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PHOSPHORUS DEFICIENCY IN *PELARGONIUM*: EFFECTS ON NITRATE AND AMMONIUM UPTAKE AND ACIDITY GENERATION

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□ *A sudden pH decline (SPD) of the substrate is an increasing problem in geranium growth systems, and the cause is unknown. In this study, we investigate whether a phosphorus (P) deficiency can cause SPD, and whether the effect is related to inhibition of ammonium (NH_4^+) and nitrate (NO_3) uptake and a corresponding shift in the cation to anion uptake balance. Geraniums (*Pelargonium x hortorum *Bailey 'Designer Dark Red) were grown in hydroponic solutions with or without P, and the hydroponics systems were located in a growth chamber programmed for light/dark temperatures of 22/18 or 26/22°C. Acidification potential was measured by the amount of base required to maintain pH at 5.8. The results indicated that much greater amounts of base were required to maintain a stable pH with P-limited plants. Using periodic exposures to $^{15}NH_4^+$ or $^{15}NO_3^-$, it was found that NO_3^- uptake was strongly inhibited as plants became P stressed. Tissue nutrient profiles showed that the NO_3^- uptake inhibition was accompanied by an increase in the cation to anion uptake ratio. Rhizosphere acidification was greater at higher temperature even though the cation and anion responses were unchanged in control plants, suggesting the involvement of carbon dioxide (CO_2) generated by root respiration. The results indicate that changes in cation and anion uptake and the associated increase in net H^+ extrusion that occur under P-stress conditions can contribute to SPD in geranium culture systems.*

Keywords: acidification, cation-anion balance, pH

INTRODUCTION

One of the largest cultural problems facing ornamental plant growers is control of root substrate pH. During crop production, pH rises and falls with irrigation water, use of acidic fertilizers, and/or from insufficient limestone

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in the substrate. The shifts in pH are usually gradual enough to be detected in time and successfully adjusted. However, there are exceptions.

Geraniums are the highest valued plant of the 2.5 billion dollar bedding plant industry (USDA, 2005). During the 1980s, many geranium producers began reporting a sporadic and unexplained decline in substrate pH. During the same time period, they began reporting frequent occurrence of high concentrations of iron (Fe) and/or manganese (Mn) in leaf tissue and the appearance of toxicity symptoms (Bachman and Miller, 1995). Logically, one would think the two effects were linked to one another. In organic-based soilless substrate such as that often used with geranium, pH has a large affect on nutrient availability (Nelson, 2003); as pH decreases, some micronutrients become more available and can become toxic. In a study with `Ringo Scarlet' geraniums, for example, substrate pH decreases from the 6.5-6.8 range to 5.2-5.5 was accompanied by ~10 fold increases in tissue Fe, Mn, and zinc (Zn) concentrations (Lee et al., 1996). Also, in a study with seedling geraniums, decreases in pH to <5.5 led to the appearance of micronutrient toxicity symptoms (Biernbaum et al., 1987). The observations indicating a `sudden pH decline' (SPD) in geranium systems and the associated micronutrient toxicities have led to substantial economic losses and, in some cases, implementation of tedious pH adjustment techniques (e.g., application of flowable limestone). Up to this time, the cause(s) of SPD in geranium culture has remained unknown.

It seems likely that geranium plants contribute to the observed rhizosphere acidification, because SPD is not limited to a particular cultural condition. The observations with micronutrients can be confusing, as some nutrient deficiencies can cause decreases in pH. Iron deficiency, for example, caused pea, sugar beet, and bean to lower the pH from 7.0 to 4.0 in 6 to 10 hours (Landsherg, 1981). The drop occurred in a nitrate (NO_3^-) - nitrogen (N) solution that normally causes substrate pH to rise. Zinc deficiency also led to substrate acidification in dicotyledonous species even when NO_3^- was the sole source of N (Cakmak and Marschner, 1990). Nonetheless, it would seem unlikely that geranium SPD is caused by Fe and Zn deficiencies. Both are applied in adequate quantities by growers as standard procedures and, as noted above, SPD seems related with micronutrient toxicity, not deficiency.

Another possible cause of geranium SPD is phosphorus (P) deficiency (Hinsinger, 2001). Growers often rely on alkaline fertilizers to anticipate and offset pH declines, and P is low or absent in the formulations (e.g., N- P_20_5-K_2O of 13-2-13, 15-0-15, 14-0-14). Studies with chickpea (Le Bot et al., 1990), barley (Rufty et al., 1991) and soybean (Rufty et al., 1993) have shown that P deficiency results in suppression of NO_3^- uptake. Because NO_3^- is the dominant anion under most nutritional conditions (Rufty, 1982; Mengel and Kirkby, 2001), a decrease in nitrate uptake might be expected to increase the cation to anion uptake ratio and cause acidification of the substrate as plants maintain electrochemical neutrality. Consistent with this notion, nutrient

media became more acidic in the experiments with chickpea (Le Bot et al., 1990). It is less clear whether P-stress affects ammonium (NH_4^+) uptake, but if NH4+ uptake were depressed less than uptake of NO3- (Schjorring, 1986), a cation to anion uptake shift and acidification still could occur. Indeed, acidification was measured with P stressed rice when N03- and NH_4^+ were both present as nitrogen sources (Kirk and Van Du, 1997). This relationship is particularly relevant with geranium, as fertilizers commonly contain both forms of inorganic nitrogen.

In the present study, we investigate geranium response to P stress and its possible contribution to SPD. Several questions are addressed: 1) Does rhizosphere pH decline when geranium plants progress into P stress? 2) If pH does decline, is it associated with adjustments in $N0_3^-$ and NH_4^+ uptake? And 3) Are there corresponding adjustments in the cation to anion uptake ratio that might lead to a downward pH change? The experiments included examination of acidification and nutrient uptake responses at a higher root temperature. Temperature fluctuations often occur in greenhouse operations, and increasing temperatures can elevate plant metabolism and have consequences for root function.

MATERIALS AND METHODS

Unrooted geranium (*Pelargonium x hortorum* Bailey `Designer Dark Red') cuttings were received on 4 dates (9/13/05, 10/12/05, 11/3/05, and 1/5/06), dipped for 30 s in 10% ZeroTol, a hydrogen peroxide based disinfectant (BioSafe Systems LLC, Glastonbury, CT, USA), and rooted for 15-16 d in 1 mM $CaSO_4$. One-hundred sixty uniformly rooted plants were distributed equally among four 200 L continuous flow hydroponic units located in a growth chamber with 9 m^2 of growing area and 2.13 m vertical clearance at The North Carolina State University Phytotron. The light period was a 9/15 h day/night period and cool white fluorescent and incandescent lamps separated from the growing area by a polycarbonate barrier supplied a photosynthetic photon flux density (PPFD) of 575±125 µmol•m^{-2}•s^{-1}. A 3 h low-light night interruption during the middle of the dark period was provided by incandescent lamps which supplied a PPFD of 25 µmol•m^{-2}•s^{-1}. CO_2 concentrations were maintained between 300-400 ppm by controlled injection of commercial grade gas. The growth chambers were programmed for one of two day/night temperature regimes; 22/18 or 26/22°C.

The control solutions consisted of 1.5 mM ammonium nitrate (NH_4NO_3), 5 mM calcium nitrate [$Ca(NO_3)_2$], 2 mM potassium nitrate (KNO_3), 1 mM monopotassium phosphate (KH_2PO_4), 1.5 mM potassium chloride (KC1), 2 mM magnesium sulfate ($MgSO_4$), 4 ppm Fe as Fe-diethylenetriaminepentaacetic acid (DTPA) (10% Fe), 9.1 µM manganese sulfate ($MnSO_4$•H_20), 0.76 µM zinc sulfate ($ZnSO_4.7H_20$), 46.3 µM boric

acid (H_3BO_3), 1.57 µM copper sulfate ($CuSO_4.5H_2O$), and 0.10µM sodium molybdate ($Na_2MoO_4.2H_2O$). The P stress treatments were established by excluding KH_2PO_4 from the nutrient solution and increasing KCl to 2.5 mM. Solution pH of the tanks was kept at 5.8 by electronic monitoring and automatic additions of 5 mM calcium hydroxide [$Ca(OH)_2$].

At specific times during the experiment (see results), six plants were removed from each hydroponic tank at about 12:00 pm and placed into aerated 500 mL beakers located within the same growth chamber. Each beaker contained solutions identical to those from which they were removed, except that the beakers contained solutions with the stable isotope ^{15}N. Half of the beaker solutions contained 15 A% $^{15}NH_4^+$ and the other half 5 A% $^{15}NO_3^-$. After 24 hours of exposure, plants were separated into roots and shoots. Roots were dipped in 1mM $CaSO_4$ for 30 s to remove ions from the apoplast (Naegle et al., 2005), and shoots were dipped in 0.1 N hydrochloric acid (HC1) to remove any external nutrients, and rinsed with deionized water. The roots and shoots were then dried in a forced-air oven at 60°C for 48 h. All leaves with petioles were removed from stems for shoot analysis. Ground tissues were analyzed for N and ^{15}N using elemental N analysis and ratio mass spectroscopy, and P, potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), Fe, copper (Cu), Mn, boron (B), and Zn content was determined with inductively coupled plasma optical emission spectroscopy (IRIS-Intrepid II, Thermo Fischer Sci., Waltham, MA, USA). All tissue concentrations are expressed on a dry weight basis.

Statistically, the experiment was a split-split-plot design with temperature treatments as the whole plot factor, plus and minus P treatments as the sub-plot factor, and 3, 11, and 19 days after transplanting (DAT) as the sub-sub-plot factor (SAS Institute, Cary, NC, USA). Error bars in figures were determined using the standard error function of Sigmaplot (SPSS Inc., Chicago, IL, USA). A single growth chamber was used for a total of 4 runs, and temperature was randomly assigned. Each run consisted of two replications of plus and minus P treatments at control or high temperature, giving a total of four replications.

RESULTS

The experimental approach involved keeping the pH stable, to minimize the potential for confounding interactions with micronutrient toxicity, and monitoring the amount of base used to assess the acidification potential of the geranium system. The amounts of base used to maintain a pH of 5.8 were different in the + and -P treatments at both temperatures (Table 1). At 22/18°C, plants grown in solutions devoid of P required 2.4 times the amount of titrating base than plants with sufficient P and there was a similar, but less dramatic effect at 26/22°C.

TABLE 1 Effect of phosphorus treatment at high (26/22°C day/night) and control (22/18) temperatures on mEq of titrating base consumed per gram plant to maintain pH of 5.8 in 200 L hydroponic tanks for 20 d

	Temperature Treatment (°C day/night)	
	Control (22/18)	High (26/22)
Phosphorus Treatment	mEq titrating base · g^{-1} dry weight plant	
Plus P	0.77	1.38
Minus P	1.83	2.33
Significance	**	***

** and *** significant at $P = 0.01$ and 0.0001 respectively.

With all plant parameters measured, it was not possible to separate temperature effects statistically. Therefore, data from the temperature treatmerits were combined. After 3 days without an external P supply, plants had noticeably lower dry weights and root to shoot ratios were increased (Figure 1). An increase in root to shoot ratio is the typical, well documented response to a nutritional stress. The growth response in the -P treatment was accompanied by a lowered P concentration in the shoots and roots (Figure 2), as well as lowered concentrations of N in shoots and, after the day three harvest, in roots as well (Figure 3).

Ammonium and Nitrate Uptake

Uptake of $^{15}NH_4^+$ and $^{15}NO_3^-$ was calculated from the ^{15}N contents in shoots and roots after 24 h of exposure to the specific ^{15}N treatments. For the three sample dates, the mean root uptake of $^{15}NH_4^+$ was lower in plants without P than in the controls, 1.19 mg compared to 1.64 mg ^{15}N per gram root dry weight (Table 2). Uptake of $^{15}NO_3^-$ also was decreased in P limited plants, to 3.31 from 5.15 mg ^{15}N. Thus, the suppression with

TABLE 2 Main effects of P treatment on $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake per gram root dry weight over a 24 hour period, and the NH_4^+ to NO_3^- uptake ratio

	Whole Plant ^{15}N Uptake		
	mg $^{15}N \cdot g^{-1}$ root dry weight		
P Treatment	$^{15}NH_4^+$	$^{15}NO_3^-$	NH_4^+ to NO_3^- Uptake Ratio
Plus P	1.64	5.15	0.323
Minus P	1.19	3.31	0.394
Significance	*	**	*
Difference in ^{15}N uptake	0.45	1.84	

* and ** Significant at $P = 0.05$ and 0.01, respectively.

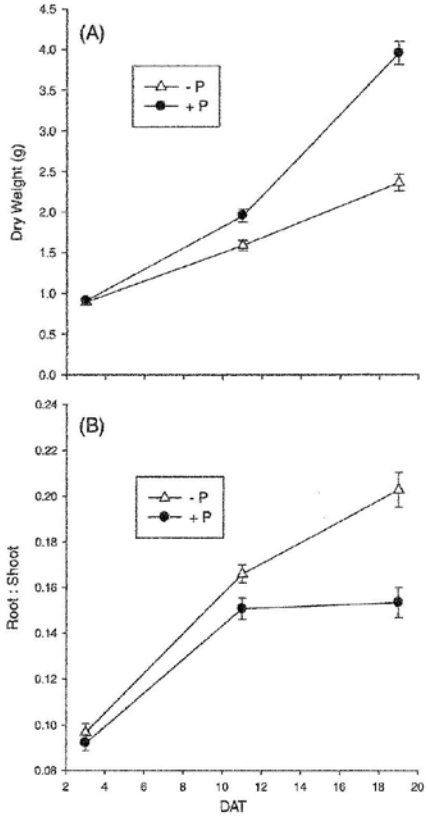

FIGURE 1 Total dry weight (A) and root to shoot dry weight ratio (B) of plants grown with or without P 3, 11, and 19 days after transplanting (DAT).

-P was four times greater than that of $^{15}NH_4^+$ in absolute terms (0.45 and 1.84 mg ^{15}N), and the $^{15}NH_4^+$ to $^{15}NO_3^-$ uptake ratio increased from 0.32 to 0.40. In addition, there was some indication that distribution of ^{15}N label between the shoot and root over the 24 h uptake period was altered by P stress. With $^{15}NH_4^+$, ~24% of the absorbed label was in the shoot in the P-limited conditions compared to 31% with the +P controls over the three treatment dates. With $^{15}N0_3^-$, 31 % of the ^{15}N was in the shoot compared to 33% with the control (data not shown).

Total Cation and Anion Uptake

To estimate the total cation to anion uptake ratio, nutrient contents were converted to meq. Of all the nutrients measured in plant tissues (N, P, K, Ca,

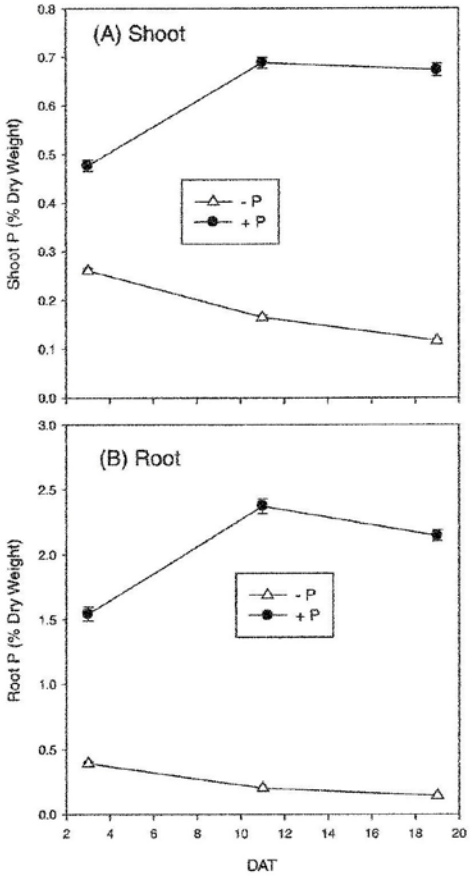

FIGURE 2 Shoot (A) and root (B) dry weight P percent of plants grown with or without P 3, 11, and 19 days after transplanting (DAT).

Mg, S, Fe, Cu, Mn, B, and Zn), the macronutrients represented ~99.0% of the total molar concentration; therefore only the macronutrients were used to evaluate changes in ion balance and the cation to anion uptake ratio. As shown in Table 3, uptake of nearly all the ions was lower with the P limited plants. The NH_4^+ to NO_3^- uptake ratios were used to estimate the proportion of total N entering in the cation or anion form. The tissue nutrient contents indicated that P-limited plants had a significantly greater cation to anion uptake ratio than the control plants that received P (Figure 4).

DISCUSSION

The results of these experiments clearly show that a P deficiency could contribute to the SPD observed with geraniums. As we had hypothesized from

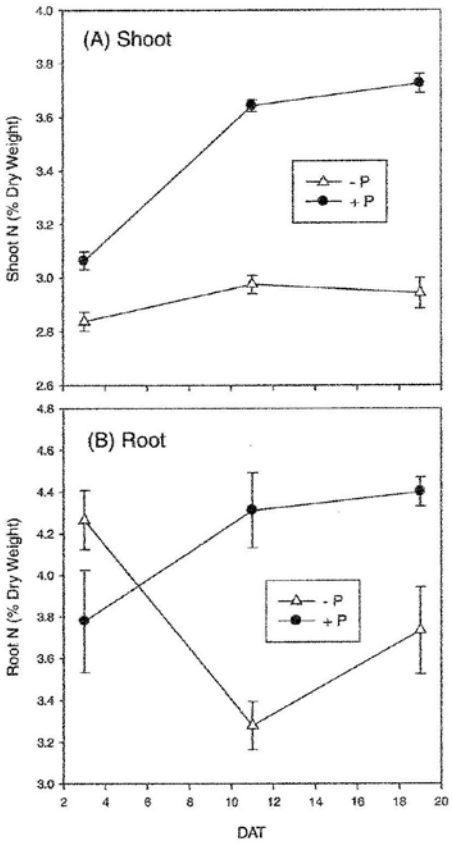

FIGURE 3 Shoot (A) and root (B) dry weight N percent of plants grown with or without P 3, 11, and 19 days after transplanting (DAT).

the results of earlier studies on P stress (Schjorring, 1986; Le Bot et al., 1990; Rufty et al., 1991, 1993), plants growing under a P limitation generated much higher amounts of acidity than control plants with an adequate P supply. The results indicated that the increase in acidity generation was associated with a shift in the cation to anion uptake ratio, and the primary controlling factor was inhibition of NO_3^- uptake (Tables 2 and 3; and Figure 4).

The conceptual basis for ion balance in plants and its relationship to transport processes in roots are solidly embedded in transport physiology literature (Hodges, 1973; Smith, 1973; Raven and Smith, 1976; Kirkby, 1981; Glass,1988,2003). Cation uptake is linked with H^+ efflux and anion uptake with H^+ influx (i.e., OH^- efflux). Thus, a decline in anion uptake greater than that in cation uptake, like that occurring here with P-stressed geranium,

TABLE 3 Main effect of P treatment 3, 11, and 19 days after transplanting (DAT) on meq of macronutrient cations and anions absorbed by roots. Data are estimated from nutrient accumulation in shoots. The amount of NH_4^+ or NO_3^- was determined by measurement of ^{15}N uptake on the sample dates and applying that ratio to the total shoot N observed on each individual DAT

	Element or total amount of ions								
	Ca	K	Mg	N-(NH_4^+)	Total cations	P	S	N-(NO_3^-)	Total anions
3 DAT					-meq				
P treatment									
Plus P	51.6	63.1	24.1	51.4	190	15.4	15.1	167.3	198
Minus P	46.5	57.8	21.9	49.6	176	8.4	14.5	153.0	176
Significance	NS	*	NS	NS	NS	*	NS	*	*
11 DAT									
P treatment									
Plus P	71.3	87.7	28.8	69.9	258	22.2	19.8	190.2	232
Minus P	50.3	67.0	22.4	66.9	207	5.3	15.4	145.6	166
Significance	***	*	**	NS	**	**	*	*	**
19 DAT									
P treatment									
Plus P	78.3	95.3	26.6	60.3	261	21.7	18.0	205.5	245
Minus P	53.1	64.5	20.8	58.3	197	3.75	14.2	150.6	169
Significance	*	**	*	NS	**	***	**	*	**

NS, *, **, *** Nonsignificant or significant at $P = 0.05$, 0.01, and 0.001 respectively.

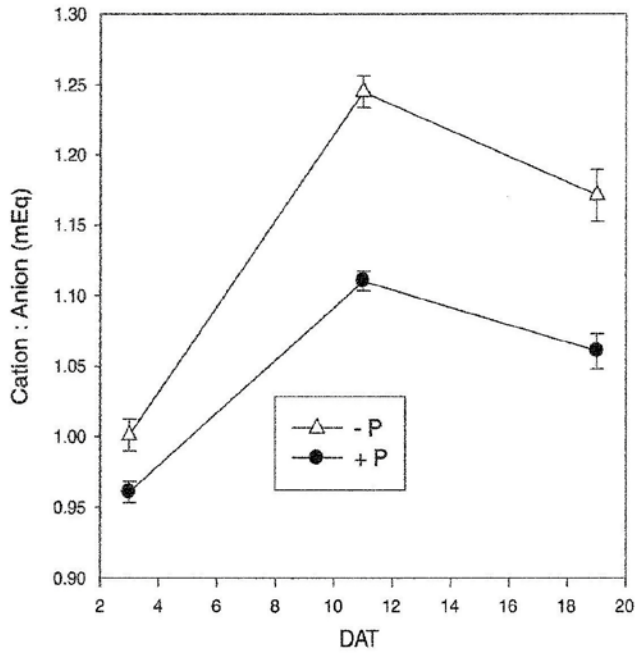

FIGURE 4 Uptake ratio of cations to anions in plants grown with or without P 3, 11, and 19 days after transplanting (DAT).

results in increased net H^+ release from the nutrient absorbing root cells.

The inhibition of NO_3^- uptake appears to follow the response profile seen with P-stressed plants in other studies. Inhibition can be measured before changes in growth can be detected, and it coincides with excess root accumulation of NO_3^- and the amino acid products of its assimilation, as translocation into the xylem is restricted (Rufty et al., 1993). Thus, the NO_3^- uptake inhibition with low P appears to involve the same feedback system that links the NO_3^- uptake rate with plant demand for N (Clarkson, 1986; Imsande and Touraine, 1994; Glass, 2003). In our study, the initial increase in root N concentration on day 3 (Figure 3) is consistent with restricted translocation of soluble N molecules into the xylem. At later sample times, root accumulation of soluble N would have been masked by the inhibition of uptake and the general decline in total N. The xylem transport inhibition could only have been resolved with separation of labeled ^{15}N fractions, which was not the level of resolution being sought in these experiments.

The suppressive regulation of NO_3^- uptake due to P deficiency would have coexisted with or been superimposed on inhibition of NO_3^- uptake by NH_4^+. Many studies have shown that the presence of NH_4^+ in nutrient media leads to decreased NO_3^- uptake, again probably involving the feedback loops controlling the NO_3^- uptake system (Breteler and Siegerist, 1984; Kronzucker et al., 1999; Aslam et al., 2001; Glass, 2003). An NH_4^+ inhibition of NO_3^- uptake would explain the cation to anion ratio being just above 1.0 in +P control plants. Dicots most often have cation to anion ratios of just under 1.0 if NO_3^- is the sole inorganic N source in nutrient media (e.g., Kirkby and Knight, 1977; Kirkby, 1981; Rufty, 1982).

Some similarities exist in the feedback loops for NO_3^- and NH_4^+ uptake (Glass, 2003), which helps to explain why uptake of both inorganic N forms were restricted under a P limitation. Of course, the NH_4^+ concentration in solution and NH_4^+ uptake were much lower than those for NO_3^-, and the absolute decrease in meq was less for NH_4^+ than for NO_3^- which led to rhizosphere acidification. Although P stress had little impact on S uptake, other studies have suggested that a close regulatory inter-relationship or `co-regulation' (Clarkson et al., 1989) may often exist among the nutrient anion transport systems (for nitrate and sulfate, see also Smith, 1980; Karmoker et al., 1991; Koprivova et al., 2000).

One of the interesting observations in these experiments was the increased generation of rhizosphere acidity with higher temperatures. This occurred even though plant growth and the cation and anion balance was not statistically different from that at the lower temperature, which implicates additional factors. The most logical explanation is that additional acidification was associated with higher root respiration and increased release of carbon dioxide (CO_2). The CO_2 would react with H_2O to form carbonic acid (H_2CO_3), which would partially dissociate into H^+ and HCO_3^-

(bicarbonate), and result in pH decline. This would also account for the increase in acidity generation at the high temperature with the +P control plants (Table 1). It should also be mentioned that phosphorus stress can lead to changes in root metabolism (Rabe and Lovatt, 1986). Increased secretion of organic acids from roots is a known occurrence with P stressed plants (Hinsinger, 2001), evidently due to increased up-regulation of PEP carboxylase activity (Raghothama, 1999). While secretion may serve to increase P availability near the root surface, the effect on root zone pH seems relatively small compared to the changes occurring in net H^+ efflux (Hinsinger, 2001).

It should be noted that there were positive and negative aspects to the experimental design used in our experiments. On the positive side, the maintenance of a stable solution pH by continual monitoring and automatic additions of base allowed assessment of the acidification potential of geranium without the confounding interactions that would come from micronutrient toxicities. On the other hand, rhizosphere acidification itself can cause adjustments in ion uptake favoring anions (Rufty, 1982). We cannot know the extent that this response would have offset the observed rates of rhizosphere acidification, if solution pH had been allowed to move downward.

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