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Phosphorus speciation in mature wheat and canola plants as affected by phosphorus supply

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Abstract
Background and aims As plants approach maturity and start to senesce, the primary sink for phosphorus (P) is the seed but it is unclear how plant P status affects the resulting P concentration and speciation in the seed and remaining plant parts of the residues. This study was established to measure how P speciation in different parts of wheat and canola is affected by plant P status.
Methods Wheat and canola grown in the glasshouse were supplied three different P rates (5, 30 and 60 kg P ha^{-1} equivalent). At physiological maturity, plants were harvested and P speciation was determined for all plant parts (root, stem, leaf, chaff/pod and seed) and rates of P application, using solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy.
Results Phytate was the dominant form of P in seed whereas orthophosphate was the dominant form of P in other plant parts. The distribution of P species varied with P status for canola but not for wheat. The phytate content of wheat chaff increased from 10 to 45 % of total P as the P rate increased. Canola pods did not show a similar trend, with most P present as orthophosphate.
Conclusions Although minor differences were observed in P speciation across the three P application

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rates and plant parts, the effect of this on P cycling from residues into soil is likely to be relatively minor in comparison to the overall contribution of these residues to soil P pools. This glasshouse experiment shows the dominant P form in crop residues that is returned to soil after harvest is orthophosphate, regardless of plant P status.

Keywords Phosphorus · Plant P status · Residues · Speciation · Organic P · Inorganic P

Abbreviations

C	Carbon
DGT	Diffuse gradient thin film
EC	Electrical conductivity
N	Nitrogen
NaOH-EDTA	Sodium hydroxide ethylenediaminetetraacetic acid
NMR	Nuclear magnetic resonance
P	Phosphorus
PBI	Phosphorus buffering index
RNA	Ribonucleic acid

Introduction

Phosphorus (P) is a highly mobile element in plants, moving readily among plant parts. The seed (grain) becomes the ultimate sink for P and at maturity may contain up to 90 % of the plant total P (Batten et al. 1986; Smith 1965). The remaining plant P is distributed among roots, stem, leaves and chaff/pod material. Phosphorus within these plant parts is eventually returned to soil where it can be released as soluble inorganic P, easily-degradable organic P or persist as more slowly available organic P.

Residue P decomposition and nutrient release are affected by residue chemical composition (concentrations of other nutrients, and availability of these nutrients to plant roots and microbes) as well as by environmental factors such as soil water status. Much information exists on the effect of crop residues with varying carbon (C):P ratios on residue P release and decomposition (Alamgir et al. 2012; Fuller et al. 1956; Kwabiah et al. 2003; Umrit and Friesen 1994; White and Ayoub 1983). Predicting when P from crop residues will be released based on these measures has proven more difficult. Many studies have used immature plant material or plant material from different plant species to achieve differences in residue C:P ratio. Two problems arise from using this information to predict the fate of P in mature crop residues post-harvest. Firstly, immature plant material may have different total P and P speciation from mature plant material. Therefore, the use of immature residues is only relevant when considering green or brown manuring crops. Secondly, depending on the source of plant material, there is the potential for differing P speciation among plant species.

Sequential chemical fractionation methods have commonly been used to speciate plant P forms. The main P forms that have been identified in plant material via this technique are inorganic P, ester P, lipids, nucleic acids, phytate and residual P (remaining unidentified P forms) (Batten and Wardlaw 1987; Chapin and Bieleski 1982; Kakie 1969; Lee et al. 1976). More recently, solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy has identified P forms in a range of mature crop residues collected from the field (Noack et al. 2012). In this study, the main P forms identified in crop residues (stem/leaf and chaff/pods) were orthophosphate, phospholipids, ribonucleic acid (RNA), pyrophosphate and phytate.

The effect of P status on P forms in plant material has been a focus for determining critical or optimal P concentrations for plant growth (Kakie 1969; Lee et al. 1976) and for leaf tissue testing (Batten and Wardlaw 1987; Bollons et al. 1997). When plant P supply is increased from the deficiency to the sufficiency range, the concentrations of major P fractions (ester, lipids, nucleic acid and inorganic P) in vegetative plant organs increase (Batten and Wardlaw 1987; Chapin and Bieleski 1982; Kakie 1969; Lee et al. 1976; Veneklaas et al. 2012; White and Ayoub 1983). Further increases in P supply result in only the inorganic P concentration increasing, where it becomes the major form of storage (Batten and Wardlaw 1987; Kakie 1969). In a study where wheat plants were supplied with either low (0.25 mM P solution) or high (1 mM) rates of P, at maturity the proportion of inorganic P to other P forms (lipid, ester and residue P) was three times greater in high-P plants compared to low-P plants (Batten and Wardlaw 1987). The leaves of wheat in the low-P treatment had translocated almost all P to other plant parts and contained a lower concentration of inorganic P. Based on these findings, we expect that crops with higher total P concentrations will leave more P in crop residues and a larger percentage of that P will be present as inorganic P (orthophosphate), which will cycle back to soil in a potentially more plant-available form.

This study aimed to measure how P speciation in different parts of wheat and canola at maturity is affected by plant P status. Wheat and canola crops constitute 60 % of Australia's current grain crop production (ABARES 2012). A better understanding of crop residue P chemistry is an important step for improving predictions of the fate of crop residue P and its contribution to soil P status and ultimately crop nutrition.

Materials and methods

Soil properties

Soil was collected to 10 cm depth from Black Point in the grain producing region of southern Australia (S34°36.776′, E137°48.599), and was classified as a Calcarosol according to the Australian Soil Classification (Isbell 1997). The soil was oven-dried at 40 °C and sieved to less than 2 mm prior to characterisation and use in the glass house experiment. Soil pH (H_2O) and electrical conductivity (EC) were measured in a 1:5 soil:solution suspension (Rayment and Higginson 1992). Calcium (Ca) carbonate content was measured according to Martin and Reeve (1955). Field capacity was measured according to Klute (1986), and total organic C according to the method of Matejovic (1997). Cation exchange capacity was measured using compulsive exchange utilising Ba^{2+}/NH_4^+ (method 15E1) and Colwell P using method 9B2 of Rayment and Higginson (1992). Phosphorus buffering index (PBI) was measured according to Moody (2007) and plant available P was measured using the diffuse gradient thin film (DGT) method outlined by Mason et al. (2010).

Black Point soil is an alkaline (pH 8.5) loam with no surface salinity issues or detectable Ca carbonate, an organic C content of 1.6 %, and cation exchange capacity of 17.9 cmol(+) kg^{-1}. The soil has a low PBI of 75 and is deficient for P according to both the Colwell (measured 3 vs. critical concentration 25 mg kg^{-1} (Moody 2007)) and DGT-P (measured 4 vs. critical concentration 60 μg L^{-1} (Mason et al. 2010)).

Glasshouse experiment

The experimental design consisted of three rates of applied P fertiliser (designed to provide deficient, adequate and luxury P status) by two species, replicated seven times. The P fertiliser rates were 5, 30 and 60 kg P ha^{-1} equivalent (10, 60 and 120 mg P pot^{-1}), applied as phosphoric acid immediately below the seed on the day of sowing. A total of 3 kg of air-dry sieved (<2 mm) soil was added to pots made of capped polyvinyl chloride drainage pipe, 17 cm long and 15 cm diameter, which was not free-draining. Basal nutrient applications of nitrogen (N) as urea equivalent to 75 kg N ha^{-1}, potassium (K) and sulphur (S) as K_2SO_4 at 40 kg K ha^{-1} and 16 kg S ha^{-1}, zinc (Zn) as $ZnSO_4.7H_2O$ at 15 kg Zn ha^{-1}, copper (Cu) as $CuSO_4.5H_2O$ at 12 kg Cu ha^{-1} and manganese (Mn) as $MnCl_2.4H_2O$ at 2 kg Mn ha^{-1} were applied as a 30 mL solution to each pot. Four pre-germinated seeds were sown in each pot and thinned to two plants per pot in the first week.

Pots were placed in a naturally lit glasshouse (median temperature 21.5 °C) and the experiment started on October 5. The pots were arranged in a completely randomised design. The positions were re-randomised every 2 weeks. Two top up applications of N were made as urea equivalent to 75 kg N ha^{-1} on day 34 and 20 kg N ha^{-1} on day 41 to ensure N was not limiting growth. All plants were harvested after physiological maturity on January 17 (104 days after sowing).

Plant shoots were oven-dried at 60ºC and separated into stem, leaves, chaff (protective casing of wheat grain) or pod (canola) and seed. Plant roots were removed from the soil, freeze dried and brushed to remove any adhering soil. Dry weights were recorded for each plant part. Sub-samples of plant material from each of the seven replicate pots were ground to <2 mm and a 0.5 g (or <0.5 in the case of wheat roots due to low dry matter production) sample was digested using concentrated HNO_3 at 140 °C (Zarcinas et al. 1987). The total P concentration in the digest was determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES). A 0.1–0.5 g sub-sample was analysed for total C and N using a CNS-2000 high temperature combustion analyser (Leco Corporation Michigan, USA) and is expressed as %C and %N on an oven-dried basis.

NaOH-EDTA extraction

Plant sub-samples from the seven replicate pots were ground and then combined (due to there being insufficient material from each replicate for analysis) prior to extraction with NaOH-EDTA using a modified method of Cade-Menun and Preston (1996) originally

developed for soil extraction. Triplicate 2.0 g subsamples of dried residue for roots, stem, wheat leaf and chaff/pods were extracted with 40 mL of 0.25 mol L^{-1} NaOH and 0.05 mol L^{-1} Na_2EDTA (or 0.5 g were extracted with 10 mL solution for plant parts with low dry matter production, wheat roots, stem and leaves) for 16 h. For wheat and canola seeds and canola leaves a larger sample:solution ratio (0.5 g: 40 mL) was used. This ratio was used for the seed samples due to the high starch content in seeds, which resulted in a highly viscous extract when the smaller ratio was used. Canola leaves contained high Ca concentrations in solution, reducing the extraction efficiency of plant P so a larger sample:solution ratio was used to overcome this. The extracts were centrifuged (1,400×*g*) for 10 min and filtered using Whatman No.42 filter paper. An aliquot (10–20 mL) from each of the triplicates was immediately frozen using liquid N and freeze-dried for NMR analysis.

NMR analysis of NaOH-EDTA extracts

Triplicate freeze-dried samples for each plant part and P rate were combined and ground; a 500-mg subsample was re-dissolved in 5 mL of deionised water, and centrifuged at 1,400×*g* for 20 min. The supernatant solution (3.5 mL) was added with methylenediphosphonic acid (MDP) (0.1 mL at a concentration of 6 g L^{-1}) and deuterium oxide (D_2O; 0.3 mL) to a 10 mm NMR tube. The resulting pH was >13. Solution ^{31}P NMR spectra were acquired at 24 °C on a Varian INOVA400 NMR spectrometer (Varian, Palo Alto, CA) at a ^{31}P frequency of 161.9 MHz. Recovery delays ranged from 40 to 60 s and were set to at least five times the T_1 (spin lattice relaxation time) values of the orthophosphate resonance determined in preliminary inversion-recovery experiments (data not presented). A 90° pulse of 25 μs was used, the free induction decay was collected for 1.0 s, and spectra were broadband ^{1}H decoupled. Between 275 and 5,295 scans were acquired. The total duration of acquisition was 5–25 h for each sample.

Quantification of P species from ^{31}P NMR spectra

The relative concentrations of P species in the NaOH-EDTA extracts were determined from ^{31}P NMR spectra using a combination of integration and deconvolution. Pyrophosphate concentrations were determined using integration alone. Integration was used to determine the combined concentration of inorganic orthophosphate and orthophosphate monoester P. The relative concentrations of P species giving rise to the numerous individual peaks in this region of the spectrum were quantified by spectral deconvolution, using a method similar to that of Bünemann et al. (2008). Each spectrum was fitted with up to 12 peaks as identified by Noack et al. (2012). These were identified as orthophosphate, α- and β-glycerophosphate, phytate (four peaks), and five peaks in the monoester region that were identified as mononucleotides, and include the 2′ and 3′ isomers that result from alkaline hydrolysis of RNA (Noack et al. 2012; Turner et al. 2003a). Each peak was defined by three parameters: the chemical shift (frequency), intensity, and the line width, which we allowed to vary in the fit. The absolute concentration of each P species (including those determined using integration alone and those determined using integration and deconvolution combined) was calculated by integration against the known concentration of the MDP that was added to each NMR tube. These values are referred to as NaOH-EDTA-extractable P.

A correction was required for some samples when determining phytate and orthophosphate concentrations due to the overlap of the phytate C-2 peak with the orthophosphate peak. For these samples, total phytate was calculated as 6/5 times the total concentration of the three observable resonances, and 1/5 of this value was subtracted from the total orthophosphate concentration (Doolette et al. 2009).

Mycorrhizal staining method

To assess arbuscular mycorrhizal colonisation, subsamples of wheat roots (~0.2 g) were cut to approximately 2 cm length before clearing in KOH 10 % and staining in a 5 % ink-vinegar solution (Vierheilig et al. 1998). Stained roots were assessed for arbuscular mycorrhizal colonisation using the gridline intersection method (Giovannetti and Mosse 1980) under a dissecting microscope at 40× magnification.

Statistical analysis

Analysis of Variance (ANOVA) was undertaken using the GENSTAT version 13 statistical package (VSN International, Rothamsted, UK). Assumptions of constant error variance (homogeneity), normality of data distribution and additivity of treatment and replicate

effects were tested for each analysis. Least significant difference (l.s.d.) between treatments was determined at <5 % significance using Fisher's protected l.s.d.

Results

Plant dry matter

Wheat plants supplied with low P produced significantly less dry matter for all plant parts compared to plants supplied the medium and high P rates, with up to five-fold increases in wheat seed (grain) yield with increasing P nutrition (Table 1). At medium and high rates of P application, dry matter production was not significantly different for any wheat plant parts, except the seed. Canola stem, pod and seed dry weight varied differently with P rates. The root and leaf dry weights were either not significantly different or only different for two of the three P rates (Table 1). Plant roots were the smallest mass of plant material, generally followed by stem and leaves, chaff/pods and seed.

Plant C:N and C:P

The C:N ratios of the plant tissue were smallest at the low rate of applied P for both crop types (Table 1). Plants supplied with medium and high P rates had similar C:N ratios for most plant parts. The C:P ratios varied with P rates and plant parts. Generally, plants at the high P rate had the smallest C:P ratios for both wheat and canola. The seed material had the smallest C:N (1:14–20) and C:P (1:68–193) ratios, followed by chaff/pod or leaf, roots and stems (Table 1).

Plant P concentrations

For all wheat plant parts except chaff, total plant P concentrations differed significantly with P rate (Table 2). Interestingly, wheat plants supplied with P at the medium rate had lower concentrations of total P than plants supplied with the low rate. Total P concentrations of canola stem and seed material were significantly different across the three P rates, whilst total P concentrations of root, leaf and pod materials were either not significantly different or only different for two of the three P rates. Plant roots contained the lowest concentrations of total P, followed by stem, leaves, chaff/pods and seed.

Extractability of plant P by NaOH-EDTA

An important consideration in interpreting NMR analysis of P speciation is the efficiency of P extraction. On average, NaOH-EDTA extraction efficiency was 93 % of total plant P measured by ICP-AES across all plant parts (Table 2). This high extraction efficiency means that NMR analysis assessed most of the P present in the various plant parts. Sodium hydroxide-EDTA-extractable P in wheat and canola plant parts ranged from 77 to 105 % for the vast majority of samples. Low extraction efficiencies were observed for leaf samples. Values >100 % were recorded for some root samples. This may be a consequence of de-mixing of homogenised root samples, which likely contained some soil material. Apparent exaction efficiencies of >100 % were attributed to some sub-samples used for total P analysis containing more of this soil (with a diluting effect) than sub-samples used for NMR analysis.

Phosphorus speciation—roots

Phosphorus forms common to both wheat and canola root extracts were orthophosphate, glycerophosphate, mononucleotides and phytate (Fig. 1). Orthophosphate and α- and β-glycerophosphate (most likely present predominantly as phospholipid in the plant material, which undergoes alkaline hydrolysis during NaOH-EDTA extraction) were the most abundant P forms in plant roots, with orthophosphate accounting for 11 to 36 % and glycerophosphate accounting for 10 to 30 % of total NaOH-EDTA-extractable P (Fig. 2). Mononucleotides comprised 14 to 22 % of P in wheat and canola root extracts. This would include ribonucleic acid (RNA) present in the plant material, which is unstable under the alkaline conditions of extraction and would be hydrolysed to a mixture of 2′- and 3′- mononucleotides). Phytate was detected at low concentrations in all root material as four resonances in the monoester region in a 1:2:2:1 ratio (Turner et al. 2003a).

The percentage of NaOH-EDTA-extractable P present as organic P ranged from 46 to 59 % in wheat and 47–66 % in canola roots (Fig. 2). The percentage of P detected as orthophosphate did not increase in wheat roots as P rate increased. The percentage of orthophosphate in canola roots increased from 35 to 53 % (25–70 mg P kg^{-1}) as P rate increased.

Table 1 Dry weight (g pot^{-1}), C: N and C: P ratios in response to rate of P application to soil for wheat and canola

P rate	Plant part	Dry weight (g pot^{-1})			C:N			C:P		
		Low	Medium	High	Low	Medium	High	Low	Medium	High
Wheat	Root	0.3a	1.6b	1.7b	53	57	48	3,995	3,115	1,560
	Stem	0.4a	5.0b	4.9b	32	155	186	2,330	5,065	3,246
	Leaves	0.8a	3.8b	4.1b	20	90	88	1,355	2,450	1,376
	Chaff	1.1a	5.4b	5.8b	41	85	85	590	955	596
	Seed	3.0a	14.5b	16.3c	14	20	20	128	172	116
	Whole plant	5.6	30.3	32.8						
Canola	Root	1.1a	2.8b	3.1b	49	82	138	5,933	3,042	3,349
	Stem	1.3a	7.9b	9.4c	40	209	231	4,226	5,945	3,028
	Leaves	2.7a	4.5b	4.8b	13	64	70	1,229	1,079	958
	Pods	3.6a	8.2b	8.9c	41	125	110	2,519	2,740	608
	Seed	3.2a	8.1b	8.5c	14	18	20	193	122	68
	Whole plant	11.9	31.4	34.7						

Data for dry weight are the mean of seven replicates and C:N and C:P are the mean of three replicates. Within a row, treatments appended by a different letter are significantly different ($P \leq 0.05$)

Pyrophosphate was detected in wheat roots comprising a further 10 to 20 % of the P in roots across the three P rates. No pyrophosphate was detected in the roots of canola.

Phosphorus speciation—stem and leaves

Both wheat and canola stem samples contained orthophosphate, glycerophosphate and phytate (Fig. 2). As P rate increased, orthophosphate as a percentage of the NaOH- EDTA-extractable P in wheat stems increased from 29 to 74 % whereas percentages of phytate decreased (from 55 to 19 %) (Fig. 2a). For canola stem material, plant P status had no consistent effect on P speciation.

Orthophosphate represented >85 % of the NaOH-EDTA-extractable P in the leaf material of both canola and wheat. The remaining 15 % of leaf P was identified

Table 2 Total plant P (mg kg^{-1}) and P extractable in NaOH-EDTA (mg kg^{-1})

P rate	Plant part	Total P (mg kg^{-1})			NaOH-EDTA-extractable P (mg kg^{-1})		
		Low	Medium	High	Low	Medium	High
Wheat	Root	57a	86b	121c	84 (146)	136 (158)	127 (105)
	Stem	180a	83b	129c	176 (98)	79 (95)	132 (103)
	Leaf	236a	153b	279c	246 (104)	225 (147)	177 (42)
	Chaff	574ab	379a	640b	442 (77)	250 (66)	582 (91)
	Seed	3176a	2384b	3519c	3,018 (95)	2,027 (85)	2,745 (78)
Canola	Root	73a	142a	131a	72 (98)	110 (77)	117 (89)
	Stem	102a	73b	143c	93 (92)	70 (96)	126 (88)
	Leaf	261a	320b	359b	242 (93)	278 (87)	312 (87)
	Pod	151a	146a	648b	127 (84)	113 (77)	674 (104)
	Seed	2902a	4753b	8416c	2,292 (79)	3,660 (77)	7,069 (84)

Data presented for total P are the mean value of seven replicates. Within a row, treatments appended by a different letter are significantly different ($P \leq 0.05$). Values in parenthesis are the percentage of total P extracted by NaOH EDTA

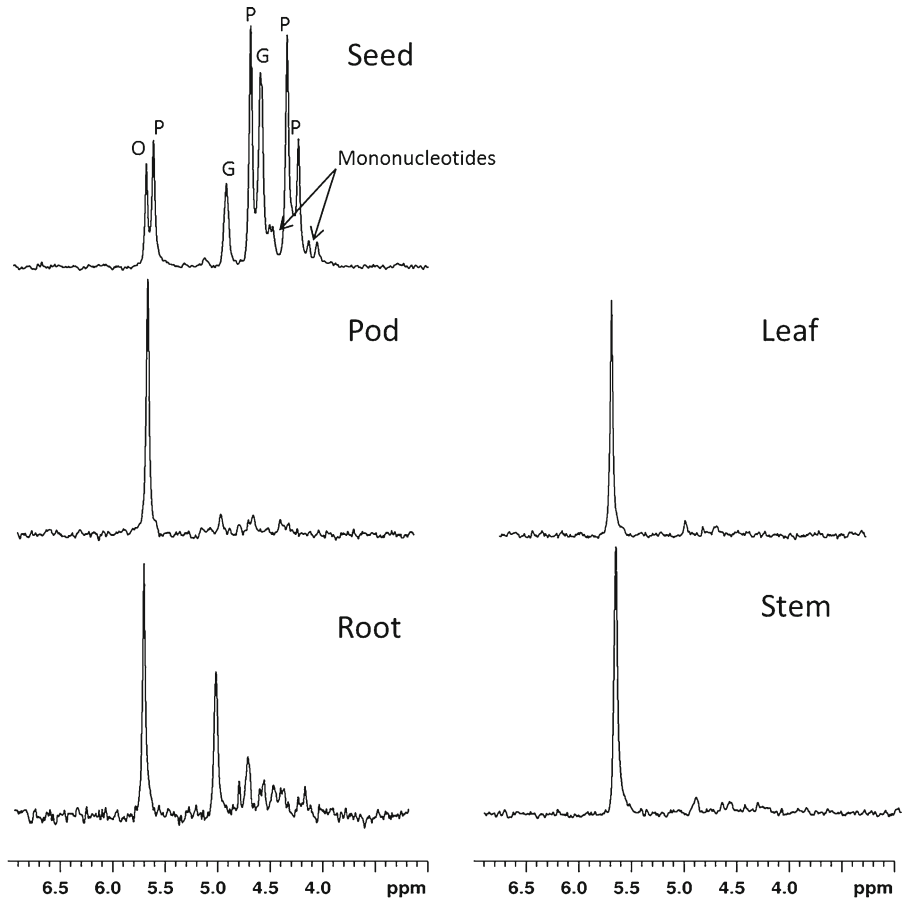

Fig. 1 Representative solution ^{31}P NMR spectra of NaOH-EDTA extracts. The spectra shown are for seed, pod, root, leaf, and stem for canola grown with low P. Peaks assigned as *O* orthophosphate, *G* glycerophosphate, *P* phytate and mononucleotides as indicated by the *arrows*

as glycerophosphate. There was no significant change in percentage of orthophosphate and glycerophosphate in leaf material across the three rates of P application for either crop type (Fig. 2).

Phosphorus speciation—chaff and pod

As with stem and leaves, orthophosphate was the most abundant P species in wheat chaff (49–87 %) and canola pods (74–81 %). Glycerophosphate and phytate were the other P forms detected in chaff and pod material. Phytate was the second most abundant P form in wheat chaff. Phytate represented 9–43 % of the NaOH-EDTA-extractable P, with this proportion increasing with increasing plant P status (Fig. 2). Glycerophosphate comprised only 4–8 % of the total wheat chaff P.

Canola pods contained lower percentages of phytate (9–13 %) and glycerophosphate (7–15 %) but a higher percentage of orthophosphate than wheat chaff. Unlike wheat chaff, plant P status did not significantly alter the percentage of the different P species identified in canola pods. A small percentage (2.5 %) of total P was detected as pyrophosphate in canola pods for the high P treatment.

Phosphorus speciation—seed

Organic P species constituted more than 90 % of the NaOH-EDTA-extractable P in the seed of both crop species examined. Phytate was the dominant form of P in both wheat and canola seeds (Fig. 2), comprising 37 to 89 % of total NaOH-EDTA-extractable P. The percentage of phytate in the wheat seeds did not change with plant P status and, on average, comprised 80 % of the total seed P across the three P rates examined. The proportion of phytate in canola seeds increased from 60 % (low P) to 75 % (medium P) to 85 % (high P) with increasing P rate. This increase in phytate was

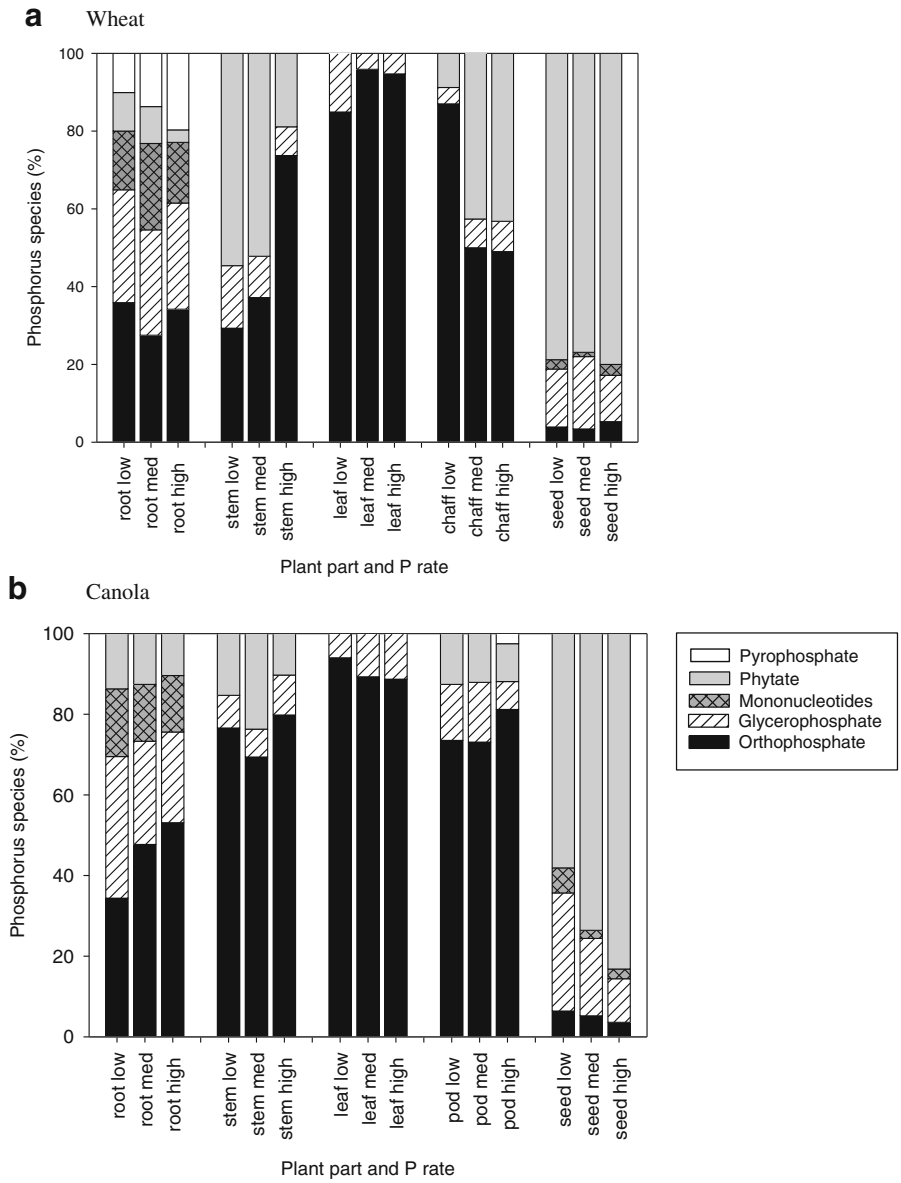

Fig. 2 Phosphorus speciation (% of P detected) as determined by solution ^{31}P NMR spectroscopy

offset by a decrease in the glycerophosphate content from 30 % (low P) to 19 % (medium P) to 11 % (high P) of the total canola seed P.

Glycerophosphate represented the second most abundant form of P in seed of both wheat and canola (Fig. 2). Mononucleotides were also identified in wheat and canola seeds, where they constituted 1–6 % of NaOH-EDTA-extractable P. Orthophosphate was present in all seeds, but constituted only 3–6 % of seed P (Fig. 2). The percentages of inorganic and organic P species in wheat seeds did not change with P nutrition. For canola seeds, however, phytate comprised an increasing proportion of total P as plant P status increased.

Mycorrhizal colonisation

All wheat roots were colonised by VA-mycorrhizae, with percentage infection ranging from 20 to 50 % of root (data not shown).

Discussion

Phosphorus status of plant material

The critical ranges for plant nutrient concentrations identified by Reuter and Robinson (1997) were used to evaluate the P status of wheat and canola plants supplied with each of the three P rates used in our experiment. Wheat grain containing 0.25–0.3 % P is categorised as 'deficient', grain containing 0.3–0.5 % P is categorised as 'adequate' and grain with >0.5 % P is categorised as having 'high/luxury' P status. On this basis, wheat material from the low and high P rates had adequate P concentrations, whereas plants supplied the medium P rate were deficient in P (Table 2). Similarly, the critical concentration ranges for mature canola seeds (Reuter and Robinson 1997) are 0.25–0.41 % P (deficient), >0.45 % (adequate) and 0.7–1.0 (high/luxury) . On this basis, canola seed P concentrations supplied the low, medium and high P rate are categorised as having had deficient, adequate and luxury P status, respectively.

The total P concentrations in the wheat plant material did not increase linearly with increasing P fertiliser rate. The relationship is often referred to as the 'Piper-Steinberg effect' (Piper 1942; Steenbjerg 1951). Wheat P concentration decreased between the low and medium rate with substantial increases in yield causing a dilution in tissue P concentration. This complicates interpretation of the data, as plants supplied the low P rate had higher P concentrations than those supplied the medium P rate. Nevertheless, the low P rate produced plants that were severely P deficient.

Phosphorus speciation varied among plant parts

At harvest (grain maturity), the wheat and canola seeds contained 75 % and 85 % of the total plant P, respectively (Table 2). Phytate was the most abundant P species in seeds, constituting up to 80 % of the NaOH-EDTA-extractable P in both seed types (Fig. 2). This is consistent with previous studies, which report 50 to 80 % of seed P in the form of phytate (Lott et al. 2002; Reddy et al. 1989). Other P forms identified in wheat and canola seeds were glycerophosphate (11–30 %), orthophosphate (4–6 %) and mononucleotides (1–6 %). Previous ^{31}P NMR-based analyses (Negassa et al. 2010; Raboy 2006) did not identify or quantify glycerophosphate and mononucleotides in mature seeds (or seed by-products) but, rather grouped them as 'other P' or 'other orthophosphate monoesters'. Plant P status had no effect on the proportion of P species detected in wheat seeds. With increasing P status of canola plants, seeds displayed luxury P storage as phytate. The vast majority of the seed of both crops is removed at harvest and so the seed usually only contributes a small fraction (on average 2–10 %) of the dry matter returned to soil (Anderson and Soper 2003; Gan et al. 2008). Differences in the amount and form of P in seeds is therefore of minor importance in understanding the cycling of P from plant residues to soil in grain cropping systems. The remaining plant dry matter (root, stem, leaves and chaff/pods), which together constitutes plant residues returned to the soil following harvest, contained 15–25 % of the total plant P.

A significant percentage of residue P that returns to soil occurred as orthophosphate (Fig. 2). Previous studies using sequential chemical fractionation methods to identify inorganic P in various plant parts reported similar percentages in mature plant material (Birch 1961; Jones and Bromfield 1969; Martin and Cunningham 1973). Birch (1961) investigated the effect of differing inorganic and organic P contents of plant residues on the transformation of residue P during decomposition. For a range of residue types, the percentage of inorganic P (cold acid extracted) ranged from 50 to 95 % of the total residue P. Slightly lower percentages (36–74 %) of orthophosphate were detected in mature stem and leaf material of a range of crop residues collected at harvest (Noack et al. 2012). This residue orthophosphate is in a form that is potentially immediately available to plant roots and microorganisms, as well as for sorption onto soil minerals.

Organic P species were detected in varying concentrations among plant parts (Fig. 2). Glycerophosphate, phytate and mononucleotides were detected in some or all plant parts. Generally, glycerophosphate comprised a higher proportion of the plant P than mononucleotides or phytate, except for wheat stem and chaff material, which contained a high proportion of phytate. Both phospholipids and nucleic acids, from which the glycerophosphate and mononucleotides in this study likely originated, have been shown to be mineralised rapidly in soils and do not contribute to the stable P pool (Harrison 1982; Islam and Ahmed 1973; Kowalenko and McKercher 1971). More recently a range of microorganisms have been found capable of degrading phytate in the laboratory (Hill and Richardson 2007). However, microbial degradation of phytate in soil will be regulated

by the ability of phytate to bind to mineral surfaces (Celi et al. 1999), and form insoluble metal complexes (He et al. 2006), thereby protecting it from microbial degradation (Turner et al. 2003b). The organic P species detected in these plant parts can thus be considered as mostly easily-degradable organic P (phospholipids, RNA and potentially phytate) with only phytate potentially contributing to a more resistant organic P pool.

Pyrophosphate was detected in wheat roots, where it constituted 7–14 % of the NaOH-EDTA-extractable P (Fig. 2a). The presence of pyrophosphate in decomposing plant material has previously been attributed to the colonisation by fungi and other microorganisms (Miltner et al. 1998; Cheesman et al. 2010). A previous study by Bünemann et al. (2008) revealed the potential for microorganisms to synthesise condensed P forms such as pyrophosphate in soils. The presence of pyrophosphate (as detected by NMR spectroscopy) was positively related to high fungal plate counts (Bünemann et al. 2008). In this experiment, the presence of pyrophosphate in wheat roots is consistent with the presence of mycorrhizal colonisation.

Phosphorus speciation varied little with P status

The relative proportions of P species were unaffected by plant P status for the majority of wheat and canola plant parts (Fig. 2). This is in contrast to many previous studies which have reported that increases in P status increase inorganic P concentrations and also increase inorganic P as a proportion of total P (Barr and Ulrich 1963; Batten and Wardlaw 1987; Chapin and Bieleski 1982; Fuller et al. 1956; Kakie 1969; Lee et al. 1976; White and Ayoub 1983). However, many of these studies involved immature plant material, with plants grown for 2–6 weeks to determine the optimum or adequate P levels required for plant growth. It is well established that immature plant material has very a different distribution of P forms compared to mature plant material (Bouma and Dowling 1982; Lewis 1992).

Few studies have examined the effect of P supply on P speciation in mature plant material (Barr and Ulrich 1963; Batten and Wardlaw 1987; Hart and Jessop 1983; Umrit and Friesen 1994). Most of these studies also reported that the concentration of inorganic P and its proportional contribution to total plant P content increased with P nutrition in mature plant material. Batten and Wardlaw (1987) grew wheat to maturity and found the concentration of inorganic P forms increased with increasing P supply. In the low P plants, 30–40 % of the P in leaves was inorganic P compared to 60 % at the higher P level. Similarly, it was found that for a pasture species (*Setaria sphacelata*), the percentage of total plant P that was acid-soluble ($0.2N\ H_2SO_4$) inorganic P increased from 63 to 92 % with increasing rates of P fertiliser in the field (Umrit and Friesen 1994). In our study, using wheat and canola, the concentration of inorganic P increased with increasing P status in many plant parts but this increase was matched by increases in other forms of P. This suggests that wheat and canola crops with deficient, adequate or luxury P status will contain different concentrations of total P but that the proportion of this P present as orthophosphate varies little.

Plant parts that were affected by P status were wheat stem and chaff and canola roots (Fig. 2). A significant percentage of P in wheat stems occurred as orthophosphate, which rose from 30 to 74 % as P rates increased, with a corresponding decrease in phytate concentration. The trend for wheat chaff was opposite to the stem, with phytate percentage rising with increasing P status. The presence of phytate in wheat stem and chaff material contrasts with previous studies which suggest excess P in plant parts other than the seed is stored as orthophosphate (Batten and Wardlaw 1987; Kakie 1969; Umrit and Friesen 1994). The factors that regulate the synthesis of phytate in plant tissue are unclear, although a recent study showed a clear association between cellular orthophosphate concentrations and phytate synthesis in non-seed tissue (Mitsuhashi et al. 2005). These authors reported that the synthesis of phytate was induced by growing *Catharanthus roseus* cells in high orthophosphate concentration solutions, while cells growing in solutions with low orthophosphate concentrations contained little or no phytate. The presence of phytate in wheat stem material may result from phytate being synthesised in response to temporarily high orthophosphate concentrations in these cells.

Implications for P cycling in grain cropping soils

The quality of crop residues in terms of C, N and P is important in understanding the decomposition of residues in soil. The C:N and C:P ratios resulting from the different rates of P application (Table 1) are in agreement with many previous papers which have shown that plants with higher total P have lower C:N and C:P ratios (e.g. White and Ayoub 1983). However, these ratios are

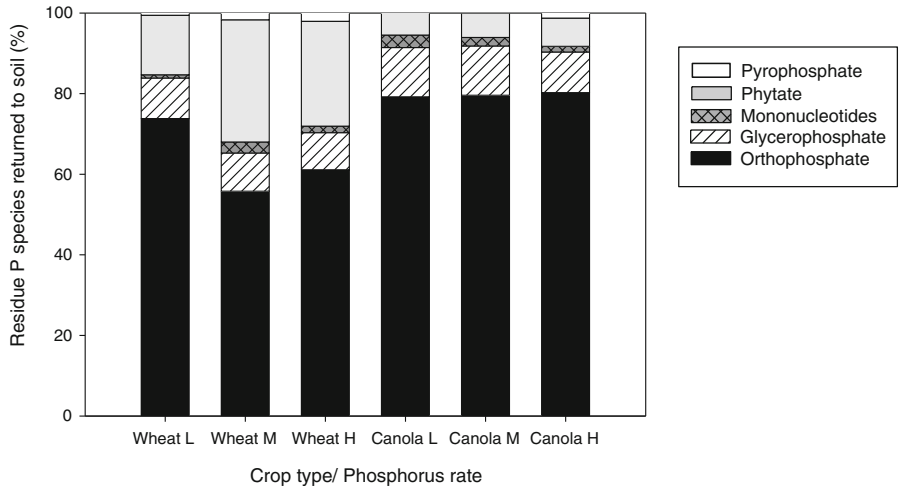

Fig. 3 The percentage of whole plant residue P speciation calculated from dry matter, P content and P speciation of non-seed plant parts (roots, stem, leaf and chaff/pod material)

not always capable of predicting residue P release (Birch 1961; Enwezor 1976), possibly due to differences in P speciation that affect availability of P released from decomposing residue for microbial degradation or for incorporation into microbial biomass.

In previous sections we have considered the P speciation of individual plant parts. The combination of non-seed plant parts (root, stem, leaves and chaff/pods) constitutes the residue pool returned to soil after the seed is harvested and removed from the field. We calculated the percentage of each P species that would be returned to soil in the residue P pool from the total P concentration, dry weight and P speciation for each plant part (Fig. 3). Although P status caused differences in P speciation in some plant parts, these differences tend to cancel out when speciation is expressed on a whole plant residue basis. Orthophosphate was the dominant P species in residues returned to soil, followed by phytate (wheat) or glycerophosphate (canola) with mononucleotides and pyrophosphate present in only very small amounts (Fig. 3). As plant P status increased, the relative percentage of these P forms remained unchanged for whole plant residues of canola, whilst changes were small and inconsistent for whole plant residues of wheat.

This improved understanding of the interaction between plant P status and P speciation in mature residues can assist with estimating the mass of each P species returned to soil in a cropping system. For example, using the medium P rate treatment for wheat and assuming an average grain yield of 3 t ha^{-1} and a harvest index of 0.4, resulting in 7.5 t dry matter ha^{-1} that would be returned to the soil as crop residues with a total content of 2 kg P ha^{-1}; 1.1 kg ha^{-1} of this P will be returned as orthophosphate (56 %), 0.6 kg ha^{-1} as phytate (30 %), 0.2 kg ha^{-1} as phospholipids (9 %), 0.05 kg ha^{-1} as RNA (3 %) and 0.05 kg ha^{-1} as pyrophosphate (2 %).

Orthophosphate is the dominant form of residue P and it has many potential fates in cropping soils. Residues remaining on the soil surface can be colonised by microorganisms, resulting in microbial uptake of orthophosphate, or this water soluble form of P can be leached from residues into the surface soil. For residue incorporated into the soil, microbial biomass can immobilise orthophosphate with the P subsequently released through mineralisation. Leached or mineralised P that ends up in the soil solution P pool will be available for uptake by plant roots and microorganisms or interactions with less labile soil P pools through sorption to soil particles or precipitation with soil minerals.

Conclusions

There was no marked change in plant P speciation with increasing plant P status across different wheat and canola parts. Stems and leaves, which contribute the bulk of post-harvest residue P, were dominated by orthophosphate. Root P was dominated by organic P but constituted only a small fraction of the post-harvest residue P derived from the plant. Although differences

in P speciation were observed in some plant parts, this effect was insignificant when expressed on a whole plant residue basis. This experiment showed the dominant P form in post-harvest crop residues is orthophosphate, regardless of plant P status.

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