THE SUITABILITY OF NATIVE WARM-SEASON GRASSES FOR EQUINE

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The suitability of native warm-season grasses for equine

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Abstract (Academic)

Introduced cool-season grasses are dominant in Virginia's grasslands, but their high digestible energy and non-structural carbohydrate (NSC) levels pose a risk for horses prone to obesity and laminitis. Native warm-season grasses (NWSG) have lower digestible energy and NSC levels that may be more suitable for horses susceptible to laminitis. The overall objectives of this research were to 1) assess voluntary intake, toxicological response, and apparent digestibility of NWSG hays fed to horses; 2) evaluate the characteristics of three NWSG species under equine grazing; and 3) evaluate establishment strategies for NWSG and wildflowers in Virginia. For the first objective, a hay feeding trial was conducted with 9 Thoroughbred geldings in a 3 x 3 Latin square design. Voluntary dry matter intake of indiangrass (*Sorghastrum nutans*) and big bluestem (*Andropogon gerardii*) hays by horses were 1.3% and 1.1% of BW/d, significantly lower than orchardgrass (*Dactylis glomerata*), an introduced cool-season grass, at 1.7% of BW/d. Biomarkers for toxicity remained within acceptable ranges for all treatments. Apparent DMD did not differ among hays, ranging from 39 to 43%. Non-structural carbohydrate levels were below the maximum recommended concentration for horses susceptible to laminitis. For the second objective, a grazing trial was conducted comparing indiangrass (IG), big bluestem (BB), and eastern gamagrass (*Tripsacum dactyloides*) (EG) yields, forage losses, changes in vegetative composition, and effects on equine bodyweight. Nine, 0.1-hectare plots were seeded with one of the three native grass treatments, and each plot was grazed by one Thoroughbred gelding in two grazing bouts, one in July and another in September 2019. Indiangrass had the highest available

forage, at 4340 kg/ha, compared with 3590 kg/ha from BB ($P < 0.0001$). Eastern gamagrass plots established poorly, and had only 650 kg/ha available forage during the experiment. Grazing reduced standing cover of native grasses in IG and BB treatments by about 30%, and trampled forage constituted 36-68% of groundcover in those plots after each grazing bout. Horses lost weight on all treatments, but tended ($P=0.09$) greater weight loss on the indiangrass treatment at 1.5 kg/d compared to 0.5 kg/d in the BB and EG treatments. For the third objective, three experiments were conducted to evaluate different strategies for establishing NWSG and wildflowers. The first experiment compared large grazed plots with or without a 2 oz/acre rate of the herbicide imazapic. Imazapic led to higher biomass and percent cover in plots seeded only with NWSG. For plots seeded with a mix of NWSG and wildflowers, imazapic reduced wildflower establishment and resulted in higher biomass and percent cover of weeds over the course of the experiment. The second experiment examined four rates of imazapic application for NWSG and wildflower establishment in small plots seeded with either NWSG or a NWSG and wildflower mix, and found biomass and percent cover of weeds was lowest at a 6 oz/acre rate, while NWSG biomass and cover did not differ between treatments. Wildflower establishment was again reduced by imazapic. The third establishment experiment compared four site preparation strategies for wildflower establishment and found tillage resulted in the most cover and biomass of wildflowers.

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Introduced cool-season grasses are dominant in Virginia's grasslands, but their high digestible energy and non-structural carbohydrate (NSC) levels pose a risk for horses prone to obesity and laminitis. Native warm-season grasses (NWSG) have lower digestible energy and NSC levels that may be more suitable for horses susceptible to laminitis. The overall objectives of this research were to 1) assess voluntary intake, toxicological response, and apparent digestibility of NWSG hays fed to horses; 2) evaluate the characteristics of three NWSG species under equine grazing; and 3) evaluate establishment strategies for NWSGs and wildflowers in Virginia. For the first objective, a hay feeding trial was conducted with 9 Thoroughbred geldings in a 3 x 3 Latin square design. Voluntary dry matter intake of indiangrass (*Sorghastrum nutans*) and big bluestem (*Andropogon gerardii*) hays by horses were 1.3% and 1.1% of BW/d, significantly lower than orchardgrass (*Dactylis glomerata*), an introduced cool-season grass, at 1.7%. Biomarkers for toxicity remained within acceptable ranges for all treatments. Apparent DMD did not differ among hays, ranging from 39 to 43%. Non-structural carbohydrate levels were below the maximum recommended concentration for horses susceptible to laminitis. For the second objective, a grazing trial was conducted comparing indiangrass (IG), big bluestem (BB), and eastern gamagrass (*Tripsacum dactyloides*) (EG) yields, forage losses, changes in vegetative composition, and effects on equine bodyweight. Nine, 0.1-hectare plots were seeded with one of the three native grass treatments, and each plot was grazed by one Thoroughbred gelding in two grazing bouts, one in July and another in September 2019. Indiangrass had the highest available forage, at 4340 kg/ha, compared with 3590 kg/ha from BB ($P < 0.0001$). Eastern gamagrass plots established poorly, and had only 650 kg/ha available forage during the experiment. Grazing reduced standing cover of native grasses in IG and BB treatments by about 30%, and trampled forage constituted 36-68% of groundcover in those plots after each grazing bout. Horses lost

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CHAPTER 1: INTRODUCTION

Introduced cool-season grasses dominate Virginia's pastures and hay fields, providing economic benefits to forage-based agriculture. However, these grasses can be unsafe for horses prone to metabolic disorders, as their high concentration of non-structural carbohydrates (NSC) can lead to insulin dysregulation and laminitis, a painful inflammatory disorder which can necessitate humane euthanasia in affected horses. Native warm-season grasses have lower concentrations of NSC that may be safer for horses susceptible to metabolic disorders or insulin dysregulation, but little is known about their potential toxicity or their nutritional suitability for horses. Additionally, research on establishment of these grasses in Virginia is nascent. The overall objectives of this research are to evaluate the potential integration of native warm-season grasses into equine forage systems, and to evaluate strategies for the establishment of these grasses.

This dissertation is organized into 6 chapters. Following the introduction, Chapter 2 reviews the literature on equine nutritional needs and grazing behavior as well as the characteristics and establishment of native warm-season grasses. Chapter 3 describes a hay feeding trial with the objective of measuring voluntary intake, apparent digestibility, and toxicological responses of horses when fed two native warm-season grasses as well as a non-native cool-season hay. Chapter 4 examines the productivity and changes in pasture botanical composition of three native warm-season grass species under equine grazing. Chapter 5 evaluates different strategies for establishing native warm-season grasses and wildflowers in a series of three experiments, two of which focus on the use of the herbicide imazapic, and a third incorporating a number of site preparation techniques for seeding these species. The final chapter presents a summary of the conclusions of these studies.

CHAPTER 2: REVIEW OF LITERATURE

The Equine Industry in Virginia

The most recent analysis of the scope of the equine industry in Virginia estimated its annual impact to be $1.2 billion (Rephann, 2011). The analysis found that the horse industry supported 16,000 jobs statewide, primarily in agriculture and services sectors. Estimates of the equine population of the Commonwealth vary widely, as the United States Department of Agriculture's Census of Agriculture (USDA) counts on-farm horses only (USDA NASS, 2019). The analysis by Rephann estimated a total population of 215,000 horses in Virginia in 2008, compared to the on-farm estimate by the USDA of about 90,000 on-farm horses for the year prior (Rephann, 2011). Recent data are available for on-farm horses only, and estimate nearly 66,000 horses in Virginia (USDA NASS, 2019). Horses used for pleasure and trail riding comprise nearly half of the equine population of the state, with show horses, breeding horses, racehorses, and horses with less common purposes such as carriage or police horses constituting the rest (Rephann, 2011). The transition from being primarily working animals in prior eras to recreational animals in the modern era has had significant implications for equine health and nutrition (Belknap & Geor, 2017).

Equine Metabolic Disorders

Thatcher and colleagues found that 51% of horses in Virginia are overweight or obese (Thatcher et al., 2008). As with humans, obesity in horses can lead to insulin resistance and inflammatory responses, though prior research indicates some horses have a higher predisposition to metabolic diseases (Geor, 2008). To determine if a horse has this predisposition, there are phenotypic signs that have been shown to be reliable indicators. Regional adiposity on the crest of the equine neck is indicative of such a predisposition, and can be measured by the Cresty Neck Score (CNS), a 0 to 5 scale wherein a score of 0 indicates a neck with no fat deposit, and 5 indicates a fat deposit so extreme it falls to one side of the horse's neck (Carter et al., 2009). The CNS should be assessed in combination with the Henneke Body Condition Score (BCS), a 1 to 9 scale in which 1 is an equine in a state of emaciation, and 9 is an obese equine (Henneke et al., 1983). When a horse has both high CNS and BCS, it indicates that a horse is at risk of equine metabolic diseases and attendant inflammatory responses (Treiber et al., 2006). One of these inflammatory responses is laminitis, a condition in which the coffin bone irreversibly rotates within the hoof of the horse, causing chronic pain and lameness.

While the exact mechanisms by which laminitis occurs aren't fully known, the causative relationship between excess rapidly fermentable carbohydrate intake and laminitis is well-established (Geor, 2010). The process begins with an excessive intake of food high in carbohydrates such as starches, fructans, sucrose, or other simple sugars. When this food reaches the small intestine, carbohydrates in excess of the small intestine's capacity to digest go on to reach the hindgut. In the hindgut, the carbohydrates are readily fermented, causing hindgut dysbiosis in the form of a proliferation of bacteria producing lactic acid, lowering hindgut pH and inducing a series of inflammatory responses. The inflammatory responses result in decreased blood flow to the legs of the horse, in turn facilitating the rotation of the coffin bone as the hoof's lamellar epithelium and extracellular matrix are destroyed (Geor, 2010).

Non-structural Carbohydrates in Forages

Grasses produce carbohydrates, oxygen, and water as the primary products of photosynthesis. Most grasses utilize a 3-carbon photosynthetic pathway utilizing only the Calvin cycle, in which ribulose-1,5-bisphosphate carboxylase (Rubisco) acts as the catalyst for converting carbon dioxide in the chloroplasts of the plant's mesophyll cells into simple carbohydrates (Chatterton et al., 1989, p. 3). The overwhelming majority of cool-season forage species—species growing primarily in spring and fall—are C3 grasses. These are the most dominant grasses for forage production in Virginia (Smith et al., 2009).

Other grasses evolved a more sophisticated 4-carbon photosynthetic process in which phosphoenolpyruvate carboxylase (PEPC) is the initial catalyst in the mesophyll, producing phosphoenolpyruvate (PEP) and oxaloacetate (OAA) which is converted into malate (Mahendra et al., 1974). Adjacent bundle sheath cells take up the malate from the mesophyll, where it is decarboxylated to increase the carbon dioxide concentration in mesophyll cells. At this point in the process, the Calvin cycle is utilized but without the oxygenation reaction seen in C3 grasses, resulting in a more efficient use of water and nitrogen in C4 grasses compared to C3 grasses (Chatterton et al., 1989).

In both C3 and C4 grasses, carbohydrates produced in excess of the plant's immediate metabolic demands are stored as carbohydrate reserves. For C3 plants, these reserves are primarily simple carbohydrates such as glucose, sucrose, and fructose. For C4 plants, these reserves can also include starches (Chatterton et al., 1989). Carbohydrate partitioning is also different in C3 versus C4 plants. In C4 plants, starch can reach a saturation point in chloroplasts, after which no more can be stored. However, in C3 plants, simple sugars have no saturation point in the vacuoles. As such, carbohydrate accumulation in C3 forages is often higher than in C4 forages (Longland & Byrd, 2006). Carbohydrates not integrated into the structure of the plant as lignin, hemicellulose, or cellulose are referred to as non-structural carbohydrates (NSC). In forage testing, NSC is comprised of water-soluble carbohydrates (WSC), and starches (McIntosh, 2006).

In C3 forage species in Virginia, such as fescue (*Festuca arundinacea*), orchardgrass (*Dactylis glomerata*), and bluegrass (*Poa pratensis*), NSC levels can reach 20% of the plant's dry weight (McIntosh, 2006). NSC levels fluctuate both seasonally and diurnally in forages, as the amount of sunlight hitting photosynthetic tissue varies (Kaufman et al., 2017; McIntosh, 2006). NSC is highest for cool-season grasses during the evening in spring and fall, as cool temperatures allow maximum photosynthetic activity in the leaf to accumulate throughout the day, whereas NSC is lowest in the morning after the plant spends the evening respiring and metabolizing the NSC, especially in summer when temperatures exceed the photosynthetically optimal range for the plant (McIntosh, 2006).

Pasture-Associated Laminitis

While high carbohydrate levels in pastures provide optimal nutrition for high-production animals like dairy and beef cattle, they risk precipitating the rapid hindgut fermentation that leads to laminitis in susceptible equines (Geor, 2008). Most laminitis cases occur in horses kept on pasture, and as pasture is the foundation of the equine diet, managing the interaction between susceptible horses and pasture is critical for preventing pasture-associated laminitis (Geor, 2009). At present, equine managers with animals susceptible to laminitis have four primary options to prevent the disease.

First, managers can remove the horses from pasture and feed hay. This requires either a low-carbohydrate hay, or soaking the hay immediately prior to feeding to leach out carbohydrates (Martinson et al., 2012). This approach also leaches out vitamins from the hay, however, and thus necessitates providing adequate vitamins and minerals in some other form. With this approach, providing a dry lot for turnout and exercise is necessary to maintain the physical and mental well-being of the horse.

Second, the manager may opt to use a grazing muzzle on the horse, which is a mask designed to reduce the quantity of grass available to the horse per bite, and thus reduce overall intake when the horse is out on pasture. A two-year study using multiple forage species found grazing muzzles reduced intake by about 30% regardless of the pasture species being grazed (Glunk et al., 2014). Another multi-season study with ponies found that pasture intake was reduced by as much as 77-83%, depending on the season of use (Longland et al., 2016). However, grazing muzzles are only effective if used constantly when the equine is on pasture, as equines with limited turnout can compensate for their shortened grazing times with greatly increased intake—the same study found that ponies can consume approximately 1% of their bodyweight in dry matter in just a 3-hour timespan (Longland et al., 2016).

Another option is to keep pastures mown to a short height in order to reduce the quantity of NSC in the forage. Mowing pastures forces plants to mobilize their carbohydrate reserves for regrowth, much as grazing would. By regularly mowing the grass and depleting these carbohydrate reserves, Siciliano and colleagues found that forage NSC levels could be reliably reduced, and glycemic and insulinemic response could also be reduced in horses on mowed pasture (Siciliano et al., 2017). However, this study did not examine the long-term persistence of the pasture grasses when subjected to defoliation stress over time, nor the weed pressure that could result from maintaining low canopy height with a stressed sward.

Finally, equine managers may completely renovate their pastures, opting to eliminate grasses high in NSC in favor of grasses with a safer level of NSC. Research conducted over two years in Maryland and Virginia with eight cool-season and six warm-season introduced turf cultivars evaluated their traffic tolerance, nutritive value, and palatability (Jaqueth, 2018). The horses showed no differences in preference between cool-season cultivars, but preferred grazing common bermudagrass (*Cynodon* dactylon), and the lone non-turf species tested, crabgrass (*Digitaria sanguinalis*). Given the lack of difference in preference among cool-season species, they determined the optimal species based on relatively lower NSC levels and higher traffic tolerance. Based on these criteria, Regenerate tall fescue, Zenith zoysiagrass (*Zoysia japonica*), and Riviera bermudagrass were selected as the optimal species (Jaqueth, 2018).

Few other studies on alternative perennial forages for horses prone to metabolic disorders have been conducted.

Native Warm-Season Grasses (NWSG)

Most of the pastures and hay fields in Virginia are dominated by cool-season grasses introduced from Eurasia. Prior to European colonization, however, Virginia had large tracts of meadows and savannahs in which NWSG and herbaceous species were the dominant plants (Stewart, 2009; Tompkins et al., 2010). Native peoples in the area used prescribed fire to keep landscapes in meadow or savannah, promoting forage species preferred by game animals, enhancing the abundance of medicinal plants, and controlling pests like ticks (Stewart, 2009). When Europeans first arrived, many described vast savannahs and meadows with tall grasses in many regions of the Mid-Atlantic.

Some of the dominant species in such savannahs were believed to include big bluestem (*Andropogon gerardii*), indiangrass (*Sorghastrum nutans*), switchgrass (*Panicum virgatum*), eastern gamagrass (*Tripsacum dactyloides*), and little bluestem (*Schizachyrium scoparium*) (Tompkins & Bridges, 2013). These grasses are bunchgrasses, meaning they grow in discrete crowns with gaps between the bases of each plant in the grassland, rather than forming a dense uninterrupted sod as other cool-season forages do. As warm-season grasses, they initiate growth weeks after the risk of frost has passed, and have the highest productivity in May, June, and July, with growth slowing in late summer as the grasses reach a reproductive state and allocate carbohydrate reserves to the root crowns for re-initiation of growth the following year. This growing season coincides with the "summer slump" in cool-season forage systems, when dominant introduced forage species are dormant, and in the case of many tall fescue varieties, highest in the toxic endophyte that impacts many grazing operations (Keyser et al., 2012). As with other forages, productivity varies by site, year, species, and management, but NWSG are known to be high-yielding even with minimal inputs, with yields in a Tennessee variety trial ranging from about 5600 kg/ha to over 13,000 kg/ha for big bluestem and indiangrass, while eastern gamagrass yields range from 6700kg/ha up to about 21,000kg/ha (Keyser et al., 2012). These yields were achieved with a meager 67kg/ha of nitrogen added.

Another potential benefit for hay producers is the season of harvest. Unlike cool-season forages, NWSG hay fields should be cut in midsummer to maximize optimal balance between yield and nutritive value (Keyser et al., 2012). This timing means that curing will take place at the hottest and sunniest time of the year, hastening the process.

Common Native Warm-season Grass Species

Big Bluestem

Big bluestem is native to an immense swathe of North America, from Central Mexico in the south to Canadian prairies in the north, and from the Rocky Mountains to the Eastern Seaboard. It typically grows 1 to 2m tall, with long arched leaves 5 to 10mm wide, and reproductive culms with digitate seedheads emerging at the nodes and apex. Emergence date varies by year and location, but in the Mid-Atlantic usually occurs around late March or early

April. Big bluestem is an arbuscular mycorrhizal obligate species, meaning it requires the presence of arbuscular mycorrhizal fungi (AMF) to survive (Wilson et al., 2012). As a result of this symbiosis, it has a fine, deep root system of around 3m at maturity, which acts as ideal habitat for the AMF with which it exchanges carbohydrates for other essential nutrients for growth—especially phosphorus (Hetrick et al., 1986). Even relative to other NWSG, big bluestem has exceptional drought tolerance (Maricle et al., 2017). Big bluestem is a short-day plant, meaning a decline in daily photoperiod triggers its reproductive tillering and seed production (Owsley, 2002).

Indiangrass

Indiangrass shares a native range with big bluestem, and is often found in mixed stands with it throughout (Silletti & Knapp, 2001). Like big bluestem, it generally grows from 1 to 2m tall in a robust crown as a bunchgrass. Leaves are a similar length and width as well, though with a rougher texture. The inflorescence is a golden paisley-shaped panicle, and seeds have long awns, giving it a furry appearance (P. Keyser, Harper, et al., 2012b). Again, like big bluestem, it emergences in mid spring in most of its range. Indiangrass is an intermediate day plant, meaning it enters a reproductive phase when day length is neither too short nor too long (Younger, 2012). Later-maturing than many other NWSG, it stays vegetative well into the summer. Its drought tolerance is evident in the range of rainfall it tolerates, from 28cm in the western portions of its range to 114cm in the eastern regions, though it isn't as drought-tolerant as big bluestem (Silletti & Knapp, 2001).

Eastern Gamagrass

Eastern gamagrass, as the name suggests, is native to the eastern half of the United States. Plant height varies widely depending on site and available water and nutrients, but it can reach up to 3 meters in height (Stubbendieck et al., 2003). Leaves can be over a meter long, and a centimeter wide with a strong white midrib. Seedheads are digitate, with fingers of corn kernel-sized seeds stacked one atop another (Stubbendieck et al., 2003). This resemblance to corn kernels is not coincidental, as gamagrass is a distant relative of corn and is thought to have contributed to the breeding of domesticated corn in Central America when crossed with corn's teosinte ancestors (Eubanks, 1997). Growing in an extremely stiff crown, gamagrass spreads rhizomatously with a dense root system, though it is not sod-forming (Stubbendieck et al., 2003). As a species with aerenchyma—oxygen-transporting rhizomes that act as a snorkel for the plants—eastern gamagrass can tolerate saturated soil conditions for long periods (Skinner et al., 2009). Concomitant to its preference for lowland sites receiving more moisture and nutrients, gamagrass is one of the few native grasses with a notable response curve to medium or high nitrogen inputs (Brejda et al., 1996), though it is still highly productive on many sites without such amendments.

Nutritional Value of NWSG

In spite of the dearth of knowledge about interactions between NWSG and horses, several studies have been conducted examining the nutritive value of NWSG for beef operations, and two for dairy cattle as well. Due to the differences in C3 and C4 grasses discussed above, we know that warm-season forages tend to be lower in carbohydrates than cool-season forages when both forages are cut at the same stage of growth. However, cattle generally do better on warm-season forages in the warmer months than they do on cool-season forages due to the "summer slump," and indeed can have high average daily gains (ADG) on NWSG in well-managed systems.

Tracy and colleagues conducted a three-year grazing trial in central Illinois comparing annual warm-season grasses (AWSG)—namely sorghum-sudan hybrids—with perennial NWSG (primarily gamagrass) in terms of both cattle performance and economics (Tracy et al., 2010). They found that while AWSGs had more nutritive value, animal performance did not actually differ between AWSGs and NWSG. In fact, productivity was also higher on NWSG treatments, and annual costs of establishment incurred to keep AWSGs in productivity year to year meant that NWSG were more economical.

Keyser and colleagues examined whether different species and mixes of NWSG would give higher ADG to cattle when interseeded with red clover (*Trifolium pretense*) or left as grass-only stands (Patrick D. Keyser et al., 2016). They found that the presence of red clover did little in terms of pasture of animal productivity, but that ADG averaged 1.25kg/day in the early season and 0.54kg/day later in the season on mixed big bluestem and indiangrass pastures. Crude protein (CP) levels were between 8-10% of the forage dry matter depending on month and year, while NDF ranged from 62-78% over the same time period. The reported gains indicate NWSG can provide adequate nutrition to bred heifers in summer months, though they also highlight, paradoxically, that NWSG tend to look like low quality forages if one goes by chemical analyses alone, rather than animal performance.

A study in Tennessee examined whether prescribed fire might improve forage nutritive values for NWSG (Mathenia, 2011). As NWSG evolved with pyrrhic herbivory and demonstrate short term boosts to yield from periodic burning, researchers burned plots at different times of the year to determine if there was an optimal timing for maximizing forage value. Plots were burned in either March, April, May, or September, with an unburned control. Burning in April was shown to have benefit to total digestible nutrients (TDN) and forage quality, though neutral detergent fiber (NDF) values remained between 66-71%. For April burns, CP on the plots ranged from 10-14%.

Animal performance on NWSG is generally higher than what would be expected based on their nutritive values determined by chemical analyses. Some hypothesize bypass proteins in the forages are at work—proteins that bypass the cow's rumen and are absorbed in the intestine (P. Keyser, Harper, et al., 2012a). Foraging theory suggests that when higher intake is possible with less work required to locate the forage, grazing efficiency is optimized (Stephens & Krebs, 1986). In other words, cattle do not have to work as hard to maximize forage intake when grazing highly productive swards. With NWSG, due to their immense productivity relative to cool-season forages, this could be another reason for high cattle gains in spite of the lower nutritional quality of NWSG. It's also possible the forages are more digestible in vivo than chemical analyses suggest, meaning animals get more nutrition per kg consumed than is predicted on the forage tests. For horses, if a bypass protein is at play, it will mean little nutritionally—horses different requirements than cattle, being hindgut fermenters with no rumen microbe protein to depend on (Cymbaluk, 1990). If the latter theories are more accurate, however, this would have implications for the net energy intake of horses on NWSG forages.

Habitat Benefits of NWSG

The environmental benefits of establishing NWSG are well researched, and include enhanced habitat for wildlife and pollinators, and improved water quality. For wildlife, NWSG pastures are primarily used for habitat restoration for grassland-nesting birds such as bobwhite quail (*Colinus virginianus*), grasshopper sparrows (*Ammodramus savannarum*), indigo buntings

(*Passerina cyanea*), and others of local or regional concern (Giuliano & Daves, 2002; Harper et al., 2015). The structure of NWSG pastures is better suited to providing cover for grassland birds and small mammals from predators and inclement weather—the bunchgrass growth habit allows for ground-level gaps between plants that animals can use to travel, while the high spreading canopy of the grasses obscures the animals from birds of prey. In contrast, sod-forming species provide no basal gaps between plants for small animals to use for travel, and canopy height and cover is generally too sparse and too weak to provide shielding from predators' eyes or inclement weather (Barnes et al., 1995).

In addition to providing cover and shelter for small birds and mammals, NWSG are also host plants for macroinvertebrates. Many skipper species—members of the *Hesperiidae* family, related to moths and butterflies—are prairie obligates, meaning they require native prairie ecosystems to survive and reproduce as species (Schlicht & Orwig, 1992). Some skipper (*Lepidoptera*) species require *Andropogon* species to lay their eggs, and their larvae feed on the grasses early in their life cycles. In one meta-analysis, Narem & Meyer found 36 *Lepidoptera* species depend on 17 species of grasses as host plants, with big bluestem being used by no fewer than 9 species as a host plant (2017). Additionally, little is known about moth species and their interactions with graminoids, meaning many more species may depend on them than is presently known (Narem & Meyer, 2017).

Water Quality and Erosion Control Benefits of NWSG

The deep fibrous root systems and strong upright growth habits of NWSG are well-suited to soil and water conservation, and research on their uses spans decades. In situation where precipitation exceeds the rate of water infiltration into the soil, excess moisture runs off the soil surface down to waterways such as rivers or ponds, carrying with it sediments and nutrients (Burwell et al., 1975). Nitrogen and phosphorus runoff contribute to eutrophication in aquatic systems, wherein excess nutrients entering the water cause algae to proliferate. When the algae decompose, oxygen in the water is reduced, and dead zones ensue as fish and other organisms suffocate in the anaerobic environment (Dodds & Smith, 2016).

When used in filter strips—strips of vegetation planted near areas vulnerable to erosion and runoff—NWSG can slow surface runoff from high rainfall events, catching sediment and reducing the nitrogen and phosphorus entering riparian systems (Skinner et al., 2009). When native bunchgrasses slow the flow of surface runoff, the water-borne sediments, nitrogen and phosphorus are given a chance to settle onto the soil surface or infiltrate into the soil, and then may be taken up by the plant later and converted into biomass (Blanco-Canqui, Gantzer, Anderson, & Alberts, 2004). Some evidence suggests NWSG are more effective as filter strips than introduced cool-season grasses like fescue. Blanco-Canqui and colleagues found that it took 4m of fescue to achieve the same filtration as 0.7m of switchgrass, for example (Blanco-Canqui et al., 2004). Lee and colleagues reported higher nitrogen and phosphorus removal by switchgrass than three cool-season introduced species in a simulated rainfall experiment in Iowa (K.-H. Lee et al., 2003).

Enhanced infiltration rates are another significant boon to NWSG when compared with more common species. The large, robust leaves and dense, deep root system act almost as funnels for precipitation: the leaves catch the precipitation, slowing the rate at which it falls to the soil surface, and the porous soil rich in organic matter created by the immense root systems acts as a sponge (Miller & Dickerson, 1999).

Production Benefits of NWSG

A wide body of research in Southern and Midwestern forage systems have demonstrated a variety of potential benefits of NWSG for hay producers and beef cattle producers. NWSGThese species are known to tolerate a wider variety of soil conditions compared to improved forages (Brejda, 2015). While optimal production of introduced cool-season grasses ideally necessitates regular soil amendments in the form of liming and the input of nitrogen, phosphorus, and potassium (NPK), NWSG are tolerant of highly acidic soils and are more efficient users of NPK. As such, producers can typically expect to produce as much or more forage biomass per acre with fewer operational inputs (Rushing et al., 2019). Another benefit—perhaps of increasing importance as anthropogenic climate change impacts seasonal precipitation patterns—is the higher water use efficiency of NWSG compared to introduced cool-season forages (Hong et al., 2013). This is an effect of both the higher water efficiency of C4 grasses compared to C3 grasses, as well as the deeper root systems of most NWSG, capable of accessing moisture deeper in the soil profile. As a result of this higher water use efficiency, combined with the higher optimal growing temperature range, NWSG are better able to tolerate drought and high summer temperatures than cool-season forages (Hong et al., 2013).

Establishment of NWSG Stands

One of the primary obstacles to adoption of NWSG is their reputation as being difficult to establish (P. Keyser et al., 2019). This reputation came from three primary challenges in establishing NWSG: equipment requirements, species and variety selection, and competition control. In terms of equipment, NWSG such as indiangrass and big bluestem require specialized seed boxes to plant, as the seeds have long awns that will not flow freely through seed boxes designed for introduced cool-season species with smooth seeds (P. Keyser, Harper, et al., 2012b).

In terms of species and variety selection, the number of NWSG species and varieties within each species which are commercially available have steadily increased over time. Germination rates are also higher than in years past due to research on seed treatments (Seymour & Seymour, 2004).

While the availability of equipment and seed source options for NWSG establishment has improved, competition control remains the single largest obstacle to NWSG establishment. Site selection is the first line of control. If converting a former row crop field into pasture, the years of weed suppression will ensure minimal competition for NWSG (P. Keyser, Harper, et al., 2012b). However, if one selects a site with bermudagrass or other warm-season perennials, control must take place prior to NWSG establishment due to the lack of herbicides targeting only introduced warm-seasons rather than all warm-season perennials (Barnes, 2004). For control in the year of establishment, seed bed preparation should include at least two applications of glyphosate prior to planting—one to kill extant perennial vegetation, and the next to eliminate the first flush of weeds from the soil seed bank (P. Keyser, Harper, et al., 2012b).

Competition control after seeding will also depend upon whether the NWSG are sown with herbaceous species or in a pure grass stand. If the latter, the herbicide imazapic may be used to suppress both cool-season graminoids and most forbs as well (Bahm & Barnes, 2011). Imazapic has a moderate residual life in the soil, with estimates of its continued presence from one to six months after application, depending on soil type and precipitation (Sheley et al., 2007). This residual action can help suppress weedy species for quite a long time after the herbicide's initial application, meaning imazapic is both a pre-and-post-emergent herbicide.

If NWSG are being seeded in combination with wildflower species for pollinator habitat, the options for post-emergent weed control are greatly reduced, as most herbicides that will eliminate weedy species will also damage the wildflowers. However, some seed companies put together seed mixes incorporating broadleaf species purported to have a higher resistance to imazapic than most common weeds, and some studies have been conducted on either pre-emergent or post-emergent application of imazapic to mixed NWSG and wildflower stands. Norcini and colleagues found that soil type and cultivar both had an impact on whether a wildflower seedling was stunted in pre-emergent applications of imazapic at two different rates (2003). Beran and colleagues found that the impact of imazapic on wildflowers was inversely proportional to weed pressure, meaning the more weeds the plant had to contend with, the higher its apparent tolerance of and benefit derived from imazapic (1999).

NWSG and Horses

Horses have been grazing NWSG since the first Spanish expeditions to what is now the Southern United States. Until the widespread conversion of native ecosystems to intensively managed agricultural lands, they would have been the primary forage base for horses in the Eastern US. To this day, many horses are kept on NWSG rangelands in extant tallgrass prairie ecosystems in places such as the Flint Hills of Kansas (Obermeyer, 2008). In Virginia, Chincoteague ponies still graze NWSG commonly found in coastal ecosystems (Furbish & Albano, 1994). In spite of this long history of equine grazing of NWSG, no nutritional trials have been conducted with horses and these forages before.

Though there has been little in the way of nutritional research, several studies have investigated the toxicological effects of certain NWSG on horses after the occurrence of clinical cases in which horses were adversely affected by the forages. Species in the *Panicum* genus, for example, have been implicated in a number of cases of hepatotoxicity in horses. Fall panicum (*Panicum dichotomiflorum*) was implicated in a case in Prince William County, Virginia, in which 14 horses fell ill and ultimately six were euthanized (Johnson et al., 2006). The horses became lethargic and lost their appetites. Blood samples revealed elevated biomarkers for hepatotoxicity, and biopsies and necropsies on liver samples from the affected horses revealed hepatic lesions. This study and previous cases have demonstrated that *Panicum* species produce steroidal saponins which are the primary toxins at play (Johnson et al., 2006; Lee et al., 2009). This toxicity can be detected in blood tests as elevated levels of aspartate aminotransferase (AST), sorbitol dehydrogenase (SDH), gamma glutamyl transferase (GGT), and alkaline phosphatase (ALP) (Johnson et al., 2006).

Other potential toxicological effects are inferential. *Sorghum* species, such as johnsongrass (*Sorghum halepense*) and annual forage sorghum varieties (*Sorghum bicolor*) have been shown to cause cystitis and ataxia in horses. Cystitis is characterized by lesions on the bladder of effected horses, while ataxia of the hind legs and urinary incontinence are evident in the later stages of the condition (Adams, 1969). The condition is caused by the chemical dhurrin, which is metabolized after grazing into hydrocyanic acid in the bloodstream of the horse. Repeated exposure to the hydrocyanic acid demyelinates axonal cells in the sacral and lumbar sections of the horse's spine, accounting for the gradual and irreversible degeneration (Morgan et al., 1990). While neither johnsongrass nor forage sorghum are NWSG, indiangrass is a distant relative of these species—indeed, "Sorghastrum" is Latin for "like sorghum." Additionally, indiangrass has been shown to contain elevated levels of dhurrin for several weeks early in the growing season, above the recommended safe level for beef cattle on pasture (Gorz et al., 1979). However, indiangrass has not been implicated in any cases of equine pasture toxicity to date, and no research on its safety for horses has been conducted.

Equine Grazing Behavior

While no nutritional assessments of NWSG for horses have been conducted, some studies of equine grazing behavior provide insight into the possible interactions of horses with these species. A meta-analysis of studies of both feral and domestic herds with free access to pasture found that horses spend 10 to 17hdaily grazing, depending on the season of the study and the location of the herd (Bott et al., 2013). This grazing is split into about 15-20 grazing bouts, split between 63-75% of daylight hours and 49-50% of nighttime hours (Bott et al., 2013). In small paddocks, horses may travel as little as 1.1km/day, while horses in larger paddocks of 16ha travel an average of 7.2 km/d (Hampson et al., 2010). In contrast, cattle in a 207 ha pasture in a Western rangeland travelled only 6.1km day, highlighting the higher mobility of grazing horses as well as the increased hoof traffic their pastures must withstand relative to cattle (Hart et al., 1993). Heightened hoof traffic also leads to increased soil compaction and trampling damage to forage (Bott et al., 2013).

Sward height also impacts equine grazing behavior. In swards with similar nutritive value but differing heights, horses selected the taller patches of sward to maximize intake per bite (Naujeck et al., 2005). However, other research demonstrates horses benefit from pastures of diverse height and digestibility, where horses can selectively graze plants of short sward height and high digestibility, alternated with intermediate sward heights and lower digestibility to maximize intake (Edouard et al., 2009). This model of alternating grazing for maximum digestibility versus grazing for maximum intake aligns with the tendency domestic equines have of grazing some patches of grass repeatedly and maintaining them at a short sward height, while allowing other portions of the pasture to grow tall (Bott et al., 2013). The landscapes created by such grazing—heterogeneous sward heights with gaps in the canopy for weedy/herbaceous species to take advantage of—can promote species richness, in the right context (Fleurance et al., 2012; Menard et al., 2002).

Summary

Paradigms centering a few economically important grass species while ignoring biodiversity and ecosystem complexity can result in ecological degradation (Fuhlendorf et al., 2012), while conservation strategies centered on the cessation of agricultural use of grasslands ignore economic and social realities as well as the potential benefits of careful agricultural stewardship (Huntsinger & Oviedo, 2014). Huntsinger and Sayre argue for a “working landscapes” perspective, which seeks to reconcile human needs and sound environmental stewardship practices (2007). This view suggests that a “both/and” approach rather than an “either/or” approach is necessary on multiple-use landscapes.

Past research demonstrates that NWSG can provide superior habitat for wildlife as well as improved soil and water quality. However, their potential integration into equine operations, whether as hay or pasture, is yet to be investigated. Additionally, given the potential obstacles to establishment of NWSG, more knowledge on effective establishment techniques specific to Virginia would contribute to their potential adoption by land managers. Our research is intended to improve the understanding of NWSG establishment strategies on Virginian landscapes, and to determine if NWSG are suitable options to meet the needs of equines susceptible to pasture-associated laminitis.

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CHAPTER 3: VOLUNTARY INTAKE, APPARENT DIGESTIBILITY AND TOXICOLOGICAL EFFECTS OF NATIVE WARM-SEASON GRASS HAYS FED TO HORSES

ABSTRACT

The objective of this research was to determine the potential suitability of two common native warm-season grasses (NWSG) for use as hay for equines. The study was a 3 x 3 Latin square, feeding indiangrass (*Sorghastrum nutans*) (IG), big bluestem (*Andropogon gerardii*) (BB), and orchardgrass (*Dactylis glomerata*) (OG) to nine (n = 9) horses (9 to 13 yrs; 569±38kg BW) for two weeks per period of the experiment. Blood samples were collected weekly to measure biomarkers for toxicity, which included albumin, alkaline phosphatase, aspartate aminotransferase, bile acid, direct bilirubin, gamma glutamyl transferase, sorbitol dehydrogenase, total bilirubin, and triglycerides. A four-day digestibility and intake trial was conducted at the end of each period. Biomarkers for toxicity remained within acceptable parameters for all treatments. Voluntary dry matter intake was greatest on the OG treatment at 1.7% BW per day, compared to 1.3% and 1.1% in the indiangrass and big bluestem treatments, respectively. Horses lost an average of 16 kg BW on BB and 21 kg BW on IG over the two weeks of each feeding trial on the NWSG hays, but gained about 4 kg on OG. Dry matter digestibility did not differ among the treatments. We conclude that these two NWSG species could be useful options for horse owners seeking a low-carbohydrate “diet” hay for horses prone to obesity and laminitis.

INTRODUCTION

More than half of horses in Virginia are overweight or obese (Thatcher et al., 2008). Obesity puts a horse at risk for serious health issues, such as insulin resistance and laminitis (Geor, 2008). Laminitis is a disease characterized by an inflammatory response damaging the lamellar layer of the horse's hooves, allowing the coffin bone to rotate (Geor, 2010). It is painful, costly to treat, and may necessitate humane euthanasia of the horse.

Although the mechanisms that precipitate laminitis are not fully understood, this inflammation in the hoof usually follows consumption of large quantities of readily-fermented carbohydrates (Geor, 2010). When a horse with a predisposition to obesity or insulin resistance consumes a large quantity of carbohydrates, the carbohydrates may prove more than the intestine can readily digest, and the bolus of carbohydrates can end up being fermented rapidly in the hindgut (McIntosh, 2006). This rapid fermentation lowers hindgut pH, altering the microbiome and causing the release of endotoxins into the bloodstream (Geor, 2010). While grains can certainly induce a laminitic response, rapidly-fermentable carbohydrate levels in pastures can also reach unsafe levels at certain times of year and under certain climatic conditions. Pasture-associated laminitis may account for nearly half of all cases in the United States (Geor, 2009).

Carbohydrates in Equine Forages

Although carbohydrates comprise the majority of a grass' mass on a DM basis, the structural carbohydrates (cellulose and hemi-cellulose) are not considered problematic for horses susceptible to laminitis (Geor, 2010). Nonstructural carbohydrates (NSC) include simple sugars, starches, and soluble fibers, and the level of NSC in a forage sample may be calculated by adding the water-soluble carbohydrate (WSC) percent and the percent of starches in the sample

(McIntosh, 2006). For horses susceptible to obesity and laminitis, it is recommended not to exceed 10-12% NSC in the diet (Geor, 2010). Cool-season grass pastures can easily exceed this limit throughout much of the year (McIntosh, 2006), and even grass hays commonly fed to horses such as orchardgrass (*Dactylis glomerata*) can have NSC concentrations above 12% (Martinson et al., 2012).

Equine managers have four primary options for preventing or mitigating high carbohydrate intake for horses on pasture. First, they may put a muzzle on the horse to restrict intake during the times of year when NSC is highest in grasses, such as spring and fall (Glunk et al., 2014). Second, they may try to reduce NSC levels in the pasture by repeatedly mowing the grass to deplete carbohydrate reserves (Siciliano et al., 2017). Third, they can replant the pasture with alternative forages thought to be safer for horses (Jaqueth, 2018). Finally, they can put the horse in a dry lot and feed hay during the spring and fall to avoid pasture NSC exposure altogether (Geor, 2009).

If an equine manager chooses the fourth option, they may still be faced with difficulty in finding a hay with suitably low NSC levels. Many resort to buying commonly available equine hays such as orchardgrass, and then soaking the hay to reduce the carbohydrate load (Martinson et al., 2012). This is an effective strategy, but also labor-intensive, and the soaking process leaches valuable vitamins and minerals from the hay. Alternative equine hay species with low NSC levels would be are needed.

Native Warm-Season Grasses

Warm-season grasses have received some attention as potential alternative forages for horses susceptible to laminitis, with studies examining the potential use of bermudagrass

(*Cynodon dactylon*), crabgrass (*Digitaria sanguinalis*), and teff (*Eragrostis tef*), among others (Jaqueth, 2018; Staniar et al., 2010). Warm-season grasses tend to have lower NSC levels than cool-season grasses due to differences in photosynthetic pathways and carbohydrate storage and allocation (Moraes et al., 2013). In spite of this interest in warm-season grasses as alternatives to common cool-season forage species for horses, we could find no digestibility or toxicological research examining the use of native warm-season grass species (NWSG) for horses.

Native warm-season grasses were once abundant in the Piedmont of Virginia as a result of Native American use of prescribed fire to promote savannahs with abundant game species (Tompkins et al., 2010). Common species native to much of the eastern United States include big bluestem (*Andropogon gerardii*), indiangrass (*Sorghastrum nutans*), and switchgrass (*Panicum virgatum*). Though research is lacking on the use of big bluestem or indiangrass as equine forages, one study examining NSC in forages under different light conditions found that NSC levels in big bluestem never exceeded 120g/kg (or 12%), suggesting some NWSG may have ideal NSC levels for horses susceptible to laminitis (Kephart & Buxton, n.d.).

Toxicity of NWSG for Horses

Though research on big bluestem or indiangrass for horses is limited, several studies have determined that the *Panicum* genus causes hepatotoxicity in horses. Several species of grass in that genus are common and widespread throughout the eastern United States, including switchgrass, fall panicum (*Panicum dichotomiflorum*), and many species commonly referred to as “panic grasses” due to their large panicle seedheads. After 14 horses at a boarding facility in Virginia fell severely ill in 2004, the cause was determined to be their hay, which was largely comprised of fall panicum (Johnson et al., 2006). A subsequent feeding trial of fall panicum to two research horses for 12 days resulted in highly elevated biomarkers for hepatotoxicity in blood samples taken from the horses, and histology revealed bile duct hyperplasia and hepatocyte swelling (Johnson et al., 2006).

Similar research with switchgrass ingestion by horses demonstrated hepatotoxicity as well, and determined diosgenin, a steroidal sapogenin, to be the primary toxin (Lee et al., 2001). While the sapogenin is believed to be metabolized in a form that crystallizes in the liver of sheep affected by *Panicum* toxicity, the mechanism by which the chemical damages the equine liver is not established, though it is thought to involve apoptosis rather than crystallization (Johnson et al., 2006).

Aside from the documented toxicity of the *Panicum* genus for horses, we could find no studies linking NWSG to toxicity in horses. However, one of the common native grasses mentioned earlier, indiangrass, is related to the *Sorghum* genus—indeed, *Sorghastrum,* indiangrass's genus, means "like a sorghum" in Latin. Sorghum species, whether annual or perennial, have been linked to cystitis ataxia in horses, a condition wherein hydrocyanic acid causes degeneration of the nervous system (Morgan et al., 1990). This results in loss of bladder control and hind leg coordination, and is irreversible and often fatal (Adams et al., 1969). The primary chemical that is hydrolyzed into hydrocyanic acid is dhurrin, which is also found in indiangrass seedlings, though in indiangrass the concentration of dhurrin declines as the plant matures (Gorz et al., 1979). Nevertheless, no cases of toxicity from indiangrass have been documented in horses, and even aged sorghum hay has been found to be safe for horses, as the toxic compound does not survive prolonged storage (Adams et al., 1969).

Objectives

This research evaluated two common NWSG species, indiangrass and big bluestem, for use as equine forages. Specific objectives are to:

1. Determine if feeding indiangrass and big bluestem causes hepatic insult to horses.
2. Determine the voluntary intake of horses fed big bluestem and indiangrass hay as compared to a common hay species, orchardgrass.
3. Compare the nutritive value and apparent digestibility of these NWSG species to a common cool-season grass species, orchardgrass, when fed as hay.

MATERIALS AND METHODS

These studies were conducted in November and December of 2018 and January of 2019 at the Middleburg Agricultural Research & Extension Center in northern Virginia. A replicated Latin square design with 3 treatments, 3 periods, and 9 horses was used. The 9 horses were Thoroughbred geldings (9-13 years) and 569±38kg BW. Horses were divided into high, medium, and low relative BW groups, and then one horse from each group was randomly assigned to each of the three squares such that each square had a similar mean BW.

The study consisted of a 10-day acclimation phase and a 4-day digestibility trial. During the first 8 days of the acclimation phase (Day 1 to Day 8), horses were housed by square in three adjacent dry lots with access to run-in sheds for shelter, ad libitum white salt and water, and were fed their treatment hay ad libitum from round bales. On Day 9, horses were moved to individual stalls (3.5m x 3.5m). for the remainder of the period where they were fed their treatment hay and again had ad libitum access to clean water and white salt. On Day 10, horses were fitted with fecal collection harnesses (Equisan Ltd, Australia) to ensure comfort and familiarity with the harness (Figure 3.1). The harness was also designed to collect urine; however urinary analyses were not conducted in this trial. From Day 9 onwards, horses had group access to a dry lot for an hour per day for exercise and social time. The digestibility trial began on Day 11, and concluded on Day 14. On Day 15, horses were turned out together into a mixed cool-season pasture for a two-week washout between experimental periods. Horses were weighed on a livestock platform scale on Day 1, Day 8, and Day 15 of each period of the experiment.

Figure 3.1. The fecal collection harness used in the experiment. The harness also collected urine, but urine analysis was not conducted in this trial.

Toxicological Trial

Blood samples were collected three times per period per horse—once on Day 1 (baseline), Day 8, and Day 15. Samples were collected intravenously from the jugular into 10-ml vaccutainer tubes, placed on ice, and driven directly to Virginia Tech's Marion DuPont Scott Equine Medical Center in Leesburg, VA, for analysis. Plasma was analyzed for 9 different markers of toxicity. The markers were selected based on past studies of *Panicum* toxicity and other common pasture-associated toxicities that caused elevated marker profiles in horse serum (Table 3.1) (Curran et al., 1996; Johnson et al., 2006). Results were forwarded to a veterinarian the same day to confirm that they were within acceptable ranges. Horses were also monitored daily for any changes in behavior that might have indicated an adverse response to the novel hays being tested.

Table 3.1. Biomarkers assessed to detect potential hepatic insult to horses fed novel NWSG hays in the study.

Albumin
Alkaline phosphatase (ALP)
Aspartate aminotransferase (AST)
Bile acid
Direct bilirubin
Gamma glutamyl transferase (GGT)
Sorbitol dehydrogenase (SDH)
Total bilirubin
Triglycerides

Digestibility and Intake

The digestibility trial was conducted the last four days of each period. Bedding was removed from stalls and fecal collection harnesses were put on each horse. Each horse was offered its treatment hay at 2.5% BW dry matter based on the BW measured on Day 8 of the experimental period. Hay DM concentration was determined by taking approximately 20 cored samples per round bale being fed, drying the samples at 135ºC for two hours, and dividing the dried weight by the original weight. Hay was split into two daily feedings at 0800 and 2000 h, and fed using hay nets. Orts were collected and weighed twice daily at 0700 and 1900 hours. Fecal collection harnesses were emptied at least 3 times daily to ensure they did not become uncomfortable for the horse, at 0600, 1400, 2000, and if needed, 0000 hr. Feces were collected in tubs lined with plastic bags which were kept shut to preserve moisture, and total fecal output was weighed for each 24-hr period starting at 2000 h the day prior to 2000 h on the day of weighing. Two, 1-kg subsamples were collected after weighing and compositing each horse's fecal output each day, and one of these samples was dried at 55ºC until it reached a constant weight to determine dry matter. The remaining sample was placed in a -20ºC freezer for storage, and later thawed at room temperature for a day, then dried at 55ºC and sent to Equi-Analytical (Ithaca, NY) for chemical analyses. Grab samples of approximately 50 g were collected from each hay bale daily as they were fed to horses, and these samples were composited and submitted to Equi-Analytical for chemical analyses as well.

Treatment Hays

Hays were obtained from two sources. The cool-season grass hay was cut on site in May 2018 at Virginia Tech's Middleburg Agricultural Research & Extension Center from a field planted with orchardgrass (cv 'HLR', Barenbrug; seed were donated by King's Agriseeds, Inc., Lancaster, PA.) The indiangrass and big bluestem hays were cut and donated in July 2018 by Ernst Conservation Seeds (Meadville, PA) from pure stands normally used for seed production.

Statistical Analysis

Nutrient compositions for the three species of hay fed were compared by one-way ANOVA. If a difference was found, Tukey's HSD was used for pairwise comparisons. Apparent digestibility was calculated by dividing the difference between average daily total nutrient intake and average daily nutrient excretion and dividing by average daily total nutrient intake. Voluntary DMI was compared using a mixed model with treatment, period, and treatment x period as fixed effects, and horse as a random effect. Biomarkers of toxicity were calculated as the overall change between values from samples taken on Day 1 (baseline) and Day 15. Changes in biomarkers of toxicity were analyzed using mixed models with treatment, period, and treatment x period as fixed effects, and horse as a random effect. Mixed models with treatment and period as fixed effects and horse as a random effect were used to analyze differences in apparent digestibility. Intake, BW changes, apparent digestibility, and biomarkers of toxicity are presented as least squares means.

RESULTS

Nutrient Composition

Digestible energy was greater in the IG hay at 1.9 Mcal/kg compared to 1.8 for both OG and BB (Table 3.2). However, NDF was also over 70 in both NWSG hays, while it was 66.3 in the OG hay. Several nutrients were also greater in the OG hay than in the NWSG hays, including Ca, P, Mg, K, and Fe. Starch was greatest in the IG hay.

Table 3.2. Mean nutrient composition values for big bluestem (BB), indiangrass (IG), and orchardgrass (OG) hay treatments. Values not connected by the same letters are significantly different at P < 0.05.

	Species		
Variable	BB (n = 6)	IG (n = 5)	OG (n = 6)
Moisture, %	5.5	5.0	6.6
Dry Matter, %	94.5	95.0	93.4
DE, Mcal/kg	1.8^{b}	1.9^{a}	1.8^{ab}
CP[1], %	8.8^{b}	7.4^{b}	13.9^{a}
ADF[2], %	49.4^{a}	44.6^{b}	41.3^{b}
NDF[3], %	76.1^{a}	74.6^{a}	66.3^{b}
Ca, %	0.2^{b}	0.3^{a}	0.4^{a}
P, %	0.2^{b}	0.2^{b}	0.3^{a}
Mg, %	0.1^{b}	0.1^{c}	0.2^{a}
K, %	2.1^{b}	1.6^{c}	2.9^{a}
Fe, PPM	80.7^{b}	97.6^{b}	197.3^{a}
Zn, PPM	21.5	19.2	16.8
Cu, PPM	5.8	6.0	7.2
Mn, PPM	66.0	84.4	75.2
Starch, %	0.4^{b}	0.7^{a}	0.6^{a}
WSC[4], %	4.0	4.7	4.4
ESC[5], %	3.2	3.2	2.2
NSC[6], %	4.4	5.4	5.0

[1] Crude Protein; [2] Acid detergent fiber; [3]Neutral detergent fiber; [4] Water-soluble carbohydrates; [5] Ethanol-soluble carbohydrates; [6] Non-structural carbohydrates

Toxicological Markers

Biomarkers for toxicity stayed within parameters deemed acceptable by our veterinarian based on accepted normal ranges and prior experience with measures from our research farm, with one exception. For one horse, biomarkers were elevated past acceptable ranges on the final sampling of period 1. The horse was immediately removed from the study and was sampled frequently to monitor biomarkers. The horse did not exhibit clinical symptoms at any time. As no other horses were affected, and the horse's biomarkers remained elevated long after removal from the hay and turnout on cool-season mixed pastures, our veterinarian determined the elevated biomarkers most likely indicated either a response to an unknown insult or a recurrence of a past medical issue. The horse was on the BB treatment; no other horse had a similar response to consuming the BB hay. Consequently, we omitted that horse from toxicological analyses. In periods 2 and 3, an alternative horse was used.

Most biomarkers did not differ among treatments (Table 3.3). Of those that did, GGT increased the most in the IG treatment and SDH decreased the most. Triglycerides were lower in BB and IG treatments than in the OG treatment.

Table 3.3. Biomarker change from baseline values in horses fed big bluestem (BB), indiangrass (IG), and orchardgrass (OG) hays. Treatment means with different letters are statistically different at $P < 0.05$. Data presented are least squares means.

	Treatment		
Biomarker	BB	IG	OG
Albumin (g/dl)	0.0	0.1	-0.5
Alkaline phosphatase (u/l)	-0.6	48.0	-8.0
Aspartate aminotransferase (u/l)	-101.1	-54.5	-57.6
Bile acid (umol/l)	-0.3	-0.4	-2.5
Direct bilirubin (mg/dl)	0.0	-0.1	0.0
Gamma glutamyl transferase (u/l)	1.0^{ab}	5.7^{a}	0.0^{b}
Sorbitol dehydrogenase (u,l)	-5.1^{ab}	-3.5^{a}	-12.9^{b}
Total bilirubin (mg/dl)	0.7	0.7	0.5
Triglycerides (mg/dl)	-3.5^{b}	-3.0^{b}	16.0^{a}

Intake

Voluntary DMI was greater for OG than either of the NWSG hays (Table 3.4). Horses lost weight on both NWSG species tested, but gained weight on OG. For all measures of intake or change in BW, there was a treatment effect, but no period effect or period × treatment interaction was detected.

Table 3.4. Dry matter intake and change in bodyweight by treatment. Treatment means with different letters are statistically different at $P < 0.05$. Data presented are least squares means.

	Treatment		
Variable	BB	IG	OG
DMI, kg/d	6.6^{b}	7.0^{b}	8.9^{a}
DMI, % of BW	1.1^{b}	1.3^{b}	1.7^{a}
Change in BW	-16.3^{ab}	-21.2^{b}	3.8^{a}

Digestibility

Apparent DM digestibility did not differ among treatments (Table 3.5). Crude protein, Ca, and starch were more digestible in IG and OG treatments than in BB. Phosphorus, Mn, and WSC apparent digestibility were higher in IG than other treatments. Apparent digestibility of CP, starch, and ethanol-soluble carbohydrates (ESC) was greater in OG than in the NWSG hays. Negative digestibility of P, Mg, Fe, Zn, and Mn for all treatments indicate greater amounts of these nutrients were excreted than ingested.

Table 3.5. Apparent digestibility of the three treatment hays fed. Treatment means with different letters are statistically different at $P < 0.05$. Data presented are least squares means.

	Species		
Digestibility, %	BB	IG	OG
Dry Matter	38.8	40.6	43.2
CP	18.3^{b}	30.1^{b}	52.4^{a}
ADF	51.3	46.7	48.5
NDF	47.6	45.7	46.5
Ca	-24.0^{b}	15.6^{a}	-13.7^{b}
P	-55.4^{a}	-30.3^{a}	-47.6^{ab}
Mg	-20.6	-23.2	-13.0
K	58.7	57.7	57.3
Fe	-213.2	-144.6	-89.3
Zn	-34.0	-29.3	-45.1
Cu	-14.6^{b}	13.3^{a}	-8.6^{b}
Mn	-77.0^{b}	-18.8^{a}	-60.0^{b}
Starch	-27.0^{b}	42.7^{a}	39.5^{a}
WSC	55.7^{b}	66.5^{a}	59.4^{b}
ESC	66.1^{b}	66.0^{b}	81.3^{a}

DISCUSSION

The objectives of this study were to determine if big bluestem and indiangrass hays induce a toxic response in horses after two weeks of feeding, and to evaluate the potential use of these species as an equine hay source. Biomarkers of toxicity did not exceed acceptable limits after two weeks. Voluntary intake of these forages was lower than orchardgrass intake in our study and lower than reported values for other species. However, this may be ideal for horse owners struggling to find optimal forages for horses prone to obesity and laminitis. Apparent digestibility of some nutrients was low or negative for the NWSG hays and orchardgrass on our study, indicating a need to supplement these forages with a ration balancer to ensure adequate intake of vitamins, minerals, and protein.

Toxicological Response

The two NWSG tested did not cause biomarkers of toxicity to exceed levels deemed safe by the veterinarian monitoring horses on this study. As discussed above, one horse on the BB treatment had elevated biomarkers, but the lack of any similar response in the other horses fed the hay as well as the continued elevated biomarkers when that horse consumed cool-season pasture during a washout and monitoring period suggest a cause other than diet. Additionally, we can find no case reports in the literature of big bluestem hay causing a toxic response in horses in spite of more than four centuries of horses and this forage species interacting.

Among biomarkers measured, only GGT increased in a NWSG hay (IG) relative to the OG treatment. GGT is an indicator of hepatic function, and changes were slight. To determine if this response indicates the potential for hepatic insult caused by IG hay, a longer feeding trial may be necessary in future research. Additionally, our results cannot rule out the possibility of cystitis-ataxia for horses consuming a diet of IG long-term, as cystitis-ataxia does not affect the liver and is diagnosed clinically rather than by blood samples. None of our horses exhibited clinical signs of cystitis-ataxia after two weeks of consuming IG; however, longer feeding trials should be conducted to determine if IG poses a risk for horses in that regard.

The declines in AST and SDH for all treatments during the feeding trial is likely a result of the horses switching from a species-rich pasture during the washout periods to a monospecific hay. On pasture, horses had exposure to greater varieties of forbs and grasses, some of which can cause hepatic insult. When fed a diet exclusively consisting of one grass species, this exposure is eliminated. A similar decline was measured in a study of acetaminophen pharmacokinetics in Thoroughbred geldings at the same facility when horses were removed from pasture and fed only hay (Mercer et al., 2019).

Triglycerides increased in the OG treatment but decreased slightly in the NWSG treatments, which can be explained by the loss of BW on the NWSG treatments and increase in BW on OG. Moderate weight loss in humans results in lower serum triglyceride levels (Andersen et al., 1995). Similarly, Suagee et al. (2013) reported a positive relationship between equine body condition and plasma triglycerides, as well as insulin concentration and plasma triglycerides. These results align with our measure of limited increases in triglycerides for horses experiencing a minor increase in BW.

Voluntary Dry Matter Intake and Nutritive Values

Big bluestem matures earlier than indiangrass, and as the fields for both treatments were cut the same week, BB hay had reached a more mature stage with a higher proportion of reproductive tillers, while IG was still vegetative. Orchardgrass hay was also in a vegetative state when cut in late spring. As hay increases in maturity, nutritional quality decreases and voluntary intake by horses declines (Staniar et al., 2010). The NWSG hays in our study were cut later than is optimal, as prolonged rain delayed opportunities for cutting and curing at the site of harvest. Nutritive values from a mixed hay field of indiangrass and big bluestem from 2010 to 2012 in

Tennessee (Keyser et al., 2012) averaged 66.8% NDF, 40.2% ADF, and 9.3% CP—substantially lower fiber concentrations and moderately higher CP than the NWSG hay in our study.

Voluntary dry matter intake (DMI) of the NWSG hays was lower than values reported for mature warm-season grasses in past studies, such as 2.1% for 'Coastal' bermudagrass (*Cynodon dactylon*) or 2.3% for Caucasian bluestem (*Bothriochloa bladhii*) (Crozier et al., 1997; LaCasha et al., 1999). Voluntary DMI for horses consuming OG was within the normal ranges of 1.5-3.1% BW described in the National Research Council's guidelines for equine nutrition, with IG falling slightly below the normal range at 1.3% and BB well below at 1.1% (Council, 2007). However, for obese horses needing reduced digestible energy intake, Virginia Cooperative Extension recommends reducing hay intake to 1-1.5% of the target BW while maintaining constant forage availability to minimize risk of gastric ulcers (Porr & Crandell, 2008).

Differences in NDF paralleled differences in intake among treatments, with OG having the lowest NDF and highest intake, and the NWSG having high NDF values and lower intake by horses. This aligns with past research demonstrating the value of NDF as a predictor of intake, with lower NDF levels predicting higher voluntary intake (LaCasha et al., 1999).

Digestibility

Dry matter digestibility (DMD) measured in our study was similar to values reported for lower-quality hay in previous research, such as 43% in coastal bermudagrass (Aiken et al., 1989), 38.5% in reed canarygrass (*Phalaris arundinacea*), and 42.1% in crested wheatgrass (*Agropyron cristatum*) (Cymbaluk, 1990). Apparent DMD of the hay treatments in our study were slightly higher than those reported by Cymbaluk (1990), but similar to values measured by Staniar et al. (2014).

That several nutrients tested had net negative apparent digestibility suggests endogenous losses exceeded the amounts of those nutrients provided by hay. As we were unable to account for endogenous losses, this limitation should be considered when interpreting our results. However, prior digestibility studies in horses reported higher apparent digestibility for several nutrients than found in our research even when endogenous losses are unaccounted for. Staniar and colleagues reported endogenous losses of phosphorus, iron, and manganese for horses on different maturities of teff hay, but reported losses were far lower than the values we observed, with apparent digestibility of -2.5% for manganese, -34.7% for iron, or -4.3% for phosphorus (Staniar et al., 2010). Crozier and colleagues reported negative apparent digestibility of P, Zn, and Fe for horses fed Caucasian bluestem, yet apparent digestibility never fell below -9% for any nutrient (Crozier et al., 1997). Endogenous losses reported by Pagan (1998) for horses of similar bodyweight to those on our study are smaller than the observed losses in our fecal analyses.

Though our hays were higher in NDF than those of the hays in the studies above, the differences were likely not great enough to account for the negative digestibility of and the nutrients in the hays analyzed in our study were similar to those reported in prior research. Additionally, nutritive differences between the BB hay and other treatments were not as pronounced as the differences in apparent digestibility between BB and the other two hays. We observed extensive sorting behavior in horses consuming the stemmier BB hay, which may partially account for the lower apparent digestibility values in that hay compared to the other two treatments if horses were consuming more digestible leaves and refusing lower quality stems. Chemical analysis of only the leaves of the BB hay may have provided a more accurate representation of the horses' diet as consumed rather than as offered

Another factor may have been contamination of either the diet of the horses or the fecal samples. As fecal samples were handled with sterile gloves and stored in sealed plastic containers at all times, opportunities for sample contamination were minimal. However, if hay was contaminated with soil in stalls, this may have led to a higher intake of minerals than would be reflected in our hay samples, which were taken directly from bales stored on a concrete floor. Stall floors were partially covered with rubber matting, and hay was fed over these mats, but portions of the stall floors were bare soil, and contamination from this source cannot be ruled out. As such, we suggest the apparent digestibility values for minerals measured in our study be viewed with caution for all species, as they fall well outside ranges reported in prior research.

Integrating NWSG into the Equine Diet

Based on published nutrient requirements (NRC, 2007), the NWSG hays fed in our study do not meet DE requirements for a mature horse at maintenance at the intake rates we observed. However, this may be advantageous for horses prone to obesity and laminitis, as reducing dietary energy and NSC in the diet of horses prone to obesity and laminitis is recommended (Geor, 2009). Additionally, the low concentration of NSC in the NWSG hays is ideal for horses susceptible to carbohydrate-induced laminitis, as they were about half the recommended maximum range of 10-12% (Geor, 2009). The loss of BW observed on NWSG hays in our study was acceptable, especially given that the horses in this study were hard-keeping off-track Thoroughbreds and the trial took place in midwinter, when horses require more energy to maintain thermal homeostasis. As such, the energy levels in these hays may be ideal for horses in need of a “diet” hay, resulting in optimal weight loss rather than either extreme loss or weight gain.

However, trace minerals and vitamins may be deficient in a diet of only BB or IG hay based on nutrient values in our hay and observed intake rates, and the ratio of Ca:P was approximately 1:1 for the BB and 3:2 for IG rather than the optimal 2:1 recommended for horses (NRC, 2007). Additionally, CP was low in the NWSG hays, indicating a necessity for protein supplementation if fed long term (Table 3.6). As such, it would be advisable for equine managers to supplement the horse's diet with a ration balancer formulated to compensate for these deficiencies. This is recommended for all horses on pasture in Virginia, however, and as such should not pose an additional challenge in nutritional management (C. A. Porr & Greiwe-Crandell, 2009).

Table 3.6. Observed intake levels and dietary availability of selected nutrients compared to their requirements for a 550kg horse at maintenance as given in the 2007 NRC publication, "Nutrient Requirements of Horses," (Council, 2007).

Hay		Intake (kg/day)	DE (Mcal)	CP (g)	Ca (g)	P (g)	K (g)
BB	Observed	6.6	11.9	581	13	13	138.6
	Balance	-3	-6.4	-112	-9	-2	111.1
IG	Observed	7.0	13.3	518	21	14	112
	Balance	-2.6	-5	-175	-1	-1	84.5
OG	Observed	8.9	16.	1237	36	27	258.1
	Balance	-0.7	-2.3	544	14	12	230.6

Impaction colic should also be considered when weighing the risks and benefits of NWSG hay. While impaction colic did not occur in the horses on this study, high fiber content in hay is a contributing factor to potential impactions. Impactions have been reported on other warm-season grasses high in fiber, such as teff and bermudagrass (Little & Blikslager, 2002; Staniar et al., 2010).

CONCLUSIONS

Big bluestem and indiangrass hay elicited no toxic effects in horses for the biomarkers we measured. Voluntary intake on NWSG hays was lower than on more common cool-season grass hays, which may be ideal for horses susceptible to metabolic disorders such as obesity and laminitis. Horse owners with animals prone to these disorders should consider integrating these two species into their equine ration in combination with a ration balancer or mineral block.

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CHAPTER 4: EQUINE GRAZING OF NATIVE WARM-SEASON GRASSES

Abstract

Virginia's pastures are dominated by non-native cool-season grass species high in carbohydrates. The high carbohydrate levels in these grasses pose dangers for horses susceptible to metabolic disorders, as they can precipitate an inflammatory response in the horse which causes the coffin bone to rotate within the hoof—a painful and irreversible disease known as pasture-associated laminitis. Warm-season perennial grasses generally have lower carbohydrate levels due to their different photosynthetic pathways and cellular structure, and are generally recommended as being safer for horses prone to pasture-associated laminitis. Our study evaluated three native warm-season grasses to determine their yield, responses to grazing, and impact on animal performance in an equine grazing setting. Nine 0.1 hectare plots were seeded with one of three native grass treatments: indiangrass (*Sorghastrum nutans*), big bluestem (*Andropogon gerardii*), or eastern gamagrass (*Tripsacum dactyloides*). Each plot was grazed by one Thoroughbred gelding in two grazing bouts in July and September 2019. Standing biomass and percent cover were assessed before and after each grazing bout. Indiangrass (IG) had the highest available forage, at 4344 kg/ha, compared with 3587 kg/ha from big bluestem (BB) ($P < 0.0001$). Eastern gamagrass (EG) plots had poor establishment, and had only 654kg/ha available forage during the grazing experiment. Grazing reduced standing cover of native grasses in IG and BB treatments by ~30%, and trampled forage constituted 36-68% of groundcover in those plots after each grazing bout. Horses lost weight on all treatments, and there was a trend for horses to lose more weight on the IG treatment at 1.5 kg/day compared to 0.5 kg/day in the other two treatments ($P = 0.09$). Native warm-season grasses offer an alternative perennial summer forage option for equine managers.

INTRODUCTION

Native grasslands and savannahs dominated by warm-season grasses and wildflowers were common in the Southeast before European colonization due to the use of prescribed fire by Native peoples (Tompkins et al., 2010). When Europeans arrived with horses, they commented upon the openness of the landscape Native peoples created, and its particular suitability for traveling by horseback (Stewart, 2009). William Byrd, traveling near the Roanoke River in the early 18th century, wrote “there is scarce a shrub in view to intercept your prospect, but grass as high as a man on horseback” (Williams, 2010).

However, since colonization by Europeans, these meadowlands were gradually replaced with forage species introduced from Eurasia and Africa. The most common introduced forage in Virginia, tall fescue (*Schedonorus arundinacea*), is high in nutrition for most classes of meat and dairy livestock in the spring, fall, and if stockpiled properly, winter. For horses, this forage may be less than optimal for a number of reasons. Tall fescue is usually infected with a fungal endophyte producing a toxic alkaloid, ergovaline, which causes reproductive issues in horses (Cross, 2015). Tall fescue and other introduced cool season grasses tend to have higher carbohydrate levels than is safe for many horses during the peak growing seasons in spring and fall. High carbohydrate concentration in the forage can induce an inflammatory response in some horses that results in a painful and incurable rotation of the coffin bone within the hoof, called laminitis (Siciliano et al., 2017).

While the exact mechanisms behind laminitis are not known, high carbohydrate concentration in forage has been shown to induce laminitic episodes (Geor, 2009). Intake of feed or forages high in readily-fermented simple carbohydrates may exceed the capacity of the horse's small intestine to metabolize, allowing the carbohydrates to pass to the hindgut (Geor, 2010). In the hindgut, these carbohydrates are fermented, causing dysbiosis in the hindgut microbiome characterized by the proliferation of lactic acid-producing bacteria, lowering hindgut pH and inducing inflammatory responses (Milinovich et al., 2010). These inflammatory responses reduce blood flow to the hoof, facilitating the rotation of the coffin bone and damage to the lamellar epithelium and extracellular matrix (Geor, 2010). This damage is irreversible, painful, costly to treat, and may require humane euthanasia.

While any ingestion of rapidly fermentable carbohydrates by susceptible horses may result in a laminitic episode, most cases of laminitis occur on pasture (Geor, 2009). As such, managing the interaction between horses and pasture is critical to prevention of this disease. At present, equine managers have four primary options in preventing pasture-associated laminitis: 1) feeding hay with low carbohydrate levels or soaking hay to reduce carbohydrates prior to feeding (Martinson et al., 2012), 2) using a grazing muzzle to reduce forage intake by the horse (Glunk et al., 2014), 3) mowing pastures regularly to reduce carbohydrate levels (Siciliano et al., 2017), or 4) replanting pastures with forage species low in carbohydrates (Jaqueth, 2018).

Warm-season grasses generally have lower levels of non-structural carbohydrates (NSC)—carbohydrates not forming the walls and membranes of the plant, but rather starch and water-soluble carbohydrates—than cool-season forages, and no fructan, a simple carbohydrate thought to play a role in laminitis (Kagan et al., 2011). Consequently, a number of studies have examined the suitability of annual or perennial warm-season forages for horses. Staniar et al. (2010) found teff hay (*Eragrostis tef*) presents adequate nutrition and low carbohydrate levels for horses. Kagan et al (2011) measured carbohydrate levels of bermudagrass (*Cynodon dactylon*) at different stages of maturity and at different times of day and found it to have suitably low carbohydrate levels to be considered safe for horses prone to laminitis. While these studies on introduced forage species have provided alternatives to tall fescue for horses, no studies have examined the use of native warm-season grasses (NWSG) in equine pasture systems.

Native warm-season grasses may have lower concentrations of non-structural carbohydrates. Data from a study examining responses to different light intensities found total non-structural carbohydrate levels in big bluestem leaf blades below 100g/kg in full sunlight (Kephart & Buxton, 1996). This is within the range of maximum non-structural carbohydrate intake of 10-12% recommended for horses prone to obesity and laminitis (Geor, 2009). Chemical analyses of indiangrass and big bluestem hays in a digestibility trial in Virginia found NSC levels no more than half the maximum range of NSC for susceptible equines (data not published). As such, they may be a valuable alternative to more common cool-season forages.

Objectives

1. To compare productivity of three NWSG species used to supply forage for equines.
2. To evaluate the short-term effects of equine grazing and trampling on NWSG swards
3. To compare changes in equine bodyweight on NWSG swards in a pasture context.

STUDY SITE AND MATERIALS AND METHODS

The study was conducted at the Virginia Tech Middleburg Agricultural Research and Extension Center in Northern Virginia in July through September 2019. Nine, 0.1-ha plots were established May 2018 in a randomized complete block design with three replicates of three treatments: indiangrass (IG), big bluestem (BB), or gamagrass (GG). Plots were established on Fauquier-Eubanks and Purcellville-Tankerville soil series (fine, mixed, active mesic Typic Hapludults). Slopes at the study site ranged from 7 to 15%.

Prior to the study, the site was managed as cool-season pasture with tall fescue (*Schedonorus arundinaceus*) as the dominant species. The site was sprayed with 4.7 l/ha glyphosate the third week of April. Two weeks later, prescribed fire was used to prepare a clean seedbed and ensure fescue mortality. On June 1, glyphosate was applied again at 2.3 l/ha to kill a flush of weedy species following the fire. The same week, IG and BB plots were seeded using a Truax FLEX-II no-till drill (COMPANY, LOCATION) at a depth of 6 mm and subsequently rolled with a water-filled roller to ensure adequate seed to soil contact. Gamagrass seeds were soaked in a 15% hydrogen peroxide (H_2O_2) solution for 18 hours to break seed dormancy (Klein et al., 2008), then were drained, rinsed, and transferred to a Great Plains 706NT seed drill (COMPANY, LOCATION). After a two-day delay due to inclement weather, the gamagrass was seeded at a depth of 2cm.

IG and BB plots were sprayed with imazapic at a rate of 0.15l/ha the week after seeding. GG plots were not sprayed with imazapic, as imazapic causes stunting and mortality in gamagrass. In the second week of July, GrazonNextHL (active ingredients: 2,4-D and aminopyralid) was applied to all plots at a rate of 2.3l/ha (A.I. rate) to control broadleaf weeds. In mid-August the same year, another application of 0.3l/ha imazapic (A.I. rate) was conducted in IG and BB plots to control crabgrass (*Digitaria sanguinalis*). No further herbicide applications were applied to the plots.

Grazing Trial

The grazing trial began in July 2019. Horses were turned out into their plots on July 10, and removed on July 24 when the majority of plots were reduced to 20- to 30-cm stubble height. Nine (n = 9) Thoroughbred geldings aged 10-14 years (median: 13) and weighing an average of 550±31 kg were grouped by weight to reduce variation in grazing and trampling pressure among treatments. Each group was assigned to one treatment per grazing bout, and then reassigned randomly to another treatment on the following bout. Each plot was grazed by one horse for the duration of the grazing bouts. Horses were each provided a shade structure for shelter from the weather, ad libitum access to water and white salt, and daily applications of fly repellant.

Horses were then turned out into fescue-dominated pastures to allow NWSG plots to regrow until all plots had reached at least 46cm height. On September 4, horses were placed back on the plots and removed on September 12, once some plots had inadequate forage to meet equine needs.

Plots were sampled for biomass and percent cover using haphazardly-placed 20-cm x 50-cm quadrats at the start of each grazing bout and shortly after the removal of horses. Biomass samples (n = 5 per plot) were hand-clipped at ground level and separated into standing NWSG and weedy species, then dried in a forced-air oven at 55ºC. Trampled biomass was also harvested from within each quadrat; however, because of the difficulty in clipping fallen NWSG, these data were discarded as unreliable. Percent cover was assessed visually as standing NWSG, grassy weeds, and broadleaf weeds (n = 10 quadrats per plot). Weeds were defined as any species not seeded in the plots. In assessments made after the end of grazing bouts, the percent cover of newly trampled NWSG was also assessed. The percent utilization—the amount of biomass of a plant removed by herbivory—of grazed standing plant specimens was estimated visually for NWSG and weeds by comparing grazed stubble to nearby ungrazed grass of the same species. Ocular estimates of utilization have been shown to have adequate accuracy for estimating the severity of herbivory on a plant (Heady, 1949).

Horses were weighed immediately before being turned out into the plots, and immediately upon removal.

Statistical Analysis

Biomass data were analyzed both separately by date, and in total using repeated measures ANOVA. NWSG biomass at the beginning of each grazing bout was categorized as "available forage," and differences between treatments were compared with repeated measures ANOVA. The difference between standing NWSG biomass at the start and finish of each grazing bout was calculated and coded as "forage removed." Forage removed was also analyzed with repeated measures ANOVA to determine differences between treatments.

Percent cover was analyzed separately by date with ANOVA and pairwise comparisons made with Tukey's HSD. Cover variables analyzed include NWSG standing cover, NWSG trampled cover, grassy weeds (GW), and broadleaf weeds (BW). Percent utilization of NWSG, GW, and BW were analyzed using an ANOVA and Tukey's HSD on combined data from both grazing bouts.

Changes in equine weight for the two grazing bouts were calculated on a per day basis to account for the differing lengths of each bout in analysis. Weight changes were compared with ANOVA.

RESULTS

Biomass: Objective I of this experiment was to determine forage yields of these NWSG species in an equine pasture setting. Available forage differed at the beginning of the grazing trial (Table 4.1). Big bluestem and IG standing biomass did not differ (about 3000 kg/ha), but the GG treatment had much lower available forage (410 kg/ha). Weedy species biomass also differed, with BB having the lowest weedy biomass at 130 kg/ha and GG having the most, at 506 kg/ha. At the end of the July grazing bout, available forage did not differ among treatments, ranging from 386 kg/ha in the GG plots to 565 kg/ha in the BB plots (Table 4.1). Weedy biomass differed again, with GG having greater weedy biomass at 580 kg/ha than both the BB and IG plots, at 62 kg/ha and 295 kg/ha, respectively.

At the beginning of the September grazing bout, IG pastures had the most available forage (1450 kg/ha) while GG again had the least (250 kg/ha). BB pasture yields were intermediate (620 kg/ha). The GG treatment again had the greater weedy species biomass, increasing to 780 kg/ha compared with 330 kg/ha and 170 kg/ha in the IG and BB treatments, respectively. After the September grazing bout, there were no differences in available forage among treatments.

Total seasonal forage availability differed among treatments, with the highest mean total available forage in the IG treatment, at 4340 kg/ha, and the lowest in the GG at 650 kg/ha. Overall forage removed also differed between treatments, with the highest mean removal on IG plots at 3880 kg/ha, and the lowest removal on GG, which had net negative removal over the course of the grazing trial, indicating an increase in biomass of about 260 kg/ha.

Table 4.1. Mean available and removed NWSG forage and weed biomass (kg/ha) for each treatment in July, September, and overall.

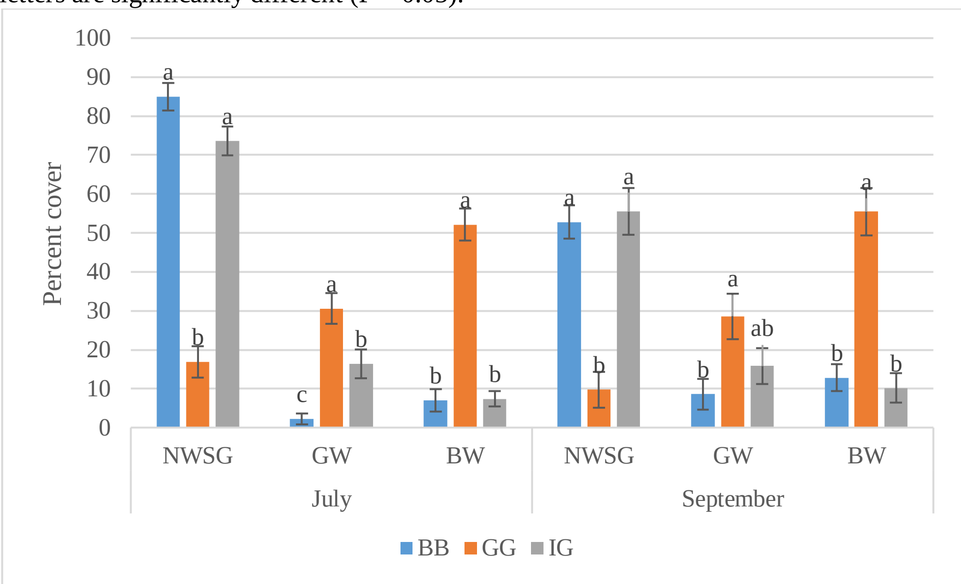
		NWSG		Weeds	
	Treatment	Available	Removed	Biomass	Removed
July	BB	2970	2400	130	70
	GG	400	20	900	310
	IG	2900	2460	510	210
September	BB	620	440	170	80
	GG	250	-280	780	240
	IG	1460	1410	330	180
Total	BB	3590	2890	300	140
	GG	650	-260	1680	550
	IG	4340	3880	830	390
P value		0.014*	0.007*	0.0025*	0.188

Effects of Equine Grazing on NWSG Swards

Objective two of this experiment was to determine the short-term effects of equine grazing on NWSG swards. BB and IG treatments had similar levels of desired species cover at the start of the experiment (85% and 74%, respectively), while GG cover was significantly lower (17%; Figure 1). Grassy weed cover was 31% in GG plots prior to grazing, while IG was half that and BB near zero. Broadleaf weeds dominated the GG plots at the beginning of the experiment at 52% cover, while BB and IG both had only 7%.

NWSG cover in September, at the start of the second grazing event, was just above 50% in the BB and IG plots and 10% in the GG plots. GG plots again had the most grassy weed cover (29%) while BB plots had a third as much (Figure 4.1). Broadleaf weeds again constituted more than 50% of GG plots and about 10% of the other two treatments.

Figure 4.1. Percent cover of native warm-season grasses (NWSG), grassy weeds (GW), and broadleaf weeds (BW) in July and September at the start of each grazing bout for the big bluestem (BB), gamagrass (GG), and indiangrass (IG) plots. Means with different letters are significantly different (P < 0.05).



After being grazed in July, over half the cover of BB and IG plots was comprised of trampled NWSG biomass, while no GG was trampled (Table 4.2). After the September grazing bout, about a third of the cover in BB plots was trampled NWSG, but IG plots once again were comprised of over 50% trampled forage from the most recent grazing bout. Again, GG plots had no measurable trampling of the native grass there.

Table 4.2. Mean percent cover of trampled NWSG by treatment following each grazing bout. Means not connected by the same letter are significantly different.

	Grazing bout	
Species	July	September
BB	67.7[a]	36.3[a]
GG	0[b]	0[b]
IG	52.5[a]	54.5[a]
P value	<0.0001*	<0.0001*

Forage utilization:

Horses utilized more standing vegetation (OR "more NWSG") in BB plots (about 34%) than in IG (17%) and GG (9%) plots (Table 4.3). Utilization of both grassy weeds and broadleaf weeds was higher in GG plots than in the other treatments, at 10.3% GW utilization in GG compared with 3.9% in IG and 0.01% in BB. BW utilization in GG plots was 2.5% while at or near zero in the other treatments.

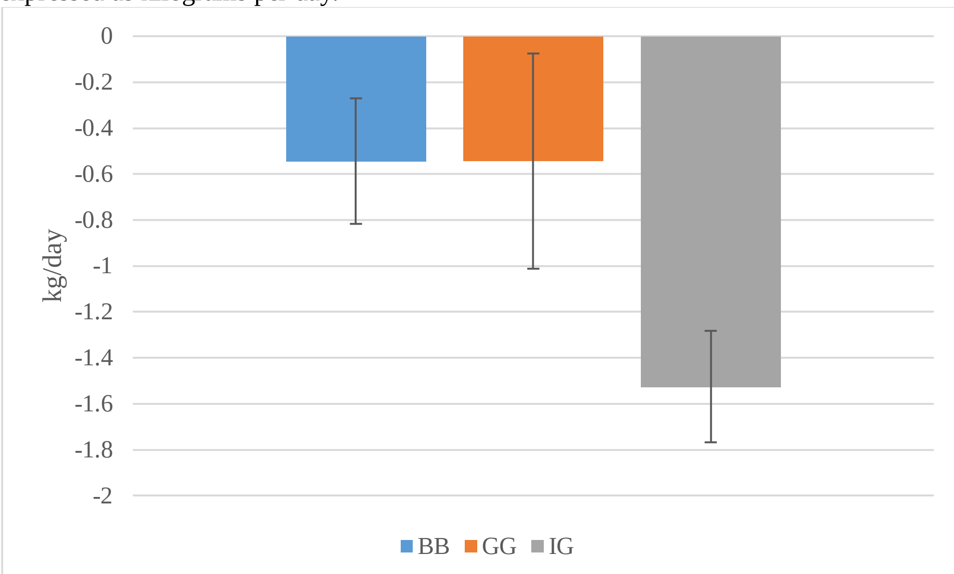
Table 4.3. Percent utilization of native warm-season grasses (NWSG), grassy weeds (GW), and broadleaf weeds (BW) in big bluestem (BB), eastern gamagrass (GG) and indiangrass (IG) pastures. Means not connected by the same letter are significantly different.

Species	NWSG	GW	BW
BB	34.4[a]	0.0[b]	0.0[b]
GG	9.1[b]	10.3[a]	2.5[a]
IG	16.6[b]	3.9[b]	0.0[b]
P value	0.0002*	<0.0001	0.0154*

Horse BW Change

Objective 3 of this study was to determine the impacts of the forage treatments on horse BW change. Horses lost weight on all treatments during the grazing bouts (Figure 4.2). Weight changes in horses did not differ among treatments; however, there was a trend for horses on the IG treatment to lose more weight than those on the BB or GG treatments, at 1.5kg/day lost on IG plots and about 0.5kg/day in the other two treatments ($P = 0.09$).

Figure 4.2. Average changes in equine bodyweight during the grazing experiment expressed as kilograms per day.



DISCUSSION

This study evaluated three NWSG species to determine their potential suitability as pasture grasses for horses in Virginia. Our results suggest both indiangrass and big bluestem could be used as pasture grasses for horses in summertime if grazed at a suitable stocking rate to ensure the long-term survival of the stand. Gamagrass plots were not sufficiently established for effective evaluation of response to grazing.

Forage Productivity

Forage biomass and regrowth in IG and BB were adequate to sustain each of the horses in this study on 0.1 hectares for about three weeks, with total available forage of 4344 kg/ha for IG and 3587 kg/ha for BB. Had the plots experienced another year of establishment prior to the grazing trial, their forage production would have been higher, as NWSG do not reach full productivity until their second or third year of establishment (Harper et al., 2011). Mature, fertilized stands of BB and IG produced 6290 and 5590 kg of dry matter/ha, respectively, in yield trials in Iowa (Hall et al., 1982). Forage yields of these two species in Tennessee typically range from 5600-9000 kg/ha (Harper et al., 2011). As these plots were unfertilized monocultures grazed a year after establishment, the lower yields relative to other studies are to be expected.

Differences in the seasonal timing of growth between species in our study may have impacted the quantity of forage regrowth between grazing bouts. The IG plots had higher regrowth than BB (940 kg/ha versus 50 kg/ha, respectively). The BB plots had produced reproductive tillers prior to the onset of the grazing trial, and may have used more of their carbohydrate reserves producing reproductive tillers than IG as a result before being grazed, limiting available reserves for regrowth. IG plots did not produce reproductive tillers until after the first grazing trial. Big bluestem generally reaches maturity earlier in the season than IG (Keyser et al., 2012). Mixing these two grasses in a pasture could provide more uniform seasonal forage distribution than managing as monocultures; however, binary mixtures of these species may not appreciably increase overall yields compared to their monocultures, as a biofuel trial in the northern Great Plains found (Hong et al., 2013).

Utilization

BB plots had approximately double the percent NWSG utilization of IG plots, and nearly quadruple that of GG plots. While no studies have compared the palatability of these NWSG species for horses, Dwyer and Sims (1964) conducted a palatability trial of common prairie species with steers and found big bluestem to be among the most palatable forages, with indiangrass intermediate among the 18 species tested. The difference in utilization may in part account for the trend for horses to lose more weight on IG plots during the grazing trial than on BB or GG plots. Though utilization of GG was lower than either of the other two species, the abundance of palatable weeds in those plots such as white clover and crabgrass, and the higher utilization of those weedy species, could in part explain the lower rate of weight loss by horses on those plots relative to IG, as many weedy species common to Virginia pastures have high nutritive value (Abaye et al., 2009).

Though logistical constraints prevented nutritive analysis of the forage species in this grazing trial, differences in nutritive value may also have factored into observed weight changes in our horses. In the digestibility trial described in Chapter 3 of this dissertation, digestible energy, neutral detergent fiber, acid detergent fiber, and crude protein did not differ between reproductive big bluestem and vegetative indiangrass cut in July, suggesting mature big bluestem may have similar nutrition to indiangrass in an intermediate growth stage. As our second grazing bout took place in September, indiangrass was mature and stemmy. BB plots were primarily fresh regrowth, and presumably more digestible. Forage samples from this grazing trial were saved in the event nutritive value may be analyzed at a later date.

Trampling

Our study did not directly measure dry matter intake of the grazing horses, and consequently determining how much of the forage removed during each grazing bout was through ingestion versus trampling is impossible. However, the disparity between total forage removed and percent utilization between species is suggestive. IG plots had on average 40%

more forage removed than BB, yet had half the percent utilized (i.e. visibly grazed), suggesting trampling may have played a larger role in forage removal in IG plots. As the grazing trial did not begin until July, both BB and IG were tall and more prone to lodging than they would have been earlier in the growing season.

Trampling damage is an important aspect of equine impacts on pasture (Bott et al., 2013). While trampling simulations have been conducted to measure traffic tolerance in turf species for horses (Jaqueth, 2018), or to determine the effect of feral horse trampling in coastal ecosystems (Turner, 1987), we found no studies quantifying pasture forage losses for actual, rather than simulated, equine trampling. A study measuring cattle trampling impacts on pasture found that a single trampling event reduced daily pasture growth from 18 kg/ha/day to 11kg/ha/day on a pasture in New Zealand, and the effect persisted for more than 7 weeks (Pande et al., 2000). Horses can move faster than cattle, and their different hoof morphology may lend itself to greater sward damage. Reductions in sward yield and vigor by equine trampling may be more pronounced.

Equine Stocking Rate

Appropriate equine stocking rates differ by site, forage species, and animal requirements, and are generally calculated by determining the dry matter intake needs of the horses, pasture productivity, and the percent of forage utilization desired (Bott et al., 2013). Voluntary dry matter intake for mature horses at maintenance ranges from 1.5-3% of bodyweight (National Research Council, 2007). A 550-kg horse would require 11 kg/day DM at an intake rate of 2% of bodyweight. Our plots were 0.1-ha each, and produced 4340 kg/ha total available forage for IG and 3860 kg/ha for BB. If potential grazing days supported were based solely on daily DMI estimates, each IG paddock should have provided about 40 days of grazing, and each BB paddock about 35 days. However, these calculations fail to account for forage losses due to trampling, as well the necessity of leaving adequate stubble height to support regrowth and long-term preservation of the sward. Both species had 80-90% forage removal after 22 days of grazing, suggesting forage removal nearly double the estimated DMI expected for horses. For NWSG pastures used in equine forage systems, calculating land area requirements strictly based on expected DMI will underestimate the amount of land needed to provide adequate forage supply. Additionally, a lower target percentage of forage removal is advisable given that 80-90% forage removal is likely to impact survival of the sward; target forage removal rates should take sward survival into account (Frost et al., 1994). Based on observed forage removal in our study, it may be more practical to base stocking rate calculations for horses grazing NWSG on about twice the estimated dry matter intake for the duration of grazing.

While prior research on the optimal stocking rate for horses on NWSG is lacking, Keyser et al. (2012) found the optimal early-season stocking density for beef cattle on big bluestem and indiangrass pastures to be about 1350-1550 kg live BW/ha, and late-season grazing to be about 1000-1350 kg live BW/ha. As our study was intended to test these forage species under stress, our stocking density was higher, at 5500kg/ha, and residual stubble height was lower than recommended for long-term stand survival (Keyser et al., 2012). For equine managers, it may be practical to start at or slightly below the stocking rate recommended for cattle to account for the excess forage loss to trampling of equines.

CONCLUSIONS

Big bluestem and indiangrass are productive species that can provide useful alternative summer forages for equine grazing systems. Gamagrass establishment was poor in this experiment but GG increased in productivity over the course of the grazing trial. For horse owners or land managers interested in forage options other than high-carbohydrate cool-season pastures, indiangrass and big bluestem provide an opportunity to optimize equine summer grazing. If grazing NWSG with horses, stocking rate determinations should take forage losses to trampling damage into account.

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CHAPTER 5: ESTABLISHMENT OF NATIVE GRASS AND WILDFLOWER STANDS IN VIRGINIA

Abstract

Virginia's pasturelands are dominated by the non-native species tall (*Schedonorus arundinacea*) Smith et al., 2009), which is toxic to livestock in the summer months (Strickland et al., 2009) and of limited value for native birds and pollinator species (Barnes et al., 1995; Jokela et al., 2016). There is a growing interest in establishing native warm-season grasses (NWSG) and wildflowers for improving summer forage productivity and wildlife and pollinator habitat. However, more research is needed to determine effective strategies land managers can use to establish these species. To that end, we carried out three experiments to investigate effective establishment methods for NWSG and wildflower mixtures. Application of imazapic, an herbicide commonly used to establish NWSG in ecosystem restoration programs, was tested in two experiments. The third investigated four seedbed preparation methods without the use of imazapic: glyphosate, glyphosate with raking to remove thatch, prescribed fire, and tillage. In the first experiment, imazapic was applied to 0.8-ha pastures containing either a mix of NWSG with or without wildflowers. All plots were grazed by beef cattle during the growing season and sampled for biomass and percent cover between May and September in 2017, 2018; only the NWSG + wildflower plots were sampled in 2019. In plots seeded with the grass and wildflower mix, those treated with imazapic had greater ($P \leq 0.012$) native grass cover (4.6% versus 1.5% in the control), and biomass (29.86g/m^2 versus 10.02 gm^2 in the control) in the second year, and between 75-96% less wildflower cover and between 74-99% less biomass for the duration of the experiment. Grass-only plots treated with imazapic had between 27-45% greater native grass

cover and 48-51% greater biomass. The second experiment compared four rates of imazapic application. In plots sown with NWSG and wildflowers, native grass biomass was greater in plots treated with 6oz/acre rate of imazapic compared to the control or plots with 2oz/acre, while native grass biomass at the 10oz/acre rate was intermediate. Weedy cover was lowest at the 6oz/acre rate at 2.4% cover and highest in the control plots at 40.5% cover, with the 2oz/acre and 10oz/acre weedy species cover values falling between. In plots sown only with NWSG, weedy species cover was higher in control plots than in any of the imazapic treatments, but biomass did not differ among plots. Native grass cover and biomass also did not differ among treatments. The third experiment examining four establishment strategies found that tillage resulted in the most wildflower biomass and cover and the lowest weed biomass and cover. In 2017, weedy species cover was lowest in the tilled plots compared to other treatments, at 49.8% compared to between 67.4% to 84.6% in the other treatments. The tilled plots also had the highest wildflower cover at 24.2%, compared to a range of 5.5% to 13.5% in the other treatments. Aboveground biomass followed the same pattern, with tilled plots averaging 86.5g/m^2 and the other treatments averaging less than half that much. The next year, the tillage treatment continued to have higher wildflower biomass than the other treatments at 75.5g/m^2 while other treatments ranged from 41.5g/m^2 in the glyphosate and rake treatment to 57.7g/m^2 in the glyphosate-only treatment. The results of these experiments indicate that imazapic use is beneficial for native grass establishment, but deleterious for wildflower establishment. If wildflower establishment is a land manager's primary goal, tillage may be a better strategy.

INTRODUCTION

Native meadows dominated by warm-season grasses and wildflowers were common in the Southeast before European colonization due to the use of prescribed fire by Native peoples (Tompkins et al., 2010). However, since colonization by Europeans, these meadowlands were gradually supplanted with introduced forage species from Eurasia and Africa.

The most common introduced forage in Virginia, tall fescue (*Schedonorus arundinacea*), is high in nutrition for most classes of livestock in the spring, fall, and if stockpiled properly, winter. However, tall fescue is often infected with a fungal endophyte producing a toxic alkaloid, ergovaline, leading to reduced gains and potentially veterinary issues for cattle in summer, costing producers billions of dollars a year in the United States (Strickland et al., 2009). Tall fescue also causes reproductive issues in horses (Cross, 2015), as well as having higher carbohydrate levels than is safe for many horses during its peak growing seasons in spring and fall (Siciliano et al., 2017). Consequently, some livestock managers are interested in incorporating novel forages into their grazing systems to mitigate fescue's drawbacks (Keyser et al., 2019).

In addition to fescue's agronomic issues, the grass presents many difficulties for maintaining or enhancing ecosystem services. Tall fescue is highly competitive, and research suggests it has an inhibitory effect on native species resulting from the inability of their seeds to detect fescue's presence, resulting in their germination into an already established fescue sward (Renne et al., 2004). This suppression of native plant species results in lower species richness and consequently lower habitat value for many species depending on native meadows, such as grassland bird species and pollinators (Barnes et al., 1995; Jokela et al., 2016). Native grasses and wildflowers coevolved with many of these species, and consequently have much higher habitat value for grassland obligate species (Washburn et al., 2000).

As a result of these disadvantages of homogenous fescue-dominated pastures, converting some fescue swards into grasslands dominated by native warm-season grasses (NWSG) and wildflowers may provide economic benefits for livestock production in summer, while enhancing the quality and diversity of habitat available for grassland obligate birds and pollinators. In spite of the potential benefits of incorporating NWSG and wildflowers into working grasslands, difficulty in establishing them remains a significant obstacle to their adoption by producers.

Establishing Native Warm-Season Grasses and Wildflowers

Native warm-season grasses have a reputation for being difficult to establish, which remains one of the largest barriers to their incorporation into agricultural operations (Keyser et al., 2019). Newly-established stands are often visibly weedy, as NWSG tend to invest much of their growth into establishing prolific root systems their first year, with aboveground growth less visible for producers. Additionally, many graziers in the South have pastures dominated by tall fescue, which is difficult to control long term without periodic management. Current recommendations by Extension programs in the South advise prolonged site preparation, with an herbicide burn-down of the site in the fall, a cover crop over the winter, and another burn-down in the spring just before planting (Keyser et al., 2012). Specialized seed drills must be used with most species because of the fluffy seeds awns—a hair-like projection attached to the seed coat—which do not flow through standard drill boxes. If no seed drill is available, land managers may broadcast seeds, but must increase their seeding rate and seed costs to maximize their chances of successful stand establishment (Keyser et al., 2012).

Given these obstacles to NWSG establishment, reducing competition from weedy species to improve the likelihood of success for land managers is vital. In other states, prairie restoration programs have successfully used the herbicide imazapic to facilitate establishment of native species, as many native warm-season grasses are resistant to its effects, while many invasive or exotic species—especially cool-season grasses such as tall fescue—are highly susceptible to it (Barnes, 2004). Imazapic is an herbicide of the imidazolinone family, characterized by a mechanism of action which inhibits amino acid synthesis plants require for growth (da Costa Marinho et al., 2019). Absorbed through leaves, stems, and roots, it can be used as a foliar spray or applied to soil for both pre-emergent or post-emergent control.

Some research on imazapic use in restoration of native grasses demonstrates that an initial application of glyphosate, followed by applying imazapic at the low rate of 0.067kg ai/ha during seeding, can be effective for weed control and seedbed preparation (Barnes, 2004). Other studies in the upper South found that a spring prescribed fire followed by a pre-emergent application of imazapic at seeding improved native grass establishment (Washburn et al., 2000). In Virginia, we could find only one small plot study on imazapic and NWSG establishment, which found the herbicide to be effective for establishing native grasses on a site in the northern Shenandoah Valley (Priest & Epstein, 2011). Given its highly variable effects and persistence depending on extant species composition and soil types, further research is needed to determine its role in NWSG and wildflower establishment in Virginia.

Objectives:

1. To evaluate effectiveness of imazapic herbicide for establishing stands of NWSG or NWSG+wildflower mixtures in a forage-livestock system.
2. To determine optimal rates of imazapic needed for successful establishment of NWSG and NWSG+wildlflower stands.
3. To explore chemical and non-chemical methods to establish wildflower stands without the use of imazapic.

STUDY SITES AND MATERIALS AND METHODS

Three discrete experiments were conducted to investigate establishment methods for NWSG and wildflowers.

Experiment I (Objective 1):

Six, 0.8-ha plots were established at the Virginia Tech Shenandoah Valley Agricultural Research and Extension Center in Virginia in June 2017. Three plots were planted with a NWSG-only mix (hereafter referred to as GO) of big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), and indiangrass (*Sorghastrum nutans*), and the remaining three with a combined NWSG and wildflower mix of the above three grasses and an additional 15 wildflower species (hereafter referred to as WF) (Table 5.1). Glyphosate was applied to the plots at a rate of 2.3L/ha prior to establishment to kill extant vegetation in preparation for seeding.

One week after the plots were planted, imazapic at a rate of 0.15L/ha (2oz/acre) was applied to a 3-m-wide swath around the perimeter of each plot. Once mixtures were established the plots were grazed by 8 mature cows between May and September in 2017, 2018, and 2019.

Thistle species were spot-sprayed with 2,4-D during the dormant seasons to prevent off-plot spread, and pokeweed was controlled manually during the growing season on plots for the same reason.

Table 5.1. The seed mixes used in Experiment I.

Native Grass Seed Mix	
Genus species	**Common name**
Andropogon gerardi	Big bluestem
Avena sativa	Oat (companion crop)
Schizachyrium scoparium	Little bluestem
Sorghastrum nutans	Indiangrass
Wildflower Seed Mix	
Genus species	**Common name**
Agastache foeniculum	Marsh blazing star
Andropogon gerardi	Big bluestem
Chamaecrista fasciculata	Partridge pea
Chrysanthemum leucanthemum	Oxeye daisy
Chrysanthemum maximum	Shasta daisy
Coreopsis lanceolata	Lanceleaf coreopsis
Desmanthus illinoensis	Illinois bundleflower
Echinacea purpurea	Purple coneflower
Gaillardia aristata	Blanketflower
Gaillardia pulchella	Indian blanket
Helianthus maximiliani	Maximilian sunflower
Liatris spicata	Anise hyssop
Linum perenne	Perennial blueflax
Ratibida pinnata	Grey-headed coneflower
Rudbeckia hirta	Black-eyed Susan
Schizachyrium scoparium	Little bluestem
Sorghastrum nutans	Indiangrass

Species density and cover data as well as dry matter yield of NWSG, wildflowers, and weeds were collected in fall 2017 and spring and summer 2018. Data collection for the NWSG plots was discontinued after 2018, but continued in the WF plots in 2019. Plant species composition was assessed visually using 10 quadrats of 0.25-m^2 placed randomly in both the herbicide treatment and control treatment of each of the plots. Aboveground biomass was measured by hand harvesting and separating quadrat samples into native grasses, weeds, and wildflowers (in the WF treatment), then drying in a forced-air forage oven at 55ºC for several days.

Experiment II (Objective 2):

Experiment two was a split plot design comparing four rates of imazapic on two seed mixes similar to those of the large plot experiments. The experiment was conducted at Virginia Tech's Shenandoah Agricultural Research & Extension Center in Raphine, Virginia. Plots were seeded in June 2018. Six blocks were seeded with either a NWSG-only mix of big bluestem, little bluestem, and indiangrass (NWSG treatment), or another combined NWSG and wildflower mix of the above three grasses and an additional 15 wildflower species (WF treatment) (Table 5.2).

Table 5.2. Seed mixes used in Experiment II.

NWSG Seed Mix	
Genus species	**Common name**
Andropogon gerardi	Big bluestem
Avena sativa	Oat (companion crop)
Schizachyrium scoparium	Little bluestem
Sorghastrum nutans	Indiangrass
Wildflower Seed Mix	
Genus species	**Common name**
Agastache foeniculum	Marsh Blazing Star
Andropogon gerardi	Big bluestem
Baptisia australis	Blue false indigo
Chamaecrista fasciculata	Partridge pea
Chrysanthemum leucanthemum	Oxeye daisy
Chrysanthemum maximum	Shasta daisy
Coreopsis lanceolata	Lanceleaf coreopsis
Desmanthus illinoensis	Illinois bundleflower
Echinacea purpurea	Purple coneflower
Gaillardia aristata	Blanketflower
Gaillardia pulchella	Indian blanket
Helianthus maximiliani	Maximilian sunflower
Linum perenne	Perennial blueflax
Ratibida pinnata	Grey-headed coneflower
Rudbeckia hirta	Black-eyed Susan
Schizachyrium scoparium	Little bluestem
Sorghastrum nutans	Indiangrass

Each block had four plots of 1.4-m × 4.6-m dimensions with rates of imazapic applied at 2oz, 6oz, and 10oz/acre rate plus a control with no imazapic. Site preparation included tillage, and seeds were broadcast by hand immediately prior to imazapic application. The seedbed was rolled to ensure adequate seed-to-soil contact.

Small plots in the imazapic rate experiment were sampled for species cover and biomass once in both 2018 and 2019 at the end of each growing season. One 0.1m^2 quadrat (20 x 50cm) was placed haphazardly in each plot away from the edges of the plot to prevent potential edge effects. Biomass was harvested by clipping to ground level, and hand-separated into native grasses, weedy species, and wildflowers (in the WF treatment only). Forage samples were dried in a forced air oven at 55ºC for several days.

Experiment III (Objective 3):

Experiment III was established at Virginia Tech's Kentland Farm in Blacksburg, Virginia in 2017 and tested a variety of establishment strategies. We used a randomized strip plot design with four blocks. Each block contained four 2 x 6-m plots each assigned to whole plot treatment of: 1) glyphosate application, 2) glyphosate with raking of thatch, 3) prescribed fire, and 4) tillage. The glyphosate treatments consisted of three applications at 2 quarts/acre—one in March, April, and May of the year of establishment. For the glyphosate + raking treatment, thatch was removed from plots with both a York rake and a mower-mounted dethatcher one week before seeding. The prescribed fire and tillage treatments were conducted the same day as the dethatching, one week prior to seeding. Tilled plots were tilled with a Kuhn EL 62 with a maximum tillage depth of 18 cm. Seeds were broadcast by hand, and included a mix of nine wildflower species and two native grass species (Table 5.3).

Table 5.3. The seed mix used in Experiment III.

Genus species	Common name
Bidens aristosa	Showy tickseed
Chamaecrista fasciculata	Partridge pea
Coreopsis lanceolata	Lanceleaf coreopsis
Coreopsis tinctoria	Plains coreopsis
Echinacea purpurea	Purple coneflower
Helianthus maximilianii	Maximilian sunflower
Monarda fistulosa	Wild bergamot
Rudbeckia hirta	Black-eyed Susan
Schizachyrium scoparium	Little bluestem
Solidago nemoralis	Gray goldenrod
Tridens flavus	Purpletop

The small plots in the experiment testing alternatives to imazapic were sampled for percent cover and density twice per growing season in 2017, 2018, and 2019 and once for biomass each September (at the end of the growing season). Cover, density, and biomass data were collected from within 1-m^2 quadrats (n=3) placed randomly on each plot. Both biomass and percent cover were further designated as either wildflowers or weeds for the analyses. Samples were dried to a constant weight in a forced-air oven at 55ºC before weighing.

Statistical Analysis

Statistical analyses were conducted with JMP®, Version 15. Cover values for desirable species were analyzed at the species level in addition to being combined into index variables (native grasses, weedy species, and wildflowers) to compare overall success of each establishment method. For Experiment I, biomass and species composition data were analyzed with t-tests within years to determine differences in cover and biomass of desirable and weedy species. Desirable species were defined as those seeded in the plots, and weeds were every other species found in the plots. For the small plot experiments, data were analyzed with ANOVA by year to compare biomass and cover of desirable and weedy species among treatments. Pairwise comparisons of imazapic rates were conducted with Tukey's HSD in Experiment II to determine the optimal rate for NWSG or wildflower establishment. Tukey's HSD was also used to compare WF establishment treatments in Experiment III. Grass-only plots and WF plots were analyzed separately as distinct experiments.

In the third experiment, NWSG were omitted from analysis because the collection team had difficulty distinguishing individual grass species. As well, a large wild population of one of the grasses in the area surrounded the plots. This complicated our ability to determine if the grass seedlings were planted or volunteers, which potentially confounded the analysis.

RESULTS

Experiment I

The objective of experiment I was to evaluate imazapic herbicide effectiveness for establishing NWSG and NWSG/WF mixtures in a forage-livestock system. In plots sown with the WF mix, percent cover and biomass of native grasses did not differ between the herbicide plots and the no-herbicide plots in 2017. Percent WF cover was greater ($P < 0.0001$) in the untreated plots than in the imazapic-treated plots (26.9% vs. 0.9% cover, respectively). Weedy presence (Table 5.4) was lower on plots treated with imazapic (13.2% vs. 35.5% cover).

Similarly, WF biomass was greater ($P = 0.0029$) in the untreated plots (65.3g/m^2) than in plots treated with imazapic (0.3g/m^2). Weedy species had lower biomass in imazapic plots at 23.9g/m^2 compared to 71.1g/m^2 in the no-imazapic plots (Table 5.5).

Table 5.4. Mean percent cover of native grasses, wildflowers, and weedy species in the large plots seeded with the native grass and wildflower mix. Cover totals do not equal 100% cover due to the omission of bare ground from this analysis.

	2017			2018			2019		
	No-imazapic	Imazapic	P value	No-imazapic	Imazapic	P value	No-imazapic	Imazapic	P value
Native grasses	2.5	2.8	.4161	1.5	4.6	0.0030*	2.7	2.0	0.4260
Wildflowers	26.9	0.9	<.0001*	44.5	11.2	<.0001*	20.5	0.9	0.0004*
Weedy species	43.2	15.0	<.0001*	40.6	60.1	0.0001*	76.4	95.4	0.0034*

* P < 0.05.

Table 5.5. Mean aboveground biomass of native grasses, wildflowers, and weeds in the large plots seeded with the native grass and wildflower mix. Values are grams per square meter (g/m^2).

	2017			2018		
	No-imazapic	Imazapic	P value	No-imazapic	Imazapic	P value
Native grasses	2.3	4.6	0.1165	10.0	29.9	0.0107*
Wildflowers	65.3	0.4	0.0029*	727.2	192.4	<.0001*
Weedy species	71.1	23.8	0.0013*	385.3	477.2	0.1934

* P < 0.05.

In 2018, NWSG cover and biomass levels were three times greater in plots treated with imazapic (Table 5.5). However, in 2019 the control plots had greater WF, NWSG, and lower weedy species than treated plotsIn plots seeded with the GO mix, imazapic plots had higher NWSG cover and lower weed cover in 2017 (Table 5.6). Weed cover was 2x higher in no-imazapic plots compared with imazapic plots in 2018.Alternatively, native grass cover was 10% higher in imazapic plots (Table 5.6).

In 2017, native grass biomass did not differ between treatments, but this changed in 2018 as native grasses had almost twice the biomass in imazapic plots than in no-imazapic plots

(Table 5.7). Imazapic suppressed weed biomass in 2017, but no treatment differences were noted for 2018.

Table 5.6. Mean percent cover of native grasses and weedy species in the large plots seeded with the native grasses only. An asterisk denotes a significant difference. Totals do not equal 100% cover due to the omission of bare ground from this analysis.

	2017			2018		
	No-imazapic	Imazapic	P value	No-imazapic	Imazapic	P value
Native grasses	9.0	16.3	0.0073*	26.0	35.8	0.0095*
Weedy species	35.5	13.2	<.0001*	25.9	12.9	0.0003*

* *P < 0.05*

Table 5.7. Mean aboveground biomass of native grasses and weeds in the large plots seeded with the native grasses only. Values are grams per square meter (g/m^2).

	2017			2018		
	No-imazapic	Imazapic	P value	No-imazapic	Imazapic	P value
Native grasses	18.99	28.16	0.1327	138.58	280.58	0.0007*
Weedy species	45.23	14.91	0.017*	228.61	192.18	0.3341

* P < 0.05

Experiment II*:* The objective of experiment II was tocompare establishment of NWSG and WF at four different rates of imazapic application. In plots sown with NWSG and wildflowers, native grass biomass was higher in plots with the 6oz/acre rate of imazapic compared to the control or plots with 2oz/acre, while native grass biomass at the 10oz/acre rate was intermediate between those two extremes. Weedy biomass and wildflower cover and biomass did not differ statistically but declined with increased imazapic rates. For example, weedy cover was lowest at the 6oz/acre rate at 2.4% cover and highest in the control plots at 40.5% cover, with the 2oz/acre and 10oz/acre weedy species cover values falling between (Figures 1 & 2).

Figure 5.1. Percent cover of wildflowers, NWSG, and weeds by imazapic rate in the plots seeded with the wildflower mix in Experiment II. Levels not connected by the same letter are significantly different. Totals do not equal 100% cover due to the omission of bare ground from this analysis.

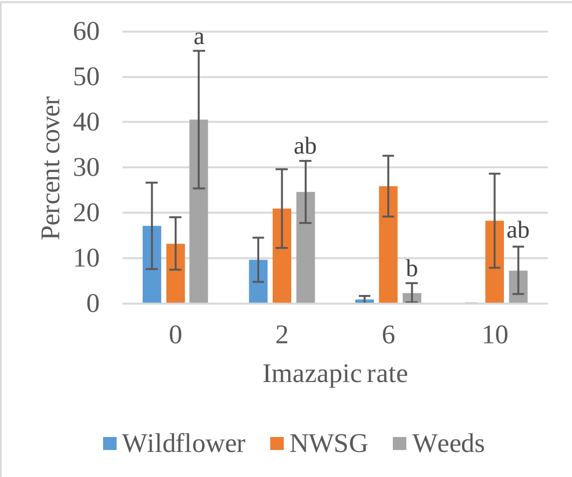
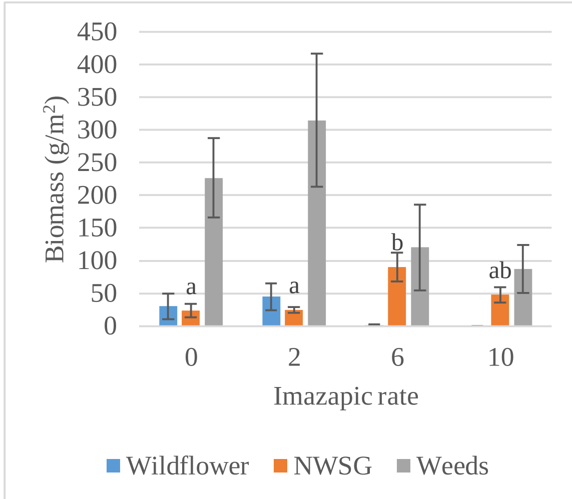


Figure 5.2. Biomass (g/m^2) of wildflowers, NWSG, and weeds by imazapic rate in the plots seeded with the wildflower mix in Experiment II. Levels not connected by the same letter are significantly different.



Of wildflower species, none established in any plot receiving the highest rate of imazapic (10oz/acre) (Figs 1 and 2) . Only black-eyed Susans (*Rudbeckia hirta*) established in a plot of 6oz/acre, and black-eyed Susan, Illinois bundleflower (*Desmanthus illinoensis*), and oxeye daisy (*Leucanthemum vulgare*) established in the 2oz/acre treatment.

In plots sown only with NWSG, imazapic effectively suppressed weeds at most applications rates, but NWSG cover and biomass did not differ among treatments (Figures 3 & 4).

Figure 5.3. Percent cover of NWSG and weeds by imazapic rate in the plots seeded only with native grasses in Experiment II. Levels not connected by the same letter are significantly different. Totals do not equal 100% cover due to the omission of bare ground from this analysis.

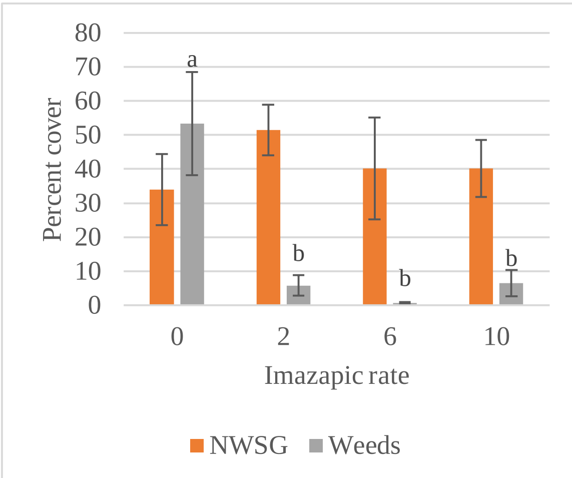
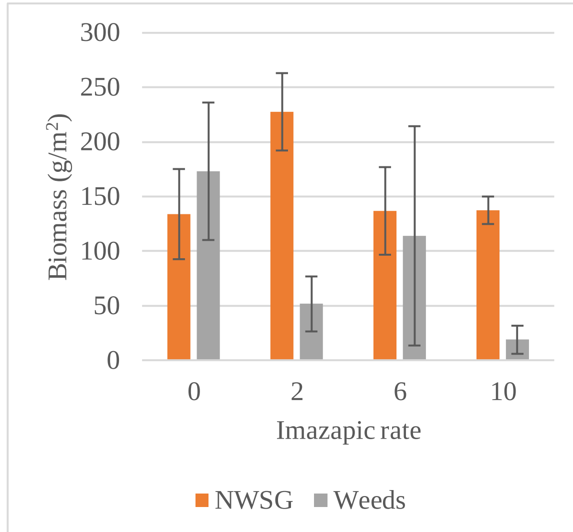


Figure 5.4. Biomass (g/m^2) of NWSG and weeds by imazapic rate in the plots seeded only with native grasses in Experiment II. Levels not connected by the same letter are significantly different.



Experiment III

The objective of experiment III was to evaluate four strategies for establishing wildflowers. In 2017, weedy species cover was lowest in the tilled plots (49%) compared with other treatments (67.4% to 84.6%) (Table 5.5). The tilled plots also had the highest wildflower cover at 24.2%, compared with a range of 5.5% to 13.5% in the other treatments. Aboveground biomass followed the same pattern, with tilled plots averaging 86.5g/m^2 and the other treatments averaging 50% less (Table 5.6). The next year, the tillage treatment continued to have higher wildflower biomass than the other treatments at 75.5g/m^2 while other treatments ranged from 41.5g/m^2 in the glyphosate/rake treatment to 57.7g/m^2 in the glyphosate-only treatment. In the final year of the experiment, 2019, biomass differences were less pronounced among treatments although wildflower biomass was greater in the tilled plots (P = 0.085) (Table 5.9). No differences in weedy biomass were noted among the treatments. Wildflower cover was highest in tilled and glyphosate treatments and lowest under prescribed fire during 2019 (Table 5.8).

Weedy species cover was still highest in the glyphosate/rake treatment and lowest under tillage in the final year, at 47.3% and 29.8% respectively.

Table 5.8. Percent cover of wildflowers and weedy species between establishment treatments in Experiment III. Levels not connected by the same letter are significantly different. Totals do not equal 100% cover due to the omission of bare ground and native grasses from this analysis.

	2017		**2018**		**2019**	
Treatment	Wildflowers	Weedy Species	Wildflowers	Weedy Species	Wildflowers	Weedy Species
Glyphosate	13.5^{b}	67.4^{b}	57.8^{b}	36.4^{b}	61.2^{ab}	33.0^{bc}
Glyphosate and Rake	5.5^{c}	84.6^{a}	41.5^{c}	55.9^{a}	51.2^{c}	47.3^{a}
Prescribed Fire	6.5^{bc}	72.4^{ab}	46.2^{bc}	45.9^{ab}	53.1^{bc}	43.1^{ab}
Tillage	24.2^{a}	49.8^{c}	75.5^{a}	18.0^{c}	65.0^{a}	29.8^{c}
P value	<0.0001*	<.00001*	<0.0001*	<0.0001*	0.0006*	0.0002*

* P < 0.05.

Table 5.9. Aboveground biomass (g/m^2) of wildflowers and weedy species between establishment treatments in Experiment III. Levels not connected by the same letter are significantly different.

	2017		**2018**		**2019**	
Treatment	Wildflowers	Weedy Species	Wildflowers	Weedy Species	Wildflowers	Weedy Species
Glyphosate	30.0^{b}	115.3^{a}	109.6^{ab}	58.1^{a}	127.2	29.1
Glyphosate and Rake	17.6^{b}	104.5^{ab}	73.6^{b}	81.9^{a}	80.1	39.3
Prescribed Fire	38.3^{b}	80.7^{b}	143.8^{ab}	75.9^{a}	115.6	30.5
Tillage	86.5^{a}	40.3^{c}	193.5^{a}	18.8^{b}	166.2	19.6
P value	0.0006*	<0.0001*	.0072*	<.0.0001*	0.085	0.4875

* P < 0.05.

DISCUSSION

This study evaluated three field experiments designed to evaluate establishment methods for native grasses and wildflowers in Virginia. Overall finding indicated that imazapic applications can aid in establishing native grasses in Virginia, but imazapic should not be used for weed control in pastures or meadows being seeded with wildflowers in the year of herbicide application. In the small plot studies, the effect of imazapic was less pronounced, though native grasses were better established in plots receiving imazapic. However, this may reflect the smaller sample size and shorter duration of that study. We also found that tillage can be an effective tool to establish wildflowers if imazapic is not used during the renovation process…

Effectiveness of Imazapic for Native Grass/Wildflower Establishment

In Experiment I, native grasses responded favorably to the weed suppression of imazapic with consistently greater biomass and cover over the course of the study. The exception was the native grasses in wildflower plots in the final year of the study, which were lower in cover and biomass in the imazapic plots relative to the control. This was likely a result of bovine grazing behavior—the control plots were more difficult to navigate for researchers and cattle alike, with the large stems of wildflower species acting as obstacles (Figure 5.5).

Figure 5.5. Large wildflowers impeded movement in the no-imazapic WF plots, potentially acting as physical barriers to cattle and allowing native grasses to escape herbivory. Photo taken at human eye-level in June, 2018.

As a result, native grasses may have been more difficult to find and eat for the cattle in the control portion of the plots relative to the easily-navigated imazapic areas, where native bunchgrasses were obvious from a distance and easy to reach. Similarly, in the second and third years of the large wildflower plots, weedy species were higher in the imazapic treatment because the suppression of wildflowers by the herbicide left a void for weedy species to fill, with the herbicide's soil residual wearing off enough by spring following the year of establishment to allow cool-season exotics such as white clover (*Trifolium repens*) and quackgrass (*Elymus repens*) to take hold.

In the no-imazapic plots, the thriving wildflowers competed with weedy species and grasses alike, suppressing both. If a land manager is more interested in establishing a pollinator meadow than a pasture, the results in the no-imazapic plots may be desirable. However, if a pasture is desired, the seed mix we used will need to be modified to increase the proportion of grasses to wildflowers.

Though the duration and size of the small plot study was limited, higher imazapic rates did not measurably or observationally result in increased establishment of native grasses relative to the lower 2oz/acre rate in grass-only plots. However, in the small plots with the seed mix of both wildflower and grass species, native grasses were higher at the 6oz and 10oz rates—rates at which almost no wildflowers were established. At the lowest imazapic 2oz/acre rate, wildflower biomass was higher than in the no-imazapic control.

Prior research on the effect of imazapic on wildflowers has been conflicting, with several studies indicating some wildflower species are resistant to imazapic, and others indicating wildflowers are generally largely suppressed by the herbicide (Beran et al., 2000; Norcini et al., 2003; Wiese et al., 2011). The results of our studies confirm the variability of such results, as imazapic use at a low rate in the large plots suppressed the establishment of wildflowers for years after establishment, but in small plots, wildflowers were successfully established at the lowest 2oz/acre rate. These differences may be a result of differing effects of imazapic in different soil types—Loux and Reese (1993) found that imidazolinone persistence was higher in lower-pH soils, but this effect was inconsistent between soil types.

Norcini and colleagues (2003) had similarly varying results in a study using native wildflowers in pots, with herbicide rate, wildflower species, potting medium, and even seed source within the same species impacting the stunting effects of imazapic on establishment. Bahm and Barnes (2011) tested the response of 22 native forb species to the pre-emergent application of two rates of imazapic (2oz and 4oz/acre) on sites in Kentucky and Indiana, finding wide variations in susceptibility between species even at low rates, and variation between sites in terms of the effects of the herbicide. Our findings in Experiment II were less variable in terms of wildflower resistance, with only three species establishing at the 2oz/acre rate of imazapic: black-eyed Susans, Illinois bundleflower, and oxeye daisy.

Research in Western rangelands reveals similar variability. A study conducted in Oregon investigating the use of imazapic for controlling exotic annuals and restoring native rangeland species also found significant variation in the impacts of imazapic between sites and species (Elseroad & Rudd, 2011). An expansive study using five rates of imazapic application in salt desert shrub and Wyoming big sagebrush ecosystems found high site and species variation in imazapic impacts as well, and the authors hypothesized that variations in precipitation, soil organic matter, and disturbance history may factor into the variability (Morris et al., 2009).

Establishment of native grass/wildflower stands without imazapic

Experiment III compared several establishment methods that excluded imazapic applications. Our results from the imazapic experiments indicate that seeding wildflowers into a seedbed prepared with imazapic is contraindicated at rates higher than 2oz/acre, and even lower rates may be deleterious given the disparity between wildflower establishment in our large plots and small plots at the same 2oz/acre rate; however, alternatives such as preparing a seedbed with tillage may be more successful in reducing weed pressure while promoting wildflower abundance. We found that among the treatments evaluated in this three-year study, tillage appeared to be most effective for successful wildflower establishment. Past research on the effectiveness of tillage as a wildflower establishment strategy is conflicting, with some studies reporting better establishment with tillage and others with no-till approaches (Aldrich, 2002; Angelella et al., 2019; Skousen & Venable, 2008). Bobwhite quail (*Colinus virginianus*) restoration projects have long advocated the periodic use of tillage or discing as an ecological disturbance to promote forbs and graminoids found to be beneficial to quail and other grassland obligate species (Madison et al., 2001). The same programs often advise periodic use of prescribed fire—which many native grass and wildflower species evolved with—in order to promote their establishment or abundance. Concordantly, our results found prescribed fire to be an effective method for site preparation if the goal is wildflower establishment.

CONCLUSIONS

Greater adoption of native grasses and wildflowers in pasture systems may help increase their provision of ecosystem services. This chapter summarized data from three field experiments designed to evaluate different ways to establish native grasses and wildflower mixtures in Virginia. Our results indicate imazapic is an effective tool for the establishment of NWSG in Virginia, but imazapic should not be used on sites where the goal is to establish wildflowers. Imazapic effectively suppresses weedy competition in the establishment year, allowing native grass stands to mature enough to outcompete weedy species without continued herbicide application. For wildflower establishment, we found tillage or prescribed fire were effective alternatives to imazapic. Indeed, in grazed plots, imazapic-treated plots had higher weed pressure by the end of our experiment due to the suppression of wildflowers by the herbicide, which left a niche for weedy species to fill. Moreover, wildflowers seemed to act as barriers to grazing of the few NWSG that established in the no-imazapic plots seeded with the WF mix. In summary, for pure stands of NWSG, imazapic is an effective tool for weed control and stand establishment; however, other seedbed preparation measures should be used when wildflower establishment is a goal.

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CHAPTER 6: SUMMARY AND IMPLICATIONS

The purpose of this research was to evaluate NWSG species for potential use in equine forage systems, as well as to assess different strategies for establishing NWSG and wildflowers in Virginia. This study is the first to evaluate NWSG as equine hay and pasture species.

Results from the digestibility trial indicate big bluestem and indiangrass are suitable forage species for horses prone to obesity and laminitis. The NWSG hays had low non-structural carbohydrate levels, well within the maximum recommended ranges for horses with metabolic disorders. Voluntary intake of NWSG hays was lower than ranges recommended for healthy horses, but were ideal for obese horses in need of moderate reduction in intake. This research will help expand options available to equine managers with metabolic horses, and can serve as a starting point for additional research into the myriad other native grass species which have not yet been evaluated for equines.

The grazing experiment found that indiangrass and big bluestem can be integrated into equine pastures, with adequate productivity even in the first year of establishment. The research also demonstrated the importance of basing equine stocking rate calculations not just on dietary dry matter requirements, but also on potential trampling damage to swards by horses. Given the different morphology and sward height of these species compared to more common cool-season species, further research to determine potential differences in grazing behavior is called for.

The establishment experiments provided useful data on effective strategies for the establishment of NWSG and wildflowers for pasture and/or wildlife habitat. Imazapic was shown to be useful for NWSG establishment, but not for wildflower establishment. Given the variable effects of imazapic reported in previous studies, more data are needed from similar experiments with a wider variety of soil types and additional species of NWSG and wildflowers to better understand the herbicide's role in establishing or restoring native grasslands in Virginia. That tillage proved effective for establishing native wildflowers in our wildflower-only establishment experiment raises as many questions as it answers, given the conflicting literature on its role in seedbed preparation for wildflower species.

Native warm-season grasses provide valuable wildlife habitat and soil and water conservation. The results of our research indicate they can also be useful forage species for equines, integrating the goals of healthier horses and healthier landscapes.

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