872.3863	1436.1931	160.9586	0.0000***
Within treatments	12	107.0730	8.9227		
Total	14	2979.4592			

LSD Procedure

TREATMENTS	CONTROL	2,4-D+PEG	PEG
CONTROL			
2,4-D+PEG	***		
PEG	***	***	

Table 3.21.4
Analysis of Variance for Fig. 3.21

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	1884.1346	942.0673	232.6624	0.0000***
Within treatments	12	48.5889	4.0491		
Total	14	1932.7235			

LSD Procedure

TREATMENTS	CONTROL	2,4-D+PEG	PEG
CONTROL			
2,4-D+PEG	***		
PEG	***	***	

Table 3.21.5
Analysis of Variance for Fig. 3.21

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	1000.3473	500.1736	167.6680	0.0000***
Within treatments	12	35.7974	2.9831		
Total	14	1036.1447			

LSD Procedure

TREATMENTS	CONTROL	2,4-D+PEG	PEG
CONTROL			
2,4-D+PEG	***		
PEG	***	***	

Table 3.22.1
Analysis of Variance for Fig. 3.22

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	297.7840	148.8920	65.7293	0.0000***
Within treatments	12	27.1828	2.2652		
Total	14	324.9668			

LSD Procedure

TREATMENTS	CONTROL	2,4-D	2,4-D+PEG
CONTROL			
2,4-D			
2,4-D+PEG	***	***	

Table 3.22.2
Analysis of Variance for Fig. 3.22

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	96.6926	48.3463	162.0443	0.0000***
Within treatments	12	3.5802	0.298		
Total	14	100.2728			

LSD Procedure

TREATMENTS	CONTROL	2,4-D	2,4-D+PEG
CONTROL			
2,4-D			
2,4-D+PEG	***	***	

Table 3.22.3
Analysis of Variance for Fig. 3.22

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	39.8846	19.9423	156.9948	0.0000***
Within treatments	12	1.5243	0.1270		
Total	14	41.4089			

LSD Procedure

TREATMENTS	2,4-D	CONTROL	2,4-D+PEG
2,4-D			
CONTROL			
2,4-D+PEG	***	***	

Table 3.22.4
Analysis of Variance for Fig. 3.22

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	25.7002	12.8501	119.5569	0.0000***
Within treatments	12	1.2898	0.1075		
Total	14	26.9900			

LSD Procedure

TREATMENTS	CONTROL	2,4-D	2,4-D+PEG
CONTROL			
2,4-D			
2,4-D+PEG	***	***	

Table 3.22.5
Analysis of Variance for Fig. 3.22

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	21.7221	10.8610	166.5372	0.0000***
Within treatments	12	0.7826	0.0652		
Total	14	22.5047			

LSD Procedure

TREATMENTS	CONTROL	2,4-D	2,4-D+PEG
CONTROL			
2,4-D			
2,4-D+PEG	***		

Table 3.23.1
Analysis of Variance for Fig. 3.23

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	158.1442	79.0721	100.6955	0.0000***
Within treatments	12	9.4231	0.7853		
Total	14	167.5674			

LSD Procedure

TREATMENTS	CONTROL	2,4-D+PEG	PREG
CONTROL			
2,4-D+PEG	***		
PEG	***		

Table 3.23.2
Analysis of Variance for Fig. 3.23

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	855.7781	427.8890	109.2832	0.0000***
Within treatments	12	46.9850	3.9154		
Total	14	902.7631			

LSD Procedure

TREATMENTS	CONTROL	2,4-D+PEG	PEG
CONTROL			
2,4-D+PEG	***		
PEG	***	***	

Table 3.23.3
Analysis of Variance for Fig. 3.23

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	758.2968	379.1484	89.6332	0.0000***
Within treatments	12	50.7600	4.2300		
Total	14	809.0568			

LSD Procedure

TREATMENTS	CONTROL	PEG	2,4-D+PEG
CONTROL			
PEG	***		
2,4-D+PEG	***		

Table 3.23.4
Analysis of Variance for Fig. 3.23

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	550.6845	275.3423	62.7822	0.0000***
Within treatments	12	52.6281	4.3857		
Total	14	603.3126			

LSD Procedure

TREATMENTS	CONTROL	PEG	2,4-D+PEG
CONTROL			
PEG			
2,4-D+PEG	***	***	

Table 3.23.5
Analysis of Variance for Fig. 3.23

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	386.0162	193.0081	38.2035	0.0000***
Within treatments	12	60.6253	5.0521		
Total	14	446.6415			

LSD Procedure

TREATMENTS	CONTROL	PEG	2,4-D+PEG
CONTROL			
PEG	***		
2,4-D+PEG	***		

Table 3.24.1
Analysis of Variance for Fig. 3.24

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	139.5291	69.7645	105.8482	0.0000***
Within treatments	12	7.9092	0.6591		
Total	14	147.4382			

LSD Procedure

TREATMENTS	CONTROL	2,4-D	2,4-D+PEG
CONTROL			
2,4-D			
2,4-D+PEG	***	***	

Table 3.24.2
Analysis of Variance for Fig. 3.24

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	7.2520	3.6260	101.7802	0.0000***
Within treatments	12	0.4275	0.0356		
Total	14	7.6796			

LSD Procedure

TREATMENTS	2,4-D	CONTROL	2,4-D+PEG
2,4-D			
CONTROL			
2,4-D+PEG	***	***	

Table 3.24.3
Analysis of Variance for Fig. 3.24

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	4.1813	2.0907	253.5545	0.0000***
Within treatments	12	0.0989	0.0082		
Total	14	4.2803			

LSD Procedure

TREATMENTS	2,4-D	CONTROL	2,4-D+PEG
2,4-D			
CONTROL			
2,4-D+PEG	***	***	

Table 3.24.4
Analysis of Variance for Fig. 3.24

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	4.5180	2.2590	139.1075	0.0000***
Within treatments	12	0.1949	0.0162		
Total	14	4.7128			

LSD Procedure

TREATMENTS	CONTROL	2,4-D	2,4-D+PEG
CONTROL			
2,4-D			
2,4-D+PEG	***	***	

Table 3.24.5
Analysis of Variance for Fig. 3.24

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	3.9097	1.9548	367.4136	0.0000***
Within treatments	12	0.0638	0.0053		
Total	14	3.9735			

LSD Procedure

TREATMENTS	2,4-D	CONTROL	2,4-D+PEG
2,4-D			
CONTROL			
2,4-D+PEG	***	***	

Table 3.25
Analysis of Variance for Fig. 3.25

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	7	928.9064	132.7009	93.0630	0.0000***
Within treatments	112	159.7037	1.4259		
Total	119	1088.6101			

LSD Procedure

TREATMENTS	1	2	3	4	5	6	7	8
CONTROL 1								
1+WATER 2								
10 PPM+WATER 3								
100 PPM+WATER 4								
10 PPM+DROUGHT 5								
100 PPM+DROUGHT 6								
1 PPM+DROUGHT 7	***	***	***	***	***	***		
DROUGHT 8	***	***	***	***	***	***	*	

Table 3.26.1
Analysis of Variance for Fig. 3.26

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	10.4378	5.2189	152.8436	0.0000***
Within treatments	24	0.8195	0.0341		
Total	26	11.2573			

LSD Procedure

TREATMENTS	CONTROL	2,4-D+PEG	PEG
CONTROL			
2,4-D+PEG			
PEG	***	***	

Table 3.26.2
Analysis of Variance for Fig. 3.26

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	140.3510	70.1755	192.4803	0.0000***
Within treatments	22	8.0209	0.3646		
Total	24	148.3719			

LSD Procedure

TREATMENTS	CONTROL	2,4-D+PEG	PEG
CONTROL			
2,4-D+PEG	***		
PEG	***	***	

Table 3.26.3
Analysis of Variance for Fig. 3.26

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	1584.0207	792.0103	149.2064	0.0000***
Within treatments	22	116.7793	5.3082		
Total	24	1700.8000			

LSD Procedure

TREATMENTS	CONTROL	2,4-D+PEG	PEG
CONTROL			
2,4-D+PEG	***		***
PEG	***		

Table 3.26.4
Analysis of Variance for Fig. 3.26

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	13442.8529	6721.4264	218.4567	0.0000***
Within treatments	22	676.8911	30.7678		
Total	24	14119.7440			

LSD Procedure

TREATMENTS	CONTROL	2,4-D+PEG	PEG
CONTROL			
2,4-D+PEG	***		
PEG	***	***	

Table 3.26.5
Analysis of Variance for Fig. 3.26

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	7286.7593	3643.3797	158.2370	0.0000***
Within treatments	22	506.5444	23.0247		
Total	24	7793.3037			

LSD Procedure

TREATMENTS	CONTROL	PEG	2,4-D+PEG
CONTROL			
PEG	***		
2,4-D+PEG	***	**	

Table 3.27.1
Analysis of Variance for Fig. 3.27

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	0.1193	0.0596	2.8819	0.0784N.S
Within treatments	21	0.4346	0.0207		
Total	23	0.5538			

Table 3.27.2
Analysis of Variance for Fig. 3.27

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	0.6661	0.3330	20.7237	0.0000***
Within treatments	18	0.2893	0.0161		
Total	20	0.9553			

bf LSD Procedure

TREATMENTS	CONTROL	2,4-D	2,4-D+PEG
CONTROL			
2,4-D			
2,4-D+PEG	*	*	

Table 3.27.3
Analysis of Variance for Fig. 3.27

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	2.0111	1.0055	117.0341	0.0000***
Within treatments	18	0.1547	0.0086		
Total	20	2.1657			

LSD Procedure

TREATMENTS	CONTROL	2,4-D	2,4-D+PEG
CONTROL			
2,4-D	***		
2,4-D+PEG	***	***	

Table 3.27.4
Analysis of Variance for Fig. 3.27

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	8.9857	4.4928	27.9223	0.0000***
Within treatments	18	2.8963	0.1609		
Total	20	11.8820			

LSD Procedure

TREATMENTS	CONTROL	2,4-D	2,4-D+PEG
CONTROL			
2,4-D	***		
2,4-D+PEG	***	*	

Table 3.27.5
Analysis of Variance for Fig. 3.27

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	22.5418	11.2709	132.6005	0.0000***
Within treatments	18	1.5300	0.0850		
Total	20	24.0718			

LSD Procedure

TREATMENTS	CONTROL	2,4-D	2,4-D+PEG
CONTROL			
2,4-D	***		
2,4-D+PEG	***		

Table 3.27.6
Analysis of Variance for Fig. 3.27

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	2	19.5122	9.7561	92.4030	0.0000***
Within treatments	18	1.9005	0.1056		
Total	20	21.4127			

LSD Procedure

TREATMENTS	CONTROL	2,4-D+PEG	2,4-D
CONTROL			
2,4-D+PEG	***		
2,4-D	***	**	

Table 3.28
Analysis of Variance for Fig. 3.28

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	1202.9693	400.9898	39.6684	0.0000***
Within treatments	36	363.9076	10.1085		
Total	39	1566.8769			

LSD Procedure

LSD Procedure

TREATMENTS	CONTROL	2,4-D	DROUGHT	2,4-D+DROUGHT
CONTROL				
2,4-D				
DROUGHT	**	**		
2,4-D+DROUGHT	***	***	***	

Chapter 4

Table 4.1
Analysis of Variance for Fig. 4.1

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	5	30946.1147	6189.2229	20.7635	0.0000***
Within treatments	24	7153.9791	298.0825		
Total	29	38100.0939			

LSD Procedure

SEEDLING ORGAN	TREATMENTS	1	2	3	4	5	6
COTYLEDONS	2,4-D+WARER 1						
COTYLEDONS	2,4-D+PEG 2						
HYPOCOTYLS	2,4-D+PEG 3						
HYPOCOTYLS	2,4-D+WATER 4	*					
ROOTS	2,4-D+WATER 5	***	***	***	***		
ROOTS	2,4-D+WATER 6	***	***	***	***		

Table 4.2
Analysis of Variance for Fig. 4.2

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	7	80820827.60	11545832.51	38.8123	0.0000***
Within treatments	32	9519324.400	297478.8875		
Total	39	90340152.00			

LSD Procedure

SEEDLING ORGAN	TREATMENTS	1	2	3	4	5	6	7	8
COTYLEDONS	2,4-D+PEG 1								
COTYLEDONS	2,4-D+WATER 2								
HYPOCOTYLS	2,4-D+PEG 3								
ROOTS	2,4-D+PEG 4								
TOTAL	2,4-D+PEG 5	*	*						
HYPOCOTYLS	2,4-D+WATER 6	**	**	*					
ROOTS	2,4-D+WATER 7	***	***	***	***	***	***		
TOTAL	2,4-D+WATER 8	***	***	***	***	***	***	**	

Table 4.3
Analysis of Variance for Fig. 4.3

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	11	21612.0501	1964.7318	7.3274	0.0000***
Within treatments	45	120612.0501	268.1336		
Total	56	33678.0643			

LSD Procedure

ORGAN	TREATMENTS	1	2	3	4	5	6	7	8	9	10	11	12
C	FROM 2,4-D+WATER TO PEG 1												
C	FROM 2,4-D+WATER TO WATER 2												
C	FROM 2,4-D+PEG TO WATER 3												
C	FROM 2,4-D+PEG TO PEG 4												
H	FROM 2,4-D+PEG TO PEG 5	*	*										
H	FROM 2,4-D+PEG TO WATER 6	**	**	*									
H	FROM 2,4-D+WATER TO WATER 7	***	***	**	*								
H	FROM 2,4-D+WATER TO PEG 8	***	***	***	**								
R	FROM 2,4-D+WATER TO PEG 9	***	***	***	**								
R	FROM 2,4-D+PEG TO WATER 10	***	***	***	**								
R	FROM 2,4-D+PEG TO PEG 11	***	***	***	**	*							
R	FROM 2,4-D+WATER TO WATER 12	***	***	***	**	*							

Table 4.4
Analysis of Variance for Fig. 4.4

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	15	49556786.41	3303785.760	18.2580	0.0000***
Within treatments	60	10856991.70	180949.8617		
Total	75	60413778.11			

LSD Procedure

ORGAN	TREATMENTS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
C	2,4-D+WATER TO PEG 1																
C	2,4-D+PEG TO WATER 2																
C	2,4-D+PEG TO PEG 3																
C	2,4-D+WATER TO WATER 4																
H	2,4-D+PEG TO PEG 5																
H	2,4-D+PEG TO WATER 6																
R	2,4-D+PEG TO PEG 7																
R	2,4-D+PEG TO WATER 8																
T	2,4-D+PEG TO PEG 9																
T	2,4-D+PEG TO WATER 10																
H	2,4-D+WATER TO WATER 11	***	***	***	**	**	**	**	**	**	**						
H	2,4-D+WATER TO PEG 12	***	***	***	***	***	***	***	***	**	**						
R	2,4-D+WATER TO WATER 13	***	***	***	***	***	***	***	***	**	**						
R	2,4-D+WATER TO PEG 14	***	***	***	***	***	***	***	***	***	***						
T	2,4-D+WATER TO PEG 15	***	***	***	***	***	***	***	***	***	***	***	***	***	***		
T	2,4-D+WATER TO WATER 16	***	***	***	***	***	***	***	***	***	***	***	***	***	***		

Table 4.5
Analysis of Variance for Fig. 4.5

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	3	49413.7164	16471.2388	22.4171	0.0000***
Within treatments	18	13225.7356	734.7631		
Total	21	62639.4520			

LSD Procedure

SEEDLING ORGAN	TREATMENTS	1	2	3	4
SHOOTS	2,4-D+WATER 1				
SHOOTS	2,4-D+PEG 2				
ROOTS	2,4-D+PEG 3	***	***		
ROOTS	2,4-D+WATER 4	***	***		

Table 4.6
Analysis of Variance for Fig. 4.6

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
Between treatments	5	4643224.563	928644.9126	14.0272	0.0000***
Within treatments	27	1787485.939	66203.1829		
Total	32	6430710.502			

LSD Procedure

SEEDLING ORGAN	TREATMENTS	1	2	3	4	5	6
SHOOTS	2,4-D+PEG 1						
SHOOTS	2,4-D+WATER 2						
ROOTS	2,4-D+PEG 3	**	**				
TOTAL	2,4-D+PEG 4	**	**				
ROOTS	2,4-D+WATER 5	***	***	**	**		
TOTAL	2,4-D+WATER 6	***	***	**	**