Assessment of first-year survival, growth, and physiological performance of seven species of graminoids within two substrate types on a green roof in the Flint Hills Ecoregion

by

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Abstract

Native vegetation on green roofs is desired due to the functions and values that indigenous plant communities provide. This includes native warm and cool season grasses. Plant selection on green roofs needs to account for the likelihood that specified species will be able to survive water stress, limited soil or substrate depths, and potentially harsh rooftop conditions. There is insufficient research on specific plant species that can perform well on an extensive green roof approximately four inches (10.16cm) deep in Manhattan, Kansas (U.S.A.). Growing conditions are largely affected by substrate characteristics such as organic matter, nutrient content, and the ability to hold moisture. The goal of this study is to lay the groundwork for the appropriate selection of native graminoids for green roofs in the Flint Hills Ecoregion, and assess the performance of two engineered substrate types used on the Experimental Green Roof (composed of three beds of different substrate depths) atop the new Architecture, Planning and Design building at Kansas State University.

The study measured plant survival, growth, and physiological performance of one native sedge and six grass species over the first growing season in two distinct types of engineered substrates. Visual assessments were used to quantify survival, and growth was determined by measuring the above-ground biomass, height, and coverage of the plants. Plant physiological performance was assessed by measuring stomatal resistance. The study was conducted for the first growing season (plants were installed on the Experimental Green Roof in October 2017 and a number of live plants were replanted in May and June 2018) with vegetation observations made and other data collected from late June to mid-October 2018. This research provides valuable baseline information for a longer-term study of this extensive green roof system.

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Chapter 1 - Introduction

Green roofs have been called "anthropogenic patches," or intentionally vegetated roofs, and are gaining worldwide popularity as a response to increasing impervious surfaces in urban areas and the resultant decrease of natural aesthetics (Sutton 2015). Green roof design and construction have been promoted as normative activities: practices that we ought to implement in order to create specific environmental, social, and economic benefits (Sutton 2015). Green roofs provide these benefits to a greater degree when vegetation and substrates fit with local climatic conditions (Cook-Patton 2015) and with the provision of effective maintenance/ management. Green roof benefits such as stormwater capture, building insulation, trapping of airborne particulates, carbon sequestration, habitat establishment, and amenity and aesthetic values, are attributed largely to green roof plants and substrates (Lundholm, Tran, and Gebert 2015). Moreover, green roofs are expected to increase the life span of the roof structures by protecting them from harmful solar radiation and ultraviolet light (Getter and Rowe 2006).

There are various factors that affect the performance of vegetation on green roofs. Establishment of live plants on green roofs requires healthy plants, properly planted, and adequate moisture to grow their roots and support above-ground biomass. Green roof plants need to be able to survive season to season climatic variations as well as specific micrometeorological conditions on a green roof, in addition to any limiting conditions related to substrate depth and composition, and water availability (Sutton et al. 2012). Various attributes related to green roof vegetation have been a matter of interest to many green roof researchers in the field.

Green roof research is a relatively new field of interest, having taken shape in the 1990s (Jim 2017). Hence, there are various factors that have not been investigated in terms of green roof vegetation and substrate, providing many research opportunities. One such factor, as noted by Sutton et al. (2012), is the long-term survival and adaptability of specific native plant species on green roofs in relation to different substrate types. In order to really understand long-term health and adaptations we first need good baseline studies. Documenting baseline conditions related to plant survival, and exploring selected tools and techniques to better understand growth and physiological performance on the new APD-EGR are the two primary purposes of this thesis.

Background and Statement of the Problem

Research on prairie grasses and forbs on steeply-sloped green roofs in Manhattan, Kansas was published in 2017 in the CitiesAlive: 15th Annual Green Roof and Green Walls Conference Proceedings (Skabelund, Decker, et al. 2017). This research revealed good coverage by native plants on sand-based green roofs (with a mostly sandy substrate mix on the West Memorial Stadium Green Roof, and a sand-Buildex substrate mix on the East Memorial Stadium Green Roof, both located at Kansas State University) with reasonably consistent supplemental irrigation over three- and two-year periods respectively. Prior to that, multi-year observations were published by Skabelund et. al in 2014, assessing the survival of native plants on a small 305 square-foot (36.70 sq.m.), three to seven-inch deep (approx. 7.5-18cm) green roof at Kansas State University (KSU). The researchers concluded that selected native plants can survive on a green roof setting in our continental climate with supplemental irrigation during prolonged dry spells, while a few species can survive for more than a few years with little or no irrigation (Skabelund, et al. 2014). This research identified the need to expand the scale of the research, increasing the number of native plant species studied (Skabelund, et al. 2014) and doing so more systematically (L. Skabelund, KSU-LARCP faculty member, pers. comm., April 2018).

Between 2015 and 2016 an experimental green roof was designed by Lee Skabelund, working with other researchers at KSU and members of the Ennead-BNIM-Confluence design team. Landscape architects at Confluence were tasked with the responsibility of completing construction drawings and documents for the Kansas State University Architecture, Planning, and Design Experimental Green Roof (APD-EGR), which was constructed in 2017. Figure 1-1 shows the 4-inch, 6-inch and 8-inch deep APD-EGR beds (with the 4-inch bed closest to the camera at the north side of this experimental green roof), and Figure 1-2 shows the 4-inch bed.

Figure 1-1: APDesign Experimental Green Roofs

(Photograph by Lee Skabelund, taken in October 2017, at the APDesign Experimental Green Roof, Kansas State University)

Figure 1-2: 4-inch (10.16cm) deep plots

(Photograph by Lee Skabelund, taken in October 2017, at the APDesign Experimental Green Roof, Kansas State University)

The research I conducted involved the initial study of one native sedge and six native grasses on the APD-EGR at Kansas State University in Manhattan, Kansas. My study employed a systematic study of selected plant species survival, growth, and physiological performance on the APD-EGR. The APD-EGR was planted in October 2017 and then partially replanted May and June 2018.

The substrate is one of the most important parts of a green roof system because most of the water holding capacity is dependent on the substrate type and depth. The substrate also accounts for much of the saturated and dry dead loads on a green roof (Sutton et al. 2012). Best, Swadek, and Burgess (2015) assert that the success of green roof plants is largely affected by the substrate capacity for water retention, adequate drainage, and nutrient availability. Substrate characteristics such as texture and organic content are essential for growing and maintaining healthy green roof plants (Best, Swadek, and Burgess 2015).

Organic and inorganic substrate components and their combinations affect plant survival, growth, and success on green roofs. Different engineered growth media have different proportions of organic and inorganic components to create particle size and mineral composition combinations that positively affect certain green roof criteria at the expense of others (Griffin 2014). For instance, a low organic substrate with high infiltration rates and porosity may not support plant growth as well as a substrate with more organic matter and higher saturated weight (Best, Swadek, and Burgess 2015).

It was unknown what substrate components and combinations best support plant performance in the continental climate of Manhattan, Kansas. Hence, this study examined plant survival, growth, and physiological performance for two engineered substrate types used on the APD-EGR: Kansas BuildEx® and rooflite® extensive mc. This research thus sought to scientifically assess these two commercial substrates in supporting the success of selected graminoids on this 24-plot “four-inch” deep experimental green roof (with actual substrate depths found to range from approximately seven to ten centimeters deep in late June 2018).

Assessing Plant Performance on the APD-EGR—Building upon Previous Studies

Farrell et al. (2013) assert that using vegetation adapted to similar environmental conditions as the specific green roof can help improve the performance of these plants. According to Anderson and Fly (1955), the soil on the upland ridges of the Flint Hills Ecoregion is thin (Anderson and Fly 1955), which was ascribed by the APD-EGR designer to be somewhat similar to a shallow 4-inch (10.16cm) extensive green roof profile (L. Skabelund, KSU-LARCP faculty member, pers. comm., April 2018). Given successful use of native plants on other green roofs at KSU, the Chicago Botanical Garden Green Roofs (Hawke 2015), and throughout North America (Dvorak & Volder 2010), there is reason to suggest that prairie vegetation will do well on green roofs, assuming that adequate moisture is provided (by precipitation and/or supplemental irrigation) to keep the roots alive during dry periods.

Since the Flint Hills tallgrass prairie is dominated by grasses (Best, Swadek & Burgess 2015), my one-growing-season study (early summer to fall 2018) evaluated the readily available and commonly used grass species selected for the APD-EGR, along with one common, dry-site sedge. Each of these seven species are native to the tallgrass prairie of the Flint Hills Ecoregion, in north-central Kansas, spanning from Marshall County in the north to Cowley County in the south, across the border into northeastern Oklahoma (“Flint Hills | GeoKansas.” n.d.). The intent of my research was to assess the success of each species from a survival, growth, and plant physiological performance standpoint on this approximately 4-inch (10.16cm) deep experimental green roof in Manhattan, Kansas.

Growth and survival, after initial establishment of live plantings, are primary indicators of plant performance on green roofs (Kazemi and Mohorko 2017). Various studies have investigated the growth and survival of green roof plants as indicators of plant selection in different climate regimes and green roof conditions (Graceson et al. 2014; Dvorak and Volder 2013; Monterusso, Rowe, and Rugh 2005; Lundholm et al. 2014; Durhman, Rowe, and Rugh 2007). Thus, for a green roof in Manhattan, Kansas, assessing survival and growth, through height and cover measurements, was highly applicable.

Several studies have also recognized physiological traits of plants as indicators for assessing which plants could succeed in coping with limited irrigation and harsh meteorological conditions on a rooftop setting. Farrell et al. (2013) assessed the physiological performance in terms of plant water status and transpiration of granite outcrop plants, including a *Sedum* spp. and other monocots, herbs, and shrubs across well-watered and water-deficient treatments. For the purpose of managing urban stormwater runoff (an objective of most green roofs in urban settings) these authors indicated that plants selected for green roofs should be both high water users and drought tolerant (Farrell et al. 2013). They also reported a list of species that performed well in the experiment conducted in southeastern Australia (Farrell et al. 2013). Ayako Nagase also reported in her dissertation, *Plant Selection for Green Roofs in the UK*, that plant health performance of green roof vegetation is necessary because it helps in species selection, and hence is necessary to maintain biodiversity (Nagase 2008).

When we know what plants can survive and grow well on a green roof, we are able to design the planting palette of the green roof with much greater confidence in the outcomes. My study is the first of its kind in the Flint Hills Ecoregion to attempt the assessment of native tallgrass prairie graminoids on green roofs based on growth, survival, and physiological responses to two commercially available substrate types, Kansas BuildEx® and rooflite® extensive mc. Each substrate has a very different composition, and it is important to determine how well these two substrates support different native plant mixes since the APD-EGR plots (or cells) each contain six species of plants (with three plants of each species in every plot). My study focuses on graminoids that are part of two of the species mixes installed on the APD-EGR, the all native plant mix and the *Sedum* and native grasses mix.

Substrate compositions can strongly affect plant performance on a green roof. Physical properties such as porosity and water holding capacity can affect how long water is retained in the substrate and can indicate whether plants adapted to saturated conditions or dry conditions will perform better on the green roof (Best, Swadek, and Burgess 2015). Plants are also affected by chemical properties such as pH and nutrient availability. Individual plants react to acidity or alkalinity of soils differently, so it is important to know the pH levels of the soil and maintain a pH-stable roof over time (Best, Swadek, and Burgess 2015).

Best, Swadek and Burgess (2015) also affirm that nutrients such as nitrogen, phosphorus, potassium, calcium, sulfur, magnesium, as well as other micronutrients all contribute to the development of green roof plants. Biological activity of subsurface bacteria, algae, mycorrhizae and protozoa is crucial for regenerating inorganic nutrients for plant use (Best, Swadek & Burgess 2015). Soil compositions must account for these physical, chemical, and biological components and their interactions.

My study assessed how two engineered soils with different components and compositions: Kansas BuildEx® and rooflite® extensive mc, affected plant survival, growth, and physiological performance of seven graminoids over the first growing season (June to October 2018) on a green roof in Manhattan, Kansas. The seven graminoids studied were *Bouteloua curtipendula*, *Bouteloua dactyloides, Bouteloua gracilis, Schizachyrium scoparium*, *Carex brevior, Koeleria pyramidata*, and *Sporobolus heterolepis.*

Scope of Study

The conducted study was complementary to research being conducted by Lee R. Skabelund on the Architecture, Planning and Design Experimental Green Roof (abbreviated as APD-EGR) at Kansas State University (KSU). My research was primarily funded by Professor Skabelund's Mary K. Jarvis Fellowship (2016-2018). An important part of Professor Skabelund's Jarvis green roof research is to assess temporal changes in vegetation over time on the APD-EGR by measuring species richness, dominance, and coverage, and to provide suggestions for the design and management of similar types of green roofs in a similar climate (Skabelund 2016).

The intent of my study is to evaluate the survival, growth and physiological performance of native grass and sedge species on green roofs in Manhattan, Kansas, and to assess plant responses to different engineered growing media. These intentions align closely to the goals of Professor Skabelund's Jarvis research. The Jarvis research focuses on plots (also called cells) within all three substrate depths—4-inch, 6-inch, and 8-inch (10.16, 15.24, and 20.32cm respectively)—all a part of the APD-EGR.

My research focuses on the 4-inch (10.16cm) substrate depth because this is the shallowest depth among the three established depths, and hence the most desirable for green roofs where structural and cost requirements are constrained. The longitudinal scope of Professor

Skabelund's ongoing APD-EGR research is planned for a minimum of three years. However, my involvement was for the first growing season only, setting a baseline and important reference for the longer-term study. Opportunities for a continuation of similar research to mine in the future is possible by other KSU researchers.

Goals of the Study

The primary goals of my single growing season green roof study are as follows:

1. ***Better understand how two specific green roof substrate types support or constrain plant survival, growth, and physiological performance of selected graminoids on an extensive green roof in Manhattan, Kansas.*** The study entailed the evaluation of plant survival, growth, and physiological performance in response to two substrate types and their characteristics.
2. ***Provide a baseline for longer-term longitudinal plant survival, growth, and physiological performance studies of seven graminoids selected for a 4-inch (10.16cm) deep experimental green roof in Manhattan, Kansas.*** Performance focused on measuring the survival, height, coverage, shoot biomass, and stomatal resistance of 192 individual plants installed as live plugs in early October 2017 or May-June 2018 in two substrate types.

Research Questions

1. Which commercial substrate type, among Kansas BuildEx® and rooflite® extensive mc, best supports the survival, growth, and physiological performance of selected grass and sedge species on the Architecture Planning and Design Experimental Green Roof (APD-

EGR) at Kansas State University during summer to fall of the first growing season (June to October 2018)?

2. Which species (from a small selection of Flint Hills-native graminoids) perform well from a plant survival and growth standpoint, when planted in one of two types of approximately 4-inch (10.16cm) deep APD-EGR substrates located in the Flint Hills Ecoregion?
3. Which species, among *Bouteloua curtipendula* (Side-oats grama) and *Schizachyrium scoparium* (Little bluestem), demonstrate better response to drought, when planted in one of two types of approximately 4-inch (10.16cm) deep APD-EGR substrates located in the Flint Hills Ecoregion?

Project Objectives

Specific objectives of the project were to assess the survival, growth, and physiological performance of seven selected graminoids in the 2018 growing season, and are described below.

1. **Survival**
   a. Measure survival rates for six species of grasses and one species of sedge in the Kansas BuildEx® and rooflite® extensive mc substrate types to assess how each substrate type supported or constrained plant survival during the first growing season (late June to October 2018).
   b. Compare survival rates of the six grasses and one sedge species to infer which species had the highest survival on the KSU APD-EGR in Manhattan, Kansas following one full growing season for each substrate type and plant species mix.

2. **Growth**

    a. Measure and compare the heights, foliar coverage, and end-of-season (November 2018) above-ground biomass of the seven graminoid species across the Kansas BuildEx® and rooflite® extensive mc substrate types.

3. **Physiological performance**

    a. Measure stomatal resistance to infer the drought stress experienced by *Bouteloua curtipendula* and *Schizachyrium scoparium*, and analyze which substrate type induced more drought stress on each plant species late spring through summer.

    b. Assess how stomatal resistance values vary among the two selected plant species and seek to understand the drought stress response of the plants.

Figure 1-3 shows a picture of the APD-EGR in October 2018.

Figure 1-3: APD-EGR plants in Fall 2018

(Photograph by Lee Skabelund, taken in October 2018, at the APDesign Experimental Green Roof, Kansas State University)

Chapter 2 - Literature Review

Green Roof History

Jim (2017) notes that modern-day green roofs, originated in Germany. The first instances of these green roofs were observed in the late 19th century (Jim 2017). Jim (2017) also describes the flat roofs at the time to be cost-efficiently waterproofed with tar, and covered with gravel to reduce flame hazards. Incidental and spontaneous vegetation growth was observed, similar in some respects to intentional green roofs designed and created today. This roof technology was later modernized as green roofs in the 1960s, when people started to seek improvements in green roof technology (Jim 2017).

The first prominent modern green roof in the United States of America was constructed on the Rockefeller Center in New York in 1936 (Getter and Rowe 2006), and was the first instance of elaborate gardens being installed on the roofs of a commercial building (Jim 2017). The Chicago City Hall is another notable green roof in the United States, which was aimed at testing and transferring knowledge about different green-roof materials, techniques, maintenance, and performance over the long-term (Jim 2017). The Chicago City Hall green roof was retrofitted on an 11-story classical revival building in 2001, with the goal to introduce green roof technology and bolster passive cooling of the city (Jim 2017). This pioneering green roof project also introduced and evaluated the planting of temperate grassland herbaceous vegetation, analogous to the natural vegetation of the region (Jim 2017). The Chicago City Hall green roof comprised of 160 species of plants all sourced from the ecoregion, and the assessment offered insights on the implications and challenges of establishing naturalistic or ecological green roofs (Jim 2017). Thus, green roofs have evolved over time from incidental sprouting of plants on rooftops to the deliberate establishment of vegetation as sustainable means of adding green space to urban environments (Jim 2017). Green roofs are currently being researched in different parts of the world to improve design, construction, and management.

Green Roof Components

A green roof is comprised of a layer of vegetation atop a layer of growing media (also called substrate). Typically the substrate layer lies on top of a waterproofing membrane and a filter fabric, with the substrate on top of a drainage filter (often a fine woven mesh fabric or filter fabric), with a combined drainage and water holding layer below. A root barrier is typically placed above a waterproofing membrane and the structure to help protect any insulation and the structural roofing system from potential root and water damage (Getter and Rowe 2006). Figure 2-1 shows a typical cross section of a green roof and its components.

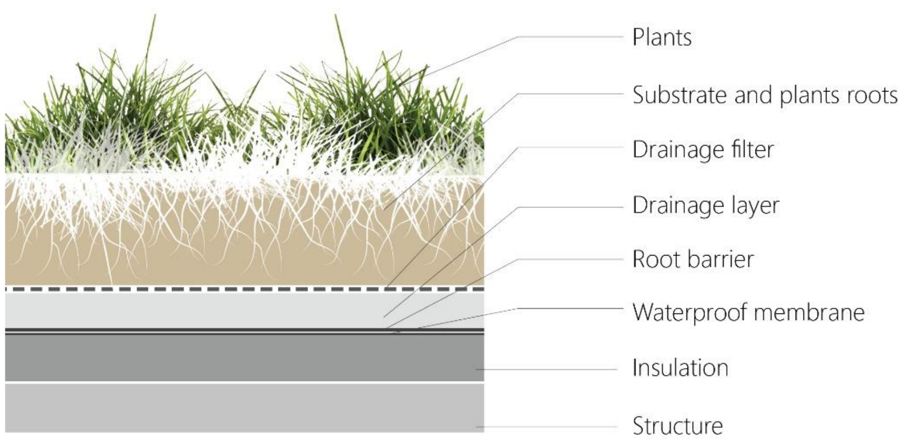

Figure 2-1: Green Roof Components

(Shrestha; adapted from Sutton 2015)

Green roofs have been categorized as extensive, intensive, and semi-intensive, based on the depth of the green roof substrate. According to Sutton (2015), Green Roofs for Healthy Cities, a non-profit organization advocating green roofs and green walls, defines the depths of extensive green roofs as less than six (6) inches (15.24cm), semi-intensive as between four (4)

(10.16cm) and eight (8) inches (20.32cm), and intensive green roofs as more than six (6) inches (15.24cm) (Sutton 2015). Designed green roof depths vary as per the structural capacity of the building or structure. Thinner substrates are desired from building load and cost savings standpoints. However, in deeper, more intensive soils, most plants perform better due to greater water holding capacity and the increased duration for which soils remain moist after rainfall or irrigation (Razzaghmanesh, Beecham, and Kazemi 2014). Durhman, Rowe and Rugh (2007) tested the survival of twenty-five succulent species in substrates with depths of 2.5cm, 5.0cm, and 7.5cm (all extensive depths) and found that the survival was greater in the 7.5cm deep substrate (Durhman, Rowe, and Rugh 2007). Thuring, Berghage, and Beattie (2010) tested two stonecrops, one ice plant, and two herbaceous perennials on a green roof in three substrate depths: 3 cm, 6 cm, and 12 cm, and found that most of the selected plants survived and produced more biomass in increased depths (Thuring, Berghage, and Beattie 2010).

The Flint Hills Ecoregion and Regional Green Roof Studies

According to Anderson and Fly (1955), the Flint Hills Ecoregion is defined by gently sloping, prairie-dominated hills of limestone and shale. Hot continental summer temperatures and cool winters (accentuated by cold arctic blasts) are prevalent in this region, and tallgrass prairie is the dominant vegetation (Anderson and Fly 1955). The soil along ridgelines of the Flint Hills are typically thin (Anderson and Fly 1955), and thus may be comparable to green roof substrates, especially in terms of the harsh growing conditions they both induce on vegetation. Figure 2-2 shows the Flint Hills Ecoregion in Kansas.

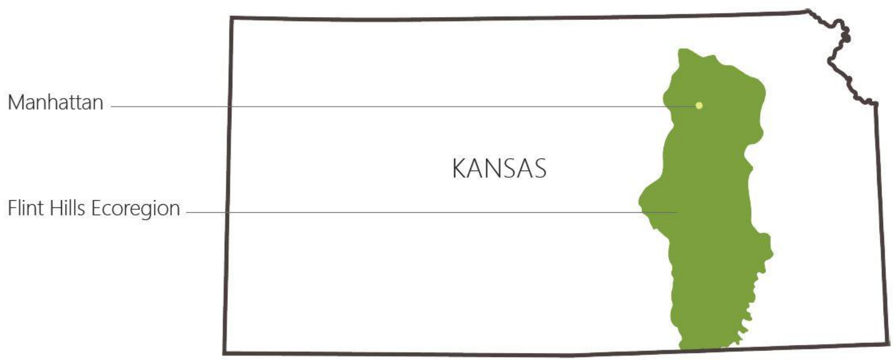

Figure 2-2: The Flint Hills Ecoregion in Kansas

(Shrestha; adapted from US Fish and Wildlife Service "Flint Hills of Kansas" n.d.)

As reported by Kazemi and Mohorko (2017), a study conducted in 2011 by Olszewski in Ambler, Pennsylvania, studied different ratios of heat-expanded clay in substrates and found that shallow substrates with heat-expanded clay dries out faster, leading them to conclude that the water holding capacity of substrates is essential for good plant performance (Kazemi and Mohorko 2017). In a similar continental climate regime, Monterusso, Rowe, and Rugh, in 2005, tested the performance of native plants and observed that grasses and perennials required deeper substrates and more supplemental irrigation in comparison to *Sedum* species (Monterusso, Rowe, and Rugh 2005). Other studies conducted in Michigan by Durhman et al. (2007), Getter et al. (2008), and Rowe et al. (2012) all advocated for deeper substrate types for better plant performance. In 2010, Thuring, Berghage, and Beattie (2010) tested two commercially available substrates in their ability to support plant survival and shoot biomass in a continental climate region of Pennsylvania, and found that the plants did not fare well in shallow substrates, and that expanded clay soils performed better than expanded shale soils (Thuring, Berghage, and Beattie 2010).

Biodiversity on Green Roofs and Native Plant Selection

Green roof studies in many parts of North America are being carried out to assess the types of vegetation that would thrive on green roofs in limited conditions of substrate depth and controlled irrigation regimes. Most green roof vegetation studies focus on *Sedum* species and suggest that these succulents do well on green roofs due to their ability to tolerate extreme drought conditions (Dvorak and Volder 2013; Durhman and Rowe 2007; Monterusso, Rowe, and Rugh 2005). However, biodiversity beyond *Sedum* species is desired on green roofs for the vigorous growth and robust animal community that they support (Cook-Patton 2015; Sutton et al. 2012). Cook-Patton also suggests that biodiverse green roofs better resist stressors like disease, herbivory, and invasion (Cook-Patton 2015).

Sutton et al. (2012) point out that green roof vegetation needs to tolerate extreme environmental conditions, including exposure to severe sun, wind, and drought conditions. Thus, green roof vegetation diversity can be limited (Sutton et al. 2012). However, the use of native plants, or regionally adapted plants, on a rooftop setting has been increasing (Butler, Butler, and Orians 2012). Butler, Butler, and Orians (2012) suggest that the use of native vegetation on green roofs is increasingly in demand for three main reasons: "(1) cultural and aesthetic arguments put forth primarily by early landscape architects, (2) an alternative to turf grass promoted by environmentally conscious landscapers, and (3) environmental reasons explored by conservation biologists." (Butler, Butler, and Orians 2012, 2). While these three co-authors recommend the use of native plants based on the logic that plants from similar "habitat templates" (Butler, Butler, and Orians 2012, 5) are better adapted to green roofs in the same regions, they are not content with the lack of transparency with which green roof researchers have used the term ‘native’ and the difference in conditions at the ground level and on a rooftop setting (Butler, Butler, and Orians 2012).

Green Roof Grasses

Various studies have tested the feasibility of grass species on green roofs. Monterusso, Rowe, and Rugh (2005) investigated eighteen native plants (four native grasses) and nine *Sedum* planted on nine green roof platforms in Michigan. Based on their observations of growth, survival and visual appearance, they concluded that *Sedum* species were more suitable for green roof applications than native plants under absence of irrigation (Monterusso, Rowe, and Rugh 2005).

Nagase and Dunnett (2010) investigated four species of grasses, along with *Sedum* and forbs, and tested these plant types for drought tolerance across three watering regimes, and in monocultures and mixes. They found that *Sedum* species were more drought tolerant than both the grasses and forbs on the green roofs they studied, with grasses and forbs performing similarly (Nagase and Dunnett 2010).

MacIvor and Lundholm (2011) researched survival, cover, and stormwater performance of plants native to the coastal regions of Atlantic Canada, and found that graminoids performed the best for all functions (MacIvor and Lundholm 2011).

In a study done by GRIT Lab researchers in Toronto, Canada, MacIvor et al. (2013) studied vegetative cover, biomass, and species diversity of two types of mixes: an all-*Sedum* mix and a grass-and-forb mix, in 33 elevated green roof modules. They found that grass-and-forb cover and biomass were greater than the *Sedum* mix in organic media, with two dominant grass species observed, but diversity declined without supplemental irrigation (MacIvor et al. 2013).

Li and Yeung (2014) report four characteristics that inherently help plants perform better on a green roof: “(1) they establish fast and reproduce efficiently; (2) they are short in height and cushion-forming or mat-forming; (3) their roots are shallow but spreading; and (4) their leaves are succulent or able to store water.” (Li and Yeung 2014, 128).

Among these various studies, some have reported a positive performance of grasses whereas others have claimed that *Sedum* species are the way to go for green roofs. The health of root systems thus seems to be essential. Many native grasses have root systems that are deep and dense, however, they have been found to adapt to shallow substrates by growing their roots horizontally (Sutton et al. 2012). This makes them reasonably adaptable to green roof conditions—assuming that sufficient water is provided to keep the roots from completely drying out and causing the plants to die.

As described by Kirkham (2014) in her book *Principles of Soil and Plant-Water Relations*, grasses are either C3 or C4, depending upon the mechanism they adopt in carbon dioxide fixation. C3 plants fix atmospheric carbon dioxide as a three-carbon compound called phosphoglyceric acid, whereas C4 plants fix atmospheric carbon dioxide as oxaloacetic acid, a four-carbon compound (Kirkham 2014e). Kirkham (2014) also states that C4 plants require relatively higher temperatures for growth. Hence, grasses that exhibit a C4 cycle are called warm-season grasses since they exhibit delayed emergence from dormancy (usually late-spring to early summer) in comparison to cool-season grasses which start to grow in early spring (Volesky et al. 2005). Warm-season grasses are also known to show better physiological responses to drought conditions (Feldhake and Boyer 1985), whereas cool-season grasses have been observed to provide more biomass (Robins 2010). Drought tolerance and the production of biomass that shades and cools green roof substrates seem to be very important.

Grasses Used on the Architecture, Planning and Design Experimental Green Roof

The grasses that have been used on the Architecture, Planning and Design Experimental Green Roof are native to the Flint Hills tallgrass prairie. Descriptions of their habits and habitats will be discussed in this section . Table 2-1 shows the dimensions of the grass species as reported by selected plant nursery websites, as well as the dimensions observed at the Chicago Botanical Garden (Hawke 2015). Figure 2-3 to Figure 2-9 show photographs of the respective graminoid species used on the APD-EGR.

Table 2-1: Sizes of the grass and sedge species (Hawke 2015; Emory Knoll Farms "Plants Archive" n.d.; Missouri Botanical Garden "Plant Finder" n.d.)

Species	**Size (height x width/ spread)**	**Chicago Botanical Garden Green Roof (height x width/spread)**
Bouteloua curtipendula	18-30 inches x 18-24 inches (Missouri Botanical Garden)	37 inches x 24 inches (in 4-inch deep substrate)
Bouteloua dactyloides (also called *Buchloe dactyloides*)	5 inches x 12 inches (Emory Knoll Farms)	3 inches x 12 inches (for *Buchloe dactyloides* 'Legacy' in 4-inch substrate)
Bouteloua gracilis	12 inches x 12 inches (Emory Knoll Farms)	18 inches x 8 inches (in 4-inch deep substrate)
Schizachyrium scoparium	36 inches x 24 inches (Emory Knoll Farms)	41 inches x 20 inches (in 8-inch substrate)
Carex brevoir	12-24 inches x 12-24 inches (New Moon Nursery and Everwilde Farms)	10 inches x 13 inches (for *Carex radiata* in 4-inch deep substrate)
Koeleria pyrammidata	12-24 inches x 9-18 inches (Missouri Botanical Garden)	24 inches x 12 inches (for *Koeleria macrantha* in 4-inch deep substrate)
Sporobolus heterolepis	24-36 inches x 24-36 inches (Missouri Botanical Garden)	37 inches x 26 inches (in 4-inch deep substrate)

***Bouteloua curtipendula* (Side-oats grama)**

Figure 2-3: *Bouteloua curtipendula* (Side-oats grama)

(Photograph by Lee Skabelund, taken in 2018, at the Seaton Hall Upper Green Roof, Kansas State University)

According to the USDA Plant Guide for side-oats grama, it "is a deep rooted, perennial grass. The plants crown will spread very slowly by means of extremely short, stout rhizomes. A mid-grass in height, it has rather wide leaves and a very distinct inflorescence consisting of a zigzag stalk with small compressed spikes dangling from it at even intervals. The short spikes dangle from one side of the stalk, thus providing the plant with its common name. In the vegetative state the grass is easily recognized by the long, evenly spaced hairs attached to the margins of the leaf near its base. Side-oats grama possesses the C-4 photosynthetic pathway common to warm-season grasses (Waller and Lewis, 1979)." (USDA NRCS "Side-oats grama" n.d., 1)

According to the USDA Plant Guide for side-oats grama, these grasses grow in dryer mid-grass prairie section of the Great Plains, which has an annual rainfall of 12-20 inches (30.5-50.8 cm). Common occurrence has been observed with blue grama (*Bouteloua gracilis*) and little bluestem (*Schizachyrium scoparium*) (USDA NRCS "Side-oats grama" n.d.). As per Leithead et al. (1971), these grasses are better adapted to calcareous and moderately alkaline soils than to acidic or neutral soils (USDA NRCS "Side-oats grama" n.d.). Side-oats grama is also adapted to sandy to clayey textured soils, and do not fare well in loose sands and dense clays, but they do the best in medium to fine texture upland soils (USDA NRCS "Side-oats grama" n.d.). They are tolerant of salinity and are moderately drought tolerant, but less than blue grama (USDA NRCS "Side-oats grama" n.d.). These grasses can also tolerate spring flooding to a certain extent, demonstrating the widest range of adaptation of any of the warm-season perennial grasses (USDA NRCS "Side-oats grama" n.d.). Side-oats grama can grow with tall grasses such as big bluestem (*Andropogon gerardii*) and switchgrass (*Panicum virgatum*), as well as shorter grasses such as buffalo grass (*Bouteloua dactyloides*) and blue grama (*Bouteloua gracilis*). (USDA NRCS "Side-oats grama" n.d.)

Bouteloua dactyloides (Buffalograss)

Figure 2-4: *Bouteloua dactyloides* (Buffalograss) plug (left) and low-growing grass on the APD-EGR with *Schizachyrium scoparium* and other green roof plants (right)

(Photographs by Lee Skabelund, taken in 2018, at the APDesign Experimental Green Roof, Kansas State University)

As described by the USDA Plant Guide (n.d.), “buffalograss is a native, warm season, stoloniferous perennial that grows 4 to 6 inches in height. The leaf blade is 1/8-inch wide and 3 to 6 inches long. The ligule is a row of short hair. The plant is dioecious. Both sexes have a spike for the seed head. The female flowers are burs partially hidden among the leaves and the male flowers have 2 or 3 short spikes on slender, erect stems (Leithead et al. 1971).” USDA NRCS “Buffalograss (*Bouteloua Dactyloides*)” n.d.)

The USDA Plant Guide for buffalograss (n.d.) reports the study of Duble (2012), and states the fact that buffalograss prefers clay soils in low to moderate amounts of rainfall, approximately 15 to 30 inches (38 to 76 cm) annually. It also tolerates alkaline soils (USDA NRCS “Buffalograss (Bouteloua Dactyloides)” n.d.). Buffalograss is generally found on dry prairies, thriving in medium to fine textured soils, as per Hatch (1995) (USDA NRCS “Buffalograss (Bouteloua Dactyloides)” n.d.). According to Gaitan-Gaitan, Buffalograss can be established by seeds, sod, plugs or stolons. Buffalograss spreads vegetatively by stolons (Gaitan-Gaitan 1995).

***Bouteloua gracilis* (Blue grama)**

Figure 2-5: *Bouteloua gracilis* (Blue grama)

(Left image: Photograph by Lee Skabelund, taken in 2018, at the APDesign Experimental Green Roof, Kansas State University; Right image: (Haddock n.d.))

“Blue grama is a major warm season grass found throughout the Great Plains. It is found on the plains, prairies, and foothills of most western states. It is short (6 to 24 inches) stature and perennial with a prolific root system. This species has some phenotypic plasticity since in the southern states it grows normally as a bunch grass, but in the northern states and in the mountains, or in areas under heavy grazing pressure it is a sod former. Phenotypic plasticity is the ability of an organism to alter its physiology or morphology in response to changes in environmental conditions (Schlichting, 1986). Blue grama possesses the C-4 photosynthetic pathway for carbon fixation (Waller and Lewis, 1979).” (USDA Plant Guide "Blue grama" n.d., 1)

Blue grama is commonly found with buffalograss (*Bouteloua dactyloides*), needle-and-thread (*Hesperostipa comata*), western wheatgrass (*Pascopyrum smithii*), and green needlegrass

(*Nassella viridula*) sagebrush (USDA NRCS "Blue grama" n.d.). It can also be found in sandier habitats with other species such as prairie sandreed and sand sagebrush (USDA NRCS "Blue grama" n.d.). Blue grama performs well in drought conditions, fairly saline, and moderately alkaline soil, and it grows well sandy to clayey soils (USDA NRCS "Blue grama" n.d.). According to the USDA Plant Guide for blue grama, this species cannot tolerate frequent submergence in water, or shade and acidic soils, and prefers precipitation between 12 to 14 inches. It thrives during the warmest part of summer (USDA NRCS "Blue grama" n.d.). Blue grama propagates by seeds and by short root stalks and tillers that expand the basal area of the plant (Phan 2000).

***Schizachyrium scoparium* (Little Bluestem)**

Figure 2-6: *Schizachyrium scoparium* (Little bluestem) on the APD-EGR

(Photograph by Lee Skabelund, taken in October 2018, at the APDesign Experimental Green Roof, Kansas State University)

According to the USDA Plant Guide for little bluestem, it “is a tufted (sometimes with short rhizomes), warm-season (C4), perennial grass broadly distributed and native to the U.S. and Canada. Because of this broad distribution, little bluestem exhibits significant ecotypic variation. Plants vary in height, color, length of leaves, flowering, and clump diameter (USDA, 1983; Uchytil, 1989). It grows from 1 to 3 feet tall with culms slightly flattened. The blades are folded, sometimes rolled inward, and smooth to hairy. They are 2 to 12 inches long, 1.5-6 mm wide, pointed with sheaths keeled and usually smooth." (USDA NRCS "Little Bluestem" 2013)

As reported by the USDA Plant Guide, as per the findings of Uchytil (1989), seed averages for Little bluestem are about 225,000 to 250,000 bearded seeds per pound. Little bluestem is tallgrass prairie increaser and mixed prairie decreaser (USDA NRCS "Little Bluestem" 2013). According to the USDA Plant Guide (2013), the plants generally occur on dry sites, primarily on upland ridges, hilltops, and steep slopes. This species also finds limey sub-irrigated sites and in prairie marshlands suitable (USDA NRCS "Little Bluestem" 2013). They occur in plant hardiness zones 3 to 9, in areas with average precipitation ranging in between 10 and 60 inches (USDA NRCS "Little Bluestem" 2013). Little bluestem spreads through tillers and short root stalks, as well as by seeds (Phan 2000).

Carex brevoir **(Short-beak sedge)**

Figure 2-7: *Carex brevior* (Fescue sedge)

(Haddock n.d.)

Carex brevior is a perennial sedge species that generally grows to a height of 1-3 ft. According to the Kansas Wildflowers and Grasses database, its culms are "erect, stiff, sharply triangular, angles rough or smooth, bases of old leaves often persisting." (Haddock "Kansas Wildflowers and Grasses - Fescue Sedge" n.d.) The leaves of the sedge have "blades 3-6, flat, ascending, shorter than culms, less than 1/6 inch wide, firm, pale green, glabrous; margins rough." (Haddock "Kansas Wildflowers and Grasses - Fescue Sedge" n.d.). The Kansas Wildflowers and Grasses database notes that the species is found in sandy prairies, meadows, ditches, and woodlands, and are most observed in dry, disturbed areas (Haddock "Kansas Wildflowers and Grasses - Fescue Sedge" n.d.). In general, *Carex* species reproduce by vegetative means through rhizomes, but also produce seeds (Bernard 1989).

***Koeleria pyrammidata* (Prairie Junegrass)**

Figure 2-8: *Koeleria pyramidata* (syn. *Koeleria macrantha*) (Prairie junegrass)

(Haddock n.d.)

The USDA Plant Guide for prairie junegrass describes it as "a highly variable, moderately long-lived, cool season perennial bunchgrass that grows 0.5 to 2 ft. tall. Clusters of narrow, markedly veined, light green to bluish green leaves grow to about 7 in. tall. The leaves are flat to in-rolled with slightly rough edges and boat-shaped, pointed tips. Erect seedheads appear as dense, pale green to purplish spikes, tapered at both ends, and 2 to 5 in. long, that are often held well above the foliage. They fluff open somewhat during flowering. There are 2 to 4 flowers per spikelet." (USDA NRCS "Prairie Junegrass" 2008)

Prairie junegrass is adapted to various soils, climates, associates itself with different native plant communities (USDA NRCS "Prairie Junegrass" 2008). According to the USDA Plant Guide (2008), prairie junegrass is mostly found on rocky to sandy loam soils with low fertility. However, it can also be seen on soils with clay, provided that drainage is adequate. The cool season grass prefers a soil pH of 6.0 to 8.0 (USDA NRCS "Prairie Junegrass" 2008). It tolerates cold, heat, drought, fire and serpentine soils, but does not do well in high salinity (USDA NRCS "Prairie Junegrass" 2008).

***Sporobolus heterolepis* (Prairie dropseed)**

Figure 2-9: *Sporobolus heterolepis* (Prairie dropseed)
(Light 2007)

Prairie dropseed is a densely tufted, perennial grass that reaches 16-34 inches in height (Haddock "Kansas Wildflowers and Grasses - Prairie Dropseed" n.d.). According to the Kansas Wildflowers and Grasses database, the culms of the species are " erect, slender, wiry, glabrous," the leaf blades are "basal and cauline; blades 8 to 16 inches long, 0.2 inch wide, filiform to involute to folded, tip tapering, glabrous, scabrous margins and midrib," and inflorescences are "panicle, open, elliptical to narrowly pyramidal, 4.5 to 8 inches long, up to 2.8 inches wide, branches ascending, 1.2 to 2.4 inches long, spikelets borne toward branch tips on short pedicels."

(Haddock "Kansas Wildflowers and Grasses - Prairie Dropseed" n.d.). Prairie dropseed is found in prairies, roadsides, woodland edges; sandy to clay loam soils (Haddock "Kansas Wildflowers and Grasses - Prairie Dropseed" n.d.). *Sporobolus heterolepis* reproduces predominantly through sexual means such as seed germination (Engstrom n.d.).

Importance of Measuring Growth

According to Kirkham (2014), water is the most important soil physical factor affecting plant growth. Thus, it is important to quantify growth to assess the effects of water stress on the plants. In such an assessment, at least one form of growth such as height, biomass, or leaf area should be measured (Kirkham 2014a). Plant growth curves serve as a means of quantifying growth over a single season, and they also help predict future growth through the use of equations (Kirkham 2014a). The growth pattern of a plant follows an s-shaped curve or a sigmoid, with five phases: (1) an initial lag phase preparatory to growth, (2) an increasing growth phase, (3) a phase when growth rate gradually decreases, (4) a point at which growth ceases as the plant reaches maturity, and (5) senescence and death of the organism (Kirkham 2014a, 5). These phases are represented in Figure 2-10.

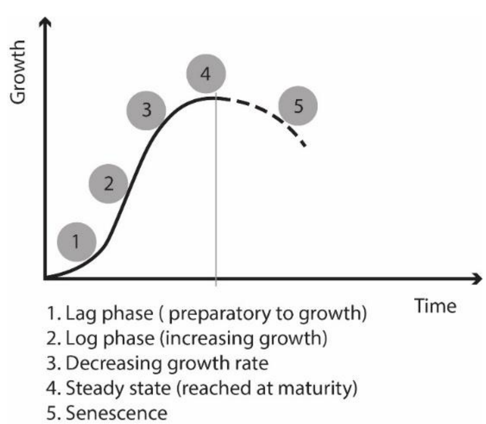

Figure 2-10: Five phases of seasonal plant growth

(Shrestha; adapted from Kirkham 2014a)

Plant Physiology and Stomatal Resistance

The physiological health of plants involves factors affecting plant life functions, including plant water relations and mineral nutrition (Ross and Salisbury 1974). It has been established that water is the most limiting abiotic (non-living) factor to plant growth and productivity, and is very important in growth, photosynthesis and the distribution of nutrients (McElrone et al. 2013). McElrone et al. (2013) assert that plants retain less than 5% of the water absorbed by the roots for growth, and disseminate the remaining 95% to the atmosphere through transpiration. In order to conduct photosynthesis and build sugars, plants absorb carbon dioxide from the atmosphere through pores in their leaf surfaces called stomata (McElrone et al. 2013). The authors also explain that the plants lose water to the atmosphere at a much higher rate than they intake carbon dioxide. The balance between evapotranspiration and photosynthesis is important in the physiological functions of plants (McElrone et al. 2013).

Water for plant use is absorbed by the roots, and then transported through the connective tissues to the leaves (McElrone et al. 2013). This transportation of water is possible because of the cohesive property of water, by means of which water molecules can stick to other water molecules through hydrogen bonds (McElrone et al. 2013). According to McElrone et al. (2013), the movement of water up to the leaves is caused by negative pressure generated by transpiration, which is the evaporation of water from the leaves to the atmosphere. Transpiration generates tension in the water columns of a plant, and the combined cohesion-tension (C-T) mechanism of the plant is the process by which water movement in plants occurs (McElrone et al. 2013). Transpiration is thus a very important part of plant health.

Stomatal resistance is the resistance to the diffusion of water vapor through the stomata of plant leaves (Kirkham 2014f, 23). When a leaf transpires, the stomatal resistance per unit leaf area is the average difference in the concentration of water vapor across the stomata divided by the rate of transpiration (Monteith, Szeicz, and Waggoner 1965). Stomatal resistance was found to be a good indicator of plant water stress for wheat and soybeans (Oosterhuis and Walker 1987). Stomatal resistance of plants depends upon light, water stress, and atmospheric temperature, among other factors (Sena, Zaidan, and Castro 2007). A mass-flow porometer, an instrument which measures the leaf resistance to flow of air, and a diffusion porometer, an instrument which measures the diffusion of water vapor from leaf stomata, are some of the ways to measure stomatal resistance (Kirkham 2014f, 23). The leaf porometer will be described in the methods section of this thesis report.

Effects of Substrate Characteristics on Green Roof Plant Performance

Substrate depth, composition, and characteristics affect plant growth and development. Green roof substrates are designed to be lightweight (Rowe 2015), so that they do not impose a threat on the structural stability of the roof. According to Young (2014), substrates used on green roofs are also shallow in depth, and free-draining, to prevent water logging and increased saturated weight. These properties of substrates may have an inverse impact on plant survival, growth and physiological status since they can cause drought stress to the plants (Young 2014). Hence, it is necessary to know the substrate depths and substrate components and their combinations in order to better understand their likely effect on the performance of green roof plants.

Various researchers have studied the effects of substrate composition on green roof plant performance. Kotsiris and colleagues tested the soil for electrical conductivity, pH, moisture content, porosity, and dry and saturated bulk densities (Kotsiris, Nektarios, and Paraskevopoulou

2012). Ntoulas et al. (2013) looked at particle size distribution, saturated and dry bulk densities, porosity, water potential, and in-situ substrate moisture of the substrates. In 2008, Emilsson studied the effects of substrate composition on vegetation development, in which he looked at organic matter, ammonium and nitrate, pH, and total nitrogen and phosphorus of the three green roof substrates that he was testing (Emilsson 2008). Young (2014) studied the physiological characteristics of *Lolium perenne* (perennial ryegrass) across three component variables of substrate: organic matter, particle size, and the presence or absence of polyacrylamide gel. (Graceson et al. 2014) carried out initial measurements of dry bulk density, total pore space, water holding capacity, air filled porosity, organic matter content, and particle size distribution and then analyzed these physical properties in relation to shoot biomass of *Sedum* and forb species.

Substrate Concepts Corresponding to Soil-Water-Plant Relations on Green Roofs

Green roof substrates are generally evaluated for their ability to hold water and support vegetation based on texture and organic matter composition. These characteristics affect the physical, chemical, and biological soil properties that correlate with the capacity for water retention, drainage, and nutrient availability, which in turn affect plant success on green roofs (Best, Swadek, and Burgess 2015). Rowe (2015) stated that substrate composition affects plant performance through water retention and nutrient availability. According to Rowe (2015), substrates should be lightweight, permanent, able to support healthy growth of plants, and should not leach nutrients.

Soil constituents

Green roof substrates normally comprise of organic and inorganic components which are mixed in proportions that allow good plant survival while adhering to the structural and functional limitations of green roofs (Griffin 2014). Typically, green roof substrates are approximately 70–95 percent minerals, with organic matter and fertilizer constituting the remainder (Best, Swadek, and Burgess 2015). According to Best, Swadek and Burgess (2015), the mineral component can be made of clay, sand, gravel, or rocks such as scoria, or modified minerals such as perlite, vermiculite, rockwool, or expanded clay, slate, and shale to reduce loads. The organic components are generally made of compost, peat, coconut coir, or decomposed sawdust or bark (Best, Swadek, and Burgess 2015). Organic matter is added for providing nutrients and moisture retention, but excess organic matter can cause the substrate to shrink (Rowe 2015).

According to Griffin (2014), substrates should be consistent and reproducible, and should provide necessary porosity, water holding capacity, and structural support to the plants. The soil particle sizes and mineral compositions affect how substrates behave in terms of porosity, water retention, as well as structural strength and compressibility, in addition to chemical properties such as cation and anion exchange capacities (Griffin 2014).

Organic matter

Organic matter on green roofs are usually a combination of compost, peat, coconut coir, or decomposed sawdust or bark, and help in providing nutrients for plant use, buffer against pH change, and retain moisture, thus increasing the water holding capacity (Best, Swadek, and Burgess 2015). According to FLL (Forschungsgesellschaft Landschaftsentwicklung

Landschaftsbau) (2002), which are guidelines developed in Germany for the planning, execution, and upkeep of green roofs, the organic matter content is related to the bulk density (mass per unit volume) of the substrate: for soils with density ≤0.8 (units assumed to be g/cm^3), total organic matter should be ≤8% by mass, and for substrates with bulk densities >0.8, total organic matter should be ≤6% by mass (FLL 2002; Griffin 2014). Best, Swadek and Burgess (2015) assert that the quantity of organic matter in substrates should correspond to climatic conditions, and give an example of tropical climates, where the rate of decomposition may be more than replenishment, which could decrease the overall substrate volume. It is also possible for organic components to break down and inhibit drainage (Best, Swadek, and Burgess 2015). Moreover, too much organic matter can result in the substrate turning hydrophobic, where inorganic components of green roof substrates are coated by water-repellent organic matter (Griffin 2014).

Particle size distribution, porosity, drainage and moisture retention

Particle size distribution, porosity, and moisture retention are all substrate characteristics that can affect plant survival and growth on green roofs. According to the American Society for Testing and Materials (2014), particle size distribution, or gradation, refers to the “proportions by dry mass of a soil distributed over specified particle-size ranges.” (ASTM 2014). Table 2-2 shows the classification of the particle sizes of soil components according to the US Department of Agriculture (USDA) as reported by Boyd and Thunjai (2002).

Table 2-2: USDA classification of soil particle sizes (Boyd, Wood, and Thunjai 2002)

Particle fraction name	**USDA**
Clay	less than 0.002
Silt	0.002 - 0.05
Very fine sand	0.05 - 0.10
Fine sand	0.10 - 0.25
Medium sand	0.25 - 0.50
Coarse sand	0.50 - 1.00
Very coarse sand	1.00 - 2.00
Gravel (fine)	2.00+
Gravel (coarse)	

Griffin (2014) asserts that particle size distribution affects porosity, and thus affects the water retention, water holding capacity, and air-filled porosity of green roof growing media. According to Latshaw, Fitzgerald, and Sutton (2009), porosity is the ratio of void to total solid volume of the substrate. Pore spaces, or the voids in between substrate particles, hold water and oxygen, both of which are important for optimum plant growth and development (Griffin 2014). A substrate with larger particles also has larger pore spaces, and a substrate made of smaller particles has smaller pore spaces (Griffin 2014). According to Latshaw, Fitzgerald, and Sutton (2009), macropores (>0.01mm) enable drainage and movement of water, air, and carbon dioxide through the substrate. Micropores (<0.01mm) hold capillary water, which is held for a longer time due to capillary action and causes slow drainage (Latshaw, Fitzgerald, and Sutton 2009). According to Best, Swadek and Burgess (2015), coarse components such as sand, lava, pumice, perlite, expanded shale, clay, slate all contain large pores, whereas finer particles such as clay and silt have more total pore space because of a greater number of smaller pores (Best, Swadek, and Burgess 2015). Green roof substrates should be comprised of a mix of large and small particles to provide adequate air space and water holding capacity (Griffin 2014). Thus, the balance of macro and micropores is important and determines the retention and movement of water in the substrate, a good balance resulting in equivalent water and air in the substrate (Latshaw, Fitzgerald, and Sutton 2009).

The proportion of air to water within the substrate profile is integral to plant survival (Griffin 2014). Pore space should constitute about 50 percent of the growing media, the remaining half should be comprised of organic content and minerals (Latshaw, Fitzgerald, and Sutton 2009). Latshaw, Fitzgerald, and Sutton (2009) assert that for optimum plant growth, the half constituted by pore space should be half air and half water to avoid excessive water-logging due to predominance of water, and to prevent drought due to too much air space. Below 10 percent air-filled porosity, plant roots cannot survive (Kirkham 2014d). According to Young (2014), the water holding capacity, or the amount of water held at field capacity of substrates, can be increased by decreasing particle size, which increases the amount of inner particle pore space, although this can increase the potential for water logging.

Thus, green roof substrates should consist of large and small sized components to balance weight, porosity, and water retention, and water holding capacity to support plant growth and effective stormwater retention while also permitting sufficient air movement for gas exchange in the root zone, as roots require a constant supply of oxygen (Griffin 2014). Porosity and moisture retention properties of substrates need to be tailored to the intent, function and climatic conditions of the green roof setting (Best, Swadek, and Burgess 2015). Best, Swadek, and Burgess (2015) give examples of hot and arid regions, where moisture retention is generally preferred for plants to provide shading and building cooling rather than evapotranspiration.

Whereas, in coastal rainy areas, infiltration and greater volume retention may be necessary for stormwater management functions (Best, Swadek, and Burgess 2015). When evaluating substrate characteristics for plant success on green roofs, substrate characteristics such as particle size, porosity, water holding capacity, and drainage are important to consider.

The FLL (Forschungsgesellschaft Landschaftsentwicklung Landschaftsbau) guidelines, which are used internationally as green roof guidelines for the planning, execution, and upkeep of green roofs, specify that air filled porosity should not be less than 10 percent (FLL 2002). The FLL Guidelines (2002) also specify that the maximum water capacity of green roof substrates should not be less than 35 percent, and should not exceed 65 percent by volume. The permeability or the ability to drain is specified, in these guidelines, to be more than 0.6 mm/min (FLL 2002) to be considered drainable.

Bulk density

Bulk density is the weight of soil per unit volume, and is expressed in g/cm^3 (USDA NRCS "Soil Quality Physical Indicator Information Sheet Series" 2008). According to Martin, Reyes, and Taguas (2017), factors such as organic matter content and compaction influence bulk density values. However, differences in bulk density values among soils are mostly because of the weights of individual substrate components and differences in particle size distributions (Martín, Reyes, and Taguas 2017).

According to Best, Swadek and Burgess (2015), the bulk density for green roofs has been set relatively low: approximately $1g/cm^3$. Sand-based soils have high porosity and infiltration rates, but are heavy with higher bulk density, whereas clay-based soils have low bulk density, but low infiltration rates as well (Best, Swadek, and Burgess 2015). For structural loading purposes, substrates with lower bulk densities would be more desirable than substrates with higher bulk densities, provided that the substrates can resist wind uplift (Griffin 2014). However, using a singular substrate constituent based on bulk density, such as clay or sand exclusively on their own, may cause restricted root growth, and poor air and water movement through the soil (USDA NRCS "Soil Quality Physical Indicator Information Sheet Series" 2008). Thus, we need combinations of multiple components for a good green roof substrate, to balance bulk density and infiltration, while providing nutrients and water to support selected vegetation.

Water holding capacity, field capacity and wilting point, and the soil moisture characteristic curve

Plant physiological performance on green roofs depends upon the water budget, which is related to the water that is available in the soil for uptake by plants, and is determined by the field capacity (FC), wilting point (WP), and available water (AW) (Kirkham 2014c). According to Kirkham (2014), the Soil Science Society of America defines the water content in soil as the amount of water by weight or volume that can be lost from the soil by drying to constant mass at 105°C (Kirkham 2014b). The field capacity is the constant value for water content in the soil at a particular depth that is reached one or two days after the soil is subjected to saturation due to precipitation or irrigation (Kirkham 2014c). According to Best, Swadek, and Burgess (2015), rainfall or irrigation moves through substrates by gravitational forces, and soil becomes saturated as its pore spaces are filled. After a precipitation or irrigation event, once all gravitational water has freely drained from the substrate system, the substrate is said to be in field capacity (Best, Swadek, and Burgess 2015).

Best, Swadek, and Burgess (2015) indicate that plants can begin extracting water at the field capacity of the substrate. The amount of water available for uptake at this time is called the available water holding capacity, which ranges from field capacity at its upper limit to permanent wilting point at its lower, drier limit (Best, Swadek, and Burgess 2015). Thus, plant available water is the difference between the field capacity and wilting point, that is available for plant uptake from the soil (Kirkham 2014c), and can affect plant survival and growth. The permanent wilting point is the amount of water per unit weight or bulk volume in the soil, expressed as a percentage, that is held so tightly by the soil that it is unavailable for uptake by the roots of the plants, thus causing the plants to wilt and eventually die (Kirkham 2014c).

The water content at field capacity and wilting point can be discerned from a soil moisture characteristic curve. A soil moisture characteristic curve is a curve plotted between volumetric water content (m3m-3) and matric potential (-m) (Tuller and Or 2005). Matric potential is the portion of water potential attributed to the adhesion of water molecules to soil particles (Tuller and Or 2005), and increases as water is removed from soil. A soil-water characteristic curve describes the amount of water retained in a soil (expressed as mass or volume water content) under equilibrium at a given matric potential (Tuller and Or 2005). Figure 2-11 shows an example of the soil water retention curve for clay, silt loam, and sand-based soils, plotted between matric potential and volumetric water content.

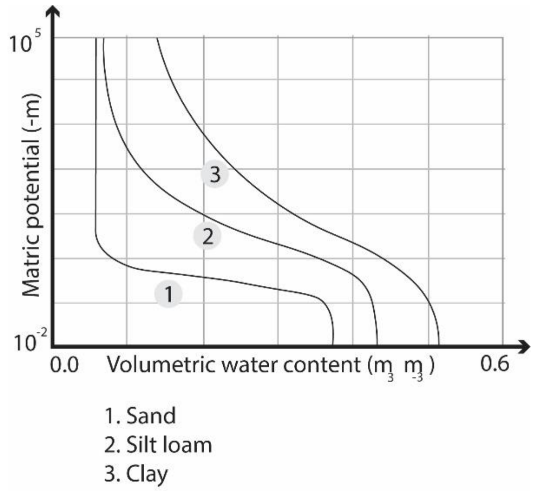

Figure 2-11: Nature of soil water characteristic curve

(Shrestha; adapted from Tuller and Or 2005)

Nutrient Availability of Green Roof Substrates

The plants on a green roof, like in normal ground-level conditions, require various nutrients to survive, develop, grow, and reproduce. According to Best, Swadek and Burgess (2015), primary nutrients required in the substrate are Nitrogen (N), Phosphorus (P), and Potassium (K). Secondary nutrients such as calcium, magnesium, and sulfur, and a suite of micronutrients required in very small amounts (boron, manganese, copper, iron, zinc, molybdenum, nickel, and chlorine) are also needed by the plants (Best, Swadek, and Burgess 2015). Plants use the macro and micronutrients for proteins and DNA or the development of cell walls, flowering structures, or for pollination (Best, Swadek, and Burgess 2015). Plants draw the nutrients from the substrate, so substrates must contain these nutrients. According to Best, Swadek and Burgess (2015), most of the macronutrients are taken up by plant roots in either cation form, and a few in the form of anions. Thus the Cation Exchange Capacity (CEC) and Anion Exchange Capacity (AEC), which are the respective amounts of positive and negative ions a soil can retain, are also important characteristics of substrates (Best, Swadek, and Burgess 2015).

In addition to the availability of essential nutrients, substrate pH also affects which plants can thrive on the roofs. Substrate pH should be within a range that allows plants to uptake nutrients from a soil, typically between 5.5 and 7.0 (Best, Swadek, and Burgess 2015). Microbes such as bacteria, algae, and mycorrhizae degrade plant and animal matter, and aid in restoring and releasing carbon, nitrogen, sulfate, phosphate, and nutrient sources for plant use (Best, Swadek, and Burgess 2015).

Effects of Substrate Depth on Green Roof Plant Performance

Green roof studies have shown that deeper substrates support better performance by some species, while some species prefer or tolerate shallow substrates very well. Kazemi and Mohorko (2017) have stated that in continental climates, *Sedum* species perform well in shallow substrate (75-100mm). *Sedum* species grown in deeper substrates showed higher survival and growth rates (in terms of coverage) in a study conducted by Durhman and colleagues (Durhman, Rowe, and Rugh 2007). VanWoert et al. (2005) also assert that deeper substrates were better than shallow ones for vegetative growth of seven *Sedum* species based on measurements of shoot dry weight accumulation. The researchers found that deeper substrates provided more growth, but required more water due to increased evapotranspiration rates (VanWoert et al. 2005). As found in research by Getter and Rowe (2008), deeper substrates have higher moisture content than shallower substrates. However, green roof substrate depth is limited to what is permissible by the structural capacity of the roof structure since it has to fulfil both horticultural and structural requirements (Thuring, Berghage, and Beattie 2010). Thus, a balance between substrate depth and loading capacity of roof structures is necessary to maintain in a green roof design.

The First Growing Season

Getter and Rowe (2008) indicate that species selection and initial establishment of plants on a green roof is critical for long-term survival and health. Especially during the early establishment period, the period between planting and first-year dormancy (Monterusso, Rowe and Rugh 2005), the plant species should be able to withstand the harsh environmental conditions of green roofs (Getter and Rowe 2008), when rooftop temperatures can be very hot due to the lack of full vegetation coverage. Skabelund, et al. found that bare green roof substrate surfaces reached temperatures greater than 60° C in early afternoon for a small integrated green roof in Manhattan, Kansas, with average subsurface temperatures greater than 33.5° C between July and August 2011, two years after its 2009 establishment (2014). In addition to considering rooftop temperatures during the establishment period, the authors suggest that plants that reduce soil erosion and impede invasion by weeds (by providing thick, full coverage) are good choices for green roofs (Getter and Rowe 2008).

Irrigation

Under ideal conditions, green roof vegetation should perform well without supplemental irrigation. However, to achieve the biodiversity beyond very drought tolerant, monotypic *Sedum* species on green roofs, at least some irrigation is necessary (MacIvor et al. 2013). VanMechelen et al. (2015) note that although irrigation on green roofs is considered unsustainable, in an urban setting other benefits such as aesthetics, plant survival, and increased thermal comfort overshadow the drawback of needing water to irrigate green roofs. Green roofs can be exposed to both high drought and rainfall conditions. During times of excessive rainfall, green roofs are expected to capture and retain stormwater and reduce load on the urban stormwater infrastructure. Farrell et al. (2013) assert that plants that can survive both drought and excessive rainfall would be ideal for green roof conditions. From a water conservation perspective, irrigation would only or at least primarily be required during the establishment period (Dvorak and Volder 2013). Nevertheless, irrigation also tempers climate, can help cool a building during the summer (VanMechelen et al. 2015), and can help create more shade-providing biomass to keep rooftop temperatures cooler, so irrigating green roofs may be beneficial for plant health and other important purposes.

Plant Survival, Growth, and Physiological Performance Studies on Green Roofs

Various methods have been adopted by different researchers to measure plant performance on green roofs. Green roof studies that evaluated survival, growth, and physiological status will be discussed in this section.

Survival

Survival is a pivotal factor in measuring the performance of plants on green roofs. Plants need to overcome extreme conditions of drought stress and winterkill to survive and grow in green roof conditions (Lundholm et al. 2014). Hence survival can be considered a baseline measure of green roof plant performance. Various studies have assessed plant survival as a means to quantify the success of plants on green roofs (Dvorak and Volder 2012; MacIvor and

Lundholm 2011; Monterusso, Rowe and Rugh 2005; Rayner et al. 2016; Razzaghmanesh, Beecham and Kazemi 2014; VanWoert et al. 2005).

In 2005, Monterusso, Rowe and Rugh (2005) tested the establishment and persistence of *Sedum* and native plant taxa to better understand what plant species will survive and flourish in the rooftop climatic conditions of Michigan. Over half of the taxa studied showed no mortality in the establishment period because the roof was being irrigated with 0.38 cm of water applied three times a day (Monterusso, Rowe and Rugh 2005). Any mortality observed, mostly in grasses and forbs, was attributed to the hot weather conditions that the plants had to incur (Monterusso, Rowe and Rugh 2005).

MacIvor and Lundholm (2011) evaluated the survival of 15 plant species native to coastal regions of Atlantic Canada, along with growth rates, and the roof cooling and stormwater retention properties of the selected plants. The graminoids, forbs, and shrubs were planted as monocultures in ten roof modules (MacIvor and Lundholm 2011). MacIvor and Lundholm (2011) found that 12 of the 15 species planted demonstrated close to 100 percent survival, with two species (a perennial tall forb and a creeping shrub) showing over 80 percent survival, and an evergreen creeping shrub showing less than two percent survival. Plant survival was evaluated and then documented by assigning “0” or “1” to plants that were dead or alive respectively (MacIvor and Lundholm 2011).

Growth

Plant growth has been established as a method of assessing green roof plant success in various green roof studies (Dvorak and Volder 2013; Lundholm et al. 2014; MacIvor and Lundholm 2011; Monterusso et al. 2005; Nagase and Dunnett 2010; Razzaghmanesh, Beecham and Kazemi 2014; Young 2014). According to Lundholm, Tran, and Gebert (2015), plant traits such as size and leaf morphology relate to strategies for differentiating species accustomed to certain environmental conditions. For instance, shorter plants, with lower maximum growth rates, generally occur in low-fertility soils (Lundholm, Tran, and Gebert 2015). Such plant traits can then be used to predict ecosystem processes such as primary production, nutrient and water uptake, and transpiration rates (Lundholm, Tran, and Gebert 2015).

As reported by Young (2014), substrate characteristics such as organic matter content, particle size, and water holding capacity affect the growth of plants on a green roof (Young 2014). Young (2014) determined the growth of *Lolium perenne* (perennial ryegrass) through shoot and root biomass measurements in his green roof study conducted in Sheffield, UK. The size and growth of green roof plants are important to measure because they are representative of the stresses on the plants such as those induced by water unavailability (Kirkham 2014a), and weather conditions on the rooftop.

Growth has been measured in various ways in several green roof vegetation studies. Monterusso, Rowe, and Rugh (2005) collected data on growth as an indicator of plant performance, along with visual appearance and survival, and found that the growth of native plant species peaked under irrigated conditions and declined when irrigation was stopped, whereas *Sedum* species were mostly unaffected by consistent water availability. Growth was measured, in this study with a plant growth index (GI), in which the researchers measured heights and widths of plants two ways and averaged the sum (Monterusso, Rowe, and Rugh 2005). They concluded that almost any plant taxa can be used on green roofs provided that they are adapted to the climate, grown in a favorable substrate type, and irrigation is available (Monterusso, Rowe, and Rugh 2005).

Dvorak and Volder (2013) assessed plant survival, devised a plant growth index and performed visual rankings of plant health, and found that the top performers in terms of survival were drought-tolerant succulents, and plants with erect morphologies. The four top survivors were *Graptopetalum paraguayense, Malephora lutea*, *Phemeranthus calycinus*, and *Manfreda maculosa*. All top performing plants had erect morphologies except *Malephora lutea* (Dvorak and Volder 2013). The researchers suggested the possibility of erect plants minimizing the amount of solar radiation intercepted, and thus reducing heat load and transpiration rates, thus favoring survival in high-light environments (Dvorak and Volder 2013). Nagase and Dunnett (2010) measured the survival, shoot and root biomass, and visual appearance of twelve plant species, which were either forbs, Sedum or grasses, and reported that greater water availability and more vegetation diversity increased overall survivability, biomass and visual appearance of the plants (Nagase and Dunnett 2010).

A study in Adelaide, Australia, by Razzaghmanesh, Beecham, and Kazemi (2014) measured the growth of Australian ground cover and grass species through measurements of horizontal cover, vertical height, and shoot and root biomass. They found that plant heights were greater in irrigated treatments than unirrigated ones, and succulent plants such as *C. rossi* with resilience to water and heat stress performed better on green roofs in Adelaide, in terms of maximum horizontal growth rate, leaf succulence (water retained in leaves), shoot biomass, and water use efficiency, and concluded that performance of vegetation depends upon appropriate combinations and depths of green roof layers (Razzaghmanesh, Beecham, and Kazemi 2014).

Plant height has been found to be positively correlated to stormwater capture on green roofs (Lundholm, Tran, and Gebert 2015; Nagase and Dunnett 2012), based on the ability of the plants to intercept, retain and transpire water. Nagase and Dunnett (2012) found that plant species with taller height, larger diameter, and more above-ground and below-ground biomass reduced water runoff more effectively than shorter plants with smaller diameter, and lesser biomass. Also, plants that provide faster establishment and coverage are typically desired on green roofs so that the number of plants required may be decreased, thus reducing the cost of planting (Monterusso, Rowe, and Rugh 2005).

Biomass studies help to determine the development of vegetation on green roofs, and can be a useful method to measure growth. Various studies have conducted biomass measurements to assess the growth of plants on green roofs (Emilsson 2008; Nagase and Dunnett 2010; Razzaghmanesh, Beecham and Kazemi 2014; VanWoert et al. 2005; Young 2014). Emilsson (2008) and VanWoert et al. (2005) conducted biomass studies to assess vegetation development on green roofs in their respective study sites in Alnarp, Sweden and East Lansing, Michigan. Emilsson (2008) evaluated the cover and biomass of nine succulent species planted in three mixes and two substrate types to assess the development of vegetation as attributed to establishment technique, species mix, and substrate design. Emilsson (2008) found *Sedum album* and *Sedum acre* to show the greatest dominance. Substrate type was shown to have an important influence on the development of plant cover, and the total succulent and biomass (Emilsson 2008). VanWoert et al. (2005) found that seven *Sedum* species in deeper substrates (6 cm deep) demonstrated higher shoot dry biomass accumulation than shallower substrates (2 cm deep) under frequent irrigation regimes (namely 2, 7, and 14 days between watering).

Based on the studies discussed above, plant height has been evaluated in my study as a measure of vertical growth of plants and foliar cover as a measure of horizontal growth. End-of-season above-ground biomass of the plants have been measured to evaluate vegetation development through the growing season. Individual methods adopted for survival, height, cover, and above-ground biomass will be discussed in Chapter 3.

Physiological performance

Plant physiological traits are important to understand in order to effectively assess the performance of plants, especially in association with substrate characteristics. Substrate characteristics such as particle size, pore space, organic matter content, and bulk density affect the amount of water available to the plants and impact the physiological traits of the plants used on a green roof (Farrell et al. 2013).

Plant physiological traits have been evaluated in several green roof studies to study plant-water-substrate relations (Farrell et al. 2013; Kotsiris, Nektarios, and Paraskevopoulou 2012; Ntoulas et al. 2013; Raimondo et al. 2015; Young 2014). Green roof substrates are designed to allow water to drain quickly. Due to shallow depth and the free draining characteristics of green roof substrates, plants usually suffer from water stress (Young 2014). Young (2014) studied the effects of substrate composition (particle size, organic matter, and the presence/absence of polyacrylamide gel) on the physiological health of *Lolium perenne* (perennial ryegrass). Young measured above and below-ground biomass as a measure of growth, in addition to shoot nitrogen content. As a measure of physiological performance chlorophyll content was also assessed (Young 2014). Young (2014) found that decreasing substrate particle size could increase substrate water holding capacity, pore volume and decrease permeability. They found that increasing the size of bricks in their substrate decreased shoot growth (Young 2014). They also observed that the type of organic matter used, such as using green waste compost instead of bark, increased *L. perenne* shoot and root biomass, chlorophyll content, and shoot nitrogen concentration, while the addition of polyacrylamide gel increased the water holding capacity of the substrate and encouraged shoot growth as well (Young 2014).

Farrell et al. (2013) tested the water use efficiency demonstrated by plants through physiological trait responses in the form of transpiration, plant water status, growth, and biomass, and they reported an association between water holding capacity of substrates and drought stress experienced by plants. The authors concluded that high water users and drought tolerant species, such as monocots (*A. milleflorum*, *S. glauca*, *D. admixta*, *L. Longifolia*, and the herb *I. axillaris*) would be ideal for green roofs (Farrell et al. 2013). Farrell et. al (2013) also assert that specific means by which plants survive drought include short-term physiological changes such as stomatal closure, and longer-term morphological changes in biomass allocation. Hence, it is helpful to know the interactions of different soil types and their characteristics with plant physiological traits as they cope with drought and water availability.

In a study conducted by the University of Messina in Trieste, Italy, Raimondo, et al. (2015) performed physiological measurements of gas exchange, leaf and xylem water potential, and plant hydraulic conductance as measures of plant performance on a Mediterranean green roof. They studied two forb species and found both to be suitable for the green roof: *A. unedo* (arbutus) and *S. officinalis* (sage). The arbutus was isohydric (demonstrating tight stomatal control of transpiration) and the sage anisohydric (keeping their stomata open longer for photosynthetic activity but continuously losing water) (Raimondo et al. 2015). The researchers concluded that substrate design is vital when specifying anisohydric plants, such as *Salvia officinalis* (sage), for green roofs to prevent foliage damage and/or desiccation under prolonged drought (Raimondo et al. 2015). While arbutus was seen to be a good choice for the green roof due to its low irrigation requirement, sage was desired to maximize transpiration-based cooling favored by a more rapid loss of water from the substrate (Raimondo et al. 2015).

Physiological status has also been defined by Kotsiris, Nektarios, and Paraskevopoulou (2012), who evaluated the growth and physiological performance of *Lavandula angustifolia* on a semi-intensive green roof in Mediterranean climatic conditions. Physiological status was measured in terms of chlorophyll a and b content, and stomatal resistance (Kotsiris, Nektarios, and Paraskevopoulou 2012). Kotsiris, Nektarios, and Paraskevopoulou (2012) asserted that substrates that stimulate higher stomatal resistance in plants would be inducing more drought stress. This finding was associated with decreased chlorophyll content, signifying reduced photosynthetic activity (Kotsiris, Nektarios, and Paraskevopoulou 2012). The researchers found that stomatal resistance values were reduced with deeper substrates, and varied with substrate type (Kotsiris, Nektarios, and Paraskevopoulou 2012).

A study involving the measurement of stomatal resistance as an indicator of physiological status of a turfgrass species was conducted by Ntoulas and the team in 2013. Stomatal resistance was measured, along with green turf cover (GTC), and normalized difference vegetation index (NDVI), in measuring the effects of substrate characteristics and depth on the growth and drought tolerance of *Zoysia matrella ‘Zeon’* in Athens (Ntoulas et al. 2013).

Based on the above-mentioned studies, stomatal resistance was used in my study as a measure of physiological performance. Stomatal resistance corresponds to the opening or closing of stomata in response to leaf water status and is indicative of the how stressed the plants are because of water deficit (Jones 2007; Ache et al. 2010). The method used for stomatal resistance measurements have been reported in Chapter 3.

The Importance of Creating Resilient, Lower Cost Green Roofs

Green roofs are important green infrastructure for any urban location. The wide array of environmental, social, and economic benefits that green roofs provide have made them a popular practice in many countries in Europe, Asia, and North America, and in many other progressive countries (Jim 2017). Most green roof research is recent, thus, many green roof attributes still need exploration. One important area of interest for green roof researchers has been maintaining biodiversity, especially native species, on a green roof (Cook-Patton 2015). This desire has caused researchers to consider the survival and growth of various vegetation types on a green roof and their performance specific to the environmental conditions on the rooftop. Plants typically need to withstand harsh environmental conditions, thin growing media, and limited water availability to survive on a green roof (Sutton 2015).

Native plants that grow vigorously given these stresses are desired. Plant species that demonstrate optimum plant-water relations (to survive drought and use water when it is in excess on the green roof) would be ideal green roof plants (Farrell et al. 2013). Examining the performance of mixed-species plant mixes on Kansas State University's APD-EGR provides one attempt to assess the success of individual plant species as they grow in relation to five other species on a green roof composed of two distinct substrate types. It is hoped that the methods and tools used during this 2018 baseline study of graminoid species survival and growth within the APD-EGR 4-inch bed will help to inform and focus future studies on this experimental green roof.

Chapter 3 - Research Setting and Methods

Research Setting

The Architecture Planning and Design Experimental Green Roof (APD-EGR) is situated above the new Seaton Hall studios at Kansas State University in Manhattan, Kansas (39.1897° N, 96.5831° W). Manhattan, Kansas has a continental climate as per the Koppen-Geiger climate classification, with average annual precipitation of 35.62 inches (904.75mm), based on 30-year averages (Knapp 2017). The highest mean maximum temperature in Manhattan, Kansas in 2008-2018 was 91.3°F (July), while lowest mean minimum temperature was -2°F (January) (National Oceanic and Atmospheric Administration 2019; Appendix-A). Appendix-A provides further information on the average temperatures found in Manhattan, Kansas, along with graphs for precipitation, wind, temperature, relative humidity, and solar radiation graphs on the APD-EGR during the 2018 study period. Figure 3-1 is a photograph taken by Professor Lee Skabelund, which shows the APD-EGR in late-June, 2018.

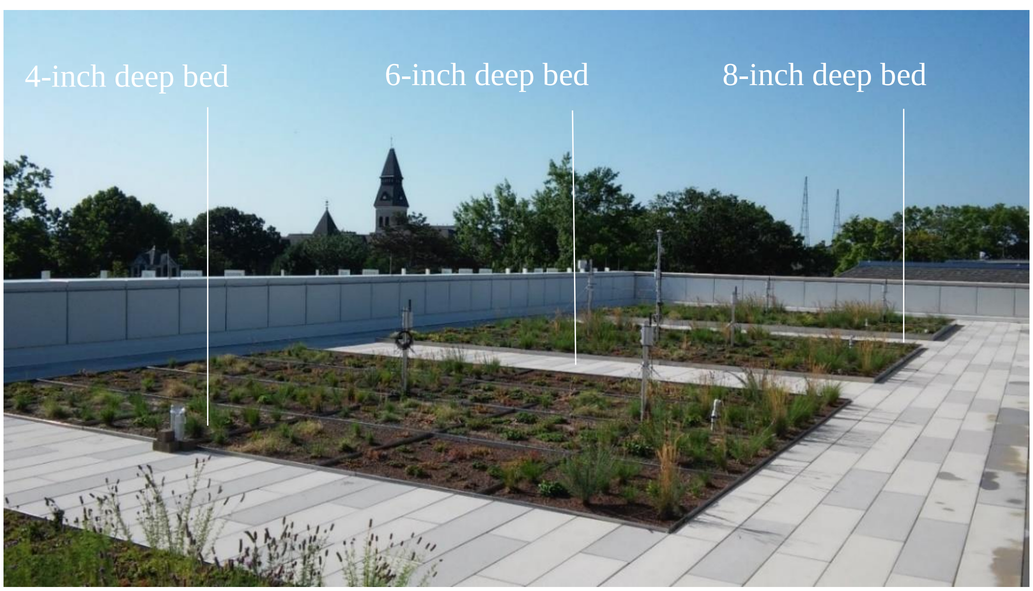

Figure 3-1: APD-EGR Green Roofs

(Photograph by Lee Skabelund, taken on June 28, 2018, at the APDesign Experimental Green Roof, Kansas State University)

Figure 3-2 illustrates a section of the APD-EGR, and shows the components of the green roof system. Vegetation has been planted atop a substrate layer, supported by a filter fabric, a gravel leveling layer, a drainage panel, a root barrier, a water-proofing membrane and insulation, all laid atop the roof structure.

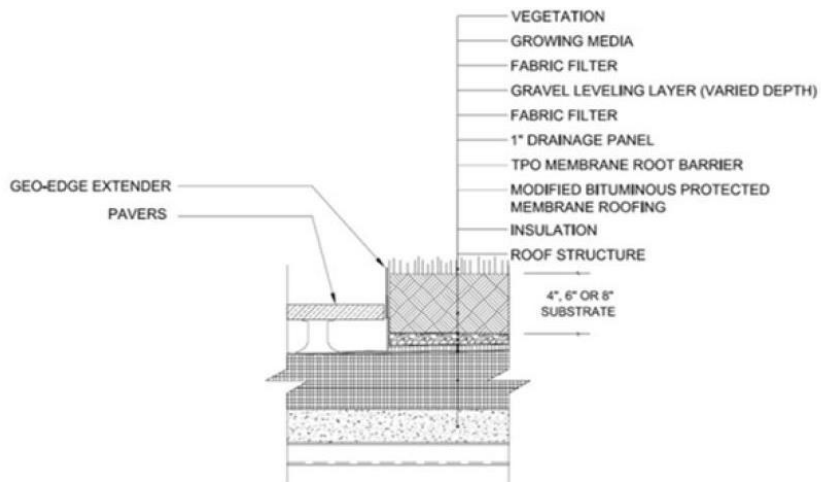

Figure 3-2: Section of APD-EGR with green roof components shown

(Shrestha; adapted from APD-EGR construction drawings)

Seventeen (17) plant species (eight *Sedum*, seven graminoids, and two forbs) were planted as live plugs in the roof plots on the APD-EGR experimental green roof at Kansas State University in Manhattan, Kansas in October 2017. Due to poor overwintering performance, 116 plants in the 4-inch bed (including 72 graminoids) were replanted in May and June 2018.

As shown in Figure 3-1, seventy-two (72) approximately 1.96 square meter experimental green roof plots were established on the roof in three substrate depths, with 24 plots in each bed of 4-inch (10.16cm), 6-inch (15.24cm), and 8-inch (20.32cm) deep substrates. My study accounted for the approximately 4-inch (10 cm) deep substrate plots (see Figure 3-3) considering that: (1) it facilitated ease of experimentation, (2) it is the shallowest depth among the three established depths, and hence the most structurally desirable and cost-effective, and (3) the plants were expected to be the most stressed in this depth. Hence, if the graminoids performed well in the 4-inch deep bed, we could assume that they would grow well in deeper media.

Figure 3-3: Approximately 4-inch (10 cm) deep plots
(Photograph by Lee Skabelund, taken on June 28, 2018, at the APDesign Experimental Green Roof, Kansas State University)

The plots were randomized with two types of substrates: Kansas BuildEx® and rooflite® extensive mc green roof media. The two green roof media had both previously been specified and used on other KSU green roofs, and both were deemed promising for growing Sedum and prairie plants on campus (personal communication with Lee Skabelund 2018).

As a part of the Mary K. Jarvis research, Professor Skabelund selected the plant species based on past precedents of plants that have done well on green roofs in other similar regions (personal communication with Lee Skabelund 2018). The plant species were selected based on a quick literature review and an evaluation of species that had performed well in Manhattan, Kansas; Omaha and Lincoln, Nebraska (personal communication by Lee Skabelund with Richard Sutton); and Ann Arbor, Michigan (personal communication by Lee Skabelund with Robert Grese). The grasses and forbs selected are native to or found within the Flint Hills Ecoregion.

Vegetation was planted on the APD-EGR in three mixes as illustrated in Table 3-1. Mix A is comprised of *Sedum* species, Mix B consists of *Sedum* and native grasses, and Mix C is comprised of native grasses and forbs. The species assigned to each mix are also reported in Table 3-1. The designer adopted a systematic numbering system in laying out the plant species in each plot. Initially, randomizing the plant mixes was attempted. According to Skabelund (personal communication in 2018), in order to avoid the undesired clustering of the same species in one location, the plants were numbered in increasing numeric and alphabetical order as shown (refer to Table 3-1; Figure 3-4).

The layout of the plant mix and substrate combinations have been randomized across the plots in four blocks, with six plots per block, and repeated plant mixes side-by-side within two distinct substrate types. Figure 3-5 shows the twenty-four (24) plots for the substrate depth of approximately four inches (10 cm), where K and R represent Kansas BuildEx® and rooflite® extensive mc, and A, B and C represent the three mixes shown in Table 3-1. Within each plot, there are three of each plant species from the corresponding mixes from Table 3-1, as shown in Figure 3-4. Since my study only evaluated graminoids, only Mix B and Mix C were studied, and are denoted by the shaded plots in Figure 3-5. Mix A was not a part of this study.

Table 3-1: Plant mixes on the Architecture Planning and Design Experimental Green Roof

Sedum species (Mix A)	**Sedum and grass mix (Mix B)**	**Native Grasses and forbs (Mix C)**
Sedum album f. murale (1)	*Bouteloua curtipendula* (1)	*Carex brevoir* (1)
Sedum ellacombeanum (2)	*Bouteloua dactyloides* (2)	*Dalea purpurea* (2)
Sedum hybridum 'immergrunchen' (3)	*Bouteloua gracilis* (3)	*Koeleria pyrammidata* (3)
Sedum kamschaticum var. floriforum 'Eeihenstephaner Gold' (4)	*Schizachyrium scoparium* (4)	*Packera obovata* (4)
Sedum sexangulare (5)	*Sedum reflexum* (5)	*Schizachyrium scoparium* (5)
Sedum spurium (6)	*Sedum rupestre* (6)	*Sporobolus heterolepis* (6)

Figure 3-4: Plant layouts for plant mixes A, B, and C

(Photographs by Allyssa Decker, taken in 2018, at the APDesign Experimental Green Roof, Kansas State University)

Numbers denoted in the figure above correspond to the numbers assigned to the plant species, as noted in Table 3-1.

← N

KA	RA	RB	KB
KB	RB	RA	KA
KC	RC	RC	KC
RB	KB	KA	RA
RA	KA	KB	RB
RC	KC	KC	RC

Figure 3-5: Plant mixes B and C in the Kansas BuildEx® (K) and the rooflite® extensive mc (R) substrates in the 4-inch deep bed

Substrates ‘K and ‘R’ stand for Kansas BuildEx® and rooflite® extensive mc respectively. Plant mixes ‘A’, ‘B’ and ‘C’ represent the three mixes respectively: *Sedum* only, *Sedum* and native grass mix, and native grasses and forbs.

The grass and sedge species that were a part of my research are listed in Table 3-2. Little bluestem was used in both the 'B' and 'C' plant mixes. The type (warm or cool season) graminoid species is indicated for each species.

Table 3-2: Grass and sedge species on the APD-EGR

Grass/sedge species	**Common name**	**Mix**	**Type of grass/sedge species**
Bouteloua curtipendula (1)	Side-oats grama	B	C4 warm season grass
Bouteloua dactyloides (2)	Buffalograss	B	C4 warm season grass
Bouteloua gracilis (3)	Blue grama	B	C4 warm season grass
Schizachyrium scoparium (4), (5)	Little bluestem	B, C	C4 warm season grass
Carex brevoir (1)	Prairie sedge	C	C3 cool season sedge
Koeleria pyrammidata (3)	Prairie junegrass	C	C3 cool season grass
Sporobolus heterolepis (6)	Prairie dropseed	C	C4 warm season grass

The APD-EGR study is a longitudinal experimental study aimed at assessing which of the selected plant mixes and substrates succeed on a green roof setting in Manhattan Kansas. My involvement was the initial part of this study spanning the first growing season of the experiment, and assessed the survival, growth, and physiological performance of the graminoids on the APD-EGR. The longitudinal study is being conducted for all of the selected species over a period of at least three years. In the 24, 4-inch (10 cm) plots with Kansas BuildEx® and rooflite® extensive mc™ substrates, as described in the 'Research setting' section and shown in Figure 3-5, there are four plots with identical substrate type and plant species layouts, indicated as KB, KC, RB, RC, where the first letters (K or R) indicate the substrates and the second letters (B or C) indicate the mixes. Figure 3-6 shows the concept map for the research with the different species, substrates, and plant mixes, as well as the methods being adopted in the study.

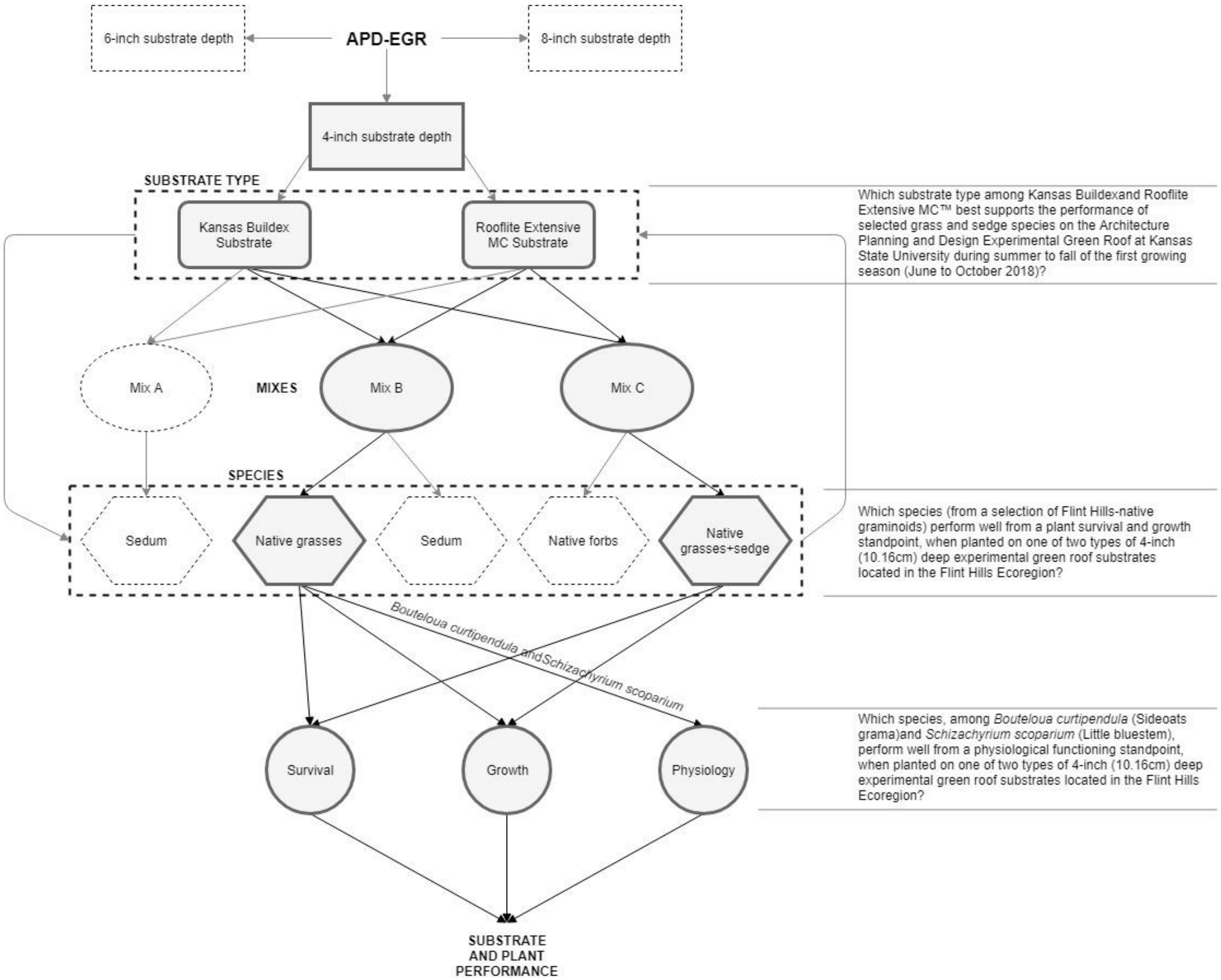

Figure 3-6: Concept map for the research

Planting and Replacement

The APD-EGR plants were initially planted in October, 2017. However, due to cold winter conditions, some poorly selected and/or planted live plants, and the short establishment period before winter dormancy, 37.5 percent of the graminoids in the 4-inch bed did not survive the winter conditions (see Appendix-B). Appendix-B shows the percentage of each plant species that survived the winter of 2017-2018. The dead plants were replaced in June 2018, after which data collection was commenced. Thus, the plants in this study have two establishment periods. An initial attempt was carried out at incorporating the two establishment dates into statistical analyses, but no consistent pattern was observed in terms of the effects due to differences in establishment on a single substrate or mix factor. Thus, establishment has been accounted for, in statistical analyses, as experimental error.

Substrate Constituents and Characteristics

According to Tim Sharp from Blueville Nursery (personal communication by Jialin Liu with Tim Sharp, dated October 1, 2018), Kansas BuildEx® is made with mason sand, fine grade peat moss, cattle manure compost mixed in equal proportions with Buildex lightweight aggregate (expanded shale). The rooflite® extensive mc substrate is a proprietary mix manufactured by Skyland LLC, and is a blend of light weight mineral aggregates and organic components such as compost approved by USCC STA. ("rooflite® extensive 800 Specifications" n.d.). Plots with the two substrates types are shown in Figure 3-7, with Mix B plants.

Figure 3-7: Kansas BuildEx® (K) and rooflite® extensive mc (R) substrates with *Sedum* and native grasses

(Photographs by Allyssa Decker, taken in 2018, at the APDesign Experimental Green Roof, Kansas State University)

Substrate chemical characteristics

In March 2018, substrate samples were collected consistently from the centers of each of the green roof plots. Samples from the same depth and substrate type were combined, and sent to the Kansas State University Soil Testing Laboratory (Manhattan, Kansas) for testing. Testing was done for nutrients, organic matter, electrical conductivity, cation exchange capacity, pH, and percentage of sand, silt and clay in the Kansas BuildEx® and rooflite® extensive mc substrates.

Table 3-3 shows the results of the soil test conducted at the KSU Soil Test Lab for the 4-inch deep bed. The nitrogen, phosphorus and the potassium values were slightly higher for Kansas BuildEx® than rooflite® extensive mc. Both of the substrates were found to be alkaline, with rooflite® extensive mc having a slightly greater pH value than Kansas BuildEx®. The rooflite® extensive mc substrate had a greater organic matter content than the Kansas BuildEx® substrate. As per the personal communication between Bryan Rutter, the lab manager of the KSU Soil test lab, and Jialin Liu (October 1, 2018), the protocols used by the KSU Soil test lab for conducting the tests are reported in Appendix-C.

Table 3-3: Soil test results for the Kansas BuildEx® and rooflite® extensive mc substrate types in the 4-inch deep APD-EGR bed (tests were conducted by KSU Soil Testing Lab in April 2018)

Substrate Characteristics	**Kansas BuildEx®**	**rooflite® extensive mc**
Cation Exchange Capacity (CECS) meq/100g	7.63	8.79
Total Nitrogen (N) %	0.76	2.14
Total Carbon (C) %	3.79	5.49
Calcium (Ca) ppm	1,256.8	1,503.2
Copper (Cu) ppm	0.3	0.6
Electrical conductivity (EC) dS/m	0.7	0.85
Magnesium (Mg) ppm	116.9	109.5
Manganese (Mn) ppm	2.0	3.2
Sodium (Na) ppm	17.8	29.3
Organic Matter (OM) loss on ignition (LOI%)	1.6	3.6
pH	7.9	8.4
Nitrate Nitrogen (NO3-N) ppm	4.7	2.1
Potassium (K) ppm	115.6	89.7
Phosphorus (P)-M ppm	85.0	59.9
Zinc (Zn) ppm	1.2	3.1
Iron (Fe) ppm	15.8	19.2
Sand %	90	86
Silt %	4	8
Clay %	6	6

Substrate physical characteristics

Substrate samples of Kansas BuildEx® and rooflite® extensive mc were obtained from Blueville Nursery Inc. (Manhattan, Kansas), and sent to Turf and Soil Diagnostics (Linwood, Kansas) in March 2019. Tests for physical characteristics of the two substrates were conducted for quantifying media density, permeability, porosity, organic particle size, pH, soluble salts, and water release characterization, including bulk density, particle density, total pore space and water release curve. The maximum media density tests were conducted as per the ASTM E2399 standards (ASTM 2019). ASTM D4972 (with CaCl2 not screened) was used for pH testing (ASTM 2018a). The air-filled porosity was tested at maximum water holding capacity, and organic matter was ashed at 550°C as per the FLL guidelines (FLL 2008). The particle size distribution testing was done as per ASTM F1632 Method B (ASTM 2018b). Additional methods utilized in substrate tests are reported in Appendix-C.

The results of the soil tests for media density, porosity, pH, electrical conductivity, and organic matter are reported in Tables 3-4 and 3-5. Table 3-6 shows the results of the particle size distribution testing, with the percentages retained in the sieves shown in Table 3-7. Figure 3-8 shows the percent of particles passed versus particle size graph based on the soil test results from Turf and Soil Diagnostics. The gray area represents the optimum particle size distribution specified by the FLL for extensive, multi-course green roofs (FLL 2002).

Table 3-4: Green roof media density test results for Kansas BuildEx® and rooflite® extensive mc substrates (tests were conducted by Turf and Soil Diagnostics, Linwood, Kansas, in March 2019)

Sample name	Water Permeability (Saturated Hydraulic Conductivity)		Initial Media Density (Application Density)		Maximum Media Density (Saturated Density)		Max. Media Water retention	Dry Media Density	
Units	in/hr	mm/min	lb/ft^3	g/cm^3	lb/ft^3	g/cm^3	%	lb/ft^3	g/cm^3
Kansas BuildEx®	0.4	0.2	103.9	1.67	110.0	1.76	29	91.2	1.46
rooflite® extensive mc	73.1	30.9	68.8	1.10	83.0	1.33	35	60.8	0.97

Table 3-5: Green roof media porosity, pH, electrical conductivity, and organic matter test results for Kansas BuildEx® and rooflite® extensive mc substrates (tests were conducted by Turf and Soil Diagnostics, Linwood, Kansas, in March 2019)

Sample name	Initial Sample Wt.	Sample Volume	Initial Sample Height	Final Sample Height	Sample Wt. After Draining	Total pore space	Air-filled Porosity	pH	Electrical Conductivity	Organic Matter
Units	kg	m^3	cm	Cm	Kg	%	%		Mmhos/cm	%
Kansas BuildEx®	3.128	0.0019	10.4	10.4	3.3	42	13	7.0	0.1	1.9
rooflite® extensive mc	2.151	0.0020	10.8	10.7	2.6	58	23	7.6	0.2	2.2

Table 3-6: Particle size distribution of the Kansas BuildEx® and rooflite® extensive mc green roof substrates. (tests were conducted by Turf and Soil Diagnostics, Linwood, Kansas, in March 2019)

				Percent Passing US Sieve (mm)					
Sample name	**Sand 2.0-0.063 mm**	**Silt 0.063-0.0002 mm**	**Clay <0.0002 mm**	**Gravel 3/8" (9.525 mm)**	**Gravel 1/8" (3.175 mm)**	**Gravel 10 (2.0 mm)**	**V. Coarse 18 (1.0 mm)**	**Medium 60 (0.025 mm)**	**V. Fine 230 (0.063) mm**
Units	%	%	%	%	%	%	%	%	%
Kansas BuildEx®	67.6	4.5	2.9	94.1	76.0	75.0	72.5	20.4	7.4
rooflite® extensive mc	52.4	5.8	1.3	100.0	69.2	59.5	41.2	13.2	6.9

Table 3-7: Percentage retained in sieve (drawn from Table 8) (tests were conducted by Turf and Soils Diagnostics, Linwood, Kansas, in March 2019)

				Percent retained in sieve						
Sample name	**Sand 2.0 -0.063 mm**	**Silt 0.063 – 0.0002 mm**	**Clay <0.000 2 mm**	**Gravel 3/8" (9.525 mm)**	**Gravel 1/8" (3.175 mm)**	**Gravel 10 (2.0 mm)**	**V. Coarse 18 (1.0 mm)**	**Medium 60 (0.025 mm)**	**V. Fine 230 (0.063 mm)**	**Passed through (0.063 mm)**
Units	%	%	%	%	%	%	%	%	%	
Kansas Buildex	67.6	4.5	2.9	5.9	18.1	1.0	2.5	52.1	13.0	7.4
rooflite® extensive mc	52.4	5.8	1.3	0	30.8	9.7	18.3	28.0	6.3	6.9

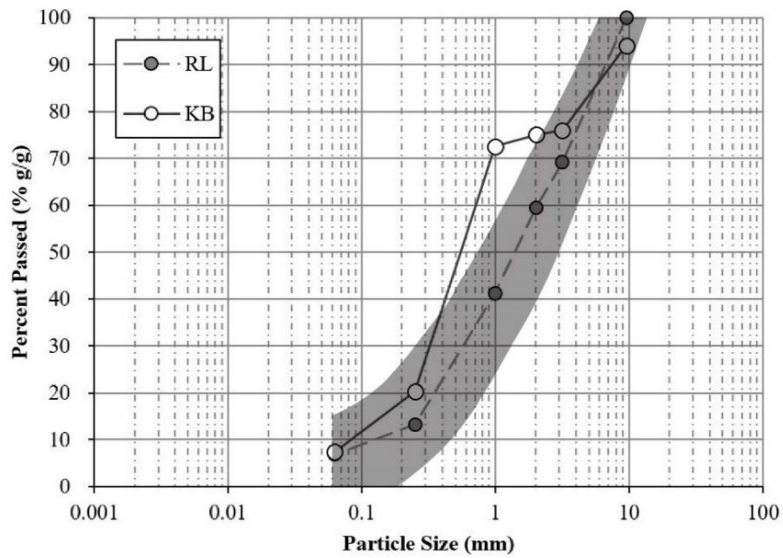

Figure 3-8: Percent passed vs. particle size graph

(adapted from graph made by Jialin Liu, in 2019)

The grey shaded area represents the optimum particle size range for extensive multi-course green roof substrates according to FLL guidelines (2002), as adapted from a graph by Jialin Liu (2019).

Table 3-8 shows the results of the soil water characterization test conducted by Turf and Soil Diagnostics, which comprises of the volumetric water content values corresponding to various tension values. This data has been plotted in Figure 3-9 as soil moisture release curves for the Kansas BuildEx® and rooflite® extensive mc substrates. The procedures adopted for these substrate tests are reported in Appendix-C.

Table 3-8: Soil water release characterization indicating volumetric moisture content (%) at different tension values (-bars) (tests were conducted by Turf and Soil Diagnostics, Linwood, Kansas, in March 2019)

Tension	**Kansas BuildEx®**	**rooflite® extensive mc**
-Bars	**Vol. Moisture Content (%)**	**Vol. Moisture Content (%)**
0	33.872	43.747
0.1	16.936	39.867
0.4	11.68	15.811
1	8.176	13.968
4	7.008	12.416
15	3.942	2.328

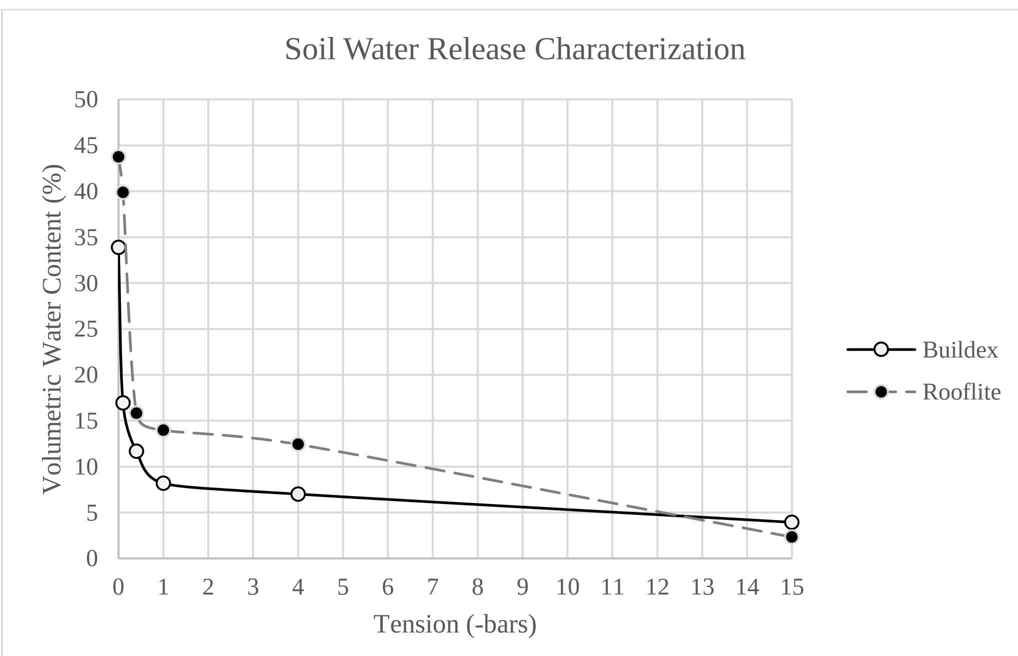

Figure 3-9: Soil water release curve for the Kansas BuildEx® and rooflite® extensive mc substrates

(adapted from tests conducted by Turf and Soil Diagnostics, Linwood, Kansas, in March 2019)

Variability in Substrate Depths within Beds

The depths of individual plots within the 4-inch bed were measured in June 2018. A total of eight (8) measurements per plot were taken and averaged to get the mean depth of each plot. The measurements taken have been reported in Appendix-D. Figure 3-8 shows a picture of Professor Lee Skabelund taking soil depth measurements using a marked rod. The average depths of the plots in the 4-inch deep bed ranged from 2.8 inches to 4.6 inches. There was no significant evidence for depth variability among treatments (substrate type, mix, substrate*mix interactions). Although pronounced effects of substrate depth variability were not observed, future tracking of plots with shallow depths is recommended. The SAS output for the statistical analyses of depth variations for the 4-inch bed has been reported in Appendix-D.

Figure 3-10: Soil depth measurements being taken by L. Skabelund (left), and soil depth measurements taken in eight locations on a rooflite® extensive mc plot (right)

(Left: Photograph by Priyasha Shrestha, Right: Photograph by Lee Skabelund, taken on June 22, 2018, at the APDesign Experimental Green Roof, Kansas State University)

Measurements were taken by tapping a measuring bar into the substrate to where it firmly touched the filter fabric, which separates the substrate from the drainage/leveling layer.

Methods for Assessing Plant Survival, Growth and Physiological Performance

The grass and sedge species were individually assessed for (1) survival, (2) growth, and (3) physiological performance across two substrate types. The methods adopted in this study are discussed below.

Survival

Survival rates were measured for each grass and sedge species by conducting visual plant counts for each graminoid species every two weeks through the growing season. Similar to the assessment performed by Rayner et al. (2016) for measuring survival, plants that had any green shoots or tissue left at the base were considered alive. Since sufficient irrigation was being provided throughout the study period, 100 percent survival was observed for the grass and sedge species in the first growing season. Survival is plant-dependent (Rayner et al. 2016), and can be deceptive when measured visually because plants may look dead, but may only be dormant. Hence, future survival measurements will need to be checked at each observation time for regenerative shoot growth (Rayner et al. 2016).

Plant growth

In the APD-EGR study, horizontal and vertical growth were measured separately in the form of coverage and height respectively, as inferred from a green roof study conducted in Adelaide, Australia (Razzaghmanesh, Beecham, and Kazemi 2014).

Plant height and coverage

Figure 3-11 shows the method adopted for plant height measurements. For the purposes of this study, plant heights of the native grass and sedge species were measured approximately two weeks apart. Heights were measured by rounding up the grass blades and extending them up vertically to get a measure of the longest grass blade or seed head (see Figure 3-11). *Bouteloua dactyloides* (buffalograss) was excluded from height assessments because it was deemed unfeasible to consistently measure height of the species. Foliar cover, i.e. vertical projection of exposed leaf area (University of Idaho College of Natural Resources 2009), of each individual plant was measured by taking overhead photographs each month, and analyzing the percent coverage in Image J (National Institutes of Health, Bethesda, Maryland, USA), and Adobe Photoshop (Adobe Inc., San Jose, CA) software (Dusza et al. 2017). Figure 3-12 shows Allyssa Decker, PhD student at Kansas State University, and green roof research team member, taking overhead images of the plots.

Figure 3-11: Plant height measurement on the APD-EGR

(Photograph by Lee Skabelund, taken in 2018, at the APDesign Experimental Green Roof, Kansas State University)

Figure 3-12: Allyssa Decker taking overhead photographs

(Photograph by Lee Skabelund, taken in 2018, at the APDesign Experimental Green Roof, Kansas State University)

The process incorporated in measuring the foliar cover of the graminoid species followed the instructions provided by Colleen Butler, a green roof researcher in New Orleans (dated October 12, 2009). Each species in each plot was segregated with the help of Image J, an image processing program, and Adobe Photoshop. Image J was used to convert the RGB image into HSB (Hue-Saturation-Brightness) stack, and then the threshold was adjusted to get a tentative black-and-white footprint of the plants. The image was then taken into Adobe Photoshop and layered against the original image to clean up and separate each plant. This image was again brought into Image J to quantify the percent foliar cover of the extracted species against the total cover of the plot. The threshold was adjusted once more and percent area was calculated using the ‘set measurements’ feature, set to ‘area fraction’ and ‘limit to threshold’, and then the ‘measure’ tool. Figure 3-13 shows the process incorporated in measuring the cover of the plants.

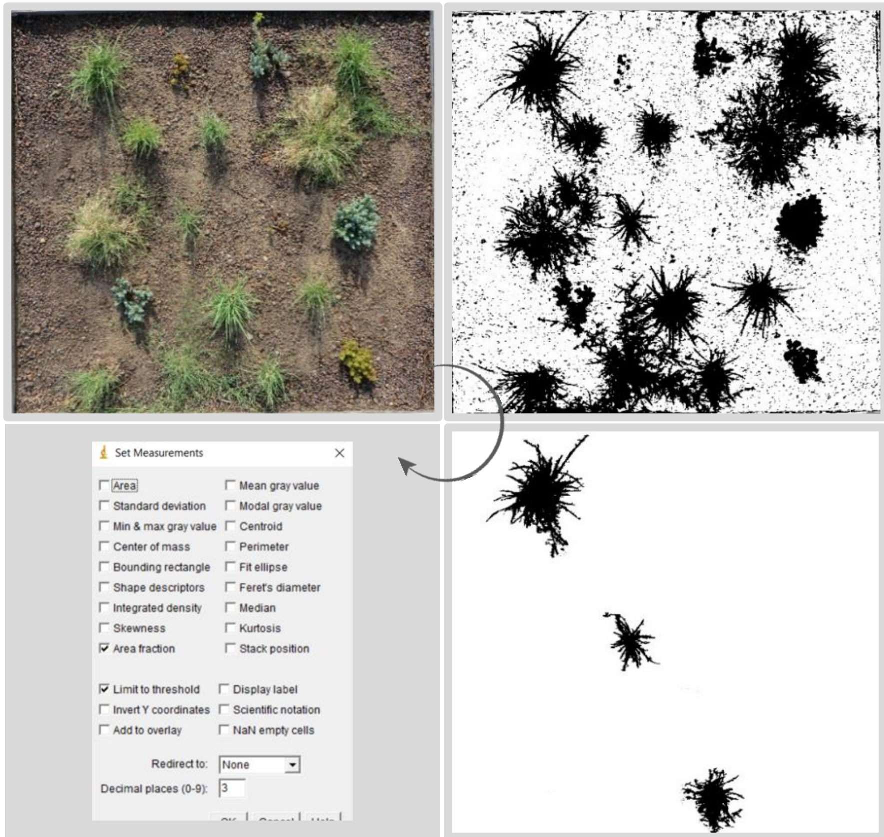

Figure 3-13 Coverage measurements using Image J and Adobe Photoshop: the extracted cover is for *Bouteloua curtipendula* in one of the 4-inch deep plots on the APD-EGR

(Shrestha; adapted from photograph by Allyssa Decker, taken in 2018, at the APDesign Experimental Green Roof, Kansas State University)

The foliar cover of each of the native grass and sedge species was documented to observe which species demonstrated greater coverage in each plot. Mean heights and foliar cover were respectively plotted against time (day of the year) to evaluate the trend in growth (Monterusso, Rowe, and Rugh 2005) for each plant species. Mean heights and foliar cover of individual species were also compared between substrate types to evaluate the relationships between substrate type and vertical and horizontal growth.

Biomass

The APD-EGR experiment also accounted for end of season above-ground biomass to assess the first-season development of vegetation and to inform differences in substrate type in supporting biomass development. For the APD-EGR study, above-ground biomass was collected by clipping the native grass and sedge species at the end of the season in November of 2018. Clipping was done to a height of approximately 2.5-inches (6.35cm) above the substrate surface. Clipped biomass for each individual graminoid species from each plot was collected in paper bags and dried in ovens at the KSU Agronomy North Farm at a near constant temperature of 60°C for a period of 72 hours to obtain their dry shoot biomass (Graceson et al. 2014). *Bouteloua dactyloides* was excluded from biomass measurements because it was deemed unfeasible to clip the species consistently at a height of 2.5 inches. Figure 3-14 is a photograph of the paper bags with biomass about to be dried in an oven at the KSU Agronomy North Farm. Shoot biomass values of the grasses were compared across the two substrates to observe probable differences in growth demonstrated by the plants in each substrate.

Figure 3-14: Paper bags with plant biomass about to be dried in a KSU North Agronomy Farm Oven

(Photograph by Jialin Liu, taken in November 2018, at the Agronomy North Farm, Kansas State University)

Plant physiological performance

Stomatal Resistance

A leaf porometer was used, in this study, to measure stomatal resistance of two grass species, *Bouteloua curtipendula* (side-oats grama) and *Schizachyrium scoparium* (little bluestem), on the APD-EGR. Water is lost mainly through the plant leaves, so it is necessary to know the extent of stomatal opening to understand how much water the plant is losing (Kirkham 2014f). Water available for plant use can be limited on green roofs with a shallow and lightweight substrate layer (Rowe 2015). Hence, it is important to assess the response of green roof plants to water deficit to comprehend their drought performance and suitability for use on green roofs.

The leaf porometer is a steady-state diffusion porometer (Model SC-1) made by METER Environment (METER Group, Inc. USA; previously Decagon Devices Inc.), which is used to quantitatively measure stomatal resistance by quantifying the diffusion of water vapor (Kirkham 2014f). A plant leaf is placed in the porometer sensor head, which measures the relative humidity and temperatures at two locations, by means of which the device calculates the stomatal resistance of the leaf (Kirkham 2014f). Jones (2014) has recorded the minimum stomatal resistance value for mesophytes (plants needing moderate water) to be within 80 to 240 s/m. According to Kirkham (2008), stomatal resistance was measured for Triticum aestivum L. 'Jagger' and values over 50 s/cm (5000 s/m) were deemed high. The maximum value of stomatal resistance has been reported to be more than 5000 s/m (Jones 2014), so any stomatal resistance value greater than 5000 s/m was to be excluded from the analysis. Figure 3-15 shows an image of an SC-1 Leaf Porometer being used to take a stomatal resistance reading on the APD-EGR.

Figure 3-15: Decagon SC-1 Leaf Porometer being used on the APD-EGR

(Photograph by Priyasha Shrestha, taken in 2018, at the APDesign Experimental Green Roof, Kansas State University)

As recommended by METER Environment, the porometer was calibrated every day before collecting data, or whenever there was more than a 15°C change in the environmental temperature in which the readings were being collected (Decagon Devices 2016). Depending on whether the stomatal resistance was to be measured on the abaxial (lower surface of the leaf), or adaxial surface (upper surface of the leaf), the leaf was placed in the sensor head so that the diffusion path/aperture was covered entirely by the leaf (Decagon Devices 2016). The readings were collected in the same time of the day since stomata go through a diurnal cycle, which is more pronounced under well-watered conditions (personal communication with Dr. Mary Beth Kirkham in June 2018).

For the APD-EGR study, the stomatal resistance of two warm-season grass species, *Bouteloua curtipendula*, and *Schizachyrium scoparium*, was compared across the two substrate types to assess the relationship between substrate type and drought stress induced on the plants.

Readings were taken on the abaxial surface, and two readings per plant were taken in each of the 4-inch deep plots comprised of Mix B. Data collection was conducted weekly for 13 weeks during Summer 2018, in the time frame of 10:00am to 1:00pm for the 4-inch plots. Irrigation on the roof was discontinued for a day before porometer readings were to be taken with the hope that a certain degree of stress could be induced on the plants, without disrupting the irrigation protocol for first-year plant establishment.

Visual assessment

In this study, a plant visual assessment was developed and used to quantify the auditor's perception of the vigor of the plant. The visual assessment scale was adapted from three published sources (Young 2014; Monterusso, Rowe, and Rugh 2005; Rayner et al. 2016). The visual assessment scale consisted of six ranks:

> 0=dead (no visible green or re-sprouting after watering); all leaves dry and shriveled
> 1=severe wilting or browning (wilted, horizontal form); sparse form; less than 25% green leaves
> 2= considerable wilting; low vigor and robustness; 25-50% green leaves
> 3=some leaves wilted or brown/reddish brown; average robustness and vigor; 50-75% green leaves
> 4=minimal (slight folding of leaf) to no wilting; robust growth and vigor; 75-95% green leaves
> 5=no wilting; very robust growth, vigorous form; 95-100% green leaves
>
> (Compare to Young 2014; Rayner et al. 2016; Monterusso et al. 2005.)

This method was highly subjective to the person rating the plants, so the results were not included in the results of this thesis report. Further development of these criteria to make the rating scale more quantifiable and reproducible is deemed necessary.

Irrigation, management, and maintenance

Green roof management requires decision-making regarding irrigation, weeding, and clipping protocols. Irrigation is extremely important on a green roof, especially during the first growing season, when the plants have to establish their roots in the green roof substrate with limited depth. The initial protocol was that during dry, hot weeks with no precipitation, the APD-EGR would be irrigated once a day for six (6) days, leaving one full-sun day for the plants to experience some level of stress prior to porometer readings. This protocol was adjusted based on precipitation so that each of the APD-EGR plots received approximately one inch (2.54 cm) of water every week either through irrigation or precipitation. Naturally, some rain events brought more than one inch of rain. During cloudy, cooler days, and hot sunny days after one or more rain event, irrigation decisions were made using soil moisture data obtained from soil moisture sensors that had been placed mid-depth in the substrates in March 2018.

Weeding was done every week to prevent seeding of the weeds. Anything apart from the plant species planted in the individual plots were considered weeds and pulled or rooted out. Clipping was done at the end of the growing season at a height of approximately 2.5 inches (6.35 cm) from the top of the substrate surface near each bunchgrass (and for the purple prairie clover) to collect, dry, and weigh above-ground biomass.

Data Analysis

According to Timothy C. Todd, Instructor in the Department of Plant Pathology at Kansas State University, “all response variables were analyzed using SAS Proc Mixed (SAS Institute Inc., Cary, NC, USA) for normally distributed data and Proc Glimmix for non-normally distributed data based on residual analysis. The experimental design was a strip plot, with substrate type and species mix as strip factors. The statistical model consisted of random block effects and fixed substrate type, species mix, and plant species within mix effects. Dependent variables included stomatal resistance, ending plant biomass and the area under the growth progress curve (AUGPC) for plant height, and coverage. Post hoc analyses of treatment means were conducted using paired t-tests ($p \leq 0.05$)" (personal communication with Timothy Todd, February 2019). Statistical analyses were conducted out by Timothy Todd.

Each bed of the experimental site incorporated a strip-plot or strip-block design, with four blocks per bed. The strip plot design was incorporated so that any confounding variables occurring due to spatial variability (for example, the direction of drainage), which might affect responses in the strip factors (substrate and mix), would be distributed uniformly among blocks. Figure 3-13 shows the four blocks in the 4-inch deep bed.

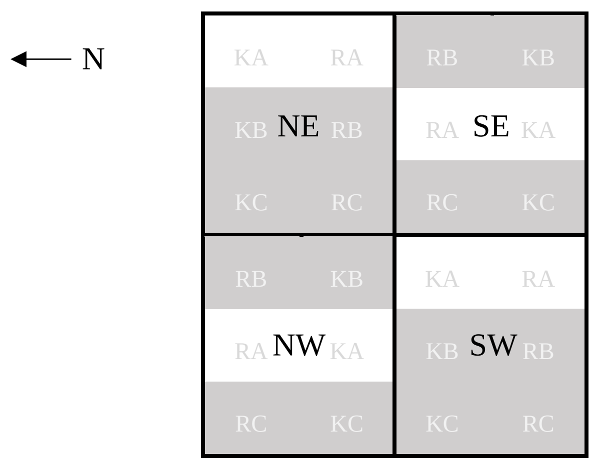

Figure 3-16: Four blocks (NE, NW, SE, SW) in the 4-inch deep bed as allocated by the strip-plot experimental design

Mixed models are used when there is a mix of fixed treatment effects and random effects (SAS Institute. "Fixed, Random, and Mixed Models :: SAS/STAT(R) 14.1 User's Guide" n.d.). The fixed effects in this study are substrate type, species mix, and plant species within mix. The random effect in this study is the block (see Figure 3-11). SAS Proc Mixed has been used to predict the values of the dependent variables in the study (variables being tested). The dependent variables in this study are stomatal resistance, ending plant biomass, and the area under the growth progress curve (AUGPC) for plant height, and for coverage. One of the assumptions for using a SAS Proc Mixed model is that the data has to follow a normal distribution, and is symmetrical around the mean (SAS Institute "PROC MIXED: Overview :: SAS/STAT(R) 9.2 User's Guide, Second Edition" n.d.). For non-normally distributed data, the Glimmix procedure was used, which assumes that the random effects are normal, but the data can have any distribution in the exponential family (SAS Institute "PROC GLIMMIX: Overview :: SAS/STAT(R) 9.2 User's Guide, Second Edition" n.d.).

Areas under the growth progress curves were chosen as dependent variables because it was a convenient way to combine multiple values (e.g. mean heights) across time, into the same index for purposes of comparison (Shiang, n.d.). The measurements of growth (height and coverage) were taken at reasonably regular intervals through the study season. Thus, AUGPC was used to integrate the growth of individual graminoid species through the season.

Chapter 4 - Results

Plant Survival

Survival rates for all graminoid species in the 4-inch bed were 100 percent because the roofs were being irrigated regularly throughout the growing season to avoid premature dieback. This perfect survival result for graminoids in the first growing season was observed for the mix of original and replanted plants within the 4-inch bed.

Plant Height

Plant height data, collected from the mid-June to early-November, was statistically analyzed as the AUGPC of six graminoid species, which demonstrated evidence of an overall main effect of substrate on plant height, with Kansas BuildEx® supporting greater vertical growth of the graminoids than the rooflite® extensive mc substrate at a 95 percent confidence level. Strong evidence of a main effect of species(mix) on plant height was also observed. *Bouteloua dactyloides* (buffalograss) was excluded from the height evaluations because it was unfeasible to consistently measure the height of the sod-forming species. Table 4-1 shows the results of the test of fixed effects (substrate, mix, substrate*mix interactions, species(mix), and substrate*species(mix) interactions) on plant height AUGPC. The complete results of the statistical analyses are reported in Appendix-E.

Figure 4-1 shows the Least Square Mean (LSM) heights (cm) versus time (day of the year) graph for the graminoids in the Kansas BuildEx® substrate and the rooflite® extensive mc substrate. The dates associated with the data points in the graph (see Figure 4-1) are June 16, June 29, July 15, July 30, August 16, September 10, September 24, October 12, and November 1 in 2018 respectively. The collective plant height AUGPC estimates for the six graminoids in

Kansas BuildEx® and rooflite® extensive mc are reported in Table 4-2. These AUGPC estimates correspond to the shaded areas under the two growth curves shown in Figure 4-1.

Table 4-1: Test of fixed effects on plant height AUGPC of the six graminoid species (excluding *Bouteloua dactyloides*)

Effect	Num DF	Den DF	F Value	Pr > F
Substrate	1	3	11.58	**0.0424***
Mix	1	3	1.83	0.2692
Substrate*Mix	1	3	1.23	0.3478
Species(Mix)	5	15	5.13	0.0061*
Substrate*Species(Mix)	5	15	2.10	0.1214

An asterisk (*) in the superscript shows significant differences between effect types (α=0.05)

Table 4-2: Plant height AUGPC estimates for the six graminoids in Kansas BuildEx® and rooflite® extensive mc substrates

Effect	Substrate	AUGPC Estimate	Standard Error	DF	T-Value	Pr > |t|	Lower	Upper
Substrate	Kansas BuildEx® (K)	7335.94	253.55	3	28.93	<.0001	6529.03	8142.85
Substrate	rooflite® extensive mc (R)	6419.34	257.27	3	24.95	0.0001	5600.59	7238.10

α=0.05

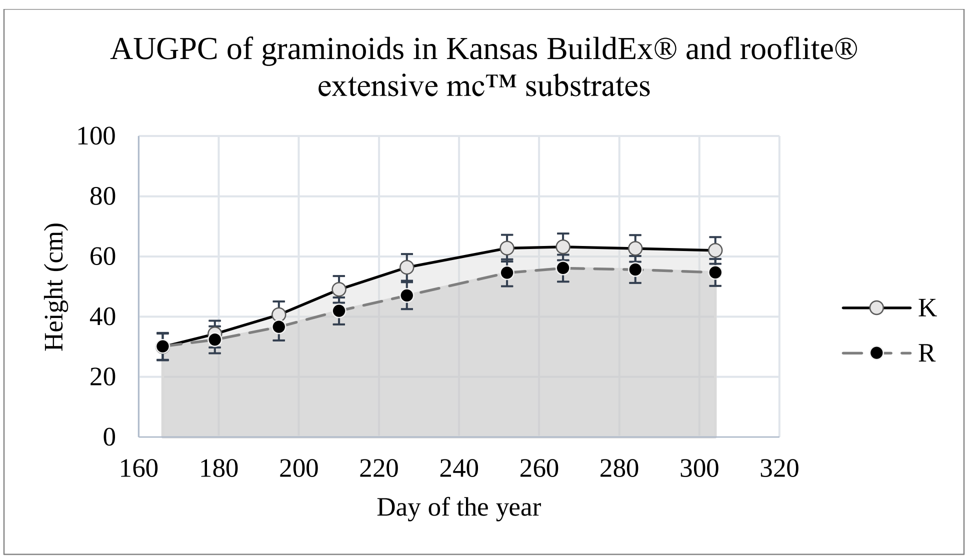

Figure 4-1: Graph depicting LSM 'Heights' versus 'Day of the year' for all six graminoids in Kansas BuildEx® (K) and rooflite® extensive mc (R) substrate types

Vertical bars denote upper and lower limits at a 95% confidence level.

Table 4-3 shows the test of effect slices on substrate*species(mix) interactions, which has been sliced by species. Thus, Table 4-3 shows the effect of substrate type on the height AUGPC of the individual species. Table 4-3 demonstrates strong evidence of a substrate effect on the heights of *Schizachyrium scoparium* in Plant Mix B and *Carex brevior* in Plant Mix C, with Kansas BuildEx® favoring greater height-based AUGPC for these two species.

Figure 4-2 is a graph comparing the height-based AUGPC estimates for individual graminoid species between Kansas BuildEx® and rooflite® extensive mc substrates. The AUGPC estimates are reported in the figure insets, and can be found along with standard errors, as a part of the complete statistical output in Appendix-E. Each of the graminoids, with the exception of *Koeleria pyramidata* (the only cool-season grass) demonstrated greater height in the Kansas BuildEx® substrate than the rooflite® extensive mc substrate.

Table 4-3: Test of effect slices on substrate*species interactions for height of the graminoids, sliced by species

Effect	Mix	Species	Num DF	Den DF	F Value	Pr > F
Substrate*Species(Mix)	B	*Bouteloua curtipendula*	1	15	1.74	0.2067
Substrate*Species(Mix)	B	*Bouteloua gracilis*	1	15	0.95	0.3454
Substrate*Species(Mix)	B	*Schizachyrium scoparium*	1	15	9.11	**0.0086***
Substrate*Species(Mix)	C	*Carex brevior*	1	15	7.29	**0.0164***
Substrate*Species(Mix)	C	*Koeleria pyramidata*	1	15	1.48	0.2426
Substrate*Species(Mix)	C	*Schizachyrium scoparium*	1	15	1.29	0.2739
Substrate*Species(Mix)	C	*Sporobolus heterolepis*	1	15	0.75	0.3994

Asterisks (*) in the superscript show significant effect of substrate type on species (α=0.05)

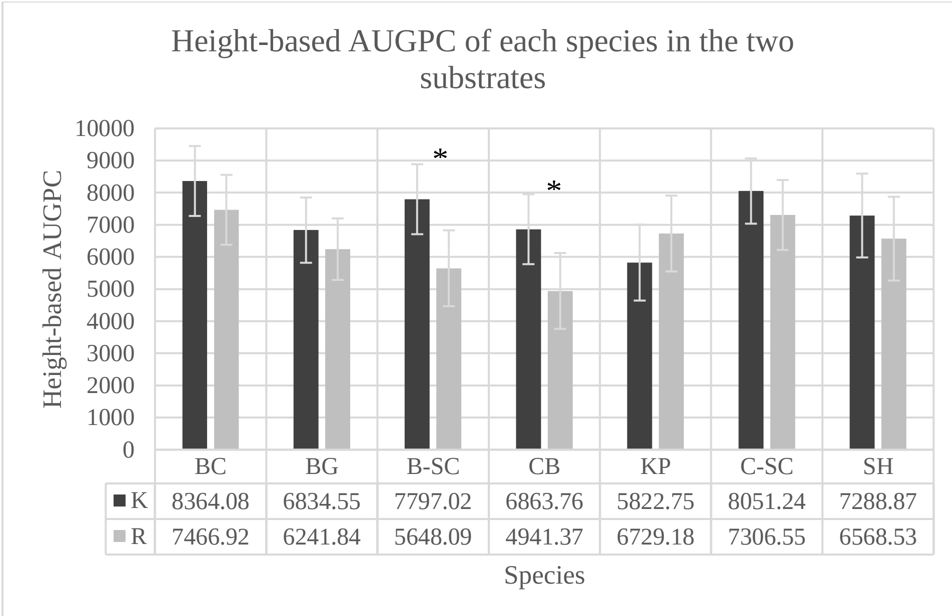

Figure 4-2: Graph depicting the height-based AUGPC estimates of individual graminoid species in the Kansas BuildEx® (K) and rooflite® extensive mc (R) substrates

Asterisks (*) show a significant effect of substrate type on species (α=0.05). Insets report the height-based AUGPC estimates for individual species across substrate type.
Vertical bars denote upper and lower confidence limits at 95%. BC=*Bouteloua curtipendula*; BG=*Bouteloua gracilis*; B-SC=*Schizachyrium scoparium* in Mix B; CB=*Carex brevior*; KP= *Koeleria pyramidata*; C-SC=*Schizachyrium scoparium* in Mix C SH=*Sporobolus heterolepis*

Table 4-4 reports the collective plant height AUGPC estimates for the grasses in Mix B and the graminoids in Mix C. Figure 4-3 illustrates the growth curves of the graminoids in each of the two mixes, obtained by plotting the least square mean (LSM) heights with time (day of the year). The shaded areas under the growth curves depicted in Figure 4-3, correspond to the AUGPC estimates reported in Table 4-4.

Table 4-4: Plant height AUGPC estimates for the graminoids in plant mixes B and C

Effect	Plant Mix	AUGPC Estimate	Standard Error	DF	T-Value	Pr > |t|	Lower	Upper
Substrate	B	7058.75	258.71	3	27.28	0.0001	6235.41	7882.09
Substrate	C	6696.53	252.65	3	26.50	0.0001	5892.48	7500.58

α=0.05

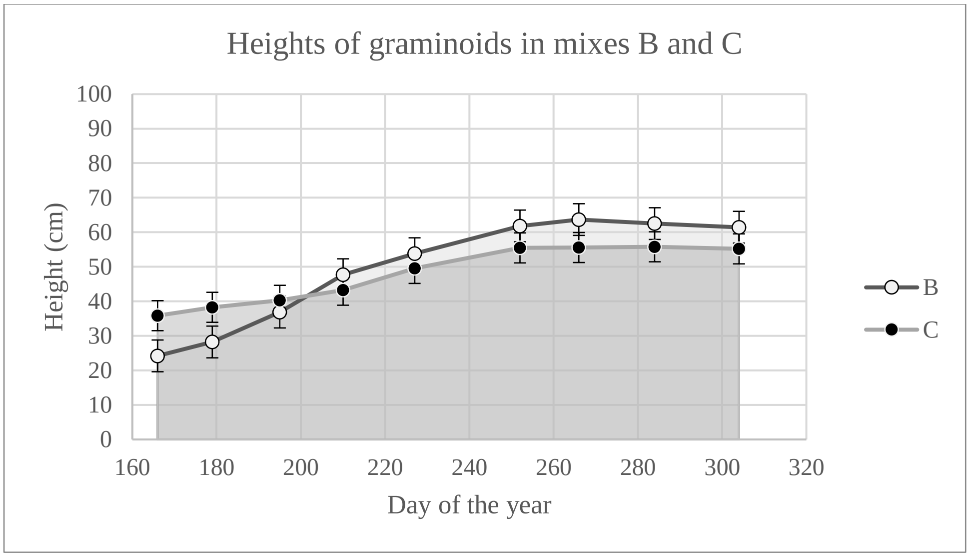

Figure 4-3: LSM 'Height' versus 'Day of the year' graph of the graminoids in Plant Mix B and Plant Mix C

Vertical bars denote upper and lower limits at a 95% confidence level.

Table 4-5 shows the plant height AUGPC estimates for the six individual species of graminoids. These estimates correspond to the shaded areas under the height-based growth curves of each species shown in Figure 4-4. Least square mean heights (in centimeters) for the individual species have been plotted against time (days of the year) in Figure 4-4, and signify the heights of the species through the study season (late June- early November).

Table 4-5: Plant height AUGPC estimate for the graminoids

Effect	Mix	Species	AUGPC Estimate	Standard error	DF	T Value	Pr > |t|	Lower	Upper
Species(Mix)	B	BC	7915.50	381.08	15	20.77	<.0001	7103.25	8727.75
Species(Mix)	B	BG	6538.20	349.55	15	18.70	<.0001	5793.14	7283.25
Species(Mix)	B	SC	6722.56	396.36	15	16.96	<.0001	5877.73	7567.38
Species(Mix)	C	CB	5902.56	396.35	15	14.89	<.0001	5057.76	6747.36
Species(Mix)	C	KP	6275.96	409.91	15	15.31	<.0001	5402.26	7149.67
Species(Mix)	C	SC	7678.90	369.60	15	20.78	<.0001	6891.12	8466.67
Species(Mix)	C	SH	6928.70	449.51	15	15.41	<.0001	5970.59	7886.81

α = 0.05

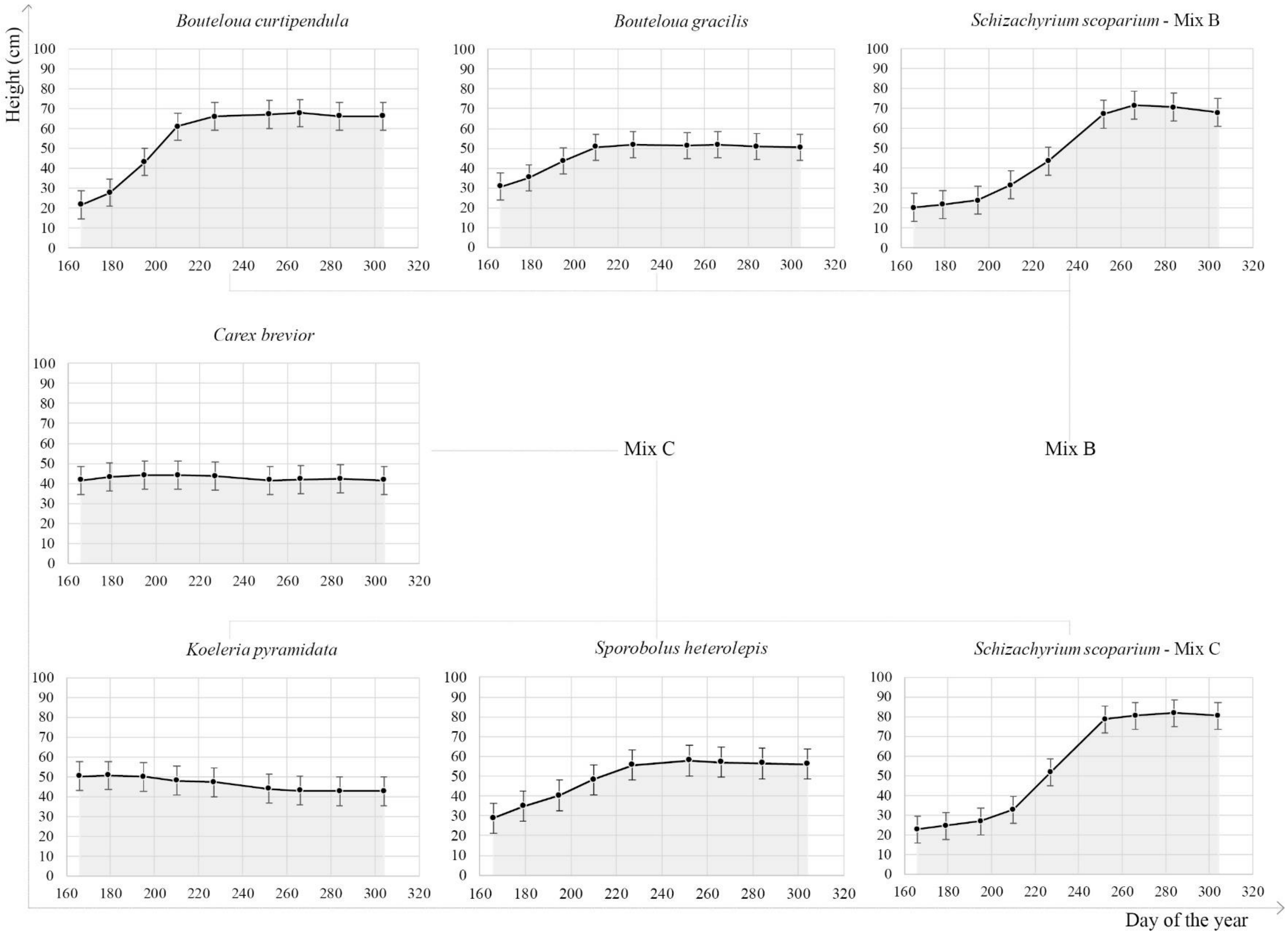

Figure 4-4: LSM 'Height' versus 'Day of the year' graphs for the graminoids through the growing season

Vertical bars denote upper and lower limits at a 95% confidence level.

Table 4-6 shows the plant height AUGPC estimates for *Schizachyrium Scoparium* in Mix B and Mix C, and the difference between the estimates is shown in Table 4-7. *Schizachyrium scoparium* was the only species common to Mixes B and C. Figure 4-5 shows the height-based growth curves of *Schizachyrium scoparium* in each of the two mixes. *Schizachyrium scoparium* showed greater vertical growth in Mix C than in Mix B, although not strongly evident at a 95% confidence level.

Table 4-6: Plant height AUGPC estimates for *Schizachyrium scoparium* (SC) in plant mixes B and C

Effect	Mix	Species	Estimate	Standard error	DF	T Value	Pr > |t|	Lower	Upper
Species(Mix)	B	SC	6722.56	396.36	15	16.96	<.0001	5877.73	7567.38
Species(Mix)	C	SC	7678.90	369.60	15	20.78	<.0001	6891.12	8466.67

α=0.05

Table 4-7: Difference in LSM of AUPGC for *Schizachyrium scoparium* (SC) in plant mixes B and C

	Mix	Sp.	Mix	Sp.	Estimate	SE	DF	T-value	Pr>|t|	Lower	Upper
Species (Mix)	B	SC	C	SC	-956.34	484.50	15	-1.97	0.0671	-1989.02	76.343

α=0.05

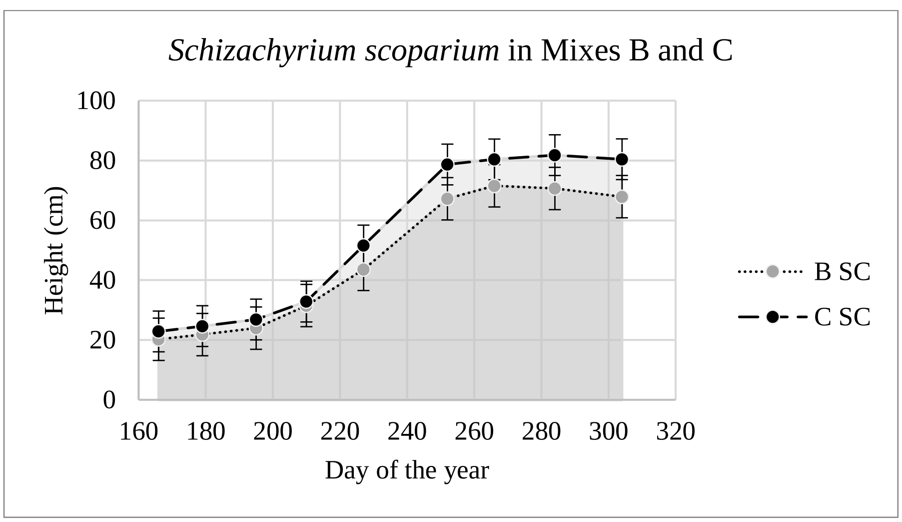

Figure 4-5: LSM 'Height' versus 'Day of the year' graph for *Schizachyrium scoparium* (SC) in plant mixes B and C

Vertical bars denote upper and lower limits at a 95% confidence level.

Plant Foliar Cover

Plant foliar cover data collected from the late-June to mid-October was statistically analyzed as the area under the growth progress curves (AUGPC) for the seven graminoid species, which demonstrated no strong evidence supporting an overall main effect of substrate on plant cover. Strong evidence for a main effect of species(mix) was observed on plant cover. Table 4-8 shows the results of the test of fixed effects (substrate, mix, substrate*mix interactions, species(mix) and substrate*species (mix) interactions) on plant cover. The collective plant cover AUPGC estimates for the Kansas BuildEx® and rooflite® extensive mc substrates are shown in Table 4-9. Figure 4-6 shows the plant cover-based growth curves for graminoids in each of the two substrates, obtained by plotting the least square mean 'Plant cover of plot' estimates with 'Day of the year. The shaded areas under the growth curves correspond to the AUGPC estimates shown in Table 4-9. The dates for the individual data points within each graph are June 26, July 11, August 10, September 12, , and October 16 in 2018. The complete results of the statistical analyses are reported in Appendix-F.

Table 4-8: Test of fixed effects on plant cover for seven graminoid species

Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Substrate	1	3	1.27	0.3421
Mix	1	3	2.03	0.2498
Substrate*Mix	1	3	1.10	0.3709
Species(Mix)	6	18	23.82	**<.0001***
Substrate*Species(Mix)	6	18	1.20	0.3498

Asterisk (*) in the superscript shows a statistically significant difference between effect types at a 95% confidence level (α=0.05)

Table 4-9: Plant cover AUPGC estimates for graminoids in Kansas BuildEx® (K) and rooflite® extensive mc (R) substrates

Effect	Substrate	Estimate	SE	DF	T- Value	Pr > |t|	Lower	Upper
Substrate	K	603.38	52.7081	3	11.45	0.0014	435.64	771.12
Substrate	R	531.05	54.1808	3	9.80	0.0023	358.62	703.48

α=0.05

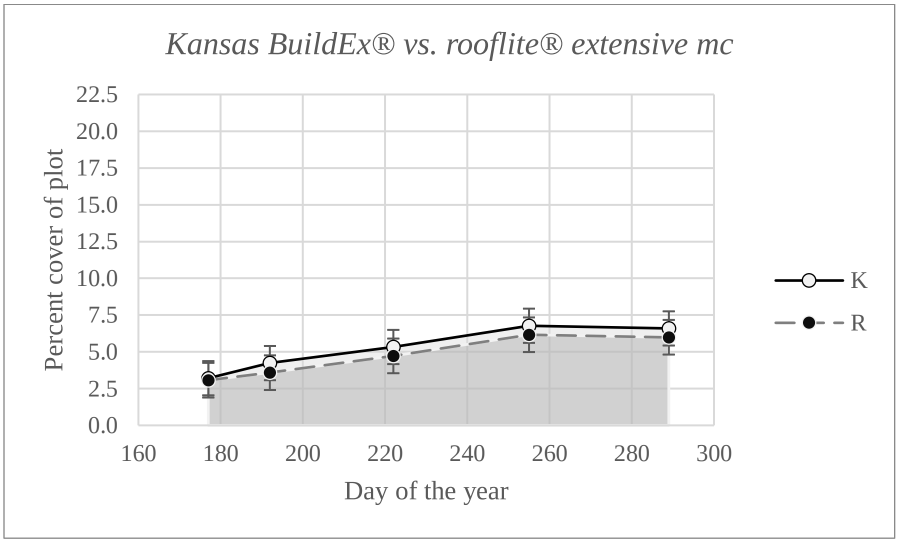

Figure 4-6: LSM 'Percent cover of plot' versus 'Day of the year' graph for the graminoids in the Kansas BuildEx® (K) and rooflite® extensive mc (R) substrates

Vertical bars denote upper and lower limits at a 95% confidence level.

Table 4-10 shows the test of effect slices on substrate*species interactions based on percent cover, which demonstrates strong evidence for substrate effect on the cover of *Bouteloua dactyloides*, with the Kansas BuildEx® substrate favoring greater cover for the species than the rooflite® extensive mc substrate. These differences are graphically illustrated in Figure 4-7, which compares the plant cover AUGPC estimates of the individual grass and sedge species in each of the two substrates: Kansas BuildEx® and rooflite® extensive mc. These AUGPC estimates can be found, with standard errors, as a part of the complete statistical output in Appendix-F.

Table 4-10: Test of effect slices on Substrate*species interactions based on percent cover, sliced by species

Effect	Mix	Species	Num Df	Den Df	F Value	Pr > F
Substrate*Species(Mix)	B	*Bouteloua curtipendula*	1	18	0.07	0.7909
Substrate*Species(Mix)	B	*Boutelous dactyloides*	1	18	9.57	**0.0063***
Substrate*Species(Mix)	B	*Bouteloua gracilis*	1	18	0.08	0.7759
Substrate*Species(Mix)	B	*Schizachyrium scoparium*	1	18	0.12	0.7344
Substrate*Species(Mix)	C	*Carex brevior*	1	18	0.09	0.7672
Substrate*Species(Mix)	C	*Koeleria pyramidata*	1	18	0.10	0.7609
Substrate*Species(Mix)	C	*Schizachyrium scoparium*	1	18	0.10	0.7556
Substrate*Species(Mix)	C	*Sporobolus heterolepis*	1	18	0.10	0.7498

** in the superscript shows a statistically significant difference between effect types at a 95% confidence level*

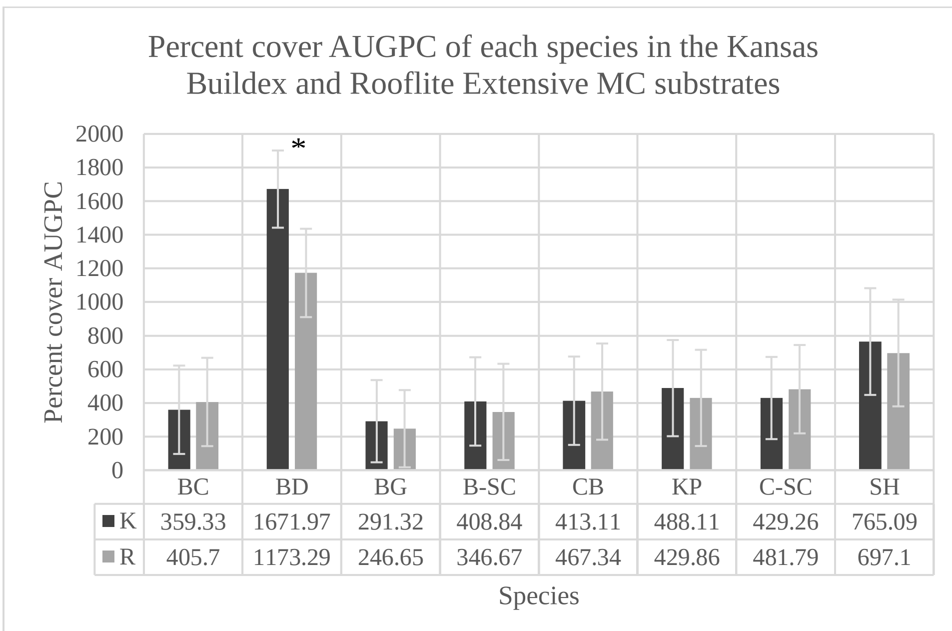

Figure 4-7: Percent cover AUGPC of each graminoid species in the Kansas BuildEx® (K) and rooflite® extensive mc (R) substrates

The asterisk (*) shows a statistically significant difference between effect types at a 95% confidence level; Vertical bars denote upper and lower limits at a 95% confidence level; Insets report the AUGPC estimates of each species based on plant cover BC=*Bouteloua curtipendula*; BD=*Bouteloua dactyloides*; BG=*Bouteloua gracilis*; B-SC=*Schizachyrium scoparium* in Mix B; CB=*Carex brevior*; KP=*Koeleria pyramidata*; C-SC=*Schizachyrium scoparium* in Mix C SH=*Sporobolus heterolepis*

Table 4-11 reports the percent plant cover AUGPC estimates for the graminoids planted in the two mixes B and C. These estimates are represented by the shaded areas under the growth curves in Figure 4-8. The growth curves have been obtained by plotting the LSM ‘Percent cover’ estimates of the graminoid species in plant mixes B and C with ‘Day of the year’.

Table 4-11: Percent plant cover AUGPC estimates for graminoids in plant mixes B and C

Effect	Mix	AUGPC Estimate	Standard Error	DF	T-Value	Pr > |t|	Lower	Upper
Substrate	B	612.97	51.3756	3	11.93	0.0013	449.47	776.47
Substrate	C	521.46	55.4822	3	9.40	0.0026	344.89	698.03

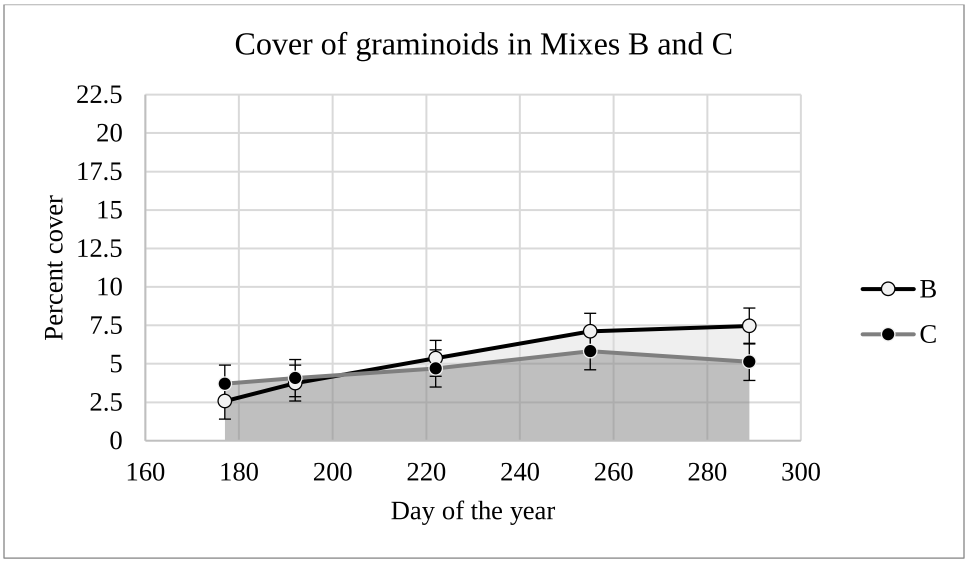

Figure 4-8: LSM ‘Percent cover’ versus ‘Day of the year’ graph of the graminoids in plant mixes B and C

Vertical bars denote upper and lower limits at a 95% confidence level

Table 4-12 shows the plant cover AUGPC estimates for the individual graminoid species. Figure 4-9 shows the growth curves for the seven graminoid species, obtained by plotting the LSM ‘Percent cover’ with ‘Day of the year. Thus, Figure 4-9, depicts the growth curves based on the cover of the species throughout the study season.

Table 4-12: Percent cover AUGPC estimates for the graminoids

Effect	Mix	Species	AUGPC Estimate	Standard error	DF	T Value	Pr > |t|	Lower	Upper
Species(Mix)	B	BC	382.51	90.6530	18	4.22	0.0005	192.06	572.97
Species(Mix)	B	BD	1422.63	85.3979	18	16.66	<.0001	1243.22	1602.05
Species(Mix)	B	BG	268.98	82.2568	18	3.27	0.0043	96.1689	441.80
Species(Mix)	B	SC	377.76	94.6485	18	3.99	0.0009	178.91	576.60
Species(Mix)	C	CB	440.23	94.6517	18	4.65	0.0002	241.37	639.08
Species(Mix)	C	KP	458.99	98.3130	18	4.67	0.0002	252.44	665.53
Species(Mix)	C	SC	455.52	87.6494	18	5.20	<.0001	271.38	639.67
Species(Mix)	C	SH	731.09	108.69	18	6.73	<.0001	502.74	959.44

α=0.05; BC=Bouteloua curtipendula; BD=Bouteloua dactyloides; BG=Bouteloua gracilis; B-SC=Schizachyrium scoparium in Mix B; CB=Carex brevior; KP=Koeleria pyramidata; C-SC=Schizachyrium scoparium in Mix C SH=Sporobolus heterolepis

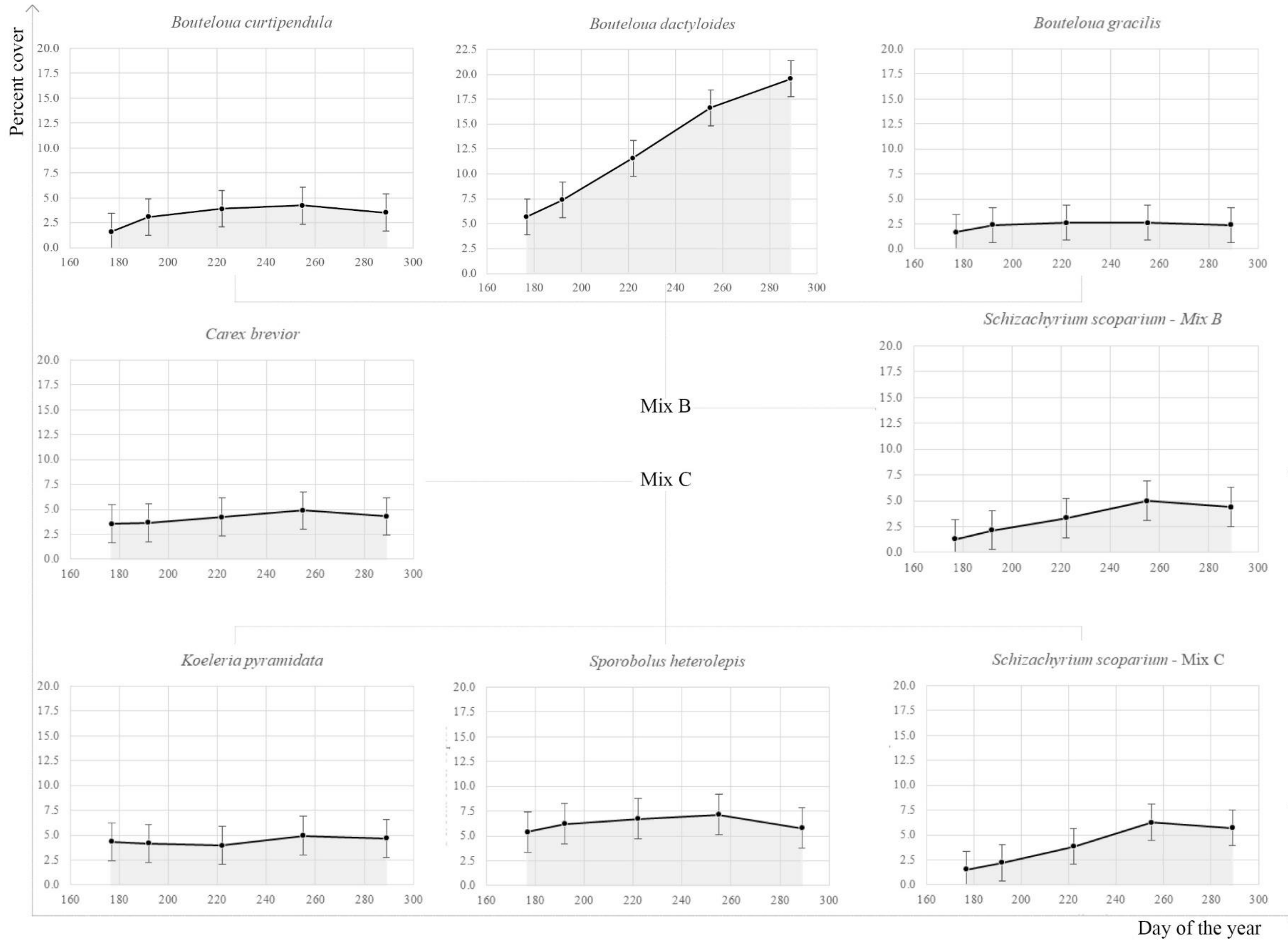

Figure 4-9: LSM 'Percent cover' versus 'Day of the year' graphs for the graminoids through the growing season

Vertical bars denote the upper and lower limits at a 95% confidence level.

Biomass

End-of-season plant biomass data collected in mid-November was analyzed for fixed effects of substrate, mix, substrate*mix interactions, species(mix), and substrate*species interactions. There was no strong evidence of an overall main effect of substrate on biomass, but there was a significant main effect of species. Table 4-13 reports the results of the test of fixed effects on biomass of six of the seven graminoid species. Table 4-14 denotes the collective LSM biomass estimates for the graminoids in the two substrates: Kansas BuildEx® and rooflite® extensive mc, and each of the two plant mixes B and C. Table 4-15 shows the sliced effect of substrate on the biomass of six graminoid species. *Bouteloua dactyloides* was excluded from the biomass study because clipping it consistently was deemed impractical because of its stoloniferous growing nature. Strong evidence of the effect of substrate type has been observed on the biomass of *Schizachyrium scoparium* in Plant Mix B, but not for other species. Complete SAS outputs for biomass analyses are reported in Appendix-G.

Table 4-13: Test of fixed effects on plant biomass of six graminoid species

Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Substrate	1	3	1.94	0.2584
Mix	1	3	3.85	0.1445
Substrate*Mix	1	3	0.16	0.7161
Species(Mix)	5	15	5.91	**0.0033***
Substrate*Species(Mix)	5	15	1.18	0.3650

α=0.05; asterisk (*) in the superscript denotes statistical significance

Table 4-14: LSM biomass estimates for the graminoids in Kansas BuildEx® and rooflite® extensive mc substrates, and plant mixes B and C (*α=0.05*)

Sub/ Mix	**Estimate**	**SE**	**DF**	**t-Value**	**Pr > |t|**	**Lower**	**Upper**	**Mean**	**SE Mean**	**Lower Mean**	**Upper Mean**
Sub K	3.0956	0.09790	3	31.62	<.0001	2.7840	3.4071	22.0995	2.1635	16.1836	30.1779
Sub R	2.9775	0.09893	3	30.10	<.0001	2.6626	3.2923	19.6380	1.9428	14.3338	26.9050
Mix B	2.9076	0.1158	3	25.11	0.0001	2.5391	3.2762	18.3136	2.1210	12.6678	26.4754
Mix C	3.1654	0.1049	3	30.18	<.0001	2.8316	3.4991	23.6978	2.4853	16.9729	33.0870

Table 4-15: Test of effect slices on substrate*species interactions for end-of-season biomass, sliced by species.

Effect	Mix	Species	Num DF	Den DF	F Value	Pr > F
Substrate*Species(Mix)	B	*Bouteloua curtipendula*	1	15	0.62	0.4434
Substrate*Species(Mix)	B	*Bouteloua gracilis*	1	15	0.13	0.7223
Substrate*Species(Mix)	B	*Schizachyrium scoparium*	1	15	6.37	**0.0234***
Substrate*Species(Mix)	C	*Carex brevior*	1	15	0.36	0.5552
Substrate*Species(Mix)	C	*Koeleria pyramidata*	1	15	0.37	0.5547
Substrate*Species(Mix)	C	*Schizachyrium scoparium*	1	15	0.38	0.5478
Substrate*Species(Mix)	C	*Sporobolus heterolepis*	1	15	0.02	0.8865

** denotes a significant effect of substrate type on species; α = 0.05*

Figure 4-10 is a graph that compares the LSM total biomass values for each species between the two substrate types. Insets show the mean biomass estimates for each species in each of the substrate types. These estimates, along with standard errors are reported in Appendix-G, as a part of the complete result of the statistical analyses.

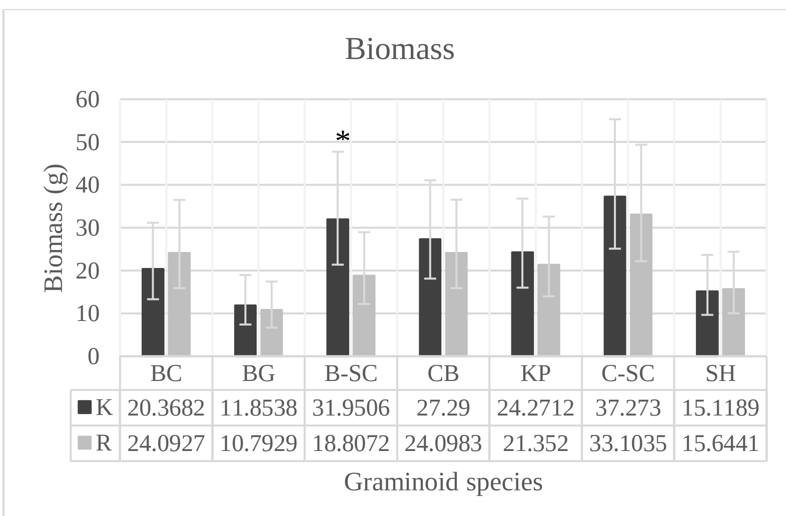

Figure 4-10: LSM biomass of the graminoid species in the Kansas BuildEx® (K) and rooflite® extensive mc (R) substrates

Asterisk (*) denotes a significant effect of substrate type on species. Vertical bars denote upper and lower limits at a 95% confidence level; BC=*Bouteloua curtipendula*; BG=*Bouteloua gracilis*; B-SC=*Schizachyrium scoparium* in Mix B; CB=*Carex brevior*; KP=*Koeleria pyramidata*; C-SC=*Schizachyrium scoparium* in Mix C SH=*Sporobolus heterolepis*

Plant Stomatal Resistance

A statistical analysis was conducted for stomatal resistance data collected from late June to September. These analyses showed that there was no strong evidence of an overall main effect of substrate on plant stomatal resistance of two warm season grasses: *Bouteloua curtipendula* and *Schizachyrium scoparium*. Stomatal resistance data was collected on June 27, July 7, July 12, July 20, July 27, August 3, August 9, August 18, August 25, September 1, September 12, September 22, and October 3 of 2018. Table 4-16 shows the LSM stomatal resistance estimates for each of the two substrates, Kansas BuildEx® and rooflite® extensive mc, and the LSM stomatal resistance estimates of the two species in the 4-inch bed. Table 4-17 shows the results of the test of fixed effects (substrate, species, substrate*species, day, substrate*day, species*day, and substrate*species*day interactions), which presents strong evidence to suggest a main effect of species on stomatal resistance. The results of the analysis are illustrated in Figure 4-11, which shows higher stomatal resistance of *Bouteloua curtipendula* (sideoats grama) as compared to *Schizachyrium scoparium* (little bluestem) over the study period. All results of the statistical analyses are reported in Appendix-H, as the complete SAS output.

Table 4-16: LSM stomatal resistance in *Bouteloua curtipendula* and *Schizachyrium scoparium* across Kansas BuildEx® and rooflite® extensive mc

Effect	Substrate	Species	Estimate	Standard error	T - value	Pr>|t|	Lower	Upper
Substrate	Kansas BuildEx®		261.33	19.5029	13.40	0.0009	199.26	323.40
Substrate	rooflite® extensive mc		261.25	19.6354	13.31	0.0009	198.77	323.74
Species		*Bouteloua curtipendula*	300.64	18.6177	16.15	<0.0001	255.08	346.19
Species		*Schizachyrium scoparium*	221.95	18.76.59	11.83	<0.0001	176.03	267.87

α = 0.05

Table 4-17: Test of fixed effects on stomatal resistance

Effect	F Value	Pr > F
Substrate	0.00	0.9976
Species	15.94	**0.0072***
Substrate*Species	1.85	0.2224
Day	5.94	<.0001*
Substrate*Day	0.42	0.9516
Species*Day	0.81	0.6394
Substrate*Species*Day	0.95	0.5005

α = 0.05; Asterisk (*) in superscript denotes a significant difference between effect types.

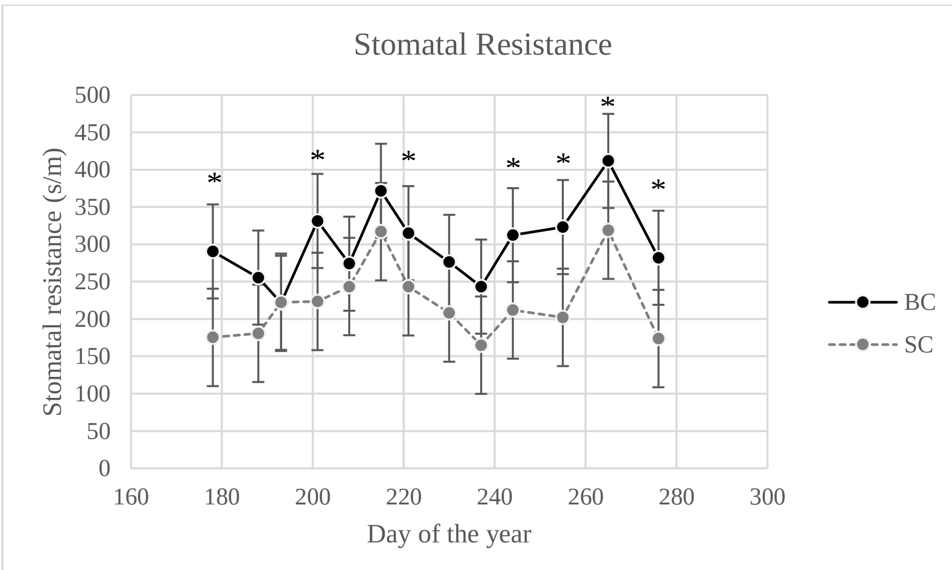

Figure 4-11: Graph of 'Stomatal resistance' versus 'Day of the year' for *Bouteloua curtipendula* (BC) and *Schizachyrium scoparium* (SC)

Vertical bars denote confidence limits at 95%. Asterisks denote significant differences between stomatal resistance in each day of measurements

Table 4-18 shows the weather conditions during the stomatal resistance data collection period. The maximum and minimum records of air temperature, relative humidity, solar radiation, wind speed, and precipitation in between a time frame of 10:00am to 2:00pm have been reported for the respective days on which data was collected. Precipitation records from twenty-four hours prior to the commencement of data collection are also reported. The meteorological data was recorded by weather station equipment supplied by METER Group Inc. (Pullman, WA, USA). Precipitation was measured using ECRN-100 High-Resolution Rain Gauge. Air temperature and relative humidity were measured by ATMOS 14, and solar radiation was measured by a PYR Solar Radiation Sensor. Data was recorded by a METER Group EM50G data logger (METER Group Inc., USA).

Table 4-18: Weather conditions during stomatal resistance readings (10:00am to 2:00pm) (data obtained from APD-EGR weather station at Kansas State University)

Date (10:00am-2:00pm)	Temperature (°C)		Relative Humidity		Solar Radiation (W/m^2)		Wind (m/s)		Precipitation (mm)		Precipitation 24 hours prior (mm)	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
June 27	28.9	32.5	0.597	0.733	86.06	942.99	0.53	1.56	0	0	0	0
July 7	27.6	32.4	0.481	0.685	126.34	932.01	0.54	1.31	0	0	0	0
July 12	32.5	37.8	0.310	0.542	84.23	939.33	1.49	2.35	0	0	0	0
July 20	27.4	31.3	0.468	0.611	49.44	922.85	0.64	1.83	0	0	0	2
July 27	23.1	29.8	0.391	0.588	67.75	921.02	0.81	1.51	0	0	0	0
Aug 3	28.0	34.4	0.309	0.461	82.40	921.02	1.95	3.97	0	0	0	0
Aug 9	28.3	35.4	0.355	0.580	64.09	990.60	0.25	0.96	0	0	0	0
Aug 18	26.8	32.2	0.424	0.664	135.50	767.21	0.56	1.03	0	0	0	0
Aug 25	28.5	33.4	0.435	0.640	76.90	855.10	1.67	2.80	0	0	0	0
Sep 1	28.4	34.0	0.453	0.633	120.85	862.43	1.31	2.78	0	0	0	0
Sep 12	22.5	27.3	0.554	0.731	53.10	703.13	0.84	2.20	0	0	0	0
Sep 22	14.1	20.9	0.491	0.734	31.13	811.16	0.65	1.62	0	0	0	0
Oct 3	26.3	31.4	0.531	0.708	45.78	703.13	3.53	5.39	0	0	0	0

Chapter 5 - Discussion and Conclusion

Discussion of Methods Used in the APD-EGR Study

Data collection was conducted late June through mid-November of 2019. Except in the case of aboveground biomass, all data collection used non-destructive approaches. Survival measurements were the most straightforward, and resulted in 100 percent survival of the grasses, which may be attributed to the ample and frequent irrigation that the team was providing.

The height measurements were easy to conduct, and made use of a meter ruler to measure the total height of the graminoids. The analysis of height excluded *Bouteloua dactyloides*, whose horizontal growth pattern restricted the consistent measurement of vertical growth for this species. Another limitation to this method and all other methods in this study was the planting time of October 2017, and the need to replant in 2018, which pushed the start date for data collection to late June, when the cool season graminoids had already grown to their full sizes. In future studies, it is recommended to initiate the data collection in early May, as was initially planned for this study.

The cover calculations were very tedious, requiring extensive time and effort to extract footprints of the graminoids, separate the footprints in Adobe Photoshop and then measure the percent cover in ImageJ again. In future studies, it is recommended to utilize the method for plant mix-level analyses, which would be achievable using Image J alone, instead of species-level analysis, which would be increasingly difficult due to overlap of leaf blades in a setting such as the APD-EGR. It is also recommended to use width as a measure of horizontal growth or methods such as the pin-frame method if individual species coverage is desired.

Stomatal resistance data was collected on a weekly basis from June to late September. Data was collected every 7-10 days, and within the same time frame of 10:00 am to 2:00 pm, in order to reduce potential variation in stomatal activity due to diurnal cycles of the stomata. This time constraint, and the prolonged time required to collect stomatal resistance data, allowed only two grasses to be assessed by this method. *Bouteloua curtipendula* (side-oats grama) and *Schizachyrium scoparium* (little bluestem) were chosen for stomatal resistance measurements, and data from these two grasses was used to compare the two types of substrates for their ability to support or constrain the physiological performance of these two grasses.

Another limitation of this method was the size of the aperture in the clamp of the leaf porometer, which needed to be covered entirely by the leaf/blade surface. This is the reason why grasses with larger leaf blades were chosen for the study. Although there may be a possibility of grouping multiple blades together for measurement, this can be tricky considering the need to orient all abaxial or adaxial leaf surfaces in the same direction of the clamp for consistency in results. The leaf porometer can be used in future studies as well, with the possibility to make an addition of broad-leaved forbs, which in the case of the APD-EGR is the *Packera Obovata*, so that comparisons can be made between the drought stress responses and water-use strategies of graminoids and forbs.

The method to collect and analyze aboveground biomass of the individual species utilized the standard procedure of collection, drying, and weighing. With the large number of plants that were analyzed, storing and transferring the plants proved difficult. Another limitation to this method was our inability to collect the biomass of the *Bouteloua dactyloides*, which grew horizontally instead of vertically and was unfeasible to clip at a constant height. Despite the limitations, biomass measurements were considered very meaningful since they integrated both vertical and horizontal growth to convey development of vegetation through the growing season.

Overall, this study establishes a baseline for the assessment of substrates and plants on the green roof in regards to soil-water-plant relations. This study introduced both common and uncommon methods in green roof research to collect, analyze, and infer results. The APD-EGR research is a longitudinal study, thus the methods chosen for this first growing season assessment were non-destructive. The methods were also selected because they were thought to be doable in the limited time available for conducting the research, and could be replicable in future research endeavors. Such a method is shown in Figure 5-1 where porometer readings are being collected. The longitudinal multi-year study envisioned is expected to help researchers determine appropriate native plant, mix and substrate selections for similar types of green roofs in the Flint Hills Ecoregion.

Figure 5-1: Stomatal resistance measurements on the APD-EGR

(Photograph by Lee Skabelund, taken in 2018, at the APDesign Experimental Green Roof, Kansas State University)

Discussion of Results

Substrate characteristics

Good drainage and substrate composition are essential for green roofs to prevent water logging, and to reduce excessive structural loads due to ponding water or heavy saturated soils (Best, Swadek, and Burgess 2015). Nevertheless, green roof substrates need to be able to retain water for plant use as well. Thus, green roof substrates need to strike a balance in regards to permeability and water holding capacity. The results of the substrate tests carried out by Turf and Soil Diagnostics Inc. showed that the rooflite® extensive mc substrate had greater maximum media water retention capacity (35%) than the Kansas BuildEx® substrate (29%), despite the rooflite® extensive mc substrate having greater water permeability (30.9 mm/min) than Kansas BuildEx® substrate (0.2 mm/min). Although it is unknown as to whether these values were significantly different for the two substrates from a statistical vantage point (refer to Table 3-4) the differences in permeability seem to point to a very important difference in how the two substrates may function and support plant growth.

Rooflite® extensive mc complies with the FLL guidelines for the planning, execution and upkeep of green roof sites, which specify a permeability value of more than 0.001 cm/s (0.6 mm/min), and a maximum water retention range of 35%-65% for multi-course extensive green roofs (FLL 2002). According to Dr. Mary Beth Kirkham, a soil is considered drainable if the saturated hydraulic conductivity is approximately 1m/day (0.69 mm/min) (personal communication by Jialin Liu with Dr. Mary Beth Kirkham, March 2019; Table 3-4). The permeability and maximum media water retention reported for Kansas BuildEx® is also less than the FLL specifications (FLL 2002; Table 3-4). In future substrate analyses of the APD-EGR it is recommended to statistically assess the differences between the permeability and moisture retention characteristics of the two substrates, for which at least three replicates are suggested. This should allow researchers to make more meaningful inferences as to which of the two substrates is more suitable for a green roof designed for stormwater management and potable water savings.

Kansas BuildEx® was observed to be a sand-based substrate, with approximately 67-68% sand as compared to the 52-53% sand in the rooflite® extensive mc, whereas the rooflite® extensive mc substrate had a greater proportion of aggregate, with approximately 40-41% as compared to the 25% in the Kansas BuildEx® substrate (refer to Table 3-6; Table 3-7). The rooflite® extensive mc substrate had total pore space of 58% and Kansas BuildEx® had total pore space of 42% (Table 3-5). Pore space should constitute about 50% of the growing media, the remaining half should be comprised of organic content and minerals (Latshaw, Fitzgerald, and Sutton 2009). This fact is corroborated by Kirkham (2014), who asserts that half of the total pore volume should be air-filled and the remaining half should be filled with water (Kirkham 2014d). The air-filled porosity for Kansas BuildEx® was 13%, which is slightly above the 10% threshold, below which plant roots cannot survive (Kirkham 2014d). The 23% air filled porosity of the rooflite® extensive mc substrate was closer to the 25% standard (refer to Table 3-5).

Figure 3-7 shows the soil water release curves plotted between volumetric water content (%) and tension (-bars) for the two substrates. The soil water release curve for the rooflite® extensive mc substrate was higher than the Kansas BuildEx® substrate, which implies that rooflite® extensive mc potentially holds more water than Kansas BuildEx®. However, to determine water available for plant use, the soil moisture characterization study would need to be supplemented with measures of water contents at field/roof capacity and wilting point capacity of the two substrates; the range between the two would give the plant-available water content for the two substrates (Best, Swadek and Burgess 2015).

The macronutrients (N, P, K) in the Kansas BuildEx® substrate in the 4-inch deep bed were slightly higher than the rooflite® extensive mc substrate, based on the substrate nutrient tests conducted by the KSU Soil Test Lab in April 2018. However, we could not ascertain whether they were in enough concentrations in the two media to substantially influence any differences in the growth of the species in those media. Moreover, due to the lack of replicates, we could not statistically analyze the observed differences between the nutrient concentrations of the two substrates (Table 3-3). Substrate nutrient contents change over time, especially when they interact with plants (Mitchell 2017), and thus it is recommended that nutrient levels be tracked in the future at the beginning and end of every growing season so that changes through the growing season may be assessed in addition to year-to-year (annual) changes to APD-EGR nutrients.

The saturated and dry media densities of the rooflite® extensive mc and Kansas BuildEx® substrate, as reported in Table 3-4, show that the Kansas BuildEx® substrate had greater weight for a given volume than the rooflite® extensive mc substrate. If substrates have lower bulk density, adequately drain, and still retain enough plant available water and nutrients to support optimum survival and growth of plants, then that substrate would typically be more suited for a green roof setting (Best, Swadek and Burgess 2015). The substrate tests conducted to date would seem to favor the rooflite® extensive mc substrate. Nevertheless, visual observations do not seem to indicate better growth in the rooflite® plots. Since plant-soil-water-nutrient relationships are complex, our research team is not able to tease out precise cause-and-effect functions and results at this time, and such functions and results are very difficult to determine without more testing and analysis (beyond the scope of this thesis).

In future studies, it is recommended to evaluate the ability of the two substrates to supply water to the plants for uptake and use. Various studies have suggested that water supplied by substrates is an important factor affecting plant survival and growth on green roofs (Farrell et al. 2013; Thuring, Berghage and Beattie 2010; VanWoert et al. 2005). Although an attempt was made in this study to evaluate the capacity of the substrates to provide water to the plants by means of a soil water characterization curve, meaningful inferences could not be made due to uncertainty regarding how water actually moves in the two substrates and how easy it would be for the plants to uptake the water. Thus, future evaluations of water potential, with respect to the field and wilting point capacities of the individual substrates, are recommended. Water potential is “the work required, per unit quantity of water, to remove an infinitesimal quantity of water from the soil to a pool of pure, free water” (Campbell and Campbell 2005, pp 1).

Plant survival, growth, and stomatal resistance

The graminoids demonstrated a 100 percent survival rate through the study period of late June to mid-November. It must be noted, however, that the plants which were studied were a mix of the ones planted in October 2017 and the ones replaced in June 2018, and this difference in establishment was accounted for as experimental error in statistical analyses. The perfect survival results observed for the graminoids were attributed to the fact that the roof was being irrigated regularly, with approximately one inch (2.54cm) of water being supplied to the plants and substrates per week via supplemental irrigation water, when rain did not supply sufficient water to the plants. The aim of the research team is to provide a consistent amount of water to each plot during the first two years of establishment. Supplemental irrigation was added based on the amount and frequency of precipitation as recorded via the APD-EGR weather station. As a result of receiving ample water throughout the first year (2018) growing season, all of the graminoids in the 4-inch deep bed survived. This is similar to the findings of Skabelund et al. (2014), who reported that native plants can survive and provide full vegetative cover on a green roof in Manhattan, Kansas with limited amounts of supplemental irrigation.

On the APD-EGR, survival needs to be assessed further in the coming growing seasons to evaluate whether the plants will survive after winter dormancy (including the rigors of winter cold and cold-season dry spells), intense summer heat, and dry periods during the summer that are more prolonged than were allowed for during 2018—with less supplemental irrigation and more stress induced or permitted. This will likely happen during the third-year growing season since the APD-EGR research team is planning for a two-year establishment period.

The statistical analysis of above-ground biomass of the graminoids did not demonstrate any strong evidence for an overall effect of substrate type on biomass (refer to Table 4-13), nor any species-specific effects of substrate on the above-ground biomass of any of the six graminoids other than *Schizachyrium scoparium* in Plant Mix B (refer to Table 4-15). A limitation to this assessment is that *Bouteloua dactyloides* was left out of the biomass study because it was not deemed meaningful to clip this low-growing stoloniferous grass at a height of 2.5 inches (6.35cm), which was the protocol set for clipping the other graminoids.

The analysis of height of the graminoids in the Kansas BuidEx® and rooflite® extensive mc substrates, however, demonstrated an overall main effect of substrate type on height of the graminoids (refer to Figure 4-1 and Table 4-1), with trends of greater plant height AUGPC observed in the Kansas BuildEx® substrate than in the rooflite® extensive mc substrate (an exception was *Koeleria pyramidata;* see Figure 4-2). The most consistent effect of substrate was observed for *Schizachyrium scoparium* in Plant Mix B, with greater height and biomass observed in Kansas BuildEx® than in rooflite® extensive mc (refer to Table 4-3 and Figure 4-2).

It is likely that the dissimilarity in the results obtained from the analyses of biomass and height occurred because the reproductive culms of the graminoids grew taller in the Kansas BuildEx® substrate than the rooflite® extensive mc substrate, but did not contribute enough to demonstrate a difference in biomass, which naturally integrated horizontal growth in addition to vertical growth (documented by measuring the tallest part of each plant measured). However, this study only accounted for the total height of the graminoids, which included the culms that bore seed-heads. Our research team attempted to track measurements of both the height of the seed-heads and the height of the tallest leaf blade during the 2018 growing season. However, it became increasingly difficult to identify the tallest leaf blade, especially once the grasses started to grow seed-heads. Thus, total height measurements were used to maintain consistency for statistical analyses. In future studies, it could be of interest to measure reproductive growth, along with vegetative growth, once the graminoids start to propagate by vegetative means, and form seed-producing physiological structures.

Because six of the seven graminoids were bunch grasses, we could not observe a strong evidence of substrate effect on the total cover of these graminoid species (refer to Figure 4-6; Table 4-8). *Bouteloua dactyloides*, however, grows horizontally via stolons, and thus demonstrated greater foliar cover in the Kansas BuildEx® substrate than in the rooflite® extensive mc substrate (as reported in Table 4-10 and as shown in Figure 4-7). Since *Bouteloua dactyloides* is known to have an affinity for both heavy clay and sandy soils, this difference in horizontal growth could be attributed to the difference in amounts of clay and sand in the Kansas BuildEx® versus the rooflite® extensive mc substrates.

Like many variables, it is too difficult to determine precise cause-and-effect using non-destructive assessment techniques. It was not possible, from this study, to make concrete assumptions as to why the graminoids showed no pronounced difference in biomass between substrate types, but exhibited overall greater height for the graminoids and greater coverage by buffalograss in the Kansas BuildEx® substrate. If precise substrate depths throughout each plot were examined, and related to the quantity of root growth for each plant species and mix, while also systematically examining organic matter and clay content, particle sizes, nutrient levels, and other important variables related to each plot, more might be said about specific plant-soil-water-nutrient interrelationships. However, this approach is generally far too time consuming and costly, and is generally not feasible for non-destructive multi-year research.

The rooflite® extensive mc substrate presumably retains more water by supplying a greater percentage of organic matter. However, the water in the substrates was likely not a limiting factor for plant growth given the well-watered APD-EGR conditions. Other potential causes for the difference in shoot height could be differences in plant-available nutrients (for example, nitrogen, phosphorus, and potassium), which have been proven to affect plant growth (Baldi et al. 2013). The nitrogen, phosphorus and potassium values were greater in the Kansas BuildEx® substrate than the rooflite® extensive mc substrate, although it is unknown whether the values are different enough to cause the observed differences in growth for the graminoids in the two substrates (refer to Table 3-3). The nutrient concentrations in substrates can vary with time depending on changes in plant community, microbial activity, substrate composition and characteristics, and the age of the roof (Buffam and Mitchell 2015). Further investigations on temporal changes in concentration levels of macro and micronutrients and their effect on plant growth and vigor could be part of future research on the APD-EGR.

Another factor that could have affected growth of the graminoids is the sub-surface temperature of the substrates, which should be of interest in future analyses. Such an investigation can make use of the METER 5TM soil moisture and temperature sensors (METER Group, Inc. USA), which have been placed approximately halfway beneath each substrate surface and the filter fabric at the base of the substrate profile within the Plant Mix C plots, in the center of each of these beds. Other abiotic factors should also be considered in relation to substrate-water-plant interrelationships, building off of ideas proposed by Skabelund, et al. (2015) and other researchers.

Height-based growth curves for the graminoid species illustrated their individual growth patterns throughout the study season. As shown in Figure 4-4, the cool season graminoids, *Carex brevior* and *Koeleria pyramidata* were already nearing their full height in late June, when data collection commenced. The warm season grasses, on the other hand, peaked in August-September, and started to decrease in height with the onset of dormancy. Thus, a mix of cool season and warm season grasses would be appropriate for providing shade on the green roof substrates, and also for maintaining visual interest throughout the growing season. Growth curves based on percent cover of the graminoids, as illustrated in Figure 4-9, showed slight increases in cover for the warm-season grasses, and reasonably comparative increases in cover for the cool-season graminoids throughout the study season. *Bouteloua dactyloides*, however, showed a relatively greater increase in cover, as induced by its stoloniferous nature (Figure 4-9).

The average heights of the grasses in Plant Mix B were smaller at the start of the study season, but outgrew the graminoids in Plant Mix C by the end of the season (see Figure 4-3).

Similar trends were observed from the horizontal growth curves of the graminoids in the two mixes (Figure 4-8). Based on height and cover analyses, strong evidence for differences in mixes was not found (refer to Table 4-1; Table 4-10).

Schizachyrium scoparium was the only species that was common in both plant mixes B and C. A difference was observed between the mean heights of *Schizachyrium scoparium* in plant mixes B and C (Figure 4-5), although the difference was not statistically significant at the 95 percent confidence level (Table 4-7). It is possible that this difference occurred due to the reduced competition that *Schizachyrium scoparium* (little bluestem) in Plant Mix C had to overcome because *Carex brevior* and *Koeleria pyramidata*, both cool season graminoids, had already grown to their full size and were not competing with little bluestem for water and nutrients as much as the other grasses in Plant Mix B. C4 plants are also said to outperform C3 plants in times of high temperature, high light levels, and limited water because C4 plants do not undergo photorespiration, and exhibit better water use (Vandegrift 2018). This interspecific mutualism within the mixes would be interesting to investigate in future growing seasons, also taking into account known or likely influences of the two forbs in Plant Mix C, and the two *Sedum* species in Plant Mix B.

An overall main effect of substrate type was not observed in regards to the mean stomatal resistance levels of *Bouteloua curtipendula* and *Schizachyrium scoparium* in the 4-inch deep green roof bed. It is important to note that the green roof was being irrigated throughout the growing season on a regular basis during the 2018 growing season. Although irrigation was ceased for 24 hours before taking stomatal resistance measurements, it is likely that the plots still retained enough water to maintain reduced stomatal resistance values for the two grasses well below 5000 s/m (Table 4-16). Stomatal resistance values over 5000 s/m are deemed high

(Kirkham 2008). However, a pronounced difference was observed between the two species, with *Bouteloua curtipendula* demonstrating greater stomatal resistance in the study period than *Schizachyrium scoparium* (refer to Table 4-17; Figure 4-11). This finding suggests that side-oats grama (*Bouteloua curtipendula*) can respond better to drought stress than little bluestem (*Schizachyrium scoparium*), although further investigations seem warranted under drought-induced conditions. This idea corresponds to observations on the KSU Seaton Hall Upper Green Roof, where side-oats grama has performed very well without irrigation since mid-August 2012 (personal communication with Lee R. Skabelund 2019).

The study of stomatal resistance can be extended into future studies, where it is recommended that sufficient drought stress be applied to the APD-EGR plants to achieve more pronounced results related to stomatal resistance or conductance, and measure the rate of passage of carbon dioxide entering, or water vapor exiting leaf stomata using a porometer (Decagon Devices 2016), as necessitated by the specific research questions being asked by future researchers.

Clearly, more in-depth investigation of substrate characteristics and plant responses beyond the first growing season are needed to more fully comprehend cause-and-effect. In combination with better understanding of substrate physical properties, substrate chemistry and nutrient levels, the study of soil biota in the different substrates and plots may be a very useful way to learn what is actually driving or limiting plant growth in each plot.

Benefits of green roof vegetation

Differences between species in terms of height, cover, and biomass are functions of the individual species-specific traits of the species. Plant species-specific traits such as height, cover, and biomass have been linked to various green roof ecosystem benefits such as stormwater retention and thermal performance (Eksi et al. 2017; Nagase and Dunnett 2012; Vandegrift 2018). Nagase and Dunnett (2012) studied different plant life forms on a green roof in England, and found that grasses performed the best from a stormwater retention standpoint, followed by forbs and *Sedum*. These researchers observed that plants with taller height, larger diameter, and larger above and below-ground biomass more effectively reduced stormwater runoff than plant species with shorter height, smaller diameter, and lesser above-ground and below-ground biomass (Nagase and Dunnett 2012). Nagase and Dunnett (2012) based the performance of vegetation in stormwater management on the ability of plants to intercept, retain, and transpire water, in addition to retention capacity of the substrate. Thus, if stormwater runoff reduction is the goal for the green roof, then the taller and more robust grasses such as *Schizachyrium scoparium,* and *Bouteloua curtipendula* would probably perform well because they can intercept water though greater exposed leaf area and high transpiration rates (Nagase and Dunnett 2012).

Another benefit of green roofs is their ability to provide thermal insulation and reduce urban heat island effects. Plant canopies can shade the roof, and can help cool the roof surface through evapotranspiration (Vandegrift 2018). According to Eksi et al. (2017), shading provided by plants decreases surface temperatures of substrates by reducing the solar radiation that reaches the substrate surface. The researchers assert that coverage also influences leaf area index and albedo, which is a coefficient that depicts the amount of solar radiation reflected by the surface (Eksi et al. 2017). Thus, plants with greater height, cover, and biomass, as well as the capacity for higher rates of evapotranspiration, are generally deemed suitable for providing thermal insulation (Vandegrift 2018; Eksi et al. 2017). Grasses such as little bluestem (*Schizachyrium scoparium*) and buffalograss (*Bouteloua dactyloides*) may be good performers for thermal insulation benefits due to their respective capacity of producing more biomass and cover. Plants that establish fast and provide greater coverage are also desired for cost and green roof management purposes.

Another major benefit of green roofs is their ability to provide habitats to insects, birds, and other wildlife. Cook-Patton establishes the importance of biodiversity on green roofs to support more diverse animal communities by maintaining more biomass (Cook-Patton 2015). This assessment implies the importance of mixes instead of monocultures, which has been corroborated by Lundholm et al. (2010), who found that combining plants of certain life-form groups, such as tall forbs, grasses and *Sedum* enhanced the ecosystem benefits obtained from the green roof (Lundholm et al. 2010). An understanding of substrate and mix interactions is important to evaluate the success of the APD-EGR and other green roofs in providing these green roof benefits. Figure 5-2 is an image of the Seaton Hall Upper Green Roof at KSU, acting as faunal habitat for butterflies.

Figure 5-2: Butterfly spotted on the Seaton Hall Upper Green Roof

(Photograph by Lee Skabelund, taken in 2010, at the Seaton Hall Upper Green Roof, Kansas State University)

Conclusion and Practical Applications

This first-growing season study was a preliminary study to assess plant survival, growth, and physiological performance of selected green roof graminoids and the ability of two commercial substrate types in supporting these and other native plants. The aims of this study evolved into exploring ways of better understanding baseline green roof conditions on the APDesign Experimental Green Roof, and providing insights and guidance for longer term monitoring of plant growth and health. Since a long-term study of green roof plants and substrates is needed to determine wise selection of green roof plant species and substrates for Manhattan, Kansas this study is seen as a small but important part of larger, ongoing research effort.

During and at the end of the first growing season, a pronounced difference between the two substrate types in terms of biomass, cover, and stomatal resistance was not observed. Even though there was an overall effect of substrate type on the height of the graminoids, as well as the cover of buffalograss (*Bouteloua dactyloides*) in the two substrates, these results alone may not be sufficient to recommend one substrate over the other. From a green roof substrate standpoint alone, the rooflite® extensive mc substrate seems to be more well-suited to a green roof setting because of its higher water retention capacity, higher permeability, lower bulk density and optimum porosity, which align better with characteristics favorable for green roofs requiring a relatively lightweight growing media to decrease structural loading.

The methods used in this study are applicable in further investigations to better understand the interactions between the plants, mixes, and substrates that support them. Widely established methods of assessing plant success on green roofs, such as survival, height and biomass measurements, as well as less commonly used methods such as the use of Image J for calculating cover, and the use of a leaf porometer for stomatal resistance (refer to Chapter 3 of this thesis), have been assessed for future use, based on their ease of implementation, applicability and the attainment of results (as discussed in the beginning of this chapter).

For instance, the measurement of stomatal resistance can be a meaningful non-destructive method to assess the drought stress that selected plants are subjected to in each of the substrates. The opportunity to relate thermal, infrared, and true-color imagery with targeted porometer readings, should be explored as this may provide a much better way to assess stress and drought tolerance of little bluestem, side-oats grama, and other species. The opportunity to create normalized difference vegetation index (NDVI) from overhead imagery could also be explored in relation to assessing plant responses to substrate types and depths, learning from the study by Ntoulas et. al. (2017). UAS imagery was taken by the research team from above the APD-EGR using a UAS in mid-July 2018 and mid-October 2018 and may be a great help in efficiently assessing plant stress and resilience.

Although pronounced discoveries have not been made on which substrate type and graminoid species are better suited for a green roof in the Flint Hills Ecoregion, this study has given direction for future research and identified limitations as well as potential areas of possible interest such as the exploration of reproductive growth and substrate temperature.

Important questions that still to be answered in relation to designing and maintaining non-sloped 4-inch green roofs include: 1) What mix of species is likely be best for a full-sun green roof in Manahattan, Kansas and the Flint Hills Ecoregion? 2) What substrates work best for this region’s climate and will support a very drought-tolerant mix of plants? 3) How much irrigation is essential to provide relatively full coverage by selected drought-tolerant vegetation?

Questions specific to longer-term management of the APD-EGR beds include: 1) What will these green roof plots look like and how will they function if they are not irrigated or weeded after the second year of establishment? 2) What should irrigation and weeding protocols be beginning in the third growing season and beyond? 3) What are the best ways to assess the pros and cons of the species and substrates selected for the three APD-EGR beds?

Being able to answer these questions would aid in the appropriate selection of specific green roof components such as vegetation and substrates for regions similar to Manhattan, Kansas, as well as improving implementation, monitoring, and management protocols for green roofs. Green roof vegetation and species mix selection depends largely on the intent of the green roof. Design intent may be aimed at maintaining aesthetics, managing stormwater, providing thermal insulation and/or introducing habitat for pollinators and birds—and plant assessment criteria will vary for each of these benefits sought. In nearly all cases, plant species need to be able to survive harsh rooftop meteorological conditions and potential limitations to water availability while exhibiting optimum growth and function to be well-suited for green roofs. The plant assessment methods introduced in this study were aimed at finding plant species that can persist and grow on a green roof in Manhattan, Kansas. They provide a baseline for long-term plant studies on the APD-EGR and could be used again in company with imagery collected using a UAS.

Assessments of vegetation performance on green roofs must be in relation to substrate characteristics, and require the understanding of plant-soil-water-nutrient interrelationships. Green roof substrate characteristics such as their physical and chemical compositions have the capacity to bolster or restrain plant performance. This study suggests that different plants exhibit varied responses to the physical and chemical properties of substrates. Thus, better understanding functions related to the bulk density of a substrate, its water-holding capacity and permeability, and the coloration of a substrate and how each of these factors relate to vital soil-water-vegetation interrelationships (for example, how color and hue impact soil moisture, temperature, evapotranspiration, and plant growth) are very important as designers seek to create appropriate plant mixes matched with selected substrate types and depths, and with the context and microclimate of each particular green roof setting.

The inability of the researchers of the APD-EGR study to fully answer the research questions suggests the importance of long-term green roof studies. Rowe (2015) corroborates this idea by stating that long-term green roof research is important because survival may change through time, eventually demonstrating reduced coverage or complete disappearance of species on a green roof due to competition, variability in climate (particularly prolonged dry periods), and other factors.

Understanding green roof changes over the a long term would not only reduce the repetition of mistakes, but also provide opportunities to track temporal changes in green roof habitats, and assess impacts of dynamic factors such as substrate moisture, composition, depth, microclimates, plant functional diversity and complexity, and maintenance practices on plant communities. In the APD-EGR research, tracking changes in species survival, plant mix coverage, and vegetation development over multiple years in relation to substrate type and depth would be a meaningful approach to further the assessment of plant mix and substrate type on the green roof.

In addition to setting baseline measures for plant and substrate evaluations for the APD-EGR, this study also started to shed light on practical applications and limitations of green roof research. The premature dieback incurred by over 37 percent of the plants after the winter of

2017-2018 highlights the importance of planting at the right time of the year (ideally spring or in late summer), installing the plants properly, and providing plants with sufficient time for root establishment. The survival of the graminoids during the first growing season validates the importance of sufficient irrigation for plant success on green roofs. On a longer term, however, providing regular and ample irrigation may not be feasible, which is why researchers need to make decisions on irrigation protocols for establishment and beyond establishment periods. Drought tolerant species should preferably be used on green roofs, and to test the hardiness of plants against drought, and to determine an optimum irrigation amount, a certain level of drought needs to be applied to green roof plants. Thus, methods such as the use of a leaf porometer to non-destructively test species for drought tolerance are important, although this method has its limitations given the amount of time required to take measurements on the ground. Ideally, green roofs should sustain healthy plant communities with little to no irrigation, and plant species and mix selection should be aimed towards this goal.

Another protocol that needs to be decided upon is weeding. Any plant species that are not a part of the initial green roof design are considered weeds (Rowe 2015). Weeds can appear on the green roof from nearby seed sources, or depending on where the substrate has been sourced from, may be existing in the substrates, although green roof substrates are frequently sterilized to avoid colonization by weeds (Rowe 2015). On the APD-EGR, weeding was carried out on a regular basis (once a week) and needs to be continued regularly as a maintenance protocol during the experimental phase of the green roof research. Weeding on a long term, however, may not be feasible, thus any long-term continuation of weeding would depend on the broad goals for the green roof design. Although some weeds may disappear with limited use of irrigation (Rowe 2015), weeds can enter a green roof where there is bare substrate.

Limitations and Future Considerations

Planting was delayed by approximately three months on the APD-EGR, which decreased the establishment period before winter, and lessened the chances of plants surviving the winter of 2017-2018 (December 2017 to March 2018). In addition, planting methods employed for some live plants were not appropriate (root bound and/or planted too high), likely making them less able to quickly establish roots in late October and November. As a result, 116 plants (including 72 graminoids) in the 4-inch bed fared very poorly and/or did not survive through the winter and were replaced in May and June of 2018. These facts are accounted for in statistical analyses as experimental error, but proper plant installation at the outset would be very helpful in limiting additional, potentially confounding factors and variables such as establishment period. It is recommended that future plantings be of the same origin, size, and quality. Planting techniques should also be consistently high quality. For example, the roots of potted root-bound plants must be cut and spread apart.

The selected species were planted as plugs, so there may have been potential influences of original potting/growing media on the initial growth of the plants. These influences may have varied among originally planted and replaced plugs. While planting plugs, it is recommended to maintain consistency in where plants are purchased, how they are grown, as well as when and how well they are installed on the green roof. An alternative to planting plugs is seeding, which has been explored by Sutton (2013) on four green roofs in Lincoln, Nebraska. Although seeding green roofs can reduce costs and potential variability caused by original growing media, use of seeding has been found to lag behind plugging by approximately two months in terms of reaching the FLL-specified 80 percent coverage (Sutton 2013).

The roof was irrigated regularly to avoid premature dieback of the plants during the first-year establishment period, due to which pronounced results for survivability and stomatal resistance could not be observed during this first growing season study. Irrigation is necessary for plants such as grasses and forbs to survive on green roofs. In his article, "Seeding green roofs with native grasses," Sutton recommended applying at least 0.50 inches to 0.75 inches water every 10 days, either through precipitation or irrigation in order for native grasses to thrive on extensive green roof systems (Sutton 2013). However, to assess survival of plants on green roof settings, a certain periods of drought stress seems to be necessary. In future APD-EGR studies researchers should limit irrigation as a means of conserving water and to more meaningfully study survival and drought tolerance of plants, and thus make much stronger inferences about plant species success on the experimental green roof. Determining appropriate water deficit treatments for the APD-EGR will be important in future years if the resilience and suitability of selected species is to be tested and documented.

Not all plot (individual planting mix cell) sizes on the APD-EGR were exactly the same size as was intended by the research design. Not all substrate depths with each bed are the same, and so within the 4-inch bed depths range from an average of approximately 2.8 inches to 4.6 inches (7.2 cm to 11.7 cm) per plot (Appendix-D). Thus, some plots have more substrate than others, and depths are not consistently even throughout the 4-inch bed, and the plant roots can probably move beneath aluminum dividers to seek after moisture. Although this is unknown, plant roots may also be able to move through and beneath the filter fabric separating each plot or cell to seek after moisture in the leveling expanded shale (gravel drainage layer) material. The manufactured rooflite® drainage layer is assumed to be consistently and correctly installed, but if there is variability in drainage layer depths and where moisture pools beneath the green roof beds, this factor could have some influence on short- and long-term plant survival and growth.

Sedum were initially planted incorrectly and had to be replanted in the correct locations and order. *Sedum* pieces were thus scattered across many plots or cells. Many agricultural weed seeds were likely brought in with the Kansas BuildEx® substrate (after being stored near a west Manhattan, Kansas farm field). Thus, active weeding throughout the first-year growing season was deemed to be essential by the research team for all of the APD-EGR beds, including the 4-inch bed. Nearby green roofs also had abundant weeds (sometimes removed prior to setting seed) which also likely added to weeds growing on the APD-EGR.

Destructive studies to examine root biomass could not be conducted due to the long-term nature of the study and the disruption that this type of approach would create for other multi-year studies being conducted on the roof. It would be possible to set up nearby green roof modules or trays using the same two substrates and plant mixes in order to investigate root growth using a destructive monitoring approach.

Some of the methods and techniques adopted in the study were subjective or inconsistent, and could have affected the results obtained, even though consistency was the intention and efforts were made to be as consistent as possible. For example, the threshold values set in ImageJ while calculating percent cover were based on the assessor's perception of whether the resultant HSB image was an accurate interpretation of the actual plant foliar cover. Moreover, when the study reached the stages nearing dormancy, the color of the plant foliage and the color of the substrate became indistinguishable by ImageJ, and manual tracing of the plants needed to be done in Photoshop, which, once again, could influence results based on accuracy (or inaccuracy) of the tracing job in Photoshop.

The use of a leaf porometer to evaluate stomatal resistance was only possible for plants with blade widths wide enough to completely cover the aperture in the device. Thus, for grasses such as *Bouteloua dactyloides*, *Bouteloua gracilis*, and *Sporobolus heterolepis*, this method may not be feasible (to ensure consistency and accuracy of measurements).

A number of other variables related to plant growth, substrate characteristics, and microclimatic dynamics would ideally be examined, but were not able to be completed or considered given the need to complete this research within a one-year timeframe. For example, it is possible to utilize of the 5TM substrate temperature and moisture sensors (METER Group Inc., USA) embedded mid-depth in the C-plots of each bed would be beneficial in tracking both sub-surface soil moisture and temperature in relation to plant performance, as well as guide irrigation decisions as had been done in the 2017-2018 growing season. Another potential area of interest could be the use of UAS (Unmanned Aerial System) imagery, such as the one shown in Figure 5-3 to assess growth and vigor on the APD-EGR, with potential to evaluate relationships of surface temperatures to plant cover of the roof (Van der Merwe et al. 2017), and stomatal resistance exhibited by plants.

Figure 5-3: UAS image taken for the APD-EGR

(Photograph by Harman Singh Sangha, taken in 2018, at the APDesign Experimental Green Roof, Kansas State University)

Although this research establishes a baseline for future APD-EGR green roof studies by assessing first-year survival, growth, and physiological performance of graminoids on the APD-EGR, additional funding, time, and expertise will be needed (including additional research personnel, equipment, and laboratory tests) to deepen our collective understanding of plant-soil-water-nutrient interrelationships over the long-term.

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Figures

Figure 2-1: Green Roof Components

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Figure 2-2: The Flint Hills Ecoregion in Kansas

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Figure 2-5: *Bouteloua gracilis* (Blue grama) plug and plant

Haddock, Mike. 1997-2019. "Blue grama." Kansas Wildflowers and Grasses. Accessed April 19, 2019. http://www.kswildflower.org/largePhotos.php?imageID=5630&aCategory=g&lastModified=2007-06-28

Figure 2-7: *Carex brevior* (Fescue sedge)

Haddock, Mike. n.d. "Fescue sedge." Kansas Wildflowers and Grasses. Accessed April 19, 2019. http://www.kswildflower.org/largePhotos.php?imageID=144&aCategory=s&lastModified=2018-02-23

Figure 2-8: *Koeleria pyramidata* (syn. Koeleria macrantha)

Haddock, Mike. "Mature junegrass." Kansas Wildflowers and Grasses. Accessed April 19, 2019. http://www.kswildflower.org/largePhotos.php?imageID=612&aCategory=g&lastModified=2007-09-09

Figure 2-9: *Sporobolus heterolepis* (Prairie dropseed)

Light, Chris. 2007. "Prairie Dropseed." as licensed under Creative Commons Attribution-Share Alike 4.0 International. Accessed April 18, 2019. https://commons.wikimedia.org/wiki/File:Prairie_Dropseed_P7280447.JPG

Figure 2-10: Five phases of seasonal plant growth

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Figure 2-11: Soil water characteristic curve

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Tables

Table 2-1: Sizes of the grass and sedge species

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Table 2-2: USDA classification of soil particle sizes

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Appendix A-Climate of Manhattan, Kansas

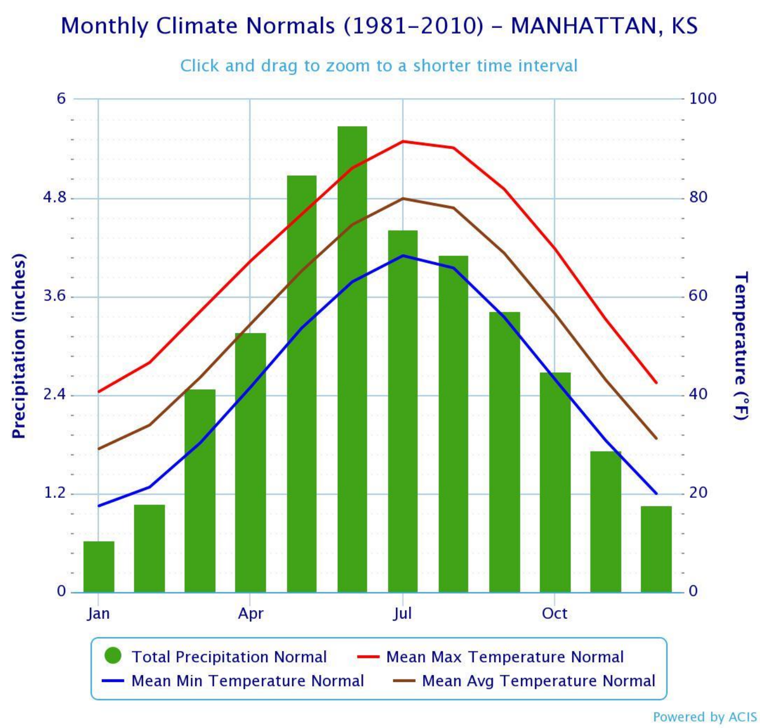

Figure A-1: 1981-2010 monthly climate normal temperatures and precipitation in Manhattan, Kansas

(Source: NOAA, https://w2.weather.gov/climate/xmacis.php?wfo=top)

Table A-1: Monthly mean maximum temperatures (°F) for Manhattan, Kansas during 2008-2018 (Source: NOAA, https://w2.weather.gov/climate/xmacis.php?wfo=top)

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
2008	42.3	41.0	53.6	62.7	76.2	87.3	89.9	86.7	78.9	68.2	55.4	40.1	65.2
2009	40.8	53.1	57.3	64.3	76.5	87.5	85.9	86.4	77.9	60.5	60.1	34.6	65.4
2010	32.3	37.5	54.8	73.4	73.7	89.1	91.2	94.2	84.0	75.1	57.5	42.0	67.1
2011	34.5	40.9	54.2	67.4	74.6	88.1	98.0	92.5	80.2	72.4	55.4	44.5	66.9
2012	48.0	48.2	70.1	72.4	82.9	89.1	99.0	89.5	80.7	67.6	60.5	45.6	71.1
2013	44.0	43.3	48.6	60.2	74.6	85.7	88.6	88.3	85.6	68.0	52.4	38.5	64.8
2014	38.8	34.6	53.5	67.0	77.4	83.8	87.9	90.5	79.7	71.5	49.3	41.4	64.6
2015	42.5	38.4	59.9	68.7	73.2	86.2	89.9	87.1	86.3	72.1	60.4	48.0	67.7
2016	38.5	51.6	64.3	70.1	73.9	90.8	90.0	86.8	83.2	74.4	63.1	40.2	68.9
2017	41.3	54.7	59.0	66.2	75.3	87.1	92.0	83.8	84.2	71.7	55.9	44.3	68.0
2018	37.9	40.5	56.5	58.9	85.8	90.9	91.7	88.3	81.0	64.5	47.0	43.9	65.6
Mean	40.1	44.0	57.4	66.5	76.7	87.8	91.3	88.6	82.0	69.6	56.1	42.1	66.8
Max	48.0 2012	54.7 2017	70.1 2012	73.4 2010	85.8 2018	90.9 2018	99.0 2012	94.2 2010	86.3 2015	75.1 2010	63.1 2016	48.0 2015	71.1
Min	32.3 2010	34.6 2014	48.6 2013	58.9 2018	73.2 2015	83.8 2014	85.9 2009	83.8 2017	77.9 2009	60.5 2009	47.0 2018	34.6 2009	64.6

Table A-2: Monthly mean minimum temperatures (°F) for Manhattan, Kansas during 2008-2018 (Source: NOAA, https://w2.weather.gov/climate/xmacis.php?wfo=top)

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
2008	-8	2	8	27	32	52	53	55	39	21	13	-6	-8
2009	0	8	9	22	40	52	50	45	35	27	22	-8	-8
2010	-10	5	15	30	37	54	57	48	42	31	17	7	-10
2011	-2	-6	17	32	36	52	69	62	43	27	19	13	-6
2012	8	7	23	35	44	45	64	52	40	26	14	6	6
2013	8	2	16	26	34	51	55	55	45	28	12	0	0
2014	-10	-5	-4	26	34	53	51	56	37	33	9	3	-10
2015	1	2	9	33	40	53	60	52	51	30	25	16	1
2016	0	17	24	30	42	56	63	55	44	33	25	-10	-10
2017	-1	14	22	32	37	55	59	54	46	24	18	-3	-3
2018	-8	1	16	16	52	56	59	58	44	29	11	12	-8
Mean	-2	4	14	28	39	53	58	54	42	28	17	3	-5
Max	8 2013	17 2016	24 2016	35 2012	52 2018	56 2018	69 2011	62 2011	51 2015	33 2016	25 2016	16 2015	6 2012
Min	-10 2014	-6 2011	-4 2014	16 2018	32 2008	45 2012	50 2009	45 2009	35 2009	21 2008	9 2014	-10 2016	-10 2016

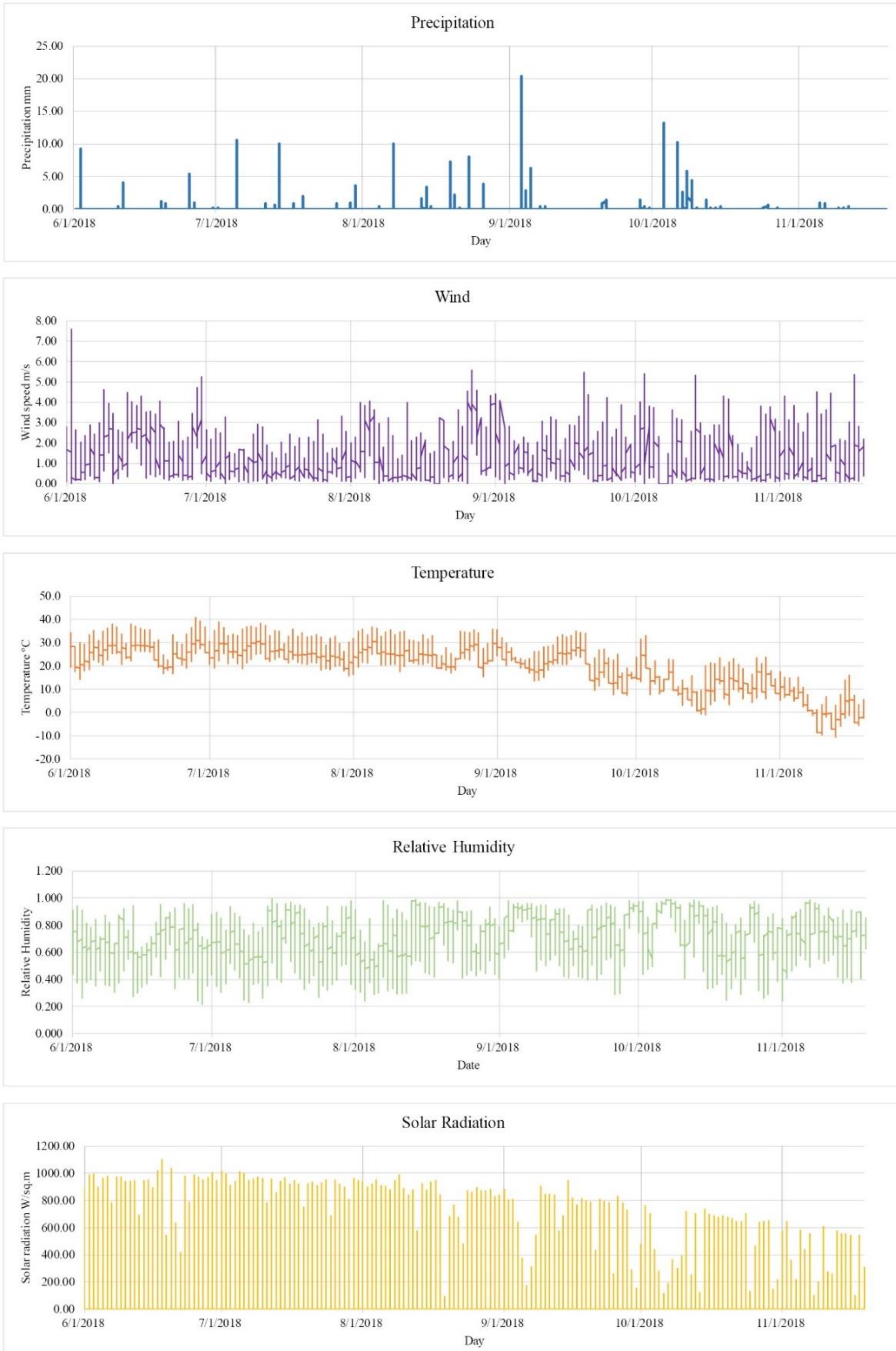

Figure A-2: Precipitation, wind speed, air temperature, relative humidity, and solar radiation graphs on the APD-EGR during the 2018 APD-EGR study period

(Source: KSU APD-EGR weather station)

Appendix B-Plant Replacement in the 4-inch APD-EGR Bed

Table B-1: Survival over the 2017-2018 winter in the 4-inch deep bed

Species	**Number survived**	**Percentage survived**	**Number replaced**	**Percentage replaced**
Bouteloua curtipendula	5	20.83%	19	79.17%
Bouteloua dactyloides	13	54.17%	11	45.83%
Bouteloua gracilis	11	45.83%	13	54.17%
Schizachyrium scoparium (B)	13	54.17%	11	45.83%
Carex brevior	21	87.50%	2	12.50%
Koeleria pyramidata	21	87.50%	3	12.50%
Schizachyrium scoparium (C)	12	45.83%	13	54.17%
Sporobolus heterolepis	24	100%	0	0%
Total	**120**	**62.5%**	**72**	**37.5%**

Appendix C-Substrate Analysis and Testing Procedures by the KSU Soil Testing Lab and Turf and Soil Diagnostics

Substrate Analysis: Per personal communications between Bryan Rutter (Manager, KSU Soil Testing Lab) and Jialin Liu (2018-2019), the following procedures were used to obtain soil test results:

"Total N and P analysis: 1 to 10 ml sample is digested with Potassium Persulfate Reagent in an autoclave and then analyzed using an Alpkem RFA for nitrate nitrogen (cadmium reduction method) and phosphorus according to:

1. Hosomi, M. and Sudu, R. 1986. Simultaneous determination of total nitrogen and total phosphorus in freshwater samples using persulfate digestion. International Journal of Environmental Studies. 27; 267-275.
2. Nelson N.S. 1987. An Acid-persulfate digestion procedure for determination of phosphorus in sediments. Communications in Soil Science Plant Analysis, 18(4); 359-369.
3. Alpkem Corporation. 1986. RFA Methodology no. A303-S170. Nitrate-Nitrite Nitrogen. Clackamas, OR 97015.

Ammonia and Nitrate Nitrogen: Alpkem RFA autoanalyzer according to:

1. Alpkem Corporation. 1986. RFA Methodology no. A303-S021. Ammonia Nitrogen. Clackamas, OR 97015.
2. Alpkem Corporation. 1986. RFA Methodology no. A303-S170. Nitrate+Nitrite Nitrogen. Clackamas, OR 97015.

Analysis of Ca, Mg, Na, Zn, Cu is done by an Inductively Coupled Plasma (ICP) Spectrometer, Model 720-ES ICP Optical Emission Spectrometer, manufactured by Varian Australia Pty Ltd, Mulgrave, Vic Australia.

Chloride analysis uses the calcium nitrate extraction and colorimetric analysis in the Mercury Thiocyanate method listed in "Recommended Chemical Soil Test Procedures for the North Central Region" on pp. 49-50 (Gelderman, R.H., Denning, J.L., and Goos, R.J.). The colorimetric assay is performed using an Alpkem RFA Methodology No. A303-S090. Water samples are not extracted in calcium nitrate but are diluted in calcium nitrate prior to analysis."

Exchangeable Cations Calcium - Magnesium - Potassium - Sodium

1. Scoop 2 g of soil for public samples. Weigh or scoop, according to submitted info sheet, 2 g of soil for research samples.
2. Dump soil into appropriate flask in K rack, tapping the scoop on the funnel to remove all of the soil from the scoop. Put LOW K CK in #12 and CK14 in #24 spot. After the samples, scoop or weigh Quality Control Samples, T-1 #1 and #13, T-2 #1 and #13, etc.
   End with LOW, HI, and Blank.
3. Dispense 20 mL of ammonium acetate (NH_4OAC) extracting solution into each flask.
   Shake at 200 epm (high) for 5 minutes at room temperature; 24 to 27°C.
4. Filter immediately through Ahlstrom 642 filter paper for calcium (Ca), potassium (K), and magnesium (Mg). Use Ahlstrom 74 filter paper if sodium (Na) was requested on samples.
5. If analyzing for potassium (K), read filtrates on atomic absorption spectrometer (AA), using appropriate K standards as listed in Table 2.5.
6. If analyzing for calcium (Ca2+), magnesium (Mg2+), and sodium (Na+), read filtrates on ICP. Pour samples from filtering racks into glass autosampler tubes and place in white ICP rack. Use appropriate cations standards as listed in Table 2.6.

References

Jones, J.B. 2001. Soil Analysis. p. 79-93. In Laboratory Guide for Conduction Soil Tests and Plant Analysis. CRC Press Boca Raton, FL.

Schollenberger, C.J. and R.H. Simon. 1945. Determination of exchange capacity and exchangeable basis in soil-ammonium acetate method. Soil Sci. 59:13-24.

Warncke D. and J.R. Brown. 1998. Potassium and Other Basic Cations. P. 31-33. In J.R. Brown (ed.) Recommended Chemical Soil Test Procedures for the North Central Region. North Central Regional Publication Number 221 (revised). Missouri Ag. Exp. Station SB 1001. Univ. of Missouri, Columbia, MO.

Cation Exchange Capacity Estimation

Summation - pH 7.0

1. Refer to Exchangeable Cation Procedure Steps (1-4). Use Ahlstrom 74 filter paper.
2. When analyzing for calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^{+}), read filtrates on ICP. Pour samples from filtering racks into glass autosampler tubes and place in white ICP rack. Use appropriate cations standards as listed in Table 2.6.
3. Open ICP cation Excel file. Multiply data by 10 (dilution factor) and subtract the blank. Verify check soils are in correct range.
4. Open CEC Calculation template. Input ICP cation values into the appropriate columns. If the SMP buffer pH is less than 7, enter the SMP buffer pH value into the H column. If the buffer pH is 7 or greater, enter 7.
5. Report values as meq/100g.

References

Chapman, H. D., "Cation Exchange Capacity," Methods of Soil Analysis, C. A. Black, ed. Agronomy Monograph No. 9, American Society of Agronomy: Inc., 1965, pp. 89l-901.

"Diagnosis and improvement of saline and alkali soils," L. A. Richards, ed., United States Department of Agriculture Handbook, No. 60, February 1954, 160 pp. Jackson, M. L., Soil Chemical Analysis--Advanced Course, 2nd ed, 11th printing. Published by author, Madison, WI, 1979, pp. 256-285.

Rich, C. I., "Removal of excess salt in cation exchange capacity determinations," Soil Science, Vol. 93, 1969, pp. 87-93.

Exchangeable Cations Calcium - Magnesium - Potassium - Sodium

1. Scoop 2 g of soil for public samples. Weigh or scoop, according to submitted information sheet, 2 g of soil for research samples.
2. Dump soil into appropriate flask in K rack, tapping the scoop on the funnel to remove all of the soil from the scoop. Put LOW K CK in #12 and CK14 in #24 spot. After the samples, scoop or weigh Quality Control Samples, T-1 #1 and #13, T-2 #1 and #13 etc.. End with LOW, HI, and Blank.
3. Dispense 20 mL of ammonium acetate (NH_4OAC) extracting solution into each flask. Shake at 200 epm (high) for 5 minutes at room temperature; 24 to 27°C.
4. Filter immediately through Ahlstrom 642 filter paper for calcium (Ca), potassium (K), and magnesium (Mg). Important: Use Ahlstrom 74 filter paper if sodium (Na) was requested on samples.
5. If analyzing for potassium (K), read filtrates on atomic absorption spectrometer (AA), using appropriate K standards as listed in Table 2.5.
6. If analyzing for calcium (Ca2+), magnesium (Mg2+), and sodium (Na+), read filtrates on ICP. Pour samples from filtering racks into glass autosampler tubes and place in white ICP rack. Use appropriate cations standards as listed in Table 2.6.

References

Jones, J.B. 2001. Soil Analysis. p. 79-93. In Laboratory Guide for Conduction Soil Tests and Plant Analysis. CRC Press Boca Raton, FL.

Schollenberger, C.J. and R.H. Simon. 1945. Determination of exchange capacity and exchangeable basis in soil-ammonium acetate method. Soil Sci. 59:13-24.

Warncke D. and J.R. Brown. 1998. Potassium and Other Basic Cations. P. 31-33. In J.R. Brown (ed.) Recommended Chemical Soil Test Procedures for the North Central Region. North Central Regional Publication Number 221 (revised). Missouri Ag. Exp. Station SB 1001. Univ. of Missouri, Columbia, MO.

DTPA Extractable Micronutrients: Zinc – Iron – Copper – Manganese

1. Scoop 10 g of soil for public samples. Weigh or scoop, according to submitted information sheet, 10 g of soil for research samples.
2. Dump soil into appropriate flask in DTPA rack using 50 mL polypropylene Erlenmeyer flasks, tapping the scoop on the funnel to remove all of the soil from the scoop. Put Blank in #12 and NAPT 2001-120 in #24 spot. After the samples, scoop or weigh Quality Control Samples, T-1 #1 and #13, T-2 #1 and #13 etc. End with NAPT CK and Blank.
3. Dispense 20 mL of DTPA extracting solution in each flask.
4. Shake for 2 hours and filter immediately through Ahlstrom 74 filter paper into filtering tubes. Refilter if extract is cloudy. Note: Samples high in Fe will have a yellow color.
5. Pour samples from filtering racks into glass autosampler tubes and place in white ICP rack. Read samples ICP unit using appropriate standards as listed in Table 2.9.

References

Khan, A. and P. N. Soltanpour, “Effect of Wetting and Drying on DTPA-extractable Fe, Zn, Mn, and Cu in Soil” Comm. Soil Sci. Plant Anal., Vol. 9, p 193-202, 1978.

Lindsay, W. L. and W. A. Norvell, “Development of a DTPA Soil Test for Zinc, Iron, Manganese, and Copper” Soil Science Amer. J., Vol. 42, p 421-428, 1978.

Soltanpour, P.N., A. Kahn, and W. L. Lindsay, “Factors Affecting DTPA-extractable Zn, Fe, Mn, and Cu from Soils” Comm. Soil Sci. Plant Anal., Vol. 17 p 797-820, 1976.

Whitney, D.A. 1998. Micronutrients: Zinc, Iron, Manganese and Copper. p. 41-44. In Ellis et al. (ed.) Recommended chemical soil test procedures for the North Central Region. North Central Regional Research Publication No. 221 (Revised). Missouri Agricultural Experiment Station SB 1001, Columbia, MO, USA.”

Substrate water release testing and other green roof media testing: Per the personal communication between Duane Otto (Turf and Soil Diagnostics) and Jialin Liu (2019), the following procedures were used to obtain the substrate water release test results and other green roof media test results:

“Our water release testing follows our lab SOP which follows ASTM D6836. We used Method C (using a pressure chamber similar to Figure 4) for tension points up to 4 bars and Method D (a Decagon DewPoint Potentiometer – see Fig. 7 in the ASTM method) for the 15 bar testing. Samples used in ASTM D6836 Method C were saturated from bottom up.

For the data on the first report sent labeled “Maximum Media Density for Dead Load Analysis”, testing methods include ASTM F1632 (particle size), ASTM D4972 (pH in $CaCl_2$), ASTM D5550 (particle density), and ASTM E2399 (saturated hydraulic conductivity and all weights and porosity data). Organic matter content was determined at 550°C per FLL Guidelines. Other than the temperature, the method is the same as ASTM D2974 Method C (which uses 440°C). Electrical conductivity is determined using a 1:5 solution following our Lab SOP, which is based on standard electrical conductivity methods.”

Appendix D-Soil Depth Measurements

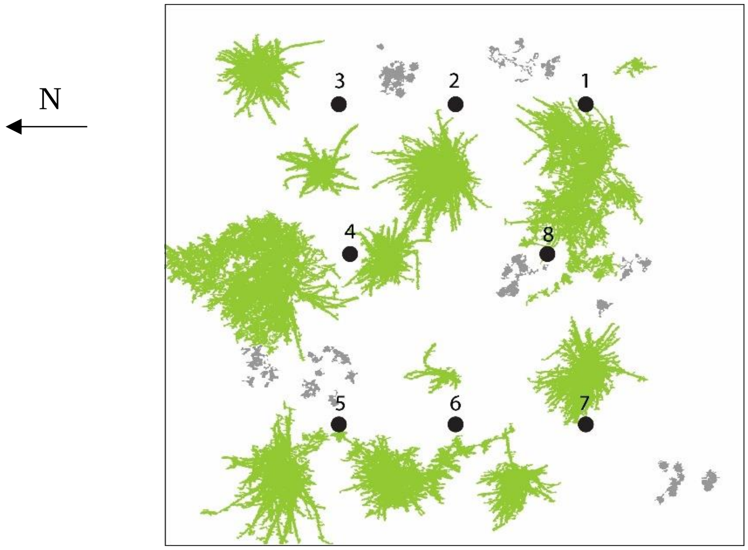

Figure D-0-1: Order of soil depth measurements taken

Table D-1: Soil depth measurements on the APD-EGR (Skabelund and Shrestha 2018)

Plot code	Depths in inches (in order taken for the 4-inch bed)								Average depth of plot (inches)
	1	2	3	4	5	6	7	8	
4-1KA	3.5	4	4.5	4	3.5	4.2	4.2	4.2	4.0
4-2KB	4.2	3.4	3.5	3.2	3.3	3.5	3.8	3.7	3.6
4-3KC	3.9	4.2	3.8	4	3.7	3.7	3.7	3.4	3.8
4-4RB	2.5	3	3	3.2	3.4	3.3	3	3.1	3.1
4-5RA	3	2.6	2.9	2.6	2.9	3	2.8	2.8	2.8
4-6RC	3.9	3.8	3.8	3.4	2.6	3	2.5	3.5	3.3
4-7KC	3.5	4.4	4.1	3.8	3.6	3.2	4	3.8	3.8
4-8KA	3.2	2.8	3.2	3.1	4.5	4	4.2	3.8	3.6
4-9KB	3.7	3.7	3.6	3.5	3.4	2.8	3.7	3.1	3.4
4-10RC	3.2	3.7	3.5	3	3.5	3.1	3.6	3.1	3.3
4-11RB	3.8	3.7	4	3.5	3.6	3.3	3.6	3	3.6
4-12RA	3.8	3.9	3.6	4	4	4	3.7	4.1	3.9
4-13RB	3.3	3.6	3.5	3.8	4.2	4	4	3.8	3.8
4-14RA	3.8	3.8	4	3.7	4.5	4.2	4.5	4	4.1
4-15RC	3.8	3.6	3.3	3.2	3.6	3.8	3.7	3.9	3.6
4-16KA	3.5	3.4	3.4	2.8	2.4	2.5	3.4	3.3	3.1
4-17KB	4.7	4.2	3.7	4	4.4	4.5	3.8	3.9	4.15
4-18KC	3.9	4	3.6	3.7	4.6	4.8	4	3.4	4.0
4-19RC	4.2	3.8	4.4	4.5	4.9	5.2	6	3.7	4.6
4-20RB	3.5	3.9	3.6	4	4.1	4	4.5	3.8	3.9
4-21RA	3.7	3.7	2.7	3.4	3.4	3	3.2	3.7	3.35
4-22KC	3.7	4	4	3.8	3.8	3.6	3.4	3.8	3.8
4-23KA	4	4.2	4.2	3.9	3.8	4	3.8	3.6	3.9
4-24KB	4.5	4.5	3.9	4.8	4	4	4	3.8	4.2

SAS output for substrate depth analyses in the 4-inch bed

The SAS System
The Mixed Procedure

Model Information	
Data Set	WORK.DATA2
Dependent Variable	DEPTH
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	Levels	Values
BLK	4	NE NW SE SW
SUBSTRATE	2	K R
MIX	3	A B C

Dimensions	
Covariance Parameters	5
Columns in X	12
Columns in Z	48
Subjects	1
Max Obs per Subject	192

Number of Observations	
Number of Observations Read	192
Number of Observations Used	192
Number of Observations Not Used	0

Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	307.90412145	
1	1	219.84000527	0.00000000

Convergence criteria met.

Covariance Parameter Estimates				
Cov Parm	Estimate	Standard Error	Z Value	Pr > Z
BLK	0.003615	0.06062	0.06	0.4762
BLK*SUBSTRATE	0.03015	0.04019	0.75	0.2266
BLK*MIX	0.1059	0.07778	1.36	0.0867
BLK*SUBSTRATE*MIX	0.03515	0.03037	1.16	0.1235
Residual	0.1388	0.01514	9.17	<.0001

Fit Statistics	
-2 Res Log Likelihood	219.8
AIC (Smaller is Better)	229.8
AICC (Smaller is Better)	230.2
BIC (Smaller is Better)	226.8

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
SUBSTRATE	1	3	1.23	0.3491
MIX	2	6	0.25	0.7835
SUBSTRATE*MIX	2	6	0.21	0.8175

Least Squares Means										
Effect	SUBSTRATE	MIX	Estimate	Standard Error	DF	t Value	Pr > ltl	Alpha	Lower	Upper
SUBSTRATE	K		3.7792	0.1471	3	25.69	0.0001	0.05	3.3110	4.2473
SUBSTRATE	R		3.6083	0.1471	3	24.53	0.0001	0.05	3.1402	4.0765
MIX		A	3.5953	0.1942	6	18.52	<.0001	0.05	3.1202	4.0705
MIX		B	3.7094	0.1942	6	19.10	<.0001	0.05	3.2342	4.1845
MIX		C	3.7766	0.1942	6	19.45	<.0001	0.05	3.3014	4.2517
SUBSTRATE*MIX	K	A	3.6594	0.2192	6	16.70	<.0001	0.05	3.1231	4.1957
SUBSTRATE*MIX	K	B	3.8375	0.2192	6	17.51	<.0001	0.05	3.3012	4.3738
SUBSTRATE*MIX	K	C	3.8406	0.2192	6	17.52	<.0001	0.05	3.3043	4.3769
SUBSTRATE*MIX	R	A	3.5312	0.2192	6	16.11	<.0001	0.05	2.9950	4.0675
SUBSTRATE*MIX	R	B	3.5813	0.2192	6	16.34	<.0001	0.05	3.0450	4.1175
SUBSTRATE*MIX	R	C	3.7125	0.2192	6	16.94	<.0001	0.05	3.1762	4.2488

Differences of Least Squares Means													
Effect	SUBSTRATE	MIX	SUBSTRATE	MIX	Estimate	Standard Error	DF	t Value	Pr > ltl	Alpha	Lower	Upper
SUBSTRATE	K		R		0.1708	0.1543	3	1.11	0.3491	0.05	-0.3204	0.6620
MIX		A		B	-0.1141	0.2570	6	-0.44	0.6728	0.05	-0.7430	0.5149
MIX		A		C	-0.1813	0.2570	6	-0.71	0.5072	0.05	-0.8102	0.4477
MIX		B		C	-0.06719	0.2570	6	-0.26	0.8025	0.05	-0.6961	0.5618
SUBSTRATE*MIX	K	A	K	B	-0.1781	0.2814	6	-0.63	0.5501	0.05	-0.8667	0.5105
SUBSTRATE*MIX	K	A	K	C	-0.1813	0.2814	6	-0.64	0.5434	0.05	-0.8698	0.5073
SUBSTRATE*MIX	K	A	R	A	0.1281	0.2033	6	0.63	0.5517	0.05	-0.3693	0.6255
SUBSTRATE*MIX	K	A	R	B	0.07812	0.3070	6	0.25	0.8076	0.05	-0.6731	0.8294
SUBSTRATE*MIX	K	A	R	C	-0.05313	0.3070	6	-0.17	0.8683	0.05	-0.8044	0.6981
SUBSTRATE*MIX	K	B	K	C	-0.00313	0.2814	6	-0.01	0.9915	0.05	-0.6917	0.6855
SUBSTRATE*MIX	K	B	R	A	0.3063	0.3070	6	1.00	0.3571	0.05	-0.4450	1.0575
SUBSTRATE*MIX	K	B	R	B	0.2562	0.2033	6	1.26	0.2543	0.05	-0.2412	0.7537
SUBSTRATE*MIX	K	B	R	C	0.1250	0.3070	6	0.41	0.6980	0.05	-0.6263	0.8763
SUBSTRATE*MIX	K	C	R	A	0.3094	0.3070	6	1.01	0.3525	0.05	-0.4419	1.0606
SUBSTRATE*MIX	K	C	R	B	0.2594	0.3070	6	0.84	0.4306	0.05	-0.4919	1.0106
SUBSTRATE*MIX	K	C	R	C	0.1281	0.2033	6	0.63	0.5517	0.05	-0.3693	0.6255
SUBSTRATE*MIX	R	A	R	B	-0.05000	0.2814	6	-0.18	0.8648	0.05	-0.7386	0.6386
SUBSTRATE*MIX	R	A	R	C	-0.1813	0.2814	6	-0.64	0.5434	0.05	-0.8698	0.5073
SUBSTRATE*MIX	R	B	R	C	-0.1313	0.2814	6	-0.47	0.6574	0.05	-0.8198	0.5573

Appendix E-Plant Height SAS Output

The SAS System

The Mixed Procedure DEPTH=4

Model Information	
Data Set	WORK.T2
Dependent Variable	AUC
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	Levels	Values
BLK	4	NE NW SE SW
SUBSTRATE	2	K R
MIX	2	B C
SPECIES	6	BC BG CB KP SC SH
ESTABLISHMENT	2	1 2

Dimensions	
Covariance Parameters	7
Columns in X	30
Columns in Z	120
Subjects	1
Max Obs per Subject	80

Number of Observations	
Number of Observations Read	80
Number of Observations Used	80
Number of Observations Not Used	0

Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	1149.33424271	
1	4	1147.48165249	0.00041136
2	3	1147.43428987	0.00000485
3	1	1147.43195413	0.00000003
4	1	1147.43193618	0.00000000

Convergence criteria met.

Covariance Parameter Estimates	
Cov Parm	Estimate
BLK	118661
BLK*SUBSTRATE	0
BLK*MIX	0
BLK*SUBSTRATE*MIX	0
BLK*SPECIES(MIX)	0
BLK*SUBST*SPECI(MIX)	0
Residual	1379176

Fit Statistics	
-2 Res Log Likelihood	1147.4
AIC (Smaller is Better)	1151.4
AICC (Smaller is Better)	1151.6
BIC (Smaller is Better)	1150.2

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
SUBSTRATE	1	3	11.58	0.0424
MIX	1	3	1.83	0.2692
SUBSTRATE*MIX	1	3	1.23	0.3478
SPECIES(MIX)	5	15	5.13	0.0061
SUBSTRA*SPECIES(MIX)	5	15	2.10	0.1214

Least Squares Means											
Effect	SUBSTRATE	MIX	SPECIES	Estimate	Standard Error	DF	t Value	Pr > ltl	Alpha	Lower	Upper
SUBSTRATE	K			7335.94	253.55	3	28.93	<.0001	0.05	6529.03	8142.85
SUBSTRATE	R			6419.34	257.27	3	24.95	0.0001	0.05	5600.59	7238.10
MIX		B		7058.75	258.71	3	27.28	0.0001	0.05	6235.41	7882.09
MIX		C		6696.53	252.65	3	26.50	0.0001	0.05	5892.48	7500.58
SUBSTRATE*MIX	K	B		7665.22	320.91	3	23.89	0.0002	0.05	6643.92	8686.51
SUBSTRATE*MIX	K	C		7006.66	309.09	3	22.67	0.0002	0.05	6023.00	7990.32
SUBSTRATE*MIX	R	B		6452.29	324.24	3	19.90	0.0003	0.05	5420.42	7484.15
SUBSTRATE*MIX	R	C		6386.40	316.68	3	20.17	0.0003	0.05	5378.59	7394.21
SPECIES(MIX)		B	BC	7915.50	381.08	15	20.77	<.0001	0.05	7103.25	8727.75
SPECIES(MIX)		B	BG	6538.20	349.55	15	18.70	<.0001	0.05	5793.14	7283.25
SPECIES(MIX)		B	SC	6722.56	396.36	15	16.96	<.0001	0.05	5877.73	7567.38
SPECIES(MIX)		C	CB	5902.56	396.35	15	14.89	<.0001	0.05	5057.76	6747.36
SPECIES(MIX)		C	KP	6275.96	409.91	15	15.31	<.0001	0.05	5402.26	7149.67
SPECIES(MIX)		C	SC	7678.90	369.60	15	20.78	<.0001	0.05	6891.12	8466.67
SPECIES(MIX)		C	SH	6928.70	449.51	15	15.41	<.0001	0.05	5970.59	7886.81
SUBSTRA*SPECIES(MIX)	K	B	BC	8364.08	510.65	15	16.38	<.0001	0.05	7275.65	9452.50
SUBSTRA*SPECIES(MIX)	K	B	BG	6834.55	476.82	15	14.33	<.0001	0.05	5818.23	7850.87
SUBSTRA*SPECIES(MIX)	K	B	SC	7797.02	510.64	15	15.27	<.0001	0.05	6708.61	8885.43
SUBSTRA*SPECIES(MIX)	R	B	BC	7466.92	510.65	15	14.62	<.0001	0.05	6378.50	8555.35
SUBSTRA*SPECIES(MIX)	R	B	BG	6241.84	449.51	15	13.89	<.0001	0.05	5283.73	7199.96
SUBSTRA*SPECIES(MIX)	R	B	SC	5648.09	553.93	15	10.20	<.0001	0.05	4467.42	6828.75
SUBSTRA*SPECIES(MIX)	K	C	CB	6863.76	510.65	15	13.44	<.0001	0.05	5775.33	7952.18
SUBSTRA*SPECIES(MIX)	K	C	KP	5822.75	553.92	15	10.51	<.0001	0.05	4642.09	7003.41
SUBSTRA*SPECIES(MIX)	K	C	SC	8051.24	476.83	15	16.88	<.0001	0.05	7034.90	9067.59
SUBSTRA*SPECIES(MIX)	K	C	SH	7288.87	611.93	15	11.91	<.0001	0.05	5984.58	8593.17
SUBSTRA*SPECIES(MIX)	R	C	CB	4941.37	553.91	15	8.92	<.0001	0.05	3760.74	6121.99
SUBSTRA*SPECIES(MIX)	R	C	KP	6729.18	553.91	15	12.15	<.0001	0.05	5548.55	7909.80
SUBSTRA*SPECIES(MIX)	R	C	SC	7306.55	510.65	15	14.31	<.0001	0.05	6218.12	8394.97
SUBSTRA*SPECIES(MIX)	R	C	SH	6568.53	611.93	15	10.73	<.0001	0.05	5264.23	7872.82

Differences of Least Squares Means														
Effect	SUBSTRATE	MIX	SPECIES	SUBSTRATE	MIX	SPECIES	Estimate	Standard Error	DF	t Value	Pr > ltl	Alpha	Lower	Upper
SUBSTRATE	K			R			916.59	266.81	3	3.44	0.0414	0.05	67.4950	1765.69
MIX		B			C		362.22	267.88	3	1.35	0.2692	0.05	-490.31	1214.75
SUBSTRATE*MIX	K	B		K	C		658.56	374.02	3	1.76	0.1765	0.05	-531.75	1848.87
SUBSTRATE*MIX	K	B		R	B		1212.93	385.36	3	3.15	0.0514	0.05	-13.4565	2439.32
SUBSTRATE*MIX	K	B		R	C		1278.81	379.69	3	3.37	0.0435	0.05	70.4560	2487.17
SUBSTRATE*MIX	K	C		R	B		554.37	376.47	3	1.47	0.2373	0.05	-643.71	1752.46
SUBSTRATE*MIX	K	C		R	C		620.25	369.20	3	1.68	0.1916	0.05	-554.71	1795.21
SUBSTRATE*MIX	R	B		R	C		65.8816	382.19	3	0.17	0.8741	0.05	-1150.42	1282.19
SPECIES(MIX)		B	BC		B	BG	1377.30	456.15	15	3.02	0.0086	0.05	405.04	2349.57
SPECIES(MIX)		B	BC		B	SC	1192.94	492.96	15	2.42	0.0287	0.05	142.23	2243.66
SPECIES(MIX)		B	BC		C	CB	2012.94	493.57	15	4.08	0.0010	0.05	960.92	3064.96
SPECIES(MIX)		B	BC		C	KP	1639.54	504.65	15	3.25	0.0054	0.05	563.91	2715.16
SPECIES(MIX)		B	BC		C	SC	236.61	472.35	15	0.50	0.6237	0.05	-770.18	1243.39
SPECIES(MIX)		B	BC		C	SH	986.80	536.61	15	1.84	0.0858	0.05	-156.96	2130.56
SPECIES(MIX)		B	BG		B	SC	-184.36	469.11	15	-0.39	0.6998	0.05	-1184.25	815.53
SPECIES(MIX)		B	BG		C	CB	635.64	469.77	15	1.35	0.1961	0.05	-365.65	1636.92
SPECIES(MIX)		B	BG		C	KP	262.23	480.83	15	0.55	0.5935	0.05	-762.64	1287.10
SPECIES(MIX)		B	BG		C	SC	-1140.70	446.44	15	-2.56	0.0220	0.05	-2092.27	-189.13
SPECIES(MIX)		B	BG		C	SH	-390.50	514.70	15	-0.76	0.4598	0.05	-1487.57	706.56
SPECIES(MIX)		B	SC		C	CB	820.00	505.06	15	1.62	0.1253	0.05	-256.52	1896.51
SPECIES(MIX)		B	SC		C	KP	446.59	515.98	15	0.87	0.4004	0.05	-653.20	1546.39
SPECIES(MIX)		B	SC		C	SC	-956.34	484.50	15	-1.97	0.0671	0.05	-1989.02	76.3430
SPECIES(MIX)		B	SC		C	SH	-206.14	547.57	15	-0.38	0.7118	0.05	-1373.27	960.98
SPECIES(MIX)		C	CB		C	KP	-373.40	514.40	15	-0.73	0.4791	0.05	-1469.82	723.02
SPECIES(MIX)		C	CB		C	SC	-1776.33	484.05	15	-3.67	0.0023	0.05	-2808.05	-744.61
SPECIES(MIX)		C	CB		C	SH	-1026.14	547.56	15	-1.87	0.0805	0.05	-2193.24	140.96
SPECIES(MIX)		C	KP		C	SC	-1402.93	494.95	15	-2.83	0.0126	0.05	-2457.89	-347.97
SPECIES(MIX)		C	KP		C	SH	-652.74	557.46	15	-1.17	0.2599	0.05	-1840.93	535.46
SPECIES(MIX)		C	SC		C	SH	750.20	528.52	15	1.42	0.1762	0.05	-376.32	1876.71

Tests of Effect Slices						
Effect	MIX	SPECIES	Num DF	Den DF	F Value	Pr > F
SUBSTRA*SPECIES(MIX)	B	BC	1	15	1.74	0.2067
SUBSTRA*SPECIES(MIX)	B	BG	1	15	0.95	0.3454
SUBSTRA*SPECIES(MIX)	B	SC	1	15	9.11	0.0086
SUBSTRA*SPECIES(MIX)	C	CB	1	15	7.29	0.0164
SUBSTRA*SPECIES(MIX)	C	KP	1	15	1.48	0.2426
SUBSTRA*SPECIES(MIX)	C	SC	1	15	1.29	0.2739
SUBSTRA*SPECIES(MIX)	C	SH	1	15	0.75	0.3994

Appendix F-Plant Cover SAS Output

The SAS System

The Mixed Procedure
DEPTH=4 NAME OF FORMER VARIABLE=TCOVER

Model Information	
Data Set	WORK.T2
Dependent Variable	AUC
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	Levels	Values
BLK	4	NE NW SE SW
SUBSTRATE	2	K R
MIX	2	B C
SPECIES	7	BC BO BG CB KP SC SH
ESTABLISHMENT	2	1 2

Dimensions	
Covariance Parameters	7
Columns in X	33
Columns in Z	132
Subjects	1
Max Obs per Subject	94

Number of Observations	
Number of Observations Read	94
Number of Observations Used	94
Number of Observations Not Used	0

Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	1139.81814740	
1	4	1139.19530058	.
2	1	1139.14629156	0.00000448
3	1	1139.14396344	0.00000002
4	1	1139.14395190	0.00000000

Convergence criteria met.

Covariance Parameter Estimates	
Cov Parm	Estimate
BLK	3174.88
BLK*SUBSTRATE	0
BLK*MIX	0
BLK*SUBSTRATE*MIX	1023.64
BLK*SPECIES(MIX)	0
BLK*SUBST*SPECI(MIX)	0
Residual	87136

Fit Statistics	
-2 Res Log Likelihood	1139.1
AIC (Smaller is Better)	1145.1
AICC (Smaller is Better)	1145.5
BIC (Smaller is Better)	1143.3

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
SUBSTRATE	1	3	1.27	0.3421
MIX	1	3	2.03	0.2498
SUBSTRATE*MIX	1	3	1.10	0.3709
SPECIES(MIX)	6	18	23.82	<.0001
SUBSTRA*SPECIES(MIX)	6	18	1.20	0.3498

Least Squares Means											
Effect	SUBSTRATE	MIX	SPECIES	Estimate	Standard Error	DF	t Value	Pr > ltl	Alpha	Lower	Upper
SUBSTRATE	K			603.38	52.7081	3	11.45	0.0014	0.05	435.64	771.12
SUBSTRATE	R			531.05	54.1808	3	9.80	0.0023	0.05	358.62	703.48
MIX		B		612.97	51.3756	3	11.93	0.0013	0.05	449.47	776.47
MIX		C		521.46	55.4822	3	9.40	0.0026	0.05	344.89	698.03
SUBSTRATE*MIX	K	B		682.87	65.8381	3	10.37	0.0019	0.05	473.34	892.39
SUBSTRATE*MIX	K	C		523.89	72.1732	3	7.26	0.0054	0.05	294.21	753.58
SUBSTRATE*MIX	R	B		543.08	68.1015	3	7.97	0.0041	0.05	326.35	759.81
SUBSTRATE*MIX	R	C		519.02	74.2303	3	6.99	0.0060	0.05	282.79	755.26
SPECIES(MIX)		B	BC	382.51	90.6530	18	4.22	0.0005	0.05	192.06	572.97
SPECIES(MIX)		B	BO	1422.63	85.3979	18	16.66	<.0001	0.05	1243.22	1602.05
SPECIES(MIX)		B	BG	268.98	82.2568	18	3.27	0.0043	0.05	96.1689	441.80
SPECIES(MIX)		B	SC	377.76	94.6485	18	3.99	0.0009	0.05	178.91	576.60
SPECIES(MIX)		C	CB	440.23	94.6517	18	4.65	0.0002	0.05	241.37	639.08
SPECIES(MIX)		C	KP	458.99	98.3130	18	4.67	0.0002	0.05	252.44	665.53
SPECIES(MIX)		C	SC	455.52	87.6494	18	5.20	<.0001	0.05	271.38	639.67
SPECIES(MIX)		C	SH	731.09	108.69	18	6.73	<.0001	0.05	502.74	959.44
SUBSTRA*SPECIES(MIX)	K	B	BC	359.33	125.07	18	2.87	0.0101	0.05	96.5727	622.08
SUBSTRA*SPECIES(MIX)	K	B	BO	1671.97	109.28	18	15.30	<.0001	0.05	1442.39	1901.56
SUBSTRA*SPECIES(MIX)	K	B	BG	291.32	116.34	18	2.50	0.0221	0.05	46.8904	535.75
SUBSTRA*SPECIES(MIX)	K	B	SC	408.84	125.07	18	3.27	0.0043	0.05	146.09	671.60
SUBSTRA*SPECIES(MIX)	R	B	BC	405.70	125.07	18	3.24	0.0045	0.05	142.94	668.46
SUBSTRA*SPECIES(MIX)	R	B	BO	1173.29	125.07	18	9.38	<.0001	0.05	910.53	1436.05
SUBSTRA*SPECIES(MIX)	R	B	BG	246.65	109.28	18	2.26	0.0367	0.05	17.0664	476.23
SUBSTRA*SPECIES(MIX)	R	B	SC	346.67	136.21	18	2.55	0.0203	0.05	60.5026	632.83
SUBSTRA*SPECIES(MIX)	K	C	CB	413.11	125.07	18	3.30	0.0040	0.05	150.35	675.88
SUBSTRA*SPECIES(MIX)	K	C	KP	488.11	136.20	18	3.58	0.0021	0.05	201.96	774.27
SUBSTRA*SPECIES(MIX)	K	C	SC	429.26	116.35	18	3.69	0.0017	0.05	184.81	673.70
SUBSTRA*SPECIES(MIX)	K	C	SH	765.09	151.11	18	5.06	<.0001	0.05	447.62	1082.55
SUBSTRA*SPECIES(MIX)	R	C	CB	467.34	136.21	18	3.43	0.0030	0.05	181.18	753.50
SUBSTRA*SPECIES(MIX)	R	C	KP	429.86	136.21	18	3.16	0.0055	0.05	143.70	716.02
SUBSTRA*SPECIES(MIX)	R	C	SC	481.79	125.07	18	3.85	0.0012	0.05	219.02	744.57
SUBSTRA*SPECIES(MIX)	R	C	SH	697.10	151.11	18	4.61	0.0002	0.05	379.63	1014.56

Differences of Least Squares Means														
Effect	SUBSTRATE	MIX	SPECIES	SUBSTRATE	MIX	SPECIES	Estimate	Standard Error	DF	t Value	Pr > ltl	Alpha	Lower	Upper
SUBSTRATE	K			R			72.3300	64.2376	3	1.13	0.3421	0.05	-132.10	276.76
MIX		B			C		91.5129	64.3002	3	1.42	0.2498	0.05	-113.12	296.14
SUBSTRATE*MIX	K	B		K	C		158.97	89.3011	3	1.78	0.1731	0.05	-125.22	443.17
SUBSTRATE*MIX	K	B		R	B		139.79	85.9485	3	1.63	0.2023	0.05	-133.74	413.32
SUBSTRATE*MIX	K	B		R	C		163.84	90.9032	3	1.80	0.1693	0.05	-125.45	453.14
SUBSTRATE*MIX	K	C		R	B		-19.1829	90.8767	3	-0.21	0.8463	0.05	-308.39	270.03
SUBSTRATE*MIX	K	C		R	C		4.8709	95.5255	3	0.05	0.9625	0.05	-299.13	308.88
SUBSTRATE*MIX	R	B		R	C		24.0539	92.4860	3	0.26	0.8116	0.05	-270.28	318.39
SPECIES(MIX)		B	BC		B	BD	-1040.12	117.00	18	-8.89	<.0001	0.05	-1285.93	-794.31
SPECIES(MIX)		B	BC		B	BG	113.53	114.61	18	0.99	0.3350	0.05	-127.25	354.31
SPECIES(MIX)		B	BC		B	SC	4.7569	123.86	18	0.04	0.9698	0.05	-255.47	264.98
SPECIES(MIX)		B	BC		C	CB	-57.7144	124.95	18	-0.46	0.6497	0.05	-320.22	204.79
SPECIES(MIX)		B	BC		C	KP	-76.4749	127.76	18	-0.60	0.5569	0.05	-344.88	191.93
SPECIES(MIX)		B	BC		C	SC	-73.0130	119.73	18	-0.61	0.5496	0.05	-324.55	178.53
SPECIES(MIX)		B	BC		C	SH	-348.58	135.81	18	-2.57	0.0194	0.05	-633.91	-63.2570
SPECIES(MIX)		B	BD		B	BG	1153.65	110.57	18	10.43	<.0001	0.05	921.36	1385.94
SPECIES(MIX)		B	BD		B	SC	1044.88	120.12	18	8.70	<.0001	0.05	792.52	1297.24
SPECIES(MIX)		B	BD		C	CB	982.41	120.95	18	8.12	<.0001	0.05	728.29	1236.52
SPECIES(MIX)		B	BD		C	KP	963.65	123.87	18	7.78	<.0001	0.05	703.40	1223.89
SPECIES(MIX)		B	BD		C	SC	967.11	115.66	18	8.36	<.0001	0.05	724.11	1210.11
SPECIES(MIX)		B	BD		C	SH	691.54	132.36	18	5.22	<.0001	0.05	413.46	969.62
SPECIES(MIX)		B	BG		B	SC	-108.77	117.86	18	-0.92	0.3683	0.05	-356.38	138.84
SPECIES(MIX)		B	BG		C	CB	-171.24	119.01	18	-1.44	0.1673	0.05	-421.27	78.7899
SPECIES(MIX)		B	BG		C	KP	-190.00	121.88	18	-1.56	0.1364	0.05	-446.07	66.0635
SPECIES(MIX)		B	BG		C	SC	-186.54	113.38	18	-1.65	0.1173	0.05	-424.75	51.6711
SPECIES(MIX)		B	BG		C	SH	-462.11	130.35	18	-3.55	0.0023	0.05	-735.98	-188.24
SPECIES(MIX)		B	SC		C	CB	-62.4713	127.82	18	-0.49	0.6309	0.05	-331.01	206.07
SPECIES(MIX)		B	SC		C	KP	-81.2318	130.58	18	-0.62	0.5417	0.05	-355.58	193.11
SPECIES(MIX)		B	SC		C	SC	-77.7699	122.74	18	-0.63	0.5343	0.05	-335.65	180.11
SPECIES(MIX)		B	SC		C	SH	-353.34	138.51	18	-2.55	0.0201	0.05	-644.33	-62.3436
SPECIES(MIX)		C	CB		C	KP	-18.7605	129.29	18	-0.15	0.8862	0.05	-290.39	252.87
SPECIES(MIX)		C	CB		C	SC	-15.2986	121.65	18	-0.13	0.9013	0.05	-270.87	240.27
SPECIES(MIX)		C	CB		C	SH	-290.87	137.58	18	-2.11	0.0487	0.05	-579.92	-1.8150
SPECIES(MIX)		C	KP		C	SC	3.4619	124.49	18	0.03	0.9781	0.05	-258.08	265.00
SPECIES(MIX)		C	KP		C	SH	-272.11	140.13	18	-1.94	0.0680	0.05	-566.50	22.2902
SPECIES(MIX)		C	SC		C	SH	-275.57	132.86	18	-2.07	0.0527	0.05	-554.70	3.5671

Tests of Effect Slices						
Effect	MIX	SPECIES	Num DF	Den DF	F Value	Pr > F
SUBSTRA*SPECIES(MIX)	B	BC	1	18	0.07	0.7909
SUBSTRA*SPECIES(MIX)	B	BD	1	18	9.57	0.0063
SUBSTRA*SPECIES(MIX)	B	BG	1	18	0.08	0.7759
SUBSTRA*SPECIES(MIX)	B	SC	1	18	0.12	0.7344
SUBSTRA*SPECIES(MIX)	C	CB	1	18	0.09	0.7672
SUBSTRA*SPECIES(MIX)	C	KP	1	18	0.10	0.7609
SUBSTRA*SPECIES(MIX)	C	SC	1	18	0.10	0.7556
SUBSTRA*SPECIES(MIX)	C	SH	1	18	0.10	0.7498

Appendix G-Plant Biomass SAS Output

The SAS System

The GLIMMIX Procedure DEPTH=4

Model Information	
Data Set	WORK.B1
Response Variable	TBIOMASS
Response Distribution	Negative Binomial
Link Function	Log
Variance Function	Default
Variance Matrix	Not blocked
Estimation Technique	Residual PL
Degrees of Freedom Method	Containment

Class Level Information		
Class	Levels	Values
BLK	4	NE NW SE SW
SUBSTRATE	2	K R
MIX	2	B C
SPECIES	6	BC BG CB KP SC SH

Number of Observations Read	56
Number of Observations Used	56

Dimensions	
G-side Cov. Parameters	6
R-side Cov. Parameters	1
Columns in X	30
Columns in Z	120
Subjects (Blocks in V)	1
Max Obs per Subject	56

Optimization Information	
Optimization Technique	Dual Quasi-Newton
Parameters in Optimization	7
Lower Boundaries	7
Upper Boundaries	0
Fixed Effects	Profiled
Starting From	Data

Iteration History					
Iteration	Restarts	Subiterations	Objective Function	Change	Max Gradient
0	0	6	54.324352676	0.95082713	154.2115
1	0	5	54.711680175	0.11798551	153.1594
2	0	2	54.720101786	0.00176393	153.141
3	0	1	54.720160894	0.00003304	153.1416
4	0	1	54.720162181	0.00002579	153.1412
5	0	1	54.720159023	0.00003319	153.1413
6	0	1	54.720160582	0.00001033	153.1414
7	0	1	54.720160069	0.00002397	153.1413
8	0	1	54.720160528	0.00002578	153.1413
9	0	1	54.720159597	0.00001878	153.1413
10	0	1	54.720160228	0.00000625	153.1413
11	0	1	54.720159926	0.00001095	153.1413
12	0	1	54.720160331	0.00001621	153.1413
13	0	1	54.720159694	0.00001110	153.1413
14	0	1	54.720160114	0.00000287	153.1413
15	0	1	54.720160001	0.00000444	153.1413
16	0	1	54.720160165	0.00000099	153.1413
17	0	1	54.720160127	0.00000411	153.1413
18	0	1	54.72015997	0.00000623	153.1413
19	0	1	54.720160205	0.00000000	153.1413

Convergence criterion PCONV=1.11022E-8) satisfied.

Estimated G matrix is not positive definite.

Fit Statistics	
-2 Res Log Pseudo-Likelihood	54.72
Generalized Chi-Square	39.96
Gener. Chi-Square / DF	0.95

Covariance Parameter Estimates		
Cov Parm	Estimate	Standard Error
BLK	0.01433	0.03011
BLK*SUBSTRATE	0	.
BLK*MIX	0.007699	0.03128
BLK*SUBSTRATE*MIX	0	0.02437
BLK*SPECIES(MIX)	0.04250	0.03464
BLK*SUBST*SPECI(MIX)	0.04395	.
Scale	0.001724	0.02904

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
SUBSTRATE	1	3	1.94	0.2584
MIX	1	3	3.85	0.1445
SUBSTRATE*MIX	1	3	0.16	0.7161
SPECIES(MIX)	5	15	5.91	0.0033
SUBSTRA*SPECIES(MIX)	5	15	1.18	0.3650

SUBSTRATE Least Squares Means												
SUBSTRATE	Estimate	Standard Error	DF	t Value	Pr > ltl	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
K	3.0956	0.09790	3	31.62	<.0001	0.05	2.7840	3.4071	22.0995	2.1635	16.1836	30.1779
R	2.9775	0.09893	3	30.10	<.0001	0.05	2.6626	3.2923	19.6380	1.9428	14.3338	26.9050

Differences of SUBSTRATE Least Squares Means									
SUBSTRATE	SUBSTRATE	Estimate	Standard Error	DF	t Value	Pr > ltl	Alpha	Lower	Upper
K	R	0.1181	0.08463	3	1.40	0.2573	0.05	-0.1513	0.3874

MIX Least Squares Means												
MIX	Estimate	Standard Error	DF	t Value	Pr > ltl	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
B	2.9076	0.1158	3	25.11	0.0001	0.05	2.5391	3.2762	18.3136	2.1210	12.6678	26.4754
C	3.1654	0.1049	3	30.18	<.0001	0.05	2.8316	3.4991	23.6978	2.4853	16.9729	33.0870

Differences of MIX Least Squares Means									
MIX	MIX	Estimate	Standard Error	DF	t Value	Pr > ltl	Alpha	Lower	Upper
B	C	-0.2577	0.1313	3	-1.96	0.1445	0.05	-0.6756	0.1602

SUBSTRATE*MIX Least Squares Means													
SUBSTRATE	MIX	Estimate	Standard Error	DF	t Value	Pr > ltl	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
K	B	2.9836	0.1321	3	22.59	0.0002	0.05	2.5632	3.4040	19.7589	2.6099	12.9778	30.0832
K	C	3.2075	0.1171	3	27.38	0.0001	0.05	2.8347	3.5803	24.7174	2.8953	17.0257	35.8839
R	B	2.8317	0.1345	3	21.06	0.0002	0.05	2.4037	3.2596	16.9740	2.2824	11.0646	26.0395
R	C	3.1233	0.1179	3	26.49	0.0001	0.05	2.7480	3.4985	22.7202	2.6787	15.6121	33.0647

Differences of SUBSTRATE*MIX Least Squares Means											
SUBSTRATE	MIX	SUBSTRATE	MIX	Estimate	Standard Error	DF	t Value	Pr > ltl	Alpha	Lower	Upper
K	B	K	C	-0.2239	0.1549	3	-1.45	0.2441	0.05	-0.7169	0.2691
K	B	R	B	0.1519	0.1319	3	1.15	0.3329	0.05	-0.2679	0.5718
K	B	R	C	-0.1397	0.1555	3	-0.90	0.4353	0.05	-0.6345	0.3552
K	C	R	B	0.3758	0.1569	3	2.39	0.0963	0.05	-0.1237	0.8753
K	C	R	C	0.08425	0.1061	3	0.79	0.4850	0.05	-0.2533	0.4218
R	B	R	C	-0.2916	0.1575	3	-1.85	0.1613	0.05	-0.7929	0.2097

SPECIES(MIX) Least Squares Means													
MIX	SPECIES	Estimate	Standard Error	DF	t Value	Pr > ltl	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
B	BC	3.0979	0.1659	15	18.68	<.0001	0.05	2.7444	3.4515	22.1523	3.6747	15.5547	31.5482
B	BG	2.4258	0.1814	15	13.37	<.0001	0.05	2.0391	2.8125	11.3109	2.0520	7.6836	16.6507
B	SC	3.1992	0.1650	15	19.39	<.0001	0.05	2.8475	3.5510	24.5133	4.0453	17.2441	34.8467
C	CB	3.2443	0.1639	15	19.79	<.0001	0.05	2.8949	3.5937	25.6445	4.2040	18.0820	36.3700
C	KP	3.1252	0.1659	15	18.84	<.0001	0.05	2.7717	3.4788	22.7649	3.7760	15.9854	32.4196
C	SC	3.5590	0.1595	15	22.31	<.0001	0.05	3.2190	3.8989	35.1264	5.6030	25.0023	49.3501
C	SH	2.7330	0.1732	15	15.78	<.0001	0.05	2.3638	3.1023	15.3793	2.6644	10.6308	22.2486

Differences of SPECIES(MIX) Least Squares Means											
MIX	SPECIES	MIX	SPECIES	Estimate	Standard Error	DF	t Value	Pr > ltl	Alpha	Lower	Upper
B	BC	B	BG	0.6722	0.2223	15	3.02	0.0085	0.05	0.1984	1.1460
B	BC	B	SC	-0.1013	0.2091	15	-0.48	0.6352	0.05	-0.5470	0.3444
B	BC	C	CB	-0.1464	0.2173	15	-0.67	0.5108	0.05	-0.6096	0.3168
B	BC	C	KP	-0.02728	0.2188	15	-0.12	0.9024	0.05	-0.4936	0.4390
B	BC	C	SC	-0.4610	0.2140	15	-2.15	0.0479	0.05	-0.9171	-0.00489
B	BC	C	SH	0.3649	0.2244	15	1.63	0.1248	0.05	-0.1134	0.8433
B	BG	B	SC	-0.7734	0.2217	15	-3.49	0.0033	0.05	-1.2459	-0.3010
B	BG	C	CB	-0.8186	0.2294	15	-3.57	0.0028	0.05	-1.3075	-0.3296
B	BG	C	KP	-0.6994	0.2308	15	-3.03	0.0084	0.05	-1.1913	-0.2076
B	BG	C	SC	-1.1332	0.2262	15	-5.01	0.0002	0.05	-1.6154	-0.6509
B	BG	C	SH	-0.3072	0.2361	15	-1.30	0.2128	0.05	-0.8105	0.1960
B	SC	C	CB	-0.04511	0.2166	15	-0.21	0.8378	0.05	-0.5069	0.4166
B	SC	C	KP	0.07400	0.2181	15	0.34	0.7391	0.05	-0.3909	0.5389
B	SC	C	SC	-0.3597	0.2133	15	-1.69	0.1124	0.05	-0.8145	0.09499
B	SC	C	SH	0.4662	0.2238	15	2.08	0.0548	0.05	-0.01080	0.9432
C	CB	C	KP	0.1191	0.2082	15	0.57	0.5758	0.05	-0.3247	0.5629
C	CB	C	SC	-0.3146	0.2032	15	-1.55	0.1425	0.05	-0.7478	0.1186
C	CB	C	SH	0.5113	0.2142	15	2.39	0.0306	0.05	0.05483	0.9678
C	KP	C	SC	-0.4337	0.2048	15	-2.12	0.0513	0.05	-0.8702	0.002755
C	KP	C	SH	0.3922	0.2156	15	1.82	0.0889	0.05	-0.06739	0.8518
C	SC	C	SH	0.8259	0.2108	15	3.92	0.0014	0.05	0.3766	1.2752

SUBSTRA*SPECIES(MIX) Least Squares Means														
SUBSTRATE	MIX	SPECIES	Estimate	Standard Error	DF	t Value	Pr > ltl	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
K	B	BC	3.0140	0.1996	15	15.10	<.0001	0.05	2.5885	3.4395	20.3682	4.0659	13.3096	31.1700
K	B	BG	2.4727	0.2206	15	11.21	<.0001	0.05	2.0026	2.9427	11.8538	2.6144	7.4080	18.9678
K	B	SC	3.4642	0.1884	15	18.39	<.0001	0.05	3.0627	3.8657	31.9506	6.0190	21.3843	47.7377
R	B	BC	3.1819	0.1948	15	16.34	<.0001	0.05	2.7668	3.5971	24.0927	4.6927	15.9070	36.4909
R	B	BG	2.3789	0.2252	15	10.56	<.0001	0.05	1.8989	2.8589	10.7929	2.4305	6.6785	17.4421
R	B	SC	2.9342	0.2025	15	14.49	<.0001	0.05	2.5026	3.3659	18.8072	3.8090	12.2138	28.9601
K	C	CB	3.3065	0.1920	15	17.22	<.0001	0.05	2.8973	3.7157	27.2900	5.2390	18.1258	41.0875
K	C	KP	3.1893	0.1952	15	16.34	<.0001	0.05	2.7732	3.6054	24.2712	4.7386	16.0091	36.7972
K	C	SC	3.6183	0.1853	15	19.53	<.0001	0.05	3.2233	4.0132	37.2730	6.9063	25.1117	55.3239
K	C	SH	2.7159	0.2100	15	12.93	<.0001	0.05	2.2683	3.1636	15.1189	3.1755	9.6627	23.6560
R	C	CB	3.1821	0.1953	15	16.29	<.0001	0.05	2.7659	3.5984	24.0983	4.7061	15.8932	36.5393
R	C	KP	3.0611	0.1985	15	15.42	<.0001	0.05	2.6381	3.4842	21.3520	4.2380	13.9864	32.5965
R	C	SC	3.4996	0.1875	15	18.66	<.0001	0.05	3.1000	3.8993	33.1035	6.2071	22.1975	49.3677
R	C	SH	2.7501	0.2087	15	13.18	<.0001	0.05	2.3052	3.1950	15.6441	3.2652	10.0265	24.4093

Tests of Effect Slices for SUBSTRA*SPECIES(MIX) Sliced By SPECIES(MIX)					
MIX	SPECIES	Num DF	Den DF	F Value	Pr > F
B	BC	1	15	0.62	0.4434
B	BG	1	15	0.13	0.7223
B	SC	1	15	6.37	0.0234
C	CB	1	15	0.36	0.5552
C	KP	1	15	0.37	0.5547
C	SC	1	15	0.38	0.5478
C	SH	1	15	0.02	0.8865

Appendix H-Plant Stomatal Resistance SAS Output

The SAS System

The Mixed Procedure DEPTH=4

Model Information	
Data Set	WORK.B1
Dependent Variable	RESIST
Covariance Structure	Variance Components
Subject Effects	BLK*SUBSTRATE, BLK*SUBSTRATE
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	Levels	Values
BLK	4	NE NW SE SW
SUBSTRATE	2	K R
SPECIES	2	BC SC
DAY	17	178 188 193 201 208 215 221 230 237 244 255 265 276 284 290 297 304

Dimensions	
Covariance Parameters	5
Columns in X	126
Columns in Z per Subject	164
Subjects	1
Max Obs per Subject	299

Number of Observations	
Number of Observations Read	391
Number of Observations Used	299
Number of Observations Not Used	92

Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	3075.36898437	
1	3	3050.48235867	0.00000966
2	1	3050.46903549	0.00000010
3	1	3050.46890057	0.00000000

Covariance Parameter Estimates		
Cov Parm	Subject	Estimate
BLK		486.03
BLK*SUBSTRATE		270.02
SPECIES	BLK*SUBSTRATE	1041.37
DAY	BLK*SUBSTRATE	0
Residual		8730.77

Fit Statistics	
-2 Res Log Likelihood	3050.5
AIC (Smaller is Better)	3058.5
AICC (Smaller is Better)	3058.6
BIC (Smaller is Better)	3056.0

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
SUBSTRATE	1	3	0.00	0.9976
SPECIES	1	6	15.94	0.0072
SUBSTRATE*SPECIES	1	6	1.85	0.2224
DAY	12	72	5.94	<.0001
SUBSTRATE*DAY	12	72	0.42	0.9516
SPECIES*DAY	12	163	0.81	0.6394
SUBSTRAT*SPECIES*DAY	12	163	0.95	0.5005

Least Squares Means											
Effect	SUBSTRATE	SPECIES	DAY	Estimate	Standard Error	DF	t Value	Pr > ltl	Alpha	Lower	Upper
SUBSTRATE	K			261.33	19.5029	3	13.40	0.0009	0.05	199.26	323.40
SUBSTRATE	R			261.25	19.6354	3	13.31	0.0009	0.05	198.77	323.74
SPECIES		BC		300.64	18.6177	6	16.15	<.0001	0.05	255.08	346.19
SPECIES		SC		221.95	18.7659	6	11.83	<.0001	0.05	176.03	267.87
SPECIES*DAY		BC	178	290.45	31.9095	163	9.10	<.0001	0.05	227.45	353.46
SPECIES*DAY		BC	188	255.46	31.9095	163	8.01	<.0001	0.05	192.45	318.47
SPECIES*DAY		BC	193	221.61	31.9095	163	6.95	<.0001	0.05	158.61	284.62
SPECIES*DAY		BC	201	331.25	31.9095	163	10.38	<.0001	0.05	268.24	394.26
SPECIES*DAY		BC	208	274.05	31.9095	163	8.59	<.0001	0.05	211.04	337.05
SPECIES*DAY		BC	215	371.64	31.9095	163	11.65	<.0001	0.05	308.63	434.65
SPECIES*DAY		BC	221	314.93	31.9095	163	9.87	<.0001	0.05	251.92	377.94
SPECIES*DAY		BC	230	276.50	31.9095	163	8.67	<.0001	0.05	213.49	339.51

SPECIES*DAY		BC	237	243.36	31.9095	163	7.63	<.0001	0.05	180.35	306.37
SPECIES*DAY		BC	244	312.21	31.9095	163	9.78	<.0001	0.05	249.20	375.22
SPECIES*DAY		BC	255	323.10	31.9095	163	10.13	<.0001	0.05	260.09	386.11
SPECIES*DAY		BC	265	411.68	31.9095	163	12.90	<.0001	0.05	348.67	474.69
SPECIES*DAY		BC	276	282.01	31.9095	163	8.84	<.0001	0.05	219.00	345.02
SPECIES*DAY		SC	178	175.34	33.0290	163	5.31	<.0001	0.05	110.12	240.56
SPECIES*DAY		SC	188	180.78	33.0290	163	5.47	<.0001	0.05	115.56	246.00
SPECIES*DAY		SC	193	222.38	33.0290	163	6.73	<.0001	0.05	157.16	287.60
SPECIES*DAY		SC	201	223.50	33.0290	163	6.77	<.0001	0.05	158.28	288.72
SPECIES*DAY		SC	208	243.48	33.0290	163	7.37	<.0001	0.05	178.26	308.70
SPECIES*DAY		SC	215	316.92	33.0290	163	9.60	<.0001	0.05	251.70	382.14
SPECIES*DAY		SC	221	243.14	33.0290	163	7.36	<.0001	0.05	177.92	308.36
SPECIES*DAY		SC	230	208.01	33.0290	163	6.30	<.0001	0.05	142.79	273.22
SPECIES*DAY		SC	237	164.91	33.0290	163	4.99	<.0001	0.05	99.6907	230.13
SPECIES*DAY		SC	244	212.07	33.0290	163	6.42	<.0001	0.05	146.85	277.29
SPECIES*DAY		SC	255	202.14	33.0290	163	6.12	<.0001	0.05	136.92	267.36
SPECIES*DAY		SC	265	318.87	33.0290	163	9.65	<.0001	0.05	253.65	384.09
SPECIES*DAY		SC	276	173.80	33.0290	163	5.26	<.0001	0.05	108.58	239.02

Tests of Effect Slices					
Effect	DAY	Num DF	Den DF	F Value	Pr > F
SPECIES*DAY	178	1	163	7.37	0.0074
SPECIES*DAY	188	1	163	3.10	0.0801
SPECIES*DAY	193	1	163	0.00	0.9856
SPECIES*DAY	201	1	163	6.45	0.0120
SPECIES*DAY	208	1	163	0.52	0.4722
SPECIES*DAY	215	1	163	1.67	0.1988
SPECIES*DAY	221	1	163	2.87	0.0924
SPECIES*DAY	230	1	163	2.61	0.1082
SPECIES*DAY	237	1	163	3.42	0.0662
SPECIES*DAY	244	1	163	5.57	0.0194
SPECIES*DAY	255	1	163	8.13	0.0049
SPECIES*DAY	265	1	163	4.79	0.0301
SPECIES*DAY	276	1	163	6.51	0.0116