CHAPTER ONE

General introduction

General introduction

Chickpea (*Cicer arietinum*) is the world's second most cultivated food legume, grown over 9.9 million ha in 2002, from the Mediterranean basin and West Asia to the Indian sub-continent (which accounts for 75% of global production), eastern Africa, Australia, and North and South America (FAO 2002). The production of chickpea in Australia began in earnest in 1978 with the release of the desi variety Tyson (Beech and Brinsmead 1980). Chickpea has been shown to be one of several pulses that are suited to the fine-textured, neutral-to-alkaline soils of the eastern cropping zone of Western Australia and eastern Australia where narrow-leafed lupin (*Lupinus angustifolius*) is poorly adapted (Siddique and Sedgley 1986; Siddique *et al.* 1993). Chickpea is seen as a crop that provides a cash income from grain, requires minimal nitrogen (N) fertiliser owing to its ability to fix atmospheric N, and in a crop rotation can improve the N nutrition and yield of subsequent cereals (Doughton *et al.* 1993).

Both the area and actual production of chickpea grew rapidly up until the 1990s when drought restricted production in Queensland and northern New South Wales (Siddique and Sykes 1997). Chickpea has fitted well into the farming systems across a broad range of environments in Australia, extending from the tropical Ord River Irrigation Area, to subtropical southern Queensland and northern New South Wales, and to the Mediterranean type environments of southern Australia. The projected areas of production for 2007 (Brown *et al.* 2007) were New South Wales (167 000 ha), Queensland (46 000 ha), Victoria (39 000 ha), Western Australia (2000 ha), and South Australia (5000 ha). A key reason for the interest in chickpea in the northern grain region of northern New South Wales and southern Queensland is the lack of alternative and profitable winter legumes suited to rotation with winter (wheat) and summer (sorghum) cereals (Whish *et al.* 2007).

Perhaps the single most important factor which contributed to the expansion of chickpea production in Australia was the opening up of export markets in the Indian sub-continent in the mid 1980s where about 80–90% of Australia's chickpea production is exported to for human consumption (Siddique and Sykes 1997). The market demand for chickpea in the Indian subcontinent and Middle East is high and an increase of 2.2% per

annum in demand for chickpea for human consumption has been reported by FAO (Siddique *et al.* 2000). Such a demand provides an opportunity to increase the production of chickpea in Australia. The majority of world chickpea exports are dominated by Australia (21.9%), Turkey (19.5%), Iran (12.5%), Mexico (12.2%) and Canada (10%), accounting for 76% of total chickpea exports in 2004 (FAO 2006).

One of the major obstacles in growing chickpea successfully is its poor ability to compete with weeds. Crop losses of 90% are possible in weedy situations (Knights 1991) and the lack of registered post-emergence herbicides for broadleaf weeds reduces the options for weed management.

There are two herbicides available for post-plant, pre-emergence use in chickpea: Balance® (75% a.i. isoxaflutole) and Gesatop® (simazine). Simazine has traditionally been the most widely used herbicide for broadleaf weed control and can provide relatively cheap control of cruciferous weeds. But the efficacy of simazine is highly dependent on receiving rainfall (20-30 mm) within 2-3 weeks of application, and weed control is often poor under drier conditions. Balance® is a non-volatile formulation and remains stable on the soil even after a prolonged dry period. Isoxaflutole has unique properties that allow the product to "recharge" when rainfall occurs, due to limited leaching. It remains active for longer periods and thus gives more consistent and reliable control of a wide range of broadleaf weeds including some difficult-to-control species. Isoxaflutole has favourable toxicological and environmental properties at low application rates (Luscombe *et al.* 1995; Vrabel *et al.* 1995). It has sufficient residual activity to control weeds over a whole season when applied at a relatively low use rate (105 g a.i. ha^{-1}), but may dissipate within a growing season and not carry over to injure rotational crops (Vrabel *et al.* 1995).

In Australia, isoxaflutole at 75 g a.i. ha^{-1} is registered for the control of several troublesome broadleaf weeds [e.g. Indian hedge mustard (*Sisymbrium orientale*), sowthistle (*Sonchus oleraceus*), capeweed (*Arctotheca calendula*), prickly lettuce (*Lactuca serriola*), wild radish (*Raphanus raphanistrum*), turnip weed (*Rapistrum rugosum*), crassula (*Crasulla* spp.), medic (*Medicago* spp.), deadnettle (*Lamium amplexicaule*), and slender celery (*Ciclospermum leptophyllum*)] in chickpea. Since isoxaflutole was registered and commercially released in Australia in 2001 as Balance®,

there has been rapid and widespread adoption of this herbicide in chickpea production systems. Over 100 000 ha of chickpeas in the northern grain region of Australia have been treated annually with isoxaflutole over the last three years. This amounts to well over half of the total chickpea cropping area in Australia.

However, there have been records of chickpea crop damage due to the application of isoxaflutole. Felton *et al.* (2004) conducted pot and field trials at Tamworth Agricultural Institute, New South Wales, Australia and reported that under some conditions this herbicide can injure even the more tolerant varieties of chickpea at an application rate of less than 75 g a.i. ha^{-1} and consequently result in a yield penalty. They also found an inverse linear relationship between the rate of isoxaflutole and the log of grain yield, where there were highly significant effects of variety, rate of herbicide, and a variety × rate of herbicide interaction. Isoxaflutole has caused considerable concern in the United States after it was released, with widespread injury occurring in maize (*Zea mays*). Bhowmik and Prostak (1996) and Obermeier *et al.* (1995) observed maize injury when isoxaflutole was applied at 158 g ha^{-1}. Sprague *et al.* (1996) also observed injury to maize from pre-emergence applications of isoxaflutole. In Canada, O'Sullivan *et al.* (2001) found considerable maize cultivar injury, combined with plant height effects, and yield reductions from isoxaflutole applied at 105 g a.i. ha^{-1}. However, in other work, isoxaflutole applied alone or in combination with herbicides such as metolachlor or atrazine did not injure maize at effective weed control rates (Luscombe *et al.* 1995; Young *et al.* 1999).

Crop injury has been observed in alkaline soils (pH greater than 7.4) and in sandy soils particularly in short-season hybrids, or when heavy rainfall has occured soon after application (Luscombe *et al.* 1995). Sprague *et al.* (1999) also noted that injury in maize was most commonly observed in coarse-textured soils with low clay and organic matter (OM) levels and was more severe with higher rates of isoxaflutole. Injury to maize was not unique to any tillage system but was dependent on site, year, and application rate of isoxaflutole (Sprague *et al.* 1999). Bhowmik *et al.* (1999) reported bleaching injury to maize in the field with a 210 g ha^{-1} rate of isoxaflutole. They found that the injury was temporary and the plants recovered within 2 to 3 weeks. They attributed the injury in their fine-textured soil (1.5% organic matter and pH 6.3) to the high rate of isoxaflutole.

Bhowmik *et al.* (1996) also did not observe any appreciable injury to maize, even at 158 g ha^{-1} of isoxaflutole, with no adverse effect on maize grain and silage yields in a field study on a Hadley fine sandy loam containing 3.2% organic matter with a pH of 6.8.

Limited information exists concerning the tolerance of chickpea genotypes to isoxaflutole under Australian conditions. There is little documented research concerning the effects of soil and environmental factors such as soil pH, organic matter, temperature and soil moisture on the degree of isoxaflutole injury to chickpea. The effects of different soil nitrate levels influencing the degree of isoxaflutole injury to chickpea growth and nodulation have not been thoroughly investigated. An improved understanding of the impact of this herbicide on nodulation and N fixation of chickpea is also important to define the potential consequences of using this herbicide in agricultural production systems where chickpea is grown to improve the N balance of legume-cereal rotations.

With this background, a project was initiated by the Cooperative Research Centre for Australian Weed Management (Weeds CRC) with the main objectives being to investigate the factors (e.g., variety, soil moisture, soil pH, organic matter, temperature, and different soil nitrate levels) that affect chickpea tolerance to isoxaflutole, and to examine the effect this herbicide has on N fixation.

CHAPTER TWO

Literature review

Literature review

Field performance of soil applied herbicides is influenced by inherent soil factors and fluctuating, interacting, variable environmental factors such as rainfall, temperature, light intensity and air humidity, all of which affect transpiration, herbicide uptake and performance. Therefore, an assessment of the factors which determine the crop tolerance to a herbicide is necessary in order to understand which factors minimise crop damage. Cultivars of specific crops resistant to particular herbicides may provide a source of improvement of weed control effectiveness. The discovery of susceptible cultivars may provide for improvements in hybrid seed production and cultivar rouging. Information of this type could also be of value in screening programmes for potential new herbicides.

Isoxaflutole, as a weed control option for maize, sugarcane (*Saccharum* spp.) and chickpea, will be reviewed. After that, the effects of genotypes and different soil and environmental factors affecting crop tolerance to the herbicide isoxaflutole will be discussed. The effects of different soil nitrate levels influencing the degree of isoxaflutole injury to chickpea growth and nodulation will also be reviewed. As chickpea-wheat rotations are extensively practised by farmers in Australia, the N balance of pulse crop-cereal rotations will also be discussed. Finally, the effects of herbicides on the nodulation and N fixation by legumes with special reference to chickpea will be assessed. The conclusions reached at the end of this review provide the basis for the approach taken in the experimental work.

2.1 Isoxaflutole

A brief outline of the key isoxaflutole properties is given here as a background to the following sections.

Properties

The chemical name of isoxaflutole is 5-cyclopropyl isoxazol-4-yl-2-mesyl-4-trifluromethylphenyl ketone. Isoxaflutole is registered under the trade name Balance® of which the active constituent isoxaflutole is 750 g kg^{-1}.

Formulation type

Formulation	Water-dispersible granules
Appearance	Grey granule
Bulk density	1.44 g mL^{-1}
Odour	No odour
Melting point	135-136°C (for active ingredient)
Vapour pressure	1×10^{-6} Pa at 25°C (for active ingredient)
Corrosiveness	Not corrosive
Poison schedule	Schedule 5
Hazchem code	Not applicable
DG Class	Not classified as a dangerous good for transport by road or rail

Isoxaflutole has relatively low solubility in all solvents useful for formulating herbicides and was initially marketed as a 75% water dispersible granule in soluble sachets (Luscombe and Pallett 1996).

Toxicological properties

Oral LD_{50} (rat)	> 5000 mg kg^{-1}
Dermal LD_{50} (rabbit)	> 2000 mg kg^{-1}
Inhalation LC_{50} (rat)	> 5.26 mg L^{-1}
Skin irritation (rabbit)	Slight irritant
Eye irritation (rabbit)	Slight to moderate irritant
Sensitisation (guinea pig)	Non-sensitising

In sub-acute and chronic studies, it was found that all species tested tolerated significant levels of isoxaflutole for prolonged periods, with few signs of toxicity (Luscombe *et al.* 1995).

Ecotoxicology

Aquatic	
Daphnia and fish acute	Non-toxic at maximum limit of water solubility
EC_{50} Eastern oyster	3.4 mg L^{-1} (96 h)
EC_{50} Mysid shrimp	18 µg L^{-1} (96 h)
Avian	
Acute oral LD_{50} quail and Mallard duck	2150 mg kg^{-1}
Beneficials	
Earthworm	Non-toxic at 1000 mg kg^{-1}
Acute oral LD_{50} honeybees	> 100 µg bee^{-1}

Isoxaflutole has been tested on a range of fish and aquatic invertebrates, on birds, and on beneficial animals such as earthworms and bees. At a maximum limit of water solubility, or at maximum regulatory doses, isoxaflutole has been shown to be non-toxic to 13 out of 15 species tested; only the Eastern oyster and the marine Mysid shrimp showed any sensitivity to isoxaflutole. Studies have also been carried out with the major soil metabolites to show that isoxaflutole will have little adverse effect on the environment (Luscombe and Pallett 1996).

Mode of action

Isoxaflutole is a pre-emergence soil applied herbicide which belongs to the isoxazole class of herbicides and used for pre-emergence control of grass and broadleaf weeds in maize and sugarcane (Luscombe and Pallett 1996; Luscombe *et al.* 1995). In plants and soil, isoxaflutole is rapidly converted to a diketonitrile (DKN) metabolite, [2-cyclopropyl-3-(2- mesyl-4- trifluoromethylphenyl)-3- oxopropanenitrile] by opening the isoxazole ring (Pallett *et al.* 1998). This DKN degrade is herbicidally active and is a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase (HPPD) (Pallett *et al.* 1998; Viviani *et al.* 1998). After herbicide application, susceptible species treated with isoxaflutole initially show a bleaching of newly developed leaves followed by growth suppression and necrosis prior to plant death. Bleaching, i.e. absence of both carotenoid

and chlorophyll pigments, of newly developed leaves results indirectly from an inhibition of carotenoid biosynthesis (Luscombe and Pallett 1996).

Isoxaflutole inhibits HPPD, which is involved in the conversion of 4-hydroxyphenylpyruvate to homogentisate, a key step in plastoquinone biosynthesis (Pallett *et al.* 1997). Inhibition of HPPD reduces the levels of plastoquinone, a cofactor of phytoene desaturase, a key enzyme of carotenoid biosynthesis (Luscombe and Pallett 1996). The reduction in plastoquinone levels resulting from the HPPD inhibition by DKN causes *in vivo* inhibition of the phytoene desaturase and this gives rise to the typical herbicidal effects, with associated accumulation of phytoene (Pallett *et al.* 1998). The pigment disruption induced by isoxaflutole in susceptible species is often associated with reduced growth and subsequent necrosis of young leaf tissues, unlike that of direct phytoene desaturase inhibitors such as diflufenican, which cause a decrease in the pigment content of leaf tissue of susceptible species without affecting growth (Barry and Pallett 1998). This additional reduced growth and necrosis is a result of either an accumulation of phytotoxic amounts of free tyrosine and/or a depletion of α-tocopherol consequential to HPPD inhibition, additional to the depletion of plastoquinone (Pallett *et al.* 1998). Isoxaflutole therefore acts similarly to the triket-one family of herbicides, which also inhibit HPPD in plants (Secor 1994). Carotenoids are important photosynthetic pigments with many different functions in plant cells, including protection from sunlight. Without carotenoids, chlorophyll is photo-oxidized and the plant dies.

- DKN works by inhibiting the production of the enzyme HPPD.
- Inhibition of HPPD stops quinone biosynthesis. Quinone is a key factor in the synthesis of carotenoid pigment.
- When the production of quinone stops, death of the choloroplasts (organelles that contain chlorophyll) occurs, eventually killing the plant.
- The loss of quinone further inhibits photosynthesis because it is needed for electron transport (the energy transferring reaction that drives photosynthesis).

Uptake and translocation

Sprague *et al.* (1999) examined the site of isoxaflutole uptake by maize. Four Pioneer maize hybrids, previously identified as tolerant or sensitive to isoxaflutole (Sprague *et al.* 1998a), were used to determine if the shoot or root was the principal site of uptake of isoxaflutole by maize. They reported that germinating maize could potentially absorb isoxaflutole or DKN via the emerging coleoptile, by the seed, or by the newly developing root system. Isoxaflutole when placed in the layer below the seed zone (Zone 1), injured all four maize hybrids by between 21 and 38%. This treatment also reduced maize height of these hybrids by between 15 and 36%, compared with the non-treated controls. Even greater maize injury was observed in three of the four hybrids when isoxaflutole was applied pre-emergence (i.e. no charcoal barrier), compared with the treatment in which isoxaflutole was placed below the seed zone (Zone 1). Maize was not significantly affected when isoxaflutole was placed immediately above the seed zone (Zone 2) and movement of the herbicide to the seeds and roots was inhibited by the charcoal layer. Sprague *et al.* (1999) further concluded that isoxaflutole is primarily absorbed through the maize roots and by the seeds and will be more phytotoxic to maize when isoxaflutole is leached into the root and seed zone. Based on visual injury ratings, maize absorption appears to be seeds plus roots > roots alone > shoot. Thus, any practice that results in greater downward movement of isoxaflutole and DKN, or greater exposure of the seed to isoxaflutole, could increase injury to maize.

Isoxaflutole can be applied from immediately after planting to just before crop emergence. As a non-volatile formulation, it remains stable on the soil even after a prolonged dry period. The herbicide is absorbed by both shoots and roots of emerging weeds as they grow and expand through the soil. Root absorption appears more important than shoot uptake. The uptake of isoxaflutole directly correlates with water uptake. As a result, isoxaflutole provides less weed control under drought conditions. Isoxaflutole is not affected by sunlight, and degradation is slow if moisture is not present. Isoxaflutole has unique properties that allow the product to "recharge" when rainfall occurs, due to limited leaching under dry conditions. This allows isoxaflutole to remain active for much longer periods than many other pre-emergence herbicides (Anon. no date).

Pallett *et al.* (1998) reported that the fate of isoxaflutole in plants is a rapid conversion to the DKN, which then undergoes further cleavage to a herbicidally inactive benzoic acid derivative; this breakdown is more rapid in tolerant maize. Following pre-emergence or root treatment, the isoxazole is rapidly absorbed by the roots and emerging shoots and the DKN is translocated throughout the entire plant, distributing both in mature and young leaves with apoplastic movement being the predominant route. The redistribution of the DKN from mature to young leaves via the phloem (i.e., symplast) also appears to occur. There were no differences in uptake and translocation patterns between maize and sensitive weeds. The major factor for the selectivity of isoxaflutole appears to be the ability of maize to more rapidly metabolise the herbicidally active DKN to inactive benzoic acid.

Young and Hart (1998) indicated that isoxaflutole is translocated in the symplast following foliar application to giant foxtail (*Setaria faberi*). Symplastic movement allows a herbicide to accumulate at the active growth sites, which they found to be sinks for ^{14}C from isoxaflutole in foliar application. Young and Hart (2000) determined the mobility of isoxaflutole in giant foxtail and quantified the extent of translocation in the symplast and apoplast when absorbed by leaf and root tissue. They concluded that the symplast and apoplast are both responsible for herbicide translocation from foliar and root uptake of isoxaflutole.

Pallett *et al.* (2001) showed that leaf disk and root uptake of both isoxaflutole and DKN are passive processes and uptake from a solution of isoxaflutole and DKN will occur until the concentration inside the plant reaches equilibrium with the external concentration. The rapid conversion of isoxaflutole to DKN inside the plant, together with the greater potential for translocation of the DKN once inside the plant (Briggs *et al.* 1982; Shone and Wood 1974) reduces the internal concentration of the isoxazole in the root and further isoxaflutole will continue to be taken up passively. Root uptake of compounds occurs when they are dissolved in the soil water; therefore those with greater water solubility should be taken up more rapidly and in greater quantity. However, this is not the situation with isoxaflutole and DKN, the former being less soluble but taken up in five to six times greater quantity than DKN. DKN exists as an anion at physiological and

soil pH which will reduce root uptake compared with the non-ionised isoxaflutole (Briggs 1984).

Metabolism

In crop

Following uptake by the plant, isoxaflutole is rapidly converted to a DKN derivative via an opening of the isoxazole ring (Pallett *et al.* 1998) and this opening can be achieved spontaneously under basic conditions with pH > 9.0 (Pallett *et al.* 2001). This DKN metabolite is commonly the active herbicidal component and is a potent inhibitor of HPPD in plants (Pallett *et al.* 1997). Pallett *et al.* (2001) demonstrated that 6h after root treatment with radio-labelled isoxaflutole, the majority of ^{14}C activity extracted from the root co-chromatographs was from DKN, with no ^{14}C activity detected as isoxaflutole. DKN is very systemic in plants and is hydrolysed to the herbicidally inactive benzoic acid derivative (Pallett *et al.* 1998). The subsequent conversion of DKN to an inactive benzoic acid derivative within 6h of herbicide treatment in maize is evidence of the selectivity mechanism of isoxaflutole, while in the susceptible velvetleaf (*Abutilon theophrasti*), no degradation of the DKN was apparent after that time (Pallett *et al.* 2001). This degradation occurs more rapidly in tolerant species such as maize and this appears to be the basis of selectivity of isoxaflutole (Luscombe and Pallett 1996).

Young and Hart (2000) conducted a controlled environment study to examine the mobility of isoxaflutole in giant foxtail which is susceptible to isoxaflutole. They identified 27, 40, and 33% of recovered ^{14}C as parent isoxaflutole, DKN, and other metabolites, respectively, 24 h after treatment from the root metabolism studies. Conversion from parent isoxaflutole to DKN was more extensive in leaf tissue than in roots with 10, 68, and 22% of recovered ^{14}C identified as parent isoxaflutole, DKN, and other metabolites, respectively, in the treated area of the leaf 24 h after treatment.

Pallett *et al.*(1998) conducted experiments where they treated maize and weed plants (grown previously in hydroponic solution) by immersing the roots once in a nutrient solution containing ^{14}C-isoxaflutole for 3, 6, 12, or 24 h (pulse treatment) to assess the fate of radio-labelled isoxaflutole in treated plants. The results shown in Table

1 compare the uptake and subsequent distribution of ^{14}C activity up to 24 h following root treatment of maize and velvetleaf with ^{14}C-isoxaflutole.

Table 1: The recovery of isoxaflutole in maize and *A. theophrasti* following treatment of roots with ^{14}C-isoxaflutole (0.5 μg mL^{-1}) in hydroponic medium

Species	Treatment period (h)	^{14}C extracted (μg g^{-1})		% isoxaflutole equivalent detected		
		Roots	Shoots	Nutrient medium	Roots	Shoots
Maize	3	0.31	0.58	99	43	4
	6	0.22	1.08	98	34	2
	12	0.21	2.26	93	21	0
	24	0.18	3.06	87	9	0
A. theophrasti	3	0.25	0.61	100	65	19
	6	0.27	1.12	94	51	7
	12	0.26	2.78	88	32	2
	24	0.22	3.34	81	14	0

Source: Pallett *et al.* (1998)

They concluded that the total ^{14}C activity expressed on a fresh weight basis of root tissue was similar in tolerant maize and susceptible velvetleaf. Overall levels of ^{14}C activity were the same at the 3 to 24 h harvests. In shoot tissues, there was a progressive accumulation of extracted ^{14}C activity from 0.6 μg g^{-1} fresh weight (3 h) to over 3 μg g^{-1} (24 h) in both species indicating xylem mobility following root uptake. Analysis of the ^{14}C activity revealed that a significant amount of parent isoxaflutole was absorbed by the root which was readily degraded; this degradation increased with the length of the treatment period. In shoot tissues very low levels of the ^{14}C activity were extracted as parent isoxaflutole during the initial treatment period and after 6 h no parent isoxazole was detected. After a 3 h exposure with maize roots, 43% of the activity in the roots was parent isoxazole, compared with 4% in the shoot.

Pallett *et al.* (1998) conducted a further metabolic study that included morning glory (*Ipomoea* spp.-moderately susceptible to isoxaflutole), in addition to the tolerant maize and susceptible velvetleaf (Table 2).

Table 2: Identity of ^{14}C-labelled products in the shoot extracts of maize, *Ipomoea* spp. and *A. theophrasti* following a 1-day exposure of roots to [*phenyl*-U-^{14}C] isoxaflutole (0.5 μg mL^{-1}) via the hydroponic medium

	Maize		*Ipomoea* spp.		*A. theophrasti*	
	1 day	1+6 days[a]	1 day	1+6 days	1 day	1+6 days
^{14}C extracted						
% total ^{14}C in shoot tissue	95	87	92	85	94	91
μg equivalent g^{-1} in shoots	3.46	3.03	2.47	2.21	1.32	1.62
% distribution of metabolites in shoots						
Diketonitrile	80	29	77	57	89	82
Benzoic acid	13	59	12	31	0	12
Unidentified	7	12	11	12	11	6

[a]After a 1-day exposure period the roots were transferred to fresh untreated medium and the plants were grown for a further 6 days period. Source: Pallett *et al.* (1998)

There was no isoxaflutole detected in the shoot extracts after 24 h treatment and DKN accounted for 80, 77, and 89% of the extracted ^{14}C activity from maize, morning glory and velvetleaf, respectively. This declined to 29, 57, and 82%, respectively, in the three species after a further 6-day metabolism period. The hydrolytic cleavage of DKN was more rapid in the tolerant maize than in the two weed species. They concluded that susceptibility is correlated to the levels of the DKN in shoot tissue. Therefore, the fate of isoxaflutole in plants is rapid conversion of the DKN, which then undergoes further cleavage to a herbicidally inactive benzoic acid derivative, which is more rapid in tolerant than in susceptible maize.

In soil

The opening of the isoxazole ring of isoxaflutole also occurs in soil following pre-emergence application (Luscombe and Pallett 1996). Isoxaflutole is considered a proherbicide because its active form, a DKN derivative, is formed after application to the soil or inside the plant following opening of the isoxazole ring (Beltran *et al.* 2003). It has been reported that desorption coupled to hydrolysis promotes reactivation of isoxaflutole herbicide's function after rainfall and contributes to the efficacy of the compound by resupplying the soil solution with a bioactive product (Taylor-Lovell *et al.* 2002; Taylor-Lovell *et al.* 2000). Taylor-Lovell *et al.* (2000) also suggested that under dry conditions isoxaflutole is likely to remain relatively stable and unavailable. Subsequent rainfall could cause rapid transformation to the bioactive DKN derivative that exhibits much less soil sorption and thus allows uptake by germinating plants.

Pallett *et al.* (2001) found that half-lives were similar in sandy loam and clay loam soils but both soil moisture and temperature influenced the persistence of isoxaflutole. The half-life of isoxaflutole was shorter with increasing moisture content because isoxaflutole needs to be in solution for conversion to DKN. At 25°C, the half-life of isoxaflutole was significantly greater at < 2% moisture content than that at 30% moisture content, and at 30% moisture content the half life was much greater at 10°C than at 25°C. The formulation of isoxaflutole also has an influence on the extent of conversion to DKN. Formulation of isoxaflutole as a 750 g kg^{-1} water dispersible granule (WDG) resulted in a 1.5-fold increase in the half-life compared with the technical material. This is likely to be due to the slower release of the isoxaflutole from the granule into solution. Isoxaflutole was far less mobile in the soil than DKN, because they found that seven days after application, activity from isoxaflutole had moved to a depth of only 5-9 cm compared with 17-19 cm with DKN. The data from their experiment revealed that isoxaflutole was retained in the surface 0-3 cm, whereas DKN was mobile down to 9 cm in the column. The herbicidal activity in the soil is regulated by soil moisture which controls the rate of solution of isoxaflutole and its conversion to DKN. The more soluble and more mobile DKN is available for uptake by the roots, whereas the less soluble isoxaflutole is taken up by surface germinating weeds. Under dry conditions, isoxaflutole persists at the surface and leads to longer-term weed control.

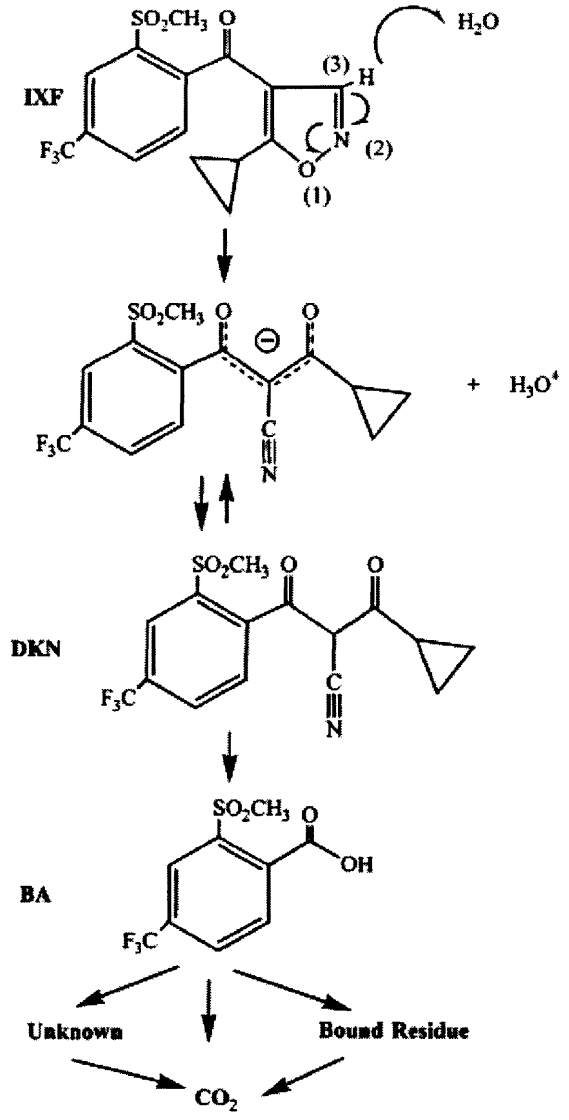
Sprague *et al.* (1999b) observed that in a spray solution of isoxaflutole at pH 7.0, 20% of the isoxaflutole was transformed into DKN after 3 h and 37% after 24 h; this degradation was faster at pH 10.0. They imbibed the maize and weed seeds for 24 h in solutions of isoxaflutole or DKN, and thereafter planted in a potting mix. Fourteen days after treatment, the height of the maize and weed plants was reduced when their seeds were imbibed in a solution of isoxaflutole; this reduction was much less when their seeds were imbibed in a solution of DKN. They concluded from the findings that isoxaflutole is more readily absorbed than DKN by seeds. On the other hand, when they applied isoxaflutole or DKN pre-emergence to soil, susceptible weeds were controlled similarly at equal rates 21 days after treatment. So, according to their study, the activity of isoxaflutole and DKN are thus equivalent when applied pre-emergence to soil.

Rouchaud *et al.* (2002) found that in the soil of the maize crops, DKN and benzoic acid were the main metabolites of isoxaflutole. They indicated that isoxaflutole was quickly transformed into DKN in soil. However during the crop growth, the transformation of isoxaflutole into DKN was never complete. The soil half-lives of isoxaflutole were between 9 and 18 days. The adsorption of isoxaflutole onto the solid phase of the soil and of its organic matter should decrease the rate of its transformation into DKN. The persistence of isoxaflutole in the soil was greater when organic fertiliser from animal origin had been applied recently. The sum of the concentrations of isoxaflutole and DKN therefore, represented the concentration of herbicide active products in the soil. The sum of the concentrations of isoxaflutole and of its metabolites DKN and benzoic acid did not account for the amount of isoxaflutole applied. The discrepancy increased with the delay after the application, showing that the acid was further metabolised in soil into common non-toxic products and ultimately into CO_2. In soil, isoxaflutole undergoes rapid conversion to DKN, and this condition is partially sensitive to light. In soil, the half-life of isoxaflutole is 20 h in light and 23 h in the dark, whereas the half-life of isoxaflutole is 40 h with aqueous photolysis conditions at 25°C in light. With low light, the photolytic half-life of DKN is over a month (Mitra *et al.* 1999).

Laboratory studies conducted by Luscombe and Pallett (1996) indicated that isoxaflutole has a relatively short half-life in soil and water. In soil, degradation proceeds by microbial degradation with CO_2 as a major product. They also reported that

isoxaflutole and its major metabolites have been shown to be potentially mobile in soil under high rainfall conditions. However, in the field, mobility and degradation occur simultaneously so that the potential to leach depends on the speed of degradation. The degradation rates of isoxaflutole and its metabolites, together with low application rates, make it unlikely that they could find their way into groundwater.

The conversion of isoxaflutole to DKN occurs rapidly under field conditions (Lin *et al.* 2000). In a field lysimeter study isoxaflutole was observed to be extremely unstable and DKN was the most predominant form of the herbicide detected in the leachate 30 h after application. The photolysis half-life of the parent isoxaflutole on or near the soil surface has been observed to be around 20-23 h (Mitra *et al.* 1999), whereas the half-life of isoxaflutole was observed to be < 9 h in an aqueous solution (250 μg L^{-1}) of pH 7.18 exposed to dim visible light (~10 μeinstein m^{-2}) at 25°C (Lin 2002). The low stability of isoxaflutole is associated with its high unequal electronic distribution in the isoxazole nucleus that results in the electron deficiency of the carbon-3 on the isoxazole ring (Beltran *et al.* 2000). Due to the electron deficiency, the proton released from the carbon-3 position has a strong potential to protonise a water molecule (pH 5.2-6) or a hydroxide ion and lead to the opening of the isoxazole ring (Beltran *et al.* 2000). Those authors reported a possible intermediate resonance stabilised enolated ion was formed before completely converting to the β-ketonitrile, DKN (Fig. 1). This high reactivity of the unsubstantiated 3-position has been observed in both alkaline and acid media (Beltran *et al.* 2000).



	Half-life in soil (days)	Partition coefficient K_{oc}*	Solubility in water (mg/L)
IXF	1.4-3	122	6.2
DKN	8-16	92	300
BA	20-60	69	8000

* K_{oc} – organic carbon partition coefficient.

Fig. 1. Degradation pathway and chemical properties of isoxaflutole (IXF) and its metabolites, diketonitrile (DKN) and benzoic acid (BA). Source: (Beltran *et al.* 2000)

2.2 Isoxaflutole and weed control

Isoxaflutole is a novel herbicide for pre-emergence control of a wide range of important broadleaf and grass weeds in maize and sugarcane (Luscombe and Pallett 1996; Luscombe *et al.* 1995). In field trials, isoxaflutole has proven to be effective against a wide spectrum of triazine-resistant weeds in maize production. It appears to perform well at relatively low dosages (11-64 g a.i. ha^{-1}) and offers season-long pre-emergence weed control (Lazo *et al.* 1997; Menendez *et al.* 1997). Isoxaflutole received conditional regulatory approval from the US Environmental Protection Agency in 1998 and was commercially introduced for the 1999 growing season in 16 key maize -producing states.

For many years maize growers have relied mainly on triazines and chloroacetanilides as the standard materials for pre-emergence control of broadleaf and grass weeds. Applied at high rates, these products have caused concerns related to toxicological and environmental safety leading to restrictions on usage. As a consequence, some European farmers, in particular, have no effective pre-emergence broadleaf weed control products at their disposal. In addition, weeds were developing resistance to the triazines which further limits their effectiveness.

Isoxaflutole provided a new opportunity for farmers to have effective pre-emergence weed control in maize. Application rates of 70-140 g a.i. ha^{-1} represent a dramatically reduced loading compared with 2 kg a.i. ha^{-1} and above for existing products. To add to the low application rates, isoxaflutole exhibits very low mammalian acute toxicity and is virtually non-toxic to aquatic vertebrates. From available evidence the persistence of isoxaflutole in the soil is unlikely to be an environmental problem. It is biodegradable, with the formation of CO_2 as the major degradation product, and is unlikely to cause concerns of environmental contamination. With a new mode of action, isoxaflutole is a herbicide with properties in line with modern toxicological and environmental requirements, and provides the farmer with broad spectrum weed control with the flexibility of application timing and less dependency on weather conditions.

In the USA trials, isoxaflutole applied pre-emergence at 105 g ha^{-1}, provided excellent control of major weed species, particularly velvetleaf. For most species efficacy was equal to or better than the standard mixture applied at a combined 4400 g ha^{-1}. The control of redroot amaranth (*Amaranthus retroflexus*) was slightly inferior to the standard

mixture; only pale pigeon grass (*Setaria glauca*) control was deficient with isoxaflutole alone. Mixtures of isoxaflutole with reduced rate chloroacetanilides or dimethenamid generally improved weed control still further and compensated for the weaknesses on redroot amaranth and pale pigeon grass. In the European trials, Luscombe *et al.*(1995) observed that isoxaflutole applied alone at 75 g ha^{-1} did not give sufficient weed control. Mixtures with reduced rate chloroacetanilides or dimethenamid gave virtually complete control of all important weed species with the exception of black bindweed (*Bilderdykia convolvulus*) which tolerated all treatments.

Isoxaflutole has also showed potential for use in sugarcane. Luscombe *et al.* (1995) also found that isoxaflutole was more active on the grass species such as alexander grass (*Brachiaria plantaginea*) and crowsfoot grass (*Eleusine indica*) than on broadleaf species [e.g. senna (*Cassia* spp.), common sida (*Sida* spp.) and morning glory]. Luscombe *et al.* (1995) further reported that sugarcane, particularly the ratoon crop, tolerated isoxaflutole at a higher rate when applied pre-emergence than post-emergence. At the pre-emergence rates of 150 g ha^{-1}, grass weed control with isoxaflutole alone was superior to that provided by the standard mixture of hexazinone and diuron. However, control of broadleaf weeds was lower than with the standard treatment. When mixed with ametryn, broad spectrum weed control of grass and broadleaf weeds was achieved which was generally better than the standard treatment. Post-emergence at the lower rate of 100 g ha^{-1}, isoxaflutole alone performed less well than the standard mixture being particularly deficient on the broadleaf weeds. However, in a mixture with ametryn, grass weed control was improved over that provided by the standard treatment. Although the mixture with ametryn gave inferior control of common sida and particularly scurvy weed (*Commelina diffusa*) compared with the standard, control of *Amaranthus* spp. and cobbler's pegs (*Bidens pilosa*) was similar to that of the standard treatment.

Isoxaflutole selectively controls both grass and broadleaf weeds in maize (Bhowmik *et al.* 1996; Rouchaud *et al.* 1998; Vrabel *et al.* 1997). Isoxaflutole applied pre or early pre-plant at 75 to 140 g ha^{-1} controls large crabgrass (*Digitaria sanguinalis*), barnyard grass (*Echinochloa crus-galli*), giant foxtail (*Setaria faberi*), green foxtail (*Setaria viridis*), fall panicum (*Panicum dichotomiflorum*), velvetleaf (*Abutilon theophrasti*), redroot pigweed (*Amaranthus retroflexus*), common ragweed (*Ambrosia*

artemisiifolia), common lambsquarters (*Chenopodium album*), Pennsylvania smartweed (*Polygonum pensylvanicum*) (Bhowmik *et al.* 1996; Luscombe *et al.* 1995; Vrabel *et al.* 1997; 1998). Isoxaflutole has provided inconsistent control of *Ipomoea* species and common cocklebur (*Xanthium strumarium*) (Curvey and Kapusta 1995; Obermeier *et al.* 1995). Control of grass species such as giant foxtail has been documented to be rate responsive to isoxaflutole applications (Bhowmik *et al.* 1996; Luscombe *et al.* 1994).

In Australia, isoxaflutole at 75 g a.i. ha^{-1} is registered for the control of several broadleaf weeds (Indian hedge mustard, sowthistle, capeweed, prickly lettuce, wild radish, turnip weed, crassula, medic, deadnettle, and slender celery etc.) in chickpea. Clarke *et al.* (2001) conducted a field trial in 2000 at Beverley, Western Australia on a red loam soil and concluded that isoxaflutole at 75 g a.i. ha^{-1} gave excellent control of Indian hedge mustard (100%), capeweed (100%) and wild radish (94%). They also indicated that isoxaflutole is expected to be initially registered on the Western Australian key weeds of Indian hedge mustard, capeweed and wild radish plus key eastern states weeds, prickly lettuce, sowthistle and turnip weed. Results across Australia over the last 4 years showed that isoxaflutole at 75 g a.i. ha^{-1} provides good broadleaf weed control. The addition of simazine improves broadleaf weed control while simazine provides some grass weed suppression.

Isoxaflutole and management of herbicide resistance

Development of resistance by weeds to a number of herbicides is becoming a major concern in the weed science community (Shaner 1995). Resistance via altered target sites has been identified for 6 classes of herbicides. Luscombe and Pallett (1996) made an assessment for the potential of resistance development to isoxaflutole by screening a large population of mutated *Arabidopsis* seeds. They did not detect any resistant mutants in their trials. Under such conditions, mutants have been obtained to sulfonylureas due to an altered target site, acetolactate synthase (Haughn and Somerville 1986). Therefore, Luscombe and Pallett (1996) concluded that the risk of weed resistance to isoxaflutole due to mutation in the target site, HPPD, is low. Consequently, isoxaflutole provides a useful tool in combating weed resistance to other widely used herbicides.

2.3 Tolerance of crops to herbicides

Screening for herbicide × cultivar interactions

Some cultivars of a crop species are more susceptible to damage from certain selective herbicides than others. This is called differential cultivar tolerance. Reductions in grain yield from the recommended rate of application are a measure of reduced field tolerance (Fryer and Makepeace 1977). Such yield losses in barley (*Hordeum vulgare*) have been demonstrated in sensitive cultivars (Lemerle *et al.* 1986) and can be as much as 50%, depending on the site and season. Farmers generally do not notice crop damage unless it causes severe symptoms and so have not considered crop tolerance in weed management decisions. However, crop damage has a major impact on optimal control strategies, especially at low weed densities (Pannell 1990). Even though companies say their products are safe, in certain circumstances they are not.

It is the responsibility of the chemical manufactures to make appropriate label warnings of cultivar susceptibility when a new herbicide is released. Ideally plant breeders should also check the reaction of new cultivars to the commonly used herbicides (Lupton 1980) but this may be an unrealistic expectation. The yield benefit of a new cultivar may be lost in some situations if it has a low tolerance to a widely used selective herbicide. In Australia, the only label warning against the use of isoxaflutole is for the cultivar Yorker in chickpea. Yorker has exhibited sensitivity to isoxaflutole in some New South Wales Department of Primary Industry trials. In 2003, Yorker displayed more visual damage in the vegetative stage than Jimbour, Howzat and Amethyst, but there was no statistically significant reduction in yield (Knights *et al.* 2007).

However, a problem with this information is that it does not give any indication of the likelihood (or risk) of crop damage in different environmental conditions. There is a need to determine the factors that cause variation in crop tolerance between seasons and sites in order to accurately predict the most efficient weed control strategies (Pannell 1990).

Effects of isoxaflutole on maize and chickpea

Isoxaflutole applied alone or in combination with other herbicides like metolachlor or atrazine did not injure maize at effective weed control rates (Luscombe *et al.* 1995; Young *et al.* 1999). However, in alkaline soils (pH greater than 7.4) and in sandy soils, crop injury has been observed, particularly in short-season hybrids or when heavy rainfall occurs shortly after application (Luscombe *et al.* 1995). Sprague *et al.* (1999) noted that injury in maize was most commonly observed in coarse-textured soils with low clay and organic matter and was more severe with higher rates of isoxaflutole. Injury to maize was not unique to any tillage systems and was dependent on site, year, and application rate of isoxaflutole (Sprague *et al.* 1999). Bhowmik *et al.* (1996) also did not observe any appreciable injury to maize, even at 158 g ha^{-1} of isoxaflutole, with no adverse effect on maize grain and silage yields in a field study on a Hadley fine sandy loam containing 3.2% organic matter with a pH of 6.8.

There have also been reports of differences in tolerance to isoxaflutole between maize hybrids. Sprague *et al.* (1998b) reported that the maize hybrids 'Pioneer 3751 (P-3751)' and 'Pioneer 3737 (P-3737)' were less tolerant to isoxaflutole than the hybrids 'Pioneer 3394 (P-3394)' and 'Pioneer 3963 (P-3963)'. Bhowmik *et al.* (1999) reported bleaching injury to maize in the field which was less than 10%, and it was found only with the 220 g ha^{-1} rate of isoxaflutole. This injury was temporary and the plants recovered within 2 to 3 weeks.

In Australia, damage of chickpea crops due to the application of isoxaflutole was first reported by Felton *et al.* (2004). From the pot and field trials conducted in 2002 at Tamworth Agricultural Institute, New South Wales, they suggested that chickpea crop damage from isoxaflutole was particularly severe with one experimental line, 91025-3021. Pot and field trials in 2003 confirmed 91025-3021 to be more sensitive than the currently recommended varieties, Amethyst, Howzat and Jimbour. However, under some conditions all varieties were damaged at the recommended rate. They also found Yorker (previously 9113-13N-2) to be more sensitive to isoxaflutole than the current varieties. Although isoxaflutole provides good control of some problem weeds in chickpea, it is important to appreciate the risks of crop injury. This often is transient but they found that under some situations there was a yield penalty even with an application less than the

recommended rate of 75 g a.i. ha^{-1}. There was also less herbicide injury to the kabuli variety Bumper than to the desi varieties Amethyst, Howzat, Jimbour and 91025-3021.

2.4 Soil and environmental factors which influence isoxaflutole bioactivity

The environmental factors that lead to differences in herbicide selectivity between seasons are air temperature, rainfall, relative humidity, light, wind, soil temperature and soil moisture. Plant physiological processes (uptake, translocation and metabolism) are influenced by these environmental factors, as well as the availability of herbicides in the soil (Beyer *et al.* 1987).

The principles of herbicide × environmental interactions are well documented in weeds (Caseley 1987; Gerber *et al.* 1983; Muzik 1976), but their effects on crop tolerance have been investigated less intensively. Being a soil-applied herbicide, it is important to understand factors that influence the fate and behavior of isoxaflutole and its degradates (i.e., DKN) in soil. Hydrolysis of isoxaflutole requires special attention because it is considered to be a proherbicide, or one that becomes active after transformation. In this case, the DKN derivative of isoxaflutole has been proven as an active form of the herbicide (Pallett *et al.* 1998), making it important to understand what conditions influence hydrolysis to form this product (Taylor-Lovell *et al.* 2000). This active DKN derivative can be formed in the soil prior to plant uptake, or after the plant has absorbed isoxaflutole. The sorption of DKN must also be considered in relationship to isoxaflutole, as the two chemicals are likely to be in the system simultaneously (Taylor-Lovell *et al.* 2000). Sorption not only is useful in retaining a pesticide in the zone where it is most likely to control weeds but it also decreases the bioavailability of the compound, a problem that may be overcome by increasing the dose rate (Leistra and Green 1990).

Hydrolysis of isoxaflutole to DKN in soil has been shown to be influenced by the soil moisture content, temperature, and pH and is primarily the result of chemical processes. However, further degradation of DKN to benzoic acid has been reported to be primarily the result of biodegradation (Beltran *et al.* 2001; Beltran *et al.* 2003). DKN is more water soluble than isoxaflutole (6.2 mg L^{-1} isoxaflutole vs 300 mg L^{-1} DKN), has a greater rate of uptake in plants, and is more mobile in soil than isoxaflutole (Pallett *et al.*

2001). As a result, the chances of crop injury and groundwater contamination due to leaching may be more likely to occur with DKN than with isoxaflutole.

Removal of herbicides from solution by sorption onto the soil surface, and desorption when water is subsequently added, are major factors controlling herbicide activity, mobility and persistence in soil (Wauchope and Koskinen 1983). If a herbicide is irreversibly bound to soil or if its desorption is very slow, its mobility and release back into the solution is negligible. The extended period of time involved in desorption of pesticides from soil suggests the possibility that some fraction of the solute may be 'irreversibly bound' to soil. This phenomenon is called hysteresis and has been reported for hydrophobic organic contaminants and herbicides (Barriuso *et al.* 1994; Celis *et al.* 1997; Huang and Weber 1998; Mersie and Seybold 1996).

Isoxaflutole acts through soil; therefore sorption plays an important role in understanding its fate and behaviour in soil. Soils are heterogeneous systems and have a tremendous capacity to adsorb chemicals. Sorption reactions offer a major mechanism for the attenuation of environmentally sensitive compounds. Sorption contributes to large-scale processes, as in the transport of chemicals from soil to groundwater, and to microscale processes, as in diffusion and biotransformation (Mitra *et al.* 1999). The removal of herbicides from solution by sorption is a major factor controlling herbicide activity, mobility, persistence and environmental fate (Wauchope and Koskinen 1983; Weber and Peter 1982). Only pesticides in solution, or readily desorbable from soil, are available for plant uptake, degradation, or transport. Pesticides that are easily desorbed would be readily available, whereas pesticides that are strongly sorbed and hysteretic during desorption would be slowly available over time or unavailable.

Sorption-desorption processes are affected by the physical and chemical properties of the pesticide and soil. Therefore, it is important to understand the influence of factors such as soil pH, organic matter, and clay content on the strength of sorption of isoxaflutole and DKN to soil. In soils low in organic matter, pesticide sorption and desorption by clay minerals may strongly influence the fate of pesticides in soil environments (Barriuso *et al.* 1994). In soil, clay and organic components are usually intimately associated such that their individual effects on sorption are difficult to separate. However, mineral surfaces may be obscured by their association with organic

colloids (Cox *et al.* 1998). The association of clay minerals and organic matter comprises a composite of swollen condensed domains (Huang and Weber 1998). These condensed domains create many micro and macro pores which sorb DKN and entrap it, leading to sorption-desorption hysteresis. Assessing the hydrolysis of isoxaflutole to DKN and the influence of the variability of soil properties within a field on sorption-desorption of both isoxaflutole and DKN will improve the understanding of the potential availability of these compounds for plant uptake, degradation, and/or off-site movement (Rice *et al.* 2004).

Effects of environmental factors

Both soil moisture and temperature influence the persistence of isoxaflutole. Moreover, biological activity had little effect on the hydrolysis of isoxaflutole, with half-lives of 1.8 and 1.4 days in sterile and nonsterile soil, respectively. However, the transformation of DKN to benzoic acid and the production of the unknown degradation products were greatly reduced in the sterile soil, suggesting one or more biologically mediated processes (Taylor-Lovell *et al.* 2002).

Effects of moisture

Taylor-Lovell *et al.* (2002) investigated the effects of several environmental factors on the dissipation, transformation, and mineralisation of isoxaflutole in laboratory incubations at University of Illinois, USA. For the moisture study, they included four different moisture treatments which were air-dry, -1500 kPa, -100 kPa, and initially air-dry (for 14 d) followed by wetting to -100 kPa. For the moisture study only, they treated the soil in bulk with ^{14}C-isoxaflutole at a concentration equivalent to the field-used rate of 105 g ha^{-1} and assuming a 15-cm mixing depth in the field. They reported from the experiment that the total recovery of radio-labelled material was 97% averaged across moisture regimes and sampling days. Mineralisation was detected in the -100 kPa soil at 7 days sampling and in the -1500 kPa soil at 14 days indicating that mineralisation was rapid with increase in moisture content. At 56 days, mineralisation increased to 9 and 28% of the applied ^{14}C in the -1500 and -100 kPa soils, respectively. No mineralisation was detected in the air-dry soil over the duration of the study, which was related to the lack of moisture for the initial hydrolysis of isoxaflutole to DKN. In the samples that were initially air-dry and

subsequently adjusted to -100 kPa at 14 days, mineralisation (0.2% of applied ^{14}C) was detected 7 days after wetting, and this increased to 11% by 56 days. Transformation of isoxaflutole to DKN, benzoic acid, and other products was stimulated by the presence of water. In air-dry soil, isoxaflutole was slowly transformed to DKN, and other products were not detected until the last sampling time (56 d). In the -1500 kPa soil, transformation of isoxaflutole to DKN was much more rapid, with DKN accounting for 68% of the extractable phase at 3 days after treatment. After 7 days, the fraction of DKN began to decline as other products were formed, presumably at the expense of this intermediate compound. The half-lives for isoxaflutole were 9.6 days in air-dry soil, 2.4 d in -1500 kPa soil, and 1.5 d in -100 kPa soil. Degradation rates began to rapidly increase after the addition of water (-100 kPa) to previously air-dry soil at 14 days after treatment. One day after re-wetting, the majority of remaining isoxaflutole had been transformed to DKN. This rapid transformation represents what could happen when rainfall occurs after application of the herbicide under dry conditions. DKN is more soluble than isoxaflutole (326 $\mu g\ mL^{-1}$ versus 6.2 $\mu g\ mL^{-1}$), thus it may be more available for plant uptake under wet conditions.

Beltran *et al.* (2003) conducted a separate study to determine the relative importance of, and factors affecting, the degradation of isoxaflutole in soil at the University of Montpellier I, France. They studied the influence of soil moisture on a sandy loam soil (pH 6.1, 9.4% clay, 73.8% sand, 16.7% silt and 1.06% organic carbon) at 30°C. The moisture contents were 17% (2 mL of isoxaflutole solution at 50 mg L^{-1}), 29% (4 mL at 25 mg L^{-1}), and 45% (8 mL at 12.5 mg L^{-1}), maintained constant throughout the experiment. They concluded from the experiment that the rate of isomerisation of isoxaflutole to DKN increased with higher moisture content. They further suggested that these results are of importance under agricultural conditions since rainfall quantity and frequency will affect the rate of formation of the active ingredient and, consequently, its migration toward deeper layers. Moreover, according to previous results (Beltran *et al.* 2000), for 30°C temperature and pH 6, the half-life of isoxaflutole in aqueous sterile solution should be 110 h; this value is higher than the half-lives determined here and confirms the catalytic effect of the soil reported by Taylor-Lovell *et al.* (2000).

Pallett *et al.* (2001) found that increasing moisture level and temperature increased the phytotoxicity symptoms in maize treated with isoxaflutole. At 25°C and < 2% moisture content, the half-life of isoxaflutole was significantly greater than that at 30% moisture content and at 30% moisture content the half life was much greater at 10°C than at 25°C. They concluded that although the half-lives were similar in sandy loam and clay loam soils, both soil moisture and temperature influence the persistence of isoxaflutole.

Effects of temperature

Taylor-Lovell *et al.* (2002) created soil biometers and maintained these at 5, 15, 25, or 35°C. They reported the total recovery of applied radioactivity averaged 97% across four temperatures when added as isoxaflutole, and 99% at 25°C when added as DKN. They found that the distribution of ^{14}C among the measured pools, however, was profoundly influenced by temperature and the rate of mineralisation was positively affected by temperature. After 56 days of incubation, 16% of the applied ^{14}C appeared as CO_2 in the 35°C treatment, whereas negligible mineralisation had occurred in the 5°C treatment. Mineralisation was detected earlier and accounted for a greater proportion of radioactivity when added as ^{14}C-DKN compared with ^{14}C added as isoxaflutole at 25°C. This suggests that conversion to DKN is an important step in the path toward mineralisation. Product formation followed the same temporal sequence (isoxaflutole > DKN > benzoic acid > unknown minor products) at each temperature; however the effect of temperature on the rate of formation of the products resulted in a different distribution of products at each temperature. At 5°C, isoxaflutole transformation was restricted primarily to DKN, which accumulated to 77% of the extractable phase by the 56-d sampling time. Accumulation of the benzoic acid and other minor products was observed when the temperature was increased to 15°C. The DKN derivative was the primary product at all temperatures throughout the study. The half-lives for dissipation of isoxaflutole were 13.9, 3.3, 1.3, and 0.8 d at 5, 15, 25, and 35°C, respectively. Formation of minor products was similar at temperatures ranging from 15 to 35°C, indicating these steps were less temperature responsive.

Beltran *et al.* (2003) conducted a study to determine the relative importance of, and factors affecting, the degradation of isoxaflutole in soil at the University of Montpellier I, France. They studied the influence of temperature using a Mediterranean clay loam soil (pH 8.0, 21.9% clay, 47.1% sand, 31.1% silt and 3.15% organic carbon) at 17% moisture content, and five different temperatures were applied: 10, 20, 30, 40 and 60°C. They concluded that the rate of decrease of isoxaflutole was higher at higher temperatures. They also evaluated the role of chemical and biological processes in isoxaflutole isomerisation and found that the destruction of biological microflora at high temperatures did not affect the reaction and confirmed the chemical character of isoxaflutole isomerisation in the soil at 17% moisture content. The formation of benzoic acid was quantitatively followed at 20, 30, 40, and 60°C. At 20 and 30°C, the amount of benzoic acid formed increased to a maximum before decreasing to the limit of quantification. This maximum was higher at 30°C than at 20°C, and occurred sooner (760 vs 1380 h), with a shorter lag phase. At 40 and 60°C, where biological activity is weakened, the amount of benzoic acid formed did not exceed 0.4 mg kg^{-1} (at 40°C), and no maximum could be determined.

Biological dependence study

Taylor-Lovell *et al.* (2002) conducted controlled laboratory experiments to examine the role of microorganisms on the degradation of isoxaflutole in soil. They reported from the studies that isoxaflutole half-lives were similar in the presence and absence of the inhibitor and DKN is formed abiotically, and that other products arise from DKN rather than from isoxaflutole. Formation of the benzoic acid derivative and other end products resulted from biological reactions. Beltran *et al.* (2003) also reported that the degradation of DKN to benzoic acid appeared to be essentially due to the biological activity of the soil.

Effects of soil factors

As a pre-emergence herbicide, isoxaflutole is applied to soils. As mentioned earlier sorption processes are important in determining the fate and behavior of the herbicide in the environment, as it controls other processes such as its transport, mobility and degradation (Bailey and White 1970; Calvet 1989; Koskinen and Harper 1990). Taylor-

Lovell *et al.* (2000) studied hydrolysis and soil sorption of isoxaflutole and its DKN degrade. They demonstrated that most (83%) of the isoxaflutole remained in solution after 24 h in a soil-free system, but only 15% remained in solution in the presence of soil. They also found that isoxaflutole was sorbed, by four soils, to a greater extent than DKN and that sorption of both compounds increased with higher soil organic matter contents. Mitra *et al.* (1999) also showed that sorption of ^{14}C-isoxaflutole residues (presumably a mixture of isoxaflutole and DKN) was mainly influenced by the organic matter content and pH, but was not influenced appreciably by clay content. In another study, Mitra *et al.* (2000) also showed that DKN had a sorptive behavior similar to that shown for ^{14}C-isoxaflutole residues, where sorption was mainly influenced by organic matter content.

Sorption of isoxaflutole has also been shown to be dependent on organic matter and not affected by pH or clay content (Beltran *et al.* 2002). Like isoxaflutole, DKN sorption has been shown to increase with increased organic matter content (Mitra *et al.* 1999; Taylor-Lovell *et al.* 2000). In contrast, Beltran and colleagues (2002) found that soil organic matter, pH and clay content had no effect on DKN soil sorption. While DKN was not shown to be affected by selected clay minerals, sorption of a variety of other herbicides has been shown to be affected by numerous clays (Cox *et al.* 1998). Moreover, Carrizosa and colleagues (2004) reported that the DKN anions form a stable chelate with cations and/or low co-ordinated lattice cations in the interlayer of organo-clays.

Effects of organic matter

Soil organic matter is the primary sorbent for hydrophobic organic compounds in the soil and isoxaflutole falls into the same category (Chiou 1989). The mechanism of release of hydrophobic compounds entails their partitioning into the organic fraction of soil. Moreover, the extent of sorption of such compounds is related to the organic matter content of the soil. However, isotherm non-linearity of isoxaflutole in soils having high organic matter content cannot be attributed to mineral surface, but can be attributed to the characteristic of soil organic matter.

Mitra *et al.* (1999) studied the sorption of isoxaflutole in five different soils. They demonstrated that sorption of isoxaflutole also increased with an increase in organic matter content, but isoxaflutole was more strongly sorbed than DKN on all soils,

probably due to lower aqueous solubility of isoxaflutole. Therefore, DKN has greater leaching potential than isoxaflutole itself in soils with low organic matter content.

Mitra *et al.* (2001) conducted sorption studies of the DKN metabolite of isoxaflutole with five soils varying in physical and chemical properties. They reported that DKN sorption was highly correlated with organic matter content and DKN sorption increased with an increase in organic matter content. Soils with relatively higher organic matter content will have greater affinity for DKN and hence, the availability and release of DKN will be less in these soils compared with soils with lower organic matter content (Mitra *et al.* 2000).

Organic carbon content is important in isoxaflutole and DKN binding (Bresnahan *et al.* 2004). Organic carbon-clay associations, along with pH, may be the main factors affecting sorption of isoxaflutole and the DKN metabolite.

Beltran *et al.* (2002) conducted a study on seven soils varying in physical and chemical properties to characterise the retention/sorption of isoxaflutole and its two main derivatives- DKN and benzoic acid. They concluded that the main parameter influencing the adsorption of isoxaflutole appeared to be the organic matter content, whereas this effect was not evident for DKN and benzoic acid.

Effects of soil pH

Soil pH and organic matter content are important factors governing the sorption of herbicides (Fontaine *et al.* 1991; Lehmann *et al.* 1992). In laboratory studies, Beltran *et al.* (2000) reported that isoxaflutole is more stable under acidic conditions than in neutral or alkaline media regardless of the prevailing temperature. Mitra *et al.* (1999) reported that isoxaflutole sorption was decreased as soil pH increased from 4.5 to 8.5. Mitra *et al.* (2001) also reported that with increase in soil pH, the sorption of DKN was decreased. The strong binding of isoxaflutole/DKN to the soil at lower pH may be due to low basicity of the isoxazole ring, which enables proton addition to DKN at low soil pH. Hence, the sorption of isoxaflutole/DKN by cation exchange is higher at low soil pH than soils at high pH. This suggests that the chances of injury are less in soils with high organic matter content and low pH as the amount of available isoxaflutole in soil solution will be reduced.

Rouchaud *et al.* (1998) reported that isoxaflutole dissipation in soils is slightly faster at pH 7.2 than at 5.5. They concluded that at pH 8.5 some of the organic matter might dissolve in the solution and contribute to the reduction of isoxaflutole/DKN sorption. Similar results have been reported by Huang and Weber (1997), where they observed background dissolved organic matter at pH 7.0.

Soil pH is a very important factor that affects sorption and degradation of herbicides. The effects of soil pH and organic matter content on flumetsulam sorption in soil are well documented (Fontaine *et al.* 1991; Lehmann *et al.* 1992), and leaching is more likely in soils with low organic matter and high pH (Kleschnick *et al.* 1992). Shaw and Murphy (1997) reported that flumetsulam mobility in soils with similar organic matter content increased as soil pH increased from 5.3 to 7.2. They attributed the greater mobility to its ionic species at pH $\geq$ 7.2. Therefore, like many other herbicides and their metabolites, sorption of isoxaflutole (Mitra *et al.* 1999) and DKN depends on organic matter and soil pH.

Effects of clay content

The clay fraction of soil and associated organic components has been shown to be responsible for the sorption of many soil-applied pesticides (Barriuso *et al.* 1994). In soil, clay and organic components are usually intimately associated such that their individual effects on sorption are difficult to separate. However, mineral surfaces may be obscured by their association with organic colloids (Cox *et al.* 1998). In soils, however, the intimate association between the individual constituents may cause some modification of their sorptive properties (Posino *et al.* 1992). Sorption of DKN in soils was found to be influenced primarily by organic matter, whereas clay components did not greatly affect sorption. Similarly, clay content appeared to have minimal effect on imazaquin sorption by soils from Alabama, USA (Goetz *et al.* 1986).

Mitra *et al.* (1999; 2001) reported that the sorption of isoxaflutole/DKN was not influenced by clay content. Rouchaud *et al.* (1998) also indicated that sorption, persistence and mobility of isoxaflutole in soils containing 2% or more organic matter were found to be correlated with organic matter content and not with the soil texture.

Carrizosa *et al.* (2004) investigated the sorption of isoxaflutole and its main degrade, DKN to natural and organo-clay derivatives. They pointed out that the transformation of isoxaflutole to DKN did not differ significantly between the clay-free, and in the presence of natural clays. They found much greater decomposition in the presence of organo-clays, suggesting sorption catalysed decomposition. Beltran *et al.* (2002) reported the same result where they did not find any correlation between the extent of adsorption and either clay content or pH of the soil, for isoxaflutole, DKN and benzoic acid.

Effects of Ca^{2+} concentration

Mitra *et al.* (1999) demonstrated minimal effect of Ca^{2+} concentration on the sorption of isoxaflutole. However, later studies by Mitra *et al.* (2001) found that concentration of Ca^{2+} had a significant effect on sorption of DKN. They corroborated that the increase in sorption of DKN at high Ca^{2+} concentrations might be due to cationic bridges. Ca^{2+} can form cationic bridges with the two oxygen atoms of the keto groups in DKN. These bridges might contribute to an increase in DKN sorption, but the role of Ca^{2+} concentration in crop injury may be ruled out.

2.5 Soil nitrate levels and isoxaflutole injury

Most Australian soils are inherently low in nitrogen (N) so the N input potential of grain legumes such as chickpea is most important (Evans 1982a). Evans (1982b) showed that increasing the soil nitrate level from 20 to 80 mg N kg^{-1} greatly reduced N_2 fixation. In other species, nodulation decreased with increasing N supply (Pal and Saxena 1975) although small amounts of available N during early growth may benefit the symbiosis (Harper 1974). Anderson *et al.* (2004) found a reduction in chickpea shoot and root biomass from the application of fertiliser N and another soil applied herbicide, chlorsulfuron. Anderson *et al.* (2004) also found a decreasing trend in nodule number of chickpea when chlorsulfuron was present in the soil. They also suggested that the addition of N fertiliser reduced the nodule weight of chickpea plants and the magnitude of the reduction was greater with the presence of chlorsulfuron in the soil. Kumar *et al.* (1981) observed a drastic reduction in nodulation of chickpea when simazine was applied to the soil surface at 1.6 and 3.2 kg ha^{-1}. Nodulation of chickpeas is known to decrease

with increasing levels of soil inorganic N (Jessop *et al.* 1984) and nodulation *per se* can be impaired in the presence of nitrate (Summerfield *et al.* 1977; Wong 1980). Rawsthorne *et al.* (1985b) also recorded a significant reduction in total nodule numbers of chickpea grown at 32.5 °C with 1.43 and 2.86 mM nitrate levels 28 days after sowing. The effects of different soil nitrate levels influencing the degree of isoxaflutole injury to chickpea growth and nodulation are not well documented.

2.6 Herbicide and legume nitrogen fixation

Legume benefits in crop rotation

Legumes, via biological N fixation, provide an alternate source of N to fertilisers (Peoples *et al.* 1995; Schwenke *et al.* 1998) so that the N balance following a legume-cereal rotation will be higher than for a cereal-cereal rotation in the same soil when addition of fertiliser N is excluded from calculations (Peoples *et al.* 1995). A N benefit may also be derived from an N-sparing effect of the legume (Chalk 1996) in which soil mineral N not taken up by legumes actively fixing N, remains available to subsequent cereal crops. However, N benefits in legume - cereal rotations have often been attributed entirely to the transfer of biologically fixed N (Munyinda *et al.* 1988). An alternative concept is that N benefits are not due to the transfer of fixed N, but can be explained by greater immobilisation of nitrate during the decomposition of cereal compared with legume residues (Green and Blackmer 1995).

Major cereal growing areas of southern Queensland and northern New South Wales have experienced declining crop yields and grain protein concentrations (Dalal *et al.* 1991; Martin *et al.* 1988). For example, mean protein concentrations in wheat (*Triticum aestivum*) in southern Queensland have fallen below 10% since 1980, thus preventing farmers from obtaining 'Prime Hard' status (13%) for their wheat crop. For improving the wheat grain yield and grain protein, the two management options are the application of nitrogenous fertiliser in a wheat monoculture (Holford *et al.* 1992; Strong *et al.* 1996) or the inclusion of a legume in rotation with wheat (Herridge *et al.* 1995).

Other benefits of legumes include control of cereal diseases. For example, Reeves *et al.* (1984) found that incidence of take-all (*Gaeumannomyces graminis*) in wheat was negligible following a lupin crop compared with 36% incidence in continuous wheat

cropping. Also, the incidence and severity of crown rot of wheat, caused by *Fusarium graminearum*, appeared to have been reduced by rotation with chickpea in southern Queensland (Wildermuth *et al.* 1992).

Benefits of including chickpea in crop rotation

Cropping systems with legumes, especially pastures, have been used successfully in southern Australia (Donald 1965) although the application of such rotation systems in Queensland has been limited, except for the pasture legume, lucerne (*Medicago sativa*) (Littler and Whitehouse 1987), and the grain legume, chickpea (Doughton 1988). Chickpea has been widely used in cereal rotations (chickpea–wheat) for long-term benefits on soil N, wheat grain yields and grain protein concentrations (Ahlawat *et al.* 1981; Doughton *et al.* 1993). Marcellos (1984) found that after one season of chickpea crop grown on an Alfisol in Tamworth, New South Wales, wheat yield in the following year increased from 1.5 t ha^{-1} in the wheat–wheat sequence to 3.0 t ha^{-1} in the chickpea–wheat sequence. The N benefits were equivalent to an application of 50 kg N ha^{-1} as ammonium nitrate. Strong *et al.* (1986a) reported a significant increase in nitrate-N concentration following 6 months of fallow after a chickpea crop. Similarly, Hossain *et al.* (1996a) measured a significantly higher mineral nitrate-N concentration after chickpea than after wheat. Agronomic efficiency of N benefits from chickpea have been calculated in terms of fertiliser N equivalents in short-term rotations (Marcellos 1984; Strong *et al.* 1986b).

Dalal *et al.* (1998) further concluded that the magnitude of wheat yield is likely to be dependent on several factors including seasonal conditions (both fallow period rainfall and in-crop rainfall), performance of the previous chickpea crop (plant biomass, grain yield, N accretion and grain N removal), soil fertility levels, tillage practices and disease incidence. Cultural practices such as sowing time of the previous chickpea crop and tillage practices affected the yields of the following wheat crops.

Dalal *et al.* (1998) recorded a small but significant increase (0.5%) in grain protein concentrations of wheat following chickpea. Felton *et al.* (1998) reported similar grain protein responses (0.6%). They concluded that supplementary applications of fertiliser N to wheat were required to increase protein concentrations of wheat to 'Prime

Hard' classification. However, residual fertiliser N left after the wheat crop decreased N_2 fixation by the following chickpea crop (Dalal *et al.* 1997).

Herbicide effects on pulse crop nodulation and nitrogen fixation

The inclusion of legumes in rotations with cereals or other crops can improve soil structure, provide a break in disease and cereal pest cycles, and increase the options available for weed control (Peoples *et al.* 1992). The presence of weeds within crops can reduce crop yields, hinder harvest operations, and contaminate produce (Powles *et al.* 1996), and herbicides are in common use for weed control in pastures and crops (Lemerle *et al.* 1996). However, residual levels of some herbicides have been found to inhibit nodulation (Eberbach and Douglas 1989; Martensson and Nilsson 1989) and N fixation (Koopman *et al.* 1995) by legumes and may have negative effects on the N balance of legume-cereal rotations.

Many cereal cropping regions of temperate Australia rely mainly upon spontaneous regeneration of pasture legumes for the input of N into the soil. Reports have appeared in the literature of herbicide-induced declines in nodulation of legumes (Bollich *et al.* 1985; Dunigan *et al.* 1972; Eberbach and Douglas 1983; Fletcher *et al.* 1957; Fletcher *et al.* 1956; Garcia and Jordan 1969; Kust and Struckmeyer 1971; Mallik and Tesfai 1985; Olume and Veatch 1969) and declines in rates of symbiotic nitrogenase activity (Bollich *et al.* 1985; Cardina *et al.* 1986; Eberbach and Douglas 1983; Mallik and Tesfai 1985; Torstensson 1975).

Mallik and Tesfai (1985) found that trifluralin, 2,4-DB, alachlor, glyphosate and metribuzin adversely affected nodulation and N fixation in soybean (*Glycine max*) when applied at 5 and 10 times normal rates. Dunigan *et al.* (1972) found that medium to high rates of trifluralin, chloramben, nitralin, and prometryn had adverse effects on nodulation by *G. max*.

Herbicide-induced declines in nodulation may be the result of injury to the legume's root system or to *Rhizobium* before or during infection. Also, the decline in nitrogenase activity may be due to a herbicide-induced reduction in supplies of photosynthates to the nodules, physiological damage to the plant root or nodules or, physiological damage to *Rhizobium* either before or after inoculation (Eberbach and

Douglas 1989). Recommended herbicide dosage rates have been shown to have a neglible effect on the growth of rhizobia (Cardina *et al.* 1986; Moorman 1986; Roslycky 1985). Although the effect of herbicides on bacterial growth is relevant, rhizobia may lose the ability to induce nodulation when exposed to pesticides before they lose the ability to multiply (Curley and Burton 1975).

Fletcher *et al.* (1957; 1956) showed that phenoxy herbicides at recommended rates could be detrimental to nodulation. Similarly, recommended rates of 2,4-DB on trefoil plants (Garcia and Jordan 1969) and trifluralin on soybean plants (Kust and Struckmeyer 1971) reduced lateral root growth and nodule formation. In both of these reports, morphological examination of root nodule tissue indicated extensive herbicide induced damage to xylem vessels but little damage to nodular bacteroids. More recent work has established that herbicide-affected legumes suffered a reduction in nitrogenase activity but not necessarily in total plant weight (Mallik and Tesfai 1985; Torstensson 1975). Results of Torstensson (1975) further indicated that it may be possible for the nodule-bacteroid complex to suffer some physiological disturbance without the plant showing any obvious signs of injury.

Herbicides which are used for weed control in legumes can have adverse effects on nodulation and N fixation (Lal 1988). There are reports that symbiotic N fixation is decreased following application of: linuron (Rennie and Dubetz 1984), alachlor, metribuzin and trifluralin to soybean (Mallik and Tesfai 1985); bentazone to kidney bean (Bethlenfalvay *et al.* 1979; Schnelle and Hensley 1990); and oxyfluorfen, linuron, metribuzin and oxadiazon to lentil (Sandhu *et al.* 1991; Sprout *et al.* 1992).

Singh and Wright (1999) examined the impact of three pre-emergence herbicides (terbutryn / terbuthylazine, trietazine / simazine and prometryn) and a post-emergence herbicide (bentazone) on nodulation, symbiotic N fixation, growth and yield of pea (*Pisum sativum*) in a growth chamber study at the University of Wales, UK. They reported that all herbicides decreased nodulation, and total root and shoot production. Moreover, herbicide application reduced leaf area and thus total photosynthate supply to the nodules, ultimately reducing total N fixed. Interestingly, however, the activity of a critical enzyme in the root nodules "nitrogenase", remained unaffected by herbicide application. These results suggested that the reduction in fixed N was not related to the

direct impact on critical biochemical processes within the nodules; rather, these authors suggested that the herbicide effect was due simply to reduced plant growth. When they examined the impact of the various herbicides on rhizobial growth under controlled laboratory conditions, they concluded that the herbicides "did not adversely affect the growth of rhizobia at the concentrations expected to be normally experienced by the rhizobia under field conditions" (Singh and Wright 2002). Moreover, the results of the experiments suggested that the decreased growth of herbicide treated plants was due to direct effects of the herbicides on peas and not due to indirect effects of the herbicides on rhizobia.

Although many studies similarly have concluded that herbicides affect N fixation largely via indirect effects on plant growth and consequent availability of photosynthate to the root nodules (Abd-Alla *et al.* 2000; Bertholet and Clark 1985; Rennie and Dubetz 1984; Sprout *et al.* 1992; Vidal *et al.* 1992), there is evidence that some pesticides might impair the ability of the rhizobia to recognise appropriate host plants. For example, Fox *et al.* (2004) reported that some pesticides can mimic naturally occurring biochemicals and thereby interfere with various biochemical signalling processes between rhizobia and appropriate host plants. As a consequence, early nodulation events can be disrupted. However, according to their research, not all pesticides had a negative impact on nodulation and the degree to which nodulation was inhibited was dependent on pesticide concentrations.

Martensson (1992) examined the impact of various herbicides on root hair formation. Rhizobia infect plant roots through root hairs and thus it was hypothesised that herbicides affecting root hair development might interfere with nodulation. He reported that some herbicides, including glyphosate, caused root hair deformations that apparently resulted in fewer nodules being formed.

Working in Australia, Gupta *et al.* (2002) reported that application of flumetsulam caused shoot yellowing and reduced plant growth. They also observed a reduction in effective nodule numbers on six-week pea roots when evaluated 10-d after herbicide application. Furthermore, they observed a partial recovery in the appearance of the plants four to five weeks after herbicide application, apparently because new nodules formed on new roots. Their observations led them to conclude that herbicide application can result

in substantial loss of nodules from the roots, likely due to the herbicide-induced stress on the plant-rhizobium symbiosis.

Herbicides normally applied to the soil may be expected to have more influence on nodulation than those applied to the foliage. Brock (1971) showed that trifluralin and carbetamide reduced nodulation and growth of red clover (*Trifolium pratense*), white clover (*Trifolium repens*), small hop clover (*Trifolium dubium*), and big trefoil (*Lotus pedunculatus*). Herbicides may influence nodulation and biological N fixation in legumes either by affecting rhizobia, the plant or both. Gaur (1980) summarised the findings of many researchers, and although he found some literature to support the theory that decreased nodulation and N fixation in leguminous plants are results of herbicide effects on the host plant, he concluded that herbicides can affect rhizobia and legume nodulation. Dinitroaniline herbicides such as trifluralin were found to inhibit growth of rhizobia bacteria and the nodulation process. Many of the summarised studies have shown that herbicides can adversely affect symbiotic N fixation.

Bollich *et al.* (1988) conducted a field experiment to determine the influence of trifluralin and pendimethalin herbicides on soybean - *Bradyrhizobium japonicum* symbiosis. They concluded that trifluralin and pendimethalin applied at rates of 1.1, 1.7, and 2.2 kg ha^{-1} delayed emergence and injured soybean seedlings grown on an Olivier silt loam soil at the Burden Research Plantation in Baton Rouge, Louisiana, USA. Nodule number, dry weight, and N fixation measured by acetylene (C_2H_2) reduction were decreased by all herbicide rates during the vegetative growth stages in 1984, with occasional decreases in nodulation noted during the late reproductive growth stage.

The acetolactate synthase (ALS) herbicides are one group of herbicides widely used throughout Australia and include the sulfonylureas, imidazolinones, and sulfonamides. Sulfonylureas are used for controlling broadleaf weeds and some grasses in cereal crops, whereas imidazolinones and sulfonamides are recommended for weed control in some legume crops and pastures (Chambers 1995). The persistence of sulfonylureas applied to cereal crops has been reported to inhibit subsequent legume crops and pastures under alkaline soil conditions, due to insufficient herbicide degradation (Gillet and Holloway 1996; Moyer *et al.* 1989; Rovira *et al.* 1993).

According to Eberbach (1993), herbicides may affect the legume-rhizobia symbiosis in a number of ways including: (i) direct effects on the host plant (e.g. reduction in root biomass, leading to fewer infection sites, or in carbohydrate supply to existing nodules); (ii) direct effects on rhizobial survival or growth, leading to a decreased potential for rhizobial infection of root hairs; (iii) an inhibition or inactivation of the biochemical signalling by either rhizobia or plants required to initiate nodule development; and/or (iv) an inhibition of nodule development by reducing the capacity for cell division. All of these possible mechanisms have the potential to reduce the efficiency of legume-rhizobia symbiotic relationship and therefore the amount of N fixed.

Effect of herbicides on nodulation and nitrogen fixation of chickpea

Kumar *et al.* (1981) working at Haryana Agricultural University, India reported that simazine and prometryn applied to the soil surface in pots at 1.6 and 3.2 kg ha^{-1} reduced chickpea leaf dry weight after 28 days and in the length of the main stem after 42 days. Dry matter accumulation in the shoot was drastically reduced with simazine but not with prometryn. All treatments retarded dry matter accumulation in the roots with time. Prometryn reduced chlorophyll content during early growth stages and simazine during later stages. They also found that the growth of the rhizobial culture was reduced with increasing concentrations (1-20 mg L^{-1}) of both simazine and prometryn. Root nodule initiation was not affected by either of the herbicides but the later production of new nodules and growth of the nodules was reduced in different degrees by various treatments. Overall nodulation was drastically reduced with simazine. Reductions in nodulation with simazine and prometryn appeared to be primarily a case of general root growth reduction. The pink pigment leghaemoglobin did not develop at all in the nodules of simazine treated plants and its concentration was not affected by prometryn treatments. The N-fixing efficiency (acetylene reduction) of the nodules was greater in the prometryn treated plants and was nil in the simazine treated ones.

When examining the effects of chlorsulfuron under laboratory conditions, Anderson *et al.* (2004) observed that even at rates equivalent to double the field rates, chlorsulfuron did not influence the growth of chickpea rhizobia. However, although rhizobial growth was not influenced, the subsequent ability of these rhizobia to form

nodules was reduced. Thus, the presence of chlorsulfuron in the soil reduced the nodulation and N fixation of the chickpea plant. Pre-exposing rhizobia to chlorsulfuron before inoculating them into pots with germinating chickpea seeds, reduced the number of nodules formed by 51%. Exposure of chickpeas and chickpea rhizobia to chlorsulfuron can adversely affect the formation and activity of symbiotic N-fixing nodules, even when only the rhizobial inoculant is exposed briefly to the herbicide. A reduction in number and weights of nodules in chickpea crops due to the application or presence of chlorsulfuron can negatively affect the potential N benefits associated with including chickpeas in rotation with cereals by (i) reducing the input of biologically fixed N into the soil/plant system and (ii) enhancing the extraction of soil mineral N.

In contrast, Martensson (1992) reported that nodulation ability was unaffected by previous exposure to chlorsulfuron. These contrasting results suggest that the impact of various herbicides on specific nodulation events may be highly dependent on specific environmental conditions, including different soil characteristics (pH, organic matter, moisture) and weather conditions.

2.7 Conclusions

Isoxaflutole is a relatively new and promising pre-emergence herbicide used for the control of a wide range of broadleaf weeds in chickpea. Since isoxaflutole was registered and commercially released in 2001 in Australia, there has been widespread adoption of this herbicide in chickpea production systems with over 100 000 hectares of chickpeas in the northern grain region of Australia treated annually over the last three years.

However, there have been reports of chickpea crop damage due to isoxaflutole. That damage was particularly severe with one experimental line, 91025-3021. Moreover, under some conditions, all varieties could be damaged at the recommended rate (75 g a.i. ha^{-1}). The variety Yorker was found to be more sensitive to isoxaflutole than the other currently used varieties. Identifying cultivars tolerant to herbicides is important to chickpea growers because it may minimise the risk of crop injury. Since chickpea genotypes varied in their response to isoxaflutole, it is important in breeding programmes to screen new genotypes according to their tolerance to isoxaflutole for selective weed control.

Herbicides used for weed control in pulses can have adverse effects on nodulation and N fixation. As a result, the subsequent wheat yields may experience a decline due to lower N economy in the field. This is an alarming situation for the whole chickpea-wheat growing areas of Australia.

Given that there is a general need to increase the competitive ability of chickpea against weeds, and particularly through varietal selection, it is important that herbicide application does not reduce the ability of chickpea to compete with weeds that escape herbicide treatments. More information is needed across a range of environments to identify more tolerant chickpea genotypes if isoxaflutole is to become a reliable weed management option for chickpea growers. With this background, it is essential to investigate factors such as variety, soil moisture, soil pH, organic matter, and temperature that affect chickpea tolerance to isoxaflutole. It is also important to assess if the effects of different soil nitrate levels influence the degree of isoxaflutole injury, and finally look at the effect of this herbicide on N fixation of chickpea.

2.8 Objectives and outline of thesis

The main objective of this study was to determine the sources of environmental variation in the field tolerance of chickpea to isoxaflutole and also find out the effect of this herbicide on nodulation and N fixation of chickpea under different inherent soil nitrate levels. This information could be used to develop guidelines for farmers on the circumstances in which damage and yield loss is likely to occur.

Therefore, the objectives of this study were to:

1. assess the sensitivity of different genotypes of chickpea to isoxaflutole;
2. examine the effects of different soil and environmental factors on chickpea tolerance to isoxaflutole;
3. assess the effects of different soil nitrate levels on the degree of isoxaflutole injury to chickpea; and
4. determine the effects of isoxaflutole on growth, nodulation and N fixation of chickpea.

CHAPTER THREE

Phytotoxic response and yield of chickpea (*Cicer arietinum*) genotypes with pre-emergence application of isoxaflutole

Manuscript submitted to the
Australian Journal of Experimental Agriculture, February 2007

Manuscript accepted June 2007

Phytotoxic response and yield of chickpea (*Cicer arietinum*) genotypes with pre-emergence application of isoxaflutole

Avishek Datta[A,C,D], Brian M. Sindel[A,C], Robin S. Jessop[A,C], Paul Kristiansen[A] and Warwick L. Felton[B]

[A]School of Rural Science and Agriculture, The University of New England, Armidale, New South Wales 2351, Australia

[B]Nyrang Avenue, Tamworth, New South Wales 2340, Australia

[C]Cooperative Research Centre for Australian Weed Management

[D]Corresponding author. Email: adatta2@une.edu.au

Short title: Effect of isoxaflutole on chickpea phytotoxicity

Abstract

Balance® (75% a.i. isoxaflutole) at 100 g ha^{-1} is registered in Australia for the control of several broadleaf weeds in chickpea. Polyhouse and field experiments were carried out to examine the tolerance of chickpea genotypes to isoxaflutole. Seven rates of isoxaflutole [0, 18.75, 37.5, 75 (recommended rate), 150, 300 and 600 g a.i. ha^{-1}] were applied to 20 genotypes in the first polyhouse experiment while in the second experiment, 16 genotypes were tested. In the field, six genotypes were treated with five herbicide rates (0, 37.5, 75, 150 and 300 g a.i. ha^{-1}). There was a strong dose response in the polyhouse experiments in visual injury ratings, plant height, and shoot and root dry weight. In general, there was less herbicide injury to the kabuli genotypes than in the desi chickpea genotypes. Chickpea genotypes Yorker, Howzat, Amethyst, Gully, 91025-3021, Jimbour, S 95425 and FLIP 94-92C exhibited higher overall mean injury rating in experiment one. Among these genotypes, shoot dry matter was reduced significantly in Amethyst, Jimbour, 91025-3021 and S 95425. Root dry matter in Amethyst, Jimbour, Yorker, 91025-3021 and S 95425 also was reduced significantly. Height was reduced significantly in all of the eight above genotypes. Although there was less overall injury level in the second polyhouse experiment, which was at lower temperatures, Howzat, Yorker, 91025-3021,

FLIP 94-92C and S 95425 again recorded high overall mean injury ratings and should be regarded as more susceptible to isoxaflutole than the other strains. In comparison, 97039-1275 and Kyabra recorded very minor injury symptoms in all the experiments and can be regarded as the most tolerant. The herbicide injury to the genotypes in the field was less than in the polyhouse although Yorker and 91025-3021 showed injury symptoms at early stages of growth. The injury symptoms were temporary and did not produce significant effects on the overall crop growth and yield. Plant breeding programmes should take into account the relative susceptibility of new chickpea genotypes to isoxaflutole.

Additional keywords: chickpea, isoxaflutole, herbicide tolerance.

Introduction

Chickpea (*Cicer arietinum*) is the world's second most cultivated food legume, grown over 9.9 million ha in 2002, from the Mediterranean basin and West Asia to the Indian subcontinent (which accounts for 75% of global production), eastern Africa, Australia, and North and South America (FAO 2002). Chickpea has been shown to be one of several pulses that are suited to the fine-textured, neutral-to-alkaline soils of the eastern cropping zone of Western Australia and eastern Australia where narrow-leafed lupin (*Lupinus angustifolius*) is poorly adapted (Siddique and Sedgley 1986; Siddique *et al.* 1993). The market demand for chickpea in the Indian subcontinent and Middle East is high and an increase of 2.2% per annum in demand for chickpea for human consumption has been reported by FAO (Siddique *et al.* 2000). Such a demand provides an opportunity to increase the production of chickpea in Australia. Chickpea is seen as a crop that provides a cash income from grain, requires minimal nitrogen (N) fertiliser through its ability to fix atmospheric N, and in a crop rotation can improve the N nutrition and yield of subsequent cereals (Doughton *et al.* 1993). One of the major obstacles in growing chickpea successfully is its poor ability to compete with weeds. Crop losses of 90% are possible in weedy situations (Knights 1991) and the lack of registered post-emergence herbicides for broadleaf weeds reduces the options for weed management.

Balance® at 100 g ha^{-1} (75% a.i. isoxaflutole) is registered for the control of several broadleaf weeds [e.g. Indian hedge mustard (*Sisymbrium orientale*), sowthistle

(*Sonchus oleraceus*), capeweed (*Arctotheca calendula*), prickly lettuce (*Lactuca serriola*), wild radish (*Raphanus raphanistrum*) and turnip weed (*Rapistrum rugosum*)] in chickpea. Since isoxaflutole was registered and commercially released in Australia in 2001, there has been rapid and widespread adoption of this herbicide in chickpea production systems. Over 100 000 ha of chickpeas in the northern grain region of Australia have been treated annually with isoxaflutole over the last three years. Its herbicidally active derivative remains stable on the soil even after a prolonged dry period. Isoxaflutole has unique properties that allow the product to "recharge" when rainfall occurs, due to limited leaching thereby allowing it to remain active for much longer periods than other herbicides. It thus gives more consistent and reliable control of a wide range of broadleaf weeds including some difficult-to-control species.

However, there have been records of chickpea crop damage due to the application of isoxaflutole. Felton *et al.*(2004) demonstrated that under some conditions this herbicide can injure the more tolerant varieties of chickpea at an application rate of less than 75 g a.i. ha^{-1} and consequently result in a yield penalty. Isoxaflutole caused considerable concern in the United States after it was released, with widespread injury occurring in corn (*Zea mays*) (Bhowmik and Prostak 1996; Sprague *et al.* 1999a). Sprague *et al.* (1999b) reported that corn injury was most common from isoxaflutole applied at high rates on soils with low clay and organic matter. In addition, corn hybrids have been shown to differ in their response to isoxaflutole (Sprague *et al.* 1999c).

This paper reports polyhouse and field experiments designed to determine the extent to which injury by isoxaflutole is related to differential sensitivity of chickpea genotypes to its application.

Materials and methods

Polyhouse experiment-1

Twenty chickpea genotypes (Table 1) were sown in 14 cm diameter plastic pots each containing ~ 1.1 kg of medium heavy clay chocolate basalt soil (0-10 cm depth, pH 6.4, organic matter 2.8%), obtained from the Laureldale Research Station, University of New England, New South Wales (NSW), Australia. The soil in each pot was pre watered to bring it to field capacity. The seeds were inoculated with the recommended *Rhizobium*

culture before sowing and three seeds were planted at a depth of 2.5-3 cm in each pot. Isoxaflutole was applied at 1 day after sowing (DAS) using a gas operated boom-sprayer through a TeeJet 11003 flat fan nozzle at a pressure of 300 kPa delivering a volume of 84 L ha^{-1}. There was a total of 20 genotypes × 7 herbicide rate treatment combinations and 3 replications of each treatment laid out in a randomised complete block design. Pots were lightly watered on alternate days to keep the soil moist. Pots also received 10 mm of simulated rainfall at 1 and 7 days after herbicide treatment (DAHT) and after that at regular intervals to avoid water stress. During the experimental period, the mean minimum and maximum temperatures in the polyhouse were 13°C and 37°C, respectively; the overall temperature was high during the study period due to unseasonably hot conditions. Seven DAS, each pot was thinned to two seedlings. Chickpea crop injury was rated visually at 35 and 45 DAHT using the European Weed Research Council (EWRC) scale (Table 2). The scoring was done on the average effect on two plants within a pot, with two operators independently scoring each pot relative to the closest unsprayed control. At 45 DAHT, plant heights were recorded by measuring from the ground level to the highest growing point. Plant shoots were harvested at the ground level on the same day and roots were hand washed. Dry weights of both shoots and roots were obtained after drying at 70°C for 48 h.

Polyhouse experiment-2

Sixteen chickpea genotypes, both desi and kabuli (Table 1), were used for a second polyhouse experiment. The experimental procedures were the same as described for experiment 1. The mean minimum and maximum temperatures during the experimental period were 9°C and 22°C, respectively; the overall temperature was lower than in experiment 1 during the study period. Plants were harvested 60 DAHT and plant height and crop injuries were rated at 35, 45 and 60 DAHT.

Field experiment-3

A field experiment was established in 2005 at the Laureldale Research Station, University of New England, Armidale, NSW. The experiment was arranged in a randomised complete block design with three replications. The treatments consisted of six desi genotypes and four herbicide rates and an untreated control (Table 1). Plots (6.6 m × 1.9 m) were sown to chickpea in 37.5 cm rows at 10 cm spacing after being inoculated with the recommended *Rhizobium* culture. The soil type was the same as that in experiments 1 and 2. Mean monthly rainfall and temperature data for the growing season at Laureldale are in Table 3.

Crop injury was rated visually 30 and 60 DAHT using the EWRC scale. The height of 10 randomly selected plants of each genotype was measured per plot from the soil surface to the highest growing point on the same day of damage assessment. Plant densities were counted on randomly selected 1-m sections of row from each plot at 30 DAHT. At maturity, yield components (number of branches plant^{-1}, number of pods plant^{-1}, number of seeds pod^{-1} and 1000-seed weight) were measured by hand harvesting randomly selected 1-m sections of row from each plot. The remainder of each plot, excluding buffers, was then harvested to obtain total grain yield.

Statistical analysis

The data for increasing rates of isoxaflutole in each experiment were fitted with a linear model in R 2.3.0 (R Development Core Team 2006) and presented with a linear regression and 95% confidence limits. *P*-values were calculated using the ANOVA function. The assumptions of ANOVA were tested for each variable by inspecting the residuals *v.* fitted plots and the quantile-quantile plots (Q-Q plots).

Table 1 Genotypes, herbicide rates, and sowing & harvest dates in experiments 1, 2 & 3

Genotypes	Isoxaflutole rate (g a.i. ha^{-1})	Sowing date	Harvest date
Experiment 1			
Desi: Kyabra, 97039-1275, Gully, Jimbour, Howzat, Amethyst, Flipper, Yorker, ICLL 87322, 91025-3021. **Kabuli:** Bumper, FLIP 94-90C, FLIP 94-92C, GCN 133-2, IG 9337, IG 96220, Kaniva, Macarena, S 95342, S 95345	0, 18.75, 37.5, 75, 150, 300 and 600	24 November 2004	8 January 2005
Experiment 2			
Desi: Kyabra, 97039-1275, Jimbour, Howzat, Amethyst, Yorker, ICLL 87322, 91025-3021. **Kabuli:** Bumper, FLIP 94-90C, FLIP 94-92C, GCN 133-2, IG 9337, IG 96220, S 95342, S 95345	0, 18.75, 37.5, 75, 150, 300 and 600	1 June 2005	1 August 2005
Experiment 3			
Desi: Kyabra, 97039-1275, Jimbour, Amethyst, Yorker, 91025-3021	0, 37.5, 75, 150 and 300	23 September 2005	30 January 2006

Table 2 Rating scale of European Weed Research Council (EWRC) used to score the level of plant tolerance following herbicide application (Sandral *et al.* 1997)

EWRC score	Plant tolerance
1	No effect
2	Very slight effects: some stunting and yellowing just visible
3	Slight effects: stunting and yellowing obvious; effects reversible
4	Substantial chlorosis and/or stunting: most effects probably reversible
5	Strong chlorosis/stunting; thinning of stand
6	Increasing severity of damage
7	Increasing severity of damage
8	Increasing severity of damage
9	All plants dead

Table 3 Mean monthly rainfall and temperature during the growing season in experiment 3

Crop month	Rainfall (mm)	Temperature (°C)	
		Min.	Max.
September 2005	100.0	2.1	13.6
October 2005	91.3	5.9	17.9
November 2005	156.8	7.2	18.6
December 2005	88.0	10.7	27.4
January 2006	72.8	14.6	27.0

Results

Experiment 1

Visible crop injury symptoms in experiment 1 included yellowing of lower branch leaves, stunting of growth, and for highly sensitive genotypes, necrosis. There was a strong dose response, and visual injury ratings, plant height, and shoot and root dry weight reduction were greater at the higher doses for all genotypes. The genotype × rate of herbicide interaction was highly significant ($P < 0.001$) for plant height recorded at 45 DAHT (Fig. 1). Significant plant height reductions occurred for all of the desi chickpea genotypes except for Kyabra, 97039-1275 and ICLL 87322. The rate of plant height reduction as measured by the slope of the line, was highest in Yorker followed by Gully, Flipper, Amethyst, Jimbour and Howzat among the desi genotypes. The plant height of kabuli genotypes generally was less affected by the application of an increased rate of herbicide. Plant height was reduced significantly only in FLIP 94-90C, FLIP 94-92C, Kaniva, Macarena and S 95425. The highest reduction in plant height was in S 95425 and FLIP 94-90C followed by FLIP 94-92C, Kaniva and Macarena among the kabuli genotypes.

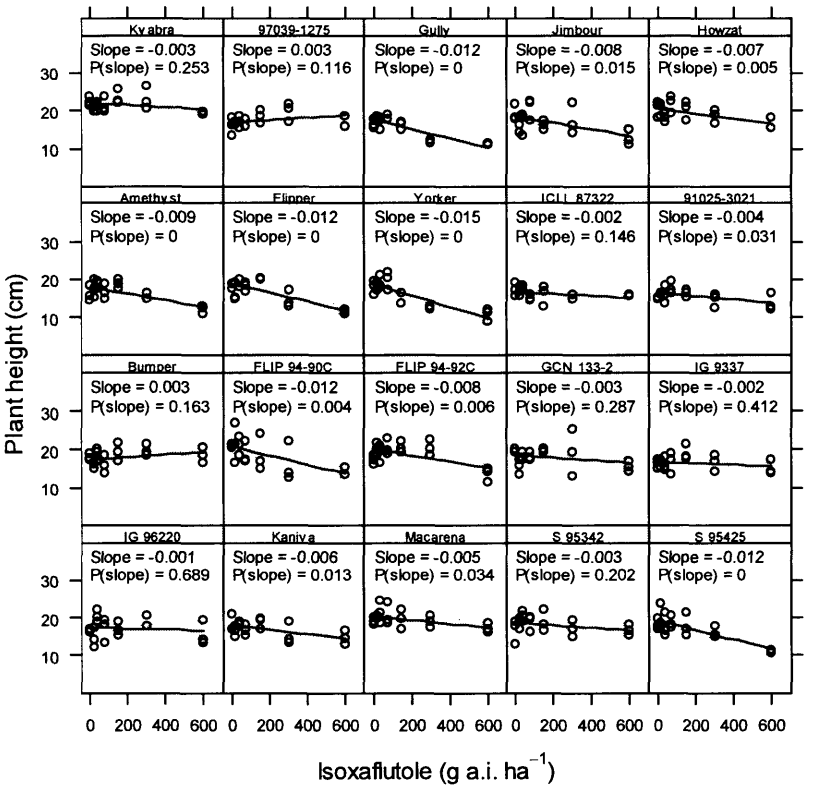


Fig. 1. Effect of increasing rates of isoxaflutole on plant height (cm) of desi (upper 10) and kabuli (lower 10) chickpea genotypes at 45 DAHT in experiment 1. Shaded areas are 95% confidence intervals of the response curve.

Shoot dry weight (Fig. 2) was significantly reduced by isoxaflutole for the desi chickpea genotypes Jimbour, Amethyst, ICLL 87322 and 91025-3021. However, reductions were not significant for most of the kabuli types except for Kaniva and S 95425. Of those with significant reductions, ICLL 87322 and 91025-3021 recorded maximum shoot dry matter reduction among the desi genotypes, whilst shoot dry matter reduction was greatest in Kaniva and S 95425 among the kabuli types.

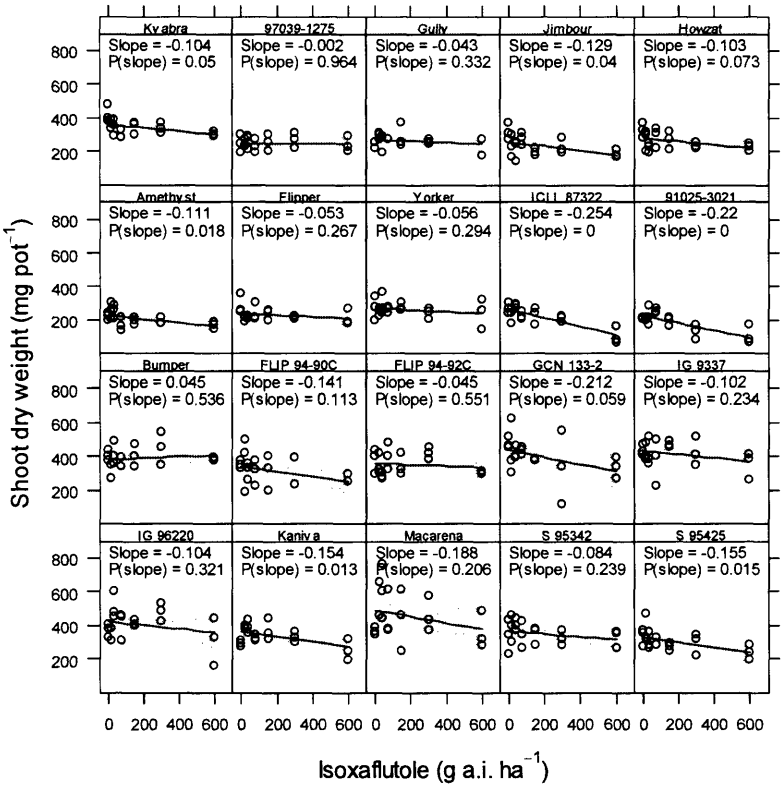


Fig. 2. Effect of increasing rates of isoxaflutole on shoot dry weight (mg pot^{-1}) of desi (upper 10) and kabuli (lower 10) chickpea genotypes at 45 DAHT in experiment 1. Shaded areas are 95% confidence intervals of the response curve.

Root dry weight reductions (Fig. 3) were significant for the desi genotypes Jimbour, Amethyst, Yorker, ICLL 87322 and 91025-3021. Among the kabuli types, only FLIP 94-90C, Kaniva and S 95425 showed significant reductions in root dry matter. Among the desi genotypes, 91025-3021 and Jimbour had the highest root dry weight reduction, whilst root dry matter reduction was greatest in FLIP 94-90C and S 95425 among the kabuli genotypes.

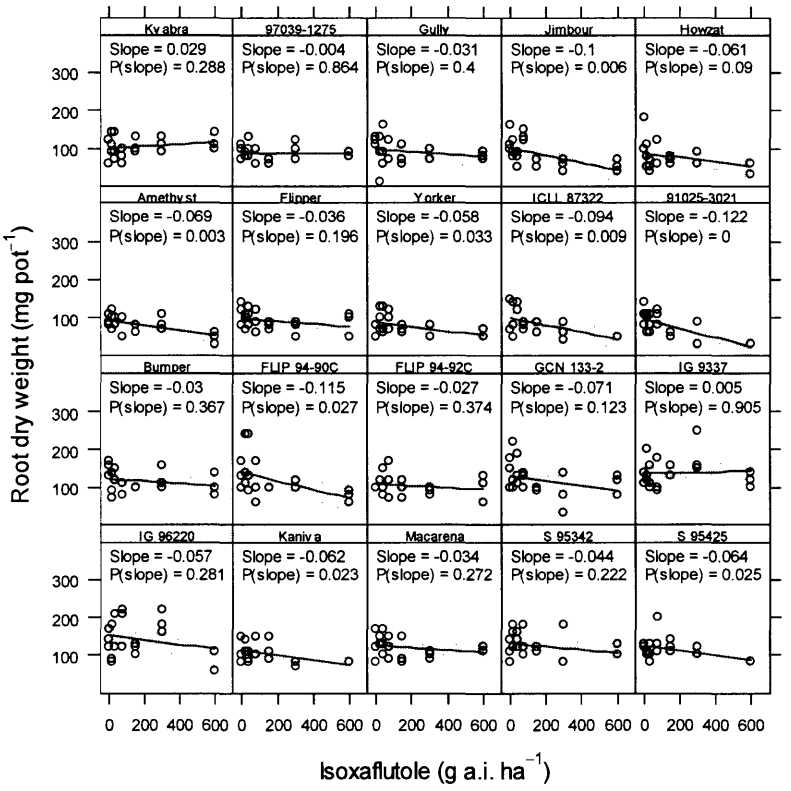


Fig. 3. Effect of increasing rates of isoxaflutole on root dry weight (mg pot^{-1}) of desi (upper 10) and kabuli (lower 10) chickpea genotypes at 45 DAHT in experiment 1. Shaded areas are 95% confidence intervals of the response curve.

Phytotoxicity symptoms first developed in pots treated with higher rates of herbicide but chickpea genotypes differed in their response. Mean injury ratings (EWRC score) for ICLL 87322 (1.6) and 97039-1275 (1.7) lines were lowest among all the genotypes tested at 35 DAHT. 91025-3021 (3.5), S 95425 (3.5), Amethyst (3.5), Gully (3.8), Howzat (4.0) and Yorker (4.5) had the most injury symptoms. Most of the other genotypes were intermediate in response. At 35 DAHT, all of the genotypes experienced injury symptoms at the recommended rate of 75 g a.i. ha^{-1} except ICLL 87322. The dose response curve was not significant for Gully, Jimbour, Flipper among the desi types, and Bumper and Macarena among the kabuli types, indicating that these genotypes showed limited increase in injury with increasing herbicide rate. There was generally less

herbicide injury in kabuli genotypes compared with desi types (Fig. 4). Injury ratings recorded 45 DAHT (data not shown) provide a similar result to that at 35 DAHT. ICLL 87322 (2.7), Kyabra (3.7), 97039-1275 (3.7) and GCN 133-2 (3.1) were among the most tolerant genotypes whilst Yorker (5.3), Amethyst (5.3), Howzat (5.1), Jimbour (4.8), Gully (4.7) and 91025-3021 (4.7) were the least tolerant genotypes. All of the other desi and kabuli genotypes were intermediate in their response to isoxaflutole.

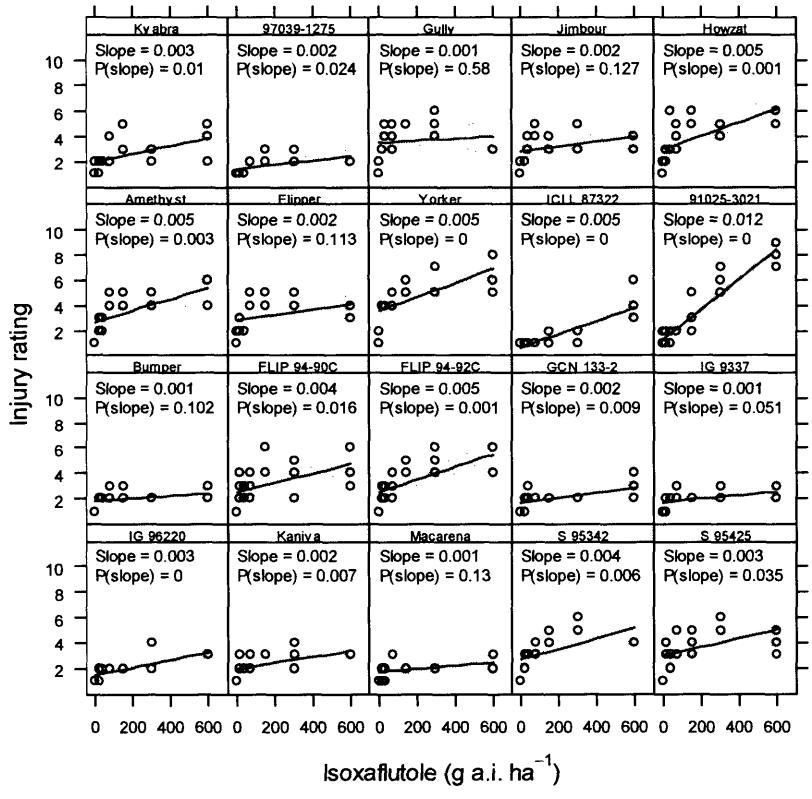


Fig. 4. Effect of increasing rates of isoxaflutole on the injury rating of desi (upper 10) and kabuli (lower 10) chickpea genotypes at 35 DAHT in experiment 1. Shaded areas are 95% confidence intervals of the response curve.

Experiment 2

In the second polyhouse experiment under cooler conditions, 97039-1275, Howzat, Amethyst, Yorker (desi types) and FLIP 94-90C, FLIP 94-92C, GCN 133-2, S 95342 and S 95425 (kabuli types) showed significant reductions in plant height with increasing herbicide rate of application at 35 DAHT (data not shown). At 45 DAHT (data not

shown), Kyabra, Howzat, Amethyst, Jimbour (desi) and FLIP 94-90C, FLIP 94-92C, GCN 133-2, S 95342, S 95425 (kabuli) showed significant height reductions while Yorker managed to recover. By 60 DAHT, several genotypes (Kyabra, 97039-1275, Jimbour, Amethyst and Yorker) that showed earlier height reductions no longer did, while others (ICLL 87322, 91025-3021, Bumper and IG 96220) remained unaffected in height thoroughout the experiment (Fig. 5).

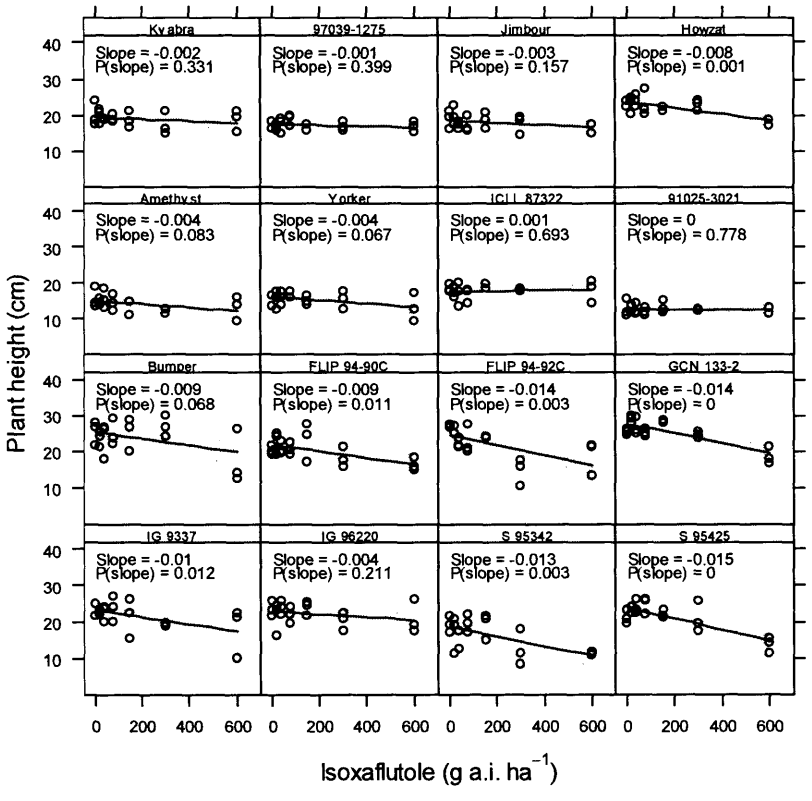


Fig. 5. Effect of increasing rates of isoxaflutole on the plant height (cm) of desi (upper 8) and kabuli (lower 8) chickpea genotypes at 60 DAHT in experiment 2. Shaded areas are 95% confidence intervals of the response curve.

A significant reduction in shoot dry weight (Fig. 6) was observed for the genotypes Amethyst, ICLL 87322, FLIP 94-92C, GCN 133-2, S 95342 and S 95425.

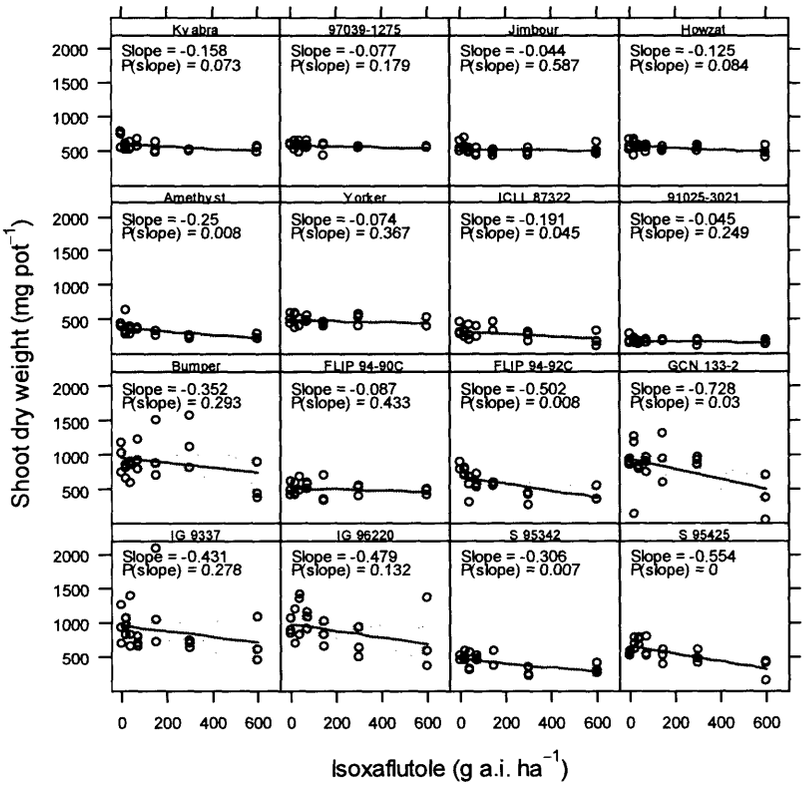


Fig. 6. Effect of increasing rates of isoxaflutole on the shoot dry weight (mg pot^{-1}) of desi (upper 8) and kabuli (lower 8) chickpea genotypes at 60 DAHT in experiment 2. Shaded areas are 95% confidence intervals of the response curve.

Root dry weight (Fig. 7) of the genotypes Jimbour, Howzat, Amethyst, Yorker, ICLL 87322 and 91025-3021 among the desi types, and FLIP 94-90C, FLIP 94-92C, GCN 133-2, IG 9337, S 95342 and S 95425 among the kabuli types were significantly affected by the applied herbicide. Of the genotypes significantly affected by isoxaflutole, root dry matter reduction was highest in Yorker, FLIP 94-92C and S 95342.

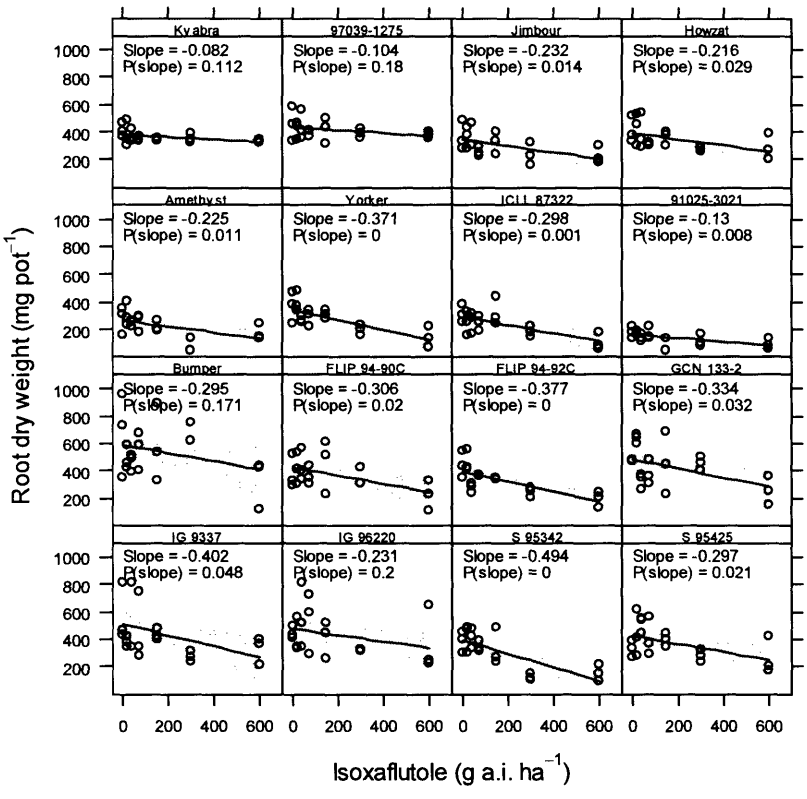


Fig. 7. Effect of increasing rates of isoxaflutole on the root dry weight (mg pot^{-1}) of desi (upper 8) and kabuli (lower 8) chickpea genotypes at 60 DAHT in experiment 2. Shaded areas are 95% confidence intervals of the response curve.

All the genotypes experienced minor injury symptoms at the recommended rate of isoxaflutole measured at 35 and 45 DAHT. The symptoms increased with the higher herbicide rates. By 60 DAHT (Fig. 8), the highest mean injury ratings at 75 g a.i. ha^{-1} were in 91025-3021 (4.3), Yorker (3.8), FLIP 94-92C (3.8) and S 95425 (3.0). Only minor injury symptoms were observed in ICLL 87322 (1.2), 97039-1275 (1.5), IG 9337 (1.7), IG 96220 (1.5) and GCN 133-2 (1.0) at the recommended herbicide rate.

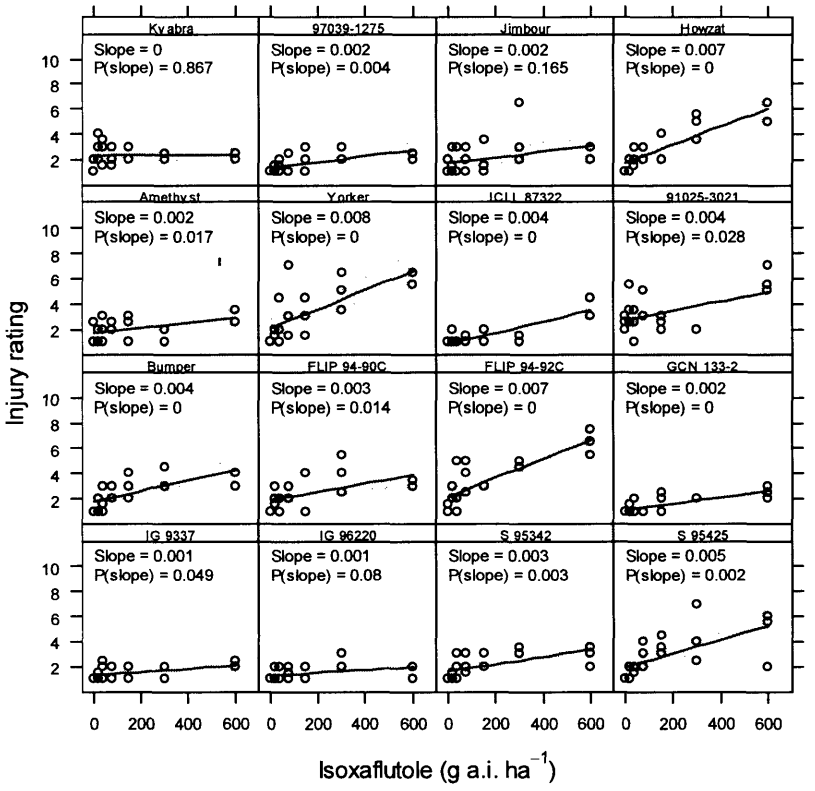


Fig. 8. Effect of increasing rates of isoxaflutole on the injury rating of desi (upper 8) and kabuli (lower 8) chickpea genotypes at 60 DAHT in experiment 2. Shaded areas are 95% confidence intervals of the response curve.

Experiment 3

Under field conditions, there was less herbicide injury to chickpea genotypes compared with those in the polyhouse experiments. Yorker (3.3) and 91025-3021 (3.0) showed injury symptoms at the recommended dose of herbicide at 30 DAHT, whereas 97039-1275 showed practically no injury symptoms even at the highest rates of herbicide (300 g a.i. ha^{-1}). Stunting and yellowing were observed at 60 DAHT with the highest rates for all genotypes except for the line 97039-1275. This injury, however, was temporary as plants later recovered. Despite there being symptoms of herbicide injury, application of isoxaflutole had no significant effect on the overall plant growth, yield components and yield (Fig. 9). Plant height reductions were not significant except for Yorker at 60 DAHT.

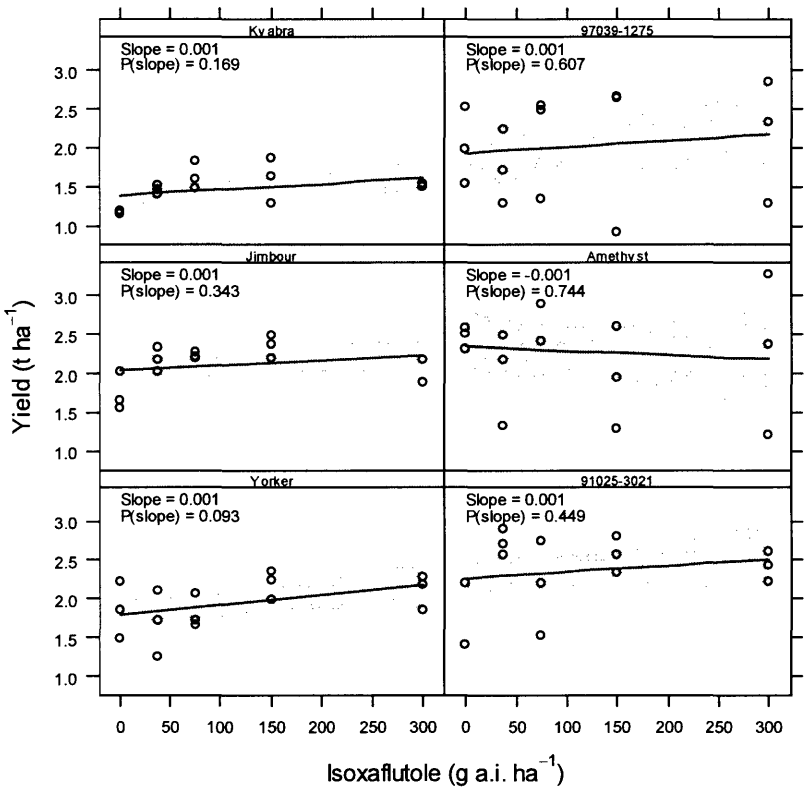


Fig. 9. Effect of increasing rates of isoxaflutole on the yield (t ha^{-1}) of desi chickpea genotypes in experiment 3. Shaded areas are 95% confidence intervals of the response curve.

Discussion

Susceptibility of chickpea to isoxaflutole damage appeared to be strongly genotype and rate dependent. At the recommended rate (75 g a.i. ha^{-1}), Yorker (EWRC score = 6.0 in experiment 1 and 3.8 in experiment 2) and 91025-3021 (EWRC score = 5.7 in experiment 1 and 4.3 in experiment 2) recorded highest injury ratings but this failed to translate into effects on plant height and shoot and root dry matter production. In general, there was less herbicide injury to the kabuli genotypes compared with the desi chickpea genotypes. These findings supported previous studies by Felton *et al.* (2004) who also found desi chickpea genotypes were more susceptible than the kabuli types. The overall injury was less in experiment 2 as compared with experiment 1 which was grown under unusually high temperatures. Studies by Pallett *et al.* (2001) found that increasing soil moisture

level and temperature increased the phytotoxicity symptoms in corn treated with isoxaflutole. At 25°C, the half-life of isoxaflutole was significantly longer at < 2% moisture content compared with 30% moisture content, and at 30% moisture content, the half life was much longer at 10°C than at 25°C.

Cultivar differences in isoxaflutole tolerance were also reported for corn (O'Sullivan *et al.* 2001; Sprague *et al.* 1999c). Obermeier *et al.* (1995) reported corn injury from isoxaflutole applied at 132 and 158 g ha^{-1}. Bhowmik and Prostak (1996) and Sprague *et al.* (1996) also observed injury to corn from pre-emergence applications of isoxaflutole. Luscombe and Pallett (1996) reported bleaching of leaf tissue and reduction in shoot height of corn when treated with isoxaflutole. Increased application rate of isoxaflutole has directly been related to corn injury and decreased shoot height (Sprague *et al.* 1999b). Visual symptoms, plant height and shoot and root dry weight reductions are all used to measure herbicide phytotoxicity (Dear *et al.* 2006; Si *et al.* 2006; Sprague *et al.* 1999c).

In plants and soil, isoxaflutole is rapidly converted to the diketonitrile metabolite by cleaving the isoxazole ring (Pallett *et al.* 1998). This diketonitrile is herbicidally active and is a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase (HPPD) that leads to indirect inhibition of carotenoid biosynthesis and gives rise to symptoms of bleaching followed by chlorosis of new growth (Pallett *et al.* 1998; Viviani *et al.* 1998). In tolerant plants, diketonitrile is hydrolysed to the herbicidally inactive benzoic acid derivative (Pallett *et al.* 1998). The rate of herbicide metabolism and deactivation is likely to have been a major factor in determining the differential tolerances of chickpea genotypes. Sprague *et al.* (1999c) reported that corn hybrids more tolerant to isoxaflutole were able to metabolise it more rapidly than the more sensitive hybrids. Previous research by Pallett *et al.* (1998) identified isoxaflutole metabolism as the primary basis for differential selectivity between the tolerant species *Z. mays* and a susceptible species *Abutilon theophrasti* (velvetleaf).

In the field experiment, isoxaflutole at rates up to 300 g a.i. ha^{-1} did not cause a significant reduction to overall plant growth and yield. The soil for the field experiment had low pH (6.4) and high organic matter (2.8%) content. The high organic matter content of the soil coupled with low pH may well have been enough to adsorb much of

the isoxaflutole overtime, although some initial injury was observed. Likewise differences in temperature and moisture under the field conditions compared with the polyhouse may have reduced the effect of isoxaflutole. As a result, isoxaflutole did not affect growth and yield of the chickpea genotypes. Bhowmik *et al.* (1999) reported bleaching injury to corn in the field with a 210 g ha^{-1} rate of isoxaflutole. They found that the injury was temporary and the plants recovered within 2 to 3 weeks. They attributed the injury to a fine-textured soil (1.5% organic matter and pH 6.3) and the high rate of isoxaflutole. Similar corn injury was reported by Sprague *et al.* (1997) in their studies in Michigan, USA in coarse-textured soils of low organic matter with an isoxaflutole rate of 158 g ha^{-1}. Bhowmik *et al.* (1996) also did not observe any appreciable injury to corn, even at 158 g ha^{-1} of isoxaflutole, with no adverse effect on corn grain and silage yields in a field study on a Hadley fine sandy loam containing 3.2% organic matter with a pH of 6.8.

Sprague *et al.* (1999) reported isoxaflutole degradation to be pH-dependent. Degradation of isoxaflutole to herbicidally active diketonitrile was greatest at pH 10 (86%) when compared with pH 4 (16%) or pH 7 (20%). Soil pH plays a key role in both sorption and degradation of isoxaflutole (Mitra *et al.* 2001). The high pH soil resulted in active herbicide formation, i.e. both precursor isoxaflutole and its active derivative, diketonitrile, thereby enhancing the phytotoxicity of the applied compound (Mitra *et al.* 2001; Sprague *et al.* 1999). Rouchaud *et al.* (1998) reported that the low basicity of the isoxazole ring should enable proton addition of isoxaflutole at low soil pH and in turn increase isoxaflutole adsorption by cation exchange and reduce the rate of isoxaflutole soil dissipation. Mitra *et al.* (1999) observed a positive correlation between soil organic matter content and isoxaflutole sorption and reported that isoxaflutole sorption increased with an increase in soil organic matter content. Chiou (1989) has reported that soil organic matter is the primary sorbent for hydrophobic organic compounds in soil such as isoxaflutole.

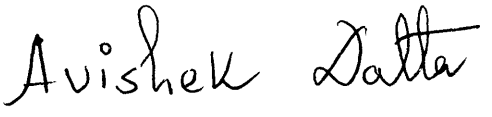
Chickpea genotypes Yorker, Howzat, Amethyst, Gully, 91025-3021, Jimbour, S 95425 and FLIP 94-92C exhibited higher overall mean injury rating in experiment one. Among these genotypes, significant shoot dry matter reductions occurred in Amethyst, Jimbour, 91025-3021 and S 95425. Amethyst, Jimbour, Yorker, 91025-3021 and S 95425

also showed significant root dry matter reductions. Furthermore, all of the eight abovementioned genotypes exhibited significant plant height reductions. Although the overall injury level was less in the second polyhouse experiment; Howzat, Yorker, 91025-3021, FLIP 94-92C and S 95425 all recorded the highest overall mean injury ratings. Genotypes Howzat, Yorker, 91025-3021, FLIP 94-92C and S 95425 should thus be regarded as most susceptible to isoxaflutole. On the other hand, 97039-1275 and Kyabra recorded very minor injury symptoms in all the experiments and should thus be regarded as most tolerant. These results were in accordance with the findings of Felton *et al.* (2004) who also recorded Yorker and 91025-3021 to be more sensitive to isoxaflutole than the currently recommended varieties. The relationship between visible herbicide injury and shoot weight reduction was not consistent for many cultivars in this study. Visible injury has likewise not always been accompanied by a reduction in shoot dry weight in other crops (Harrison and Keinath 2003). In the field, chickpea plants may have more capacity to grow out of such symptoms.

Since chickpea genotypes varied in their response to isoxaflutole, it is important that breeding programmes screen new chickpea genotypes according to their tolerance to isoxaflutole for selective weed control. More research needs to be conducted across a range of environments to identify more tolerant chickpea genotypes if isoxaflutole is to become a reliable weed management option for chickpea growers. Future experiments will examine the effects of pH and soil organic matter on sensitivity of chickpea genotypes to isoxaflutole.

Statement of Originality:

All the work contained within this paper is the original research of the PhD candidate, Avishek Datta.

Candidate: 

Principal Supervisor: 

Statement of Contribution by Others:

This paper has been prepared by the PhD candidate, Avishek Datta. All coauthors are either PhD supervisors (Sindel, Jessop, Felton) or statistical advisor (Kristiansen) and have only contributed to this paper to the extent that would normally be expected of such roles. All coauthors have given their consent for having their contributions to this paper included in the thesis and accept the student's contribution as indicated in the Statement of Originality.

Candidate: 

Principal Supervisor: 

CHAPTER FOUR

The effects of soil pH and organic matter on chickpea (*Cicer arietinum*) genotype sensitivity to isoxaflutole

Manuscript submitted to Plant and Soil, April 2007

Manuscript accepted with minor revision on August 2007

The effects of soil pH and organic matter on chickpea (*Cicer arietinum*) genotype sensitivity to isoxaflutole

Avishek Datta[1,3,4], Brian M. Sindel[1,3], Paul Kristiansen[1], Robin S. Jessop[1,3] & Warwick L. Felton[2]

[1]School of Environmental and Rural Science , The University of New England, Armidale, New South Wales 2351, Australia. [2]Nyrang Avenue, Tamworth, New South Wales 2340, Australia. [3]Cooperative Research Centre for Australian Weed Management.
[4]Corresponding author. E-mail: adatta2@une.edu.au

Keywords: isoxaflutole, soil pH, organic matter, *Cicer arietinum*, herbicide tolerance.

Abstract

Glasshouse experiments were conducted to investigate the effects of soil pH and organic matter on chickpea (*Cicer arietinum*) tolerance to isoxaflutole applied pre-emergence at 0, 75 (recommended rate) and 300 g a.i. ha^{-1}. For the soil pH study, the variables examined were 2 desi chickpea genotypes (97039-1275 as a tolerant line and 91025-3021 as a sensitive line) and 4 pH levels (5.1, 6.9, 8.1, and 8.9). For the soil pH and organic matter experiment, 2 other desi genotypes (Yorker as a sensitive and Kyabra as a tolerant cultivar) were evaluated for 2 different levels of soil organic matter (2.7% and 3.6%) with 4 pH levels (6.6, 7.6, 8.3 and 9.1). The results demonstrated differential tolerances among chickpea genotypes to isoxaflutole at different rates, soil pH and organic matter levels. Isoxaflutole applied pre-emergence resulted in increased phytotoxicity with increases in soil pH and herbicide rate. Even the most tolerant chickpea genotype was damaged when exposed to higher pH and herbicide rates, as indicated by increased leaf chlorosis and significant reductions in plant height, and shoot and root dry weight. The effects were more severe with the sensitive genotype. The combined studies of soil pH and organic matter indicated that the root dry weight reductions and phytotoxic symptoms were most acute for chickpea on a low organic matter soil compared with a high organic matter soil. Chickpea cultivar performed comparatively well in the higher organic matter soil

compared with the lower organic matter soil having the same pH for atleast one growth parameter (root dry weight). The susceptibility of chickpea to this herbicide depends on genotype, soil pH and organic matter, which should be taken into account in breeding new lines, and in the agronomy of chickpea production.

Introduction

Chickpea (*Cicer arietinum*) is an important grain legume in Australia and is often grown in rotation with cereal crops, offering the advantage of cereal disease control, a possible increase in soil fertility, and a cash grain crop. One of the major obstacles in growing chickpea successfully is their poor ability to compete with weeds. Crop losses of 90% are possible in weedy situations (Knights 1991) and the lack of registered post-emergence herbicides for broadleaf weeds reduces the options for weed management.

Isoxaflutole at 75 g a.i. ha^{-1} is newly registered in Australia for the control of several troublesome broadleaf weeds in chickpea. It is a pre-emergent systemic soil applied herbicide, and has a relatively short half-life in soil (average 28 days), degrading via hydrolysis and microbial degradation (Luscombe *et al.* 1995). Isoxaflutole has favourable toxicological and environmental properties at low application rates (Luscombe *et al.* 1995; Vrabel *et al.* 1995). Isoxaflutole has sufficient residual activity to control weeds for the length of the growing season when applied at 105 g a.i. ha^{-1} in the field, but dissipates within a growing season and does not carry over to injure rotational crops (Vrabel *et al.* 1995).

However, there have been records of chickpea crop damage due to the application of isoxaflutole. Felton *et al.* (2004) demonstrated that under some conditions this herbicide can injure even the more tolerant varieties of chickpea at an application rate of less than 75 g a.i. ha^{-1} and consequently result in a yield penalty. Mitra *et al.* (2001) and Taylor-Lovell *et al.* (2000) have reported that isoxaflutole and its herbicidally active metabolite diketonitrile are most labile in soils with low organic matter and high pH. Mitra *et al.* (1999) showed that the sorption of isoxaflutole and diketonitrile was mainly influenced by the organic matter content and pH, but was not influenced appreciably by soil texture, i.e. clay content. Bresnahan *et al.* (2004) reported that organic carbon content is important in isoxaflutole and diketonitrile binding. Organic carbon-clay associations,

along with pH, may be the main factors affecting sorption of isoxaflutole and the diketonitrile metabolite.

Previous polyhouse trials with different chickpea genotypes treated with varying rates of isoxaflutole showed the genotypes Kyabra and 97039-1275 to be amongst the most tolerant and Yorker and 91025-3021 to be more sensitive genotypes to the herbicide (Datta *et al.* 2006). However, limited information exists on the effect of soil pH and organic matter on isoxaflutole injury to chickpea. The objectives of this research were to (1) evaluate tolerance to the application of isoxaflutole across a range of soil pH and organic matter levels and (2) determine if these effects vary between sensitive and more tolerant desi chickpea genotypes.

Materials and methods

Glasshouse experiment 1 - soil pH

The effect of isoxaflutole was compared with the desi chickpea breeding lines, 97039-1275 (isoxaflutole tolerant) and 91025-3021 (isoxaflutole sensitive), grown in soil in a glasshouse at four pH levels. A medium clay black vertosol soil (pH 6.8, 3.3% organic matter, 58% clay, 12% silt and 30% sand) was collected from a field site at the McMaster Research Station, Warialda, New South Wales (NSW), Australia. The pH levels (1:5 soil/water) of this soil were adjusted to 4.9 - 5.1, 6.8 - 6.9, 7.9 - 8.1 and 8.8 – 8.9. For increasing the pH, fixed amounts of a 60% $Ca(OH)_2$ and 40% $Mg(OH)_2$ mixture were added in two plastic bags each containing 500 g of soil. After mixing the soil thoroughly with the powder, water was added. To decrease the pH, a fixed volume of 1 M H_2SO_4 was added to each soil sample. The plastic bags were then placed in a 14 cm diameter plastic pot and kept in a glasshouse at 15 to 25°C for a 4 week pH equilibration period during which the soil was watered at regular intervals and the pH constantly monitored until it remained unchanged after 3 consecutive measurements. Five *Rhizobium* inoculated chickpea seeds were sown at a depth of 2.5-3 cm in the adjusted soil (1 kg). There was a total of 2 genotype × 4 pH level × 3 herbicide rate treatment combinations and 3 replications of each treatment laid out in a completely randomised design. Isoxaflutole was applied 1 day after sowing (DAS) at 0, 75 (recommended rate) and 300 g a.i. ha^{-1} equivalent using a gas-operated boom-sprayer through a TeeJet 11003 flat fan nozzle at a

pressure of 300 kPa delivering a volume of 84 L ha^{-1}. Seven DAS each pot was thinned to contain three chickpea seedlings. Pots were lightly watered every alternate day to keep the soil moist; pots also received 10 mm of simulated rainfall at 1 and 7 DAS and after that at regular intervals to avoid water stress. Chickpea crop injury was rated visually at 35 days after herbicide treatment (DAHT) using the scale of 1-9 where 1 = no leaf chlorosis, 5 = 50% of leaves chlorotic with stunting and 9 = total loss of plants. Plant heights were also recorded 35 DAHT by measuring the height from the ground level to the furthest extremity of the longest branch. At 35 DAHT, plants were harvested and dry weights of shoots and roots as well as tap root length were measured.

Glasshouse experiment 2 - soil pH and organic matter

The relative performance of two commercial cultivars, Kyabra (isoxaflutole tolerant) and Yorker (isoxaflutole sensitive), was evaluated for a soil (black vertosol) having different organic matter (OM) status. The soil was collected from an area at the McMaster Research Station, Warialda, NSW, with a long cropping history (suggesting low OM), and an adjacent native pasture (suggesting higher OM). The OM for the long cropping history was 2.7% whilst for native pasture was 3.6%. Other relevant properties of the two soils were similar (original pH was 7.5 – 7.6 and soil particle analysis was 58% clay, 12% silt and 30% sand). The pH levels of these two soils were then adjusted to 6.5 - 6.6, 7.5 - 7.6, 8.1 - 8.3 and 8.8 - 9.1. The experiment consisted of a completely randomised design containing the 2 × 3 × 2 × 4 factorial combinations of 2 genotypes (isoxaflutole tolerant and sensitive cultivar), 3 isoxaflutole rates (0, 75 and 300 g a.i. ha^{-1}), 2 OM levels (3.6 and 2.7%) and 4 pH levels (6.6, 7.6, 8.3 and 9.1), with three replications. The experimental procedures were the same as described for experiment 1. At 35 DAHT, plants were harvested and plant height, injury ratings, and dry weights of shoots and roots were measured.

Statistical analysis

Results were analysed using the analysis of variance (ANOVA) function of R 2.3.0 (R Development Core Team 2006) and P values < 0.05 were considered significant. The zero isoxaflutole rate was not included in the analysis of injury rating in both experiments due to the constant value for those treatments. Variances were checked by plotting

residual vs. fitted values to confirm the homogeneity of the data. No transformations were necessary. Means for significant treatment effects were separated based on standard errors (SE). The treatment combination means presented for a variable are based on the highest order of factorial combination that is significant in the ANOVA. Where this is less than the maximum factorial combination, the tables have been generated by pooling the data across the non significant factors.

Results

Glasshouse experiment 1 - soil pH

Pre-emergence application of isoxaflutole caused injury and reduced plant height in both chickpea genotypes. Visible crop injury symptoms included yellowing of leaves on the lower and middle branches, shedding of leaves, stunting, and for the sensitive genotype, necrosis. The three-way interaction (genotype × isoxaflutole rate × pH) was significant ($P < 0.05$) for the injury ratings recorded at 35 DAHT (Table 1). There was a strong pH response with injury ratings being greater at higher herbicide doses on high pH soils. Both genotypes had injury symptoms at 35 DAHT (Table 2). At the recommended rate of herbicide (75 g a.i. ha^{-1}) increasing pH increased leaf chlorosis for the tolerant line but the effect of increasing pH on leaf chlorosis was more pronounced for the sensitive line. At the high rate of isoxaflutole, death of the sensitive line occurred at high pH, while injury was much less at pH 5.1. In the tolerant line minimal damage occurred at pH 5.1 but damage occurred with increasing pH.

Table 1 Significance levels[a] in three-way ANOVA of the effects of genotype (tolerant and sensitive line), isoxaflutole rate (0, 75, 300 g a.i. ha^{-1}) and soil pH (5.1, 6.9, 8.1, 8.9), on the injury rating, plant height, shoot and root dry weight and tap root length of chickpea (experiment 1)

Terms	Injury rating	Plant height (cm)	Shoot dry weight (g pot^{-1})	Root dry weight (g pot^{-1})	Tap root length (cm)
Isoxaflutole (I)	***	ns	***	***	**
pH	***	***	***	***	**
Genotype (G)	***	***	***	***	ns
I × pH	ns	**	***	**	**
G × I	ns	***	*	ns	ns
G × pH	**	*	**	**	ns
G × I × pH	*	***	**	ns	ns

[a](***) $P < 0.001$; (**) $P < 0.01$; (*) $P < 0.05$; ns = not significant

Table 2 Effect of the rate of isoxaflutole on the injury rating[a] of a tolerant and sensitive chickpea genotype as affected by pH 35 days after herbicide treatment (experiment 1)

Genotype	Isoxaflutole (g a.i. ha^{-1})	Injury rating			
		pH 5.1	pH 6.9	pH 8.1	pH 8.9
Tolerant line	0[b]	1.0	1.0	1.0	1.0
	75	1.7	1.0	2.3	3.0
	300	2.0	2.8	5.7	6.7
Sensitive line	0[b]	1.0	1.0	1.0	1.0
	75	2.0	4.7	5.8	7.0
	300	5.0	7.3	9.0	9.0
SE			0.61		

[a] crop injury was rated visually using the scale of 1-9 where 1 = no leaf chlorosis, 5 = 50% of leaves chlorotic with stunting and 9 = total loss of plants; [b] the constant injury rating means (1.0) for isoxaflutole rate 0 g a.i. ha^{-1} were excluded from the analysis

The three-way interaction was highly significant ($P < 0.001$) for plant height at 35 DAHT (Table 1). Plant height was reduced by 25% in the tolerant line and 37% in the

sensitive line with increased soil pH without herbicide application (Table 3). When the higher herbicide rates were applied to the sensitive genotype, plant heights were reduced more at neutral to alkaline pH (up to 41% for the recommended rate, and 56% for the higher rate). The tolerant genotype also had significant reductions in plant height with increased soil pH (up to 23% for the recommended rate, and 50% for the higher rate). At any particular pH plant height of the tolerant line was little affected by increased herbicide rate. In contrast plant height of the sensitive line was reduced by 53% at pH 6.9 and 36% at pH 8.9.

Table 3 Effect of the rate of isoxaflutole on the plant height of a tolerant and sensitive chickpea genotype as affected by pH 35 days after herbicide treatment (experiment 1)

Genotype	Isoxaflutole (g a.i. ha^{-1})	Plant height (cm)			
		pH 5.1	pH 6.9	pH 8.1	pH 8.9
Tolerant line	0	14.9	18.1	13.3	13.7
	75	14.9	14.7	15.3	11.4
	300	16.4	23.1	15.5	11.5
Sensitive line	0	12.0	16.6	10.8	10.5
	75	12.5	14.1	11.0	8.4
	300	15.0	7.8	7.9	6.7
SE			1.52		

The three-way interaction was highly significant ($P < 0.01$) for shoot dry weight (Table 1). Shoot dry weight was reduced significantly in the tolerant genotype (Table 4) with increased soil pH (up to 32% for the recommended rate and 68% for the higher rate). Change in shoot dry weight in response to increasing herbicide rate was not significant for the tolerant genotype up to pH 8.1 but was significant at pH 8.9. The damage was more severe in the sensitive genotype (up to a 67% reduction for the recommended rate, and 81% for the higher rate with increased soil pH). The reduction in shoot dry weight in response to higher herbicide rates for the sensitive genotype was significant for the whole pH range except for pH 5.1.

Table 4 Effect of the rate of isoxaflutole on the shoot dry weight of a tolerant and sensitive chickpea genotype as affected by pH 35 days after herbicide treatment (experiment 1)

Genotype	Isoxaflutole (g a.i. ha^{-1})	Shoot dry weight (g pot^{-1})			
		pH 5.1	pH 6.9	pH 8.1	pH 8.9
Tolerant line	0	0.23	0.34	0.19	0.26
	75	0.22	0.25	0.23	0.17
	300	0.24	0.37	0.20	0.12
Sensitive line	0	0.13	0.23	0.12	0.15
	75	0.15	0.14	0.07	0.05
	300	0.16	0.07	0.03	0.03
SE		0.031			

Both genotypes behaved in a similar way for different herbicide rate and pH levels with respect to root dry weight (Table 1) as the three-way interaction was not significant ($P \geq 0.05$). The two-way interaction of genotype and herbicide rate was also not significant ($P \geq 0.05$). The two-way interactions involving pH (genotype × pH and isoxaflutole rate × pH), however, were highly significant for root dry weight ($P < 0.01$). At acid pH, root dry weight of chickpea was not affected by herbicide application (Table 5).

Table 5 Effect of the rate of isoxaflutole on the root dry weight of chickpea as affected by pH 35 days after herbicide treatment (experiment 1)

Isoxaflutole (g a.i. ha^{-1})	Root dry weight (g pot^{-1})			
	pH 5.1	pH 6.9	pH 8.1	pH 8.9
0	0.14	0.21	0.11	0.11
75	0.12	0.10	0.05	0.05
300	0.11	0.09	0.04	0.02
SE		0.016		

Root dry weight was reduced by 52 - 55% at neutral and alkaline soil conditions with the recommended herbicide rate. At pH 8.9, root dry weight was reduced by 82% with the higher rate of herbicide. There was no significant difference in chickpea root dry weight between the recommended and higher herbicide rates across the range of pHs. Root dry weight in the tolerant line was highest at pH 6.9 whereas it was the same in the sensitive line at pH 5.1 and 6.9 (Table 6). Root dry weight of the tolerant and sensitive genotypes was reduced by between 45 and 50% with increased soil pH above 6.9.

Table 6 Effect of increasing soil pH on the root dry weight of a tolerant and sensitive chickpea genotype 35 days after herbicide treatment (experiment 1)

Genotype	Root dry weight (g pot^{-1})			
	pH 5.1	pH 6.9	pH 8.1	pH 8.9
Tolerant line	0.14	0.18	0.08	0.07
Sensitive line	0.09	0.09	0.06	0.05
SE		0.013		

The three-way interaction was not significant ($P \geq 0.05$) for tap root length (Table 1). The two-way interaction of isoxaflutole rate × pH was highly significant ($P < 0.01$). Tap root length without herbicide treatment was not uniformly affected by changes in soil pH across chickpea genotypes (Table 7). At the recommended herbicide rate, increasing pH progressively decreased tap root length by up to 49% whereas with the higher rate of herbicide this reduction was 68%. At pH 8.9, tap root length was reduced by 40% with the recommended rate of herbicide.

Table 7 Effect of the rate of isoxaflutole on the tap root length of chickpea as affected by pH 35 days after herbicide treatment (experiment 1)

Isoxaflutole (g a.i. ha^{-1})	Tap root length (cm)			
	pH 5.1	pH 6.9	pH 8.1	pH 8.9
0	22.9	32.2	26.0	23.2
75	27.2	21.9	15.9	13.9
300	28.0	19.1	11.1	9.0
SE		3.76		

Glasshouse experiment 2 - soil pH and organic matter

Chickpea injury rating was affected by an interaction between genotype × pH ($P < 0.001$) (Table 8). Both cultivars showed more injury symptoms at high pH than at low pH with the addition of herbicide (Table 9). The injury rating of the tolerant cultivar with the herbicide was 3.2 at pH 6.6 whereas the rating was 4.3 at pH 9.1. The injury symptoms were much more acute with the sensitive cultivar ranging from 4.4 at low pH to 6.5 with higher pH. Injury rating of chickpea was also influenced by highly significant main effects of isoxaflutole rate ($P < 0.001$) and OM ($P < 0.001$). The injury symptoms of chickpea were increased with increasing herbicide rate (Table 10). With the recommended herbicide rate, the injury rating was 4.0 whereas the injury rating was 5.3 with the higher herbicide rate. The herbicide injury symptoms were significantly greater in chickpea in the lower OM soil compared with the higher OM soil (5.0 versus 4.3 respectively with the herbicide).

Table 8 Significance levels[a] in four-way ANOVA of the effects of genotype (tolerant and sensitive cultivar), isoxaflutole rate (0, 75, 300 g a.i. ha^{-1}), organic matter (2.7, 3.6%) and soil pH (6.6, 7.6, 8.3, 9.1), on the injury rating, plant height, and shoot and root dry weight of chickpea (experiment 2)

Terms	Injury rating	Plant height (cm)	Shoot dry weight (g pot^{-1})	Root dry weight (g pot^{-1})
Genotype (G)	***	***	***	***
Isoxaflutole (I)	***	***	***	***
Organic matter (OM)	***	ns	ns	***
pH	***	***	***	***
G × I	ns	***	***	*
G × OM	ns	ns	ns	ns
G × pH	***	*	ns	ns
I × OM	ns	ns	ns	ns
I × pH	ns	*	ns	ns
OM × pH	ns	ns	ns	*
G × I × pH	ns	ns	*	ns
G × I × OM	ns	ns	ns	ns
G × OM × pH	ns	ns	ns	ns
I × OM × pH	ns	ns	ns	ns
G × I × OM × pH	ns	ns	ns	ns

[a](***) $P < 0.001$; (**) $P < 0.01$; (*) $P < 0.05$; ns = not significant

Table 9 Effect of increasing soil pH on the injury rating[a] of a tolerant and sensitive chickpea genotype 35 days after herbicide treatment (experiment 2). The constant injury rating means (1.0) for isoxaflutole rate 0 g a.i. ha^{-1} were excluded from the analysis

Genotype	Injury rating			
	pH 6.6	pH 7.6	pH 8.3	pH 9.1
Tolerant cultivar	3.2	3.2	3.7	4.3
Sensitive cultivar	4.4	5.4	6.4	6.5
SE		0.21		

[a] crop injury was rated visually using the scale of 1-9 where 1 = no leaf chlorosis, 5 = 50% of leaves chlorotic with stunting and 9 = total loss of plants

Table 10 Effect of the rate of isoxaflutole and soil organic matter (OM) on the injury rating[a] of chickpea 35 days after herbicide treatment (experiment 2)

Isoxaflutole (g a.i. ha^{-1})	Injury rating
0[b]	1.0
75	4.0
300	5.3
SE	0.11
OM (%)	
2.7	5.0
3.6	4.3
SE	0.11

[a] crop injury was rated visually using the scale of 1-9 where 1 = no leaf chlorosis, 5 = 50% of leaves chlorotic with stunting and 9 = total loss of plants; [b] the constant injury rating means (1.0) for isoxaflutole rate 0 g a.i. ha^{-1} were excluded from the analysis

In regard to plant height, isoxaflutole rate × pH ($P < 0.05$), genotype × pH ($P < 0.05$) and isoxaflutole rate × genotype ($P < 0.001$) were significant interactions (Table 8). Plant height reductions between the zero and recommended herbicide rates were not significant across chickpea genotypes at any pH (Table 11). However, at the higher

herbicide rate (300 g a.i. ha^{-1}), plant height was significantly reduced compared with the zero rate with increasing pH. At pH 7.6, plant height was reduced by 23% with the 300 g a.i. ha^{-1} rate. Plants of the tolerant cultivar were tallest at pH 7.6 while those of the sensitive cultivar were similar at pH 6.6 and 7.6 (Table 12). Height of both cultivars declined with strongly alkaline conditions. Plant height of the tolerant cultivar was unaffected with increasing herbicide rate whereas it was reduced by 9 and 41% with the recommended and higher herbicide rates respectively in the case of the sensitive cultivar (Table 13).

Table 11 Effect of the rate of isoxaflutole on the plant height of chickpea as affected by pH 35 days after herbicide treatment (experiment 2)

Isoxaflutole g a.i. ha^{-1}	Plant height (cm)			
	pH 6.6	pH 7.6	pH 8.3	pH 9.1
0	18.1	19.4	17.0	16.1
75	18.2	20.6	18.1	17.3
300	16.4	15.0	14.3	13.4
SE		0.71		

Table 12 Effect of increasing soil pH on the plant height of a tolerant and sensitive chickpea genotype 35 days after herbicide treatment (experiment 2)

Genotype	Plant height (cm)			
	pH 6.6	pH 7.6	pH 8.3	pH 9.1
Tolerant cultivar	20.8	22.4	19.9	18.4
Sensitive cultivar	14.4	14.2	13.0	12.8
SE		0.58		

Table 13 Effect of the rate of isoxaflutole on the plant height of a tolerant and sensitive chickpea genotype 35 days after herbicide treatment (experiment 2)

Isoxaflutole (g a.i. ha^{-1})	Plant height (cm)	
	Tolerant cultivar	Sensitive cultivar
0	19.0	16.3
75	22.2	14.9
300	19.9	9.7
SE	0.50	

The three-way interaction (genotype × isoxaflutole rate × pH) was significant ($P < 0.05$) for chickpea shoot dry weight (Table 8). Shoot dry weight (Table 14) reductions were generally significant for the tolerant cultivar between 0 and 300 g a.i. ha^{-1} herbicide rate but there were no significant differences between the recommended and higher rates. Shoot dry weight was reduced more in the case of the sensitive cultivar compared with the tolerant cultivar with increasing herbicide rate and pH. Shoot dry weight of the sensitive cultivar was reduced significantly at the recommended rate of herbicide for the pH $\geq$ 7.6 but there was no effect on the tolerant cultivar under similar situations. At the lower pH (6.6), the reduction in shoot dry weight of the sensitive cultivar was not significant with the recommended rate of herbicide. Shoot dry weight of the sensitive cultivar was reduced by 31% with the recommended herbicide rate and by 47% with the higher herbicide rate at pH 8.3. With the recommended herbicide rate, shoot dry weight was reduced by 40% when the pH was increased from 6.6 to 9.1.

Table 14 Effect of the rate of isoxaflutole on the shoot dry weight of a tolerant and sensitive chickpea genotype as affected by pH 35 days after herbicide treatment (experiment 2)

Genotype	Isoxaflutole (g a.i. ha^{-1})	Shoot dry weight (g pot^{-1})			
		pH 6.6	pH 7.6	pH 8.3	pH 9.1
Tolerant cultivar	0	0.59	0.51	0.47	0.45
	75	0.47	0.49	0.46	0.41
	300	0.50	0.50	0.40	0.37
Sensitive cultivar	0	0.38	0.41	0.36	0.31
	75	0.35	0.31	0.25	0.21
	300	0.24	0.21	0.18	0.17
SE			0.034		

Root dry weight of chickpea was influenced by interactions between soil pH × OM ($P < 0.05$) and genotype × isoxaflutole rate ($P < 0.05$) (Table 8). Root dry weight was reduced in the lower OM soil as compared with higher OM soil but this varied with pH from 32% reduction at pH 7.6 to 20% at pH 9.1 (Table 15). Root dry weight reduction was greater for the sensitive cultivar with increasing herbicide rate than the tolerant cultivar (Table 16). Root dry weight was reduced by 45% with the recommended rate of herbicide in the case of the sensitive cultivar, whereas it was 24% in the case of the tolerant cultivar with the same herbicide rate.

Table 15 Effect of increasing soil pH on the root dry weight of chickpea genotype as affected by organic matter (OM) 35 days after herbicide treatment (experiment 2)

OM (%)	Root dry weight (g pot^{-1})			
	pH 6.6	pH 7.6	pH 8.3	pH 9.1
2.7	0.23	0.17	0.23	0.21
3.6	0.29	0.25	0.25	0.26
SE		0.013		

Table 16 Effect of the rate of isoxaflutole on the root dry weight of a tolerant and sensitive chickpea genotype 35 days after herbicide treatment (experiment 2)

Isoxaflutole (g a.i. ha^{-1})	Root dry weight (g pot^{-1})	
	Tolerant cultivar	Sensitive cultivar
0	0.37	0.29
75	0.28	0.16
300	0.21	0.10
SE	0.012	

Discussion

These experiments showed that chickpea genotypes differ in their sensitivity to isoxaflutole and that soil pH can have a major effect on the degree of injury. Organic matter also influenced the degree of injury. These findings support research that has shown differential tolerance of chickpea to isoxaflutole (Felton *et al.* 2004). The herbicidal activity of isoxaflutole in susceptible species is associated with the development of a characteristic bleaching of foliar tissue following treatment (Pallett *et al.* 2001). The biochemical target of isoxaflutole is the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), inhibition of which results in a depletion of plastoquinone, which is a co-factor of phytoene desaturase (Pallett *et al.* 1998; Viviani *et al.* 1998). This results in a depletion of carotenoids and an absence of chloroplast development in emerging foliar tissues which appear bleached and stunted (Luscombe and Pallett 1996). In plants, soil, or water, a diketonitrile derivative is produced by opening of the isoxazole ring of isoxaflutole (Pallett *et al.* 1998; Viviani *et al.* 1998) and this diketonitrile is the herbicidally active form known to inhibit HPPD in plants. The rate of herbicide metabolism may be a major factor in determining the differential tolerances of chickpea genotypes. Sprague *et al.* (1999c) reported that corn (*Zea mays*) hybrids more tolerant to isoxaflutole were able to metabolize it more rapidly than the more sensitive hybrids. Previous research by Pallett *et al.* (1998) has identified isoxaflutole metabolism as the primary basis for differential selectivity between the tolerant species corn and a susceptible species *Abutilon theophrasti* (velvetleaf).

Injury symptoms of chickpea generally increased and plant dry weight decreased as herbicide rate and pH increased irrespective of the genotype. The more tolerant genotype showed symptoms of injury at high pH levels at the recommended rate of isoxaflutole and the symptoms translated into reductions in plant height and dry weights of shoots and roots. The effects of high soil pH on the sensitive genotype were more severe. Chickpea cultivar performed comparatively well in higher organic matter soil compared with the lower organic matter soil having the same pH for atleast one growth parameter (root dry weight). The soil organic matter levels of 2.7 and 3.6% were probably not sufficiently different to have a large effect on herbicide activity. The effect of soil organic matter warrants further investigation. Sprague *et al.* (1999a) reported isoxaflutole degradation to be pH-dependent. Degradation was greatest at pH = 10 (86%) when compared with pH = 4 (16%) or pH = 7 (20%). Soil pH plays a key role in both sorption and degradation of isoxaflutole (Mitra *et al.* 2001). The high pH soil resulted in active herbicide formation, i.e., both the precursor isoxaflutole and its active derivative, diketonitrile, thereby enhancing the phytotoxicity of the applied compound (Mitra *et al.* 2001; Sprague *et al.* 1999a). Rouchaud *et al.* (1998) found that isoxaflutole dissipation in soils was slightly faster at pH 7.2 than at pH 5.5. They concluded that at pH 8.5 some of the organic matter might dissolve in the solution and contribute to the reduction of isoxaflutole/diketonitrile sorption. The low basicity of the isoxazole ring may also enable proton addition of isoxaflutole at low soil pH and in turn increase isoxaflutole adsorption by cation exchange and reduce the rate of isoxaflutole soil dissipation.

In laboratory studies, it has been reported that isoxaflutole is more stable under acidic conditions than in neutral or alkaline media regardless of the prevailing temperature (Beltran *et al.* 2000). Mitra *et al.* (1999) demonstrated that sorption of isoxaflutole increased with an increase in organic matter content, but isoxaflutole was more strongly sorbed than diketonitrile on all soils, probably due to lower aqueous solubility of isoxaflutole. Soils with relatively higher organic matter content will have greater affinity for diketonitrile and hence, the availability and release of diketonitrile will be less in these soils compared with soils with lower organic matter content (Mitra *et al.* 2000). Mitra *et al.* (2001) also reported that the sorption of diketonitrile increased with an increase in organic matter content and a decrease in soil pH suggesting less herbicide

injury to a crop in soils with high organic matter and low pH. Soil pH is a very important factor that affects sorption and degradation of herbicides. For example, the effects of soil pH and organic matter content on flumetsulam sorption in soil are well documented (Fontaine *et al.* 1991; Lehmann *et al.* 1992), and leaching is more likely in soils with low organic matter and high pH (Kleschnick *et al.* 1992). Shaw and Murphy (1997) reported that flumetsulam mobility in soils with similar organic matter content increased as soil pH increased from 5.3 to 7.2. They attributed the greater mobility to its ionic species at $pH \geq 7.2$.

In order to achieve effective weed control in chickpea using the herbicide isoxaflutole without sustaining significant crop injury, care is needed in selecting tolerant genotypes, and in avoiding high pH and low organic matter soils. It is likely that when a new herbicide is released (Vrabel *et al.* 1995) it may not have been sufficiently tested under a wide range of conditions to identify potential problems.

Statement of Originality:

All the work contained within this paper is the original research of the PhD candidate, Avishek Datta.

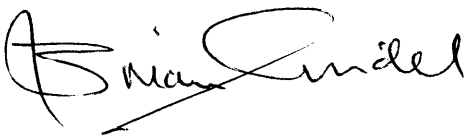
Candidate: 

Principal Supervisor: 

Statement of Contribution by Others:

This paper has been prepared by the PhD candidate, Avishek Datta. All coauthors are either PhD supervisors (Sindel, Jessop, Felton) or statistical advisor (Kristiansen) and have only contributed to this paper to the extent that would normally be expected of such roles. All coauthors have given their consent for having their contributions to this paper included in the thesis and accept the student's contribution as indicated in the Statement of Originality.

Candidate: 

Principal Supervisor: 

CHAPTER FIVE

The effects of temperature and soil moisture on chickpea (*Cicer arietinum*) genotype sensitivity to isoxaflutole

Manuscript submitted to Weed Research, April 2007

(currently under review)

The effects of temperature and soil moisture on chickpea (*Cicer arietinum*) genotype sensitivity to isoxaflutole

Avishek Datta[A,C,D], Brian M. Sindel[A,C], Robin S. Jessop[A,C], Paul Kristiansen[A] and Warwick L. Felton[B]

[A]School of Rural Science and Agriculture, The University of New England, Armidale, New South Wales 2351, Australia

[B]Nyrang Avenue, Tamworth, New South Wales 2340, Australia

[C]Cooperative Research Centre for Australian Weed Management

[D]Corresponding author. Email: adatta2@une.edu.au

Abstract

Controlled environment experiments were used to determine the tolerance of chickpea to isoxaflutole under a range of temperature and soil moisture levels. For the soil moisture study, the variables examined were 2 desi chickpea (*Cicer arietinum*) genotypes (Kyabra as a tolerant cultivar and Yorker as a sensitive cultivar), 3 soil moisture levels (50, 75 and 100% of field capacity-FC) with 3 herbicide rates [0, 75 (recommended rate) and 300 g a.i. ha^{-1}]. For the temperature and soil moisture study, the variables examined were 2 other desi chickpea genotypes (97039-1275 as a tolerant line and 91025-3021 as a sensitive line), 3 temperature regimes (20/5, 30/15, and 35/25°C), 2 soil moisture conditions (50 and 100% FC) with the same 3 herbicide rates. The results demonstrated differential tolerances among chickpea genotypes to isoxaflutole at different rates, temperatures and soil moisture levels. Increasing temperature and soil moisture content made the susceptible chickpea genotype more vulnerable to isoxaflutole damage. The soil moisture study also pointed out that isoxaflutole caused more damage to chickpea in terms of increased leaf chlorosis and reduction in shoot height and dry matter production with increasing moisture content. The sensitivity of chickpea to isoxaflutole depends on existing temperature and moisture content and the chances of crop damage were enhanced with increasing temperature and moisture levels.

Introduction

Isoxaflutole is a new soil applied herbicide which belongs to the isoxazole class of herbicides and used for pre-emergence control of grass and broadleaf weeds in maize (*Zea mays*) and sugarcane (*Saccharum* spp.) (Luscombe and Pallett 1996; Luscombe *et al.* 1995). In Australia isoxaflutole at 75 g a.i. ha^{-1} is registered for the control of several troublesome broadleaf weeds [Indian hedge mustard (*Sisymbrium orientale*), sowthistle (*Sonchus oleraceus*), capeweed (*Arctotheca calendula*), prickly lettuce (*Lactuca serriola*), wild radish (*Raphanus raphanistrum*), turnip weed (*Rapistrum rugosum*), crassula (*Crasulla* spp.), Medic (*Medicago* spp.), deadnettle (*Lamium amplexicaule*), and slender celery (*Ciclospermum leptophyllum*)] in chickpea (*Cicer arietinum*). When applied pre-emergence or early pre-plant, relatively low rates of isoxaflutole provide effective control of important broadleaf and grass weeds of maize (Luscombe *et al.* 1995).

However, there have been records of chickpea crop damage due to the application of isoxaflutole. Felton *et al.* (2004) demonstrated that under some conditions this herbicide can injure varieties of chickpea at an application rate of less than 75 g a.i. ha^{-1} and result in a yield penalty. The influence of factors like temperature and soil moisture on the degree of isoxaflutole injury to chickpea is not well documented. Pallett *et al.* (2001) found that both soil moisture and temperature influenced the persistence of isoxaflutole in soil and reported that increasing moisture level and temperature increased the phytotoxicity symptoms in maize treated with isoxaflutole. It has been shown that, for some herbicides, weather (Hallgren 1989; Lundkvist 1997; Minkey and Moore 1996) and temperature, in particular, can significantly affect herbicide activity (Caseley 1987). Controlled environment experiments have demonstrated a strong influence of temperature on the activity of many herbicides including MCPA + dichloprop (Jensen and Kudsk 1988; Kudsk and Kristensen 1992), chlorsulfuron (Nalewaja and Woznica 1985), thifensulfuron (Kudsk *et al.* 1990) and ioxynil (Merritt 1984). Soil moisture content is also an important factor in the amount of herbicide injury (Loston and Penner 1990).

Previous polyhouse trials with different chickpea genotypes treated with varying rates of isoxaflutole showed genotypes 97039-1275 and Kyabra to be amongst the most tolerant and 91025-3021 and Yorker to be more sensitive genotypes to the herbicide

(Datta *et al.* 2006). The objectives of this study were to (1) examine possible interactions between isoxaflutole at different temperature and soil moisture levels and (2) determine if these effects vary between sensitive and more tolerant desi chickpea genotypes.

Materials and methods

Growth chamber experiment 1 - temperature and soil moisture

This experiment was carried out in growth chambers at the University of New England, Armidale, New South Wales (NSW), Australia using one isoxaflutole tolerant (97039-1275) and one sensitive chickpea genotype (91025-3021). The soil in each plastic pot (diameter 14 cm) was pre watered to bring to field capacity before sowing the seeds. Five *Rhizobium* treated seeds were sown in each pot filled with 1 kg of black vertisol soil (2.7% organic matter, pH 7.5, 58% clay, 12% silt and 30% sand) from the McMaster Research Station, Warialda, NSW, Australia. Isoxaflutole was applied 1 day after sowing (DAS) at 0, 75 (recommended rate) and 300 g a.i. ha^{-1} equivalent using a gas-operated boom-sprayer through a TeeJet 11003 flat fan nozzle at a pressure of 300 kPa delivering a volume of 84 L ha^{-1}. The experiment consisted of a completely randomised design containing the 2 × 3 × 3 × 2 factorial combinations of 2 genotypes (isoxaflutole tolerant and sensitive line), 3 isoxaflutole rates (0, 75 and 300 g a.i. ha^{-1}), 3 temperature regimes (20/5, 30/15 and 35/25°C) and 2 soil moisture contents (50% and 100% of field capacity-FC), with four replications. The temperatures were chosen to cover the range of growing conditions of chickpea from planting to harvest. The pots were kept in a glasshouse (25/15°C) for one week for germination. Before transferring the pots to the growth chambers, the moisture contents were adjusted to the two levels. Each pot was thinned to contain three chickpea seedlings at 10 DAS. Three temperature controlled growth cabinets (photoperiod approximately 12 h day/12 h night) were used to create the 3 temperature regimes. On every alternate day moisture levels were checked and returned to the desired level up to harvest. Plant heights were recorded at 35 days after herbicide treatment (DAHT) by measuring the height from the ground level to the furthest extremity of the longest branch. Injury rating was assessed on a scale of 1-9 where 1 = no leaf chlorosis, 5 = 50% of leaves chlorotic with stunting and 9 = total loss of plants. The scoring was done on the average effect on two plants within a pot, with two operators

independently scoring each pot relative to the closest unsprayed control. Plants were then harvested at 35 DAHT and dry weights of shoots and roots were measured after drying for 48 hours at 80°C.

Growth chamber experiment 2 - soil moisture

This experiment was carried out in growth chambers at the University of New England, Armidale, NSW. The desi chickpea cultivars Yorker (sensitive to isoxaflutole) and Kyabra (tolerant to isoxaflutole) were used for this experiment. The soil in each plastic pot (diameter 14 cm) was pre watered to bring it to FC before sowing. Five *Rhizobium* treated seeds were sown in each pot filled with the same soil as described in experiment 1. Isoxaflutole was applied 1 DAS at 0, 75 and 300 g a.i. ha^{-1} equivalent as described in experiment 1. There were 2 genotye × 3 isoxaflutole rate × 3 soil moisture treatment combinations and 3 replications of each treatment laid out in a completely randomised design. The pots were then kept in a glasshouse (25/15°C) for one week for germination. Before transferring the pots to the growth chambers, the moisture content of the pots was adjusted to 50%, 75% and 100% FC. Ten DAS each pot was thinned to contain three chickpea seedlings. The temperature of the growth cabinet was adjusted to 30°C day and 15°C night (photoperiod approximately 12 h day/12 h night). On alternate days the moisture level of each pot was adjusted to the desired level. Plants were harvested at 35 DAHT and plant height, injury ratings, and dry weights of shoots and roots were measured.

Statistical analysis

Results were analysed using the analysis of variance (ANOVA) function of R 2.3.0 (R Development Core Team 2006) and P values < 0.05 were considered significant. The zero isoxaflutole rate was not included in the analysis of injury rating in both experiments due to the constant value for those treatments. Variances were checked by plotting residual vs. fitted values to confirm the homogeneity of the data. No transformations were necessary. Means for significant treatment effects were separated based on standard errors (SE). The treatment combination means presented for a variable are based on the highest order of factorial combination that is significant in the ANOVA. Where this is less than

the maximum factorial combination, the tables have been generated by pooling the data across the non significant factors.

Results

Growth chamber experiment 1- temperature and soil moisture

Visible crop injury symptoms included yellowing of lower branch leaves, stunting of growth, and for the sensitive genotype, necrosis. Chickpea injury rating was affected by interactions between genotype × temperature × moisture ($P < 0.01$) and genotype × isoxaflutole rate ($P < 0.001$) (Table 1). The tolerant line produced very minor injury symptoms irrespective of any moisture levels and temperature regimes (Table 2). The injury was much more acute in the sensitive line with higher temperature and moisture content. The injury rating was 3.4 with 20/5°C and 100% FC and 4.5 with 30/15°C and 100% FC but at 35/25°C and 100% FC, the sensitive line had a severe injury rating of 5.4. With the recommended rate of herbicide, the injury rating of the tolerant line was 1.5 whereas the rating was 3.0 for the sensitive line (Table 3). The injury rating increased significantly in the sensitive line with increased herbicide rate but not in the tolerant line.

Table 1 Significance levels[a] in four-way ANOVA of the effects of genotype (tolerant and sensitive line), isoxaflutole rate (0, 75, 300 g a.i. ha^{-1}), temperature (35/25, 30/15, 20/5°C) and soil moisture (FC, 50% FC), on the injury rating, plant height, and shoot and root dry weight of chickpea (experiment 1)

Terms	Injury rating	Plant height (cm)	Shoot dry weight (g pot^{-1})	Root dry weight (g pot^{-1})
Genotype (G)	***	***	***	***
Isoxaflutole (I)	***	***	***	***
Temperature (T)	***	***	***	***
Moisture (M)	***	***	***	***
G × I	***	***	***	***
G × T	***	ns	ns	***
G × M	***	***	***	***
I × T	ns	ns	ns	*
I × M	ns	**	***	***
T × M	ns	***	***	***
G × I × T	ns	ns	ns	*
G × I × M	ns	***	**	**
I × T × M	ns	ns	ns	**
G × T × M	**	ns	ns	***
G × I × T × M	ns	***	ns	ns

[a](***) $P < 0.001$; (**) $P < 0.01$; (*) $P < 0.05$; ns = not significant

Table 2 Effect of moisture on the injury rating[a] of a tolerant and sensitive chickpea genotype as affected by temperature 35 days after herbicide treatment. The constant injury rating means (1.0) for isoxaflutole rate 0 g a.i. ha^{-1} were excluded from the analysis

Genotype	Moisture levels	Injury rating		
		Temperature regime (°C)		
		35/25	30/15	20/5
Tolerant line	FC[b]	1.7	1.5	1.5
	50% FC	1.8	1.0	1.1
Sensitive line	FC	5.4	4.5	3.4
	50% FC	2.0	1.8	1.3
SE			0.35	

[a] crop injury was rated visually using the scale of 1-9 where 1 = no leaf chlorosis, 5 = 50% of leaves chlorotic with stunting and 9 = total loss of plants; [b] Field capacity

Table 3 Effect of increasing rates of isoxaflutole on the injury rating[a] of a tolerant and sensitive chickpea genotype 35 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Injury rating	
	Tolerant line	Sensitive line
0[b]	1.0	1.0
75	1.5	3.0
300	1.7	5.2
SE	0.14	

[a] crop injury was rated visually using the scale of 1-9 where 1 = no leaf chlorosis, 5 = 50% of leaves chlorotic with stunting and 9 = total loss of plants; [b] the constant injury rating means (1.0) for isoxaflutole rate 0 g a.i. ha^{-1} were excluded from the analysis

The four-way interaction (genotype × isoxaflutole rate × temperature × moisture) was highly significant ($P < 0.001$) for plant height (Table 1). Isoxaflutole had no effect on plant height (Table 4) within any of the temperature or moisture treatments with the tolerant line at 35 DAHT. At 50% FC increasing herbicide rate in each of the temperature

regimes did not affect plant height of the sensitive line whereas at 100% FC increasing herbicide rate did reduce height and the reduction was increased with increasing temperature (30 and 36% plant height reduction in 35/25°C and 30/15°C temperature regime respectively as compared with 16% in 20/5°C regime at 100% FC). The effect of increased temperature on plant height at 100% FC was reduced by 25% at the recommended herbicide rate, but so too was the height reduced by 25% in the ansence of herbicide.

Table 4 Effect of increasing rates of isoxaflutole on the plant height of a tolerant and sensitive chickpea genotype as affected by temperature and moisture 35 days after herbicide treatment (experiment 1)

Genotype	Isoxaflutole (g a.i. ha^{-1})	Moisture levels	Plant height (cm)		
			Temperature regime (°C)		
			35/25	30/15	20/5
Tolerant line	0	FC[a]	15.8	19.2	22.2
		50% FC	7.2	7.7	14.0
	75	FC	17.7	17.4	23.0
		50% FC	7.4	9.4	13.4
	300	FC	16.4	20.8	19.8
		50% FC	6.7	7.9	13.7
Sensitive line	0	FC	14.5	18.7	19.0
		50% FC	6.6	8.8	13.5
	75	FC	14.3	18.9	19.1
		50% FC	6.1	8.3	13.6
	300	FC	10.2	12.0	15.9
		50% FC	6.6	8.6	11.9
SE				1.11	

[a] Field capacity

Chickpea shoot dry weight was influenced by interactions between genotype × isoxaflutole rate × moisture ($P < 0.01$) and temperature × moisture ($P < 0.001$) (Table 1). Isoxaflutole had no effect on shoot dry weight (Table 5) of the tolerant line in either of the moisture treatments. The sensitive line recorded significant shoot dry weight reduction with increasing herbicide rate at 100% FC - by 29% with the recommended herbicide rate and 67% with the higher rate of herbicide. At 50% FC, shoot dry weight of the sensitive line was only marginally affected with increasing herbicide rate.

Table 5 Effect of increasing rates of isoxaflutole on the shoot dry weight of a tolerant and sensitive chickpea genotype as affected by moisture 35 days after herbicide treatment (experiment 1)

Genotype	Isoxaflutole (g a.i. ha^{-1})	Shoot dry weight (g pot^{-1})	
		Moisture levels	
		FC[a]	50% FC
Tolerant line	0	0.35	0.11
	75	0.33	0.12
	300	0.33	0.10
Sensitive line	0	0.21	0.09
	75	0.15	0.08
	300	0.07	0.06
SE		0.014	

[a] Field capacity

Shoot dry weight of chickpea was generally reduced at higher temperature and particularly under drier conditions at 50% FC (Table 6).

Table 6 Effect of moisture on the shoot dry weight of chickpea as affected by temperature 35 days after herbicide treatment (experiment 1)

Temperature regime (°C)	Shoot dry weight (g pot^{-1})	
	Moisture levels	
	FC[a]	50% FC
35/25	0.20	0.05
30/15	0.26	0.09
20/5	0.26	0.14
SE	0.01	

[a] Field capacity

All four three-way interactions for root dry weight of chickpea were significant (Table 1). However, those which best demonstrate treatment effects in combination with herbicide are genotype × isoxaflutole rate × moisture ($P < 0.01$) and genotype × isoxaflutole rate × temperature ($P < 0.05$). Root dry weight of the tolerant genotype was unaffected with increasing herbicide rate in either moisture treatment although root dry weight was much higher at FC than 50% FC (Table 7). With the recommended rate of herbicide root dry weight of the sensitive genotype was significantly reduced (67%) at 100% FC and reduced again when the herbicide was increased to 300 g a.i. ha^{-1}. In contrast, at the recommended rate of herbicide root dry weight was unaffected under 50% FC but there was a significant drop in root dry weight from the recommended rate to the higher rate (300 g a.i. ha^{-1}).

Table 7 Effect of increasing rates of isoxaflutole on the root dry weight of a tolerant and sensitive chickpea genotype as affected by moisture 35 days after herbicide treatment (experiment 1)

Genotype	Isoxaflutole (g a.i. ha^{-1})	Root dry weight (g pot^{-1})	
		Moisture levels	
		FC[a]	50% FC
Tolerant line	0	0.21	0.06
	75	0.19	0.06
	300	0.20	0.06
Sensitive line	0	0.18	0.06
	75	0.06	0.07
	300	0.01	0.03
SE		0.017	

[a] Field capacity

Increasing herbicide rate had no effect on root dry weight of the tolerant genotype in any of the temperature regimes though growth was optimal at 30/15°C and generally reduced at either higher or lower temperatures (Table 8). Root dry weight of the sensitive genotype was reduced by 69% at 30/15°C with the recommended herbicide rate at the temperature where growth was generally considered optimal, while at the higher temperature regime reductions in shoot dry weight were not significant. At the lower temperature regime (20/5°C), root dry weight was unaffected with the recommended herbicide rate, but was reduced significantly at 300 g a.i. ha^{-1}.

Table 8 Effect of increasing rates of isoxaflutole on the root dry weight of a tolerant and sensitive chickpea genotype as affected by temperature 35 days after herbicide treatment (experiment 1)

Genotype	Isoxaflutole (g a.i. ha^{-1})	Root dry weight (g pot^{-1})		
		Temperature regime (°C)		
		35/25	30/15	20/5
Tolerant line	0	0.08	0.19	0.13
	75	0.09	0.16	0.13
	300	0.07	0.20	0.14
Sensitive line	0	0.05	0.16	0.14
	75	0.03	0.05	0.11
	300	0.02	0.02	0.04
SE			0.021	

Growth chamber experiment 2 - soil moisture

The three-way interaction (genotype × isoxaflutole rate × moisture) was highly significant ($P < 0.01$) for injury rating recorded at 35 DAHT (Table 9). The tolerant cultivar had very minor phytotoxic symptoms with 100% FC and recommended rate of herbicide (Table 10). The injury symptoms increased with increasing soil moisture and herbicide rate. The injury was much more acute in the sensitive cultivar so that symptoms were obvious even at 50% FC. At 100% FC, the sensitive cultivar had severe injury (5.7) at the recommended herbicide rate while injury was comparatively less at 75% (3.2) and 50% FC (1.9).

Table 9 Significance levels[a] in three-way ANOVA of the effects of genotype (tolerant and sensitive cultivar), isoxaflutole rate (0, 75, 300 g a.i. ha^{-1}) and moisture (FC, 75% FC, 50% FC), on the injury rating, plant height, and shoot and root dry weight of chickpea (experiment 2)

Terms	Injury rating	Plant height (cm)	Shoot dry weight (g pot^{-1})	Root dry weight (g pot^{-1})
Genotype (G)	***	***	***	***
Isoxaflutole (I)	***	***	***	***
Moisture (M)	***	***	***	***
G × I	*	***	ns	ns
G × M	**	***	*	ns
I × M	**	**	***	***
G × I × M	**	*	ns	ns

[a](***) $P < 0.001$; (**) $P < 0.01$; (*) $P < 0.05$; ns = not significant

Table 10 Effect of increasing rates of isoxaflutole on the injury rating[a] of a tolerant and sensitive chickpea genotype as affected by moisture 35 days after herbicide treatment. The constant injury rating means (1.0) for isoxaflutole rate 0 g a.i. ha^{-1} were excluded from the analysis (experiment 2)

Genotype	Isoxaflutole (g a.i. ha^{-1})	Injury rating		
		Moisture levels		
		FC[b]	75% FC	50% FC
Tolerant cultivar	0	1.0	1.0	1.0
	75	1.7	1.0	1.0
	300	4.5	2.5	1.0
Sensitive cultivar	0	1.0	1.0	1.0
	75	5.7	3.2	1.9
	300	7.6	6.5	3.7
SE			0.47	

[a] crop injury was rated visually using the scale of 1-9 where 1 = no leaf chlorosis, 5 = 50% of leaves chlorotic with stunting and 9 = total loss of plants; [b] Field capacity

The three-way interaction (genotype × isoxaflutole rate × moisture) was significant for plant height ($P < 0.05$) recorded at 35 DAHT (Table 9). Increasing herbicide rate had no effect on plant height of the tolerant cultivar 35 DAHT even at the higher soil moisture levels (Table 11). There was a significant reduction in plant height of the sensitive cultivar at the higher moisture content when herbicide rate was increased. The increasing herbicide rate had no effect on plant height for the sensitive cultivar at 50% FC. But height reduction of the sensitive cultivar with increasing herbicide rate was significant with 300 g a.i. ha^{-1} at 75% FC and at 75 g a.i. ha^{-1} at 100% FC. Height was reduced by 16% with the sensitive cultivar at the recommended rate and 100% of FC.

Table 11 Effect of increasing rates of isoxaflutole on the plant height of a tolerant and sensitive chickpea genotype as affected by moisture 35 days after herbicide treatment (experiment 2)

Genotype	Isoxaflutole (g a.i. ha^{-1})	Plant height (cm)		
		Moisture levels		
		FC[a]	75% FC	50% FC
Tolerant cultivar	0	29.1	29.2	16.8
	75	30.9	30.7	17.1
	300	28.8	28.9	17.3
Sensitive cultivar	0	27.0	20.4	12.3
	75	22.7	20.3	14.2
	300	12.2	13.6	11.8
SE			1.87	

[a] Field capacity

Shoot dry weight was influenced by the interactions between isoxaflutole rate × moisture ($P < 0.001$) and genotype × moisture ($P < 0.05$) (Table 9). Shoot dry weight of chickpea did not decrease with increasing herbicide rate at 50% FC but the reduction was highly significant at 75 and 100% of FC (Table 12). Shoot dry weight was reduced by 28% at 100% FC and 25% at 75% FC with the recommended herbicide rate. At 75% FC,

the recommended and higher herbicide rates had similar effects on shoot dry weight. Only at 100% FC did the higher herbicide rate reduce shoot dry weight more than the recommended herbicide rate.

Table 12 Effect of increasing rates of isoxaflutole on the shoot dry weight of chickpea as affected by moisture 35 days after herbicide treatment (experiment 2)

Isoxaflutole (g a.i. ha^{-1})	Shoot dry weight (g pot^{-1})		
	Moisture levels		
	FC[a]	75% FC	50% FC
0	0.76	0.61	0.23
75	0.55	0.46	0.22
300	0.38	0.37	0.22
SE		0.052	

[a] Field capacity

Shoot dry weight of the tolerant cultivar was similar under 100 and 75% FC but was reduced significantly with 50% FC (Table 13). In comparison, shoot dry weight of the sensitive cultivar was reduced significantly (by 29%) when the moisture content declined from 100% FC to 75% FC.

Table 13 Effect of moisture on the shoot dry weight of a tolerant and sensitive chickpea genotype 35 days after herbicide treatment (experiment 2)

Genotype	Shoot dry weight (g pot^{-1})		
	Moisture levels		
	FC[a]	75% FC	50% FC
Tolerant cultivar	0.64	0.60	0.25
Sensitive cultivar	0.49	0.35	0.19
SE		0.043	

[a] Field capacity

Root dry weight of chickpea was significantly affected by an interaction between isoxaflutole rate × moisture ($P < 0.001$) (Table 9). Root dry weight of chickpea was unaffected by increasing isoxaflutole rate at 50% FC, while the reductions in root dry weight were significant at 75 and 100% FC (Table 14). Root dry weight was reduced up to 38% with the recommended rate at both 75 and 100% of FC.

Table 14 Effect of increasing rates of isoxaflutole on the root dry weight of chickpea as affected by moisture 35 days after herbicide treatment (experiment 2)

Isoxaflutole (g a.i. ha^{-1})	Root dry weight (g pot^{-1})		
	Moisture levels		
	FC[a]	75% FC	50% FC
0	0.40	0.38	0.16
75	0.25	0.24	0.13
300	0.10	0.12	0.13
SE		0.026	

[a] Field capacity

Discussion

These experiments showed that both soil moisture and temperature influence the degree of chickpea sensitivity to isoxaflutole. Furthermore, chickpea genotypes differed in their tolerance to isoxaflutole. Inhibition in the shoot growth of a sensitive chickpea cultivar was observed with higher temperature, moisture content and herbicide rate but the tolerant cultivar did not produce any significant reduction in shoot height with any of the studied temperatures and soil moisture levels. Kaur *et al.* (2004) observed inhibition in the shoot growth of *Phalaris minor* when grown in soil treated with 0.5 and 1 mg L^{-1} isoxaflutole. Luscombe & Pallett (1996) reported bleaching of leaf tissue and reduction in shoot height of maize (*Zea mays*) when treated with isoxaflutole. Increased application rate of isoxaflutole has directly been related to maize injury and decreased shoot height (Sprague *et al.* 1999).

Following uptake by the plant, isoxaflutole is rapidly converted to a diketonitrile (DKN) derivative via an opening of the isoxazole ring (Pallett *et al.* 1998) and this

opening can be achieved spontaneously under basic conditions with pH > 9.0 (Pallett *et al.* 2001). This DKN metabolite is commonly the active herbicidal component and is a potent inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD) in plants (Pallett *et al.* 1997). It has been shown that phytotoxic symptoms resulting from isoxaflutole application (due to inhibition of carotenoid biosynthesis) are photobleaching of the chlorophyll and destruction of the photosynthetic apparatus (Sprague *et al.* 1999). In tolerant plants, DKN is hydrolysed to the herbicidally inactive benzoic acid derivative (Pallett *et al.* 1998). The rate of herbicide metabolism and deactivation is likely to have been a major factor in determining the differential tolerances of chickpea genotypes. Sprague *et al.* (1999c) reported that maize hybrids more tolerant to isoxaflutole were able to metabolize it more rapidly than the more sensitive hybrids. Previous research by Pallett *et al.* (1998) identified isoxaflutole metabolism as the primary basis for differential selectivity between the tolerant species *Z. mays* and a susceptible species *Abutilon theophrasti* (velvetleaf). Visual symptoms and shoot height are parameters that have been recommended to assess herbicide toxicity, for example, isoxaflutole alone and with metolachlor/benoxacor and acetochlor/MON-13900 on maize (Sprague *et al.* 1999c). In the present study, shoot height measurement was taken as a bioassay parameter to assess isoxaflutole phytotoxicity. Isoxaflutole inhibits the activity of the enzyme HPPD which converts 4-hydroxyphenylpyruvate to homogentisate (Pallett *et al.* 1997). This results in the inhibition of meristematic tissue, which is responsible for the reduction in shoot height.

We observed more root biomass reduction than shoot biomass under increased temperature, soil moisture and herbicide rate. With increasing soil water content more isoxaflutole is dissolved for plant uptake in the soil water system. The internal concentration of isoxazole is reduced in the root due to rapid conversion of isoxaflutole to DKN coupled with greater potential for translocation of the DKN once inside the plant (Briggs *et al.* 1982; Shone and Wood 1974). The process continues until the concentration inside the plant reaches equilibrium with the external concentration of isoxaflutole (Pallett *et al.* 2001). As a result, root absorption of isoxaflutole occurs more predominantly than shoot uptake. Sprague *et al.* (1999) also found that maize absorbtion of isoxaflutole followed the order of seeds plus roots > roots alone > shoot. Roots came

into direct contact with the soil-applied isoxaflutole and therefore perhaps exhibited effects earlier than the shoots. Bhowmik *et al.* (2001) reported up to 80% reduction in shoot and root dry weight of oilseed rape (*Brassica napus*) with isoxaflutole at 4.4 and 9.0 μM concentration. They further confirmed that with the increase in concentration of isoxaflutole the dry weights of both shoot and root decreased. Kaur *et al.* (2004) also observed a significant reduction in shoot fresh biomass of *P. minor* when it was grown in soil treated with 0.5 and 1 mg L^{-1} isoxaflutole.

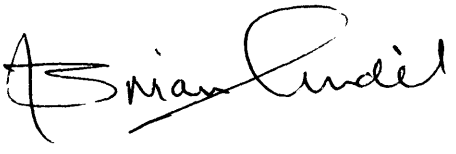
At the higher temperature regime and moisture content the sensitive line had severe injury. At the recommended rate of herbicide and higher moisture content the sensitive line showed acute phytotoxic symptoms while plant death occurred at higher doses. These findings support Pallett *et al.* (2001) who found that increasing moisture level and temperature increased the phytotoxicity symptoms in maize treated with isoxaflutole. At 25°C, the half-life of isoxaflutole was significantly greater at < 2% moisture content than that at 30%, and at 30% moisture content the half life was much greater at 10°C than at 25°C. Dhareesank *et al.* (2005) observed the phytotoxic activity of the soil-applied herbicide pethoxamid [2-chloro-*N*-(2-ethoxyethyl)-*N*-(2-methyl-1-phenyl-1-propanyl) acetamide], TKC-94, on the plant growth of rice (*Oryza sativa* cv. Kiyohatamochi) seedlings under different soil moisture conditions and concluded that the phytotoxic activity of pethoxamid mixed with soil on the shoot and root growth of rice seedlings was uppermost under the highest soil moisture condition and it decreased with declining soil moisture content, while the inhibition was greater on the root growth than the shoot growth. Taylor-Lovell *et al.* (2002) also reported that transformation of isoxaflutole to DKN, benzoic acid, and other products was stimulated by the presence of water. They also showed that the rate of mineralisation of isoxaflutole to DKN and other end products was positively affected by temperature. They recorded higher mineralisation of isoxaflutole at high temperature whereas negligible mineralisation occurred with the lower temperature. Beltran *et al.* (2003) concluded that the rate of isomerisation of isoxaflutole to DKN increased with higher temperature and moisture content.

Isoxaflutole could be used as an effective herbicide option to control problematic broadleaf weeds if more isoxaflutole tolerant chickpea genotypes are grown. Isoxaflutole may cause crop damage in a situation where prevailing temperature is high and rainfall is likely soon after spraying of herbicide.

Statement of Originality:

All the work contained within this paper is the original research of the PhD candidate, Avishek Datta.

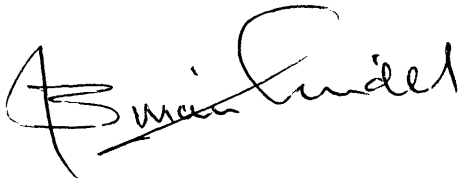
Candidate: 

Principal Supervisor: 

Statement of Contribution by Others:

This paper has been prepared by the PhD candidate, Avishek Datta. All coauthors are either PhD supervisors (Sindel, Jessop, Felton) or statistical advisor (Kristiansen) and have only contributed to this paper to the extent that would normally be expected of such roles. All coauthors have given their consent for having their contributions to this paper included in the thesis and accept the student's contribution as indicated in the Statement of Originality.

Candidate: 

Principal Supervisor: 

CHAPTER SIX

Effects of isoxaflutole on the growth and nodulation of chickpea (*Cicer arietinum*), using isoxaflutole tolerant and sensitive cultivars and different soil nitrogen rates

Manuscript in preparation for Weed Science

Effects of isoxaflutole on the growth and nodulation of chickpea (*Cicer arietinum*), using isoxaflutole tolerant and sensitive cultivars and different soil nitrogen rates

Avishek Datta[1,3,4], Brian M. Sindel[1,3], Paul Kristiansen[1], Robin S. Jessop[1,3] & Warwick L. Felton[2]

[1]School of Environmental and Rural Science, The University of New England, Armidale, New South Wales 2351, Australia. [2]Nyrang Avenue, Tamworth, New South Wales 2340, Australia. [3]Cooperative Research Centre for Australian Weed Management.
[4]Corresponding author. E-mail: adatta2@une.edu.au

Key words: isoxaflutole, soil nitrate, *Cicer arietinum*, phytotoxicity, growth, nodulation.

Abstract

Isoxaflutole at 75 g a.i. ha^{-1} is registered for the control of several broadleaf weeds in chickpea (*Cicer arietinum*), an important grain legume crop in Australia. An experiment was carried out to examine the growth and nodulation response of one isoxaflutole tolerant and one sensitive chickpea cultivar under a range of soil nitrate (NO_3^-) concentrations (0, 0.75, 1.5, 3.0 and 6.0 mM) and herbicide rates [0, 75 (recommended rate) and 300 g a.i. ha^{-1}]. The sensitive chickpea cultivar was more susceptible to isoxaflutole damage with increasing herbicide rate and nitrate concentrations. Some damage was observed with the tolerant cultivar at the highest herbicide rate and nitrate concentrations. In general, isoxaflutole decreased shoot height, shoot dry weight, root dry weight, nodule number, nodule dry weight and average nodule weight of the sensitive cultivar. Root dry weight of the sensitive cultivar was reduced with the application of 6 mM nitrate whereas nodule dry weight was decreased with the entire nitrate concentrations. Nodule dry weight of the sensitive cultivar was reduced by 86% at 6 mM nitrate level without any herbicide incorporation. Herbicide applied at the recommended rate reduced nodule dry weight of chickpea by 51% at 6mM nitrate level. Shoot height of

the tolerant cultivar was little affected with the increasing herbicide rates. Shoot and root dry weights of the tolerant cultivar were reduced by 18 and 24% respectively at the recommended herbicide rate. Nodule number of the tolerant cultivar was decreased by 30% at the recommended rate of herbicide. Nodule dry weight and average nodule weight were also decreased with increasing nitrate concentrations.

Introduction

In the major grain growing areas of north-eastern Australia, continuous cereal cropping has caused severe depletion of soil nitrogen (N) on a range of vertisols (Dalal and Mayer 1986). This has led to decreasing grain protein concentrations and yield (Dalal *et al.* 1991). As a result, N fertiliser use has increased in the region (Strong *et al.* 1996), and there is currently much interest in sustainable cropping systems involving legumes as a source of N. Chickpea (*Cicer arietinum*) has been used in short-term rotations with cereals (chickpea–wheat) in northern New South Wales (Marcellos 1984) and southern Queensland (Doughton *et al.* 1993) for long-term benefits in soil N levels, wheat grain yields and grain protein concentrations. Marcellos (1984) found that after one season of chickpea crop grown on an Alfisol in Tamworth, New South Wales, wheat yield in the following year increased from 1.5 t ha^{-1} in the wheat–wheat sequence to 3.0 t ha^{-1} in the chickpea–wheat sequence. The N benefits were equivalent to an application of 50 kg N ha^{-1} as ammonium nitrate. But the symbiotic N fixation in legumes associated with rhizobia is inhibited by nitrate N applied to the soil (Daimon and Yoshioka 2001). Nitrite metabolised from nitrate assimilation has been considered as a possible inhibitor in relation to root hair deformation (Abdel-Waheb *et al.* 1996), formation of the oxygen diffusion barrier (Vessey *et al.* 1988), and inactivation of leghaemoglobin (Kanayama *et al.* 1990). Inhibition by the limited photosynthate translocated to the nodules of nitrate-fed plants [e.g. field pea (*Pisum sativum*), subterranean clover (*Trifolium subterraneum*) and cowpea (*Vigna unguiculata*)] has also been suggested as a carbohydrate deprivation hypothesis (Streeter 1986).

Most Australian soils are inherently low in N so the N input potential of grain legumes such as chickpea is most important (Evans 1982a). Evans (1982b) showed that increasing the soil nitrate level from 20 to 80 mg N kg^{-1} greatly reduced N_2 fixation of

chickpea. In other species, it has generally been found that nodulation decreases with increasing N supply (Pal and Saxena 1975) although small amounts of available N during early growth may benefit the symbiosis (Harper 1974). The inclusion of legumes in rotations with cereals or other crops can improve soil structure, provide a break in disease and cereal pest cycles, and increase the options available for weed control (Peoples *et al.* 1992). However, residual levels of some herbicides have been found to inhibit nodulation (Eberbach and Douglas 1989; Martensson and Nilsson 1989) and N fixation (Koopman *et al.* 1995) by legumes and may have negative effects on the N balance of legume-cereal rotations.

In Australia, isoxaflutole at 75 g a.i. ha^{-1} is registered for the control of several broadleaf weeds in chickpea. Isoxaflutole is a relatively new pre-emergence soil applied herbicide which belongs to the isoxazole class of herbicides and used for pre-emergence control of grass and broadleaf weeds in maize (*Zea mays*) and sugarcane (*Saccharum* spp.) (Luscombe and Pallett 1996; Luscombe *et al.* 1995). In plants and soil, isoxaflutole is rapidly converted to a diketonitrile (DKN) metabolite, [2- cyclopropyl-3-(2- mesyl-4-trifluoromethylphenyl)-3- oxopropanenitrile], by opening the isoxazole ring (Pallett *et al.* 1998). This DKN degrade is herbicidally active and is a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase (Pallett *et al.* 1998; Viviani *et al.* 1998). After herbicide application, susceptible species treated with isoxaflutole initially show a bleaching of newly developed leaves followed by growth suppression and necrosis prior to plant death. Bleaching, i.e. absence of both carotenoid and chlorophyll pigments, of newly developed leaves results indirectly from an inhibition of carotenoid biosynthesis (Luscombe and Pallett 1996).

However, there have been records of chickpea crop damage due to the application of isoxaflutole. Felton *et al.* (2004) demonstrated that under some conditions this herbicide can injure the more tolerant varieties of chickpea at an application rate of less than 75 g a. i. ha^{-1} and consequently result in a yield penalty. The effects of different soil nitrate levels influencing the degree of isoxaflutole injury to chickpea growth and nodulation are not well documented. Previous field and polyhouse trials with a wide range of different chickpea genotypes treated with varying rates of isoxaflutole showed cultivar Kyabra to be amongst the most tolerant and Yorker to be one of the most

sensitive cultivars to the herbicide (Datta *et al.* 2007). Therefore, the objectives of this research were to (1) assess the growth and nodulation of two chickpea cultivars with and without isoxaflutole, and (2) examine possible interaction effects of isoxaflutole on the growth and nodulation of chickpea at various nitrate levels.

Materials and methods

Experimental design

The experiment consisted of a randomised complete block design with 5 × 2 × 3 factorial combinations: 5 NO_3^- concentrations (0, 0.75, 1.5, 3.0 and 6.0 mM), 2 chickpea genotypes (the isoxaflutole tolerant cultivar - Kyabra, and the sensitive cultivar - Yorker) and 3 isoxaflutole rates (0, 75, and 300 g a.i. ha^{-1}). There were four replications.

Plant materials and growth conditions

The experiment was conducted under glasshouse conditions at the University of New England, Armidale, New South Wales, Australia. Five seeds of the chickpea cultivar were grown in a free-draining sand system without any N content in a 14 cm diameter plastic pot. The seeds were inoculated with the recommended *Rhizobium* culture before sowing and seeds were planted at a depth of 2.5-3 cm. Glasshouse conditions were maintained at 25°C day and 15°C night. Isoxaflutole was applied at 1 day after sowing (DAS) using a gas operated boom-sprayer through a TeeJet 11003 flat fan nozzle at a pressure of 300 kPa delivering a volume of 84 L ha^{-1}. At 7 DAS, plants were thinned to three per pot. Pots were flushed with 300 mL of distilled water after sowing the seeds, and 150 mL of distilled water after spraying the herbicide to facilitate proper mixing of the herbicide in the system. Pots were flushed with 150 mL of distilled water or 150 mL of nutrient solution on alternate days after sowing so that regular flushing of solution through the pots occurred. The nutrient solution contained 0, 0.75, 1.5, 3.0 and 6.0 mM NO_3^- as KNO_3. Other macro and micro nutrients used as a basal treatment were: 0.25 mM $CaCl_2$, 0.125 mM K_2SO_4, 0.5 mM $MgSO_4.7\ H_2O$, 0.13 mM KH_2PO_4, 0.13 mM K_2HPO_4, 22.4 µM Fe-EDDHA [Ethylenediame-di(*o*-hydroxyphenylacetic acid)], 1.2 µM $MnSO_4.H_2O$, 0.08 µM $ZnSO_4.7\ H_2O$, 0.05 µM $CuSO_4.5\ H_2O$, 0.002 µM $CoSO_4.7\ H_2O$, and 0.02 µM $Na_2MoO_4.2H_2O$. These NO_3^- rates were based according to the fact that large

concentrations of inorganic N (in excess of 14.3 mM) were wholly detrimental to symbiotic effectiveness of several grain legumes (Rawsthorne *et al.* 1985a). In contrast, smaller concentrations of nitrate (1.43 mM) were helpful to improve nodulation, N fixation and seed yields by nodulated plants (Eaglesham *et al.* 1983; Minchin *et al.* 1981). To describe quantitatively the sensitivity of the symbiosis to the concentration of N applied, an exploratory investigation (Minchin *et al.* unpublished data) revealed that 3.57 mM NO_3^- was supra-optimal for nodulation and N fixation in chickpeas.

Measurements

Plants were harvested at 35 days after herbicide treatment (DAHT). Before harvest, plant heights were recorded by measuring the height from the ground level to the extremity of the longest branch. The numbers of nodules per plant were counted after hand-washing the roots. Dry weights of nodules, shoots and roots were determined after drying for 48 hours at 80°C. Average nodule weight was calculated from nodule weights and nodule numbers.

Statistical analysis

Results were analysed using the analysis of variance (ANOVA) function of R 2.3.0 (R Development Core Team 2006) and P values ≤ 0.05 were considered significant. Variances were checked by plotting residual versus fitted values to confirm the homogeneity of the data. No transformations were necessary. Means for significant treatment effects were separated based on standard errors (SE). The treatment combination means presented for a variable are based on the highest order of factorial combination that is significant in the ANOVA. Where this is less than the maximum factorial combination, the tables have been generated by pooling the data across the non significant factors.

Results

Plant height

The genotype × isoxaflutole rate × nitrate interactions were not significant for the plant height ($P \geq 0.05$) 35 DAHT suggesting that the trend of variation in plant height of both cultivars to herbicide rates was the same under different concentrations of nitrate (Table

1). The isoxaflutole rate × genotype interaction was significant ($P < 0.001$) indicating that the cultivars differed in their tolerance to isoxaflutole. Plant height of the tolerant cultivar was slightly reduced (7%) with the recommended herbicide rate (75 g a.i. ha^{-1}) but there was no significant difference in plant height of the tolerant cultivar between the recommended and higher herbicide rates (Table 2). Plant height of the sensitive cultivar was significantly reduced (32%) with the addition of recommended rate of herbicide. At the higher rate of herbicide (300 g a.i. ha^{-1}), plant height of the sensitive cultivar was reduced by 39%.

Table 1 Significance levels[a] in three-way ANOVA of the effects of genotype (tolerant and sensitive cultivars), isoxaflutole rate (0, 75, 300 g a.i. ha^{-1}) and NO_3^- concentrations (0, 0.75, 1.5, 3.0, 6.0 mM), on the plant height, shoot and root dry weight, nodule number, nodule dry weight and average nodule weight of chickpea

Terms	Plant height (cm)	Shoot dry weight (g pot^{-1})	Root dry weight (g pot^{-1})	Nodule number	Nodule dry weight (mg pot^{-1})	Average nodule weight (mg)
Isoxaflutole (I)	***	***	***	***	***	**
NO_3^- (N)	ns	ns	***	***	***	***
Genotype (G)	***	***	***	*	***	***
I × N	ns	ns	ns	ns	***	ns
I × G	***	*	*	*	ns	ns
N × G	ns	ns	*	ns	***	**
I × N × G	ns	ns	ns	ns	ns	ns

[a](***) $P < 0.001$; (**) $P < 0.01$; (*) $P < 0.05$; ns = not significant

Table 2 Effect of increasing rates of isoxaflutole on the plant height of a tolerant and sensitive chickpea cultivar 35 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Plant height (cm)	
	Tolerant cultivar	Sensitive cultivar
0	17.1	15.3
75	16.0	10.5
300	15.3	9.4
SE	0.51	

Shoot dry weight

The tolerant and susceptible cultivars showed different responses to isoxaflutole rates (Table 1) in shoot dry weight as indicated by the significant isoxaflutole rate × genotype interaction ($P < 0.05$). The addition of nitrate did not significantly increase shoot dry weight of the chickpea cultivar. The presence of isoxaflutole at the recommended rate significantly decreased shoot dry weight (Table 3) of the tolerant cultivar by 18%. But the higher rate of herbicide did not have any further inhibitory effect on shoot dry weight of the tolerant cultivar. At the recommended rate of herbicide, shoot dry weight of the sensitive cultivar was reduced by 29%. With the 300 g a.i. ha^{-1} rate, the reduction in shoot dry weight was more severe (45%) in the sensitive cultivar.

Table 3 Effect of increasing rates of isoxaflutole on the shoot dry weight of a tolerant and sensitive chickpea cultivar 35 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Shoot dry weight (g pot^{-1})	
	Tolerant cultivar	Sensitive cultivar
0	0.41	0.38
75	0.34	0.27
300	0.32	0.21
SE	0.018	

Root dry weight

Chickpea root dry weight was influenced by the interactions between genotype × isoxaflutole rate and genotype × nitrate concentrations ($P < 0.05$) (Table 1). Root dry weight of the sensitive cultivar was reduced by 48% with the recommended herbicide rate and 58% with the higher herbicide rate (Table 4). With the recommended herbicide rate, the tolerant cultivar had a 24% reduction in root dry weight but with the higher rate of herbicide the reduction was about 34%. Root dry weight of the tolerant cultivar was greater under 0 and 0.75 mM nitrate levels but was reduced significantly with increasing nitrate levels (Table 5). Root dry weight of the tolerant cultivar was 46% lower when 6.0 mM nitrate was present, compared with those grown without nitrate. Root dry weight of the sensitive cultivar was unaffected with the increasing level of nitrate (up to 3.0 mM level) but also significantly reduced (29%) with 6.0 mM nitrate level as compared with the nil nitrate treatment.

Table 4 Effect of increasing rates of isoxaflutole on the root dry weight of a tolerant and sensitive chickpea cultivar 35 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Root dry weight (g pot^{-1})	
	Tolerant cultivar	Sensitive cultivar
0	0.21	0.19
75	0.16	0.10
300	0.14	0.08
SE	0.011	

Table 5 Effect of increasing nitrate concentrations on the root dry weight of a tolerant and sensitive chickpea cultivar 35 days after herbicide treatment

Nitrate concentrations (mM)	Root dry weight (g pot^{-1})	
	Tolerant cultivar	Sensitive cultivar
0	0.22	0.14
0.75	0.20	0.14
1.5	0.17	0.13
3.0	0.15	0.11
6.0	0.12	0.10
SE	0.015	

Nodule number

Nodule number of chickpea was significantly affected by a 2-way interaction between genotype × isoxaflutole rate ($P < 0.05$) (Table 1). Nodule number of the tolerant cultivar was significantly decreased (30%) with the recommended herbicide rate and the highest reduction (36%) occurred with 300 g a.i. ha^{-1} rate (Table 6). The rate of reduction in nodule number of the sensitive cultivar was more as compared with the tolerant cultivar with increasing herbicide rate. At the recommended rate of herbicide the nodule number of the sensitive cultivar was reduced by 32% and the highest reduction (59%) occurred with the 300 g a.i. ha^{-1} rate. There was also a significant main effect of the presence of nitrate ($P < 0.001$) on the estimate of nodule number of chickpea (Table 1). Nodule number of chickpea was unaffected with the addition of 0.75 mM nitrate level but was reduced significantly with the incorporation of ≥ 1.5 mM nitrate concentrations (Table 7). Chickpea nodule number was decreased by 43 and 61% with 3.0 and 6.0 mM nitrate levels respectively compared with zero nitrate treatment.

Table 6 Effect of increasing rates of isoxaflutole on the nodule number of a tolerant and sensitive chickpea cultivar 35 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Nodule number	
	Tolerant cultivar	Sensitive cultivar
0	26.5	26.1
75	18.6	17.9
300	16.9	10.9
SE	1.73	

Table 7 Effect of increasing nitrate concentrations on the nodule number of chickpea 35 days after herbicide treatment

Nitrate concentrations (mM)	Nodule number
0	26.0
0.75	24.0
1.5	22.0
3.0	15.0
6.0	10.3
SE	1.58

Nodule dry weight

The dry weight of nodules was influenced by genotype × nitrate ($P < 0.001$) and isoxaflutole rate × nitrate ($P < 0.001$) interactions (Table 1). The addition of increasing concentrations of nitrate significantly reduced nodule dry weight of both chickpea cultivars (Table 8). Nodule dry weight of the tolerant cultivar was reduced by 26, 38, 75, and 90% at 0.75, 1.5, 3.0, and 6.0 mM nitrate levels respectively. In contrast, nodule dry weight of the sensitive cultivar was reduced by 32, 42, 72, and 86% with the addition of 0.75, 1.5, 3.0, and 6.0 mM nitrate levels respectively. Herbicide applied at the recommended rate reduced nodule dry weight of chickpea (Table 9) by 37% (without nitrate), 43% (0.75 mM), 40% (1.5 mM), 43% (3.0 mM) and 51% (6.0 mM). Nodule dry

weight was reduced by 53 and 65% with the higher herbicide rate (300 g a.i. ha^{-1}) at zero and 0.75 mM nitrate levels respectively. The nodule dry weight reduction was similar at the recommended and higher herbicide rates under ≥ 1.5 mM nitrate levels.

Table 8 Effect of increasing nitrate concentrations on the nodule dry weight of a tolerant and sensitive chickpea cultivar 35 days after herbicide treatment

Nitrate concentrations (mM)	Nodule dry weight (mg pot^{-1})	
	Tolerant cultivar	Sensitive cultivar
0	29.7	20.2
0.75	22.2	13.9
1.5	18.4	11.8
3.0	7.6	5.7
6.0	3.2	3.0
SE	1.57	

Table 9 Effect of increasing rates of isoxaflutole on the nodule dry weight of chickpea as affected by nitrate concentrations 35 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Nodule dry weight (mg pot^{-1})				
	Nitrate concentrations (mM)				
	0	0.75	1.5	3.0	6.0
0	35.5	28.1	21.3	10.1	5.1
75	22.4	16.1	12.6	5.8	2.5
300	17.0	9.9	11.3	4.0	1.7
SE	1.92				

Average nodule weight

There was a significant genotype × nitrate rate interaction ($P < 0.01$) for the average weight of nodules (Table 1). Average nodule weight was reduced with increasing nitrate levels for both the tolerant and the sensitive cultivar (Table 10). Increasing nitrate level decreased average nodule weight of the tolerant cultivar by 25% (0.75 mM), 29% (1.5

mM), 63% (3.0 mM) and 74% (6.0 mM). The average nodule weight of the sensitive cultivar was reduced by 22% (0.75 mM), 25% (1.5 mM), 51% (3.0 mM) and 63% (6.0 mM). The rate of average nodule weight reduction was higher in the tolerant cultivar as compared with the sensitive one. There was also a significant main effect of the presence of isoxaflutole ($P < 0.01$) on the average nodule weight of chickpea (Table 1). Average nodule weight of chickpea was significantly reduced (18%) with the addition of the recommended rate of herbicide (Table 11). The effects of recommended and higher herbicide rate were similar on average nodule weight.

Table 10 Effect of increasing nitrate concentrations on the average nodule weight of a tolerant and sensitive chickpea cultivar 35 days after herbicide treatment

Nitrate concentrations (mM)	Average nodule weight (mg)	
	Tolerant cultivar	Sensitive cultivar
0	1.17	0.77
0.75	0.88	0.60
1.5	0.83	0.58
3.0	0.44	0.38
6.0	0.31	0.29
SE	0.082	

Table 11 Effect of increasing rates of isoxaflutole on the average nodule weight of chickpea 35 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Average nodule weight (mg)
0	0.72
75	0.59
300	0.57
SE	0.045

Discussion

Significant inhibition in the shoot growth of the sensitive chickpea cultivar was observed under the entire range of herbicide rates, but the increasing herbicide rate had a very minor effect on shoot height of the tolerant cultivar. Kaur *et al.* (2004) also observed inhibition in the shoot growth of *Phalaris minor* when grown in soil treated with 0.5 and 1 mg L^{-1} isoxaflutole. Luscombe and Pallett (1996) reported bleaching of leaf tissue and reduction in shoot height of maize (*Zea mays*) when treated with isoxaflutole. Increased application rate of isoxaflutole has directly been related to maize injury and decreased shoot height (Sprague *et al.* 1999). In tolerant plants, diketonitrile is hydrolysed to the herbicidally inactive benzoic acid derivative (Pallett *et al.* 1998). The rate of herbicide metabolism and deactivation is likely to have been a major factor in determining the differential tolerances of chickpea genotypes. Sprague *et al.* (1999c) reported that maize hybrids more tolerant to isoxaflutole were able to metabolise it more rapidly than the more sensitive hybrids. Pallett *et al.* (1998) identified isoxaflutole metabolism as the primary basis for differential selectivity between the tolerant species *Z. mays* and a susceptible species *Abutilon theophrasti* (velvetleaf). In the present study, shoot height measurement was taken as a bioassay parameter to assess isoxaflutole phytotoxicity. Isoxaflutole inhibits the activity of the enzyme 4-hydroxyphenylpyruvate dioxygenase which converts 4-hydroxyphenylpyruvate to homogentisate (Pallett *et al.* 1997). This results in the inhibition of meristematic tissue, which is responsible for the reduction in shoot height.

We observed more shoot biomass reduction in the sensitive cultivar compared with the tolerant cultivar with increasing herbicide rate. We also found more root biomass reduction than shoot biomass under increased nitrate levels and herbicide rates. Roots came into direct contact with the soil-applied isoxaflutole and therefore probably exhibited effects earlier than the shoots. The addition of increased levels of nitrate failed to alleviate the shoot biomass loss experienced from the application of isoxaflutole. The reduction in root biomass in the presence of isoxaflutole may have decreased the ability of chickpea to absorb nutrients and contributed to the lack of response to nitrate in the presence of isoxaflutole. The reduction in root biomass in response to the presence of isoxaflutole was enhanced when nitrate was added at higher concentrations. This further

reduction may be due to an accumulation of carbohydrates and free amino acids, along with an increase in fermentation and a decrease in respiration, leading to growth inhibition (Gaston *et al.* 2002). Anderson *et al.* (2004) also found a reduction in chickpea root biomass from the application of fertiliser N and another soil applied herbicide, chlorsulfuron. Bhowmik *et al.* (2001) reported up to 80% reduction in shoot and root dry weight of oilseed rape (*Brassica napus*) with isoxaflutole at 4.4 and 9.0 μM. They further corroborated that with the increase in concentration of isoxaflutole the dry weights of both shoot and root were decreased.

Application of isoxaflutole reduced the number of nodules of both chickpea cultivars. Increasing levels of nitrate also decreased the overall nodule numbers in chickpea. Nodule dry weight of both cultivars was decreased with increasing nitrate levels, with or without herbicide application. The lower number of nodules in the presence of isoxaflutole suggests an impedance of nodule formation, whereas low nodule weight points to an effect on nodule development or maintenance. Anderson *et al.* (2004) also found a decreasing trend in nodule number of chickpea when chlorsulfuron was present in the soil. They also suggested that the addition of N fertiliser reduced the nodule weight of chickpea plants and the magnitude of the reduction was greater with the presence of chlorsulfuron in the soil. Kumar *et al.* (1981) observed a drastic reduction in nodulation of chickpea when simazine was applied to the soil surface at 1.6 and 3.2 kg ha^{-1}. Nodulation of chickpeas is known to decrease with increasing levels of soil inorganic N (Jessop *et al.* 1984) and nodulation *per se* can be impaired in the presence of nitrate (Summerfield *et al.* 1977; Wong 1980). Rawsthorne *et al.* (1985b) also recorded a significant reduction in total nodule numbers of chickpea grown at 32.5°C with 1.43 and 2.86 mM nitrate levels 28 days after sowing.

Wheat cropping systems in Australia rely strongly on legume fixed N for sustained yields. If grain legumes are to be as effective as a pasture ley phase, legumes such as chickpea must prove their capacity to enhance soil N status under a range of inherent soil N levels. The results of this study, although undertaken in an experimental sand based system that was low in organic matter and had low pH and continuous flushing, suggest that higher nitrate levels coupled with isoxaflutole had a detrimental effect on the general growth and nodulation of both chickpea cultivars. But the response

of the isoxaflutole tolerant cultivar was better than the sensitive one for at least some growth parameters. For effective weed control in chickpea using the herbicide isoxaflutole under different soil nitrate levels without sustaining significant crop injury, tolerant genotypes will need to be selected to incur less crop injury and better N economy for the succeeding cereal crops.

Statement of Originality:

All the work contained within this paper is the original research of the PhD candidate, Avishek Datta.

Candidate: 

Principal Supervisor: 

Statement of Contribution by Others:

This paper has been prepared by the PhD candidate, Avishek Datta. All coauthors are either PhD supervisors (Sindel, Jessop, Felton) or statistical advisor (Kristiansen) and have only contributed to this paper to the extent that would normally be expected of such roles. All coauthors have given their consent for having their contributions to this paper included in the thesis and accept the student's contribution as indicated in the Statement of Originality.

Candidate: 

Principal Supervisor: 

CHAPTER SEVEN

Effect of isoxaflutole on the growth, nodulation and nitrogen fixation of chickpea (*Cicer arietinum*)

Manuscript in preparation for Australian Journal of Agricultural Research

Effect of isoxaflutole on the growth, nodulation and nitrogen fixation of chickpea (*Cicer arietinum*)

Avishek Datta[1,3,4], Brian M. Sindel[1,3], Robin S. Jessop[1,3], Paul Kristiansen[1] & Warwick L. Felton[2]

[1]School of Environmental and Rural Science, The University of New England, Armidale, New South Wales 2351, Australia. [2]Nyrang Avenue, Tamworth, New South Wales 2340, Australia. [3]Cooperative Research Centre for Australian Weed Management.
[4]Corresponding author. E-mail: adatta2@une.edu.au

Key words: isoxaflutole, *Cicer arietinum*, phytotoxicity, growth, nodulation, nitrogen fixation.

Abstract

A controlled environment experiment was carried out to examine the growth, nodulation response and nitrogen (N) fixation of one isoxaflutole tolerant and one sensitive chickpea cultivar under different soil nitrate (NO_3^-) levels (0 and 1.5 mM), rhizobia treatments (seeds were inoculated with rhizobia and uninoculated control) and herbicide rates [0 and 75 (recommended rate) g a.i. ha^{-1}] at 2 harvest times (21 and 42 days after herbicide treatment). The tolerant cultivar recovered from the early inhibition of shoot growth at the second harvest whereas the sensitive cultivar was unable to compensate this loss. The tolerant cultivar also compensated for the early root dry weight loss with isoxaflutole at the second harvest whereas shoot dry weight was unaffected with isoxaflutole at both harvests. The loss was consistent in the sensitive cultivar at both harvests. In general, shoot dry weight of both cultivars was increased with the addition of nitrate. However, higher root dry weight reduction than shoot dry weight reduction was noticed when isoxaflutole was applied. At the second harvest, nodule number of chickpea was reduced in the presence of isoxaflutole and nitrate treatments. Nodule dry weight of the tolerant cultivar was unchanged whereas it was decreased in the sensitive cultivar with

isoxaflutole. Total plant N of both the tolerant and sensitive cultivars was increased with the addition of nitrate with or without isoxaflutole compared with plants grown without nitrate. Isoxaflutole only reduced total N of the sensitive cultivar when nitrate was added. At the recommended rate of isoxaflutole, the chemical reduced the amount of fixed N by both the tolerant and sensitive cultivars while the intensity of reduction was greater in the sensitive cultivar. The amount of fixed N was also reduced in the sensitive cultivar when nitrate was added.

Introduction

Chickpea has been shown to be one of several pulses that are suited to the fine-textured, neutral-to-alkaline soils of the eastern cropping zone of both Western Australia and eastern Australia where narrow-leafed lupin (*Lupinus angustifolius*) is poorly adapted (Siddique and Sedgley 1986; Siddique *et al.* 1993). Chickpea is seen as a crop that provides a cash income from grain, requires minimal nitrogen (N) fertiliser through its ability to fix atmospheric N, and in a crop rotation can improve the N nutrition and yield of subsequent cereals (Doughton *et al.* 1993). The inclusion of legumes in rotations with cereals or other crops can improve soil structure, provide a break in disease and cereal pest cycles, and increase the options available for weed control (Peoples *et al.* 1992). The presence of weeds within crops can reduce crop yields, hinder harvest operations, and contaminate produce (Powles *et al.* 1996), and herbicides are in common use for weed control in pastures and crops (Lemerle *et al.* 1996). One of the major obstacles in growing chickpea successfully is its poor ability to compete with weeds. Crop losses of 90% are possible in weedy situations (Knights 1991) and the lack of registered post-emergence herbicides for broadleaf weeds reduces the options for weed management. In Australia, isoxaflutole at 75 g a.i. ha^{-1} is registered for the control of several broadleaf weeds [e.g. Indian hedge mustard (*Sisymbrium orientale*), sowthistle (*Sonchus oleraceus*), capeweed (*Arctotheca calendula*), prickly lettuce (*Lactuca serriola*), wild radish (*Raphanus raphanistrum*), turnip weed (*Rapistrum rugosum*), crassula (*Crasulla* spp.), medic (*Medicago* spp.), deadnettle (*Lamium amplexicaule*), and slender celery (*Ciclospermum leptophyllum*)] in chickpea.

One of the principal benefits of growing a legume crop is the symbiotic fixation of N. Thus it is important to consider any possible effects herbicide application could have, either directly on rhizobium species, or indirectly by affecting the rhizobium-plant symbiosis (Bertholet and Clark 1985). However, residual levels of some herbicides have been found to inhibit nodulation (Eberbach and Douglas 1989; Martensson and Nilsson 1989) and N fixation (Koopman *et al.* 1995) by legumes and may have negative effects on the N balance of legume-cereal rotations. Mallik and Tesfai (1985) found that trifluralin, 2,4-DB, alachlor, glyphosate and metribuzin adversely affected nodulation and N fixation in soybean (*Glycine max*) when applied at 5 and 10 times normal rates. Dunigan *et al.* (1972) also found that medium to high rates of trifluralin, chloramben, nitralin, and prometryn had adverse effects on nodulation by *G. max*. Herbicide-affected legumes suffered a reduction in nitrogenase activity but not necessarily in total plant weight (Mallik and Tesfai 1985; Torstensson 1975).

Singh and Wright (1999) examined the impact of three pre-emergence herbicides (terbutryn / terbuthylazine, trietazine / simazine and prometryn) and a post-emergence herbicide (bentazone) on nodulation, symbiotic N fixation, growth and yield of pea (*Pisum sativum*) and reported that all pre-emergence herbicides decreased nodulation, total nitrogenase activity, net photosynthesis, root and shoot dry weight, N content and seed yield. Kumar *et al.* (1981) found that root nodule initiation of chickpea was not affected by either simazine or prometryn herbicide but the later production of new nodules and growth of the nodules was reduced in different degrees by various treatments. Overall nodulation was drastically reduced with simazine. They concluded that the reductions in nodulation with simazine and prometryn appeared to be primarily a case of general root growth reduction. When examining the effects of chlorsulfuron on chickpea under laboratory conditions, Anderson *et al.* (2004) observed that even at rates equivalent to double the field rates, chlorsulfuron did not influence the growth of chickpea rhizobia. However, although rhizobial growth was not influenced, the subsequent ability of these rhizobia to form nodules was reduced. Thus, the presence of chlorsulfuron in the soil reduced the nodulation and N fixation of chickpea plants.

According to Eberbach (1993), herbicides may affect the legume-rhizobia symbiosis in a number of ways including: (i) direct effects on the host plant (e.g.

reduction in root biomass, leading to fewer infection sites, or in carbohydrate supply to existing nodules); (ii) direct effects on rhizobial survival or growth, leading to a decreased potential for rhizobial infection of root hairs; (iii) an inhibition or inactivation of the biochemical signalling by either rhizobia or plants required to initiate nodule development; and/or (iv) an inhibition of nodule development by reducing the capacity for cell division. All of these possible mechanisms have the potential to reduce the efficiency of the legume-rhizobia symbiotic relationship and therefore the amount of N fixed.

Previous field and polyhouse trials with different chickpea genotypes treated with varying rates of isoxaflutole showed that the cultivar Kyabra was amongst the most tolerant and Yorker was one of the most sensitive cultivars to the herbicide (Datta *et al.* 2007). An increased understanding of the influence of isoxaflutole on growth, nodulation, and N fixation of isoxaflutole tolerant and susceptible chickpea genotypes will assist in defining the potential impacts of using this herbicide in chickpea production systems. Therefore, the objectives of this research were to (1) assess the growth, nodulation, and N fixation of chickpea with and without isoxaflutole, and (2) determine if these effects vary between isoxaflutole sensitive and more tolerant desi chickpea genotypes.

Materials and methods

Experimental design

The experiment was set up as a randomised complete block design with 2 × 2 × 2 × 2 factorial combinations: 2 rhizobia treatments (seeds were either inoculated with rhizobia or left as an uninoculated control), 2 NO_3^- levels (0 and 1.5 mM), 2 chickpea cultivars (the isoxaflutole tolerant cultivar - Kyabra and the sensitive cultivar - Yorker), and 2 isoxaflutole rates [0 and 75 g a.i. ha^{-1} (recommended rate)]. There were four replications.

Plant materials and growth conditions

The experiment was carried out in growth chambers at the University of New England, Armidale, New South Wales (NSW), Australia using one isoxaflutole tolerant (Kyabra) and one sensitive chickpea cultivar (Yorker). The pots were placed in a controlled-

environment room with 25°C maximum and 15°C minimum temperatures (photoperiod approximately 12 h day/12 h night), and light intensity of approximately 800 μmol m^{-2} s^{-1}. Chickpea seeds were sterilised to kill any existing bacteria by immersion in 0.2% $HgCl_2$ for 3 min followed by six rinses in sterile water. Five seeds of the chickpea cultivar were grown in a free-draining sand system without any N content in a 14 cm diameter plastic pot. The sand medium was sterilised by heating for 8 hours at 80°C. Pots were also sterilised by soaking overnight with sodium hypochlorite (available chlorine: 4% w/v). The seeds with inoculation treatment were inoculated with the recommended *Rhizobium* culture before sowing and seeds were planted at a depth of 2.5-3 cm. Isoxaflutole was applied at 1 day after sowing (DAS) using a gas operated boom-sprayer through a TeeJet 11003 flat fan nozzle at a pressure of 300 kPa delivering a volume of 84 L ha^{-1}. At 7 DAS, plants were thinned to three per pot. Pots were watered with 150 mL of distilled water after sowing the seeds, and 150 mL of distilled water after spraying the herbicide to facilitate proper mixing of the herbicide in the system. Pots were flushed with 150 mL of distilled water or 150 mL of nutrient solution on alternate days after sowing so that regular flushing of solution through the pots occurred. The nutrient solution contained 0 and 1.5 mM NO_3^- (N treatment) as KNO_3. Other macro and micro nutrients used as a basal treatment were: 0.25 mM $CaCl_2$, 0.125 mM K_2SO_4, 0.5 mM $MgSO_4.7\ H_2O$, 0.13 mM KH_2PO_4, 0.13 mM K_2HPO_4, 22.4 μM Fe-EDDHA [Ethylenediame-di(*o*-hydroxyphenylacetic acid)], 1.2 μM $MnSO_4.H_2O$, 0.08 μM $ZnSO_4.7\ H_2O$, 0.05 μM $CuSO_4.5\ H_2O$, 0.002 μM $CoSO_4.7\ H_2O$, and 0.02 μM $Na_2MoO_4.2H_2O$.

Measurements

Plants were harvested at 21 and 42 days after herbicide treatment (DAHT). At each harvest, plant height, shoot and root dry weight, tap root length, nodulation (nodule number, dry weight and average nodule weight), and amount of N (shoot N, root N and total N) were determined. The amount of N taken up by the plant was also calculated. Before harvest, plant heights were recorded by measuring from the ground level to the highest growing point. Plant shoots were harvested at the ground level and roots were then hand washed. The numbers of nodules per plant were counted after hand-washing the roots. Dry weights of nodules, shoots and roots were determined after drying for 48

hours at 80°C. Average nodule weight was calculated from nodule weights and nodule numbers.

Dried chickpea seeds, roots, and shoots were ground to fine powder of < 0.05 mm size using a mechanical grinder and analysed for shoot, root and total N with a Carlo Erba NA1500 solid sample analyser. An estimate of the amount of N fixed by the chickpea plants (42 DAHT) was made for plants grown without the addition of fertiliser N according to the following equation (Anderson *et al.* 2004).

N fixed = (Total N of inoculated plants – Seed N) - (Total N of uninoculated plants – Seed N)

Statistical analysis

Results were analysed using the analysis of variance (ANOVA) function of R 2.3.0 (R Development Core Team 2006) and P values ≤ 0.05 were considered significant. Variances were checked by plotting residual versus fitted values to confirm the homogeneity of the data. No transformations were necessary. Means for significant treatment effects were separated based on standard errors (SE). The treatment combination means presented for a variable are based on the highest order of factorial combination that is significant in the ANOVA. Where this is less than the maximum factorial combination, the tables have been generated by pooling the data across the non significant factors. The uninoculated treatments were included as controls to check for contamination. The data for nodule number, nodule dry weight, and average nodule weight were not normally distributed because of the high frequency of zero values in the uninoculated treatment. As a result, the uninoculated treatments for these parameters were not included to generate ANOVA due to the constant value for those treatments. To reduce the number of tables presented, in places where main effects were significant, the data are presented in text locations only and not in table form.

Results

Effects of isoxaflutole on plant growth

Plant height

At 21 days after herbicide treatment (DAHT), chickpea plant height was affected by interactions between isoxaflutole rate × cultivar ($P < 0.001$) and isoxaflutole rate ×

rhizobia treatment ($P < 0.01$). Plant height of the tolerant cultivar was reduced by 8% with the recommended rate of herbicide (75 g a.i. ha^{-1}) whereas the reduction was 36% for the sensitive cultivar with the same herbicide rate (Table 1).

Table 1 Effect of isoxaflutole on the plant height of a tolerant and sensitive chickpea cultivar 21 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Plant height (cm)	
	Tolerant cultivar	Sensitive cultivar
0	18.5	18.4
75	17.0	11.7
SE	0.47	

Chickpea plant height under the rhizobia treatment was reduced by 17% with the recommended herbicide rate at 21 DAHT. In contrast, under the non-rhizobia treatment plant height was reduced by 27% with the same herbicide rate (Table 2). While there was no difference in height between rhizobia treatments in the nil herbicide treatment, plant height was reduced significantly in the uninoculated control where herbicide was applied.

Table 2 Effect of isoxaflutole on the plant height of chickpea as affected by rhizobia treatments 21 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Plant height (cm)	
	With rhizobia	Uninoculated control
0	18.3	18.5
75	15.2	13.5
SE	0.47	

At 42 DAHT, chickpea plant height was influenced by interactions between isoxaflutole rate × cultivar ($P < 0.001$) and cultivar × rhizobia treatment ($P < 0.05$). Plant height of the tolerant cultivar was unaffected with the recommended herbicide rate but

plant height of the sensitive cultivar was reduced by 29% with the same herbicide rate (Table 3).

Table 3 Effect of isoxaflutole on the plant height of a tolerant and sensitive chickpea cultivar 42 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Plant height (cm)	
	Tolerant cultivar	Sensitive cultivar
0	21.4	21.8
75	20.6	15.5
SE	0.52	

Plant height of the tolerant cultivar was similar under both rhizobia and uninoculated treatments (Table 4). Plant height of the sensitive cultivar was reduced by 7% in the uninoculated treatment compared with the rhizobia treatment. Plant height of chickpea was also influenced by a significant main effect of nitrate ($P < 0.05$). With 1.5 mM nitrate, plant height of chickpea was 20.3 cm which was a 5% increase compared with plants grown without nitrate (19.3 cm).

Table 4 Effect of rhizobia treatments on the plant height of a tolerant and sensitive chickpea cultivar 42 days after herbicide treatment

Inoculation treatment	Plant height (cm)	
	Tolerant cultivar	Sensitive cultivar
With rhizobia	20.8	19.3
Uninoculated control	21.2	17.9
SE	0.52	

Shoot dry weight

At 21 DAHT, shoot dry weight of chickpea was significantly affected by a three-way interaction among isoxaflutole rate, cultivar, and rhizobia treatment ($P < 0.05$). Shoot dry

weights of the tolerant and sensitive cultivars were similar under both rhizobia and uninoculated treatments without any herbicide application (Table 5). But shoot dry weight of the tolerant cultivar was unaffected by the application of herbicide in both inoculation treatments. Shoot dry weight of the sensitive cultivar on the other hand was decreased by 48% with the application of herbicide in the uninoculated treatment.

Table 5 Effect of isoxaflutole on the shoot dry weight of a tolerant and sensitive chickpea cultivar as affected by rhizobia treatments 21 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Shoot dry weight (g pot^{-1})			
	Tolerant cultivar		Sensitive cultivar	
	With rhizobia	Uninoculated	With rhizobia	Uninoculated
0	0.26	0.22	0.25	0.27
75	0.22	0.18	0.21	0.14
SE		0.021		

At 42 DAHT, shoot dry weight of chickpea was influenced by interactions between isoxaflutole rate × cultivar ($P < 0.05$) and cultivar × nitrate ($P < 0.05$). Shoot dry weight of the tolerant cultivar was unaffected with the recommended rate of herbicide but shoot dry weight of the sensitive cultivar was reduced by 22% with the same herbicide rate (Table 6).

Table 6 Effect isoxaflutole on the shoot dry weight of a tolerant and sensitive chickpea cultivar 42 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Shoot dry weight (g pot^{-1})	
	Tolerant cultivar	Sensitive cultivar
0	0.53	0.50
75	0.51	0.39
SE	0.027	

Shoot dry weight of the tolerant cultivar was unchanged with the addition of 1.5 mM nitrate whereas shoot dry weight of the sensitive cultivar was increased by 34% with the same amount of nitrate (Table 7).

Table 7 Effect of nitrate on the shoot dry weight of a tolerant and sensitive chickpea cultivar 42 days after herbicide treatment

Nitrate concentration (mM)	Shoot dry weight (g pot^{-1})	
	Tolerant cultivar	Sensitive cultivar
0	0.50	0.38
1.5	0.54	0.51
SE	0.027	

Root dry weight

At 21 DAHT, the root dry weight of chickpea was influenced by an interaction between isoxaflutole rate × cultivar ($P < 0.01$). Root dry weight of the tolerant cultivar was reduced by 27% with the recommended rate of herbicide. In contrast, the root dry weight of the sensitive cultivar was decreased by 61% with the same herbicide rate (Table 8).

Table 8 Effect of isoxaflutole on the root dry weight of a tolerant and sensitive chickpea cultivar 21 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Root dry weight (g pot^{-1})	
	Tolerant cultivar	Sensitive cultivar
0	0.15	0.18
75	0.11	0.07
SE	0.012	

At 42 DAHT, root dry weight of chickpea was influenced by a three-way interaction among isoxaflutole rate × cultivar × rhizobia treatment ($P < 0.05$). Root dry weight of the tolerant cultivar was similar with the recommended rate of herbicide under

both inoculated and uninoculated treatments (Table 9). Whereas root dry weight of the sensitive cultivar was significantly reduced under both inoculated and uninoculated treatments with the recommended rate of herbicide. Inoculation with rhizobia only had a significant effect at zero herbicide rate in the sensitive cultivar where it reduced root dry weight. Root dry weight was reduced by 50 and 63% under inoculated and uninoculated treatments respectively with the recommended herbicide rate. Root dry weight of chickpea was also affected by a highly significant main effect of nitrate ($P < 0.01$). Root dry weight of chickpea with 1.5 mM nitrate was 0.27 g which was a 15% increase compared with the plants grown without nitrate (0.23 g).

Table 9 Effect of isoxaflutole on the root dry weight of a tolerant and sensitive chickpea cultivar as affected by rhizobia treatments 42 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Root dry weight (g pot^{-1})			
	Tolerant cultivar		Sensitive cultivar	
	With rhizobia	Uninoculated	With rhizobia	Uninoculated
0	0.30	0.31	0.26	0.32
75	0.27	0.30	0.13	0.12
SE		0.023		

Tap root length

At 21 DAHT, tap root length of chickpea was influenced by an interaction among nitrate × cultivar × rhizobia treatments ($P < 0.01$). Under the rhizobia treatment, tap root length of the tolerant cultivar was reduced by 13% with the addition of 1.5 mM nitrate but with increasing nitrate level tap root length was unaffected under the uninoculated treatment (Table 10). Tap root length of the sensitive cultivar was similar with the addition of 1.5 mM nitrate under both inoculated and uninoculated treatments but inoculation with rhizobia increased tap root length by 13% only at 1.5 mM nitrate treatment. There was also a highly significant main effect of isoxaflutole rate on the tap root length of chickpea ($P < 0.001$). Tap root length with isoxaflutole was 19.6 cm, which represented a 11% reduction when compared with plants grown without isoxaflutole (22.0 cm).

At 42 DAHT, tap root length of chickpea was only affected by a highly significant main effect of isoxaflutole rate ($P < 0.01$). Tap root length of chickpea was 23.8 cm without the herbicide but it was reduced by 8% when isoxaflutole was present at the recommended rate (21.9 cm).

Table 10 Effect of nitrate on the tap root length of a tolerant and sensitive chickpea cultivar as affected by rhizobia treatments 21 days after herbicide treatment

Nitrate concentrations (mM)	Tap root length (cm)			
	Tolerant cultivar		Sensitive cultivar	
	With rhizobia	Uninoculated	With rhizobia	Uninoculated
0	21.6	19.6	21.0	22.0
1.5	19.1	19.9	23.0	20.3
SE		1.08		

Effects of isoxaflutole on nodulation

Nodule number

At 21 DAHT, nodule number of chickpea was influenced by an interaction among isoxaflutole rate × cultivar × nitrate ($P < 0.05$). Nodule number of the tolerant cultivar was similar under the 0 and 1.5 mM nitrate levels without any herbicide but was reduced significantly (50%) only in the 1.5 mM nitrate level with the recommended rate of herbicide (Table 11). Nodule number of the sensitive cultivar was reduced by 39% when nitrate was added at zero herbicide rate but was reduced by 47% with the recommended herbicide rate only at 0 mM nitrate level; it was similar under 1.5 mM nitrate level with the same herbicide rate.

At 42 DAHT, nodule number of chickpea was significantly affected by isoxaflutole rate ($P < 0.05$) and nitrate ($P < 0.001$). Nodule number of chickpea grown in the presence of isoxaflutole was 43.9 which represented a reduction of 15% when compared with plants grown in the absence of isoxaflutole, which had a nodule number of 51.8. Nodule number was also significantly reduced (by 29%) when nitrate was added

as compared with the plants grown without nitrate. Mean nodule number of chickpea was 56 and 39.7 under 0 and 1.5 mM, respectively.

Table 11 Effect of isoxaflutole on the nodule number of a tolerant and sensitive chickpea cultivar as affected by nitrate 21 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Nodule number			
	Tolerant cultivar		Sensitive cultivar	
	0 mM nitrate	1.5 mM nitrate	0 mM nitrate	1.5 mM nitrate
0	17.3	15.9	21.7	13.2
75	13.8	8.0	11.5	14.8
SE		3.15		

Nodule dry weight

At 21 DAHT, nodule dry weight of chickpea was significantly affected by isoxaflutole rate ($P < 0.001$). Nodule dry weight of chickpea was 17 mg pot^{-1} without any herbicide application but was 12 mg pot^{-1} when isoxaflutole was applied; this represented a 29% reduction in nodule dry weight.

At 42 DAHT, nodule dry weight of chickpea was influenced by a two-way interaction between isoxaflutole rate × cultivar ($P < 0.05$). Nodule dry weight of the tolerant cultivar was unaffected with the recommended rate of herbicide whereas it was significantly reduced (53%) in the sensitive cultivar with the same herbicide rate (Table 12). Nodule dry weight of chickpea was also significantly reduced by nitrate ($P < 0.01$) from 59.8 mg pot^{-1} to 46.4 mg pot^{-1}, with the addition of 1.5 mM nitrate, a reduction of 22%.

Table 12 Effect of isoxaflutole on the nodule dry weight of a tolerant and sensitive chickpea cultivar 42 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Nodule dry weight (mg pot^{-1})	
	Tolerant cultivar	Sensitive cultivar
0	63.3	65.6
75	52.5	31.1
SE	6.02	

Average nodule weight

At 21 DAHT, average nodule weight of chickpea was influenced by a significant main effect of isoxaflutole rate ($P < 0.05$). Average nodule weight of chickpea was 0.69 mg without any herbicide compared with 0.51 mg when isoxaflutole was applied at the recommended rate; this represented a 26% reduction of average nodule weight with the application of herbicide.

At 42 DAHT, average nodule weight of chickpea was affected by a two-way interaction between isoxaflutole rate × cultivar ($P < 0.01$). Average nodule weight of the tolerant cultivar was similar with or without the recommended rate of herbicide but it was significantly reduced (42%) in the sensitive cultivar with the addition of herbicide (Table 13).

Table 13 Effect of isoxaflutole on the average nodule weight of a tolerant and sensitive chickpea cultivar 42 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Average nodule weight (mg)	
	Tolerant cultivar	Sensitive cultivar
0	1.21	1.31
75	1.16	0.76
SE	0.101	

Effects of isoxaflutole on plant nitrogen (N)

Shoot N

At 21 DAHT, shoot N of chickpea was influenced by a three-way interaction among isoxaflutole rate, cultivar, and rhizobia treatment ($P < 0.01$). Shoot N of the tolerant cultivar was similar under the rhizobia and uninoculated treatments with the control and recommended rates of herbicide (Table 14). However, the sensitive cultivar had an increase (29%) in shoot N with the recommended herbicide rate under the rhizobia treatment whereas it was similar in the uninoculated treatment with the same herbicide treatment.

Table 14 Effect of isoxaflutole on the shoot nitrogen of a tolerant and sensitive chickpea cultivar as affected by rhizobia treatments 21 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Shoot nitrogen (mg pot^{-1})			
	Tolerant cultivar		Sensitive cultivar	
	With rhizobia	Uninoculated	With rhizobia	Uninoculated
0	7.5	6.6	6.3	6.7
75	7.1	7.1	8.1	6.1
SE		0.59		

At 42 DAHT, shoot N of chickpea was affected by a nitrate × isoxaflutole rate × cultivar interaction ($P < 0.01$). Shoot N of the tolerant cultivar was increased by 20% with the addition of 1.5 mM nitrate without any herbicide application but it was unaffected with the recommended herbicide rate at both 0 and 1.5 mM nitrate levels (Table 15). Shoot N of the sensitive cultivar was increased by 67% with 1.5 mM nitrate without any herbicide. Shoot N of the sensitive cultivar was similar under the herbicide treatments at 0 mM nitrate level but was slightly decreased (14%) at 1.5 mM nitrate level when isoxaflutole was applied at the recommended rate.

Table 15 Effect of isoxaflutole on the shoot nitrogen of a tolerant and sensitive chickpea cultivar as affected by nitrate 42 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Shoot nitrogen (mg pot^{-1})			
	Tolerant cultivar		Sensitive cultivar	
	0 mM nitrate	1.5 mM nitrate	0 mM nitrate	1.5 mM nitrate
0	8.5	10.2	7.0	11.7
75	7.9	11.2	8.0	10.1
SE		0.77		

Root N

At 21 DAHT, root N of chickpea was influenced by an interaction between isoxaflutole rate × cultivar ($P < 0.001$). Root N of the tolerant cultivar was reduced by 16% with the recommended herbicide rate whereas the reduction was 42% in the sensitive cultivar with the same herbicide rate (Table 16).

Table 16 Effect of isoxaflutole on the root nitrogen of a tolerant and sensitive chickpea cultivar 21 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Root nitrogen (mg pot^{-1})	
	Tolerant cultivar	Sensitive cultivar
0	2.86	3.30
75	2.39	1.92
SE	0.170	

At 42 DAHT, root N of chickpea was affected by two-way interactions between isoxaflutole rate × cultivar ($P < 0.001$), isoxaflutole rate × nitrate ($P < 0.05$), and rhizobia treatment × cultivar ($P < 0.05$). Root N of the tolerant cultivar was unaffected with the herbicide whereas it was significantly reduced (42%) in the sensitive cultivar with the same herbicide rate (Table 17). At the recommended herbicide rate, root N was far higher in the tolerant cultivar than the sensitive cultivar.

Table 17 Effect of isoxaflutole on the root nitrogen of a tolerant and sensitive chickpea cultivar 42 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Root nitrogen (mg pot^{-1})	
	Tolerant cultivar	Sensitive cultivar
0	3.36	3.51
75	3.14	2.02
SE	0.155	

Root N of chickpea was increased by 33% with the addition of 1.5 mM nitrate without herbicide and was reduced by 20 and 28% at 0 and 1.5 mM nitrate levels, respectively, with the addition of the recommended herbicide rate (Table 18).

Table 18 Effect of isoxaflutole on the root nitrogen of chickpea as affected by nitrate 42 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Root nitrogen (mg pot^{-1})	
	0 mM	1.5 mM
0	2.95	3.91
75	2.36	2.80
SE	0.155	

Root N of the sensitive cultivar was similar under both rhizobia and uninoculated treatments but was reduced by 14% in the tolerant cultivar in the uninoculated treatment (Table 19). When rhizobia were present, the tolerant cultivar had higher root N level than the sensitive cultivar.

Table 19 Effect of rhizobia treatments on the root nitrogen of a tolerant and sensitive chickpea cultivar 42 days after herbicide treatment

Inoculation treatments	Root nitrogen (mg pot^{-1})	
	Tolerant cultivar	Sensitive cultivar
With rhizobia	3.49	2.75
Uninoculated	3.01	2.77
SE	0.155	

Total N

At 21 DAHT, total N of chickpea was affected by a three-way interaction among isoxaflutole rate × cultivar × rhizobia treatment ($P < 0.05$). Total N of the tolerant cultivar was unaffected with the recommended herbicide rate under both rhizobia and uninoculated treatments (Table 20). Total N of the sensitive cultivar was significantly reduced (22%) with the recommended herbicide rate in the uninoculated treatment whereas herbicide rate had no effect in the rhizobia treatment.

Table 20 Effect of isoxaflutole on the total nitrogen of a tolerant and sensitive chickpea cultivar as affected by rhizobia treatments 21 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Total nitrogen (mg pot^{-1})			
	Tolerant cultivar		Sensitive cultivar	
	With rhizobia	Uninoculated	With rhizobia	Uninoculated
0	10.5	9.2	9.5	10.1
75	9.5	9.5	10.1	7.9
SE	0.78			

At 42 DAHT, total N content of chickpea was influenced by a three-way interaction among isoxaflutole rate × cultivar × nitrate treatments ($P < 0.05$). Total N content of the tolerant cultivar was increased by 23% with the addition of 1.5 mM nitrate without any herbicide application but, total N content was similar under 0 and 1.5 mM

nitrate levels at the recommended herbicide rate compared with the zero herbicide rate (Table 21). Total N content of the sensitive cultivar was increased by 57% with the addition of 1.5 mM nitrate without any herbicide application but at the 0 mM nitrate level the total N content was similar between the two herbicide levels. Total N content of the sensitive cultivar was significantly reduced by 21% with the recommended herbicide rate at 1.5 mM nitrate. Total N content of chickpea showed a significant main effect of rhizobia treatment ($P < 0.001$); total N content of chickpea was 13.23 mg pot^{-1} with the rhizobia treatment whereas it was 11.41 mg pot^{-1} with the uninoculated treatment, which was 14% lower compared with the rhizobia treatment.

Table 21 Effect of isoxaflutole on the total nitrogen content of a tolerant and sensitive chickpea cultivar as affected by nitrate 42 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Total nitrogen (mg pot^{-1})			
	Tolerant cultivar		Sensitive cultivar	
	0 mM nitrate	1.5 mM nitrate	0 mM nitrate	1.5 mM nitrate
0	11.4	14.0	10.0	15.7
75	10.8	14.5	9.8	12.4
SE		0.82		

Effects of isoxaflutole on fixed nitrogen (N)

At 42 DAHT, the amount of N fixed by chickpea was influenced by the two-way interactions between isoxaflutole rate × cultivar ($P < 0.05$) and cultivar × nitrate ($P < 0.001$). The recommended rate of isoxaflutole significantly reduced the amount of fixed N by both the tolerant and sensitive cultivars but the reduction was higher in the sensitive cultivar (42%) compared with the tolerant cultivar (37%) (Table 22). Nevertheless, the sensitive cultivar fixed more N in absolute terms compared with the tolerant cultivar at zero and the recommended herbicide rate. So although the sensitive cultivar was affected more by the herbicide, it was still a better fixer of N.

Table 22 Effect of isoxaflutole on the amount of fixed nitrogen of a tolerant and sensitive chickpea cultivar 42 days after herbicide treatment

Isoxaflutole (g a.i. ha^{-1})	Amount of fixed nitrogen (mg pot^{-1})	
	Tolerant cultivar	Sensitive cultivar
0	1.66	2.89
75	1.05	1.68
SE	0.175	

The tolerant cultivar fixed 41% more N at the 1.5 mM nitrate level compared with the zero nitrate treatment whereas the amount of fixed N of the sensitive cultivar was reduced by 32% with the addition of 1.5 mM nitrate (Table 23).

Table 23 Effect of nitrate on the amount of fixed nitrogen of a tolerant and sensitive chickpea cultivar 42 days after herbicide treatment

Nitrate concentration (mM)	Amount of fixed nitrogen (mg pot^{-1})	
	Tolerant cultivar	Sensitive cultivar
0	1.13	2.70
1.5	1.59	1.83
SE	0.175	

Discussion

Effects of isoxaflutole on plant growth

Inhibition of shoot growth of both the tolerant and sensitive chickpea cultivars was observed when treated with the recommended rate of isoxaflutole at the first harvest. The tolerant cultivar recovered from the early inhibition of shoot growth at the second harvest whereas the sensitive cultivar failed to compensate the loss. Kaur *et al.* (2004) observed a significant reduction in shoot height of the sensitive littleseed canarygrass (*Phalaris minor*) when grown in soil treated with 0.5 and 1 mg L^{-1} isoxaflutole. But the authors did not notice any significant difference in shoot height of the tolerant wheat (*Triticum*

aestivum) when grown in soil treated with 0.05, 0.1, and 0.5 mg L^{-1} isoxaflutole compared with the control. Luscombe and Pallett (1996) reported stunting of shoots and bleaching of leaf tissue of maize (*Zea mays*) when treated with isoxaflutole. It has been shown that phytotoxic symptoms resulting from isoxaflutole application (due to inhibition of carotenoid biosynthesis) are photobleaching of the chlorophyll and destruction of the photosynthetic apparatus (Sprague *et al.* 1999). Isoxaflutole inhibits the activity of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD) which converts 4-hydroxyphenylpyruvate to homogentisate, a key step in plastoquinone biosynthesis (Pallett *et al.* 1997). This results in the inhibition of meristematic tissue, which is responsible for the reduction in shoot height of susceptible species.

The tolerant cultivar compensated for the early root dry weight loss with isoxaflutole at the second harvest whereas shoot dry weight was unaffected with isoxaflutole at both harvests. The loss was consistent in the sensitive cultivar at both harvests. Shoot dry weights of both cultivars, in general, were increased with the addition of nitrate. However, higher root dry weight reduction than shoot dry weight reduction was noticed when isoxaflutole was applied. Tap root length of chickpea was also decreased with the recommended rate of herbicide. In plants, soil, or water, a diketonitrile (DKN) derivative is produced by opening of the isoxazole ring of isoxaflutole (Luscombe and Pallett 1996; Sprague *et al.* 1999). DKN is the active principle of the herbicide and acts by the inhibition of HPPD, a specific enzyme affecting carotenoid synthesis (Viviani *et al.* 1998). This DKN undergoes degradation to an inactive benzoic acid derivative in treated plants and the extent of this degradation is correlated to the degree of susceptibility, being most rapid in tolerant maize and slowest in susceptible *Abutilon theophrasti* (Pallett *et al.* 1998). The rate of isoxaflutole metabolism and deactivation is likely to have been a major factor in determining the differential tolerances of chickpea genotypes. Sprague *et al.* (1999c) reported that maize hybrids more tolerant to isoxaflutole were able to metabolise it more rapidly than the more sensitive hybrids. Roots came into direct contact with the soil applied isoxaflutole and therefore probably exhibited effects earlier than the shoots. The reductions in shoot and root dry weights of the sensitive cultivar due to addition of isoxaflutole may have reduced the ability of the plant to absorb nutrients and contributed to the lack of response to fertiliser N in the

presence of isoxaflutole. Anderson *et al.* (2004) also found up to 56% reduction in chickpea shoot dry weight when another soil applied herbicide (chlorsulfuron) was applied equivalent to 10% of the field application rate after 6 weeks of growth. The above authors also reported that the addition of N fertiliser increased shoot biomass of chickpea by 23% in the absence of chlorsulfuron.

Root dry weight of the sensitive cultivar was significantly reduced under both inoculated and uninoculated treatments with isoxaflutole. Anderson *et al.* (2004) also reported that the presence of chlorsulfuron in the soil reduced chickpea root biomass at both 3 and 6 weeks of growth while the reduction was greater after 6 weeks. Bhowmik *et al.* (2001) also reported up to an 80% reduction in shoot and root dry weight of susceptible oilseed rape (*Brassica napus*) with isoxaflutole at 4.4 and 9.0 mM. Swarcewicz *et al.* (2002) reported up to 64% growth reduction in the root length and 94% reduction in the root dry weight of oilseed rape from isoxaflutole when applied at 15.0 μg a.i. kg^{-1}.

Effects of isoxaflutole on nodulation

At the second harvest, the number of nodules on chickpea was reduced in the presence of isoxaflutole and nitrate treatments. Nodule dry weight of the tolerant cultivar was unchanged whereas it was decreased in the sensitive cultivar with the recommended rate of isoxaflutole. Average nodule weight was also reduced in the sensitive cultivar when isoxaflutole was applied. The lower number of nodules in the presence of isoxaflutole suggests an impedance of nodule formation, whereas low nodule dry weight and average nodule weight point to an effect on nodule development or maintenance. Anderson *et al.* (2004) found a decreasing trend in nodule number of chickpea when chlorsulfuron was present in soil. These authors suggested that the addition of N fertiliser reduced the nodule weight of chickpea and the magnitude of the reduction was greater with the presence of chlorsulfuron in the soil. Kumar *et al.* (1981) observed a drastic reduction in nodulation of chickpea when simazine was applied to the soil surface at 1.6 and 3.2 kg ha^{-1}. Nodulation of chickpeas is known to decrease with increasing levels of soil inorganic N (Jessop *et al.* 1984) and nodulation *per se* can be impaired in the presence of nitrate (Summerfield *et al.* 1977; Wong 1980). Rawsthorne *et al.* (1985b) also recorded a

significant reduction in total nodule numbers of chickpea crop grown at 32.5°C with 1.43 and 2.86 mM nitrate levels 28 days after sowing.

Herbicide-induced reductions in nodulation may be the result of injury to the legume's root system, or to *Rhizobium* before or during infection. The decline in nitrogenase activity may also be due to a herbicide-induced reduction in supplies of photosynthates to the nodules, physiological damage to the plant root or nodules or, physiological damage to *Rhizobium* either before or after inoculation (Eberbach and Douglas 1989). Recommended herbicide dosage rates have been shown to have only a negligible effect on the growth of rhizobia (Cardina *et al.* 1986; Moorman 1986; Roslycky 1985). Although the effect of herbicides on bacterial growth is relevant, rhizobia may lose the ability to induce nodulation when exposed to pesticides before they lose the ability to multiply (Curley and Burton 1975).

Effects of isoxaflutole on plant nitrogen (N)

At the second harvest, shoot N of the tolerant cultivar was increased with the addition of nitrate while isoxaflutole had no effect on shoot N either in nitrate or zero nitrate treatments. Shoot N of the sensitive cultivar was also increased with added nitrate but was reduced with isoxaflutole application only at the 1.5 mM nitrate level. Root N of the sensitive cultivar was decreased with isoxaflutole whereas isoxaflutole had no effect on root N of the tolerant cultivar. In general, root N of chickpea was increased with the addition of nitrate without isoxaflutole but isoxaflutole reduced root N under both nitrate and zero nitrate treatments. Total N of both the tolerant and sensitive cultivars was increased with the addition of nitrate with or without isoxaflutole compared with plants grown without nitrate. But isoxaflutole only reduced total N of the sensitive cultivar when nitrate was added. The plant N relationships may have been affected by isoxaflutole in the sensitive cultivar and addition of N fertiliser was unable to overcome the problem imposed by isoxaflutole. Anderson *et al.* (2004) reported that the addition of N fertiliser resulted in a large increase in chickpea plant N when rhizobia was pre-exposed to chlorsulfuron. They also concluded that the pre-exposure of rhizobia to chlorsulfuron led to reduced N concentrations in chickpea plants.

Effects of isoxaflutole on fixed nitrogen (N)

The recommended rate of isoxaflutole reduced the amount of fixed N by both the tolerant and sensitive cultivars while the reduction was greater in the sensitive cultivar. The amount of fixed N was also reduced in the sensitive cultivar when nitrate was added. The decreased amount of fixed N with isoxaflutole is probably associated with lower nodulation, nodule dry weight, total N content and root and shoot growth. Anderson *et al.* (2004) reported a 70% reduction in the amount of N fixed by chickpea plants when chlorsulfuron was present in the soil compared with plants grown without chlorsulfuron. All herbicides decreased photosynthetic productivity, thereby potentially restricting the amount of photosynthate available for nodulation and N fixation (Singh and Wright 1999). However, it is possible that herbicides decreased the nodulation capacity of rhizobia (Madhavi *et al.* 1993; Martensson 1992).

A reduction in the amount of fixed N in chickpea crops due to the application of isoxaflutole can negatively affect the potential N benefits associated with including chickpeas in rotation with cereals. Grain legume crops are often associated with an N 'sparing' effect in which soil mineral N not taken up by legumes actively fixing N, remains available to subsequent cereal crops (Ahmad *et al.* 2001; Chalk 1998; Strong *et al.* 1986a). The performance of the tolerant chickpea cultivar was comparatively better for at least some growth, nodulation and N fixation parameters. The addition of fertiliser N did not alleviate the residual herbicide effect; additions of fertiliser N may also lead to greater reductions in N fixation. Further experiments are required to determine whether the adverse effects of the herbicide observed in this early growth study occur to the same degree under field conditions and continue towards grain harvest, thus influencing grain yields.

Statement of Originality:

All the work contained within this paper is the original research of the PhD candidate, Avishek Datta.

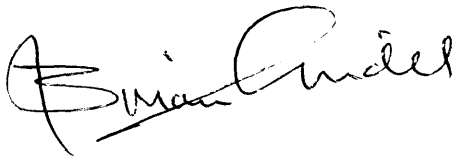
Candidate: 

Principal Supervisor: 

Statement of Contribution by Others:

This paper has been prepared by the PhD candidate, Avishek Datta. All coauthors are either PhD supervisors (Sindel, Jessop, Felton) or statistical advisor (Kristiansen) and have only contributed to this paper to the extent that would normally be expected of such roles. All coauthors have given their consent for having their contributions to this paper included in the thesis and accept the student's contribution as indicated in the Statement of Originality.

Candidate: 

Principal Supervisor: 

CHAPTER EIGHT

General conclusions

General conclusions

Introduction

Chickpea seedlings are slow to emerge and develop. They are poor competitors with weeds because of their open canopy structure and slow growth. Even moderate weed infestations can result in severe yield losses and harvesting problems. A lack of effective broadleaf weed control options in chickpea has been a major hindrance to the expansion of the chickpea industry in the northern grain region of Australia. The commercial release of isoxaflutole is viewed as a major step forward for the chickpea industry by many farmers. However, there have been records of chickpea crop damage due to the application of isoxaflutole. Under some conditions, all varieties might be damaged at the recommended rate (75 g a.i. ha^{-1}) of herbicide. It has also been found that herbicides which are used for weed control in pulses can have adverse effects on nodulation and nitrogen fixation. As a result, the subsequent wheat yields may experience a decline due to lower nitrogen economy in the field. This is an alarming situation for chickpea growing areas of Australia. This research project entitled "Factors affecting the sensitivity of chickpea (*Cicer arietinum*) to isoxaflutole and its effect on nitrogen fixation" was undertaken to examine the various factors which affect chickpea tolerance to the herbicide and to examine the effect this herbicide has on nitrogen fixation of chickpea.

Research findings

Differential response of chickpea genotypes to isoxaflutole

Identifying cultivars tolerant to herbicides is important to chickpea growers because it may minimise the risk of crop injury. Susceptibility of chickpea to isoxaflutole damage appeared to be strongly genotype and rate dependent. From a range of polyhouse and field trials it can be concluded that chickpea genotypes Howzat, Yorker, 91025-3021 (desi types), FLIP 94-92C and S 95425 (kabuli types) were more susceptible to isoxaflutole damage. In comparison, the desi genotypes 97039-1275 and Kyabra recorded very minor injury and can be regarded as the most tolerant. Other genotypes - Gully, Jimbour, Amethyst, Flipper, ICLL 87322 (desi types) and Bumper, FLIP 94-90C, GCN 133-2, IG

9337, IG 96220, Kaniva, Macarena, S 95342 (kabuli types) – are intermediate in their response to isoxaflutole. In general, there was less herbicide injury to the kabuli genotypes as compared with the desi chickpea genotypes. It is suggested that plant breeding programmes take into account the relative susceptibility of new chickpea genotypes to isoxaflutole. Farmers should avoid the most sensitive genotypes and instead use the most tolerant genotypes when they are using isoxaflutole as a weed control option in chickpea.

Role of soil pH and organic matter on chickpea genotype sensitivity to isoxaflutole

It is likely that when a new herbicide is released it may not have been tested under a sufficiently wide range of conditions to identify all potential problems. The present findings demonstrated differential tolerances among chickpea genotypes to isoxaflutole at different rates, soil pH and organic matter levels. Isoxaflutole caused increased phytotoxicity in chickpea with increase in soil pH and herbicide rate. Even the most tolerant chickpea genotype was damaged when exposed to higher pH and herbicide rates. The effects were more severe with the sensitive genotypes. The phytotoxic symptoms were more acute on a low organic matter soil compared with higher organic matter soil. Chickpea cultivar performed comparatively well in the higher organic matter soil compared with the lower organic matter soil having the same pH. The susceptibility of chickpea to this herbicide depends on soil pH and organic matter, which should be taken into account during the breeding of new lines, and in the agronomy of chickpea production. In order to achieve effective weed control in chickpea using isoxaflutole without sustaining significant crop injury, care is needed in selecting tolerant genotypes, and in avoiding high pH and low organic matter soils. This could be important since some soils used for chickpea production (e.g. south eastern Australia) may have high pH levels (in excess of pH 8.5, 1:5 soil:water) and relatively low levels of organic matter.

Role of temperature and soil moisture on chickpea genotype sensitivity to isoxaflutole

The results demonstrated differential tolerances among chickpea genotypes to isoxaflutole at different temperature and soil moisture levels. Increasing temperature and moisture content made the susceptible chickpea genotype more vulnerable to isoxaflutole damage, while isoxaflutole caused more damage to chickpea generally with increasing

moisture content. Isoxaflutole may cause crop damage in situations with high temperatures and where rainfall is likely soon after herbicide spraying. These factors also need to be considered in making recommendations about the use of this herbicide.

Effects of isoxaflutole on chickpea under differenet soil nitrogen levels

Many wheat cropping systems in Australia rely on legume fixed N for sustained grain yields. If grain legumes are to replace the pasture ley phase, legumes such as chickpea must prove their capacity to enhance soil N status under a range of inherent soil N levels. The results of this study suggest that higher nitrate levels coupled with isoxaflutole had a detrimental effect on the general growth and nodulation of both tolerant and sensitive chickpea cultivars. But the response of the isoxaflutole tolerant cultivar was better than the sensitive one for at least some growth parameters. Tolerant genotypes of chickpea will need to be selected when using the herbicide isoxaflutole under variable soil nitrate levels to incur less crop injury and have better nitrogen economy for the succeeding cereal crops.

Effects of isoxaflutole on nitrogen fixation capacity of chickpea

The recommended rate of isoxaflutole reduced the amount of fixed nitrogen by both the tolerant and sensitive cultivars while the intensity of reduction was greater in the sensitive cultivar. The amount of fixed nitrogen was also reduced in the sensitive cultivar when nitrate was added. A reduction in the amount of fixed nitrogen in chickpea crops due to application of isoxaflutole can negatively affect the potential N benefits associated with including chickpeas in rotation with cereals. Pulse crops are often associated with a nitrogen 'sparing' effect in which soil mineral nitrogen not taken up by legumes actively fixing nitrogen, remains available to subsequent cereal crops. The performance of the tolerant chickpea cultivar was comparatively better for at least some growth, nodulation and nitrogen fixation parameters. The addition of fertiliser nitrogen did not alleviate the residual herbicide effect; additions of fertiliser nitrogen may lead to greater reductions in nitrogen fixation.

This research project improved our understanding of the factors that affect the sensitivity of chickpea varieties to isoxaflutole and its effects on nodulation and nitrogen

fixation. The research findings will increase farmers' awareness regarding chickpea tolerance to isoxaflutole and provide guidelines for adjustment of rates for minimisation of crop injury and maximisation of the benefits of pulses in the rotation. By understanding the effects of soil characteristics on chickpea isoxaflutole tolerance, guidelines for the control of troublesome weeds in chickpea in particular situations may be improved. The study is also the precursor to understanding the effects of herbicides such as isoxaflutole on the competitive ability against weeds of apparently tolerant crops.

Due to the limited time period of this programme, most of the experiments were conducted under glasshouse and growth chamber conditions. One field experiment was undertaken to assess the differential sensitivity of chickpea to isoxaflutole but was limited to one set of soil and environmental conditions. Hence, more research needs to be undertaken across a range of environments to identify tolerance levels under field conditions and provide more reliable weed management advice for chickpea growers.

References

Abd-Alla, M. H., Omar, S. A., and Karanzha, S. (2000). The impact of pesticides on arbuscular mycorrhizal and nitrogen-fixing symbioses in legumes. *Applied Soil Ecology* **14**, 191-200.

Abdel-Waheb, A. M., Zahram, H. H., and Abd-Alla, M. H. (1996). Root-hair infection and nodulation of four grain legumes as affected by the form and application time of nitrogen fertiliser. *Folia Microbiologica* **41**, 303-308.

Ahlawat, I. P. S., Singh, A., and Saraf, C. S. (1981). Effects of winter legumes on the nitrogen economy and productivity of succeeding cereals. *Experimental Agriculture* **17**, 57-62.

Ahmad, T., Hafeez, F. Y., Mahmood, T., and Malik, K. A. (2001). Residual effect of nitrogen fixed by mungbean (*Vigna radiata*) and blackgram (*Vigna mungo*) on subsequent rice and wheat crops. *Australian Journal of Experimental Agriculture* **41**, 245-248.

Anderson, A., Baldock, J. A., Rogers, S. L., Bellotti, W., and Gill, G. S. (2004). Influence of chlorsulfuron on rhizobial growth, nodule formation, and nitrogen fixation with chickpea. *Australian Journal of Agricultural Research* **55**, 1059-1070.

Anon., (no date). Balance®: For the selective control of certain broadleaf weeds in chickpeas. Bayer CropScience, Australia Technical Guide, 2nd Edition, pp. 1-15.

Bailey, G. W., and White, J. L. (1970). Factors influencing the adsorption, desorption and movement of pesticide in soil. *Residue Review* **32**, 29-92.

Barriuso, E., Laird, A., Koskinen, W. C., and Dowdy, R. H. (1994). Atrazine desorption from smectites. *Soil Science Society of America Journal* **58**, 1632-1638.

Barry, P., and Pallett, K. E. (1998). The effect of diflufenican on pigment levels in carotenogenic systems. *In European Weed Research Society Symposium*, Wagennigen, The Netherlands, p. 51.

Beech, D. F., and Brinsmead, R. B. (1980). Tyson: a chickpea (*Cicer arietinum* L.) cultivar for grain production. *Journal of the Australian Institute of Agricultural Science* **46**, 127-129.

Beltran, E., Fenet, H., Cooper, J. F., and Coste, C. M. (2000). Kinetics of abiotic hydrolysis of isoxaflutole: influence of pH and temperature in aqueous mineral buffered solutions. *Journal of Agriculture and Food Chemistry* **48**, 4399-4403.

Beltran, E., Fenet, H., Cooper, J. F., and Coste, C. M. (2001). Kinetics of chemical degradation of isoxaflutole: influence of the nature of aqueous buffers (alkanoic acid/sodium salt *vs* phosphate). *Pest Management Science* **57**, 366-371.

Beltran, E., Fenet, H., Cooper, J. F., and Coste, C. M. (2002). Influence of the physical and chemical properties of soil on the retention process of isoxaflutole and its two main derivatives. *Weed Research* **42**, 385-393.

Beltran, E., Fenet, H., Cooper, J. F., and Coste, C. M. (2003). Fate of isoxaflutole in soil under controlled conditions. *Journal of Agriculture and Food Chemistry* **51**, 146-151.

Bertholet, J., and Clark, K. W. (1985). Effect of trifluralin and metribuzin on faba bean growth, development, and symbiotic nitrogen fixation. *Canadian Journal of Plant Science* **65**, 9-21.

Bethlenfalvay, G. J., Norris, R. F., and Phillips, D. A. (1979). Effect of bentazon, a Hill reaction inhibitor, on symbiotic nitrogen-fixing capability and apparent photosynthesis. *Plant Physiology* **63**, 213-215.

Beyer, E. M., Duffy, M. J., and Schlueter, D. D. (1987). Sulfonylurea herbicides. *In Herbicides: Chemistry, Degradation and Mode of action.* (P. C. Kearney and D. K. Karfman, eds.), Vol. 3, Marcel Dekker, New York, pp. 117-183.

Bhowmik, P. C., and Prostak, R. G. (1996). Activity of EXP 31130A in annual weed control in field corn. *In Weed Science Society of America Abstract*, Vol. 36, p. 13.

Bhowmik, P. C., Vrabel, T. E., Prostak, R., and Cartier, J. (1996). Activity of RPA 201772 in controlling weed species in field corn. *In Proceedings of the Second International Weed Control Congress*, Vol. 2, Copenhagen, Denmark, pp. 807-812.

Bhowmik, P. C., Kushwaha, S., and Mitra, S. (1999). Response of various weed species and corn (*Zea mays*) to RPA 201772. *Weed Technology* **13**, 504-509.

Bhowmik, P. C., Swarcewicz, M. K., and Mitra, S. (2001). Plant bioassay for isoxaflutole in soil. *In Proceedings of the 18th Asian Pacific Weed Science Society Conference*, Standard Press of China, Beijing, China, pp. 663-670.

Bollich, P. K., Dunigan, E. P., and Jadi, A. W. M. (1985). Effects of seven herbicides on N_2 (C_2H_2) fixation by soybeans. *Weed Science* **33**, 427-430.

Bollich, P. K., Dunigan, E. P., Kitchen, L. W., and Taylor, V. (1988). The influence of trifluralin and pendimethalin on nodulation, N_2 (C_2H_2) fixation, and seed yield of field-grown soybeans (*Glycine max*). *Weed Science* **36**, 15-19.

Bresnahan, G. A., Koskinen, W. C., Dexter, A. G., and Cox, L. (2004). Sorption-desorption of 'aged' isoxaflutole and diketonitrile degradate in soil. *Weed Research* **44**, 397-403.

Briggs, G. G., Bromilow, R. H., and Evans, A. A. (1982). Relationship between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. *Pesticide Science* **13**, 495.

Briggs, G. G. (1984). Factors affecting the uptake of soil-applied chemicals by plants and other organisms. *In BCPC Monograph No. 27, British Crop Protection Conference* (R. J. Hance, ed.), Farnham, Surrey, UK, pp. 35-40.

Brock, J. L. (1971). Effects of the herbicides trifluralin and carbetamide on nodulation and growth of legume seedlings. *Weed Research* **12**, 150-154.

Brown, A., Drum, F., Haseltine, C., and Lawrance, L. (2007). "Australian Crop Report No. 141, ABARE, Canberra."

Calvet, R. (1989). Adsorption of organic chemicals in soils. *Environmental Health Perspectives* **83**, 145-177.

Cardina, J., Hartwig, N. L., and Lukezic, F. L. (1986). Herbicide effects on crownvetch rhizobia and nodule activity. *Weed Science* **34**, 338-343.

Carrizosa, M. J., Rice, P. J., Koskinen, W. C., Carrizosa, I., and Hermosin, M. D. C. (2004). Sorption of isoxaflutole and DKN on organoclays. *Clays and Clay Minerals* **52**, 341-349.

Caseley, J. C. (1987). Effects of weather on herbicide activity. *In Proceedings of the Eighth Australian Weeds Conference*, Sydney, New South Wales. Council of Australian Weed Science Societies, pp. 386-394.

Celis, R., Cornejo, J., Hermosin, M. C., and Koskinen, W. C. (1997). Sorption-desorption of atrazine and simazine by model soil colloidal components. *Soil Science Society of America Journal* **61**, 436-443.

Chalk, P. M. (1996). Nitrogen transfer from legumes to cereals in intercropping. *In Roots and Nitrogen in Cropping Systems of the Semi-arid Tropics.* (O. Ito, C. Johansen, J. J. Adu-Gyamfi, K. Katayama, J. V. D. K. Kumar Rao and T. J. Rego, eds.), Japan International Research Centre for Agricultural Sciences: Tsukuba, pp. 351-374.

Chalk, P. M. (1998). Dynamics of biologically fixed N in legume-cereal rotations: a review. *Australian Journal of Agricultural Research* **49**, 303-16.

Chambers, A. (1995). "Field crop herbicide information: detailed information on Australian field crop herbicides." (Rutherglen Research Institution, Australia: Rutherglen, Victoria).

Chiou, C. T. (1989). Theoretical considerations of the partition uptake of non-ionic compounds by soil organic matter. *In Reactions and Movement of Organic Chemical in Soil* (B. L. Sawhney and K. Brown, eds.), SSSA, Madison, Wisconsin, pp. 1-29.

Clarke, M., Hodgson, J., and Price, L. (2001). Balance - a new broadleaf herbicide for the chickpea industry. *In Crop Updates (Weeds) Western Australia*, pp. 91-92.

Cox, L., Koskinen, W. C., and Yen, P. Y. (1998). Changes in sorption of imidacloprid with incubation time. *Soil Science Society of America Journal* **62**, 342-347.

Curley, R. L., and Burton, J. C. (1975). Compatibility of *Rhizobium japonicum* with chemical seed protectants. *Agronomy Journal* **67**, 807-808.

Curvey, S. E., and Kapusta, G. (1995a). Corn weed control with pre-emergence EXP 31130A. *In Research Report of North Central Weed Science Society*, Vol. 52, pp. 164-165.

Daimon, H., and Yoshioka, M. (2001). Response of root nodule formation and nitrogen fixation activity to nitrate in a split-root system in pea nut (*Arachis hypogaea* L.). *Agronomy and Crop Science* **187**, 89-95.

Dalal, R. C., and Mayer, R. J. (1986). Long term trends in fertility of soils under continuous cultivation and cereal cropping in southern Queensland. I. Overall changes in soil properties in winter cereal yields. *Australian Journal of Soil Research* **24**, 265-279.

Dalal, R. C., Strong, W. M., Weston, E. J., and Gaffney, J. (1991). Sustaining multiple production systems. 2. Soil fertility decline and restoration of cropping lands in sub-tropical Queensland. *Tropical Grasslands* **25**, 173-180.

Dalal, R. C., Strong, W. M., Doughton, J. A., Weston, E. J., McNamara, G. T., and Cooper, J. E. (1997). Sustaining productivity of a Vertisol at Warra, Queensland, with ferlisers, no-tillage or legumes. 4. Nitrogen fixation, water use and yield of chickpea. *Australian Journal of Experimental Agriculture* **37**, 667-676.

Dalal, R. C., Strong, W. M., Weston, E. J., Cooper, J. E., Wildermuth, G. B., Lehane, K. J., King, A. J., and Holmes, C. J. (1998). Sustaining productivity of a Vertisol at Warra, Queensland, with ferlisers, no-tillage or legumes. 5. Wheat yields, nitrogen benefits and water-use efficiency of chickpea-wheat rotation. *Australian Journal of Experimental Agriculture* **38**, 498-501.

Datta, A., Sindel, B. M., Jessop, R. S., Birchall, C., and Felton, W. L. (2006). Differential response of chickpea genotypes to isoxaflutole. *Communications in Agricultural and Applied Biological Sciences* **71 (3a)**, 733-742.

Datta, A., Sindel, B. M., Jessop, R. S., Kristiansen, P., and Felton, W. L. (2007). Phytotoxic response and yield of chickpea (*Cicer arietinum*) genotypes with pre-emergence application of isoxaflutole. *Australian Journal of Experimental Agriculture* **47 (12)**, 1460-1467.

Dear, B. S., Sandral, G. A., and Wilson, B. C. D. (2006). Tolerance of perennial pasture grass seedlings to pre- and post-emergent herbicides. *Australian Journal of Experimental Agriculture* **46**, 637-644.

Dhareesank, A., Kobayashi, K., and Usui, K. (2005). Phytotoxic activity of pethoxamid in soil under different moisture conditions. *Weed Biology and Management* **5**, 197-202.

Donald, C. M. (1965). The progress of Australian agriculture and the role of pastures in environmental change. *Australian Journal of Science* **27**, 187-197.

Doughton, J. A. (1988). Grain legumes and nitrogen in Queensland cropping systems. *In Proceedings of the Queensland Crop Production Conference* (J. P. Thompson and J. A. Doughton, eds.), Queensland Department of Primary Industries: Brisbane, pp. 46-54.

Doughton, J. A., Vallis, I., and Saffigna, P. G. (1993). Nitrogen fixation in chickpea. I. influence of prior cropping or fallow, nitrogen fertiliser and tillage. *Australian Journal of Agricultural Research* **44**, 1403-1413.

Dunigan, E. P., Frey, J. P., Allen, L. D. J., and McMahon, A. (1972). Herbicidal effects on the nodulation of *Glycine max* (L.) Merrill. *Agronomy Journal* **64**, 806-808.

Eaglesham, A. R. J., Hassouna, S., and Seegers, R. (1983). Fertiliser-N effects on N fixation by cowpea and soybean. *Agronomy Journal* **75**, 61-66.

Eberbach, P. (1993). The effect of herbicides and fungicides on Legume-*Rhizobium* symbiosis. *In Pesticide Interactions in Crop Production: Beneficial and Deleterious Effects* (J. Altman, ed.), CRC Press: London.

Eberbach, P. L., and Douglas, L. A. (1983). Persistence of glyphosate in a sandy loam. *Soil Biology and Biochemistry* **15**, 485-487.

Eberbach, P. L., and Douglas, L. A. (1989). Herbicide effects on the growth and nodulation potential of *Rhizobium trifoli* with *Trifolium subterraneum* L. *Plant and Soil* **119**, 15-23.

Evans, J. (1982a). Symbiosis, nitrogen and dry matter distribution in chickpea (*Cicer arietinum*). *Experimental Agriculture* **18**, 339-351.

Evans, J. (1982b). Nitrate reduction and dinitrogen fixation in chickpea. *In Proceedings of the 2nd Australian Agronomy Conference*, Wagga Wagga, New South Wales, p. 294. (Australian Society of Agronomy, Griffin Press, South Australia).

FAO (2002). 'FAOSTAT.' (FAO: Rome). http://apps.fao.org/default.htm.

FAO (2006). 'FAOSTAT.' (FAO: Rome). http://apps.fao.org/default.htm.

Felton, W. L., Marcellos, H., Alston, C. L., Martin, R. J., Backhouse, D., Burgess, L. W., and Herridge, D. F. (1998). Chickpea in wheat-based cropping systems of northern New South Wales. II. Influence on biomass, grain yield, and crown rot in the following wheat crop. *Australian Journal of Agricultural Research* **49**, 401-407.

Felton, W. L., Knights, T. D., Haigh, B. M., and Hardsen, S. (2004). Tolerance of chickpea to isoxaflutole. *In Fourteenth Australian Weeds Conference*, Wagga Wagga, New South Wales. (B. M. Sindel and S. B. Johnson, eds.) pp. 257-260. (Weed Science Society of New South Wales, Sydney)

Fletcher, W. W., Dickenson, P. B., and Raymond, J. C. (1956). The effect of certain hormone herbicides on the growth and nodulation of *Trifolium repens sylvestre* in aseptic culture. *Phyton* 7, 121-130.

Fletcher, W. W., Dickenson, P. B., Forrest, J. D., and Raymond, J. C. (1957). The effect of soil applications of certain substituted phenoxyacetic and phenoxybutyric acids on growth and nodulation of *Trifolium repens sylvestre*. *Phyton* **9**, 41-46.

Fontaine, D. D., Lehmann, R. G., and MIller, J. R. (1991). Soil adsorption of neutral and anionic forms of sulfonamide herbicide, flumetsulam. *Journal of Environmental Quality* **20**, 759-762.

Fox, J. E., Starcevic, M., Jones, P. E., Burow, M. E., and McLachlan, J. A. (2004). Phytoestrogen signalling and symbiotic gene activation are disrupted by endocrine-disrupting chemicals. *Environmental Health Perspectives* **112**, 672-677.

Fryer, J. D., and Makepeace, R. J. (1977). The principles of weed control in cereals. *In Weed Control Handbook*. Vol. 1, Blackwell Scientific Publications, London.

Garcia, M. M., and Jordan, D. C. (1969). Action of 2, 4-D and dalapon on the symbiotic properties of *Lotus cornicularis* (birdsfoot trefoil). *Plant and Soil* **30**, 317-334.

Gaston, S., Zabalza, A., Gonzalez, E. M., Arrese-Igor, C., Aparicio-Tejo, P. M., and Royuela, M. (2002). Imazethapyr, an inhibitor of the branched-chain amino acid

biosynthesis, induces aerobic fermentation in pea plants. *Physiologia Plantarum* **114**, 524-532.

Gaur, A. C. (1980). Effects of pesticides on symbiotic nitrogen fixation by legumes. *Indian Journal of Microbiology* **20**, 362-370.

Gerber, H. R., Nyffeler, A., and Green, D. H. (1983). The influence of rainfall, temperature, humidity and light on soil and foliage-applied herbicides. *Aspects of Applied Biology* **4 (Influence of environmental factors on herbicide performance and crop and weed biology)**, 1-14.

Gillet, D. L., and Holloway, R. E. (1996). The effects of sulphonylurea herbicide on annual medic in alkaline soil. *In Workshop Paper: Farming Systems Developments*, CRC for Soil and Land Management, Adelaide, South Australia, pp. 77-78.

Goetz, A. J., Wehtje, G., Walker, R. H., and Hajek, B. (1986). Soil solution and mobility characterisation of imazaquin. *Weed Science* **34**, 788-793.

Green, C. J., and Blackmer, A. M. (1995). Residue decomposition effects on nitrogen availability to corn following corn or soybean. *Soil Science Society of America Journal* **59**, 1065-1070.

Gupta, V., Roget, D., and Davoren, B. (2002). Nitrogen fixation by grain legumes in the low rainfall Mallee soils - Potential effects of herbicide application. Grainzone Research Update 2002, Grains Research and Development Corporation.

Hallgren, E. (1989). Infuence of temperature and relative humidity at application on the effect of weed control in spring and winter cereals with MCPA, MCPA + dichlorprop and oxtril 4. Field trials. *In Proceedings of the 30th Swedish Crop Protection Conference. Weeds and Weed Control*, Uppsala, Sweden, pp. 203-222.

Harper, J. E. (1974). Soil symbiotic nitrogen requirement for optimum soybean production. *Crop Science* **14**, 255-260.

Harrison, H. F., and Keinath, A. P. (2003). Glasshouse assessment of clomazone response of US pumpkin cultivars. *Crop Protection* **22**, 795-798.

Haughn, G. W., and Somerville, C. R. (1986). Sulfonylurea-resistant mutants of *Arabidopsis thaliana*. *Molecular and General Genetics* **204**, 430-434.

Herridge, D. F., Marcellos, H., Felton, W. L., Turner, G. L., and Peoples, M. B. (1995). Chickpea increases soil-N fertility in cereal systems through nitrate sparing and N_2 fixation. *Soil Biology and Biochemistry* **27**, 545-551.

Holford, I. C. R., Doyle, A. D., and Leckie, C. C. (1992). Nitrogen response characteristics of wheat protein in relation to yield response and their interactions with phosphorus. *Australian Journal of Agricultural Research* **43**, 969-986.

Hossain, S. A., Dalal, R. C., Waring, S. A., Strong, W. M., and Weston, E. J. (1996a). Comparison of legume-based cropping systems at Warra, Queensland. 1. Soil nitrogen and organic carbon accretion and potentially mineralisable nitrogen. *Australian Journal of Soil Research* **34**, 273-287.

Huang, W., and Weber, W. J., Jr. (1997). A distributed reactivity model for sorption by soils and sediments . 10. Relationships between desorption, hysteresis, and the chemical characteristics of organic domains. *Environmental Science and Technology* **31**, 2562-2569.

Huang, W., and Weber, W. J., Jr. (1998). A distributed reactivity model for sorption by soils and sediments. 11. Slow concentration-dependent sorption rates. *Environmental Science and Technology* **32**, 3549-3555.

Jensen, P. K., and Kudsk, P. (1988). Prediction of herbicide activity. *Weed Research* **28**, 473-478.

Jessop, R. S., Hetherington, S. J., and Hoult, E. H. (1984). The effect of soil nitrate on the growth, nodulation and nitrogen fixation of chickpeas (*Cicer arietinum*). *Plant and Soil* **82**, 205-214.

Kanayama, Y., Watanabe, I., and Yamamoto, Y. (1990). Inhibition of nitrogen fixation in soybean plants supplied with nitrate. 1. Nitrite accumulation and formation of nitrosylleghaemoglobin in nodules. *Plant and Cell Physiology* **31**, 341-346.

Kaur, H., Inderjit., and Bhowmik, P. C. (2004). Phytotoxicity of isoxaflutole to *Phalaris minor* Retz. *Plant and Soil* **258**, 161-168.

Kleschnick, W. A., Gerwick, B. C., Carson, C. M., Monte, W. T., and Sanders, S. W. (1992). DE-498, a new acetolactate synthase inhibiting herbicide with multicrop selectivity. *Journal of Agriculture and Food Chemistry* **40**, 1083-1085.

Knights, E. (1991). Chickpea. *In New Crops, Agronomy and Potential of Alternative Crop Species*. (R. S. Jessop and R. L. Wright, eds.), Inkata Press: Melbourne, pp. 27-38.

Knights, T., Moore, K., and Cumming, G. (2007). Yorker, desi chickpea, agronomic management. *In Pulse Variety Management Package*, Pulse Australia and New South Wales Department of Primary Industries.

Koopman, D. J., Tow, P. G., Reeves, T. G., and Gibson, A. H. (1995). Soil acidification, chlorsulfuron application and *Rhizobium meliloti* as factors in lucerne yield decline. *Soil Biology and Biochemistry* **27**, 673-677.

Koskinen, W. C., and Harper, S. S. (1990). The retention process: Mechanisms. *In Pesticides in the Soil Environment* (H. H. Cheng, ed.), Soil Science Society of America, Madison, Wisconsin, pp. 51-77.

Kudsk, P., Olesen, T., and Thonke, K. E. (1990). The influence of temperature, humidity and simulated rain on the performance of thiameturon-methyl. *Weed Research* **30**, 261-269.

Kudsk, P., and Kristensen, J. L. (1992). Effect of environmental factors on herbicide performance. *In Proceedings of the First International Weed Control Congress*, Melbourne, Australia, pp. 173-186.

Kumar, S., Pahwa, S. K., Promila, K., and Sharma, H. R. (1981). Effect of simazine and prometryn on the growth and nodulation of chickpea (*Cicer arietinum* L.). *Journal of Agricultural Science* **97**, 663-668.

Kust, C. A., and Struckmeyer, B. E. (1971). Effects of trifluralin on growth, nodulation, and anatomy of soybeans. *Weed Science* **19**, 147-152.

Lal, S. (1988). Effects of pesticides on *Rhizobium*-legume symbiosis. *In Pesticides and Nitrogen Cycles*. (R. Lal and S. Lal, eds.), Vol. 3, CRC Press, Boca Raton, Florida, pp. 47-128.

Lazo, M., Lopez de Medina, F., Sardina, J. L., and Gomez-Arnau, J. (1997). Isoxaflutole, a new corn herbicide from Rhone-Poulenc. *In Proceedings of the 6th Congress of the Spanish Weed Science Society*, Valencia, Spain, pp. 383-387.

Lehmann, R. G., Miller, J. R., Fontaine, D. D., Laskowski, D. A., Hunter, J. H., and Cordes, R. C. (1992). Degradation of sulfonamide herbicide as a function of soil sorption. *Weed Research* **32**, 564-567.

Leistra, M., and Green, R. E. (1990). Efficacy of soil applied herbicides. *In Pesticides in the Soil Environment: Processes, Impacts and Modelling* (H. H. Cheng, ed.), Soil Science Society of America: Madison, WI, pp. 401-428.

Lemerle, D., Kidd, C. R., and Read, B. J. (1986). Tolerance of barley cultivars to post-emergence herbicides. *Australian Journal of Experimental Agriculture* **26**, 383-392.

Lemerle, D., Yuan, T. H., Murray, G. M., and Morris, S. (1996). Survey of weeds and diseases in cereal crops in the southern wheat belt of New South Wales. *Australian Journal of Experimental Agriculture* **36**, 545-554.

Lin, C. H., Lerch, R. N., Li, Y. X., Thurman, E. M., Garrett, H. E., and George, M. F. (2000). Determination of isoxaflutole and its metabolites in water, soil, and plant material using high-performance liquid chromatography and mass spectrometry. *In Proceedings for the International Workshop on Pesticide 2000: Harmonisation of Pesticide Management, Regulation, Monitoring and Evaluation.* (S. S. Wong, ed.), UPAC-TACTRI/ COA, Taichung, Taiwan, p. 229.

Lin, C. H. (2002). Bioremediation capacity of five forage grasses for atrazine, balance (isoxaflutole) and nitrate removal., Ph. D. dissertation, University of Missouri, Columbia.

Littler, J. W., and Whitehouse, M. J. (1987). Effects of pasture on subsequent wheat crops on a black earth soil of the Darling Downs. III. Comparison of nitrogen from pasture and fertiliser sources. *Queensland Journal of Agriculture and Animal Science* **44**, 1-8.

Loston, R., and Penner, D. (1990). Factors affecting chloroacetanilide injury to corn (*Zea mays*). *Weed Technology* **4**, 904-906.

Lundkvist, A. (1997). Influence of weather on the efficacy of dichlorprop-P/MCPA and tribenuron-methyl. *Weed Research* **37**, 361-371.

Lupton, F. G. H. (1980). The plant breeder's reactions to herbicide tolerance in cereals. *In Proceedings of the 1980 British Crop Protection Conference-Weeds*, British Crop Protection Council, pp. 873-875.

Luscombe, B. M., Vrabel, T. E., Paulsgrove, M. D., Cramp, S., Cain, P., Gamblin, A., and Millet, J. C. (1994). RPA 201772: a new broad spectrum pre-emergence herbicide for corn. *In Proceedings of the North Central Weed Science Society*, Vol. 49, pp. 57-58.

Luscombe, B. M., Pallett, K. E., Loubiere, P., Millet, J. C., Melgarejo, J., and Vrabel, T. E. (1995). RPA 201772: a novel herbicide for broadleaf and grass weed control in maize and sugarcane. *In Brighton Crop Protection Conference - Weeds*, BCPC, Farnham, Surrey, UK, pp. 35-40.

Luscombe, B. M., and Pallett, K. E. (1996). Isoxaflutole for weed control in maize. *Pesticide Outlook* 7, 29-32.

Madhavi, B., Anand, C. S., Bharathi, A., and Polasa, H. (1993). Effects of pesticide on growth of rhizobia and their host plants during symbiosis. *Biomedical and Environmental Sciences* **6**, 89-94.

Mallik, M. A. B., and Tesfai, K. (1985). Pesticidal effect on soybean-rhizobia symbiosis. *Plant and Soil* **85**, 33-41.

Marcellos, H. (1984). Influence of prior crops of chickpeas, fababeans and lupins on wheat. *The Journal of the Australian Institute of Agricultural Science* **50**, 111-113.

Martensson, A. M., and Nilsson, A. K. (1989). Effects of chlorsulfuron on *Rhizobium* grown in pure culture and in symbiosis with alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*). *Weed Science* **37**, 445-450.

Martensson, A. M. (1992). Effects of agrochemicals and heavy metals on fast-growing *rhizobia* and their symbiosis with small-seeded legumes. *Soil Biology and Biochemistry* **24**, 435-445.

Martin, R. J., McMillan, M. G., and Cook, J., B. (1988). Survey of farm practices of the northern wheat belt of New South Wales. *Australian Journal of Experimental Agriculture* **28**, 499-509.

Menendez, J., De Prado, A., and Gomez-Arnau, J. (1997). Control of s-triazine-resistant biotypes with the new herbicide isoxaflutole. *In Proceedings of the 6th Congress of the Spanish Weed Science Society*, Valencia, Spain, pp. 89-92.

Merritt, C. R. (1984). Influence of environmental factors on the activity of ioxynil salt and ester applied to *Stellaria media*. *Weed Research* **24**, 173-182.

Mersie, W., and Seybold, C. (1996). Adsorption and desorption of atrazine, deethylatrazine, deisopropylatrazine and hydroxyatrazine on levy wetland. *Journal of Agriculture and Food Chemistry* **44**, 1925-1929.

Minchin, F. R., Summerfield, R. J., and Neves, M. C. P. (1981). Nitrogen nutrition of cowpeas (*Vigna unguiculata*): effects of timing of inorganic nitrogen applications on nodulation, plant growth and seed yield. *Tropical Agriculture* (Trinidad) **58**, 1-12.

Minkey, D. M., and Moore, J. H. (1996). Estimating dose-response curves for predicting glyphosate use rates in Australia. *In Proceedings of the Eleventh Australian Weeds Conference*, Melbourne, Australia, pp. 166-169.

Mitra, S., Bhowmik, P. C., and Xing, B. (1999). Sorption of isoxaflutole by five different soils varying in physical and chemical properties. *Pesticide Science* **55**, 935-942.

Mitra, S., Bhowmik, P. C., and Xing, B. (2000). Sorption and desorption of diketonitrile metabolite of isoxaflutole in soils. *Environmental Pollution* **108**, 183-190.

Mitra, S., Bhowmik, P. C., and Xing, B. (2001). Physical and chemical properties of soil influence the sorption of the diketonitrile metabolite of RPA 201772. *Weed Science* **49**, 423-430.

Moorman, T. B. (1986). Effects of herbicides on the survival of *Rhizobium japonicum* strains. *Weed Science* **34**, 628-633.

Moyer, J. R., Esau, R., and Kozub, G. C. (1989). Chlorsulfuron persistence and response of legumes in an alkaline soil. *Journal of Environmental Science and Health* **B24**, 37-56.

Munyinda, K., Karamanos, R. E., and O'Halloran, I. P. (1988). Yields of wheat in rotation with maize and soybeans in Zambia. *Canadian Journal of Plant Science* **68**, 747-753.

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

Nalewaja, J. D., and Woznica, Z. (1985). Environment and chlorsulfuron phytotoxicity. *Weed Science* **33**, 395-399.

Obermeier, M. R., Slack, C. H., Martin, J. R., and Witt, W. W. (1995). Evaluations of EXP 31130A- a new pre-emergence corn herbicide. *In Proceedings of the North Central Weed Science Society*, Vol. 50, pp. 25-26.

Olume, J. W. K., and Veatch, C. (1969). Organic matter-amiben interaction on nodulation and growth of soybean. *Weed Science* **17**, 264-265.

O'Sullivan, J., Thomas, R. J., and Sikkema, P. (2001). Sweet corn (*Zea mays*) cultivar sensitivity to RPA 201772. *Weed Technology* **15**, 332-336.

Pal, U. R., and Saxena, M. C. (1975). Responses of soybean to symbiosis and nitrogen fertilisation under humid subtropical conditions. *Experimental Agriculture* **11**, 221-226.

Pallett, K. E., Little, J. P., Veerasekaran, P., and Viviani, F. (1997). Inhibition of 4-hydroxyphenylpyruvate dioxygenase: the mode of action of the herbicide RPA 201772 (isoxaflutole). *Pesticide Science* **50**, 83-84.

Pallett, K. E., Little, J. P., Sheekey, M., and Veerasekaran, P. (1998). The mode of action of isoxaflutole I. Physiological effects, metabolism, and selectivity. *Pesticide Biochemistry and Physiology* **62**, 113-124.

Pallett, K. E., Cramp, S. M., Little, J. P., Veerasekaran, P., Crudace, A. J., and Slater, A. E. (2001). Isoxaflutole: the background to its discovery and the basis of its herbicidal property. *Pest Management Science* **57**, 133-142.

Pannell, D. J. (1990). The economics of herbicide application for weed control: deterministic, stochastic and tactical analysis., Ph. D. thesis, University of Western Australia.

Peoples, M. B., Brockwell, J., and Bergersen, F. J. (1992). Factors affecting nitrogen fixation: scope for improvement? *In Transfer of Biologically Fixed Nitrogen to Wheat.* (J. F. Angus, ed.), GRDC: Canberra, ACT.

Peoples, M. B., Herridge, D. F., and Ladha, J. K. (1995). Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production? *Plant and Soil* **174**, 3-28.

Posino, A., Liu, W., and Gessa, C. (1992). Influence of organic matter and its clay complexes on metolachlor adsorption on soil. *Pesticide Science* **36**, 283-286.

Powles, S. B., Preston, C., Bryon, I. B., and Jutsum, A. R. (1996). Herbicide resistance: impact and management. *Advances in Agronomy* **58**, 57-93.

R Development Core Team (2006). R: *A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna.

Rawsthorne, S., Hadley, P., Summerfield, R. J., and Roberts, E. H. (1985a). Effects of supplemental nitrate and thermal regime on the nitrogen nutrition of chickpea (*Cicer arietinum* L.) I. Growth and development. *Plant and Soil* **83**, 265-277.

Rawsthorne, S., Hadley, P., Summerfield, R. J., and Roberts, E. H. (1985b). Effects of supplemental nitrate and thermal regime on the nitrogen nutrition of chickpea (*Cicer arietinum* L.) II. Symbiotic development and nitrogen assimilation. *Plant and Soil* **83**, 279-293.

Reeves, T. G., Gllington, A., and Brooke, H. D. (1984). Effects of lupin-wheat rotations on soil fertility, crop disease and crop yields. *Australian Journal of Experimental Agriculture and Animal Husbandry* **24**, 595-600.

Rennie, R. J., and Dubetz, S. (1984). Effect of fungicides and herbicides on nodulation and N_2 fixation in soybean fields lacking indigenous *Rhizobium japonicum*. *Agronomy Journal* **76**, 451-454.

Rice, P. J., Koskinen, W. C., and Carrizosa, M. J. (2004). Effect of soil properties on the degradation of isoxaflutole and the sorption-desorption of isoxaflutole and its diketonitrile degrade. *Journal of Agriculture and Food Chemistry* **52**, 7621-7627.

Roslycky, E. B. (1985). Sensitivity and adaptation of selected rhizobia and agrobacteria to paraquat. *Canadian Journal of Soil Science* **65**, 555-562.

Rouchaud, J., Neus, O., Callens, D., and Bulcke, R. (1998). Isoxaflutole herbicide soil persistence and mobility in summer corn and winter wheat crops. *Bulletin of Environmental Contamination and Toxicology* **60**, 577-584.

Rouchaud, J., Neus, O., Eelen, H., and Bulcke, R. (2002). Soil metabolism of isoxaflutole in corn. *Archives of Environmental Contamination and Toxicology* **42**, 280-285.

Rovira, A. D., Neate, S. M., and Warren, R. A. (1993). The effect of glean residues on growth and nodulation of medic in an alkaline dark brown sandy loam. *In Proceedings of the 7th Australian Agronomy Conference*, Adelaide. (The Australian Society of Agronomy, Parkville, Victoria.)

Sandhu, P. S., Dhingra, K. K., Bhandari, S. C., and Gupta, R. P. (1991). Effect of hand-hoeing and application of herbicides on nodulation, nodule activity and grain yield of *Lens culinaris* Med. *Plant and Soil* **135**, 293-296.

Schnelle, M. A., and Hensley, D. L. (1990). Effects of pesticides upon nitrogen fixation and nodulation by dry bean. *Pesticide Science* **28**, 83-88.

Schwenke, G. D., Peoples, M. B., Turner, G. L., and Herridge, D. F. (1998). Does nitrogen fixation of commercial, dryland chickpea and faba bean crops in north-west New South Wales maintain or enhance soil nitrogen? *Australian Journal of Experimental Agriculture* **38**, 61-70.

Secor, J. (1994). Inhibition of barnyard grass 4-hydroxyphenylpyruvate dioxygenase by sulcotrione. *Plant Physiology* **106**, 1429-1433.

Shaner, D. L. (1995). Studies on mechanisms and genetics of resistance: their contribution to herbicide resistance mechanism. *In Brighton Crop Protection Conference - Weeds*, Vol. 2, pp. 537-545.

Shaw, D. R., and Murphy, G. P. (1997). Adsorption and relative mobility of flumetsulam. *Weed Science* **45**, 573-578.

Shone, M. G. T., and Wood, A. V. (1974). A comparison of the uptake and translocation of some organic herbicides and a systemic fungicide in barley. I. Absorption in relation to physico-chemical properties. *Journal of Experimental Botany* **25**, 390-400.

Si, P., Sweetingham, M. W., Buirchell, B. J., Bowran, D. G., and Piper, T. J. (2006). Genotypic variation in metribuzin tolerance in narrow-leafed lupin (*Lupinus angustifolius* L.). *Australian Journal of Experimental Agriculture* **46**, 85-91.

Siddique, K. H. M., and Sedgley, R. H. (1986). Chickpea (*Cicer arietinum* L.), a potential grain legume for south-western Australia: seasonal growth and yield. *Australian Journal of Agricultural Research* **37**, 245-261.

Siddique, K. H. M., Walton, G. H., and Seymour, M. (1993). A comparison of seed yields of winter grain legumes in Western Australia. *Australian Journal of Experimental Agriculture* **33**, 915-922.

Siddique, K. H. M., and Sykes, J. (1997). Pulse production in Australia past, present and future. *Australian Journal of Experimental Agriculture* **37**, 103-111.

Siddique, K. H. M., Brinsmead, R. B., Knight, R., Knights, E. J., Paull, J. G., and Rose, I. A. (2000). Adaptation of chickpea (*Cicer arietinum* L.) and faba bean (*Vicia faba* L.) to Australia. *In Linking Research and Marketing Opportunities for Pulses in the 21st Century*. (R. Knight, ed.), Kluwer: Dordrecht, The Netherlands pp. 289-303.

Singh, G., and Wright, D. (1999). Effects of herbicides on nodulation, symbiotic nitrogen fixation, growth and yield of pea (*Pisum sativum*). *Journal of Agricultural Science* **133**, 21-30.

Singh, G., and Wright, D. (2002). *In vitro* studies on the effects of herbicides on the growth of rhizobia. *Letters in Applied Microbiology* **35**, 12-16.

Sprague, C. L., Kells, J. J., and Penner, D. (1996). Weed control and corn tolerance with RPA 201772. *In Proceedings of the North Central Weed Science Society*, Vol. 51, pp. 50-51.

Sprague, C. L., Kells, J. J., and Penner, D. (1997). Effect of application timing on corn tolerance and weed control with isoxaflutole. *In Weed Science Society of America Abstract*, Vol. 37, p. 5.

Sprague, C. L., Kells, J. J., and Penner, D. (1998a). Basis for differential tolerance of corn hybrids to PRA 201772. *In Proceedings of the North Central Weed Science Society*, Vol. 53, p. 138.

Sprague, C. L. and Penner, D. (1998b). Basis for differential tolerance of four corn hybrids to RPA 201772. *In Proceedings of North Central Weed Science Society*, Vol. 53, p. 94.

Sprague, C. L., Penner, D., and Kells, J. J. (1999). Important considerations for RPA 201772 utility. *Weed Technology* **13**, 814-820.

Sprague, C. L., Kells, J. J., and Penner, D. (1999a). Weed control and corn (*Zea mays*) tolerance from soil-applied RPA 201772. *Weed Technology* **13**, 713-725.

Sprague, C. L., Penner, D., and Kells, J. J. (1999b). Enhancing the margin of selectivity of RPA 201772 in *Zea mays* with antidotes. *Weed Science* **47**, 492-497.

Sprague, C. L., Penner, D., and Kells, J. J. (1999c). Physiological basis for tolerance of four *Zea mays* hybrids to RPA 201772. *Weed Science* **47**, 631-635.

Sprout, S. L., Nelson, L. M., and Germida, J. J. (1992). Influence of metribuzin on the *Rhizobium leguminosarum*-lentil (*Lens culinaris*) symbiosis. *Canadian Journal of Microbiology* **38**, 343-349.

Streeter, J. G. (1986). Effect of nitrate on acetylene reduction activity and carbohydrate composition of *Phaseolus vulgaris* nodules. *Physiologia Plantarum* **68**, 294-300.

Strong, W. M., Harbison, J., Nielsen, R. G. H., Hall, B. D., and Best, E. K. (1986a). Nitrogen availability in a Darling Downs soil following cereal, oilseed and grain legume crops. 1. Soil nitrogen accumulation. *Australian Journal of Experimental Agriculture* **26**, 347-351.

Strong, W. M., Harbison, J., Nielsen, R. G. H., Hall, B. D., and Best, E. K. (1986b). Nitrogen availability in a Darling Downs soil following cereal, oilseed and grain legume crops. 2. Effects of residual soil nitrogen and fertiliser nitrogen on subsequent wheat crops. *Australian Journal of Experimental Agriculture* **26**, 253-259.

Strong, W. M., Dalal, R. C., Weston, E. J., Cooper, J. E., Lehane, K. J., King, A. J., and Chicken, C. J. (1996). Sustaining productivity of a Vertisol at Warra, Queensland, with fertilisers, no-tillage or legumes. 2. Long term fertiliser N needs to enhance wheat yields and grain protein. *Australian Journal of Experimental Agriculture* **36**, 665-674.

Summerfield, R. J., Dart, P. J., Huxley, P. A., Eaglesham, A. R. J., Minchin, F. R., and Day, J. M. (1977). Nitrogen nutrition of cowpea (*Vigna unguiculata*). I. Effects of applied nitrogen and symbiotic nitrogen fixation on growth and seed yield. *Experimental Agriculture* **13**, 129-147.

Swarcewicz, M. K., Bhowmik, P. C., and Mitra, S. (2002). Plants response to isoxaflutole residues in soil. *Electronic Journal of Polish Agricultural Universities* **5**.

Taylor-Lovell, S., Sims, G. K., Wax, L. M., and Hassett, J. J. (2000). Hydrolysis and soil adsorption of the labile herbicide isoxaflutole. *Environmental Science and Technology* **34**, 3186-3190.

Taylor-Lovell, S., Sims, G. K., and Wax, L. M. (2002). Effects of moisture, temperature and biological activity on the degradation of isoxaflutole in soil. *Journal of Agriculture and Food Chemistry* **50**, 5626-5633.

Torstensson, L. (1975). Effects of bentazon and dinoseb on soil microorganisms and on the *Rhizobium leguminosarum* symbiosis. *Swedish Journal of Agricultural Research* **5**, 177-183.

Vessey, J. K., Walsh, K. B., and Layzell, D. B. (1988). Can a limitation in phloem to nodules account for the inhibitory effect of nitrate on nitrogenase activity in soybean? *Physiologia Plantarum* **74**, 137-146.

Vidal, D., Martinez, J., Bergareche, C., Miranda, A. M., and Simon, E. (1992). Effect of methabenzthiazuron on growth and nitrogenese activity in *Vicia faba*. *Plant and Soil* **144**, 235-245.

Viviani, F., Little, J. P., and Pallett, K. E. (1998). The mode of action of isoxaflutole II. Characterisation of the inhibition of carrot 4-hydroxyphenylpyruvate dioxygenase by the diketonitrile derivative of isoxaflutole. *Pesticide Biochemistry and Physiology* **62**, 125-134.

Vrabel, T. E., Jensen, J. O., Wrucke, M. A., and Hicks, C. (1995). EXP 31130A: a new pre-emergent herbicide for corn. *In Proceedings of the North Central Weed Science Society*, Vol. 50, pp. 24-25.

Vrabel, T. E., Cartier, J. P., and White, M. (1997). Performance of isoxaflutole in pre-emergence and preplant applications in conventional tillage corn. *In Proceedings of the Northeastern Weed Science Society*, Vol. 51, p. 53.

Vrabel, T. E., Veilleux, D. P., and Gemma, C. M. (1998). Isoxaflutole performance in tank mixes and pre mixes in corn. *In Proceedings of the Northeastern Weed Science Society*, Vol. 52, p. 114.

Wauchope, R. D., and Koskinen, W. C. (1983). Adsorption-desorption equilibria of herbicides in soil: thermodynamic perspective. *Weed Science* **31**, 504-512.

Weber, J. B., and Peter, C. J. (1982). Adsorption, bioactivity, and evaluation of soil tests for alachlor, acetochlor, and metolachlor. *Weed Science* **30**, 14-20.

Whish, J. P. M., Castor, P., and Carberry, P. S. (2007). Managing production constraints to the reliability of chickpea (*Cicer arietinum* L.) within marginal areas of the northern grains region of Australia. *Australian Journal of Experimental Agriculture* **58**, 396-405.

Wildermuth, G. B., Tinline, A. D., and McNamara, R. B. (1992). Assessment of yield loss caused by common root rot in wheat cultivars in Queensland. *Australian Journal of Agricultural Research* **43**, 43-58.

Wong, P. P. (1980). Nitrate and carbohydrate effects on nodulation and nitrogen fixation (acetylene reduction) activity of lentil (*Lens esculenta* Moench). *Plant Physiology* **66**, 78-81.

Young, B. G., and Hart, S. E. (1998). Optimising foliar activity of isoxaflutole on giant foxtail (*Setaria faberi*) with various adjuvants. *Weed Science* **46**, 397-402.

Young, B. G., Hart, S. E., and Simmons, F. W. (1999). Pre-emergance weed control in conventional-till corn (*Zea mays*) with RPA 201772. *Weed Technology* **13**, 471-477.

Young, B. G., and Hart, S. E. (2000). Mobility of RPA 201772 in *Setaria faberi*. *Weed Science* **48**, 188-192.