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## **DOCTOR OF PHILOSOPHY**

**Studies of the biological activity of a novel herbicide (Triasulfuron) including studies of mobility and persistence in soils.**

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STUDIES ON THE BIOLOGICAL ACTIVITY OF A NOVEL HERBICIDE  
(TRIASULFURON) INCLUDING STUDIES OF MOBILITY AND PERSISTENCE  
IN SOIL.

A thesis presented for the degree of Philosophiae Doctor in the  
University of Wales.

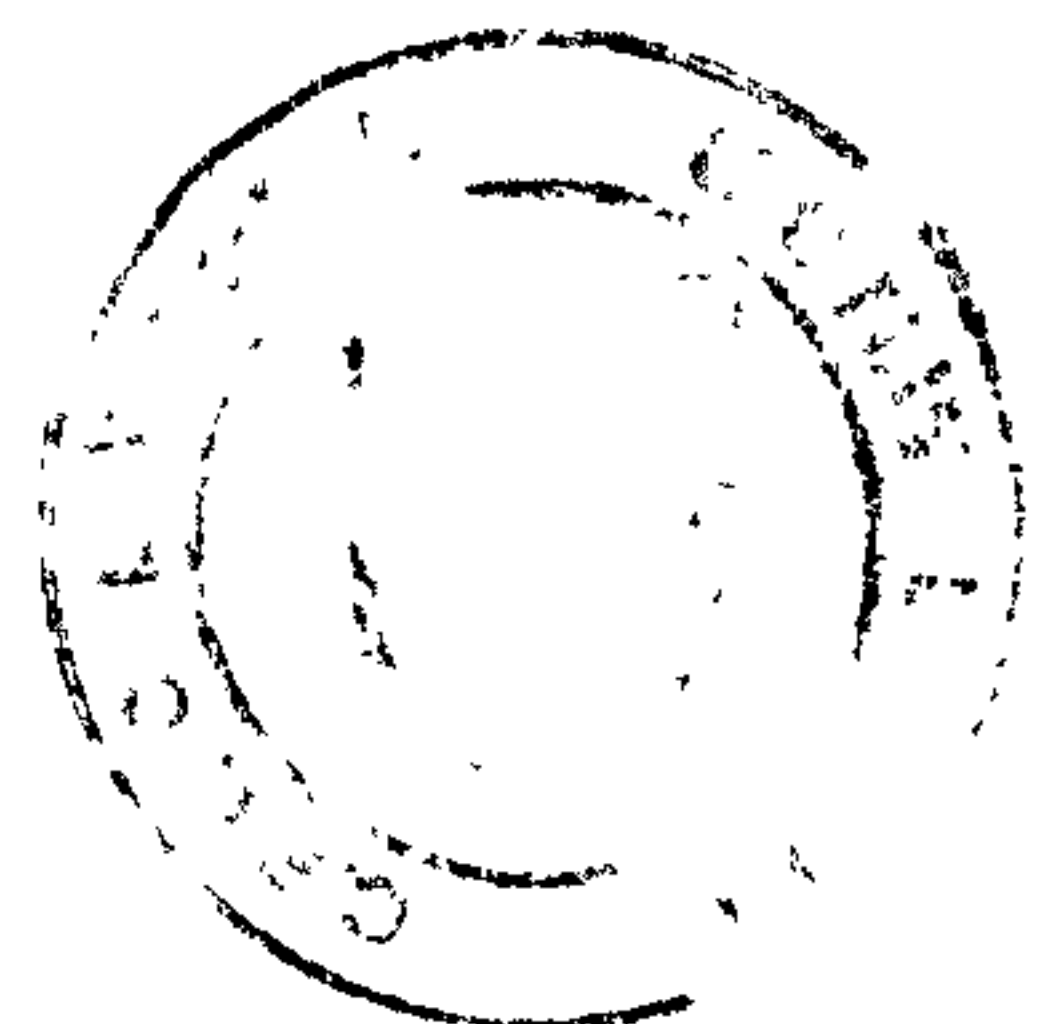
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To my parents.

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## SUMMARY.

Triasulfuron {3-(6-methoxy-4-methyl-1,3,5-triazin-2-yl)-1-[2-(2-chloroethoxy)-phenylsulfonyl]-urea} is a new pre- and post-emergence herbicide developed for the control of broad-leaved weeds and some grasses in small grain cereals at rates of 10-20g $\text{ha}^{-1}$ .

Oilseed rape, pea, broad bean, Senecio vulgaris, Veronica persica, Lolium perenne, Poa annua, Poa trivialis and Poa pratensis were severely affected by pre-emergence and post-emergence applications of triasulfuron but wheat and barley were much more resistant. The development of injury symptoms was generally slow and was characterized by an initial growth retardation followed by chlorosis and necrosis with death occurring 3-4 weeks after application. The herbicide had a flat dose-response curve throughout the investigations.

Wheat and barley showed greater tolerance to post-emergence than to pre-emergence applications. Pre-emergence applications of triasulfuron reduced tiller numbers in barley and wheat but application of the herbicide at the 2-3 leaf stage stimulated the outgrowth of tiller buds but this effect was temporary and was not the result of visible physical damage to the shoot meristem. There was no intra-specific variability between cultivars of wheat and barley. Grain yield and other yield components of spring barley were not affected by post-emergence treatments of triasulfuron.

Triasulfuron had high activity through the soil and both the roots and subterranean shoots of developing seedlings absorbed the herbicide from the soil. The bioactivity and mobility of the herbicide down the soil profile was inversely related to the organic matter content of the soil. The amount and frequency of rainfall directly influenced the rate of leaching of the herbicide down the soil profile.

Comparisons of the rates of disappearance of triasulfuron in autoclaved and non-autoclaved soils suggested the involvement of a biological degradation pathway. Triasulfuron disappeared faster at 30°C than at 10°C and higher moisture levels enhanced the rate of breakdown.

Studies on the mode of action of triasulfuron indicated that the herbicide acts by inhibiting cell division in susceptible plants. Evidence suggested that the inhibition occurred during interphase rather than during the mitotic sequence. The addition of a 1:1 mixture of isoleucine and valine to the treatment solution prevented the inhibition of cell division at the root tips of broad bean.

CHAPTER 1.

GENERAL INTRODUCTION.

## GENERAL INTRODUCTION.

One of the significant contributions to the rapid development of modern agriculture has been the use of selective herbicides. Their introduction in the 1940's led to many changes in the patterns of crop production. In many areas of the world, chemicals replaced hand and mechanical methods of weed control (Eichers and Andrilena, 1979). However, whilst crop production has increased at a rate far in excess of the increase in market demand in developed countries, food production is still low in most developing countries where the use of herbicides for weed control is limited. The continuously expanding world population, coupled with the serious food deficits experienced annually in many less developed areas of the world, make it increasingly important for herbicides to be fully exploited to advance human welfare through increased production of food and raw materials.

The use of herbicides however, has some associated problems, notably the creation of unemployment, risk to the environment and changes in weed problems. For example, the replacement of dicotyledonous weeds by grasses as a result of the continuous use of the chlorophenoxy compounds (Ashton and Crafts, 1973) is well known and so also is the serious and widespread resistance of a number of broad-leaf weeds and grasses to the triazine herbicides (LeBaron and Gressel, 1982). Many or all of the substituted ureas, s-triazines, uracils, picloram and the benzoic acid derivatives are known to have long persistence in the soil (Ashton and Crafts, 1973) and therefore have the potential of limiting the types of subsequent crops that can be grown following their continuous use.



Herbicides may be classified into non-selective types, which kill all vegetation or selective types which are applied to suppress or kill weeds without injuring the crop. Some herbicides are effective only at their points of contact with the weed while others have to be applied to the foliage or to the soil before they are absorbed and translocated by the foliage, roots or both, to sites of action which are usually remote from the site of application or uptake. Climatic factors such as temperature, humidity and rainfall and edaphic factors like soil type and soil moisture can have large influences on the phytotoxicity and selectivity of herbicides.

In spite of the already impressive number of chemicals used for weed control [about 80 chemicals have been approved for use in the United Kingdom alone (Anon, 1985)], new herbicides are still being introduced. Detailed studies of the selectivity, mode of action and persistence of these chemicals are needed.

Triasulfuron, {3-(6-methoxy-4-methyl-1,3,5-triazin-2-yl)-1-[2-(2-chloroethoxy)-phenylsulfonyl]-urea}, code-named CGA 131'036, is a new herbicide developed for use in small grain cereals and which is active through both foliage and soil. It was introduced by Ciba-Geigy agrochemicals in late 1985 and it belongs to the sulfonylurea class of chemicals which have high activity at extremely low doses. In its pure form, it is a white crystalline solid with a molecular weight of 401.83, a melting point of 186°C and vapour pressure of  $7.5 \times 10^{-13}$  mm Hg at 20°C and pH 7; it is slightly soluble in most organic solvents. The acute oral and dermal toxicities (LD<sub>50</sub>) to rats are greater than 5000 and 2000 mgKg<sup>-1</sup> respectively. The commercial product is formulated as water dispersible granules (Amrein and

Gerber, 1985). The chemical formula of triasulfuron is given in Figure 1.1.

The herbicide is applied from the two-leaf stage to the booting stage of cereals at rates between 10 and 20g ai ha<sup>-1</sup>. There is pre-emergence activity on and in the soil which provides season long control of late germinating weeds (Amrein and Gerber, 1985). Triasulfuron is compatible with substituted urea herbicides; and mixtures of triasulfuron and isoproturon {N,N-dimethyl-N'-[4-(1-methylethyl)phenyl]urea} or chlorotoluron {N'-(3-chloro-4-methylphenyl)-N,N-dimethylurea} controlled Bromus sterilis L. and Alopecurus myosuroides Huds. in cereals when applied post-emergence (Hobson and Ryan, 1987).

Selectivity occurs when herbicide-treated weeds are inhibited in their growth or killed while the crop remains relatively unharmed (Holly, 1976). The sulfonyleurea herbicides are particularly effective in controlling broad leaved weeds in cereals with some degree of activity on some grasses. Post-emergence applications of triasulfuron provided excellent selective activity against a wide spectrum of dicot weeds in European winter and spring cereals with some partial effects on grasses like Apera spica-venti (L.) Beauv. and Lolium spp. (Amrein and Gerber, 1985).

Practical selectivity may be achieved when a herbicide is applied in an orchard or plantation in such a way that only the weeds growing under the crop are sprayed thus leaving the crop unaffected. In some situations, practical selectivity may be achieved when a contact herbicide is applied to control emerged weeds before the crop emerges. However, real selectivity is most often dependent on biological differences between the crop and the weed (Sagar, 1968)



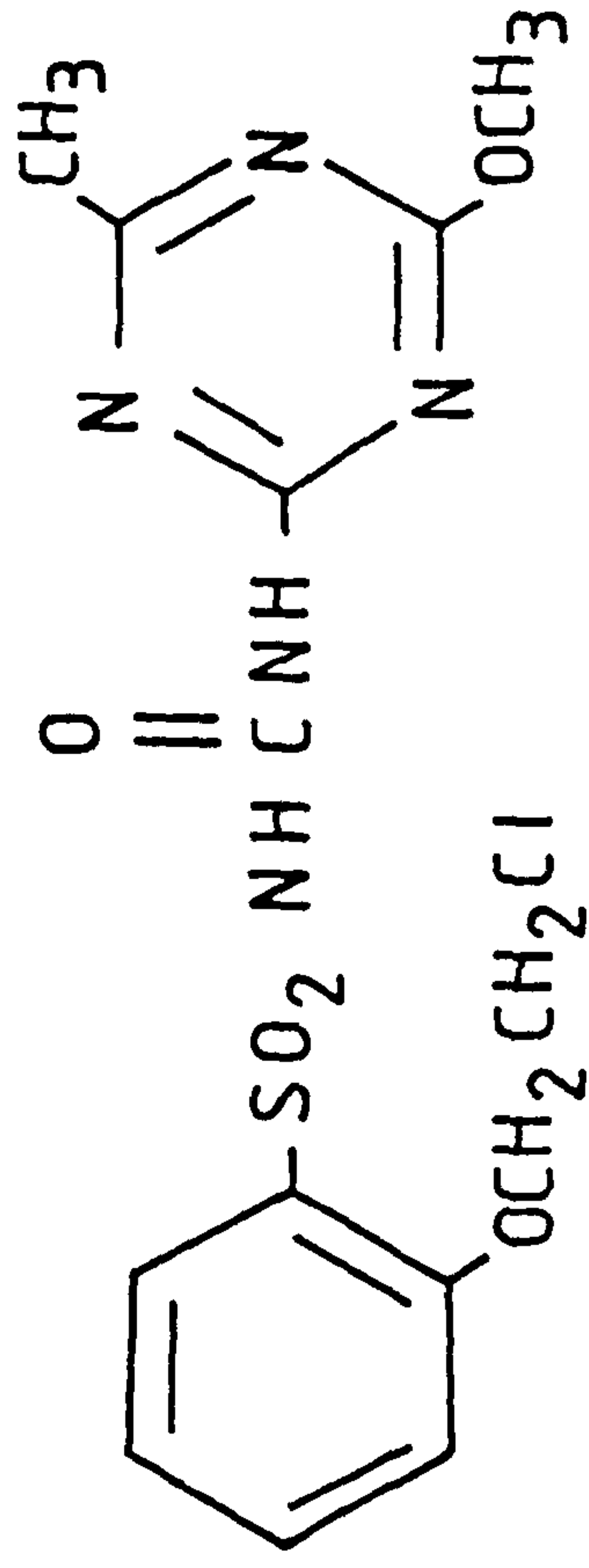


FIG.1.1. CHEMICAL FORMULA OF TRIASULFURON

and as such any variations in, for example, herbicide retention and penetration into the shoot could result in selectivity being achieved. Feeny and Colby (1968) found eight times as much chloroxuron {N'-[4-(4-chlorophenoxy)phenyl]-N,N-dimethylurea} in the leaves of susceptible annual morning glory (Ipomoea purpurea L.) than in the tolerant soybean [Glycine max (L.) Merr. which had been similarly treated. Differences in the rate of entry of ioxynil (4-hydroxy-3,5-diiodobenzonitrile) into leaves of mustard (Sinapis alba L.), barley (Hordeum vulgare L.) and pea (Pisum sativum L.) was a contributory factor to selectivity between these species (Davies et al., 1968). Whilst investigating the selectivity of asulam{methyl[(4-aminophenyl)sulfonyl]carbamate} between wild oat (Avena fatua L. and linseed (Linum usitatissimum L.), Hebbit (1969) found an increase in retention per unit fresh weight with increasing age of the susceptible wild oat but retention decreased with age of the tolerant linseed. In separate studies, Kirkwood et al.(1966; 1968; 1972) noted that the resistance of broad bean (Vicia faba L.) to MCPB {4-(4-chloro-2-methylphenoxy)butanoic acid) is partly due to the inability of the compound to pass through the cuticle as readily as MCPA {(4-chloro-2-methylphenoxy)acetic acid} to which broad bean is susceptible. Similar conclusions have been reached by Neidermeyer and Nalewaja (1970) for barban {4-chloro-2-butynyl-3-chlorophenyl carbamate} in the selectivity between wild oats and wheat (Triticum aestivum L.), Sharma and Vanden Born (1973) for picloram {4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid} between barley and soybean and by Hosaka and Takagi (1987) for sethoxydim {2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one} between

maize (Zea mays L.) and pea.

Differences in translocation and in accumulation of herbicide at the sites of action may also contribute towards selectivity. This form of selectivity was demonstrated when Chenopodium album L., a susceptible species, was shown to absorb and extensively translocate both MCPA and MCPB whereas Polygonum convolvulus L., which is resistant to both herbicides, failed to translocate these chemicals out of the treated leaves (Kirkwood et al., 1966). Similar observations have been made for soybean where marked variations in the translocation and accumulation of bentazon {3-(1-methylethyl)-(1H)-2,1,3-benzon-thiadiazin -4(3H)-one 2, 2-dioxide} in the active sites of different cultivars was related to selectivity (Hayes and Wax, 1975).

In some situations selectivity is based on the ability of plants to metabolize inactive compounds into active derivatives of that compound. A classical example is the conversion of 2,4-DB {4-(2,4-dichlorophenoxy)butanoic acid} and MCPB to the potent 2,4-D and MCPA in susceptible species (Wain, 1954) but not in resistant species. Based on the same principle, MCPA, an active herbicide was inactivated in Galium aparine L. through a biochemical mechanism whereby the side chain of the herbicide molecule was split off thus rendering it harmless to the plant (Leafe, 1962).

Another basis for selectivity is the differential adsorption or binding of herbicides at metabolically inactive sites within the plant. Some molecules may interact or be absorbed at less sensitive sites during translocation and thereby rendered incapable of exerting a biological effect (Brian 1958). Leaves of burcucumber (Sicyos angulatus L.) and oats (Avena sativa L.) treated with 2,4-D did not



show any serious injury due to the immobilization of the herbicide in the treated leaves but in the susceptible cocklebur (Xanthium spp.), the herbicide was translocated from the treated leaves and caused severe injury to the whole plant (Dexter et al., 1971).

Selectivity may be achieved as a result of differences in the rate of detoxification of herbicides in plants. Many plants owe their resistance to the rapid rates at which they can detoxify such compounds. Red currants (Ribes rubrum L.) detoxifies 2,4-D much more rapidly than does the more susceptible black currant (Ribes nigrum L.) (Luckwill and Lloyd-Jones, 1960). Likewise, the resistance of maize to simazine {6-chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine} (Hamilton and Moreland, 1962) and atrazine {6-chloro-N ethyl-N'-(1-methylethyl)-1,3,5 triazine-2,4-diamine} (Shimabukuro et al., 1971) has been ascribed to a biochemical mechanism which breaks down the phytotoxic molecule to non-toxic derivatives. In other studies, differences in the detoxification of propanil {N-(3,4-dichlorophenyl)propanamide} (Frear and Shimabukuro, 1970); dicamba {3,6-dichloro-2-methoxybenzoic acid} (Chang and Vanden Born, 1971) and diclofop {(+) -2-[4-(2,4-dichlorophenoxy) phenoxy]propanoic acid} (Shimabukuro et al., 1979; Donald and Shimabukuro, 1980) was the basis for selectivity between resistant and susceptible species.

The selectivity of the sulfonylurea herbicides has been found to be due to differential detoxification of the active ingredient in resistant and susceptible species. In studies in which similar quantities of <sup>14</sup>C-chlorosulfuron {2-chloro-N-[[4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide} were applied to leaves of wheat, wild oat, sugar beet (Beta vulgaris L.) and cotton

(Gossypium hirsutum L.), less than 10% of the labelled herbicide was recovered from wheat or wild oat whereas 80 and 97% was unmetabolized in the susceptible cotton and sugar beet plants respectively (Sweetzer et al., 1982). Likewise, Hageman and Behrens (1984) reported that only 7% of absorbed chlorsulfuron was metabolized in the susceptible velvetleaf (Abutilon theophrasti Medic.) after 72 hours but the resistant eastern black nightshade (Solanum ptycanthum Dun.) had metabolized 81% of the absorbed chlorsulfuron within the same period.

Another (theoretical) basis for selectivity may occur when the susceptible species has a specific and essential biochemical process which is absent in the resistant species. Toxicity of a herbicide to that process could result in selectivity being achieved (Roberts, 1982).

If absorption of a herbicide is mainly by the roots, selectivity can occur if crop and weed roots are of different depths (Holly, 1976). This type of selectivity, often referred to as depth protection, depends on the relative absence of movement of the chemical in the soil profile. Selectivity, based on depth protection will therefore be at risk in very porous soils where there is excessive rainfall or where the herbicide has high solubility. In addition, the physical chemistry of the herbicide will play a major part in mobility through the process of adsorption. Because of these limiting factors, in all situations where depth protection is important in selectivity, herbicide recommendations require depths of untreated soil above the crop seed to be specified (Walker, 1980). Herbicide application under tree crops has on many occasions been successful due to the existence of depth protection.



Even though both the roots of a crop and a weed may be localized in the same horizon within the soil, selectivity could still be achieved because of differences in the absorption of the herbicide by the roots. Freeman et al. (1964) with simazine, Appleby et al. (1965) with diallate {S-(2,3-dichloro-2-propenyl) bis(1-methylethyl) carbamothioate}, Hargroder and Rogers (1974) and Devlin et al. (1987) with metribuzin {4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one} and Basler et al. (1978) with prometryne {N,N'-bis(1-methylethyl)-6-methylthio-1,3,5-triazine-2,4-diamine} have all related the selectivities of these herbicides to their greater uptake by roots in susceptible rather than in resistant species. However, no such relationship between selectivity and uptake by the roots was found with chlorpropham {1-methylethyl 3-chlorophenylcarbamate} (Prendeville, 1968) or triallate {S-[2,3,3-trichloro-2-propenyl)bis(1-methylethyl)carbamo-thioate} (Thiele and Zimdahl, 1976).

In some cases selectivity may be explained by differences in the ability of some species to retain absorbed herbicides in their roots with very little translocation to the sensitive areas of the shoot where the herbicide may have its site of action. Examples of this type of selectivity have been reported with the diphenylethers (Shimotori and Kuwatsuka, 1978; Vanstone and Stobbe, 1978); norflurazon {4-chloro-5-(methylamino)-2-(3-(trifluoromethyl)phenyl)-3(2H)-pyridazone} (Strang and Rogers, 1975) and fluridone {1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenoxy]-2-nitrobenzoate} (Bernard et al., 1978).

Several studies have shown that uptake of herbicide from the soil by young shoots of seedlings may occur and selectivity under these

circumstances has been reported. Differential uptake of the thiocarbamates (Appleby et al., 1965; Parker, 1966; Nalewaja, 1968; Prendeville, 1968), atrazine and trifluralin {2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzidineamine} (Knake et al., 1967), dinitroanilines (Barrentine and Warren, 1971), acetamides (Chandler et al., 1974; Narsaiah and Harvey, 1977) by the emerging shoot of young seedlings caused more damage to susceptible rather than to resistant species. This type of selectivity is sometimes attributed to variations in the development and length of the mesocotyl or first internode, the coleoptile (Parker, 1963, 1966) and the crown node (Baker, 1960) of various grasses. In the susceptible species, the region of most active uptake by young developing shoots remains in the soil for a relatively longer period and as a result contact with the herbicide is over a longer period whereas the sensitive regions of the resistant species emerge rapidly out of the herbicide-treated zone with a relatively shorter period of contact with the herbicide.

The performance of a soil applied herbicide in relation to the depth at which it is placed in the soil is determined by the chemical and physical properties of the chemical and the soil, climatic factors and the site through which the herbicide is absorbed into the plant. Several workers have observed a reduction in herbicide injury to weeds or crop species with increasing depth of incorporation, which in some cases has been ascribed to a dilution effect (Friesen et al., 1962; Parker, 1963; Knake et al., 1967; Savage and Barrentine, 1969; Narsaiah and Harvey, 1977). Conversely, studies with EPTC {S-ethyl dipropylcarbamothioate} (Waldrep and Freeman, 1964; Menges and Hubbard, 1966), several thiocarbamates (Hauser, 1965), chloramben {3-amino-2,5-dichlorobenzoic acid} (Sommerville and



Wax, 1971) and butylate {S-ethyl bis(2-methylpropyl)carbamothioate} (Wright and Rieck, 1974) showed increasing phytotoxicity to various plants following increased depths of incorporation. Okafor et al. (1983a) reported maximum phytotoxicity from dinitramine {N<sup>3</sup>,N<sup>3</sup>-diethyl-2,4-dinitro-6-(trifluoromethyl)-1,3-benzenediamine} to bean (Phaseolus vulgaris L.) seedlings which were sown into a zone into which the herbicide had been incorporated and injury to the plant increased as the distance between the point of contact with the herbicide and the soil surface increased.

The soil is a complex and a dynamic system and because of this the behaviour of soil applied herbicides is influenced to a large extent by several properties of the soil. Many researchers have studied the effects of organic matter (Sheets, 1958; Upchurch, 1958; Sheets et al., 1962; Upchurch et al., 1966; Grover, 1966; Stevenson, 1972; Weber et al., 1974; Harrison et al., 1976) and clay (Coggins and Crafts, 1959; Greenland, 1965; Bailey et al., 1968; Weber, 1970) on the activity of herbicides. In all these studies, soil organic matter was found to exert the most significant influence on the activity of soil applied herbicides and high negative correlations have been obtained between the activities of herbicides like the phenylureas (Sheets, 1958), s-triazines (Upchurch and Mason, 1962; Sheets et al., 1962; Hayes, 1970; Rahman and Matthews, 1979), the substituted ureas (Harris and Sheets, 1965); dinitroanilines (Horowitz et al., 1974; Weber et al., 1974; Okafor et al., 1983b) and the organic matter content of soils. It should be however noted that for a few herbicides ( eg. diquat {6,7-dihydrodipyrido[1,2- $\alpha$ :2',1'-c]pyrazinedium ion}, paraquat {1,1'-dimethyl-4,4'-bipyridinium ion}



and glyphosate {N-(phosphonomethyl) glycine} ), it is the clay fraction of the soil which is significantly more important than the organic matter fraction (Coats et al., 1966; Weber and Scott, 1966; Calderbank and Tomlinson, 1968; Sprankle et al., 1975).

Mersie and Foy, (1985) examined the activity of chlorsulfuron in six different soils and concluded that organic matter was the soil variable with the greatest influence on chlorsulfuron phytotoxicity. In all the six soils, an inverse relationship between chlorsulfuron bioactivity and soil organic matter was established but there was no significant relationship between soil clay content and chlorsulfuron toxicity. These results were confirmed by Anderson and Humburg (1987) whose studies revealed that organic matter levels had the largest influence on the duration of chlorsulfuron bioactivity and leaching in the soil. However, Eleftherohorinos et al. (1985) did not find any effect on the availability, distribution or persistence of chlorsulfuron, applied at normal field rates on soils amended with either mushroom compost, chopped straw, wheat straw ash or their combinations and hence concluded that the performance of chlorsulfuron is not likely to be affected by changes in soil organic matter typical of arable cropping with cultivations.

In general, adsorption of herbicide by soil constituents is believed to be the major reason for reduced toxicity of herbicides in soil. Adsorption is the reversible fixation of a dissolved or vapourous substance on or in the surfaces of a solid or liquid (Hartley, 1976). The concentration of a herbicide in the soil solution normally depends on how strongly it is adsorbed onto soil surfaces and the position of the equilibrium between adsorbed and free chemical determines the availability of the herbicide to plants,

its effectiveness and the rate at which it moves in the soil (Hartley, 1960; Holly, 1964). Adsorption isotherms are normally used to describe the extent of herbicide adsorption in different kinds of soil and they are well predicted by the empirical Freundlich equation which relates the amount of herbicide adsorbed to the amount remaining in solution (Hamaker and Thompson, 1972). The Freundlich equation is given by the relationship:  $X=KC^{1/n}$  where X is the amount of adsorbate (herbicide) per unit of adsorbent ( $\mu\text{g g}^{-1}$ ); C is the equilibrium adsorbate concentration ( $\mu\text{g cm}^{-3}$ ) while K and n are constants (Weber et al., 1986).

Desorption, which is the release of adsorbed material can occur in soils normally when the interaction between the adsorbate and the adsorbent causes a repulsion of the adsorbate from the surfaces of the adsorbent (Hamaker and Thompson, 1972; Roberts, 1982). Withdrawal of chemical from soil solution by for example, uptake by plant roots also causes desorption. The adsorption of chlorsulfuron to soil is usually stronger at low pH than at high pH whilst desorption normally increases with an increasing pH resulting in a higher phytotoxicity (Mersie and Foy, 1985).

Many residual herbicides are applied pre-emergence onto the surface of the soil or during the early stages of plant growth and rainfall or irrigation is relied upon to move the herbicide into the soil before it can be absorbed by the roots or other subterranean organs of the weeds. Knowledge of the relative ease with which a herbicide moves down the soil profile is therefore essential in understanding its efficacy, suitability for use as a selective herbicide and potential for contaminating ground water. Many



investigators have demonstrated that the movement of herbicides in the soil depends on several properties of the soil such as organic matter (Upchurch and Pierce, 1958; Hurtt et al., 1958; Harris, 1966; Gray and Weierich, 1968; Smith and Meggit, 1970a; Mersie and Foy, 1986), soil pH (Hurtt et al., 1958; Bailey et al., 1968; Best and Weber, 1974) and soil permeability, porosity and structure (Helling, 1971b). Others have also shown that movement in the soil is influenced by the water solubility, ionizability or vapour pressure of the herbicide (Harris, 1967; Wu and Santelmann, 1975; Weber and Whitacre, 1982). Furthermore, the amount, intensity and frequency of rainfall affects the rate of movement of herbicides in the soil profile (Upchurch and Pierce, 1957; Wiese and Davis, 1964; Weber and Whitacre, 1982). Gray and Weierich (1968) studied the movement of five thiocarbamate herbicides in five different soils and concluded that the depth of leaching in mineral soils were directly correlated with their solubility in water but inversely correlated with organic matter or clay content of the soil. However in an earlier study, the movement of four s-triazine herbicides was more related to their adsorption in the soil than to their water solubilities (Harris, 1966). Cationic herbicides like the bipyridiniums are amongst the least mobile herbicides due to their strong ionic bonding to the cation exchange complex of soil colloids but other herbicides such as picloram, bromacil {5-bromo-6-methyl-3-(1-methylpropyl)-2,4(1H,3H) pyrimidine dione}, dicamba and fenuron {N,N-dimethyl-N'-phenylurea} are highly mobile in soils because of their low adsorption to soil colloids (Weber et al., 1986).

Some sulfonylurea herbicides tend to be strongly adsorbed in soil and are thus less mobile whilst others like chlorsulfuron and

metsulfuron methyl {2-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino]carbonyl]sulfonyl]benzoic acid} are relatively mobile (Beyer et al., 1987). In general, the mobility of chlorsulfuron in the soil increases with increasing soil pH (Nicholls and Evans, 1985; Fredrickson and Shea, 1986; Mersie and Foy, 1986) and decreasing soil organic matter (Mersie and Foy, 1986; Beyer et al., 1987; Nicholls, 1988).

Many researchers have used soil columns (Upchurch and Pierce, 1958; Gray and Weierich, 1968; Brockman and Duke, 1977; Grover, 1977; Huggenberger and Ryan, 1985) to study the movement of herbicides in soil. Others have used soil thin-layer chromatography (Helling and Turner, 1968; Helling, 1971a; Wu and Santelmann, 1975) or soil thick-layer chromatography (Gerber et al., 1970; Wu and Santelmann, 1975) but Wu and Santelmann (1975) found herbicide leaching by the three different methods to be comparable. Huggenberger and Ryan, (1985) studied chlorsulfuron movement in loam soil packed into plastic columns and subjected to  $20\text{g ai ha}^{-1}$  of chlorsulfuron and a total of 265mm natural rainfall over a period of 5 months. They observed that the herbicide was leached to a depth of 10cm after 5 months but substantial quantities were still detected in the top 5cm of the soil. Another study with columns packed with a silt loam soil demonstrated that chlorsulfuron is capable of moving vertically upwards with rising capillary water (Nilsson, 1985). Nilsson therefore concluded that a relatively mobile sulfonylurea herbicide like chlorsulfuron which had earlier been leached beyond the root zone might re-enter the upper layers from deeper layers of the soil profile. Beyer et al. (1987) however suggested that, despite the high



mobility of some of the sulfonyleurea herbicides under certain soil and climatic conditions, their exceptionally low rates and low toxicities made it unlikely that they would pose problems of ground water pollution.

The movement and persistence of herbicides in soils is normally determined by bioassay or chemical analysis. Each of these methods has advantages and limitations and in spite of many improvements in the analytical methods, bioassays continue to be widely used in herbicide research because of their relative simplicity and versatility (Horowitz, 1976). A bioassay is a means of using living organisms to determine quantitatively the biologically active concentration of a herbicide known to be present in a material; or determining the presence or absence of a particular herbicide (Hance and Mckone, 1976; Lavy and Santelmann, 1986). In using bioassays, it is always considered that the species used will show an injury response proportional to the herbicide concentration and that the response obtained will be reproducible. Several researchers have used pre-germinated maize seedlings as bioassay species to estimate the concentrations of chlorsulfuron by measuring the lengths of roots or shoots after several days of growth in the material containing the unknown concentration of the herbicide (Hsiao and Smith, 1983; Anderson and Barret, 1985; Mersie and Foy, 1985). Other bioassay species like pea (Ray, 1984; Joshi et al., 1985;), lettuce (Lactuca sativa L.) (Walker and Brown, 1983a), Sinapis alba (Caseley, 1982) and Lolium multiflorum (L.) Lam. (Huggenberger and Ryan, 1985) have been used to estimate the amount or establish the presence or absence of chlorsulfuron in the soil.

The persistence of a herbicide in the soil is desirable during

the critical period of weed control but persistence beyond this can pose problems with regard to potential damage to the following crop especially if such a crop is susceptible to the herbicide. In addition, resistance to degradation of a herbicide in the soil could create problems of injury to non-target organisms as well as movement out of the intended zone of activity. Ideally, a soil applied herbicide should retain its activity long enough to provide satisfactory weed control but not so long that soil residues after crop harvest limit the nature of subsequent crops which can be grown (Hurle and Walker, 1980).

Herbicides are mainly degraded in the soil by a combination of microbiological and chemical processes and the rate at which the decomposition takes place is governed by soil type and environmental conditions. Factors such as organic matter content of the soil (Donaldson and Foy, 1965; Smith and Meggit, 1970a; Walker and Thompson, 1977), soil pH (Nearpass, 1965; Corbin and Upchurch, 1967; Ladlie et al., 1976); temperature (Zimdahl et al., 1970; Hyzack and Zimdahl, 1974; Gingerich and Zimdahl, 1976) and soil moisture (Meikle et al., 1973; Usoroh and Hance, 1974; Zimdahl and Gwynn, 1977) play important roles in determining the rates of microbial degradation of many herbicides.

In several studies, pH, temperature and soil moisture have been the principal factors influencing the dissipation of the sulfonylurea herbicides. The microbial breakdown of chlorsulfuron (Anderson and Barret, 1985; Joshi et al., 1985; Mersie and Foy, 1985; Thirunarayanan et al., 1985; Fredickson and Shea, 1986) and metsulfuron (Anderson and Barret, 1985) was enhanced at lower rather



than at higher soil pH. Other studies also showed that the disappearance of chlorsulfuron in the soil increased with increasing temperature and soil moisture (Walker and Brown, 1983a; Anderson and Barret, 1985; Joshi et al., 1985) but not with increasing levels of organic matter (Sampson et al., 1982).

Generally, microbial degradation of herbicides has been studied by measuring and comparing herbicide persistence in soils that have been autoclaved, fumigated, gamma-irradiated or treated with microbial inhibitors to reduce microbiological activity (Kaufman and Kearney, 1976), with soils which have not been sterilized. Biodegradation is considered to be the difference between residual herbicide in the sterile and non-sterile soils. Microbial degradation of some compounds is characterized by a lag phase with little or no breakdown after the application of the herbicide, followed by a phase of rapid disappearance of the compound (Kaufman and Kearney, 1976). Herbicides which follow this pattern of breakdown are usually of short persistence (Hurle and Walker, 1980; Roberts, 1982) and they include 2,4-D (Audus, 1951, 1960), dalapon {2,2-dichloropropanoic acid} (Kaufman, 1964), chlorpropham (Kaufman and Kearney, 1965); MCPA (Fryer and Kirkland, 1970) and propham {1-methylethylphenylcarbamate} (Kaufman and Blake, 1973). Other compounds follow a pattern, where the rate of degradation is proportional to the concentration of the herbicide in the soil with no lag phase and such compounds are usually of long persistence (Hurle and Walker, 1980; Roberts, 1982). First order kinetics, given by the equation:  $C=C_0 e^{-kt}$ , where C is the initial concentration and k the rate constant, is used to interpret results following this pattern of breakdown. A straight line relationship is usually obtained for plots of the logarithms of

herbicide concentrations against time. Half-lives (the time taken for 50% of the herbicide to degrade) are derived from the curves (See Hurle and Walker, 1980).

There is enough evidence to show that, soil micro-organisms rapidly degrade repeated applications of certain herbicides with reduced lag periods. Hurle and Rademacher (1970) compared the breakdown of DNOC {2-methyl-4,6-dinitro-phenol} and 2,4-D in soil treated for the first time and soil from field plots treated annually over a period of twelve years and found that 2,4-D dissipation was more rapid in previously treated soil than in soil treated for the first time whereas pre-treatment had no effect on the rates of dissipation of DNOC. The rapid disappearance of subsequent applications has been attributed to the preponderance of larger active populations of adapted organisms in the previously treated soil ( Kaufman and Kearney, 1976). Similar promotions of degradation of repeated application of herbicides have been demonstrated for various phenoxyalkanoic compounds (Audus, 1951, 1960; Kirkland and Fryer, 1966), endothal {7-oxabicyclo[2.2.1] heptane-2,3-dicarboxylic acid} (Horowitz, 1966), dalapon (Thiegs, 1955; Leasure, 1964), and prophan (Kaufman and Blake, 1973) but not for simazine or linuron {N'-(3,4-diclorophenyl)-N-methoxy-N-methylurea} (Fryer and Kirkland, 1970).

Tillering is an important phenomenon in the growth of grasses and cereals. Tillers develop from buds in the axils of expanded leaves and the sequence of production and arrangement follow well defined patterns. The apical meristem has a dominant influence over the growth and development of axillary buds (Philips, 1975), with



dominance increasing as the seedling grows through the vegetative to the reproductive phase (Jewiss, 1972). Apical dominance is believed to be due to competition for nutrients between the apical and lateral buds and to the effects of plant hormones (Philips, 1975). In view of this, any effect on the apical meristem in the form of injury, death or a disturbance in the balance of plant hormones will lead to the loss of the dominance. In most cases such effects are expressed through the release of lateral buds from dormancy.

Plant growth regulators have been widely exploited to increase tillering in cereals by using them to break apical dominance. For example, tiller bud outgrowth was stimulated when ancymidol ( $\alpha$ -cyclopropyl-4-methoxy- $\alpha$ -(pyrimidin-5-yl)benzyl alcohol) was applied to sorghum [Sorghum bicolor (L.) Moench.] (Isbell and Morgan, 1982). Some herbicides show such growth regulating properties when applied at low doses to cereals and grasses. Glyphosate, applied at sub-lethal doses increased tillering in quackgrass [Agropyron repens (L.) Beauv.] (Caseley, 1972; Coupland and Caseley, 1975). The responses induced by glyphosate application to sorghum and wheat has been given much attention (Baur et al., 1977). Basal bud outgrowth was stimulated in wheat at 27°C but not at 16°C and for sorghum, bud outgrowth was stimulated at 21°C following glyphosate application. An examination of the apex of sorghum did not show any injury, indicating that the glyphosate-induced basal bud development was not the result of the release of apical dominance that occurs when a meristem is injured or killed. A further study related the basal bud stimulation to the auxin-cytokinin balance in the base of the stem (Baur, 1979). In an earlier study, cacodylic acid (dimethylarsinic acid) applied to yellow foxtail [Setaria glauca (L.) Beauv.] and

goosegrass [Eleusine indica (L.) Gaertn.] caused increased tillering in both species (Taylorson, 1966).

A herbicide may affect plant growth through the inhibition of photosynthesis, respiration, cell division, nucleic acid and protein synthesis or lipid synthesis. However, because of multi-action effects and variable responses to the range of herbicide concentrations, especially under in vitro conditions, some difficulty in determining the exact primary site of action frequently arises (Fletcher and Kirkwood, 1982).

Herbicide effects on photosynthesis may occur through interference with the reproduction, development, structure and integrity of chloroplasts, the light reactions involved in the conversion of light energy to chemical energy or the biosynthetic pathways which culminate in the synthesis of photosynthetic products (Moreland and Hilton, 1976). Herbicides such as fluometuron { N,N-dimethyl-N'-[3-(trifluoromethyl)phenyl]urea } (Bruinsma, 1965), dichlormate {3,4-dichlorobenzyl methylcarbamate} (Herret and Berthold, 1965) and haloxydine {3,5-dichloro-2,6-difluoro-4-pyridinol} (Slater, 1968; Dodge and Lawes, 1972) are known to interfere with the normal development of chloroplasts whilst the structure of chloroplasts is affected by glyphosate (Campbell et al., 1973), some of the substituted ureas and the bipyridiniums (Anderson and Thomson, 1973). On the other hand, herbicides may affect the photosynthetic mechanism by acting as electron transport inhibitors (eg. s-triazines, carbamates and uracils), uncouplers (eg. perfluidone), energy transfer inhibitors (eg. 1,2,3-thiadiazolylphenylurea), inhibitory uncouplers (eg. dinitrophenols)



or electron acceptors (eg. bipyridiniums) [See Moreland, 1967; Corbertt, 1974; Moreland and Hilton, 1976].

Respiration may be affected through interference with glycolysis, the pentose phosphate pathway, the tricarboxylic acid cycle, oxidative phosphorylation or the electron transport system. Herbicides may act as uncoupling agents, inhibitors of energy transfer or electron transport or inhibitory uncouplers. The phenoxy-acids, carbamates, benzoic acids, picloram and the nitriles are known to interfere with respiration (See Kirkwood, 1976; Moreland, 1980; Fletcher and Kirkwood, 1982).

Many herbicides are known to affect cell division through interference with the synthesis or active transport of precursors required for DNA synthesis during interphase, the modification of the chemical and physical properties of DNA, interference with spindle formation or function or the formation of cell walls (Moreland, 1980). The dinitroanilines, diallate, pronamide {3,5-dichloro(N-1,1-dimethyl-2-propynyl)benzamide} (Linck, 1976) and more recently chlorsulfuron (Ray, 1982, 1984; Rost, 1984) and metsulfuron (Doig et al., 1983) have been found to inhibit cell division. Some of the anilides (Duke, 1967) nitrophenols (Gruenhagen and Moreland, 1971), the chloroacetamides (Jaworski, 1969; Duke et al., 1975) and the carbamates (Mann et al., 1965) have been shown to inhibit nucleic acid and protein synthesis. Other herbicides such as TCA {trichloroacetic acid} (Kollattukudy, 1968), dinoseb {2-(1-methylpropyl)-4,6-dinitro phenol} (St. John and Hilton, 1973) and ethofumesate {(+)-2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulfonate} (Leavitt et al., 1978) are believed to inhibit lipid biosynthesis and the deposition of epicuticular or cuticular

waxes.

Plant growth results from the process of cell division, cell enlargement and cell differentiation and hence an interference in any of these processes might lead to an inhibition in growth. Some herbicides reduce plant growth by disrupting one or all of these processes but a very common site of action for many herbicides which act as growth inhibitors is shoot and root tip meristems. The shoot and root meristems are the growing points of plants and are made up of numerous cells which are actively progressing through the cell cycle. The cell cycle consists of the interphase and the mitotic stages; prophase, metaphase, anaphase and telophase (Howard and Pelc, 1953). Certain specific enzymes, proteins and RNAs in addition to oxygen, water and a source of energy are required for the progression of the cell division cycle. In view of this, lack of any of these requirements will affect cell cycle progression (Rost, 1977).

A number of herbicides including the carbamates, thiocarbamates, trifluralin and nitralin {4-(methylsulfonyl)-2,6-dinitro-N,N-dipropylaniline} (Cartwright, 1976), the chloroacetamides (Dhillon and Anderson, 1972; Deal and Hess, 1980) and the sulfonylureas (Ray, 1982; Doig et al., 1983) are known to inhibit cell division. Studying the effects of trifluralin on mitosis at the root tips of soybean, Talbert (1965) observed that dividing cells were arrested at the prophase stage a few hours after treatment and the inhibition was accompanied by abnormally enlarged cells which were multinucleate. In another study, Gentner and Burk (1968) treated corn root tips with nitralin and observed after twenty four hours that mitosis had stopped at metaphase with the treated root tips exhibiting enlarged



and multinucleate cells without the formation of spindles or cell walls. However in other studies, the major effect has been a reduction in the number of cells in each stage of mitosis, indicating that the site of action is an event preceding mitosis and as such the effect is rather an inhibition of entry into mitosis. Ray (1982), demonstrated this phenomenon with root tips of Vicia faba treated with chlorsulfuron. He obtained an 87% reduction in the number of dividing cells in treated root tips but did not find any aberrant mitotic figures and the frequency distribution of the various mitotic figures did not show any significant difference from that of the root tips of control plants. He therefore concluded that the cells were not inhibited at the mitotic phase but rather at some other stage in the cell cycle. Similar observations were made with cinmethylin {exo-1-methyl-4-(1-methylethyl)-2-[(2-methylphenyl)methoxy]-7-oxabicyclo [2.2.1]heptane} on oat root tips (Mahmound and Hess, 1986) when substantial reductions of mitotic figures at all stages were obtained in treated roots with an insignificant number of aberrant figures. The authors therefore suggested that inhibition by cinmethylin was due to an interference with events during interphase and not a result of a disruption of the mitotic sequence.

Many herbicides which inhibit cell division also inhibit cell enlargement. However, in situations where cell enlargement is the only process that is inhibited, plants will still be retarded in their growth. It has been shown that inhibition of cell division by trifluralin in soybean and corn is accompanied by increased enlargement of cells and in cucumber (Cucumis sativus L.), reduction in root growth was attributed to inhibition of cell elongation by propachlor {2-chloro-N-(1-methylethyl)-N-phenyl acetamide} (Duke,

1967). Herbicidal disruption of cell division in the primary meristems is, in most cases, followed by excessive vacuolation and precocious differentiation of cells in a disorganized pattern. Such responses in root meristems have been observed with trifluralin (Bayer et al., 1967), bromacil {5-bromo-6-methyl-3-(1-methylpropyl)-2,4(1H,3H)pyrimidinedione} (Ashton et al., 1968) and pronamide (Peterson and Smith, 1971).

The studies reported in the present thesis address aspects of the selectivity, mode of action and persistence of triasulfuron. The thesis is divided into seven chapters. Between the General Introduction and the General Conclusions are five chapters each prefaced by their own specific introductions. The first of these, (Chapter 2) records studies on the selectivity of triasulfuron at both inter- and intra-specific levels. Chapter 3 reports studies made to examine an effect of triasulfuron on tiller production in barley whilst the fourth chapter is a record of studies on the activity of triasulfuron in soil with particular reference to placement, organic matter and the mobility as influenced by rainfall and organic matter. In Chapter five, studies on the degradation of triasulfuron in soil are reported. The sixth chapter is a record of studies on cell division at root tips of seedlings treated with triasulfuron and amino acids alone and in combination.

CHAPTER 2.

SELECTIVITY.



## INTRODUCTION

For a herbicide to be toxic, it must make contact with the foliage, roots or both, enter the plant and move to a site of action within the plant. A clear understanding of these processes exposes some of the bases of selectivity. Selectivity is achieved when a herbicide, applied at the same dose and under the same conditions to a crop and a weed reaches and disrupts essential processes in the weed but not in the crop (Holly, 1976). Selectivity is a relative phenomenon since it is influenced by, for example, environmental conditions, the rate at which the herbicide is applied and the state of growth of the plant. Some types of selectivity can therefore be lost by changes in the environmental conditions or increases in herbicide concentration.

Selectivity may be achieved when sprays of a contact herbicide are directed to control weeds with extreme care taken to avoid contact with the crop as occurs when emerged weeds are controlled with a contact herbicide before the emergence of the crop; when herbicide application is carried out during a dormant period in the development of the crop (to avoid the period of active growth at which the crop might be susceptible); or when placing the herbicide in the top layers of the soil to control shallow rooted annual weeds whilst the deeply sown crop is protected from the effects of the herbicide (Holly, 1976; Roberts, 1982). Differences in the morphology and anatomy of plants could result in situations where broad-leaved weeds with horizontal leaves retain more spray than say grasses with more erect leaves thus making the former more susceptible in practice to some herbicides. The waxiness of some leaf surfaces could lead to



less retention of herbicide spray whilst the thickness of the cuticle of some leaves may result in low or slower penetration and hence a reduction in herbicide injury (Roberts, 1982).

At the cellular level, selectivity can occur through the immobilization of herbicide molecules at less sensitive sites within a tolerant plant (Dexter et al., 1971), differential translocation of the herbicide to the sensitive sites within the plant (Feeny and Colby, 1968), biochemical conversion of the toxic herbicide molecules to non-phytotoxic forms within the tolerant plant (Luckwill and Lloyd-Jones, 1960, Hamilton and Moreland, 1962; Shimabukuro et al., 1971) or conversely, the conversion of less toxic chemicals like MCPB {4-(4-chloro-2-methylphenoxy)butanoic acid} and 2,4-DB {4-(2,4-dichlorophenoxy)butanoic acid} to the more potent MCPA {(4-chloro-2-methylphenoxy)acetic acid} and 2,4-D {(2,4-dichlorophenoxy)acetic acid} in susceptible species whereas the tolerant plants do not have the biochemical mechanism to effect such lethal synthesis (Wain, 1954). Several aspects of selectivity were discussed in the General Introduction (Chapter 1) and the general principles behind the various types of selectivity have been reviewed frequently (See eg. Ashton and Crafts, 1973; Holly, 1976; Roberts, 1982).

The sulfonylurea herbicides are applied at extremely low rates to control broad-leaved weeds and some grasses in small grain cereals and the tolerance of the crop to chlorsulfuron {2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzene sulfonamide} is believed to be due to an ability to rapidly detoxify the herbicide through hydroxylation and conjugation reactions (Sweetzer et al., 1982; Hageman and Behrens, 1984). Amrein and Gerber

(1985), reported that both winter and spring wheat and barley tolerated post-emergence applications of  $20\text{g ai ha}^{-1}$  triasulfuron {3-(6-methoxy-4-methyl-1,3,5-triazin-2-yl)-1-[2-(2-chloroethoxy)-phenyl sulfonyl]urea} without any reduction in crop vigour or yield. In earlier studies with chorsulfuron, wheat, barley and oats were not affected by post-emergence applications at the rate of  $160\text{g ai ha}^{-1}$  (Richardson et al., 1980). However Foley (1986), observed that barley was only marginally tolerant to soil residues and pre-emergence applications of chlorsulfuron and that when roots of both barley and wheat seedlings at the 1.5- to 2-leaf growth stages were exposed to nutrient solution containing 35mM chlorsulfuron, growth was significantly reduced in barley after 24h whereas wheat showed no growth reduction after 3 days.

Although selectivity may often be found between species, it is not uncommon for cultivars of the same species to exhibit differences in susceptibility or tolerance to a particular herbicide. Such intraspecific differences in response to various herbicides have been reported for both wheat and barley. Several workers have reported that wheat cultivars differed in response to 2,4-D (Foster, 1949; Shaw and Willard, 1949; Derscheid, 1951; Elliot et al., 1975), chlorotoluron {N'-(3-chloro-4-methylphenyl)-N,N-dimethylurea} (Tottman et al., 1975), atrazine {6-chloro-N'-(-methylethyl)-1,3,5-triazine-2,4-diamine} (Brinkman et al., 1980) and difenzoquat {1,2-methyl-3,5-diphenyl-1H-pyrazolium} (Miller and Nalewaja, 1974; Anderson and Arnold, 1975; Pallet and Caseley, 1980; Pallet, 1984). Derscheid et al. (1952), showed that differential responses to 2,4-D occurred between barley cultivars. Hodgson et al. (1964), studied the responses of several cultivars of barley to eight different



herbicides and observed that barley cultivars of 'Smyrna' parentage were more resistant to yield reduction by 2,4-D, MCPA or barban {4-chloro-2-butynyl 3-chlorophenylcarbamate} than were other cultivars. Similar differential responses of barley cultivars to barban (Evans, 1960; Fiddian, 1962) atrazine (Brinkman et al., 1980) and to diclofop {(+)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid}, difenzoquat and metribuzin {4-amino-6-(1,1-dimethylethyl-3-methylthio)-1,2,4-triazin-5(4H)-one} (Clay et al., 1988) and for several other crops and weeds including soybean (Hardcastle, 1974) and potato (Solanum tuberosum L.) [Graf and Ogg, 1976] to metribuzin and Lolium perenne L. cultivars to paraquat {1,1'-dimethyl-4,4-bipyridinium ion} (Faulkner and Harvey, 1981) have been reported.

Intraspecific variability within wheat and barley cultivars has been reported with some of the sulfonylurea herbicides. Hageman and Behrens (1981) recorded some differences within spring wheats, barleys and oats cultivars following pre-emergence or post-emergence application of chlorsulfuron. Likewise, large differences in the sensitivities between barley cultivars to post-emergence treatments (Lemerle et al., 1986) and pre-emergence treatments with chlorsulfuron in spring wheat cultivars (Bowran and Blacklow, 1987) have been reported. Other workers however failed to find any differences in crop vigour or yield among barley and wheat cultivars treated with pre-emergence or post-emergence applications of chlorsulfuron (Hageman and Behrens, 1979; O'Sullivan, 1982), triasulfuron (Amrein and Gerber, 1985) and DPX L5300 {Methyl 2-[3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-3-methylureidosulfonyl] benzoate} (Muntan and Bencivelli, 1987). The above examples point to

the fact that selectivity may depend on variations in the genetic constitution within plant species and knowledge of such intraspecific variations could be important for breeding herbicide-tolerant cultivars.

When the study was begun, very little was known about the response of different cultivars of wheat and barley to the herbicide. Studies were therefore made on the response of various species of crops and weeds as well as the response of some cultivars of wheat and barley to triasulfuron and these are reported in this chapter.



EXPERIMENT 2.1: EFFECTS OF TRIASULFURON ON THE GERMINATION AND EARLY SEEDLING DEVELOPMENT OF SOME CROP AND WEED SEEDS.

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**Materials and Methods.**

The following seeds of crops and weeds were sown in petri-dishes lined with Whatman's No. 3 filter paper on 25 October 1985;

Winter wheat cv Galahad - 30 seeds per petri-dish

Lolium perenne cv. S321, Talbot - 50 seeds per petri-dish

Poa trivialis - 50 seeds per petri-dish

Trifolium repens cv. NZ, Huia - 100 seeds per petri-dish

Cabbage (Brassica oleracea var capitata cv. April) -100 seeds per petri-dish.

Carrot (Daucus carota cv. Chantenay red-core) - 100 seeds per petri-dish.

Five doses of triasulfuron\* and a control viz; 0 (deionized water), 0.0625, 0.125, 0.25, 0.50 and 1.0g ai l<sup>-1</sup> were prepared. Aliquots (5 ml) of the prepared solutions were added to the petri-dishes which were arranged in a completely randomized design and placed in an incubator at 15°C. The experiment was replicated three times. The seeds were observed daily for germination and seedling development. The protrusion of the radicle was used as an index of germination whilst the protrusion of the plumule was used as an index of seedling development. Daily germination counts were made for ten days except for carrot where recording was continued for 20 days.

After the tenth day, samples of six seedlings each from all treatments of wheat and Lolium perenne were randomly selected and lengths of the longest root, coleoptile and first leaf were measured.

\*(20% a.i. water dispersible granules)

The roots and coleoptile of wheat and Lolium perenne were then observed under a microscope at a magnification of x40.

In a similar experiment set up on 10 November 1985, 100 seeds each of Veronica persica, Senecio vulgaris and Capsella bursa-pastoris were sown into 9cm diameter petri-dishes as described above. Aliquots (5ml) of the herbicide solutions at the concentrations of 0, 0.05, 0.075 or 0.10g ai l<sup>-1</sup> were added to the petri-dishes and incubated at 25°C. The experiment was replicated three times. Germination counts were made after 10 days and samples of 10 seedlings from all treatments were selected and lengths of longest roots were determined. The data were processed by analysis of variance using the Minitab statistical package and comparison between treatment means was done by Tukey's Honestly Significant Difference test (HSD).

## Results.

For Poa trivialis and carrot, radicle emergence was depressed with increases in the dose of the herbicide (Table 2.1). The emergence of the radicles of wheat, cabbage, Trifolium repens and Lolium perenne was not inhibited. However, the emergence of the coleoptile in Lolium perenne was adversely affected by all the herbicide doses but most especially 0.25, 0.50 and 1.0 gl<sup>-1</sup> (Table 2.2). The emergence of coleoptiles of Poa trivialis and of the cotyledons of carrot was also reduced by the herbicide.

Although the percentage emergence of roots and shoots in wheat, cabbage and Trifolium repens was high, the herbicide reduced the lengths of the longest root, coleoptile and first leaf of Lolium perenne and wheat (Tables 2.3 and 2.4). The tips of the roots of

these species showed brownish colouration especially at the higher doses viz; 0.5 and 1.0  $\text{gl}^{-1}$ . There were no readily observed morphological differences in the coleoptiles.

In the second experiment, germination of Veronica persica and Senecio vulgaris seeds were severely inhibited at all doses of the herbicide but those of Capsella bursa-pastoris were unaffected (Table 2.5). Lengths of the longest roots of Veronica persica were significantly reduced below the controls. Although lengths of roots were reduced at all doses of the herbicide for Senecio vulgaris and at 0.075 and 0.10  $\text{gl}^{-1}$  for Capsella bursa-pastoris, the differences were not significant (Table 2.6).



Table 2.1: Effect of triasulfuron on the germination (protrusion of radicle) of some crop and weed seeds. (Results are expressed as percentage of control values).

Herbicide dose (gl <sup>-1</sup> )	Wheat	Cabbage	Carrot	<u>Lolium perenne</u>	<u>Poa trivialis</u>	<u>Trifolium repens</u>
Control - 0	100.00	100.00	100.00a	100.00	100.00a	100.00
0.0625	100.00	100.00	80.08a	97.76	80.83a	100.00
0.125	98.90	99.05	69.24ab	94.99	77.65a	100.00
0.25	98.90	98.70	67.16ab	93.60	48.33b	100.00
0.50	98.90	96.60	62.00b	96.37	41.68b	98.28
1.00	100.00	96.60	57.86b	92.91	15.83c	99.00

Table 2.2: Effect of triasulfuron on seedling development (coleo-tile and cotyledon emergence) of some crop and weed seeds. (Results are expressed as percentage of control values).

Herbicide dose (gl <sup>-1</sup> )	Wheat	Cabbage	Carrot	<u>Lolium perenne</u>	<u>Poa trivialis</u>	<u>Trifolium repens</u>
Control - 0	100.00	100.00	100.00a	100.00a	100.00a	100.00
0.0625	100.00	100.00	20.84b	55.64b	75.11a	100.00
0.125	98.84	98.58	16.90b	49.22b	73.37a	98.14
0.25	97.73	98.23	12.39b	34.24b	44.03b	100.00
0.50	98.84	97.17	2.82b	24.25bc	39.70bc	98.14
1.00	98.84	96.81	0.56b	13.55c	16.39c	100.00

Values in a column sharing the same letter are not significantly different at  $P < 0.05$ .

Table 2.3 Effect of triasulfuron on lengths (cm) of longest root, coleoptile and first leaf of Lolium perenne, 12 days after treatment.

Herbicide dose (gl <sup>-1</sup> )	Length of longest root	Length of coleoptile	Length of first leaf
Control - 0	4.73a	3.56a	1.93a
0.0625	1.47b	1.30b	1.71ab
0.125	1.23b	0.85c	1.71ab
0.25	0.89c	0.23d	1.59abc
0.50	0.39c	0.13d	1.50bc
1.00	0.36c	0.03d	1.34c

Table 2.4 Effect of triasulfuron on lengths (cm) of longest root, coleoptile and first leaf of wheat 10 days after treatment.

Herbicide dose (gl <sup>-1</sup> )	Length of longest root	Length of coleoptile	Length of first leaf
Control - 0	7.07a	3.05a	2.54a
0.0625	2.55b	2.61a	2.50ab
0.125	2.81b	1.62b	2.19bc
0.25	2.47b	1.27b	2.14c
0.50	2.07b	1.39b	1.74d
1.00	2.07b	1.00b	1.80d

Values in a column sharing the same letter are not significantly different at  $P < 0.05$ .

Table 2.5: Effect of triasulfuron on the germination (protrusion of radicle) of some weed seeds 10 days after treatment. (Results are expressed as percentage of control values)

Herbicide dose <sub>1</sub> (gl <sup>-1</sup> )	<u>Veronica persica</u>	<u>Senecio vulgaris</u>	<u>Capsella bursa-pastoris</u>
Control - 0	100.0a	100.0a	100.0
0.05	38.6b	30.3b	98.5
0.075	32.0b	20.2b	88.7
0.10	24.5b	12.5b	89.0

Table 2.6: Length (cm) of longest root per seedling 12 days after treatment.

Herbicide dose <sub>1</sub> (gl <sup>-1</sup> )	<u>Veronica persica</u>	<u>Senecio vulgaris</u>	<u>Capsella bursa-pastoris</u>
Control - 0	3.11a	0.72	0.41
0.05	0.51b	0.19	0.62
0.075	0.25b	0.47	0.26
0.10	0.26b	0.20	0.19

Values in a column sharing the same letter are not significantly different at  $P < 0.05$ .



**EXPERIMENT 2.2:            EFFECTS OF TRIASULFURON ON THE GROWTH OF WHEAT,  
BARLEY AND OILSEED RAPE.**

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**Materials and Methods.**

On 26 November 1985, seeds of Winter wheat cv. Galahad, Winter barley cv. Maris otter, and Oilseed rape cv. English Giant Lair were sown at a rate of 20 seeds per pot into John Innes No.1 compost ( pH 6.8; organic matter, 7.0 %). Average greenhouse day temperatures were 15°C (day) and 10°C (night).

Triasulfuron was applied at four stages; post-sowing pre-emergence (28 November), post-emergence (5 December), post-emergence (12 December) and post-emergence (19 December) at rates of 0, 10, 15, 20 or 30g ai ha<sup>-1</sup> in 200 lha<sup>-1</sup> of water. Application was made with an Oxford precision sprayer at a pressure of 1.4 kgcm<sup>-2</sup>. Watering was by coarse overhead irrigation from a watering can. The pots were arranged in a completely randomized design and the treatments were replicated three times. Emerged seedlings were counted one and two weeks after the pre-emergence application. Plant height was recorded two weeks after each application and whole plant dry weights were determined after drying the material at 70°C for 48h. The data were processed as described in Experiment 2.1.

**Results.**

**Pre-emergence application.**

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The emergence of seedlings of wheat was delayed following pre-emergence treatments of 15-30 gha<sup>-1</sup> but this effect was not found with barley. Emergence of oilseed rape was significantly reduced at

all doses of the herbicide but there was no evidence of a dose-related response (Tables 2.7 and 2.8). Similarly, two and three weeks after application the dry weights of the herbicide-treated plants of oilseed rape were much lower than those of the control but there was no dose-related response. However, for both wheat and barley two weeks after treatment, all herbicide doses had reduced dry weights below those of the control and there was some evidence that higher doses had greater effect (Table 2.9). Similar results were recorded for barley three weeks after pre-emergence application when dry weights were significantly reduced below the control at all doses of the herbicide (Table 2.10).

#### First post-emergence application.

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All doses of the herbicide reduced the dry weights of barley below that of the control, two and three weeks after treatment; but the response was not dose-related. Wheat did not respond (Tables 2.11 and 2.12). Growth of oilseed rape was inhibited by the herbicide with the treated plants showing chlorosis and necrosis. Treated plants were completely dead four weeks after treatment.

#### Second post-emergence application.

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All doses of the herbicide reduced the dry weight of barley and oilseed rape below that of the control two and three weeks after treatment but this response was not dose-related. Barley plants treated at 20 and 30 gha<sup>-1</sup> produced several tillers and showed symptoms of leaf trapping (newly emerged leaves curled, with the tips

enclosed in the sheaths). Wheat did not respond to the herbicide treatments (Tables 2.13 and 2.14). Treated plants of oilseed rape showed symptoms of chlorosis and necrosis with death occurring four weeks after application.

### Third post-emergence application

The dry weights of both wheat and barley were not significantly reduced by the herbicide treatments two and three weeks after application (Tables 2.15 and 2.16). However, the barley plants produced small tillers at doses of 20 and 30 gha<sup>-1</sup>. Some plants showed symptoms of leaf trapping. Growth of oilseed rape was inhibited at all doses of the herbicide with the plants dying four weeks after application.



Table 2.7: Percentage emergence of seedlings one week after pre-emergence application.

Herbicide <sub>1</sub> dose (gha <sup>-1</sup> )	Wheat	Barley	Oilseed rape
Control - 0	96.65a	96.65	98.35a
5	91.65a	98.35	56.65b
10	83.35a	95.00	56.65b
15	61.65b	95.00	46.65b
20	56.65b	93.35	58.35b
30	58.35b	86.65	65.00b

Table 2.8: Percentage emergence of seedlings two weeks after pre-emergence application.

Herbicide dose (gha <sup>-1</sup> )	Wheat	Barley	Oilseed rape
Control - 0	96.55a	98.35	98.35a
5	93.35ab	98.35	58.35b
10	91.65ab	100.00	60.00b
15	80.00bc	95.00	46.65b
20	73.35c	95.00	58.35b
30	81.65abc	91.65	65.00b

Values in a column sharing the same letter are not significantly different at  $P < 0.05$ .

Table 2.9: Dry weight per plant (g) two weeks after pre-emergence treatment.

Herbicide dose (g ha <sup>-1</sup> )	Wheat	Barley	Oilseed rape
Control - 0	0.039a	0.041a	0.031a
5	0.030b	0.026b	0.006b
10	0.023bc	0.021bc	0.006b
15	0.021c	0.015c	0.005b
20	0.015c	0.015c	0.005b
30	0.015c	0.013c	0.006b

Table 2.10: Dry weight per plant (g) three weeks after pre-emergence treatment.

Herbicide dose (g ha <sup>-1</sup> )	Wheat	Barley	Oilseed rape
Control - 0	0.045	0.045a	0.025a
5	0.048	0.037bc	0.006b
10	0.049	0.038ab	0.006b
15	0.045	0.033bc	0.005b
20	0.039	0.028c	0.006b
30	0.041	0.034bc	0.007b

Values in a column sharing the same letter are not significantly different at  $P < 0.05$ .

Table 2.11: Dry weight per plant (g) two weeks after first post-emergence treatment.

Herbicide dose ( $\text{gha}^{-1}$ )	Wheat	Barley
Control - 0	0.121	0.133a
5	0.110	0.097b
10	0.097	0.084b
15	0.083	0.093b
20	0.093	0.092b
30	0.105	0.083b

Table 2.12: Dry weight per plant (g) three weeks after first post-emergence treatment.

Herbicide dose ( $\text{gha}^{-1}$ )	Wheat	Barley
Control - 0	0.133	0.142a
5	0.130	0.113bc
10	0.114	0.105bc
15	0.110	0.119ab
20	0.101	0.083c
30	0.123	0.086c

Values in a column sharing the same letter are not significantly different at  $P < 0.05$ .



Table 2.13: Dry weight per plant (g) two weeks after second post-emergence treatment.

Herbicide dose ( $\text{gha}^{-1}$ )	Wheat	Barley
Control - 0	0.109bc	0.142a
5	0.128a	0.107b
10	0.110abc	0.117b
15	0.093c	0.109b
20	0.113ab	0.105b
30	0.103bc	0.100b

Table 2.14: Dry weight per plant (g) three weeks after second post-emergence treatment.

Herbicide dose ( $\text{gha}^{-1}$ )	Wheat	Barley	Oilseed rape
Control - 0	0.172	0.213a	0.298a
5	0.181	0.151bc	0.044b
10	0.140	0.170b	0.042b
15	0.152	0.147bcd	0.040b
20	0.208	0.130cd	0.036b
30	0.157	0.124d	0.046b

Values in a column sharing the same letter are not significantly different at  $P < 0.05$ .

Table 2.15: Dry weight per plant (g) two weeks after third post-emergence treatment.

Herbicide dose ( $\text{gha}^{-1}$ )	Wheat	Barley	Oilseed rape
Control - 0	0.175	0.221	0.182
5	0.198	0.205	0.119
10	0.153	0.179	0.098
15	0.191	0.162	0.092
20	0.168	0.182	0.120
30	0.157	0.161	0.085

Table 2.16: Dry weight per plant (g) three weeks after third post-emergence treatment.

Herbicide dose ( $\text{gha}^{-1}$ )	Wheat	Barley
Control - 0	0.223b	0.297
5	0.299a	0.274
10	0.252ab	0.225
15	0.210b	0.200
20	0.217b	0.188
30	0.187b	0.203

Values in a column sharing the same letter are not significantly different at  $P < 0.05$ .

**EXPERIMENT 2.3: THE RESPONSE OF SOME CULTIVARS OF WHEAT AND BARLEY TO TRIASULFURON.**

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**Materials and Methods.**

On 16 February 1986, seeds of three varieties each of wheat and barley viz; Winter wheat cv. Galahad, cv. Longbow and cv. Norman, Winter barley cv. Maris Otter, cv. Panda and Spring barley cv. Arthos were sown at a rate of 15 seeds per 12.5cm pots into John Innes No.1 compost (organic matter, 9.2% and pH, 6.6). Greenhouse temperature was 15°C (day) and 10°C (night).

Triasulfuron was applied at four stages; post-sowing pre-emergence (17 February), post-emergence (24 February), post-emergence (3 March) and post-emergence (10 March) at doses of 0, 10, 15 and 20g ai ha<sup>-1</sup> in 200 lha<sup>-1</sup> of water. Spraying was done with an Oxford precision sprayer at a pressure of 1.4 kgcm<sup>-2</sup>. Plants were watered by coarse overhead irrigation and the pots were arranged in a randomized design with three replicates. Emerged seedlings were counted two weeks after the pre-emergence application. Number of leaves, number of tillers, length of longest leaf blade and leaf blades, shoot and root dry weights per plant were determined three weeks after each application after drying at 70°C for 48h. The data were analysed as described in Experiment 2.1. Only the pooled results of all varieties of wheat and barley are presented in this chapter. Detailed results of the individual varieties of barley and wheat are presented in Appendix 1.



## Results.

### Pre-emergence application.

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The emergence of seedlings of all varieties of wheat and barley was not inhibited by the pre-emergence application of the herbicide (Table 2.17). However the number of leaves and tillers per plant, lengths of the longest leaf blade and the dry weights of the leaf blades and shoot of both wheat and barley when all the varieties were pooled together, were lower at all doses of the herbicide than those of the control three weeks after sowing (Tables 2.18 and 2.22). Some of the plants of all varieties of wheat and barley showed symptoms of leaf trapping.

### Post-emergence application one week after sowing.

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In barley, there were more leaves and tillers at all doses of the herbicide than in the control but the differences were not significant. The tillers were however smaller as compared to those of the control. Wheat showed such response only at the dose of  $20\text{g ha}^{-1}$ . The lengths of the longest leaf blades of all the varieties of wheat and barley were significantly reduced below those of the control at all doses of the herbicide four weeks after sowing but the response was not dose-related (Table 2.19). There were symptoms of leaf trapping in the barley plants. The dry weights of the leaf blades of the pooled varieties of wheat and barley were significantly lower than those of the control at doses of 15 and  $20\text{ gha}^{-1}$  four weeks after sowing but the shoot and root dry weights were not significantly different (Table 2.23).

Post-emergence application two weeks after sowing.

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Barley had significantly more leaves and tillers at all doses of the herbicide than it did in the absence of the herbicide five weeks after sowing. Similarly, all doses of the herbicide reduced the lengths of the longest leaf blade of all varieties of wheat and barley below those of the control five weeks after sowing but the response was not dose-related (Table 2.20). Some of the herbicide-treated plants of barley showed symptoms of leaf trapping and the tillers, though numerous, were very tiny as compared to those of the control. The dry weights of leaf blades, shoot and root of both wheat and barley were not significantly different five weeks after sowing (Table 2.24).

Post-emergence application three weeks after sowing.

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Although the herbicide-treated plants of barley and wheat produced more leaves and tillers than those of the control six weeks after sowing, the differences were not significant. Similarly, the lengths of the longest leaf blade of the herbicide treated plants of both barley and wheat were not significantly different from those of the control (Table 2.21). Some of the herbicide-treated plants of barley showed symptoms of leaf trapping and the tillers were smaller than those of the control. The dry weights of the leaf blades, shoot and root of the pooled varieties of wheat and barley were not significantly different six weeks after sowing (Table 2.25).

Generally, there were no striking differences in tolerance to the herbicide between the various varieties of wheat and barley. Visual

observations however showed that the varieties of wheat were more tolerant to the effects of the herbicide than those of barley.



Table 2.17: Percentage emergence of seedlings two weeks after pre-emergence application. (Results have been expressed as percentage of control values).

Herbicide dose ( $\text{gha}^{-1}$ )	Wheat	Barley
Control - 0	100.00	100.00
10	101.30	91.13
15	101.20	98.57
20	96.73	96.93

Table 2.18: Mean number of leaves, length of longest leaf blade and tillers per plant three weeks after pre-emergence application. (Results have been expressed as percentage of control values).

Herbicide dose ( $\text{gha}^{-1}$ )	Leaves per plant		Length of longest leaf blade		Tillers per plant	
	Wheat	Barley	Wheat	Barley	Wheat	Barley
Control-0	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
10	82.20b	94.03a	58.40b	43.83b	52.93b	29.93b
15	74.00b	82.77ab	42.10b	31.87b	41.66b	44.90b
20	74.23b	65.60b	33.23b	25.90b	43.53b	26.47b

Values in a column sharing the same letter are not significantly different at  $P < 0.05$ .

Table 2.19: Mean number of leaves, length of longest leaf blade and tillers per plant four weeks after sowing. (First post-emergence application: Results have been expressed as percentage of control values).

Herbicide dose ( $\text{gha}^{-1}$ )	Leaves per plant		Length of longest leaf blade		Tillers per plant	
	Wheat	Barley	Wheat	Barley	Wheat	Barley
Control-0	100.00	100.00	100.00a	100.00a	100.00	100.00
10	105.30	118.67	70.07b	55.97b	139.30	135.43
15	89.97	106.00	65.73b	51.07b	101.10	114.60
20	86.63	110.10	55.33b	45.27b	95.50	121.03

Table 2.20: Mean number of leaves, length of longest leaf blade and tillers per plant five weeks after sowing. (Second post-emergence application: Results have been expressed as percentage of control values).

Herbicide dose ( $\text{gha}^{-1}$ )	Leaves per plant		Length of longest leaf blade		Tillers per plant	
	Wheat	Barley	Wheat	Barley	Wheat	Barley
Control-0	100.00	100.00b	100.00a	100.00a	100.00	100.00b
10	118.37	142.03a	76.20b	72.43b	128.83	182.13a
15	112.87B	167.57aA	77.47b	73.20b	123.10B	234.27aA
20	117.80	138.93a	71.07b	72.00b	127.33B	212.33aA

Values in a column sharing a common lower-case letter and values in a row sharing a common capital letter do not differ significantly at  $P < 0.05$ .

Table 2.21: Mean number of leaves, length of longest leaf blade and tillers per plant six weeks after sowing. (Third post-emergence application: Results have been expressed as percentage of control values).

Herbicide dose ( $\text{gha}^{-1}$ )	Leaves per plant		Length of longest leaf blade		Tillers per plant	
	Wheat	Barley	Wheat	Barley	Wheat	Barley
Control-0	100.00	100.00	100.00	100.00	100.00	100.00
10	127.43	149.40	88.50	92.10	158.50	151.30
15	134.67	134.70	85.10	91.97	169.07	164.73
20	127.77	141.50	87.23	93.30	162.53	156.20

Table 2.22: Mean dry weight of leaf blades, shoot and root per plant three weeks after pre-emergence application. (Results have been expressed as percentage of control values).

Herbicide dose ( $\text{gha}^{-1}$ )	Leaf blades		Shoot		Root	
	Wheat	Barley	Wheat	Barley	Wheat	Barley
Control-0	100.00a	100.00a	100.00a	100.00a	100.00	100.00
10	54.63b	32.40b	76.97ab	42.27b	136.87	89.13
15	39.83b	18.89b	49.37b	33.80b	130.73	80.80
20	27.43b	13.93b	44.83b	31.47b	100.00	75.67

Values in a column sharing the same letter are not significantly different at  $P < 0.05$ .



Table 2.23: Mean dry weight of leaf blades, shoot and root per plant four weeks after sowing. (First post-emergence application: Results have been expressed as percentage of control values).

Herbicide dose <sub>-1</sub> (gha <sup>-1</sup> )	Leaf blades		Shoot		Root	
	Wheat	Barley	Wheat	Barley	Wheat	Barley
Control-0	100.00a	100.00a	100.00	100.00	100.00	100.00
10	78.37ab	65.87ab	90.77	57.17	91.67	62.40
15	68.87b	56.53b	73.80	54.50	82.93	70.13
20	60.53b	60.20b	55.90	50.47	51.37	98.70

Table 2.24: Mean dry weight of leaf blades, shoot and root per plant five weeks after sowing. (Second post-emergence application: Results have been expressed as percentage of control values).

Herbicide dose <sub>-1</sub> (gha <sup>-1</sup> )	Leaf blades		Shoot		Root	
	Wheat	Barley	Wheat	Barley	Wheat	Barley
Control-0	100.00	100.00	100.00	100.00	100.00	100.00
10	83.87	81.97	77.63	73.20	114.07	62.20
15	83.83	93.00	79.10	69.90	107.47	59.17
20	81.73	75.13	80.80	65.37	78.83	56.87

Values in a column sharing the same letter are not significantly different at P < 0.05.

Table 2.25: Mean dry weight of leaf blades, shoot and root per plant six weeks after sowing. (Third post-emergence application: Results have been expressed as percentage of control values).

Herbicide dose <sub>-1</sub> (gha <sup>-1</sup> )	Leaf blades		Shoot		Root	
	Wheat	Barley	Wheat	Barley	Wheat	Barley
Control-0	100.00	100.00	100.00	100.00	100.00	100.00
10	101.03	96.33	92.17	70.07	133.33	85.80
15	94.83	95.17	85.97	77.73	147.87	79.10
20	90.80	99.50	81.77	70.47	115.87	78.07

**EXPERIMENT 2.4: EFFECTS OF TRIASULFURON ON SEEDLING GROWTH AND DEVELOPMENT OF WHEAT, LOLIUM PERENNE AND VERONICA PERSICA.**

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**Materials and Methods.**

On 12 June 1986, twenty seeds of Winter wheat cv. Norman and fifty each of Lolium perenne and Veronica persica were sown in 12.5cm plastic pots filled with John Innes No. 1 compost. Triasulfuron was applied at rates of 0, 10, 15 or 20g ai ha<sup>-1</sup> in 200 lha<sup>-1</sup> of water at four growth stages viz; post-sowing pre-emergence, post-emergence (2 weeks after sowing), post-emergence (3 weeks after sowing) and post-emergence (4 weeks after sowing). The herbicide was applied with an Oxford precision sprayer at a pressure of 1.4 kgcm<sup>-2</sup>. The pots were arranged in a completely randomized design with three replications and kept in a greenhouse with temperatures of 20 ± 3°C (day) and 15 ± 2°C (night) and daylength of 16h supplemented by 400W High pressure sodium lamps. Watering was by overhead irrigation from a watering can. The number of emerged seedlings in the pre-emergence treatment were counted three weeks after sowing and the shoot dry weights per pot of wheat, Lolium perenne and Veronica persica were determined eight weeks after sowing, after drying in an oven at 70°C for five days. The data were processed as described in Experiment 2.1.

**Results.**

Generally, injury to the seedlings occurred at a much faster rate in the pre-emergence, the first and second post-emergence treatments than it did in the third post-emergence treatment with both Lolium perenne and Veronica persica.



The leaves of Lolium perenne seedlings became chlorotic a week after the post-emergence application and gradually turned necrotic with death occurring three or four weeks after each application. Seedling growth of Veronica persica appeared to have stopped, one week after receiving the herbicide and some of the leaves developed a yellow or purple colouration and later became necrotic. Most of the plants in the first and second post-emergence treatments showed these symptoms but they were less severe in the third post-emergence treatment where some few plants were unaffected.

Triasulfuron had inhibited seedling emergence of Veronica persica and Lolium perenne at all doses up to three weeks after the pre-emergence application but that of wheat was not inhibited (Table 2.26). The shoot dry weight of wheat was not affected by any of the post-emergence treatments but in the pre-emergence treatment there was an indication that the herbicide had an effect although the reductions were not significant when compared individually with the controls (Table 2.27). Eight weeks after spraying, the shoot dry weights of Lolium perenne and Veronica persica were markedly reduced below those of the controls at all doses of the herbicide and at all stages of application (Tables 2.28 and 2.29).

Table 2.26: Seedling emergence of wheat, Lolium perenne and Veronica persica 3 weeks after pre-emergence application. (Results are expressed as percentage of control values.)

Herbicide dose (gha <sup>-1</sup> )	Wheat	<u>Lolium perenne</u>	<u>Veronica persica</u>
Control - 0	100.00	100.00a	100.00a
10	105.13A	1.70bB	4.07bB
15	102.73A	0.00bB	0.00bB
20	109.87A	1.70bB	1.70bB

Table 2.27: Total shoot dry weight (g) of wheat 8 weeks after sowing.

Herbicide dose (gha <sup>-1</sup> )	Stages of application.			
	Pre-emergence	Post-emergence 2 weeks after sowing	Post-emergence 3 weeks after sowing	Post-emergence 4 weeks after sowing
Control-0	13.81	14.07	14.71	13.76
10	11.60	12.87	13.89	14.05
15	11.32	14.61	14.30	14.56
20	11.13B	14.94AB	14.25AB	15.44A

Values in a column sharing a common lower-case letter and values in a row sharing a common capital letter do not differ significantly at P < 0.05.

Table 2.28: Total shoot dry weight (g) of Lolium perenne 8 weeks after sowing.

Herbicide dose (g ha <sup>-1</sup> )	Stages of application.			
	Pre-emergence	Post-emergence 2 weeks after sowing	Post-emergence 3 weeks after sowing	Post-emergence 4 weeks after sowing
Control-0	3.76	5.36	5.41	4.13
10	0.00	0.13	0.58	1.13
15	0.00	0.16	0.53	1.79
20	0.00	0.16	0.70	0.74

Table 2.29: Total shoot dry weight (g) of Veronica persica 8 weeks after sowing.

Herbicide dose (g ha <sup>-1</sup> )	Stages of application.			
	Pre-emergence	Post-emergence 2 weeks after sowing	Post-emergence 3 weeks after sowing	Post-emergence 4 weeks after sowing
Control-0	2.18	2.39	1.79	2.94
10	0.00	0.04	0.13	0.07
15	0.00	0.09	0.20	0.04
20	0.00	0.20	0.06	0.41



**EXPERIMENT 2.5: EFFECTS OF TRIASULFURON ON THE GERMINATION AND GROWTH OF THREE SPECIES OF POA.**

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**Materials and Methods.**

Fifty seeds each of Poa annua, Poa pratensis and Poa trivialis were sown into 12.5cm diameter plastic pots filled with John Innes No. 1 compost on 30 August, 1986. Triasulfuron was applied at four stages viz; post-sowing pre-emergence, post-emergence 2 weeks after sowing, post-emergence 3 weeks after sowing and post-emergence 4 weeks after sowing, at the rates of 0, 10, 15 or 20 g ai ha<sup>-1</sup> in 200 lha<sup>-1</sup> of water. The herbicide was applied with an Oxford precision sprayer at a pressure of 1.4 kgcm<sup>-2</sup>. The experiment was laid out as a completely randomized design with three replicates and kept in a heated greenhouse with temperatures of 15 + 2°C (day) and 10 + 2°C (night) and a minimum daylength of 16h maintained with 400W High pressure sodium lamps.

Three weeks after sowing, the numbers of emerged seedlings in the pre-emergence treatments were counted. At harvest, eight weeks after sowing, the above ground parts of seedlings in each pot were clipped at soil level and their dry weights determined after drying in an oven at 70°C for 48h. The data were processed by analysis of variance using the Genstat statistical package and comparison between treatment means was done by Tukey's HSD test.

**Results.**

Seedlings of all the three species showed signs of chlorosis two weeks after the post-emergence applications and almost all seedlings in the first post-emergence treatments were completely dead five

weeks after applying the herbicide. Although most seedlings in the third post-emergence treatments were severely chlorotic at the time of harvest, some few seedlings still had green leaves and appeared unaffected. The pre-emergence and first post-emergence treatments caused more damage to the seedlings than did the second and third post-emergence treatments.

Seedling emergence of all the three species was severely inhibited at all doses of the herbicide (Table 2.30). Similarly, the shoot dry weights of all the species were significantly reduced below their control values at all doses of the herbicide and at all four stages of application (Tables 2.31, 2.32, 2.33 and 2.34).

Table 2.30: Number of emerged seedlings 3 weeks after pre-emergence treatment.

Herbicide dose ( $\text{gha}^{-1}$ )	<u>Poa annua</u>	<u>Poa pratensis</u>	<u>Poa trivialis</u>
Control - 0	39.70	22.00	19.30
10	0.33	0.00	0.00
15	0.00	0.00	0.00
20	0.00	0.33	0.00

Table 2.31: Shoot dry weight (g) 8 weeks after pre-emergence treatment. (Results are expressed as percentage of control values).

Herbicide dose ( $\text{gha}^{-1}$ )	<u>Poa annua</u>	<u>Poa pratensis</u>	<u>Poa trivialis</u>
Control - 0	100.00	100.00	100.00
10	0.00	0.00	0.00
15	0.00	0.00	0.00
20	0.00	0.00	0.00



Table 2.32: Shoot dry weight (g) 8 weeks after sowing (Triasulfuron was applied 2 weeks after sowing: results have been expressed as percentage of control values).

Herbicide dose ( $\text{gha}^{-1}$ )	<u>Poa annua</u>	<u>Poa pratensis</u>	<u>Poa trivialis</u>
Control - 0	100.00a	100.00a	100.00a
10	13.50b	13.80b	12.00b
15	15.40b	9.10b	12.90b
20	12.20b	14.70b	11.10b

Table 2.33: Shoot dry weight (g) 8 weeks after sowing (Triasulfuron was applied 3 weeks after sowing: results have been expressed as percentage of control values).

Herbicide dose ( $\text{gha}^{-1}$ )	<u>Poa annua</u>	<u>Poa pratensis</u>	<u>Poa trivialis</u>
Control - 0	100.00a	100.00a	100.00a
10	17.40b	31.90b	31.30b
15	28.10b	22.50b	36.60b
20	19.40b	39.70b	26.00b

Values in a column sharing the same letter are not significantly different at  $P < 0.05$ .

Table 2.34: Shoot dry weight (g) 8 weeks after sowing (Triasulfuron was applied 4 weeks after sowing: results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	<u>Poa annua</u>	<u>Poa pratensis</u>	<u>Poa trivialis</u>
Control - 0	100.00a	100.00a	100.00a
10	24.90b	20.20b	35.00b
15	30.70b	39.10b	35.30b
20	35.60b	21.00b	34.40b

Values in a column sharing the same letter are not significantly different at P < 0.05.

**EXPERIMENT 2.6: EFFECTS OF TRIASULFURON ON THE GROWTH OF POA ANNUA FROM SEEDS COLLECTED FROM NATURAL POPULATIONS.**

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**Materials and Methods.**

Seedlings of Poa annua from Treboth Botanical Gardens, U.C.N.W were transplanted on 20 June 1987 into six plastic trays filled with John Innes No.2 compost and kept in a greenhouse at Pen-y-ffridd Experimental Station, U.C.N.W at  $20 \pm 3^{\circ}\text{C}$  (day) and  $15 \pm 2^{\circ}\text{C}$  (night) and minimum daylength of 16h maintained with 400W High pressure sodium lamps. The seedlings were watered as required and the seeds produced from them were collected, bulked and stored at room temperature. A preliminary germination test showed high and uniform germination of the seeds when they were incubated in the dark at  $10^{\circ}\text{C}$ .

On 16 November 1987, samples of 100 seeds were sown into 9cm diameter plastic pots filled with John Innes No.2 compost. The pots were placed in an incubator in the dark at  $10^{\circ}\text{C}$  for 2 weeks when they were transferred to the greenhouse. Triasulfuron was applied to different sets of plants at three post-emergence stages viz: 3-leaf (10 December 1987); 4-leaf (16 December 1987) or 5-leaf (23 December 1987) at 0, 10 or 20 g ai ha<sup>-1</sup> in 200 lha<sup>-1</sup> of water. The herbicide was applied with an Oxford precision sprayer at a pressure of 1.4 kgcm<sup>-2</sup>. The experiment was laid out in a completely randomized design with four replicates.

At harvest, 9 weeks after sowing, the above-ground parts of the seedlings were clipped at soil level and their dry weights determined after drying at  $70^{\circ}\text{C}$  for 48h. The data were processed as described in



## Experiment 2.5.

### Results.

Growth of the herbicide-treated plants was severely inhibited and the leaves of most treated plants showed signs of chlorosis two weeks after applying the herbicide. At harvest, nine weeks after sowing, all plants which had received the herbicide at the 3-leaf stage were completely dead; those treated at the 4-leaf stage were severely necrotic although a few had some green leaves. Most plants which had been treated at the 5-leaf stage were severely chlorotic at the time of harvest, but a few which had received the lower dose had some green leaves and had produced inflorescences.

Shoot dry weights were significantly reduced below the control values at all doses of the herbicide and at all stages of application (Table 2.35). By the time of harvest, triasulfuron had caused more damage to the seedlings treated at the 3 and 4-leaf stages than those treated at the 5-leaf stage.

Table 2.35: Shoot dry weight (g) of Poa annua seedlings 9 weeks after sowing.

Herbicide dose (gha <sup>-1</sup> )	Stages of application		
	3-Leaf	4-Leaf	5-Leaf
Control - 0	3.13a	3.35a	3.37a
10	0.18d	0.45cd	1.00b
20	0.19d	0.49cd	0.80bc

Values sharing the same letter are not significantly different at P < 0.05.

**EXPERIMENT 2.7: EFFECTS OF TRIASULFURON ON THE GROWTH OF PEA AND BROAD BEAN.**

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**Materials and Methods.**

On 13 November 1987, three seeds each of Vicia faba cv. Aquadulce Claudia and Pisum sativum cv. Kelvedon Wonder were sown into 14cm diameter plastic pots filled with John Innes No.1 compost. Triasulfuron was applied at two stages viz; post-sowing pre-emergence and post-emergence two weeks after sowing at the rates of 0, 10 or 20 g ai ha<sup>-1</sup> in 200 lha<sup>-1</sup> of water. The herbicide was applied with an Oxford precision sprayer at a pressure of 2.1 kgcm<sup>-2</sup>. The experiment was laid out as a completely randomized design with three replicates and kept in a heated greenhouse with temperatures of 20 ± 2°C (day) and 15 ± 3°C (night) and a minimum daylength of 16 hours maintained by high pressure sodium lamps (400W).

At harvest, five weeks after sowing, the plants were carefully uprooted from the soil, their roots washed free of soil and their heights recorded. The dry weights of whole plants were then determined after drying in an oven at 70°C for 48h. The data were processed as described in Experiment 2.5.

**Results.**

Triasulfuron severely inhibited the emergence of both broad bean and pea seedlings. None of the seedlings in the pre-emergence treatment had emerged two weeks after applying the herbicide. However, when some seedlings eventually emerged, they were very unhealthy and their growth was very slow. Plants which had received the post-emergence treatment started showing signs of chlorosis



between 7 (pea) and 10 (broad bean) days after application and at the final harvest, the leaves of the pea plants were severely necrotic whilst some parts of the leaves, stem and base of the broad bean plants had some black colouration.

The pre-emergence treatments resulted in reduced plant height and dry weight of both species below their respective control values. Similar results were obtained for the post-emergence treatments where plant heights and dry weights at all doses of the herbicide were significantly lower than their controls in both species (Tables 2.36 and 2.37).

Table 2.36: Heights (cm) and dry weights (g) plant<sup>-1</sup> of pea seedlings 5 weeks after sowing.

Herbicide dose (gha <sup>-1</sup> )	Height plant <sup>-1</sup>		Dry weight plant <sup>-1</sup>	
	Pre-emergence	Post-emergence 2 weeks after sowing	Pre-emergence	Post-emergence 2 weeks after sowing
Control-0	20.6	21.8a	0.21	0.22a
10	0.0	9.7b	0.00	0.11b
20	2.2	11.5b	0.01	0.11b

Table 2.37: Heights (cm) and dry weights (g) plant<sup>-1</sup> of broad bean seedlings 5 weeks after sowing.

Herbicide dose (gha <sup>-1</sup> )	Height plant <sup>-1</sup>		Dry weight plant <sup>-1</sup>	
	Pre-emergence	Post-emergence 2 weeks after sowing	Pre-emergence	Post-emergence 2 weeks after sowing
Control-0	55.9	57.4a	1.10	1.06a
10	15.3	30.7b	0.10	0.60b
20	2.9	37.8b	0.08	0.78b

Values of plant height or dry weight sharing the same letter are not significantly different at P < 0.05.

## DISCUSSION

Triasulfuron inhibited germination (protrusion of the radicle) of carrot, Poa trivialis, Veronica persica and Senecio vulgaris when applied to seeds sown in petri-dishes whereas germination of wheat, cabbage, Trifolium repens and perennial ryegrass was not affected (Tables 2.1 and 2.5). Early seedling development, measured by the protrusion of the coleoptile or cotyledons was inhibited in all species except wheat, cabbage and Trifolium repens but subsequent growth of these seedlings was markedly reduced; for wheat and perennial ryegrass, the lengths of longest roots and coleoptiles of treated seedlings were reduced (Tables 2.2, 2.3 and 2.4). In greenhouse studies, although some seeds of oilseed rape, pea and broad bean germinated following pre-emergence applications of triasulfuron, seedling emergence was severely inhibited. In most cases the seedlings either failed to emerge or the few that emerged soon died. This response to triasulfuron was not observed for wheat or barley (Tables 2.7, 2.8, 2.17, 2.26 and 2.30). These results confirm the observations that triasulfuron inhibits growth of young seedlings and may also inhibit germination of some species (Amrein and Gerber, 1985).

In a germinating seed, many physiological and biochemical processes occur. Generally the whole is characterized by initial water imbibition leading to the release of enzymes followed by the synthesis of proteins and RNA after which elongation growth may proceed. Some of the enzymes may be released or activated from existing proteins eg. amylopectin glucosidase or may be synthesized afresh eg.  $\alpha$ -amylase. Some inhibitors of RNA and protein synthesis



are known to inhibit these latter enzymes thereby disrupting the mobilization of stored food reserves in the endosperm or cotyledon upon which germination and early seedling growth depends (Swain and Dekker, 1969). Tepper et al. (1967) stated that DNA synthesis is not critical for the germination of seeds since germination occurs in seeds where DNA synthesis and mitotic activity have been artificially blocked. Ray (1980) showed with pea and corn root tips, that the immediate effect of chlorsulfuron, a sulfonylurea herbicide, is the inhibition of cell division followed by the inhibition of other physiological processes like protein and RNA synthesis, respiration and photosynthesis. It is therefore possible that during the early stages of germination, triasulfuron might have acted on some of the events involved in protein and RNA synthesis or respiration, leading to disruption of the mobilization of food reserves and hence to the inability of the radicles of certain species to emerge.

Davidson (1966) has reported that DNA synthesis and mitotic activity normally begin after the emergence of the radicle. Since a major effect of triasulfuron on young seedlings was found to be an inhibition of growth immediately after germination, there is the likelihood that interference with cell division and/or cell elongation might have caused the inhibition of growth and the subsequent deaths of young emerged seedlings of the susceptible species. The same argument can be used to explain the reduction in length of roots and coleoptiles of wheat and perennial ryegrass seedlings raised in petri-dishes (Tables 2.3 and 2.4). Similar effects on germination and early seedling growth have been reported for other herbicides such as trifluralin {2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzen-amine and diphenamid {N,N-dimethyl- $\alpha$ -phenyl

benzeneacetamide) which had very little effect on the germination of seeds of several weed species but severely retarded or killed the young seedlings (Grover, 1965). Ray (1980) and O'Sullivan (1982) have reported that although seed germination is not usually inhibited and cotyledons usually unfold normally following pre-emergence applications of chlorsulfuron, subsequent growth is greatly affected and in most cases, true leaves fail to emerge.

It is quite common for differential absorption of herbicides to occur between seeds of different species. Haskell and Rogers (1960) for example demonstrated that the absorption of chloramben {3-amino-2,5-dichlorobenzoic acid}, simazine {6-chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine} and amitrole {1H-1,2,4-triazol-3-amine} was faster in seeds of soybean than of maize (Zea mays) but seeds of jimsonweed (Datura stramonium L.) scarcely absorbed any of these herbicides. The fact that triasulfuron prevented germination of seeds of some species but not others could be due to differences in the rates of absorption. Richardson et al. (1980) using chlorsulfuron at a rate of 25g ha<sup>-1</sup> and Richardson and West (1986) using 50 or 200g ha<sup>-1</sup> thiameturon recorded high pre-emergence activity of these sulfonylurea herbicides on small seeded annuals.

In general, wheat and barley showed greater tolerance to triasulfuron at all the stages of application than did the broad-leaved and other grass species used in the present studies (see Experiments 2.2, 2.4, 2.5 and 2.7) although the pre-emergence and early post-emergence applications retarded early seedling growth of both species. Differential spray retention, absorption, translocation within the plant and metabolism of herbicides are general mechanisms



responsible for differential plant responses to foliar-applied herbicides. The selectivity of the sulfonylurea herbicides is believed to be based on the detoxification of the herbicide molecule to inactive products within the resistant plant. Sweetzer et al. (1982) showed that 24h after applying similar quantities of  $^{14}\text{C}$ -chlorsulfuron to the leaves of wheat, wild oat, sugar beet and cotton, less than 10% of the radioactivity was recovered in the tolerant wheat and wild oat plants whereas 80 and 97% of the herbicide was unmetabolized in the susceptible cotton and sugar beet plants respectively. Hageman and Behrens (1984) measured spray retention, absorption and translocation of chlorsulfuron in Eastern black nightshade (Solanum ptycanthum Dun.), a tolerant species and velvet-leaf (Abutilon theophrasti Medic.), a susceptible species. They found that the differences in retention, absorption and translocation were inadequate to account for the large differences in tolerance observed between the two species. However, studies on the metabolism of  $^{14}\text{C}$ -chlorsulfuron within the two species showed that in Eastern black nightshade, 69.9 and 81% of the absorbed chlorsulfuron was metabolized within 24 and 72h respectively but only 7.1% of the absorbed chlorsulfuron was metabolized in velvet-leaf within 72h. In field studies, triasulfuron has been reported to have excellent selectivity between the small grain cereals wheat and barley and a number of broad-leaved weeds and grasses such as Apera spica-venti and perennial ryegrass at the rates of  $10\text{--}20\text{g ha}^{-1}$  applied at the 2-3 leaf stage (Amrein and Gerber, 1985).

The results of Experiments 2.2, 2.3 and 2.4 showed a trend for increase in tolerance to triasulfuron in both wheat and barley as the seedlings increased in age. Pre-emergence applications caused



significant growth reductions in both species but the effects decreased considerably when the herbicide was applied from the 2-leaf stage onwards (Tables 2.13, 2.14, 2.15, 2.16, 2.24, 2.25 and 2.27). In the susceptible broad-leaved and grass species, later applications of the herbicide resulted in delay in the appearance of phytotoxic symptoms and death of plants (see Experiments 2.4, 2.5, 2.6 and 2.7). It is well known that cuticular and epicuticular waxes on plant surfaces play a major role in the retention of herbicide spray on the surfaces of leaves. Most often, the nature of leaf surfaces change as plants advance in age mainly because of the formation and deposition of waxes. In young leaves, wax deposition may be incomplete and they may retain more spray than mature leaves (Roberts, 1982). It is possible that the greater tolerance of wheat and barley to triasulfuron with age might be partly due to poorer retention and lower penetration of the herbicide as the ratio of older to younger leaves increases.

The first symptom observed in all the experiments following post-emergence applications of triasulfuron was inhibition of growth manifested by reductions in leaf and root lengths and stunted growth. This effect was usually followed by severe chlorosis and necrosis but in Veronica persica, some leaves developed a purple colouration whilst some black lesions were observed on leaves and stems of treated broad bean seedlings. In general, the development of phytotoxic symptoms and death of plants was slow; complete death of treated plants normally occurred 3 to 4 weeks after applying the herbicide. These observations are similar to those made in field trials by Amrein and Gerber (1985). Similar symptoms have been

described for chlorsulfuron (Richardson et al., 1980) and thiameturon (Richardson and West, 1986). Although early seedling growth was usually retarded in barley and wheat following pre-emergence applications, the seedlings of these species did not develop any particular phytotoxic symptoms.

Studying the effects of chlorsulfuron on wheat, barley, oats and a number of broad-leaved and grass species, Richardson et al. (1980) found that the major symptom was a powerful inhibition of the apical meristem. It is possible that the inhibition of processes such as cell division and cell elongation at the apical meristems might in part, be responsible for the cessation of growth and eventual death of susceptible species. Detailed studies on the effects of triasulfuron on shoot meristem and on cell division at root tips are reported in Chapters 3 and 6 respectively.

In Experiment 2.3, the effects of triasulfuron on three different cultivars of wheat and of barley were studied. No differences in cultivar response to the herbicide were observed. However, from visual observations, wheat cultivars appeared to be slightly more tolerant to triasulfuron than were barley cultivars, particularly, after pre-emergence applications. Amrein and Gerber (1985) have also reported an absence of differences between cultivars of wheat and barley following the application of triasulfuron in field trials. They, however pointed out that wheat was more tolerant than barley to pre-emergence applications. Hageman and Behrens (1981) observed reductions in barley root mass when a field application of  $250\text{g ha}^{-1}$  chlorsulfuron was made but wheat and oats were unaffected. Foley (1986) reported that barley was less tolerant than wheat when the roots of both species, at the 1.5- and 2-leaf stages were exposed to



nutrient solution containing 35mM chlorsulfuron. Similarly, post-emergence application of thiameturon at rates of 60 or 120 g ai ha<sup>-1</sup> were found to be more toxic to winter barley than to winter wheat cultivars. (Sionis et al., 1985). However there are conflicting reports on the response of different cultivars of wheat and barley to chlorsulfuron. For example, O'Sullivan (1982) neither observed any visible injury during the growing season nor reduction in yield due to herbicide treatments when 100 or 200g ha<sup>-1</sup> chlorsulfuron was applied at the one-, three- or five-leaf stages to four barley and two wheat cultivars. On the other hand, Bowran and Blacklow (1987) using inhibition of rates of elongation of the third leaf of seedlings to identify differences in sensitivity to chlorsulfuron of sixteen cultivars of spring wheat following an application of the herbicide at a rate of 40µg kg<sup>-1</sup> of soil, found that the cultivars Timstein and Gabo were more tolerant and Sonora and Miling more sensitive to the herbicide.

The results of Experiments 2.2 and 2.3 showed that wheat and barley seedlings produced more tillers than did the controls when triasulfuron was applied post- but not pre-emergence. Indeed pre-emergence treatments led to reductions in tiller numbers (Tables 2.18, 2.19, 2.20 and 2.21). The stimulation of tillering in wheat and barley has not previously been reported with any of the sulfonylurea herbicides. It is a well known phenomenon in cereals and grasses that an injury (physical or physiological) at the shoot meristem usually results in the loss of apical dominance leading to the release of dormant buds from dormancy. It is possible that triasulfuron might have damaged the shoot meristem leading to the loss of dominance and



hence the production of extra tillers. Further studies on the multi-tillering response of wheat and barley to triasulfuron are reported in Chapter 3.

Another characteristic property of triasulfuron observed in all the experiments was a flat-dose response curve with plants showing only very small increases in phytotoxicity in response to higher doses. The reason for this relative lack of dose-related response cannot be readily explained but similar flat-dose response curves have been reported for other sulfonylurea herbicides. Foley (1986) for example demonstrated that 7nM chlorsulfuron applied to barley roots caused reductions in the fresh weight, dry weight and lengths of roots but increasing the concentration above 7nM did not cause further reductions.

CHAPTER 3.

EFFECTS ON TILLERING.

## INTRODUCTION.

Tillering is one of the most distinct features of the development of temperate cereals because of the major role it plays in helping the crop to fully exploit the environment. Apart from contributing significantly to yield, tillering is important in compensating for low sowing rate, poor establishment and the effects of pests and diseases (Jewiss, 1972; Kirby and Faris, 1972).

Tillers develop from buds in the axils of leaves of temperate cereals and their emergence follows an ordered sequence which is closely related to the number of leaves on the main shoot. Usually only a proportion of tiller buds grow and develop into leafy tillers whilst the remainder do not grow beyond the bud stage. It is believed that the apical meristem of the main shoot is the main organ responsible for the suppression of the outgrowth of tiller buds (Sachs and Thimann, 1967; Phillips, 1975). Some evidence in support of the apical dominance phenomenon was demonstrated when tillering was stimulated in barley and teosinte plants following an artificial damage to the shoot apex but this effect was reversed when auxin was applied to the damaged apex (Leopold, 1949). Some workers have also reported that other growth hormones such as gibberellins (Evans et al., 1964; Kirby and Faris, 1972) and abscisic acid (Harrison and Kaufmann, 1980) inhibit the outgrowth of tiller buds. On the other hand, it has been shown that the application of cytokinin has an over-riding effect on the inhibitory action of auxin on lateral buds (Wickson and Thimann, 1958) and its application to leaves, buds and roots was found to promote prolific tiller bud outgrowth in cereals (Harrison and Kaufmann, 1980; Isbell and Morgan, 1982). It has been



suggested that changes in the synthesis of apical auxin modified by nutrient level is the major factor controlling the outgrowth of tiller buds (Aspinall, 1961) but others believe that the most important factor is rather the levels of endogenous auxin and cytokinin and that the strength of apical dominance is determined by the balance between the two growth hormones ( Sachs and Thimann, 1964; Phillips, 1975; Harrison and Kaufman, 1980; Isbell and Morgan, 1982).

Many factors including temperature, light, soil moisture and nutrients, plant density and chemical applications are known to have a large influence on the growth and development of tillers but the degree to which these factors affect tillering depends also on the genotypic constitution of the plant.

Several workers have shown that high rates of sowing are not conducive to the growth of tiller buds whereas tillering is enhanced at low plant densities (Kirby, 1967; Kirby and Faris, 1972; Darwinkel, 1978; Colvill and Marshall, 1981). It has been suggested that when cereals are sown at high densities, interplant competition occurs, which is manifested by a low proportion of tiller buds growing and a high percentage of tiller deaths whereas the availability of adequate resources enable plants, sown at low densities, to produce more tillers (Darwinkel, 1978). However, cereals sown at low rates with subsequent profuse tillering may not necessarily produce increased grain yield since intra-plant competition may operate to reduce the number of ear bearing tillers.

Stimulatory effects following the application of sub-toxic doses of some herbicides have often been reported. For example, in cereals, simazine {6-chloro-N,N-diethyl-1,3,5-triazine-2,4-diamine} applied at

sub-lethal concentrations increased the growth of corn (Freney, 1965), winter barley and winter wheat (Bastin et al., 1970) and oats (Steerbjerg et al., 1972) whilst low doses of 2,4-D {2,4-dichlorophenoxy acetic acid} resulted in increased protein contents and yield of wheat (Huffaker et al., 1967). Barban {4-chloro-2-butanyl 3-chlorophenyl carbamate} has been shown to increase the protein content of oats when applied at sub-lethal levels (Wiedman and Appleby, 1972). Similarly, Thonke and Kudsk (1986) obtained significant increases in the growth of Sinapis alba following the application of  $0.02\text{g ai ha}^{-1}$  triasulfuron at  $7.2^{\circ}\text{C}$  and 60 or 75% relative humidity. These examples support earlier suggestions that substances that typically inhibit a biological process will often stimulate that process at sufficiently low concentrations (Thimann, 1956).

Some chemicals, particularly plant growth regulators have been used to induce tillering in some cereals. For example, the application of ancymidol to sorghum (Isbell and Morgan, 1982), CCC {2-chloroethyl) trimethyl-ammonium ion} (Koranteng and Matthews, 1982) and 'Terpal' {mepiquat chloride + ethephon} (Jensen and Andersen, 1981; Woodward, 1986) to barley resulted in increased tillering and in some cases grain yield was increased. Some herbicides have been found to stimulate tillering when applied at sub-lethal quantities. For example, more tillers were released from dormancy when glyphosate was applied at sub-lethal doses to Elymus repens (L.) Gould (Caseley, 1972; Coupland and Caseley, 1975). Similarly, Baur et al. (1977) observed increased tillering in sorghum at  $21^{\circ}\text{C}$  and in wheat at  $27^{\circ}\text{C}$  when the plants were treated with sub-lethal concentrations



of glyphosate. In another study, Chandrasena and Sagar (1984) obtained increased numbers of tillers in Elymus repens (L.) Gould following soil applications of fluazifop-butyl ( $\pm$ )-2-[4-[[5-(trifluoromethyl)-2-pyridinyl] oxy] phenoxy] propanoic acid} at concentrations of less than  $0.3 \text{ kg ha}^{-1}$ . Pallet (1984), studied the responses of three winter wheat cultivars to difenzoguat and obtained increased tiller numbers and reduced main shoot height in all the three cultivars six weeks after applying the recommended dose of  $1 \text{ kg ai ha}^{-1}$  or twice the recommended dose ( $2 \text{ kg ai ha}^{-1}$ ) of the herbicide at the 3 to 4 leaf stages. In the above examples, the stimulation of tillering by the herbicide was attributed to loss of apical dominance leading to the release of tiller buds from dormancy.

Even though there are known examples where increased tillering following the application of plant growth regulators led to increased grain yield in barley (Koranteng and Matthews, 1982), there is very little or no evidence to show that such multi-tillering responses induced by the application of some herbicides had beneficial effects on grain yield of cereals.

As reported in Experiment 2.3 of Chapter 2, tillering was induced by triasulfuron in both wheat and barley with the latter showing the greatest effect when both crops were treated at the 1, 2 or 3 leaf growth stage. The experiments reported in this Chapter were conducted to investigate: the responses of other cultivars of barley to the herbicide, the pattern of tiller initiation and growth at different densities of triasulfuron-treated seedlings and also to find out whether there were any effects on yield and yield components of plants grown to maturity. Studies were also conducted to find out whether the site of application of the herbicide was important in the



response and also whether late applications of the herbicide could induce tillering. Furthermore, attempts were made to find out whether there is any link between the multi-tillering response shown in barley and apical control.

**EXPERIMENT 3.1: EFFECTS OF TRIASULFURON ON SOME VARIETIES OF BARLEY.**

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**Materials and Methods.**

On 10 September 1986 seeds of four varieties of barley viz: Winter barley cv Maris Otter, cv. Panda and Spring barley cv. Triumph and cv. Atem were sown at a rate of 10 seeds per 14cm diameter plastic pot filled with John Innes No.1 compost.

On 28 September 1986 (18 days after sowing), triasulfuron at 0, 15 or 20 g ai in 200 l of water was sprayed onto the plants to the point of run-off using a small hand sprayer. The pots were kept in a heated greenhouse at  $20 \pm 2^{\circ}\text{C}$  (day) and  $15 \pm 2^{\circ}\text{C}$  (night) with minimum daylength of 16h maintained with supplementary light (400W High pressure sodium lamps). The experiment was laid out as a completely randomized design with five replicates. The numbers of tillers per pot were counted one week after spraying and at harvest 3 weeks later when the dry weights of shoots were determined after drying at  $70^{\circ}\text{C}$  for 48h. The data were subjected to analysis of variance using the Genstat statistical package and comparisons between treatment means was done by Tukey's Honestly Significant Difference (HSD) test.

**Results.**

Generally the winter barley cultivars produced more tillers per plant than did the spring cultivars. One week after application, winter barley cv. Panda had significantly more tillers at all doses of the herbicide than it did in the absence of the herbicide. Similar trends were seen for spring barleys cv. Triumph and cv. Atem but the

differences were not significant (Table 3.1). Although the herbicide-treated plants of all the cultivars produced more tillers than their untreated controls 4 weeks after application, the differences were not significant (Table 3.2).

Triasulfuron did not affect the shoot dry weight per plant in any of the four cultivars (Table 3.3).



Table 3.1: Number of tillers per plant one week after applying triasulfuron.

Herbicide <sub>1</sub> dose(g ha <sup>-1</sup> )	Winter barley cv. Maris Otter	Winter barley cv. Panda	Spring barley cv. Triumph	Spring barley cv. Atem	Mean
Control - 0	3.03ab	2.48bc	2.00d	1.63d	2.28
15	2.93abc	3.23a	2.23cd	2.20cd	2.64
20	2.95abc	3.38a	2.43bcd	2.30cd	2.76
Mean	2.97	3.03	2.22	2.04	

Values sharing a common letter do not differ significantly at  $P < 0.05$ .

Table 3.2: Number of tillers per plant two weeks after applying triasulfuron.

Herbicide <sub>1</sub> dose(g ha <sup>-1</sup> )	Winter barley cv. Maris Otter	Winter barley cv. Panda	Spring barley cv. Triumph	Spring barley cv. Atem	Mean
Control - 0	6.58	4.50	2.70	2.30	4.02b
15	7.50	5.60	3.85	2.80	4.94ab
20	7.63	5.63	4.15	3.03	5.11a
Mean	7.23a	5.24b	3.57c	2.71c	

Values in a row or a column sharing a common letter do not differ significantly at  $P < 0.05$ .

Table 3.3: Dry weight per plant four weeks after applying triasulfuron.

Herbicide dose (g ha <sup>-1</sup> )	Winter barley cv. Maris Otter	Winter barley cv. Panda	Spring barley cv. Triumph	Spring barley cv. Atem	Mean
Control - 0	1.21	1.54	1.41	1.50	1.42
15	1.23	1.46	1.35	1.45	1.37
20	1.26	1.56	1.41	1.41	1.41
Mean	1.24b	1.52a	1.39ab	1.45a	

Values sharing a common letter do not differ significantly at  $P < 0.05$ .

**EXPERIMENT 3.2: EFFECTS OF TRIASULFURON ON THE GROWTH OF BARLEY GROWN AT DIFFERENT DENSITIES.**

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**Materials and Methods.**

On 27 November 1986, seeds of Winter barley cv. Maris Otter were sown at rates of 1, 5, 10, or 15 seeds per 14cm diameter plastic pot into John Innes No.1 compost. Triasulfuron was applied post-emergence (2-leaf stage) on 10 December or (3-leaf stage) on 18 December 1986 on separate groups of plants at doses of 0, 10, 15 or 20 g ai ha<sup>-1</sup> in 200 lha<sup>-1</sup> of water. Spraying was carried out with an Oxford precision sprayer at a pressure of 2.1 kgcm<sup>-2</sup>. All other experimental conditions were similar to those described in Experiment 3.1. The plants were watered by coarse overhead irrigation and the pots were arranged in a completely randomized design. There were five replicates.

Tiller numbers were counted five and eight weeks after sowing. Eight weeks after sowing, the numbers of leaves were recorded and the dry weights of shoots determined after drying the material in an oven at 70°C for 48h. The data were processed as described in Experiment 3.1.

**Results.**

Some of the plants which had received 20 g ai ha<sup>-1</sup> showed symptoms of leaf trapping one or two weeks after each application. Five weeks after sowing, all plants treated with herbicide had more tillers than did controls but the numbers of tillers varied inversely with density (Tables 3.4 and 3.5). Similar results were obtained eight weeks after sowing when the herbicide-treated plants had more



leaves and tillers than did the controls although significant differences were obtained only between the highest dose and the controls at the second application. Plants grown singly in pots always had more tillers and leaves than those grown at higher densities (Tables 3.6, 3.7, 3.8 and 3.9).

Plants grown singly had significantly higher individual shoot dry weights than those grown at higher densities. The only significant reductions in dry weight due to the herbicide treatments were recorded for doses of 15 and 20 g ai ha<sup>-1</sup> in the first and second post-emergence applications respectively (Tables 3.10 and 3.11).

Table 3.4: Number of tillers per plant 5 weeks after sowing. (Triasulfuron was applied 2 weeks after sowing).

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Plant density				Mean
	One plant per pot	5 plants per pot	10 plants per pot	15 plants per pot	
Control - 0	3.20	2.84	1.88	1.12	2.26b
10	4.60	2.92	2.44	2.38	3.08a
15	4.40	3.28	3.40	2.74	3.46a
20	5.00	3.12	3.06	2.12	3.33a
Mean	4.30a	3.04b	2.70bc	2.09c	

Table 3.5: Number of tillers per plant 5 weeks after sowing. (Triasulfuron was applied 3 weeks after sowing).

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Plant density				Mean
	One plant per pot	5 plants per pot	10 plants per pot	15 plants per pot	
Control - 0	4.80	2.56	2.08	1.40	2.71b
10	4.20	2.76	2.22	1.74	2.73b
15	5.40	3.28	2.88	1.84	3.35ab
20	5.00	4.76	3.34	2.04	3.78a
Mean	4.85a	3.34b	2.63bc	1.76c	

Values in a row or a column sharing a common letter do not differ significantly at P < 0.05.

Table 3.6: Number of tillers per plant 8 weeks after sowing. (Triasulfuron was applied 2 weeks after sowing).

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Plant density				Mean
	One plant per pot	5 plants per pot	10 plants per pot	15 plants per pot	
Control - 0	11.00	4.88	2.52	1.64	5.01
10	13.40	5.60	4.08	2.68	6.44
15	10.80	6.16	5.78	4.38	6.78
20	11.80	7.00	4.64	3.22	6.67
Mean	11.75a	5.91b	4.26b	2.98b	

Table 3.7: Number of tillers per plant 8 weeks after sowing. (Triasulfuron was applied 3 weeks after sowing).

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Plant density				Mean
	One plant per pot	5 plants per pot	10 plants per pot	15 plants per pot	
Control - 0	16.60	5.08	2.46	1.76	6.48b
10	12.80	7.28	4.84	2.54	6.87ab
15	14.60	7.20	5.02	3.36	7.55ab
20	13.80	8.24	6.24	4.42	8.18a
Mean	14.45a	6.95b	4.64b	3.02b	

Values in a row or a column sharing a common letter do not differ significantly at  $P < 0.05$ .



Table 3.8: Number of leaves per plant 8 weeks after sowing. (Triasulfuron was applied 2 weeks after sowing).

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Plant density				Mean
	One plant per pot	5 plants per pot	10 plants per pot	15 plants per pot	
Control - 0	38.60	18.60	10.74	7.06	18.75
10	47.00	22.40	15.72	9.76	23.72
15	39.40	22.88	19.66	13.76	23.93
20	45.00	24.78	17.26	11.86	24.73
Mean	42.50a	22.17b	15.85b	10.61b	

Table 3.9: Number of leaves per plant 8 weeks after sowing. (Triasulfuron was applied 3 weeks after sowing).

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Plant density				Mean
	One plant per pot	5 plants per pot	10 plants per pot	15 plants per pot	
Control - 0	52.80	18.84	10.30	7.06	22.25b
10	46.60	24.36	16.30	9.16	24.11ab
15	54.40	25.08	17.46	11.90	27.21ab
20	55.00	27.60	19.76	13.34	28.93a
Mean	52.20a	23.97b	15.96b	10.37b	

Values in a row or a column sharing a common letter do not differ significantly at  $P < 0.05$ .

Table 3.10: Dry weight (g) per plant 8 weeks after sowing. (Triasulfuron was applied 2 weeks after sowing).

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Plant density				Mean
	One plant per pot	5 plants per pot	10 plants per pot	15 plants per pot	
Control - 0	1.96	0.95	0.57	0.41	0.97a
10	2.11	0.85	0.53	0.39	0.97a
15	1.19	0.69	0.45	0.32	0.66b
20	1.82	0.64	0.37	0.33	0.79ab
Mean	1.77a	0.78b	0.48b	0.36b	

Table 3.11: Dry weight (g) per plant 8 weeks after sowing. (Triasulfuron was applied 3 weeks after sowing).

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Plant density				Mean
	One plant per pot	5 plants per pot	10 plants per pot	15 plants per pot	
Control - 0	2.64	0.95	0.57	0.42	1.15a
10	2.39	0.87	0.53	0.41	1.05ab
15	2.15	0.90	0.51	0.41	0.99ab
20	1.82	0.76	0.49	0.37	0.86b
Mean	2.25a	0.87b	0.53b	0.40b	

Values in a row or a column sharing a common letter do not differ significantly at P < 0.05.

**EXPERIMENT 3.3: EFFECTS OF TRIASULFURON ON TILLER GROWTH AND DEVELOPMENT OF WINTER BARLEY.**

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**Materials and Methods.**

On 4 December 1986, seeds of Winter barley cv. Maris Otter were sown into 14cm diameter plastic pots filled with John Innes No.1 compost. One week after sowing, the seedlings were thinned to densities of one or 15 plants per pot and the single plants and one plant each in the centre of the higher density pots (15 plants per pot) were tagged.

On 24 December 1986, the numbers of tillers and leaves on each tagged plant and the lengths of the leaf blades and sheaths of the third leaf on the main shoot were recorded. Triasulfuron at 0 or 20 g ai ha<sup>-1</sup> in 200 l ha<sup>-1</sup> of water was then sprayed onto the plants using an Oxford precision sprayer at a pressure of 2.1 kgcm<sup>-2</sup>. One week after applying the herbicide, emerging tillers were identified and grouped into families [viz: T1, T2, T3, T4, T5, CT (coleoptile tillers) ], labelled with plastic coloured rings and counted. The number of leaves on the main shoot and on the tillers and the lengths of the third, fourth and fifth leaf blades and sheaths of the main shoot (MS) were recorded at frequent intervals. The experiment was made in a greenhouse at 20 ± 2°C (day) and 15 ± 3°C (night) with daylength of 16h maintained with supplementary light (400W High pressure sodium lamps). The plants were watered by coarse overhead irrigation and the pots were arranged in a randomized block design with five replicates.

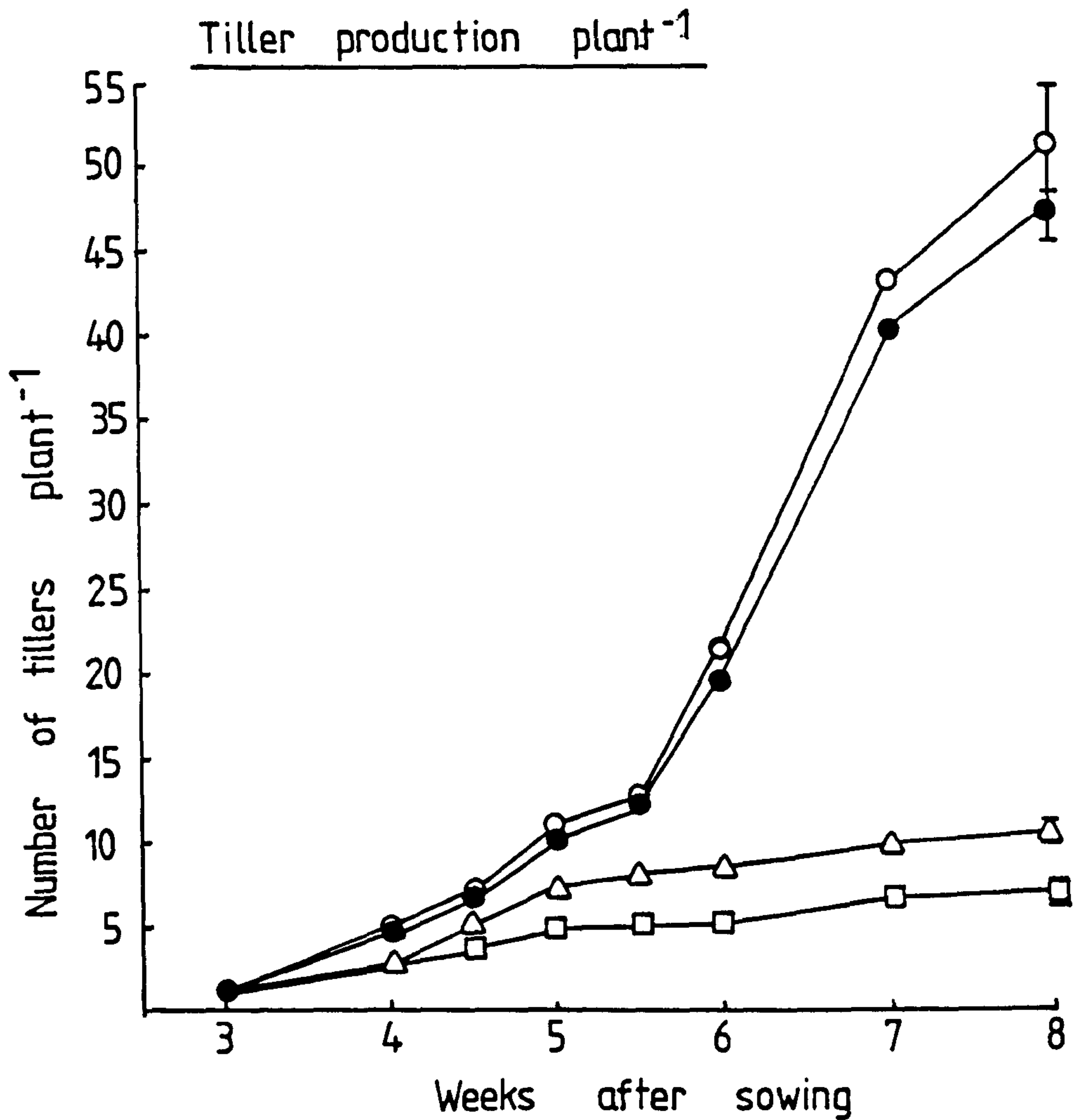
At the final harvest, 8 weeks after sowing, the dry weights of the main shoot and of individual tiller groups were determined by



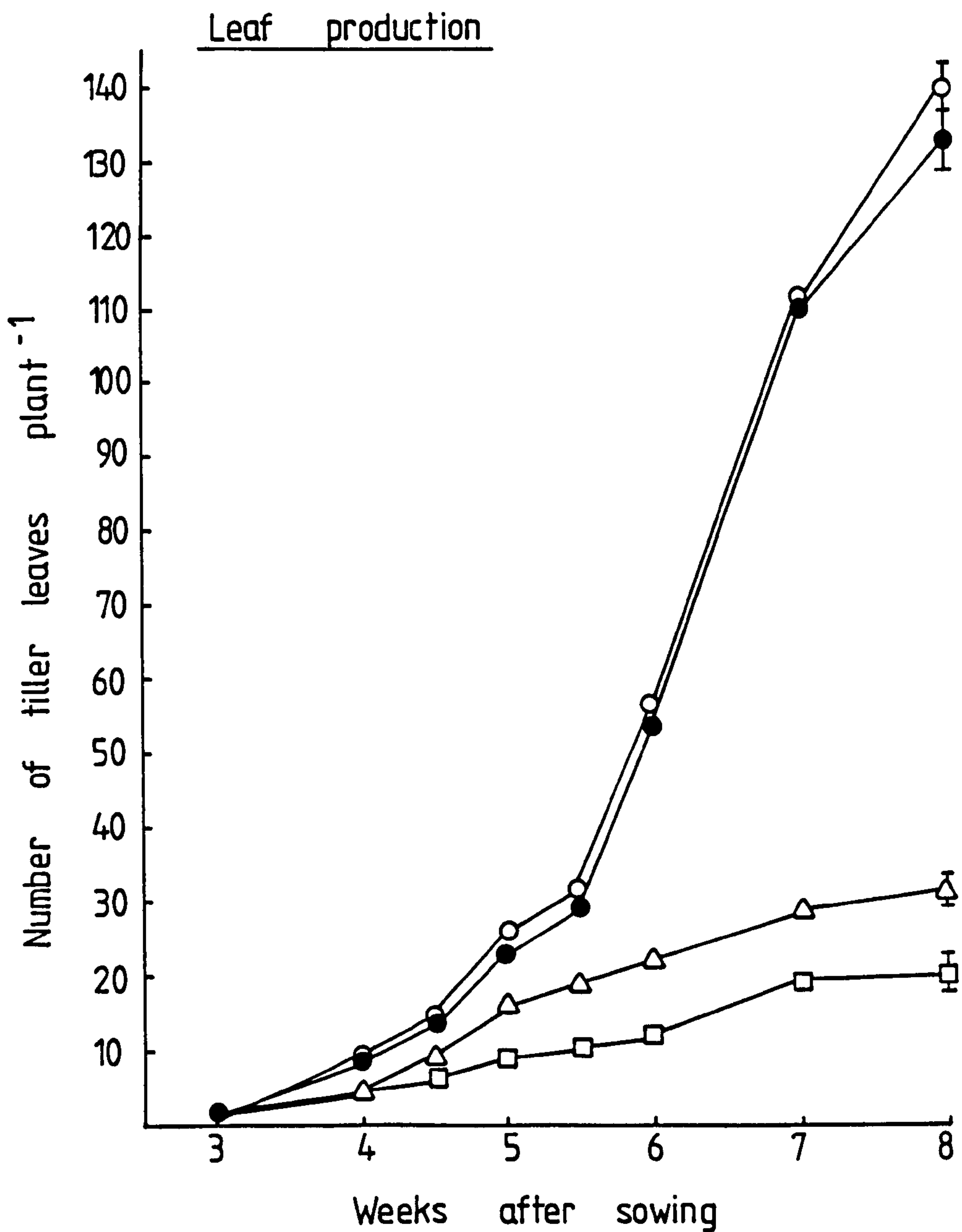
drying in an oven at 70°C for 48h.

## Results.

Triasulfuron increased leaf and tiller production one week after application and by the final harvest tiller production was increased by 7.8% and 34.6% and leaf production by 7.4% and 35.9% at the low density (one plant pot<sup>-1</sup>) and the high density (15 plants pot<sup>-1</sup>) respectively (Fig. 3.1; 3.2). Similarly, leaf production on the main shoot of treated plants showed increases over controls of 2.8% and 9.6% at the low and high densities respectively (Fig. 3.3). The additional tillers in the treated plants were due mainly to the greater production of tillers by the T1, T2, T3, and T4 families and the emergence of more coleoptile and higher order tillers (Fig. 3.5). Triasulfuron reduced the lengths of the leaf blades and sheaths of the third, fourth and fifth leaves on the main shoot (Tables 3.12, 3.13, 3.14, 3.15, 3.16 and 3.17). The total dry weight plant<sup>-1</sup> was significantly lower for plants grown at the high rather than at the low density but there were no significant differences in the dry weights between treated and control plants (Fig. 3.4). The patterns of distribution of tillers, leaves and the dry weights of the main shoot and individual tiller groups were similar at both low and high densities (Figs. 3.5, 3.6 and 3.7).

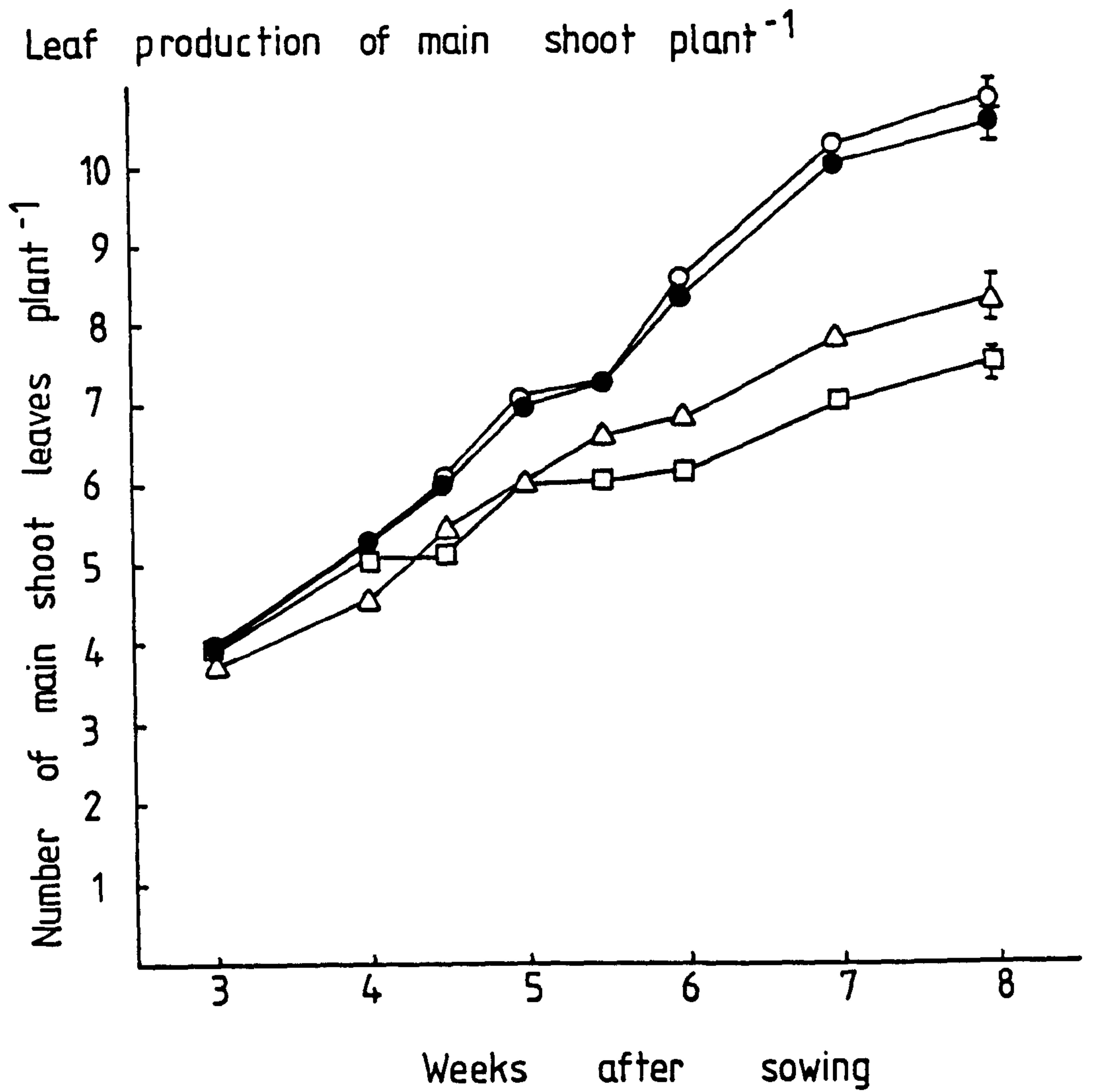


**FIG. 3.1:** Effect of Triasulfuron on winter barley sown at low and high densities (—●— low density, control; —○— low density, 20 g ha<sup>-1</sup>); —□— high density, control; —△— high density, 20 g ha<sup>-1</sup>): tiller production plant<sup>-1</sup>.

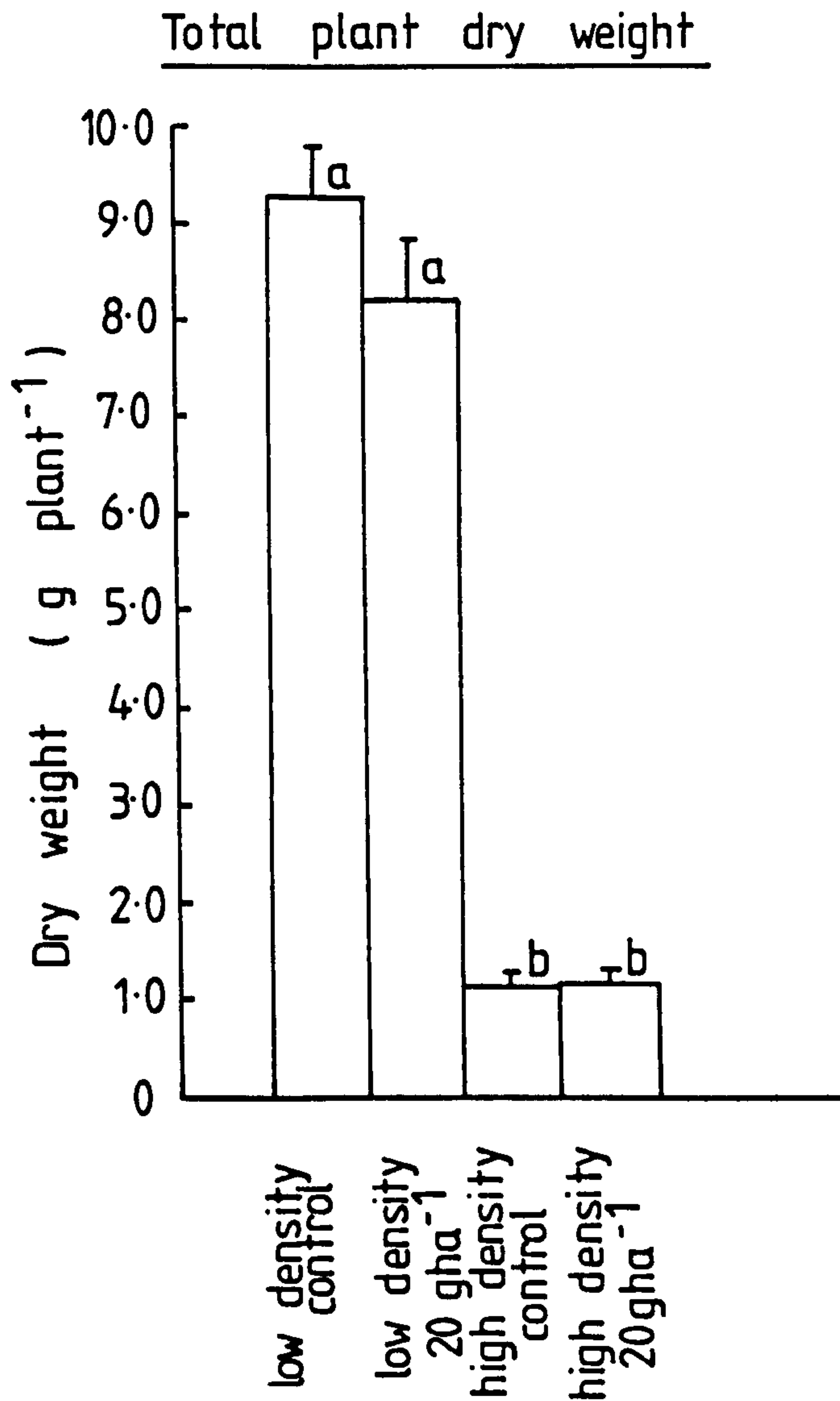


**FIG. 3.2:** Effects of Triasulfuron on winter barley sown at low and high densities (—●— low density, control; —○— low density, 20 g ha<sup>-1</sup>; —□— high density, control; —△— high density, 20 g ha<sup>-1</sup>): leaf production of tillers plant<sup>-1</sup>.



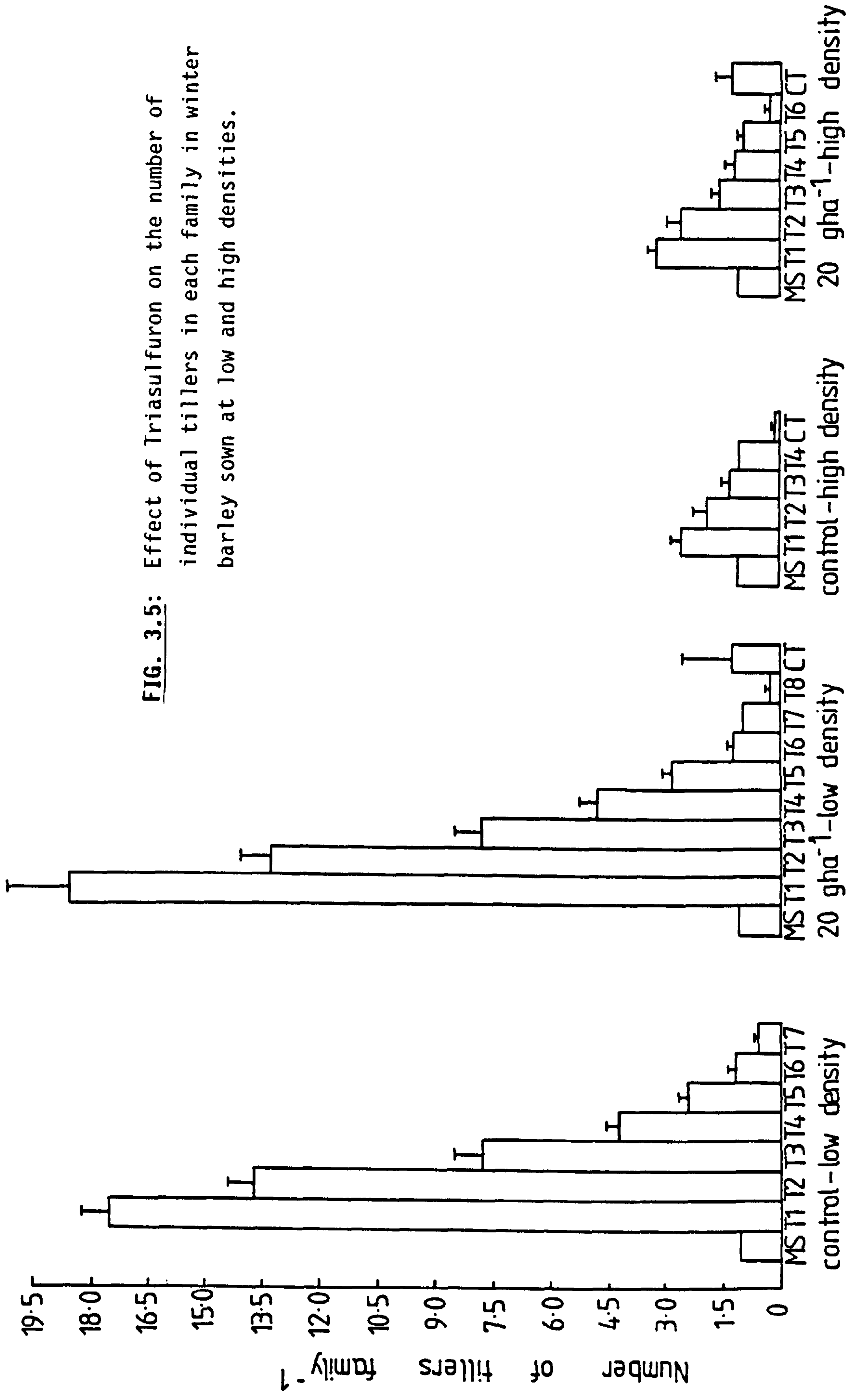


**FIG. 3.3:** Effect of Triasulfuron on winter barley sown at low and high densities ( ● low density, control; ○ low density, 20 g ha<sup>-1</sup>; □ high density, control; △ high density, 20 g ha<sup>-1</sup>): leaf production of main shoot plant<sup>-1</sup>.

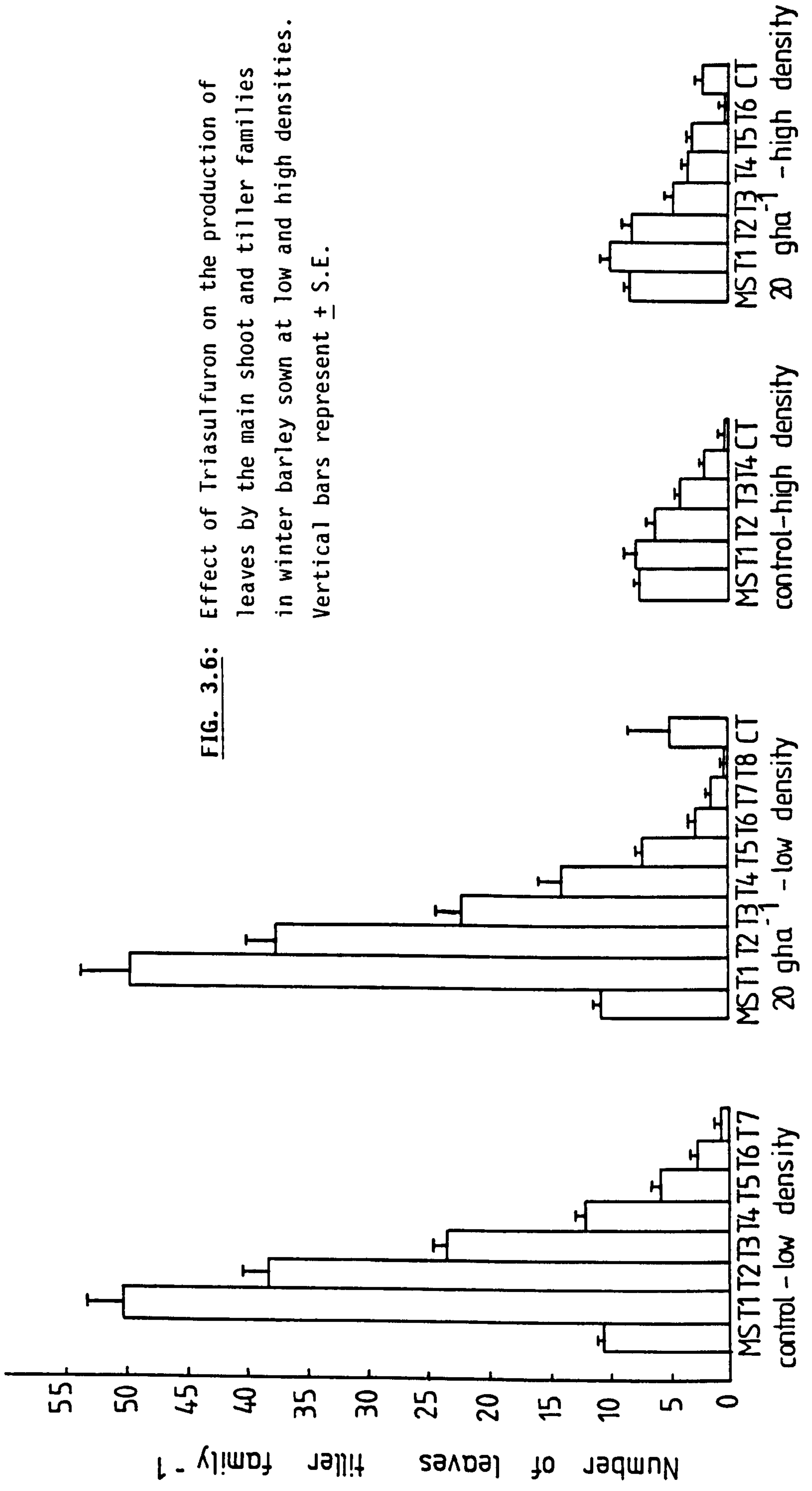


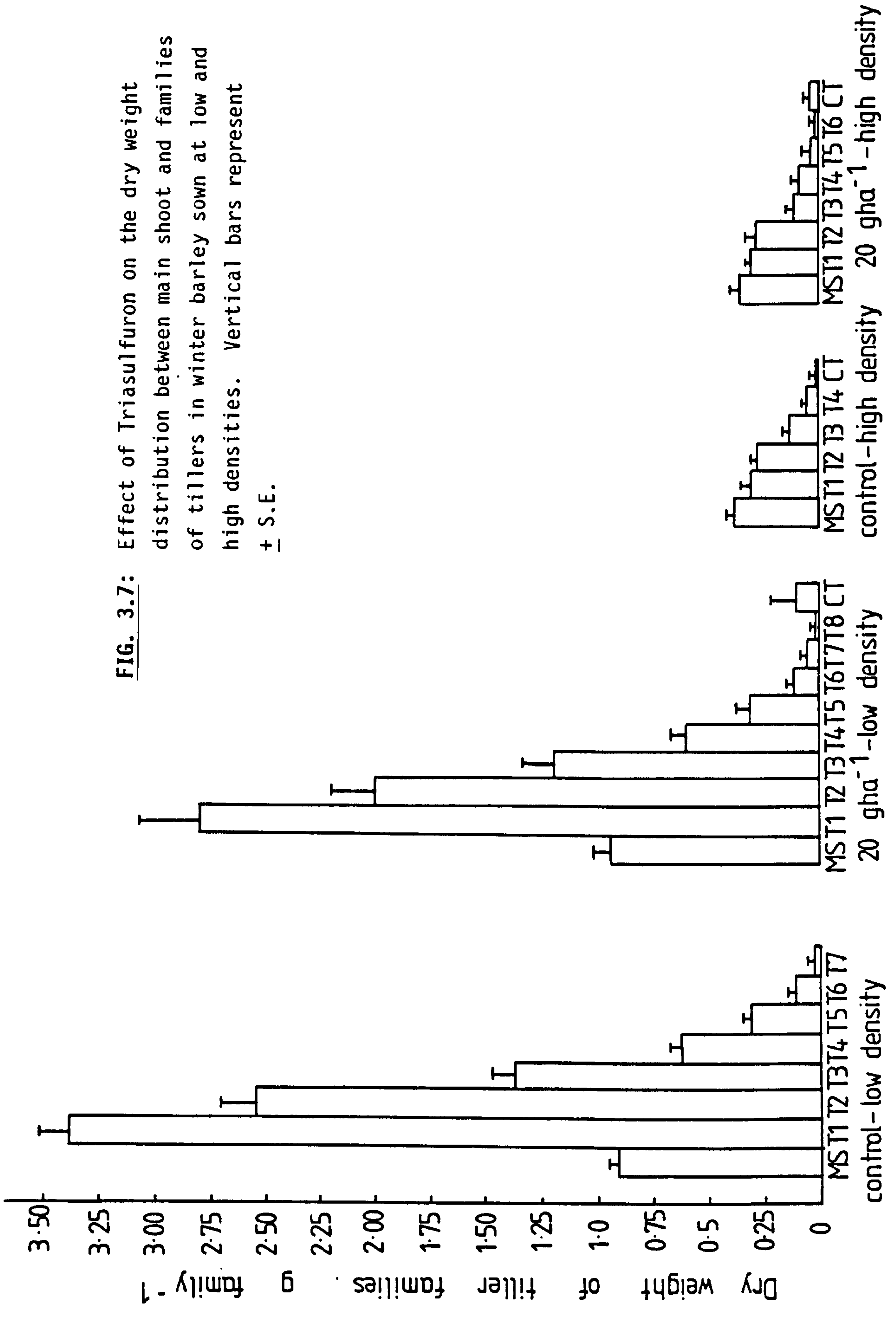
Vertical bars represent  $\pm$  S.E.

**FIG. 3.4:** Effect of Triasulfuron on winter barley sown at low and high densities: total dry weight plant<sup>-1</sup> 8 weeks after sowing.









**FIG. 3.7:** Effect of Triasulfuron on the dry weight distribution between main shoot and families of tillers in winter barley sown at low and high densities. Vertical bars represent  $\pm$  S.E.

Table 3.12: Length (cm) of third leaf of main shoot 6 weeks after sowing.

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Plant density		Mean
	One plant per pot	15 plants per pot	
Control - 0	28.33	28.41	28.32
20	27.35	25.59	26.47
Mean	27.79	27.00	

Table 3.13: Length (cm) of fourth leaf of main shoot 6 weeks after sowing.

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Plant density		Mean
	One plant per pot	15 plants per pot	
Control - 0	30.71	29.22	29.97a
20	26.21	22.97	24.59b
Mean	28.46	26.09	

Values sharing a common letter do not differ significantly at  $P < 0.05$ .

Table 3.14: Length (cm) of fifth leaf of main shoot 6 weeks after sowing.

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Plant density		Mean
	One plant per pot	15 plants per pot	
Control - 0	32.86a	29.78ab	31.32
20	26.32b	18.44c	22.38
Mean	29.59	24.11	

Table 3.15: Length (cm) of third leaf sheath of main shoot 6 weeks after sowing.

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Plant density		Mean
	One plant per pot	15 plants per pot	
Control - 0	5.40ab	6.50a	5.95
20	5.36ab	4.61b	4.99
Mean	5.38	5.55	

Values sharing a common letter do not differ significantly at  $P < 0.05$ .



Table 3.16: Length (cm) of fourth leaf sheath of main shoot 6 weeks after sowing.

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Plant density		Mean
	One plant per pot	15 plants per pot	
Control - 0	6.92a	7.31a	7.12
20	5.61b	4.76b	5.18
Mean	6.26	6.03	

Table 3.17: Length (cm) of fifth leaf sheath of main shoot 6 weeks after sowing.

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Plant density		Mean
	One plant per pot	15 plants per pot	
Control - 0	8.33	7.86	8.10a
20	6.50	4.85	5.68b
Mean	7.41a	6.36b	

Values in a row or a column sharing a common letter do not differ significantly at  $P < 0.05$ .

**EXPERIMENT 3.4: EFFECTS OF SITE OF APPLICATION OF TRIASULFURON ON THE GROWTH OF BARLEY.**

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**Materials and Methods.**

On 26 December 1986, three seeds of winter barley cv. Maris Otter were sown into each of 90, 12.5cm diameter plastic pots filled with John Innes No.1 compost. The seedlings were thinned to two per pot one week after sowing and a week later when the plants had two fully expanded leaves, solutions of triasulfuron at the rates of 0, 10 or 20 g ai in 200 l of water were applied either to the soil at volumes of 0, 25 or 50 ml pot<sup>-1</sup> or sprayed onto the above ground foliage (with the soil covered with vermiculite), or to the distal or basal parts of the foliage with the parts which were not to receive the herbicide covered with aluminium foil or to both the foliage and soil. The foliage treatments were applied to the point of run-off using a small hand sprayer. The vermiculite and aluminium foils were removed after the spray on the foliage had dried. The experiment was designed as a randomized block with five replicates and was made in a heated greenhouse with temperatures of 20 ± 2°C (day) and 15 ± 2°C (night) and a minimum daylength of 16h maintained with supplementary light (400W High pressure sodium lamps). Watering was by overhead irrigation using a watering can.

At harvest 6 weeks after sowing, the numbers of leaves and tillers per pot were recorded. Shoot dry weights per pot were determined after drying the material in an oven at 70°C for 48h.

In another experiment set up on 19 March 1987, five seeds of winter barley cv. Maris Otter were sown into each of 50, 12.5cm diameter plastic pots containing John Innes No.1 compost and were

thinned to three seedlings per pot a week after sowing. Experimental procedures were the same as those for the first experiment except that the herbicide was applied when the plants had an average of three fully expanded leaves (3 weeks after sowing) at the higher rates of 0, 20 or 40 g ai in 200 l of water. This experiment had only one soil treatment where the herbicide was given in 20 ml pot<sup>-1</sup>.

At harvest 8 weeks after sowing, the numbers of leaves, tillers and shoot dry weight per pot were determined. Data for both experiments were processed as previously described in Experiment 3.1.

### Results.

Generally, there was no obvious difference in phytotoxicity between the above-ground foliage treatment and the distal or basal treatments to the foliage. It was evident in both experiments that the soil applications caused more reduction in growth than did any of the other treatments. The symptoms of leaf trapping was observed in the treated plants two weeks after applying the herbicide and it appeared to be more severe in the soil treatments.

In the first experiment, significantly higher number of leaves were found at all doses of the herbicide than in the control (Table 3.18). More tillers were recorded when the herbicide was applied to the whole or distal parts of foliage at the dose of 20 g ha<sup>-1</sup> than were recorded in the soil treatment (50 ml pot<sup>-1</sup>). Although treated plants in all treatments had more tillers than their respective controls, the differences were not always significant (Table 3.19). The plants that had received soil treatments had low shoot dry

weights, at all doses of the herbicide, than did the controls. Plants in the foliage plus soil treatments at the highest dose had lower shoot dry weights than the control (Table 3.20).

The results of the second experiment were similar to those of the first. There were no marked differences related to the site of deposition of the spray on the foliage (distal, basal or overall). More leaves and tillers were produced in the herbicide-treated plants than in the controls in all the treatments and at all doses of the herbicide. Shoot dry weight was reduced significantly only in the soil treatment at the dose of  $20 \text{ gha}^{-1}$  (Tables 3.21, 3.22 and 3.23).



Table 3.18: Number of leaves per pot of barley seedlings 6 weeks after sowing.

Site of application	Herbicide dose (gha <sup>-1</sup> )			Mean
	Control-0	10	20	
Soil (25ml pot <sup>-1</sup> )	66.20	78.00	67.20	70.47
Soil (50ml pot <sup>-1</sup> )	67.40	64.20	63.60	65.07
Foliage (above ground)	68.60	73.80	81.60	74.67
Soil + Foliage	67.00	76.00	79.80	74.27
Foliage (distal parts only)	66.80	74.70	79.40	73.53
Foliage (basal parts only)	65.20	76.80	79.00	73.67
Mean	66.87b	73.87a	75.10a	

Table 3.19: Number of tillers per pot of barley seedlings 6 weeks after sowing.

Site of application	Herbicide dose (gha <sup>-1</sup> )			Mean
	Control-0	10	20	
Soil (25ml pot <sup>-1</sup> )	18.80ab	21.80ab	20.20ab	20.27
Soil (50ml pot <sup>-1</sup> )	20.40ab	16.20b	16.60b	17.73
Foliage (above ground)	19.20ab	21.60ab	23.80a	21.53
Soil + Foliage	19.40ab	21.20ab	23.00ab	21.20
Foliage (distal parts only)	19.20ab	20.60ab	23.60a	21.13
Foliage (basal parts only)	18.80ab	23.00ab	21.80ab	21.20
Mean	19.30	20.73	21.50	

Values sharing a common letter do not differ significantly at  $P < 0.05$ .

Table 3.20: Shoot dry weight per pot of barley seedlings 6 weeks after sowing.

Site of application	Herbicide dose (gha <sup>-1</sup> )			Mean
	Control-0	10	20	
Soil (25ml pot <sup>-1</sup> )	4.41a	2.47cd	1.56de	2.82
Soil (50ml pot <sup>-1</sup> )	4.47a	1.37de	1.14e	2.32
Foliage (above ground)	4.83a	4.45a	4.73a	4.67
Soil + Foliage	4.20a	3.77ab	2.81bc	3.60
Foliage (distal parts only)	4.43a	4.29a	4.44a	4.39
Foliage (basal parts only)	4.06a	4.95a	4.30a	4.44
Mean	4.40	3.55	3.16	

Table 3.21: Number of leaves per pot of barley seedlings 8 weeks after sowing.

Site of application	Herbicide dose (gha <sup>-1</sup> )			Mean
	Control-0	20	40	
Soil (20ml pot <sup>-1</sup> )	96.00	142.20	-	119.10
Foliage (above ground)	98.60	125.80	-	112.20
Soil + Foliage	98.20	128.60	-	113.40
Foliage (distal parts only)	97.60	-	121.20	109.40
Foliage (basal parts only)	97.00	-	132.80	114.90
Mean	97.48b	132.30a	127.00a	

Values sharing a common letter do not differ significantly at P < 0.05.

Table 3.22: Number of tillers per pot of barley seedlings 8 weeks after sowing.

Site of application	Herbicide dose (gha <sup>-1</sup> )			Mean
	Control-0	20	40	
Soil (20ml pot <sup>-1</sup> )	27.80	41.20	-	34.50
Foliage (above ground)	25.80	37.80	-	31.80
Soil + Foliage	27.40	38.80	-	33.10
Foliage (distal parts only)	28.00	-	38.80	33.40
Foliage (basal parts only)	29.80	-	40.40	35.10
Mean	27.76b	39.27a	39.60a	

Table 3.23: Shoot dry weight (g) per pot of barley seedlings 8 weeks after sowing.

Site of application	Herbicide dose (gha <sup>-1</sup> )			Mean
	Control-0	20	40	
Soil (20ml pot <sup>-1</sup> )	4.15a	2.60b	-	3.38
Foliage (above ground)	4.71a	4.50a	-	4.61
Soil + Foliage	4.83a	4.06a	-	4.70
Foliage (distal parts only)	4.43a	-	4.51a	4.47
Foliage (basal parts only)	4.79a	-	4.55a	4.67
Mean	4.58	3.89	4.53	

Values sharing a common letter do not differ significantly at  $P < 0.05$ .

**EXPERIMENT 3.5: EFFECTS OF TRIASULFURON ON SHOOT MERISTEM OF BARLEY.**

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**Materials and Methods.**

On 24 January 1987, five seeds of winter barley cv. Maris Otter were sown into each of eight, 12.5cm diameter plastic pots filled with John Innes No.1 compost. The pots were placed on a bench in the School of Plant Biology, U. C. N. W., with temperatures of  $15 \pm 2^{\circ}\text{C}$  (day) and  $10 \pm 2^{\circ}\text{C}$  (night) and a minimum daylength of 16 hours maintained with a light bank which comprised of six 1.5m 65/80W fluorescent tube plus two 400W mercury vapour lamps, giving a light reading of  $123.6 \mu\text{mols m}^{-2} \text{s}^{-1}$ . Three weeks after sowing when the plants had three fully expanded leaves, solutions of triasulfuron at the rates of 0 and 20 g ai in 200 l of water were sprayed onto the plants using a small hand sprayer.

One, 3, 7, or 14 days after applying the herbicide, four plants from treated and untreated pots were randomly selected and carefully uprooted from the pots and their roots were washed free of soil. The roots were then cut off and the shoot apices carefully dissected using a sharp mounted needle, a blade and a small pair of scissors, a pair of forceps and plasticine for holding the small shoots while the youngest leaves were removed from the bud. The dissections were carried out with the aid of a Nikon binocular stereoscopic microscope at a magnification of x10 with a low voltage lamp providing additional illumination. After exposing the shoot meristems, the number of primordia on each were counted and the apices were then mounted under a photomicroscope and photographs were taken. The lengths of the apices measured from the tip to the base were then



determined by comparing them with the photograph of a standard graticule (1.0mm long) taken at the same magnification.

In a second experiment, five seeds of winter barley cv. Maris Otter were sown on 10 March 1987, into each of eight 14cm diameter plastic pots containing John Innes No.1 compost. The pots were placed in a heated greenhouse with temperatures of  $20 \pm 2^{\circ}\text{C}$  (day) and  $15 \pm 2^{\circ}\text{C}$  (night) and a minimum daylength of 16 hours maintained with supplementary light (400W High pressure sodium lamps). Three weeks after sowing when the plants had four fully expanded leaves, solutions of triasulfuron were applied at rates of 0 or 30g ai in 200 l of water using a hand sprayer.

One, 2, 3 or 4 weeks after applying the herbicide, four plants each from the treated and untreated pots were randomly selected, dissected and photographed following the same procedures as in the first experiment.

## Results.

In the first experiment, there were no differences in the number of primordia between the treated and untreated plants. However, the herbicide appeared to have caused reductions in the lengths of the shoot meristems of the treated plants from the first day up to 14 days after applying the herbicide (Table 3.24 and 3.25). No readily observed abnormality on the treated meristems was recorded (Plates 3.1 and 3.2).

The higher rate of the herbicide used in the second experiment did not result in any increased effects on the meristems (Plates 3.3 and 3.4). There were no consistent differences in the number of

primordia between treated and untreated shoot meristems. The lengths of the treated meristems were slightly lower than those of the untreated up to the third week after applying the herbicide but any differences had disappeared by the end of the fourth week (Table 3.26 and 3.27).

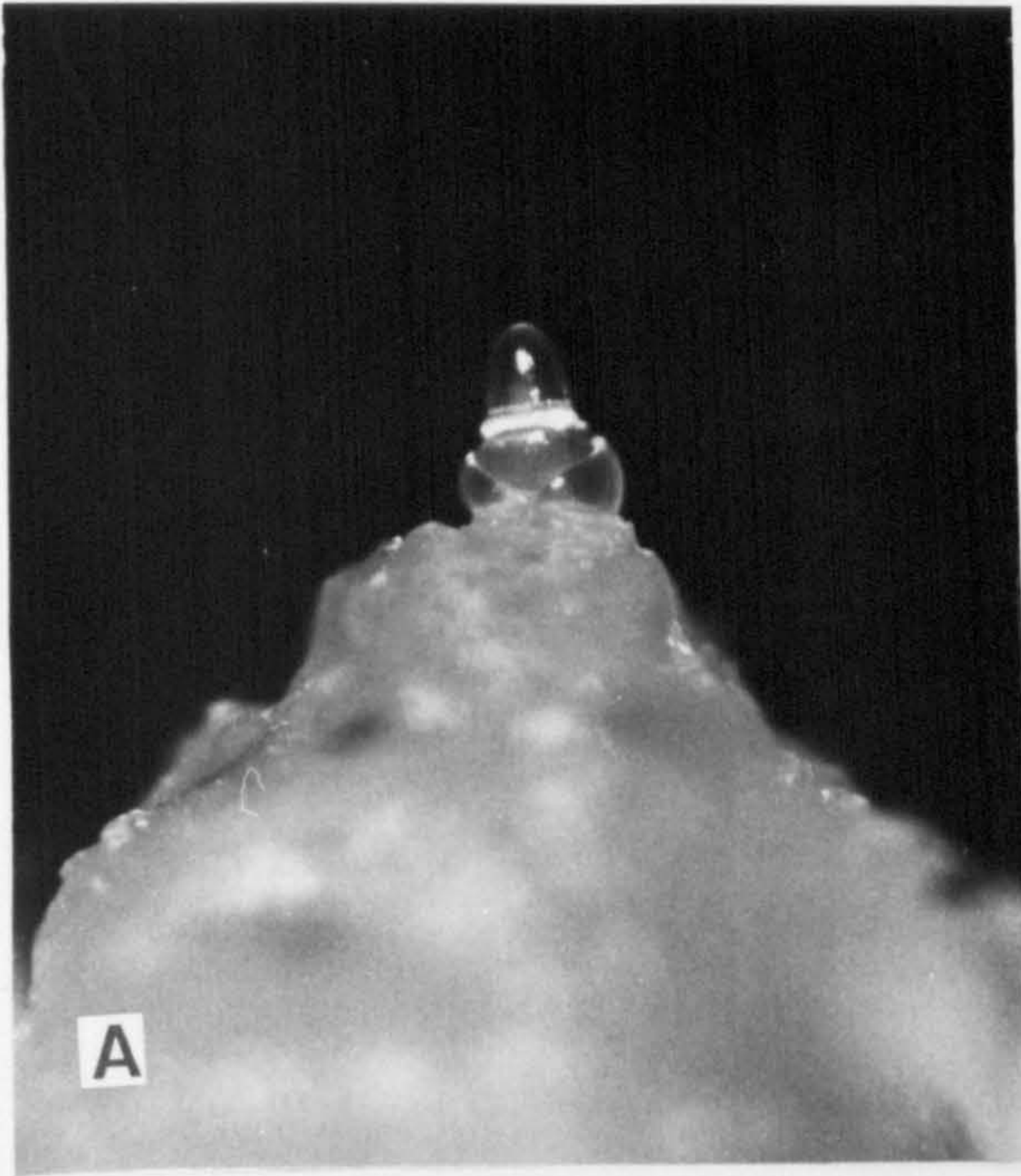
Table 3.24: Number of primordia on shoot meristem of barley treated at 3-fully unfolded leaf stage.

Time after treatment	Herbicide dose (gha <sup>-1</sup> )	
	Control - 0	20
24 hours	6.0	6.0
3 days	6.5	6.3
7 days	6.5	6.5
14 days	7.8	8.3

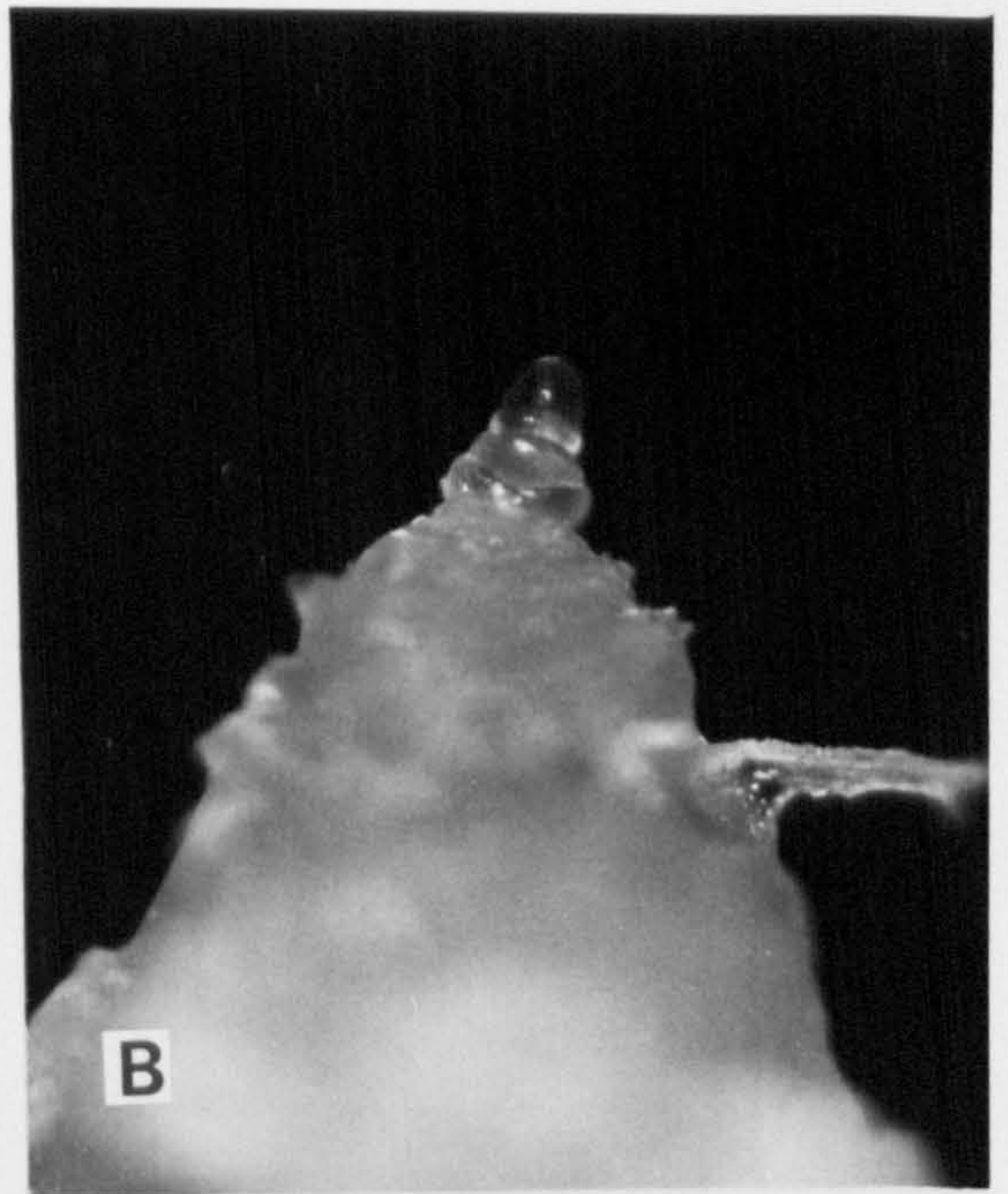
Table 3.25: Length (mm) of shoot meristem of barley treated at 3-fully unfolded leaf stage.

Time after treatment	Herbicide dose (gha <sup>-1</sup> )	
	Control - 0	20
24 hours	0.30 ± 0.01	0.23 ± 0.02
3 days	0.42 ± 0.02	0.34 ± 0.01
7 days	0.44 ± 0.02	0.37 ± 0.02
14 days	0.48 ± 0.03	0.47 ± 0.02

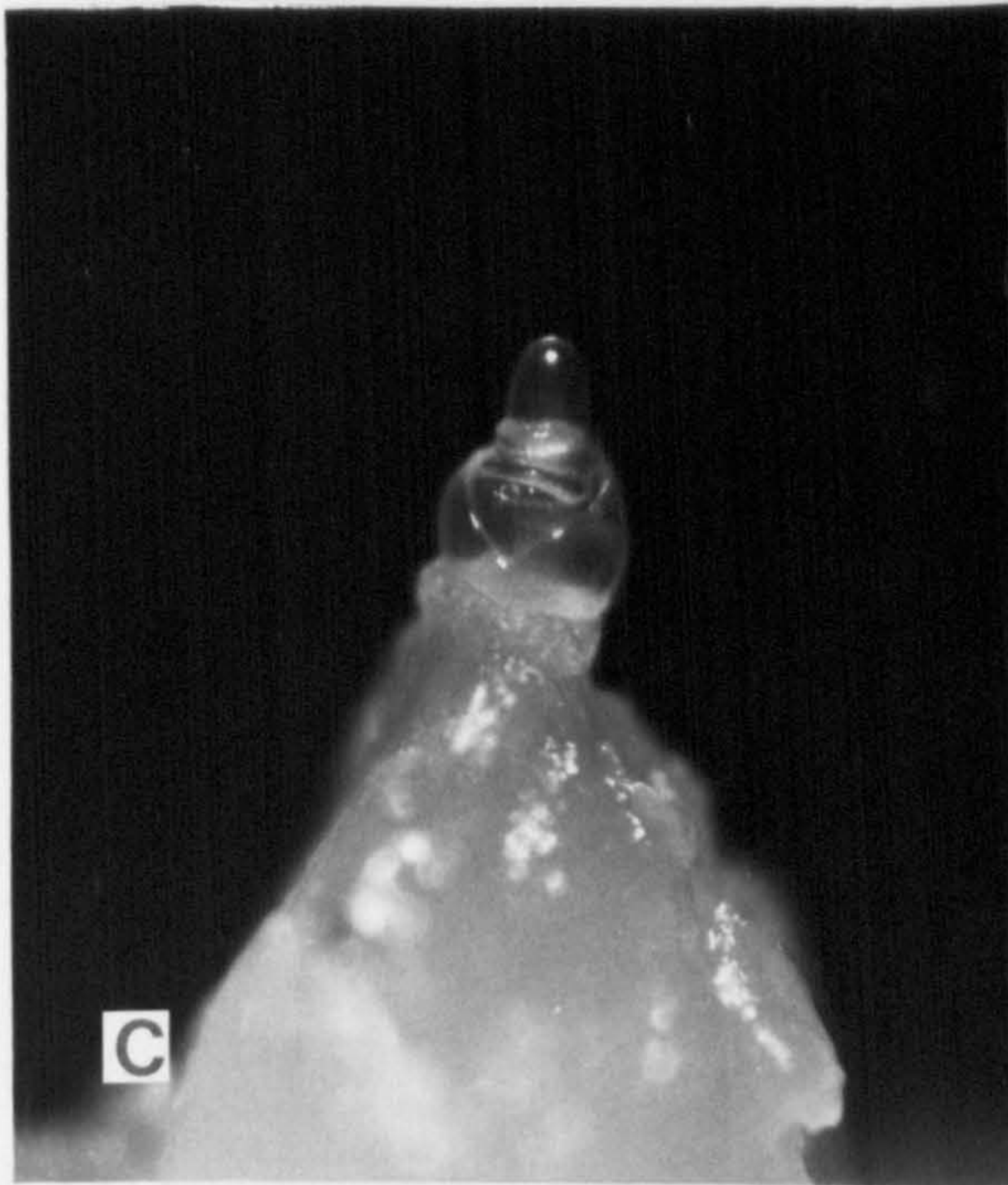




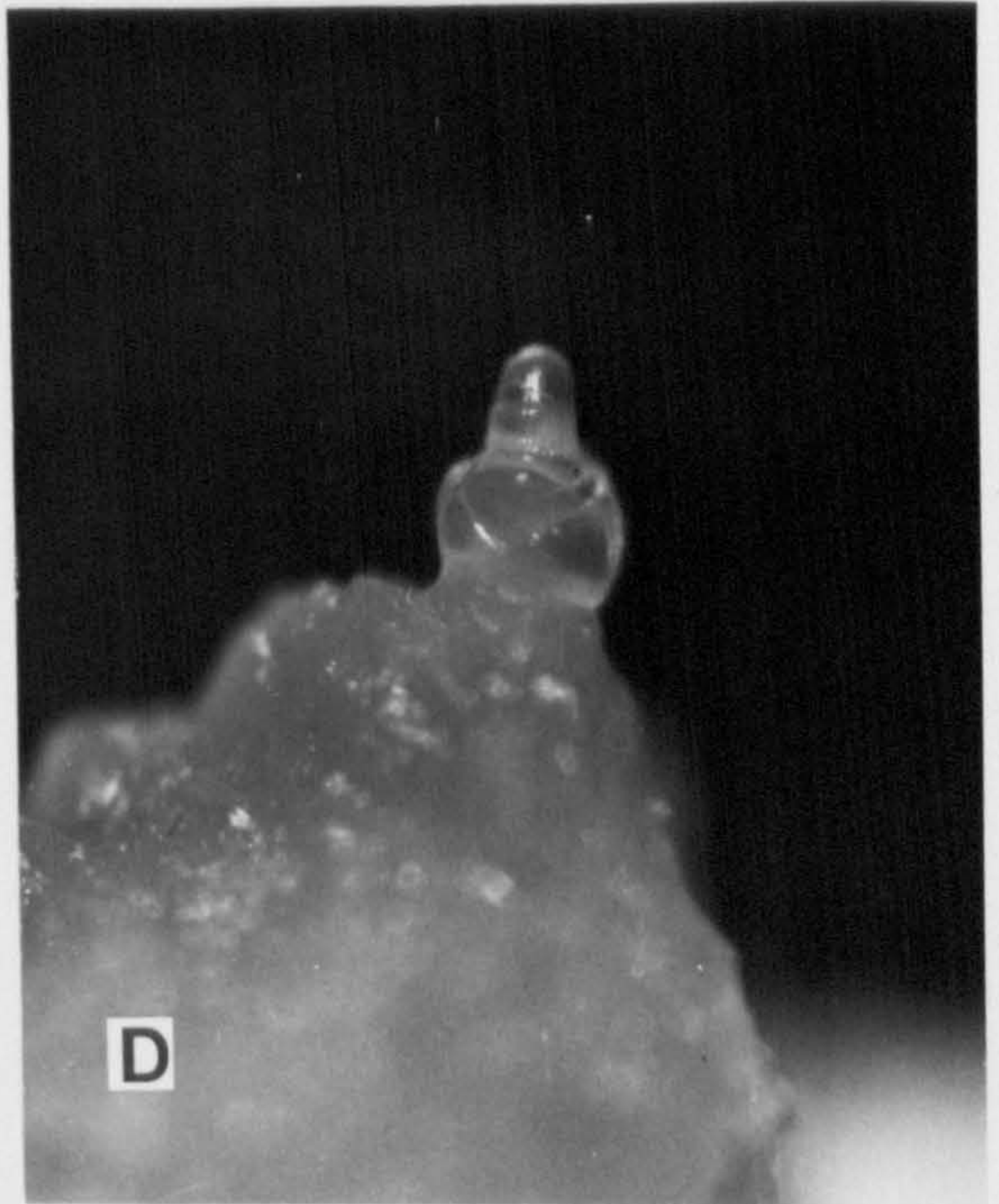
Control - 24h.



Treated - 24h.



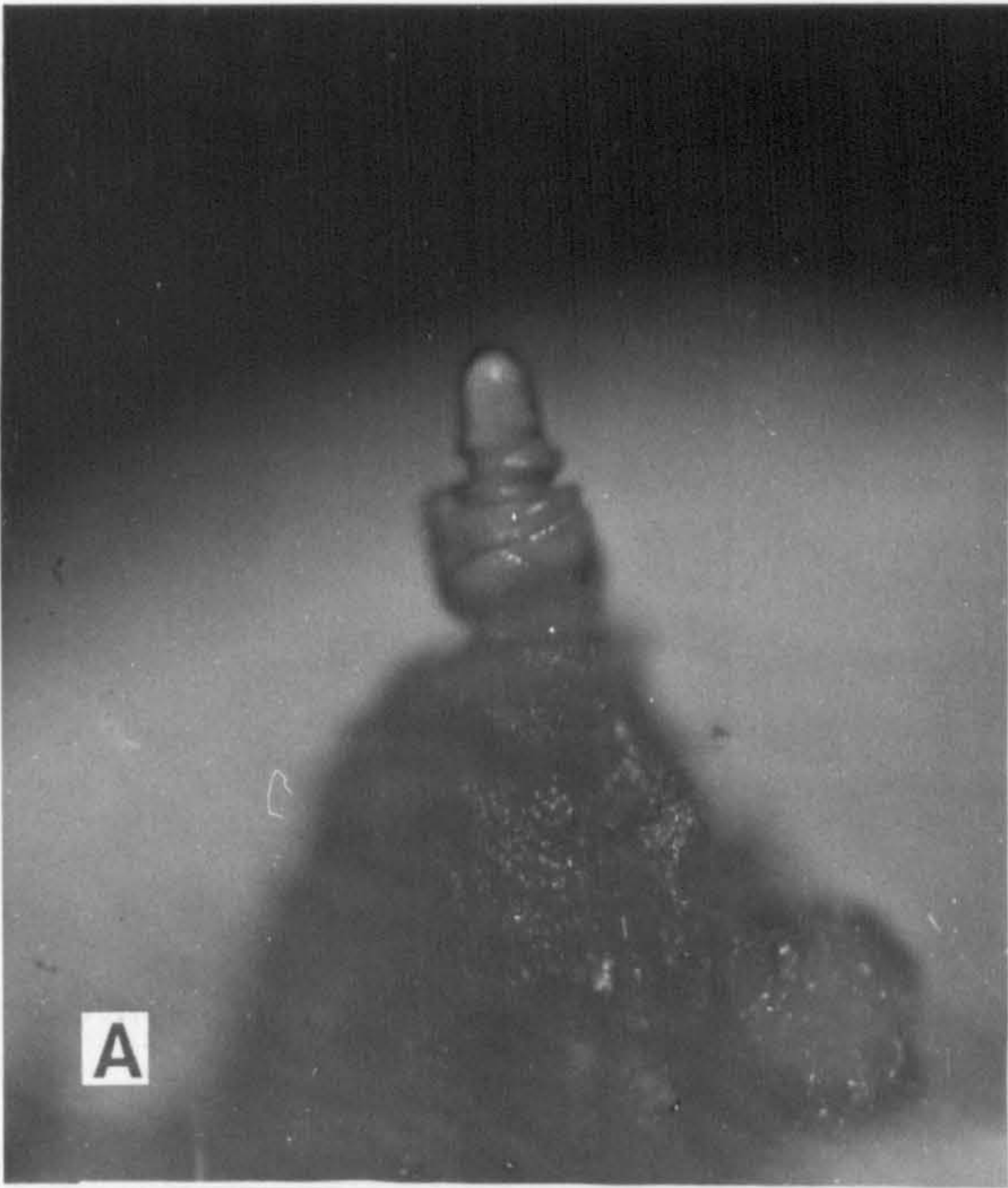
Control - 3 days.



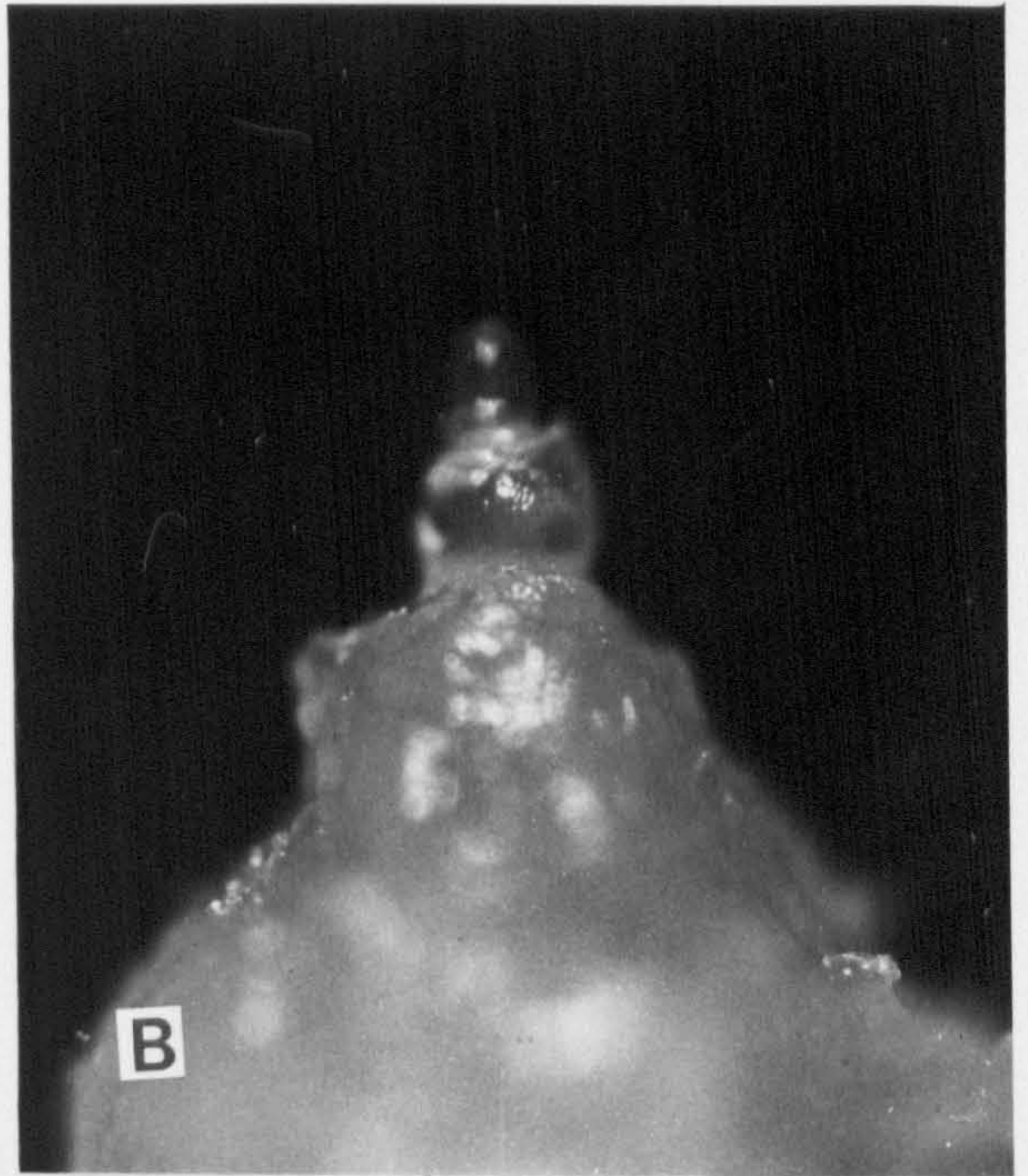
Treated - 3 days.

Plate 3.1: Shoot meristems of barley, 24h or 3 days after the application of  $20 \text{ gha}^{-1}$  triasulfuron at the 3-fully unfolded leaf stage. Scale bar  $\text{—|—} = 0.2\text{mm}$ .

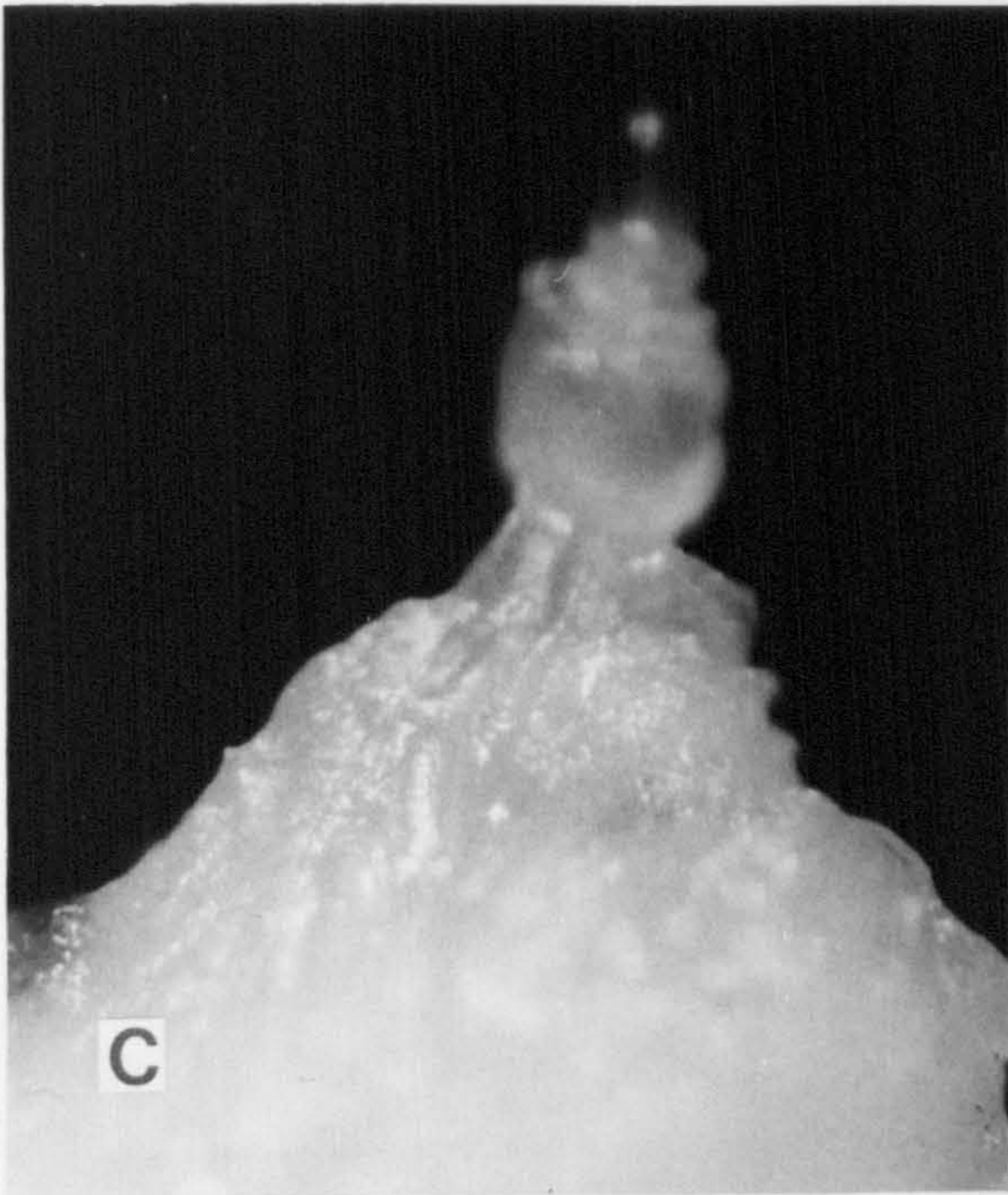




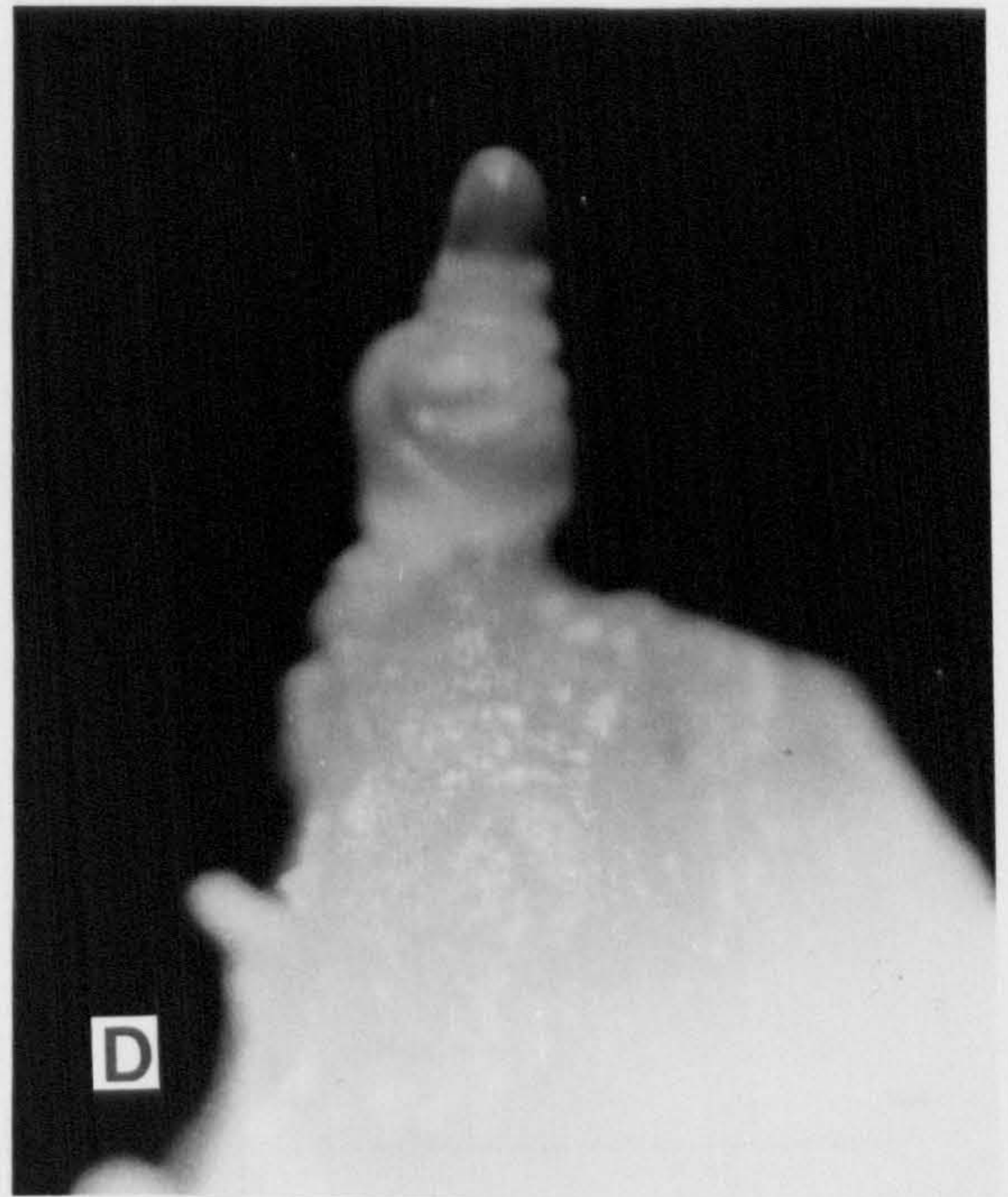
Control - 7 days.



Treated - 7 days.



Control - 14 days.



Treated - 14 days.

Plate 3.2: Shoot meristems of barley, 7 or 14 days after the application of  $20 \text{ gha}^{-1}$  triasulfuron at the 3-fully unfolded leaf stage. Scale bar  $\text{—|—} = 0.2 \text{ mm}$ .



Table 3.26: Number of primordia on shoot meristem of barley treated at 4-fully unfolded leaf stage.

Time after treatment	Herbicide dose (gha <sup>-1</sup> )	
	Control - 0	30
7 days	9.0	9.3
14 days	11.7	11.3
21 days	13.3	13.3
28 days	14.3	14.7

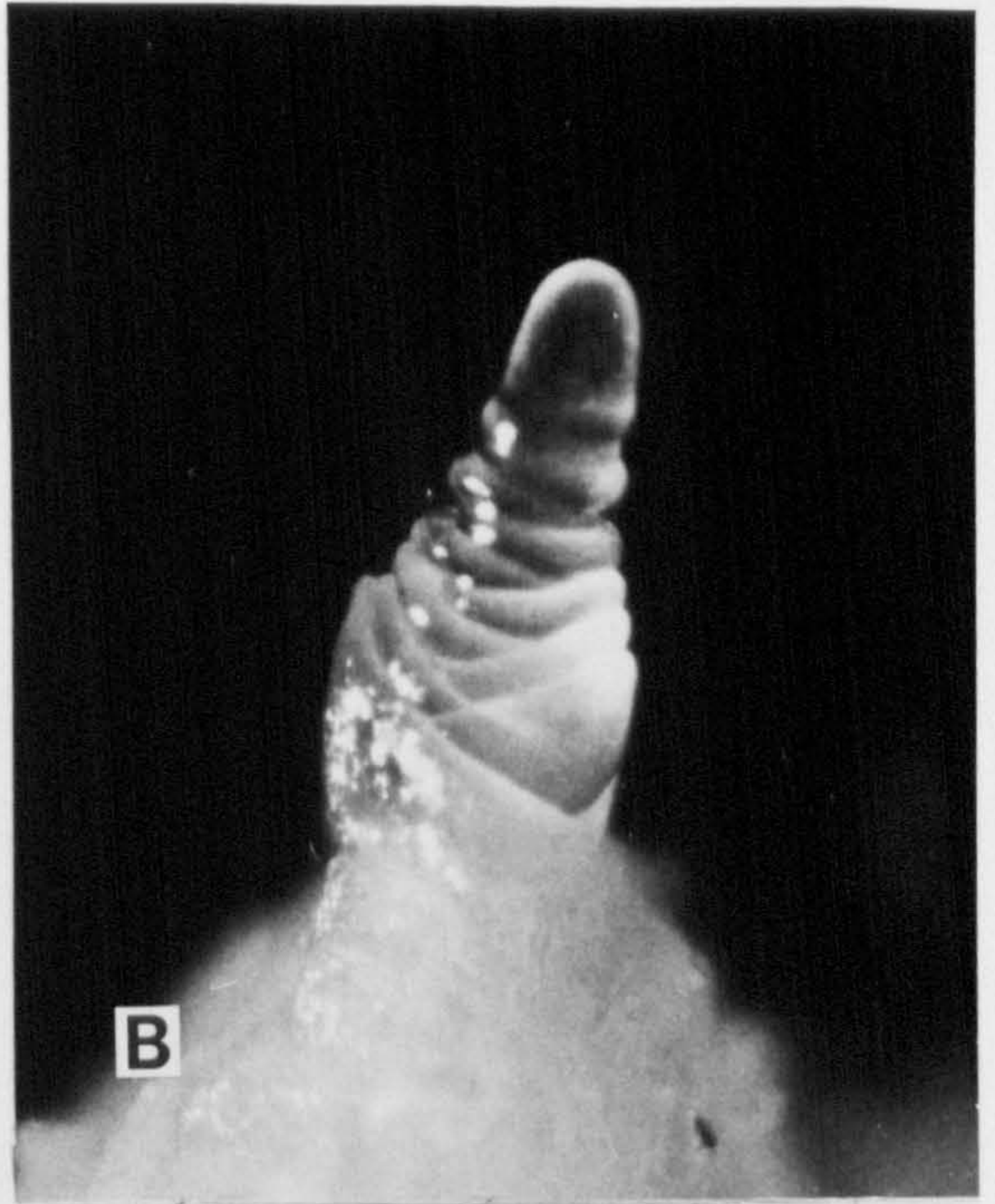
Table 3.27: Length (mm) of shoot meristem of barley treated at 4-fully unfolded leaf stage.

Time after treatment	Herbicide dose (gha <sup>-1</sup> )	
	Control - 0	30
7 days	0.61 ± 0.05	0.60 ± 0.07
14 days	0.77 ± 0.06	0.75 ± 0.03
21 days	0.83 ± 0.07	0.81 ± 0.02
28 days	0.85 ± 0.05	0.86 ± 0.03

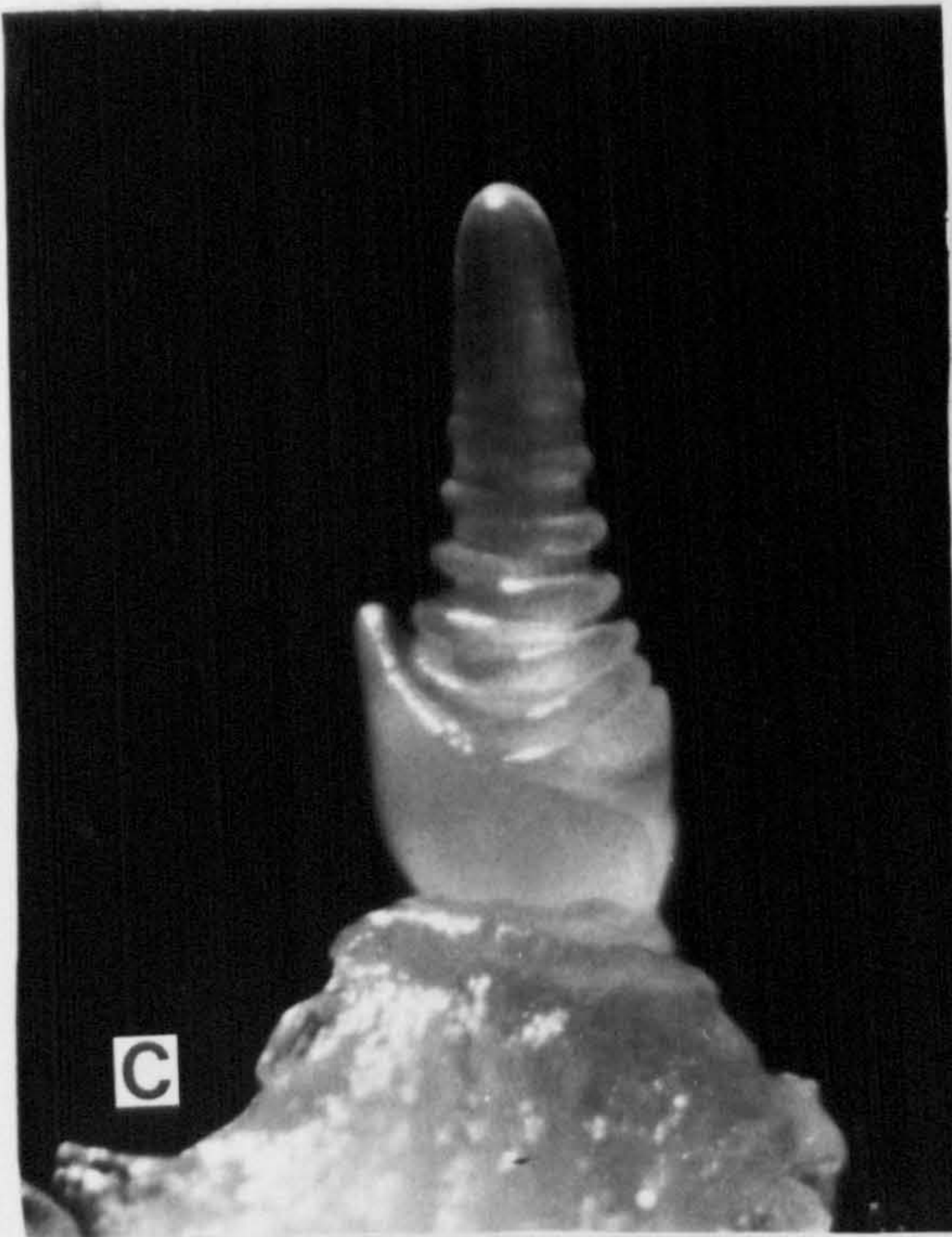




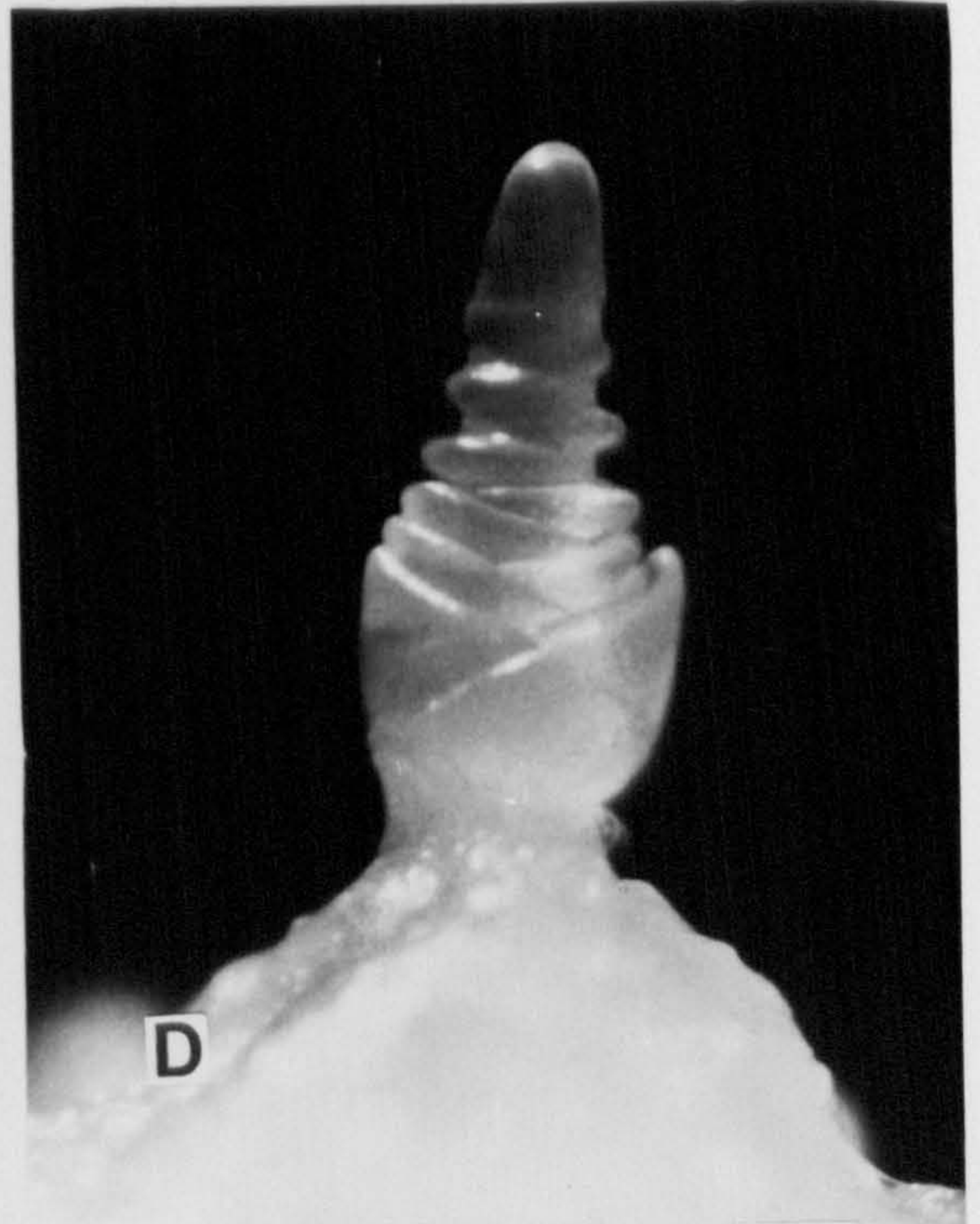
A  
Control - 7 days.



B  
Treated - 7 days.



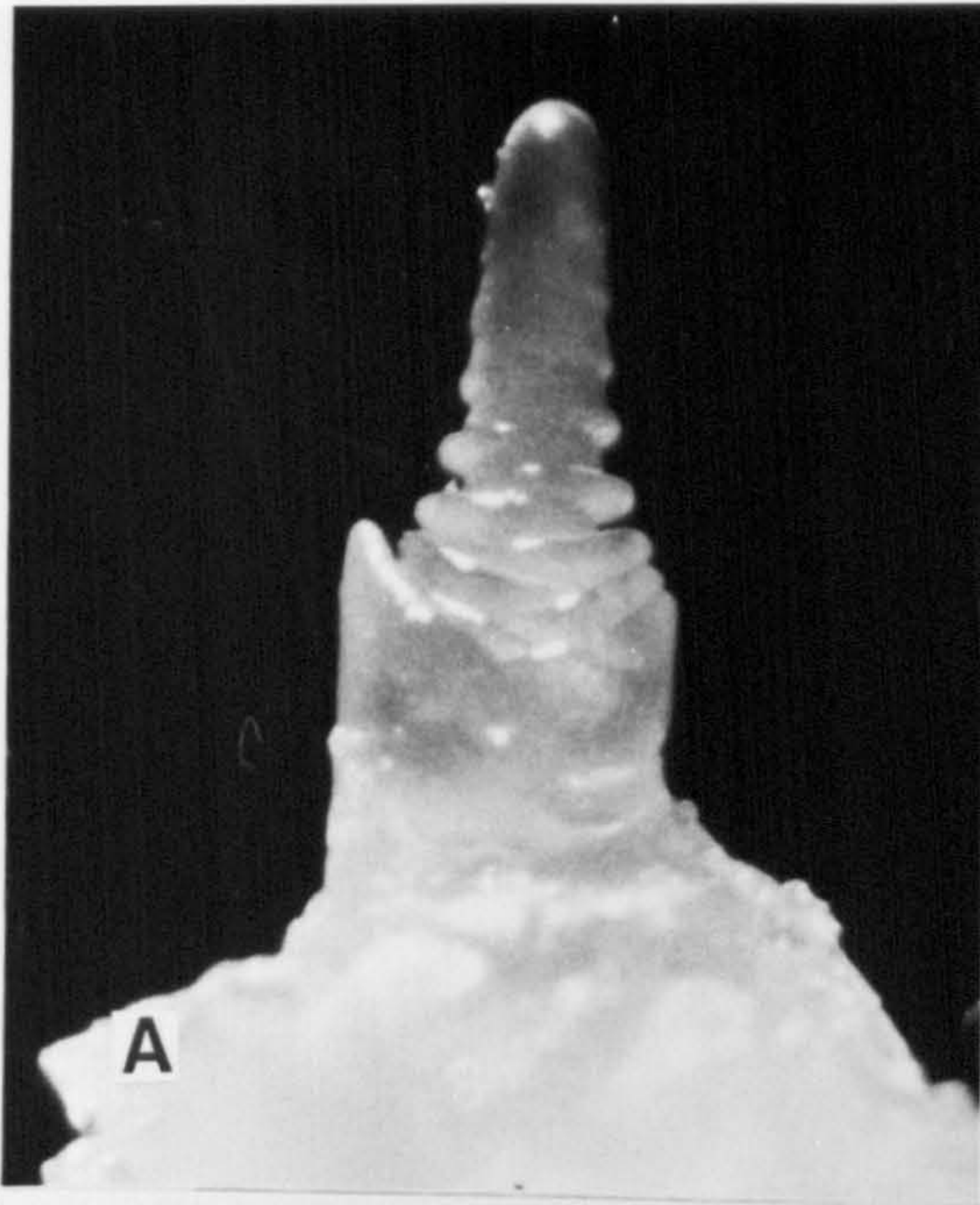
C  
Control - 14 days.



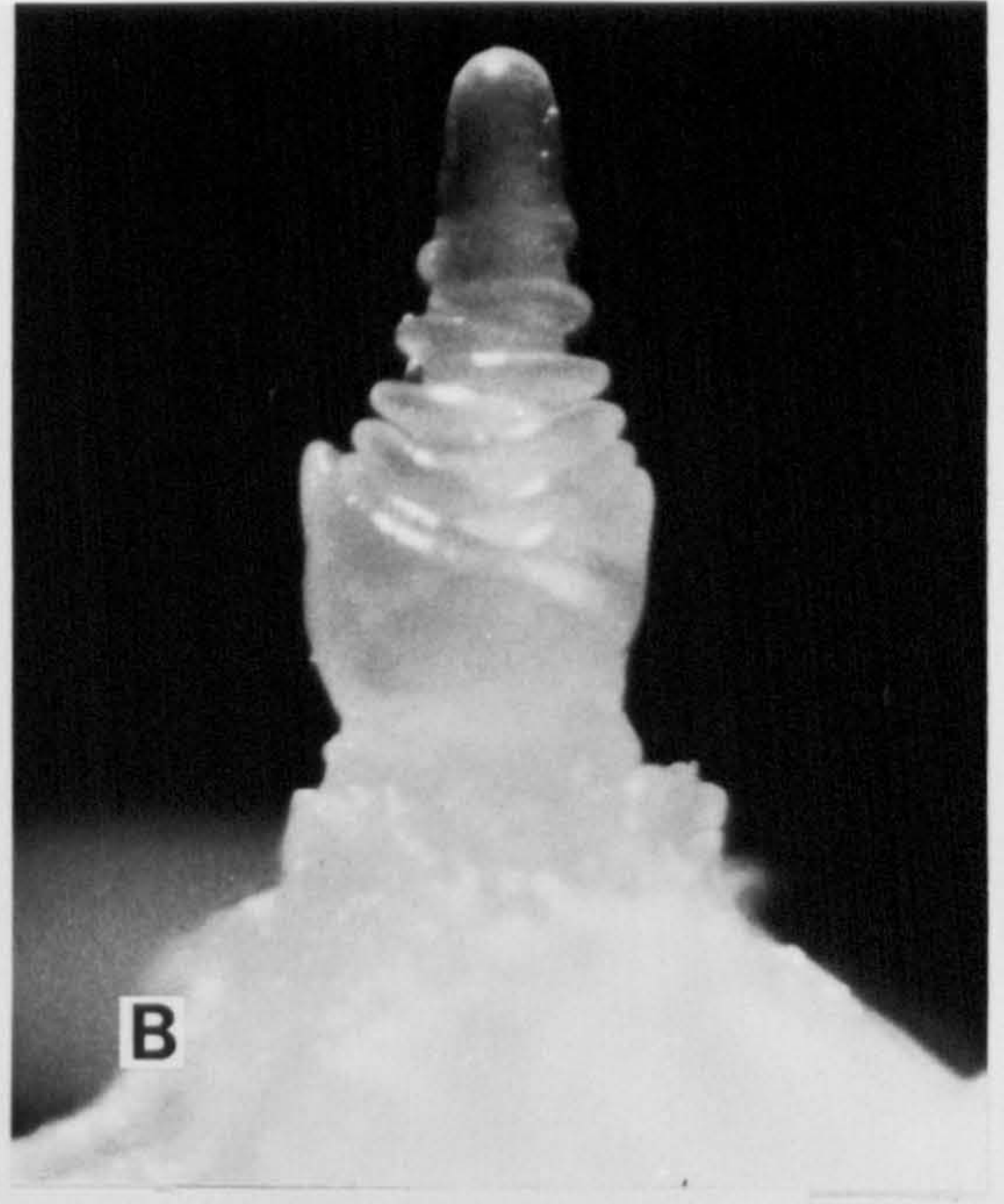
D  
Treated - 14 days.

Plate 3.3: Shoot meristems of barley, 7 or 14 days after the application of  $30 \text{ gha}^{-1}$  triasulfuron at the 4-fully unfolded leaf stage. Scale bar  $\text{—} = 0.2\text{mm}$ .

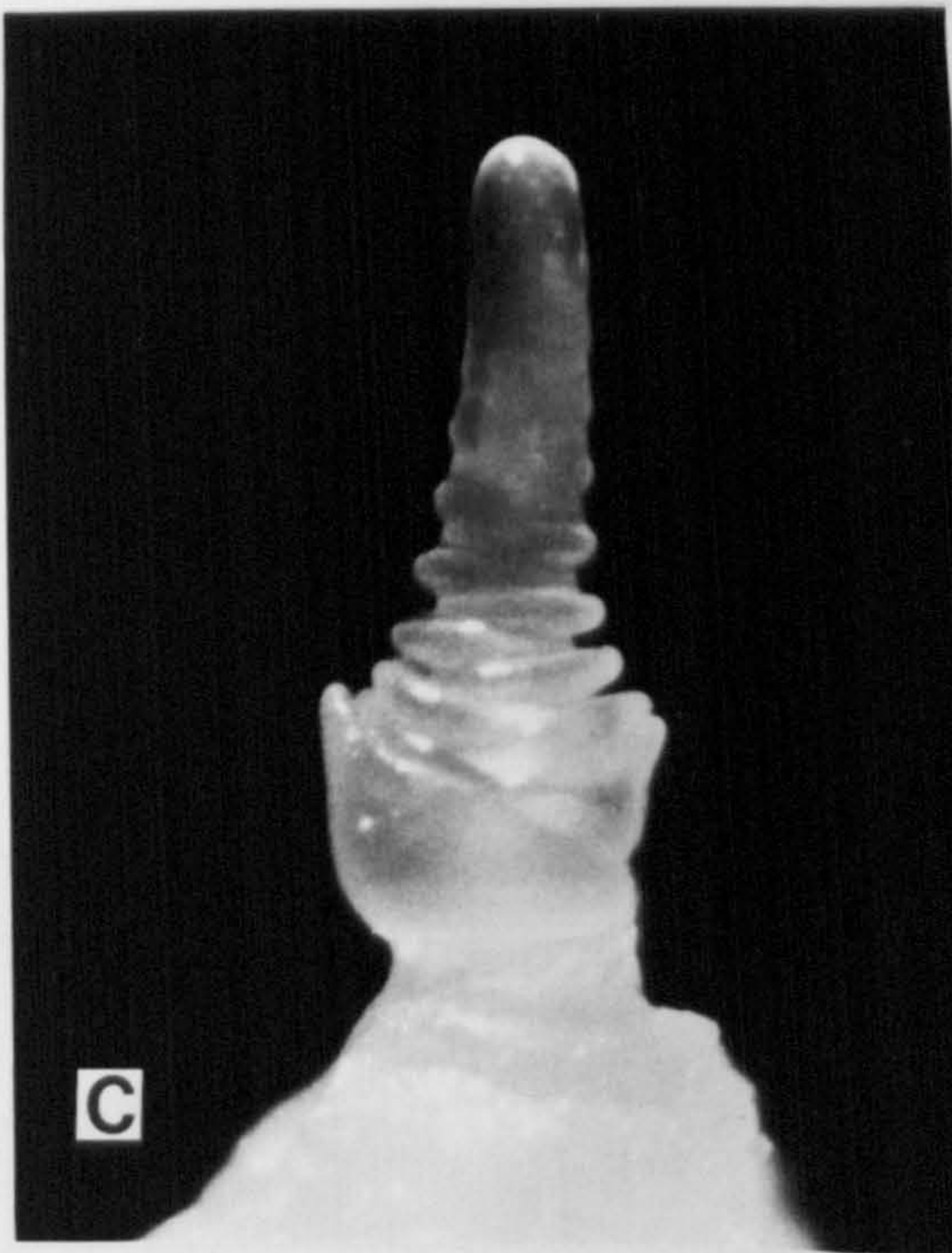




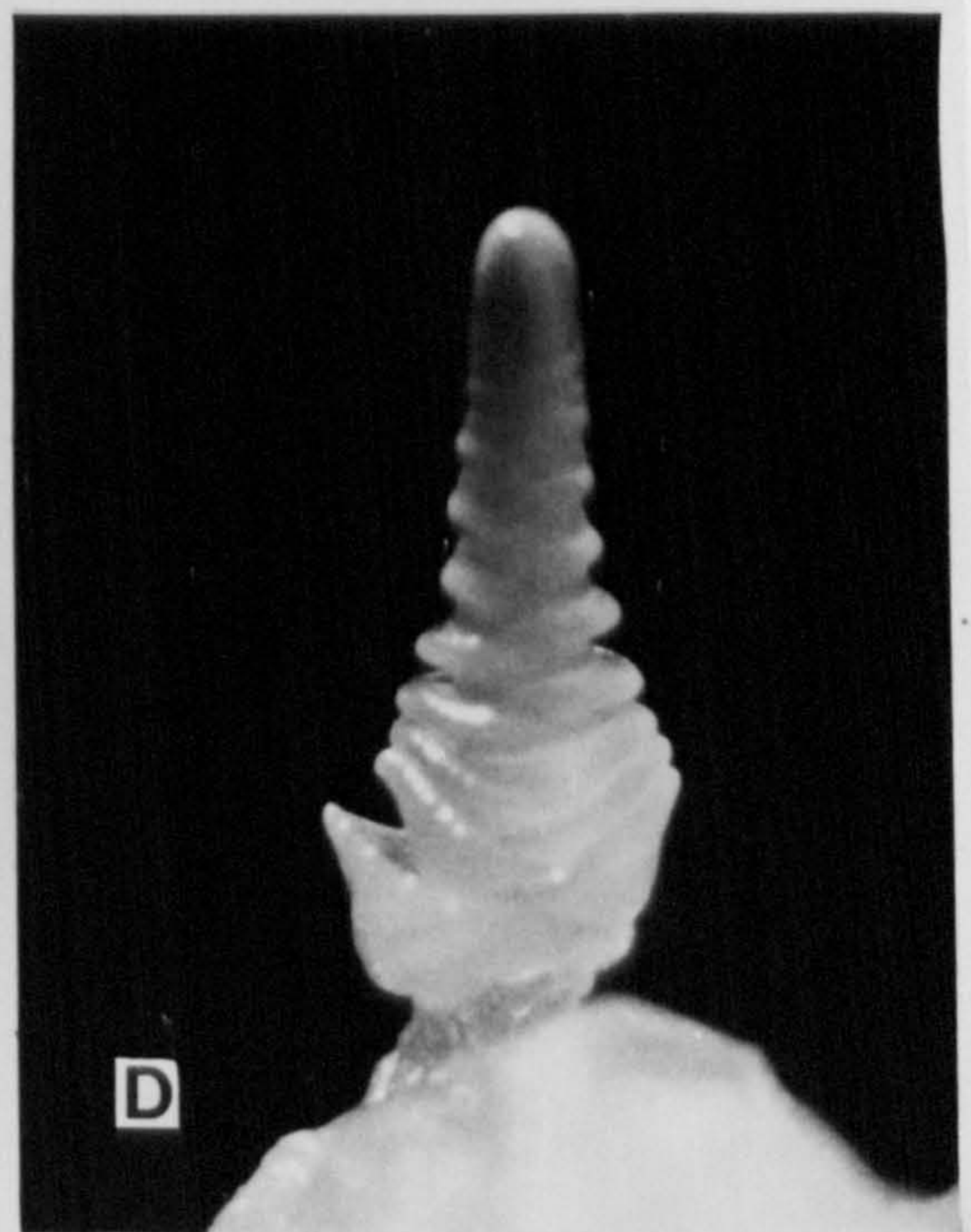
A  
Control - 21 days.



B  
Treated - 21 days.



C  
Control - 28 days.



D  
Treated - 28 days.

Plate 3.4: Shoot meristems of barley, 21 or 28 days after the application of  $30 \text{ gha}^{-1}$  triasulfuron at the 4-fully unfolded leaf stage. Scale bar  $\text{—|—|} = 0.2\text{mm}$ .



**EXPERIMENT 3.6: EFFECTS OF LATE APPLICATION OF TRIASULFURON ON THE GROWTH OF BARLEY.**

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**Materials and Methods.**

On 5 March 1987, seeds of Winter barley cv. Maris Otter were sown into 14cm diameter plastic pots filled with John Innes No.1 compost. The seedlings were thinned one week after sowing to densities of one or 15 plants pot<sup>-1</sup>.

On 30 April 1987 (8 weeks after sowing), the numbers of tillers on the single plants and on the central plant in the high density pots (15 plants pot<sup>-1</sup>) were recorded. Triasulfuron at 0, 10, 15, or 20 g ai ha<sup>-1</sup> in 200 lha<sup>-1</sup> of water was then sprayed onto the plants using an Oxford precision sprayer at a pressure of 2.1 kgcm<sup>-2</sup>. All other experimental conditions were similar to those described in Experiment 3.1. The experiment was laid out as a randomized block with five replicates.

At harvest, 12 weeks after sowing, the numbers of tillers of the single plants pot<sup>-1</sup> and of the central plants in the high density treatments were recorded and their dry weights determined after drying at 70°C for 48h. The data were processed using the same procedures as described in Experiment 3.1.

**Results.**

Late application of triasulfuron did not stimulate the production of more tillers although the numbers of tillers per plant before and after application of the herbicide were significantly higher for plants grown at low rather than high density (Tables 3.28 and 3.29).

Similarly, the herbicide treatments did not reduce the dry

weights of the plants by comparison with controls; the dry weights of individual plants grown at the lower density were significantly greater than those at the higher density (Table 3.30).



Table 3.28: Number of tillers per plant of winter barley cv. Maris Otter 8 weeks after sowing (prior to triasulfuron application).

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Plant density		Mean
	One plant per pot	15 plants per pot	
Control - 0	50.6	6.2	28.4
10	50.0	6.4	28.2
15	39.6	6.4	23.0
20	50.2	5.8	28.0
Mean	47.6	6.2	

Table 3.29: Number of tillers per plant of winter barley cv. Maris Otter 12 weeks after sowing (4 weeks after applying triasulfuron).

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Plant density		Mean
	One plant per pot	15 plants per pot	
Control - 0	67.0	6.6	36.8
10	64.4	6.6	35.5
15	54.6	6.4	30.5
20	58.8	5.8	32.3
Mean	61.2	6.4	

Table 3.30: Dry weight (g) per plant of winter barley cv. Maris Otter 12 weeks after sowing (4 weeks after applying triasulfuron).

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Plant density		Mean
	One plant per pot	15 plants per pot	
Control - 0	26.1	2.3	14.2
10	28.0	2.1	15.0
15	24.8	2.3	13.5
20	25.0	2.1	13.6
Mean	26.0a	2.2b	

Values sharing a common letter do not differ significantly at  $P < 0.05$ .

**EXPERIMENT 3.7: EFFECTS OF TRIASULFURON ON GROWTH AND YIELD OF SPRING BARLEY CV. EGMONT.**

---

**Materials and Methods.**

On 19 September 1986, three seeds of spring barley cv. Egmont were sown into each of 40, 21cm diameter plastic pots containing John Innes No.1 compost. The seedlings were thinned to one per pot a week after sowing and were sprayed using an Oxford precision sprayer at a pressure of  $1.4 \text{ kg cm}^{-2}$  with solutions of triasulfuron at the rates of 0, 10, 15 or  $20 \text{ g ai ha}^{-1}$  in  $200 \text{ l ha}^{-1}$  of water when they had an average of two fully expanded leaves (ca. 2 weeks after sowing). The experiment was designed as a randomized complete block with ten replicates and kept in a greenhouse with temperatures of  $15 \pm 2^\circ\text{C}$  (day) and  $10 \pm 2^\circ\text{C}$  (night) and a minimum daylength of 16h supplemented by 400W High pressure sodium lamps. Watering was by overhead irrigation using a watering can. Weekly records were taken of the appearance and identity of tillers on five randomly selected replicate plants and these were harvested 6 weeks after sowing when the dry weights of main shoot and of tiller groups [viz; primary and secondary tillers of T1, T2, T3, TALL (higher order tillers) and CT (coleoptile tillers) ] were determined. Long Ashton nutrient solution was applied to the remaining five replicates and these were allowed to grow to maturity.

At harvest, 6 months after sowing, the numbers of tillers, ears, grains per plant and grains per ear were recorded. Dry weights of grains and straw (leaves plus stem) per plant were determined after drying in an oven at  $70^\circ\text{C}$  for 5 days. The data were analysed using the Genstat statistical package.



## Results.

By the time of the first harvest, 6 weeks after sowing, slightly higher numbers of leaves and of tillers appeared to have been produced at the dose of  $10 \text{ gha}^{-1}$  than in the other treatments including the control (Table 3.31). The dry weights of the main shoot, groups of T1, T2, T3, CT and TALL (other tillers) were little affected by the herbicide at  $10 \text{ gha}^{-1}$  but at  $20 \text{ gha}^{-1}$  the dry weights were significantly reduced below those of the controls. There were some reductions in dry weights of some tillers (T1, TALL) in plants treated at  $15 \text{ gha}^{-1}$ . Significantly lower above-ground dry weights were obtained at herbicide doses of 15 and  $20 \text{ gha}^{-1}$  than from control plants. Most of the treated plants produced coleoptile tillers; these were absent in the control plants (Table 3.32).

At the final harvest, total numbers of tillers, ears, grains, grains per ear, dry weight of grains and mean dry weight per grain of the treated plants were not significantly different from those of the control. The dry weights of straw were however significantly lower than that of the control at all doses of the herbicide. The harvest index was slightly but not significantly higher for the plants that had been treated with the herbicide than it was in the control plants (Table 3.33).

Table 3.31: Number of leaves and tillers of spring barley cv. Egmont seedlings 6 weeks after sowing. (Results are expressed as percentage of control values).

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Leaves per plant	Tillers per plant
Control - 0	100.0	100.0
10	120.0	127.8
15	104.6	99.1
20	105.3	98.3

Table 3.32: Dry weights (g) of main shoot and tillers 6 weeks after sowing.

Herbicide <sub>1</sub> dose(gha <sup>-1</sup> )	Main shoot	Coleoptile tiller	First tiller	Second tiller	Third tiller	Other tillers	Total
Control-0	0.95a	-	1.60a	1.11a	0.65a	0.62a	4.97a
10	0.86ab	0.88	1.07ab	0.80ab	0.59ab	0.64a	4.84a
15	0.49ab	0.41	0.80b	0.70ab	0.45ab	0.29b	3.14b
20	0.41b	0.67	0.79b	0.60b	0.35b	0.21b	3.03b

Values in a column sharing a common letter do not differ significantly at  $P < 0.05$ .

Table 3.33: Growth and yield parameters of spring barley cv. Egmont 6 months after sowing.

Growth and yield parameters	Herbicide dose (gha <sup>-1</sup> )				HSD
	Control-0	10	15	20	
Number of tillers plant <sup>-1</sup>	77.0	63.3	60.0	65.0	21.3
Number of ears plant <sup>-1</sup>	60.7	51.3	46.0	49.0	19.8
Number of grains plant <sup>-1</sup>	1162.0	1014.0	824.0	841.0	356.0
Number of grains ear <sup>-1</sup>	19.1	19.8	17.9	17.2	2.9
Total weight of grains(g) plant <sup>-1</sup>	47.3	44.9	35.5	39.4	13.1
Hundred grain weight (g) plant <sup>-1</sup>	4.69	4.76	4.72	4.98	0.9
Dry weight of straw (g) plant <sup>-1</sup>	113.5a	81.7b	72.8b	73.5b	25.4
Harvest index	0.29	0.36	0.33	0.35	

Values sharing a common letter do not differ significantly at P < 0.05.



**EXPERIMENT 3.8: EFFECTS OF TRIASULFURON ON GROWTH AND YIELD OF SPRING BARLEY CV. TRIUMPH.**

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**Materials and Methods.**

Another experiment similar to Experiment 3.7 was set up on 28 April 1987. Two seeds of spring barley cv. Triumph were sown into each of twenty 14cm diameter plastic pots containing John Innes No.1 compost. The seedlings were thinned to one per pot a week after sowing and were sprayed using an Oxford precision sprayer at a pressure of  $1.4 \text{ kg cm}^{-2}$  with solutions of triasulfuron at the rates of 0, 10, 15 or 20 g ai ha<sup>-1</sup> in 200 lha<sup>-1</sup> of water when they had three fully expanded leaves (2 weeks after sowing). All experimental procedures and conditions were similar to those of Experiment 3.7 except that the present experiment was made in a greenhouse with temperatures of  $20 \pm 2^{\circ}\text{C}$  (day) and  $15 \pm 3^{\circ}\text{C}$  (night). The experiment was designed as randomized block with five replicates.

Weekly records of leaves and tiller numbers of seedlings were taken up to the end of the fifth week after sowing. Long Ashton nutrient solution was applied to the pots 8 weeks after sowing. At harvest 15 weeks after sowing, the numbers of tillers, ears, grains per plant and grains per ear were recorded. Dry weights of grains and straw (leaves plus stem) per plant were determined after drying in an oven at  $70^{\circ}\text{C}$  for 5 days.

**Results.**

Five weeks after sowing, the number of tillers was slightly lower than those of the control at all doses but leaf production was marginally higher at 15 gha<sup>-1</sup> than in the other treatments including

the control (Table 3.34).

At the final harvest, the total numbers of tillers, ears, grains, mean dry weight per hundred grains were not significantly different from those of the control. Total grain dry weight per plant were slightly lower at the highest dose but dry weight of straw per plant were significantly reduced below that of the control at 15 and 20  $\text{gha}^{-1}$ . The harvest index was slightly but not significantly, higher for the plants that had been treated with the herbicide than it was with the control plants (Table 3.35).

Table 3.34: Number of leaves and tillers of spring barley cv. Triumph seedlings 5 weeks after sowing. (Results are expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Leaves per plant	Tillers per plant
Control - 0	100.0	100.0
10	92.8	92.0
15	105.5	96.6
20	92.5	88.5

Table 3.35: Growth and yield parameters of spring barley cv. Triumph 15 weeks after sowing.

Growth and yield parameters	Herbicide dose (g ha <sup>-1</sup> )				HSD
	Control-0	10	15	20	
Number of tillers plant <sup>-1</sup>	30.8	28.6	26.8	23.8	7.1
Number of ears plant <sup>-1</sup>	28.4	27.0	25.0	22.4	6.7
Number of grains plant <sup>-1</sup>	520.0	530.0	496.0	450.0	86.1
Number of grains ear <sup>-1</sup>	18.3	19.3	19.8	20.1	2.2
Total weight of grains (g) plant <sup>-1</sup>	25.3ab	25.8a	24.0ab	22.0b	3.7
Hundred grain weight (g) plant <sup>-1</sup>	4.99	4.95	4.96	5.03	0.4
Dry weight of straw (g) plant <sup>-1</sup>	27.3a	25.6ab	22.6b	21.5b	4.4
Harvest index	0.48	0.50	0.52	0.51	

Values in a row sharing a common letter do not differ significantly at P < 0.05.



## DISCUSSION.

Triasulfuron applications to barley seedlings at the 2- or 3-leaf stages stimulated tiller bud outgrowth and this effect was usually accompanied by reductions in growth of the main shoot and the lengths of leaf blades and sheaths. However, shoot dry weights of seedlings were not always reduced by the herbicide treatments (see Experiments 3.1, 3.2, 3.3 and 3.4). These results confirm similar observations made in Experiment 2.3 of Chapter 2.

The development of lateral buds is under the control of the main shoot apex (Sachs and Thimann, 1967; Phillips, 1975; Harrison and Kaufman, 1982) and physical damage to, or removal of, the shoot apex usually leads to prolific outgrowth of tiller buds in cereals and grasses (Leopold, 1949; Laidlaw and Berrie, 1974). Several workers have proposed that growth hormones are responsible for maintaining apical dominance at the shoot apex and some studies have confirmed that a balance in the levels of endogenous auxin and cytokinin is the most important factor controlling the outgrowth of tiller buds (Sachs and Thimann, 1964; Sachs and Thimann, 1967; Phillips, 1975; Isbell and Morgan 1982). Others have proposed that competition within the plant between the shoot apex and lateral buds for a limited supply of nutrients used in growth determines whether or not a bud will grow and develop into a tiller (Phillips, 1969; McIntyre, 1977). On the other hand, Aspinall (1961) has suggested that both apical auxin and nutrients may be involved in controlling the growth of tiller buds. However, studies on the partition of assimilates in Lolium perenne and Lolium temulentum plants showed that apical meristems require only small quantities of assimilates for continued growth (Ryle,

1970) and as a result a similar demand from axillary buds should not materially affect the supply of nutrients (Jewiss, 1972).

Pallet (1984) applied difenzoquat at rates of one (recommended field rate), 2 or 4 kg ai ha<sup>-1</sup> to seedlings of three winter wheat cultivars: Hobbit, Maris Huntsman and Score, at the 3- to 4-leaf stage. With the 1 kg ha<sup>-1</sup> treatment, tiller numbers were increased by 180, 130 and 30% for Score, Maris Huntsman and Hobbit respectively, 6 weeks after application. In addition, the herbicide reduced main shoot height by 80, 35 and 8% in Score, Hobbit and Maris Huntsman respectively and lamina lengths and foliage dry weights were reduced in all the three cultivars. Similar results were reported in earlier studies where post-emergence applications of difenzoquat increased the number of tillers and reduced main shoot height, lamina lengths and dry weight of the susceptible Sicco cultivar of wheat but not of the tolerant Butler cultivar (Pallet and Caseley, 1980). In both studies, the responses were attributed to differences in the inhibition of DNA synthesis in the apical meristem. Bakar (1980) obtained increased numbers of diminutive tillers in oat seedlings following foliar application of ethofumesate {(+)-2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulfonate} at a rate of 20 µg ai cm<sup>-3</sup> and suggested that an interruption of apical growth, in particular, the process of cell division might have led to the response. In the present studies, it is possible that triasulfuron might have disrupted some essential physiological processes at the shoot apex thereby slowing down the growth of the main shoot which eventually led to loss or reduction of apical dominance and thereby, secondarily, to the growth of tiller buds. With the growth of axillary tillers, competition for nutrients might have developed to



the extent that the growth rate of the main shoot was retarded.

Attempts were made in Experiment 3.5 to elucidate the role of the apical meristem in inducing tillering in barley plants treated with triasulfuron. Herbicide at concentrations of 20 or 30g ai ha<sup>-1</sup> did not affect the number of primordia on the shoot meristems but length of the meristem was slightly reduced 24h after treatment although this effect had disappeared by the end of the fourth week after treatment (Tables 3.24, 3.25, 3.26 and 3.27). No morphological abnormality was observed on the treated shoot meristems. These results suggest that the multi-tillering response of barley seedlings to triasulfuron was not due to any physical damage to the shoot apex. Baur et al. (1977) found that the application of sub-lethal doses of glyphosate to sorghum and wheat stimulated the production of basal buds at 21 and 27°C respectively. However, these authors were unable to relate the stimulation of bud outgrowth to any physical damage to the shoot apex since histochemical studies revealed that the apical meristem in treated plants was viable and bud release was not due to the death of, or visible injury to the shoot apex. Further studies led to the conclusion that the outgrowth of basal buds induced by glyphosate in sorghum was related to the auxin-cytokinin balance in the base of the stem (Baur, 1979). Although, in the present studies, only small differences were observed between the lengths of the treated and control meristems, and only for a short time, it is possible that such apparently small effects reflect disruption of some physiological or biochemical events at the shoot apex.

Generally, winter barley cultivars produced more leaves and tillers than did cultivars of spring barley (Experiment 3.1). Winter



cereal cultivars are known to produce more leaves than spring cultivars and therefore have more axillary sites for buds to develop into tillers (Jewiss, 1972; Kirby and Appleyard, 1984). In addition, different cultivars of barley usually exhibit differences in maximal and final tiller number (Thorne, 1962; Kirby, 1967) and this was generally evident in the present studies (Table 3.2). The differential cultivar responses with respect to leaf and tiller numbers did not appear to be reflected in responses to the herbicide treatments.

In Experiment 3.2 and 3.3, tillering was suppressed at high density (Tables 3.4, 3.5, 3.6, 3.7, 3.8, 3.9 and Fig. 3.1). The most plausible explanation appears to be competition for nutrients, water or light and was reflected in the lower shoot dry weights of seedlings sown at high densities as compared to those sown at low densities (Tables 3.10, 3.11 and Fig. 3.4). High plant density has been reported to have repressive effects on tillering in barley (Kirby, 1967; Kirby and Faris, 1972) and Aspinall (1961) has demonstrated that nutrient supply has a great influence on tiller number of barley. In the treatments where barley was grown at low density resources for plant growth might have been adequate enabling plants to attain maximal growth whereas interplant competition might have occurred in the densely sown treatments resulting in reduced growth.

The stimulation of tillering by triasulfuron at the low (one plant per pot) and high (15 plants per pot) densities gave a completely different picture when the data were expressed as percentage of controls. For example, in Experiment 3.3, the number of tillers, five weeks after applying triasulfuron had increased by 7.8%

and 34.6% and leaf production by 7.4 and 35.9% at the low and the high densities respectively (Fig. 3.1 and 3.2). This suggests that some form of interaction between plant density and the herbicide might have occurred.

Late application of the herbicide to 8-week old seedlings failed to induce growth of tiller buds (Tables 3.28 and 3.29). This was probably due to the fact that at the time of application, reproductive growth and stem elongation had begun: cessation of tillering usually coincides with this stage of growth (Aspinall, 1961; Jewiss, 1972). Coupled with the evidence from Chapter 2 that pre-emergence applications of triasulfuron led to reductions in tillering, these results lend further support to the general conclusion that the developmental stage of plants at the time of application of herbicides determines its precise effect on growth and development (Cartwright, 1976; Van Andel et al., 1976).

Leaf and tiller production and shoot dry weight were unrelated to the site of application of triasulfuron (Tables 3.18, 3.19, 3.21 and 3.22). No differences in leaf and tiller numbers or dry weights were recorded between applications to either the distal or basal parts of leaves. This result did not follow the generally accepted view of increased effects of herbicides when they are applied to the base of the lamina. Holly (1960) for example, showed that the efficacy of barban on wild oats was increased when the herbicide was applied to the base of the first leaf. Thompson et al. (1970) reported an improved performance of atrazine when the herbicide was applied to the base of the lamina of leaves of maize. Coupland et al. (1978) found an increase in the efficacy of barban and difenzoquat applied



to the lamina base of wild oat plants but application of  $1 \mu\text{g}\mu\text{l}^{-1}$  glyphosate to the lamina base of Agropyron repens at the 3-fully unfolded leaf stage did not improve the performance of the herbicide. In all these studies, the authors assigned the increased efficacy of the herbicides to the presence of lesser amounts or different types of epicuticular wax on the basal parts of laminae as opposed to the apical parts, the proximity of the lamina base to the site of action at the growing point or the more favourable conditions of humidity in the basal areas leading to longer drying times for the spray in this region.

It was quite obvious from Experiment 3.4 that triasulfuron has high activity through the soil: indeed dry weights of seedlings were reduced only in the soil and in the foliage plus soil treatments and not in the foliage treatments. It can be argued that triasulfuron would have been available for uptake in the soil for a much longer period than on foliage thus ensuring continuous absorption by the roots and consequently, much higher accumulation at the growing point. Barley has been reported to be only marginally tolerant to soil residues of chlorsulfuron (Foley, 1986). It is also possible that slower metabolism by the roots than by the foliage or shoots might have allowed greater accumulation of the herbicide at the growing point. For example, it has been reported that the shoots of barley and wheat metabolize DPX-A7881 {methyl 2-[(4-ethoxy-6-methylamino-1,3,5-triazine-2-yl)=carbamoyl sulphanoyl benzoate} much more rapidly than do the roots where the enzymes necessary for detoxification are apparently lacking (Hutchison et al., 1987). Foley (1986) applied  $^{14}\text{C}$ -chlorsulfuron to the roots of wheat and barley seedlings and found after 24h that metabolism of the herbicide



absorbed by the roots was slower in barley (77%) than in wheat (89%) although he concluded that this variation may not be enough to account for the greater sensitivity of barley roots exposed to chlorsulfuron.

Dry weight of straw was the only yield component which was consistently reduced in Experiments 3.7 and 3.8. Grain yield of barley and other yield components were not significantly affected by the herbicide (Tables 3.33 and 3.35). These results are in agreement with those made by Amrein and Gerber (1985) who found no yield reductions in six field trials of winter barley and two of winter wheat following the application of 15 or 30g ai ha<sup>-1</sup> triasulfuron on weed-free trial sites. In the present studies single plants were raised in large pots and were supplied with nutrients. Tiller production was generally high in both treated and control plants and there was very little evidence of stimulation of tiller buds by the herbicide (Table 3.31 and 3.34). It is possible that the rapid rate of growth of the plants might have led to a dilution of the concentration of the herbicide or its breakdown as the plants matured thus causing less effects.

Evidence from the studies in the present chapter and those of Chapter 2 have consistently shown that the stimulation of bud outgrowth by triasulfuron occurs only during the early post-emergence stages of growth of barley and this response which appeared to be temporary may disappear before the crop matures without any significant influence on grain yield.

CHAPTER 4.

MOBILITY IN SOIL.

## INTRODUCTION.

Triasulfuron was developed to provide pre-emergence and post-emergence weed control against a number of broad-leaved weeds and some grasses in crops of wheat and barley. However, because pre-emergence applications are less tolerated by barley than by wheat, the recommended time of application proposed by the manufacturer is post-emergence between the 2 and 3-leaf growth stages of the crop (Amrein and Gerber, 1985 ;Anon., 1986). The results of the experiments reported in Chapter 2 and Chapter 3 (Experiment 3.4) showed that entry through the soil plays a key role in herbicidal activity following pre-emergence and early post-emergence applications. Because of this high activity of triasulfuron in the soil, it becomes essential to understand its behaviour in the soil as well as its interactions with soil components.

The extent of injury to plants growing in soil containing herbicide residues may depend on where in the soil profile the herbicide is located. Many soil acting herbicides can be used selectively by sowing crop seeds relatively deeply in the soil with a layer of untreated soil separating them from the herbicide layer higher in the profile. Most weed seeds are located near the surface and 95% of the weed seeds that give rise to seedlings are in the top 2.5cm of the soil. This mechanism, which is often referred to as depth protection (Gerber and Guth, 1973), positional selectivity (Dunham and Crawford, 1973) or spatial selectivity (Riley and Morrod, 1976) is designed to avoid the penetration of the herbicide in significant concentrations into the soil zones that are critical to



the safety of the crop. The success of this type of physical selectivity of herbicides between crops and weeds depends to a large extent on the water solubility of the herbicide, amount and intensity of rain or irrigation water and the texture and structure of the soil (Robinson, 1965; Kratky and Warren, 1971; Toth and Milham, 1975; Walker, 1980).

It is generally accepted that organic matter is the most important soil property governing the activity of most herbicides in soil although clay content and other soil components are also important especially for some herbicides (Sheets, 1958; Upchurch and Mason, 1962; Grover, 1966; Bailey et al., 1968; Weber, 1970; Rahman and Matthews, 1979; Kozak and Weber, 1983). Many researchers have reported a high negative correlation between herbicide activity and soil organic matter content (Grover, 1966; Weber, 1970; Harrison et al., 1976; Mapplebeck and Waywell, 1983; and Okafor et al., 1983b) and in all these studies, adsorption of herbicides onto organic colloids is believed to be the principal reason for the reduction in bioactivity. Mersie and Foy (1985) and Anderson and Humburg (1987) have reported an inverse relationship between chlorsulfuron {2-chloro-N-[[ (4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl] benzenesulfonamide} phytotoxicity and soil organic matter but such a relationship has not yet been examined for triasulfuron.

Leaching is one of the important factors affecting the performance of soil applied herbicides. The effectiveness of a soil applied herbicide may increase as it is leached from the soil surface into the soil zone where weed seeds are germinating (Anderson et al., 1968). However, excessive leaching due to heavy rainfall or the application of large amounts of irrigation water may modify

performance when the herbicide is leached to such a depth that uptake by weed seedlings does not occur or is reduced or prevented because of dilution through the soil profile (Ashton, 1961; Nishimoto et al., 1969) or the herbicide is moved in high concentrations to the zone of uptake by the crop where damage to the crop may occur. (Klingman, 1961; Anderson et al., 1968). Studies on the movement of herbicides in soil are therefore useful for understanding the performance of soil applied herbicides against weeds, safety to the crop and for the evaluation of the potential risks of polluting ground and surface water (Leistra, 1980). In addition, leaching data are useful for predicting and understanding the behaviour of herbicides under various rainfall conditions and in different types of soil (Gray and Weierich, 1968).

The movement of herbicides has been studied using modified thin layer chromatographic techniques such as the soil thin layer (Helling and Turner, 1968) the soil thick layer techniques ( Gerber et al., 1970) or modified soil columns originally designed to measure water and nutrient movement through soil ( Gardner and Brooks, 1957; Stockinger et al., 1965 and Williams, 1968). Wu and Santelmann (1975) used the three different soil leaching techniques to study the movement of four herbicides in soil and found them to be comparable. However among the three methods, leaching of herbicides through soil columns is the one which best approximates to actual field conditions and it allows the investigator to vary the amounts and increments of water additions over different time periods (Weber and Whitacre, 1982).

Herbicides may be moved in any direction depending on the flow of



water (Ashton, 1961; Harris, 1966). In soil leaching column studies, water may be added to the top of a vertically held column and carried through the soil by gravity (Majka and Lavy, 1977; Weber and Peeper, 1982; Weber et al., 1986), added to the bottom of the column and subirrigated by capillary forces (Harris, 1967, 1969) or added under pressure to a horizontal column (Davidson et al., 1968). It has been suggested that the amount of water entering the soil as a function of time is the most important aspect of water and chemical movement in soil (Stockinger et al., 1965). This was demonstrated in an experiment where large amounts of water (51cm) applied continuously under saturated-flow conditions over a short period of time (3 hours) moved 81% of applied terbuthiuron { N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N'-dimethylurea } through a soil column (30cm long). Application of the same total amount of water in small amounts (1.2cm day<sup>-1</sup>) under unsaturated flow conditions over a period of 40 days resulted in only 2.5% of the herbicide passing through the whole 30cm column (Weber and Whitacre, 1982).

Whether or not a herbicide is leached out of the soil in drainage water and the rate at which this occurs depends on several factors other than the amount and intensity of rainfall or the rate of application of irrigation water (Roberts, 1982). Several of these factors were identified in the General Introduction (Chapter 1). It has been shown in many investigations that the organic matter content of the soil appears to be the most consistent factor affecting herbicide movement because of its high capacity to take herbicides out of the soil solution (Jordan and Day, 1962; Harris and Warren, 1964; Harris and Sheets, 1965; Lambert et al., 1965; Roberts and Wilson, 1965; Eshel and Warren, 1967; Gray and Weierich, 1968; Smith



and Meggit, 1970a; Mersie and Foy, 1986). Adsorption of the herbicide molecules by organic colloids is believed to be responsible for retarding the movement of herbicides down the soil profile since only that fraction which is unadsorbed is free to move with the soil water (Roberts, 1982). Other factors such as soil pH (Bailey et al., 1968; Best and Weber, 1974; Mersie and Foy, 1986; Nicholls, 1988) and the solubility of the herbicide (Harris, 1967; Gray and Weierich, 1968; Rodgers, 1968; Wu and Santelmann, 1975; Weber and Whitacre, 1982) have been found to influence the mobility of some herbicides in soil. Nicholls (1988) has however pointed out that lipophilicity is the most important physico-chemical property influencing the movement of non-ionised pesticides through the soil whilst water solubility is usually only an important factor in leaching for a few moderately polar solids with high melting points.

The mobility in soil of some of the sulfonylurea herbicides has been studied and the results show a range from those with low mobility and tight soil binding like bensulfuron methyl to those that are relatively mobile like chlorsulfuron and metsulfuron methyl {2-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoic acid} (Beyer et al., 1987). In several of these studies soil pH and organic matter were found to be the principal factors influencing the mobility of the sulfonylurea herbicides, with mobility generally increasing with increasing soil pH and decreasing organic matter (Nicholls and Evans, 1985; Fredrickson and Shea, 1986; Mersie and Foy, 1986; Beyer et al., 1987).

Since triasulfuron is very active through the soil when applied pre-emergence or early post-emergence, the location and the

availability and mobility of its residues in soil are likely to be of direct relevance to its phytotoxic action. However, there is very little information in the literature on its soil activity or mobility in the soil. The first two experiments reported in the present section were designed to study the influence of placement in the soil and organic matter content on the herbicidal activity of triasulfuron whilst the remaining experiments were devoted to studies on the movement of triasulfuron in soil as influenced by time, amount and frequency of simulated rain and the organic matter content of soil.

**Materials and Methods.**

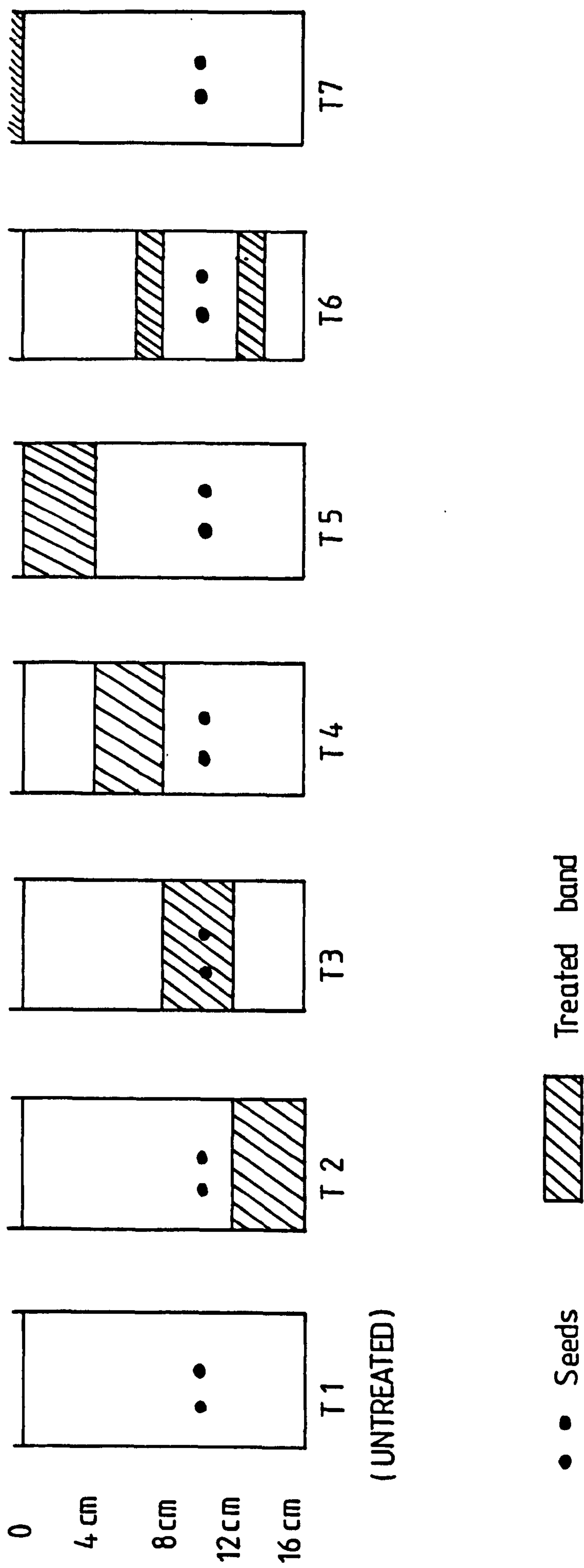
On 8 May 1986, thirty seeds each of wheat cv. Norman and barley cv. Maris Otter were sown into plastic pots (21.5cm diameter; 19.0cm depth) containing John Innes No. 1 compost. All the seeds were sown 6cm from the bottom of the pots as shown in Figure 4.1 irrespective of the depth of placement of a 4cm deep band of soil in which triasulfuron had been incorporated at a dose of 15 g ai ha<sup>-1</sup> in 200 lha<sup>-1</sup> of water. In one treatment, the 4cm band was split into two x 2cm bands of soil and placed 2cm above and below the zone where the seeds were sown and in another treatment the herbicide was sprayed onto the surface of the soil. The control pots contained untreated soil. All the seedlings had to pass through 10cm of soil to emerge. The experiment was laid out in five randomized blocks.

At harvest, six weeks after sowing, the number of emerged seedlings, the lengths of the second leaves and the numbers of leaves and tillers per pot were determined. Roots of seedlings in each pot were washed free of soil and the dry weights of roots and shoots were determined after drying at a temperature of 70°C for 48h. The data were processed by analysis of variance using the Genstat statistical package and comparison between treatment means was done by Tukey's HSD test.

**Results.**

In general, the greatest phytotoxicity of triasulfuron was observed where the seeds had been sown in the treated zone (T3) and





**FIG. 4.1:** Depth of placement of 4 cm band of soil containing Triasulfuron in soil profile.

where the seeds had been sown with treated bands 2cm above and below (T6).

In barley, significantly fewer seedlings emerged from seeds in the treated zone (T3) than in all the other treatments. Although a similar response was observed in wheat, the differences were not significant (Table 4.1). In both species, fewer leaves and tillers were produced by seedlings in T3 and T6 and the lengths of the second leaves of T3 and T6 in barley and T3 in wheat were also significantly reduced below those of the control (Tables 4.2, 4.3 and 4.4). The same trend was observed with shoot and root dry weights where shoot dry weights of both species in T3 (seeds sown in the treated zone) and T6 (bands of herbicide above and below the seeds) were significantly lower than those of the control (Table 4.5). Lower root dry weights were obtained for wheat in T3, T4 and T6 but in barley a significant reduction was obtained only in T3 (Table 4.6). There were never any significant differences between T5 and T7.

Table 4.1: Seedling emergence of wheat and barley 6 weeks after sowing. (Results are expressed as percentage of control values).

Treatments	Wheat	Barley
T1- Untreated	100.0	100.0a
T2	121.6	114.8a
T3	87.6	33.5b
T4	115.3	127.2a
T5	103.3	133.9a
T6	112.4	129.5a
T7	103.3	115.1a

Table 4.2: Number of leaves per pot of wheat and barley seedlings 6 weeks after sowing.

Treatments	Wheat	Barley
T1- Untreated	203.4a	230.6a
T2	196.2a	226.0a
T3	91.8b	42.8b
T4	184.2a	209.0a
T5	188.2a	233.4a
T6	108.8b	157.6a
T7	157.0ab	172.2a

Values in a column sharing a similar letter do not differ significantly at  $P < 0.05$ .



Table 4.3: Number of tillers per pot of wheat and barley seedlings 6 weeks after sowing.

Treatments	Wheat	Barley
T1- Untreated	48.4a	63.0a
T2	43.0ab	52.2a
T3	11.8c	9.3c
T4	38.2ab	44.4ab
T5	40.2ab	55.4a
T6	12.0c	27.0b
T7	24.8b	41.4ab

Table 4.4: Lamina length (cm) of second leaves of wheat and barley seedlings 6 weeks after sowing.

Treatments	Wheat	Barley
T1- Untreated	16.5a	17.5a
T2	16.1a	15.2ab
T3	13.2b	7.7c
T4	15.6ab	15.7ab
T5	15.3ab	16.3a
T6	15.6ab	11.9b
T7	14.8ab	14.5ab

Values in a column sharing a similar letter do not differ significantly at  $P < 0.05$ .

Table 4.5: Shoot dry weight (g) per pot of wheat and barley seedlings 6 weeks after sowing.

Treatments	Wheat	Barley
T1- Untreated	9.44a	10.11a
T2	9.37a	8.33ab
T3	2.51c	1.27c
T4	7.96ab	6.68ab
T5	8.68a	9.25a
T6	4.24b	4.77bc
T7	5.54abc	6.17ab

Table 4.6: Root dry weight (g) per pot of wheat and barley seedlings 6 weeks after sowing.

Treatments	Wheat	Barley
T1- Untreated	3.35a	2.57a
T2	2.88ab	2.45a
T3	0.75c	1.19b
T4	1.11c	1.88ab
T5	1.41bc	2.01ab
T6	0.90c	1.68ab
T7	1.34bc	1.43ab

Values in a column sharing a similar letter do not differ significantly at  $P < 0.05$ .

**EXPERIMENT 4.2: EFFECTS OF SOIL ORGANIC MATTER ON THE PHYTOTOXICITY OF TRIASULFURON.**

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**Materials and Methods.**

On 9 June 1986, 14cm diameter plastic pots were filled with either quarry sand, peat or quarry sand-peat mixtures containing 5, 10 or 20 % w/w commercial horticultural peat (pH 5.7). Twenty seeds of winter barley cv. Maris Otter were sown into the different planting media at a depth of 2cm. The soil in each pot was watered to field capacity and then maintained at between 75% and 100% field capacity throughout the period of the experiment by watering from above as necessary. Solutions of triasulfuron at doses of 0, 10, 15 and 20 g ai ha<sup>-1</sup> in 200 lha<sup>-1</sup> of water were applied pre-emergence using an Oxford precision sprayer at a pressure of 1.4 kgcm<sup>-2</sup>. The experiment was laid out in a randomized complete block design with three replicates and kept in a greenhouse with day temperatures of 15 ± 2°C and 10 ± 2°C at night and a minimum daylength of 16h maintained with 400W High pressure sodium lamps. Three weeks after sowing, each pot was supplied with 50ml of Long Ashton nutrient solution.

At harvest six weeks after sowing, the numbers of emerged seedlings and of leaves, lengths of the second leaf and shoot and root dry weights per pot were determined.

A second experiment was set up with the same barley cultivar on 21 July 1986. Experimental procedures were as already described except that the medium in each pot was supplied with 50ml of Long Ashton nutrient solution two and three weeks after sowing. At harvest five weeks after sowing, whole plant dry weights per pot were



determined after drying the material at a temperature of 70°C for 48h. Data for both experiments were analysed as described in Experiment 4.1.

## Results.

The emergence of seedlings was not inhibited and similarly leaf production of treated plants was not significantly different from the controls (Tables 4.7 and 4.8).

The lengths of the second leaves were significantly reduced below that of the control at all doses of the herbicide in the sandy soil and at the dose of 20 gha<sup>-1</sup> in the soil containing 5% organic matter (Table 4.9). In the sand, lower shoot dry weights were obtained and the dry weight of roots was significantly lower than that of the control at all doses of the herbicide (Tables 4.10 and 4.11). Plants which received the herbicide treatments in the sandy soils had fewer branch roots than did control plants.

In the second experiment, the maximum reduction in total dry weight of seedlings was obtained in the sand. There was evidence of a reduction in phytotoxicity with increasing organic matter content (Table 4.12).

Table 4.7: Emergence of barley seedlings 6 weeks after sowing. (Results are expressed as percentage of control values).

Herbicide dose ( $\text{gha}^{-1}$ )	Percentage Organic Matter					Mean
	0 (Sand)	5	10	20	100	
Control - 0	100.0	100.0	100.0	100.0	100.0	100.0
10	113.7	90.0	100.1	100.2	96.7	100.1
15	104.2	90.9	102.0	103.7	86.4	97.4
20	103.6	97.5	98.0	97.0	86.1	96.5
Mean	105.4	94.6	100.0	100.2	92.3	

Table 4.8: Number of leaves per pot of barley seedlings 6 weeks after sowing. (Results are expressed as percentage of control values).

Herbicide dose ( $\text{gha}^{-1}$ )	Percentage Organic Matter					Mean
	0 (Sand)	5	10	20	100	
Control - 0	100.0	100.0	100.0	100.0	100.0	100.0
10	118.5	107.9	104.7	95.6	84.2	102.2
15	109.2	87.2	94.8	111.6	77.8	96.1
20	118.1	124.4	132.5	113.0	84.0	114.4
Mean	111.1	104.8	108.0	105.1	86.5	

Table 4.9: Lamina length of second leaves of barley 6 weeks after sowing. (Results are expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Percentage Organic Matter					Mean
	0 (Sand)	5	10	20	100	
Control-0	100.0a	100.0	100.0	100.0	100.0	100.0
10	65.4bc	77.5abBC	100.0AB	106.2A	94.7AB	88.8
15	63.8bB	89.2abAB	86.1AB	104.0A	93.2A	87.3
20	58.3bc	71.3bBC	76.2BC	110.8A	94.3AB	82.2
Mean	71.9	84.5	90.6	105.2	95.5	

Table 4.10: Shoot dry weight per pot of barley seedlings 6 weeks after sowing. (Results are expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Percentage Organic Matter					Mean
	0 (Sand)	5	10	20	100	
Control - 0	100.0a	100.0	100.0	100.0	100.0	100.0
10	51.3ab	67.0	92.7	100.4	76.7	77.6
15	52.3abB	72.8AB	74.3AB	113.7A	66.3AB	75.9
20	41.5bB	57.8B	71.0AB	111.2A	81.9AB	72.7
Mean	61.3	74.4	84.5	106.3	81.2	

Values in a column sharing a common lower-case letter and values in a row sharing a common capital letter do not differ significantly at  $P < 0.05$ .



Table 4.11: Root dry weight per pot of barley seedlings 6 weeks after sowing. (Results are expressed as percentage of control values).

Herbicide dose ( $\text{gha}^{-1}$ )	Percentage Organic Matter					Mean
	0 (Sand)	5	10	20	100	
Control - 0	100.0a	100.0a	100.0	100.0	100.0	100.0
10	44.9bc	52.5bBC	94.3AB	100.2A	94.4A	77.7
15	38.8bc	65.4abBC	87.2AB	120.1A	81.2ABC	78.5
20	29.4bc	43.6bBC	58.7ABC	101.8A	84.9AB	63.7
Mean	53.3	65.4	85.0	105.0	90.6	

Table 4.12: Whole plant dry weight of barley seedlings 5 weeks after sowing. (Results are expressed as percentage of control values).

Herbicide dose ( $\text{gha}^{-1}$ )	Percentage Organic Matter					Mean
	0 (Sand)	5	10	20	100	
Control - 0	100.0	100.0	100.0	100.0	100.0	100.0
10	69.1	86.2	99.0	96.6	104.8	91.2
15	64.9B	62.8B	70.5B	106.4AB	138.8A	88.7
20	59.0	67.5	88.9	101.4	109.3	85.2
Mean	73.2	79.1	89.6	101.1	113.2	

Values in a column sharing a common lower-case letter and values in a row sharing a common capital letter do not differ significantly at  $P < 0.05$ .

**EXPERIMENT 4.3: EFFECTS OF DAILY APPLICATION OF SIMULATED RAINFALL ON THE MOBILITY OF TRIASULFURON.**

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**Materials and Methods.**

Plastic tubes, 6cm in diameter and 30cm long were cut 2.5, 7.5, 12.5, 17.5 and 22.5cm from the top to produce six smaller columns, 0-2.5, 2.5-7.5, 7.5-12.5, 12.5-17.5, 17.5-22.5 and 22.5-30cm which were re-sealed together with a waterproof plastic tape to recreate the whole column. On 27 February 1987, the tubes were packed with John Innes No.2 compost (pH 6.4) to a bulk density of  $0.93 \text{ gcm}^{-3}$  and the columns supported vertically in 18cm diameter plastic pots containing sand. The pots were arranged on a greenhouse bench with temperatures of  $20 \pm 2^{\circ}\text{C}$  (day) and  $15 \pm 3^{\circ}\text{C}$  (night) and 16h daylength maintained with 400W High pressure sodium lamps and the soil in the columns was brought to field capacity.

Triasulfuron solutions at the rates of 0 or  $100 \text{ mg ai l}^{-1}$ , which is equivalent to 0 or  $350 \text{ g ai ha}^{-1}$  in  $200 \text{ lha}^{-1}$  of water were delivered to the soil surface at 1 ml per column. Beginning 24h after applying the herbicide, the columns were leached with 40ml of simulated rain (equivalent to 14mm of rain  $\text{day}^{-1}$ ) every day for four weeks using a small hand sprayer. At one, 3, 7, 14, 21 or 28 days after applying the herbicide, the distribution of the herbicide in ten soil columns was determined by bioassay after separating them into the units indicated above and placing the soil in small plastic trays into which ten seeds each of oilseed rape were sown. The trays were placed in a greenhouse with temperatures of  $20 \pm 2^{\circ}\text{C}$  (day) and  $15 \pm 3^{\circ}\text{C}$  (night) and a daylength of 16h maintained with supplementary

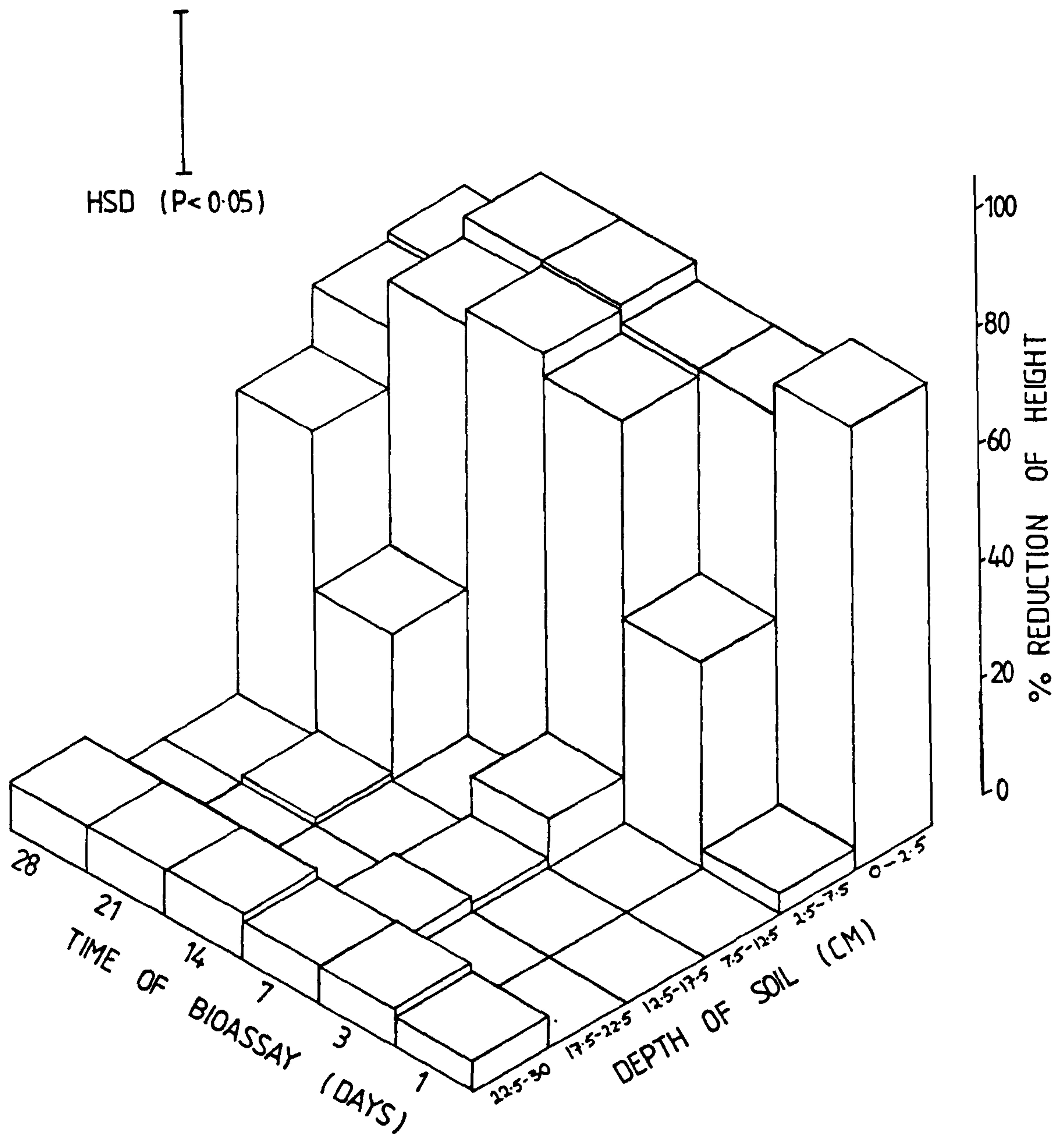
light (400W High pressure sodium lamps).

At harvest, three weeks after sowing the rape, plant heights were measured and dry weights determined after drying the material in an oven at 70°C for 48h. The experiment was designed as a randomized block with five replicates and the data were analysed using the Genstat statistical package. Comparison between treatment means was done by Tukey's HSD test.

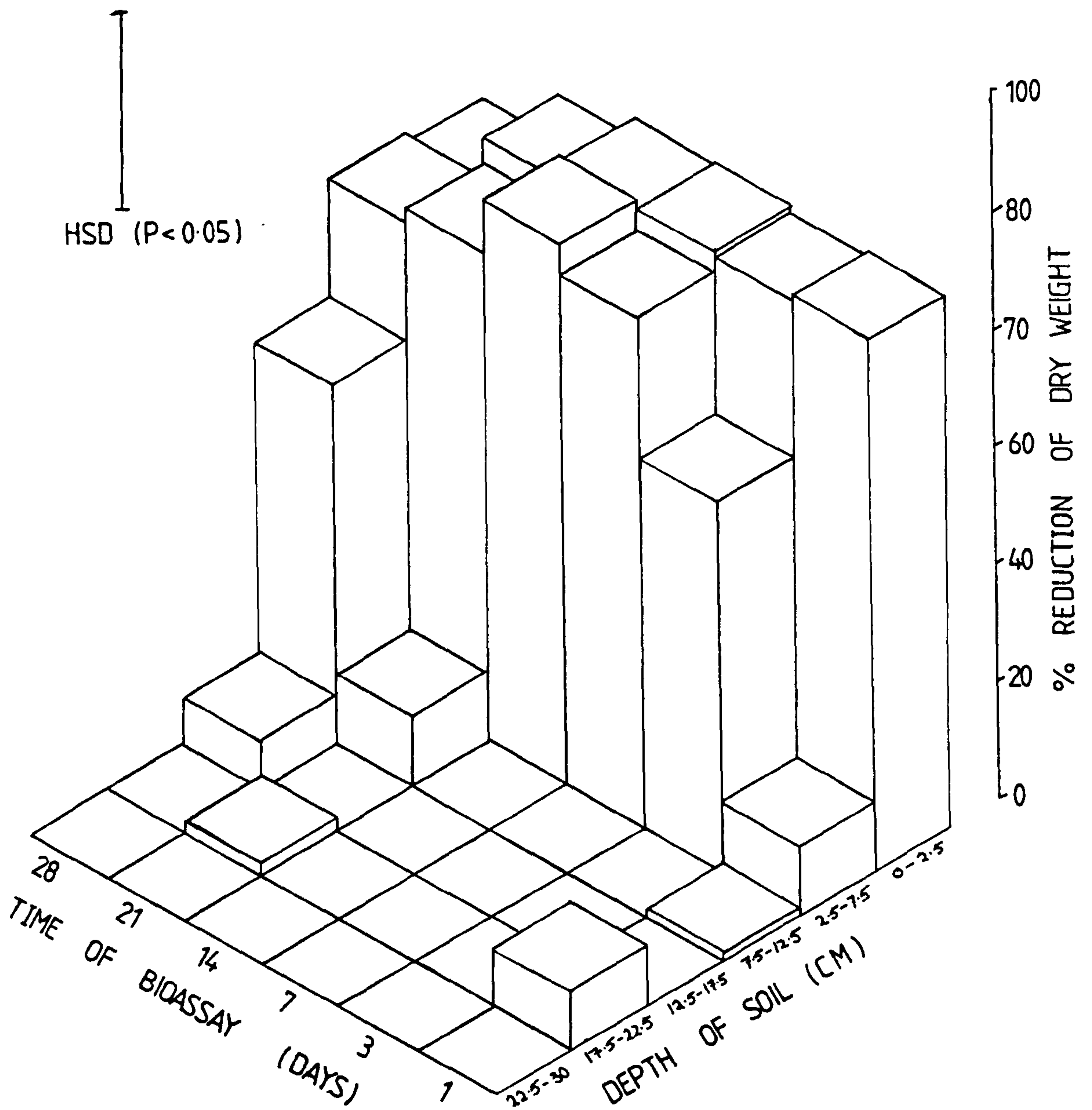
### Results.

Twenty four hours after application, the herbicide was located primarily in the top 2.5cm of the profile. Three days after application, significant levels of herbicide were detected in the zone 2.5-7.5cm as well as in the 0-2.5cm zone. A similar pattern of distribution was also recorded 7 and 14 days after application of the herbicide (Figs. 4.2 and 4.3). However after 21 days, evidence was obtained [from the plant height data (Fig. 4.2) ] of significant levels of herbicide in the 7.5-12.5cm zone. The shoot dry weights were not significantly reduced on soils taken from the 7.5-12.5cm zone (Fig. 4.3). At the final harvest, 28 days after application, significant percentage reductions of both height and shoot dry weight were recorded at the depths of 0-2.5, 2.5-7.5 and 7.5-12.5cm showing that after 4 weeks of daily leaching with 14mm of simulated rain, triasulfuron leached to a depth of at least 7.5cm (Figs. 4.2 and 4.3).





**FIG. 4.2:** Movement of Triasulfuron, applied to the surface, down the soil profile following daily application of 14.0 mm simulated rainfall. Data are percentage reduction from control plants of heights of oilseed rape seedlings used as bioassay (3 weeks after sowing).



**FIG. 4.3:** Movement of Triasulfuron, applied to the surface, down the soil profile following daily application of 14.0 mm simulated rainfall. Data are percentage reduction from control plants of dry weights of oilseed rape seedlings used as bioassay (3 weeks after sowing).

**EXPERIMENT 4.4: MOBILITY OF TRIASULFURON IN SOIL UNDER NATURAL CONDITIONS.**

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**Materials and Methods.**

Plastic tubes were prepared in the same way as described in Experiment 4.3. On 14 April 1987, the tubes were packed with John Innes No. 2 compost (pH 6.3) to a bulk density of  $0.99 \text{ gcm}^{-3}$  and were then supported vertically in 18 cm plastic pots containing sand. The soil in the columns was brought to field capacity by surface irrigation and subsequent drainage for 24h.

On 15 April 1987, solutions of triasulfuron at the rates of 0 or  $100 \text{ mg ai l}^{-1}$  (equivalent to 0 or  $350 \text{ g ai ha}^{-1}$  in  $200 \text{ lha}^{-1}$  of water) were delivered to the soil surface at 1 ml per column. The columns were then placed in the open near the weather station at Pen-y-ffridd Experimental Station, U. C. N. W., Bangor where the soil surfaces were exposed to outdoor temperatures and natural rainfall. Rainfall and air and soil temperatures were monitored throughout the experiment (Table 4.13). At monthly intervals the distribution of the herbicide in ten soil columns were determined by bioassay as described in Experiment 4.3. Plant heights and shoot dry weights of oilseed rape seedlings were determined 3 weeks after sowing. The total duration of the experiment was six months and it was designed as a randomized block with five replicates. All the results were expressed as percentage reductions of their untreated control values and subjected to analysis of variance as described in Experiment 4.3.

**Results.**

The results show progressive and continuing leaching of the

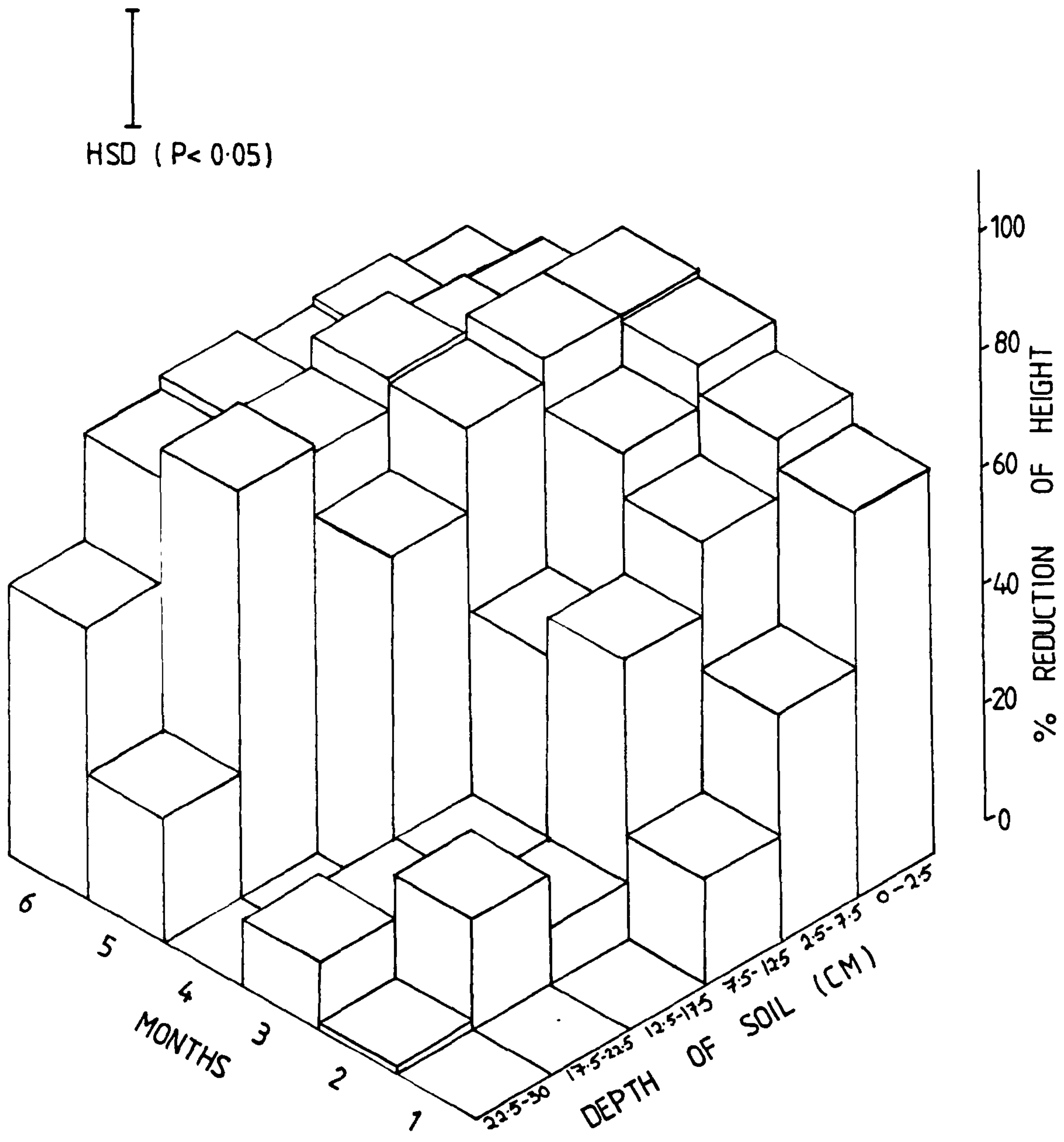


herbicide down to the 22.5-30.0cm zone over six months of exposure to natural rainfall.

One month after application, plant heights and shoot dry weights were significantly reduced in the zones 0-7.5cm and at both two and three months after application, significantly higher percentage reductions of height and shoot dry weight were also obtained from soil in the 12.5cm zone (Figs. 4.4 and 4.5). At four months after application, percentage reductions of height and dry weight were significant in all zones from 0-17.5cm. Further leaching to the 17.5-22.5cm zone was detected five months after application (Figs. 4.4 and 4.5). At the final harvest, 6 months after application, the herbicide was detected in all the segments of the soil columns (Figs. 4.4 and 4.5).

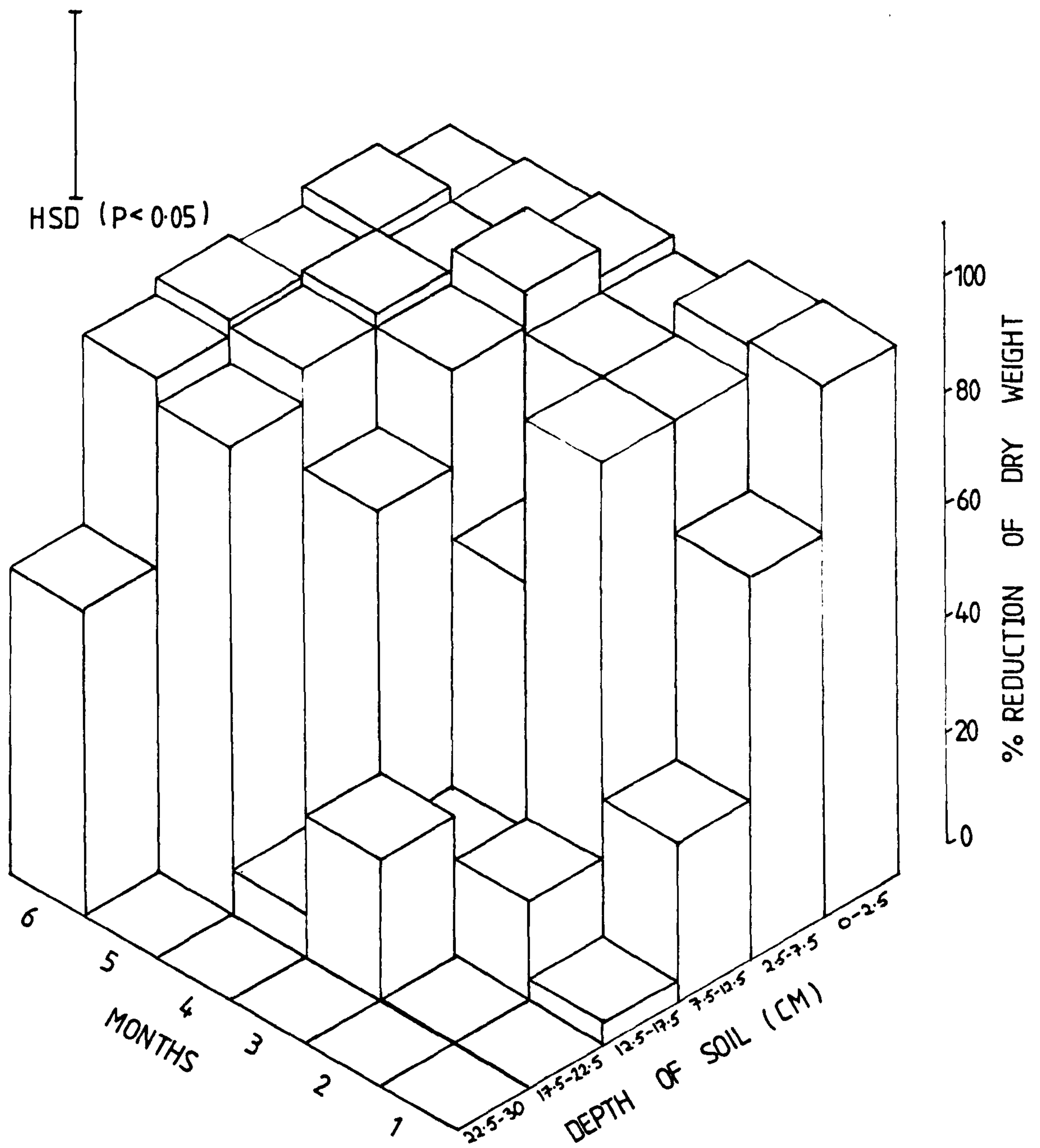
Table 4.13: Monthly rainfall, air and soil temperatures from April to October, 1987.

Months	Rainfall (mm)		Maximum temperatures°C		
	Total per month	Daily average	Air	Soil(10cm)	Soil(20cm)
15 April- 14 May	36.0	1.2	15.4	11.6	11.3
15 May- 14 June	62.0	2.0	14.1	12.0	12.2
15 June- 14 July	63.0	2.1	18.7	16.5	16.0
15 July- 14 August	108.5	3.5	17.0	15.4	15.6
15 August- 14 September	117.8	3.8	18.4	15.6	15.9
15 September- 14 October	141.0	4.7	15.1	11.3	12.3



**FIG. 4.4:** Movement of Triasulfuron, applied to the surface, down the soil profile under natural conditions. Data are percentage reduction from control plants of heights of oilseed rape seedlings used as bioassay (3 weeks after sowing).





**FIG. 4.5:** Movement of Triasulfuron, applied to the surface, down the soil profile under natural conditions. Data are percentage reduction from control plants of dry weights of oilseed rape seedlings used as bioassay (3 weeks after sowing).

**EXPERIMENT 4.5: EFFECTS OF AMOUNT AND FREQUENCY OF SIMULATED RAIN ON MOBILITY OF TRIASULFURON.**

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**Materials and Methods.**

Plastic columns were prepared in the same way as described in Experiment 4.3. On 20 May 1987, the tubes were uniformly packed with John Innes No.2 compost (pH 6.3) to a bulk density of  $1.14 \text{ g cm}^{-3}$  and supported vertically in 18cm diameter plastic pots containing sand. The pots were arranged on a greenhouse bench and the soil in the columns was brought to field capacity.

On 21 May 1987, solutions of triasulfuron at the rates of 0 or  $100 \text{ mg ai l}^{-1}$  (equivalent to  $350 \text{ g ai ha}^{-1}$  in  $200 \text{ l ha}^{-1}$  of water) were delivered to the soil surface at 1 ml per column. Beginning 24h after applying the herbicide, the soil columns were leached with either 10, 20 or 40ml of simulated rain (equivalent to 3.5, 7.0 or 14.0mm of rain respectively) either every day or every 4 days for 4 weeks using a small hand sprayer.

At the end of the fourth week, the distribution of the herbicide in each segment of the columns were determined by bioassay in the same way as described in previous experiments. Growth conditions were the same as those described in Experiment 4.3. The experiment was designed as randomized block with three replicates.

At harvest, 3 weeks after sowing, plant heights were measured and dry weights determined after drying the material in an oven at  $70^{\circ}\text{C}$  for 48h. The data were expressed as percentage reductions of the appropriate untreated controls and were processed in the same way as described for previous experiments.

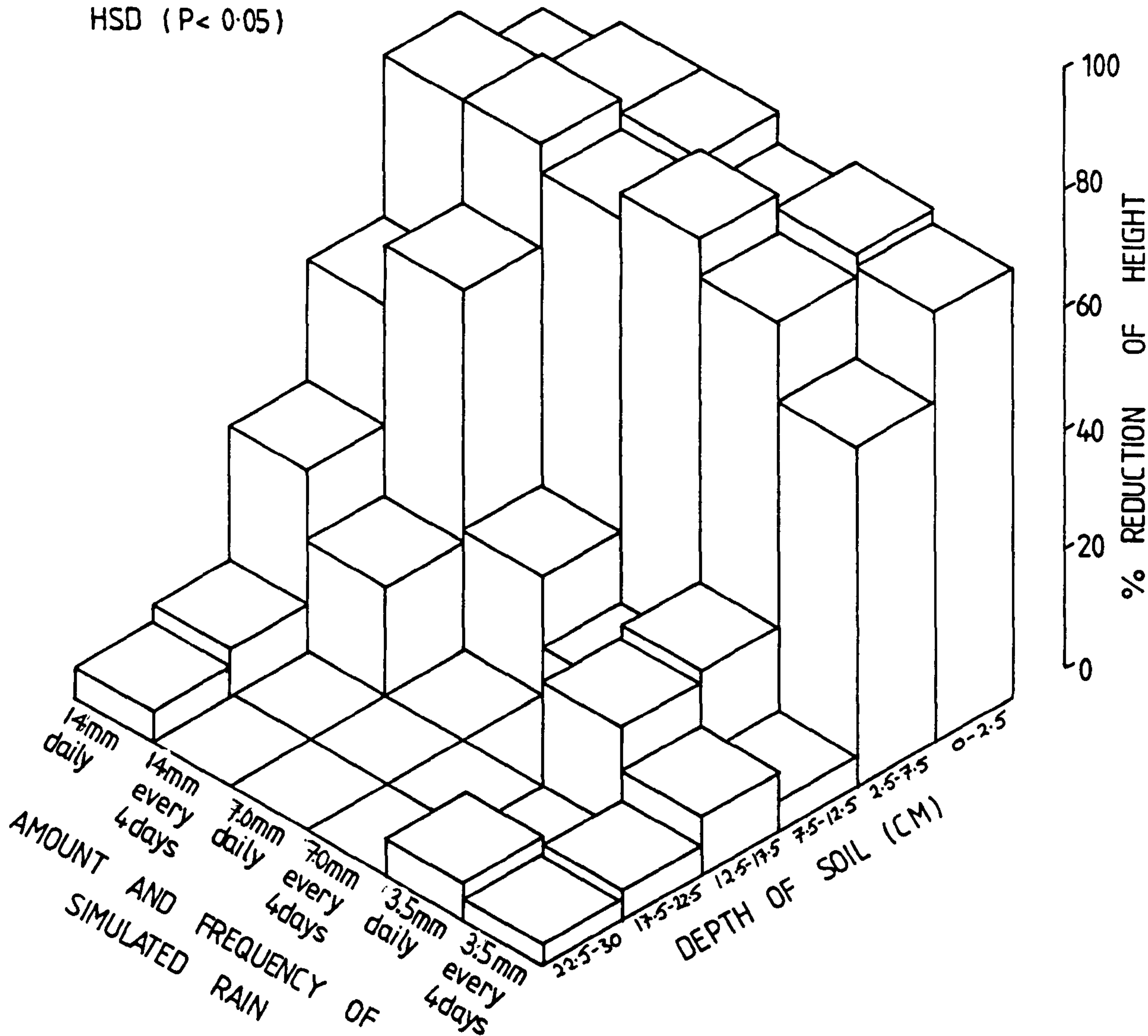
## Results.

Generally, the higher rainfall of 14.0mm caused more leaching of the herbicide down the soil profile than did either the 3.5 or 7.0mm treatments and there was a slight trend towards greater downward movement of the herbicide when simulated rain was given daily rather than at 4-day intervals.

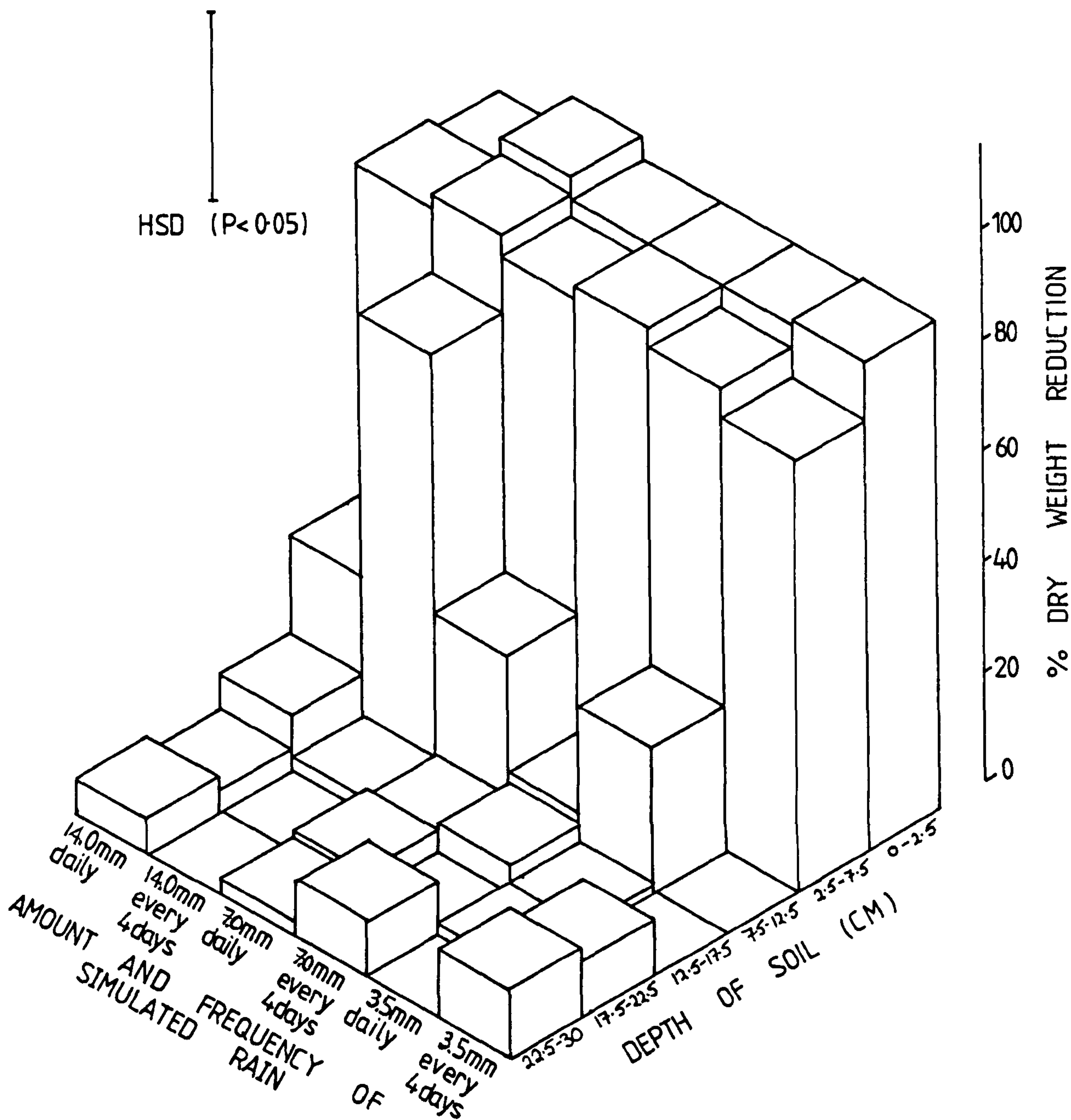
Four weeks after applying the herbicide, plant heights and shoot dry weights were significantly reduced in the zones 0-7.5cm following the application of 3.5mm rain either daily or every 4 days. There were some indications of further leaching into the 7.5-12.5cm zone in the tubes which had received 3.5mm rain daily. Similar results were obtained when 7.0mm simulated rain was applied either daily or at 4-day intervals (Figs. 4.6 and 4.7). Significant reductions of height were recorded in the 0-12.5cm zone following the application of 14.0mm rain daily or every 4 days (Fig. 4.6) and the same amount of rain resulted in reduced dry weights in the 0-12.5cm zone when applied at 4-day intervals but significant reductions of dry weight were obtained only in the 0-7.5cm zone of tubes which had received 14.0mm rain daily (Fig. 4.7).



HSD (P < 0.05)



**FIG. 4.6:** Movement of Triasulfuron, applied to the surface, down the soil profile following the application of 3.5, 7.0 or 14.0 mm simulated rain every day or every 4 days over a period of 4 weeks. Data are percentage reductions from control plants of height of oilseed rape seedlings used as bioassay (3 weeks after sowing).



**FIG. 4.7:** Movement of Triasulfuron, applied to the surface, down the soil profile following the application of 3.5, 7.0 or 14.0 mm simulated rain every day or every 4 days over a period of 4 weeks. Data are percentage reductions from control plants of dry weight of oilseed rape seedlings used as bioassay (3 weeks after sowing).

**EXPERIMENT 4.6: EFFECTS OF DIFFERENT LEVELS OF SOIL ORGANIC MATTER ON THE MOBILITY OF TRIASULFURON.**

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**Materials and Methods.**

Plastic tubes were prepared in the same way as described in Experiment 4.3. On 4 May 1987, the columns were packed with either quarry sand, peat or quarry sand-peat mixtures containing 5, 10 or 20 % w/w commercial horticultural peat (pH 4.4) before being embedded for support in 18cm diameter plastic pots containing sand. The pots were then arranged on a greenhouse bench with temperatures of  $20 \pm 2^{\circ}\text{C}$  (day) and  $15 \pm 3^{\circ}\text{C}$  (night) and 16h daylength maintained with 400W High pressure sodium lamps and the soil in the columns were brought to field capacity.

On 5 May 1987, solutions of triasulfuron at the rates of 0 or 100mg ai  $\text{l}^{-1}$  (equivalent to 0 or 350g ai  $\text{ha}^{-1}$  in 200  $\text{lha}^{-1}$  of water) were delivered to the soil surface at 1 ml per column. Beginning 24h after applying the herbicide, the columns were leached with 40 ml of simulated rain (equivalent to 4mm of rain  $\text{day}^{-1}$ ) every day for four weeks using a small hand sprayer. After the fourth week, the distribution of the herbicide in the soil columns was determined by bioassay after separating the soil columns into the zones described in Experiment 4.3. The soils were placed in small plastic trays into which ten seeds each of oilseed rape were sown. The trays were placed in a greenhouse with temperatures of  $20 \pm 2^{\circ}\text{C}$  (day)  $15 \pm 2^{\circ}\text{C}$  (night) and minimum daylength of 16h maintained with supplementary light (400W High pressure sodium lamps). The heights of plants and the dry weights of their shoots were determined three weeks after sowing. The experiment was designed as a randomized block with three replicates.



A second experiment, similar to the first, was set up on 24 July 1987. The preparation of the columns and all other experimental procedures and conditions were the same as in the previous experiment except that the columns were packed with either quarry sand or quarry sand-peat mixtures containing 1, 2, 3, 4 or 5 % w/w commercial horticultural peat. After 4 weeks of leaching with 14mm day<sup>-1</sup> of simulated rain, the distribution of the herbicide in the columns was determined by the bioassay previously described. The experiment was laid out as a randomized block with three replicates. The results of both experiments were expressed as percentage reductions from their appropriate untreated controls and processed as previously described in other experiments.

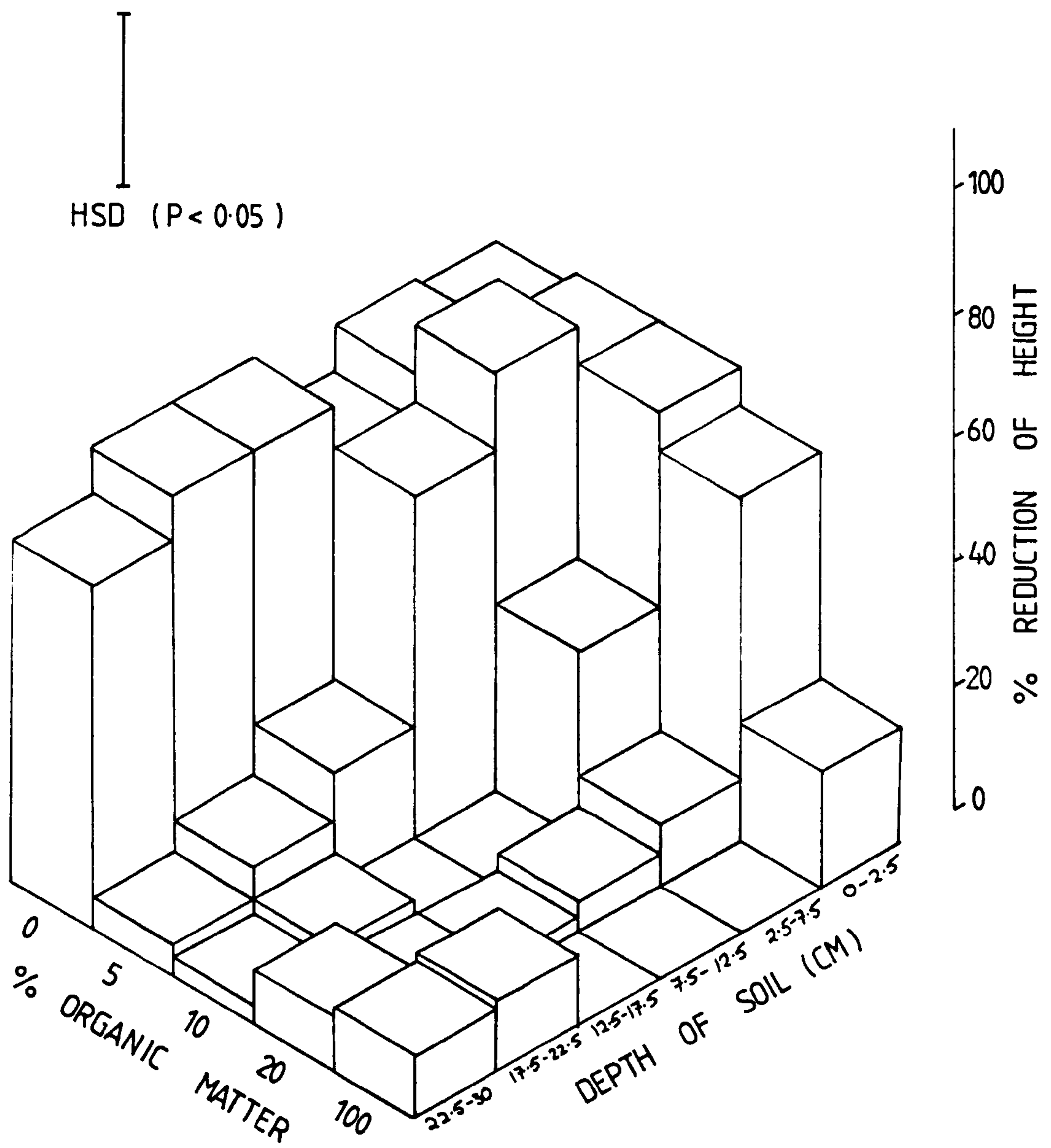
### Results.

The results of both experiments showed clear evidence of a reduction in the rate of leaching of the herbicide with increasing organic matter in the soils.

In both experiments, significant reductions in plant height and shoot dry weights were recorded in all the zones of the columns containing quarry sand (0 % organic matter) indicating leaching down to at least 22.5cm in 4 weeks (Figs. 4.8, 4.9, 4.10 and 4.11). In the first experiment, in the soil with 5 % organic matter, significant reductions of plant height and shoot dry weight were obtained down to the 7.5-12.5cm zone but in the soil with 10 % organic matter although plant height and weight were reduced in the 0-2.5cm layer there was only a slight indication of the presence of the herbicide in the 2.5-7.5cm zone (Figs. 4.8 and 4.9). In the medium with 20 % organic


matter, both plant heights and shoot dry weights of the seedlings were significantly reduced only in the top 0-2.5cm layer (Figs 4.8 and 4.9). In the columns containing 100 % peat, the bioassay was unsatisfactory, the oilseed rape plants being unaffected by the herbicide in all the samples (Figs. 4.8 and 4.9).

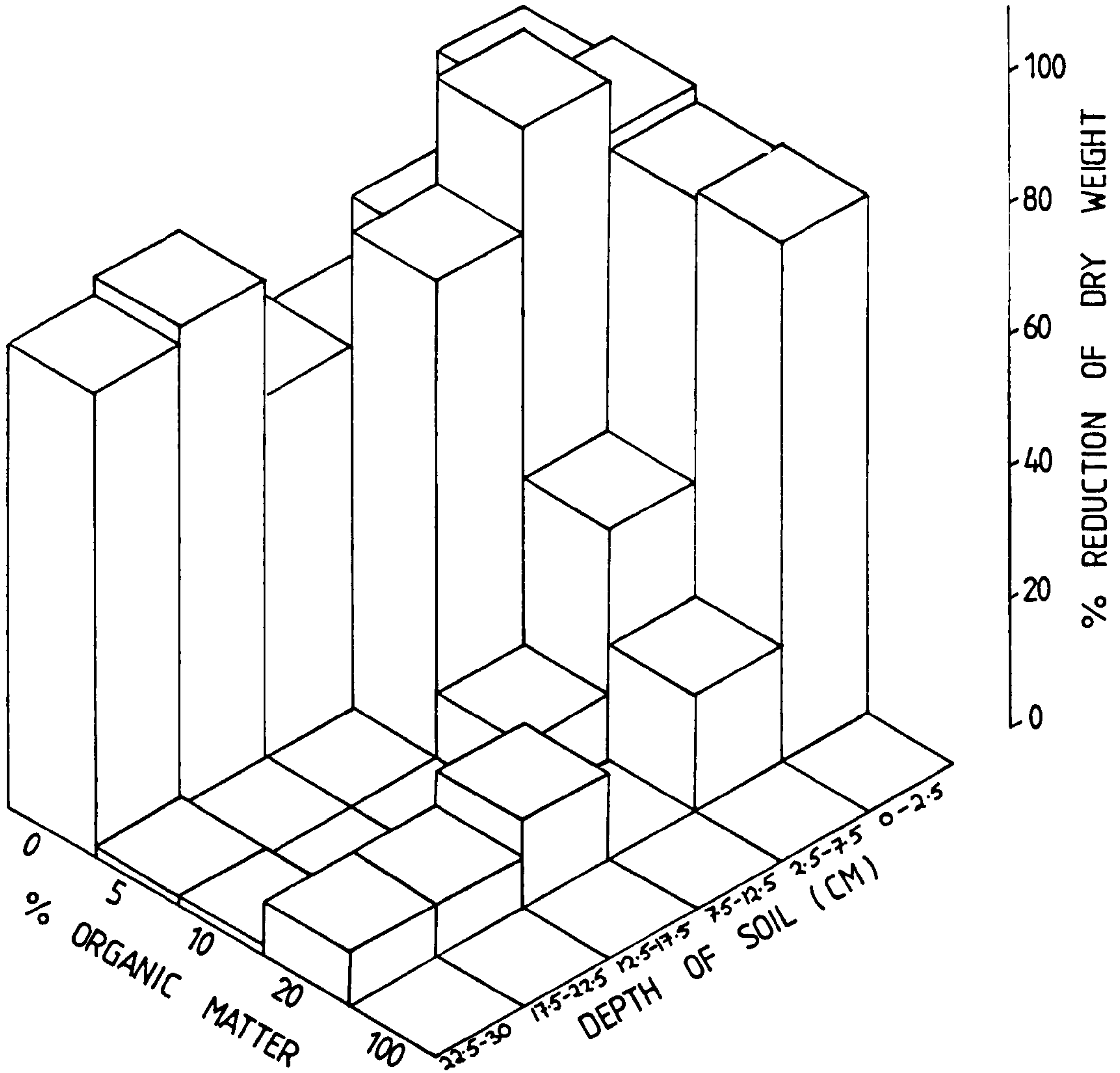
In the second experiment, using soils with 1, 2 and 3 % organic matter, large reductions in plant height (Fig. 4.10) and weight (Fig. 4.11) were obtained from soils in all the depth zones. At 4 % organic matter content, percentage reductions of height were significant down to and including the 7.5-12.5cm zone (Fig. 4.10) and dry weights were reduced in the 12.5-17.5cm layer (Fig. 4.11) The results obtained from the 5 % organic matter medium were similar to those of the first experiment where plant height and shoot dry weight reductions were significantly greater in the 0-2.5, 2.5-7.5, 7.5-12.5 zones than at depths 12.5-17.5, 17.5-22.5 and 22.5-30.0cm (Figs. 4.10 and 4.11).



**FIG. 4.8:** Effect of organic matter on the movement of Triasulfuron, applied to the surface, down the soil profile following daily application of 14.0 mm simulated rainfall for 4 weeks. The data are percentage reduction from control plants of heights of oilseed rape seedlings used as bioassay (3 weeks after sowing).

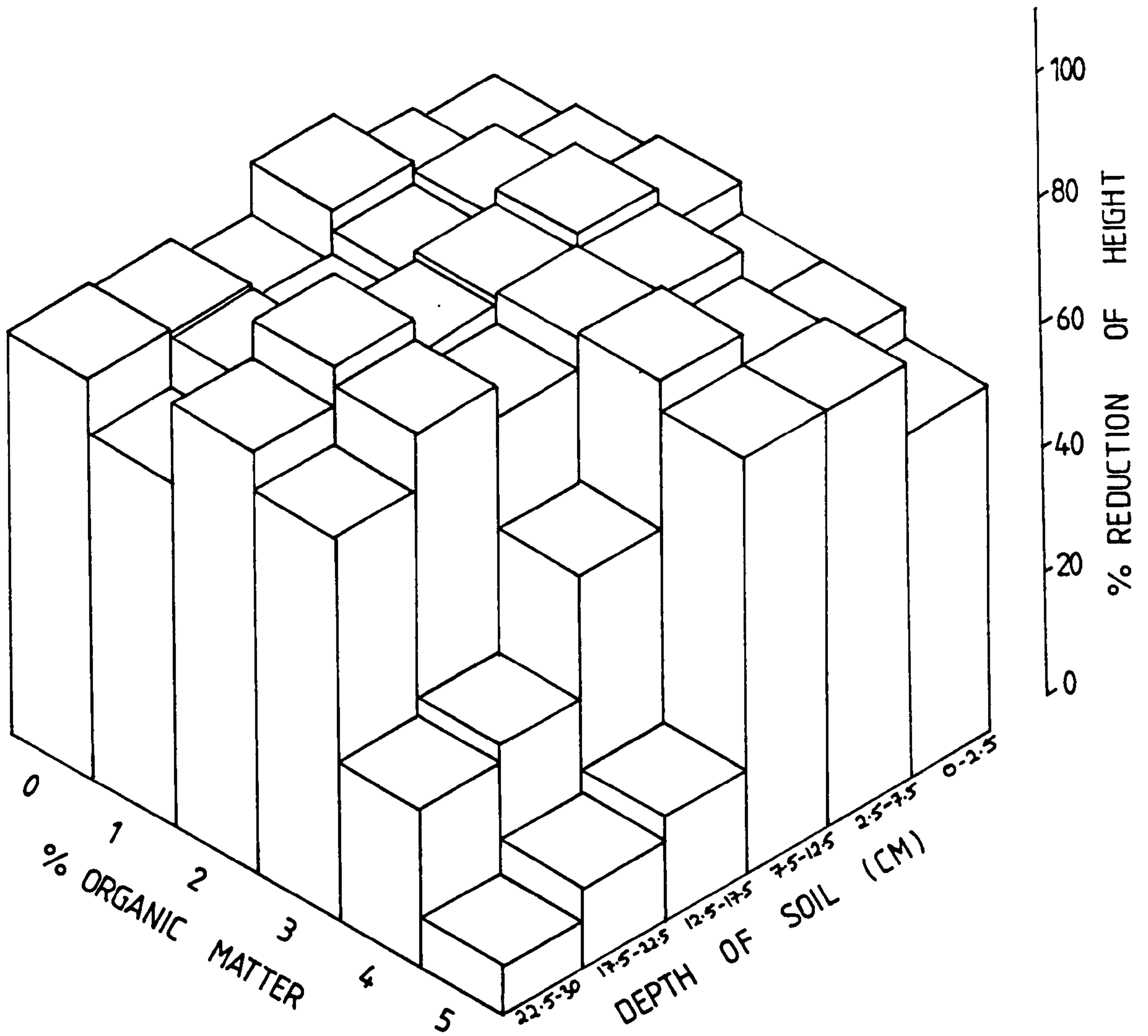


  
 HSD (P < 0.05)

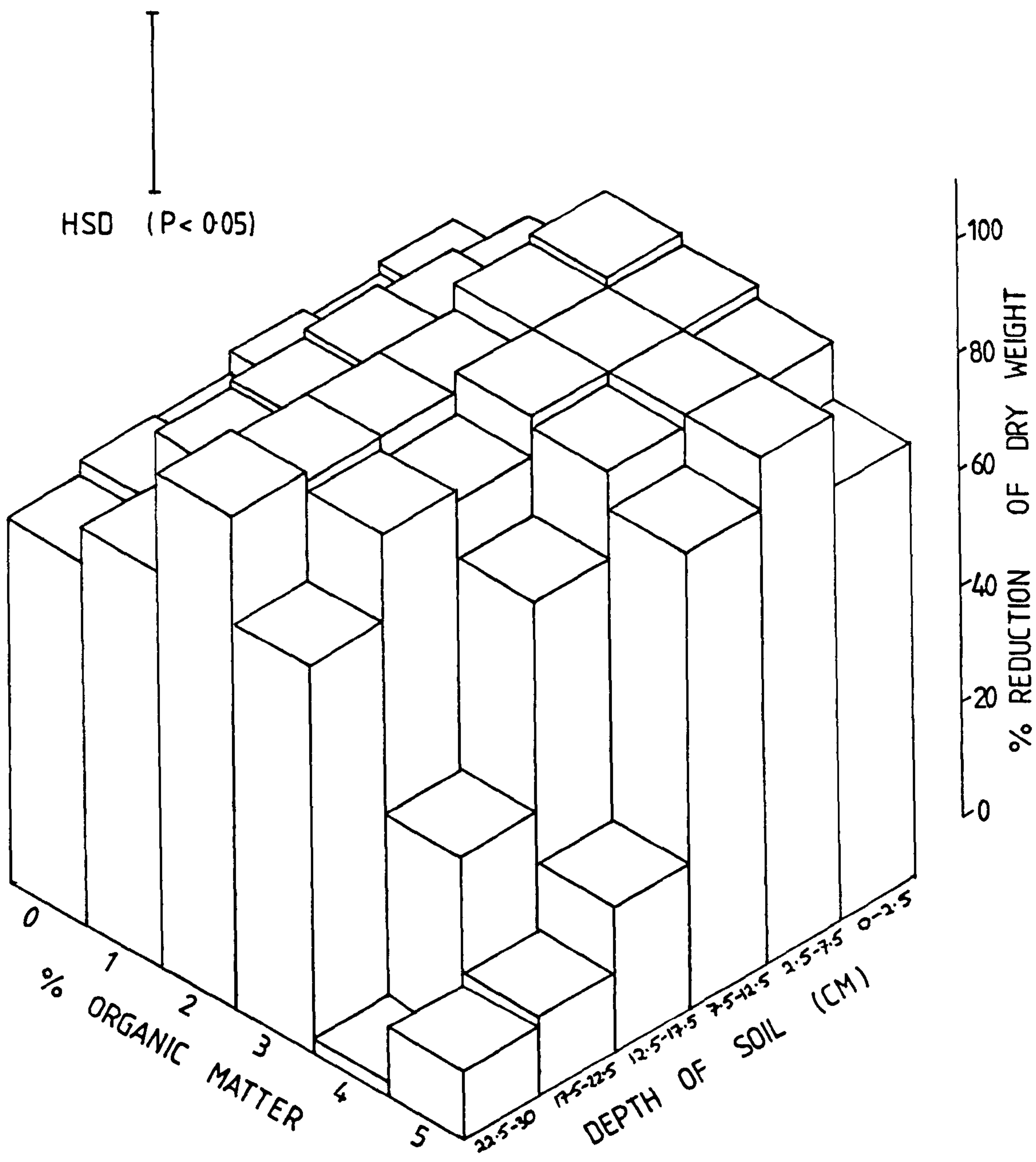


**FIG. 4.9:** Effect of organic matter on the movement of Triasulfuron, applied to the surface, down the soil profile following a daily application of 14.0 mm simulated rainfall for 4 weeks. The data are percentage reduction from control plants of dry weights of oilseed rape seedlings used as bioassay (3 weeks after sowing).

HSD (P<0.05)



**FIG. 4.10:** Effect of low levels of organic matter on the movement of Triasulfuron, applied to the surface, down the soil profile following daily application of 14.0 mm simulated rainfall for 4 weeks. The data are percentage reduction from control plants of heights of oilseed rape seedlings used as bioassay (3 weeks after sowing).



**FIG. 4.11:** Effect of low levels of organic matter on the movement of Triasulfuron, applied to the surface, down the soil profile following daily application of 14.0 mm simulated rainfall for 4 weeks. The data are percentage reduction from control plants of dry weights of oilseed rape seedlings used as bioassay (3 weeks after sowing).



## DISCUSSION.

In Experiment 4.1, the position of herbicide placement relative to seed placement had a significant effect on the growth of wheat and barley. The design of the experiment made it possible for triasulfuron to be placed either in the shoot zone (T4, T5 and T7), the root zone (T2) or both the shoot and root zones where the seeds were sown within the herbicide-treated band of soil (T3) or placed 2cm above and below the seeds (T6) (Fig. 4.1). The greatest phytotoxicity to wheat and barley seedlings by the herbicide was observed in T3 and T6. In these treatments, growth of seedlings was severely depressed, particularly in T3, where seedling emergence, numbers of leaves and tillers, lamina length of second leaves and shoot and root dry weights were significantly reduced below the respective controls in both wheat and barley (Tables 4.1, 4.2, 4.3, 4.4, 4.5 and 4.6). These results indicate that both the subterranean shoot and the roots of a developing seedling are involved in the uptake of triasulfuron from the soil.

The phytotoxicity of some pre-emergence herbicides is usually increased when they are placed close to the site of uptake. Prendeville et al. (1967) for example showed that simazine was more phytotoxic to pea seedlings when placed in the root- rather than the shoot-zone. They also demonstrated that root exposure to chlorpropham severely inhibited root growth although with little effect on shoot fresh weight. Other studies however, revealed that shoot uptake and consequently phytotoxicity of herbicides such as the thiolcarbamates (Parker, 1963, 1966; Appleby et al., 1965; Prendeville et al., 1967) and the chloroacetamides (Knake and Wax, 1968; Nishimoto et al.,

1969; Narsaiah and Harvey, 1977) was much higher when they were placed in the shoot rather than the root zone of young seedlings. When maize was grown hydroponically in the presence of chlorsulfuron, the fresh weights of the roots, which were in direct contact with the herbicide were more reduced than the fresh weights of the shoots (Ray, 1982). O'Leary and Prendeville (1985) exposed separately roots, shoots or both roots and shoots of maize seedlings over a period of 21 days to chlorsulfuron at a rate of  $0.07 \text{ mgKg}^{-1}$  dry soil, and observed that although both shoot and root growth was severely inhibited in the three treatments, the effect was much greater when the herbicide was placed in both the root and shoot zones. In addition, the growth of shoot and roots was retarded when the crown root node, growing point and lower stem were exposed to chlorsulfuron-treated soil. Exposure of the shoot above the growing point to the treated soil resulted in only slight inhibition of the shoot but not of roots. The authors therefore concluded that the inhibition of growth by chlorsulfuron might be due to the sensitivity of the meristematic regions of the shoot and of the root to the herbicide. Usually, there is very little elongation of the mesocotyl of wheat and of barley and therefore in T3 and T6, the sensitive apical regions might have been exposed to the herbicide over a longer period than in the other treatments, thus leading to increased retardation of growth. Since the apical regions of the shoot and roots are important sites for physiological processes such as cell division and elongation, a disruption of one or both events might have resulted in the poor growth of seedlings in T3 and T6. Richardson et al. (1980) have suggested that the apical meristem is a major site of action of chlorsulfuron and cell division at the



meristematic regions of root tips of plants susceptible to chlorsulfuron is often blocked leading to reduced growth (Ray, 1980, 1982). Furthermore, it is possible that absorption of the herbicide through the seed coat might have retarded the rate of mobilization of food reserves to the developing shoots and roots of wheat and barley seedlings. It has already been shown in Chapter 2 that although germination of wheat and barley seeds was not affected by pre-emergence applications of triasulfuron, subsequent seedling growth was retarded and this might explain the low number of emerged seedlings in T3 (Table 4.1).

Contrary to the findings of O'Leary and Prendeville (1985) with chlorsulfuron, the placement of triasulfuron in the root zone (T2) did not result in significant growth reductions. This may be due to the fact that the herbicide was placed in the bottom 4cm layer of soil in the pot and therefore might have been leached from the treated zone and out of the pot thus reducing the concentration available for root uptake. In addition, there was little chance of the shoot apex coming into direct contact with the herbicide as compared to T3 and T6 where both the root and shoot zones were exposed to the herbicide.

The emergence of seedlings, numbers of leaves and tillers, lamina lengths and dry weights of shoot were not significantly affected in T4, T5 and T7 (shoot zone exposure). This may be partly accounted for by the fact that elongation of the mesocotyl of both species did not occur or was slow and as a result the sensitive shoot apex was protected by mature sheathing leaf bases before it entered the treated layer or before it came into contact with leached herbicide.



There was however, some evidence of leaching of the herbicide in these treatments where root dry weights, especially of wheat were substantially reduced even though bands of untreated soil separated the herbicide-treated layer from the seed (Table 4.6). Since watering was by overhead irrigation, the herbicide might have been moved down from the treated layer into the root zone. Okafor et al. (1983) working with dinitramine also found that root growth of French beans was inhibited in spite of the presence of untreated soil protecting the seeds from the treated layer. They suggested that leaching of the herbicide out of the treated layer either by mass flow, in solution or in the vapour phase into the root zone might have caused the inhibition of root growth.

There were no significant differences between direct applications of the herbicide onto the surface of the soil (T7) and incorporation of the herbicide into the top soil (T5). This result suggests that triasulfuron could be applied directly onto the soil without the need for incorporation into the soil, which most often increases the cost of application.

Growth of barley seedlings was greatly reduced by triasulfuron when the plants were grown in quartz sand but there was very little effect on those grown in soils containing high levels of organic matter (Tables 4.9, 4.10, 4.11 and 4.12). These results followed the generally accepted view that the phytotoxicity of most herbicides is often negatively correlated with organic matter content of the soil. Mersie and Foy (1985) compared the phytotoxicity of chlorsulfuron in six soils with organic matter contents ranging from 1.5 to 7.2% and obtained an inverse relationship between the bioactivity of the herbicide and organic matter. Palm et al. (1980) have also noted high

activity of chlorsulfuron in gravelly sand soils with less than 1% organic matter. It is generally believed that adsorption of herbicide onto soil organic matter causes a reduction in the concentration of the herbicide available for bioactivity through the soil solution. High levels of organic matter will therefore lead to greater adsorption thus reducing the amount of herbicide available for biological activity. It has however been pointed out that the adsorption of chlorsulfuron in soils containing high organic matter is reversible and is pH-dependent with desorption increasing with an increase in soil pH (Mersie and Foy, 1985). A similar conclusion was made when adsorption of chlorsulfuron in two different soils was found to decrease markedly as pH increased (Nicholls and Evans, 1985).

There was some evidence to show that the adsorption of triasulfuron in soil containing 5% or less organic matter was not strong since growth of seedlings in such soils was retarded by the herbicide. In general, basic herbicides tend to be strongly adsorbed by soil organic matter (Weber et al., 1969) and clay minerals (Weber, 1970) whereas acidic herbicides are moderately adsorbed on organic matter and in relatively low amounts on clay (Carringer and Weber, 1974). Most of the sulfonylurea herbicides are weak acids (Beyer et al., 1987) and are therefore likely to behave as other acidic herbicides. However, a few sulfonylurea herbicides such as bensulfuron methyl (Beyer et al., 1987) and DPX-A7881 {methyl 2-[(4-ethoxy-6-methylamino-1,3,5-triazin-2-yl)=carbamoylsulphanoyl] benzoate} (Hutchison et al., 1987) are strongly adsorbed onto organic matter in soil resulting in much less biological activity



from soil treatments. It would seem reasonable to suggest from the present studies that the weak binding of triasulfuron in soils containing 5% or less organic matter might have led to an increase in the concentration of herbicide available for uptake by the plants and may partly account for the high activity of triasulfuron in soil observed in Chapters 2 and 3. Another consequence of such weak adsorption of triasulfuron in soils containing low levels of organic matter is a great potential for movement through the soil. This hypothesis was examined in detail in Experiment 4.6.

In the mobility studies (Experiments 4.3, 4.4, 4.5 and 4.6), bioassay procedures were used to determine the presence and distribution of triasulfuron in the soil profile. This was indicated by reduced plant growth (plant height and shoot dry weight) of oilseed rape in treated columns as compared to those sown in soils from untreated columns. A major advantage of this method was the assurance that only the phytotoxic component of the herbicide molecule present in the soil was being measured. Oilseed rape was chosen as the indicator species for these experiments as it had previously shown excellent sensitivity to triasulfuron (see Chapter 2).

Application of a total of 392mm simulated rain ( $14\text{mm day}^{-1}$ ) over 28 days (Experiment 4.3) resulted in significant reductions of both height and shoot dry weight of oilseed rape at the depths of 0-2.5cm, 2.5-7.5cm and 7.5-12.5cm (Figs. 4.2 and 4.3). Because of the high sensitivity of oilseed rape to triasulfuron, it must be concluded that the herbicide had been leached through the 0-7.5cm zones since the bioassay revealed its presence within the 7.5-12.5cm layer. In a similar study, Mersie and Foy (1986) packed four different soils



(Acredale silt loam, Cullen clay loam, Roanoke sandy loam and Kenansville loamy sand) representing a range of organic matter, clay content and pH in 35cm columns. Following the application of 40 gha<sup>-1</sup> chlorsulfuron and a total of 16.8cm of water (1.2cm day<sup>-1</sup>) to the surface of the soil over 14 days, the authors observed the least movement of chlorsulfuron (5cm) in Acredale silt loam soil which had a relatively low pH and high organic matter content; but in Kenansville loamy sand which had high pH and low organic matter content, the herbicide was leached to a depth of at least 25cm. They therefore concluded that the mobility of chlorsulfuron in soil appeared to be negatively and positively correlated with organic matter and pH respectively. These results are in partial agreement with those obtained for triasulfuron in Experiment 4.6 where herbicide movement down the soil profile was found to be inversely proportional to organic matter content (Figs. 4.8, 4.9, 4.10 and 4.11). The herbicide was leached through almost the whole 30cm column in soils containing 0-3% organic matter after the application of 20 gha<sup>-1</sup> triasulfuron and a total of 392mm simulated rain (14mm day<sup>-1</sup> for 28 days) (Figs. 4.10 and 4.11) but downward movement of the herbicide was increasingly retarded as the percentage of organic matter increased. Anderson and Humburg (1987) applied chlorsulfuron at a rate of 35 or 70 gha<sup>-1</sup> to four different soils in field experiments and found that organic matter and pH had a large influence on the duration of bioactivity and leaching of chlorsulfuron. Mobility of triasulfuron in soil containing 5% organic matter followed a pattern similar to that of John Innes No.2 compost in Experiment 4.3, where plant growth reductions were recorded down

to the 0-12.5 cm zones. The simplest explanation for these results is that adsorption of triasulfuron onto soil organic matter reduced the concentration of herbicide available in the soil solution as the percentage of organic matter increased thereby leading to less downward movement whereas weak binding of the herbicide in soils containing low levels of organic matter might have increased the amount of herbicide in the soil solution resulting in more leaching.

Soil pH appears to be one of the most important factors influencing the mobility of the sulfonylurea herbicides in soil. At low soil pH, most of the sulfonylurea molecules are in the neutral undissociated state and are more lipophilic and therefore less soluble in water but when the pH increases to that of normal soil (pH 6 to 7) and above, ionization of the herbicide molecules occurs leading to less lipophilicity and increased water solubility (Beyer et al., 1987; Nicholls et al., 1987). Since the John Innes No.2 compost used in Experiment 4.3 had a pH of 6.3, there must have been a slight shift in the equilibrium towards the water-soluble ionized form of triasulfuron leading to its greater availability in the soil solution and consequently its greater downward movement with soil water. In general, mass flow and diffusion are the mechanisms by which herbicides move in soil. However since diffusion usually occurs slowly over several months (Lavy, 1970), mass flow is often the mechanism primarily responsible for bulk herbicide transport. It is therefore likely that triasulfuron moved from the top layers into the deeper layers of the soil in the mass flow of water through the soil.

When the herbicide treated soil columns and their untreated controls were exposed to outdoor temperatures and natural rainfall (Experiment 4.4), there was a progressive and continuing leaching of



the herbicide down to a depth of at least 22.5cm over six months (Figs. 4.4 and 4.5). The importance of amount and frequency of rainfall in moving triasulfuron down the soil profile was evident in this experiment. For example, during the first month of exposure, total rainfall of 36mm was recorded and the herbicide was detected mainly in the 2.5-7.5 zone (Figs. 4.4 and 4.5) whereas in Experiment 4.3, where 392mm of simulated rain ( $14\text{mm day}^{-1}$ ) was applied over the same period, the herbicide was detected in the 0-12.5cm zones (Figs. 4.2 and 4.3). However with more rainfall during the subsequent months, the herbicide was progressively leached down the profile and was detected in all the segments after six months of exposure. It is worth pointing out that the abnormally heavy rainfall between 15 July and 14 October, 1987 (Table 4.13) was well above the monthly average of 84mm (calculated from 1982 to 1986: see Appendix 2) and might have contributed to the extent of movement of the herbicide through the whole soil column during the six month period. A similar study was made by Huggenberger and Ryan (1985) when they applied  $20\text{ gha}^{-1}$  chlorsulfuron to a loam soil packed into plastic columns and left in the open. After the soil columns had received a total of 265mm rainfall over a period of 5 months, the authors detected the herbicide down to a depth of 10cm but substantial quantities were still present in the top 5cm of the soil. The results of the present studies also showed that there were still highly phytotoxic concentrations of triasulfuron in the top 2.5cm layer (Figs. 4.4 and 4.5) six months after application thus confirming the persistence of this herbicide. It has been reported that chlorsulfuron may move vertically with rising capillary water during periods of net upward



flow of soil water (Nilsson, 1985). It is therefore possible that triasulfuron would behave in a similar manner by re-entering the upper layers from the deeper layers of the soil during periods of high evaporation. A direct implication of this coupled with persistence is that, as most weeds emerge from the top 2cm layer of the soil (Chancellor, 1964), germination and growth of weeds will continue to be suppressed over a relatively long period following soil applications of triasulfuron. On the other hand, such relatively long persistence of triasulfuron in the soil may limit the types of rotational crops that can be grown. Detailed studies on the degradation of triasulfuron are presented in Chapter 5.

In Experiment 4.5, more leaching of triasulfuron down the soil profile occurred when 14mm of simulated rain was applied than when applications of 3.5mm or 7.0mm rain were made (Figs. 4.10 and 4.11). This result confirmed the observations made in Experiment 4.4 where leaching of triasulfuron was strongly influenced by the amount of rainfall. O'Sullivan (1982) applied 500 gha<sup>-1</sup> chlorsulfuron and a total of 38mm or 75mm of simulated rain to silt loam soil packed into plastic columns. He observed that after 6 days the herbicide had been leached to a depth of 11cm or 17cm in columns which received the low and high amounts of rain respectively. It has been demonstrated in field studies that the amount of rainfall is one of the major environmental factors influencing the mobility of chlorsulfuron in soil (Anderson and Humburg, 1987; Nicholls et al., 1987).

There was very little difference in the mobility of triasulfuron between daily application of simulated rain or application once every 4 days. However, the importance of frequency of rainfall in moving herbicides down the soil profile has been demonstrated for other

herbicides. For example, in an experiment where 51cm of water was applied continuously under saturated-flow conditions over a 3-hour period, 81% of applied terbuthiuron was moved through a 30cm long soil column but application of the same total amount of rain at 1.2cm per day under unsaturated-flow conditions over 40 days resulted in only 2.5% of the applied herbicide passing through the whole soil column (Weber and Whitacre, 1982).

The results of the studies in this chapter indicate that triasulfuron has a moderate affinity for organic matter and in coarse textured soils with low organic matter and under conditions of high rainfall can be leached from the top soil into the root zone of crops. As a class, the sulfonyleureas are generally characterized as relatively mobile compounds in soil, and depending on rainfall, net soil water movement and degree of soil drainage, this mobility can be agronomically and environmentally important (Beyer et al., 1987). In addition, the results suggest that some triasulfuron may persist for relatively long periods in the soil to provide season long control of weeds although such persistence may pose some problems to following crops in a rotation.

CHAPTER 5.

DEGRADATION IN SOIL.



## INTRODUCTION

Herbicides may be applied directly to the soil or enter it as run-off, drift from target area, wash-off from treated plants or as constituents of crop or animal residues in the soil (Kaufman and Kearney, 1976). Once herbicides have entered the soil, an important aspect of their subsequent behaviour is the length of time for which they and their phytotoxic breakdown products persist. This is particularly important for those herbicides which have high biological activity through the soil.

Herbicides may be lost from soils by physical removal of the unchanged molecule through uptake by plants, leaching, volatilization or by degradation through photodecomposition and chemical or microbial processes (Klingman, 1961; Roberts, 1982). However, for most compounds, chemical and microbiological degradation in the soil is the major route of herbicide loss (Walker and Allen, 1984). The rate of microbial breakdown of pesticides in soil depends on the availability of the chemicals to the micro-organisms which can degrade them as well as the quantity and activity level of those micro-organisms (Frehse and Anderson, 1983). Edaphic factors such as organic matter and clay contents, moisture level, temperature, pH, aeration and nutrient status, which normally influence the quantity and activity level of micro-organisms are important in determining the rates of microbial degradation of herbicides (Hurle and Walker, 1980; Frehse and Anderson, 1983). Briggs (1976) has however pointed out that factors associated with the inherent degradability of the particular molecular structure are also important in determining the rates of pesticide degradation.

Although it is well known that soils containing high organic matter often have high microbiological activity (Frehse and Anderson, 1983), there is ample evidence to show that in most soils, adsorption of many herbicides increases with an increase in soil organic matter. Smith and Meggit (1970b) with chloridazon {5-amino-4-chloro-2-phenyl pyridazin-3(2H)-one}, Moyer et al. (1972) with atrazine {6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine} and chlorthiamid {2,6-dichloro(thiobenzamide)} and Hurle and Lang (1981) with napropamide {N,N-diethyl-2-(1-naphthalenyloxy) propamide} obtained low rates of degradation of these herbicides as the organic matter contents of the soil increased. Similarly, degradation of simazine (Walker et al., 1983) and napropamide (Walker and Brown, 1983b) decreased with increasing clay contents of the soil. In these examples, increased adsorption and hence reduced availability of the herbicides for degradation was the main reason given for the low rates of herbicide dissipation. Contrary to these findings, other researchers have reported rapid degradation of dicamba {3,6-dichloro-2-methoxybenzoic acid} (Donaldson and Foy, 1965) and linuron {N'-(3,4-dichlorophenyl)-N-methoxy-N-methylurea} (Walker and Thompson, 1977) in soils with high organic matter content. The authors attributed the faster rate of loss of the herbicide to the increased microbial activity due to the high soil organic matter levels. Hurle and Walker (1980) have pointed out that, although some chemicals may be degraded slowly in soils containing high organic matter or clay fractions, their biological activity may be low in such soils because of increased adsorption.

Soil pH may influence degradation through changes in adsorption



of the chemical, changes in the population of micro-organisms or through effects on the compound if its stability depends on pH (Walker and Allen, 1984). Several herbicides including prometryne {N,N'-bis(1-methylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine} (Best and Weber, 1974), metribuzin {4-amino-6(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one}, (Ladlie et al., 1976) and napropamide (Walker and Brown, 1983b) have been found to degrade slowly with decreasing soil pH due to increased adsorption and hence reduced availability to micro-organisms of the herbicides at low pH levels. With other herbicides such as simazine (Nearpass, 1965), atrazine (Best and Weber, 1974; Hiltbold and Buchanan, 1977) and chlorsulfuron {2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl] benzenesulfonamide} (Joshi et al., 1985; Thirunayaranan et al., 1985; Fredrickson and Shea, 1986), degradation was enhanced at lower soil pH levels due to increased rates of hydrolysis of these chemicals.

In general, rates of degradation of most herbicides have been found to increase with increasing temperature and moisture content of the soil. Available evidence show that herbicide degradation rates are faster in moist soils with high temperatures due to the promotion of microbiological activity. Meikle et al., (1973) with picloram {4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid}, Hyzack and Zimdahl, (1974) with metribuzin, Usoroh and Hance (1974) with linuron and Zimdahl and Gwynn (1977) with three dinitroanilines have reported increased degradation of these herbicides with increasing temperatures and moisture levels up to field capacity. Likewise, chlorsulfuron (Walker and Brown, 1983a; Anderson and Barret, 1985; Joshi et al., 1985; Thirunarayanan et al., 1985) and triasulfuron {3-



(6-methoxy-4-methyl-1,3,5-triazin-2-yl)-1-[2-(2-chloroethoxy-phenylsulfonyl)-urea} (Amrein and Gerber, 1985) have been found to degrade faster under increased temperature and moisture conditions. Anderson (1985) however observed that the degradation of metsulfuron-methyl {2-[[[4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl amino] sulfonyl]benzoic acid} was unaffected by soil moisture at 16°C or 24°C in loam soil but soil moisture enhanced the breakdown of the herbicide in sand at 24°C.

Kinetic analysis of herbicide degradation may be used to predict the rates of disappearance and the residual life of herbicides in the soil. The breakdown of some of the sulfonylurea herbicides has been found to follow first-order kinetics (Walker and Brown, 1983a; Amrein and Gerber, 1985; Thirunarayanan et al., 1985). Palm et al., (1980) reported that in the field, the half-life of chlorsulfuron in the soil is between 4 and 8 weeks and in laboratory studies, Amrein and Gerber, (1985) reported that the half-life of triasulfuron in soil at 75% moisture and pH 6.4 was 20 and 14 days at 21°C and 30°C respectively. DPX-M6316 (thiameturon) {Methyl 3-(3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)ureidosulfonyl) thiopene-2-carboxylate} has been reported to have a half-life of 0.5-2 days at 30°C (Brown et al., 1987). Repeated applications of thiameturon to soil did not diminish the rapid rate of degradation of the herbicide.

The sulfonylurea herbicides are believed to undergo degradation by both chemical and biological mechanisms (Joshi et al., 1985; Beyer et al., 1987). The previous experiments in Chapters 2, 3 and 4 revealed that triasulfuron has high activity in soil and because of this, it was essential to investigate further its behaviour in soil

with particular reference to the length of time its residues remain in the soil. The experiments reported in the present chapter were conducted to study the microbiological degradation of triasulfuron in soil. The first experiment investigated the effects of temperature and moisture content on the disappearance of the herbicide from sterile and non-sterile soils whilst the second experiment was designed to study the degradation of subsequent applications of triasulfuron in soil which had previously been treated with the herbicide.

**EXPERIMENT 5.1: EFFECTS OF TEMPERATURE AND SOIL MOISTURE ON THE DEGRADATION OF TRIASULFURON IN NON-AUTOCLAVED AND AUTOCLAVED SOILS.**

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**Materials and Methods.**

Top soil was collected to a depth of 10cm from a plot at Pen-y-ffridd Experimental Station, U. C. N. W. on 21 August 1987 and was air-dried and passed through a 3mm sieve. The soil was obtained from a plot which had had a minimum of 3 years fallow. The last crop raised on the plot was potato and a combination of paraquat and linuron had been used to control weeds. The physical and chemical characteristics of the soil are presented in Table 5.1.

On 28 August 1987, solutions of triasulfuron (2.5ml volume) were added to 2.5kg-batches of air-dried autoclaved soil (autoclaved at a pressure of  $1 \text{ kg cm}^{-2}$  and  $120^{\circ}\text{C}$  for 4h.) or to non-autoclaved soil to produce a dry soil concentration of  $0.08 \text{ mg ai kg}^{-1}$ . The soil was thoroughly mixed and moistened to either 25%, 75% or 100% of field capacity and then placed in polythene bags which were sealed and incubated in the dark at 10 or  $30^{\circ}\text{C}$ . The bags were opened at weekly intervals for 30 minutes to allow gaseous exchange and then weighed and when necessary water added to restore the initial moisture levels. Each treatment had its untreated control and the experiment was carried out in duplicate.

Three soil samples (each ca. 80g) were removed from each bag 0, 7, 30, 60, 90, 120 or 150 days after incubation and the disappearance of the herbicide from the soil samples were followed by using the rapid bioassay method of Parker (1964) which depends on the root



inhibition of a selected test species grown in petri-dishes. Generally, the procedures for sampling the soil for bioassay were not strictly aseptic but considerable attention was given to avoiding contamination of soil with particles from other samples.

For the bioassay, five pre-germinated seeds of maize cv. Anko, with the radicles just beginning to emerge, were placed in a row across the surface of approximately 80g of treated or untreated soil contained in 9cm diameter plastic petri-dishes. The radicles were all aligned in one direction and the seeds were pushed into the soil so that the tops were even with the soil. The lids were placed on the dishes and they were firmly taped. The dishes were then mounted at an angle of  $15^{\circ}$  in the dark at  $20^{\circ}\text{C}$  for 5 days so that the roots would grow down against the lids. The lengths of the longest roots at the end of the incubation period were expressed as percentages of the lengths of the roots of appropriate control plants. Each bioassay included a comparison with a standard or reference series of dishes containing maize seedlings exposed to known concentrations of the herbicide viz; 0, 0.0005, 0.001, 0.002, 0.005, 0.01, 0.015, 0.02, 0.03, 0.04, 0.06 or  $0.08\text{mg ai kg}^{-1}$ . Standard curves were derived by plotting the percentage root lengths against the herbicide concentrations. Percentage root lengths of seedlings grown in samples containing unknown concentrations were then converted to values for amounts of herbicide remaining in the soil using the standard curve.

The results of the standard bioassays at each sampling date generally showed increased inhibition of root extension as the concentration of the herbicide increased from 0.0005 to  $0.08\text{ mg ai kg}^{-1}$  although only small differences were obtained between the

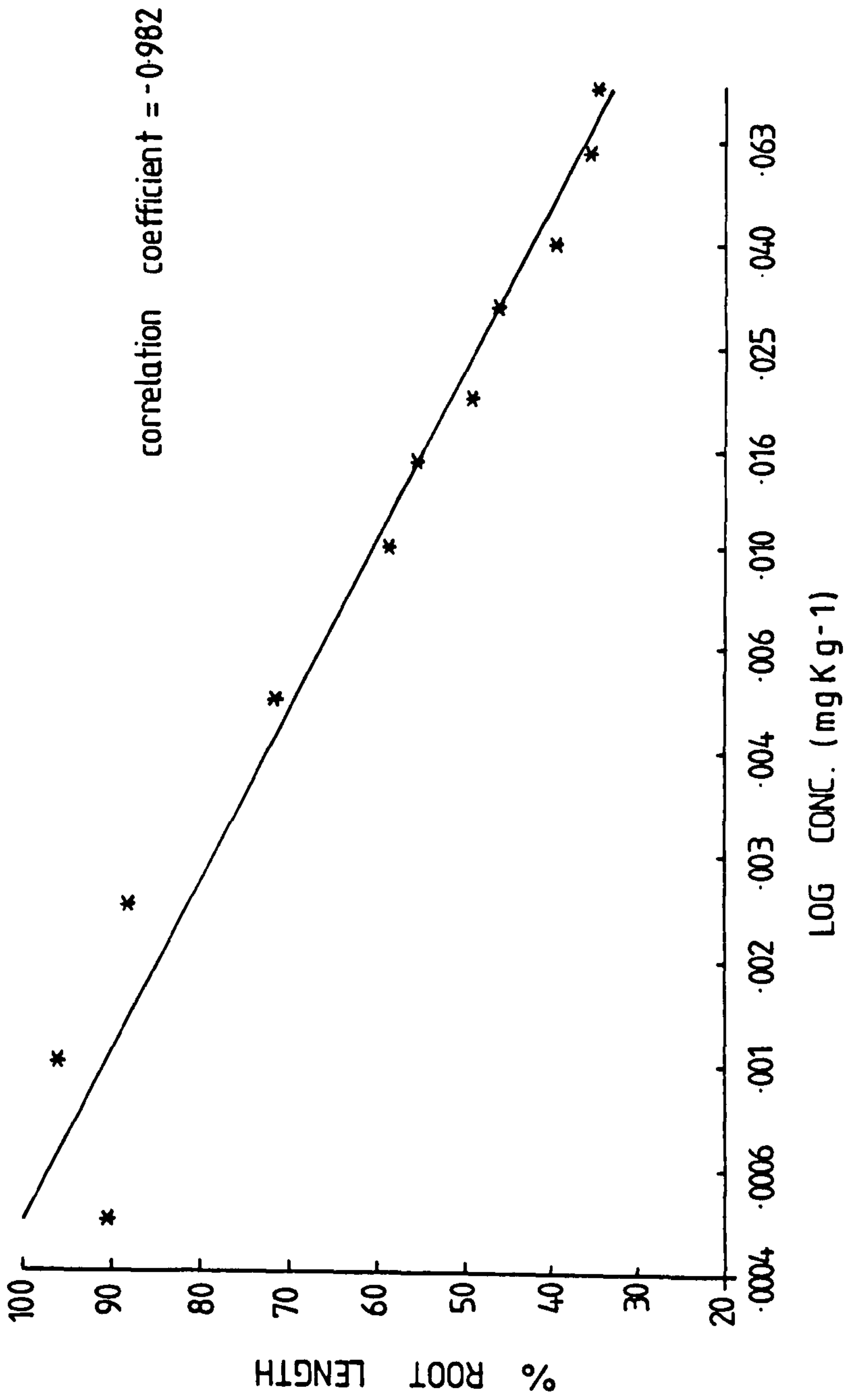
concentration of 0.04 and 0.08mg ai kg<sup>-1</sup>. Due to some inconsistencies in the values obtained at lower concentrations of 0.0005 and 0.001mg ai kg<sup>-1</sup>, the results of all the reference bioassays were pooled together to obtain mean values from which an overall bioassay curve was derived. A plot of root lengths (expressed as percentage of control values) against log herbicide concentrations showed a linear relationship with a highly significant ( $P < 0.01$ ) correlation coefficient of -0.982 (Fig. 5.1). The herbicide concentration resulting in a 50% root inhibition ( $GR_{50}$ ) was approximately 0.02mg ai kg<sup>-1</sup>.

### Results.

Degradation of triasulfuron was generally slow at 10°C although it was faster in the non-autoclaved than in the autoclaved soil. There was virtually no breakdown of the herbicide in the autoclaved soils stored at 10°C and with moisture contents of 25 and 75% after 150 days whereas soils with 100% moisture and stored at the same temperature had a residual concentration of 0.042mg ai kg<sup>-1</sup> at that time (Fig. 5.2). Significant linear correlation coefficients ( $P < 0.05$ ) were obtained from plots of log concentrations of the remaining herbicide versus time for the non-autoclaved soils stored at 10°C and rate constants (K) derived from the slopes of the lines were used to calculate half-lives. Values of 182.9, 112.0 and 68.0 days were obtained for soils with moisture contents of 25, 75 and 100% respectively (Table 5.2). After 150 days of incubation, residual herbicide concentrations of ca. 0.022, 0.008 and 0.004mg ai kg<sup>-1</sup> were detected in the non-autoclaved soil stored at 10°C and with moisture contents of 25, 75 and 100 % respectively (Fig. 5.2).

Degradation was rapid at 30°C and at all moisture levels in the non-autoclaved soil. The half-life values of 26.5, 25.6 and 30.8 days for soils with moisture contents of 25, 75 and 100% respectively were not very different from each other (Table 5.2). After 90 days of incubation, only trace amounts of the herbicide was detected at all moisture levels for the non-autoclaved soil (Fig. 5.3). Breakdown of the herbicide in the autoclaved soil was also faster at 30°C than at 10°C. Half-life values of 62.5, 54.2 and 42.0 days were recorded at moisture levels of 25, 75 and 100% respectively (Table 5.3). After 90 days of incubation, a residual concentration of 0.002mg ai kg<sup>-1</sup> was detected in the autoclaved soil with 100% moisture but at 25 and 75% moisture levels the values were 0.015 and 0.009mg ai kg<sup>-1</sup> respectively. However after 150 days of incubation, these values had dropped to 0.002mg ai kg<sup>-1</sup> for autoclaved soil at 25% moisture and to 0.001mg ai kg<sup>-1</sup> for autoclaved soil with 75% moisture (Fig. 5.3).

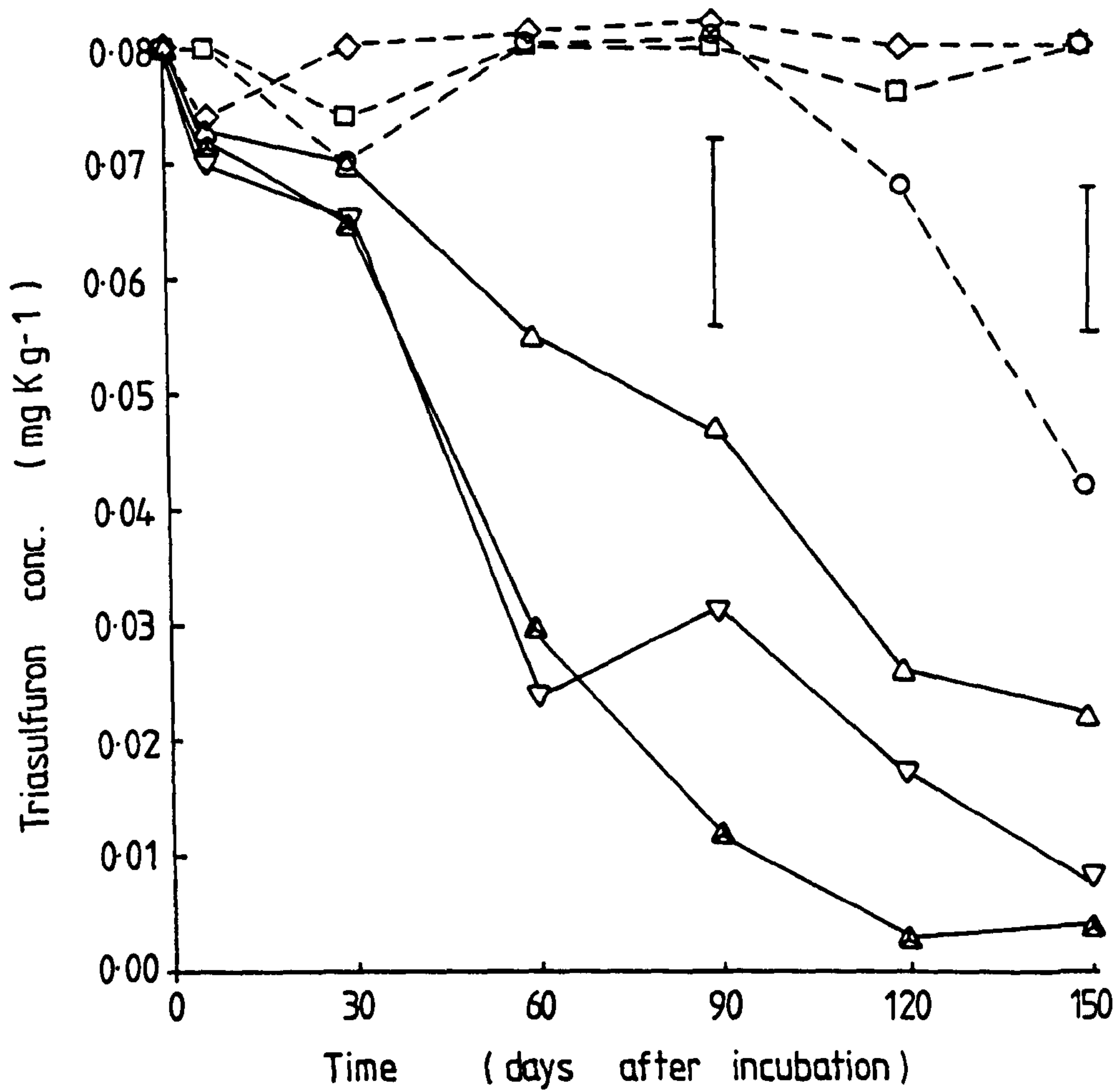




**FIG. 5.1:** Standard bioassay curve, derived from inhibitions of root extension of maize by different concentrations of Triasulfuron after 5 days of incubation at 20°C.

DEGRADATION OF TRIASULFURON

TEMPERATURE = 10°C



**FIG. 5.2:** Degradation of Triasulfuron in non-autoclaved and autoclaved soils with moisture contents of 25, 75 or 100% of field capacity at 10°C. Vertical bars represent HSD,  $P < 0.05$ . Key: --□-- autoclaved (25% moisture); --◇-- autoclaved (75% moisture); --○-- autoclaved (100% moisture); —△— non-autoclaved (25% moisture); —▽— non-autoclaved (75% moisture); —▲— non-autoclaved (100% moisture).

Table 5.1: Properties of the soil used in the investigation.

Origin	pH	Organic carbon by the Walkley-Black method (%)	Textural analysis		
			Sand	Silt	Clay
Pen-y-ffridd Experimental Station, U.C.N.W.	5.2	5.95	85.4	10.5	4.1

Table 5.2: First-order rate constants (K), half-lives ( $t_{50}$ ) and correlation coefficients of triasulfuron degradation in non-autoclaved soils.

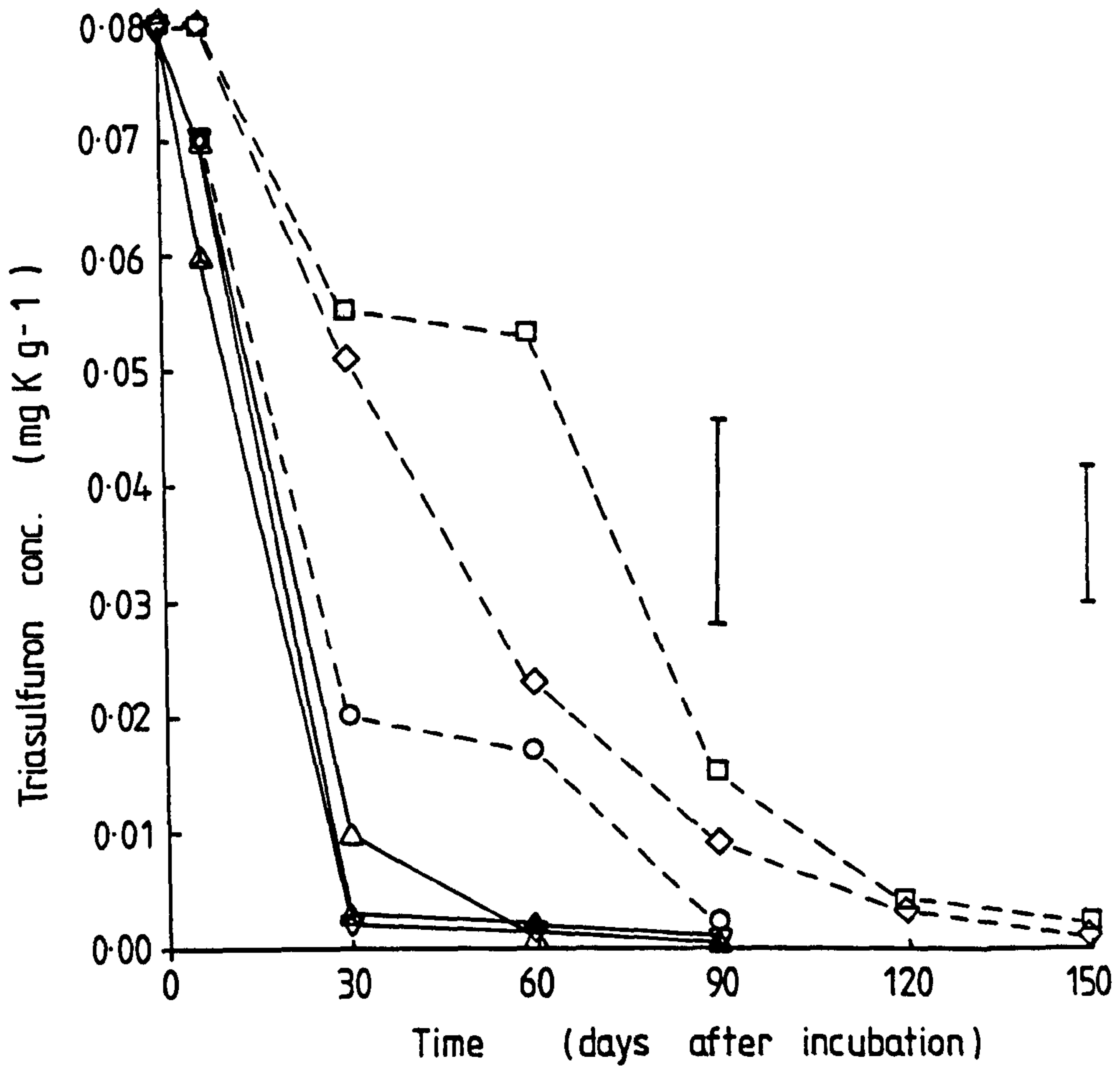
Temperature and moisture content (% of field capacity).	Rate constant ( $\text{day}^{-1}$ )	Half-life ( $t_{50}$ : days)	Correlation coefficient (r)
10°C + 25% moisture	$3.79 \times 10^{-3}$	182.9	-0.973*
10°C + 75% moisture	$6.19 \times 10^{-3}$	112.0	-0.961*
10°C + 100% moisture	$1.02 \times 10^{-2}$	68.0	-0.968*
30°C + 25% moisture	$2.62 \times 10^{-2}$	26.5	-0.986*
30°C + 75% moisture	$2.71 \times 10^{-2}$	25.6	-0.941*
30°C + 100% moisture	$2.25 \times 10^{-2}$	30.8	-0.930*

Correlation coefficient values followed by an asterik (\*) indicate significance at  $P < 0.05$ ).



DEGRADATION OF TRIASULFURON

TEMPERATURE = 30 °C



**FIG. 5.3:** Degradation of Triasulfuron in non-autoclaved and autoclaved soils with moisture contents of 25, 75 or 100% of field capacity at 30°C. Vertical bars represent HSD, P<0.05. Key: ---□--- autoclaved (25% moisture); --◇-- autoclaved (75% moisture); --○-- autoclaved (100% moisture); —△— non-autoclaved (25% moisture); —▽— non-autoclaved (75% moisture); —▲— non-autoclaved (100% moisture).

Table 5.3: First-order rate constants (K), half-lives ( $t_{50}$ ) and correlation coefficients of triasulfuron degradation in autoclaved soils.

Temperature and moisture content (% of field capacity).	Rate constant ( $\text{day}^{-1}$ )	Half-life ( $t_{50}$ : days)	Correlation coefficient (r)
10°C + 25% moisture	$2.00 \times 10^{-6}$	-	-0.008
10°C + 75% moisture	$8.90 \times 10^{-5}$	-	-0.448
10°C + 100% moisture	$1.29 \times 10^{-3}$	-	-0.722
30°C + 25% moisture	$1.11 \times 10^{-2}$	62.5	-0.965*
30°C + 75% moisture	$1.28 \times 10^{-2}$	54.2	-0.992*
30°C + 100% moisture	$1.65 \times 10^{-2}$	42.0	-0.962*

Correlation coefficient values followed by an asterik (\*) indicate significance at  $P < 0.05$ ).

**EXPERIMENT 5.2:    DEGRADATION OF TRIASULFURON IN PREVIOUSLY  
TREATED NON-AUTOCLAVED AND AUTOCLAVED SOILS.**

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**Materials and Methods.**

A second experiment was set up using the remaining non-autoclaved (with 25, 75 or 100% moisture) and autoclaved (with 100% moisture) soils which had been incubated at 30°C in Experiment 5.1. These soils had lost considerable amounts of the initial concentration of the herbicide after 90 days of incubation.

On 4 December 1987, solutions of triasulfuron (1.0 ml) were added to 1kg-batches of the soils to produce a concentration of approximately 0.08mg ai kg<sup>-1</sup> on an air-dry basis. The herbicide was thoroughly mixed into the soil and then placed in polythene bags which were incubated in the dark at 30°C. All other experimental procedures and conditions were similar to those of Experiment 5.1. All treatments had their untreated controls and they were in duplicate. Two samples of approximately 80g treated or untreated soil were removed from each bag 0, 3, 7, 15, 30 or 60 days after incubation had began and the disappearance of the herbicide was followed using the bioassay procedure already described in Experiment 5.1. Percentage root lengths of seedlings grown in samples containing the unknown concentrations were converted to values for amounts of herbicide remaining in the soil using the standard bioassay curve.

**Results.**

The patterns of degradation of triasulfuron in the non-autoclaved and autoclaved soils were similar to those of Experiment 5.1.

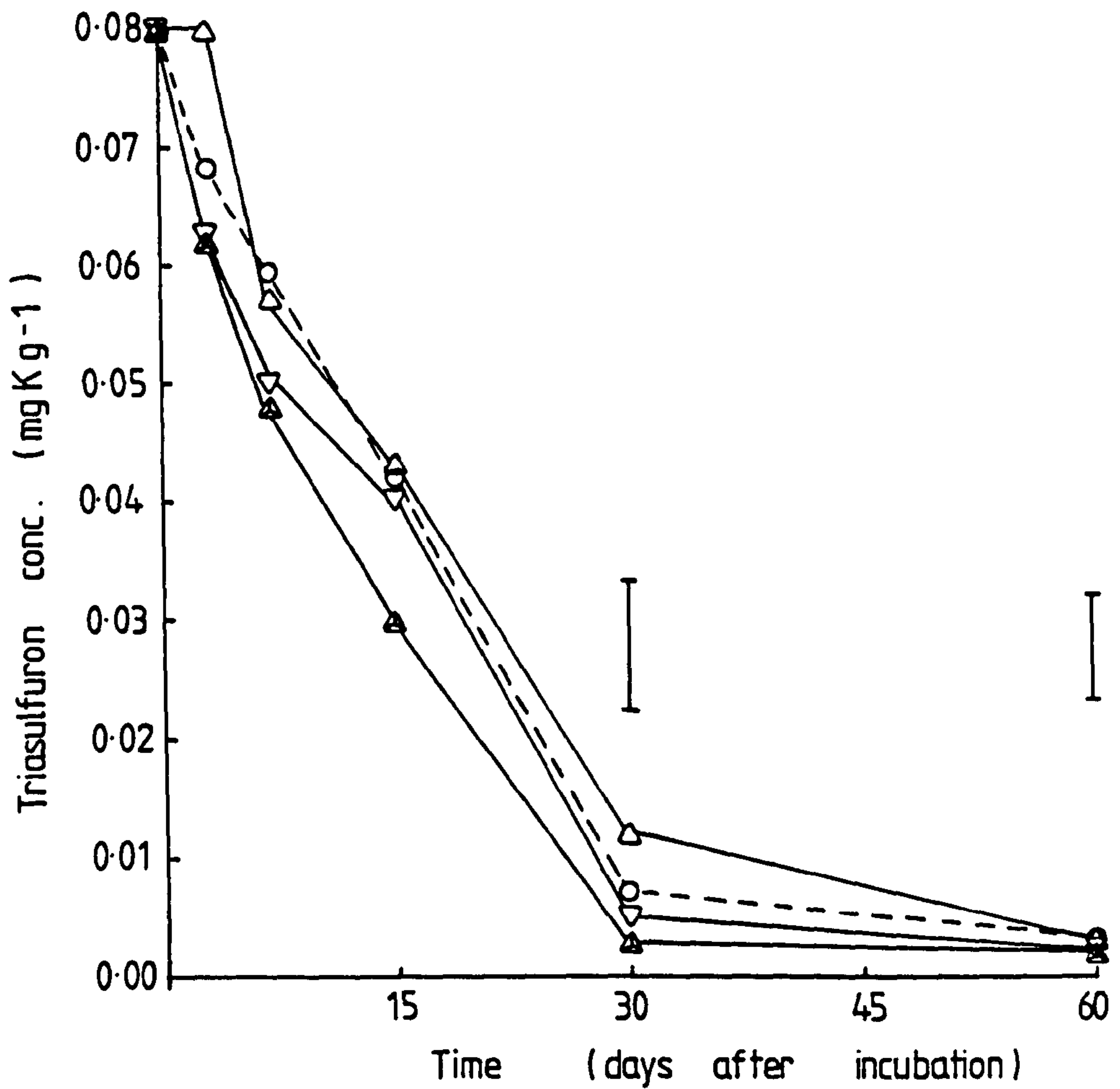


Generally degradation appeared to be rapid during the first 30 days of incubation followed by a gradual disappearance up to 60 days after incubation. Significant linear correlation coefficients ( $P < 0.05$ ) were recorded for all the plots of log concentration of residual herbicide against time. The time necessary for half the original concentration of the herbicide to decompose was considerably reduced in the non-autoclaved and autoclaved soils containing 100% moisture whilst that of non-autoclaved soil containing 75% moisture was only marginally reduced (Table 5.4) as compared to Experiment 5.1. After 60 days of incubation, considerable amounts of the herbicide had disappeared with only ca. 0.003, 0.002 and 0.002mg ai kg<sup>-1</sup> remaining in the non-autoclaved soils with moisture levels of 25, 75 and 100% respectively whereas ca. 0.003mg ai kg<sup>-1</sup> was detected in the autoclaved soil containing 100% moisture (Fig. 5.4).

Generally, there were no significant differences in the rate of breakdown of triasulfuron between the previously treated non-autoclaved and autoclaved soils used in this experiment.

DEGRADATION OF TRIASULFURON IN PREVIOUSLY TREATED SOILS.

TEMPERATURE = 30 °C



**FIG. 5.4:** Degradation of Triasulfuron in previously treated non-autoclaved (—) and autoclaved (-----) soils with moisture contents of 25, 75 or 100% of field capacity at 30°C. Vertical bars represent HSD,  $P < 0.05$ . Key: ---○--- autoclaved soil (100% moisture); —△— non-autoclaved soil (100% moisture); —▽— non-autoclaved soil (75% moisture); —△— non-autoclaved soil (25% moisture).

Table 5.4 First-order rate constants (K), half-lives ( $t_{50}$ ) and correlation coefficients of triasulfuron degradation in previously treated non-autoclaved and autoclaved soils.

Temperature and moisture content (% of field capacity).	Rate constant ( $\text{day}^{-1}$ )	Half-life ( $t_{50}$ : days)	Correlation coefficient (r)
<b>Non-autoclaved soil</b>			
30°C + 25% moisture	$2.48 \times 10^{-2}$	28.0	-0.994*
30°C + 75% moisture	$2.83 \times 10^{-2}$	24.5	-0.967*
30°C + 100% moisture	$2.89 \times 10^{-2}$	24.0	-0.938*
<b>Autoclaved soil</b>			
30°C + 100% moisture	$2.25 \times 10^{-2}$	30.8	-0.969*

Correlation coefficient values followed by an asterik (\*) indicate significance at  $P < 0.05$ ).



## DISCUSSION

The patterns of breakdown of triasulfuron in sterile and non-sterile soils were followed by using the method of inhibition of root extension of maize seedlings. A modified version of this maize root-bioassay procedure had been previously used to quantitatively estimate chlorsulfuron residues in soil (Hsiao and Smith, 1983). The relatively fast root growth of maize, coupled with its ability to withstand the removal of soil under tap water with little damage prior to root length determinations made it a suitable indicator species for the present experiments. From preliminary experiments on the response of maize root extension to different concentrations of triasulfuron, a 5-day incubation period was found to give the most consistent results and was therefore used as the standard time of incubation throughout the studies.

In agreement with other studies on the degradation of the sulfonylurea herbicides, Figures 5.2 and 5.3 showed a faster dissipation of triasulfuron in the non-autoclaved soil than in the autoclaved soil. Joshi et al. (1985) also found that the degradation of chlorsulfuron was consistently faster in non-sterilized soil than in soil sterilized with either ethylene oxide or gamma irradiation. The authors obtained further proof of the importance of soil microbes in the degradation of chlorsulfuron when they found that rapid degradation of the herbicide had been restored in sterile soil following re-innoculation with indigenous soil micro-organisms. In addition, they showed that a suspension of numerous micro-organisms including actinomycetes, fungi and bacteria isolated from non-sterilized soil actively metabolized 95% of  $^{14}\text{C}$ -chlorsulfuron in pure

culture within 11 days but only 5% degradation of a similar quantity of  $^{14}\text{C}$ -chlorsulfuron occurred in an autoclaved microbial suspension over the same period. Similarly, thiameturon has been reported to undergo rapid degradation by soil micro-organisms and several fungi, bacteria and actinomycetes isolated from fresh field soils were found to degrade readily the herbicide in pure culture (Brown et al., 1987). The present results amply demonstrate that soil microbes are at least, partly involved in the breakdown of triasulfuron since herbicide loss was generally faster in the non-autoclaved than in the autoclaved soil at all moisture and temperature conditions. There was virtually no degradation of triasulfuron in the autoclaved soil incubated at  $10^{\circ}\text{C}$ , with moisture contents of 25 and 75% of field capacity but some dissipation of the herbicide was observed in the autoclaved soil with 100% moisture (field capacity) (Fig. 5.2). On the other hand, the breakdown of the herbicide in the autoclaved soils incubated at  $30^{\circ}\text{C}$  proceeded at a relatively fast rate at all moisture levels (Fig. 5.3). It is possible that decomposition of the herbicide by chemical processes might have been responsible for much of the disappearance of the herbicide in these soils since the autoclaving would have either killed or drastically reduced the microbial population. Triasulfuron (Amrein and Gerber, 1985) and indeed almost all the sulfonylurea herbicides (Beyer et al., 1987) are believed to undergo degradation by both microbiological and chemical processes.

A rapid disappearance of triasulfuron was noted during the first 30 days of incubation followed by a constant and slower rate of dissipation especially in the non-autoclaved soil at  $30^{\circ}\text{C}$  (Fig. 5.3).



In non-autoclaved soils incubated at 30°C and containing 25, 75 or 100% moisture, 87.5, 97.5 and 96.3% respectively of the initial concentration of the herbicide had disappeared by the end of the first 30 days after incubation had began but only 11.9, 2.1 and 2.7% of the initial concentration respectively disappeared during the following 60 days (Fig. 5.3). A similar pattern of breakdown was obtained for chlorsulfuron in 4 different soils when a rapid disappearance of the herbicide was observed during the first 15 days of incubation followed by a constant and slower rate in all the soils (Thirunarayanan et al., 1985). Duffy et al. (1987) have also pointed out that an important feature of the degradation curves obtained from studies with sulfonylurea herbicides in non-sterile microbially active soils in laboratory experiments is an initial rapid decay followed by a period of slower degradation. The authors noted that, for chlorsulfuron, the transition to the slower breakdown phase usually occurred after 2 to 4 weeks. Hamaker and Goring (1976) proposed a two-compartment model to explain this pattern of herbicide breakdown in soil. They suggested that a large proportion of a herbicide is usually available for rapid degradation immediately after application. However with time, a proportion of the compound becomes adsorbed and therefore less is available and thus disappearance slows down eventually reaching a steady state and proceeding at a rate determined by the amount of herbicide present in the available pool. This pattern of breakdown has been reported for other herbicides. For example, herbicides such as metribuzin {4-amino-6-tert-butyl-3-(methylthio)-as-triazin-5(4H)-one} (Hyzack and Zimdahl, 1974), trifluralin { $\alpha$ ,  $\alpha$ ,  $\alpha$ -trifluoro-2,6-dinitro-N-N-dipropyl-p-toluidine}, fluchloralin {N-(2-chloroethyl)-2,6-dinitro-N-



propyl-4-(trifluoromethyl)anilide} and pendimethalin {N-(*n*-ethyl-propyl)-3,4-dimethyl-2,6-dinitro-benzeneamine} showed an initial rapid loss followed by a slower rate of dissipation (Savage and Jordan, 1980). The soil used in the present studies had a relatively high organic carbon content and therefore a proportion of triasulfuron might have been adsorbed and the subsequent slow release into the soil solution might have resulted in a reduced rate of degradation by the soil micro-organisms after the initial 30 days of incubation.

The rapid initial breakdown of triasulfuron could also have been caused by the method used in the present studies whereby triasulfuron was added to air-dry soil followed by wetting which probably led to a flush of microbial activity. Stevenson (1956) observed an increase in microbial activity in re-moistened air-dried soil. However, Jenkinson and Powlson (1976) discussed the effects of mechanical disturbance and air drying on microbial respiration and concluded that these effects can either be stimulatory or inhibitory.

Temperature and soil moisture affected the rate of degradation of triasulfuron in both non-autoclaved and autoclaved soils. In general, the breakdown of the herbicide proceeded at a much slower rate at 10°C than it did at 30°C (Fig. 5.2 and 5.3). The degradation of the herbicide was interpreted by first order kinetics and much longer half-lives were obtained at the lower temperature (10°C) than at 30°C (Tables 5.2 and 5.3). With the exception of the non-autoclaved soil incubated at 30°C, the degradation rate also generally increased directly with moisture content (Tables 5.2 and 5.3). These results were not unexpected since it is generally accepted that the rates of both microbiological and chemical breakdown of herbicides are

markedly influenced by temperature and moisture content. The results of the present studies lend further credence to those obtained in Experiment 4.4 of Chapter 4 where residues of triasulfuron were detected in all segments of herbicide-treated 30cm soil columns after exposing them to a total of 528mm natural rain over 6 months. Average maximum soil temperature (10cm depth) over this period (15 April to 14 October 1987) was 13.7°C and this relatively low temperature might have contributed to the slow rate of dissipation of the herbicide over the 6-month period. In an earlier study on the degradation of triasulfuron in soils with pH 6.4, and 25, 50 or 75% moisture at 21 or 35°C, the rate of herbicide loss proceeded at a faster rate at 35°C and the rate of breakdown followed first order kinetics (Amrein and Gerber, 1985). The same authors obtained the shortest half-lives of 20 and 14 days at 21°C and 35°C respectively in soil containing 75% moisture whereas 73 and 34 days were obtained at 21°C and 35°C respectively for soils containing 25% moisture. In comparison with the present studies, half-lives of 112 and 25.6 days were recorded at 10°C and 30°C respectively in non-autoclaved soils (pH 5.2) containing 75% moisture whilst 182.9 and 26.5 days were needed at 10°C and 30°C respectively for soils with 25% moisture. Increased soil moisture and temperature have also been reported to enhance the breakdown of chlorsulfuron in different soils (Walker and Brown, 1983a; Joshi et al., 1985; Thirunarayanan et al., 1985 and Fredrickson and Shea, 1986). However increased soil moisture did not enhance the degradation of metsulfuron in loam soil at 16 or 24°C but that of sand was accelerated at 24°C (Anderson, 1985).

Soil pH has been identified in several studies as one of the major factors affecting the persistence of the sulfonylurea



herbicides. For example, Peterson and Arnold (1985) found less persistence of chlorsulfuron at locations with pH of less than 5.5 than near pH 7. In general, degradation of the sulfonylurea herbicides has been reported to be enhanced by acid pH (Beyer et al., 1987). Results from degradation studies conducted in four soils under laboratory conditions at 75% of field capacity and at 20°C gave half-lives of 38.1, 60.2, 82.0 and 99.0 days for soils with pH 6.2, 7.1, 7.7 and 8.1 respectively when the calculation included time-zero (Thirunarayanan et al., 1985). Similarly, Flom et al. (1986) also demonstrated the effect of pH on chemical degradation when they observed that six weeks after incubating autoclaved soil samples at 30°C with moisture content of 80% of field capacity, approximately 90% of <sup>14</sup>C-chlorsulfuron applied to samples of soil at pH 4 were converted to the primary metabolites whereas in soil at pH 9, over 90% was the parent chlorsulfuron molecule. Joshi et al. (1985) have pointed out that the dissipation of chlorsulfuron is generally faster in acidic soil where both metabolism by microbes and chemical hydrolysis contribute to its degradation; but in alkaline soils, chemical hydrolysis is slow and dissipation of chlorsulfuron proceeds primarily by microbial processes. The rapid breakdown of triasulfuron in the soil used in the present studies might as well have been influenced by the acidic pH of the soil (pH 5.2) which provided favourable conditions for microbial and possibly chemical degradation in the non-autoclaved soil and chemical degradation in the autoclaved soil.

In Experiment 5.2, subsequent addition of triasulfuron to autoclaved and non-autoclaved soil in which previous application of



the herbicide had decomposed did not affect the pattern of breakdown. The pattern of herbicide loss (Fig. 5.4) was similar to that of Experiment 5.1 (Fig. 5.3) and was characterized by a rapid initial degradation followed by a steady rate of dissipation. Sixty days after incubation had began, the levels of herbicide residues in the non-autoclaved soils were similar to those obtained in Experiment 5.1 after the same period (Figs. 5.3 and 5.4). In a similar study, Brown et al. (1987) observed an exceptionally rapid microbial degradation of thiameturon with 0.5 to 2-day half-lives in several fresh field soils covering a wide range of pH, organic matter, sand, silt and clay compositions. However, repeat applications of the herbicide to the soils did not diminish or enhance this rapid degradation. The half-life of the herbicide in the autoclaved soil containing 100% moisture was considerably reduced to 30.8 days (Table 5.4: calculated after 60 days of incubation) compared with 42 days recorded in Experiment 5.1 (Table 5.3: calculated after 90 days of incubation). The difference may be partly due to the calculation of the half-lives over different time intervals. Taking into consideration the slower rate of degradation of triasulfuron which occurred after the first 30 days of incubation, it follows logically that calculations from first order plots over a shorter period may give shorter half-lives. Thirunarayanan et al. (1985) made a similar conclusion when they found that the half-lives of chlorsulfuron were 38.1, 60.2, 82.0 and 99.0 days for soils at pH 6.2, 7.1, 7.7 and 8.1 respectively when the calculation included time-zero but half-lives calculated from slopes of first order plots beginning at day 15 (after initial rapid degradation) were 88.8, 105.0, 135.8 and 143.3 days respectively for the same pH levels. There were no significant differences between

the rates of degradation of herbicide in autoclaved soil (with 100% moisture) and the non-autoclaved soils in the present experiment. This might partly be explained by the fact that microbial populations might have proliferated and become active with time in the autoclaved soil and hence dissipation of the herbicide might have occurred by both chemical and microbial processes.

In conclusion, the present studies have shown that microbial decomposition is partly responsible for the disappearance of triasulfuron from soils and the rate of such breakdown is markedly affected by temperature and soil moisture. A direct implication of this is that in soils which are warm and moist and possibly with low pH, the breakdown of triasulfuron may occur at a faster rate but in cold soils, herbicide losses may be slower with the possibility of residues persisting over a longer period.

CHAPTER 6.

EFFECTS ON CELL DIVISION.



## INTRODUCTION.

One of the characteristic symptoms of the effects of triasulfuron on susceptible plants is inhibition of growth. As reported in Chapter 2, the first obvious symptom of the phytotoxicity of triasulfuron in susceptible species is growth inhibition expressed by reduced plant height and stunted appearance. Other morphological symptoms such as chlorosis and necrosis appear later.

Cell division is one of the processes that is basic to the growth of plants for without new cells being produced growth will ultimately stop. Many cells in root and shoot tip meristematic regions go through continuous cell division cycles. Each cycle is a sequential progression of steps composed of the interphase which is made up of G1 (Gap1), S (DNA synthesis) and G2 (Gap2) and mitosis (M). G1 and G2 represent the stages during which the cells become metabolically prepared for passing through DNA synthesis and mitosis respectively (Howard and Pelc, 1953). The mitotic stage consists of prophase, metaphase, anaphase and telophase. These events are related in the sense that, for one stage to be entered, the one preceding it must be completed and the stages are controlled by certain specific requirements which were identified in the General Introduction (Chapter 1). Inhibition of any of the requirements by environmental factors such as temperature, oxygen, biotic, radiation, nutritional and chemical stress either by scarcity or excess will affect the cell cycle (Rost, 1977).

Chemicals may affect cell division in one of three ways. They may be: pre-prophase inhibitors which arrest cell cycle progression at some stage of interphase (G1, S or G2) thereby inhibiting the passage

of cells into mitosis, chemicals which interfere with the synthesis or the orientation of the mitotic spindle thus disrupting the controlled movement of mitotic chromosomes or chemicals which inhibit cytokinesis by preventing the formation of cell plate and cell wall between daughter cells resulting in the formation of binucleate cells (Kihlman, 1966; Rost, 1977). Some herbicides such as the chloroacetamides (Deal and Hess, 1980) and cinmethylin {exo-1-methyl-4(1-methylethyl)-2-[(2-methylphenyl)methoxy]-7-oxabicyclo [2.2.1]heptane} (Mahmound and Hess, 1986) have been reported to inhibit growth by inhibiting entry of cells into mitosis. Other herbicides like the dinitroanilines are known to disorganize nuclear division and the formation of cell plate and cell wall (HacsKaylo and Amato, 1968; Hess and Bayer, 1974; Parka and Soper, 1977).

The sulfonylurea herbicides such as chlorsulfuron (Ray, 1980; 1982), thiameturon {3-(3-(4 methoxy-6-methyl-1,3,5-triazin-2-yl) ureidosulphonyl)thiopene-2-carboxylate} (Sionis et al., 1985) and DPX-L5300 {Methyl 2-[3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-3-methylureidosulphonyl]benzoate} (Ferguson et al., 1985) are believed to inhibit plant growth by disrupting cell division. Chlorsulfuron has been demonstrated to inhibit cell division in broad bean and corn root tips at concentrations of  $1 \text{ mg l}^{-1}$  and  $2.8 \text{ nM}$  respectively (Ray, 1980; 1982). Ray (1982) reported that although the mitotic indices of chlorsulfuron-treated root tips of broad bean were significantly reduced below those of the control root tips, the frequency distribution of the various mitotic stages in the treated root tips did not show any significant change over that of the untreated root tips. He suggested that chlorsulfuron inhibition of cell division



occurred at some stage other than the mitotic in the cell cycle.

Attempts to discover the primary site of action of chlorsulfuron showed that the basic processes of the plant cell such as respiration, photosynthesis, protein and RNA synthesis were significantly affected only when the herbicide concentration was higher than that required to inhibit cell division (Ray, 1982). Subsequent studies with pea root tips revealed that chlorsulfuron inhibits cell cycle progression by blocking both G1 and G2 phases without interfering directly with mitosis and DNA synthesis (Rost, 1984). In another study, Ray (1984) showed with pea plants that acetolactate synthase, a key enzyme of the common biosynthetic pathway of valine, isoleucine and leucine was inhibited in vitro by chlorsulfuron and the addition of valine and isoleucine together protected cultured and whole plants against the growth inhibition induced by chlorsulfuron. He concluded that the basis for the effect of chlorsulfuron on cell division, was the inhibition of the biosynthesis of the essential amino acids valine and isoleucine. Similarly, Chaleef and Mauvais (1984) used tobacco (Nicotiana tabacum L.) mutants resistant to chlorsulfuron and sulfometuron methyl {2-[[[(4,6-dimethyl-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoic acid} to confirm that acetolactate synthase is the site of action of these herbicides in higher plants.

Contrary to these findings, Giardina et al. (1987) applied the same concentrations of chlorsulfuron, valine and isoleucine as did Ray (1984), to three genotypes of pea and two genotypes of maize but found the treatments ineffective in reducing the growth inhibition induced by chlorsulfuron even though the enzyme acetolactate synthase was inhibited in vitro by the herbicide in both pea and maize. These



authors therefore suggested that the mechanism of action of chlorsulfuron may involve more than one site of activity.

In studying the effects of chemicals on cell division, the most common procedure is to categorize the cells in a root tip meristem into interphase, prophase, metaphase, anaphase, telophase or aberrant. Changes in the frequency of these stages in relation to untreated roots can reveal important information with respect to the site of action of the herbicide in the cell cycle sequence. Normally, when the frequency of cells in mitosis decreases, the site of action is deduced to be on a process occurring during G1, S or G2 and the effect is an inhibition of mitotic entry whilst abnormal mitotic figures in herbicide-treated roots are usually held to indicate a disruption in the mitotic phase (Hess, 1982).

Very little has been reported on the mechanism of action of triasulfuron in plants. However, because of its strong inhibition of growth in susceptible species, attempts were made to study the effects of the herbicide on cell division at the root tips of broad bean (a susceptible species) and barley (a tolerant species) as well as the influence of the amino acids, valine and isoleucine in preventing the inhibition of cell division.

**EXPERIMENT 6.1: EFFECTS OF TRIASULFURON ON THE MITOTIC INDEX AND DISTRIBUTION OF MITOTIC STAGES IN ROOT TIPS OF VICIA FABEA.**

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**Materials and Methods.**

On 7 October 1987, seeds of Vicia faba cv. Bunyard's Exhibition were germinated for 72 h in moist vermiculite at 30°C in the dark. Seedlings were then transferred into 9cm diameter petri-dishes lined with Whatman No.3 filter paper and containing 15ml of 0 (de-ionized water), 2 or 100 mg l<sup>-1</sup> triasulfuron solution. The dishes were kept at 20°C in the dark for 6 h after which 1cm long root tips were excised and using a method described by Dyer (1979), the root tips were processed for examination of mitotic figures.

The excised roots were fixed for 5 min in a solution made up of absolute ethanol, chloroform, glacial acetic acid and formalin in the ratios of 10:2:2:1 respectively. After fixation, the root tips were briefly washed in 70% ethanol and then heated at 60°C for 5 min in 1M HCL. They were then transferred into water and 1mm long root tips were cut on a slide and tapped and teased with a metal rod in a drop of 60% lacto-propionic orcein. A second drop of the stain was then added and a coverslip was lowered onto the preparation. The coverslip was tapped gently with a needle point to disperse the material and then pressed through a blotting paper. The preparation was left for 24 h.

Squashes of five treated and five untreated root tips were examined under a Leitz Wetzlar light microscope at a magnification of x400. A graticule was fitted to one of the eyepieces. Counts of actively dividing and non-dividing cells were made in squares (each

300 $\mu$ m x 300 $\mu$ m) at five different locations on each slide. The mitotic index (number of dividing cells per 100 cells) and the frequency distribution of the various stages of mitosis were determined.

On 17 October 1987, the experiment was repeated and the procedures already described were followed for the determination of the mitotic index and frequency distribution of the mitotic figures. The diameters of 25 randomly selected nuclei and nucleoli of non-dividing cells per preparation were measured using a graticule fitted to one eye-piece. Six squash preparations of treated and of untreated controls were examined.

## Results.

All the stages of mitosis viz; prophase, metaphase, anaphase and telophase were observed in both treated and untreated root tips. However there were more actively dividing cells per unit area in the preparations for the untreated root tips than for the treated root tips. The mitotic indices were consequently much lower in roots treated with 2 or 100 mg l<sup>-1</sup> triasulfuron solution than in those of the untreated root tips. In both experiments, the higher concentration of 100 mg l<sup>-1</sup> appeared to have caused slightly more inhibition of cell division than did the lower concentration. The frequency distribution of the various mitotic stages in the treated root tips did not show any significant change over that found in the untreated root tips (Tables 6.1 and 6.2). Apart from a few mitotic figures in the treated root tips where thickened and shortened chromosomes were observed, no other obvious abnormalities of the dividing cells were recorded. The diameters of the nuclei and nucleoli of root tips treated with 2 and 100 mg l<sup>-1</sup> triasulfuron were



slightly less than those of the controls (Table 6.3).

Table 6.1: Effects of 2 or 100 mg l<sup>-1</sup> triasulfuron on the mitotic index and distribution of mitotic stages in root tips of Vicia faba.

Herbicide dose (mg l <sup>-1</sup> )	Mitotic index per 100 cells (Mean ± S.E)	Percentage distribution			
		Prophase	Metaphase	Anaphase	Telophase
Control	9.6 ± 1.0	44.7	24.2	16.5	14.6
2	4.0 ± 0.6	48.6	27.8	11.1	12.5
100	2.8 ± 0.8	63.0	16.0	12.0	9.0

Table 6.2: Effects of 2 or 100 mg l<sup>-1</sup> triasulfuron on the mitotic index and distribution of mitotic stages in root tips of Vicia faba.

Herbicide dose (mg l <sup>-1</sup> )	Mitotic index per 100 cells (Mean ± S.E)	Percentage distribution			
		Prophase	Metaphase	Anaphase	Telophase
Control	12.8 ± 0.7	42.9	24.7	15.8	16.7
2	4.8 ± 0.6	54.2	21.1	13.2	11.6
100	3.2 ± 0.5	46.3	30.6	11.1	12.0

Table 6.3: Effects of 2 or 100 mg l<sup>-1</sup> triasulfuron on the diameter of nuclei and nucleoli of root tips of Vicia faba.

Herbicide dose (mg l <sup>-1</sup> )	Diameter of nuclei (μm) (Mean ± S.E)	Diameter of nucleoli (μm) (Mean ± S.E)
Control	15.6 ± 0.4	9.1 ± 0.3
2	14.3 ± 0.2	7.4 ± 0.3
100	13.7 ± 0.2	6.8 ± 0.3

**EXPERIMENT 6.2: EFFECTS OF TRIASULFURON ON THE MITOTIC INDEX AND DISTRIBUTION OF MITOTIC STAGES IN ROOT TIPS OF WINTER BARLEY.**

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**Materials and Methods.**

On 3 November 1987, seeds of winter barley cv. Maris Otter were germinated for 72 h on moist Whatman No. 3 filter paper in 9cm diameter petri-dishes at 20°C in the dark. The seedlings were transferred into fresh petri-dishes lined with Whatman No. 3 filter paper and containing 15ml of 0 (de-ionized water) or 2 mg l<sup>-1</sup> triasulfuron solution. The petri-dishes were kept for 6 h at 20°C in the dark after which root squashes were prepared for examination using the procedures outlined in Experiment 6.1. Squashes of seven treated and ten untreated control root tips were examined.

**Results.**

Triasulfuron at a concentration of 2 mg l<sup>-1</sup> did not inhibit cell division in winter barley cv. Maris Otter. Mitotic indices of 8.2 and 7.8 respectively, were recorded for the untreated and treated root tips. Similarly, the frequency distribution of the various stages of mitosis in the untreated and treated root tips were not different from each other (Table 6.4).



Table 6.4: Effects of 2 mg l<sup>-1</sup> triasulfuron on the mitotic index and distribution of mitotic stages in root tips of winter barley cv. Maris Otter.

Herbicide dose (mg l <sup>-1</sup> )	Mitotic index per 100 cells (Mean ± S.E)	Percentage distribution			
		Prophase	Metaphase	Anaphase	Telophase
Control	8.2 ± 1.1	47.7	25.3	15.8	11.2
2	7.8 ± 1.2	47.8	21.2	16.1	14.9

**EXPERIMENT 6.3: CELL DIVISION IN PREVIOUSLY TREATED (TRIASULFURON) VICIA FABA ROOT TIPS AFTER 24 AND 48 HOURS IN HERBICIDE-FREE VERMICULITE.**

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**Materials and Methods.**

On 20 December 1987, seeds of *Vicia faba* cv. Bunyard's Exhibition were sown in moist vermiculite and kept for 72h at 30°C in the dark. The seedlings were transferred into 9cm diameter petri-dishes lined with Whatman No.3 filter paper and containing 15ml of 0 (de-ionized water) or 2 mg l<sup>-1</sup> triasulfuron solution. The dishes were kept for 6h at 20°C in the dark. The seedlings were then removed and washed several times with de-ionized water and carefully transplanted into moist vermiculite which was kept for 24 or 48h at 20°C in the dark. Squashes of five root tips of each treatment were prepared and the mitotic index, the frequency distribution of the mitotic figures and the diameter of nuclei and nucleoli of 25 randomly selected non-dividing cells per squash were determined using the methods previously described.

**Results.**

Cell division in roots previously treated for 6h with 2 mg l<sup>-1</sup> triasulfuron did not recover after 24 or 48h in herbicide-free moist vermiculite. A much lower mitotic index of 1.3 was recorded after 48h than after 24h (3.2) in the moist vermiculite. There were no marked differences between the frequency distributions of the mitotic stages in the treated and untreated root tips even though fewer anaphase and telophase stages were observed in the treated root tips after 24h whereas there were more prophase figures in the treatment

which was kept in moist vermiculite for 48 h (Tables 6.5 and 6.6). Triasulfuron slightly reduced the diameter of the nuclei and nucleoli of treated roots below those of the control (Table 6.7).

Table 6.5: Cell division in previously treated ( $2 \text{ mgl}^{-1}$  triasulfuron solution for 6 h) Vicia faba roots after 24 h in herbicide-free vermiculite.

Herbicide dose ( $\text{mgl}^{-1}$ )	Mitotic index per 100 cells (Mean $\pm$ S.E)	Percentage distribution			
		Prophase	Metaphase	Anaphase	Telophase
Control	12.3 $\pm$ 1.1	46.6	20.4	18.6	14.4
2	3.2 $\pm$ 1.0	50.7	36.8	6.9	5.6



Table 6.6: Cell division in previously treated ( $2 \text{ mgl}^{-1}$  triasulfuron solution for 6 h) Vicia faba roots after 48 h in herbicide-free vermiculite.

Herbicide dose ( $\text{mgl}^{-1}$ )	Mitotic index per 100 cells (Mean $\pm$ S.E)	Percentage distribution			
		Prophase	Metaphase	Anaphase	Telophase
Control	12.2 $\pm$ 0.4	44.3	23.1	17.1	15.6
2	1.3 $\pm$ 0.9	60.7	16.4	11.5	11.5

Table 6.7: Diameter of nuclei and nucleoli of previously treated Vicia faba roots after 24 or 48 h in herbicide-free vermiculite.

Herbicide dose ( $\text{mgl}^{-1}$ )	24 h.		48 h.	
	Diameter of nuclei ( $\mu\text{m}$ ) (Mean $\pm$ S.E)	Diameter of nucleoli ( $\mu\text{m}$ ) (Mean $\pm$ S.E)	Diameter of nuclei ( $\mu\text{m}$ ) (Mean $\pm$ S.E)	Diameter of nucleoli ( $\mu\text{m}$ ) (Mean $\pm$ S.E)
Control	15.6 $\pm$ 0.3	8.8 $\pm$ 0.3	14.7 $\pm$ 0.5	9.1 $\pm$ 0.3
2	13.2 $\pm$ 0.3	7.1 $\pm$ 0.3	12.9 $\pm$ 0.3	6.7 $\pm$ 0.3

**EXPERIMENT 6.4: THE INFLUENCE OF ISOLEUCINE AND VALINE IN PREVENTING OR REVERSING THE INHIBITION OF MITOTIC ENTRY IN TRIASULFURON-TREATED VICIA FABA ROOT TIPS.**

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**Materials and Methods.**

On 3 January 1988, seeds of Vicia faba cv. Aquadulce Claudia were germinated using the methods described in the earlier experiments. The seedlings were treated with 15 ml of 0 (de-ionized water); 0.02 or 2  $\text{mg l}^{-1}$  triasulfuron solution; a 1:1 mixture of isoleucine (100 $\mu\text{M}$ ) and valine (100 $\mu\text{M}$ ); 0.02  $\text{mg l}^{-1}$  triasulfuron plus a 1:1 mixture of isoleucine and valine at 100 $\mu\text{M}$  or 2  $\text{mg l}^{-1}$  triasulfuron plus a 1:1 mixture of 100 $\mu\text{M}$  isoleucine and valine. The dishes were kept for 6 h at 20°C in the dark after which root squashes were prepared and the mitotic indices and frequency distributions were determined using the procedures described in Experiment 6.1. Five squashes from each treatment were examined.

A second experiment similar to the above was started on 10 January 1988 using the same variety of V. faba. The germinated seedlings were treated with 0 (de-ionized water); 1  $\text{mg l}^{-1}$  triasulfuron; a 1:1 mixture of isoleucine and valine at 100  $\text{mg l}^{-1}$  or 1  $\text{mg l}^{-1}$  triasulfuron plus a 1:1 mixture of isoleucine and valine at 100  $\text{mg l}^{-1}$ . The seedlings were exposed to the treatments for 6 h at 20°C in the dark after which five squashes per each treatment were prepared and examined.

In another experiment started on 18 January 1988, seeds of V. faba cv. Aquadulce Claudia were germinated as previously described. They were treated with 15 ml of either de-ionized water; 1  $\text{mg l}^{-1}$

triasulfuron; a 1:1 mixture of isoleucine ( $50 \text{ mgl}^{-1}$ ) plus valine ( $50 \text{ mgl}^{-1}$ ) or they were first treated with  $1 \text{ mgl}^{-1}$  triasulfuron for 6h and then transferred into a 1:1 mixture of isoleucine ( $50 \text{ mgl}^{-1}$ ) and valine ( $50 \text{ mgl}^{-1}$ ) and kept at  $20^{\circ}\text{C}$  in the dark for 18 h. Five root squashes per each treatment were prepared and examined following the procedures already outlined.

This experiment was repeated on 26 January 1988 without including the treatment which consisted of treating the seedlings with a 1:1 mixture of isoleucine and valine at  $50 \text{ mgl}^{-1}$ . Five squashes per each treatment were prepared and examined.

### Results.

Triasulfuron at concentrations of  $0.02$  or  $2 \text{ mgl}^{-1}$  reduced the mitotic index below that of the control. The mitotic index of roots treated with a 1:1 mixture of isoleucine and valine at  $100\mu\text{M}$  was not affected. Similarly, the mitotic index was not different from that of controls, in roots treated with triasulfuron plus a 1:1 mixture of  $100\mu\text{M}$  isoleucine and valine. The frequency distributions of the mitotic stages of all the treatments were not different from those of the control (Table 6.8).

In the second experiment,  $1 \text{ mgl}^{-1}$  triasulfuron reduced the mitotic index below that of the control whereas the mitotic index of roots treated with a 1:1 mixture of isoleucine and valine at  $100 \text{ mg l}^{-1}$  was not affected. The mitotic index of root tips which had been treated with  $1 \text{ mgl}^{-1}$  triasulfuron plus a 1:1 mixture of isoleucine and valine at  $100 \text{ mgl}^{-1}$  was lower than that of the control but was much higher than that of roots treated with triasulfuron alone (Table 6.9). Similar results were obtained when the concentration of



isoleucine and valine was reduced to 50 mg l<sup>-1</sup> (Tables 6.10 and 6.11).

There appeared to be some reversal of triasulfuron inhibition of cell division when roots previously treated with triasulfuron were transferred into a 1:1 mixture of isoleucine and valine at 50 mg l<sup>-1</sup>. The mitotic index obtained for this treatment was lower than that recorded for the control but was higher than that of roots treated with triasulfuron alone (Tables 6.10 and 6.11).

Table 6.8: Effects of isoleucine and valine, triasulfuron and their combination on mitosis of root tips of Vicia faba.

Herbicide and/or Amino acid dose	Mitotic index per 100 cells (Mean $\pm$ S.E)	Percentage distribution			
		Prophase	Metaphase	Anaphase	Telophase
Control	6.0 $\pm$ 1.3	63.7	19.4	8.3	8.3
0.02 (mg l <sup>-1</sup> ) triasulfuron	4.4 $\pm$ 0.7	68.5	10.8	16.2	4.5
2 (mg l <sup>-1</sup> ) triasulfuron	3.8 $\pm$ 0.5	59.8	18.4	13.8	8.1
100 $\mu$ M valine + isoleucine	6.7 $\pm$ 0.6	59.7	13.4	12.8	14.1
0.02 (mg l <sup>-1</sup> ) triasulfuron + 100 $\mu$ M valine + isoleucine	5.5 $\pm$ 1.2	47.2	20.4	18.5	13.9
2 (mg l <sup>-1</sup> ) triasulfuron + 100 $\mu$ M valine + isoleucine	5.9 $\pm$ 0.9	64.9	20.6	6.9	7.6

Table 6.9: Effects of isoleucine and valine, triasulfuron and their combination on mitosis of root tips of Vicia faba.

Herbicide and/or Amino acid dose	Mitotic index per 100 cells (Mean $\pm$ S.E)	Percentage distribution			
		Prophase	Metaphase	Anaphase	Telophase
Control	8.5 $\pm$ 1.3	54.7	16.8	16.8	11.8
1.0 mgl <sup>-1</sup> triasulfuron	2.7 $\pm$ 0.4	59.6	26.9	7.7	5.8
100 mgl <sup>-1</sup> valine + isoleucine	8.3 $\pm$ 1.1	52.5	18.9	13.9	14.8
1.0 mgl <sup>-1</sup> triasulfuron + 100 mgl <sup>-1</sup> valine + isoleucine	5.3 $\pm$ 0.5	58.1	22.6	9.7	9.7



Table 6.10: Effects of isoleucine and valine, triasulfuron and their combination on mitosis of root tips of Vicia faba.

Herbicide and/or Amino acid dose	Mitotic index per 100 cells (Mean $\pm$ S.E)	Percentage distribution			
		Prophase	Metaphase	Anaphase	Telophase
Control	7.9 $\pm$ 0.7	47.1	16.1	20.1	16.7
1.0 mg l <sup>-1</sup> triasulfuron	3.7 $\pm$ 0.9	58.8	18.8	13.8	8.8
50 mg l <sup>-1</sup> valine + isoleucine	7.1 $\pm$ 1.3	44.7	23.5	17.4	14.4
1.0 mg l <sup>-1</sup> triasulfuron + 50 mg l <sup>-1</sup> valine + isoleucine	6.6 $\pm$ 1.3	57.5	21.2	10.6	10.6
1.0 mg l <sup>-1</sup> triasulfuron (first) <sub>1</sub> + 50 mg l <sup>-1</sup> valine + isoleucine (later).	5.5 $\pm$ 0.9	58.7	19.8	12.2	9.3

Table 6.11: Effects of isoleucine and valine, triasulfuron and their combination on mitosis of root tips of Vicia faba.

Herbicide and/or Amino acid dose	Mitotic index per 100 cells (Mean $\pm$ S.E)	Percentage distribution			
		Prophase	Metaphase	Anaphase	Telophase
Control	10.4 $\pm$ 0.7	44.5	22.5	16.1	16.9
1.0 mg l <sup>-1</sup> triasulfuron	4.9 $\pm$ 0.6	67.9	19.5	8.8	3.8
1.0 mg l <sup>-1</sup> triasulfuron + 50 mg l <sup>-1</sup> valine + isoleucine	7.5 $\pm$ 0.6	62.0	15.0	11.3	11.7
1.0 mg l <sup>-1</sup> triasulfuron (first) <sub>1</sub> + 50 mg l <sup>-1</sup> valine + isoleucine (later).	6.8 $\pm$ 1.4	52.5	23.7	14.0	9.8

## DISCUSSION.

Vicia faba was used throughout the present studies to establish the effects of triasulfuron on cell division since it was one of the several broad-leaved species which showed high sensitivity to the herbicide in the selectivity studies reported in Chapter 2. The use of high concentrations of triasulfuron could have induced secondary effects unrelated to the primary mode of action and in view of this, lower doses (viz: 0.02, 1 or 2  $\text{mg l}^{-1}$ ), which are several times below the recommended field rate were used in all the studies with the exception of Experiment 6.1 where a higher dose of 100  $\text{mg l}^{-1}$  (equivalent to 20g ai  $\text{ha}^{-1}$ ) was included in the treatments. In general, substantial reductions of the mitotic indices of herbicide-treated root tips were obtained at the low concentrations.

In Experiment 6.1, 2 and 100  $\text{mg l}^{-1}$  triasulfuron reduced the mitotic indices of the root tips of broad bean by 58 and 70.8% respectively after exposing them to these concentrations for 6h (Table 6.1). A similar effect was observed when the experiment was repeated; the mitotic indices were inhibited by 62.5 and 75% after 6h of treatment with 2 or 100  $\text{mg l}^{-1}$  respectively (Table 6.2) and the diameters of the nuclei and nucleoli of non-dividing cells in herbicide-treated root tips were slightly reduced below those of the controls (Table 6.3). In both experiments, the frequency distribution of the various mitotic stages in treated root tips were not significantly different from those of the untreated root tips (Tables 6.1 and 6.2). These findings show that the severe retardation in growth of susceptible species following the application of triasulfuron (see Chapter 2), is largely due to the disruption of



cell division at the meristematic regions. On the other hand, triasulfuron at a concentration of  $2 \text{ mg l}^{-1}$  did not inhibit cell division at the root tips of winter barley after 6 h of treatment (Table 6.4) thus confirming the greater relative tolerance of this species to the herbicide.

In a similar study, Ray (1980, 1982) treated corn root tips for 6h with  $2.8 \times 10^{-6} \text{ M}$  chlorsulfuron (equivalent to  $1 \text{ mg l}^{-1}$ ) and obtained an 80 to 90% inhibition of cell division in the treated root tips. The same author also demonstrated that the mitotic indices in broad bean root tips treated with a similar concentration of chlorsulfuron was reduced by 87% but the various mitotic stages did not show any change in frequency from those of the controls. He therefore concluded that the cells were not inhibited at the mitotic but rather at some other stage in the cell cycle. In another study, applications of cinmethylin at concentrations of  $1 \times 10^{-7}$ ,  $1 \times 10^{-6}$  or  $1 \times 10^{-5} \text{ M}$  significantly reduced the mitotic indices at the root tips of oats after 12 or 18h of treatment (Mahmound and Hess, 1986). The authors observed that the occurrence of all mitotic figures after treatments of 12 or 18h duration had been reduced by more than 70% in root tips treated with  $1 \times 10^{-6}$  or  $1 \times 10^{-5} \text{ M}$  cinmethylin. In addition, they observed that the number of aberrant division figures was insignificant and hence suggested that the inhibition of cell division by the herbicide was not due to a disruption of the mitotic sequence but rather was the result of an interference with events during interphase. Similar findings of inhibitions of cell division at the root tips of corn by sethoxydim {2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one} have recently

been reported and these were attributed to interference with the entry of cells into mitosis rather than a disruption of the mitotic sequence (Hosaka and Takagi, 1987). In the present studies, only a small number of aberrant mitotic figures was observed and coupled with the fact that there were only small differences in the frequency distribution of the mitotic stages between treated and control root tips, it would seem reasonable to suggest that the inhibition of cell division in broad bean root tips by triasulfuron must have occurred during interphase rather than during the mitotic stage. Studies by Rost (1984) with chlorsulfuron confirmed that, the inhibition of cell division in pea root meristem cells was due to a block in the G1 and G2 phases of interphase.

In Experiment 6.3, mitosis at the root tips of broad bean, previously treated for 6 h with 2 mg l<sup>-1</sup> triasulfuron failed to recover after 24 or 48 h in herbicide-free moist vermiculite. Since it takes approximately 18 h for cells in broad bean root tips to complete one cell division cycle (Dyer, 1979), the recovery times of 24 and 48 h were designed to find out whether active cell division could resume in roots which had been previously treated with the herbicide. The results showed that after 24 and 48 h in a medium which did not contain the herbicide, the mitotic indices of treated roots were reduced by 74 and 89% respectively (Tables 6.5 and 6.6) indicating an increased inhibition of cell division with time. Sweetzer et al. (1982) reported that when <sup>14</sup>C-chlorsulfuron was applied to the leaves of tolerant species such as wheat, wild oats and barley, the herbicide was metabolized with less than 10% remaining after 24 h but 80 and 97% of the herbicide was unmetabolized over the same period when similar quantities were



applied to leaves of the susceptible cotton and sugar beet plants respectively. The increased inhibition of cell division in the treated roots of broad bean in spite of the potential recovery periods of 24 and 48h in a herbicide-free growth medium might well be due to the inability of the broad bean roots to rapidly detoxify the herbicide.

There were no effects on mitosis when the roots of broad bean were treated for 6 h with a 1:1 mixture of isoleucine and valine or in combination with triasulfuron (Tables 6.8 and 6.9). In addition, there appeared to be some reversal of triasulfuron inhibition of cell division when roots previously treated with the herbicide were transferred after 6 h to a solution containing 1:1 mixture of isoleucine and valine for 18 h. Although the mitotic index of root tips which received this treatment was lower than that of the control, it was greater than that of roots treated with triasulfuron alone, indicating a partial resumption of cell division (Tables 6.10 and 6.11). These results are in agreement with those reported in earlier studies with chlorsulfuron. Ray (1984) for example, reported that a combination of 100  $\mu\text{M}$  each of valine and isoleucine completely protected cultured pea roots from growth inhibition in the presence of up to 280  $\mu\text{M}$  chlorsulfuron. He also demonstrated this effect on whole plants when he found that pea seedlings grew normally when the seeds were germinated in a growth medium containing 28 nM chlorsulfuron and 100  $\mu\text{M}$  each of valine and isoleucine, suggesting that the effect of the herbicide on plant growth was due to an interference with the production of these amino acids. Ray concluded that the effect on plant growth caused by the application of



chlorsulfuron was due to the inhibition of acetolactate synthase, an important enzyme for the synthesis of valine, isoleucine and leucine. Subsequent studies by Rost and Reynolds (1985) confirmed that, chlorsulfuron-induced inhibition of mitotic entry in pea root tips could be prevented with the addition of 100  $\mu$ M each of valine and isoleucine to the treatment solution.

It is evident from the present results that the mode of action of triasulfuron is similar to that of chlorsulfuron: indeed symptoms expressed by susceptible plants (see Chapter 2 ) following the application of triasulfuron were similar to those reported for chlorsulfuron (Ray, 1980; Richardson et al., 1980). It must therefore be concluded from these results that triasulfuron acts by inhibiting cell division in susceptible plants leading to severe growth retardation and subsequent death of these plants.

CHAPTER 7.

GENERAL CONCLUSIONS.

## GENERAL CONCLUSION.

The studies reported in the present thesis have attempted to provide some basic information on the selectivity, mobility and persistence in soil and mode of action of triasulfuron.

In Chapter 2, triasulfuron was found to inhibit the growth of young seedlings of several broad-leaved and grass species as well as the germination of some species. Broad-leaved species such as Senecio vulgaris, oilseed rape, broad bean and pea showed high sensitivity to the herbicide but Veronica persica was only moderately affected. Lolium perenne, Poa annua, Poa trivialis and Poa pratensis were severely affected by pre-emergence and early post-emergence applications of the herbicide. Late applications of the herbicide led to delay in the development of injury symptoms and death of susceptible plants. On the other hand, wheat and barley tolerated post-emergence applications of triasulfuron but pre-emergence applications retarded early growth of seedlings of both species and a few plants showed symptoms of leaf trapping. Both wheat and barley plants showed greater tolerance to post-emergence applications of the herbicide as they increased in age. In agreement with the findings of Amrein and Gerber (1985), it was obvious that the best time for the application of triasulfuron in wheat and barley was from the 3-leaf stage onwards. The use of  $^{14}\text{C}$ -triasulfuron to study the penetration, translocation and metabolism of the herbicide in susceptible and tolerant species will give a better understanding of the basis of selectivity of this herbicide.

The first obvious symptom after the application of triasulfuron to susceptible plants was the cessation of growth. This was followed



by severe chlorosis and necrosis but in Veronica persica, some leaves developed a purple colouration whilst some black lesions were found on the leaves and stems of treated broad bean seedlings. The development of phytotoxic symptoms was generally slow and death of susceptible plants normally occurred 3 to 4 weeks after applying the herbicide. These symptoms were similar to those described for other sulfonylurea herbicides (Ray, 1980; Richardson et al., 1980; Richardson and West, 1986).

The nature of the symptoms developed by susceptible plants after receiving doses of triasulfuron suggested that the herbicide had interfered with some essential physiological processes at the apical meristem. This hypothesis led to the studies in Chapter 6 where the effects of triasulfuron on cell division at the root tips of broad bean and winter barley were examined. Triasulfuron substantially reduced the mitotic indices at the root tips of broad bean after only 6 h of treatment with herbicide concentrations well below the recommended field rate thus confirming that the severe inhibition of growth of susceptible species by the herbicide was at least partly due to an interference with cell division in the meristematic regions. Cell division at the root tips of winter barley was not affected thus confirming the relative tolerance of this species to the herbicide.

Throughout the studies, triasulfuron was shown to have a flat-dose response curve with plants showing only small increases in phytotoxicity in response to higher concentrations of the herbicide. This behaviour of the herbicide could not be readily explained but similar observations has been made with chlorsulfuron (Palm et al.,

1980).

There were no differences in the responses of different cultivars of wheat and barley used in the present studies to triasulfuron. However, from visual observations, wheat cultivars were slightly more tolerant than barley cultivars particularly to pre-emergence treatments. These findings confirmed those of Amrein and Gerber (1985) who also reported greater tolerance of wheat than of barley to pre-emergence applications of triasulfuron in field trials. There is however, a need to test more cultivars of both species before definite conclusions can be reached.

Post-emergence applications of triasulfuron at the 2- and 3-leaf stages stimulated bud outgrowth in wheat and barley although the effect appeared to be slightly greater in the latter (Chapters 2 and 3). This response was usually accompanied by reduced height of the main shoot which suggested that the effect was probably due to activities of the herbicide at the main shoot apex. However, the shoot apex did not show any readily observed morphological abnormalities when winter barley seedlings were treated with triasulfuron at the 3-leaf stage thus indicating that the partial loss of apical dominance was not due to any readily observed physical damage to the shoot apex. However, the lengths of meristems of herbicide-treated plants were slightly reduced but this effect had disappeared by the end of the fourth week after application. It is essential for further studies to be made to establish the effects of triasulfuron on the levels of endogenous auxin and cytokinin in treated plants as opposed to control plants since it is well known that a shift in the balance between these growth hormones usually leads to loss of apical dominance and consequently the outgrowth of



dormant buds.

The stimulation of tiller bud outgrowth was unrelated to the site of application of triasulfuron. The application of the herbicide to either the distal, basal parts of leaves or to the whole foliage of barley seedlings led to increased tiller numbers but there were no differences in tiller numbers between the treatments.

Late application of triasulfuron to barley plants failed to induce tiller bud outgrowth. This result, coupled with evidence that pre-emergence applications of the herbicide caused reductions in tiller numbers of barley seedlings led to the conclusion that the developmental stage of the seedlings at the time of application of the herbicide was important in the stimulation of tiller bud outgrowth.

Different cultivars of barley exhibited differences in maximum tiller numbers but these differential responses were not due to the herbicide treatments. The herbicide however, induced the growth of extra tillers in all the cultivars of spring and winter barley tested. Tiller production was suppressed at high densities whilst plants grown at low densities tillered freely due to the availability of adequate resources for growth. When expressed as percentage increases of controls however, the stimulation of tiller outgrowth caused by triasulfuron appeared to be unexpectedly higher at high rather than at low densities.

Grain yield and other yield components, with the exception of straw dry weight of barley were not significantly affected when triasulfuron was applied at the 2- or 3-leaf growth stages thus confirming earlier reports that yield of European small grain cereals



is not affected by applications of triasulfuron (Amrein and Gerber, 1985).

The activity of triasulfuron through the soil was generally high; soil applications of the herbicide severely inhibited germination and/or subsequent growth of seedlings in all the relevant studies. In Chapter 4, the position of herbicide placement relative to seed placement had a significant effect on the growth of wheat and barley. Seedlings of these species showed the greatest effect when both the shoot and roots were exposed to a band of soil into which triasulfuron had been incorporated thus confirming the importance of both the subterranean shoot and the roots of developing seedlings in absorbing triasulfuron from the soil. There were no significant differences between direct applications of the herbicide onto the surface of the soil and incorporation of the herbicide into the surface layer of the soil.

Some evidence of leaching of the herbicide was obtained when a band of soil containing triasulfuron, localized in the shoot zone, reduced root dry weights even though bands of untreated soil separated the herbicide-treated layer from the seed. This result led to detailed studies on the mobility of triasulfuron in soil. It was demonstrated that adsorption of the herbicide onto organic matter was not strong in soils with low organic matter levels. In general, an inverse relationship between the bioactivity of triasulfuron and organic matter was obtained. Leaching of the herbicide down the soil profile was greatest in soil containing low levels of organic matter and downward movement of the herbicide was increasingly retarded as the percentage of organic matter increased.

A total of 392mm of simulated rain, applied over 28 days under

unsaturated flow conditions moved triasulfuron from the top soil down the profile of soil columns packed with John Innes No. 2 compost to a depth of at least 7.5cm. Similar observations were made when triasulfuron was progressively leached to a depth of at least 22.5cm in the profile of soil columns when exposed to outdoor temperatures and a total of 528mm natural rain over 6 months. The rates of leaching in this experiment appeared to be dependent on the amount and frequency of rainfall. It was suggested that the exceptionally high rainfall during the second half of the experiment might have contributed to the extent of leaching of the herbicide from the top layers of the soil. In spite of the fact that substantial amounts of the herbicide were leached into deeper layers of the soil over the six month period, phytotoxic residues were still present in the top layers indicating that weeds would continue to be suppressed over relatively long periods following soil applications of the herbicide. It was concluded that due to the moderate affinity of triasulfuron for organic matter, there is a likelihood that the herbicide would be leached into deeper layers of the soil under conditions of high rainfall. It has been stated that the sulfonylurea herbicides are relatively mobile compounds in the soil and depending on rainfall, net soil water movement and degree of soil drainage, the mobility can be agronomically and environmentally important (Beyer et al., 1987). Further studies on the movement of the herbicide in undisturbed soil in the field over a much longer period are needed.

It was shown in Chapter 5 that soil micro-organisms are at least partly responsible for the degradation of triasulfuron in soil. The disappearance of triasulfuron in non-autoclaved soil was several



times faster than in autoclaved soils at all moisture and temperature levels. Since the herbicide did disappear slowly in the autoclaved soil especially at 30°C despite the fact that the soil was rendered virtually sterile by the autoclaving, it was logical to conclude that the decomposition of the herbicide in these soils proceeded by chemical processes; indeed the sulfonurea herbicides have been reported to undergo both microbiological and chemical degradation (Beyer et al., 1987). In future studies, the isolation and identification of micro-organisms in non-sterilized soil in which triasulfuron has been degraded would lend further credence to these findings.

The pattern of breakdown of triasulfuron in non-autoclaved soil at 30°C was characterized by a rapid rate of loss during the first 30 days of incubation followed by a constant and slower rate. Similar patterns of breakdown have been reported for chlorsulfuron (Thirunarayanan et al., 1985; Duffy et al., 1987). Possible reasons for this pattern of breakdown were fully discussed in Chapter 5. Repeat applications of the herbicide did not affect the pattern of breakdown.

Temperature and soil moisture affected the rate of degradation of triasulfuron in both non-autoclaved and autoclaved soils. In general, breakdown of the herbicide was faster at high rather than at low temperatures and as a result, much shorter half-lives were recorded in soils incubated at high rather than at low temperatures. The slow rate of disappearance of the herbicide at low temperatures may account for the presence of phytotoxic levels of triasulfuron in the top layers of soils six months after application (see Chapter 4 ) since the average soil temperature (depth 10cm) over this period was



only 13.7°C. The study showed that in warm soils with adequate moisture, losses of triasulfuron would proceed at a much faster rate but on the other hand, residues of the herbicide could persist over long periods in cold soils.

It was established using broad bean root tips that triasulfuron acts by inhibiting cell division in susceptible species and evidence was obtained to show that the disruption of cell division by the herbicide occurred during interphase rather than during the mitotic sequence. Furthermore, the addition of a 1:1 mixture of isoleucine and valine to the treatment solution reduced the inhibition of cell division at the root tips of broad bean; an indication that a block in the synthesis of these amino acids must have been responsible for the disruption of cell division and consequently, the inhibition of growth of susceptible species. Similar findings have been previously reported for chlorsulfuron (Ray, 1984). A preliminary study on the recovery of cell division in root tips of broad bean by exposing them to a 1:1 mixture of isoleucine and valine after pre-treatment with triasulfuron was made. The results showed a partial recovery of cell division in the root tips: this area of study deserves further examination.

The studies have demonstrated that triasulfuron is a potentially useful herbicide for the control of weeds in wheat and barley when applied post-emergence. However, because of its high activity through the soil even at low doses, soil residues following post-emergence application are important and as a consequence, autumn applications would ensure weed-free conditions well into spring. However, such long residual activity might restrict the types of following crops

that could be grown. Since degradation is enhanced by high temperatures, residues in the soil would be likely to be less following post-emergence applications in spring.

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**APPENDIX.**



App. Table 1.1: Percentage emergence of seedlings two weeks after pre-emergence application.

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0	100.0	100.0	100.0	100.0	100.0
10	102.4	105.7	95.9	96.1	80.0	109.3
15	100.2	105.7	97.8	99.5	93.0	103.2
20	95.6	105.3	89.2	98.9	91.5	100.4

App. Table 1.2: Number of leaves per plant three weeks after pre-emergence application (Results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0a	100.0a	100.0	100.0	100.0a	100.0
10	88.0ab	82.2b	76.4	89.1	88.8a	104.2
15	71.7bc	70.0bcC	80.3BC	77.8BC	67.6abc	102.9A
20	83.7abA	61.1cAB	77.9A	63.6AB	45.3bB	87.9A

Values in a column sharing a common lower-case letter and values in a row sharing a common capital letter are not significantly different at  $P < 0.05$ .

App. Table 1.3: Length of longest leaf blade per plant three weeks after pre-emergence application (Results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
10	56.0b	64.9b	54.3b	38.3b	45.0b	48.2b
15	39.6b	42.4b	44.3b	32.8b	25.3b	37.5b
20	37.5b	27.4c	34.8b	21.4b	25.6b	30.7b

App. Table 1.4 : Number of tillers per plant three weeks after pre-emergence application (Results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0	100.0a	100.0a	100.0a	100.0a	100.0
10	60.0	61.0b	37.8b	28.4b	21.4b	40.0
15	45.0	30.6b	49.4b	47.4b	20.6b	66.7
20	51.7	30.6b	48.3b	13.3b	33.3b	32.8

Values in a column sharing a common letter are not significantly different at  $P < 0.05$ .

App. Table 1.5: Dry weight of leaf blades per plant three weeks after pre-emergence application (Results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
10	55.2bA	54.2bA	54.5bA	20.3bB	33.8bAB	43.1bAB
15	40.9b	30.1b	48.5b	14.6b	15.6b	26.4b
20	33.8b	18.7b	29.8b	9.5b	11.9b	20.4b

App. Table 1.6: Dry weight of shoot per plant three weeks after pre-emergence application (Results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0a	100.0	100.0	100.0a	100.0a	100.0a
10	73.0abAB	104.7A	53.2B	31.9bC	47.6bB	47.3bB
15	52.5b	43.2	52.4	28.9b	38.1b	34.7b
20	52.2b	39.9	42.4	24.1b	35.6b	34.7b

Values in a column sharing a lower-case small letter and values in a row sharing a common capital letter are not significantly different at  $P < 0.05$ .



App. Table 1.7: Dry weight of roots per plant three weeks after pre-emergence application (Results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0	100.0	100.0	100.0	100.0	100.0
10	77.8B	86.1B	246.7A	44.4B	100.4B	122.6B
15	77.8B	61.1B	253.3A	61.1B	96.8B	84.5B
20	88.9	61.1	150.0	41.7	57.9	127.4

App. Table 1.8: Number of leaves per plant four weeks after sowing (First post-emergence application: results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0	100.0	100.0	100.0	100.0	100.0
10	108.6AB	97.4B	109.9AB	131.1A	124.8A	100.1AB
15	86.0	92.5	91.1	111.5	117.0	89.5
20	72.8B	77.0B	110.1AB	102.3AB	119.9A	108.1AB

Values in a row sharing a common capital letter are not significantly different at  $P < 0.05$ .

App. Table 1.9: Length of longest leaf blade per plant four weeks weeks after sowing (First post-emergence application: results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
10	57.5b	73.9ab	78.8b	57.6b	54.6b	55.7b
15	68.4b	71.0b	57.8b	56.2b	51.4b	45.6b
20	50.8b	48.9b	66.3b	56.6b	37.3b	41.9b

App. Table 1.10: Number of tillers per plant four weeks after sowing (First post-emergence application: results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0	100.0	100.0	100.0	100.0	100.0
10	162.6	104.7	150.6	126.5	149.2	130.6
15	98.1	83.8	121.4	104.8	122.9	116.1
20	70.0	86.5	130.0	88.9	132.5	141.7

Values in a column sharing a common letter are not significantly different at P < 0.05.

App. Table 1.11: Dry weight of leaf blades per plant four weeks after sowing (First post-emergence application: results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0	100.0a	100.0	100.0	100.0a	100.0a
10	73.9	65.3ab	95.9	70.6	60.1b	66.9b
15	73.1	57.3b	76.2	69.8	53.2b	46.6c
20	52.8	40.9b	87.9	57.2	36.5b	86.9ab

App. Table 1.12: Dry weight of shoot per plant four weeks after sowing (First post-emergence application: results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0	100.0	100.0	100.0a	100.0a	100.0a
10	88.9	75.3	107.1	43.1b	71.3ab	57.1b
15	75.8	71.4	74.2	60.5b	56.5b	46.5b
20	59.7	46.4	61.6	82.4ab	41.1b	27.9b

Values in a column sharing a common letter are not significantly different at P < 0.05.



App. Table 1.13: Dry weight of roots per plant four weeks after sowing (First post-emergence application: results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0	100.0	100.0	100.0	100.0	100.0
10	95.2	54.8	125.0	52.8	53.5	80.9
15	122.9	55.9	70.0	50.0	68.3	92.1
20	61.0	29.8	63.3	61.1	96.9	138.1

App. Table 1.14: Number of leaves per plant five weeks after sowing (Second post-emergence application: results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0	100.0	100.0	100.0b	100.0b	100.0
10	116.4AB	131.9AB	106.8B	140.6aAB	154.7aA	130.8AB
15	104.4B	125.8AB	108.4B	188.8aA	175.3aAB	138.6AB
20	110.3	124.6	118.5	150.8a	139.7a	126.3

Values in a column sharing a common lower-case letter and values in a row sharing a common capital letter are not significantly different at  $P < 0.05$ .

App. Table 1.15: Length of longest leaf blade per plant five weeks after sowing (Second post-emergence application: Results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
10	75.8b	72.4b	80.4b	70.4b	84.3b	62.6b
15	73.9b	76.2b	82.3b	71.6b	76.1b	71.9b
20	63.4b	79.5b	70.3b	70.1b	71.3b	74.6b

App. Table 1.16: Number of tillers per plant five weeks after sowing (Second post-emergence application: results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0	100.0	100.0	100.0b	100.0b	100.0b
10	122.4	156.7	107.4	177.9a	194.0a	174.5ab
15	105.9	146.7	116.7	257.0a	228.6a	217.2a
20	116.3	150.0	115.7	250.4a	179.2a	207.8a

Values in a column sharing a common letter are not significantly different at  $P < 0.05$ .

App. Table 1.17: Dry weight of leaf blades per plant five weeks after sowing (Second post-emergence application: results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0	100.0	100.0	100.0	100.0	100.0
10	89.9	77.4	84.3	84.8	90.6	70.5
15	93.7	80.8	77.0	116.3	95.5	87.2
20	85.5	90.8	68.9	89.0	64.1	72.3

App. Table 1.18: Dry weight of shoot per plant five weeks after sowing (Second post-emergence application: results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0	100.0	100.0	100.0a	100.0a	100.0
10	74.8	79.6	78.5	82.7b	85.4ab	51.5
15	84.7	78.5	74.1	79.2b	77.8b	52.7
20	76.6	84.3	81.5	71.7b	63.9b	60.5

Values in a column sharing a common letter are not significantly different at  $P < 0.05$ .



App. Table 1.19: Dry weight of root per plant five weeks after sowing (Second post-emergence application: results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0	100.0	100.0	100.0	100.0	100.0
10	116.7	87.7	137.8	53.4	77.8	55.4
15	100.0	91.3	131.1	42.4	77.8	57.3
20	75.0	61.5	100.0	49.9	68.9	51.8

App. Table 1.20: Number of leaves per plant six weeks after sowing (Third post-emergence application: results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0	100.0	100.0	100.0	100.0	100.0
10	135.9	131.9	114.5	182.9	124.3	141.0
15	117.8	149.3	136.9	164.6	130.5	109.0
20	118.7	140.3	123.9	137.3	145.7	141.5

App. Table 1.21: Length of longest leaf blade per plant six weeks after sowing. (Third post-emergence application: Results have been expressed as percentage of control values).

Herbicide dose g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0a	100.0	100.0	100.0	100.0a	100.0a
10	90.3b	87.3	87.9	93.1	95.5b	87.7b
15	83.2b	89.3	82.8	95.9	92.9b	87.1b
20	89.8b	87.8	84.1	95.8	93.2b	90.0b

App. Table 1.22: Number of tillers per plant six weeks after sowing. (Third post-emergence application: results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0	100.0b	100.0	100.0	100.0	100.0
10	163.0	183.4a	129.1	186.7	165.3	101.9
15	130.0	209.1a	168.1	210.0	171.8	112.4
20	142.7	199.8a	145.1	153.3	155.9	159.4

Values in a column sharing a common letter are not significantly different at P < 0.05.

App. Table 1.23: Dry weight of leaf blades per plant six weeks after sowing (Third post-emergence application: results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0	100.0	100.0	100.0	100.0	100.0
10	117.6	99.1	86.4	109.2	95.3	84.5
15	84.5	117.1	82.9	108.7	86.2	90.6
20	89.6	100.6	82.2	103.2	87.1	108.2

App. Table 1.24: Dry weight of shoot per plant six weeks after sowing (Third post-emergence application: results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0ab	100.0	100.0a	100.0	100.0	100.0a
10	113.2a	98.8	64.5b	83.7	71.4	55.1b
15	72.2b	107.3	78.4ab	80.5	104.7	48.0b
20	84.7ab	91.8	68.8b	69.9	82.6	58.9b

Values in a column sharing a common letter are not significantly different at P < 0.05.



App. Table 1.25: Dry weight of roots per plant six weeks after sowing (Third post-emergence application: results have been expressed as percentage of control values).

Herbicide dose (g ha <sup>-1</sup> )	Winter Wheat cv. Galahad	Winter Wheat cv. Longbow	Winter Wheat cv. Norman	Winter Barley cv. Maris Otter	Winter Barley cv. Panda	Spring Barley cv. Arthos
Control-0	100.0	100.0	100.0	100.0	100.0	100.0
10	177.7A	145.0AB	77.3BC	130.9AB	71.4BC	55.1C
15	127.7B	209.2A	106.7BC	84.7BC	104.7BC	47.9C
20	101.3	130.0	104.3	92.7	82.6	58.9

Values in a row sharing a common capital letter are not significantly different at  $P < 0.05$ .

App. Table 2.1: MONTHLY TOTAL AND AVERAGE DAILY RAINFALL AT PEN-Y-FFRIDD FIELD STATION, U.C.N.W. BANGOR: 1982.

Month	Monthly Total Rainfall (mm).	Average Daily Rainfall (mm).
January	60.8	1.96
February	72.2	2.58
March	97.0	3.13
April	19.9	0.66
May	34.0	1.11
June	70.8	2.36
July	60.8	1.96
August	109.9	3.55
September	114.9	3.83
October	139.5	4.50
November	193.2	6.44
December	121.0	3.90

Annual Total Rainfall for 1982 = 1094.00 mm.

Daily Average Rainfall for 1982 = 3.00 mm.

App. Table 2.2: MONTHLY TOTAL AND AVERAGE DAILY RAINFALL AT PEN-Y-FFRIDD FIELD STATION, U.C.N.W. BANGOR: 1983.

Month	Monthly Total Rainfall (mm).	Average Daily Rainfall (mm).
January	156.8	5.06
February	45.3	1.62
March	108.9	3.51
April	60.6	2.02
May	77.0	2.48
June	68.7	2.29
July	57.5	1.92
August	109.8	3.54
September	138.7	4.62
October	161.7	5.22
November	47.8	1.59
December	158.8	5.12

Annual Total Rainfall for 1983 = 1191.60 mm.

Daily Average Rainfall for 1983 = 3.25 mm.



App. Table 2.3: MONTHLY TOTAL AND AVERAGE DAILY RAINFALL AT  
PEN-Y-FFRIDD FIELD STATION, U.C.N.W. BANGOR: 1984.

Month	Monthly Total Rainfall (mm).	Average Daily Rainfall (mm).
January	297.0	9.58
February	58.0	2.00
March	55.4	1.79
April	16.3	0.54
May	50.9	1.64
June	50.1	1.67
July	20.6	0.67
August	62.1	2.00
September	148.8	4.96
October	155.4	5.01
November	144.2	4.81
December	105.5	3.40

Annual Total Rainfall for 1984 = 1164.30 mm.

Daily Average Rainfall for 1984 = 3.17 mm.

App. Table 2.4: MONTHLY TOTAL AND AVERAGE DAILY RAINFALL AT  
PEN-Y-FFRIDD FIELD STATION, U.C.N.W. BANGOR: 1985.

Month	Monthly Total Rainfall (mm).	Average Daily Rainfall (mm).
January	70.2	2.27
February	38.6	1.39
March	58.0	1.87
April	87.9	2.93
May	61.9	2.00
June	104.0	3.47
July	94.4	3.05
August	138.7	4.47
September	69.5	2.32
October	64.1	2.07
November	134.3	4.48
December	150.4	4.85

Annual Total Rainfall for 1985 = 1072.00 mm.

Daily Average Rainfall for 1985 = 2.93 mm.

App. Table 2.5: MONTHLY TOTAL AND AVERAGE DAILY RAINFALL AT PEN-Y-FFRIDD FIELD STATION, U.C.N.W. BANGOR: 1986.

Month	Monthly Total Rainfall (mm).	Average Daily Rainfall (mm).
January	125.0	4.03
February	19.2	0.69
March	111.5	3.60
April	83.0	2.77
May	67.8	2.19
June	40.6	1.35
July	91.2	2.94
August	78.2	2.52
September	100.0	0.33
October	96.2	3.10
November	175.6	5.85
December	-	-

Total Rainfall (from January to November) for 1986 = 988.30 mm.

Daily Average Rainfall for 1986 = 2.67 mm.