Glyphosate residues in Australian soils and implications for crop growth

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Abstract
Glyphosate [N-(phosphonomethyl)-glycine] is a broad spectrum weedkiller used widely in Australia and across the globe. It is generally degraded rapidly in soils by microbes, but can also become bound in soil via its phosphonic acid moiety, competing with phosphorus (P) for binding sites. We have recently measured residues of glyphosate and its main breakdown product AMPA in a range of Australian cropping soils, but it is not known whether the residue levels are likely to cause phytotoxicity to crops. We conducted a glasshouse study to determine critical glyphosate residue concentrations in a Tenosol that are associated with phytotoxicity to wheat and lupin plants in the presence and absence of P fertiliser. Wheat plants were not affected by glyphosate concentrations of up to 14 mg/kg in soils at sowing in the absence of P fertiliser, but when soluble-P was applied to soil at 20 kg/ha shoot biomass reductions occurred at glyphosate concentrations exceeding 6.75 mg/kg. Lupin plants were more sensitive than wheat, and biomass was reduced when soil glyphosate residues at sowing were 6.5 mg kg^{-1} or higher in the absence of P fertiliser, and 1.2 mg/kg or higher when P fertiliser was applied (20 kg P/ha). Levels of around 1 mg/kg glyphosate observed in the survey of Australian cropping soils suggest that sensitive crops in certain paddocks may be negatively affected by glyphosate when P fertiliser is applied at sowing.

Keywords
Herbicide residues, lupin, wheat, sandy soil, Arenosol, Tenosol.

Introduction
Glyphosate is the most widely used herbicide in the world because it controls a broad spectrum of weeds, has a relatively low cost of production, has low mammalian toxicity (Rose et al. 2016) and plays a key role in no-tillage farming systems (Duke and Powles, 2008). The use of glyphosate is also increasing with the emergence of glyphosate-resistant crops, with an estimated 56% of glyphosate usage associated with glyphosate-resistant crops (Benbrook 2016).

In addition to the concerns that have been raised about the prevalence of glyphosate use and residues in food products (Bøhn et al. 2014) and glyphosate resistant weed populations (Powles, 2008), a recent survey of Australian cropping soils frequently detected glyphosate residues in the topsoil (0-10cm) and the 10-30cm horizon (M. Rose unpublished). Thirty percent of the topsoil samples contained glyphosate residues greater than 0.25 mg/kg, with the highest level detected at 1.3 mg/kg (M. Rose, unpublished data). Because glyphosate is often sprayed as a knockdown prior to sowing, it is likely that a large proportion of these residues arise from the most recent application, which is at times only days before crops are sown. Nevertheless, given that a number of studies have shown that glyphosate toxicity can be induced in crop plants when P fertiliser is applied to soils containing glyphosate residues, we investigated whether the residue levels observed in the survey of Australian cropping soils may be detrimental to subsequent crop growth by determining critical glyphosate residue concentrations in a Tenosol that are associated with phytotoxicity to wheat and lupin plants in the presence and absence of P fertiliser.

Methods
Soil was collected from the top 30 cm horizon of a Tenosol at Wongan Hill research station, WA and was transported to NSW DPI's Wollongbar Primary Industries Institute and sieved to 2 mm. Soil tests were undertaken at the NATA-accredited testing laboratories at DPI Wollongbar using methods described in Rayment and Lyons (2011). Briefly, the soil had a pH of 5 ($CaCl_2$), total carbon 0.3%, total nitrogen 0.03%, Colwell P concentration of 5.8 mg/kg and a Phosphorus Buffer Index of 15.

Basal nutrients (except P) were pipetted onto the surface of 20 kg samples of sieved, air-dried soil (2.8% moisture) as per Rose et al. (2007) and the soil was air-dried for 24 h before nutrients were mixed

thoroughly. Glyphosate was then applied to the appropriate 20 kg soil sample at rates equivalent to 0, 0.83, 2.5, 7.5, 22.5 and 67.5 L Roundup CT/ha, to give approximate soil concentrations of 0.25, 0.75, 2.25, 6.75, 20.25 mg glyphosate /kg soil in the top 10 cm. Each 20 kg soil sample was then wet to 50% water holding capacity (WHC, 19.05% v/w) and transferred to a covered container prior to incubation for 28 d at room temperature. Glyphosate was then extracted from soil using 0.6 M KOH and extract cleanup was performed on a strong-anion exchange column (Bond Elut SAX, Agilent) before filtration (0.2 um) into glass vials. Isotopically-labelled glyphosate (^{13}C, ^{15}N) was added to soils prior to extraction as an internal standard. Underivatised glyphosate was separated on a porous graphitic carbon column (Hypercarb, Thermofisher) and quantified via MS/MS (Micromass, Waters) according to Pereira (2006).

On 25th July 2015, 500 g sieved, air-dried soil was added into 70-mm-diameter, 200-mm deep, free draining pots for the subsoil. Subsequently, 25 mL deionised water was then added to each pot to achieve a subsoil moisture content of 50% WHC. The top section of each pot was then filled with 285 g of the 28 d-incubated soil (equivalent to 250 g air-dried soil) which had either had P fertiliser applied - equivalent to 20 kg/ha P on a pot soil surface area basis – as a liquid solution (15 mL of 4.1 mg/L P solution where P was supplied as K_2HPO_4), or had no P applied (15 mL DI water only). Four seeds of either inoculated lupin cv. Jenabillup or wheat cv. Cunderdin were then sown 15 mm deep into each pot. Each P treatment x crop species x glyphosate residue treatment was replicated three times. Pots were laid out in a randomised block design in a glasshouse at NSW DPI, Wollongbar and pots within a block were re-randomised at each watering event. Plants were thinned to two seedlings per pot at 8 d after sowing (DAS). Pots were watered to weight every 3 d for the first 14 d and then every 2 d for the remainder of the experiment.

Plants were harvested at 48 DAS by severing the shoots approximately 2 mm above the soil surface. Shoots were oven-dried for 5 d at 60°C prior to weighing. Shoot biomass data were fitted with quadratic models, since sigmoidal dose-response curves could not be generated due to lack of response at high dose rates.

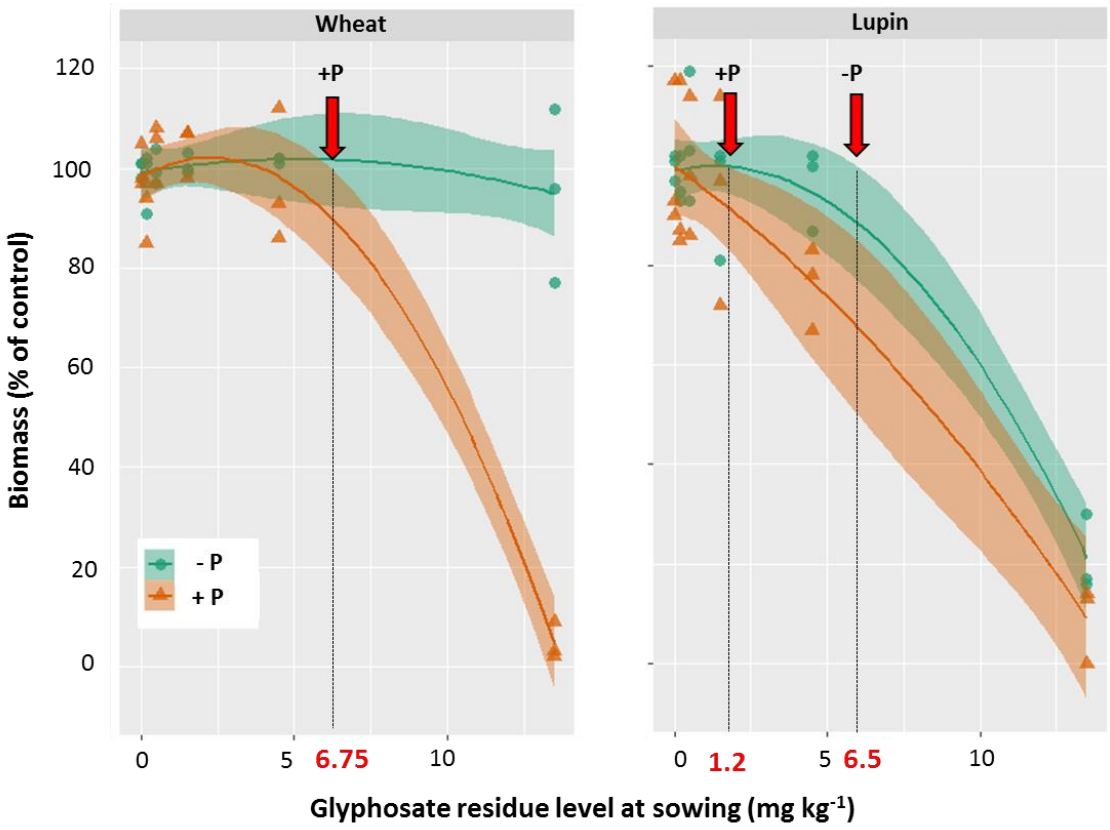

Figure 1. Wheat and lupin shoot biomass at 48 days after sowing in response to glyphosate residues in soil. Orange and green shaded areas represent 95% confidence intervals of the model estimate.

Results and Discussion

Glyphosate levels in the soil at sowing ranged from 0-14.8 mg/kg soil, which equates to around 50-75 % of the glyphosate originally added in each treatment with higher percentages of degradation at lower dose rates. This range encompasses the residue range from 0-1.3 mg/kg observed in Australian cropping soils (M. Rose, unpublished data). Glyphosate residues in soil had no impact on wheat shoot biomass at 48 DAS up to concentrations of 14 mg/kg when no P fertiliser was applied at sowing (Figure 1).

However, when the equivalent of 20 kg P/ha was supplied, wheat shoot biomass was significantly reduced at glyphosate residue levels above 6.75 mg/kg in soil (Figure 1). Lupins were more susceptible to glyphosate residues and, based on a quadratic model, shoot biomass reductions were observed when soil glyphosate concentrations were at or above 6.5 mg/kg in the absence of P fertiliser or were at or above 1.2 mg/kg when P fertiliser was applied at sowing (Figure 1). The exacerbation of shoot growth impairment when P fertiliser is applied is consistent with earlier reports with tomatoes and soybeans (Cornish 1992; Bott et al. 2011), likely a result of phosphate anions displacing glyphosate sorbed to soils (Sprankle et al. 1975).

Conclusion

The study demonstrates that residues of glyphosate observed in some Australian cropping soils of around 1 mg/kg may be detrimental to susceptible crop species planted into these residues when P fertiliser is applied at sowing. However, we applied P in a liquid for to provide uniform P application to the topsoil as per earlier studies (Bott et al. 2011), whereas the reality in many cropping systems if that P fertiliser is applied as granules either broadcast onto the soil surface and incorporated, or banded below the seeding row at sowing. Whether the same toxicity effects are observed when P is banded in a narrow volume of soil is yet to be determined. More research is also needed to explore potential interactions in a range of soils for other crops, particularly other legumes and oilseeds.

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