RESPONSE OF WHEAT CULTIVARS TO CHLORSULFURON AND THE EFFECT OF NITROGEN AVAILABILITY

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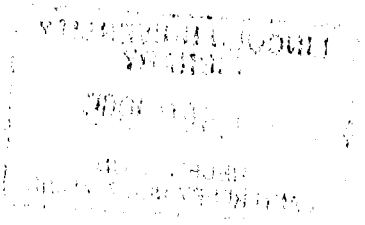

To my wife Shirin
for all her encouragement,
support and patience

Abstract of a thesis submitted in fulfilment

of the requirements for the Degree of Doctor of Philosophy

Response of Wheat Cultivars to Chlorsulfuron and the Effect of Nitrogen Availability

Farhad Dastgheib

Sensitivity of wheat cultivars to the herbicide chlorsulfuron was tested and mechanisms to explain their differential response determined. In addition, a sensitive wheat cultivar, Rongotea, was selected and the effect of different levels of nitrogen on its response to chlorsulfuron was evaluated, and the mode of action of chlorsulfuron in this sensitive wheat cultivar was investigated.

Thirteen wheat cultivars showed differential sensitivity to post-emergence applications of chlorsulfuron at rates from 0 to 90 g a.i. ha^{-1}. Five cultivars were chosen and tested in further experiments under glasshouse and field conditions. Cultivar 'Kotare' was found to be tolerant of chlorsulfuron and showed no injury symptoms at application rates of 15 or 60 g a.i. ha^{-1}. Cultivars 'Lancer' and 'Rongotea' showed early damage in pot and field experiments at both rates of the herbicide, and there were yield reductions in pot experiments. Cultivars 'Abele' and 'Jasper' were intermediate in their response to chlorsulfuron.

Retention, uptake, translocation and distribution pattern of chlorsulfuron was similar between the five cultivars studied. Metabolism of chlorsulfuron was found to be more rapid in 'Kotare' than 'Lancer' or 'Rongotea'. Within 48 h of application, Kotare metabolised 92.2% of ^{14}C-chlorsulfuron, while Lancer and Rongotea metabolised only 43.5% and 63% of the herbicide,

respectively. The concentration of chlorsulfuron in young tissues of Kotare, Lancer and Rongotea at this time was calculated as 1.2, 31.9 and 15.6 ng g^{-1} dry weight, respectively. It was concluded that differential rates of metabolism were the main basis for differences in sensitivity to chlorsulfuron between the wheat cultivars tested.

Rongotea wheat showed greater reduction in dry weight following chlorsulfuron application when grown at high (5 mol m^{-3}) than low (1 mol m^{-3}) nitrate. No differences were found in retention, uptake, translocation or metabolism of the herbicide between plants grown at either nitrate level. Moreover, plants grown at low nitrate and transferred to high nitrate at spraying showed similar growth reductions to plants grown at high nitrate throughout. It was concluded that low nitrate prior to spraying does not result in increased tolerance to chlorsulfuron and that another mechanism must be involved.

Chlorsulfuron limited the capacity of wheat to respond to additional nitrate. This was not through an interference with uptake or assimilation of nitrogen as these processes were not affected by chlorsulfuron. Sprayed plants accumulated reduced N indicating an inability to utilize assimilated nitrogen for growth. Supplying branched chain amino acids (BCAA), which are associated with the mode of action of chlorsulfuron, partially overcame the restriction on growth imposed by chlorsulfuron. This was not a specific BCAA effect as supplying glutamine plus glutamate gave a similar response.

Leaf extension, acetolactate synthase (ALS) activity, valine content and its proportion of the total free amino acid pool was decreased rapidly following chlorsulfuron application. This is strong evidence that the initial effect of chlorsulfuron in restricting growth of Rongotea wheat was through inhibiting the activity of ALS. Moreover, plants kept on high nitrate following spraying

accumulated nitrate to toxic levels. This was shown to be the main reason for decreased growth in the long term. Plants kept on low nitrate and transferred to high nitrate three weeks after spraying could respond to nitrate. In addition, it was found that ALS activity returned to normal at this time. It is likely therefore, that accumulation of nitrate in high-nitrate-fed plants was related to the effect of chlorsulfuron on the target enzyme. However, other possibilities are not discounted.

Keywords : Chlorsulfuron; Sulfonylurea; Herbicide selectivity; Wheat; *Triticum aestivum*; Cultivar; Acetolactate synthase; ALS; Branched chain amino acids; Nitrogen; Nitrate.

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LIST OF HERBICIDES AND HERBICIDE MODIFIERS

COMMON NAME [a] OR DESIGNATION	CHEMICAL NAME
2,4-D	(2,4-dichlorophenoxy)acetic acid
AC 243,997	see imazapyr
ametryn	N-ethyl-N'-(1-methylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine
atrazine	6-chloro-N-ethyl-N'-(methylethyl)-1,3,5-triazine-2,4-diamine
bensulfuron	2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]methyl]benzoic acid
bentazon	3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide
benzoylprop	N-benzoyl-N-(3,4-dichlorophenyl)-DL-alanine
bromoxynil	3,5-dibromo-4-hydroxybenzonitrile
buturon	3-(4-chlorophenyl)-1-methyl-1-(1-methylprop-2-ynyl) urea
chlorimuron	2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoic acid
chlorsulfuron	2-chloro-N-[[4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide
chlorotoluron	3-(3-chloro-p-tolyl)-1,1-dimethylurea
Citowett [R]	alkylarylpolyglycol ether
clopyralid	3,6-dichloro-2-pyridinecarboxylic acid

cyometrinil	(Z)-a-[(cyanomethoxy)imino] benzenzacetonitrile
DDCA	N,N-diallyl-2,2-dichloroacetamide
diclofop	(±)-2-[4-(2,4-dichlorophenoxy) phenoxy]propanoic acid
diphenamid	N,N-dimethyl-a-phenyl benzeneacetamide
DNOC	4,6-dinitro-o-cresol
DPX-V9360 (Accent ^{R})	2-((4,6-dimethoxy-pyrimidin-2-yl-aminocarbonyl)aminosulfonyl)-N,N-dimethyl-3-pyridinecarboxamide
DPX-4189	see chlorsulfuron
EPTC	S-ethyl dipropyl carbamothioate
ethametsulfuron	2-[[[[[4-ethoxy-6-(methylamino)-1,3,5,-triazin-2-yl]amino] carbonyl]amino]sulfonyl]benzoic acid
fenoxaprop	(±)-2-[4-[(6-chloro-2-benzoxazolyl) oxy]phenoxy]propanoic acid
fluazifop	(±)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid
fluchloralin	N-(2-chloroethyl)-2,6,dinitro-n-propyl-4-(trifluoromethyl)benzenamine
glyphosate,	N-(phosphonomethyl)glycine
Glean ^{R}	see chlorsulfuron
haloxyfop	2-[4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid

imazapyr	(±)2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid
imazaquin	2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-quinolinecarboxylic acid
MCPA	(4-chloro-2-methylphenoxy)acetic acid
metsulfuron	2-[[[[(4-methoxy-6-methyl-1,3,5,-triazin-2-yl)amino]carbonyl] amino]sulfonyl]benzoic acid
metolachlor	2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide
metribuzin	4-amino-6-(1,1-dimethyethyl)-3-(methylthio)-1,2,4-triazin-5(4h)-one
NA	1,8-naphthalic anhydride
nitralin	4-(methylsulfonyl)-2,6-dinitro-n,n- dipropylaniline
paraquat	1,1'-dimethyl-4,4'-bipyridinium ion
picloram	4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid
primisulfuron	2-[[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl] amino]sulfonyl]benzoic acid
sethoxydim	2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one
simazine	6-chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine
sulfometuron	2-[[[[(4,6-dimethyl-2-pyrimidinyl)amino]carbonyl] amino]sulfonyl]benzoic acid

TCA	trichloroacetic acid
thifensulfuron	3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylic acid
triasulfuron	1-[2-(2-chloroethoxy)phenylsulfonyl]-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl) urea
tribenuron	2-[[[[(4-methoxy-6methyl-1,3,5-triazin-2-yl)methylamino] carbonyl]amino]sulfonyl]benzoic acid
trifluralin	2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzenamine
UAN	urea ammonium nitrate
vernolate	s-propyl dipropylcarbamothioate

a) Common names are given as acids.
R) Trade name

LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AA	amino acids
AHAS	acetohydroxyacid synthase
ALS	acetolactate synthase
acetyl-CoA	acetyl coenzyme A
BCAA	branched chain amino acids
DAS	days after spraying
dpm	disintegrations per minute
DWt	dry weight
FWt	fresh weight
g	gram, or centrifugal force
g a.i. ha^{-1}	grams active ingredient per hectare
GLN	glutamine
GLU	glutamate
h	hour
ha	hectare
iso.	isoleucine
kPa	kilopascal
leu.	leucine
mol m^{-3}	moles per cubic meter (millimolar)
mmol m^{-3}	millimoles per cubic meter (micromolar)
μmol m^{-2} s^{-1}	micromoles per square meter per second
N	nitrogen; used for different forms of nitrogen in the tissue, such as reduced N, nitrate N, etc.
pptn.	precipitation
TLC	thin layer chromatography
v:v	volume to volume (by volume)
val.	valine
ZGS	Zadoks Growth Scale

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PART ONE

CHAPTER 1

CHAPTER 1

A Review of Literature

1.1 Introduction

Since the introduction of selective organic herbicides in 1945, the science of weed control has always faced one major problem; the need for herbicides with enhanced overall performance. This requires greater selectivity for the crop while maintaining adequate efficacy on target weed species. Moreover, modern herbicides must also be safe in the environment (Percival and Baker, 1991). Selectivity has sometimes been achieved through employment of selective application methods, but economic control of a wide range of weeds in a crop often requires biological selectivity. Achieving such selectivity has proved to be a challenging task over the past decades.

Some analysts have claimed that the crop protection industry is approaching technical maturity as fewer new products are being discovered and developed (Hill, 1982). This may be true, especially considering the market growth rate of crop protection chemicals which has been falling continuously from 10% and 6.3% in 1960s and 1970s, respectively, to 2.7% in 1980s with still a lower growth rate of only 2.3% being forecasted by 1995 (Goosey, 1992). Moreover, development costs of new pesticides have been rising sharply, and more stringent toxicological and environmental safety regulations have been introduced (Parry, 1989). At the same time, farmers are still facing many problems for economic and safe control of pests (including weeds), and changes in pest populations call for continuous improvements and changes in plant protection measures. Given all these points, weed scientists have the difficult responsibility of testing techniques and developing innovative strategies to solve problems in wide ranging and dynamic weed-crop-environment situations.

One major concern with the use of all agricultural chemicals, including herbicides, is their effect on the environment. Ecologists and environmental scientists have warned about the harmful effects of chemicals. In many areas of the world, soil physical and chemical properties are affected, surface and ground water is contaminated, and the populations of beneficial organisms have been reduced drastically due to the unwise use of pesticides (Muller, 1988; Cohen, 1990). In addition, weed scientists have found that repeated use of some herbicides results in an ecological shift in weed populations to tolerant species. Resistant biotypes of normally sensitive species have also evolved. The development of resistance has been most rapid in monoculture situations, where only one herbicide with high persistence was used (Gressel, 1990a & b). Such changes in weed populations were reported as early as two decades ago (Ryan, 1970), and now include resistance of many broadleaf and grass species to different classes of herbicides (Bandeen, Stephenson and Cowett, 1982; Gressel *et al.*, 1982; McKinley, 1990; Primiani, Cotterman and Saari, 1990; Thill *et al.*, 1990). Given these concerns, there should not be sole reliance on chemicals for weed control. In addition efficient use of herbicides must be practiced without detrimental effects on the environment.

Consistent search for practices which give the best weed control for the least risk to the crop and the environment has resulted in development of Integrated Weed Management Systems (IWMS), which are used as a part of Integrated Pest Management (IPM) strategies. By definition, IWMS includes the use of multi-pest resistant, high yielding, well-adapted crop varieties that resist weed competition; precision placement and timing of fertilizer application to give the crop a differential advantage in comparison with weeds; and the use of different weed control methods including chemical ones (Shaw, 1982; Blair and Parochetti, 1982). When used in combination with other methods, herbicides will be used much less frequently and at lower rates than when used alone.

In the absence of a selective herbicide, better use can be made of the available products through the use of herbicide antidotes. The use of antidotes, also referred to as protectants or safeners, helps enhance the physiological tolerance of crops to herbicides which may not be adequately selective (Stephenson and Ezra, 1983). For instance, 1,8-naphthaiic anhydride (NA), can increase the tolerance of maize (*Zea mays* L.) to chlorsulfuron (Parker, Richardson and West, 1980; Hatzios, 1984, Sweetser, 1985). A more successful antidote for maize is N,N-diallyl-2,2-dichloroacetamide (DDCA), designated as R-25788. This antidote is included in the formulation of EPTC to make it selective in maize (Parker, 1983).

Stephenson and Ezra (1983) predicted that new antidotes may emerge in the future from studies on the structure of functional groups of herbicides or from the utilization of antagonistic effects of some herbicides. For instance, clopyralid pretreatment completely eliminated the herbicidal symptoms of picloram in rapeseed (*Brassica napus* L.) plants (Hall and Soni, 1989). Chow *et al.* (1989) reported that the mixtures of growth regulator herbicides and some graminicides such as fenoxaprop ethyl could be used: 1) to reduce crop injury and thus extend the graminicide usage in cereals, and 2) to broaden the control spectrum of both grass and broadleaf weeds. More recently, promising results have been reported with the use of microbial herbicide safeners which can protect maize from EPTC and some other thiocarbamate herbicides (Nagy, Kecskes and Nagy, 1991)

A major advance in selective chemical weed control has occurred in recent years through the development of herbicide-resistant crops. Genes responsible for resistance to a particular herbicide in tolerant biotypes or mutants of bacteria, fungi and higher plants have been located by molecular biology. Advanced techniques are then used to transfer the genes to desired crops.

Such genetically engineered crops can tolerate phytotoxic doses of the herbicide. The technique makes it possible to extend the use of chemicals with favourable herbicidal and environmental characteristics to cover not only major crops but also minor crops for which no selective herbicide is available (Mazur and Falco, 1989). Three basic approaches have been taken for the engineering of herbicide resistance in plants. In the first approach, the biochemical target site of the herbicide, usually a protein, is altered so that it does not bind to the herbicide molecule. The second approach involves overproduction of the target protein. These two methods are useful if the herbicide has a single well-defined target site, as is the case with triazine, glyphosate, sulfonylurea and imidazolinone herbicides. The third approach for developing herbicide resistant crops is to enhance detoxification of the herbicide by transferring the genes encoding enzymes responsible for herbicide metabolism. This method has been used for developing resistance against 2,4-D and bromoxynil (Botterman and Leemans, 1988; Lyons *et al.*, 1989; Stalker, 1989).

It seems obvious that advancement in chemical weed control is impossible without fundamental information about plants. Detailed knowledge of plant physiological and biochemical processes and interactions associated with the activity and selectivity of herbicides will be required to improve the performance of existing herbicides and introduction of new compounds (Percival and Baker, 1991). Extensive and intensive research is needed to explore the complicated plant processes at cellular and sub-cellular levels. Modern researchers need to employ model systems which allow for rapid, precise and in-depth examination of such plant processes. This has been made possible through the use of cell suspension culture and non-differentiated callus of various plants. These systems have the advantages of uniformity, reproducibility and ease of manipulation. They are especially attractive in the study of herbicide mode of action and metabolism as they allow the use of smaller quantities of radiolabelled herbicides, require fewer steps in herbicide extraction, give higher purity of metabolite preparations, and permit evaluation

of metabolism dissociated from the complicating factors of cuticular penetration and translocation (Swisher and Weimer, 1986; Mersie and Foy, 1987; Powell and Rees, 1989). Moreover, much information about the mode of action of herbicides has been obtained through work with lower plant forms and bacteria. For example, the first indication of the site of action of sulfonylurea herbicides was obtained from work on *Salmonella typhimurium* (LaRossa and Schloss, 1984). Bacteria have many biochemical processes in common with higher plants but have the advantage of a simpler, better defined cell and DNA structure. However, the results should always be verified with plant cells, as mechanisms of uptake and a number of metabolic pathways are different between the two systems (Powell and Rees, 1989).

In summary, advanced technology has helped in the search for improved herbicides and their use. Herbicides with greater efficacy which can be applied at reduced rates are a reality. The general trend in herbicides developed over the last fifty years indicates that the application rates per hectare are falling from several kilograms of DNOC in 1930s to around one kilogram of 2,4-D or other herbicides in 1950s (Parry, 1989). The sulfonylurea herbicides have application rates in the order of 10-20 g a.i. ha^{-1}. Discovery of the target site of such potent compounds has resulted in rapid developments in weed science, and also has contributed to basic science by increasing the understanding of the physiological and biochemical mechanisms of plants. Modern agricultural chemistry is benefiting from this knowledge by using it for synthesis and development of compounds with specific modes of action. Such a rational approach helps improve the low 'hit rate' which is often a constraint in the empirical approach to herbicide development (Moberg and Cross, 1990). In future, innovations in chemistry coupled with advancements in biological fields may make it possible to design highly selective herbicides and specifically resistant crops for higher and more economic production.

1.2 Overview of sulfonylurea herbicides

Although the first sulfonylurea compound which showed some herbicidal properties was reported in 1966, it was only in the mid-1970s that active work on this group of compounds began. However, due to the exceptionally high activity of these compounds, large research programmes were focused on their synthesis, and by 1989 more than 375 patents had been issued for sulfonylurea herbicides covering tens of millions of potential compounds (Brown, 1990; Hay, 1990).

The general structure of sulfonylurea herbicides is given in Figure 1.1. There are three distinct parts to the structure: an aryl group, a nitrogen containing heterocycle, and a sulfonylurea bridge between the two.

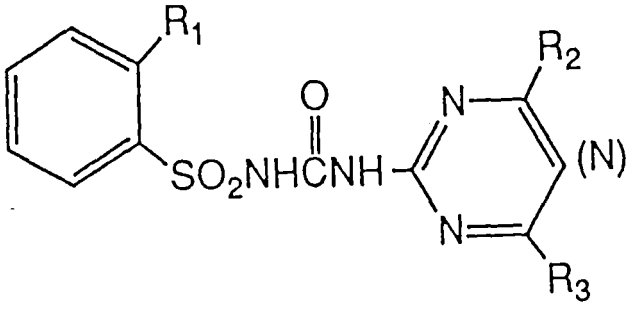


Figure 1.1 General structure of sulfonylurea herbicides (after Brown, 1990). R_1, R_2 and R_3 are specific to individual compounds, for chlorsulfuron R_1= Cl, R_2= -CH_3 and R_3= -OCH_3 .

Important sulfonylurea herbicides developed so far have a phenyl group as the aryl portion and a triazine or a pyrimidine as the heterocyclic portion of the molecule (Beyer *et al.*, 1988; Ray, 1989). Compounds developed so far in this group generally have very low toxicity; which is related to their primary mode of action in influencing amino acid metabolism (See section 1.3.3).

The acute oral LD_{50} values of these herbicides in rats are greater than 4100 mg/kg (Beyer *et al.*, 1988).

The sulfonylureas have extremely high herbicidal activity, with use rates in the range of 2-75 g a.i. ha^{-1}. These herbicides are potent and rapid inhibitors of plant growth (Ray,1989; Brown 1990). For example, root growth in maize was inhibited by as little as 1 ppb (2.8 nM) chlorsulfuron (Ray, 1982a). Another characteristics of sulfonylureas is their rapid effect on plant growth. Chlorsulfuron significantly reduced the growth rate in corn leaves within 2 h of treatment (Ray, 1982a). Visual symptoms of phytotoxicity can be seen in plants treated with sulfonylurea herbicides within 1 to 2 days. However plant death might occur after a week or more and may be accompanied by secondary symptoms such as anthocyanin formation, loss of leaf nyctinasty, abscission, vein discoloration, terminal bud death, necrosis and chlorosis (Beyer *et al.*, 1988; Brown, 1990).

The first commercial sulfonylurea herbicide was chlorsulfuron which was marketed in 1982 for broad spectrum weed control in cereals. Several other compounds soon followed with a wide range of uses. A list of these is given in Table 1.1.

Table 1.1 Some of the important sulfonylurea herbicides*

Common name	Trade name(s)	Uses	Recommended rate (g a.i. ha)
Chlorsulfuron	Glean	Cereals	4-26
	Telar	Non-crop land	13-158
Sulfometuron methyl	Oust	Non-crop land	70-840
Metsulfuron methyl	Ally, Gopper	Cereals	1.8-8
	Escort	Non-crop land	2.6-126
Chlorimuron ethyl	Classic	Soybean, lucerne	8-13
Bensulfuron methyl	Londax	Rice	20-75
Tribenuron	Granstar	Cereals	12
	Express	Pasture	Spot application
Thifensulfuron-methyl	Harmony	Cereals	10-35
		Soybean	4-6
Triasulfuron	Amber, Logran	Cereals	10-40
Ethametsulfuron		Oilseed rape	15-20
Primisulfuron-methyl		Corn	20-40
DPX-V9360	Accent	Corn	35-70

* After Beyer *et al.*, 1988 and Brown, 1990.

1.3 Chlorsulfuron

1.3.1 General

Chlorsulfuron (2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]-benzene sulfonamide) is the active ingredient in the herbicides Glean and Telar. Glean is recommended for use in cereals such as wheat, barley, oats, rye and triticale, while Telar is used for industrial or non-crop weed control. Chlorsulfuron gives effective control of many broadleaf weeds and suppresses a few grass weeds at rates of 10-25 g a.i. ha^{-1} (Levitt *et al.* 1981; Palm, Riggleman and Allison, 1980; Beyer *et al.* 1988).

1.3.2 Mode of action

Ray (1982a & b, 1984) examined different physiological processes and biochemical reactions which could have been involved in the mode of action of chlorsulfuron. Dose dependence and time course experiments showed that chlorsulfuron at very low concentrations (1 ppb) significantly inhibited root growth and at higher concentrations (10 ppb) reduced shoot growth within 2 h of treatment (Ray, 1982a).

Bioassays employing plant hormones were used to evaluate the effect of chlorsulfuron on cell expansion. Concentrations of chlorsulfuron which effectively stopped growth had no effect on auxin-, cytokinin-, or gibberellin-induced cell enlargement. In contrast, cell division was found to be very sensitive to chlorsulfuron. Mitotic index of *Vicia faba* roots was reduced 87% with 1 ppb chlorsulfuron (Ray, 1982a).

As further evidence for the effect of chlorsulfuron on cell division, incorporation of ^{3}H-thymidine into DNA of corn roots was shown to be inhibited very rapidly at concentrations as low as 10 ppb

(Ray, 1982a). Further research showed, however, that this was not due to a direct interference with plant DNA synthesis (Ray, 1982b). Important plant metabolic processes such as photosynthesis, respiration, protein and RNA synthesis, and lipid synthesis were not affected by concentrations of chlorsulfuron required to inhibit plant growth and cell division (De Villiers, Vandenplas and Koch, 1980; Ray, 1982a). Inhibition of the above processes can therefore only be a secondary effect of chlorsulfuron action.

The effect of chlorsulfuron on cell division was further characterized by Rost (1984), who studied the cell cycle in pea (*Pisum sativum* L.) roots in the presence of the herbicide. The progression of dividing cells into mitosis (M) was stopped soon after chlorsulfuron treatment, and 4 h after treatment the entry of cells into the DNA synthesis phase was inhibited. Therefore, Rost (1984) suggested that chlorsulfuron imposed two blocks on cell cycle progression: a primary block in G_2 (pre-mitotic phase) and a secondary block in G_1 (pre-DNA synthesis phase). There was no direct effect on the mitotic apparatus itself.

1.3.3 Site of action

Although the above studies established that chlorsulfuron and other sulfonylurea herbicides are rapid and potent inhibitors of cell division, their site of action at a biochemical level was not identified. The first insight into this problem came from studies involving bacteria. LaRossa and Schloss (1984) showed that growth of *Salmonella typhimurium* in the presence of valine was inhibited by sulfometuron methyl. This inhibition could be reversed by isoleucine, but not by the other amino acids. This suggested the herbicide interfered with the biosynthesis of branched-chain amino acid (BCAA): valine, leucine and isoleucine. The authors showed that sulfometuron methyl inhibited acetolactate synthase (ALS) isozyme II.

The discovery of the site of action of sulfonylurea herbicides in bacteria was soon extended to higher plants. Ray (1984) demonstrated that as little as 2.8 nM (1 ppb) chlorsulfuron significantly inhibited root growth in excised pea roots, while 28 nM chlorsulfuron inhibited growth of the whole plant. Testing 20 different amino acids, he found that the only group of amino acids which alleviated chlorsulfuron-induced growth inhibition was that of BCAA. In fact, the addition of only valine and isoleucine to the culture medium restored growth to normal levels, even in the presence of up to 280 nM (0.1 ppm) chlorsulfuron. Thus, inhibition of ALS was the site of action of chlorsulfuron in peas. In addition to peas, ALS from other plants was also shown to be sensitive to chlorsulfuron. Acetolactate synthase extracted from wheat, a tolerant species, is as sensitive to chlorsulfuron as that of susceptible species such as peas (Ray, 1984). Evidence from other systems soon followed. For example, chlorsulfuron-induced growth inhibition in soybean (*Glycine max* (L.) Merr.) cell suspension culture was reversed by the addition of BCAA (Scheel and Casida, 1985). Tracer studies with ^{15}N demonstrated that chlorsulfuron inhibited the incorporation of ^{15}N into valine, leucine and isoleucine in *Lemna minor* L. (Rhodes *et al.*, 1987).

In addition to the biochemical data reported above, genetic evidence also confirmed that ALS was the primary site of action of chlorsulfuron in plants. Tobacco (*Nicotiana tabacum* L.) mutants resistant to chlorsulfuron were isolated and were shown to have an altered form of ALS which was insensitive to chlorsulfuron and sulfometuron methyl. The altered form of ALS cosegregated with the resistant phenotype (Chaleff and Mauvais, 1984; Chaleff and Ray, 1984). Recently, differences in ALS levels in the roots of maize inbred lines were shown to explain their differential sensitivity to chlorsulfuron (Forlani *et al.*, 1991), and the development of resistance in several weed species as well as in sugarbeet (*Beta vulgaris* L.) to sulfonylurea herbicides was correlated with a form of ALS that was less sensitive to inhibition by the herbicides (Hall and Devine, 1990; Devine, Marles and Hall, 1991; Hart, Saunders and Penner, 1992; Saari *et al.*, 1992).

1.3.3.1 Acetolactate synthase and its inhibitors

Acetolactate synthase (ALS; E.C. 4.1.3.18), also known as acetohydroxyacid synthase (AHAS), is the first enzyme common to the synthesis of the branched chain amino acids: valine, leucine and isoleucine (Appendix 1A). In higher plants, ALS is located in plastids in roots and in chloroplasts in leaves (Miflin, 1974; Jones, Young and Leto, 1985). The enzyme is present only in low quantities in higher plants and becomes unstable upon extraction. This makes its purification and characterization a very difficult task (Durner and Böger, 1989; Durner, Gailus and Böger, 1991). Most of the information about ALS is therefore based on the enzyme extracted from lower plants. In bacteria, ALS has three major isozymes, designated ALS I, II, and III, which are encoded by separate genes designated ilvB, ilvG, and ilvHI, respectively (Ray, 1989).

Three major classes of herbicides, with totally different chemical structures, have been found to inhibit ALS. These include sulfonylureas (LaRossa and Schloss, 1984; Ray, 1984), imidazolinones (Anderson and Hibberd, 1985; Shaner and Reider, 1986; Pillmoor and Caseley, 1987) and triazolopyrimidines, also known as sulphonanilides (Gerwick *et al.* 1990). Moreover, efforts are being directed towards designing more compounds with potential for inhibition of BCAA synthesis either via ALS or other enzymes of the pathway (Hawkes, Howard and Pontin, 1989; Moberg and Cross, 1990; Schloss and Aulabaugh, 1990).

1.3.3.2 Acetolactate synthase inhibition and herbicidal activity

Despite overwhelming evidence for ALS as the site of action of chlorsulfuron, the exact mechanism by which the inhibition of ALS activity results in an inhibition in cell division is not understood (Ray, 1989; Brown, 1990; Moberg and Cross, 1990). In a series of papers, Rost (1984), Rost and Reynolds (1985) and Robbins and Rost (1987) reported that the inhibition of cell division by chlorsulfuron in excised pea roots was completely reversed by the addition of

isoleucine and valine. These authors concluded that BCAA might be involved in cell cycle regulation and hypothesized the involvement of a cell cycle specific RNA which depends upon BCAA. An inhibition in biosynthesis of the amino acids would deplete this species of RNA, which in turn would stop cell division, quite specifically.

Further research by Rost *et al.* (1990), however, questioned the validity of this assumption. The authors reported similar reductions in BCAA levels in cases where reductions in mitotic index were quite different. Moreover, some workers reported that the chlorsulfuron-induced inhibition of root growth in maize and pea and of K^+ uptake in maize root apical segments was not alleviated by the addition of BCAA (Rubin and Casida, 1985; Giardina, De Agazio and Grego, 1987). These observations suggest that a reduction in the BCAA pool size by itself can not explain an inhibition in cell division.

1.3.3.3 The possible alternative sites of action

Other mechanisms have been proposed to explain the phytotoxic effect of ALS inhibition. LaRossa, Van Dyk and Smulski (1987) reported that certain mutants of *Salmonella typhimurium* were inhibited by sulfonylureas even in the presence of BCAA. The authors showed that α-ketobutyrate, an intermediate in BCAA biosynthesis, was accumulated in these cells to toxic levels. This was proposed as the mechanism of action of sulfonylureas in bacteria. The accumulation of α-ketobutyrate also induced several secondary responses in bacterial cells, including the inhibition of acetyl-CoA synthesis, which is inhibitory to growth (LaRossa *et al.* 1987). This hypothesis has not yet been tested in higher plants, but some evidence has been found in its favour. Rhodes *et al.*(1987) demonstrated the accumulation of α-amino-n-butyrate, a non-protein amino acid, in *Lemna minor* L. after adding chlorsulfuron to the growth medium but did not report on the toxicity of this intermediate. It has also been shown that sodium butyrate,

which may be derived from α-ketobutyrate, arrests the cell cycle in *Pisum sativum* L. root tips in interphase (Tramonto, De Costanza and De Lillo, 1989).

The possibility of a chlorsulfuron effect on plasma membranes has been investigated. Chlorsulfuron inhibited K^+ influx through the plasmalemma in root apical segments of maize. Moreover, maize root segments pre-treated with 10 μM chlorsulfuron lost the capacity to recover from cutting injury by washing. It is known that cutting causes a rapid increase in membrane permeability (De Agazio and Giardina, 1984). De Agazio and Giardina (1987) demonstrated that the fusicoccin stimulation of K^+ uptake and H^+ extrusion was inhibited by chlorsulfuron in maize root apical segments when applied to the whole seedlings, but not when applied to excised root tips. Fusicoccin is a fungal phytotoxin which acts directly on the plasma membrane. The authors hypothesized that chlorsulfuron causes an alteration at the plasmalemma involving the fusicoccin binding sites. This effect was not a consequence of the inhibition of ALS induced by chlorsulfuron. The activity of ALS in excised maize root tips pre-treated with chlorsulfuron was reduced markedly, while the fusicoccin-stimulated K^+ uptake was not inhibited. These reports indicate the possibility of other sites of action for chlorsulfuron, possibly involving the plasmalemma.

More recently, Giardina and Carosi (1990) reported the effects of chlorsulfuron on free polyamine content and on growth of maize seedlings. Chlorsulfuron resulted in a 60% decrease in the spermidine (the most abundant polyamine) content in the root tips. In contrast, the polyamine contents in the basal root zone, which essentially lacks cell division activity, was unaffected. Polyamines are intimately involved in the regulation of cell progression through the cell cycle. As such, the authors hypothesized that the depletion of spermidine content, induced by chlorsulfuron, could be responsible for the inhibition of cell division leading to growth inhibition.

Sulfonylureas appear to cause a decrease in the level of total soluble protein without directly affecting protein synthesis (Ray, 1982a; Clayton and Reynolds, 1991). It has been suggested that the decrease in protein level might be a consequence of the inhibition of ALS enzyme (Hawkes, Howard and Pontin, 1989). Presumably the plant responds to amino acid starvation by increasing the turnover rate of the protein pool in the meristem. However, Royuela *et al.* (1991) found that in wheat (*Triticum aestivum* L.), accumulation of free amino acids and increases in relative proportion of some of them were observed even when decrease in BCAA contents could hardly be measured.

With regard to the research reviewed above, the exact mechanism of action of chlorsulfuron and other sulfonylurea herbicides remains unclear.

1.3.4 Basis of selectivity

There is a 4000-fold difference in tolerance to chlorsulfuron between sensitive and tolerant species. Such a great difference could not be explained by differences in penetration or translocation of the herbicide (Sweetser *et al.*, 1982). Moreover, the sensitivities of the ALS enzymes extracted from plants highly sensitive and highly tolerant to chlorsulfuron were similar (Ray, 1984). On the other hand, the rate of metabolism of chlorsulfuron was highly correlated with its tolerance in different species. Sweetser *et al.* (1982) found that nearly 97% of ^{14}C-chlorsulfuron recovered after 24 h from a sensitive species such as sugarbeet was the parent compound. In contrast, in wheat leaves, only 5% of the ^{14}C was chlorsulfuron. Sweetser *et al.* (1982) further studied the metabolic pathway of ^{14}C-chlorsulfuron in wheat using high performance liquid chromatography (HPLC) and mass spectrometric analysis. A major ^{14}C-metabolite was identified as the 5-hydroxyphenyl analogue of chlorsulfuron. According to the model proposed by the authors (Figure 1.2) chlorsulfuron is first metabolised in wheat and other tolerant grasses to the 5-hydroxyphenyl intermediate. This metabolite is still active against ALS,

but is rapidly conjugated to glucose, thus becoming inactive (Sweetser *et al.*, 1984; Brown, 1990). Erbes (1984) characterized the enzyme system involved in chlorsulfuron metabolism. An oxygenase and a UDP-glucosyl transferase are involved in the hydroxylation and conjugation steps respectively (Figure 1.2). According to the above studies, metabolism was proposed as the basis for selectivity in cereals.

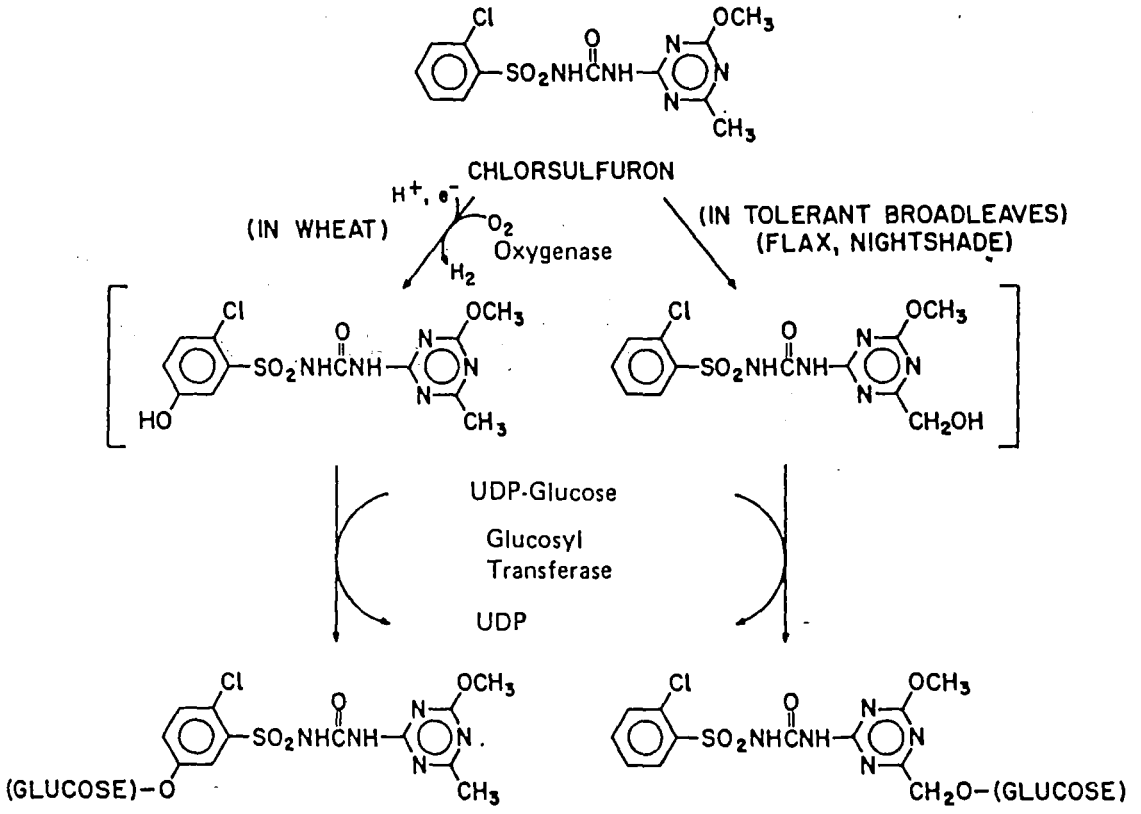


Figure 1.2 Proposed metabolic pathways of chlorsulfuron in wheat and tolerant broadleaf species (after Beyer *et al.*, 1988)

Furthermore, metabolism was found to be the basis of selectivity in tolerant broadleaf plants. The tolerant species such as flax (*Linum usitatissimum* L.), black nightshade (*Solanum nigrum* L.) and eastern black nightshade (*S.ptycanthum* Dun.) were able to metabolise chlorsulfuron rapidly, while the majority of chlorsulfuron remained unmetabolised in the sensitive velvetleaf (*Abutilon theophrasti* Medic.) (Hageman and Behrens, 1984; Hutchison, Shapiro and Sweetser, 1984). Cell culture studies showed that leafy spurge (*Euphorbia esula* L.) cells metabolised all of the applied ^{14}C-chlorsulfuron within 72 h, while Canada thistle (*Cirsium arvense* (L.) Scop.) metabolised less than 2% (Swisher and Weimer, 1986). This explained differences in chlorsulfuron phytotoxicity in these two species. In another study monoploid callus tissue of white potato (*Solanum phureja* Juz.& Buk.) had metabolised less than 5% of ^{14}C-chlorsulfuron 48 h after application, while its growth was inhibited 55% (Mersie and Foy, 1987). It was found that the pathway for metabolism of chlorsulfuron in tolerant broadleaf plants was different from that of grasses (Hutchison *et al.*, 1984). In broadleaf species, hydroxylation and conjugation with sugar occurs on the triazine ring rather than the phenyl ring (Figure 1.2).

In contrast to the above reports, metabolism was not always responsible for the tolerance of plants to chlorsulfuron. Both wheat and barley (*Hordeum vulgare* L.) rapidly metabolise chlorsulfuron, but barley is only marginally tolerant to pre-emergence application of the herbicide (Foley, 1986). Goatley *et al.* (1990) reported that the small difference in the rate of degradation of chlorsulfuron was not adequate to explain the difference in response to the herbicide between the tolerant Kentucky bluegrass (*Poa pratensis* L.) and the sensitive tall fescue (*Festuca arundinacea* Schreb.)

1.3.5 Uptake and translocation

Chlorsulfuron is absorbed by both roots and foliage of plants. Its rate of uptake depends upon the site of application and the species. Sweetser *et al.* (1982) reported values for percentage

penetration of ^{14}C-chlorsulfuron into the leaves of various species within 24 h, ranging from 56% in cotton (*Gossypium hirsutum* L.) to 97.7% in wild mustard (*Sinapis arvensis* L.). Velvetleaf and eastern black nightshade absorbed 38% and 44% of the applied ^{14}C-chlorsulfuron in 24 h (Hageman and Behrens, 1984). Canada thistle absorbed 39% of the applied ^{14}C-chlorsulfuron within 48 h and up to 75% after 144 h, while leaves of perennial sow thistle (*Sonchus arvensis* L.) absorbed only 30% of the applied dose after 144 h (Devine and Vanden Born, 1985; Peterson and Swisher, 1985). Leys and Slife (1988) found that wild garlic (*Allium vineale* L.) absorbed less than 4% of the applied chlorsulfuron in 12 h. The uptake continued to rise linearly and reached 62.9% after 144 h.

From the limited number of reports available on root uptake of chlorsulfuron, it appears that root applied chlorsulfuron is absorbed much more slowly than foliage applied herbicide. When ^{14}C-chlorsulfuron was added to the nutrient solution of Canada thistle, only 16% of the applied dose was absorbed after 48 h, compared to 39% in the foliage application (Peterson and Swisher, 1985). Wheat and barley roots absorbed 3.7% and 1.9%, respectively, of the applied ^{14}C-chlorsulfuron after three days. When the herbicide was applied to leaf 3 of wheat and barley, 16.2% and 14% of the applied radioactivity was absorbed within the same period, respectively (Foley, 1986). In the same way, penetration of ^{14}C-chlorsulfuron into roots of Kentucky bluegrass and tall fescue was 10.3% and 9%, respectively, of the applied radioactivity within 96 h, while during the same period 27% and 31.3% of the applied dose penetrated into leaves of the grasses (Goatley *et al.*, 1990).

Devine, Bestman and Vanden Born (1987) studied the pattern and mechanism of chlorsulfuron penetration into excised pea roots. Chlorsulfuron was absorbed rapidly by fresh pea root, but not by dead root, from the bathing solution and was accumulated against a concentration gradient. This suggests the importance of an intact plasma membrane in the uptake of chlorsulfuron. The authors showed that uptake of chlorsulfuron increased linearly over a wide range of

concentrations, which indicates that it is taken up by a passive, nonfacilitated process. Membrane permeability to sulfonylureas depends on the compound's relative lipophilicity and pKa (Brown, 1990). Chlorsulfuron has a low dissociation constant (pKa=3.6), thus it is a weak acid and pH greatly affects its water solubility and partition coefficient (Beyer *et al.*, 1988). As such, at low pH the cell membrane is much more permeable to chlorsulfuron because the greater proportion of its molecules are in the undissociated form, which is more lipophilic. The alkaline pH within the cytoplasm results in its accumulation inside the cell through an acid-trapping mechanism (Beyer *et al.*, 1988; Brown, 1990).

A review of the reports on chlorsulfuron shows that the translocation of this herbicide in plants is ambimobile. The rate of translocation after foliage application is very species dependent. Sweetser *et al.* (1982) reported that, depending on the species, 1.1% to 17.6% of the foliar applied ^{14}C-chlorsulfuron moved to other leaves and stem within 24 h, while much smaller percentages of the applied dose moved to roots. In another study 48.6% and 68.3% of the absorbed ^{14}C-chlorsulfuron was translocated out of the treated velvetleaf and eastern black nightshade leaves, respectively, 24 h after application (Hageman and Behrens, 1984). In Canada thistle, 10% and 21%, respectively, of the applied ^{14}C-chlorsulfuron was translocated from the treated leaf, 48 h and 144 h following application (Devine and Vanden Born, 1985; Peterson and Swisher 1985). Leys and Slife (1988) found that 144 h after application of ^{14}C-chlorsulfuron to wild garlic, 17% of the applied radioactivity translocated out of the treated leaf.

The low phloem mobility of chlorsulfuron in several species, such as Canada thistle, perennial sow thistle, tartary buckwheat (*Fagopyron tartaricum* (L.) Gaertn.) and field pennycress (*Thlaspi arvense* L.) has been reported, and reasons for it have been investigated. In field pennycress, for example, 24 h after the foliar application of ^{14}C-chlorsulfuron, 12% of the applied dose had been absorbed, of which 7% to 23% was exported out of the treated leaf (Vanden Born *et al.*, 1988). This corresponded to 0.84% to 2.75% of the applied amount. Such low phloem mobility is

surprising considering chlorsulfuron's ability to penetrate the plasmalemma and accumulate in the symplast (Devine *et al.*, 1987). In fact, as a weak acid, chlorsulfuron is expected to become trapped in the anionic form once it enters the alkaline sieve tube / companion cell complex and thus to be available for transport (Devine *et al.*, 1987; Beyer *et al.*, 1988). Devine, Bestman and Vanden Born (1990) recovered most of the applied chlorsulfuron close to the site of entry into the leaf rather than around the leaf tip or leaf margins. This ruled out the possibility of leakage of chlorsulfuron out of the phloem into the xylem. Further research showed that chlorsulfuron reduced transport of assimilates from treated leaves within 6 h of its application, with no immediate or direct effect on carbon dioxide fixation. The low phloem mobility of chlorsulfuron was therefore explained by a self-limitation effect on phloem translocation (Vanden Born *et al.*, 1988; Bestman, Devine and Vanden Born, 1990; Devine *et al.*, 1990; Geiger and Bestman, 1990).

1.4 Crop tolerance and cultivar sensitivity

Selectivity of any herbicide depends on the differential sensitivity between weeds and the crop for which the herbicide is recommended. If the margin of tolerance between the crop and weeds is narrow, selectivity might be lost under unfavourable conditions. Although chlorsulfuron has excellent selectivity characteristics, up to 4000-fold difference in tolerance between sensitive and tolerant species (Sweetser *et al.*, 1982), some damage to crops may occur if certain environmental and plant conditions prevail. A major reason for the possible loss of herbicide selectivity can be found in variations among genotypes. Intraspecific differences in tolerance to other herbicides are well known (Lemerle *et al.*, 1985a & b, 1986; Martin, Worthington and Gray, 1987; Wilkinson, 1988). Several reports that suggest it occurs for chlorsulfuron as well are reviewed below.

Palm *et al.* (1980), reviewing the early reports on chlorsulfuron performance, did not find any cultivar differences in wheat and barley for tolerance to chlorsulfuron. Most reports compare only the final yield as a measure of tolerance and neglect any possible early damage. Cornwell

and Lane (1981) reported that apart from slight chlorosis in some experiments, various wheat cultivars grown in New Zealand, including Rongotea, Oroua, Takahe, Karamu, 1004-76, Tiritea and Kopara, showed no yield reduction following foliar applications of 9 to 75 g a.i. ha^{-1} chlorsulfuron. Hageman and Behrens (1981) reported that spring wheat cultivars tolerated foliar application of chlorsulfuron up to a rate of 0.25 kg a.i. ha^{-1}, while durum wheat (*Triticum durum* Desf.) showed 18% yield reduction at the same high rate.

Further research with chlorsulfuron has produced an increasing list of sensitive cultivars. Significant yield reductions have been reported for winter wheat following foliar applications of chlorsulfuron at 35 or 70 g a.i. ha^{-1} (Anderson, 1986; Wicks, Nordquist and Schmidt, 1987). Stage of plant development appears to be an important determinant of crop selectivity. Chlorsulfuron at 50 g a.i. ha^{-1} reduced plant height and yield of a winter wheat cultivar when applied at three-leaf stage or at mid-boot stage, but not when applied at fully tillered stage (Martin, Miller and Alley, 1989; Ferreira, Baker and Pepper, 1990).

Pre-emergence application of chlorsulfuron, even at the recommended rate, is not tolerated by all wheat cultivars (Bowran, Blacklow and Boyd, 1984; Lemerle *et al.*, 1985a; Blacklow and Pheloung 1987; Royuela, Munoz-Rueda and Gonzales-Murua, 1990). Royuela *et al.* (1990) observed no phytotoxic response from application of chlorsulfuron to a spring wheat cultivar up to 30 g a.i. ha^{-1}, however a winter wheat cultivar showed injury symptoms following chlorsulfuron application at 20 g a.i. ha^{-1}. Bowran and Blacklow (1987) proposed the inhibition of the rate of elongation of the third leaf of wheat, as a non-destructive measure of sensitivity to chlorsulfuron following pre-emergence application. Based on this measure, the authors identified two spring wheat cultivars, Sonora and Miling, as sensitive to chlorsulfuron. Bowran (1990a) reported that chlorsulfuron applied pre-emergence at 15 g a.i. ha^{-1} reduced the yield of some wheat cultivars, while at 30 g a.i. ha^{-1} all cultivars examined showed significant yield reductions.

1.5 Environmental factors affecting the performance of sulfonylurea herbicides

Environment can affect the performance of many herbicides. Air temperature, relative humidity, irradiance, precipitation, wind, soil temperature and moisture are common environmental factors affecting plant responses to herbicide applications (Devine, 1988). While there have been numerous studies on the effect of these variables on the efficacy of herbicides, only a few reports are available on chlorsulfuron. However, variations in the performance of chlorsulfuron and other sulfonylurea herbicides have been noticed in different years and at different locations (Lemerle, Leys and Kidd, 1986 & 1987; Ferreira *et al.*, 1990). The general observations indicate that stressed plants have a reduced capacity to metabolise the herbicide and may show injury symptoms. Thus, crop injury may be magnified by the application of chlorsulfuron in mid-winter, when the crop is stressed by cold temperatures or diurnal freezing and thawing (Ferreira *et al.*, 1990). The interactive nature of environmental factors with each other and also with plant factors, and the inconsistency of reports on chlorsulfuron phytotoxicity to crops makes it hard to find general rules for the effect of environment on chlorsulfuron selectivity. Nevertheless, some of the available reports on climatic and edaphic factors on the performance of chlorsulfuron and other sulfonylurea herbicides will be reviewed.

1.5.1 Climatic factors

High relative humidity (95-100%) after chlorsulfuron application increased its phytotoxicity to kochia (*Kochia scoparia* (L.) Schard.) and green foxtail (*Setaria viridis* (L.) Beauv.). Increased temperature from 10 °C or 20 °C to 30 °C reduced chlorsulfuron toxicity at high but not at low relative humidity (Nalewaja and Woznica, 1985). The effect of chlorsulfuron on wheat was greater if cool temperatures followed application (Caseley, 1987). In contrast to the above reports, Buchanan, Gillespie and Swanton (1990) did not find any reduction in the herbicidal

activity of the sulfonylurea herbicide, ethametsulfuron under different temperature and relative humidity regimes.

Ferreira *et al.* (1990) used visual wheat injury data from 13 experiments to calculate correlation coefficients to identify factors that influenced wheat tolerance to post emergence application of chlorsulfuron at 26 g a.i. ha^{-1}. The best correlation was the negative correlation with mean daily minimum air temperature during the first week after treatment. The authors observed more injury to crop at experimental sites where the mean daily minimum temperature during this critical period was -6 °C versus 0 °C. This can be explained by a slower metabolism of chlorsulfuron at lower temperatures (Beyer *et al.*, 1988). The study by Ferreira *et al.* (1990) also showed that wide diurnal temperature flactuation may increase the potential for crop injury.

Reviewing various reports on rainfall does not lead to a consistent pattern of its effect on chlorsulfuron performance. Up to 4 mm of simulated rainfall immediately after foliar application reduced chlorsulfuron phytotoxicity to kochia and green foxtail. The loss in chlorsulfuron effect decreased as the time interval between application and simulated rainfall was increased (Nalewaja and Woznica, 1985). On the contrary, grain yield loss in barley and wheat following a foliar application of chlorsulfuron was greater in wet years than in dry ones (Lemerle *et al.*, 1987 & 1990). The inconsistency in the results from these reports can perhaps be explained given that chlorsulfuron is effective both through foliage and soil. Probably, the amount of simulated rainfall in the former study was not enough to wash chlorsulfuron off the foliage into the root zone. In another study, no correlations were found between amount or timing of rainfall with chlorsulfuron injury to wheat (Ferreira *et al.*, 1990).

1.5.2 Edaphic factors

Chlorsulfuron concentration and bioactivity in soil are affected by a complex of edaphic factors among which are: soil texture, soil pH, cation exchange capacity, soil temperature, soil microbial content and activity, soil organic matter content, soil moisture and nutrient status (Beyer *et al.*, 1988). These factors interact to affect chlorsulfuron concentration in the soil solution over time which determines crop tolerance in the same season as well as chlorsulfuron persistence, which may limit the growth of some rotational crops. Several reports have studied the persistence of chlorsulfuron in soil. While Palm *et al.* (1980) reported a half life of less than 2 months for chlorsulfuron in soil from many different locations, later studies showed that chlorsulfuron can remain in the soil for much longer. Sugarbeet showed serious injury 26 months after application of chlorsulfuron (Brewster and Appleby, 1983). Smith and Hsiao (1985) found that 3% to 16% of the applied chlorsulfuron could remain in the top soil after one year. Soil pH appears to be an important factor in chlorsulfuron persistence. The half life of chlorsulfuron in the soil was 89 days at pH 6.2 and 144 days at pH 8.1 (Thirunarayan, Zimdahl and Smika, 1985). Wiese, Wood and Chenault (1988) reported that at pH 6.5, chlorsulfuron which had been applied to wheat at 35 g a.i. ha^{-1} did not affect a sorghum (*Sorghum bicolor* (L.) Moench.) crop sown 16 months later. At pH 7.5 or above, however, sorghum showed injury up to 25 months after chlorsulfuron application. Contrary to the above reports, a study on the responses of barley and wheat to chlorsulfuron at 3 sites in 3 seasons showed no direct relationship between soil pH and dry weight reduction (Lemerle *et al.*, 1990). It may be that under field conditions other factors have a greater influence on chlorsulfuron performance than soil pH.

Among the soil physical and chemical properties studied by Mersie and Foy (1985), organic matter was the soil factor most highly correlated with chlorsulfuron toxicity. There was an inverse relationship between organic matter content and chlorsulfuron activity. Percentage of organic matter correlated negatively with pH and positively with cation exchange capacity. No

significant relationship between clay content and chlorsulfuron toxicity was observed; which suggested a low affinity of chlorsulfuron to clay. In the same way, Shea (1986) reported that chlorsulfuron has a limited affinity for soil adsorption and could be highly mobile in soil solution.

Photolysis and volatilization of chlorsulfuron are of minor importance in its dissipation. Thus, dissipation largely depends on chemical hydrolysis and microbial breakdown, both of which are enhanced by warmer temperatures (Beyer *et al.*, 1988). Joshi, Brown and Romesser (1985) showed that hydrolysis of chlorsulfuron was most rapid in acidic soils, whereas in alkaline soils the rate of breakdown was reduced. Moreover, soil sterilization significantly reduced breakdown of chlorsulfuron indicating the importance of soil microorganisms in its degradation. These authors identified several soil microorganisms active in chlorsulfuron decomposition: *Streptomyces griseolus*, a soil actinomycete, and two fungi, *Aspergillus niger* and *Penicillium* sp. were able to degrade chlorsulfuron rapidly.

Soil moisture has been shown to influence the phytotoxicity of sulfonylurea herbicides. For instance, waterlogging increased the sensitivity of wheat to chlorsulfuron (Papalia and Blacklow, 1984). This might have been due to both a reduced ability of stressed plants under waterlogged conditions to metabolise chlorsulfuron and a reduced breakdown of the herbicide by soil microorganisms under anaerobic conditions. Anderson (1985) found that increasing soil water from 0.15 to 0.20 kg kg^{-1} reduced chlorsulfuron degradation and bioactivity in loam soil. At the other extreme, water stressed plants were not effectively controlled by chlorsulfuron (Nalewaja and Woznica, 1985), and reduced herbicidal activity of the sulfonylurea herbicide, ethametsulfuron was noticed when application was followed by low soil moisture (Buchanan *et al.*, 1990).

More detailed studies on chlorsulfuron dissipation in soil have revealed that its degradation curve is biexponential and does not fit first order kinetics (Duffy *et al.*, 1987; Brown, 1990). This

behaviour is characterized by an initial rapid decay, lasting for about 2 to 4 weeks, followed by a period of slower breakdown. In sterilized soil, the degradation curve follows first order kinetics. The biphasic kinetics in soil is explained through a connected two-compartment model. Following application, chlorsulfuron is in the 'available compartment', which is subject to both chemical hydrolysis and microbial degradation. With time, the herbicide diffuses into a 'protected compartment' which makes it unavailable to microbial activity. Therefore, dissipation follows a slower rate as only chemical hydrolysis can degrade the herbicide molecules.

The complicated nature of the soil environment and the presence of inter-relationships amongst soil variables affecting chlorsulfuron persistence have made it difficult to predict the residual activity of the herbicide. It is therefore often necessary to determine the concentration of chlorsulfuron in the soil which might affect the following crop. Analytical methods for determining chlorsulfuron concentration are difficult and time consuming, while immunoassay methods and the use of radioisotopes are unsuitable for routine field work (Blacklow and Pheloung, 1991). This has encouraged development of bioassay procedures for determination of chlorsulfuron residues in the soil. These methods generally involve comparing the reduction in root or shoot growth of sensitive species grown in the soil with that caused by known concentrations of chlorsulfuron. The detection level for bioassay techniques is generally very low, ranging from 0.125 to 10 $\mu g\ kg^{-1}$ (ppb) (Hsiao and Smith, 1983; Groves and Foster, 1985; Eleftherohorinos, 1987; Günther, Rahman and Pestmer, 1989; Sunderland, Santelmann and Baughman, 1991). Even lower detection limits (0.01 $\mu g\ kg^{-1}$) have been reported using highly sensitive species (Duffy *et al.*, 1987). As direct bioassay techniques have a limited range of sensitivity and sometimes require dilutions of the soil, some workers have developed bioassay methods based on soil extraction (Morishita *et al.* 1985; Blacklow and Pheloung, 1991).

There is only very limited information on the effect of mineral nutrients on the performance of sulfonylurea or other groups of herbicides. Reports on the effect of soil nitrogen level on the

activity of other herbicides are reviewed in the introduction to Chapter 4 and generally indicate a reduction in activity for several herbicides at lower nitrogen levels. The published work on the interactive effect of soil nutrients and chlorsulfuron activity is limited to very few reports. Nalewaja and Woznica (1985) found that an increase in soil nitrogen from 20 to 140 ppm increased the phytotoxicity of chlorsulfuron to green foxtail. Bowran and Blacklow (1987) found that the response of some wheat cultivars to nitrogen and phosphorous was inhibited by chlorsulfuron applied to the soil. At the same time, a few workers have studied the effect of foliarly applied nutrients as spray additives to sulfonylurea herbicides. As the foliar uptake of sulfonylurea herbicides is very low, this approach might have implications in enhancing the performance of the herbicides and reducing their application rates. Uptake and translocation of chlorimuron ethyl increased when urea ammonium nitrate (UAN) was added to the spray solution and this resulted in improved weed control (Fielding and Stoller, 1990). Similarly, UAN increased the uptake of thifensulfuron in velvetleaf from 15% to 30% (Becket and Stoller, 1991).

1.6 Research objectives

Chlorsulfuron is a major cereal herbicide which has replaced the traditional chemical weed control practices in many wheat growing areas. The preceding review of literature shows that although much progress has been made in understanding the translocation, metabolism, mode of action, and soil behaviour of chlorsulfuron, some important aspects of its activity have not been fully investigated. Of particular significance is the problem of crop damage which can occur under certain conditions. In Australia, where chlorsulfuron is usually used pre-emergence, many farmers have experienced poor crop growth and yellowing in certain wheat cultivars (Bowran, 1990b). In New Zealand, chlorsulfuron is recommended as a post-emergence treatment, and cases of reduced crop vigour and plant damage have been observed with some wheat cultivars, especially on highly fertilized soils, in flooded areas, or when cold temperatures followed application (R.J. Field and G. Iggo, Pers.comm., 1988). As suggested by previous workers

(Lemerle *et al.*, 1985a; Royuela *et al.*, 1990), there is a great need to define clearly plant and environmental conditions which can bring about a loss of chlorsulfuron selectivity, if damage to crops is to be avoided.

Differences between cultivars in their response to chlorsulfuron have already been shown in the literature, but the basis for the differences has not been thoroughly investigated. Such an investigation was undertaken as the first objective in the present study. It aimed primarily to screen important wheat cultivars grown in New Zealand for their sensitivity to chlorsulfuron and to establish the basis for any differences which might be found between them. Fluorometry and radioisotope techniques have been employed to investigate whether any differences exist in the amount of chlorsulfuron reaching the site of action which may explain the differences in sensitivity between cultivars.

Field observations have indicated increased sensitivity of certain wheat cultivars where high rates of nitrogen have been applied to the soil (G. Iggo, 1988; Pers. Comm.). So far, however, only a few reports have been published on this subject (Nalewja and Woznica, 1985; Bowran and Blacklow, 1987). Considering the mode of action of chlorsulfuron and other sulfonylurea herbicides, full examination of the nitrogen-herbicide interaction is warranted. Sulfonylurea herbicides inhibit ALS activity and thus the biosynthesis of BCAA, a latter step in the nitrogen assimilation pathway in plants. An in-depth study of the relationship between supplemental nitrogen and chlorsulfuron phytotoxicity to wheat was undertaken as the second objective of this study.

The suitable plant material for the second part of the study would be a sensitive cultivar which clearly showed the interactive effect of chlorsulfuron and external nitrogen. This cultivar was selected based on the findings in the first part of the study. Investigating the interactive effects of supplemental nitrogen and chlorsulfuron efficacy was a primary objective. Studies on

understanding the physiological reasons for this interaction were planned using two approaches. Firstly, using radioisotope techniques to study the uptake, translocation and metabolism of chlorsulfuron in wheat at different nitrogen concentrations. Secondly, the use of whole plant nutrition studies to allow investigation of the effect of chlorsulfuron on wheat supplied with different mineral or organic forms of nitrogen, including different groups of amino acids. In addition to a detailed study of nitrogen metabolism, some other major physiological processes have been examined to investigate their probable role in mediating plant response to chlorsulfuron. Furthermore, a full examination of the effect of chlorsulfuron on its target enzyme, ALS, and on amino acid composition of plants was carried out in order to describe the mechanism of action of chlorsulfuron in a sensitive wheat cultivar. The overall objective of the study was to determine the importance of external factors, specifically available soil nitrogen on the efficacy and mode of action of chlorsulfuron against a range of wheat cultivars. The greatest emphasis was placed on understanding the mechanism of herbicide action and secondarily on how this information could be used to improve the overall use of chlorsulfuron for weed control in wheat crops.

PART TWO

CHAPTER 2

CHAPTER 3

CHAPTER 2

Differential Response of Wheat Cultivars to Chlorsulfuron

2.1 Introduction

Chlorsulfuron is a highly active herbicide recommended for weed control in cereals at rates less than 20 g a.i. ha^{-1}. It can be used either pre- or post-emergence to the crop (Campion, 1982), although only post-emergence application is recommended in New Zealand. Reports about the response of cereal cultivars to chlorsulfuron are contradictory. Initial reports indicated no differences in tolerance to chlorsulfuron between cultivars of winter and spring wheat, durum wheat, barley and oat (Hageman and Behrens, 1979; Hageman and Behrens, 1981; Palm *et al.*, 1980). Several workers reported early injury symptoms such as chlorosis, stunting or leaf abscission but observed no yield reduction (Cornwell and Lane, 1981; Hageman and Behrens 1981). However, Campion (1982) stated that chlorsulfuron was not to be used on some wheat cultivars due to their sensitivity. Significant yield reduction in some cultivars was found when chlorsulfuron was sprayed post-emergence to winter wheat at rates of 35 to 70 g a.i. ha^{-1} (Anderson, 1986; Wicks *et al.*, 1987; Ferreira *et al.*, 1990). In addition reports from Australia have indicated that pre-emergence application of chlorsulfuron can result in growth and yield reduction in some wheat cultivars but not in others (Bowran *et al.*, 1984; Blacklow and Pheloung, 1987; Bowran and Blacklow, 1987; Bowran, 1990a).

There is a need to define the effect of chlorsulfuron on wheat cultivars in a wide range of environments. This information will be required in making decisions on the optimum cultivar-weed control strategy for each location. The objectives of this study were: firstly, to evaluate tolerance to chlorsulfuron of some wheat cultivars used in New Zealand, and secondly to separate the effect of root versus shoot uptake on chlorsulfuron phytotoxicity after plants had received an aerial spray of the herbicide.

Some of the environmental conditions affecting chlorsulfuron performance have been studied and soil nitrogen availability has been reported to enhance differences observed in the rate of leaf

extension between sensitive and tolerant wheat cultivars (Bowran *et al.*, 1984; Bowran and Blacklow, 1987). A detailed study of the interacting effects of nitrogen and chlorsulfuron on cultivar Rongotea is presented in the third part of this thesis. A third objective of the present study was to evaluate the response of the wheat cultivars to external nitrogen when sprayed with chlorsulfuron.

2.2 Materials and Methods

2.2.1 Effect of different rates of chlorsulfuron on 13 wheat cultivars grown in the glasshouse

Thirteen wheat cultivars were selected for initial screening of their sensitivity to chlorsulfuron. The cultivars were Abele, Crossbow, Dartagnan, Jasper and Pegasus, nominated as winter cultivars with a strong vernalization requirement and late maturity characteristics; Karamu, Lancer, Norseman and Otane defined as spring cultivars which are insensitive to daylength, have no vernalization requirement, and are of early maturity type; and Advantage, Kotare, Oroua and Rongotea which are intermediate cultivars with no vernalization requirement but with daylength sensitivity (Anonymous, 1987a & b). Seeds were germinated on moist paper towels and uniform seven-day-old seedlings of all cultivars were planted individually into 1300-mm volume plastic pots filled with Templeton silt loam soil. These were maintained in a glasshouse with a min./max. temperature of 16/30 °C during the growing period. Mercury lamps extended the daylength to 14 h. Seed germination and transplanting of seedlings were staggered to allow all cultivars to reach the three-leaf stage: (Zadoks Growth Scale (ZGS) 13), (Zadoks, Chang and Konzak, 1974; Tottman and Makepeace, 1979), at approximately the same time, when a foliar application of chlorsulfuron was made at 0, 30, 60, and 90 g a.i. ha^{-1}.

Chlorsulfuron was applied with a CO_2 pressurized sprayer fitted with two teejet 8001 nozzles which delivered 250 l of water ha^{-1} at 275 kPa. Citowett surfactant was added to the spray solution at a concentration of 0.25 ml l^{-1}. A randomized complete block design with a factorial combination of 13 cultivars and four chlorsulfuron rates was used. All treatment combinations were replicated five times. Pots were watered regularly and their shoot dry weights determined at harvest by drying to constant weight at 70 °C for two days. The cultivars Abele, Crossbow, Dartagnan and Jasper did not produce seed heads and were harvested 148 days after spraying

(DAS) when the foliage was dry. Other cultivars were harvested at maturity (ZGS 92) which varied between 88 and 138 DAS.

2.2.2 Effect of root vs. foliage uptake on the phytotoxicity of chlorsulfuron to five wheat cultivars

Seeds of Abele, Jasper, Kotare, Lancer and Rongotea were sown on 10 July 1989 into 200-mm diameter black plastic (planter) bags containing 9 kg of Templeton silt loam soil (Appendix 2A), held at field capacity. Pots were maintained outdoors and thinned to a final stand of five plants per pot. On 12 August 1989 when plants had reached the three-leaf stage (ZGS 13), they received an application of chlorsulfuron at 0, 15, or 60 g a.i. ha^{-1}, as previously described. The soil surface of half the pots was temporarily covered with 10 mm of vermiculite to prevent the herbicide from reaching the soil. The experiment was a randomized complete block design consisting of a factorial combination of five cultivars, three herbicide rates and two methods of application (with or without soil cover). The thirty treatments were replicated five times. Plants were harvested 43, and 161 DAS. At each harvest, two plants were hand-clipped at the soil surface and their dry weights determined. An intermediate harvest at 62 DAS was taken when one plant was harvested from each pot. At the final harvest when all plants came to maturity (ZGS 92), seed heads were removed and hand threshed to determine grain yield and its components. The number of spikelets, and number of grains per ear were counted and used in equation 2.1 to determine the number of grains $spikelet^{-1}$.

$$\text{Grains spikelet}^{-1} = \frac{\text{Grains ear}^{-1}}{\text{Spikelets ear}^{-1}} \qquad (2.1)$$

2.2.2.1 Residual Activity of Chlorsulfuron in the Soil

The aim of this experiment was to determine the residual activity of chlorsulfuron in the soil. Samples were collected from the top 30 mm of soil in each pot from the experiment described in section 2.2.2 on 1 March 1990 (203 DAS). Soil from pots having a similar spraying treatment was mixed and a composite sample taken for bioassay according to the procedure described by Groves and Foster (1985).

A sample of 260 g of soil at 5% moisture was placed in 350-ml volume plastic pots in which the drainage holes were sealed with parafilm. Maize seeds (cv. PX 9199 hybrid) were soaked under running water overnight and germinated in moist paper towels in the dark at 21 °C for 72 h. This allowed for selection of seedlings with uniform root length (20 ± 1 mm). Two seedlings were planted in each pot at a depth of 10 mm. Pots were watered to 80% field capacity and wrapped in aluminium foil to minimize evaporation. Pots were maintained at 21 °C and their water content kept at 80% field capacity by regular weighing and watering. Root lengths were measured to the nearest millimeter after eight days and the mean of the two seedlings in each pot was used for analysis.

Chlorsulfuron residues were determined from a standard curve which showed a linear relationship between percentage inhibition of root growth and natural logarithm (ln) of chlorsulfuron concentration (Appendix 2B). Standard concentrations of chlorsulfuron in the soil were made by adding the herbicide in 10 ml distilled water to a known weight of unsprayed soil to give concentrations of 0, 0.25, 0.50 and 1.0 $\mu g\ kg^{-1}$. Soil was brought to 40% field capacity before adding chlorsulfuron to avoid any possible adsorption of the herbicide on dry soil colloids (Groves and Foster, 1985). After thorough mixing, the soil was placed in plastic pots and two maize seedlings were planted in each pot as described above. Pots were maintained at 80% field capacity and mean root length was determined after eight days.

2.2.3 Effect of chlorsulfuron on growth and yield of five wheat cultivars growing in the field

The experiment was carried out on a Wakanui silt loam soil at the Lincoln University research farm. A soil incubation test (Quin, Drewitt and Stephen, 1982) showed that the soil contained equivalent of 113 kg ha^{-1} inorganic and 47 kg ha^{-1} mineralisable nitrogen. An additional 20 kg N ha^{-1} was added in the form of calcium nitrate soon after crop emergence.

Seeds of Abele, Jasper, Kotare, Lancer and Rongotea were drilled in 0.15-m rows with an Oyjord Cone seeder (Walter and Wintersteiger, Austria) on 3 August 1990. Plot size was 2.1 x 5 m with 0.9 m between adjacent plots. Sowing rates were adjusted for seed size and germination percentage to achieve a population of 260 plants m^{-2}. A plant count at the two-leaf stage on 11 September 1990 showed a population range of 294 to 309 plants m^{-2} for the different cultivars (Appendix 2C).

Chlorsulfuron at 0, 15 and 60 g a.i. ha^{-1} was applied on 21 September 1990 when plants were at the three- to four-leaf stage (ZGS 13-14). Chlorsulfuron was applied with a motorized sprayer fitted with four Teejet 11001 nozzles on a two-m boom which delivered 200 l water ha^{-1} at 310 kPa. Citowett was added to the spray solution at a concentration of 0.25 ml l^{-1}. Weeds in all unsprayed control plots were removed by hand on 20-23 October 1990. Growth of wheat canopy soon covered the inter-row spaces so that no further weeding was necessary. The experiment was a factorial combination of five cultivars and three chlorsulfuron rates and was laid-out in a randomized complete block design with four replicates.

There were four harvests during the growing season as described in Table 2.1. At harvests 1 to 3, plants from two 0.1 m^2 quadrats, placed at random, were pulled out of soil, cleaned and kept in a

cold room at 4 °C until processing. The number of tillers per plant and shoot dry weight were determined. The fourth (final) harvest was made at two dates according to maturity of the cultivars and the data were combined. At the final harvest plants from five 0.2 m^2 quadrats for each plot were cut to ground level for determination of yield components and shoot dry weight. The number of ears was counted in each quadrat sample and the number of spikelets determined on a random subsample of 20 ears. The entire quadrat sample was then threshed in a Kurtpelz stationary thresher, and cleaned using a Westrup seed dresser. Grain weight and straw weight (all non-grain parts) were determined after drying at 70 °C for 2 days. Mean kernel weight was then determined for each quadrat sample. Number of grains per ear and per spikelet was derived from the above values using equation 2.1 and 2.2:

$$\text{Grains ear}^{-1} = \frac{\text{Weight of grains ear}^{-1}}{\text{Grain weight}} \qquad (2.2)$$

Table 2.1 Growth stage of wheat cultivars in Experiment 2.2.3 at different harvests.

Harvest	Date	DAS	Plant growth stage
1	12 Oct. 1990	21	6-leaf stage, main stem plus 4-5 tillers (25)*
2	28 Nov. 1990	68	Kotare, Lancer and Rongotea at anthesis (65)
3	18 Dec. 1990	88	Abele and Jasper at anthesis (65)
4A	27 Jan. 1991	128	Kotare, Lancer and Rongotea at maturity (92)
4B	3 Feb. 1991	135	Abele and Jasper at maturity (92)

* Numbers in brackets represent the stage of plant development according to Zadoks Growth Scale.

2.2.4 Effect of Chlorsulfuron on wheat cultivars grown at Low and High Nitrate regimes

Uniform seven-day-old seedlings of cultivars Abele, Jasper, Kotare, Lancer, Rongotea and Sonora were planted individually into 800-ml volume pots containing vermiculite/perlite mixture (1:1, v:v) and fed regularly with a basal nutrient solution (Appendix 2D) containing 1.0 mol m^{-3} potassium nitrate. Potassium sulphate was used to balance the K^+ level between the two nitrate regimes. Plants were maintained in a controlled environment cabinet at 12 h daylength, 70% relative humidity and day/night temperatures of 20/10 °C. Lighting was provided by white fluorescent plus incandescent tubes which gave a photosynthetic photon flux density of approximately 300 μmol m^{-2} s^{-1} at the pot surface.

When plants reached the three-leaf stage (ZGS 13), half were grown on high (5 mol m^{-3}) nitrate and the other half continued to receive low (1 mol m^{-3}) nitrate for the rest of their growing period. At this stage a foliar application of chlorsulfuron was applied at 0, 15 or 60 g a.i. ha^{-1}, as described in section 2.2.1.

The experiment was a randomized complete block design consisting of a factorial combination of three chlorsulfuron rates and two nitrate levels, replicated five times. At 52 DAS plants were harvested and plant height, number of tillers and shoot dry weight measured.

2.2.5 Plant material and data analysis

Seeds were obtained from the following sources: Plant breeders NZ Ltd. (Advantage); Pyne, Gould and Guiness NZ Ltd. (Pegasus and Karamu); Challenge seeds, Christchurch, NZ (Abele, Crossbow, Jasper, Kotare, Norseman and Rongotea); Hodder and Tolley (Oroua and Otane). Sonora was a gift from Australian Winter Cereals Collection, Tamworth, NSW, Australia.

In the experiments described in this chapter data were examined by analysis of variance using the SAS statistical programme (SAS Institute Inc., Cary, NC, USA). The effects described have a probability of at least $p < 0.05$ unless otherwise stated. Means of sprayed plants were compared with that of the control using Dunnet's procedure (Steel and Torrie, 1980; Damon and Harvey, 1987). As the aim of the experiments described in this chapter was to determine the phytotoxicity of chlorsulfuron to wheat cultivars in comparison with a zero-dose control, Dunnet's procedure provided a simple and realistic statistic for such comparisons (Chew, 1976). The Dunnet's statistic, designed as 'd' is presented with the means for each cultivar at different chlorsulfuron application rates.

2.3 Results

2.3.1. Effect of different rates of chlorsulfuron on 13 wheat cultivars grown in the glasshouse

Significant reductions in shoot dry weight occurred for Lancer at all rates of chlorsulfuron and for Rongotea at rates greater than 30 g a.i.ha^{-1} (Table 2.2). No significant reduction in shoot dry weight was observed for other cultivars. When averaged over all chlorsulfuron rates, Lancer and Rongotea showed the highest (48.9% and 44%, respectively) reductions in shoot dry weight compared to their respective controls (Table 2.2).

2.3.2. Effect of root versus foliage uptake on the response of five wheat cultivars to chlorsulfuron

All cultivars except Kotare, showed significant reductions in dry weight 43 DAS with 60 g a.i.ha^{-1} chlorsulfuron sprayed on foliage plus soil (Table 2.3). Abele and Jasper showed significant reductions with both methods of spraying at 60 g a.i. ha^{-1} chlorsulfuron. Shoot dry weight in Abele and Rongotea was reduced significantly even at 15 g a.i. ha^{-1}, when chlorsulfuron was applied to foliage plus soil. Rongotea showed detachment of leaf 4 at the base of the leaf sheath when 60 g a.i. ha^{-1} chlorsulfuron was applied to foliage plus soil (Plate 2.1).

Most cultivars showed recovery from early damage in the succeeding harvests. At 62 DAS, dry weight reductions were significant only for Lancer and Rongotea where foliage plus soil surface had been sprayed. This reduction occurred for Lancer with both rates of chlorsulfuron and for Rongotea at the higher rate only (Table 2.3). The reduction was highest for Rongotea and shoot dry weight was only 37.4% of that in the control.

Table 2.2 Effect of different rates of chlorsulfuron on shoot dry weight (g) of 13 wheat cultivars in the glasshouse.

Cultivar	Chlorsulfuron rate (g a.i. ha^{-1})					Percentage of control[a]
	0	30	60	90	d	
Abele	1.12	1.17	1.29	0.91	0.694	100.3
Advantage	1.06	1.33	1.24	0.93	0.893	110.1
Crossbow	1.24	1.10	1.02	1.10	0.876	86.6
Dartagnan	1.26	1.34	1.27	0.83	0.623	91.0
Jasper	1.11	1.02	0.72	0.82	0.592	76.9
Karamu	1.04	0.80	0.77	0.67	0.846	71.8
Kotare	1.65	1.01	1.06	1.13	0.866	64.6
Lancer	2.06	1.20*	0.96*	1.00*	0.815	51.1
Norseman	1.62	1.47	1.44	1.14	0.862	83.3
Oroua	1.72	1.54	1.21	1.16	1.139	75.7
Otane	1.75	1.51	1.17	0.95	1.108	69.2
Pegasus	1.74	1.65	1.30	0.97	1.139	75.1
Rongotea	1.75	1.28	0.66*	0.99*	0.760	56.0

a) Average shoot dry weight of sprayed plants for all chlorsulfuron rates as percentage of unsprayed control.

* An asterisk indicates significant reduction in shoot dry weight compared with the control based on Dunnet's procedure (d) at $p < 0.05$.

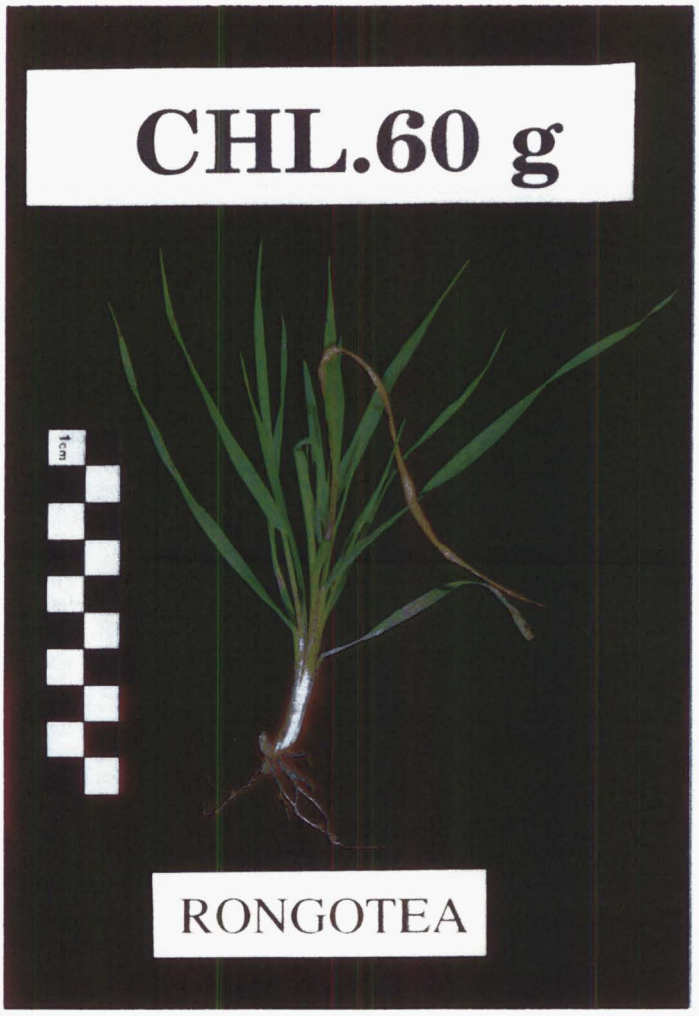


Plate 2.1 Detachment of leaf 4 in wheat cv. Rongotea sprayed with chlorsulfuron at 60 g a.i. ha^{-1}.

Table 2.3 Effect of chlorsulfuron applied to foliage alone (F) or to foliage plus soil surface (F+S) on shoot dry weight (g) of wheat cultivars at different harvests and on grain yield (g).

Cultivar	Rate	Days after spraying						Grain yield	
		43		62		161			
		F	F+S	F	F+S	F	F+S	F	F+S
Abele	**0**	**0.96**		**2.06**		**4.77**		**5.57**	
	15	**0.76**	**0.72***	**1.93**	**1.69**	**4.21**	**4.37**	**5.20**	**4.94**
	60	**0.72***	**0.61***	**1.61**	**1.58**	**4.91**	**5.02**	**5.96**	**5.68**
	d	0.211		0.965		0.936		1.073	
Jasper	**0**	**1.06**		**2.24**		**4.88**		**6.20**	
	15	**0.92**	**0.90**	**2.31**	**2.73**	**4.90**	**5.34**	**6.41**	**6.06**
	60	**0.78***	**0.65***	**1.55**	**1.55**	**3.96**	**4.56**	**6.27**	**5.60**
	d	0.241		1.027		1.349		1.530	
Kotare	**0**	**1.15**		**2.36**		**4.59**		**5.02**	
	15	**0.88**	**0.98**	**2.23**	**2.44**	**4.46**	**5.49**	**5.09**	**5.86**
	60	**0.96**	**0.86**	**1.89**	**2.40**	**5.76**	**4.98**	**5.00**	**5.65**
	d	0.312		1.050		1.330		1.381	
Lancer	**0**	**1.02**		**2.89**		**3.92**		**5.32**	
	15	**0.97**	**0.96**	**2.52**	**1.43***	**3.74**	**4.03**	**4.67**	**5.34**
	60	**0.77**	**0.61***	**2.23**	**1.37***	**3.78**	**3.36**	**4.94**	**4.10***
	d	0.341		1.288		0.995		1.050	
Rongotea	**0**	**0.94**		**2.06**		**4.27**		**4.44**	
	15	**0.78**	**0.68***	**2.02**	**1.54**	**3.98**	**3.49***	**3.68**	**3.56**
	60	**0.69**	**0.45***	**1.38**	**0.77***	**3.77**	**3.47***	**3.83**	**3.45***
	d	0.250		0.978		0.783		0.943	

* An asterisk indicates a significant reduction compared with the control based on Dunnet's procedure (d) at $p < 0.05$.

At maturity the only cultivar with a significant reduction in shoot dry weight was Rongotea which showed greater than 18% reduction when foliage plus soil had been sprayed with either 15 or 60 g a.i. ha^{-1} chlorsulfuron (Table 2.3). Reductions in grain yield were significant for Lancer and Rongotea when sprayed with 60 g a.i. ha^{-1} chlorsulfuron applied to foliage plus soil surface. This treatment produced only 77.1% and 77.7% of the grain yield in the controls for Lancer and Rongotea, respectively. Chlorsulfuron application at 15 g a.i. ha^{-1} resulted in 20% reduction in grain yield of Rongotea, however this reduction was not significant. The significant reductions in the yield of Lancer and Rongotea were associated with significant reductions in the number of spikelets ear^{-1}. None of the other yield components were significantly affected in any of the cultivars studied (Appendix 2E).

2.3.2.1 Residual Activity of chlorsulfuron in the soil

Maize root growth was inhibited severely when grown in soil that had been sprayed with chlorsulfuron 203 days previously (Table 2.4). A 38.9% and 43.8% reduction in root length of maize seedling was observed when the soil surface had been sprayed with chlorsulfuron at 15 and 60 g a.i. ha^{-1}, respectively. These values corresponded to 0.39 and 0.47 $\mu g\ kg^{-1}$ chlorsulfuron in the soil, as estimated by use of the standard curve (Appendix 2B). No chlorsulfuron residue was detected when the soil surface was not exposed to spraying.

Table 2.4 Inhibition of maize root length following growth in soil from chlorsulfuron treatments applied to foliage alone (F) or to foliage plus soil surface (F+S). Soil samples were taken 203 days after spraying.

Initial chlorsulfuron rate (g a.i. ha^{-1})	Application method	Root length (mm)	Reduction in root length (%)	Chlorsulfuron residue ($\mu g\ kg^{-1}$)[a]
0	--	144	--	--
15	F	117	18.8	n.d.[b]
	F+S	88*	38.9	0.39
60	F	121	16.0	n.d.
	F+S	81*	43.8	0.47
d		43.4		

a) Chlorsulfuron residue was estimated from a standard curve (Appendix 2B).

b) n.d.= not detectable, estimated chlorsulfuron residue was smaller than the lowest standard concentration used.

* An asterisk indicates a significant reduction compared with the control based on Dunnet's procedure (d) at $p < 0.05$.

2.3.3 Effect of chlorsulfuron on growth and yield of five wheat cultivars in the field

Shoot dry weight measured 21 DAS showed significant reductions at 60 g a.i. ha^{-1} chlorsulfuron in all cultivars except Kotare (Table 2.5). The reduction was greatest for Rongotea which produced a dry weight of only 70.2% of the control. Rongotea also showed a significant reduction in shoot dry weight at 15 g a.i. ha^{-1} chlorsulfuron. Detachment of leaf 4 was observed in Rongotea sprayed with the high rate of chlorsulfuron.

Table 2.5 Effect of chlorsulfuron on shoot dry weight (g) per plant in wheat cultivars 21 days after spraying.

Cultivar	Chlorsulfuron rate (g a.i. ha^{-1})			d
	0	15	60	
Abele	0.38	0.31	0.29*	0.073
Jasper	0.32	0.27	0.25*	0.046
Kotare	0.44	0.39	0.36	0.093
Lancer	0.48	0.45	0.39*	0.050
Rongotea	0.47	0.40*	0.33*	0.060

* An asterisk indicates a significant reduction compared with the control based on Dunnet's procedure (d) at $p < 0.05$.

At 68 DAS, Rongotea sprayed with 60 g a.i. ha^{-1} chlorsulfuron showed a reduction in shoot dry weight (significant at $p < 0.10$) and produced only 83% as much dry weight as that of the control (Appendix 2F_I). None of the other cultivars showed any reduction in shoot dry weight at this time. No significant reductions in shoot dry weight were observed in any cultivars at either 88 DAS (Appendix 2F_I) or at final harvest (Appendix 2F_II). Chlorsulfuron did not influence the number of ears per unit area, the number of grains per spikelet or the individual grain weight (Appendix 2F_III). In Rongotea and Lancer, there were significant reductions in the number of spikelets per ear but no significant reduction in grain yield.

2.3.4 Effect of chlorsulfuron on wheat cultivars grown at low and high nitrate concentrations

Shoot dry weight of unsprayed plants increased with an increase in nitrate concentration from 1 to 5 mol m^{-3} for all cultivars (Figure 2.1). At 15 g a.i. ha^{-1} chlorsulfuron, the only cultivars which showed a significant increase in dry weight at high nitrate were Kotare and Lancer. At 60 g a.i. ha^{-1} chlorsulfuron, Kotare was the only cultivar with a significant increase in dry weight when

grown at the high nitrate concentration. There was a decrease in shoot dry weight of Abele and Sonora at the high nitrate treatment when sprayed with 60 g a.i. ha^{-1} chlorsulfuron.

Plant height showed a similar trend to shoot dry weight for all cultivars, but the number of tillers did not always follow a similar pattern (Appendix 2G). There was an increase in the number of tillers at high nitrate for Abele, Jasper and Kotare, irrespective of chlorsulfuron rate. With Lancer, Rongotea and Sonora there was a greater increase in the number of tillers at 5 mol m^{-3} nitrate when plants received the higher rate of the herbicide (Appendix 2G).

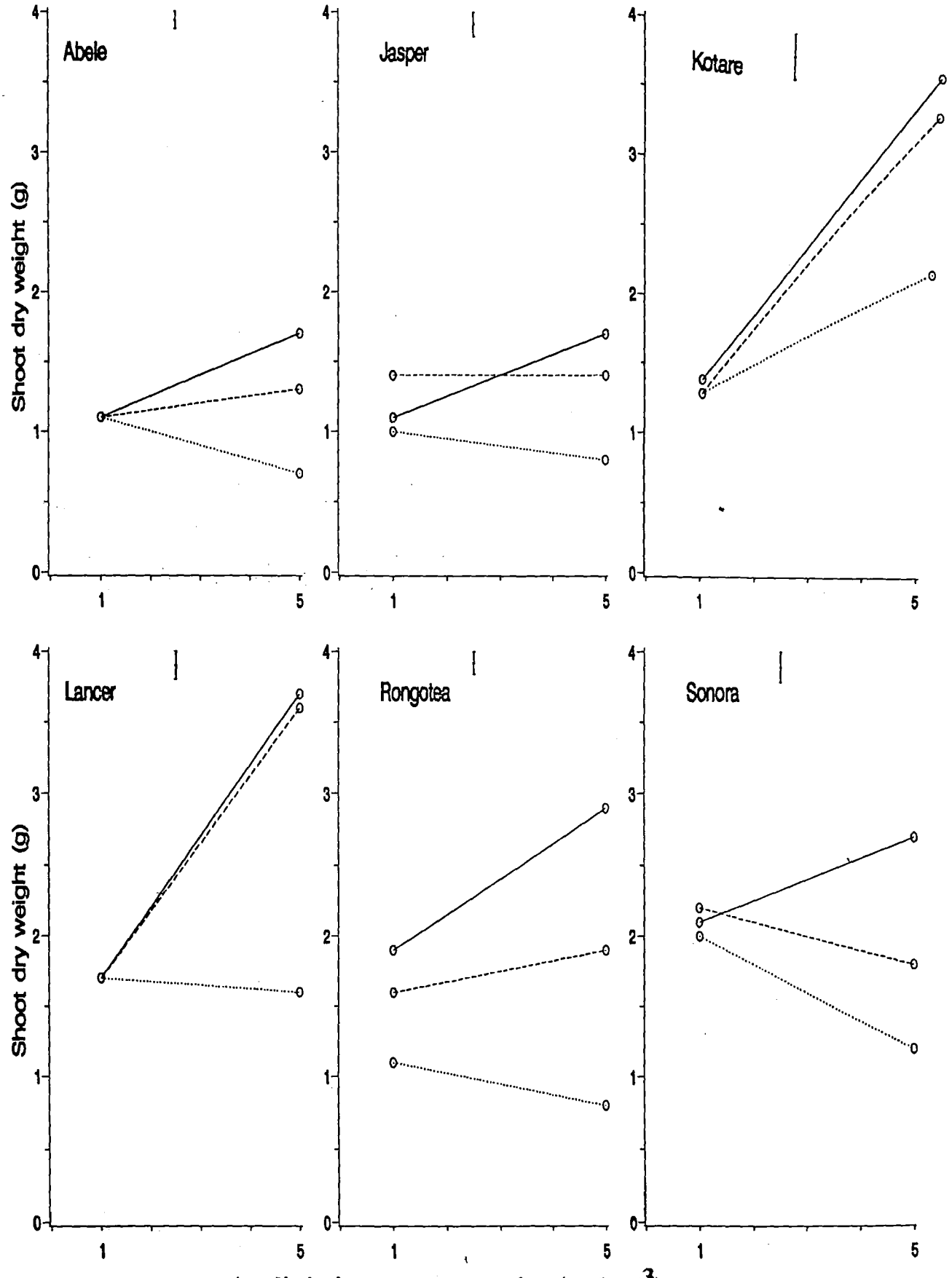


Figure 2.1 Effect of chlorsulfuron at 0 (——), 15 (- - -) or 60 (.....) g a.i. ha^{-1} on shoot dry weight of wheat cultivars grown at 1 or 5 mol m^{-3} nitrate concentration. Error bars are SEm.

2.4 Discussion

A series of experiments conducted under varying environmental conditions showed that cultivars Lancer and Rongotea were sensitive to a foliar application of chlorsulfuron. In contrast Kotare was tolerant to chlorsulfuron application. There were greater reductions in shoot dry weight or yield for sensitive cultivars at 60 g a.i. ha^{-1} chlorsulfuron compared to the recommended rate of 15 g a.i. ha^{-1}. This demonstrates the potential for serious crop damage to sensitive cultivars under practical field conditions where recommended rates may be exceeded. The differences in tolerance to chlorsulfuron were independent of the maturity type of cultivars. For example, the sensitive Rongotea and the tolerant Kotare are both defined as intermediate cultivars, while Rongotea and Lancer were both sensitive to chlorsulfuron but are intermediate and early maturity types, respectively.

Chlorsulfuron damage to Lancer and Rongotea was greater and longer lasting when foliage plus soil surface received the herbicide (Table 2.3). This probably confirms the importance of root uptake in the response to chlorsulfuron. It has been shown that chlorsulfuron can readily be leached in a silt loam soil (O'Sullivan, 1982), and it can be absorbed by roots (Ray, 1982a). The greater phytotoxicity of chlorsulfuron when both foliage plus soil received the herbicide could be related to both a higher amount of chlorsulfuron immediately available to the plant, and to a continuous supply of the herbicide from the soil. Hall *et al.* (1985) showed that soil spray deposit was an important component of the activity of picloram, clopyralid, chlorsulfuron and metsulfuron against Canada thistle regrowth under greenhouse conditions. In the experiment reported in Section 2.3.2, apparently chlorsulfuron was leached into the soil from the spray deposits on the soil surface with irrigation water. Leaching of chlorsulfuron coupled with its persistence seem to be important factors in its phytotoxicity to Lancer and Rongotea. Residual concentrations of 0.39 $\mu g\ kg^{-1}$ and 0.47 $\mu g\ kg^{-1}$ chlorsulfuron were found in the treated soil 203 days after application of 15 and 60 g a.i. ha^{-1}, respectively (Table 2.4). After approximately the

same period, Royuela *et al.* (1990) detected chlorsulfuron residues of 0.12 and 0.28 $\mu g\ kg^{-1}$ following pre-emergence application of 20 and 30 g a.i. ha^{-1} to a silt loam soil. For comparison, chlorsulfuron concentrations of 0.125 and 0.4 $\mu g\ kg^{-1}$ were found to be injurious to maize and lentil (*Lens culinaris* Medic.) roots, respectively (Groves and Faster, 1985; Blacklow and Pheloung, 1991). Susceptible crosses of maize had a GR_{20} (concentration required to inhibit root length by 20%) of only 0.07 $\mu g\ kg^{-1}$ (Landi, Vicari and Catizone, 1989).

Although it was not an initial aim of the experiment to determine the starting concentration of chlorsulfuron in the soil, it is apparent that much higher concentrations than the values detected 203 DAS were present in the treated soil. Other workers have reported a range of 7-24 $\mu g\ kg^{-1}$ chlorsulfuron following an application of 10-35 g a.i. ha^{-1} to a sandy loam (Blacklow and Pheloung, 1991) or 8.3-33.2 $\mu g\ kg^{-1}$ chlorsulfuron following an application of 20-80 g a.i. ha^{-1} to a silt loam soil (Royuela *et al.*, 1990). The results of the bioassay assist in the explanation of the greater phytotoxicity of wheat when foliage plus soil were sprayed than when only the foliage was sprayed. These results showed that chlorsulfuron had a long residual activity under the experimental conditions. The half life of chlorsulfuron at pH 5.8 was 176 days at 20 °C and 47 days at 30 °C. Moreover, half life increased with pH up to pH 9.0 (Blacklow and Pheloung, 1991). The silt loam soil used in Experiment 2.2.2 had a pH of 6.2 (Appendix 2C), and the mean soil temperature was below 10 °C for two months following the herbicide application (Appendix 2H). Under these conditions chlorsulfuron dissipation is expected to be slow. Royuela *et al.* (1990) also reported that the rate of dissipation of chlorsulfuron was much slower in cold months, immediately following its application to a winter wheat crop, than the warmer months towards the end of the growing season.

The detachment of the emerging leaf (leaf 4) of Rongotea following chlorsulfuron application observed in these experiments is similar to previous reports on wheat (Hageman and Behrens, 1981) and velvetleaf (Hageman and Behrens, 1984). Reductions in plant dry weight were always

greater at the earlier harvests and all cultivars showed considerable recovery from the early damage (Tables 2.3 and 2.5, Appendix 2F). Similarly, Royuela *et al.*, (1990) found that phytotoxic effects from chlorsulfuron were less evident at the heading stage than at tillering in winter wheat. This could be accounted for by a greater concentration of chlorsulfuron at early stages, both within the plant following foliar uptake and from soil-available herbicide. Shortly after spraying, the concentration of chlorsulfuron inside the plant will decrease as a result of herbicide metabolism (Sweetser *et al.*, 1982). Chlorsulfuron concentration in the soil will also decrease in time due to chemical hydrolysis and microbial breakdown (Beyer *et al.*, 1988; Brown, 1990). Cereals have a strong compensatory growth ability and can recover if the early damage is mild and growth conditions are favourable. The early growth reductions in Rongotea with 15 g a.i. ha^{-1} chlorsulfuron were therefore overcome by further growth. In comparison, Rongotea sprayed with 60 g a.i. ha^{-1} could recover only under more favourable conditions in the field experiment.

The only component of yield affected by chlorsulfuron in these experiments was the number of spikelets ear^{-1}. Some workers have reported on the effect of chlorsulfuron on yield components including grain weight and number of grains $spike^{-1}$ (Tanaka and Anderson, 1985), number of spikelets ear^{-1} (Bowran *et al.*, 1984), and number of ears m^{-2} (Bowran *et al.*, 1984; Royuela *et al.*, 1990). Initiation of spikelets is an early event in the apical development of wheat. It corresponds to the formation of double ridges and continues until the terminal spikelet is formed (Kirby, 1981; Thorne, 1982; Baker and Gallagher, 1983). Dissection of wheat seedlings at the three-leaf stage (ZGS 13) showed the formation of double ridges in the apical meristem. It was at this stage that chlorsulfuron was applied and the concentration of chlorsulfuron in the plant and also in the soil could have been expected to be high for some time after application. Thus it is likely that chlorsulfuron could affect the development of spikelet primordia through an interference with cell division, which is reported to be its primary mode of action (Ray, 1989).

From the initial chlorsulfuron damage to sensitive cultivars, significant yield reductions might have been anticipated. This occurred in the pot experiment but not in the field. The lack of chlorsulfuron effect on the yield under the field conditions could be explained in two ways. First, a greater amount of chlorsulfuron might have been available to roots in the pot experiments than in the field. Root uptake was a significant component of chlorsulfuron activity against the pot grown plants (see above discussion). The low ratio of soil volume to root length and the closer proximity of the major portion of the roots and the herbicide deposits at the soil surface might have resulted in a greater availability of chlorsulfuron to roots in the pots. A second explanation for the lack of serious yield reduction in the field could be the plasticity of yield components and the compensatory growth ability of wheat. Grain yield is the sum of contributions made by various components and the plant can often compensate for unsatisfactory development of one component by promotion of another. In the field, Lancer and Rongotea showed a significant increase in the number of tillers 21 DAS following application of chlorsulfuron (Appendix 2F_IV), and a non significant increase in the number of ears m^{-2} (Appendix 2F_III). These changes may be the basis of compensation for the reduction in the number of spikelets ear^{-1}.

The increase in the number of tillers could be due to one or both of the following effects. Early development of tillers is supported by photoassimilates from the main shoot (Lauer and Simmons, 1985). A slower growth rate in the main stem apical meristem induced by greater concentrations of chlorsulfuron might have caused a shift in assimilate transport to the tiller buds which gave them an earlier start with a better chance of survival. In addition, lower concentrations of chlorsulfuron in the tiller buds might have had a stimulatory effect. The stimulation of vegetative growth in the young seedlings of several species by sublethal doses of chlorsulfuron is documented in the literature (Beyer *et al.*, 1988; Landi *et al.*, 1989).

It may also be suggested that cultivars Lancer and Rongotea, though sensitive to chlorsulfuron at an early stage, have adequate compensatory growth capacity to overcome the damage and escape a

potential yield loss when grown under favourable conditions. However, this might not be the case for all sensitive cultivars. The extent of compensation probably depends upon the cultivar and the environment (Lemerle *et al.*, 1985). For example, a cultivar with low-tillering capacity may not compensate for reductions in other yield components initiated by herbicide application. This can result in serious yield reductions, especially under conditions which might further limit its compensatory growth ability. Reports on chlorsulfuron effects show an inhibition of tillering or a reduction in the number of ears m^{-2} in some cultivars (Bowran *et al.*, 1984; Vanakitmongkol and Blacklow, 1990; Royuela *et al.*, 1990). An inability to produce tillers may be a reason why some workers have found significant yield reductions with chlorsulfuron at rates of 15-35 g a.i. ha^{-1} (Anderson, 1986; Bowran, 1990a), while others did not observe any effect on yield from chlorsulfuron even up to 250 g a.i. ha^{-1} despite early damage (Hageman and Behrens, 1981).

Increases in shoot dry weight, number of tillers and plant height obtained with supplemented nitrate in control plants of Experiment 2.3.4 are in accordance with the previous reports on positive effect of nitrogen (Dougherty *et al.*, 1975; Goh and Haynes, 1986). The magnitude of the response to nitrate was different between cultivars, but could not be attributed to differences in maturity type. Differences in the effect of nitrogen fertilizer on wheat cultivars have been reported (Kozlowska-Ptaszynska, 1983; Lal, 1984). The response of most cultivars to nitrate was limited when sprayed with chlorsulfuron, and this was related to their tolerance of chlorsulfuron as determined in the pot and field experiments. Thus, there was no effect on the response of Kotare to increased nitrate at 15 g a.i. ha^{-1} chlorsulfuron and only a small reduction in its response to nitrate at 60 g a.i. ha^{-1} chlorsulfuron (Figure 2.1). In comparison, the responses of Rongotea and Sonora were greatly inhibited by both rates of chlorsulfuron, and Lancer showed a strong inhibition at 60 g a.i. ha^{-1} chlorsulfuron.

In summary, the results in this chapter demonstrate that some wheat cultivars have low levels of tolerance to chlorsulfuron. It is important to find explanations for differential sensitivity to

chlorsulfuron between cultivars. Such a study is undertaken in Chapter 3 of this thesis. Moreover, differences were found in the effect of chlorsulfuron on the response of wheat cultivars to nitrate supplementation (Figure 2.1). Similarly, Bowran and Blacklow (1987) reported that sensitive wheat cultivars like Sonora grown in chlorsulfuron-treated soil failed to respond to increases in soil nitrogen as measured by the rate of leaf extension. Increased phytotoxicity of chlorsulfuron with added nitrogen was also reported for green foxtail (Nalewaja and Woznica, 1985). At present, there is insufficient information in the literature to warrant a full description of the nature of this effect. The primary site of action of chlorsulfuron is reported to be an inhibition of the enzyme acetolactate synthase which is the first enzyme in the biosynthesis of branched chain amino acids (Ray, 1984). Several other secondary effects have been observed with chlorsulfuron. A full investigation of chlorsulfuron effect on wheat, cv. Rongotea, grown at different levels of nitrogen is the subject of the third part of this thesis.

CHAPTER 3

The Mechanism of Differential Response of Wheat Cultivars to Chlorsulfuron

3.1 Introduction

Variations in the performance of a foliar-applied herbicide have been associated with differential spray deposit, uptake and translocation or metabolism within the plant (Hathway, 1986; Owen, 1989; Hess and Falk, 1990; Devine and Vanden Born, 1991). Variations in one or more of these events may cause changes at the target site in the concentration of the herbicide in toxic form. This may induce biochemical responses leading to changes in plant growth and structure (Ashton and Crafts, 1981). Wheat cultivars showed differential tolerance to the application of chlorsulfuron (Chapter 2). Similar differences in sensitivity to chlorsulfuron amongst cultivars have been reported by various workers (Hageman and Behrens, 1981; Anderson, 1986; Wicks *et al.*, 1987; Bowran and Blacklow, 1987). However, the physiological reasons for such differences in chlorsulfuron activity have not been elucidated. Research with other herbicides shows the relationship between some of the above mentioned events and herbicide efficacy, although responses are often herbicide and species-specific, as explained below.

Differences in tolerance among some cereal crop cultivars to several herbicides have been reported to be due to differential rates of uptake, translocation or metabolism. Variations in the response of three winter wheat cultivars to post-emergence application of difenzoquat were reported to be due to differences in spray retention and the accumulation of the herbicide in the apical meristem (Pallet, 1984). Different translocation rates were partly responsible for variations in phytotoxicity of bensulfuron-methyl in rice cultivars (Pyon *et al.*, 1987). Degradation of chlorotoluron was more rapid in tolerant than sensitive varieties of cereals (Ryan and Owen, 1983). In another study tolerant wheat cultivars metabolised ethiozin more rapidly than sensitive cultivars (Fedtke and Schmidt, 1988). The significance of metabolism as a basis for tolerance to different herbicides has been the subject of several reviews (Hathway, 1986; Owen, 1989).

As the primary site of action of chlorsulfuron is in meristematic tissues (Ray, 1989), its absorption by and translocation from the intercepting leaves to these tissues are essential steps in determining phytotoxicity. Some workers have tried to attribute differences in chlorsulfuron sensitivity among species to differential rates of retention, penetration, translocation or metabolism of the herbicide. Sweetser *et al.*, (1982) reported that differences in uptake and translocation were not responsible for the differences in phytotoxicity of chlorsulfuron observed between tolerant cereals and sensitive broadleaf weeds. Similarly, retention, uptake and translocation were reported to be comparable between susceptible velvetleaf and tolerant eastern black nightshade (Hageman and Behrens, 1984). In contrast, metabolism was found to be the basis of selectivity in both grasses and tolerant broadleaf species. Wheat metabolised about 95% of ^{14}C-chlorsulfuron in 24 h while a sensitive plant such as sugarbeet could metabolise only 3% of the applied chlorsulfuron in the same period (Sweetser *et al.*, 1982). Among the tolerant broadleaf species, flax, black nightshade and eastern black nightshade were reported to be able to metabolise chlorsulfuron rapidly (Hutchison *et al.*, 1984).

Very little published information is available to correlate sensitivity of different crop cultivars to chlorsulfuron, or other sulfonylurea herbicides, with their uptake, translocation or metabolism of the herbicide. Blacklow and Pheloung (1987) reported that detached leaves of sensitive wheat cultivars metabolised chlorsulfuron more slowly than tolerant cultivars. Matthews *et al.*, (1990) found some evidence to correlate resistance to chlorsulfuron in an annual ryegrass (*Lolium rigidum* L.) biotype to its ability to oxidise the herbicide to a less active catabolite. Similarly, Harms *et al.* (1990) found that differential metabolism was the mechanism of tolerance of maize inbred lines to the sulfonylurea herbicide primisulfuron. The objective of the present study was to investigate the possible mechanisms for differential sensitivity of wheat cultivars to foliar application of chlorsulfuron. Thus retention of chlorsulfuron on the foliage, its penetration into the leaves, its translocation and distribution inside the plant and its rate of metabolism within the plant were compared in five of the wheat cultivars which have been shown to have different degrees of tolerance to chlorsulfuron (Chapter 2).

3.2 MATERIALS AND METHODS

3.2.1 Retention of chlorsulfuron by foliage.

Seeds of wheat cultivars Abele, Jasper, Kotare, Lancer and Rongotea were sown on 10 June 1989 in 700-ml volume plastic pots containing Templeton silt loam soil. These were kept outdoors and thinned to two seedlings per pot. The experiment was a randomized complete block design with four replicates.

At the three-leaf stage (ZGS 13) retention of chlorsulfuron on the foliage was determined as described by Richardson (1984). Plants were sprayed with 15 g a.i. ha^{-1} chlorsulfuron as described in section 2.2.1. The herbicide solution contained Citowett surfactant (0.25 ml l^{-1}) and was saturated with fluorescein dye (0.05 g l^{-1}) which was compatible with the herbicide solution. After 30 minutes, when foliage was dry, plants were cut at the base and the dye was washed off the foliage by shaking for 30 seconds in a plastic bag containing 30 ml of a solution of 5 mol m^{-3} sodium hydroxide. The washings were filtered through glass micro fibre discs (Whatman GF/C) and a sample was taken for fluorimetry (Schimadzu RF-540 spectrofluorophotometer). The excitation and the emission wavelengths were 450 and 515 nm, respectively. The amount of the dye in the wash solutions was determined by comparison with a standard curve which was linear in the concentration range used (Appendix 3A). After washing the foliage, all leaves were passed through an area meter (Lambda LI 3100) and total leaf area measured. The leaves were then oven dried at 70 °C for 48 h for dry weight determination.

3.2.2 Uptake and translocation of ^{14}C-chlorsulfuron

Wheat seedlings from cultivars Abele, Jasper, Kotare, Lancer and Rongotea were grown individually in 350-ml volume plastic pots filled with Templeton silt loam soil to which the equivalent of 50 kg N ha^{-1} in the form of calcium nitrate was added. Pots were maintained in a controlled environment cabinet as previously described (section 2.2.5). The experiment was a randomized complete block design with four replicates.

Radiolabelled chlorsulfuron was applied at the three-leaf stage (ZGS 13). Ten μl ^{14}C-chlorsulfuron was applied as 35-40 droplets with a micro-syringe to the adaxial surface of the lamina of leaf 2 in an area approximately 30 mm long and 15 mm from the ligule. Chlorsulfuron [Carbonyl-^{14}C]; specific activity 1016.1 MBq $mmole^{-1}$ (76.8 μCi mg^{-1}) and radioactive purity of 99.0%, was formulated by dissolving in 0.025% Citowett solution in deionized water to give a final concentration of 1850 Bq (0.05 μCi) in 10 μl. This was approximately equivalent to the concentration of chlorsulfuron used in other experiments at the recommended rate of 15 g a.i. ha^{-1}. The ^{14}C-chlorsulfuron was applied 3 h after the start of the photoperiod.

Uptake of ^{14}C-chlorsulfuron was measured 12 h and 48 h after application of the radiolabel. At each time, the treated lamina was detached and washed with 20 ml 0.025% Citowett solution. A 1-ml aliquot of the wash-off solution was added to 10 ml Bray's scintillation cocktail (Appendix 3B) and the radioactivity was determined by liquid scintillation spectrometry (Philips PW 4700). Uptake was calculated from the difference between the applied dpm and the dpm in the wash solution and expressed as a percentage of the applied radioactivity.

Translocation and distribution of chlorsulfuron in different wheat cultivars was assessed 12 h and 48 h after application. At each time, the treated lamina was detached and washed. Plants were then divided into various fractions namely; leaf 1, lamina of leaf 2, sheath of leaf 2, young untreated tissue (which included leaves 3 and 4, tillers and apical meristem) and root. These were freeze-dried, weighed and kept frozen (-18 °C) until combusted in a sample oxidizer (R.J. Harvey, OX-300). The $^{14}CO_2$ from combusted samples was trapped in 12 ml of carbon 14

cocktail (R.J. Harvey Co.) and dpm values were determined by liquid scintillation spectrometry as described above. The oxidizer was calibrated using a commercial ^{14}C-labelled radioactive organic standard solution (Packard Spec Chec). The total amount of ^{14}C-chlorsulfuron translocated out of the treated area was calculated as a percentage of the amount applied (Translocation A) and also as a percentage of the radioactivity recovered from plant, excluding the wash off (Translocation B) according to equations 3.1 and 3.2, respectively:

$$\text{Translocation A} = \frac{(\text{Applied dpm} - (\text{wash off dpm} + \text{dpm in treated area})) * 100}{\text{Applied dpm}} \quad (3.1)$$

$$\text{Translocation B} = \frac{(\text{Total dpm recovered from plant} - \text{dpm in treated area}) * 100}{\text{Total dpm recovered from plant}} \quad (3.2)$$

In addition, the concentration of radioactivity in different plant parts was determined.

3.2.3 Metabolism of chlorsulfuron

Plant culture and growing conditions were the same as described for Experiment 3.2.2. Experimental design was a randomized complete block with four replicates.

Ten microlitres of ^{14}C-chlorsulfuron, formulated as previously described, was applied as 18-20 droplets with a micro-syringe to the adaxial laminar surfaces of each of leaves 2 and 3 in similar positions to those described in Section 3.2.2. Two plants were paired as one plot, hence, the total amount of radioactivity applied per plot was 7400 Bq (0.2 μCi). This helped detection of radioactive metabolites in the untreated plant parts. Plants were harvested 12 and 48 h after application of the label. At each harvest the untreated young parts (all the untreated leaves and stem except for leaf 1) from both plants were pooled together. All samples obtained in this way were freeze-dried and kept at -18 °C until extraction of radioactivity.

Metabolism of chlorsulfuron was studied following the procedure described by Foley (1986) with some modifications. The leaves were cut into small sections and homogenized with mortar and pestle in 5 ml of 80% acetone and centrifuged at 10,000 g for 10 min, and the supernatant was collected. The residue was extracted three more times and centrifuged. The supernatants were combined and dried *in vacuo* using a speed vac concentrator (Savant SVC-200H) and suspended in 35-40 μl of 80% acetone. A sample of approximately 30 μl was spotted on a pre-coated 50 x 200 mm silica gel thin layer chromatography (TLC) plate (T-7270, 250 μm thickness, Sigma Chemical Company). A 5-10 μl sample of the resuspended plant extract was put in a vial with 10 ml Bray's cocktail and assayed for radioactivity by liquid scintillation spectrometry. The extracted plant residue was also put in scintillation fluid for determination of ^{14}C by liquid scintillation spectrometry. Less than 1% of the total radioactivity was found in plant residues indicating a highly effective extraction procedure. The TLC plates were developed to a 160-mm solvent front in a chloroform: acetic acid (19:3, v:v) solvent system. Parallel TLC plates with 1-5

μl ^{14}C-chlorsulfuron were developed similarly as standards. All plates were scanned by a radiochromatogram scanner (Packard 7201) and different metabolites were located on the scanner output for calculation of Rf values.

Other solvents were tested for development of TLC plates. A sample of authentic ^{14}C-chlorsulfuron did not move from the point of origin in toluene and moved only slightly in toluene:acetone (1:1, v/v). Both dichlormethane:methanol:9N ammonium hydroxide (72:25:3, v/v/v) and chloroform:acetic acid (19:3, v/v) solvent systems were able to move ^{14}C-chlorsulfuron from the origin, but the latter solvent system produced a sharper, clearer peak (Appendix 3C) and was used in all metabolism experiments. The distribution of radioactivity from tissue extracts amongst different metabolites and parent chlorsulfuron was assessed by comparing the peaks on the radiochromatogram charts of plant samples and standard charts. Peaks having the same Rf values as those of the major peak on the standard plates were considered to be the parent chlorsulfuron and all other peaks were considered as metabolites. The radioactive zones corresponding to the parent chlorsulfuron and metabolites were scraped from the plates and put in scintillation vials containing 10 ml of Bray's cocktail and quantified by liquid scintillation spectrometry. Quenching of radioactivity by TLC material was negligible. Metabolism of chlorsulfuron in plant samples was calculated as the percentage of total radioactivity extracted from the plate. The percentage distribution of radioactivity between chlorsulfuron and metabolites was determined by analysis of both liquid scintillation counter data and by use of a chart integrator attached to the chromatogram scanner. The two sets of data were similar.

3.2.4 Chlorsulfuron concentration in young tissues following a foliar application

By considering data on retention, distribution and metabolism of chlorsulfuron, obtained in the different experiments, it was possible to calculate the amount of un-metabolised chlorsulfuron reaching the young tissues following a foliar application of 15 g a.i. ha^{-1}, using equation 3.3.

$$A = \frac{R * C * D_y * P}{\text{Dry weight}} \qquad (3.3)$$

where R is the retention of spray solution by plant foliage in μl $plant^{-1}$ (Table 3.1), C is the concentration of chlorsulfuron in the spray solution (0.06 μg μl^{-1}), D_y is distribution of radioactivity in young plant tissues as percentage of applied dose (Appendix 3D), P is the amount of parent chlorsulfuron as a percentage of the total (derived from Table 3.5) and A is the concentration of un-metabolised chlorsulfuron in young tissues in μg g^{-1} dry weight. Dry weights of individual plant parts are given in Appendix 3E.

3.2.5 Statistical Analysis

All experiments were carried out twice and only the typical results are presented. All data were subject to analysis of variance using SAS statistical package (SAS institute Inc. Cary, N.C.). Counts of radioactivity and percentage values were transformed using square root and arcsine transformation respectively, before analysis. Since the results were similar for the untransformed and transformed data only the former are presented (Finney, 1973). Means for cultivars were compared using Duncan's Multiple Range Test at $p < 0.05$ level.

3.3 Results

3.3.1 Retention of chlorsulfuron by foliage

There were differences in total leaf area of wheat cultivars at the time of application which affected the total herbicide retention by their foliage (Table 3.1). For example, Rongotea and Kotare had a larger leaf area than Abele. As a result, the amount of herbicide deposit on Abele leaves was smaller than that for Rongotea and Kotare. However, when herbicide retention was expressed per unit leaf area or dry weight, no differences were found amongst the wheat cultivars tested (Table 3.1).

Table 3.1 Retention of chlorsulfuron and total leaf area at spraying for 5 wheat cultivars

Cultivar	Leaf area (cm^2)	Retention		
		$\mu l\ plant^{-1}$	$\mu l\ cm^{-2}$ leaf area	$\mu l\ g^{-1}$ dry wt.
Abele	9.17b*	8.60c	0.94a	181.51a
Jasper	9.88ab	9.53bc	0.98a	196.96a
Kotare	11.51a	10.78ab	0.95a	207.64a
Lancer	9.89ab	9.85bc	1.00a	178.43a
Rongotea	11.92a	11.25a	0.95a	188.17a

* Means in each column followed by the same letter are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

3.3.2 Uptake and translocation of ^{14}C-chlorsulfuron

At 12 h after application, uptake was low in both Kotare and Rongotea, while Abele, Jasper and Lancer had greater uptake values (Figure 3.1). Kotare and Rongotea showed lower uptake values than other cultivars 48 h after application. There was an increase in penetration of chlorsulfuron

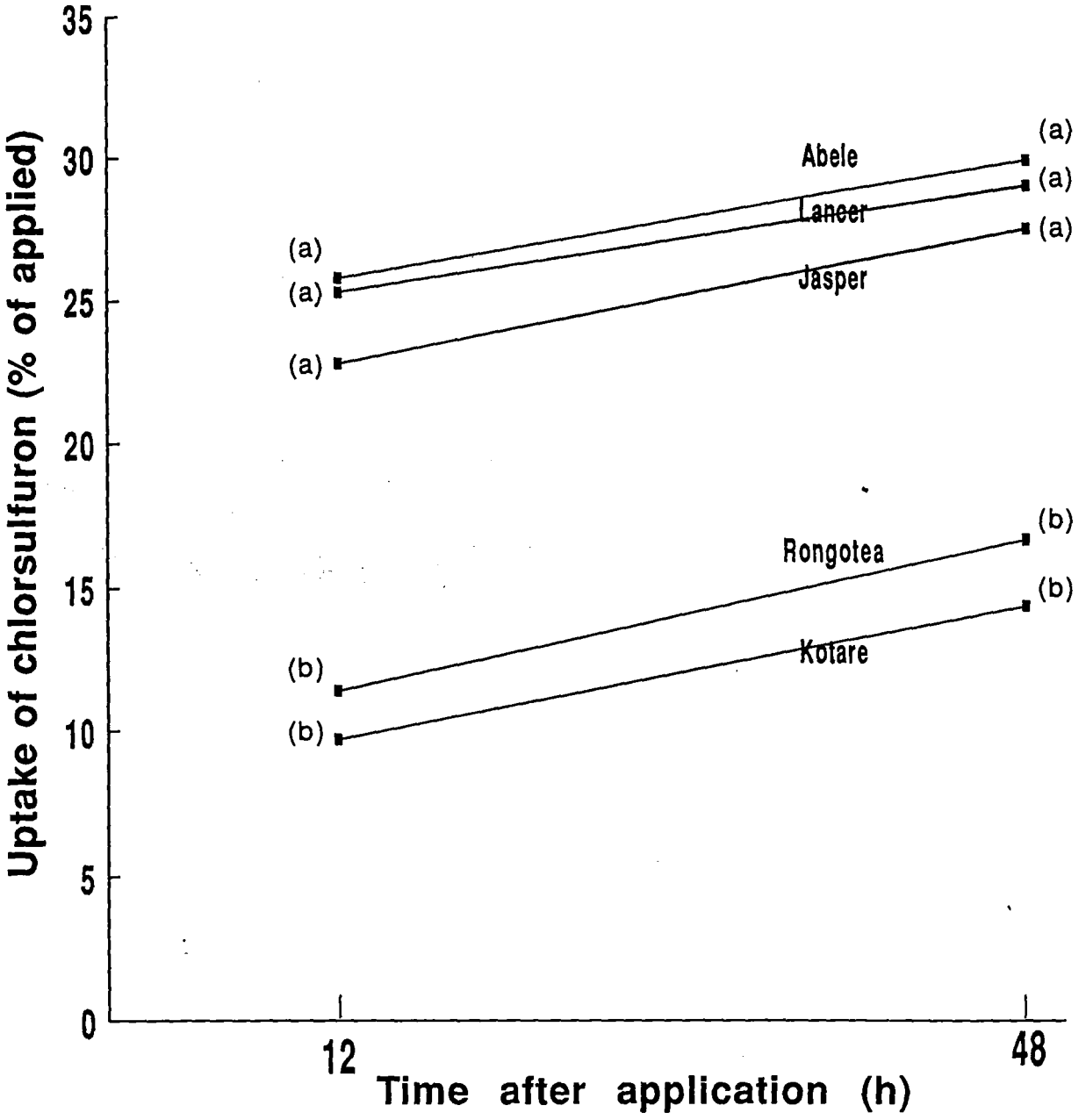


Figure 3.1 Absorption of ^{14}C-Chlorsulfuron by wheat cultivars 12 and 48 h after application to leaf 2 lamina. Similar letters in brackets at each application time indicate that uptake values are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

into the foliage with time in all cultivars, but clearly the rate of uptake had declined after the initial period. Uptake of chlorsulfuron, averaged over all cultivars tested, was 19% and 23% (SEm=0.7) of the applied dose at 12 h and 48 h after application, respectively.

Translocation of ^{14}C-chlorsulfuron out of the treated area is shown in two different ways in Table 3.2. As a percentage of the applied dose (Translocation A), movement of ^{14}C was lower in Kotare and Rongotea than Abele, Jasper and Lancer at both times of harvest. At 48 h after application Kotare and Rongotea had translocated 11.8% and 12.8%, respectively, of the applied dose out of the treated lamina. Other cultivars had translocated more than 23% of the applied dose during the same period. There were differences in the recovery of radioactivity between cultivars. In Kotare and Rongotea 90-92% of the radioactivity was recovered, whereas only 78-80% was recovered in other cultivars. When recovery of radioactivity was taken into account in calculating translocation A values, differences between cultivars were largely eliminated (Table 3.2).

When translocation was calculated as a percentage of the total radioactivity recovered from plant (Translocation B), no difference was found among wheat cultivars 12 h after application (Table 3.2). At 48 h after application, Abele had a significantly lower value for translocation as a percentage of the recovered radioactivity from the plant, than Lancer or Rongotea. At this time other cultivars had similar translocation values.

Table 3.2 Translocation of ^{14}C-chlorsulfuron in five wheat cultivars 12 and 48 h after application of the radiolabel to leaf 2 lamina. Values in brackets are corrected for the differences in recovery of radioactivity between cultivars.

	(Translocation A)[a]		(Translocation B)[a]	
Cultivar	12 h	48 h	12 h	48 h
Abele	21.6a* (8.7a)	23.7a (8.9ab)	11.8a	10.4b
Jasper	20.4a (8.3ab)	24.0a (8.7b)	10.0a	13.3ab
Kotare	8.2b (8.2b)	11.8b (8.5b)	13.6a	14.4ab
Lancer	21.5a (8.6ab)	24.1a (9.3a)	11.6a	18.1a
Rongotea	8.6b (8.4ab)	12.8b (8.9ab)	13.1a	18.5a

a) Translocation A = translocation as a percentage of the applied dose.
Translocation B = translocation as a percentage of radioactivity recovered from plant.

* Means in each column followed by the same letter are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

Distribution of radioactivity in various plant parts of wheat cultivars is shown in Table 3.3. At both harvest times leaf 2 lamina (the treated area) contained significantly more radioactivity than any other plant part. Across the cultivars, there was no difference in the amount of radioactivity recovered from leaf 2 lamina or young tissues, 12 h after application. At the same time, roots of Abele and Kotare had more radioactivity than those of Lancer.

At 48 h after application, Abele had more radioactivity in the leaf 2 lamina fraction (treated area) than Rongotea or Lancer. In contrast, the young tissue fraction of Rongotea contained more ^{14}C than similar fractions in Abele, Jasper and Kotare. At the same time roots of Abele contained less radioactivity than those of Lancer and Rongotea (Table 3.3).

Table 3.3 Distribution of radioactivity in various parts of wheat cultivars 12 and 48 h after application of ^{14}C-chlorsulfuron to leaf 2 lamina. Values are percentage of total radioactivity recovered from the plant.

Time after application	Plant part	Abele	Jasper	Kotare	Lancer	Rongotea	SEm
12 h	Leaf 1	0.18b*	0.17b	0.44a	0.15b	0.72a	0.129
	Leaf 2 lamina	88.18a	89.99a	86.62a	88.42a	86.87a	1.560
	Leaf 2 sheath	0.82b	0.80b	1.36a	1.12ab	1.53a	.145
	Young tissue	3.76a	4.74a	6.03a	6.34a	6.35a	0.856
	Root	7.06a	4.29bc	5.77ab	3.96c	4.53bc	0.670
48 h	Leaf 1	0.36a	0.41a	0.68a	0.53a	0.57a	0.107
	Leaf 2 lamina	89.60a	86.72ab	85.64abc	81.89bc	81.51bc	1.745
	Leaf 2 sheath	1.38a	1.60a	2.37a	1.70a	2.27a	0.431
	Young tissue	5.93b	7.04b	7.08b	9.24ab	10.94a	0.921
	Root	2.95c	4.43bc	4.23bc	6.65a	4.72b	0.576

* Means in each row followed by the same letter are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

There were significant differences between cultivars in the concentration of radioactivity 12 h after application in all plant parts except for leaf 1 and the roots (Table 3.4). Kotare had the lowest values for concentration of ^{14}C in leaf 2 lamina (the treated area), and the concentration of radioactivity in its young tissue was significantly smaller than similar fractions in Lancer and Rongotea. As there was no difference between cultivars in the distribution of radioactivity in either leaf 2 lamina or young tissue 12 h after application (Table 3.3), differences in concentration of ^{14}C were mainly due to variations in dry weight (Appendix 3E).

At the second harvest, 48 h after application, significant differences were found between cultivars in the concentration of radioactivity in all plant fractions (Table 3.4). The concentration of ^{14}C in leaf 2 lamina fraction was greatest for Abele and Lancer and smallest for Kotare. Kotare also had a lower concentration of radioactivity in the young tissue fraction than Lancer or Rongotea and a lower concentration of radioactivity in roots than Lancer. Most plant parts in Kotare had larger dry weight values than their similar fractions in other cultivars 48 h after application (Appendix 3E). This in part accounted for the lower values for the concentration of radioactivity in tissue fractions of this cultivar.

Table 3.4 Concentration of ^{14}C in various fractions of wheat cultivars 12 and 48 hours after application of ^{14}C-chlorsulfuron to leaf 2 lamina. Values are Bq μg^{-1} dry weight.

Time after application	Plant part	Abele	Jasper	Kotare	Lancer	Rongotea	SEm
12 h	Leaf 1	8.3a*	6.7a	5.0a	8.3a	10.0a	3.50
	Leaf 2 lamina	3163.0a	2315.0b	698.0c	3293.0a	1780.0b	306.67
	Leaf 2 sheath	128.0ab	80.0bc	50.0c	177.0a	118.0b	18.16
	Young tissue	43.0bc	43.0bc	23.0c	88.0a	57.0b	9.43
	Root	58.0a	28.0a	20.0a	35.0a	25.0a	13.17
48 h	Leaf 1	23.3b	20.0b	11.9b	40.0a	16.7b	5.12
	Leaf 2 lamina	4947.0a	3178.0b	144.0c	4977.0a	2688.0b	678.33
	Leaf 2 sheath	255.0ab	235.0ab	145.0b	392.0a	248.0ab	57.50
	Young tissue	100.0bc	82.0bc	52.0c	183.0a	113.0b	18.27
	Root	38.0b	38.0b	25.0b	93.0a	37.0b	12.62

* Means in each row followed by the same letter are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

3.3.3 Metabolism of ^{14}C-chlorsulfuron

Significant differences in metabolism of chlorsulfuron were found between wheat cultivars tested (Table 3.5). At 12 h after application, Kotare was the most efficient cultivar in metabolising chlorsulfuron while Lancer and Rongotea showed the lowest metabolism. At 48 h after application, Abele, Jasper and Kotare had metabolised more than 88% of the chlorsulfuron. In contrast, Lancer and Rongotea metabolised only 43.5% and 63% of the chlorsulfuron respectively, during the same period.

Table 3.5 Percentage metabolism of ^{14}C-chlorsulfuron in the young untreated tissues of wheat cultivars 12 and 48 hours after application.

Cultivar	12 h	48 h
Abele	56.8b*	88.1a
Jasper	61.2b	90.2a
Kotare	87.2a	92.2a
Lancer	19.8c	43.5c
Rongotea	20.1c	63.0b

* Means in each column followed by the same letter are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

Three metabolites, besides chlorsulfuron, were isolated from leaf extracts of wheat cultivars. There was no attempt to identify the metabolites but according to their lower Rf values (Table 3.6, Fig. 3.2), they were more polar than chlorsulfuron. The ^{14}C-chlorsulfuron standard applied to TLC plates migrated with an Rf value of 0.80 in chloroform : acetic acid (19:3, v:v). Peaks of different magnitudes were detected at the same Rf value when extracts from different cultivars were developed in the same solvent system (Figure 3.2). Chlorsulfuron was not transformed during extraction and chromatography. This was demonstrated by adding ^{14}C-chlorsulfuron to untreated wheat leaves immediately prior to extraction. Samples prepared in this way produced similar charts to standards.

Table 3.6 Major ^{14}C-compounds detected in plant extracts following application of ^{14}C-chlorsulfuron to wheat

Compound	Rf value
Peak 1	0.0
" 2	0.25
" 3	0.60
" 4 (Chlorsulfuron)	0.80

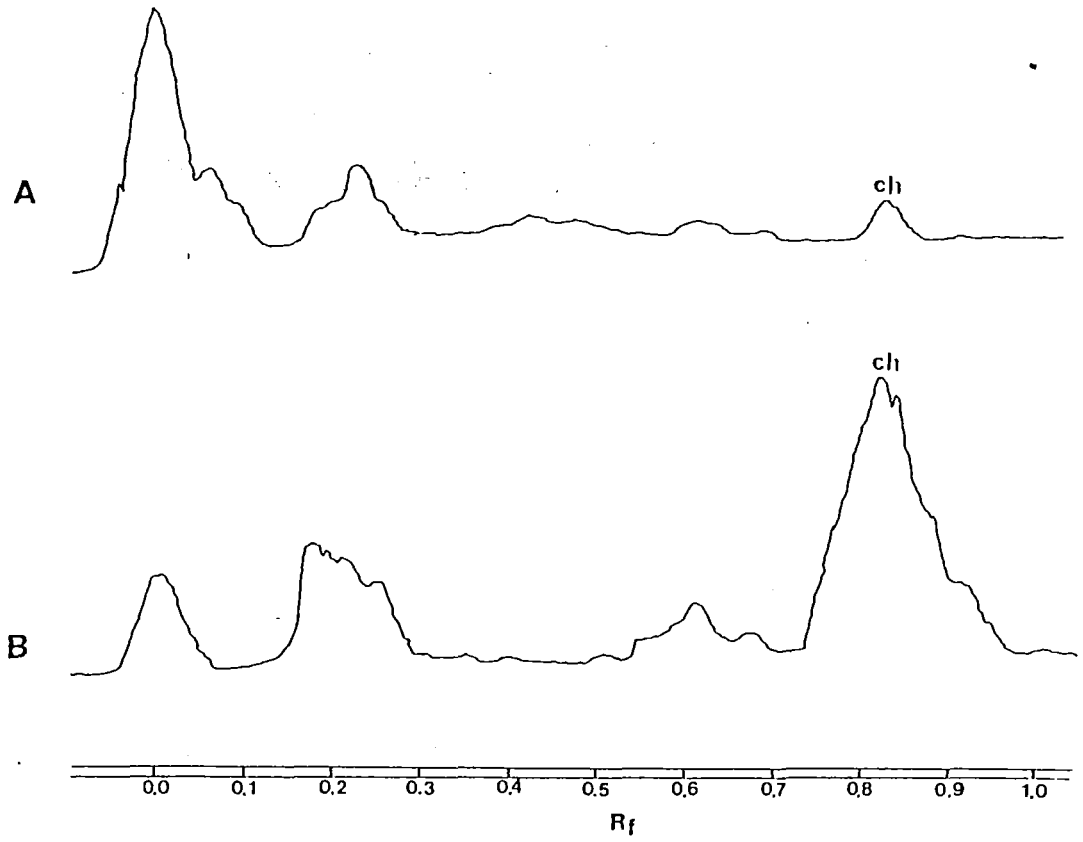


Figure 3.2 Typical radiochromatograms of Kotare (A) and Rongotea (B) tissue extracts 12 h after application of ^{14}C-chlorsulfuron. Parent chlorsulfuron peak is designated as 'ch'.

3.3.4 Chlorsulfuron concentration in young tissue following a foliar application

Table 3.7 shows the calculated values for the concentration of total ^{14}C (chlorsulfuron plus metabolites) and un-metabolised chlorsulfuron in the young tissues following a foliar application of chlorsulfuron at the recommended rate of 15 g a.i. ha^{-1} (see equation 3.3). It was estimated that 48 h after spraying, Rongotea and Lancer would have had 42.2 and 56.5 ng g^{-1} dry weight of chlorsulfuron and metabolites. The calculated value for Kotare was 15.3 ng g^{-1} at the same time (Table 3.7).

The differences between cultivars were much larger when metabolism was not accounted for. Twelve hours after spraying it was estimated that in cultivars Rongotea and Lancer there would be 15.5 and 22.3 ng chlorsulfuron g^{-1} dry weight while in Kotare there would have been only 1.1 ng g^{-1}. Similar differences between cultivars were observed 48 h after spraying. At this time Rongotea and Lancer have calculated values of 15.6 and 31.9 ng chlorsulfuron g^{-1} dry weight respectively, compared with 1.2 ng g^{-1} dry weight for Kotare (Table 3.7).

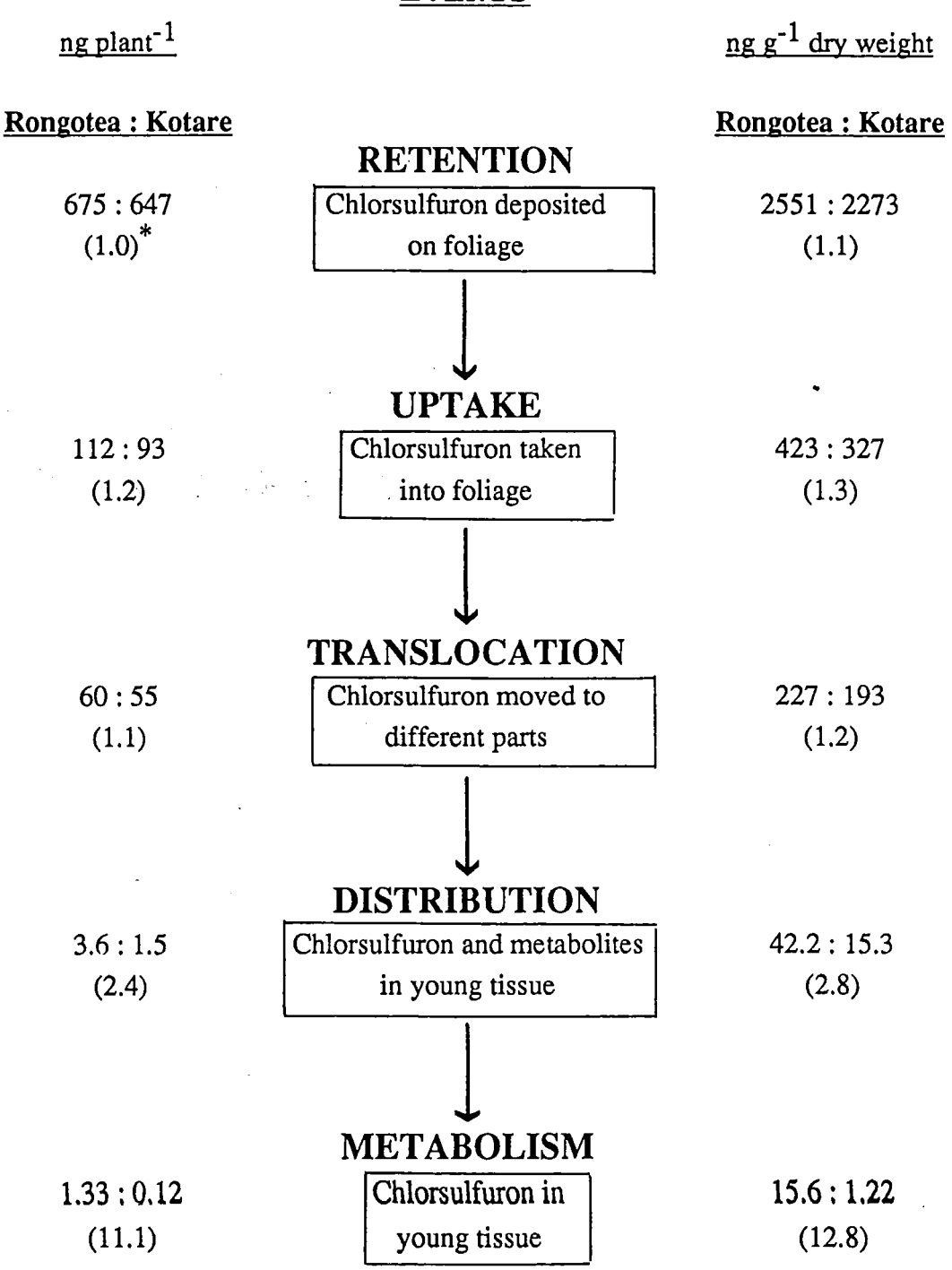
Table 3.7 Concentration of total ^{14}C and parent chlorsulfuron (ng g^{-1} dry weight) in the young tissues of wheat cultivars 12 and 48 h after spraying.

	Total ^{14}C		Parent chlorsulfuron	
Cultivar	12 h	48 h	12 h	48 h
Abele	11.3bc	28.0bc	5.0b	3.3c
Jasper	14.2bc	25.9bc	5.5b	2.6c
Kotare	8.3c	15.3c	1.1b	1.2c
Lancer	27.8a	56.5a	22.3a	31.9a
Rongotea	20.0ab	42.2ab	15.5a	15.6b

* Means in each column followed by the same letter are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

In order for chlorsulfuron to affect plant growth, it must be absorbed and translocated to the site of action in meristematic tissues at high enough concentrations. Various steps (events) involved in this process are summarized in Figure 3.3, and the amounts of chlorsulfuron available to the plant, both on per plant and on dry weight bases, are calculated for two extreme cultivars (Kotare and Rongotea) 48 h after a foliar application. No differences were observed between the two cultivars in retention, uptake and translocation of chlorsulfuron. Distribution of chlorsulfuron and metabolites to young tissues of Rongotea was 2.4 and 2.8 times greater than for Kotare, based on a per plant and per dry weight basis, respectively. Differential metabolism of chlorsulfuron by these cultivars greatly increased the difference between the cultivars in the concentration of the herbicide in meristematic tissues. Thus, 48 h after spraying, young tissues of Rongotea contained 11.1 and 12.8 times as much chlorsulfuron as similar tissues of Kotare on per plant and on dry weight basis, respectively (Figure 3.3).

Figure 3.3 Diagram showing the amount of chlorsulfuron at different events involved in herbicide application. Values are calculated for Kotare and Rongotea wheat 48 h after a foliar application of 15 g a.i. ha^{-1}.



* Values in brackets are the ratio of Rongotea : Kotare in the amounts of chlorsulfuron available at each event.

3.4 Discussion

Retention of chlorsulfuron was not different between wheat cultivars tested when calculated on the basis of leaf area or plant dry weight (Table 3.1). The small difference between Abele and Rongotea in the amount of chlorsulfuron deposited on the foliage was mainly due to the difference in leaf area between the cultivars and is not likely to be a reason for differences in cultivar sensitivity. Herbicide deposition on plant foliage is dependent on leaf orientation and the affinity of the surface for the spray solution (Kraatz and Andersen, 1980). It seems that differences amongst cultivars in plant geometry or surface properties were not large enough to result in differences in spray retention. Similarly, Hageman and Behrens (1984) reported that two broadleaf species; velvetleaf and eastern black nightshade, which are different in their sensitivity to chlorsulfuron, retained similar amounts of the herbicide.

Currently the most common method of studying uptake, translocation or metabolism of a herbicide is through its application in radiolabelled form, which makes subsequent recovery possible. Although some workers have expressed concern about the applicability of results obtained from isotope studies to actual field conditions, valuable information can be obtained by using such techniques. The major criticism of most radioisotope studies is that localized application of discrete droplets in the range of 0.2 - 1 μl (inflight diameter of > 725 μm) is far from real situations where much smaller droplets, in the range of 100 - 500 μm diameter, are applied to plant foliage (Field, 1983; Devine, 1988). Although this is a valid criticism, some reports indicate that the droplet size may not be of critical importance. For example, Merritt (1982) reported similar phytotoxicity from identical doses of glyphosate applied in droplets of 0.004 or 0.03 μl to *Raphanus sativa* L. and *Avena fatua* L. Similarly, there was no difference in the uptake of glyphosate when applied as 1 or 0.05 μl droplets to *Lolium perenne* L. (Bishop, 1987). In the radioisotope studies described in this thesis the smallest droplet size which could be

delivered from a microsyringe (0.25 μl) was applied in order to bring the results closer to typical applications from an hydraulic sprayer.

Recovery of ^{14}C-chlorsulfuron from wheat cultivars was incomplete and variable. Some other reports also indicate incomplete recovery of radioactivity after foliage applications of ^{14}C-chlorsulfuron to some species (Blair and Martin, 1988). For Canada thistle recovery of radioactivity was 87% after 48 h (Peterson and Swisher, 1985) and 84% after 144 h (Devine and Vanden Born, 1985). The loss of radioactivity found in the present study could have been through one or more of the following ways.

The herbicide could have been lost off the leaf surface, due to microbial degradation or chemical hydrolysis (see discussion below). Volatilization is not likely as chlorsulfuron has a vapour pressure of only 4.6 x 10^{-6} mm Hg at 25 °C (Beyer *et al.*, 1988). A second possible route for loss of radioactivity from application of ^{14}C-chlorsulfuron could be chemical hydrolysis. In the hydrolysis reaction water attacks the carbonyl carbon in the sulfonylurea bridge, which leaves as carbon dioxide (Appendix 3F). The pH controls the rate of sulfonylurea bridge hydrolysis through its effect on ionization, with faster hydrolysis occurring at acidic pH values. Hydrolysis occurs for sulfonylurea herbicides in both soil and water and it might occur inside the plant as well. The apoplast has an acidic pH of 4.5-5.0, compared with the symplast which has a pH of 7.0 to 7.5 (Hess, 1985). Chlorsulfuron molecules passing through the apoplast are expected to be in neutral form which makes them 250-1000 times more susceptible to hydrolysis than the anionic form (Brown, 1990). As the ^{14}C-chlorsulfuron used in the present study was carbonyl-labelled, hydrolysis of sulfonylurea bridge will result in loss of radioactivity in the form of $^{14}CO_2$.

The third mechanism for loss of radioactivity of chlorsulfuron might be through root exudation. This has been reported for other herbicides (Hall and Swanton, 1988). For chlorsulfuron, three days after the application of ^{14}C-herbicide to leaf 3 of hydroponically grown wheat and barley,

69.3% and 78.4% of the absorbed radioactivity, respectively was found in the nutrient solution (Foley, 1986). This indicates a basipetal movement for chlorsulfuron which is in agreement with its phloem mobility. As a weak acid, the chlorsulfuron molecule will be trapped in the anionic form once it enters the alkaline phloem environment (Beyer *et al.*, 1988). Chlorsulfuron is not likely to leak out of the phloem and into the xylem (Devine *et al.*, 1990). This gives it a greater chance of basipetal movement towards the roots. There was no attempt to investigate the loss of radioactivity through root exudation in the present work.

Recovery of radioactivity was similar for the tolerant cultivar Kotare and the sensitive cultivar Rongotea, thus it is not likely to affect conclusions reached in this study. However, it is suggested that recovery of radioactivity should be taken into account in radioisotope work, as it might influence the results of translocation studies, as discussed below.

Wheat cultivars fell into two groups according to their uptake of chlorsulfuron. Abele, Jasper and Lancer showed higher uptake values than Kotare and Rongotea at 12 and 48 h after application (Figure 3.1). The difference in chlorsulfuron uptake between the two groups of cultivars could have been due to differences in their surface characteristics. Chlorsulfuron molecules exist both in neutral-- lipophilic and ionized-- hydrophilic forms (Beyer *et al.*, 1988). Thus chlorsulfuron entry into the cuticle may be via both hydrophilic and lipophilic pathways. Thickness and composition of cuticle and epicuticular wax, as well as the presence of polar (hydrophilic) routes and the frequency of trichomes are among the important surface characteristics which affect the uptake of herbicides (Price, 1982; Hess, 1985). In the present study, however, differences in uptake did not account for differences in sensitivity to chlorsulfuron among the wheat cultivars tested as the tolerant Kotare and the sensitive Rongotea had similar values for uptake (Figure 3.1).

Despite differences between cultivars in the percentage uptake of chlorsulfuron at each time, the rate of uptake between 12 h and 48 h was similar for all cultivars (Figure 3.1). For all cultivars,

there was an initial rapid uptake of chlorsulfuron, up to 12 h following application, followed by a slower phase. As explained later in Chapter 4, chlorsulfuron uptake following the initial phase depends on maintenance of a concentration gradient across the cuticle, which is dictated by transport of absorbed material out of the treated area. Translocation of chlorsulfuron, as a percentage of total radioactivity recovered from the plant, was not significantly different between cultivars (Table 3.2), which explains the similarity in the rate of uptake.

Absorption of chlorsulfuron by plant foliage depends upon the species and a rather wide range has been reported in the literature for chlorsulfuron absorption (see section 1.3.5). In the present study chlorsulfuron absorption by leaf 3 of wheat 48 h after application ranged from 14.4% of the applied dose for Kotare to 30% of the applied dose for Abele (Figure 3.1). Similar uptake values have been reported for some cereals and grasses. Foley (1986) reported that wheat and barley absorbed 16.2% and 14% of ^{14}C-chlorsulfuron, respectively, three days after the application to leaf 3. Kentucky bluegrass and tall fescue absorbed 14.6% and 24.4% of chlorsulfuron, respectively, after 48 h (Goatley *et al.*, 1990). These values are not in agreement with the study by Sweetser *et al.* (1982), which reported an uptake of 68.7% of the applied dose for wheat within 24 h. Apart from differences in environmental and formulation factors between these studies, in the latter report ^{14}C-chlorsulfuron had been applied to leaf 1 at an early growth stage which may have facilitated its uptake.

There are several methods for presenting data on translocation of herbicides in plants. Translocation can be expressed in terms of: 1) the total amount of radioactivity applied to the plant, 2) as a percentage of radioactivity absorbed by the plant or 3) as a percentage of radioactivity recovered from the plant. In most radioisotope studies it is customary to use only one method for presentation of results. It is often difficult to compare the results from different reports when the bases for presenting data are different. In this study translocation of chlorsulfuron is presented both as a percentage of the applied dose and as a percentage of the

radioactivity recovered from plant (see Materials and Methods for calculation methods). Translocation as a percentage of the applied dose provides useful information from a practical point of view, while data based on the radioactivity recovered from the plant are more effective estimates of the actual amounts of herbicide in the plant and are physiologically more meaningful.

There is an apparent discrepency in translocation values presented in Table 3.2. Values for translocation as a percentage of radioactivity recovered from the plant (Translocation B) were apparently low with respect to the translocation as a percentage of the applied dose (Translocation A) and the uptake values (Figure 3.1). This was due to the fact that calculation of translocation A usually results in an overestimation of the actual values, because it does not consider the losses of radioactivity during the course of the experiment. These losses may be from the wash off and the treated area. The greater the loss of radioactivity, the greater will be the extent of overestimation in calculating translocation A values. In this study, Abele, Jasper and Lancer showed lower recoveries (78-80%) than Kotare and Rongotea (90-92%), therefore, the discrepency between translocation A and translocation B values seem to be greater for the former cultivars. When the dpm values obtained for the leaf wash and the treated area were adjusted for recovery, the variability in translocation A data between cultivars was greatly reduced and the discrepency with translocation B was removed (Table 3.2). This analysis demonstrates the significance of losses of radiolabelled material in uptake and translocation studies. In publication, losses of radioactivity of up to 20% are commonly considered acceptable (Thompson, Sanders and Pallet, 1986). Unfortunately, some published work does not report the recovery of radioactivity. The results of these studies, and when losses are greater than 20%, should be regarded with caution.

Corrected values obtained for translocation of chlorsulfuron in wheat cultivars ranged from 8.2% of the applied dose for Kotare 12 h after application to 9.3% of the applied dose for Lancer 48 h after application (Table 3.2). Similarly, Sweetser *et al.*, (1982) reported a total translocation of 8.7% of the applied ^{14}C-chlorsulfuron for wheat within 24 h. Both lower and higher

translocation values have been reported for different species (Foley, 1986; Devine *et al.*, 1990; Goatley *et al.*, 1990). The proposed explanations for the low phloem mobility of chlorsulfuron in some suceptible species are presented in Chapter 1.

The pattern of distribution of radioactivity among plant parts following the application of ^{14}C-chlorsulfuron (Table 3.3) can be explained by the relative sink strengths. Thus, most of the radioactivity exported out of leaf 2 was recovered in actively growing plant parts such as young shoot tissues and roots. This agrees with the hypothesis of phloem mobility of weak acids such as chlorsulfuron (Chapter 1). Leaf 1 which was fully expanded imported very little radioactivity. This indicates that there was little apoplastic movement of chlorsulfuron as symplastic movement is usually in the export direction in mature leaves (Devine and Hall, 1990).

Significant differences were found between wheat cultivars in their ability to metabolise chlorsulfuron. Metabolism of chlorsulfuron by Kotare was always greater than that of Lancer and Rongotea (Table 3.5). The tolerant cultivar Kotare could metabolise 87.2% and 92.2% of the applied chlorsulfuron within 12 and 48 h, respectively. These results agree with the general findings of Sweetser *et al.*, (1982) and Bestman *et al.*, (1987).

Although different chlorsulfuron metabolites recovered from plant extracts were not identified, they were more polar than chlorsulfuron (Table 3.6). Chlorsulfuron metabolites in wheat including a short-lived hydroxylated derivative and a phenyl-glycoside conjugate are both more polar than the parent chlorsulfuron (Sweetser *et al.*, 1982, Bestman *et al.*, 1987). The origin peak found in wheat extracts in this study has been reported also for Canada thistle extracts separated in a similar solvent system (Peterson and Swisher, 1985) and it probably represents one or more highly polar metabolites of chlorsulfuron. It has been noted that glycosides need not represent terminal herbicide metabolites and may be subjected to further modification (Owen, 1989). In general glycosidation may contribute to detoxification by virtue of the enhanced water solubility

of the products which facilitates their disposal in the vacuole. Moreover, in the case of chlorsulfuron metabolism, the glycoside conjugate is herbicidally inactive (Sweetser *et al.*, 1982; Owen, 1989).

The concentration of chlorsulfuron in young tissues of sensitive cultivars Lancer and Rongotea 48 h after an application of 15 g a.i. ha^{-1} were calculated to be 31.9 and 15.6 ng g^{-1} dry weight, respectively (Table 3.7), equivalent to 15.7 and 7.8 nM, respectively (Appendix 3G), taking into account a tissue fresh weight : dry weight ratio of approximately six. Ray (1984) reported that inhibition of growth was detectable for excised pea roots grown in culture with as little as 2.8 nM chlorsulfuron, while 28 nM of the herbicide resulted in complete growth inhibition of germinating seeds. The calculated values reported in this chapter are for the young tissues which include expanding leaves and tillers as well as the apices. Only a small number of cells are dividing compared to the bulk of growing tissue and it is likely that the actual concentration of chlorsulfuron in meristems could have been greater than the overall values reported for young tissues. However, wheat is a tolerant species to chlorsulfuron and even sensitive cultivars like Lancer or Rongotea can metabolise it, albeit not as rapidly as others (Table 3.5). The initial high concentrations of chlorsulfuron in the apices will eventually decrease to non-effective levels. It seems possible though, that if the initial concentration is high enough for a sustained period of time, the plant cannot recover from the damage. Chlorsulfuron is a very rapid inhibitor of growth and can reduce growth of maize seedlings within 2 h (Ray, 1989). A more in-depth study on the mode of action of chlorsulfuron in the sensitive cultivar Rongotea is presented in the third part of this thesis, in an effort to elucidate the physiological nature of the chlorsulfuron damage.

The diagram of chlorsulfuron concentrations in the plant at each event following a foliar application (Figure 3.3), makes it easier to separate the effect of these events and rank them. Based on the data provided, differential metabolism is the main reason for differences in sensitivity between Rongotea and Kotare. Smaller differences between cultivars in distribution of

chlorsulfuron among plant parts or uptake by the foliage do not seem to account, by themselves, for differential sensitivity between cultivars. These differences, however, might contribute to greater sensitivity of a cultivar which has a low capacity to detoxify chlorsulfuron.

PART THREE

CHAPTER 4

CHAPTER 5

CHAPTER 6

CHAPTER 4

Effect of Chlorsulfuron on Wheat cv. Rongotea Grown at Different Nitrate Concentrations

4.1 INTRODUCTION

The growth response of some wheat cultivars to added nitrate was limited when sprayed with chlorsulfuron and this was related to their tolerance to the herbicide (Chapter 2). Chlorsulfuron is a selective herbicide for wheat and its selectivity is based on rapid metabolism (Sweetser *et al.*, 1982; Bestman, Devine and Vanden Born, 1987). However, it was demonstrated in Chapter 3 that sensitive wheat cultivars like Lancer and Rongotea have low metabolism of chlorsulfuron and can accumulate the active herbicide in their growing tissues at concentrations that are more than an order of magnitude greater than in tolerant cultivars. The site of action of chlorsulfuron in sensitive species has been reported as the inhibition of the biosynthesis of branched chain amino acids through its effect on the enzyme acetolactate synthase (Ray, 1984; Ray 1989). It is not known, however, whether this is the cause for the observed limitation in response to additional nitrogen.

Nitrogen is a major plant nutrient and an essential element for plant growth. It constitutes an essential part of the structure of most organic compounds, especially amino acids and hence proteins and enzymes. The major form of inorganic nitrogen available to plants in most agricultural soils is nitrate which is either added directly to the soil or is produced by the action of soil micro-organisms on other mineral fertilizers such as ammonia and urea (Haynes 1986). In natural habitats the concentration of nitrate in the soil solution is usually around 1 mol m^{-3} or less, with 0.1 mol m^{-3} not uncommon (Novoa and Loomis, 1981; Andrews, 1986). In arable soils in temperate regions, nitrate concentration can be as high as 20 mol m^{-3} due to fertilizer application (Russel, 1973; Andrews, 1986).

Herbicides are an essential component of modern agriculture. They are normally applied to crops at an early growth stage at which time available nitrogen levels in the soil are usually relatively

high due to cultivation and fertilization (Goh and Haynes, 1986). Mixed applications of fertilizers and herbicides are also practiced in many situations for reasons of economy (Martens, Burnside and Cramer, 1978; Randall, Wells and Hanway, 1985). Information is required on the possible interactive effects between common herbicides and important nutrients such as nitrogen. Very little research has been conducted in this area. Addition of nitrogen to soybean or cowpea (*Vigna unguiculata* L.) plants treated with nitralin or diphenamid resulted in an increase in dry weight and N content of shoots, while nitrogen supplementation affected only N concentration in trifluralin-treated soybeans (Behran *et al.*, 1979). In experiments with sugarbeet, application of NPK fertilizer increased the efficacy of TCA against susceptible weeds, but reduced its activity against resistant weeds (Nepochatov and Zimovskaya, 1976). Churchill and Klepper (1979) grew wheat seedling on nitrate, ammonia or zero nitrogen and treated them with ametryn, a photosynthetic inhibitor. Nitrate-grown plants treated with ametryn accumulated nitrate and were dead after 10 days, while treated plants grown on ammonia or zero-nitrogen survived.

More recently, several studies have been reported on the performance of glyphosate and herbicides of the polycyclic alkanoic acid group on plants grown at different nitrogen levels (Dickson, Andrews and Field, 1988; Andrews *et al.* , 1989; Dickson *et al.*, 1990). In general the activity of these herbicides was reduced when plants were grown at low compared with high nitrogen concentrations. For instance when cultivated oat (*Avena sativa* L. cv. Amuri) was sown in the field with 200 kg N ha^{-1}, application of diclofop-methyl and fluazifop-butyl resulted in 54.5% and 100% reduction in seed head dry weight, respectively. On the contrary plants which did not receive any nitrogen fertilizer showed a reduction of 1.3% and 47.3% with diclofop-methyl and fluazifop-butyl application, respectively (Dickson *et al.*, 1988). Andrews *et al.* (1989a) reported that cultivated oat sprayed with diclofop-methyl was severely damaged at 10 mol m^{-3} nitrate but was tolerant of the herbicide at 1 mol m^{-3} nitrate. Similarly, under both controlled environment and field conditions, glyphosate was less phytotoxic to oats grown at low levels of nitrogen (Dickson *et al.*, 1990).

There is only a very limited amount of information on the interactive effect of nitrogen and chlorsulfuron or other sulfonylurea herbicides. Nalewaja and Woznica (1985) found that an

increase in soil nitrogen level from 20 to 140 ppm increased the phytotoxicity of chlorsulfuron to green foxtail. Damage to wheat, cv. Sonora, has been reported in soils rich in nitrogen and phosphorous from soil applications of chlorsulfuron (Bowran, Blacklow and Boyd, 1984). Chlorsulfuron inhibited the responses of wheat cv. Sonora (Bowran and Blacklow, 1987) and Rongotea (Dastgheib, Andrews and Field, 1989) to nitrogen although the relationship of this effect of the herbicide to its site of action in the plant is not clearly understood.

The environment in which a plant is growing is of key importance in its growth and development which might in turn affect its interception, retention, penetration, translocation and distribution, and finally metabolism of herbicides (Caseley and Coupland, 1985). Some plant parameters which may be influenced by the environment include leaf area, leaf orientation, surface characteristics of the leaf e.g. waxiness and cuticle development, which affect retention and uptake of herbicides (Sagar *et al.*, 1982; Hess, 1985; Peregoy *et al.*, 1990). Translocation and metabolism of herbicides are affected by plant physiological and biochemical processes and the interaction of environmental factors on these needs to be defined (Cole, 1983; Coupland, 1989).

Among a variety of environmental factors which affect herbicide performance, temperature, relative humidity, irradiance and precipitation have been studied in reasonable detail (Gerber, Nyffeler and Green, 1983; Caseley, 1987; Devine, 1988), while only a few studies have been reported on the effects of soil nutrients on herbicide activity. Regimbal and Martin (1985) reported that the addition of 84 kg ha^{-1} nitrogen in the fall prior to a spring treatment of 0.3 kg ha^{-1} picloram improved control of leafy spurge (*Euphorbia esula* L.). Efficacy of glyphosate on bermudagrass (*Cynodon dactylon* L.) control was increased with nitrogen fertilizer and this was attributed to greater translocation (Whitwell and Santelmann, 1978). Graftstrom and Nalewaja (1988) found that soil fertility increased fluazifop-butyl performance against green foxtail and this could be explained by greater uptake and translocation of the herbicide. However in another study, uptake of fluazifop-butyl in oats was found to be unaffected by nitrate level while its translocation was greater in plants grown at relatively high nitrate levels (Dickson *et al.*, 1990).

Haque, Weisgerber and Klein (1977) grew wheat plants in nutrient solutions which were either complete or deficient in one of the elements; N,P,K or Mg. The mineral deficiencies reduced the uptake and translocation of ^{14}C-buturon and increased both its metabolism and efflux from the roots. Low concentration of nutrients reduced metabolism of ^{14}C-benzoylprop ethyl in hydroponically grown wild oat (*Avena fatua* L.) which resulted in greater phytotoxicity (Hill and Stobbe, 1978).

No information is available on the effect of soil nutrients on retention, uptake, translocation or metabolism of chlorsulfuron. Increasing levels of soil nitrogen might have an impact on one or more of these events. For example, plant height, leaf area and leaf angle can be influenced by nitrogen supply (Novoa and Loomis, 1981). Moreover, thickness, composition and permeability of leaf waxes can be affected by nitrogen (Kirkwood, 1987). These changes could result in differences in the deposition, uptake and translocation of chlorsulfuron.

Furthermore, nitrogen can affect both the source and the sink capacities in plants and it has a major effect on photosynthesis (Novoa and Loomis, 1981). Chlorsulfuron movement in the plant following foliar absorption is with photoassimilates in the phloem (Beyer *et al.*, 1988). Such relationship could lead to differences in translocation of chlorsulfuron between low and high nitrate plants. Other key physiological processes are affected by nitrogen. For instance, rates of respiration, amino acid and protein synthesis and the amount of chlorophyll, total soluble protein and Rubisco protein as well as the activities of many important enzymes are dependent upon nitrogen supply (Lawlor *et al.*, 1987a & b). Such changes in physiological status of the plant may lead to differences in the metabolism of chlorsulfuron.

The third part of this thesis (Chapters 4 to 6) aims to describe the mode of action of chlorsulfuron in wheat under conditions conducive to damage. For this purpose the sensitive cultivar Rongotea as identified in Chapter 2 was selected. The experiments described in this chapter had two main objectives. Firstly, to determine the effect of chlorsulfuron on wheat cv. Rongotea grown at different nitrate concentrations. Secondly, to investigate whether differences observed in the

response of Rongotea to chlorsulfuron when grown at low and high nitrate could be explained by differential herbicide retention, uptake, translocation or metabolism.

4.2 MATERIALS AND METHODS

4.2.1 Effect of chlorsulfuron on wheat grown at low and high nitrate concentrations

Uniform seven-day-old seedlings of wheat cv. Rongotea were planted individually into 700-ml-volume plastic pots filled with a vermiculite /perlite mixture (1:1, v:v) and irrigated regularly with a basal nutrient solution (Appendix 2D) containing either 1 or 10 mol m^{-3} nitrogen as potassium nitrate. Plants were maintained in a controlled environment cabinet as described in Section 2.2.5. When plants reached the five-leaf stage, (ZGS 15), chlorsulfuron was applied at 0, 15 or 60 g a.i. ha^{-1} as described in Section 2.2.1. All treatments were replicated five times and arranged in a randomized complete block design.

Forty days after spraying (DAS) plants were harvested and shoot and root dry weight determined. Shoots were freeze dried and a sample of approximately 0.10 g used for chlorophyll determination. Pigments were extracted in 90% acetone saturated with magnesium carbonate using a hand homogenizer. After centrifugation at 5000 rpm in a Heareus Christ centrifuge (model Digifuge) for 5 minutes absorbances were measured at 645, 665 and 750 nm (for turbidity correction) using a Shimadzu uv-160 A spectrophotmeter. Chlorophyll concentration was calculated using equations from Strickland and Parsons (1972). These equations are given below:

$$\text{Chlorophyll A} = \frac{(11.6 * A_{665} - 1.3 * A_{645})\ v}{1000 * DWt} \qquad (4.1)$$

$$\text{Chlorophyll B} = \frac{(19.1 * A_{645} - 4.7 * A_{665})\ v}{1000 * DWt} \qquad (4.2)$$

In the above equations chlorophyll a and chlorophyll b concentrations are in mg g^{-1} dry weight, v is the volume (ml) of 90% acetone and DWt is the leaf dry weight used in the extraction. Turbidity was corrected for by subtracting the absorbance at 750 nm.

4.2.2 Effect of chlorsulfuron on wheat grown at a range of nitrate concentrations

Plant culture and experimental design were similar to the previous experiment except that 1300-ml-volume pots were used and plants were maintained in a glasshouse with 14-h daylength and max/min. temperatures of 30/15 °C during the growth period. There were five nitrate treatments; 1, 5, 10, 25, and 50 mol m^{-3}. When plants reached the three-leaf stage (ZGS 13), chlorsulfuron was applied at 0, 15 and 60 g a.i.ha^{-1} as described in Section 2.2.1. All treatments were replicated five times and there were two harvests. The first harvest was at 21 DAS when plants were at stem elongation stage and had six to seven leaves on the main stem (ZGS 16-17). At this harvest shoot dry weight was determined. The second harvest was at maturity, when all plant parts had turned yellow (ZGS 92). Time was allowed for plants in all treatments to reach this stage and they were harvested at 135 DAS, when shoot dry weight and seed head dry weight were determined.

4.2.3 Effect of chlorsulfuron on dry matter accumulation of wheat grown at low and high nitrate concentrations

Growing conditions and plant culture were similar to the experiment described in Section 4.2.1. There were two nitrate treatments; 1 and 5 mol m^{-3}. Chlorsulfuron was applied at the three leaf stage (ZGS 13) at 0 or 15 g a.i. ha^{-1} as described in Section 2.2.1. There were four harvest times in the experiment; 1, 3, 7, and 27 DAS. At each harvest root and shoot dry weight were determined. There were four replicates in the experiment which was arranged as a randomized complete block design.

4.2.4 Retention of chlorsulfuron by the foliage of wheat grown at low and high nitrate concentrations

Plant culture and growing conditions were the same as described in Section 4.2.1, except that 350-ml-volume pots were used. Seedlings of Rongotea wheat were grown on either 1 or 5 mol m^{-3} nitrate and were sprayed at the three-leaf stage (ZGS 13). All plants were sprayed with chlorsulfuron at a rate of 15 g a.i. ha^{-1} as described in Section 2.2.1. The herbicide solution contained Citowett surfactant at a concentration of 0.25 ml l^{-1} and was saturated with fluorescein dye (0.05 g l^{-1}). Herbicide retention on the foliage was determined by fluorimetry as described in Section 3.2.1. The experiment was carried out twice with either 14 or eight replicates, arranged in randomized complete blocks.

4.2.5 Uptake and translocation of ^{14}C-chlorsulfuron in wheat grown at low and high nitrate concentrations

Two experiments were carried out to determine the foliar uptake of ^{14}C-chlorsulfuron by wheat and the pattern of its distribution under two nitrate treatments. As there were some small differences in the methods employed in the two experiments, they will be described separately. The results of the preliminary experiment, however, will be presented in Appendix 4C to avoid repetition.

4.2.5.1 Preliminary experiment

Wheat seedlings cv. Rongotea were grown in 350-ml-volume pots as previously described (Section 4.2.1.1). There were two nitrate treatments; 1 and 5 mol m^{-3} and 8 replicates. These were arranged in a randomized complete block design. When plants reached the four-leaf stage (ZGS 14), ten 0.5 μl droplets of ^{14}C-chlorsulfuron were applied uniformly with a microsyringe to the upper surface of the lamina of leaf 3 in a region approximately 30 mm long and 15 mm from the ligule. The ^{14}C-chlorsulfuron was formulated as previously described (Section 3.2.2) to give a final activity of 1036 Bq (0.028 μCi) in 5 μl. Assessment of herbicide uptake was done 24 h after application. The lamina of leaf 3 was detached and washed first with 25 ml 0.025% Citowett solution (termed water wash hereafter) and then with 10 ml acetone to determine the amount of ^{14}C-chlorsulfuron deposited in the cuticular wax (acetone wash). A 1-ml aliquot of the water wash was added to 10 ml Bray's scintillation cocktail (Appendix 3B) and the number of disintegrations per minute (dpm) counted by liquid scintillation spectrometry (LKB Wallac 1219 Rackbeta). Similarly radioactivity in the acetone wash was determined after allowing acetone to evaporate, then adding 1 ml 0.025% Citowett solution and 10 ml of Bray's cocktail. Uptake was calculated as a percentage of applied dose using equation 4.3:

(4.3)

$$\text{Uptake} = \frac{(\text{Applied dpm} - (\text{dpm in water wash} + \text{dpm in acetone wash})) * 100}{\text{Applied dpm}}$$

For the translocation and distribution study, plants were divided into various fractions immediately after washing the treated area. Plant fractions included leaf 1, leaf 2, lamina of leaf 3, sheath of leaf 3, young tissue (comprising leaf 4 plus apex and tillers) and roots. Samples were dried in an oven at 70 °C for 48 h and combusted in a sample oxidizer (see Section 3.2.2) and the radioactivity determined by liquid scintillation spectrometry, as described above. Translocation, distribution and concentration of radioactivity in different fractions were determined as described in Section 3.2.2.

4.2.5.2 Time course experiment

Plant culture, growing conditions and formulation of ^{14}C-chlorsulfuron were similar to the preliminary experiment. The experiment was a randomized complete block design with 7 replicates. At the three-leaf stage of plant growth (ZGS 13), all plants were given a foliar spray of chlorsulfuron at 15 g a.i ha^{-1} immediately prior to applying the radiolabel. Ten µl ^{14}C-chlorsulfuron, radioactivity 2405 Bq (0.065 µCi), was applied as 35-40 µl droplets to the lamina of leaf 2 in a similar position as described in Section 4.2.5.1. Uptake and translocation of chlorsulfuron was assessed 24, 48 and 168 h after the application of the radioactive material. At each time, the treated lamina was detached and washed with 25 ml 0.025% Citowett solution. It was found in the preliminary experiment that the radioactivity removed by acetone wash was less than 1% of that of the water wash (Appendix 4C), and therefore only a water wash was used in this and further experiments. The amount of radioactivity in the water wash was determined by liquid scintillation spectrometry as previously described and percentage uptake of the total applied radioactivity was calculated as the difference between the applied dpm and the dpm in the wash solution. For the translocation study, plants were divided into leaf 1, leaf 2 lamina, leaf 2

sheath, young tissue (which included leaf 3 and younger leaves, tillers and apex) and root fractions. These were freeze-dried and kept frozen (-18 °C) until combustion and quantification by liquid scintillation spectrometry (see Section 4.2.5.1). Translocation, distribution and concentration of radioactivity in different fractions were determined as described in Section 3.2.2.

4.2.6. Metabolism of ^{14}C-chlorsulfuron in wheat grown at low and high nitrate concentrations

Plant culture, growing conditions and formulation of ^{14}C-chlorsulfuron were similar to the preliminary experiment (Section 4.2.5.1). The experiment was a randomized complete block design with 5 replicates. At the three-leaf stage (ZGS 13), immediately after a foliar application of chlorsulfuron at 15 g a.i. ha^{-1}, 7.5 μl of ^{14}C-chlorsulfuron was applied with a microsyringe to each of the adaxial lamina surfaces of leaf 2 and leaf 3. The total amount of radioactivity applied per plant was 6660 Bq (0.18 μCi). Plants were harvested 24, 48 and 168 h after the application of the labelled chlorsulfuron. At each harvest, the treated leaf laminae were detached and washed with 25 ml 0.025 % Citowett solution. These were then freeze-dried and kept frozen (-18 °C) until extraction for determination of chlorsulfuron metabolism. Metabolism of chlorsulfuron was studied as described previously (Section 3.2.3). Previous data showed that there was good agreement between the data obtained through calculating the areas under different peaks on radiochromatograms and those obtained using liquid scintillation spectrometry for quantifying the radioactivity in different zones on TLC plates (Section 3.2.3). Therefore, in this experiment only the former method was employed. Relative areas under the peaks were calculated by counting the number of integrations corresponding to each curve, following scanning of plates on a Packard Radiochromatogram Scanner (model 7201).

4.2.7 Statistical Analysis

All data were subject to analysis of variance using SAS statistical package (SAS institute Inc. Cary, N.C.) and standard error of mean values (SEm) presented to indicate variability of data. Counts of dpm and percentage values were transformed using square root and arcsine transformation respectively, before analysis. Since the results were similar for the untransformed and transformed data only the former are presented (Finney, 1973). Values for radioactivity are presented in units of bequerel (Bq). The effects described have a probability level of $p < 0.01$.

4.3. RESULTS

4.3.1 Effect of chlorsulfuron on wheat grown at low and high nitrate concentrations

In the absence of chlorsulfuron, plant growth increased significantly with increasing nitrate concentration (Table 4.1). Shoot dry weight of wheat grown at 10 mol m^{-3} nitrate was more than twice that of plants grown at 1 mol m^{-3} nitrate. Similarly, root dry weight increased 1.7 times in plants grown at 10 mol m^{-3} compared with plants grown at 1 mol m^{-3} nitrate. When plants were sprayed with chlorsulfuron, at either 15 or 60 g a.i. ha^{-1}, no significant increase in shoot or root dry weight was obtained with added nitrate. At 1 mol m^{-3} nitrate, shoot dry weight did not show any change with chlorsulfuron application at up to 4 times the recommended rate (60 g a.i. ha^{-1}). At 10 mol m^{-3} nitrate, however, shoot dry weight decreased significantly with both rates of chlorsulfuron and the reduction was greater at the higher rate. Chlorsulfuron effect on root dry weight was similar to its effect on shoot dry weight (Table 4.1).

Table 4.1 Effect of different chlorsulfuron rates on wheat grown at 1 and 10 mol m^{-3} nitrate.

Nitrate (mol m^{-3})	Chlorsulfuron (g a.i. ha^{-1})	Shoot dry weight (g)	Root dry weight (g)	Chlorophyll concentration (mg g^{-1} DWt)
1	0	1.60	1.33	1.35
	15	1.55	1.19	1.18
	60	1.57	0.96	1.07
10	0	3.36	2.21	2.99
	15	2.01	1.26	2.10
	60	1.79	0.93	1.68
SEm		0.212	0.151	0.180

Chlorophyll concentration was higher in the control plants grown at 10 mol m^{-3} nitrate compared to those grown at 1 mol m^{-3} nitrate (Table 4.1). Chlorophyll concentration in plants grown at 1 mol m^{-3} nitrate was unaffected by chlorsulfuron. For plants grown at 10 mol m^{-3} nitrate, chlorophyll concentration was decreased significantly by chlorsulfuron and the reduction was greatest at the higher rate of the herbicide. These plants showed chlorotic spots on the young leaves a few days after spraying which progressed with time.

4.3.2 Effect of chlorsulfuron on wheat grown at a range of nitrate concentrations

Three weeks after spraying, shoot dry weight of control plants increased as external nitrate concentration increased from 1 to 5 mol m^{-3}, then decreased with further increases in nitrate concentration over the range used (Figure 4.1A). At 15 g a.i. ha^{-1} chlorsulfuron, shoot dry weight decreased sharply when the concentration of applied nitrate increased from 1 to 10 mol m^{-3}, then decreased slightly with additional nitrate thereafter. Chlorsulfuron at 15 g a.i. ha^{-1} decreased the shoot dry weight of wheat in the range 1 to 25 mol m^{-3} nitrate as compared with unsprayed plants. The greatest reduction in shoot dry weight with the lower rate of chlorsulfuron (47.8%) occurred at 5 mol m^{-3} nitrate. At 60 g a.i. ha^{-1} chlorsulfuron, shoot dry weight of wheat remained unchanged over the entire range of applied nitrate. Chlorsulfuron at 60 g a.i.ha^{-1} caused a reduction in shoot dry weight at all nitrate concentrations relative to unsprayed controls (Figure 4.1A).

At the second harvest, shoot dry weight of control plants increased with increased applied nitrate concentration from 1 to 5 mol m^{-3}, then decreased with increased nitrate concentration thereafter (Figure 4.1B). The same pattern was observed at both rates of chlorsulfuron. Chlorsulfuron at 15 g a.i. ha^{-1} did not affect the shoot dry weight of plants growing at 1 mol m^{-3} nitrate. The same rate of chlorsulfuron caused significant reductions in shoot dry weight at nitrate concentrations in the range 5 to 25 mol m^{-3}. At the higher rate, chlorsulfuron caused a reduction in shoot dry

weight at all nitrate concentrations. The difference between unsprayed and sprayed plants was greatest at 5 mol m^{-3} nitrate for both rates of chlorsulfuron used and a greater growth reduction was observed with the high rate (Plate 4.1). Variations in seed head dry weight showed similar results to those obtained for changes in dry weight (Appendix 4A).

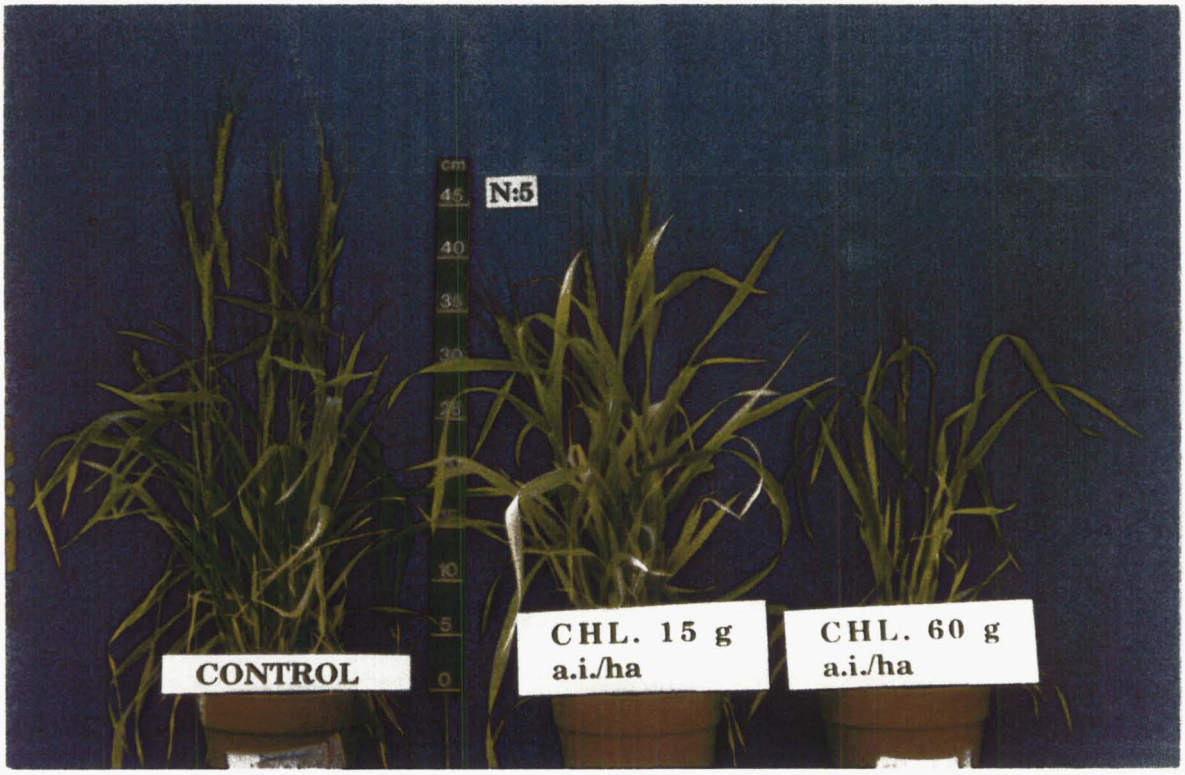


Plate 4.1 Effect of chlorsulfuron at 15 and 60 g a.i. ha^{-1} on wheat grown at 5 mol m^{-3} nitrate 67 days after spraying.

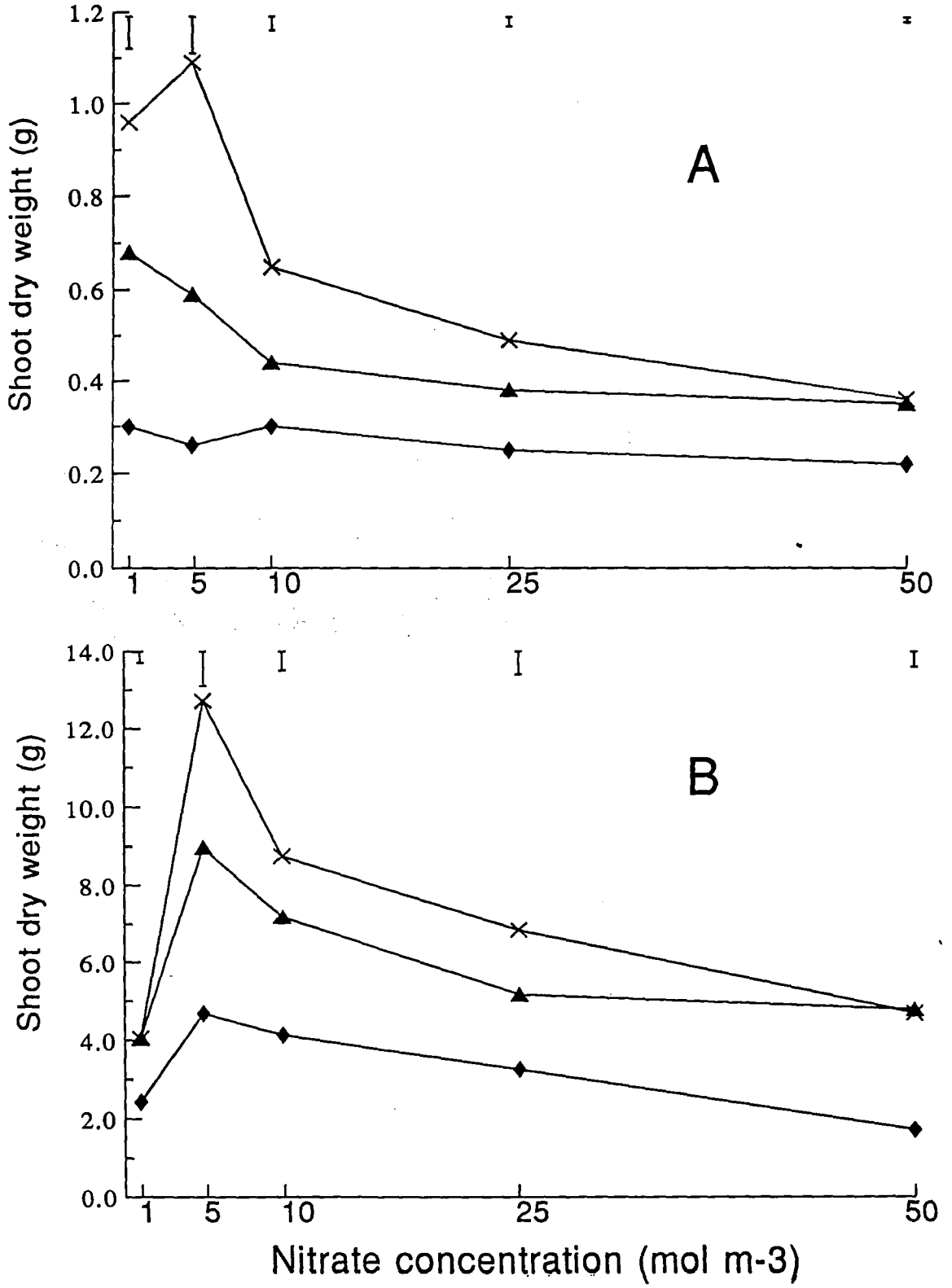


Figure 4.1 Effect of chlorsulfuron on shoot dry weight of wheat grown at different nitrate concentrations, 21 (A) and 135 (B) days after spraying with 0 (×), 15 (▲) or 60 (◆) g a.i. ha^{-1}. Error bars are SEm at each nitrate concentration.

4.3.3 Effect of chlorsulfuron on dry matter accumulation of wheat grown at low and high nitrate concentrations.

Shoot dry weight of control plants was not different between low and high nitrate concentrations at one or three DAS (Figure 4.2A). At seven DAS, (22 days after planting), there was a small but significant increase in shoot dry weight for control plants grown at 5 mol m^{-3} nitrate compared to plants at 1 mol m^{-3} nitrate. The difference in shoot dry weight between nitrate treatments increased over time and at 27 DAS, shoot dry weight of control plants growing at 5 mol m^{-3} nitrate was 1.8 times those growing at 1 mol m^{-3} nitrate. Shoot dry weight of sprayed plants at each nitrate regime did not show any significant reduction compared to the control plants, up to day 7. At 27 DAS chlorsulfuron caused a reduction of 13.6% in shoot dry weight of plants at 1 mol m^{-3} nitrate, while the reduction in shoot dry weight at 5 mol m^{-3} nitrate was 56.4% compared to their respective controls.

Root dry weight of unsprayed plants was not different between low and high nitrate treatments up to seven DAS (Figure 4.2B). Only at the final harvest at 27 DAS, was root dry weight of plants growing at 5 mol m^{-3} nitrate significantly greater than that of plants growing at 1 mol m^{-3} nitrate. Chlorsulfuron application did not have any significant effect on root dry weight up to day seven after spraying. At 27 DAS values for root dry weight of sprayed plants were significantly smaller than the respective values for the control plants at each nitrate level. There was a greater reduction in root dry weight with chlorsulfuron at 5 mol m^{-3} compared to 1 mol m^{-3} nitrate.

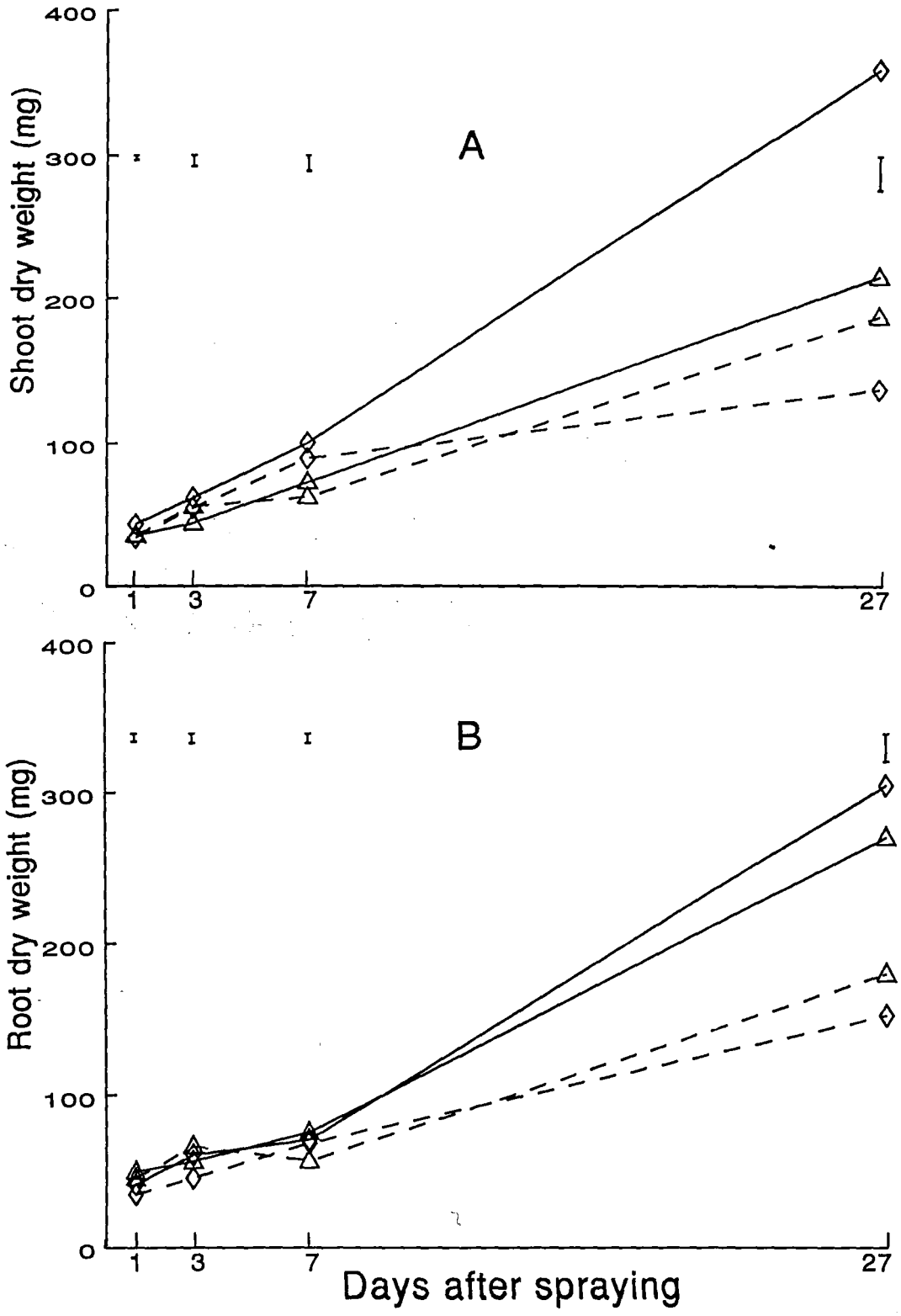


Figure 4.2 Effect of chlorsulfuron (15 g a.i. ha^{-1}) on shoot (A) and root (B) dry weight of wheat grown at 1 (△) or 5 (◇) mol m^{-3} nitrate. Solid lines are controls. Error bars are SEm at each time.

4.3.4 Retention of chlorsulfuron by the foliage of wheat grown at low and high nitrate concentrations

Table 4.2 shows that wheat plants grown at 5 mol m-3 nitrate retained more spray than plants grown at 1 mol m-3 nitrate on a per plant basis. However, when values were corrected for leaf area and dry weight, no differences were found between plants grown at 1 and 5 mol m-3 nitrate. The repeat experiment (Appendix 4B) showed similar results.

Table 4.2 Retention of chlorsulfuron spray on the foliage of wheat grown at two nitrate levels.

Nitrate ($mol\ m^{-3}$)	µl of spray		
	per plant	per cm^2 leaf area	per g dry weight
1	13.8	1.2	201.3
5	18.5	1.3	240.4
SEm	1.23**	0.15	17.56

** Double asterisk indicates a significant F test at $p < 0.01$.

4.3.5 Uptake and translocation of ^{14}C-chlorsulfuron in wheat grown at low and high nitrate concentrations

There was no difference in the recovery of radioactivity between the two nitrate treatments at any time. Recovery values averaged over nitrate treatments were 95.9% and 90.2% of the applied amount 24 h and 48 h after application, respectively. Recovery of radioactivity was lower 168 h after application and only 82.3% of the applied dose could be recovered.

Uptake of ^{14}C-chlorsulfuron was similar between low and high nitrate plants throughout the experimental period (Figure 4.3). Uptake was relatively low after 24 h, and only 10.3% and

10.7% of the applied ^{14}C-chlorsulfuron were absorbed by the lamina of leaf 2 in plants grown at 1 and 5 mol m^{-3} nitrate, respectively. Values for uptake continued to rise in both nitrate treatments and 48 h after application reached 16.0% and 18.7% of the applied radioactivity in low and high nitrate plants, respectively. There was a further increase in the amount of ^{14}C-chlorsulfuron absorbed after this time and at 168 h after application, the uptake values were 21.8% and 21.2% of the applied material. However, the rate of increase in uptake was slower after 48 h than during the initial period (Figure 4.3). The repeat experiment showed no difference in uptake of chlorsulfuron 24 h after application to plants growing at 1 and 5 mol m^{-3} nitrate (Appendix 4C).

Translocation of ^{14}C-chlorsulfuron, both as a percentage of the applied dose (translocation A) and as a percentage of the radioactivity recovered from the plant (translocation B), is presented in Table 4.3. The total amount of ^{14}C-chlorsulfuron translocated out of the treated lamina was not different between 1 and 5 mol m^{-3} nitrate at any time throughout the experiment, irrespective of the calculation method. Only 5.8% and 6.3% of the applied ^{14}C-chlorsulfuron moved out of the treated area 24 h after application to plants grown at 1 and 5 mol m^{-3} nitrate, respectively. Translocation continued over the experimental period and at 168 h after application reached 15.2% and 15% of the applied material in low- and high-nitrate plants, respectively. The rate of translocation declined after 48 h (Table 4.3). Values of translocation as a percentage of the total radioactivity recovered from plant ranged from 13.7% and 13.3% for low and high nitrate treatments, respectively, 24 h after application to 24% and 23.9% for low and high nitrate treatments respectively, 168 h after application (Table 4.3). Similarly, in the repeat experiment no difference in translocation was found between the two nitrate treatments 24 h after the application of ^{14}C-chlorsulfuron (Appendix 4C).

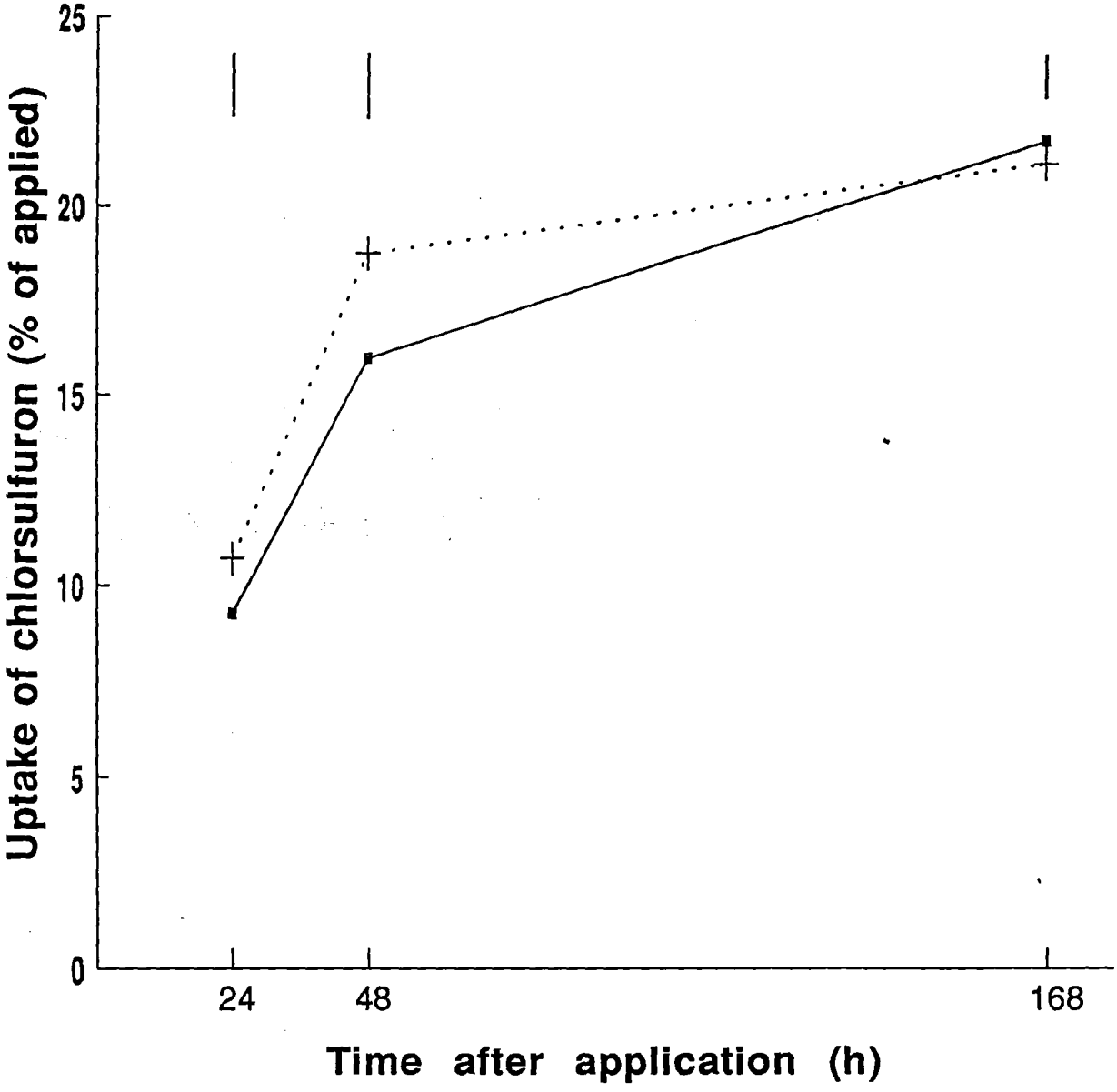


Figure 4.3 Uptake of ^{14}C-chlorsulfuron by leaf 2 lamina of wheat grown at 1 (—) or 5 (·····) mol m^{-3} nitrate. Error bars are SEm at each time.

Table 4.3 Translocation of radioactivity in wheat grown at two nitrate levels, 24, 48 and 168 h after application of ^{14}C-chlorsulfuron to leaf 2 lamina.

Nitrate (mol m^{-3})	Translocation A			Translocation B		
	24 h	48 h	168 h	24 h	48 h	168 h
1	5.8	10.4	15.2	13.7	16.1	24.0
5	6.3	10.0	15.0	13.3	14.5	23.9
SEm	0.60	2.29	3.44	1.05	1.26	1.50

a) Translocation A = translocation as a percentage of the applied dose,
Translocation B = translocation as a percentage of radioactivity recovered from plant, (see Section 3.2.2).

Distribution of ^{14}C to various plant parts, as a percentage of the radioactivity recovered from plant, is shown in Table 4.4. Treated laminae contained far greater amounts of radioactivity than other parts, throughout the experiment. There was no difference in the amount of ^{14}C-chlorsulfuron in the treated lamina between plants grown at 1 and 5 mol m^{-3} nitrate. Very little radioactivity was detected in leaf 1, but young tissue imported a significant amount of the applied ^{14}C-chlorsulfuron. Distribution of radioactivity in all plant parts was similar between low and high nitrate at all times of measurement (Table 4.4).

The pattern of differences in concentration of the radiolabelled chlorsulfuron in various plant fractions was similar to that of the distribution of total ^{14}C (Table 4.5). Concentration of radiolabelled herbicide was the smallest in leaf 1 and the greatest in leaf 2 lamina, the treated fraction, throughout the experimental period. No differences were observed between plants grown at 1 and 5 mol m^{-3} nitrate in the concentration of radioactivity in their tissue fractions, 24 and 48 h after application. However, at 168 h after application, except for leaf 1 and the treated lamina, the concentration of radioactivity was greater in all fractions when plants had been grown at low, compared to high nitrate levels.

Table 4.4 Distribution of radioactivity in various plant parts 24, 48 and 168 h after application of ^{14}C-chlorsulfuron to leaf 2 lamina of wheat plants grown at low (1 mol m^{-3}) or high (5 mol m^{-3}) nitrate. Values are percentages of the total radioactivity recovered from plant.

Plant part	24 h		48 h		168 h	
	Low	High	Low	High	Low	High
Leaf 1	**0.23**	**0.30** (0.098)[a]	**0.13**	**0.10** (0.011)	**0.52**	**0.43** (0.054)
Leaf 2 lamina	**86.25**	**86.44** (2.162)	**84.00**	**85.29** (0.987)	**76.03**	**76.19** (1.567)
Leaf 2 sheath	**1.03**	**0.91** (0.102)	**4.45**	**3.19** (0.711)	**5.26**	**5.28** (0.388)
Young tissue	**7.50**	**8.44** (0.627)	**8.54**	**9.03** (0.545)	**12.05**	**13.27** (1.519)
Root	**4.85**	**4.15** (0.476)	**3.26**	**3.52** (0.721)	**6.15**	**5.29** (0.843)

a) Values in brackets are SEm for each plant part at each time.

Table 4.5 Concentration of radioactivity in various plant parts 24, 48 and 168 h after application of ^{14}C-chlorsulfuron to leaf 2 lamina of wheat grown at low (1 mol m^{-3}) or high (5 mol m^{-3}) nitrate. Values are Bq μg^{-1} dry weight.

Plant part	24 h		48 h		168 h	
	Low	High	Low	High	Low	High
Leaf 1	**60.0**	**48.3** (4.99)[a]	**16.7**	**16.7** (1.50)	**50.0**	**40.0** (11.83)
Leaf 2 lamina	**6398.3**	**6443.3** (266.33)	**11696.7**	**11473.3** (1160.99)	**13705.0**	**10093.3** (928.00)
Leaf 2 sheath	**1306.7**	**1336.7** (136.17)	**1371.7**	**1505.0** (143.67)	**573.3**	**280.0** (45.17)**
Young tissue	**263.3**	**188.3** (32.33)	**218.3**	**208.3** (28.67)	**143.3**	**78.3** (6.83)**
Root	**78.3**	**76.7** (6.99)	**58.3**	**90.0** (13.33)	**103.3**	**70.0** (6.67)**

a) Values in brackets are SEm for each plant part at each time.

Double asterisks indicate a significant F test at $p < 0.01$ between the two nitrate levels for each particular measurement.

4.3.6 Metabolism of ^{14}C-chlorsulfuron in wheat grown at low and high nitrate concentrations

No difference was observed in metabolism of ^{14}C-chlorsulfuron between low and high nitrate plants at any time during the experiment (Figure 4.4). At 24 h after application 40.2% and 45.4% of chlorsulfuron had been metabolised in plants growing at 1 and 5 mol m^{-3} nitrate, respectively. Metabolism continued throughout the experimental period, though at a slower rate and reached 80.1% and 84.4% in 1 and 5 mol m^{-3} nitrate plants, respectively, 168 h after application (Figure 4.4).

The pattern of distribution of radioactivity on TLC plates was similar to that obtained in the metabolism study reported in Chapter 3. Authentic ^{14}C-chlorsulfuron migrated on TLC plates with an Rf value of 0.80 after development to a 160-mm solvent front in chloroform : acetic acid (19:3, v:v) solvent system. Other metabolites had lower Rf values and were located in similar positions as reported previously (Table 3.6).

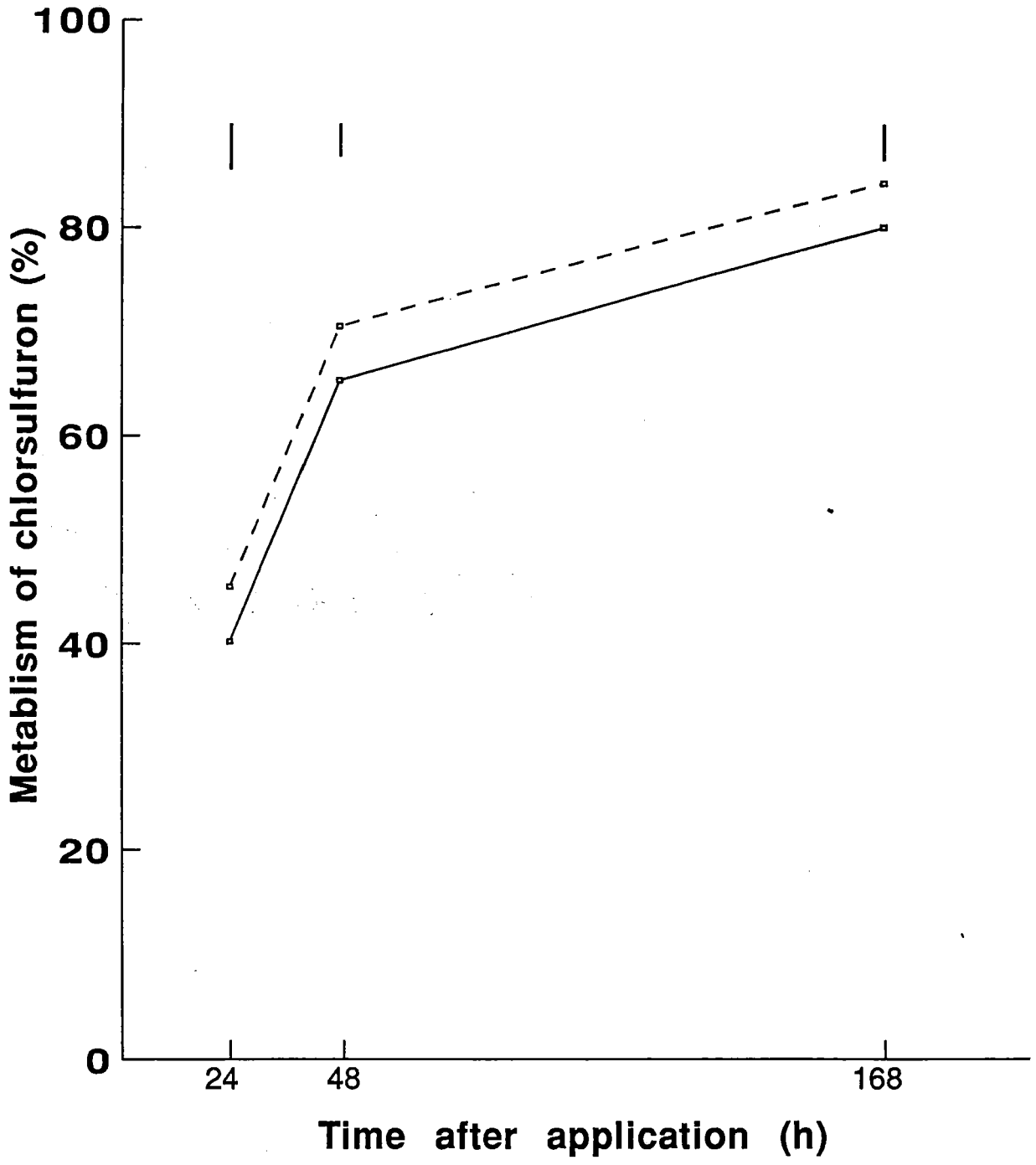


Figure 4.4 Metabolism of chlorsulfuron in wheat grown at 1 (——) or 5 (– – –) mol m^{-3} nitrate at different times after application. Error bars are SEm at each time.

4.4 Discussion

Experiments described in Sections 4.3.1 and 4.3.2 were designed to determine if there were any differences in chlorsulfuron effect on wheat cv. Rongotea when grown at different nitrate concentrations. The greatest growth of both control plants and plants sprayed with 15 g a.i. ha^{-1} chlorsulfuron was obtained at 5 mol m^{-3} nitrate (Figure 4.1). However, the greatest reduction in growth with chlorsulfuron application, relative to control plants, also occurred at 5 mol m^{-3} nitrate. The reduction in shoot dry weight of Rongotea plants grown at 5 mol m^{-3} nitrate, by chlorsulfuron treatment was a medium-long term response and increased with time (Figure 4.2). However, this does not rule out a short term effect of chlorsulfuron as will be shown in Chapter 6. Dry matter accumulation is a gradual process. The greater reduction in dry matter at 27 DAS than at the earlier harvests indicates that the herbicide effect persisted for a period of time and limited plant growth.

It is appropriate to contrast chlorsulfuron and some other herbicides in their effects on plants grown at different nitrogen levels. In the case of diclofop-methyl, fluazifop butyl and glyphosate, plant dry weight or seed head dry weight of cultivated oat sprayed at the recommended rate was much lower at high than at low nitrogen availability. Sprayed plants at high nitrogen died in most cases, while plants at low nitrogen survived (Dickson, Andrews and Field, 1988; Andrews *et al.*, 1989a; Dickson *et al.*, 1990). In the case of chlorsulfuron, plants sprayed at the recommended rate had greater dry weight and seed head dry weight when grown at high than at low nitrogen (Figure 4.1, Appendix 4A). However, their growth was reduced significantly compared to the controls. The data presented in Sections 4.3.1 - 4.3.3 showed that chlorsulfuron limited the capacity of wheat cv. Rongotea to respond to increased external nitrate concentration. This confirms the conclusion reached for several wheat cultivars including Rongotea in Chapter 2. The limitation imposed by chlorsulfuron in the response of sensitive wheat cultivars to nitrogen has been documented for the extension rate of leaf 3 following a pre-emergence application of the

herbicide (Bowran and Blacklow, 1987), but had not been shown previously for dry matter production.

For plants growing at low and high nitrate, it was reasonable to expect differences in one or more of the steps leading from herbicide application to the accumulation at the site of action (see Section 4.1). However, there was no indication from the data presented in this chapter that differences observed in the sensitivity of wheat grown at low and high nitrate concentration to chlorsulfuron was related to a difference in the concentration of the herbicide within the plants. Retention of the herbicide per unit leaf area or per unit dry weight (Table 4.2), the amount of chlorsulfuron absorbed by leaves (Figure 4.3), translocation and distribution pattern of chlorsulfuron to various plant parts (Tables 4.3 and 4.4) and its metabolism (Figure 4.4) were all similar between plants grown at 1 and 5 mol m^{-3} nitrate.

In the present study, nitrate levels were selected to represent the concentrations within the range likely to occur in agricultural soils (Russel, 1973; Andrews, 1986). The major part of the response to nitrogen for wheat and some other graminaceous species, at least for leaves 1 to 3, has been reported to occur at concentrations below 1 mol m^{-3} (Thomas, 1983; Andrews *et al.*, 1991). Total plant dry weight was not different for Rongotea grown at 1 or 5 mol m^{-3} nitrate up to the second day after spraying (Appendix 4D). Differences in the growth rate of plants at the two nitrate concentrations used in this study became apparent only in the longer term measurements (Appendix 4D, Section 4.3.3). It follows that small differences in growth of plants at 1 and 5 mol m^{-3} nitrate were not large enough to result in differences in uptake, translocation or metabolism of chlorsulfuron. Therefore, the growth reduction in plants at 5 mol m^{-3} nitrate, following chlorsulfuron application must have other physiological explanations. This issue will be considered in Chapters 5 and 6.

The concentration of ^{14}C in different plant parts remained similar between low and high nitrate plants 24 and 48 h after application (Table 4.5). There was a significant increase in this measurement at 168 h after application in leaf 2 sheath, young tissue and roots of plants grown at low nitrate compared to those at high nitrate. Since there was no difference between the two nitrate treatments in the distribution of radioactivity to plant parts at this time (Table 4.4), the higher concentration of ^{14}C in the young parts of low nitrate plants could be attributed to their lower dry weights compared to the corresponding parts in high nitrate plants (Appendix 4D).

Values found for uptake, translocation, distribution and metabolism of chlorsulfuron in Rongotea grown at either nitrate regime were comparable to the values obtained for the same cultivar in the previous radioisotope experiment reported in Chapter 3. Similar values have been reported in the literature for ^{14}C-chlorsulfuron studies on wheat (Sweetser *et al.*, 1982; Foley, 1986).

The absorption pattern of chlorsulfuron over time showed a sharp increase at the start followed by a reduction in rate after 48 h (Figure 4.3). Similarly other workers have found that the absorption of chlorsulfuron begins to slow after a certain period (Hageman and Behrens, 1984; Devine and Vanden Born, 1985). This is probably due to two sets of factors. Firstly, absorption is dependent upon translocation. Devine *et al.* (1987) suggested that chlorsulfuron is taken up by a passive, non-facilitated process. Immediately following application, a steep gradient exists across the cuticle which enhances diffusion. This continues, provided that translocation of the absorbed molecules maintains the concentration gradient (Price, 1983; Caseley and Coupland, 1985). After a certain period, however, chlorsulfuron reaches the growing sinks in the meristems and inhibits their activity. This will result in a reduced sink strength and a consequent reduction in transport out of the treated leaves which in turn will cause a reduced uptake across the cuticle. There is also evidence in the literature for a direct effect of chlorsulfuron on assimilate transport in the phloem (Devine *et al.*, 1990; Geiger and Bestman, 1990). Secondly, diffusion usually proceeds in an aqueous environment. As the water evaporates from the spray droplet, the concentration of the

active ingredient will increase, facilitating its diffusion across the cuticle (Caseley and Coupland, 1985). Further evaporation will eventually result in precipitation of an increasing proportion of the deposit until equilibrium is attained and no further uptake occurs (Price, 1983).

In summary, the data presented in this chapter showed that the effect of chlorsulfuron on wheat was more severe when plants were grown at high than at low nitrate (Sections 4.3.1 - 4.3.3). It was expected that retention, uptake, translocation or metabolism of chlorsulfuron would reveal differences between the two nitrate regimes to explain the variation in sensitivity to the herbicide. However, the data showed that this was not the case and the values obtained for the above parameters were similar for plants grown at low and high nitrate (Sections 4.3.4 - 4.3.6). Plants grown at 1 and 5 mol m^{-3} nitrate could metabolise only 40.2% and 45.4% of chlorsulfuron, respectively, within 24 h (Figure 4.4). The data presented in this chapter and Chapter 3 indicate that Rongotea has a low capacity to metabolise chlorsulfuron irrespective of the growth medium or the nitrate levels tested. Other reports indicate that the effect of chlorsulfuron is very rapid. Growth of maize seedlings was severely reduced within 2 h following chlorsulfuron treatment (Ray, 1989). This suggests that plants grown at low nitrate could be damaged but the effect was only apparent in plants grown at high nitrate (Figure 4.1). It is concluded that chlorsulfuron limits the ability of Rongotea wheat to respond to additional nitrate as was found for some other sensitive cultivars (Section 2.3.4). Further research is required to determine the physiological basis for this limitation and its relationship to the site of action of chlorsulfuron.

CHAPTER 5

Chlorsulfuron Effects on Uptake and Assimilation of Nitrate in Wheat cv. Rongotea Supplied Nitrate or Amino Acids as a Nitrogen Source

5.1 Introduction

Chlorsulfuron limits the capacity of Rongotea wheat to respond to additional nitrate as demonstrated in Chapter 4. A similar limitation in response to nitrogen has been shown for the rate of leaf extension of some other sensitive wheat cultivars following a pre-emergence application of chlorsulfuron (Bowran and Blacklow, 1987). At a biochemical level, chlorsulfuron has been shown to inhibit the activity of acetolactate synthase (ALS), the first enzyme in the biosynthetic pathway of the branched chain amino acids (BCAA) : valine, leucine and isoleucine (Ray, 1984; Chaleff and Mauvais, 1984). Previous reports on the ability of additional BCAA to protect higher plants from chlorsulfuron injury are inconsistent. Ray (1984) added 20, L-amino acids, in groups based on their common biosynthetic pathways, to the excised root culture of peas in the presence of chlorsulfuron. The only group of amino acids which alleviated chlorsulfuron-induced growth inhibition was BCAA. Furthermore, he showed that addition of valine plus isoleucine alone gave protection against the herbicide both in excised root culture and whole plant culture of peas. Rost and Reynolds (1985) found that the addition of valine and isoleucine to pea root tips treated with chlorsulfuron prevented the inhibition of cell division. Scheel and Casida (1985) obtained similar results with soybean cell cultures. Inhibition of assimilate transport induced by chlorsulfuron in some sensitive species was overcome by supplementation of BCAA (Vanden Born *et al.*, 1988; Devine *et al.*, 1990). Contrary to the above reports the addition of BCAA was ineffective in preventing chlorsulfuron-induced growth inhibition in pea and maize roots (Rubin and Casida, 1985; Giardina *et al.*, 1987). With regard to these and other reports (see Section 1.3.3.3), the possibility of other mechanisms of chlorsulfuron action or some unknown effects of ALS inhibition exist.

Chlorsulfuron may affect uptake and use of soil minerals by plants. Tanaka and Anderson (1985) reported that application of chlorsulfuron with diclofop-methyl, barban or difenzoquat to durum

wheat resulted in large yield losses. They attributed this to a lower nitrogen content in treated plants at anthesis. Chlorsulfuron has been reported to interfere with absorption and movement of calcium in maize (Crowley and Prendeville, 1985), and to reduce zinc uptake in wheat (Bowran, Porritt and Madin, 1987; McLay and Robson, 1992; Osborne and Robson, 1992). In another study, chlorsulfuron was found to increase chloride efflux from roots of a sensitive wheat cultivar and this was interpreted as an effect on root integrity (Vanakitmongkol and Blacklow, 1990). It is therefore necessary to determine if chlorsulfuron application can affect the uptake or assimilation of nitrogen by plants.

Chlorsulfuron has been found to affect photosynthesis only at concentrations several orders of magnitude greater than the concentration required to inhibit cell division (De Villiers *et al.*, 1980; Ray, 1982a). Therefore, inhibition of photosynthesis was ruled out as a primary mode of action of chlorsulfuron. However, an effect on photosynthesis might be important in growth restriction of Rongotea plants grown at high nitrate, since it could indirectly affect nitrogen metabolism (Wallsgrove *et al.*, 1983; Turpin and Weger, 1990).

In the first two experiments described in this chapter, wheat, cv. Rongotea seedlings grown at two levels of external nitrate were supplied with BCAA and treated with chlorsulfuron. Chemical analysis was carried out to determine the amount of different forms of nitrogen in plant tissues. The primary objectives of the experiments were to determine: 1) if chlorsulfuron effects on the response of Rongotea to additional nitrogen were due to reduced nitrate uptake and/or assimilation, and 2) if addition of BCAA could overcome the limitation on growth caused by chlorsulfuron.

Following the findings of the initial experiments, two other experiments were carried out to determine: 1) if effects of valine, leucine and isoleucine in overcoming chlorsulfuron-induced damage were specific BCAA effects, and 2) if decreased growth or accumulation of nitrate was related to a reduction in photosynthetic rate.

5.2 Materials and Methods

5.2.1 Plant culture and growing conditions

Uniform seven-day-old seedlings of wheat cv. Rongotea were planted individually in 1300-ml volume plastic pots filled with vermiculite/perlite mixture (1:1, v:v). Plants were maintained in a controlled environment cabinet as described in Section 2.2.4. All pots were flushed every two days with a basal nutrient solution (Appendix 2D) containing the appropriate nitrogen treatment. Potassium concentration was made equal in all treatments by the addition of potassium sulphate. When plants reached the three-leaf stage (ZGS 13), chlorsulfuron was applied as described in Section 2.2.1.

5.2.2 Effect of BCAA on the response of wheat to chlorsulfuron.

Two experiments were carried out. In the first experiment, two methods of supplementing BCAA: either via roots or via leaf sheaths were compared. The second experiment examined the effects of BCAA, supplied via roots, on growth and content of different forms of nitrogen in wheat sprayed with chlorsulfuron.

Experiment 1: There were six nitrogen treatments; low and high nitrate (1 and 5 mol m^{-3}), plus and minus BCAA (valine, leucine and isoleucine) added either as a drench with each irrigation or injected into the sheaths of leaves one to three. Each of the amino acids was at a concentration of 1 mol m^{-3} in the drench and 10 mol m^{-3} when injected (100-150 μl per leaf sheath). All drench treatments were applied every two days starting from one day after planting. The leaf sheath treatment was started five days after planting and was applied every two days afterwards. Chlorsulfuron was applied at 0, 15 and 60 g a.i. ha^{-1}. Plants were harvested 28 days after spraying (DAS) and shoot dry weights determined after drying at 60 °C for two days.

Experiment 2: There were six nitrogen treatments initially. These were low (1 mol m^{-3}) and high (5 mol m^{-3}) nitrate plus 0, 1 or 3 mol m^{-3} of each of the three BCAA. Chlorsulfuron was

applied at 0 or 15 g a.i. ha^{-1}. Immediately after spraying, some plants supplied with low nitrate were transferred to high nitrate, giving a seventh nitrogen treatment. Plants were harvested 45 DAS, divided into root and shoot for dry weight determination. Dried material was then ground (0.10 mm mesh), and an aqueous extract of a 20-50 mg sample analysed for nitrate, nitrite and ammonium as described by Mackereth, Heron and Talling (1978). Total N was determined in 100 mg samples by Kjeldahl digestion. Ammonium produced was subsequently measured by the indophenol blue colorimetric method using a Technicon AA II autoanalyser. Reduced N was determined as the difference between total N and nitrate N plus nitrite N.

5.2.3 Effect of supplementing different groups of amino acids on the response of wheat to chlorsulfuron.

In this experiment, plants supplied 1 mol m^{-3} nitrate were taken to the three-leaf stage (ZGS 13) and sprayed with 0 or 15 g a.i. ha^{-1} chlorsulfuron. Four nitrogen treatments were introduced one day prior to spraying. These were low nitrate (1 mol m^{-3}), high nitrate (5 mol m^{-3}), low nitrate plus 4 mol m^{-3} nitrogen as BCAA (1.33 mol m^{-3} of each) or low nitrate plus 4 mol m^{-3} nitrogen as glutamine and glutamate (GLN/GLU, 1.33 mol m^{-3} of each). Shoot dry weight was determined as above, 37 days after spraying.

5.2.4 Effect of chlorsulfuron on photosynthetic rate of wheat.

Plants were supplied 1 or 5 mol m^{-3} nitrate and sprayed with chlorsulfuron as in Experiment 5.2.4. Photosynthetic rate (net CO_2 uptake) was determined for the 2 youngest fully expanded leaves of all plants *in situ* 2, 7, 11, 19 and 27 days after the herbicide application. Measurements were taken with a portable Infra Red Gas Analyser (IRGA; ADC limited), using a 56-mm-long leaf chamber which tightly enclosed the central portion of the leaf lamina about 20 mm above the ligule. All measurements were taken under constant air flow (4 ml s^{-1}) and light intensity (300 μmol m^{-2} s^{-1}). In the differential mode, the IRGA gave readings of the difference in CO_2 concentration between the leaf chamber and the ambient air. This figure, adjusted for temperature and flow rate was used to calculate the rate of CO_2 uptake. Performance of the IRGA was

evaluated prior to measurements at each time by running it with an empty chamber to ensure a differential reading of zero, and also by measuring the photosynthetic rate of leaves under a range of flow rates (4-8 ml s^{-1}) and irradiance levels (200-400 μmol m^{-2} s^{-1}) as recommended by Field, Ball and Berry (1989).

5.2.5 Experimental design and data analysis

All experiments described in this chapter had five replicates and were arranged in randomized complete block designs. All data were subject to analysis of variance using SAS statistical package (SAS Institute Inc., Cary, N.C.) and standard errors of mean values presented to indicate variability of data. All effects discussed have a probability of $p < 0.01$.

5.3 Results

5.3.1 Effect of BCAA on the response of wheat to chlorsulfuron.

Experiment 1: For all nitrogen treatments shoot dry weight was lower at 60 than at 0 or 15 g a.i. ha^{-1} chlorsulfuron (Table 5.1). At 60 g a.i. ha^{-1} chlorsulfuron, shoot dry weight was unaffected by nitrogen treatment and all results described are for the recommended field rate of chlorsulfuron (15 g a.i. ha^{-1}). Regardless of BCAA treatment, sprayed plants were unable to respond to nitrate. Application of BCAA through the leaf sheath did not increase the shoot dry weight of sprayed or unsprayed plants. As a root drench at both levels of nitrate, BCAA caused a significant increase in shoot dry weight of unsprayed plants. Although sprayed plants did not respond to increased level of nitrate, shoot dry weight almost doubled when BCAA were added to the roots at low nitrate. Shoot dry weight of sprayed plants given low nitrate plus amino acids was approximately 80% that of comparable unsprayed plants and almost 85% that of unsprayed plants given high nitrate alone. Sprayed plants given high nitrate also responded to additional BCAA but their shoot dry weight was similar to that for plants given low nitrate plus BCAA.

Table 5.1 Effect of BCAA supplied through leaf sheath or roots on shoot dry weight (g) of wheat sprayed with chlorsulfuron at low (1 mol m^{-3}) and high (5 mol m^{-3}) nitrate.

Chlorsulfuron (g a.i. ha^{-1})	BCAA treatment					
	None		Leaf sheath		Roots	
	Low	High	Low	High	Low	High
0	**0.79** (0.038)[a]	**1.81** (0.143)	**0.88** (0.026)	**1.33** (0.131)	**1.92** (0.176)	**2.37** (0.212)
15	**0.76** (0.053)	**0.87** (0.118)	**0.68** (0.029)	**0.56** (0.087)	**1.51** (0.118)	**1.21** (0.040)
60	**0.45** (0.048)	**0.35** (0.023)	**0.42** (0.013)	**0.44** (0.059)	**0.51** (0.036)	**0.45** (0.056)

a) Values in brackets are SEm.

Experiment 2: Total plant dry weight was greater for unsprayed plants than for sprayed plants for all nitrogen treatments, except high nitrate plus 9 mol m^{-3} BCAA, which regardless of herbicide treatment showed marked chlorosis and leaf damage and stopped growing before completion of the experiment (Table 5.2). The reduction in growth caused by chlorsulfuron was greater at high than at low nitrate. Control and sprayed plants transferred from low to high nitrate were similar to comparable plants given high nitrate throughout. The effect of chlorsulfuron was greater with high nitrate alone than with low nitrate plus BCAA. For unsprayed plants, plant dry weight was similar with high nitrate or with low nitrate plus 3 mol m^{-3} BCAA, but for sprayed plants, plant dry weight was greater with the amino acid treatment. Also for unsprayed plants, plant dry weight was greater with high nitrate plus 3 mol m^{-3} BCAA than with low nitrate plus 9 mol m^{-3} BCAA, but for sprayed plants the opposite was true.

Table 5.2 Effect of nitrate supply and branched chain amino acids (BCAA) on total plant dry weight, total nitrogen (N) and reduced N per plant of wheat sprayed with chlorsulfuron.

Nitrate (mol m-3)	BCAA[a]	Dry weight (g)		Total N (mg)		Reduced N (mg)	
		Control	Sprayed	Control	Sprayed	Control	Sprayed
1	0	**2.24** (0.091)[b]	**1.44** (0.042)	**22.2** (1.12)	**20.6** (0.50)	**21.8** (0.91)	**20.4** (0.54)
5	0	**5.10** (0.440)	**2.10** (0.301)	**114.4** (5.23)	**78.0** (5.41)	**112.6** (5.72)	**67.2** (8.71)
1	3	**5.72** (0.235)	**4.63** (0.372)	**83.9** (5.02)	**76.3** (5.01)	**83.7** (5.02)	**76.2** (5.04)
5	3	**8.31** (0.542)	**3.67** (0.456)	**171.0** (8.63)	**130.3** (7.74)	**169.8** (8.92)	**113.0** (10.31)
1	9	**6.99** (0.291)	**5.88** (0.643)	**178.8** (8.02)	**161.8** (6.51)	**178.5** (8.04)	**161.5** (6.51)
5	9	**0.66** (0.064)	**0.54** (0.072)	**33.3** (6.91)	**25.4** (4.74)	**40.1** (8.71)	**35.2** (7.74)
1→5	0	**5.15** (0.182)	**1.47** (0.231)	--	--	--	--

a) concentrations of BCAA are 0, 3 and 9 mol m^{-3} total of valine, leucine and isoleucine.

b) Values in brackets are SEM.

At low nitrate or low nitrate plus BCAA, total N or reduced N were not significantly different for sprayed and unsprayed plants (Table 5.2). At high nitrate, total plant N and total plant reduced N were 30% and 40% lower in sprayed than in unsprayed plants, respectively. Similar reductions with chlorsulfuron occurred with the high nitrate plus BCAA treatment.

Changes in shoot dry weight with nitrogen treatments (Table 5.3) followed the same pattern as plant dry weight (Table 5.2). For sprayed and unsprayed plants additional nitrate or BCAA caused an increase in reduced N content of shoots (Table 5.3). Chlorsulfuron increased reduced N content of shoots in all treatments except that of 9 mol m^{-3} BCAA. For plants at low nitrate, regardless of BCAA treatment, nitrate N content was in the range 0.02-0.05 for control and sprayed plants. Chlorsulfuron caused a marked increase in nitrate N content of plants grown at high nitrate alone or high nitrate plus 3 mol m^{-3} BCAA. Ammonium N content constituted only 2-3% of reduced N content in all treatments except high nitrate plus 9 mol m^{-3} BCAA in which ammonium N content was higher (7.7%). Chlorsulfuron did not affect ammonium N content of shoots. For all nitrogen treatments nitrite N was undetectable (<0.02 $\mu g\ g^{-1}$ dry weight) in shoots.

Treatment effects on dry weight and reduced N, nitrate N, nitrite N and ammonium N contents of roots were similar to shoots (Table 5.4). Similar results were obtained in a repeat experiment (Appendix 5A).

Table 5.3 Effect of nitrate supply and BCAA on dry weight (g), reduced N, nitrate N and ammonium N content (mg g-1 dry weight) of shoot of wheat sprayed with chlorsulfuron at 15 g a.i. ha-1.

Nitrate	BCAA	Dry weight (g)		Reduced N		Nitrate N		Ammonium N	
(mol m-3)[a]		Control	Sprayed	Control	Sprayed	Control	Sprayed	Control	Sprayed
1	0	1.09 (0.054)[b]	0.67 (0.027)	11.2 (0.30)	17.6 (0.71)	0.05 (0.005)	0.05 (0.010)	0.34 (0.027)	0.36 (0.028)
5	0	3.00 (0.248)	1.33 (0.167)	28.4 (0.68)	37.8 (1.35)	0.26 (0.118)	9.49 (2.950)	0.58 (0.049)	0.77 (0.049)
1	3	3.20 (0.111)	2.47 (0.221)	18.1 (1.12)	21.3 (0.83)	0.05 (0.022)	0.02 (0.008)	0.42 (0.025)	0.44 (0.028)
5	3	4.91 (0.262)	2.37 (0.246)	26.7 (1.26)	40.0 (2.14)	0.12 (0.035)	5.88 (1.728)	0.50 (0.013)	0.81 (0.010)
1	9	4.47 (0.123)	3.41 (0.356)	32.1 (1.86)	35.1 (4.04)	0.04 (0.011)	0.04 (0.022)	0.52 (0.027)	0.72 (0.091)
5	9	0.43 (0.040)	0.35 (.057)	79.2 (3.40)	78.5 (3.52)	2.22 (0.161)	2.23 (0.186)	6.06 (0.343)	6.02 (0.352)

a) concentrations of BCAA are 0, 3 and 9 mol m^{-3} total of valine, leucine and isoleucine.

b) Values in brackets are SEM.

Table 5.4 Effect of nitrate supply and BCAA on dry weight (g), reduced N, NO3 N and ammonium N content (mg g-1 dry weight) of roots of wheat sprayed with chlorsulfuron at 15 g a.i. ha-1.

Nitrate	BCAA	Dry weight (g)		Reduced N		Nitrate N		Ammonium N	
(mol m-3)[a]		Control	Sprayed	Control	Sprayed	Control	Sprayed	Control	Sprayed
1	0	**1.15** (0.039)[b]	**0.77** (0.019)	**8.6** (0.07)	**11.3** (0.44)	**0.03** (0.011)	**0.17** (0.020)	**0.07** (0.028)	**0.11** (0.020)
5	0	**2.10** (0.199)	**0.77** (0.139)	**13.9** (0.67)	**15.9** (0.58)	**0.60** (0.210)	**8.24** (2.390)	**0.20** (0.051)	**0.27** (0.052)
1	3	**2.52** (0.116)	**2.16** (0.158)	**10.3** (0.52)	**11.2** (0.81)	**0.05** (0.020)	**0.03** (0.020)	**0.08** (0.030)	**0.10** (0.031)
5	3	**3.40** (0.265)	**1.30** (0.209)	**11.1** (0.20)	**15.6** (0.72)	**0.20** (0.118)	**4.61** (0.941)	**0.12** (0.021)	**0.28** (0.031)
1	9	**2.52** (0.129)	**1.87** (0.295)	**14.2** (1.80)	**15.3** (4.02)	**0.06** (0.018)	**0.09** (0.008)	**0.20** (0.038)	**0.37** (0.091)
5	9	**0.23** (0.026)	**0.19** (.029)	**28.5** (1.12)	**34.5** (1.11)	**3.52** (0.161)	**3.66** (0.192)	**0.76** (0.075)	**0.65** (0.077)

a) concentrations of BCAA are 0, 3 and 9 mol m^{-3} total of valine, leucine and isoleucine.

b) Values in brackets are SEM.

5.3.2 Effect of supplementing different groups of amino acids on the response of wheat to chlorsulfuron.

In this experiment shoot dry weight for all nitrogen treatments was greater for unsprayed plants than for sprayed plants (Table 5.5). Similar to other experiments presented in this chapter and Chapter 4, the effect of chlorsulfuron on shoot dry weight was greater at high nitrate than at low nitrate or low nitrate plus BCAA. For control and sprayed plants, shoot dry weight was as great with GLN/GLU as with BCAA. The repeat experiment showed similar results (Appendix 5B).

Table 5.5 Effect of nitrate supply, branched chain amino acids (BCAA) and glutamine plus glutamate (GLN/GLU) on shoot dry weight of wheat sprayed with chlorsulfuron.

Nitrate	Amino acids	Shoot dry weight (g)	
	(mol m^{-3})[a]	Control	Sprayed
1	0	**0.93** (0.06)[b]	**0.75** (0.09)
5	0	**2.24** (0.33)	**0.67** (0.13)
1	4 as BCAA	**2.40** (0.08)	**1.50** (0.21)
1	4 as GLN/GLU	**2.75** (0.08)	**1.61** (0.12)

a) Concentrations of amino acids are 0 and 4 mol m^{-3} as total nitrogen in each group.
b) Values in brackets are SEm.

5.3.3 Effect of chlorsulfuron on photosynthetic rate of wheat.

For control plants throughout the experiment, photosynthetic rate per unit area was similar at low and high nitrate (Table 5.6). Chlorsulfuron did not affect photosynthetic rate of plants grown at low nitrate. For plants grown at high nitrate, chlorsulfuron did not affect the photosynthetic rate until 19 DAS. At this time, the photosynthetic rate of leaf 4 was not affected by chlorsulfuron,

while there was a decrease in photosynthetic rate of leaf 5 in the sprayed plants. At 27 DAS, significant reductions were found in photosynthetic rate of sprayed plants at high nitrate for both leaves examined. Repeat experiment showed similar results (Appendix 5C).

Table 5.6 Shoot dry weight (g) and photosynthetic rate (μmol m^{-2} s^{-1}) of wheat grown at 1 or 5 mol m^{-3} nitrate at different times after the application of chlorsulfuron.

Nitrate (mol m^{-3})	Rate[a]	Shoot dry weight[b]	Day 2	Day 7		Day 11		Day 19		Day 27	
			Leaf3	Leaf3	Leaf4	Leaf3	Leaf4	Leaf4	Leaf5	Leaf5	Leaf6
1	0	1.20	10.25	10.32	10.60	10.07	10.87	8.56	9.24	8.94	8.58
	15	1.10	9.15	9.45	9.35	8.92	9.72	9.30	9.02	7.87	9.23
5	0	3.44	9.91	10.42	9.67	10.16	9.20	8.91	8.71	7.76	8.85
	15	2.12*	11.03	10.69	9.17	10.26	9.19	8.85	5.61*	2.43*	5.87*
SEm		0.130	0.244	0.302	0.225	0.225	0.263	0.222	0.421	0.682	0.385

a) Chlorsulfuron rate (g a.i. ha^{-1}).
b) Shoot dry weight was taken 35 days after application.
* An asterisk indicates significant reduction from the control at $p < 0.01$.

5.4 Discussion

The first experiment presented in Section 5.3.1 aimed to determine an effective method of feeding BCAA to whole plants. Previous reports on feeding amino acids to higher plants have employed the technique either in tissue culture (Ray, 1984), cell culture (Scheel and Casida, 1985) or nutrient solution (Vanden Born *et al.*, 1988). Some workers have supplied amino acids to whole plants but studied the response in the short term only. Rubin and Casida (1985) added BCAA in sterilized sand culture containing corn or pea seedlings and measured root elongation for five days. Giardina *et al.* (1987) studied the effect of supplementing valine and isoleucine in sterile medium on growth of maize in the presence of chlorsulfuron up to 10 days. Growth of Rongotea wheat was markedly improved by supplementing BCAA to the roots but not to the leaf sheaths (Table 5.1). Therefore, the root drench method was used in subsequent experiments.

The objectives of the second experiment were to determine if the limitation on the response of Rongotea to additional nitrogen imposed by chlorsulfuron was due to reduced nitrate uptake and/or assimilation, and if effects of chlorsulfuron on Rongotea could be overcome by addition of BCAA. For all nitrogen treatments in the second experiment, except 9 mol m^{-3} BCAA, total plant dry weight was greater for unsprayed plants than for sprayed plants (Table 5.2). Addition of BCAA gave greater growth of sprayed plants at low or high nitrate but did not give complete protection against the herbicide. Similarly, Scheel and Casida (1985) reported that growth inhibition in soybean cell cultures induced by chlorsulfuron was only partially reversed by BCAA supplied after the treatment. In another report, growth of maize seedlings treated with the imidazolinone herbicide, imazapyr, and supplied with BCAA was only 80% of the control plants (Shaner and Reider, 1986). As shown in Chapter 4, chlorsulfuron limited the ability of Rongotea to respond to nitrate. Plants transferred from low to high nitrate at spraying were similar to plants given high nitrate throughout. This shows that low nitrate prior to spraying does not result in increased tolerance to the herbicide. This is consistent with findings from the radioisotope

studies. Data presented in Section 4.3.2 showed that there was no difference in retention, uptake, translocation or metabolism of chlorsulfuron in Rongotea grown at low and high nitrate.

At high nitrate, total plant N was approximately 30% lower in sprayed than in unsprayed plants (Table 5.2), thus, less nitrate was taken up. Total plant reduced N at high nitrate was approximately 40% lower in sprayed plants than in unsprayed plants (Table 5.2). For all nitrogen treatments, nitrite was undetectable and ammonium constituted 1-3% of reduced N in root and shoot and its levels were unaffected by chlorsulfuron. Therefore, at high nitrate, chlorsulfuron caused a 40% decrease in nitrate assimilation. This decrease is likely to have been part of the cause of the reduction in dry weight at high nitrate. However, for sprayed plants, total N and total reduced N were similar with the high nitrate and low nitrate plus 3 mol m^{-3} BCAA, but total plant dry weight was twice as great with the latter (Table 5.2). Therefore, the main factor limiting growth of sprayed plants at high external nitrate was not decreased nitrate uptake or assimilation but was an inability to utilize increased reduced N to produce dry matter. This resulted in increased reduced N content (mg g^{-1} dry weight) in both root and shoot (Tables 5.3 and 5.4). The possibility that reduced N accumulation inhibits growth must be considered. For sprayed plants, reduced N content was similar with 5 mol m^{-3} nitrate or 1 mol m^{-3} nitrate plus 9 mol m^{-3} BCAA, but shoot dry weight was 2.5 times greater with the BCAA treatment (Table 5.3). Therefore, increased reduced N content is unlikely to be the reason for decreased growth of nitrate-fed plants in comparison with those supplied BCAA.

With regard to mode of action of the herbicide, increased reduced N content at high nitrate in comparison with low nitrate plus BCAA may have been due to amino acid accumulation caused by decreased BCAA production and hence decreased synthesis of specific proteins (Rhodes *et al.*, 1987). Chlorsulfuron also caused an increase in reduced N content of root and shoot of plants supplied BCAA (Tables 5.3, 5.4, Appendix 5A). This may have been due to inadequate BCAA reaching the tissues or other effects of the herbicide such as; decreased cell division and hence

decreased sink activity, inhibition of translocation processes between mature tissues and growing regions (Bestman *et al.*, 1990; Devine *et al.*, 1990), or another effect related to inhibition of ALS activity (LaRossa *et al.*, 1987). These possibilities are discussed more fully in Chapter 6 where aspects of mode of action of chlorsulfuron, such as ALS activity are considered.

Within the plant, nitrate is reduced to ammonium before being assimilated into amino acids. Glutamine and glutamate are considered to be the primary products of ammonium assimilation (Andrews 1986; Layzell, 1990; Figure 5.1).

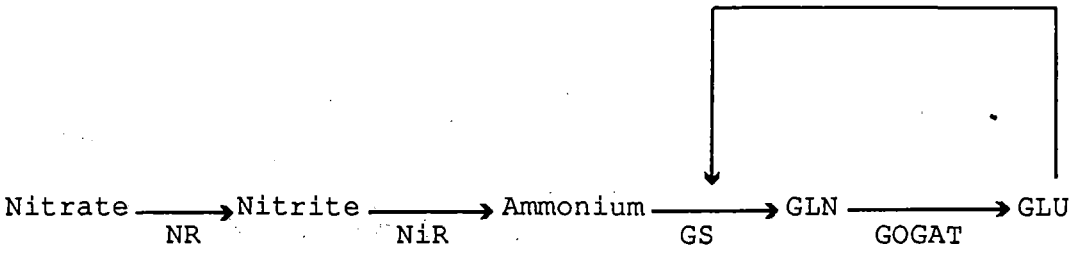


Figure 5.1 Pathway of nitrate assimilation in plants (after Schrader and Thomas, 1981 and Layzell, 1990). NR: nitrate reductase, NiR: nitrite reductase, GS: glutamine synthetase: GOGAT: glutamate synthase.

If increased tolerance to chlorsulfuron with additional valine, leucine and isoleucine, in comparison with nitrate, is a specific BCAA effect, then addition of GLN/GLU to sprayed plants should have no effect. This was tested in Experiment 5.3.2. For all nitrogen treatments, shoot dry weight was greater for unsprayed plants than for sprayed plants (Table 5.5). Similar growth was obtained for the sprayed plants which received either BCAA or GLN/GLU. This indicates that increased tolerance of Rongotea to chlorsulfuron with additional BCAA is not a specific BCAA effect. If this is the case, it must then be asked why reduced N produced via nitrate assimilation cannot be utilized to produce dry matter in sprayed plants? One possible reason is that nitrate accumulation (Table 5.3, 5.4) inhibits growth. Andrews *et al.*, (1992) found that for wheat and some other temperate cereals additional nitrate above 5 mol m^{-3} resulted in an increase in nitrate content accompanied by a decrease in plant dry weight. Zhen and Leigh (1990) determined a

threshold value for internal nitrogen concentration above which nitrate accumulated rapidly. This threshold was lower for roots than shoots. The possibility that nitrate accumulation is the main factor causing decreased growth of nitrate-fed plants in comparison with those fed BCAA is tested in Chapter 6.

Many of the reactions of nitrogen metabolism depend on photosynthetically produced ATP and reductants (Wallsgrove *et al.*, 1983; Turpin and Weger, 1990), and nitrate has been found to accumulate under conditions in which photosynthesis is inhibited (Andrews, Love and Sprent, 1989). Also, photosynthesis inhibitor herbicides were found to cause an increase in nitrate accumulation (Fedtke, 1982). In the present study, photosynthetic rate per unit area was not inhibited until three weeks after spraying (Table 5.6). This is in agreement with previous reports on chlorsulfuron effects on photosynthesis (De Villiers *et al.*, 1980; Beyer *et al.*, 1988). Photosynthetic rate of unsprayed plants were similar for 1 and 5 mol m^{-3} nitrate regimes (Table 5.6) Nitrogen availability is expected to increase growth and physiological response of plants (Lawlor *et al.*, 1987a & b). However, for a range of cereals including wheat, the major part of the response to N has been reported to occur at concentrations below 1 mol m^{-3} (Thomas, 1983; Andrews *et al.*, 1991).

In conclusion, the data presented in this chapter showed that the limitation in response of wheat to additional nitrate imposed by chlorsulfuron is a result of an inability to utilize the assimilated nitrogen to produce dry matter (Table 5.2). This can result in an increase in reduced N and nitrate N content of the tissues (Tables 5.3 and 5.4). The growth reduction with chlorsulfuron was partially overcome when nitrate in the medium was replaced with either BCAA or GLN/GLU (Table 5.5). This shows that the effect of BCAA in overcoming the herbicide effect is not specific to BCAA, and suggests that part of the growth reduction is related to nitrate accumulation. The effect of chlorsulfuron on photosynthesis was tested and discounted as a probable cause for nitrate accumulation and growth restriction (Table 5.6). The data also showed

that growth of sprayed plants supplied amino acids was still lower than that of the control plants (Tables 5.2 and 5.5). This is contrary to the reports which obtained complete alleviation of chlorsulfuron effects in susceptible species by addition of BCAA (Ray, 1984 and 1989; Rost and Reynolds, 1985). Further work is required to determine if decreased growth of Rongotea with chlorsulfuron correlates with decreased acetolactate synthase activity and decreased levels of BCAA, and if BCAA supplied via the nutrient medium were translocated to the shoot.

CHAPTER 6

Study of the Mechanism of Action of Chlorsulfuron in Wheat cv. Rongotea

Introduction

Chlorsulfuron has been shown to inhibit the activity of acetolactate synthase (ALS) in a range of broadleaf and grass species including wheat (Ray, 1989). This enzyme catalyses the first common reaction in the synthesis of the branched chain amino acids (BCAA): valine, leucine and isoleucine. For several species, the content of BCAA and/or the proportion of total amino acids as BCAA have been found to decrease with chlorsulfuron treatment (Scheel and Casida, 1985; Rhodes *et al.*, 1987; Royuela *et al.*, 1991). The general tolerance to chlorsulfuron by temperate cereals appears to be related to its rapid metabolism to non toxic products and not to insensitivity of ALS to the herbicide (Sweetser *et al.*, 1982; Ray, 1989). Recently chlorsulfuron was found to increase total free amino acid content and decrease ALS activity and BCAA content in wheat and maize, but the concentration of chlorsulfuron required to cause these effects was greater for wheat (Royuela *et al.*, 1991). Chlorsulfuron appears not to inhibit protein synthesis in maize and pea (Ray, 1982a; Clayton and Reynolds, 1991).

Differences in chlorsulfuron sensitivity between wheat cultivars appears to be related to rate of metabolism of the herbicide (Chapter 3). In Chapters 4 and 5, it was shown that chlorsulfuron limits the capacity of the sensitive wheat cultivar Rongotea to respond to nitrate. Decreased growth with chlorsulfuron was associated with increases in nitrate and reduced N content of root and shoot. Addition of BCAA partially overcame the chlorsulfuron restriction in growth indicating that an inability to synthesize BCAA is a factor causing the herbicide effect. However, reduced N also accumulated in sprayed plants supplied BCAA and the amino acids glutamine and glutamate were as effective as BCAA in overcoming the chlorsulfuron effect. It was concluded that (a) decreased growth with chlorsulfuron was due to an inability to utilize increased reduced N to produce dry matter, and (b) increased tolerance of wheat to chlorsulfuron with BCAA was not a specific BCAA effect. It was proposed that nitrate accumulation may be the main factor

causing decreased growth of nitrate-fed plants in comparison with those supplied BCAA. The objectives of the experiments described in this chapter were : 1) to determine chlorsulfuron effects on leaf extension, ALS activity and levels of BCAA in Rongotea wheat, 2) to test if BCAA supplied to roots were transported to the shoot, 3) to determine if nitrate accumulation in plant tissues could be a cause of growth reduction, and 4) if nitrate accumulation in sprayed plants was due to decreased nitrate reductase activity (NRA).

6.2 Materials and Methods

6.2.1 Plant culture and growing conditions

Uniform seven-day-old seedlings of wheat cv. Rongotea were planted individually in 800-ml volume plastic pots filled with vermiculite/perlite mixture (1:1, v:v). Plants were maintained in a controlled environment cabinet as described in Section 2.2.4. All pots were flushed every two days with a basal nutrient solution (Appendix 2D) containing the appropriate nitrogen treatment. Potassium concentration was made equal in all treatments by the addition of potassium sulphate. When plants reached the three-leaf stage (ZGS 13), chlorsulfuron was applied as described in Section 2.2.1.

6.2.2 Effect of chlorsulfuron on leaf extension, ALS activity and branched chain amino acid content in wheat

All plants were started on a basal nutrient solution (Appendix 2D) containing 1 mol m^{-3} potassium nitrate and transferred to their appropriate nitrogen treatment one week prior to chlorsulfuron application. The nitrogen treatments were 5 mol m^{-3} nitrate and 1 mol m^{-3} nitrate plus 4 mol m^{-3} BCAA (1.33 mol m^{-3} of each of valine, leucine and isoleucine). Plants were harvested 7 and 28 days after chlorsulfuron application (DAS), and shoot fresh weight and dry weight determined. Leaf extension was measured every two days starting from one day prior to spraying. Leaf length was taken from the leaf tip to the ligule of the leaf two positions below (Dastgheib *et al.*, 1990).

Acetolactate synthase activity of shoots was assayed at one and seven days after chlorsulfuron application following the method described by Ray (1984) with some modifications. Young shoots (0.4 g) were homogenized on ice in 2 ml of extraction buffer, pH 7.5 (Appendix 6A), with 0.2 g polyvinylpyrrolidone (PVP). The homogenate was strained through two layers of muslin and centrifuged at 15,000 g at 4 °C for 15 minutes in a Sorval RC-5 refrigerated centrifuge. Two ml of the supernatant was desalted on a Sephadex G25M column (PD10, Pharmacia) equilibrated

with cold desalting buffer, pH 7.5 (Appendix 6A). The resuspended enzyme extract was kept on ice until assay.

Assay was carried out using 200 µl enzyme extract to which was added sequentially 50 µl solutions of assay buffer (50 mol m^{-3} potassium dihydrogen orthophosphate, pH 7.0) containing either 500 mol m^{-3} pyruvate or 0.1 M magnesium sulphate, 2.5 mol m^{-3} thiamine pyrophosphate (TPP) and 0.2 mol m^{-3} flavin adenine dinucleotide (FAD). The final mixture was made to 500 µl with the addition of the basic assay buffer and incubated at 30 °C for 1 h after which the reaction was stopped by the addition of 50 µl of 6 N sulphuric acid. The acidified reaction mixture was incubated at 60 °C for 15 minutes. The acetoin produced was determined as described by Westerfeld (1945). Following the sequential addition of 100 µl of 5 N sodium hydroxide, 150 µl of 0.5% creatine and 150 µl of 5% α-naphthol, freshly prepared in 2.5 N sodium hydroxide, the reaction mixture was incubated at 60 °C for 1 h for colour development. Samples were centrifuged at 2500 g in a Heraeus Christ centrifuge (model Biofuge B) and the absorbances determined at 530 nm. Acetoin content of the samples was determined by comparing with a standard curve which was linear in the concentration range used (Appendix 6B). Background colour was determined from blank samples which received sulphuric acid before the enzyme extract.

Protein content was determined by the method of Bradford (1976). A subsample (20 µl) of the enzyme extract was made to 0.5 ml with desalting buffer and 5 ml of protein reagent (Appendix 6C) added and mixed. Absorbance was measured at 595 nm and compared with a standard curve (Appendix 6B) using bovine serum albumin (BSA) solutions.

The content of 12 amino acids namely valine, leucine, isoleucine, aspartic acid, glutamic acid, glycine, alanine, lysine, arginine, phenylalanine, tyrosine and methionine were determined in shoot and xylem sap samples at one, four and seven DAS. Xylem sap was collected from detopped shoots over a 4-6 h period. Shoot and sap samples were kept frozen at -18 °C until assay time. Shoots were finely ground in liquid nitrogen and a sample of approximately 5 mg was put into a Kimble tube and 400 µl citrate buffer, pH 3.0, added and extracted with 3-4 bursts

of sonication over 1 h. The extract was centrifuged at 13,000 rpm, supernatant removed and the pellet extracted with a further 150 µl buffer and centrifuged. Supernatants were combined and cleaned via Sep Pak columns and made to 10 ml with deionized water. Xylem sap samples were thawed and volumes measured and extracted as above. A sample of shoot or sap extracts (60 µl) was used for amino acid assay by HPLC. Standard solutions of all amino acids mentioned above were used for determination of retention time, and the content of BCAA and their proportions relative to the total content of the above 12 amino acids were calculated.

A repeat experiment was carried out in which plants were started on nitrogen treatments two days prior to chlorsulfuron application.

6.2.3 Effect of chlorsulfuron on nitrate accumulation in wheat grown at a range of applied nitrate concentrations

There were seven nitrate concentrations: 0.5, 1.0, 2.5, 5.0, 7.5, 10.0 and 15.0 mol m^{-3} nitrate. Plants were sprayed with 0 or 15 g a.i. ha^{-1} chlorsulfuron at the three-leaf stage. Plants were harvested 42 DAS. Nitrate reductase activity (NRA) was determined on fresh subsamples of shoots of sprayed and unsprayed plants supplied 1 or 5 mol m^{-3} nitrate. An *in vivo* NRA assay was used as described by Andrews *et al.* (1984). Shoot samples (0.4 g) were vacuum infiltered at 8 kPa for 10 minutes with 100 mol m^{-3} sodium phosphate buffer (pH 7.6) containing 3% propan-1-ol and 50 mol m^{-3} potassium nitrate. After removal of a time zero sample (0.5 ml), the mixtures were incubated at 30 °C for 20 minutes in a shaking water bath in the dark and then a further 0.5 ml sample was taken. Both samples were analysed for nitrite by colorimetry as described in Section 5.2.1., and NRA activity was calculated as the difference between the two. Shoot dry weight and reduced N, nitrate N and nitrite N contents were then determined for all plants as described in Section 5.2.2.

6.2.4 Effect of chlorsulfuron on response of wheat to additional nitrate at different times after spraying

Plants supplied 1 mol m^{-3} nitrate were transferred to 5 mol m^{-3} nitrate at 1, 2 or 3 weeks after chlorsulfuron application. Shoot dry weight and nitrate content of leaves were determined 50 DAS as described in Section 5.2.2.

6.2.5 Experimental design and data analysis

Experiments described in this chapter had five replicates and were arranged in randomized complete block designs. Amino acid contents were determined on three replicates made by pairing plants. All data were subject to analysis of variance using SAS statistical package (SAS Institute Inc., Cary, N.C.) and standard errors of mean values presented to indicate variability of data. All effects discussed have a probability of $p < 0.05$.

6.3 Results

6.3.1 Effect of chlorsulfuron on leaf extension, ALS activity and branched chain amino acid content in wheat

During the first seven days after chlorsulfuron application, leaf extension rates for leaf 4 of control plants were similar with nitrate or BCAA as nitrogen source (Figure 6.1). Chlorsulfuron caused a rapid decrease in leaf extension rate regardless of nitrogen treatment. Within one day of spraying, chlorsulfuron caused a 27-30% reduction in the extension rate of leaf 4, regardless of nitrogen treatment. For sprayed plants, leaf extension rate was as great with nitrate as with BCAA.

For control plants, shoot fresh weight seven DAS and shoot dry weight 28 DAS were similar with nitrate or BCAA (Table 6.1). Regardless of nitrogen treatment, chlorsulfuron caused a substantial decrease in shoot fresh weight seven DAS and in shoot dry weight 28 DAS. For sprayed plants, shoot fresh weight seven DAS was similar with nitrate or BCAA but shoot dry weight 28 DAS was greater with BCAA.

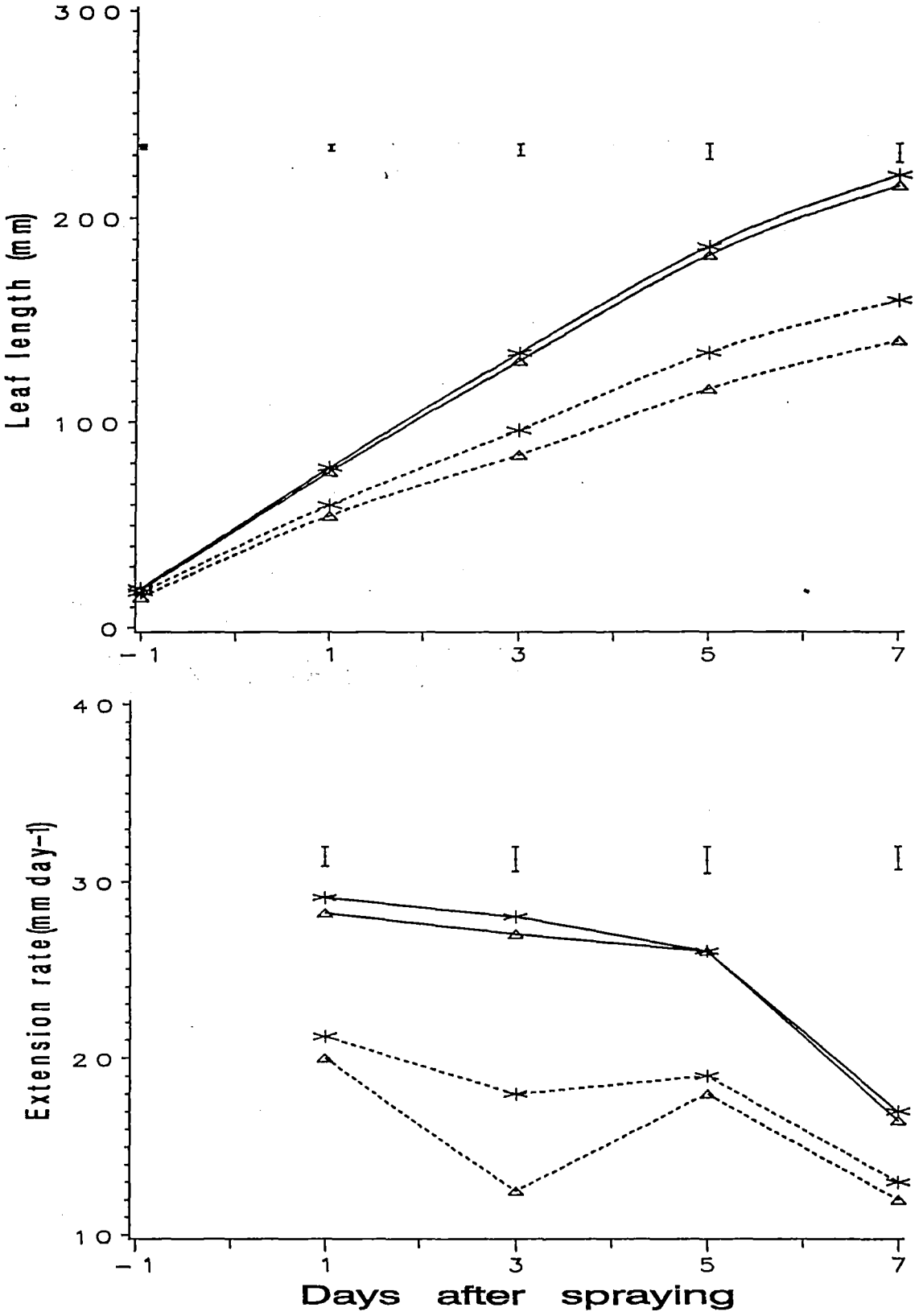


Figure 6.1 Leaf length (A) and extension rate (B) of leaf 4 of wheat supplied 5 mol m^{-3} nitrate (✳) or 1 mol m^{-3} nitrate plus 4 mol m^{-3} branched chain amino acids (△), in control plants (solid lines) and plants sprayed with 15 g a.i. ha^{-1} chlorsulfuron (broken lines). Error bars are SEm.

Table 6.1 Effect of chlorsulfuron on shoot fresh weight and shoot dry weight of wheat supplied high nitrate or low nitrate plus branched chain amino acids (BCAA).

Nitrate	BCAA	Fresh weight (g)[a]		Dry weight (g)[a]	
(mol m^{-3})		Control	Sprayed	Control	Sprayed
5	0	**1.27** (0.11)[b]	**0.69** (0.04)	**1.44** (0.25)	**0.44** (0.06)
1	4	**1.04** (0.07)	**0.70** (0.08)	**1.35** (0.09)	**0.65** (0.06)

a) Fresh and dry weight were taken 7 and 28 days after chlorsulfuron application, respectively.

b) Values in brackets are SEm.

For control plants, one and seven days after chlorsulfuron application, ALS activity was similar for plants supplied nitrate or BCAA (Table 6.2). Regardless of nitrogen supply, ALS activity was approximately 80-90% lower in sprayed plants than in unsprayed plants one DAS. Seven days after spraying, ALS activity was approximately 55-75% lower in sprayed than in unsprayed plants. In a separate experiment, ALS activity in plants grown at 5 mol m^{-3} nitrate was found to decrease substantially following chlorsulfuron application but was similar to control plants three weeks after spraying (Table 6.3). Protein content was unaffected by nitrogen form supplied or chlorsulfuron (Table 6.2).

Table 6.2 Effect of chlorsulfuron (15 g a.i. ha^{-1}) on specific activity of ALS (nmol mg^{-1} protein h^{-1}) and on protein content (mg g^{-1} fresh weight) of shoots of wheat supplied high nitrate or low nitrate plus branched chain amino acids (BCAA), extracted one or seven days after spraying.

Nitrate	BCAA	Specific activity				Protein content			
		Day one		Day seven		Day one		Day seven	
(mol m^{-3})		Control	Sprayed	Control	Sprayed	Control	Sprayed	Control	Sprayed
5	0	190.9	33.7	318.3	83.5	11.00	9.75	8.75	10.75
1	4	155.2	18.9	305.0	132.9	9.50	8.75	8.50	9.00
SEm		19.98	3.63	19.66	9.93	0.475	0.950	0.875	1.450

Table 6.3 Effect of chlorsulfuron (15 g a.i. ha^{-1}) on specific activity of ALS (nmol mg^{-1} protein h^{-1}) of shoots of wheat supplied high nitrate during the first three weeks after spraying.

chlorsulfuron (g a.i. ha^{-1})	Days after spraying				
	1	4	7	14	21
0	258.5	268.7	375.6	500.2	395.0
15	54.2	128.1	144.3	301.5	370.5
SEm	22.51	29.01	35.20	30.50	40.23

For control and sprayed plants, one, four or seven DAS, concentrations of BCAA in shoot tissue were similar for plants supplied nitrate or BCAA (Table 6.4). Regardless of form of nitrogen supplied, shoot valine content was lower in sprayed plants than in unsprayed plants except for plants supplied nitrate seven DAS. Chlorsulfuron did not affect levels of leucine or isoleucine in shoots. In most cases the concentration of total free amino acids determined was higher in sprayed plants than the controls (Table 6.4). Chlorsulfuron caused significant reductions in valine proportion of the total amino acids measured regardless of nitrogen form supplied (Table 6.5).

For control plants, one, four or seven DAS, concentrations of BCAA in xylem sap and the proportion of amino acids as BCAA were similar for plants supplied nitrate or BCAA (Tables 6.6 and 6.7). Chlorsulfuron increased the xylem sap concentration of total free amino acids in BCAA-fed plants one DAS and in both nitrogen treatments seven DAS. In general, chlorsulfuron caused a decrease in xylem sap valine content, but this effect was usually not significant. However, in all cases, the proportion of amino acids as valine was significantly lower in sprayed than in unsprayed plants (Table 6.7). Chlorsulfuron also caused a decrease in the proportion of amino acids as leucine and isoleucine in nitrate-fed plants one DAS and in both nitrogen treatments seven DAS.

Similar effects of chlorsulfuron on fresh and dry weight, leaf extension, ALS activity and BCAA content were obtained in the repeat experiment (Appendix 6D).

Table 6.4 Effect of chlorsulfuron on concentration (nmol mg^{-1} dry weight) of branched chain amino acids and total free amino acids determined (Total AA) in shoots of wheat at different times after application.

Nitrate	BCAA		Day one				Day four				Day seven			
(mol m^{-3})		Rate[a]	Val.	Iso.	Leu.	Total AA	Val.	Iso.	Leu.	Total AA	Val.	Iso.	Leu.	Total AA
5	0	0	2.2	1.1	1.7	43.2	2.6	1.0	1.7	52.8	2.3	0.7	1.1	51.5
		15	1.6*	1.2	1.8	59.3*	1.8*	1.1	1.7	58.0*	2.2	0.8	1.3	50.2
1	4	0	2.5	1.1	1.8	40.1	2.4	1.0	1.5	41.4	2.7	1.1	1.7	39.5
		15	1.8*	1.3	1.8	52.0*	1.6*	0.9	1.3	47.5*	1.7*	0.9	1.5	45.6*
SEm			0.22	0.08	0.14	2.82	0.18	0.10	0.17	1.92	0.24	0.06	0.07	1.69

a) Chlorsulfuron rate (g a.i. ha^{-1}).

* An asterisk indicates significant reduction from the control at $p < 0.05$.

Table 6.5 Effect of chlorsulfuron on percentage of the total amino acids as branched chain amino acids in shoots of wheat at different times after application.

Nitrate (mol m^{-3})	BCAA	Rate[a]	Day one			Day four			Day seven		
			Val.	Iso.	Leu.	Val.	Iso.	Leu.	Val.	Iso.	Leu.
5	0	0	5.1	2.4	4.0	4.9	1.9	3.1	4.4	1.4	2.2
		15	2.7*	2.0	3.1	3.1*	1.8	2.8	4.5	1.6	2.6
1	4	0	6.3	2.8	4.7	5.7	2.3	3.6	6.9	2.8	4.3
		15	3.5*	2.5	3.5	3.4*	1.9	2.8	3.8*	2.1	3.4
SEm			0.53	0.26	0.47	0.47	0.12	0.16	0.43	0.11	0.12

* An asterisk indicates significant difference from the control at $p < 0.05$.

Table 6.6 Effect of chlorsulfuron on concentration (mmol m^{-3}) of branched chain amino acids and total free amino acids determined (Total AA) in xylem sap of wheat at different times after application.

Nitrate (mol m^{-3})	BCAA	Rate[a]	Day one				Day four				Day seven			
			Val.	Iso.	Leu.	Total AA	Val.	Iso.	Leu.	Total AA	Val.	Iso.	Leu.	Total AA
5	0	0	371.2	58.8	64.9	1605.0	469.2	100.1	116.7	2063.9	657.2	180.8	207.1	2153.0
		15	140.5*	38.8	32.3	1992.0	305.8	96.0	111.6	2239.7	637.9	228.1	291.5	5594.5*
1	4	0	384.7	101.8	93.0	1808.5	568.7	159.7	208.9	2333.6	733.2	197.2	244.1	2049.0
		15	342.2*	124.2	183.8	2370.4	272.0	137.9	229.0	2280.7	336.8	81.0	123.1	3472.5*
SEm			5.50	15.05	16.08	74.12	64.58	54.86	64.91	151.86	188.05	38.85	55.12	280.25

* An asterisk indicates significant reduction from the control at $p < 0.05$.

Table 6.7 Effect of chlorsulfuron on percentages of the total amino acids determined as branched chain amino acids in xylem sap of wheat at different times after application.

Nitrate	BCAA		Day one			Day four			Day seven		
(mol m^{-3})		Rate[a]	Val.	Iso.	Leu.	Val.	Iso.	Leu.	Val.	Iso.	Leu.
5	0	0	23.3	3.7	4.1	22.5	4.6	5.3	30.5	8.5	9.8
		15	7.1*	1.8*	1.6*	12.6*	3.9	4.5	13.3*	4.4*	5.8*
1	4	0	21.5	4.1	5.0	24.4	6.8	8.9	35.8	9.6	11.9
		15	14.5*	6.7	7.7	11.1*	6.1	10.3	9.7*	3.0*	4.4*
SEm			0.460	0.353	0.330	2.46	1.71	2.00	1.37	0.27	0.22

* An asterisk indicates significant reduction from the control at $p < 0.05$.

6.3.2 Effect of chlorsulfuron on nitrate accumulation in wheat grown at a range of external nitrate concentrations

Shoot dry weight of control plants increased with increased applied nitrate concentration from 0.5 to 5 mol m^{-3}, then decreased with increased nitrate supply thereafter (Figure 6.2A). For sprayed plants, shoot dry weight increased with nitrate supply from 0.5 to 2.5 mol m^{-3}, then decreased with additional nitrate thereafter. The greatest reduction in shoot dry weight of sprayed plants compared to the controls was observed at 5 mol m^{-3} nitrate, which is similar to the results presented in Section 4.3.2.

Reduced N content of shoots of control and sprayed plants increased sharply with increased applied nitrate from 1 to 7.5 mol m^{-3} then increased slightly with additional nitrate thereafter (Figure 6.2B). At 5 mol m^{-3} nitrate reduced N content was approximately twice as great in sprayed plants as in control plants. Nitrate N content of control plants remained low (<0.13 mg g^{-1} dry weight) at applied nitrate concentrations from 0.5 to 5 mol m^{-3} then increased sharply with additional nitrate from 5.0 to 7.5 mol m^{-3} (Figure 6.2C). For sprayed plants, nitrate N content increased sharply with increased applied nitrate from 2.5 to 7.5 mol m^{-3}. At 5 mol m^{-3} applied nitrate, nitrate N content was 25 times greater for sprayed plants than for controls. For all applied nitrate concentrations, nitrite N was undetectable in plants (<0.02 μg g^{-1} dry weight).

Chlorsulfuron did not affect NRA of shoots at 1 or 5 mol m^{-3} applied nitrate (Table 6.8). Similarly, in a repeat experiment, NRA of shoots of plants grown at 5 mol m^{-3} applied nitrate was unaffected by chlorsulfuron. Values for NRA in the repeat experiment were 904.2 and 894.5 nmol g^{-1} fresh weight h^{-1} (SEm=113.8), for control and sprayed plants, respectively.

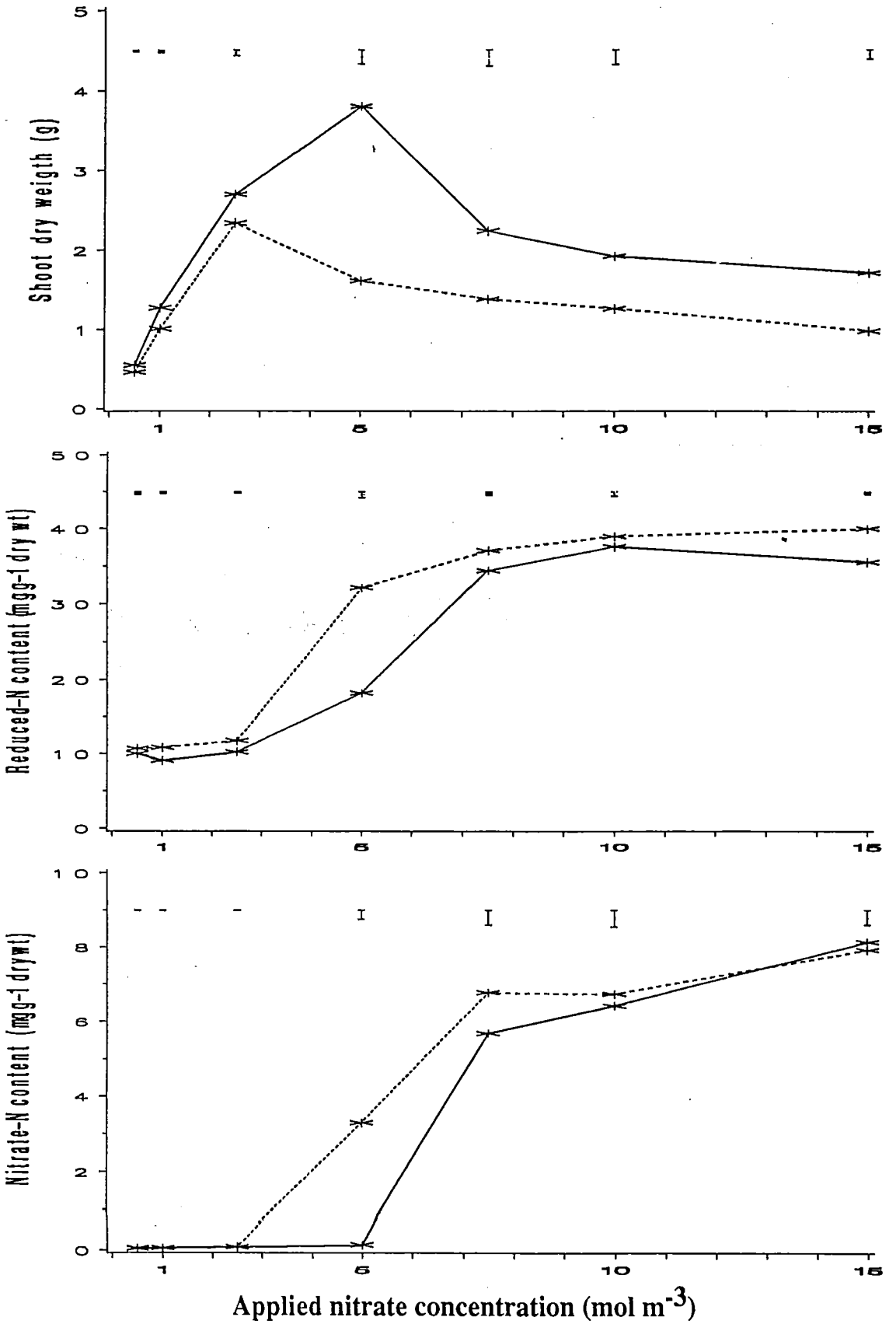


Figure 6.2 Shoot dry weight (A), reduced N content (B) and nitrate N content (C) of wheat grown at different nitrate concentrations and sprayed with chlorsulfuron at 0 (solid lines) or 15 g a.i. ha^{-1} (broken lines). Error bars are SEm.

Table 6.8 Effect of chlorsulfuron on nitrate reductase activity (nmol nitrite g^{-1} fresh weight h^{-1}) of shoots of wheat grown at 1 and 5 mol m^{-3} nitrate.

Nitrate (mol m^{-3})	Control	Sprayed
1	223.4	296.7
5	994.9	971.7
SEm	82.47	

6.3.3 Effect of chlorsulfuron on response of wheat to additional nitrate at different times after spraying

Shoot dry weight of control plants was not affected by the time they were transferred to the high nitrate regime (Table 6.9). In contrast, for sprayed plants, shoot dry weight increased with increased time to transfer to high nitrate. Chlorsulfuron caused significant reductions in shoot dry weight of plants transferred to high nitrate at one or two weeks after application. For plants transferred to high nitrate three weeks after application, no significant reduction in shoot dry weight was obs[illegible] with chlorsulfuron.

Values for nitrate N content of unsprayed plants were low (0.11 to 0.38 mg g^{-1} dry weight) and similar regardless of time to transfer to high nitrate regime (Table 6.9). Chlorsulfuron caused a substantial increase in nitrate N content of plants transferred to high nitrate one or two weeks after spraying, but did not affect nitrate N content of plants transferred to high nitrate three weeks after spraying. A repeat experiment showed similar results (Appendix 6E).

Table 6.9 Effect of chlorsulfuron on shoot dry weight (g) and nitrate N content of wheat transferred from 1 to 5 mol m^{-3} nitrate regime at different times after spraying. Measurements were taken 50 DAS

Time of transfer	Shoot dry weight		Nitrate N content	
	Control	Sprayed	Control	Sprayed
One week	**2.49** (0.112)[a]	**0.77** (0.120)	**0.38** (0.049)	**1.87** (0.340)
Two weeks	**2.50** (0.082)	**1.07** (0.098)	**0.11** (0.021)	**1.78** (0.378)
Three weeks	**2.15** (0.061)	**1.95** (0.060)	**0.17** (0.071)	**0.20** (0.097)

a) Values in brackets are SEm.

6.4 Discussion

It was shown previously that in the long term (28-45 days), growth of chlorsulfuron-sprayed plants was greater with BCAA than with nitrate as a nitrogen source (Chapter 5). It was also shown that the amino acids glutamine and glutamate were as effective as BCAA in overcoming the chlorsulfuron-induced restriction in growth and it was concluded that increased tolerance of wheat to chlorsulfuron with BCAA was not a specific BCAA effect. Regardless of nitrogen form supplied, decreased growth with chlorsulfuron was associated with increased reduced N content (Tables 5.3 and 5.4). Nitrate accumulated to very high levels in sprayed plants grown at high nitrate, and it was proposed that this may be the main factor causing decreased growth of nitrate fed plants in comparison to those supplied BCAA (Section 5.3.1). The experiments described in this chapter were designed to gain greater understanding of the mode of action of chlorsulfuron in Rongotea wheat.

The general tolerance to chlorsulfuron by temperate cereals appears to be related to its rapid metabolism to non-toxic products and not to insensitivity of their ALS to the herbicide (Sweetser *et al.*, 1982; Ray, 1989; Royuela *et al.*, 1991). Similarly, it was shown in Chapter 3 that differences in chlorsulfuron tolerance between wheat cultivars were related to their rate of metabolism of the herbicide (Table 3.5). For a range of species including wheat, the content of BCAA and/or the proportion of amino acids as BCAA have been found to decrease with chlorsulfuron treatment (Scheel and Casida, 1985; Rhodes *et al.*, 1987; Royuela *et al.*, 1991). Royuela *et al.* (1991) could not find differences in extractable ALS activity between control and chlorsulfuron treated wheat and maize plants and suggested that this was possibly due to weak enzyme-herbicide binding. Other reports, however, have demonstrated reductions in ALS activity following the application of chlorsulfuron or other ALS inhibiting herbicides (Matthews *et al.*, 1990; Stidham and Shaner, 1990) and it has also been reported that these herbicides might permanently inactivate ALS (Durner, Gailus and Böger, 1991). In the present study on Rongotea,

ALS activity and valine content in shoots were found to decrease significantly within one day of spraying with chlorsulfuron regardless of nitrogen form supplied (Tables 6.2 and 6.4). Also, leaf extension rate, which was used as a rapid indicator of chlorsulfuron effect on growth (Bowran and Blacklow, 1987), decreased substantially within one day of spraying (Figure 6.1). This is strong evidence that the initial effect of chlorsulfuron on growth of Rongotea is due to decreased ALS activity. Addition of BCAA to the rooting medium did not counter the decrease in valine content as they did not reach the shoot in high enough quantities or for long enough period (Tables 6.4 and 6.6, Appendices 6D_IV and 6D_VI).

During the first seven DAS, leaf extension rate was substantially lower for sprayed plants than for unsprayed plants regardless of the form of nitrogen supplied (Figure 6.1). Also, for sprayed plants, leaf extension rate was as great with nitrate as with BCAA showing that supplementing BCAA in the short term does not protect against chlorsulfuron damage in wheat. Seven DAS shoot fresh weight for sprayed plants was as great with nitrate or BCAA treatment, but 28 DAS shoot dry weight was greater with BCAA as was found previously (Tables 5.3 and 6.1). Experiment 6.3.2 was carried out to determine if chlorsulfuron caused nitrate accumulation in Rongotea to levels which could inhibit growth. For control plants, reductions in shoot dry weight started at 7.5 mol m^{-3} applied nitrate (Figure 6.2). This was accompanied by a marked increase in nitrate content of the shoots. For sprayed plants there was a significant reduction in shoot dry weight at 5 mol m^{-3} applied nitrate accompanied by an increase in nitrate content. Reduced N also increased with chlorsulfuron, however, data presented in Chapter 5 showed that this was unlikely to be the reason for decreased growth (Section 5.4). Therefore, the data are consistent with the proposal that nitrate accumulation is the main cause of decreased growth of sprayed plants grown at high nitrate in comparison with BCAA as a nitrogen source.

One possible reason for accumulation of nitrate could have been a decrease in nitrate reductase activity (NRA). Pronina and Ladonin (1985) reported that linuron and bentazon caused a

decrease in NRA which resulted in nitrate accumulation in susceptible plants. This possibility was tested for chlorsulfuron but no inhibition of NRA was observed in Rongotea (Table 6.8). In Experiment 6.3.1, the activity of ALS was found to return to normal three weeks after spraying (Table 6.3). The finding that at this time sprayed plants supplied low nitrate showed normal growth response to additional nitrate with no nitrate accumulation is strong evidence that for nitrate-fed plants, nitrate accumulation is the main cause of decreased growth and suggests that this nitrate accumulation is related to decreased ALS activity.

In summary, the data presented in this chapter demonstrated that chlorsulfuron caused a rapid inhibition in leaf extension and in the activity of the target enzyme, ALS, in Rongotea wheat (Figure 6.1, Table 6.2). This was accompanied by an increased level of the total free amino acid pool (Table 6.4), which is consistent with increased content of reduced N reported in Chapter 5 (Table 5.3). Significant reductions in valine content and its proportion of the total free amino acid pool were observed in sprayed plants (Tables 6.4 and 6.5). The data reported here showed that the mode of action of chlorsulfuron in a sensitive wheat cultivar like Rongotea is likely to be similar to susceptible species. Moreover, the data showed that chlorsulfuron application can result in nitrate accumulation in plants grown at high nitrate. This is the main cause of the damage to these plants in medium to long term (one month or more after spraying). Plants grown on low nitrate or amino acids do not accumulate nitrate to toxic levels and their growth is reduced less severely in the short term. These plants can respond to additional nitrate with no apparent growth inhibition when the effect of chlorsulfuron is over (Tables 6.3 and 6.9).

PART FOUR

GENERAL DISCUSSION

APPENDICES

REFERENCES

PUBLISHED AND SUBMITTED PAPERS

CHAPTER 7

General Discussion

The sulfonylurea herbicide, chlorsulfuron, which is used for weed control in cereal crops is known to have wide selectivity margins between sensitive and tolerant plants. However, damage has been observed in certain wheat and barley cultivars following chlorsulfuron application (Anderson, 1986; Wicks *et al.*, 1987; Bowran, 1990a), and environmental factors have been suggested as having a major effect on the tolerance of crops to the herbicide (Lemerle *et al.*, 1986 and 1987; Ferreira *et al.*, 1990). These reports indicate that chlorsulfuron must be adequately tested against recommended crop cultivars under a wide range of environmental conditions to assess the potential risk of crop damage. In the present work, selectivity of chlorsulfuron in wheat was evaluated in relation to genetic variation and nitrogen availability. Different sources of nitrogen supplementation were used to investigate the mechanism of action of chlorsulfuron in a sensitive wheat cultivar.

Cultivar differences were observed in their tolerance to chlorsulfuron under glasshouse and field conditions (Chapter 2). The effect of chlorsulfuron was more pronounced and more persistent in pot experiments compared to the field experiment. This difference can be partly explained by the greater contribution of herbicide spray deposits reaching the soil surface in the pots. It was shown that following a foliar application, chlorsulfuron spray deposits leached into the soil and persisted for several months and were thus available to plant roots (Table 2.4). It is likely that the roots of pot grown plants had a greater access to spray deposits as a result of being confined in a smaller soil volume than plants grown in the field. Moreover, plants grown under more favourable conditions in the field, specifically in terms of soil volume and irradiance, had a greater potential to compensate for the damage they received at an earlier stage, probably through an increase in the number of tillers (Appendix 2F_IV).

Differences in the response to chlorsulfuron between Kotare (tolerant cultivar) and Lancer or Rongotea (sensitive cultivars) were correlated to differential rates of metabolism of the herbicide

(Table 3.5). It is appropriate to compare the bases of tolerance to chlorsulfuron or other sulfonylurea herbicides in wheat cultivars reported here and in weed biotypes and inbred lines of susceptible crops which have developed resistance to these herbicides. Development of resistance in susceptible species has been reported to be due to reduced sensitivity of the target site rather than an increase in herbicide metabolism (see Section 1.3.3). No differences were observed in the rates of ^{14}C-chlorsulfuron uptake, translocation and metabolism or in the ALS specific activity between susceptible and resistant biotypes of several weed species to the herbicide (Saari, Cotterman and Primiani, 1990; Saari *et al.*, 1992). In the comparison of wheat cultivars undertaken in this study, the activity of ALS enzyme extracted from shoots of Kotare, Lancer and Rongotea was similar (Appendix 7A). Moreover, chlorsulfuron application resulted in similar inhibition of ALS activity in these cultivars 24 h after application (Appendix 7A). This is similar to the report on chlorsulfuron susceptible and resistant biotypes of annual ryegrass (Matthews *et al.*, 1990). Thus, it has been concluded that the large difference between the tolerant and the sensitive cultivars in the rate of metabolism (Table 3.5) is the main factor determining the differential sensitivity of the wheat cultivars studied.

Efforts were directed towards an understanding of physiological mechanisms involved in the differences in response of wheat to chlorsulfuron under different nitrate regimes. No differences were found in retention, uptake, translocation or metabolism of chlorsulfuron in wheat grown at either 1 or 5 mol m^{-3} nitrate (Chapter 4). It was shown that plants grown at low nitrate and transferred to high nitrate at spraying were affected by chlorsulfuron to the same extent as plants grown at high nitrate throughout (Table 5.2). It was concluded that chlorsulfuron limited the capacity of wheat to respond to increased applied nitrate. Limitation in response of sensitive wheat cultivars to additional nitrogen has been reported for leaf extension (Bowran and Blacklow, 1987). This is the first report which documents this limitation for dry matter production and investigates the physiological reasons for it.

The limitation to respond to nitrogen imposed by chlorsulfuron could have been through an interference with uptake and/or assimilation of nitrogen. Detailed examination of these processes in wheat showed no direct effect on nitrogen uptake or assimilation (Section 5.3.1). Reduced N

was accumulated in sprayed plants regardless of nitrogen treatment, indicating an inability to utilize assimilated nitrogen for growth. It was concluded that inability to use reduced N was the main reason for decreased growth with chlorsulfuron. It is unlikely that accumulation of reduced N by itself is a cause of reduced growth as the levels measured in sprayed plants were not toxic (Table 5.3, Section 5.4). Increased content of reduced N was consistent with the finding that total free amino acid content increased in sprayed plants (Table 6.4) and indicated that the effect was related to the mode of action of chlorsulfuron.

It was shown that chlorsulfuron application to Rongotea rapidly inhibited ALS activity and resulted in a decrease in valine content (Section 6.3.1). At the same time leaf extension was reduced in sprayed plants. This is strong evidence that the effect of chlorsulfuron in restricting growth of a sensitive wheat cultivar like Rongotea is similar to susceptible species (Ray, 1989), and results from low metabolism of the herbicide (Table 3.5). Plants grown on high nitrate following spraying accumulated nitrate in their tissues to toxic levels which resulted in further damage (Figure 6.2). When high nitrate was replaced with other nitrogen sources such as BCAA or GLN/GLU (Tables 5.2 and 5.5), or plants were supplied low nitrate during a critical period of approximately three weeks following chlorsulfuron application, this damage could be avoided and plants could respond to additional nitrate with no nitrate accumulation (Table 6.9). The mechanism for decreased growth at high nitrate is not known. Possible reasons include an ion imbalance, competition between the nitrate assimilation pathway and dry matter production for energy derived from photosynthesis and iron deficiency related to hydroxyl ion/ organic acid production during the nitrate assimilation process (Andrews *et al.*, 1989).

Previous research has shown that supplementation of BCAA can overcome the effects of sulfonylurea or other ALS inhibiting herbicides (Section 5.1). Most of the reports where recovery or protection from the herbicides has been obtained, have used the technique in cell culture, tissue culture or with excised root tips (Ray, 1984; Rost and Reynolds, 1985; Scheel and Casida, 1985). In comparison, studies with whole plants have not always been successful in overcoming the herbicidal effects (Rubin and Casida, 1985; Giardina *et al.*, 1987). None of the reports have measured the concentration of BCAA in plant tissues. In experiments described in Chapters 5

and 6, growth of control plants given 1 mol m^{-3} nitrate plus 4 mol m^{-3} BCAA (5 mol m^{-3} total nitrogen) was always at least as good as that for plants given 5 mol m^{-3} nitrate; with both treatments producing greater growth than plants grown at 1 mol m^{-3} nitrate (Tables 4.2, 5.1, 5.2 and 6.1). This indicates that BCAA were taken up and utilized by plants. However, data in Tables 6.4 and 6.6 showed no differences in concentration of valine, leucine or isoleucine in plants supplied nitrate alone or nitrate plus BCAA as nitrogen sources. There was some indication that BCAA supplied to the roots were transported to the shoots for a short period following the commencement of feeding, but their concentrations dropped to the levels of plants given nitrate alone shortly afterwards. This evidence is found by comparing values measured at one DAS (three days after commencement of feeding) with those measured four or seven DAS (Appendices 6D_IV & 6D_VI). Plants usually demonstrate interconversion of amino acids through transamination activity (Givan, 1980), and it seems likely that the levels of amino acids were adjusted through this process. If BCAA were transported to the shoots, then why did they not prevent the effect of chlorsulfuron on leaf extension? One possible explanation is that BCAA may have been converted to other chemical forms in mature tissues and did not reach the meristematic tissues, where the demand for amino acids is likely to be greatest due to the requirements of cell division. Mature tissue appears to have a greater capacity for such interconversion, as it has a large supply of proteins (enzymes) and energy (Shaner, 1991).

If nitrate accumulation is likely to occur under a set of specific crop growing conditions, a recommendation from this study would be to delay addition of nitrogen fertilizers for a 3-4 week period following chlorsulfuron application. Agronomic studies are consistent with this recommendation as fertilizer application for cereals in New Zealand is recommended to coincide the commencement of tillering to increase yield and around flag leaf emergence to increase grain protein levels (Greenwood, Quin and Sinclair, 1984; Scott, Martin and Stevenson, 1992), and reports from other countries show that splitting nitrogen dressing in winter wheat can promote the efficient use of nitrogen (Spiertz and De Vos, 1983).

The finding that plants grown at low nitrate could respond to additional nitrate three weeks after spraying (Table 6.9) and that ALS activity had returned to normal levels at this time (Table 6.3),

indicate that the accumulation of nitrate observed in this study may be related to chlorsulfuron's effect on the target enzyme. However, this needs to be tested adequately and the physiological and biochemical mechanisms involved need to be defined. Another possibility is that the inhibitory effect of chlorsulfuron on assimilate transport which has been reported for some species (Vanden Born *et al.*, 1988; Devine *et al.*, 1990) may result in accumulation of reduced N and nitrate in mature tissues. This may be a direct effect on phloem loading (Geiger and Bestman, 1990) and/or an indirect effect through inhibition of cell division resulting in decreased sink activity (Shaner, 1991; Stidham, 1991).

The effects of chlorsulfuron on ALS activity, total free amino acid content and valine content of wheat are in agreement with the literature on the mode of action of the herbicide in susceptible species (Ray, 1984; Scheel and Casida, 1985; Royuela *et al.*, 1991). However, it is not understood how these biochemical events can result in growth inhibition and herbicidal effect (Moberg and Cross, 1990; Rost *et al.*, 1990). The possibility of inhibition of specific RNAs or proteins involved in cell division has been proposed as a link between the biochemical and growth responses observed following chlorsulfuron treatment (Shaner and Reider, 1986; Robbins and Rost, 1987). In addition, accumulation of toxic intermediates in the BCAA biosynthesis pathway has been documented in bacteria and plants following the application of sulfonylurea herbicides (LaRossa *et al.*, 1987; Rhodes *et al.*, 1987). The possibility of their involvement in the mode of action of sulfonylurea herbicides has been supported by some workers (Rost *et al.*, 1990; Clayton and Reynolds, 1991). Further research is needed to determine the mechanisms involved.

The limitation in response to nitrogen imposed by chlorsulfuron was observed for several wheat cultivars (Figure 2.1). Greater growth and yield reductions than those found in this study, have been reported following chlorsulfuron application to some wheat and barley cultivars (Lemerle *et al.*, 1987; Bowran, 1990a). Future research should investigate the effect of chlorsulfuron on nitrogen assimilation and accumulation of reduced N and nitrate in sensitive cultivars under a variety of environmental conditions. Of particular importance are the conditions which stress plants and decrease their capacity for herbicide detoxification, such as cold temperature or waterlogging following application. In addition, conditions restricting photosynthetic ability of

plants might enhance nitrate accumulation as nitrogen metabolism is dependent upon a supply of energy and reductants from photosynthesis (Wallsgrove *et al.*, 1983; Turpin and Weger, 1990). Moreover, some environmental conditions may have a direct effect on plant enzymes including ALS. Waterlogging, for instance, reduced ALS activity in wheat (Kueh, Caseley and Bond, 1989), which may render the plant more vulnerable to herbicides that inhibit this enzyme.

The dependence of herbicide efficacy on environmental factors is well known (Caseley, 1987), but the exact conditions for each case need to be defined. Under the conditions used in the present experiments, the effect of chlorsulfuron on wheat was greater at 5 than at 1 mol m^{-3} nitrate (Figures 2.1 and 4.1). Other workers have reported the importance of soil pH, soil temperature, organic matter content, amount of rainfall and waterlogging on the efficacy of chlorsulfuron (see Chapter 1). The enhancement of chlorsulfuron's effect on sensitive cultivars by these factors has implications for plant breeding programmes. The specification of these and other environmental factors can be utilized to provide the best conditions for screening wheat cultivars for their tolerance to chlorsulfuron as well as to other herbicides.

The complexity of climatic and edaphic factors and the interactive nature of their effects calls for development of computer models which simulate the performance of chlorsulfuron and other sulfonylurea herbicides in the field. Expert systems have been developed which integrate large information bases to provide specific weed control recommendations and to predict the possible side effects of herbicides (e.g. Günther *et al.*, 1992). In addition, computer models have been developed for predicting soil residues of sulfonylurea herbicides which may damage following crops (Duffy *et al.*, 1987; Walker and Welch, 1989). It is desirable to have a computer-based information system capable of simulating crop responses to sulfonylurea herbicides under different conditions. However, greater understanding of these herbicides and their interaction with the key external factors is a pre-requisite for developing such systems.

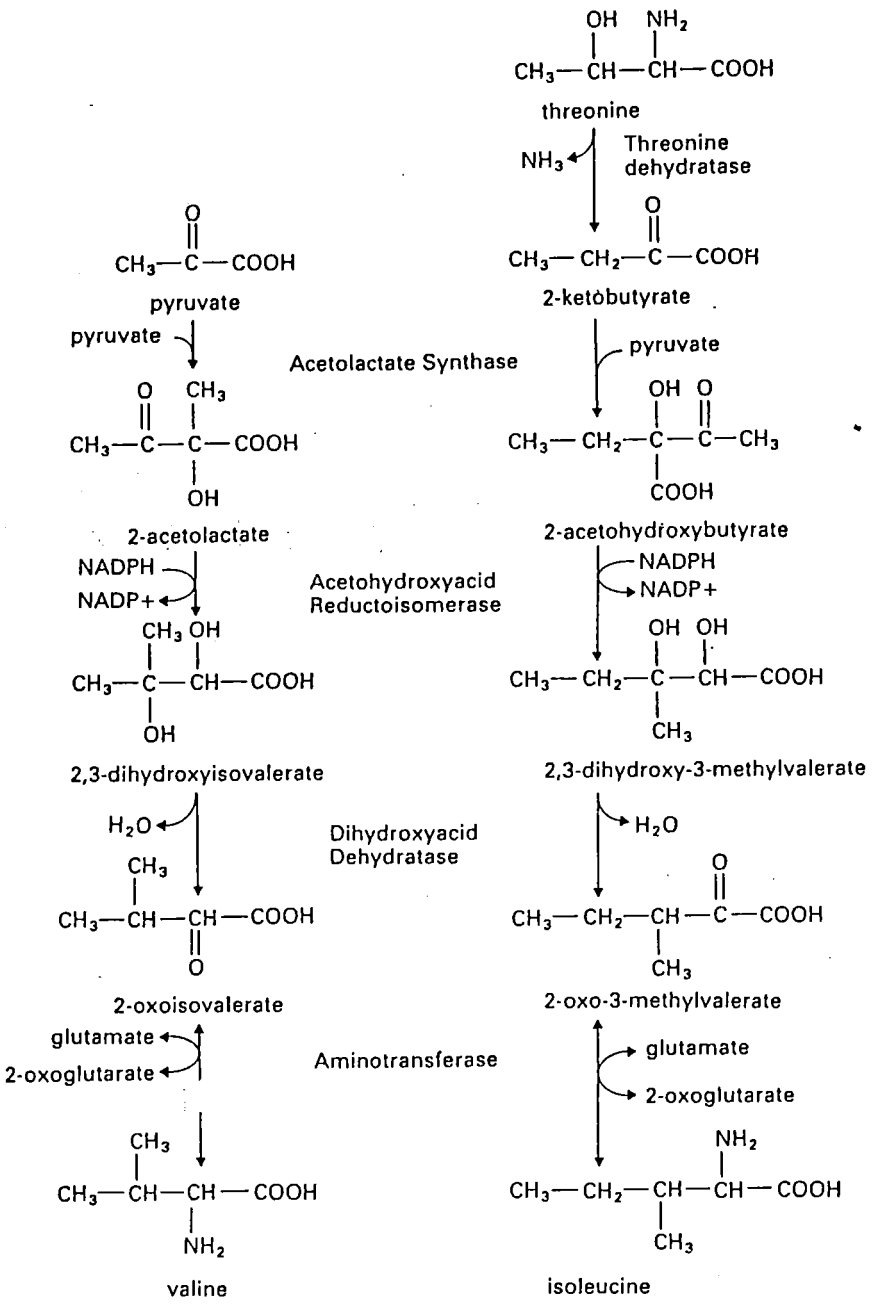
Sensitive wheat cultivars showed a low capacity to metabolise chlorsulfuron regardless of growing substrate or nitrogen level used (Table 3.5, Figure 4.5). This demonstrates the influence of genetic factors on biochemical responses of plants to the herbicide. The enzyme system

responsible for the metabolic inactivation of chlorsulfuron includes an oxygenase and a glucosyltransferase (Erbes, 1984). Research using DNA mapping is required to define the genetic constitution of extreme cultivars encoding the enzymes involved in the metabolism of sulfonylurea herbicides. This information could then be used to enhance the production of enzymes through genetic engineering to promote herbicide metabolism in order to improve the tolerance of sensitive cultivars to sulfonylurea compounds. Another approach to improving the tolerance of desirable cultivars which are sensitive to sulfonylurea herbicides might be through the use of herbicide antidotes. Sweetser (1985) reported that degradation of chlorsulfuron and metsulfuron methyl to inactive metabolites in wheat was improved by naphthalic anhydride and cyometrinil. Further work is required to increase the understanding of the physiological and biochemical mechanisms involved in the herbicide-antidote interaction.

In summary, this study showed that differences in wheat cultivars in their tolerance to chlorsulfuron were related to their ability to metabolise the herbicide. A greater concentration of chlorsulfuron in young tissues of the sensitive cultivar, Rongotea (Figure 3.3), resulted in an inhibition of the activity of the target enzyme, ALS, and reductions in valine content and leaf extension of sprayed plants (Section 6.3.1). These data in conjunction with previous reports (Ray, 1989) lead to the conclusion that inhibition of ALS activity is the primary mode of action of chlorsulfuron in Rongotea. Furthermore, this study showed that chlorsulfuron affected nitrogen utilization, an effect which had not been adequately described previously. Other sulfonylurea herbicides and other ALS inhibitors, such as imidazolinones and triazolopyrimidines, have the potential to influence nitrogen use and metabolism in crop plants. This will open an area of research which needs to be explored for a better understanding of mode of action of these important chemicals, as well as for predicting the response behaviour of crop cultivars to these herbicides.

APPENDIX 1A

Biosynthetic pathways of branched chain amino acids (After Cobb, 1992).



APPENDIX 2A

Properties of soil used in different experiments described in Chapter 2.

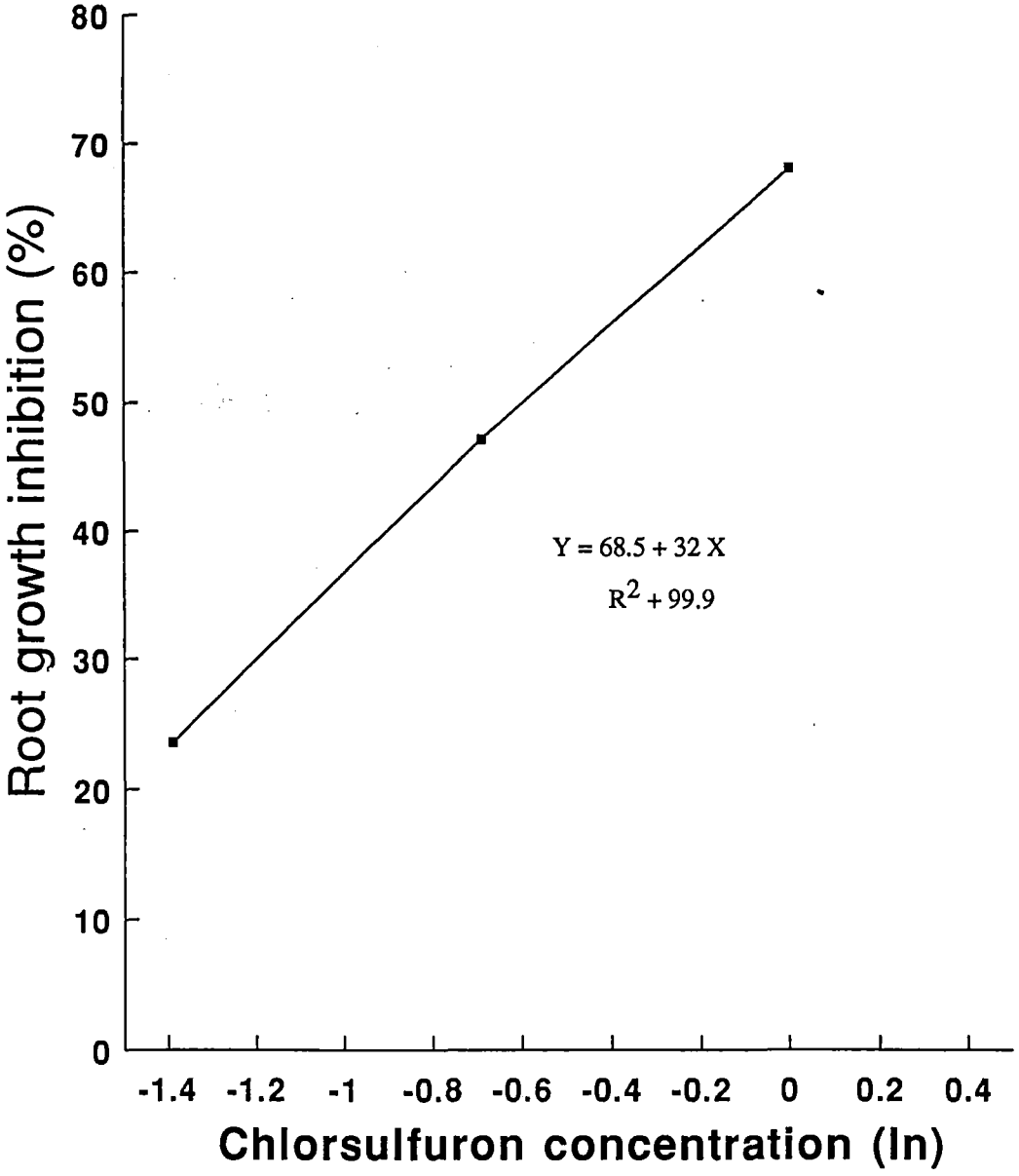
Experiment	Soil texture	pH in water	Organic matter (%)	CEC [a]	Total nitrogen [b]
2.2.1 and 2.2.2	Templeton silt loam	6.1	3.4	14.2	59
2.2.3	Wakanui silt loam	6.2	1.5	7.1	82

a) milliequivalent 100 g^{-1}

b) μg N g^{-1} air dried soil

APPENDIX 2B

Standard curve for determination of chlorsulfuron residues in the soil used in Experiment 2.2.2.1. Each data point is the mean of five replicates.



APPENDIX 2C

Details of seed characteristics used for calculation of sowing rates for wheat cultivars in the field (Experiment 2.2.3).

Cultivar	Germination (%)	100 kernel weight (g)	Sowing rate ($g\ m^{-2}$)	Plant population*
Abele	92	4.41	12.5	296
Jasper	91	4.95	14.1	305
Kotare	88	4.75	14.0	309
Lancer	92	4.22	11.9	294
Rongotea	98	4.59	12.2	305
SEm				7.6

* Number of plants per m^{-2} counted 10 days prior to spraying at ZGS 12.

APPENDIX 2D

The list and concentrations of nutrients used in Experiment 2.2.4.

Macronutrients	Concentration ($mol\ m^{-3}$)
$CaSO4, 2H_2O$	3.0
KH_2PO_4	3.0
K_2HPO_4	0.3
$MgSO_4, 7H_2O$	2.4
KNO_3	as stated

Micronutrients	Concentration ($mmol\ m^{-3}$)
H_3BO_3	5.0
$C_6H_5O_7Fe, 5H_2O$	5.0
NaCl	10.0
$MnSO_4, 4H_2O$	1.0
$Na_2MoO_4, 2H_2O$	0.5
$CuSO_4, 5H_2O$	0.1
$ZnSO_4, 5H_2O$	0.1
$CoSO_4, 6H_2O$	0.02

APPENDIX 2E

Effect of chlorsulfuron applied to foliage alone (F) or to foliage plus soil (F+S) on components of grain yield in wheat cultivars in Experiment 2.2.2.

		Number of ears per plant		Number of spikelets per ear		Number of grains per spikelet		Grain weight (mg)	
Cultivar	Rate[a]	F	F+S	F	F+S	F	F+S	F	F+S
Abele	**0**	**2.9**		**20.5**		**2.7**		**36.1**	
	15	**2.5**	**3.0**	**21.2**	**19.5**	**2.8**	**2.5**	**36.1**	**35.2**
	60	**2.9**	**2.8**	**21.5**	**21.2**	**2.7**	**2.6**	**35.6**	**37.0**
	d	0.81		2.08		0.44		3.51	
Jasper	**0**	**3.4**		**19.0**		**2.4**		**40.1**	
	15	**3.9**	**3.5**	**17.7**	**18.5**	**2.3**	**2.4**	**41.5**	**39.11**
	60	**3.6**	**3.5**	**18.9**	**17.5**	**2.2**	**2.3**	**41.4**	**40.0**
	d	0.87		1.14		0.36		4.81	
Kotare	**0**	**3.7**		**16.7**		**2.1**		**39.5**	
	15	**3.8**	**4.4**	**17.0**	**16.8**	**2.0**	**2.1**	**39.1**	**37.0**
	60	**4.0**	**3.9**	**17.8**	**16.6**	**1.9**	**2.2**	**38.0**	**39.8**
	d	0.97		1.50		0.39		3.71	
Lancer	**0**	**3.2**		**16.2**		**2.5**		**41.0**	
	15	**3.1**	**3.4**	**15.8**	**15.6**	**2.4**	**2.6**	**40.0**	**39.8**
	60	**3.2**	**2.9**	**15.4**	**13.8***	**2.5**	**2.5**	**40.2**	**40.9**
	d	0.72		1.52		0.20		2.99	
Rongotea	**0**	**3.3**		**15.8**		**1.9**		**45.2**	
	15	**2.9**	**3.3**	**15.4**	**14.9**	**1.8**	**1.6**	**45.7**	**45.0**
	60	**3.0**	**3.0**	**15.6**	**14.2***	**1.8**	**1.8**	**45.9**	**45.3**
	d	0.92		1.50		0.28		4.39	

a) Chlorsulfuron rate (g a.i. ha^{-1}).

* An asterisk indicates a significant reduction compared to the control based on Dunnet's procedure (d) at $p < 0.05$.

APPENDIX 2F_I

Effect of chlorsulfuron on shoot dry weight (g) per plant of wheat cultivars 68 and 88 days after spraying (DAS) in the field (Experiment 2.2.3).

		Chlorsulfuron rate (g a.i. ha^{-1})			
DAS	Cultivar	0	15	60	d
68	Abele	3.14	3.23	3.15	0.759
	Jasper	2.95	2.60	2.79	0.551
	Kotare	3.59	3.69	3.47	0.533
	Lancer	4.27	4.12	4.00	0.738
	Rongotea	3.65	3.25	3.03+	0.579
88	Abele	6.06	5.56	5.18	1.087
	Jasper	5.22	5.12	4.55	0.920
	Kotare	6.55	5.96	6.18	1.178
	Lancer	7.52	6.99	7.20	2.798
	Rongotea	5.99	5.82	4.84	1.460

+ A plus sign indicates a significant reduction at $p < 0.10$.

APPENDIX 2F_II

Effect of chlorsulfuron on grain yield and straw weight of wheat cultivars at the final harvest in the field (Experiment 2.2.3)

Measurement	Cultivar	Chlorsulfuron rate (g a.i. ha^{-1}) 0	15	60	d
Grain yield	Abele	748.2	757.7	631.2	167.39
($g\ m^{-2}$)	Jasper	720.3	672.0	660.4	96.22
	Kotare	595.0	547.4	596.5	130.02
	Lancer	806.5	778.7	784.2	127.62
	Rongotea	538.6	543.7	493.4	232.15
Straw					
dry weight	Abele	1070.5	1104.2	984.1	193.99
($g\ m^{-2}$)	Jasper	873.1	921.9	856.2	68.35
	Kotare	977.0	982.6	967.4	85.66
	Lancer	936.1	916.8	905.2	110.71
	Rongotea	861.9	812.6	783.7	114.82

APPENDIX 2F_III

Effect of chlorsulfuron on the components of grain yield in wheat cultivars in the field Experiment 2.2.3).

Cultivar	Rate[a]	Ears m^{-2}	Spikelets ear^{-1}	Grains $spikelet^{-1}$	Grain weight (mg)
Abele	0	568.5	17.7	1.8	41.5
	15	585.3	18.2	1.8	39.7
	60	559.4	17.0	1.6	34.6
	d	114.68	1.28	0.24	5.93
Jasper	0	591.0	17.0	1.9	38.5
	15	625.5	16.5	1.8	37.1
	60	591.8	16.3	1.8	37.6
	d	59.58	0.86	0.13	3.18
Kotare	0	652.5	14.5	1.6	40.4
	15	723.3	14.1	1.4	37.4
	60	723.0	14.1	1.4	40.7
	d	90.91	0.79	0.24	4.93
Lancer	0	537.0	15.6	2.6	41.3
	15	596.3	14.5*	2.5	38.2
	60	607.9	14.0*	2.5	39.8
	d	85.08	0.97	0.24	5.24
Rongotea	0	610.5	14.5	1.6	36.2
	15	653.3	14.0	1.6	37.7
	60	654.5	13.1*	1.6	34.6
	d	112.54	0.62	0.21	5.97

a Chlorsulfuron rate (g a.i. ha^{-1}).

* An asterisk indicates a significant reduction compared with the control based on Dunnet's procedure (d) at $p < 0.05$.

APPENDIX 2F_IV

Effect of chlorsulfuron on number of tillers per plant in wheat cultivars 21, 68 and 88 days after spraying (DAS) in the field (Experiment 2.2.3).

		Chlorsulfuron rate (g a.i. ha^{-1})			
DAS	Cultivar	0	15	60	d
21	Abele	6.0	5.7	5.7	1.66
	Jasper	4.5	4.2	4.2	0.76
	Kotare	4.1	4.8	4.8	0.79
	Lancer	2.9	3.4*	3.6*	0.46
	Rongotea	3.5	4.2*	4.7*	0.69
68	Abele	2.7	2.9	3.0	0.38
	Jasper	2.9	2.8	2.9	0.40
	Kotare	2.9	3.0	3.0	0.26
	Lancer	2.5	2.6	2.7	0.48
	Rongotea	2.6	2.9	3.0	0.43
88	Abele	2.8	2.8	2.8	0.35
	Jasper	3.0	3.0	2.8	0.40
	Kotare	3.1	3.3	3.4	0.60
	Lancer	2.8	2.8	3.0	0.97
	Rongotea	3.0	3.1	2.9	0.69

* An asterisk indicates a significant reduction compared with the control based on Dunnet's procedure (d) at $p < 0.05$.

APPENDIX 2G

Effect of chlorsulfuron on number of tillers and plant height of wheat cultivars grown at low (L) or high (H) nitrate (Experiment 2.2.4).

Cultivar	Rate[a]	No.of tillers plant^{-1}		Height (cm)	
		L	H	L	H
Abele	**0**	**6.0**	**9.2**	**30.4**	**46.6**
	15	**5.4**	**8.4**	**28.8**	**42.4**
	60	**5.4**	**7.0**	**28.8**	**30.8**
	SEm	0.92		1.83	
Jasper	**0**	**5.8**	**10.0**	**27.0**	**39.8**
	15	**5.8**	**11.0**	**33.6**	**36.2**
	60	**5.2**	**17.6**	**34.6**	**20.6**
	SEm	1.23		4.53	
Kotare	**0**	**7.4**	**12.2**	**39.2**	**54.8**
	15	**5.8**	**13.8**	**38.0**	**56.0**
	60	**6.2**	**14.4**	**35.0**	**52.0**
	SEm	0.82		2.34	
Lancer	**0**	**3.2**	**6.8**	**55.6**	**67.0**
	15	**3.8**	**6.4**	**58.4**	**65.6**
	60	**4.2**	**10.8**	**59.6**	**44.4**
	SEm	1.00		2.83	
Rongotea	**0**	**5.4**	**9.0**	**29.8**	**42.2**
	15	**4.8**	**9.2**	**31.0**	**38.2**
	60	**7.2**	**15.6**	**25.2**	**21.8**
	SEm	1.90		1.61	
Sonora	**0**	**2.8**	**4.0**	**63.4**	**57.2**
	15	**2.8**	**4.6**	**60.0**	**50.8**
	60	**3.4**	**12.4**	**53.6**	**32.4**
	SEm	0.50		2.71	

a) Chlorsulfuron rate (g a.i. ha^{-1}).

APPENDIX 2H

Meteorological data

Mean monthly air and soil temperatures (° C), and total precipitation (mm) during the period of study (July 1989-January 1990) for Experiment 2.2.2.

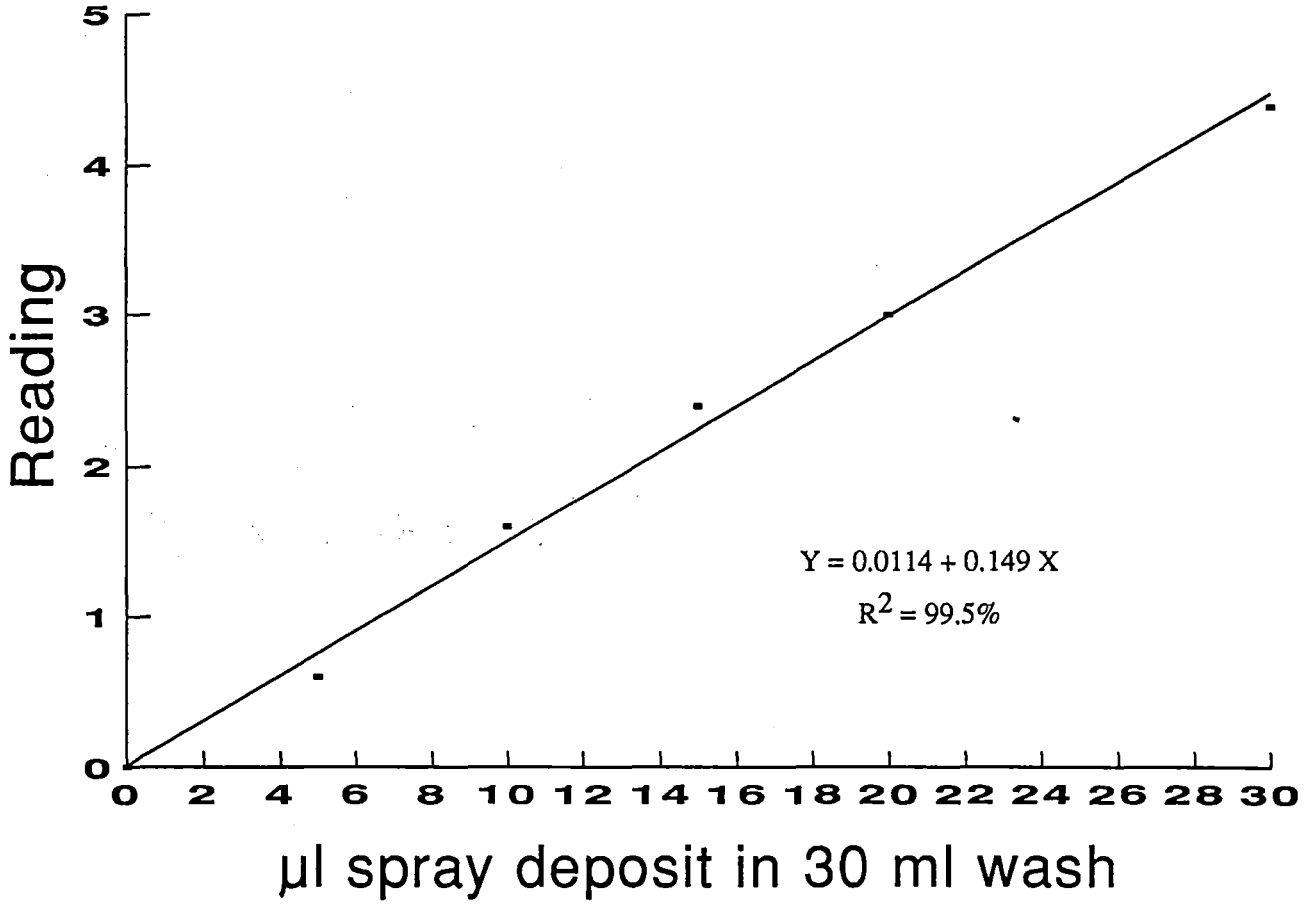
Variable	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
Mean air temp.	5.4	8.3	10.1	12.4	14.2	15.4	17.6
Mean soil temp.	3.1	6.0	8.7	10.7	13.8	15.1	17.0
Total pptn.	49.7	47.4	27.2	93.0	32.3	78.6	20.1

Mean monthly air and soil temperatures (° C), and total precipitation (mm) during the period of study (July 1989-January 1990) for Experiment 2.2.3.

Variable	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Mean air temp.	7.2	8.3	11.1	12.7	15.9	16.7	15.2
Mean soil temp.	5.2	6.1	9.3	11.9	14.6	15.9	15.2
Total pptn.	138.5	47.9	41.6	51.0	99.2	67.2	69.0

APPENDIX 3A

Standard curve for determining chlorsulfuron retention on the foliage. Each data point is the mean of four replicates.



APPENDIX 3B

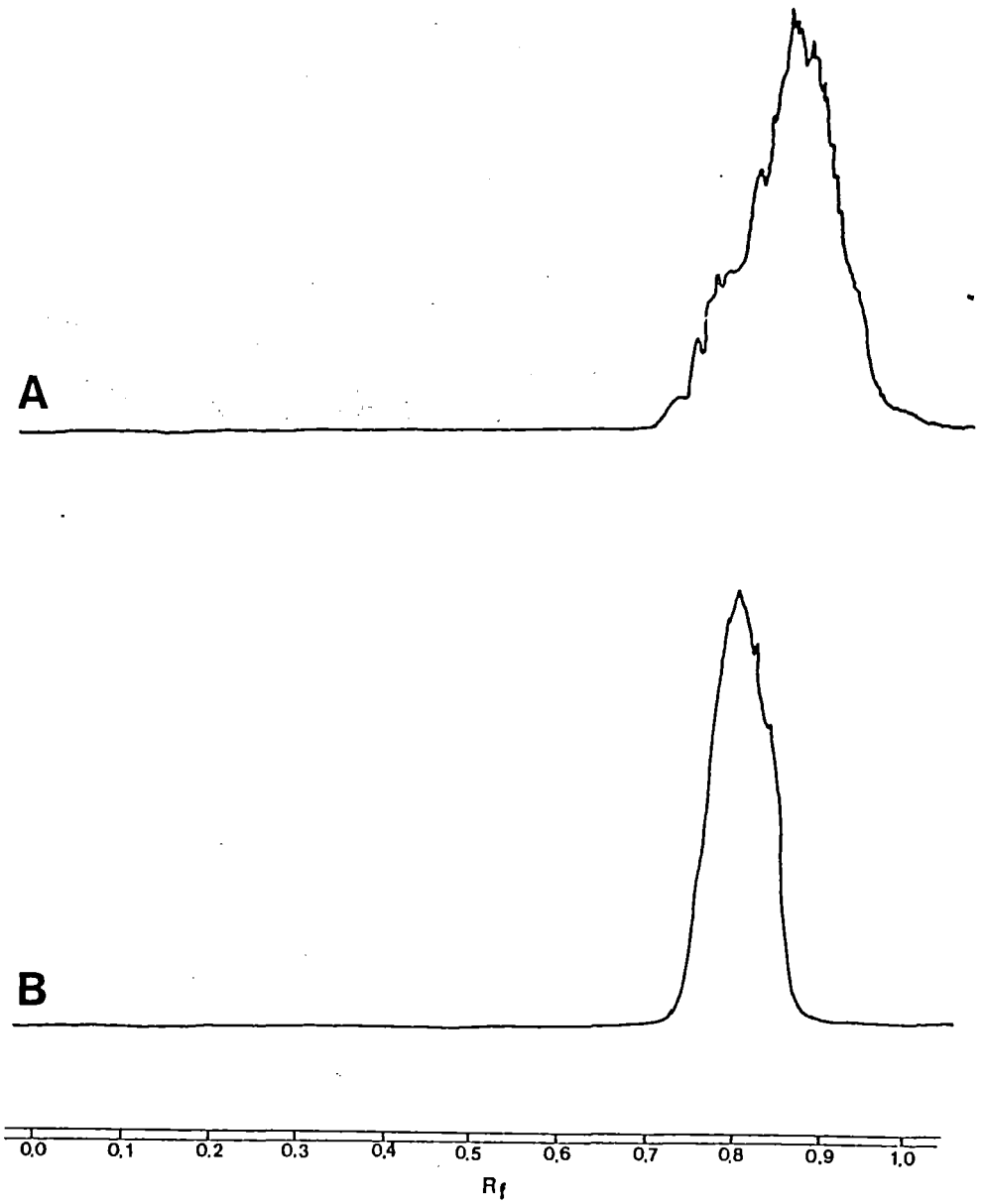
Bray's scintillation cocktail (After Chapman and Ayrey, 1981)

PPO	20	g
POPOP	1	g
Naphthalene	300	g
Ethylene glycol	100	ml
Methanol	500	ml

Make up to 5 l with 1,4-Dioxan

APPENDIX 3C

Radiochromatograms of ^{14}C-chlorsulfuron developed in (A) dichlormethane : methanol : 9N ammonium hydroxide (72:25:3) and (B) chloroform : acetic acid (19:3) solvent systems.



APPENDIX 3D

Distribution of radioactivity in various parts of wheat cultivars 12 and 48 h after application of ^{14}C-chlorsulfuron to leaf 2 lamina. Values are percentage of applied radioactivity.

Time after application	Plant part	Abele	Jasper	Kotare	Lancer	Rongotea
12 h	Leaf 1	0.01b*	0.01b	0.01b	0.01b	0.02a
	Leaf 2 lamina	4.26a	2.47b	1.61c	3.83a	2.81b
	Leaf 2 sheath	0.04ab	0.02c	0.03bc	0.05a	0.05a
	Young tissue	0.18b	0.13b	0.11b	0.27a	0.21ab
	Root	0.35a	0.12a	0.11a	0.17a	0.15a
48 h	Leaf 1	0.03a	0.02a	0.02a	0.03a	0.03a
	Leaf 2 lamina	6.31a	3.62c	2.66d	5.03b	3.83c
	Leaf 2 sheath	0.09a	0.07a	0.08a	0.11a	0.11a
	Young tissue	0.40ab	0.30b	0.23b	0.57a	0.53a
	Root	0.20b	0.19b	0.13b	0.41a	0.22b

* Means in each row followed by the same letter are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

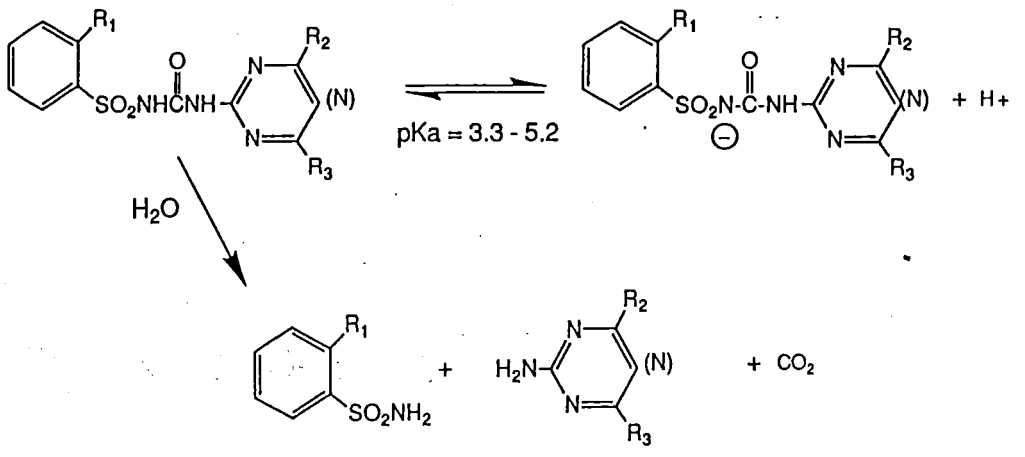
APPENDIX 3E

Dry weight (mg) of individual plant parts of wheat cultivars 12 and 48 h after application of ^{14}C-chlorsulfuron.

Time after application	Plant part	Abele	Jasper	Kotare	Lancer	Rongotea
12 h	Leaf 1	20.3b*	14.6b	36.8a	14.4b	35.1a
	Leaf 2 lamina	25.5bc	19.8c	40.4a	21.5bc	29.2b
	Leaf 2 sheath	5.6c	5.3c	9.7a	5.1c	7.9b
	Young tissue	82.2ab	52.2c	85.9a	57.5bc	73.0a
	Root	108.3a	73.7c	97.5ab	88.5bc	113.0a
48 h	Leaf 1	19.4b	17.3b	34.6a	15.5b	28.7a
	Leaf 2 lamina	25.0b	21.9b	34.8a	20.0b	26.5b
	Leaf 2 sheath	5.8b	5.1b	9.9a	5.3b	7.9a
	Young tissue	74.6a	67.1a	98.3a	59.6a	85.3a
	Root	95.3a	92.1a	107.0a	89.5a	116.2a

* Means in each row followed by the same letter are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

APPENDIX 3F



Sulfonylurea herbicide ionization and hydrolysis (after Brown, 1990). R_1, R_2 and R_3 are specific to individual compounds, for chlorsulfuron R_1= Cl, R_2= -CH_3 and R_3= -OCH_3 .

APPENDIX 3G

Concentration of un-metabolised chlorsulfuron in the young tissues of wheat cultivars 12 and 48 h after spraying.

	Parent chlorsulfuron (nM)	
Cultivar	12 h	48 h
Abele	2.43c*	1.51c
Jasper	2.76c	1.19c
Kotare	0.50c	0.58c
Lancer	10.70a	15.72a
Rongotea	7.16b	7.77b

* Means in each column followed by the same letter are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

APPENDIX 4A

Seed head dry weight (g) of wheat grown at different nitrate concentrations and sprayed with chlorsulfuron. Data are from Experiment 4.2.2.

Chlorsulfuron (g a.i. ha^{-1})	Nitrate (mol m^{-3})				
	1	5	10	25	50
0	6.4	8.8	3.5	2.8	2.2
15	7.1	6.2	3.7	3.3	2.8
60	3.1	3.9	2.6	1.7	1.1
SEm	0.63	0.71	0.23	0.35	0.28

APPENDIX 4B

Retention of chlorsulfuron spray on the foliage of wheat grown at two nitrate levels. Repeat experiment in Section 4.2.4

Nitrate (mol m^{-1})	µl of spray		
	per plant	per cm^2 leaf area	per g dry weight
1	10.0	1.57	290.6
5	16.5	1.64	288.9
SEm	1.31**	0.082	24.01

**Double asterisk indicates a significant F test at $p < 0.01$.

APPENDIX 4C

Results of the preliminary experiment on uptake, translocation and distribution of ^{14}C-chlorsulfuron in wheat. Data from experiment 4.2.5.1.

Uptake and translocation of ^{14}C-chlorsulfuron, 24 h after application to the lamina of leaf 3 of wheat grown at 1 or 5 mol m^{-3} nitrate. Data are percentages of the applied radioactivity.

Nitrate (mol m^{-3})	Percentage of ^{14}C recovered in		Translocation
	Water wash	Acetone wash	
1	87.7	0.7	10.0
5	86.6	0.7	11.4
SEm	0.88	0.13	0.61

Distribution of radioactivity in various parts of wheat plants 24 h after application of ^{14}C-chlorsulfuron to leaf 3 lamina of plants grown at 1 or 5 mol m^{-3} nitrate. Values are percentages of the radioactivity recovered from plant.

Nitrate (mol m^{-3})	Leaves 1 and 2	Leaf 3 lamina	Leaf 3 sheath	Young tissue	Root
1	0.6	84.1	4.4	4.5	6.5
5	0.5	86.6	3.1	4.6	5.5
SEm	0.05	1.07	0.50	0.29	0.85

APPENDIX 4D

Dry weight (mg) of various plant parts 24, 48 and 168 h after application of ^{14}C-chlorsulfuron to leaf 2 lamina of wheat grown at low (1 mol m^{-3}) and high (5 mol m^{-3}) nitrate. Data from Experiment 4.2.5.2.

	24 h		48 h		168 h	
Plant part	Low	High	Low	High	Low	High
Leaf 1	**9.4**	**9.5** (0.91)[a]	**10.1**	**10.7** (0.74)	**11.0**	**14.0** (0.91)
Leaf 2 lamina	**13.3**	**14.6** (1.50)	**13.5**	**16.8** (1.43)	**18.5**	**26.4*** (1.54)
Leaf 2 sheath	**4.5**	**4.4** (0.35)	**5.4**	**5.2** (0.29)	**6.4**	**7.0** (0.42)
Young tissue	**48.3**	**68.5****(2.28)	**58.3**	**93.9***(9.05)	**129.9**	**254.2****(11.63)
Root	**71.2**	**55.3** (5.10)	**79.4**	**76.2** (7.21)	**134.2**	**168.8*** (6.71)
Total	146.7	152.3	166.7	202.8	299.0	470.4**

a) Values in brackets are SEm for each plant part at each time.

* Single and double asterisks indicate a significant difference at $p < 0.05$ or $p < 0.01$, respectively, between the two nitrate levels for each particular measurement.

APPENDIX 5A

Effect of nitrate supply and branched chain amino acids (BCAA) on dry weight (g), reduced N and nitrate N content (mg g-1 dry weight) of shoot of wheat sprayed with chlorsulfuron at 15 g a.i. ha-1. Repeat experiment for Section 5.3.1.

Nitrate	BCAA	Dry weight		Reduced N		Nitrate N	
(mol m-3)		Control	Sprayed	Control	Sprayed	Control	Sprayed
1	0	1.27	1.01	8.9	10.9	0.05	0.05
5	0	3.80	1.61	18.2	32.1	0.13	3.30
1	4	3.65	2.05	18.3	30.5	0.12	0.62
SEm		0.150		0.62		0.150	

APPENDIX 5B

Effect of nitrate supply, branched chain amino acids (BCAA) and glutamine plus glutamate (GLN/GLU) on shoot dry weight of wheat 25 days after spraying with chlorsulfuron. Repeat experiment for Section 5.3.2.

Nitrate	Amino acids	Shoot dry weight (g)	
(mol m^{-3})[a]		Control	Sprayed
1	0	**0.50** (0.032)[b]	**0.48** (0.033)
5	0	**0.90** (0.041)	**0.50** (0.025)
1	4 as BCAA	**0.99** (0.051)	**0.80** (0.081)
1	4 as GLN/GLU	**1.25** (0.056)	**1.00** (0.041)

a) Concentrations of amino acids are 0 and 4 mol m^{-3} as total nitrogen in each group.

b) Values in brackets are SEm.

APPENDIX 5C

Shoot dry weight (g) and photosynthetic rate (μmol m^{-2} s^{-1}) of leaves of wheat grown at 1 or 5 mol m^{-3} nitrate, at different times following the application of chlorsulfuron.

Nitrate (mol m^{-3})	Rate[a]	Shoot dry weight	Day 8	Day 20		Day 31	
			Leaf3	Leaf3	Leaf4	Leaf3	Leaf4
1	0	0.78	12.0	9.0	10.1	10.1	11.0
	15	0.59	10.3	8.1	9.3	8.5	9.7
5	0	1.85	11.0	8.6	9.0	10.0	9.8
	15	0.63*	9.3	8.1	6.7*	7.7*	5.1*
SEm		0.091	0.90	0.43	0.44	0.56	0.50

* An asterisk indicates significant reduction from the control at $p<0.05$.

Appendix 6A

Formulations of extraction and desalting buffer solutions used for ALS assay.

Extraction buffer

1. Basic stock solution, pH 7.5

KH_2PO_4	50 mol m^{-3}
$MgSO_4, 7H_2O$	1 mol m^{-3}
Ethanediol	10% by volume
Triton X100	0.05% by volume

2. Chemicals to be added just before use

Pyruvate	10 mol m^{-3}
Thiamine pyrophosphate (TPP)	0.5 mol m^{-3}
Flavin adenine dinucleotide (FAD)	10 mmol m^{-3}
L-leucine	1 mol m^{-3}
L-valine	1 mol m^{-3}

Desalting buffer

1. Basic stock solution, pH 7.5

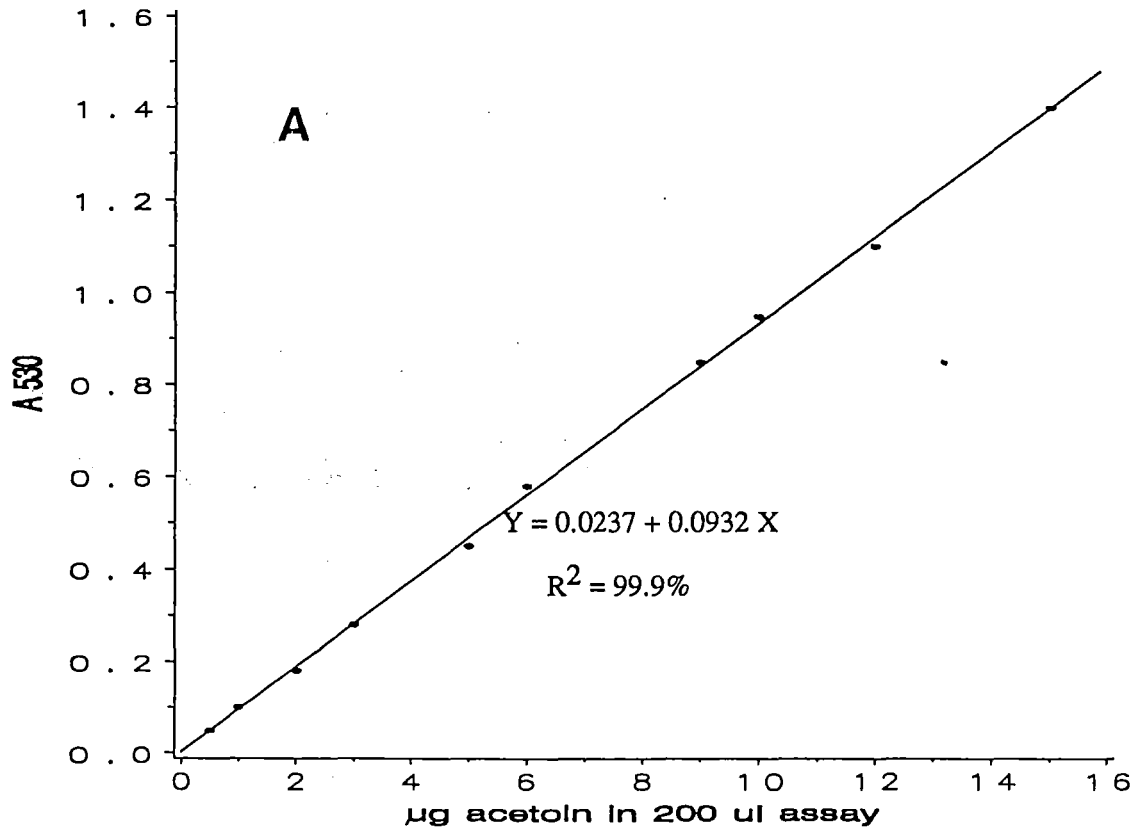
KH_2PO_4	50 mol m^{-3}
$MgSO_4, 7H_2O$	1 mol m^{-3}
Ethanediol	30% by volume

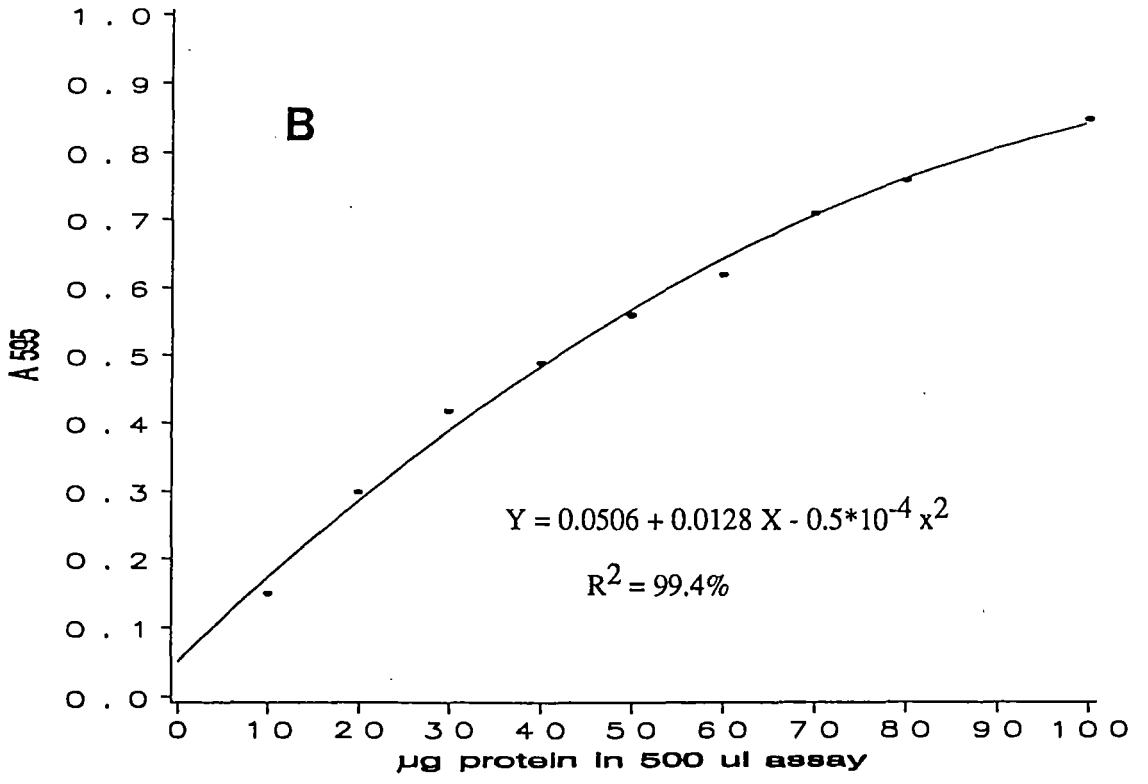
2. Chemicals to be added just before use

Pyruvate	10 mol m^{-3}

Appendix 6B

standard curves for ALS assay (A) and for protein assay (B). each data point is the mean of ten different measurements.





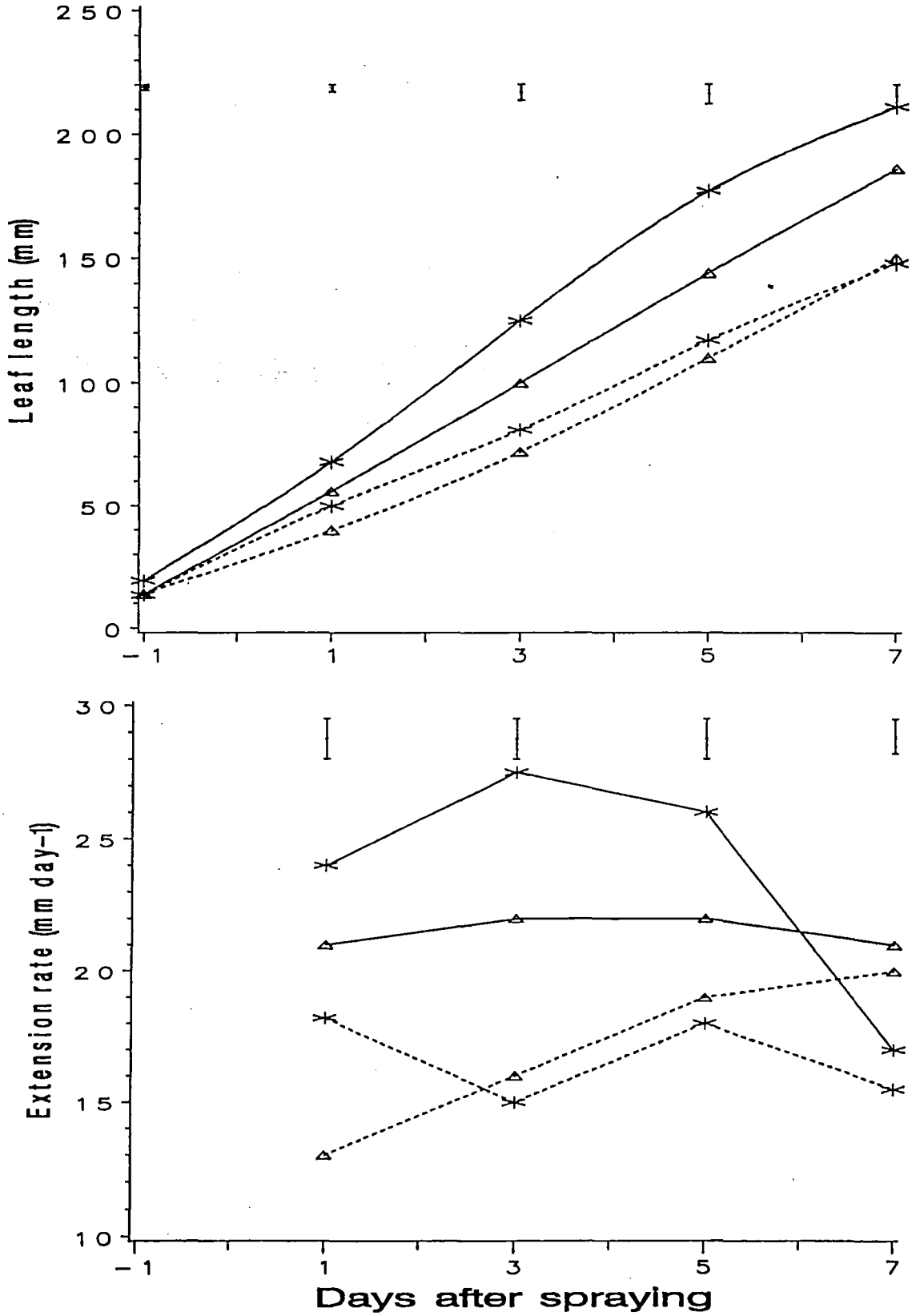
Appendix 6C

Formulation of Coomasie reagent used for protein assay (After Bradford, 1976).

Procedure: Coomasie Brilliant Blue (100 mg) is dissolved in 50 ml of 95% ethanol and 100 ml of 85% phosphoric acid is added. The solution is made to 1 l with distilled water and filtered.

Appendix 6D_I

Leaf length (A) and extension rate (B) of leaf 4 of wheat supplied 5 mol m^{-3} nitrate ($\ast$) or 1 mol m^{-3} nitrate plus 4 mol m^{-3} branched chain amino acids ($\triangle$), in control plants (solid lines) and plants sprayed with 15 g a.i. ha^{-1} chlorsulfuron (broken lines). Error bars are SEm. Repeat experiment for data in Figure 6.1.



APPENDIX 6D_II

Effect of chlorsulfuron on shoot fresh weight and shoot dry weight of wheat supplied high nitrate or low nitrate plus branched chain amino acids (BCAA). Repeat experiment for data in Table 6.1.

Nitrate	BCAA	Fresh weight (g)[a]		Dry weight (g)[a]	
(mol m^{-3})		Control	Sprayed	Control	Sprayed
5	0	**1.06** (0.07)[a]	**0.53** (0.03)	**1.15** (0.12)	**0.43** (0.04)
1	4	**1.02** (0.05)	**0.65** (0.06)	**1.09** (0.06)	**0.65** (0.05)

a) Fresh and dry weight were taken 7 and 28 days after chlorsulfuron application, respectively.

b) Values in brackets are SEm.

APPENDIX 6D_III

Effect of chlorsulfuron (15 g a.i. ha^{-1}) on specific activity of ALS (nmol mg^{-1} protein h^{-1}) and on protein content (mg g^{-1} fresh weight) of shoots of wheat supplied high nitrate or low nitrate plus branched chain amino acids (BCAA), extracted one or seven days after spraying. Repeat experiment for data in Table 6.2.

Nitrate	BCAA	Specific activity				Protein content			
		Day one		Day seven		Day one		Day seven	
(mol m^{-3})		Control	Sprayed	Control	Sprayed	Control	Sprayed	Control	Sprayed
5	0	172.5	32.5	348.5	118.6	8.70	8.75	7.25	10.25
1	4	142.62	25.8	282.8	106.1	9.95	8.30	7.50	7.40
SEm		13.77	5.20	24.93	6.40	1.055	0.960	0.705	1.255

APPENDIX 6D_IV

Effect of chlorsulfuron on concentration (nmol mg^{-1} dry weight) of branched chain amino acids and total free amino acids determined (Total AA) in shoots of wheat at different times after application. Repeat experiment for data in Table 6.4.

Nitrate	BCAA		Day one				Day four				Day seven			
(mol m^{-3})		Rate[a]	Val.	Iso.	Leu.	Total AA	Val.	Iso.	Leu.	Total AA	Val.	Iso.	Leu.	Total AA
5	0	0	2.68	1.15	2.09	46.7	2.4	0.77	1.20	50.1	2.58	0.93	1.52	50.9
		15	1.81	1.17	1.69	59.5*	1.0*	0.91	1.49	60.2*	2.00	0.82	1.53	59.2
1	4	0	6.71	4.41	5.98	40.4	2.38	1.10	1.67	44.5	1.63	0.63	1.14	32.5
		15	3.67*	3.43	4.65	50.8	1.2*	1.21	1.59	48.9	1.32	0.63	1.24	37.4
SEm			0.769	0.555	0.638	2.66	0.310	0.215	0.343	2.20	0.215	0.108	0.075	3.43

* An asterisk indicates significant reduction from the control at $p < 0.05$.

APPENDIX 6D_V

Effect of chlorsulfuron on percentage of the total amino acids as branched chain amino acids in shoots of wheat at different times after application. Repeat experiment for data in Table 6.5.

Nitrate	BCAA		Day one			Day four			Day seven		
(mol m^{-3})		Rate[a]	Val.	Iso.	Leu.	Val.	Iso.	Leu.	Val.	Iso.	Leu.
5	0	0	5.63	2.43	4.48	4.73	1.54	2.41	5.02	1.82	2.97
		15	2.65	1.98	2.85	1.61*	1.64	2.73	3.43*	1.39	2.60
1	4	0	18.52	8.36	11.27	5.30	2.42	3.70	5.00	1.93	3.51
		15	7.25*	6.69	9.16	3.68*	2.51	3.31	3.54*	1.68	3.31
SEm			0.900	0.900	0.828	0.839	0.482	0.759	0.393	0.123	0.20

* An asterisk indicates significant reduction from the control at $p < 0.05$.

APPENDIX 6D_VI

Effect of chlorsulfuron on concentration (nmol m^{-3}) of branched chain amino acids and total free amino acids determined (Total AA) in xylem sap of wheat at different times after application. Repeat experiment for data in table 6.6.

Nitrate (mol m^{-3})	BCAA	Rate[a]	Day one				Day four				Day seven			
			Val.	Iso.	Leu.	Total AA	Val.	Iso.	Leu.	Total AA	Val.	Iso.	Leu.	Total AA
5	0	0	390.5	53.6	49.9	1563.9	398.8	96.6	77.2	1673.2	594.9	149.2	172.6	2775.5
		15	127.8*	57.6	48.9	2241.3	87.9*	29.2	30.0	1648.5	270.4*	102.2	133.6	2785.5
1	4	0	997.5	333.8	263.1	2625.0	254.7	78.9	61.4	1340.5	442.5	142.2	174.3	1976.5
		15	998.4	584.2	470.6	3395.5	89.7*	46.4	37.3	1065.9	235.1	105.2	129.5	2254.5
SEm			79.18	57.80	49.11	316.01	53.39	22.23	29.07	152.35	67.76	22.20	27.12	196.32

* An asterisk indicates significant reduction from the control at $p < 0.05$.

APPENDIX 6D_VII

Effect of chlorsulfuron on percentages of the total amino acids determined as branched chain amino acids in xylem sap of wheat at different times after application. repeat experiment for data in Table 6.7.

Nitrate	BCAA		Day one			Day four			Day seven		
(mol m^{-3})		Rate[a]	Val.	Iso.	Leu.	Val.	Iso.	Leu.	Val.	Iso.	Leu.
5	0	0	24.9	3.2	3.4	24.1	4.4	5.4	21.4	5.4	6.3
		15	6.3*	2.2	2.8	6.2*	1.9*	1.9*	10.1*	3.5*	4.6*
1	4	0	29.2	10.0	12.7	18.5	4.7	6.0	22.2	7.1	8.7
		15	32.0	14.1	17.6	9.4*	3.5	4.4	10.9*	4.4*	5.4*
SEm			4.38	1.13	1.64	1.86	0.58	0.76	1.44	0.08	0.11

* An asterisk indicates significant reduction from the control at $p < 0.05$.

APPENDIX 6E

Effect of chlorsulfuron on shoot dry weight (g) of wheat transferred from 1 to 5 mol m^{-3} nitrate regime at different times after spraying. Measurements were taken 54 DAS. Repeat experiment for Section 6.3.3.

Time of transfer	Shoot dry weight	
	Control	Sprayed
One week	**3.57** (0.241)[a]	**1.32** (0.230)
Three weeks	**3.33** (0.151)	**3.00** (0.120)

a) Values in brackets are SEm.

APPENDIX 7A

Specific activity of acetolactate synthase (nmol mg^{-1} protein h^{-1}) extracted from shoots of wheat cultivars 24 h after spraying with chlorsulfuron.

Chlorsulfuron rate (g a.i. ha^{-1})	Kotare	Lancer	Rongotea
0	**190.6** (25.11)	**176.9** (19.80)	**221.8** (27.75)
15	**46.5** (9.50)	**30.4** (9.24)	**46.9** (10.82)

a) Values in brackets are SEm.

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