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Effects of Acute Moisture Stress on Creeping Bentgrass Cuticle Morphology and Associated Effects on Foliar Nitrogen Uptake

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***Abstract*. Foliar fertilization is a common practice to deliver nitrogen (N) to turfgrasses. The mechanisms of foliar applied nutrient uptake, particularly the effects of the leaf cuticle layer, have not been clearly characterized in turfgrasses. The objectives of this study were to determine the effect of acute moisture stress on the morphological and compositional components of the cuticle and the resulting effect on foliar-applied N absorption. Creeping bentgrass (*Agrostis stolonifera* L.) was irrigated to return 100% or 50% evapotranspiration rate (ET) for 10 days to examine cuticular modifications resulting from acute moisture stress and foliar N uptake with and without a surfactant. Acute water stress increased the total cuticle wax by 11%, mostly as a result of the compound 1-hexacosanol, and increased crystalloid density creating a rougher leaf surface. The 50% ET treatment significantly reduced recovery of ^{15}N-labeled urea by 14%, which was attributed to the increased total cuticle wax and crystalloid density making the surface less receptive to foliar applications. The surfactant addition to the urea solution increased ^{15}N-labeled urea recovery by 21% and absorption of ^{15}N in 50% ET plants to levels consistent with the 100% ET plants. These results suggest that acute moisture stress modifies the cuticle wax load and morphology, thereby hindering foliar absorption; however, a surfactant addition can help to mitigate this effect and increase absorption of N.**

Drought is a major environmental stress worldwide resulting in decreased plant productivity and diminished plant health. Climate model predicts this to become a more severe problem in the future (Farooq et al., 2012). Drought stress causes cellular dehydration, loss of turgor pressure, and ion toxicity (Bartels and Sunkar, 2005). Plants' protective responses to water deficiency can include stomatal closure to reduce transpiration (Sharp and Davies, 1989), leaf rolling/orientation to reduce water loss and heat exposure (Kao and Forseth, 1992), and osmotic adjustment to reduce water potential (Delauney and Verma, 1993). In addition to these drought tolerance strategies, cuticle augmentation in response to water deficit has been well documented in a number of plant species, specifically cotton (*Gossypium hirsutum* L.) (Bondada et al., 1996), Arabidopsis [*Arabidopsis thaliana* (L.) Heynh.] (Kosma et al., 2009), tree tobacco (*nicotiana glauca* L.) (Cameron et al., 2006), soybeans [*Glycine max* (L.) Merr.] (Kim et al., 2007a), sesame (*Sesamum indicum* L.) (Kim et al., 2007b), rose (*Rosa ×hybrids*) (Jenks et al., 2001), citrus (Bondada et al., 2001), and peanut (*Arachis hypogaea* L.) (Samdur et al., 2003).

The plant cuticle is a continuous extracellular membrane located on the above-ground organs of most higher plants. Only roots and secondary plant tissue as well as some mosses are devoid of this protective barrier (Koch and Ensikat, 2008). The main function of the cuticle is to protect against uncontrolled water loss to the atmosphere through transpiration (Burghardt and Riederer, 2006; Cameron et al., 2006; Riederer and Schreiber, 2001). Secondary characteristics of the cuticle include antiadhesive properties, repelling water, particles, pathogens, and other molecules, which could hinder the uptake of foliar applications of nutrients and pesticides in an agricultural system (Bargel et al., 2006; Koch et al., 2008). The plant cuticle is comprised of two main portions: the cutin, which provides structure, and waxes, which provide protective functions to the plant. Many of the protective functions of the cuticle, especially repellency, can be attributed to the cuticular and epicuticular waxes that develop on the plant surface. The chemical composition of cuticular waxes is a mixture of aliphatic and aromatic components comprised of various combinations of long chain alkanes, fatty acids, primary and secondary alcohols, aldehydes, and ketones, the proportions of which are dependent on species, developmental stage, and organ (Bargel et al., 2006; Jetter et al., 2006; Riederer and Markstadter, 1996). Epicuticular waxes form thin two-dimensional films and/or three-dimensional structures, depending on chemical composition (Koch et al., 2008). Crystalloids are common three-dimensional structures and are characterized as granules, plates, platelets, rodlets, threads, and tubules (Barthlott et al., 1998; Jeffree, 2006). The three-dimensional structures add roughness to the cuticle making it more hydrophobic to foliar applications.

Foliar fertilization is widespread in turfgrass maintenance programs because of the labor efficiency and cost-effectiveness resulting from the ability to tank-mix and apply the fertilizer concurrently with additional chemicals. Low-rate application of nutrients to turfgrasses in standard intervals promotes uniform growth that increases playability and aesthetics (Bowman, 2003). Increased canopy color, leaf N concentration, and leaf micronutrient concentrations were reported from creeping bentgrass (*Agrostis stonlonifera* L.) fertilized frequently using liquid solutions (Schlossberg and Schmidt, 2007). However, the combination of liquid and granular fertilizers provides the best turfgrass quality and reduces total fertilizer input (Totten, 2006; Totten et al., 2008). Minimal N losses from volatilization were documented on creeping bentgrass and hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × C. *transvaalensis* Burtt Davy cv. TifEagle] putting greens applied with foliar urea applications, providing evidence of lower environmental impact by foliar fertilization (Stiegler et al., 2011).

Nitrogen absorption with various N sources and factors affecting uptake have been studied on various species in the past. Stiegler et al. (2013) found ^{15}N-labeled urea uptake was superior to other tested N sources, where absorptions levels ranged from 31% to 56%

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of N sources applied after 8 h. Absorption of ^{15}N-labeled urea was variable through summer months ranging from 36% to 69% on a creeping bentgrass putting green, which it was suggested could partly have been associated with increased cuticle wax loads (Stiegler et al., 2011). In ryegrass (*Lolim perenne* L.), foliar-applied ^{15}N-labeled urea was found to be absorbed by 30.3% and 53.1% by new and old leaves after 48 h, respectively. A significant negative relationship was found with urea uptake and epicuticular wax amounts in citrus leaves and cotton (Bondada et al., 1997, 2001). Aqueous pores (greater than 1 nm) have been demonstrated on plant cuticles and provide entry of water and small molecules into the plant (Schönherr, 2006). There is also evidence of a stomatal pathway for the uptake of foliar-applied solutions. Eichert and Goldbach (2008) found significant differences in N uptake with stomatal aperture and stomatous vs. astomatous leaf surfaces, indicating the role of stomata in foliar uptake.

The use of adjuvants has also been demonstrated to increase the uptake of foliar-applied chemicals in several plant species under various environmental conditions (Fernandez et al., 2006; Liu, 2004; Neal et al., 1990). For example, surfactant added to potassium nitrate applications increased potassium content in cotton leaves compared with applications with water alone (Howard et al., 1998; Howard and Gwathmey, 1994). Rawluk et al. (2000) documented a 25% increase in N recovery when a nonionic surfactant was added to foliar applications of ^{15}N-labeled urea in wheat (*Triticum aestivum* L.). Surfactant added to ^{14}C-labeled glyphosate applied to barnyardgrass increased the uptake, movement, and herbicide activity (Kirkwood et al., 2000). The addition of adjuvants has become a common practice to increase uptake and reduce losses of foliar-applied fertilizers and pesticides (Wang and Liu, 2007).

Creeping bentgrass is the most widely used turfgrass species for putting greens. There is currently a lack of research examining the influence of water stress to the cuticle layer within the species. Data are also lacking to determine if morphological or compositional changes in the cuticle from acute moisture stress influence foliar N uptake and if added surfactant can amend those changes. The objectives of this study were to 1) determine creeping bentgrass cuticle total wax, chemical composition, and morphology; 2) determine the influence of drought stress on creeping bentgrass cuticle total wax, chemical composition, and morphology; and 3) quantify foliar absorption of ^{15}N-labeled urea with and without a surfactant.

Materials and Methods

Experimental units. This study was conducted in a growth room at The Clemson University Greenhouse Facilities, Clemson, SC. A mixture of 50:50 seed established (in 2010) ‘A1-A4’ creeping bentgrass putting green plugs (51.61 cm^2) were harvested from the nursery green at Thornblade Country Club, Greenville, SC, on 4 Apr. 2011 and 2 June 2011, washed thoroughly to remove sand and organic matter from the root zone, and roots cut to 10 cm. Plants were transplanted into 51.61-cm^{-2} diameter × 18.41-cm depth plastic pots (Elite 300 series) with free drainage, an 85:15 (sand:peat) root zone mixture, and placed in the growth room. The average temperature and relative humidity were 21 °C and 31%, respectively, under a 12-h photoperiod with 350 to 450 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation at canopy height by AgroSun Gold 1000-W sodium/halide lamps (Maryland Hydroponics, Laurel, MD). The creeping bentgrass was established for 30 d. During this acclimation period, the bentgrass was fertilized every 2 weeks with a 10N–1.3P–4.2K liquid fertilizer (Progressive Turf, Birmingham, AL) at a rate of 0.98 g N/m^2 with a backpack sprayer and mowed at a height of 1.27 cm three times per week with electric clippers. After treatments commenced, the bentgrass was only mowed once to ensure sufficient plant material for the analysis of cuticle layer and irrigated daily with respective treatments.

ET was determined gravimetrically over a 72-h period (Bremer, 2003; Wherley and Sinclair, 2009). Pots were weighed at 800 every 24 h with a digital scale (Weigh-Tronix PC-220, Fairmont, MN). Irrigation was applied to replace either 100% or 50% of the daily average ET to represent non-stressed and water-stressed conditions, respectively. Irrigation treatments were administered for 10 d. At this point, some mild wilting could be seen in plants with 50% ET treatment.

Cuticle composition and morphology analysis. Leaf tissue was collected on Day 11 for cuticle analysis. Solvent extractable cuticle analysis and quantification were conducted according to the methods of Jenks et al. (1995) with the following modifications. One gram of fresh leaf clippings was harvested from each pot and submerged in 25 mL of hexane for 50 s to extract the cuticle layer. Extracts were evaporated to dryness with a N_2 stream. The residue was then re-suspended in ≈1 mL of hexane and vortexed to incorporate the entire sample. The extract was transferred into a conical vial and evaporated to dryness with a N_2 stream, for a second time, and the dried residue was prepared for gas chromatography (GC) by derivatization using bis(trimethylsilyl)-acetimide (Sigma-Aldrich, St. Louis, MO) at 100 °C for 20 min. Samples were analyzed with GC using an Agilent Technologies GC 7890A equipped with an Agilent Technologies 5975C Mass Spectrometer (MS). The initial temperature was 50 °C held for 2 min, then increased 40 $°C \cdot min^{-1}$ to 200 °C, then increased 3 $°C \cdot min^{-1}$ to 300 °C, where it remained for 6 min. The GC was equipped with a DB5 MS 20 m × 250 μm × 0.25-μm film column with helium used as a carrier gas. Two microliters of each sample were injected with the injector temperature set at 250 °C and a constant flow of 1 $\mu m \cdot min^{-1}$. Quantification and analysis of the cuticle composition were based on Electron Impact Mass Detector (EIMD) scanning from 40 to 500 atomic mass units after the detector was initiated at 5 min. The quantification of constituents was determined based on comparison of the internal standard tetracosane (10 μg) added during sample extraction. To compare retention times and mass spectra data, three chemical composition standards commonly found in plant cuticles were analyzed separately: 1) a set of alkanes (C_{21}-C_{40}; Sigma Aldrich); 2) a primary alcohol (C_{26}; Sigma Aldrich); and 3) a fatty acid (C_{26}; Sigma Aldrich). Cuticle constituent identification was based off standards and spectra data in the Wiley NIST library. Leaf surface area was calculated on 10 d using fresh clipping samples and a surface area-to-weight ratio for each pot was determined using a WinRhizo scanner (Regent, Canada).

The creeping bentgrass cuticle morphology was studied with scanning electron microscopy (SEM) (Hitachi SU6600 Field Emission SEM) at the Clemson Electron Microscopy Laboratory in Pendleton, SC. Leaves were harvested and fixed with carbon tape on aluminum stubs with adaxial side up. Leaves were allowed to air-dry for 12 to 24 h and sputtercoated with platinum before imaging (Pathan et al., 2008). Images were acquired from areas with relatively flat surfaces, avoiding vascular tissue. Description of crystalloids is based on terminology by Barthlott et al. (1998) and Jeffree (2006).

The evaluation of crystalloid density used the methodologies of Beattie and Marcell (2002) with the following modifications. SEM images were acquired at 10,000 × magnification and analyzed with NIS-Elements (Nikon Instruments Inc., Melville, NY). Images were subjected to a uniform adjustment of brightness and contrast before analysis. Percent leaf area covered by crystalloids was determined based on percentage of pixels above a pre-determined level of brightness threshold.

^{15}N foliar absorption. ^{15}N-labeled urea (ICON Isotopes, NJ) was applied to pots at 0.732 g N/m^2 using a spray chamber at the Clemson University Greenhouse Facility calibrated to deliver 561 $L \cdot ha^{-1}$. A nonionic surfactant (Precision Laboratories, Waukegan, IL) was added at a rate of 0.125% v/v to half of the pots with the ^{15}N-labeled urea solution. Applications occurred on the tenth day of irrigation treatments and tissue harvesting 24 h after applications. Samples were rinsed thoroughly with deionized water before harvesting to remove any residual fertilizer on leaf tissue. Analysis of isotopic ^{15}N in leaf tissue samples and fertilizer applied was determined at the University of Illinois at Urbana–Champaign using the automated Rittenburg technique (Mulvaney et al., 1990) on a Nuclide/MAAS 3-60-RMS double mass spectrometer (Nuclide Corporation, Bellefonte, PA). ^{15}N-labeled urea solution enrichment was tested and found to be 2.75 atom % ^{15}N (average of four subsamples).

Statistical analysis. The experimental design was a completely randomized design with six replications. Factors were irrigation and surfactant with two treatment levels per factor. The experiment was repeated and data presented were pooled because no significant interactions were found between trials. Treatment effects were analyzed using JMP Version 10.0 statistical software (SAS, Cary, NC) with analysis of variance (ANOVA) at $\alpha = 0.05$. Treatment effects were analyzed with ANOVA at $\alpha = 0.05$.

Results and Discussion

Cuticle composition and morphology analysis. The solvent extractable wax composition of the creeping bentgrass leaf cuticle was composed of primary alcohols (91.8%), an aldehyde (3.5%), fatty acids (1.8%), alkanes (less than 1.0%), and a group of unidentified compounds (1.9%). Jetter et al. (2006) reported that many *Poaceae* species have large quantities of their cuticles composed of mainly β-diketones or primary alcohols, including 1-dotriacontanol C_{32} in maize (*Zea mays* L.) (Ristic and Jenks, 2002), 1-octacosanol, C_{28} in wheat (*Triticum aestivum* cv. Naturastar L.) (Koch et al., 2006), and 1-hexacosanol, C_{26} in creeping bentgrass (Stiegler et al., 2010). Our results are consistent with those reported by Stiegler et al. (2010) where 1-hexacosanol, C_{26}, was the major constituent (88%) in the leaf cuticles of creeping bentgrass. The second largest constituent was hexacosanal, a C_{26} aldehyde, at 3.5% of the total cuticle wax.

Creeping bentgrass irrigated to replace 50% ET had significantly greater total cuticle wax (25.7 $\mu g \cdot dm^{-2}$) compared with bentgrass irrigated to replace 100% ET (23.1 $\mu g \cdot dm^{-2}$, $P = 0.034$; (Table 1). An increase in total cuticle wax of plants receiving reduced water has been previously documented (Bondada et al., 1996; Cameron et al., 2006; Kim et al., 2007a; Kosma et al., 2009), which is attributed as a defense mechanism to reduce water loss from the plant.

Plants increase their cuticular wax in response to abiotic changes with minimal to no change in composition (Koch and Ensikat, 2008). Creeping bentgrass irrigated to replace 50% ET had greater primary alcohol, fatty acid, and alkane content than bentgrass irrigated to replace 100% ET. There was no difference between irrigation treatments for the aldehyde, hexacosanal ($P = 0.8377$), despite being the second most present compound (Table 1). Among the primary alcohol compounds, the 50% ET treatment had significantly greater content of four of the six primary alcohol compounds, specifically 1-tetracosanol, 1-hexacosanol, 1-octacosanol, and 1-triacontanol than the 100% ET (Fig. 1). Similarly, the 50% ET treatment had greater content in five fatty acids (eicosanoic acid, docosanoic acid, tetracosanoic acid, hexacosanoic acid, and octacosanoic acid) in comparison with the 100% ET treatment (Fig. 1). Alkanes represented less than 1% of the entire cuticle yet had the greatest number of different compounds (Fig. 1). The majority of alkane compounds showed increases with the 50% ET treatment, but only three were significantly greater: nonacosane, hentriacontane, and tritriacontane (Fig. 1).

Cuticle morphology. Epicuticular crystalloids were found on the creeping bentgrass leaf surface with the underlying cuticle being relatively smooth. The crystalloid shape found on the cuticle layer was irregular membranous platelets also known as primary alcohol plates. The crystalloids were thin with irregular serrated edges and ranged from 1 to 3 μm tall and 1 to 3 μm wide (Fig. 2C). Crystalloid morphology has a direct relationship with chemical composition of the cuticle as crystalloids arise from self-assembly (Bargel et al., 2006; Barthlott et al., 1998; Jeffree et al., 1975). It was found that 91% of the cuticle of creeping bentgrass was composed of primary alcohols and the leaves were covered in epicuticular wax structures. This was expected because a high percentage of alcohols in the cuticle leads to leaves covered in epicuticular wax structures (Jetter et al., 2006). Crystalloid shapes fall under very broad categories and can be slightly modified by the chemical composition of the cuticle (Jeffree, 2006). Creeping bentgrass cuticle irrigated to replace 50% ET had a slight difference in overall shape and increased size (2 to 4 μm tall and 1 to 4 μm wide) of crystalloids compared with cuticles from bentgrass irrigated to replace 100% ET (Fig. 2F). The irrigation treatment also increased crystalloid density for the 50% ET regime compared with the 100% ET (Table 1). The density of epicuticular waxes of tree tobacco has also been demonstrated to increase as a result of periodic drying events (Cameron et al., 2006). Under low air humidity, Koch et al. (2006) reported that crystalloid density increased as well as wax amount, subsequently decreasing the wettability of *Brassica oleracea* plant leaves. Because chemical composition has a direct effect on crystalloid shape, it is postulated that the minor changes in the fatty acids and alkanes resulted in the minor morphological changes in the crystalloid shape. Similar to Koch et al. (2006) finding, an increase in crystalloid density could result in a decrease in leaf wettability, leading to reduced foliar absorption.

^{15}N foliar absorption. Creeping bentgrass irrigated to replace 50% ET had 14% less ^{15}N-labeled urea compared with bentgrass irrigated to replace 100% ET (Table 1). This result is consistent with Bondada et al. (1997, 2001), which found a negative relationship between the amount of cuticle wax and the foliar absorption of ^{15}N-labeled urea on cotton and citrus leaves. Yoshimitsu et al. (2002) using precise silicon wafers found that as surface roughness increased the contact angle of a water droplet increased, thus reducing adhesion. It is postulated a similar response of the increase in total cuticle wax and increase in density and size of cuticle crystalloids of the bentgrass irrigated to replace 50% ET could increase surface roughness, which would subsequently decrease the wettability of the cuticle, leading to decreased absorption of foliar-applied urea observed here.

Table 1. Total percent ^{15}N recovery, total cuticle wax, cuticle wax groups, and crystalloid density for creeping bentgrass leaves as affected by the main effects of irrigation and surfactant.

Main effects	^{15}N recovery (%)	Total wax	Primary alcohols	Fatty acids	Alkanes	Aldehyde	Unknown	Crystalloid density (%)
		($\mu g \cdot dm^{-2}$)						
Irrigation								
100% ET	28.91	23.21	21.34	0.42	0.15	0.82	0.45	43.35
50% ET	24.92	25.93	23.76	0.53	0.20	0.85	0.50	59.37
Surfactant								
Non-surfactant	24.37	24.22	22.16	0.46	0.18	0.79	0.46	N/A
Surfactant	29.47	24.91	22.81	0.50	0.17	0.87	0.49	N/A
ANOVA								
Source of variation								
I	*	*	*	***	**	NS	NS	***
S	**	NS	NS	NS	NS	NS	NS	N/A
I*S	NS	NS	NS	NS	NS	NS	NS	N/A

*Significant at the 0.05 *P* level.
**Significant at the 0.01 *P* level.
***Significant at the 0.001 *P* level.
NS = nonsignificant.
ET = evapotranspiration rate; ANOVA = analysis of variance; NA = not analyzed.

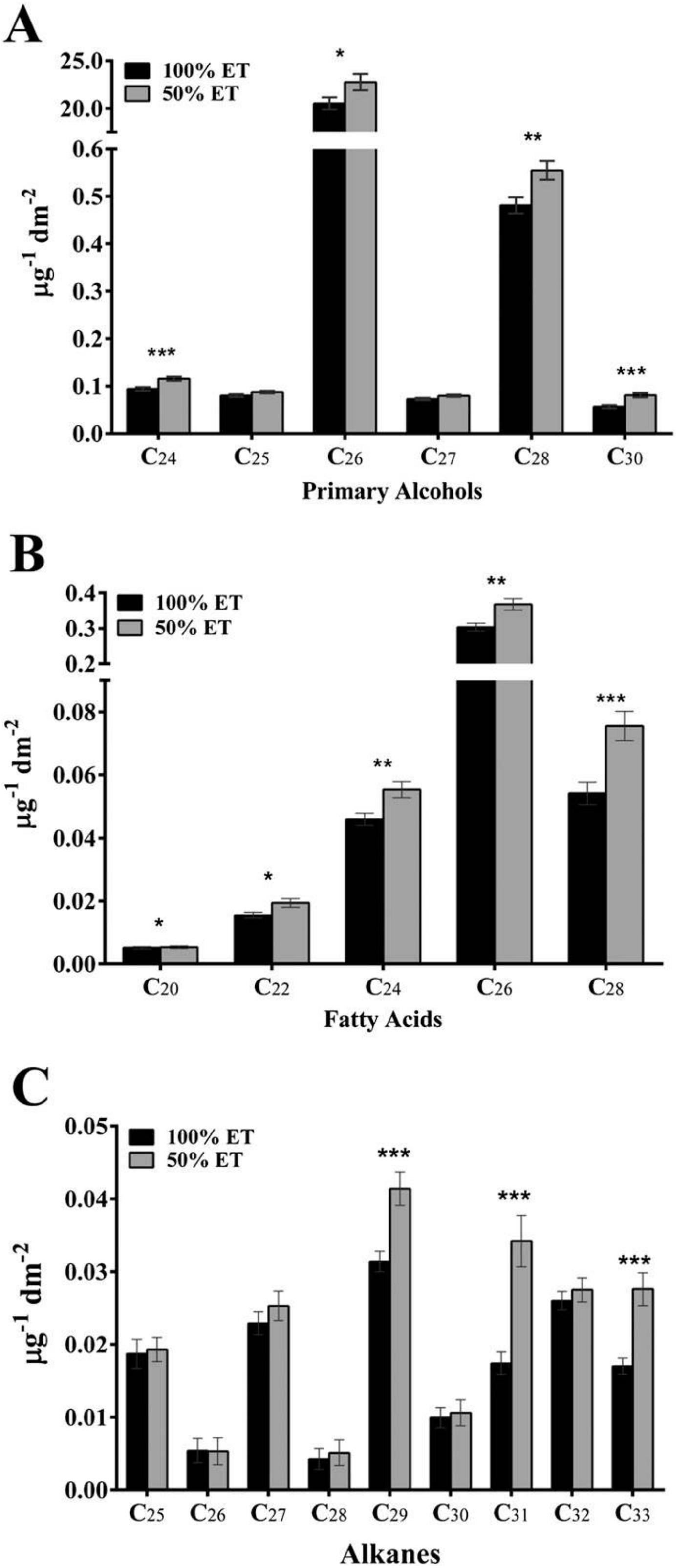

Fig. 1. Creeping bentgrass cuticle wax coverage by irrigation treatment. (**A**) Individual primary alcohols found in the creeping bentgrass cuticle by irrigation treatment. Y-axis break from 0.6 to 17.5 $\mu g^{-1} \cdot dm^{-2}$ (C_{24} = 1-tetracosanol; C_{25} = 1-pentocosanol; C_{26} = 1-hexacosanol; C_{27} = 1-heptacosanol; C_{28} = 1-octacosanol; C_{30} = 1-triacontanol). (**B**) Individual fatty acids found in creeping bentgrass cuticle by irrigation treatment. Y-axis break from 0.09 to 0.20 $\mu g^{-1} \cdot dm^{-2}$ (C_{20} = eicosanoic acid; C_{22} = dococanoic acid; C_{24} = tetracosanoic acid; C_{26} = hexacosanoic acid; C_{28} = octadecanoic acid). (**C**) Individual alkanes found in creeping bentgrass cuticle by irrigation treatment (C_{25} = pentacosane; C_{26} = hexacosane; C_{27} = heptacosane; C_{28} = octacosane; C_{29} = nonacosane; C_{30} = tricontane; C_{31} = hentriacontane; C_{32} = dotriacontane; C_{33} = tritriacontane) *, **, *** denote significant differences $P <$ 0.05, 0.01, and 0.001, respectively.

Another possible cause of decreased foliar absorption is the stomatal aperture being reduced as a result of water stress in the 50% ET treatments. Using nanoparticles, Eichert et al. (2008) found penetration through the stomata of leek (*Allium porrum* L.) and broad bean (*Vicia faba* L.) using confocal microscopy. Eichert and Goldbach (2008) found uptake of NH_4^+ or NO_3^- with ^{13}C-labeled sucrose was significantly affected by stomatal aperture. In another study, Eichert and Burkhardt (2001) determined that not only stomatal aperture, but stomatal density had a significant effect on foliar uptake. Reduced foliar absorption documented from bentgrass irrigated to replace 50% ET could partially be attributed to stomatal closure in a plant response to conserve water. Additionally, plants reduce photosynthesis rates when under water stress, which could lead to a depressed sink for N in the 50% ET treatments. Foyer et al. (1998) and Reguera et al. (2013) found water stress reduced photosynthesis resulting in a reduction of carbon and N assimilation in rice (*Oryza sativa japonica* 'Kataake') and maize plants, respectively.

The addition of a surfactant to the urea solution increased ^{15}N recovery by 21% in creeping bentgrass leaves compared with the no surfactant treatment (Table 1). An increase of foliar uptake of chemicals when surfactants are applied in conjunction with the chemical has been well documented (Fernandez et al., 2006; Howard and Gwathmey, 1994; Liu, 2004; Neal et al., 1990). Fernandez et al. (2006) observed a reduction in surface tension and increase in iron concentration in peach when a surfactant was added to the solution. Liu (2004) determined that adding surfactants to the solution increased glyphosate and 2,4-D uptake in broad bean, wheat, and lambsquarter (*Chenopodium album* L.). Foliar uptake of potassium in field-grown cotton was greater when a surfactant was added (Howard and Gwathmey, 1994). In comparison, Neal et al. (1990) documented that adding a surfactant did not increase control of crabgrass (*Digitaria ischaemem* Schreb.) in cool-season turfgrass under well-irrigated conditions, but that efficacy improved with a surfactant where irrigation was limited. In the present study, applying the nonionic surfactant increased foliar absorption of urea for both of the irrigation treatments (Table 1).

Conclusions

More frequent and intense droughts have resulted in water restriction to non-essential uses of water, including irrigating turfgrasses. As a cool-season plant, creeping bentgrass requires more water than warm-season turfgrasses (McCarty, 2011). It is hypothesized that creeping bentgrass would be less tolerant of acute moisture stress if water restrictions were implemented. A better understanding of the response of creeping bentgrass under reduced water conditions and

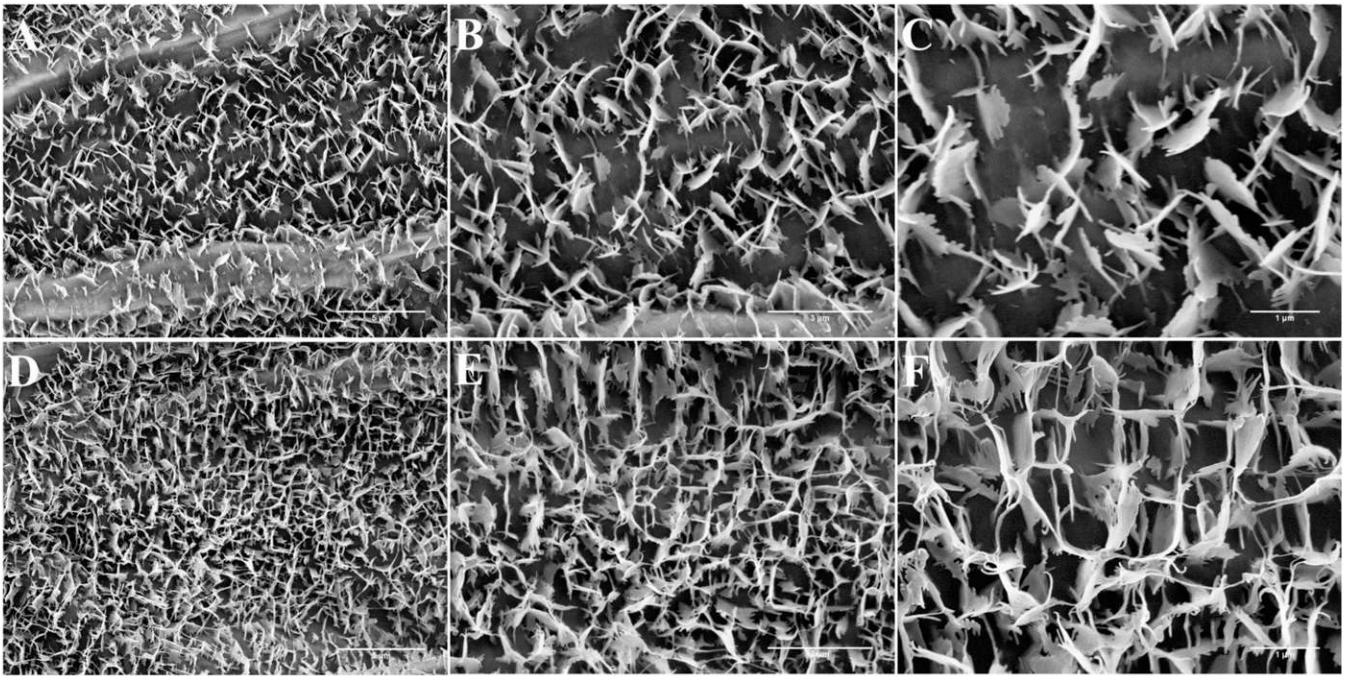

Fig. 2. Scanning electron micrographs of creeping bentgrass cuticle layer under irrigation regimes. (**A–**C) Cuticle morphology of 100% evapotranspiration rate (ET) treatment at 5,000, 10,000, and 20,000 ×, respectively. (**D–F**) Cuticle morphology of 50% ET treatment at 5,000, 10,000, and 20,000 ×, respectively.

identifying enhanced management options would allow for the development of techniques to ensure a healthy, high-performance, and aesthetically pleasing sward. Limiting irrigation to replacing 50% ET significantly increased total cuticle wax, density, and size of cuticle crystalloid structures on creeping bentgrass. Foliar uptake of ^{15}N was significantly decreased as a result of the reduced irrigation treatment, which could be attributed to modifications in the cuticle layer making it less receptive to foliar-applied solutions. The evidence provided in this study suggests that adding a surfactant aided uptake of urea, even at 50% ET level, and it could be considered a good practice to include a surfactant when foliar feeding mildly water-stressed turf. Recommendations include applying solutions when bentgrass is being irrigated to replace 100% ET or adding a nonionic surfactant to a urea solution to improve uptake efficiency when not being irrigated to replace 100% ET. Future research should investigate different irrigation regimes and different abiotic stresses such as heat and water quality, longer experimental periods, and different surfactant chemistries.

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