

**Impact of Shade on Cool-Season Forage Mixtures
for the Mid-Atlantic Region**

Kelly Marie Mercier

Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfilment of the requirements for the degree of

Master of Science
in
Crop and Soil Environmental Sciences

Christopher D. Teutsch, Co-Chair

John H. Fike, Co-Chair

Benjamin F. Tracy

John F. Munsell

Brian D. Strahm

February 24, 2017

Blacksburg, VA

Key words: silvopasture, yield, nutritive characteristics, ergot alkaloids, botanical diversity

Impacts of Shade on Cool-Season Forage Mixtures for the Mid-Atlantic Region

Kelly Marie Mercier

ABSTRACT (academic)

Silvopastures integrate trees, forages, and livestock. Tall fescue, the dominant forage in much of the U.S., harbors an endophyte that produces toxic ergot alkaloids. Diluting the sward with other forages can reduce ergot alkaloid concentrations, but it is unknown how shade impacts alkaloid production and productivity of different forage mixtures. This study tested the effects of increasing shade and mixture complexity on sward yield, botanical composition, nutritive characteristics and ergot alkaloid concentrations. Slatted shade structures created 30, 50, and 70% shade compared to full sun. Three forage mixtures were evaluated (SIMPLE = tall fescue and white clover; INTERMEDIATE = SIMPLE + orchardgrass and red clover; and COMPLEX = INTERMEDIATE + Kentucky bluegrass, birdsfoot trefoil, and alfalfa).

Fifty and 70% shade reduced yield while red clover and orchardgrass dominated shaded swards. Birdsfoot trefoil, Kentucky bluegrass, and white clover did not perform well in any treatment. Nutritive value declined beneath shade in spring and fall. Sward ergot alkaloid concentration increased beneath shade in simple mixtures because of greater proportions of tall fescue. In the intermediate and complex mixtures, ergot alkaloids were diluted by other forage species and was not affected by shade. This illustrates the importance of incorporating multiple species into the sward. Low light levels may not have been sufficient to meet the forages' high energetic demands in the spring. Even though total forage production or nutritive value may be sacrificed during part of the year, this may be compensated for by diverse swards diluting ergot alkaloid concentrations.

ABSTRACT (general audience)

Silvopastures are an integrated land use practice that incorporate trees into pasture systems. Well-managed silvopastures have the potential to improve tree, forage, and/or livestock production, but local climate, individual system components, and management result in a wide range of conditions that can variably influence each element's production. Therefore, we designed a study to evaluate a range of shade levels and forage mixtures in order to gain a better understanding of how each factor influences forage production in silvopastures. Shade levels ranged from 0 to 70% shade while forage mixtures contained 2 to 7 species. Responses of interest were yield and quality of shaded forage mixtures as compared to mixtures grown in full sun. Forage yields were reduced at moderate and heavy shade levels, and red clover and orchardgrass were dominant species in shaded mixtures, while white clover, Kentucky bluegrass and birdsfoot trefoil scarcely contributed to pasture yield. Forage quality somewhat declined in spring and fall, but negative factors such as toxicity were reduced when more diverse mixtures were planted. This research illustrates the importance of using site-adapted, shade tolerant forages in silvopasture systems, while also demonstrating that tree canopy management is necessary in order to maintain adequate yields.

Acknowledgements

I've already given personal thanks to everyone who has helped make this research a success. 99% of them aren't going to read this, so they won't be offended if I don't mention them all by name. But whether it was for advice, help with manual labor, or preserving my mental sanity, they know who they are. And I must give my sincerest thanks and apology to any and all who helped move my shade structures and lived to tell and/or complain about it. Sorry about all the torn flesh and articles of clothing. I believe I was the only one who came away unscathed. You all shouldn't have gotten so close to those man-eaters.

Special thanks must go out to everyone who helped put the grants together and to the NRCS CIG and Southern SARE for funding this crazy adventure that I would not have been able to undertake otherwise. My co-advisors, Dr. Chris Teutsch and Dr. John Fike deserve extra thanks for putting up with me and editing the crap out of my 3rd grade level writing, because clearly I do not do my best work after three beers.

And lastly, thanks go out to any family or friends who are actually reading this. Thanks for showing interest in the last two and a half years of my life, beyond just asking, "What were you studying again?".

Table of Contents

Abstract.....	ii
Acknowledgements.....	iii
Chapter 1: Introduction	1
Literature Cited	3
Chapter 2: Literature Review and Objectives.....	5
Introduction to Agroforestry and Silvopastures	5
Silvopasture Establishment.....	6
Shade Effects on Environmental Characteristics and Forage Production	7
Tall Fescue in Grazing Systems.....	14
Factors Affecting Alkaloid Concentration in Tall Fescue Pastures.....	18
Ecologic and Agronomic Benefits of Botanical Biodiversity	23
Summary and Objectives	25
Literature Cited	27
Chapter 3: Materials and Methods.....	40
Site Description	40
Experimental Design and Layout	40
Plot Management.....	42
Measurements	42
Data Analysis	45
Literature Cited	46
Chapter 4: Production and Composition of Cool-Season Forage Mixtures in Response to Shade.....	48
Abstract	48
Introduction	49
Procedures	52
Site Description	52
Experimental Design and Layout.....	52
Plot Management.....	54
Measurements	55
Data Analysis	57
Results and Discussion	57
Yield	58
Botanical Composition	63
Simple Mixture	63
Intermediate Mixture	63
Complex Mixture	64

Botanical Diversity.....	66
Sward Height	68
Groundcover.....	70
Sward Density.....	71
Soil Temperature and Moisture	71
Summary and Implications.....	73
Literature Cited	75
Chapter 5: Ergot Alkaloid Concentration and Nutritive Characteristics of Shade-Grown Forage	
Mixtures	80
Abstract	80
Introduction	81
Procedures	85
Site Description	85
Experimental Design and Layout.....	86
Plot Management.....	87
Measurements	88
Data Analysis	89
Results and Discussion	90
Forage Nutritive Characteristics.....	90
Crude Protein.....	90
Acid Detergent Fiber.....	91
Neutral Detergent Fiber	91
Total Digestible Nutrients.....	91
Ergot Alkaloid Concentration	94
Summary and Implications.....	95
Literature Cited	97
Chapter 6: Summary and Conclusions	
Tables	106
Figures.....	115

List of Tables

Table 1. Forage mixtures and seeding rates	106
Table 2. Summary of the frequency and timing of measurements taken.....	107
Table 3. Significance of variables from ANOVA analysis of annual and seasonal yield.....	108
Table 4. Significance of variables from ANOVA analysis of botanical composition	109
Table 5. Significance of variables from ANOVA analysis of diversity, plot height, groundcover, compressed sward height, and compressed sward density.....	110
Table 6. Significance of variables from ANOVA analysis of soil temperature and moisture.....	111
Table 7. Significance of variables from ANOVA analysis of ergot alkaloid concentrations for sward and tall fescue	112
Table 8. The effect of shade on soil moisture in 2016.....	113
Table 9. Forage nutritive characteristics for each treatment averaged over the two year study	114

List of Figures

Figure 1. Average temperature and rainfall for Blackstone, VA, in 2015 and 2016.....	115
Figure 2. Aerial and schematic views of plot layout.....	116
Figure 3. Portable shade structures.....	117
Figure 4. Annual yield response to shade treatments averaged over the two year study	118
Figure 5. Seasonal yield responses to shade treatments	119
Figure 6. Impact of shade on the yield of simple, intermediate, and complex mixtures for the summer of 2016 harvests	120
Figure 7. Seasonal yield responses to mixture treatments	121
Figure 8. Simple mixture components' responses to shade in 2015.....	122
Figure 9. 2016: Simple mixture components' responses to shade	123
Figure 10. 2015: Intermediate mixture components' responses to shade	124
Figure 11. Intermediate mixture components' responses to shade in 2016	125
Figure 12. Complex mixture components' responses to shade in 2015	126
Figure 13. 2016: Complex mixture components' responses to shade	127
Figure 14. Impact of shade on the simple, intermediate, and complex mixtures' desired species diversity in 2015.....	128
Figure 15. 2016: Desired species diversity response to shade and mixture treatments in spring and fall.....	129
Figure 16. Impact of shade on simple, intermediate, and complex mixtures' desired species diversity in summer 2016	130
Figure 17. Total species diversity (planted species + weed species) response to shade and mixture treatments.....	131
Figure 18. Functional group diversity (cool-season grasses, warm-season grasses, legumes, and forbs) response to shade and mixture treatments.....	132
Figure 19. Plot height response to shade and mixture treatments.....	133
Figure 20. Groundcover response to shade and mixture.....	134
Figure 21. Compressed sward height response to shade and mixture during August and November 2016	135
Figure 22. Compressed sward density response to shade and mixture treatments.....	136
Figure 23. Seasonal soil temperature responses to shade treatments.....	137
Figure 24. Shade effect on monthly maximum soil temperature	138
Figure 25. Soil moisture response to shade treatments in 2016.....	139
Figure 26. Seasonal crude protein response to shade treatments and mixture.....	140
Figure 27. Seasonal acid detergent fiber responses to shade treatments.....	141
Figure 28. Neutral detergent fiber response to shade and mixture treatments	142
Figure 29. Seasonal total digestible nutrient response to shade treatments	143

Figure 30. Shade effects on mixture for ergot alkaloid concentration for entire sward 144
Figure 31. Total ergot alkaloid response to shade and mixture on a “per fescue plant” basis.. 145

Chapter 1. Introduction

Globally, farmers produce more than enough food to support our current population based on per capita food availability, which means that the amount of kilocalories produced exceeds the amount required per person per day (FAO, 2012). However, this level of production may not be able to sustain the world's growing population while maintaining environmental quality. Major concerns stemming from agricultural intensification include water quantity and quality, pesticide resistance, soil erosion, and habitat loss and degradation (Tilman et al., 2002). Scientists around the world are working to improve agricultural efficiency without compromising environmental quality in order to be able to feed the growing population for years to come.

Sustainable agriculture can be defined many ways, but the American Society of Agronomy (1989) defined it as follows: "Sustainable agriculture is one that, over the long-term, enhances environmental quality and the resource base on which agriculture depends, provides for basic human food and fiber needs, is economically viable, and enhances the quality of life for farmers and society as a whole." Much commercial production is under monoculture cultivation, but research has shown that polycultures have the potential to increase yields while utilizing resources more efficiently, both of which contribute to sustainable systems (Minns et al., 2001).

Agroforestry is an example of polyculture as it integrates trees into crop or livestock systems (Gold and Mason, 2015). Some of the aspects of agroforestry that contribute to sustainable agriculture objectives are efficiently utilizing resources, reducing inputs, and conserving soil and water resources (Mason et al., 2014; Peters, 2000).

Beef cattle production is an important component of the nation's agriculture industry and contributes over \$700 million in revenue each year in Virginia alone (NASS, 2017). The well-developed cattle industry in Virginia and other states provides ample opportunity to incorporate cattle production into agroforestry. The primary forage base supporting more than 8.5 million cows in the southeastern United States is tall fescue, a shade tolerant cool-season grass with potential for use in agroforestry systems (Ball et al., 2007; Buckner et al., 1979; Lin et al., 1999). Unfortunately, the mutualistic endophyte that gives tall fescue many of its desirable qualities also produces toxic ergot alkaloids that negatively impact livestock performance (Stuedemann and Hoveland, 1988). Cattle grazing endophyte infected tall fescue exhibit symptoms of heat stress and frequently seek shade (Ball et al., 2007).

Most shade on many farms is found near riparian areas (Higgins et al., 2011). Unrestricted access to these areas can cause environmental damage in the form of stream bank erosion and water pollution (Armour et al., 1991). Fencing out streams removes the immediate cause of this environmental degradation, but also limits livestock access to shade and the cooling effect of water.

Silvopastures, one of the five North American agroforestry practices, intentionally integrate trees into a managed forage-livestock production system and are designed to improve sustainability by increasing total farm productivity while returning social, ecological, and economical benefits (Clason and Sharrow, 2000). When managed properly, incorporating trees into grazing systems could also provide high quality grazing as well as shade for livestock. However, there is limited information on how the shade will impact forage mixtures in the mid-Atlantic region of the United States, particularly whether forage quantity and quality will be

affected. Therefore, this study was designed using increasing levels of shade and forage mixture complexity to address these questions.

Literature Cited

American Society of Agronomy. 1989. Agronomy news. Madison, Wisconsin.

Armour, C.L., D.A. Duff, and W. Elmore. 1991. The effects of livestock grazing on riparian and stream ecosystems. *Fisheries* 16:7-11.

Ball, D.M., C.S. Hoveland, and G.D. Lacefield. 2007. Southern forages. 4th ed. International Plant Nutrition Institute, Norcross, GA.

Buckner, R.C., J.B. Powell, and R.V. Frakes. 1979. Historical development. p. 1-8. *In*: R.C. Buckner and L.P. Bush (eds.), Tall Fescue. Agron. Monogr. 20. ASA, CSSA, SSSA, Madison, WI.

Clason, T.R. and S.H. Sharrow. 2000. Silvopastoral practices. p. 119-147. *In*: H.E. Garret, W.J. Rietveld, and R.F. Fisher (eds.) North American agroforestry: an integrated science and practice. ASA-CSSA-SSSA. Madison, WI.

Food and Agriculture Organization of the United Nations, editor. 2012. FAO in the 21st century: ensuring food security in a changing world. Food and Agriculture organization of the United Nations, Rome.

Gold, M. and A. Mason. 2015. Defining Agroforestry. p. 9-15. *In*: Training manual for applied agroforestry practices, 2015 edition. University of Missouri Center for Agroforestry, Columbia, MO.

Higgins, S.F., C.T. Agouridis, and S.J. Wightman. 2011. Shade options for grazing cattle. University of Kentucky Cooperative Extension Bull. AEN-99. Lexington, KY.

Lin, C.H., R.L. McGraw, M.F. George, and H.E. Garrett. 1999. Shade effects on forage crops with potential in temperate agroforestry practices. *Agrofor. Sys.* 44:109-119.

Mason, A., D. Wallace, and R. Straight. 2014. An overview of agroforestry. *Agroforestry Notes* (1). USDA National Agroforestry Center, Lincoln, NE.

Minns, A., J. Finn, A. Hector, M. Caldeira, J. Joshi, C. Palmborg, B. Schmid, M. Scherer-Lorenzen, E. Spehn, A. Troumbis and the BIODEPTH project. 2001. The functioning of European grassland ecosystems: potential benefits of biodiversity to agriculture. *Outlook Agric.* 30(3):179-185.

NASS. 2017. 2015 State agriculture overview: Virginia. USDA. Accessed 8 February 2017.
https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=VIRGINIA.

Peters, S.M. 2000. Agroforestry-an integration of land use practices. University of Missouri Center for Agroforestry, Columbia, MO.

Stuedemann, J.A. and C.S. Hoveland. 1988. Fescue endophyte: history and impact on animal agriculture. J. Prod. Agric. 1(1):39-44.

Tilman, D., K.G. Cassman, P.A. Matson, R. Naylor, and S. Polasky. 2002. Agricultural sustainability and intensive production practices. Nature 418:671-677.

Chapter 2. Literature Review

Introduction to Agroforestry and Silvopastures

Agroforestry is a term used to describe a set of land use practices that incorporate trees or shrubs with crops or livestock in a way that enhances beneficial interactions between the individual components (Gold and Mason, 2015). The five North American agroforestry practices are windbreaks, riparian buffers, forest farming, alley cropping, and silvopasture (Peters, 2000). Agroforestry is not a new practice, but since the late 1970's and early 1980's it has been gaining more attention because of both socioeconomic and ecological issues including global deforestation and the inability of the resource-poor to utilize the new technologies that came with the Green Revolution (Nair, 1993).

Agroforestry systems have the potential to support sustainable agricultural production via more efficient resource utilization, reduced inputs, soil and water conservation, increased crop production and economic diversification, and improved wildlife habitat, aesthetics, and carbon sequestration (Mason et al., 2014; Peters, 2000). The land equivalency ratio (LER), the ratio of polyculture production divided by monoculture production on the same unit of land, can be a useful tool for evaluating agroforestry's potential for overyielding (Vandermeer, 1981). In general, agroforestry systems, which by definition have multiple species and are considered polycultures, can have higher total production than monocultures because of more efficient resource utilization, giving them LER greater than one.

Silvopasture, one of the five North American agroforestry practices, intentionally incorporates trees into an intensively managed integrated forage-livestock production system (Gold and Mason, 2015). There are many environmental and economic benefits to

silvopastures, including shade and shelter for livestock, improving water quality and infiltration, recharging aquifers, boosting biodiversity, and providing wildlife habitat, more efficient nutrient cycling, increased aesthetic value and enhanced income stability by integrating long- and short-term revenue streams (Fike et al., 2004; Gold and Mason, 2015; Mosquera-Losada & Rigueiro-Rodriguez, 2013; Walter, 2015). Silvopastures, as a sub-discipline of agroforestry, share the same beneficial attributes: the potential for low input, resource use efficient systems that may foster increased production and profitability (Clason and Sharrow, 2000).

Silvopasture Establishment

Silvopastures are often created by planting trees into an existing pasture or by thinning a forest stand and planting forages under the remaining tree canopy (Walter, 2015). The first method of establishment allows for control of tree species selection and planting density, but takes a longer time to establish a tree stand capable of providing shade to livestock. Common tree arrangements include rows, randomly scattered individuals, or scattered clumps of trees (Peters, 2000). When thinning existing stands, tree stumps and spatial arrangement can inhibit use of large equipment necessary for planting and fertilizing (Blazier et al., 2008). Leaf litter accumulation can also inhibit seed to soil contact, creating a patchy appearance of forage establishment (Facelli and Pickett, 1991).

In some cases, forestry mulchers are used to grind any remaining woody material (limbs, stumps, etc.) down to the soil surface in order to facilitate site access with seed drills and large agricultural equipment used for fertilization and seedbed preparation. Mulching results in an influx of carbon to the system that needs to be decomposed by microbes. Microbes must

maintain an average carbon (C) to nitrogen (N) ratio (C:N) of approximately 8.6:1 (high N requirement) in order to decompose forest litter, although the exact ratio varies over time and between species (Kirchman, 2012). Because the added material has a such a high C:N, microbes will need to extract N from the soil in order to facilitate decomposition, thus competing with establishing forages (Hodge et al., 2000; Wheedon et al., 2009; Yang and Luo, 2010).

Soil pH and fertility may present additional challenges for forage establishment.

Piedmont forest soils of the southeastern United States are often acidic and relatively infertile, and are typically less productive than the region's agricultural lands (Baker, 2009). Southern Piedmont forest soils typically have low concentrations of N and phosphorus (P) and a pH of around 4-5, while improved forages generally require medium to high soil fertility and a pH of 6 or higher (Della-Bianca and Wells, 1967; Furiness et al., 2011; Metz et al., 1970; Peterson and Brann, 2009). Large amounts of costly soil amendments may be required to adjust soil pH and fertility to a level that will support improved forages.

Shade Effects on Environmental Characteristics and Forage Production

Compared to typical pastures, forages in silvopasture systems are subject to different environmental conditions due to the presence of trees. Karki and Goodman (2010, 2015) consistently reported lower values for wind speeds, air temperatures, relative humidity, solar radiation, photosynthetically active radiation (PAR), and evapotranspiration in mature silvopastures as compared to open pastures. Feldhake (2002) reported warmer surface temperatures during frost-prone nights underneath silvopastures, which could lead to an extended grazing season by supporting earlier green-up and later dormancy. This phenomenon

was also observed by Kallenback et al. (2006). Pierson et al. (1990) and Perry et al. (2009) found that cheatgrass (*Bromus tectorum*) (Pierson et al., 1990), big bluestem (*Andropogon gerardii*), smooth brome grass (*Bromus inermis*) and birdsfoot trefoil (*Lotus corniculatus*) (Perry et al., 2009) exhibited delayed maturity beneath shade, and Smith (1942) observed that Canada bluegrass grown underneath silvopastures remained in a vegetative state throughout the Ohio summers. These findings indicate lengthened periods of high forage quality in silvopasture systems, because it is well-known that forage maturity is the main driver of forage nutritive characteristics (Ball et al., 2007).

Silvopastures can also alter the below-ground conditions. Karki and Goodman (2015) reported lower soil moisture under mature loblolly-pine (*Pinus taeda* L.) silvopastures compared to open pastures due to an increased water uptake from the entire system. In a young silvopasture the same authors (2013) found the opposite to be true; soils in silvopastures with young longleaf-pine (*Pinus palustris* Mill.) had greater soil moisture than open pastures. These findings show the difference between the tree age and their impacts on forage competition for moisture resources, in that young trees seem to not compete as strongly for soil moisture as older, larger trees. Soil temperature was also affected and was lower in mature silvopastures, but higher in young silvopastures relative to neighboring open pastures (Karki and Goodman, 2010; 2013; 2015).

The microclimate of silvopasture systems also can alter and improve the botanical composition of the forage understory. Holochek et al. (1981) demonstrated increased botanical diversity underneath a tree canopy as compared to an open system. Neel et al. (2008) showed an increase of introduced forages beneath lightly shaded silvopastures over a 3-year time

period. The increase in desirable forages displaced native forages and reduced bare ground coverage (Neel et al., 2008). Smith (1942) also observed more weeds in control plots as compared to black locust (*Robinia pseudoacacia*) or black walnut (*Juglans nigra*) trees.

In some instances, the compositional change may be more complex and dynamic over time, as exhibited by Dahlan et al. (1993) where, as oil palm plantations matured, the species composition shifted with fewer broadleaved species and legumes occurring under trees of increasing age. Ehret et al. (2015) demonstrated white clover's (*Trifolium perenne*) inability to persist underneath dense shade, and Burner and Brauer's (2003) experiment showed a decline in tall fescue (*Festuca arundinacea*) and a shift from cool-season species to warm-season species during the warmer months in silvopastures. They attributed the loss of fescue to stresses associated with low soil moisture and limited management and the botanical shift to standard seasonal variation. Monk and Gabrielson's (1985) experiment of artificial shade effects on an abandoned field in South Carolina showed a drastic reduction in total species composition and a shift towards more perennial as compared to annual species under shaded conditions. A similar phenomenon occurred in Spain where perennial species abundance increased at the expense of annual species abundance (Mosquera-Losada et al., 2009). This could be due to some perennial root systems being more developed and therefore more tolerant of summer drought stress than annuals (Mapfumo et al., 2002).

One factor that may influence shade tolerance is whether the forage follows the C₃ or C₄ photosynthetic pathway. One difference between these pathways in regards to light utilization is that C₃ species are light saturated at lower light intensities than C₄ species (Stier and Gardner, 2008). This means that once a plant reaches its light saturation point, photosynthesis becomes

carbon limited rather than light limited, and it can no longer increase its photosynthetic rate as light intensity increases (Levai and Veres, 2013).

Based on these factors, C_4 species tend to be less shade-tolerant because they are able to utilize more sunlight than C_3 plants, and warm-season (C_4) plants generally have reduced growth under shade as compared to cool-season (C_3) species (Lin et al, 1999; Kephart et al., 1992). However, warm-season plants may have an advantage underneath shaded conditions in hotter climates when high temperatures induce photorespiration in cool-season species (MacAdam and Nelson, 2003).

Another advantage C_4 plants have is increased N and water use efficiency (Ghannoum et al., 2011). Perhaps the system where this would be of most importance is under mature silvopastures where large trees may compete with the understory forages for nutrients and moisture. The ability of C_4 species to be highly productive using less water than C_3 species could result in their increased persistence in water-limited environments, but may not be beneficial under adequate moisture.

In addition, each individual forage species responds differently to shaded conditions. However, some common responses to shade include increased leaf area and stem height, and decreased root growth, tiller number, and stem dry weight, and lower leaf dry weight and thickness (Allard et al., 1991; Corre, 1983; Kephart et al., 1992; Wong et al., 1985). Plants that exhibit these characteristics tend to look tall and spindly with larger and thinner leaves than their sun-grown counterparts.

When the plant is growing in shaded conditions, it is allocating more assimilated resources, such as carbon, nutrients, and water, to aboveground growth in order to promote

photosynthesis in a light limited environment, resulting in diminished root growth capacity (Poorter et al., 2012). Many shade-grown forages regrow after defoliation by utilizing carbon stocks stored in roots, as well as by relying on the photosynthetic capacity of remaining green material (Belesky, 2005). If the carbon stores in the roots are already limited, these plants may have slower rates of regrowth after a grazing event if light is limited. Forage species that are not well adapted to shade will likely have a reduced lifespan in silvopasture systems where the compound stresses of reduced light levels and herbivory will strain the plants' resources as compared to more shade-tolerant species. These factors may warrant longer rest intervals and/or less intense defoliation.

Silvopastures all over the world create different environmental dynamics and production based on their different soil types, forages, trees, and livestock components. The modified microclimate found in silvopastures may impact forage productivity especially, and there are conflicting findings on whether or not silvopastures reduce, maintain, or increase forage yields as compared to open pasture counterparts.

Many studies have documented reduced yields in shade or silvopastures as compared to open systems (Bambo et al., 2009; Barro et al., 2008; Belesky, 2005; Buergler et al., 2005; Burner, 2003; Feldhake et al., 2010; Kallenbach et al., 2006; Lin et al., 1999; Neel and Belesky, 2015; Neel et al., 2008; Obispo et al., 2013; Peri et al., 2001; Perry et al., 2009). But several studies have found increased yields in similar systems (Belsky et al., 1993; Buergler et al., 2005; Frost and McDougald, 1989; Garrett and Kurtz, 1983; Lin et al., 1999; Wilson et al., 1990). Additionally, some studies found no difference in forage yield between open and moderately shaded pastures (Burner and Brauer, 2003; Lin et al., 1999). Some of this disparity may be

attributed to differing tree stand density or age, as evidenced in a poplar silvopasture in New Zealand, where 5-year-old trees had no effect on forage yields (Guevara-Escobar et al., 2007), but reductions were seen in trees of 8 to 11 years of age in a similar site (Douglas et al., 2001).

As mentioned previously, each silvopasture system is unique. To understand some of the variation in forage production among shade conditions, Buergler et al. (2005) measured forage production under 7- and 8-year-old black walnut and honeylocust (*Gleditsia triacanthos*) trees planted at different densities. Forage yields were about 16% greater in moderately shaded sites, while forage yield was similar between the high shade and low shade sites. The authors suggested that heavy shade and tree competition reduced forage yield while very light shade did not provide enough canopy and soil cooling to benefit cool-season grass growth. Although the authors hypothesize that forage growth would be most benefited during hot Southern summers, most of this yield gain occurred in spring.

Yield is not the only indicator of potential production of silvopasture systems, however. Forage nutritive characteristics also are responsible for determining animal performance (Sollenberger and Vanzant, 2011), which can be another measure of silvopasture productivity. As with yield, the literature contains inconsistencies about whether shade in silvopastures has beneficial, detrimental, or neutral effects on forage nutritive characteristics.

Often, forage production in shaded environments has been associated with increased forage nutritive value in terms of increased crude protein (Bambo et al., 2009; Barro et al., 2008; Belesky et al., 2009; Burner, 2003; Kallenbach et al., 2006; Perry et al., 2009; Smith, 1942), reduced acid detergent fiber and neutral detergent fibers (ADF and NDF) (Kallenbach et al., 2006), increased in vitro digestibility (Barro et al., 2008; Kephart and Buxton, 1993; Obispo

et al., 2013) and greater forage mineral concentrations (Buerghler et al., 2006). In contrast, shade-grown forages may have higher ADF (Karki et al., 2009), lower total non-structural carbohydrates (TNC) (Belesky et al., 2006; Belesky et al. 2009; Buerghler et al., 2006), and increased cellulose and lignin concentrations (Hight et al., 1968). Belesky et al., (2006) suggested that some of these results may reflect a mechanism by which cool-season plants enhance light uptake in shaded systems. This involves distributing a higher percentage of N to leaf tissue, thus increasing the number of chloroplasts and the amount of chlorophyll for absorbing sunlight and converting it to energy. Because this N is largely unbound and occurs concomitant with low amounts of total non-structural carbohydrates due to reduced photosynthetic activity, overall forage quality may be reduced (Belesky et al., 2006).

Although nutritive value is commonly used as a measure of forage quality, perhaps a more direct approach to measuring forage production and quality in silvopastures is animal performance (Ball et al., 2007). Overall animal performance is a reflection of diet, animal genetics, management, and environment (National Research Council, 1996). Silvopasture systems throughout the world differ in many of these regards, so it would follow that animal performance can be variable from system to system.

Findings have shown beef heifer (Kallenbach et al., 2006), beef steer (Obispo et al., 2013), hair sheep (Fannon-Osborne, 2012), and sheep (Neel and Belesky, 2015; Neel et al., 2003) gains to be similar in silvopastures and open pastures. In contrast, cattle grazing oil palm silvopastures achieved greater productivity than their counterparts grazing open pastures (Chen, 1990).

In some instances, animal gains have been lower under shade or in silvopasture systems. Total ewe lamb gain and lamb gain per hectare were reduced under shade (Peri et al., 2001), sheep gain was reduced when fed shade grown forages (Hight et al., 1968), live weight gain of sheep per hectare in silvopastures was half of open pasture systems (Peri et al., 2001), and wool production was reduced in silvopasture systems as compared to open pasture (Bird et al., 2010).

All of these differences in yield, quality, or animal gains point to interactions that are occurring between microclimate moderation effects on the animal and forages as well as productivity differences that are inherent to each unique system. Specific factors that could be influencing these differences are tree species and spacing, time of year the study were conducted, and the forage species and animal species/breeds present.

Tall Fescue in Grazing Systems

Tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.) is the dominant forage base for much of the United States' improved cool-season forage systems and particularly for the transition zone in the humid East (Buckner et al., 1979; West and Waller, 2007). Tall fescue is thought to have been introduced from Europe as a contaminant of meadow fescue, which was commonly used for pasture plantings (Hoveland, 2009; Vinall, 1909).

The most widely planted fescue cultivar, 'Kentucky 31', was developed from a local ecotype found growing on a hill farm in Kentucky in 1931. The plant showed extraordinary agronomic qualities, one of which was the ability to extend the grazing season, and was quickly

adopted by many farmers (Hoveland, 2009). Historically Kentucky 31 fescue was widely used because of its productivity, persistence, and tolerance to a wide range of environmental and management-related stressors (USDA, 2006). Later it gained popularity for turf and soil stabilization in both agriculture and construction (Hoveland, 2009). Some specific beneficial qualities of tall fescue include relatively easy establishment, pest and drought tolerance and the ability to withstand close and frequent grazing (Ball et al, 2007; West and Waller, 2007).

Many of fescue's desirable qualities are attributed to its mutualistic association with the *Epichloë coenophiala* ((Morgan-Jones & W. Gams) C.W. Bacon & Schardl) endophyte, a fungus that lives within the plant (Arachevaleta et al., 1989; Bacon et al., 1977; Latch, 1993; Read and Camp, 1986). However, the fungus also produces alkaloids that are toxic to several classes and species of livestock. Maladies in cattle include increased body temperature, decreased weight gains, and lower conception rates, as well as several other symptoms collectively known as "fescue toxicosis" (Ball et al., 2007; Bush and Buckner, 1973; Porter, 1995; Stuedemann and Hoveland, 1988; Thompson and Stuedemann 1993). Concentrations above critical thresholds result in losses in performance and are estimated to cost the cattle industry more than \$1 billion annually (Roberts and Andrae, 2010).

Studies aimed at quantifying the amount of tall fescue toxins in forage that cause fescue disorders report a range of thresholds. Rottinghaus et al. (1991) reference unpublished data by Garner that found a threshold of 200 ppb of ergovaline. Tor-Agbidye et al. (2001) reported 400-750 ppb, and Stamm et al. (1994) concluded that up to 475 ppb of ergovaline is safe. Vazquez de Aldana et al. (2003) reported the accepted threshold is 400 ppb. Alkaloid concentrations vary throughout the year. High levels occur in the spring when seed heads are produced,

followed by general reductions in the summer, and a second peak in the fall which coincides with increased plant growth. Lowest levels occur in the winter when plants are dormant or semi-dormant (Agee and Hill, 1994; Ball et al., 1995, Rottinghaus et al., 1991). This seasonal variation leads to different mitigation strategies during different times of the year.

When tall fescue is rapidly growing in the spring decreasing fertility inputs or changing sources can reduce the amount of ergot alkaloids produced within the plant. Phosphorus and N (especially as ammonium as compared to nitrate) applications typically increase alkaloid concentrations (Arechavaleta et al., 1992; Azevedo et al., 1993; Rottinghaus et al., 1991).

While reducing fertility may compromise yields, another alternative is to increase pasture diversity or offer supplements, providing a dilution effect (Roberts et al., 2009). Adding clovers or other grass species that do not produce ergot alkaloids allows livestock to consume a variety of forages and therefore reduce their alkaloid intake. This strategy can be very effective, sometimes resulting in animal performance that is similar to animals grazing non-infected tall fescue (Petriz et al., 1980). However, other studies have given conflicting results, thereby implicating other factors such as environment and management regimes as having strong importance (Stuedemann and Seman, 2005).

Ergot alkaloids vary seasonally, so grazing pastures when concentrations are low (early spring and late fall/winter) and deferring grazing when alkaloid concentrations are high (late spring and early fall) may reduce alkaloid consumption and consequent stress on livestock (Adcock et al., 1997; Lane et al., 2000). Fall stockpiling is also a good way to utilize tall fescue because alkaloid concentrations in the forage decreases throughout the winter (Kallenbach et al., 2003). As concentrations of ergot alkaloids are very high in seedheads, clipping pastures in

the spring before they fully develop reproductive tillers and keeping pastures in a vegetative state throughout the growing season may also reduce total alkaloid concentrations (Rottinghaus et al., 1991; Stuedemann and Seman, 2005).

Removing the endophyte from tall fescue eliminates the risk of the common maladies associated with “toxic” tall fescue (Ball et al., 2007). However, endophyte-free tall fescue varieties do not have the positive agronomic traits common to fescue with the plant-fungal mutualism. Many productivity factors are sacrificed including plant vigor and persistence (Ball et al., 2007).

In order to keep the endophytic benefits while also reducing or eliminating the symptoms of fescue toxicosis, breeders used endophyte strains that did not produce ergovaline (Bouton et al., 2002). The result is a plant that remains hardy and vigorous under stressful conditions but does not negatively impact animal performance (Bouton et al., 2002). An increasing number of commercial varieties are available including Jesup MaxQ, Texoma MaxQ2, Lacefield MaxQ2 (Pennington Seed, Madison, GA), BarOptima PLUS E34 (Barenbrug USA, Tangent, OR), DuraMax GOLD, Martin2 647 and Tower 647 (DLF International Seeds, Halsey, OR) and ArkShield (Mountain View Seeds, Salem, OR). This is currently the “gold standard” for tall fescue pastures, and animals grazing novel varieties perform similar to endophyte free varieties, while the plant still retains the benefits of endophyte association (Ball et al., 2007). However, animals have been shown to increase intake of novel endophyte fescue by 25% (Bondurant et al., 2001). This increase in intake is due to the lack of negative feedback to the animal that is associated with ingesting toxic tall fescue (Bondurant et al., 2001). High levels of

forage intake necessitates more intensive pasture management in order to maintain sward health and stability.

Converting or renovating pastures is costly and takes land out of production while new pastures are establishing. This option may also be undesirable for highly erodible land whereby removing existing vegetation can be considered an environmental hazard. Additionally, endophyte infected seed may be reintroduced to pastures by hay or equipment. For more information on converting toxic to novel endophyte tall fescue pastures see Roberts and Andrae (2010).

Factors Affecting Alkaloid Concentration in Tall Fescue Pastures

Using perennial ryegrass, Rasmussen et al. (2007b) positively correlated endophyte abundance with alkaloid concentrations for peramine, lolitrem B and janthitrems, but not for ergovaline. Also in ryegrass, Spiering et al. (2005) reported that endophyte abundance accounted for 20% of the variation of ergovaline, while Ball et al. (1995) found that regression R^2 values for endophyte alkaloid levels as related to endophyte concentrations ranged from 0.54 to 0.68. Therefore, high levels of endophyte have the potential to result in high total alkaloid concentrations, although perhaps not for ergovaline specifically. Thus, factors affecting endophyte abundance could affect alkaloid production as well.

Hiatt and Hill (1997) regressed ergot alkaloid concentrations with mycelia protein mass in tall fescue. Results for both leaf sheath and blade suggest that other factors besides fungal abundance determine ergot alkaloid concentrations. Norriss et al. (2007) also tried to correlate hyphal abundance to alkaloid concentration in tall fescue. They evaluated two AR542

endophyte-infected cultivars selected for grazing tolerance, high endophyte density, and insect tolerance. One variety, 'Resolute', displayed increased loline and peramine as well as increased hyphal density. The other variety, 'Quantum', displayed similar, though non-significant patterns. They concluded that hyphal density may, in part, lead to increased alkaloid concentrations within the plant, but reiterated both that breeding increased tolerance to stressors may have inadvertently shifted cultivar selection towards higher alkaloid production, and that other factors are important in alkaloid expression.

Several factors have been shown to impact alkaloid levels in the plant. High N, P, and carbohydrate content in perennial ryegrass cultivars decreased endophyte and alkaloid concentration in the plant (Rasmussen et al., 2007a and 2007b). The authors speculate the reduction in total alkaloids could have been caused by either 1) a direct correlation between endophyte density and alkaloid concentrations, or by 2) the treatments decreasing the rate of alkaloids produced on a per endophyte basis (Rasmussen et al., 2007a and 2007b).

In contrast, high N was shown to increase ergovaline/ergovalinine in ryegrass (Lane et al., 1997) and ergot alkaloids in tall fescue as well, but the extent was somewhat dependent on N source (Arechavaleta et al., 1992). High rates of N fertilizer as both potassium nitrate and ammonium chloride also increased ergot alkaloid levels in greenhouse-grown tall fescue to similar degrees, indicating that N source may not make a difference (Lyons et al., 1986). A 15-16-17 NPK fertilizer application increased endophyte density in tall fescue (Mack and Rudgers, 2008).

Soil P also can increase ergot alkaloid levels in tall fescue plants, although this response appears dependent on levels in soil solution and plant tissue. In a greenhouse study, alkaloid

levels in shoots increased as soil P increased from 17 to 50 mg P/kg but concentrations decreased at 96 mg P/kg; in contrast, root alkaloid concentrations increased linearly with P availability (Malinowski et al., 1998). The authors concluded that endophyte association aids the plant by supporting greater photosynthate allocation to roots, thus increasing P uptake in infertile soils and supporting higher ergot alkaloid production (Malinowski et al., 1998).

Climatic variables play a role in endophyte and alkaloid production in tall fescue. Water stress was shown to increase ergovaline/ergovalinine in ryegrass (Lane et al., 1997), but under extreme moisture ergovaline concentration was not significantly different before and after flooding events (Arechavaleta et al., 1989). In climate manipulation studies, endophyte infection frequency in tall fescue was increased under elevated atmospheric CO₂ (Brosi et al., 2011). However, even though more plants exhibited endophyte association, individual plant ergovaline and loline concentrations were reduced (Brosi et al., 2011). Increases in atmospheric temperatures reduced tall fescue populations but increased their ergot alkaloid concentrations by 30-40% in fall-harvested tissues (McCulley et al., 2014). In contrast, Lane et al. (1997) increased daytime temperatures by 15°C and nighttime temperatures by 5°C in a greenhouse and did not see any effect of increased temperature on ryegrass alkaloids.

Biotic stressors may also play a role in alkaloid expression in tall fescue. Mack and Rudgers (2008) examined the interactions between mycorrhizal colonization and fungal endophytes in tall fescue plants. Mycorrhizae had no effect on the endophyte, but the endophyte reduced mycorrhizal colonization by 50% which may be related to one or more of the following factors: 1) alkaloids may serve as a defense mechanism against mycorrhizal infection, 2) reduced need for mycorrhizae because endophyte presence supports increased

nutrient acquisition, and 3) endophytes have closer proximity to carbohydrate food sources (in above-ground tissues) than the root-associated mycorrhizae. With respect to this third point, if there is a set amount or proportion of carbohydrates that the plant can allocate to its mutualistic associations, then it would follow that presence in the leaves (where carbohydrates are produced) would provide the endophyte opportunity to capture those carbohydrates before they move to the roots where the mycorrhizae are located.

Integrating silvopastures into fescue-based production systems may offer several ways to mitigate the effects of fescue alkaloids. Along with shaded conditions being able to lower animal body temperature and make a more comfortable habitat for grazing livestock (Buffington et al., 1983; Higgins et al., 2011), the altered microclimate under trees may favor the growth of other cool-season grasses and legumes as shown by Buerger et al. (2005).

Incorporating other desirable forage species into pastures has been shown to offset some of the negative effects of fescue toxicosis, which could indirectly reduce the alkaloids at the sward level by diluting the amount of toxic fescue available for animal consumption. The dilution effect was documented by Petriz et al. (1980) on a farm in southern Indiana where cows and calves grazing mixed tall fescue-legume pastures gained more weight than those grazing fescue pastures, and was slightly better than those grazing orchardgrass pastures (Ball et al., 2007). Cow pregnancy rates followed the same trend (Petriz et al., 1980). Other research has shown similar results when comparing pure tall fescue pastures with mixed fescue-legume pastures in regards to animal productivity (Burns et al., 1973; Harris et al., 1972; Hoveland et al., 1981; McMurphy et al., 1990).

Reduced available sunlight also may have the potential to indirectly limit alkaloid synthesis (Belesky et al., 2009). Because tall fescue feeds the fungus with products from photosynthesis (Bacon and White, 2000), it would follow that alkaloid levels should be greatest when plant nutrients are in abundant supply, such as when growing conditions support high plant productivity and photosynthetic activity. In support of this claim, Belesky et al., (2009) demonstrated slightly reduced ergot alkaloid quantity in shade-grown tall fescue as a function of reduced photosynthates supplied to the fungus.

Alternatively, Davitt et al. (2010) showed that under very heavy shade tall fescue increased its endophyte density by 86%, however ergot alkaloids were not quantified in this study. The authors suggest that plants need other defenses such as alkaloid production against herbivory because of less structural defenses produced in shade grown plants as compared to open-grown plants. Based on these results, there could be an irradiance threshold that a plant needs to experience in order to increase the amount of photosynthate partitioned to the endophyte in light limited environments.

Defoliation has been shown to increase ergovaline concentrations in *Festuca rubra* (Bazely et al., 1997), but reduced the total alkaloid yield of tall fescue by half as compared to uncut plants (Belesky and Hill, 1997). Belesky and Hill (1997) suggest periods of rest, such as stockpiling, have the potential to increase alkaloid concentrations in plants while intense defoliation regimes should reduce alkaloid production. They also hypothesize that regrowing vegetation requires carbohydrate stores which then cannot be partitioned to the fungus for alkaloid production. Therefore, once the plant regrows (or is stockpiled for a length of time) and can rely more on energy acquired from photosynthesis it can partition more energy to the

endophyte (Belesky and Hill, 1997). However, once the stockpiled forage stops growing and goes dormant, ergovaline concentrations decline steadily throughout the winter and are drastically reduced by approximately 85% by the end of the stockpiling season (Feb-Mar) (Kallenbach et al., 2003).

Further evidence supporting this hypothesis is generally shown when total non-structural carbohydrates (TNC) are low, alkaloid production is low as well, and increases in TNC then leads to increases in alkaloids (Belesky and Hill, 1997). The reduced irradiance of the silvopasture microclimate consistently decreases forage TNC (Belesky et al. 2006; Belesky et al., 2009; Buerger et al., 2006). These simple sugars, along with amino acids and vitamins from the apoplasm, feed the endophyte, which could explain why low concentrations of TNC equate to lower alkaloid production (Bacon and White, Jr., 2000).

Ecologic and Agronomic Benefits of Botanical Biodiversity

Environmental biodiversity is essential to global ecosystem stability and well-being (Hooper et al., 2005). Diverse plant communities provide many benefits and ecosystem services, such as increased productivity, soil water and nitrogen retention, environmental resilience, weed suppression, and carbon sequestration (Kirwan et al., 2007; McNally et al., 2015; Minns et al., 2001). Improving biodiversity on farms not only benefits farm productivity, but also can improve our ecosystems (Minns et al., 2001).

Conventional agriculture is most commonly based on monoculture production practices, (Minns et al., 2001). Improving forage production generally results in a loss of biodiversity (Peeters, 2004). Usually, in order to keep diversity high, systems must limit inputs including

fertilizer (Isselstein et al., 2005). Otherwise, agronomic species will outcompete native vegetation under high fertility and management, and the use of chemical herbicides reduces the competition from weeds (Isselstein et al., 2005; Minns et al., 2001). The converse is true in unmanaged systems where more species tend to be present under low to moderate amounts of nutrients (the humpback curve), rather than the high-nutrient input of managed agricultural systems (Adams, 2010).

A multinational research effort put forth by Hector et al. (1999) showed that on average, throughout Europe declining species diversity resulted in reduced productivity of grassland ecosystems. They also reported that reduced functional diversity within a given number of species led to reduced productivity (especially when legumes were excluded from the sward) and reduced species richness resulted in a reduction of plant cover. Bonin and Tracy (2012) demonstrated a similar phenomenon using warm-season prairie plants in the USA where a yield benefit for warm-season grasses occurred with the inclusion of multiple species as compared to monocultures.

However, in the Northeast USA a three-species cool season mixture dominated by white clover and tall fescue outperformed diverse mixtures of up to 15 species (Tracy and Sanderson, 2004). This example contradicts other findings about biodiversity and productivity relationships, but showcases the success of agricultural selection for high-yielding cultivars that can produce as much or more than unimproved native plants. The authors emphasized that the increased sward stability was due to the inclusion of legumes in the sward, and Minns et al. (2001) stated that legumes can have a “keystone” effect on ecosystems. That is, their ability to fix N can drastically alter community processes as compared to swards without N-fixing species.

Similarly, Skinner et al. (2006) reported that increased yield benefits from complex mixtures were a result of including high-yielding forages, rather than purely a biodiversity effect.

Animal production can also benefit from grazing diverse pastures. Totty et al. (2013) reported increased milk production (16.9 vs. 15.0 kg/day for dairy cows grazing a 5-species mixture as compared to a 2-species mixture, even though the cows did not increase daily dry matter intake. Phillips and James (1998) also showed a 3.2-kg/day increase in milk production for dairy cows grazing a grass-clover biculture as compared to a grass monoculture. Similar effects were observed in a collection of studies evaluating beef steer gains (Burns and Standaert, 1985). Steers increased average daily gain by 0.14 kg by grazing on grass-legume pastures as compared to grass pastures fertilized with N (Burns and Standaert, 1985). Dierking et al. (2010) also reported increased gains on grass-legume pastures as compared to monoculture fescue pastures.

In other cases, animals were not more productive on more diverse pastures. Soder et al. (2006) grazed dairy cows on mixtures of 2 to 9 species and found no difference in milk yield for all pastures. Lambs grazing botanically diverse pastures even had lower gains than when grazing legume- or ryegrass-based pastures (Lourenco et al., 2007). Finally, Tracy and Faulkner (2006) grazed cow-calf pairs and found no difference in gains between 3-, 5-, or 8-species forage mixtures.

Summary and Objective

Silvopastures show potential to increase productivity and revenue for farms in the southeastern U.S. However, shade provided by the trees in silvopastures may reduce plant

productivity and dry matter yield. The negative impact on dry matter may be offset by increases in nutritive value, botanical shifts to more desirable forage species, and an altered microclimate that benefits both plants and animals during the summer months. As compared to typical pastures, the silvopasture microclimate generally has lower wind speeds and a moderated temperature. This mild environment may improve forage quality, but the combination of reduced light levels and herbivory may strain plants' resources leading to reduced stand persistence.

An important concept in silvopastures, as with any farming system, is to utilize components that work well with the existing climate and landscape. Tall fescue is particularly well-adapted and is extensively utilized in the Southeast. Tall fescue contains a mutualistic fungal endophyte. As part of the symbiosis, the grass feeds the endophyte products from photosynthesis. In return, the endophyte produces chemical compounds that confer tolerance to many environmental and management stressors.

However, some of these ergot alkaloid compounds, lead to fescue toxicosis in livestock, a malady that costs the cattle industry \$1+ billion each year. Abiotic factors such as soil fertility, seasonality, and climate influence toxin levels within the plant. Grazing management can also affect the amount of alkaloids livestock ingest. Deferring grazing when alkaloid concentrations are highest and appropriately timing fertilizer application are common mitigation strategies.

One of the most effective ways to mitigate fescue toxicosis is to dilute the amount of total ergot alkaloids present in a sward by planting a diverse array of forages. Biological diversity can increase production and improve ecosystem service provisioning, both of which are key functions of successful silvopasture systems. High-yielding shade tolerant forage

cultivars can be utilized in silvopastures in conjunction with tall fescue to dilute the effects of fescue toxicosis. However, it is unclear as to whether the shaded environment of silvopastures will affect alkaloid production within the plant or whether the sward botanical composition would shift to be dominated by species more shade tolerant than tall fescue. Recognizing and understanding all of these relationships is important for making sound management decisions for successful silvopasture production systems. Therefore, the objective of this study is to evaluate the impact of increasing shade on productivity, nutritive characteristics, and ergot alkaloid concentration of forage mixtures of varying species complexity.

Literature Cited

- Adams, J. 2010. Species richness: patterns in the diversity of life. Springer Science & Business Media, Chichester, UK.
- Adcock, R.A., N.S. Hill, H.R. Boerma, and G.O. Ware. 1997. Sample variation and resource allocation for ergot alkaloid characterization in endophyte-infected tall fescue. *Crop Sci.* 37:31-35.
- Agee, C.S. and N.S. Hill. 1994. Ergovaline variability in *Acremonium*-infected tall fescue due to environment and plant genotype. *Crop Sci.* 34:221-226.
- Allard, G., C.J. Nelson, and S.G. Pallardy. 1991. Shade effects on growth of tall fescue: I. leaf anatomy and dry matter partitioning. *Crop Sci.* 31:163-167.
- Arechavaleta, M., C.W. Bacon, C.S. Hoveland, and D.E. Radcliffe. 1989. Effect of the tall fescue endophyte on plant response to environmental stress. *Agron. J.* 81:83-90.
- Arechavaleta, M., C.W. Bacon, R.D. Plattner, C.S. Hoveland, and D.E. Radcliffe. 1992. Accumulation of ergopeptide alkaloids in symbiotic tall fescue grown under deficits of soil water and nitrogen fertilizer. *Appl. Environ. Microbiol.* 58:857-861.
- Azevedo, M.D., R.E. Welty, A.M. Creag, and J. Bartlett. 1993. Ergovaline distribution, total nitrogen and phosphorus content of two endophyte-infected tall fescue clones. p. 59-62. *In*: D.E. Hume, G.C.M. Latch, and H.S. Easton (eds.) Proc. 2nd Int. Symp. on

- Acremonium*/Grass Interactions, Palmerston North, New Zealand. 4-6 February, 1992. AgResearch, Grassland Research Centre, Palmerston North.
- Bacon, C.W. and J.F. White, Jr. 2000. Physiological adaptations in the evolution of endophytism in the Clavicipitaceae. p. 237-261. *In*: C. W. Bacon and J.F. White (eds.) Microbial endophytes. CRC Press, New York.
- Bacon, C.W., J.K. Porter, J.D. Robbins, and E.S. Luttrell. 1977. *Epichloë typhina* from toxic tall fescue grasses. *Appl. Environ. Microb.* 34:576–581.
- Baker, J.C. 2009. Soils of Virginia. p. 69-74. *In*: Agronomy Handbook, Publication 424-100. Virginia Cooperative Extension, Blacksburg, VA.
- Ball, D.M., C.S. Hoveland, and G.D. Lacefield. 2007. Southern forages. 4th ed. International Plant Nutrition Institute, Norcross, GA.
- Ball, O.J.P., R.A. Prestidge, and J.M. Sprosen. 1995. Interrelationships between *Acremonium lolii*, peramine, and lolitrem B in perennial ryegrass. *Appl. Environ. Microbiol.* 61:1527-1533.
- Bambo, S.K., A.R. Blount, J. Nowak, A.J. Long, R.O. Myer, and D.A. Huber. 2009. Annual cool-season forage nutritive value and production after overseeding into bahiagrass sod in silvopastoral systems. *Agroecol. Sustain. Food Sys.* (previously *J. Sustain. Agric.*) 33:917-934.
- Barro, R.S., J.C. de Saibro, R.B. de Medeiros, J.L.S. da Silva, A.C. Varella. 2008. Forage yield and nutritive value of cool-season annual forage grasses shaded by *Pinus elliottii* trees and at full-sun. *Revista Brasileira de Zootecnia (Brazilian J. Anim. Sci.)* 37:1721-1727.
- Bazely, D.R., M. Vicari, S. Emmerich, L. Filip, D. Lin, and A. Inman. 1997. Interactions between Herbivores and Endophyte-Infected *Festuca rubra* from the Scottish Islands of St. Kilda, Benbecula and Rum. *J. Appl. Ecol.* 34:847-860.
- Belesky, D.P. 2005. Growth of *Dactylis glomerata* along a light gradient in the central Appalachian region of the eastern USA: I. dry matter production and partitioning. *Agrofor. Sys.* 65:81-90.
- Belesky, D.P. and N.S. Hill. 1997. Defoliation and leaf age influence on ergot alkaloids in tall fescue. *Annals of Bot.* 79:259-264.
- Belesky, D.P., J.M. Ruckle, L.P. Bush. 2009. Microsite conditions influence nutritive value characteristics of a tall fescue cultivar devoid of, or infected with a native, or a novel non-ergogenic endophyte. *Environ. Exp. Bot.* 67:284-292.

- Belesky, D.P., N.J. Chatterton, and J.P.S. Neel. 2006. *Dactylis glomerata* growing along a light gradient in the Central Appalachian Region of the Eastern USA: III. Nonstructural carbohydrates and nutritive value. *Agrofor. Sys.* 67(1):55-51.
- Belsky, A.J., S.M. Mwangi, and J.M. Duxbury. 1993. Effects of widely spaced trees and livestock grazing on understory environments in tropical savannas. *Agrofor. Sys.* 24:1-20.
- Bird, P.R., J.D. Kellas, T.T. Jackson, and G.A. Kearney. 2010. *Pinus radiata* and sheep production in silvopastoral systems at Carngham, Victoria, Australia. *Agrofor. Sys.* 78:203-216.
- Blazier, M.A., L.A. Gaston, T.R. Clason, K.W. Farrish, B.P. Oswald, and H.A. Evans. 2008. Nutrient dynamics and tree growth of silvopasture systems: impact of poultry litter. *J. Environ. Qual.* 37:1546-1558.
- Bondurant, J.A., M.A. McCann, J.S. McCann, J.H. Bouton, C.S. Hoveland, R.H. Watson, and J.G. Andrae. 2001. Steer grazing behavior on endophyte-free, toxic endophyte-infected, and non-toxic endophyte-infected (MaxQ) tall fescue. *J. Anim. Sci.* 79(Suppl. 1):457.
- Bonin, C.L. and B.F. Tracy. 2012. Diversity influences forage yield and stability in perennial prairie plant mixtures. *Agric. Ecosyst. Environ.* 162:1-7.
- Bouton, J.H., G.C.M. Latch, N.S. Hill, C.S. Hoveland, M.A. McCann, R.H. Watson, J.A. Parish, L.L. Hawkins, and F.N. Thompson. 2002. Reinfection of tall fescue cultivars with non-ergot alkaloid-producing endophytes. *Agron. J.* 94:567-574.
- Brosi, G.B., R.L. McCulley, L.P. Bush, J.A. Nelson, A.T. Classen and R.J. Norby. 2011. Effects of multiple climate change factors on the tall fescue-fungal endophyte symbiosis: infection frequency and tissue chemistry. *New Phytol.* 189:797-805.
- Buckner, R.C., J.B. Powell, and R.V. Frakes. 1979. Historical development. p. 1-8. *In*: R.C. Buckner and L.P. Bush (eds.), *Tall Fescue*. *Agron. Monogr.* 20. ASA, CSSA, SSSA, Madison, WI.
- Buffington, D.E., R.J. Collier, and G.H. Canton. 1983. Shade management systems to reduce heat stress for dairy cows in hot, humid climates. *Trans. ASAE* 26:1798-1802.
- Buergler A. L., J. H. Fike, J. A. Burger, C. M. Feldhade, J. R. McKenna, and C. D. Teutsch. 2005. Botanical composition and forage production in an emulated silvopasture. *Agron. J.* 97:1141-1147.
- Buergler A. L., J. H. Fike, J. A. Burger, C. M. Feldhade, J. R. McKenna, and C. D. Teutsch. 2006. Forage nutritive value in an emulated silvopasture. *Agron. J.* 98:1265-1273.
- Burner, D.M. 2003. Influence of alley crop environment on orchardgrass and tall fescue herbage. *Agron. J.* 95:1163-1171.

- Burner, D.M. and D.K. Brauer. 2003. Herbage response to spacing of loblolly pine trees in a minimal management silvopasture in southeastern USA. *Agrofor. Sys.* 57:69-77.
- Burns, J.C., L. Goode, H.D. Gross, and A.C. Linnerud. 1973. Cow and calf gains on ladino clover-tall fescue and tall fescue, grazed alone and with coastal bermudagrass. *Agron. J.* 65:877-880.
- Burns, J.C. and J.E. Standaert. 1985. Productivity and economics of legume-based vs. nitrogen fertilized grass-based pasture in the United States. *In: R.F. Barnes (ed.) Forage legumes for energy-efficient animal production: Proc. Trilateral Workshop, Palmerton North, New Zealand. 30 April-4 May. USDA-ARS, Washington, DC.*
- Bush, L. and R.C. Buckner. 1973. Tall fescue toxicity. p. 99-112. *In: A.G. Matches (ed.) Antiquality components of forages. CSSA Spec. Publ. 4. Madison, WI.*
- Chen, C.P. 1990. Cattle productivity under oil palm in Malaysia. *In: Proc. ACIAR Workshop on Forages for Plantation Crops, 27-29 June, 1990. Udayana University, Bali, Indonesia.*
- Clason, T.R. and S.H. Sharrow. 2000. Silvopastoral practices. p. 119-147. *In: H.E. Garret, W.J. Rietveld, and R.F. Fisher (eds.) North American agroforestry: an integrated science and practice. ASA-CSSA-SSSA. Madison, WI.*
- Corre, W.J. 1983. Growth and morphogenesis of sun and shade plants I. the influence of light intensity. *Acta Botanica Neerlandica* 32(1/2):49-62.
- Dahlan, I., Y. Yamada, and M.D. Mahyuddin. 1993. Botanical composition and models of metabolizable energy availability from undergrowth in oil palm plantations for ruminant production. *Agrofor. Sys.* 24:233-246.
- Davitt, A.J., M. Stansberry, and J.A. Rudgers. 2010. Do the costs and benefits of fungal endophyte symbiosis vary with light availability? *New Phytol.* 188:824-834.
- Della-Bianca, L. and C.G. Wells. 1967. Some chemical properties of forest soils in the Virginia-Carolina piedmont. U.S. Forest Service Research Paper SE-28.
- Dierking, R.M., R.L. Kallenbach, and I.U. Grun. 2010. Effect of forages species on fatty acid content and performance of pasture-finished steers. *Meat Sci.* 85:597-605.
- Douglas G.B., A.S. Walcroft, B.J. Wills, S.E. Hurst, A.G. Foote, K.D. Trainor, and L.E. Fung. 2001. Resident pasture growth and the micro-environment beneath young, widespaced poplars in New Zealand. *In: Proc. N. Z. Grassl. Assoc.* 63:131-138.

- Ehret, M., R. Graß, and M. Wachendorf. 2015. The effect of shade and shade material on white clover/perennial ryegrass mixtures for temperate agroforestry. *Agrofor. Sys.* 89:557-570.
- Facelli, J.M. and S.T.A. Pickett. 1991. Plant litter: its dynamics and effects on plant community structure. *Bot. Rev.* 57(1):1-32.
- Fannon-Osborne, A.F. 2012. Hair sheep production in temperate, deciduous Appalachian silvopastures. M.S. thesis. Virginia Polytechnic Institute and State University, Blacksburg.
- Feldhake, C.M. 2002. Forage frost protection potential of conifer silvopastures. *Agric. Meteorol.* 112:123-190.
- Feldhake, C.M., J.P.S. Neel, and D.P. Belesky. 2010. Establishment and production from thinned mature deciduous-forest silvopastures in Appalachia. *Agrofor. Sys.* 79:31-37.
- Fike, J.H., A.L. Buergler, J.A. Burger, and R.L. Kallenbach. 2004. Considerations for establishing and managing silvopastures. Online. *Forage and Grazinglands*. doi:10.1094/FG-2004-1209-01-RV.
- Frost, W.E. and N.K. McDougald. 1989. Tree canopy effects on herbaceous production of annual rangeland during drought. *J. Range Manage.* 42:281-283.
- Furiness, C., E. Cowling, L. Allen, R. Abt, D. Frederick, K. Zering, R. Campbell. 2011. Using animal manures in forest fertilization. North Carolina Cooperative Extension.
- Ghannoum, O., J.R. Evans, and S. von Caemmerer. 2011. Nitrogen and water use efficiency of C₄ plants. p. 129-146. *In*: A.S. Raghavendra and R.F. Sage (eds.) C₄ photosynthesis and related CO₂ concentrating mechanisms. Springer, Dordrecht, Netherlands.
- Gold, M. and A. Mason. 2015. Defining Agroforestry. p. 9-15. *In*: Training manual for applied agroforestry practices, 2015 edition. University of Missouri Center for Agroforestry, Columbia, MO.
- Guevara-Escobar, A., P.D. Kemp, A.D. Mackay, and J. Hodgson. 2007. Pasture production and composition under poplar in a hill environment in New Zealand. *Agrofor. Sys.* 69:199-213.
- Harris, R.R., E.M. Evans, J.K. Boseck, and W.B. Webster. 1972. Fescue, orchardgrass, and coastal bermudagrass grazing for yearling beef steers. *Alabama Agric. Exp. Stn. Bull.* 432.
- Hector, A., B. Schmid, C. Beierkuhnlein, M.C. Caldeira, M. Diemer, P.G. Dimitrakopoulos, J.A. Finn, H. Freitas, P.S. Giller, J. Good, R. Harris, P. Hogberg, K. Huss-Danell, J. Joshi, A. Jumpponen, C. Korner, P.W. Leadley, M. Loreau, A. Minns, C.P.H. Mulder, G. O'Donovan,

- S.J. Otway, J.S. Pereira, A. Prinz, D.J. Read, M. Scherer-Lorenzen, E.-D. Schulze, A.-S.D. Siamantziouras, E.M. Spehn, A.C. Terry, A.Y. Troumbis, F.I. Woodward, S. Yachi, and J.H. Lawton. 1999. Plant diversity and productivity experiments in European grasslands. *Science* 286:1123-1127.
- Hiatt III, E.E. and N.S. Hill. 1997. *Neotyphodium coenophialum* mycelia protein and herbage mass effects on ergot alkaloid concentration in tall fescue. p. 167-169. *In*: C.W. Bacon and N.S. Hill (eds.) *Neotyphodium/grass interactions: Proc. 3rd Int. Symp. Acremonium/Grass Interactions*, 28-31 May, 1997, Athens, GA.
- Higgins et al. 2011. Shade options for grazing cattle. University of Kentucky Cooperative Extension Bull. AEN-99. Lexington, KY.
- Hight, G.K., D.P. Sinclair, and R.J. Lancaster. 1968. Some effects of shading and of nitrogen fertilizer on the chemical composition of freeze-dried and oven-dried herbage, and on the nutritive value of oven-dried herbage fed to sheep. *N. Z. J. Agric. Res.* 11:286-302.
- Hodge, A., D. Robinson, and A. Fitter. 2000. Are microorganisms more effective than plants at competing for nitrogen? *Trends Plant Sci.* 5:304-308.
- Holochek, J.L., M. Vavra, and J. Skovlin. 1981. Diet quality and performance of cattle on forest and grassland range. *J. Anim. Sci.* 53:291-298.
- Hooper, D.U., F.S. Chapin, III, J.J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J.H. Lawton, D.M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A.J. Symstad, J. Vandermeer, and D.A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol. Monogr.* 75(1):3-35.
- Hoveland, C.S. 2009. Origin and history. p. 3-10. *In*: H.A. Fribourg, D.B. Hannaway, and C.P. West (eds.) *Tall fescue for the twenty-first century*. Agron. Monogr. No. 53. ASA, CSSA, and SSSA. Madison, WI.
- Hoveland, C.S., R.R. Harris, E.E. Thomas, E.M. Clark, J.A. McGuire, J.T. Eason, and M.E. Ruf. 1981. Tall fescue with ladino clover or birdsfoot trefoil as pasture for steers in northern Alabama. *Alabama Agric. Exp. Stn. Bull.* 530.
- Isselstein, J., B. Jeangros, and V. Pavlu. 2005. Agronomic aspects of biodiversity targeted management of temperate grasslands in Europe-a review. *Agron. Res.* 3(2):139-151.
- Kallenbach, R.L., G.J. Bishop-Hurley, M.D. Massie, G.E. Rottinhaus, and C.P. West. 2003. Herbage mass, nutritive value, and ergovaline concentration of stockpiled tall fescue. *Crop Sci.* 43:1001-1005.

- Kallenbach, R.L., M.S. Kerley, and G.J. Bishop-Hurley. 2006. Cumulative forage production, forage quality and livestock performance from an annual ryegrass and cereal rye mixture in a pine-walnut silvopasture. *Agrofor. Sys.* 66:43-53.
- Karki, U. and M.S. Goodman. 2010. Landscape use by cattle in silvopasture versus open pasture. *Agrofor. Sys.* 78:159–168.
- Karki, U. and M.S. Goodman. 2013. Microclimate differences between young longleaf-pine silvopasture and open-pasture. *Agrofor. Sys.* 87:303-310.
- Karki, U. and M.S. Goodman. 2015. Microclimatic differences between mature loblolly-pine silvopasture and open-pasture. *Agrofor. Sys.* 89:319-325.
- Karki, U., M.S. Goodman, and S.E. Sladden. 2009. Nitrogen source influences on forage and soil in young southern-pine silvopasture. *Agric. Ecosys. Environ.* 131:70-76.
- Kephart, K.D. and D.R. Buxton. 1993. Forage quality responses of C₃ and C₄ perennial grasses to shade. *Crop Sci.* 33:831-837
- Kephart, K.D., D.R. Buxton, and S.E. Taylor. 1992. Growth of C₃ and C₄ perennial grasses under reduced irradiance. *Crop Sci.* 32:1033-1038.
- Kirchman, D.L. 2012. *Processes in microbial ecology.* Oxford University Press, New York.
- Kirwan, L., A. Luscher, M.T. Sebastao, J.A. Finn, R.P. Collins, C. Porqueddu, A. Helgadottir, O.H. Baadshaug, C. Brophy, C. Coran, S. Dalmanndottir, I. Delgado, A. Elgersma, M. Fothergill, B.E. Frankow-Lindberg, P. Golinski, P. Grieu, M. Gustavsson, M. Hoglind, O. Huguenin-Elie, C. Iliadis, M. Jorgensen, Z. Kadzieuliene, T. Karyotis, T. Lunnan, M. Malengier, S. Maltoni, V. Meyer, D. Nyfeler, P. Nykanen-Kurki, J. Parente, H.J. Smit, U. Thumm, and J. Connolly. 2007. Evenness drives consistent differences in intensive grassland systems across 28 European sites. *J. Ecol.* 95:530-539.
- Lane, G.A., B.A. Tapper, E. Davies, D.E. Hume, G.C.M. Latch, D.J. Barker, H.S. Easton, and M.P. Rolston. 1997. Effect of growth conditions on alkaloid concentrations in perennial ryegrass naturally infected with endophyte. p. 179-182. *In:* C.W. Bacon and N.S. Hill (eds.) *Neotyphodium/grass interactions: Proc. 3rd Int. Symp. Acremonium/Grass Interactions*, 28-31 May, 1997, Athens, GA.
- Lane, G.A., M.J. Christensen, and C.O. Miles. 2000. Coevolution of fungal endophyte with grasses: the significance of secondary metabolites. p. 341-388. *In:* C.W. Bacon and J. White (eds.) *Microbial endophytes.* Marcel Dekker, New York, NY.
- Latch, G.C.M. 1993. Physiological interactions of endophytic fungi and their hosts. Biotic stress tolerance imparted to grasses by endophytes. *Agric. Ecosyst. Environ.* 44:143-156.

- Levai, L. and S. Veres. 2013. Applied plant physiology. University of Debrecen, Service Sciences Methodology Centre, Debrecen, Hungary.
- Lin, C.H., R.L. McGraw, M.F. George, and H.E. Garrett. 1999. Shade effects on forage crops with potential in temperate agroforestry practices. *Agrofor. Sys.* 44:109-119.
- Lourenco, M., G. Van Ranst, S. De Smet, K. Raes, and V. Fievez. 2007. Effect of grazing pastures with different botanical composition by lambs on rumen fatty acid metabolism and fatty acid pattern of *longissimus* muscle and subcutaneous fat. *The Animal Consortium* 1:537-545.
- Lyons, P.C., R.D. Plattner, and C.W. Bacon. 1986. Occurrence of peptide and clavinet ergot alkaloids in tall fescue grass. *Science* 232:487-489.
- MacAdam, J.W. and C.J. Nelson. 2003. Physiology of forage plants. p. 73-79. *In*: R.F. Barnes, C.J. Nelson, M. Collins, and K.J. Moore (eds.) *Forages: the science of grassland agriculture*, 4th ed. Iowa State Press, Ames, IA.
- Mack, K.M.L. and J.A. Rudgers. 2008. Balancing multiple mutualists: asymmetric interactions among plants, arbuscular mycorrhizal fungi, and fungal endophytes. *Oikos* 117(2):310-320.
- Malinowski, D.P., D.P. Belesky, N.S. Hill, V.C. Baligar, and J.M. Fedders. 1998. Influence of phosphorus on the growth and ergot alkaloid content of *Neotyphodium coenophialum*-infected tall fescue (*Festuca arundinacea* Schreb.). *Plant and Soil* 198:53-61.
- Mapfumo, E., M.A. Naeth, V.S. Baron, A.C. Dick, and D.S. Chanasyk. 2002. Grazing impacts on litter and roots: perennial versus annual grasses. *J. Range Manage.* 55:16-22.
- Mason, A., D. Wallace, and R. Straight. 2014. An overview of agroforestry. *Agroforestry Notes* (1). USDA National Agroforestry Center, Lincoln, NE.
- McCulley, R.L., L.P. Bush, A.E. Carlisle, H. Ji, and J.A. Nelson. 2014. Warming reduces tall fescue abundance but stimulates toxic alkaloid concentrations in transition zone pastures of the U.S. *Front. Chem.* 2:1-14.
- McMurphy, W.E., K.S. Lusby, S.C. Smith, S.H. Muntz, and C.A. Strasia. 1990. Steer performance on tall fescue pastures. *J. Prod. Agric.* 3:100-102.
- McNally, S.R., D.C. Laughlin, S. Rutledge, M.B. Dodd, J. Six, and L.A. Schipper. 2015. Root carbon inputs under moderately diverse sward and conventional ryegrass-clover pasture: implications for soil carbon sequestration. *Plant and Soil* 392:289-299.

- Metz, L.J., C.G. Wells, and P.P. Kormanik. 1970. Comparing the forest floor and surface soil beneath four pine species in the Virginia piedmont. USDA Forest Service Res. Paper SE-55.
- Minns, A., J. Finn, A. Hector, M. Caldeira, J. Joshi, C. Palmborg, B. Schmid, M. Scherer-Lorenzen, E. Spehn, A. Troumbis and the BIODDEPTH project. 2001. The functioning of European grassland ecosystems: potential benefits of biodiversity to agriculture. *Outlook Agric.* 30(3):179-185.
- Monk, C.D., and F.C. Gabrielson, Jr. 1985. Effects of shade, litter and root competition on old-field vegetation in South Carolina. *Bull. Torrey Bot. Club* 112:383-392.
- Mosquera-Losada, M.R. and A. Rigueiro-Rodriguez. 2013. Silvopasture: a combination of grasslands and trees to green livestock production. p. 475-477. *In: A. Helgadottir and A. Hopkins (eds.) The role of grasslands in a green future, threats and perspectives in less favoured areas. Proc. 17th Symp. Eur. Grassl. Fed., Akureyri, Iceland. 23-26 June 2013.*
- Mosquera-Losada, M.R., S. Rodriguez-Barreira, M.L. Lopez-Diaz, E. Fernandez-Nunez, A. Rigueiro-Rodriguez. 2009. Biodiversity and silvopastoral system use change in very acid soils. *Agric. Ecosyst. Environ.* 131:315-324.
- Nair, P.K.R. 1993. An introduction to agroforestry. Kluwer Academic Publishers, Dordrecht, Netherlands.
- National Research Council. 1996. Nutrient requirements of beef cattle: update 2000: 7th revised edition. The National Academies Press, Washington, D.C.
- Neel, J.P.S. and D.P. Belesky. 2015. Herbage production, nutritive value, and animal productivity within hardwood silvopasture, open and mixed pasture systems in Appalachia, United States. *Grass and Forage Sci.* Online. doi: 10.1111/gfs.12211.
- Neel, J.P.S., C.M. Feldhake, and D.P. Belesky. 2003. Forage nutritive value and performance of lambs in a silvo-pastoral system. p. 303-307. *In: K.A. Cassida, D. Belesky, J. Berdahl, J. Jennings, D. Kee, T. Terril, M. Wiedenhoef, and M. Williams (eds.) Vol. 12 Proc. Am. Forage Grassl. Council., Lafayette, Louisiana,*
- Neel, J.P.S., C.M. Feldhake, and D.P. Belesky. 2008. Influence of solar radiation on the productivity and nutritive value of herbage of cool-season species of an understory sward in a mature conifer woodland. *Grass and Forage Sci.* 63:38-47.
- Norris, M.G., T.J. Frost-Smith, and J.I.M. Sutherland. 2007. Increasing endophyte alkaloid expression in tall fescue by selecting for increased endophyte hyphal density. *Proc. N. Z. Grassl. Assoc.* 69:197-200.

- Obispo, N.E., Y. Espinoza, J.L. Gil, F. Ovalles, E. Cabrera, and M.J. Perez. 2013. Relationship of shade proportion in the pasture with yield, quality of forage and daily weight gain in steers. *Revista Científica, Facultad de Ciencias Veterinarias, Univ. Zulia (Scientific Journal, Faculty of Vet. Sci., Univ. Zulia)* 23:531-536.
- Peeters, A. 2004. *Wild and sown grasses*. Blackwell Publishing. Rome, Italy.
- Peri, P.L., A.C. Varella, R.J. Lucas, and D.J. Moot. 2001. Cocksfoot and Lucerne productivity in a *Pinus radiata* silvopastoral system: a grazed comparison. p. 139-147. *In: Proc. N. Z. Grassl. Assoc., Waikato, New Zealand*. 30 Oct.-1 Nov. 2001.
- Perry, M.E.L., W.H. Schacht, G.A. Ruark, and J.R. Brandle. 2009. Tree canopy effect on grass and grass/legume mixtures in eastern Nebraska. *Agrofor. Sys.* 77:23-35.
- Peters, S.M. 2000. *Agroforestry-an integration of land use practices*. University of Missouri Center for Agroforestry, Columbia, MO.
- Peterson, P.R., and D.E. Brann. 2009. Forage crops. p. 69-74. *Agronomy Handbook*, Virginia Cooperative Extension, Blacksburg, VA.
- Petriz, D.C., V.L. Lechtenberg, and W.H. Smith. 1980. Performance and economic returns of beef cows and calves grazing grass-legume herbage. *Agron. J.* 72:581-584.
- Phillips, C.J.C., and N.L. James. 1996. The effects of including white clover in perennial ryegrass swards and the height of mixed swards on the milk production, sward selection and ingestive behavior of dairy cows. *J. Anim. Sci.* 67:195. Abstract.
- Pierson, E.A., R.N. Mack, and A. Black. 1990. The effect of shading on photosynthesis, growth, and regrowth following defoliation for *Bromus tectorum*. *Oecologia* 84:534-543.
- Poorter, H., K.J. Niklas, P.B. Reich, J. Oleksyn, P. Poot, and L. Mommer. 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytol.* 193:30-50.
- Porter, J.K. 1995. Analysis of endophyte toxins: fescue and other grasses toxic to livestock. *J. Anim. Sci.* 73:871-880.
- Rasmussen, S., A.J. Parsons, Q. Liu, H. Xue, and J.A. Newman. 2007a. High nutrient supply and carbohydrate content reduce endophyte and alkaloid concentration. *N. Z. Grassl. Assoc.: Endophyte Symp.* 69:135-138.
- Rasmussen, S., A.J. Parsons, S. Bassett, M.J. Christenson, D.E. Hume, L.J. Johnson, R.D. Johnson, W.R. Simpson, C. Stacke, C.R. Voisey, H. Xue, and J.A. Newman. 2007b. High nitrogen

- supply and carbohydrate content reduce fungal endophyte and alkaloid concentration in *Lolium perenne*. *New Phytol.* 173:787-797.
- Read, J.C. and B.J. Camp. 1986. The effect of the fungal endophyte *Acremonium coenophialum* in tall fescue on animal performance, toxicity, and stand maintenance. *Agron. J.* 78:848-850.
- Roberts, C. and J. Andrae. 2010. Fescue toxicosis and management. ASA, CSSA, and SSSA, Madison, WI. doi: 10.2135/2010.fescuetoxicosis.
- Roberts, C.A., G.D. Lacefield, D. Ball, and G. Bates. 2009. Management to optimize grazing performance in the northern hemisphere. p. 85-100. *In*: H.A. Fribourg, D.B. Hannaway, and C.P. West (eds.) Tall fescue for the twenty-first century. *Agron. Monogr. No.* 53. ASA, CSSA, and SSSA, Madison, WI.
- Rottinghaus, G.E., G.B. Garner, C.N. Cornell, and J.L. Ellis. 1991. HPLC method for quantitating ergovaline in endophyte-infested tall fescue: seasonal variation of ergovaline levels in stems with leaf sheaths, leaf blades, and seed heads. *J. Agric. Food Chem.* 39:112-115.
- Skinner, R.H., M.A. Sanderson, B.F. Tracy, and C.J. Dell. 2006. Above- and belowground productivity and soil carbon dynamics of pasture mixtures. *Agron. J.* 98:320-326.
- Smith, R.M. 1942. Some effects of black locusts and black walnuts on southeastern Ohio pastures. *Soil Sci.* 53:385-389.
- Soder, K.J., M.A. Sanderson, J.L. Stack, and L.D. Muller. 2006. Intake and performance of lactating cows grazing diverse forage mixtures. *J. Dairy Sci.* 89:2158-2167.
- Sollenberger, L.E., and E.S. Vanzant. 2011. Interrelationships among forage nutritive value and quantity and individual animal performance. *Crop Sci.* 51:420-432.
- Spiering, M.J., G.A. Lane, M.J. Christensen, and J. Schmid. 2005. Distribution of the fungal endophyte *Neotyphodium lolii* is not a major determinant of the distribution of fungal alkaloids in *Lolium perenne* plants. *Phytochemistry* 66:195-202.
- Stamm, M.M., T. DelCurto, M.R. Horney, S.D. Brandyberry, and R.K. Barton. 1994. Influence of alkaloid concentration of tall fescue straw on the nutrition, physiology, and subsequent performance of beef steers. *J. Anim. Sci.* 72:1068-1075.
- Stier, J.C. and D.S. Gardner. 2008. Shade stress and management. p. 447-471. *In*: M. Pessarakli (ed.) *Handbook of turfgrass management and physiology*. CRC Press, Boca Raton, FL.
- Stuedemann, J.A. and C.S. Hoveland. 1988. Fescue endophyte: history and impact on animal agriculture. *J. Prod. Agric.* 1(1):39-44.

- Stuedemann, J.A. and D. H. Seman. 2005. Integrating genetics, environment, and management to minimize animal toxicoses. p. 305-326. *In*: C.A. Roberts, C.P. West, and D.E. Spiers (eds.) *Neotyphodium* in cool-season grasses. Blackwell Publishing, Oxford, UK.
- Thompson, F.N. and J.A. Stuedemann. 1993. Pathophysiology of fescue toxicosis. *Agric. Ecosyst. Environ.* 44:263-281.
- Tor-Agbidye, J., L.L. Blythe, and A.M. Craig. 2001. Correlation of endophyte toxins (ergovaline and lolitrem B) with clinical disease: fescue foot and perennial ryegrass staggers. *Vet. Hum. Toxicol.* 43:140-146.
- Totty, V.K., S.L. Greenwood, R.H. Bryant, and G.R. Edwards. 2013. Nitrogen partitioning and milk production of dairy cows grazing simple and diverse pastures. *J. Dairy Sci.* 96:141-149.
- Tracy, B.F. and D.B. Faulkner. 2006. Pasture and cattle responses in rotationally stocked grazing systems sown with differing levels of species richness. *Crop Sci.* 46:2062-2068.
- Tracy, B.F. and M.A. Sanderson. 2004. Productivity and stability relationships in mowed pasture communities of varying species composition. *Crop Sci.* 44:2180-2186.
- USDA NRCS Plant Materials Program. 2006. Tall fescue. Plant fact sheet. USDA NRCS. Accessed 27 October 2016. https://plants.usda.gov/factsheet/pdf/fs_loar10.pdf.
- Vandermeer, J. 1981. The interference production principle: an ecological theory for agriculture. *BioScience* 31(5):361-364.
- Vazquez de Aldana, B.R., I. Zabalgoceazcoa, A. Garcia Ciudad, and B. Garcia Criado. 2003. Ergovaline occurrence in grasses infected by fungal endophytes of semi-arid pastures in Spain. *J. Sci. Food Agric.* 83:347-353.
- Vinall, H.N. 1909. Meadow fescue; its culture and uses. USDA Farmers Bull. 361. Government Printing Office, Washington.
- Walter, D. 2015. Silvopasture. p. 50-66. *In*: Training manual for applied agroforestry practices, 2015 edition. University of Missouri Center for Agroforestry, Columbia, MO.
- West, C.P. and J.C. Waller. 2007. Forage systems for humid transition areas. p. 313-321. *In*: Forages. Vol. 2, 6th ed. Iowa State Press, Ames.
- Wheedon, J.T., W.K. Cornwell, J.H.C. Cornelissen, A.E. Zanne, C. Wirth, and D.A. Coomes. 2009. Global meta-analysis of wood decomposition rates: a role for trait variation among tree species? *Ecol. Letters* 12:45-56.

- Wilson, J.R., K. Hill, D.M. Cameron, and H.M. Shelton. 1990. The growth of *Paspalum notatum* under the shade of a *Eucalyptus grandis* plantation canopy or in full sun. Trop. Grassl. 24:24-28.
- Wong, C.C., H. Rahim, and M.A. Mohd. Sharudin. 1985. Shade tolerance potential of some tropical forages for integration with plantations: I. grasses. MARDI Res. Bull. 13(3):225-247.
- Yang, Y. and Y. Luo. 2010. Carbon:nitrogen stoichiometry in forest ecosystems during stand development. Global Ecol. Biogeography 20:354-361.

Chapter 3. Materials and Methods

Site Description

This research was conducted at Virginia Tech's Southern Piedmont Agricultural Research and Extension Center (SPAREC) near Blackstone, VA (37.091889, -77.963632). Soil series at this site are predominantly Seneca sandy loam (Fine-loamy, mixed, thermic Aquic Hapludults) with a small proportion of Durham coarse sandy loam (Fine-loamy, siliceous, semiactive, thermic Typic Hapludults) and are considered prime farmland with capability units of IIe-4 (Soil Survey Staff, 2016). Weather data were collected onsite at SPAREC using a WatchDog 2900ET weather station (Spectrum Technologies, Aurora, IL). Average yearly temperature for SPAREC is 14.5 °C with 112 cm of relatively evenly distributed rainfall per year. Average temperature and rainfall for the duration of the study can be found in Fig. 1.

Experimental Design and Layout

The experimental design was a randomized complete block design with four replications and a factorial treatment arrangement. Treatments included varying levels of shade that could be found in silvopastures (30, 50 and 70% shade) compared to a full sun control and forage species mixture complexity (simple, intermediate and complex). Field position was used as a blocking factor. Plots were 2.74 m x 3.05 m. Alleys between blocks were 3.05 m and distance between plots within blocks was 1.83 m (Fig. 2).

Shade structures were built to create artificial shade over individual plots. Structures were modified from Varella et al. (2011) who concluded that slatted shade structures more closely represented the dappled shade of silvopastures as compared to the uniform light

distribution of shade cloth. A portable elevated frame of 2.44 x 3.66 x 0.91 m was constructed using 2"x4" dimensional lumber. Structures were deliberately built to be larger than the plots. This ensured that plots would be covered with the desired amount of shade throughout the day, even when the sun was not directly overhead.

Snow fence was secured to the tops and sides of the structures to create 50% shade. Thirty percent knitted shade cloth (Agriculture Solutions, Strong, ME) was added to the 50% shade structures to create the 70% shade environment. Even though the dappled effect was reduced by using shade cloth, extra slats were not added to structures in order to maintain structural integrity. Half of the slats in the snow fence were removed to create 30% shade (Fig. 3). Shade levels were validated by simultaneously recording PAR beneath structures using a line quantum sensor (LI-COR, Lincoln, NE) and in adjacent open areas using a PAR sensor (Onset, Bourne, MA). Shade levels were calculated as follows: $100 - (\text{PAR under structure} / \text{PAR in open sun})$.

Forage mixtures, cultivars and seeding rates are described in Table 1. Mixture species were as follows: SIMPLE = tall fescue (*Schedonorus arundinaceus*) and white clover (*Trifolium repens*); INTERMEDIATE = SIMPLE + orchardgrass (*Dactylis glomerata*) and red clover (*T. pretense*); and COMPLEX = INTERMEDIATE + Kentucky bluegrass (*Poa perenne*), alfalfa (*Medicago sativa*), and birdsfoot trefoil (*Lotus corniculatus*). Species included in the mixtures were chosen because they are adapted to the North-South transition zone. All of these species utilize the C₃ photosynthetic pathway which has been shown to be more shade tolerant than C₄ pathway (Kephart et al., 1992). Some of these species have been shown to exhibit shade

tolerance when grown in monoculture (Lin et al., 1999), but they have not been evaluated as part of a mixture, and may express different competitiveness under low light conditions.

Plot Management

A conventional seedbed was prepared by moldboard plowing, disking, field cultivating, and cultipacking. Soil samples were collected to a depth of 10 cm, and initial soil test levels were 14 ppm extractable P and 69 ppm extractable K. Samples were analyzed at Virginia Tech's Soil Testing Laboratory using the Mehlich I procedure (Maguire and Heckendorn, 2009). Prior to seeding, 34, 134, and 157 kg/ha of N, P₂O₅, and K₂O, respectively, was incorporated into the seedbed based on soil test recommendations (Maguire and Heckendorn, 2015). After the initial fertilization, no other amendments were added to the plots.

In April 2015, plots were seeded using a small plot walk-behind cultipack-type seeder (Carter Manufacturing, Brookston, IN). Shade structures were put in place immediately after planting to simulate the shade of forests. Structures were removed the morning of harvest and placed back on plots within 24 h of harvest.

Forages were harvested when they reached a height of approximately 35 cm and were allowed to rest for at least four weeks between cuttings. Harvest occurred only twice in 2015 (15 Jun and 6 Aug) in order to let the forages establish healthy root systems. Harvest occurred five times in 2016 (26 Apr, 26 May, 27 Jun, 12 Aug and 3 Nov).

Measurements

Note: Not all measures were collected at each harvest. Measurement frequency and timing are shown in Table 2.

Plot height. Plot heights were measured using a standard measuring stick. Heights were determined using the average height of standing planted vegetation. Measurements were recorded at five points in each plot, one in the approximate center and one each approximately 0.5 m from the inside of each corner. The average of the five measurements is reported for each plot.

Yield and dry matter. A small plot harvester (Winterstieger, Salt Lake City, UT) was used to harvest a 1.5-m x 3-m (4.5 m²) swath through the center of each plot. The freshly cut material was weighed with load cells on the harvester. A 250-g subsample was weighed fresh and oven dried for three days in a forced air oven at 60°C. Dried subsamples were reweighed to determine percent dry matter (DM) using the following formula: $([\text{kg dry weight} / \text{kg fresh weight}] * 100)$. Total yield was calculated as kg DM/ha using the following equation: $(\text{kg fresh weight} / 4.5 \text{ m}^2) * \% \text{ DM} * (10,000 \text{ m ha}^{-1} / 4.5 \text{ m}^2 \text{ plot}^{-1})$.

Nutritive value concentrations. The same 250 g subsample was used to determine nutritive value concentrations. Dried subsamples were ground sequentially to pass through 2 and 1 mm screens using Wiley (Thomas Wiley, Philadelphia, PA) and Cyclone (Udy Corporation, Fort Collin, CO) mills, respectively. Crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) were estimated using near infrared reflectance spectroscopy (Foss North America, Eden Prairie, MN) with a robust equation for fresh hay and forage. Total digestible nutrients (TDN) were calculated using the following equation from Virginia Tech's former Forage Testing Lab: $\text{TDN} = 100.32 - 1.118 * \text{ADF}$.

Botanical composition. A second 250-g subsample was collected at harvest to determine botanical composition. Subsamples were hand separated into each individual planted species and weed components in both years; weeds were further separated into individual species in 2016. Botanical components were then oven dried following the above procedures and each component's percentage of the sward on a DM basis for each plot was calculated using the following equation: $100 * (\text{individual component mass} / \text{total component mass})$.

Ergot alkaloid concentration. A third and final 250 g subsample was collected upon harvest, placed in a sealed Ziplock bag, and immediately put on ice. Upon return from the field, samples were frozen until freeze drying could occur. Samples were freeze dried for ten days using the following cycle: days 1-3 at -30°C, days 4-5 at -17°C, days 6-7 at 0°C, day 8 at 18°C, and days 9-10 at 23.9°C. Freeze dried samples were quantitatively analyzed for total ergot alkaloid concentrations using a phytoscreen PT ergot alkaloid ELISA kit (Agrinostics, Athens, GA). These values were then divided by the proportion of tall fescue in each plot to determine total ergot alkaloid concentration for the tall fescue plant.

Soil temperature and moisture. Two replications of soil temperature (12-bit temperature smart sensor, Onset, Bourne, MA) and soil moisture (Soil Moisture Smart Sensor, Onset, Bourne, MA) sensors were installed in a subset of intermediate mixture plots in May 2016 (Fig. 2). Measurements were recorded every 15 minutes using a datalogger (HOBO Micro Station, Onset, Bourne, MA). Daily average, high, and low, and monthly average high and average low temperatures were calculated and compared. Soil moisture data were separated and analyzed by rainfall event. Soil moisture differences over the course of the growing season were evaluated as well.

Sward diversity. Shannon's diversity index (H') was used to measure sward and functional group diversity using the following equation: $H' = -\sum p_i \ln p_i$ where p_i is the proportion of individuals (or weight, in our case) for each i^{th} species (Magurran, 1988). Higher numbers equate to higher biological diversity.

Cover rating. Following harvest and before shade structures were replaced, a Likert-type scaling system was used to qualitatively measure groundcover in each plot. The scaling was as follows: 1 = very poor, 2 = poor, 3 = fair, 4 = good, 5 = very good.

Sward density. A rising plate meter (Filip's Folding Plate Pasture Meter, Jenquip, Feilding, New Zealand) was used to measure compressed sward height before each harvest. Plate meter recordings were taken by holding the plate meter above the forage canopy and lowering it until the center rod touched the ground. Twenty measurements were taken per plot. The numbers on the counter corresponded to centimeters of compressed forage height. The difference between the starting and ending counts was divided by 20 to give the average compressed forage height per plot. Plot yield (kg/ha) was divided by compressed forage height (cm) to give compressed forage density (kg/ha/cm).

Data Analysis

SAS 9.4 software (SAS Institute, Cary, NC) was used to analyze data. The General Linear Model procedure was used to generate an ANOVA table. If treatment (shade or mixture) by year interactions were not significant, data was presented across years. When year by treatment interactions occurred, data was analyzed and presented by year. Season by treatment interactions were also evaluated within and across years along with shade by

mixture interactions. When appropriate, interaction means are presented and regressions or standard errors were calculated. Regression analysis was performed using SigmaPlot 11.0 (Systat Software, San Jose, CA), and standard errors were calculated using the stderr option for lsmeans in SAS.

Fisher's protected least significant difference post-hoc test was used to separate means for all data except soil moisture. Moisture data were analyzed in SAS 9.4 using the MIXED procedure. Shade was considered a fixed effect, and Kenward Roger denominator degrees of freedom were calculated using the ddfm = kr operation. The pdiff option was used to separate soil moisture means. All differences were considered significant at an alpha level of 0.05.

Literature Cited

- Google. 2016. 37.091889, -77.963632. Online at <https://www.google.com/maps/place/37%C2%B005'30.8%22N+77%C2%B057'49.1%22W/@37.0918872,-77.9811416,14z/data=!3m1!4b1!4m5!3m4!1s0x0:0x0!8m2!3d37.091889!4d-77.963632>. Accessed 22 November 2016.
- Kephart K.D., D.R. Buxton, and S.E. Taylor. 1992. Growth of C₃ and C₄ perennial grasses in reduced irradiance. *Crop Sci.* 32:1033–1038.
- Lin, C.H., R.L. McGraw, M.F. George, and H.E. Garrett. 1999. Shade effects on forage crops with potential in temperate agroforestry practices. *Agrofor. Syst.* 44:109-119.
- Maguire, R.O. and S.E. Heckendorn. 2009. Laboratory procedures: Virginia Tech Soil Testing Laboratory. Virginia Cooperative Extension publication 452-881. Blacksburg, VA.
- Maguire, R.O. and S.E. Heckendorn. 2015. Soil test recommendations for Virginia. Virginia Cooperative Extension. Blacksburg, VA.
- Magurran, A.E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, NJ.

Soil Survey Staff. 2016. Web Soil Survey. USDA-NRCS. Online at <http://websoilsurvey.nrcs.usda.gov/>. Accessed 22 November, 2016.

Varella, A.C., D.J. Moot, K.M. Pollock, P.L. Peri, and R.J. Lucas. 2011. Do light and alfalfa responses to cloth and slatted shade represent those measured under an agroforestry system? *Agrofor. Syst.* 81:157-173.

Chapter 4: Production and composition of cool-season forage mixtures in response to shade

Abstract

Silvopasture systems intentionally integrate trees, forages, and livestock. The shade from trees moderates the forage microclimate in a way that may affect forage productivity. The objective of this study was to evaluate the impact of increasing shade levels on the yield and botanical composition of forages of increasing mixture complexity that have the potential to be used in Virginia silvopastures. The study was conducted at Virginia Tech's Southern Piedmont Agricultural Research and Extension Center located near Blackstone, VA. The experimental design was a randomized complete block using four replications. Slatted shade structures created three different light levels (30, 50, and 70% shade) and were compared to a full sun control. Three cool-season forage mixtures were evaluated (SIMPLE = tall fescue and white clover; INTERMEDIATE = SIMPLE + orchardgrass and red clover; and COMPLEX = INTERMEDIATE + Kentucky bluegrass, birdsfoot trefoil, and alfalfa). Yield was reduced at 50 and 70% shade, and showed no difference between mixtures, regardless of forage diversity. During spring and fall, seasonal yields were reduced beneath shade, but intermediate and simple mixture yields were increased at 30% shade during summer once forages were established. Most likely, this occurred due to reduced irradiance during the periods where cool-season species need the most resources to sustain high growth rates, whereas during the hottest part of the year when cool-season forage growth is reduced, shade tolerant species thrived and remained vegetative under the modified microclimate of the 30% shade treatment. During this time even intermediate mixtures under 50% shade yielded as much as open sun counterparts. Once forages established, orchardgrass increased while weeds and white clover decreased in

intermediate and complex mixtures. Red clover also comprised a significant proportion of the swards beneath shade. In the simple mixtures, tall fescue increased under shade while white clover decreased. Although orchardgrass is not particularly well-adapted to Virginia, silvopastures may be an effective way to integrate this and other marginally adapted cool-season grasses into grazing systems.

Introduction

The term 'agroforestry' describes a set of land use practices that incorporate trees or shrubs with crops or livestock in a way that enhances beneficial interactions between the individual components (Gold and Mason, 2015). Silvopasture, one of the five North American agroforestry practices, intentionally incorporates trees into an intensively managed integrated forage-livestock production system (Gold and Mason, 2015). There are many potential environmental and economic benefits to silvopastures, including shade and shelter for livestock, improved water quality and infiltration, greater aquifer recharge, increased biodiversity, providing wildlife habitat, more efficient nutrient cycling, increasing aesthetic value, and enhancing income stability by integrating long- and short-term revenue streams (Fike et al., 2004; Gold and Mason, 2015; Mosquera-Losada & Rigueiro-Rodriguez, 2013; Walter, 2015).

Due to the presences of trees, forages in silvopasture systems are subject to different microclimatic conditions than forages grown in open pastures. These conditions include lower wind speeds, air temperatures, relative humidity, solar radiation, and evapotranspiration (Karki and Goodman, 2010; 2015). Silvopasture systems around the world are subject to varied

microclimatic conditions and production outputs based on their different environmental and edaphic conditions and the corresponding forage, tree, and livestock components.

The presence of shade and the modified microclimate found in silvopastures may impact forage productivity and quality (Perry et al., 2009). However, there are conflicting findings on whether or not silvopastures reduce, maintain, or increase forage yields as compared to open pasture counterparts. Many studies have documented reduced yields under shade or silvopastures as compared to open systems (Bambo et al., 2009; Barro et al., 2008; Belesky, 2005; Burner, 2003; Feldhake et al., 2010; Kallenbach et al., 2006; Lin et al., 1999; Neel and Belesky, 2015; Neel et al., 2008; Obispo et al., 2013; Peri et al., 2001; Perry et al., 2009). But several studies have found increased yields in similar systems (Belsky et al., 1993; Buerghler et al., 2005; Frost and McDougald, 1989; Garrett and Kurtz, 1983; Lin et al., 1999; Wilson et al., 1990). In other studies, no differences in forage yield was observed between open and moderately shaded pastures (Burner and Brauer, 2003; Lin et al., 1999). Some of this disparity may be attributed to differing tree ages, as evidenced in a poplar silvopasture in New Zealand, where 5-year-old trees had no effect on forage yields (Guevara-Escobar et al., 2007), but reductions were seen in trees of 8-11 years of age in a similar site (Douglas et al., 2001).

In addition, each forage responds differently to shaded conditions. However, in general, some common responses to the shade of silvopastures include increased leaf area and stem height, and decreased root growth, tiller number, stem dry weight, and leaf dry weight and thickness (Allard et al., 1991; Corre, 1983; Kephart et al., 1992; Wong et al., 1985). Plants that exhibit these characteristics may look tall and spindly with larger and thinner leaves than their sun-grown counterparts.

When the plant is growing in shaded conditions it will allocate more assimilated resources, such as carbon, nutrients, and water, to above-ground growth in order to promote photosynthesis in a light-limited environment, resulting in diminished root growth capacity (Poorter et al., 2012). Many shade-grown forages utilize carbon stocks stored in roots to regrow after defoliation, in addition to relying on the photosynthetic capacity of remaining green material (Belesky, 2005). If the pool of available carbon stores in the roots is limited by lower root mass and volume, these plants likely will require more time to regenerate new leaves after a grazing event. Without appropriate grazing management, the compound stresses of low light and defoliation, coupled with limited root reserves, could potentially reduce their lifespan in the sward as compared to more shade-tolerant species.

One way to maintain forage aboveground primary productivity in the shaded environment of silvopastures is by planting diverse, shade-tolerant swards. Diverse plant communities provide many benefits and ecosystem services, including increased productivity, soil water and nitrogen retention, environmental resilience, weed suppression, and carbon sequestration (Kirwan et al., 2007; McNally et al., 2015; Minns et al., 2001). Improving biodiversity on farms can not only benefit farm productivity, but can also improve our ecosystems (Minns et al., 2001).

The objective of this study was to evaluate the effect of increasing shade and mixture diversity on the yield and botanical composition of cool-season forage mixtures that have the potential to be used in silvopasture systems. Our hypotheses were that: 1) forage yields would not be sacrificed at or below 50% shade, 2) more diverse mixtures would result in increased forage yields, 3) high levels of shade would alter the botanical composition to favor species

more tolerant of limited irradiance, 4) shaded plots would grow taller and have less groundcover than their unshaded counterparts, regardless of mixture, and 5) sward density would be equal among shaded and unshaded treatments, however more complex plots would have higher density than simple mixtures.

Procedures

Site Description

This research was conducted at Virginia Tech's Southern Piedmont Agricultural Research and Extension Center (SPAREC) located near Blackstone, VA (37.091889, -77.963632). Soil series at this site are predominantly Seneca sandy loam (Fine-loamy, mixed, thermic Aquic Hapludults) with a small proportion of Durham coarse sandy loam (Fine-loamy, siliceous, semiactive, thermic Typic Hapludults) and are considered prime farmland with capability units of Ile-4 (Soil Survey Staff, 2016). Weather data were collected onsite at SPAREC using a WatchDog 2900ET weather station (Spectrum Technologies, Aurora, IL). Average yearly temperature for SPAREC is 14.5 °C with 112 cm of relatively evenly distributed rainfall per year. Average temperature and rainfall for the duration of the study can be found in Fig. 1.

Experimental Design and Layout

The experimental design was a randomized complete block design with four replications and a factorial treatment arrangement. Treatments included varying levels of shade that could be found in silvopastures (30, 50 and 70% shade) compared to a full sun control and forage species mixture complexity (simple, intermediate and complex). Field position was used as a

blocking factor. Plots were 2.74 m x 3.05 m. Alleys between blocks were 3.05 m and distance between plots within blocks was 1.83 m (Fig. 2).

Shade structures were built to create artificial shade over individual plots. Structures were modified from Varella et al. (2011) who concluded that slatted shade structures more closely represented the dappled shade of silvopastures as compared to the uniform light distribution of shade cloth. A portable elevated frame of 2.44 x 3.66 x 0.91 m was constructed using 2"x4" dimensional lumber. Structures were deliberately built to be larger than the plots. This ensured that plots would be covered with the desired amount of shade throughout the day, even when the sun was not directly overhead.

Snow fence was secured to the tops and sides of the structures to create 50% shade. Thirty percent knitted shade cloth (Agriculture Solutions, Strong, ME) was added to the 50% shade structures to create the 70% shade environment. Even though the dappled effect was reduced by using shade cloth, extra slats were not added to structures in order to maintain structural integrity. Half of the slats in the snow fence were removed to create 30% shade (Fig. 3). Shade levels were validated by simultaneously recording PAR beneath structures using a line quantum sensor (LI-COR, Lincoln, NE) and in adjacent open areas using a PAR sensor (Onset, Bourne, MA). Shade levels were calculated as follows: $100 - (\text{PAR under structure} / \text{PAR in open sun})$.

Forage mixtures, cultivars and seeding rates are described in Table 1. Mixture species were as follows: SIMPLE = tall fescue (*Schedonorus arundinaceus*) and white clover (*Trifolium repens*); INTERMEDIATE = SIMPLE + orchardgrass (*Dactylis glomerata*) and red clover (*T. pretense*); and COMPLEX = INTERMEDIATE + Kentucky bluegrass (*Poa perenne*), alfalfa

(*Medicago sativa*), and birdsfoot trefoil (*Lotus corniculatus*). Species included in the mixtures were chosen because they are adapted to the North-South transition zone. All of these species utilize the C₃ photosynthetic pathway which has been shown to be more shade tolerant than C₄ pathway (Kephart et al., 1992). Some of these species have been shown to exhibit shade tolerance when grown in monoculture (Lin et al., 1999), but they have not been evaluated as part of a mixture, and may express different competitiveness under low light conditions.

Plot Management

A conventional seedbed was prepared by moldboard plowing, disking, field cultivating, and cultipacking. Soil samples were collected to a depth of 10 cm, and initial soil test levels were 14 ppm extractable P and 69 ppm extractable K. Samples were analyzed at Virginia Tech's Soil Testing Laboratory using the Mehlich I procedure (Maguire and Heckendorn, 2009). Prior to seeding, 34, 134, and 157 kg/ha of N, P₂O₅, and K₂O, respectively, was incorporated into the seedbed based on soil test recommendations (Maguire and Heckendorn (2015). After the initial fertilization, no other amendments were added to the plots.

In April 2015, plots were seeded using a small plot walk-behind cultipack-type seeder (Carter Manufacturing, Brookston, IN). Shade structures were put in place immediately after planting to simulate the shade of forests. Structures were removed the morning of harvest and placed back on plots within 24 h of harvest.

Forages were harvested when they reached a height of approximately 35 cm and were allowed to rest for at least four weeks between cuttings. Harvest occurred only twice in 2015 (15 Jun and 6 Aug) in order to let the forages establish healthy root systems. Harvest occurred five times in 2016 (26 Apr, 26 May, 27 Jun, 12 Aug and 3 Nov).

Measurements

Note: Not all measures were collected for each harvest. Measurement frequency and timing are shown in Table 2.

Plot height. Plot heights were measured using a standard measuring stick. Heights were determined using the average height of standing planted vegetation. Measurements were recorded at five points in each plot, one in the approximate center and one each approximately 0.5 m from the inside of each corner. The average of the five measurements is reported for each plot.

Yield and dry matter. A small plot harvester (Wintersteiger, Salt Lake City, UT) was used to harvest a 1.5-m x 3-m (4.5 m²) swath through the center of each plot. The freshly cut material was weighed with load cells on the harvester. A 250-g subsample was weighed fresh and oven dried for three days in a forced air oven at 60°C. Dried subsamples were reweighed to determine percent dry matter (DM) using the following formula: $([\text{kg dry weight} / \text{kg fresh weight}] * 100)$. Total yield was calculated as kg DM/ha using the following equation: $(\text{kg fresh weight} / 4.5 \text{ m}^2) * \% \text{ DM} * (10,000 \text{ m ha}^{-1} / 4.5 \text{ m}^2 \text{ plot}^{-1})$.

Botanical composition. A second 250-g subsample was collected at harvest to determine botanical composition. Subsamples were hand separated into each individual planted species and weed components in both years; weeds were further separated into individual species in 2016. Botanical components were then oven dried following the above procedures and each component's percentage of the sward on a DM basis for each plot was calculated using the following equation: $100 * (\text{individual component mass} / \text{total component mass})$.

Soil temperature and moisture. Two replications of soil temperature (12-bit temperature smart sensor, Onset, Bourne, MA) and soil moisture (Soil Moisture Smart Sensor, Onset, Bourne, MA) sensors were installed in a subset of intermediate mixture plots in May 2016 (Fig. 2). Measurements were recorded every 15 minutes using a datalogger (HOBO Micro Station, Onset, Bourne, MA). Daily average, high, and low, and monthly average high and average low temperatures were calculated and compared. Soil moisture data were separated and analyzed by rainfall event. Soil moisture differences over the course of the growing season were evaluated as well.

Sward diversity. Shannon's diversity index (H') was used to measure sward and functional group diversity using the following equation: $H' = -\sum p_i \ln p_i$ where p_i is the proportion of individuals (or weight, in our case) for each i^{th} species (Magurran, 1988). Higher numbers equate to higher biological diversity.

Cover rating. Following harvest and before shade structures were replaced, a Likert-type scaling system was used to qualitatively measure groundcover in each plot. The scaling was as follows: 1 = very poor, 2 = poor, 3 = fair, 4 = good, 5 = very good.

Sward density. A rising plate meter (Filip's Folding Plate Pasture Meter, Jenquip, Feilding, New Zealand) was used to measure compressed sward height before each harvest. Plate meter recordings were taken by holding the plate meter above the forage canopy and lowering it until the center rod touched the ground. Twenty measurements were taken per plot. The numbers on the counter corresponded to centimeters of compressed forage height. The difference between the starting and ending counts was divided by 20 to give the average

compressed forage height per plot. Plot yield (kg/ha) was divided by compressed forage height (cm) to give compressed forage density (kg/ha/cm).

Data Analysis

SAS 9.4 software (SAS Institute, Cary, NC) was used to analyze data. The General Linear Model procedure was used to generate an ANOVA table. If treatment (shade or mixture) by year interactions were not significant, data was presented across years. When year by treatment interactions occurred, data was analyzed and presented by year. Season by treatment interactions were also evaluated within and across years along with shade by mixture interactions. When appropriate, interaction means are presented and regressions or standard errors were calculated. Regression analysis was performed using SigmaPlot 11.0 (Systat Software, San Jose, CA), and standard errors were calculated using the stderr option for lsmeans in SAS.

Fisher's protected least significant difference post-hoc test was used to separate means for all data except soil moisture. Moisture data were analyzed in SAS 9.4 using the MIXED procedure. Shade was considered a fixed effect, and Kenward Roger denominator degrees of freedom were calculated using the ddfm = kr operation. The pdiff option was used to separate soil moisture means. All differences were considered significant at an alpha level of 0.05.

Results and Discussion

The 2015 growing season showed similar average temperature with 13 cm more rainfall as compared to the 63-year average for the site. In 2016, the temperature was approximately 1°C warmer and rainfall was 8 cm above the 63-year average (Fig. 1). Year x treatment

interactions occurred for most parameters ($P < 0.05$), therefore data will be presented by individual years where appropriate. Significance for variable responses for individual parameters are presented in Tables 3-6.

Yield

Annual forage yield was not different under 30% as compared to the full sun control but was reduced by 21 and 36% under the 50 and 70% shade treatments, respectively ($P < 0.001$). Mixture affected annual yield with intermediate mixes having the greatest yields (6710 kg DM/ha) followed by complex (6280 kg DM/ha) and simple mixtures (6090 kg DM/ha) (Fig. 4). In 2016, annual yields were 182% greater than 2015 (data not shown) given the greater number of harvests of the more mature forage stands.

Averaged across both years, mixture did not affect annual forage yield ($P > 0.05$) (Fig. 4). In the Northeast U.S., a three-species cool-season mixture dominated by white clover and tall fescue outperformed diverse mixtures of up to 15 species (Tracy and Sanderson, 2004). This example contradicts other findings about biodiversity and productivity relationships, but showcases the success of agricultural selection for high yielding cultivars that can produce as much or more than unimproved native plants. The authors emphasized that increased sward stability came from including legumes in the sward. Legumes can have a “keystone” effect on ecosystems, meaning that their ability to fix nitrogen can drastically alter community processes as compared to swards without N-fixing species (Minns et al., 2001). Similarly, Skinner et al. (2006) reported that increased yield benefits from complex mixtures were a result of including high-yielding forages, rather than purely a biodiversity effect. This study utilized both improved grasses and legumes in all mixtures, which may have been the reason that no annual yield

differences occurred between mixtures. Planting proven high-yielding shade tolerant forages in silvopastures in simple or intermediate mixtures likely will produce long-lasting productive forage stands.

There was a mixture x year and a shade x year interaction for seasonal forage yield, therefore 2015 and 2016 seasons were analyzed separately ($P < 0.04$). Mixture effects occurred for spring and fall 2016 ($P < 0.002$, $P < 0.001$), but not during spring or summer of 2015. This may have been due to extremely high weed components in all mixtures (Fig. 7).

In spring of 2015, the 30% shade treatment yielded the same as under the 0 and 50% shade treatments, but more than forage yields from under the 70% shade (Fig. 5). During summer 2015, forage yields were greatest under the full sun and 30% shade treatments (3610 and 3370 kg DM/ha) while the 50 and 70% shade treatments were reduced (2430 and 2140 kg DM/ha) ($P < 0.001$). In spring and fall 2016 yield declined as shade increased ($P < 0.001$) (Fig. 5).

In the summer of 2016, there were shade x mixture interactions for seasonal yield ($P < 0.001$) (Fig. 6). Complex and intermediate mixtures were positively affected by 30% shade in summer. Plots yielded approximately 3780 kg DM/ha in full sun, increasing to approximately 5270 and 4490 kg DM/ha for intermediate and complex mixtures at 30% shade, respectively, and then steadily decreasing with increasing shade to 2370 kg DM/ha under 70% shade. Yields did not differ by mixture under full sun, but unlike the intermediate and complex mixtures, yields of the simple mixture declined steadily across shade treatments. Yields did not differ by mixture under 70% shade (Fig. 6).

Both intermediate and complex mixtures contained red clover which comprised approximately 48 and 36% of the sward under 30% shade in the intermediate and complex

mixtures, respectively. The wet spring seemed to facilitate abundant clover growth that carried over into the summer months. This may have resulted in the elevated yields observed at 30% shade.

During spring 2016, simple mixtures yielded approximately 22% less than both intermediate and complex mixtures (2790 kg vs. 3190 kg DM/ha), both of which were dominated by orchardgrass and red clover (Fig. 7). In the fall of 2016, the opposite occurred where simple mixtures (1770 kg DM/ha) yielded approximately 36% more than the intermediate and complex mixtures (1300 kg DM/ha) (Fig. 7). In the fall, the majority of these swards were comprised of tall fescue and dead or dying crabgrass. This is important to note because it demonstrates tall fescue's late-season productivity and its subsequent suitability for fall stockpiling (Ball et al., 2007). The crabgrass found in these plots was the result of its invasion into swards with low diversity during the summer months.

Annual yield reductions did not occur until shade levels reached 50%, therefore, these forage mixtures show potential for lightly to moderately shaded silvopastures in the transition zone of the United States. The general consensus in the literature is that silvopastures affect forage yields to varying degrees based on each system's unique environmental factors and individual system components, such as forage species and tree age, species, spacing and orientation/arrangement (Buergler et al., 2005; Pent and Fike, 2016). For example, more mature or closely spaced trees may have a negative effect on forage yields as compared to younger or more widely spaced trees by providing more shade and/or moisture competition (Douglas et al., 2001; Guevara-Escobar et al., 2007; Karki and Goodman, 2013; Karki and Goodman 2015). Warm-season forages may also be more prone to yield reductions in

silvopastures as they generally are not as shade tolerant as cool-season species (Kephart et al., 1992).

Alternatively, some silvopastures have shown increased forage yields as compared to open systems, such as those under a moderately shaded silvopasture in Appalachia (Buerger et al., 2005; Pent and Fike, 2016). In drought-prone areas of California, tree canopy presence increased native annual forage yields (Frost and McDougald, 1989). The authors suggest the shade provided by the canopy reduces evaporative losses from the soil, thereby retaining more soil moisture for the forages to utilize as compared to open grasslands. Although forage yields in this study were not reduced under 30% shade, there were no trees in the system to compete for soil moisture, which may occur in actual silvopasture systems.

When evaluating seasonal yield responses to shade in the first growing year (2016), during the periods where cool-season species have highest rates of growth (spring and fall) seasonal yield declined as shade increased. The opposite occurred in the cool-seasons in Appalachia where Buerger et al. (2005) found yields to be highest under medium-density as compared to high density shade or low density shade (which they state was comparable to open systems). They claimed soil temperatures in the low density/open treatments were too high to support maximum cool-season forage growth. However, the soil temperature differences between full sun and shade in our study was much more drastic in the summer time than during spring or fall, with differences of up to 5.5°C during the hottest months. Perhaps temperatures were not high enough during the spring and fall of this study to detrimentally affect forages grown in full sun, and the decreased light reduced their photosynthetic capacity during the times they have highest growth rates and biggest energy needs.

Conversely, during the summer intermediate and complex mixtures exhibited highest yields under 30% shade. During the summer, photosynthesis of C₃ species becomes less efficient because their photorespiration rates increase due to elevated temperatures (McDonald, 2003). Again, this contradicts the findings of Buerger et al. (2005) which generally found the same forage yields in low/no shade as moderately shaded pastures during the summer months. Feldhake et al. (2010) also found approximately 60% yield reductions in silvopastures as compared to open pastures during the summer, although they attribute this to soil quality and historical management differences between the two sites. These conflicting findings reiterate the idea that individual systems will affect forages in different ways.

Red clover and orchardgrass were the dominant forages in the intermediate and complex mixtures under 30% shade during the summer (data not shown). This result was somewhat surprising because previous data has shown that red clover showed decreased yields under shading in Missouri (Lin et al., 1999). However, their shade levels were 0, 50 and 80%, which does not include a lightly shaded treatment. Another study found red clover to be light saturated at 1500 foot-candles, which equates to approximately 15% full sunlight, meaning it should be fairly shade tolerant (Bula, 1960). Delate et al. (2005) stated that forages like red clover with an upright growth habit tend to be more shade tolerant than their prostrate counterparts because they rely more on root reserves to facilitate regrowth. However, as shown by this study and by Lin et al. (1999), heavy shade drastically limits the light available for photosynthesis, thus reducing the plants' capacity for optimal yields.

These results illustrate the importance of using species well-adapted to individual systems. Even though mixture had no effect on annual yield over the course of the entire

experiment, understanding individual species' contributions to seasonal yield responses can be useful in grazing and pasture management and when designing forage systems to more uniformly supply forage throughout the grazing season.

Botanical Composition

Each mixture treatment varied in amount of species planted. Therefore, each mixture was individually evaluated for botanical composition differences occurring from shade treatments, and not compared to other mixtures. Shade x year interactions occurred for many species in all mixtures, perhaps due to an abundance of weeds in the first year as compared to the second year, so shade effects on mixtures were evaluated for each year separately.

Simple Mixture: In 2015, as a percent of the sward, tall fescue showed increased growth under all shade levels as compared to full sun (mean of 45% vs. 21%) ($P < 0.01$), white clover performed best under 30% shade, followed by full sun, and 50 and 70% shade (7.3, 4.4, 2.7 and 2.8%) ($P < 0.04$), and weeds were greatest under full sun (75%) and were reduced by an average of 32% under all shade levels ($P < 0.02$) (Fig. 8).

In 2016, tall fescue was reduced by 36% in full sun as compared to shade (38.5% vs. mean of 60.5%) ($P < 0.001$). Weeds were not affected but averaged 34% ($P > 0.70$) (Fig. 9a). White clover showed shade x season interactions in 2016 ($P < 0.01$). In spring and summer, white clover proportions were reduced by 80% to less than 10% of the sward DM under all shade levels as compared 36% in full sun. In fall of 2016, white clover proportions were not affected by shade, but dropped below 5% for all light treatments ($P > 0.50$) (Fig. 9b).

Intermediate Mixture: In 2015, tall fescue's proportion in the sward increased from 6.4% (full sun) to 14% (70% shade) ($P < 0.03$). White clover was not affected by shade treatment but

comprised less than 2% of the sward ($P > 0.14$). Red clover comprised the greatest proportion of the sward at 50% shade (28% of sward DM compared to an average of 16% for all other treatments) ($P < 0.02$). Weeds were reduced beneath shade as compared to full sunlight with lowest weed proportions occurring under 50 and 70% shade (65% vs. 36%) ($P < 0.01$) (Fig. 10a). Orchardgrass showed shade x season interactions ($P < 0.001$) where in spring proportions were greatest at 50 and 70% shade (37.7 and 31.4%) and decreased at 30% shade and full sun (22 and 17%) ($P < 0.01$), and in summer proportions increased with increasing shade, from 6% in full sun to 30% in 70% shade ($P < 0.001$) (Fig. 10b).

In 2016, tall fescue was not affected by shade treatments ($P > 0.21$). Orchardgrass was greatest at 50 and 70% shade (41%) compared to full sun and 30% shade (21%) ($P < 0.001$). White clover was reduced under all shade levels as compared to full sun (2% vs. 8%) ($P < 0.001$). Red clover was moderately affected by shade treatment with lowest proportions at 70% shade compared to all other treatments (18% vs. 28%) ($P < 0.09$), and weeds declined as shade increased (32% in full sun to 15% under 70% shade) ($P < 0.01$) (Fig. 11).

Complex Mixture: In 2015, tall fescue proportions were greatest under 50% shade (18%) followed by 70% shade (13%), 30% shade (8%) and full sun (5%) ($P < 0.001$). Orchardgrass was present in the highest proportion in 50 and 70% shade (35%) followed by 30% shade (22%) and full sun (10%) ($P < 0.001$). White clover proportions were not affected by shade treatment and averaged 1.2% ($P > 0.05$) (data not shown). Red clover exhibited highest proportions under 30 and 50% shade (13%) while proportions declined under 70% shade and full sun (5%) ($P < 0.01$). Alfalfa proportions were not affected by shade treatment and made up 10% of the sward dry

matter ($P < 0.09$). Weeds were most abundant in full sun plots (73%) and declined by an average of 55% for all shade levels ($P < 0.001$) (Fig. 12).

In 2016, tall fescue proportions were greatest in full sun (25%) followed by 50% shade (21%) and 30 and 70% (16% average) ($P < 0.02$). Orchardgrass proportions increased as shade increased with 13% in full sun and 47% under 70% shade ($P < 0.001$). White clover showed higher proportions in full sun as compared to all shade treatments (10% vs. 2%) ($P < 0.001$) (data not shown). Alfalfa was greatest under 30% shade (8%) followed by 50% (4%) and full sun and 70% shade (2.7%) ($P < 0.01$). Stands contained greater proportions of weeds in full sun (34%) compared to 20% for all shade levels ($P < 0.05$) (Fig. 13a). Red clover showed shade x season interactions ($P < 0.01$), where in summer proportions were increased at 30 and 50% shade (36%) compared to full sun and 70% shade (14%) ($P < 0.001$), but there were no differences in spring and fall ($P > 0.09$) (Fig. 13b). Kentucky bluegrass and birdsfoot trefoil never exceeded 1% of the sward, regardless of year or shade treatment (data not shown).

Once forages were established, tall fescue increased under shade while white clover decreased in the simple mixture. When orchardgrass and red clover were added to the simple mixtures, orchardgrass dominated swards at 50 and 70% shade while weeds and red clover were the dominant species at 0 and 30% shade, although orchardgrass still comprised approximately 20% of the sward. During both spring and summer in 2016, red clover was a significant component of complex mixtures, but was not during fall, possibly due to its biennial/weak perennial life cycle (Abaye, 2010).

Orchardgrass, weeds and tall fescue, were the primary components of all shaded plots in the complex mixtures, while full sun mixtures were dominated by tall fescue and weeds.

Many weed species were warm season grasses, primarily from the *Digitaria* genus. Warm-season species have been shown to be generally less shade tolerant because of their higher light saturation point as compared to C₃ species (Kephart et al., 1992; Stier and Gardner, 2008).

These results indicate that tall fescue, orchardgrass, and red clover will be suitable for silvopastures in the Southeast U.S., with the recognition that red clover will need to be reseeded every two to four years. Alfalfa may also be included in mixtures to increase diversity and nitrogen fixation capabilities. Orchardgrass, tall fescue, and alfalfa were shown to be shade tolerant when grown in monoculture beneath shade cloth in Missouri (Lin et al., 1999). This study corroborates those results while also demonstrating these species remain competitive when grown in polyculture.

Lin et al. (1999) found white clover to be shade tolerant when grown in monoculture, but in our study, white clover was not a significant part of the sward. Perhaps when grown in a mixed sward more robust and upright species would maintain a competitive advantage by outgrowing and overtopping the white clover, thus capturing more of the incoming sunlight, and further reducing the total photosynthetically active radiation available to these plants. There is a correlation between nitrogen fixation capabilities and legume dry matter yield, and both red clover and alfalfa comprised greater proportions of the sward and have the capacity to fix more nitrogen than white clover (Carlsson and Huss-Danell, 2003). Therefore, white clover may not be the best choice for silvopastures in the southern U.S.

Botanical Diversity

In 2015, there was a shade x mixture interaction for desired species (planted species only) diversity ($P < 0.001$) where the simple mixture diversity was consistently lower than the

intermediate and complex mixtures for all light levels. Diversity of desired species for both intermediate ($P < 0.02$, $R^2 = 0.26$) and complex ($P < 0.001$, $R^2 = 0.57$) mixtures showed a quadratic response to increasing shade level where shade presence increased desired species diversity, however simple mixture diversity showed no response to increasing shade (Fig. 14).

Results from 2016 were separated by season due to significant mixture x season and shade x season interactions ($P < 0.01$). In spring, diversity was increased with mixture complexity but reduced with increasing level of shade ($P < 0.001$) (Fig. 15a). During the summer the simple mixture had lower diversity than intermediate and complex mixtures and increasingly lower diversity with increasing shade (quadratic response; $P < 0.001$; $R^2 = 0.59$). Desired diversity (H') was greater in the complex mixture, with greatest separation among treatments under 30% shade (Fig. 16). In fall of 2016, shade did not affect desired species diversity ($P > 0.23$), but this response was increased ($P < 0.001$) with mixture complexity (Fig. 15b).

Total species diversity (weeds and planted species; only calculated in 2016) of the plots was reduced as shade increased ($P < 0.001$). In contrast, species diversity increased as mixture complexity increased and mixture ($P < 0.001$) (Fig. 17).

Diversity of functional groups (cool-season grasses, warm-season grasses, legumes, and forbs) was only calculated for 2016 using Shannon's diversity index. Functional group diversity was affected by shade ($P < 0.001$) but not by mixture ($P > 0.50$). As shade increased functional group diversity decreased. At 70% shade functional group diversity was reduced by 20% as compared to full sun (Fig. 18).

As expected, intermediate and complex mixtures had greater desired species diversity than simple mixtures because they were planted with a more diverse seed mix. When planting mixtures of four or more shade tolerant species there should be no sacrifice in desired species diversity in silvopasture systems.

When weed species are included in the diversity calculation, total species diversity increased with increasing mixture complexity and decreased with all shade treatments as compared to full sun. Functional group diversity was not affected by mixture but decreased as shade increased. Similar to our findings, Sparks et al. (1996) also found that diversity was compromised as shade increased in English woodlands.

From a biological standpoint, losing functional diversity may result in declines in productivity (Tilman et al., 1997), and may have negative implications in regards to ecosystem functioning as botanical functional composition is linked to ecosystem processes (Diaz and Cabido, 2001). From an agronomic view, however, the use of high yielding cultivars and specific functional groups such as legumes may more than compensate for plot-level diversity (Tracy and Sanderson, 2004). However, including trees in the whole system will add another element to total botanical and functional diversity, and has the potential to increase wildlife diversity as well (Husak and Grado, 2001).

Sward Height

Spring sward heights responded similarly to shade and mixture treatments in 2015 and 2016, thus these data are presented over years (Fig. 19a). Sward heights under shade (mean of 40 cm) were greater than sward heights from plots in full sun (32 cm) ($P < 0.001$). Simple

mixtures (33 cm) were also an average of 17% shorter than intermediate and complex mixes (40 cm) ($P < 0.001$) (Fig. 19a).

Sward heights in summer and fall were measured only in 2016 and were affected by a treatment x season interaction ($P < 0.001$), thus data are presented by season. During the summer, all mixtures averaged 27 cm when grown in full sun but mixture heights varied by shade treatment (shade x mixture interaction; $P < 0.001$). Heights of the complex mixture increased to 44 cm under 30% shade, but declined slightly to an average of 40 cm under 50 and 70% shade. The intermediate mixture was tallest (50 cm) under 30% shade, and then decreasing in height under 50 and 70% shade treatments (42 and 37 cm, respectively). Sward height of the simple mixture gradually increased with shade, from 30 cm under 30% shade to 34 cm under 50% shade. A slight decrease to 32 cm in height occurred for simple mixtures under 70% shade (Fig. 19b). Although a shade x mixture interaction occurred ($P < 0.03$) for fall as well, the general trend was for increased plot heights under shade (30 cm) as compared to full sun (24 cm) (Fig. 19c).

Similar to results from Eriksen and Whitney (1981), overall plot heights increased under shade as compared to full sunlight. Intermediate and complex mixtures had taller swards under shade than the simple mixtures in the spring and summer. The favorable growing conditions coupled with shade's effect on stem elongation undoubtedly supported tall and robust red clover populations. However, tall fescue and orchardgrass were harvested at the vegetative stage. Visually, both grasses had very long leaves, but few, if any, stems were present. Leaf elongation is a common response to shading as Allard et al. (1991) and Peri et al. (2007) showed for tall fescue and orchardgrass, respectively. Therefore, it is unlikely that the

increases in sward height were due to grass stem elongation, but were more likely due to increased heights of red clover.

Groundcover

Groundcover ratings were only performed in June, August and November 2016. Both shade ($P < 0.001$) and mixture ($P < 0.001$) affected cover. As shade increased, groundcover decreased, with a 30% reduction from full sun to 70% shade (4.58 vs. 3.27) (Fig. 20). Using monocultures of various grasses and legumes, Firth et al. (2002) also saw reduced groundcover when increasing shade levels from 79 to 97.3% shade. Although these levels are outside the range of our treatments, these results indicate that our mixtures most likely would have continued decreased groundcover at extremely high shade levels. The simple mixture had the highest cover rating while the intermediate had the lowest (4.23 vs. 3.4). The complex mixture fell between the two (Fig. 20).

Simple mixtures and mixtures in full sun had the most groundcover. Often these were the weediest treatments. Planting only two cool-season species left niches to be filled, facilitating the establishment of a weedy warm-season grass. In our plots there was abundant crabgrass (*Digitaria* spp.) during the summer months. Crabgrass has a tendency to fill in bare ground in agriculture fields, but as a warm-season grass it is inherently not as shade tolerant as its cool-season grass counterparts based on their respective light saturation levels (Kephart et al., 1992; Lin et al, 1999; Stier and Gardner, 2008).

Less groundcover has been associated with reduced environmental services, such as soil conservation and water infiltration potential (Ziegler and Giambelluca, 1998). Although shaded systems may reduce the understory groundcover, including trees into a forage production

system will slow the raindrops, reducing their kinetic energy and allowing more time for water to infiltrate into the soil rather than running off the soil surface (Walter et al., 2015).

Sward Density

Compressed sward height measures were performed only in August and November 2016. Significant treatment x season interactions occurred ($P < 0.05$), therefore, data are presented by season. During the summer, only shade affected compressed sward height ($P < 0.001$). Greatest measures occurred beneath 30% shade, followed by full sun, 50% and 70% shade treatments (29, 26, 23, and 18) (Fig. 21a). During the fall, both shade ($P < 0.001$) and mixture ($P < 0.001$) affected compressed sward height (Fig. 21b). Compressed heights were similar for both full sun and 30% shade, and then decreased with each additional shade level. Greatest heights were observed with the simple mixtures, while the intermediate and complex mixtures were reduced by an average of 14%.

Compressed sward height and yield were used to calculate compressed sward density. Overall, as shade increased, compressed sward density decreased ($P < 0.01$). Mixture had no effect on compressed sward density ($P > 0.09$) (Fig. 22). The greater density of the full sun and 30% shade treatments can be attributed to increased yields and greater groundcover as compared to the more heavily shaded treatments. One thing to note is that this measure was only collected during the summer and fall of 2016 when crabgrass was a prominent sward component. Crabgrass effectively covered much of the bare ground that was found in simple mixtures and full sun plots.

Soil Temperature and Moisture

Throughout the 2016 growing season, daily minimum ($P < 0.03$), maximum ($P < 0.001$) and average ($P < 0.001$) soil temperatures were affected by shade. Daily temperature minimums and averages were highest with full sun and 30% shade and declined with 50 and 70% shade (Fig. 23). During the evening shade has less of an effect on soil temperature because there is no sunlight available to be shaded. Shade effects were most pronounced on daily soil temperature highs, which were greatest in full sun (33.8 °C) and declined as shade increased (29.3 °C) (Fig. 23). Effects of shade on soil temperatures were greatest during the hottest summer months (July - September) and less apparent during the cooler June and October months (shade x month interaction; $P < 0.001$) (Fig. 24).

Increased soil temperature in the root zone likely promoted increased root respiration of sun-grown forages, as root respiration is temperature dependent (Lambers et al., 1996). Up to 50% of the CO₂ fixed by a plant during photosynthesis may be lost via root respiration, with averages of around 25% CO₂ loss for grasses (Lambers et al., 1996). Perhaps one reason for the lack of decrease in yields of the 30% shade treatment was due to cooler soil temperatures providing less root respiration and more energy reserves for forage regrowth.

Soil moisture differences among treatments were compared between each of the twelve rainfall events that occurred throughout the 2016 season (Fig. 25; Table 3). Soil moisture levels in ten out of the twelve periods were affected by shade ($P < 0.001$). For eight of those ten periods, soil moisture in the full sun treatment plots was less than in all of the shade treatments (Table 3). Averaged over all periods, the mean of percent soil moisture beneath all shaded treatments was 11% greater than that in the full sun plots.

Because the soils and the swards within shaded plots were cooler, both evaporative and transpirational demands on soil moisture would have been lower. Lower forage production with higher levels of shade would also have played a role in greater moisture with shade by reducing water demands for growth. In addition, the shade structures may have affected wind speed in the forage canopy which could have increased relative humidity, putting a dampening effect on evaporative losses in a manner similar to the effects of trees on silvopasture microclimates.

These soil results are consistent with other data from silvopasture systems. In young silvopastures in which the trees were not taking up much moisture, Karki and Goodman (2013) found greater soil moisture in silvopastures as compared to open pastures. In the same study, as well as others (Karki and Goodman, 2010; 2015), lower soil temperatures were also observed beneath silvopastures compared to open systems. However, once trees mature their water demand will increase with the potential to decrease overall soil moisture in silvopasture systems (Karki and Goodman, 2015).

Summary and Implications

Although yield reductions may occur under moderate and heavy shade, in settings with low levels of shade (e.g., newly established silvopastures with small trees that do not produce heavy shade or compete strongly for soil and water resources), forage yields per unit area should be comparable to that from open pastures. However overall yields may be reduced because some of the area is taken up by trees. Once trees mature and canopies begin to close,

forage yields will likely decline. However, increased shade may benefit livestock in regards to comfort and performance.

Although plot height generally increased with shade, this was not associated with increased yields. Rather, it likely reflected the phenotypic plasticity of shade-grown forages which often exhibit decreased tiller numbers, longer internode lengths, and reduced overall plant dry weight. Although more productive, the compressed sward heights of open plots were lower in summer; this reflects crabgrass invasion in open plots, which contributed to yield increases, and the fact that crabgrass has a more decumbent growth form in full-sun environments.

Orchardgrass is not typically as well-suited to Virginia's hot climate, but it was able to yield very well under shade. This could be in part due to its shade tolerant nature, but also to reduced soil temperatures and increased moisture beneath all shaded treatments. The milder microclimate may have contributed to its ability to outyield tall fescue, a species known to be hardy even in extreme conditions. Silvopastures may be an effective way to integrate high quality cool-season grasses that may not normally persist in the South into grazing systems.

Maintaining relatively low shade levels and planting improved, shade-adapted forage mixtures that include both cool-season grasses and legumes should supply similar levels of forage production as typical full-sun systems. However, despite forage yield reductions beneath some shade levels, there is potential to maintain animal gains equivalent to that in open systems (Neel and Belesky, 2015; Pent and Fike, 2016), especially in the summertime when livestock are generally heat-stressed.

Literature Cited

- Abaye, A.O. 2010. Identification and adaptation: common grasses, legumes and non-leguminous forbs of the Eastern United States. Virginia Tech, Blacksburg, VA.
- Allard, G., C.J. Nelson, and S.G. Pallardy. 1991. Shade effects on growth of tall fescue: I. leaf anatomy and dry matter partitioning. *Crop Sci.* 31:163-167.
- Ball, D.M., C.S. Hoveland, and G.D. Lacefield. 2007. Southern forages. 4th ed. International Plant Nutrition Institute, Norcross, GA.
- Bambo, S.K., A.R. Blount, J. Nowak, A.J. Long, R.O. Myer, and D.A. Huber. 2009. Annual cool-season forage nutritive value and production after overseeding into bahiagrass sod in silvopastoral systems. *Agroecol. Sustain. Food Sys.* (previously *J. Sustain. Agric.*) 33:917-934.
- Barro, R.S., J.C. de Saibro, R.B. de Medeiros, J.L.S. da Silva, A.C. Varella. 2008. Forage yield and nutritive value of cool-season annual forage grasses shaded by *Pinus elliottii* trees and at full-sun. *Revista Brasileira de Zootecnia (Brazilian J. Anim. Sci.)* 37:1721-1727.
- Belesky, D.P. 2005. Growth of *Dactylis glomerata* along a light gradient in the central Appalachian region of the eastern USA: I. dry matter production and partitioning. *Agrofor. Sys.* 65:81-90.
- Belsky, A.J., S.M. Mwonga, and J.M. Duxbury. 1993. Effects of widely spaced trees and livestock grazing on understory environments in tropical savannas. *Agrofor. Sys.* 24:1-20.
- Buergler A. L., J. H. Fike, J. A. Burger, C. M. Feldhade, J. R. McKenna, and C. D. Teutsch. 2005. Forage nutritive value in an emulated silvopasture. *Agron. J.* 98:1265-1273.
- Bula, R.J. 1960. Vegetative and floral development in red clover as affected by duration and intensity of illumination. *Agron. J.* 52:74-77.
- Burner, D.M. 2003. Influence of alley crop environment on orchardgrass and tall fescue herbage. *Agron. J.* 95:1163-1171.
- Burner, D.M. and D.K. Brauer. 2003. Herbage response to spacing of loblolly pine trees in a minimal management silvopasture in southeastern USA. *Agrofor. Sys.* 57:69-77.
- Carlsson, G. and K. Huss-Danell. 2003. Nitrogen fixation in perennial forage legumes in the field. *Plant Soil* 253:353-372.
- Corre, W.J. 1983. Growth and morphogenesis of sun and shade plants I. the influence of light intensity. *Acta Botanica Neerlandica* 32(1/2):49-62.

- Delate, K., E. Holzmueller, D.D. Davis Frederick, C. Mize, and C. Brummer. 2005. Tree establishment and growth using forage ground covers in an alley-cropped system in Midwestern USA. *Agrofor. Syst.* 65:43-52.
- Diaz, S. and M. Cabido. 2001. Vive la difference: plant functional diversity matters to ecosystem processes. *Trends Ecol. Evol.* 16(11):646-655.
- Douglas G.B., A.S. Walcroft, B.J. Wills, S.E. Hurst, A.G. Foote, K.D. Trainor, and L.E. Fung. 2001. Resident pasture growth and the micro-environment beneath young, widespaced poplars in New Zealand. *In: Proc. N. Z. Grassl. Assoc.* 63:131–138.
- Eriksen, F.I. and A.S. Whitney. 1981. Effects of light intensity on growth of some tropical forage species. I. Interaction of light intensity and nitrogen fertilization on six forage grasses. *Agron. J.* 73:427-433.
- Feldhake, C.M., J.P.S. Neel, and D.P. Belesky. 2010. Establishment and production from thinned mature deciduous-forest silvopastures in Appalachia. *Agrofor. Sys.* 79:31-37.
- Fike, J.H., A.L. Buerger, J.A. Burger, and R.L. Kallenbach. 2004. Considerations for establishing and managing silvopastures. Online. *Forage and Grazinglands*. doi:10.1094/FG-2004-1209-01-RV.
- Firth, D.J., R.M. Jones, L.M. McFadyen, B.G. Cook, and R.D.B. Whalley. 2002. Selection of pasture species for groundcover suited to shade in mature macadamia orchards in subtropical Australia. *Trop. Grassl.* 36:1-12.
- Frost, W.E. and N.K. McDougald. 1989. Tree canopy effects on herbaceous production of annual rangeland during drought. *J. Range Manage.* 42:281-283.
- Garrett, H.E. and W.B. Kurtz. 1983. An evaluation of the black walnut-tall fescue pasture management system. p. 838-840. *In: J.A. Smith and V.W. Hayes (eds.) Proc. Int. Grassl. Congr., XIV, Lexington, KY. Westview Press, Boulder, CO.*
- Gold, M. and A. Mason. 2015. Defining Agroforestry. p. 9-15. *In: Training manual for applied agroforestry practices, 2015 edition. University of Missouri Center for Agroforestry, Columbia, MO.*
- Guevara-Escobar, A., P.D. Kemp, A.D. Mackay, and J. Hodgson. 2007. Pasture production and composition under poplar in a hill environment in New Zealand. *Agrofor. Sys.* 69:199-213.
- Husak, A.L. and S.C. Grado. 2001. Monetary and wildlife benefits in a silvopastoral system. *Journal Article FO144. Forest and Wildlife Research Center, Mississippi State University.*

- Kallenbach, R.L., M.S. Kerley, and G.J. Bishop-Hurley. 2006. Cumulative forage production, forage quality and livestock performance from an annual ryegrass and cereal rye mixture in a pine-walnut silvopasture. *Agrofor. Sys.* 66:43-53.
- Karki, U. and M.S. Goodman. 2010. Landscape use by cattle in silvopasture versus open pasture. *Agrofor. Sys.* 78:159–168.
- Karki, U. and M.S. Goodman. 2013. Microclimate differences between young longleaf-pine silvopasture and open-pasture. *Agrofor. Sys.* 87:303-310.
- Karki, U. and M.S. Goodman. 2015. Microclimatic differences between mature loblolly-pine silvopasture and open-pasture. *Agrofor. Sys.* 89:319-325.
- Kephart, K.D., D.R. Buxton, and S.E. Taylor. 1992. Growth of C₃ and C₄ perennial grasses under reduced irradiance. *Crop Sci.* 32:1033-1038.
- Kirwan, L., A. Luscher, M.T. Sebastao, J.A. Finn, R.P. Collins, C. Porqueddu, A. Helgadottir, O.H. Baadshaug, C. Brophy, C. Coran, S. Dalmannsdottir, I. Delgado, A. Elgersma, M. Fothergill, B.E. Frankow-Lindberg, P. Golinski, P. Grieu, M. Gustavsson, M. Hoglind, O. Huguenin-Elie, C. Iliadis, M. Jorgensen, Z. Kadzieuliene, T. Karyotis, T. Lunnan, M. Malengier, S. Maltoni, V. Meyer, D. Nyfeler, P. Nykanen-Kurki, J. Parente, H.J. Smit, U. Thumm, and J. Connolly. 2007. Evenness drives consistent differences in intensive grassland systems across 28 European sites. *J. Ecol.* 95:530-539.
- Lambers, H., O.K. Atkin, and I. Scheurwater. 1996. Respiratory patterns in roots in relation to their functioning. p. 323-362. *In*: Y. Waisel, A. Eschel and U. Kafkaki (eds.) *Plant roots: the hidden half*. Marcel Dekker, New York.
- Lin, C.H., R.L. McGraw, M.F. George, and H.E. Garrett. 1999. Shade effects on forage crops with potential in temperate agroforestry practices. *Agrofor. Sys.* 44:109-119.
- Maguire, R.O. and S.E. Heckendorn. 2009. Laboratory procedures: Virginia Tech Soil Testing Laboratory. Virginia Cooperative Extension publication 452-881. Blacksburg, VA.
- Maguire, R.O. and S.E. Heckendorn. 2015. Soil test recommendations for Virginia. Virginia Cooperative Extension. Blacksburg, VA.
- Magurran, A.E. 1988. *Ecological diversity and its measurement*. Princeton University Press, Princeton, NJ.
- McDonald, M.S. 2003. *Photobiology of higher plants*. John Wiley & Sons, Ltd. Chichester, England.

- McNally, S.R., D.C. Laughlin, S. Rutledge, M.B. Dodd, J. Six, and L.A. Schipper. 2015. Root carbon inputs under moderately diverse sward and conventional ryegrass-clover pasture: implications for soil carbon sequestration. *Plant and Soil* 392:289-299.
- Minns, A., J. Finn, A. Hector, M. Caldeira, J. Joshi, C. Palmborg, B. Schmid, M. Scherer-Lorenzen, E. Spehn, A. Troumbis and the BIODDEPTH project. 2001. The functioning of European grassland ecosystems: potential benefits of biodiversity to agriculture. *Outlook Agric.* 30(3):179-185.
- Mosquera-Losada, M.R. and A. Rigueiro-Rodriguez. 2013. Silvopasture: a combination of grasslands and trees to green livestock production. p. 475-477. *In: A. Helgadottir and A. Hopkins (eds.) The role of grasslands in a green future, threats and perspectives in less favoured areas. Proc. 17th Symp. Eur. Grassl. Fed., Akureyri, Iceland. 23-26 June 2013.*
- Neel, J.P.S. and D.P. Belesky. 2015. Herbage production, nutritive value, and animal productivity within hardwood silvopasture, open and mixed pasture systems in Appalachia, United States. *Grass and Forage Sci.* Online. doi: 10.1111/gfs.12211.
- Neel, J.P.S., C.M. Feldhake, and D.P. Belesky. 2008. Influence of solar radiation on the productivity and nutritive value of herbage of cool-season species of an understory sward in a mature conifer woodland. *Grass and Forage Sci.* 63:38-47.
- Obispo, N.E., Y. Espinoza, J.L. Gil, F. Ovalles, E. Cabrera, and M.J. Perez. 2013. Relationship of shade proportion in the pasture with yield, quality of forage and daily weight gain in steers. *Revista Científica, Facultad de Ciencias Veterinarias, Univ. Zulia (Scientific Journal, Faculty of Vet. Sci., Univ. Zulia)* 23:531-536.
- Pent, G.J. and J.H. Fike. 2016. Sheep performance, grazing behavior, and body temperatures in silvopasture systems. *In: Proc. Am. Forage and Grassl. Counc. Baton Rouge, LA, January 13-15, 2016.*
- Peri, P.L., A.C. Varella, R.J. Lucas, and D.J. Moot. 2001. Cocksfoot and Lucerne productivity in a *Pinus radiata* silvopastoral system: a grazed comparison. p. 139-147. *In: Proc. N. Z. Grassl. Assoc., Waikato, New Zealand. 30 Oct.-1 Nov. 2001.*
- Peri, P.L., D.J. Moot, P. Jarvis, D.L. McNeil, and R.J. Lucas. 2007. Morphological, anatomical, and physiological changes of orchardgrass leaves grown under fluctuating light regimes. *Agron. J.* 99:1502-1513.
- Perry, M.E.L., W.H. Schacht, G.A. Ruark, and J.R. Brandle. 2009. Tree canopy effect on grass and grass/legume mixtures in eastern Nebraska. *Agrofor. Sys.* 77:23-35.

- Poorter, H., K.J. Niklas, P.B. Reich, J. Oleksyn, P. Poot, and L. Mommer. 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytol.* 193:30-50.
- Skinner, R.H., M.A. Sanderson, B.F. Tracy, and C.J. Dell. 2006. Above- and belowground productivity and soil carbon dynamics of pasture mixtures. *Agron. J.* 98:320-326.
- Soil Survey Staff. 2016. Web Soil Survey. USDA-NRCS. Online at <http://websoilsurvey.nrcs.usda.gov/>. Accessed 22 November, 2016.
- Sparks, T.H., J.N. Greatorex-Davies, J.O. Mountford, M.L. Hall, and R.H. Marrs. 1996. The effects of shade on the plant communities of rides in plantation woodland and implications for butterfly conservation. *For. Ecol. Manage.* 80:197-207.
- Stier, J.C. and D.S. Gardner. 2008. Shade stress and management. p. 447-471. *In*: M. Pessaraki (ed.) *Handbook of turfgrass management and physiology*. CRC Press, Boca Raton, FL.
- Tilman, D., J. Knops, D. Wedin, P. Reich, M. Ritchie, and E. Siemann. 1997. The influence of functional diversity and composition on ecosystem processes. *Science* 277:1300-1302.
- Tracy, B.F. and M.A. Sanderson. 2004. Productivity and stability relationships in mowed pasture communities of varying species composition. *Crop Sci.* 44:2180-2186.
- Varella, A.C., D.J. Moot, K.M. Pollock, P.L. Peri, and R.J. Lucas. 2011. Do light and alfalfa responses to cloth and slatted shade represent those measured under an agroforestry system? *Agrofor. Syst.* 81:157-173.
- Walter, D. 2015. Silvopasture. p. 50-66. *In*: Training manual for applied agroforestry practices, 2015 edition. University of Missouri Center for Agroforestry, Columbia, MO.
- Walter, D., S. Jose, and D. Zamora. 2015. Alley cropping. p. 31-49. *In*: Training manual for applied agroforestry practices, 2015 edition. University of Missouri Center for Agroforestry, Columbia, MO.
- Wilson, J.R., K. Hill, D.M. Cameron, and H.M. Shelton. 1990. The growth of *Paspalum notatum* under the shade of a *Eucalyptus grandis* plantation canopy or in full sun. *Trop. Grassl.* 24:24-28.
- Wong, C.C., H. Rahim, and M.A. Mohd. Sharudin. 1985. Shade tolerance potential of some tropical forages for integration with plantations: I. grasses. *MARDI Res. Bull.* 13(3):225-247.
- Ziegler, A.D. and T.W. Giambelluca. 1998. Influence of revegetation efforts on hydrologic response and erosion, Kaho'olawe Island, Hawai'i. *Land Degrad. Develop.* 9:189-206.

Chapter 5. Ergot alkaloid concentration and nutritive characteristics of shade grown forage mixtures

Abstract

Trees have been incorporated into traditional forage-livestock systems to create what is known as silvopastures. Tree presence in silvopasture systems provide a unique microclimate to the understory forages. Tall fescue is the dominant forage base for Virginia and much of the Southeast and shows shade-tolerance potential. Tall fescue harbors a mutualistic fungal endophyte that produces ergot alkaloids, compounds that cause toxicity issues in livestock. Diluting the sward with other forage species is a common mitigation strategy. It is unknown how shade will affect forage quality and ergot alkaloid concentrations in mixtures of increasing species complexity. This study was conducted at Virginia Tech's Southern Piedmont Agricultural Research and Extension Center located near Blackstone, VA. The experimental design was a randomized complete block with a two factor factorial treatment arrangement. Treatments were shade level and mixture complexity. Slatted shade structures created three different light levels (30, 50, and 70% shade) and were compared to a full sun control. Three cool-season forage mixtures were evaluated (SIMPLE = tall fescue and white clover; INTERMEDIATE = SIMPLE + orchardgrass and red clover; and COMPLEX = INTERMEDIATE + Kentucky bluegrass, birdsfoot trefoil, and alfalfa). Nutritive value declined beneath shade in spring and fall, most likely because the filtered light could not supply enough energy to meet the rapid growth rates of cool-season forages during these times. Crude protein was high enough to meet metabolic requirements for dry and lactating cows, and steers. However, during the summer steers may need energy supplementation in order to attain acceptable production goals. Total sward ergot alkaloid concentration was elevated beneath shade in the

simple mixtures because they had greater proportions of tall fescue. In the intermediate and complex mixtures, fescue was diluted with other forage species and ergot alkaloid concentration was minimally affected by shade. This illustrates the importance of incorporating multiple species into the sward to dilute the alkaloid concentration.

Introduction

Silvopasture intentionally incorporates trees into an intensively managed integrated forage-livestock production system (Gold and Mason, 2015). There are many environmental and economic benefits to silvopastures, including shade and shelter for livestock, improving water quality and infiltration, recharging aquifers, boosting biodiversity, and providing wildlife habitat, more efficient nutrient cycling, increased aesthetic value and enhanced income stability by integrating long- and short-term revenue streams (Gold and Mason, 2015; Fike et al., 2004; Mosquera-Losada & Rigueiro-Rodriguez, 2013; Walter, 2015). These systems have the potential for a combination of low inputs and resource use efficiency that may foster increased production and profitability (Clason and Sharrow, 2000).

Compared to typical pastures, forages in silvopasture systems are subject to different environmental conditions due to the presence of trees. Karki and Goodman (2010, 2015) consistently reported lower values for wind speeds, air temperatures, relative humidity, solar radiation, photosynthetically active radiation (PAR), and evapotranspiration in mature silvopastures as compared to open pastures. Feldhake (2002) reported warmer surface temperatures during frost-prone nights underneath silvopastures, which could lead to an extended grazing season. Pierson et al. (1990) and Perry et al. (2009) each found different

forages that exhibited delayed maturity beneath shade, and Smith (1942) claimed that grasses grown underneath silvopastures remained in a vegetative state throughout the Ohio summers. These findings indicate lengthened periods of high forage quality in silvopasture systems, because it is well-known that forage maturity is the main driver of forage nutritive characteristics (Ball et al., 2007).

Often, shade and/or silvopastures have been shown to increase forage nutritive value by way of increasing crude protein (Bambo et al., 2009; Barro et al., 2008; Belesky et al., 2009; Burner, 2003; Kallenbach et al., 2006; Perry et al., 2009; Smith, 1942) or by maintaining or reducing acid detergent fiber (ADF) and neutral detergent fiber (NDF) (Kallenbach et al., 2006). Several studies contradict these findings by reporting that shading results in higher ADF and NDF (Lin et al., 2001; Karki et al., 2009), lower total non-structural carbohydrates (TNC) (Belesky et al., 2006; Belesky et al. 2009; Buergler et al., 2006), and fewer soluble carbohydrates and increased cellulose and lignin concentrations (Hight et al., 1968). Belesky et al., (2006) suggested that some of these results may be caused by a mechanism that some cool-season plants use to enhance light uptake in shaded systems, which involves distributing a higher percentage of nitrogen to leaf tissue, thus increasing the amounts of chloroplasts that are responsible for absorbing sunlight and converting it to energy. This nitrogen is largely unbound and coupled with low amounts of total non-structural carbohydrates facilitated by reduced photosynthetic activity may lead to some reduction in forage quality (Belesky et al., 2006).

Tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.) is the dominant forage base for not only the transition zone, but for much of the United States' improved forage systems (Buckner et al., 1979; West and Waller, 2007). Kentucky 31, a local ecotype of tall

fescue found on a farm in Kentucky in 1931, showed extraordinary agronomic qualities including productivity, persistence, and tolerance to a wide range of environmental and management-related stressors (USDA-NRCS Plant Materials Program, 2006).

Many of fescue's desirable qualities can be attributed to its mutualistic association with the *Epichloë coenophiala* endophyte, a fungus that lives within the plant (Arachevaleta et al., 1989; Latch, 1993; Read and Camp, 1986). However, the fungus also produces alkaloids. Thompson and Stuedemann (1993) have compiled a substantial list of evidence suggesting that these alkaloids are linked to several maladies in cattle, including increased body temperature, decreased weight gains, and lower conception rates, as well as several other symptoms collectively known as "fescue toxicosis" (Ball et al., 2007; Bush and Buckner, 1973; Porter, 1995; Stuedemann and Hoveland, 1988). Concentrations above critical thresholds result in losses in performance and are estimated to cost the cattle industry more than \$1 billion annually (Roberts and Andrae, 2010).

Climatic variables play a role in endophyte and alkaloid production in tall fescue. Water stress was shown to increase ergovaline/ergovalinine in ryegrass (Lane et al., 1997). Predicted increases in atmospheric temperatures were shown to reduce tall fescue populations but increase their ergot alkaloid concentrations by 30-40% (McCulley et al., 2014).

Integrating silvopastures into fescue-based production systems may offer several ways to mitigate the effects of fescue alkaloids. Along with shaded conditions being able to lower animal body temperature and make a more comfortable habitat for grazing livestock (Buffington et al., 1983; Higgins et al., 2011), the altered microclimate under trees may favor the growth of other cool-season grasses and legumes as shown by Buergler et al. (2005).

Incorporating other desirable forage species into pastures has been shown to offset some of the negative effects of fescue toxicosis, which could indirectly reduce the alkaloids at the sward level by diluting the amount of toxic fescue available for animal consumption. The dilution effect was documented by Petriz et al. (1980) on a farm in southern Indiana where cows and calves grazing mixed tall fescue-legume pastures gained more than those grazing fescue pastures, and was slightly better than those grazing orchardgrass pastures (Ball et al., 2007). Cow pregnancy rates followed the same trend (Petriz et al., 1980). Other research has shown similar results when comparing pure tall fescue pastures with mixed fescue-legume pastures in regards to animal productivity (Burns et al., 1973; Harris et al., 1972; Hoveland et al., 1981; McMurphy et al., 1990).

Reduced available sunlight also may have the potential to indirectly limit alkaloid synthesis (Belesky et al., 2009). Because tall fescue feeds the fungus with products from photosynthesis (Bacon and White, 2000), it would follow that alkaloid levels should be greatest when plant nutrients are in abundant supply, such as when growing conditions support high plant productivity and photosynthetic activity. In support of this claim, Belesky et al., (2009) demonstrated slightly reduced ergot alkaloids in shade-grown tall fescue as a function of reduced photosynthates supplied to the fungus.

Alternatively, Davitt et al. (2010) showed that under very heavy shade tall fescue increased its endophyte density by 86%, however ergot alkaloids were not quantified in this study. The authors suggest that plants need other defenses such as alkaloid production against herbivory because of less structural defenses produced in shade grown plants as compared to open-grown plants. Based on these results, there could be an irradiance threshold a plant

needs to experience in order to increase the amount of photosynthate partitioned to the endophyte in light limited environments.

The objective of this study was to understand the influences of shade and forage mixture complexity on nutritive characteristics and ergot alkaloid concentrations of forages with the potential for use in silvopasture systems. Our hypotheses were that increasing shade would increase crude protein and fiber, but would reduce ergot alkaloid concentrations. We also hypothesized that increasing forage mixture complexity would increase forage nutritive characteristics while decreasing ergot alkaloid concentrations.

Procedures

Site Description

This research was conducted at Virginia Tech's Southern Piedmont Agricultural Research and Extension Center (SPAREC) located near Blackstone, VA (37.091889, -77.963632). Soil series at this site are predominantly Seneca sandy loam (Fine-loamy, mixed, thermic Aquic Hapludults) with a small proportion of Durham coarse sandy loam (Fine-loamy, siliceous, semiactive, thermic Typic Hapludults) and are considered prime farmland with capability units of Ite-4 (Soil Survey Staff, 2016). Weather data were collected onsite at SPAREC using a WatchDog 2900ET weather station (Spectrum Technologies, Aurora, IL). Average yearly temperature for SPAREC is 14.5 °C with 112 cm of relatively evenly distributed rainfall per year. Average temperature and rainfall for the duration of the study can be found in Fig. 1. The 2015 growing season showed similar average temperature with 13 cm more rainfall as compared to

the 63-year average for the site. In 2016, the temperature was approximately 1°C warmer and rainfall was 8 cm above the 63-year average.

Experimental Design and Layout

The experimental design was a randomized complete block design with four replications and a factorial treatment arrangement. Treatments included varying levels of shade that could be found in silvopastures (30, 50 and 70% shade) compared to a full sun control and forage species mixture complexity (simple, intermediate and complex). Field position was used as a blocking factor. Plots were 2.74 m x 3.05 m. Alleys between blocks were 3.05 m and distance between plots within blocks was 1.83 m (Fig. 2).

Shade structures were built to create artificial shade over individual plots. Structures were modified from Varella et al. (2011) who concluded that slatted shade structures more closely represented the dappled shade of silvopastures as compared to the uniform light distribution of shade cloth. A portable elevated frame of 2.44 x 3.66 x 0.91 m was constructed using 2"x4" dimensional lumber. Structures were deliberately built to be larger than the plots. This ensured that plots would be covered with the desired amount of shade throughout the day, even when the sun was not directly overhead.

Snow fence was secured to the tops and sides of the structures to create 50% shade. Thirty percent knitted shade cloth (Agriculture Solutions, Strong, ME) was added to the 50% shade structures to create the 70% shade environment. Even though the dappled effect was reduced by using shade cloth, extra slats were not added to structures in order to maintain structural integrity. Half of the slats in the snow fence were removed to create 30% shade (Fig. 3). Shade levels were validated by simultaneously recording PAR beneath structures using a

line quantum sensor (LI-COR, Lincoln, NE) and in adjacent open areas using a PAR sensor (Onset, Bourne, MA). Shade levels were calculated as follows: $100 - (\text{PAR under structure} / \text{PAR in open sun})$.

Forage mixtures cultivars, and seeding rates are described in Table 1. Mixture species were as follows: SIMPLE = tall fescue (*Schedonorus arundinaceus*) and white clover (*Trifolium repens*); INTERMEDIATE = SIMPLE + orchardgrass (*Dactylis glomerata*) and red clover (*T. pretense*); and COMPLEX = INTERMEDIATE + Kentucky bluegrass (*Poa perenne*), alfalfa (*Medicago sativa*), and birdsfoot trefoil (*Lotus corniculatus*). Species included in the mixtures were chosen because they are adapted to the North-South transition zone. All of these species utilize the C₃ photosynthetic pathway which has been shown to be more shade tolerant than C₄ pathway in (Kephart et al., 1992). Some of these species have been shown to exhibit shade tolerance when grown in monoculture (Lin et al., 1999), but they have not been evaluated as part of a mixture, and may express different competitiveness under low light conditions.

Plot Management

A conventional seedbed was prepared by moldboard plowing, disking, field cultivating, and cultipacking. Soil samples were collected to a depth of 10 cm, and initial soil test levels were 14 ppm extractable P and 69 ppm extractable K. Samples were analyzed at Virginia Tech's Soil Testing Laboratory using the Mehlich I procedure (Maguire and Heckendorn, 2009). Prior to seeding, 34, 134, and 157 kg/ha of N, P₂O₅, and K₂O, respectively, was incorporated into the seedbed based on soil test recommendations (Maguire and Heckendorn, 2015). After the initial fertilization, no other amendments were added to the plots.

In April 2015, plots were seeded using a small plot walk-behind cultipack-type seeder (Carter Manufacturing, Brookston, IN). Shade structures were put in place immediately after planting to simulate the shade of forests. Structures were removed the morning of harvest and placed back on plots within 24 h of harvest.

Forages were harvested when they reached a height of approximately 35 cm and were allowed to rest for at least four weeks between cuttings. Harvest occurred only twice in 2015 (15 Jun and 6 Aug) in order to let the forages establish healthy root systems. Harvest occurred five times in 2016 (26 Apr, 26 May, 27 Jun, 12 Aug and 3 Nov).

Measurements

Nutritive Value Concentrations. A 250 g subsample was taken from each plot to determine dry matter and nutritive value concentrations. Samples were weighed fresh and oven dried for three days in a forced air oven at 60°C and reweighed to determine percent dry matter (DM) using the following formula: $([\text{kg dry weight} / \text{kg fresh weight}] * 100)$. Dried subsamples were ground sequentially to pass through 2 and 1 mm screen using Wiley (Thomas Wiley, Philadelphia, PA) and Cyclone (Udy Corporation, Fort Collin, CO) mills, respectively. Crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) were estimated using near infrared reflectance spectroscopy (Foss North America, Eden Prairie, MN) with a robust equation for fresh hay and forage. Total digestible nutrients (TDN) were calculated using the following equation from Virginia Tech's former Forage Testing Lab: $\text{TDN} = 100.32 - 1.118 * \text{ADF}$.

Ergot Alkaloid Concentration. A second 250 g subsample was collected upon harvest, placed in a sealed Ziplock bag, and immediately put on ice. Upon return from the field, samples

were frozen until freeze drying could occur. Samples were freeze dried in an 18 x 40 Flower Dryer (SP Scientific, Gardiner, NY) for ten days using the following cycle: days 1-3 at -30°C, days 4-5 at -17°C, days 6-7 at 0°C, day 8 at 18°C, and days 9-10 at 23.9°C. Dried samples were ground using the above procedure and quantitatively analyzed for total ergot alkaloid concentrations using a phytoscreen PT ergot alkaloid ELISA kit (Agrinostics, Athens, GA). These values were then divided by the proportion of tall fescue in each plot to determine total ergot alkaloid concentration for the tall fescue plant. Samples were collected for all harvests, however only June 2015 – June 2016 results are presented as August and November 2016 samples were not analyzed by the time of this writing.

Data Analysis

SAS 9.4 software (SAS Institute, Cary, NC) was used to analyze data. The General Linear Model procedure was used to generate an ANOVA table. If treatment (shade or mixture) by year interactions were not significant, data was presented across years. When year by treatment interactions occurred, data was analyzed and presented by year. Season by treatment interactions were also evaluated within and across years along with shade by mixture interactions. When appropriate, interaction means are presented and regressions or standard errors were calculated. Regression analysis was performed using SigmaPlot 11.0 (Systat Software, San Jose, CA), and standard errors were calculated using the stderr option for lsmeans in SAS.

Fisher's protected least significant difference post-hoc test was used to separate means for all data except soil moisture. Moisture data were analyzed in SAS 9.4 using the MIXED procedure. Shade was considered a fixed effect, and Kenward Roger denominator degrees of

freedom were calculated using the $ddf = kr$ operation. The `pdiff` option was used to separate soil moisture means. All differences were considered significant at an alpha level of 0.05.

Results and Discussion

Forage Nutritive Characteristics

The weighted average for CP, ADF, NDF, and TDN were analyzed by both season and year. No treatment x year interaction occurred for any nutritive entity; therefore, results were averaged over both years ($P > 0.10$). Although there were no year x treatment interactions, to gain an understanding of seasonal nutritive changes, data were also analyzed by season of harvest (spring, summer, or fall) across both years. When separated by year, all nutritive characteristics exhibited shade x season interactions in both years ($P < 0.01$). Significance for variable responses for individual parameters are presented in Table 7.

Crude Protein: Overall, neither shade nor mixture affected crude protein levels throughout the study ($P > 0.58$). Average values were 19.9% which meets the nutritional requirements of 200 kg steers and dry and lactating cows (Table 9). In 2015, CP was only affected by shade during summer where, as shade increased, so did CP (12.7% in full sun to 16.1% in 70% shade) ($P < 0.001$) (Fig. 26a). In spring and summer 2016, CP was affected by mixture ($P < 0.001$, $P < 0.01$) where the simple mix had lower CP than the intermediate and complex mixtures (spring: 17.1, 19.2, and 19.0% for the simple, intermediate, and complex, respectively; summer: 16.4, 17.6, and 17.3% for the simple, intermediate, and complex, respectively). In fall of 2016, shade increased CP from 15.4% in full sun to 18.8% in 70% shade ($P < 0.01$) (Fig. 26a).

Acid Detergent Fiber: Acid detergent fiber was not affected by either shade or mixture when averaged over the two year study ($P > 0.15$) (Table 4). In 2015, ADF was affected by shade for both spring and summer ($P < 0.001$, $P < 0.01$). During the spring all shade treatments exhibited higher ADF than the full sun treatment (34% vs. 31%). In summer 2015, as shade increased ADF decreased (37% in full sun to 35% under 70% shade). Acid detergent fiber trends were the same in spring 2016, with slightly lower averages (31% under all shade vs. 28% in full sun) ($P < 0.001$). In summer 2016, ADF was highest in 30 and 50% shade (35%) and declined slightly with full sun and 70% shade (34%). Shade did not affect ADF in fall 2016, ($P > 0.06$) (Fig. 27). Acid detergent fiber ranged from 29-36% and was not affected by mixture in any season over both years ($P > 0.16$) (data not shown).

Neutral Detergent Fiber: Averaged over years, NDF was not affected by mixture complexity ($P > 0.40$), but increased with increasing shade (50% in full sun to 54% under 70% shade) ($P < 0.001$) (Table 4). When analyzed by year and season, in 2015, NDF was only affected by shade ($P < 0.001$) (Fig. 28a) and mixture ($P < 0.02$) (Fig. 28b) in spring. During spring 2015, lower NDF was observed in full sun as compared to all shade levels (47% vs. 53%). In 2016 for both spring ($P < 0.001$) and summer ($P < 0.001$), as shade increased so too did NDF (spring: 44% in full sun to 54% under 70% shade; summer: 51% in full sun to 56% under 70% shade) (Fig. 28a). Mixture did not affect NDF for any season in 2016 ($P > 0.40$) (Fig. 28b).

Total Digestible Nutrients: Averaged over both years, TDN was not affected by shade or mixture ($P > 0.15$, $P > 0.28$) (Table 4). When analyzed by seasons in each year, in spring 2015, TDN was reduced from 66% in full sun to 62.5% in shade ($P < 0.001$). In summer 2015, the opposite occurred where, as shade increased, so did TDN (59 to 62% TDN from full sun to 70%

shade) ($P < 0.01$). Total digestible nutrient values acted the same in spring 2016, as in spring 2015, ranging from 69% in full sun to 66% under all shaded treatments ($P < 0.001$). In summer 2016, TDN was highest in full sun and 70% shade (63%) with a slight decrease under 30 and 50% shade (61%) ($P < 0.01$) (Fig. 29). Total digestible nutrients ranged from 60-68% throughout the study and was not affected by mixture during any season in both years ($P > 0.16$) (data not shown).

Overall, there were no shade or mixture effects when evaluating forage quality when averaged over both years. However, when analyzing seasonal changes for both years of the study, during the spring the general trend was for reduced forage quality as shade increased. Some of these results may be explained by decreased photosynthetic capacity resulting in lower TNC concentrations and an increase in cell wall/fiber components as shown by Lin et al. (2001). Perhaps during the time when cool-season forages have their highest growth rates they are more affected by a decrease in potential photosynthetic activity resulting from shading.

Results for the summer in the establishment year pointed towards increased forage quality with increasing shade, which is consistent with Kallenbach et al.'s (2006) findings in Missouri, but this trend did not occur in year two. In 2016, there was 4 percentage units difference between full sun and shaded treatments prior to summer harvests, and although soil moisture data was not collected in 2015, rainfall was slightly below average prior to summer harvest. Environmental factors were roughly equivalent, which would not explain this discrepancy.

In summer 2015, weeds were reduced and orchardgrass was greatly increased beneath shade. Weed nutritive values were not individually analyzed, but the large proportions of

crabgrass in full sun as compared to shade may have resulted in decreased plot nutritive value as compared to orchardgrass-dominated shade plots, as cool-season grasses are generally more digestible than warm-season grasses (Ball et al., 2007). Orchardgrass was always cut at the vegetative state, therefore it should not have shown dramatic decreased forage quality due to maturity. Differences may not have been as apparent in summer 2016 as there was no difference in weediness under shade as compared to full sun and orchardgrass increases were not as dramatic as in 2015.

Forage quality during the fall of the second year was mostly not affected, and soil moisture trends were similar to summer 2016. In an Appalachian silvopasture, Buerger et al. (2006) reported overall similar nutritive characteristics between low/no shade, moderate shade, and high shade systems for both spring and summer, so perhaps there are other factors that need to be considered that we did not measure that would help describe these disparities.

Seasonal crude protein levels were always high enough to meet growing steer requirements regardless of shade level. However, seasonal TDN was only able to meet steer requirements under all shade levels during the spring and fall of 2016, otherwise they were not sufficient by approximately four percentage points. Although steers may need extra energy supplementation at these levels, dry and lactating beef cows' energy requirements would be met throughout the year.

Many other studies have shown increases in crude protein in response to shade (Bambo et al., 2009; Barro et al., 2008; Belesky et al., 2009; Burner, 2003; Kallenbach et al., 2006; Perry et al., 2009; Smith, 1942). Lin et al. (2001) reported similar findings for individual cool season grass species, but not for legumes. Perhaps one reason for the lack of increases in CP in

response to shade in this study is due to the fact that the swards were mixed to include both grasses and legumes. Legumes tend to have higher CP content than grasses (Church and Pond, 1988), so by including substantial proportions of legumes in the swards that typically do not show CP increases as shade increases, the effect on grasses may have been masked (Lin et al., 2001). Perhaps sampling the entire sward for total nutritive concentration may have hidden a shade effect on the nutritive status of individual species.

Ergot Alkaloid Concentration

No year x treatment interactions occurred for sward total ergot alkaloid concentration ($P > 0.07$). However, a shade x mixture interaction occurred where the intermediate and complex mixtures showed no correlation between shade level and ergot alkaloid concentration and stayed within the range of 363 – 676 ppb ($P < 0.03$). Total ergot alkaloids in the simple mixture increased from full sun to 70% shade (1069 to 2184 ppb) (Fig. 30).

This phenomenon can be attributed to the large quantities of tall fescue in the simple mixture as compared to the other two mixtures. As shade increased, tall fescue replaced either white clover or weeds in the simple mixtures (see Fig. 8 and 9, Chapter 3). Therefore, when tall fescue is abundant in swards, the total sward ergot alkaloid concentration will be elevated as well. Ergot alkaloids were reduced by approximately half in full sun systems and by nearly three quarters under shade by incorporating other forage species into the mixture.

When estimating total ergot alkaloid concentration for tall fescue alone, a mixture x year interaction occurred and data were analyzed by individual year ($P < 0.01$). In 2015, fescue ergot alkaloids were highest at 0, 30, and 70% shade and were reduced under 50% shade ($P < 0.03$). Mixture also affected fescue alkaloids in 2015 where the complex mixtures showed

greater alkaloid concentrations than both simple and intermediate mixtures ($P < 0.01$) (Fig. 31a). In 2016, neither mixture nor shade affected fescue ergot alkaloid concentrations, but average concentrations were approximately 50% lower than 2015 ($P > 0.40$) (Fig. 31b).

Belesky and Hill (1997) demonstrated that ergot alkaloid concentration increased as total non-structural carbohydrates increased. Buergler et al. (2006) and Belesky et al. (2006 & 2009) showed decreasing total non-structural carbohydrates under shade. It would follow that shaded plants should exhibit lower TNC and lower ergot alkaloid concentrations than their sun-grown counterparts.

Ergot alkaloids were reduced beneath high shade during the establishment year. Perhaps when plants are young without many energy reserves they may allocate more photosynthates to promote their own growth rather than aiding the endophyte as suggested by Belesky and Hill (1997). During the second year there was no relationship between shade level and ergot alkaloid concentration. These results may indicate that once the plant establishes, alkaloid concentrations may not be affected by the reduced irradiance. To our knowledge, there are no other findings as to whether shade alters total ergot alkaloid concentration in tall fescue. To better understand the mutualism between tall fescue and its symbiotic endophyte, future studies should focus on quantifying ergot alkaloid concentrations, endophyte abundance, and resource transfer between plant and fungus.

Summary and Implications

Sward nutritive value varied throughout the course of the experiment and seemed to be more affected by botanical composition than by environmental factors during the summers.

Although even during the summer when TDN and CP were the lowest for the year, values were always high enough to meet the metabolic requirements for lactating cows. However, during the spring, quality reductions may occur beneath shade when forages have their highest energy requirements, but values are still sufficient to support lactating cows.

Nutritive value should not be the only determinant of silvopasture suitability for grazing systems in the Southeast. Pent and Fike (2016) and Kallenbach et al. (2006) showed similar or greater gains for livestock grazing silvopastures compared to open pastures despite the differences in forage yield and quality. These authors speculate that tree presence moderates the microclimate in both summer and winter to reduce temperature stress on the animals, thereby compensating for either reductions in forage yield or quality.

Total ergot alkaloids in the sward only increased in shaded simple mixtures because the proportion of tall fescue was higher in these treatments. Once forages became established (2016), there were no differences in ergot alkaloid concentrations in tall fescue with regards to shade level or mixture complexity. This demonstrates the importance of diluting the sward with other non-alkaloid producing species, as total sward ergot alkaloid concentration was reduced by 70% when two or more species were added to a simple fescue-clover forage mixture.

In addition to our findings, other studies have shown improvements in livestock performance (animal gains and pregnancy rates) on cattle grazing fescue-legume as compared to fescue only pastures (Burns et al., 1973; Harris et al., 1972; Hoveland et al., 1981; McMurphy et al., 1990; Petriz et al., 1980). In addition to our own, these results show the importance of

diluting the sward with other species that do not produce ergot alkaloids in order to facilitate competitive exclusion of alkaloid production.

When managed with rotational stocking, these shaded forage mixtures should be able to provide adequate nutrition throughout most of the year for both dry and lactating cows. The inclusion of red clover and orchardgrass shows promising potential to decrease total ergot alkaloid concentration of shaded swards, as they both exhibited greater shade tolerance and competitiveness as compared to tall fescue when all three species were grown together in a mixture. The shaded nature of silvopastures may also modify the microclimate in a way that improves livestock comfort, which may also help alleviate some heat-stress associated with fescue toxicosis.

Literature Cited

- Arechavaleta, M., C.W. Bacon, C.S. Hoveland, and D.E. Radcliffe. 1989. Effect of the tall fescue endophyte on plant response to environmental stress. *Agron. J.* 81:83-90.
- Bacon, C.W. and J.F. White, Jr. 2000. Physiological adaptations in the evolution of endophytism in the Clavicipitaceae. p. 237-261. *In*: C. W. Bacon and J.F. White (eds.) *Microbial endophytes*. CRC Press, New York.
- Ball, D.M., C.S. Hoveland, and G.D. Lacefield. 2007. *Southern forages*. 4th ed. International Plant Nutrition Institute, Norcross, GA.
- Bambo, S.K., A.R. Blount, J. Nowak, A.J. Long, R.O. Myer, and D.A. Huber. 2009. Annual cool-season forage nutritive value and production after overseeding into bahiagrass sod in silvopastoral systems. *Agroecol. Sustain. Food Sys.* (previously *J. Sustain. Agric.*) 33:917-934.
- Barro, R.S., J.C. de Saibro, R.B. de Medeiros, J.L.S. da Silva, A.C. Varella. 2008. Forage yield and nutritive value of cool-season annual forage grasses shaded by *Pinus elliottii* trees and at full-sun. *Revista Brasileira de Zootecnia (Brazilian J. Anim. Sci.)* 37:1721-1727.

- Belesky, D.P. and N.S. Hill. 1997. Defoliation and leaf age influence on ergot alkaloids in tall fescue. *Ann. Bot.* 79:259-264.
- Belesky, D.P., J.M. Ruckle, L.P. Bush. 2009. Microsite conditions influence nutritive value characteristics of a tall fescue cultivar devoid of, or infected with a native, or a novel non-ergogenic endophyte. *Environ. Exp. Bot.* 67:284-292.
- Belesky, D.P., N.J. Chatterton, and J.P.S. Neel. 2006. *Dactylis glomerata* growing along a light gradient in the Central Appalachian Region of the Eastern USA: III. Nonstructural carbohydrates and nutritive value. *Agrofor. Sys.* 67(1):55-51.
- Buckner, R.C., J.B. Powell, and R.V. Frakes. 1979. Historical development. p. 1-8. *In*: R.C. Buckner and L.P. Bush (eds.), *Tall Fescue*. Agron. Monogr. 20. ASA, CSSA, SSSA, Madison, WI.
- Buffington, D.E., R.J. Collier, and G.H. Canton. 1983. Shade management systems to reduce heat stress for dairy cows in hot, humid climates. *Trans. ASAE* 26:1798-1802.
- Buergler A. L., J. H. Fike, J. A. Burger, C. M. Feldhade, J. R. McKenna, and C. D. Teutsch. 2005. Botanical composition and forage production in an emulated silvopasture. *Agron. J.* 97:1141-1147.
- Buergler A. L., J. H. Fike, J. A. Burger, C. M. Feldhade, J. R. McKenna, and C. D. Teutsch. 2006. Forage nutritive value in an emulated silvopasture. *Agron. J.* 98:1265-1273.
- Burner, D.M. 2003. Influence of alley crop environment on orchardgrass and tall fescue herbage. *Agron. J.* 95:1163-1171.
- Burns, J.C., L. Goode, H.D. Gross, and A.C. Linnerud. 1973. Cow and calf gains on ladino clover-tall fescue and tall fescue, grazed alone and with coastal bermudagrass. *Agron. J.* 65:877-880.
- Bush, L. and R.C. Buckner. 1973. Tall fescue toxicity. p. 99-112. *In*: A.G. Matches (ed.) *Antiquity components of forages*. CSSA Spec. Publ. 4. Madison, WI.
- Church, D.C. and W.G. Pond. 1988. *Basic animal nutrition and feeding* (3rd ed.). John Wiley and Sons, New York.
- Clason, T.R. and S.H. Sharrow. 2000. Silvopastoral practices. p. 119-147. *In*: H.E. Garret, W.J. Rietveld, and R.F. Fisher (eds.) *North American agroforestry: an integrated science and practice*. ASA-CSSA-SSSA. Madison, WI.
- Davitt, A.J., M. Stansberry, and J.A. Rudgers. 2010. Do the costs and benefits of fugal endophyte symbiosis vary with light availability? *New Phytol.* 188:824-834.

- Feldhake, C.M. 2002. Forage frost protection potential of conifer silvopastures. *Agric. Meteorol.* 112:123-190.
- Fike, J.H., A.L. Buegler, J.A. Burger, and R.L. Kallenbach. 2004. Considerations for establishing and managing silvopastures. Online. *Forage and Grazinglands*. doi:10.1094/FG-2004-1209-01-RV.
- Gold, M. and A. Mason. 2015. Defining Agroforestry. p. 9-15. *In: Training manual for applied agroforestry practices, 2015 edition.* University of Missouri Center for Agroforestry, Columbia, MO.
- Harris, R.R., E.M. Evans, J.K. Boseck, and W.B. Webster. 1972. Fescue, orchardgrass, and coastal bermudagrass grazing for yearling beef steers. *Alabama Agric. Exp. Stn. Bull.* 432.
- Higgins et al. 2011. Shade options for grazing cattle. University of Kentucky Cooperative Extension Bull. AEN-99. Lexington, KY.
- Hight, G.K., D.P. Sinclair, and R.J. Lancaster. 1968. Some effects of shading and of nitrogen fertilizer on the chemical composition of freeze-dried and oven-dried herbage, and on the nutritive value of oven-dried herbage fed to sheep. *N. Z. J. Agric. Res.* 11:286-302.
- Hoveland, C.S., R.R. Harris, E.E. Thomas, E.M. Clark, J.A. McGuire, J.T. Eason, and M.E. Ruf. 1981. Tall fescue with ladino clover or birdsfoot trefoil as pasture for steers in northern Alabama. *Alabama Agric. Exp. Stn. Bull.* 530.
- Kallenbach, R.L., M.S. Kerley, and G.J. Bishop-Hurley. 2006. Cumulative forage production, forage quality and livestock performance from an annual ryegrass and cereal rye mixture in a pine-walnut silvopasture. *Agrofor. Sys.* 66:43-53.
- Karki, U. and M.S. Goodman. 2010. Landscape use by cattle in silvopasture versus open pasture. *Agrofor. Sys.* 78:159-168.
- Karki, U. and M.S. Goodman. 2015. Microclimatic differences between mature loblolly-pine silvopasture and open-pasture. *Agrofor. Sys.* 89:319-325.
- Karki, U., M.S. Goodman, and S.E. Sladden. 2009. Nitrogen source influences on forage and soil in young southern-pine silvopasture. *Agric. Ecosys. Environ.* 131:70-76.
- Kephart K.D., D.R. Buxton, and S.E. Taylor. 1992. Growth of C₃ and C₄ perennial grasses in reduced irradiance. *Crop Sci.* 32:1033-1038.
- Lane, G.A., M.J. Christensen, and C.O. Miles. 2000. Coevolution of fungal endophyte with grasses: the significance of secondary metabolites. p. 341-388. *In: C.W. Bacon and J. White (eds.) Microbial endophytes.* Marcel Dekker, New York, NY.

- Latch, G.C.M. 1993. Physiological interactions of endophytic fungi and their hosts. Biotic stress tolerance imparted to grasses by endophytes. *Agric. Ecosyst. Environ.* 44:143-156.
- Lin, C.H., R.L. McGraw, M.F. George, and H.E. Garrett. 2001. Nutritive quality and morphological development under partial shade of some forage species with agroforestry potential. *Agrofor. Sys.* 53:269-281.
- Maguire, R.O. and S.E. Heckendorn. 2009. Laboratory procedures: Virginia Tech Soil Testing Laboratory. Virginia Cooperative Extension publication 452-881. Blacksburg, VA.
- Maguire, R.O. and S.E. Heckendorn. 2015. Soil test recommendations for Virginia. Virginia Cooperative Extension. Blacksburg, VA.
- McCulley, R.L., L.P. Bush, A.E. Carlisle, H. Ji, and J.A. Nelson. 2014. Warming reduces tall fescue abundance but stimulates toxic alkaloid concentrations in transition zone pastures of the U.S. *Front. Chem.* 2:1-14.
- McMurphy, W.E., K.S. Lusby, S.C. Smith, S.H. Muntz, and C.A. Strasia. 1990. Steer performance on tall fescue pastures. *J. Prod. Agric.* 3:100-102.
- Mosquera-Losada, M.R. and A. Rigueiro-Rodriguez. 2013. Silvopasture: a combination of grasslands and trees to green livestock production. p. 475-477. *In: A. Helgadottir and A. Hopkins (eds.) The role of grasslands in a green future, threats and perspectives in less favoured areas. Proc. 17th Symp. Eur. Grassl. Fed., Akureyri, Iceland. 23-26 June 2013.*
- Pent, G.J. and J.H. Fike. 2016. Sheep performance, grazing behavior, and body temperatures in silvopasture systems. *In: Proc. Am. Forage and Grassl. Council. Baton Rouge, LA, January 13-15, 2016.*
- Perry, M.E.L., W.H. Schacht, G.A. Ruark, and J.R. Brandle. 2009. Tree canopy effect on grass and grass/legume mixtures in eastern Nebraska. *Agrofor. Sys.* 77:23-35.
- Petriz, D.C., V.L. Lechtenberg, and W.H. Smith. 1980. Performance and economic returns of beef cows and calves grazing grass-legume herbage. *Agron. J.* 72:581-584.
- Pierson, E.A., R.N. Mack, and A. Black. 1990. The effect of shading on photosynthesis, growth, and regrowth following defoliation for *Bromus tectorum*. *Oecologia* 84:534-543.
- Porter, J.K. 1995. Analysis of endophyte toxins: fescue and other grasses toxic to livestock. *J. Anim. Sci.* 73:871-880.
- Read, J.C. and B.J. Camp. 1986. The effect of the fungal endophyte *Acremonium coenophialum* in tall fescue on animal performance, toxicity, and stand maintenance. *Agron. J.* 78:848-850.

- Roberts, C. and J. Andrae. 2010. Fescue toxicosis and management. ASA, CSSA, and SSSA, Madison, WI. doi: 10.2135/2010.fescuetoxicosis.
- Smith, R.M. 1942. Some effects of black locusts and black walnuts on southeastern Ohio pastures. *Soil Sci.* 53:385-389.
- Stuedemann, J.A. and C.S. Hoveland. 1988. Fescue endophyte: history and impact on animal agriculture. *J. Prod. Agric.* 1(1):39-44.
- Thompson, F.N. and J.A. Stuedemann. 1993. Pathophysiology of fescue toxicosis. *Agric. Ecosyst. Environ.* 44:263-281.
- USDA NRCS Plant Materials Program. 2006. Tall fescue. Plant fact sheet. USDA NRCS. Accessed 27 October 2016. https://plants.usda.gov/factsheet/pdf/fs_loar10.pdf.
- Varella, A.C., D.J. Moot, K.M. Pollock, P.L. Peri, and R.J. Lucas. 2011. Do light and alfalfa responses to cloth and slatted shade represent those measured under an agroforestry system? *Agrofor. Syst.* 81:157-173.
- Walter, D. 2015. Silvopasture. p. 50-66. *In*: Training manual for applied agroforestry practices, 2015 edition. University of Missouri Center for Agroforestry, Columbia, MO.
- West, C.P. and J.C. Waller. 2007. Forage systems for humid transition areas. p. 313-321. *In*: Forages. Vol. 2, 6th ed. Iowa State Press, Ames.

Chapter 6. Summary

Silvopastures incorporate trees into forage/livestock systems utilizing intensive management strategies and are designed to reap the benefits of synergistic interactions between the tree, forage, and livestock components. As compared to their open pasture counterparts, silvopastures have been shown to increase total system outputs, enhance environmental services, and be aesthetically pleasing. In the Southeast U.S., another incentive for silvopasture use is to provide shade for livestock during the summer months when cattle are more prone to heat stress.

The microclimate created in silvopastures can vary greatly due to differences in individual soil types, understory vegetation, livestock, and tree components. Therefore, many findings provide conflicting results as to whether silvopastures have a positive or negative effect on forage quantity and quality.

The forage that is most commonly utilized in the transition zone of the U.S. is tall fescue, a hardy, shade tolerant cool-season grass. Many of the traits that make fescue desirable arise from its mutualistic association with an endophytic fungus. In addition to enhancing fescue's tolerance to a wide range of stressors, the fungus produces chemical compounds that are harmful to livestock, resulting in fescue toxicosis. Cattle plagued by fescue toxicity often fail to shed winter coats and experience vasoconstriction, resulting in heat stress. This decreases growth rates and reproductive performance. These symptoms, among others associated with fescue toxicosis, cost the livestock industry over \$1 billion annually.

Integrating trees into fescue-based pastures may offer some relief from fescue toxicosis symptoms by alleviating livestock heat stress and by shifting botanical composition away from

fescue dominance to a more diverse forage base, thus diluting the amount of toxins available for consumption. In addition, shading may help decrease alkaloid production by reducing the amount of photosynthates partitioned to the fungus, a hypothesis that had not previously been tested.

In order to better understand how shade may influence forages in silvopasture systems, this research evaluated the impact of increasing shade intensity on forage mixtures of varying species complexity. Each mixture contained tall fescue and up to six additional species. This allowed us to evaluate both shade and pasture composition effects on several forage productivity and quality parameters.

Results indicated that yield reductions may occur in moderately to heavily shaded silvopastures, but forage productivity under lightly shaded silvopastures can match or exceed those found in open pastures, especially when utilizing several shade tolerant species. Increased plot height beneath shade did not translate to increased yields, and if using a standard grazing stick as a measure of forage availability, caution will need to be used so that forage biomass is not overestimated since stands are less dense beneath heavy shade.

Birdsfoot trefoil, Kentucky bluegrass, and white clover have previously shown some shade tolerance, but they did not perform well in our shaded mixtures. In general, these species each made up less than 2% of shaded swards. Tall fescue showed increased yields beneath shade in simple mixtures, but red clover and orchardgrass dominated shaded swards when included in intermediate and complex mixtures. This demonstrates a potential opportunity to integrate cool-season forage species that may not be as well-suited to Virginia's

hot summers into grazing systems. This opportunity arises from the ability of trees to modify the microclimate through shading, making the environment more favorable for these species.

Like seasonal yield, some nutritive values also declined beneath shade in spring and fall. The filtered light may not have supplied enough energy to meet the rapid growth rates of cool-season forages during these times. However, light shade has the potential to increase yields (especially during the summer months), maintain forage quality, and extend the grazing season of cool-season forages into both the summer and winter months due to the moderated microclimate. Throughout the study, crude protein was high enough to meet metabolic requirements for all beef animal classes, which is often the case with cool-season dominated pastures. However, during the summer steers may need energy supplementation in order to attain acceptable production goals whether grazing open or shaded plots.

A more diverse sward led to depressed ergot alkaloid concentrations. At the plant level, there were no increases in ergot alkaloid concentration once forages became established. During the first year of the study, fescue alkaloid concentrations could have been reduced under shade because the plant may not have been able to allocate as many resources to the fungus when it needed energy to meet its own needs. This may be similar to the effect that shade had on nutritive quality where low light levels may not have been sufficient to meet growth demands.

Even though our results show that total forage production or nutritive value may be sacrificed during some portion of the year, silvopasture systems exhibit many other redeeming factors. One potential benefit of incorporating trees into a grazing system is the ability to

provide a cooler grazing environment for livestock during the summer. This aspect is especially desirable for farms with fescue toxicity concerns.

The possibility of maintaining or improving animal gains, diversifying income, and improving ecosystem services makes silvopastures intriguing. However, information regarding design and economic feasibility of incorporating silvopastures into traditional grazing systems in the Mid-Atlantic region is lacking. Future research and demonstrations addressing these issues are key to facilitating adoption of silvopastures in the Mid-Atlantic region.

Table 1. Forage mixtures and seeding rates.

Mixture and species	Cultivar	Seeding rate	Seed Coating	Pure Seed	Germ	Hard seed	PLS
		kg PLS/ha					
<u>Simple Mixture</u>							
Tall fescue (<i>Schedonorus arundinaceus</i>)	Kentucky-31 E+	28.0	0.00	1.00	0.85	n/a	0.85
White clover (<i>Trifolium repens</i>)	Will	2.2	0.34	0.66	0.80	0.10	0.53
Total seed		30.2					
<u>Intermediate Mixture</u>							
Tall fescue (<i>Schedonorus arundinaceus</i>)	Kentucky-31 E+	13.4	0.00	1.00	0.85	n/a	0.85
Orchardgrass (<i>Dactylis glomerata</i>)	Benchmark Plus	6.7	0.38	0.61	0.85	n/a	0.52
White clover (<i>Trifolium repens</i>)	Will	2.2	0.34	0.66	0.80	0.10	0.53
Red clover (<i>Trifolium pratense</i>)	Cinnamon Plus	6.7	0.34	0.66	0.80	0.10	0.53
Total seed		29.1					
<u>Complex Mixture</u>							
Tall fescue (<i>Schedonorus arundinaceus</i>)	Kentucky-31 E+	11.2	0.00	1.00	0.85	n/a	0.85
Orchardgrass (<i>Dactylis glomerata</i>)	Benchmark Plus	6.7	0.38	0.61	0.85	n/a	0.52
Kentucky bluegrass (<i>Poa pratensis</i>)	Balin	4.5	0.00	0.98	0.77	n/a	0.75
White clover (<i>Trifolium repens</i>)	Will	1.1	0.34	0.66	0.80	0.10	0.53
Red clover (<i>Trifolium pratense</i>)	Cinnamon Plus	2.2	0.34	0.66	0.80	0.10	0.53
Alfalfa (<i>Medicago sativa</i>)	Evermore	2.2	0.34	0.66	0.80	0.10	0.53
Birdsfoot trefoil (<i>Lotus corniculatus</i>)	Norcen	2.2	0.00	0.98	0.77	0.13	0.75
Total seed		30.2					
<u>Border Packets</u>							
Tall fescue (<i>Schedonorus arundinaceus</i>)	Kentucky-31 E+	28.0	0.00	1.00	0.85	n/a	0.85
Total seed		28.0					

Table 2. Summary of the frequency and timing of measurements taken.

Variable	Harvest Date						
	Jun '15	Aug '15	Apr '16	May '16	Jun '16	Aug '16	Nov '16
Plot Height	X	—	X	X	X	X	X
Yield	X	X	X	X	X	X	X
Dry Matter	X	X	X	X	X	X	X
Nutritive Concentrations	X	X	X	X	X	X	X
Botanical Composition	X*	X*	X	X	X	X	X
Ergot Alkaloid Concentrations	X	X	X	X	X	X	X
Soil Temperature and Moisture	—	—	X	X	X	X	X
Sward Diversity	*	*	X	X	X	X	X
Ground Cover Rating	—	—	—	—	X	X	X
Sward Density	—	—	—	—	—	X	X

* weeds were not separated into species; only planted species diversity was calculated

Table 3. Significance of variables from ANOVA analysis of annual and seasonal yield

<i>Response</i>	<i>Variable (Step 1)</i>							<i>Variable (Step 2 if interaction occurred in step 1)</i>			
	rep (year)	year	mix	shade	mix*year	shade*year	mix*shade	rep	mix	shade	mix*shade
Annual Yield	*	***	ns	***	ns	ns	ns				
Seasonal Yield											
Spring	**	***	**	***	*	ns	ns				
2015								*	ns	*	ns
2016								ns	ns	*	ns
Summer	**	*	ns	***	ns	*	***				
2015								**	ns	***	ns
2016								*	ns	***	*
Fall (2016)								*	***	***	ns

* = < 0.05; ** = < 0.01; *** = < 0.001

Table 4. Significance of variables from ANOVA analysis of botanical composition.

<i>Response</i>	<i>Variable (Step 1)</i>				<i>Variable (Step 2 if interaction occurred in step 1)</i>	
	rep (season)	season	shade	shade*season	rep	shade
Botanicals 2015						
Simple						
Tall Fescue	ns	ns	**	ns		
White Clover	ns	ns	*	ns		
Weeds	ns	ns	*	ns		
Intermediate						
Tall Fescue	ns	*	*	ns		
White Clover	*	ns	ns	ns		
Weeds	ns	ns	**	ns		
Orchardgrass	ns	***	***	***		
Spring					ns	***
Summer					ns	***
Red Clover	ns	***	*	ns		
Complex						
Tall Fescue	ns	**	***	ns		
White Clover	ns	ns	ns	ns		
Weeds	ns	*	***	ns		
Orchardgrass	ns	**	***	ns		
Red Clover	ns	**	**	ns		
Kentucky Bluegrass	ns	**	*	ns		
Alfalfa	ns	ns	ns	ns		
Birdsfoot Trefoil	ns	ns	ns	ns		
Botanicals 2016						
Simple						
Tall Fescue	*	***	***	ns		
White Clover	ns	***	***	**		
Spring					ns	***
Summer					ns	***
Fall					ns	ns
Weeds	ns	***	ns	ns		
Intermediate						
Tall Fescue	*	***	ns	ns		
White Clover	*	*	***	ns		
Weeds	ns	***	**	ns		
Orchardgrass	**	***	***	ns		
Red Clover	ns	***	ns	ns		
Complex						
Tall Fescue	*	***	*	ns		
White Clover	ns	*	***	ns		
Weeds	ns	***	*	ns		
Orchardgrass	ns	***	***	ns		
Red Clover	ns	***	*	**		
Spring					ns	ns
Summer					*	***
Fall					ns	ns
Kentucky Bluegrass	ns	*	*	ns		
Alfalfa	ns	ns	**	ns		
Birdsfoot Trefoil	ns	ns	ns	ns		

* = < 0.05; ** = < 0.01; *** = < 0.001

Table 5. Significance of variables from ANOVA analysis of diversity, plot height, groundcover, compressed sward height, and compressed sward density.

Response	Variable (Step 1)							Variable (Step 2 if interaction occurred in step 1)							Variable (Step 3 if interaction occurred in step 2)			
	rep (year)	year	mix	shade	mix*year	shade*year	mix*shade	rep (season)	season	mix	shade	mix*season	shade*season	mix*shade	rep	mix	shade	mix*shade
Desired Species Diversity	**	**	***	**	ns	***	***											
2015								*	ns	***	***	ns	ns	***				
2016								*	***	***	***	**	***	**				
Spring															ns	***	***	ns
Summer															ns	***	*	*
Fall															*	***	ns	ns
Response	Variable (Step 1)							Variable (Step 2 if interaction occurred in step 1)										
	rep (season)	season	mix	shade	mix*season	shade*season	mix*shade	rep	mix	shade	mix*year	shade*year	mix*shade					
Total Species Diversity (2016)	***	***	***	*	ns	ns	ns											
Functional Group Diversity (2016)	***	***	ns	**	ns	ns	ns											
Plot Height	**	***	***	***	***	***	**											
Spring (2015 & 2016)								**	***	***	ns	ns	ns					
Summer (2016)								*	***	***	na	na	***					
Fall								*	**	*	***	na	na	*				
Groundcover (Summer & Fall 2016)	ns	ns	***	***	na	na	na											
Compressed Sward Height	ns	***	**	***	*	***	ns											
August 2016								ns	ns	***	na	na	ns					
November 2016								ns	***	***	na	na	ns					
Compressed Sward Density (Aug & Nov '16)	***	***	ns	**	ns	ns	ns											

* = < 0.05; ** = < 0.01; *** = < 0.001

Table 6. Significance of variables from ANOVA analysis of soil temperature and moisture.

<i>Response</i>	<i>Variable (Step 1)</i>				<i>Variable (Step 2 if interaction occurred in step 1)</i>	
	rep (month)	month	shade	shade*month	rep	shade
Minimum Soil						
Temperature (2016)	ns	***	*	ns		
Average Soil						
Temperature (2016)	ns	***	***	ns		
Maximum Soil						
Temperature (2016)	ns	***	***	***		
June					ns	ns
July					ns	*
August					ns	*
September					ns	**
October					ns	ns
Soil Moisture (2016)						
Rain Event 1	na	na	***	na		
Rain Event 2	na	na	***	na		
Rain Event 3	na	na	***	na		
Rain Event 4	na	na	***	na		
Rain Event 5	na	na	***	na		
Rain Event 6	na	na	***	na		
Rain Event 7	na	na	***	na		
Rain Event 8	na	na	***	na		
Rain Event 9	na	na	***	na		
Rain Event 10	na	na	ns	na		
Rain Event 11	na	na	ns	na		
Rain Event 12	na	na	***	na		

* = < 0.05; ** = < 0.01; *** = < 0.001

Table 7. Significance of variables from ANOVA analysis of ergot alkaloid concentrations for sward and tall fescue.

<i>Response</i>	<i>Variable (Step 1)</i>							<i>Variable (Step 2 if interaction occurred in step 1)</i>						
	rep (year)	year	mix	shade	mix*year	shade*year	mix*shade	rep (harvest)	harvest	mix	shade	mix*harvest	shade*harvest	mix*shade
Sward Ergot Alkaloid Concentration	***	ns	***	**	ns	ns	*							
Tall Fescue Ergot Alkaloid Concentration	***	***	**	*	**	ns	ns							
2015								***	***	**	*	ns	ns	ns
2016								***	ns	ns	ns	ns	ns	ns
		<i>Variable (Step 1)</i>												
<i>Response</i>	rep	mix	shade	mix*shade										
Crude Protein														
Spring 2015	**	ns	ns	ns										
Summer 2015	ns	ns	***	ns										
Spring 2016	*	***	**	ns										
Summer 2016	*	**	ns	ns										
Fall 2016	*	ns	**	ns										
Acid Detergent Fiber														
Spring 2015	***	ns	***	ns										
Summer 2015	ns	ns	**	ns										
Spring 2016	ns	ns	***	ns										
Summer 2016	ns	ns	**	ns										
Fall 2016	*	ns	ns	ns										
Neutral Detergent Fiber														
Spring 2015	ns	*	***	ns										
Summer 2015	ns	ns	ns	ns										
Spring 2016	ns	ns	***	ns										
Summer 2016	***	ns	***	ns										
Fall 2016	*	ns	ns	ns										
Total Digestible Nutrients														
Spring 2015	***	ns	***	ns										
Summer 2015	ns	ns	**	ns										
Spring 2016	ns	ns	***	ns										
Summer 2016	ns	ns	**	ns										
Fall 2016	*	ns	ns	ns										

* = < 0.05; ** = < 0.01; *** = < 0.001

Table 8. The effect of shade on soil moisture in 2016. Shade treatments containing the same letter within each rainfall event are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

Rainfall Event	Shade Level (%)			
	0	30	50	70
1 5/20-5/26	B	B	A	A
2 5/27-6/2	B	A	A	A
3 6/3-6/14	B	A	A	A
4 6/15-6/21	B	A	A	A
5 6/22-7/5	B	A	A	A
6 7/6-7/12	B	A	A	A
7 7/13-7/29	B	A	A	A
8 7/30-9/1	B	A	A	A
9 9/2-9/19	C	B	B	A
10 9/20-9/24			ns	
11 9/25-10/6			ns	
12 10/7-10/29	C	A	AB	B

Table 9. Forage nutritive characteristics for each treatment averaged over the two year study. Treatments within either shade or mixture level containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

Treatment	Nutritive Characteristic			
	CP*	ADF	NDF	TDN
0% Shade	19.5 a	32.4 a	50.4 c	64.1 a
30% Shade	19.4 a	33.5 a	52.9 b	62.8 a
50% Shade	20.3 a	33.0 a	53.2 ab	63.4 a
70% Shade	20.4 a	32.6 a	54.5 a	63.9 a
Simple mix	19.9 a	32.9 a	52.7 a	63.5 a
Intermediate mix	20.5 a	33.3 a	53.2 a	63.1 a
Complex mix	19.2 a	32.5 a	52.3 a	64.0 a

* CP = crude protein, ADF = acid detergent fiber, NDF = neutral detergent fiber, TDN = total digestible nutrients

** Beef cattle requirements: dry cow = 7.5% CP, 50% TDN
lactating cow = 11% CP, 60% TDN
200 kg steer = 12% CP, 65% TDN

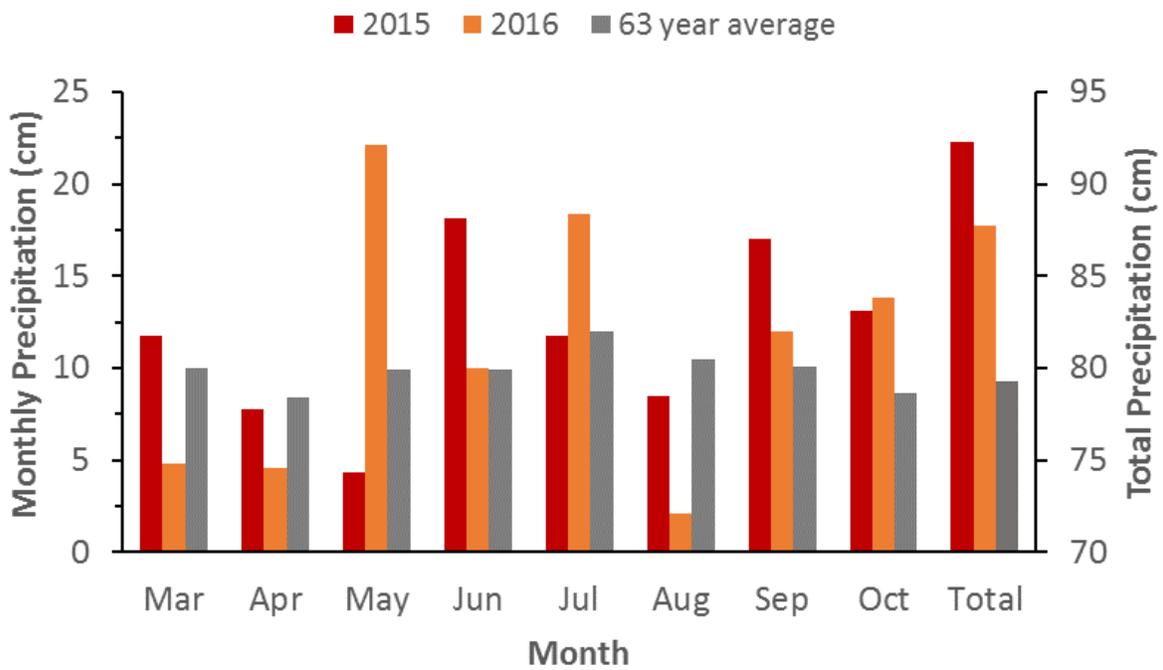
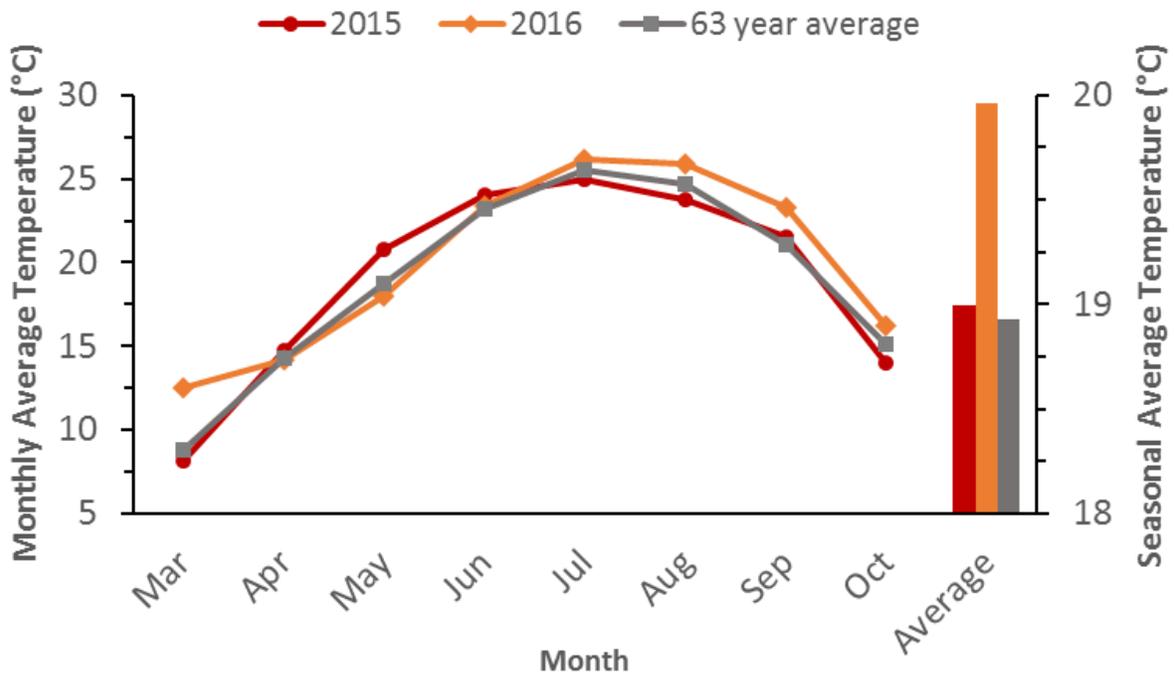


Figure 1. Average temperature and rainfall for Blackstone, VA, in 2015 and 2016.



Figure 3. Portable shade structures: 30% shade (top), 50% shade (middle), 70% shade (bottom).

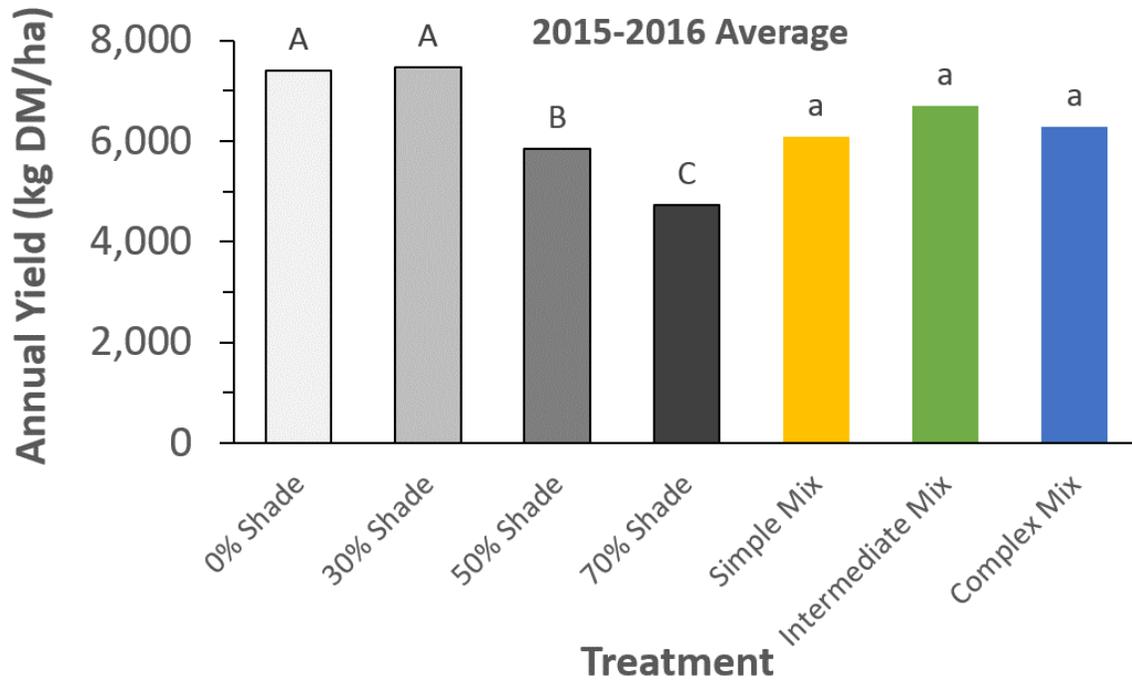


Figure 4. Annual yield response to shade and mixture main effects averaged over the two year study. Bars in each treatment category (shade or mixture) with the same letters are not different according to Fisher's protected least significant difference ($\alpha = 0.05$).

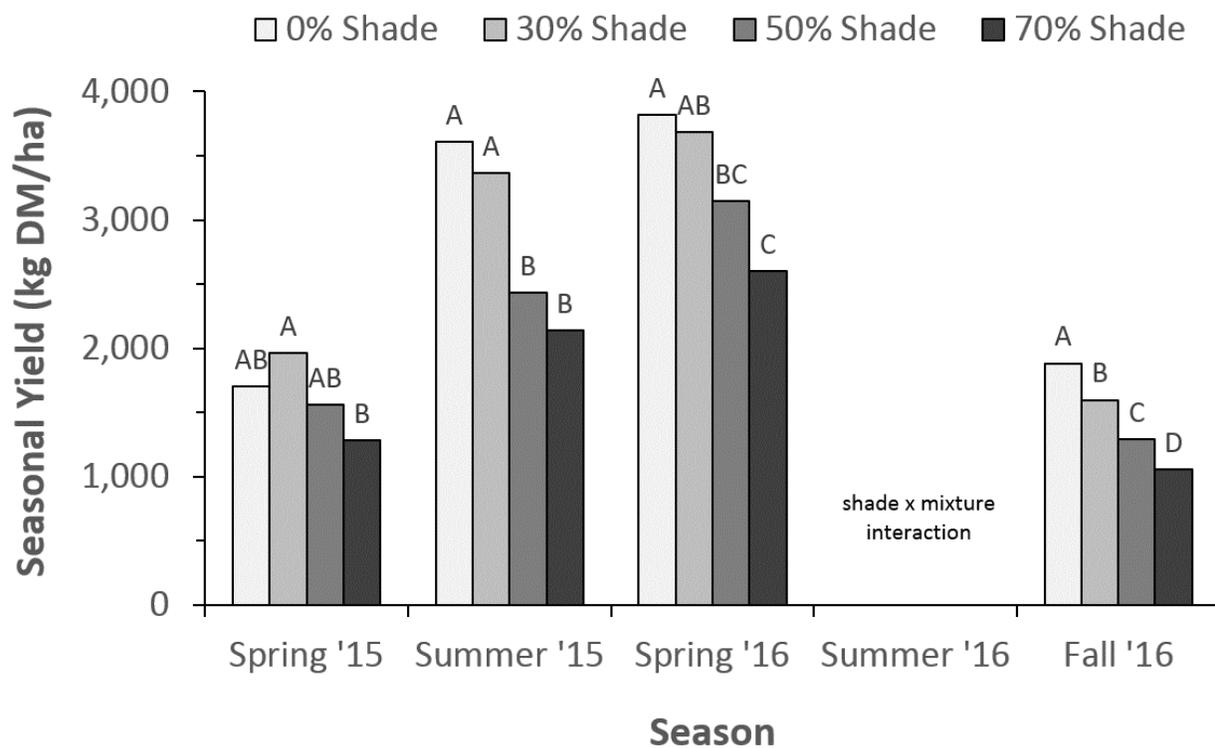


Figure 5. Seasonal yield responses to shade treatments. Treatments within seasons containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

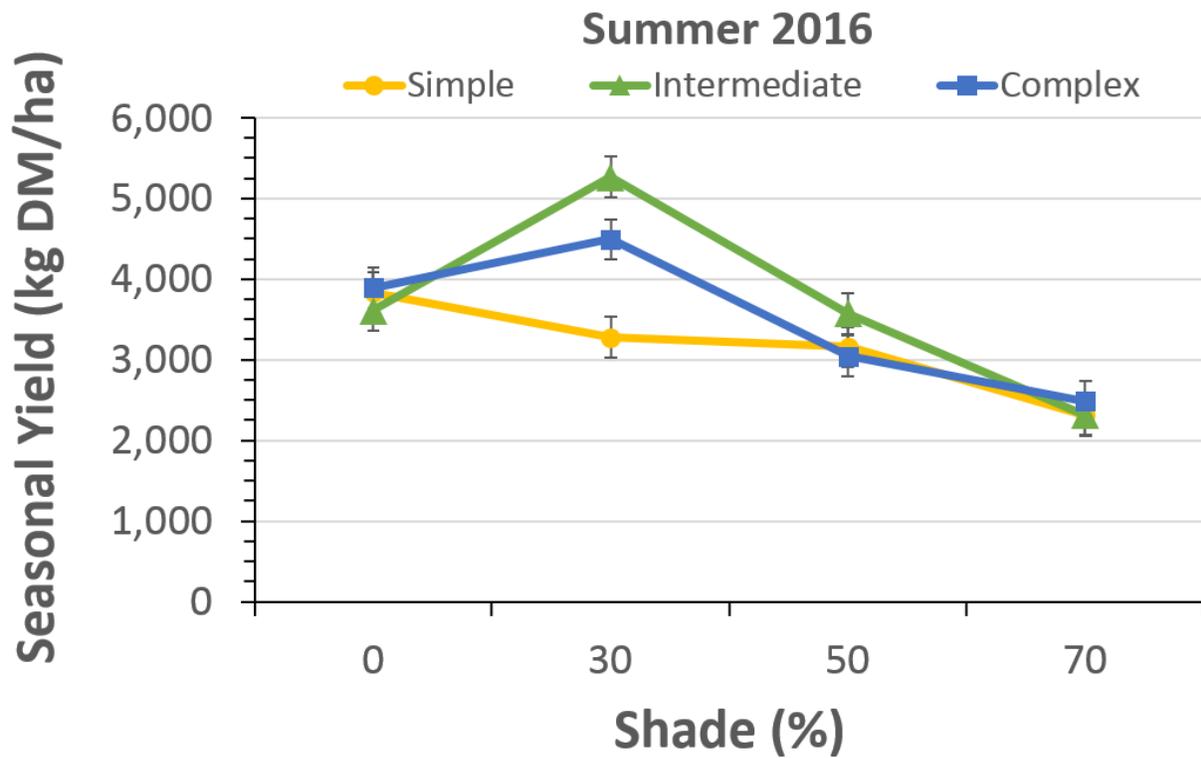


Figure 6. Impact of shade on the yield of simple, intermediate, and complex mixtures for the summer of 2016 harvests (standard error = 125.5 kg DM/ha).

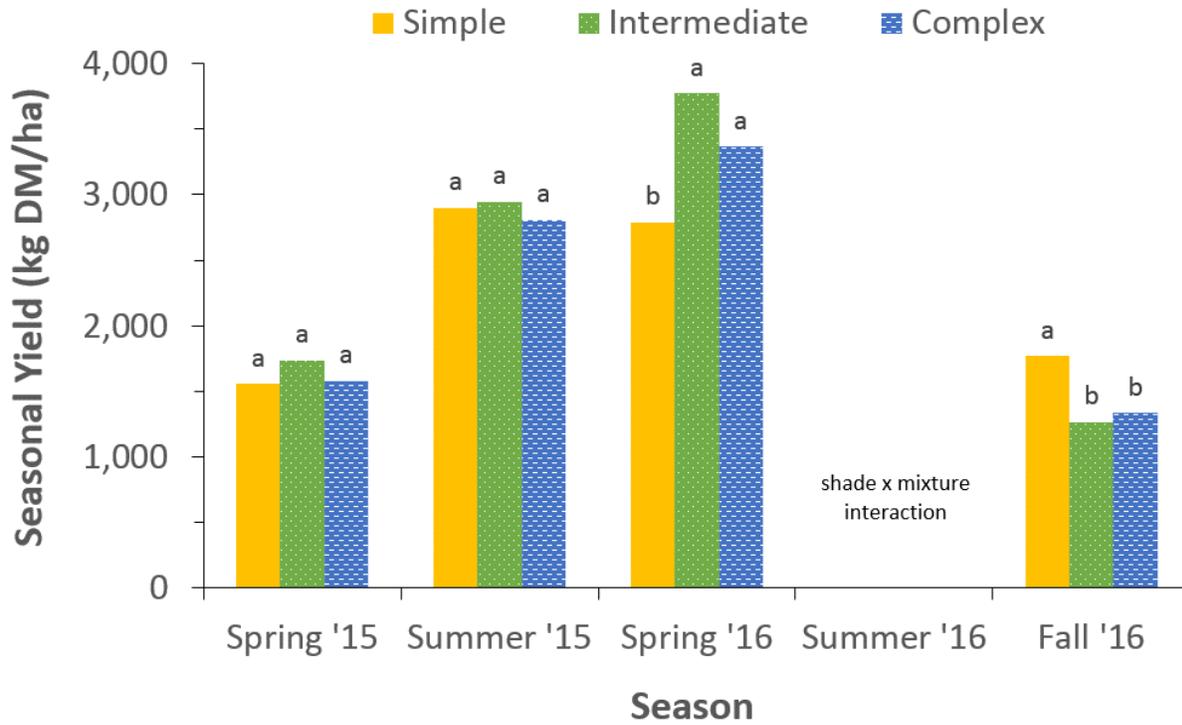


Figure 7. Seasonal yield responses to mixture treatments. Treatments within seasons containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

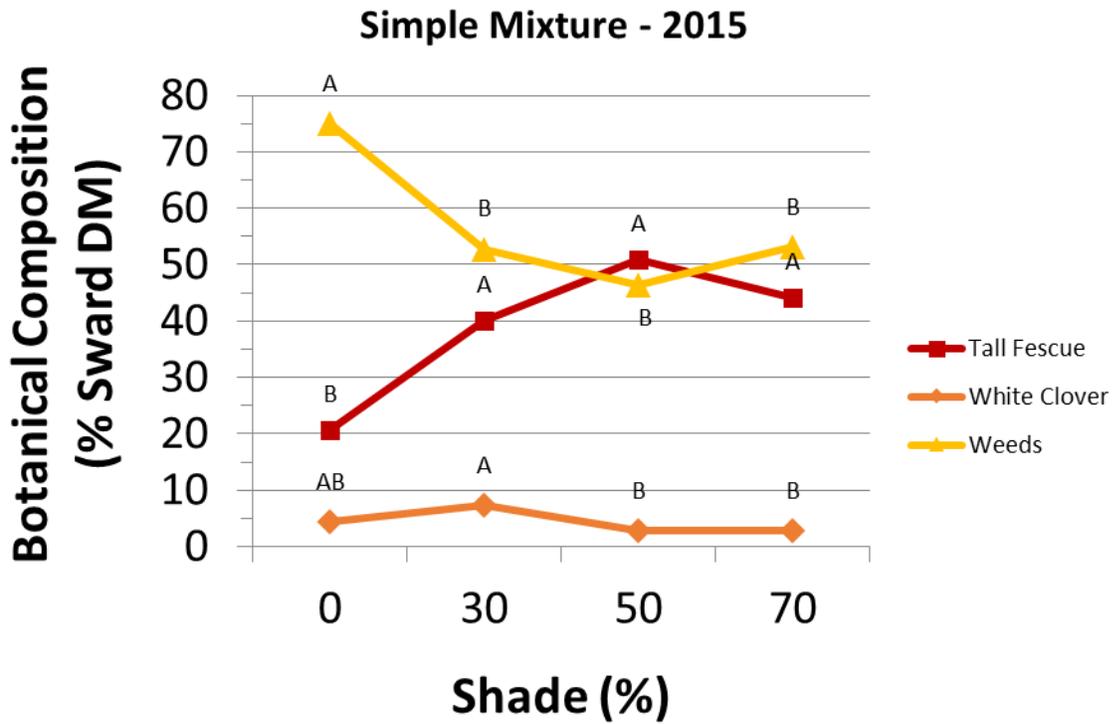


Figure 8. Simple mixture components' responses to shade in 2015. Treatments within forage species containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

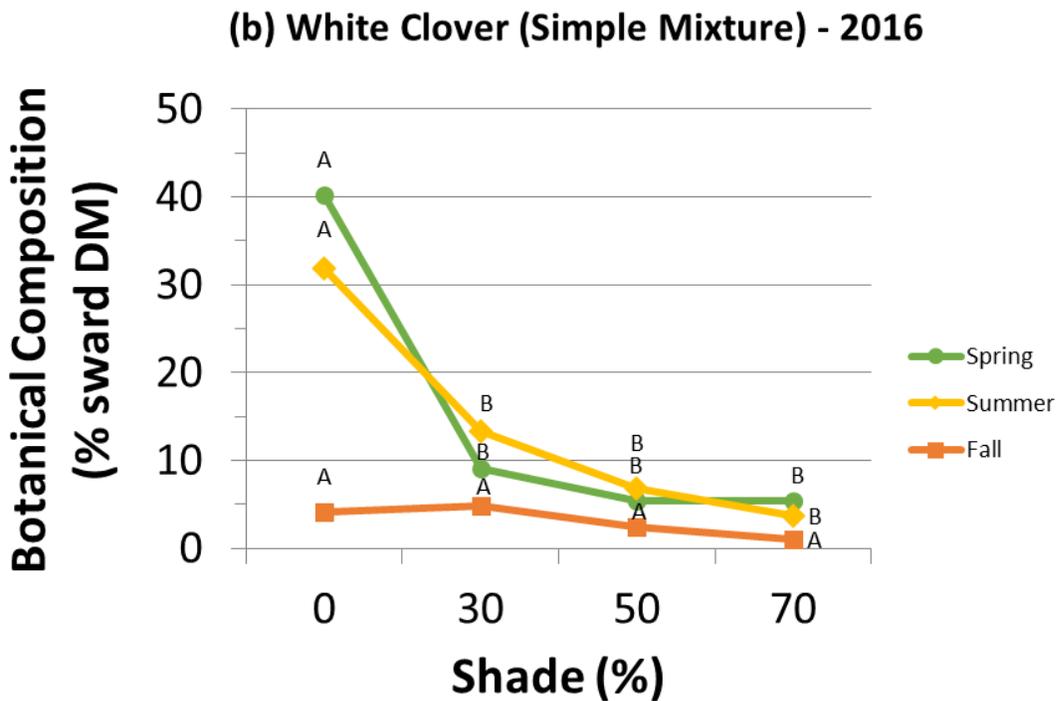
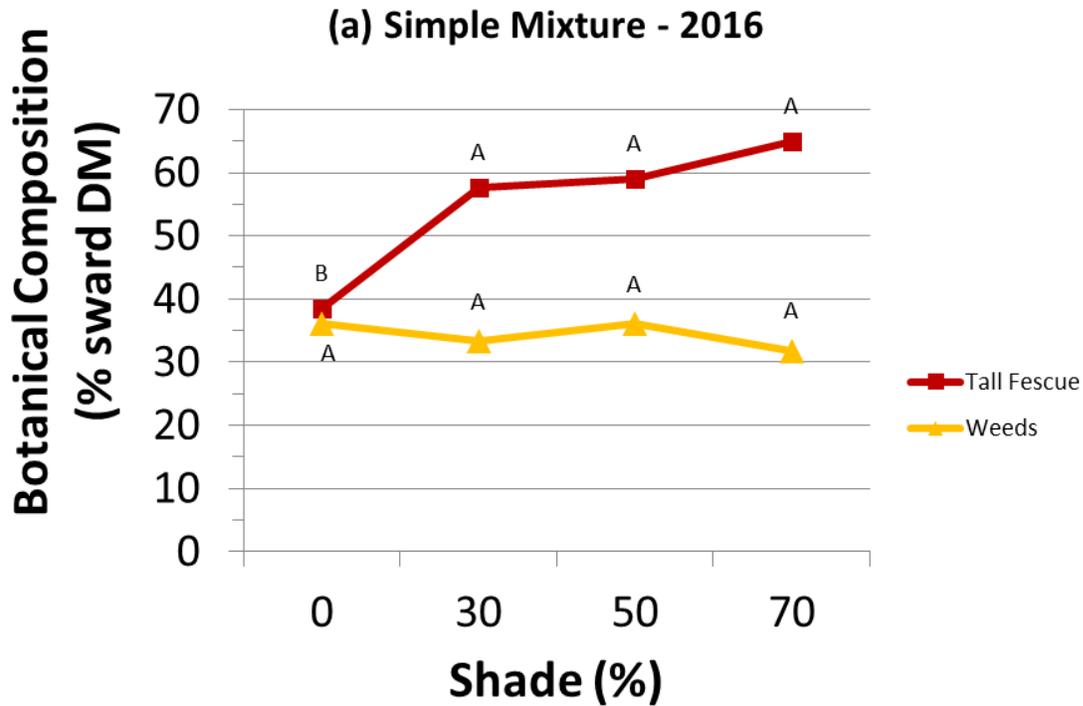


Figure 9. 2016: Simple mixture components' responses to shade (a). White clover showed a shade x season interaction and is separated by season ($P < 0.01$) (b). Treatments within forage species or season containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

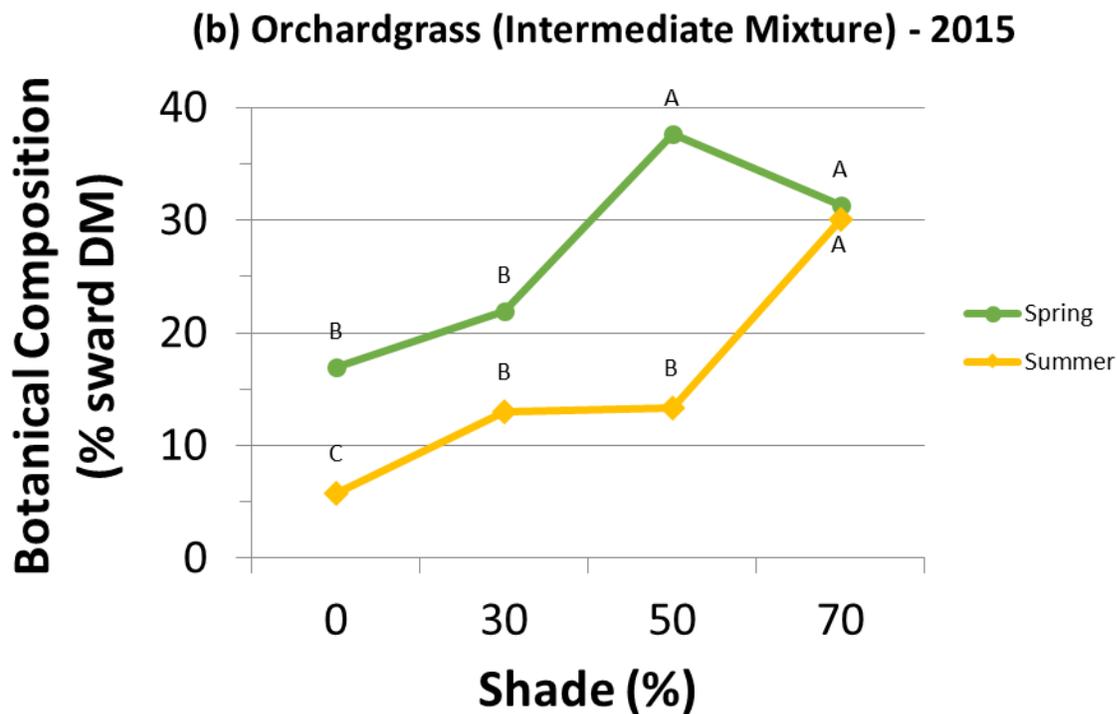
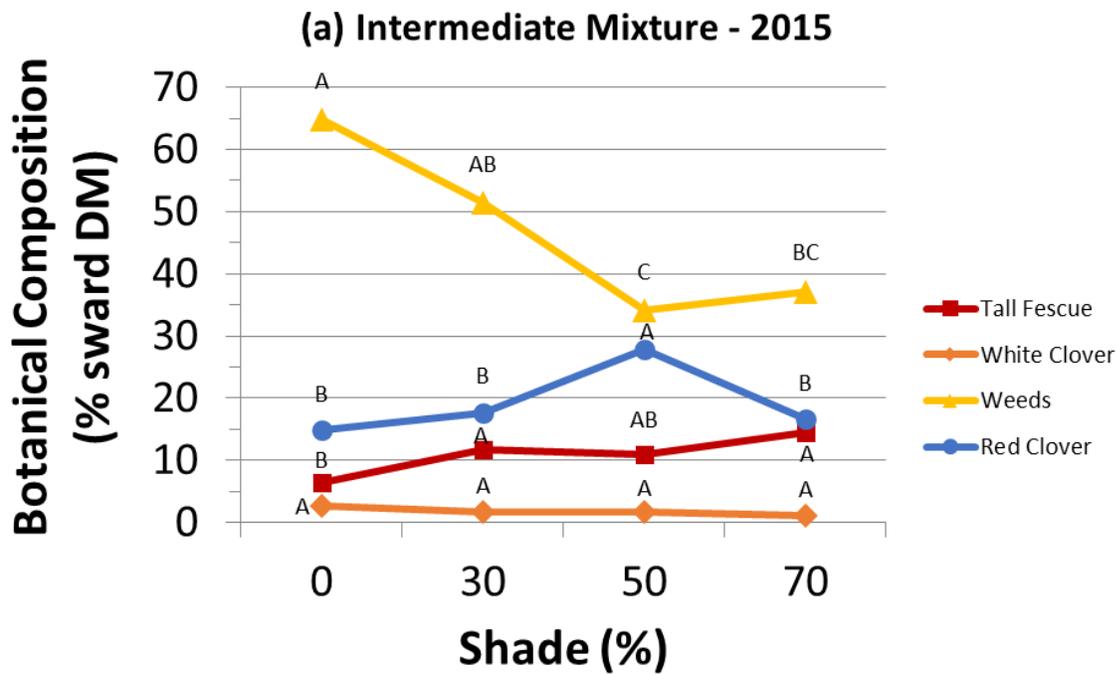


Figure 10. 2015: Intermediate mixture components' responses to shade (a). Orchardgrass showed a shade x season interaction and is separated by season ($P < 0.001$) (b). Treatments within forage species or season containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

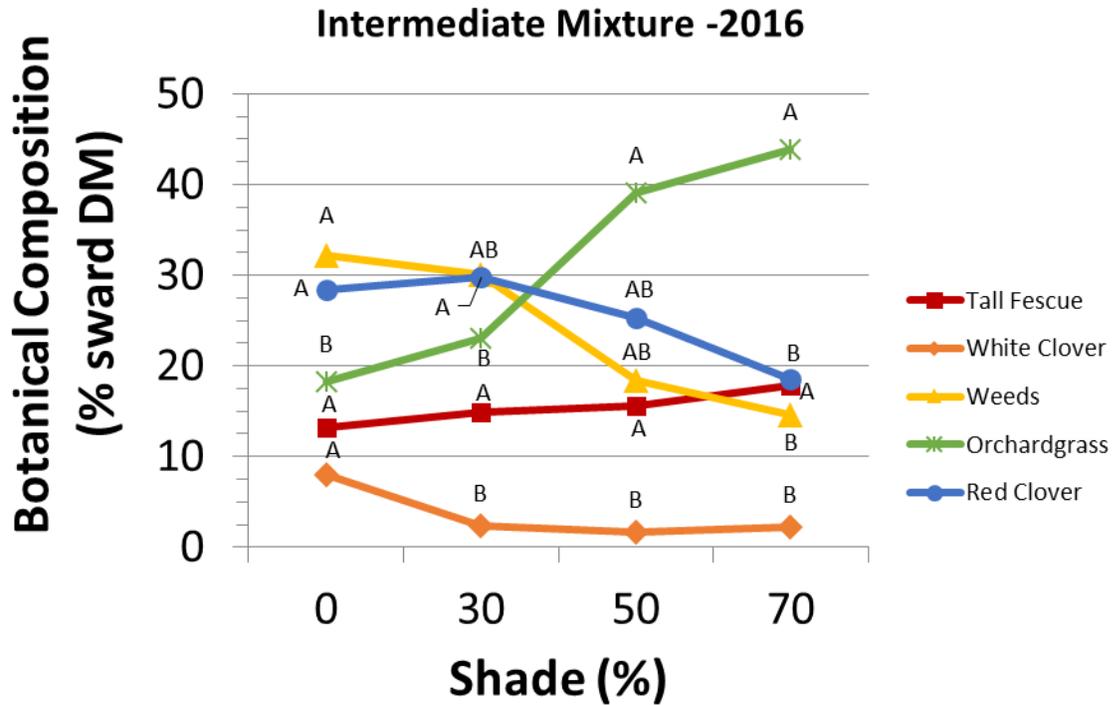


Figure 11. Intermediate mixture components' responses to shade in 2016. Treatments within forage species containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

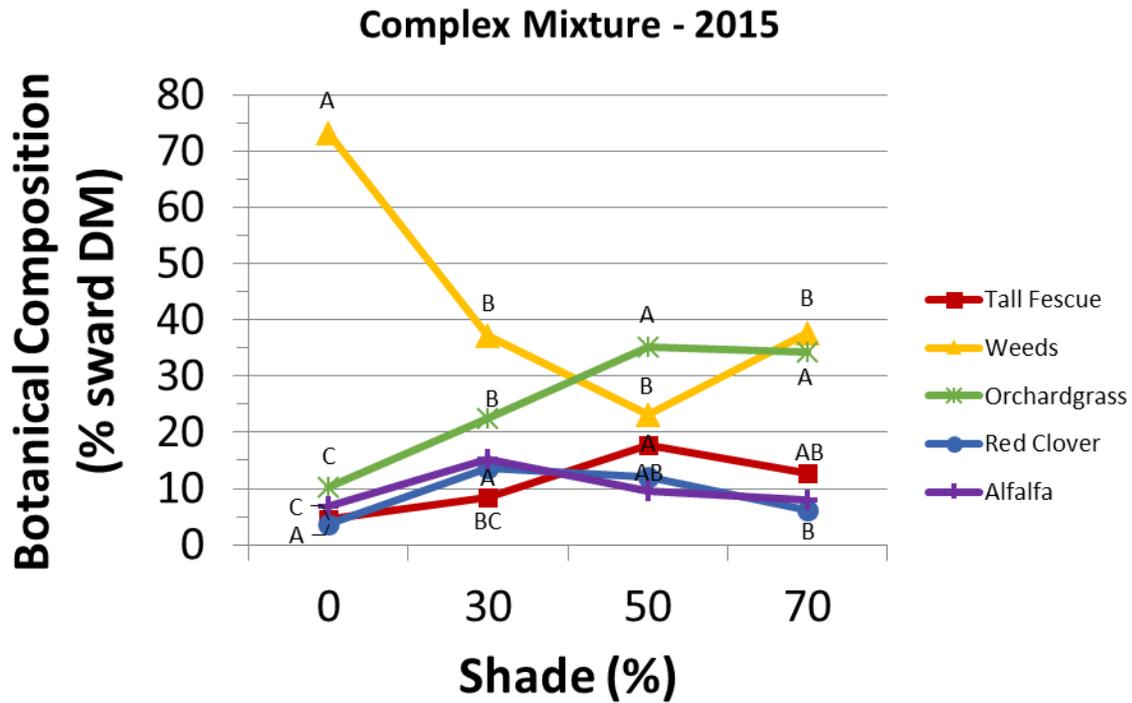


Figure 12. Complex mixture components' responses to shade in 2015. Treatments within forage species containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$). Alfalfa proportions were not different under all shade levels, therefore letter designations were not given ($P < 0.09$). White clover, Kentucky bluegrass, and birdsfoot trefoil never comprised more than 2% of the sward under any treatment and are not shown.

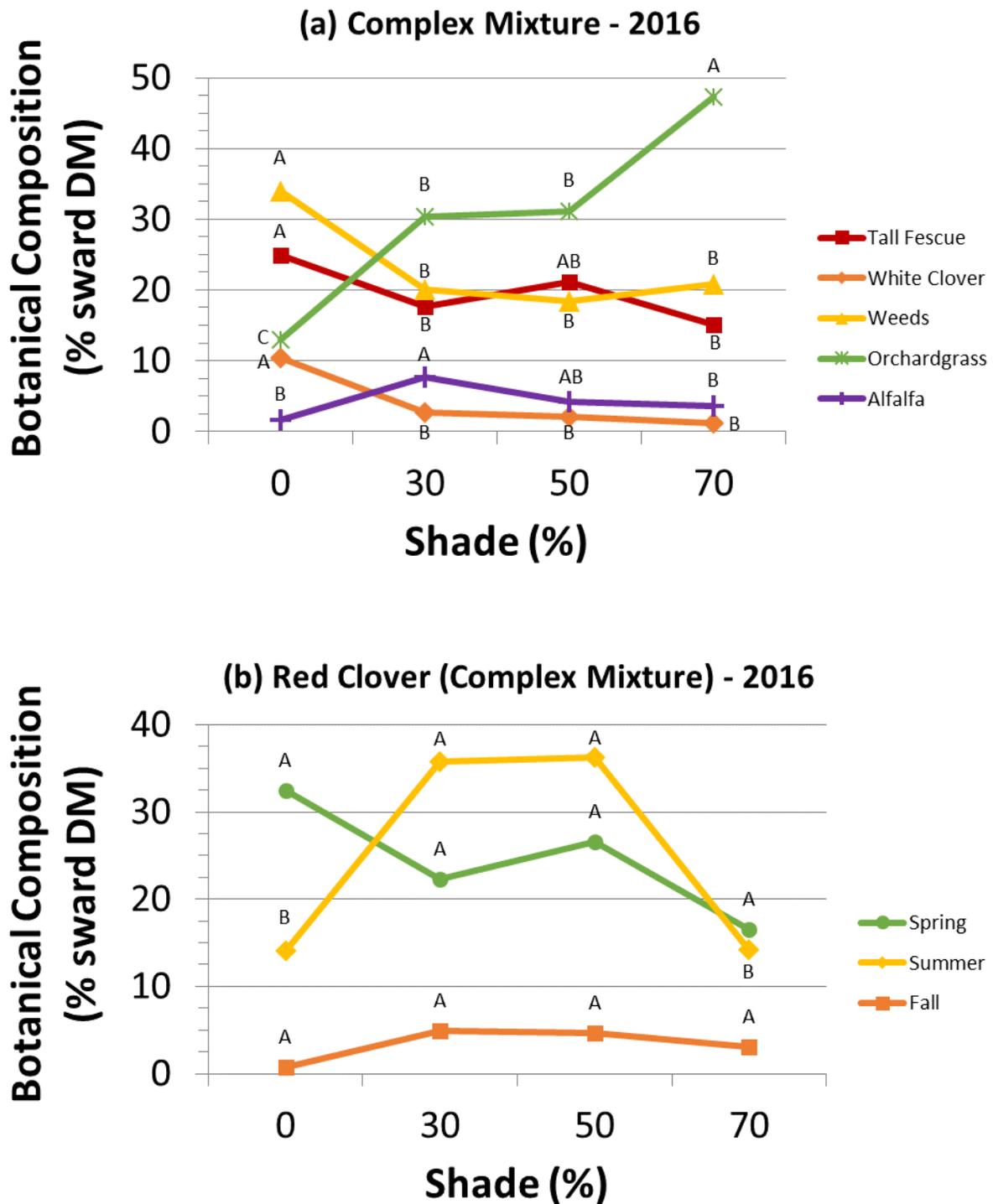


Figure 13. 2016: Complex mixture components' responses to shade (a). Red clover showed a shade x season interaction and is separated by season ($P < 0.01$) (b). Treatments within forage species or seasons containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$). Kentucky bluegrass and birdsfoot trefoil never comprised more than 1% of the sward under any treatment and are not shown.

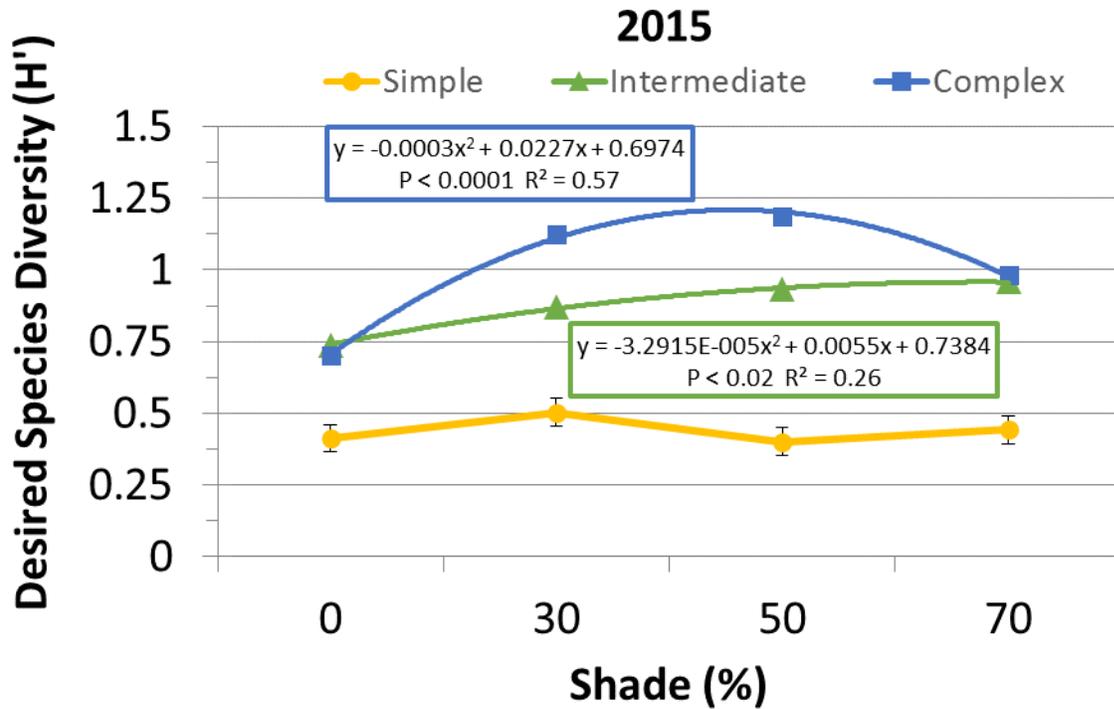


Figure 14. Impact of shade on the simple, intermediate, and complex mixtures' desired species diversity in 2015. Simple mixture responses to shade treatments neither fit a quadratic ($P > 0.70$) nor a linear curve ($P > 0.90$).

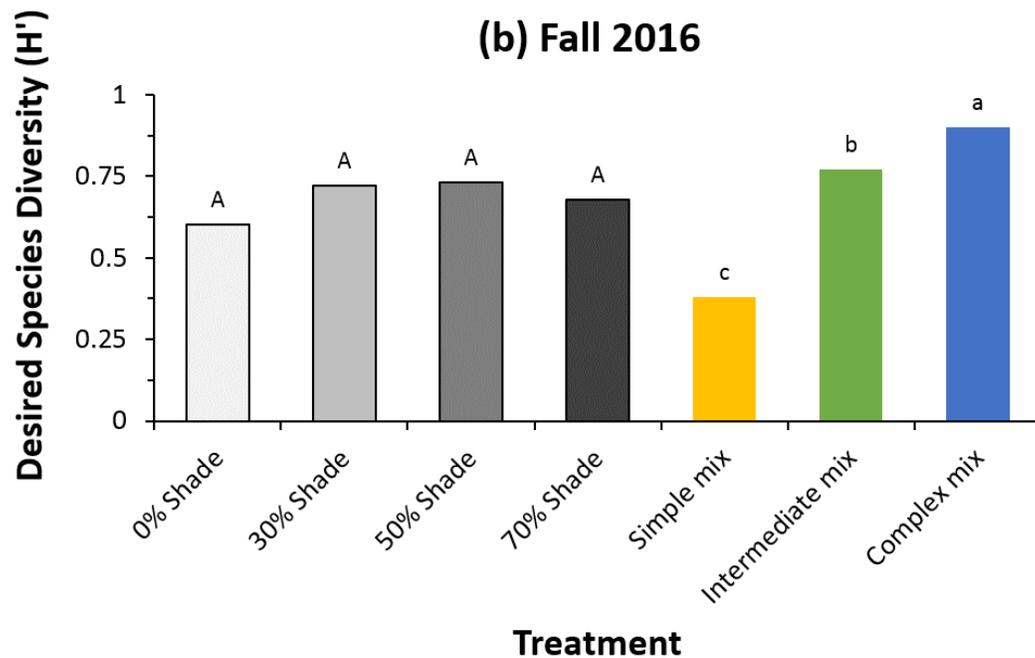
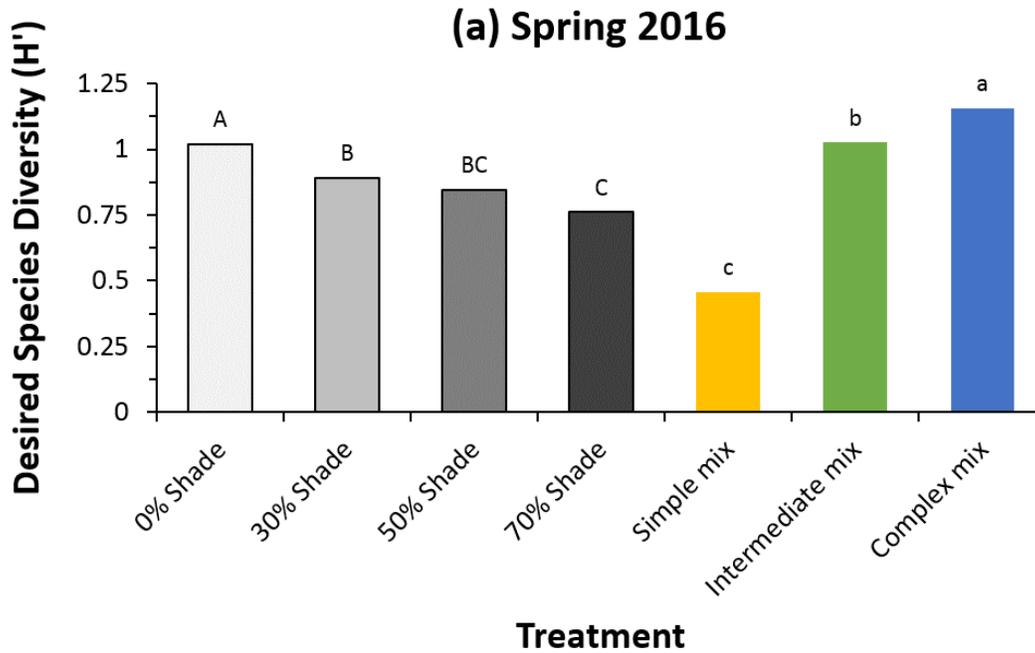


Figure 15. 2016: Desired species diversity response to shade (gray bars) (spring: $P < 0.001$; fall: $P > 0.23$) and mixture (colored bars) (spring: $P < 0.001$; fall: $P < 0.001$) main effects in spring (a) and fall (b). Treatments within shade level (0-70%) or mixture (simple, intermediate, complex) containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

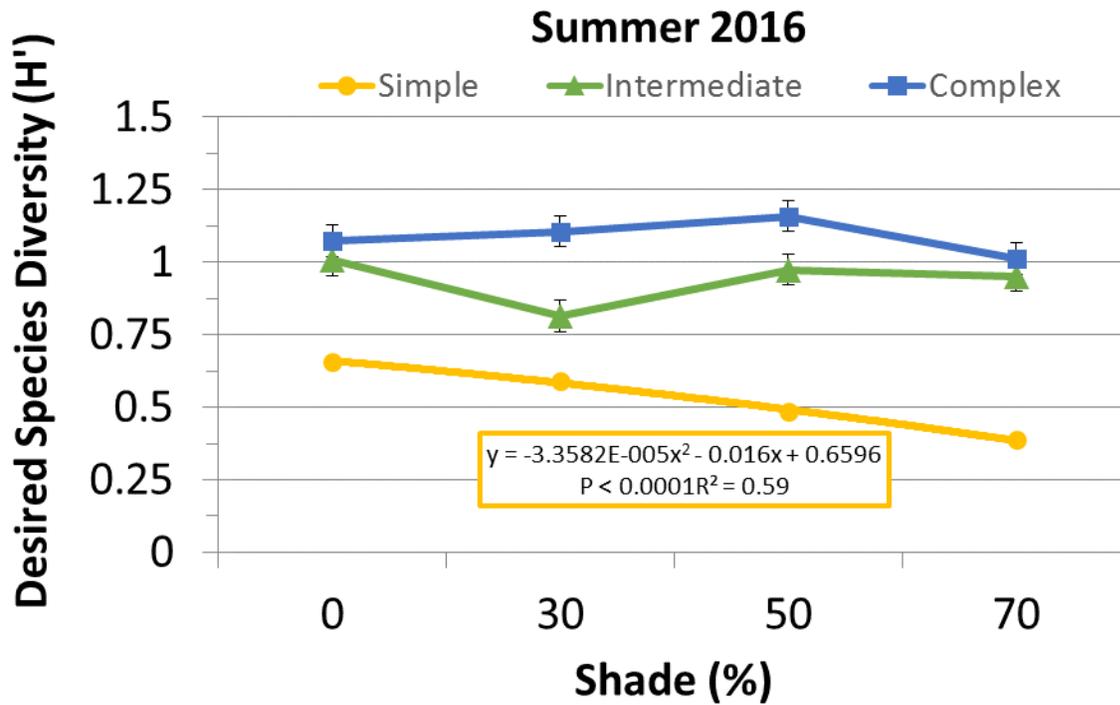


Figure 16. Impact of shade on simple, intermediate, and complex mixtures' desired species diversity in summer 2016. Intermediate and complex mixture responses to shade treatments neither fit a quadratic ($P > 0.24$ and $P > 0.46$) nor a linear curve ($P > 0.82$ and $P > 0.74$).

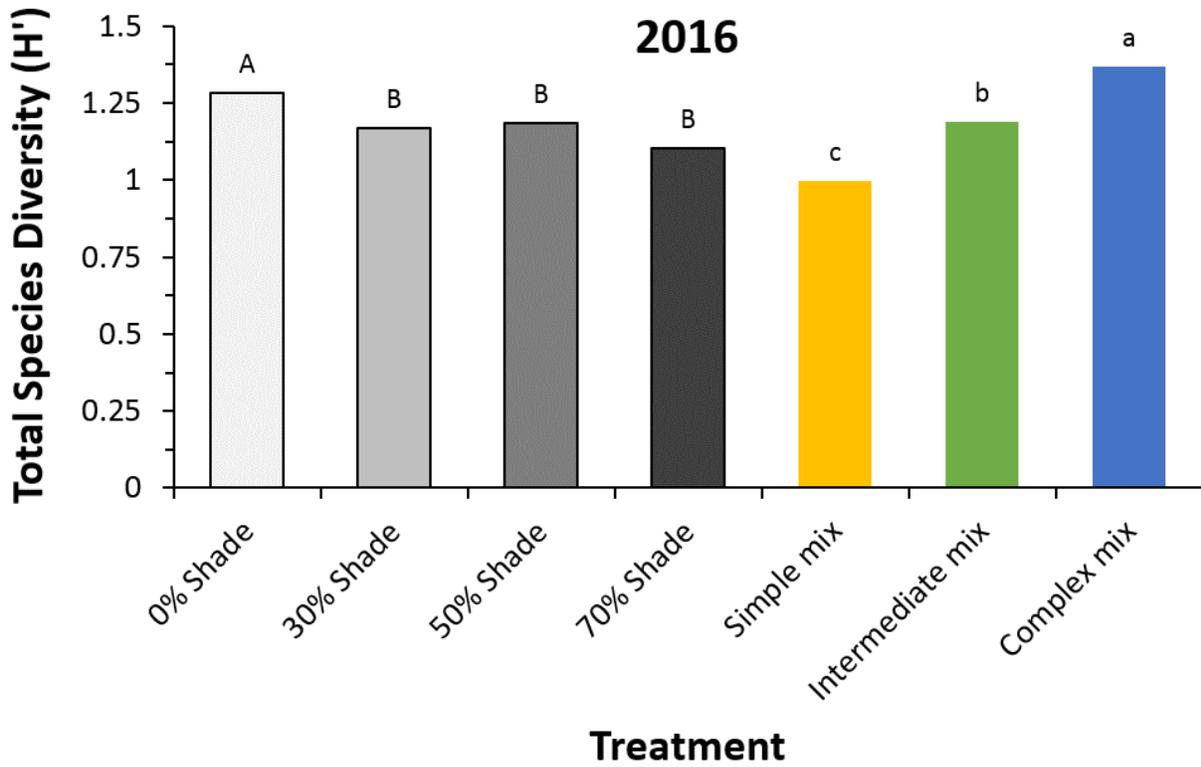


Figure 17. Total species diversity (planted species + weed species) response to shade (gray bars) ($P < 0.001$) and mixture (colored bars) ($P < 0.001$) main effects. Treatments within shade level (0-70%) or mixture (simple, intermediate, complex) containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

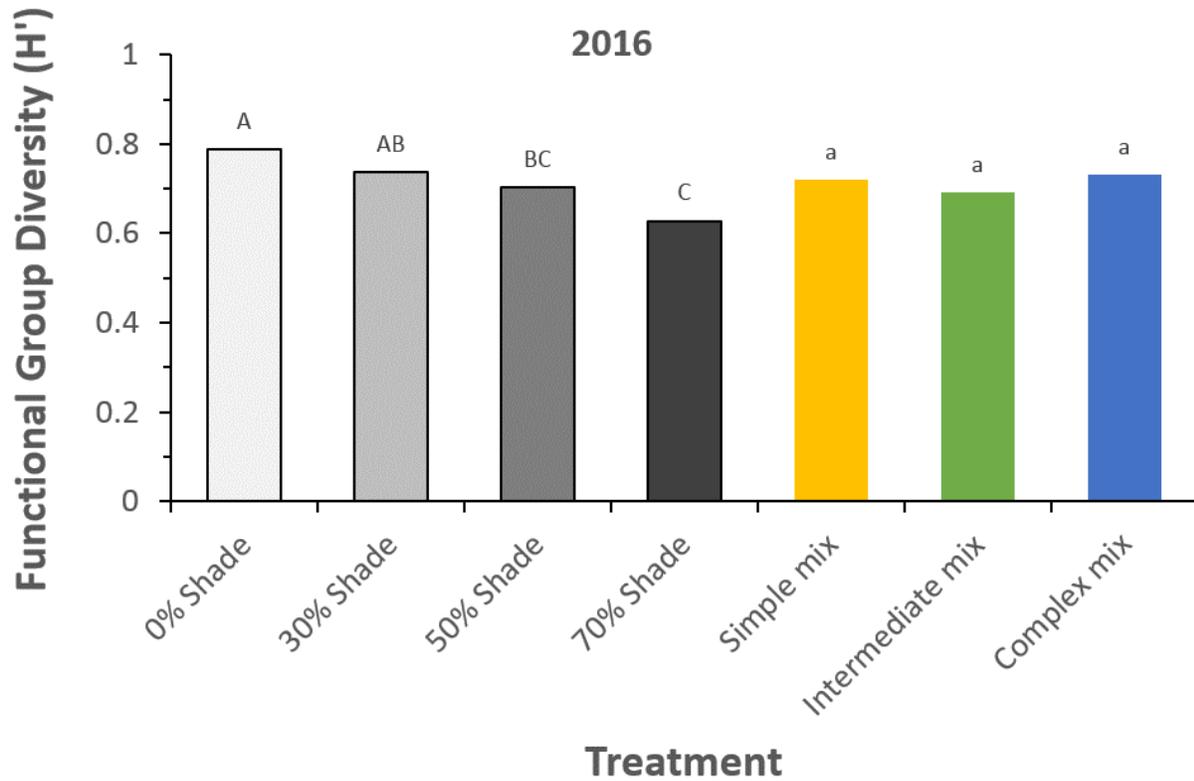


Figure 18. Functional group diversity (cool-season grasses, warm-season grasses, legumes, and forbs) response to shade (gray bars) ($P < 0.001$) and mixture (colored bars) ($P > 0.50$) main effects. Treatments within shade level (0-70%) or mixture (simple, intermediate, complex) containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

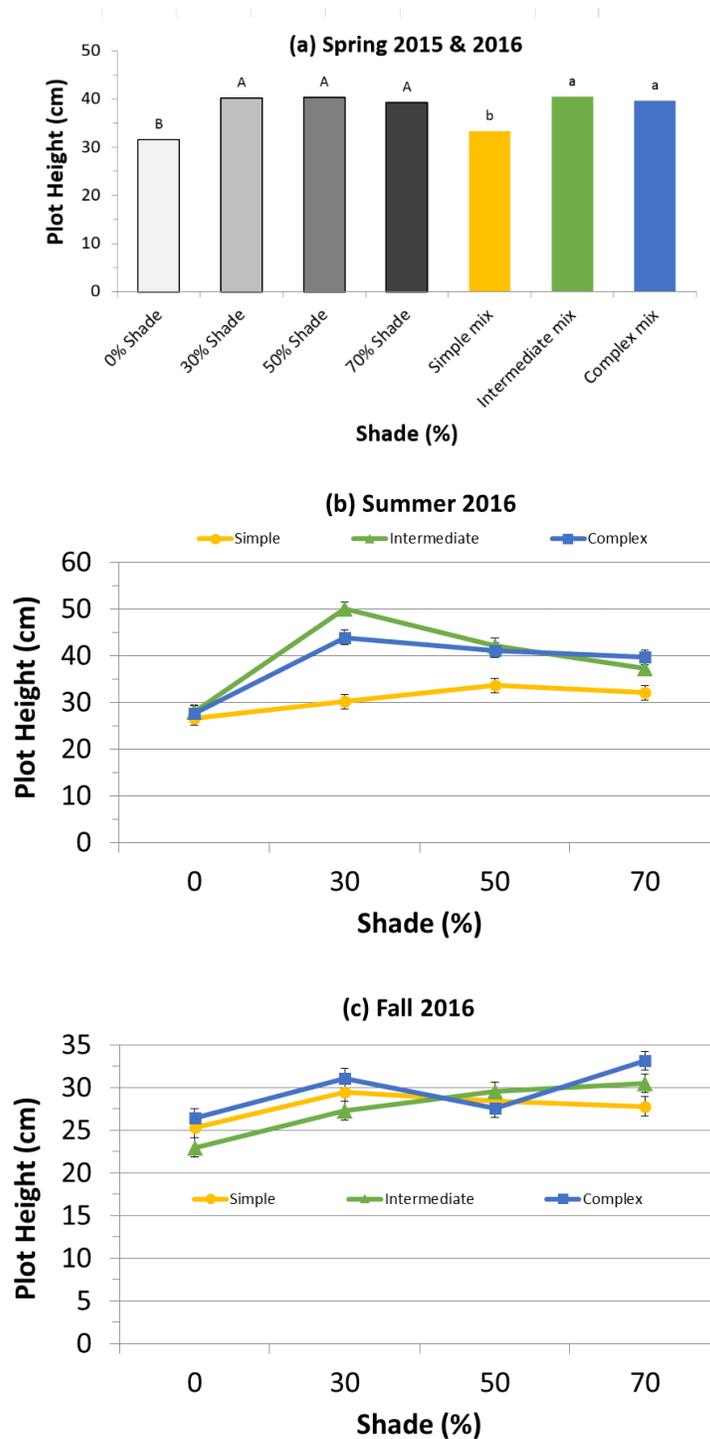


Figure 19. Plot height response to shade (gray bars) ($P < 0.001$) and mixture (colored bars) ($P < 0.001$) main effects during spring (a). Treatments within shade level (0-70%) or mixture (simple, intermediate, complex) containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$). Impact of shade on simple, intermediate, and complex mixtures' plot height for summer ($P < 0.001$, standard error = 1.55) (b) and fall ($P < 0.03$, standard error = 1.10) (c).

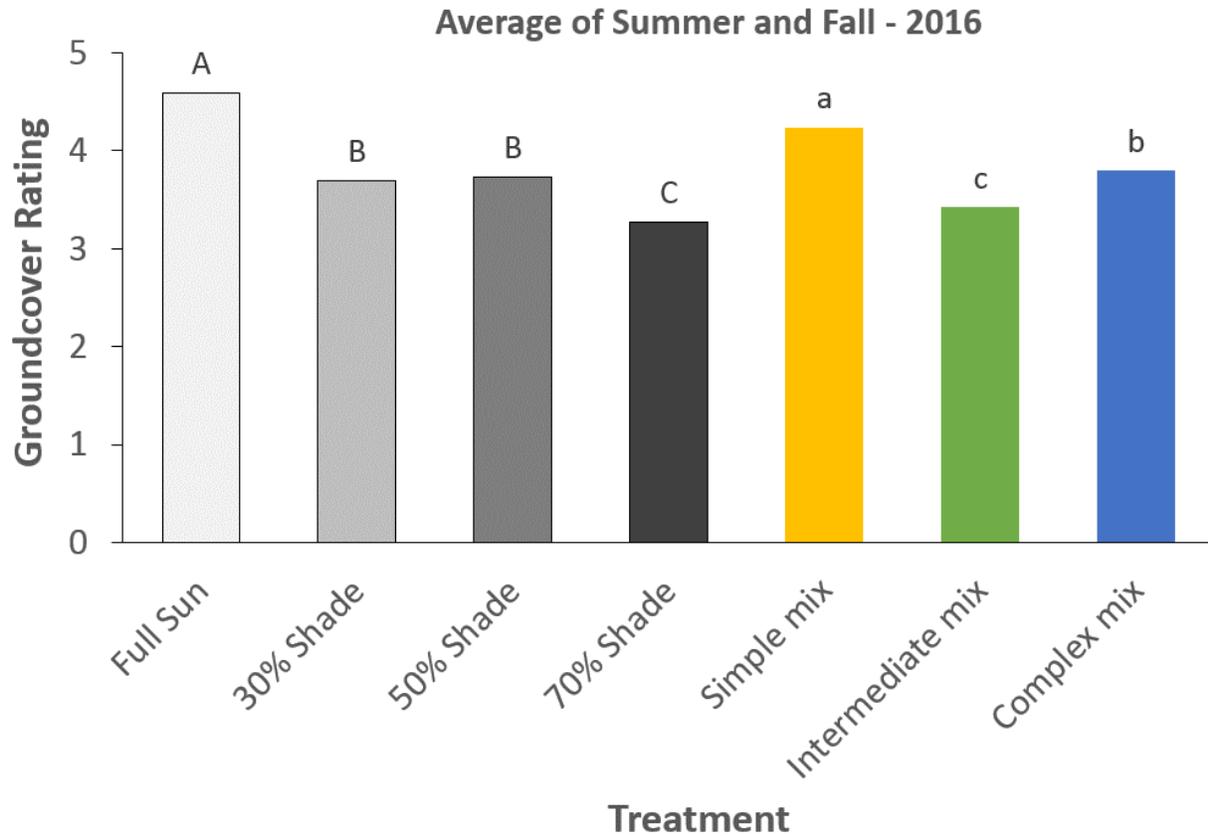


Figure 20. Groundcover response to shade ($P < 0.001$) and mixture ($P < 0.001$) main effects. Likert-type ratings are as follows: 1 = very poor, 2 = poor, 3 = fair, 4 = good, 5 = very good. Treatments within shade level (gray bars; 0-70%) or mixture (colored bars; simple, intermediate, complex) containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

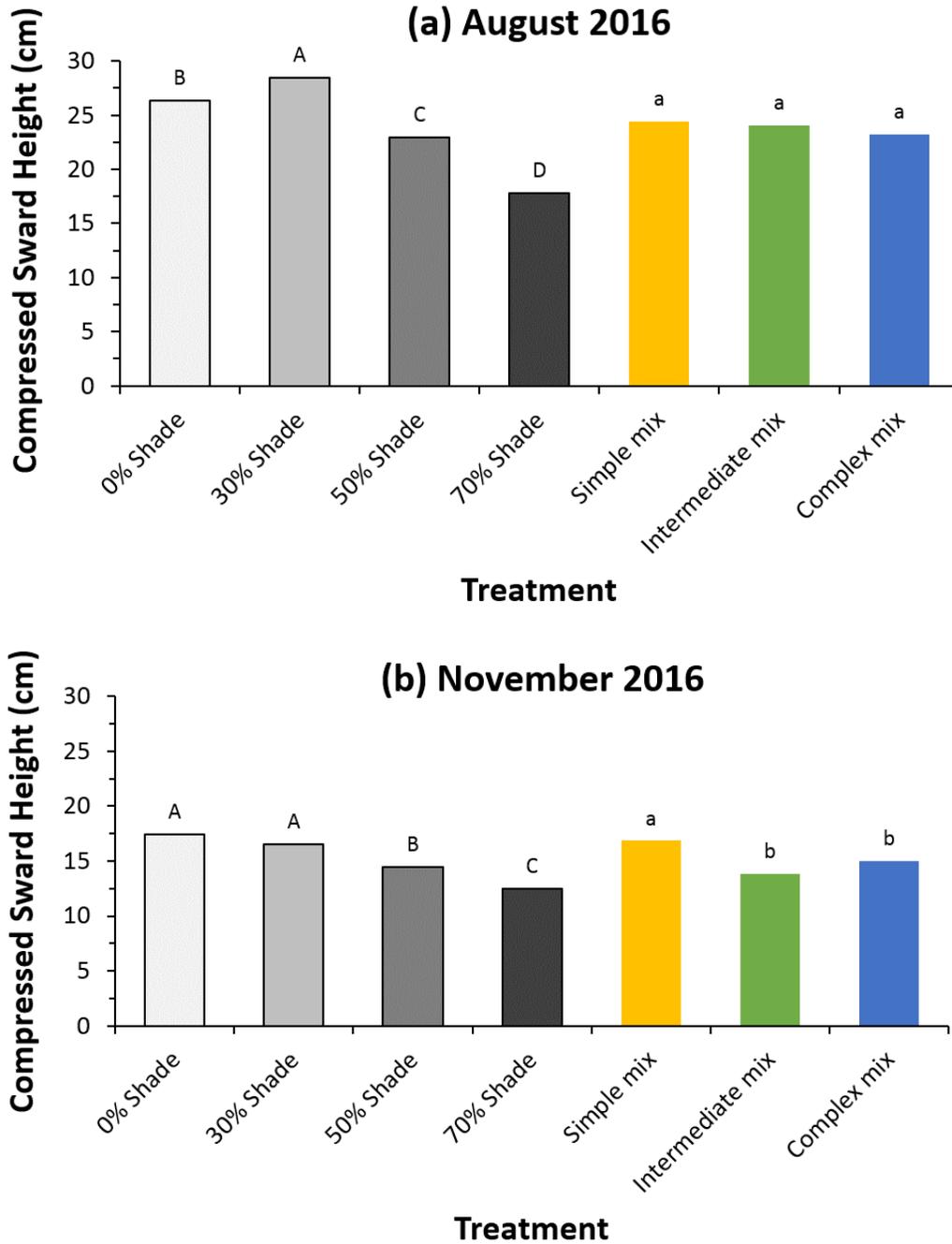


Figure 21. Compressed sward height response to shade (gray bars) (August 2016: $P < 0.001$; November 2016: $P < 0.001$) and mixture (colored bars) (August 2016: $P < 0.001$; November 2016: $P < 0.001$) main effects during August 2016 (a) and November 2016 (b). Treatments within shade or mixture containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

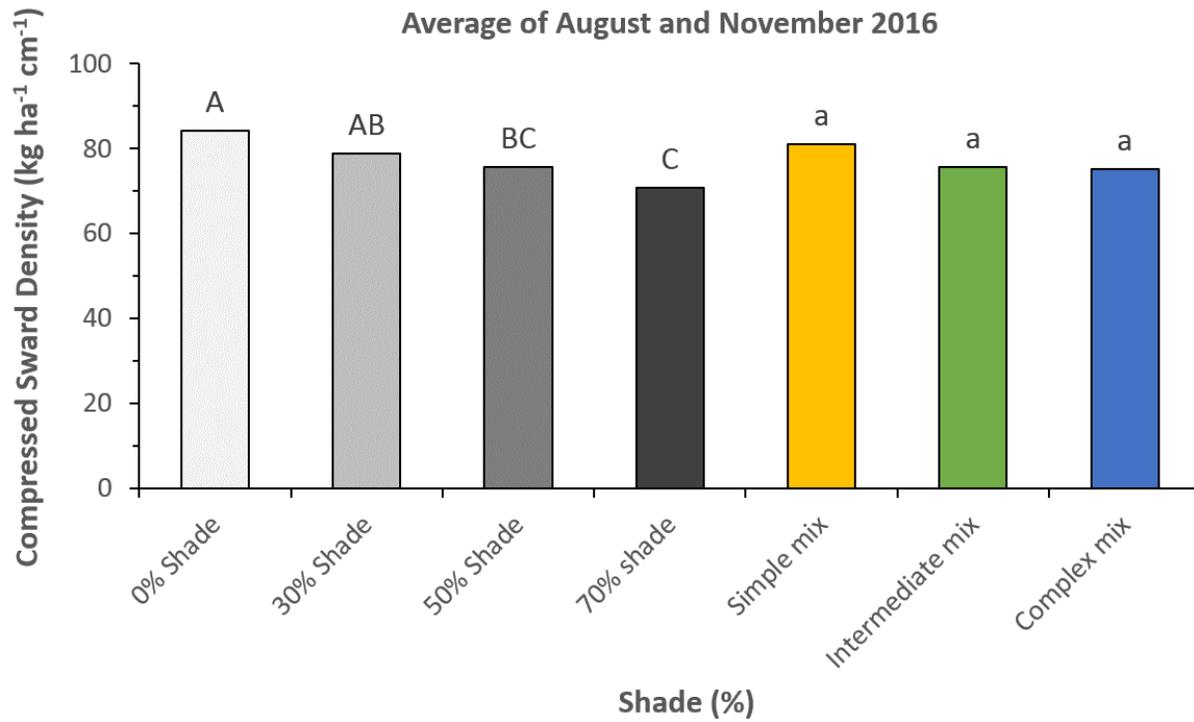


Figure 22. Compressed sward density response to shade and mixture main effects. Treatments within shade level (0-70%) or mixture (simple, intermediate, complex) containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

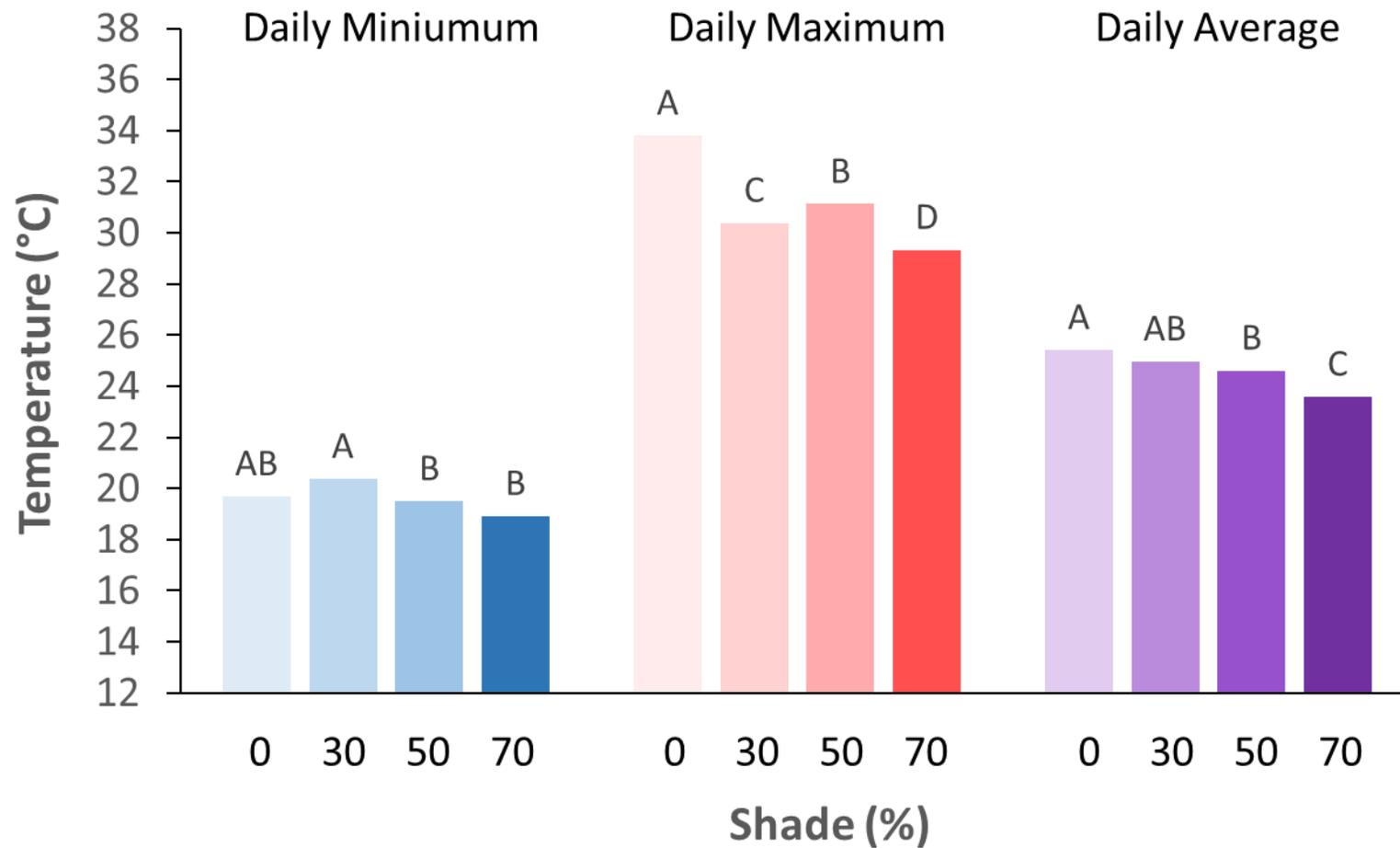


Figure 23. Seasonal soil temperature responses to shade treatments (daily minimum: $P < 0.03$; daily maximum: $P < 0.001$; daily average: $P < 0.001$). Treatments within temperature category (daily minimum, maximum, average) containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

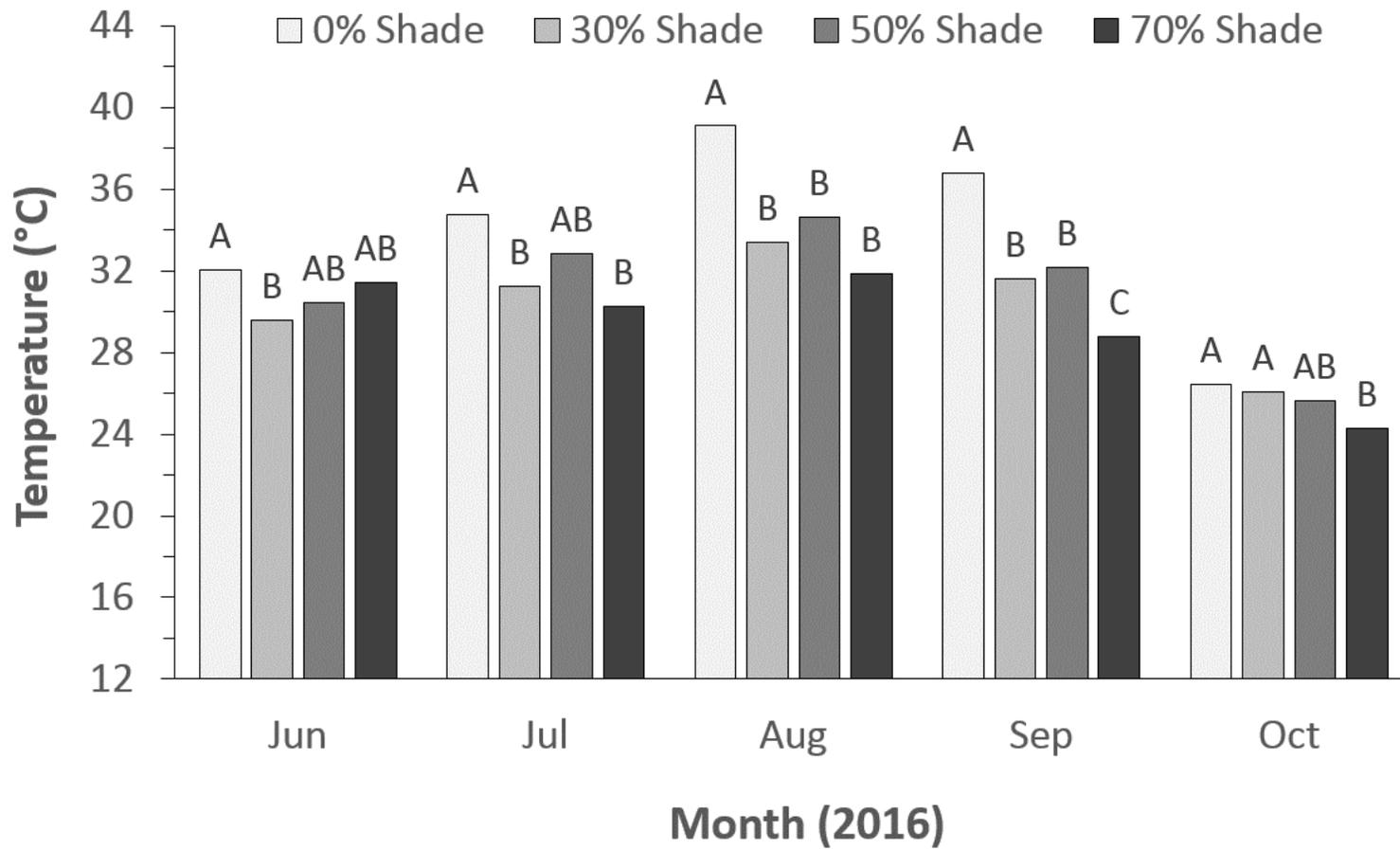


Figure 24. Shade effect on monthly maximum soil temperature. Treatments within each month containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

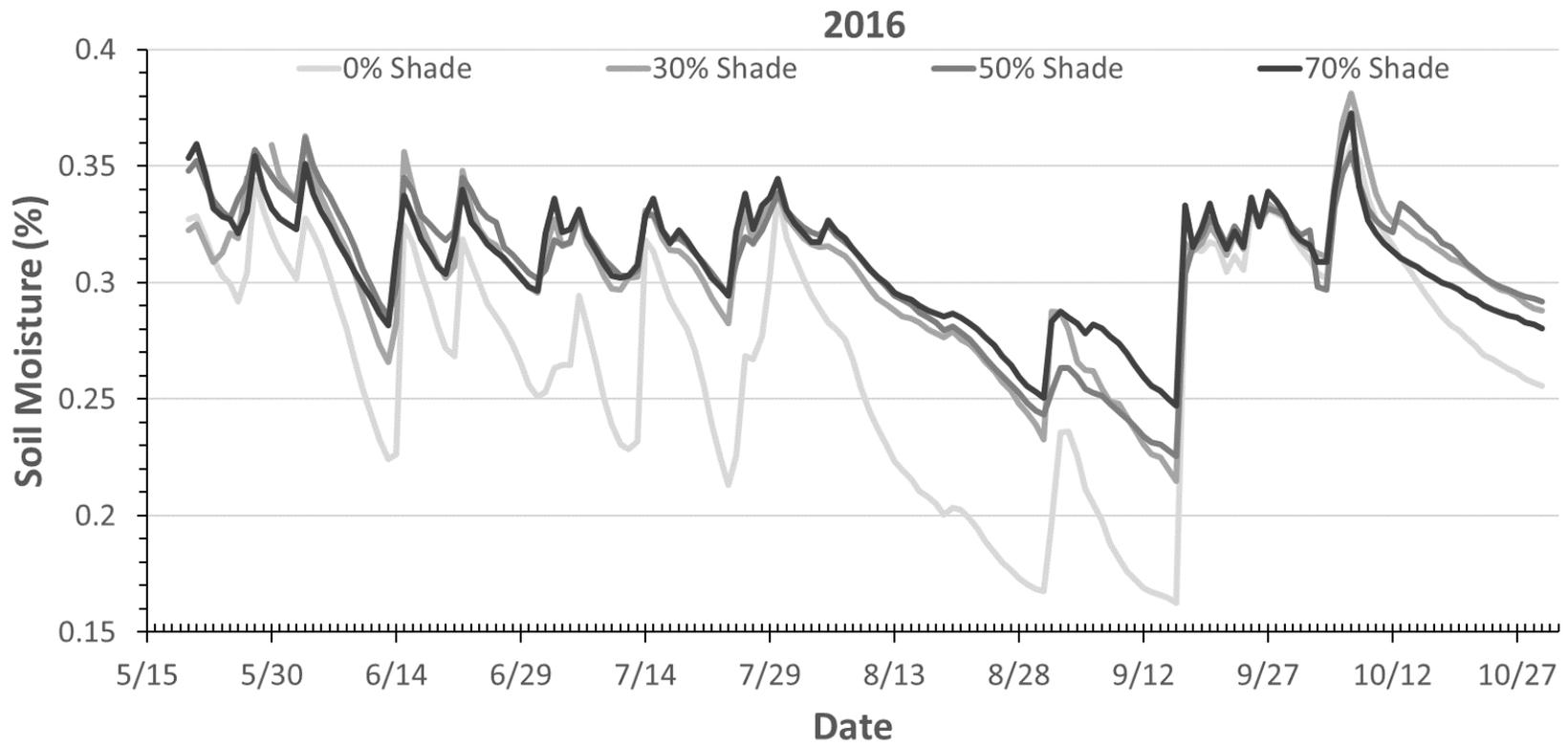


Figure 25. Soil moisture response to shade treatments in 2016.

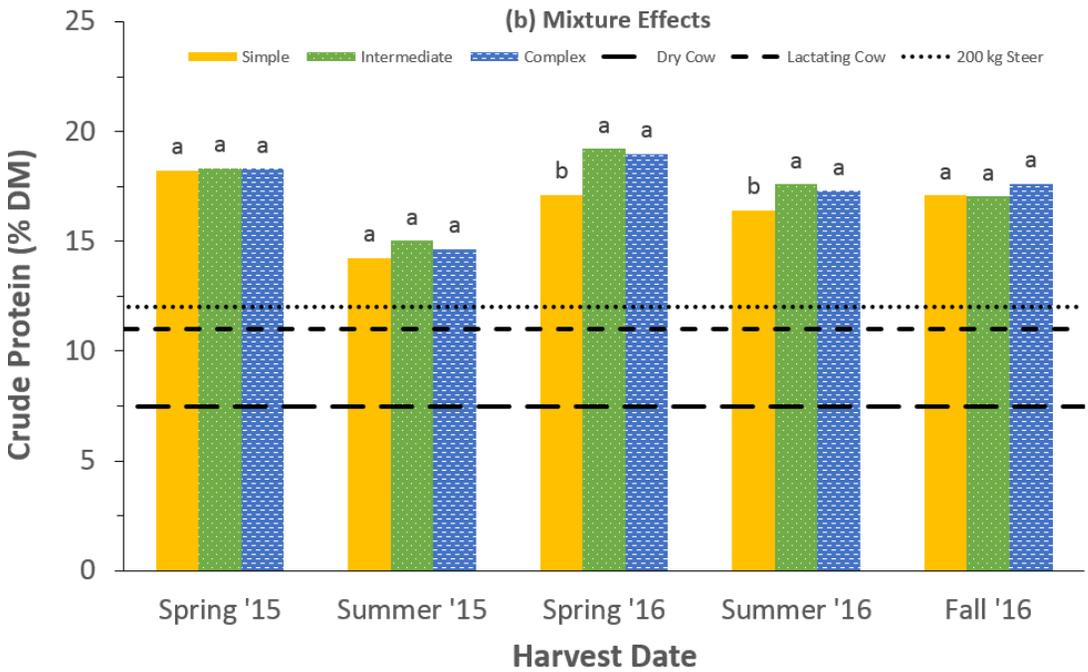
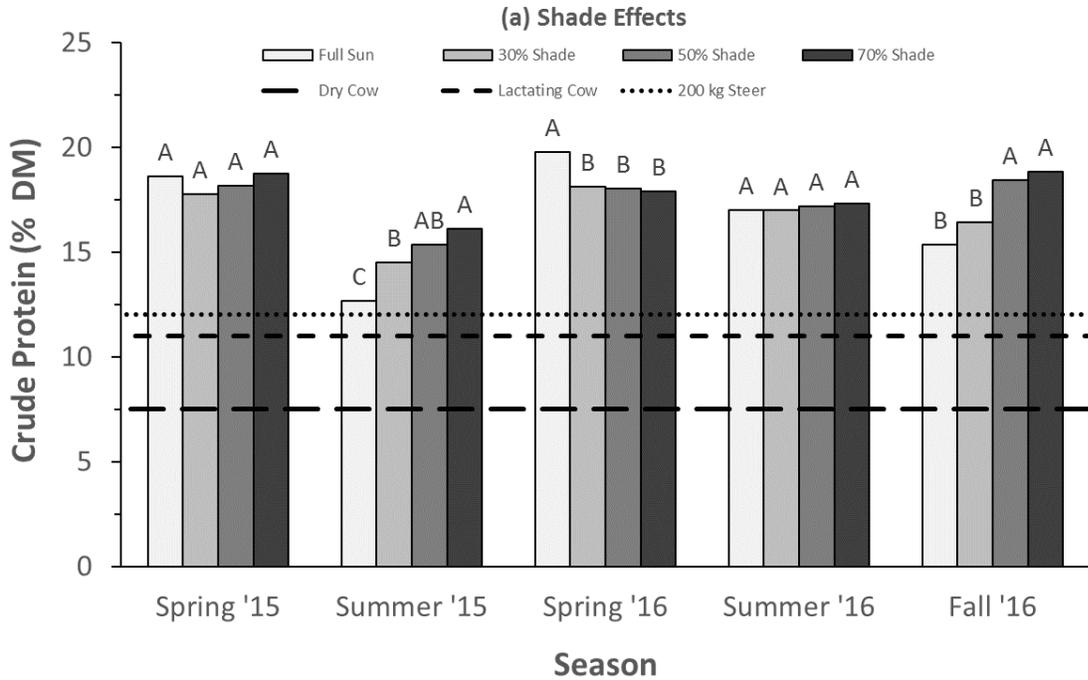


Figure 26. Seasonal crude protein response to shade treatments (a) and mixture (b). Treatments within season and year containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$). Requirements for different classes of livestock are as follows: dry cow = 7.5% CP, lactating cow = 11% CP, 200 kg steer gaining 0.77 kg/day = 12% CP.

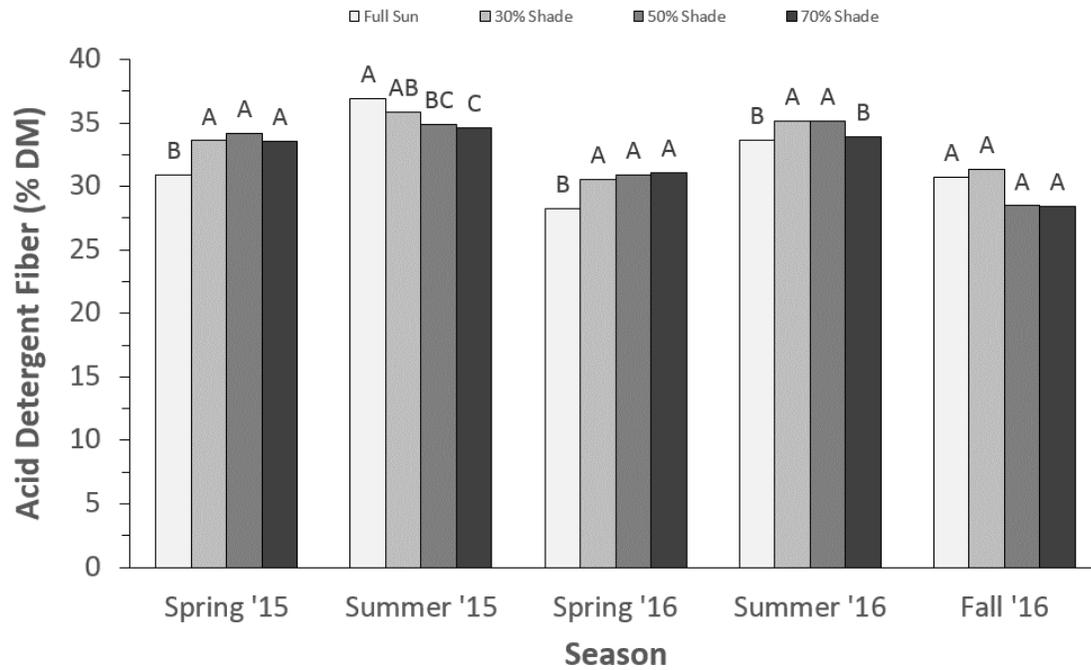


Figure 27. Seasonal acid detergent fiber responses to shade treatments. Treatments within seasons containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

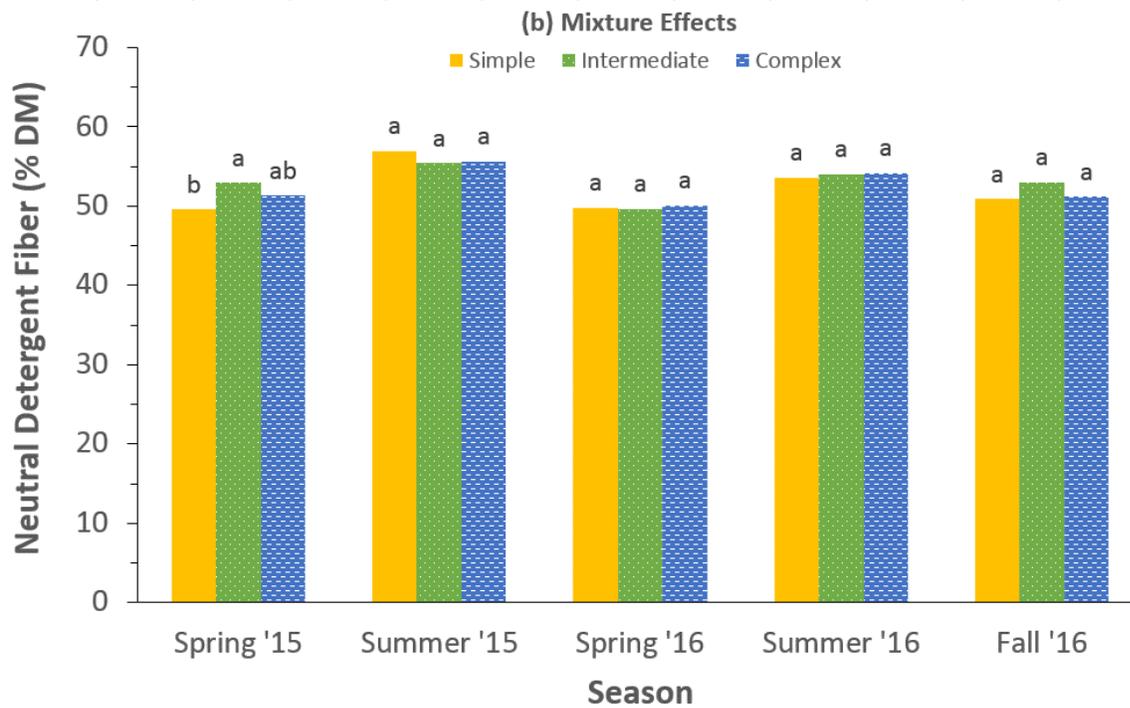
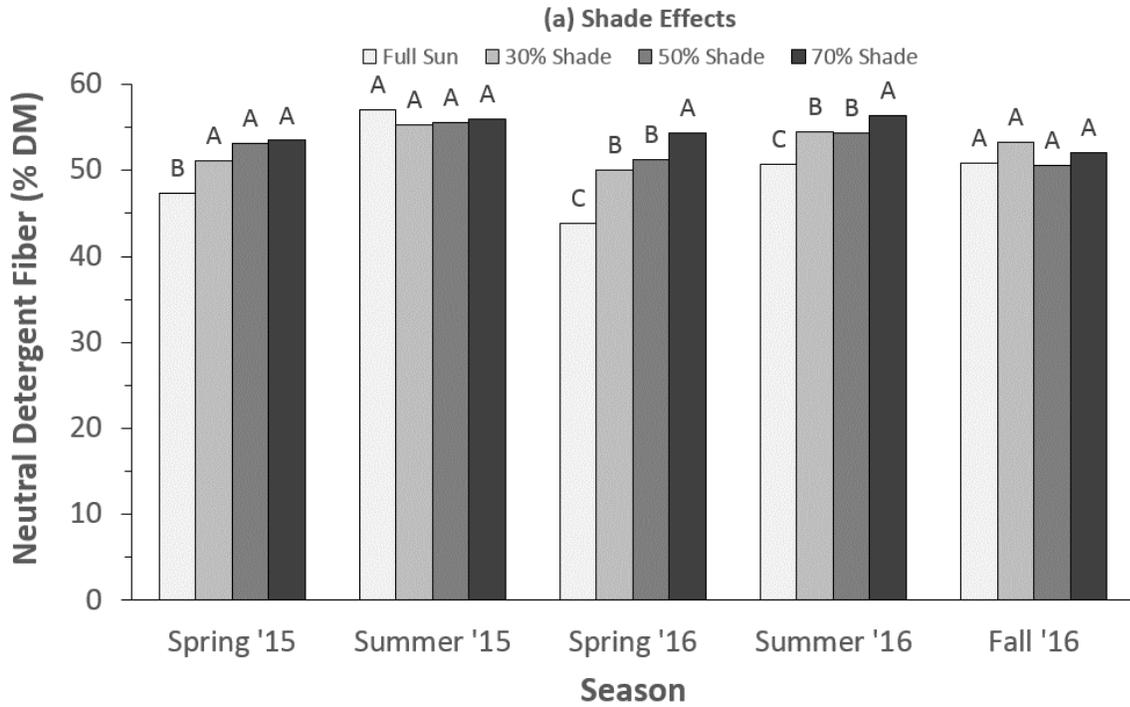


Figure 28. Neutral detergent fiber response to shade (a) and mixture (b) treatments. Treatments within season and year containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$).

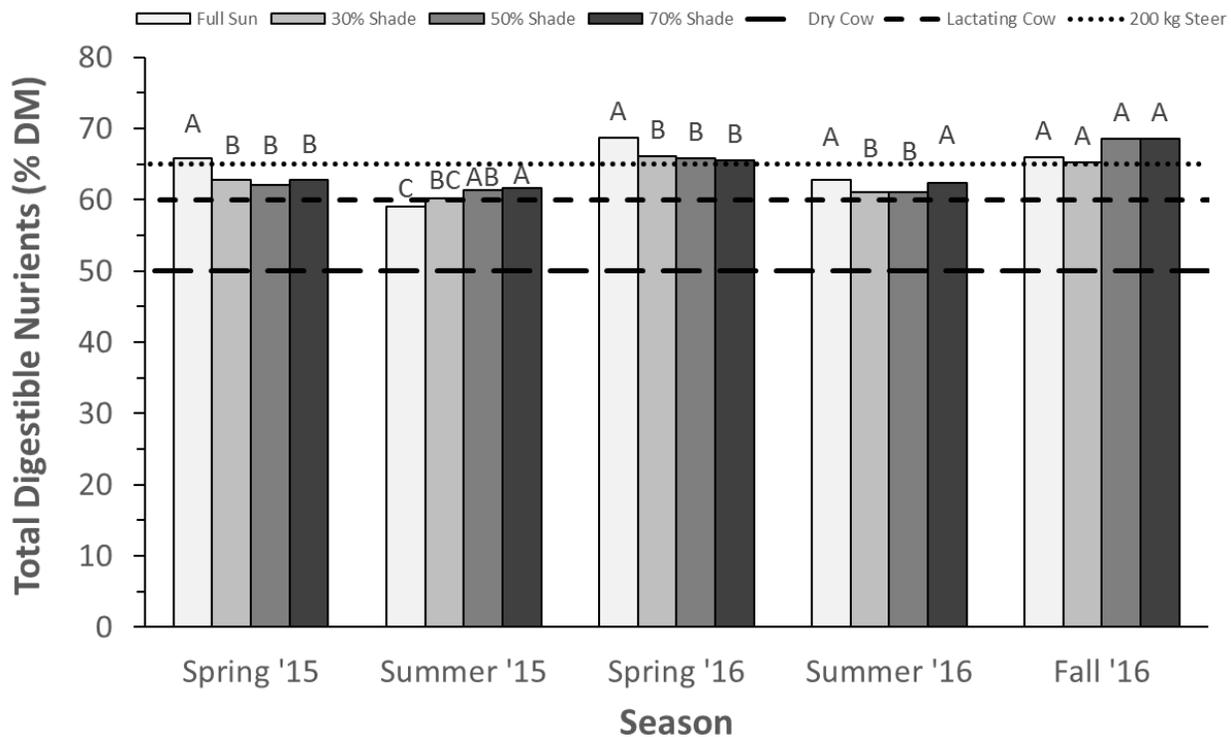


Figure 29. Seasonal total digestible nutrient response to shade treatments. Treatments within seasons containing the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$). Requirements for different classes of livestock are as follows: dry cow = 50% TDN, lactating cow = 60% TDN, 200 kg steer gaining 0.77 kg/day = 65% TDN.

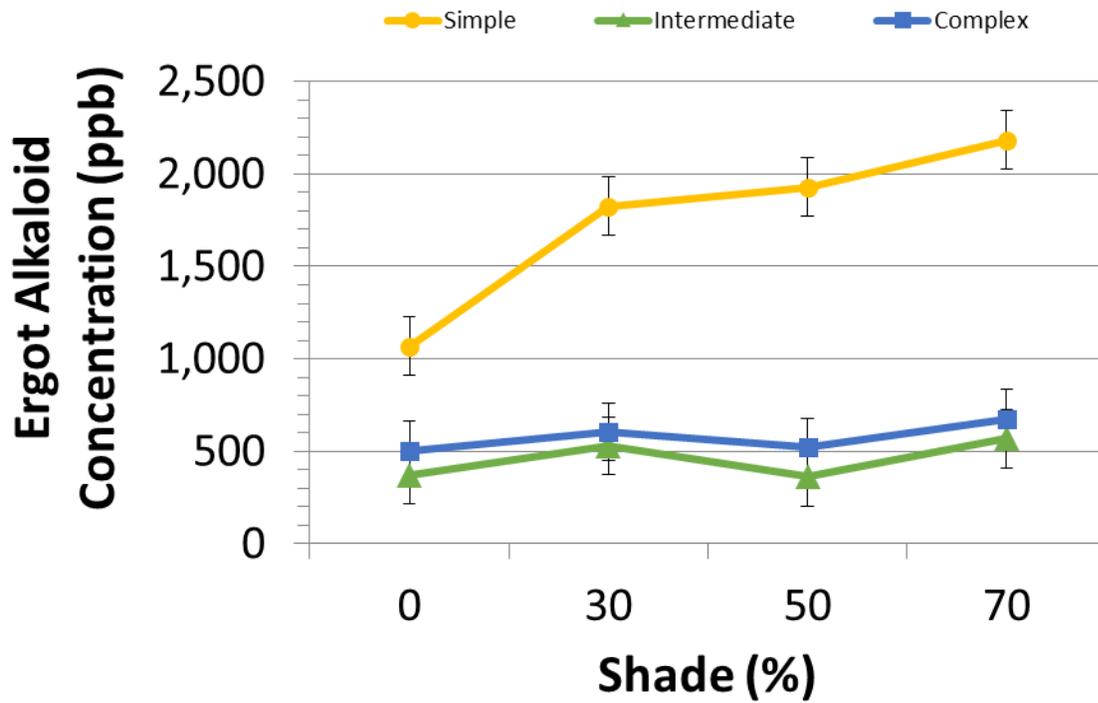


Figure 30. Shade effects on mixture for ergot alkaloid concentration for entire sward. Standard error = 157 ppb.

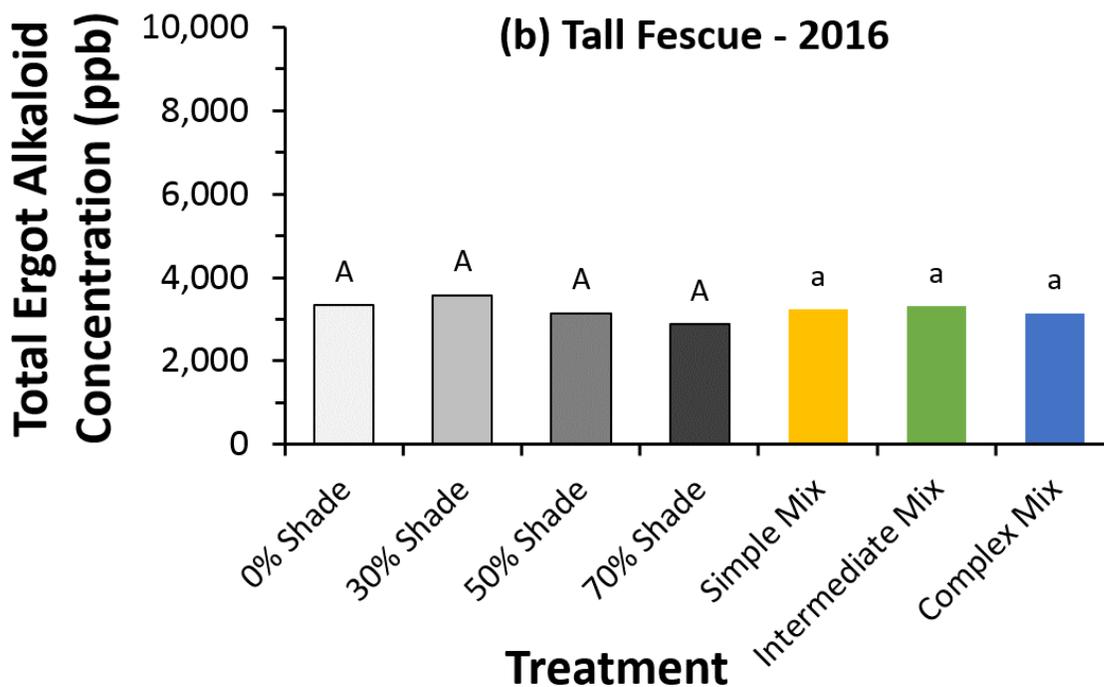
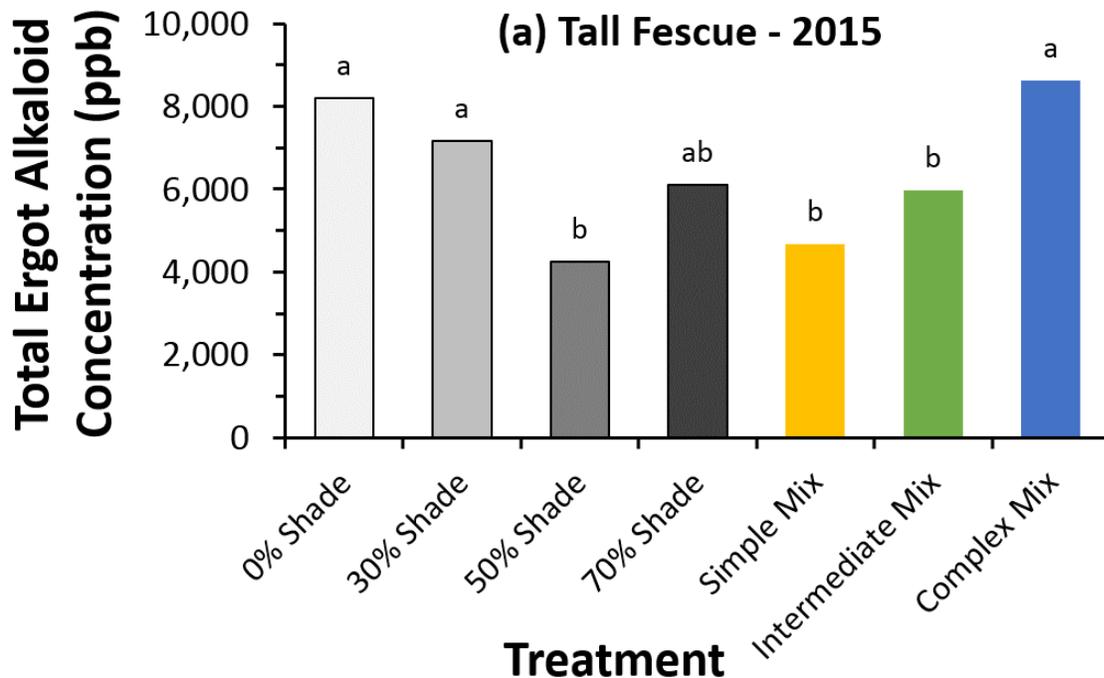


Figure 31. Total ergot alkaloid response to shade and mixture main effects on a “per fescue plant” basis. Treatments within seasonal category containing the same letter are not significantly different according to Fisher’s protected least significant difference ($\alpha = 0.05$)