ABSTRACT

SERMONS, SHANNON MICHELLE. Abiotic Resource Allocation by Turfgrass Plants During Times of Transition or Stress. (Under the direction of Dr. Thomas Rufty).

Understanding how plants respond to their environment and the inputs that are necessary for growth are foundational principles of agriculture. In the turfgrass transition zone, which includes North Carolina, cool-season grasses like tall fescue (*Festuca arundinacea* Schreb.) experience heat and drought stress during the summer months, while warm-season grasses like common bermudagrass (*Cynodon dactylon* (L.) Pers.) face specific challenges associated with returning to active growth following winter dormancy. These environmental stresses affect management strategies and input requirements. In this dissertation, three chapters examine the intersection between environmental stresses and management inputs in turfgrasses, with specific attention to the transition-zone concerns of heat and drought stress in cool-season tall fescue, and winter dormancy and nitrogen issues in post-dormancy warm-season bermudagrass.

Two sets of experiments examined factors influencing water requirements by tall fescue. First, two environmental factors, temperature and vapor pressure deficit (VPD), were evaluated for their roles in influencing physiological control over transpiration. Temperature and VPD were found to interact; plants could mitigate water loss at high VPD when temperature was optimal, but high temperatures impaired the regulatory mechanism. Some acclimation was seen after extended exposure to harsh conditions. Then, the relationship between growth and VPD in influencing transpiration was explored. Again, VPD proved to be the dominant factor in water loss.

A third experiment examined nitrogen pools, and the roles of internal and external nitrogen sources, in bermudagrass as it emerged from dormancy. Because nitrogen is integral for plant growth, it well may be a limiting factor for bermudagrass growth in spring. The most important internal reserve of nitrogen was in the stolon tissue; nitrogen emptying from stolons was adequate to drive shoot growth for about two weeks post-dormancy. Roots were active almost immediately. Pre-existing roots were able to take up fertilizer nitrogen within a few days. New roots, though small in size, had a much higher nitrogen uptake rate than did the older roots and were able to take up large quantities of nitrogen by the second week post-dormancy.

Abiotic Resource Allocation by Turfgrass Plants During Times of Transition or Stress

by
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A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Crop Science

Raleigh, North Carolina

2014

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DEDICATION

This effort is dedicated to my wonderful family, especially my husband, Brent McCraven, and my parents, Bill and Linda Sermons, for always believing in me.

BIOGRAPHY

Shannon M. Sermons, originally from Plymouth, North Carolina, is a graduate of the North Carolina School of Science and Mathematics and The Florida State University. She also holds an M.S. in Crop Science from North Carolina State University. Shannon works as a research technician for the USDA Agricultural Research Service. Shannon and her husband, Brent McCraven, live in Raleigh with their furry kids and are expecting the arrival of their first human child in November 2014.

ACKNOWLEDGMENTS

This academic pursuit has been supported and influenced by many friends and colleagues. Brent McCraven has been the best husband imaginable, helping with whatever needed doing- helping me with taking measurements and recording data, keeping our household functional, and being my sounding board. My fellow students, especially Laura Vance, Dr. Tom Seversike, and Dr. Mandeep Riar, have sparked many lively discussions and helped so much with brainstorming, proofreading, and double-checking calculations. Dr. Tom Sinclair's students, postdocs, and visiting scientists asked the hard questions in our many lab meetings, making us all dig for the truth. The fabulous faculty at NCSU has provided a deep and wide intellectual experience; some of the most stellar among these educators are Dr. Cavell Brownie, Dr. Dean Hesterberg, Dr. Paul Murphy, Dr. Steve Spiker, Dr. David Shew, and Dr. Heike Winter-Sederoff. Any student having the opportunity to learn from any of these excellent professors should do so (and soon- some have already retired!). The Crop Science department has some really great people; I think especially of the several directors of graduate programs who have served during my years as a graduate student- Dr. David Danehower, Dr. Randy Wells, and Dr. Dan Bowman- and the graduate secretaries, and Mrs. Kathy Kelly who knows how to get things done.

Finally, I thank my committee members Dr. Tom Sinclair, Dr. Bill Hoffmann, and Dr. Ed Fiscus, for their diverse perspectives, and my committee chair, Dr. Tom Rufty, for pushing everyone around him to look at the "big picture".

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INTRODUCTION

Over the thousands of years that humans have intentionally managed plant growth, various strategies have been employed in agriculture and horticulture to improve yield and quality. One of these strategies is selecting species, and genotypes within each species, that are well suited to the climate where they are grown. Another main strategy is providing inputs that encourage optimal growth. As these strategies have been further studied, the field of plant breeding has advanced the science of variety development. Simultaneously, the fields of agronomy and plant physiology have defined the inputs that are necessary for plant growth, discovering that the most-frequently-limiting components, those that are required in the largest amounts, are water and nitrogen (Haynes, 1986; Sinclair and Sinclair, 2010)

These basic principles, selecting varieties suited for their environment and providing adequate inputs, are still foundational to plant management. This dissertation specifically explores the intersection of environment and management in turfgrass. Bell (2011) observed that management of turfgrass is easiest when the type of turf is well-matched to the climate where it is grown. When the match is poor, management becomes more complex and managers must supply the components that are naturally lacking. In principle, the obvious solution would be to simply choose the correct variety for the climate and allow nature to do all the work. However, in practice, a perfectly-suited variety for a given environment does not always exist. Further complicating the matter, weather is not constant. In addition to the obvious seasonal variations, longer-term climate changes are causing shifts in plant hardiness zones. The difficulties in matching variety with climate are highly evident in the turfgrass transition zone of the mid-latitude United States.

The transition zone between warm/humid and cool/humid climate zones is one of the most challenging regions for turf management (Fry and Huang, 2004), which can easily be understood based on the different growth habits of turfgrass types. Cool-season species, which typically have C3 carbon fixation, perform well in northern regions of the US. Warm-season species, typically C4 types, grow well in the southern US. The C4 metabolic pathway is often found in plants that are adapted to warm, dry climates because they use the efficient enzyme PEP carboxylase to initially bind CO_2, sequestering Rubisco in bundle sheath cells where wasteful photorespiration (which can be especially damaging in high temperatures) is less likely to occur (Sage, 2004). The transition zone is well-suited to neither turfgrass type, causing heat stress and drought symptoms in cool-season grasses during the summer, and low-temperature stress in warm-season grasses during the winter. Some species within each type can perform in the transition zone, but they must be carefully managed to mitigate stresses associated with growing in a marginally-suited climate. North Carolina is squarely in the transition zone, at the northern edge of the warm humid region, bordering on the cool humid region.

Like other cool-season turfgrasses, growth of tall fescue (*Festuca arundinacea* Schreb.) declines as temperatures approach 25°C, but tall fescue is less damaged by heat and drought than most cool-season turfgrasses (Burns and Chamblee, 1979; Hill et al., 1985). Older, pasture-type varieties such as Kentucky-31, which was released in 1943, are more robust against these stresses than newer, finer-textured, turf types. Thus, the pasture types remain popular in turf applications (Bell, 2011; Fry and Huang, 2004; Meyer and Watkins, 2003).

A large part of fescue's heat and drought resistance may actually be due to avoidance mechanisms associated with an extensive root system. Deep rooting allows access to greater reserves of water, which directly decreases the exposure to water limitations and places the temperature-sensitive roots in cooler portions of the soil profile. Access to additional water also indirectly counteracts heat stress by facilitating transpirational cooling of the shoots. Still, tall fescue suffers during North Carolina summers (Spak et al., 1993), where daytime temperatures typically exceed 30°C. One reason may be that deep rooting is obstructed by the highly acidic soils common to this area, which can be below the optimal pH range of tall fescue, especially in the deeper layers (Foy and Murray, 1998). Thus, the primary mechanism of drought avoidance by production of deep root systems would not be functional.

An alternative approach to increasing fescue drought avoidance could be management based. Some work has suggested that drought tolerance of turfgrasses can be enhanced by application of plant growth regulators such as trinexapac-ethyl (e.g. Jiang and Fry, 1998; McCann and Huang, 2007). While it is logical that a reduction in growth would concurrently decrease evapotranspiration, results have been mixed, with some experiments indicating lower water use following application of TE, but others finding no change (Ervin and Koski, 2001; Wherley and Sinclair, 2009b). Clearly, despite improvements in development of varieties and management tools, the performance of cool-season tall fescue in the warm southeastern summers continues to be challenged by environmental stressors of high temperature and limited water.

Of the turf-type warm-season grasses, common bermudagrass (*Cynodon dactylon* (L.) Pers.) has better cold tolerance than most. Though many varieties of warm-season grasses

can survive winters in the transition zone, they do not grow and thrive year-round, instead experiencing a dormant period as temperatures approach freezing (Taliaferro, 2003). Leaves senesce in autumn and the remaining stolon, rhizome, node, and root tissues maintain a low level of activity until spring, when regrowth begins.

The seasonal nature of bermudagrass growth presents management challenges, especially in proper application of nitrogen fertilizer. Nitrogen is the most-limiting mineral nutrient, being necessary for building proteins, nucleic acids, and chlorophyll (Fry and Huang, 2004). Southeastern soils are naturally N-deficient, so nitrogen must be applied regularly to facilitate growth of new tissues. At the same time, over-application can pose an environmental danger for water quality due to leaching and runoff. Clearly, proper nitrogen nutrition is very important. This is especially true, and especially difficult to implement, during bermudagrass's seasonal transition from dormancy to active growth. Nitrogen is needed to drive the progression to rapid growth but uptake capacity is limited.

In this dissertation, three chapters examine the intersection between environmental stresses and management inputs in turfgrasses, with specific attention to the transition-zone concerns of heat and drought stress in cool-season tall fescue, and winter dormancy and nitrogen issues in post-dormancy warm-season bermudagrass.

Chapter 1. Temperature Influences the Ability of Tall Fescue to Control Transpiration in Response to Atmospheric Vapor Pressure Deficit.

Vapor pressure deficit is one of the main environmental drivers of water loss from plant leaves. The ability of plants to control water loss during periods of high atmospheric demand

(high VPD) is an important physiological mechanism for efficiently using limited water supplies during drought. Some C3 grasses have been shown to have this ability (Sinclair et al., 2007; Wherley and Sinclair, 2009c), presumably due to stomatal closure at high VPD. However, evidence from experiments in wheat and barley indicates that high temperature may inhibit the VPD response (Bunce, 2000).

To address the question of whether temperature modifies the ability of tall fescue to conserve water during times of high atmospheric demand (i.e. high VPD), the separate roles of temperature and VPD in driving evapotranspiration were explored through two sets of experiments. First, factorial experiments allowed independent manipulation of both temperature and VPD and determination of their impact on transpiration, and then sequential application of environmental changes allowed characterization of potential plant acclimation and adjustments in evapotranspiration controls. This chapter was published in Functional Plant Biology 2012, 39:979-986.

Chapter 2. Assessing Transpiration Estimates in Tall Fescue: The Relationship Between Transpiration, Growth, and Vapor Pressure Deficits.

Many approaches have been used to estimate water requirements of grasses. One that appears promising is based on the relationship between growth and water loss, which relies on the physics of gas exchange through the stomatal pore: influx of carbon dioxide for growth is paired with the concurrent efflux of water vapor (Sinclair et al., 2014). This relationship is of interest not only because of the utility of predicting water requirements, but also because of

the possibility for reducing water requirements by using management techniques to slow turf growth.

These experiments addressed the questions of whether shoot growth of tall fescue was related with its water use, and whether evapotranspiration could be decreased by intentionally slowing shoot growth. Growth of tall fescue was manipulated by application of the plant growth regulator trinexapac-ethyl, lowering of nitrogen fertility, and exposure to different temperature regimes. In addition, the growth manipulation treatments were applied in hydroponics to more precisely assess the potential effects on growth of all plant parts.

Chapter 3. The Role of Internal and External Nitrogen Pools in Bermudagrass Growth During Spring Emergence from Dormancy.

Adequate nitrogen supply is critical for growth of bermudagrass, but over-application can be environmentally damaging, so matching supply with demand is important for maximizing plant health and minimizing negative environmental impacts (Barton and Colmer, 2006). Applying this principle can be challenging due to the seasonal winter dormancy of bermudagrass. Nitrogen applied to dormant bermudagrass is highly susceptible to leaching because, without active growth, only about 13% of that applied is taken up (Wherley et al., 2009a). In contrast, actively growing bermudagrass during the late spring (coupled with the closely-associated soil microbial population) absorbed over 80% of applied nitrogen over a similar 2-week time period. The capacity of the bermudagrass system to take up nitrogen during the transition from dormancy to vigorous growth has not been determined. Post-dormancy fertilization strategies have emerged for bermudagrass over the past two decades,

but the physiological basis for their validity is missing. Post-dormancy nitrogen relations are extremely complicated. Internal nitrogen reserves may be an important nitrogen source for growth processes, as can nitrogen taken up from the rhizosphere as the uptake capacity increases.

The purpose of experiments in Chapter 3 is to more clearly outline the roles of internal nitrogen pools and newly acquired (fertilizer) nitrogen in growth of bermudagrass as it emerged from dormancy over time. Regrowth of dormant bermudagrass was observed in carefully controlled conditions, with or without exposure to ^{15}N isotopically-labelled nitrate fertilizer, and destructive harvests over time allowed determination of growth and nitrogen partitioning in all tissues. The results are coupled with those of experiments conducted by collaborator Dr. Ben Wherley, which specifically examined uptake capabilities of old and young developing roots in the post-dormancy period.

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CHAPTER 1

Temperature Influences the Ability of Tall Fescue to Control Transpiration in Response to Atmospheric Vapor Pressure Deficit

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Abstract. Water availability for turfgrass systems is often limited, and likely to become more so in the future. These experiments examined the ability of tall fescue (*Festuca arundinacea* Schreb.) to control transpiration with increasing vapor pressure deficit and whether control was influenced by temperature. The first studies were in steady-state conditions at two temperatures (21°C and 27°C) and two vapor pressure deficits (1.2 and 1.8 kPa). At the lower temperature, water use was similar at both VPDs, indicating a restriction of transpiration at high VPD. At 27°C, transpiration control at high VPD was weakened, and root growth also declined; both responses increase susceptibility to water-deficit stress. Another series of experiments examined the physiological stability of the transpiration

control. Temperature and VPD were adjusted in a stepwise manner and transpiration measured across a range of VPD in the days following environmental shifts. The results indicated that VPD control acclimated to the growth environment, with adjustment to drier conditions becoming evident after about 1 week. Control was again more effective at cool than at hot temperatures. The results, collectively, indicate that transpiration control by this cool season grass is most effective in the temperature range where it is best adapted.

Additional keywords: drought, evapotranspiration, K_c

INTRODUCTION

Water availability has become one of the most critical issues in management of turfgrass systems. Many factors associated with turfgrass water use remain ambiguous, none more than the ability of plants to control water losses. Understanding plant controls is an essential element in the selection and development of drought-tolerant turfgrass varieties.

One of the most obvious drivers of water loss from plants is the humidity status of the surrounding atmosphere. The evaporative potential of any surface, including the plant leaf, is determined by the vapor pressure deficit (VPD) of the air around it. The importance of VPD has been recognized since the early attempts to predict evapotranspiration (ET) rates, as shown by inclusion of VPD or both temperature and air water content (represented by measurements of relative humidity, vapor pressure, or dewpoint temperature) in ET equations (Penman 1948; Tanner and Pelton 1960; Fritschen 1965). An important aspect of

VPD that was overlooked, however, is that it may trigger physiological mechanisms in plants that control transpiration and thus water loss from the turfgrass system.

The primary gateways for water release from plants are the stomata embedded in the leaf surface. During times of high atmospheric VPD, when transpiration potential exceeds the ability of plants to supply water to leaves, water loss can be mitigated by closing stomata. Recent research indicates that plants can have different sensitivities to increasing VPD; some exhibit little control, while others strongly inhibit transpiration as VPD increases. Plant VPD response differences have been demonstrated among genotypes in some crop species (Fletcher *et al.* 2007; Sadok and Sinclair 2009; Devi *et al.* 2010; Gholipoor *et al.* 2010; Fiscus *et al.* 2012) and are viewed as a key in the development of drought-tolerant varieties.

Recent evidence indicates that cool-season (C_3) turfgrasses may possess a VPD-sensitive water control mechanism. In a study of tall fescue (*Festuca arundinacea* Schreb.), transpiration did not increase when VPD increased (opposite to the result that would be predicted by calculations of ET) and growth decreased (Sinclair *et al.* 2007). Other experiments demonstrated stable transpiration rates in several C_3 grasses with stepwise increases in VPD (Wherley and Sinclair 2009). The absence of increases in transpiration in the high-VPD environments suggest stomatal closure, with the restriction of leaf gas exchange in high-VPD environments also limiting carbon dioxide intake and growth (Sinclair *et al.* 2007).

An important unknown in the plant VPD response is the influence of temperature. Because the highest VPD conditions often occur in hot summer months, an ideal drought tolerance trait would remain engaged as temperature increases. If not, the ability of plants to

control water loss would be compromised at the time when it would be most needed. It is difficult to sort out interactions between plant VPD responses and temperature from existing literature, because interactions are often confounded experimentally. Indeed, temperature is sometimes adjusted to achieve a range of VPD conditions (Ebdon *et al.* 1998; Al-Faraj *et al.* 2001). But in one series of experiments with the C_3 grasses wheat and barley, stomatal conductance decreased as VPD increased at low temperatures but not at high temperatures (Bunce 2000, refer to ambient CO_2 treatment), which suggests that temperature can affect the operation of the VPD response mechanism.

A main purpose of this study was to investigate the effect of VPD on transpiration by tall fescue and determine whether the response was modified by high temperature. The experiments were conducted in two climate controlled systems: outdoor plant environment chambers with sustained control of temperature and humidity and an indoor system that allowed stepwise, independent adjustment of VPD and temperature in the growth environment. The relationship between transpiration and growth was also examined in the outdoor chamber experiments. While some effects on growth would be expected from changes in leaf gas exchange in response to VPD, temperature may also independently affect growth processes.Specifically, high temperatures have been observed to reduce root growth of cool-season grass (Jiang and Huang 2000), and less-extensive rooting is known to impair performance of turfgrasses when water availability is limited (Qian *et al.* 1997; Ebdon and Kopp 2004; McCann and Huang 2008).

MATERIALS AND METHODS

Transpiration by tall fescue was first assessed while conditions were held constant for several weeks in a factorial experiment, in which two levels of VPD and two temperatures (for a total of four VPD * temperature treatments) allowed for differentiation of individual and interacting effects. In addition, acclimation of transpiration control was examined at intervals after altering the VPD and/or temperature of the growth environment. Transpiration control, which is the restriction of water loss at high VPD, was determined at each time interval by measuring transpiration during short exposures to different VPD. These short exposures may be thought of as snapshot "VPD challenges".

Long-term transpiration and growth

Tall fescue (*Festuca arundinacea* Schreb.) cv. 'Kentucky-31' was seeded at a rate of 58.6 g m^{-2} into 22.5-cm diameter pots containing approximately 6 L of sandy loam field soil. The pots were located in a greenhouse at the Method Road facility at North Carolina State University for a seven-week establishment period, during which they were clipped once or twice per week, fertilized weekly with a complete nutrient solution, and preventatively treated once with azoxystrobin fungicide (Heritage TL, Syngenta Crop Protection, Greensboro, NC). After the establishment period, the pots were moved to the USDA-ARS Plant Science Research Unit field site at Inwood Road in Raleigh, NC, where they were placed in eight small (2.44 m x 1.52 m) temperature- and humidity-controlled Outdoor Plant Environment Chambers (OPECs) (Flowers *et al.* 2007). The OPECs were programmed for four different combinations of temperature and vapor pressure deficit (VPD), with two

chambers at each condition: 21°C & 1.2 kPa (52% relative humidity), 21°C & 1.8 kPa (28% RH), 27°C & 1.2 kPa (66% RH), 27°C & 1.8 kPa (50% RH). Three pots were placed into each chamber, for a total of six pots per environmental treatment. The turf was maintained in these environments for four weeks prior to the measurement period.

At the beginning of the measurement period, each pot was watered well and allowed to drain overnight, then placed into another pot lined with a plastic bag to prevent drainage. Thereafter, only measured quantities of water were added, in amounts equivalent to evapotranspiration (determined gravimetrically). To maintain consistent nutrient availability, dilute soluble liquid fertilizer was added twice weekly during the measurement period.

Turf was trimmed with hand shears to a height of 6.4 cm at three- to six-day intervals during the 27-day measurement period. Clippings from each pot were collected, dried, and weighed. After the final trimming, shoot tissue remaining below clipping height (verdure) and root tissue were collected, dried, and weighed.

Data were analyzed using Proc GLIMMIX in SAS (SAS Institute, Cary, NC). In some cases, significant VPD*temperature interactions were found; where that occurred, comparisons were made based on VPD effects within each temperature level and temperature effects within each VPD level. Where no VPD*temperature interaction was found, the main effects of VPD and temperature are reported (Table 1).

Acclimation of transpiration controls

Tall fescue cv. 'Kentucky-31' was seeded into cylindrical pots of 10-cm diameter and 30-cm height, using the same seeding rate and field soil as in the initial experiments in the OPEC

chambers. A ceramic cup was placed inside the bottom of each pot, surrounded with a layer of diatomaceous earth and connected to a vacuum pump by a tube through the pot wall. Periodically, a vacuum of about 20 kPa was applied, ensuring adequate drainage and minimizing the likelihood of anaerobic effects. The lip of each pot was a flange that allowed for attachment of the micro-environment chambers during measurement periods. The pots were located in a growth room at the Southeastern Plant Environment Laboratory at North Carolina State University for a 12-week establishment period, during which they were clipped and fertilized with a complete nutrient solution once or twice per week. Approximately 600 to 700 µmol m^{-2} s^{-1} of light was provided by fluorescent and incandescent bulbs. During the initial growth phase, the growth room was maintained at 21°C and a VPD of ~ 0.75 kPa. Later in the experiment, new growth conditions were established by changing the temperature of the growth room and adjusting VPD with a dehumidifier (model GD55S, Fantech, Lenexa KS): the second growth condition was 21°C, 1.8 kPa, and the third was 29°C, 3.0 kPa (see timeline in Fig. 5).

Evapotranspiration was measured during 60- to 90-minute periods on individual days spaced throughout the several months of growth. Before the first measurement day, a layer of gravel was placed on the soil surface to reduce evaporation, and on the day prior to each measurement date the fescue was clipped to a 5 cm height, fed with nutrient solution, watered thoroughly, and drained overnight by engagement of the vacuum.

On each measurement day, the micro-environment chambers were affixed to the pots. Each of the 6-L capacity clear plastic chambers contained a temperature and humidity sensor/logger and a small fan, and was attached to a tube providing air flow from a

compressor. Different VPD measurement ranges were achieved by varying air flow rate into each chamber and by placing a tube of desiccant in the air line. Each VPD condition was applied for a 30- to 60-minute adjustment period, then pots were weighed before and after a further 60- to 90-minute exposure to that condition to determine water loss, i.e. transpiration. Two to three different VPD ranges were tested on 12 pots on each measurement day. After the day's measurements, the micro-environmental chambers were removed and the pots were returned to their growth environment. Measurements were generally conducted within 2°C of the growth condition, except for the last set of conditions where measurements were made at a cooler range (see timeline in Fig. 5 for timing of growth conditions and measurements).

Data from two to three measurement days were combined. Analysis was similar to that previously reported for transpiration-vs.-VPD measurements (Fletcher *et al.* 2007), with data grouped into clusters, each containing four data points, and the mean transpiration and VPD of each cluster used for regression of a two-segment model using GraphPad Prism (GraphPad Software Inc., San Diego, CA).

RESULTS

Long-term transpiration and growth

In the OPECs, water loss and growth were measured over several weeks at two temperatures and two VPD levels. If the direct physical effect of the VPD treatments on water diffusion from the leaf were predicted (i.e., a state with no mechanisms for plant control over water loss from plant leaves), plants exposed to 1.8 kPa would lose 50% more water than those at 1.2 kPa due to the proportional relationship between transpiration and the leaf-atmosphere

vapor pressure differential (Lambers *et al.* 2008). However, this was not the observed result; instead, at the cooler temperature of 21°C (Fig. 1a), plants at high VPD had a small, but statistically insignificant, reduction in water loss versus those at low VPD, clearly indicating control of transpiration. At the higher temperature of 27°C (Fig. 1b), high VPD led to greater water loss. But, the loss was only 23% more at 1.8 kPa than at 1.2 kPa or about half of the predicted increase. Thus, while the water loss indicated some control over transpiration at the higher temperature, it was less effective than that at the cooler temperature.

A restriction of water loss with increasing VPD typically is correlated with a reduction in stomatal conductance, which would depress water vapor loss, but also carbon dioxide influx. For that reason, growth might be expected to be affected similarly to water loss. The effects of VPD and temperature on growth were measured by collecting clippings over time and by collecting all plant tissues at the experiment's conclusion. In the case of clipping dry mass, a suppression of growth appeared to occur. At the cool temperature, where water restriction was evident at 1.8 kPa, clipping mass was reduced by 57% at 1.8 kPa compared with 1.2 kPa (Fig. 2a). At higher temperature, where less water restriction was observed, clipping dry mass was not depressed by VPD (Fig. 2b). However, upon examination of root and verdure mass, a different effect of VPD and temperature on growth becomes apparent. Both root and verdure had similar or even slightly greater mass at 1.8 kPa compared to 1.2 kPa in both temperatures (Fig. 3). Note, verdure and root mass are much larger than clipping mass in part because they were collected at the conclusion of the experiment, thus their masses include tissue produced during the establishment, acclimation, and measurement periods (a total of seven weeks before environmental treatments were

imposed and eight weeks after); clippings were discarded during the establishment and acclimation periods, so only clippings produced during the four-week measurement period are represented (Fig. 3). However, taking all growth data under consideration, the results gave no indication that a reduction in growth occurred at the higher VPD; if anything, there appeared to be a slight (but not statistically significant) increase.

Because tall fescue is a cool-season turfgrass, the effect of temperature on transpiration and growth is also of interest, separate from VPD. The effect of temperature on transpiration can be seen in comparisons between temperatures within each VPD treatment. When VPD was low, temperature had no significant effect on water loss, with the 27°C treatment having just 8% more water loss than the 21°C treatment (compare 1.2 kPa treatment between Fig. 1a and 1b and see temperature effect in Table 1), but when VPD was high, water loss was 42% higher at 27°C than at 21°C (Table 1, $P<0.01$; also compare 1.8 kPa treatment between Fig. 1a and 1b). Both shoot growth and root growth were affected by temperature. Clipping mass increased with higher temperature in both VPD treatments, with a 19% increase at 1.2 kPa and 182% at 1.8 kPa. All other growth parameters were decreased by high temperature. Verdure was 8 and 9% lower in the high-temperature treatment at 1.2 and 1.8 kPa respectively, and root mass was decreased by 44 and 36%, which was largely responsible for a 33 and 26% reduction in total plant mass at the higher temperature (Table 1; comparisons between Fig. 3a and 3b).

Acclimation of transpiration controls

Tall fescue plants were exposed to a sequence of VPD and temperature conditions to determine whether the growth environment influenced their ability to respond to VPD, and whether transpiration controls were adjusted when the growth environment changed. The measurements were conducted in individual micro-environment chambers that allowed measurement of transpiration over a range of VPD. As demonstrated in the example in Figure 4, higher VPD in the measurement chamber generally correlated with greater water loss from plants. The relationship between VPD and transpiration, however, often was not constant. At low VPD, each unit of increase in VPD yielded a larger increase in transpiration than occurred in the higher VPD range (refer to slope A vs. slope B; Fig. 4). To estimate the extent of the adjustment in transpiration control, the ratio of slope B to slope A can be calculated and the VPD of the breakpoint between the two segments determined. A lower ratio indicates more effective control over transpiration at high VPD and a lower breakpoint indicates that transpiration begins to be restricted at a lower VPD.

In these experiments, adjustments in transpiration responses to VPD were followed as plants were exposed to a series of growth environments (Fig. 5, timeline & a-f). When plants had been exposed to cool, very humid conditions (VPD 0.75 kPa) for an extended period of time, they exhibited little control over transpiration when challenged with increasing VPD in the micro-environment chambers (Fig. 5a, g). After one to four days of exposure to a drier growth condition (VPD 1.8 kPa), the response to VPD had changed little (Fig. 5b, g). But, during the second week of exposure, a restriction of transpiration became evident when plants were challenged with the increasing VPD, with a severe drop in slope occurring as

VPD increased above 1.45 kPa (Fig. 5c, g). Statistical analyses confirmed that a two-segment model was not significantly different than a linear model during the initial four days of exposure to dry conditions ($P>0.05$), but it was significant in the second week when the slope at high VPD declined sharply ($P<0.001$). This led to a corresponding decline in the calculated slope ratio.

Then, plants were shifted into a warm environment of 29°C with an even higher VPD (3.0 kPa). Within the first week of exposure, the slope ratio increased somewhat and the breakpoint increased noticeably to 1.92 kPa, indicating less effective control over transpiration (Fig. 5d, g). By the next week, the parameters had changed slightly, with breakpoint reduced to 1.84 kPa and slope ratio reduced only a little. When the plants were then subjected to a VPD challenge at a cool temperature (23°C), very effective transpiration control was evident (5f). A similar slope ratio was maintained as before, but the breakpoint (i.e. sensitivity to VPD) dropped to 1.25, the lowest value measured in any of the conditions. Other runs of the experiment have shown that the strong VPD-sensitive response seen in a cool-temperature VPD challenge with plants acclimated to a warm, dry growth environment (similar to Fig. 5f) was lost after the plants were placed in a cool, low VPD growth environment for several days. The VPD response profile then returned to that observed with cool, low VPD-acclimated plants (data not shown; refer to Fig. 5a).

DISCUSSION

These experiments examined tall fescue that was supplied with adequate water so that the effects of vapor pressure deficit and temperature on water loss from leaves could be isolated.

The results are generally consistent with those from numerous recent studies showing that plants can control transpiration in response to VPD (Fletcher *et al.* 2007; Sadok and Sinclair 2009; Devi *et al.* 2010; Gholipoor *et al.* 2010). Our results provide two new insights into plant regulation of transpiration. The first is that the VPD responses are strongly influenced by temperature, with controls less effective in high temperature ranges. The second insight is that the plant transpiration responses to high VPD are dynamic and not a fixed or stable physiological property. Plants appeared to acclimate to the VPD of the growth environment.

The temperature dependence of transpiration control was obvious in the longer-term experiments. When the growth temperature was 21°C, despite a VPD increase of 50% to 1.8 kPa, water loss was nearly identical to that at 1.2 kPa. In contrast, at 27°C transpiration control was present, but it was noticeably less effective and water loss was 23% greater at 1.8 than at 1.2 kPa. And in the shorter-term growth room experiments, sensitivity to VPD decreased (increasing 'breakpoint') when plants were moved from 21 to 29°C and increased (lower 'breakpoint') when plants were moved from 29 to 23°C (Fig. 5).

Collectively, the responses to VPD indicated that control over transpiration was most effective when fescue was in its optimal temperature range. Cool-season grasses have long been known to exhibit optimal growth in the range of 15-23°C (see classic review by Beard 1973). This cool temperature adaptation was borne out in our longer-term experiments where growth was measured, as plants were considerably larger at 21 than 27°C (Fig. 3).

Less effective regulation over transpiration and more rapid water movement through plants at higher temperatures implies that cooling and the protection of heat sensitive metabolic processes becomes a physiological priority. The role of transpiration in plant

cooling is a basic tenet in plant physiology, of course (Nobel 1974), and it has been referred to as an important physiological mechanism of turfgrasses at high temperatures (Feldhake *et al.* 1984; Bonos and Murphy 1999). From this perspective, it is logical that decreased stomatal conductance has been associated with increased leaf temperature under conditions of limited water availability (Al-Faraj *et al.* 2001; Blonquist *et al.* 2009).

The VPD acclimation response, i.e. an increase in the effectiveness of transpiration control, was most evident when plants grown at 0.75 kPa were moved into 1.8 kPa (Fig. 5b-c). No control over water loss could be detected over the range of VPD in the first four days after transfer, but a distinct breakpoint was evident in the second week. Only a slight acclimation could be detected after the next shift - one week of exposure to 3.0 kPa (Fig. 5e). In that case, one might assume that the response toward transpiration control at high VPD was counteracted to some extent by an opposite response to the high temperature. And then, in plants that had acclimated to hot, dry conditions (refer to Fig. 5f), the highly effective control over transpiration that was observed in the cool VPD challenge dissipated with time as plants acclimated to the cool, low-VPD growth environment (returned to a pattern similar to that shown in Fig. 5a).

The observations that plant VPD responses are influenced by the temperature at the time when measurements are taking place and by acclimation to the VPD of the plant growth environment have important consequences for studies comparing species or genotypes within a species (Fletcher *et al.* 2007; Sadok and Sinclair 2009; Devi *et al.* 2010; Gholipoor *et al.* 2010). While not dismissing the value of observations that control over transpiration occurs, and that the controls can vary among genetically distinct plants, our results imply that such

plant comparisons must be viewed with caution. Quite clearly, precise environmental control before and during the measurement period is necessary to avoid artifacts due to acclimation.

It should be noted that growth data from the long-term study did not support a fixed relationship between growth and water use in tall fescue such as that proposed for crop plants (Sinclair *et al.* 1984). A fixed relationship assumes proportionate changes in water vapor loss and CO_2 uptake as stomata open or close. Our results indicated a limitation on transpiration at high VPD in both growth temperatures (Fig. 1), and the clipping data appeared to validate the prediction of a linkage with gas exchange (Fig. 2). But, plants grown at 1.8 kPa actually had the same or slightly greater total mass compared with those at 1.2 kPa. Also, greater whole plant growth (due to increased root growth) was evident at 21°C even though transpiration was limited to a much greater degree (Fig. 3). The observations, together, suggest a separate temperature effect on growth, independent of VPD; i.e. fixation of carbon was limited even when stomatal openings freely permitted gas exchange.

Possible Mechanisms

Many of the structural and metabolic mechanisms observed to cause plant hydraulic adjustments in response to drought (Maseda and Fernandez 2006) could contribute to the temperature effects and acclimations in the VPD responses observed in our experiments. Anatomical limitations such as narrower xylem diameter or lower stomatal density, for example, could contribute to reductions in plant hydraulic conductivity. Canopy-wide expression of anatomical changes would be limited by the rate of leaf growth and senescence, as developing younger leaves would progressively replace older leaves (Fournier

et al. 2005) and dominate the response profile for the canopy. Those types of adjustment could contribute to the acclimation responses that required a period of a week to be expressed. The temperature effects, however, occurred too rapidly to be caused by structural changes.

A previous report of rapid changes in VPD response of maize at increased temperature proposed that a suite of biochemical mechanisms could be responsible (Yang *et al.* 2012). High temperature can quickly increase membrane permeability and water viscosity (as reviewed in Sack and Holbrook 2006), and it can also affect activity of aquaporins, which are thought to facilitate the great majority of cell-to-cell water movement (Tyerman *et al.* 2002). A growing body of evidence indicates that the effect of temperature on activity of aquaporins is mediated through changes in abundance and gating. For example, a decrease in aquaporin synthesis was observed with increasing temperature in broccoli roots (Iglesias-Acosta *et al.* 2010), while temperature changes led to reversible phosphorylation-associated gating that controlled water movement in tulip petals (Azad *et al.* 2004). Reduced root hydraulic conductivity of cucumber and rice at low temperature has been attributed to activity of aquaporins (Lee and Chung 2005; Murai-Hatano *et al.* 2008), and water movement through maize root aquaporins was maximized at 20°C, which was the treatment nearest to the growth temperature (Ionenko *et al.* 2010). While the mechanisms of aquaporin action *in situ* remain largely unknown, the rapid changes in water flow and responses to VPD with shifts of fescue to higher temperature obviously align with the time frames for fine and coarse controls of protein systems like those of aquaporins.

Implications for the Field

Our results help to explain some of the reasons for poor health of this cool-season grass in warm months, where high temperature often co-occurs with high VPD. With diminished control over transpiration, fescue plants would rapidly deplete available soil water, and decreased root growth would limit access to water within the soil profile. The root growth inhibition observed at 27°C may be relatively mild compared to the degree of inhibition that would occur at higher temperatures common in summer months even in temperate regions. Development of fescue varieties with drought tolerance must pursue enhanced ability to retain stomatal sensitivity to VPD and maintain root growth during periods of high temperature.

The results from our studies have important implications for estimates of irrigation requirements. A typical strategy is to estimate plant evapotranspiration (ET_c) and then replace that amount of water with irrigation. Estimations of ET_c are based on multiplication of two factors: potential evapotranspiration (ET_o), and a correction factor (K_c) for the particular crop ($ET_c = K_c * ET_o$). Determining ET_o can be as simple as measuring evaporation from a standard-size pan (e.g. Kneebone and Pepper 1982; Bastug and Buyuktas 2003; Short and Colmer 2007), or it can be calculated based on equations such as the Penman-Monteith or Blaney-Criddle, incorporating measurements of the physical environment such as windspeed, solar radiation, temperature, and humidity (e.g. Jones *et al.* 1984; Kerr *et al.* 1996). Values of K_c are empirically derived, usually by measuring water loss from lysimeters, and they are assumed to be a constant for particular species. Theoretically, this approach appears sound because it incorporates environmental factors and

a crop specific coefficient. In practice, though, K_c measurements have been extremely variable. K_c for turfgrasses has been found to vary among species (Kneebone and Pepper 1982; Ervin and Koski 1998; Short and Colmer 2007), and among cultivars of the same species (Salaiz *et al.* 1991), and K_c is affected by management techniques and nutritional status (Devitt *et al.* 1992; Carrow 1995; Brown *et al.* 2001). Our results further demonstrate that, because physiological mechanisms controlling water loss in plants are responsive to VPD as well as temperature and length of exposure to the VPD of the growth environment, K_c cannot be considered a constant. Within one particular field of turfgrass, interactions with the environment can lead to changes in transpiration on a minute-by-minute scale, in effect, changing K_c values dynamically. This may explain the difficulty in defining a consistent K_c value for a species or variety. According to our results, treating K_c as a constant would result in overestimation of water requirements during cool, dry weather due to water conservation by plants, but underestimation during short periods of warm weather due to relaxation of transpiration controls. Though the equations incorporate VPD as a physical factor influencing *potential* evapotranspiration (ET_o), the complexity of the plant responses of fescue may prohibit accurate calculation of actual plant evapotranspiration (ET_c) in ever-changing natural environments.

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Table 1. Significance (*P*-values) of temperature and VPD effects on water use and components of growth. For parameters that did not have significant temperature*VPD interactions ($P>0.05$), overall main effects of VPD and of temperature are reported. For each parameter that had a significant ($P<0.05$) interaction of temperature*VPD, overall effects of VPD and temperature are not reported; instead, separate effects are reported for VPD within each temperature treatment and for temperature within each VPD treatment.

	Over time		Cumulative final harvest			
	Water use	Clippings	Clippings	Verdure	Root	Total
VPD						
Overall	-	-	-	0.13	0.14	0.12
at 21°C	0.31	<0.0001	<0.0001	-	-	-
at 27°C	0.018	0.88	0.79	-	-	-
Temperature						
Overall	-	-	-	0.063	0.0050	0.0040
at 1.2 kPa	0.30	0.047	0.059	-	-	-
at 1.8 kPa	0.0033	<0.0001	<0.0001	-	-	-

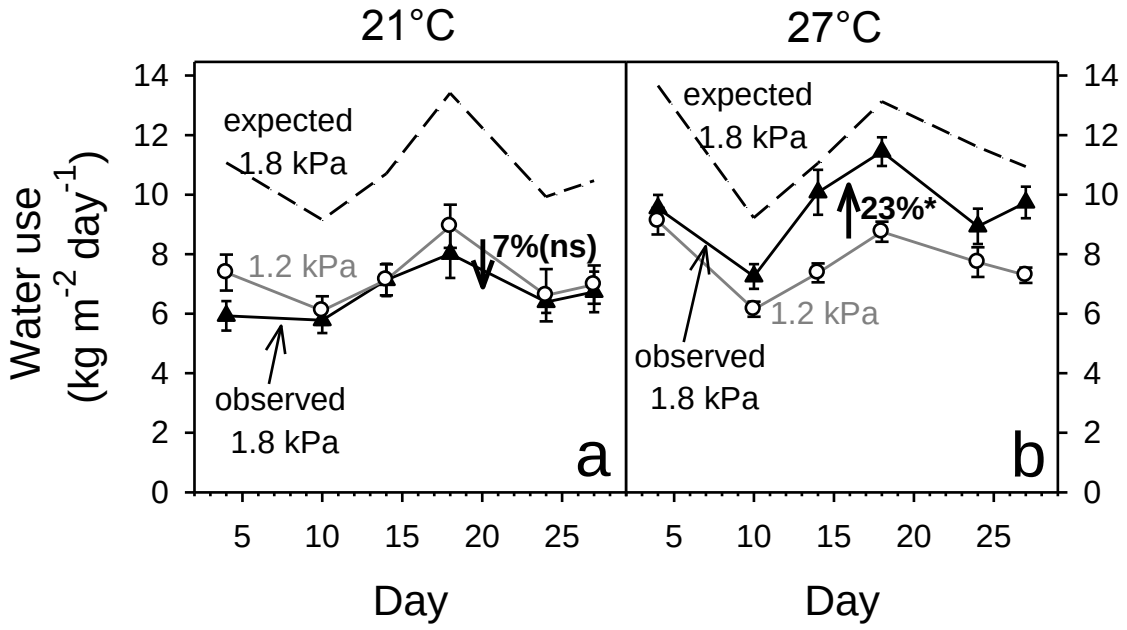


Figure 1. Water loss per m^2 of pot surface area per day by fescue in four environmental conditions: temperatures of (a) 21°C or (b) 27°C , and VPDs of —○—1.2 kPa or —▲— 1.8 kPa. If there were no restrictions in water loss by plants at increased VPD, then the water loss at 1.8 kPa would be 50% greater than that at 1.2 kPa; these calculated values are represented by dashed lines. The increase or decrease in water loss at 1.8 kPa (compared with 1.2 kPa) is indicated by an arrow and value (%) within each panel with significance indicated:*, $P<0.05$. Error bars in all figures represent standard error of the mean.

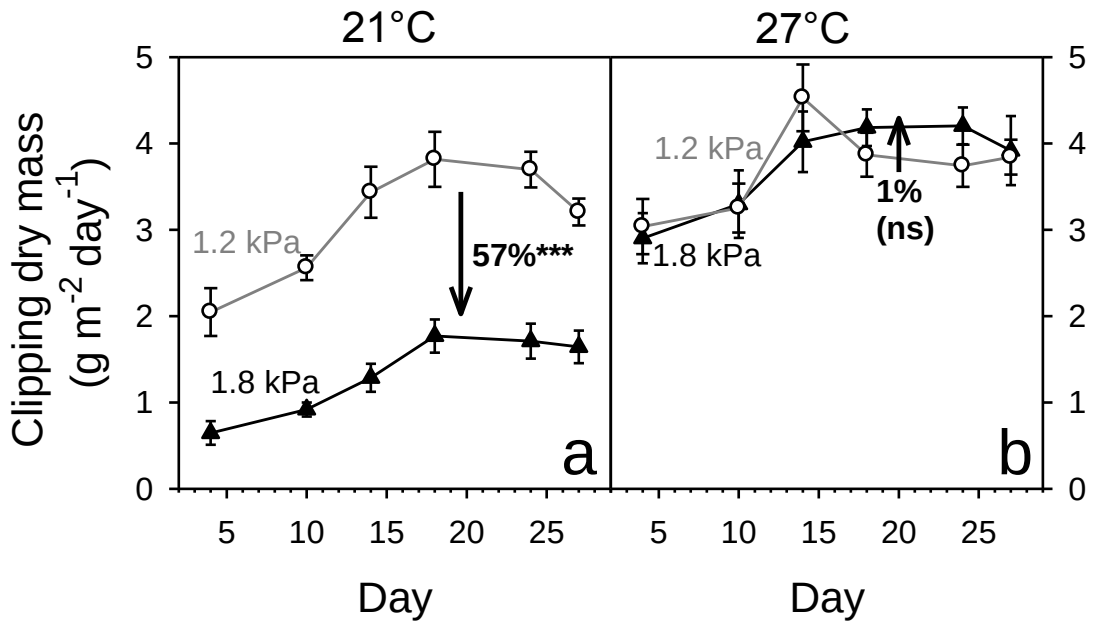


Figure 2. Dry mass of clippings produced per m^2 of pot surface area per day by tall fescue exposed to four environmental conditions, temperatures of (a) 21°C or (b) 27°C , and VPDs of —○— 1.2 kPa or —▲— 1.8 kPa. The increase or decrease in clipping dry mass at 1.8 kPa (compared with 1.2 kPa) is indicated by an arrow and value (%) within each panel with significance indicated: ***, P<0.0001.

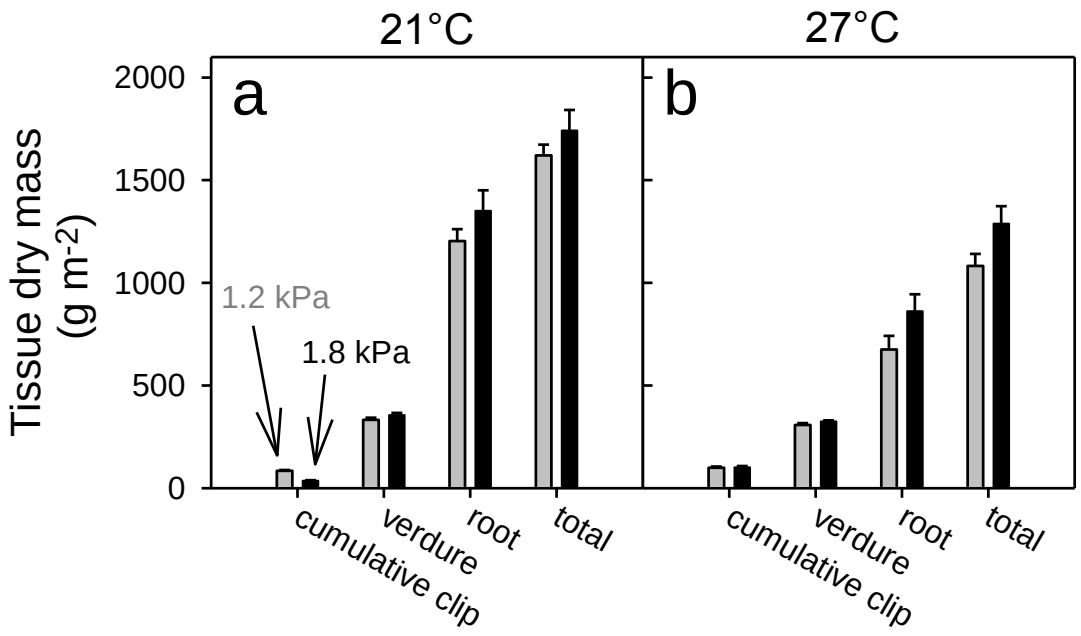


Figure 3. Dry mass of tissues produced per m^2 of pot surface area per day by tall fescue exposed to four environmental conditions, temperatures of (a) 21°C or (b) 27°C, and VPDs of 1.2 kPa (grey bars) or1.8 kPa (black bars). Cumulative clippings were collected only during the measurement period, while verdure and roots were collected at the conclusion of the experiment and include tissue produced during the establishment period and the measurement period.

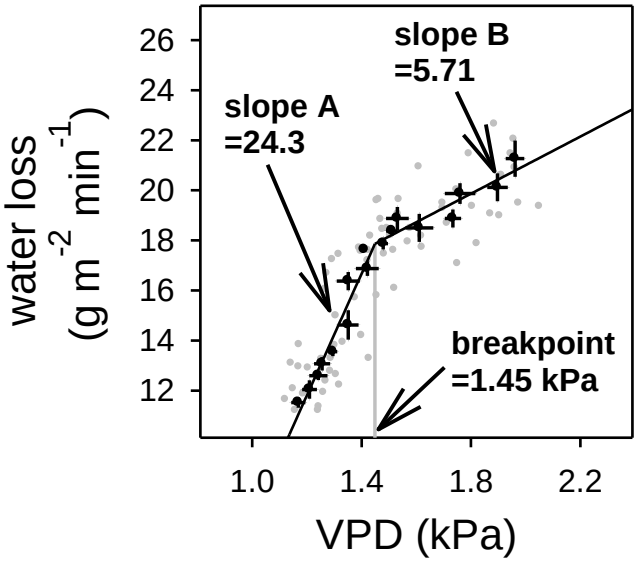
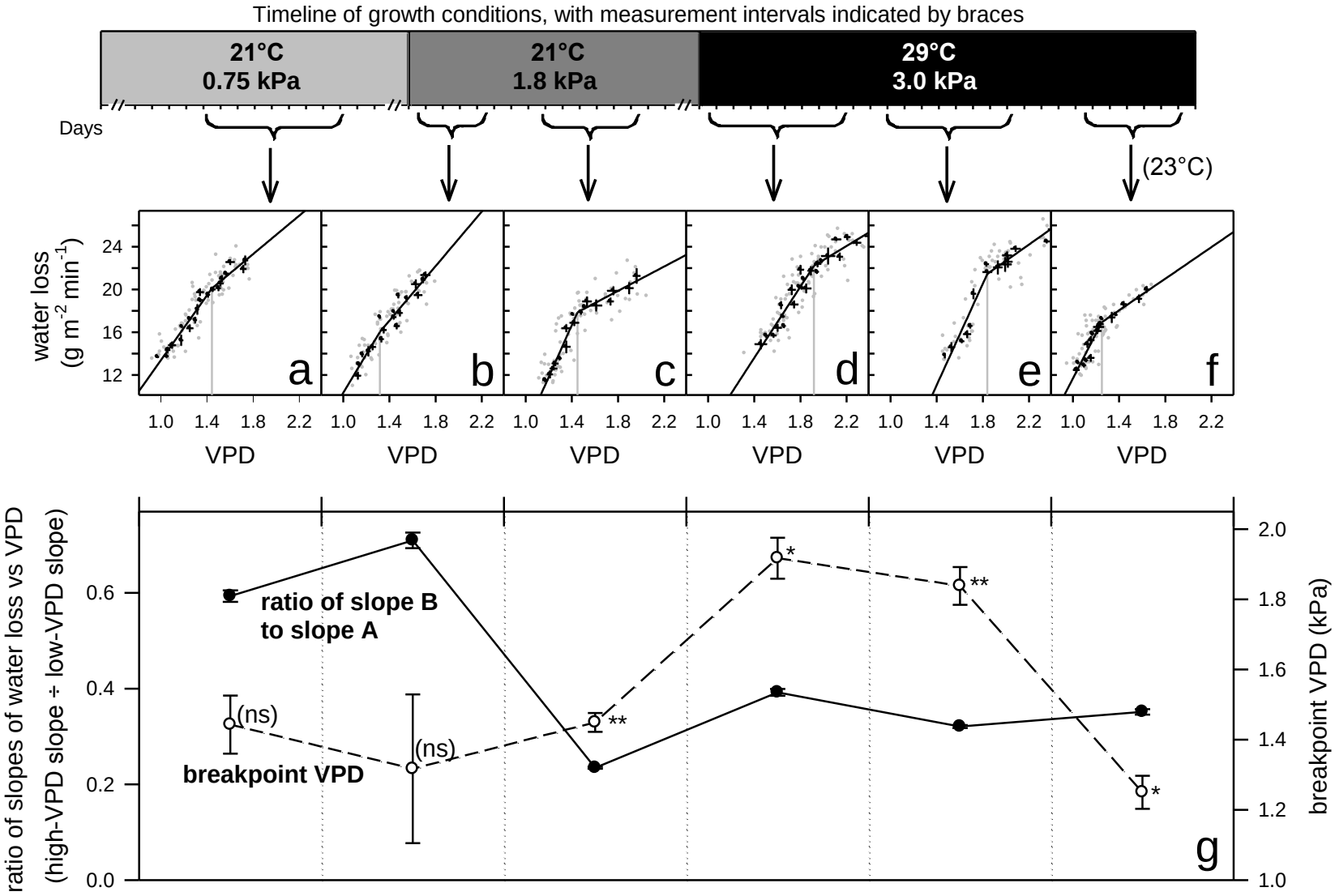


Figure 4. A representative figure of water loss (per m^2 of pot surface area per minute) vs. VPD. Parameters that indicate water conservation at increasing VPD are (1) breakpoint VPD, with a lower value indicating more sensitivity to VPD and (2) ratio of slopes, calculated as slope B ÷ slope A, with a lower value indicating stronger conservation at high VPD. Individual data are shown in grey, and the grouped means and standard errors are shown in black.

Figure 5. Water loss vs. VPD and associated parameters. Plants were subjected to a sequence of growth conditions (timeline). Water loss was measured over a range of VPD at particular times during the exposure to the conditions (a-f). Measurements (a-e) were conducted at temperatures similar to growth conditions, except for the final measurement (f), which was measured at a cooler temperature. Breakpoint VPD and the ratio of slope B to slope A (as shown in Figure 4) were calculated for each set of measurements (g). Significance of the breakpoint is indicated to the right of each point: * indicates $P<0.01$, ** indicates $P<0.001$; where breakpoint was not significant, the data were equally well fit by a linear model, but the two-segment model is shown for comparison.


Timeline of growth conditions, with measurement intervals indicated by braces
21°C
0.75 kPa
21°C
1.8 kPa
29°C
3.0 kPa
Days
(23°C)
water loss
(g m-2 min-1)
24
20
16
12
a
b
c
d
e
f
1.0 1.4 1.8 2.2
VPD
ratio of slopes of water loss vs VPD
(high-VPD slope ÷ low-VPD slope)
0.6
0.4
0.2
0.0
ratio of slope B
to slope A
(ns)
(ns)
**
*
**
*
breakpoint VPD
g
2.0
1.8
1.6
1.4
1.2
1.0
breakpoint VPD (kPa)

CHAPTER 2

Assessing Transpiration Estimates in Tall Fescue: The Relationship Between Transpiration, Growth, and Vapor Pressure Deficits.

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Abbreviations: ET, evapotranspiration; k_c and k_s, plant-specific constants in water use calculations; pET, potential evapotranspiration; TE, trinexapac-ethyl; VPD, vapor pressure deficit

Abstract. Limitations in water availability for irrigation due to droughts and water-use regulation necessitate increased understanding of water use efficiency by grasses. This study evaluated the relationship between evapotranspiration (ET) and shoot growth of tall fescue (*Festuca arundinacea* Schreb.), assessing the validity of an equation to estimate ET based on environmental and plant growth parameters. Experiments examined differences in ET when tall fescue was grown over a range of temperatures and vapor pressure deficits (VPD) and growth was slowed by low nutrition and the growth regulator trinexapac-ethyl. Temperatures ranged from 22/18°C to 26/22 and 30/26°C. In each temperature environment, treatments

caused large changes in clipping mass but transpiration remained similar. These results, and others from hydroponic experiments, showed no effect of the treatments on whole-plant growth, demonstrating that whole-plant growth data are essential to accurately estimate plant water use over short time periods. The compelling positive correlation from our experiments was between transpiration and VPD, especially within each temperature environment. There were indications that plant control over transpiration decreased with increasing temperature. An increase in VPD from 1 to 2 kPa in the coolest temperature caused an increase in ET of about 50%, but the increase was over 80% in warmer temperatures. The instability of physiological control over transpiration highlights the potential limitations of equations using a single constant to represent the plant-specific component of transpiration.

INTRODUCTION

Tall fescue is a cool-season grass commonly used as a turfgrass in lawns, parks, and athletic fields, and also as forage for grazing livestock. Because of its versatility, at least 14 million hectares of tall fescue are grown in the United States (Buckner et al., 1979). In many areas, water availability is an important limiting factor for growth and health of grasses in both urban and traditional agricultural settings. Despite improvements in irrigation efficiency, irrigation demands per unit of land area are likely to increase in the coming decades due to higher evapotranspiration rates and more sporadic rainfall, both associated with climate change. This increased need for irrigation water will occur even as water availability becomes more limited because of increased demand for other uses (Brown et al., 2013; Trenberth et al., 2007). Given the likelihood of more intense government regulation and

increased cost of water for landscape irrigation, greater understanding of physiological factors controlling transpiration rates and more accurate prediction of irrigation needs are imperative for grasses like tall fescue.

The water requirements of crop plants generally have been estimated by the potential for evapotranspiration (pET). The most common approach has involved use of the Penman or Penman-Monteith Equations (Allen et al., 1998). These equations require empirical measurements to resolve two main components: environmental conditions such as temperature, humidity, wind speed, and solar radiation are used to estimate the energy-balance parameters underlying the Penman equation; and a coefficient (k_c), which varies by crop species and plant status, scales for actual evapotranspiration rate relative to the theoretical Penman's value. The assumption that a constant can be used to represent the plant-specific component of water loss appears to be an empirical weakness in the approach. Indeed, considerable variation always has been encountered in studies that attempted to define crop-specific coefficients in different climatic conditions, plant developmental stages, or seasons during the year (e.g. Brown et al., 2001; Carrow, 1995; Devitt et al., 1992; Piccinni et al., 2009; Wherley et al., 2014).

Another approach for estimating the water requirement of crop plants is to correlate water use with plant growth. This relationship, proposed by deWit (1958), has been modified and used extensively by others (e.g. Bierhuizen and Slatyer, 1965; Sinclair, 2012; Tanner and Sinclair, 1983). Most recently, the relationship has been expressed by Sinclair et al. (2014) as

$$\text{Transpiration} = \text{Growth} \left(\frac{\text{VPD during photoperiod}}{k} \right) \qquad \text{(Equation 1)}$$

where transpiration and growth are in units of mass, and atmospheric vapor pressure deficit (VPD) and the constant k are in units of pressure, i.e. Pascals. For clarity in this manuscript, this k value will be referred to as "k_s" to differentiate it from Penman's k_c. The Sinclair approach relies on the principle that when stomata are open, water vapor diffuses out of the leaf while carbon dioxide simultaneously diffuses inward. Thus, the amount of plant matter constructed from incoming CO_2 is quantitatively correlated with transpiration rate. The only environmental variable directly included in the equation is VPD. Because VPD defines the gradient for water diffusion out of the leaf, it determines the amount of water exchanged per unit of CO_2. The other key component of the model is k_s, which depends on the efficiency of CO_2 intake and subsequent dry mass production from assimilated CO_2. The value of k_s is defined for each species, depending on specific characteristics of its carbon assimilation pathway and the biochemical composition of tissues produced.

The Sinclair approach for estimating water use has been applied to field crops such as sorghum, barley, and wheat (Abbate et al., 2004; Hammer et al., 1997; Kemanian et al., 2005), and seems to accurately estimate seasonal water requirements for producing different yield levels. Also, actual transpiration by four warm-season grasses, periodically measured during a three year period, was positively correlated with transpiration computed from Equation 1, but with growth estimated from incident solar radiation and calculated radiation use efficiency (Sinclair et al., 2014). Lower transpiration by plants in high-VPD conditions was accompanied by a concurrent decrease in growth, responses consistent with a linked restriction of leaf water vapor and CO_2 exchange (Sinclair et al., 2007). Thus, an

accumulating amount of evidence supports the physiological basis for the Sinclair model and its use in predicting water use in the field.

In this study with tall fescue, we further evaluate the Sinclair approach for estimating transpiration, in this case transpiration in a turfgrass system. In a series of greenhouse and growth chamber experiments, fescue is exposed to different temperature environments and levels of nutrition, and applications of a growth regulator. Using the environmental variation, we attempted to answer three questions. First, can transpiration be accurately estimated from the Sinclair model when growth is measured as clipping mass? For the approach to successfully predict irrigation needs over intervals of hours or days, it must rely on a readily measurable growth factor. Because whole plants are not harvested in turfgrass systems, clipping production must serve as a proxy for total plant growth. A second question is, if the relationship between clipping mass and transpiration is not consistent, can transpiration be accurately predicted from differences in VPD, the other key element of the Sinclair equation? It is conceivable that VPD or 'sink strength' for water loss (Sinclair et al., 2014) could be the dominant factor in the equation, decreasing the importance of precise growth measurements. Third, to understand the limitations of using grass clippings to estimate fescue growth, does dry matter partitioning readily shift from above to inaccessible below-ground tissues? Studying mass partitioning in hydroponics, under treatments similar to the greenhouse experiments, allowed accurate measurement of all tissues, including verdure and roots. A key feature of this study was the imposition of treatments, including vapor pressure deficit, temperature, fertility, and spraying with trinexapac-ethyl, as approaches to alter shoot growth, total plant growth, or both.

MATERIALS AND METHODS

Greenhouse experiments

Two experiments were conducted. For Experiment A, seeds of tall fescue cultivar 'Kentucky-31' were sown on September 26 2008 at a rate of 61±3 g m^{-2} into 9-L pots (surface area 363 cm^2) containing a sandy loam field soil (69% sand, 18% silt, 13% clay). The pots were placed in a temperature-controlled greenhouse at the North Carolina State University Phytotron (the Southeastern Plant Environment Laboratory) that was set to 26°C during the day and 22°C at night. During a seven-week establishment period, the turf was clipped weekly to a height of 10 to 12 cm. A soluble fertilizer solution (36-6-6 N-P_2O_5-K_2O Miracle-Gro lawn food, Scotts Miracle-Gro Products, Inc, Marysville, OH) was added weekly, supplying nitrogen at 2.44 g m^{-2} wk^{-1} during the first three weeks after emergence and half that rate thereafter.

Experiment B used the same seeding rate and soil, but seeds were sown into 11-L (570 cm^2 surface area) pots in the Method Road greenhouses at NCSU (at approximately 30 °C day/24 °C night) on April 10 2008, where they remained for eight weeks before transfer to the NCSU Phytotron greenhouses. As in Experiment A, plants were clipped regularly and the same rate of soluble complete fertilizer solution was applied during the establishment period.

Azoxystrobin fungicide (Heritage TL, Syngenta Professional Products, Greensboro, NC) was applied preventatively in both experiments, and spinosad (Conserve SC, Dow AgroSciences LLC, Indianapolis, IN) and bifenazate (Floramite, Chemtura AgroSolutions, Lawrenceville, GA) were used to control insects and mites in Experiment B.

Three temperatures and three growth-modulation regimes (high fertility, high fertility plus the gibberellic acid inhibitor trinexapac-ethyl [TE], and low fertility) were imposed on the established turf cultures. There were seven replicates of each treatment in Experiment A and five in Experiment B. Temperature treatments were imposed for an acclimation period of two to three weeks before the first measurements by dividing the pots among three different greenhouses programmed for day/night temperatures of 30/26, 26/22, or 22/18°C. Fertility treatments were established by varying the concentration of liquid fertilizer that was applied on each clipping date (once or twice per week in Experiment A, twice per week in Experiment B). The fertilizer product was the same as used during the establishment period, but the dilution was adjusted so that the low-fertility treatment received a nitrogen rate of 0.24 g m^{-2} at each clipping date and the other treatments received 0.61 g m^{-2} in each application. The trinexapac-ethyl (Primo MAXX, Syngenta Professional Products, Greensboro, NC) treatment was applied using a CO_2 backpack sprayer delivering 38 mg active ingredient per m^2 in Experiment A and 114 mg m^{-2} in Experiment B, first at 8 to 11 days before beginning measurements and again 16 to 20 days later. Experiment A was conducted in winter (measurements collected during December and January), while Experiment B was conducted during the summer (measurements during June and July).

Water loss was measured daily by weighing each pot. At the beginning of the experimental measurement period, all pots were watered well and allowed to drain overnight, then placed inside another pot lined with a plastic bag to retain any additional water. Each pot was weighed to determine its initial well-watered mass. During the experimental period, the pots were weighed daily and water was added to restore each pot to 250 g (Experiment

A) or 400 g (Experiment B) less than the initial well-watered mass; the deficit prevented oversaturation of the soil in the absence of drainage.

Turfgrass was clipped carefully once or twice per week at a height of 3 cm above the rim of the pot, approximating a mowing height of 6 cm. Clippings were collected, and both fresh and oven-dry mass were measured. For analysis purposes, water use by each pot was summed over intervals corresponding to the clipping intervals, so that the water use and clipping production could be compared, quantitatively, for the same measurement periods.

At the conclusion of Experiment A, eight weeks after treatments began, all shoot tissue remaining below the clipping height (verdure) was removed at the soil surface and roots were washed free of soil; both fresh and dry mass of all tissues were measured. Temperature and humidity in each greenhouse were measured at 15-minute intervals throughout both experiments (WatchDog Model 150, Spectrum Technologies, Plainfield, IL), which allowed calculation of VPD.

Hydroponic experiment

Seeds of tall fescue cultivar 'Kentucky-31' were evenly distributed at a rate of 58.6 g m^{-2} in plastic cups with 3.1 cm diameter and porous plastic mesh bottoms. The culture cups were placed into six 12-L continuous-flow hydroponic units so that the seeds were in contact with solution, and clear plastic wrap covered the top of each cup until seed germination. Air temperature was set to 26/22°C and light was provided at a PPFD of 400-700 µmol m^{-2} s^{-1} by a combination of halogen and metal halide lamps. During the 49-d establishment period, plants were grown in a complete nutrient solution (at half concentration for the first four weeks of growth, then brought to full concentration of: 1.2 mM $KN0_3$, 500 µM KH_2P0_4, 1

mM $CaS0_4$, 1 mM $MgS0_4$, 620 nM H_3BO_3, 120 nM $MnCl_2$, 110 nM $ZnS0_4$, 130 nM $CuS0_4$, 3 nM $NaMo0_4$, and 70 µM iron as Sequestrene 330 [Sprint 330, Becker Underwood Inc., Ames, IA]. Leaf tissues were clipped level with the top of each cup (2.5 cm shoot height) as needed to maintain a shoot height of less than 4 cm. One week prior to the first measurement, roots were trimmed with scissors to a length of 3 cm.

Six hydroponic units were used to establish six different growth conditions, a factorial design of three root temperature treatments and two nitrogen fertility levels. Temperature treatments were established one day prior to the first measurement, with two units at each root temperature (20, 25, and 30 °C). Low nitrogen treatments were implemented by allowing the nitrogen level in one unit of each temperature treatment to deplete from the initial full concentration and then supplying only a small amount of N during the experimental period (100 µmol NO_3^- L^{-1} wk^{-1}). The high-N treatments were maintained at 600 to 1200 µM NO_3^-. Solution NO_3^- and PO_4^- concentrations were measured one to two times per week by HPLC (Dionex, Sunnyvale, CA) and all nutrients aside from NO_3^- were provided in adequate quantities to all treatments.

Within each growth condition, one of two spray treatments (water control or trinexapac-ethyl) was applied to all cups in each hydroponic unit, for a total of five replicate cups in each temperature/nutrient/spray treatment. Spray treatments were implemented 4 d prior to the first measurement date and again two weeks later by applying either a solution delivering 38 mg m^{-2} TE in 76 mL m^{-2} of water, or the same volume of deionized water, using a plastic trigger spray bottle.

Clippings were collected from each cup every 3 to 5 d for 3 wk, for a total of five clipping measurements. After the final collection of clippings, the remaining verdure was separated from the roots. The fresh and dry mass of all tissues was measured.

Data from all experiments were analyzed using SAS software (SAS Institute, Cary, NC). Clipping mass and water use over time were treated as split-block, repeated measures data and analyzed using PROC MIXED for the greenhouse experiment and PROC GLIMMIX for the hydroponic experiment. Final dry mass data from the hydroponic experiment were analyzed using PROC GLM. Comparisons of the relationship between water use and VPD in the greenhouse experiment were made using PROC NLMIXED.

RESULTS

Greenhouse shoot growth and water use

Though greenhouse temperatures were the same in both experiments, VPD was very different (Table 1). Because Experiment A was conducted in winter, when air generally contains relatively little water vapor, the VPD range was relatively high (1.97 to 2.81 kPa); the humid summer air during Experiment B generated lower VPDs (1.15 to 2.47 kPa). In each experiment, VPD increased with increased greenhouse temperature, as would be expected.

The temperature of the growth environment noticeably influenced the mass of tall fescue clippings. Comparing growth responses of plant supplied high N, a smaller mass of clippings was produced by plants in the cool environment compared to those in the medium temperature in experiments A and B (Table 1; $P<0.05$). In Experiment A, the average

clipping dry mass decreased from 1.58 g m^{-2} d^{-1} in the medium temperature to 1.36 g m^{-2} d^{-1} in the cool temperature (P<0.05). In Experiment B the decrease was from 1.88 to 1.34 g m^{-2} d^{-1} (P<0.01). An interesting difference between the two experiments can be seen in a comparisons of growth in the moderate and high temperatures. In Experiment A, the temperature increase from 26/22°C to 30/26°C resulted in an increase in the average clipping production from 1.58 to 1.91 g m^{-2} d^{-1} (P<0.05). But in Experiment B, the same temperature difference led to decreased growth, from 1.88 to 1.10 g m^{-2} d^{-1} (P<0.05). Thus, the effect of high temperature on shoot growth of tall fescue was inconsistent. This may be related to the difference in VPD between the experiments: in Experiment A the 26/22°C and 30/26°C treatments produced similar VPD levels, but in Experiment B the warmer greenhouse had a VPD almost 1 kPa higher than the moderate temperature greenhouse (refer to VPD in Table 1).

Low N and application of TE suppressed growth, as intended (P<0.001). To easily see the relative magnitude of the decreases, data are expressed as a percentage of a selected reference treatment, the high-N treatment in the moderate 26/22°C temperature (values in Table 1). In the low-N treatment of Experiment A, clipping mass at 22/18°C was only 57% of the reference, at 26/22°C the decrease was to 72%, and at 30/26°C clipping mass decreased to 81% of the reference (Table 2, Fig. 1; P<0.001 for all comparisons). This was true in Experiment B also, as clippings decreased to 37% of the reference at 22/18°C, to 61% at 26/22°C, and to 49% of the reference at 30/26°C (P<0.001 for all comparisons). In all cases, the effects of temperature on clipping production by the nitrogen-deprived plants were similar to the temperature effects observed at high N. Similarly, the TE treatment strongly

restricted shoot extension, with low clipping production occurring in all temperatures in both experiments. In Experiment A, addition of TE caused a decrease in average clipping production to 15% of the reference at 22/18°C, 21% at 26/22°C, and 28% at 30/26°C (Table 2, Fig. 1; $P<0.001$ for all comparisons). In Experiment B, the TE-associated decrease in clipping production was to 26% of the reference at 22/18°C, 29% at 26/22°C, and 28% at 30/26°C ($P<0.001$ for all comparisons).

Measurements of water use by tall fescue growing in the various conditions again indicated that temperature was important. Plants consistently lost more water with increasing temperature ($P<0.001$). In Experiment A plants growing in 22/18°C used 6.23 kg m^{-2} d^{-1} or about 70% of the average in the 26/22°C greenhouse (8.58 kg m^{-2} d^{-1}), while plants at the higher temperature of 30/26°C had an evapotranspiration rate of 10.4 kg m^{-2} d^{-1}, about 120% of that at 26/22°C benchmark (Table 1). Similarly in Experiment B, transpiration at the lower temperature was decreased to about 90% of that at 26/22°C (4.94 vs 5.66 kg m^{-2} d^{-1}) and increased to about 145% at the higher temperature (8.39 kg m^{-2} d^{-1}). Unlike the clipping measurements, water use showed very small effects of the growth-suppression treatments. Comparison of mean water use (Table 1) and water use over time (Fig. 2) indicated that differences in evapotranspiration among the nitrogen and TE treatments were small and inconsistent. Though some differences were significant (in Experiment A, low N and TE treatments had lower water use than the high N reference, $P<0.001$; in Experiment B, the TE treatment did not have overall lower water use than the high N reference, neither treatment affected water use in the 26/22°C greenhouse, and both treatments had higher water loss than the high-N reference in the 30/26°C greenhouse), the effects were so small, mainly within

±9% of the high-N treatment, that temperature effects clearly were dominant in influencing water loss.

Quantitative relationships. With measurements of water use, clipping production, and environmental conditions available, it was possible to assess the theoretical relationships among parameters. First, water use was highly correlated with VPD (Fig. 3); however, the relationship varied somewhat with temperature, independent of the evaporative gradient imposed by VPD. In the lowest temperature, 22/18°C, the regression results indicated an increase in transpiration rate of 1.98 kg m^{-2} d^{-1} for each 1 kPa increase in VPD (regression slope). In the warmer greenhouses, a higher slope was observed, with transpiration increasing by 3.35 to 3.37 kg m^{-2} d^{-1} for each kPa of VPD increase. The r^2 values were between 0.68 and 0.89 and the higher slopes for the warmer temperatures were different ($P<0.05$) from that at the lower temperature. The difference in slope indicates that temperature affects plant water regulation. Theoretically, a doubling of VPD (for example, from 1 kPa to 2 kPa), would double evaporation from the leaf surface in the absence of plant regulation. In reality, a doubling of VPD increased ET by only 50% within the cool temperature treatment, indicating a strong regulation of water loss, but in the warm temperature treatment ET increased by about 80 to 90% as VPD doubled, indicating a weakened control.

The correlation between evapotranspiration and clipping production was evaluated in the same fashion (figure not shown). These two factors were found to have a poor relationship, with r^2 less than 0.03 in each temperature treatment.

The more complex relationship among growth, transpiration, and VPD was evaluated by rearranging Equation 1 as:

$$\text{growth}\left(g*m^{-2}\right) = k_s(Pa) * \frac{\text{water use } (g*m^{-2})}{\text{VPD (Pa)}} \qquad \text{(Equation 2)}$$

This illustrates that, when growth of fescue (here estimated by clipping mass) is represented on the ordinate of a graph and evapotranspiration divided by VPD is represented on the abscissa, the constant k_s should be equivalent to the slope of the regression (Fig. 4). In the scatter of data in Figure 4, one can readily see differences among the nitrogen and TE treatments (regressions fit to the combined treatments within each temperature had $r^2 < 0.13$).

Due to the poor regression results in Figure 4, linear regressions were applied to the data from the high-nitrogen treatment within each temperature and the remaining treatments compared to this treatment. If the growth:transpiration relationship were consistent across temperatures, the slope, k_s, would be the same in all three panels; if it were consistent among growth-suppression treatments, all points in each panel would fall along the high-N regression. In examining the high-N regression in each of the three temperatures, two trends are apparent. First, the fit of the regression improves as temperature increases (r^2, from coolest to warmest treatment, is 0.094, 0.42, and 0.55 [Fig. 4a, 4b, 4c, respectively]), although all r^2 values are low, indicating high variability. Second, the slope of the regression, or k_s, increases with temperature (k_s, from coolest to warmest treatment, is 0.13, 0.85, and 0.99). Both of these trends indicate that shoot growth and (water use/VPD) are more closely

correlated as temperature increases, and importantly, that the relationship between shoot growth and (water use/VPD) is not consistent across different environmental conditions.

In addition to the impact of temperature, the effects of the growth-suppression treatments were examined. Within each temperature, both the TE and low nitrogen treatments fall below the high-N regression. This indicates that, though shoot growth was restricted by the low N and TE treatments, the concurrent reduction in water use that would be predicted by the theoretical model did not occur.

Hydroponic growth measures

One explanation for the lack of a consistent positive correlation between shoot growth and water use could be that shoot growth does not reflect growth of the whole fescue plant. If that were true then quantification of verdure and root mass, along with that of shoots, would be necessary for accurate assessment of the relationship between growth and (water use/VPD). Unfortunately, due to high variability in verdure and especially root mass in the solid soil media, our attempts to assess whole plant growth in the pot system have not been successful (data not shown). A more complete exploration of the effects of treatments on growth of all plant parts was thus pursued in hydroponic studies where variation is much smaller.

In this experimental system (versus the greenhouse study), all plant tissues were easily recoverable from the system. In addition, any changes in growth due to the growth-control treatments were magnified by having a shorter establishment period than in the greenhouse and by clipping the roots prior to beginning treatments, so that a larger

percentage of the final harvest of shoot and root material was reflective of growth that occurred during the treatment period.

In the hydroponic study, all treatments experienced the same aerial environment. Variation in root temperature in the range studied, 20 to 30°C, had little effect on growth (the 20°C root temperature did produce a slightly lower mass of clippings than the 25 and 30°C treatments, $P<0.05$, but temperature did not have a significant effect on production of other tissues). However, the growth-suppression treatments had a greater effect on tissue production. As seen in the greenhouse experiments, clipping production during the experimental period was decreased by application of TE and by withholding nitrogen ($P<0.001$; Fig. 5). However, root and verdure tissues reflected more complex adjustments in growth due to the growth control treatments.

Application of TE increased verdure mass in all temperatures by 14 to 24%, largely offsetting the reduction in clipping production. Thus, the total mass of all tissue produced by the TE-treated fescue was not significantly different from the high-N reference treatment (Fig. 6). The low-nitrogen treatment did not have increased verdure production, but instead had a much higher root mass than the high-nitrogen reference (Fig. 6); root mass was nearly doubled by the low-nitrogen treatment in all three temperatures. Summing all tissues, the increase in root mass balanced the decrease in clippings, so total plant mass showed no reduction due to the low nitrogen treatment over the 3 week period (Fig. 6).

The results from growth in hydroponics indicate that the growth-suppression treatments, low N and TE, reduced extension of shoot tissue into the clipping zone but caused no effect on the total mass of tissue produced by tall fescue plants over a period of

several weeks (P=0.81). During this time period, nitrogen concentration of shoot and root tissues did drop by about 50% in the low-nitrogen treatment as tissue growth continued while nitrogen nutrition was withheld (data not shown); obviously, as nitrogen stores become progressively depleted over time the growth rate would eventually slow, so these results cannot be extrapolated to a more extended time frame.

DISCUSSION

An obvious conclusion from these experimental results is that transpiration by fescue over relatively short time periods cannot be accurately predicted by an equation that depends solely on shoot growth, i.e. harvested clippings, for an estimate of plant CO_2 and water vapor exchange. The relationship between shoot growth and (evapotranspiration/VPD) was extremely inconsistent among the different treatments, as indicated by large differences among the three temperature regimes and within each temperature (Fig. 4). In addition to the very low r^2 values, the calculated k_s (slope) varied with temperature, and the low-N and TE treatments did not follow the same patterns as the high-N treatment. The response profiles indicated that shoot growth could vary greatly but transpiration varied to a much smaller extent.

One explanation for the inconsistency in the growth and evapotranspiration/VPD relationship is that clipping mass does not consistently relate to whole plant growth. As shown in the hydroponic experiments, when fescue is subjected to different temperatures (in this case, variable root temperatures), low nutrition, and growth regulator treatments, changes in partitioning of mass can occur, shifting tissue production to verdure and roots without

affecting total plant mass. Moreover, when shoot growth was restricted, the partitioning adjustments were not consistent. In one case (low N), root growth was increased, and in the other (growth regulator TE), verdure increased. The hydroponics results, of course, cannot be directly applied to growth in a greenhouse with soil, or to the field. The point, though, is that these kinds of growth adjustments readily occur and obviously would undermine maintenance of a stable relationship between shoot growth and transpiration.

As an important aside, a practical implication from our experiments is that management strategies to depress shoot growth are unlikely to lead to lower transpiration. Previous studies have sometimes, but not always, found low nutrient levels and plant growth regulators to be associated with decreased evapotranspiration (Ebdon et al., 1999; Ervin and Koski, 2001; Shearman and Beard, 1973; Wherley and Sinclair, 2009a). We find no indication that lowering N fertility or application of growth regulators would have a significant impact on irrigation requirements.

The importance of plant response to VPD

The dominant relationship found in our data sets was a highly significant positive correlation between water use and VPD (Fig. 3). In all temperatures and growth-altering treatments, evapotranspiration increased when plants were exposed to the higher evaporative potential inherent to higher-VPD conditions. Thus, more accurate estimates of fescue transpiration must be anchored within the prevailing influence of VPD, and the ability of plants to respond to it.

In the case of grasses, short-term experiments have shown that cool-season grasses exhibit transpiration control with increasing VPD (Wherley and Sinclair, 2009b). More recent experiments confirmed that this type of mechanisms is present in fescue (Sermons et al., 2012). Longer-term experiments such as those in the current study do not allow precise definition of adjustments in transpiration control. Nonetheless, our results clearly indicate that tall fescue restricted water loss in response to increasing VPD. An increase in VPD from 1 to 2 kPa would be expected to cause doubling of transpiration if no restraint were present, but transpiration was increased only 50% in the cool greenhouse and about 80 to 90% in the higher two temperatures (Fig. 3).

The differential restraint of water loss in the cool and higher temperatures is a key observation; the data clearly indicated that transpiration was not as well controlled at the higher temperature. Less effective transpiration control with increasing temperature has been observed in short-term VPD experiments with tall fescue (Sermons et al., 2012), and also with maize (Yang et al., 2012) and soybean (Seversike et al., 2013). Fescue is a cool season grass that is generally grown in the middle to northern areas of the U.S. (Sleper and West, 1996). More effective transpiration control in temperatures like the 22/18°C used here aligns with its optimal adaptation range, and one might expect that effectiveness would continue to decline if temperatures were increased beyond our high temperature of 30/26°C. We know very little about the mechanistic basis for the VPD responses. Physiologically, transpiration control could involve factors like hydraulic limitations in water movement to the stomata, which may be caused by low vascular conductance in roots or leaves (Choudhary and Sinclair, 2014; Sinclair et al., 2008), or perhaps by gating of water movement through

aquaporins (Devi et al., 2012). But, pursuit of mechanisms is particularly intimidating because plant control and temperature interactions, themselves, are not stable. In the most recent studies with fescue (Sermons et al., 2012) and maize (Yang et al., 2012), plants acclimated to changing VPD and temperature conditions over time.

Complications in estimating transpiration

Considering the dynamic nature of the plant control mechanisms responding to atmospheric variables of VPD and temperature, it seems unlikely that one could define a reliable constant representing plant control over water loss, such as the k_c in the Penman-Monteith (k_c) equation. In situ expression of transpiration control almost certainly would differ season-to-season in a particular geographical region and among different climatic regions like those with arid environments and others with episodic rainfall. And, of course, different genotypes within a species cannot be depended on to respond similarly to their environment. Thus, it should not be surprising that research closely examining k_c has not isolated a repeatable, single value (e.g. Brown et al., 2001; Carrow, 1995; Devitt et al., 1992; Piccinni et al., 2009; Wherley et al. 2014).

Theoretically, regardless of changes in temperature and plant control, as long as whole plant growth (CO_2 uptake) remained coupled with water loss (transpiration), a k_s value would remain stable. One could envision that within a moderate range of temperatures, say 18 to 28°C for fescue, the equation may accurately predict transpiration if some modification of the equation occurs to account for below-ground growth, a task that is not impossible if fertility and chemical management conditions are well defined. Much more daunting is the

progressive decoupling of growth and water use as temperatures increase into the upper range of adaptation and beyond. Growth would be depressed to greater extents while transpiration continued to increase with increasing VPD but with less effective control. For the equation to become more precise in those circumstances, high temperature would need to be defined as a stress condition similar to depleted soil water or plant nitrogen status (Sinclair et al., 2014); then its effect on radiation use efficiency could be calculated and incorporated into the equation. Considering the dynamic nature of plant controls, along with constantly changing micro-environmental atmospheric conditions that occur in the field, a substantial amount of imprecision could persist in estimating water use over short time periods.

ACKNOWLEDGMENTS

Thanks to Dr. Consuelo Arellano for statistical analysis and Mike Jennette, William Blount, and the staff of the NCSU Phytotron for their help in executing the experiments.

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Table 1. Clipping dry mass and water use of high-nitrogen tall fescue in both greenhouse experiments averaged over all dates and pots within each temperature treatment, and photoperiod VPD averaged over all dates within each run. With standard error of the mean. The 26/22°C, high-N treatment, shown in bold lettering, is the baseline reference for calculating all values presented in Figures 1 and 2 and Tables 2 and 3.

Temperature	Experiment	Clipping dry mass of High N trt ($g*m^{-2}*d^{-1}$)	Water used by High N trt ($kg*m^{-2}*d^{-1}$)	Photoperiod VPD (kPa)
22/18°C	A	1.36 ± 0.05	6.23 ± 154	1.97 ± 0.10
	B	1.34 ± 0.10	4.94 ± 132	1.15 ± 0.03
26/22°C	A	**1.58 ± 0.04**	**8.58 ± 164**	2.47 ± 0.09
	B	**1.88 ± 0.13**	**5.66 ± 191**	1.52 ± 0.07
30/26°C	A	1.91 ± 0.08	10.4 ± 194	2.81 ± 0.14
	B	1.10 ± 0.13	8.39 ± 386	2.47 ± 0.08

Table 2. Dry mass of clippings produced by tall fescue in both greenhouse experiments, expressed as a percent of the 26/22°C high-nitrogen treatment from the same run, averaged over all dates. With standard error of the mean.

		Clipping dry mass (as % of High N, 26/22°C)		
Temperature	Experiment	High N	Low N	High N + TE
22/18°C	A	87 ± 4	57 ± 3	15 ± 3
	B	77 ± 5	37 ± 5	26 ± 6
26/22°C	A	**100** ± 4	72 ± 5	21 ± 3
	B	**100** ± 5	61 ± 8	29 ± 4
30/26°C	A	121 ± 5	81 ± 6	28 ± 2
	B	56 ± 6	49 ± 7	28 ± 3

Table 3. Water used by tall fescue in both greenhouse experiments, expressed as a percent of the 26/22°C high-nitrogen treatment from the same run, averaged over all dates. With standard error of the mean.

		Water used (as % of High N, 26/22°C)		
Temperature	Experiment	High N	Low N	High N + TE
22/18°C	A	72 ± 2	69 ± 2	67 ± 2
	B	90 ± 3	70 ± 3	87 ± 3
26/22°C	A	**100** ± 1	95 ± 1	96 ± 1
	B	**100** ± 5	91 ± 1	97 ± 2
30/26°C	A	120 ± 1	116 ± 1	119 ± 1
	B	146 ± 6	160 ± 3	158 ± 4

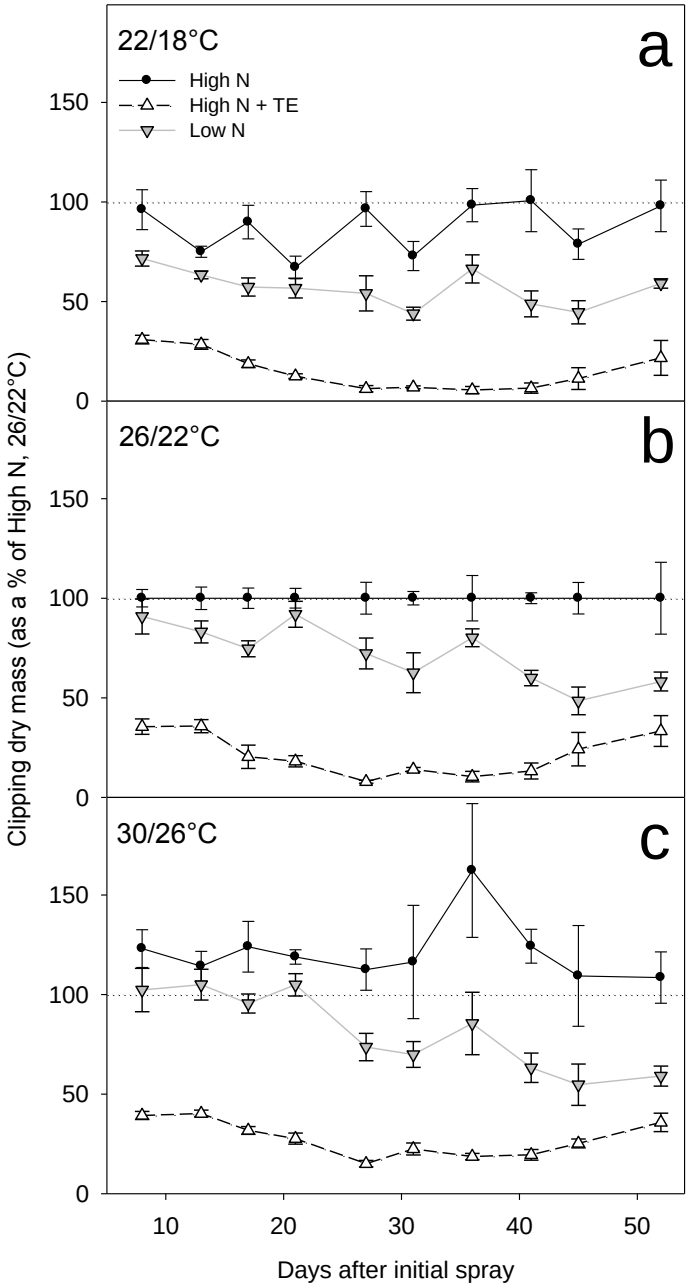


Figure 1. Dry mass of clippings from tall fescue plants in three temperature regimes: (a) 22/18°C, (b) 26/22°C, and (c) 30/26°C and three growth-control regimes: ● high N, △ high N + Trinexepac-ethyl , and ▼ low N. Data are from Experiment A and are presented as a percent of the 26/22°C, high-N reference treatment. Error bars represent s. e.of the mean.

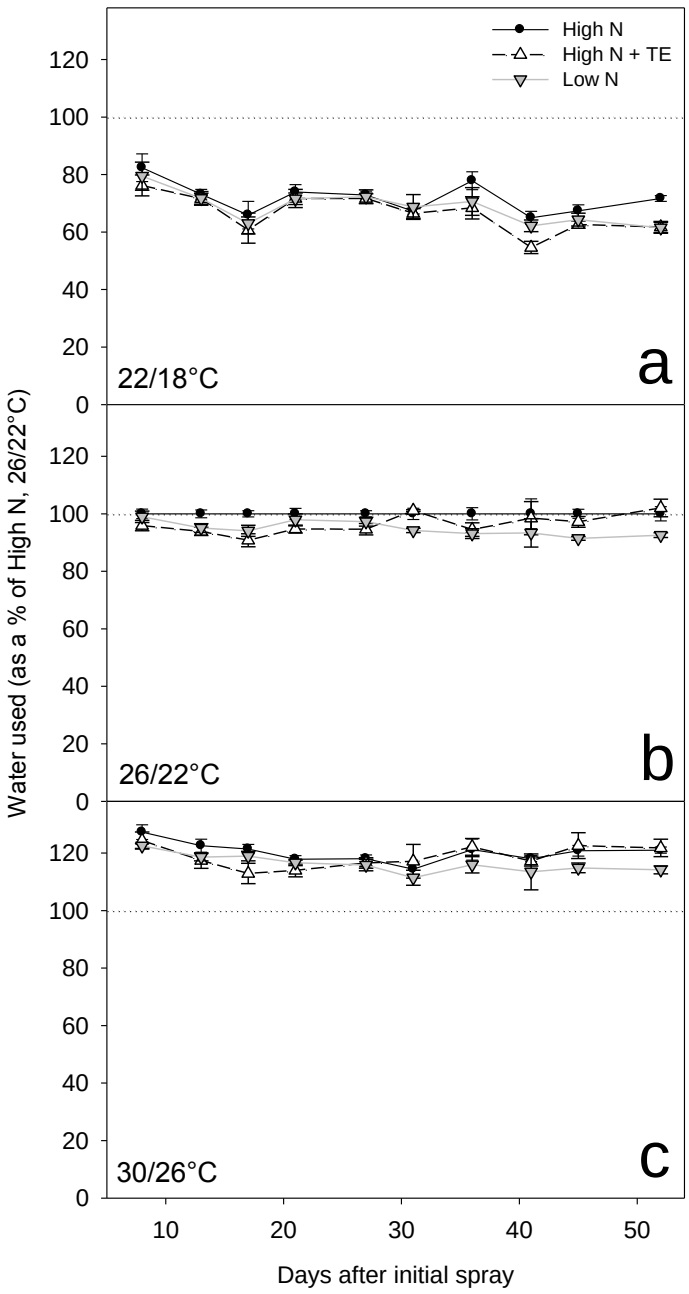


Figure 2. Water used (transpired + evaporated) by tall fescue plants in three temperature regimes: (a) 22/18°C, (b) 26/22°C, and (c) 30/26°C and three growth-control regimes: ● high N, △ high N + TE , and ▼ low N. Data are from Experiment A and are presented as a percent of the 26/22°C, high-N reference treatment. Error bars represent s. e. of the mean.

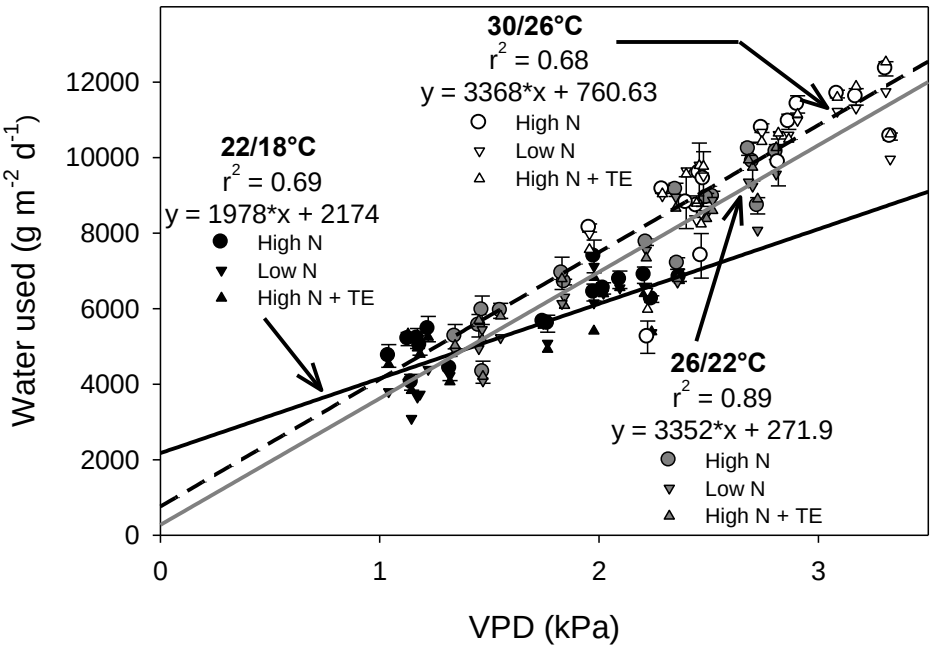
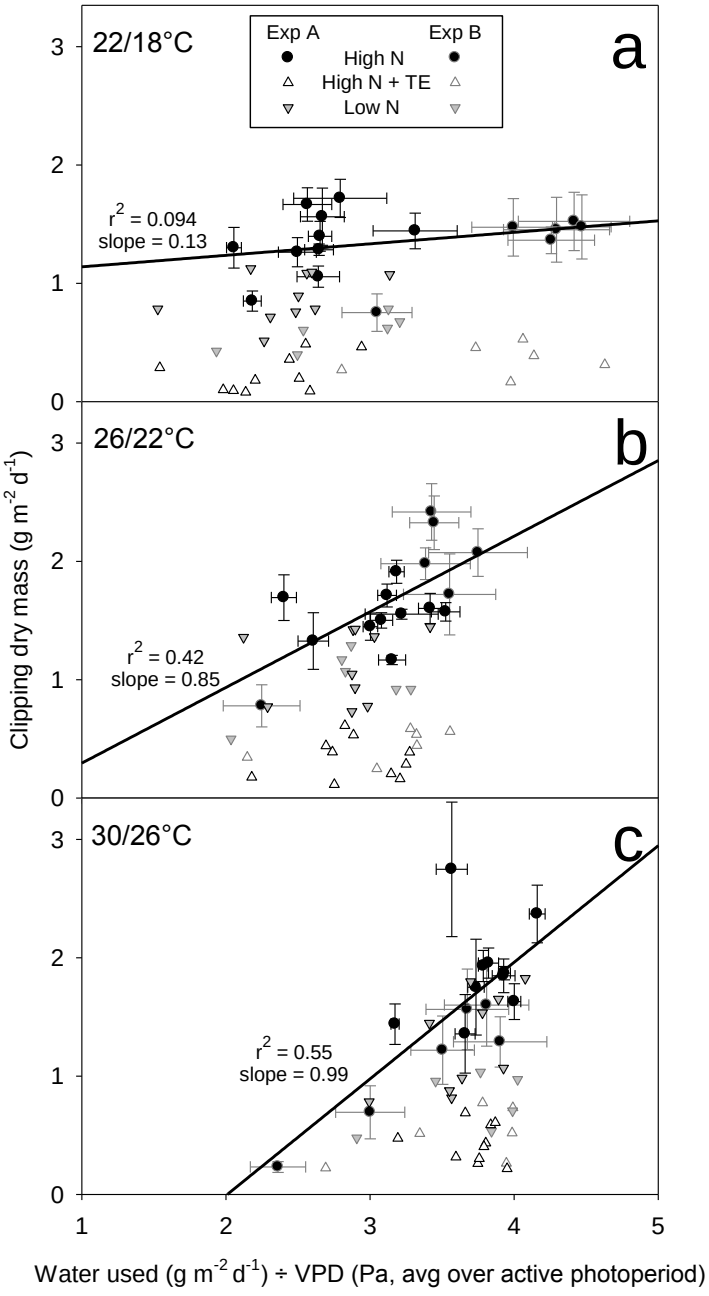


Figure 3. Water use vs VPD in three temperature regimes: solid symbols represent 22/18°C, gray symbols represent 26/22°C, and empty symbols represent 30/26°C. Symbol shape reflects the three different growth-control regimes: ● high N, ▼ low N, and ▲ high N + Trinexepac-ethyl. Regressions were fitted to all treatments combined (high N, low N, and high N + TE) within each temperature. Error bars, shown only for the high-N treatment, represent s. e. of the mean.

Figure 4. Clipping dry mass vs. water use / VPD in three temperature regimes: (a) 22/18°C, (b) 26/22°C, and (c) 30/26°C and three different growth-control regimes: ● high N, △ high N + Trinexepac-ethyl , and ▼ low N. Black-bordered symbols represent Experiment A; gray-bordered symbols represent Experiment B. A linear regression was fit to the high-nitrogen treatment in each panel and the slope of the fitted line corresponds with "k_s" (Equation 2 in text). Each point represents the mean of all replicates of a treatment from one measurement interval as described in the Methods. Error bars, shown only for the high-N treatment, represent s. e. of the mean.


22/18°C
Exp A
Exp B
High N
High N + TE
Low N
a
r2 = 0.094
slope = 0.13
26/22°C
b
r2 = 0.42
slope = 0.85
30/26°C
c
r2 = 0.55
slope = 0.99
Clipping dry mass (g m-2 d-1)
Water used (g m-2 d-1) ÷ VPD (Pa, avg over active photoperiod)

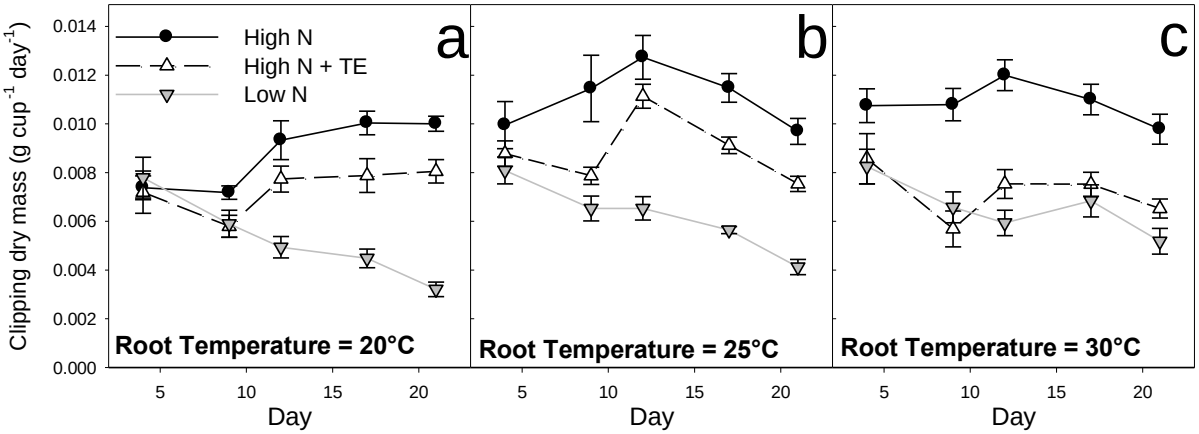


Figure 5. Dry mass of tall fescue clippings over time when grown in hydroponics at three root temperatures: 20°C (a), 25°C (b), and 30°C (c) and three growth-control treatments: ● high N, △ high N + Trinexepac-ethyl , and ▼ low N. Error bars represent s. e. of the mean.

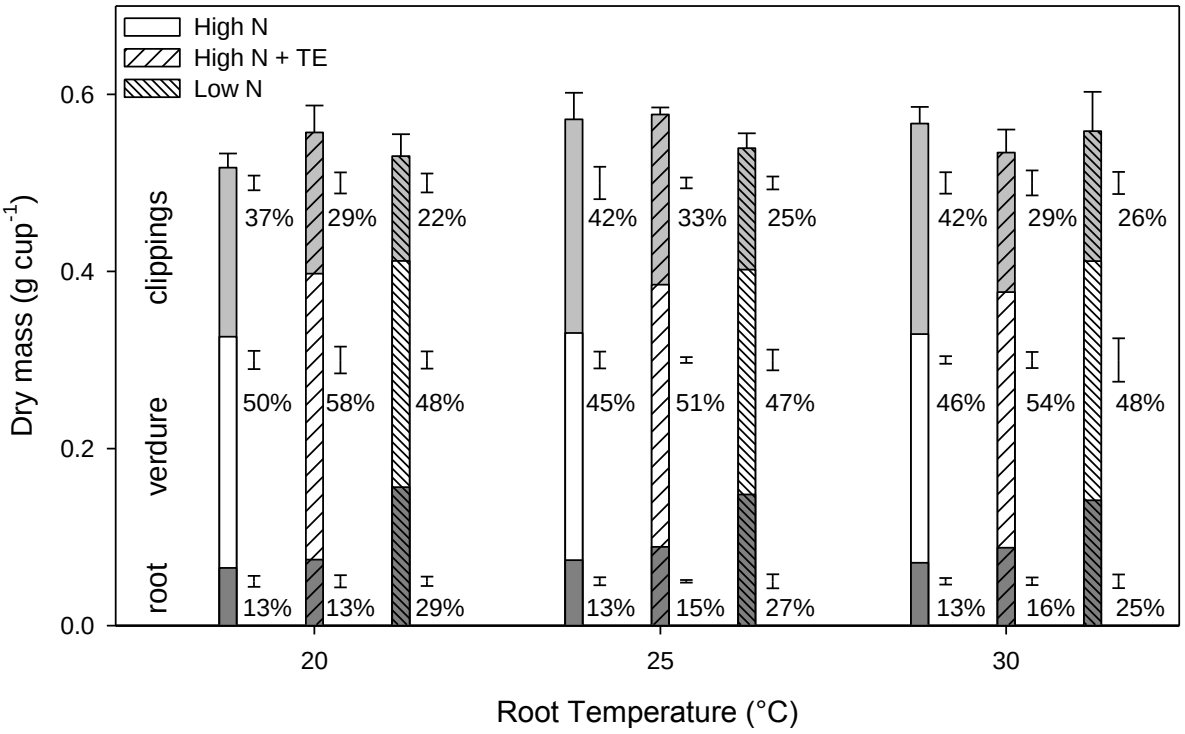


Figure 6. Dry mass of tall fescue grown in hydroponics at three root temperatures and three growth-control treatments, presented as a sum of root and verdure tissue collected at the experiment's conclusion and all clippings collected during the experimental period. To the right of each bar, the fractional contribution of each component is indicated along with an error bar representing the standard error of the mean for the component. The error bar at the top of each stacked bar represents s. e. of the mean for total mass.

CHAPTER 3

The Role of Internal and External Nitrogen Pools in Bermudagrass Growth During Spring Emergence from Dormancy

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Abstract. As bermudagrass (*Cynodon dactylon* (L.) Pers.) transitions from winter dormancy to active growth in spring, nitrogen is required as essential in the growth of new tissues. In these studies, the relative contributions of internally stored nitrogen and that taken up by preexisting and newly produced roots were examined. Dormant bermudagrass was transferred to a nutrient solution culture system and allowed to emerge from dormancy. Cultures were grown either with a non-nitrogen-containing solution or with one amended with ^{15}N-labeled nitrate, which allowed tracking of endogenous and exogenous N pools in all tissues over time. Nitrogen stored in stolons was the largest N source for early growth of new tissues. Though mass increased at the same rate in both treatments for 3 wks of growth, the un-fertilized treatment showed early signs of nitrogen depletion: low N concentration in

tissues, slowed leaf elongation, and fewer but longer roots. Preexisting roots were active almost immediately, and new roots were produced quickly and had even higher N uptake rates.

INTRODUCTION

Bermudagrass (*Cynodon dactylon* (L.) Pers.) is commonly used in the southern U.S. for lawns, recreation areas, and golf courses. A warm-season C4 perennial, bermudagrass experiences dormancy during the temperate winters in the northern part of its range, returning to active growth each spring (Taliaferro, 2003).

In considering nitrogen use in bermudagrass systems, two opposite perspectives arise. First, nitrogen is required by plants in larger amounts than any other soil nutrient and is generally recognized as the pace-setter of the growth process (Haynes, 1986); however, most soils, especially the highly weathered soils typical in much of the southern US, do not provide adequate nitrogen for turfgrass growth (Turner and Hummel, 1992). Thus, fertilizer nitrogen must be applied to turfgrass systems.

The other perspective on nitrogen use relates to environmental concerns over degradation of water supplies. If more nitrogen is applied than can be readily taken up by grasses, leaching into groundwater or runoff into surface water can occur. Limiting nitrogen applications to amounts equal to turfgrass uptake is a key for prevention of nitrogen loss and environmental damage (Barton and Colmer, 2006). In practice, proper management of nitrogen in bermudagrass is complicated, as nitrate leaching potential can vary widely depending on turf age and time of year. Several months after sodding, for example, nearly

half of applied N was leached from a common bermudagrass system, but by the following year, less than one percent was lost to leaching (Bowman et al., 2002). Also, previous research focusing on seasonal dormancy found that dormant bermudagrass took up only 13% of applied nitrate over a two-week period in January, leaving the remainder highly susceptible to leaching, but by May, about 8 wks after green-up, the turf was able to take up over 70% of applied N within 10 days of application, with another 13% bound in the closely associated soil microbial fraction (Wherley et al., 2009).

Optimizing fertilizer application is especially challenging in early spring, when bermudagrass is emerging from winter dormancy. Current recommendations typically advise that ½ to 1 lb of nitrogen per 1,000 ft^2 (2.44 to 4.88 g m^{-2}) should be applied several weeks after bermudagrass "greens up" (Bruneau, 2000; Hale and Burgess, 2005; Taylor and Gray, 1999). The recommendation assumes that internal nitrogen pools will be adequate for initial growth processes, while avoiding excessive nitrate loading of a system when bermudagrass has a limited uptake ability. Up to this time, however, little research has examined the physiological processes that underlie nitrogen use during this important period.

A bermudagrass phytomer is composed of several anatomical components that survive winter dormancy and interact during green-up. Nodes contain meristematic tissues that generate new leaves and roots (Stiff and Powell, 1974). But, as is typical for plant growth centers, meristematic tissues are made up mostly of small undifferentiated cells that possess little if any nitrogen storage capacity. Thus, continuous cell division, along with formation of essential macromolecules such as proteins and chlorophyll, requires nitrogen import, either from mobilized tissue nitrogen or from concurrent nitrogen uptake by roots.

The plant tissues most likely to contribute nitrogen for new growth during spring green-up are rhizomes and stolons, the internodal stem tissues containing layers of parenchyma cells with the capacity for nutritional storage (Rogers et al., 1976). Carbohydrate storage in stolons has been correlated with rapid spring green-up (Macolino, 2010); based on enhanced spring green-up with fall nitrogen application, nitrogen may also serve an important role in winter survival and emergence from dormancy (Goatley, et al., 1994; Richardson, 2002). Nitrogen may be stored in stem tissues (stolons and rhizomes), and possibly in roots. Pre-existing roots may also supply N for early spring growth by rapidly taking up available soil nitrogen, although their role has been debated and remains unclear (DiPaola, 1982). Roots of some grasses have been found to provide important reserves of nitrogen for spring re-growth (Gloser, 2002), although this is not the case for all species (Bausenwein et al., 2001).

The experiments described in this manuscript were conducted to further understanding of nitrogen relations during bermudagrass emergence from winter dormancy. By use of dormant phytomers grown in a controlled system and supplied with ^{15}N-labeled nitrate, it was possible to profile nitrogen movement from internal pools to new growth and evaluate the ability of roots to take up nitrogen during this important seasonal transition period. The results provide a physiological framework for understanding fertility recommendations during post-dormancy growth.

MATERIALS AND METHODS

Growth and nitrogen allocation study

Plant material. A sod cutter was used to collect dormant, established, common bermudagrass turf, including roots and soil to a depth of 10 cm, from the Sandhills Research Station in Jackson Springs, NC in March of 2010. The turf system, previously described by Wherley (2007), was typically managed by mowing at a 2 cm height and application of complete fertilizer from May to September. Segments, each containing similar-sized nodes and roots, were separated from the washed sod by cutting through the internode tissue 1 cm from the node, so that each growth unit (phytomer) consisted of a node, root, and two 1-cm sections of internode (stolons only, not rhizomes). Some nodes showed shoot emergence at the time of collection; these were not selected for use in the experiment.

Culture conditions. The collected phytomers were placed into eight continuous-flow solution culture (SC) units in a growth chamber. The nutrient solution in each unit consisted of 800 µM $CaSO_4$, 300 µM $MgSO_4$, 150 µM KH_2PO_4, 310 nM H_3BO_3, 60 nM $MnCl_2$, 55 nM $ZnSO_4$, 65 nM $CuSO_4$, 3 nM Na_2MoO_4. Every three to four days, 25 µmol L^{-1} $FeSO_4$ was added to each unit. Half of the units also contained 600 µM KNO_3 enriched to 3 atom percent of ^{15}N, while the others had non-isotopically-enriched 300 µM K_2SO_4. Plastic cups with mesh bottoms supported the node and internode tissues just above the nutrient solution with roots extending into the solution. The chamber temperature was maintained at 30/22°C with a 10-hour daylength and the solution temperature was held at 26°C and a pH of 6.5.

Measurements. A subset of eight phytomers was destructively harvested on day zero (placement into the solution culture units), and eight more from each nitrogen treatment were

sampled on days 3, 6, 10, 14, and 21 after initiation. On each collection date, the number of shoots on each phytomer (those that were harvested as well as those remaining in the solution culture units) was recorded. For the harvested phytomers, length of each shoot (measured from the node to the tip of the longest leaf blade), and number of leaves on each shoot were also recorded, along with number and length of newly produced roots. Each phytomer was dipped several times into 1 mM $CaSO_4$ to rinse off nutrients, then a razor blade was used to separate the tissues into node, internode, old root (brown color allowed identification of roots that were present at the time the phytomer was collected from the field), new roots (white color), and individual shoots (Fig. 1). The parts were individually dried at 60°C and stored in a dessicator until further analysis.

Data from preliminary studies allowed for estimation of tissue nitrogen content and ^{15}N enrichment. ^{15}N can be expressed in several ways: $\delta^{15}N$ represents differences in ^{15}N compared with the normal background atmospheric concentration on a ‰, or per-mil basis, and atom % ^{15}N, or $A\%^{15}N$, is a direct representation of ^{15}N as a percent of all N in the sample. Analysis required approximately 20 µg of nitrogen per sample, thus samples smaller than 1 mg were supplemented with ground citrus leaf standard (2.7% N; $\delta^{15}N = 3.8$, which is close to the normal, unenriched, background level of ^{15}N). Accuracy can be diminished in large samples and when size varies greatly from one sample to the next, so those with mass greater than 10 mg were subsampled (roots and shoots were cut with scissors, nodes and internodes cut with a razor blade). Samples were placed into tin capsules, weighed on a microbalance, and then analyzed at the Stable Isotope Mass Spectrometry Laboratory at Kansas State University. The 3 atom % enrichment of nitrogen in the nutrient solution

provided a very good contrast between endogenous and exogenous pools of nitrogen, based on ^{15}N measurements of the tissues. Tissues from phytomers given no external N had very small $\delta^{15}N$ values, 2.8 ± 0.6, while those provided external N ranged from $\delta^{15}N = 296 \pm 50$ on day 3 to $\delta^{15}N = 3370 \pm 220$ on day 21 (if all N had been derived from the enriched source, $\delta^{15}N$ would have been about 7400). Percent nitrogen and $\delta^{15}N$ for the samples, subsamples, and supplemented samples were used to calculate the amount of endogenous and exogenous nitrogen in each tissue.

Old and new root activity study

Separate experiments were conducted to examine the ability of roots to take up nitrogen. Procedures were generally similar to those described above, with bermudagrass turf collected from the same field site. A full description is given by Wherley (2007). ^{15}N-nitrate uptake from solution was measured with unaltered phytomers (referred to as intact-root treatment, having node, internode, and old root tissues, along with any shoot and new root tissues that developed during the study), and also with some phytomers that had all old roots removed at the beginning of the study (new-root-only treatment), and some with all new roots excised throughout the study (old-root-only treament). As in the previously described study, tissues were destructively harvested over time and mass, N content, and ^{15}N enrichment measured.

Statistical analysis

All statistical analyses of data were conducted using SAS 9.3 (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) for all continuous variables such as mass and length on each

sampling date was performed using the GLM PROCEDURE with means comparisons conducted between the two nitrogen treatments. The REG PROCEDURE was used to fit a regression comparing nitrogen content of internode and shoot tissues. Analysis of variance (ANOVA) for discrete data such as leaf and root counts was performed using the GLIMMIX PROCEDURE with underlying data distribution defined as Poisson distribution. Because of the variability in the data, differences in the nitrogen allocation study are discussed at $P<0.10$. For the root activity study, multiple comparison of means was performed using Tukey's Honest Significant Difference (HSD) at the probability level of 0.05.

RESULTS

Phytomer growth

Without external N. Growth was measured as increased phytomer mass over time as bermudagrass emerged from dormancy. In the –N treatment, total dry mass increased from 20±3 mg to 37±5 mg during the 21-day experiment. Most of this increase was due to shoot production (Fig. 2a), which contributed 10±2 mg, accounting for two-thirds of the new tissue accumulation. Any changes in mass of old tissues were relatively small, with the internode dry mass dropping by 2.6 mg (Fig. 2b), the node maintaining a dry mass of 3.8±0.5 mg, and the old root having high variability and no clear pattern of change, with an average dry mass of 5.1±1.0 during the experiment. The new roots were responsible for the remainder of the total increase, contributing half as much as the shoots, at 5.1±1.2 mg (Fig. 2c).

With external N. The trajectory of shoot growth suggested that application of nitrogen fertilizer increased the growth rate, but the shoot mass was not significantly different

between the +N and –N treatments even at day 21 (P=0.11) (Fig. 2a). Overall phytomer mass for the +N treatment, at 39±6 mg on day 21, was similar to the –N treatment. As in the –N treatment, node and old root mass in the +N treatment were fairly steady over time at 3.8±0.5 and 5.3±1.0, respectively, while internode mass dropped by nearly 5 mg (Fig. 2b). New roots contributed 4.6±1.6 mg in the +N treatment (Fig. 2c), similar to the –N treatment.

A comparison. Shoot tissue exhibited a larger increase in mass over time than any other tissue, but the difference between +N and –N treatments was not statistically significant. Rate of shoot initiation also showed no difference between treatments, as a similar number of shoots were produced in the +N and –N treatments throughout the entire experiment, ending with 1.75 to 2 shoots per phytomer (Fig. 3a). Similarly, leaf production, with 6.5±0.6 leaves per phytomer in the –N treatment, versus 8.4±1.0 in the +N treatment (Fig. 3b), was not statistically different. However, closer examination of the individual shoots reveals that those in the –N treatment were significantly shorter: by day 21, Shoot 1, the first initiated and therefore largest shoot on each phytomer, was 44±4 mm long in the –N treatment compared to 70±6 mm in the +N treatment (Fig. 3c, P<0.01).

Development of new roots also showed some small differences between treatments. Mass of new roots was the same in both the +N and –N treatments, and the number of new roots was also similar, with the exception of the final harvest date when the +N treatment had 2.6±0.5 new roots per phytomer, versus 1.4±0.2 in the –N treatment (Fig. 3d, P=0.10). Even so, the total length of new roots per phytomer was identical in both treatments (Fig. 3e) because the length of each individual new root in the –N treatment tended to be greater than in the +N treatment (Fig. 3f, P<0.05 at day 21).

Nitrogen partitioning

Without external N. By virtue of their large increases in mass, new shoots and roots were the major sinks for nitrogen (Fig. 4a-c). In the absence of applied nitrogen, growth was primarily associated with N transfer from the internode, which contributed ~60% of its original N by day 21. In contrast, the node and old root nitrogen pools were smaller than the internode pool and stayed fairly constant, averaging 52±8 and 62±10 µg N. Nitrogen accumulation in the shoot and nitrogen depletion in the internode were quantitatively similar (Fig. 5, slope = 0.94), suggesting that the internode served as the main storage reserve of nitrogen for spring shoot growth.

With external N. Supplying external N allowed the growing shoot to accumulate triple the amount of nitrogen (557±135 vs. 184±34 µg, $P<0.05$) of its unfertilized counterpart, but nitrogen accumulation in the new root did not differ significantly (113±33 vs. 56±11 µg, $P=0.13$) (Fig. 4a,c). The additional nitrogen in the growing tissues of the +N treatment was not due to greater net depletion in the original tissues, as the internode, node, and old root tissues had similar N content over time in both treatments (with the exception of old root at days 10 and 14, which in the +N treatment contained significantly more N than did the –N treatment, $P<0.05$). A closer examination of nitrogen from internal (endogenous) pools and N taken up from the ^{15}N-labelled fertilizer, however, reveals more complex movement (Fig. 6). During early growth, the main changes were, as expected, a decline in endogenous N in the internode and an increase in N, originating from internal pools, in the shoot. By Day 10, fertilizer N began to accumulate in the original tissues and also began to contribute to shoot growth. As root growth increased after day 14, the majority of N in new roots was from

newly acquired N (P<0.05). By the final harvest, a significant portion of nitrogen in the older tissues had been replaced with fertilizer N, which constituted 28% of the total nitrogen in the internode and about 40% in the node and old root.

Nitrogen status of tissues. Clearly, nitrogen content of new tissues was very different in the +N and –N treatments, showing a greater contrast than was seen in the tissue mass. Another way to examine the relationship of nitrogen with growth is to consider the nitrogen concentration in the tissues. In the –N cultures, the new tissues, shoots and new roots, had a high nitrogen concentration soon after initiation, but N concentration declined as the tissues continued to grow (Table 1a). With added N, initial N in the new tissues was similarly high, but the dilution over time was less.

Nitrogen uptake potential of old and new roots

The role of old and new roots in taking up nitrogen during emergence from dormancy must be considered. As in the allocation study, mass of old roots did not change much over time, while mass of new roots increased (Fig. 7a). Importantly, these data showed that uptake of fertilizer nitrogen began within the first week, and that the old-root-only treatment was able to take up nearly as much nitrogen as the intact-root treatment (Fig. 7b).

While mass of the new roots was small, their rate of nitrogen uptake was very high: 2.3 ± 1.6 μg N mg^{-1} root DW hr^{-1} during the final measurement interval, versus 0.12 ± 0.04 and 0.10 ± 0.05 μg N mg^{-1} root DW hr^{-1} in the old-root-only and intact-root treatments (Fig. 7c). Thus, compared with the intact-root treatment, the new-root-only treatment was able to take up 60% as much nitrogen despite having only 13% as much root tissue (Fig. 7b).

Statistical comparisons were challenging due to high variability and the small number of measurement dates that included all treatments, but in the interval between day 6 and day 10, the new-root-only treatment did have a significantly higher rate of N uptake than did the other treatments ($P<0.01$), confirming the high level of N uptake activity.

DISCUSSION

This experiment assessed changes in internal nitrogen partitioning and the ability to take up external N during the first three weeks of bermudagrass emergence from dormancy, a time of rapid adjustment to active growth. Emergence from dormancy is a process of activation of existing tissues and the subsequent production of new shoots and roots. The production of the new shoots and their development of chlorophyll is synonymous, physiologically, with "greening up" in a bermudagrass system.

The profiles of tissue nitrogen content clearly show the importance of internode nitrogen for new growth, particularly when the external supply is absent. Because of the nitrogen requirement for cell division and synthesis of macromolecules in the meristematic areas, and the relatively low amount of nitrogen in the meristematic centers themselves, there must be a continual supply of nitrogen for growth to occur. Without externally supplied nitrogen, the nitrogen in internode tissues was steadily depleted over the 21 day experiment in an amount quantitatively similar to that accumulating in the developing leaves. Any contribution of node and old root nitrogen to shoot growth would have been small, relative to the contribution from internodes. A similar depletion of internode N occurred when external nitrogen was supplied. Though fertilizer N did accumulate in the internode (by the end of the

21 days, 30% of the total internode N content was comprised of fertilizer N), the total N content declined in exactly the same quantity as in internodes of the –N treatment. Since the decline was not offset or prevented by external uptake of nitrogen and mixing with the original internal nitrogen pool, the depletion pattern appeared to indicate that the internode was 'programmed' to empty of nitrogen.

The importance of internodes for winter storage, in this case storage of nitrogen, that we observed aligns well with previous observations that stolon tissue can also store carbohydrates that contribute to spring green-up (Macolino 2010). As is the case with carbohydrates, our results imply that increased internode storage of nitrogen over winter is responsible for the instances where fall fertilization leads to faster green-up in the spring (Goatley et al, 1994; Richardson, 2002). We do not know the form of nitrogen being stored in the internodes during winter dormancy, but rapid mobilization during the transition out of dormancy suggests either that nitrogen is in a form that is easily transported, e.g. nitrate and amino acids (Dunn and Nelson, 1974), or that substantial amounts of proteases are present to break down larger protein molecules. Our results add to the mixed results regarding the importance of rhizomes and stolons for winter nitrogen storage in grasses (Gloser, 2002; Perry and Moser, 1974)

The amount of nitrogen supplied from the internode was sufficient to drive growth similar to that with an external supply until the end of the 3-week experiment. Nonetheless, the decline in tissue N concentrations and changes in shoot and root morphology compared to bermudagrass supplied with external nitrogen demonstrated that nitrogen was becoming deficient about 10 days to two weeks into the experiment. A limitation in vertical shoot

growth, fewer new roots, and elongated roots all are indicative of nitrogen deficiency in higher plant systems (Drew et al., 1973; Rufty et al, 1984).

When nitrogen was applied externally, uptake of fertilizer nitrogen began immediately, but the amount of uptake was relatively small for the first week. Old roots were mainly responsible for ^{15}N uptake initially, as new root growth was limited during the first two weeks of the experiment. The increase in ^{15}N uptake, and movement of the absorbed N to developing leaves, coincided with the increased rate of shoot growth as the experiment progressed. This reflected, at least in part, increasing contributions from the developing new roots. The root activity study indicated that the new roots had the capacity for very rapid uptake per unit of root mass.

The increase in ^{15}N-nitrate uptake by old roots over time in the growth and N allocation study suggests that uptake systems were under feedback control (refer to Clarkson, 1986; Glass, 2003). According to shoot and root 'Interdependence Theory', this is what one would expect (Rufty, 1998). As shoot development proceeded and the growth potential of the plant increased, de-repression of the nitrogen uptake systems and higher uptake would follow. The root activity study also indicates involvement of higher-level plant control over nitrogen uptake. Comparison of the combined N uptake by the old-root-only and new-root-only treatments, 778±179 µg, versus the uptake of the intact-root treatment, 524±105 µg, suggests that root uptake of nitrogen in the intact phytomer was under feedback control. By comparison, our observed range of N uptake rates was similar to the range seen in *Lolium multiflorum* roots, about 0.2 to 0.8 µg N mg^{-1} root DW hr^{-1}, when uptake rates were manipulated by localized exposure of some roots to a supply of NO_3^- (Laine et al., 1998).

Implications for the field

It is always difficult to extend results from a controlled setting to the field environment. Nonetheless, physiological events occurring with green-up of the bermudagrass in our system seems entirely consistent with descriptions from the field. Internal nitrogen reserves, primarily mobilization of nitrogen in internode tissues, were sufficient to support new leaf development for about 10 days to two weeks. Thereafter, green-up proceeded without external nitrogen, but early symptoms of nitrogen deficiency were observed. The nitrogen uptake capacity of the bermudagrass was increasing at 10 to 14 days, just as growth was beginning its rapid growth phase.

From the progression of physiological events, the normal recommendations of fertilizing bermudagrass about 2 weeks after first green-up seem appropriate. The bermudagrass is morphologically able to respond to nitrogen at that time, and relatively efficient uptake would minimize the likelihood of nitrogen leaching. However, this approach could be detrimental if the amount of nitrogen applied exceeds the rate of bermudagrass uptake. In that case, slow-release fertilizers certainly would have an important role (Guertal and Howe, 2012; Quiroga-Garza 2001).

ACKNOWLEDGMENTS

Thanks to Mike Jennette for his expert technical assistance, Troy Ocheltree at SIMSL for careful analysis of our samples, and the Sandhills Research Station (especially Mark Thompson) for providing the dormant bermudagrass.

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Table 1. N concentration in new tissues of phytomers relying on internal N reserves and those supplied external N. Values in bold lettering showed significant differences ($P<0.05$) between the +N and –N treatments.

	%N			
	--------shoots--------		--------new roots--------	
day	- N	+ N	- N	+ N
0				
3	3.35 ± 0.07	3.55 ± 0.13		
6	4.12 ± 0.22	4.38 ± 0.33		3.13
10	3.98 ± 0.34	4.39 ± 0.14	**2.61 ± 0.04**	**3.21**
14	3.65 ± 0.27	3.97 ± 0.35	2.29 ± 0.28	3.15 ± 0.18
21	**2.06 ± 0.43**	**3.16 ± 0.19**	**1.52 ± 0.30**	**2.66 ± 0.16**

Table 2. N concentration in old tissues of phytomers relying on internal N reserves and those supplied external N. Values in bold lettering showed significant differences ($P<0.05$) between the +N and –N treatments.

	%N					
	--------node--------		--------internode--------		--------old roots--------	
day	- N	+ N	- N	+ N	- N	+ N
0	1.59 ± 0.08	1.59 ± 0.08	1.88 ± 0.16	1.88 ± 0.16	1.56 ± 0.13	1.56 ± 0.13
3	1.58 ± 0.05	1.79 ± 0.25	**1.85 ± 0.13**	**1.40 ± 0.12**	1.31 ± 0.09	1.23 ± 0.07
6	1.64 ± 0.10	1.27 ± 0.15	1.72 ± 0.21	1.26 ± 0.10	1.20 ± 0.10	1.30 ± 0.15
10	1.29 ± 0.12	1.14 ± 0.07	1.21 ± 0.21	1.40 ± 0.16	**1.13 ± 0.12**	**1.92 ± 0.10**
14	1.40 ± 0.11	1.13 ± 0.07	1.33 ± 0.21	1.56 ± 0.21	**1.25 ± 0.08**	**1.57 ± 0.08**
21	0.93 ± 0.12	1.05 ± 0.04	**0.88 ± 0.09**	**1.19 ± 0.09**	**1.29 ± 0.06**	**1.60 ± 0.13**

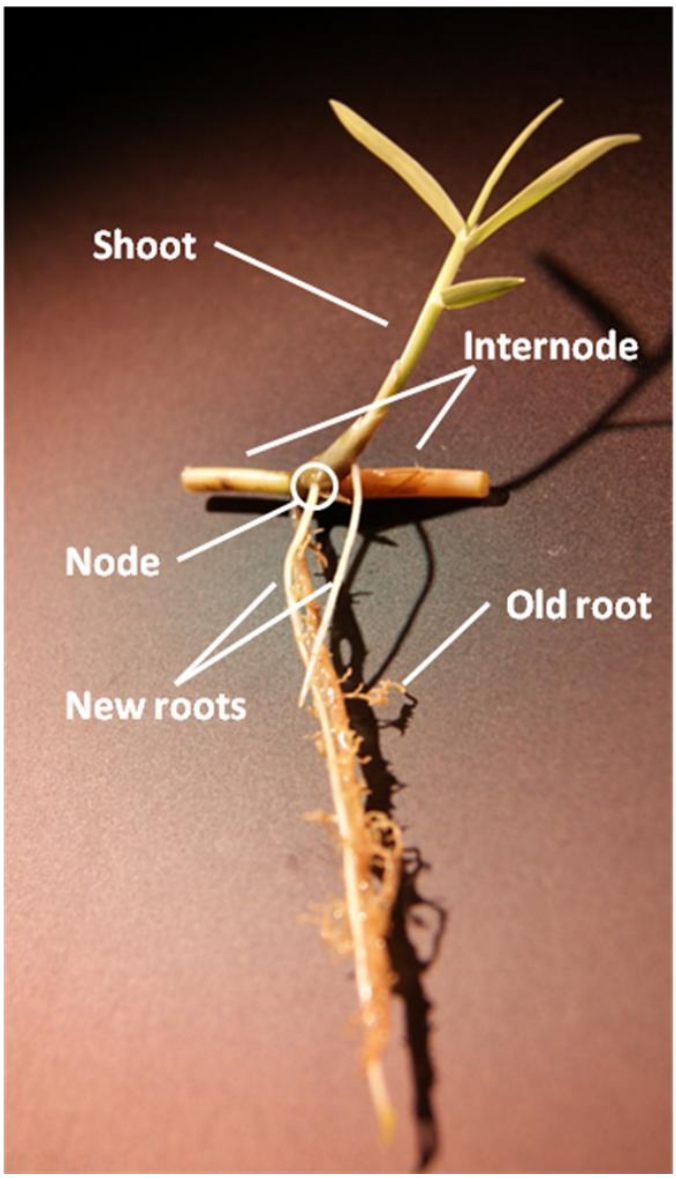

Figure 1. Photograph of a phytomer at Day 14. Mass and nitrogen measurements were made on individual tissues: the original tissues of node, internode, and old root were separated, and within the new tissues, new roots and each shoot (one for this phytomer) were collected separately. The node is obscured in this photo by the new growth, but it was easily distinguished after removal of the shoot and roots.

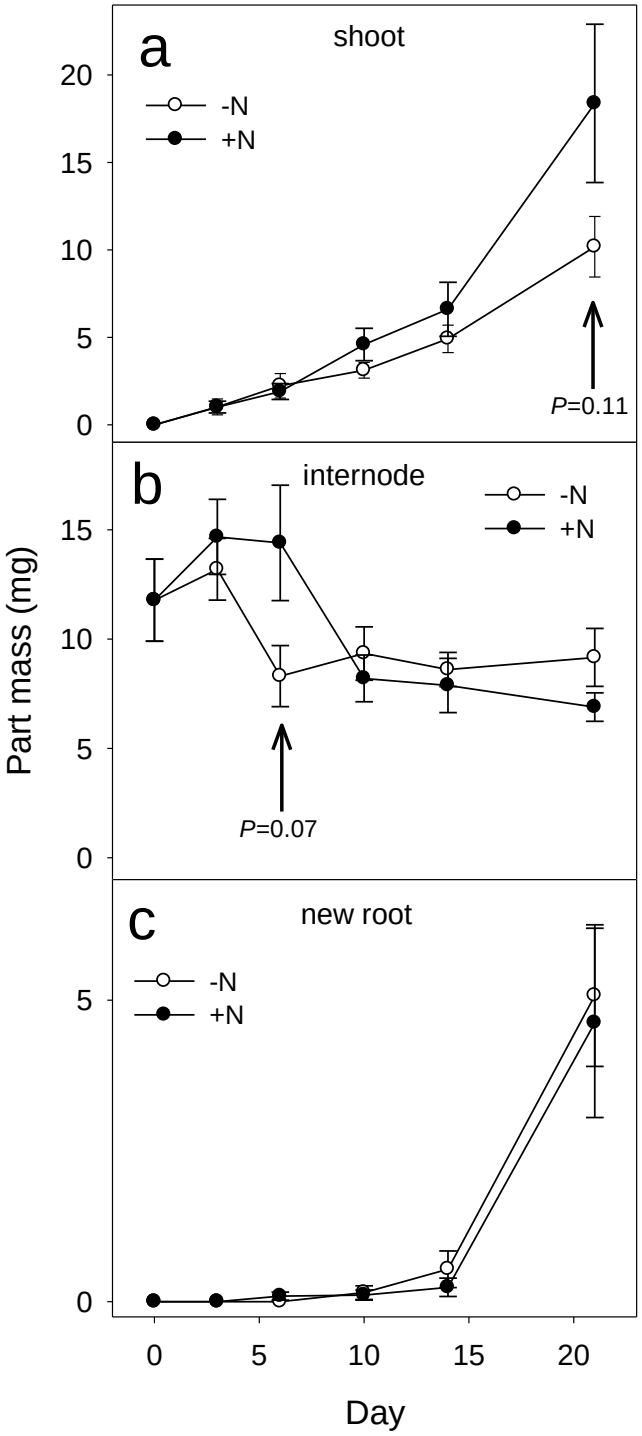

Figure 2. Mass of (a) shoots, (b) internode, and (c) new roots over time. Note that the y-axis scale differs among panels.

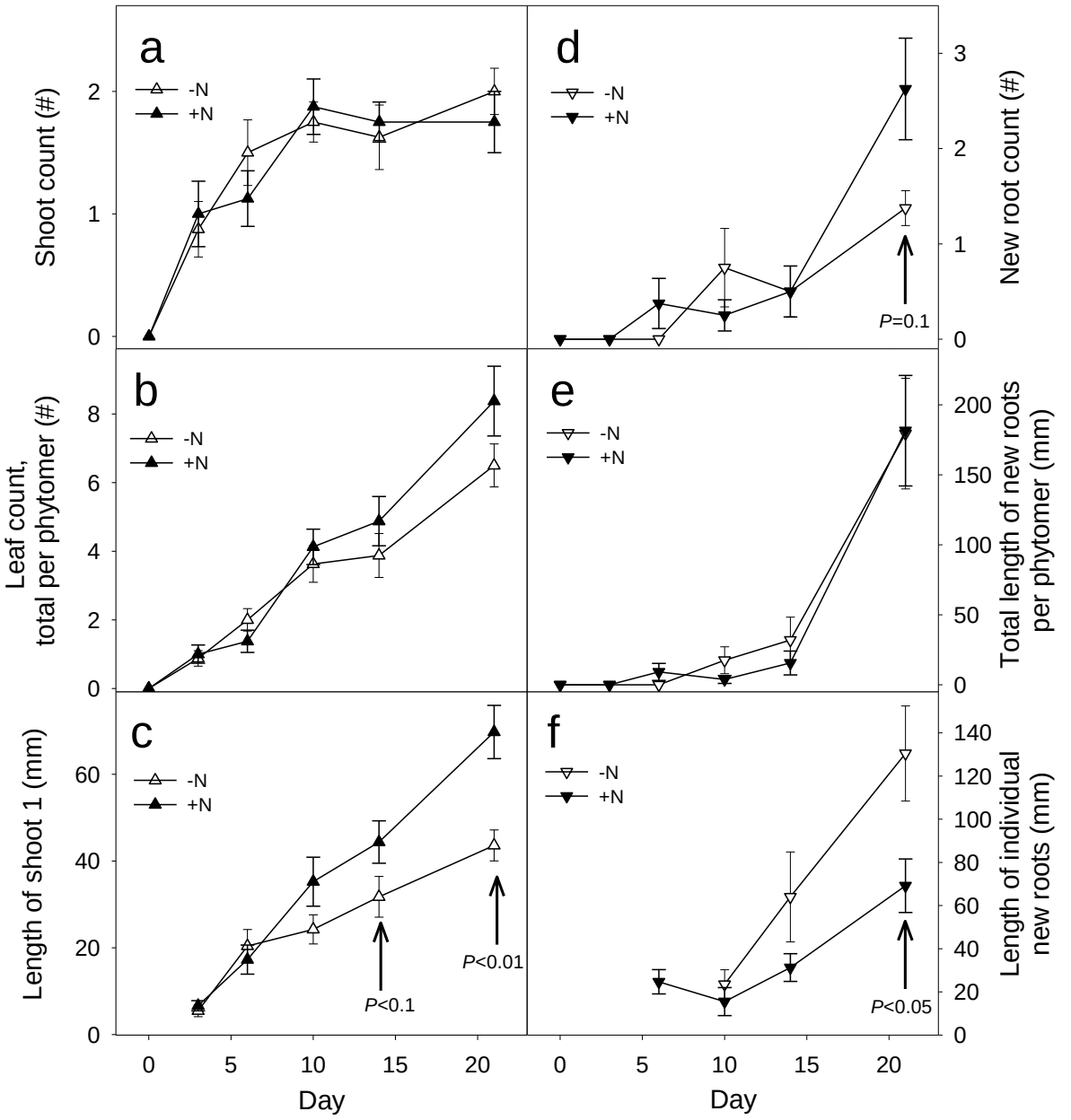

Figure 3. Measurements of new tissue growth: (a) number of shoots, (b) number of leaves, (c) length of first shoot to emerge, (d) number of new roots, (e) total length of new roots, and (f) average length of individual new roots.

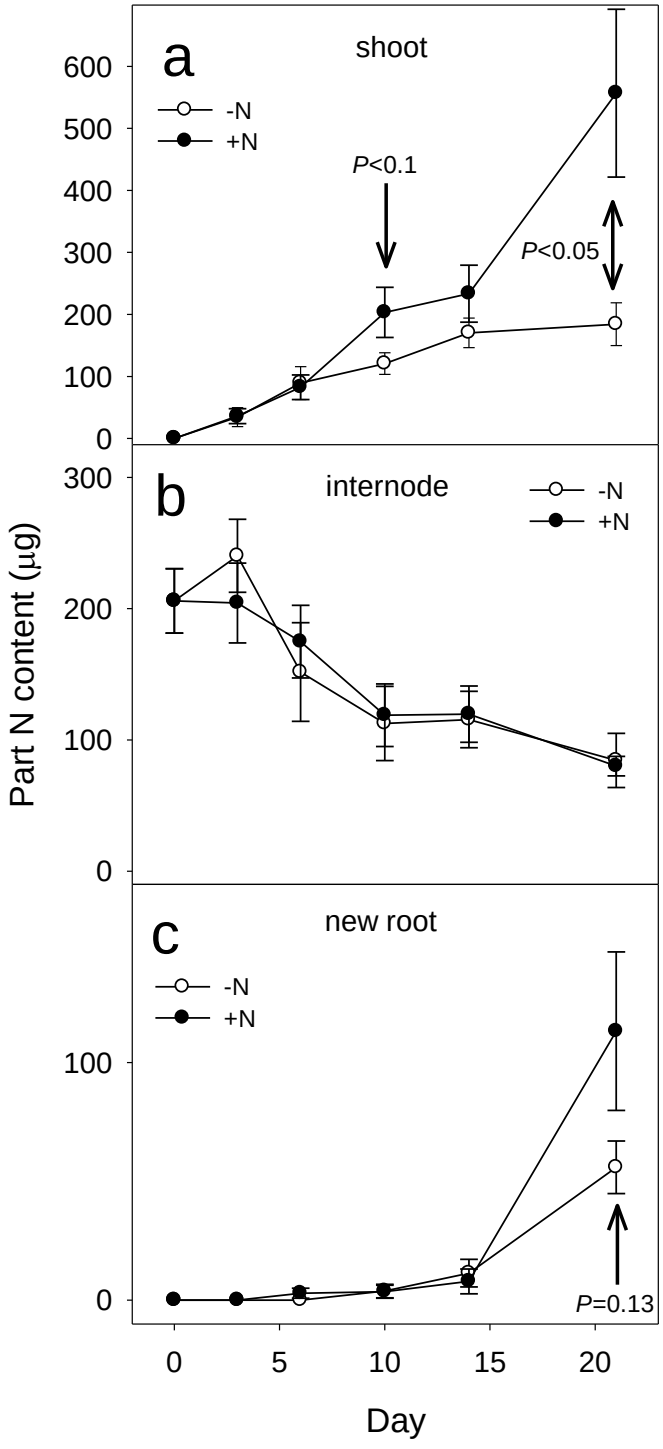

Figure 4. Nitrogen content of (a) shoots, (b) internode, and (c) new roots over time. Note that the y-axis scale differs among panels.

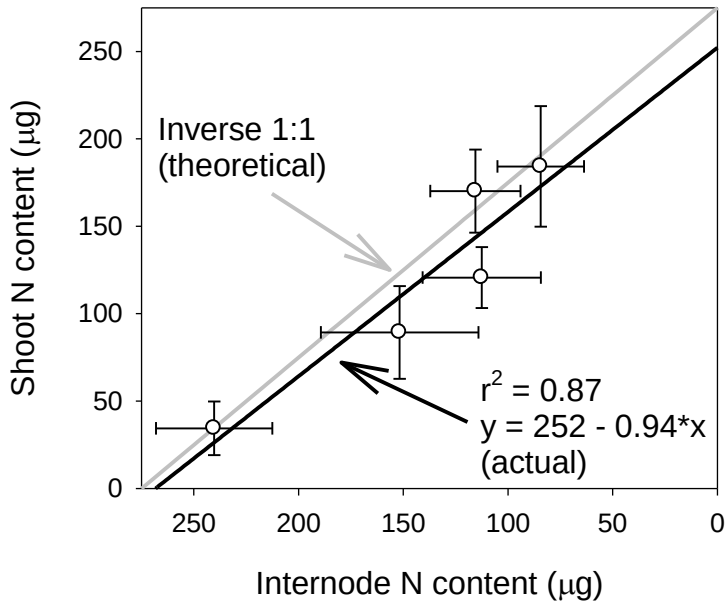

Figure 5. Nitrogen content of shoots and internode of -N treatment only (no external nitrogen applied). Inverse 1:1 line is shown for comparison.

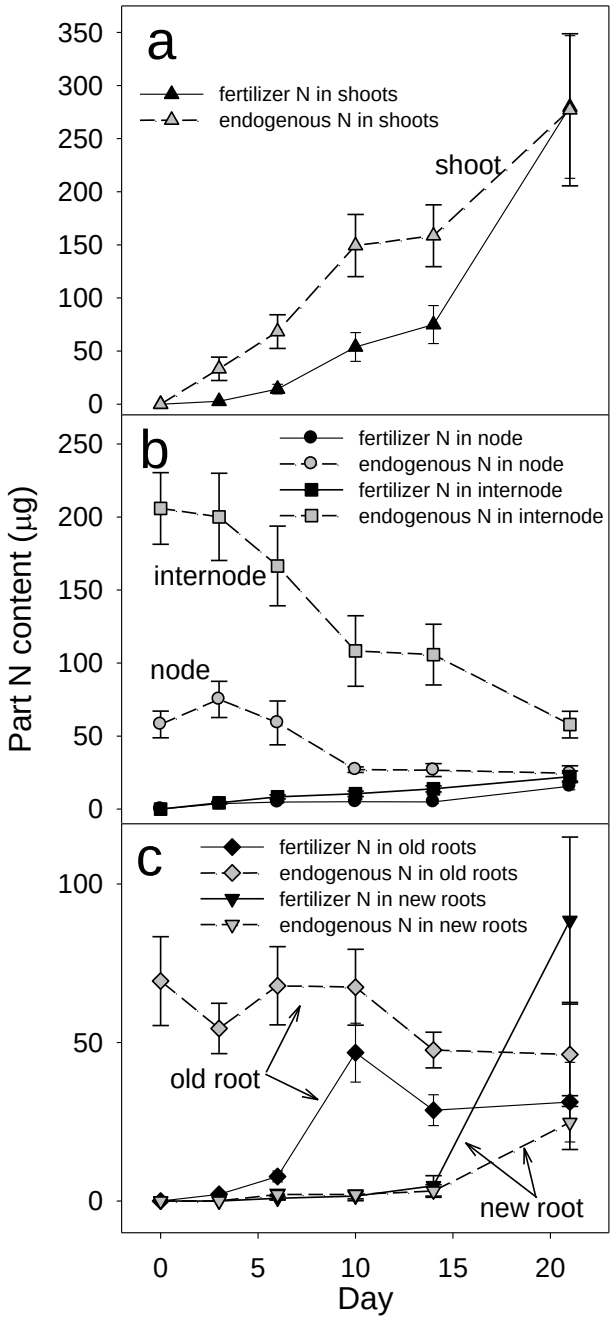

Figure 6. Amount of nitrogen derived from endogenous phytomer reserves and from newly acquired fertilizer nitrogen in each tissue: (a) shoots, (b) internode and node, and (c) old roots and new roots. Amount of nitrogen from each source was calculated from ^{15}N measurements. Only the +N treatment is shown. Note that the y-axis scale differs among panels.

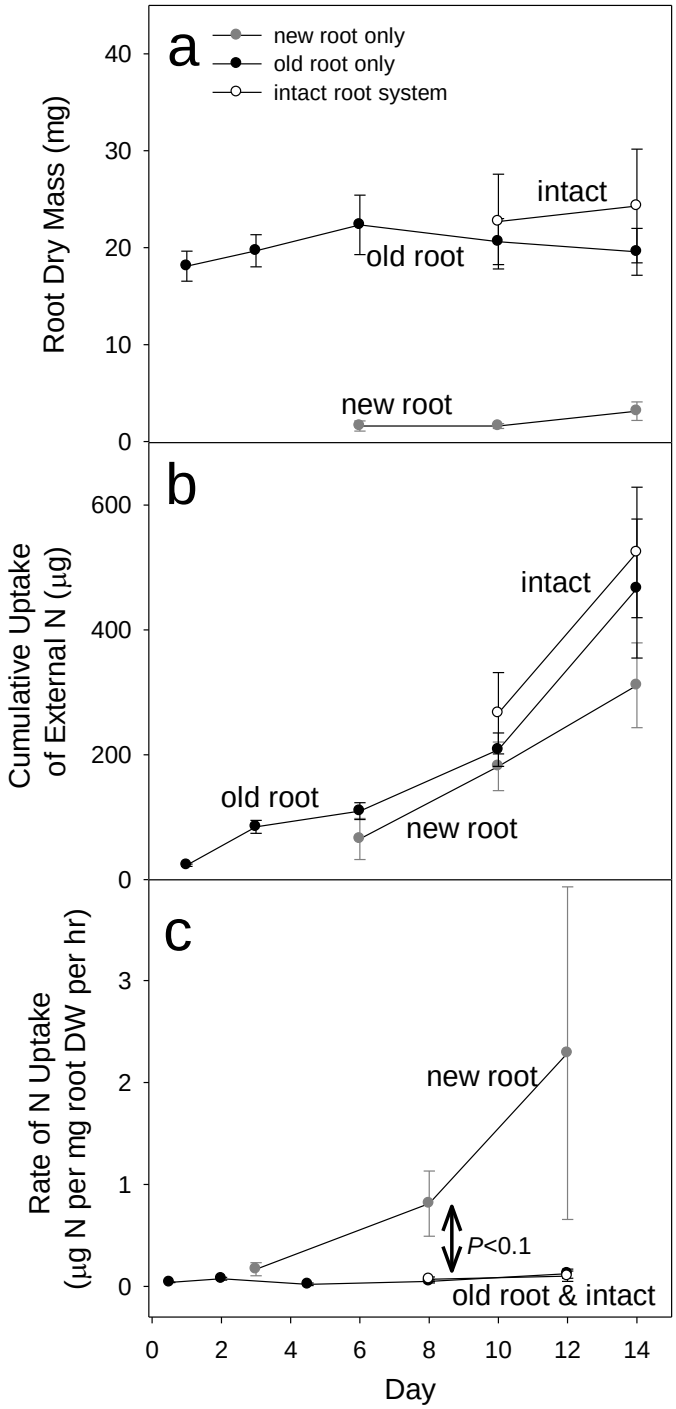

Figure 7. (a) Root mass, (b) phytomer N originating from external sources, and (c) calculated rate of N uptake efficiency from the nitrogen uptake study.