Evaluation of interseeded forbs and pasture composition in Alabama cool-season grazing systems

by

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Abstract

Diversification of Southeastern pasture systems with cool-season annual forbs has been proposed as a low-input pathway toward improved sward composition and reduced enteric methane emissions through plant secondary metabolite (PSM) contributions. Two studies evaluated whether candidate PSM-producing forbs could be reliably established in a southeastern production system without negative animal impacts. A two-year grazing trial at the Sand Mountain Research and Extension Center in Crossville, Alabama compared 'AU Merit' hairy vetch (*Vicia villosa* Roth), 'Florida broadleaf' mustard (*Brassica juncea* (L.) Czern.), and 'Purple Top' turnip (*Brassica rapa* L.) interseeded into 'KY31' tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.). A small-plot study evaluated twelve (nine additional) cool-season forb species across three site-years. Additionally, a third methodological study compared two non-destructive methods of estimating botanical composition against hand-separation of clipped forage samples. Hairy vetch was the only species to reliably establish in both the grazing study and small-plot studies, increasing the planted forb fraction (18.7% of standing dry matter biomass; $P < 0.0001$) above the control, lowering the weed fraction (10.4% of standing dry matter biomass; $P < 0.0001$) below turnip paddocks, and increasing crude protein (14.9%; $P = 0.0056$) above brassica treatments without measurable effects on animal health or performance. Point-step transects produced the strongest non-destructive association with forb mass ($R^2 = 0.75$; $P < 0.0001$). These results support hairy vetch as a low-input diversification candidate for Southeastern tall fescue pastures.

Artificial Intelligence (AI) Use Disclosure Statement

In the preparation of this thesis, the following Artificial Intelligence (AI) tools were used: Claude Cowork (Anthropic PBC, San Francisco, CA). This tool was used primarily to organize and categorize scientific resources for ease of access in the literature review process. The author acknowledges full responsibility for the intellectual content of this work and has ensured that all AI-assisted sections have been reviewed and revised for accuracy and appropriate academic style. All AI-generated content was reviewed and validated for relevance, appropriateness, and accuracy before incorporation into the final document to maintain scholarly integrity of this research.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

ac	Acre(s)
ADF	Acid detergent fiber
ADG	Average daily gain
ADL	Acid detergent lignin
a.e.	Acid equivalents
AOAC	Association of Official Analytical Collaboration
AU	Auburn University
BBREC	Black Belt Research and Extension Center
BLM	Bureau of Land Management
BUN	Blood urea nitrogen
BW	Body weight
Ca	Calcium
CBC	Complete blood count
CEC	Cation exchange capacity
CH_4	Methane
cm	Centimeter(s)
cmol	Centimole(s)
CO_2	Carbon dioxide
CP	Crude protein
CRD	Completely randomized design
CTRL	Control
Cu	Copper
CV	Coefficient of variance
CVAS	Cumberland Valley Analytical Services
d	Day(s)
df	Degree(s) of freedom
DM	Dry matter
DMD	Dry matter disappearance
DMI	Dry matter intake
DWR	Dry-weight rank
E+	Toxic-endophyte tall fescue
E-	Endophyte-free tall fescue
EDTA	Ethylenediaminetetraacetic acid
et al.	And others
FA	Forage allowance
fL	Femtoliter(s)
g	Gram(s)
GE	Gross energy
GHG	Greenhouse gas(ses)
GLIMMIX	Generalized Linear Mixed Model
GPA	Gain per area
h	Hour(s)
H_2SO_4	Sulfuric acid
ha	Hectare(s)
HCT	Hematocrit
hd	Head
HGB	Hemoglobin
IACUC	Institutional Animal Care and Use Commitee
IVDDM	In-vitro digestible dry matter
IVDMD	In-vitro dry matter digestibility
IVTD	In-vitro true digestibility
K	Potassium
kcal	Kilocalorie(s)
kg	Kilogram(s)
L	Liter(s)
lb	Pound(s)
LSM	Least squares means
LYM	Lymphocyte
m	Meter(s)
Mcal	Megacalorie(s)

MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
ME	Metabolizable energy
mg	Milligram(s)
Mg	Megagram(s)
mmol	Millimole(s)
mo	Month(s)
MONO	Monocyte
MPV	Mean platelet volume
min	Minute(s)
MJ	Megajoule(s)
mL	Milliliter(s)
mm	Millimeter(s)
N	Nitrogen
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acids
NEg	Net energy for gain
NEm	Net energy for maintenance
NEU	Neutrophil
ng	Nanogram
NH_3	Ammonia
NOAA	National Oceanic and Atmospheric Administration
NRCS	Natural Resources Conservation Service
OM	Organic matter
P	Phosphorus
pg	Picogram
pH	pH
PLS	Pure live seed
PLT	Platelet
ppm	Parts per million
PROC	Procedure
PSM	Plant secondary metabolite
PVC	Polyvinyl chloride
R^2	Coefficient of determination
RBC	Red blood cell(s)
RCBD	Randomized complete block design
RDW	Red blood cell distribution width
REG	Regression
RMSE	Root mean square error
rpm	Revolutions per minute
s	Second(s)
S	Sulfur
SD	Standard deviation
SE	Standard error
SEM	Standard error of the mean
SF_6	Sulfur hexafluoride
SMCO	S-methylcysteine sulfoxide
SMREC	Sand Mountain Research and Extension Center
TF	Tall fescue
TVREC	Tennessee Valley Research and Extension Center

CHAPTER I: REVIEW OF LITERATURE

1.1 Tall Fescue Systems

1.1.1 Role of Tall Fescue in Southeastern Grazing Systems

Tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons) is the dominant cool-season perennial forage species across the southeastern United States and serves as the foundation of most forage-based beef production systems in the region (Ball et al., 2015; Roberts & Andrae, 2004; Sleper & West, 1996). Its widespread adoption reflects exceptional persistence under variable environmental and management conditions, including drought, grazing pressure, and poor fertility (Sleper & West, 1996). This persistence is a critical factor in its economic value because it does not need frequent renovation and can maintain a productive stand with relatively low input requirements.

The persistence of tall fescue is closely tied to its morphology and physiology. Its deep, extensive root system enhances water and nutrient acquisition under stress (Sleper & West, 1996) and contributes to soil structure and long-term soil carbon accumulation (Franzluebbers & Poore, 2021). Tall fescue is a C3 grass that exhibits a bimodal seasonal growth pattern, with peak biomass accumulation occurring during the spring and fall (Ball et al., 2015). These periods align with key phases of livestock nutrient demand, including lactation in cow-calf systems and backgrounding in stocker systems. However, the decline in productivity during summer months due to high temperatures creates a seasonal limitation in forage availability and addressing this limitation using complementary forages, conserved feeds, or adaptive grazing strategies is a longstanding management challenge in the Southeast (Freeman et al., 2019).

1.1.2 Plant-Endophyte Symbiosis and Tall Fescue Toxicosis

The symbiotic association between tall fescue and the fungal endophyte *Epichloë coenophiala* is the defining feature of this species' performance in the Southeast (Roberts & Andrae, 2004). The endophyte lives intercellularly in plant tissues and concentrates in seeds, persisting from one plant generation to the next. In return for plant-supplied nutrients and physical protection, the endophyte produces a suite of ergot alkaloids including ergovaline, ergovalinine, ergocornine, and ergocryptine. These confer resistance to insect herbivory and grazing pressure, as well as enhancing drought and heat tolerance (Klotz, 2015; Roberts & Andrae, 2004; Strickland et al., 2011). Ergovaline, the most abundant and most closely associated with animal impacts, is typically present at concentrations ranging from 100 to over 1,000 ng g^{-1} DM in heavily infected 'KY 31' tall fescue stands with concentrations several-fold greater in seedheads than in vegetative tissue (Klotz, 2015; Strickland et al., 2011).

The ergot alkaloids which benefit the tall fescue plant, are also responsible for the condition of fescue toxicosis in grazing ruminants. Alkaloids act on dopaminergic, serotonergic, and adrenergic receptors, causing vasoconstriction and endocrine disruption, manifesting as reduced voluntary intake, depressed weight gain, impaired reproductive performance, and disrupted thermoregulation (Klotz, 2015; Roberts & Andrae, 2004; Strickland et al., 2011). Quantitative consequences of fescue toxicosis on stocker performance have been well characterized. McMurphy et al. (1990) reported steer average daily gain (ADG) depression of approximately 0.2 – 0.4 kg d^{-1} on toxic endophyte ('E+') tall fescue compared with endophyte-free ('E-') tall fescue, with the amount of depression scaled with intake of ergovaline (Stuedemann & Hoveland, 1988). Vasoconstriction limits blood flow to the extremities and as a result, animals consuming E+ fescue cannot dissipate heat efficiently and become disproportionately vulnerable to heat stress. Behavioral responses such as reduced grazing time and shade-seeking compound the loss of intake under spring and summer heat (Browning, 2003; Johnson et al., 2019). Aiken et al. (2009) directly quantified the vasoconstrictive component of toxicosis using Doppler ultrasound. In crossbred (Angus × Brangus) heifers (345 ± 19 kg) fed E+, E-, or E+/E- tall fescue seed, caudal artery luminal area, peak systolic velocity, mean velocity, and serum prolactin all responded negatively to the presence of alkaloids over a 9-d experimental period and measurable changes appeared within 27 hours of initial E+ exposure (Aiken et al., 2009). Pavao-Zuckerman et al. (1999) further documented that this same alkaloid pressure interacts with enteric methane (CH_4) production. Their study reported daily CH_4 emissions of 95 – 200 g d^{-1} for steers and 150 – 240 g d^{-1} for cows on tall fescue and showed that steers grazing E+ tall fescue with interseeded clover emitted 20% less CH_4 kg^{-1} BW d^{-1} than steers grazing pure E+, E-, or mixed E+/E- pastures.

Mitigation strategies for tall fescue toxicosis fall into two broad categories: plant-based and animal-based interventions. Plant-based interventions include replacement of toxic stands with novel-endophyte (NE) cultivars that retain some agronomic persistence without producing toxic alkaloids (Poore & Horner, 2024). Adoption of these varieties has been limited by renovation costs commonly exceeding \$2,000 – \$4,000 ha^{-1} (Poore & Horner, 2024) and by uncertainty about the longevity of NE stands under continuous grazing (Roberts & Andrae, 2004; Stuedemann & Hoveland, 1988).

Some animal-based intervention strategies include dilution with non-toxic species or supplements, providing shade, and grazing management to avoid mature seedheads (Johnson et al., 2019; Vibart et al., 2007). Vibart et al. (2007) used fresh E+ tall fescue in continuous culture to demonstrate that ruminal fermentation patterns were minimally altered by the presence of the endophyte at low grain supplementation levels, but that high grain inclusion (450 g kg^{-1} dry matter (DM)) altered CH_4 production patterns across all three grass treatments. This implies that energy supplementation can modify fermentation enough to interact with toxic endophyte effects. Trotta et al. (2018) used an in vitro fermentation system with corn supplementation of tall fescue hay and found that corn-based supplementation increased true DM digestibility and digestible energy and reduced acetate proportion ($P < 0.001$) and increased propionate proportion ($P < 0.001$). This confirms that diet manipulation can shift fermentation away from acetate-favoring pathways characteristic of high-fiber fescue diets. Despite these tools, E+ tall fescue remains the dominant cool-season forage in the Southeast, and low-input strategies that improve animal performance on existing stands are still needed. Interrante et al. (2012) compared tall fescue with annual legume mixtures (TF-Leg: arrowleaf clover (*Trifolium vesiculosum* Savi) + hairy vetch (*Vicia villosa* Roth) + field pea (*Pisum sativum* L.)) against tall fescue fertilized at 112 kg ha^{-1} (TF-N) over three grazing seasons in Oklahoma. The TF-N system produced more grazing days (429 vs. 334 d ha^{-1}; $P = 0.03$) than TF-Leg, but stocker ADG was similar between systems (0.70 kg d^{-1}; $P = 0.61$) and total gain was similar (296 vs 237 kg ha^{-1}; $P = 0.10$), indicating that legume integration can substitute for synthetic N fertility while maintaining individual animal performance, just at a lower per-hectare carrying capacity in this study.

1.1.3 Nutritional and Seasonal Constraints

Animal performance in tall fescue systems is shaped by the interaction of forage nutritive value, voluntary intake, and the depressive effects of ergot alkaloids. As tall fescue matures through spring and into summer, structural carbohydrates (hemicellulose, cellulose, and lignin) accumulate, raising neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) concentration and lowering digestibility (McMurphy et al., 1990). McMurphy et al. (1990) reported that NDF concentrations in tall fescue herbage commonly increase from approximately 50% to over 65% from spring vegetative growth through reproductive maturity, with ADF following a parallel increase from approximately 30% to over 35% across the same window. Higher fiber loads slow ruminal passage and increase rumen fill, which further constrains voluntary intake. This relationship has been long-proven with the negative correlation between NDF concentration and dry matter intake (Allen, 2000; Van Soest, 1994). Allen (2000) reviewed short-term intake regulation in dairy cattle and found the physical fill of the reticulorumen as the primary constraint, with NDF intake capped at 1.0 – 1.2% of body weight. Galyean and Defoor (2003) extended these principles to feedlots, demonstrating that roughage source and amount interact with concentrate composition to determine dry matter intake. This may explain why cattle on high-fiber, low-quality forages like mature tall fescue may have limited intake even when total forage availability is non-limiting. These energy supply shortfalls have the largest downstream impact on growing and lactating animals, whose nutrient demands are much less forgiving (Galyean & Defoor, 2003; Van Soest, 1994).

Diversification with legumes or higher-quality forbs offers one method to offset the seasonal decline in nutritive value of grass-based systems (Sanderson et al., 2004, 2016). Sanderson et al. (2004) provided some initial framework for this argument and Sanderson et al., again in 2016, demonstrated it quantitatively by comparing simple (2-species) vs. complex (5+ species) commercial pasture mixtures. They reported that more diverse mixtures supported greater total dry matter productivity and improved nutritive value across multiple sites and years. For example, as the number of species in a mixture increased from 2 species to 6 species, the mean forage biomass within the sward increased from 7,197 kg DM ha^{-1} to 9,788 kg DM ha^{-1}.

Tall fescue's bimodal seasonal growth has compounding effects with relative plant maturity when it comes to nutritive value (Ball et al., 2015). Spring growth is generally high in digestibility and crude protein, but summer brings the pressures of heat stress and toxicosis. Fall regrowth, particularly under stockpiling, can offer improved nutritive value when temperatures are cooler and seedheads are absent (Poore & Drewnoski, 2010). The recurring spring-to-summer decline in forage quality is a defining feature of this system and a primary target for management.

1.1.4 Methane Emissions in Forage-Based Systems

Enteric CH_4 production is an inherent consequence of ruminal fermentation, representing both an energetic loss to the animal and a contribution to greenhouse gas emissions from livestock systems. Methane is produced in the rumen by methanogenic archaea that utilize hydrogen generated during the fermentation of carbohydrates, particularly the structural carbohydrates that are dominant in forage-based diets (Beauchemin et al., 2008; Min et al., 2020). Mature tall fescue diets are typically high in fiber and low in digestibility; these conditions favor acetate production over propionate and because acetate generation releases more hydrogen per unit of substrate, these diets tend to produce greater methane per unit of dry matter intake (Iqbal et al., 2008; Pavao-Zuckerman et al., 1999).

Methane emissions must be evaluated within the broader context of system efficiency. Methane intensity (emissions per unit of animal product) is heavily influenced by intake and animal performance. DeRamus et al. (2003) demonstrated that improved grazing management can reduce methane intensity even when total methane production is unchanged. Eugène et al. (2021) summarized that methane yield (g CH_4 kg^{-1} DMI) decreases as forage digestibility increases in both dairy cattle and sheep, and that methane intensity (g CH_4 kg^{-1} milk) also decreases with greater forage digestibility for dairy cattle. These relationships imply that management intervention that raises diet digestibility, including forb diversification, has the potential to reduce methane intensity even where total emissions are unchanged.

Nutritional strategies to reduce emissions have focused on shifting fermentation toward propionate via more digestible diets (Beauchemin et al., 2009; Eugène et al., 2021) and also on utilizing plant secondary metabolites such as condensed tannins and glucosinolates that directly inhibit methanogens (Iqbal et al., 2008; Machado et al., 2022; Puchala et al., 2005). Gere et al. (2024) measured enteric CH_4 emissions with the sulfur hexafluoride (SF_6) tracer technique in beef steers grazing two systems: a hairy vetch + annual ryegrass (*Lolium multiflorum* Lam.) + forage radish (*Raphanus sativus* L.) cover crop mixture (CC) and an alfalfa (*Medicago sativa* L.) + tall fescue pasture (AFP). Steers grazing the cover crop mixture produced 29% less CH_4 in g d^{-1} (119.1 vs. 167.1 g d^{-1}; $P < 0.05$) and 36% less CH_4 yield (4.3 vs. 6.7 g kg^{-1} DMI) than steers on the alfalfa + fescue system. Average daily gain, dry matter intake (DMI), and CH_4 intensity (g CH_4 kg^{-1} ADG) did not differ between treatments. Li et al. (2025) evaluated 33 pasture mixtures across 3 years for in vitro CH_4 yield and reported that CH_4 yield was negatively correlated with saponin and condensed tannin concentrations in herbage, with chicory-based mixtures combining high productivity, high feed value, and low CH_4 yield. Pavao-Zuckerman et al. (1999) similarly reported that interseeded clover into E+ tall fescue reduced steer CH_4 output by 20% on a per kilogram of body weight basis. Integrating legumes into grass-based systems has therefore been consistently associated with improved nitrogen retention and reductions in methane (Gere et al., 2024; Li et al., 2025; MacAdam et al., 2022).

1.1.5 The Case for System Diversification

Diversifying the botanical composition of tall fescue pastures is one of the few tools that may simultaneously improve animal performance and lower methane intensity (Min et al., 2020). The performance and environmental limitations described above arise in part because tall fescue pastures are typically managed as monocultures. Monocultures are inherently constrained in their ecosystem function. They lack complementary rooting depths, nutrient acquisition pathways, and canopy architectures that allow diverse plant communities to use resources more efficiently and to resist disturbances from drought, pests, and disease (Machado et al., 2022; Sanderson et al., 2007; Tracy & Sanderson, 2004). Simpler plant communities also support narrower soil microbial population diversities and provide less support for biodiversity at the field and environmental scale (Jaramillo et al., 2021; Rook et al., 2003).

Perennial grasses provide important ecosystem services: soil stabilization, enhanced water infiltration, and carbon sequestration (Franzluebbers & Poore, 2021; Sleper & West, 1996). Adding nitrogen-fixing legumes can reduce dependence on synthetic fertilizer while simultaneously improving forage nutritive value and animal performance (MacAdam et al., 2022; Sanderson et al., 2016). Non-leguminous forbs broaden the functional diversity further by contributing distinct rooting strategies, producing plant secondary metabolites, and offering seasonal growth patterns that no single grass or legume can supply alone (Soder et al., 2007; Villalba et al., 2023).

1.2 Forb Integration into Perennial Pastures

1.2.1 Morphology and Physiology of Forage Forbs

Forbs are broadleaf forages that are comprised by a broad and functionally diverse group of herbaceous, dicotyledonous species whose morphology, physiology, and chemistry differ substantially from grasses (Ball et al., 2015). Many forb species have broader leaves, contrasting leaf-to-stem architecture, and distinct rooting strategies. All these influence light interception, water and nutrient acquisition, animal diet selection, and ecosystem function within mixed swards (Belesky et al., 1999; Bellangue et al., 2024; Grace et al., 2018). Two families of plants, *Fabaceae* (legumes) and *Brassicaceae* (brassicas), represent the contrasting functional groups most often considered for incorporation into grass-based pasture systems.

Leguminous forbs are distinguished by their symbiotic association with *Rhizobium* bacteria and the resulting capacity for biological nitrogen fixation. This relationship can substantially reduce reliance on synthetic N fertilizer, a particularly relevant benefit in tall fescue systems that are otherwise heavily dependent on external N inputs to maintain productivity (MacAdam et al., 2022; Sollenberger & Collins, 2017). Beyond N-fixation, legumes generally have greater tissue N, lower fiber concentrations, and greater digestibility than grasses. Their inclusion tends to improve sward nutritive value and support improved livestock performance (Beuselinck et al., 1994; Sollenberger & Collins, 2017). Mortenson et al. (2005) reported that yellow-flowered alfalfa interseeded sites produced 42 – 143% greater live aboveground biomass than adjacent native rangeland controls. Alfalfa CP averaged 16 – 18% and contributed significant N to the resident grass through fixation and nutrient recycling in the soil.

Persistence of legumes in pasture systems is one of the biggest challenges in their successful management and adoption. Legume stand longevity depends on adequate establishment, root and crown morphology, life-cycle traits, carbohydrate reserves, disease and insect tolerance, reseeding ability, soil pH and nutrient status, and tolerance to defoliation under grazing or haying (Beuselinck et al., 1994). Across the Southeast, successful establishment depends critically on planting date, winter temperatures, frost timing, soil, moisture, soil fertility, and competition from the resident sod (Guretzky et al., 2012; Hoveland et al., 1981).

Other broadleaf forages that do not fall into the legume family are considered non-leguminous and can play useful roles in pasture systems. Chicory (*Cichorium intybus* L.) is one example of a useful pasture forb that falls outside the legume family. Its deep taproot supports productivity under high air temperatures and low soil moisture, and it can sustain forage quality through summer when shallow-rooted cool-season grasses decline (Belesky et al., 1999; Skinner et al., 2010).

Brassicas are a family of plants that also differ from legumes in many functional dimensions. They lack the capacity for symbiotic N fixation, but compensate with rapid establishment, vigorous cool-season growth, high digestibility, and the ability to produce substantial biomass when perennial grasses are least productive (Barry, 2013; De Ruiter et al., 2009; Dillard et al., 2020). Species within the family differ in the way biomass is partitioned. ‘Purple Top’ turnip (*Brassica rapa* L.) and forage-type radish (*Raphanus sativus* L.) allocate biomass to both leaf tissue and enlarged belowground storage organs, while kale (*Brassica oleracea* L.) and rape (*Brassica napus* L.) produce predominantly aboveground leaf and stem biomass that is well-suited to grazing (Barry, 2013; De Ruiter, 2009). ‘Broadleaf’ mustard (*Brassica juncea* (L.) Czern.) establishes quickly and competes well during early growth, making it useful for filling short-term forage gaps but also more management-intensive in mixed-species systems (De Ruiter, 2009). These morphological contrasts influence dry matter production, animal selectivity, and competitive interactions within the grass-forb matrix.

Brassicas are low in structural fiber and rapidly fermented in the rumen, so they are most effective when grazed in combination with grasses or other roughage rather than as a sole diet (Barry, 2013). Barry (2013) cautions that brassicas grazed as a sole diet by young ruminants typically produce growth substantially below expectations from nutritive value. Brassicas also contain glucosinolates, S-methylcysteine sulfoxide (SMCO), and other sulfur-containing compounds that have been shown to potentially negatively influence palatability, animal metabolism, and methane production (Barry, 2013). They are best suited as a strategic dietary component that complements, rather than replaces, the perennial grass base (Barry, 2013; Dillard et al., 2020).

Taken together, the functional diversity among forbs allows targeted matching of plant traits to system limitations. Legumes can contribute fixed nitrogen to the system from their symbiotic association with *Rhizobium* bacteria as well as improved forage nutritive value (Beuselinck et al., 1994; Sollenberger & Collins, 2017). Brassicas can contribute high digestibility and rapid cool-season growth, filling seasonal gaps (Barry, 2013; Dillard et al., 2020); and chicory contributes rooting depth and drought resilience (Belesky et al., 1999; Skinner et al., 2010). Through species selection and management, forbs can meaningfully improve the productivity and sustainability of perennial pastures (Bellangue et al., 2024).

1.2.2 Agronomic Considerations for Forb Establishment

Successful establishment in an existing perennial sward is one of the primary practical barriers to integration. In tall fescue pastures, the resident grass competes strongly for light, water, nutrients, and space (Bellangue et al., 2024; Hoveland et al., 1981). Getting seed in the ground is only the first of many decisions that govern emergence, persistence, and productivity (Bellangue et al., 2024). Species-specific recommendations for the cool-season annual forbs most relevant to Southeastern integration are summarized in Table 1.1.

Hoveland (1981) showed that legume establishment in tall fescue improves substantially when grass competition is reduced before seeding. Mowing the resident sod to 2 – 5cm, light tillage, and chemical suppression with non-residual herbicides [typically N-(phosphonomethyl)glycine (glyphosate) at 0.85 – 1.1 kg ae ha^{-1} for fall seeding] are routinely used to create microsites for seedling emergence and early growth (Bellangue et al., 2024; Hoveland et al., 1981; Stevens et al., 1981; Zajicek et al., 1986). Additionally, site preparation, vegetation control, and choice of planting strategy (i.e. broadcast, frost-seeding, no-till drill) are critical steps for successful forb establishment in pastures (Ball et al., 2015; Bellangue et al., 2024). The relative importance of sod suppression scales with the vigor of the resident grass. In well-established KY 31 tall fescue stands and bermudagrass (*Cynodon dactylon* [L.] Pers.) sods, the standing crop and thatch layer at planting can be thick enough to exclude small-seeded forbs even when the seed is placed precisely (Bellangue et al., 2024; Hoveland et al., 1981; Templeton & Taylor, 1975).

The use of a no-till drill is generally preferred in forage systems because it places seed precisely and improves seed-to-soil contact without major soil disturbance (Leep et al., 2003). Soil moisture, planting depth, and surface residue then become the key constraints to emergence (Hoveland et al.,1981). Recommended planting depth can vary by ~5-fold across species (Ball et al., 2015; Sollenberger & Collins, 2017). Brassicas (mustard (*Brassica juncea* (L.) Czern.), turnip (*Brassica rapa* L.), radish (*Raphanus sativus* L.), rape (*Brassica napus* L.), kale (*Brassica oleracea* L.)) and chicory (*Cichorium intybus* L.)

require very shallow placement at 0.6 – 1.3 cm because of their small seeds and limited cotyledon reserves (De Ruiter et al., 2009). Small-seeded legumes such as crimson (*Trifolium incarnatum* L.), berseem (*Trifolium alexandrinum* L.), and white clovers (*Trifolium repens* L.) and burr medic (*Medicago polymorpha* L.) perform best at the same 0.6 – 1.3 cm depth. Alfalfa (*Medicago sativa* L.) is recommended at 0.6 – 1.9 cm, larger seeded hairy vetch tolerates 1.3 – 3.8 cm depth, and white lupin (*Lupinus albus* L.) tolerates 2.5 – 5.0 cm placement (Ball et al., 2015; Guretzky et al., 2012; Sollenberger & Collins, 2017). Across all species, planting depth and seed-to-soil contact are consistently the most important agronomic factors. Planting too shallow risks desiccation or animal predation, while planting too deep exhausts seed reserves before emergence (Bellangue et al., 2024; Leep et al., 2003).

Seeding rate influences stand density, canopy development, interspecific competition, and animal grazing behavior (De Ruiter et al., 2009; Stefanski et al., 2010). Recommended pure live seed (PLS) rates for Southeastern conditions vary greatly across forb species. Seeding rates for common forb species used as forages can be found in Table 1.1 (Ball et al., 2015; Guretzky et al., 2012; Sollenberger & Collins, 2017). In mixed pastures, higher rates may improve canopy closure and weed suppression but also intensify intraspecific competition and shift botanical balance (Bellangue et al., 2024; Stefanski et al., 2010). Rates that are too low risk failure to establish a persistent stand, particularly when residual tall fescue or weed seedbanks rapidly occupy open ground (Hoveland et al., 1981).

Fertility management diverges sharply between legumes and brassicas, and pH tolerances vary dramatically among forbs. This is a critical consideration for Southeastern producers working with naturally acidic Coastal Plain and Piedmont soils (Beuselinck et al., 1994; Sollenberger & Collins, 2017). Alfalfa is among the species of forbs that are highly sensitive to soil pH (Ball et al., 2015). Berseem clover and crimson clover tolerate a slightly lower pH (> 6.0), burr medic prefers neutral to slightly alkaline soils (6.0 – 7.5) and can dominate on calcareous sites (Ball et al., 2015). Hairy vetch is more pH-tolerant (> 5.5) but its performance still benefits from liming when pH is below 6.0. White lupin is the most acid-tolerant of the legumes considered in this study (5.0 – 6.5), which reflects its origin in acidic Mediterranean soils (Ball et al., 2015; Beuselinck et al., 1994; Guretzky et al., 2012; Leep et al., 2003; Sollenberger & Collins, 2017). Brassicas tolerate the broadest pH range (5.5 – 7.5) but are most productive at near-neutral pH (De Ruiter et al., 2009).

Beyond pH, legumes are sensitive to soil phosphorus (P) and potassium (K) and require species-appropriate *Rhizobium* inoculant for nodulation and nitrogen (N) fixation (Beuselinck et al., 1994; Sollenberger & Collins, 2017). Warren et al. (2023) demonstrated that hairy vetch establishment in unamended hayfields can be constrained by soil fertility, especially soil K. In a no-till interseeding study, an intermediate rate of wood wash amendment (8,967 kg ha^{-1}) increased hairy vetch biomass by 8.5 – 12.4 times relative to tilled and unamended controls. Soil K alone explained 42% of the variability in hairy vetch biomass, emphasizing that even “reliable” species like vetch are sensitive to baseline fertility conditions (Warren et al., 2023).

Excess N can shift competitive advantage toward grasses, depressing legume establishment because grasses tend to be more responsive to N fertilization than legumes (Sollenberger & Collins, 2017). Brassicas, by contrast, are very N-responsive and convert available N rapidly into vegetative biomass. This characteristic supports their use as a short-term forage gap filler, but means their performance is closely tied to fertilizer inputs (De Ruiter et al., 2009; Dillard et al., 2020).

Adequate soil moisture is essential for germination and root extension, and many cool-season forb stands fail when fall or winter rainfall does not align with the optimal planting window (Bellangue et al., 2024; Guretzky et al., 2012). Optimal soil temperatures for cool-season forb germination are typically in the 10 – 20°C range, with brassicas requiring slightly warmer conditions (15 – 25°C) for rapid germination and small-seeded legumes tolerant of cooler conditions (8 – 15°C) (Ball et al., 2015; De Ruiter et al., 2009). In Southeastern production conditions, the late-September through mid-November planting window typically aligns with declining soil temperatures from peak summer values toward optimal forb-germination ranges, but rainfall during this period is inconsistent and introduces lots of variability in establishment success year-to-year and site-to-site (Bellangue et al., 2024; Guretzky et al., 2012).

Persistence depends on plant traits, defoliation, pest pressure, and competition (Beuselinck et al., 1994). Selective grazing reduces the abundance of palatable species over time unless management mitigates that pressure (Grace et al., 2018). Chicory establishment under grazed conditions can be particularly challenging. Skinner (2010) evaluated chicory reestablishment into multi-species perennial pastures and reported mortality rates of 77-84% by early October of the seeding year. This showed that even under controlled experimental conditions, fewer than one in four planted chicory seedlings persisted through the first season. Brassicas are typically utilized as annuals, so long-term persistence is not necessarily the goal because their presence year-to-year depends on annual reseeding (Barry, 2013). In contrast, hairy vetch has been documented to persist in established perennial pastures across multiple seasons through self-seeding when allowed to set seed before it is grazed or chemically killed. This provides a partial path to multi-year stand maintenance (Templeton & Taylor, 1975).

The same disturbance, fertility, and seedbed conditions that favor desirable forbs also favor undesirable broadleaf weeds emerging from the soil seedbank (Bellangue et al., 2024; Zajicek et al., 1986). Selective post-emergence and residual herbicides used to manage weeds may also damage establishing forbs. Residual carryover from prior broadleaf-active products can suppress forb establishment for one or more growing seasons after application (McCurdy et al., 2013).

1.2.3 Forage Yield and Seasonal Productivity

A central reason to incorporate forbs into perennial pastures is their potential to redistribute biomass production across the year. In cool-season systems, forage supply concentrates in spring and fall with production lulls in summer and winter (Ball et al., 2015). Introducing species with different temperature, moisture, and fertility responses can broaden the production curve and address gaps that perennial grasses alone cannot fill (Belesky et al., 1999; Grace et al., 2018). The forage contribution of forbs is often as much about when biomass is produced as it is about how much.

Forage brassicas excel at short-term, high-quality biomass production. Their feeding value comes from their inherent digestibility (dry matter disappearance (DMD) 0.81 – 0.89; metabolizable energy (ME) 12.1 – 14.1 MJ kg^{-1} dry matter (DM); Barry, 2013) and from their capacity to accumulate biomass rapidly under cool-season conditions (Barry, 2013; De Ruiter et al., 2009). Brassicas respond strongly to fertility and can be put in rotations and grazing plans to fill seasonal gaps in biomass production. Dillard et al. (2020) reported brassica DM yields of 734 – 861 DM ha^{-1} over two fall seasons in Pennsylvania. This was more than double the DM yield of annual ryegrass (344 kg DM ha^{-1}) under the same conditions. There were also similar advantages in CP and net energy yields per hectare. Villalobos and Brummer (2015) documented that planting date is the dominant management factor for brassica biomass, showing that a delay in planting from early to mid-August reduced fall yield by 4,222 kg ha^{-1} in their Colorado study.

Legumes contribute differently; rather than producing short-lived biomass peaks, legumes improve sward productivity through complementary canopy structure, nitrogen contribution, and improved nutritive value across the grazing season (Beuselinck et al., 1994; Sollenberger & Collins, 2017). Hairy vetch is among the most vigorous cool-season annual legumes and can contribute substantial biomass in annual or interseeded systems. Guretzky et al. (2012) reported 3,513 kg DM ha^{-1} for hairy vetch across multiple southern Great Plains site-years which was comparable to field pea (3,210 kg DM ha^{-1}) and substantially greater than berseem clover (1,138 kg DM ha^{-1}) or crimson clover (1,071 kg DM ha^{-1}). Mortenson et al. (2005) showed that long-term legume integration provides overall biomass benefits as well as quality benefits. In mixed-grass rangeland interseeded with yellow-flowered alfalfa, sites interseeded in 1965, 1987, and 1998 produced 68%, 143%, and 42% greater live aboveground biomass respectively than adjacent native control sites. Alfalfa contributed 1,489 of 2,969 kg ha^{-1} live biomass on the 1965 interseeded site. Crimson and berseem clovers can produce dense cool-season biomass with less management than alfalfa which is more reliant on careful site management to maintain productivity and persistence (Leep et al., 2003; Sollenberger & Collins, 2017).

1.2.4 Nutritive Value and Animal Performance

The nutritive value of a mixed pasture involves the contribution of each plant species, weighted by botanical composition and animal selection. Legumes typically raise sward CP and lower NDF and ADF relative to grasses (Beuselinck et al., 1994; Sollenberger & Collins, 2017), while brassicas contribute high digestibility and metabolizable energy with low structural fiber (Barry, 2013; Dillard et al., 2020). The effect of forb inclusion on diet quality depends on the proportion of forb in the available biomass, the proportion of forb consumed by the animal, and interactions with the resident grass, including any effects from ergot alkaloids.

Reported gains in pasture CP from legume integration into tall fescue are well-documented, particularly with hairy vetch, white and red clovers, and alfalfa (Dobson et al., 1976; Hoveland et al., 1981; Interrante et al., 2012; Templeton & Taylor, 1975). Brassicas can match or exceed legumes for CP on a single-species basis but vary by tissue type, where leaf material has substantially greater CP content than storage roots (Barry, 2013; Villalobos & Brummer, 2015). Barry (2013) reported that forage brassicas commonly achieve DMD of 0.81 – 0.89 and metabolizable energy concentrations of 12.1 – 14.1 MJ ME

kg^{-1} DM. These values are greater than most grass-based pastures or legumes, which confirms brassicas' role as one of the highest-quality cool-season forage classes available. Villalobos and Brummer (2015) evaluated nine brassica cultivars stockpiled for fall grazing in Colorado and reported fiber content of 19.0 – 25.2% (NDF), CP content of 18.6 – 25.5%, and in vitro true digestibility (IVTD) of 85.5 – 92.9%. These values are well above the nutritional requirements of all classes and stages of beef cattle. Dillard et al. (2020) reported that net energy concentrations for lactation (NE_L 1.73 – 1.79 Mcal kg^{-1}), gain (NEg 1.04 – 1.11 Mcal kg^{-1}), and maintenance (NEm 1.65 – 1.72 Mcal kg^{-1}) in canola and forage rape exceeded those of annual ryegrass ($P < 0.001$), and total nutrient yields (CP 176 – 204 vs. 88 kg DM ha^{-1}; NE_L 1,200 – 1,500 vs. 555 Mcal ha^{-1}) were also greater. These results make the case for brassica integration as a fall-window quality and biomass accumulation enhancement, particularly relevant to the cool-season forage gap in Southeastern beef systems.

In tall fescue systems, legume inclusion has been linked to improved average daily gain (ADG), gain per area, and reproductive performance compared with monoculture and E+ stands (Hoveland et al., 1981; Interrante et al., 2012). The amount of animal-performance response to forb integration is variable across studies. It reflects the interaction of forb species, inclusion rate, baseline forage availability, and animal class. Interrante et al. (2012) compared tall fescue with annual legume mixtures (TF-Leg) against tall fescue fertilized with 112 kg N ha^{-1} and found similar stocker ADG between systems as well as total gains, although the N-fertilized treatment achieved a greater number of total grazing days (429 vs. 334 d ha^{-1}).

Brassica supplementation in integrated grass systems has been associated with improved milk yield in dairy systems and improved gain in stocker systems (Barry, 2013; Dillard et al., 2020; Villalobos & Brummer, 2015). Reid (1994) evaluated brassica grazing systems for sheep and reported that lamb ADG on brassicas was generally higher than on stockpiled tall fescue or orchardgrass (*Dactylis glomerata* L.)-red clover (*Trifolium pratense* L.) pastures. ‘Tyfon’ Chinese cabbage (*Brassica oleracea* L.) produced 241 – 330 g d^{-1} ADG and ‘Forage Star’ turnip produced 197 – 275 g d^{-1} although the overall ADG range and year-to-year variability was substantial (19 – 330 g d^{-1} ADG).

Indices like blood urea nitrogen (BUN) and non-esterified fatty acids (NEFA) provide complementary views of animal nutritional status in grazing studies. Blood urea nitrogen reflects ruminal protein degradation and the balance between dietary protein and energy. Elevated BUN in legume-rich diets can indicate an excess of rapidly degradable protein relative to the amount of fermentable energy (Trotta et al., 2018). Non-esterified fatty acid reflects negative energy balance and adipose mobilization, providing an early signal of an energy deficit that body weight changes alone may miss (Gunter et al., 2019). These indices have become standard measurements in grazing trials that evaluate forage effects on animal performance.

1.2.5 Ecosystem Services

Beyond animal performance, forb integration can provide many ecosystem services that monoculture grass pastures may not. These include nitrogen cycling, soil structure and carbon storage, water infiltration, biodiversity support, and resilience to drought and pest pressure (Franzluebbers & Poore, 2021; Machado et al., 2022).

Biological N fixation by legumes is the most quantified ecosystem service of pasture forbs. Symbiotic *Rhizobium* associations can supply meaningful plant-available nitrogen, reducing reliance on synthetic fertilizer inputs. Legume residues also recycle N to companion grasses through mineralization, providing N throughout the season (MacAdam et al., 2022; Sollenberger & Collins, 2017).

Diverse perennial grass systems support continuous root activity, contributing to soil aggregation, infiltration, and stable soil carbon (Franzluebbers & Poore, 2021). Franzluebbers and Poore (2021) evaluated soil organic carbon and nitrogen accrual under tall fescue management over a 25-year period and reported soil organic C sequestration of 0.75 Mg C ha^{-1} yr^{-1} and total soil N sequestration of 75 kg N ha^{-1} yr^{-1}. These values translate to about 19 Mg C ha^{-1} and 1,875 kg N ha^{-1} accrued over the full study. Forbs with contrasting rooting strategies like deep-rooted chicories, fibrous-rooted clovers, and storage-rooted brassicas explore different parts of the soil profile and alter the spatial distribution of root inputs to soil organic matter (Jaramillo et al., 2021).

1.2.6 Plant Secondary Metabolites

Plant secondary metabolites (PSMs) are non-nutritive, low-molecular-weight compounds produced by plants for defense, signaling, and abiotic stress tolerance (Villalba et al., 2023). In forage systems they exert influence on animal nutrition, health, and environmental footprint despite occurring at low concentrations in the plant (Iqbal et al., 2008; Machado et al., 2022; Min et al., 2020; Villalba et al., 2023). The main classes relevant to forb-diversified pastures are condensed tannins, glucosinolates and related sulfur-containing compounds, and isoflavones and other phenolics.

Condensed tannins bind dietary protein in the rumen, reducing ruminal degradation and increasing protein flow to the small intestine (Beauchemin et al., 2009; MacAdam & Villalba 2015). Concentrations vary widely among forage species. Most cool-season legumes contain only trace amounts (typically < 0.5% of DM), while specialty legumes like sericea lespedeza (*Lespedeza cuneata* [Dum. Cours.] G. Don), birdsfoot trefoil (*Lotus corniculatus* L.), and sainfoin (*Onobrychis viciifolia* Scop.) contain tannins at concentrations of 4 – 18% of DM (MacAdam & Villalba, 2015; Min et al., 2020; Puchala et al., 2005). Puchala et al. (2005) directly compared methane emissions in goats consuming high-tannin sericea lespedeza (17.7% condensed tannins, 10.3% CP, 64.5% IVDMD) versus a low-tannin crabgrass (*Digitaria sanguinalis* [L.] Scop.)-tall fescue mixture (0.5% tannins, 13.0% CP, 75.3% IVDMD). They reported that methane emissions on a quantity per day and g CH_4 per kg DMI basis were significantly lower for the lespedeza despite its lower digestibility. Min et al. (2020) summarized that condensed tannin concentrations between 2 and 6% of DM are generally associated with measurable reductions in methane production with minimal depression of voluntary intake or fiber digestibility. Concentrations above 8% will reduce intake and protein digestibility (Min et al., 2020; Villalba et al., 2023). This relationship means that species selection and inclusion rate can be meaningful factors in managing diverse swards for lower methane production.

Brassicas contain glucosinolates, S-methylcysteine sulfoxide (SMCO), and related sulfur-containing compounds (Barry, 2013). Concentrations vary by species, cultivar, plant tissue, and sulfur fertility. S-methylcysteine sulfoxide is greatest in swede (*Brassica napus* L.) and kale (*Brassica oleracea* L.) and lowest in turnip and rape. This corresponds to Barry

(2013) and the reported effects of brassica diets on lamb growth (lamb ADG of 95 g d^{-1} on swedes and 120 g d^{-1} on kale vs. 173 g d^{-1} on turnips and 225 g d^{-1} on rape). Barry (2013) further reported that low-SMCO kale (achieved through low sulfur fertilization), eliminated a typical 6-week period of suppressed lamb growth observed on standard kale. This suggests fertility acts as an important management tool in regulating the presence and effects of PSMs in brassicas. Practical use of brassicas should emphasize inclusion alongside grasses rather than the sole diet, management of sulfur and nitrogen fertility to limit SMCO and nitrate accumulation, and gradual adaptation of animals to brassica inclusion in diet. Glucosinolates do have some antimethanogenic effects under some conditions, which can contribute to reducing methane emissions from systems that include brassicas (Dillard et al., 2017; Iqbal et al., 2008; Machado et al., 2022; Min et al., 2020).

Red clover and several other legume species contain isoflavones such as biochanin A, formononetin, genistein, and daidzein at concentration ranging from 0.2 – 1.5% of DM. Biochanin A is the dominant isoflavone in red clover at 0.1 – 0.5% of DM (MacAdam & Villalba, 2015; Villalba et al., 2023). These compounds have been studied for their potential to mitigate fescue toxicosis through interaction with vasoconstriction pathways activated by ergot alkaloids. Isoflavones may counteract reductions in blood flow associated with tall fescue and improve animal performance under E+ grazing systems (MacAdam & Villalba, 2015; Villalba et al., 2023). Henson et al. (2025) also found a reduction in rumen temperature of steers fed red clover as 10% of DMI and a greater temperature reduction with 20% and 30% red clover inclusion compared to a 100% E+ tall fescue control diet.

Isoflavone concentrations vary substantially with species, cultivar, plant tissue, and stage of maturity, which complicates the ability to predict an effective dose or inclusion rate in a pasture. This variability is one reason red clover inclusion has produced inconsistent toxicosis-mitigation results across studies.

Saponins are amphiphilic glycosides found in higher concentrations in alfalfa, chicory, and some other forbs (1 – 3% of DM in alfalfa). Like tannins, saponins can disrupt ruminal microbial populations and reduce methane production (Min et al., 2020). Li et al. (2025) evaluated 33 pasture mixtures over 3 years for in vitro methane yield and reported that methane yield was negatively correlated with both saponin and condensed-tannin concentrations in herbage. They identified chicory-based mixtures as ones that combine higher productivity, higher feed value, and lower methane yield. This work supports saponin- and tannin-rich forbs as targets for diversified pasture methane mitigation.

1.3 Weeds and Botanical Composition in Diverse Pastures

1.3.1 Weed Ecology in Forb-Diversified Systems

Weeds in pastures are conventionally defined as undesirable plants competing with sown forages, but in forb-diversified systems, the boundary between “desirable forb” and “weed” can blur (Ball et al., 2015). Many Southeastern pasture weeds are forbs themselves. Broadleaf species like buttercup (*Ranunculus spp.* L.), henbit (*Lamium amplexicaule* L.), shepherd’s purse (*Capsella bursa-pastoris* [L.] Medik.), dandelion (*Taraxacum officinale* F.H. Wigg.), marestail (*Conyza canadensis* [L.] Cronquist), and chickweed (*Stellaria media* [L.] Vill.) all occupy the same ecological niches as planted forage forbs. They respond similarly to sod disturbance, fertility, and seeding-rate decisions made at forb establishment (Bellangue et al., 2024; Bunton et al., 2020; Zajicek et al., 1986).

Marten et al. (1987) directly compared the nutritive value of nine perennial forb and grass weeds against alfalfa and smooth bromegrass (*Bromus inermis* Leyss.) in Minnesota field trials and demonstrated that several pasture broadleaf weeds carry inherent nutritive value that is comparable to or exceeds cultivated forages. They reported that in vitro digestible dry matter (IVDDM) of dandelion ranged from 736 to 835 g kg^{-1} DM across maturity stages, which exceeded that of alfalfa (489 to 788 g kg^{-1} DM). Jerusalem artichoke (*Helianthus tuberosus* L.) reached 814 – 860 g kg^{-1} DM at early maturity and Canada thistle (*Cirsium arvense* [L.] Scop.), hoary alyssum (*Berteroa incana* [L.] DC.), and perennial sowthistle (*Sonchus arvensis* L.) all produced IVDDM values comparable to or exceeding smooth bromegrass. Crude protein concentrations followed a similar pattern. Vegetative dandelion had 17.2 – 20.3%, Jerusalem artichoke had 18.3 – 28.6%, curly dock (*Rumex crispus* L.) had 17.4 – 27.9%, and Canada thistle had 14.7 – 27.6%. By comparison, alfalfa ranged from 14.2 – 27.0% and smooth bromegrass was between 6.5 and 22.9%. Neutral detergent fiber (NDF) concentrations in vegetative dandelion (252 – 328 g kg^{-1} DM) were less than half that of smooth bromegrass (471 – 667 g kg^{-1} DM) at similar maturity. These findings create a complicated picture of pasture biodiversity and blur the line between what constitutes a desirable or undesirable broadleaf species. Even some of those plants typically classified as weeds can improve, rather than deplete, whole-sward nutritive value.

Bunton et al. (2020) provided a comprehensive quantitative analysis of how soil chemistry, groundcover, and grazing pressure influence weed incidence in Southeastern pastures. They surveyed 63 Missouri pastures from 2015 – 2017 with 14-day sampling intervals between mid-April through late September and used a stepwise linear regression to relate 25 weed species' densities to soil pH, soil P, K, Mg, Ca, S, Zn, Mn, Cu, tall fescue groundcover, total forage groundcover, and stocking density. Soil pH was the single strongest predictor of total weed pressure. Through stepwise linear regression, they found a 1-unit increase in soil pH was associated with 146 fewer weeds ha^{-1} averaged across all species along the entire pH scale. Annual broadleaf weed density decreased by 1,572 ha^{-1} per unit pH, perennial grass weed density decreased by 2,224 ha^{-1} per unit pH, and brush species by 1,410 ha^{-1} per unit pH. Increases of 1% in tall fescue groundcover were associated with each decrease of 54 weeds ha^{-1}. There was 1% additional total forage groundcover with each decrease of 87 weeds ha^{-1} and a 1-animal-unit ha^{-1} increase in stocking density with 764 fewer weeds ha^{-1} on average. The most commonly occurring weed species were horsenettle (*Solanum carolinense* L.) in 100% of pastures, common ragweed (*Ambrosia artemisiifolia* L.) in 97% of pastures, yellow foxtail (*Setaria pumila* [Poir.] Roem. & Schult.) in 86% of pastures, dandelion in 85% of pastures, and broadleaf plantain (*Plantago major* L.) in 77% of pastures. Some species reached densities of 16,000 – 24,000 plants ha^{-1} in heavily infested sites. These relationships help inform a framework for cultural weed management and emphasize that liming can both support integration of desirable forbs while reducing the occurrence of undesirable forbs (Bunton et al., 2020).

Sod disturbance to create microsites for forb establishment also exposes weed seedbanks (Bellangue et al., 2024; Zajicek et al., 1986). The composition of those weeds that may emerge depends on field history, prior herbicide use, grazing intensity, and seedbank persistence (Bunton et al., 2020; McCurdy et al., 2013). Herbin et al. (2020) demonstrated that weed competition during establishment can substantially depress the nutritive value of resulting pasture stands. Most broadleaf-selective, post-emergence herbicides used in pastures damage planted forbs as much as they damage broadleaf weeds. Residual herbicides can persist in the system and prevent forb establishment for months to years in that area (McCurdy et al., 2013). McCurdy et al. (2013) evaluated four *Trifolium* species against eleven common broadleaf herbicides and reported that imazaquin (2-[(RS)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]quinoline-3-carboxylic acid); 2,4-D (2,4-dichlorophenoxy~ acetic acid); 2,4-DB (4-(2,4-dichlorophenoxy)butanoic acid); and triclopyr ([(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid) produced height reductions in clovers. Field herbicide history must be evaluated before forb integration is attempted and weed-management plans for forb-inclusive paddocks must rely more on cultural, mechanical, and biological control methods.

Grazing animals influence weed populations through differential consumption of plant species. Highly palatable broadleaves will reduce in density while unpalatable species will increase in density over time under continuous grazing pressure (Grace et al., 2018; Soder et al., 2007). Bunton et al. (2020) reported that cattle actively avoided patches with dense weed cover, and an earlier study by Sather et al. (2013) found that cattle spent 1.5- to 4.9-fold greater time grazing in herbicide-treated, weed-free areas within the same pasture. This suggests that weedier areas suffer from under-utilization and that the spatial distribution and species composition of weeds in the pasture is reinforced by cattle grazing behavior. In forb-inclusive pastures, this selectivity may suppress palatable sown forbs faster than resident weeds because those sown forbs are preferentially grazed, leaving the less palatable broadleaf weeds to persist (Carlassare & Karsten, 2003; Harris et al., 1968).

1.3.2 Quantifying Botanical Composition

Botanical composition is the most important variable used to describe a diversified pasture quantitatively. It is essential to have accurate, repeatable measurements to relate species composition to forage nutritive value and animal response. Kallenbach et al. (2015) reviewed the methodological landscape for measuring plants in pastures and emphasized that the chosen method must be well suited to the research objective, the scale of the area, and the available resources. Three broad classes of methods are currently described across the literature: hand separation of clipped samples, in-field point-step transects, and digital image counts.

The standard reference method is to clip a fixed-area quadrat to ground level, sort the harvested biomass into pre-defined categories (grass, legume, brassica, weed, dead material), and weigh each of those categories dry (Baxter et al., 2017; Burt et al., 2022). This approach traces to foundational rangeland research literature, where destructive harvest of a small fixed-area sample was established as the quantitative standard against which all other composition methods are validated (Kallenbach et al., 2015; Sanderson et al., 2016). Hand separation produces direct mass-based estimates of botanical composition and remains the standard reference, but its main limitations are labor cost, inherent destructiveness, and limited spatial coverage. Small quadrat sizes (typically 0.1 – 0.5 m^2) and just a few sampling points per paddock bring up constraints to precision in mixed-species and diverse swards (Kallenbach et al., 2015; Sanderson et al., 2016; Burt et al., 2022).

To reduce the labor burden associated with full hand-separation while preserving the estimate on a mass basis, the dry-weight-rank (DWR) method of Mannetje and Haydock (1963) was developed. The DWR method estimates botanical composition by ranking the three species contributing the greatest dry mass within each quadrat on a 1-2-3 basis without requiring physical separation. Specifically, for each quadrat clipped, someone assigns rank 1 to the species contributing the greatest dry weight, and ranks 2 and 3 to the species with the second and third greatest contribution to dry weight respectively. The rank frequency is summed across many quadrats and multiplied by derived factors of 70.19, 21.08, and 8.73 for ranks 1, 2, and 3 respectively. This achieves an estimate of the dry-weight proportion for each species in the sward (Mannetje & Haydock, 1963). The factors were derived from 63 equations across 15 data sets in which DWR ranks and hand-separated dry-weight percentages were available. The method was validated in four separate field trials against the reference values from hand-separation, and no great differences between the two methods were detected. They suggested using 50 – 100 quadrats per pasture for stable estimates, but reported that quadrat size was not critical to the accuracy of the estimate. One limitation of this method is that the maximum dry-weight percentage able to be estimated for any one species is 70.2% (rank-1 factor), meaning this method is best used in scenarios where the dominant species inhabits less than this proportion of the sward.

Point-based methods record the species occurring at fixed points or intervals along a transect which provides species frequency, or cover-based estimates of botanical composition. The point-step method of Evans and Love (1957), where someone walks along a transect and records the species nearest the toe of the boot at fixed paces (typically every 1 – 3 paces over 100 – 200 points per transect), was developed on California annual rangelands and remains widely used today. Evans and Love (1957) showed that point-step estimates of cover correlated well with quadrat-based reference values for dominant species, and required much less field time for each unit area sampled. One limitation is that those involved in sampling must be well-trained on a standardized procedure to minimize between-human biases.

Further expansion on cover-based estimates have included the Daubenmire (1959) method which uses a fixed rectangular frame (typically 20 × 50 cm) within which someone assigns each species to one of six cover classes (0 – 5%, 5 – 25%, 25 – 50%, 50 – 75%, 75 – 95%, 95 – 100%) and class assignments are averaged across several frames to estimate cover. The wheel-point method of Tidmarsh & Havenga (1955) uses a wheel to drop points onto the sward at fixed intervals, making point selection more reproducible than visual estimation alone. The frequency grid of Vogel & Masters (2001) is a 5 × 5-cell metal frame (25 cells per placement) developed specifically for measuring grassland establishment success. Four frame placements are taken (totaling 2.25 m^2), and the proportion of cells containing at least one seeded plant is multiplied by 0.4 to provide an estimate of plants per m^2. The U.S. Interagency Technical Reference *Sampling Vegetation Attributes* (USDA-BLM-NRCS, 1999) consolidates these methods (DWR, Daubenmire, line intercept, etc.) into the standard government protocol for rangeland and pasture vegetation measurement.

Point and transect methods are far less labor-intensive than hand-separation, they scale well to larger areas, and capture spatial patterns more accurately. They estimate cover or frequency rather than contribution by mass, and cannot be directly compared to hand-separation values. Some limitations of these methods are standardized personnel training, bias regarding plant growth habit (upright vs. prostrate), and likelihood of missing species with more minor contributions (Kallenbach et al., 2015; Tracy & Sanderson, 2004).

Image-based methods capture overhead photographs of fixed-area quadrats and use either manual annotation, point-grid overlays, or automated computer-vision classification to estimate cover by species or functional group. Image methods have two key advantages over field-based point and transect methods: they reduce field time and they generate a permanent record that can be reclassified or calibrated against reference data. Rayburn (2014) provided a relevant validation of digital photography for measuring legume content in pastures. He took photos at 24 points in a rotationally stocked pasture and paired each sample with a hand-separated reference. He found that this method's estimates of grass and legume proportion did not differ from hand-separated values, although it did underestimate forb fraction. Calibration R^2 values ranged from 0.45 – 0.98 depending on cover and species. His recommendations for the method are > 12 photos per pasture, > 100 points per photo, with > 12 paired hand-separated samples for calibration. Baxter et al. (2017) adapted the method from Rayburn (2014) to estimate legume content in grass-legume pastures using 300 random points on a digital image uploaded to PowerPoint. They found this method produced the highest predictive accuracy for non-destructive estimation of legume content ($R^2 = 0.78$).

No single method is superior across all production and research contexts (Kallenbach et al., 2015; Baxter et al., 2017; Burt et al., 2022). Hand-separation is typically treated as the reference for biomass proportion, but is somewhat impractical at landscape and production scales. The DWR method reduces that labor burden while preserving the mass-based estimate (Mannetje & Haydock, 1963). In-field point-step and frame-based methods scale up to the production level well, but have their own biases (Daubenmire, 1959; Evans & Love, 1957; Tidmarsh & Havenga, 1955; Vogel & Masters, 2001). Digital image methods are rapidly improving and provide a permanent visual record, but are sensitive to canopy structure and require calibration against reference biomass measurements (Rayburn, 2014; Baxter et al., 2017; Burt et al., 2022).

1.4 Research Objectives

The research described in the remainder of this thesis addresses the three following objectives:

1. Quantify the biomass production, nutritive value, and animal performance response of cool-season annual forbs interseeded into endophyte-infected tall fescue under continuous grazing at the Sand Mountain Research and Extension Center (Chapter II, grazing study, 2025-2026).

2. Characterize the establishment dynamics of twelve cool-season annual forb species under controlled small-plot conditions across three site-years (Chapter II, small-plot forb establishment study).

3. Compare three methods for quantifying pasture botanical composition: physical hand-separation of clipped samples, in-field point-step transects, and digital image counts using measurements within the 2026 grazing study (Chapter III).

Table 1.1. Recommended seeding rates, planting depths, and soil pH ranges for cool-season annual forb species.

Species[1]	kg PLS ha^{-1}	Planting depth (cm)	pH range
Hairy vetch (*Vicia villosa)*	16.8 – 28.0	1.3 – 3.8	> 5.5
Turnip (*Brassica rapa*)	3.4 – 6.7	0.6 – 1.3	5.5 – 7.5
Mustard (*Brassica juncea*)	3.4 – 6.7	0.6 – 1.3	5.5 – 7.5
Crimson clover (*Trifolium incarnatum*)	5.6 – 11.2	0.6 – 1.3	> 6.0
Berseem clover (*Trifolium alexandrinum*)	9.0 – 13.4	0.6 – 1.3	> 6.0
Burr medic (*Medicago polymorpha*)	5.6 – 11.2	0.6 – 1.9	6.0 – 7.5
Alfalfa (*Medicago sativa*)	9.0 – 13.4	0.6 – 1.9	6.5 – 7.0
White lupin (*Lupinus albus*)	67.2 – 112	2.5 – 5.0	5.0 – 6.5
Radish (*Raphanus sativus*)	3.4 – 6.7	0.6 – 1.3	5.5 – 7.5
Kale (*Brassica oleracea*)	3.4 – 5.6	0.6 – 1.3	5.5 – 7.5
Forage rape (*Brassica napus*)	3.4 – 6.7	0.6 – 1.3	5.5 – 7.5
Chicory (*Cichorium intybus*)	3.4 – 5.6	0.6 – 1.3	> 5.5

[1]Compiled from Ball et al. (2015), Guretzky et al. (2012), Sollenberger & Collins (2017), De Ruiter et al. (2009), Beuselinck et al. (1994), and Leep et al. (2003). Rates are pure live seed (PLS).

CHAPTER II: AGRONOMIC AND ANIMAL PERFORMANCE OF COOL-SEASON ANNUAL FORBS INTERSEEDED INTO TALL FESCUE

2.1 Introduction

Beef production across the southeastern United States is supported by perennial cool-season grasses, mainly tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.). These systems contribute to enteric methane emissions from grazing ruminants, which is of increasing concern due to methane's contributions to global climate change as a greenhouse gas (Beauchemin et al., 2008; Eugène et al., 2021; Pavao-Zuckerman et al., 1999). Diversifying grass-monoculture swards through integrating cool-season forbs has been proposed as a low-input path to improve sward composition, enhance forage quality, and reduce methane emissions through the contribution of plant secondary metabolites (PSM) such as condensed tannins and glucosinolates (Min et al., 2020; Puchala et al., 2005; Villalba et al., 2023). Hairy vetch (*Vicia villosa* Roth) and forage brassicas are species of interest in this context because both carry PSM with potential antimethanogenic activity and because both have been evaluated in beef cattle production systems (Barry, 2013; De Ruiter et al., 2009; Dillard et al., 2020; Gere et al., 2024).

The rationale for forb diversification is well established, but producers face several practical questions before adopting these species more widely. Among them is whether forbs can be reliably established into existing grass sods with a no-till drill under the conditions typical of Southeastern pasture systems and whether their incorporation into animals' diets comes with any negative effects. Establishment is a prerequisite for measuring forage quality, animal performance, or any methane emissions (Bellangue et al., 2024; Hoveland et al., 1981; Templeton & Taylor, 1975; Warren et al., 2023).

The two experiments described in this chapter were designed as proof of concept for Southeastern forb integration. The objectives were to quantify the effects of three cool-season annual forb species (hairy vetch, mustard [*Brassica juncea* (L.) Czern.], and turnip [*Brassica rapa* (L.)]) interseeded into toxic-endophyte (E+) tall fescue on forage biomass, botanical composition, nutritive value, and animal performance over two consecutive grazing seasons at the Sand Mountain Research and Extension Center (SMREC) and evaluate the establishment potential of a broader set of twelve cool-season forb species under small-plot conditions across three site-years representing distinct soil and climate regions of Alabama. It was hypothesized that PSM-producing forbs could be successfully established by no-till drill into existing tall fescue and bermudagrass (*Cynodon dactylon* [L.] Pers.) sods under field conditions typical of Southeastern production; interseeding forbs into existing pastures would shift sward botanical composition toward greater forb biomass and improve overall pasture nutritive value; and inclusion of forbs would not produce measurable negative effects on animal average daily gain, blood metabolites, or complete blood count parameters relative to monoculture grass controls.

2.2 Materials and Methods

2.2.1 Grazing Study

The project was approved by the Auburn University Institutional Animal Care and Use Committee (IACUC Protocol #2025-5555).

The grazing study was conducted across two years (2025 and 2026) at the Auburn University Sand Mountain Research and Extension Center (SMREC), near Crossville, Dekalb County, Alabama (34°16’59”N,85°57’11”W). The site lies on the Sand Mountain plateau in Northeast Alabama within the Appalachian Plateau physiographic region. Soils at the experimental site are classified as Wynnville-Nauvoo fine sandy loams (fine-loamy, siliceous, subactive, thermic Glossic Fragiudults) at 2 to 6% slopes and Hartsells fine sandy loams (fine-loamy, siliceous, subactive, thermic Typic Hapludults) at 6 to 10% slopes according to Web Soil Survey (Natural Resources Conservation Service, United States Department of Agriculture). Soils were tested by the Auburn University Soil, Forage, and Water Testing Laboratory ahead of the study and determined to be Soil Group 2 (ECEC = 4.6-9.0 $cmol_c\ kg^{-1}$). The existing forage base was comprised primarily of ‘KY 31’ tall fescue, with bermudagrass present in some areas and volunteer annual ryegrass (*Lolium multiflorum* Lam.) present at a moderate frequency.

The study was conducted as a completely randomized design (CRD) each year, with four treatments randomly assigned to 12 paddocks (*n* = 3 paddocks per treatment). Those treatments were a control (CTRL) where only the existing tall fescue sward was maintained, hairy vetch (VETCH), mustard (MUST), and turnip (TURN). Each forb treatment was interseeded annually via no-till drill along with supplemental tall fescue in the fall season prior to spring grazing.

Pre-trial composite soil samples (0-10 cm) were collected from each paddock on March 25, 2025 using a Wintex 3000 automatic soil sampler (Wintex Agro, Bancroft, IA). Samples were analyzed in the Auburn University Soil Testing Laboratory by Mehlich-1 extraction. Soil pH averaged 5.85 across all paddocks (range: 5.5-6.2) and nutrient status indicated very high soil phosphorus (P), high potassium (K), high magnesium (Mg), and high calcium (Ca) across all paddocks according to classification based on the Nutrient Recommendation Tables for Alabama crops (Mitchell & Huluka, 2012).

Daily air temperature and precipitation data were obtained from the SMREC weather station for the duration of each season (establishment – termination of grazing). Long-term climate at this site is characterized by annual precipitation of approximately 1,400 mm distributed throughout the year, and a mean annual temperature of approximately 14°C (NOAA).

Forb treatments and supplemental tall fescue were established in each paddock by no-till drilling into the existing tall fescue sward in each of two consecutive seasons. Year 1 (2024-2025 season) was planted between November 11 and December 6, 2024. Year 2 (2025-2026 season) was planted between November 3 and November 7, 2025. Establishment in both years followed the same procedure. Each paddock was mowed to approximately 5 cm immediately prior to drilling, and seeds were placed at a target depth of 6-13 mm using a no-till drill at 19-cm spacing. Tall fescue seed was interseeded into every paddock, including controls, at the time of forb establishment at a rate of 28 kg PLS ha^{-1} to supplement the existing perennial sward and maintain consistency across paddocks. Forbs were interseeded at a rate recommended by Ball et al. (2015) to achieve a

30% inclusion rate based on sward biomass. Previous work was also done in Alabama (Siziba et al., 2026) to determine optimal monoculture broadcast seeding rates for these forb species. The chosen cultivar for hairy vetch was ‘AU Merit’ and it was seeded at a rate of 16.8 kg PLS ha^{-1}. ‘Florida Broadleaf’ mustard was planted at a rate of 5.6 kg PLS ha^{-1}. ‘Purple Top’ turnip was seeded at a rate of 5.6 kg PLS ha^{-1}.

Heavy weed pressure in Year 1 encouraged alternative management in CTRL for Year 2 to create a “broadleaf-free” environment. Prior to the initiation of grazing in 2026, an application of DuraCor® herbicide (florpyrauxifen-benzyl + aminopyralid; Corteva Agriscience, Indianapolis, IN) was made to CTRL paddocks at a rate of 1.46 L product ha^{-1} on March 4, 2026 using a UTV sprayer.

In each grazing season, 36 yearling beef heifers (primarily Angus crossbred) were sourced from the resident herd at the SMREC as well as from the resident heard at the Tennessee Valley Research and Extension Center (TVREC; Belle Mina, AL) and transported to SMREC. In Year 1, 25 heifers came from TVREC and 11 came from SMREC. In Year 2, 23 heifers came from TVREC and 13 came from SMREC. Animals were allocated to paddocks following a pre-trial weighing each year and stratified by the initial body weight (BW) to balance starting body weight (BW) across treatments (2025: 341.6 ± 6.50 kg starting BW; 2026: 344.6 ± 6.77 kg starting BW).

Grazing was initiated each year once the standing biomass across paddocks reached a threshold of approximately 1,000 kg DM ha^{-1}. Pre-grazing standing biomass in the paddock was estimated using a grazing stick. Grazing was terminated each year once the forage allowance, calculated as the ratio of available pasture dry matter (kg DM) to total animal body weight (kg BW), fell below a threshold of 1:1 across the entire paddock area. Forage allowance was monitored throughout each season with each forage sampling (every 14 days) and each animal weigh date (every 28 days). If forage allowance deviated from the 1:1 target, the paddock area was altered using temporary electric poly-wire fencing to restore forage allowance within 10% of the target forage allowance (FA). This strategy maintained stocking density and forage allowance without altering animal numbers per paddock so that treatment comparisons could be made under comparable forage availability. In Year 2, no adjustments were made to paddock size during the grazing season. Animals had ad libitum access to fresh water, shade, and free-choice mineral salt. Standard herd and health management and animal welfare monitoring followed Auburn University protocols and Beef Quality Assurance practices.

In each year, forage samples were collected at the initiation of grazing and on bi-weekly intervals thereafter. On each sampling date within each paddock, six 0.16 m^2 (40 × 40 cm) quadrats made of polyvinyl chloride (PVC) were placed at randomly selected points, excluding heavier-use areas and bare spots. All standing biomass within each quadrat was clipped to a 2.5-cm stubble height, placed in a paper bag, and transported directly to the lab at Auburn University.

Four of the six quadrat samples were used for dry matter biomass accumulation determination. Wet weights of forage were recorded immediately upon return to the lab, and then samples were dried at 55°C in a Thermo Scientific Heratherm OGS750 forced-air dryer to a constant mass (> 72 hr) and dry weights were recorded. Dry matter biomass was calculated as average dry weight per quadrat divided by quadrat area and reported on a kg ha^{-1} basis.

Two quadrat samples per paddock were used for botanical composition by hand separation (Kallenbach, 2015). The biomass in these samples was hand-sorted into functional-group categories (grass, forb, weed). Each fraction was put in a separate paper bag, weighed wet, and then dried at 55°C in a forced-air dryer to constant mass, and weighed dry. Botanical composition was expressed as the percentage contribution of each functional group to dry matter biomass within the samples. In Year 2, botanical composition was also determined through in-field point-step transects and digital image analysis. Further explanation of these methods and comparisons between them can be found in Chapter III.

In Year 2, forage sampling also differed with the addition of grazing exclusion cages. Prior to grazing, three, 1.4-m^2 metal cages were constructed in each paddock and distributed evenly across the entire paddock to exclude animals from grazing within. Forage samples were taken from these "ungrazed" zones at 28-day intervals to assess forage growth and botanical composition under no grazing pressure. Ungrazed forage samples were collected twice at initiation and termination of the grazing season.

Dried forage samples from all dates were ground in a Wiley Hammer mill (Thomas Scientific, Chadds Ford Township, PA), first through a 2-mm sieve screen, and then ground again through a 1-mm sieve screen. The total contents from each sample date were then composited by paddock and stored at ambient room temperature for further analysis.

Forage dry matter (DM) content was determined by drying 1-g ground sub-samples at 105°C for at least 12 h in a forced-air oven. Organic matter (OM) content was determined by burning those dried samples at 500°C for at least 4 h in a muffle furnace. Organic matter content was calculated as ash weight subtracted from dry weight, then divided by dry weight (AOAC Official Method 942.05).

Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined sequentially using ANKOM 2000 and ANKOM Delta Fiber Analyzers (ANKOM Technology, Macedon, NY) according to the methods of Vogel et al. (1999) for NDF and ADF and the methods of the AOAC for ADL. Neutral detergent fiber was determined with amylase pretreatment, ADF was determined on residues from NDF analysis, and ADL was determined by 72% sulfuric acid extraction of ADF residues. All fiber values were corrected for residual ash and reported on a dry matter basis.

Crude protein was determined by combustion-method nitrogen analysis (Dumas method, AOAC 990.03) by Cumberland Valley Analytical Services (CVAS, Waynesboro, PA). Sub-samples of the 1-mm particle size were shipped from each paddock on each sampling from both years. Crude protein was calculated as nitrogen content (N) × 6.25 and reported on a dry matter basis.

Gross energy was determined by bomb calorimetry using a LECO AC-600 calorimeter (LECO Corporation, St. Joseph, MI) on 0.2-g sub-samples. Each sample was analyzed in duplicate, and reps with a coefficient of variation (CV) greater than 5% were repeated.

Heifers were weighed individually before the trial each year at their original research station as well as upon initiation of the grazing trial. Follow-up weighing was repeated every 28 d throughout the grazing season each year. In total, there were three weigh-dates in Year 1: April 1 (initiation), May 1, and May 23 (termination), 2025. In Year 2, body weights were collected only on March 24 (initiation) and April 24 (termination), 2026. Average daily gain (ADG) was calculated as (final BW – initial BW) ÷ days on trial. Forage allowance (FA) was calculated for each paddock-date combination from the most recently obtained dry matter biomass and total paddock BW measurements. Stocking density (SD) was calculated as the average kg BW per hectare across the entire season for each treatment. Gain per area (GPA) was calculated as the total kg BW gained per hectare across the entire season for each treatment.

In each year, blood samples were collected via jugular venipuncture upon initiation and termination of grazing. Blood from each animal was collected into three vacutainers, two of which contained EDTA anti-coagulant. Samples were transported to the lab on ice immediately following collection. One of the vacutainers containing anti-coagulant was transported to the Auburn College of Veterinary Medicine Clinical Pathology Lab for complete blood count (CBC) analysis. Complete blood count analyses were conducted to monitor animal health and ensure that incorporating cool-season forbs into grazing systems did not adversely affect normal physiological function. Measurements in CBC analyses provide a broad assessment of hematological status, including immune response, red blood cell dynamics, and potential indicators of stress or disease.

The remaining two vacutainers were brought to the Auburn University Animal Sciences and Forages Research Team lab in Upchurch Hall and centrifuged at 4,000 rpm for 15 min at room temperature. The resulting blood plasma and serum supernatant from each respective tube was pipetted off into smaller aliquots and stored at -80°C until analysis. Blood urea nitrogen (BUN) content in the serum was determined for each animal through a colorimetric assay detection kit from Thermo Fisher Scientific (Product: EIABUN, Thermo Fisher Scientific, Auburn, AL). Non-esterified fatty acid (NEFA) content in the serum was determined for each animal through a colorimetric assay detection kit from RayBiotech (Product: MA-NEFA-2, RayBiotech, Peachtree Corners, GA).

All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). Forage variables and animal variables were analyzed using a generalized linear mixed model approach in PROC GLIMMIX. The model included fixed effect of treatment while paddock was treated as the experimental unit. Year was included in the model as a random effect and both years' data were analyzed together. For animal variables measured at multiple points within each year, individual animal (identified by tag number) within each paddock was included as a random effect, with treatment as the fixed effect of interest. Treatment least-squares means were separated using the Tukey-Kramer post-hoc test to make pair-wise comparisons between all treatments. Treatment differences with $P < 0.05$ were considered significant.

2.2.2 Small Plot Forb Study

This complementary small-plot study was conducted across three site-years: the Black Belt Research and Extension Center (BBREC) in 2024 – 2025, and the TVREC and SMREC in 2025-2026.

The Black Belt site at the BBREC (32°28'38.35"N 87°14'46.77"W, Marion Junction, Dallas County, AL) was evaluated between October 2024 and May 2025 for establishment of interseeded forbs. Soils at the site were classified as Houston clays (very fine, smectitic, thermic Oxyaquic Hapluderts) with 1 to 5% slopes. The existing perennial sward consisted of mature, E+ tall fescue. The Tennessee Valley site at the TVREC (34°41'11.33"N 86°52'40.06"W, Belle Mina, Limestone County, AL) was evaluated between October 2025 and March 2026. Soils at the site were classified as Etowah silt loams (fine-loamy, siliceous, semiactive, thermic Typic Paleudults) with 0% slope. The existing sward consisted of mature, E+ tall fescue. The Sand Mountain site at the SMREC (34°16'59.74"N 85°57'12.34"W, Crossville, Dekalb County, AL) was evaluated between October 2025 and March 2026. Soils at the site were classified as Wynnville-Nauvoo fine sandy loams (fine-loamy, siliceous, subactive, thermic Glossic Fragiudults) with 2 to 6% slopes. The existing sward consisted of a heavy thatch layer of bermudagrass, and abundant volunteer annual ryegrass.

A randomized complete block design (RCBD) was used at each site, with four blocks of 12 cool-season forb species (n = 48 plots per site-year). Each individual plot contained one forb species and measured 1.5 m × 6.1 m directly adjacent to one another on the long side within each block and with 1.5-m alleys between blocks.

Twelve cool-season annual forb species (six leguminous and six non-leguminous) were interseeded into the existing sward at each site at pure live seed (PLS) rates calibrated to a target forb contribution of 30% of standing biomass at peak forb expression. Pure live seed rates were determined in 2024 for the Black Belt site based on the certified seed tag from each species. In 2025, for the TVREC and SMREC sites, PLS was determined based on germination tests conducted in-house according to the Association of Official Seed Analysts (AOSA; *Rules for Testing Seeds*; Volume 1., 2019). Pure live seed was calculated as a percent where PLS (%) = (Purity (%) × Total Germination (%)) / 100 and final seeding rate was calculated as Rate = PLS Rate (kg ha^{-1}) / PLS (%). Recommended PLS seeding rates were determined based on *Southern Forages* (Ball et al., 2015) and Siziba et al. (2026).

At the BBREC, plots were seeded on October 30, 2024 using a Great Plains (Great Plains Ag, Salina, KS) no-till drill with a cone-seeder attachment calibrated to 6.1 m plot length. At the SMREC, plots were seeded on October 16, 2025 using an Almaco (ALMACO, Nevada, IA) no-till drill with a cone-seeder attachment calibrated to a 6.1 m plot length. At the TVREC, plots were seeded on October 23, 2025 using the same Almaco (ALMACO, Nevada, IA) no-till drill from SMREC with the same settings. All species were planted at the same depth at ~1 cm within one week following a close mowing (5 cm) of the existing sward.

Legumes evaluated were hairy vetch ('AU Merit'; *Vicia villosa* Roth; 16.8 kg PLS ha^{-1}), burr medic (variety not specific [VNS]; *Medicago polymorpha* L.; 6.72 kg PLS ha^{-1}), alfalfa ('Bulldog 505'; *Medicago sativa* L.; 7.84 kg PLS ha^{-1}), berseem clover (VNS; *Trifolium alexandrinum* L.; 8.96 kg PLS ha^{-1}), crimson clover ('Dixie'; *Trifolium incarnatum* L.; 11.2 kg

PLS ha^{-1}), and white lupin (VNS; *Lupinus albus* L.; 67.2 kg PLS ha^{-1}). Non-legumes evaluated were mustard ('Florida broadleaf'; *Brassica juncea* (L.) Czern.; 5.6 kg PLS ha^{-1}), turnip ('Purple Top'; *Brassica rapa* L.; 5.6 kg PLS ha^{-1}), radish ('Driller'; *Raphanus sativus* L.; 5.6 kg PLS ha^{-1}), kale ('Bayou'; *Brassica oleracea* L.; 5.6 kg PLS ha^{-1}), rape ('Winfred'; *Brassica napus* L.; 5.6 kg PLS ha^{-1}), and chicory ('Forb-feast'; *Cichorium intybus* L.; 4.5 kg PLS ha^{-1}).

Establishment was evaluated visually at each site-year during the winter and early spring (approximately 60 to 150 days after planting), with each species' establishment classified per plot on a visual indexed scale of 0 - 5 based on visible seedling emergence, persistence, and competitive presence within the row. A score of 0, 1, 2, 3, 4, or 5 translated to failed, trace, poor, moderate, good, and excellent, respectively. Due to insufficient and highly variable establishment across site-years, the originally planned biomass harvests, nutritive-value analysis, and animal preference grazing trial were not conducted. This study is reported as a categorical establishment across species and site-years. Data were analyzed with a Chi square frequency test using PROC FREQ in SAS Version 9.4 and results were reported as differences between species.

2.3 Results and Discussion

2.3.1 Grazing Study

Conditions during the two establishment and grazing windows differed substantially and provide important context for the variation between years. The 2024 establishment period was characterized by an exceptionally dry October 2024 as seen in Table 2.1 (6.35 mm precipitation vs. 98.55 mm 30-year average; -94%). December 2024 was also drier than the 30-year average. The establishment window during 2025 was also much drier than the 30-year average as seen in Table 2.1. In both years, grazing periods were drier than the 30-year average, especially in 2026, where April 2026 only saw 36.6 mm, 72% lower than the average. Temperature trends for both trial periods can be seen in Table 2.2. Average air temperatures tended to stay slightly above the 30-year average throughout the entire trial period in both years, with the exception of January 2025 which was cooler than the 30-year average (Table 2.2).

Pre-trial composite soil samples (0-10 cm; collected March 25, 2025) showed baseline pH ranging from 5.5 to 6.2 across the 12 paddocks (mean 5.85 ± 0.07). Mehlich-1 extractable nutrients were uniform across paddocks. Phosphorus was very high and potassium, magnesium, and calcium were all high according to classification based on the Nutrient Recommendation Tables for Alabama crops (Mitchell & Huluka, 2012). The relatively low soil pH sat below the threshold of 6.0 for optimal establishment of crimson clover, berseem clover, and alfalfa. Bunton et al. (2020) surveyed 63 Missouri pastures and reported that a 1-unit increase in soil pH was associated with 146 fewer weeds ha^{-1} averaged across all species and 1,572 fewer annual broadleaf weeds ha^{-1}. With relatively low soil pH and no soil amendments at SMREC in both years, as well as dry establishment and grazing conditions, it is no surprise that weed interference and forb establishment was less than ideal and highly variable.

Forage biomass production did not differ among treatments (Table 2.3). It is likely that overall forb contribution between years did not reach a threshold sufficient to contribute significantly to biomass accumulation. In Year 1, all forbs were present in some proportion, but all below 10% of the stand. In Year 2, hairy vetch was the only forb that established, but was an average of 31.6% of standing biomass. Year was treated as a random effect because both years of the study were considered a random sample of the range of environmental conditions under which treatments could be applied, rather than as the specific primary variable of interest. Modeling year as random accounted for the variability among years resulting from differences in weather and other uncontrolled environmental factors. This allowed treatment effects to be estimated independently from year-to-year variation.

Treatment had an effect on all botanical composition components. Control paddocks contained the greatest grass fraction and were greater than turnip paddocks with vetch and mustard as intermediates (P = 0.0036; Table 2.4). All paddocks were subject to supplemental overseeded tall fescue in both years, and control paddocks received an application of DuraCor® prior to Year 2 that likely contributed to the greater measured grass proportion and absence of some weed pressure seen in paddocks with forb treatments that could not be treated with broadleaf herbicide.

Hairy vetch paddocks contained a greater forb fraction than MUST, TURN, and CTRL paddocks (*P* ≤ 0.0021; Table 2.4). Hairy vetch establishment was variable between years, with Year 2 reaching target establishment of 30% of standing biomass. The brassica species had modest establishment in Year 1 (still below 10% standing biomass) but had a total stand failure in Year 2 (< 5% forb). One of the primary reasons for this poor establishment may have been dry conditions around or directly after planting seen in Table 2.1. Emerging seedlings require adequate rainfall for successful establishment (Bellanuge et al., 2024). Late planting may have also contributed to the poor establishment of brassica species in both years. Interseeding occurred in mid-to-late November in Year 1 and early November in Year 2. In both cases, this is later than the preferred time-frame of early-to-mid October (Ball et al., 2015). Brassicas planted later in the fall are vulnerable to frost since they have not accumulated enough energy to withstand harsher temperatures (Barry, 2013).

The proportion of weed biomass differed among treatments (*P* < 0.0001; Table 2.4). Turnip paddocks contained a greater weed fraction than all other treatments. The high weed pressure in TURN paddocks is consistent with low establishment success in Year 1, as well as a total stand failure in Year 2. Lower weed fractions in CTRL and VETCH paddocks may be partly explained by the DuraCor® application to control paddocks prior to Year 2, as well as vigorous establishment and canopy closure in VETCH paddocks in Year 2 (McCurdy et al., 2013; Bunton et al., 2020). Hairy vetch was the only treatment in this trial that significantly shifted sward composition toward desirable forbs while simultaneously suppressing weed pressure. Brassica treatments produced no consistent compositional advantage over control paddocks and eliminated the ability for a beneficial herbicide application to control for undesirable broadleaf weeds.

Crude protein content differed among treatments (*P* = 0.0056; Table 2.5). Hairy vetch paddocks had greater CP content than MUST and TURN paddocks, with CTRL as an intermediate (CTRL *P* = 0.4150). Interseeding of hairy vetch produced greater CP than either brassica treatment (*P* ≤ 0.0279). Hairy vetch had a greater forb fraction than that of either brassica treatment (*P* ≤ 0.0021). The superior establishment, paired with the greater tissue N characteristic of legume species, is may be part of what drove the improved CP with vetch incorporation (Beuselinck et al., 1994; Sollenberger & Collins, 2017). The lesser CP observed under both brassica treatments compared to the CTRL may be attributed to the poor establishment of the forb species or the reduced grass proportion in mustard and especially turnip paddocks compared to the control. Turnip paddocks also had greater proportion of undesirable weeds such as buttercup (*Ranunculus spp.* L.), henbit (*Lamium amplexicaule* L.), buckhorn plantain (*Plantago coronopus* L.), and curly dock (*Rumex crispus* L.), that may have negatively impacted CP levels depending on several factors like leaf-stem ratio and maturity (Marten et al., 1987).

Neutral detergent fiber , ADF, and ADL did not differ among treatments (NDF *P* = 0.5259; ADF *P* = 0.8215; ADL *P* = 0.3137; Table 2.5). The absence of treatment differences in fiber fractions among treatments is likely due to the low contribution of forbs to overall sward biomass. If forbs, particularly brassicas, had been present in greater proportions within the sward, we may expect to have seen reduced overall fiber (Barry, 2013; Villalobos & Brummer, 2015).

Organic matter did not differ among treatments (*P* = 0.7304; Table 2.5), suggesting that inclusion of forbs did not alter the overall balance between organic components and mineral ash. Organic matter represents the total combustible fraction of forage, and it is less responsive to species composition than other nutritive value parameters (Ball et al., 2001). Shifts in CP content seen with the inclusion of hairy vetch represent shifts within the OM composition rather than shifts in OM content overall.

Average daily gain did not differ among treatments (*P* = 0.7205; Table 2.6). Values were between 1.05 kg hd^{-1} d^{-1} for CTRL and 0.86 kg hd^{-1} d^{-1} for TURN. Although forage nutritive value in the form of enhanced CP was improved with the inclusion of hairy vetch, this did not translate to a measurable improvement in animal gains.

Over the course of the trial, forage allowance remained non-limiting. Average forage allowance across all treatments was 1.25 kg DM per kg animal BW, and there was no effect of treatment on forage allowance (*P* = 0.2868; Table 2.6). This means that animal intake was not inhibited by availability of DM biomass. This may help explain why animal ADG was not different between treatments (Sollenberger & Vanzant, 2011). Total biomass was also not different between treatments, showing that the incorporation of forbs did not increase forage availability. Forage allowance was also modulated through temporary electric fencing to reduce between-paddock forage allowance variance. Stocking density (SD) and gain per area (GPA) were also not affected by treatment (SD *P* = 0.3833; GPA *P* = 0.4722; Table 2.6).

Another reason for the absence of treatment effects on animal performance despite improvements in nutritive value with the inclusion of forbs is that the forb fraction remained relatively low. Forbs' overall contribution to the diet may not have been substantial enough to translate to measured benefits in ADG despite measured benefits to nutritive value. The trial period was also relatively short: 52 days in Year 1 and 31 days in Year 2. A longer trial period would have exposed animals to treatments for longer, where more potential effects may have been realized.

However, these findings are consistent with the literature on legume-fescue grazing trials. Interrante et al. (2012) similarly reported equivalent ADG between stocker steers grazing tall fescue + annual legume mixtures and N-fertilized monoculture tall fescue across three grazing seasons in Oklahoma. Hoveland et al. (1981) and Templeton & Taylor (1975) also documented that legume integration into established tall fescue produces only modest, variable, or undetectable animal performance responses. The improvements mainly came in sward diversity, soil N status, and grazing-day capacity.

It is worth noting that there may be system-level benefits to moderate forb integration beyond ADG that are operating. Diversification of tall fescue pastures with forbs that are rich in PSM has been shown to reduce enteric methane emissions per unit of intake (Pavao-Zuckerman et al., 1999; Gere et al., 2024; Puchala et al., 2005; Min et al., 2020). Also, soil health parameters were not considered within this experiment, but diversification of pastures with several plant functional groups through forb integration may impact long-term soil health positively.

Blood metabolite changes from initial to final sampling did not differ between treatments (BUN $P = 0.2479$; NEFA $P = 0.5482$; Table 2.7). Even though VETCH had greater CP content than TURN and MUST paddocks, no differences in BUN circulating through the blood serum of animals subjected to each of those treatments were measured. Legume-rich diets have greater rapidly degradable protein according to Trotta et al. (2018), but animal intake of legumes in paddocks containing hairy vetch could not be determined. It may be that the modest establishment of hairy vetch (18.9% of standing biomass) was not in great enough proportion to see elevated nitrogen levels in the blood serum. Changes in NEFA levels were predominantly negative, indicating that most treatments supported a transition towards improved energy status across the grazing period (Gunter et al., 2019). Mustard is the exception, where changes in NEFA status were near zero. Despite these nuances, there were no differences in the change in NEFA levels between treatments. Complete blood count (CBC) analysis showed no differences among treatments for any parameter measured (Table 2.8).

2.3.2 Small Plot Forb Study

Results from all three site-years were combined and subjected to a Chi square frequency analysis to determine if there were establishment differences among species. A total of 144 plots were evaluated (3 sites × 12 species × 4 blocks) on a scale from 0 to 5 (0 – failed, 1 – trace, 2 – poor, 3 – moderate, 4 – good, 5 – excellent). Of those 144 plots, 113 (78.5%) failed to establish any detectable forb stand. The chi-square test indicated a species × establishment interaction ($\chi^2 = 205.40$, df = 55, $P < 0.0001$; Figure 2.1). Cramer's V was 0.534, which indicates a moderately strong species effect on establishment outcome. The Mantel-Haenszel chi-square test for a linear trend across the establishment scale (failed → trace → poor → moderate → good → excellent) was also significant (χ^2_MH = 5.34, df = 1, *P* = 0.021). This indicates that the effect of species operated as an ordinal trend rather than as a random phenomenon.

Figure 2.1 shows the distribution of establishment scores across species combined at all three site-years. It demonstrates the superior establishment of hairy vetch interseeded into existing grass sod across a variety of environmental conditions. It also indicates the majority of species did not establish or established poorly under the same management where hairy vetch thrived. Some factors that may have contributed to poor establishment are a later-than-optimal planting date, as well as drought conditions around the time of planting (Bellangue et al., 2024). This experiment supports the idea that hairy vetch is a reliable forb species for use as an interseeded forage in grass-dominant pastures. However, for many other species, further research is required to understand the specific management and environmental conditions that promote successful establishment in this production context.

2.4 Conclusions

Hairy vetch emerged as the only cool-season forb species in this study to reliably establish and produce a measurable shift in sward composition under unamended Southeastern field conditions. Across two consecutive grazing seasons at SMREC, hairy vetch paddocks contained the greatest forb fraction and the least weed fraction of any treatment, and produced greater whole-sward CP than either brassica treatment. The small-plot forb study extended this pattern across three site-years and twelve species. Hairy vetch was the only species to establish a stand at every site, while all five brassicas, chicory, and burr medic failed in 12 of 12 plots each. The chi-square frequency analysis confirmed that species establishment success was strongly non-random with respect to species, with hairy vetch as the primary contributor to this effect.

Despite the measurable improvements in forage nutritive value and botanical composition under hairy vetch interseeding, individual animal performance did not differ among treatments. Average daily gain, blood urea nitrogen, non-esterified fatty acids, and complete blood count parameters were not affected by treatment. From a proof-of-concept standpoint, the absence of treatment effects on animal health parameters shows that hairy vetch can be incorporated into the grazing diet at an average of 18.7% without producing negative effects on animal performance, blood metabolism, or health status. This demonstrates that forb integration at modest fractions can be accomplished in a production context without compromising animal outcomes.

Brassica integration, by contrast, was not consistently successful under the unamended protocol used in this study. Brassica species failed to establish at any small plot site-year, and brassica forb fractions in the grazing study were modest in 2025 (mustard 7.6%, turnip 4.0%) and totally absent in 2026. Producers seeking to incorporate brassicas into existing tall fescue stands in this way should target an early-fall planting window. If planting occurs later, there may be risk of low establishment success which may therefore require management interventions like sod suppression, N fertilization, close attention to thatch and residue, and lime amendment.

Hairy vetch can be recommended as a reliable interseeding option for low-input diversification of Southeastern tall fescue pastures. Economic feasibility was not evaluated directly in this study, but the low-input nature of this approach (no lime, no fertilizer, no herbicide on forb treatments, single annual no-till seeding) is consistent with adoption by producers operating under cost constraints and narrow profit margins that are characteristic of Southeastern cow-calf and stocker operations. Hairy vetch seed cost per acre is also modest relative to alternative legume options such as alfalfa and white lupin (Ball et al., 2015), and its contribution to soil nitrogen may help partially offset future fertilizer expenditure. This contribution to plant-available soil N still needs to be quantified. Future work should evaluate the effect of repeated hairy vetch interseeding on soil nitrogen and grass productivity over multiple seasons, quantify the enteric methane response to vetch inclusion, and identify management interventions to make brassica integration more reliable. Future work should also aim to investigate the individual species contributions to pasture nutritive value and animal performance to identify the effect therein of cool-season grasses, common weeds, and desirable forbs.

Table 2.1. Total monthly precipitation (mm) at the Sand Mountain Research and Extension Center (SMREC) across both trial years

Year[1]	Month	Precip. (mm)[2]	30-yr Avg. Precip. (mm)	Δ from 30-yr Avg.
2024	September	82.6	113.8	-27.5%
	October	6.4	98.6	-93.6%
	November	102.1	120.9	-15.5%
	December	81.8	155.7	-47.5%
2025	January	70.6	138.9	-49.2%
	February	102.6	125.7	-18.4%
	March	111.5	141.0	-20.9%
	April	113.3	131.6	-13.9%
	May	108.2	108.2	-16.9%
	September	67.6	113.8	-40.6%
	October	101.3	98.6	+2.8%
	November	30.7	120.9	-74.6%
	December	48.3	155.7	-69.0%
2026	January	81.5	138.9	-41.3%
	February	91.7	125.7	-27.1%
	March	97.8	141.0	-30.6%
	April	36.6	131.6	-72.2%

[1]Data compiled from National Oceanic and Atmospheric Administration (NOAA).

[2]Precip. = precipitation

Table 2.2. Average monthly air temperature (°C) at the Sand Mountain Research and Extension Center (SMREC) across both trial years.

Year[1]	Month	Temperature (°C)	30-yr Avg. Temp. (°C)
2024	September	22.1	21.2
	October	17.2	15.4
	November	13.4	9.7
	December	6.8	6.1
2025	January	2.0	4.6
	February	8.2	6.6
	March	13.6	10.6
	April	17.7	14.8
	May	19.8	18.9
	September	22.2	21.2
	October	16.2	15.4
	November	11.6	9.7
	December	6.8	6.1
2026	January	4.7	4.6
	February	8.5	6.6
	March	15.1	10.6
	April	18.2	14.8

[1]Data compiled from National Oceanic and Atmospheric Administration (NOAA).

Table 2.3. Forage biomass (kg ha^{-1}) in tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.) paddocks interseeded with different forb species.

Treatment	Biomass (kg ha^{-1})
Hairy vetch	2683
Control	2042
Turnip	1986
Mustard	1920
SEM[1]	245.2
P-value	0.1083

[1]SEM = standard error of the mean

Table 2.4. Botanical composition (%) of tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.) paddocks interseeded with different forb species.

Treatment	Grass (%)[1]	Forb (%)	Weed (%)
Control	83.2[a]	0.0[b]	16.8[b]
Hairy vetch	70.9[ab]	18.7[a]	10.4[b]
Mustard	69.6[ab]	4.8[b]	25.7[b]
Turnip	53.8[b]	2.5[b]	43.7[a]
SEM[2]	5.49	2.68	4.66
P-value	0.0036	< 0.0001	< 0.0001

[1]Superscript letters indicate treatments that are different

[2]SEM = standard error of the mean

Table 2.5. Nutritive value of tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.) paddocks interseeded with different forb species.

Parameter[1]	Control	Hairy vetch	Mustard	Turnip	SEM[2]	*P*-value
CP (%)[3]	13.3[ab]	14.9[a]	11.4[b]	12.0[b]	0.73	0.0056
OM (%)	91.9	91.5	91.9	91.7	0.31	0.7304
NDF (%)	55.2	53.4	52.9	51.2	1.90	0.5259
ADF (%)	29.1	29.8	28.3	28.5	1.21	0.8215
ADL (%)	3.9	4.7	3.7	4.6	0.46	0.3137

[1]CP = crude protein, OM = organic matter, NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin

[2]SEM = standard error of the mean

[3]Superscript letters indicate treatments that are different

Table 2.6. Animal performance of yearling heifers grazing tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.) paddocks interseeded with different forb species.

Parameter[1]	Control	Hairy vetch	Mustard	Turnip	SEM[2]	*P*-value
ADG (kg hd^{-1} d^{-1})	1.05	0.95	0.95	0.86	0.121	0.7205
FA (kg DM:kg BW)	1.25	1.45	1.17	1.15	0.408	0.2868
SD (kg ha^{-1})	1,462.2	1,667.7	1,465.4	1,453.1	254.53	0.3833
GPA (kg ha^{-1})	58.5	59.0	50.3	45.8	20.63	0.4722

[1]ADG = average daily gain, FA = forage allowance, SD = stocking density, GPA = gain per area
[2]SEM = standard error of the mean

Table 2.7. Blood metabolites of yearling heifers grazing tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.) paddocks interseeded with different forb species.

Treatment	ΔBUN (mg dL^{-1})[1]	ΔNEFA (μM)
Hairy vetch	+4.45	+2.17
Mustard	+2.21	-55.51
Control	+2.04	-131.56
Turnip	+1.34	-163.54
SEM[2]	1.047	86.206
P-value	0.2479	0.5482

[1]BUN = blood urea nitrogen, NEFA = non-esterified fatty acids

[2]SEM = standard error of the mean

Table 2.8. Complete blood count (CBC) parameters of yearling heifers grazing tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.) paddocks interseeded with different forb species.

Parameter[1]	Control	Mustard	Turnip	Hairy vetch	SEM[2]	*P*-value
ΔWBC (10^3 μL^{-1})	+0.67	+0.82	+0.72	+0.52	0.40	0.9647
ΔNEU (10^3 μL^{-1})	-0.36	+0.24	+0.07	-0.21	0.30	0.5057
ΔLYM (10^3 μL^{-1})	+0.89	+0.22	+0.43	+0.65	0.35	0.5686
ΔMONO (10^3 μL^{-1})	-0.12	-0.11	-0.18	-0.13	0.06	0.8134
ΔEOS (10^3 μL^{-1})	+0.18	+0.39	+0.32	+0.15	0.10	0.2848
ΔBAS (10^3 μL^{-1})	+0.06	+0.08	+0.09	+0.06	0.03	0.8913
ΔRBC (10^6 μL^{-1})	+0.43	-0.68	-0.12	+0.45	0.48	0.3509
ΔHGB (g dL^{-1})	+1.19	-0.35	+0.71	+1.20	0.54	0.2306
ΔHCT (%)	+2.92	-1.12	+5.98	+2.75	2.59	0.3187
ΔMCV (fL)	+0.94	+2.43	+2.19	+0.85	0.91	0.5196
ΔMCH (pg)	+0.52	+0.86	+0.97	+0.50	0.37	0.7442
ΔMCHC (g dL^{-1})	+0.36	-0.09	+0.36	+0.45	0.25	0.4745
ΔRDW (%)	+0.21	+0.03	-0.33	+0.72	0.64	0.7592
ΔPLT (10^3 μL^{-1})	+105.22	+67.17	-17.67	+48.50	44.53	0.3062
ΔMPV (fL)	+0.01	-0.11	+0.05	-0.22	0.20	0.8738

[1]WBC = white blood cell count, NEU = neutrophil count, LYM = lymphocyte count, MONO = monocyte count, EOS = eosinophil count, BAS = basophil count, RBC = red blood cell count, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, RDW = red blood cell distribution width, PLT = platelet count, MPV = mean platelet volume

[2]SEM = standard error of the mean

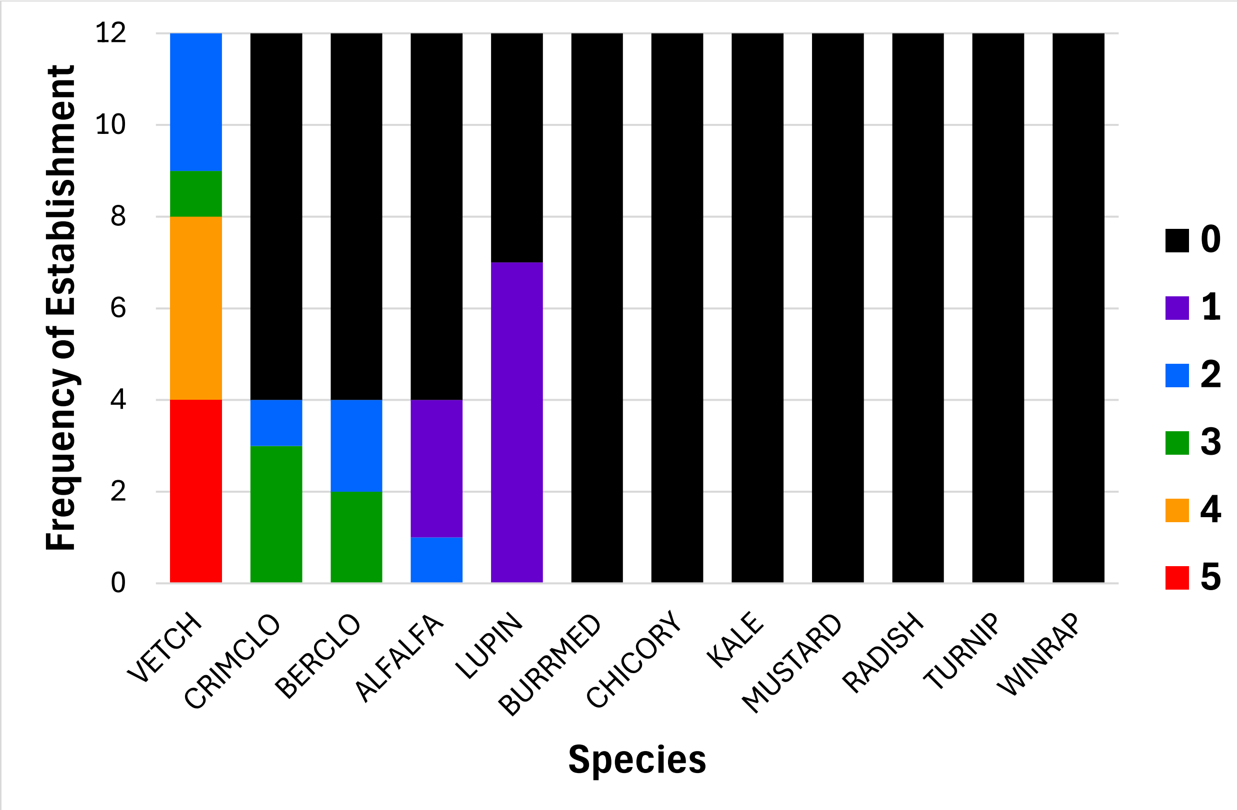

Figure 2.1. Establishment frequency of 12 species of cool-season forbs interseeded into perennial grass sod across three site-years.

Each species was planted in 12 plots total, and each plot was rated for establishment success on a scale from 0 to 5 where scores of 0, 1, 2, 3, 4, and 5 represented failed, trace, poor, moderate, good, and excellent establishment, respectively. Colored components of each species' bar in the chart correspond to the number of plots that established with each rating based on the legend on the right side of the figure. Species identities were as follows: 'VETCH' = hairy vetch (*Vicia villosa* Roth), 'CRIMCLO' = crimson clover (*Trifolium incarnatum* L.), 'BERCLO' = berseem clover (*Trifolium alexandrinum* L.), 'ALFALFA' = alfalfa (*Medicago sativa* L.), 'LUPIN' = white lupin (*Lupinus albus* L.), 'BURRMED' = burr medic (*Medicago polymorpha* L.), 'CHICORY' = chicory (*Cichorium intybus* L.), 'KALE' = kale (*Brassica oleracea* L.), 'MUSTARD' = mustard (*Brassica juncea* (L.) Czern.), 'RADISH' = radish (*Raphanus sativus* L.), 'TURNIP' = turnip (*Brassica rapa* L.), 'WINRAP' = rape (*Brassica napus* L.).

CHAPTER III: A COMPARISON OF METHODS FOR QUANTIFYING BOTANICAL COMPOSITION IN SOUTHEASTERN TALL FESCUE GRAZING SYSTEMS

3.1 Introduction

Pasture botanical composition is an important variable in grazing system research because it links agronomic management outcomes with dietary inputs for grazing animals. The methods used to estimate composition in pasture systems (hand-separation, in-field point-step transects, and digital image analysis) and their respective strengths and biases are reviewed in Chapter I, Section 1.3.2. Hand-separation provides direct mass-based estimates and serves as the primary reference but is labor-intensive and cannot cover as much area as other methods. Transect and image-based methods are less labor-intensive and provide repeatable, scalable estimates, but they produce cover and frequency-based outputs that cannot be directly compared to mass-based estimates. They have to be related to the mass-based reference before they can be used as an estimate for composition measurements in research or for on farm practical purposes (Kallenbach et al., 2015; Rayburn, 2014; Tracy & Sanderson, 2004; Baxter et al., 2017).

Direct paired comparisons of multiple non-destructive methods against hand-separation within a single trial are uncommon, particularly in Southeastern cool-season pasture systems where complex species mixtures including tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.), bermudagrass (*Cynodon dactylon* [L.] Pers.), forbs, and weeds make classification complex. These comparisons are needed because choice of method in future grazing-system research depends on an understanding of how each sampling approach performs for each functional group in realistic field conditions.

The objective of this study was to compare two non-destructive methods, in-field point-step transects and digital image analysis, against hand-separation as the reference method for estimating grass, forb, and weed fractions of pasture composition in a Southeastern tall fescue grazing system. It was hypothesized that both non-destructive methods would provide useful estimates of functional-group composition relative to hand separation and method performance would vary by functional group, reflecting the visual contrast and spatial growth pattern of grasses, forbs, and weeds within the sward.

3.2 Materials and Methods

This study was conducted in 2026 within the 12 grazed paddocks of the cool-season forb grazing trial at the Sand Mountain Research and Extension Center (SMREC). Full site description, paddock layout, treatments, and grazing management are provided in Chapter II, Section 2.2.1.

Botanical composition was estimated using three methods on the same sampling dates and within the same paddocks across the 2026 grazing season. Each method was applied to all 12 paddocks on each of three sampling dates, generating n = 36 paired observations per functional group (grass, forb, weed). All three methods were performed on the same day at each paddock to minimize any differences between estimates.

Hand-separation followed the protocol used in the grazing study (Chapter II, Section 2.2.1, *Forage Sampling*). In brief, within each paddock and sample date, standing biomass from two 0.16 m^2 (40 × 40 cm) quadrats was clipped to a 2.5-cm stubble height, returned to the laboratory, sorted into grass, planted forb, and weed fractions, dried at 55°C to constant mass, and weighed. Functional-group percentages of standing biomass were calculated as the dry weight of each fraction divided by the total dry weight per sample.

Point-step transects followed the method of Evans and Love (1957). Within each paddock and sample date, the observer walked one transect diagonally across the paddock and recorded the single species directly in contact with a single, pre-identified point on the toe of the boot every six paces, totaling 50 points per paddock. Each point was classified as grass, planted forb, weed, or bare ground. Functional-group percentages were calculated from the proportion of points falling in each category.

Six representative sample points from each paddock were chosen and subjected to digital image analysis. Sample points were stratified to be evenly distributed across the entire paddock and to avoid heavy-use areas and fencelines which would not represent the predominant forage base of the paddock. A 0.16 m^2 quadrat made of polyvinyl chloride (PVC) was used to define the sample area. Images were taken with a Kodak Pixpro WPZ2 digital camera (Eastman Kodak Company, Rochester, NY) mounted to a monopod at a height of 1.25 m. Following the method described by Baxter et al. (2017), the monopod was tilted to center the camera over the quadrat and leveled with respect to the ground before recording the image. Images were saved as a .JPEG file.

Images were manually cropped to the exact 0.16 m^2 sample area prior to analysis. Digital image analysis followed the photo point count procedure described by Rayburn

(2014) and Baxter et al. (2017), with minor modifications. A point count grid of the capital letter “V” was made on a standard PowerPoint slide (4:3) by typing 6 rows of 6 Vs with 1.5-line spacing and three spaces between characters at Calibri font size 66, totaling 36 letters. The Vs yielded three points per letter, totaling 108 points per image for categorization. Each point was assigned as grass, planted forb, weed, or bare ground, and data were saved in Microsoft Excel (Microsoft Corporation, Redmond, WA) for statistical analysis.

Method performance was evaluated using linear regression (PROC REG, SAS Version 9.4; SAS Institute, Cary, NC) with hand-separation percentages as dependent variables and the corresponding non-destructive estimates as predictors. Single-predictor models were fit separately for each non-destructive method against each functional group. Two-predictor models including both transect and digital image inputs were also fit to evaluate whether the methods contributed complementary information. Coefficient of determination (R^2), root mean square error (RMSE), parameter estimates, and *P*-values are reported for each model. Significance was declared at $\alpha = 0.05$.

3.3 Results and Discussion

Point-step transects provided cover-based estimates of functional-group composition with lower per-sample labor cost than hand-separation. Regression of hand-separation grass percentage on transect grass percentage showed a strong linear relationship ($P < 0.0001$), with the transect explaining 41% of the variation in hand separated grass mass (Figure 3.1; $R^2 = 0.41$; slope = 0.92; SE = 0.19). The slope indicated that transect-based grass cover scaled around one-to-one with grass mass with the intercept near zero (intercept = 0.19; $P = 0.07$). Transect-based estimates produced the strongest fit to hand-separation for the planted forb fraction (Figure 3.3; $R^2 = 0.75$; slope = 0.93; SE = 0.09; $P < 0.0001$) with an intercept at essentially zero (intercept = 0.01; $P = 0.56$). This relationship indicated that transect-based forb cover was an excellent predictor of hand-separated forb mass across the range of observations. For weed estimation, the transect explained 47% of the variation in hand-separated weed mass (Figure 3.5; $R^2 = 0.47$; slope = 0.81; SE = 0.15; $P < 0.0001$). The accuracy of the transect method may have been improved further through more transects per paddock and more points per transect. Evans & Love (1957) were the pioneers of this sampling method and recommend between 300-500 points in an area to maximize the precision of the estimate. This study's objective was to detect differences among methods over time rather than total sample precision, so 50 points per paddock were used to balance labor with precision in this evaluation.

Digital image analysis produced cover estimates with comparable or slightly weaker fit to hand-separation than the transect method, depending on functional group. The image method explained 38% of the variation in hand-separated grass mass (Figure 3.2; $R^2 = 0.38$; slope = 0.71; SE = 0.16; $P < 0.0001$) and 56% of the variation in hand-separated forb mass (Figure 3.4; $R^2 = 0.56$; slope = 1.02; SE = 0.15; $P < 0.0001$). For weed estimation, the image method explained 44% of the variation in hand-separated weed mass (Figure 3.6; $R^2 = 0.44$; slope = 0.85; SE = 0.17; $P < 0.0001$) which is comparable to the performance of the transect method for the weed functional group.

Both non-destructive methods produced linear relationships with hand-separation across all three functional groups ($P < 0.0001$). Across the three functional groups, transect-based estimates produced greater single-predictor R^2 than image-based estimates for two of three comparisons (grass and forb). Slopes for both methods were near 1.00 for forb and weed, indicating that the non-destructive cover estimates scaled about one-to-one with mass for these functional groups. The image method consistently produced slopes below 1.00 for grass (0.71), meaning that this method tended to underestimate grass mass at higher grass fractions. This may reflect canopy interference and overlap in an overhead photo where the lower canopy is covered by the upper canopy. Baxter et al. (2017) used the same digital image method described in this study, but with 300 evaluation points per photo. They found the digital image method to be superior to the other non-destructive sampling techniques they evaluated. The digital image method used in this thesis may have been improved with more images per paddock, more reference quadrats, and more points per image.

There were clear differences in the performance of methods based on functional group. Forb estimation was the most successful for both non-destructive methods (transect R^2 = 0.75; image R^2 = 0.56), which reflects the greater visual contrast between planted forbs (i.e. hairy vetch) and the grass-sod base. Grass estimation produced the weakest fit (transect R^2 = 0.41; image R^2 = 0.38) which may be because grass fractions tended to be high (> 60%) and without much variance across paddocks. At greater grass proportions, cover-based estimates may underestimate overall grass contribution to biomass due to canopy structures as aforementioned. Weed estimation produced an intermediate fit (transect R^2 = 0.47; image R^2 = 0.44), with comparable performance between the two non-destructive methods.

Both weed-biomass regression models exhibited heteroscedasticity, with residual variance increasing at greater weed fraction (Figures 3.5 and 3.6). At low weed fractions, both non-destructive methods produced estimates tightly clustered around hand-separation values. At levels above approximately 25 to 30%, residual variance expanded. This pattern may reflect the complexity of weed biomass at greater fractions in that weed-dominated paddocks carry a more complex mixture of species that were complicated to quantify accurately.

One important consideration for interpretation of the forb R^2 is that most of the observed paddocks contained no planted forb biomass due to low establishment of brassica species described in Chapter II. This produced a cluster of zero-zero observations that increased the leverage of the regression and forcing the line of best fit through the origin. The slope near 1.00 and intercept near zero (0.01) indicated the relationship was not very skewed by these observations. Overall, this reflects both non-destructive sampling methods' ability to predict forb fractions where forbs are present as well as to correctly identify where they are absent.

The two-predictor models that included both transect and image inputs simultaneously improved fit for grass ($R^2 = 0.50$; both $P < 0.05$) and weed ($R^2 = 0.53$; both $P < 0.05$), but not for forb ($R^2 = 0.76$; transect $P < 0.05$; image $P = 0.24$). This indicates that the two non-destructive methods provided complementary information for predicting grass and weed estimates, but that the transect method had most of the explanatory power for forb estimation.

Each method involves trade-offs between labor cost, spatial coverage, repeatability, and accuracy. Hand-separation is the reference method and provides direct mass-based estimates but is also labor-intensive and limited in its spatial coverage. Point-step transects provide cover-based estimates at lower per-sample labor cost, scale efficiently to larger areas, and produced the strongest agreement with hand-separation for the planted forb fraction in this study. The biggest limitations are an observer's knowledge of plant species and that the consistency of classification requires standardized training and can be influenced if there are several species at the observation point. Digital image analysis provides cover-based estimates with a permanent visual record of each sample point. This enables reclassification, calibration, and better comparison. Its main limitations are canopy layers blocking one another and dependence on lighting, conditions at the sampling time, and image quality.

3.4 Conclusions

Both in-field point-step transects and digital image analysis produced useful estimates of pasture functional-group composition when regressed against hand-separation as the reference method. Across the three functional groups evaluated (grass, forb, and weed), point-step transects produced greater single-predictor R^2 than digital image analysis for two of three comparisons (grass and forb), with the strongest agreement observed for the forb fraction ($R^2 = 0.75$; near 1:1 slope). Two-predictor models that combined transect and image inputs improved fit for grass and weed estimation but offered no improvement for forb estimation, where the transect explained most of the variation in hand-separated estimations of forb mass. All single- and two-predictor models were significant ($P < 0.0001$).

For Southeastern pasture diversification research, where accurate estimation of forb biomass is a variable of great interest, point-step transects can be recommended as a labor-efficient, non-destructive method that will produce a reliable approximation of hand-separated forb mass under the conditions of this experiment. Digital image analysis is still a viable alternative, but it should be recognized that overhead photos may underestimate grass at higher fractions due to canopy overlap. Future work could improve image method performance by incorporating machine learning classification to reduce observer dependence. This work should also be replicated across additional site-years under a variety of environmental and management conditions to evaluate the robustness of the methods.

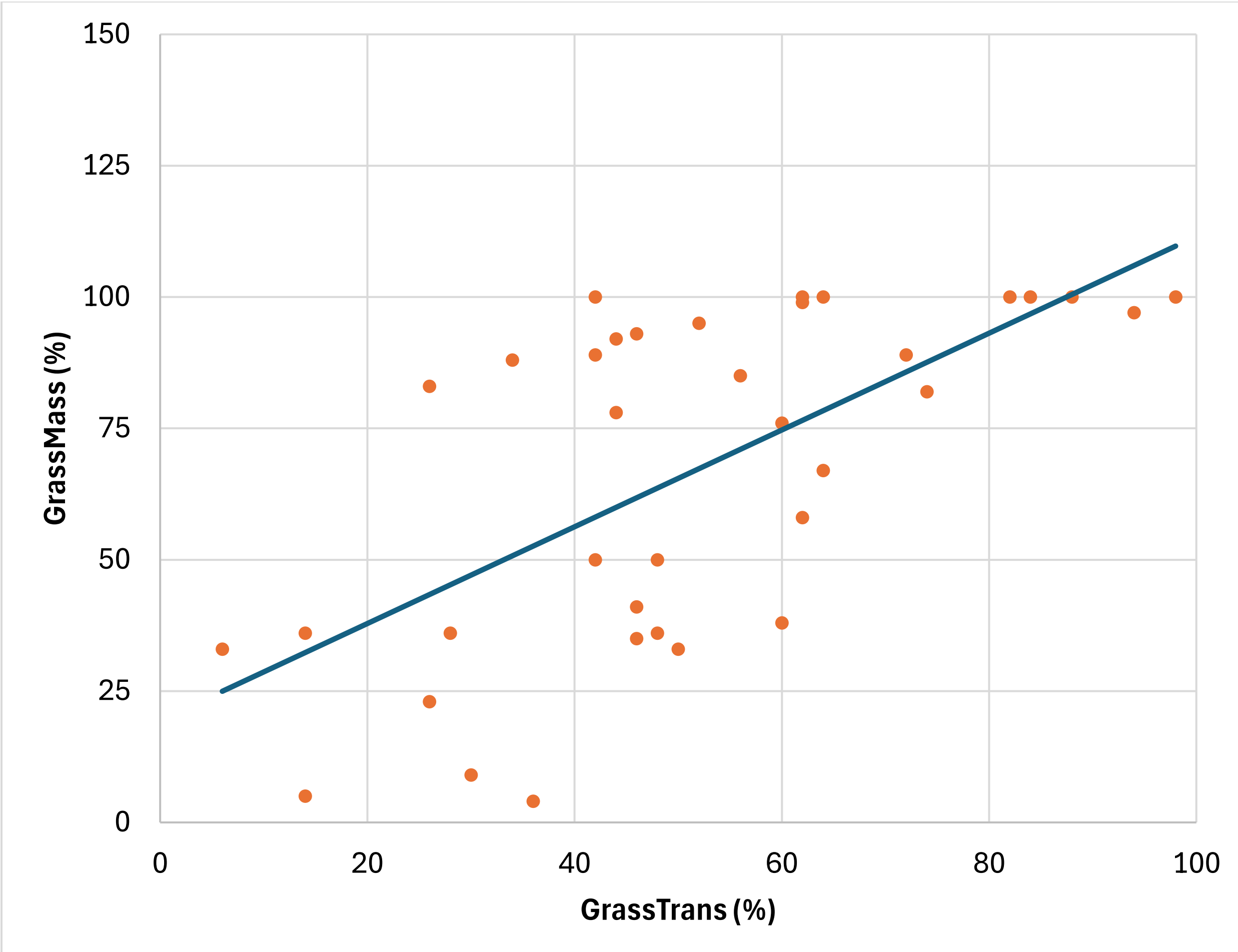

Figure 3.1. Relationship between hand-separated grass mass and point-step transect grass cover for 36 paired observations from the Sand Mountain Research and Extension Center.

GrassMass = grass fraction of standing biomass determined by hand-separation of clipped quadrat samples (% dry matter basis); GrassTrans = grass cover fraction determined by point-step transect (% of points classified as grass). The solid line represents the simple linear regression of GrassTrans on GrassMass (regression line: y = 0.92x + 19.4; R^2 = 0.41; $P < 0.0001$).

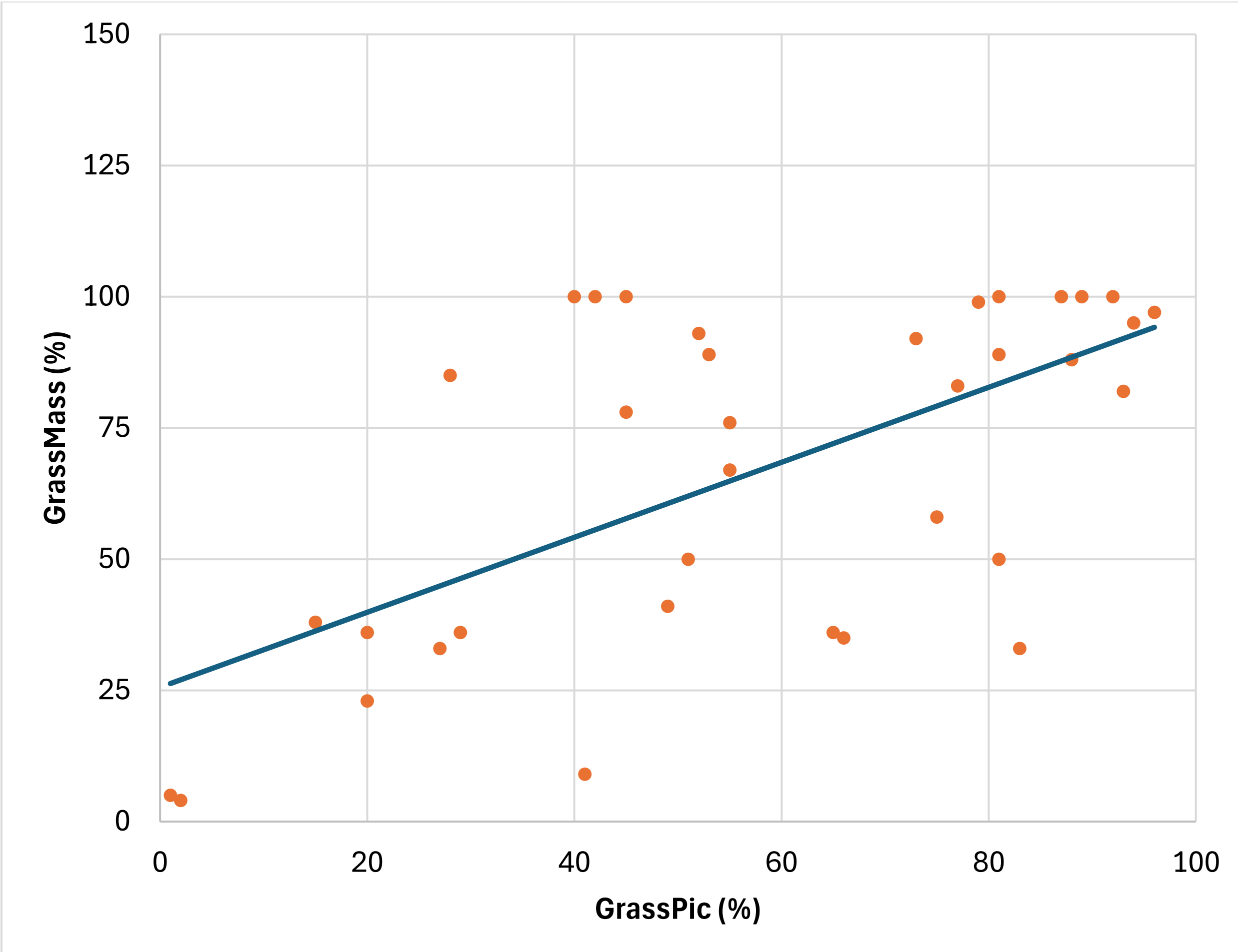

Figure 3.2. Relationship between hand-separated grass mass and digital image grass cover for 36 paired observations from the Sand Mountain Research and Extension Center.

GrassMass = grass fraction of standing biomass determined by hand-separation of clipped quadrat samples (% dry matter basis); GrassPic = grass cover fraction determined by digital image analysis (% of classification points identified as grass). The solid line represents the simple linear regression of GrassPic on GrassMass (regression line: y = 0.71x + 25.5; $R^2 = 0.38$; $P < 0.0001$).

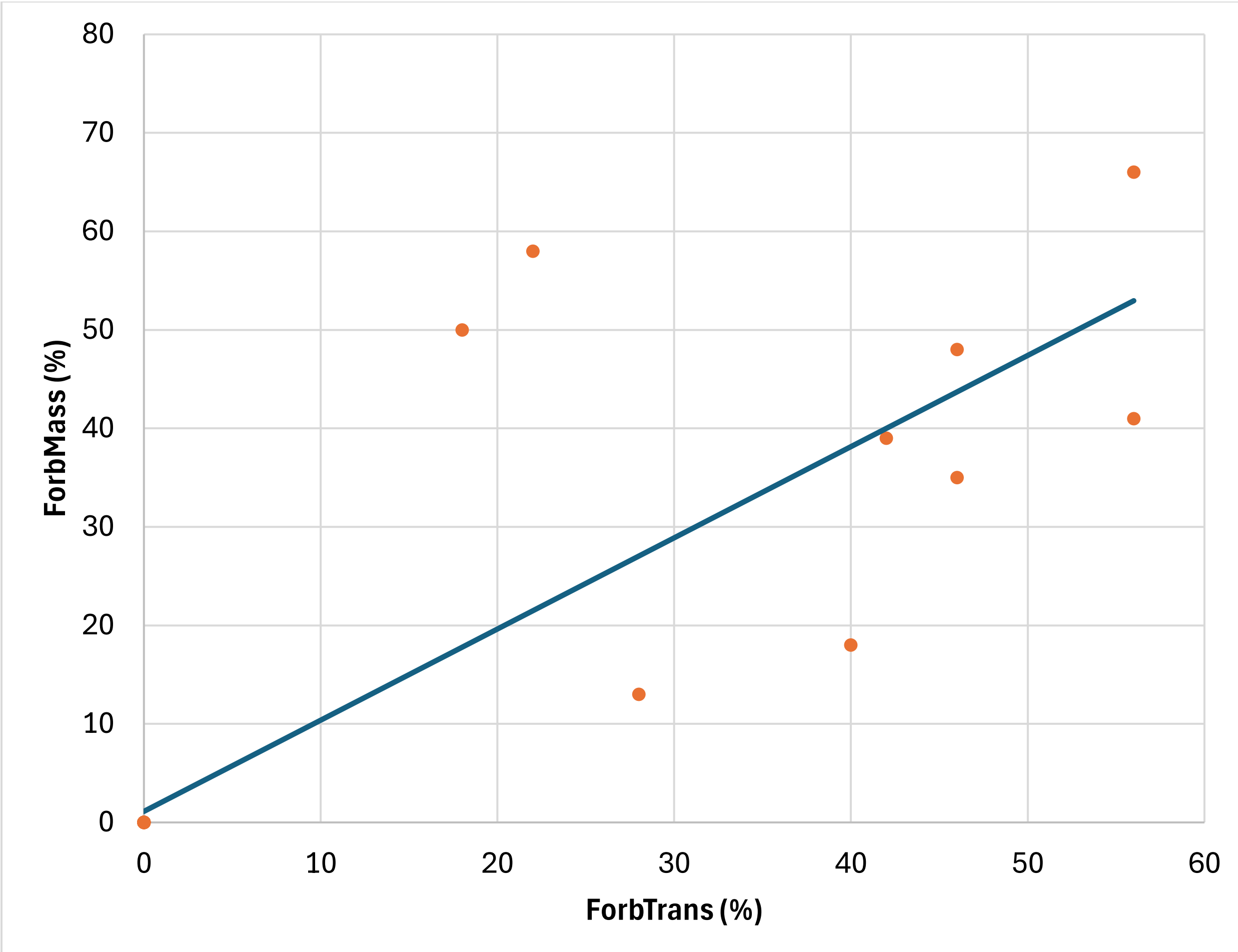

Figure 3.3. Relationship between hand-separated forb mass and point-step transect forb cover for 36 paired observations from the Sand Mountain Research and Extension Center.

ForbMass = forb fraction of standing biomass determined by hand-separation of clipped quadrat samples (% dry matter basis); ForbTrans = forb cover fraction determined by point-step transect (% of points classified as forb). The solid line represents the simple linear regression of ForbTrans on ForbMass (regression line: y = 0.93x + 0.01; R^2 = 0.75; P < 0.0001).

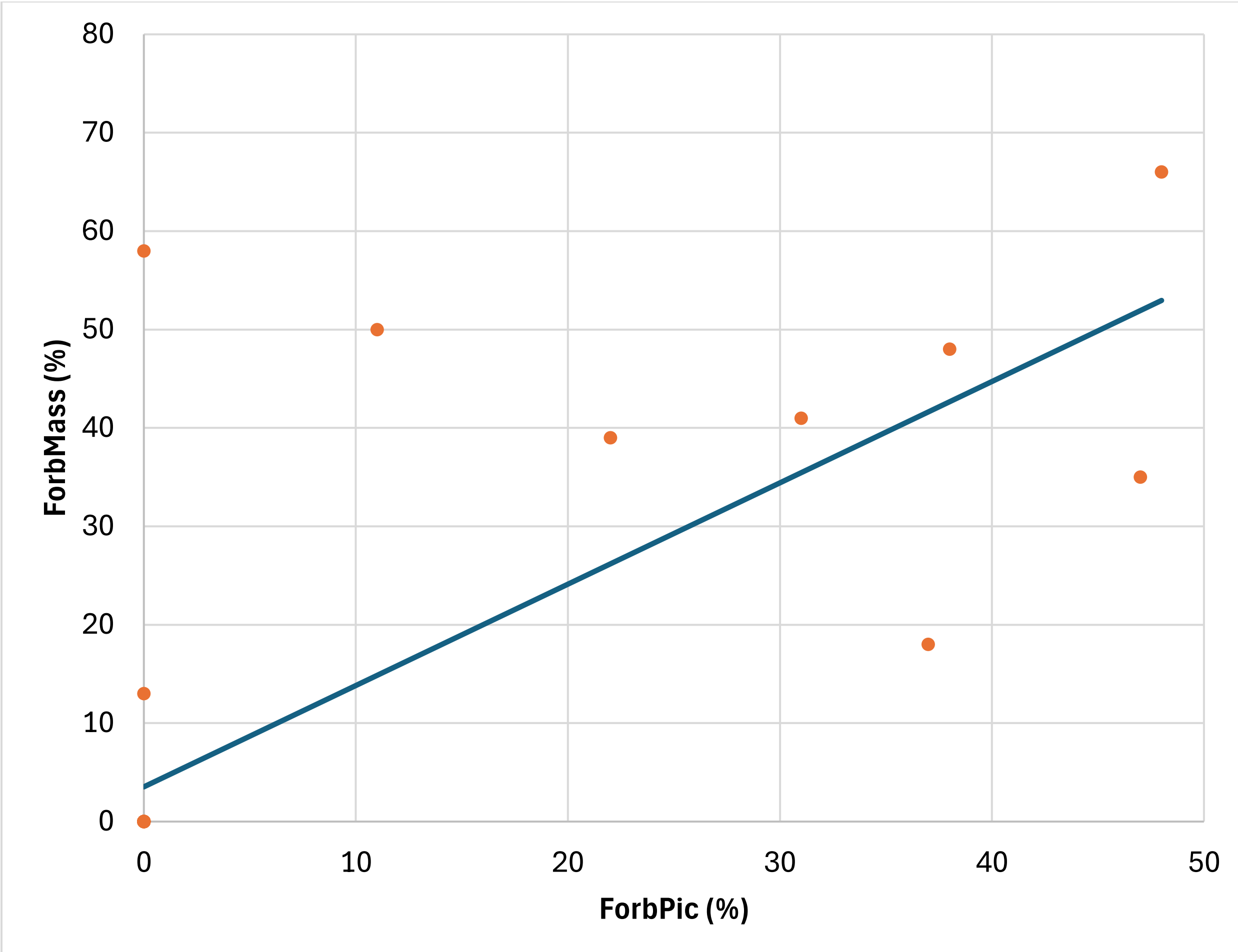

Figure 3.4. Relationship between hand-separated forb mass and digital image forb cover for 36 paired observations from the Sand Mountain Research and Extension Center.

ForbMass = forb fraction of standing biomass determined by hand-separation of clipped quadrat samples (% dry matter basis); ForbPic = forb cover fraction determined by digital image analysis (% of classification points identified as forb). The solid line represents the simple linear regression of ForbPic on ForbMass (regression line: y = 1.02x + 0.03; R^2 = 0.56; $P < 0.0001$).

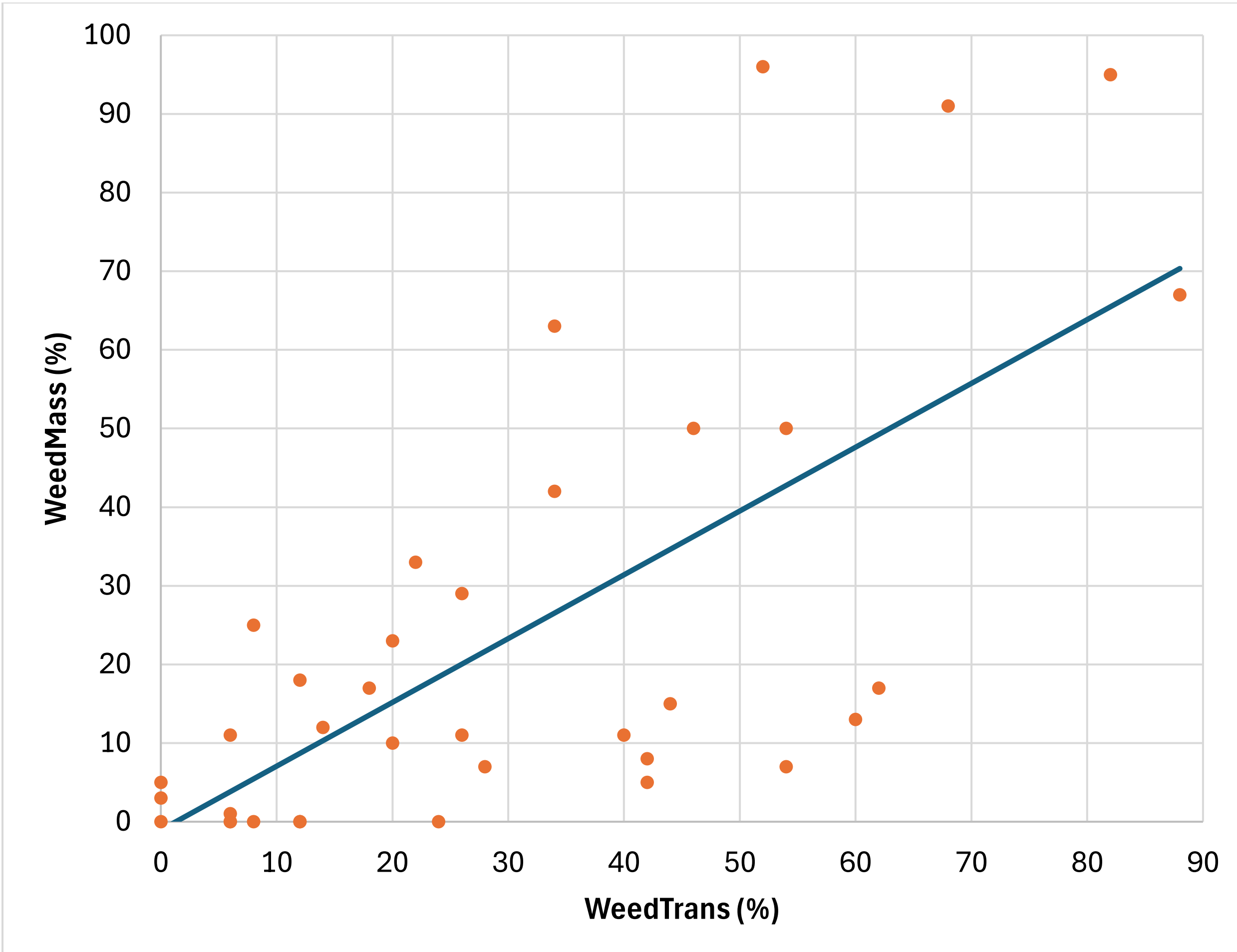

Figure 3.5. Relationship between hand-separated weed mass and point-step transect weed cover for 36 paired observations from the Sand Mountain Research and Extension Center.

WeedMass = weed fraction of standing biomass determined by hand-separation of clipped quadrat samples (% dry matter basis); WeedTrans = weed cover fraction determined by point-step transect (% of points classified as weeds). The solid line represents the simple linear regression of WeedTrans on WeedMass (regression line: $y = 0.81x - 0.01$; $R^2 = 0.47$; $P < 0.0001$).

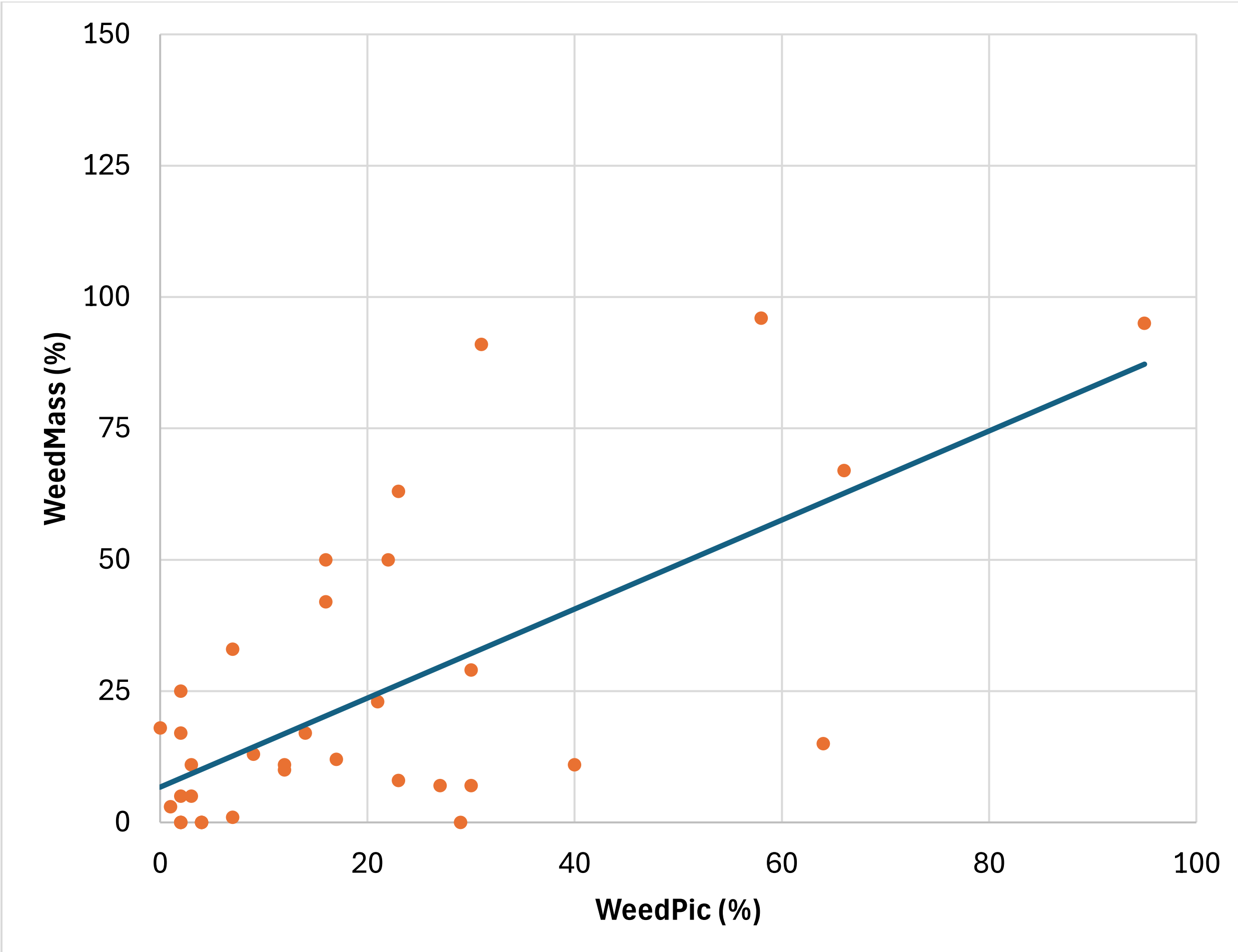

Figure 3.6. Relationship between hand-separated weed mass and digital image weed cover for 36 paired observations from the Sand Mountain Research and Extension Center.

WeedMass = weed fraction of standing biomass determined by hand-separation of clipped quadrat samples (% dry matter basis); WeedPic = weed cover fraction determined by digital image analysis (% of classification points identified as weeds). The solid line represents the simple linear regression of WeedPic on WeedMass (regression line: y = 0.85x + 0.07; R^2 = 0.44; $P < 0.0001$).

Implications of Research

Across three complementary studies, hairy vetch (*Vicia villosa* Roth) emerged as the only species to consistently establish and produce a measurable shift in sward composition under Deep South conditions. Neither animal performance or health differed among treatments. These findings establish a foundation for future work on the methane mitigation, soil nitrogen, and ecosystem-service outcomes that justify pursuing pasture diversification at scale.

Hairy vetch can also be recommended as a viable low-input option for diversifying existing Southeastern tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.) pastures. The approach evaluated in this thesis fits within the management context of a typical cow-calf or stocker operation within the region. Producers should expect year-to-year variability in establishment success and may not be able to expect immediate per-animal weight-gain advantages. The benefits of hairy vetch integration are found at the system level through improved sward composition and forage nutritive value rather than as immediate animal productivity gains. Brassica integration under the conditions evaluated here was unreliable and cannot be recommended without further research on potential establishment success with management intensification. For producers operating under cost constraints, hairy vetch integration can be a step toward pasture diversification that does not require substantial investment. The approach used in these studies was accessible and supports widespread adoption potential.

References

Aiken, G. E., J. R. Strickland, M. L. Looper, L. P. Bush, and F. N. Schrick. 2009. Hemodynamics are altered in the caudal artery of beef heifers fed different ergot alkaloid concentrations. J. Anim. Sci. 87(6):2142–2150.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83(7):1598–1624.

Ball, D. M., M. Collins, G. Lacefield, N. Martin, D. Mertens, K. Olson, D. Putnam, D. Undersander, and M. Wolf. 2001. Understanding forage quality. American Farm Bureau Federation Publication 1(01):1–15.

Ball, D. M., C. S. Hoveland, and G. D. Lacefield. 2015. Southern forages. 5th ed. International Plant Nutrition Institute, Norcross, GA.

Barry, T. N. 2013. The feeding value of forage brassica plants for grazing ruminant livestock. Anim. Feed Sci. Technol. 181(1):15–25.

Baxter, L. L., C. P. West, C. P. Brown, and P. E. Green. 2017a. Comparing nondestructive sampling techniques for predicting forage mass in alfalfa–tall wheatgrass pasture. Agron. J. 109(5):2097–2106.

Baxter, L., C. West, P. Brown, and P. Green. 2017b. Nondestructive determination of legume content in grass–legume pastures. Crop Forage Turfgrass Manag. 3(1):cftm2016.12.0088.

Beauchemin, K., M. Kreuzer, F. O'Mara, and T. McAllister. 2008. Nutritional management for enteric methane abatement: a review. Aust. J. Exp. Agric. 48(2):21–27.

Beauchemin, K. A., T. A. McAllister, and S. M. McGinn. 2009. Dietary mitigation of enteric methane from cattle. CABI Rev. 1–18.

Belesky, D. P., J. M. Fedders, K. E. Turner, and J. M. Ruckle. 1999. Productivity, botanical composition, and nutritive value of swards including forage chicory. Agron. J. 91(3):450–456.

Bellangue, D., J. Barney, M. Flessner, J. Kubesch, M. O'Rourke, B. Tracy, and J. L. Reid. 2024. Site preparation and planting strategies to improve native forb establishment in pasturelands. Agronomy 14(11):2676.

Beuselinck, P. R., J. H. Bouton, W. O. Lamp, A. G. Matches, M. H. McCaslin, C. J. Nelson, L. H. Rhodes, C. C. Sheaffer, and J. J. Volenec. 1994. Improving legume persistence in forage crop systems. J. Prod. Agric. 7(3):311–322.

Booth, D. T., S. E. Cox, and R. D. Berryman. 2006. Point sampling digital imagery with 'SamplePoint'. Environ. Monit. Assess. 123(1):97–108.

Browning, R., Jr. 2003. Tall fescue endophyte toxicosis in beef cattle: clinical mode of action and potential mitigation through cattle genetics. Beef Improvement Federation 35th Annual Meeting Proceedings.

Bunton, G., Z. Trower, and K. W. Bradley. 2020. Relationships between soil, forage, and grazing parameter effects on weed incidence in Missouri pastures. Weed Technol. 34(3):408–415.

Burt, J., L. Baxter, and J. J. Tucker. 2022. Evaluating nondestructive forage sampling techniques in alfalfa–bermudagrass mixtures in the southeastern United States. Crop Forage Turfgrass Manag. 8(2):e20194.

Carlassare, M., and H. Karsten. 2003. Species population dynamics in a mixed pasture under two rotational sward grazing height regimes. Agron. J. 95(4):844–854.

Daubenmire, R. F. 1959. Canopy coverage method of vegetation analysis. Northwest Sci. 33:39–64.

De Ruiter, J., D. R. Wilson, S. Maley, A. L. Fletcher, T. Fraser, W. R. Scott, S. Berryman, A. Dumbleton, and W. Nichol. 2009. Management practices for forage brassicas. Forage Brassica Development Group, Christchurch, New Zealand.

DeRamus, H. A., T. C. Clement, D. D. Giampola, and P. C. Dickison. 2003. Methane emissions of beef cattle on forages: efficiency of grazing management systems. J. Environ. Qual. 32(1):269–277.

Dillard, S. L., A. N. Hafla, A. I. Roca-Fernández, A. F. Brito, M. D. Rubano, and K. J. Soder. 2017. Effect of feeding warm-season annuals with orchardgrass on ruminal fermentation and methane output in continuous culture. J. Dairy Sci. 100(2):1179–1188.

Dillard, S. L., E. D. Billman, and K. J. Soder. 2020. Assessment of forage brassica species for dairy and beef-cattle fall grazing systems. Appl. Anim. Sci. 36(2):157–166.

Dobson, J. W., C. D. Fisher, and E. R. Beaty. 1976. Yield and persistence of several legumes growing in tall fescue. Agron. J. 68(1):123–125.

Eugène, M., K. Klumpp, and D. Sauvant. 2021. Methane mitigating options with forages fed to ruminants. Grass Forage Sci. 76(2):196–204.

Evans, R. A., and R. M. Love. 1957. The step-point method of sampling — a practical tool in range research. J. Range Manage. 10(5):208–212.

Fahey, G. C., Jr., ed. 1994. Forage quality, evaluation, and utilization. American Society of Agronomy, Madison, WI.

Franzluebbers, A. J., and M. H. Poore. 2021. Tall fescue management and environmental influences on soil, surface residue, and forage properties. Agron. J. 113:1–18.

Freeman, S. R., M. H. Poore, and J. T. Green. 2019. Enhancing the adoption of stockpiling tall fescue and managed grazing. Forage Grazinglands.

Galyean, M. L., and P. J. Defoor. 2003. Effects of roughage source and level on intake by feedlot cattle. J. Anim. Sci. 81(14_suppl_2):E8–E16.

Gere, J. I., A. Restovich, A. Vanzin, V. Faverín, M. Pighín, R. Bualó, V. González, R. Cardín, S. Crelier, C. Faverín, and V. T. Tieri. 2024. Enteric methane emission from cattle grazing systems with cover crops and legume–grass pasture. Animals 14(15):2230.

Grace, C., M. B. Lynch, H. Sheridan, B. Lott, R. Fritch, and T. M. Boland. 2018. The effect of increasing pasture species on herbage production, chemical composition, and utilization under intensive sheep grazing. Grass Forage Sci. 73(4):852–864.

Gresta, F., A. Oteri, S. Scordia, A. Costale, C. Cosentino, M. Anastasi, and M. Allegra. 2023. White lupin (*Lupinus albus* L.), an alternative legume for animal feeding in the Mediterranean area. Agriculture 13(2):434.

Gunter, P. A., B. W. Smith, K. J. Soder, S. L. Dillard, A. F. Brito, and K. M. Daniel. 2019. Nitrogen delivery method and monensin effects on productivity, nutritive value, and animal performance of grazing dairy cattle. Anim. Feed Sci. Technol. 257:114270.

Guretzky, J. A., D. R. Mooney, and J. J. McGrew. 2012. Adaptation of annual forage legumes in the southern Great Plains. Crop Sci. 52(5):2331–2340.

Harris, W. 1968. Some factors affecting change in botanical composition in a ryegrass–white clover pasture. N.Z. J. Agric. Res. 11(1):15–38.

Henson, M. B. 2025. Evaluation of the effects of grass–legume mixtures on beef cattle in the Deep South. Ph.D. Diss. Auburn University, Auburn, AL.

Herbin, A. L., G. M. Bertelsen, K. A. Albrecht, and M. Liebig. 2020. Weed effects on the establishment and nutritive value of pastures with different botanical compositions. Agron. J. 112(6):4827–4839.

Hornick, J. L., C. Van Eenaeme, O. Gérard, I. Dufrasne, and L. Istasse. 2000. Mechanisms of reduced and compensatory growth. Domest. Anim. Endocrinol. 19(2):121–132.

Hoveland, C. S. 1981. Seeding legumes into tall fescue sod. Auburn University, Alabama Agricultural Experiment Station, Auburn, AL.

Hume, D. E., and J. L. Brock. 1997. Morphology of tall fescue (*Festuca arundinacea*) and perennial ryegrass (*Lolium perenne*) plants in pastures under sheep and cattle grazing. J. Agric. Sci. 129(1):19–31.

Interrante, S. M., J. T. Biermacher, J. K. Rogers, B. C. Tomar, T. J. Butler, and J. P. Muir. 2012. Production and economics of steers grazing tall fescue with annual legumes or fertilized with nitrogen. Crop Sci. 52(3):1414–1422.

Iqbal, M. F., Y. F. Cheng, W. Y. Zhu, and B. Zeshan. 2008. Mitigation of ruminant methane production: current strategies, constraints and future options. World J. Microbiol. Biotechnol. 24:2747–2755.

Jaramillo, D. M., J. C. Sheridan, J. C. Dubeux, Jr., E. R. S. Santos, M. Ruiz-Moreno, and N. DiLorenzo. 2021. Enhancing the sustainability of temperate pasture systems through more diverse seed mixtures. Crop Forage Turfgrass Manag. 7(2):e20121.

Johnson, K. A., A. M. Sproul, S. J. Boddicker, K. J. Hand, B. J. Heins, and W. P. Weiss. 2019. Feeding strategies and shade type for growing cattle grazing endophyte-infected tall fescue pastures during summer in the Pacific Northwest. Prof. Anim. Sci. 35(6):540–548.

Jones, R. M., and J. N. G. Hargreaves. 1979. Improvements to the dry-weight-rank method for measuring botanical composition. Grass Forage Sci. 34(3):181–189.

Kallenbach, R. L. 2015. Describing the dynamic: measuring and assessing the value of plants in the pasture. Crop Sci. 55(6):2531–2539.

Klotz, J. L. 2015. Activities and effects of ergot alkaloids on livestock physiology and production. Toxins 7(8):2801–2821.

Leep, R. H., J. A. Andresen, and J. R. Jeranyama. 2003. Steps to successful no-till establishment of forages. Michigan State University Extension, East Lansing, MI.

Li, G. D., G. M. Lodge, J. M. Brennan, and W. J. Pluske. 2025. Agronomic performance, herbage quality, methane yield and methane emission potential of pasture mixtures. Agriculture, Ecosystems & Environment 380:109042.

Louhaichi, M., M. M. Borman, and D. E. Johnson. 2001. Spatially located platform and aerial photography for documentation of grazing impacts on wheat. Geocarto Int. 16(1):65–70.

MacAdam, J. W., and J. J. Villalba. 2015. Beneficial effects of temperate forage legumes that contain condensed tannins. Agriculture 5(3):475–491.

MacAdam, J. W., L. Solomon, T. C. Griggs, and S. Boyer. 2022. Increased nitrogen retention and reduced methane emissions of beef cattle grazing legume vs. grass irrigated pastures. Animals 12(3):304.

Machado, J. M., F. C. F. Lopes, P. Henrique Mazza Rodrigues, and L. G. R. Pereira. 2022. Strategies to mitigate the emission of methane in pastures: enteric methane: a review. Aust. J. Crop Sci. 16(4):541–550.

Marten, G. C., C. C. Sheaffer, and D. L. Wyse. 1987. Forage nutritive value and palatability of perennial weeds. Agron. J. 79(6):980–986.

McCurdy, J. D. 2013. Differential response of four *Trifolium* species to common broadleaf herbicides: implications for mixed grass–legume swards. Weed Technol. 27(1):123–128.

McMurphy, W. E., M. E. Ahring, and D. F. Stephens. 1990. Steer performance on tall fescue pasture. J. Prod. Agric. 3(1):113–116.

Min, B. R., S. Solaiman, H. F. Waldrip, D. Parker, R. W. Todd, and D. Brauer. 2020. Dietary mitigation of enteric methane emissions from ruminants: a review of plant tannin mitigation options. Anim. Nutr. 6(3):231–246.

Mitchell, CC. & Huluka, G. 2012. Nutrient recommendation tables for Alabama crops.

Mortenson, M. C., G. E. Schuman, L. J. Ingram, V. Nayigihugu, and B. W. Hess. 2005. Forage production and quality of a mixed-grass rangeland interseeded with *Medicago sativa* ssp. *falcata*. Rangel. Ecol. Manag. 58(5):505–513.

Omokanye, A., A. Lardner, A. Iwaasa, T. McAllister, and S. Acharya. 2021. Alternative forage feeds for beef cattle in northwestern Alberta, Canada: forage yield and nutritive value of forage brassicas and forage cereals. J. Agric. Sci. 159(5–6):399–410.

Pavao-Zuckerman, M. A., J. C. Waller, T. Ingle, and H. A. Fribourg. 1999. Methane emissions of beef cattle grazing tall fescue pastures at three levels of endophyte infestation. J. Environ. Qual. 28(6):1963–1969.

Mannetje, L. 't, and K. P. Haydock. 1963. The dry-weight-rank method for the botanical analysis of pasture. Grass Forage Sci. 18(4):268–275.

Poore, M. H., and M. E. Drewnoski. 2010. Utilization of stockpiled tall fescue in winter grazing systems for beef cattle. Prof. Anim. Sci. 26(2):142–149.

Poore, M. H., D. W. Hancock, and J. C. Burns. 2023. Economics and other factors affecting the adoption of novel endophyte technology. Forages 2:25–35.

Prigge, J. L., L. M. Smith, B. F. Tracy, and J. L. Reid. 2024. Native forbs provide pollinator resources and improve forage nutrient composition in cool-season pastures. Rangel. Ecol. Manag.

Prigge, J. L., L. M. Smith, B. F. Tracy, and J. L. Reid. 2025. Interseeded native forbs resilient under variable grazing regimen. Agron. J.

Puchala, R., B. R. Min, A. L. Goetsch, and T. Sahlu. 2005. The effect of a condensed tannin-containing forage on methane emission by goats. J. Anim. Sci. 83(1):182–186.

Rayburn, E. B. 2014. Measuring legume content in pastures using digital photographs. Forage Grazinglands 12.

Rayburn, E. B., E. R. McCarty, and W. M. Brown. 2021. Validating a simple mechanistic model that describes weather impact on pasture growth. Agron. J. 113(3):2533–2546.

Reid, R. L., G. A. Jung, J. M. Cox-Ganser, B. F. Rybeck, and E. C. Townsend. 1994. Evaluation of brassicas in grazing systems for sheep: I. Quality of forage and animal performance. J. Anim. Sci. 72(7):1823–1831.

Ridley, J. R., S. R. Wilkinson, and P. R. Eberhart. 1963. Relationship of animal preference to botanical composition of irrigated pasture. Agron. J. 55(6):512–514.

Roberts, C., and J. Andrae. 2004. Tall fescue toxicosis and management. Crop Manage. 3(1):1–18.

Rook, A. J., B. Dumont, J. Isselstein, K. Osoro, M. F. WallisDeVries, G. Parente, and J. Mills. 2003. Grazing and pasture management for biodiversity benefit. Biol. Conserv. 119(2):137–150.

Sanderson, M. A., R. H. Skinner, D. J. Barker, G. R. Edwards, B. F. Tracy, and D. A. Wedin. 2004. Plant species diversity and management of temperate forage and grazing land ecosystems. Crop Sci. 44(4):1132–1144.

Sanderson, M. A., S. C. Goslee, K. J. Soder, R. H. Skinner, B. F. Tracy, and A. Deak. 2007. Plant species diversity, ecosystem function, and pasture management — a perspective. Can. J. Plant Sci. 87(3):479–487.

Sanderson, M. A., R. H. Skinner, S. C. Goslee, R. M. Sullivan, and K. J. Soder. 2016. Productivity, botanical composition, and nutritive value of commercial pasture mixtures. Agron. J. 108(1):93–100.

Sather, B. C., J. D. Sevy, S. M. Petersen, and M. K. Petersen. 2013. Evaluation of cattle grazing distribution in response to weed and legume removal. Range. Beef Cow Symp. Proc. 326.

Siziba, C. 2026. Suitability of secondary metabolite-producing forbs for southeastern beef cattle production systems. M.S. Thesis. Auburn University, Auburn, AL.

Skinner, R. H. 2010. Reestablishing chicory into multi-species perennial pastures. Forage Grazinglands.

Sleper, D. A., and C. P. West. 1996. Tall fescue. In: L. E. Moser, D. R. Buxton, and M. D. Casler, ed. Cool-Season Forage Grasses. Agron. Monogr. 34. ASA, CSSA, SSSA, Madison, WI. p. 471–502.

Soder, K. J., A. J. Rook, M. A. Sanderson, and S. C. Goslee. 2007. Interaction of plant species diversity on grazing behavior and performance of livestock grazing temperate region pastures. Crop Sci. 47(1):416–425.

Sollenberger, L. E., and E. S. Vanzant. 2011. Interrelationships among forage nutritive value and quantity and individual animal performance. Crop Sci. 51(2):420–432.

Sollenberger, L. E., and M. Collins. 2017. Legumes for southern areas. In: M. Collins, C. J. Nelson, K. J. Moore, and R. F. Barnes, ed. Forages, Vol. 1: An Introduction to Grassland Agriculture. 7th ed. Wiley-Blackwell, Hoboken, NJ. p. 191–214.

Stefanski, E. P., E. M. Henry, and J. R. Caradus. 2010. Effects of sowing rate and grazing management of forage rape (*Brassica napus*) on grazing behavior and herbage utilization by dairy cattle. Anim. Prod. Sci. 50(11):1024–1031.

Stevens, R., and S. B. Monsen. 1981. Interseeding and transplanting shrubs and forbs into grass communities. Range. 3(2):55–58.

Strickland, J. R., M. L. Looper, J. C. Matthews, C. F. Rosenkrans, Jr., M. D. Flythe, and K. R. Brown. 2011. Board-invited review: St. Anthony's Fire in livestock: causes, mechanisms, and potential solutions. J. Anim. Sci. 89(5):1603–1626.

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

Templeton, W. C., Jr., and T. H. Taylor. 1975. Performance of bigflower vetch seeded into bermudagrass and tall fescue swards. Agron. J. 67(1):69–72.

Tidmarsh, C. E. M., and C. M. Havenga. 1955. The wheel-point method of survey and measurement of semi-open grasslands and Karoo vegetation in South Africa. Bot. Surv. Mem. 29. Government Printer, Pretoria, South Africa.

Tracy, B. F., and M. A. Sanderson. 2004. Forage productivity, species evenness and weed invasion in pasture communities. Agric. Ecosyst. Environ. 102(2):175–183.

Trotta, R. J., S. L. Dillard, K. J. Soder, B. J. Heins, A. N. Hafla, and A. F. Brito. 2018. Effects of source and level of dietary energy supplementation on in vitro digestibility and methane production of grass-based diets. Anim. Feed Sci. Technol. 244:65–75.

USDA–BLM–NRCS. 1999. Sampling vegetation attributes. Interagency Technical Reference 1734-4. U.S. Department of the Interior, Bureau of Land Management, National Applied Resource Sciences Center, Denver, CO.

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. 2nd ed. Cornell University Press, Ithaca, NY.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74(10):3583–3597.

Vibart, R. E., C. R. Tavares, A. F. Brito, J. C. Burns, and W. A. Hartschuh. 2007. Varying endophyte status and energy supplementation of fresh tall fescue in continuous culture. Anim. Feed Sci. Technol. 137(3–4):231–248.

Villalba, J. J., I. Mereu, and S. A. Atwood. 2023. Integrating plant secondary metabolites and foraging behavior to enhance animal nutrition and health. Animals 13(7):1184.

Villalobos, L., and J. E. Brummer. 2015. Forage brassicas stockpiled for fall grazing: yield and nutritive value. Crop Forage Turfgrass Manag. 1(1):cftm2015.0166.

Vogel, K. P., J. F. Pedersen, S. D. Masterson, and J. J. Toy. 1999. Evaluation of a filter bag system for NDF, ADF, and IVDMD forage analysis. Crop Sci. 39(1):276–279.

Vogel, K. P., and R. A. Masters. 2001. Frequency grid — a simple tool for measuring grassland establishment. J. Range Manage. 54(6):653–655.

Warren, A. R., M. R. Schomberg, J. J. Halvorson, M. L. Cabrera, A. Bary, J. G. Hill, R. D. Lee, and T. M. Webster. 2023. Effects of soil amendments on hairy vetch no-till interseeded into a hayfield. Agron. J. 115(4):2197–2210.

Zajicek, J. M. 1986. Direct seeding of selected forbs into an established grassland. M.S. Thesis. Utah State University, Logan, UT.