Regulation of Root Distribution and Depth by Phosphorus Localization in *Agrostis stolonifera*

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**Abstract.** Root distribution in turfgrass systems influences drought tolerance and resource competition with undesirable species. We hypothesized that spatial localization of phosphorus (P) supply would permit manipulation of turfgrass root distribution. To test this hypothesis, creeping bentgrass (*Agrostis stolonifera* L.) plants were exposed to and Jonathan P. Lynch

Healthy root systems are essential to maintain turfgrass quality in the demanding environment of golf greens. Increased rooting of creeping bentgrass on golf greens can increase drought tolerance (DaCosta and Huang, 2006), nitrogen uptake (Bowman et al., 1998), and the general health of the turfgrass. When rooting is limited, like in compacted soils, more shallowly rooted species such as annual bluegrass (*Poa annua* L.) can become invasive (Klecka, 1937). Deeper-rooted creeping bentgrass would also reduce the need for supplemental irrigation because the plant would be able to extract water from deeper in the root zone. The ability to regulate root distribution in turfgrass systems would therefore be an important tool for turfgrass managers.

Phosphorus (P) availability regulates a variety of plant characteristics, including shoot development, leaf expansion, water use, reproductive phenology, and root development. Plants grown in low-P soils typically have increased root-to-shoot ratios (Lynch and Beebe, 1995) and a decrease in bentgrass root weight was observed when a complete fertilizer was applied as opposed to a fertilizer without P (Holt and Davis, 1948). Low P availability increases the intensity of leaf color in creeping bentgrass, which improves visual quality (Waddington et al., 1978). Pellet and Roberts (1963) noted that high rates of P led to the deterioration of Kentucky bluegrass during drought, whereas adequate P levels were needed for rapid recovery after drought. Evidence from these studies suggests that P availability can be optimized to improve several aspects of turfgrass stress tolerance and quality.

Conventional P fertilizers deliver high doses of P (mmol levels) for a limited time as a result of pellet dissolution. The ideal situation in turf would be to hold the P availability at a constant low level (μmol levels) as would be found in natural soils. A novel fertilizer, originally developed for growing horticultural crops in soilless media (Lin et al., 1996; Lynch et al., 1990), could provide a method for studying P placement in the root zone and eventually controlling P levels to manipulate plant growth. This fertilizer is a solid phase-buffered P fertilizer consisting of phosphate adsorbed to small particles of aluminum oxide (Al-P) and can be mixed into sand culture to maintain P levels at low concentrations (μmol levels) over an extended period of time (Coltman et al., 1982; Elliott, 1989; Lynch et al., 1990). The solid alumina releases micromolar concentrations of P, acting as a buffer, using equilibrium exchange between solid phosphate and solid phase-adsorbed phosphate. In this way, it mimics the P buffering that occurs in soil through complex chemical and biological mechanisms (Comerford, 1998) and the alumina can be used to provide optimal P nutrition over time. This is in contrast to slow-release fertilizers, which can maintain adequate P concentrations in the root zone over extended periods of time but release P as a function of pellet dissolution rather than plant requirements and do not prevent P leaching in sandy soils (Havis and Baker, 1985).

Research with soilless media indicates that addition of Al-P at 1% of the volume of the dry medium is sufficient for optimal plant performance of horticultural crops and for vegetable production in the field (Brown et al., 1999; Lin et al., 1996; Tanaka et al., 2006). Preliminary work with turf species demonstrated that creeping bentgrass grew satisfactorily in 80% sand:20% peat with 1% Al-P as the sole source of P (Lyons et al., 2000). Because soluble P concentrations remain very low with buffered P sources, negligible amounts of P are lost even if large quantities of water flow through the medium. In the systems that have been tested, leaching has been reduced to less than 1% of that in conventionally fertilized systems (Borch et al., 1998; Lin et al., 1996).

The placement of P deeper in the root zone may permit deeper rooting, thereby improving heat tolerance, recuperative ability, drought resistance, and overall turfgrass health. The objective of these experiments was to determine whether creeping bentgrass roots responded to spatial P supply and to determine whether deeper rooting could be encouraged by placing Al-P deeper in a sand-based root zone.

**Materials and Methods**

**Plant material.** All experiments used ‘Pennlu’, a vegetatively propagated cultivar of creeping bentgrass (Anonymous, 1954). Initially, mature tillers were transplanted (one each) into Conainers (Stuewe and Sons Inc., Corvallis, OR), 2.5 cm in diameter and 15 cm deep, filled with silica sand conforming to USGA specifications (Green Section Staff, 1993) to allow for root growth and ease of washing when they were later transplanted into the split root apparatus for the horizontal split root experiment. The same source of sand was used for both experiments. The experiment was initiated before the tillers began to vegetatively reproduce. Tillers from the original stand, maintained in the greenhouse, were used in the vertical P localization experiment.
Horizontal split root. The experiment was conducted in Summer 2000 in a climate-controlled greenhouse at 18 to 22 °C with a minimum daily photoperiod of 12 h (lat. 40.79° N, long. 77.86° W). The experiment used an apparatus that allowed the roots to be separated horizontally into two different 2.5-cm diameter Conetainers filled with sand. The separation was accomplished by using 1.25-cm polyvinyl chloride (PVC) plumbing junctions. Tillers were removed from the Conetainers, the sand was washed from the roots, and the existing root mass was separated so that approximately equal amounts of roots were placed through a T junction and an elbow joint on each side directing the roots into a Conetainer (Fig. 1A). After transplanting the plants, the PVC T junction was filled with silica sand to hold moisture and to protect the top portion of the roots from desiccation.

Immediately after transferring the plants, nutrient treatments were randomly applied to the root growth tubes on each side of the apparatus. Three treatments were applied: plants received nutrient solution containing P 1) in both sides of the root zone (HH); 2) with no P on either sides of the root zone (LL); or 3) with P on one side and nutrient solution without P on the other (HL). Nutrient solutions were added twice weekly and the plants were watered daily or as needed. The nutrient solutions were a complete nutrient solution providing 4.4 mmol L⁻¹ nitrogen (3.88 mmol L⁻¹ NO₃⁻, 0.50 mmol L⁻¹ NH₄⁺), 1.5 mmol L⁻¹ potassium, 0.5 mmol L⁻¹ P, 0.94 mmol L⁻¹ calcium, 0.25 mmol L⁻¹ magnesium, and 0.25 mmol L⁻¹ sulfur and a phosphorus-free solution that provided 4.4 mmol L⁻¹ nitrogen (3.88 mmol L⁻¹ NO₃⁻, 0.50 mmol L⁻¹ NH₄⁺), 1.5 mmol L⁻¹ potassium, 1.19 mmol L⁻¹ calcium, 0.25 mmol L⁻¹ magnesium, and 0.25 mmol L⁻¹ sulfur. Both solutions were supplemented with a complete micronutrient solution containing 23 μmol L⁻¹ boron, 1.85 μmol L⁻¹ copper, 22 μmol L⁻¹ iron, 11 μmol L⁻¹ manganese, 1.5 μmol L⁻¹ molybdenum, and 10 μmol L⁻¹ zinc.

The plants were transferred on 15 June and data collected 15 Aug. The plants were harvested by clipping the roots at the end of the PVC elbows where they entered the 2.5-cm diameter root growth tube. The shoots were clipped at the top of the PVC T joint. The root mass contained within the PVC elbows and T joint was not measured because it was difficult to remove completely. The roots from each growth tube were washed and both roots and plant shoots were dried at 70 °C for 48 h. For analysis of tissue P concentration, subsamples of the dried samples were ground and ashed at 495 °C for 10 h. The ash was dissolved in 4 mL of 100 mmol L⁻¹ HCl and analyzed for P spectrophotometrically (Murphy and Riley, 1962).

The experiment used a completely randomized design with five replicates per treatment per harvest. Differences in root mass and P concentration between the two sides of the split root system were analyzed using a paired Student's t-test. Differences in shoot mass and shoot P concentration were evaluated using Fisher's protected least significant difference (LSD).

Vertical localization of phosphorus. The vertical P localization experiment was a 9-week growth study in which plants were grown in 38-cm deep, 10-cm diameter PVC pots in an attempt to simulate a golf green root zone. The pots were filled with 8 cm of pea gravel and then topped with 30 cm of sand, both conforming to USGA specifications for golf greens (Green Section Staff, 1993). The control contained sand throughout the root zone. The other treatments included sand mixed with Al-P fertilizer buffer the soil solution at 30 μmol L⁻¹ P with two different application techniques, a homogeneous mix (1% Al-P) and a 10-cm deep band starting 20 cm below the surface (1% Al-P band) (Fig. 1B). The Al-P was mixed into the sand at a rate of 10 g L⁻¹ (1% weight by volume). Alumina-P granules fall within the particle size distribution of the sand used within the experiments and have not been shown to significantly alter soil physical properties or pH at the concentrations used in these experiments. Buffering capacity was determined by placing 1 g of the material in 20 mL of deionized water, agitating, and measuring the P concentration of the solution after 24 h (Murphy and Riley, 1962).

All pots were filled in three steps: the 10 cm of gravel was placed into each pot and then the first 10 cm of sand or sand + Al-P was filled and allowed to settle with two waterings over 24 h and then the remaining 20 cm of the root zone was filled with the appropriate material. The pots were then irrigated daily for 2 weeks to allow for settling. Pots were topped off if needed. The three treatments reported in this experiment are the control, 1% Al-P mixed, and 1% Al-P band. The plants were transplanted from a maintained stand of Pennlu creeping bentgrass from a greenhouse. Three to four tillers were planted in each pot on 16 and 17 Dec. 2002, and the pots were placed in a
climate-controlled greenhouse (18 to 22 °C with natural light supplemented to maintain a minimum of a 12-h daylength). The plants were irrigated with tap water for 1 week and then irrigated daily with nutrient solution. The control received a complete nutrient solution providing 4.4 mmol L⁻¹ nitrogen (3.88 mmol L⁻¹ NO₃⁻, 0.50 mmol L⁻¹ NH₄⁺), 1.5 mmol L⁻¹ potassium, 0.5 mmol L⁻¹ P, 0.94 mmol L⁻¹ calcium, 0.25 mmol L⁻¹ magnesium, and 0.25 mmol L⁻¹ sulfur and the Al-P-amended treatments received a nutrient solution without P providing 4.4 mmol L⁻¹ nitrogen (3.88 mmol L⁻¹ NO₃⁻, 0.50 mmol L⁻¹ NH₄⁺), 1.5 mmol L⁻¹ potassium, 1.19 mmol L⁻¹ calcium, 0.25 mmol L⁻¹ magnesium, and 0.25 mmol L⁻¹ sulfur. Both solutions were supplemented with a complete micronutrient solution described previously.

The plants were mowed to a height of 5 cm on 13 Jan. and the first of 10 weekly harvests was performed on 15 Jan. 2003. The plants were clipped just below the sand surface to assure that both the crown and stem tissue were included in the shoot mass. The tillers were separated and counted and the shoots were dried at 60 °C and weighed. The shoots were then ashed at 500 °C and analyzed for P concentration as described previously. After the seventh week, the tillers were no longer counted as a result of the large number present in each pot. The entire root zone was harvested for the first 7 weeks. In Weeks 8 to 9, the root zone was split into the top 15 cm and the bottom 15 cm. The two portions of the root zone were then washed separately. The roots were washed by hand using a sieve, were dried at 60 °C, and weighed. Samples were ashed at 605 °C until the organic matter was burned off (10 h). The ash weight was then subtracted from the dry weight to adjust for sand in the sample resulting in an ash-free dry weight for the root samples.

The experiment used a completely random design and four replicates per treatment were harvested on each harvest date. Statistical analysis of the data was performed using JMP (SAS, Cary, NC). Differences in growth parameters and P concentration were analyzed by analysis of variance. When warranted, Fisher’s protected LSD was used for means separation.

Results and Discussion

Horizontal split root. The plants receiving no P in either side of the root zone had a greater than 50% reduction in shoot growth compared with treatments receiving P (Fig. 2). The treatment receiving P on only one side of the root zone had shoot growth that was similar to the growth of the plants receiving P on both sides of the root zone (Fig. 2). Root growth was reduced in the “no P” treatment providing evidence of P deficiency resulting in reduced mass of the entire plant (Fig. 3). Root mass in the side of the root zone receiving P (0.309 g) was greater than the side receiving no P (0.188 g).

These results show that creeping bentgrass alters its root architecture with a non-uniform supply of P as observed in several species (reviewed in Hodge, 2004; Robinson, 1994). The mechanism for this preferential root allocation is believed to be increased lateral branching of fine roots from parent roots in zones of enriched nitrogen or P availability (Huang and Eisenstat, 2000). Root proliferation is not always the response to localized nutrient supply. In some instances, increased uptake rates can confer a similar advantage to the plant and may have been selected for in crop plants because the carbon expense of increased uptake capacity is lower than root proliferation (Robinson, 2001). Despite being selected for growth in the uniform environment of golf greens (Huff and Landschoot, 1999), creeping bentgrass appears to be in the category of plants that respond to localized P supply with root proliferation. This is possibly because breeding of creeping bentgrass has not generally selected for increased shoot growth and maximum reproductive ability at the cost of root proliferation as with many grain crops. In addition, the breeding history of creeping bentgrass is relatively short in comparison with annual
crops such as wheat (*Triticum aestivum* L.) and beans (*Phaseolus* L.), which have been selected annually over thousands of years.

The tissue P concentration of turfgrass species varies widely. Waddington and Zimmerman (1972) reported that creeping bentgrass had an average tissue P concentration of 7.6 mg·g⁻¹. In a later study, creeping bentgrass was found to have tissue P levels of 5 mg·g⁻¹ and no change in growth occurred when tissue P levels were found as high as 8.4 mg·g⁻¹ (Waddington et al., 1978). Jones (1980) concluded that the sufficiency range for most turfgrass species is 3.0 to 5.5 mg·g⁻¹. In the present study, shoot P levels were high compared with commonly reported sufficiency levels, although deficiency symptoms (purple stolons and leaves) were observed in the LL-treated plants that had tissue P concentrations of ≈ 5 mg·g⁻¹. Tissue P concentration paralleled growth responses to the treatments (Fig. 4). The shoots from the “no P” treatment had 30% of the P concentration of the treatments containing P. The P-treated plants had equivalent P concentration in the shoots regardless of the presence of spatial heterogeneity. The P concentration of roots in the high P side of the HL treatment was 17% greater than roots in the “no P” side. Moreover, the P concentration of roots in the “no P” side of the HL treatment was greater than the P concentration of roots in the treatment (LL) receiving no P in either side of the root zone.

**Vertical localization of phosphorus.** In the vertical localization of P, experiment differences in shoot characteristics caused by P treatments did not begin to appear until after Week 6 of the experiment when the plants receiving the complete nutrient solution began to have greater shoot mass than plants in both Al-P-amended treatments (P < 0.0006). This delay in response may have been because the plants were transplanted from an environment adequate in P. Shoot growth of the control treatment was three- to five-fold greater than that of the two treatments using Al-P as the sole source of P (Fig. 5). This same pattern was observed in the tiller densities measured throughout the first 7 weeks of the experiment (data not shown). Reduced shoot growth may be the result of a significant reduction in the P concentration of the shoots in the Al-P-treated plants throughout the experiment (Fig. 6). Shoot tissue P concentration of the control treatment was significantly higher in Weeks 3 to 6 than Weeks 1 and 2 and then declined during the latter weeks (Weeks 8 and 9) of the experiment as the plants grew larger than those in the Al-P treatments. The Al-P-treated plants maintained consistent shoot P concentration throughout the experiment (Fig. 6) and never showed typical P deficiency symptoms, a purplish leaf color that is darkest at the crown (Turner and Hummel, 1992), which was observed in the low P treatments of the horizontal split root experiment. The tissue P levels (peaking at 11.1 mg·g⁻¹ in Week 3 and declining to 2.9 mg·g⁻¹ in Week 9) of control treatment plants in the vertical P

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**Fig. 5.** Shoot (A) and root mass (B) of creeping bentgrass during a 9-week growth experiment in response to a complete nutrient solution and phosphorus-free nutrient solutions with alumina-bound phosphorus fertilizer mixed throughout the root zone and banded in the lower 10 cm of a 30-cm root zone. Error bars represent se.

**Fig. 6.** Phosphorus concentration of creeping bentgrass shoots during a 9-week growth experiment in response to a complete nutrient solution and phosphorus-free nutrient solutions with alumina-bound phosphorus fertilizer mixed throughout the root zone and banded in the lower 10 cm of a 30-cm root zone. Error bars represent se.
localization experiment could be considered very high based on the numbers reported in the literature and may be evidence supporting the concept that most turfgrass species are “luxury consumers” of nutrients when nutrients are available in large amounts (Hull, 1992). The Al-P-treated plants may have become P-deficient with tissue P levels falling below 1.8 mg g$^{-1}$ in Weeks 8 and 9 of the experiment. Despite the differences in shoot growth, root growth for the 9 weeks of the experiment appeared to be identical among treatments with the exception of Week 5 when the root growth in both Al-P-amended pots was lower than the control ($P = 0.0361$) and in Week 7 when the plants in the 1% Al-P-banded pot had less than half of the root mass of the control treatment and the 1% Al-P mixed treatment ($P = 0.0120$). The root-to-shoot ratios of the Al-P-treated plants were significantly greater ($\approx 100\%$) than those of plants in the control treatment (Fig. 7). This increase in root-to-shoot ratios is likely because the plants grown in the Al-P-amended media produced lower shoot mass while maintaining similar root growth as plants in the control treatment (Fig. 5). The Al-P treatment increased root-to-shoot ratios over the control treatment appears to be a response by the plants to obtain greater amounts of P by exploiting more of the Al-P throughout the root zone or in the bottom 10 cm of the root zone. In most natural soils, nutrient bioavailability, particularly that of P, is greatest at the surface of the soil (Chu and Chang, 1966; Pothuluri et al., 1986; Pregitzer et al., 1993). Bean plants with shallower basal root angles are more competitive for surface P (Lynch and Brown, 2001; Rubio et al., 2003) presumably because of increased root length density near the soil surface where P is present.

The increase in root-to-shoot ratio observed in the Al-P-treated pots that ranged from 0.22 to 0.45 from Week 3 on compared with the control pots that ranged from 0.06 to 0.15 (Fig. 7) could be the result of the reduced overall size of the plants in the Al-P-treated pots. Within a cultivar (Larcher, 1995) and across broad taxonomic ranges (Enquist and Niklas, 2002), smaller, less mature plants have a tendency to have higher root-to-shoot ratios (Wilson, 1988). The plant in the Al-P-treated pots exhibited reduced shoot growth, but the similarities in the root mass among all treatments in Weeks 8 and 9 (Fig. 4) show that the carbon allocation between roots and shoots in the Al-P-treated plants was being shifted from shoots to roots in contrast to being a function of reduced plant growth as was observed in the horizontal split root experiment (Figs. 2 and 3).

For the last 2 weeks of the experiment, root growth in the top 15 cm and bottom 15 cm of the growth tubes was measured. Plants in both Al-P treatments had the same root mass in the top half of the root zone. The control plants had significantly ($P < 0.05$) less root mass below 15 cm of depth (0.15 g) compared with the plants in the Al-P treatments (0.35 g and 0.43 g) in Week 8 despite having equal or greater total root mass than both of the Al-P-treated plants. The root zones with the Al-P banded in the lower portion of the root zone had greater root mass in the lower half of the root zone (0.56 g) than both the control (0.22) and the Al-P mixed (0.35 g) treatments in Week 9 (Fig. 8). The control plants were given P in a nutrient solution applied to the surface of the pots, which mimics typical turfgrass growth environments. The addition of the Al-P to soil in the bottom half of the pot inverted the P availability regime that is typically found in soils. It was shown that mixing Al-P throughout the root zone could increase root mass below 15 cm of depth but that banding the Al-P deeper in the root zone would result in an even greater root mass below the 15-cm
depth compared with that observed in the other treatments. Deep root systems are important for healthy turf in that they allow the plant to obtain water from deeper in the soil profile (Huang and Gao, 2000). This allows leaf stomates to remain open supporting continued transpirational cooling and carbon fixation during times of periodic heat stress and drought. Future research may explore the effects of Al-P as the sole source of P on the ability of creeping bentgrass to survive temporary drought conditions experienced during the late afternoon on golf greens.

Other than encouraging deeper root growth, another potential benefit of providing P deeper in the root zone using Al-P is the inhibition of annual bluegrass invasion into creeping bentgrass. Increasing P availability has been shown to encourage annual bluegrass invasion on golf greens (Waddington et al., 1978) and to favor annual bluegrass growth in mixed stands with creeping bentgrass turf (Kuo, 1993). If P were only available below 20 cm of depth, annual bluegrass would not be able to obtain P necessary for growth because annual bluegrass found on golf greens has very little root mass below 12 cm of depth (Lyno, unpublished data). It is also possible that placing P deeper in the root zone may select for more deeply rooted ecotypes of annual bluegrass. Although this may increase the ability of annual bluegrass to invade creeping bentgrass, it may also eliminate some of the undesirable plant characteristics related to the shallow root system typically found with annual bluegrass.

Sand-based root zones used in golf course greens construction are ideal for resisting problems associated with compaction but lack the ability to provide proper nutrition, and increased nutrients are needed when the greens are young to promote proper growth and turfgrass quality (McCellan et al., 2007). Generally, it is believed that P loss occurs primarily through runoff. Although runoff from turfgrass can occur, turfgrass systems are generally considered good at minimizing P surface runoff unless a dramatic rainfall event occurs (Kaufman and Watschke, 2007; Linde and Watschke, 1997; Shuman, 2002). Although it is widely believed P leaching from sand-based golf greens is rare (McCellan et al., 2007; Sartain and Brown, 1998), it has been shown that in sandy soils, P leaching can be substantial, especially during establishment (Shuman, 2001). The use of Al-P has been shown to significantly reduce P leaching in sandy soils (Borch et al., 1998, 2003) and although leaching was not measured in this experiment, the ability of buffered P to reduce P escape is significant. In addition, the increased amount of deeper roots will allow the plant to possibly capture more nitrogen potentially reducing nitrate leaching (Bowman et al., 1998).

Conclusions

Our results show that creeping bentgrass roots proliferate in soil domains with greater P bioavailability, and the use of a buffered P source to localize P supply deeper in the soil profile results in deeper bentgrass rooting. The buffered P source reduced shoot growth but not root growth. The production of bentgrass stands with deeper roots and reduced shoot growth would be advantageous in many turfgrass systems by reducing the need for mowing while increasing drought tolerance and decreasing the fitness of shallow-rooted weeds such as moss (Bromus argenteus Huds.) and annual bluegrass. Although not measured directly in this study, the use of buffered P sources substantially reduces P leaching from inert media such as sand (Borch et al., 2003). The use of buffered P sources appears to offer several advantages over conventional turfgrass fertilization.

The response of creeping bentgrass to spatial P supply creates an opportunity to increase root-to-shoot ratios and increase overall rooting depth. The effects of altered root architecture on stress tolerance still needs to be explored. In addition to providing adequate but not excessive P, buffered P has the potential to minimize nutrient pollution by reducing the P that can move through sand-based root zones. The feasibility of using Al-P in turfgrass applications should be explored under field conditions to determine its efficacy.

Literature Cited

McCellan, T.A., R.C. Shearman, R.E. Gaussoin, and SSSA, Madison, WI.
McCellan, T.A., R.C. Shearman, G.L. Horst, and D.B.