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Uptake of zinc and phosphorus by plants is affected by zinc fertiliser material and arbuscular mycorrhizas

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Abstract

Background and Aims

Water solubility of Zn fertilisers affects their plant availability. Further, simultaneous application of Zn and phosphorus (P) fertiliser can have antagonistic effects on plant Zn uptake. Arbuscular mycorrhizas (AM) can improve plant Zn and P uptake. We conducted a glasshouse experiment to test the effect of different Zn fertiliser materials, in conjunction with P fertiliser application, and colonisation by AM, on plant nutrition and biomass.

Methods

We grew a mycorrhiza-defective tomato genotype (*rmc*) and its mycorrhizal wild-type progenitor (76R) in soil with six different zinc fertilisers ranging in water solubility (Zn sulphate, Zn oxide, Zn oxide (nano), Zn phosphate, Zn carbonate, Zn phosphate carbonate), and supplemental P. We measured plant biomass, Zn and P contents, mycorrhizal colonisation and water use efficiency.

Results

Whereas water solubility of the Zn fertilisers was not correlated with plant biomass or Zn uptake, plant Zn and P contents differed among Zn fertiliser treatments. Plant Zn and P uptake was enhanced when supplied as Zn phosphate carbonate. Mycorrhizal plants took up more P than non-mycorrhizal plants; the reverse was true for Zn.

Conclusions

Zinc fertiliser composition and AM have a profound effect on plant Zn and P uptake.

Introduction

Zinc (Zn) is an essential micronutrient in biological systems. It is estimated that nearly 50% of the world's important cereal-growing soils have low levels of plant available Zn (Cakmak 2002; Graham and Welch 1997), and around 30% of the world's population is affected by Zn deficiency (Alloway 2008). As a consequence, Zn is considered the most yield-limiting micronutrient in some areas of the world (Fageria 2010). The essentiality of Zn in crop production, coupled with its severe deficiency in some of the world's principal agricultural soils, has increased awareness of the importance of Zn in crop production in recent years (Fageria 2010). To this end, agronomic biofortification in the form of Zn fertiliser application, has become an important agricultural practice to increase the delivery of Zn to crop tissues (Cakmak 2008).

Zinc fertiliser for agricultural purposes can be bought as a standalone product, typically as hydrated Zn sulphate ($ZnSO_4.7H_2O$) or Zn oxide (ZnO). There is also an increasing range of macronutrient fertilisers that can act as carriers for Zn, such as Zn-NPK, Zn-coated superphosphate or Zn-coated urea (Grewal 2010; Shivay et al. 2008; Ortas 2012; Mortvedt and Gilkes 1993; Milani et al. 2012). Zinc sulphate and Zn oxide are the most common Zn materials used as fertilisers; however, other sources such as Zn phosphate and Zn carbonate are also used (Alloway 2008; Fageria 2010). The water solubility of a Zn fertiliser is an important factor in its agronomic effectiveness (Milani et al. 2012). Plant availability of Zn in soil is strongly correlated with water solubility of the compound, in that more water-soluble compounds confer higher amounts of plant Zn availability and uptake (Mortvedt 1992; Amrani et al. 1999; Shaver et al. 2007). The solubility of Zn fertilisers ranges widely; whereas

Zn sulphate is water-soluble, Zn oxide, Zn carbonate and Zn phosphate are all, to varying degrees, water-insoluble (Alloway 2008; Boawn et al. 1957) which markedly affects their use as fertilisers. Plant availability of Zn in soil is also altered by a number of edaphic factors, the most important determinant being soil pH (Broadley et al. 2007). Specifically, an increase in soil pH decreases the availability of Zn for plant uptake (Marschner 1995; Fageria 2010). Other factors that influence plant availability of Zn in soil include; soil organic matter content, clay content, soil moisture, microbial activity in the rhizosphere, and macronutrient concentrations (discussed below) (Alloway 2008). Understanding the complex chemical behaviour of Zn in soils is an important aspect of ensuring the efficient use of Zn-containing fertilisers.

Given that many of the world's soils are both Zn and P deficient (Ortas 2012; Vance et al. 2003; Alloway 2008), there is increasing interest in the simultaneous and effective delivery of both nutrients to crops via fertiliser application (Mortvedt and Gilkes 1993). However, the application of macronutrient fertilisers high in phosphorus (P), can significantly decrease plant Zn availability, and thus uptake from the soil (Ryan et al. 2008; Zhang et al. 2012), due to complex Zn-P interactions that alter both soil and plant factors (Marschner 1995; Robson and Pitman 1983), now discussed in turn.

In the soil solution, there are a number of mechanisms that drive the decrease in available Zn under P fertilisation; however, they are not yet well understood (Alloway 2008). For example, there are several possible ways that Zn could be adsorbed under P-fertilisation, including changes in pH, and bonding of Zn to oxides and hydroxides of iron (Fe) and aluminium (Al), among others (Barrow 1987; Loneragan et al. 1979). In addition, cations added with, and H^+ ions generated by phosphate salts, may inhibit Zn absorption from the solution (Loneragan and Webb 1993). Plant uptake of Zn is also dependent upon plant-based factors such as production of phytosiderophores, expression of Zn transporters and mycorrhizal associations (Marschner 1993), which may be modified by soil P conditions (see below). Additionally, increased growth due to P fertilisation can lead to a dilution of Zn *in planta* (Loneragan et al. 1979). Because many fertiliser products contain both P and Zn, these issues are especially important, and there is a need to find a way to facilitate effective delivery of both P and Zn to crop tissues, simultaneously.

The capacity of plants to acquire Zn can be significantly improved through the formation of arbuscular mycorrhizas (AM). Around 80% of plants form AM, including a range of important cereal, and horticultural crops (Smith and Read 2008). These mutualistic relationships between plants and a specialised group of soil fungi, are especially important when the soil is Zn deficient (Rengel 1999). Considering the rising demand for Zn fertiliser, exploitation of AM's capacity to enhance plant Zn uptake has been suggested to provide at least part of the solution to Zn deficiency in agricultural soils, in conjunction with fertiliser application (Ortas 2012). Additionally, AM have been shown to lower rhizosphere pH, which can result in an increase of plant-available soil Zn (Li et al. 1991; Mohammad et al. 2005). However, it has been well established that infection of roots by AMF is suppressed by soil P fertilisation, which is another mechanism behind the P-induced Zn deficiency discussed above (Loneragan and Webb 1993). Colonisation by arbuscular mycorrhizal fungi also offers other benefits, such as an increase in pathogen resistance of the host plant (Perrin 1990), water use efficiency (WUE) (Al-Karaki 1998) and improvement of soil structure in the rhizosphere (Barea et al. 2002), which may lead to improvements in crop yield and nutrition. Furthermore, there is evidence that the WUE of plants may be improved with correction of Zn deficiency (Khan et al. 2003). Given that AM can improve plant Zn acquisition, it may follow that improvements in WUE of AM plants may be related to improvements in Zn nutrition; however, to our knowledge, the potential link between AM, Zn and WUE has not been directly investigated.

While there are a range of potential Zn materials that can be used as fertilisers, and naturally occurring Zn in the environment can be found in many forms, most research of AM effects on plant Zn acquisition uses Zn which has been added to the soil as $ZnSO_4.7H_2O$. Therefore, we need to look at other Zn fertilisers, to investigate how efficiently they can deliver Zn to plant tissues, and how AM modify the uptake and delivery of Zn to the plant. This is also important in the context of fertilisers that seek to supply Zn and P together.

To explore the link between AM and the water solubility of Zn fertilisers, we conducted a fully factorial glasshouse experiment using six different Zn materials with solubilities spanning five orders of magnitude, to fertilise the soil, in concentrations sufficient, but not toxic, to plants. Five Zn fertilisers were chosen on the basis of their use in agricultural practice, and one novel Zn compound

(Zn phosphate carbonate) was trialled as a fertiliser following its recent characterisation and possible role in Zn homeostasis in mammalian systems (Turney et al. 2012). These amorphous nanosized materials are uniquely formed from Zn^{2+} only in the presence of both carbonate and phosphate ions in solution. We grew a mycorrhiza-defective mutant tomato genotype (*rmc*) (Barker et al. 1998) and compared it to its wild-type progenitor (76R), to investigate how plant Zn uptake and nutrition was further modified by mycorrhizal colonisation.

Specifically, we hypothesised that:

1. Differences in water solubility of Zn compounds in soil would directly affect their plant availability in soil, and thence, the capacity of plants to acquire Zn;
2. With decreasing Zn availability, AM would improve the capacity of plants to acquire Zn from the soil;
3. Zinc fertilisers that contain P would reduce availability of Zn to the plant; and
4. That the WUE of plants would be greater with increasing Zn availability in the soil, and that improvements in plant Zn nutrition associated with the formation of AM would also increase plant WUE.

Methods

Soil and plants

Plastic, free-draining pots were filled with 1 kg of a 80:20 (*W/W*) sand/field soil mixture. The field soil was collected from Wallenjoe Swamp State Game Park located in Victoria, Australia (lat = -36.471935, long = 144.868512). This soil is classified as a grey vertisol (Martin 2007), and has a pH of 6.4 ± 0.4, a total C content of 19 ± 11 g kg^{-1}, a total N content of 2 ± 1 g kg^{-1}, and has low concentrations of plant available (Colwell) P (Colwell 1963) (12.8 ± 7.4 mg P kg^{-1} soil) and DTPA extractable Zn (Lindsay and Norvell 1978) (1.2 ± 0.7 mg Zn kg^{-1} soil). In our earlier work we have found this soil to have a high AMF inoculum potential (Cavagnaro and Martin 2011; Watts-Williams and Cavagnaro 2012). The sand used was a coarse washed river sand. The soil-sand mixture, which is referred to as "soil" hereafter, is well suited to soil nutrient addition studies, as it has low baseline nutrient concentrations (plant available [Colwell] P concentration was 3.5 ± 0.1 mg P kg^{-1} soil, and

DTPA extractable Zn concentration was 0.2 ± 0.0 mg Zn kg^{-1} soil), and allows for the easy isolation of root material.

To investigate the effect of different chemical forms of soil Zn upon plant growth and nutrition, the soil was amended with a range of Zn compounds, as follows:

Six soil Zn treatments were established by adding Zn compounds (see Table 1), ranging in solubility and particle size, to the soil at a rate of 25 mg Zn kg^{-1} soil. Compounds were then mixed thoroughly through the soil. The rate of Zn addition used in this study was found to be sufficient and not toxic to plants, as previously shown by the application of Zn sulphate (Watts-Williams and Cavagnaro 2012). Supplemental P (as $CaHPO_4.2H_2O$) was added so that the total addition of P to the soil was 25 mg P kg^{-1} soil, in order to provide good growth without inhibiting mycorrhizal colonisation (Watts-Williams et al. 2013). Soil P addition was adjusted accordingly for Zn materials already containing P (Zn phosphate and Zn phosphate carbonate); thus, in all treatments, the same amount of P was added to the soil.

Seeds of the reduced mycorrhizal colonisation tomato (*S. lycopersicum*) (Barker et al. 1998) mutant (*rmc*, hereafter) and its mycorrhizal wild-type progenitor *S. lycopersicum* cv. 76R (76R, hereafter) were surface-sterilised (Cavagnaro et al. 2010) and directly sown into pots (three seeds per pot) containing soil amended with Zn and P. Plants were thinned to one per pot after one week. Each treatment was replicated five times, giving a total of 60 pots. The plants were grown in a controlled environment glasshouse on the Monash University Clayton campus. Conditions in the glasshouse during the experimental period (October – December 2012) were as follows: Light levels during daylight hours (16 hr day length) averaged 272 ± 41 μmol photons m^{-2} s^{-1}, and the temperature was 23.4 ± 0.4 °C during the day and 20.1 ± 0.7 °C at night. All plants were watered with 1/10 strength modified Long-Ashton solution (P and Zn omitted, Cavagnaro et al. 2001) to 60% field capacity until harvest (see below). Water additions were recorded at each watering event (thrice weekly), in order to calculate water use efficiency (WUE). The plants were arranged in the glasshouse as a randomised complete block design, with the position of plants in the glasshouse rotated on a weekly basis.

Harvesting and analysis

There was one destructive plant harvest 8 weeks after planting, when plants were at the vegetative (pre-flowering) growth stage, as follows. Plants were removed from the pots by careful washing with water. All the shoots and a sub-sample of the roots were oven-dried before shoot dry weight (SDW) and root dry weight (RDW) were determined. The dried plant material (ground to a fine powder) was digested in 4:1 nitric acid:hydrogen peroxide, and analysed by radial view inductively coupled plasma-optical emission spectroscopy (Waite Analytical Services, Urrbrae, South Australia). A second weighed sub-sample of fresh roots were used for assessment of mycorrhizal colonisation using the gridline intersection method (150 intersects per sample) (Giovannetti and Mosse 1980), after roots were cleared with KOH (10% *W/V*) (Phillips and Hayman 1970) and stained with ink and vinegar (Vierheilig et al. 1998). Measurements of available (Colwell) P (Colwell 1963) and DTPA extractable Zn (Lindsay and Norvell 1978) were made on soil samples with Zn and P fertilisers added at the start of the experiment (See Table 2).

Calculation and data analysis

Plant water use efficiency (WUE) was calculated as grams of dry plant biomass produced per litre of water applied to the pot (Khan et al. 2003). Plant Zn and P content were calculated as concentration of Zn and P (as milligrams per kilogram) in the shoots/roots multiplied by total root/shoot biomass (kilograms).

Properties analysed by two-factor ANOVA were: SDW, RDW, shoot Zn content, root Zn content, total Zn content, total P content and WUE. Factors in the analysis were *Genotype* and *Zn fertiliser*. Mycorrhizal colonisation was analysed by a student's t-test, in the 76R genotype only. Where the two-way interaction or the *Zn fertiliser* main effect was significant, pairwise comparisons were made using Tukey's honestly significant difference (HSD, Zar 2007). Where the main effect of *Genotype* was significant, pairwise comparison was made using a student's t-test. All data were analysed using JMP statistical software (version 10.0.0).

Results

Mycorrhizal colonisation

Roots of the *rmc* genotype were effectively non-colonised by AMF (0.27 ± 0.34 % averaged across all *rmc* plants, data not shown), consistent with our earlier studies using this genotype and soil (Cavagnaro and Martin 2011; Watts-Williams et al. 2013). In contrast, the roots of the 76R genotype were well colonised (Table 3), therefore the effect of Zn fertiliser on colonisation of this genotype was considered separately from the *rmc* genotype. Colonisation of the 76R roots by AMF ranged from 24.0 to 34.5%; there were, however, no significant differences in AM colonisation among Zn addition treatments (Tables 3 & 4).

Plant biomass

Average plant shoot dry weight (SDW) ranged from 2.7 to 3.2 g across all treatments (Figure 1). Analysis of SDW data revealed a significant two-way interaction between *Genotype* and *Zn fertiliser* (Table 4). Specifically, the average SDW of both genotypes in the Zn carbonate treatment were significantly smaller than the 76R Zn phosphate treatment, with all other treatments intermediate.

Water use efficiency

Water use efficiency (WUE) differed among Zn fertiliser treatments, irrespective of genotype (Table 3), with the WUE of plants in the Zn carbonate treatment significantly lower than all other treatments (Tables 3 & 4). There was no effect of *Genotype* on WUE.

Plant zinc content

Zinc contents (Figure 2) of plant shoots were higher in *rmc* plants compared to 76R plants, and differed between Zn addition treatments, as indicated by a significant two-way interaction between *Genotype* and *Zn fertiliser* (Table 4). Specifically, Zn contents were highest in the Zn phosphate carbonate treatment, but lowest in the Zn phosphate and Zn carbonate treatments for the 76R and *rmc* treatments respectively. A similar pattern was seen for shoot Zn concentrations (data not shown, but compare Figures 1 and 2).

For roots, Zn contents did not differ between genotypes, but did between *Zn fertiliser* treatments, irrespective of *Genotype* (Table 4, Figure 2). When pooled over genotypes, root Zn contents were highest in the Zn phosphate carbonate, Zn sulphate and Zn oxide addition treatments, which were significantly higher than in the Zn oxide (nano) and Zn carbonate treatments, which in turn were significantly higher than in the Zn phosphate treatment. Similar results were found when root Zn concentrations were considered (data not shown, but compare Figures 1 and 2).

When whole plant Zn contents (Figure 2) were considered (i.e. shoot Zn + root Zn), Zn contents were significantly higher in *rmc* than 76R plants, irrespective of *Zn fertiliser* treatment (0.79 ± 0.03 and 0.70 ± 0.03 mg Zn $plant^{-1}$, respectively; Table 4). When *Zn fertiliser* treatments were considered, pooled over genotypes (Table 4), mean total Zn content was significantly higher in the Zn phosphate carbonate treatment than all other Zn fertiliser treatments, except Zn sulphate, and total Zn content was significantly lower in the Zn phosphate treatment than in all other treatments, with the exception of Zn carbonate.

Plant phosphorus content

When total plant P content was considered, there was a significant main effect of *Genotype* and of *Zn fertiliser* (Table 4). Specifically, the total P content of 76R plants was significantly higher than that of *rmc* plants (5.20 ± 0.08 and 3.76 ± 0.07 mg P $plant^{-1}$, respectively; pooling Zn fertiliser treatment). When the significant main effect of *Zn fertiliser* was considered, plants grown on the soil amended with Zn phosphate carbonate had significantly higher total P content than all other treatments (pooling genotype). Conversely, the plants grown on soils amended with Zn oxide (nano), or Zn carbonate, had significantly lower total P content than all other treatments. For both shoot and root P contents, the same patterns were seen as for total plant P contents and so are not described in further detail here (see Figure 3, Table 4).

Discussion

The results presented here clearly demonstrate that the form of Zn applied to the soil has a large impact on plant Zn nutrition, as has been shown previously (Whiting et al. 2001; Amrani et al. 1999; Mortvedt 1992; Boawn et al. 1957). Importantly, we found that the provision of Zn along with P, in the presence of carbonate (i.e. as Zn phosphate carbonate), had a large positive effect on plant Zn and P nutrition. In fact, plant Zn content in the plants provided with Zn phosphate carbonate, was similar to those that were supplied the highly water-soluble Zn sulphate in the presence of an equivalent soil concentration of P. Interestingly, whereas AM provided a benefit to plants in terms of P acquisition, the same was not true for Zn. Together the results highlight the importance of the physical and chemical nature of Zn fertilisers, and are now discussed in the context of plant Zn and P nutrition, while also considering the effects of mycorrhizal colonisation.

Plant nutrition - effects of Zn fertiliser

The effects of Zn fertiliser on plant nutrition were interesting and complex. Because of the importance of effective co-supply of P and Zn to plants, we considered how Zn fertiliser affected plant P, as well as Zn uptake. Plant P content/uptake was highest in the plants in the Zn phosphate carbonate treatment. Thus, the Zn phosphate carbonate fertiliser allowed for the addition of both P and Zn to the soil, whilst minimising the antagonistic effects on uptake of either nutrient. So, while the Zn phosphate and Zn carbonate fertilised plants had low Zn contents (ie. soil Zn was unavailable in the presence of P), it appears that having both phosphate and carbonate in the fertiliser (Zn phosphate carbonate) allowed plants to take up both Zn and P effectively (ie. soil Zn was available in the presence of P). Consequently, we consider this as a potential useful formulation for adding P and Zn to the soil simultaneously. Zn sulphate and Zn oxide also performed well when plant P uptake was considered, however their plant P contents were significantly lower than that of those fertilised with Zn phosphate carbonate.

Zn sulphate and Zn phosphate carbonate fertilisers performed similarly in terms of delivery of Zn to the whole plant, with Zn oxide close behind. Given that biomass between treatments was very similar, it is apparent that some Zn fertilisers were taken up more effectively by the plant, than others. Generally, Zn phosphate and Zn carbonate fertilisers performed poorly, in terms of plant Zn content.

Mycorrhizal colonisation

The overall low colonisation rate across all mycorrhizal plants is consistent with previous studies using the same soil and tomato genotypes, and similar additions of soil P and Zn (Watts-Williams and Cavagnaro 2012). Colonisation of roots by AMF was generally not affected by any of the Zn addition treatments. Increasing soil Zn concentrations have been shown to have positive (Lee and George 2005; Zhu et al. 2001), neutral (Diaz et al. 1996; Ortas et al. 2002; Cavagnaro et al. 2010), and negative (Bi et al. 2003; Chen et al. 2004; Gildon and Tinker 1983; Watts-Williams and Cavagnaro 2012; Watts-Williams et al. 2013) effects on mycorrhizal colonisation. Although it was predicted that differences in Zn availability of the fertilisers would affect colonisation, this prediction was not supported in this study. It is likely that magnitude of difference in Zn availability between treatments was not large enough to affect colonisation. It is more likely that in this study, soil P availability, which is considered to be the main edaphic factor regulating levels of AM colonisation (Smith and Read 2008; Ryan et al. 2000), and was consistent across all Zn fertiliser treatments, had more control over mycorrhizal colonisation than soil Zn availability.

Plant nutrition – effects of AM

Whereas mycorrhizal plants had significantly higher P contents than their non-mycorrhizal counterparts, the reverse was true for Zn. This indicates that AM were functional at least in terms of P uptake (ie. there was a positive mycorrhizal P response). In this study, the benefits of plants taking up luxury P are unclear, as the mycorrhizal plants were not larger than the non-mycorrhizal as would be expected when P contents are higher. However, it has been shown in tomatoes that higher uptake of P, as a result of being mycorrhizal, increased plant reproductive fitness in several ways, including increased flower production, fruit mass and seed number, and that improvement in reproductive traits were greater than improvements in vegetative traits (Poulton et al. 2002). Thus, there may have been an advantage in the reproductive fitness of the mycorrhizal plants grown in this study, as a result of higher P uptake than the non-mycorrhizal plants, which would have manifested if the plants had been harvested later. The reduced uptake of Zn by the mycorrhizal plants relative to the non-mycorrhizal plants may be due to the addition of P fertiliser to the soil. It has been shown previously that the provision of P fertiliser to mycorrhizal plants decreases Zn uptake, relative to non-mycorrhizal plants (Goh et al. 1997). Also, we would expect that if the Zn fertiliser treatments had been added to the soil at concentrations considered deficient, rather than sufficient, that we would see a clear Zn uptake benefit of being mycorrhizal, as in earlier studies (Watts-Williams et al. 2013). It is important to note that soil pH was lowered by growing plants in the soil, however the mycorrhizal plants did not lower soil pH more than the non-mycorrhizal plants (data not shown).

Plant biomass

It was expected that water solubility of the Zn fertilisers would correlate positively with plant biomass, as found in other studies (Amrani et al. 1999; Mortvedt 1992); however, this was not the case. Biomass was the same, without differences between fertiliser treatments, or genotype. While this translates to no benefit in terms of biomass accumulation, there were apparent nutritional benefits associated with the addition of some of the fertilisers. It is important to note that it is likely that all plants benefited in terms of biomass from the application of soil P and Zn fertiliser. In an earlier study, the addition of P and Zn fertiliser in the same amount as those in the present study, compared to no fertiliser addition, demonstrated a strong positive growth response (Watts-Williams et al. 2013).

Fertiliser properties

As with biomass, water solubility of Zn fertilisers has been shown to correlate positively with Zn uptake in plants (Amrani et al. 1999; Mortvedt 1992). However, there was no correlation between the water solubility of the Zn fertilisers in this study, and plant Zn uptake. In fact, the two fertilisers that contributed to the greatest plant Zn uptake had the highest (Zn sulphate) and a very low (Zn phosphate carbonate) water solubility. While there is little published open literature on the Zn phosphate carbonate material, we can speculate that in the presence of P fertiliser, this material exhibits equivalent soil solubility to that of Zn sulphate, lending it to enhanced availability and thus uptake by plants. Furthermore, it will be important to quantify the uptake of Zn fertilisers from various pools in the soil. This may be addressed by using a radioactive or stable isotope of Zn with the addition of the fertilisers, in order to trace the uptake of Zn from the soil into the plant. Interestingly, DTPA extractable Zn values were similar for all Zn fertiliser treatments, and did not correlate with plant Zn uptake. Therefore, in this experiment we found that neither water solubility, nor DTPA Zn extractability of Zn fertilisers were a useful indicator of a plant's ability to take up soil Zn.

Water use efficiency

Earlier studies have shown positive links between water use efficiency (WUE) and AM (Al-Karaki 1998; Kaya et al. 2003), and WUE and increasing soil Zn supply (Khan et al. 2003). Therefore, we hypothesised that the mycorrhizal genotype, and plants supplied with highly soluble Zn fertiliser (Zn sulphate), would have improved WUE over the non-mycorrhizal genotype, and plants supplied with less soluble Zn fertilisers. In this study, we saw no difference between genotypes, in terms of WUE.

Additionally, soil Zn was not deficient in any of the Zn fertiliser treatments, so any effect of Zn fertiliser solubility on WUE that we hypothesised might exist, was rendered less important. Interestingly, plants fertilised with Zn carbonate, a relatively insoluble compound, had significantly lower WUE than plants fertilised with any other compound. However, plants fertilised by other relatively insoluble Zn products (ie. Zn phosphate) did not display lower WUE, and this may be a result that deserves further investigation, using a wide range of agronomically important plant species.

Conclusions and implications

The dual application of P and Zn fertiliser can have antagonistic effects on plant uptake of both nutrients, most deleteriously on the uptake of Zn (Verma and Minhas 1987; Burleson et al. 1961; Cakmak and Marschner 1987). Therefore, effective co-supply of P and Zn to crops is important (Mortvedt and Gilkes 1993). The Zn phosphate carbonate material used in this experiment may be useful in addressing this problem, due to its ability to increase plant availability of both P and Zn, relative to other Zn fertilisers. Further investigation into the use of Zn fertilisers that can deliver Zn to plants in the presence of P fertiliser will be of particular interest. It is important to note that this novel form of Zn/P fertiliser may have unexpected effects on the biology or chemistry of the soil, that may account for the observed results. Thus, investigation into effects of the Zn phosphate carbonate fertiliser on soil microbiology and chemistry, as well as plant nutrition, will be important. Equally, it will be important to extend this work to include a wider range of crop species, especially those grown in regions where Zn deficiency in human diets is a major concern. Effective use of AM may be of further benefit to uptake of P and Zn, but P-Zn interactions that occur when the nutrients are taken up by AM, must be considered.

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