Interaction of pre-emergent herbicides and crop residues in Western Australian no-tillage systems

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B. Sc. Agricultural Engineering (Plant Protection)

M. Sc. History of Applied Science

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The research content of this thesis consists of four scientific papers that includes one published paper (Chapter 3) and three manuscripts prepared for later publication (Chapters 4, 5 and 6). The work associated with the production of these manuscripts in this thesis is my own, as are the introductory (Chapter 1 and 2) and concluding chapter (Chapter 7). The contribution of the co-authors is associated with the initial research directions, research planning and editorial input in various versions of the drafts of the manuscripts and the thesis. The reference list have been placed at the end of the thesis and the details of the research manuscripts are outlined below:

Details of the work:

Location in thesis: Chapter 3

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Chapter 4: Rainfall leaching herbicide from wheat residue into the soil is greatest with pyroxasulfone and least with trifluralin (under internal review, soon to be sent to a journal for publication).

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Chapter 6: Effect of wheat residue height, amount and orientation on pyroxasulfone interception, leaching, and weed control efficacy.

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Abstract

Pre-emergent herbicides play an important role in cropping systems by providing broad-spectrum, residual, weed control. These herbicides are applied to the soil and can be incorporated by rainfall, irrigation, tillage or, in the case of no-tillage (NT) systems, by the seeding operation. Prosulfocarb, pyroxasulfone, and trifluralin are the most common pre-emergent herbicides used in Western Australia in NT systems and are commonly used in agriculture world-wide. However, these herbicides can be intercepted by stubble, which may reduce weed control in NT cropping systems.

The first objective of this study was to determine the effect of rainfall intensity, amount and timing on leaching of prosulfocarb, pyroxasulfone and trifluralin from crop residue. The second objective was to investigate the effect of crop residue type, age and moisture content on the sorption and leaching (desorption) of these herbicides. The third objective was to determine the effect of residue height, amount and orientation on pyroxasulfone interception, leaching and weed control efficacy.

Prior to performing the experiments, a bioassay was developed to assess the bioavailability of the three herbicides in crop residues and a sandy-loam soil. The developed bioassay used annual ryegrass shoot inhibition for relatively low suspected concentrations of herbicide and cucumber shoot inhibition for higher rates.

A range of controlled environment chamber, glasshouse and field experiments were then conducted to assess the efficacy of these three pre-emergent herbicides using the bioassay. A computerised rainfall simulator was used to simulate different rainfall amounts of varying intensity and occurrence. Experiments included a range of rainfall treatments, crop residue amounts, conditions, types and ages. In addition, field experiments studied the effect of four heights of standing residue and four quantities of flat residue on pyroxasulfone interception and leaching.

This project increased our understanding of the effect of rainfall amount and intensity on the leaching of prosulfocarb, pyroxasulfone and trifluralin from crop residues. At higher rainfall amounts, the herbicide leached from the crop residue into the soil soon after application, and more with rain in one event rather than multiple events. However, the intensity of rainfall had no effect. Pyroxasulfone leached easily from the residue into the soil for up to 14 days after its application to potentially offer good weed control,
prosulfocarb leached from the residue for about 7 seven days, while only a small amount of trifluralin leached from stubble with rain one day after application.

Less herbicide leached from crop residue into the soil after rainfall when prosulfocarb and trifluralin were applied to wet residue rather than dry residue, but the initial moisture condition did not affect the leaching of pyroxasulfone from residue. Higher quantities (up to 4 t/ha) of wheat residue resulted in greater herbicide interception. Simulated rainfall improved herbicide activity in the soil, particularly for pyroxasulfone, highlighting the effectiveness of rainfall for good weed control with pyroxasulfone in high residue NT systems. Therefore pyroxasulfone is highly recommended as a pre-emergent herbicide in NT systems when the amount of residue on the soil surface exceeds 2 t ha\(^{-1}\), and prosulfocarb could also be used for amounts less than 2 t ha\(^{-1}\). Barley and wheat residues intercepted more herbicide than an equivalent amount of canola, chickpea or lupin residue, which was largely due to the increased ground cover with the cereal residues. 

The effect of residue age on herbicide interception and leaching was relatively small and variable. Overall, more herbicide reached the soil when sprayed on one-year-old residue than new residue, which was largely due to reduced groundcover with aged residue. Overall, the amount of herbicide reaching the soil through crop residue was strongly related to the ground cover percentage. The linear relationship, between ground cover percentage and growth of bioassay species, explained about 75\% of the variation in the data when the herbicides were modelled separately.

The field experiment showed that taller standing residue, generally resulted in decreased spray coverage at the soil surface and weed control efficacy, although this was only significant between nil stubble and 30 cm cut height. Increased amounts of horizontal wheat residue on the soil surface significantly reduced the amount of herbicide reaching the soil surface, which reduced the efficacy of annual ryegrass control in the field. The effect was marked when the amount of residue increased from 2 t ha\(^{-1}\) to 4 t ha\(^{-1}\) of horizontal wheat residue.
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Chapter 1

General introduction

1.1 Conservation agriculture

In traditional farming systems, conventional tillage (CT) is the main practice used to prepare seedbeds and control weeds, with 100% of the soil surface being disturbed multiple times during the season. Moreover, crop residues are often grazed, burned or manually removed to assist cultivation. These practices reduce soil health and soil tilth, and destroy soil structure (D’Emden, Llewellyn & Burton 2006; Kassam et al. 2012; Chauhan, Gill & Preston 2006).

Conservation agriculture (CA) is an agricultural system developed to improve soil fertility, crop production and profitability, and has been adopted widely by Australian farmers and others around the world over the last 30 years (Kassam et al. 2012; Llewellyn, D’Emden & Kuehne 2012). This system is based on three main components: minimum soil disturbance, diverse crop rotation, and permanent soil coverage with crop residues (Friedrich, Derpsch & Kassam 2012; Fuentes et al. 2009; Kassam et al. 2012; Brouder & Gomez-Macpherson 2014; Giller et al. 2015; Serraj & Siddique 2012). The implementation of all three principles over a long period is considered the key to enhancing soil health under CA (Kassam et al. 2012). No-tillage (NT) fits within CA and is defined by Crabtree (2010) as the seeding of crops without any type of land preparation, with less than 20% soil disturbance and full retention of crop residues or stubble on the soil surface. The initial adoption of NT in Australia was mainly to reduce wind erosion on cultivated soils and to reduce input costs (D’Emden, Llewellyn & Burton 2006; Llewellyn, D’Emden & Kuehne 2012). The estimated adoption rate of this system across Western Australia is currently around 90% (Crabtree 2010; Llewellyn, D’Emden & Kuehne 2012).

A diverse crop rotation is an important component of CA which helps to reduce disease and weed populations and to promote nitrogen fixation by legumes (Flower & Braslin 2006). However, the adoption of continuous cereals is increasing in the south-west of Australia due to higher profitability (Flower & Braslin 2006), as a result of higher yields.
and lower risk of crop failure compared to legumes, relatively cheap nitrogen fertiliser and, until recently, good weed management options.

The retention of crop residues is a focus of NT systems in Australia (Roper, Gupta & Murphy 2010). However, Scott et al. (2010) and Kirkegaard et al. (2014) found that many Australian farmers are pragmatic about retaining crop residue. Nonetheless, Flower and Braslin (2006) reported that keeping the soil surface covered with residue was more important than reducing tillage under NT systems. According to Kassam et al. (2012), the soil surface should be 100% covered with retained stubble to provide sufficient material to maintain soil organic matter levels. As a result of the combined impact of minimal soil disturbance, crop rotation and permanent residue retention, crop yields under CA increase significantly in comparison to CT systems (Fuentes et al. 2009; Dalal et al. 2011). However, yield reductions with CA, increased labour requirements when herbicides are not used and insufficient soil coverage as a result of low productivity and livestock feeding with crop residues has been reported in Africa (Giller et al. 2009).

1.2 Crop residue in no-tillage systems

There are differing opinions regarding the value of crop residues and their benefits to cropping systems (Prasad & Power 1991). There are many gains from keeping the soil surface covered with permanent plant residue, for example, protecting soil from wind and water erosion, maintaining soil water content, increasing soil organic matter (SOM) and improving soil structure (Locke, Zablotowicz & Weaver 2006a; Haddad et al. 2013). Crop residues protect soil against the forces of raindrop impact and wind shear and influence radiation balance and energy fluxes reducing the rate of evaporation from the soil (Wilhelm et al. 2004). As a result of the combined impact of permanent plant residue retention and minimal soil disturbance in NT systems, the capacity and activity of soil microbial biomass increases when compared to CT approaches (Roper, Gupta & Murphy 2010; Fuentes et al. 2009; Dalal, Henderson & Glasby 1991; Gupta et al. 1994) due to the increased availability of soil water and organic carbon (Locke & Bryson 1997). Permanent residue retention increases soil water conservation compared to systems where the residue is removed or incorporated (Fuentes et al. 2009; Dalal et al. 2011). In addition, crop residues also influence water dynamics, infiltration, runoff (Prasad & Power 1991; Sommer et al. 2014). For instance, retention of crop residues on the soil surface increased water infiltration and soil water levels under NT compared to where stubble was removed.
or at low levels in field conditions. This contributed to yield increases of up to 30% (Govaerts et al. 2007; Sommer et al. 2012; Ward et al. 2012). Crop residues on the soil surface may also play a crucial role in reducing the germination of weed seeds, due to the reduction of light underneath the residue, and the allelopathic effects of the residue (Nichols et al. 2015; Farooq et al. 2011).

Some negative effects of crop residue retention have been reported, especially with large amounts of residue on the soil surface. For instance, machinery blockages can occur which delay the sowing operation and cause variation in sowing depth (Kirkegaard et al. 2014). Reduced crop seedling growth, increased foliar disease and soil N immobilisation have been observed under residue retention (Rainbow & Derpsch 2011; Fenster 1977; Thompson 1992). Moreover, research has revealed that the growth of both crop and weed species are influenced by many factors related to residue retention (Ashworth, Desbiolles & Tola 2010). These factors include poor contact of seeds with soil (Blackshaw et al. 2009), reduced air and soil temperature (Ball et al. 1997), increased root disease (Cook & Haglund 1991) and release of phytotoxins from plant materials (Bruce et al. 1999). In addition, NT is a system which excludes tillage prior to sowing, therefore, there is more reliance on the use of herbicides for weed control (Nichols et al. 2015).

Crop residue retention plays an important role in CA farming systems from the perspective of enhancing soil quality and increasing crop production. According to Kirkegaard et al. (2014), the average wheat yield in Australia is about 2.2 t ha\(^{-1}\) which produces around 3.3 t ha\(^{-1}\) crop residue. Retaining a reasonable amount (\(\geq 4\) t ha\(^{-1}\)) of crop residues on the soil surface will play an important role in suppressing weed seed germination. However, retaining crop residues on the soil surface may intercept applied herbicides and reduce their effectiveness in weed control (Chauhan, Gill & Preston 2006), which is the focus of this research.
Chapter 2

Review of related literature

2.1 Introduction

The increase in crop residue in CA systems may alter herbicide dissipation in soil and in turn may affect the efficacy of weed control. Mechanisms of herbicide dissipation include; sorption, degradation, movement in leachate or surface runoff, volatilisation and plant uptake. These mechanisms can be influenced by NT practices which affect soil characteristics (Locke, Zablotowicz & Weaver 2006a). The effect of residue management techniques on the fate of herbicides has been studied for many herbicides such as alachlor (Locke, Gaston & Zablotowicz 1996), atrazine (Levanon et al. 1994), bentazone (Wagner et al. 1996), chlorimuron-ethyl (Reddy, Zablotowicz & Locke 1995), metolachlor (Levanon et al. 1994) and metribuzin (Locke & Harper 1991). Different patterns of herbicide degradation in soil have been compared in NT and CT soils, and depend on the herbicide, soil characteristics with herbicide history. For example, chlorimuron-ethyl is sorbed to crop residues more than to soil particles (Zablotowicz, Locke & Smeda 1998). This is in turn will make it less degradable when sprayed in NT compared to CT.

There are two main categories of herbicides used in cropping systems: pre-emergent and post-emergent herbicides. The efficacy of weed management can be enhanced by knowing the physical and chemical differences of herbicides and how to use them correctly during the growing season. Pre-emergent herbicides play an important role in cropping systems by providing broad-spectrum residual weed control. Applied to the soil they can provide effective early weed control, often controlling multiple germinations to reduce early weed competition with crops. They are applied to the soil, and incorporated by cultivation, rainfall or irrigation (Kleemann et al. 2015; Haskins 2012). Post-emergent herbicides are used after crop and weed emergence and work by traveling down the plant stem and into the root system. They are applied directly to weeds and usually need to be applied several times (not same herbicide) throughout the growing season (Beckie et al. 1999). This review includes a discussion of the fate of herbicides in NT systems with residue retention, including herbicide interception, degradation, and movement. This
Chapter 2: Review of related literature

review will elaborate on three pre-emergent herbicides ( trifluralin (Treflan®), pyroxasulfone (Sakura®) and prosulfocarb (Arcade®)) which are the most commonly used in Western Australia in NT systems for wheat production. The review will also describe bioassays for herbicides, as a method of measuring their bioavailability and identify any knowledge gaps. Common and chemical names for herbicides mentioned in this review are shown in Table 2.1.

<table>
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<tr>
<th>Common name</th>
<th>Chemical name</th>
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<tr>
<td>Acetochlor</td>
<td>2-chloro-N-ethoxymethyl-6’-ethylacet-o-toluidide.</td>
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<td>Alachlor</td>
<td>2-chloro-2’,6’-diethyl-N-methoxymethylacetonilide.</td>
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<tr>
<td>Atrazine</td>
<td>6-chloro-N²-ethyl-N²-isopropyl-1,3,5-triazine-2,4-diamine.</td>
</tr>
<tr>
<td>Bentazon</td>
<td>3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide</td>
</tr>
<tr>
<td>Chlorimuron</td>
<td>2-(4-chloro-6-methoxy-pyrimidin-2-ylcarbamoylsulfamoyl)benzoic acid.</td>
</tr>
<tr>
<td>Chlorimuron-ethyl</td>
<td>ethyl 2-(4-chloro-6-methoxy-pyrimidin-2-ylcarbamoylsulfamoyl)benzoate.</td>
</tr>
<tr>
<td>Cyanazine</td>
<td>2-(4-chloro-6-ethylamino-1,3,5-triazin-2-ylamino)-2-methylpropiononitrile.</td>
</tr>
<tr>
<td>Dimethenamid-P</td>
<td>(S)-2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)acetamide.</td>
</tr>
<tr>
<td>Fluometuron</td>
<td>1,1-dimethyl-3-(α,α,α-trifluoro-m-tolyl)urea.</td>
</tr>
<tr>
<td>Isoproturon</td>
<td>3-(4-isopropylphenyl)-1,1-dimethylurea. Or 3-p-cumenyl-1,1-dimethylurea.</td>
</tr>
<tr>
<td>Metribuzin</td>
<td>4-amino-6-tert-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one. Or 4-amino-6-tert-butyl-3-methylthio-1,2,4-triazin-5(4H)-one.</td>
</tr>
<tr>
<td>Paraquat</td>
<td>1,1’-dimethyl-4,4’-bipyridinium.</td>
</tr>
<tr>
<td>Picloram</td>
<td>4-amino-3,5,6-trichloropyridine-2-carboxylic acid. Or 4-amino-3,5,6-trichloropicolinic acid.</td>
</tr>
<tr>
<td>Prosfocarb</td>
<td>S-benzyl dipropyl(thiocarbamate)</td>
</tr>
<tr>
<td>Pyroxasulfone</td>
<td>5-(difluoromethoxy)-1-methyl-3-(trifluoromethyl)pyrazol-4-ylmethyl 4,5-dihydro-5,5-dimethyl-1,2-oxazol-3-yl sulfone. Or 3-[(5-(difluoromethoxy)-1-methyl-3-(trifluoromethyl)pyrazol-4-ylmethylsulfonyl]-4,5-dihydro-5,5-dimethyl-1,2-oxazole.</td>
</tr>
<tr>
<td>Trifluralin</td>
<td>α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine.</td>
</tr>
</tbody>
</table>

### 2.2 Fate of herbicides in no-tillage systems with residue retention

The fate of herbicide can be grouped into three main mechanisms: (1) interception on the stubble or in the crop canopy which may be available for soil incorporation and
Chapter 2: Review of related literature

subsequent weed control; (2) movement in leachate or surface runoff, volatilisation and plant uptake; and (3) degradation when a herbicide undergoes a chemical reaction in the presence of sunlight or microorganisms and is unavailable for weed control (Congreve 2015). To fully understand how pre-emergent herbicides work, it is crucial to understand their chemical and physical characteristics, the soil type and how they interact and are broken down in the environment (Congreve 2015).

2.2.1 Herbicide interception

Crop residues on the surface of uncultivated soils in CA systems can intercept much of the applied herbicide and reduce the amount of chemical which reaches the weed seeds or seedlings, thereby affecting herbicide efficacy, persistence, and fate (Ghadiri, Shea & Wicks 1984; Gaston, Boquet & Bosch 2001; Chauhan, Gill & Preston 2006). However, the residues can play a short-term role where they intercept herbicides, which are then transferred to the soil surface by rainfall, macropore flow or volatilisation (Unger 1994). For instance, crop residue can intercept 15-80% of the applied herbicide which may account for the reduction in herbicide effectiveness sometimes observed in CA systems (Chauhan, Gill & Preston 2006). However, other studies showed that plant residues did not significantly reduce herbicide efficacy (Erbach & Lovely 1975; Robison & Wittmuss 1973). For example, the efficacy of a mixture of alachlor and atrazine in controlling weeds was not affected by surface mulching when recommended rates of herbicide were used (Chauhan, Gill & Preston 2006). Similarly, Erbach and Lovely (1975) reported that 6.2 t ha⁻¹ of non-chopped corn stems did not significantly reduce the effectiveness of weed control using recommended rates of atrazine; this may be due to high recommended rates of alachlor and atrazine or difference in binding to organic matter (SOM). Research on the retention of atrazine on standing and flat wheat residue (average flat = 3.4 t ha⁻¹ and standing = 3 t ha⁻¹ covering 80–90% of the soil surface) showed that, directly after application of 1.7 kg ha⁻¹ atrazine, almost 60% of the sprayed chemical had been intercepted by the residue and 40% reached the surface soil under the residue (Ghadiri, Shea & Wicks 1984). Factors such as water rate for spraying also affect efficacy, for example, increasing total volume rates from 30 L ha⁻¹ to 150 L ha⁻¹ enhanced the efficacy of trifluralin and pyroxsulfone in controlling rigid ryegrass (Lolium rigidum Gaudin) in NT Western Australian systems (Borger et al. 2013).

The type or composition of crop residues plays an important role in their interaction with
herbicides. Dao (1991) reported that the lignin content of plant stubble may be responsible for most of its sorptive capacity while cellulose, the more abundant plant material, has little impact (Dao 1991). Furthermore, older, partially-decomposed straw appears to adsorb more herbicide than fresh straw (Unger 1994). This may be due to decomposition of cellulose and other plant elements, thereby exposing the more reactive lignin compounds (Unger 1994). In contrast, Grover (1971) found that picloram was not adsorbed on wheat stubble or cellulose but was highly adsorbed on SOM. It was proposed that physical-chemical mechanisms like the sorption of the herbicides by lignocellulose may reduce the effective solution concentration, thereby curbing bioavailability and, in turn, biodegradation (Zablotowicz, Locke & Smeda 1998). Reddy et al. (1995; 1997) showed that sorption of chlorimuron and cyanazine on hairy vetch (Vicia villosa Roth), rye (Secale cereale L.) and Italian ryegrass (Lolium multiflorum Lam., hereafter referred to as annual ryegrass) residues increased with the degree of residue decay and this was not completely reversible (Gaston, Boquet & Bosch 2001).

Walker and Crawford (1968) studied the effect of organic matter on the adsorption of triazine herbicides by soils and residue and found that fresh plant material was not particularly adsorptive. Fluometuron intercepted by the growing crop was least sorbed (sorption coefficient or KD = 11 L kg⁻¹) compared with vetch and wheat residues, where (KD = 17 L kg⁻¹) (Gaston, Boquet & Bosch 2001). Furthermore, sorption of fluometuron to cover crop residue, collected after four weeks of spraying with paraquat, was greater than sorption in the soil due to the larger surface area and number of sorption sites in crop residues (Locke, Zablotowicz & Gaston 1995).

The role of wheat residue retention on the effectiveness of acetochlor, alachlor and metolachlor was studied by Banks and Robinson (1986) who found 50% or less of the sprayed herbicides reached the soil surface with horizontal stubble amounts of 1.12 t ha⁻¹ or more. Sprinkler irrigation (13 mm) at 6.5 mm min plot⁻¹ applied by a hand-held sprinkler immediately after herbicide application enhanced the amount of herbicide washed into the soil by 15–20%. The amount of metolachlor retained on the residue was greater than that for acetochlor or alachlor. Locke et al. (1996) found alachlor sorption in Dundee soils was time-dependent and well-described by a kinetic model which allowed slowly reversible partitioning between methanol-extractable and unextractable fractions.

Bentazone sorption to soil was weak (Freundlich adsorption coefficient, Kf values were 0.15 – 0.57 µg l⁻¹ (cm³/h g⁻¹, and Freundlich exponent, n values = 0.77 – 1.11). However,
isoproturon sorption was stronger than bentazone in all studied soils \( K_f = 1.2 - 3.2 \mu g^{1-n} (cm^3)^n g^{-1}, n = 0.84 - 0.94 \) (Larsbo et al. 2009). The Freundlich parameter values for bentazone and isoproturon were similar to those reported by Gaston et al. (1996), Walker et al. (1999), and Boivin et al. (2005). Under reduced tillage (RT) with shallow cultivation depths (0–5 cm), the sorption of both bentazone and isoproturon were stronger compared with conventional treatments with greater cultivation depths (10 — 20 cm) (Larsbo et al. 2009).

Sorption of pyroxasulfone, s-metolachlor, and dimethenamid-p was investigated by Westra (2012) on 25 different soil types. The order of sorption was pyroxasulfone = dimethenamid-p < s-metolachlor with sorption coefficient values of 1.7, 2.3 and 4 kg L\(^{-1}\), respectively (Westra 2012). The lower sorption coefficient values for pyroxasulfone compared to s-metolachlor and dimethenamid-p indicates it would be more available in the soil, increasing its weed control effectiveness (Mueller & Steckel 2011; Westra 2012). SOM concentration was most closely correlated with sorption of all three herbicides across the different soils tested (Rahman 1976; Carringer, Weber & Monaco 1975; Westra 2012).

Herbicide interception is greater under CA practices. The level of interception depends on the amount and nature of crop residues, and the climatic conditions, such as application time and first rainfall. Interception of herbicides by crop residues may curb their movement into the underlying soil and relatively little is known about their transformations in residues (Zablotowicz, Locke & Smeda 1998), especially for pre-herbicides such as trifluralin, pyroxasulfone and prosulfocarb.

### 2.2.2 Herbicide movement

The movement of herbicides, which is dependent on their mobility and persistence, can be expressed in three different processes: volatilisation, leaching and runoff. A study on the transfer of atrazine from standing and flat wheat stubble showed that, three weeks after application and 50 mm of rainfall, atrazine on standing and flat residue declined by 90 and 63% respectively while the amount of herbicide in the soil doubled. Nine weeks after application, there was no atrazine left in the residue (Ghadiri, Shea & Wicks 1984). Williams and Wicks (1978) revealed that 70% of the applied atrazine reached the soil surface within the initial 90 days in an NT system when 85% of the soil surface was covered by crop residues. Moreover, the 30% of soil-applied atrazine intercepted by
stubble was lost by volatilisation or degradation and did not reach the soil (Williams & Wicks 1978). Therefore, volatilisation probably plays an important role in the fate of atrazine intercepted by retained residues (Unger 1994). Similar results were found by Banks and Robinson (1982) where most of the applied metribuzin leached from residue. Others have found interception and leaching of metribuzin from retained stubble was a critical factor affecting herbicide efficacy and concentration in the soil (Unger 1994). However, Banks and Robinson (1986) found only 15–20% of sprayed acetochlor, alachlor, and metolachlor washed off wheat residue, when 13 mm of sprinkler irrigation was applied.

Fluometuron was not immediately washed off a growing crop or vetch or wheat stubble, indicating the type of residue has some effect on how quickly herbicides can be leached from the residue (Gaston, Boquet & Bosch 2001). The quantity of fluometuron washed off the residues can be calculated from sorption coefficient (KDs). However, the increased quantity of fluometuron washed off by continued simulated rainfall indicates that sorption KDs may increase as the crop residue decomposes (Gaston, Boquet & Bosch 2001).

The degree of volatilisation losses of herbicides sprayed onto crop residue is uncertain, with few studies reported. Wet residues had lower sorption capacity for herbicides than the soil underneath (Unger 1994). Also, the capacity of a herbicide to volatilise from standing straw is relatively high (Unger 1994; Willis et al. 1983). As a result volatilisation losses were large with the combination of the standing, wet residue (Unger 1994). However, once the herbicide contacted the soil, the chance of volatilisation decreased because sorption was large, particularly in soils with more organic matter (Unger 1994). The loss of trifluralin by volatilisation from soil water was negatively correlated to its concentration, with big losses within 12-hours compared to 8-hours. Trifluralin losses by evaporation were greater from a saturated soil than from a soil at field capacity due to the availability of free liquid for evaporation, soluble trifluralin in the liquid and competition of water with herbicide for the adsorption sites (Bardsley, Savage & Walker 1968). Under dry soil, trifluralin had poor control on rigid ryegrass due to the lower incorporation of herbicide under cloddy conditions. However, at other sites in Western Australia, where the soil was sandier, trifluralin performed better under dry conditions (Minkey & Ashworth 2012). Placing trifluralin in the soil at 1.27 cm depth reduced volatilisation of the herbicide compared to applying it to the soil surface (Bardsley, Savage & Walker
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1968; Chauhan, Gill & Preston 2006). Trifluralin volatilizes with time of exposure to air and its losses increase when irradiated with mercury-vapour light or sunlight (Wright & Warren 1965). Hence, in CT systems, trifluralin is commonly applied before sowing and incorporated by a thin covering of soil by the seeder, thereby minimising exposure to sunlight and reducing losses (Carter 2000; Wright & Warren 1965).

The contrasting results of herbicide movement under CA systems may be explained by initial soil (water content and temperature) and climatic (rainfall intensity, interval between application and first rainfall) conditions which play a large role in the dynamics of water and solutes. More clarification on herbicide leaching from residues is needed. Most studies highlight the short-term and local nature of movement mechanisms, making them difficult to specify. Local research is important in developing comprehensive models for herbicide recommendations (Alletto et al. 2010).

2.2.3 Herbicide degradation

The degradation of herbicides under CA systems has been reported. The three ways that are mainly used to evaluated management impacts on herbicide degradation are: (1) in vitro laboratory studies under controlled conditions, (2) laboratory column studies using intact soil cores, and (3) field degradation studies (Locke, Zablavitowicz & Weaver 2006a).

Herbicide degradation rate is expressed by half-life per day which is the time required for the amount of herbicide to fall to half its initial value. Trifluralin can be broken down in field soils to 10–15% of original levels within 6–12 months of application (Probst et al. 1967). Trifluralin is prone to volatilization, photo-degradation and other losses when it is intercepted by residue (Chauhan, Gill & Preston 2006).

Brown et al. (1994) found that increased organic matter from either long-term NT or a hairy vetch residue had no impact on fluometuron degradation. Similarly, Brown et al. (1996) revealed that the rate of fluometuron degradation was not affected by NT or vetch residue retention under field conditions. Zablavitowicz et al. (2000) found increased fluometuron sorption and decreased degradation in NT compared with CT soils. Degradation by N-demethylation of fluometuron in ryegrass stubble can progress as quickly as in soil if there is adequate soil moisture (Zablavitowicz, Locke & Smeda 1998). Fluometuron adsorption was mainly on SOM but there was no clear impact of organic matter on the fluometuron degradation rate. Fluometuron degradation was quicker at soil pH > 6 than at pH ≤ 5 probably due to a change in microbial activity and/or microbe
population at the lower soil pH (Brown et al. 1994). Other studies found a cover crop had little or no role in the degradation rates of alachlor where the half-life in both CT and ZT was 6.5 days in the glasshouse, which was less than reported in the field (Locke, Gaston & Zablotowicz 1996). Zablotowicz et al. (1998) reported the microbial activity in crop residue, that had been killed by herbicide, was higher than in the soil underneath; however, the breakdown of 2,4-D was lower in the crop residue compared to the soil.

According to Larsbo et al. (2009), degradation of bentazone and isoproturon mostly followed first-order kinetics (non-linear regression, $R^2 > 0.92$ for all soils). Bentazone degradation was fast, with degradation rate constants of 0.046–0.25 d$^{-1}$, which mean half-lives of 2.8–15.1 days. However, degradation rate constants for isoproturon were 0.038–0.08 d$^{-1}$, which mean half-lives of 7.9 – 18.5 days (Larsbo et al. 2009). The bentazone degradation rates were higher than those reported elsewhere (Huber & Otto 1994; Bergström, Jarvis & Stenström 1994; Leistra et al. 2001; Li et al. 2008), those of isoproturon were similar to previous studies (Walker et al. 2001; Sonia Rodríguez-Cruz, Jones & Bending 2006). The degradation rates for both bentazone and isoproturon were higher in reduced-tillage at 0–5 cm depth (RT$0_{-5cm}$) compared to conventional tillage at 10–20 cm depth (CT$10_{-20cm}$) due to the higher organic matter contents in the top RT$0_{-5cm}$

Figure 2.1. The relationship between the processes conditioning the fate of pesticides in soil, water and air and the soil factors modified by tillage operations (Alletto et al. 2010)
soil than the soil from CT_{10-20} cm depths (Larsbo et al. 2009).

Investigation of pyroxasulfone and s-metolachlor degradation and movement was carried out at two field sites (Nunn fine clay loam, and Olney fine sandy loam) in northern Colorado. Half-life values ranged from 47–134 days and from 39–63 days for pyroxasulfone and s-metolachlor, respectively (Westra 2012; Westra et al. 2014).

Particular attention should be paid to the soil sampling strategy with respect to degradation of herbicides (Alletto et al. 2010). The potential for biodegradation of herbicides by crop residues and non-biological degradation e.g. photo-degradation, and the interaction between sorption and bioavailability needs to be understood to better explain the impacts of crop residue management on herbicide efficacy and environmental fate (Zablotowicz, Locke & Smeda 1998). Figure 2.1 shows the relationships between the processes conditioning the fate of pesticides in soil, water and air and the soil factors modified by tillage operations (Alletto et al. 2010).

In summary, there has been considerable research on the loss of soil–active herbicides under conventional systems, but much less so under CA systems, with high levels of crop residue, and the results available from the few studies under taken so far have been inconsistent. In addition, little work has been done under Australian conditions and that done elsewhere, under different environments, may not be valid under local situations with different crops, soils and climate (Chauhan, Gill & Preston 2006). For example, Borger et. al (2013) stated that there is a lack of information available to evaluate the effect of residue height/amount on the efficacy of trifluralin and pyroxasulfone. As crop residues are a key component of NT systems in Western Australia, further research is needed to understand the effect of residue height, quantity, orientation, physical condition, age and residue type on the three most widely used, pre-emergent herbicides (trifluralin, pyroxasulfone and prosulfocarb). It is also important to understand herbicide sorption and leaching from residues by rainfall under local conditions.

2.3 Pre-emergent herbicides

Early control of weeds is crucial for maximising crop yield because early–emergent weeds during the cropping season have the greatest competition effect on yield (Borger et al. 2010). Therefore, pre-emergent herbicides play an important role in our cropping systems. In Western Australian cropping systems, pre-emergent herbicides are sprayed onto the soil before seeding and then incorporated during the seeding operation (Haskins
Chapter 2: Review of related literature

2012; Boutsalis, Gill & Preston 2014). Due to the continued evaluation of herbicides, Australian farmers have been encouraged to adopt alternative weed management approaches, including new pre-emergent herbicides (Congreve 2015). Trifluralin, prosulfocarb and pyroxasulfone are the most commonly used pre-emergent herbicides in Western Australia in NT systems, so this review will focus on them.

Table 2.2. Properties of selected herbicides used in Australian grains production (GRDC, Pre-emergent herbicides, Fact sheet)

<table>
<thead>
<tr>
<th>Mode of action</th>
<th>Chemical name</th>
<th>Active</th>
<th>Dinitroanilines</th>
<th>Thiocarbamates</th>
<th>Isoxazolines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td></td>
<td>trifluralin</td>
<td>prosulfocarb</td>
<td>pyroxasulfone</td>
<td></td>
</tr>
<tr>
<td>Chemical name</td>
<td>α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Common trade name</td>
<td></td>
<td>Treflan®</td>
<td>Arcade®</td>
<td>Sakura®</td>
<td></td>
</tr>
<tr>
<td>Solubility1</td>
<td>0.22 (low)</td>
<td>13.2 (low)</td>
<td>3.49 (low)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volatility2</td>
<td>9.5 (volatile)</td>
<td>0.79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Persistence3</td>
<td>35-375 (av. 170)</td>
<td>7-13 (av. 10)</td>
<td>16-22 (av. 22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil Sorption4</td>
<td>13400 (very high)</td>
<td>2000 (high)</td>
<td>223 (medium)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mobility in the soil5</td>
<td>15800 (non-mobile)</td>
<td>1367-2340 (non-mobile)</td>
<td>16-119 (av. 95)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degradation6</td>
<td>181</td>
<td>12</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary breakdown pathway</td>
<td>Subject to photo-degradation and volatility loss if not incorporated. Slow microbial degradation in the soil, faster under waterlogged conditions.</td>
<td>Microbial</td>
<td>Microbial via cleavage of the methyl-sulfone bridge (Australian &amp; Veterinary Medicines 2011)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suitability for use in high stubble loads</td>
<td>Maybe (stubble will tie up products. Use higher label rates)</td>
<td>Yes (Will wash off stubble)</td>
<td>Yes (Will wash off stubble)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) is a herbicide for pre-emergent control of annual grasses (e.g. rigid ryegrass) and certain broadleaf (e.g. redroot)

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1 Solubility in water (mg/L @ 20°C)
2 Vapour pressure (mPa @ 25°C)
3 Persistence measured as average DT50 (days for 50% decomposition) under field situations.
4 Soil Sorption (Koc mL/g)
5 Mobility in the soil – propensity for soil binding using average KOC value to determine rating
6 Degradation (half-life per days)
pigweed (*Amaranthus retroflexus*) weeds in certain horticultural and agricultural crops. Trifluralin prevents the growth of emerging shoots and roots of weeds. It is a dinitroaniline herbicide in the mode of action Group D which binds to the major microtubule protein tubulin, causing a loss of microtubule structure and inhibiting mitosis. Often this can be seen as swelling/clubbing of the roots tips as the cells cannot divide or elongate (Congreve & Cameron 2014). The herbicide is most effective when incorporated into the soil or when a spray droplet directly hits a weed seed on the soil surface.

Proisulfocarb (S-benzyl dipropyl(thiocarbamate) is a residual pre-emergent herbicide for the control of rigid ryegrass and other grass and broadleaf weeds in wheat, barley, chickpeas, faba beans, field peas, lentils, lupins, and potatoes. It is a thiocarbamate herbicide in the mode of action Group J which inhibits fat synthesis within the plant, and affecting waxy cuticle formation which is important for water loss prevention from cells. Cell elongation and division are also thought to be impaired (Congreve & Cameron 2014; Abulnaja & Harwood 1991).

Pyroxsulfone  (5-(difluoromethoxy)-1-methyl-3-(trifluoromethyl)pyrazol-4-ylmethyl 4,5-dihydro-5,5-dimethyl-1,2-oxazol-3-yl sulfone. Or 3-[5-(difluoromethoxy)-1-methyl-3-(trifluoromethyl)pyrazol-4-ylmethylsulfonyl]-4,5-dihydro-5,5-dimethyl-1,2-oxazole) is a residual, soil-applied, pre-emergent herbicide for the control of rigid ryegrass, barley grass (*Hordeum leporinum*), annual phalaris (*Phalaris minor*), silver grass (*Vulpia bromoides*) and toad rush (*Juncus Bufonius*) and the suppression of certain grass weeds in wheat (not durum wheat) and triticale. Pyroxsulfone is an isoxazoline herbicide in the mode of action Group K which inhibits cell division and long chain fatty acids (Congreve & Cameron 2014; Tanetani et al. 2009). Common properties of trifluralin, prosulfocarb and pyroxsulfone (Congreve & Cameron 2014) are summarised in Table 2.2.

### 2.4 Herbicide bioassay

Chemical analysis approaches are appropriate for detecting herbicides in soil or plant residues (Szmigielski, Johnson & Schoenau 2014). However, plant bioassays can be more useful as they provide information on the bioavailability of herbicides, which may be better indicators of herbicide efficacy or residual effects (Szmigielski, Johnson & Schoenau 2014). Although there are some negative aspects of bioassays, such as variable responses with different plant species or soil type, they are commonly responsive to low
concentrations of available herbicide (Szmigielski, Johnson & Schoenau 2014). There are many bioassay procedures reported including those in soil, on leaves and petri dish assays (Camper 1986). Various plant species have been utilized as bioassay indicators. The plant species tested should be susceptible to very small amounts of herbicide present in the growth medium and reveal a gradual increase in sensitivity as the amount of herbicide is increased (Camper 1986).

Sensitive species for trifluralin bioassays include: morning-glory (Ipomoea alba L.), alfalfa (Medicago sativa L.), velvetleaf (Abutilon theophrasti Medik.), oat (Avena sativa L.), barley (Hordeum vulgare L.), cucumber (Cucumis sativus L.) (Camper 1986), sorghum (Eshel & Warren 1967) and green foxtail (Setaria viridis L.P.Beauv.) (Beckie et al. 1990). Sugar beet (Beta vulgaris L.), canola (Brassica napus L.) and oriental mustard (Brassica juncea L.) have been used as a bioassay for pyroxasulfone in soils (Szmigielski, Johnson & Schoenau 2014) while annual ryegrass has been used for prosulfocarb (Nègre et al. 2006).

2.5 Conclusion

Weed control is a major challenge to cropping in Western Australia and herbicides are one of the largest costs in broad-acre cropping systems (D’Emden, Llewellyn & Burton 2008). Crop residues are a key component of the NT cropping system and farmers are encouraged to retain as much residue on the surface as possible, both to prevent erosion and to store soil moisture. Therefore, crop residue in the NT system can be considered the interface between the soil and atmosphere and can intercept a significant amount of herbicide. Crop residues are considered detrimental to herbicide efficacy as they can intercept chemicals and prevent them from reaching their target (either the soil or emerging weeds) thereby reducing their efficacy (Sadeghi, Isensee & Shelton 1998; Locke et al. 2008). Also, a reduction in effective herbicide dose may speed the development of herbicide resistance in weeds (O’Connell & Allard 2004; Busi, Neve & Powles 2013).

Some NT systems in Australia retain all crop residues over summer, and stubble from one or two previous seasons may be present at sowing. Australian farmers are uncertain about the fate of herbicides and their performance under NT systems (Chauhan, Gill & Preston 2006). Further research is needed to clearly understand the effect of NT systems on herbicide efficacy (Chauhan, Gill & Preston 2006). Most studies in the scientific literature
are from overseas, with little work done in Australia on the persistence of herbicides under various cropping systems. Trifluralin is a widely used soil-active herbicide to control weeds in NT grain crops in Australia (Chauhan, Gill & Preston 2006). Two other pre-emergent herbicides pyroxasulfone and prosulfocarb have more recently been registered for use in Australia and are widely used to control annual ryegrass in NT cropping systems.

It is critical to know the fate of herbicides intercepted by crop residues. There is little information on the mechanisms of sorption and the subsequent release of trifluralin, pyroxasulfone and prosulfocarb from different residues of crops grown in Western Australia. As a result, it is unknown if the applied herbicide remains active on the residue, is deactivated by photo-degradation, or can be washed off into the soil with potential weed control benefits or crop phytotoxicity. The literature has no information on residue type, age or degradation status in relation to herbicide sorption and the required rainfall intensity to leach herbicides (e.g. trifluralin, pyroxasulfone, prosulfocarb) from such residue (e.g. lupin, canola, chickpea, barley), especially under the Australian rain-fed Mediterranean climate.

Therefore, the overall objective of this research was to investigate the interception of trifluralin, pyroxasulfone and prosulfocarb by different crop residue amounts, types and ages and the effect of rainfall in washing off the chemicals into the soil. Part of this research required the development of bioassays to quickly and cheaply assess the bioavailability of the three herbicides in both crop residue and soil.
Chapter 3

A bioassay for prosulfocarb, pyroxasulfone and trifluralin
detection and quantification in soil and crop residues

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Abstract

Three experiments were conducted to develop a bioassay method for assessing the bioavailability of prosulfocarb, pyroxasulfone and trifluralin in both crop residue and soil. In preliminary experiments, annual ryegrass (\textit{Lolium multiflorum} Lam.), cucumber (\textit{Cucumis sativus} L.) and beetroot (\textit{Beta vulgaris} L.) were tested as bioassay plant species for the three pre-emergent herbicides. Four growth parameters (shoot length, root length, fresh weight and dry weight) were measured for all plant species. Shoot-length inhibition was identified as the most responsive growth parameter to the herbicide application rates.
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Annual ryegrass was the most sensitive species to all tested herbicides, whereas beetroot and cucumber had lower and similar sensitivity to shoot inhibition for the three herbicides. The bioassay species performed similarly in wheat and canola residues collected a few days after harvest. In bioassay calibration experiments, dose–response curves were developed for prosulfocarb, pyroxasulfone and trifluralin in a sandy loam soil typical of the grain belt of Western Australia and with wheat residue. The developed bioassay uses ryegrass shoot inhibition for relatively low suspected concentrations of herbicide, and cucumber shoot inhibition for higher rates. The bioassay was validated by spraying the three herbicides separately onto wheat residue and soil and comparing the concentrations derived from chemical analysis with those from the bioassay. All of the linear correlations between concentrations derived from chemical analyses and the bioassays were highly significant. These results indicate that the bioassay calibration curves are suitable for estimating herbicide concentrations in crop residue collected soon after harvest and a sandy-loam soil, low in organic matter.

**Additional keywords:** conservation farming, ED50, log-logistic model, no-tillage, root length inhibition.

### 3.1 Introduction

Pre-emergent herbicides play an important role in cropping systems by providing broad-spectrum, residual control of weeds. They offer effective early weed control, often killing multiple germinations to reduce early weed competition with crops. These herbicides are applied to the soil and can be incorporated by rainfall, irrigation, tillage or, in the case of no-tillage systems, by the seeding operation (Haskins 2012; Rainbow & Derpsch 2011; Kleemann et al. 2015). Prosulfocarb (s-benzyl dipropyl thiocarbamate), pyroxasulfone (3-[5-(Difluoromethoxy)-1-methyl-3-trifluoromethyl]pyrazol-4-yl methyl sulfonyl]-4,5-dihydro-5,5-dimethyl-1,2-oxazole) and trifluralin (a,a,a-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) are the most common pre-emergent herbicides used in Western Australian in no-tillage systems (Haskins 2012; Congreve & Cameron 2014; Boutsalis, Gill & Preston 2014). They are also commonly used in agriculture worldwide (Bailly 2012; Szmigielski, Johnson & Schoenau 2014; Mangin 2016).

No-tillage has been widely adopted in southern Australian cropping systems, where wheat
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(Triticum aestivum L.), canola (Brassica napus L.) and grain legumes are the main crops (Derpsch et al. 2010; Harries, Anderson & Hüberli 2015). Crop-residue retention plays an important role in these systems from the perspectives of erosion control, improved water infiltration and building soil organic matter (Farooq et al. 2011). However, these residues can create a physical barrier that prevents the herbicides from reaching the soil (Carbonari et al. 2016). Also, the herbicides can chemically react with the residues, affecting their bioavailability (Locke & Bryson 1997; Locke, Zablotsowicz & Weaver 2006b). Therefore, it is crucial to understand the fate and efficacy of herbicides applied in no-tillage systems, especially with large amounts of surface residue.

Numerous studies have examined the movement and adsorption of herbicides in soil (Eshel & Warren 1967; Carter 2000; Locke, Zablotsowicz & Weaver 2006b; Larsbo et al. 2009; Alletto et al. 2010; Westra et al. 2014). However, few have focused on the interception or adsorption of pre-emergent herbicides on crop residues and their subsequent desorption and washing off the residue, which could impact herbicide efficacy (Banks & Robinson 1982; Banks & Robinson 1986; Ghadiri, Shea & Wicks 1984; Carbonari et al. 2016). The methods used in those studies usually involved chemical analysis of herbicide residues in the soil or crop residue, which can be complex, time-consuming and expensive, and may not relate to the impact of the herbicide on weed control. By contrast, bioassays are an important means of quantitative analysis of herbicides in soil and water (Blair & Martin 1988; Rahman, James & Mortimer 1988b; Eshel & Warren 1967; Lavy & Santelmann 1986a). Bioassays are relatively simple but require a few weeks for germination of the test-plant species (Eshel & Warren 1967). These assays can also determine the bioavailability of herbicides in soil, which is useful for agricultural research.

Bioassays have been developed for trifluralin in both water and soil media, and test species have included: morning glory (Ipomoea alba L.), lucerne (Medicago sativa L.), velvetleaf (Abutilon theophrasti Medik.), sorghum (Sorghum bicolor L.), oats (Avena sativa L.), barley (Hordeum vulgare L.), cucumber (Cucumis sativus L.) (Camper 1986) and green foxtail (Setaria viridis L. P.Beauv.) (Rahman & Ashford 1970; Jacques & Harvey 1974; Beckie et al. 1990). Bioassay methods using annual ryegrass (Lolium multiflorum Lam.) have evaluated the bioavailability of prosulfocarb in different soil
types (Nègre et al. 2006). The behaviour of pyroxasulfone in five typical Canadian prairie soils was assessed in a 7-day shoot-length bioassay with sugar beet (Beta vulgaris L.) (Szmigielski, Johnson & Schoenau 2014). However, none of these studies have evaluated herbicide activity after interception by crop residues.

The aim of the present study was therefore to develop a bioassay method for prosulfocarb, pyroxasulfone and trifluralin in both crop residues and soil. To do this, annual ryegrass, cucumber and beetroot (Beta vulgaris L.) were tested as bioassay indicators for the three pre-emergent herbicides. The bioavailability of the herbicides was compared in soil and two different crop-residue types, wheat and canola. Chemical analyses for these herbicides were used to validate the bioassay method.

### 3.2 Materials and methods

Three series of experiments were done to develop this bioassay. Initial experiments were used to select the appropriate herbicide application rates and bioassay plant species, followed by experiments to: (i) develop calibration curves for these herbicides in crop residues and soil, and (ii) validate the bioassays by comparing herbicide concentrations estimated by the bioassay with those from chemical analyses.

#### Table 3.1 Properties of the soil used in the bioassays

<table>
<thead>
<tr>
<th>pH</th>
<th>OM</th>
<th>CEC</th>
<th>Al</th>
<th>Ca</th>
<th>K</th>
<th>Mg</th>
<th>Na</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
</tr>
</thead>
<tbody>
<tr>
<td>(CaCl₂)</td>
<td>(%)</td>
<td>(cmol/kg)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(cmol/kg)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>4.4</td>
<td>1.8</td>
<td>2.96</td>
<td>0.02</td>
<td>2.75</td>
<td>0.14</td>
<td>0.45</td>
<td>0.07</td>
<td>74</td>
<td>12</td>
<td>14</td>
</tr>
</tbody>
</table>

#### 3.2.1 Preliminary experiments

The preliminary bioassay tested the three herbicides prosulfocarb (Arcade, 800 g a.i. L⁻¹; Syngenta Crop Protection, Postfach, Switzerland), pyroxasulfone (Sakura, 850 g a.i. kg⁻¹; Bayer Crop Science, Leverkusen, Germany) and trifluralin (Treflan, 480 g a.i. L⁻¹; Dow AgroSciences, Indianapolis, IN, USA) in three types of growth media (soil, wheat residue and canola residue). Three plant species were used: herbicide susceptible annual ryegrass, cucumber, and beetroot. Sandy loam soil was collected from the soil surface (0–10 cm) of a farmer’s field near Cunderdin, Western Australia (31.5842°S, 117.327038°E). The soil was air-dried and passed through a 2-mm sieve prior to analysis by Soil Science
Laboratories of The University of Western Australia and CSBP Soil and Plant Laboratory (www.csbp-fertilisers.com.au) for texture, pH (CaCl2), cation exchange capacity and organic carbon (Rayment & Lyons 2011) (Table 3.1). Dry wheat and canola residues were collected shortly after harvest from the same Cunderdin field site. After being sprayed with herbicide, as described below, the residue was immediately ground into small particles by using a mechanical plant material grinder (Retsch, Haan, Germany).

### 3.2.1.1 Experimental design and management

The experiments were conducted in a randomised complete block (RCB) design and with four replications of six herbicide application rates × three bioassay species × three media. Plastic trays of dimensions 340 mm by 285 mm by 50 mm, with no holes, were used to hold the crop residues or soil, with the latter in Petri dishes placed in the trays. The trays were uniformly covered by 39 g unground crop residue (wheat or canola), equivalent to a coverage (by area) of ~4 t ha⁻¹, or they contained four Petri dishes (9 cm in diameter), each with 50 g dry soil. Commercial formulations of the three herbicides were applied to the trays containing the residue or soil by using a twin nozzle laboratory sprayer fitted with 1108 01 flat-fan spray jets (TeeJet Technologies, Glendale Heights, IL, USA) delivering herbicide in 117.1 L ha⁻¹ of water at 210 kPa, travelling at 3.6 km h⁻¹. The herbicides were tested at rates above and below recommended field rates in Western Australia, which were 2000, 100 and 960 g a.i. ha⁻¹ for prosulfocarb, pyroxasulfone and trifluralin, respectively (Boutsalis et al. 2014) (Table 3.2).

<table>
<thead>
<tr>
<th></th>
<th>Application rates</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CP L ha⁻¹</td>
<td>CP g ha⁻¹</td>
<td>CP L ha⁻¹</td>
</tr>
<tr>
<td></td>
<td>a.i. g ha⁻¹</td>
<td>a.i. g ha⁻¹</td>
<td>a.i. g ha⁻¹</td>
</tr>
<tr>
<td><strong>Proslufocarb</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>400</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>800</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1600</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2400</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3200</td>
<td>240</td>
</tr>
<tr>
<td><strong>Pyroxasulfone</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>26</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>51</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>102</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>180</td>
<td>204</td>
</tr>
<tr>
<td><strong>Trifluralin</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>300</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>600</td>
<td>1200</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1200</td>
<td>1800</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2400</td>
<td>2400</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After herbicide application, the crop residues were ground, as described previously, placed in plastic bags and stored for a few days at ~20°C until used in the bioassays. To minimise sample contamination, the grinder was cleaned after each batch, using a vacuum and then an air compressor. The Petri dishes with soil were covered and stored at ~20°C.
For the bioassay, residue (5 g) or soil (50 g) was placed in a Petri dish and then in a controlled environment room. Six seeds from one species were placed 1 cm deep in the media (soil or residue) in each dish, and the dishes placed onto shelves equipped with cool white fluorescent lamps (LUMILUX Model L36W/840; OSRAM, Munich, Germany). Photosynthetically active radiation at the top of the Petri dishes was 109±5 µmolm⁻²s⁻¹ with a 12-h photoperiod. Room air temperature was maintained at 25±2°C (light) and 22.5±1°C (dark), and the relative humidity was 70±10%. The plants were hand-watered on a daily basis by adjusting soil or residue moisture to near field capacity (Somasegaran & Hoben 1985) with fresh deionised water.

The seedlings were harvested 7 days after sowing and the soil or crop residue was washed away with running tap water. Shoot length, root length, and whole plant fresh and dry weight were measured, and percentages shoot and root length inhibition calculated from the untreated control for each media using the following formula (Szmigielski, Johnson & Schoenau 2014):

\[
\text{Inhibition} \% = (1 - \frac{L_t}{L_0}) \times 100\%
\]  

[1]

where \(L_t\) is the shoot or root length measured in the herbicide treated soil or crop residue and \(L_0\) is the shoot or root length in the untreated soil or crop residue. The GI50 (i.e. 50% growth inhibition relative to the untreated control) was calculated by using the log-logistic method described below (see Data analyses).

### 3.2.2 Calibration experiments

Calibration experiments were conducted with the same three pre-emergent herbicides applied at seven rates (Table 3.3), based on the preliminary experiments, to create suitable dose–response curves for bioassays of herbicide concentration in a sandy loam soil (typical of the grain belt of Western Australia) and on crop residue (which would be collected and ground for the bioassay). Each herbicide was tested in a separate experiment, and the rates of each herbicide applied to the soil and crop residue varied. For these calibration experiments, a known amount of herbicide was added, using a pipette, to each Petri dish, which contained either 50 g soil or 5 g ground wheat residue. The herbicides were not sprayed onto whole residue because it would be difficult to determine how much was intercepted by the residue. Annual ryegrass and cucumber were
used as indicator plants for the tested herbicides, with five seeds of the same species planted 1 cm deep in each Petri dish. There were four replications of each treatment (herbicide rates × media) arranged in a RCB design. The Petri dishes were placed in the controlled environment room, and the procedure described for the preliminary experiments was followed.

Table 3.3 The rates of three pre-emergent herbicides (active ingredient) added to Petri dishes containing soil or ground wheat residue in the calibration experiments.

<table>
<thead>
<tr>
<th></th>
<th>Soil (AR) †</th>
<th>Soil (CU) †</th>
<th>Wheat residue (AR) †</th>
<th>Wheat residue (CU) †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prosulfocarb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 g ha⁻¹</td>
<td>0 mg kg⁻¹</td>
<td>0 mg kg⁻¹</td>
<td>0 g ha⁻¹</td>
<td>0 mg kg⁻¹</td>
</tr>
<tr>
<td>16</td>
<td>0.2 g ha⁻¹</td>
<td>0.3 g ha⁻¹</td>
<td>39 g ha⁻¹</td>
<td>5 mg kg⁻¹</td>
</tr>
<tr>
<td>24</td>
<td>0.3 g ha⁻¹</td>
<td>3.0 g ha⁻¹</td>
<td>79 g ha⁻¹</td>
<td>10 mg kg⁻¹</td>
</tr>
<tr>
<td>39</td>
<td>0.5 g ha⁻¹</td>
<td>6.0 g ha⁻¹</td>
<td>197 g ha⁻¹</td>
<td>25 mg kg⁻¹</td>
</tr>
<tr>
<td>118</td>
<td>1.5 g ha⁻¹</td>
<td>12 g ha⁻¹</td>
<td>393 g ha⁻¹</td>
<td>50 mg kg⁻¹</td>
</tr>
<tr>
<td>236</td>
<td>3.0 g ha⁻¹</td>
<td>18 g ha⁻¹</td>
<td>786 g ha⁻¹</td>
<td>100 mg kg⁻¹</td>
</tr>
<tr>
<td>472</td>
<td>6.0 g ha⁻¹</td>
<td>24 g ha⁻¹</td>
<td>1062 g ha⁻¹</td>
<td>135 mg kg⁻¹</td>
</tr>
<tr>
<td>Pyroxasulfone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 g ha⁻¹</td>
<td>0 mg kg⁻¹</td>
<td>0 mg kg⁻¹</td>
<td>0 g ha⁻¹</td>
<td>0 mg kg⁻¹</td>
</tr>
<tr>
<td>0.8</td>
<td>0.01 mg kg⁻¹</td>
<td>0.1 mg kg⁻¹</td>
<td>0.4 g ha⁻¹</td>
<td>0.05 mg kg⁻¹</td>
</tr>
<tr>
<td>2.0</td>
<td>0.02 mg kg⁻¹</td>
<td>0.2 mg kg⁻¹</td>
<td>0.8 g ha⁻¹</td>
<td>0.1 mg kg⁻¹</td>
</tr>
<tr>
<td>3.0</td>
<td>0.04 mg kg⁻¹</td>
<td>0.3 mg kg⁻¹</td>
<td>4.0 g ha⁻¹</td>
<td>0.5 mg kg⁻¹</td>
</tr>
<tr>
<td>6.0</td>
<td>0.08 mg kg⁻¹</td>
<td>0.4 mg kg⁻¹</td>
<td>8.0 g ha⁻¹</td>
<td>1.0 mg kg⁻¹</td>
</tr>
<tr>
<td>8.0</td>
<td>0.10 mg kg⁻¹</td>
<td>0.6 mg kg⁻¹</td>
<td>12 g ha⁻¹</td>
<td>1.5 mg kg⁻¹</td>
</tr>
<tr>
<td>9.0</td>
<td>0.12 mg kg⁻¹</td>
<td>0.8 mg kg⁻¹</td>
<td>16 g ha⁻¹</td>
<td>2.0 mg kg⁻¹</td>
</tr>
<tr>
<td>Trifluralin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 g ha⁻¹</td>
<td>0 mg kg⁻¹</td>
<td>0 mg kg⁻¹</td>
<td>0 g ha⁻¹</td>
<td>0 mg kg⁻¹</td>
</tr>
<tr>
<td>16</td>
<td>0.2 mg kg⁻¹</td>
<td>4.0 mg kg⁻¹</td>
<td>16 g ha⁻¹</td>
<td>2.0 mg kg⁻¹</td>
</tr>
<tr>
<td>79</td>
<td>1.0 mg kg⁻¹</td>
<td>6.0 mg kg⁻¹</td>
<td>47 g ha⁻¹</td>
<td>6.0 mg kg⁻¹</td>
</tr>
<tr>
<td>315</td>
<td>4.0 mg kg⁻¹</td>
<td>8.0 mg kg⁻¹</td>
<td>94 g ha⁻¹</td>
<td>12 mg kg⁻¹</td>
</tr>
<tr>
<td>629</td>
<td>8.0 mg kg⁻¹</td>
<td>10 mg kg⁻¹</td>
<td>550 g ha⁻¹</td>
<td>70 mg kg⁻¹</td>
</tr>
<tr>
<td>786</td>
<td>10 mg kg⁻¹</td>
<td>12 mg kg⁻¹</td>
<td>865 g ha⁻¹</td>
<td>110 mg kg⁻¹</td>
</tr>
<tr>
<td>1180</td>
<td>15 mg kg⁻¹</td>
<td>15 mg kg⁻¹</td>
<td>1494 g ha⁻¹</td>
<td>190 mg kg⁻¹</td>
</tr>
</tbody>
</table>

†Soil (AR) = Annual ryegrass as a bioassay indicator grown in soil, Soil (CU) = Cucumber as a bioassay indicator grown in soil, Wheat residue (AR) = Annual ryegrass as a bioassay indicator grown in ground wheat residue, Wheat residue (CU) = Cucumber as a bioassay indicator grown in ground wheat residue.

3.2.3 Chemical analysis and bioassay validation

The third set of experiments consisted of the same three pre-emergent herbicides applied at seven rates (Table 3.4). Each herbicide was tested in a separate experiment. The spray application procedure of the experiments was similar to that used in the preliminary
experiment, with herbicides sprayed over trays either containing Petri dishes with 50 g soil or covered with whole wheat residue at a rate equivalent to ~4 t ha\(^{-1}\) (39 g per tray). There were four replications of each treatment (herbicide rate \times\) media) arranged in RCB design. In total, eight Petri dishes for each dose of each herbicide with soil were used (four Petri dishes for chemical analyses and four Petri dishes for the bioassay), and after spraying the herbicide, half of them were sent directly to the Separation Science and Metabolomics Laboratory (SSML), Murdoch University, where they were stored at -40°C until the chemical analysis was done. The other half were stored at -20°C until used for the bioassays. For the residue, the material was separated into two, with half placed in UV-stabilised plastic bags and sent directly to SSML to be stored at -40°C for later chemical analysis. The other half of the wheat residue was ground, placed into plastic bags and stored at -20°C until used for the bioassays.

For chemical analyses, herbicides were extracted by using a buffered QuEChERS sample preparation adapted from Lehotay et al. (2005), with samples of 10 g soil and 2.5 g homogenised crop residue. Quantitation of analytes was achieved by using a Bruker 451-GC interfaced with a Bruker Scion triple quadrupole mass spectrometer and a CP-8400 auto-sampler (Bruker Daltonics, Bremen, Germany). Chromatographic separation was achieved with a Restek Rtx-5MS 5% diphenyl and 95% dimethylpolysiloxane capillary column, 30m by 0.25mm ID, protected by a 10-m Integra-Guard column (Restek, Bellefonte, PA, USA). Column temperature was initially held at 80°C for 3 min and increased to 150°C at a rate of 30°C min\(^{-1}\). The temperature was further increased to 300°C at a rate of 50°C min\(^{-1}\) with a final hold for 12 min. Ultra-high purity (UHP) helium was the carrier gas at a flow rate of 1.1mL min\(^{-1}\). For tandem mass spectrometry (MS/MS), UHP argon was used as the collision gas. The mass spectrometer was operated in electron ionisation mode at ~70 eV and the temperatures of the transfer line and source were 250°C and 200°C, respectively. Data acquisition and processing were performed using MS Workstation version 8.0 (Bruker Daltonics). Results were calculated by using matrix matched calibration standards. Acenaphthene-d10, chrysene-d12 and phenanthrene-d10 were used as the extraction efficiency internal standards and triphenyl phosphate was used as the instrument performance standard.
For the bioassay, annual ryegrass and cucumber were used as indicator plants for all tested herbicides, and 10 seeds, five of each species, were planted 1 cm deep in each dish, containing either 50 g soil or 5 g wheat residue. The shoot and root length and wet and dry mass were determined after 7 days as previously described.

### 3.2.4 Data analyses

Statistical analyses of the data from preliminary experiments were done using GENSTAT 12th Edn (VSN International, Hemel Hempstead, UK), and the data were tested for the assumptions of normality and homogeneity of variances. An exponential curve (asymptotic regression) was fitted for seedling shoot or root length or dry weight inhibition (Y):

\[
Y = A + B (R^X) \tag{2}
\]

where A is the plateau that the curve approaches as X increases, A + B is associated with the intercept, R corresponds to the rate of decrease and X is the herbicide dose.

Shoot length, which was the only measurement presented for the calibration and validation experiments, was regressed against herbicide dose, with the lower limit being zero, using a three-parameter log-logistic model as suggested by (Knezevic, Streibig & Ritz 2007):

\[
Y = D / (1 + \exp[B(\log X - \log E)]) \tag{3}
\]

where Y is the response (shoot length), D is the upper limit, B is the relative slope of the line around E, which is the effective dose that provides 50% weed control (also known as ED\textsubscript{50}), and X is the herbicide dose. The upper limit, D, corresponds to the response of the
Figure 3.1 Shoot length inhibition of annual ryegrass, beetroot and cucumber grown for seven days in response to different application rates (mg a.i. kg\(^{-1}\)) of prosulfocarb (a, d), pyroxasulfone (b, e) and trifluralin (c, f) in soil (a – c) and wheat residue (d – f). Bars are SE of the mean (n=4).
untreated control. The analysis of dose–response curves and ED$_{50}$ values was performed separately for each herbicide by using the open-source statistical software R 3.3.0 (R Foundation for Statistical Computing, Vienna) and drc package as described by (Knezevic, Streibig & Ritz 2007; Ritz, Kniss & Streibig 2015). For the validation experiments, Pearson’s correlation coefficient was used to assess the strength of the linear relationship between herbicide concentration determined by chemical analysis and that estimated by bioassay. The data were log10-transformed before analysis, to satisfy the assumption of bivariate normal distribution, and the null hypothesis of no correlation was tested. Regression confidence intervals (95%) were used to assess whether the slopes were different from one and the intercepts different from zero. For the bioassay, annual ryegrass was used to estimate the herbicide concentrations, except when the plants died and the cucumber data were used.

**3.3 Results and discussion**

A significant amount of pre-emergent herbicide can be intercepted by crop residue on the soil surface in no-tillage systems, which affects the efficacy of the herbicide. Therefore, it is important to measure how much herbicide is intercepted by the residues and whether some of the chemical is leached onto the soil by rainfall. To do this, we developed bioassays to measure a range of concentrations of pyroxasulfone, prosulfocarb and trifluralin in both soil and crop residues.

**3.3.1 Preliminary experiments**

Of the four plant parameters, shoot length and root length were more responsive to the herbicides than fresh weight (data not shown) and dry weight (Figures 3.1–3.3), which agrees with previous findings (Beckie et al. 1999; Nègre et al. 2006; Szmigielski, Johnson & Schoenau 2014). After 7 days, no significant differences were observed between the two crop-residue media (wheat and canola residues) for the species tested. Therefore, only data from the soil and the wheat residue were plotted.
Figure 3. 2 Root length inhibition of annual ryegrass, beetroot and cucumber grown for seven days in response to different application rates (mg a.i. kg\(^{-1}\)) of prosulfocarb (a, d), pyroxasulfone (b, e) and trifluralin (c, f) in soil (a – c) and wheat residue (d – f). Bars are SE of the mean (n=4).
Although the two residue types showed similar responses to the three herbicides tested, the material used was collected a few days after harvest. It is expected that decayed or aged crop residue would show increased sorption of herbicides, as the more ‘labile’ fractions such as cellulose decomposed, leaving the more recalcitrant lignin, which has been associated with the retention of herbicides, e.g. metribuzin in wheat straw (Dao 1991). In addition, the different physical structure of wheat straw and canola straw collected soon after harvest may alter the efficacy in the field. For equivalent weight of straw, wheat is likely to intercept more herbicide than canola owing to the finer material—stems associated with wheat residues. In addition, residue that has been trampled (horizontal) is likely to intercept more herbicide than standing residue and this would reduce herbicide efficacy.

In most combinations of germination media and herbicide, the responses of shoot and root length were similar (Figures 3.1 and 3.2), except for prosulfocarb with beetroot, where shoot length inhibition was much greater than root inhibition for both soil and wheat residue (Figure 3.1a, d vs Figure 3.2a, d, respectively). In addition, for trifluralin with cucumber, shoot length inhibition was greater than root inhibition for wheat residue. Therefore, shoot length was selected as the most sensitive parameter to assess herbicide activity in subsequent experiments. Nègre et al. (2006) reported significant inhibition of annual ryegrass shoot length at lower concentrations of prosulfocarb (0.1–0.25 mg a.i. L⁻¹) compared with root length (0.5 mg a.i. L⁻¹). Similarly, for pyroxasulfone, Szmigielski et al. (2014) reported greater shoot length inhibition (65%) than root-length inhibition (40%) in 7-day old sugar beet. Beckie et al. (1990) also found that shoots were more susceptible than roots to trifluralin. Moreover, the sensitivity of shoot growth to trifluralin compared with root growth is due to sensitivity of the coleoptilar node, as demonstrated in green foxtail (another grass weed) (Rahman & Ashford 1970; Appleby & Bernal 1989).

On the other hand, dry-weight inhibition (Figure 3.3) of the three plant species in the bioassay showed more variation between the combinations of plant species and media. For prosulfocarb, dry-weight inhibition was less than shoot inhibition in all species (Figure 3.3 vs Figure 3.1). For pyroxasulfone, dry-weight inhibition was less than shoot- and root-length inhibition for wheat residue, and the difference was smaller for soil. For trifluralin, dry-weight inhibition was markedly less than root- and shoot-length inhibition.
Figure 3. Dry weight inhibition of annual ryegrass, beetroot and cucumber grown for seven days in response to different application rates (mg a.i. kg\(^{-1}\)) of prosulfocarb (a, d), pyroxasulfone (b, e) and trifluralin (c, f) in soil (a – c) and wheat residue (d – f). Bars are SE of the mean (n=4).
in beetroot and cucumber. Thus, for the three species tested in the bioassay, dry-weight inhibition was not as useful as shoot- or root-length inhibition in assessing the bioavailability of prosulfocarb, pyroxasulfone and trifluralin in soil and wheat residue (Figures 3.1–3.3).

Annual ryegrass was the most sensitive of the test species to the herbicides tested; beetroot and cucumber had lower and similar sensitivities with regard to shoot inhibition from the three herbicides (Figure 3.1). Likewise, root length was inhibited more in annual ryegrass than in beetroot and cucumber from the addition of all herbicides (Figure 3.2).

For annual ryegrass, prosulfocarb, pyroxasulfone and trifluralin caused 100% shoot- and root-length inhibition, relative to the control, at application rates of 41, 0.7 and 23 mg a.i. kg⁻¹ in soil and 784, 12.5 and 146 mg a.i. kg⁻¹ in wheat residue, respectively. Therefore, annual ryegrass was controlled in soil at application rates only 5%, 6% and 16% of those needed in wheat residue, for prosulfocarb, pyroxasulfone and trifluralin, respectively. Annual ryegrass shoot and root length were most sensitive to pyroxasulfone, moderate to trifluralin and least sensitive to prosulfocarb (Figures 3.1–3.2). When dry weight was measured, annual ryegrass still was most sensitive to pyroxasulfone, but least sensitive and with a more variable response to prosulfocarb (Figure 3.3).

None of the herbicide rates caused 100% shoot inhibition for cucumber or beetroot, with inhibition reaching a plateau of ~60% at the highest rates tested, except for pyroxasulfone in soil, where beetroot showed ~80% shoot inhibition (Figure 3.1). For root length, inhibition varied from >10% to 60% for beetroot and from 20% to 70% for cucumber at the highest rates tested (Figure 3.2). For dry weight of beetroot and cucumber, inhibition ranged from 10% to 50% at the highest rates tested (Figure 3.3).

For the subsequent calibration experiments, annual ryegrass was selected as the bioassay species for lower rates of herbicide and cucumber for higher rates at which no annual ryegrass survived. Wheat was selected as the crop residue type. Based on the previous results, herbicide rates were adjusted and the maximum rate for the annual ryegrass bioassay was set to the rate at which 100% inhibition occurred in that species. For the cucumber bioassay, although 100% inhibition was not achieved for any herbicide in the preliminary experiment, the maximum rates were kept close to recommended field rates.
Figure 3.4 Herbicide dose–response curves in soil (black and red) and wheat residue (green and blue) determined in the seven day annual ryegrass [a1-c1: black and green] and cucumber [a2-c2: red and blue] shoot length bioassay. a1-a2) prosulfocarb, b1-b2) pyroxasulfone, and c1-c2) trifluralin. The regression lines were calculated using equation 3 and the parameter values are shown in Table 3.5.
Chapter 3: A bioassay for prosulfocarb, pyroxasulfone and trifluralin

3.3.2 Calibration experiments

As expected, shoot length decreased with increasing herbicide concentration (Figure 3.4). When the data were fitted using Equation 3, a lack-of-fit (95%) test was not significant for any of the curves, indicating that the three-parameter log-logistic model was appropriate; therefore, regression parameters with ED50 values (50% control) were determined (Table 3.5, Figure. 3.4).

The ED50 values varied with germination media and bioassay species (Table 3.5). The ED50 for soil was always markedly less than for wheat residue, by a factor of between 17 (pyroxasulfone with cucumber) and 150 (trifluralin with cucumber), therefore showing greater herbicide phytotoxicity at equivalent herbicide rates. This is to be expected because of the organic nature of the wheat residue. For example, Knezevic et al. (2009) found that soils with more organic matter were more likely to adsorb the chemical (KIH-485/pyroxasulfone) than those with less organic matter. Similarly, Mitra et al. (1999) showed that sorption of isoxaflutole in five soils increased with increasing organic matter.

Phytotoxicity of all herbicides against annual ryegrass was always greater than for cucumber. Vera et al. (2001) and Nègre et al. (2006) reported annual ryegrass control at very low concentrations of prosulfocarb (0.5 mg L\(^{-1}\)). In the present study, pyroxasulfone was always more phytotoxic than prosulfocarb at equivalent rates, and in soil, this was 16-fold for annual ryegrass and 17-fold higher for cucumber. Pyroxasulfone was 20-fold more phytotoxic than trifluralin to annual ryegrass in soil, but trifluralin was slightly more phytotoxic for cucumber in soil (Table 3.5). Boutsalis et al. (2014) reported 98% control of rigid ryegrass with pre-emergent applications of pyroxasulfone, compared with 59% with prosulfocarb and 39% with trifluralin. The effectiveness of pyroxasulfone in controlling L. rigidum was also reported by Walsh et al. (2011).

3.3.3 Chemical analyses and bioassay validation

It was difficult to obtain suitable soil and residue ‘blank’ samples for the chemical analyses, because these herbicides appeared to be ubiquitous in the field. Untreated soil and stubble samples were initially screened, and the soil samples with the lowest baseline level of the three herbicides were then selected as blanks for a matrix-matched standard generation. Chemical analyses of the control soil samples showed a background level of
trifluralin \((0.08 \text{ mg kg}^{-1})\) and prosulfocarb \((0.08 \text{ mg kg}^{-1})\), with a lower concentration of pyroxasulfone \((0.008 \text{ mg kg}^{-1})\). For the stubble, control samples showed low concentrations for pyroxasulfone \((0.03 \text{ mg kg}^{-1})\) and trifluralin \((0.03 \text{ mg kg}^{-1})\) with a higher level observed of prosulfocarb \((0.7 \text{ mg kg}^{-1})\) (Table 3.6).

Table 3. 5 Regression parameters for the three parameter log-logistic model \((Y = D/1 + \exp [B(\log X \ - \ \log E)])\) for shoot length \((Y)\) against the log of herbicide rate, for combinations of soil or wheat residue and the test species of annual ryegrass or cucumber. Values in brackets are ± SE \((n=4)\). See also Figure 3.4.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Herbicide</th>
<th>Regression Parameters†</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil/ryegrass</td>
<td>Prosulfocarb</td>
<td>0.84 (0.09)</td>
<td>47.71 (2.02)</td>
<td>0.48 (0.07)</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>Pyroxasulfone</td>
<td>1.09 (0.14)</td>
<td>60.14 (2.85)</td>
<td>0.03 (0.00)</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>Trifluralin</td>
<td>0.96 (0.11)</td>
<td>57.08 (2.63)</td>
<td>0.60 (0.13)</td>
<td>0.79</td>
</tr>
<tr>
<td>Soil/cucumber</td>
<td>Prosulfocarb</td>
<td>0.56 (0.13)</td>
<td>32.69 (2.15)</td>
<td>3.81 (1.51)</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Pyroxasulfone</td>
<td>0.73 (0.25)</td>
<td>31.22 (2.84)</td>
<td>0.23 (0.08)</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>Trifluralin</td>
<td>0.92 (0.30)</td>
<td>33.60 (2.44)</td>
<td>8.34 (1.78)</td>
<td>0.81</td>
</tr>
<tr>
<td>Wheat residue/ryegrass</td>
<td>Prosulfocarb</td>
<td>0.86 (0.07)</td>
<td>71.55 (1.98)</td>
<td>10.59 (1.09)</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>Pyroxasulfone</td>
<td>0.78 (0.08)</td>
<td>69.35 (2.88)</td>
<td>0.23 (0.04)</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>Trifluralin</td>
<td>1.01 (0.14)</td>
<td>52.60 (2.69)</td>
<td>13.24 (2.63)</td>
<td>0.86</td>
</tr>
<tr>
<td>Wheat residue/cucumber</td>
<td>Prosulfocarb</td>
<td>0.51 (0.26)</td>
<td>35.81 (2.31)</td>
<td>571.54 (408.44)</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>Pyroxasulfone</td>
<td>0.36 (0.10)</td>
<td>36.06 (2.81)</td>
<td>4.01 (1.63)</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>Trifluralin</td>
<td>0.38 (0.15)</td>
<td>36.06 (2.58)</td>
<td>159.26 (78.89)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

†B = slope of the curve around \(ED_{50}\) values, \(D = \) upper limit of the curve (average level in untreated control), and \(E = ED_{50}\), the herbicide concentration corresponding to 50% inhibition. *\(R^2\): these values were calculated by plotting shoot length \((Y)\) against herbicide rate \((X)\) using equation (2).

All of the linear correlations between concentrations derived from chemical analyses and the bioassays

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Herbicide</th>
<th>Regression Parameters†</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Prosulfocarb</td>
<td>Application rate ((\text{g ha}^{-1}))</td>
<td>0</td>
<td>64</td>
<td>128</td>
<td>248</td>
</tr>
<tr>
<td></td>
<td>Detected ((\text{mg kg}^{-1}))</td>
<td>Soil</td>
<td>0.08</td>
<td>0.4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wheat residue</td>
<td>0.7</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Pyroxasulfone</td>
<td>Application rate ((\text{g ha}^{-1}))</td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Detected ((\text{mg kg}^{-1}))</td>
<td>Soil</td>
<td>0.008</td>
<td>0.03</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wheat residue</td>
<td>0.03</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Trifluralin</td>
<td>Application rate ((\text{g ha}^{-1}))</td>
<td>0</td>
<td>30</td>
<td>60</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Detected ((\text{mg kg}^{-1}))</td>
<td>Soil</td>
<td>0.08</td>
<td>0.3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wheat residue</td>
<td>0.03</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>
were highly significant, ranging from $P < 0.008$ for trifluralin in soil to $P < 0.001$ for the remaining correlations. The correlation coefficients ranged from 0.54 for trifluralin in soil to 0.93 for trifluralin in residue (Table 3.7). The weakness of the correlation coefficient ($r = 0.54$) between the bioassay and chemical analysis for trifluralin for the soil was possibly due to variability as a result of the volatile nature of trifluralin, especially compared with other two herbicides. Grass et al. (1994) and Bedos et al. (2006) reported that 64–96% of trifluralin was volatilised within the first 48 h after spraying herbicide. The intercepts were not significantly different from zero and the slopes were not significantly different from unity, except for trifluralin in soil, where the 95% confidence intervals for the slope were 0.17 and 0.96.

These results indicate that the bioassay calibration curves are suitable for estimating herbicide concentrations in crop residue collected soon after harvest and in a relatively sandy soil low in organic matter. Older residue, or soils with more clay and/or organic matter, may have different adsorption characteristics (Szmigielski, Johnson & Schoenau 2014; Dao 1991), and therefore, appropriate bioassay response curves will be required.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Media</th>
<th>Coefficient</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prosulfocarb</td>
<td>Soil</td>
<td>0.91</td>
<td>0.79 (-0.21)</td>
<td>1.19 (0.08)</td>
</tr>
<tr>
<td></td>
<td>Wheat residue</td>
<td>0.73</td>
<td>0.57 (-0.14)</td>
<td>1.09 (0.69)</td>
</tr>
<tr>
<td>Pyroxasulfone</td>
<td>Soil</td>
<td>0.85</td>
<td>0.78 (-0.26)</td>
<td>1.36 (0.34)</td>
</tr>
<tr>
<td></td>
<td>Wheat residue</td>
<td>0.89</td>
<td>0.73 (-0.08)</td>
<td>1.15 (0.21)</td>
</tr>
<tr>
<td>Trifluralin</td>
<td>Soil</td>
<td>0.54</td>
<td>0.17 (-0.01)</td>
<td>0.96 (0.52)</td>
</tr>
<tr>
<td></td>
<td>Wheat residue</td>
<td>0.93</td>
<td>0.82 (-0.30)</td>
<td>1.15 (0.22)</td>
</tr>
</tbody>
</table>

Table 3.7 Pearson correlation coefficients between herbicide concentrations derived from chemical analysis and that from bioassay for the validation experiment, along with 95% confidence intervals for the slope and intercept (brackets are intercept intervals).

A bioassay was developed for assessing herbicide bioavailability in both crop residues and sandy loam soil for the main pre-emergent herbicides used in Western Australian no-tillage systems. This will be particularly useful for investigating the impact of stubble condition on the interception and efficacy of pre-emergent herbicides in no-tillage systems, because stubble can prevent the herbicides from reaching the soil (Carbonari et al. 2016). The bioassay could be used where concentrations of herbicide are suspected to be relatively low, through to about twice the recommended field rates.
Rainfall leaching herbicide from wheat residue into the soil is greatest with pyroxasulfone and least with trifluralin

Abstract

No-tillage (NT) with stubble retention is a widely used cropping system for soil conservation and yield benefits. The NT farming system in southern Australia relies heavily on herbicides for weed management, but heavy crop residues may have a negative impact on the activity of pre-emergent herbicides applied. Any herbicide intercepted by the crop residue may not reach the soil surface without timely rainfall and may dissipate due to volatilisation, photo-degradation and/or microbial activity.

Two experiments were carried out to investigate the interception of prosulfocarb, pyroxasulfone, and trifluralin herbicide by wheat residue and retention following simulated rainfall. The first experiment had four simulated rainfall amounts (0, 5, 10, and 20 mm), three intensities (5, 10, and 20 mm h\(^{-1}\)) and five application times (immediately after spraying, 6 h, 1, 7, and 14 days after spraying). In the second experiment, 20 mm of rainfall was applied at 10 mm h\(^{-1}\) in either 4 × 5 mm rainfall events over two days, 2 × 10 mm rainfall events over one day, or a single 20 mm rainfall event, with a no-rainfall control treatment. Bioassays were used to assess the herbicide activity/availability in the soil and remaining on the residue, using cucumber and annual ryegrass as indicator plants.

At higher rainfall amounts, most of the herbicide leached from the stubble into the soil with rainfall soon after chemical application, more so with rain in one event rather than multiple events. However, the intensity of rainfall had no effect. Pyroxasulfone leached easily from the residue to the soil with rainfall applied up to 14 days after spraying to potentially offer good weed control, prosulfocarb had an intermediary leaching effect up to seven days, while only a small amount of trifluralin leached from stubble after rain.

**Keywords:** No-tillage, Pre-emergent herbicide, Rainfall simulation, Bioassay species, herbicide interception.
Chapter 4: Rainfall leaching herbicide from wheat residue

4.1. Introduction

Conservation agriculture, with minimal or no soil tillage and maximum residue retention, known as no-tillage (NT) with residue retention, is widely practised in Australia and many regions around the world (Verhulst et al. 2010; Kassam et al. 2012; Serraj & Siddique 2012). The NT system is heavily dependent on herbicides for weed control (Nichols et al. 2015), but heavy crop residues may have a negative impact on the activity of these herbicides (Carbonari et al. 2016). Herbicides can be intercepted by the crop residue (Ghadiri, Shea & Wicks 1984; Gaston, Boquet & Bosch 2001; Chauhan, Gill & Preston 2006; Selim, Zhou & Zhu 2003) and may not reach the soil surface without timely rainfall to wash them off the crop residues into the soil below, where they will be available to control germinating weeds (Banks & Robinson 1982; 1986; Ghadiri, Shea & Wicks 1984; Reddy et al. 1995; Shaner 2013; Carbonari et al. 2016). For example, crop residues have been shown to intercept 15–80% of the applied herbicide, which may account for the reduction in herbicide effectiveness sometimes observed in NT systems (Chauhan, Gill & Preston 2006). The herbicide remaining on the residue may also dissipate due to volatilisation, photo-degradation and/or microbial degradation (Congreve 2015; Aslam et al. 2015). The rainfall amount and intensity can significantly impact herbicide wash-off from residue and its movement and dissipation in NT soils (Beulke et al. 2002; Edwards et al. 1992; Zhou et al. 2003; Singh, Kloeppel & Klein 2002). On the other hand, a number of studies reported intensity does not affect wash off rather the total amount of rain is important. However, rainfall intensity may influence herbicide movement but possibly not dissipation (McDowell et al. 1985; Willis & McDowell 1987; Willis et al. 1992). In addition, the frequency of rainfall events affects moisture levels (Rusinamhodzi et al. 2011) and the variations in drying and wetting periods influence microbial processes occurring at the residue–soil interface (Coppens et al. 2006). Westra et al. (2014) showed that the dissipation half-life of pyroxsulfone (5-(difluoromethoxy)-1-methyl-3-(trifluoromethyl) pyrazol-4-ylmethyl 4,5-dihydro-5,5-dimethyl-1,2-oxazol-3-yl sulfone) ranged from 47 to 134 days and appeared to be influenced by soil type and the movement of herbicides below 30 cm following rainfall. The mobility of the herbicides was dependent on the site–year conditions with pyroxsulfone moving further down the soil profile than S-metolachlor (2-chloro-N-(6-ethyl-o-tolyl)-N-[(1RS)-2-methoxy-1-methylethyl] acetamide).
Pre-emergent herbicides are intended to be applied to the soil, and many require incorporation by rainfall, irrigation, tillage or, in the case of NT systems, the seeding operation (Rainbow & Derpsch 2011; Kleemann et al. 2015; Haskins 2012). The activity of pre-emergent herbicides applied to crop residues depends not only on the physicochemical properties of the herbicides, but the amount and origin of the crop residues, spray volume, and the period prior to the first rainfall event after application and the duration of following rainfall events (Lamoreaux, Jain & Hess 1993; Rodrigues 1993; Watts & Hall 1996). A study on the transfer of atrazine (6-chloro-\(N^2\)-ethyl-\(N^4\)-isopropyl-1,3,5-triazine-2,4-diamine) from standing and flat wheat stubble showed that, three weeks after application and 50 mm of rainfall, atrazine declined by 90% on standing residue and 63% on flat residue, while the amount of herbicide in the soil doubled. Nine weeks after application, there was no atrazine left in the residue (Ghadiri, Shea & Wicks 1984). Williams and Wicks (1978) showed that 70% of the applied atrazine reached the soil surface within the initial 90 days in an NT system when 85% of the soil surface was covered by crop residues. For pre-emergent herbicides, such as atrazine, pyroxasulfone, and metolachlor, most of the chemicals will wash off the residue with as little as 5 mm of rainfall (Shaner 2013; Prueger, Hatfield & Sauer 1999). However, differences exist depending on the type of crop residue and the herbicide. Some herbicides, such as metolachlor, can volatilise from the crop residue if rainfall does not occur soon after application (Shaner 2013; Prueger, Hatfield & Sauer 1999).

Commonly used pre-emergent herbicides for weed control in southern Australia are prosulfocarb (S-benzyl dipropyl (thiocarbamate)), pyroxasulfone and trifluralin (\(a,\alpha,\alpha\)-trifluoro-2,6-dinitro-\(N,N\)-dipropyl-p-toluidine). This region has a Mediterranean-type climate, with mild, wet winters and hot, dry summers. NT with crop residue retention is the predominant cropping system (Derpsch et al. 2010; Harries, Anderson & Hüberli 2015) with pre-emergent herbicides applied and only partially incorporated by seeding. Many farmers now start seeding prior to seasonal rain under dry soil conditions (sometimes called dry-seeding) (Fletcher et al. 2015). Therefore, it is important to understand the longevity of pre-emergent herbicides on stubble and their interaction with rainfall. The main objectives of this research were to determine (1) the effect of rainfall timing, amount and intensity and (2) the effect of single and multiple rainfall events on leaching of prosulfocarb, pyroxasulfone and trifluralin from wheat stubble.
Chapter 4: Rainfall leaching herbicide from wheat residue

4.2. Materials and methods

Two experiments were conducted to determine the effect of rainfall on leaching of prosulfocarb, pyroxasulfone and trifluralin from wheat stubble. The first experiment focused on rainfall amount, intensity and timing, while the second compared single and multiple rainfall events. Briefly, the herbicides were sprayed onto plastic trays containing soil in Petri dishes that were covered with wheat residue. Simulated rainfall was then applied to the trays, and the amount of herbicide remaining in the residue and leached into the soil was determined by bioassays.

4.2.1 The soil and crop residue

The soil was typical of the Western Australian wheatbelt and was collected from the surface (0–10 cm) of a farm paddock in the Cunderdin area of Western Australia (−31.58442° S, 117.327038° E). The soil was air-dried and passed through a 2-mm sieve, with a sample analysed for texture, pH (CaCl₂), CEC and organic carbon, using the methods of Rayment & Lyons (2011), the Soil Science Laboratories of University of Western Australia, Perth, and the CSBP Soil and Plant Laboratory (www.csbp-fertilisers.com.au). The soil was acidic and classified as a sandy loam (Table 4.1).

<table>
<thead>
<tr>
<th>pH (CaCl₂)</th>
<th>OM (%)</th>
<th>CEC</th>
<th>Al (%)</th>
<th>Ca (cmol(+)kg⁻¹)</th>
<th>K (%)</th>
<th>Mg (%)</th>
<th>Na (%)</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.4</td>
<td>1.8</td>
<td>2.96</td>
<td>0.02</td>
<td>2.75</td>
<td>0.14</td>
<td>0.45</td>
<td>0.07</td>
<td>74</td>
<td>12</td>
<td>14</td>
</tr>
</tbody>
</table>

Dry wheat stubble was collected shortly after harvest from the same Cunderdin field as the soil was collected. After being sprayed with herbicide (described below) and the various rainfall applications, the wheat residue was then air-dried, ground into small particles (to be used as a germination media) using a mechanical plant material grinder (www.retsch.com), and then used in the bioassay. After grinding, the length of about 85% of the particles ranged from 1–4 mm. To minimise sample contamination, the grinder was cleaned after each batch, using a vacuum and then air compressor (forced-air blower).

4.2.2 Herbicide application

Commercial formulations of the three pre-emergent herbicides were applied at
recommended field rates: prosulfocarb 2000 g a.i. ha\(^{-1}\) (Arcade® 80-EC), pyroxasulfone 102 g a.i. ha\(^{-1}\) (Sakura®, 850-WG) and trifluralin 960 g a.i. ha\(^{-1}\) (Treflan® 480-EC) (Boutsalis, Gill & Preston 2014). The herbicides were applied using a twin-nozzle laboratory sprayer fitted with 110° 01 flat-fan spray jets (Tee jet™) delivering 117.1 L ha\(^{-1}\) at 210 kPa, travelling at a speed of 3.6 km h\(^{-1}\).

### 4.2.3 Rainfall simulation set up and calibration

The rainfall simulator was based on a design used by Meyer (1960) and Hermsmeier et al. (1963). Four pillar legs supported the rainfall simulator, which was 2.4 m above the ground and consisted of a metal frame shaped in a truncated pyramid (3.10 × 2.70 m at the base, 3.0 × 0.26 m at the top, 2.5 m high). The simulated rainfall was applied through three VeeJet flat-fan nozzles (Spraying Systems, Wheaton, IL) that were turned back and forth through 45° by a motor, at a pre-determined rate. A pressure gauge on top of the water inlet manifold was used to regulate the flow of water to the nozzles, and the wait-time of the nozzles at the end-point could be altered to vary the rainfall application rate. Three nozzle types were used to achieve the three different rainfall intensities of 5, 10 and 20 mm h\(^{-1}\) (VeeJet 8020, 8050, and 80100) using a constant pressure of 80 KPa. The rainfall simulator was calibrated prior to the experiment by placing four rain gauges on the ground below the nozzles to catch the rainfall and to determine the resulting rainfall amount (mm) and intensity (mm h\(^{-1}\)). The process was repeated until the desired rainfall amounts and intensities were achieved.

### 4.2.4 Rainfall treatments

The trials were conducted at The University of Western Australia School of Agriculture and Environment facilities (–31.9812° S, 115.8199° E). Four Petri dishes, each representing one replication, containing 50 g of dry soil, were placed onto plastic seedling trays; the trays and Petri dishes were uniformly covered with an equivalent of 4 t ha\(^{-1}\) wheat residue (the average amount expected to be found in the field) and sprayed with one of the three herbicides at the recommended field rate or not sprayed (untreated control). In the first experiment, simulated rainfall was applied in one of four amounts (0, 5, 10, or 20 mm), three different intensities (5, 10, or 20 m h\(^{-1}\)) and five application times after spraying the herbicides (immediately after spraying (0), 0.25, 1, 7, or 14 days). In the second experiment, the herbicides were applied over the residue and this was immediately followed by four rainfall treatments
Chapter 4: Rainfall leaching herbicide from wheat residue

arranged in a randomised block with four replications: (1) no rainfall, or 20 mm of rainfall applied as (2) 4 × 5 mm rainfall events over two days, (3) 2 × 10 mm rainfall events over one day, or (4) a single 20 mm rainfall event. For the second experiment, the rainfall treatments were applied at an intensity of 10 mm h⁻¹.

After spraying, the Petri dishes with soil were placed in a –20°C freezer prior to the bioassays. The wheat residue was air-dried and ground, as previously described, and placed in plastic bags and stored at –20°C prior to the bioassays.

4.2.5 Bioassay conditions

The method for the bioassays was previously described by Khalil et al. (2018). Briefly, the bioassays were conducted in a growth room on shelves equipped with LUMILUX® cool white fluorescent lamps (Model L36W/840, OSRAM), with photosynthetically active radiation (PAR) at the top of the plants of 109 µmol m⁻² s⁻¹ (SD ±5 µmol m⁻² s⁻¹), and a 12-hour photoperiod. The air temperature was maintained at 25/22.5°C (SD ±2/1°C) during the light/dark period. Relative humidity in the room was 70% (SD±10%). Petri dishes (9 cm diameter) were filled with either 50 g soil or 5 g ground wheat residue, and five seeds each of annual ryegrass (Lolium multiflorum) and cucumber (Cucumis sativus L.) were planted in the same Petri dish at 1 cm depth. The plants were hand watered on a daily basis by adjusting the moisture of the medium to near field capacity with deionised water (Somasegaran & Hoben 1985). After seven days, the media was washed from the plants with running tap water and the plants removed for shoot length measurements. The percent shoot length inhibition from the untreated control (UTC) was calculated for each media using the formula [shoot length (% of untreated control) = Lₜ × (100/L₀)], where Lₜ is the shoot length measured in the herbicide-treated soil or wheat residue, and L₀ is the shoot length in the untreated soil or wheat residue.

4.2.6 Data analysis

Each herbicide and bioassay species were analysed separately, and both the annual ryegrass and cucumber data are shown, as in some instances no annual ryegrass germinated. The data were tested for normality and homogeneity of variance before conducting ANOVA on the shoot length data, using GenStat 12 (Payne et al. 2009), to test for significance at P ≤ 0.05.

For the first experiment, a three-way ANOVA was performed (rainfall intensity × rainfall...
amount × rainfall timing) and a one-way ANOVA for the second experiment, to compare the four treatments.

4.3. Results and Discussion

Rainfall generally leached the herbicides from the residue into the soil, resulting in increased shoot growth of the bioassay species in the residue and a corresponding decrease in shoot growth in the soil, as the concentrations of herbicide was greater. Overall, annual ryegrass was more sensitive to the three herbicides than cucumber, particularly for pyroxasulfone (Figures 4.1–4.4). In the first experiment, there were no interactions between rainfall intensity and rainfall amount or rainfall timing (P= 0.937), so the mean results for each rainfall intensity are shown (Figure 4.1). There was a significant interaction between rainfall amount and rainfall timing on the leaching of intercepted herbicides from wheat residue (Figures 4.2–4.4).

Figure 4.1 Shoot length (% of untreated control) of bioassay species (annual ryegrass and cucumber) grown in wheat residue and soil after spraying prosulfocarb, pyroxasulfone and trifluralin followed by different rainfall intensities (5, 10 and 20 mm h⁻¹). Bars show LSD at P = 0.05 for comparisons within rainfall intensities for each media and herbicide, where significant differences were found.
4.3.1 Rainfall intensity

Rainfall intensity had no consistent effect on the leaching of herbicide from the residue into the soil, although there was a trend for the intensity of 20 mm h\(^{-1}\) to leach more prosulfocarb and trifluralin than the lower intensities, but not for pyroxasulfone (Figure 4.1). Also, there were more phytotoxic levels of herbicide (i.e. reduced shoot length) in the residue compared with the soil for all intensities for prosulfocarb, but not for pyroxasulfone and trifluralin. Varying the intensity of rainfall between 5 mm h\(^{-1}\) and 20 mm h\(^{-1}\) had minor and inconsistent effects on herbicide leaching from the residue to the soil. Willis et al. (1987; 1992) and McDowell et al. (1985) also found that wash-off of insecticides from plant canopies depended on rainfall amount and was not affected by rainfall intensity.

4.3.2 Rainfall amount and timing

All three herbicides had interactions between rainfall amount and timing (P = 0.015) (Figures 4.2–4.4). For prosulfocarb, higher amounts of rainfall generally leached more herbicide from the residue, resulting in increased growth of the bioassay plants in the residue, even after 14 days. There was a corresponding reduction in bioassay plant growth in the soil, except at 14 days, when there was no difference between the rainfall amounts (Figure 4.2). Leaching of pyroxasulfone from the residue followed a similar pattern to prosulfocarb, except that few annual ryegrass germinated at any rainfall amount after 14 days, showing the sensitivity of this species to pyroxasulfone. There was a corresponding reduction in bioassay plant growth in the soil with rainfall amount, which still occurred with rainfall 14 days after herbicide application (Figure 4.3).
Chapter 4: Rainfall leaching herbicide from wheat residue

Figure 4.2 Shoot length (% of untreated control) of bioassay species grown in a) wheat residue, b) soil after spraying prosulfocarb followed by different amounts and timing of rainfall. Bars show LSD at $P = 0.05$ for comparisons within each bioassay species.
Figure 4.3 Shoot length (% of untreated control) of bioassay species grown in a) wheat residue, b) soil after spraying pyroxasulfone followed by different amounts and timing of rainfall. Bars show LSD at P = 0.05 for comparisons within each bioassay species.
Chapter 4: Rainfall leaching herbicide from wheat residue

Much of the sprayed herbicide was intercepted by the crop residue as seen in the reduced shoot length in the residue compared with the soil. This effect was most evident for pyroxasulfone when no simulated rainfall was applied (Figure 4.3). This is particularly so as Khalil et al. (2018) showed that the ED$_{50}$ for these bioassay species in the soil was markedly less than in the residue by between 17-fold (pyroxasulfone) and 150-fold (trifluralin), therefore much more herbicide was required to reduce shoot length in residue than soil. Also, differences in rainfall amounts from 5 to 20 mm were not as obvious with pyroxasulfone as with prosulfocarb, indicating that it was easily leached.

For trifluralin, the annual ryegrass data showed that rainfall had no effect on herbicide leaching from the residue beyond one day after herbicide application. The cucumber data indicated that increasing amounts of rainfall tended to leach more herbicide from the residue for up to 14 days, although differences were small compared to the other herbicides. The soil data (shoot length of bioassay plant species) for trifluralin was more variable, but there was little additional leached chemical in the soil with rainfall after about one day after herbicide application (Figure 4.4). The herbicide sprayed on the wheat residue lost efficacy over time, as shown by increased shoot length, and this loss could be due to degradation, photolysis or volatilization; although pyroxasulfone appeared to change the least. Trifluralin on the wheat residue appeared to have almost disappeared by 14 days after application, whereas the other two herbicides were still bioactive on the residue (Figures 4.2a–4.4a). Grass et al. (1994) and Bedos et al. (2006) reported that up to 96% of trifluralin was volatilised within the first 48 h following herbicide application. Generally, for all tested herbicides, increasing amounts of rainfall from 5 to 20 mm applied soon after spraying leached more herbicide from the wheat residue, although the corresponding decrease in shoot growth in the soil from the increased rainfall was not as evident and changed over time. Pyroxasulfone seemed to leach well with as little as 5 mm of rainfall, but prosulfocarb generally required more rainfall to leach herbicide into the soil. Rainfall reduced shoot length in the soil up to 7 days after application with prosulfocarb and up to 14 days with pyroxasulfone. No rainfall was applied beyond 14 days and it is possible that significant amounts of pyroxasulfone could still be washed from residue into the soil after this period. There appeared to be little trifluralin leached into the soil with rainfall one day after spraying. Similarly, Carbonari et al. (2016) reported that the first 20 mm of simulated rainfall was responsible for washing off most of the sulfentrazone sprayed onto sugarcane residue.
Figure 4.4 Shoot length ( % of untreated control) of bioassay species grown in a) wheat residue, b) soil after spraying trifluralin followed by different amounts and timing of rainfall. Bars show LSD at $P = 0.05$ for comparisons within each bioassay species.
Chapter 4: Rainfall leaching herbicide from wheat residue

Carbonari et al. (2016) concluded that time of rainfall occurrence was a crucial factor affecting the availability of herbicides applied to crop residue. Similarly, Granovsky et al. (1994) reported that a strong rainfall event soon after atrazine application washed off more of the chemicals intercepted by crop residues into the soil.

Bedos et al. (2006) showed that volatilisation of trifluralin was very high immediately after application, but it declined in the following 24 h. The efficacy of trifluralin dropped by 62% by delaying incorporation for 48 h after application due to surface volatilisation (Grass, Wenclawiak & Rüdel 1994). Moreover, Haskins (2012) and Selim et al. (2003) reported that crop residues on the soil surface would make some herbicides inert, such as trifluralin, and recommended using higher label rates of these herbicides in conservation farming systems.

Despite trifluralin having the longest half-life of the three herbicides (35–375 days), it is subject to volatilisation and photo-degradation, especially with surface application without incorporation. The half-life of pyroxasulfone and prosulfocarb are lower (16 – 90 days) (Gennari et al. 2002; Haskins 2012). Nonetheless, pyroxasulfone appeared the best of the three herbicides tested for conservation farming systems, especially for high residue situations, as a significant amount of herbicide leached from the residue with rainfall 14 days after application. Prosulfocarb was intermediate between pyroxasulfone and trifluralin, as some herbicide leached into the soil with rainfall after seven days. This is maybe related to greater leaching of pyroxasulfone. Or it is maybe due to greater biological activity of pyroxasulfone than other two herbicides. Further studies should be conducted to determine how much longer pyroxasulfone will leach from crop residue into the soil.

4.4.2 Multiple rainfall events

All rainfall treatments (≥5 mm) leached some prosulfocarb, pyroxasulfone and trifluralin from residue as seen in the increased shoot length in the residue bioassay compared with nil rainfall (Figure 4.5). Nonetheless, the residue remained phytotoxic to annual ryegrass for prosulfocarb and pyroxasulfone. Although there were some anomalies, applying the 20 mm of rainfall over the residue in a single event immediately after herbicide application generally gave better control (reduced shoot length) of the bioassay species in the soil than applying the same amount of rainfall in multiple events (4 × 5 mm events over two days or 2 × 10 mm events over one day), but the differences were not large and in some instances not significant (Figure 4.5). Therefore, even 5 mm of rainfall can effectively leach herbicide
into the soil. For trifluralin, rainfall over the residue after spraying had little beneficial effect for controlling the plants growing in the soil below. For prosulfocarb with annual ryegrass, all three rainfall amount treatments reduced annual ryegrass shoot growth compared with 0 mm, while for cucumber only the 20 mm treatment differed significantly, with reduced shoot growth in the soil (Figure 4.5). It would be expected that shoot growth in the residue would be greater with 20 mm of rainfall applied as a single event, as more herbicide leached into the soil; however, in many instances, this was not the case. This can be explained by the effect of a longer period of wet residue, with the multiple rainfall events, which may have increased the loss of herbicide. Aslam et al. (2015) showed that under a light and frequent rainfall regime, S-metolachlor dissipation in crop residues was quicker than a heavy and infrequent rain regime. This was due to wetter surface conditions, where crop residue decomposition was also faster. Microbial decomposition of crop residues is highly affected by water dynamics (rainfall) and temperature at the soil–residue interface (Coppens et al. 2006; Taylor-Lovell, Sims & Wax 2002; Isensee & Sadeghi 1995; Issa & Wood 2005).
Chapter 4: Rainfall leaching herbicide from wheat residue

Unger (1994) reported that crop residues that are often wet have low sorption capacity for pesticides and are less aerodynamically stable than the soil underneath, therefore have greater potential to dissipate.

4.5. Conclusion

Some herbicide leached from the residue with as little as 5 mm of rainfall, although higher rainfall amounts generally leached more herbicide from the residue. The sooner the rainfall occurred after herbicide application, the greater the amount of herbicide leached. There were no differences between rainfall intensities. Multiple rainfall events (4 × 5 mm over two days) leached slightly less of the intercepted herbicide from the wheat residue than a single event of 20 mm.

Rainfall was very effective at leaching pyroxasulfone from the residue into the soil, even in heavy residues (4 t ha⁻¹) when rainfall occurred up to 14 days after herbicide application. Rainfall leached less prosulfocarb, and this only occurred with rain up to 7 days after application of the chemical. Trifluralin leached the least or was lost from the residue, with little improvement in ‘weed’ control when rainfall occurred one day after herbicide application.
Chapter 5

Effect of crop residues on interception and activity of prosulfocarb, pyroxasulfone, and trifluralin

Abstract

Crop residue retention is one of the most important components of conservation agriculture, due to its many benefits for soil and crop productivity. In the south west of Australia, where no-tillage (NT) with crop residue retention is widely adopted, weed management relies heavily on herbicides. However, thick crop residues on the soil surface at seeding time can intercept the pre-emergent herbicides and reduce their weed control efficacy. Three experiments were conducted to investigate the effect of crop residue moisture, amount, type and age on the interception and subsequent leaching of prosulfocarb, pyroxasulfone, and trifluralin from the residue into soil. Experiment 1 studied the effect of residue moisture — wet or dry wheat residue at the time of herbicide application — on the leaching of herbicides following rainfall. Experiment 2 evaluated the effect of different amounts of wheat residue (0, 1, 2 and 4 t ha$^{-1}$) on herbicide interception. Experiment 3 evaluated herbicide interception and subsequent leaching of the chemicals after rainfall for different types of residue (wheat, barley, canola, chickpea, and lupin) and residue ages (new or one year-old).

Bioassays, using cucumber and annual ryegrass as indicator plants, were used to assess herbicide activity/availability in the soil and on the residue. Less chemical leaching from crop residue into the soil after rainfall occurred when prosulfocarb and trifluralin were applied to wet residue than dry residue, but the initial moisture condition had not affect the leaching of pyroxasulfone from residue. If practically possible, farmers should minimise spraying prosulfocarb and trifluralin into wet crop residue otherwise they may need to increase the application dose to compensate for leaching losses. Greater amounts of wheat residue intercepted more herbicide, which increased considerably from 2 to 4 t ha$^{-1}$ of residue. After simulated rainfall to wash the herbicide into the soil, complete control of annual ryegrass occurred only with bare soil for trifluralin, bare soil and 1 t ha$^{-1}$ of residue for prosulfocarb, and all residue amounts for pyroxasulfone. Therefore
Chapter 5: Effect of crop residues on interception

pyroxasulfone is recommended as a pre-emergent herbicide in NT systems when the amount of residue on the soil surface exceed 2 t ha\(^{-1}\) and prosulfocarb is recommended for amounts less than 2 t ha\(^{-1}\).

Barley and wheat residues intercepted more herbicide than an equivalent amounts of canola, chickpea or lupin residue, which was largely due to the increased ground cover with cereal residues. The effect of residue age on herbicide interception and leaching was relatively small and variable. Overall, more herbicide reached the soil when sprayed on one-year old residue than new residue, which was largely due to reduced ground cover with aged residue. A strong positive linear relationship existed between ground cover percentage and growth of bioassay species, indicating lower herbicide availability in the soil. The linear relationship explained about 75\% of the variation in the data when the herbicides were modelled separately. This means that farmers can use crop residue ground cover to assess potential herbicide interception and not be concerned about residue type or age. Simulated rainfall leached the herbicides into the soil, particularly for pyroxasulfone, highlighting the importance of rainfall for good weed control in high residue NT systems.

**Keywords**

Pre-emergent herbicides, crop residue condition, crop residue amount, crop residue type, bioassay.

**5.1 Introduction**

Conservation agriculture (CA) is a production system widely adopted around the world, particularly in Australia. Crop residue retention is one of the pillars of CA, along with zero or minimum mechanical soil disturbance and diverse crop rotations (Verhulst et al. 2010; Kassam et al. 2012; Serraj & Siddique 2012). No-tillage (NT) with residue retention on the soil surface is a form of CA system that has been highly adopted in Western Australia (WA) (Kassam et al. 2012; Llewellyn, D’Emden & Kuehne 2012). The presence of crop residues on the soil surface protects the soil from erosion, conserves soil moisture, and builds up soil organic carbon (SOC) for crop production (Unger & Wiese 1979; Prasad & Power 1991; Roper, Gupta & Murphy 2010; Farooq & Siddique 2015).

Crop residues on the soil surface also play a role in reducing herbicide efficacy in NT systems (Banks & Robinson 1982; Donald, Gail & Burnside 1986) because they can
Chapter 5: Effect of crop residues on interception

intercept a considerable amount of herbicide at the time of application (Banks & Robinson 1982; Ghadiri, Shea & Wicks 1984; Bauman & Ross 1983). For example metolachlor activity in the soil declined with high amounts of wheat residue (Banks and Robinson (1983). Even after rainfall, crop residue retained atrazine (Ghadiri, Shea & Wicks 1984) and metolachlor (Banks & Robinson 1983; Strek & Weber 1981; Strek & Weber 1982). The interception of herbicides by crop residue and consequent reduction in the amount of herbicide reaching the soil, would be expected to reduce the efficacy of herbicides in weed control. However, Prihar et al. (1975) found that plots with maize residue had better weed control, than those without maize residue, irrespective of atrazine application. Soil covered with residue had better weed control with alachlor application than uncovered soil (Liebl & Worsham 1983), but this may be due to the residue smothering the weeds, which compensates for the reduction in herbicide reaching the soil (Crutchfield, Wicks & Burnside 1986). In addition, Day (1968) reported that weeds smothering by crop residue may have weakened the seedlings of weeds (not able to kill them) so that it was easier for the herbicide to control them.

Several factors affect whether herbicides are intercepted by crop residues on the soil surface at application time, some being herbicide-related (physio-chemical properties), while others are related to the properties of the crop residues. For example, wet residues have lower sorption capacity for herbicides than the soil underneath (Unger 1994). The capacity of a herbicide to volatilise is relatively high for standing straw compared with horizontal straw (Unger 1994). As a result, herbicide losses increased when combined with standing, wet residue (Unger 1994). However, once the herbicide contacted the soil, the chance of volatilisation decreased due to increased sorption, particularly in soils with more organic matter (Unger 1994).

Studies conducted by Lal (1976) and De-Silva and Cook (2003) suggested that 4–6 t ha$^{-1}$ of residue is enough to improve rainfall infiltration and reduce soil erosion. Banks and Robinson (1986) reported that the amount of acetochlor, alachlor, and metolachlor detected in the soil progressively declined as the amount of wheat residue on the soil surface increased from 0 to 6.7 t ha$^{-1}$. However, the percent cover may be more closely related to herbicide interception than the amount of residue; the conversion of residue into coverage for different crops has not been well researched (Morrison, Prunty & Giles 1985).

The type and composition of crop residues also influences the interaction with herbicides.
Dao (1991) reported that the lignin content of plant stubble might be responsible for most of its sorptive capacity while cellulose, the more abundant plant material, has little impact (Dao 1991). Furthermore, older, partially-decomposed straw appears to adsorb more herbicide than fresh straw (Unger 1994). This may be due to the decomposition of cellulose and other plant elements, thereby exposing the more reactive lignin compounds (Unger 1994). In contrast, Grover (1971) found that picloram was not adsorbed on wheat stubble or cellulose but was highly adsorbed on SOM. The physical–chemical mechanisms, such as the sorption of herbicides by lignocellulose may reduce the effective solution concentration, thereby curbing bioavailability and biodegradation (Zablodowicz, Locke & Smeda 1998). The sorption of chlorimuron and cyanazine increased with the degree of residue decay on hairy vetch (Vicia villosa Roth), rye (Secale cereale L.) and ryegrass (Lolium multiflorum Lam.) residues Reddy et al. (1997; 1995; 1995) and was not completely reversible (Gaston, Boquet & Bosch 2001).

The main pre-emergent herbicides currently used in NT systems are prosulfocarb, pyroxasulfone and trifluralin (Boutsalis, Gill & Preston 2012; Saini et al. 2015). They were introduced to control grasses and small-seeded broadleaves in corn (Zea mays L.), soybean (Glycine max (L.) Merr.), sunflower (Helianthus annus L.), and field pea in Canada (Tanetani 2011; Tanetani et al. 2009; Tidemann et al. 2014; Mangin 2016), United Kingdom (Bailly 2012), United States and Australia (Busi & Powles 2016; Busi 2014; Busi et al. 2012; Tanetani et al. 2009). No research has been conducted to investigate the effect of crop residue conditions on the interception and activity of these pre-emergent herbicides. As stubble retention is a key component of NT systems, it is important to understand the impact of crop residue amount and type on the activity of these herbicides, especially after rainfall. In this study, prosulfocarb, pyroxasulfone and trifluralin were sprayed onto crop residues followed by simulated rainfall to test the hypotheses that: 1) initially wet residue will leach less herbicide into the soil than initially dry residue; 2) increased residue amount will intercept more herbicide, which will leach into the soil after rainfall; 3) crop residue type will influence the amount of herbicide leached and 4) older residue will leach less herbicide than fresh new residue.

5.2 Materials and Methods

Three experiments were conducted to investigate the effect of simulated rainfall on prosulfocarb, pyroxasulfone, and trifluralin leaching from the different crop residues
treatments into the soil. Experiment 1 examined the effect of crop residue moisture at the time of herbicide application on the sorption and leaching of these three herbicides. Experiment 2 evaluated the effect of the amount of wheat residue on herbicide interceptions and leaching and Experiment 3 evaluated the effect of crop residue type and age on herbicide interception and leaching.

5.2.1 Experimental design and management:

The three experiments were conducted at The University of Western Australia, School of Agriculture and Environment facilities (−31.9812° S, 115.8199° E). Four Petri dishes, each containing 50 g of dry soil, were placed onto plastic trays, which were then covered with the crop residue treatment and sprayed with one of three herbicides, except for an unsprayed control. The rates of herbicide were 2000 g a.i. ha⁻¹ of prosulfcarb, 102 g a.i. ha⁻¹ pyroxasulfone and 960 g a.i. ha⁻¹ trifluralin, which were the recommended field rates (Boutsalis, Gill & Preston 2014). After spraying, 20 mm of simulated rainfall at 10 mm hr⁻¹ was applied, except for the rainfall control (nil rainfall). The crop residues were then air-dried for a few hours and ground into small particles using mechanical plant material grinder (www.retsch.com). To minimise sample contamination, the grinder was thoroughly cleaned after each batch with a vacuum and then air compressor to blow air through the grinder. The particle size of the ground residue was determined by sieving 50 g of unsprayed material, with most residues ranging from 2 to 4 mm. The ground residue was placed in plastic bags and stored, along with the Petri dishes of soil, for a few days at −20°C until being used in a bioassay to determine bioavailability. The bioassays were conducted with susceptible annual ryegrass (Lolium multiflorum Lam., Dargo, Irwin Hunter Seeds, Unit11, 88 Forrest St, Cottesloe, WA 6011, www.irwinhunter.com.au), and cucumber (Cucumis sativus L., Long Green Supermarket, Mr. Fothergill’s Seeds, 15B Walker St, South Windsor NSW 2756, www.mrfothergills.com.au), because annual ryegrass was found to be sensitive to low concentrations and cucumber relatively high concentrations (that kill the ryegrass) of the three herbicides, which was reported in Chapter 3 (Khalil et al. 2018).

Each experiment had four randomised complete blocks. In Experiment 1, the residue treatments were equivalent to 4 t ha⁻¹ of wheat residue (measured dry). Water was sprayed onto the residue for the wet treatment (500 ml which was equivalent to 5 mm of rainfall). Crop residue was wet when sprayed with herbicides. Experiment 2 compared wheat
residue amounts of 0, 1, 2 and 4 t ha\(^{-1}\). In Experiment 3, the equivalent of 4 t ha\(^{-1}\) of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), canola (*Brassica napus* L.), chickpea (*Cicer arietinum* L.), and lupin (*Lupinus angustifolius* L.) residue were compared at two ages — recent (collected a few days after harvest) and aged (one-year-old). Further details are described below.

### 5.2.2 The residue and soil (germination media in the bioassay)

The soil was collected from the surface (0–10 cm) of a farmer’s field near Cunderdin, WA (–31.5844° S, 117.3270° E). The soil was air-dried and passed through a 2-mm sieve prior to analysis (Soil Science Laboratories of The University of Western Australia, Perth and CSBP Soil and Plant Laboratory (www.csbp-fertilisers.com.au). The soil was a sandy loam (74% sand, 12% silt, 14% clay), with pH (CaCl\(_2\)) of 4.4, 2.96 cmol(+)kg\(^{-1}\) CEC, exchangeable cations of 2.75 Ca, 0.14 K, 0.45 Mg, 0.07 Na cmol(+)kg\(^{-1}\), and 1.8% organic carbon (*Rayment & Lyons 2011*).

Different dry crop residues (wheat, barley, canola, chickpea, and lupin) were collected from the same Cunderdin field as the soil and another nearby field (–31.641311° S, 117.243087° E), which had different rotations. Two different ages of residue — recent/new collected shortly after harvest and aged (collected from the residue remaining from the previous season, which was before the current harvest) — were thoroughly mixed by hand (each type and age kept separate) and a representative dry sample was ground and sieved with a 1-mm sieve. A 10 g sample of the residue was sent to Forage Laboratory/One Dairy (www.dairyone.com) for analyses of lignin content, using the ANKOM Technology Method 9 (Method for Determining Acid Detergent Lignin in the Daisy\(^{\text{II}}\) Incubator) (www.ankom.com). Briefly, for the lignin analysis, 0.5 g samples were weighed into filter bags and digested for 75 minutes in 2 L of Acid Detergent Fibre (ADF) solution (20 g cetyl trimethyl ammonium bromide (CTAB) to 1L 1.00 N H\(_2\)SO\(_4\)) in an ANKOM A200 Digestion Unit. Samples were rinsed three times with boiling water for 5 minutes in filter bags followed by a 3 minute acetone soak and drying at 105°C for 2 h. The filter bags were then re-weighed after cooling. Following this, the filter bags were covered by 72% w/w H\(_2\)SO\(_4\) for 3 h in ANKOM Daisy\(^{\text{II}}\) Incubator at ambient temperature, rinsed with water, dried and weighed as previously described (Dairy-One-Forage-Laboratory 2015).

After applying the residue treatments to the trays, the ground cover percentage was
estimated using a digital photograph of the tray and the “Agronomist Panel” option of ASSESS 2.0 Image Analysis Software, (Lamari 2008).

5.2.3 Herbicide application

The herbicides were applied in a spray cabinet, using a twin-nozzle laboratory sprayer fitted with 110° 01 flat-fan spray jets (Tee jet™) delivering herbicide in 117.1 L ha⁻¹ of water at 210 kPa, travelling at a speed of 3.6 km h⁻¹.

5.2.4 Rainfall simulation set up and calibration

The rainfall simulator was based on a design by Meyer (1960) and Hermsmeier et al. (1963). The structure consisted of a metallic frame shaped in a truncated pyramid (3.10 m × 2.70 m at the base, 3.0 m × 0.26 m at the top, 2.5 m high) built up with 32–40 mm diameter tubes of galvanised iron. Four pillar legs supported the rainfall simulator to the ground. A laptop computer was used to control a motor at the top of the frame via an RS-232 communication port. The simulated rainfall was applied through three HB1/24–80° VeeJet flat fan nozzles (Spraying Systems, Wheaton, IL) attached to a pipe 2.4 m above the ground that was turned back and forth by the motor through 45°, at a pre-determined rate. Troughs collected water at the end-point of each rotation and returned it to the storage tank. The wait-time of the nozzles at the end-point could be altered to vary the rainfall application rate. To achieve the three different rainfall intensities a range of different nozzle sizes (VeeJet 8020, 8050, and 80100) were used. A pressure gauge on top of the water inlet manifold was used to regulate and monitor the flow of water to the nozzles. The rainfall simulator was calibrated prior to the experiment by placing four rain gauges on the ground below the nozzles to catch the rainfall and determine the resulting rainfall amount (mm) and the intensity (mm h⁻¹). The process was repeated until the desired rainfall amounts and intensities achieved. Simulated rainfall was applied at constant rates of 10 mm h⁻¹ with a pressure of 80 kPa to apply 20 mm. Simulated rainfall was applied for 120 minutes.

5.2.5 Bioassay conditions

The bioassay conditions were previously reported by (Khalil et al. 2018) in Chapter 3 of this thesis. Briefly, the experiment was conducted in a 3 m × 4 m growth room. A rack of three shelves equipped with LUMILUX® cool white fluorescent lamps (Model L36W/840, OSRAM). Photosynthetically active radiation (PAR) at the top of the plants
was 109 µmol m$^{-2}$ s$^{-1}$ (SD ±5 µmol m$^{-2}$ s$^{-1}$) with a 12-h photoperiod. Room air temperature was maintained at 25/22.5°C (SD ±2/1°C) during the light/dark period. Relative humidity in the room was 70% (SD±10%). The bioassay plants (annual ryegrass and cucumber) were planted at 1 cm depth in 9 cm diameter and 1.5 cm deep Petri dishes filled with either soil or ground wheat residue. The plants were hand watered on a daily basis by adjusting the media moisture to near field capacity (Somasegaran & Hoben 1985) with fresh deionized water. Shoot length of each plant was measured 7 d after sowing, following removal from the Petri dishes and washing away the soil or crop residue with running tap water. Shoot length as a percentage of the untreated control (UTC) was calculated for each media using the formula \[ \text{Shoot length (\% of untreated control)} = L_d \times (100/L_0) \] where \( L_d \) is the shoot length measured in the herbicide-treated soil or wheat residue and \( L_0 \) is the shoot length in the untreated soil or wheat residue.

### 5.2.6 Data analysis

Each combination of herbicide, bioassay medium and bioassay species was analysed separately; the annual ryegrass and cucumber data are both shown, as in some instances no annual ryegrass germinated. The data were tested for normality and homogeneity of variance before conducting ANOVA on the shoot length data, using GenStat 12 (Payne et al. 2009), to test for significance at \( P \leq 0.05 \).

In Experiment 1, a one-way ANOVA was conducted to compare the three treatments [dry residue, wet residue, and treated control (sprayed with herbicide but no rainfall applied)]. In Experiment 2 a two-way ANOVA was performed to compare the different amounts of wheat residue × rainfall. In Experiment 3, a three-way ANOVA was performed (crop residue type × crop residue age × rainfall).

A series of linear regression analyses for percent ground cover against cucumber shoot length (\% of untreated control, as a measure of herbicide activity) in the soil was conducted. The cucumber bioassay was used as no annual ryegrass germinated for many of the treatments, due to relatively high herbicide concentrations. Firstly, a regression of the cucumber (in soil) data without simulated rainfall was performed, followed by a regression where the data were classified (grouped) by herbicide. Then regressions were undertaken separately for each herbicide with the data classified by rainfall (vs nil rainfall).
5.3 Results and Discussion

5.3.1 Experiment 1. Crop residue moisture

The differences in herbicide leaching between wet and dry residue were not always clear-cut. Virtually no annual ryegrass germinated with any of the herbicides in the residue bioassay media, indicating that phytotoxic levels remained on the residue even after rainfall (Figure 5.1). The cucumber data showed that rainfall leached some herbicide from the residue relative to the treated control (with no rainfall applied) and that less herbicide leached with initially wet than dry residue, although no significant differences were evident for pyroxasulfone. The same trend occurred for the soil bioassays, where less herbicide tended to reach the soil with wet than dry residue, although again no significant differences were evident for pyroxasulfone. These results indicate that in an NT system, with crop residue covering the soil, more prosulfocarb and trifluralin reaches the soil after rainfall when the chemicals are sprayed onto dry rather than wet residue. The moisture status of the residue has little effect on the amount of pyroxasulfone that reaches the soil after subsequent rainfall. From a practical point of view and to minimise the loss of
intercepted herbicides, NT farmers should avoid spraying prosulfocarb and trifluralin onto wet crop residue otherwise they may need to increase the herbicide application rate to compensate for losses when sprayed onto wet residues. Erbach and Lovely (1975) reported that wetting corn residue before or after alachlor application had no significant effect on foxtail millet control.

5. 3. 2 Experiment 2. Residue amount

Annual ryegrass did not germinate in the wheat residue, even with simulated rainfall, except for trifluralin after rainfall but only to <10% of the untreated control (Figure 5.2a). Overall, shoot length of cucumber in wheat residue generally increased with residue amount, indicating a dilution of the herbicide as residue amounts increased. Shoot length of cucumber grown in the residue medium was shorter in the absence of rainfall, due to higher concentrations of herbicide remaining on the residue.

In the absence of simulated rainfall, the growth of the bioassay species in the soil increased with increasing amount of residue, with a marked increase in shoot length from 2 to 4 t ha⁻¹ (Figure 5.2b). This is due to greater interception of herbicide by increased residue amount.

Rainfall, which leached herbicide from the residue into the soil, generally reduced annual ryegrass shoot length in the soil for all residue rates, but especially at 4 t ha⁻¹ (Figure 5.2b). Prosulfocarb killed all the annual ryegrass in the soil after rainfall at 0 and 1 t ha⁻¹ of residue, whereas pyroxasulfone killed all the annual ryegrass even at 4 t ha⁻¹ of residue. Trifluralin only achieved 100% kill at 0 t ha⁻¹ of residue, but there was >70% shoot inhibition up to 2 t ha⁻¹ residue. The cucumber bioassay in soil was much less sensitive, and rainfall only reduced shoot length with pyroxasulfone (Figure 5.2).

This suggests that pyroxasulfone should be used as a pre-emergent herbicide in NT systems when the amount of flat residue on the soil surface exceeds about 2 t ha⁻¹ but for residue amounts less than 2 t ha⁻¹, prosulfocarb could also be used. Trifluralin should be used when it can be incorporated into the soil either by rainfall immediately after application or with seeding machinery. Reduced amounts of herbicide reaching the soil are due to interception by crop residue (Banks & Robinson 1982; Bauman & Ross 1983; Erbach & Lovely 1975; Ghadiri, Shea & Wicks 1984). Banks and Robinson (1983) reported that increased levels of wheat residue on the soil surface decreased the amount of acetochlor, alachlor and metolachlor in the soil, which reduced weed control and that
Figure 5.2 Effect of wheat residue rate (0–4 t ha\(^{-1}\)) on shoot length (% of untreated control) of annual ryegrass and cucumber grown in a) wheat residue (note: 0 t ha\(^{-1}\) had no residue in the bioassay) and b) soil bioassay media, after spraying prosulfocarb, pyroxsulfone and trifluralin followed by 20 mm (+) or nil (−) rainfall. Bars show LSD at \(P = 0.05\) for comparisons within the same bioassay species and herbicide.
Chapter 5: Effect of crop residues on interception

13 mm of subsequent irrigation washed 15–20% of the original herbicide application into the soil. Also, the residue retained more metolachlor than acetochlor or alachlor. Other studies have shown that plant residues do not significantly reduce weed control efficacy. For example, application of 1.5 times the recommended rate of metolachlor to wheat straw levels up to 6.8 t ha⁻¹ did not reduce weed control efficacy, as the straw itself provided some measure of weed control and compensated for the lesser amounts of herbicide reaching the soil (Crutchfield, Wicks and Burnside 1986). In our study, the physical effect of crop residues on weed suppression could not be tested in the bioassay. In other research, the efficacy of a mixture of alachlor and atrazine in controlling weeds was not affected by surface mulching when recommended rates of herbicide were used (Chauhan, Gill & Preston 2006). Similarly, Erbach and Lovely (1975) reported that 6.2 t ha⁻¹ of non-chopped corn stems did not reduce the effectiveness of weed control when using recommended rates of atrazine or alachlor, but did when lower herbicide rates were used. Presumably, chopping the straw would increase the proportion of ground covered by the residue, which would reduce weed control efficacy. Therefore, a key factor when using herbicides with high levels of crop residue is to ensure that sufficiently high rates of the chemical are used along with high carrier/spray volumes, as shown by Borger et al. (2013). These authors found that increasing the carrier volume from 30 to 150 L ha⁻¹ improved the average control of annual ryegrass by trifluralin and pyroxasulfone from 53 to 78%. Further research should be conducted to assess optimal water volumes and herbicide rates for the different pre-emergent herbicides to achieve good weed control at different crop residue levels.

5. 3. 3 Experiment 3. Residue type and age

The ability of crop residue to adsorb and retain intercepted herbicide is related to its lignin concentration (Dao 1991). Older residues (left in the field for a year) of chickpea, canola, and lupin had higher lignin concentrations than new residues (collected shortly after harvest). In contrast, the lignin concentration of older cereal residues (wheat and barley) was similar to the recently collected material (Table 5.1), which is likely due to the relatively slow decomposition of cereals, as shown by Reinertsen (1984).
Ground cover percentage, with the same amount of residue, varied with both residue type and age (Table 5.2). Aged residue had less ground cover than fresh residue for canola, chickpea and lupin, whereas the differences were relatively small for cereal residues. Lupin stubble had the least ground cover, followed by chickpea, canola and the cereals, which had >90% ground cover for the 4 t ha\(^{-1}\) of residue applied. Leonard (1993) reported that 50% ground cover was achieved with 1 t ha\(^{-1}\) cereal residue, 2 t ha\(^{-1}\) lupin residue and 3 t ha\(^{-1}\) of canola residue. Leonard (1993) and Leys and Heinjus (1991) reported 100% ground cover for wheat residue and 80% for lupin residue at 6 t ha\(^{-1}\) (standing and flat) in the field. Therefore, it would be expected that lupin, chickpea and canola residue would have less impact on herbicide efficacy than an equivalent amount of cereal residue.

Table 5.1 Dry matter and lignin concentration for different residue types and ages.

<table>
<thead>
<tr>
<th>Crop residue type</th>
<th>Age</th>
<th>Dry matter (%)</th>
<th>Lignin (% DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chickpea</td>
<td>Aged*</td>
<td>93.4</td>
<td>18.8</td>
</tr>
<tr>
<td>Chickpea</td>
<td>Recent*</td>
<td>92.4</td>
<td>12.9</td>
</tr>
<tr>
<td>Canola</td>
<td>Aged</td>
<td>93.3</td>
<td>16.1</td>
</tr>
<tr>
<td>Canola</td>
<td>Recent</td>
<td>93.6</td>
<td>12</td>
</tr>
<tr>
<td>Lupin</td>
<td>Aged</td>
<td>92.2</td>
<td>14.3</td>
</tr>
<tr>
<td>Lupin</td>
<td>Recent</td>
<td>91.1</td>
<td>10</td>
</tr>
<tr>
<td>Wheat</td>
<td>Aged</td>
<td>92.2</td>
<td>6.8</td>
</tr>
<tr>
<td>Wheat</td>
<td>Recent</td>
<td>92.8</td>
<td>9.1</td>
</tr>
<tr>
<td>Barley</td>
<td>Aged</td>
<td>92.6</td>
<td>6.6</td>
</tr>
<tr>
<td>Barley</td>
<td>Recent</td>
<td>93.1</td>
<td>7.1</td>
</tr>
</tbody>
</table>

* Recent residue collected shortly after the harvest
+ Aged residue left in the field for a year

Table 5.2 Ground cover percentage of different crop residue types and ages estimated by ASSESS 2.0 (Lamari 2008)

<table>
<thead>
<tr>
<th>Crop residue type</th>
<th>Barley</th>
<th>Wheat</th>
<th>Canola</th>
<th>Chickpea</th>
<th>Lupin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crop residue age</td>
<td>Recent</td>
<td>100</td>
<td>99</td>
<td>92</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Aged</td>
<td>99</td>
<td>91</td>
<td>42</td>
<td>45</td>
</tr>
</tbody>
</table>

5.3.3.1 Herbicide remaining on the residue

Shoot length declined in the annual ryegrass bioassays in the residue medium and, in some instances plants were killed by the relatively high concentration of herbicide retained in the crop residues, especially with no rainfall (Figures 5.3a–5.5a). As a result, this study focused on the cucumber bioassay results for the crop residue media.
For prosulfocarb remaining in the residue, there was an interaction between residue type, age and rainfall, P-values were 0.020 for annual ryegrass and 0.017 for cucumber (Figure 5.3a). Overall, barley and wheat residues produced the longest cucumber shoot lengths and lupin the shortest, indicating highest concentrations of ‘bioavailable’ prosulfocarb.
were retained in lupin residue. Martin et al. (1978) reported that corn residue (6 – 8.9 t ha\(^{-1}\)) retained 50% and 30% of tested herbicides (alachlor, atrazine, cyanazine and propachlor) after applying 10 mm and 35 mm of water, respectively. Dang et al. (2016) also reported that sugarcane residue (5 t ha\(^{-1}\)) retained 20% of sprayed herbicides (atrazine, ametryn, diuron, and hexazinone) after 30 mm of rainfall. This indicates that different amounts of sprayed herbicides can be retained by different crop residues.

In this study, the canola and chickpea results were variable. In the absence of rainfall, aged wheat and chickpea residue had greater shoot length than the equivalent new residue, and the reverse occurred with canola. Age of material had little effect on prosulfocarb activity in barley and lupin residue. Rainfall increased cucumber growth the most with chickpea residue, for both aged and new material, but only had a small effect on cucumber shoot length with barley residue and an intermediate with wheat and canola residues. Rainfall increased shoot length in new lupin residue but had little effect on aged lupin residue.

For pyroxsulfone remaining in the residue, cucumber shoot length followed a similar pattern to prosulfocarb where barley had the greatest shoot length in most instances, particularly with new residue, followed by wheat, canola and chickpea (Figure 5.4a). Likewise, lupin had the least shoot length. The differences between aged and new residue were relatively small, although aged residue tended to have longer shoots, except for barley, indicating less herbicide was available in the residue. This was particularly in the case for wheat and chickpea, with and without rainfall, and for lupin with no rainfall. Rainfall generally increased shoot length and had the greatest effect on chickpea residue, a smaller effect on barley residue and little effect on aged lupin residue.

As per the other two herbicides, trifluralin generally had the greatest or equal shoot length with barley residue and smallest with lupin residue (Figure 5.5a). With no rainfall, aged chickpea residue had longer shoots than new residue, and the reverse occurred with canola. Age had little effect on the other crop residue types. Rainfall generally increased shoot length including aged lupin residue, which for lupin was unlike the other herbicides. Rainfall also had little effect on cucumber growth in canola residue.

In summary, herbicide remaining on the residue was influenced most by crop residue type. In general, barley residue had the greatest or equal cucumber shoot length (as a percentage of the equivalent untreated residue) followed by wheat, canola, chickpea and
lupin residues. This indicates that the barley residue had the least bioavailable herbicide and the lupin residue had the most. Although, the shoot length of bioassay plant species grown in wheat, canola and chickpea residue was variable and influenced slightly more by herbicide, age and rainfall. The effect of residue age was smaller than residue type.
Figure 5. Effect of crop residue type and age on shoot length (% of untreated control) of ryegrass and cucumber in a) crop residue and b) soil, after spraying with trifluralin followed by 20mm (+) or nil (–) rainfall. LSD bars show interactions between rainfall and age of residue at $P = 0.05$ for comparisons within the same bioassay species.

Older residue tended to have greater shoot length than new residue, although the effect of age was minimal with barley and lupin residue and the reverse was true for canola residue, except for pyroxsulfone. Rainfall generally increased shoot length of cucumber, indicating some herbicide was washed off the residue into the soil below. The rainfall
effect was greatest with chickpea residue and least for barley residue. Rainfall did not affect aged lupin residue, except with trifluralin.

5.3.3.2 Herbicide in the soil

There were interactions between residue type, age and rainfall, with the responses greater for annual ryegrass than cucumber (Figure 5.3b). For prosulfocarb in the absence of rainfall, barley and wheat residues had the greatest or equal shoot length, canola residue was intermediate and lupin and chickpea residues the least, although aged lupin residue had similar shoot length as aged canola residue (Figure 5.3b). Aged chickpea and canola residue had shorter shoots in soil than new residue, although the differences were small, but significant. Rainfall generally reduced shoot length more with wheat than barley residue and this was particularly noticeable for aged wheat residue. Rainfall reduced shoot length to a smaller degree with the other residue types and ages.

When fields contain similar amounts of residue, prosulfocarb may be least effective for weed control when applied to barley residue than the other residue types. While wheat residue had a similar effect to barley residue in the absence of rainfall, rainfall improved prosulfocarb efficacy more in wheat than barley residue. The effects of residue age on weed control efficacy of prosulfocarb were relatively small.

For pyroxsulfone, the bioassay species in the soil generally had the greatest shoot length with barley and wheat residues, followed by canola, chickpea and lupin. The effect of age was relatively small and nonsignificant (P-values were 0.464 for annual ryegrass and 0.138 for cucumber), but older residue consistently produced shorter shoots in cucumber than new residue, suggesting more herbicide reached the soil under older residue. Pyroxsulfone killed all the annual ryegrass in the soil after rainfall for all residue types and ages and, unlike prosulfocarb, rainfall effectiveness did not differ between barley and wheat residue (Figure 5.4b). Rainfall also reduced shoot length in cucumber with both aged and new residue, although the effect was relatively small for aged residue.

In the absence of rainfall, the effect of trifluralin and residue type on shoot length was similar to the other herbicides with the barley and wheat residues generally having the greatest and chickpea and lupin residues the least shoot length; implying that more herbicide reached the soil under chickpea and lupin residues (Figure 5.5b). With no rainfall, aged residue produced smaller shoots compared with new residue, but the effect was variable and relatively small. Like the other herbicides, shoot length generally
Figure 5.6 Relationship between shoot length (% untreated control) and ground cover (across the different residue types and ages) for a) prosulfocarb, b) pyroxasulfone and c) trifluralin.
declined with rainfall, but the results were more variable for trifluralin; for example, rainfall did not affect shoot length with fresh wheat residue or aged barley residue (Figure 5.5b).

Considering all three herbicides, shoot length in the soil was generally greatest or equal for barley residue followed by wheat residue, intermediate but variable for canola residue, and least for chickpea and lupin residues. This suggests that more herbicide reached the soil under chickpea and lupin residues and this was mainly a function of ground cover. A linear regression of ground cover percentage against cucumber shoot length in the soil (% of untreated control) for all data (across all herbicides, residue types and ages) without simulated rainfall showed a significant, but weak, relationship with 36% of the variance accounted for. However, this increased to 75% of the variance when the regression included herbicide as a factor i.e. separate linear regressions for each herbicide (Table 5.3).

Further linear regression analysis, on individual herbicides, compared simulated rainfall with the nil rainfall control. As expected, the regression line for rainfall was lower than that of nil rainfall, because the plants were smaller due to more herbicide washing off the residue into the soil (Figure 5.6 and Table 5.3). Only pyroxasulfone had a significant interaction with rainfall (i.e. the slopes of the rain and nil rainfall lines differed significantly), with the slope of the regression decreasing from 0.51 in the absence of rainfall to 0.27 following rainfall (Table 5.3). This demonstrates the effectiveness of rainfall to reduce the impact of residue and improve the efficacy of pyroxasulfone, especially in the presence of thick crop residues (Figure 5.6). The value for the slope of the prosulfocarb regression was relatively low compared with the other herbicides, even without rainfall, indicating that ground cover level had less effect on the efficacy of this herbicide. However, it may also indicate that cucumber is less sensitive to changes in concentration of prosulfocarb than the other herbicides, which was also observed in Chapter 3. The cucumber bioassay was used as no annual ryegrass germinated for many of the treatments, due to relatively high herbicide concentrations. Overall, residue age only had a small effect on the amount of herbicide reaching the soil, with slightly more herbicide reaching the soil under aged than new residue. In the absence of rainfall this would most likely be related to ground cover, with aged residue having lower ground cover percentage than new residue, with the differences more evident for canola, chickpea and lupin residues.
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Table 5. 3 Linear regressions of ground cover percentage against cucumber shoot length in the soil (% of untreated control) for a) all data with no simulated rainfall and herbicide (prosulfocarb, pyroxasulfone and trifluralin) as the grouping factor and b) prosulfocarb, c) pyroxasulfone and d) trifluralin comparing simulated rainfall (rain vs nil) as the grouping factor

<table>
<thead>
<tr>
<th>Regression description</th>
<th>Grouping factor</th>
<th>Intercept</th>
<th>Intercept P-value</th>
<th>Slope</th>
<th>Slope P-value</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) All data with nil rainfall</td>
<td>No grouping factor (single line)</td>
<td>Single line</td>
<td>9.2</td>
<td>0.048</td>
<td>0.49</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>Herbicide as grouping factor</td>
<td>Prosulfocarb</td>
<td>11.6</td>
<td>0.020</td>
<td>0.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pyroxasulfone</td>
<td>1.9</td>
<td>0.700</td>
<td>0.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trifluralin</td>
<td>14.2</td>
<td>0.005</td>
<td>0.69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>b) Prosulfocarb</td>
<td>No grouping factor (single line)</td>
<td>Single line</td>
<td>6.58</td>
<td>0.016</td>
<td>0.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Rainfall as grouping factor</td>
<td>Nil rain</td>
<td>10.02</td>
<td>0.047</td>
<td>-0.04</td>
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<td></td>
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<td>Rain</td>
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<td>0.236</td>
<td>0.30</td>
<td>&lt;0.001</td>
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<td></td>
<td></td>
<td>Nil rain</td>
<td>10.03</td>
<td>&lt;0.001</td>
<td></td>
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<tr>
<td>c) Pyroxasulfone</td>
<td>No grouping factor (single line)</td>
<td>Single line</td>
<td>6.58</td>
<td>0.016</td>
<td>0.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Rainfall as grouping factor</td>
<td>Nil rain</td>
<td>-6.18</td>
<td>0.406</td>
<td>0.24</td>
<td>0.017</td>
</tr>
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<td></td>
<td></td>
<td>Rain</td>
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<td>0.127</td>
<td>0.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nil rain</td>
<td>1.9</td>
<td>0.717</td>
<td>0.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>d) Trifluralin</td>
<td>No grouping factor (single line)</td>
<td>Single line</td>
<td>7.66</td>
<td>0.088</td>
<td>0.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Rainfall as grouping factor</td>
<td>Nil rain</td>
<td>12.99</td>
<td>0.140</td>
<td>-0.092</td>
<td>0.429</td>
</tr>
<tr>
<td></td>
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<td>Rain</td>
<td>4.39</td>
<td>0.345</td>
<td>0.74</td>
<td>&lt;0.001</td>
</tr>
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<td></td>
<td></td>
<td>Nil rain</td>
<td>10.39</td>
<td>0.021</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Prosulfocarb – slopes not significantly different, therefore estimate of parallel lines given.
‡ Pyroxasulfone – slopes significantly different, therefore estimate of separate lines given.
* Trifluralin – slopes not significantly different, therefore estimate of parallel lines given.

The effect of lignin concentration (between residue types and ages) on the amount of herbicide washing off the residue was difficult to determine, as lignin was confounded with ground cover percentage. Nonetheless, the effect appeared to be minimal as the
response to rainfall was generally similar for aged and new residue (data not shown). This analysis suggests little chemical difference (the ability of residue to adsorb and retain herbicide) between the residues such that farmers simply need to know the residue ground cover, irrespective of crop type. It also suggests that lignin content is not as important as earlier suggested, at least for the three chemicals tested.

5.4 Conclusion

The application of 20 mm of rainfall leached herbicide from the residue into the soil. Rainfall after herbicide application improved the efficacy by leaching intercepted herbicide into the soil beneath crop residues. For instance, 100% annual ryegrass control was achieved with pyroxasulfone after rainfall, even at 4 t ha\(^{-1}\) residue. In a NT system with crop residue covering the soil, more prosulfocarb and trifluralin reached the soil after rainfall when the chemicals are sprayed onto initially dry compared with wet wheat residue. In contrast, the moisture status of the residue at the time of herbicide application had little effect on the amount of pyroxasulfone that reached the soil after subsequent rainfall. Therefore, farmers may need to increase prosulfocarb and trifluralin dose to compensate for losses when spraying onto wet residues, as less material will wash into the soil (for weed control) after rainfall. In the absence of simulated rainfall, the growth of the bioassay species in the soil increased with increasing residue amount, with a marked increase in shoot length from 2 to 4 t ha\(^{-1}\). Pyroxasulfone is well suited as a pre-emergent herbicide in NT systems when the amount of residue laying flat on the soil surface exceeds 2 t ha\(^{-1}\) and prosulfocarb when the amount of residue laying flat on the soil surface is less than 2 t ha\(^{-1}\). Aged residue of canola, chickpea and lupin generally had less ground cover than fresh residue, while the differences were relatively small for aged and recent cereals residues. Lupin stubble had the least ground cover, followed by chickpea, canola and the cereals, which had > 90% ground cover at 4 t ha\(^{-1}\) of residue.

Herbicide remaining on the residue was influenced most by crop residue type. In general, the barley residue had greatest or equal cucumber shoot length (as a percentage of the equivalent untreated residue) followed by wheat, canola, chickpea and lupin residues. With 4 t ha\(^{-1}\) of crop residue, the least herbicide reached the soil with barley residue followed by wheat with canola residue intermediate but variable, and chickpea and lupin residues the most. There was a strong positive linear relationship between ground cover percentage and growth of the bioassay species i.e. less herbicide reached the soil with
increased ground cover. Rainfall increased the amount of herbicide reaching the soil, especially with pyroxasulfone, highlighting the effectiveness of rainfall to wash pyroxasulfone from the residue into the soil for good weed control in high residue NT systems. Further research should be conducted to assess optimal water and herbicide rates for the different pre-emergent herbicides to achieve good weed control at different ground cover percentages of crop residue.
Chapter 6

Effect of wheat residue height, amount and orientation on pyroxasulfone interception, leaching, and weed control efficacy

Abstract

The effect of wheat (Triticum aestivum L.) residue level on pyroxasulfone interception, leaching and weed control efficacy was studied in a no-tillage cropping system at Cunderdin and the UWA Shenton Park Research Station, Western Australia, in 2015 and 2016. Two wheat residue treatments [horizontal wheat residue at four levels (0, 1, 2, 4 t ha\(^{-1}\)) \times vertical standing wheat residue at four heights (0, 10, 20, 30 cm)] were established in the field. Pyroxasulfone [3-[5-(Difluoromethoxy)-1-methyl-3-(trifluoromethyl) pyrazol-4-yl methylsulfonyl]-4,5-dihydro-5,5-dimethyl-1,2-oxazole] was applied as a pre-emergent herbicide at the recommended rate of 100 g a.i. ha\(^{-1}\) before planting. Generally, cutting standing residue higher resulted in decreased spray coverage at the soil surface and weed control efficacy, although this was only significant between nil stubble and 30 cm cut height. Percent coverage of sprayed pyroxasulfone, measured by water sensitive paper, declined with increasing amount of horizontal wheat residue on the soil surface. Therefore, increased amounts of wheat residue on the soil surface (horizontal residue) intercepted more herbicide, significantly reduced the efficacy of pyroxasulfone in controlling annual ryegrass in the field and decreased pyroxasulfone concentrations in the soil.

Key words

Conservation agriculture, no-tillage, pre-emergent herbicide, bioassay.
6.1 Introduction

Conservation agriculture (CA) is a widely adopted cropping system comprising minimal soil disturbance, diversified crop rotations and crop residue retention (Hobbs, Sayre & Gupta 2008). Promotion of CA with crop residue retention on the soil surface offers soil fertility benefits, and additional biomass for grazing in integrated crop–livestock systems (Kassam et al. 2012; Conceição, Dieckow & Bayer 2013; Sulc & Franzluebbers 2014). Crop residues are useful for erosion control, water conservation, increased soil organic matter levels, improved soil structure (Unger & Wiese 1979; Prasad & Power 1991; Roper, Gupta & Murphy 2010; Farooq & Siddique 2015), and weed suppression (Unger 1975). Weed suppression by crop residues results from different chemical and physical factors. Lower soil temperatures and shading are physical effects of crop residues that reduce weed growth (Wesson & Wareing 1967). Allelopathy, toxic microbial products, and increased/decreased in soil pH are chemical effects of crop residues that improve weed control (Elliott, McCalla & Waiss 1978; Kimber 1973; Liebl & Worsham 1983).

Crop residue on the soil surface can intercept a considerable amount of herbicide at the time of application (Banks & Robinson 1982; Bauman & Ross 1983; Ghadiri, Shea & Wicks 1984). Significant quantities of atrazine (Ghadiri, Shea & Wicks 1984) and metolachlor (Banks & Robinson 1982; Strek & Weber 1981; Strek & Weber 1982) were intercepted and retained by crop residues after many rainfall events. The interception and retention of herbicide by crop residues reduces the amount of herbicide that reaches the soil, which is likely to reduce their weed control efficacy. Weed control, however, varies when herbicides are applied to soils with and without crop residues. Banks and Robinson (1983) reported that the activity of metolachlor declined with large amounts of wheat residue on the soil surface at application. In contrast, Prihar et al. (1975) found that weed control with or without atrazine increased on plots with crop residue, relative to those without crop residue. Liebl and Worsham (1983) reported that covered soil had better weed control using alachlor than bare soil in some situations. This may be related to the nature of the herbicide, and is unlikely to occur with herbicides that volatilise from crop residue such as the dinitroaniline herbicide trifluralin (Parochetti & Hein 1973; Bedos et al. 2006).

Early control of weeds is crucial for maximising crop yield because weeds that emerge early in the cropping season have the greatest competition effect on yield (Borger et al.
Chapter 6: Effect crop residue height, amount and orientation on pyroxasulfone interception

2010). Therefore, pre-emergent herbicides play an essential role in CA cropping systems. The evolution of herbicide resistance in weed populations is a major driver of change in weed management strategies (Congreve 2015). In Western Australian cropping systems, pre-emergent herbicides are sprayed onto the soil before seeding and then incorporated during the seeding operation (Haskins 2012; Boutsalis, Gill & Preston 2014). Trifluralin, prosulfocarb and pyroxasulfone are the most common pre-emergent herbicides used in Western Australia in no-tillage (NT) systems. Pyroxasulfone is a residual, soil-applied, pre-emergent herbicide for the control of annual ryegrass (Lolium rigidum Lam.), barley grass (Hordeum leporinum), annual phalaris (Phalaris minor), silver grass (Vulpia bromoides) and toad rush (Juncus Bufonius) and the suppression of certain grass weeds in wheat (not durum wheat) and triticale. Pyroxasulfone is an isoxazoline herbicide with a Group K mode of action, which inhibits cell division and long chain fatty acids (Congreve & Cameron 2014; Tanetani et al. 2009).

Lafond et al. (1992) reported significant economic and crop management advantages, such as faster harvest and easier seeding when crops were cut high at harvest then seeded between the residue rows in the following season. Swella et al. (2015) studied the effect of standing crop residue height and horizontal crop residue amount on the capture of rainfall, evaporation from the soil surface and spatial variability of soil water across the standing residue rows. They found that high rates of horizontal residue combined with tall-standing residue maximised soil water content after high rainfall events (between 20 and 50 mm) when compared with lower rates of residue (Swella et al. 2015). A positive effect of cutting crop residue tall in NT systems is the maintenance of a favorable microclimate for plants (Aase & Siddoway 1980; Cutforth & McConkey 1997).

This study investigated the effect of standing crop residue height and the amount of horizontal crop residue on pyroxasulfone interception, leaching from the residue, and weed control efficacy. We hypothesised that: (1) high residue rates, particularly horizontal residue, will reduce the concentration of pyroxasulfone reaching the soil surface and that subsequent rainfall would mitigate this effect by leaching herbicide from the residue into the soil; and (2) more herbicide would reach the soil under tall-standing residue than an equivalent amount of horizontal surface residue and, therefore, have greater weed control efficacy. This research will provide farmers and advisors with more data that will enable them to maximise pre-emergent herbicides efficacy for better weed control in NT systems and thereby for improved crop production in rainfed
Mediterranean-type environments.

### 6.2 Materials and Methods

#### 6.2.1 Site and trial design

Three experiments were conducted with two at Cunderdin in 2015 and 2016 (31°35′03.9″S, 117°19″37.3″E and 31°38′28.7″S 117°14′36.4″E, respectively) and the third at Shenton Park in 2016 (The University of Western Australia 31°56′59.1″S, 115°47′37.1″E). The year before the experiments were implemented, wheat was grown and harvested to leave a standing residue cut to about 30 cm in height. All experiments were in randomised blocks arranged as split plots with four wheat residue heights (standing residue) as the main plots and the amount of horizontal residue as subplots. The size of the main and subplots and treatment layout is detailed in Figure 6.1. The stubble row spacing was 30 cm at Cunderdin and 25 cm at Shenton Park.

The standing wheat residue heights of 0, 10, 20, and 30 cm and the four horizontal wheat residue rates of 0, 1, 2, and 4 t ha\(^{-1}\) were established in May 2015 and 2016. The amount of wheat residue present in each plot before imposing the treatments was determined (Whitfield 1982) and the designated height and amount of wheat residue obtained by pulling, clipping, or raking to remove excess straw or by adding wheat residue by hand spreading. After setting up the different wheat residue treatments (horizontal and...
standing), the ground cover percentage at herbicide application was estimated from a digital photograph of each plot and the “Agronomist Panel” option of ASSESS 2.0 Image Analysis Software, (Lamari 2008). The weight of standing wheat residue (t ha⁻¹) was estimated by cutting and removing the standing stubble at ground level from seven samples in a 40 cm length of row from each main plot. The samples were weighed and converted into t ha⁻¹ using the row spacing. Petri dishes of soil were placed in the plots, along with water-sensitive paper (described later) and all plots were sprayed in May with pyroxasulfone (Sakura®, 850-WG) at 118 g ha⁻¹ (100 g a.i. ha⁻¹) prior to sowing. The herbicide was applied with a field sprayer (9 m wide) fitted with GA110° 02 flat-fan HYPRO® nozzles (GUARDIAN AIR™) delivering 80 L ha⁻¹ at 3 bars, travelling at a speed of 12 km h⁻¹. At Cunderdin, the horizontal stubble was removed and the wheat seeded at 80 kg ha⁻¹ between stubble rows at 30 cm wide spacing using a NDF SA550 single-disc combine seeder with press wheels on 10 May 2016, then the stubble was replaced. However, the experimental site at Shenton Park, was not seeded.

6.2.2 Herbicide interception

Spray coverage from pyroxasulfone application was assessed by placing water-sensitive paper cards (7.6×2.6 cm from Hardi Australia) between and within the rows in each plot at two locations (inter-row and on-row under wheat residue) at the three sites (Cunderdin 2015, Shenton Park and Cunderdin 2016) (Figure 6.2). After spraying, the cards were collected and air-dried. Scanning software was used to create digital images of the cards at a resolution of 1,200 dots per square inch. The SnapCard Spray App was used to assess the percent coverage of spray droplets on each card (Nansen, Emery & Garel 2014). The program was set up to scan 75% of the card area (in the center). Percent card cover is a recognised technique for assessing high spray volumes and imaging systems due to its consistency (Thériault, Salyani & Panneton 2001; Fox et al. 2003). Since spread factor was not taken into account, this method provides a comparative rather than an actual indication of spray coverage.

The amount of herbicide intercepted by the residue and then leached into the soil with, subsequent rainfall, was assessed by estimating the herbicide concentration in the residue and the soil below at various times using a bioassay (Khalil et al. 2018). To do this, Petri dishes (9 cm diameter) containing 50 g of soil were placed on the soil surface adjacent to standing residue rows and underneath horizontal wheat residue (Figure 6.2). The soil used
in the Petri dishes was typical of the Western Australian grainbelt (acidic, sandy loam), which was collected from the surface (0–10 cm) of a farm paddock near to the Cunderdin site (31°35′03.9″S, 117°19′37.3″E). The soil was air-dried and passed through a 2-mm sieve and then analysed (Soil Science Laboratories of The University of Western Australia, Perth and CSBP Soil and Plant Laboratory (www.csbp-fertilisers.com.au)) for texture (74% sand, 12% silt, 14% clay), 4.4 pH (CaCl₂), 2.96 cmol(+)kg⁻¹ CEC (0.02 Al, 2.75 Ca, 0.14 K, 0.45 Mg, 0.07 Na) and 1.8% organic carbon (Rayment & Lyons 2011).

The first set of Petri dishes was collected after herbicide application and before sowing or the first rainfall. In 2015, further sampling occurred only once after herbicide application as the experiment was discontinued due to the disturbance of residue treatments by the planter. For the Cunderdin and Shenton Park sites in 2016, a second set of unsprayed Petri dishes containing soil replaced the first set, which was collected and replaced by third and fourth sets after each rainfall event. Only first set of Petri dishes were sprayed with herbicide, then removed to be replaced by second, third, and fourth set to assess leachate from crop residue into the soil. Rainfall amount at each site was recorded for the duration of the experiment using a tipping bucket gauge.

Samples of horizontal and standing wheat residue were taken at the same sampling times as the Petri dishes. Depending on the height of the residue, between 25–100 cm row-length of standing wheat residue was cut at the soil surface in each plot, or removed for the horizontal residue treatment, to obtain 25 g of residue from each plot. The wheat residue was air-dried and ground into small particles (~85% of the particles ranged from 1–4 mm) using a mechanical plant material grinder (www.retsch.com). To minimise sample contamination, the grinder was cleaned after each batch, using a vacuum and then air compressor blower. The ground wheat residue samples and Petri dishes containing soil were placed in plastic bags and stored at −20°C prior to conducting bioassays to determine the concentration of pyroxasulfone remaining in the soil and the wheat residue.

6.2.2 Herbicide bioassays

The method for the bioassays is described in chapter 3 (2018). Briefly, the bioassays were conducted in a growth room on shelves equipped with LUMILUX® cool white fluorescent lamps (Model L36W/840, OSRAM), with photosynthetically active radiation at the top of the plants of 109 µmol m⁻² s⁻¹ (SD ± 5 µmol m⁻² s⁻¹), and a 12-hour photoperiod. The air temperature was maintained at 25/22.5°C (SD ± 2/1°C) during the light/dark period.
Relative humidity was 70% (SD ± 10%). For the bioassay, five seeds each of annual ryegrass (*Lolium multiflorum* L.) and cucumber (*Cucumis sativus* L.) were planted in the same Petri dish at 1 cm depth into either 50 g of soil or 5 g of ground wheat residue. The plants were hand watered daily with deionised water by adjusting the moisture of the medium to near field capacity (Somasegaran & Hoben 1985). After seven days, the media was washed from the plants with running tap water and the plants removed for shoot length measurements. The percent shoot length inhibition from the untreated control (UTC) was calculated for each media using the formula \[ \text{shoot length (\% of untreated control)} = \frac{L_t}{L_0} \times (100) \], where \( L_t \) is shoot length measured in herbicide-treated soil or wheat residue, and \( L_0 \) is shoot length in untreated soil or wheat residue.

### 6.2.3 Weed control

To assess the effect of wheat residue amount and architecture on weed control efficacy of pyroxasulfone, a total of 32 seeds of susceptible annual ryegrass were planted in each plot before herbicide application at two positions (32 seeds in each position) within each plot (Figure 6.2). The annual ryegrass was counted twice (after emergence and one month later) and averaged across both positions.

Figure 6.2 Diagram showing location of annual ryegrass planting, Petri dishes with soil, sampling of standing and flat residue, and water sensitive card. For visual ease, the photograph represents the treatment with 30 cm standing residue and 0 t ha\(^{-1}\) (amount).
6.2.4 Data analysis

Data from the different sites and years (Cunderdin 2015, Shenton Park 2016 and Cunderdin 2016) were used for analysis where the full data sets were available. The data were tested for normality and homogeneity of variance before performing ANOVA, using GenStat 12 (Payne et al. 2009), with differences considered significant $P \leq 0.05$. Data from all sites/years were used for percent coverage from the spray cards. The analysis tested the four way interaction between sampling location, within and between rows (i.e. on-row or inter-row in a plot) $\times$ residue amount (horizontal residue sub-plot) $\times$ residue height (standing residue main plot); the design was nested from the smallest to largest units as location(sampling)/horizontal/standing/block/site.

For the annual ryegrass counts, data from Shenton Park 2016 and Cunderdin 2016 were considered in the same analysis. As numerous measurements were taken from the same plots, a repeated measures ANOVA was performed.

Pyroxasulfone concentrations (mg kg$^{-1}$) in the soil were estimated from the bioassay shoot length data, using the dose response equations developed in Chapter 3 (Khalil et al. 2018). The concentration data from each experiment were analysed separately and a repeated measures ANOVA performed where multiple measurements were made on the same site/plot data.

3. Results and Discussion

3.1 Rainfall

The first sampling at both sites occurred after herbicide application and before the rain. Subsequent samplings were repeated after each of three rainfall events (21.8, 77.8, and 67.2 mm) at Shenton Park 2016 and two rainfall events (39.2 and 24.4 mm) at Cunderdin 2016 (Figure 6.3).
3.2 Residue percent coverage

Ground cover percentage of different combinations of horizontal and vertical wheat residue were averaged across experimental sites and summarised in Table 6.1.

Table 6.1 Ground cover percentage (%) of different combinations of horizontal wheat residue amounts and vertical residue heights estimated by ASSESS 2.0 (Lamari 2008). Values are means across all sites. LSD = 20.63 at P-value = 0.05

<table>
<thead>
<tr>
<th>Wheat residue height (cm)</th>
<th>Wheat residue amount (t ha(^{-1}))</th>
<th>0 t ha(^{-1})</th>
<th>1 t ha(^{-1})</th>
<th>2 t ha(^{-1})</th>
<th>4 t ha(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 cm</td>
<td></td>
<td>0</td>
<td>40</td>
<td>43</td>
<td>91</td>
</tr>
<tr>
<td>10 cm</td>
<td></td>
<td>12</td>
<td>41</td>
<td>75</td>
<td>97</td>
</tr>
<tr>
<td>20 cm</td>
<td></td>
<td>28</td>
<td>47</td>
<td>77</td>
<td>89</td>
</tr>
<tr>
<td>30 cm</td>
<td></td>
<td>37</td>
<td>72</td>
<td>87</td>
<td>100</td>
</tr>
</tbody>
</table>

The amounts of standing wheat residue (t ha\(^{-1}\)) are summarised in Table 6.2, with higher weights with taller residue.

Table 6.2 The amount of standing wheat residue (t ha\(^{-1}\)) available on the soil surface at Cunderdin 2016 and Shenton Park 2016

<table>
<thead>
<tr>
<th></th>
<th>Shenton Park 2016</th>
<th>Cunderdin 2016</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 cm</td>
<td>10 cm</td>
<td>20 cm</td>
</tr>
<tr>
<td>Shenton Park 2016</td>
<td>0</td>
<td>0.6</td>
<td>0.81</td>
</tr>
<tr>
<td>Cunderdin 2016</td>
<td>0</td>
<td>0.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Average</td>
<td>0</td>
<td>0.6</td>
<td>1</td>
</tr>
</tbody>
</table>

3.3 Water-sensitive paper (percent spray coverage)

The only significant interaction found was between horizontal residue amount (0–4 t ha\(^{-1}\))
and sampling location i.e. between and within row (on or inter-row) \( (P = 0.031) \). With no horizontal residue, there appeared to be greater spray coverage at the inter-row location than in the stubble row (on-row). As expected, the percent coverage of sprayed pyroxasulfone decreased with increasing amounts of horizontal residue on the soil surface (Figure 6.4), due to herbicide interception by residue on the soil surface, which is similar to the findings of other studies (Crutchfield, Wicks & Burnside 1986; Ghadiri, Shea & Wicks 1984; Bauman & Ross 1983; Banks & Robinson 1982; Erbach & Lovely 1975). Borger et al. (2010) also reported increased percent spray coverage with greater spray volumes. The least spray coverage, occurred when the water-sensitive card was placed in the standing wheat residue row with 4 t ha\(^{-1}\) of flat residue on the soil surface, although this was similar to the inter-row position (Figure 6.4).

Across all sites and both seasons, cutting standing residue to different heights only had a small effect on pyroxasulfone spray coverage, with slightly lower coverage at the 30 cm cut height (by about 3–4%), which was significantly different to the 20 cm and 0 cm heights (Figure 6.5). Overall, the amount of horizontal wheat residue on the soil surface
accounted for most of the herbicide interception relative to different vertical residue heights (Figures 6.4 and 6.5).

3.4 Annual ryegrass counts (weed control efficacy)

There was no interaction between the amount of horizontal wheat residue and the height of standing wheat residue on pyroxasulfone efficacy in controlling annual ryegrass. The efficacy of pyroxasulfone in controlling annual ryegrass at both sites declined significantly when the amounts of horizontal wheat residue increased. This was shown by the increased number of annual ryegrass plants with increasing amounts of horizontal wheat residue on the soil surface, due to the increased interception of herbicide which prevented the herbicide from reaching the soil surface (Figure 6.6). Banks and Robinson (1983) reported that crop residue reduced the amount of metolachlor in the soil after overhead irrigation. Erbach and Lovely (1975) found that the quantity of corn residue on the soil surface at the time of herbicides application had some impact on weed control with atrazine and alachlor in the field. In contrast, other results have shown no effect of
crop residues on reducing weed control efficacy. For example, Crutchfield et al. (1986) and Prihar et al. (1975) reported that weed populations and weed biomass decreased with increasing wheat residue level and metolachlor rate. Crutchfield et al. (1986) also reported that wheat residue had no effect on weed control, even with half the recommended rate of metolachlor. This may be due to a smothering effect of the residue weeds, which compensates for the reduction of herbicide in the soil (Crutchfield, Wicks & Burnside 1986). They also reported that sufficient weed control occurred with 3.7–7.5 t ha$^{-1}$ of wheat residue combined with half the recommended rate of metolachlor. In the current study, amounts of horizontal wheat residue were generally below these levels, except at the highest rate of 4 t ha$^{-1}$. In addition, Day (1968) reported that smothering of weeds by crop residues might weaken weed seedlings (but not kill them) so that it was easier for the herbicide to control them.

The lower weed control efficacy with increased residue amount is in contrast to the previous chapters, which reported high pyroxasulfone efficacy on annual ryegrass, even

Figure 6.6 Density of annual ryegrass (plant m$^{-2}$) as a function of increasing amount of horizontal wheat residue (0–4 t ha$^{-1}$) at both sites (Cunderdin and Shenton Park) in 2016. Bars show LSD at $P = 0.05$ for comparisons of interaction effects between counting date and amount of horizontal wheat residue across both sites.
Chapter 6: Effect crop residue height, amount and orientation on pyroxasulfone interception

with relatively high amounts of crop residue. These differences are likely due to the controlled conditions for the previous experiments, where the soil under the crop residue was placed into ‘ideal’ conditions for the bioassay, which provided adequate water at all times. Also, the indicator plants in the bioassay were grown in a medium with herbicide uniformly mixed throughout. In the current field study, the annual ryegrass roots may have grown deeper into untreated soil. Also, no rain fell prior to the first count date, therefore lower herbicide efficacy is expected, due to insufficient water for herbicide uptake. With subsequent rainfall, the efficacy then improved at the second count date.

The population of annual ryegrass declined between the two counting dates when an interaction effect occurred between counting dates of annual ryegrass and the horizontal wheat residue amount (Figure 6.6). This reduction in annual ryegrass count between the two dates is mainly attributed to fact that pyroxasulfone has little effect on germination but does affect subsequent growth, which then leads to plant death (Tanetani et al. 2009); it also has a residual effect (Knezevic et al. 2009; Boutsalis, Gill & Preston 2014; Yamaji et al. 2014). Also, rainfall between the dates may have activated more herbicide in the soil and, in the case of horizontal residue, leached additional herbicide from the residue into the soil.

![Figure 6.7](image-url)

**Figure 6.7** Population of annual ryegrass (of 32 seeds planted), as a function of standing wheat residue at different heights (0–30 cm) averaged across both sites (Cunderdin and Shenton Park) in 2016. Overall means are presented (P < 0.001). Bar shows LSD at P = 0.05 for comparisons of chopped wheat residue across both sites.

The efficacy of pyroxasulfone in controlling annual ryegrass under field conditions across
both sites declined slightly with increasing residue height, although this was only significant between no residue (0 cm) and 30 cm cut height (Figure 6.7).

3.5 Pyroxasulfone concentrations in the soil estimated by bioassay

At Cunderdin 2015, where soil sampling only occurred once (after herbicide application and before any rainfall) the estimated concentration of pyroxasulfone in the soil underneath the crop residue declined significantly with increasing amounts of horizontal wheat residue on the soil surface; 4 t ha\(^{-1}\) of horizontal residue had significantly lower concentrations than bare soil and 1 t ha\(^{-1}\) (Figure 6.8). The same effect was observed at both sites in 2016. A significant interaction occurred between the amount of horizontal residue and soil sampling time. This was because there was significantly higher pyroxasulfone concentration in the soil with the lower amounts of residue compared with higher amounts of residue for the first timing and no significant differences for the later timings as concentrations diminished; although, there was the same trend with higher concentrations present at lower amounts of residue.

Crutchfield et al. (1986) reported that the differences between wheat residue levels decreased with time, but the decreasing trend was still present at each sampling date. The
later the soil was sampled after rainfall events, the less pyroxasulfone was found in the soil (less washed off wheat residue into the soil underneath by rainfall). Other researchers reported similar findings (Strek & Weber 1981; Strek & Weber 1982): metolachlor concentrations in the soil decreased with increasing crop residue on the soil surface at 2, 8, and 18 weeks after herbicide application, which was probably due to the retention of metolachlor on the residue or volatilisation from the residue.

Managing crop residues in NT systems is a compromise between protecting the soil surface with ground cover and optimising residue at seeding time. The height of residue cover and its horizontal profile can be important for minimising wind erosion. Standing plant material is more effective than flat residues for run-off and wind erosion control, as it is less likely to be carried away due to its intact roots (Bowman & Scott 2009). Horizontal residue, measured as ground cover, also reduces or eliminates water run-off, allowing more time for water infiltration. Nonetheless, horizontal residue cover is effective only if it is not carried away with the run-off; so the effectiveness of flat residue is enhanced by the presence of some standing crop residue (Bowman & Scott 2009).

Tall pastures and crops (>30 cm), and their standing residues, are most effective at minimising wind erosion. If cereal stubble is standing (30–60 cm tall), 20 to 30% cover is required to reduce the risk of erosion, as the standing stalks greatly reduce the wind speed at the soil surface (Findlater & Riethmuller 2000). With horizontal residue, about 50% of the surface should be covered to control wind erosion. This is approximately 0.75–1 t ha⁻¹ of cereal residues or 1.5 kg ha⁻¹ of lupin residue (Carter 2002). Much higher levels are required for wind erosion control if the plant residues are easily blown by the wind (for example, field pea stubble). Precision agriculture systems allow growers to sow through standing stubble using wider row spacings and inter-row sowing (Bowman & Scott 2009). An ‘acceptable’ horizontal stubble rate for weed control purposes, and ‘acceptable’ stubble rate for erosion control can overlap to simultaneously achieve the two goals, especially as standing crop residues are better than horizontal ones at reducing erosion. Taller crop residues will also leave less horizontal residue, resulting in better herbicide efficacy herbicide coverage and improved weed control efficacy.

6.4 Conclusion

The efficacy of pyroxasulfone, the pre-emergent herbicide commonly used in NT systems was investigated in field experiments at two sites over two growing seasons (2015–2016).
There was no interaction between horizontal and standing vertical wheat residues on the efficacy of pyroxasulfone for controlling annual rye grass. Horizontal residue had the greatest impact on pyroxasulfone interception. Percent cover of sprayed pyroxasulfone, herbicide concentration in the soil and weed control efficacy declined significantly with increasing amounts of horizontal wheat residue on the soil surface, especially with 4 t ha\(^{-1}\) of residue. Generally, taller standing residue resulted in decreased spray coverage at the soil surface and weed control efficacy, although this was only significant between nil stubble and 30 cm cut height.

Herbicides differ in their suitability for use in NT systems and crop residues vary in their effectiveness at covering the soil. Cereal (small grains) residues are more effective at providing soil cover per unit weight compared with other crop residues (Greb 1967), although this is likely to decrease herbicide efficacy. Crop residue will intercept herbicides on application, but this process alone will not always reduce weed control, as results with other herbicides and crop residues have been found to vary from those observed in these experiments. The total effect of crop residues on weed control will rely on many factors, since crop residues affect weed growth differently.
Crop residue retention is considered an important component of conservation agriculture (CA), due to its many benefits to soil and crop productivity (Verhulst et al. 2010; Kassam et al. 2012; Serraj & Siddique 2012). Increased crop residues on the soil surface along with their management techniques may impact the fate of herbicides such as alachlor (Locke, Gaston & Zablotowicz 1996), atrazine (Levanon et al. 1994), bentazone (Wagner et al. 1996), chlorimuron-ethyl (Reddy, Zablotowicz & Locke 1995), metolachlor (Levanon et al. 1994) and metribuzin (Locke & Harper 1991) in the soil and their efficacy in weed control. Three main possible mechanisms of herbicide dissipation in NT systems (interception, movement, degradation) were discussed. Some herbicides might be washed off residue by rainfall, increasing their efficacy in the soil. The literature review revealed considerable research on the loss of soil-active herbicides under conventional systems, but much less under CA systems with high levels of crop residue, and the few reports were inconsistent. In addition, little work has been undertaken in Australian conditions and research done elsewhere in different environments, may not be valid under local situations with different crops, soils and climate (Chauhan, Gill & Preston 2006). Prior to this study, the interaction of pre-emergent herbicides and crop residues in Western Australian NT systems had not been documented and herbicides that have been studied are not the herbicides commonly used in WA. For instance, Borger et Al. (2013) reported a lack of information to evaluate the effect of residue height/amount on the efficacy of trifluralin and pyroxasulfone under NT systems. With the crucial role of crop residues in NT systems in WA, further research was conducted to better understand interception and leaching of prosulfocarb, pyroxasulfone and trifluralin, three commonly used pre-emergent herbicides, under NT systems with residue retention. The focus was on the interception and leaching of the herbicides from crop residues by rainfall under local conditions. In order to assess the interception and leaching of the herbicides, bioassays for the three herbicides in soil and crop residues were first developed (Chapter 3).

The main research questions arising from the review of literature presented in chapter 2 were:
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What is the effect of rainfall amount, intensity and timing on the leaching of prosulfocarb, pyroxasulfone and trifluralin from crop residue? (Chapter 4).

What is the effect of crop residue type and age on the sorption and leaching of prosulfocarb, pyroxasulfone and trifluralin? (Chapter 5).

What is the effect of residue height, amount and orientation on pyroxasulfone interception and leaching into the soil and its weed control efficacy? (Chapter 6).

This general discussion includes a summary of the main findings in each research chapter and their practical implications from the perspective of improving the efficacy of pre-emergent herbicides in NT farming systems in WA. The chapter concludes with future research priorities.

Key observations and management implications

Chapter 3: A bioassay for prosulfocarb, pyroxasulfone and trifluralin detection and quantification in soil and crop residues

Key Findings:
1. Annual ryegrass (Lolium multiflorum Lam) was the most sensitive species to all tested herbicides; beetroot (Beta vulgaris L.) and cucumber (Cucumis sativus L.) had lower and similar sensitivities to the three herbicides for shoot inhibition.
2. The developed bioassay uses annual ryegrass shoot inhibition at relatively low suspected concentrations of herbicide, and cucumber shoot inhibition for higher rates.
3. The herbicide concentrations derived from chemical analyses and the bioassays had highly significant linear correlation.
4. The results indicated that the bioassay calibration curves are suitable for estimating herbicide concentrations in crop residue collected soon after harvest and in a sandy loam soil that is low in organic matter.

Methods of chemical analyses of herbicide residues in the soil or crop residue are used in most studies. These methods are sophisticated, time-consuming and expensive. In contrast, relatively simple (Eshel & Warren 1967) bioassays are important for the quantitative analysis of herbicides in soil and water and the determination of herbicide bioavailability in soil, which is useful for agricultural research (Blair & Martin 1988; Rahman, James & Mortimer 1988a; Eshel & Warren 1967; Lavy & Santelmann 1986b). A two-plant species bioassay was developed and validated to assess the bioavailability in
crop residue and sandy loam soil for the main pre-emergent herbicides used in Western Australian NT systems as a function of shoot length inhibition of the two bio-indicators (annual ryegrass, cucumber). The developed bioassay will be useful for investigating the effect of crop residue condition on the interception and efficacy of pre-emergent herbicides in NT systems. The bioassay could be used where herbicide concentrations are relatively low to approximately twice the recommended field rates.

Chapter 4: Rainfall leaching herbicide from wheat residue into the soil is greatest with pyroxasulfone and least with trifluralin

**Key Findings:**
1. Most of the herbicide was leached from crop residue into the soil at higher rainfall amounts occurring soon after herbicide application, and in one event, rather than multiple events.
2. Rainfall intensity had little effect on the amount of herbicide that leached from the residue into the soil.
3. Rainfall was very effective at leaching pyroxasulfone from the residue into the soil to provide good weed control, even with heavy residues of 4 t ha\(^{-1}\) when rainfall occurred up to 14 days after herbicide application.
4. Rainfall was less effective at leaching prosulfocarb; improved weed control (from the leached chemical) only occurred with rain up to 7 days after herbicide application.
5. Rainfall was least effective in leaching trifluralin from the residue into the soil, with little improvement in weed control when rainfall occurred one day after herbicide application.

Pre-emergent herbicides should be applied to the soil, and many require incorporation by rainfall, irrigation, tillage or, in the case of NT systems, the seeding operation (Rainbow & Derpsch 2011; Kleemann et al. 2015; Haskins 2012). The activity of pre-emergent herbicides applied to crop residues depends not only on the physicochemical properties of herbicides, but the amount and origin of crop residues, spray volume, and the period prior to the first rainfall event after application and the duration of following rainfall events (Lamoreaux, Jain & Hess 1993; Rodrigues 1993; Watts & Hall 1996). Many farmers now start seeding prior to the seasonal rain, under dry soil conditions (Fletcher et al. 2015). Therefore, it is important to understand the longevity of pre-emergent herbicides on crop residues and their interaction with subsequent rainfall following herbicide application.
In this research some herbicide leached from the residue with as little as 5 mm of rainfall, although higher rainfall amounts generally leached more herbicide. The greatest amount of herbicide leaching from the residue into the soil occurred with rainfall soon after herbicide application, compared with later rainfall. Rainfall intensity had no significant effect on herbicide leaching from residue for any of the herbicides. Multiple rainfall events (four 5-mm events over two days) leached slightly less of the intercepted herbicide from the wheat residue compared with the same amount of rainfall in a single event. Granovsky et al. (1994) reported that a strong rainfall event soon after atrazine application compared with low rainfall events washed more of the chemicals intercepted by crop residue into the soil. Our study highlighted that pyroxasulfone leached easily from the residue into the soil for up to 14 days to potentially offer good weed control, prosulfocarb had intermediate leaching, while only a small amount of trifluralin leached from stubble with rain one day after application. In situations where a considerable amount of residue is on the soil surface, it is recommended that pyroxasulfone is used or irrigation (where available) is applied immediately after herbicide application.

Chapter 5: Effect of crop residues on the interception and activity of prosulfocarb, pyroxasulfone, and trifluralin

Key Findings:

1. Less chemical leaching from crop residue into the soil after rainfall occurred when prosulfocarb and trifluralin were applied to wet residue than dry residue but the initial moisture condition did not affect the leaching of pyroxasulfone from residue.
2. Increased amounts of wheat residue intercepted more herbicide, with a large increase in interception from 2 to 4 t ha$^{-1}$.
3. After simulated rainfall, 100% annual ryegrass death in the bioassay occurred only with bare soil for trifluralin, bare soil and 1 t ha$^{-1}$ of residue for prosulfocarb, and all residue treatments up to 4 t ha$^{-1}$ for pyroxasulfone.
4. Barley and wheat residues intercepted more herbicide than an equivalent amount of canola, chickpea or lupin residue, which was largely due to the increased ground cover with cereal residues.
5. More herbicide reached the soil when sprayed on one-year old residue compared with new residue, which was largely due to reduced ground cover with aged residue.
6. *There was a strong positive linear relationship between ground cover percentage and growth of bioassay species. Therefore ground cover percentage was a better measure of potential herbicide interception by residue than amount of residue.*

Herbicide intercepted by crop residues at application is impacted by several of factors (physio-chemical properties of herbicides and crop residue properties). For instance, wet residue had a lower sorption capacity for herbicides than the soil underneath (Unger 1994). Banks and Robinson (1986) reported that the amount of acetochlor, alachlor, and metolachlor reaching the soil declined as the amount of wheat residue on the soil surface increased from 0 to 6.7 t ha⁻¹. However, Morrison et al. (1985) reported that percent ground cover might be more closely related to herbicide interception than the amount. Dao (1991) reported that the lignin content of plant stubble might be responsible for most of its sorptive capacity while cellulose, the more abundant plant material, has little impact. Furthermore, older, partially decomposed straw appears to adsorb more herbicide than fresh straw (Unger 1994).

The results of this study showed that in an NT system with crop residue covering the soil, more prosulfocarb and trifluralin reached the soil after rainfall when the herbicides were sprayed onto initially dry residue than the wet residue. In contrast, the moisture condition of the residue at the time of herbicide application had little effect on the amount of pyroxasulfone that reached the soil after subsequent rainfall. Practically, farmers should minimise spraying prosulfocarb and trifluralin into wet crop residue, otherwise they may need to increase the application dose to compensate for the loss of herbicide when sprayed onto wet residue. In the absence of simulated rainfall, the growth of the bioassay species in the soil increased with increasing residue amount and there was a marked increase in shoot length as residue quantity increased from 2 to 4 t ha⁻¹ as a result of the reduced herbicide concentration. The reduction in the amount of herbicide reaching the soil was due to crop residue interception (Banks & Robinson 1982; Bauman & Ross 1983; Erbach & Lovely 1975; Ghadiri, Shea & Wicks 1984). This suggests that pyroxasulfone as a pre-emergent herbicide in NT system is recommended when the amount of horizontal residue on the soil surface exceeds 2 t ha⁻¹. In cases where horizontal residue on the soil surface is less than 2 t ha⁻¹, prosulfocarb could also be used. Aged residue generally had less ground cover than fresh residue for canola, chickpea and lupin, but the differences were relatively small for cereal residues. Lupin stubble had the least ground cover, followed by chickpea, canola and cereals, which had >90% ground cover with 4 t ha⁻¹ of applied
residue. Herbicide remaining on the residue was partially influenced by crop residue type. With 4 t ha\(^{-1}\) of crop residue, the least herbicide reached the soil with barley residue followed by wheat residue, canola residue was intermediate but variable, and the most herbicide reached the soil with chickpea and lupin residues. Ground cover percentage and growth of bioassay species had a strong positive linear relationship. Rainfall increased the amount of herbicide reaching the soil, especially with pyroxasulfone. This highlights the effectiveness of rainfall to wash pyroxasulfone from the residue into the soil, for good weed control in high residue NT systems. For instance, 100% annual ryegrass control was achieved with pyroxasulfone after rainfall, even with 4 t ha\(^{-1}\) of residue.

**Chapter 6: Effect of wheat residue height, amount and orientation on pyroxasulfone interception, leaching, and weed control efficacy in the field**

**Key Findings:**

1. The amount of horizontal residue had a greater effect on the interception of herbicide than the cut height of the stubble.
2. Increased amounts of horizontal wheat residue on the soil surface significantly:
   a. Reduced the proportion of pyroxasulfone reaching the soil surface.
   b. Reduced the efficacy of pyroxasulfone for controlling annual ryegrass in the field, with the most herbicide intercepted (higher weed population) with 4 t ha\(^{-1}\) of horizontal wheat residue.
   c. Reduced the concentrations of pyroxasulfone in the soil
3. Taller standing residue intercepted more herbicide than shorter, although only stubble cut to 30 cm in height intercepted significantly more herbicide than no stubble present.

The interception and retention of herbicide by crop residues reduces the amount of herbicide reaching the soil, which is expected to reduce their efficacy in controlling weeds. In Western Australian cropping systems, pre-emergent herbicides are sprayed onto the soil before seeding and then incorporated during the seeding operation (Haskins 2012; Boutsalis, Gill & Preston 2014).

This study revealed that there was no interaction between horizontal and vertical wheat residue on the efficacy of pyroxasulfone in controlling annual ryegrass under NT farming system. The most dominant factor impacting the amount of pyroxasulfone reaching the soil surface and weed control efficacy was the amount of horizontal wheat residue on the soil surface. Increased amounts of horizontal stubble intercepted more herbicide and
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reduced weed control efficacy, especially at 4 t ha$^{-1}$. Taller of standing wheat residue intercepted more pyroxasulfone relative to shorter heights, but only a 30 cm cut height was significantly different to bare soil. The estimated concentrations of pyroxasulfone in the soil declined significantly with increased amounts of horizontal wheat residue on the soil surface, but only the 30 cm cut height was significant. Nonetheless, the amount of horizontal residue had a greater effect than stubble height. Therefore, farmers could improve their weed control in high residue NT systems by cutting the stubble higher, thereby leaving less horizontal material on the soil surface. In addition, partially grazing paddocks by sheep is probably the main cause of large amounts of horizontal crop residues (i.e. trampling). Herbicides differ in their suitability for use in NT systems, and crop residues vary in their effectiveness at covering the soil. Cereal (wheat and barley) residues are highly effective at providing soil cover per unit weight compared with other crop residues (Greb 1967); therefore they will intercept more herbicide. The results for other herbicides and crop residues may vary from those observed in these experiments. Crop residue will intercept herbicide, but this process alone does not always reduce weed control. The total effect of crop residue on weed control relies on many factors, since crop residues affect weed growth differently.

Our research may give farmers and advisors more confidence to alter residue management to maximise pre-emergent herbicide efficacy for better weed control in NT systems, which may be important for crop production in rainfed Mediterranean-type environments.

7.1 Future research priorities

This study has identified some research opportunities, which are summarised below:

1. Investigate the implication and suitability of the developed bioassay in Chapter 3 for other pre-emergent herbicides used in NT farming systems.

2. Test and validate this bioassay by assessing samples from different locations and farming backgrounds (Chapter 3).

3. If possible, repeat the experiments in Chapter 4 under field conditions and sample the soil in the field to better understand the effect of rainfall on herbicide leaching. Also, intercepted herbicide losses (photodegradation, biodegradation, and wash off by rain) from crop residue need further research to assess how much is lost through each pathway.

4. Determine for how long pyroxasulfone, relative to trifluralin and prosulfocarb, will
leach from crop residue into the soil (Chapter 4).

5. Assess the optimal rates of different pre-emergent herbicides for good weed control at different ground cover percentages of crop residue. Herbicide rates could then be adjusted (within the boundaries of the current herbicide label) to account for residue levels present in different fields (Chapter 5).

6. Further investigation of the field experiment in Chapter 6 to better understand and quantify other factors (seeding operation, dry conditions after herbicide application, and strong rainfall events after herbicide application). More pre-emergent herbicides could be tested under the same experimental conditions to assess their behaviour.

### 7.2 Final remark

The research within this PhD study highlighted the significant capacity of crop residue to intercept and prevent the tested pre-emergent herbicides from reaching the soil surface to better control weeds. With this knowledge, it is possible to promote management practices to counter the reduction in herbicide efficacy when sprayed onto crop residues in NT systems. Therefore, this thesis is a significant contribution to the development of sustainable weed control strategies. This research identified the rainfall effect on leaching intercepted pre-emergent herbicides, and the effect of crop residue conditions and types on the sorption and leaching of pre-emergent herbicides, and orientation in the field on the efficacy of pyroxasulfone in controlling annual ryegrass. As a result of this research it should be possible to increase the efficacy of pre-emergent herbicides applied in NT systems with residue retention on the soil surface. NT systems with crop residue retention have been widely adopted in Australia and worldwide, and weed control is considered the most problematic issue in Australian grain growing regions. However, if the information in this thesis used by industry to improve the efficacy of pre-emergent herbicides, then weeds will be more successfully controlled in Australian NT systems with crop residue retention.
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