Foliar Fertilisation of Wheat Plants with Phosphorus

A thesis submitted to The University of Adelaide

in fulfilment of the requirements for the degree of Doctor of Philosophy

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Abstract

Phosphorus (P) is an important macronutrient essential for plant growth. Broadacre cropping often requires additional inputs of mineral P fertiliser to grow profitable crops. Current management practice is to apply all fertiliser P at sowing. If the conditions under which foliar applied P could reliably increase grain yield are met, foliar application of P could be used as an in-season management strategy to top up P supply of wheat. This could be of significant benefit to farmers to reduce risk in regions with variable climate.

Through a series of plant experiments under controlled environmental conditions, this thesis investigated plant physiological (leaf wettability and growth stage) and foliar formulation (form of P, P concentration, adjuvant choice and pH) factors affecting the efficacy of foliar P uptake and translocation. The first experiment investigated the influence of leaf side and its corresponding wettability on the uptake and translocation of foliar applied P. The second and third experiments examined the effect of adjuvants on the wettability of wheat leaves and the associated uptake and translocation of foliar applied P (from phosphoric acid) after a few days and when harvested at maturity. The last experiment investigated the effect of foliar formulations differing in pH, P source and adjuvant, on wheat growth and uptake and translocation of P.

A number of methods and techniques were used throughout the thesis. Investigations on the effect of leaf morphology on uptake and wettability involved the use scanning electron microscopy. Wettability of leaves by both water and fertilisers was characterised using contact angle measurements with a combination of static, advancing, receding and spreading contact angles over time measured. Uptake and translocation of the foliar applied fertilisers was quantified through the use of dual or single labelling isotopic tracer techniques.

Absorption and subsequent translocation of foliar applied P was higher for the adaxial (upper) leaf side despite it being more difficult to wet than the abaxial (lower) side. When the foliar P concentration was increased the contribution of foliar P to plant P uptake increased but was translocated away from the site of application at a lower efficiency, likely due to the higher scorch experienced by the leaves at higher concentrations. Importantly, the morphology of the wheat leaf influenced both the retention and contact angle of the fertiliser on the leaf surface and the uptake and subsequent translocation of the foliar applied P. Foliar application of P at ear emergence had higher absorption and subsequent translocation of P than when applied at anthesis.

The inclusion of a surfactant in the foliar P formulation is essential because wheat leaves are difficult to wet. Application of foliar P without a surfactant resulted in lower levels of fertiliser retention on leaves. When applied with phosphoric acid the choice of adjuvant affected the spreading dynamics and leaf wetting area but did not affect the foliar uptake of P. The yield response to foliar applied phosphoric acid was inconsistent despite the uptake and translocation being the same for all formulations that included a surfactant. The timing of application was more important than surfactant choice with higher translocation of foliar applied P when it was applied at flag leaf emergence compared to tillering.

While increases in P uptake by wheat plants with foliar application of phosphoric acid were consistent, increases in plant growth and yield were not. Although foliar P from phosphoric acid was absorbed, only a small proportion was translocated. Specific combinations of adjuvant and P sources other than phosphoric acid were able to increase both plant P uptake and peak biomass. These foliar fertilisers ranged in associated cations (potassium, sodium and ammonium phosphates) and pH (2.2, 4.3, 6.5 and 8.7). Increases in plant P uptake did not always translate to biomass increases with translocation of foliar applied P playing a more crucial role than foliar uptake of P.

This thesis has made important progress in our understanding of the effects of wheat leaf morphology, leaf wettability and crop phenology on the recovery of foliar applied P fertilisers in wheat plants. The processes of retention, absorption and translocation of foliar-applied P have proven important for inducing positive biomass and grain yield responses and this has been achieved using several foliar P formulations. However, a single characteristic of the formulation that optimises these processes has not been identified and as a result prediction of the exact scenarios when positive responses of wheat to foliar-applied P should occur has not been achieved. It appears that there is some plasticity in the response by wheat plants to additional P supplied via the leaves and some remaining uncertainty about the effects of scorch that are influencing the predictability of the response. Field validation is required to ascertain whether the positive response found in controlled experiments can be replicated when environmental conditions are more varied and unpredictable.

Declaration

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I would like to dedicate my thesis to my Grandmas. Although they may not have understood my research, they still asked how it was going whenever they saw me. It was difficult to lose them during my PhD, but I am sure they would have been proud of all I have achieved.

List of publications and presentations

Peer-reviewed research articles

Fernández V., Guzmán P., Peirce, C., McBeath T., Khayet M., McLaughlin M. J., 2014, Effect of wheat phosphorus status on leaf surface properties and permeability to foliar applied phosphorus, Plant and Soil 384, 7-20, DOI 10.1007/s11104-014-2052-6

Peirce C. A. E., McBeath T. M., Fernández V. and McLaughlin M. J., 2014, Wheat leaf properties affecting the absorption and subsequent translocation of foliar applied phosphoric acid fertiliser. Plant Soil 384, 37-51, DOI 10.1007/s11104-014-2245-z

Peirce C. A. E., Priest C., McBeath T. M., McLaughlin M. J., 2015, Uptake of phosphorus from surfactant solutions by wheat leaves: spreading kinetics, wetted area, and drying time. Soft Matter 12, 209-218, DOI: 10.1039/c5sm01380a

Abstracts from presentations in scientific meetings

Fernández V., Guzmán P., Peirce, C., McBeath T., Khayet M. and McLaughlin M. J., 2013, Effect of phosphorous nutrition on wheat leaf surface properties, XVII. International Plant Nutrition Colloquium, Istanbul, Turkey, 19th-22nd August 2013. (poster presentation)

Peirce C., Facelli E., McBeath T., McLaughlin M. J., 2015, 'Topping up' wheat with foliar P: getting the right combination of P formulation and adjuvant, 2015 Agronomy Conference, Hobart, Tasmania, Australia 20th-24th September 2015 (short oral presentation)

Peirce C. A. E., Priest C., McBeath T.M. and McLaughlin M.J., 2015, Wetting and Uptake of Phosphorus Foliar Fertilizer by Wheat Leaves. The Australian Colloid and Interface Symposium, Hobart, Tasmania, Australia 1st-5th February 2015. (poster presentation)

Peirce C., Priest C., Facelli E., McBeath T., McLaughlin M. J., 2014, The effect of adjuvant on leaf wetting and uptake of fluid foliar P fertilizers for wheat. Fluid Forum, Scottsdale, Arizona, USA 17th-18th February 2014. (oral presentation)

Peirce, C., Fernández V., McBeath T., McLaughlin M. J and Guzmán P., 2013, Foliar uptake of phosphorus by wheat is greater from the adaxial than the abaxial leaf side, XVII. International Plant Nutrition Colloquium, Istanbul, Turkey, 19th-22nd August 2013. (poster presentation)

Industry publications

Facelli E., McBeath T., Peirce C., McLaughlin M., Hunt E., Montalvo D., 2015, 'Topping Up' Wheat with Foliar P – Does it work? In Proceedings of the 2015 Fluid Forum, Scottsdale, Arizona, USA, Fluid Fertilizer Foundation, Manhattan, Kansas, USA.

McBeath T., Facelli E., Peirce C., McLaughlin M., Hunt E., 2015 "Topping Up" Wheat with Foliar P – Does it work? In Proceedings of the GRDC Grains Research Update for Advisors, Adelaide, SA, 2015

Peirce C. A. E., Facelli E., McBeath T. M., McLaughlin M. J., 2014, Tactical foliar phosphorus (P) fertilisation of dryland crops in WFN Farm Bulletin Spring Editions 2014, Wimmera Farming Network

Peirce C., Priest C., Facelli E., McBeath T., McLaughlin M. J., 2014, The effect of adjuvant on leaf wetting and uptake of fluid foliar P fertilizers for wheat. In Proceedings of the 2014 Fluid Forum, Scottsdale, Arizona, USA, Fluid Fertilizer Foundation, Manhattan, Kansas, USA

Structure of the thesis

This thesis is presented in the publication format and includes papers that are have been published or prepared for submission to a journal.

Chapter 1 introduces the thesis and gives an overview and general discussion on the rationale behind why we were interested in researching foliar fertilisation of wheat with phosphorus. It also provides an overview of the literature relevant to my research as put together for my initial research proposal in July 2012. As a result, more recent publications are not included in the literature review but are discussed where relevant in the discussion sections of the subsequent chapters. This chapter concludes with the aims and objectives of my thesis.

Chapter 2 describes an experiment published in *Plant and Soil* investigating the uptake and translocation of foliar applied phosphoric acid to the adaxial (upper) and abaxial (lower) sides of wheat leaves. The influence of leaf morphology and structure on both wettability and uptake of foliar fertilisers was explored.

Chapter 3 presents the results of collaboration with the Ian Wark Institute at the University of South Australia, which investigated the spreading and wettability of the phosphoric acid based formulations containing different adjuvants on wheat leaves and the initial uptake and translocation of P seven days after foliar application. This paper investigated the dynamics of wetting of wheat leaves by various phosphoric acid based formulations and has been published in the interdisciplinary journal *Soft Matter*.

Chapter 4 comprises a paper that follows and expands on Chapter 3 by investigating the effect of timing of application and the use of adjuvant with phosphoric acid on the wettability and surface structure of wheat leaves, as measured by contact angles and scanning electron microscopy, in combination with the uptake and translocation of five phosphoric acid formulations that differed in choice of adjuvant measured using isotopic techniques. The plants were grown through to maturity which allowed measurement of the final sink of the foliar applied P and the resultant yield effect.

Chapter 5 presents the last experiment focussing on the evaluation of fertiliser formulation through a plant experiment grown through to peak biomass with 21 different P formulations tested (seven P products × three adjuvants) that varied in both pH and associated cations. This experiment was designed due to a lack of consistent biomass results and low translocation achieved with phosphoric acid.

Chapter 6 summarises the main findings of my thesis and concludes with future recommendations for work in this area.

In the Appendix is a preliminary paper published in *Plant and Soil* on which I am third co-author on the effect of plant P status on leaf wettability and foliar P uptake. This study, conducted in collaboration with Victoria Fernandez, a visiting plant physiologist from Spain, was instrumental in developing my understanding of plant surfaces and microscopy techniques early in my PhD candidature. Importantly, this paper demonstrated that foliar applied P will not be effective for correcting severely deficient plant P status and should only be used under conditions of marginal soil P status.

Chapter 1

Introduction and Literature Review

Introduction

Phosphorus (P) is a macronutrient essential for plant growth. However, many soils have low P availability due to low P concentrations and/or high retention of P by soil surfaces (Marschner and Rengel 2012). A deficiency in P will result in poor growth and reduced crop yield. In order for there to be sufficient soil P available for crops, fertilisers are used to increase the available pool of P in the soil (Hedley and McLaughlin 2005). Fertiliser that is applied to soil is readily sorbed and a large proportion of the added P, whether in a liquid or granular form, will undergo chemical reactions that initially bind the P and remove it from the available pool (Hedley and McLaughlin 2005). This P is often referred to as residual P and although it may only be sparsely available for the current crop, it can contribute substantially over a longer period of time to future crops. Due to the low P efficiency of soil-based P fertiliser in the season of application; a high application rate is required so that crops do not suffer P deficiency. The increasing cost of P fertilisers means there is a large cost for fertiliser at the beginning of the growing season to ensure that crops have the best chance at producing high yields. Unlike soil-based applications, foliar applications of nutrients potentially pose a more tactical fertilisation option as the fertiliser can be applied as needed in seasons of higher yield potential when extra P resources are in demand. This management approach can potentially provide cost benefits to farmers if it were to reliably allow smaller applications of P to soil at sowing.

In Australia, wheat is the most important broadacre crop accounting for over half of Australia's grain production. Over the five years from 2007/8 to 2011/12, Australia produced over 23 million tonnes of grain from wheat crops per year (Land and Commodities 2014). Over that same period, 55 % (AU$6.13 billion) of annual production from the Australian grains industry came from wheat. Although Australian wheat only accounts for 3 % of the world wheat production, it represents around 15 % of the world wheat trade annually and is exported to over 40 countries (ABS 2006). Due to its importance in Australian agriculture, this thesis will focus on the application of foliar P fertilisers to wheat crops.

For a foliar application to be successful, there are number of processes that occur before the fertiliser can reach the internal cells of the plant. In order, these are deposition, retention, uptake and translocation (Zabkiewicz 2000). The relative efficiencies of these individual processes can compound to result in a vastly different overall efficiency of the spray process (Table 1). The process of deposition relates mainly to the act of foliar spraying and determines the amount of the spray that reaches the plant surface. Retention is then related to the wettability of the leaf surface and is the proportion of the deposited spray that adheres to the surface. Uptake of the fertiliser is the proportion of the spray retained on the leaf that is

able to pass through the leaf cuticle to reach the internal cells. Finally translocation relates to how much of the nutrient is able to be mobilised in the plant and moved away from the site of application. These four processes are influenced by factors relating to the plant, the formulation of the spray and both the environment in which the fertiliser is sprayed and the environmental conditions during plant growth.

Table 1: Possible spray application efficiency for a herbicide application (Zabkiewicz 2007)

Spray efficacy processes	Process efficiency (%)	System efficiency (%)
Deposition	80-95	80-95
Retention	10-100	8-95
Uptake	30-80	2.4-76
Translocation	10-50	0.24-38

Three main overarching groups of factors (plant factors, formulation related factors and environmental factors) all inter-relate and work together to influence the efficacy of a foliar spray (Fernández and Eichert 2009). Plant-related factors are generally associated with the morphology of the leaf surface, which can govern the ability of leaves to retain and absorb foliar sprays. These plant factors can vary with plant species, leaf side, nutrient status of the plant, position on the plant and leaf age. The main formulation factors of interest are those that control the retention, uptake and translocation of nutrients namely; the use of an adjuvant (surfactant, oil, humectant, etc.) and its associated mode of action, the pH of the formulation and the form of P used. Environmental factors are hard to control but conditions for optimal deposition, retention and uptake are mostly related to temperature and relative humidity, which affect the effective time sprays stay on the leaf and are available for uptake. Environmental factors also include the soil type and ability of a soil to provide nutrients to the plant. It should be noted that the efficiency of foliar applications is also limited by the surface cover of the crop (available leaf surface area for nutrient acquisition, which relates to the deposition process), which is in turn related to the growth stage and timing of application.

This literature review will focus on foliar P applications to wheat plants; however, some comparisons and examples will be drawn from studies on other plant species, mainly crops, and for other nutrients. This review will summarise the processes involved in foliar application efficacy, the current state of knowledge on the pathways of foliar uptake and investigate some of the plant, environmental and fertiliser formulation variables that have limited the effectiveness of foliar P applications in past studies. This review was conducted at the start of the thesis prior to starting experimental work and updated at the end of the thesis. As a result, recently published studies (post 2012) will be discussed in the subsequent chapters and manuscripts rather than in this literature review. It should be noted that both prior and post this literature review being carried out, a number of review papers and books

have been published on foliar fertilisation in general (Eichert 2013; Eichert and Fernández 2012; Fageria et al. 2009; Fernández and Eichert 2009; Fernández et al. 2013) and more specifically relating to foliar P (Noack et al. 2011).

Plant demand for phosphorus

The demand for plant P and the total P content of wheat plants change over the life cycle of the plant. Phosphorus is essential early in the crop's growth to establish plant vigour and as a result, soil fertilisation with P at the start of the growing season is necessary to ensure good crop establishment and replace P exported from previous crops. The use of foliar P application as a complete replacement for soil application is unlikely to be effective due to the high P demand of crop plants, especially at the start of the growing season when there is little leaf surface area for uptake of foliar-applied P (Batten et al. 1986). However its use as a supplement to soil application to enhance yields is still under debate due to the variability in yield response to foliar-applied P under field conditions.

The yield of wheat is limited by P deficiency through slowing the rate of tiller emergence and therefore reducing the overall number of tillers (Rodriguez et al. 1999). Phosphorus deficiency often delays the onset of anthesis compared to plants with sufficient P status (Rodriguez et al. 1998). Phosphorus deficiency can also reduce the elongation rate of leaves and therefore reduce the total leaf area of wheat plants and total dry weight of the plants (Rodriguez et al. 2000; Rodriguez et al. 1998). Thus wheat plants that are deficient in P will have less leaf area available to intercept foliar sprays compared to P-sufficient plants, which will in turn reduce the effectiveness of foliar P applications. This is irrespective of the ability of P-deficient plants to absorb and translocate foliar-applied P (see the section Nutrient status of the plant).

Although plant demand for P is high during the early stages (vegetative stages) of wheat growth, this does not mean there is no demand later in the season. Wheat plants are particularly efficient at remobilising P from senescing leaves and redistributing it to the grain during anthesis with 20-90 % of the P in grain at maturity coming from P re-translocated from other plant parts (Batten and Wardlaw 1987). Although this accounts for a large proportion of the P in the grain, P uptake from the soil accumulates rapidly between stem elongation and anthesis, continuing through to dough development of the grain (Waldren and Flowerday 1979) with dry matter accumulation continuing until maturity (Figure 1). Likewise Römer and Schilling (1986) showed that around 50 % of P in the plant accumulated after stem elongation (2^{nd} node visible). Plant uptake of P from soil is often limited by P diffusion to the root,

particularly as the soil dries out (Marschner and Rengel 2012). Foliar P has been suggested as a technique to overcome this issue of P scarcity in dry soil, which often occurs later in the growing season (Sutton et al. 1983). Some authors have suggested that additional P applied after flag leaf expansion does not result in any increases in yield due to P within the plants being re-translocated more effectively by plants that were supplied with P-free nutrient solution compared to a P-containing nutrient solution after flag leaf expansion (Peng and Li 2005). Likewise Batten et al. (1986) found that supply of P beyond anthesis increased the plant P content, P content of the grain and tiller dry matter but did not increase the grain yield. However Sutton et al. (1983) suggested that although maximum dry matter was achieved with P supplied up until the first node growth stage, for maximum grain yield winter wheat plants required a supply of P up until the grains were in the soft dough stage. Mohamed and Marshall (1979) also found that for Spring wheat, almost half of the total P that accumulated in the plant occurred after anthesis. There appears to be plant demand for P later in the season, but it is still a question of whether additional fertiliser P will result in an increase in grain yield particularly when applied foliarly.

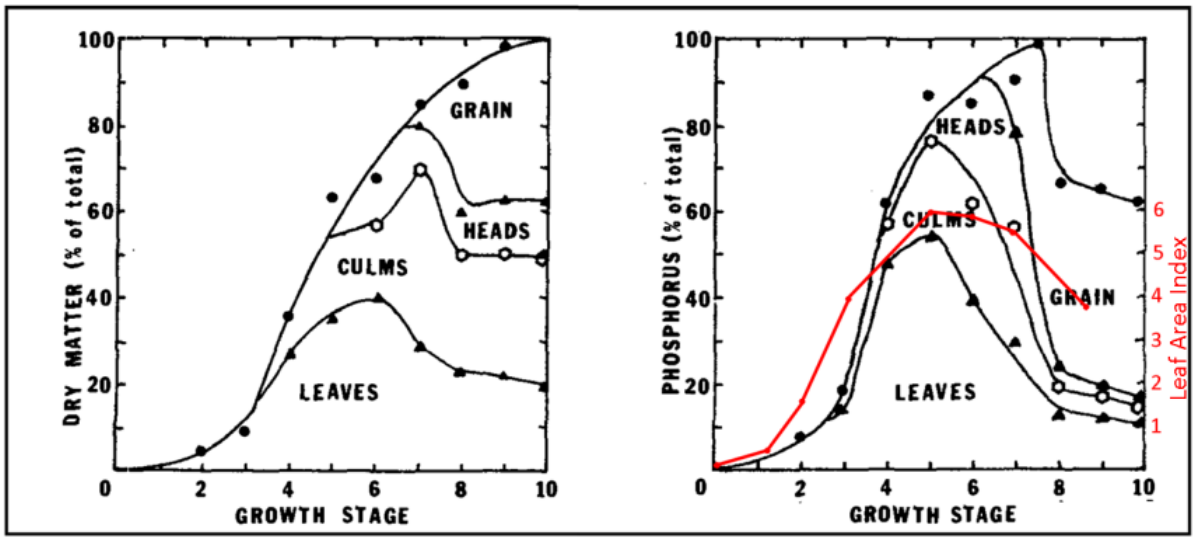

Figure 1: Distribution of dry matter (left) and phosphorus (right) in a winter wheat crop at different growth stages (0 germination, 2 stem elongation, 4 booting, 6 anthesis, 8 dough development, 10 maturity) from (Waldren and Flowerday 1979). Leaf area index (LAI) redrawn in red from (Laaboudi and Mouhouche 2012) on the graph of P accumulation.

Interception of foliar P

For a foliar spray to reach its intended target (the crop) there needs to be a sufficient canopy to intercept the spray before it reaches the soil. One way to describe the canopy cover of a crop is the leaf area index (LAI). The LAI is defined as the ratio between the total leaf area (of one side of the leaf) per unit land area (i.e. m^2 m^{-2}). As the leaves of wheat are fairly erect, a LAI of 1 does not necessarily correspond to complete ground cover with canopy

closure for winter wheat crops in European conditions estimated to occur at a LAI of about 3 (Scotford and Miller 2004). Canopy closure will depend on the spacing of rows and plant density and as a result can vary depending on farmer practice. LAI increases over the growing season; often rapidly between Zadoks 31 (first node, (Zadoks et al. 1974)) where LAI can be less than 2, and Zadoks 39 (flag leaf ligule visible) to a maximum LAI at Zadoks 59 (ear completely emerged) before it decreases again as the leaves start to senesce (Scotford and Miller 2004).

Figure 1 shows the comparison of how P uptake (from the soil) and LAI change as a wheat crop progresses through its growth stages. The substantial increase in LAI between tillering and ear emergence corresponds with a sharp increase in P content, particularly after stem elongation. This suggests there is potential for foliar application in this window to influence the P uptake and status of the plant.

For foliar application of urea, a LAI for wheat between 2 and 4 was adequate for most of the foliar spray to be retained by wheat leaves (Thorne and Watson 1955). Since foliar nitrogen (N) is mobile in the soil, the loss of the spray to the soil is a less critical factor reducing the efficiency of the spray, unlike P which is poorly mobile in soil. It is likely that canopy closure is required for foliar P sprays to be most effective and allow the maximum interception of sprays. Past agronomic studies have suggested that the optimum timing for foliar application to broadacre crops (including wheat) occurs between canopy closure and anthesis (Benbella and Paulsen 1998; Girma et al. 2007; Mosali et al. 2006).

The leaf surface and foliar pathways

Structure of the leaf

Although there are many differences between leaves of different plant species, the general structure is often fairly similar (Figure 2). The outer surface of aerial plant parts including leaves is covered by the cuticle which is a lipid layer that protects the inside of the plant leaf from desiccation. The cuticle layer varies in thickness from <0.1 -10 µm and is mainly composed of the polymer cutin, and/or cutan (Holloway 1993). There can also be waxes deposited on (epicuticular) or interspersed (intracuticular) in this matrix (Jeffree 2006). The epicuticular waxes generally consist of an amorphous layer that covers the cuticle, and may also contain a crystalline wax layer on top of this. It is these crystalline waxes that often limit the penetration of water and solutes into leaves (Wang and Liu 2007). In particular, wheat leaves have been reported to be highly water-repellent due to the microcrystalline form of the epicuticular wax (Gaskin and Holloway 1992). In contrast, intracuticular waxes are

considered to be much more polar than epicuticular waxes and are not such a barrier to water permeability (Bukovac and Norris 1967). The top part of the cuticle is called the cuticle proper and is distinguished from the lower cuticular layer by the absence of polysaccharides. The cuticular layer has an increased polarity compared to the epicuticular wax layer and the cuticle proper due to the presence of polysaccharides which extend from the plant cell wall below. As a result, the cuticle forms an asymmetric membrane which increases in polarity as the distance increases from the often highly hydrophobic outer surface.

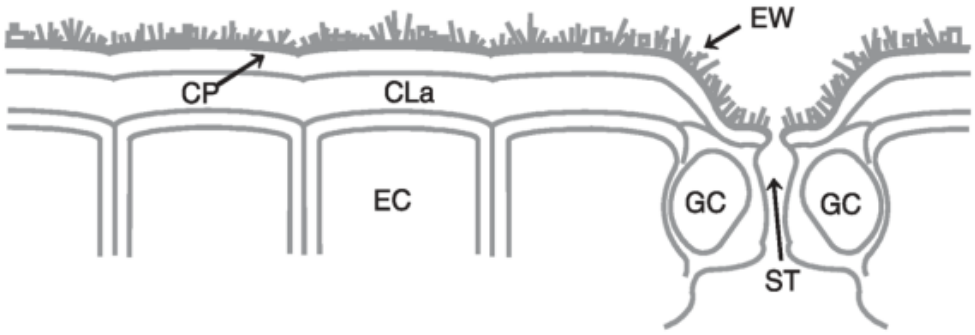

Figure 2: Schematic cross section of a plant leaf showing: epicuticular waxes (EW), cuticle proper (CP), cuticular layer (CLa), stomata (ST), guard cells (GC) and epidermal cells (EC); (Shepherd and Griffiths 2006).

The epidermis also contains specialised pores called stomata, which can be present on both the adaxial (upper side) and abaxial (lower side) of the leaf and may contribute to foliar uptake as a separate pathway alternate to diffusion through the bulk cuticle. A leaf can be classified as hypostomatal (stomata on only one side) or amphistomatal (stomata on both leaf sides), while a leaf side that does not have stomata is referred to as astomatous. Stomata are responsible for gas exchange between the plant and the atmosphere and open and close in response to internal signalling of the plant in response to external factors. Around the stomatal pores are guard cells and subsidiary cells that help to regulate stomatal opening (Figure 2). Stomatal distribution varies markedly among species. Some plants have astomatous adaxial and stomatous abaxial leaves whilst others have stomata on both the adaxial and abaxial surfaces. The number of stomata, shape and general cuticle surface characteristics vary with plant species; therefore foliar uptake for one species is not indicative for all species. In particular, there has been little examination of foliar pathways of nutrient uptake for wheat (*Triticum aestivum)*, despite it being a staple food crop. This may in part be due to the difficulty of isolating the cuticular membrane from the underlying tissues (Jeffree 2006).

On some leaves there are also trichomes or hairs that differ in morphology (density, shape, height to width ratio and orientation) and function depending on the plant species. Although trichomes have not been conclusively shown as a separate foliar pathway, higher foliar absorption has been shown around the base of trichomes (Fernández and Eichert 2009). They

can also play a significant role in reducing leaf wettability and the retention of liquids on leaves (Brewer and Nunez 2007). Trichomes range from simple unicellular structures to more complex multi-cellular structures and glandular trichomes which secrete chemicals (Wagner et al. 2004). There can be multiple types of trichomes with different functions present on one species. For example, maize leaves produce prickle hairs, macrohairs (much longer trichomes) and bicellular microhairs (Martin and Glover 2007). The function of simple trichomes include reduced predation from both insects and herbivores (due to leaf roughness), temperature regulation (due to altering the microclimate around stomata), increased light reflectance and subsequent reduction in water loss and a reduction in leaf wettability due to the roughness of the surface (Gutschick 1999; Wagner et al. 2004). Glandular trichomes are more complex and can have the same function as simple trichomes with the added effect of secreting substances which can immobilise insects, be toxic to fungi and bacteria, or attract pollinators depending on the chemical secreted. Wheat leaves of some cultivars have been shown to have small simple trichomes (prickle hairs) on the leaves composed of silicon (Tripathi et al. 2012) particularly on drought tolerant cultivars (Doroshkov et al. 2011).

Function of the cuticle in nutrient uptake

The main function of the cuticle is to minimise water loss and effectively keep the plant internal tissues separate from the external environment. This means minimising the loss of water and nutrients from within plant parts to the outer environment, but also minimising the uptake of chemicals and compounds through leaves.

In order for foliar fertilisers to be taken up by plants, they must be able to adhere to the leaf surface before being able to penetrate the cuticle and enter the leaf cytoplasm. Foliar uptake may occur through a number of pathways including through the cuticle, *via* stomata, trichomes or other specialised epidermal cells or through cracks or imperfections in the cuticle surface (Tukey et al. 1961) (Figure 3). The importance of these different pathways for nutrient uptake is debated, but generally only stomatal and cuticular pathways are studied. Depending on the purpose of the investigation, studies may be conducted on intact plants, detached leaves or leaf discs, epidermal strips or isolated cuticular membranes (see the section Methods to study foliar P effectiveness). Although we are interested in total leaf uptake, to gain a better understanding of the mechanisms of uptake, studies generally try to identify the individual pathways separately from each other.

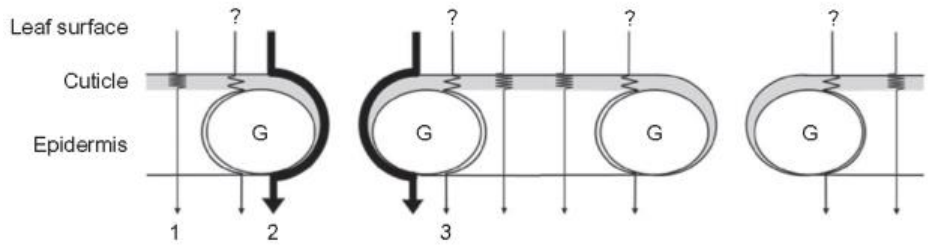

Figure 3: Schematic diagram of the possible pathways for foliar fertilisers through the leaf surface: 1. penetration through the cuticle, 2. penetration of the stomatal pore, 3. penetration of the peristomatal cuticle as discussed in the section "stomatal pathway". G: guard cell (Eichert and Fernández 2012).

Cuticular pathway

Despite the phrase 'foliar uptake' being used throughout the literature, the penetration of solutes through the cuticle is a diffusive process driven by concentration gradients rather than an active uptake process. It is not until the nutrients (in our case P) reach the internal cells that the process becomes an active one. Due to the lipophilic nature of the membrane, the uptake of polar or hydrophilic compounds is much lower than the uptake of lipophilic compounds (Schönherr 2006). However, it has been suggested that hydrophilic uptake is still greater than would be expected for a simple diffusive process to be the only mechanism involved. This has led to the hypothesis that a physically distinctive pathway is present, termed 'polar pores' or 'aqueous pores' (Schönherr 1976a; Schönherr 2000). Schönherr (2000) suggested that these pores are generated by hydration of the cuticle where water molecules are adsorbed to polar moieties within the cuticular membrane. This causes swelling within the cuticle that then allows the formation of a distinct pathway available for polar solutes. However, evidence for this is indirect as the polar pores are estimated to have an average pore radius between 0.3 and 0.5 nm (Schönherr 2006), which is smaller than can be visualised by microscopy (Koch and Ensikat 2008).

To remove the influence of stomata on uptake when examining the cuticular pathway, studies generally use astomatous cuticular membranes. This involves chemically or enzymatically isolating the cuticular membrane from the underlying cell wall. Numerous studies have used isolated membranes (Karbulková et al. 2008; Kirsch et al. 1997; Schönherr 1976b; Schonherr and Schreiber 2004); however, there is debate over whether the isolation procedure alters the structure and/or permeability of the cuticle. For example, Schonherr and Schreiber (2004) found that only 30-40 % of isolated cuticular membranes passed a leak and imperfection test before use. In contrast, Kirsch et al. (1997) found that enzymatic isolation did not alter the permeability of the cuticle, with permeability of intact leaves and isolated cuticles of three tree species not being significantly different to each other. Despite the debate over the validity of the technique, the process is a non-destructive method and allows the same isolated membrane to be used multiple times. It also allows direct quantification of

penetration rates across the cuticle through recovery of a solute applied to the outer side of the membrane in a receiver solution placed on the inner side of the membrane. Unfortunately it is not a technique that can be used for all plant species, especially those containing stomata or trichomes. In particular, there has been difficulty in isolating the cuticular membrane from the underlying cell wall of grass species, including wheat (Jeffree 2006), without causing significant damage to the membrane. Although isolated membrane studies are useful to examine the mechanisms of cuticle penetration whilst controlling humidity and concentration factors, they disregard the impact stomata can have on foliar uptake. Studies on astomatous cuticular membranes therefore have less relevance to plants with stomatous cuticles such as wheat.

Since there is a high variability in leaf uptake rates between species, and most studies focus on isolating one pathway of uptake, it is difficult to quantify the importance of the cuticular pathway compared to other pathways. The importance of this pathway will also change according to the nature of the solute in question. The cuticular pathway is the main pathway for the penetration of lipophilic compounds, but its importance to hydrophilic compounds is unclear. However, Riederer and Schreiber (2001) suggest that most of the water diffuses through the lipophilic cuticular pathway as individual molecules whilst only a minor fraction penetrates through polar pores.

Stomatal pathway

The role of stomata in uptake of hydrophilic substances has been a matter of debate. This pathway was originally disregarded due to the work of Schönherr and Bukovac (1972) who found that spontaneous infiltration of stomata by water (mass flow) could not occur due to the architecture of the stomatal pore. The critical surface tension of the *Zebrina* leaf surface used in their study was 25-30 dyne cm^{-1} which meant any liquid with a surface tension higher than this (i.e. water which has a surface tension of 72.6 dyne cm^{-1}) would not be able to enter the substomatal cavity spontaneously unless an external pressure was applied. Despite this, many studies have found higher penetration of hydrophilic solutes through leaves (in the absence of surfactants which decrease the surface tension of the solute) both in the presence of stomata and in response to stomatal opening (Eichert and Burkhardt 2001; Eichert and Goldbach 2008; Eichert et al. 1998; Eichert et al. 2008). Although foliar uptake has been correlated with stomatal density, not all stomata on a leaf contribute to foliar uptake (i.e. are penetrable) (Eichert and Burkhardt 2001). Unsurprisingly, diurnal variation has been reported for foliar uptake rates of iron chelates into stomatous leaves (Schlegel et al. 2006). However, Schönherr and Bukovac (1978) attributed increased penetration of solutes around stomata to be a result of increased permeability of the cuticle over guard cells of open stomata, compared to the

cuticle over the bulk leaf surface. Another proposed mechanism is that penetration of stomata by solutes occurs *via* diffusion along the pore surfaces without infiltrating the pore itself (Eichert et al. 2008). Regardless of the exact mechanism, qualitative rather than quantitative measurements of stomatal uptake are generally employed by use of imaging methods. This is due to the difficulty in isolating total uptake between stomatal and cuticular pathways. Such imaging methods include radiolabelled or fluorescent tracers, or the use of metal salt precipitates. One such method used to study the stomatal pathway is the use of uranine, a fluorescent anionic dye. Eichert et al. (1998) used uranine to trace uptake on the abaxial side of leek epidermal strips. They found that when stomata were open, uranine uptake over a 24 hour period was 30 times higher than when stomata were closed and the highest concentration of the dye was seen in the centre of the stomatal pore. Although the dye uptake was localised to stomatal pores, only a small proportion of stomatal pores appear to be penetrated by uranine (Eichert and Burkhardt 2001).

Despite most of the studies examining the stomatal pathway of uptake being qualitative studies, quantitative measurements have been attempted. Generally this is done by attempting to block either the cuticular or stomatal pathway; however this can be difficult to do. Sargent and Blackman (1962) assumed that forced closure of stomata through darkness ensured stomata were no longer able to contribute to foliar uptake. However, Eichert et al. (2008) used fluorescent polystyrene particles in suspension to show that even at small apertures, i.e. below 0.5 µm where stomata are considered closed (Fernández and Eichert 2009), solutes could still penetrate.

Ultimately, the influence of the stomatal pathway is of importance if the species being investigated has a high stomatal density. A species with higher stomatal density will therefore be more reliant on the stomatal pathway as it is likely to contribute more to overall foliar uptake. Whether the stomata will be the main pathway is unclear, however Karbulková et al. (2008) have reported for two different plant species that the permeability of stomatous cuticular membranes is significantly higher than the permeability of astomatous cuticular membranes. This pathway will also be of importance if there are differences in the leaf physiology between the adaxial and abaxial leaf sides causing heterogeneous total uptake of nutrient by the leaf. External factors will have more influence on the stomatal pathway than the cuticular pathway. For example, water stress, low light, high temperature and lower humidity will all result in stomatal closure and decreased stomatal uptake in response to decreasing transpiration and water loss from leaves.

The efficacy of total foliar uptake and the particular pathways through the leaf is ultimately governed by factors relating to the plant surface itself, the properties of the formulation being

used and the impact of the environmental conditions on both the plant and the formulation. The efficacy of foliar application can further be divided into a few main processes that will determine the proportion of a spray that reaches the leaf surface (deposition), adheres to the leaf (retention), penetrates through the outer cuticle (uptake) and is transported within the plant (translocation) to provide a beneficial effect on either growth or plant health. The following section discusses these four important processes.

Processes governing the uptake and translocation of foliar-applied P within the target plant

As mentioned in the Introduction, there are four main processes that determine the uptake and movement of foliar P within the target plant, namely deposition, retention, uptake and translocation. These four processes govern the effect of the foliar-applied spray on the P nutrition of the target plant.

Deposition

Deposition can be defined as the physical amount of the fertiliser or active ingredient in a spray application (kg ha^{-1}) that reaches the plant surface. The rate of spray that is applied in the field is always going to be higher than the amount that reaches the leaf surface. This is due to both formulation factors and plant factors. In particular, the formulation factors involved relate to many operational parameters e.g. spray release height, carrier volume, type of nozzle used, spray pressure and droplet size/spectrum, which in turn will also affect the amount of spray drift. The variability in deposition as a result of changes in these factors is large (see Table 1 in the Introduction section) and as a result only the influence of droplet size is covered in this literature review (see the Droplet size section). For more detailed discussion on the influence of operational spray conditions/factors, see the following reviews (Hilz and Vermeer 2013; Knoche 1994; Lake 1977; Spanoghe et al. 2007; Spillman 1984). The influence of plant factors relates to both the crop architecture and the orientation of leaves in the canopy. These factors are due to the actual surface area available for spray interception as discussed (see the Interception of foliar P section).

In research experiments, one way to limit the variability that is inherent in the deposition process is to use a targeted application of foliar fertilisers. This can be through application of the fertiliser as drops, painting the fertiliser onto the leaf, or dipping the leaf into the fertiliser solution. These methods are often used in association with radioactive tracers allowing a full mass balance measurement of absorbed nutrient, nutrient runoff and surface-adhered nutrient.

Retention

The process of retention of foliar fertilisers on leaf surfaces is first governed by the adhesion of the liquid to the leaf surface. Retention is then the overall amount of fertiliser that is captured on the plant by either initial or subsequent contact of the drops with the surface i.e. drops that will initially adhere to the leaf or drops that are initially reflected (bounce) and are then subsequently caught by lower foliage (Zabkiewicz 2000). The initial adhesion of fertilisers is related to the properties of the foliar fertiliser (in particular the surface tension of the solution (see the Adjuvants section), the size and velocity of the drops (see the Droplet size section) and the wettability of the leaves.

Wettability

The presence of hydrophobic waxes can make leaves inherently difficult to wet. It is not only the presence of waxes but also the composition and surface roughness, often at multiple scales, which interact to determine the wettability of a leaf (Koch et al. 2008). The degree of wetting can vary remarkably between plant species; from superhydrophilic leaves like the rainforest species *Calathea zebrina* (Koch et al. 2008) to the superhydrophobic Lotus leaf *Nelumbo nucifera* (Barthlott and Neinhuis 1997). Wheat leaves have often been classified as hydrophobic due to the presence of crystalline epicuticular waxes (Holloway 1969; Koch et al. 2006a; Netting and von Wettstein-Knowles 1973), but the presence of trichomes may also play a role by increasing the surface roughness of the leaves (Brewer et al. 1991).

The main method for quantifying the wettability of leaves is through measuring the contact angle (CA, α°) of water on the leaf surface (Koch et al. 2008). The contact angle measurement allows classification of the surface into two broad categories, hydrophilic (CA less than 90°) or hydrophobic (CA more than 90°). A recent study by Fernández et al. (2011) investigated the pubescent surface of peach fruit and characterised the effect of waxes and trichomes on wettability. This appears to be one of the first plant studies to measure the static CA of three liquids; water, glycerol and diidomethane, to calculate the free energy, polarity and work of adhesion of the leaf surface. Fernández et al. (2011) found that the mechanical removal of trichomes from the peach surface decreased the surface energy through increasing the contact between the deposited liquids and polar groups on the peach surface. Since wheat leaves are known to have trichomes on at least the adaxial leaf side, the characterisation of the polarity of the leaf surface is important.

In addition to the CA, a measurement of CA hysteresis allows further classification refinement for superhydrophobic and superhydrophilic states (Figure 4). The CA hysteresis represents the range of CA a drop will have on the surface and is the difference between the advancing and receding CA. It is the CA hysteresis which controls whether a drop is likely to

roll off the surface (Taylor 2011). As such, superhydrophobic surfaces have a high CA (more than 150°) with low CA hysteresis. A superhydrophilic surface will result in complete wetting and a resulting CA of less than 10°. However, most studies on the wettability of wheat leaves (Holloway 1969; Netting and von Wettstein-Knowles 1973) have only measured the static CA (which represents a CA somewhere between the receding and advancing CA) with no indication of CA hysteresis. As a result, some vital information is missing on the wettability of wheat leaves.

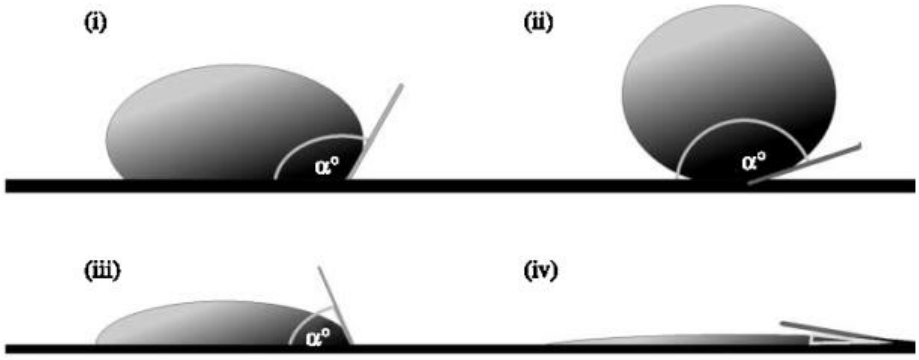

Figure 4: Representation of the four classes of surface wettability (i) hydrophobic (90° < CA < 150°), (ii) superhydrophobic (CA > 150°), (iii) hydrophilic (10° < CA < 90°) and (iv) superhydrophilic or complete wetting (CA < 10°) (Koch and Barthlott 2009).

Uptake

The uptake process is critical for the foliar spray to be effective. This is the process of the nutrient or active ingredient in the spray passing through the cuticle by either the cuticular or stomatal pathway and reaching the internal cells of the plant. It is therefore subject to all the factors which will be discussed later (see the section Factors affecting foliar efficacy). Plant factors include the presence of waxes, stomata and trichomes which may slow or hinder foliar uptake and their relative abundances, which differ with growth stage, leaf side, cultivar and nutrient status of the plant. Formulation factors are particularly important as they are often optimised to ensure that uptake by the plant occurs. These relate mainly to the influence of adjuvants on the penetration of the cuticle through droplet spreading and hence drying time of the droplet, and wetting of the leaf to ensure good contact between the solution and the leaf. Environmental factors include the presence of rain, which may wash the foliar spray off the leaf, and relative humidity, temperature and wind, which will increase the rate of droplet drying (Zabkiewicz 2000).

Translocation

The last process of translocation occurs once the nutrient is taken up by the plant and is the process of it moving away from the site of absorption to other plant parts. This process is therefore an active one which relates to the mobility of the nutrient within the plant and

whether it is transported in the xylem or phloem. Transport of foliar-applied P is thought to occur mainly in the phloem of plants (Biddulph and Markle 1944; Biddulph 1956; Bukovac and Wittwer 1957) and as mentioned earlier (see the section Plant demand for phosphorus), is efficiently translocated in wheat plants. In a dual labelling study with ^{14}C and ^{32}P it was shown that the movement and demand for carbohydrate mainly determined the movement of P, rather than P translocation being determined by the strength of the P sink (Marshall and Wardlaw 1973). However Martin (1982) showed that when the phloem is interrupted by steam girdling the stem, retranslocation of P, N and magnesium (Mg) but not potassium (K) from the leaves to the grain still occurred *via* the xylem. The sinks for P will be different depending on the growth stage of the plant - either the growing plant parts (roots, tillers, new leaves) early in the plant's growth cycle or the grain as the plant approaches maturity.

Translocation is related to the growth stage of the plant and whether the site of absorption is a source or sink for that nutrient. This is visually represented in the autoradiographs taken by Koontz and Biddulph (1957) of bean plant leaves of different ages (Figure 5). When ^{32}P was applied to a mature leaf (Figure 5a) it was readily translocated around the plant to other leaves and the roots, whereas when it was applied to an immature leaf (Figure 5d) there was no translocation out of the treated leaf.

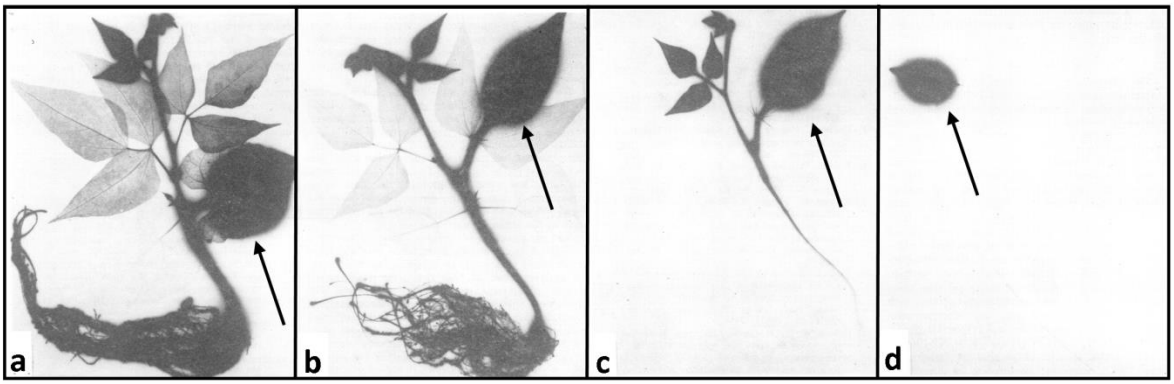

Figure 5: Translocation of ^{32}P from foliar-applied fertiliser when applied to the leaves (treated leaves indicated by arrows) of Bean plants of different ages. a: mature unifoliate leaf, b: mature terminal leaf of the first trifoliate leaf, c: young terminal leaf of the second trifoliate leaf, d: immature terminal leaf of the third trifoliate leaf; the darker the image, the more ^{32}P translocated (adapted from Koontz and Biddulph (1957).

Factors affecting foliar efficacy

Armed with an understanding of the key processes that are required for a foliar-applied spray to influence the P nutrition of the target plant, there are three broad categories of factors that will determine the efficacy of foliar applications: plant factors, formulation factors and environmental factors. These three categories all interact within plant studies to influence the effectiveness of foliar application and can cause difficulty in interpreting results and

comparing between different studies when multiple factors are uncontrolled within the same study.

Plant factors

The efficacy of foliar applications can vary as a result of a number of different plant factors by affecting the leaf wettability (retention), uptake and translocation of the fertiliser in the leaf. These plant factors relate to the surface morphology and structure of the leaves and include the age of the leaf, how the leaf surface changes over time, which side of the leaf is exposed to the foliar spray, the nutrient status of the leaf and even differences in the leaf surface between both species and cultivars. One reason that it is difficult to compare studies is due to different responses to foliar P from different plant species. For instance, Barel and Black (1979a) found that for a number of different P compounds, soybeans could only tolerate 66-75% of the P concentration that corn could without causing significant leaf damage.

Changes in leaf properties with age

The effect of optimal timing of a foliar spray is not only related to the P needs of the crop throughout the growing season, but also the ability of leaves of different ages to take up foliar-applied chemicals. Generally, young partially expanded leaves are considered more permeable to foliar application than older leaves (Sargent and Blackman 1962; Thorne 1958); however the physical damage that occurs with age *via* insect interaction and particle abrasion can reduce the barrier function of leaves rendering them more permeable. On the other hand, a reduction in stomatal opening with age may decrease the opportunity for solute uptake *via* the stomatal pathway (Fernández et al. 2008).

The growth stage of crops will also be of importance in the timing of foliar applications. Arif et al. (2006) investigated the difference in yield between spraying a mixed nutrient solution at tillering, tillering and jointing (two sprays) or tillering, jointing and booting (three sprays) for wheat. They found that maximum grain yield was achieved using two or three foliar sprays. Whether this was due to the multiple sprays used or the timing of sprays is unclear. Mosali et al. (2006) compared a foliar spray of 2 kg P ha^{-1} at three different growth stages; second node of stem formed (Zadoks 32, (Zadoks et al. 1974)), heads emerging (Zadoks 50) and anthesis (Zadoks 65) and found the best wheat yield response to foliar P was when it was applied during anthesis (Zadoks 65). It appears that for cereal crops, the best timing for foliar application of P to increase yield is from canopy closure to anthesis (Benbella and Paulsen 1998; Girma et al. 2007; Mosali et al. 2006).

Another study examined the wettability of soybean leaves through contact angle measurements of water on different leaves on the plant and plants at different growth stages. Puente and Baur (2011) found that wettability of adaxial surfaces of soybean leaves was greater near the top of the plant than for leaves near the base and with increasing development stage from the first leaf unfolded to tillering/four side shoots visible. Although studies have examined the differences in foliar uptake or wettability of leaves with growth stage or leaf age, what is missing is the link between this and the leaf physiological properties. There is also a lack of information on the relative importance of the different leaves to the uptake of nutrients. For a plant at any point in time, there will be leaves of different ages, which may differ in their wettability (Ellis et al. 2004). As the plant matures through its growth stages, there is also the influence of deposition relating to canopy cover and leaf area. Ellis et al. (2004) attributed a decrease in variability of retention of various liquids in mature outdoor wheat plants compared to younger indoor plants to three main factors: the water-repellence of leaves decreased with age, there was more damage to the leaf surface of outdoor plants than indoor ones, and as the plants grew, the increase in canopy density reduced spray lost to the ground. So, although younger leaves may be more permeable to foliar sprays at early growth stages, the effective leaf area for spray retention is much smaller and therefore the foliar fertiliser efficiency is likely reduced.

Adaxial and abaxial surfaces

In many plant species, the adaxial (upper) side of the leaf has fewer stomata than the abaxial (lower) side of the leaf. In fact, many studies that have investigated differences between adaxial and abaxial surfaces have been on hypostomatous leaves (Karbulková et al. 2008; Schlegel et al. 2006; Will et al. 2012). A review by Wójcik (2004) reported it is generally accepted that the abaxial side of leaves has more rapid nutrient uptake than the adaxial side. However, Cook and Boynton (1952) suggest that the rate of uptake is different due to different uptake mechanisms between the sides. Although the abaxial leaf sides of McIntosh apples had a significantly higher uptake of urea in a two hour period (59.6 to 12.5 %), when the absorption period was extended to 72 hours, the difference in total uptake had reduced (84.5 to 49 %). This was due to a steady rate of uptake from the adaxial leaf side over the absorption period.

Higher abaxial uptake rates have been attributed to the higher number of stomata on the abaxial leaf side and a thinner cuticular membrane layer (Hull 1970). For wheat this stomatal trend does not seem to occur. Teare et al. (1971) found that the stomatal frequency of a selection of species and cultivars from the *Triticum* genus was always highest on the adaxial surfaces of leaves. They also found that there were differences in stomatal frequency with the

age or position of the leaf on the plant and the stomatal frequency decreased from the leaf base towards the tip of individual leaves. It remains to be seen whether the lower stomatal frequency on the abaxial side of wheat leaves will decrease the nutrient uptake compared to the adaxial side, or whether there are other factors that will also influence nutrient uptake. However, a recent study by Will et al. (2012) found that the abaxial side of both soybean (amphistomatal species) and lychee (hypostomatal species) had higher boron (B) uptake and translocation than the adaxial side. Likewise Thorne (1958) found that there was higher ^{32}P uptake from the abaxial side of swede leaves than the adaxial side; however no information was given on differences in stomatal density or epicuticular waxes between the different sides.

There are also likely to be differences in the amount, morphology and composition of waxes between leaf sides. These differences will affect leaf wettability and ultimately foliar penetration rates of adaxial and abaxial leaf surfaces. For example. Jetter et al. (2000) found abaxial leaf surfaces of *Prunus laurocerasus* yielded much more cuticular wax than the adaxial surface, and there were differences in wax composition, particularly the higher relative amount of alkanes in waxes from the abaxial surface.

Cultivar effects

Differences in leaf characteristics between cultivars can include the presence or absence of trichomes, differences in stomatal density and aperture, and differences in both composition and abundance of waxes. One possible reason for there not being many descriptive studies on leaf physical traits of wheat is due to the variability that occurs between cultivars. These leaf traits relate to the morphology of the leaves which in turn affects the wettability and subsequent uptake and translocation of foliar-applied fertilisers and crop chemicals. Despite there being differences in leaf morphology between wheat cultivars, foliar uptake studies only occasionally compare cultivars within the same study and sometimes different cultivars are used at different locations. For example, Ahmed et al. (2006) studied the growth and yield response of two wheat cultivars (Sids-1 and Sakha-69) to application of a multi-nutrient foliar fertiliser and found differences in growth and yield responses to the foliar fertiliser between the cultivars – however, because they provided no information regarding the morphology of the cultivars it is difficult to ascertain if this was related to the morphology or due to other factors. In a foliar N study, differences in dry matter and N accumulation between wheat cultivars was attributed to the N demand of the plant and differences in the genetic ability of the cultivar to accumulate N in the grain (Sarandon and Gianibelli 1992). However, as this study did not use a tracer (which would allow partitioning of the uptake and translocation

between foliar and soil sources) it is not certain whether the foliar uptake and translocation of N was higher from one cultivar compared to another.

Another trait which differs between wheat cultivars relates to the waxiness of the leaves and classification of glaucousness. Glaucousness is the physical manifestation of epicuticular waxes that gives rise to the bluish-white colour of some leaves (Richards et al. 1986). It is a characteristic that is beneficial in drought-affected areas due to its positive correlation with water-use efficiency and yield (Richards et al. 1986). It is also correlated with higher surface reflectance which may reduce tissue temperature (Johnson et al. 1983). The structure and abundance of epicuticular waxes of glaucous cultivars is different to non-glaucous cultivars (Johnson et al. 1983). Barber and Netting (1968) found that glaucous lines of wheat had significant amounts of β-diketones in the wax whilst non-glaucous lines had only a trace. Since different wax composition may change the permeability of the leaf, the degree of variation in wheat leaf permeability of cultivars should be further investigated, especially in reference to the glaucous characteristic.

Nutrient status of the plant

Recent work has examined the effect of plant nutrient status on foliar uptake of the same nutrient. It was found that B-deficient soybean plants had reduced uptake of foliar B due to irreversible structural and morphological changes to the leaf surface (Will et al. 2011). Likewise, Fernández et al. (2008) suggested that changes in leaf physiological and structural properties in response to iron (Fe) deficiency may alter the barrier properties (the topography of the leaf surface, amount of soluble cuticular lipids, weight of the abaxial cuticle, size of the guard cells etc.) and therefore solute permeability of peach and pear leaves. This is of importance for foliar nutrition as applications are often made in response to nutrient deficiencies of the plant. In the case of foliar P applications, it is likely that such applications would be made to plants having a marginal P status rather than a severe deficiency, due to the practical limitations of the number of sprays (and hence amount of P) that can be applied and the limitation of leaf salt load (scorch) at high doses of P. There has been little work on the influence of P status on foliar P uptake. However, Clarkson and Scattergood (1982) found that P-stressed barley leaves were more responsive to foliar applications of P than control plants. In this study barley seedlings were grown in nutrient solution before transferring some plants to a P free solution for seven days before foliar application of ^{32}P-labelled KH_2PO_4. Twice as much foliar-applied P was absorbed by mature barley leaves compared to the control. This finding for P foliar uptake contradicts the studies on reduced B and Fe foliar uptake when plants were deficient in those nutrients.

Formulation factors

There are a number of formulation variables that will affect the uptake of foliar P fertilisers. These include the concentration of the nutrient, form of P compounds in the fertiliser (i.e. whether ionic or non-ionic), the pH of the solution and the presence of other compounds within the mixture.

Nutrient concentration

The relationship between the penetration rate of an applied solute and its concentration is not entirely clear (Fernández and Eichert 2009). For Fe chelates, increasing the concentration from 2.5 to 5 mM decreased the penetration rate of Fe (uptake as a % of applied h^{-1}) by a factor of 2.2 (Schlegel et al. 2006). Although increasing the concentration of P in the foliar solution increases the amount of nutrient penetration into the leaf, it is not a linear relationship. In an experiment using ^{32}P-labelled sodium phosphate, doubling the concentration of foliar-applied P only resulted in a small increase in the percentage of fertiliser absorbed (Thorne 1958). The concentrations previously used in isotopic tracer studies and studies investigating uptake pathways are usually low, often below 1 M (Fernández and Eichert 2009). This is in part due to many studies being for micronutrients which are needed by plants in lower concentrations than macronutrients; however studies with foliar P have also used low concentrations. For example Koontz and Biddulph (1957) applied sodium phosphate at concentrations of 0.3-10 mM NaH_2PO_4 and Bouma (1969) applied phosphoric acid at concentrations of 10-30 mM H_3PO_4. For macronutrients including N and P, the high plant requirements of the nutrients have resulted in high concentrations tested in field studies with positive yields generally occurring for rates between 1.5 and 4 kg P ha^{-1} (Noack et al. 2011). If this is dissolved in 100 L ha^{-1} of water, then the P concentrations will be 0.5 to 1.3 M. Dilution volumes used in past studies have ranged from 25 L ha^{-1} (Arif et al. 2006) to 750 L ha^{-1} (Garcia and Hanway 1976). However, it is not always easy to compare concentrations used in previous studies as field application rates are expressed as kg P ha^{-1} and in some cases the dissolved volume of the spray is not stated (Harder et al. 1982a; b; Mosali et al. 2006).

Due to the much higher concentrations being used in field studies compared to isotopic tracer and pathway studies, it is not surprising that one of the main barriers to foliar P application is the concentration that can be applied without causing scorch or leaf burn. Higher P rates lead to a higher nutrient and salt loading, which can lead to leaf damage ranging from necrotic spots to complete defoliation (Eichert and Fernández 2012). Many studies have observed leaf burn after the application of foliar P droplets or sprays with some

studies having such severe leaf burn that there is a decrease in yield due to a reduction in photosynthesis when the leaves die (Barel and Black 1979b; Koontz and Biddulph 1957).

Form of P, associated cations and formulation pH

Different P compounds have been investigated for foliar application with varying degrees of success. Phosphorus can be applied in the orthophosphate form (PO_4^{3-}, $H_2PO_4^-$, HPO_4^{2-}) or as a poly phosphate (pyrophosphate, tripolyphosphate or more complex phosphates). The ionisation of orthophosphate depends on the pH of the solution as phosphate has 3 distinct pKa values (Figure 6). At each pKa value, ionic species will be in equilibrium with the proportion of the two species present at that pH being 1:1 (i.e. at a pH of 2.2 50 % of the species will be present as H_3PO_4 and 50 % as the dissociated $H_2PO_4^-$. Below a pH of 2.2, phosphoric acid will (H_3PO_4) dominate, between a pH of 2.2 and 7.2 $H_2PO_4^-$ will dominate, between a pH of 7.2 and 12.3 HPO_4^{2-} will dominate and above a pH of 12.3 the phosphate will be completely dissociated (PO_4^{3-}). The degree of dissociation may play a role in the ability of P to penetrate the cuticle and therefore influence the best formulation pH for foliar P absorption.

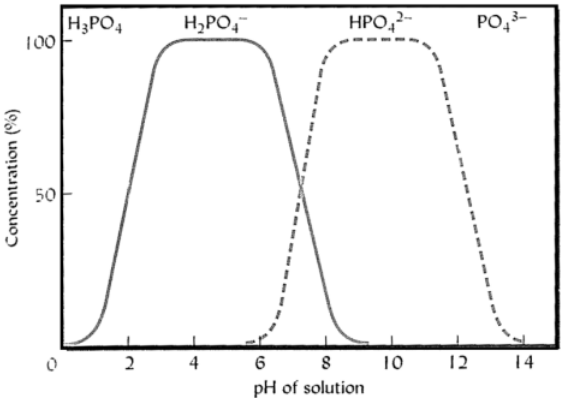

Figure 6: Dissociation of phosphoric acid as a function of pH (Brady and Weil 2002).

Despite orthophosphate being the plant-available form of P, it appears that polyphosphate forms can also penetrate the leaf surface (Barel and Black 1979b). Barel and Black (1979a) investigated a number of different P compounds and found that the concentration of tri- and tetra-polyphosphates that could be applied to corn and soybeans was two and a half to three times higher than the concentration that could be applied as orthophosphate without substantial leaf burn. Further studies in the greenhouse and in the field to assess the plant growth response found that with the exception of tripolyphosphate (which caused severe leaf burn), all the P compounds tested increasing the yield of soybeans over the control (Barel and Black 1979b). However, when phosphoric acid and ammonium polyphosphate (APP) were tested at the same P concentration in the glasshouse at field applicable rates, orthophosphate (as phosphoric acid) significantly increased wheat yield whereas APP did not (McBeath et al.

2011). In this study, yield increases for wheat with foliar phosphoric acid plus an adjuvant occurred in one of two soils used, increasing the grain yield by 25 % compared to the control.

The accompanying cation to orthophosphate affects the uptake of P from the foliar fertiliser. Koontz and Biddulph (1957) ran a number of experiments altering the concentration of P in applied drops and investigating the influence of pH and cation on the absorption and translocation of foliar-applied P. For solutions applied at a concentration of 10 mM P, the H_3PO_4 solution translocated only 4.7 % of the applied P (with significant leaf burn) compared to 6 % for both ammonium and diammonium phosphate. In comparison, there were differences in the translocation of both sodium phosphates and potassium phosphates depending on the pH of the solution (Figure 7). Overall Koontz and Biddulph (1957) found that the foliar translocation of P in Red kidney bean leaves decreased in the following order: sodium phosphate (NaH_2PO_4) > dipotassium phosphate (K_2HPO_4) > tripotassium phosphate (K_3PO_4) = disodium phosphate (Na_2HPO_4) = monoammonium phosphate ($NH_4H_2PO_4$) = diammonium phosphate ($(NH_4)_2HPO_4$) > phosphoric acid (H_3PO_4) (which caused leaf scorch) > monopotassium phosphate (KH_2PO_4) = trisodium phosphate (Na_3PO_4).

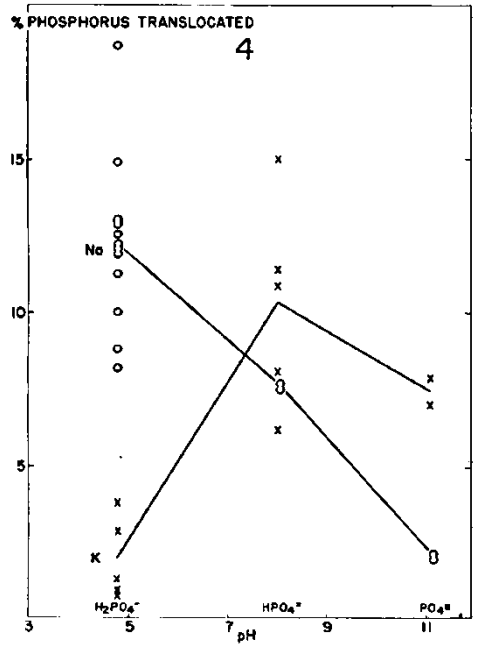

Figure 7: Percentage of applied foliar P translocated out of the treated leaf from red kidney bean plants within 24 hours after spray application of 0.2ml of ^{32}P-labelled sodium (o) and potassium (x) phosphates. Individual replicates are displayed with the average represented by lines (Koontz and Biddulph 1957).

It is not always easy to distinguish between the effect of pH and the effect of associated cation with some studies not even attempting to separate them (Koontz and Biddulph 1957). The results from Koontz and Biddulph (1957) suggest that it is not a simple process of lower pH resulting in higher uptake and translocation, given that at a pH of 5, NaH_2PO_4 had the highest translocation (about 12 % applied isotope movement out of the treated area) whilst

KH_2PO_4 had the lowest (about 2.5 %). A number of agronomic studies have also been conducted with mixed foliar nutrients, in particular NPK+ sulfur (S) foliar application rather than just a single P application (Ahmed et al. 2006; Alston 1979; Arif et al. 2006; Giskin and Efron 1986). This can cause difficultly in interpreting whether the response by the plant to the foliar spray is a result of one particular nutrient or the combination. Alston (1979) attributed the yield response of wheat to foliar application of NP to be the result of the N rather than the P in the spray.

As well as leaf burn from nutrient loading, a low solution pH may cause damage to the leaf surface. Plant cuticles are poly-electrolytes with isoelectric points around 3 (Schönherr and Huber 1977); therefore the pH of the foliar-applied fertiliser may affect the phytotoxicity of the treatment and the rate of both nutrient penetration and translocation away from the application site. Since it has been documented that a pH of 2-3 facilitates more rapid uptake of P (Bouma 1969; Tukey et al. 1961) there is a need to balance the pH for optimal uptake with the leaf burn to find an appropriate formulation pH. For example, Bouma (1969) found that adjusting a phosphoric acid solution with potassium hydroxide to achieve a pH of 5 (forming KH_2PO_4) compared to an original pH of 2.5 resulted in a reduction of foliar P uptake by subterranean clover plants after 24 hours (measured after washing the leaves) from 58 % to 40 % of what was applied. Reed and Tukey (1978) also showed that for K, sodium (Na), ammonium (NH_4^+) and calcium (Ca) phosphates applied at a concentration of 25 mM on chrysanthemum leaves, foliar absorption was highest at a pH of 2 (pH adjusted with HCl for each pH unit between 2 and 10 except for calcium phosphate, which was only from pH 2-5). It has been suggested by Wittwer and Teubner (1959) that the increased uptake of P at a low pH is a result of penetration of the cuticle by undissociated molecules of H_3PO_4, which at a pH of 2 would be the dominant form of P.

Adjuvants

Adjuvants comprise a wide range of chemicals that are added to foliar-applied solutions to increase the penetration of the leaf surface by the active ingredient or modify the spray characteristics (Hazen 2000). They can be divided into a number of categories depending on their function and mode of action. These include pH buffers, oils, humectants (which increase the retention time of solutes on the leaf) and surfactants. Surfactants (short for surface active agent) are one of the largest and most widely used groups (Wang and Liu 2007) and aid foliar uptake by lowering the surface tension of the solution, which decreases the CA on the leaf and increases the liquid spread and leaf coverage. Surfactants are compounds that have a hydrophilic head group and a hydrophobic tail. They can be non-ionic, anion, cationic or zwitterionic but for use in sprays, non-ionic surfactants are more widely used because they are

not sensitive to pH (Fernández and Eichert 2009). Some of the most common non-ionic surfactants are based on ethylene oxide (EO) as the hydrophilic component. The relative ratio between the hydrophilic and hydrophobic (or lipophilic) moieties of a surfactant determines its hydrophile/lipophile balance (HLB) (Hess and Foy 2000). The higher the HLB, the more water soluble the surfactant is and the higher its EO content (if the hydrophilic component contains ethylene oxide).

A large proportion of the published studies investigating adjuvants have been for use with pesticides and herbicides. However, the properties of pesticide and herbicide formulations are different to nutrients, in particular due to many pesticides and herbicides being lipophilic rather than hydrophilic. Unlike foliar fertiliser studies, studies on the use of adjuvants with pesticides and herbicides are common for wheat (Gaskin and Holloway 1992). A number of studies have compared the EO content of the surfactant and its effect on foliar uptake of herbicides, nutrients and the surfactants themselves (Holloway and Edgerton 1992; Liu 2003; 2004; Stock et al. 1992; Stock et al. 1993). Surfactants with high EO content enhance foliar uptake of hydrophilic compounds whilst surfactants of low EO content are preferred for lipophilic herbicides (Liu 2004). However, Stock et al. (1992) found that the uptake by wheat leaves of lower EO compounds was faster than high EO compounds. There were also differences in the effectiveness of surfactants between plant species. Stock et al. (1993) found that uptake enhancement from surfactant use was greatest for waxy wheat leaves compared to less waxy field bean leaves. In fact the use of adjuvants in general appears to be more crucial for hydrophobic leaves with larger increases in efficacy of foliar uptake for plants that are initially difficult to wet compared to those that are easy to wet (Knoche 1994; Taylor 2011).

Stein and Storey (1986) investigated the influence 46 adjuvants had on leaf burn and the uptake of P (as diammonium phosphate) and N (as urea and diammonium phosphate) in soybean leaves. The phytotoxicity of the adjuvants generally increased with increasing concentration; however, only glycerol (which acts as a humectant) was effective in increasing P and N uptake (by increasing the N and P concentration in the plant shoots compared to the control). Likewise, Fernández et al. (2006) investigated 80 Fe foliar fertiliser formulations based on combinations of different Fe-compounds, surfactants and adjuvants. Of these 80 formulations, 26 were considered appropriate for foliar sprays as assessed by leaf wetting, surface tension and negligible interactions between the components. From the studies investigating adjuvants, it is apparent that no single adjuvant will be suitable for increasing the spread of the chemical, delaying drying of the formulation and increasing the permeability of the cuticle for all formulation-plant combinations (Noack et al. 2011).

Droplet size

When foliar sprays are applied, drops are formed when liquid is atomised through a hydraulic nozzle (Hilz and Vermeer 2013). As a result, the atomisation process produces a droplet spectra which varies according to the type of spray nozzle (Figure 8), the operating pressure used and the properties of the spray solution (Spanoghe et al. 2007). The main spray solution characteristic of interest is the surface tension of the solution which is reduced by the addition of surfactants. Drop size in studies is normally described in one of two ways , either by the median droplet diameter or by the proportion of drops prone to drifting (classified as below 100 µm (Spanoghe et al. 2007) or 150-200 µm (Hewitt 2008)). Sprays produce a range of different droplet sizes rather than a uniform volume and these can range from less than 100 µm diameter to 1 mm, although 60-80 % of drops are usually below 0.5 mm in diameter (Hewitt 2008). The effect of adjuvants on drop size is complex both increasing and decreasing the median droplet diameter and proportion of driftable drops under different conditions (Spanoghe et al. 2007).

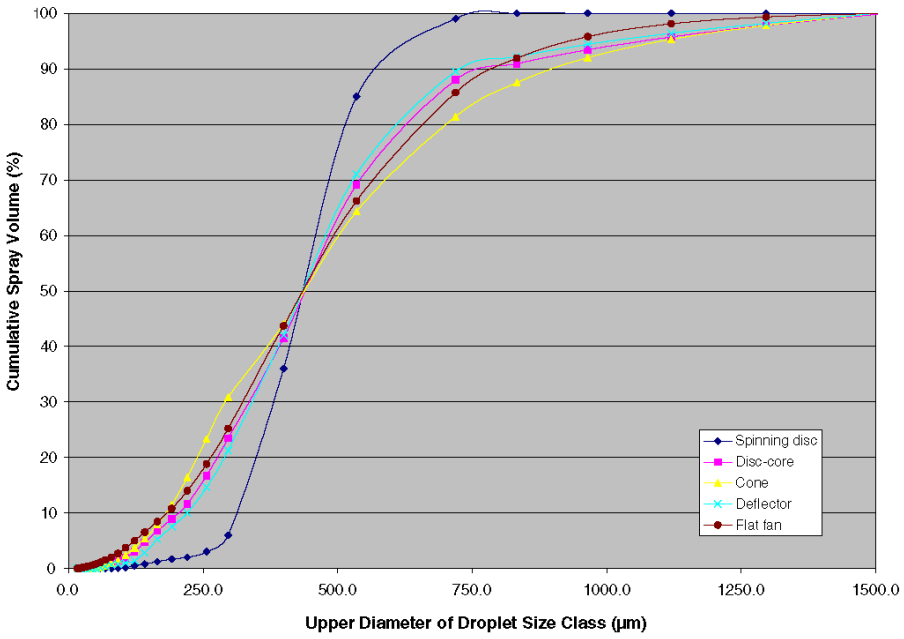

Figure 8: Cumulative volumetric droplet size for different nozzle types (Hewitt 2008).

Drop size is relevant to both the deposition and retention processes. This is because driftable (small) drops are unlikely to be deposited in the targeted area, particularly due to the influence wind has on their flight path. When there is no wind, droplet motion is only downwards; however, with increasing wind the trajectory of drops can approach horizontal (Spillman 1984). Very large drops may not be retained by plant leaves and therefore bounce or shatter on first impact with the leaf surface. In some cases the shattered or bouncing drops will be retained on second impact (Taylor 2011) or with leaves lower in the canopy, but if

drops are too large, they may be lost to the soil. For example, Lake (1977) found that only the smallest drops (100 µm diameter) applied to young barley and wild oats leaves were retained readily (50-90 % retention) with larger drops (200-600 µm diameter) retained less (generally less than 20 % retention).

Environmental Factors

The main environmental factors that can affect the efficacy of foliar application are temperature, relative humidity and light, which are all interrelated. These factors are difficult to control especially in field situations although with knowledge of the importance of these factors, recommendations can be made ensure optimal spraying conditions to maximise foliar uptake. To investigate the effect of these environmental factors, studies are usually performed under controlled conditions. In addition to these factors, the soil type and ability of the soil to provide nutrients to the plant can also be classified as environmental factors. These soil factors will influence the growth of the plant, particularly its nutrient status as discussed earlier (see the section Nutrient status of the plant).

Temperature, humidity and light

The long-term environmental conditions during plant growth can influence the leaf morphology and structure as well as the rate of physiological processes. This is often related to the composition and amount of waxes on the leaves as influenced by temperature and humidity (Koch et al. 2006b), although it may also influence the size of the plants and overall growth when also combined with the availability of water and nutrients. In the short-term, suboptimal conditions immediately prior, during and after foliar application can also affect the uptake process itself. Low humidity will decrease the hydration of the cuticle and may induce stomatal closure. Karbulková et al. (2008) investigated the effect of humidity on two plants of contrasting climates, a temperate climate (*Hedera helix*) and a subtropical climate (*Zamioculcas zamiifolia*). They found that the astomatous cuticles of both plants were more permeable to water when grown in humid air compared to dry air. The rate of foliar uptake is particularly limited in arid and semi-arid climates due to the combination of low humidity and high temperature.

Light stimulates stomatal opening and as a result will affect the stomatal pathway. Increased foliar uptake rates have been shown when application occurred during light compared to dark conditions (Sargent and Blackman 1962). For this reason, it is suggested that foliar spraying occurs early morning rather than at night. Increased temperature often increases chemical processes including diffusion which relates to the rate of foliar uptake.

Increased foliar uptake and translocation has been shown when temperatures were increased from less than 10° to 30° C although often declined again at higher temperatures (Swanson and Whitney 1953).

In addition to the direct effect of environmental conditions on the uptake process, they can also act on the foliar formulation itself. Higher temperatures result in more rapid drying of the solution and hence reduced time for uptake to occur. Higher temperature will also lower surface tension and viscosity of the solution but increase the solubility of salts which are all important parameters of the foliar formulation (Fernández and Eichert 2009). The effect of humidity on the formulation also influences the drying of the formulation and relates to the point of deliquescence (POD) of the salts in the solution.

Methods to study foliar P effectiveness

Throughout the literature on foliar-applied nutrient uptake, a wide range of different methods have been used to qualify and quantify the effectiveness of foliar applications. These range from detailed studies on the mechanisms of uptake through both stomatal and cuticular pathways, to field studies with agronomic measurements of yield and plant health. This section summarises some of the important methods available for studying the efficacy of foliar application.

Measuring uptake pathways

Identifying whether ionic uptake was possible and the pathways for preferential uptake was initially measured with ionic fluorescent dyes or metal precipitates. For both these methods the initial work involved visualisation of sites of preferential penetration of the leaf by the dye, or sites where the precipitation of insoluble metal salts accumulated. As discussed by Fernández and Eichert (2009), one of the earliest studies to use this technique was Strugger (1939) who used berberine sulphate (a cationic dye), which strongly fluoresces when it binds to the cell wall (this is only able to occur once the dye penetrates the outer cuticle). This study was able to visualise the accumulation of the dye in the cuticular ledges of guard cells, the anticlinal walls and at the base of trichomes. Since this initial study, other authors haves used an anionic fluorescent dye (Na-3-hydroxy-5,8,10-pyrenestrisulfonate) (Dybing and Currier 1959; Dybing and Currier 1961) , the anionic fluorescent dye uranine (Na_2 fluorescein) (Eichert and Burkhardt 2001; Eichert and Goldbach 2008; Eichert et al. 1998) or suspensions of fluorescent polystyrene particles that have had their surface carboxylate-modified (Eichert et al. 2008).

The visualisation of insoluble metal salt precipitates in cuticular ledges of guard cells provided further evidence of sites of preferential foliar uptake of polar and ionic solutes. The metal precipitation technique involves applying soluble silver nitrate ($AgNO_3$) to the outer side of a plant cuticular membrane and sodium chloride (NaCl) to the inner side. When the Ag^+ and Cl^- meet within the isolated cuticles they precipitate to form AgCl indicating the presence of polar pores or preferential areas of Ag^+ penetration (Schreiber et al. 2006). In a similar experiment, Schlegel et al. (2005) applied $AgNO_3$ to the surface of intact stomatous broad bean leaves and used the Cl^- within the native plant tissue to cause the precipitation reaction. Once again the precipitates were found at the base of trichomes, and in guard cells.

Techniques to visualise and identify the foliar pathways are sometimes conducted on intact plants but may also be conducted on detached or pieces of plant leaves to minimise the influences of plant growth factors and ensure a constant concentration over time (often by immersing the leaf section completely). These include:

- Isolated cuticular membranes – the astomatous cuticular membranes (CM) is either chemically or enzymatically detached from the underlying cell walls. This allows mechanistic studies to be carried out on the ability of solutes to diffuse through the cuticle. It allows control of concentration gradients and environmental conditions i.e. temperature and humidity. It also enables direct quantification of penetration rates without bias due to washing procedures (as necessary for intact plants) as the nutrient or molecule of interest is directly measured in the receiver solution on the internal side of the cuticular membrane. It also allows the same membrane to be used multiple times provided it is not damaged (Fernández and Eichert 2009).
- Isolated leaf disks – using a cork borer, sections of leaf are cut from the bulk leaf and the disks are put in a Petri dish with a moistened strip of filter paper. The chosen solute is applied to a small section of the leaf disk through a plastic tube (adhered to the leaf surface with petroleum jelly) to ensure a constant concentration of the solute (i.e. minimise increase in concentration due to drops drying) over the course of the experiment (Sargent and Blackman 1962).
- Epidermal strips – epidermal strips involve mechanically, often with forceps, stripping the epidermal layer of the leaf from the underlying mesophyll tissue (Fischer 1968). This is done after pre-cutting a small section (strip) of the leaf on three sides. Epidermal strips can then be used in a similar way to isolated CM in conjunction with an ion exchange membrane as explained by Eichert and Burkhardt (2001) (Figure 9).

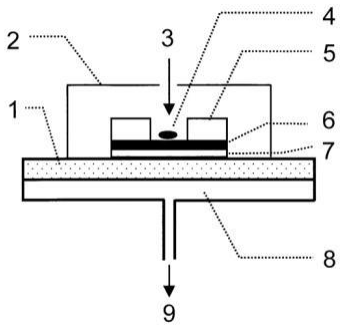

Figure 9: Model system for experiments with epidermal strips (ES). 1, ceramic plate; 2, plastic lid; 3, air inlet; 4, droplet of test solution; 5, PTFE ring with 8 mm hole; 6, ES; 7, ion exchange membrane (IEM); 8, water tank; 9, tube to water reservoir 80 cm below plate surface (Eichert and Burkhardt 2001).

Measuring uptake and translocation using isotopes

The two radioactive isotopes of phosphorus (^{32}P and ^{33}P) in addition to the naturally occurring isotope (^{31}P) allow for the study of foliar uptake directly using isotopic tracers. Early foliar P work with radioisotopes used autoradiographs to visualise the movement of foliar-applied P within the plant. Although this allowed comparison of how quickly the isotope labelled nutrient was able to be taken up by the plant and be translocated, it did not allow quantification of the amount. Use of ^{32}P in early work has been summarised and forms the basis for most studies on the influence of different plant and formulation factors for foliar P (Tukey et al. 1961; Tukey et al. 1956). Many of the early studies involved application of single or multiple ^{32}P labelled drops to the leaves of a variety of different plant species including red kidney bean (Koontz and Biddulph 1957), soybean (Barrier and Loomis 1957), clover (Bouma 1969) and chrysanthemum (Reed and Tukey 1978). These studies measured the effect of a range of different factors including formulation pH, adjuvants, temperature, and application method (spray vs. vein injection vs. drops). These studies provided valuable information on how some of the plant and environmental factors affected the initial rates of uptake and translocation. Since these studies were only conducted over a small length of time, generally hours to days, they did not provide any information on the long-term translocation of foliar P out of the treated area or the effect on yield. Since the early work, only one isotopic study has applied this technique with plants grown through to maturity (McBeath et al. 2011).

Measuring plant responses using agronomic techniques

For many studies on foliar application in the absence of using an isotope, the effectiveness of foliar application was measured by the plant nutritional and growth response. Initially this is due to an increase in concentration of the nutrient in the plant tissues but later on can be measured as the yield of harvestable plant parts. As leaves were rarely washed before analysis (at least in the case of field studies) in many studies it is not possible to distinguish between

foliar retention, uptake and translocation (Ahmed et al. 2006; Girma et al. 2007; Haq and Mallarino 1998; 2000; Leach and Hameleers 2001; Sherchand and Paulsen 1985). For a plant response to be measured in the absence of a tracer, control plants are required to compare an unfertilised plant with a fertilised plant (i.e. increase in concentration from the control to the fertilised plant). This difference method is not as reliable as a direct tracer due to variability in growth and nutrient concentrations within a plant species, independent of the nutritional effect.

Field based studies have been used to a large extent to investigate factors including nutrient formulations, rates, timing of application, and use of adjuvants to increase uptake for various crops under different climatic conditions and across different soil types. Although a substantial number of trials have been reported (see Table 2 and Noack et al. (2011)), a large number of variables have been investigated often with a combination of nutrients, which can make it difficult to attribute the agronomic response to foliar P alone and can make comparisons between studies very difficult. The majority of these studies were also conducted on soils with sufficient to optimum soil P status which meant foliar P application generally had little effect on yield (Noack et al. 2011). Only a small proportion of these agronomic studies have been on broadacre grain crops with a much larger proportion focussing on horticultural plants and fruits. The crop species that have been studied include soybean (Barel and Black 1979a; b; Garcia and Hanway 1976; Haq and Mallarino 1998; 2000; Mallarino et al. 2005; Mallarino et al. 2001; Syverud et al. 1980), corn (Barel and Black 1979b; Girma et al. 2007; Giskin and Efron 1986; Harder et al. 1982a; Leach and Hameleers 2001; Ling and Silberbush 2002), clover (Bouma 1969) and wheat (Ahmed et al. 2006; Arif et al. 2006; Benbella and Paulsen 1998; McBeath et al. 2011; Mosali et al. 2006).

For foliar P, in addition to the increase in P concentration of the plant, yield responses are critical to the application of the technique. Yield responses to foliar P fertilisation of wheat in agronomic studies have been variable with a large proportion of studies finding only one or two treatments result in a significant yield increase (Table 2). For example, McBeath et al. (2011) found that only foliar phosphoric acid plus adjuvant resulted in a 25 % grain yield response for glasshouse grown wheat in one of the two soils with marginal P status used. Mosali et al. (2006) also found that only 50 % of their trials showed significant response to foliar P, despite soil P tests being below 100 % sufficiency at all three sites studied. In the study, foliar P rates were varied from 0-4 kg P ha^{-1} in the first and second year with additional foliar P rates of 8, 12, 16 and 20 kg P ha^{-1} in the third year. Foliar applications occurred at either Zadoks 32 (second node), Zadoks 50 (heads emerging) or Zadoks 65 (anthesis) with or without initial soil P (starter fertiliser). However, comparing between the three soil sites and three seasons was made difficult by using different wheat varieties. It is likely that a

combination of methods and techniques are needed along with better knowledge of the wheat leaf surface, since the morphology plays a significant role in the retention and uptake processes. The combination of techniques will help us to understand the processes that govern the uptake and translocation of foliar applied P and to develop the right formulations and conditions for foliar application before testing foliar P using field-based techniques.

Table 2: Summary of the literature for yield responses of wheat to foliar P treatments.

Foliar rates (kg ha^{-1})	Foliar formulation	Grain yield response	Author
P: 0, 6-12 N: 0, 55- 110*	P, N and NP as urea and phosphoric acid	Glass house: not significant for foliar P under low water stress through to 66 % increase for foliar NP under low water stress conditions	(Alston 1979)
P: 1.65	Ammonium polyphosphate, NP blend, phosphoric acid	Glass house: -17 % with NP blend + adjuvant in one soil to 25 % for phosphoric acid + adjuvant in the other soil	(McBeath et al. 2011)
P: 0, 2.6, 5.2; OR P: 0, 0.2, 0.5, 0.9	Potassium phosphate, β-glycerophosphate, tripolyphosphate, phytic acid OR Potassium phosphate	Field trial: not significant for phytic acid and tripolyphosphate to 1059 kg ha^{-1} (48 %) response to potassium phosphate	(Sherchand and Paulsen 1985)
P: 0, 2.2, 4.4, 6.6	Potassium phosphate	Field trial: not significant to 1040 kg ha^{-1} (22 %)	(Benbella and Paulsen 1998)
P: 0, 1, 2, 4	Potassium phosphate	Field trial: -447 kg ha^{-1} (-15 %) to 837 kg ha^{-1} (45 %) Application at Zadoks 32 generally gave better grain yield than Zadoks 50 or 65	(Mosali et al. 2006)
P: 0.4, 0.8, 1.2#	Complete foliar fertiliser 'Dogoplus' – N, P (9 %), K, Zn, Fe, Mn, Mg, Cu, S, B	Field trial: 4 % at the lowest application rate to 20 % at the highest application rate	(Ahmed et al. 2006)

*Rates for Alston (1979) take into account estimated foliar interception of 15-30 % of spray to give range of applied rate
#Approximate rates split between two applications based on rates of 0.3, 0.6 and 0.9 % with 300L feddan^{-1} applied each time

Conclusions and objectives of this thesis

Although there is a significant body of work on foliar fertilisation, I have identified the following gaps in the literature:

- There is limited information on the surface properties of wheat leaves which is important since it will govern the penetration of applied solutes including;

    - Detailed studies on the physiological properties of wheat leaves between the adaxial and abaxial surfaces,
    - Studies on the physiological properties of wheat leaves at different leaf ages and growth stages.
- Detailed studies on the influence of plant P status on both uptake and translocation of foliar-applied P have not been completed;
- There is a lack of comprehensive studies on the interaction between wheat leaf properties, formulation properties and the subsequent absorption and translocation including;
    - The influence adjuvants have on spreading of foliar P formulations on wheat leaves, the role adjuvants play in retention of foliar fertiliser, and the initial uptake and translocation of foliar P as measured after a few days,
    - The influence of leaf wettability at different phenological growth stages and the interaction between the leaf surface and the foliar P formulation that includes different adjuvants on the retention of foliar fertilisers, foliar uptake, translocation and subsequent plant growth and grain yield, and
- A study on the interaction between foliar P source and adjuvant and subsequent effect on the foliar uptake, translocation of P (at field applicable rates) and plant growth of wheat has not been conducted.

There is an opportunity to use an inter-disciplinary approach to investigate these factors that have limited our understanding and interpretation of previous studies. Although there are a number of studies on foliar P fertilisation, results have been variable in part due to the inability to work towards capturing the four key processes in a single body of work. Comparison between studies has been difficult due to the large range of factors studied with numerous variables both within and between experiments. For many studies, at least one treatment has resulted in a significant yield increase; however this is only a small proportion of the number of treatments investigated. Studies have also not used an integrated approach, investigating either agronomic response or mechanisms of plant uptake under controlled environment conditions. Reliance on understanding of the leaf properties of other species is unwise due to variability between species. Once there is a better understanding of these properties for wheat, fertiliser formulation may be modified to enhance foliar uptake. The fertiliser formulation should take into account the interaction between formulation components (adjuvant and nutrients) and the effect of formulation pH on foliar uptake. Finally

the interaction of the plant surface with the fertiliser formulation requires further investigation.

Although there have been studies using radioactive tracers, many have only involved short-term uptake and translocation at low rates or have used single drops not applicable to field application rates. This PhD thesis focussed on using a combination of techniques including utilisation of scanning electron microscopy, measuring contact angles of water and fertilisers on wheat leaves, both single and double isotopic labelling techniques (^{32}P and ^{33}P) of the fertilisers and plant growth studies under controlled conditions to examine the surface morphology, wettability, foliar uptake, subsequent translocation and effect of foliar application of P on wheat growth.

Hence, the main objectives of this study were to investigate:

- How the leaf structure, physiology and morphology differs between the adaxial (upper) and abaxial (lower) leaf surfaces of wheat and its effect on foliar P uptake and subsequent translocation of P applied as phosphoric acid (Chapter 2);
- How the addition of different surfactants to phosphoric acid will affect the wettability of the formulation on wheat leaves and whether differences in the wettability of the formulations affects the initial foliar uptake and translocation of P (Chapter 3);
- How the addition of different surfactants and a humectant to phosphoric acid applied at two different growth stages will affect the wettability of the formulation and whether differences in the wettability of the formulations affects grain yield, foliar uptake and translocation of P when grown through to maturity (Chapter 4); and
- The effect of adjuvant on other P sources which range in both pH and associated cations on peak biomass of wheat and foliar uptake and translocation (Chapter 5).

To make the effects of soil nutrient supply on wheat leaf physiology and hence foliar P uptake consistent across all studies, the same wheat cultivar and the same P-responsive soil was used throughout, at a level of soil P shown to be marginally deficient for wheat growth. An experiment was also conducted in collaboration with Dr Victoria Fernández (Technical University of Madrid) on the effect of P status of the plant (deficient, marginally deficient, sufficient) on the morphology and wettability of wheat leaves, plant P uptake and translocation (see Appendix).

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Chapter 2

Wheat leaf properties affecting the absorption and subsequent translocation of foliar-applied phosphoric acid fertiliser

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Chapter 3

Uptake of phosphorus from surfactant solutions by wheat leaves: Spreading kinetics, wetted area, and drying time

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Chapter 4

The timing of application and inclusion of a surfactant is important for absorption and translocation of foliar phosphoric acid by wheat leaves

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The timing of application and inclusion of a surfactant is important for absorption and translocation of foliar phosphoric acid by wheat leaves

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Abstract

In order for foliar application of phosphorus (P) fertiliser to be successful, the first barrier to overcome is the adhesion of the fertiliser to the leaf. Given that the surfaces of wheat leaves are covered in waxes and leaf hairs, they are inherently difficult to wet. The timing of foliar fertiliser application is important because the characteristics of wheat leaves that control the wettability of the leaf and the demand for P change as the plant progresses through the different stages of plant maturity. Adjuvants, in particular surfactants, are used to help reduce the surface tension of the fertiliser solution and consequently improve the wettability of the leaves. It is unknown which adjuvant will give the best improvement in wheat leaf wettability and whether this will in turn increase the uptake of the foliar fertiliser. Once the formulation has adhered to the leaf, it requires uptake and translocation to other plant parts in order to influence the P nutrition of the plant. In this study, we measured the leaf wettability, uptake and translocation of foliar applied phosphoric acid in combination with five different adjuvants when applied to wheat at either early tillering or flag leaf emergence. Although there was significant uptake of phosphoric acid in combination with all adjuvants that contained a surfactant, only one treatment (LI700 applied at flag leaf emergence) resulted in an increase in grain yield and two treatments (LI700 and Genapol X-080 applied at tillering) resulted in a decrease in grain yield when grown through to harvest. This is despite the wettability, as measured by contact angles, of all foliar fertilisers being markedly different. However, the translocation of P from foliar sources, measured using a direct isotopic tracing technique, was greater when applied at a later growth stage than when applied at tillering despite the leaf surface properties that affect wettability being similar across all leaves at both growth stages.

Introduction

Phosphorus (P) is an essential nutrient needed for plant growth but due to chemical reactions in soil it has low immediate availability and limited efficiency in the year of fertiliser application. Fertiliser P requirement of crops in Mediterranean cropping systems is highly dependent on seasonal rainfall. As a result, fertiliser can be a high risk input cost for farmers, especially in areas of southern Australia with variable rainfall (Kingwell 2011). Current best practice is to apply all P fertiliser at sowing, banded below the seed, at a time when predicting rainfall driven yield potential can be unreliable (McLaughlin et al. 2011). Applying all P fertiliser at sowing, when the season ahead is unknown, increases the risk associated with the fertiliser investment. Hence the potential to use an in-season foliar P top-up as a tactical management technique in response to favourable seasonal conditions is attractive. A tactical management approach has been used for nitrogen (N) to increase grain protein content but has been inconsistent at increasing grain yields (Bly and Woodard 2003; Gooding and Davies 1992; Varga and Svecnjak 2006; Woolfolk et al. 2002). Foliar application with micronutrients has been used extensively especially in horticulture to alleviate nutrient deficiencies and maintain plant health (Fernández and Eichert 2009), but use with macronutrients both in horticulture and, cropping systems has been limited.

In order for foliar fertilisation to be successful the nutrients must be able to penetrate the outer protective layer of the leaf (either directly diffusing through the cuticle or indirectly passing through stomata and cuticular cracks) to reach the internal cells of the plant (Fernández and Eichert 2009). To then be beneficial to the plant, there must be movement from the site of application to other plant cells (translocation). One benefit of P in this respect is that it is a mobile element in the phloem and is readily transported and redistributed around the plant, unlike other nutrients including calcium and boron which are relatively phloem immobile (White 2012). It has also been shown that the P can be translocated not only in the phloem but also the xylem (Martin 1982) and as a result at maturity, depending on the P status of the plant, translocation of P from other plant parts can account for 20 to 90 % of the P in the grain (Batten and Wardlaw 1987; Peng and Li 2005). Before both uptake and translocation can occur however, the foliar fertiliser must adhere to the leaf.

The wettability of plant leaves as well as the ability of leaves to absorb and translocate foliar-applied fertilisers can vary with individual leaf age and crop growth stage (Puente and Baur 2011; Sargent and Blackman 1962; Troughton and Hall 1967). In addition to the implication this has for uptake of foliar fertilisers, there must also be demand of the nutrient at the growth stage corresponding to when the spray is applied for the application to be beneficial. The maximum possible interception of foliar sprays is controlled by the crop cover and area of

leaf available to intercept the spray. For P, this is essential because of the limited mobility of P in soil, particularly if the P is surface applied where the soil can dry out, thus further limiting P movement (Marschner and Rengel 2012). The supply of P is critical during early plant growth with wheat yields substantially reduced if P supply is limited (Grant et al. 2001). For maximum grain yield to be achieved, P uptake is required until heading (Boatwright and Viets 1966) or anthesis (Batten et al. 1986) with supply post-anthesis suggested to have no effect on grain yields. This is despite substantial soil P uptake occurring after anthesis in some studies (Mohamed and Marshall 1979). Crop P uptake from soil accumulates rapidly between stem elongation and anthesis which also coincides with the period of maximum leaf area (Waldren and Flowerday 1979). Past studies have suggested the optimal timing of foliar P application is from canopy closure to anthesis (Noack et al. 2011).

The adhesion of foliar fertilisers is particularly important for wheat leaves compared to some other broadacre crops. This is because wheat plants have been shown to have leaves that are particularly difficult to wet due to the surface roughness induced by extracuticular waxes and leaf hairs (trichomes) (de Ruiter et al. 1990; Holloway 1969; Netting and von Wettstein-Knowles 1973). The surface roughness can induce hydrophobicity where the contact angle of water to the leaf surface can be as high as 160 degrees, with little resultant adhesion of water (Fernández et al. 2014). It has been suggested that improved wetting of foliar fertiliser on leaves (i.e. a small contact angle measured at the leaf-fertiliser interface), will increase the rate of uptake for foliar-applied fertilisers (Fernández and Eichert 2009). It follows that poorer wetting (higher contact angles) will result in lower uptake rates of the fertiliser formulation.

To improve the efficacy of foliar uptake, the addition of adjuvants to the fertiliser formulation is often required (Fernandez and Eichert, 2009). Adjuvants are defined as any material that is added to a spray solution to enhance uptake of the active ingredient of the spray, whether the spray be a fertiliser, herbicide or pesticide, or to modify the spray characteristics (Hazen 2000). Adjuvants can include oils, pH buffers, surfactants, humectants, or mixtures which can contain multiple modes of action (Somervaille et al. 2014). The addition of oils, which can act as a penetrating agent, to sprays, and the addition of humectants and surfactants (generally with a low hydrophile/lipophile balance (HLB)) are common for lipophilic herbicides and pesticides, which are often sparingly soluble in water (Hazen 2000; Somervaille et al. 2014). The use of pH buffers is also important for herbicides due to greater effectiveness of many active ingredients at a low pH compared to higher pH (Somervaille et al. 2014). Of more relevance to foliar fertilisers is the use of surfactants especially those with a high HLB, which work by lowering the surface tension of the formulation to improve spreading and adhesion of the fertiliser on the leaf surface and therefore increase the leaf area in contact with the fertiliser (Fernández and Eichert 2009). In addition to their effect on wettability, there is also

some evidence that surfactants themselves penetrate the plant cuticle or increase the hydration of the cuticle and as a result also increase the rate of foliar uptake (Hess and Foy 2000). Due to the hydrophobic nature of the plant cuticle, surfactants with a low HLB are able to absorb into the cuticle better than those with a high HLB (Hess and Foy 2000). Humectants may also be useful in improving uptake of foliar fertilisers. This is because humectants increase the drying time of aqueous sprays (Hazen 2000), which is essential as foliar uptake only occurs when the fertiliser is in a liquid form (Fernández and Eichert 2009). There are a large number of adjuvants which are commercially available for use in combination with agrochemicals, however many provide no label recommendations for use with foliar-applied fertilisers (Somervaille et al. 2014). There is potential for incompatibility between P-containing components and adjuvants in the formulation.

To measure the effect of adjuvants on uptake and translocation of foliar-applied phosphoric acid, two commercial adjuvant mixtures, two laboratory grade surfactants and one laboratory grade humectant were applied in combination with ^{33}P-labelled phosphoric acid at two different growth stages to wheat plants grown under controlled conditions. These two growth stages were chosen to represent a time of high P requirement by the plant (early tillering) and towards the end of peak P demand when there is more cover and opportunity for interception (flag leaf emergence). This study aimed to investigate whether the choice of adjuvant influences the uptake and final sink of foliar-applied P when plants were grown through to maturity and also whether any of the foliar formulations resulted in an increase in wheat yield when grown on a soil with marginal soil P availability.

Experimental Section

Soil collection and chemical properties

The soil used in this study was classified as a Calcarosol according to the Australian Soils Classification (Isbell 2002) and was collected from an agricultural site near Black Point on the Yorke Peninsula in South Australia (S34°36.776', E137°48.599). It has a sandy loam A horizon which overlies calcareous substrate. Soil characteristics have been described in Peirce et al. (2014). Briefly, the soil is a loam with a pH of 8.5 that has no detectable calcium carbonate or surface salinity issues. The cation exchange capacity (CEC) is 17.9 cmol kg^{-1} and it has an organic carbon content of 1.6 g kg^{-1}. The soil is classified as P-deficient with plants grown in the soil likely to be P-responsive (measured Colwell-P 3 mg kg^{-1}, PBI 75 and DGT-P 4 µg L^{-1}) (McBeath et al. 2007).

Wheat growth conditions

Plants were grown in pots of 15 cm diameter and 17 cm depth that were not free-draining and held a total of 3 kg soil pot^{-1}. Before sowing, the soil was wetted to 15 % of field capacity (Klute 1986) and the following basal nutrients were mixed through the soil: potassium (K) as K_2SO_4 at 200 mg K pot^{-1} (113 kg K ha^{-1}), magnesium (Mg) as $MgSO_4.7H_2O$ at 50 mg Mg pot^{-1} (28 kg Mg ha^{-1}), zinc (Zn) as $ZnSO_4.7H_2O$ at 30 mg Zn pot^{-1} (17 kg Zn ha^{-1}), copper (Cu) as $CuSO_4.5H_2O$ at 24 mg Cu pot^{-1} (14 kg Cu ha^{-1}), manganese (Mn) as $MnCl_2$ at 4 mg Mn pot^{-1} (2 kg Mn ha^{-1}) and the total sulfur (S) applied in these reagents equated to 175 mg S pot^{-1} (57 kg S ha^{-1}). The soil was watered to 70 % field capacity and P and nitrogen (N) were added to the soil as a band 2 cm beneath the seed at a rate of 12 mg P pot^{-1} (6.6 kg P ha^{-1}) as phosphoric acid and 150 mg N pot^{-1} (85 kg N ha^{-1}) as urea before sowing. At early tillering, 18 days after sowing (DAS) an additional 75 mg N pot^{-1} as urea and 7.5 mg of Zn as $ZnSO_4.7H_2O$ was applied in solution to the soil surface and watered in.

Four seeds of wheat (*Triticum aestivum* cv. Axe) that had been germinated a few days prior were sown in each pot at 1 cm depth and thinned at the two-leaf growth stage by leaving the two most uniform seedlings per pot. Immediately after sowing, the surface of the pot was covered with 80 g of alkathene granules to minimise evaporation from the soil. Pots were watered every two days to maintain 80 % field capacity before increasing watering frequency to every day from early booting. Plants were grown in a controlled environment room (20 °C/15 °C day/night cycle of 12 h each) and their positions randomised every week. The plants were moved to the glasshouse 67 DAS to ripen and watering was suspended 89 DAS, two weeks before harvest. Average growing conditions in the glass house were 23.2 °C with 60.9 % relative humidity (RH) (minimum 15.9 °C 13.7 % RH and maximum 35.8 °C 92.5 % RH).

Adjuvants

In this study we used five different adjuvants including two commercial products, two pure surfactants and a humectant, namely: LI 700®, Agral®, Genapol® X-080, Triton™ X-100 and glycerol. LI 700® is an acidifying and penetrating mixture with 35 % w v^{-1} propionic acid (CAS No. 79-09-4), 35 % w v^{-1} soyal phospholipids (CAS No. 8002-43-5) and 10-30 % w v^{-1} non-ionic surfactant. It also acts as a pH buffer by acidifying the spray solution. Agral® is a spray additive with 63 % w v^{-1} nonyl phenol ethylene oxide condensate (non-ionic surfactant) (CAS No. 9016-45-9). Genapol® X-080 is a pure non-ionic surfactant of polyethylene glycol monoalkyl ether (CAS No. 9043-30-5). Glycerol is a simple polyol (CAS No. 56-81-5) which is hygroscopic and therefore acts as a humectant. Triton™ X-100 is a non-ionic surfactant of p-tertiary-octophenoxy polyethyl alcohol (CAS No. 9002-93-1).

Microscopy

Wheat leaves corresponding to the treated leaves from the foliar application experiment were collected at both early tillering (Zadoks 21) and when the flag leaf collar was visible/late flag leaf emergence (Zadoks 39 (Zadoks et al. 1974)) and small sections from the middle of the leaf (avoiding the mid-rib) were cut and fixed for scanning electron microscopy (SEM). The plant samples were fixed and vacuum infiltrated in 0.25 % gluteraldehyde, 4.0 % paraformaldehyde in phosphate buffer solution (PBS) with 4% sucrose at a pH of 7.2 overnight. Samples were then rinsed in PBS and 4 % sucrose three times before post fixing in 2 % osmium tetroxide in PBS for 1 h. They were then washed and progressively dehydrated in an ethanol series: 70 % (two changes of 15 min), 90 % (two changes of 15 min), and 100 % ethanol (three changes of 15 min). Samples were then critical point dried in a Bal-tec CPD 030 Critical Point Dryer, mounted on a stub and coated with a 5 nm layer of platinum. Images were taken on a Philips XL20 scanning electron microscope under high vacuum at 10 kV and a working distance of 10 mm. Stomatal and trichome densities were calculated from analysing images taken by SEM.

Contact angle measurements on leaves

The static advancing and receding contact angle of water and fertilisers was measured on the adaxial (upper) side of leaves from wheat plants at growth stages corresponding to the two foliar application timings (Zadoks 21 and Zadoks 39, (Zadoks et al. 1974)). Measurements were made using the sessile drop method (with the needle in) and calculated based on observation of the profile of small water droplets (1-2 µl) (DataPhysics, OCAH 200) as described in Forsberg et al. (2010). The initial droplet volume was brought into contact with the surface, increased slowly until the contact line advanced, and then stopped before measurement. In the same way the liquid volume of the drop deposited onto the surface was decreased until the contact angle receded, and then was stopped before measurement. All contact angle measurements were made on the mid-section (length-wise) of the leaf between the leaf edge and mid-vein. To do this, sections of the leaf were cut and stuck to glass slides with double-sided tape. Care was taken to avoid damage to the leaf surface: the leaf was only handled on its edges away from where contact angle measurements were made. Unlike water, adjuvant drops were allowed to relax for 20 s before contact angle measurements were taken. This was due to the dynamics of the adjuvants at the leaf surface causing the drop to spread over time rather than remain a static contact angle. A time of 20 s was chosen to allow a more reproducible angle to be measured (Peirce et al. 2015). A final contact angle was not measured but has been shown for three of the five adjuvants to effectively reach zero if allowed enough time to spread (Peirce et al. 2015). For fertilisers on leaves, the receding

contact angle could not be measured as the droplet was not observed to recede from the leaf surface, i.e. the receding contact angle was effectively 0° in every case except for glycerol. Contact angle values reported for fertilisers are the average of 12 measurements taken over three leaves (corresponding to those treated in the plant experiment) and contact angle values for water are the average of 15-25 measurements taken over all leaf sections.

Foliar uptake and translocation

Foliar treatments consisted of five adjuvants (one concentration each) at two different foliar application timings to give ten treatment groups with five replicates in each group. A further five replicates were included without foliar P application as a control and another five replicates for destructive measurements (contact angles and SEM as described above). This gave a total of 60 pots.

The two foliar applications were 21 DAS corresponding to plants at early tillering (Zadoks GS21) and 32 DAS corresponding to plants with the flag leaf collar visible (Zadoks 39 (Zadoks et al. 1974)). The rate of foliar applied P for all treatments was 3.4 mg of P pot^{-1} as phosphoric acid, equivalent to approximately 1.9 kg P ha^{-1} at a watering rate of 100 L ha^{-1} (based on pot surface area). The concentration of adjuvant used depended on the adjuvant in question but was the label rate for Agral® and LI 700® (0.1 and 0.3 % w v^{-1} respectively) and consistently 0.1 % w v^{-1} for the other 3 adjuvants. Each foliar fertiliser was labelled with carrier-free ^{33}P in the orthophosphate form to give a spike rate of 0.5 MBq pot^{-1} at application. Before foliar P application, the soil surface was covered with plastic wrap to ensure any drops that did not adhere to the leaves would not reach the soil surface. The foliar fertilisers were applied mid-morning to the five most prominent fully expanded leaves for each plant at the time of application. This corresponded to two leaves on the tillers and three on the main stem at both timings to give ten leaves per pot. Drops were applied with a micropipette to the adaxial leaf side totalling 177 µl pot^{-1} split between all the leaves. Drop size was consistent between all the treatments (4-5 µl) except glycerol which, due to the difficulty in detaching the droplets from the micropipette, were much larger (average of 12 µl and 10 µl for the two timings respectively). The estimated loss of foliar fertiliser through droplet movement (non-adherence to the leaf) was recorded by visual observation of each deposited drop. All treated leaves were marked for easy identification and three days after application each treated leaf was scored for leaf burn according to a modification of the method of Stein and Storey (1986), namely 1 = no effect, 2 = slight surface burn on the treated area without cell collapse, 3= slight to heavy burn on the treated area only with some cell collapse, 4 = heavy surface burn extending between treated areas, 4.5 = the same as 4 with leaf tip senescence, and 5= leaf dead and not functioning.

Above-ground plant parts were harvested at maturity when grains were ripe (Zadoks 92, (Zadoks et al. 1974)). Plant parts were harvested 1 cm from above the soil surface and divided into the following sections before washing: treated leaves; untreated leaves; heads; and stems. Each of these plant parts was washed for 30 s in 100 mL of 0.05 % w v^{-1} Triton™ X-100 + 0.1 M HCl then rinsed in RO water for 20 s and DI water for 20 s (Fernández et al. 2014). The first washing solution was kept for analysis of total P by inductively coupled plasma - atomic emission spectroscopy (ICP-AES) and ^{33}P activity on the beta counter. All parts were dried in an oven at 60 °C for 72 h. Plant parts were weighed and the grain was separated from the chaff. All plant parts were digested in boiling nitric acid and analysed for P by ICP-AES (Zarcinas et al. 1987). A 2 mL sample of the digest was added to a vial with 10 mL of scintillation fluid (EcoScint) and counted on a Perkin Elmer Quanta Smart liquid scintillation analyser (Model Tri-Carb B3110TR). All counts were blank corrected and corrected for decay to a single time point.

Calculations and statistical analysis

The amount of foliar P absorbed (P uptake) was expressed as a percentage and calculated as the amount of ^{33}P recovered in washed plant parts divided by the ^{33}P in the applied fertiliser. The translocation was also expressed as a percentage of the total ^{33}P in the applied fertiliser and consisted of the ^{33}P recovered in all washed plant parts minus the treated leaves divided by the ^{33}P in the applied fertiliser.

$$\%\ foliar\ uptake\ = \frac{33P_{in\ plant\ parts\ from\ fertiliser}\left(\frac{mg\ P}{pot}\right)}{33P_{in\ foliar\ fertiliser}\left(\frac{mg\ P}{pot}\right)} \times 100$$

$$\%\ foliar\ translocation\ = \frac{P_{in\ plant\ parts\ from\ fertiliser}\left(\frac{mg\ P}{pot}\right) - P_{in\ treated\ leaves}\left(\frac{mg\ P}{pot}\right)}{P_{in\ foliar\ fertiliser}\left(\frac{mg\ P}{pot}\right)} \times 100$$

The plant P derived from the foliar fertiliser was simply the ^{33}P radioactivity of the washed plant parts divided by the specific activity (SA) of the foliar fertiliser.

$$P\ derived\ from\ foliar\ fertiliser\left(\frac{mg}{pot}\right) = \frac{33P_{radioactivity\ in\ plant\ parts}\left(\frac{Bq}{pot}\right)}{SA_{of\ foliar\ fertiliser}\left(\frac{Bq}{mg\ P}\right)}$$

Statistical analysis was performed by analysis of variance (ANOVA) in the Genstat® V.15 statistical package. Both the normality of distribution and constant error variance assumptions were tested for each analysis. Differences between treatments were determined by least significant difference (l.s.d.) at the 5 % significance level using Fisher's protected l.s.d. The

treatment structure run in ANOVA for all analysis that included controls (dry weight and P uptake) was foliar/(timing × adjuvant) where foliar = yes (all treatments) or no (controls), timing = early tillering or flag leaf emergence and adjuvant = Glycerol, Agral®, LI 700®, Triton™ X-100 or Genapol® X-080. The treatment structure for all other analysis undertaken in ANOVA was adjuvant × timing.

Results

Plant growth

The foliar application of phosphoric acid with LI 700® at flag leaf emergence produced the only positive grain yield response of 12 % more grain than the control (Table 1). Conversely, when the LI 700®treatment was applied at early tillering, it produced 22 % less grain than the control. The foliar application of phosphoric acid in combination with Genapol® X-080 also resulted in a decrease in grain yield of 12 % when applied at early tillering. There were no differences between treatments in total above-ground plant biomass or stem biomass at harvest (Table 1). Only the LI 700® treatment applied at early tillering had significantly lower leaf and chaff biomass than the control, corresponding with the loss of grain yield. There was also a significant effect of timing of application for the weight of stems, leaves and whole plants. Plants fertilised at early tillering had lower stem and whole plant biomass than the control. Foliar application at flag leaf emergence did not result in any differences in biomass compared to the control plants. Neither 1000-grain weight nor grain number (grand mean of 157 pot^{-1}) showed any differences between treatments (Table 2). There were also no differences in the P content or P concentration of the grain between any of the treatments (Table 2).

Table 1: Effect of foliar treatments on shoot dry weight.

	Grain	Chaff	Stems	Leaves	Whole plant
			(g pot^{-1})		
Foliar.Adjuvant.Timing					
Control (no foliar)	5.24 ^{bc}	1.94 ^{ab}	2.23	1.79 ^{abc}	11.20
Early tillering (Z21)					
Glycerol	5.55 ^{abc}	1.98 ^{ab}	2.07	1.79 ^{abc}	11.40
LI 700®	4.09 ^{e}	1.43 ^{c}	1.48	1.34 ^{d}	8.34
Triton™ X-100	5.10 ^{cd}	1.86 ^{ab}	1.93	1.67 ^{bcd}	10.56
Agral®	5.66 ^{ab}	1.94 ^{ab}	1.90	1.76 ^{abc}	11.27
Genapol® X-080	4.63 ^{de}	1.76 ^{bc}	1.79	1.58 ^{cd}	9.77
Flag leaf emergence (Z39)					
Glycerol	5.53 ^{abc}	2.05 ^{ab}	2.47	1.97 ^{ab}	12.03
LI 700®	5.88 ^{a}	2.21 ^{a}	2.28	2.08 ^{a}	12.46
Triton™ X-100	5.32 ^{bc}	1.89 ^{ab}	2.18	1.91 ^{abc}	11.29
Agral®	5.19 ^{bc}	1.80 ^{abc}	1.95	1.79 ^{abc}	10.74
Genapol® X-080	5.52 ^{abc}	1.97 ^{ab}	1.94	1.87 ^{abc}	11.31
LSD ($p \leq 0.05$)	*0.54*	*0.43*	*n.s.*	*0.33*	*n.s.*
	Foliar.Timing				
No foliar application	5.24	1.94	2.23 ^{a}	1.79 ^{ab}	11.20 ^{a}
Early tillering *(Z21)*	5.01	1.79	1.84 ^{b}	1.63 ^{b}	10.27 ^{b}
Flag leaf emergence *(Z39)*	5.49	1.98	2.17 ^{ab}	1.93 ^{a}	11.57 ^{a}
LSD ($p \leq 0.05$)	*n.s.*	*n.s.*	*0.35*	*0.26*	*0.86*

Statistical differences within a column and treatment design indicated with different letters (p≤0.05, LSD in table)

Table 2: Effect of foliar treatments on grain number, P content and P concentration.

	Grains pot^{-1}	Grain P content *mg pot^{-1}*	Grain P concentration *mg kg^{-1}*
Foliar.Adjuvant.Timing			
Control (no foliar)	159	12.5	2400
Early tillering (Z21)			
Glycerol	162	14.4	2594
LI 700®	125	11.4	2803
Triton™ X-100	156	14.4	2838
Agral®	176	17.1	3011
Genapol® X-080	140	13.1	2850
Flag leaf emergence (Z39)			
Glycerol	166	14.4	2619
LI 700®	174	15.9	2654
Triton™ X-100	155	14.1	2717
Agral®	152	14.8	2862
Genapol® X-080	163	14.7	2660
LSD ($p \leq 0.05$)	*n.s.*	*n.s.*	*n.s.*
Grand Mean	**157**	**14.3**	**2728**

Statistical differences within a column indicated with different letters (p≤0.05, LSD in table)

There was no relationship between the scorch score and either the above ground dry weight or grain weight (data not shown). There were differences in scorch scores between adjuvants and due to timing. The scorch score for all treatments except glycerol was high causing visible necrosis and senescing of at least some leaves within each pot when scorch was measured

three days after foliar application (Figure 1). There was both an adjuvant effect due to the lower scorch from glycerol treatments and a timing effect with application of foliar fertiliser at the later timing resulting in less severe scorch.

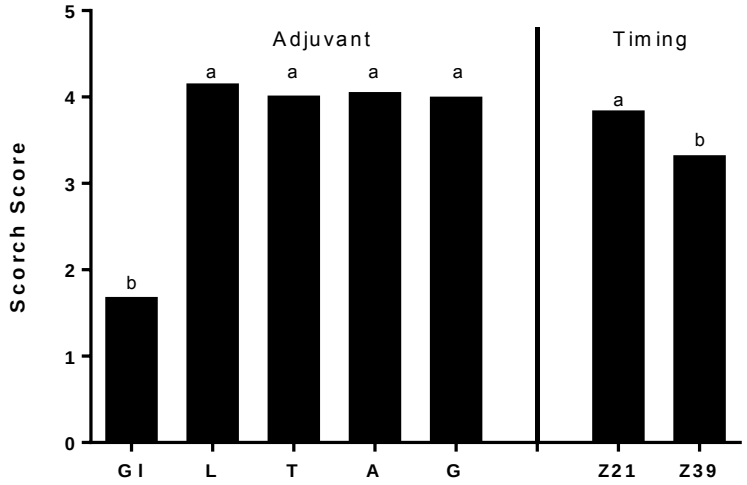

Figure 1: Average scorch score for adjuvants and timing; there was no significant adjuvant by timing interaction, Treatments: Gl-Glycerol, L-LI 700®, T-Triton™ X-100, A-Agral® and G- Genapol® X-080. Statistical differences between average scorch score for adjuvant effect ($p \leq 0.05$, l.s.d. 0.27) and timing effect ($p \leq 0.05$, l.s.d. 0.17) indicated on graph with different letters.

Plant surface and contact angles

Figure 2 shows the adaxial leaf side of wheat leaves taken by scanning electron microscope corresponding to the growth stages at which foliar P was applied. The leaves shown also correspond to leaves that had foliar fertilisers applied to them, namely the largest fully expanded leaf tiller, and the second (L2) and third leaf (L3) counting up from the base of the main stem (MS). For the second application timing, another leaf from the main stem corresponding to the penultimate main stem leaf (i.e. leaf below the flag leaf), L4, was also treated but was similar to L3 (Figure 2f). Although there appear to be slightly different densities of stomata and trichomes ranging from 42-65 stomata mm^{-2} and 13-42 trichomes mm^{-2} across the treated leaves (Table 3), this is likely to be natural variation as the wettability (measured by advancing and receding contact angles of water) was not significantly different between the leaves or the timings (grand mean of 162° and 154° for advancing and receding contact angles respectively).

Table 3: Number of stomata and trichomes mm^{-2} ± standard deviation on the adaxial side of leaves representative of foliar-treated leaves (counted using scanning electron microscopy). Leaf number (L2 etc.) corresponds to the leaf count from the base of the main stem upwards.

Timing	Leaf	Stomata	Trichomes
		No. mm^{-2}	
Early tillering (Z21)	L2	56 ± 12	16 ± 4
	L3	46 ± 7	21 ± 8
	tiller	49 ± 7	37 ± 4
Flag leaf emergence (Z39)	L2	42 ± 12	13 ± 4
	L3	59 ± 4	20 ± 7
	L4	65 ± 7	42 ± 6
	tiller	56 ± 9	37 ± 15
Average both timings		**55 ± 12**	**27 ± 13**

When we measured the contact angles of the fertiliser treatments on leaves at the two different growth stages it became apparent that once again there was not a growth stage or timing effect but there was a formulation treatment effect (Figure 3). However, when fertiliser drops of phosphoric acid with glycerol were deposited on the growing leaves, significantly more drops did not adhere when applied at early tillering compared to flag leaf emergence (Table 4; estimated run-off). Contact angles measured 20s after the formulation came into contact with the leaf showed that, with the exception of glycerol, all adjuvant treatments significantly decreased the advancing contact angle of the drop, but to different degrees depending on the adjuvant ranging from 111° for LI 700® to 0° for Genapol® X-080 (Figure 3a). The receding contact angle for all these treatments (except glycerol) was also effectively zero as the drop could not be removed from the leaf once it was deposited (Figure 3b). All these adjuvants also had a spreading dynamic, continuing to spread on the leaf surface until the drop dried out. For glycerol however, there was only a small surfactant effect, with both advancing and receding contact angles similar to water although slightly lower than water when applied at flag leaf emergence (advancing 158° ± 4, receding 153° ± 9).

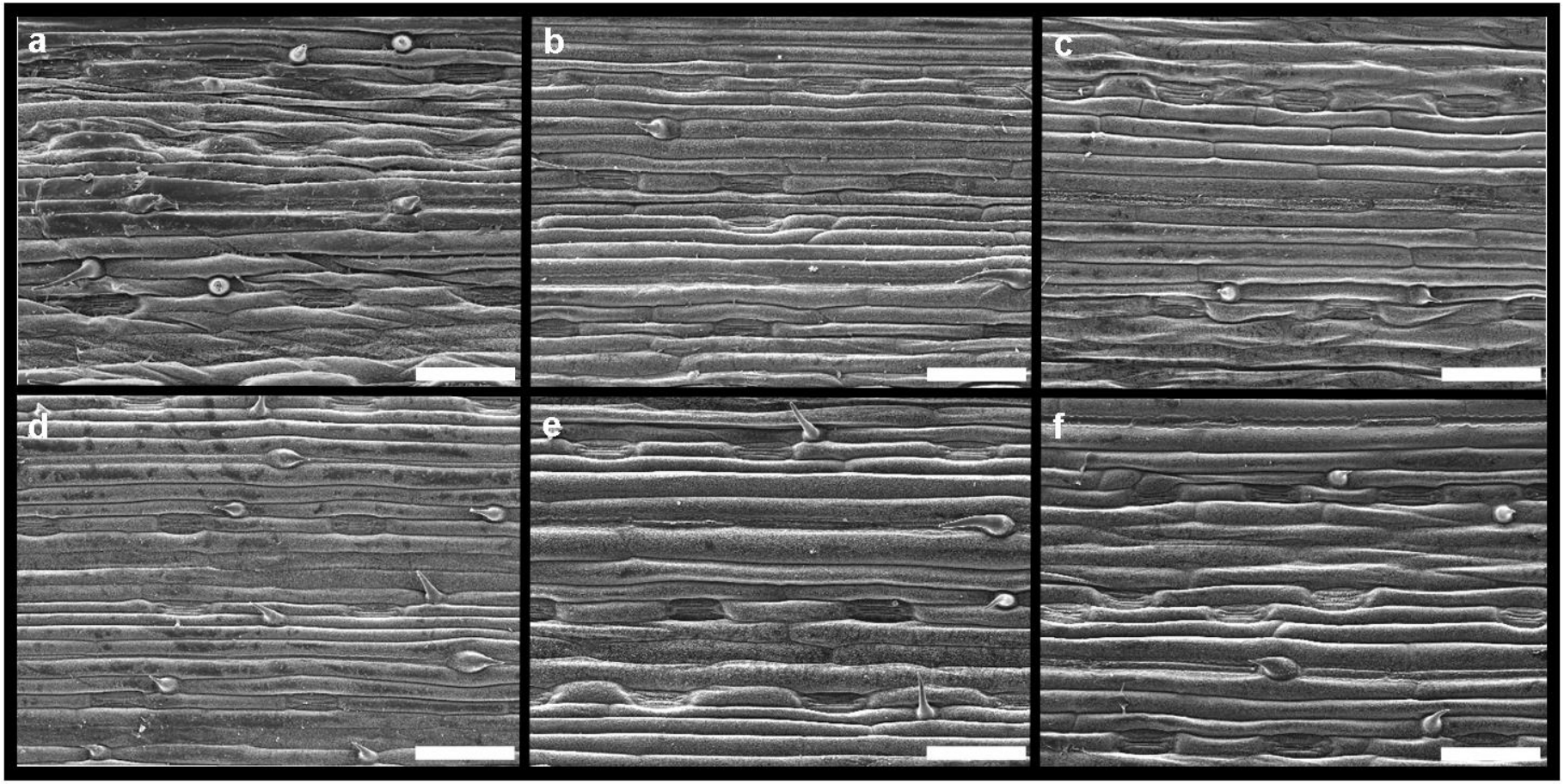

Figure 2: Scanning electron microscope images of the adaxial side of wheat leaves: (a-c) at early tillering Z21, (d-f) and at flag leaf emergence Z39. (a and d) leaf on first tiller, (b and e) Leaf 2 from main stem base, (c and f) Leaf 3 from main stem base; scale bar = 100 µm

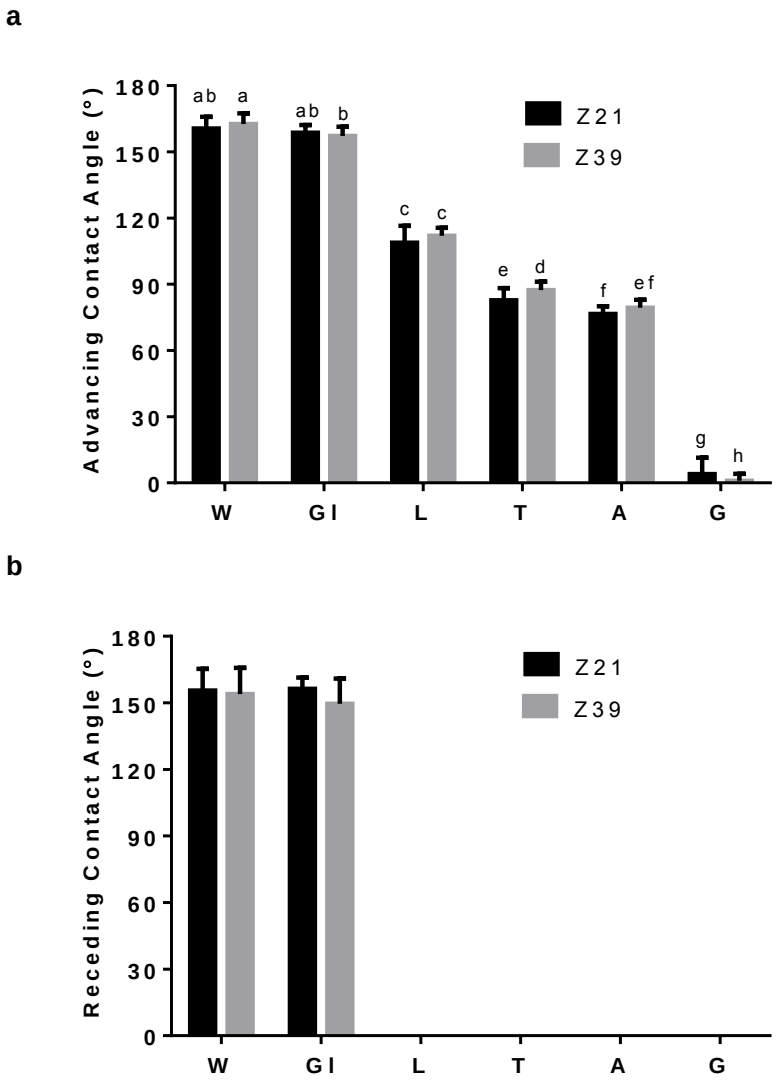

Figure 3: Average (a) advancing and (b) receding contact angle on adaxial side of fully expanded wheat leaves (tiller and main stem leaves) at 20 s for water and each of the adjuvants at both foliar timings (+/- standard deviation), Treatments: W-water, Gl-Glycerol, L-LI 700®, T-Triton™ X-100, A-Agral® and G- Genapol® X-080. Statistical differences between advancing contact angles indicated on graph with different letters ($p \leq 0.05$, l.s.d. 3.95)

Plant P uptake and translocation

Despite foliar treated plants receiving additional P in the foliar fertiliser, the total P uptake of the plants and P derived from the soil was not significantly higher than the control plants (Figure 4). However, there were differences in the uptake of P derived from the foliar source between foliar treatments. At both timings, the glycerol treatment had significantly less foliar P in the plants than all the other foliar treatments with less P derived from the foliar application when applied at early tillering compared to flag leaf emergence. A timing effect also occurred for the foliar LI 700® treatments with the application at early tillering having significantly more P derived from the foliar application than when applied at flag leaf emergence.

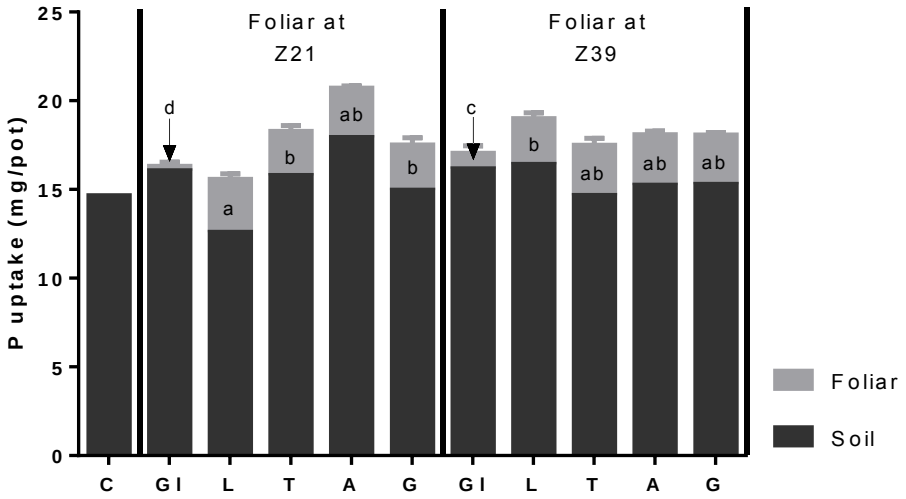

Figure 4: Source of P taken up by above-ground plant parts. Treatments: C-control, Gl-Glycerol, L-LI 700®, T-Triton™ X-100, A-Agral and G- Genapol® X-080. Statistical differences between foliar P treatments (at both times) indicated on graph with different letters ($p \leq 0.05$ l.s.d 0.37).

The uptake of foliar P as a percentage of P applied was similar for all adjuvant treatments across both timings (averaging 79.6 %) except for glycerol treatments, which were considerably lower (Table 4). For glycerol treatments, there was higher uptake at the second timing (27.4 compared to 7.8 %) due to higher drop adhesion (lower estimated loss due to run-off 80.1 % compared to 61.8 % for the two timings respectively; Table 4) suggesting that leaves were more wettable at the second timing despite the contact angle data not showing differences between timings (Figure 3). In all cases, only a small percentage of the foliar fertiliser P that adhered to the leaves was washed off, with the smallest percentage from glycerol treatments, but in all cases less than 5 % (Table 4). Any fertiliser not recovered as plant uptake, in the washings or estimated as run-off loss was classified as unrecovered foliar fertiliser P. Although there were no differences between treatments, this accounted for 10-27 % of the foliar P applied.

Table 4: Foliar fertiliser recovery in the plant, washing solution and run-off from different foliar treatments. Estimated run-off loss calculated by visual observation of number of drops that did not adhere to the leaf.

	Plant P uptake	P in wash	Run-off (estimated)	Residual
	Phosphorus (as a % of foliar fertiliser P applied)			
Adjuvant.Timing				
Early tillering (Z21)				
Glycerol	7.8 ^{d}	0.3 ^{f}	80.1 ^{a}	11.9
LI 700®	81.0 ^{ab}	3.0 ^{b}	0.5 ^{c}	15.4
Triton™ X-100	82.4 ^{ab}	4.5 ^{a}	0.5 ^{c}	12.5
Agral®	81.6 ^{ab}	2.7 ^{bc}	1.8 ^{c}	12.2
Genapol® X-080	82.1 ^{ab}	3.0 ^{b}	0.0 ^{c}	14.9
Flag leaf emergence (Z39)				
Glycerol	27.4 ^{c}	0.9 ^{ef}	61.8 ^{b}	9.8
LI 700®	71.4 ^{b}	1.2 ^{def}	0.4 ^{c}	27.0
Triton™ X-100	83.5 ^{a}	1.9 ^{cde}	0.5 ^{c}	14.1
Agral®	79.8 ^{ab}	2.1 ^{bcd}	1.8 ^{c}	16.4
Genapol® X-080	74.9 ^{ab}	2.7 ^{bc}	0.9 ^{c}	21.5
LSD ($p \leq 0.05$)	*11.7*	*1.03*	*5.2*	*n.s.*

Statistical differences within a column indicated with different letters (p≤0.05, LSD in table)

There was both an adjuvant and timing effect but not an interaction for foliar translocation of P expressed as a percentage of applied foliar P (Figure 5). Due to the reduced uptake of P in the glycerol treatment (due to fertiliser not adhering to the leaf), glycerol-treated plants also had lower total translocation, and translocation to the grain, chaff and stem from the foliar treated area than the other adjuvant treatments. There were no differences in either total translocation (averaging 43 %), or translocation to individual plant parts between the other four adjuvants (which all contained surfactants). The total translocation and translocation to all plant parts except the leaves was also higher when applied at the later timing, flag leaf emergence, than at tillering. For all treatments regardless of adjuvant used or timing, the largest sink for translocated P was the grain when harvested at maturity.

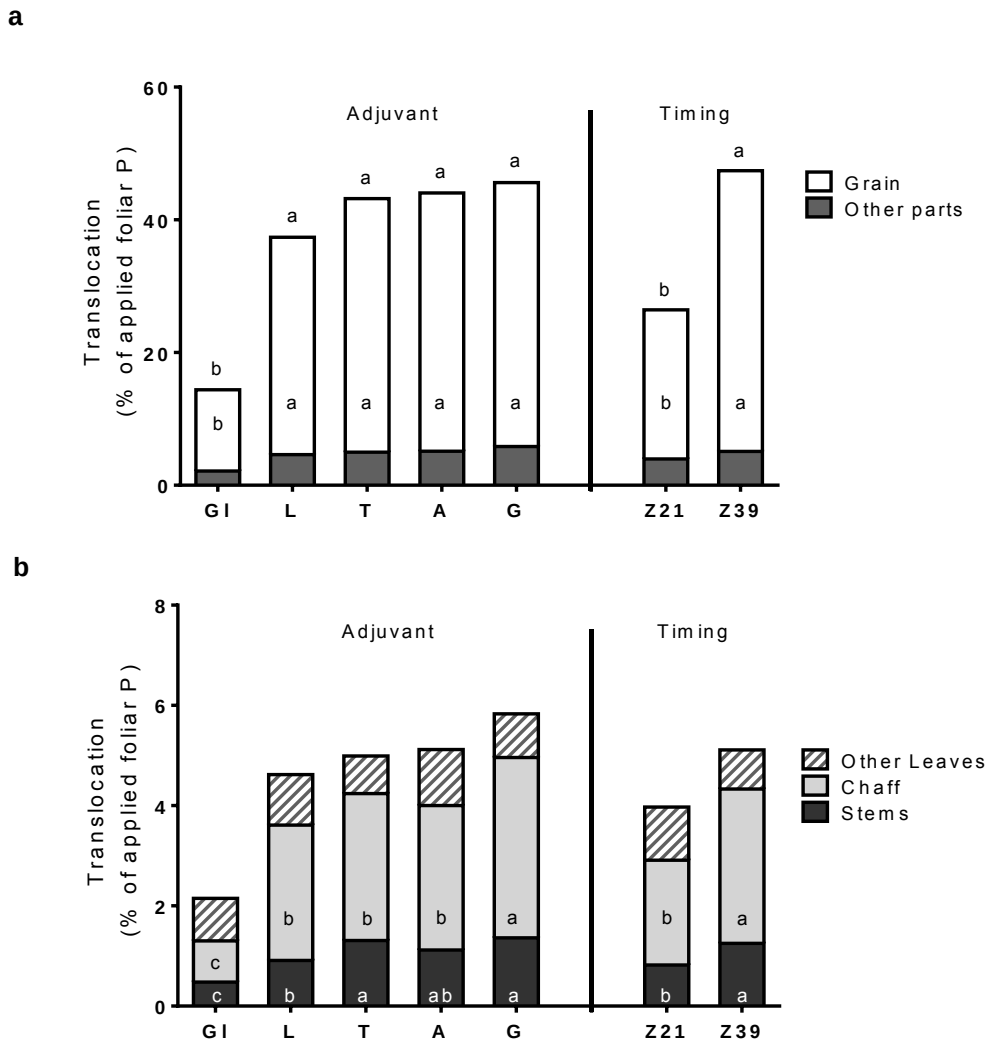

Figure 5: Translocation of foliar P to above-ground plant parts as a percentage of applied fertiliser; (a) total translocation and translocation to grain vs. the other plant parts, (b) expansion of translocation to other plant parts/ Treatments: C-control, Gl-Glycerol, L- LI 700®, T-Triton™ X-100, A-Agral® and G-Genapol® X-080. Statistical differences within an effect and plant part for foliar P translocation indicated on graph with different letters ($p \leq 0.05$; for adjuvant effect: total translocation l.s.d. 6.0, grain l.s.d. 5.2, other leaves n.s., chaff l.s.d. 0.6, stems l.s.d. 0.3; for timing effect: total translocation l.s.d. 3.8, grain l.s.d. 3.3, other leaves n.s., chaff l.s.d. 0.4, stems l.s.d. 0.2)

Discussion

Yield response

Yield response to foliar applied phosphoric acid depended on the growth stage at which it was applied. Application of phosphoric acid in combination with either LI 700® or Genapol® X-080 at early tillering caused a reduction in grain weight. When the LI 700® treatment was applied at flag leaf emergence, it increased the grain weight as was also found in one of two soils tested by McBeath et al. (2011). In the study of McBeath et al. (2011) the same foliar treatment at a rate equivalent to 1.65 kg P ha^{-1} applied at flag leaf emergence produced a grain yield increase of 25 %. The soil they used had a higher initial available P status (C_{DGT} 81 µg

L^{-1} and Colwell P 29 mg kg^{-1}) compared to our soil and hence had bigger plants and larger total grain weight increases than found in our study.

The P concentrations in the grain of both control plants and foliar-treated plants (generally <3000 mg kg^{-1}) suggest that the plants had marginal P status (Reuter and Robinson (1997). However, Elliott et al. (1997) found the critical P concentration for grain P at maximum grain yield is between 2100 and 2400 mg kg^{-1}. Our control plants had grain P concentrations of 2400 mg kg^{-1}, very close to the critical concentration, with all foliar treatments lifting the concentration above this critical value. It appears that the yield response to phosphoric acid with LI 700® did not increase the P concentration in the grain to adequate status according to the accepted standard set out by Reuter and Robinson (1997).

In both our study and the McBeath et al. (2011) study, the roots were not harvested as it was outside the scope of the study. As a result, we cannot confirm whether the foliar application stimulated root growth. It is likely that the yield increase noted in McBeath et al. (2011) was due to a stimulation of the root pathway, due to the increase in P content of the plants (compared to the control) being higher than the amount applied in the foliar fertiliser. Any increases in P uptake from the soil from foliar treatments would be a result of stimulation of the root pathway possibly due to increased root biomass (as shown by Asen et al. (1953) for foliar P application to chrysanthemum plants) and therefore root P uptake. However in our study even though one treatment resulted in a grain yield increase and two treatments resulted in a grain yield decrease, this was not shown to be due to the root pathway being stimulated. In all foliar treatments except the LI 700® treatment at early tillering, even though there appeared to be higher P uptake from the soil for each foliar treatment compared to the control, there were no significant differences. It may be that due to the low P status of the Black Point soil (both available and total), the foliar application was not able to stimulate root uptake of P as the amount of available P in the soil was too small.

The degree of scorch was not correlated with yield. However, scorch was very high for all treatments that had drops of fertiliser adhering to the leaves (all fertilisers except the glycerol treatment). It is likely that the scorch score was lower for glycerol only because most of the drops did not adhere to the leaves. The scorch measured in this experiment is unlikely to be a result of the adjuvants themselves, but more likely a combined effect of the low pH and the salt load of the fertiliser solutions, which resulted in scorch scores similar to those described in Peirce et al. (2014) when phosphoric acid was applied at rates equivalent to 1.0 and 2.6 kg ha^{-1}. Although the scorch was not correlated to yield, there is a possibility that the scorch inhibited any potential yield increases that may have resulted from the foliar P application. Reductions in yield with foliar application of P have often been attributed to scorch for a

number of different crops (Barel and Black 1979a; b; Parker and Boswell 1980). This could be a direct result of decreased photosynthesis of the plant due to leaf damage (Fageria et al. 2009). The reduction in yield could also be as a result of the formulation causing general or localised cell death (phytotoxicity) due to the rapid uptake of components of the formulation into the plant cells, as has been documented for herbicides (Zabkiewicz 2000). As a result of this rapid uptake, the localised death of the leaf cells can in turn reduce the ability of the cells to translocate P and other nutrients from the treated leaves to other plant parts.

Wettability

Although a control foliar treatment with phosphoric acid only was not included in this experiment, it is likely to have resulted in P uptake, translocation and yield results similar to the glycerol treatments due to the low adhesion of the drops on wheat leaves. We considered including a no adjuvant foliar treatment but decided against it due to the difficulty in applying the drops to the wheat leaves without them rolling off (Fernández et al. 2014). The inclusion of an organosilicone surfactant which induces super-spreading of the formulation and promotes stomatal infiltration (Stevens 1993; Stevens et al. 1991) was also considered in this experiment but excluded due to the instability of the product at a low pH (Stevens 1993) as would be the case in a formulation containing phosphoric acid.

As has been shown in this study and other studies (Fernández et al. 2014; Peirce et al. 2014; Peirce et al. 2015), the adaxial side of wheat leaves, to which we applied the foliar fertilisers to in our study, was difficult to wet. Due to the high advancing contact angle and low hysteresis (difference between advancing and receding contact angles), the adaxial leaf side is sometimes classified as superhydrophobic (Lafuma and Quere 2003). This indicates that water and fertilisers with a surface tension similar to water have difficulty adhering to the leaf surface, resulting in loss of foliar fertiliser and reduced uptake efficiency. In the absence of a surfactant, the contact angle measurements suggest that fertiliser drops were in a Cassie-Baxter state (Cassie and Baxter 1944) where the drops rested on top of the surface structures (waxes and trichomes). The addition of an adjuvant that contained a surfactant (all adjuvants in this study except glycerol) resulted in a reduction in both the advancing and receding contact angle when compared to water or phosphoric acid alone. In all cases except glycerol, the contact angle reduction resulted in fertiliser drops changing to a Wenzel wetting state (Wenzel 1936) where the drop penetrated into the surface structure of the leaves resulting in difficulty removing the drop and a receding contact angle of zero. It also means that drops were unlikely to roll off once attached to the plant.

However, during application of fertiliser to intact leaves a small percentage of droplet loss was observed (Table 4). This estimated run-off may be a slight overestimation as only partial

loss of drops (a small film of the drop remained on the surface) occurred while we assumed loss of the full volume of the drop. Although the contact angles of glycerol were not statistically different for the two timings, the estimated loss through drops not adhering was significantly higher at the earlier timing. However, in order for any of the droplets of glycerol to adhere at all, the volume of the droplet had to be increased 2-3 times the volume of the other fertilisers, which would result in droplet flattening due to gravity and a lower contact angle than measured due to the differences in volume (Shirtcliffe et al. 2010). The large size of the glycerol fertiliser drops would be much higher than the size of spray droplets and therefore not relevant when compared to field application.

The difference in wettability between the adjuvants is expected as they have different properties. The two commercial adjuvants Agral® and LI 700® are somewhat unknown as manufacturers do not disclose the exact formulation. Agral® had one active ingredient that is a non-ionic surfactant. It is also made up of 39 % non-hazardous (and undisclosed) ingredients. LI 700® is a mixture of propionic acid and soyal phospholipids with multiple modes of action, including claims to both acidify the solution and aid penetration of the cuticle. Due to the emulsion nature of the formulation, homogeneity within the solution was difficult to achieve and resulted in higher variability for contact angles measured with this solution. Genapol® X-080 is a non-ionic surfactant, which greatly reduces the surface tension (27 mN m^{-1} at 0.1 % (Khayet and Fernández 2012) compared to 72.8 mN m^{-1} for water) of the fertiliser to allow complete wetting of the leaf surface. Triton™ X-100, although also a surfactant, does not reduce the contact angle as drastically as Genapol® X-080. Although there were differences in wettability, the uptake was not affected by the choice of adjuvant with the exception of glycerol and is consistent with the results of Peirce et al. (2015). This may be due to the penetrating ability of the phosphoric acid itself, as evidenced by the high leaf burn that occurs as the P penetrated the leaf surface for the fertiliser treatments that adhered to the leaf.

Foliar P uptake and translocation

The uptake of foliar P is in agreement with a recent study which investigated the influence of adjuvants on leaf wettability and the initial uptake and translocation of P from phosphoric acid formulations (Peirce et al. 2015). In this study, which harvested the plants seven days after foliar application, there were no differences in uptake between adjuvants with up to 98% uptake by the plants. If in this longer term study the unrecovered P is assumed to be located in the roots, there were similar uptake rates (94-98 %) for all adjuvants containing surfactants across both timings. These uptake results are much higher than rates for lower P concentrations and with other P compounds as found by Reed and Tukey (1978) (1-23 % uptake from 25 mM phosphates of potassium, sodium, ammonium and calcium applied to

chrysanthemum leaves). It is hard to compare uptake between our study and most other studies as many excluded the foliar-treated area as they could not distinguish between absorbed P and P on the outside of the leaf (Bukovac and Wittwer 1957; McBeath et al. 2011). The high uptake rates in our study are not surprising as the method of application was a targeted droplet application rather than a spray. In order for the fertiliser to be labelled with ^{33}P and safely applied with known accuracy of the application rate, drops were deposited carefully on the leaves rather than sprayed as would be done under field conditions. The potential efficacy of the spray process is particularly affected by the first process involved, deposition. Depending on the environmental (wind, temperature) and spray (nozzle choice, operating pressure etc.) conditions, 5-20 % of the spray may not reach the plant surface and can be lost as spray drift or can evaporate before reaching the target (Zabkiewicz 2007).

Previous studies for fertilisers (Fernández et al. 2006; Koontz and Biddulph 1957; McBeath et al. 2011; Rolando et al. 2014), herbicides and pesticides (Baker et al. 1992; Gaskin and Holloway 1992; Stock et al. 1992) have shown that adjuvants can have either a positive or negative effect compared to a control by influencing the uptake of the active ingredient, efficacy or yield. For example, Koontz and Biddulph (1957) tested nine different adjuvants in combination with sodium phosphate and found that none of them increased and two anionic surfactants (Tergitol 7 and Vatsol OTB) decreased the translocation of P from red kidney beans. In contrast, Fisher and Walker (1955) noted a seven-fold increase in the apparent absorption of potassium phosphate with the addition of Triton X-100 by McIntosh apple leaves. The studies for fertilisers however were rarely done on wheat and often conducted for plants with unknown leaf wettability (Koontz and Biddulph 1957). In the case of easy to wet leaves, the need for a surfactant in the spray solution may not be essential, unlike for wheat. In our study the role of the adjuvant was to reduce the surface tension of the solution and allow it to adhere to the leaf. The adjuvant choice (excluding glycerol) did not change the uptake or translocation of foliar applied P between treatments. This shows that not only the adjuvant needs to be taken into account with studies on uptake, but also the leaf surface properties need to be considered.

The reduced translocation observed when foliar application occurred during tillering may be attributed to the higher phytotoxicity of the formulation at this early growth stage or the reduced ability of the tiller leaves, at their early stage of development, to translocate nutrients out of the leaves. This is consistent with a study by Koontz and Biddulph (1957) which showed that immature bean leaves did not translocate any ^{32}P to other plant parts within 24 hours compared to fully expanded leaves, which showed rapid translocation. Sargent and Blackman (1962) also found an inverse relationship between the maturity of *Phaseolus vulagris* (French bean) leaves and the rate of 2,4-D with potassium phosphate penetration. It

may be that the rapid uptake of foliar applied P by the wheat leaves at the earlier timing resulted in more severe scorch and a reduction in the translocation of P out of the leaves. A younger leaf will also still be a sink for P rather than acting as a source of P for re-translocation. If damage occurs between the timing of foliar application and the leaf changing to a source phase, there may be a reduction of translocation when grown through to maturity.

The addition of glycerol as one of the treatments was included due to Stein and Storey (1986) showing that, for soybeans, this was the only adjuvant that helped to increase the uptake of P and N into the leaves. If a surfactant had been included in the glycerol treatment to lower the surface tension of the solution there may have been an increase in foliar P uptake, but due to the low adhesion of the drops, any potential increase in uptake due to the humectant properties of glycerol was negated. Using the proportion of adhered drops that were translocated, (i.e. translocation as a percentage of uptake) the overall translocation was greater than 80 % for glycerol compared to an average of 40 % when applied at tillering and 70 % when applied at ear emergence for the other surfactants. This could be due to the glycerol treatment significantly increasing the time the fertiliser remained in solution - some glycerol drops were still present three days after application while the other surfactants dried within one or two hours. The larger drop size for the glycerol treatment would also have affected the available time for uptake and may have contributed to the higher translocation when expressed as a percentage of foliar uptake.

It is plausible that the grain response measured for the LI 700® treatment occurred due to the humectant properties of the adjuvant compared to the other treatments. The humectant properties arise from the soyal phospholipid part of the LI 700® adjuvant which slows the rate of droplet drying and allows it to stay in solution longer compared to other surfactants. Peirce et al. (2015) also reported a much longer drying time of LI 700® compared to Genapol® X-080 but a similar time to Agral®. For this reason, it may be the combination of longer drying time and reduced spread of the droplet (meaning a lower area of the plant scorched and therefore lower phytotoxicity) which resulted in a positive yield response to phosphoric acid in combination with LI 700®. Interestingly, the yield response did not correspond to higher uptake or translocation and must therefore have been a result of a complex plant response to P from the foliar source. As a result, we suggest further investigation into the role of humectants in combination with surfactants is warranted.

Conclusions

The foliar application of phosphoric acid in combination with the adjuvant LI 700® produced an increase in grain yield when applied at flag leaf emergence but a decrease in grain yield when applied at early tillering. The foliar application of phosphoric acid with Genapol X-080 at early tillering also resulted in a yield decrease. The addition of glycerol to phosphoric acid had low fertiliser droplet retention on the leaves as would also be expected for phosphoric acid without an adjuvant, and reduced P uptake. All other foliar treatments had high P uptake regardless of whether they were applied at early tillering or flag leaf emergence. However, translocation of foliar P from the treated leaves to other plant parts was reduced when applied at early tillering rather than flag leaf emergence and is likely due to the high scorch and reduced ability of leaf cells to re-translocate P to other plant parts. From this study it is apparent that for phosphoric acid applied to wheat leaves, the foliar P formulation must contain a surfactant, which lowers the surface tension of the formulation, to allow retention of the fertiliser on the leaves. The choice of surfactant is not important for either foliar P uptake or translocation even though different surfactants reduced the contact angle of the fertiliser on the leaves to different degrees. However, it is likely that a formulation which is retained on the leaf (surfactant properties) and stays in solution for longer (humectant properties) will be more likely to produce a positive yield response under controlled environmental conditions. The timing of application appears to be more important than the adjuvant choice with early application resulting in leaf damage, which reduced the plant's ability to both photosynthesise and translocate nutrients around the plant.

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Chapter 5

Phosphorus fertiliser formulations for foliar application to enhance phosphorus nutrition and biomass production in wheat

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Phosphorus fertiliser formulations for foliar application to enhance phosphorus nutrition and biomass production in wheat

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Keywords

Foliar uptake, phosphorus source, adjuvant, isotopic tracer, biomass response

Abstract

Aims

The aim of this study was to test a range of phosphorus (P) formulations with varying pH, accompanying cation and adjuvant for their effectiveness as foliar fertilisers for wheat plants. The effectiveness was tested as the change in biomass and P uptake of wheat plants grown to anthesis as well as the recovery and translocation of the foliar-applied fertiliser using a ^{33}P tracer.

Methods

A total of 21 foliar fertilisers consisting of seven different P sources (laboratory reagents and commercial products) in combination with three adjuvants were evaluated in a pot experiment under controlled conditions. Wheat plants were grown in a P-responsive soil and foliar fertilisers were applied at the flag leaf visible growth stage (GS37). The effect of the ^{33}P-labelled formulations on plant growth, foliar P uptake and translocation were evaluated when harvested at the end of anthesis (GS68).

Results

Foliar P uptake was high for most formulations (>80 %), but more importantly, translocation of foliar applied P was positively associated with P uptake and plant biomass. There was no consistent effect of solution pH, adjuvant or accompanying cation on fertiliser effectiveness.

Conclusions

Foliar P is a highly effective pathway for P uptake by plants. Foliar uptake and translocation were not related to formulation pH or associated cations as positive biomass responses occurred across a pH range of 2.2 to 8.7 and for P associated with potassium, nitrogen and sodium.

Introduction

Phosphorus (P) is a macronutrient essential for plant growth with wheat yield limited by P deficiency through slowing the rate of tiller emergence and therefore reducing the overall number of tillers (Rodriguez et al. 1999). Fertiliser P that is applied to soil is readily sorbed and a large proportion of the added P will undergo chemical reactions that initially bind the P and remove it from the available pool (Hedley and McLaughlin 2005). Due to the low P efficiency of soil-based P fertiliser in the season of application, a high application rate is often required on soils where soil P reserves are low. For soils where P has been managed such that P levels are at a maintenance phase (Weaver and Wong 2011), the fertiliser requirement is often marginal and dependent on in-season rainfall (McBeath et al. 2012). In Mediterranean systems like southern Australia, application of P to soils occurs at sowing. As a result, it does not allow the subsequent climatic conditions to be taken into consideration. With increasing costs of fertiliser P, this fertiliser input represents a large capital investment that could potentially be managed more effectively.

Foliar application of P is a management strategy that can be used in-season to increase the P status of the plants and possibly increase yields during seasons of higher yield potential (and in response to favourable climatic conditions). It may also allow a reduction in starter P inputs to the soil that could provide cost benefits to farmers. However, the efficiency of foliar application is variable due to the interactions that occur between the plant, the fertiliser formulation and the environment (Fernández and Eichert 2009). These interactions can influence the processes of deposition, retention, uptake and translocation of the foliar fertiliser, which will all determine the overall efficacy of the foliar P application. As a result, achieving consistent yield responses to foliar applied P has been elusive (Noack et al. 2011). In a previous study, we measured a 25 % grain yield response of wheat to foliar applied phosphoric acid in the growth room in one of the two soils evaluated (McBeath et al. 2011). Further work focussed on the use of phosphoric acid as the P source due to this initial yield response and the ready availability of the product to farmers. However, yield responses to phosphoric acid have been inconsistent. This is despite the foliar uptake of P from phosphoric acid being high (greater than 90 % of the applied P) (Peirce et al. 2015).

There is scope to investigate the effectiveness of other P formulations as foliar fertilisers since the accompanying cation and formulation pH can affect foliar P uptake (Koontz and Biddulph 1957). Previous studies have investigated the influence of replacing hydrogens from phosphoric acid with either ammonium, sodium or potassium and also altering the pH (Tukey et al. 1956). It is generally accepted that a low formulation pH of 2-3 facilitates more rapid uptake of foliar P (Bouma 1969; Swanson and Whitney 1953; Tukey et al. 1961) although it

is often associated with necrotic spots and high leaf burn. For this reason, it is difficult to distinguish between the effect of pH and the effect of associated cation, with most studies looking at the combined effect (Koontz and Biddulph 1957). The results from Koontz and Biddulph (1957) suggest that it is not a simple process of a lower pH resulting in higher uptake and translocation given that at a pH of 5, NaH_2PO_4 had the highest translocation of P away from the treated area whilst KH_2PO_4 had the lowest. Furthermore, most previous studies that investigated the effect of pH and accompanying cation were conducted at P concentrations well below those used in the field (Bouma 1969; Koontz and Biddulph 1957; Tukey et al. 1961).

In addition to the source of P used, foliar fertilisation often includes the use of an adjuvant. An adjuvant is defined as any chemical added to a foliar spray solution that modifies the spray characteristics of the solution or aids in increasing the penetration of the leaf by the active ingredient (Hazen 2000). As a result, there are a large number of different chemicals which are classified as adjuvants including, oils, humectants, pH buffers and surfactants. The use of adjuvants is especially important for wheat crops, due to the hydrophobic nature of the wheat leaves rendering them difficult to wet with hydrophilic foliar sprays (Holloway 1969; Netting and von Wettstein-Knowles 1973; Peirce et al. 2015). The use of adjuvants can initially influence the deposition of the foliar fertiliser (through altering the spray characteristics including the droplet size) on the plant foliage (Spanoghe et al. 2007; Zabkiewicz 2007). Adjuvants also play a large role in increasing the retention of the sprays (particularly for surfactants, which lower the surface tension of the formulation) and aiding the uptake of the foliar applied P through increasing the diffusion of the active ingredient or increasing the hydration of the cuticle and hence its water permeability (Hess and Foy 2000). The role of adjuvants in the translocation of foliar P once it enters the plant cells is still relatively unknown although Stolzenberg et al. (1982) have shown there was little movement of a labelled surfactant and its metabolites away from the initial application site. It is therefore likely that adjuvants do not play a significant role once the P enters the leaf. Although the type of adjuvant was not important for either uptake or translocation of foliar-applied P when used in combination with phosphoric acid as the P source (Peirce et al. 2015), it is not known whether interactions may occur between adjuvants and other P sources to render them more or less effective.

We investigated, using isotopic tracer techniques, whether the source of P and solution pH influences wheat biomass response and foliar P uptake and translocation from seven different P products (commercial and laboratory grade) in combination with three commercial adjuvants applied to foliage at field applicable rates.

Methods

Experimental Design

The experiment was set up to investigate the uptake and translocation of seven different foliar P sources using different products in combination with three adjuvants belonging to different classes (Table 1). These products included phosphoric acid (treatment 1), two ammonium phosphate products (treatments 3 and 4), analytical grade sodium (Na) phosphate (treatment 6) and three potassium (K) phosphates; an acidic K-phosphate (treatment 2), an analytical grade K-phosphate (treatment 5) and a K-phosphate plus citrate (treatment 7). This gave a total of 21 P fertilisers (P product × adjuvant). Hereafter foliar P products and adjuvants will be referred to by treatment number (1-7) and adjuvant number (A-C). In addition to the fertiliser treatments, controls were set up with no foliar P application (treatment 0) to compare the effect of the foliar fertiliser on plant growth when harvested at peak biomass. This gave a total of 96 pots. The experimental design was a completely randomised design with four replicates of each foliar fertiliser treatment and 12 replicates of the nil foliar control.

Table 1: Phosphorus products and adjuvants evaluated in the growth room.

Treatment No.	**Products**	**N : P : K (w/w)**	**Manufacturer**	**pH of undiluted product**
1	*Phosphoric acid	0 : 26.9 : 0	Redox Pty Ltd	1.5
2	*PeKacid®	0 : 26.5 : 16.7	ICL Fertilizers	N/A
3	#Ammonium phosphate (MAP)	12.2 : 27.0 : 0	BDH Chemicals Pty Ltd	N/A
4	*Maxi-Phos 16 Neutral®	7.8 : 12.5 : 0	Spraygro Liquid Fertilizers	6.0-7.0
5	#Potassium Phosphate	0 : 22.8 : 28.7	Merck Pty Ltd	N/A
6	#Sodium Phosphate	0 : 22.5 : 0	BDH Merck Pty Ltd	N/A
7	*Pick 15-42®	0 : 9.4 : 26.3	Spraygro Liquid Fertilizers	10.0-11.0
Adjuvant No.	**Adjuvant**	**Composition**		**pH of 1% solution**
A	Hasten®	esterified and emulsified canola oil and non-ionic surfactants	Victorian Chemical Company Pty Ltd	7^
B	LI700®	soyal phospholipids and propionic acid	Nufarm Australia Ltd	3
C	Spreadwet 1000®	alcohol alkoxylate surfactant	SST Australia Pty Ltd	6-8

*Commercially available fertiliser #Analytical grade reagent ^pH of undiluted product

Growth Conditions

Wheat plants (*Trit*icum *aestivum* cv. Axe) were grown in 1.5 kg of soil in pots with a diameter of 10 cm and depth of 17 cm that were not free-draining. Plants were grown in a P-responsive soil (DGT-P 4 µg L^{-1}, Colwell P 3 mg kg^{-1} and total P 48 mg kg^{-1}) from Black Point, South Australia (S 34°36.776′, E137°48.599) with a surface soil pH of 8.5 and

additional soil characteristics as described in Peirce et al. (2014). Before sowing, the soil was wetted to 5 % w w^{-1} (field capacity (FC) was 22.2 % w w^{-1} as measured according to the method of Klute (1986)) with the following basal nutrients mixed through the soil using a mixer: nitrogen as $CO(NH_2)_2$ at 75 mg N pot^{-1}, P as H_3PO_4 at 4.8 mg P pot^{-1} (equivalent to 6 kg P ha^{-1} based on pot surface area), potassium as K_2SO_4 at 100 mg K pot^{-1}, magnesium as $MgSO_4.7H_2O$ at 25 mg Mg pot^{-1}, zinc as $ZnSO_4.7H_2O$ at 15 mg Zn pot^{-1}, copper as $CuSO_4.5H_2O$ at 12 mg Cu pot^{-1}, manganese as $MnCl_2.4H_2O$ at 2 mg Mn pot^{-1}, and total sulphur (S) applied in these reagents equating to 81 mg S pot^{-1}. Additional N and K were added to the basal nutrients (including control pots) to balance the N and K applied in the foliar fertilisers (Table 2).

Table 2: Foliar fertiliser formulation pH (of the applied solution including water) and nutrients added in basal soil and foliar applied fertilisers. Treatments are defined as the product number followed by the adjuvant where A is Hasten®, B is LI700® and C is Spreadwet 1000®.

Treatment	**pH**	**added in foliar fertiliser**		**added to soil**	
	(foliar applied fertiliser)	**N (mg pot^{-1})**	**K (mg pot^{-1})**	**N (mg pot^{-1})**	**K (mg pot^{-1})**
1A	1.4	0.0	0.0	76.1	104.5
1B	1.4	0.0	0.0	76.1	104.4
1C	1.4	0.0	0.0	76.1	104.5
2A	2.2	0.0	1.2	75.5	103.3
2B	2.2	0.0	1.2	75.5	103.3
2C	2.2	0.0	1.2	75.5	103.3
3A	4.3	0.7	0.0	75.4	104.5
3B	4.2	0.7	0.0	75.4	104.5
3C	4.3	0.7	0.0	75.4	104.5
4A	4.3	1.0	0.0	75.1	104.5
4B	4.2	1.0	0.0	75.1	104.5
4C	4.3	1.0	0.0	75.1	104.5
5A	4.4	0.0	2.0	76.1	102.5
5B	4.3	0.0	2.0	76.1	102.5
5C	4.4	0.0	2.0	76.1	102.5
6A	6.5	0.0	0.0	76.1	104.5
6B	6.5	0.0	0.0	76.1	104.5
6C	6.5	0.0	0.0	76.1	104.5
7A	8.7	0.0	4.5	76.1	100.0
7B	8.6	0.0	4.5	76.1	100.0
7C	8.7	0.0	4.5	76.1	100.0

After a week of equilibration, the soil was added to the pots and wetted to 70 % FC before sowing four pre-germinated seeds per pot at a depth of 1 cm. Immediately after sowing the surface of the pot was covered with 50 g of alkathene granules to minimise evaporation from

the soil surface and the soil was wetted to 80 % FC. When the plants were at the two-leaf growth stage they were thinned to the two most uniform seedlings per pot. Plants were watered by weight every two days to maintain 80 % FC and were grown in a controlled environment room (20 °C/15 °C day/night cycle of 12 h each for 41 days, 23 ^{o}C/15 ^{o}C thereafter until harvest at 62-65 DAS) with their positions randomised every few days. To ensure N was not limiting, at 15 and 27 DAS 25 mg N pot^{-1} was applied to the surface and watered in.

Foliar Fertiliser Application

Foliar fertilisers were applied 34 or 35 DAS (due to large number of treatments, application occurred over two days with two of four blocks treated each day) at Zadoks growth stage flag leaf visible (GS37 (Zadoks et al. 1974)). The P in the fertilisers was labelled with carrier-free ^{33}P (in the orthophosphate form) tracer to give an activity of 144 kBq pot^{-1} at application. Fertilisers were applied with an Eppendorf Multipette M4® (which controlled droplet size) as 40 × 2 µl drops to give an application rate of 1.6 mg P pot^{-1} equivalent to 2 kg P ha^{-1} in 100 L ha^{-1} total volume (0.65M P). Fertiliser application was spread between the five leaves on the main stem excluding the flag leaf provided they were healthy. If a leaf was not healthy, had been broken or already senesced, it was excluded from the foliar application. The location of foliar P application was marked to allow easy identification at harvest. Before foliar P application, the soil surface was covered with plastic wrap to ensure any drops that did not adhere to the leaves would not reach the soil surface. Visual observation of drops not adhering to the plant was noted, with loss of the whole drop volume assumed.

Plants were harvested at the end of anthesis rather than growing the plants through to maturity to ensure isotope activity was above detection limits. It has been shown that biomass, particularly head (spike) biomass at this growth stage is well correlated with final yield if favourable growth conditions (i.e. water, light, temperature) are maintained (Savin and Slafer 1991). Above-ground plant material was harvested and separated into heads, treated leaves, tillers and the main stem with the remaining leaves attached. All plant parts, including controls, were washed according to the method outlined in Fernandez et al. (2014) to remove fertiliser P not taken up by the leaves. After washing, treated leaves were scanned and images used to quantify the scorch from the fertiliser (mm^2 drop^{-1}) using the software program Image J. The total number of tillers per pot was counted with the growth stage noted for each tiller. Plant parts were then dried in an oven at 60 °C for 72 h before being weighed, ground and digested in boiling nitric acid and analysed for P by inductively coupled plasma - atomic emission spectroscopy (ICP-AES) (Zarcinas et al. 1987). A 2 mL sample of the digest was added to a vial with 10 mL of scintillation fluid (Ultima Gold™ AB) and isotope activity

determined using a Perkin Elmer Quanta Smart liquid scintillation analyser (Model Tri-Carb B3110TR). Washing solutions were also retained to analyse both total and radioactive P by both ICP-AES and liquid scintillation counting. All counts were corrected for decay to a single time point.

Calculations and Statistical Analysis

Total P in the plant was calculated as the sum of P (mg pot^{-1}) in all harvested plant parts after washing. The total P was then divided between P derived from the soil and seed or from the labelled foliar fertiliser. Phosphorus derived from the foliar fertiliser was calculated according to Equation 1.

$$P_{in\ plant\ part\ from\ foliar\ fertiliser}\left(\frac{mg\ P}{pot}\right) = \frac{P_{in\ washed\ plant\ part}\left(\frac{Bq}{pot}\right)}{Specific\ activity_{foliar\ fertiliser}\left(\frac{Bq}{mg\ P}\right)} \quad (1)$$

Using the washing solution, we could also calculate the amount of P that was not absorbed by the plant by dividing the activity of the washing solution by the specific activity of the foliar fertiliser. Absolute recovery of the foliar fertiliser was then calculated as the sum of what was recovered in the plant parts and in the washing solution as a percentage of the applied fertiliser (Equation 2).

$$\%\ fertiliser\ recovery\ = \frac{P_{in\ all\ plant\ parts\ from\ fertiliser}\left(\frac{mg\ P}{pot}\right) + P_{in\ washing\ solution}\left(\frac{mg\ P}{pot}\right)}{P_{added\ in\ foliar\ fertiliser}\left(\frac{mg\ P}{pot}\right)} \times 100 \quad (2)$$

Both foliar P uptake and foliar P translocation were expressed as a percentage of the applied foliar fertiliser recovered in the plant parts after they were washed (Equations 3 and 4). Translocation was calculated as the percentage of foliar-applied P recovered in all the plant parts at harvest excluding the treated leaves (Equation 4).

$$\%\ foliar\ uptake\ = \frac{P_{in\ all\ plant\ parts\ from\ fertiliser}\left(\frac{mg\ P}{pot}\right)}{P_{added\ in\ foliar\ fertiliser}\left(\frac{mg\ P}{pot}\right)} \times 100 \quad (3)$$

$$\%\ foliar\ translocation\ = \frac{P_{in\ all\ plant\ parts\ from\ fertiliser}\left(\frac{mg\ P}{pot}\right) - P_{in\ treated\ leaves}\left(\frac{mg\ P}{pot}\right)}{P_{added\ in\ foliar\ fertiliser}\left(\frac{mg\ P}{pot}\right)} \times 100 \quad (4)$$

Analysis of variance (ANOVA) was carried out using Genstat® V.15 statistical package for all data using a one-way ANOVA for the 22 treatments (control treatment and 21 foliar fertiliser treatments) except for the scorch data which were not normally distributed. Assumptions of distribution normality and constant variance error were tested for all data analysed. Least significant difference (l.s.d.) between treatments was calculated using Fisher's protected l.s.d. at the 5 % significance level. For scorch data, the ANOVA and pairwise comparisons were undertaken using PerMANOVA (Anderson 2005), at the 5 % significance level.

Results

Plant growth and scorch

There were differences in above-ground dry weight between treated plants and the controls (no foliar fertiliser, treatment 0) (Figure 1a). Three treatments resulted in an increase in the total above-ground biomass compared to the control, namely product 2 (acidic K-phosphate) with adjuvant C, product 6 (Na-phosphate) with adjuvant A and product 7 (K-phosphate plus citrate) with adjuvant C. These three treatments (2C, 6A and 7C) had higher tiller biomass compared to the control, which corresponded with higher number of viable tillers (GS45 or later) that would grow through to grain (Figure 1b). In all cases, the heads associated with tillers were not fully emerged at harvest (ranged from seedling stage through to late booting where awns were visible). There were no differences in the overall number of tillers between treatments (data not shown). Although no treatments had reduced tiller biomass compared to the control, fertilisers 4B and 7B had fewer than one tiller on average per pot (Figure 1b). There were five treatments that had greater head biomass compared to the controls, namely product 3 with adjuvant A, product 6 with adjuvant B and product 7 with all three adjuvants.

There was no scorch associated with the control plants or for product 5 (analytical reagent K-phosphate) with either adjuvant A or adjuvant B (Figure 2). Visual observation of the leaves from treatment 5A and 5B indicated that rather than scorching leaves, the foliar fertiliser dried as a crystalline deposit on the leaf surface. This crystalline deposit was subsequently removed during the washing step, as confirmed by analysis of wash solutions (Table S 1). For all other foliar fertilisers, the scorch area gave an accurate representation of where the drops were deposited and spread on the leaves, although not all drops produced scorch or the same degree of scorch (for representative photos of scorch, see Figure S 1). The scorch did not appear to be directly related to pH or associated cations, however the most acidic (Product 1) and most basic (Product 7) products had high scorch across all three adjuvants. For the other five products (2-6) there were differences in scorch depending on the associated adjuvant. In all cases the scorch with adjuvant C (non-ionic surfactant) was highest, indicating the drops spread more on the leaf surface than for the other two adjuvants. The scorch was not correlated with biomass response since the scorch area ranged from 0.10 mm^2 $drop^{-1}$ for treatment 6A to 0.23 mm^2 $drop^{-1}$ for treatment 2C despite both of these treatments recording an increase in biomass compared to the control.

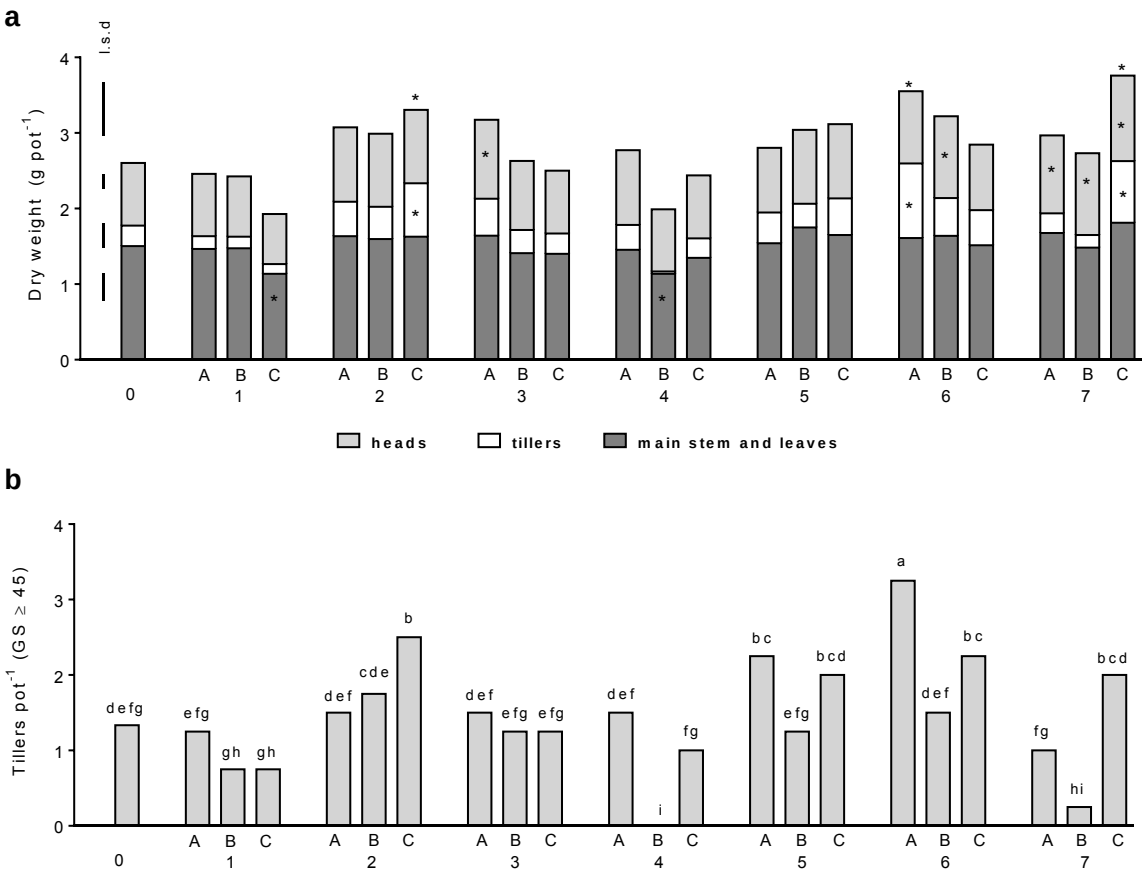

Figure 1: (a) Total above-ground biomass (g pot^{-1}) for wheat plants harvested at the end of anthesis that were fertilised with (seven products, see Table 1) or without foliar P (control) at Zadoks GS 37 in combination with three adjuvants. Significant differences compared to the control indicated by an asterisk (*) for total (above the bars), tiller, head and main stem and leaves (on the bars) dry weights ($p \leq 0.05$, n=4 for all foliar treatments, n=12 for control, l.s.d. indicated on graph: total 0.70, heads 0.18, tillers 0.31, main stem and leaves 0.36) (b) Number of tillers per pot that had reached the growth stage of booting (GS 45) or later when plants were harvested at the end of anthesis. Different letters above the bars indicate significant differences in tiller number ($p \leq 0.05$, n=4 for all foliar treatments, n=12 for control, l.s.d. 0.67)

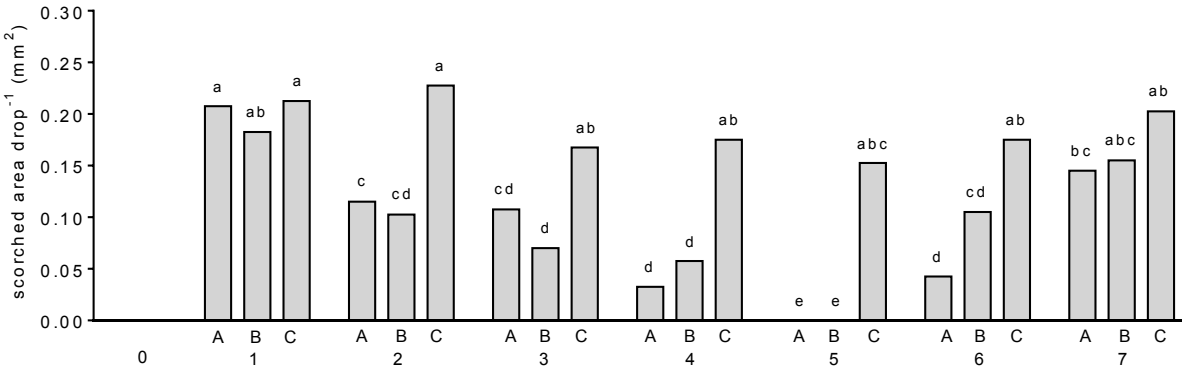

Figure 2: Average scorch area (mm^2) for the seven foliar products in combination with the three adjuvants as measured at harvest (see Table 1 for description). Scorch area represents the scorched area caused by a 2 µL drop applied to the leaf which was replicated 40 times per wheat plant. Different letters above the bars indicate significant differences in scorched area (PerMANOVA $p \leq 0.05$, n=4).

Recovery of foliar P, P uptake and translocation

There was high foliar P uptake for most of the 21 treatments (Figure 3). In most cases, nearly all of the recovered foliar fertiliser was located within the plant parts (Figure 3 and Table S 1), only a small proportion (0-3 %) of the fertiliser was observed to not adhere to the leaves (data not shown). Most of the products also had a large proportion of the foliar P translocated out of the treated leaves (Figure 3). The main translocation sink across all treatments was the head accounting for between 5 and 49 % of the applied fertiliser P (Table S 1). There was only one P product, Product 5 (analytical grade K-phosphate) that had a substantial proportion of the foliar fertiliser (8-32 %) washed off the leaves and this was the only product with more than 5 % of the fertiliser recovered in the washings (Table S 1). For treatments 5A and 5B, the high recovery of foliar P in the washings corresponded to no scorch on the leaves as mentioned above (Figure 1b). The treatments that had lower foliar uptake (less than 80 %) and lower overall recovery of the spike (the sum of plant uptake and washed off P from Table S 1) were fertilisers 3A, 4A, 5A, 5B, 5C and 6A. There were no common associated cation properties for these treatments as they represented ammonium phosphate, K-phosphate and Na-phosphate products. These treatments were often associated with the oil-based adjuvant A. The pH of five of these treatments (3A, 4A, 5A, 5B and 5C) was around 4.3 whilst the pH of 6A was 6.5. However, overall the foliar uptake of P was not directly related to pH with high uptake across the full range of formulation pH values tested.

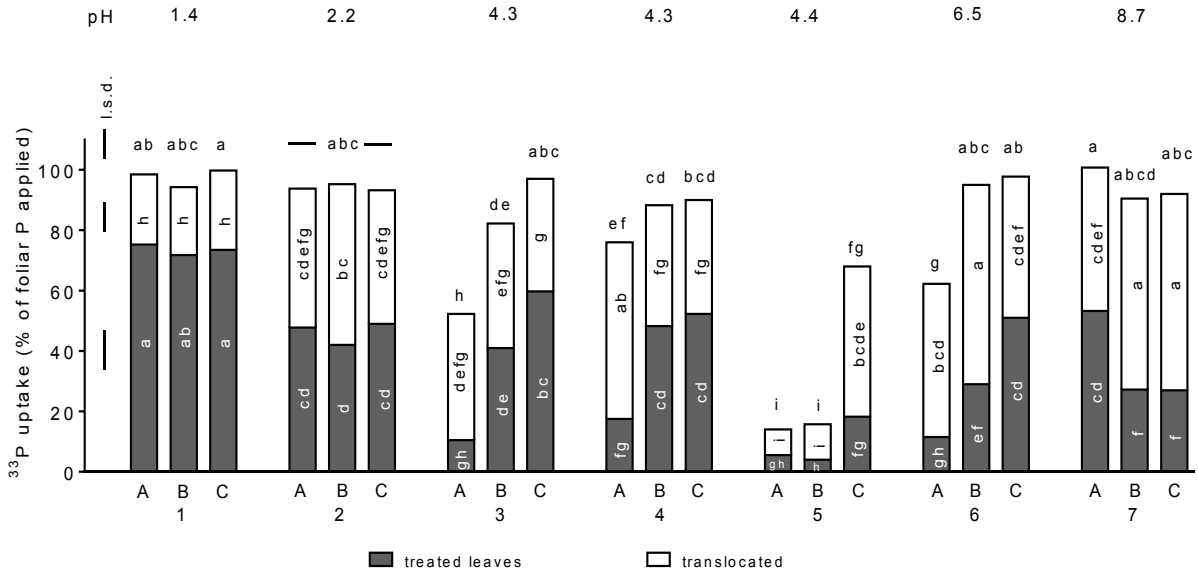

Figure 3: Plant foliar P uptake (% of foliar P applied) from the seven foliar products in combination with three adjuvants (see Table 1 for description). Uptake is divided between the amount that remained in the treated leaves when harvested at the end of anthesis and the fertiliser that was translocated to other above-ground plant parts. Different letters above the bars indicate significant differences in total foliar uptake, different letters within bars indicate significant differences in translocated foliar P or treated leaves P ($p \leq 0.05$, n=4, l.s.d. indicated on graph: treated leaves 13, translocated 10, total uptake 10). The average formulation pH for each product is provided above the graph.

Of the products that were commercially sourced (Products 1, 2, 4 and 7) the uptake of foliar P was high regardless of which adjuvant they were applied with (76-100 % of applied amount) (Figure 3) and even though they varied in their chemical components with pH ranging from 1.4 to 8.7 and associated cations of phosphoric acid, ammonium phosphate and K-phosphate. The products that were analytical grade reagents (Products 3, 5 and 6) had variable uptake results (ranging from 14 % for treatment 5A to 98 % for 6C) with uptake within a product being higher when used in combination with Adjuvant C (surfactant) than Adjuvant A (oil-based). There were no common features between the analytical reagents with each product having a different pH and different associated cation. Strikingly, Product 1 (phosphoric acid) differed from all other products in the amount of foliar P that was translocated to other plant parts (Figure 3). Only 22-26 % of the applied P was translocated with the remainder (72-75 %) located in the treated leaves at harvest. In comparison, all other products had a significantly higher proportion of their P translocated out of the treated leaves (38-82 % of the foliar P recovered in the plant parts). Products 1-3 showed no difference between adjuvants with the same translocation of P to the heads, regardless of the adjuvant used. For Products 4, 6 and 7, translocation to the heads varied depending on the adjuvant used. Translocation to the stems accounted for 2-13 % of the foliar applied P with no differences between adjuvants for each product with the exception of Product 5. Translocation to the tillers was related to a positive tiller biomass response in comparison to the controls, namely treatments 2C, 6A and 7C, while for other treatments translocation to the tillers was generally less than 5 % of the foliar fertiliser applied.

Due to the contribution of P from the foliar fertilisers, the plant P content in the whole plant was greater than the control for most of the treatments (15 of the 21 treatments) (Table 3). Treatments that had higher total P content in the treated leaves generally had higher whole plant P content. The increase in P content appeared to be driven by the increased P concentration of the plant since all 15 treatments that had higher P contents than the control also had higher P concentrations than the control (Table S 2). The contribution of foliar P to the total plant P varied from 5 % for treatment 5A, which had low foliar uptake (14 %) to 41 % for treatment 4B which had high foliar P uptake (88 %) but low soil-derived P. Treatments with a positive biomass response had foliar-derived P accounting for between 19 % and 36 % of the whole plant P.

The treated leaves of plants from foliar treatment 1 (phosphoric acid) had the highest P content derived from both the foliar and soil P sources of all the treatments leading to a total P content 4.4-4.9 times the control (Table 3). Most foliar treatments had higher total P content in the treated leaves compared to the control as would be expected due to the contribution of

extra P from the foliar fertiliser (Table 3). It appeared that the oil-based adjuvant A allowed better translocation of foliar P to the other plant parts for some formulations as shown by lower fertiliser derived P in the treated leaves. For treatments 5A and 5B, the lower fertiliser derived P in the treated leaves was a result of lower foliar uptake.

The total P content for both foliar and soil sources in all other plant parts was higher for a number of treatments compared to the control (Table 3). All treatments which had a tiller biomass response (2C, 6A and 7C; see Figure 1) also had higher total plant P content. Only one other treatment (6B) that produced a head biomass response (Figure 1a) had a higher plant P content than the control. Treatments 2A, 2B and 5C also had an increase in plant P content in all other plant parts even though the increased P content did not produce a biomass response.

Only a few treatments resulted in increases in the concentration of measured macronutrients (P, K and S) compared to the control (Tables S 2, S 3, and S 4). Increases in P concentration (Table S 2) followed the same trends for increases in P content (Table 3). Increases in the K concentration of whole shoots compared to the control occurred for only two treatments (6A and 7C; see Table S 3) with only one of those treatments containing K in the foliar fertiliser. Despite three products containing K, none of the treatments increased the K concentration of the treated leaves. One treatment (6A) increased the S concentration whilst another treatment (4C) decreased the S concentration of whole shoots compared to the control (Table S 4). Treatment 6A was the only treatment to increase the concentration of all measured macronutrients in whole shoots.

Table 3: Plant P content (mg per pot) as P derived from foliar fertiliser and P derived from the soil and seed for the different above-ground plant parts when harvested at the end of anthesis. For foliar P contributions, different letters within columns indicate significant differences in the average foliar contribution to the plant part, for soil and total P contribution, significant differences compared to the control (0) are indicated by an asterisk (*) ($p \leq 0.05$, n=4 for all foliar treatments, n=12 for control, l.s.d. indicated in the table)

Treatment		Untreated plant parts			Treated Leaves			Whole Plant		
		Foliar	Soil	Total	Foliar	Soil	Total	Foliar	Soil	Total
0			2.93	2.93		0.37	0.37		3.30	3.30
1	A	0.36 ^{h}	3.23	3.58	1.16 ^{a}	0.65 *	1.80 *	1.51 ^{abcd}	3.88	5.39 *
	B	0.34 ^{h}	2.83	3.17	1.10 ^{ab}	0.63 *	1.73 *	1.44 ^{abcde}	3.45	4.90 *
	C	0.41 ^{h}	2.29	2.69	1.13 ^{ab}	0.50	1.63 *	1.54 ^{abc}	2.78	4.32 *
2	A	0.76 ^{cdef}	3.19	3.96 *	0.79 ^{cd}	0.51	1.30 *	1.56 ^{ab}	3.71	5.26 *
	B	0.88 ^{bcd}	3.04	3.92 *	0.70 ^{d}	0.43	1.13 *	1.58 ^{a}	3.48	5.06 *
	C	0.73 ^{defg}	3.38	4.11 *	0.82 ^{cd}	0.50	1.31 *	1.55 ^{ab}	3.87	5.42 *
3	A	0.65 ^{fg}	3.01	3.66	0.16 ^{gh}	0.37	0.53	0.81 ^{j}	3.38	4.19
	B	0.64 ^{fg}	2.94	3.58	0.64 ^{de}	0.40	1.04 *	1.28 ^{fg}	3.34	4.62 *
	C	0.58 ^{g}	2.50	3.08	0.93 ^{bc}	0.40	1.33 *	1.51 ^{abcd}	2.90	4.41 *
4	A	0.92 ^{abc}	2.41	3.33	0.27 ^{fg}	0.27	0.55	1.20 ^{gh}	2.68	3.88
	B	0.63 ^{fg}	1.68 *	2.30	0.76 ^{cd}	0.31	1.07 *	1.39 ^{cdef}	1.98 *	3.37
	C	0.59 ^{g}	2.61	3.20	0.82 ^{cd}	0.47	1.29 *	1.41 ^{bcdef}	3.08	4.49 *
5	A	0.14 ^{i}	3.44	3.57	0.09 ^{gh}	0.46	0.55	0.22 ^{k}	3.90	4.12
	B	0.18 ^{i}	3.29	3.47	0.06 ^{h}	0.50	0.56	0.24 ^{k}	3.79	4.03
	C	0.78 ^{cdef}	3.30	4.08 *	0.29 ^{fg}	0.44	0.73 *	1.07 ^{hi}	3.74	4.81 *
6	A	0.82 ^{bcde}	3.51	4.34 *	0.19 ^{gh}	0.43	0.62	1.01 ^{i}	3.95	4.95 *
	B	1.06 ^{a}	2.75	3.81 *	0.47 ^{ef}	0.26	0.73 *	1.53 ^{abc}	3.01	4.54 *
	C	0.75 ^{def}	2.94	3.69	0.82 ^{cd}	0.41	1.23 *	1.57 ^{a}	3.35	4.92 *
7	A	0.70 ^{efg}	2.73	3.44	0.79 ^{cd}	0.50	1.29 *	1.50 ^{abcde}	3.23	4.73 *
	B	0.94 ^{ab}	2.16	3.11	0.41 ^{f}	0.27	0.67 *	1.35 ^{efg}	2.43	3.78
	C	0.97 ^{ab}	3.19	4.16 *	0.40 ^{f}	0.42	0.83 *	1.37 ^{def}	3.61	4.99 *
l.s.d. ($p \leq 0.05$)		*0.16*	*0.83*	*0.87*	*0.46*	*0.15*	*0.26*	*0.15*	*0.92*	*0.96*

Discussion

Our study shows that foliar applied P is effectively taken up by wheat leaves leading to an increase in biomass for some treatments when harvested at the end of anthesis that may translate to an increase in grain yield. Overall, these responses do not seem highly dependent on source of P (pH, accompanying cations) or adjuvant type with the exception of analytical grade K-phosphate (treatment 5).

Overall biomass, uptake and translocation responses

Some of the foliar fertilisers produced positive anthesis biomass responses compared to the control. In particular, the product Pick 15-42 (K-phosphate plus citrate) with all three

adjuvants produced the most consistent head biomass increase over the control. All increases in tiller biomass from foliar treatments were due to an increase in the number of viable tillers that were in a booting stage at harvest and would have produced more grain compared to the control. The tillering response is the most plastic yield trait in response to varying environmental conditions (Sadras and Slafer 2012). As tillering is limited by P deficiency through slowing leaf emergence and reducing the maximum rate of tiller emergence (Rodriguez et al. 1999), the additional P supplied *via* foliar application for treatments PeKacid® + Spreadwet 1000®, sodium phosphate + Hasten® and Pick 15-42® + Spreadwet 1000® (2C, 6A and 7C) helped to improve the P nutrition of the plant and promote further tillering. An increase in biomass due to increased uptake of P from foliar application has also previously been reported (McBeath et al. 2011; Mosali et al. 2006; Sherchand and Paulsen 1985).

The high efficiency of the foliar pathway found in this study is consistent with other studies which used foliar P at field applicable concentrations (McBeath et al. 2011; Peirce et al. 2015; Peirce et al. 2014). The efficiency of foliar P uptake was not affected much by formulation with only analytical grade K-phosphate having much lower efficiency due to crystallisation of the fertiliser on the surface, which was subsequently washed off the leaves. Even though high recovery efficiencies were achieved by most foliar treatments, most of the P in plants at harvest was derived from the soil (70-80 %) with foliar P providing 20-30 % of total plant P for most treatments. It is expected that higher P uptake from the foliar fertiliser and therefore more P resources for the plant to grow, would lead to above-ground biomass increases compared to the control, particularly if the tissue P concentration was low. The P concentration of whole shoots for the control treatment was below the threshold for P adequate plants when harvested at anthesis according to Elliott et al. (1997) (measured 824 mg kg^{-1} (Table S 2) compared to the threshold of 1600 mg kg^{-1}). A number of foliar P treatments were able to significantly increase the P concentration of whole shoots but none of them raised the concentration above the critical threshold. It has been shown that severely P deficient plants are not able to absorb foliar applied P whilst marginally P deficient plants and P sufficient plants could absorb foliar applied P (Fernández et al. 2014). In our study, although we were working at a marginally P deficient plant status, it is apparent that the amount of P that was foliar applied was not sufficient to increase the plants to a sufficient P level. Despite all plants being classed as P deficient, a few fertiliser treatments produced biomass responses and most treatments had high foliar uptake. As a result of only above-ground plant parts being harvested in this study, there is the potential for total plant uptake and translocation to be higher if some of the foliar applied P was translocated to the roots. It is

possible that the lower foliar P recoveries for some of the treatments (3A, 4A, 5A, 5B, 5C, 6A) may have been a result of foliar P translocated to the roots.

For a foliar application to be effective, once it is absorbed by the leaf, it must be able to move to the growing plant parts and be utilised for growth. Phosphorus is a nutrient that has been shown to be very effectively translocated from senescing plant parts to the grain when grown through to maturity (Batten et al. 1986). In our study, the expected sinks for foliar translocation were the flag leaf, head and tillers given the foliar P was applied before the transition to reproductive stages and plants were harvested well into the reproductive stages when translocation is high and can be the dominant P process rather than P uptake from soil (Grant et al. 2001; Peng and Li 2005; Waldren and Flowerday 1979). For all foliar treatments, translocation occurred to all three of these sinks with the majority of foliar P translocating to the head. The foliar treatments that produced a positive head biomass response generally had both higher total and foliar P contents in the head, as also noted by McBeath et al. (2011), although not all treatments with high total P contents produced a biomass response. This is consistent with the literature (Batten et al. 1986; Sherchand and Paulsen 1985) and reflects the complex responses of wheat yield to foliar applied P (Noack et al. 2011).

In our study, there was no adjuvant or product that was consistently best for all combinations in terms of total dry matter yield or P uptake and translocation. This indicates that there are interactions that may occur between products and adjuvants to reduce the efficacy of a foliar fertiliser. However, predicting these interactions and subsequent outcomes before application is not yet possible as discussed by Fernández and Eichert (2009). The choice of adjuvant for products with more acidic or basic pH (products 1, 2 and 7) was not important and showed no differences within the products for foliar uptake and translocation. The adjuvants chosen in this study represented different classes of adjuvants (i.e. an oil with a low hydrophile/lipophile balance (HLB), a surfactant with a high HLB and a lecithin mixture) with different modes of action. It is possible that for the products 1, 2 and 7 (phosphoric acid, PeKacid® and Pick 15-42®), which have the most acidic or basic pHs of all products, the only requirement needed from the adjuvant is an ability to reduce the surface tension of the formulation allowing the fertiliser to adhere to the leaf (i.e. surfactant properties). For these products, the scorch produced by the low or high pH may be able to act as a penetrating agent allowing high uptake of the P in the formulation. This mechanism was also discussed in Tukey et al. (1961) regarding the early work of Barinov and Ratner who discovered that 0.1 M phosphoric acid entered the leaf rapidly due to destruction of the protoplasm in epidermal cells and alteration of the cutin in the waxes. Alternatively, the high uptake associated with these products may have led to greater scorching as it penetrated the outer leaf surface.

A reduction in biomass due to scorch from the foliar fertiliser is not uncommon (Barel and Black 1979; Gooding and Davies 1992; Parker and Boswell 1980). Scorch is often the justification given for why foliar fertilisation with macronutrients is not feasible. In our study, we measured noticeable scorch of the leaves at the point of foliar application for most treatments. Given the amount of scorch observed (Figure 2 and Figure S 1), the lack of biomass depression with foliar fertiliser application (only within the stem and leaves for two treatments) was surprising. It is possible that there are opposing forces from the scorch reducing plant productivity and foliar- applied nutrients increasing plant productivity, which results in no biomass response compared to the control for some of the treatments. Our measurement of scorch in this experiment gave an approximation of the drop spread as often reported for crop protection studies (Forster et al. 2004; Lake 1977; Liu 2003) but did not provide information on the degree of scorch. For example, the type of scorch produced by phosphoric acid was very severe (produced more necrotic tissue) and visually different to the most alkaline product (Pick 15-42®) even though they had the same leaf area affected by scorch. This may help to explain why there were differences in the translocation of foliar P out of the treated leaves between these treatments.

The high P concentration (0.65 M) used in our study is consistent with rates used in field studies (Benbella and Paulsen 1998; Mosali et al. 2006; Sherchand and Paulsen 1985) but much higher than those used in transport and cuticular studies on foliar uptake (Bouma 1969; Koontz and Biddulph 1957; Thorne 1957; Thorne 1958). This is because application was designed to represent what is commercially possible for wheat production in the growing conditions of southern Australia (yield potential less than 5 t/ha, with multiple foliar applications not feasible). As a result of the relatively high P concentration of the foliar fertiliser, it is not surprising that we encountered scorch with nearly every foliar treatment. However, the scorch of this targeted application is likely much higher than that achieved in the field. This is due to the larger drop size of 2 µl used in this study compared to that achieved with a boom spray. At the concentration of salts that were applied, it is possible that the scorch associated with the salt load is increasing the rate of uptake by damaging the leaf cuticle itself. Since the only treatments that produced no scorch had foliar uptake 3.5-6.7 times less than any of the other foliar treatments; it is possible that the scorch created by the fertiliser created an additional pathway to allow the fertiliser to enter the leaf cells in this experiment. Despite the scorch damage, a large proportion of the foliar P was able to be re-translocated to other plant parts for most foliar treatments.

The effect of pH, adjuvant and accompanying cations

Product 1 (phosphoric acid) had high overall uptake across all formulations with the three different adjuvants. However, only 22-26 % was translocated out of the treated leaves. It is likely that the small amount of foliar P that was translocated, regardless of the high uptake, is responsible for the lack of biomass response for this product. This low translocation is consistent with our previous work at similar foliar P rates (Peirce et al. 2015; Peirce et al. 2014). The higher foliar and soil P located in the treated leaves compared to other treatments including the control (Table 3) showed that not only was translocation of foliar P out of the treated leaves hindered in this treatment but also translocation of P that originated from the soil. It may be that the scorch associated with the low pH of phosphoric acid rendered the plant cells unable to re-translocate P from the treated leaves. In previous work, a biomass response to phosphoric acid was found in one of the two soils tested (McBeath et al. 2011) and although that study did not separate out uptake and translocation, at maturity up to 90 % of the foliar fertiliser applied was located in the grain.

There were three products that contained phosphate in combination with potassium, namely Product 2 (PeKacid®), Product 5 (analytical grade K-phosphate) and Product 7 (Pick 15-42®). The best performing of these three products was Product 7, which consistently increased plant biomass across the three adjuvants. It also had high foliar uptake (> 90%) and translocation (47-65 %) of P. This product is a commercial K-phosphate and K-citrate solution that had an average pH of 8.7 when applied to the plants in combination with the adjuvants. Not only did all three treatments show a positive head biomass response but treatment 7C also had a strong positive tiller response. This was despite high scorch associated with the product across all three adjuvants. The acidic K-phosphate solution (Product 2) with Adjuvant C also produced a positive biomass response with tillers and total biomass. Once again, all three treatments with Product 2 had high foliar uptake (>90 %) and translocation (44-53 %) even though only one treatment produced a biomass response. In contrast, the analytical reagent K-phosphate had very low uptake and translocation with all the adjuvants, particularly with Adjuvants A (Hasten®) and B (LI700®), which produced no scorch on the leaves. It is not surprising that there was low uptake with Adjuvants A and B as a crystalline deposit formed on the leaf as the fertiliser dried. Reed and Tukey (1978) also observed decreased absorption for foliar fertilisers that dried as salt deposits on the leaf. They observed that for K-phosphates, the highest absorption by chrysanthemum leaves was at a pH of 2 (similar to Product 2 in our study) with absorption decreasing as the pH increased to 7, after which absorption increased again (Tukey et al. 1956). Those absorption trends with pH are consistent with our study which found highest absorption for K-phosphates for the products with either low or high pH. Interestingly, several field studies have documented wheat yield increases with application of

K-phosphate (Benbella and Paulsen 1998; Mosali et al. 2006; Sherchand and Paulsen 1985). However, these studies did not indicate the pH of the applied foliar fertilisers, but it would be expected to be similar to Product 5 in our study if prepared with high quality water. It is not known whether these yield responses were in response to the P or K in the fertilisers as the K applied in the foliar fertilisers was not balanced in the soil as was done in our study (Table 2) and little information was provided on the K status of the plants. Yield responses in the study by Mosali et al. (2006) were often documented in a field site with low soil K levels identified. In our study, the responses are unlikely to be due to the foliar K as the whole shoot K concentrations of the control plants were adequate (measured 1.6% (Table S 3) against critical values of 1.5 % at Zadoks GS50 and 0.9 % at Zadoks GS75 (Reuter and Robinson 1997)).

For the products that contained phosphate in combination with nitrogen (ammonium) (Products 3 and 4), only one treatment produced a positive head biomass response, treatment 3A. This is despite both products having the same pH. The only difference between the products was the ratio of N:P. When applied at the same P rate of 1.6 mg pot^{-1}, Product 3 added 0.7 mg N pot^{-1} to the leaves while Product 4 added 1 mg N pot^{-1} to the leaves. Despite the different amounts of N added in the foliar fertilisers, scorch was not different between the products. It is therefore difficult to say why the products did not produce the same biomass response. Foliar uptake was variable for the products ranging from 52 % to 97 %, but in all cases the translocation of foliar P was high (37-59 %). In a study on the foliar uptake of P by bean leaves comparing treatments across the pH range of 2-7 and the accompanying cations of K, Na and ammonium, ammonium phosphate solutions at a pH of 2-3 performed best (Tukey et al. 1956). Likewise, Reed and Tukey (1978) found that ammonium phosphate absorption was highest at pH 2 and was much lower (only 5-8 %) at all other pH values. Despite their results, we found quite high uptake of P from the ammonium phosphates suggesting that at higher concentrations, total foliar absorption increases.

Sodium phosphate (Product 6) was the second best performing product overall. It produced either a positive tiller and total biomass response or a head biomass response depending on whether it was applied in combination with Adjuvants A or B. Foliar uptake ranged from 62-98 % with high translocation particularly for 6B (66 %). Not many studies have examined sodium phosphate as a foliar P source. Tukey et al. (1956) showed that sodium absorption and translocation to the roots of bean plants was highest at a pH of 2-3 and second highest at a pH of 5 when the foliar fertilisers were applied at pHs ranging from 2 to 7 although the amount of P absorbed as a percentage of applied was not discussed. Reed and Tukey (1978) also found that P absorption using sodium phosphate reduced as the pH increased (from 22 % at a pH of 2 to 5 % at a pH of 6), percentage absorption values which are significantly lower than

measured in our study. Our results are comparable to Thorne (1957) who found 79-87 % of foliar applied P from sodium phosphate was recovered in the plant parts of swede and sugar-beet plants.

The inconsistent results regarding the use of adjuvants and their effect on foliar uptake and yield make generalisations difficult. The use of the adjuvant to increase the retention of the fertilisers on the leaves was performed equally well by all three adjuvants. In all cases, less than 3 % of the foliar fertiliser volume for each treatment was not retained by the leaves. However, the spread of the droplets was different between the fertilisers with those in combination with Adjuvant C (Spreadwet 1000®) having larger spread and hence higher scorch area per drop than those with the other two adjuvants. This is not surprising as Adjuvant C is a pure non-ionic surfactant which is used to increase the spreading of the fertiliser drops. We have shown in previous work, that uptake of foliar applied P was high regardless of the adjuvant used provided the adjuvant reduced the surface tension of the fertiliser to allow it to be retained on the leaf surface (Peirce et al. 2015). The second way adjuvants can influence the efficacy of a foliar application is their role in increasing plant uptake of the foliar P in the formulation. Given that the three adjuvants were chosen based on their belonging to different classes, it would be expected that they may influence the uptake by different mechanisms. As Adjuvant A (Hasten®) is an esterified oil with non-ionic surfactants, it would be expected to have components with both a low and high hydrophile/lipophile balance (HLB). Adjuvant B (LI700®) is an emulsion of soyal phospholipids and propionic acid (with some surfactants) which, apart from likely having both a high and low HLB, also claims to acidify the formulation. Given the low concentration of LI700 in the fertiliser formulation and the high pH buffering capacity of phosphate in solution, we saw negligible pH differences between formulations as a result of the adjuvants (Table 2). Adjuvant C (Spreadwet 1000®) is a pure non-ionic surfactant likely to have only a high HLB. Adjuvants with a high HLB have been documented to be more effective at increasing uptake of hydrophilic active ingredients (Stock et al. 1993), as is the case for foliar fertilisers. This is due to increases in the hydration of the plant cuticle to increase the diffusion of the nutrient through the cuticle (Hess and Foy 2000). Adjuvants with a high HLB are thought to influence the uptake of foliar applied chemicals through increasing the fluidity of the waxes on and in the cuticle as they diffuse easily through the lipophilic cuticle (Hess and Foy 2000). Another way that the adjuvants could increase the uptake of foliar P is through their humectant properties, i.e. their ability to delay drying of the droplets. Although drying times were not measured in this study, it is likely that both Adjuvants A and B delayed drying of the fertilisers compared to adjuvant C, which helped to increase the time available for foliar uptake of P. The trade-off between delayed drying and greater droplet spread has

been discussed and attributed to the lack of differences in uptake of foliar phosphoric acid (Peirce et al. 2015).

Conclusions

The plant uptake of P from foliar-applied solutions was high. Except for reagent grade K-phosphate, most formulations averaged plant uptake of 80-95 % of P applied. The foliar route is therefore an effective pathway for P acquisition by crops and exceeds efficiency of the fertiliser-soil-root pathway. However, given that foliar P can only be applied later in the plant growth cycle, when LAI is high enough to intercept sprayed solutions, the percentage of P in the plant derived from the foliar fertiliser is generally smaller than that taken up from soil. Biomass responses at anthesis, and the efficiency of foliar P uptake and translocation were not consistently related to fertiliser pH, adjuvant type or accompanying cation. However, P applied as phosphoric acid appears to be poorly translocated from treated leaves, perhaps due to serious scorch damage to leaves. Grain yield responses to foliar P application may be possible, but are dependent on product formulation. Field experimentation is required to validate if foliar fertilisation is a viable pathway for tactically providing P to wheat, and consistently increasing grain yields, in marginally P deficient soils.

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Supplementary Information

Figure S 1: Photos of leaf scorch on growing (intact) leaves taken 4 days after foliar treatment with P products in combination with 3 different adjuvants (38-39DAS).

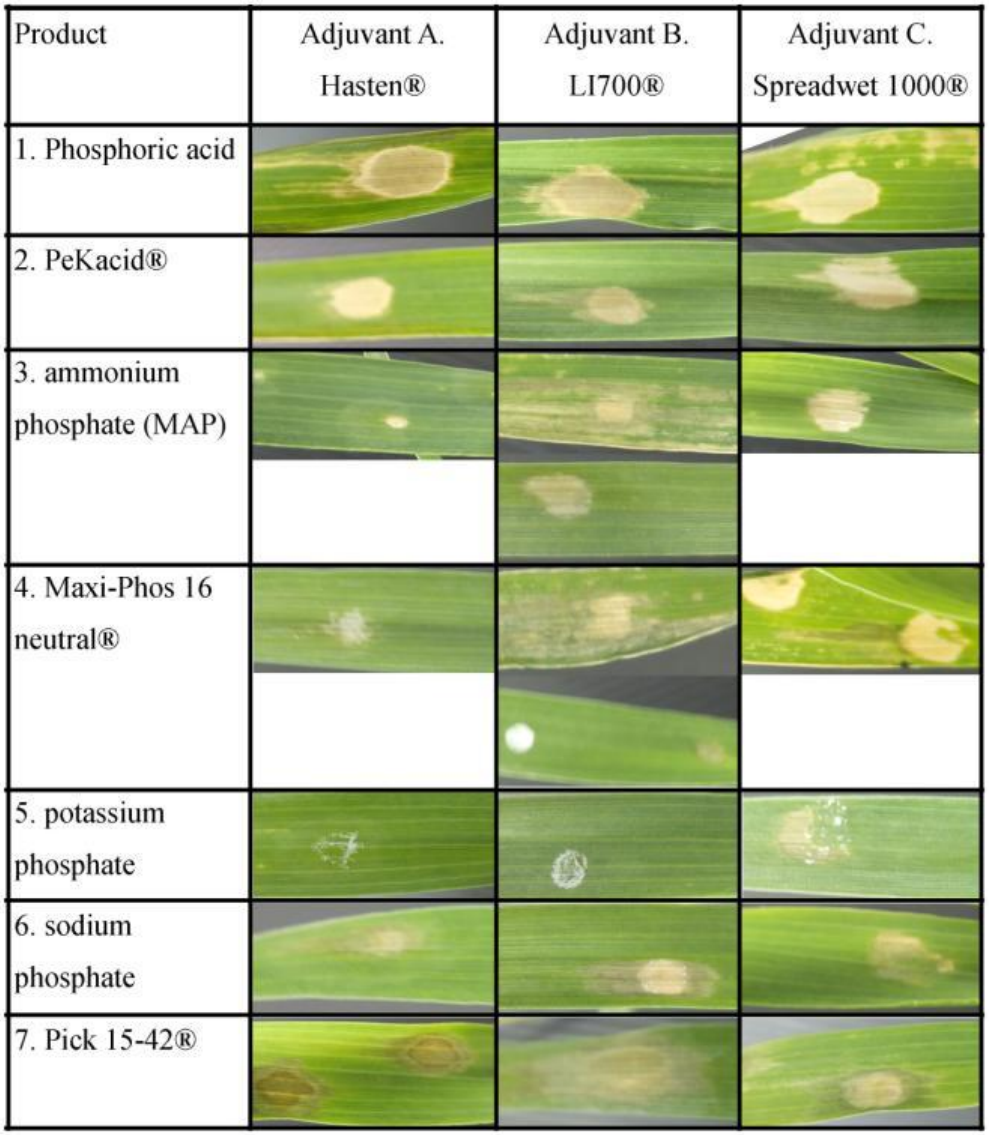

Table S 1: Isotope recovery of foliar applied P (% of P applied) for the 7 foliar products in combination with the 3 adjuvants when harvested at the end of anthesis. Total translocation is the percentage of spike recovered in all plant parts except the treated leaves. "Washed off" is the percentage of spike recovered in the washing solution. Different letters within columns indicate significant differences in the average spike recovery for that component ($p \leq 0.05$, n=4, l.s.d. indicated in the table).

Treatment		Heads	Tillers	Stem and Untreated Leaves	Treated Leaves	Total Translocation	Total Plant (Uptake)	Washed Off
1	A	16 h	1 e	6 fg	75 a	23 h	98 ab	1 gh
	B	16 h	1 e	5 gh	72 ab	22 h	94 abc	0 h
	C	21 gh	1 e	5 gh	74 a	26 h	100 a	1 gh
2	A	32 def	4 de	10 bcd	48 cd	46 cdefg	94 abc	2 fg
	B	38 bcd	4 de	11 abc	42 d	53 bc	95 abc	2 fg
	C	30 ef	6 cd	7 efg	49 cd	44 cdefg	93 abc	1 gh
3	A	30 ef	5 cd	8 def	10 gh	42 defg	52 h	5 d
	B	29 ef	4 de	9 cde	41 de	41 efg	82 de	3 ef
	C	25 fg	3 de	9 cde	60 bc	37 g	97 abc	2 fg
4	A	41 bc	5 cd	12 ab	17 fg	59 ab	76 ef	4 de
	B	32 def	1 e	8 def	48 cd	40 fg	88 cd	2 fg
	C	26 fg	3 de	8 def	52 cd	38 fg	90 bcd	2 fg
5	A	5 i	1 e	2 i	6 gh	9 i	14 i	32 a
	B	8 i	1 e	3 hi	4 h	12 i	16 i	23 b
	C	34 cde	5 cd	11 abc	18 fg	50 bcde	68 fg	8 c
6	A	30 ef	11 ab	9 cde	12 gh	51 bcd	62 g	2 fg
	B	45 ab	8 bc	13 a	29 ef	66 a	95 abc	1 gh
	C	31 def	5 cd	11 abc	51 cd	47 cdef	98 ab	3 ef
7	A	34 cde	3 de	11 abc	53 cd	47 cdef	100 a	1 gh
	B	49 a	3 de	12 ab	27 f	63 a	91 abcd	1 gh
	C	40 bc	12 a	13 a	27 f	65 a	92 abc	1 gh
l.s.d. ($p \leq 0.05$)		*8*	*4*	*3*	*13*	*10*	*10*	*2*

Table S 2: Phosphorus concentration (mg kg^{-1}) of plant parts at harvest. Significant differences compared to the control are indicated by an asterisk (*) ($p \leq 0.05$, n=4 for all foliar treatments, n=12 for control, l.s.d. indicated in the table)

Treatment		Head	Tillers	Stem and Untreated Leaves	Treated leaves	All Untreated parts	Whole Shoots
		mg P kg^{-1} of plant					
0		2,086	1,682	735	741	975	824
1	A	2,655 *	2,550 *	1,014 *	3,534 *	1,194	1,347 *
1	B	2,527 *	2,118	869	3,510 *	1,056	1,225 *
1	C	2,637 *	2,297 *	822	3,649 *	897	1,080 *
2	A	2,345	2,147	868	2,504 *	1,319 *	1,315 *
2	B	2,412	1,672	842	2,213 *	1,308 *	1,264 *
2	C	2,302	1,731	747	2,486 *	1,370 *	1,355 *
3	A	2,100	1,161 *	741	1,091	1,219	1,047
3	B	2,503 *	1,433	875	2,362 *	1,194	1,155 *
3	C	2,221	1,565	904 *	3,032 *	1,027	1,103 *
4	A	2,090	1,377	785	1,181	1,111	969
4	B	2,115	1,255	677	2,729 *	768	842
4	C	2,334	1,894	842	2,779 *	1,066	1,122 *
5	A	2,197	1,848	901 *	1,106	1,191	1,030
5	B	2,119	1,675	744	1,009	1,157	1,008
5	C	2,251	1,794	900 *	1,380 *	1,360 *	1,202 *
6	A	2,272	1,428	756	1,205	1,445 *	1,239 *
6	B	2,125	1,356	722	1,475 *	1,271 *	1,134 *
6	C	2,332	1,757	871	2,487 *	1,231	1,230 *
7	A	2,088	1,572	758	2,476 *	1,145	1,182 *
7	B	2,063	996 *	658	1,501 *	1,035	944
7	C	1,925	1,295	717	1,506 *	1,387 *	1,247 *
l.s.d. ($p \leq 0.05$)		*391*	*520*	*165*	*612*	*290*	*239*

Table S 3: Potassium concentration (mg kg^{-1}) of plant parts at harvest. Significant differences compared to the control are indicated by an asterisk (*) ($p \leq 0.05$, n=4 for all foliar treatments, n=12 for control, l.s.d. indicated in the table)

Treatment		Head	Tillers	Stem and Untreated Leaves	Treated leaves	All Untreated parts	Whole Shoots
		mg K kg^{-1} of plant					
0		9,003	29,404	33,042	30,186	16,304	15,974
1	A	7,952	31,949	27,414 *	31,753	12,582	13,499
1	B	8,793	29,958	30,823	29,682	13,773	13,992
1	C	11,123	34,145	33,403	28,220	11,673	11,958
2	A	8,445	28,748	30,908	32,954	18,518	18,191
2	B	9,387	27,964	31,067	34,665	18,251	18,101
2	C	8,269	28,221	28,158 *	34804 *	19,549	19,427
3	A	10,246	26,560	34,070	30,661	20,890	19,456
3	B	10,907	22,639	35,965	28,434	17,607	16,376
3	C	10,578	27,056	37,317	29,862	17,109	16,122
4	A	11,553 *	20,551 *	38,894 *	30,605	19,463	18,097
4	B	11,491 *	16,519 *	34,137	25,830	11,971	11,509
4	C	10,369	25,503	35,364	29,094	15,873	15,316
5	A	8,161	32,769	34,940	34,476	18,897	18,453
5	B	8,626	29,466	33,205	33,988	19,191	19,098
5	C	8,613	31,070	35,994	30,766	21,172	19,959
6	A	8,557	27,036	31,655	30,856	22,982 *	21,203 *
6	B	10,254	25,533	36,592	25,141 *	22,086 *	19,629
6	C	9,076	30,399	37,216	28,204	20,007	18,498
7	A	9,565	21,235	33,596	34,300	18,009	18,050
7	B	10,986	18,161 *	32,463	31,534	16,117	15,621
7	C	8,461	25,899	34,003	33,186	24,859 *	23,197 *
l.s.d. ($p \leq 0.05$)		*2,157*	*8,515*	*4,621*	*4,533*	*5,319*	*4,535*

Table S 4: Sulfur concentration (mg kg^{-1}) of plant parts at harvest. Significant differences compared to the control are indicated by an asterisk (*) ($p \leq 0.05$, n=4 for all foliar treatments, n=12 for control, l.s.d. indicated in the table)

Treatment		Head	Tillers	Stem and Untreated Leaves	Treated leaves	All Untreated parts	Whole Shoots
		mg S kg^{-1} of plant					
0		2,123	2,629	2,873	5,815	1,810	2,077
1	A	2,324	2,880	3,021	5,371	1,756	2,003
1	B	2,099	2,185	2,751	4,665	1,570	1,755
1	C	1,999	3,129	2,619	4,266 *	1,247	1,425
2	A	2,011	2,516	2,692	6,180	2,025	2,325
2	B	1,909	2,318	2,594	5,741	1,916	2,168
2	C	2,072	2,740	2,751	6,306	2,306	2,591
3	A	1,788	2,013	2,445	5,495	1,891	2,103
3	B	2,231	2,302	2,904	5,420	1,826	1,972
3	C	1,757	1,979	2,960	4,565 *	1,601	1,707
4	A	1,706	1,642 *	2,704	5,818	1,696	1,939
4	B	1,512 *	1,049 *	2,272 *	4,322 *	1,003 *	1,188 *
4	C	1,929	2,075	3,021	5,160	1,635	1,830
5	A	2,231	2,995	3,408 *	7,314 *	2,231	2,582
5	B	2,202	2,411	2,935	6,967	2,139	2,569
5	C	2,021	2,902	3,088	6,976	2,282	2,635
6	A	1,989	2,634	2,883	7,102 *	2,514 *	2,800 *
6	B	1,757	1,876 *	2,588	5,440	1,945	2,123
6	C	2,014	2,495	3,119	5,455	2,030	2,195
7	A	1,815	1,641 *	2,677	4,942	1,813	2,012
7	B	1,502 *	1,620 *	2,163 *	4,525 *	1,372	1,543
7	C	1,738	2,140	2,824	6,218	2,452	2,689
l.s.d. ($p \leq 0.05$)		*419*	*734*	*524*	*1,190*	*654*	*658*

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Chapter 6

Conclusions, recommendations and future outlook

Main findings and conclusions

Foliar application of phosphorus (P) is a potential management strategy that allows tactical application of fertiliser in favourable seasons. However, both field and glasshouse grown plants have shown variable responses to foliar P application (Noack et al. 2011). The aims of this thesis were:

- to systematically explore the morphological factors of wheat leaves that control retention (wettability) and absorption (uptake) of foliar P solutions;
- to use this knowledge to examine the role of adjuvants in the formulation to enhance retention and absorption;
- to understand factors including growth stage and foliar P concentration that influence the translocation of absorbed foliar P; and
- to use this knowledge to test the effectiveness of a range of formulations that vary in solution pH, accompanying cation and adjuvant.

This thesis approached the topic of foliar fertilisation with P using a multidisciplinary approach to investigate some of the main processes that govern the efficacy of foliar P application. The novel approach integrated measurements and observations of leaf surface structure and wettability of wheat leaves through contact angle measurements with the absorption and uptake of foliar P measured using isotopically labelled P in the foliar fertilisers. Although this isotopic approach had been used previously for single drops and at low concentrations (Bouma 1969; Bukovac and Wittwer 1957; Reed and Tukey 1978), it had only been conducted once with multiple drops at field relevant rates with plants grown for longer than a few days after foliar application (McBeath et al. 2011). This approach allowed the efficiency of foliar application (uptake and translocation as a percentage of fertiliser applied) and the relative contribution of foliar P uptake to total plant P uptake to be quantified. Using these combined methods I investigated how the characteristics of the wheat leaf surface influenced the retention, uptake and translocation of foliar-applied P and quantified the efficiency of foliar application.

In collaboration with visiting scientist Dr Victoria Fernandez from the Technical University of Madrid, it was shown that severely P deficient wheat plants did not absorb foliar-applied P due to the irreversible structural and morphological changes that were induced by P deficiency ((Fernández et al. 2014); see Appendix). No detectable foliar uptake occurred for plants grown at a severely deficient P status. However, marginally P deficient wheat plants were capable of absorbing and translocating foliar-applied P, although absorption was lower than for plants grown at a sufficient P status. This finding was similar to

the work of Will et al. (2011) who found that the foliar absorption of boron by soybean leaves was significantly reduced by boron deficiency, but contradictory to the findings of Clarkson and Scattergood (1982) who found that P-stressed barley leaves absorbed foliar-applied P twice as rapidly as leaves that were not P-stressed. Due to the importance of the morphology of the leaves in governing the foliar pathways and efficiency of uptake, my first experiment examined the morphology and physiology of the different sides of the wheat leaf to gain a better understanding of the surfaces and how surface morphology might affect P acquisition by the leaf. Plants were grown at a marginally deficient plant P status. The marginal status was obtained from dose response curves for wheat grown in the P responsive soil used for all experiments.

From this first experiment (Chapter 2), I found that the trichome and stomatal densities of wheat leaves varied with leaf side. The densities of both trichomes and stomata were higher for the adaxial (upper) leaf side than for the abaxial (lower) leaf side. The wettability of wheat leaves was inversely related to the trichome density with higher fertiliser and water adhesion for the abaxial (lower) leaf side, in agreement with the relationship shown by Fernández et al. (2014). While the abaxial leaf side was more wettable than the adaxial side, the absorption of foliar-applied P was less, and higher absorption and subsequent translocation of foliar-applied P (as phosphoric acid) was measured for P applied to the adaxial leaf side. These findings support the theory that stomata provide an important and dominant pathway for foliar uptake of hydrophilic solutes (Fernández and Eichert 2009). It is therefore likely that if fertilisers are applied at times when the stomata are closed (at night or on hot days in response to high temperatures and low humidity), uptake and translocation of P from the fertiliser will be reduced compared to when the stomata are open. This experiment also suggests that trichomes may play an important role in foliar uptake and provide an additional pathway, likely due to higher permeability around the base of the trichomes as suggested by Tukey et al. (1961). From my study it was therefore concluded that the morphology of wheat leaves plays a crucial role in the efficiency of foliar-applied P through affecting both the absorption of P and the wettability of the leaves.

In addition to the difference in uptake and translocation of foliar-applied P from phosphoric acid between the two leaf sides, Chapter 2 also reports an investigation of the influence of foliar P rate or concentration (0.3, 0.6 or 1.1 M) and timing on P uptake. The foliar timings chosen were at ear emergence and anthesis, as it is well known that root uptake of P at early growth stages (Römer and Schilling 1986) is important for crop establishment and a substantial leaf area is required to maximise interception of the foliar fertiliser by the leaves. At the high P concentrations used in this study, phosphoric acid caused significant scorch at the site of application but while the degree of scorch increased with increasing foliar

P rates, it did not affect the yield or biomass of the plants. Although previous studies have used similar concentrations and rates of foliar P (Benbella and Paulsen 1998; Sherchand and Paulsen 1985), only one previous study used isotopically labelled P to trace the fate of the foliar-applied P at concentrations relevant to field application (McBeath et al. 2011). As the P concentration was increased, translocation as a percentage of P recovered in the plant was reduced, but the amount of foliar P translocated was still higher than when the lower P concentration was applied. There was also a difference in absorption and subsequent translocation of foliar-applied P with timing of the foliar application. Absorption and subsequent translocation of foliar-applied P was higher when fertilisers were applied at ear emergence compared to mid-anthesis. Previously, results in the literature suggested that foliar application during grain filling could be used to maximise yields when soil P was limited (Sutton et al. 1983) and delay leaf senescence (Benbella and Paulsen 1998). My findings help to refine the window of opportunity for foliar application of P to before anthesis. The main sink for foliar-applied P regardless of timing was the mature grain or head as also shown by Marshall and Wardlaw (1973) and McBeath et al. (2011), suggesting that once the foliar-applied P enters the leaf it is remobilised efficiently. This is consistent with the literature that shows remobilisation of P within the plants to the grain plays a significant role at later growth stages (Grant et al. 2001).

From Chapter 2, it became clear that the wettability of wheat leaves and the interaction between the leaf surface and the P formulation was an important parameter that required further investigation. This led to collaboration with Dr Craig Priest from the University of South Australia to measure the interaction between the fertiliser solution and wheat leaves using the sessile drop technique to measure advancing and receding contact angles. This measurement technique allowed me to investigate how the wettability of leaves varied with growth stage and how the inclusion of different adjuvants at varying concentrations influenced both the initial contact angle and spreading of the drop on the wheat leaf surface. Utilising isotopic tracing techniques I was able to measure whether the wettability of wheat leaves and the contact angles of the fertilisers on the leaves influenced the uptake and translocation of the foliar-applied fertiliser.

Both Chapter 3 and Chapter 4 investigated the influence of leaf wettability and the use of adjuvants on the uptake and translocation of foliar-applied phosphoric acid. In Chapter 3, the wetting of wheat leaves was explored in great detail with advancing and receding contact angles measured for phosphoric acid in combination with three different adjuvants ranging in concentration from 0.01 to 0.3 % w v^{-1}. The advancing and receding contact angles and contact angle hysteresis (difference between the advancing and receding contact angles) represent the largest and smallest angles present between the drop and the leaf surface and are

parameters that control whether a drop is likely to adhere or roll off. In addition to these contact angles (measured after 20 s), which differed for the three adjuvants, it was discovered that the dynamics of droplet spreading were faster for some adjuvants than others. As a result, the contact angle of the drops was also measured as a function of time to investigate these dynamics. Tanner's law was applied to the data to investigate the mechanism of spreading and Wenzel's equation was used to estimate the roughness factor of wheat leaves, which influences wetting behaviour. The leaves of wheat plants were found to be difficult to wet in the absence of a surfactant. The inclusion of a surfactant in the foliar formulation was essential to obtain a high contact area between the fertiliser and the leaf, which in turn led to higher foliar uptake (compared to the only treatment that did not include a surfactant, glycerol in Chapter 4). However, for phosphoric acid, uptake and total translocation was similar regardless of the surfactant used, both over the short term (7 days) as investigated in Chapter 3, and when grown through to maturity as investigated in Chapter 4, despite different initial wetting (contact angles) achieved by the adjuvants. This finding plays a critical role in informing farmer's practice for foliar spraying of fertilisers. Even low concentrations of surfactants in the foliar spray will allow the fertiliser solution to adhere to the leaf surface increasing the efficacy of the foliar application process through increased retention on the leaves. Interestingly though, an increase in droplet spreading through use of a stronger surfactant (Genapol X-080®), which reduced the surface tension of the fertiliser considerably compared to water and the other surfactants, did not result in higher uptake of foliar P. This contradicts common sense that would lead to the conclusion that greater spreading (and higher contact area between the fertiliser and the leaf surface) would result in higher uptake. It therefore supports the notion that drying time (which is longer for drops with higher contact angles) is just as important as good contact of the fertiliser on the leaf to enable high foliar uptake. The use of humectants which delay droplet drying could therefore be an important research area for foliar P as has also been highlighted for uptake of foliar-applied calcium (Blanco et al. 2010) and iron (Fernández et al. 2006).

In Chapter 4, in addition to the investigation of whether the choice of adjuvant influenced the wetting, foliar uptake and translocation of phosphoric acid, plants were also grown through to maturity to identify whether a grain yield benefit could be achieved with foliar application. I found that the foliar uptake and translocation of phosphoric acid did not directly influence the grain yield of wheat. Even though uptake and translocation were similar with different adjuvants in combination with phosphoric acid, only one combination resulted in a yield increase and two combinations resulted in a yield decrease. The positive yield response may be a result of the surfactant and humectant properties of the LI700® adjuvant combining to both reduce the surface tension of the fertiliser, which allowed it to adhere to the wheat

leaf, and to increase the time the fertiliser drop remained a liquid. Hence the P solution was available for uptake over a longer period of time compared to use of the other adjuvants, which only contained active-ingredients with a surfactant mode of action. The influence of foliar timing was again investigated, but with application occurring earlier at tillering and flag leaf emergence rather than when tested in Chapter 2. This was in response to the lack of differences between biomass for the two later timings and a postulated inability of the foliar P to influence either tillering or head size at these late growth stages. Moving foliar application to the earlier growth stages of tillering and flag leaf emergence would allow foliar P to be applied when P demand is high and capable of influencing the physiological components of wheat that affect grain yield. Foliar application of P at tillering reduced translocation to untreated plant parts compared to application at flag leaf emergence. The decreased translocation at tillering appeared to be related to scorch, with higher scorch ratings for foliar application when applied at tillering compared to flag leaf emergence, although it may also have been a result of the reduced ability of younger leaves to translocate P as was also found by Koontz and Biddulph (1957) for bean leaves. The higher scorch was not surprising since the same amount of foliar P was applied over a smaller leaf area (as the plants were younger and smaller). There was also a grain yield depression for one foliar P treatment when foliar P was applied at this early growth stage. The results from this study helped to further refine the window of opportunity for foliar application of P to after tillering and before anthesis. It should however be noted that if foliar P fertilisers were sprayed rather than applied as drops, the overall efficacy of the foliar application is likely to be lower due to reduced interception of the spray by the foliage. The scorch to the leaves would also change due to drop size which could affect the efficiency of uptake and translocation. In this respect, it is therefore necessary to validate this result in the field under commercial sprayer conditions.

From the previous experiments, I found that a foliar application rate equivalent to 2 kg P ha^{-1} in 100 L ha^{-1} fluid volume (0.65M P) applied at flag leaf emergence was capable (phosphoric acid with the adjuvant LI700® treatment only) of increasing the yield of wheat. Due to the lack of a consistent increase in wheat grain or biomass yield when foliar P was applied as phosphoric acid and the finding that only a small percentage of phosphoric acid translocated from the site of application, a range of other products was explored in Chapter 5 for their potential as foliar-applied P fertilisers. Formulations with different pH, accompanying cation and adjuvants were evaluated in terms of P absorption, translocation and biomass response. The most promising foliar timing and rate that produced a biomass response in the previous experiment were used in this experiment and the plants were harvested at anthesis.

Higher translocation of foliar-applied P from PeKacid®, ammonium phosphate, sodium phosphate, and Pick 15-42® in combination with adjuvants resulted in an increase in plant biomass compared to a no-foliar control. Neither foliar uptake, nor translocation were related to solution pH or associated cations as individual parameters because positive biomass responses occurred for fertiliser formulations that varied in both pH ranging from 2.2 to 8.7, and for phosphate associated with potassium, nitrogen and sodium (although the use of commercial products meant the design was not fully factorial). This contradicts the common misconception that foliar uptake of P is highest at a low pH of 2-3 (Bouma 1969; Tukey et al. 1961), as it is the combined effect of pH and associated cation that determines the foliar uptake efficiency and subsequent biomass response. It is difficult to separate the effect of pH and associated cation as there always needs to be a balancing cation in solution if protons are to be neutralised. Overall, my results regarding formulation pH and associated cation were fairly consistent with work of Tukey et al. (1956) and Koontz and Biddulph (1957) although the efficiency of foliar uptake of P was much higher in my study. The only product with particularly low foliar uptake, analytical grade potassium phosphate, crystallised on the leaf surface, which supports the observation of Reed and Tukey (1978) who also noticed lowest uptake rates for solutions that crystallised on the leaf.

In comparison to the other products, the foliar-application of phosphoric acid had high P absorption but low translocation, which may have been a result of leaf burn that caused leaf damage. The scorch measured as area per leaf did not convey the severity of the scorch (e.g. chlorotic vs. necrotic) and visual observation suggested that the phosphoric acid caused a more severe form of scorch that may have inhibited translocation of P. While phosphoric acid was found to have similar efficacy regardless of the adjuvant used (Chapter 3 and 4), some other products performed better with a particular adjuvant. This resulted in Hasten®, LI700® and Spreadwet 1000® all providing biomass increases in combination with at least one P product but none of these adjuvants was consistently better across all P forms. This finding is in agreement with the work of Fernández et al. (2006) who found that it is not yet possible to predict if negative interactions will occur between the foliar nutrient and adjuvant and therefore which foliar formulation will perform the best. This experiment demonstrated the importance of all three key processes for foliar P to influence wheat productivity; retention, absorption and translocation.

Uncertainties

Although significant progress has been made in furthering our understanding of the influence of wheat leaf morphology, leaf wettability, the importance of translocation of foliar-applied P and the identification of some promising foliar P formulations, there are still a number of uncertainties that prevent the prediction of which combination of formulation and application factors will result in consistent yield increases in wheat. The complexity of the interaction between environmental conditions (soil and climate related) and plant characteristics (P status, growth stage) makes a reliable prediction of foliar P requirement difficult. It is also possible that the balance between applying enough foliar P to produce a yield response and using rates that do not cause scorch to a level that reduces the photosynthetic capacity of the leaves cannot be achieved. In this case, the application of multiple sprays may be warranted, but increasing the number of sprays increases the cost of application and may negate any perceived cost-benefits of foliar application. Finally, it could be that there is plasticity in the response of wheat plants to P application. Phosphorus concentrations of healthy wheat plants can vary and at the time of foliar application, additional P may not influence grain yield parameters. In a number of the chapters in this thesis, although plants were grown at a marginal P status, which resulted in plant P concentrations of control plants being below the critical threshold, foliar P application was still unable to raise the P concentration above the threshold. In this case, perhaps there is an inability to supply enough P through foliar application even at this marginal status. Additional P application may only help to increase the P concentration and P content of the marginally deficient wheat plant, but not biomass and grain yield. The results from this thesis suggest that there is insufficient evidence for reducing starter P applications to the soil and substituting with foliar P applications in seasons of higher yield potential. Perhaps the best fit for foliar P fertilisers is as a tactical application in response to transient P deficiency in soils as induced by drying out of the soil, however this should be investigated further.

Future research direction/priorities

There are a number of opportunities for further research as a result of gaps identified in the process of exploring the effect of wheat leaf morphology, wheat growth stage, P dose, adjuvants and other formulation factors:

- Not all the foliar-applied P was recovered in the controlled environment room studies. Although we postulated that the incomplete recovery of ^{32}P or ^{33}P was located in the roots, this was not confirmed. In particular, the lower total isotope

recovery (sum of plant recovery and washing solution) in Chapter 5 of potassium phosphate would suggest substantial translocation of foliar P to the roots occurred. Since it is likely that for a substantial yield benefit to occur, the application of foliar P must stimulate root P uptake as noted by McBeath et al. (2011) in their study (since the increase in P from the foliar fertiliser alone cannot always cover the increased P uptake by the plant), the analysis of root P and even root biomass would substantially improve our understanding of why foliar P may work in some conditions. For example, if a 1.5 kg P ha^{-1} foliar spray was to increase the grain yield of a crop by 0.5 t ha^{-1} and grain P concentration was 0.3 % (at the lower end of adequate according to the critical values identified by Reuter and Robinson (1997)), then 100 % of the foliar P would need to be translocated to the grain to cover the increase in yield. In addition to harvesting roots, it may also be beneficial to investigate whether there is a negative feedback mechanism between foliar uptake and root uptake much in the same way as mycorrhizal inoculation can down-regulate Pi transporters in the plant roots (Smith and Smith 2012). This may occur only under some conditions, but may help to explain the variability in yield response to foliar-applied P.

- It would be interesting to look at the effect of different soils on the balance between soil and foliar uptake of P. My thesis used only one soil to ensure the variability in plant response that can occur between different soils was minimised. It is likely that the plant response to foliar P will change not only in response to the availability of P in the soil, as found by McBeath et al. (2011), but also in response to other soil factors including the soil microbiological properties which were disturbed in my study due to air-drying prior to use. The soil: foliar fertiliser uptake balance could be investigated in either a dual-labelling study (^{32}P for the foliar fertiliser and ^{33}P for the soil) or with parallel/duplicate treatments where one pot has ^{33}P labelled foliar fertiliser and the other pot ^{33}P labelled soil.
- All the experiments in this thesis were conducted using the wheat cultivar Axe. However, as shown in Chapter 2 and the Appendix, leaf morphology can vary substantially with P status and leaf side. This morphology, in turn, will affect both the wettability of the leaves and the foliar uptake of the fertiliser. It is therefore likely that different cultivars will have different responses to foliar-applied P, particularly drought-tolerant cultivars which are known to have higher trichome densities and wax coverage (Doroshkov et al. 2011; Johnson et al. 1983). In order to help control variables including the P use efficiency (PUE) of the plants, it may be beneficial to investigate differences by using isogenic (or near-isogenic) lines.

There are isogenic lines available that differ in the glaucous characteristic (Johnson et al. 1983; Richards et al. 1986).

- The positive yield response that we found for phosphoric acid in combination with LI700® (Chapter 4), despite similar uptake and translocation rates compared to the other surfactants, poses the question of why this treatment resulted in a yield increase while the others did not. It may be that the combination of humectants and surfactant properties inherent in LI700® caused this yield response. We would suggest that further investigation into the combination of humectants and surfactants is warranted, but using controlled combinations of humectant and surfactant ingredients rather than using commercial products. The isotopic tracing techniques utilised in this thesis could again be used to trace the movement of the labelled foliar P in combination with the adjuvants themselves using ^{14}C labelling techniques (Shafer and Bukovac 1987). Measurements of spread and drying times should also be utilised to identify correlations between these factors and uptake of foliar-applied P.
- I found a number of formulations that may provide positive yield responses in the field if applied at the optimal timing of post-tillering and pre-anthesis (Chapter 5). As a result, there is a need for field validation of the efficacy of the products to see whether the positive biomass response found in the controlled environment room translates to a grain yield response in the field. In addition to the field testing of products, field testing of the timing of applications is also important due to differences in the duration and progression of growth stages between wheat plants grown in controlled environmental conditions and the field.
- If the degree of scorch is negatively affecting the photosynthetic capacity of wheat leaves and negating any possible yield increases from the foliar application, it may be worth investigating the use of multiple foliar applications with lower rates (i.e. splitting the 2 kg P ha^{-1} over two or three applications). This may help to reduce scorch, although there is then the difficulty of fitting multiple sprays within the optimum growth stage window.
- Additional experimentation to determine the mechanisms of scorch is warranted. It is likely to be a complex area of work as the negative effects of scorch need to be considered alongside the possible benefit scorch provides in reducing the hydrophobicity of the leaves and possibly allowing greater uptake of P into the internal cells of the leaf. This could be through initially scorching the leaf (i.e. with weak acids) and then applying isotopically labelled P (dual labelling technique) at a

non-injurious concentration both on and off scorched parts of the leaf. This technique could help to determine whether the scorch is aiding the foliar uptake process or not.

- A further area of investigation is whether foliar fertilisation could be used to address transient P deficiencies as is the case during dry periods where the surface soil dries out, P diffusion is limited and P uptake is restricted. This would only be the case if there was sufficient water at depth to ensure the plants had adequate yield potential to make use of the extra P nutrition. However, this strategy may be challenging as a water stressed plant will close its stomata to preserve water and this is likely to limit the uptake of foliar P. This idea could be explored by working with deep pots (that could be irrigated at depth when necessary) which enabled the soil surface to dry out but still provided enough subsoil moisture to maintain a high yield potential.

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Appendix

Effect of wheat phosphorus status on leaf surface properties and permeability to foliar-applied phosphorus

In the first 6 months of my PhD, we had a plant physiologist, Dr Victoria Fernandez from Spain visit our laboratory as part of a collaboration with CSIRO. As part of her visit, we undertook some research which resulted in the following publication. These results helped to direct the rest of my PhD and are therefore an important part of my work although I was not the first author. As a result they have been included as an appendix and the results are referred to throughout both the literature review and my PhD chapters.

Fernández V., Guzmán P., Peirce, C., McBeath T., Khayet M., McLaughlin M. J., 2014, Effect of wheat phosphorus status on leaf surface properties and permeability to foliar-applied phosphorus, *Plant and Soil* 384, 7-20, DOI 10.1007/s11104-014-2052-6

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Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Courtney Peirce
Contribution to the Paper	Co-author on the manuscript Grew and looked after wheat plants, undertook 32P-labelled leaf dipping experiment and analysis of data, contributed to methods, results and discussion sections and to evaluate and edit the manuscript.
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Name of Co-Author	Victoria Fernández
Contribution to the Paper	Senior author on the manuscript Performed preliminary blotting experiment, SEM and TEM examination, wax extraction and contact angle measurements and interpreted data. Wrote manuscript and acted as corresponding author.
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Contribution to the Paper	Performed preliminary blotting experiment, SEM and TEM examination, wax extraction and contact angle measurements, interpreted data and wrote manuscript.
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Contribution to the Paper	Helped with interpretation of contact angle data and to evaluate and edit the manuscript.
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