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Leaf morphology explains the disparity between annual bluegrass and creeping bentgrass growth under foliar fertilization

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Abstract: On golf courses planted to creeping bentgrass, invasion of annual bluegrass is a constant concern. To analyze if nitrogen fertilization manipulation could bias growth to creeping bentgrass, both grasses were fertilized either through foliar or soil application with either urea or ammonium sulfate and the impact on shoot and root growth measured. Ammonium sulfate resulted in greater overall growth for both species. Foliar application resulted in greater shoot growth for annual bluegrass and soil application resulted in greater root growth for creeping bentgrass. Leaf samples, as well as multiple leaf samples collected from golf courses, were examined microscopically for potential routes for foliar nutrient uptake: stomata and aqueous pores. No statistical difference was observed in the stomatal number between the two species but annual bluegrass possessed more aqueous pores. The enhanced ability of annual bluegrass to benefit from foliar fertilization may aid in its encroachment on highly managed golf greens.

Key words: annual bluegrass (Poa annua L.); aqueous pores; creeping bentgrass (Agrostis stolonifera L.); foliar fertilization; leaf morphology; nitrogen fertilization; turfgrass growth

Introduction

Annual bluegrass (Poa annua L.) is a pervasive weed on creeping bentgrass (Agrostis stolonifera L.) putting greens (Lush 1989). Controlling the invasion of annual bluegrass is a primary concern for golf superintendents because of the reduced stress tolerance of annual bluegrass compared to creeping bentgrass (Vargas and Turgeon 2003). Annual bluegrass is highly susceptible to diseases and is less resilient to heat and drought stresses (Beard 2002; McCullough, Liu, and McCarty 2005). Traditionally, plant growth regulators (PGRs) such as trinexapac-ethyl, mefluidide or ethephon have been used to reduce seed head emergence and thereby reduce growth of annual bluegrass in creeping bentgrass stands (Danneberger, Branham and Vargas 1987; Eggens et al. 1989; Johnson and Murphy 1996). However, there are limitations for the effectiveness of these chemicals and not every herbicide is acceptable on golf courses. Chemicals such as mefluidide have erratic effectiveness and can cause turf injury (Danneberger, Branham and Vargas 1987; McMahon and Hunter 2012). Treatment of creeping bentgrass with ethephon has been associated with a negative impact on putting green quality and ball roll distance (McCullough, Liu, and McCarty 2005). Although the herbicide bispyribac-sodium has been shown to control annual bluegrass in creeping bentgrass fairways, it has also been shown to be harmful to creeping bentgrass (Lycan and Hart 2006; Dernoeden, McDonald, and Kaminski 2008). While newer chemistries such as methiozolin can provide excellent control of annual bluegrass in creeping bentgrass, they require multiple yearly applications (Askew and McNulty 2014). Herbicides have also been shown to persist in the soil where they can affect future growth (Kaminski, Dernoeden, and Bigelow 2004). In most of the world, the use of certain PGRs and numerous herbicides is banned. In response to regulatory bans and restrictions, golf superintendents have begun to focus on management practices to control annual bluegrass invasion. Nutrient management strategies thus far have been shown to have limited effect on annual bluegrass populations (Waddington et al. 1978; Varco and Sartain 1986). Although the foliar application of iron (Fe) and magnesium (Mg) was shown to reduce annual bluegrass populations in seeded sites, it was ineffectual on golf course fairways (Bell, Odorizzi, and Danneberger 1999). While nutrient manipulation holds promise for annual bluegrass management, to date there are no completely effective nutrient management strategies. This may be due in part to limited empirical data from such strategies on the physiological effects on creeping bentgrass and annual bluegrass growth. Exploitation of differences between the two species is a propitious avenue for the control of annual bluegrass invasion. Creeping bentgrass is considered a deep-rooted species (Lyons, Landschoot, and Huff 2011) and annual bluegrass a shallow-rooted species (Hutchinson and Seymour 1982). Deep-rooted plants benefit from an ability to absorb more water and more nitrogen (N) (McMurtrie et al. 2012; Rogers and Benfey 2015). Therefore, it is reasonable to hypothesize that creeping bentgrass may benefit more from soil-applied nutrients than annual bluegrass.

Foliar N fertilization is routine in turfgrass maintenance programs owing to its ease of application and its compatibility with the application of other turf care products such as fungicides and PGRs. On golf courses, foliar N fertilizer is applied every 10 to 17 days, allowing for fine control of plant growth for uniform growth and a reduction in the amount of fertilizer applied throughout the year (Bowman 2003; Schlossberg and Schmidt 2007; Totten et al. 2008). There is the added benefit of low rates of N fertilization leading to a reduction in the environmental impact of excess N (Wesely, Shearman, and Kinbacher 1985; Orbovic et al. 2001; Dong et al. 2005). While there are clear advantages to foliar fertilization, the combination of granular (soil-applied) and foliar fertilization reduces total N input while producing the best turfgrass quality for creeping bentgrass (Totten et al. 2008).

Foliar fertilization requires the absorption of nutrients across the leaf cuticle, a thin layer of wax and lipids embedded in a cutin matrix that provides a barrier between the plant and the environment (Riederer and Friedmann 2006; reviewed in Fernández and Brown 2013). Polar and lipophilic transport systems within the cuticle allow molecules such as foliar fertilizers to move from the external to the internal environment. The cuticle thus influences foliar absorption and is a dynamic structure affected by leaf age and environmental factors (Hull, Morton, and Wharrie 1975; Bondada, Oosterhuis, and Norman 1997; Bondada, Syvertsen, and Albrigio 2001). The increase in cuticle wax on creeping bentgrass leaves in response to moisture stress has been shown to negatively affect foliar absorption of N (Bethea et al. 2014). Lipid-soluble neutral molecules diffuse through lipophilic pathways while hydrophilic molecules penetrate through aqueous pores (Schönherr 2006; Eichert 2013). Fatty acid chains composed of carboxyls and hydroxyls in the hydrophobic portion of the cuticle disassociate leaving an open space (the aqueous pore) for molecules to enter (Schönherr 1976; Tyree, Scherbatskoy, and Tabor 1990; Schreiber 2005). The number of aqueous pores depends on the cuticle type, number of stomata or trichomes and pH of the solution applied to the cuticle (Schönherr 1976; Schreiber 2006; Eichert 2013). The uptake of nutrients occurs through both stomata as well as aqueous pores (Schönherr 2006; Eichert and Goldbach 2008; Eichert 2013). Previous research has shown that the number of stomata and trichomes is correlated to the absorption of water and different solutes (Benzing et al. 1976; Tan, Ikeda, and Oda 1999; Schlegel and Schönherr 2002; Schreiber 2006; Eichert et al. 2008). To date there are no studies that examine the morphological characteristics of stomatal number and trichome frequency of creeping bentgrass or annual bluegrass in relation to foliar nutrient uptake. The uptake of N is dependent on its chemical form. The low cost and rapid absorption of urea make it the most commonly used N foliar fertilizer worldwide (Wesely, Shearman, and Kinbacher 1985; Bowman and Paul 1992; Orbovic et al. 2001; Witte et al. 2002). Foliar absorption of urea is thought to be more efficient than that of other N compounds due to its smaller size and neutral charge (Wittwer, Bukovac, and Tukey 1963; Schönherr 1976). A recent study showed that N absorption ranges from 36–69% of N applied as foliar urea (Stiegler, Richardson, and Karcher 2011). Unlike urea that remains in its original form in water, ammonium sulfate dissociates in water and the cationic ammonium ion is absorbed by the plant (Wittwer, Bukovac, and Tukey 1963). In a foliar uptake study of N sources by creeping bentgrass, the absorption of the cationic form of N (ammonium ion) was not significantly different from that of urea in two of three studies unlike the uptake of anionic nitrate that was consistently low (Stiegler et al., 2013). However, other studies in creeping bentgrass (Henning, Mulvaney, and Branham 2013) and in perennial ryegrass (Bowman and Paul 1992) have observed no difference in the foliar absorption of urea, ammonium or nitrate. Studies thus far have focused only on N uptake and here we are examining the growth effects resulting from two N sources and application methods.

There is limited research on the effects of fertilization method (foliar versus soil applied) on plant traits in turfgrasses including root and shoot growth or if these effects vary among species. We hypothesized that creeping bentgrass might have a growth advantage when N was soil applied as it has a deeper root system. We further hypothesized that foliar-applied urea would be absorbed more efficiently by the cuticle due to its smaller size and neutral charge than the cationic ammonium ion from ammonium sulfate, and would thereby more positively impact plant growth. The objectives of this research were twofold. The first objective was to examine changes in plant growth when creeping bentgrass and annual bluegrass were fertilized with either foliar or soil-applied N in the form of either urea or ammonium sulfate. The second objective resulted directly from data generated from the first objective and was to microscopically examine the leaf morphologies of creeping bentgrass and annual bluegrass to ascertain if there were any differences that could account for the disparity between the two species in foliar absorption of N.

Materials and methods

Foliar fertilization experiment

Creeping bentgrass (L-93) was planted into trays of silica sand in May 2008. Cores of annual bluegrass were harvested from a golf green at the Guelph Turfgrass Institute (GTI), Guelph, Ontario, Canada. The cores contained approximately 80% annual bluegrass and 20% creeping bentgrass. After proper identification, annual bluegrass tillers were transplanted into a tray with silica sand. A single tiller of each species was transplanted to 16 polyvinyl chloride (PVC) tubes with a diameter of 8 cm for a total of 32 tubes. The PVC tubes contained 10 cm of gravel topped with 30 cm of silica sand meeting United States Golf Association (USGA) recommendations to mimic a putting green root zone (USGA, 2004). A plastic tube of 15 cm in length was inserted to the soil level to allow application of water and/or fertilizer. The tops of all PVC tubes were covered with aluminum foil, with the tiller protruding from the foil, to ensure that foliar-applied fertilizer remained on the plant and was not washed into the soil. Plants were grown in a greenhouse under natural light supplemented with a light photosynthetic photon flux density of 70 µmol photons m^{-2} s^{-1} at canopy height from 400 W high pressure sodium bulbs to provide light 16 hr a day for the length of the experiment.

Fertilizer treatments

Two 18-3-18 [N-Phosphorus (P)-Potassium (K)] fertilizer solutions were prepared for the experiment. The urea-based and the ammonium sulfate-based (N) fertilizer solutions both contained monopotassium phosphate (P) and potash (K). Fertilizer was applied weekly at a rate of 3.79 L per 100 m^2 at a concentration of 45.56 g of N per liter. All application methods received the same amount of N per application throughout the experiment. For foliar application of fertilizer, 50 mL of deionized water was injected through the 15 cm plastic tube inserted to the soil of the PVC tube before 0.2 mL of fertilizer solution was sprayed over the plants. The sprayer was calibrated before the foliar fertilization by weighing a circle of filter paper (8 cm in diameter) before and immediately after spraying water. For soil-applied fertilizer, 50 mL of deionized water and 0.2 mL of fertilizer were mixed and injected through the 15 cm plastic tube inserted to the soil of the PVC tube. Aqueous magnesium sulfate (0.6%) was added to all PVC tubes at week four to eliminate potential sulfur deficiency as a cause for any growth differences. Replicates for experiments were temporally independent and pooled for analysis.

Sampling and measurements

Fertilizer application was maintained for eight weeks prior to plant harvest. Plants were separated into root and shoot biomass and tillers counted. Plant material was then rinsed under tap water to remove residual fertilizer. Roots were washed over a 1 mm sieve to eliminate associated soil particles. Biomass was determined after drying at 70ºC for six days and recorded. For shoot material, total Kjeldahl N (TKN) was analyzed colorimetrically by spectrophotometry (Astoria A2 auto-analyzer, Astoria Pacific International, Oregon, USA) after H_2SO_4-Se-K_2SO_4 digestion (Sen Tran and Simard 1993).

Experimental design and statistical analyses

The experimental design was a three-way factorial (species, application method and fertilizer type) randomized with four replicates. Analyses of variances (ANOVA) were performed using the MIXED procedure in SAS (version 9.1) (SAS Inc. Carey, NC, USA). Fisher's protected LSD tests were done (Steel, Torrie, and Dickey 1997) when interactions were significant at $\alpha = 0.05$.

Plant material for microscopy

Leaf blades were examined microscopically for any morphological differences between creeping bentgrass and annual bluegrass leaves. Both creeping bentgrass and annual bluegrass leaf samples harvested at the end of the original experiment were examined as well as samples from plants collected from golf greens at three different sites in Guelph, Ontario, Canada. Collected plants were regrown to ensure that leaf tissue was harvested at eight weeks from fully expanded leaves from growing plants. Two of the three sites are golf courses (Victoria Park East, Cutten Club) and samples of the third site were taken from two GTI research greens. At each golf course, four plants of each species were collected from four separate greens for a total of 16 samples per golf course. At the GTI, four plants of each species were collected from two separate greens for a total of eight samples. Plants were harvested with a knife and placed in plastic bags with a wet paper towel to maintain soil moisture. Each tiller was transferred into a pot filled with sand and placed in a greenhouse with a temperature cycle of 24ºC/16ºC day/night and a 16 hr photoperiod. The samples were placed under automatic misters (15 s/hr) and fertilized by watering with a 20-20-20 (N-P-K) solution weekly. Plants were clipped once a week to a height of 6 cm.

Stomatal count

For tissue samples, a 2-cm section of leaf blade was selected between the sheath and the leaf tip from fully expanded leaf blades of growing plants. All procedures were conducted at room temperature during the day under normal room lighting after Rice, Glenn, and Quisenberry (1979) with the following modifications. To obtain average stomatal count, clear nail polish was painted onto the leaf blade surface fixed at its ends with tape on a microscope slide. Once the nail polish dried, it was peeled from the leaf blade and placed on a slide with a drop of water and a cover slip, which was sealed with nail polish. Four counts were taken from each peel under a compound microscope (Olympus BX51) at 200x, and photographed with an Olympus DP71 camera (Olympus Corporation, Tokyo, Japan). The stomatal count was an average per 1 mm^2 . The same procedure was used for both creeping bentgrass and annual bluegrass species on adaxial and abaxial surfaces.

Localization of aqueous pores with silver nitrate using light and scanning electron microscopy (SEM)

To identify locations of aqueous pores, silver nitrate (AgNO3) was applied to the leaf surface (Lord, Green, and Emino 1979; Schlegel, Sch€onherr, and Schreiber 2005). Sites of silver (Ag) precipitate in the apoplast or cytoplasm are indicative of sites of selective permeability. For light microscopy, $AgNO_3$ uptake was performed on cleared leaves (pigment removed) of both species. Leaf blade samples were prepared as above. A 2-cm section of a leaf blade was selected between the sheath and the leaf tip. The leaves were cleared by submergence in 5.25% sodium hypochlorite solution for 4 hr at room temperature (21ºC), and were then rinsed twice in deionised water for 5 min. Cleared leaves were washed with hand soap in place of a wetting agent to reduce surface tension of the cuticle and upon rinsing, placed on filter paper in a petri dish. After 40 mL of a 0.02 M solution of $AgNO_3$ was placed on the samples, the closed petri dish was incubated at room temperature for 1 hr. The leaves were then rinsed with deionised water to remove excess $AgNO_3$, and mounted on a microscope slide with a drop a glycerol. A cover slip was placed on top and sealed with nail polish. Slides were viewed under a compound microscope (Olympus BX51) and photographed with an Olympus DP71 camera at 200X (Olympus Corporation, Tokyo, Japan).

Leaf samples prepared for SEM were not cleared but were treated with AgNO3 as above. Samples were left to dehydrate for 24 hr prior to being mounted on an SEM aluminum stub. Images were captured on microscopes that did not require a sputter coating of gold. Annual bluegrass samples were analyzed using the Hitachi Tabletop Microscope TM-1000 SEM (Hitachi High-Technologies Corporation, Tokyo, Japan) and creeping bentgrass using the Fei Phenom SEM (Fei Company, Hillsboro, USA).

Results

Nitrogen fertilization experiments

The number of tillers arising from the single tiller at the start of the experiment was determined as a gross indication of the effectiveness of N fertilization. Here a strong fertilizer solution effect was noted with ammonium sulfate fertilized plants of both species producing more tillers regardless of application method ($p = .0002$). A more limited but significant species effect was noted with annual bluegrass generating more tillers than creeping bentgrass ($p = .026$).

Analysis of shoot dry mass revealed statistically significant effects of fertilization solution (N source), application method and an interaction between species and application method (Table 1). With fertilizer type pooled, a difference between application methods was observed for creeping bentgrass where soil application resulted in greater shoot dry mass than foliar application (Figure 1a). Application method did not affect the shoot dry mass of annual bluegrass but under foliar fertilization, the shoot dry mass of annual bluegrass was significantly greater than that of creeping bentgrass (Figure 1a). The type of fertilizer was consequential. Shoot dry mass was higher when species, creeping bentgrass and annual bluegrass pooled were fertilized with ammonium sulfate as opposed to urea (Table 1; Figure 1b). Nitrogen analysis, TKN, revealed a significant increase in shoot N concentration for plants (species pooled) grown with foliar-applied fertilizer (2.17%) compared to soil-applied fertilizer (1.78%) ($p = .025$).

Table 1. Summary of ANOVA results for shoot and root dry mass measured on creeping bentgrass and annual bluegrass submitted to various fertilization solutions and application methods. ndf = numerator degrees of freedom.

Source of Variation (Fixed)	ndf	Shoot Dry Mass F	Shoot Dry Mass P	Root Dry Mass (Above 6 cm) F	Root Dry Mass (Above 6 cm) P	Root Dry Mass (Below 6 cm) F	Root Dry Mass (Below 6 cm) P
Species (S)	1	0.23	0.633	12.53	0.002	11.47	0.002
Fertilization solution (F)	1	27.81	<0.001	8.26	0.008	0.70	0.004
Application method (A)	1	4.63	0.042	4.23	0.051	7.28	0.013
S x F	1	1.30	0.265	0.19	0.670	2.63	0.118
F x A	1	4.05	0.056	10.79	0.003	4.56	0.043
S x A	1	5.07	0.034	2.89	0.102	2.48	0.129
S x F x A	1	1.84	0.181	0.55	0.586	4.77	0.018

The effect on root growth was greater overall for both creeping bentgrass and annual bluegrass when ammonium sulfate was soil applied compared to all other conditions (p = .043). Since creeping bentgrass is considered a deep-rooted species (Lyons, Landschoot, and Huff 2011) and annual bluegrass a shallow-rooted species (Hutchinson and Seymour 1982), root dry mass was separated into that in the top 6 cm of the soil and that below 6 cm in order to ascertain whether the N fertilization permutations resulted in differences between the two species. As would be predicted, root dry mass distribution was significantly affected by species (p = .005). Creeping bentgrass fertilized with soil-applied ammonium sulfate had greater root dry mass in the upper 6 cm (p = .004) compared to all other conditions (data not shown). When fertilization solution and application method are pooled, root dry mass in the top 6 cm was still greater for creeping bentgrass (Figure 2a). While the deep-rooted creeping bentgrass had 50% of its total root mass in the upper 6 cm of the soil profile (0.171 g (SE = 0.03)) the shallow-rooted annual bluegrass had 66% of its total root mass (0.117 g (SE = 0.025)) in the upper 6 cm. Root dry mass in the upper 6 cm was affected not only by species but also by fertilization solution with an interaction between fertilization solution and application method (Table 1). Plants of both species fertilized with ammonium sulfate showed a 20% increase in root dry mass compared to those fertilized with urea, although this effect was not observed under foliar application ($p = .001$) (Figure 2b). Creeping bentgrass had more root dry mass in the upper 6 cm under soil-applied fertilizer 0.20 g (SE = 0.03) compared to foliar fertility 0.13 g (SE = 0.01) (Figure 2a).

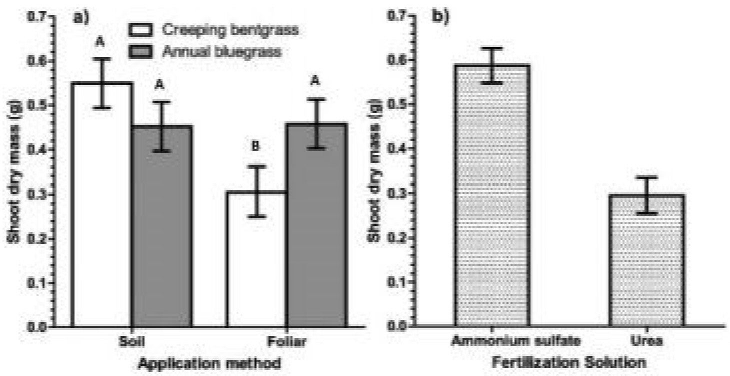

Figure 1. (a) The effect of application method of nitrogen fertilizers on the shoot dry mass of creeping bentgrass and annual bluegrass with the fertilizer type pooled, (b) the effect of fertilizer type on shoot dry mass when species and application method are pooled. Error bars represent standard error of four replicates in a factorial experiment. Values with the same letter are not statistically different at the 0.5% level.

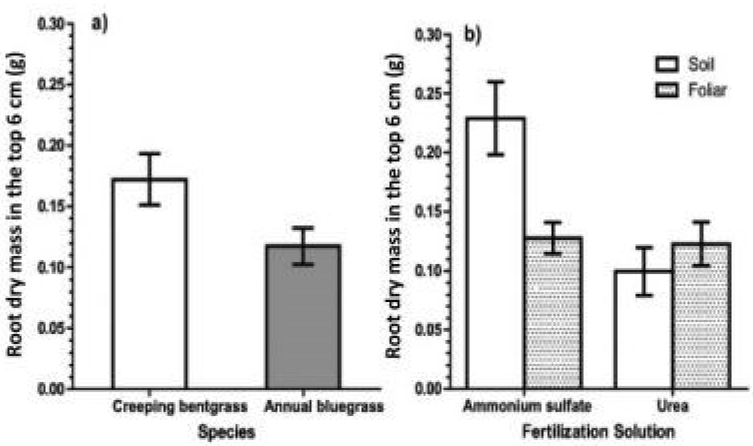

Figure 2. (a) Root dry mass in the top 6 cm of creeping bentgrass and annual bluegrass with fertilization solution and application method pooled, (b) the root dry mass in the top

6 cm of soil- and foliar-applied ammonium sulfate and urea (species pooled). Error bars represent standard error of four replicates in a factorial experiment.

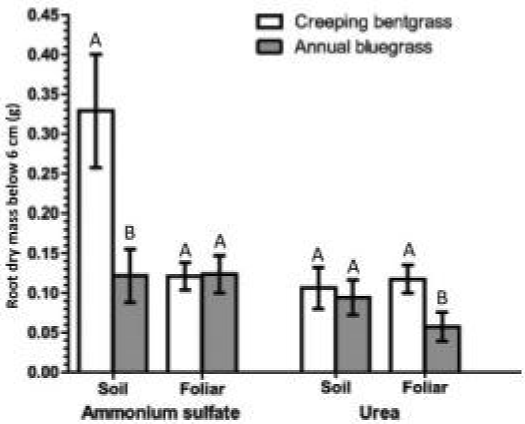

Figure 3. Root dry mass below 6 cm of creeping bentgrass and annual bluegrass when ammonium sulfate and urea are applied to foliage or soil. Error bars represent standard error of four replicates in a factorial experiment. Values with the same letter are not statistically different at the 0.5% level.

The root dry mass in the upper 6 cm of the soil for annual bluegrass was not significantly affected by application method. For root dry mass below 6 cm, species, fertilization solution and application method were all significant (Table 1). Roots in the lower portion of the soil profile fertilized with ammonium sulfate had a 19% increase in root mass over those fertilized with urea (Figure 3). Further, there is a statistically significant interaction between fertilization solution and application method (Table 1). Below 6 cm, creeping bentgrass again had a higher root dry mass under soil-applied ammonium sulfate compared to all other conditions (Figure 3).

Stomatal counts

No difference between species and populations was found for stomatal count for either the adaxial (p = .805) or the abaxial (p = .173) surfaces. The adaxial leaf surface had a mean count of 122.39 (+/- 42.13) stomates per mm^2 and the abaxial side 109.19 (+/- 46.66) stomates per mm^2.

Localization of aqueous pores

The black precipitate of silver chloride that forms at sites of $AgNO_3$ absorption reveals the locations of aqueous pores in leaves (Schreiber 2005). Precipitate was observed close to both trichomes and stomata.

Some cultivars of creeping bentgrass such as L-93 are known to have trichomes or "barbed-like" extensions from the waxy cuticular layer (Bonos, Casler and Meyer 2004; Williams and Harrell 2005). These structures were present on the margins of the creeping bentgrass leaves (Figure 4c). Annual bluegrass also possessed these structures and they were present on more of the leaf surface at the margins, midrib and veins. A higher absorption of $AgNO_3$ was observed to be concentrated at the base and head of these barbs on annual bluegrass leaves compared to creeping bentgrass leaves (Figure 4a). Silver deposits were observed on creeping bentgrass barbs or trichomes but not to the same degree as on annual bluegrass.

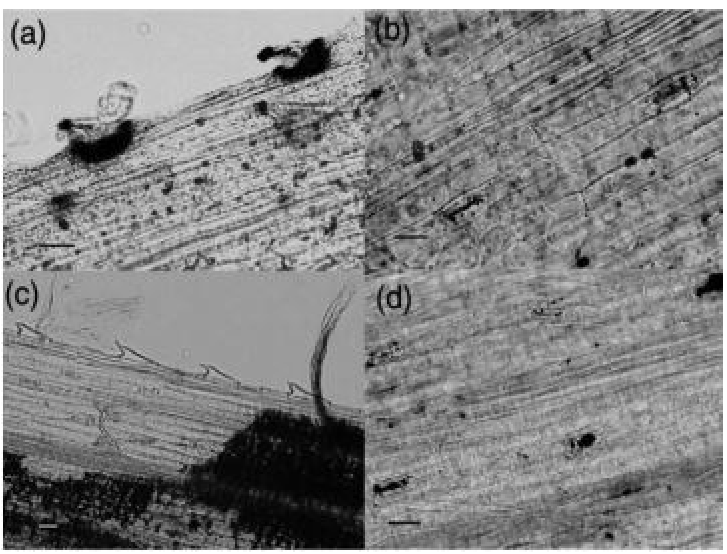

Figure 4. Bright field photomicrographs of cleared leaves of annual bluegrass (a, b) and creeping bentgrass (c, d) after treatment with silver nitrate. Characteristic silver precipitates are shown on barbs (a, c) and stomata (b, d).

Both creeping bentgrass and annual bluegrass had aqueous pores near the stomata (Figure 4b and d). On cleared leaf samples, the black precipitate appeared to be concentrated between the guard cells and not outside them. The concentration of silver precipitate with the guard cells of the stomata is even more striking when viewing the SEM micrographs (Figure 5). Both creeping bentgrass and annual bluegrass species had consistent morphological traits regardless of the population (genotype/ecotype). The greater amount of silver precipitate on annual bluegrass leaves as compared to creeping bentgrass leaves would correlate to annual bluegrass leaves having a greater number of aqueous pores.

Discussion

The exploitation of species differences between creeping bentgrass and annual bluegrass in response to N fertilization rests on the accumulation of empirical data of plant growth. It was surprising that by every measure ammonium sulfate resulted in the greatest growth regardless of application method. Our hypothesis that the deep-rooted creeping bentgrass would have a growth advantage over shallowrooted annual bluegrass when N was soil applied was upheld. The advantage of annual bluegrass shoot growth over creeping bentgrass under foliar N fertilization correlates with the greater number of aqueous pores available for N absorption observed on the leaves of annual bluegrass.

Ammonium sulfate outperformed urea in growth stimulation of both the species. It was expected that urea would be absorbed more efficiently due to its smaller size and neutral charge than the cationic ammonium ion from ammonium sulfate. Other studies have observed no difference in N uptake between urea and ammonium (Bowman and Paul 1992; Henning, Mulvaney, and Branham 2013; Stiegler et al. 2013). The work presented here is not directly comparable to those studies as plant growth served as an indirect indicator of N absorption. It should be noted that the addition of aqueous magnesium sulfate to all pots ensured that any growth discrepancies were not attributable to a sulfur deficiency since urea as an N source lacks the sulfur component of ammonium sulfate. In tea, sulfur deficiency can cause poor shoot growth that results in less absorption of nitrogen (Ruan and Gerendás 2015).

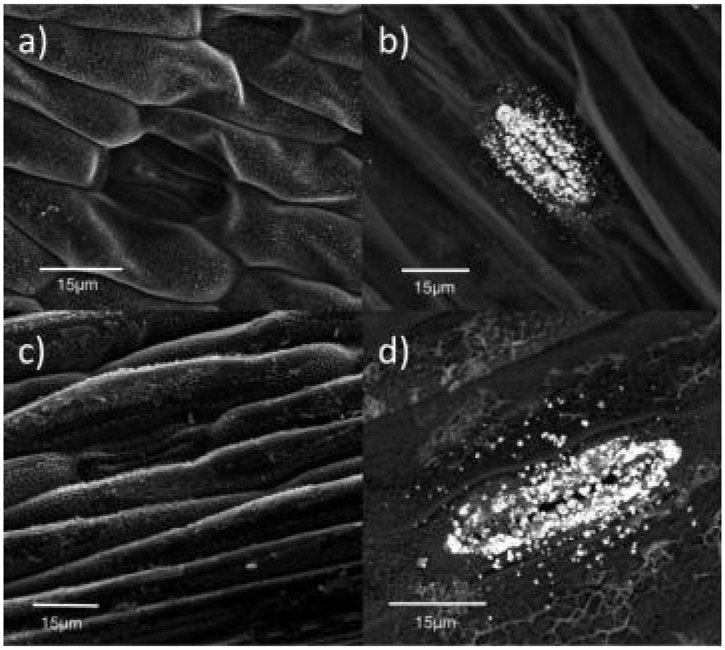

Figure 5. SEM photomicrographs of uncleared leaves of annual bluegrass (a, b) and creeping bentgrass (c, d) after treatment with silver nitrate. Stomata of untreated samples (a, c) lack the characteristic silver precipitates are shown on treated samples (b, d).

Our results showed that shoot dry mass was higher when grass species were fertilized with ammonium sulfate, regardless of the application method. In general, the greatest impact on shoot growth was observed when N fertilizer was soil applied. Creeping bentgrass exhibited a clear preference, based on dry shoot mass, for soil-applied fertilizer. Foliar application of N fertilizer clearly resulted in annual bluegrass achieving greater shoot growth than creeping bentgrass. When creeping bentgrass and annual bluegrass are competing for dominance, the predilection of creeping bentgrass for fertilizer applied to the soil may in fact bias growth toward annual bluegrass under foliar application of N fertilizer. The concentration of N in shoots as measured by TKN analysis revealed higher concentrations under foliar application with species pooled. Within the confines of those data, one could not draw any direct correlation between N concentrations in shoots and shoot dry mass. Just as fertilization with ammonium sulfate resulted in greater shoot growth as measured by shoot dry mass for both the species; it also resulted in a greater number of tillers for both the species underscoring the greater growth response of both species to this N form. It should be noted that the effect was greater for annual bluegrass than creeping bentgrass. This is interesting because it speaks to the invasive potential of annual bluegrass in a course planted to creeping bentgrass. More work would need to be done to discern if tiller number for annual bluegrass would vary under soil versus foliar application of ammonium sulfate.

The impact on growth observed in shoot dry mass was also observed in the root dry mass. The root to shoot ratio was similar for plants treated with foliar and soil applied fertilizers (data not shown). Again, greater overall growth was observed for ammonium sulfate-treated plants than for urea-treated plants. The root systems of the deep-rooted creeping bentgrass (Lyons, Landschoot, and Huff 2011) and shallow-rooted annual blue grass (Hutchinson and Seymour 1982) are known to differ in their depth. These observations were consistent when plants were treated with soil-applied fertilizer. However, root systems of creeping bentgrass and annual bluegrass treated with foliar-applied fertilizer were of similar size. Normally only 10–20% of the root system is needed when there is an adequate supply of water and nutrients (Marschner 2002). Under foliar-applied fertilizers, plants can require even less of that root system for N uptake as N is supplied directly onto the leaves. Witte et al. (2002) demonstrated that little N was moved into the root system of potato plants treated with foliar applications of N. A limit in N in the roots may be problematic in the summer when root growth is impaired by warm temperatures (Huang, Liu, and Xu 2001). Under foliar fertilization, creeping bentgrass no longer has the advantage of a deeper root system over annual bluegrass. For that reason, soil application of N fertilizer would be recommended for creeping bentgrass as the resultant greater root mass would relate to a strong competitive advantage for drought tolerance. In terms of overall quality for creeping bentgrass, it has been shown that a combination of soil and foliar-applied N works best (Totten et al. 2008).

Microscopic analysis of creeping bentgrass and annual bluegrass leaves revealed a potential explanation for foliar-applied fertilizer resulting in a greater shoot dry mass for annual bluegrass as compared to creeping bentgrass. Foliar uptake of nutrients occurs through stomata as well as aqueous pores (Schönherr 2006; Eichert and Goldbach 2008; Eichert 2013). No statistical difference was observed between the turfgrass species studied here for either adaxial or abaxial stomatal counts. Care was taken to harvest leaves of similar position and age as stomatal density is influenced not only by species and cultivar, but also by leaf age and position (Shearman and Beard 1972). The number of stomata observed here is in tight agreement with that observed for creeping bentgrass at putting green height (Williams and Harrell 2005). Green, Beard, and Casnoff (1990) also reported similar adaxial and abaxial stomatal counts for creeping bentgrass and annual bluegrass. Therefore, the greater effect of foliar fertilization on annual bluegrass as compared to creeping bentgrass shoot dry mass cannot be solely attributed to the number of stomata. Instead the greater shoot dry mass of annual bluegrass may be the result of increased N absorption through aqueous pores associated with "barb-like" extensions observed on the leaf cuticle. These extensions extrude from the waxy cuticular layer and resemble trichomes and have been associated with absorption of foliar solutions (Schreiber 2006). While these "barb-like" structures have also been observed on creeping bentgrass (Williams and Harrell 2005), we have detected them on annual bluegrass in multiple locations: at the margins, midribs and veins of the leaf surface. The absorption of $AgNO_3$ at the base and the head of these structures is similar to that observed for trichomes of Vicia faba leaves (Schlegel, Schönherr, and Schreiber 2005). We theorize that the higher $AgNO_3$ absorption at the site of the annual bluegrass barbs mimics the higher absorption of N and could explain the greater shoot dry mass of this species compared to creeping bentgrass when fertilized with a foliar solution. There are two distinct ecotypes of annual bluegrass: one annual and one short lived perennial (Lush 1989; Darmency and Gasquez 1997) and many genotypes across this range of reproductive strategies (Zontek 1987; Huff 2004). Interestingly, observations of the leaf morphology of this species were independent of ecotype or genotype. The potential modifications of leaf morphology due to manipulation of fertilization regime would be noted when comparing leaf data from the experiment to that from the field. Plants harvested from the field were all exposed to the same rate and source of N, K and P. Leaf age is also known to impact nutrient absorption with older leaves absorbing less due to an increase in cuticle thickness (Bondada, Syvertsen, and Albrigio 2001). Care was taken to harvest freshly expanded leaf tissue so that cuticle thickness related to leaf age would not be an issue. Therefore, we propose that the shoot growth and tiller generation advantage of annual bluegrass under foliar fertilization results from having a greater number of aqueous pores for nutrient absorption. The leaf morphologies of the creeping bentgrass ecotypes/genotypes examined were also similar to one another regardless of the population studied or where the samples originated: either from the N experiment or the field. Clearly, the next step would be to repeat the experiment in the field, but the fact remains that the species specific differences in leaf morphology were not consequent to N fertilization manipulation.

In conclusion, the results of this research reveal that the type of N used for fertilization was significant with both creeping bentgrass and annual bluegrass achieving greater shoot and root dry mass when fertilized with ammonium sulfate versus urea. While species did not affect the preference for the type of fertilizer, the impact of application method on shoot and root dry mass was species specific. Here we provide evidence that that foliar application of N fertilizer creates a bias for annual bluegrass growth whereas soil fertilization creates a bias toward creeping bentgrass growth. The manipulation of N fertilization provides novel approach and a promising control strategy for managing annual bluegrass encroachment in golf greens planted to creeping bentgrass.

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References

Askew, S. D., and B. M. S. McNulty. 2014. Methiozolin and cumyluron for reemergence annual bluegrass (*Poa annua*) control on creeping bentgrass (*Agrostis stolonifera*) putting greens. Weed Technology 28:535–42. doi:10.1614/WT-D-14-00018.1.

Bell, G., E. Odorizzi, and K. Danneberger. 1999. Reducing populations of annual bluegrass and roughstalk bluegrass in creeping bentgrass fairways: A nutritional approach. Weed Technology 13:829–34.

Beard, J. B. 2002. Turf management for golf courses. 2nd ed. Chelsea, Michigan: Ann Arbor Press.

Benzing, D. H., K. Henderson, B. Kessel, and J. Sulak. 1976. The absorptive capacities of bromeliad trichomes. American Journal of Botany 63:1009–14. doi:10.2307/2441760.

Bethea, F. G., Jr., D. Park, A. Mount, N. Menchyk, and H. Liu. 2014. Effects of acute moisture stress on creeping bentgrass cuticle morphology and associated effects on foliar nitrogen uptake. HortScience 49:1582–87.

Bondada, B. R., D. M. Oosterhuis, and R. J. Norman. 1997. Cotton leaf age, epicuticular wax, and nitrogen15 absorption. Crop Science 37:807–11. doi:10.2135/cropsci1997.0011183X003700030019x.

Bondada, B. R., J. P. Syvertsen, and L. G. Albrigio. 2001. Urea nitrogen uptake by citrus leaves. HortScience 36:1061–65.

Bonos, S. A., M. D. Casler, and W. A. Meyer. 2004. Plant responses and characteristics associated with dollar spot resistance in creeping bentgrass. Crop Science 44:1763–69. doi:10.2135/cropsci2004.1763.

Bowman, D. C., and J. Paul. 1992. Foliar absorption of urea, ammonium, and nitrate by perennial ryegrass turf. Journal of the American Society for Horticultural Science 117:75–79.

Bowman, D. C. 2003. Daily vs. periodic nitrogen addition affects growth and tissue nitrogen in perennial ryegrass turf. Crop Science 43:631–38. doi:10.2135/cropsci2003.0631.

Danneberger, T. K., B. E. Branham, and J. M. Vargas. 1987. Mefluidide applications for annual bluegrass seedhead suppression based on degree-day accumulation. Agronomy Journal 79:69–71. doi:10.2134/agronj1987.00021962007900010014x.

Darmency, H., and J. Gasquez. 1997. Spontaneous hybridization of the putative ancestors of the allotetraploid Poa annua. New Phytologist 136:497–501. doi:10.1046/j.1469-8137.1997.00772.x.

Dernoeden, P. H., S. McDonald, and J. E. Kaminski. 2008. Creeping bentgrass and perennial ryegrass seedling tolerance to bispyribac-sodium. Horticultural Science 43:2186–90.

Dong, S., D. Neilsen, G. Neilsen, and L. Fuchigami. 2005. Foliar N application reduces soil NO3-N leaching loss in apple orchards. Plant and Soil 268:357–66. doi:10.1007/s11104-004-0333-1.

Eggens, J. L., C. P. M. Wright, D. P. Murr, and K. Carey. 1989. The effect of ethephon on annual bluegrass and creeping bentgrass growth. Canadian Journal of Plant Science 69:1353–57. doi:10.4141/cjps89-163.

Eichert, T., and H. E. Goldbach. 2008. Equivalent pore radii of hydrophilic foliar uptake routes in stomatous and astomatous leaf surfaces – further evidence for a stomatal pathway. Physiologia Plantarum 132:491–502. doi:10.1111/j.1399-3054.2007.01023.x.

Eichert, T., A. Kurtz, U. Steiner, and H. E. Goldbach. 2008. Size exclusion limits and lateral heterogeneity of the stomatal foliar uptake pathway for aqueous solutes and water-suspended nanoparticles. Physiologia Plantarum 134:151–60. doi:10.1111/j.1399-3054.2008.01135.x.

Eichert, T. 2013. Foliar nutrient uptake – of myths and legends. In Acta Horticulturae 984: Proceedings of the VIIth IS on Mineral Nutrition of Fruit Crops, eds. S. Poovarodom and S. Yingjajaval, 69–76. Fernández, V., and P. H. Brown. 2013. From plant surface to plant metabolism: The uncertain fate of foliar-applied nutrients. Frontiers in Plant Science 4:289. doi:10.3389/fpls.2013.00289.

Green, R. L., J. B. Beard, and D. M. Casnoff. 1990. Leaf blade stomatal characterizations and evapotranspiration rates of 12 cool-season perennial grasses. Horticultural Science 25:760–1.

Henning, S. W., R. L. Mulvaney, and B. E. Branham. 2013. Factors affecting foliar nitrogen uptake by creeping bentgrass. Crop Science 53:1778–83. doi:10.2135/cropsci2012.11.0650.

Huang, B., X. Liu, and Q. Xu. 2001. Supraoptimal soil temperatures induced oxidative stress in leaves of creeping bentgrass cultivars differing in heat tolerance. Crop Science 41:430–5. doi:10.2135/cropsci2001.412430x.

Huff, D. 2004. Developing annual bluegrass cultivars for putting greens. September 22, 2015. USGA Turfgrass and Environmental Research Online 3:1–8. http://usgatero.msu.edu/tero_browse.html#2004.

Hull, H. M., H. L. Morton, and J. R. Wharrie. 1975. Environmental influences on cuticle development and resultant foliar penetration. The Botanical Review 41:420–51. doi:10.1007/BF02860832.

Hutchinson, C., and G. Seymour. 1982. Poa annua L. Journal of Ecology 70:887–901. doi:10.2307/2260111. Johnson, B. J., and T. R. Murphy. 1996. Suppression of a perennial subspecies of annual bluegrass (*Poa annua spp. reptans*) in a creeping bentgrass (*Agrostis stolonifera*) greens with plant growth regulators. Weed Technology 10:705–9.

Kaminski, J. E., P. H. Dernoeden, and C. A. Bigelow. 2004. Creeping bentgrass seedling tolerance to herbicides and paclobutrazol. Horticultural Science 39:1126–9.

Lord, W. G., D. W. Green, and E. R. Emino. 1979. Absorption of silver nitrate and lead nitrate into leaves of McIntosh apple. Canadian Journal of Plant Science 59:137–42. doi:10.4141/cjps79-019. Lush, M. 1989. Adaptation and differentiation of golf course populations of annual bluegrass (*Poa annua*). Weed Science 37:54–9.

Lycan, D. W., and S. E. Hart. 2006. Seasonal effects on annual bluegrass control in creeping bentgrass with bispyribacsodium. Weed Technology 20:722–7. doi:10.1614/WT-04-314R2.1.

Lyons, E. M., P. J. Landschoot, and D. R. Huff. 2011. Root distribution and tiller densities of creeping bentgrass cultivars and greens-type annual bluegrass cultivars in a putting green. HortScience 46:1411–7.

Marschner, H. 2002. Mineral nutrition of higher plants. 2nd ed. New York, United States: Academic Press. McCullough, P. E., H. B. Liu, and L. B. McCarty. 2005. Ethephon and gibberellic acid inhibitors influence creeping bentgrass putting green quality and ball roll distances. Horticultural Science 40:1902–3.

McMahon, G., and A. Hunter. 2012. Determination of effects of plant growth regulators on Agrostis stolonifera and Poa annua. Acta Horticulturae 937:161–8. doi:10.17660/ActaHortic.2012.937.19.

McMurtrie, R. E., C. M. Iversen, R. C. Dewar, B. E. Medlyn, T. Näsholm, D. A. Pepper, and R. J. Norby. 2012. Plant root distributions and nitrogen uptake predicted by a hypothesis of optimal root foraging. Ecology and Evolution 2 (6):1235–50. doi:10.1002/ece3.266.

Orbovic, V., D. Archor, P. Petracek, and J. P. Syvertsen. 2001. Air temperature, humidity, and leaf age affects penetration of urea through grapefruit leaf cuticles. Journal of the American Society for Horticultural Science 126:44–50.

Riederer, M., and A. Friedmann. 2006. Transport of lipophilic non-electrolytes across the cuticle. In Annual plant reviews, Vol. 23, Biology of the plant cuticle, eds. M. Riederer and C. Muller, 250 –79. Oxford, United Kingdom: Blackwell Publishing.

Rice, J. S., E. M. Glenn, and V. L. Quisenberry. 1979. Rapid method for obtaining leaf impressions in grasses. Agronomy Journal 71:894–6. doi:10.2134/agronj1979.00021962007100050045x.

Rogers, E. D., and P. N. Benfey. 2015. Regulation of plant root system architecture: Implications for crop advancement. Current Opinion in Biotechnology 32:93–8. doi:10.1016/j.copbio.2014.11.015

Ruan, J., and J. Gerendás. 2015. Absorption of foliar-applied urea-15N and the impact of low nitrogen, potassium, magnesium and sulfur nutritional status in tea

(Camellia sinensis L.) plants. Soil Science and Plant Nutrition 61:653–63. doi:10.1080/00380768.2015.1027134.

Schlegel, T., and J. Schönherr. 2002. Selective permeability of cuticles over stomata and trichomes to calcium chloride. Acta Horticulturae 549:91–6. doi:10.17660/ActaHortic.2002.594.7.

Schlegel, K., J. Schönherr, and L. Schreiber. 2005. Size selectivity of aqueous pores in stomatous cuticles of Vicia faba leaves. Planta 221:648–55. doi:10.1007/s00425-005-1480-1.

Schlossberg, M. J., and J. P. Schmidt. 2007. Influence of nitrogen rate and form on quality of putting greens cohabited by creeping bentgrass and annual bluegrass. Agronomy Journal 99:99–106. doi:10.2134/agronj2006.0136.

Schönherr, J. 1976. Water permeability of isolated cuticular membranes: The effects of pH and cations on diffusion, hydrodynamic permeability and size of polar pores in the cutin matrix. Planta 128:113–26. doi:10.1007/ BF00390312.

Schönherr, J. 2006. Characterization of aqueous pores in plant cuticles and permeation of ionic solutes. Journal of Experimental Botany 57 (11):2471–91. doi:10.1093/jxb/erj217.

Schreiber, L. 2005. Polar paths of diffusion across plant cuticles: New evidence for an old hypothesis. Annals of Botany London 95:1069–73. doi:10.1093/aob/mci122.

Schreiber, L. 2006. Characterisation of polar paths of transport in plant cuticle. In Biology of the plant cuticle, annual plant review, eds. M. Riederer and C. Muller, vol. 23, 280–91. Oxford, United Kingdom: Blackwell Publishing.

Sen Tran, T., and R. R. Simard. 1993. Mehlich III-extractible elements. In Soil sampling and methods of analysis, ed. M. R. Carter, 43–9. Boca Raton, Florida, USA: Lewis Publishers.

Shearman, R. C., and J. B. Beard. 1972. Stomatal density and distribution in Agrostis as influenced by species, cultivar, and leaf blade surface and position. Crop Science 12:822–3. doi:10.2135/cropsci1972.0011183X001200060031x.

Steel, R. G. D., J. H. Torrie, and D. A. Dickey. 1997. Principles and procedures of statistics: A biometrical approach. Boston, Massachusetts, USA: McGraw-Hill Publishing Company.

Stiegler, J. C., M. D. Richardson, and D. E. Karcher. 2011. Foliar nitrogen uptake following urea application to putting green turfgrass species. Crop Science 51:1253–60. doi:10.2135/cropsci2010.06.0377.

Stiegler, J. C., M. D. Richardson, D. E. Karcher, T. L. Roberts, and R. J. Norman. 2013. Foliar absorption of various inorganic and organic nitrogen sources by creeping bentgrass. Crop Science 53:1148–52. doi:10.2135/cropsci2012.08.0511.

Tan, X., H. Ikeda, and M. Oda. 1999. Absorption, translocation, and assimilation of foliar-applied urea compared with nitrate and ammonium in tomato plants. Soil Science and Plant Nutrition 45:609–16. doi:10.1080/ 00380768.1999.10415824.

Totten, F., H. Liu, L. McCarty, C. Baldwin, D. Bienlenberg, and J. Toler. 2008. Efficiency of foliar versus granular fertilization: A field study of creeping bentgrass performance. Journal of Plant Nutrition 31:972–82. doi:10.1080/ 01904160802043171.

Tyree, M., T. Scherbatskoy, and C. Tabor. 1990. Leaf cuticles behave as asymmetric membranes. Plant Physiology 92:103– 9. doi:10.1104/pp.92.1.103.

USGA Green Section Staff. 2004. USGA Recommendations for a method of putting green construction. http:// www.usga. org/turf!course construction/ green articles/USGA Recommendations for a Method of Putting Green Construction. pdf.

Varco, J. J., and J. B. Sartain. 1986. Effects of phosphorus, sulfur, calcium hydroxide, and pH on the growth of annual bluegrass. Soil Science Society of America Journal 50:128–32. doi:10.2136/sssaj1986.03615995005000010025x.

Vargas, J. M., Jr., and A. J. Turgeon. 2003. Poa annua: Physiology, culture and control of annual bluegrass. Hoboken, New Jersey, USA: John Wiley and Sons.

Waddington, D. V., T. R. Turner, J. M. Duich, and E. L. Moberg. 1978. Effect of fertilization on ‘Penncross’creeping bentgrass. Agronomy Journal 70:713–8. doi:10.2134/agronj1978.00021962007000050005x.

Wesely, R., R. Shearman, and E. Kinbacher. 1985. Foliar N-uptake by eight turfgrasses grown in a controlled environment. Journal of the American Society for Horticultural Science 110:612–4.

Williams, D., and M. Harrell. 2005. Leaf surface morphology among Agrostis species and cultivars and correlation with dollar spot severity. USGA Turfgrass and Environmental Research Online 4:1–6. http://usgatero.msu.edu/v04/n10.pdf (accessed September 22, 2015).

Witte, C., S. Tiller, M. Taylor, and H. Davies. 2002. Leaf urea metabolism in potato. Urease activity profile and patterns of recovery and distribution of 15N after foliar urea application in wild-type and urease-antisense transgenics. Plant Physiology 128:1129–36. doi:10.1104/pp.010506.

Wittwer, S. H., M. J Bukovac, and H. B. Tukey. 1963. Advances in foliar feeding of plant nutrients. In Fertilizer technology and use, ed. M. H. McVickar, 429–55. Madison, Winsconson, USA: SSSA.

Zontek, S. 1987. The continuing saga of Poa annua. USGA Green Section Record 25:1–5. http://gsrpdf.lib.msu.edu/ticpdf. py?file D /1980s/1987/870501.pdf (accessed September 22, 2015).