SOIL CRC

Performance through collaboration

Developing knowledge and tools to better manage herbicide residues in soil

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Dr Michael Rose

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CONTENTS

PROJECT PARTICIPANTS

Michael Rose, NSW Department of Primary Industries
Lukas Van Zwieten, NSW Department of Primary Industries
Josh Rust, NSW Department of Primary Industries
Scott Petty, NSW Department of Primary Industries
Richard Bell, Murdoch University
Gavan McGrath, Murdoch University
Win Win Pyone, Murdoch University
Simon Yeap, Murdoch University
Terry Rose, Southern Cross University
Lee Kearney, Southern Cross University
Amanda Cook, Department of Primary Industries and Regions, SA
Naomi Scholz, Ag Innovation and Research Eyre Peninsula
Mark Stanley, Ag Innovation and Research Eyre Peninsula
Kelly Angel, Birchip Cropping Group
David Minkey, Western Australian No-Tillage Farmers Association

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The Soil CRC recognises the value of knowledge exchange and the importance of objective peer review. It is committed to encouraging and supporting its research teams in this regard.

The author(s) confirm(s) that this document has been reviewed and approved by the project's steering committee and by its program leader. These reviewers evaluated its:

- Originality
- Methodology
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- compliance with ethical guidelines
- conclusions against results
- conformity with the principles of the Australian Code for the Responsible Conduct of Research (NHMRC 2018), and provided constructive feedback which was considered and addressed by the author(s).

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Herbicides, carryover, plant-back, soil sorption, phytotoxicity, pesticide residues

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EXECUTIVE SUMMARY

Herbicides are widely used in Australian agricultural systems for weed control. Some herbicides can persist in soil long enough to carry over from one cropping season to the next, which can pose a risk of injuring the following crop. There are currently few resources (i.e. information, tools, services) available to farmers to help determine if herbicide residues are causing yield losses. In this project we aimed to develop new knowledge and tools for managing herbicide residues, including: i) defining herbicide concentration in soils that cause damage to crops (i.e. 'toxicity thresholds'); ii) developing new methods to diagnose herbicide injury in crops; iii) assessing rapid methods to determine herbicide sorption in soil, which regulates herbicide fate and bioavailability; iv) predicting herbicide persistence in soil over time; and v) determining whether herbicide residue mixtures are influencing crop performance. We generated 80 new herbicide toxicity thresholds; derived new pedotransfer functions to predict sorption of three priority herbicides in soil; created and validated a new model to predict herbicide persistence in soil; and conducted over 30 field surveys and designed experiments to measure the fate and effects of herbicide residues in soil. The information was translated to farmers, agronomists, and researchers through annual field days at three different experimental sites, and over 10 conference presentations, research booklets, and journal articles. Feedback from growers and agronomists suggests that information generated in this project would help build confidence in decision making to avoid herbicide residue damage, by better diagnosing when, where, and how herbicides are persisting in soil and causing crop damage. More research and translation is now required to validate findings from this project under a wider range of conditions and establish a testing service for herbicide residues in soil and plant samples.

OBJECTIVES

The overall purpose of this research project was to minimise crop damage from herbicide carryover in soil, thereby improving yield and soil productivity, through the following aims:

i) to determine phytotoxicity thresholds for a range of crops exposed to five priority herbicides, based on new measures of bioavailable herbicide residues in soil

ii) to improve identification of herbicide damage by biochemical fingerprinting (metabolomics/MIR) of affected plants

iii) to develop a new tool based on infrared spectroscopy (MIR) that can predict sorption and degradation of five priority herbicides in a range of soil types

iv) to validate a site-specific model that can predict the phytotoxicity of five priority herbicides remaining in soil at a designated time after application across the range of climates, weather and soil types from the CRCs Grower Groups

v) to quantify the extent of yield loss caused by herbicide residues in a minimum of three different environments.

Through achieving these aims we sought to help fulfil the wider long-term objectives of:

i) eliminating acute and chronic crop losses due to residual herbicides in soil

ii) empowering farmers to make sound soil management decisions related to herbicides to improve their agronomic flexibility, and facilitate opportunistic cropping or pasture breaks

iii) providing farmers with tools and models to enable intercropping and cover cropping while maintaining effective weed control.

RESULTS

i) Herbicide toxicity thresholds for the effect of soilborne residues on seedling shoot biomass were developed for 6 different herbicides in sand and soil, for 8 different crop types. Crop tolerance rankings were determined for each of these herbicides.

ii) Critical damage thresholds for herbicide in plant leaf tissue were defined for 6 different herbicides, which enabled leaf testing under field conditions to diagnose whether and/or which herbicides were causing crop injury.

iii) The sorption of three herbicides with contrasting physicochemical properties (diuron, imazapyr and pyroxasulfone) was determined on 48 different soil types. Using this data, we developed new pedotransfer functions based on MIR spectroscopy combined with machine learning to predict sorption of these herbicides across a range of soil types.

iv) The persistence of imazapyr, clopyralid, diuron and pyroxasulfone was monitored at several different sites over two cropping seasons. Monitoring data showed that persistence was strongly related to rainfall, where <100 mm of rainfall in the 180 days post application led to greater persistence of herbicides in soil and higher risk to following crops. A stochastic solute transport model was developed to predict retention and mobilisation of herbicides in soils by accounting for the inherent variability in climate. The model was applied to a field trial measuring imazapyr concentrations in shallow, 0 – 10 cm and deeper, and 10 – 30 cm soils. The model reproduced the observed concentrations well with parameters that were relatively consistent between sites. Differences in the cumulative rainfall between sites looked to significantly influence the vertical distribution and the annual carryover of the herbicide. A 100-year simulation using a calibrated model was then applied to estimate the likelihood of carryover of herbicide exceeding a threshold.

v) Replicated, designed field experiments were established at three different sites to determine the effect of herbicide residue mixtures on soil health relative to control treatments where herbicide use was withdrawn. These experiments were monitored over two seasons. At the Victorian site, no significant effects were measured; at the SA site, hand-weeding (no herbicide) control plots yielded significantly lower than plots receiving full farmer practice herbicide application in one season; and in the WA site, diuron application in one season reduced crop yields relative to control (hand-weeded) plots. This highlights the site-specific nature of herbicide benefits and potential costs, and reinforces the need for greater tools and knowledge to help avoid potential crop injury caused by herbicides.

NEXT STEPS	TIMING
• Initiate provision of a soil and plant tissue testing service for herbicide residues in soil	• Within 6 -12 months
• Conduct a pilot-scale sampling and testing scheme on over 50 farms to validate and/or refine findings from this project over a wider environmental range	• Over next 1-2 cropping seasons
• Continue defining toxicity thresholds and bioavailability models for new and challenging herbicides	• Annually as required over next 5 years
• Continue development and validation of model to predict herbicide persistence in soil and plant-back effect	• Over next 3 years

INTRODUCTION

Herbicides are widely used in Australian cropping systems to control weeds and maximise yields. This is generally preferable to tillage for weed control because it retains soil structure and preserves soil organic matter. However, herbicide residues in soils can limit crop performance if they are not managed correctly.

It is difficult for growers and advisors to know whether herbicide residues will cause issues, because the persistence and behaviour of these residues depends on numerous site-specific factors, including soil and climatic conditions. There are currently few tools to assist growers in determining the level of herbicide residues present, and whether or not they are negatively affecting soil and crop performance. The overall objective of this project was to develop new knowledge and tools to better understand the factors regulating herbicide persistence and bioavailability, giving farmers increased confidence in crop choice, timing of sowing, and herbicide management to ensure soil and crop performance is not limited by herbicide residues. This was achieved by addressing the following five specific aims:

Aim 1: To determine phytotoxicity thresholds for a range of crops exposed to five priority herbicides, based on new measures of bioavailable herbicide residues in soil.

Aim 2: To improve identification of herbicide damage by biochemical fingerprinting (metabolomics/MIR) of affected plants.

Aim 3: To develop a new tool based on infrared spectroscopy (MIR) that can predict sorption and degradation of five priority herbicides in a range of soil types. Current methods to assess sorption and bioavailability are time consuming, costly, and require technical laboratory skills and access.

Aim 4: To validate a site-specific model that can predict the phytotoxicity of five priority herbicides remaining in soil at a designated time after application across the range of climates, weather, and soil types from the CRC's Grower Groups.

Aim 5: To quantify the extent of yield loss caused by herbicide residues in a minimum of three different environments. Previous research has shown that herbicide residue mixtures are often present in soil when the (main) winter crop is planted, but the effect of these residue mixtures is not known. Replicated field trials at three sites involving herbicide withdrawal (i.e. no herbicide, manual weeding), compared with farmer practices involving application of multiple herbicides, are required to address this knowledge gap.

The outcome will be a more informed workforce equipped to react to variable environmental and soil conditions, thereby reducing risk, and increasing crop diversity, yields, and economic returns at a lower environmental cost.

1. BACKGROUND

1.1 PREVIOUS RESEARCH & LITERATURE

Herbicides are a valuable tool for controlling weeds and realising crop yield potential. Weeds were recently estimated to cost $3.3 billion in Australian cropping systems alone (Llewellyn et al. 2016). Herbicide application is the main method for weed control in Australian cropping systems and accounts for over 80% of weed control costs (Llewellyn et al. 2016). Herbicide use has increased over the last two decades due to a number of reasons, including widespread adoption of minimum or no-till cropping, increased use in fallows due to the realisation of the importance of stored soil water in fallows, selection of herbicide-tolerant crop cultivars to enable more effective weed control, and decreasing cost of herbicides relative to manual labour (Llewellyn et al. 2016; Gianessi 2013; Haggblade et al. 2017; Malone and Foster 2019). The primary driver of increased herbicide use has been to reduce soil tillage and limit the associated soil degradation involving soil organic matter loss, structural decline, and increased soil erosion (Hobbs et al. 2008). Although increased herbicide use is not mandated for no-till systems (Friedrich and Kassam 2012), there is a tendency for more herbicides to be used in these systems for efficient weed control (Malone and Foster 2019).

Another driver for the changes in herbicide-use practices has been the recently evolving threat of herbicide-resistant weeds (Heap 2014). Repeated use of only a single herbicide active ingredient, or active ingredients with the same mode of action, puts selection pressure on weed populations and can result in the development of weeds with high tolerance to those herbicides (Powles and Yu 2010). In order to counter this development of herbicide-resistant weed populations, many growers are now diversifying and rotating the spectrum of herbicides used, and/or increasing herbicide application rates to ensure no survivors. This often involves the use of ‘double-knock’ tactics, in which two different weed management strategies (often two different herbicide chemical groups) are deployed over the course of 3-10 days to control potential weed survivors (Beckie et al. 2019).

A recent survey of agronomic management indicated that a wide range of herbicides are being used, and some herbicides are being used on more than one occasion in a season (Harries et al. 2020). Many agronomists now spend a significant amount of time planning crop rotations and weed management strategies to lower the weed seed bank and ensure herbicides with different modes of action can be used over time to prevent herbicide resistance in weeds. To help growers and agronomists plan these herbicide application strategies, there is a significant amount of information on the efficacy of individual herbicides or herbicide combinations for controlling specific weeds during fallow periods, or in-crop, without impacting the health of the growing crop – often in the form of industry management guides (e.g. Congreve and Cameron 2019). Regulatory requirements also ensure that some additional knowledge is available on the fate of these herbicides in soils, and the safety of soilborne herbicides residues to soil organisms (Rose et al. 2016) and non-target plants, including subsequent crops.

Nevertheless, there remains a significant challenge to ensure that herbicides do not carry over or accumulate in soil from one season to the next. Despite the best planning, unforeseen environmental, economic, or management events can alter the course of the cropping rotation, leaving growers exposed to soilborne herbicide residue legacies that can unknowingly impact future crops or constrain their crop options. Managing herbicide carryover in soil is particularly important where future cash crops or cover crops are sensitive to persistent herbicides used in the previous crop (e.g. when a legume follows a cereal crop)

(Cornelius and Bradley 2017). The likelihood of carryover or buildup of a herbicide residue in soil is governed by the rate of breakdown or dissipation of the active ingredient in comparison to the rate and frequency of application (Curran 2016). Environmental and edaphic factors, particularly those that influence soil moisture content and microbial activity, strongly regulate herbicide persistence in soil (McGrath et al. 2019).

A number of research studies have focussed on the potential accumulation of glyphosate residues, because the rapid increase in glyphosate use of over the past two decades (Benbrook 2016) has led to concerns of 'pseudopersistence' as the frequency of use exceeds its rate of degradation in soil (Primost et al. 2017). Several regional or national surveys of pesticide residues have also frequently detected residues and transformation products from other herbicide chemical classes, including s-triazines, chloroacetanilides, and diflufenican (Hvězdová et al. 2018; Silva et al. 2019; Tan et al. 2020; Geissen et al. 2021; Pelosi et al. 2021; Riedo et al. 2021). Our recent soil survey of over 80 paddocks over two season (Rose et al. 2022) also found at least one herbicide (or herbicide metabolite) residue at all sites, with a median of 6 analytes detected in 2015 and 7 analytes detected in 2016. The most frequently detected residues were glyphosate and its primary breakdown product aminomethylphosphonic acid (AMPA), in 87 and 100%, respectively, of topsoil (0–10 cm) samples in 2015, and 67 and 93% of samples in 2016. Residues of 2,4-dichlorophenoxyacetic acid, trifluralin, diflufenican, and diuron were also detected in >30% of topsoil samples in both seasons. Unfortunately, risk assessment of herbicide toxicity to crop growth based on measured soil concentrations is challenging, because there are very few publicly-available toxicity threshold values. This knowledge gap limits our ability to diagnose whether current herbicide-use practices are sustainable (Fischer and Connor 2018) and if they will limit crop diversification practices in the future (Kumar et al. 2020). From a grower or agronomist's point of view, this means that even if they take and submit soil for herbicide residue analysis, they will struggle to interpret the relevance of the test results because there is no data to which it can be compared.

Anecdotally, Soil CRC Grower Groups have identified herbicide carryover in soil as a potential factor in the decline of crop or pasture performance. This mirrors recent evidence from a GRDC/NSW DPI co-funded project which indicated the presence of mixtures of herbicide residues in cropping soils at sowing, and other reports of herbicide damage in Australian cropping systems (Yates et al. 2014). Growers urgently need evidence-backed guidance on the site-specific persistence of herbicides to allow for flexible crop selection and avoidance of plant-back damage, and field-validated information on the potential long-term effects of herbicide residues on soil and crop health.

1.2 GAPS IN CURRENT KNOWLEDGE

Specific challenges include:

- A number of commercial service providers can measure herbicide residue in soil, but there are very few publicly-available toxicity thresholds to enable interpretation of soil tests. A greater number of publicly-available phytotoxicity thresholds are essential for understanding whether soilborne herbicide residues pose a risk to crops.
- Grain growers and consultants sometimes observe poor crop growth or crop injury but cannot identify the cause. Misidentification of the issue can lead to money being spent on solutions that will not fix the problem, or the problem recurring over time. New methods are therefore needed to correctly identify if, when, and which herbicide residues are causing damage.

- Predicting herbicide fate, including bioavailability of measured residues in soil, requires site-specific knowledge of herbicide sorption to soil. Herbicide sorption is the extent to which herbicides are bound to soil, limiting their movement in soil water, and uptake by soil organisms including plants. Current methods to assess sorption and bioavailability are time consuming, costly, and require technical laboratory skills and access. New methods are required to rapidly predict soil-specific sorption at minimum cost, to help inform herbicide fate models; and to help interpret herbicide residue tests in the context of phytotoxicity thresholds in different soil types.
- General information on the longevity and persistence of herbicides is available on product labels, international databases, and some published studies, but quantitative site-specific information for growers and agronomists in Australia is sparse. Data and tools are needed for showing growers and agronomists the *quantitative* variation that can occur in herbicide persistence due to variation in rainfall and soil properties in their farming environment. Although there are models that can predict herbicide behaviour, these models are generally designed for regulatory purposes, and are highly technical to populate and run, and require substantial input of measured soil and environmental properties. The previously-mentioned GRDC/NSW DPI project developed an 'alpha' model version of a model to predict herbicide persistence, but it was not validated under field conditions. New modelling tools that are easy to run and need fewer input parameters are required so that they can be used by agronomists, growers, and agricultural researchers to calibrate their own understanding of herbicide behaviour under local conditions.
- Previous research has shown that herbicide residue mixtures are often present in soil when the (main) winter crop is planted, but the effect of these residue mixtures is not known. It is difficult to determine the long-term effect of herbicide residue mixtures, since nearly all broadacre agricultural systems have already experienced repeated application of various herbicide over the last few decades. Thus, an alternative approach is required, involving 'herbicide withdrawal', where herbicides cease being used, and soil and crop health is monitored with respect to ongoing herbicide application under normal farmer practice.

In this project, grower groups identified a dearth of information and specific knowledge gaps for a number of key herbicides, including:

- Limited understanding of persistence and fate of the newly-registered herbicide pyroxasulfone
- Concern about the repeated use of imidazolinones herbicides (imazapyr and imazamox) with long residual half-lives when using imidazolinone-tolerant crops, with unknown consequences due to potential (pseudo) accumulation
- Unknown effects of herbicide residue mixtures including widespread diuron residues identified in Southern-Australian cropping systems in a recent soil survey (Rose et al. 2022)
- Difficultly in managing residues of clopyralid when growing highly sensitive grain-legume crops.

2. METHODOLOGY

2.1 HERBICIDE RESIDUE PHYTOTOXICITY THRESHOLDS

2.1.1 Outline of dose-response treatments

Over the course of the project, numerous bioassays were conducted with different combinations of herbicides, crops and soil types (Table 2.1.1).

Table 2.1.1. Experimental bioassays conducted during this project.

Herbicide	Soil type	Crops	Experimental Location
Clopyralid	Sand	Wheat, barley, canola, field pea, lentil, lupin, chickpea, faba bean	Wollongbar, NSW
Clopyralid	Sand	Wheat, canola, field pea, lentil, lupin, chickpea	Murdoch University, WA
Clopyralid	Alkaline red loam (Minnipa, SA)	Wheat, barley, canola, field pea, lentil, lupin, chickpea, faba bean	Wollongbar, NSW
Clopyralid	Neutral clay loam (Birchip, WA)	Wheat, barley, canola, field pea, lentil, lupin, chickpea, faba bean	Wollongbar, NSW
Imazapyr	Sand	Wheat, canola, field pea, lentil, lupin, chickpea, faba bean	Wollongbar, NSW
Imazapyr	Alkaline red loam (Minnipa, SA)	Wheat, barley, canola, field pea, lentil, lupin, chickpea	Wollongbar, NSW
Imazapyr	Neutral clay loam (Birchip, WA)	Wheat, barley, canola, field pea, lentil, lupin, chickpea	Wollongbar, NSW
Diuron	Sand	Wheat, canola, field pea, lentil, lupin, chickpea	Murdoch University, WA
Diuron	Sandy loam (Meckering, WA)	Wheat, canola, field pea, lentil, lupin, chickpea	Murdoch University, WA
Pyroxasulfone	Sand	Wheat, canola, field pea, lentil, lupin, chickpea	Murdoch University, WA
Pyroxasulfone	Sandy loam (Meckering, WA)	Wheat, canola, field pea, lentil, lupin, chickpea	Murdoch University, WA
Trifluralin	Sand	Wheat, canola, field pea, lentil, lupin, chickpea	Murdoch University, WA
Propyzamide	Sand	Wheat, canola, field pea, lentil, lupin, chickpea	Murdoch University, WA

2.1.2 Phytotoxicity bioassays

To determine thresholds under maximum bioavailability (minimum sorption), washed pool sand or soil was spiked with increasing concentrations of herbicide using a rotating drum mixer. The target herbicide concentrations were designed to cover a range of concentrations that might be expected in the field, from <10% of the recommended label rate, to 3 times the label rate (Table 2.1.1). Herbicides were prepared from stock solutions and spiked onto soils by diluting stock solutions to appropriate concentrations to bring sand/soil moisture content to 20% - 40% of field capacity.

Bioassays at Wollongbar Primary Industries Institute were conducted in square pots (65 mm by 65 mm and 160 mm depth) with drainage holes in the bottom covered by a thin square (10 mm) of polyurethane foam. Pots were filled with herbicide-spiked sand or soil to a depth of 140 mm, and wet up to 80% of field capacity moisture content. Seeds of wheat (*Triticum aestivum* L. Scepter), barley (*Hordeum vulgare* L. La Trobe), canola (*Brassica napus* L. Diamond), lupin (*Lupinus angustifolia* L. PBA Batemen), field pea (*Pisum sativum* L. PBA Butler), chickpea (*Cicer arietinum* L. Slasher), faba bean (*Vicia faba* L. Nasma), and lentil (*Lens culinaris* L. PBA Bolt) were sown and soil moisture was maintained to 80% of field capacity every second day by watering to weight from the top (Figure 1). Plants were grown in a climate-controlled glasshouse with temperatures set to 15°C at night (6 pm – 6 am) and 25°C day (6 am – 6 pm).

Plants were harvested at 21 days after sowing. Shoots were cut at the soil surface, weighed, dried at 60°C for 2 days, and then re-weighed to determine dry weight. Roots were washed from the soil, blotted dry, and weighed. Roots were stored in 20% ethanol at 4°C prior to measuring root length using WhinRhizo.

2.1.3 Herbicide analysis

Prior to sowing, subsamples of spiked soil/sand were collected and analysed to confirm target herbicide residue concentrations. Herbicide concentrations were determined by extracting using QuEChERS methodology and subsequent analysis by LC-MS/MS or GC-MS. Analytical details are given in Appendix A.

2.1.4 Statistical analysis

Dose-response thresholds were determined by fitting shoot dry weight data to soil herbicide concentrations using 4 parameter log-logistic curves using the package ‘drc’ (Ritz et al. 2015) in the R statistical software environment (R Core Team 2022).

2.2 HERBICIDE DAMAGE FINGERPRINTING

2.2.1 Plant material for fingerprinting

Plant (shoot) material from dose-response bioassays conducted in 2.1.3 were dried at 60°C and ground. Because there was a limited mass of shoot material for each replicate, all replicate subsamples were pooled for each specific herbicide dose. Pooled samples (0.2-1.0g) were accurately weighed in to a 50mL polypropylene centrifuge tubes and extracted in 2mL deionised water and 10mL acetonitrile by blending with a Polytron PT 2500E homogenizer.

2.2.2 Metabolomics analysis

To test the potential for metabolomic analysis to identify herbicide injury thresholds, lupins exposed to soilborne clopyralid residues were extracted as per herbicide analysis methods. Acetonitrile extracts (1mL) were evaporated under a stream of nitrogen at 40°C. Dried extracts were reconstituted in 0.5mL of ethyl acetate and derivatised at 40°C with 50uL of Mt-BSTFA. Samples (1uL) were injected into an Agilent 5890 GC equipped with an Agilent 5977E MSD and an Agilent 7693A automated liquid sampler (ALS).

2.2.3 Herbicide analysis

Herbicide concentrations in plant leaf tissue were determined by extracting using QuEChERS methodology and subsequent analysis by LC-MS/MS or GC-MS. Because of the high load of matrix background, which can result in analyte suppression or enhancement, matrix-matched standards were used for quantification. Analytical details are given in Appendix A.

2.3 HERBICIDE SORPTION IN CROPPING SOILS

2.3.1 Soil sampling and characterisation

Soil samples were taken from 30 paddocks from the Grower Group partners at two depths, 0-10 and 10-30cm prior to sowing in 2019. These sites included paddocks being used for field studies in [4] and [5]. Soil samples were couriered to NSW DPI where they were air dried and sieved < 2mm. Soil physicochemical properties were characterised by standard wet chemical techniques in a NATA-accredited facility. Infra-red reflectance spectroscopy was conducted on finely-ground subsamples using a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific) equipped with a KBr beamsplitter. Absorption spectra of bulk soils were trimmed from 600 to 4,000 cm−1 to obtain the mid-infrared spectral region.

2.3.2 Sorption kinetics

Sorption kinetics were determined for each herbicide at a single-concentration representative of the concentration found in soil (top 5 cm) after a high label rate application. Replicate soil slurries (10 for each herbicide x soil combination) were spiked with the appropriate amount of herbicide and duplicate samples were destructively analysed at 0.5, 2, 4, 8, 24 and 48 hours after shaking at 150 rpm. Kinetic curves were fit using the package *drc* (Ritz et al. 2015) in R software via the negative exponential function AR.2(). Control without soil were also analysed and demonstrated negligible sorption (<10%) to the experimental apparatus (centrifuge tubes, microfilters).

2.3.3 Sorption isotherms (linear range)

Initially, soil sorption coefficients were determined by spiking duplicate soil samples with the appropriate amount, and shaking until equilibrium was established (24 hours) before herbicide analysis of the dissolved fraction. However, large coefficients of variation were observed for some soils, so sorption isotherms were repeated for all soils by spiking with 0, 10, 50 or 250 ng/g of herbicide to construct linear isotherms over a range of concentrations. Sorption isotherms were measured as per OECD guidelines (OECD 2000) after optimising sorption kinetics (time to reach equilibrium, 18 hours) and soil to solution concentrations (1:2 soil: solution). After centrifuging, the concentration of herbicide remaining in the soil solution was determined by LC-MS/MS, and the amount of herbicide sorbed calculated by difference. Plots of sorbed concentration versus dissolved concentration were fit with Linear sorption isotherms to calculate sorption coefficients.

2.3.4 Statistical analysis

Sorption coefficients (K_d) for each soil were tabulated together with physicochemical and MIR data. Pairwise correlations for K_d and other physicochemical properties were assessed by using the pairs() function in R (R Core Team 2022). Subsequently, the ability of linear regression (LR) and generalised additive models (GAM) using physicochemical properties predict K_d was compared against machine-learning-based predictions using MIR spectra as input.

LR and GAMs were constructed with K_d as the response variable and pH, cation exchange capacity (CEC), electrical conductivity (EC), clay content, P buffer index (PBI), and organic C (OC) as predictor variables. The importance of predictor variables was initially explored by fitting all possible second-order variable combinations, using the *glmulti* package (Calcagno 2020) in R. The ‘best’ model was selected from the top 10 models with lowest AIC values as having a parsimonious number of predictors. Important predictors for LR identified with *glmulti* were used as a guide to fit and assess multiple GAMs containing combinations of those predictors as smooth or tensor terms. Potential models were compared using AIC scores and the model with lowest AIC selected as the optimum model.

Prediction of K_d using MIR and machine-learning was undertaken using the framework provided by Wadoux et al. (2021). Briefly, this involves a number of steps including importation of spectra, exploratory visualisation, pre-processing, calibration, and validation of potential models. Spectra were imported into R as .csv files using the files2SpecraObject function and then trimmed to wavenumbers between 1000 and 4000 cm^{-1}. Smoothing, centring and scaling were achieved using a Savitzky-Golay (SG) filter with a window size of 11 and second-polynomial and Standard Normal Variate (SNV) transformation with resampling window of 5 nm. These operations were performed with custom functions written and provided by Wadoux et al. (2021), modified as necessary to process our data.

2.4 PERSISTENCE OF HERBICIDES IN CROPPING SOILS

2.4.1 Field sites

The persistence of priority herbicides (clopyralid, imazapyr/imazamox, diuron, and pyroxasulfone) was measured at a number of different field locations. These fields were selected in consultation with grower groups and their members, and are therefore centred in three locations: the central WA wheatbelt (WANTFA); the Eyre Peninsula SA (AIR EP, PIRSA); and the Victorian Mallee (BCG). Sites were selected on the basis that at least one of the priority herbicides was applied, and that the site was accessible for soil sampling throughout the growing season until the following year. A list of the sites monitored is given in Table 2.4.1.

Table 2.4.1. Locations of herbicide persistence field monitoring sites in 2019.

Site	Location	Latitude	Longitude	Soil Clay (%)	Soil pH (CaCl2)	Soil OC (%)
BCG1	Kinnabulla	35.868481	142.68362	14	7.4	1.1
BCG4	Brim	36.07534	142.55353	19	7.5	1.0
BCG6	Lawloit	36.41274	141.41711	2	4.5	0.8
BCG9	Horsham	36.71469	142.16702	9	7.6	1.0
BCG10	Jil Jil	35.821949	142.94572	29	8.0	1.0
SA1	Minnipa	32.82805	135.15244	6	7.8	1.0
SA2	Poochera	32.60609	134.78525	6	7.9	1.0
SA3	Minnipa	32.96545	135.07353	<1	7.0	0.2
SA4	Minnipa	32.89067	135.20055	8	7.7	1.2
SA5	Mt Cooper	33.09702	134.5390	10	7.1	1.7
WA1	Bolgart	31.30122	116.61591	<1	6.3	0.7
WA2	Cunderdin	31.57033	117.27561	TBC	6.2	1.2

2.4.2 Herbicide application and soil sampling

Herbicides were applied as per farmer practice. The rates and timing of herbicide application, together with the crop type and sowing date, are given in Table 2.4.2.

Table 2.4.2. Locations of herbicide persistence field monitoring sites in 2019.

Site	Location	Priority herbicide	Date of application	Active ingredient rate (g/ha)
BCG1	Kinnabulla	Imazapyr	1/06/2019	11
		Imazamox	1/06/2019	25
		Clopyralid	1/06/2019	10
BCG4	Brim	Imazamox	2/08/2019	20
		Imazapyr	2/08/2019	9
BCG6	Lawloit	Imazamox	2/08/3019	20
BCG9	Horsham	Imazapic	6/06/2019	21
		Imazapyr	6/06/2019	7
BCG10	Jil Jil	Imazamox	22/07/2019	20
		Imazapic	22/07/2019	9
SA1	Minnipa	Clopyralid	23/07/2019	45
SA2	Poochera	Clopyralid	25/06/2019	15
SA3	Minnipa	Diuron	16/05/2019	270
		Clopyralid	23/07/2019	27
SA4	Minnipa	Pyroxasulfone	8/05/2019	100
		Clopyralid	19/06/2019	30
SA5	Mt Cooper	Clopyralid	4/07/2019	27
WA1	Bolgart	Pyroxasulfone	28/05/2019	100
WA2	Cunderdin	Diuron	8/07/2019	450

Soil samples were taken in the week prior to application of the herbicide and then at increasing time intervals after herbicide application. Sampling times were nominally 1, 7, 21, 42, 84, 168 and 364 days after herbicide application, although the exact timing at each site depended on the logistics of site accessibility. Sampling was undertaken at four points at the corners of a set 50 m by 50 m grid located at a random position within the paddock. These points were GPS logged and sampling occurred within 5m of this position at each sampling time. Samples were taken to 30cm and split into two depths: 0-10 and 10-30 cm. Samples were then kept cool until receival at Wollongbar Primary Industries Institute, where they were air-dried at 40°C before being sieved and frozen until analysis. The target herbicides were analysed using the methods described in Appendix A.

2.5 EFFECT OF HERBICIDE WITHDRAWAL ON CROP GROWTH AND YIELD

2.5.1 Outline of field experiments

Three field experiments were established to determine whether a reduction in herbicide use can reduce herbicide residue carryover, improve soil health, and increase crop yields. Field experiments were established by the grower groups in the project team and focussed on herbicides commonly used by local growers. The field sites were maintained and monitored for 2 cropping seasons (2019, 2020).

2.5.2 Description of the WANTFA experiment

The WANTFA experiment was established at Cunderdin, WA. Treatments at this site included a control (i.e. herbicide withdrawal – no herbicide applied), diuron, trifluralin, pyroxasulfone and trifluralin + pyroxasulfone (Table 2.5.2a).

Table 2.5.2a. Experimental treatments at the WANTFA site. Numbers in brackets are herbicide application rates (g a.i. ha $^{-1}$).

Trt No.	Seeding	In-crop	Summer Fallow
1	Nil	Nil	Nil
2	Pyroxasulfone	Farmer Practice	Farmer Practice
3	Diuron	Farmer Practice	Farmer Practice
4	Trifluralin	Farmer Practice	Farmer Practice
5	Pyroxasulfone + Trifluralin	Farmer Practice	Farmer Practice

The experiment was a randomised block design, with 3 replicates per treatment. Plot sizes were 2m long by 1m wide. Weeds were removed from the control plots by hand weeding every 4-8 weeks as required, with other trial details given in Table 2.5.2b.

Table 2.5.2b. Trial details at the WANTFA site.

Details	2019	2020
Crop (variety)	Barley (Clearfield Spartacus)	Wheat (Scepter)
Sowing date	11 May 2019	5 May 2020
Fertiliser inputs	KTill 80, UAN 50	Ktill 80 UAN 80
Harvest date	December	December
GSR (mm)	195	268

2.5.3 Description of the AIR EP/PIRSA experiment

The PIRSA experiment was established at Minnipa Agricultural Centre, SA. Treatments at this site included a control (i.e. herbicide withdrawal – no herbicide applied) and different pre-emergent, in-crop, and summer fallow herbicide treatments (Table 2.5.3a).

Table 2.5.3a. Experimental treatments at the AIR EP/PIRSA site. Numbers in brackets are herbicide application rates (g a.i. ha $^{-1}$).

Trt No.	Seeding	In-crop	Summer Fallow
1	Nil	Nil	Nil
2	Glyphosate +Trifluralin	Nil	Farmer practice
3	Glyphosate	Clopyralid	Farmer practice
4	Trifluralin	Clopyralid	Farmer practice
5	Glyphosate +Trifluralin	Clopyralid	Farmer practice
6	Glyphosate +Trifluralin	Clopyralid + Diflufenican/MCPA	Farmer practice
7	Glyphosate +Trifluralin	Clopyralid + Diflufenican/MCPA	Nil
8	Pyroxasulfone	Nil	Farmer practice

The experiment was a randomised block design, with 3 replicates per treatment. Plot sizes were 12 m long by 2 m wide. Weeds were removed from the control plots by hand weeding approximately every 4-8 weeks as required, with other trial details given in Table 2.5.3b.

Table 2.5.3b. Trial details at the AIR EP/PIRSA site.

Details	2019	2020
Crop (variety)	Barley (Clearfield Spartacus)	Wheat (Scepter)
Sowing date		6th May
Fertiliser inputs		DAP (60 kg/ha)
Harvest date		
GSR (mm)		

2.5.4 Description of the BCG experiment

The BCG experiment was established at Cuyro, Vic. Treatments at this site included a control (i.e. herbicide withdrawal – no herbicide applied) and different pre-emergent, in-crop, and summer fallow herbicide treatments (Table 2.5.4a).

Table 2.5.4a – Experimental treatments at the BCG site. Numbers in brackets are herbicide application rates (g a.i. ha $^{-1}$).

Trt No.	Seeding	In-crop	Summer Fallow
1	Nil	Nil	Nil
2	Glyphosate +Trifluralin	Nil	Farmer practice
3	Glyphosate	Clopyralid	Farmer practice
4	Trifluralin	Clopyralid	Farmer practice
5	Glyphosate +Trifluralin	Clopyralid	Farmer practice
6	Glyphosate +Trifluralin	Clopyralid + Diflufenican/MCPA	Farmer practice
7	Glyphosate +Trifluralin	Clopyralid + Diflufenican/MCPA	Nil
8	Pyroxasulfone	Nil	Farmer practice

The experiment was a randomised block design, with 3 replicates per treatment. Plot sizes were 12 m long by 2 m wide. Weeds were removed from the control plots by hand weeding approximately every 4-8 weeks as required, with other trial details given in Table 2.5.4b.

Table 2.5.4b. Trial details at the BCG site.

Details	2019	2020
Crop (variety)	Barley (Clearfield Spartacus)	Wheat (Scepter)
Sowing date		6th May
Fertiliser inputs		DAP (60 kg/ha)
Harvest date		
GSR (mm)		

2.5.5 Soil and plant sampling

At all sites, soil samples were taken from each plot every 3 months, nominally in March, June, September, and December. Triplicate cores per plot taken to 30 cm and split into two depths: 0-10 and 10-30 cm. Samples were then kept cool until receival at Wollongbar Primary Industries Institute, where they were air-dried at 40°C before being sieved and frozen until analysis. The target herbicides were analysed using the methods described in Appendix A

Plant samples were taken at harvest for biomass and yield measurements by taking cuts of 2 m lengths of row from central rows, and measuring fresh and dry biomass and grain yield. Data was converted to mass per hectare based on row numbers per m^2.

2.6 SOILBORNE HERBICIDE RESIDUES AT SOWING – A PILOT STUDY

2.6.1 Experimental locations and sampling

Grower groups called for expression of interest from their member growers to submit topsoil (0-10cm) samples taken from paddocks with a history of diuron, clopyralid or imidazolinone herbicide application where there were concerns of potential carryover. A total of 13 paddocks were selected for the pilot study (Table 2.6.1).

Table 2.6.1. Pilot study locations.

Site code	Location	GPS	Target Compound	Sample Date
SA1	Elliston	S 33.61599 E 134.88672	Clopyralid	10-Mar-2021
SA2	Elliston	S 33.61599 E 134.88672	Imidazolinone	10-Mar-2021
SA3	Buckleboo	S 32.95485 E136.21164	Imidazolinone and Clopyralid	17-Mar-2021
SA4	Minnipa	S 32.82943 E135.14563	Imidazolinone	15-Mar-2021
SA5	Minnipa	S 32.82139 E135.15506	Clopyralid	15-Mar-2021
SA6	Koongawa	S 33.30725 E 135.86652	Imidazolinone	17-Mar-2021
SA7	Koongawa	S 33.320988 E 135.86903	Imidazolinone	17-Mar-2021
SA8	Minnipa	S 33.06878 E135.05864	Pyroxasulfone	17-Mar-2021
VIC1	Lawloit	S36.43321, E141.42356	Imidazolinone	17-Mar-2021
VIC2	Jil Jil	S35.77898, E42.97085	Imidazolinone	26-Mar-2021
WA1	Kojunup	S33.9721, E117.2026	Pyroxasulfone, diuron	6-Apr-2021
WA2	Kojunup	S33.9721, E117.2026	Imidazolinone	6-Apr-2021
WA3	Kojunup	S33.9721, E117.2026	Clopyralid	6-Apr-2021

2.6.2 Soil sampling, submission

Four soil samples from each paddock were taken from a 50 x 50m grid in March-April 2021, where each sample was a composite of three homogenised subsamples. Samples were transported to NSW DPI Wollongbar in insulated foam boxes with ice bricks, and analysed for herbicides using a modified QuECHERs extraction and LC-MS/MS or GC/MS quantification. In addition, spike-recoveries were conducted for each soil sample for quality control purposes.

2.6.3 Reporting and feedback

Dispatch, receival, extraction, and reporting dates for each sample were collated in order to ascertain representative turnaround times. Reports were emailed back to growers via grower groups which included information on how to interpret the tests. This included information on clopyralid and imazapyr toxicity thresholds (ED_{20}) for plant biomass for different crop species.

3. RESULTS

3.1 HERBICIDE RESIDUE PHYTOTOXICITY THRESHOLDS

3.1.1 Assessment of dose rates

Initial experiments in sand demonstrated that the doses chosen were appropriate for constructing dose-response curves from no effect through to maximum effect (plant death) for sensitive species (Figure 3.1.1a, left; Figure 3.1.1b). As expected, this was not the case with tolerant species (Figure 3.1.1a, right), where no or minimal toxicity effects were observed/measured.

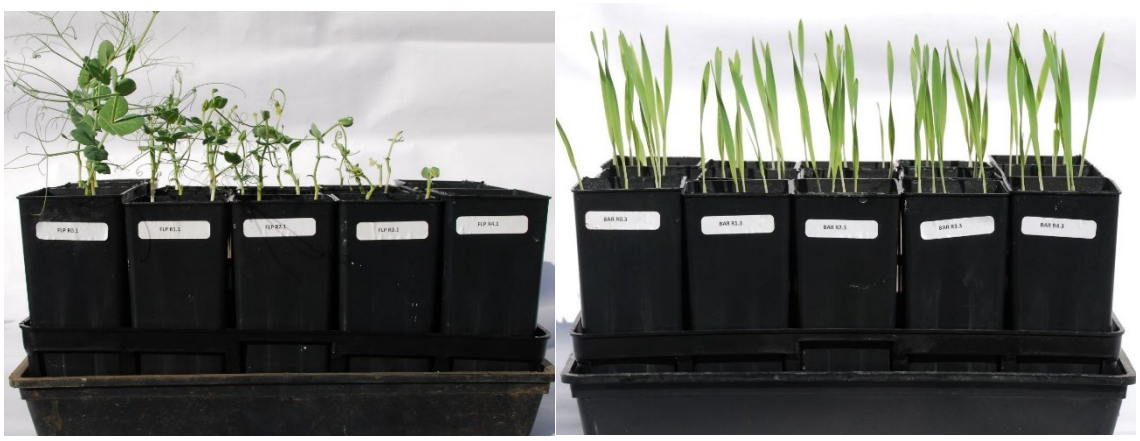

Figure 3.1.1a. Phytotoxicity dose-response of clopyralid residues in sand against: (Left) field peas (sensitive); and (Right) barley (tolerant). Within each photo, the clopyralid dose increases from control (no clopyralid) on the far left, to label rate (~ 50 µg kg^{-1}) on the far right.

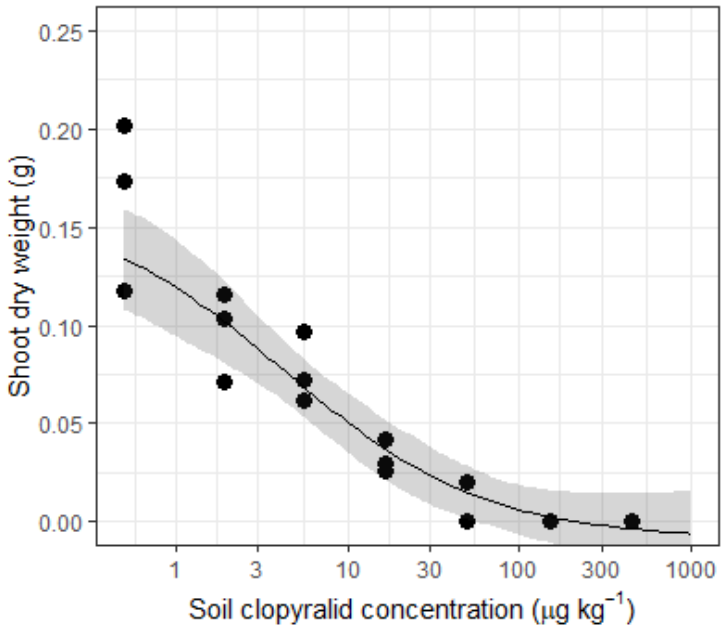

Figure 3.1.1b. Example dose-response curve, for field pea in sand.

3.1.2 Bioassay results for clopyralid

Dose-response experiments for clopyralid were conducted in sand at Wollongbar Primary Industries Institute and Murdoch University using the same methodology, to check the reproducibility of toxicity thresholds. Good agreement between thresholds were obtained across the two sites (Table 3.1.2).

Clopyalid bioassays were repeated in a sandy loam soil from Minnipa, SA, and Curyo, Vic, to determine potential alterations to toxicity thresholds due to soil specific interaction (primarily sorption, but also potential transformation/transportation of clopyralid in the soil profile and/or alteration to plant root growth). Significant attenuation of phytotoxicity was observed in both soils (e.g. Figure 3.1.2), with the thresholds for 20% biomass reduction for legume species being 1.5-50 times greater in soil compared with the sand.

Figure 3.1.2 Phytotoxicity dose-response of clopyralid residues toward field peas in: (Left) sand; and (Right) soil (sandy loam, Minnipa, SA). Within each photo, the clopyralid dose increases from control (no clopyralid) on the far left, to label rate (~ 50 µg kg^{-1}) on the far right.

Overall, bioassays showed that wheat, barley and canola were all tolerant to soil residues of clopyralid at rates representative of label recommendations (Table 3.1.2). This was expected since clopyralid is registered for use in-crop for the crop species. The legumes tested were all sensitive, with the order of tolerance (from least to most sensitive in terms of shoot biomass at 21 days being lentil ~ field pea < chickpea < faba bean < lupin [Table 3.1.2]).

Table 3.1.2. Phytotoxicity thresholds (µg kg^{-1}) for 20% shoot biomass reduction (ED_{20}) for different crop species growing in sand or soil spiked with clopyralid.

Species	Sand (WPII)	Sand (MU)	Minnipa Soil	Birchip Soil (15% clay)
Lentil	0.5	0.2	3.4	2.5
Field pea	0.6	0.2	1.9	31
Lupin	8.8	0.3	54	>100
Chickpea	0.5	0.8	6.2	8.7
Faba bean	3.2	nd	25	38
Wheat	>100	>100	>100	>100
Barley	>100	nd	>100	>100
Canola	>100	>100	>100	>100

3.1.3 Bioassay results for imazapyr

Dose response bioassays for imazapyr were conducted for eight crop species in washed sand and two soil types. Imazapyr showed significant toxicity to wheat, barley and canola, whereas legumes were generally tolerant to label-rate applications, with the exception of faba beans (Table 3.1.3). Faba bean germination in the two soil types was low in all treatments (including controls), so thresholds could not be determined in those soil types. Although canola was most sensitive in the washed pool sand, wheat was the most sensitive crop in both soil types tested.

Table 3.1.3 Phytotoxicity thresholds (µg kg^{-1}) for 20% shoot biomass reduction (ED_{20}) for different crop species growing in sand or soil spiked with imazapyr.

Species	Sand	Minnipa Soil	Birchip Soil (15% clay)
Lentil	>30	>30	>30
Field pea	21	>30	>30
Lupin	19	>30	>30
Chickpea	>30	>30	>30
Faba bean	3.7	ND	ND
Wheat	1.0	1.3	4.2
Barley	ND	1.7	5.5
Canola	0.1	3.9	6.2

3.1.4 Bioassay results for diuron

Dose response bioassays for diuron were conducted for six crop species in washed sand and a sandy soil from Meckering, WA. Diuron dose-response curves were characterised by a much sharper shoot biomass toxicity threshold in soil than clopyralid and imazapyr (see Figure 3.1.4 as an example). This often resulted in complete plant death when rates were < 5 times the ED_{50}.

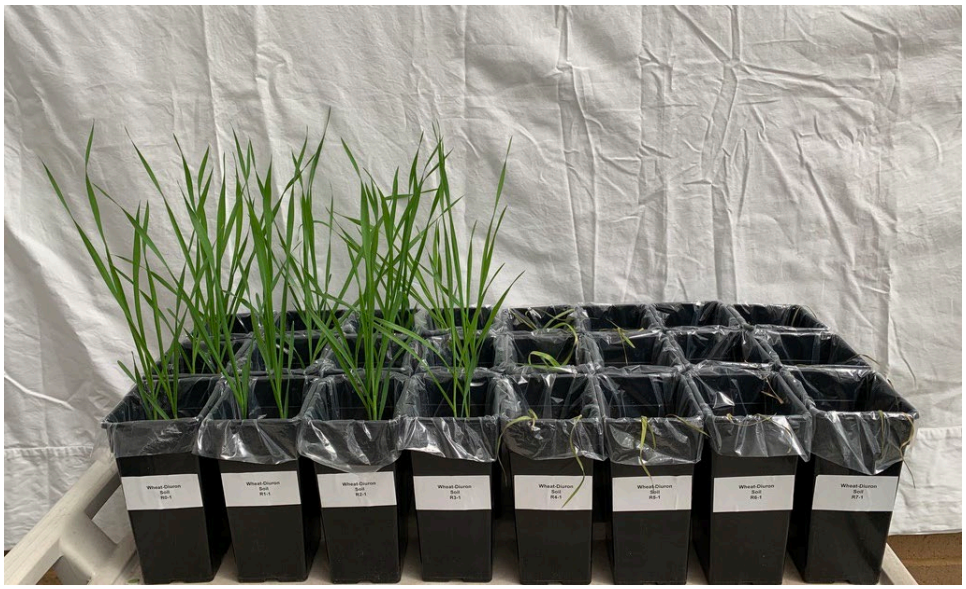

Figure 3.1.4 Diuron phytotoxicity to wheat at increasing rates in Meckering soil.

Lupins were the most tolerant to diuron residues in soil, followed by field pea > wheat ~ chickpea > canola ~ lentil (Table 3.1.4). Canola and lentil were particularly susceptible in washed sand, confirming the buffering effect of organic matter in soil.

Table 3.1.4. Phytotoxicity thresholds (µg kg^{-1}) for 20% shoot biomass reduction (ED_{20}) for different crop species growing in sand or soil spiked with diuron.

Species	Sand	Meckering Soil
Lentil	0.1	24
Field pea	8.3	99
Lupin	40	199
Chickpea	18.5	40
Wheat	4.1	77
Canola	0.2	42

3.1.5 Bioassay results for pyroxasulfone

As with diuron, dose-response bioassays for pyroxasulfone were conducted for six crop species in washed sand and a sandy soil from Meckering, WA. Field peas and chickpeas were the most tolerant to pyroxasulfone residues in soil, followed by wheat ~ lentil ~ lupin > canola (Table 3.1.5, Figure 3.1.5). This confirms label plant-back requirements which show that canola is the most susceptible to pyroxasulfone residues in soil.

Figure 3.1.5. Pyroxasulfone phytotoxicity to canola at increasing rates in Meckering soil.

Table 3.1.5. Phytotoxicity thresholds (µg kg^{-1}) for 20% shoot biomass reduction (ED_{20}) for different crop species growing in sand or soil spiked with pyroxasfulone.

Species	Sand	Meckering Soil
Lentil	294	104
Field pea	>567	149
Lupin	7	104
Chickpea	>567	298
Wheat	19	144
Canola	1.9	28

3.1.6 Bioassay results for trifluralin and propyzamide

Trifluralin and propyzamide are two additional, commonly-used herbicides in Australian cropping systems, particularly in WA and SA. Dose-response bioassays in sand were conducted for these two herbicides to get a preliminary assessment of potential toxicity ranges to different crop species. All species had similar tolerance to trifluralin in sand, however wheat was considerably more susceptible to propyzamide residues than the other crop species (Figure 3.1.6, Table 3.1.6).

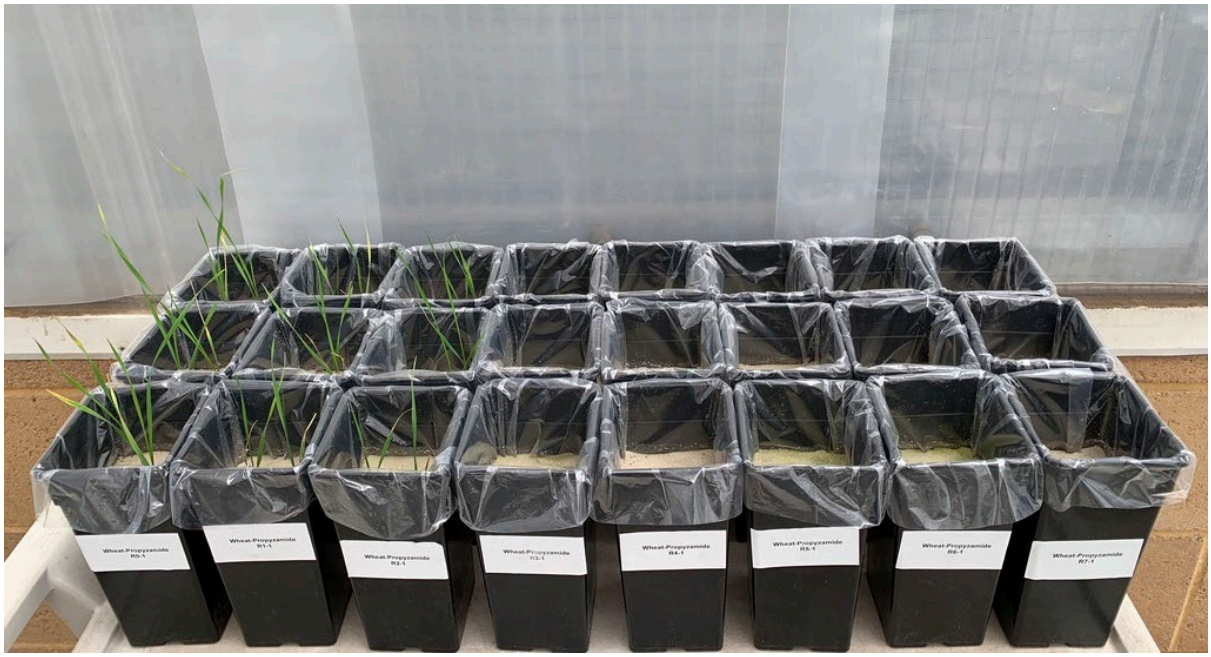

Figure 3.1.6 Propyzamide phytotoxicity to wheat at increasing rates in Meckering soil.

Table 3.1.6 Phytotoxicity thresholds (µg kg^{-1}) for 20% shoot biomass reduction (ED_{20}) for different crop species growing in sand spiked with increasing doses of trifluralin or propyzamide

Species	Trifluralin	Propyzamide
Lentil	328	>1000
Field pea	257	>1000
Lupin	496	248
Chickpea	550	>1000
Wheat	266	50
Canola	386	188

3.1.7 Additional data

Additional data for different responses (e.g. root length, root dry weight) and thresholds (e.g. ED_{50}) are also available for all crop-soil-herbicide combinations provided. This data set is still being compiled and will be included in draft manuscripts for peer-reviewed publication.

3.2 HERBICIDE DAMAGE FINGERPRINTING

3.2.1 Leaf tissue analysis of clopyralid injury in lupins

Lupins were selected as being a representative of a susceptible legume species. Leaf tissue was analysed for lupins growing in two contrasting soils (BCG1 or SA1) that had been spiked with increasing concentrations of clopyralid. Concentrations of clopyralid increased linearly in lupin leaf tissue in response to increasing concentrations in soil. This relationship was not significantly different between the two contrasting soil types (Figure 3.2.1).

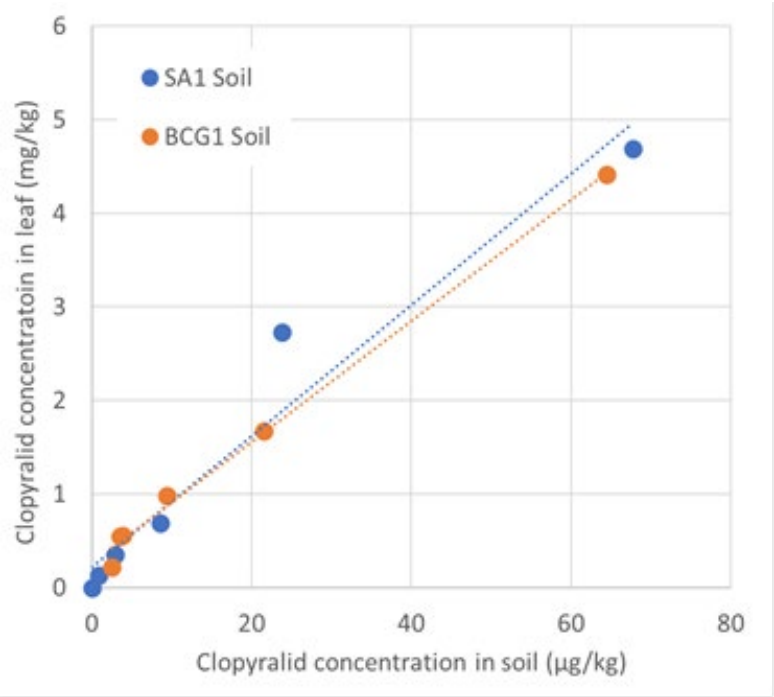

Figure 3.2.1. Relationship between concentration of clopyralid detected in lupin leaf tissue relative to the concentration of clopyralid in soil.

3.2.2 Leaf tissue analysis of other herbicide-crop combinations

Leaf tissue concentrations were also analysed in multiple crops for the priority herbicides diuron, propyzamide, trifluralin and pyroxasulfone (Table 3.2.2). These values can now be used as a reference for leaf tissue samples being tested for herbicide residues, showing indicative toxicity threshold concentrations that could be causing crop damage.

Table 3.2.2. Concentration of priority herbicides in leaf tissue of different crop species in sand or soil (Meckering sandy loam) at soil concentrations that are phytotoxic (approximately ED_{20}).

Soil	Herbicide	Crop	SDW ED_{20}	Target soil Concentration (µg/kg)	Active Ingredient Leaf tissue concentration (µg/kg)	Metabolite leaf tissue concentration (mg/kg)
Meckering	Diuron	Canola	42	30	0.23	
Meckering	Diuron	Canola	42	50	0.28	
Sand	Diuron	Canola	0.2	0	0	
Sand	Diuron	Canola	0.2	30	0.56	
Meckering	Diuron	Chickpea	40	30	0.10	
Meckering	Diuron	Chickpea	40	50	0.35	

Sand	Diuron	Chickpea	19	0	0	
Sand	Diuron	Chickpea	19	30	1.78	
Meckering	Diuron	Field pea	99	100	1.55	
Meckering	Diuron	Field pea	99	290	6.82	
Sand	Diuron	Field pea	8.3	0	0	
Sand	Diuron	Field pea	8.3	30	3.46	
Meckering	Diuron	Lentil	24	0	0.00	
Meckering	Diuron	Lentil	24	30	0.26	
Sand	Diuron	Lentil	0.11	0	0	
Sand	Diuron	Lentil	0.11	30	2.84	
Meckering	Diuron	Lupin	199	100	0.05	
Meckering	Diuron	Lupin	199	290		
Sand	Diuron	Lupin	40	40	0.12	
Sand	Diuron	Lupin	40	80	0.33	
Meckering	Diuron	Wheat	77	50	0.31	
Meckering	Diuron	Wheat	77	100	0.86	
Sand	Diuron	Wheat	4.1	0	0	
Sand	Diuron	Wheat	4.1	30	4.08	
Sand	Propyzamid	Canola	188	100	1.57	
Sand	Propyzamid	Canola	188	310	2.79	
Sand	Propyzamid	Lupin	248	100	1.99	
Sand	Propyzamid	Lupin	248	310	4.77	
Sand	Propyzamid	Wheat	50	30	0.93	
Sand	Propyzamid	Wheat	50	50	1.17	
Meckering	Pyroxasulfone	Canola	28	24	<LOR [1]	1.29
Meckering	Pyroxasulfone	Canola	28	72	<LOR	3.40
Sand	Pyroxasulfone	Canola	1.9	0	<LOR	1.72
Sand	Pyroxasulfone	Canola	1.9	7	<LOR	7.22
Meckering	Pyroxasulfone	Lentil	104.4	72	<LOR	0.74
Meckering	Pyroxasulfone	Lentil	104.4	216	<LOR	1.02
Sand	Pyroxasulfone	Lentil	293.5	189	<LOR	6.53
Sand	Pyroxasulfone	Lentil	293.5	378	<LOR	6.71
Meckering	Pyroxasulfone	Lupin	104	72	<LOR	<LOR
Meckering	Pyroxasulfone	Lupin	104	216	<LOR	<LOR
Sand	Pyroxasulfone	Lupin	6.9	7	<LOR	0.73
Sand	Pyroxasulfone	Lupin	6.9	10.5	<LOR	0.88
Meckering	Pyroxasulfone	Wheat	145	72	<LOR	<LOR
Meckering	Pyroxasulfone	Wheat	145	216	<LOR	<LOR
Sand	Pyroxasulfone	Wheat	19	10.5	<LOR	10.05
Sand	Pyroxasulfone	Wheat	19	21	<LOR	6.22
Sand	Trifluralin	Canola	386	375	0.25	
Sand	Trifluralin	Canola	386	1125	1.14	
Sand	Trifluralin	Chickpea	551	375	0.03	
Sand	Trifluralin	Chickpea	551	1125	0.14	
Sand	Trifluralin	Field pea	257	125	0.02	
Sand	Trifluralin	Field pea	257	375	0.09	

Sand	Trifluralin	Lentil	327.5	125	<LOR	
Sand	Trifluralin	Lentil	327.5	375	<LOR	
Sand	Trifluralin	Lupin	496	375	0.06	
Sand	Trifluralin	Lupin	496	1125	0.17	
Sand	Trifluralin	Wheat	366	125	<LOR	
Sand	Trifluralin	Wheat	366	375	<LOR	

1 LOR = Limit of Reporting. This depends on sample mass and analytical limit of detection.

3.3 HERBICIDE SORPTION IN CROPPING SOILS

3.3.1 Soil physicochemical characteristics

Soils were sampled, processed and analysed for physicochemical characteristics. Example data are shown below in soils from Birchip Cropping Group (Table 3.3.1).

Table 3.3.1. Physicochemical characteristics of top (0-10 cm) and mid (10-30cm) soil profiles in collaborating farmer paddocks, to be used for MIR calibration of sorption isotherms.

Property	Units	BCG 1-top	BCG 1-mid	BCG 2-top	BCG 2-mid	BCG 3-top	BCG 3-mid	BCG 4-top	BCG 4-mid	BCG 5-top	BCG 5-mid
pH (Water)	pH	8.2	9.4	7.8	9.0	8.8	9.0	8.4	9.1	7.4	8.8
pH ($CaCl_2$)	pH	7.4	8.2	7.1	8.2	8.0	8.2	7.5	8.0	6.8	7.8
EC	dS/m	0.23	0.34	0.06	0.13	0.12	0.12	0.21	0.35	0.17	0.22
Sulfur (KCl_{40})	mg/kg	4.0	7.3	4.1	2.4	4.0	3.5	4.1	5.7	7.0	8.0
Colwell P	mg/kg	41	7.6	27	7.7	23	4.2	24	4.4	24	6.3
P Buffer Index		120	170	16	76	76	150	110	140	120	130
Organic C	%	1.1	0.59	0.3	0.14	0.61	0.29	0.97	0.54	1.5	0.47
Total N	%	0.13	0.08	0.04	0.02	0.07	0.04	0.13	0.07	0.16	0.06
Cl	mg/kg	4.4	32	<2	2	3.5	2.1	23	130	18	7.7
B	mg/kg	1.4	4.1	0.66	2.2	0.71	1.1	1.6	4	2.7	4.1
Cu	mg/kg	0.95	1	0.12	0.28	0.19	0.41	0.76	0.72	0.68	0.45
Zn	mg/kg	0.83	<0.1	2.2	<0.1	1.2	<0.1	0.56	<0.1	0.3	<0.1
Mn	mg/kg	12	6.1	5.5	1.1	3.7	1.9	6.6	2.1	15	4.3
Fe	mg/kg	9.3	11	8.7	6.7	2.2	5.1	4.2	3.8	21	10
Al	c_{mol}/kg	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Ca	c_{mol}/kg	18	16	2.9	8.4	11	11	18	16	17	12
K	c_{mol}/kg	1.5	0.81	0.41	0.69	0.98	0.81	1.8	0.98	1.5	0.65
Mg	c_{mol}/kg	6.6	11	1.2	4.9	1.5	3.5	6.5	9.7	6.7	7.9
Na	c_{mol}/kg	1.7	6	0.07	0.38	0.05	0.1	2.1	5.7	1.2	2.5
CEC (effective)	c_{mol}/kg	27	34	4.5	14	13	16	29	32	26	23
Ca/Mg ratio		2.6	1.5	2.5	1.7	7.4	3.3	2.8	1.6	2.5	1.5
Exch. Ca	% ECEC	64	47	64	58	81	72	64	49	64	53
Exch. K	% ECEC	5.6	2.4	8.9	4.8	7.3	5.1	6.4	3	5.9	2.8
Exch. Mg	% ECEC	24	32	25	34	11	22	23	30	25	34

Exch. Na	% ECEC	6.1	18	1.5	2.7	0.41	0.63	7.4	18	4.5	11
Clay	% w/w	14	25	4	18	9	16	19	31	18	32
Silt	% w/w	11	16	4	6	5	11	13	16	19	19
Fine Sand	% w/w	61	38	32	32	35	31	43	30	42	31
Coarse sand	% w/w	14	21	60	44	51	42	25	23	21	18
Gravel	% w/w	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

The soil samples provided adequate coverage across key physicochemical variables, including soil pH, clay content, and OC content (Figure 3.3.1). There was a slight bias towards more alkaline, low C, and sandier soil types. These soils are generally higher-risk soils because of their lower herbicide-binding potential.

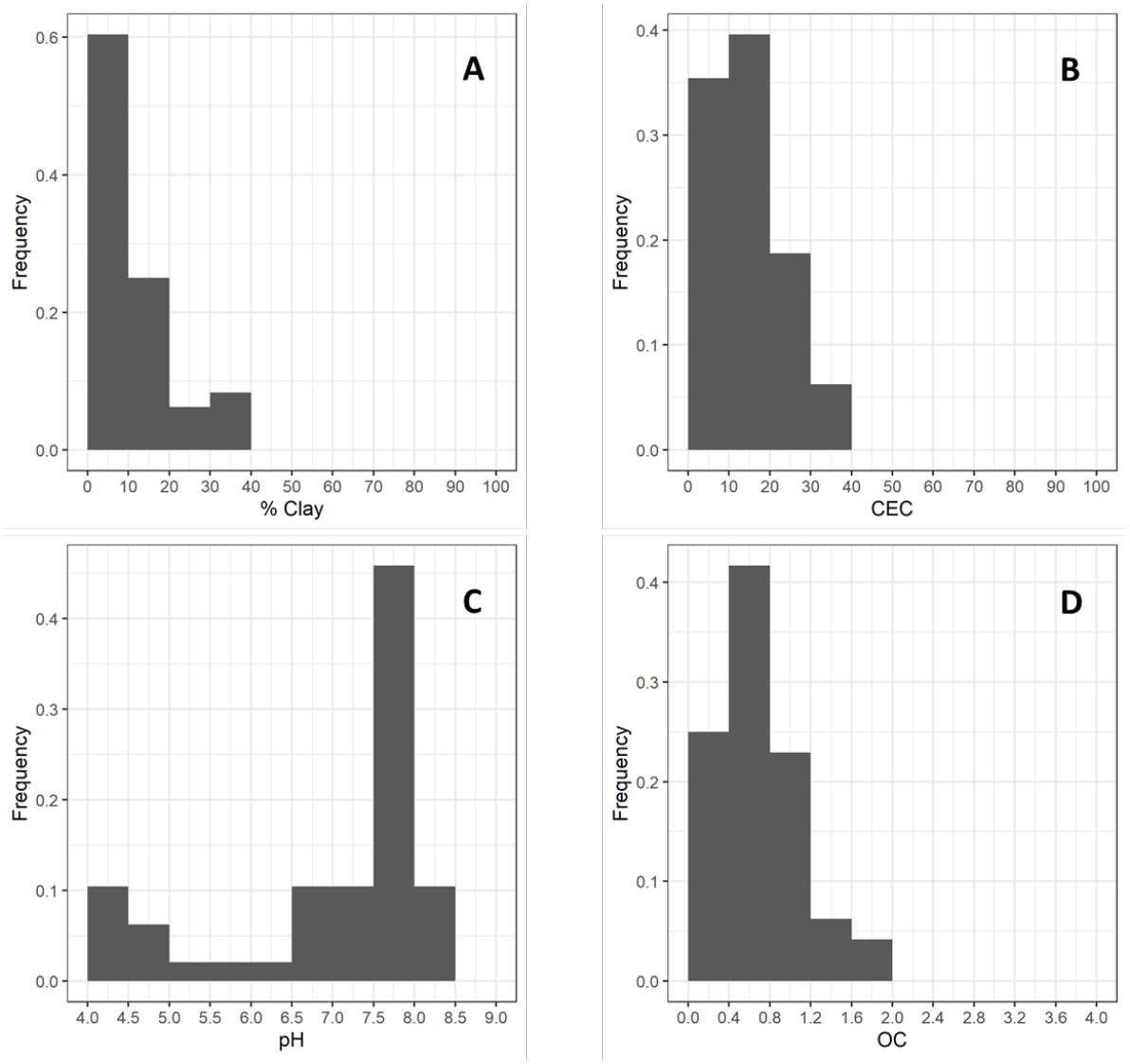

Figure 3.3.1. Frequency distributions of key soil physicochemical properties of the soils used in the sorption experiments, including a) Clay content, b) cation exchange capacity, c) pH_{CaCl2} and d) organic C.

3.3.2 Optimisation of sorption equilibrium time

Initial kinetics experiments were conducted on three different soil types to establish the time required to reach equilibrium, as per OECD protocols (OECD 2000). All herbicides reached equilibrium by 12 hours in the three different soils (data for imazapyr shown in Figure 3.3.2). Subsequent sorption experiments were conducted for 24 hours to ensure equilibrium was reached whilst minimising potential for degradation that could occur under longer shaking duration.

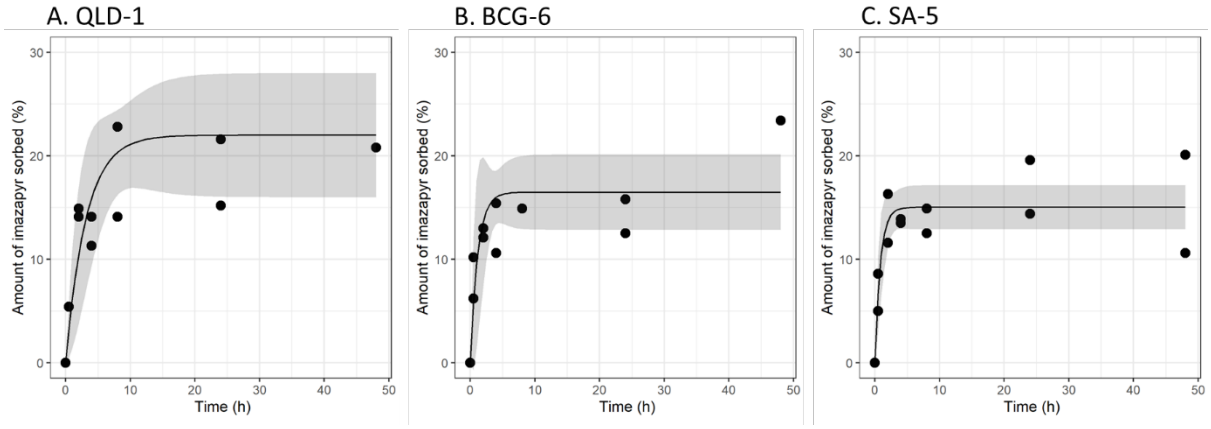

Figure 3.3.2. Sorption kinetics of imazapyr onto 3 contrasting soils

3.3.3 Sorption isotherms and equilibrium partition coefficients (K_d) for diuron, imazapyr and pyroxasulfone

Within the range of concentrations tested for each herbicide, representing likely concentrations in the topsoil, isotherms for all soils were adequately described ($r^2 > 0.8$) by linear fits (see examples in Figure 3.3.3). This allowed for the calculation of simple linear partition co-efficients (K_d), which are shown for each soil in Table 3.3.3.

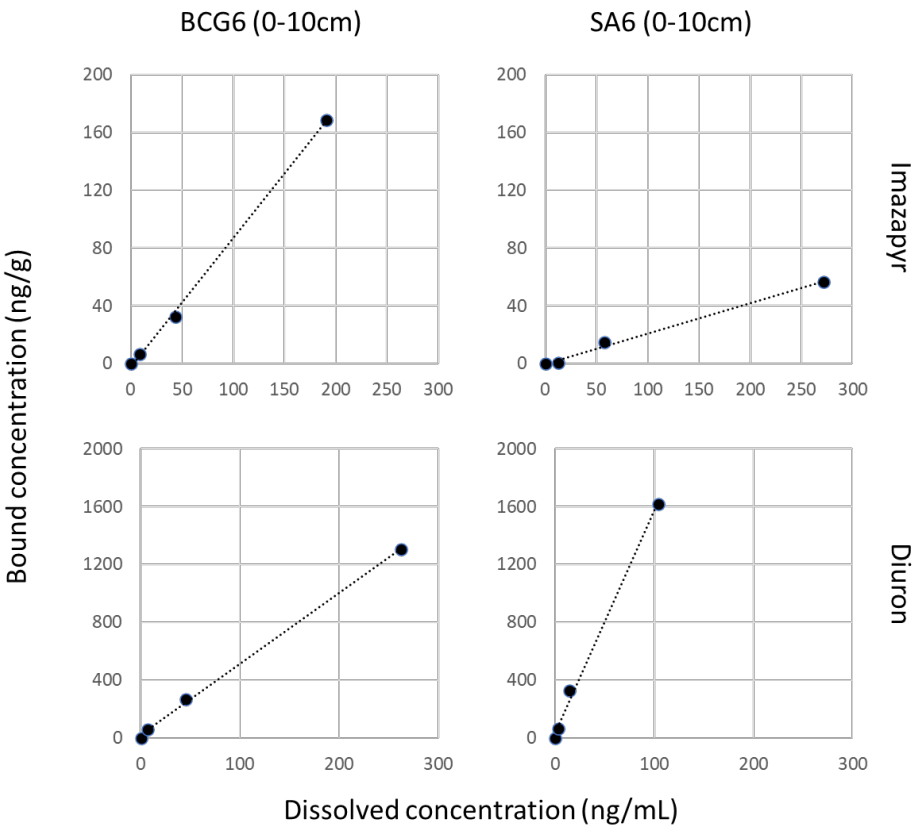

Figure 3.3.3. Frequency distributions of key soil physicochemical properties of the soils used in the sorption experiments, including a) Clay content, b) cation exchange capacity, c) pH_{CaCl2} and d) organic C.

Table 3.3.3. Herbicide sorption partition coefficients for soils from grower group members

Soil ID	Site	Depth	Imazapyr K_d	Diuron K_d	Pyroxasulfone K_d
CRC1	BCG1	0-10	0.56	10.7	2.7
CRC2	BCG1	10-30	0.41	4.0	1.1
CRC3	BCG2	0-10	0.37	2.1	1.0
CRC4	BCG2	10-30	0.25	0.2	0.8
CRC5	BCG3	0-10	0.22	1.8	0.5
CRC6	BCG3	10-30	0.31	0.9	0.5
CRC7	BCG4	0-10	0.56	6.1	1.4
CRC8	BCG4	10-30	0.49	1.6	0.7
CRC9	BCG5	0-10	0.44	20.1	0.4
CRC10	BCG5	10-30	0.32	9.4	2.2
CRC11	BCG6	0-10	0.89	4.9	1.6
CRC12	BCG6	10-30	0.61	1.0	0.6
CRC13	BCG7	0-10	0.33	3.2	0.3
CRC14	BCG7	10-30	0.30	1.4	0.8
CRC15	BCG8	0-10	0.27	5.0	0.2
CRC16	BCG8	10-30	0.36	2.6	0.7
CRC17	BCG9	0-10	0.67	7.4	0.3
CRC18	BCG9	10-30	0.58	8.5	1.7
CRC19	BCG10	0-10	0.75	8.2	0.9

CRC20	BCG10	10-30	0.65	4.3	0.9
CRC21	SA1	0-10	0.70	6.2	2.0
CRC22	SA1	10-30	0.16	2.6	1.2
CRC23	SA2	0-10	0.25	3.6	1.4
CRC24	SA2	10-30	0.30	2.1	1.8
CRC25	SA3	0-10	0.41	1.6	1.0
CRC26	SA3	10-30	0.34	0.8	0.1
CRC27	SA4	0-10	0.16	4.4	1.4
CRC28	SA4	10-30	0.40	2.0	0.1
CRC29	SA5	0-10	0.22	2.3	3.2
CRC30	SA5	10-30	0.14	12.4	1.4
CRC31	SA6	0-10	0.21	15.3	5.0
CRC32	SA6	10-30	0.35	5.9	1.8
CRC33	SA7	0-10	0.11	3.5	0.3
CRC34	SA7	10-30	0.31	2.6	0.2
CRC35	SA8	0-10	0.20	5.8	0.2
CRC36	SA8	10-30	0.24	3.9	1.5
CRC37	SA9	0-10	0.19	8.1	0.3
CRC38	SA9	10-30	0.20	4.1	0.1
CRC39	SA10	0-10	0.25	6.5	1.0
CRC40	SA10	10-30	0.24	0.8	0.3
CRC41	WA6	0-10	0.12	1.5	1.1
CRC42	WA7	0-10	0.34	3.9	0.7
CRC43	WA8	0-10	0.17	4.8	0.3
CRC44	WA8	10-30	0.28	4.9	0.3
CRC45	WA9	0-10	0.72	3.5	0.3
CRC46	WA9	10-30	0.67	3.2	0.3
CRC47	WA10	0-10	0.59	2.3	0.1
CRC48	WA10	10-30	0.53	3.0	0.5

Imazapyr sorption coefficients were low, ranging from 0.1 – 0.9 L kg^{-1}. That the K_d values were all <1 indicates that imazapyr is very mobile and that soil type effects on bioavailability will be minor compared with many other herbicides with K_d > 1. The K_d values determined here are within the range of those determined in other studies: 0.07 L kg^{-1} - 0.19 L kg^{-1} for 5 Alabama soils (Wehtje et al. 1987) and <0.1-2.8 L kg^{-1} for several Argentinean soils (Gianelli et al. 2014; Porfiri et al. 2015).

Diuron sorption coefficients ranged over two orders of magnitude, from 0.2 – 20 L $kg^{—1}$, with more than 90% of the soils exhibiting K_d > 1 L kg^{-1}. The K_d values determined here are similar to those determined in other studies. For example, 0.1 – 91.5 L kg^{-1} determined for a set of 98 Australian soils by Forouzangohar et al. (2008), with a median of 4.5 and mean of 8.5.

Pyroxasulfone sorption coefficients were intermediate, between that of imazapyr and diuron, and ranged from 0.2-3.0 L kg^{-1}. There is very little data on pyroxasulfone sorption in soils.

3.3.4 Prediction of imazapyr sorption onto soils

The soil sorption coefficients determined here were significantly (inversely) correlated to soil pH (Figure 3.3.4a), similar to previous research demonstrating significant negative correlations with pH and positive correlations with OC and clay content (see Gianelli et al. 2014 for discussion). It is likely that the limited variation in physiochemical properties (i.e. predominantly low OC, neutral-alkaline soils) and the low K_d values precluded us from other potential relationships with clay or OC.

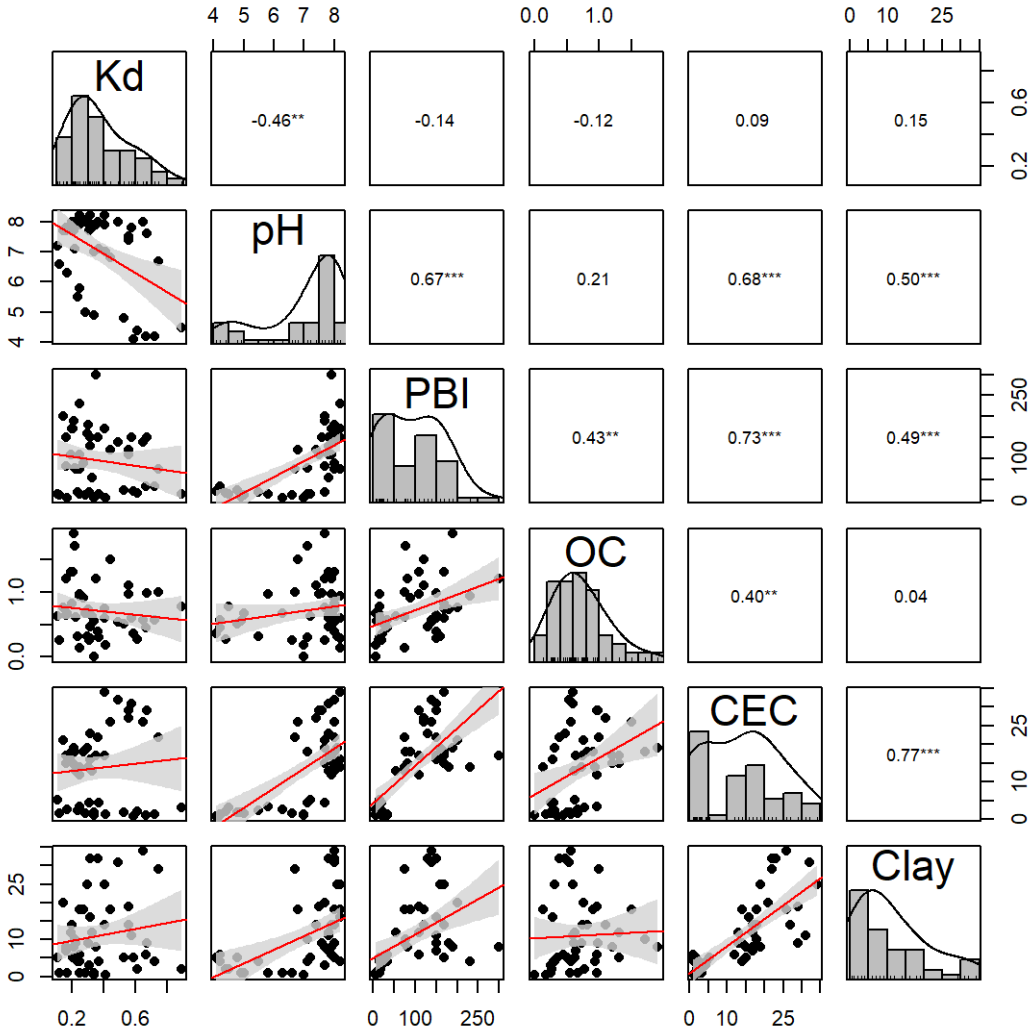

Figure 3.3.4a. Relationship between imazapyr K_d and key soil physicochemical properties.

Linear regression with multiple soil properties found that inclusion of pH, cation exchange capacity, and OC in the model improved predictions of imazapyr sorption (Figure 3.3.4b) compared to single correlations. These soil properties are measured as part of most soil tests, which means that the sorption coefficient (K_d) for imazapyr can be estimated for soil types where these data already exist. The use of a generalised additive model only marginally improved the r^2 of the model but did to improve the root mean-square error (RMSE) or mean absolute error (MAE).

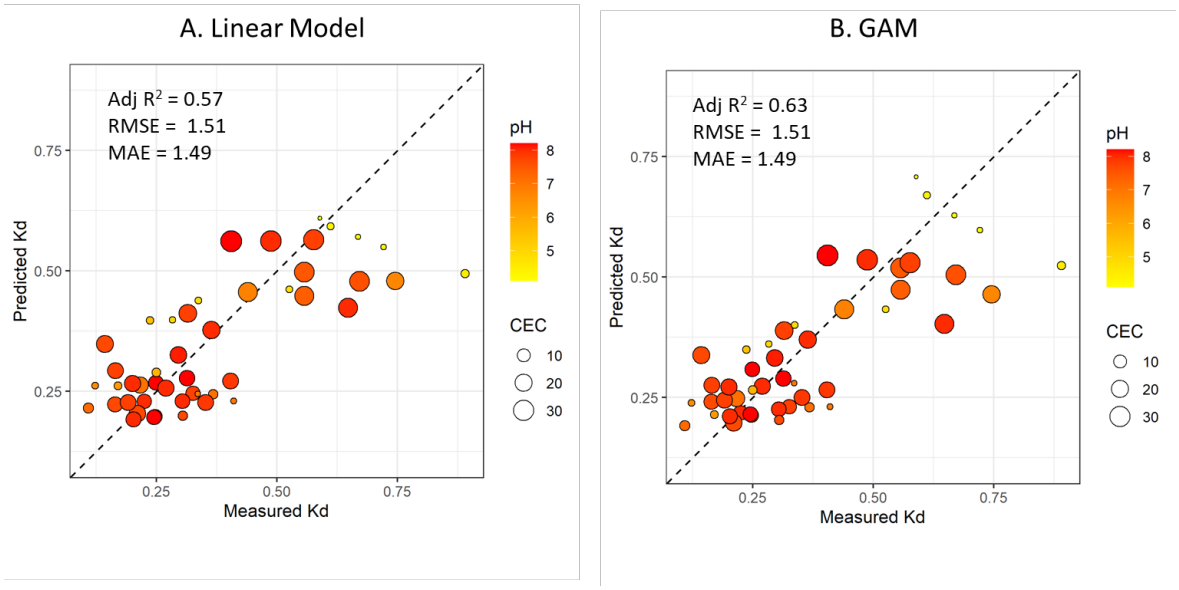

Figure 3.3.4b. Measured versus best fit predictions of imazapyr soil sorption coefficients for 48 contrasting soils, using linear regression (A) or generalised additive models (B). Factors in the best linear model included pH, OC, CEC, all of which were significant (P<0.01). The dashed line represents the 1:1 line of perfect model fit.

3.3.5 Prediction of diuron sorption onto soils

The soil sorption coefficients determined here were significantly positively correlated to soil OC content (r^2 = 0.75) (Figure 3.3.5a), similar to previous research (see Forouzangohar et al. 2008 for discussion).

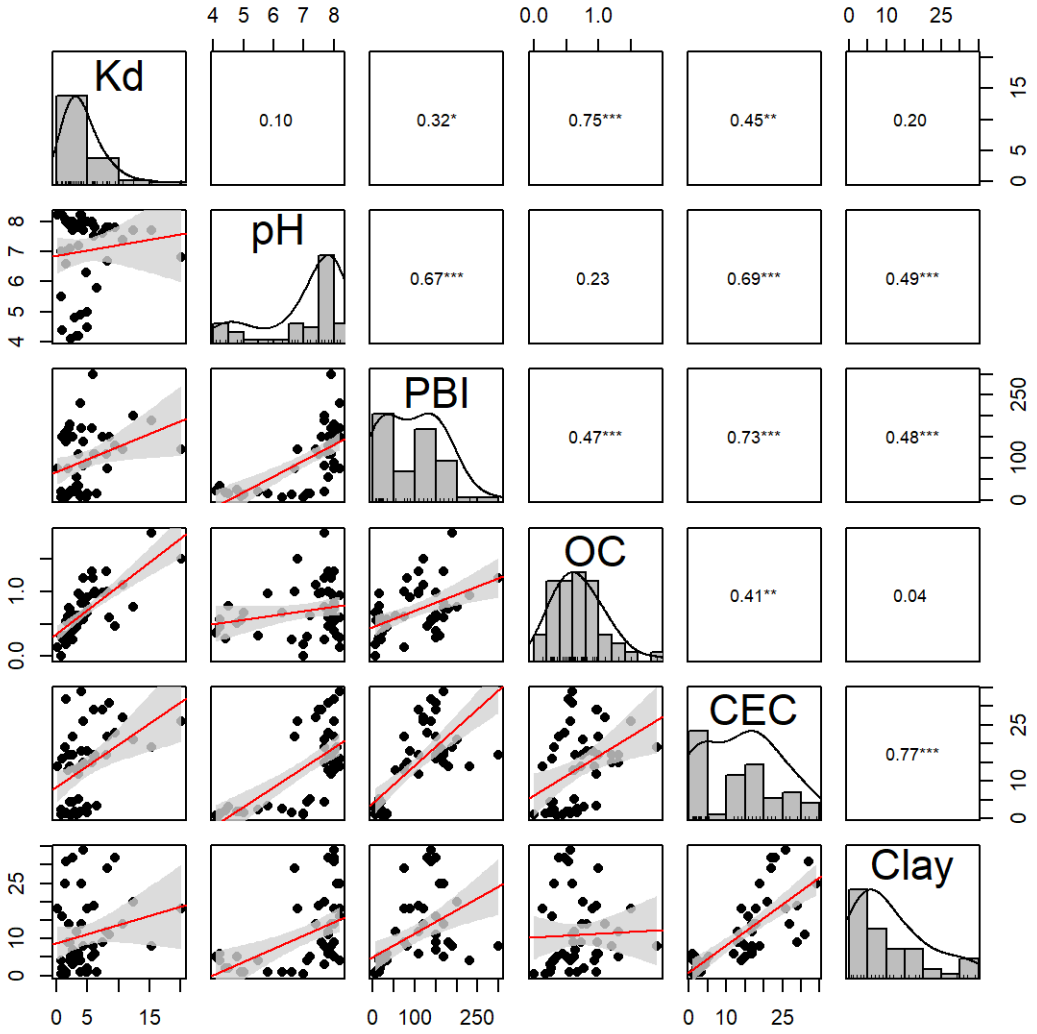

Figure 3.3.5a. Relationship between diuron K_d and key soil physicochemical properties.

Linear regression with multiple soil properties found that inclusion of cation exchange capacity and OC in the model improved predictions of diuron sorption (Figure 3.3.5b) compared to single correlations. As with the imazapyr predictions, the use of a generalised additive model slightly improved the r^2 and RMSE values but did not change the MAE.

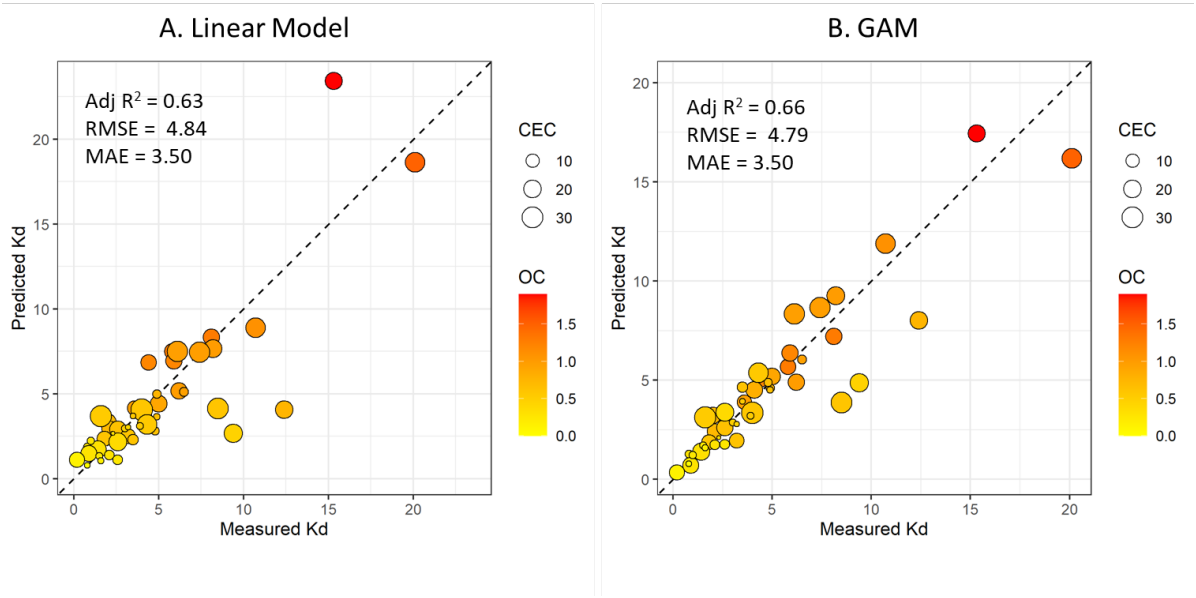

Figure 3.3.5b. Measured versus best fit predictions of diuron soil sorption coefficients for 48 contrasting soils, using linear regression (A) or generalised additive models (B). Factors in the best linear model included pH, OC, CEC, all of which were significant (P<0.01). The dashed line represents the 1:1 line of perfect model fit.

3.3.6 Prediction of pyroxasulfone sorption onto soils

Pyroxasulfone sorption coefficients were determined for 22 soils this quarter, with another 26 soils pending statistical analysis. Sorption coefficients ranged were higher than for imazapyr, but lower than diuron, with a range between 0.1 – 3.2 L kg^{-1}. The coefficients determined here are similar to those determined in other studies. For example, 0.3 – 9.6 L kg^{-1} determined for a set of 25 soils from USA by Westra et al. (2014), with a mean of 1.7 L kg^{-1}.

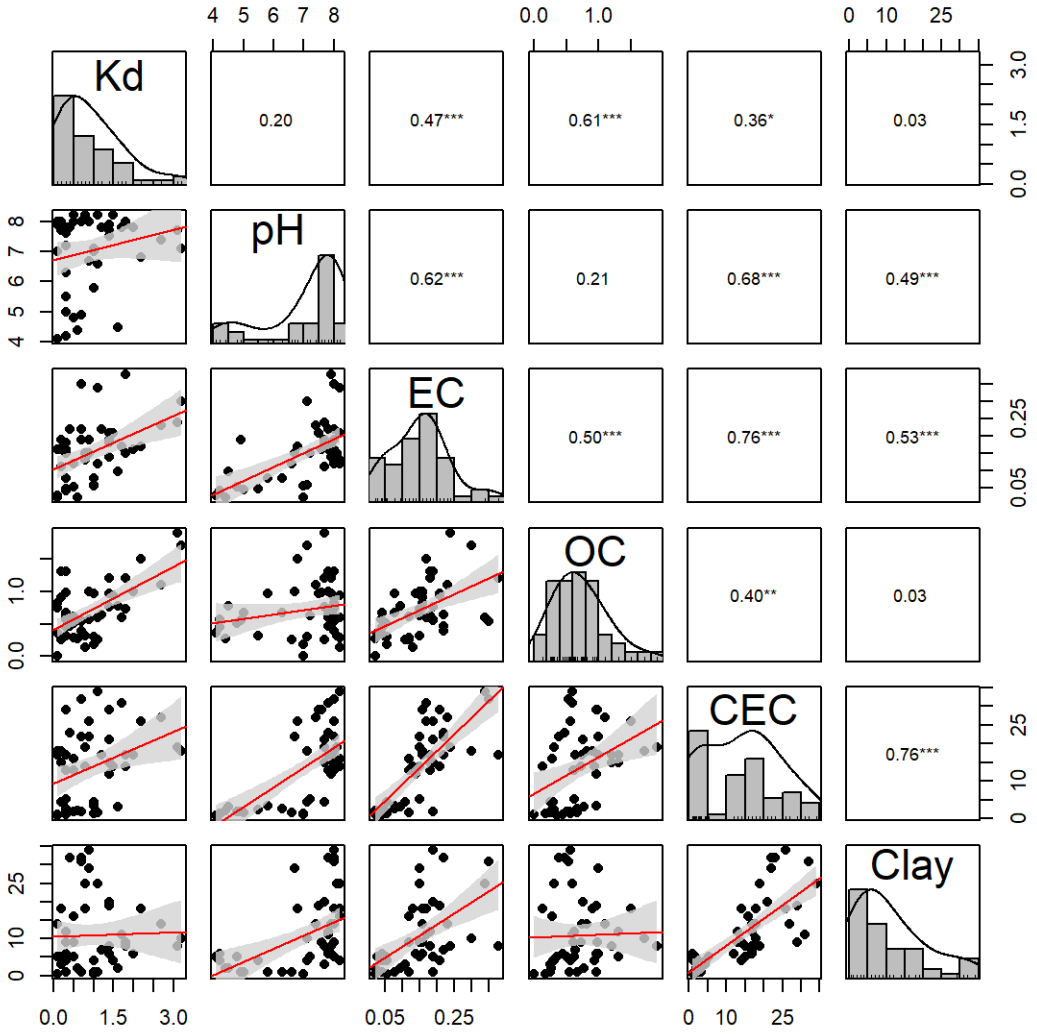

Figure 3.3.6a. Relationship between pyroxasulfone K_d and key soil physicochemical properties

The soil sorption coefficients determined here were significantly positively correlated to soil OC content (r^2 = 0.61) (Figure 3.3.6a, similar to previous research; see Westra et al. 2014). We also found a positive correlation with electrical conductivity (r^2 = 0.47) which when included in the linear regression model with multiple soil properties improved predictions of pyroxasulfone sorption (Figure 3.3.6b) compared to single correlations.

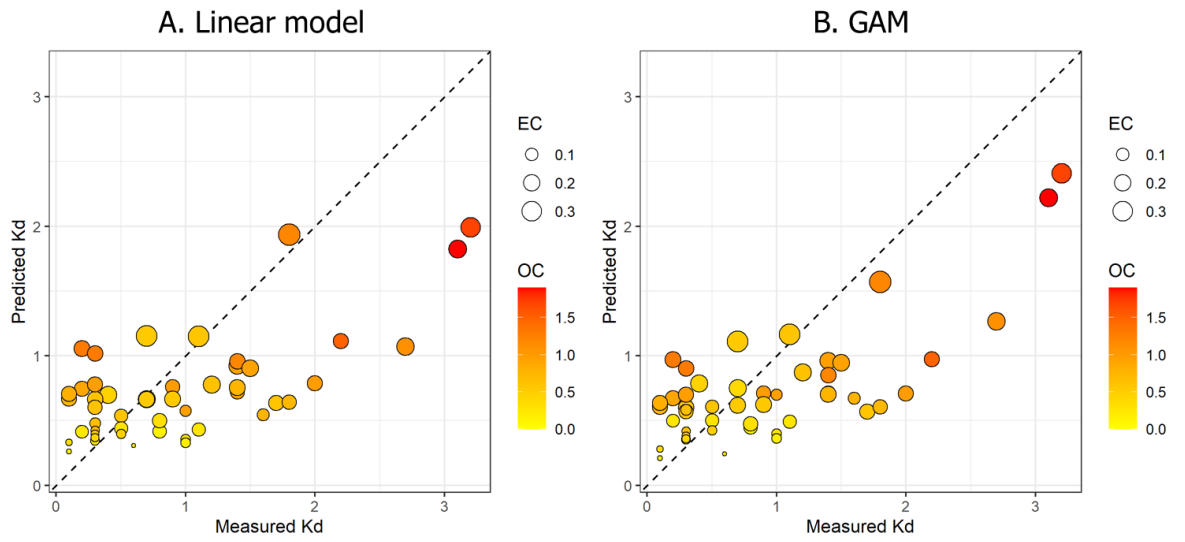

Figure 3.3.6b. Measured versus best fit predictions of diuron soil sorption coefficients for 48 contrasting soils, using linear regression (A) or generalised additive models (B). Factors in the best linear model included OC and EC which were significant (P<0.01). The dashed line represents the 1:1 line of perfect model fit.

3.3.7 Prediction of herbicide sorption onto soils using MIR spectroscopy

Mid-infrared reflectance spectroscopy was performed on all soils that were used for sorption studies of imazapyr, diuron and pyroxasulfone. Initially we calibrated and validated the use of MIR for predicting soil OC, as this has been previously demonstrated to give consistently accurate prediction across various soil collections. This demonstrated high fidelity of the model to measured OC content in both calibration and validation sets (Figure 3.3.7a)

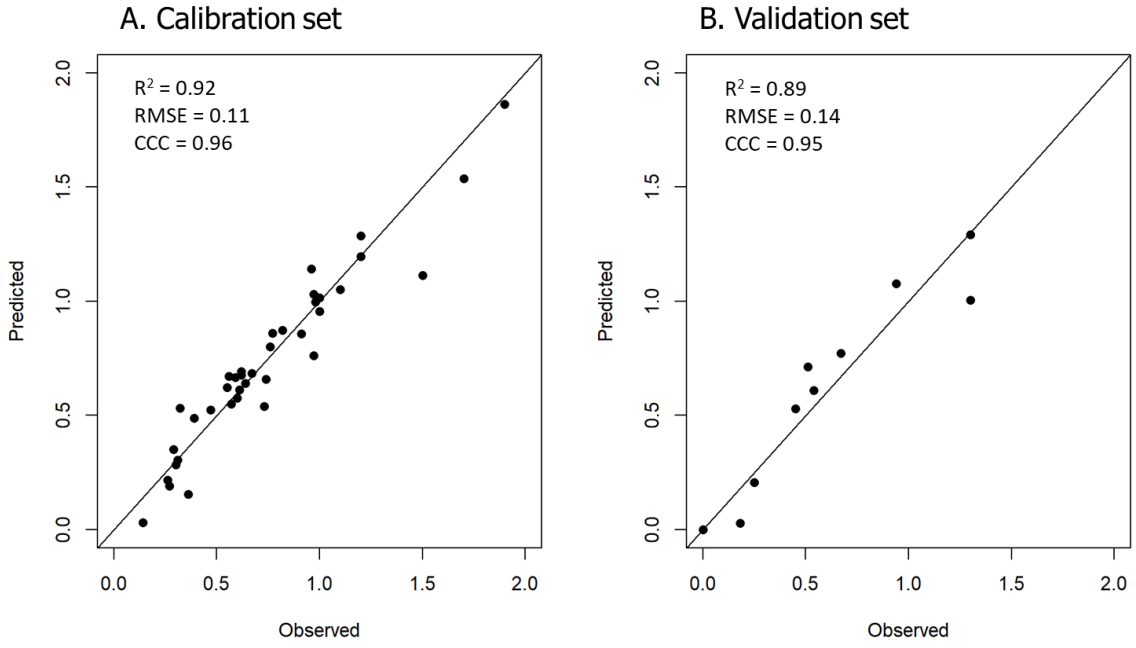

Figure 3.3.7a. Measured versus best fit predictions of OC content for 48 contrasting soils used for determining herbicide sorption. The solid line represents the 1:1 line of perfect model fit.

We subsequently used cubist-based model fitting to predict sorption coefficients for imazapyr, diuron and pyroxasulfone (Figs. 3.3.7b, 3.3.7c, and 3.3.7d, respectively). The prediction for diuron was very good; likely a consequence of the strong dependence of glyphosate on OC content for sorption. The model fits for imazapyr and pyroxasulfone were fair, but probably only useful for screening purposes rather than accurate estimates of K_d. This is probably a consequence of the very low binding of these two compounds, constrained set of soils with generally low clay and OC content, and confined pH ranges. The relationship of pyroxafulfone sorption with EC found in the physicochemical modelling suggests that MIR may not be very good at picking up soluble salts and therefore provides a relatively poor fit for pyroxasulfone.

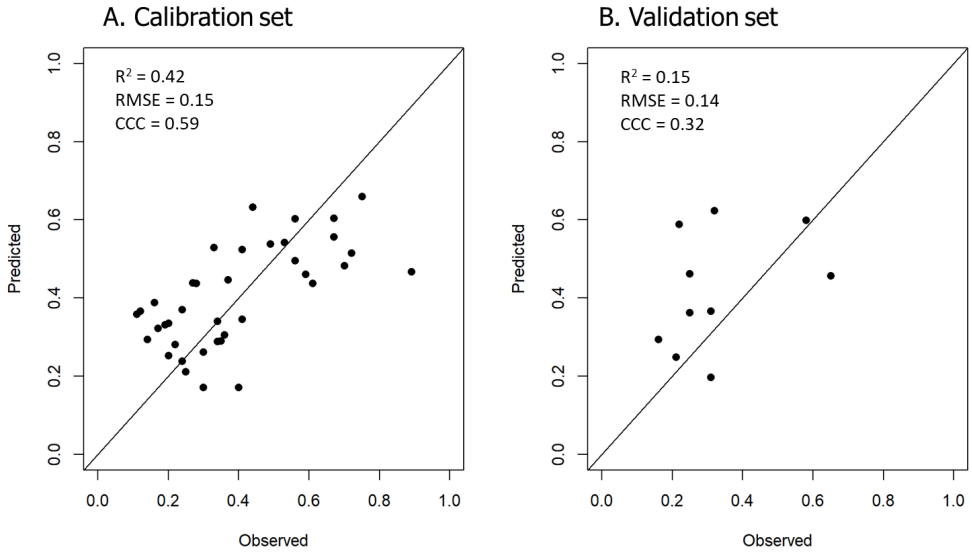

Figure 3.3.7b. Measured versus best fit predictions of imazapyr sorption for 48 contrasting soils used for determining herbicide sorption. The solid line represents the 1:1 line of perfect model fit.

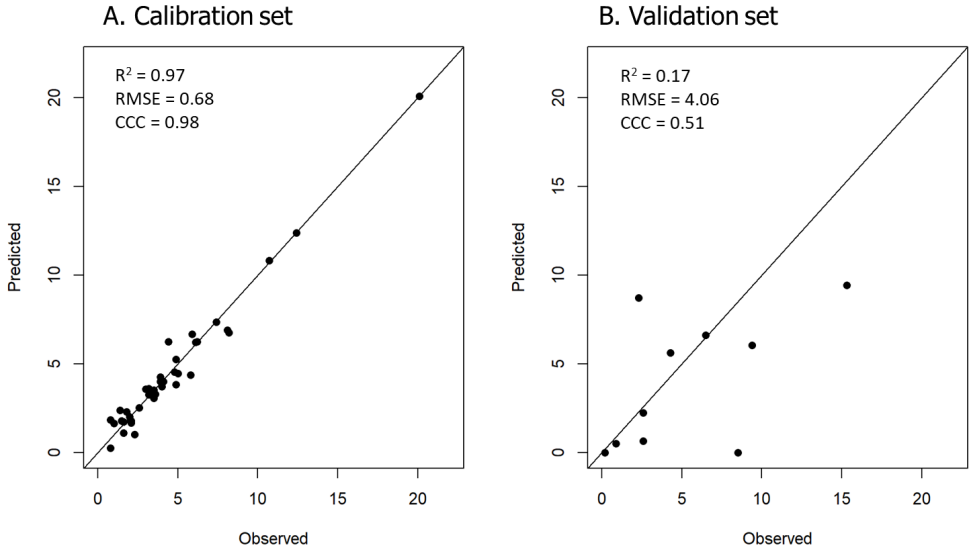

Figure 3.3.7c. Measured versus best fit predictions of diuron sorption for 48 contrasting soils used for determining herbicide sorption. The solid line represents the 1:1 line of perfect model fit.

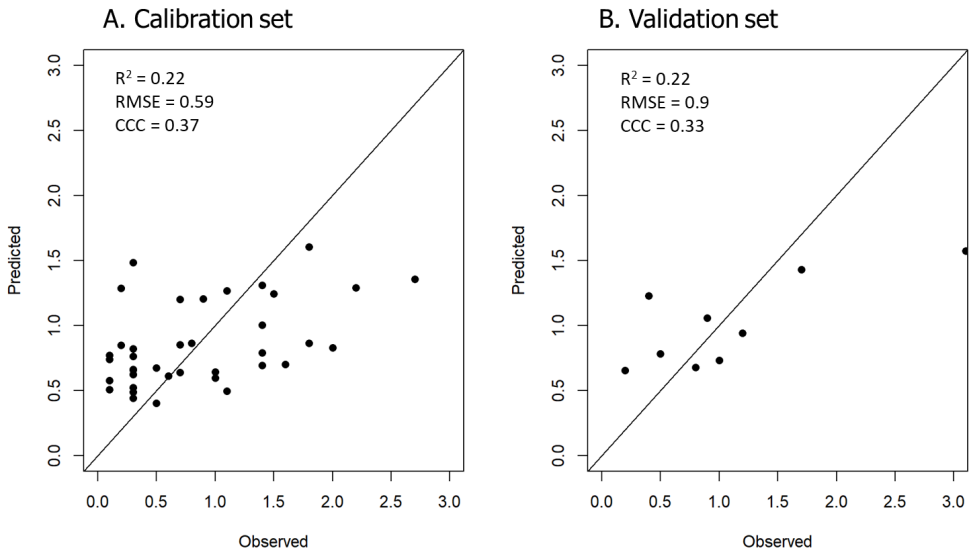

Figure 3.3.7d. Measured versus best fit predictions of pyroxasulfone sorption for 48 contrasting soils used for determining herbicide sorption. The solid line represents the 1:1 line of perfect model fit.

3.4 PERSISTENCE OF HERBICIDES IN CROPPING SYSTEMS

3.4.1 Herbicide persistence at BCG sites

There was significant variation in the persistence of imazapyr at the four different sites where imazapyr was applied and measured in 2019-2020. Dissipation of imazapyr at Horsham and Kinnabulla was relatively rapid (Table 3.4.1; Figure 3.4.1), where > 150 mm rain was recorded in the six months following application.

Table 3.4.1. Soil properties, imazapyr application date and imazapyr dissipation at four monitoring sites.

Location	Soil Clay (%)	Soil pH	Soil OC (%)	Date of application	Precipitation 0-180 d post-spray (mm)	Estimated 1st-order half-life (d)
Horsham	9	7.6	1.0	6/06/2019	230	11
Kinnabulla	14	7.4	1.1	1/06/2019	150	13
Jil Jil	29	8.0	1.0	22/07/2019	94	118
Brim	19	7.5	1.0	2/08/2019	99	320

In contrast, the two sites where imazapyr persistence was >100 days (Jil Jil and Brim) had <100 mm rain for the 6-month period following application. Both these sites also have a higher clay content, which may increase sorption and therefore decrease the herbicide bioavailability for microbial breakdown. The lower amount of rainfall also restricts microbial activity and can therefore lead to longer herbicide persistence in soil. The pesticide properties database (Lewis et al., 2015) lists the field half-life of imazapyr as 6-142, supporting our results that there is large variation across different sites.

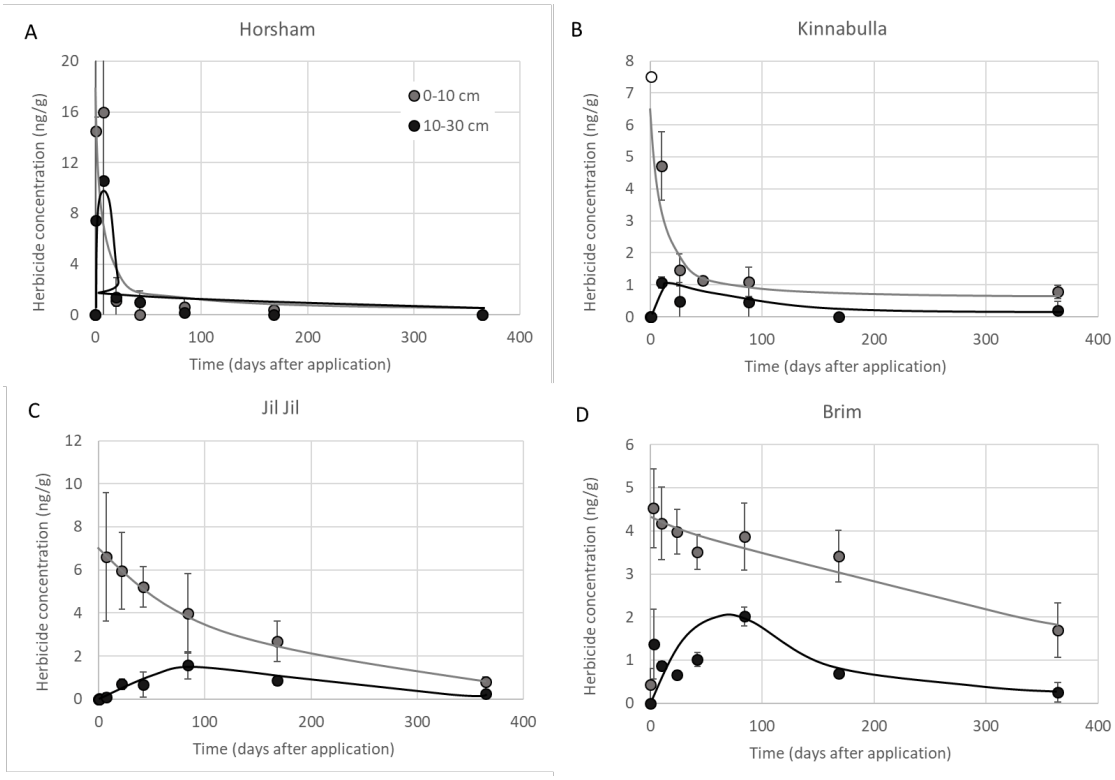

Figure 3.4.1. Imazapyr residue concentrations in 0-10 cm layer (grey points) and 10-30 cm layer (black points) at four BCG monitoring sites. Points represent average residue levels of three field replicates. Smoothed splines have been overlaid as a visual guide and are not statistical model fits.

3.4.2 Herbicide persistence at SA sites

There was some variation in the persistence of clopyralid at the four different sites monitored from mid-2019 to mid-2020 (Figure 3.4.2). At all sites except Poochera, baseline residues of clopyralid were detected at 1-2 ng/g prior to the application of clopyralid in-crop in 2019. After clopyralid application, concentrations in topsoil (0-10 cm) increased to maximum levels of 12-18 ng/g, depending on the site. At the Minnipa sites, there was a steady decline in clopyralid over the 364 days after application, with approximately 30% of the clopyralid remaining at the 6-month sampling in January 2020. In contrast, at the Poochera and especially the Mt Cooper sites, dissipation was faster in the initial 3 months to day 84, to the point where clopyralid could no longer be detected in topsoil at Mt Cooper. However, clopyralid residues increased again at both sites at the 6-month sampling date and remained detectable (but low) at 1-2 ng/g by 364 days.

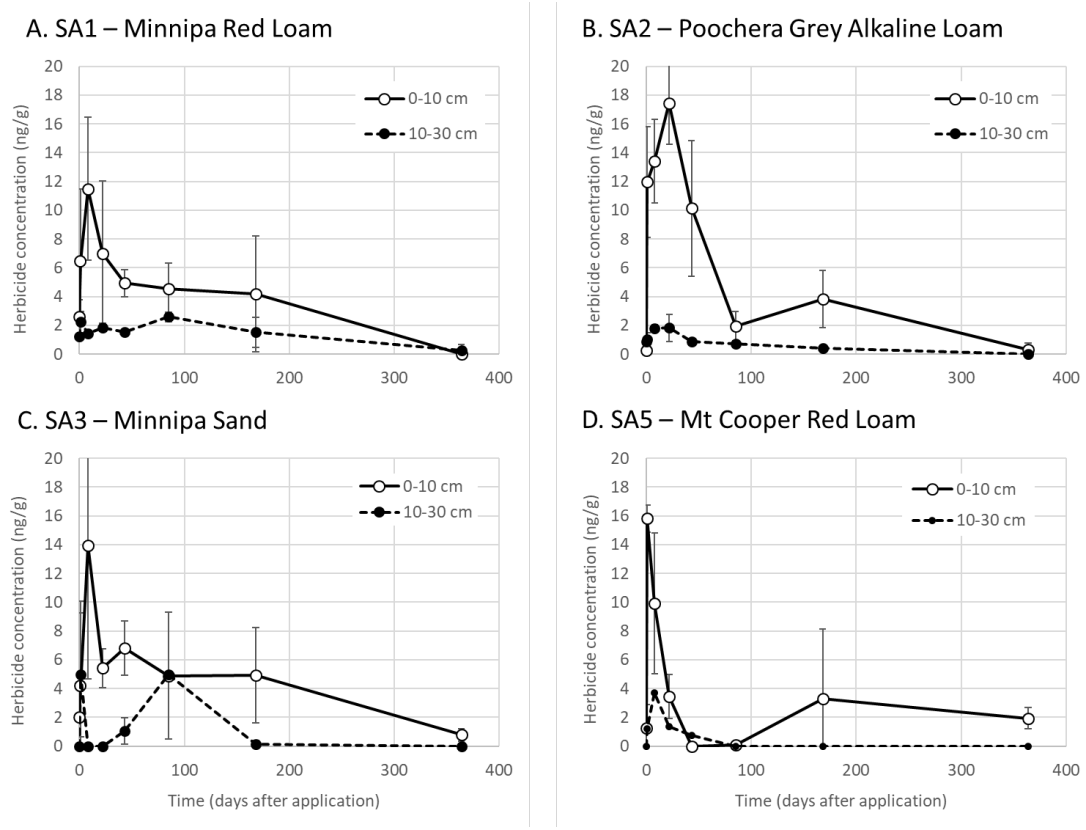

Figure 3.4.2. Clopyralid residue concentrations in 0-10 cm layer (white points, solid line) and 10-30 cm layer (black points, dashed line) at four EP monitoring sites. Points represent average residue levels of three field replicates, error bars are standard deviations. Line are a visual guide and are not statistical model fits.

Another important point to note is the high variation in clopyralid concentrations at each time point, particularly at 168 days after spraying. Repeat analysis of the same soil sample (i.e. lab replicate) showed that analytical variation was low, suggesting that there is high variation across field replicates. This means that although the average concentration in one paddock could be 3.5 ng/g (i.e. Mt Cooper SA5 at 168 days), the actual concentrations at different points across that paddock could vary from 0 – 10 ng/g or more.

In the second season of clopyralid persistence monitoring 2020-21, clopyralid dissipation was significantly faster than the previous season. Six months after clopyralid application, the average clopyralid concentration in topsoil (0-10 cm) at three of the four sites was below 1.0 ng/g, which is the limit of quantification of the analytical method (Table 3.4.2). Residues were only consistently detected at low levels (average of 1.1 ng/g) at the SA3 Minnipa site. These values are significantly lower than the levels of clopyralid in samples taken at a similar time in the previous season, 6 months after application in 2019 (Table 3.4.2). Concentrations of clopyralid in the 10-30 cm soil depth were also below the limit of quantification (< 1.0 ng/g).

Table 3.4.2 Concentration of clopyralid in topsoil (0-10 cm) at different sites taken in January 2020 (previous season results) and January 2021 (this season results), at approximately 6 months after clopyralid application.

Year of soil sample	ID	Location	Clopyralid application date	Rate (g/ha)	Rainfall 0-180 d post-spray (mm)	Mean clopyralid concentration @ 180 d after application (ng/g) (n=3)	Higher risk crops
2020	SA1	Minnipa	25 June 2019	45	120	4.2	Lentil, Field pea
2020	SA2	Poochera	25 June 2019	30	75	3.8	Lentil, Field pea
2020	SA3	Minnipa	23 July 2019	27	108	4.9	Lentil, Field pea
2020	SA5	Mt Cooper	4 July 2019	24	117	3.3	Field pea
2021	SA1	Minnipa	23 June 2020	40	204	<1.0	-
2021	SA2	Poochera	16 July 2020	18	217	<1.0	-
2021	SA3	Minnipa	6 July 2020	27	158	1.1	-
2021	SA4	Kimba	16 July 2020	60	214	<1.0	-

The clopyralid concentrations in the January 2021 soil samples were lower than all legume toxicity thresholds and were unlikely to affect any crops during the 2021 season. This contrasts with the clopyralid concentrations detected in the January 2020 samples, which may have impacted lentil or field pea seedlings sown at those sites.

3.5 HERBICIDE WITHDRAWAL EXPERIMENTS

3.5.1 BCG Site

In 2019 there was no influence on crop performance of Spartacus CL barley from herbicide use (Table 3.5.1a). This is as would be expected as the crop type used has recommended-use patterns to clopyralid, and is tolerant of the use of imazamox and imazapyr (Intervix) as part of a herbicide program. This variety is commonplace in the cropping program in this area as it gives flexibility to manage weeds with unconventional chemistries due to breeding for crop tolerance.

Table 3.5.1a. Crop biomass and yield results from variety and herbicide treatments in 2019.

BCG	**Spartacus Barley CL**			
Trt No.	**Seeding**	**In-crop**	**Biomass (T/ha)**	**Yield (T/ha)**
1	Nil	Nil	15.5	6.27
2	Farmer practice	Imazamox/Imazapyr	15.8	6.30
3	Farmer practice	Clopyralid	15.8	6.26
4	Farmer practice	Imazamox/Imazapyr	15.4	6.20
5	Farmer practice	Clopyralid	15.8	6.27
Pr (>F)			0.94	0.61
HSD			2.1	0.19

In January 2020, approximately six months after the application of herbicides in the 2019 barley crop, residues of imazapyr/imazamox and clopyralid were still detectable in plots receiving each of the herbicides. However, by April 2020 clopyralid was no longer detectable (<1 ng/g in all but one plot) and imazapyr/imazamox in the top 10 cm of soil had declined to approximately 4 ng/g. By the time of sowing, (15th May 2020) residues of all focus herbicides had declined to levels below the estimated ED_{20} for lentils.

Biomass and yield harvest at the end of 2020 showed that there was no statistically significant difference in performance between lentil varieties and herbicide treatments (Table 3.5.1b). There was a trend towards lower yield in the withdrawal plots, probably a consequence of less-efficient weed control (i.e. hand-weeding) reducing crop performance. There was no significant interaction noted so only main effects are presented. Although it could have been expected that Lontrel or Intervix would have influenced one or both varieties in terms of performance, the seasonal conditions over the period of the trial were conducive to sufficient breakdown of the herbicides (Figure 3.5.1), limiting the effect on crop performance as discussed above.

Nodulation and N fixation effects of herbicide treatment were also measured and showed no difference as a result of herbicide treatments (data not shown). This is not surprising given the favourable conditions that likely allowed enough breakdown and lack of effect on biomass and crop yield. However herbicidal effects that influence pulse crops' ability to nodulate should remain a consideration, as it may have flow on effects to subsequent crops due to increased nitrogen requirements to meet the needs of the crop. Further work in this area needs to be undertaken to understand the usually unmeasured effects of herbicide use that may have longer term impacts on the system.

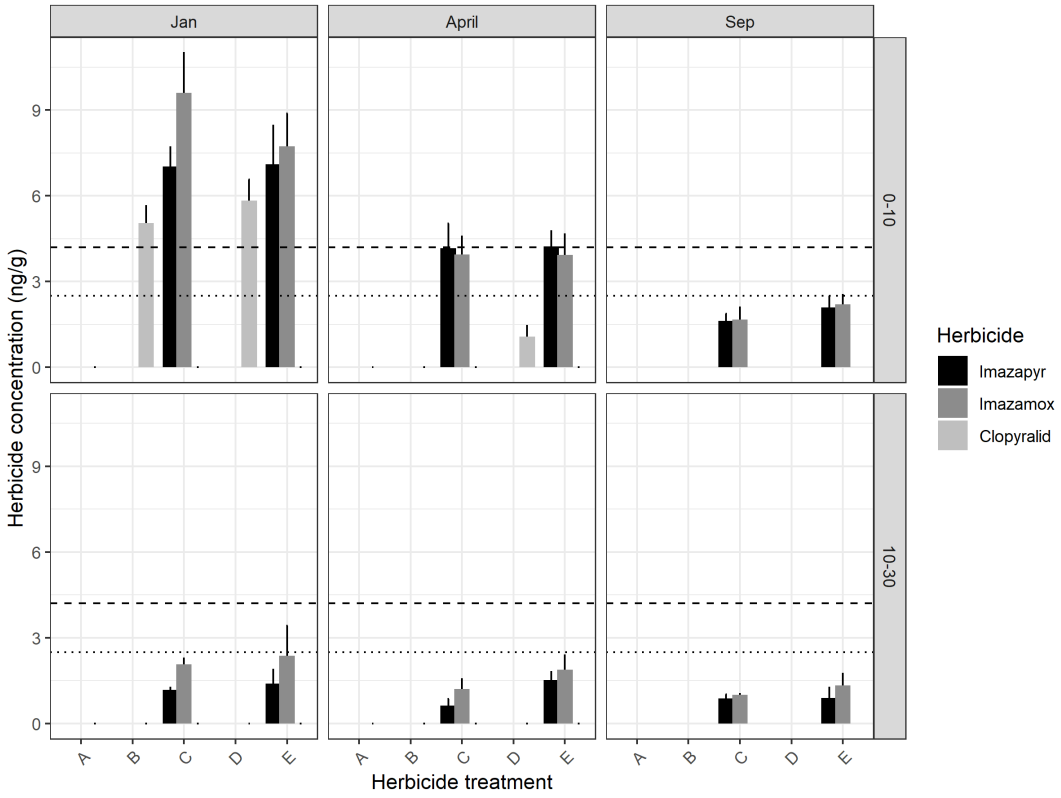

Figure 3.5.1. Herbicide concentration over time in 2020. Treatments were: A = Nil herbicide; B = Clopyralid in crop – no summer spray; C = Imazamox/imazapyr in crop – no summer spray; D = Clopyralid in crop + summer farmer practice; E = Imazamox/imazapyr in crop + summer farmer practice. The dashed line represents the ED_{20} for imazapyr toxicity to wheat and the dotted line represents the ED_{20} for clopyralid toxicity to lentil.

Table 3.5.1b. Crop biomass and yield results from variety and herbicide treatments in 2020.

	Crop Biomass (t/ha)	**Grain Yield (t/ha)**
Variety		
Hallmark XT	1.20	2.21
Jumbo 2	1.15	2.18
Herbicide Treatment		
Nil Herbicide	1.19	1.91
Clopyralid in crop – no summer spray	1.17	2.36
Imazamox/imazapyr in crop – no summer spray	1.16	2.27
Clopyralid in crop + summer farmer practice	1.11	2.12
Imazamox/imazapyr in crop + summer farmer practice	1.23	2.32
Sig. Diff. **LSD (P=0.05)**	**NS** **NS**	**NS** **NS**

3.5.2 Herbicide withdrawal experiment – Minnipa (SA) Site

In 2019 there was no influence of herbicide treatment on crop performance of Spartacus CL barley from herbicide use (Table 3.5.2a). This as expected as the herbicide applied were all registered for use in paddock preparation (pre-sowing) and in-crop for barley.

Table 3.5.2a. Crop biomass and yield results from different herbicide treatments at Minnipa, SA, in 2019.

SARDI	**2019 Spartacus Barley CL**			
Trt No.	**Seeding**	**In-crop**	**Biomass (T/ha)**	**Yield (T/ha)**
1	Nil	Nil	4.13	1.35
2	Glyphosate +Trifluralin	Nil	5.03	1.84
3	Glyphosate	Clopyralid	4.86	1.88
4	Trifluralin	Clopyralid	4.60	1.60
5	Glyphosate +Trifluralin	Clopyralid	4.84	1.77
6	Glyphosate +Trifluralin	Clopyralid + Diflufenican/MCPA	4.75	1.77
7	Glyphosate +Trifluralin	Clopyralid + Diflufenican/MCPA	4.64	1.82
8	Pyroxasulfone	Nil	4.35	1.70
Pr (>F)			0.25	0.23
HSD			1.19	0.70

In 2020, there was a significant effect (P<0.05) of herbicide treatment on yield (but not biomass) (Table 3.5.2b). Plots receiving full herbicide control, including fallow, pre-seeding and in-crop sprays (as per recommended practice), delivered significantly higher yields than treatments that did not receive fallow sprays, or the pre-emergent residual herbicide trifluralin.

Table 3.5.2b. Crop biomass and yield results from different herbicide treatments at Minnipa, SA, in 2020.

SARDI	**2020 Sceptre Wheat**				
Trt No.	**2019/20 Fallow**	**Seeding**	**In-crop**	**Biomass (T/ha)**	**Yield (T/ha)**
1	Nil	Nil	Nil	2.63	1.61 b
2	Glyphosate	Glyphosate +Trifluralin	Nil	2.91	1.70 ab
3	Glyphosate	Glyphosate	Clopyralid	2.49	1.65 b
4	Glyphosate	Trifluralin	Clopyralid	2.63	1.65 b
5	Glyphosate	Glyphosate +Trifluralin	Clopyralid	2.59	1.72 ab
6	Glyphosate	Glyphosate +Trifluralin	Clopyralid + Diflufenican/MCPA	2.47	**1.87 a**
7	Nil	Glyphosate +Trifluralin	Clopyralid + Diflufenican/MCPA	2.38	1.68 ab
8	Nil	Pyroxasulfone	Nil	2.76	1.63 b
	Pr (>F)			0.34	0.015
	HSD			0.75	0.20

3.5.3 Herbicide withdrawal experiment – Cunderdin (WA) Site

In 2019 there was no influence of herbicide treatment on crop performance of Spartacus CL barley from herbicide use (Table 3.5.3a). This as expected as the herbicide applied were all registered for use in paddock preparation (pre-sowing) and in-crop for barley.

Table 3.5.3a. Crop biomass and yields from different herbicide treatments at Cunderdin, WA, in 2019.

WANTFA	**2019 – Sceptre Wheat**			
Trt No.	**Seeding**	**In-crop**	**Biomass (T/ha)**	**Yield (T/ha)**
1	Nil	Nil	4.83	1.71
2	Pyroxasulfone	Farmer Practice	4.97	1.76
3	Diuron	Farmer Practice	4.98	1.80
4	Trifluralin	Farmer Practice	4.43	1.77
5	Pyroxasulfone + Trifluralin	Farmer Practice	4.91	1.79
Pr (>F)			0.65	0.92
HSD			1.36	0.37

In 2020, there was a significant effect (P<0.05) of herbicide treatment on yield (but not biomass) (Table 3.5.3b). Crops not receiving any herbicide had significantly higher yield (3.75 T/ha) than plots receiving diuron as a pre-emergent application (2.93 T/ha), by approximately 25%. Diuron is more mobile than pyroxasulfone and trifluralin, and it is possible that rain following sowing washed diuron into the crop row and caused some damage.

Table 3.5.3b. Crop biomass and yields from different herbicide treatments at Cunderdin, WA, in 2019.

WANTFA	**2019 – Sceptre Wheat**			
Trt No.	**Seeding**	**In-crop**	**Biomass (T/ha)**	**Yield (T/ha)**
1	Nil	Nil	6.93	3.75 a
2	Pyroxasulfone	Farmer Practice	7.15	3.65 ab
3	Diuron	Farmer Practice	6.11	2.93 b
4	Trifluralin	Farmer Practice	6.71	3.54 ab
5	Pyroxasulfone + Trifluralin	Farmer Practice	6.81	3.45 ab
Pr (>F)			0.26	0.05
HSD			1.47	0.81

3.6 SOILBORNE HERBICIDE RESIDUES AT SOWING – A PILOT STUDY

The average turnaround time between sample receival and reporting of results to the growers was 1 month. This is a conservative estimate of how long a commercial service would take to process samples and report results but suggests that sampling should take place at least 6 weeks prior to sowing.

Herbicides were detected in all but one sample batch submitted to the laboratory, even though some samples were only analysed for 6 different herbicides. It is likely that an

expanded suite of herbicide analytes would have detected more herbicides, based on results of previous soil surveys (Rose et al. 2022).

Four out of the seven paddocks sampled in South Australia contained negligible or non-detectable residues of the herbicides of concern. However, three of the paddocks contained residues that could potentially affect some crops (Table 3.6a). Paddock SA1 contained residues of clopyralid that were around the concentration that could affect lentil and field pea growth (i.e. ED_{20} of 2 - 4 ng/g), but this paddock was planted to canola which is very tolerant to clopyralid (ED_{20} of >100 ng/g). Paddock SA2 contained residues of both imazapyr and imazapic at similar levels. The concentrations of imazapyr in this paddock were slightly higher than the toxicity threshold for wheat predicted to cause 20% shoot biomass reduction in seedlings. The other sites contained residues of imazapic, for which there were no available toxicity thresholds at this time.

Table 3.6a. Herbicide residue concentrations (ng/g) and potential crop effect in the seven paddocks participating in the pilot study.

ID	Target	Rep.	Herbicide residue concentration (ng/g)	Higher risk crops	Crop planted
SA 1	Clopyralid	1	4.1	Lentil, Field pea	Canola
		2	2.1		
		3	2.0		
		4	<LOD		
SA2	Imazapyr/ Imazapic	1	2.0/2.4	Wheat, Barley	Wheat (Scepter)
		2	1.8/3.2		
		3	2.0/2.2		
		4	<LOD/<LOD		
SA3	Clopyralid	1	trace	Negligible risk	Lentil (Hurricane XT)
		2	trace		
		3	trace		
		4	trace		
SA4	Imazapyr/ Imazapic	1	<LOD	Negligible risk	
		2	<LOD		
		3	<LOD		
		4	<LOD		
SA5	Clopyralid	1	<LOD	Negligible risk	
		2	<LOD		
		3	<LOD		
		4	<LOD		
SA6	Imazapic	1	2.0	Data not available	Wheat (Scepter)
		2	1.2		
		3	1.0		
		4	<LOD		
SA7	Imazamox	1	<LOD	Negligible risk	Wheat (Scepter)
		2	<LOD		
		3	<LOD		
		4	<LOD		

In Victoria the target herbicides were detected but only at low levels (Table 3.6b). One grid sample at Lawloit (2.4 ng/g imazapyr) exceeded barley and wheat thresholds on sandy soils, but the other grid samples from the same paddock contained low or undetectable levels.

Table 3.6b. Mean (and range) concentrations (ng/g) of imidazolinone residues taken at four grid points within target paddocks in SA and Vic.

ID	Target	Rep.	Herbicide residue concentration (ng/g)	Higher risk crops	Crop planted
Vic 1	Imazapyr/ imazamox/	1	tr/2.4	Negligible risk	Wheat (Scepter)
		2	<LOD/<LOD		
		3	<LOD/<LOD		
		4	<LOD/<LOD		
Vic 2	Imazapyr/ imazamox/ Imazapic	1	1.6/tr	Wheat, Barley	Wheat (Scepter)
		2	tr/3.6		
		3	<LOD/<LOD		
		4	2.4/5.4		

In Western Australia, the target herbicides were detected at sites WA1 and WA3, but only at low levels (Table 3.6c). It is unlikely that any of these residues would have affected the crop planted.

Table 3.6c. Mean (and range) concentrations (ng/g) of imidazolinone residues taken at four grid points within target paddocks in SA and Vic.

ID	Target	Rep.	Herbicide residue concentration (ng/g)	Higher risk crops	Crop planted
WA 1	Pyroxasulfone, diuron	1	tr/3	Negligible risk	Canola
		2	tr/4		
		3	tr/<LOD		
		4	Sample missing		
WA 2	Imazapyr/ imazamox/	1	<LOD/<LOD	Negligible risk	Wheat
		2	<LOD/<LOD		
		3	<LOD/<LOD		
		4	<LOD/<LOD		
WA 3	Clopyralid	1	2.4	Lentils, chickpeas	Lupin
		2	2.2		
		3	Tr		
		4	2.4		

4. DISCUSSION

4.1 HERBICIDE RESIDUE PHYTOTOXICITY THRESHOLDS

Growers and consultants have expressed interest in being able to send soil samples for herbicide residue analysis to gauge whether a crop is likely to suffer herbicide damage. Methods for total soil herbicide residue analysis are well established, and several commercial laboratories offer this service, but predicting if a toxicity risk exists is still a challenge. This is because there are very few toxicity threshold values for common broadacre crops available in the public domain. In a recent soil survey and review of the relevance of herbicide residues to crop toxicity, less than five thresholds were identified for each of the priority herbicides under investigation in this project (Rose et al. 2022).

This project has now generated additional herbicide residue phytoxicity thresholds for 80 different herbicide-crop-soil combinations, including six different herbicides for five or more crop species. This is a significant increase in knowledge considering the recent review (Rose et al. 2022) found a total of only 138 herbicide-crop-soil phytotoxicity thresholds from international literature for crops grown in the Australian grains industry. These thresholds can be used directly by agronomists as a rough guide for interpreting soil herbicide residue tests. Thresholds generated in sand demonstrate a 'worst-case' scenario, such that if the residue level for the herbicide of concern is lower than the sand threshold, then the likelihood of damage from that herbicide is negligible. Thresholds generated in soil provide a more realistic guide to the range of residue levels that may cause damage. Although the range of thresholds for a single herbicide may vary by a factor of 5 across different soil types, they still provide a context for interpreting a soil herbicide test.

The value of these thresholds can also be maximised by combining threshold data for specific soil types with estimates of herbicide bioavailability (e.g. sorption partition coefficients, K_d) in these soils. Work in a complementary GRDC-funded project has shown that there is a significantly empirical relationship between phytotoxicity thresholds and herbicide K_d. Although these relationships could not be confirmed in this project due to a limited number of soils being used, our data can be aggregated with other data to improve on these estimates.

4.2 DAMAGE FINGERPRINTING

Along with testing soil for herbicide residues prior to sowing, grower and consultants have expressed a desire for more accurate identification of the causes for poor crop growth after emergence. Currently, diagnosis of potential herbicide damage is mainly achieved via matching visual crop observations to classic physiological symptoms caused by herbicide carryover. Several manuals/websites are available with pictorial information to help growers and consultant make this diagnosis (e.g. https://www.agric.wa.gov.au/mycrop/diagnosing-contact-herbicide-damage-cereals). Although successful diagnosis can be made when damage is serious and correspond to classic physiological symptoms, there are numerous cases where: i) sublethal damage presents as 'ill-thrift' with few defined symptoms; ii) other stresses cause symptoms similar to classic herbicide carryover or drift symptoms (e.g. virus); iii) multiple stressors acting together cause different physiological symptoms; or iv) new herbicides are being used or farmers/agronomists cannot attribute damage to herbicides due to lack of experience.

Leaf testing offers an alternative or complementary method for helping diagnose potential herbicide carryover and damage. For most herbicides, there is information available on their

fate within certain plant species, in terms of if or how quickly the herbicides are metabolised, and what the metabolic degradation products are. However, this information is not available for all crop species. Furthermore, there is very little quantitative information that links herbicide concentrations with plant tissue to toxicity thresholds. There are a few exceptions that have mainly been driven by issues of spray drift, for example thresholds for 2,4-D and dicamba in cotton and soybeans (Andersen et al. 2004) and glyphosate in spring wheat (Wiersma and Durgan 2017).

Initially, we hypothesised that metabolomics (i.e. the analysis of naturally occurring metabolites essential for plant function) under different herbicide stress would provide a means to accurately identify if and when a herbicide was at significant levels to disrupt crop biochemical functioning. This has previously been demonstrated for glyphosate residues in soil affecting the biochemistry of wheat seedlings (Claassens et al. 2019). However, metabolomics requires expensive and highly-technical analytical and statistical processing methods to give accurate predictions regarding crop damage (Claassens et al. 2019). Instead, pilot experiments showed that for most susceptible crops, herbicide residues accumulated to levels that were quantifiable in crop leaf tissue, and that these concentrations could be linked to toxicity thresholds in terms of biomass reduction compared to health control plants.

Of the 6 herbicides used for generating soilborne herbicide toxicity thresholds, we were able to quantify the parent compound for all except for pyroxasulfone in the leaf tissue. The concentration of herbicide in the leaf tissue increased with increasing soil dose, implying that the traditional non-linear regression models (e.g. log-logistic) may enable leaf concentration to be used to estimate damage (e.g. biomass reduction). This was demonstrated for lupins exposed to clopyralid, since there was a strong relationship between soil concentration and leaf concentration. Unlike soil testing, which requires pedotransfer functions to estimate bioavailability and potential damage, the leaf tissue concentration is a direct measure of plant exposure, and should therefore enable a greater understanding and more accurate estimate of potential production losses across the grains industry caused by herbicide residues.

There is very little information available in the literature on herbicide residues in crop leaf tissue, and to our knowledge, a framework or service focussed on leaf tissue testing for herbicide residues has not previously been proposed but warrants further investigation.

4.3 HERBICIDE SORPTION TO SOIL

Herbicide sorption to soil is a key parameter that regulates how much herbicide is bioavailable in the soil over time. Although herbicide sorption varies widely from soil to soil, the current ‘gold-standard’ databases (e.g. Pesticide Properties Database, University of Hertfordshire) provide data for only one or a small number of soils. Furthermore, there is currently no reliable way to predict these parameters from more easily-measured soil properties, meaning that currently for each different soil a specific empirical lab study is required. Recent research has shown the infrared reflectance spectroscopy can be calibrated across large soil datasets to predict physicochemical properties of interest, including soil texture, total soil carbon and soil C fractions, pH and P-buffering index – which is a measure of P-sorption in soil. Previous research has also demonstrated that infrared reflectance spectroscopy can also be used to predict sorption of organic compounds, including herbicides such as atrazine (Kookana et al. 2008) and diuron (Forouzangohar et al. 2008).

In this project we tested the ability of linear regressions and machine learning to predict the sorption of 3 priority herbicides onto soils types found in collaborating farmer groups regions. Linear regressions (including generalised additive models) using routinely-analysed physicochemical properties (including pH, OC and CEC) were able to predict sorption reasonably well for all herbicides, but the use of more advanced ML methods coupled with MIR improved these predictions. This agrees with previous research (e.g. Kookana et al. 2014) that reviewed and discussed the benefits of using MIR to integrate soil properties and predict sorption.

Nevertheless, both modelling approaches have strengths and drawbacks which means that they can be tailored for different uses. Simple models based purely on one or several routinely-measured physicochemical properties, such as OC, pH and clay content, are easily transferable and user-friendly and can therefore be quickly adopted and applied with only basic technical knowledge. Although MIR requires greater technical knowledge, once it is established, it can provide faster and more cost-effective estimates of sorption for multiple herbicides and other soil properties. An increasing number of commercial laboratories are now offering services that involve NIR/MIR prediction of soil physicochemical characteristics. For herbicide sorption to be adopted as a routine output for commercial laboratories, a greater number of samples will be required to improve the calibration of the model. Although we have demonstrated a proof-of-concept with <50 samples, it is likely that a minimum of 100-200 samples will be required to capture sufficient soil-herbicide variation to make it MIR prediction widely applicable.

4.4 HERBICIDE PERSISTENCE IN CROPPING SOILS

4.4.1 Clopyralid

The persistence of clopyralid in soils of southern Australian farming systems is of interest to farmers because: i) it is widely used for controlling difficult broadleaf weeds, including capeweed (*Arctotheca calendula*) and skeleton weed (*Chondrilla juncea*); and ii) several high-value pulses, such as lentils, are particularly sensitive to clopyralid residues.

The results from two seasons of monitoring clopyralid residues in the summer fallow prior to sowing demonstrate the dominant influence that rainfall has on dissipation of herbicides with residual properties like clopyralid. Despite some variation in the persistence of clopyralid at the four different sites monitored from mid-2019 to mid-2020, clopyralid was detected at 2-8 ng/g at all sites 168 days after spraying. In this season, <120 mm of rain was recorded at each site over the six-month period. In contrast, during the 2020-21 season, rainfall was 50-100% higher than the previous season at each of the sites. This meant that although residue levels were still detectable at 84 days after application, residues had fully dissipated to negligible levels by 168 days after application. This data reinforces product labels that emphasise the importance of rainfall, particularly in the summer months, for sufficient clopyralid breakdown to ensure protection for subsequent crops.

The half-lives measured in our field experiments (~10-100 days) are similar to those measured in other studies, which ranged from 10-47 days at 85% field capacity and 10-30°C in three soil (clay, clay loam, and sandy loam) under lab conditions (Smith and Ubin 1989) and 30-70 days under field conditions at three different locations in Canada (Pik et al. 1977). The importance of soil moisture was also highlighted by Pik et al. (1977), who found that dissipation was fastest in moist soils and was greatly reduced during dry and cold periods.

Interestingly, at the Poochera and the Mt Cooper sites in the first season, clopyralid residues appeared to be remobilised and remained detectable (but low) at 1-2 ng/g by 364 days. This pattern fits field observations (and product labels) that clopyralid can be released from crop

stubble where clopyralid herbicides have been applied in crop (Wells 2002). Thus, even though clopyralid breakdown/dissipation from soil can be rapid in some soils where rainfall and organic matter is sufficient (e.g. Mt Cooper, SA5), clopyralid residues may still be present in soil at sowing of the following crop, due to remobilisation of bound residues. This poses a unique challenge for the management of clopyralid, such that soil samples taken in areas where the stubble load is low may not accurately represent the risk of phytotoxicity in areas where stubble loads are high. It is therefore recommended that if pre-sowing soil tests are to be used for risk assessment of clopyralid carryover to following crops, then crop stubble needs to be retained when taking soil samples, and areas of high and low stubble load should be included in the sampling regime.

4.4.2 Imazamox/imazapyr

Imazamox and imazapyr are commonly applied together as a commercial product (e.g. Intervix ®). Their use has increased in Australia during the last decade, particularly for control of grassy weeds in Clearfield ® (imidazolinone-tolerant, IT) crop varieties (Kleemann and Gill 2009) and more recently for weed control in summer fallows (Daniel 2016). These herbicides are relatively persistent in the soil, with field half-lives of up to 410 days (Lewis et al. 2016). Carryover and crop phytotoxicity have been observed in Australia (Hollaway et al. 2002) and elsewhere (Alister and Kogan 2005) and there is concern that repeated use in IT crops could lead to accumulation in the soil (Mark Congreve, personal communication).

In our field experiments we measured imazapyr half-lives of 11-320 days. As with clopyralid, imazapyr dissipation was much greater at sites receiving higher amounts of rainfall. It is likely that the Horsham, Kinnabulla, and Jil Jil sites would not have experienced any plant-back toxicity in the 2020 season, as levels of imazapyr (and imazamox where it was co-applied) were < 1ng/g by sowing in 2020. Imazapyr residue levels were between 3-4 ng/g at sowing in 2020 at the Brim site, and it is possible that these concentrations would have posed a low level of phytotoxicity risk, given that the ED_{20} for wheat in a similar BCG soil was 4.2 ng/g. Leaf tissue sampling of the 2020 crop at Brim would have provided additional evidence for whether or not phytotoxicity was present, however we were unable to take and analyse these samples.

4.4.3 Modelling herbicide persistence

The modelling approach presented here proved capable of reproducing observed herbicide residues across a range of sites. The large volume of soil samples needed to be better accounted for in the model by integrating results over the sampling depth. Usually, model calibration is applied to a modelled concentration occurring at a specified depth. Due to the moderate sorption of Imazapyr it appeared to be largely retained near the soil surface after application in these soils so the integration method appeared to be necessary to better reproduce observations.

The CLT model with a stochastic description of climate was also developed by Jury and Gruber (1989), however there are several differences to the present work. Firstly, they adopted a climate model based on rainfall but failed to demonstrate how this rainfall probability distribution could be translated into cumulative infiltration. Here we applied a simple threshold based upon an interception threshold which led to a natural filtering of the probability distribution with modified parameters. In application to the field trial, we also applied an evaporation threshold which filtered daily rainfall to estimated infiltration. Given the similarity of solute transport parameters between sites this looks to have been a reasonable approach. Secondly, Jury and Gruber also did not demonstrate their model's ability to reproduce observed data. Lastly, there was strong seasonality in rainfall and evapotranspiration at the field trial sites. The CLT with cumulative infiltration demonstrated

its ability to account for this seasonality. Few other applications of the CLT have to our knowledge been applied in climates with such strong seasonality.

The model needs some further modifications to better account for the behaviour of other herbicides. Clopyralid for example is known to strongly bind to plant material and to slowly release back to soil during the decomposition of target plants (Lewis et al. 2014). The CLT might be able to account for this in two ways. The first, given a release rate time series, the equations above could be evaluated using a convolution integral. Even the joint probability density function (pdf) could be calculated in a similar manner. A second approach could be to add a third 'dummy' layer to represent the decomposing plant source. Jury and Roth (1990) illustrate how to extend the CLT to an arbitrary number of distinct layers with separate parameters in a straightforward manner.

Solute transport models like this usually are not applied by agronomists or restoration ecologists. Here we developed from the model an empirical relationship between annual rainfall and herbicide carryover which might be a simple method to assess risks. Together with verification via sampling and analysis of concentrations in soil this approach could perhaps be the simplest and most practical method to develop a useful risk tool.

4.5 EFFECT OF HERBICIDE WITHDRAWAL ON CROP PRODUCTIVITY

A recent soil survey of herbicides in Australian grain cropping soils showed that there is a median of 6-7 different herbicide residues detectable in each paddock at the time of sowing (Rose et al. 2022). Our pilot-scale study in this project confirmed that multiple herbicide residues are a feature of grain-cropping soils. Although there are some toxicity thresholds available to assess the potential risks of individual herbicides, the potential combined effects of multiple herbicide residues on crop productivity are rarely discussed or investigated. This is despite the fact that herbicide synergism and antagonism are well-known for their ability to enhance or inhibit weed control. For example, El-Nahhal and Hamdona (2015) demonstrated both synergistic and antagonistic effects of binary mixtures of the herbicides alachlor, bromacil and diuron on several crops. Similarly, Dear and Sandral (1999) showed the combination of bromoxynil and diflufenican was significantly more toxic to lucerne and clover than when applied singly.

Because of the cost and complexity of deriving multiple, interactive toxicity thresholds, we designed 'herbicide withdrawal' experiments at three different grower group sites to enable assessment of herbicide residue mixtures on crop health versus control treatments where herbicides were not used but weeds were controlled by physical, low-disturbance hand-weeding. The herbicides examined varied across sites according to which herbicides were commonly used by farmers in each region.

Our results over two years of study found limited effects of herbicides on crop yields compared with hand-weeded controls where herbicides were not used. The exception was a 25% reduction in wheat yield in 2020 caused by a pre-sowing diuron application. In this instance, it is likely that a 10 mm rainfall in the two days immediately following sowing washed diuron into the seeding rows and inhibited wheat growth. However, no damage was observed for the other, less mobile herbicides, trifluralin and pyroxasulfone. Such an occurrence is a known risk when applying these herbicides, especially in sandy soils where even slightly mobile herbicides can move with the wetting front in soil following rainfall and result in crop injury to sensitive crops (Congreve and Cameron, 2019). With the development of the herbicide damage fingerprinting tools (Aim 2 of this project), we would now be able to take leaf tissue samples if damage was expected to confirm (or reject) the hypothesis that diuron was the cause for poor growth.

Despite the observation of probable herbicide toxicity at the Bolgart site in WA, the reverse was true for wheat growing at the Minnipa (SA) site in 2020. Here, the herbicide withdrawal control treatment yielded significantly lower (by ~ 16%) than the full herbicide treatment plots that received fallow, pre-sowing and in-crop herbicide applications. This demonstrates that even timely mechanical weed control (i.e. hand-weeding every 4-6 weeks in this study) cannot fully protect crop yield loss from weed competition as compared with a comprehensive herbicide program. That the treatment plots receiving the greatest herbicide load returned the highest yield reinforces the reason why growers use herbicides in the first place, and that if used appropriately, herbicides can increase yields. Thus, even if herbicide residues do periodically result in crop damage, the incentive to continue using herbicides to maximise yields under good management and environmental conditions remains.

Because of the time frame of this project, the effect of withdrawing herbicides from the cropping system could only be investigated over a two-year period. It is possible that long-term cumulative effects of previous herbicide use were not detected over this short timeframe, and that longer-term monitoring may be necessary to resolve this study limitation. The BCG site will continue operation as a long-term field site to study the effects of repeated herbicide use versus herbicide withdrawal over additional seasons, through the new 'Microbial Indicators' project in program 2 of the Soil CRC (project 2.1.008). Importantly this project will further investigate the potential effects of herbicides on soil biology and function and how this might contribute to crop health and yield.

4.6 PILOT-SCALE STUDY OF SOIL TESTING SERVICE

A pilot-scale service to analyse herbicide residues in soil prior to sowing was offered to collaborating grower groups in early 2020. This was conducted to evaluate the real-life applicability of herbicide analysis and toxicity thresholds methods generated in this project, and the feasibility of sampling design, logistics, testing, and reporting back to growers. The study also provided information on the likelihood of herbicide carryover and potential crop damage in paddocks where growers were concerned about potential herbicide carryover.

Of the twelve sample batches tested, we detected the target herbicide (i.e. herbicide of concern) in eight. By comparing the detected field residue concentrations with glasshouse-generated toxicity values, we were able to assess relative risks of plat-back toxicity to different crops. Of the eight paddocks where the target herbicide was detected, only four were assessed to pose a risk to certain crops, but only 2 of these paddocks were planted to potentially sensitive crops. Paddock SA2 contained residues of both imazapyr and imazapic and the concentrations of imazapyr were slightly higher than the toxicity threshold for wheat predicted to cause 20% shoot biomass reduction in seedlings. Paddock Vic2 also contained residues of imazapyr one replicate sample that exceeded the ED_{20} toxicity threshold for wheat in sandy soils. Whether or not toxicity would have occurred would have depended on conditions leading up to sowing (which could have stimulated further breakdown before establishment) and conditions during the growing period, including whether other stresses were present (e.g. micronutrient deficiencies). Follow-up conversations with the grower at site SA1 (Aug 2021) suggested that there was no observable toxicity, however, it is difficult to conclude whether a slight reduction would have occurred since there were no 'controls' in place to test this. Although there were also detections of imazapic in paddock SA6, recent findings from a complementary GRDC project suggest that ED_{20} values for imazapic toxicity to wheat are >10 ng/g, so it is unlikely that there would have been observable toxicity.

These findings concur with our recent soil herbicide residue survey and risk assessment that although herbicide residues are often present and at times may pose a risk to sensitive crops, growers are usually aware of these risks and actively minimise risks by planting

tolerant crops. Nevertheless, feedback from growers participating in the study suggest that an analytical residue testing service was ‘very useful’ for giving additional confidence to decision making. Even if the results of the testing would not change a grower’s practice, interpretable testing results ‘provided peace of mind’ and reduced stress regarding decision making.

5. CONCLUSION

We successfully addressed all of our original aims, which together illustrate new opportunities for growers and agronomists to identify and avoid crop injury from herbicide residues in soil. Dose-response experiments in sand identified the minimum levels of diuron, imazapyr, clopyralid, pyroxasulfone, trifluralin, and propyzamide required to reduce crop biomass. These experiments also provided tolerance rankings for different crops, giving growers information about which crops can be selected as more robust options when residues are present. Follow-up dose-response experiments in soil demonstrated that residue concentration thresholds are higher in soil than sand, where soil properties regulate sorption and therefore bioavailability. We value-added to these dose-response experiments by analysing herbicide concentrations in crop leaf tissue at levels where damage became significant (i.e. around the ED_{20} threshold, where crop leaf biomass is reduced by 20%). Agronomists and growers can now interpret soil and leaf tissue tests when herbicide damage is suspected, for the priority herbicides investigated in this project. Prior to this project, soil and leaf residue testing was of little use because few threshold values were available to allow accurate interpretation of the tested concentrations.

Because of the importance of herbicide sorption in regulating herbicide movement and bioavailability in soil, we aimed to improve the speed, affordability, and accuracy of predicting the herbicide sorption coefficient, K_d, across different soil types. We demonstrated that both soil physicochemical properties and MIR spectroscopy can be used to adequately estimate sorption for three herbicides of differing chemical characteristics. The developed models can be used in conjunction with soil testing for herbicide residue concentrations to predict toxicity thresholds on different soil types. They can also be used for site-specific parameterisation of herbicide fate models to predict herbicide persistence over time after application, under different soil and weather conditions.

This was demonstrated through the monitoring and modelling of different herbicides (imazapyr, clopyralid, diuron, pyroxasulfone) at different grower group sites over two seasons. Monitoring data showed that persistence was strongly related to rainfall, where <100 mm of rainfall in the 180 days post application led to greater persistence of herbicides in soil and higher risk to following crops. A revised probabilistic model was developed and calibrated with monitoring data; this model reinforced the strong influence of rainfall and soil infiltration in the dissipation of herbicides. This model can be used to simulate the effects of rainfall on herbicide dissipation, which was demonstrated for imazapyr dissipation using 100 years of historical rainfall data.

Finally, we investigated the effects of multiple herbicide residues on crop health at three different locations. We found little evidence of crop damage over two seasons, but we were able to demonstrate through soil testing and comparison with crop-toxicity thresholds that herbicide dissipation at the experimental sites was sufficient to avoid toxicity to the planted crops. These experiments also highlighted the trade-offs between herbicide selection and adequate weed control, since significantly higher yields were measured at one site when full herbicides application (fallow weed control, pre-crop, and in-crop herbicides) compared with hand-weeded control plots where no herbicides were applied.

In conclusion, this project demonstrated that monitoring of herbicide residue concentrations in soil and plant tissue provides valuable information to growers and agronomists that can be practically applied to help reduce crop damage by herbicide residues. More work is now needed to expand this information to other herbicides and validate the work through farmer-participatory research. This will hasten adoption of soil and leaf tissue testing for improved herbicide management and crop health.

6. RECOMMENDATIONS

- Pilot-scale testing of our soil and plant tissue testing framework by consultants and farmers is now needed to validate our approach for predicting herbicide residue damage across a wider cropping area
- More information on how herbicide residue effects on crop seedling biomass might translate to effects on yield is required
- New herbicides are being registered with different chemistry and behaviour to previously-registered herbicides, and information on toxicity thresholds and bioavailability are required for these new herbicides
- There are still unresolved questions around potential effects of herbicide residue mixtures on soil and crop health
- Effort is needed to integrate these findings with commercial service providers for herbicide residue testing, or provide an independent testing laboratory

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APPENDIX A HERBICIDE ANALYSIS

1. Reagents and standards

Unless otherwise specified, all reagents were of AR grade or equivalent, and water refers to Type-1 H_2O (milli-Q water). Instrument calibration standards, internal standards, and fortification standards used for calibration and recovery estimates were prepared from certified reference standards, purchased from Novachem, Australia.

Sodium hydroxide, hydrochloric acid, acetonitrile, and methanol were purchased from Thermofisher Australia. MtBSTFA derivatization reagent (N-methyl-N-tert-butyldimethylsilyltrifluoroacetamide + 1% tert-Butyldimethylchlorosilane) (product number 00942-1ML-KC) and citric acid were purchased from Sigma-Aldrich. Dispersive solid-phase extraction salts (QuEChERS EN method) were purchased from Agilent Australia (product number 5982-0650).

2. Soil extraction

Soil samples (5g) were weighed into 50mL polypropylene centrifuge tubes and spiked with 100µL of imazaquin and 2,4-dichlorphenylacetic acid (1000 ng/mL) as herbicide surrogates. Samples were wet with 4mL of milliQ water and allowed to stand for 5 min. The pH was adjusted to >12 by the addition of 1mL of 5M NaOH to hydrolyse conjugated acid herbicides before the addition of 10mL of acetonitrile. Samples were extracted by shaking at 40°C for 45 minutes, after which they were neutralised to pH7 with 5M HCl (using a pH indicator test strip to ensure neutralisation). QuEChERS EN buffer salts were then added to each container, as well as 1mL of 50% w/v citric acid, which was found to improve recovery of acidic analytes (See Table A1.1). Samples were vigorously shaken by hand for 10 sec before centrifuging at 3000xG for 10 min. A 5 mL aliquot of the supernatant was taken into a 10 mL glass tube and blown down to dryness under N_2 gas (for imidazolinone, diuron, pyroxasulfone, trifluralin and propyzamide analysis), reconstituted in 1 mL of acetonitrile and filtered through a syringe filter (nylon, 0.45 µm) into a 2 mL glass chromatography vial. Another 1 mL sample was taken from the soil extract supernatant and dried under N_2 gas in a 2 mL glass chromatography vial (for derivatisation and analysis of clopyralid by GC-MS).

Table A1.1. Recoveries of acid herbicides using modified QuEChERS method involving addition of citric acid (in soil SA2).

Compound Name	Wt. CA (g)	Spike Level (ng/g)	Av.Recovery (%)	No reps	Std. Dev.
Imazamox	0.0	100	33	3	17
	0.5	100	47	3	7
	1.0	100	81	3	15
	1.5	100	111	3	4
Imazapic	0.0	100	29	3	17
	0.5	100	43	3	8
	1.0	100	77	3	18
	1.5	100	101	3	5
Imazapyr	0.0	100	29	3	20
	0.5	100	43	3	8
	1.0	100	72	3	10
	1.5	100	106	3	3
Imazethapyr	0.0	100	30	3	16
	0.5	100	44	3	8
	1.0	100	71	3	12
	1.5	100	93	3	4

3. Analysis of diuron and imidazolinone herbicides

Imidazolinone herbicides (imazapyr, imazamox and imazapic) and diuron were analysed by LC-MS/MS. The LC-MS/MS used was a Waters Quattro Micro™ Micromass (Milford, MA, USA), equipped with a Waters 2795 Separation Module and an electrospray ionisation interface. A 10 µL injection was made and analytes were separated on an Atlantis T3 (C18, 3 um, 100 mm x 2.1mm i.d.) column set at 25°C. The mobile phase consisted of 100% A (0.1% formic acid in 10% methanol/water) for 0.1 min, to 30% A and 70% B (0.1% formic acid in methanol) at 3 min, then 100% B at 10 min, held for 2 min and returned to 100% A at 13 min and held to 15 min. Analytes were detected and quantified by integration of peak areas at two mass transitions according to Table A1.2.

Table A1.2. Optimised LC-MS/MS conditions for detection and quantification of polar analytes

Compound	Retention time (min)	Mode	Dwell time (s)	Transitions	Cone (V)	Collision Energy (V)
Imazapyr	5.8	P	0.1	262 > 149	32	27
				262 > 217 *	32	18
Imazamethabenz	5.97	P	0.1	275.1 > 144.1 *	30	32
				275.1 > 89.1	30	25
Imazamox	6.19	P	0.1	306.1 > 163.1	32	30
				306.1 > 193.1 *	32	26
Imazapic	6.23	P	0.1	276.2 > 163.1	30	23
				276.2 > 231.2 *	30	17
Diuron	8	P	0.1	232.8>72.1	28	18
				232.9>160.3 *	28	24
Diuron-D6 (IS)	8	P	0.1	239.1 > 77.7	28	18
				239.1 > 159.9	28	24

4. Analysis of clopyralid

Dried samples for clopyralid analysis were reconstituted in 0.5 mL of ethyl acetate and then derivatised with 50µL of MtBSTFA solution. Derivatisation was allowed to proceed for 30 min at room temperature prior addition of the internal standard (trifluralin-D14) and injection into the GC-MS. The GC-MS system was comprised of an Agilent 7890 GC and 5975 Mass spectrometers equipped with HP-5MS column, 30 m × 0.25 mm, 0.25 µm film thickness. The MS was run in EI mode at 70eV, with the MS transfer line set to 280°C. A splitless 1 µL injection was made into the inlet, which was set at 250°C. The initial oven temperature was set at 50°C for 1 min, then ramped to 125°C at 25°C/min, then ramped to 250°C at 10°C/min, then ramped to 320°C at 50°C/min and held for 7 min. The total run time was 24.9 min. Clopyralid (and the surrogate 2,4-DP) were detected as their tert-butyl dimethyl silyl derivatives, using product ions detailed in Table A1.3, and quantitated by reference to a certified reference standard mix of clopyralid and 2,4-DP containing the required amount of internal standard.

5. Analysis of pyroxasulfone, trifluralin and propyzamide

Pyroxasulfone, trifluralin, and propyzamide were analysed by GC-MS, using the same system, column and temperature program described above. In order to overcome matrix effects, a two-layer sandwich injection was used, which involved withdrawing 1 µL of sample, followed by withdrawing 0.2 µL of analyte protectant solution, then injection into the GC-MS. The analyte protectant solution consisted of 10 mg/mL of xylitol, sorbitol and D-(-)gluconic acid-δ-lactone in 50% acetonitrile. This was found to dramatically increase sensitivity. Pyroxasulfone, trifluralin, and propyzamide were identified and quantified using product ions detailed in Table A1.3.

Table A1.3. Optimised GC-MS conditions for detection and quantification of polar analytes

Compound	Retention time (min)	Mode	Dwell time (s)	Quant (m/z)	Qual I (m/z)	Quantification ion II (m/z)
Trifluralin-D14 (IS)	9.7	EI (70eV)	0.1	251	267	315
Trifluralin	9.75	EI (70eV)	0.1	306	264	290
Propyzamide	10.99	EI (70eV)	0.1	173	175	255
Pyroxasulfone	11.95	EI (70eV)	0.1	229	179	
Clopyralid-TBDMS	11.07	EI (70eV)	1.1	250	146	248
2,4-DP-TBDMS	11.52	EI (70eV)	2.1	261	263	159

6. Quality control

Limits of detection (LOD), limits of quantification (LOQ), calibration ranges, linearity, and recovery data are provided in Table A1.4. Each batch of analyses (maximum 30 samples per batch) contained a minimum of one lab reagent blank, one control blank (using a soil known to contain no herbicide residues), one fortified recovery test (in duplicate, by spiking in known concentrations of analyte mixtures), and at least one sample duplicate.

Table A1.4. Limits of reporting (µg/kg) and recovery data for all analytes

Group[1]	Analyte	Method	Calibration range (ng/mL)	Linearity (r2)	LOD[2] (µg/kg)	LOQ[3] (µg/kg)	Spike level (µg/kg)	Recovery (n=10)	CV (n=7)
2	Imazapyr	LC-MS/MS	1-100	0.998	0.4	1.3	4-40	112	20
	Imazamox	LC-MS/MS	1-100	0.999	0.5	1.4	4-40	120	17
	Impazapic	LC-MS/MS	1-100	0.998	0.9	2.9	4-40	118	10
3	Trifluralin	GC-MS	5-200	0.995	10	20	20	85%	13%
4	Clopyralid	GC-MS	2-200	0.999	1	3	4-40	96%	25%
5	Diuron	LC-MS/MS	5-200	0.993	10	20	20	106%	14%
15	Pyroxasulfone	GC-MS	5-200	0.993	10	20	20	118%	14%

[1] Mode of action group according to the Herbicide Resistance Action Committee (HRAC, 2022)

[2] The limit of detection (LOD) is the lowest concentration level of a pure standard that can be detected to be statistically different from a blank with 99% confidence. As part of our method validation, a 99% confidence LOD was calculated by area of the lowest standard divided by slope of the calibration curve times the factor of the ion ratio of the least abundant qualifier ion.

[3] The limit of quantification (LOQ) is what can be detected and quantified with 99% confidence in an actual sample. In this case, the minimum area of an actual sample used for LOQ calculation was three times the area of the closest retention time peak in the blank sample.