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DOI link to the version of record on the publisher's site

Dempsey, J.J., Wilson, I., Spencer-Phillips, P.T. and Arnold, D.L. (2022) 'Uptake and translocation of foliar applied phosphite and its effect on growth and development in cool season turfgrass', *Journal of Plant Nutrition*.

23 February 2022

Uptake and translocation of foliar applied phosphite and its effect on growth and development in cool season turfgrass

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Abstract

Phosphate (PO_4^{3-}, Pi) is the sole phosphorus (P) containing compound utilised for plant growth, leading to the widespread use of Pi containing fertilisers. An alternative form of P, phosphite (PO_3^{3-}, Phi) has increasingly been used in many crop systems, including amenity turfgrass, not only as a nutrient source but also as a pesticide and biostimulant. There are, however, conflicting reports of the efficacy and benefits of Phi as a source of P. This study was conducted to determine the rate of uptake, translocation and fate of Phi when applied as a foliar treatment to amenity turfgrass, and to assess its role as a source of P nutrition, determining the effect Phi treatments have on turfgrass growth, P deficiency responses, tissue and soil P accumulations. Analysis of Phi treated turfgrass using High Performance Ion Chromatography determined that Phi is rapidly taken up and translocated, that sequential Phi treatments lead to cumulative increases in meristematic tissues, an increase in soil P levels and no *in planta* conversion to Pi. In P sufficient rootzones (> 35 ppm), foliar applied Phi increased biomass in shoot, crowns, and roots, but also led to a reduction in root to shoot ratios. In phosphorus deficient rootzones (< 5 ppm), foliar applied Phi led to growth reductions in leaf, crown and root, and repression of P deficiency responses.

Keywords

Phosphite, Phosphate, Phosphorus, Turfgrass, Ion chromatography

Introduction

Phosphorus (P) is a major plant nutrient used in many metabolic processes, and because P is only found in combinations with other elements, phosphate (PO_4^{3-}, Pi) is the sole P-containing nutrient important for optimal plant growth. The majority of cultivated crops require regular inputs of Pi containing fertilisers (Raghothama and Karthikeyan, 2005).

However, an alternative form of P, phosphite (PO_3^{3-}, Phi) has increasingly been used, not only as a nutrient source but also as a pesticide and biostimulant in many crop systems, including amenity turfgrass (Fernando *et al*., 2015). The ability of Phi to control numerous plant diseases caused by Oomycetes, particularly of the genera *Peronospora, Plasmopara, Phytophthora* and *Pythium*, has been well documented (Lobato *et al*., 2010; Silva *et al.*, 2011; Burra *et al.*, 2014). Phosphite has also proven effective in reducing *Microdochium nivale* infection in amenity turfgrass (Dempsey *et al.*, 2012; Mattox *et al*., 2020).

The role of Phi as a source of P nutrition and its effects on plant growth however are more contentious. There are conflicting data regarding the efficacy and benefits of Phi as a source of P nutrition. Some studies report Phi application led to enhanced growth responses (Lovatt, 1990a; Rickard, 2000; Vincelli and Dixon, 2005). However, the majority of studies concluded that, although Phi is readily taken up and is highly mobile within a plants vascular system, it cannot be used directly as a nutrient source and therefore cannot complement or substitute Pi fertiliser (Saindrenan *et al.*, 1985; Ouimette and Coffey, 1988; Roos *et al*., 1999; Thao and Yamakawa, 2009; Borza *et al.*, 2014). Other studies have shown that the presence of Phi can inhibit Pi deficiency compensatory responses (Ticconi *et al.*, 2001). Enhanced root growth or an increase in root to shoot ratios are definitive responses to P limitation and these were strongly inhibited by Phi in *Brassica nigra* (Carswell *et al.*, 1996). Furthermore, Fabricio *et al.* (2012) concluded that foliar applied Phi caused harmful effects to *Phaseolus vulgaris* growing in P-limited soil. As with many cultivated plants, turfgrasses require Pi as a regular fertiliser input. Phosphite is commonly used in turfgrass management programmes, but there are few studies on the effect Phi treatment has on turfgrass growth, and no published data on the uptake, accumulation and fate of Phi following application. Research into Phi specifically as a turfgrass fertiliser by Butler *et al.* (2009) investigated the effects of Phi and Pi treatments on *Agrostis stolonifera* in a greenhouse study by measuring weekly changes in grass dry weights, leaf tissue phosphorus content and root dry weights. It was concluded that Phi applications have limited influence on turfgrass growth and development, when applied to a newly sown turfgrass sward.

With regards to the effect of Phi on turfgrass quality, field trials by Horvath *et al*. (2007) at a number of locations in the United States assessed the impact of a range of Phi products on *A.*

stolonifera. Results showed that no Phi product consistently provided a significant improvement in turf quality or colour. Enhanced turfgrass quality following sequential treatment with Phi was reported, however, by Cook *et al*. (2006) on a mixed sward of *A. stolonifera* and *P. annua* and by Dempsey and Owen (2010) on an *A. stolonifera* sward. Phosphite has also been shown to inhibit the *in vitro* mycelial growth of *M. nivale*, a major pathogen of amenity turfgrass (Dempsey *et al.*, 2018) and to suppress disease symptoms in the field (Dempsey *et al.*, 2012; Mattox *et al.*, 2020). If Phi's mode of inhibition involves the suppression of *M. nivale* hyphal growth *in planta*, it is therefore of interest to assess the uptake, translocation and fate of foliar applied Phi.

The aims of this research, therefore, were to: determine and describe the uptake, vascular translocation, accumulation and fate of Phi in treated turfgrass tissues; assess the value of Phi as a source of P nutrition in turfgrass; and determine the effect Phi treatment has on turfgrass growth, P deficiency responses and tissue and soil P accumulations.

Materials and methods

Establishment and growth conditions of greenhouse turfgrass samples

Three turfgrass species, *Agrostis stolonifera* L. variety Shark, *Lolium perenne* L. variety Bargold and *Poa annua reptans* L. variety Truputt were established and maintained in greenhouses. All samples were sown in 110 mm diameter poly-vinyl chloride (PVC) pipes cut to 300 mm lengths, filled with rootzone sand complying with Sports Turf Research Institute (STRI) recommendations for golf green construction in the UK (Baker, 2005). The growth vessels were maintained in greenhouses in Kildare, Ireland, under natural light and temperature conditions during the trial periods from January 2011 to September 2014. All were seeded at the optimum rate for the particular species (Turgeon, 2005; Butler *et al*., 2009) and allowed to establish before commencement of experimental procedures. Turfgrass growth was maintained through the trial period with regular inputs of soluble urea, giving annual nutritional inputs (ANI) of 60 kg N ha^{-1}, with all other nutritional inputs supplied as part of treatment applications. Minimal irrigation inputs were applied via a hand-held hose to replace water lost through evapotranspiration.

Turfgrass treatments and tissue sample collection

Foliar treatments of potassium phosphite (KH_2PO_3, Phi), potassium phosphate (KH_2PO_4, Pi) and potassium chloride (KCL, as control) were applied sequentially, at rates and timings as required by the research protocols, using 5 l pressure sprayers, operating at 6 bars, calibrated to deliver 400 l ha^{-1}. Phi and Pi treatments were prepared by titrating 1 M solutions phosphorous and phosphoric acids with 6 M reagent-grade potassium hydroxide (KOH) to adjust to pH 6.5. KCl treatments were prepared from commercially available potassium chloride. Leaf tissues were collected using scissors, crowns were harvested by removing the leaf tissues, then slicing the crowns from the roots using a knife. Roots were collected by placing the rootzone into a 2 mm sieve and washing with water until all rootzone soil was removed. All tissues were dried at 60^0 C for 48 h prior to analyses.

Uptake, translocation and accumulation of phosphite and phosphate in turfgrass

Phosphite was applied as a foliar treatment to *A. stolonifera* and *P. annua* in February 2011 and July 2012, at a rate of 0.35 g PO_3^{3-} m^{-2}. Harvesting of leaf and root tissues was at 0, 6, 12, 24, 36, 48, 60, 72, 84 and 96 h post application (p.a.) and 0, 1, 2, 3, 4, 5 and 6 weeks p.a. Tissue content of Phi and Pi was measured by High Performance Ion Chromatography (HPIC), using a modified version of a technique published by Roos *et al*. (1999); all analyses were carried out by OEW Laboratories, Cornwall, UK. The ion chromatograph consisted of a Dionex ICS100 ion chromatograph equipped with an IonPac AG9-HC Guard Tube (4 x 50 mm), IonPac AS9-HC Analytical Column (unheated 4 x 250 mm), ASRS300 Suppressor (4 mm), DS6 Heated Conductivity Cell, and a 25 µl injection loop. The eluent was 9 mM sodium carbonate (99.999%), degassed and pressurised to 1 bar, flowing at 1 ml min^{-1} (approximately 2200 psi) with a single back pressure loop. Method run time was set to 18 minutes. Prior to tissue analyses, a Pi standard (as PO_4^{3-} w/v) was prepared from sodium Pi monobasic anhydrous (H_2NaO_4P) and >18.2 Mohm deionised water, and a Phi standard (as PO_3^{3-} w/v) was prepared from sodium Phi dibasic pentahydrate (Na_2 (PHO_3).$5H_20$. Standard mixed solutions were prepared at 12.5, 25, 50, 100, 200, 500 and 1000 ppm w/v of both PO_4^{3-} and PO_3^{3-}. The ion chromatograph was calibrated by 12.5, 25, 50, 100, 200, 500 and 1000 ppm mixed Pi/Phi standards. The calibration curve was not linear over this calibration range, as a cubic curve was found to give a better fit.

Samples of 0.5 g of finely ground turfgrass leaf and roots were weighed into 15 ml polypropylene centrifuge tubes and agitated for 2 min with 10.0 ml of sterile distilled water. he mixture was allowed to extract overnight at ambient temperature. The samples were agitated again for 2 min prior to analysis. Samples were taken up in 2 ml disposable syringes from the centrifuge tubes and manually injected into the ion chromatograph, through 0.47 µm syringe filters, into the sample loop of the Dionex HPIC system, using a 9 mM sodium carbonate eluent. The solutions did not require any additional dilution. Results were adjusted for the weights of extracted samples and reported as ppm of dried tissue weight. To evaluate the effect of sequential Phi foliar treatments on turfgrass tissue and rootzone P levels, PO_3^{3-}, was applied at 0.35 g square meter at monthly intervals, to *A. stolonifera* and *P. annua* from July 2012 to July 2014. Leaf and root tissues were collected at 6, 12 and 24 month intervals, one week post-treatment application and analysed for Phi content.

Rootzone samples were collected prior to and at the conclusion of the 24 month trial period and analysed for treatment effect on nutrient status using techniques shown in Table 1.

Phosphite as a source of phosphorus nutrition and effects on P deficiency responses

To assess the properties of Phi as a source of phosphorus (P) nutrition for turfgrass growing in two different soil P levels and to determine its effect on turfgrass development, foliar treatments were applied to *L. perenne* and *P. annua* bi-weekly, over a six-month period. Two soil P levels were used, P-deficient and P-sufficient, where P deficient corresponded to 5 ppm and P sufficient 38 ppm (Mehlich, 1984). Treatments of Phi and Pi applied at 0.35 g m^{-2} PO_3^{3-} and PO_4^{3-} and KCl were applied from March to September 2013, to give 13 applications in total. Treatment effect on shoot growth was determined by the cumulative dry weights of clippings of leaf tissues in excess of the selected height of cut of 5 mm. Crowns and roots were collected at the end of the trial and weighed for dry mass determination and calculation of root to shoot ratios. Root to shoot ratios were calculated by dividing the mean dry root weights by the mean dry shoot weights. Shoot, crown, and root dry masses were analysed for P content.

Data analysis

All treatments, unless otherwise stated, were randomised with six replications. Prior to any analyses, residuals were tested to ensure the assumptions of the one-way Anova were satisfied. Outliers were assessed by inspection of a boxplots, Shapiro-Wilk's test determined normality (Shapiro and Wilke, 1965) and homogeneity of variances assessed by Levene's test (Levene, 1960). Phosphite and Pi accumulations were analysed using two-way Anova with dependent variables of Phi and Pi accumulation in turfgrass tissues and independent variables of turfgrass species, plant tissues and timing of data collection. Tukey HSD post hoc analyses at $p = 0.05$ separated any differences. Phosphate tissue and rootzone accumulations were analysed using Paired-samples t-test at $p = 0.05$. Two-way Anova analysed treatment effect on leaf, crown and root development, root to shoot ratios and tissue P levels with dependent variables of tissue dry weight and independent variables of turfgrass species, plant tissues and treatments. Tukey HSD post hoc analyses at $p = 0.05$ separated significant differences. All data analysis was performed using the statistical program SPSS Statistics 21.

Results

Uptake, translocation and accumulation of phosphite and phosphate in turfgrass

Phosphite uptake in greenhouse samples of *A. stolonifera* and *P. annua* in February 2011, when mean air temperatures were 7.6 ^{o} C, was determined using HPIC analyses. Phosphite accumulation in leaf tissues 6 h p.a. was 3191 ppm in *A. stolonifera* and 3085 ppm in *P. annua*. Accumulation in leaf tissues peaked 48 h p.a. with 4886 ppm and 5071 ppm in *A. stolonifera* and *P. annua* respectively. Leaf tissue amounts in both turfgrass species gradually declined and at 96 h p.a. were 4270 ppm in *A. stolonifera* and 4534 ppm in *P. annua* 96 h p.a., = (Fig. 1). One week p.a. leaf tissue accumulations in *A. stolonifera* were 3332 ppm and 4395 ppm in *P. annua.* At the conclusion of the assessment period, 6 weeks p.a., Phi amounts had decreased to 496 ppm and 862 ppm in *A. stolonifera* and *P. annua* respectively (Fig. 2). Translocation of foliar applied Phi to the root systems was observed in both turfgrass species, with accumulations 117 ppm and 373 ppm at 6 and 96 h p.a. in *A. stolonifera* and 96 ppm and 385 ppm 6 and 96 h p.a. in *P. annua* (Fig. 1). Phosphite root accumulations in *A. stolonifera* peaked at 479 ppm two weeks p.a. with amounts declining over the following four weeks to 81 ppm, at six weeks p.a. Phosphite amounts in *P. annua* roots peaked earlier than in *A.*

stolonifera with 376 ppm at one week p.a. with amounts declining to 163 ppm, at six weeks p.a. (Fig. 2).

Results from the July 2012 series of analyses showed a similar pattern in Phi take up as that in the February 2011 study. Higher greenhouse mean air temperatures of 22.3 ^{o} C gave rise to higher turfgrass growth and subsequent increased uptake rate. Phosphite accumulation in leaf tissues at 6 h p.a. were 3265 ppm in *A. stolonifera* and 3194 ppm in *P. annua*. Accumulation in leaf tissues peaked 48 h p.a. with 5520 ppm and 5418 ppm in *A. stolonifera* and *P. annua* respectively. As in the February analyses, leaf tissue amounts declined over the six-week assessment period, with 4314 ppm at 96 h p.a. in *A. stolonifera* and 4452 ppm in *P. annua* (Fig. 3). One week p.a. leaf tissue accumulations in *A. stolonifera* were 3451 ppm, and 3387 ppm in *P. annua.* At the conclusion of the assessment period, 6 weeks p.a., Phi amounts had decreased to 261 ppm and 218 ppm in *A. stolonifera* and *P. annua* respectively (Fig. 4).

As in the February 2011 study, following foliar treatment with Phi, root accumulations were considerably less than in the leaf tissues. Phosphite accumulations in *A. stolonifera* were 108 ppm and 441 ppm at 6 and 96 h p.a., and 101 ppm and 328 ppm at 6 and 96 h p.a. in *P. annua* (Fig. 3). Phosphite root accumulations in *A. stolonifera* peaked at 463 ppm two weeks p.a. with amounts declining over the following four weeks to 256 ppm six weeks p.a. Phosphite amounts in *P. annua* roots peaked later than in *A. stolonifera* with 457 ppm three weeks p.a., with amounts declining to 313 ppm six weeks p.a. (Fig. 4).

Determination of PO_4^{3-} in phosphite treated turfgrass tissues

Determination of PO_4^{3-} levels was an important part of this study, as the question of *in planta* conversion of PO_3^{3-} to PO^{3-} needed to be examined. In *A. stolonifera* Pi leaf amounts during the February study decreased significantly from 8656 ppm to 8390 ppm. Pi levels in the root tissues followed a similar trend, with amounts decreasing significantly from 1436 ppm to 1314 ppm. During the July assessments, Pi leaf levels increased, but not significantly from 8287 ppm to 8327 ppm. Pi levels in the root tissues, however, did increase significantly , from 1397 ppm at the start to 1558 ppm at the conclusion (Fig. 5).

In *P. annua*, Pi levels during the February study increased significantly from 8234 ppm to 9127 ppm. Pi levels in the root tissues did not change significantly with amounts at the start of the study of 1113 ppm, and 1110 ppm at the conclusion. During the July assessments, Pi

levels in the leaf decreased significantly from 8361 ppm at the start to 7917 ppm. Pi levels in the root tissues also decreased significantly from 1235 ppm1104 ppm (Fig. 5).

Accumulation of phosphite in turfgrass tissues following sequential treatments over two years.

Significant differences in Phi accumulations in leaf and root tissues were determined in both *A. stolonifera* and *P. annua* following monthly Phi treatment applied sequentially between July 2012 and July 2014. In *A. stolonifera,* Phi amounts in leaf tissues were 3590 ppm in January 2013, significantly greater than both the July 2013 level of 3272 ppm and the July 2014 level of 3468 ppm, which was significantly greater than the July 2013 figure of 3272 ppm. In *P. annua,* Phi amounts in leaf tissues were 4078 ppm in January 2013, significantly greater than both the July level of 2013 3573 ppm and the July 2014 level of 3712 ppm, which was significantly greater than the July 2013 value (Fig. 6).

In root tissues of *A. stolonifera* Phi amounts were 490 ppm in January 2013, significantly less than both the July 2013 level of 753 ppm and the July 2014 level of 835 ppm, with the July 2014 level significantly greater than the July 203 level. In *P. annua,* Phi amounts were 693 ppm in January 2013, significantly greater than both the July 2013 level of 655 ppm and the July 2014 level of 662 ppm; there were no significant differences between the July 2013 and July 2014 amounts (Fig. 6).

Rootzone nutrient analyses following sequential phosphite treatments over two years

Rootzone nutrient levels, as determined by the analytical methods described in Table 1, prior to the start of the two-year treatment programme and at the conclusion of the study are shown in Table 2. The cation exchange capacity (C.E.C.) status of these rootzones was shown to be extremely low with mean values of 8.0 meq/100g (Table 2). Sequential applications of P in the form of either Phi or Pi, significantly increased soil P levels in the rootzones of both turfgrass species compared to levels prior to treatment applications. In *A. stolonifera* rootzones, P levels following Phi treatments increased significantly from 37 to 51 ppm. In Pi treated rootzones, levels increased significantly from 37 ppm to 40 ppm. P levels in Phi treated rootzones were significantly greater than Pi treated rootzones. In *P. annua* rootzones P levels following Phi treatments increased significantly from 37 to 57 ppm. In Pi treated

rootzones levels increased from 37 ppm to 44 ppm. P levels in Phi treated rootzones were significantly greater than Pi treated rootzones (Table 2).

The Phi was applied combined with potassium (K) as potassium phosphite, so changes in rootzone K levels were of interest. In *A. stolonifera* rootzones K levels following Phi treatments increased significantly from 88 ppm to 109 ppm and from 88 ppm to 105 ppm following Pi treatments. K levels in Phi treated rootzones were significantly greater than the Pi treated rootzones In *P. annua* rootzones, K levels increased significantly from 88 ppm to 104 ppm following Phi treatments and from 88 ppm to 110 ppm following Pi treatments. K levels in Phi treated rootzones were significantly greater than levels in the Pi treated samples (Table 2).

Effects of phosphite treatment on leaf, crown, and root development in *L. perenne* and *P. annua* growing in phosphorus sufficient rootzones

In *L. perenne,* Phi treatment significantly increased dry weights in leaf, crown and root tissues, compared with Pi and KCl treated plants (Fig. 7). Dry weight of leaf cuttings was significantly greater following Phi treatment at 3.62 g, compared to Pi at 3.15 g and KCl at 3.13 g. There were no significant differences between the Pi and KCl treatments. Crown dry weights were significantly greater following Phi treatment at 17.29 g, than both Pi at 13.81 g and KCl at 13.35 g. The Pi treatments were significantly greater than KCl. Root dry weights were significantly greater following Phi treatment at 8.59 g, than both Pi at 7.29 g and KCl at 8.10 g. The KCl treated root dry weights were significantly greater than the Pi treated tissues.

In *P. annua,* Phi treatment significantly increased dry weights leaf, crown and root tissues, compared with Pi and KCl treated plants (Fig. 7). Dry weight of leaf cuttings was significantly greater following Phi treatment at 3.79 g, compared to Pi at 3.45 g and KCl at 2.83 g, with Pi treated leaf weights significantly greater than the KCl . Crown dry weights were significantly greater following Phi treatment at 14.55 g, than both Pi at 11.40 g and KCl at 10.32 g, with Pi treatments significantly greater than KCl . Root dry weights were significantly greater following Phi treatment at 5.93 g, compared to both Pi at 5.03 g and KCl at 5.60 g. The KCl treated root dry weights were significantly greater than the Pi treated roots.

Effects of phosphite treatment on leaf, crown, and root development in *L. perenne* and *P. annua* growing in phosphorus deficient rootzones

In *L. perenne,* Phi treatment significantly reduced dry weights in leaf, crown and root tissues, compared with Pi and KCl treated plants (Fig. 8). Dry weights of leaf cuttings were significantly less following Phi treatment at 1.75 g, compared to Pi at 3.04 g and KCl at 2.39 g. The Pi treated leaf dry weights were significantly greater than the KCl. Crown dry weights were significantly less following Phi treatment at 7.11 g, compared to Pi at 9.01 g and KCl at 8.34 g, with the Pi treatments significantly greater than KCl. Root dry weights were significantly reduced following Phi treatment at 5.45 g, compared to Pi at 7.86 g and KCl at 7.21 g. The root dry weight following Pi treatments were significantly greater than KCl (Fig. 8).

In *P. annua* dry weights of *P. annua* leaf cuttings were significantly less following Phi treatment at 1.46 g, compared to Pi at 2.94 g and KCl at 2.15 g. The leaf dry weights from the Pi treated plants were significantly greater than the KCl. Crown dry weights were significantly less following Phi treatment at 5.92 g, than both the Pi at 8.09 g and KCl at 7.46 g, with the Pi treatments significantly greater than KCl . Root dry weights were significantly reduced following Phi treatment at 3.76 g, compared to Pi at 5.73 g and KCl at 4.96 g. The root dry weight following Pi treatments were significantly greater than KCl (Fig. 8).

Effect of phosphite treatments on the root to shoot ratios of *L. perenne* and *P. annua*

Root to shoot ratios in *L. perenne*, growing in both rootzone types were significantly lower in the Phi treated plants than the Pi and KCl treated plants. In the P sufficient rootzone the KCl treatments producing the highest ratios at 0.607, significantly greater than the Pi treatments of 0.528, and both were significantly greater than the Phi treated ratio of 0.495 (Fig. 9). In the *L. perenne* growing in the P deficient rootzone, Pi treatments produced the highest ratios at 0.873, significantly greater than the KCl
at 0.862, and both were significantly greater than the Phi treated ratios of 0.765 (Fig. 9).

Root to shoot ratios in *P. annua* growing in both rootzone types also displayed significantly lower ratios in the Phi treated plants compared to the Pi and KCl (Fig. 9). In the P sufficient rootzones the KCl treatments producing the highest ratios at 0.540, significantly greater than the Pi treatments of 0.442, and both were significantly greater than the Phi treated ratios of

298 0.408, (Fig. 9). In the *P. annua* growing in the P deficient rootzones the Pi treatments
299 produced the highest ratios at 0.707, significantly greater than the KCl treatments of 0.663,
300 and both were significantly greater than the Phi treated ratios of 0.637 (Fig. 9).

301 **Discussion**

302 Prior to this study, there were no published data on the uptake and accumulation of Phi in
303 turfgrasses. The most relevant data on the foliar application of nutrients in turfgrasses
304 reported on the uptake and accumulation of major and minor nutrients, in particular nitrogen.
305 These studies have shown that in turfgrasses most nutrients are rapidly taken up, but the
306 speed of uptake varies in correlation with nutrient compound size (Bowman and Paul, 1989;
307 Gaussoin *et al.*, 2009; Stiegler *et al.*, 2009). Other studies, however, have shown the uptake
308 of Phi in other plant systems (Thao and Yamakawa, 2010; Borza *et al.*, 2014) and protocols
309 presented for the determination of Phi accumulation in plant tissues by Saindrenan (1985)
310 and Berkowitz *et al.* (2011), and the method adapted for this study (Roos *et al.*, 1999)**.**
311 The HPIC analyses reported here produced significant and novel data. The data show that
312 Phi, following foliar application to *A. stolonifera* and *P. annua*, is rapidly taken up into the
313 leaf tissues and within hours detectable in the roots. This confirms phloem mobility and
314 suggests full symplastic ambimobility. The first set of treatments and analyses commenced in
315 February 2011, during a period of low turfgrass growth and metabolism. Uptake into the leaf
316 tissues during this period was rapid, with 65% in *A. stolonifera* and 61% in *P. annua* of the
317 maximum accumulation achieved within 6 h of application (Fig. 1). The level of Phi within
318 the leaf tissues peaked at 48 h p.a. and by 96 h p.a. levels had declined in both turfgrass
319 species. Over the full 6 week study period it was shown that Phi levels progressively declined
320 and by 6 weeks p.a. had reduced to 10% of the maximum accumulation in *A. stolonifera* and
321 to 17% of the maximum in *P. annua* leaf tissues (Fig. 2).
322 Following the first series of studies, it was thought that uptake could be significantly affected
323 by growth conditions, therefore the second series of experiments was during a period of
324 increased metabolic activity during July 2012. The results of this second study were similar
325 to the first with regard to rapid take up and translocation rates but confirmed that Phi uptake
326 was increased during periods of greater growth potential. In *A. stolonifera* for example, leaf
327 accumulations 48 p.a. were 5520 ppm in July compared to 4886 ppm in the February

assessments (Figs 1 and 2). Similarly, in *P. annua* the Phi leaf accumulations at 48 p.a. were 5418 in July compared to 5071 ppm in February (Figs 1 and 2).

As well as an increased uptake during the July period, it was determined that there was a more rapid decline in leaf amounts during periods of higher turfgrass growth rates. In *A. stolonifera* Phi amounts in leaf tissues 6 weeks p.a. had decreased from 5520 ppm to 261 ppm, 5% of the maximum accumulation, compared to 10% of the maximum during the February study. Similarly, in *P. annua*, amounts in leaf tissues 6 weeks p.a. had dropped from 5520 ppm to 261 ppm, 5% of the maximum accumulation, compared to 17% in February (Figs 3 and 4).

Demonstration of symplastic mobility, in that the foliar applied Phi translocated and was detected at 6 hours p.a. in the roots of treated turfgrasses, was a significant outcome of this study. Although the maximum root accumulations, 479 ppm in *A. stolonifera* (February 2011) and 457 ppm in *P. annua* (July 2012) (Figs 2 and 4), were much less than in the leaf tissues, it remains a significant result, as no other compound used for pathogen suppression in turfgrasses demonstrates symplastic ambimobility and this is the first time that this mobility has been reported in these turfgrass species.

These data are of particular significance to turfgrass managers, who utilise Phi as part of their nutritional and disease prevention programs. Many apply Phi on a 2 to 3-week cycle, prior to and during periods of high disease pressure, as Phi treatments have been shown to suppress *M. nivale* incidence (Dempsey *et al*, 2012; Mattox *et al.*, 2020). The results here would indicate that this cycle of sequential Phi applications would maintain Phi levels in the leaf within the range of 3000 to 3500 ppm. The mechanisms of disease suppression by Phi have not been fully determined, but one possibility or factor in the disease incidence reduction is that Phi has a direct effect on the *in vitro* growth of *M. nivale* (Dempsey *et al*., 2018). Therefore, the presence of Phi in the turfgrass tissues could slow the infection progress, allowing the turfgrass increased time to initiate and deliver defense responses.

Phosphite accumulation following sequential treatments over two years

Phosphite treatment gave rise to rapid uptake and accumulations in all turfgrass tissues, but the fate and persistence of foliar applied Phi following long term applications needed to be addressed. During the two year treatment programme of this study, tissue Phi levels for *A.*

stolonifera and *P. annua*, one week post treatment application at 6, 12, and 24 months following trial commencement, were determined using HPIC analyses. Over the two years of sequential applications, Phi in leaf tissues remained at constant levels, varying only with time p.a. and the metabolic rate as governed by seasonal growth rates, thus showing no evidence of a systemic buildup in these tissues. This does not infer that Phi is metabolised, de-graded biochemically or as shown in Fig. 5, converted to Pi, but rather is physically removed, as part of the on-going mowing regime, typical of amenity turf maintenance. However, there was an increasing accumulation of Phi in root systems. Following uptake of Phi via leaf tissues, it is translocated within hours to the root systems of both turfgrass species and remained detectable throughout the six-week trial periods (Figs 1 to 4). Sequential applications of Phi, over two years, gave rise to significantly increasing levels of root accumulation in *A. stolonifera* and to a lesser extent, in *P. annua* (Fig. 6). This lower accumulation of Phi in *P. annua* following long term applications could be due to the shorter lifespan of *P. annua*, compared to the perennial *A. stolonifera*, with the root systems senescing more rapidly in *P. annua*. This is evidenced further by the increased levels of P found in the rootzones of *P. annua* compared to *A. stolonifera* (Table 2). The senescence of any turfgrass tissues which contained Phi accumulations would give rise, over time, to increased levels of soil P. This would also be the case, although to a lesser extent, with leaf tissue, which although in the case of golf greens are collected during mowing, would eventually contribute to increased soil P content.

Rootzone phosphorus accumulations

The use of P containing fertilisers is a contentious issue worldwide, with some regions allowing P applications only after confirmation of P deficiency from soil test analyses. Therefore, the effect of long-term sequential application of Phi on the P status of rootzone soils was an important factor in these studies. Over two years of sequential treatments, the Phi and Pi applications supplied equivalent amounts of P, however soil P levels in rootzones of both turfgrass species receiving Phi increased significantly. The rootzone of *A. stolonifera* receiving Phi increased by 38% from a base level of 37 ppm to 51 ppm. The P level increase

in the *P. annua* rootzone was even greater at 54%, from 37 ppm to 57 ppm. Over the same period, soil P levels following sequential Pi applications also increased but by a lesser extent. The rootzone of *A. stolonifera* receiving Pi increased by 8%, from 37 ppm to 40 ppm, whilst in the *P. annua* rootzone the increase was 19%, from 37 ppm to 44 ppm (Table 2).

This significantly higher level of rootzone P accumulation following Phi treatments is of interest. It could be due to Phi being locked into the rhizosphere by soil microorganisms. Oxidation of Phi to Pi in soil relies on microbial activity, requiring the absorption and uptake of Phi by soil bacteria and subsequent oxidation to Pi. This however, is a slow process with a half-life of several months (Mcdonald *et al.*, 2001). Phosphorus in the rootzone following Pi treatment would be less persistent and more easily leached, bearing in mind the C.E.C. status of these rootzones is extremely low with mean values of 8.0 meq/100g (Table 2). Whatever the reason, the steady increase of soil P levels following sequential Phi treatments may pose a problem for turfgrass management. Higher levels of soil P are often attributed to increased proclivity of *P. annua*, a species, which although dominant in many golf greens, is widely regarded as an undesirable weed species. This therefore in an area which requires further and more extensive study.

Phosphite to phosphate conversion *in planta*

Determination of PO_4^{3-} levels following Phi treatment was an interesting part of this study, as the question of *in planta* conversion of PO_3^{3-} to PO_4^{3-} is often raised, with numerous commercial suppliers claiming Phi as a source of P nutrition following *in planta* conversion of Phi to plant usable Pi. The results here were conclusive, the level of Pi in leaf and root tissues were determined as part of the HPIC analyses. In *A. stolonifera* leaf and root tissues, during both assessment periods, there was a clear determination that there was no *in planta* conversion of Phi to Pi, as there was no significant increase in Pi levels in the six weeks following Phi treatment. In *P. annua*, during the February assessments, there was a significant increase in Pi levels in leaf tissues from 8234 ppm to 9127 ppm, with no change in the Pi levels in the roots. The results from the July assessments, however, determined significant reductions in Pi levels in both and root tissues. Despite the Pi increase in *P. annua* leaf tissues during the February analyses it can be concluded from both studies that the

application of Phi does not lead to *in planta* conversion to Pi, a conclusion that is strongly supported by the results shown in Fig. 8 and which is further discussed below.

Phosphite effects on turfgrass growth in phosphorus deficient and sufficient rootzones

There are numerous published studies examining the role of Phi as a supplier of P nutrition, with no clear consensus regarding its efficacy. There are reports of both beneficial and detrimental effects on plant growth following Phi treatment (Thao and Yamakawa, 2009; Fernando *et al*., 2015). The present study determined significant differences in growth responses, both positive and negative in *L. perenne* and *P. annua* following Phi treatment, in both the P deficient and P sufficient rootzones. Phi and Pi chemically are very similar, and both are acquired by plants via Pi transporters (Varadarajan, 2002; Jost *et al*., 2015), but this similarity ends at the level of uptake and translocation. As determined above, Phi is not converted into Pi in plants, therefore it cannot enter the biochemical pathways in which Pi is assimilated. A second point is that as Phi competes with Pi for uptake via the same plant transport system (Carswell *et al.*, 1996; Varadarajan, 2002; Danova-Alt *et al.*, 2008; Jost *et al*., 2015), this would lead to a reduction of usable P, leading to further Pi depletion. It was surmised prior to the start of these studies that in P limited situations Phi treatment would not supply P in a form that could be metabolised by plants, and in fact would inhibit growth as P deficiency responses would not be initiated. The results here confirmed that hypothesis, as it was determined that Phi, when applied under P limited situations does in fact inhibit growth, as shown in Fig. 8. Furthermore, that the KCl treatment gave rise to increased growth compared to the Phi treatment is evidence that Phi also suppressed the P deficiency responses in both species. These results agree with the findings of Ticconi *et al.* (2001) who concluded that Phi inhibited P deficiency compensatory responses in *Arabidopsis thaliana*, and Fabricio *et al.* (2012) who determined foliar-applied Phi caused harmful effects to plants growing in P-limited soils.

The conclusion that Phi suppressed deficiency responses is further supported by the root dry weight (Fig. 8) and the root to shoot ratio (Fig. 9) data. Varadarajan (2002) determined that Phi suppressed many of the definitive responses to P limitation, such as enhanced root growth and increased root to shoot ratios. The results here show that while there were significant differences in the root mass and root to shoot ratios between the KCl and Pi treatments, there

447 was significantly less root growth and reduced ratios in the Phi treated plants, compared to
448 both other treatments. These findings agree with work where plants grown in P limited
449 conditions are reported to be highly sensitive to Phi, displaying toxicity symptoms such as
450 leaf chlorosis and stunted growth (McDonald *et al.*, 2001; Ratjen and Gerendas, 2009; Thao
451 and Yamakawa, 2009). These results give clear evidence that in amenity turfgrass
452 management, Phi should only be applied under conditions of sufficient P levels for the target
453 species involved.
454 There is no evidence in the literature to support the metabolism of Phi as a P source or it's *in*
455 *planta* conversion to a plant useable form of P, and this is strongly supported by the results
456 determined here following Phi treatment in the P deficient rootzones. Taking that into
457 account therefore, the results of Phi treatments to turfgrass growing in a P sufficient
458 rootzones were surprising. Phi treatment significantly increased leaf, crown and root biomass,
459 compared with Pi and KCl treated plants (Fig. 7). While there are no published data of Phi
460 increasing turfgrass growth there are numerous reports of improved turfgrass quality
461 following sequential applications of Phi (Vincelli and Dixon, 2005; Horvath *et al.*, 2007;
462 Dempsey and Owen, 2010). Turf quality is defined as the degree to which a turf sward
463 conforms to an agreed standard that is a composite of uniformity, shoot density, leaf texture,
464 growth habit, smoothness, and colour (Horvath *et al*., 2007). Research with plant systems
465 other than turfgrass however have reported enhanced growth responses following Phi
466 treatment (Lovatt, 1990b; Albrigo, 1999; Rickard, 2000), but the reasons for the enhanced
467 growth responses are not explained. Lovatt and Mikkelsen (2006) suggest Phi-enhanced
468 growth may be a growth-regulatory or phytohormonal factor, effecting sugar metabolism,
469 stimulation of the shikimic acid pathway, or internal hormonal and chemical changes. Zhang
470 *et al*. (2011) concluded that while *Microcystis aeruginosa* could not utilise Phi as a sole P
471 nutrient at any concentration, when Phi was supplied simultaneously with Pi it increased cell
472 numbers and chlorophyll content. In their review of the biostimulant activities of Phi,
473 Fernando *et al.* (2015) concluded that Phi can be used as a biostimulant which will enhance
474 plant growth via activation of molecular, biochemical and physiological responses, but these
475 positive responses require and are attenuated in the presence of Pi.
476 Root growth and development is a crucial component of all plants, but can be especially so
477 for turfgrass, which in golf greens is maintained under highly stressed situations. Root

development can determine how the turfgrass plant reacts in situations which can seriously impact on the viability and even survival of the sward. Abiotic and biotic challenges, such as drought, traffic related wear and disease pressure, are constantly endangering the plants and a well-developed root system can often be the major influencing factor in the turfgrass plants success. In this study, when the root to shoot ratios were calculated (Fig. 9), it was shown Phi treatments produced the lowest mean ratio of roots to shoots in both *A. stolonifera* and *P. annua,* in either the P sufficient or the P deficient rootzone situation. These ratios are a direct indication of the number of roots per shoot, with the higher ratios showing the greater volume of root growth per plant. What this indicates is that in a P sufficient situation, while Phi treatment gave rise to increased growth of shoots, crowns and roots, compared with Pi and KCL treatments, the enhanced above ground growth was at the expense of the development of the root systems. The reason for this is not clear and would require further study. In the P deficient rootzones, root to shoot ratios were also significantly reduced by Phi treatments. This however was to be expected and consistent with the research by Carswell *et al.* (1996), which concluded that Phi treatments to P limited plants decreased the root to shoot ratios significantly, a conclusion supported by this current research.

Conclusions

This study determined that Phi is rapidly taken up and translocated by turfgrass, and that sequential applications applied on a 3-week cycle would maintain leaf tissue accumulations of approximately 3000 ppm. Long-term sequential Phi treatments maintain leaf tissue accumulations but can lead to cumulative increases in meristematic tissues such as roots and can cause increases in soil P levels. In P sufficient rootzones foliar-applied Phi increased biomass in all plants, but also led to a reduction in root to shoot ratios. In P deficient rootzones foliar-applied Phi does not supply a usable form of P and furthermore deficiency responses were repressed.

As well as producing novel and significant data, this research also gave rise to a number of issues which require further study. These include the long term effect of sequential applications of Phi on soil P accumulations and availability, increased accumulations in meristematic tissues and reduction in root to shoot ratios. Research over a longer time frame

than in this study could assess these issues, using a wider range of turfgrasses, growing in rootzones with varying physical and chemical properties.

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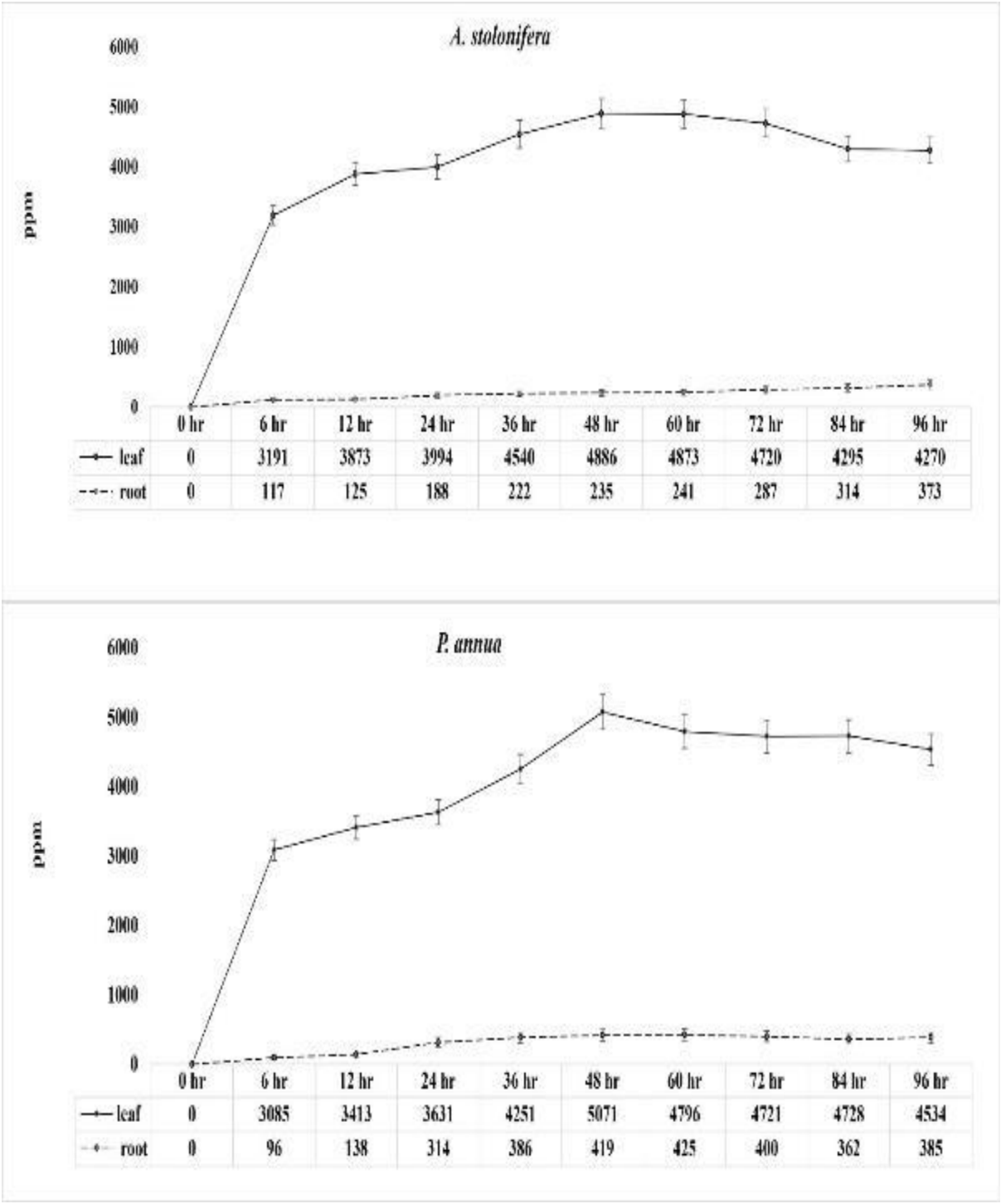

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Figure 1. Uptake and accumulation of Phi in *A. stolonifera* and *P. annua* leaf and root tissues, from 0 to 96 hours post application of Phi at a rate of 0.35 g PO_3^{3-} m^{-2}, in February 2011. Bars indicate 95% confidence intervals, n=6.

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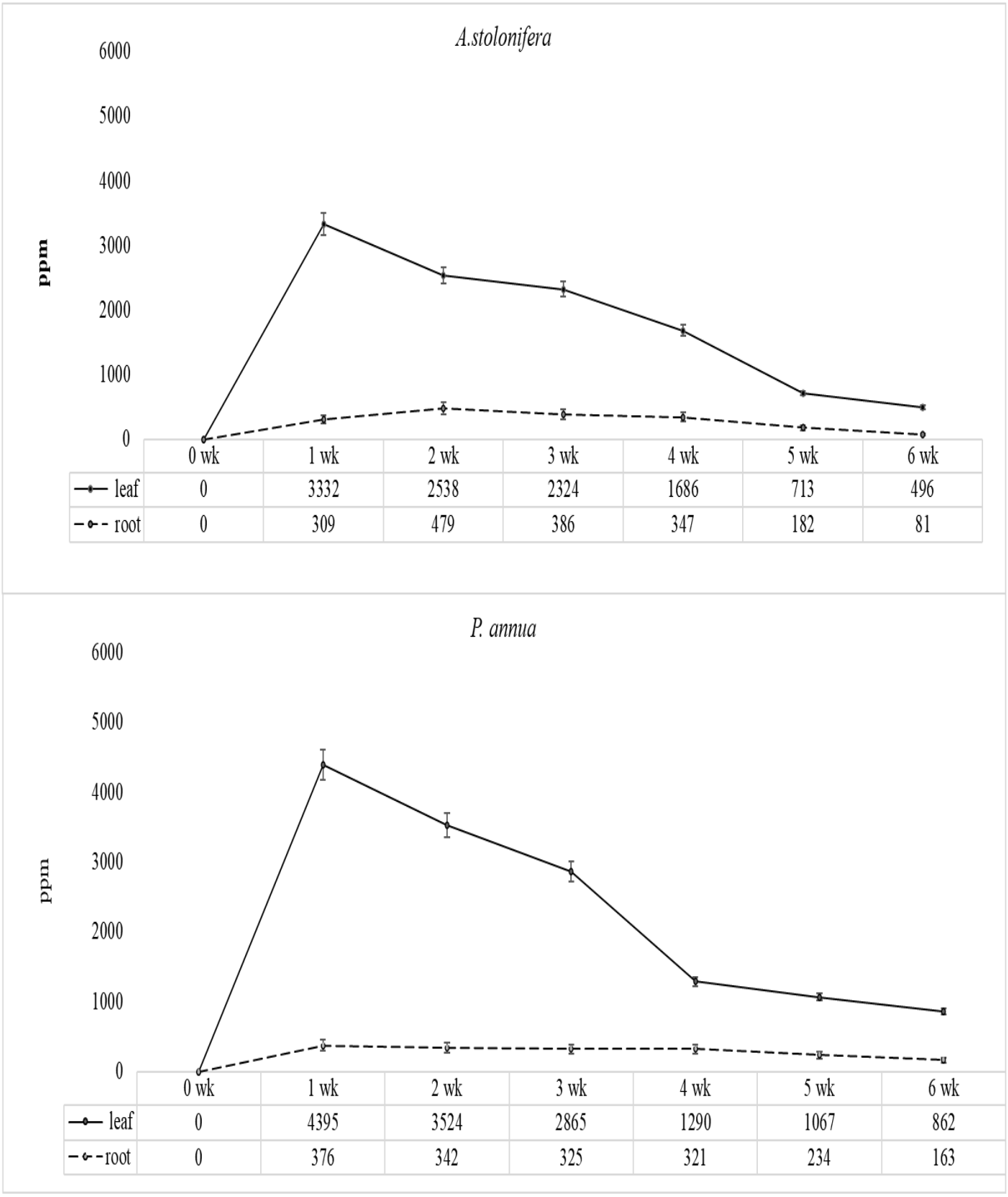

Figure 2. Uptake and accumulation of Phi in *A. stolonifera* and *P. annua* leaf and root tissues, from 0 to 6 weeks post application of Phi at a rate of 0.35 g PO_3^{3-} $m^{-2,}$ in February 2011. Bars indicate 95% confidence intervals, n=6.

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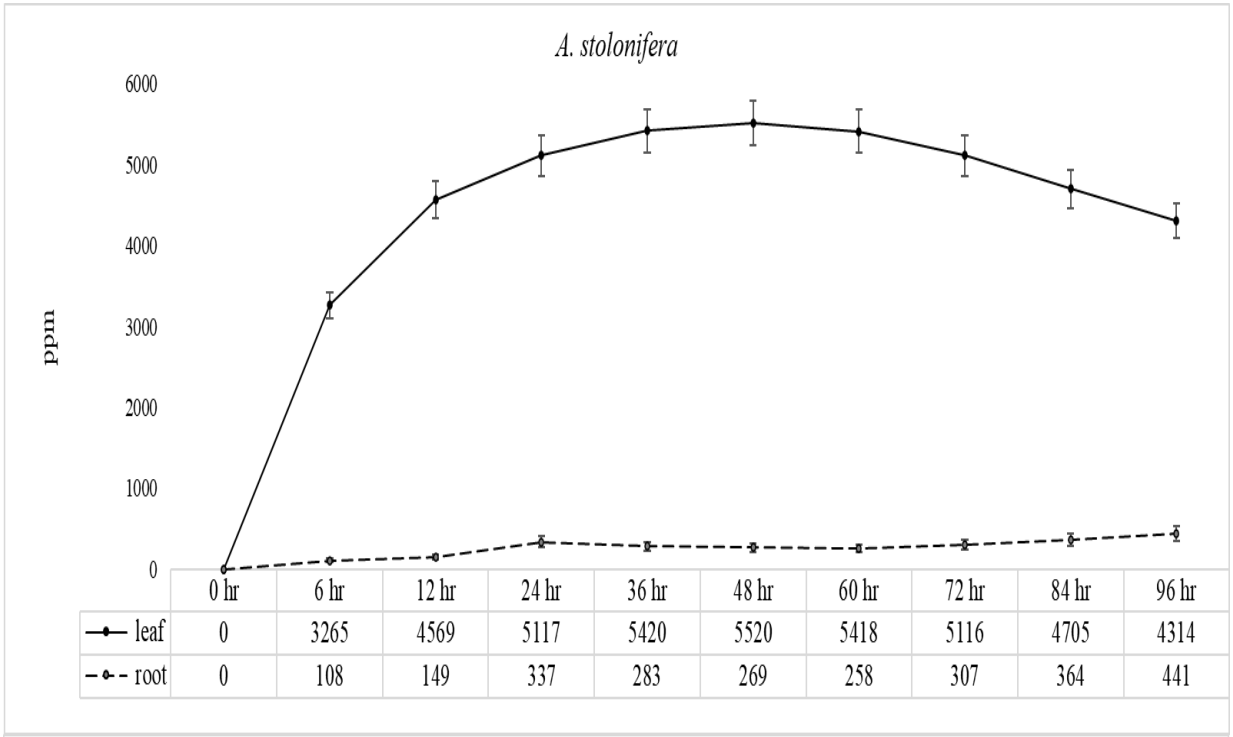
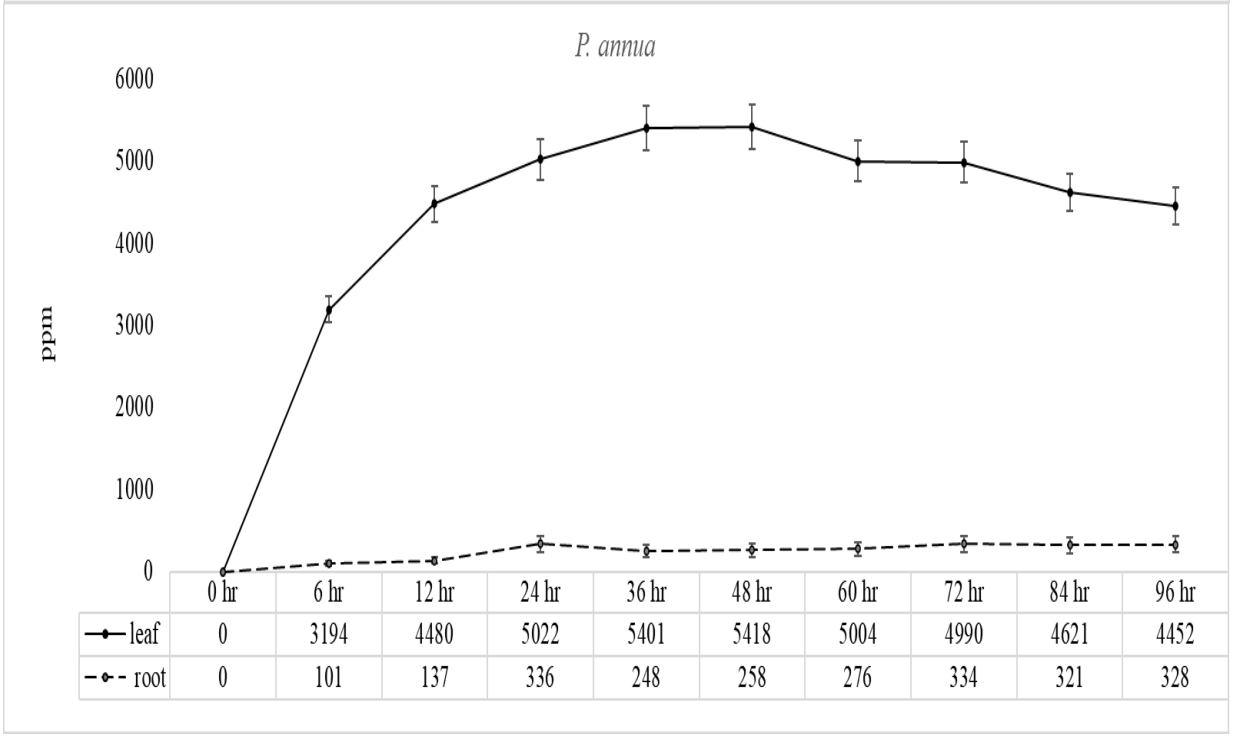


	0 hr	6 hr	12 hr	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr
leaf	0	3265	4569	5117	5420	5520	5418	5116	4705	4314
root	0	108	149	337	283	269	258	307	364	441



	0 hr	6 hr	12 hr	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr
leaf	0	3194	4480	5022	5401	5418	5004	4990	4621	4452
root	0	101	137	336	248	258	276	334	321	328

Figure 3. Uptake and accumulation of Phi in *A. stolonifera* and *P. annua* leaf and root tissues, from 0 to 96 hours post application of Phi at a rate of 0.35 g PO_3^{3-} $m^{-2,}$ in July 2012. Bars indicate 95% confidence intervals, n=6.

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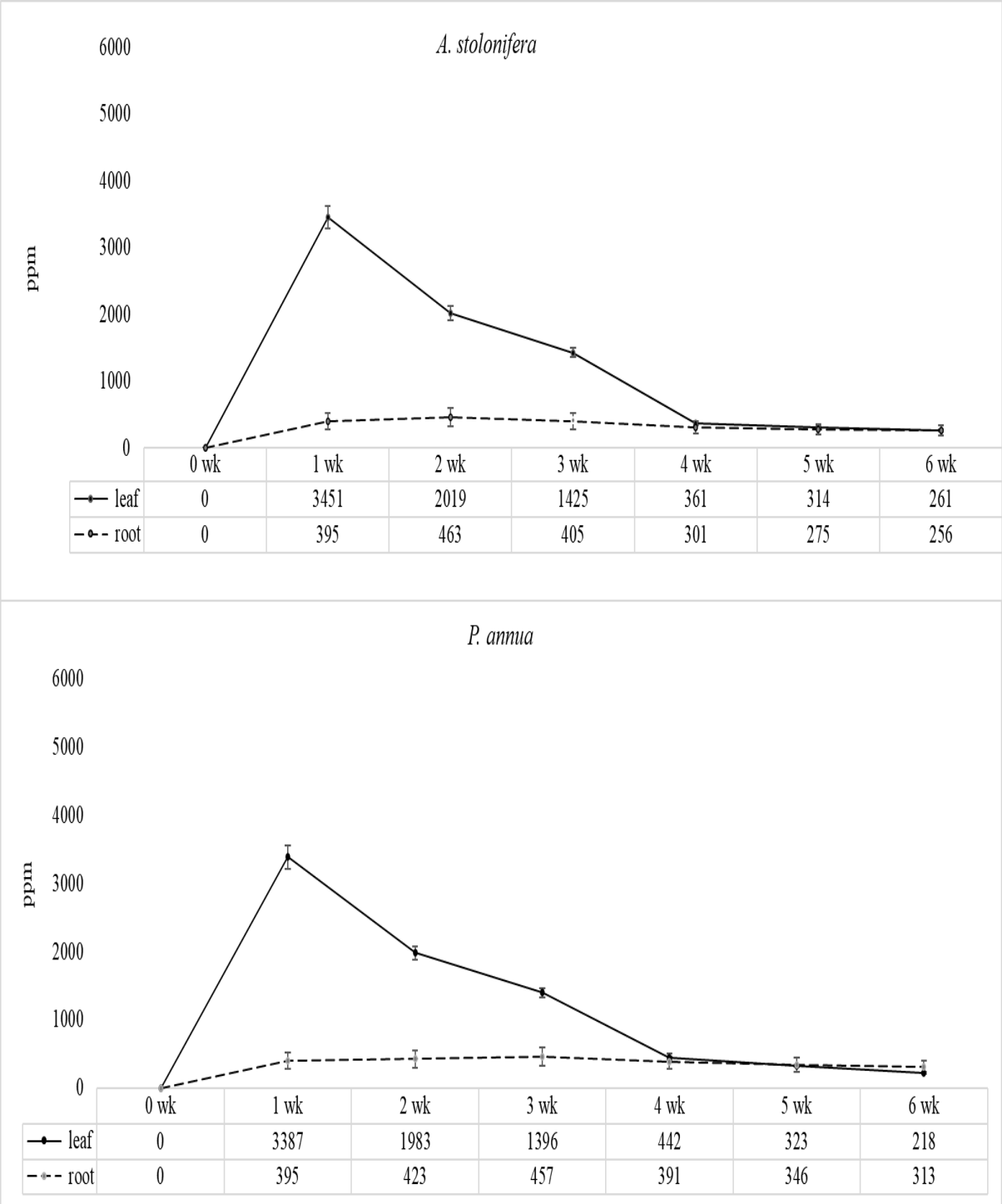

Figure 4. Uptake and accumulation of Phi in *A. stolonifera* and *P. annua* leaf and root tissues, from 0 to 6 weeks post application of Phi at a rate of 0.35 g PO_3^{3-} $m^{-2,}$ in July 2012. Bars indicate 95% confidence intervals, n=6.

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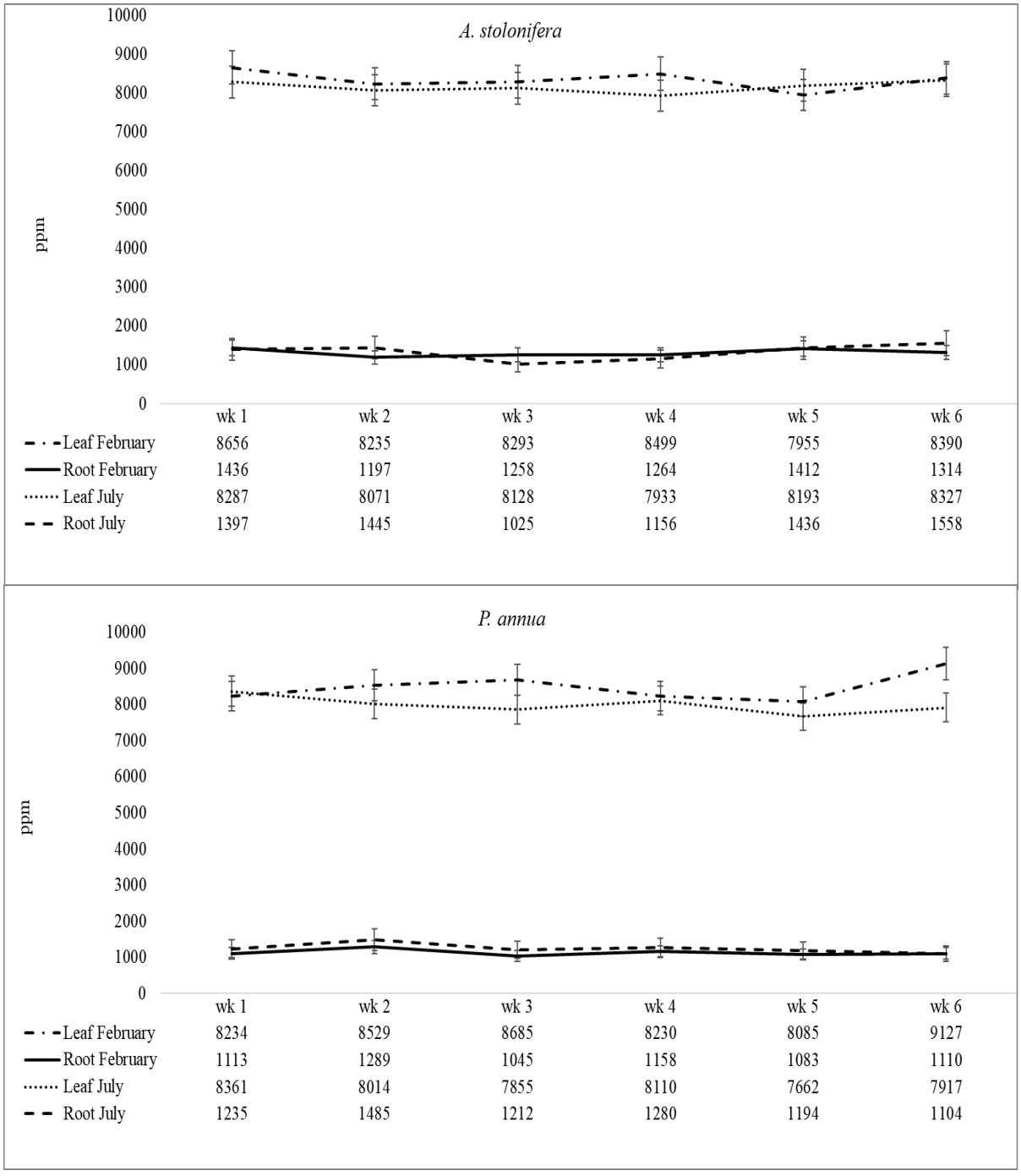

Figure 5. Pi amounts in leaf and root tissues of *A. stolonifera* and *P. annua*, six weeks post treatment with Phi at a rate of 0.35 g PO_3^{3-} m^{-2}, in February 2011 and July 2012. Bars indicate 95% confidence intervals, n=6.

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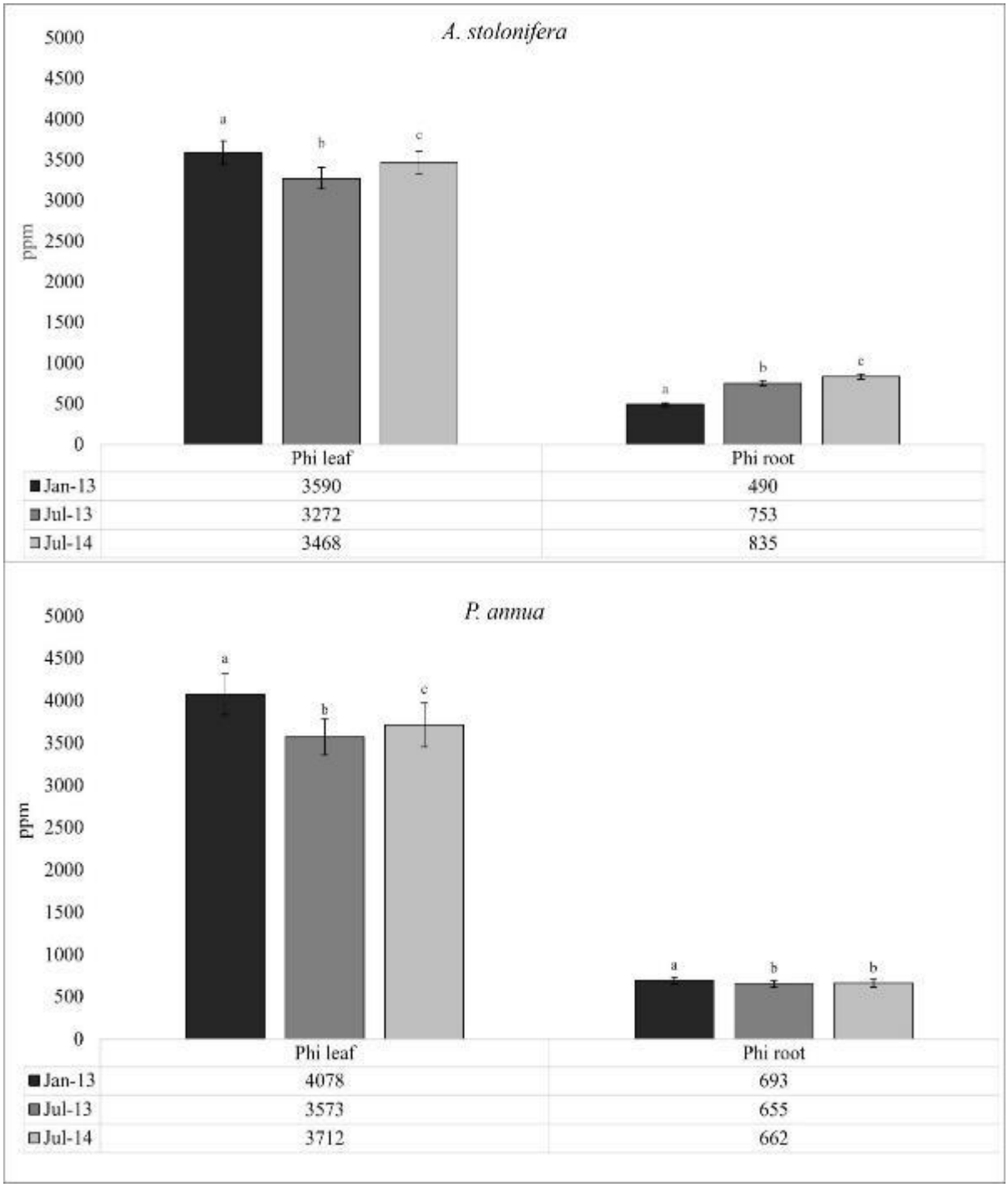

Figure 6. Phi accumulations in leaf and root tissues of *A. stolonifera* and *P. annua* following sequential monthly applications of Phi, at a rate of 0.35 g PO_3^{3-} m^{-2}, between July 2012 and July 2014. Data were recorded one week post treatment application at 6, 12 and 24 months from commencement of treatments. Bars indicate 95% confidence limits; letters indicate significant differences in the accumulation of Phi in tissues between treatments for each month as determined by Tukey HSD at p = 0.05.

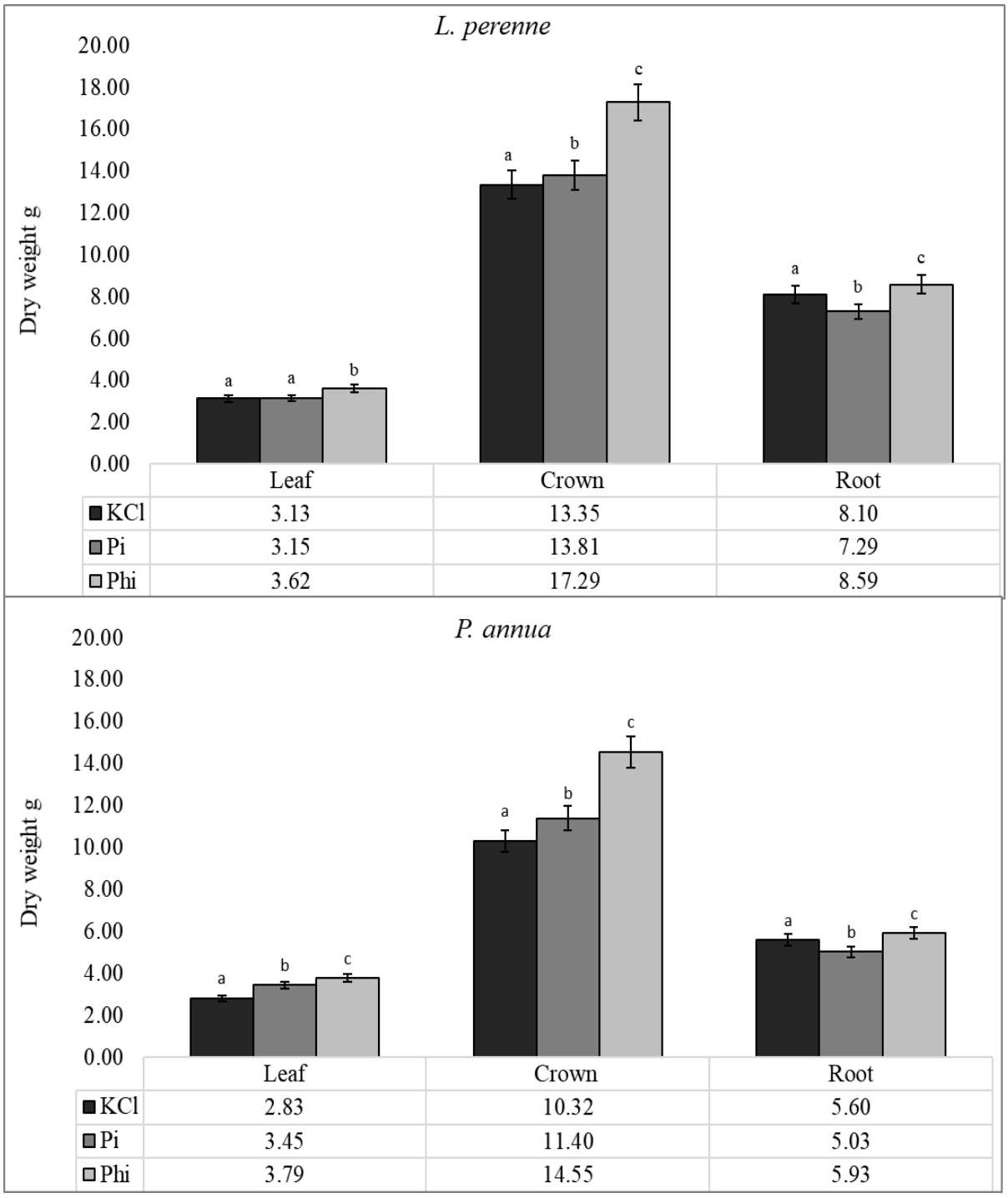

Figure 7. Effect on the growth of leaf, crown, and root tissues of *L. perenne* and *P. annua*, growing in a P sufficient rootzone (P > 38 ppm), following sequential treatments over a six-month period, of phosphate, phosphite and potassium chloride (control). Bars are 95% confidence intervals, n=6. Letters indicate significant differences within tissue type as determined by Tukey HSD post hoc analyses at p = 0.05.

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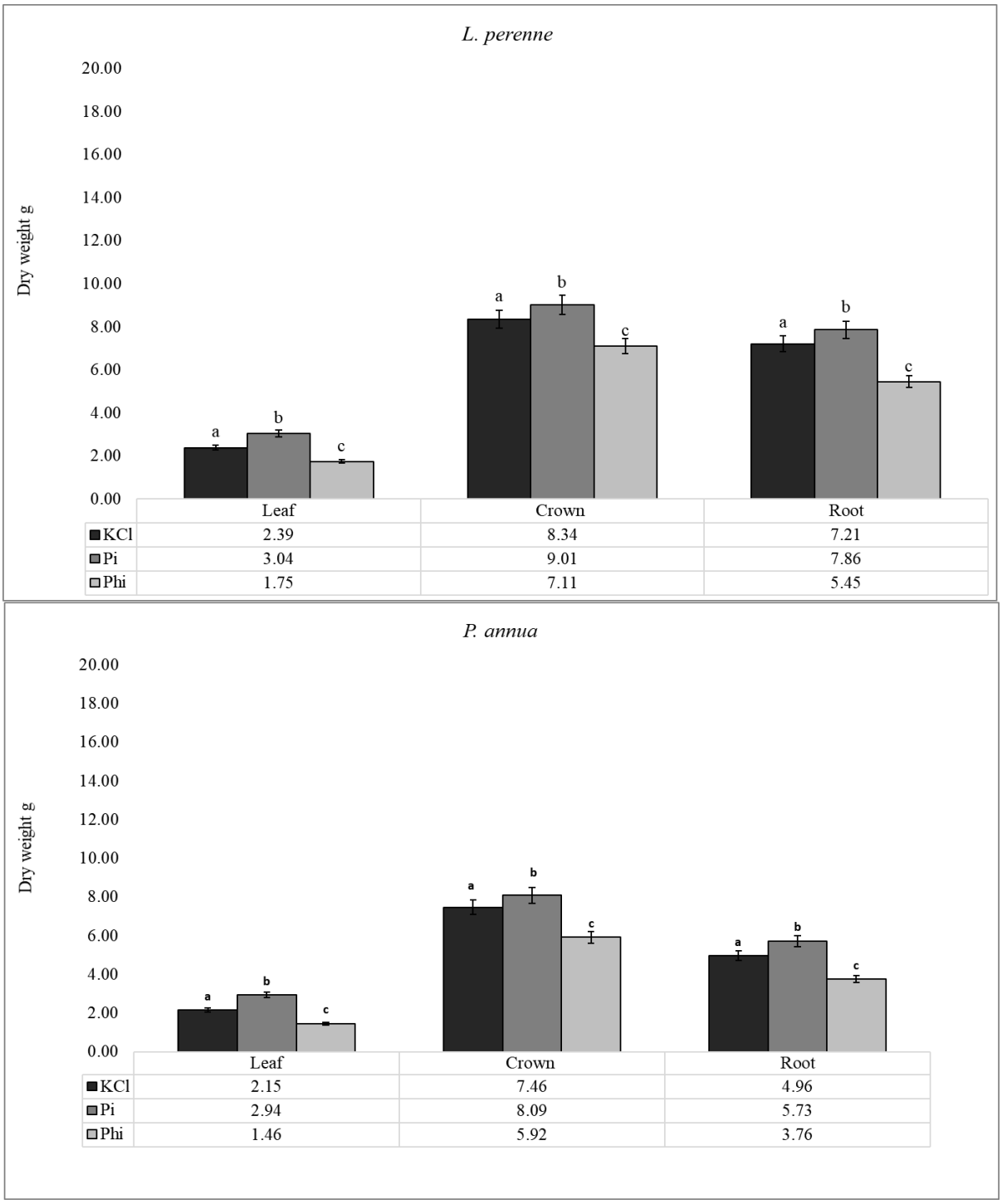

Figure 8. Effect on the growth of leaf, crown, and root tissues of *L. perenne* and *P. annua*, growing in a P deficient rootzone (P < 5 ppm), following sequential treatments over a six-month period, of phosphate, phosphite and potassium chloride (control). Bars are 95% confidence intervals, n=6. Letters indicate significant differences within tissue type as determined by Tukey HSD post hoc analyses at p = 0.05.

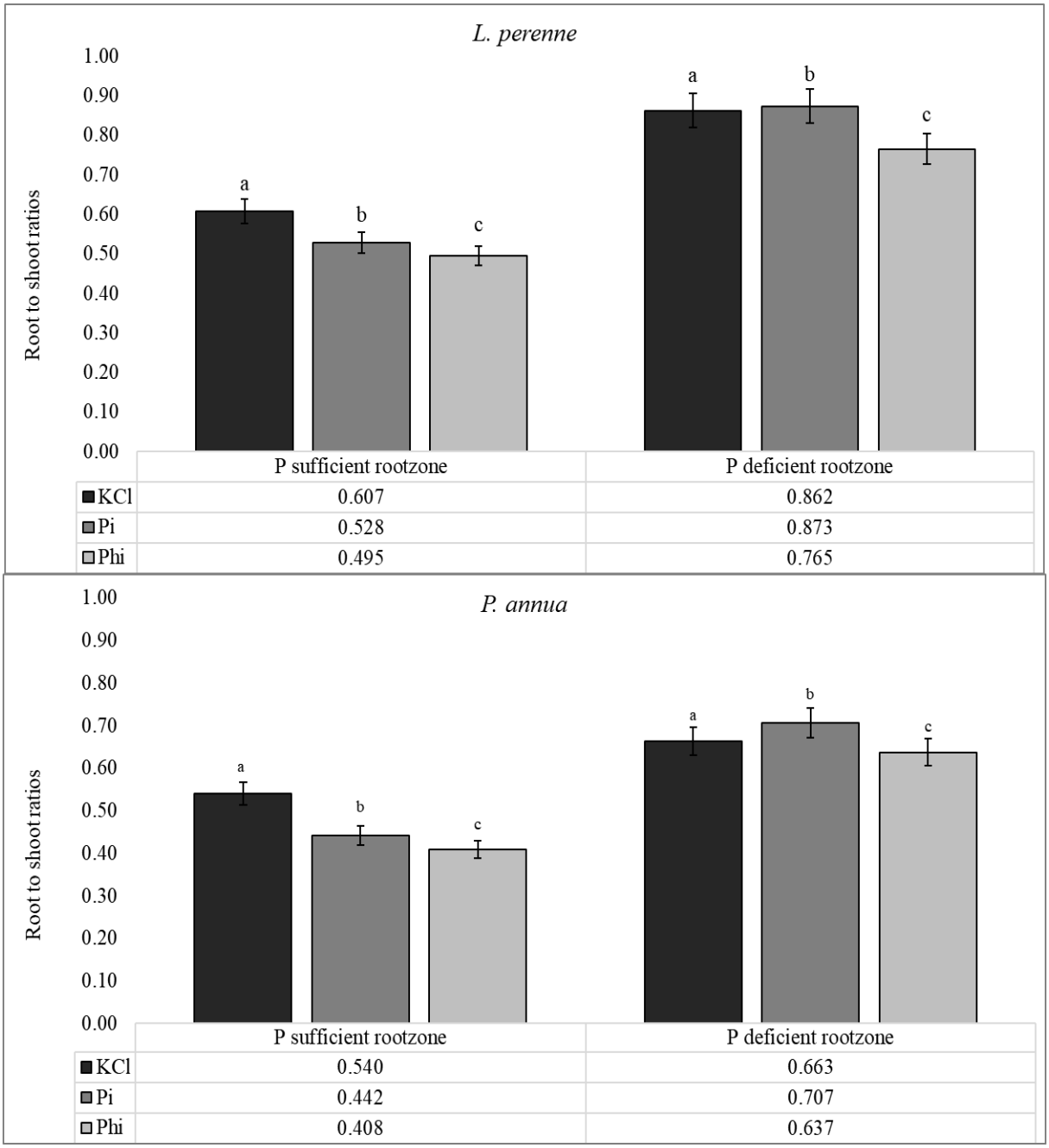

Figure 9. Effect on root to shoot ratios of *L. perenne* and *P. annua* growing in P sufficient (P > 38 ppm) and P deficient (P < 5 ppm) rootzones, following sequential treatments over a six-month period, of phosphate, phosphite and potassium chloride (control). Bars are 95% confidence intervals, n=6. Letters indicate significant differences within tissue type, as determined by Tukey HSD post hoc analyses at p = 0.05.

651 Table 1. Description of analytical methods used to determine rootzone properties and nutrient levels.

Element	**Unit**	**Digestion Extractant**	**Analytical Technique**
Nitrogen	ppm	Sulphuric/orthophosphoric acid	Kjeldhal distillation CNS analyser
Phosphorus	ppm	Mehlick 3 solution	Solution spectrophotometry
Potassium	ppm	1M Ammonium acetate @ pH 7.0	Atomic absorption spectrometer
Magnesium	ppm	1M Ammonium acetate @ pH 7.0	Inductively coupled plasma atomic emission spectrometer (ICP-AES)
Iron	ppm	0.005 M EDTA disodium salt	ICP-AES
Calcium	ppm	1M Ammonium acetate @ pH 7.0	ICP-AES
Cation Exchange	Meq/100g	1 M ammonium acetate	Summation of extracted cations (K, Mg, Ca, Na, H)

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Table 2 Rootzone nutrient content (ppm) and Cation Exchange Capacity (C.E.C.), prior to the start of treatments in July 2012 and at the conclusion of treatments in July 2014.

Treatment		N	P	K	Mg	Fe	Ca	C.E.C.
A. stolonifera rootzone							667	
Jul-12	Phi	6.5	37	88	46	280	1510	7.7
	Pi	6.5	37	88	46	280	1510	7.7
Jul-14	Phi	7.5	51	109	71	328	1443	7.9
	Pi	7.2	40	105	79	282	1422	8.0
P. annua rootzone								
Jul-12	Phi	6.5	37	88	46	280	1510	7.7
	Pi	6.5	37	88	46	280	1510	7.7
Jul-14	Phi	7.9	57	104	73	277	1373	7.9
	Pi	6.8	44	110	77	304	1404	8.1