

AMERICAN ELDERBERRY (*Sambucus nigra* subsp. *canadensis*) BIOMASS NUTRITIVE
VALUE ASSESSMENT AS A POTENTIAL FEED SOURCE FOR RUMINANT LIVESTOCK

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

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ABSTRACT

The perennial, fruit-bearing elderberry plant has been used by humans for a variety of purposes throughout recorded history. Elderberries ripen and are ready for harvest in mid to late summer. After harvest, the leaves stay on the plant into the fall. This study examines the viability of using the post-harvest, latent American elderberry (*Sambucus nigra* subsp. *canadensis*) leaf biomass as a feed source for ruminant livestock by analyzing its nutritive value as measured by dry matter, organic matter, neutral detergent fiber, acid detergent fiber, acid detergent lignin, and crude protein. Six cultivars of elderberry were analyzed over two seasons at two locations in Missouri. There was little consistency observed within plant nutrient content between the two locations when looking at individual cultivars, suggesting that growing conditions may have a larger effect on leaf biomass quality than cultivar traits. Similar to other forages, the analyses in this study showed an increase over the growing season in neutral detergent fiber content ($P < 0.001$), with the lowest concentration in May ($22.5 \pm 1.30\%$), and the highest in August ($27.3 \pm 1.31\%$). An expected decrease in crude protein content was observed over the growing season ($P < 0.001$), with the highest concentration in May ($24.8 \pm 0.50\%$), and the lowest in September ($10.6 \pm 0.54\%$). When compared to other tree fodders such as willow (*Salix* sp.) and mulberry (*Morus* sp.), elderberry leaf biomass displayed a comparable nutrient profile. Analysis of four cyanogenic glycosides known to be present in elderberry juice shows only dhurrin concentration differed between cultivars at both locations ($P = 0.007$). This study found that the sampled biomass had a maximum potential cyanogenic glycoside concentration of $117.4 \mu\text{g/g}$, but the average concentration was much lower at $12.1 \mu\text{g/g}$, which is well below the recommended

threshold for acute cyanide toxicity of 500 $\mu\text{g/g}$. This suggests that American elderberry leaf biomass likely presents a low risk of toxicity for ruminant livestock throughout the growing season when grown under study conditions.

I. Introduction & Literature Review

Introduction

Throughout history, the elderberry plant has been used by humans for a variety of purposes. A 2021 study examining human transition to agriculture showed that calcified dental plaque of human remains from the Late Mesolithic era (10,000 – 8,000 BCE) contained traces of elderberry DNA (Ottoni et al., 2021). In 400 BCE, Hippocrates, the father of modern medicine, referred to elderberry as his “medicine chest,” and other ancient Greek and Roman healers extolled the healing properties of the plant (Krawitz et al., 2011). There are ten species of elderberry spread across the globe. The two most common species utilized in commercial production (Byers et al., 2014) are American elderberry (*Sambucus nigra* subsp. *Canadensis*) which is native to North America, and black elderberry (*Sambucus nigra* subsp. *Nigra*) which is native to Eurasia. American elderberry is a fruit-bearing shrub in the Viburnaceae family that is native to eastern North America and most of the continental United States with the exception of Nevada, Utah, Idaho, Oregon, and Washington (Taylor, 2023).

The market for American elderberry is rapidly expanding in the United States and across the globe. According to financial reports on the burgeoning elderberry market, the global market for nutraceutical products containing elderberry extracts is currently valued at 1.1 billion US dollars and is projected to expand by 8.9% year over year and reach a valuation of 2.9 billion US dollars in the next decade (FMI, 2023). The primary drivers for this expansion are the use of berries and flowers in a variety of nutraceutical products due to the high levels of antioxidants contained in the plant (Byers, 2005; Cernusca et al., 2012). Elderberries are also used in food products such as juice, jelly, jam, liqueur (Byers

et al., 2014), and natural food coloring (Cernusca et al., 2018). American elderberry was included in the 2017 USDA Census of Agriculture for the first time and data from that census showed that approximately 790 acres of land in the United States were in elderberry production (Athearn et al., 2021). The newly released 2022 USDA Census of Agriculture shows that elderberry production acreage has increased over 100% since 2017 to 1682 acres (USDA, 2024).

Elderberries in the Northern hemisphere ripen and are ready for harvest from mid-August to mid-September. After harvest, the leaves stay on the plant well into the fall. The standard management practice for elderberry is to coppice the plants after the growing season in preparation for spring regrowth (Byers et al., 2014). An unanswered question in the body of academic research is whether the leaf material of elderberry plants can be used as a forage source for ruminant livestock following flower or berry harvest. There is an opportunity for elderberry producers to leverage this resource as supplemental feed for ruminant livestock and increase their total revenue potential.

Conventional Agriculture Externalities

Contemporarily, conventional agricultural systems are focused on creating commodity surpluses that can be used to generate profits and capital in accordance with modern economic theory (Brewer, 2008; Nicholls, 1963). Many negative externalities that affect the environment and society are associated with conventional agricultural practices. Eutrophication of surface waters and leaching of nutrients into groundwater are attributable to excessive fertilization of monoculture row cropping fields (Cassuto & Saville, 2012). Runoff and leaching problems can also be caused by faulty waste lagoons in industrial livestock production (Marks, 2001; Nicole, 2013). Yearly tillage and the

removal of living roots from crop fields outside of the growing season contribute to extensive loss of topsoil from wind and water erosion (Thaler et al., 2021). Frequent use of pesticides and failure to change pesticide modes of action in conventional agricultural systems has given rise to pesticide resistant insects (Reid & McKenzie, 2016; Wilen et al., 2022).

The concept of negative externalities has been known for a century and was pioneered in the 1920 book, *The Economics of Welfare*, by economist Arthur Pigou (Pigou, 1920). Since that time, there have been extensive studies on the valuation of positive and negative externalities created by the conventional agricultural sector (Baum & Kozera-Kowalska, 2019; Novikova, 2014; Tegmeier & Duffy, 2004). A recent study from the European Union (Pieper et al., 2020) that quantifies the external costs of greenhouse gas emissions in agriculture shows that it would be necessary to assign a surcharge for animal products (146%), dairy products (91%), and plant products (25%) in order to internalize the externality costs. Organic plant products would require the lowest surcharge at 6%. While the negative externalities of conventional agriculture have long been well known, the costs of those externalities are not commonly calculated as part of production costs or end consumer prices and are instead absorbed by the environment and society at large.

Agroecological Transitions through Agroforestry

Utilization of agroforestry practices can ameliorate many negative externalities associated with traditional production systems. In differentiation to conventional agriculture, the framework of agroforestry is defined as intensive land management that optimizes the physical, biological, ecological, economic, and social benefits arising from

biophysical interactions created when trees and/or shrubs are deliberately combined with crops and/or livestock (Garrett et al., 2022). A primary tenet within the discipline of agroforestry is that the agricultural practices should be intentional, intensive, integrative, and interactive (Garrett et al., 2022). While the academic discipline of agroforestry was created over 40 years ago, Indigenous communities across the globe have been utilizing similar integrated techniques to produce food, fiber, fuel, and livestock for millennia (Brandle et al., 2004; Olofson, 1983).

One of the primary goals of agroforestry systems is the diversification of agricultural ecosystems. Contrary to the streamlined efficiency of conventional agricultural production through monoculture and specialization, agroforestry practices are predominantly focused on biological diversity within production systems by integrating perennial trees and shrubs with crops and/or livestock (Santos et al., 2022). While iterations of modern agroforestry practices have been developed and utilized by Indigenous cultures for millennia and are still in use across the globe (Mpanga et al., 2021), the academic approach to agroforestry science and implementation is a relatively recent development. The initial agroforestry practice that was created and adopted into conventional agriculture in response to the Dust Bowl of the 1930s was the use of windbreaks to reduce the effects of wind erosion (Garrett et al., 2022). While the term agroforestry had not yet been coined, windbreaks follow the basic tenets of the discipline by stabilizing soil structure and reducing wind speeds with the integration trees in an agricultural setting. Other more recently employed practices such as silvopasture, alley cropping, riparian buffers, forest farming, and urban food forests meet the same criteria for multifunctional land use.

Economic Benefits

The integration of trees and shrubs into crop and livestock production systems attempts to provide economic, environmental, and social benefits for producers. Economically, agroforestry offers farm operators the opportunity to diversify their production outputs over the short, medium, and long term (Cialdella et al., 2023; Kassie, 2017). A row cropping or livestock operation can add nut or fruit trees and thereby create short-term revenue from the annual commodity (cash crop or livestock), medium-term revenue from the nut or fruit harvest, and long-term revenue through the sale of mature trees as saw logs for milled lumber and veneers. Diversification of outputs can protect producers from wild market swings and variance in seasonal production output (Boakye et al., 2021; Lin, 2011). In addition, the planting of trees for saw logs is a capital investment that can accrue wealth for subsequent generations or can increase the value of land in a future sale (Tankersly, 2006). The implementation of agroforestry practices can be used by producers as a marketing tool. By leveraging environmental and social benefits of agroforestry such as improved biodiversity, increased community engagement, and enhanced aesthetics, producers can command a price premium for their products by marketing directly to environmentally and socially conscious consumers and retailers (Rittenhouse, 2016). Diversity through agroforestry practices has the ability to make farming operations more stable and profitable for farm operators.

Environmental Benefits

The inclusion of trees and shrubs in crop and/or livestock production can create beneficial environmental outcomes (Pantera et al., 2021; Willmott et al., 2023). There are several means in which a diversified selection of species increases the long term

sustainability of an ecosystem. Soil health can be maintained or improved through enhanced nutrient cycling and by reducing soil loss due to erosion (Dollinger & Jose, 2018; Muchane et al., 2020). The root systems of trees and shrubs can increase infiltration and retention of water in the soil profile (Wang et al., 2015; Zhang et al., 2019). The inclusion of trees can also create microclimatic changes in an ecosystem, making conditions under the canopy cooler in the summer and warmer in the winter (Lasco et al., 2014). This reduces variability in upper soil horizon temperatures which further increases water retention (Kovacs et al., 2020) while allowing ample photosynthetically active radiation to reach the understory. This can facilitate equitable output of warm and cool season grasses when compared to open pasture (Castillo et al., 2020). The diversity of species in an agricultural system can also reduce the need of expensive, off-farm inputs such as fertilizers (Tsufac et al., 2022) and pesticides (Sakar et al., 2018) through increased nutrient cycling and the inclusion of “trap crops” as a preferred food source for problem pests. Chemical fertilizers can run off from fields and pastures, causing eutrophication of surface waters, and groundwater can be contaminated as excess nutrients leach through the soil profile into the water table (Henryson et al., 2020), but this non-point source pollution can be reduced by agroforestry buffers (Garrett et al., 2022). Beyond the direct environmental impact from the application of fertilizers and pesticides, the production process of these chemicals is very energy intensive. Ammonia synthesis for nitrogen fertilizer creates approximately 2% of worldwide greenhouse gas emissions (Daramola & Hatzell, 2023). As referenced previously, agroforestry can alleviate many of the negative environmental issues associated with

conventional agriculture, and these environmental benefits can be translated into economic benefits for producers by marketing to concerned consumers.

Social Benefits

There are several important social benefits derived from agroforestry practices. Agroforestry implementation by landowners is an excellent means to support land stewardship (Hastings et al., 2021). The extractive nature of agriculture can be managed sustainability while ensuring that future generations will have access to productive farmland. Agroforestry practices are known to be more labor intensive than conventional systems. While this labor cost can be considered a negative aspect of agroforestry, it also offers the opportunity to bolster rural communities by creating employment prospects and increased incomes in areas that have been socially depressed by the relocation of job opportunities into urban centers (Mukhlis et al., 2022). Past studies have shown that communities have a willingness to pay for aesthetic improvements created by agroforestry practices such as riparian buffers and silvopasture (Mercer et al., 2017; Shrestha & Alavalapati, 2004). Agroforestry also provides a platform for the acknowledgement and implementation of traditional ecological knowledge found in the land use systems of Indigenous cultures (Rossier & Lake, 2014). The inclusion of knowledge sourced outside of the western academic and agricultural sphere can help reduce social biases related to ethnicity and educational attainment. Increased adoption of agroforestry practices and the associated economic, environmental, and social benefits can set an example that encourages conventional producers to follow the lead of agroforesters.

Practices

Agroforestry is defined as, “intensive land-use management that optimizes the benefits (physical, biological, ecological, economic, social) from biophysical interactions created when trees and/or shrubs are deliberately combined with crops and/or livestock” (Garrett et al., 2022). There are four specific criteria—known as the four I’s—that must be met in order to differentiate agroforestry from conventional practices. First, the practices must be intentional. Agroforestry systems must be designed so that they yield multiple outputs that are managed as a whole unit instead of a singular focus on the subsequent parts. Second, the practices must be intensive. The practices are intensively managed to preserve their functionality through the use of labor inputs for cultivation, fertilization, irrigation, pruning, thinning and livestock management. Third, the practices must be integrated. The individual elements are combined into an integrated management system designed to achieve the goals of the producer. Finally, the practices must be interactive. Agroforestry actively seeks to manipulate and facilitate interactions among individual components of the system in order to produce multiple outputs while creating positive economic, environmental, and social benefits. There are six individual agroforestry practices that will be briefly described in the following section and all relevant information is sourced from the third edition of *North American Agroforestry: An Integrated Science and Practice* (Garrett et al., 2022).

Silvopasture

Silvopasture is the intentional combination of trees and/or shrubs with pasture based forages and rotationally grazed livestock production (Garrett et al., 2022). There are two distinct varieties of silvopasture. Plantation silvopasture refers to planting trees in

existing pasture while woodland silvopasture involves the thinning of existing tree stands to allow sunlight to reach the forages in the understory. The shade from trees improves animal welfare by lowering heat stress, reducing wind speeds, and moderating the microclimate under the canopy which lessens temperature variability. Additionally, the trees create short and long term revenue potential through the sale of fruit and nut crops as well as high value saw logs for lumber and veneers.

Alley Cropping

Alley cropping involves planting trees in rows with spacing in between to allow for the production of a cash crop and the necessary space for harvesting equipment (Garrett et al., 2022). Valuable hardwood and nut trees such as oak, walnut and pecan are generally grown in these systems. The associated cash crop and nut harvest can provide consistent yearly income while the trees mature to a point where they can be harvested and sold as valuable saw logs. An additional benefit to alley cropping is the alteration of the microclimate which allows for the cultivation of sensitive crops that would struggle in an open field.

Riparian Buffers

Riparian and upland buffers are strips of vegetation, consisting of trees, shrubs, grasses, and forbs that are designed to function as a single unit (Garrett et al., 2022). Riparian buffers are used to separate farmland and bodies of water. They can be used to reduce runoff from fields and pastures, stabilize eroded stream banks, provide shade to streams to reduce water temperature while producing profitable outputs. They also create habitat and travel corridors for terrestrial wildlife.

Windbreaks

Windbreaks are planted and managed rows of trees that were originally implemented in the United States in response to the Dust Bowl in the early 20th century as a means to slow wind speeds and reduce erosion (Garrett et al., 2022). They are still used for this purpose today. Windbreaks are also used to protect sensitive crops from damage. Much in the same manner as previously listed agroforestry practices, windbreaks also provide shelter for livestock and habitat for wildlife.

Forest Farming

Forest farming is the cultivation of high value specialty crops that thrive in the altered microclimate of a forest understory (Garrett et al., 2022). Species such as ginseng and log grown shiitake are ideal crops for this system as they are shade tolerant and perform well in these conditions. The trees that make up the canopy are managed as high value timber for lumber and veneers.

Urban Food Forests

Since the advent of the academic discipline of agroforestry, the five previous practices comprised the entirety of the list. Recently, a sixth system, urban food forests, was added to the list of official practices. “Urban food forests are defined as: 1) The intentional use of perennial food-producing plants to improve the sustainability and resilience of urban communities; 2) A food forest is an edible, perennial, polyculture system that is designed and managed to mimic multistory forest structures and to function like a natural, self-sustaining forest” (Garrett et al., 2022). Urban food forests can be a valuable tool in managing the challenges of sustainability, food insecurity, and poverty. It

can also increase community engagement through the shared responsibility of food production and can act as a gathering place for community driven events.

Limitations of Agroforestry

Time

A primary challenge for adopters of agroforestry practices is the inherent time lag between implementation and the harvest of tree products. Even the fastest maturing fruit trees will take a minimum of three years to produce a harvestable crop, with many varieties taking five years or more (Warmund, 2016). Nut trees can take more than ten years to produce a harvestable crop and many nut tree varieties are alternate bearing whereby they produce a heavy crop one year and a small crop the following year (Andersen & Shahid, 2022; Webber et al., 2022). The time lag from planting to harvest can be a significant loss of productive land for farmers and may limit enthusiasm for agroforestry adoption. If the focus of the agroforestry practices is to produce hardwood saw logs for lumber and veneers, the lag time from planting to harvest in a plantation style agroforestry system can take decades, if not generations (Zon et al., 1929). While the impact of this time lag can be lessened by the utilization of existing trees on marginal lands, production of hardwood saw logs can be viewed as a long term capital investment that will increase property value but will not generate revenue until some point in the distant future (Tankersly, 2006). Because of the delay in financial compensation for fruit, nut, and lumber products, it is important that profitable short term revenue generation can be achieved through annual or perennial cash crops and livestock sales.

Complexity and Training

As agricultural production has become more specialized and less diversified, farmers have developed specialized skill sets generally devoted to a select few species of plants or animals. Agroforestry requires a broad knowledge base of a multitude of plant and animal species as well as the competitive and facilitative interactions between those species (Kiyani et al., 2017). This can be a significant challenge for entrenched producers that have devoted their professional lives to mastering singular system management. A potential avenue to ameliorate these issues is to encourage farmers to partner with university based extension services (Scherr, 1991). In doing so, farmers can gain access to experts and valuable information that can assist in the transition to agroforestry production. An alternate means to increase agroforestry adoption is to reach out to new farmers who are inherently less specialized and inform them of the economic, environmental, and social benefits of agroforestry.

This training and knowledge not only apply to existing farmers, but also to some members of the extension community as well. Foresters generally dissuade livestock managers from grazing woodlands by stating that grazing can damage the value of timber stands. The primary reasons given for this damage are soil compaction, limited seedling regeneration, and interference with nutrient cycling (Mercker & Smith, 2019). While these are legitimate concerns in a continuous grazing system, properly managed rotational grazing and the protection of desired seedlings with tree cages (Byrnes et al., 2018; Lehmkuhler et al., 2003) can greatly reduce these concerns. Education and collaboration between producers and extension services will be vital for increasing the understanding and adoption of agroforestry.

Labor Inputs

Because agroforestry is an integrated system of production that requires a broad knowledge base in order to be effectively managed, it can necessitate labor inputs not normally associated with conventional production systems. While growing trees in a specifically forestry based application requires relatively minimal labor inputs after the initial planting, agroforestry production demands constant observation and adjustment (Smith et al., 2022). It is necessary to routinely evaluate the canopy cover of tree stands to continue ample production of cash crops and/or forages in the understory (Garrett et al., 2004). Additionally, the inclusion of rotationally grazed livestock will require frequent moves and more active management than would be needed in a timber or row cropping production system. In contrast with conventional agriculture practices, agroforestry utilizes increased human labor inputs for the establishment of trees and shrubs and the associated canopy maintenance necessary to ensure ample photosynthetically active radiation reaches the understory (Hoagland et al., 2010; McCrae, 2024). This can be expensive and thereby limit the acceptance and adoption of agroforestry by conventional producers. The expected return for these increased labor inputs is a price premium for differentiated agroforestry products (Cernusca et al., 2018). Further expansion of existing markets for agroforestry products will be helpful in alleviating producer concerns related to recouping labor costs. Overall, the limitations to agroforestry adoption can be communicated to conventional producers while also detailing the potential profitability increases through price premiums gained by meeting consumer demands for environmental and social responsibility.

Forage Quality

Utilization of high quality forages in an agroforestry-based livestock production system can increase productive capacity and profitability for producers while further alleviating the impact of the aforementioned limitations. Forage quality is a generalized, colloquial term that encompasses measurable metrics such as nutrient density, digestibility, palatability, intake, and anti-quality factors (Ball, 2001; Buxton et al., 1995; Coleman, 2003; Lee, 2018). Typically, there is a positive relationship between crude protein (CP) content and forage quality, and a negative relationship between forage quality and the fiber constituents in the forage (George et al., 2001; Randall et al., 2023). As forage plants mature into the reproductive stage of growth, energy resources are directed towards flower and seed production, which lowers available resources in other parts of the plant (Mazis et al., 2023). Additionally, as forage plants reach and progress through reproductive maturity, there is a corollary increase in fiber constituents, and the higher fiber content reduces forage quality (Buxton & Redfearn, 1997). There is a narrowing of leaf to stem ratio as the plant progresses through reproductive maturity. The lower digestibility of stems and the increased ratio of stem to leaf material leads to a decrease in overall forage quality. Environmental factors such as precipitation (Patton et al., 2007; Perotti et al., 2021), soil fertility (Horrocks & Vallentine, 1999; Ward, 1959), altitude (Escobar et al., 2020; Melo et al., 2022), temperature (Hart et al., 2022; Lee et al., 2017), and light intensity related to latitude (Kumar et al., 2022) can cause variable rates of maturation and quality in forage species.

Importance of Forage Quality

Average daily gain is a simple measure of the amount of weight an animal has gained over a specified period of time. An ideal average daily gain for stocker beef cattle is approximately two pounds per day (Hancock, 2011). While this number is easily achieved by voluntary intake of cool season forages during the spring flush of growth, maintaining these gains during summer and winter months when forage growth slows or ceases can be challenging. In order to provide livestock with quality forages throughout the entirety of the year, producers can employ a variety of strategies. During the summer, pastures planted with warm season grasses and legumes can create a nutritive forage base while cool season forages are dormant (Duiker & Williamson, 2019). During the fall, a late flush of cool season forages can occur. While this growth is not nearly as robust as spring growth, this late growth can be “stockpiled” for use as fresh forage throughout the fall and into the winter months (Hartman, 2021). When all fresh forages are exhausted, maintenance of a nutritive diet for ruminants will necessitate the introduction of stored feeds such as hay, silage, and grains. The utilization of these three strategies can ensure that average daily gains are relatively consistent throughout the entirety of the calendar year.

There are two differing calculation methods used to quantify the nutritive value of a given forage or feed supplement. The first of these, known as relative feed value (RFV), was developed in 1978 by the Forage Analysis Subcommittee of the Hay Marketing Task Force organized by the American Forage and Grassland Council (Rohweder et al., 1978). This metric is derived from ruminant dry matter intake (DMI) and digestible dry matter (DDM). The formula for relative feed value is as follows:

$$RFV = (DMI \times DDM) / 1.29$$

The value 1.29 was chosen to make full bloom alfalfa (*Medicago sativa*) the baseline forage with an RFV of 100. RFV was used as an industry standard until 2002 when the concept of relative feed quality (RFQ) was created (Moore & Undersander, 2002). The authors' reasoning for innovating this new formula was the inconsistent correlation between DMI and neutral detergent fiber (NDF). Relative feed quality utilizes total digestible nutrients (TDN) instead of DDM to create the formula:

$$RFQ = (DMI \times TDN) / 1.23$$

The authors state that there is a need for an alternative metric because RFV can give unacceptable estimations of voluntary DMI and TDN and is not always applicable across a broad range of forage types. The addition of RFQ did not invalidate RFV but added another tool for forage analysts to qualify the nutritive values of a wide array of forage types. While RFV and RFQ are generally used as marketing tools in stored forage sales, these indices can also be useful in evaluating unusual or atypical forages (Tassone et al., 2022).

An important aspect of milk production for pasture-based dairy operations is the availability of high quality forages. A University of Missouri report notes that the vital factors to consider when planning a dairy production system are forage quality, forage yield, forage distribution, and stand persistence (Kallenbach, 2014). Forage quality, as described previously, is a combination of voluntary DMI and DDM and/or TDN. Forage yield is the amount of expected forage produced per acre over the growing season while forage distribution refers to the times during a calendar where a given forage will produce acceptable levels of DMI and TDN. For instance, cool season grasses and legumes

distribute their growth peaks in the spring and fall while warm season forages exhibit a single growth spike in the hottest months of the summer (Ruh et al., 2018). Stand persistence is the ability of individual plants in a stand to persist year after year. This indicates the ability of a species to reseed itself if given the opportunity to go to seed. Stand persistence also refers to plants that do not reseed well but are long lived perennials that will survive year after year. Forage quality, forage yield, forage distribution, and stand persistence are all vital factors in producing a consistent forage base for livestock (Kallenbach, 2014).

In addition to the benefits of high quality forages for average daily gains and milk production, reproductive efficiency is enhanced through access to consistent, nutritive forages (Kerley, 2004; Mwangi et al., 2019). Pregnancy for cows creates a demand for a diet of up to 50% higher energy density than normal. Failure to maintain an intake of highly nutritive forages during pregnancy can result in reduced calf vigor, the inability to breed back the following season, and diminished lactation following pregnancy. Nutritive forages with consistent availability are the most important tool for livestock managers to operate an effective breeding program.

There are several anti-quality components of forages that will have a negative impact on overall livestock health and productivity. These anti-quality components consist of nutritional inadequacy, mineral deficiency, and toxicity (Gore Allen & Segarrs, 2001). The exact economic impact of deficient forages is difficult to exactly quantify, but losses to productive output, reproduction, and even death can be devastating for profit margins. Without the use of supplements, low quality forages will increase production time which further impacts economic returns (Llewellyn, 2012). As the quality of a given

forage will vary throughout the growing season, it is important that livestock managers are aware of potential nutrient deficiencies, and necessary adjustments to feed supplementation are implemented (Wiedmeier, 2001). When attempting to establish a novel ruminant feed source such as American elderberry, it is necessary to fully evaluate the importance of forage quality.

Nutrient Concentration

Structural carbohydrates are a primary nutritive component of forages that must necessarily be quantified when analyzing forage quality. These structural carbohydrates make up the cell wall of plant cells and are separated into three main categories as neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). The Van Soest method of fiber analysis for NDF involves the removal of all plant cell components such as protein, sugars, pectin, and lipids, but leaves behind cellulose, hemicellulose, and lignin (Van Soest et al., 1991). NDF is composed of hemicellulose, cellulose, and lignin, and it provides a proxy for the “bulkiness” of a forage. On average, cattle will consume between 1% and 3% of their total body weight per day, with that percentage gradually increasing as the quality of the forage increases (Belyea et al., 1993). Acid detergent fiber contains cellulose and lignin. The Van Soest method for ADF analysis removes the hemicellulose following NDF analysis with a mild sulfuric acid based detergent (Van Soest et al., 1991). There is an inverse relationship between ADF and total digestible nutrients (TDN), where higher ADF content results in lower TDN. Acid detergent lignin analysis removes cellulose, leaving only lignin which is essentially the woody part of a plant. It is considered to be indigestible and is passed through the rumen and expelled in the feces (Harlan, 1991). Typically, as the percent of NDF, ADF,

and ADL increases, the quality of the forage decreases (Belyea et al., 1993; George et al., 2001; Randall et al., 2023). The combination and analysis of these three structural carbohydrates can provide information on the overall quality of a forage, its nutrient density, and the amount of a forage that is expected to be consumed over time. It is vitally important that a livestock manager is aware of these structural carbohydrates when planning a high quality forage base.

Crude protein (CP) is composed of amino acids and is important for many functions in the body of ruminant livestock (Van Dung et al., 2013; Xia et al., 2018). In forage analysis, CP is a measurement of nitrogen in a forage. While CP is not reflective of the amount of actual protein contained in a given forage, as it also contains non-protein nitrogen, it provides a vital component for estimating forage quality. Both protein, which is used for growth, reproduction, and lactation, as well as non-protein nitrogen, which is used by bacteria for proper rumen function, are important for healthy and productive livestock (Cappellozza, 2013). Legumes generally contain higher percentages of CP than grasses and the addition of legumes into pasture grasses and stored forages can increase the CP content in a ruminants diet (Quintero-Anzueta et al., 2021; Solomon, 2022).

Forage Anti-Quality Factors

There are several factors that negatively affect forage quality and can cause decreased animal health, potentially even causing death. Many common plants such as jimsonweed (*Datura stramonium*) and poison hemlock (*Conium maculatum*) contain toxic alkaloids that have frequently killed livestock (Cortinovis & Caloni, 2015). Some plants contain secondary metabolites known as condensed tannins. Livestock are known to have palatability issues with plants containing condensed tannins because of their bitter

flavor (Besharati et al., 2022). While tannins are generalized as anti-quality factors, there is also evidence that tannins provide benefits such as reducing methane emissions generated through enteric fermentation (Naumann et al., 2017). Plants containing phytoestrogen can cause numerous issues when consumed by livestock (Adams, 1995; Wyse et al., 2022). These metabolites can interfere with the development and function of reproductive organs as well as causing dietary disorders that impact overall animal health. Cyanogenic glycosides (CNG) are found in many different plants. This compound is non-toxic until it is hydrolyzed into hydrogen cyanide (or prussic acid) during the digestion process. Acute cyanide toxicity restricts cellular respiration and can lead to rapid death (Allison, 2019; Cope, 2021). Johnsongrass (*Sorghum halepense*) and Sudangrass (*Sorghum bicolor*) are relatively common warm season grasses known to contain cyanogenic compounds (Giantin et al., 2024).

Considering each of the above factors can greatly increase the forage quality of livestock pastures and thereby increase animal performance, health, and welfare while improving economic outcomes for livestock producers.

Fodder Banks

The use of fodder banks to feed livestock is relatively commonplace globally in tropical small landholder systems. By planting fast growing perennial trees and shrubs for livestock consumption, small scale landowners are able to supplement their grazing management and remain productive throughout the year (Franzel et al., 2014; Idan et al., 2023; Kubkomawa, 2019). Fodder banks are generally planted along field edges or contours in the ground and can assist in erosion mitigation (Hà et al., 2012). Livestock

are either allowed to graze the plants directly or the fodder can be cut and taken to where the livestock are located.

The primary reason for utilizing fodder banks is the ability of livestock managers to meet the nutritional needs of their animals during the dry season. By planting fast growing perennial trees and shrubs, the deep tap roots of the plant can access groundwater that is unavailable to the more shallow roots of grasses and herbaceous plants (Kubkomawa, 2019). If the perennial trees and shrubs are allowed to properly mature in order to establish acceptable tap root depth, they will respond well to being cut back for supplemental feed. Several studies have examined tree fodders such as mulberry (Garcez et al., 2018; Yao et al., 2000) and willow (Hussein et al., 2022; Martin, 2019). These studies reported the nutritive values of these fodders and showed them to be acceptable for maintaining adequate ruminant nutrition. This method of forage production provides an economically feasible means for low income farmers to manage their herds between wet seasons.

There are several key benefits for small scale farms implementing this production practice (Franzel et al., 2014). The first, and likely most important, benefit is increased production and income from cattle. A trial in this study showed an overall increase in dairy production when cows were fed a steady 2 kg/day diet of calliandra throughout the year. This production and income increase was realized with small ruminants as well. Once the appropriate tree and shrub species have been selected, they are relatively easy to grow and require minimal investment of land resources. Additionally, fodder banks can provide useful supplies such as firewood, poles, and stakes. They also provide services such as natural fencing, erosion control, and improvements to soil fertility. There are a

few issues with establishing fodder banks that mostly involve knowledge of practices. There needs to be a viable means of distributing the necessary information to landholders, so they are aware of the appropriate species to select, how to plant them, and how to manage them for productivity and longevity.

American Elderberry (Sambucus nigra subsp. canadensis) as an Alternative Livestock Forage

While research related to the dietary information of elderberries for humans is robust (Domínguez et al., 2021; Osman et al., 2023; Sidor & Gramza-Michałowska, 2015), there is very little, if any, research on the nutrition and safety of using elderberry leaf material as a fodder source for ruminant livestock. The information directly related to this topic is effectively anecdotal and has little empirical data to back up the claims. The 1963 book, *California Range Brushlands and Browse Plants* makes several mentions of elderberry species being used as a forage source. The authors claim that, “Certain species of elderberry...are browsed little or not at all until the first killing frost in the fall has blackened and killed the succulent foliage” (Sampson & Jeperson, 1963). This book is specifically talking about blue elderberry (*Sambucus nigra* subsp. *cerulea*) when stating that, “Blue elderberry is a useful range plant but is not equally palatable at all seasons... Browse rating. Good for goats; good to fair for sheep; good to poor for deer; fair for cattle; and fair to poor for horses.” A study in 1983 on late season grazing of riparian communities noted that, “Cattle utilization of palatable shrubs such as blue elderberry (*Sambucus cerulea*)...was heavy, and was often greater than 100% of the current year’s growth” (Kauffman et al., 1983). The USDA plant guide for blue elderberry also quotes the 1963 rangelands book saying, “Blue elderberry is a useful range plant for domestic

livestock” (USDA, 2016). While the researched information is testimonial and appears to be mostly related to blue elderberry instead of American elderberry, this is evidence that academic investigators have observed voluntary livestock consumption of elderberry leaf material without reporting injury or death to the livestock. It would not be prudent to design a forage production system prior to the addition of empirical data but these anecdotal reports provide justification to examine elderberry as a ruminant feed source.

Cyanogenic Glycosides

According to the *Merck Veterinary Manual*, pasture forages are considered safe for ruminant consumption when the plants contain cyanogenic glycoside (CNG) concentrations less than 220 $\mu\text{g/g}$ on a wet weight basis and less than 500 $\mu\text{g/g}$ on a dry weight basis (Cope, 2021). Cyanogenic glycosides are plant secondary metabolites that are broken down into hydrogen cyanide through hydrolysis (Appenteng et al., 2021). Intact CNGs are non-toxic, but herbivory (mastication, digestion) will break down the compounds and hydrolyze them into cyanide. In high enough concentrations, acute cyanide poisoning will inactivate cellular respiration and lead to a rapid death (Allison, 2019). A study by Appenteng et al. examined all parts of American elderberry fruit: seeds, stem, skin, pulp, juice, and ripe/unripe whole berries. The authors specifically analyzed these berries for the presence of four CNGs: amygdalin, linamarin, dhurrin, and sambunigrin (Appenteng et al., 2021). This study showed that all of the individual plant parts analyzed contained CNGs. While the concentration of identified cyanogens in the plant biomass was generally considered to be low, the highest concentrations were found in the berry stems and unripe berries.

A 2017 study by Senica et al. examined phenolic and CNG variation in European elderberry (*Sambucus nigra* subsp. *nigra*) at different altitudes across the growing season (Senica et al., 2017). The authors showed that higher elevations increased concentrations of the CNG sambunigrin. The study also noted that higher mean air temperatures reduced the concentrations of sambunigrin. Additionally, the authors shared previous research on white clover (*Trifolium repens*) that mirrored this temperature related variance in cyanogenic concentration (Stochmal & Oleszek, 1997).

Cyanogenic Glycosides in Elderberry Leaf Material

A 2000 study focused on the cyanogenic compounds in the leaves of American elderberry (Buhrmester et al., 2000). The researchers examined thirty individual plants across nine different plots of wild elderberry and only found that two plots had potentially dangerous concentrations of CNGs. Gas chromatography identified the specific compound as (S)-sambunigrin. This study showed that the examined American elderberry plants produced CNGs in the leaf material.

There is a distinct gap in the body of scientific literature related to the use of elderberry biomass as a potential feed source for ruminant livestock. As a productive perennial species in temperate agroforestry systems, elderberry management practices offer a window of opportunity to integrate ruminant livestock in order to consume post-harvest elderberry leaf biomass. Anecdotally, this biomass is a browsable food resource for ruminants, however, there are numerous resources that declare elderberry unsafe for consumption by ruminant livestock due to the presence of cyanogens (Arnold, 2021; Carlson, 2013; Llewellyn, 2014; Smith et al., 2014). While American elderberry fruit and juice is known to contain trace amounts of CNGs, the concentration in leaf biomass and

its potential impact on ruminant health has not been thoroughly explored. As such, this study aims to analyze the nutritive value and CNG concentration of American elderberry leaf biomass in order to ascertain its potential for use as a safe and nutritious ruminant feed source.

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II. American Elderberry (*Sambucus nigra* subsp. *canadensis*) Biomass as a Supplemental Feed Source for Ruminant Livestock

Introduction

Background

American elderberry (*Sambucus nigra* subsp. *canadensis*) is a hearty, fruit bearing species from the Viburnaceae family that is native to most of the continental United States and southeastern Canada. It thrives in a variety of soil types and soil moisture regimes (Bulatovic-Danilovich, 2022). American elderberry has been used by the Indigenous peoples of North America for centuries. These uses include food, fiber, fuel, medicine, tools, instruments, and toys (Charlebois et al., 2010). Contemporarily, American elderberry has grown in popularity for its use in juices, jams, jellies, baked goods, wines, and liqueurs. Additionally, the global market for nutraceutical products containing elderberry extracts is currently valued at 1.1 billion US dollars and is projected to expand by 8.9% year over year and reach a valuation of 2.9 billion US dollars in the next decade (FMI, 2023). With the anticipated market growth of elderberry products over the next decade, it is expected that there will be a corollary increase in the amount of acreage devoted to elderberry production.

The standard management practice for American elderberry is to coppice the plants after the growing season in preparation for spring regrowth (Byers et al., 2014). Leaves remain on the plant through the time of berry harvest until seasonal leaf abscission in the fall. While elderberry is browsed by the native ruminant, white tail deer (Shrestha & Lubell, 2015), it is also known to contain cyanogenic glycosides (CNG)

(Appenteng et al., 2021; Buhrmester et al., 2000; Senica et al., 2017). Latent elderberry leaf biomass following berry harvest is potentially useful for livestock managers, yet the safety and utility as a feed supplement for ruminants has not been thoroughly analyzed. There are several outdated, anecdotal references in the body of literature that refer to domesticated livestock consuming elderberry as a forage source. A 1983 study on riparian plant communities reported that cattle heavily browsed blue elderberry (*Sambucus nigra* subsp. *cerulea*) and often consumed more than 100 % of seasonal plant growth (Kauffman et al., 1983). The 1963 book, *California Range Brushlands and Browse Plants* described blue elderberry as a seasonally palatable forage, and classified its browse rating as fair for cattle, good for goats, and good to fair for sheep (Sampson & Jeperson, 1963). While these testimonies may encourage producers to experiment with elderberry as a supplemental feed source, empirical data that verifies its nutritive value and safety is currently unavailable.

Forage quality considers the nutrient density, digestibility, palatability, intake, and anti-quality factors of a given forage (Ball, 2001; Buxton et al., 1995; Lee, 2018). Environmental factors such as precipitation (Patton et al., 2007; Perotti et al., 2021), soil fertility (Horrocks & Vallentine, 1999; Ward, 1959), altitude (Escobar et al., 2020; Melo et al., 2022), temperature (Hart et al., 2022; M. A. Lee et al., 2017), and light intensity related to latitude (Kumar et al., 2022) can cause variable rates of growth and overall forage quality in a given forage species. The same environmental factors of precipitation (Rolbiecki et al., 2018), soil fertility (Thomas et al., 2013), altitude (Senica, et al., 2017), temperature (Ferreira et al., 2023), and light intensity (Osman et al., 2023) have been demonstrated to affect growth characteristics in both American and European elderberry.

Objective and Hypothesis

The objective of this study is to determine the viability of utilizing American elderberry leaf biomass as a supplemental feed source for ruminant livestock by analyzing the nutrient constituents and CNG content over the course of two growing seasons across six different cultivars grown at two different locations. Considering previous research on the CNG content of American elderberry biomass, we predict that detectable but minimal concentrations of CNGs will be found in the examined biomass. Additionally, past testimonials by field observation have shown that elderberry species might be a valuable forage for ruminant livestock. We predict that the nutritive value of American elderberry will be acceptable as a seasonal feed source for use in ruminant livestock production.

Methods

Study Site

This study utilized American elderberry grown at two locations. The first location is the Horticulture and Agroforestry Research Farm (HARF) in New Franklin, Missouri (39.01604, -92.75164). The Natural Resources Conservation Service (NRCS, 2023) has defined this Major Land Resource Area (MLRA) as Central Mississippi Valley Wooded Slopes, Western Part (115b), and the ecoregion is listed as River Hills (72f). This area is comprised of loess covered rolling hills that frequently form ravines as upland, low order streams converge into the Missouri River. The Web Soil Survey shows that the location is primarily Menfro silt loam (USDA, 2023). This farm location receives a mean annual precipitation of 1060 mm and has a freeze-free period of approximately 205 days (USDA, 2024; USDA, 2023). Temperatures at HARF were normal during the sampling

months in both years compared to a 10 year average (University of Missouri Extension, 2024; Table 1). Precipitation totals at HARF were slightly above normal in 2021 (1224 mm) when compared to the 30 year average (1060 mm), but the month of June (298 mm) was much higher than the 30 year average for June (127 mm). In 2022 during the months of June, July, August, and September, precipitation totals were 55-65% lower than the 30 year average, and the yearly precipitation total for HARF (739 mm) was much lower than the 30 year average (USDA, 2024; Table 2).

The second growing location is the Southwest Research Center (SWC) in Mount Vernon, Missouri (37.07338, -93.87934). The Natural Resources Conservation Service (NRCS, 2023) has defined this Major Land Resource Area (MLRA) as Springfield Plain (116b), and the ecoregion is listed as Springfield Plateau (39a). This area is characterized as a savannah-like transition zone between the Cherokee Plain and the forested Ozark Highlands. The Web Soil Survey shows an approximately even distribution of Creldon, Hoberg, and Gerald silt loams, as well as Keeno gravelly silt loam soil types (USDA, 2023). This farm location receives a mean annual precipitation of 1163 mm, and a freeze-free period of approximately 210 days (USDA, 2024; USDA, 2023). The mean temperatures during sampling months at SWC were normal compared to a 10 year average (Table 1). Precipitation totals at SWC were well below the 30 year average in August and September of 2021, and June, July, and September of 2022 (Table 2).

Experimental Design

This study utilized a completely randomized design for six different cultivars, and sampling design was randomized across both growing locations across regular time intervals. As such, treatments for this study included cultivar, sample date, and location.

These plots were originally established in 2015 and 2016 at SWC and HARF, respectively. The plots in the initial study examined elderberry flower harvest and the subsequent effects on berry production (McGowan, 2017). Each of the six cultivars were derived from undomesticated varieties and bred to be the cultivars Bob Gordon, Kelly 7-14, Ozark, Pocahontas, Rogersville, and Wyldewood. Each location consisted of 96 total plots, with 16 plots for each cultivar. The individual plots were considered the experimental unit, and for each sample date, cultivar was replicated twice at each location. The plots were spaced at 3 m between the rows and 2.4 m between each plot.

During plot establishment in 2015 and 2016, the soil substrate at each location was amended to increase P and K levels as well as balance the soil pH for elderberry production (Byers et al., 2014). The plot management was intended to be comparable between each location in relation to fertilization, irrigation, pest management, weed control, and pruning as illustrated in the 2014 report, *Growing and Marketing Elderberries in Missouri* (Byers et al., 2014). However, significantly higher weed pressure was observed at HARF.

Sampling Procedure

Leaf biomass samples were collected each month at both locations from May through September in 4-week intervals during 2021 and 2022. American elderberry has a pinnately compound leaf structure with seven to eleven elliptic leaflets attached to a central rachis. Every four weeks from May through September, randomly assigned plots were harvested by clipping the rachis between the compound leaf and the petiole. The uppermost portion of the plant, the inflorescence, was not harvested in this procedure to avoid compromising berry production.

Each plot was composed of three separate parent plants and was subdivided into A, B, and C subplots. These sub-samples were to ensure a representative collection from each parent plant and were combined into one plot sample after initial dry matter analysis. The collected samples of four to ten leaves, depending on leaf size, were sealed in appropriately labeled plastic bags and stored in an iced cooler while in the field. An aggregate sample of approximately twenty leaves from the entire plot was collected for CNG analysis. Four extra plots of each cultivar were retained at both locations and were utilized as alternates if a particular plot was underproducing. Following collection, samples were then moved from cold storage in the field to a standard chest deep freezer and kept at or below -18°C at the HARF facility in preparation for analysis.

Forage Analysis

Each step in the forage analysis was conducted in duplicate to produce accurate data output. Samples for 60°C dry matter analysis were weighed on a balance scale (A&D Engineering, Model EK-30KL balance, Ann Arbor, MI, USA), and recorded to 0.1g at HARF. The samples were then dried in a mechanical convection horizontal air-flow oven (Blue M Electric, POM-1406F, Watertown, WI, USA) at 60°C for 48 hours and weighed back. The 60°C dry matter (DM) percentage was calculated as follows:

$$60^{\circ}\text{C DM}\% = ((\text{dry weight}/\text{wet weight}) * 100)$$

A mean 60°C DM percentage for the entire sample plot was generated as an average of subplot samples A, B, and C. Following the drying process, subplot samples A, B, and C were aggregated and ground through a 1 mm screen in a laboratory mill (Thomas Scientific, Thomas-Wiley Model 4 mill, Chadds Ford Township, PA, USA). The samples were then stored in sterile sampling bags (Fisher Scientific, #14-955-186, Hampton, NH,

USA) and transported to the University of Missouri-Columbia campus for further analysis.

Ground partial DM samples were used to analyze total DM by weighing sample material to 0.5g (+/- 0.05g) on a balance scale (Fisher Scientific, Accu-124 precision balance, Hampton, NH, USA) and recorded to 0.0001g. The samples were then placed in ceramic crucibles and dried at 100°C for 24 hours in a general-purpose drying oven (Fisher Scientific, Model 6925 isotemp oven, Hampton, NH, USA). After drying, the samples were weighed back. A 100°C DM% and total DM% was calculated using the equations:

$$100^{\circ}\text{C DM}\% = ((100^{\circ}\text{ DM weight}/60^{\circ}\text{ DM weight}) * 100)$$
$$\text{Total DM}\% = ((60^{\circ}\text{ DM}\%/100^{\circ}\text{ DM}\%) * 100)$$

Following the total DM analysis, organic matter (OM) analysis was completed by moving the sample containing crucibles into a muffle furnace (Thermo Fisher Scientific, Lindberg/BlueM 5.3L B2 Moldatherm muffle furnace, Waltham, MA, USA). The temperature was set to 600°C for six hours. After allowing the oven to cool to less than 200°C, the samples were removed, cooled in a desiccator, and recorded to 0.0001g.

Ash% and OM% were calculated as follows:

$$\text{Ash}\% = ((\text{ash weight}/100^{\circ}\text{C DM weight}) * 100)$$
$$\text{OM}\% = (100 - \text{ash percent})$$

The Van Soest method was used sequentially for all fiber analysis in this study. Neutral detergent fiber (NDF) analysis involves the removal of all plant cell components such as protein, sugars, pectin, and lipids, but leaves behind cellulose, hemicellulose, and lignin (Van Soest et al., 1991). The sample material was weighed to 0.5g (+/- 0.05g) and sealed in filter bags (Ankom Technology, F57 filter bag, Macedon, NY, USA). The filter

bags were placed into a fiber analyzer (Ankom Technology, A200 fiber analyzer, Macedon, NY, USA) and washed with a mild detergent. After rinsing with 80-100°C distilled water and acetone, the filter bags were air dried overnight to evaporate the acetone, and then placed in a 100°C oven for 2-4 hours. After weighing the bags, the NDF% was calculated as follows:

$$NDF\% = ((\text{washed bag weight} / \text{initial bag weight}) * 100)$$

Following the removal of specific cell components through NDF analysis, the prepared bags were then used for the acid detergent fiber (ADF) procedure. The protocol used the same equipment and drying procedures as NDF analysis but used a stronger detergent with sulfuric acid. This process removed the hemicellulose that was left over from NDF analysis. After the filter bags were washed and dried, the ADF% was calculated as follows:

$$ADF\% = ((\text{washed bag weight} / \text{post-NDF bag weight}) * 100)$$

Following the removal of hemicellulose through ADF analysis, the filter bags were then used for the acid detergent lignin (ADL) procedure to remove cellulose. The prepared filter bags were placed in one-gallon jars and 500 ml of 72% sulfuric acid was added to cover the bags. The jars containing filter bags of sample material were placed in an in vitro agitator (Ankom Technology, Daisy Incubator, Macedon, NY, USA) for 3 hours. The jars were then drained, and the bags were rinsed in tap water until the pH was neutral. The bags were then rinsed with acetone and dried identically to the NDF and ADF procedures. After fully drying, ADL% was calculated as follows:

$$ADL\% = ((\text{washed bag weight} / \text{post-ADF bag weight}) * 100)$$

Sample materials were analyzed for crude protein (CP) content by Dairy One in Ithaca, NY. A nitrogen analyzer (Leco Corporation, CN628 elemental analyzer, St. Joseph, MI, USA) was used to determine crude protein content of the elderberry leaf biomass samples. This process utilized the combustion-based Dumas Method (Simonne et al., 1997) whereby samples are heated to between 800-900°C in the presence of pure oxygen. This produces nitrogen oxide, carbon dioxide, and water. The gases are then separated and removed to isolate and detect the concentration of elemental nitrogen.

Cyanogenic Analysis

Elderberry leaf biomass samples were analyzed for the cyanogenic glycosides (CNGs) amygdalin, linamarin, dhurrin, and sambunigrin at the Metabolomics Center on the University of Missouri-Columbia campus. Elderberry leaves were lyophilized and ground to a fine powder using a mortar and pestle. The powder samples (10 ± 0.06 mg) were accurately weighed into glass vials. Extractions were carried out by adding 1.0 mL of 80% methanol/20% water containing internal standard umbelliferone (18 μ g/mL). All samples were sonicated and then agitated for 18 h at room temperature. Afterward, the samples were centrifuged at 3500 rpm for 20 min. The supernatants (~ 900 μ L) were transferred into Eppendorf tubes and centrifuged at 15000 rpm for 15 min in a benchtop high-speed microcentrifuge (2400-37, VWR, Radnor, PA). Supernatants were transferred into glass vials and dried using a RapidVap evaporator (Labconco Corporation, Kansas City, MO). Extracts were reconstituted in methanol before solid-phase extraction (SPE) and LC-MS/MS analysis. The SPE purifications were carried out using Oasis® HLB 1cc (30-mg) extraction cartridges.

Quantitative measurements of CNGs were performed using a Waters ACQUITY UPLC system coupled to a Xevo TQ-XS triple quadrupole mass spectrometer (UHPLC-MS/MS). Chromatographic separations were performed using an ACQUITY UPLC HSS T3 column (1.8 μ M, 2.1 \times 100 mm) and a flow rate of 0.5 mL/min. Mobile phases A and B were 2 mM ammonium formate in water and 100% acetonitrile, respectively. The column temperature was set at 45 °C and the injection volume was 2 μ L. The analysis time was 18 min and the elution gradient for mobile B was as follows: initially equilibrated at 1% B; 0-12 min, 1-12% B; 12-12.5 min, 12-95% B; 12.5-14.5 min, 95% B; 14.5-15 min, 95-5% B; 15-18 min, 5-1% B.

The mass spectra were acquired in positive electrospray ionization (ESI) mode. Detection of all analytes was performed using multiple reactions monitoring (MRM) mode. The source temperature and desolvation temperature were maintained at 150 °C and 500 °C, respectively. Nitrogen and argon were used as the desolvation gas (1000 L/h) and collision gas (0.14 ml/min), respectively. The MRM transitions were optimized as follows.

	Compound	Rt (min)	Ion type	Transition (m/z)	Cone (V)	Collision (eV)
1.	Linamarin	3.34	Quantifier	265.40 > 180.01	4	8
			Qualifier	265.40 > 162.92	4	10
2.	Dhurrin	6.40	Quantifier	329.45 > 131.90	8	10
			Qualifier	329.45 > 84.80	8	26
3.	Amygdalin	9.89	Quantifier	475.46 > 84.87	24	30
			Qualifier	475.46 > 144.92	24	20
4.	(R)-Prunasin	11.14	Quantifier	313.45 > 180.01	20	8
			Qualifier	313.45 > 144.92	20	12
5.	Sambunigrin	11.34	Quantifier	313.45 > 180.01	20	8
			Qualifier	313.45 > 144.92	20	12

Calibration curves were generated using standard compounds purchased from Sigma and SCBY. MassLynx software (version 4.1, Waters, Milford, MA, USA) was used for instrument control and data processing. Data processing and quantification were processed using Waters QuanLynx software.

Data Analysis

This completely randomized design examined location (SWC, HARF), cultivar (Bob Gordon, Kelly 7-14, Ozark, Pocahontas, Rogersville, and Wyldewood) and sampling date (May, June, July, August, and September) as fixed effects, with sample year (2021, 2022) as a random effect. The overall effect of independent variables (location, cultivar, and sampling date) on mean DM, OM, NDF, ADF, ADL, and CP were tested for analysis of variance (ANOVA) using the GLIMMIX procedure of the SAS statistical software program (version 9.4). The four cyanogenic compounds were tested using analysis of variance (ANOVA) in R statistical software program (R version 3.6.2). Significant interactions ($\alpha = 0.05$) were evaluated within all levels, and significance was determined if $P \leq 0.05$, with tendencies toward significance declared when $P \leq 0.10$ and > 0.05 . A post hoc Tukey test was then conducted to identify significant differences among treatments.

Results

Forage Analysis

When analyzing the DM concentrations of elderberry leaf biomass, there were observed two-way interactions between location by date ($P < 0.001$; Figure 1) and location by cultivar ($P < 0.001$; Figure 2). When comparing interactions between location

and date ($P < 0.001$), DM increases steadily throughout the growing season at both locations. The DM content was higher at HARF in May, not different in June, and higher at SWC in July, August, and September. The DM concentrations were different at SWC and HARF ($P < 0.001$) when comparing interactions between location and cultivar. The cultivars Wyldewood and Pocahontas were not different at SWC and HARF. Bob Gordon, Kelly 7-14, and Rogersville had higher DM content at SWC, and Ozark had higher DM content at HARF.

When analyzing OM concentrations, there was a three-way interaction ($P = 0.017$; Table 3) between location, sample date, and cultivar. The only difference for Kelly 7-14 between locations occurred in June ($P = 0.047$), where OM was higher at SWC ($94.05 \pm 0.41\%$) compared to HARF ($92.97 \pm 0.41\%$). Differences between locations in June were also observed in the Wyldewood cultivar ($P = 0.012$), with OM being higher at SWC ($94.27 \pm 0.41\%$). Differences in the Ozark cultivar between locations occurred in August ($P = 0.012$) with OM content at SWC being higher ($92.34 \pm 0.41\%$) than at HARF ($90.69 \pm 0.41\%$). The cultivars Bob Gordon ($92.44 \pm 0.35\%$) and Pocahontas ($91.12 \pm 0.35\%$) were different at HARF only in the month of May ($P = 0.003$). Those same cultivars were different at SWC only in June ($P = 0.003$), July ($P < 0.001$) and August ($P = 0.036$). The OM content was higher at SWC for these two cultivars across all sampling dates except for Pocahontas in July where it was higher at HARF ($90.09 \pm 0.41\%$) than at SWC ($89.74 \pm 0.41\%$). Unlike the other cultivars, Rogersville was consistently higher in OM at SWC compared to HARF throughout the growing season (May: $P = 0.010$, June: $P < 0.001$, July: $P = 0.003$, August: $P < 0.001$, and September: $P < 0.001$).

When analyzing the NDF, ADF, and ADL, the means show that over the course of the growing season, NDF ($P < 0.001$; Figure 3), ADF ($P < 0.001$; Figure 4), and ADL ($P < 0.001$; Figure 5) all display a consistent increase in concentration through August, and a declining concentration following the completion berry production into September. The highest fiber concentrations were observed in August for NDF ($27.3 \pm 1.31\%$) and ADF ($20.4 \pm 1.43\%$), and the lowest in May for NDF ($22.5 \pm 1.30\%$) and ADF ($15.8 \pm 1.41\%$). The highest ADL concentration was observed in July ($11.1 \pm 1.01\%$), and the lowest in May ($7.06 \pm 1.00\%$).

When examining fiber constituents by cultivar, there was a difference in NDF concentrations ($P < 0.001$; Figure 6), ADF had a tendency towards difference between cultivars ($P = 0.056$; Figure 7) and ADL was not different between cultivars ($P = 0.161$; Figure 8). The highest NDF concentration was observed in Bob Gordon ($26.6 \pm 1.33\%$), and the lowest was found in Wyldewood ($23.9 \pm 1.32\%$). The highest ADF concentration was observed in Rogersville ($19.6 \pm 1.43\%$), and the lowest was found in Wyldewood ($17.9 \pm 1.43\%$). The highest ADL concentration was observed in Rogersville ($9.9 \pm 1.02\%$), and the lowest was found in Bob Gordon ($8.7 \pm 1.02\%$).

By location, only NDF was different ($P < 0.001$; Figure 10), while ADF ($P = 0.197$; Figure 11) and ADL ($P = 0.607$; Figure 12) showed no variation. The fiber constituent concentrations at SWC for NDF ($25.9 \pm 1.27\%$), ADF ($18.7 \pm 1.40\%$), and ADL ($9.4 \pm 0.98\%$) were higher than what was found at HARF for NDF ($24.4 \pm 1.27\%$), ADF ($18.3 \pm 1.40\%$), and ADL ($9.3 \pm 0.98\%$).

When analyzing CP, only location by date had a tendency to be different ($P = 0.054$; Figure 13). While observed CP was higher for each sampling date at SWC than at

HARF, there was a progressive reduction in CP at both locations throughout the growing season. Crude protein content in August and September at HARF were not different, and July, August, and September at SWC were also not different from each other. The SWC had its highest CP concentration in May ($24.8 \pm 0.50\%$) and lowest ($16.2 \pm 0.57\%$) in September. The highest CP concentration at HARF was found in May ($18.5 \pm 0.49\%$) and lowest at ($10.6 \pm 0.54\%$) in September. The means for CP content show a difference ($P < 0.001$; Figure 9) between the six examined cultivars. Bob Gordon had the highest CP content ($17.8 \pm 0.47\%$) and was identical to Rogersville ($17.2 \pm 0.46\%$) and Pocahontas ($16.5 \pm 0.46\%$). Kelly 7-14 ($16.4 \pm 0.47\%$), Ozark ($15.9 \pm 0.46\%$), and Wyldewood ($15.7 \pm 0.45\%$) were different from Bob Gordon.

Cyanogenic Analysis

Elderberry leaf biomass from the cultivars Bob Gordon and Ozark was analyzed for the cyanogenic compounds amygdalin, linamarin, dhurrin, and sambunigrin. There was a location by cultivar interaction observed for dhurrin concentration ($P = 0.007$; Figure 14). At SWC, dhurrin content for Bob Gordon was $2.03 \pm 0.88 \mu\text{g/g}$, and Ozark was $4.43 \pm 0.82 \mu\text{g/g}$. At HARF, dhurrin content for Bob Gordon was $3.86 \pm 0.82 \mu\text{g/g}$, and Ozark was $11.32 \pm 0.82 \mu\text{g/g}$. The Ozark cultivar at HARF was different from Ozark at SWC and Bob Gordon at HARF and SWC.

When examining the means by date, there was no difference in amygdalin content ($P = 0.464$; Figure 15). Amygdalin concentration ranged between $4.93 \pm 2.87 \mu\text{g/g}$ in September and $0.18 \pm 2.87 \mu\text{g/g}$ in June. There was no difference in linamarin content ($P = 0.487$; Figure 16) by date. Linamarin content was numerically highest in June at $1.54 \pm 0.86 \mu\text{g/g}$ and dropped to a low of $0.20 \pm 0.86 \mu\text{g/g}$ in July. There was no difference in

sambunigrin content ($P = 0.633$; Figure 17) by date. Sambunigrin concentrations were spread between $9.92 \pm 4.27 \mu\text{g/g}$ in September, and $3.31 \pm 4.75 \mu\text{g/g}$ in August.

When examining the means by cultivar, there was no difference in amygdalin content ($P = 0.320$; Figure 18). The recorded values for amygdalin content were $1.90 \pm 1.91\mu\text{g/g}$ in Bob Gordon, and $0.54 \pm 1.82\mu\text{g/g}$ in Ozark. There was no difference in linamarin content ($P = 0.321$; Figure 19) by cultivar. The linamarin concentration in Bob Gordon was $0.79 \pm 0.57\mu\text{g/g}$, and $0.25 \pm 0.55\mu\text{g/g}$ in Ozark. There was no difference in sambunigrin content ($P = 0.636$; Figure 20) by cultivar. Sambunigrin content was $5.55 \pm 2.83\mu\text{g/g}$ in Bob Gordon, and $4.28 \pm 2.70\mu\text{g/g}$ in Ozark.

When examining the means by location, there was no difference in amygdalin content ($P = 0.398$; Figure 21). Amygdalin concentration was $2.25 \pm 1.91\mu\text{g/g}$ at SWC, and $0.66 \pm 1.82 \mu\text{g/g}$ at HARF. There was no difference in linamarin content ($P = 0.202$; Figure 22) by location. Linamarin content at HARF was $0.88 \pm 0.55 \mu\text{g/g}$, and the $0.15 \pm 0.57 \mu\text{g/g}$ at SWC. There was no difference in sambunigrin content ($P = 0.479$; Figure 23) by location. The documented sambunigrin concentrations were $5.91 \pm 2.83\mu\text{g/g}$ at SWC, and $3.94 \pm 2.70\mu\text{g/g}$ at HARF.

Discussion

Forage physiology and subsequent nutritive value changes continually throughout the growing season (Buxton & Redfearn, 1997; George et al., 2001; Mazis et al., 2023; Randall et al., 2023). Forages will typically have an increase in dry matter and fiber concentrations as the plant reaches reproductive maturity (Buxton & Redfearn, 1997). Conversely, crude protein concentrations in a forage will be highest early in the vegetative stage and will decrease throughout the growth cycle as the plant reaches

reproductive maturity (Ball, 2001; Idan et al., 2023; Mazis et al., 2023). The analysis of nutrients in this study shows similar seasonal changes in nutrient constituents for American elderberry leaf biomass. While this seasonal change in nutrient content was expected, there was an observed difference between the nutrient constituents at HARF and SWC. While this difference could be caused by incongruent management practices between locations, it could also be environmental. The region where HARF is located experienced a severe drought in the summer of 2022 (Missouri Department of Natural Resources, 2024). The months of June, July, August, and September received 55-65% less precipitation than normal when compared to the 30 year average (University of Missouri Extension, 2024; USDA, 2024). As expected, crude protein concentration consistently decreased at each location over the five sample dates. Dry matter concentrations consistently increased at each location over the five sample dates. Fiber constituents (NDF, ADF, ADL) consistently increased over the first four sample dates (May, June, July, August), but then returned to pre-July levels on the last sampling date in September following the completion of berry production. Considering that no live animal trials were performed in this study, it was necessary to closely compare the analyzed elderberry leaf biomass with other proven grasses, legumes, stored forages, and tree fodders in order to qualify its potential efficacy as a feed supplement.

During the dry season and in times of drought, it is possible to supplement pasture forages with plant biomass from tree and shrub fodder banks (Idan et al., 2023; Kubkomawa, 2019). This practice is more frequently used in the tropical and sub-tropical systems than in temperate areas (Franzel et al., 2014). Two popular species utilized for this purpose are mulberry (*Morus* sp.) and willow (*Salix* sp.). Mulberry has been shown

to be a highly nutritive fodder source for ruminant livestock (Sanchez, 2000). A 2018 study from the Universidade Estadual de Londrina in Brazil analyzed the nutritive value of mulberry plant biomass as a supplemental feed source for ruminant livestock (Garcez et al., 2018). The mulberry biomass was analyzed as fresh fodder, silage, and a mixed ration. The fresh mulberry was sampled in the spring (October) at the Federal University of Paraná in Brazil. The nutrient content of fresh mulberry was 25.4% DM, 34.0% NDF, 26.1% ADF, and 17.0% CP. Elderberry leaf biomass sampled in the present study in May, a comparable seasonal date to October in Brazil, had higher CP and lower NDF and ADF than this mulberry biomass, indicating that elderberry leaf biomass is potentially higher in forage quality than this mulberry fodder.

A 2000 study from the College of Animal Sciences at Zhejiang University (Yao et al., 2000) reported nutrient concentrations of four cultivars of mulberry leaves in the spring and fall. The cultivars Tuantou Heyebai, Husang No.9, Tongxiangqing, and Nongsang No.8 are the primary cultivars used in China for silkworm production. Although the growing locations are not latitudinally identical, the China location is 31°N and the Missouri locations are 37 and 39°N, the most equitably comparable dates to the present study are May 29 and September 30. In May, the mulberry biomass contained 26.6% DM, 42.9% NDF, and 20.2% CP. In September, it contained 33.8% DM, 47.2% NDF, and 18.9% CP. Similar to the previous study on mulberry biomass, NDF content was much lower in elderberry leaf biomass and CP content was equal or slightly lower at both sampling dates. Again, this suggests that elderberry fodder has equitable or higher forage quality when compared to this mulberry fodder.

A report published by the Food and Agriculture Organization (FAO) of the United Nations in the *World Animal Review* journal concludes by stating that mulberry, “is comparable or better than many other forage plants in terms of nutritional value and yield of digestible nutrients per unit of area” (Sanchez, 2000). The cultivars that were fully reported showed average values of 28.5% NDF, 22.0% ADF and 18.4% CP, and is again reflective of the comparative similarity in nutrient content between American elderberry leaf biomass and mulberry biomass.

Willow is also known to be an excellent supplemental fodder source for ruminant livestock during times of short and long term drought when typical pasture and range forages are dormant or underproducing (Moore et al., 2003; Pitta et al., 2007). Feed supplementation with willow has shown positive benefits to the health and animal performance of ruminant livestock (Seidavi et al., 2020). A thorough nutrient analysis of six willow cultivars in the early season (June/July) and late season (August/September) provides data for use in comparison to elderberry leaf biomass (Martin, 2019). The mean nutrient concentrations in willow leaves during the early (June/July) sampling period was 39.5% DM, 35.4% NDF, 25.1% ADF, and 14.5% CP. Mean nutrient concentrations in willow leaves in the late (August/September) sampling period was 43.7% DM, 35.3% NDF, 25.0% ADF, and 14.5% CP. Elderberry leaf biomass had lower NDF and ADF content and equitable or higher CP than willow leaves in both the early and late sampling periods. This demonstrates that elderberry biomass also has similar forage quality to the willow leaves sampled in this study.

A 2022 study exploring nitrogen uptake and nutritional composition of willow provides additional data for comparison to elderberry leaf biomass (Hussein et al., 2022).

This study differed from the previously referenced studies on fodder species by combining leaf material with stems averaging 4 mm in diameter. The examined willow biomass mean nutrient concentrations were reported for both the unfertilized control plots and the fertilized plot treatments. The control plot willow contained 47.0% NDF, 40.3% ADF, and 4.5% CP. The fertilized plot willow contained 47.1% NDF, 38.8% ADF, and 19.5% CP. Again, this shows an equitable comparison between the nutritive values of elderberry leaf biomass and willow biomass.

Atypical of other studies on tree and shrub fodder, the Hussein et al. study also calculated relative feed value (RFV) of willow biomass. An estimate of dry matter digestibility and intake is calculated using NDF and ADF values to generate an RFV. It is a forage quality index that is based on the nutritive value of full bloom alfalfa where the RFV equals 100 (Rohweder et al., 1978). While this index was initially created to evaluate grass and legume hay and may not be perfectly applicable to tree and shrub fodders, it has been shown as a useful tool for evaluating unusual or atypical forages (Tassone et al., 2022). The formula for RFV calculation is as follows:

$$\begin{aligned}RFV &= (DDM * DMI) / 1.29 \\ DDM &= 88.9 - (0.779 * \%ADF) \\ DMI &= 120 / \%NDF\end{aligned}$$

The RFV of willow biomass in the Hussein study was reported as 114.4 RFV for the control plots and 117.8 RFV for the fertilized plots. The same formula can be applied to calculate the RFV of the first willow study, as well as two of the mulberry studies in which both NDF and ADF values were recorded. The first willow study (Martin, 2019) had an RFV of 182.6. The mulberry sampled and analyzed by Garcez et al. had an RFV of 187.6 (Garcez et al., 2018). The Sanchez report published by the FAO shows mulberry

biomass had an RFV of 234.2 (Sanchez, 2000). The mean RFV of elderberry leaf biomass in the current study was calculated to be 276.1. Overall, the nutrient composition of elderberry biomass, as specifically illustrated here through the RFV metric, compares favorably with other species of tree and shrub fodder utilized in ruminant livestock production.

American elderberry leaf biomass also compares favorably with traditional grass and legume pasture forages. A 2015 study examined the nutritive value of both tall fescue (*Lolium arundinaceum*) and perennial ryegrass (*Lolium perenne*). Samples were collected and analyzed in both May and November (Kaufononga, 2015). The average NDF (~45%) and ADF (25-30%) content in tall fescue and perennial ryegrass stayed relatively static through both sampling dates. Crude protein content was higher at the early sampling date (25-28%) than what was observed in the fall (~18%). Elderberry leaf biomass has much lower fiber content and slightly lower CP content than these C3 grasses, which indicates that elderberry fodder likely has equitable forage quality in comparison to this tall fescue and perennial ryegrass.

A 2022 study of five C4 warm season grasses examined the nutritive value of switchgrass (*Panicum virgatum*), bermudagrass (*Panicum virgatum*), eastern gamagrass (*Tripsacum dactyloides*), crabgrass (*Digitaria sp.*), and a big bluestem (*Andropogon gerardii*) mix (Keyser et al., 2022). Nutrient content of this collection of C4 grasses ranged between 60-68% NDF, 38-42% ADF, and 9-12% CP. For comparison, elderberry leaf biomass has much lower fiber content and higher CP, and likely has higher forage quality than these C4 grasses.

A 2019 study analyzed the nutrient concentration of alfalfa (*Medicago sativa*) at three separate stages of maturity (Guo et al., 2019). The alfalfa examined in this study during the bud stage had a nutrient composition of 32.4% NDF, 22.7% ADF, and 21.7% CP. During the initial bloom stage, this alfalfa contained 34.5% NDF, 24.4% ADF, and 20.1% CP. In full bloom, this alfalfa contained 37.3% NDF, 26.3 ADF, and 19.4% CP. Alfalfa and elderberry leaf biomass both exhibit progressive seasonal increase in fiber content and a decrease in CP content. Elderberry biomass contains lower fiber and slightly lower CP content than alfalfa, which indicates that these two species are potentially equitable in forage quality.

A report published by the College of Agricultural, Consumer and Environmental Sciences at New Mexico State University on hay quality listed the quality standards for grass, legume, mixed hay, and alfalfa only hay (Marsalis et al., 2009). The classification system for grass, legume, and mixed hay uses the term “prime” as its highest category. Elderberry leaf biomass qualifies as prime for the fiber and RFV components, but the CP content was one category lower than the prime classification. The classification system for alfalfa only hay uses the term “supreme” as its highest category. Elderberry leaf biomass was ranked as supreme for NDF, ADF, and RFV. The CP content of elderberry biomass falls below the rank of supreme and was classified as “good” when compared to alfalfa only hay. Overall, the nutritive value of American elderberry leaf biomass compares favorably with other traditional cool and warm season grasses and legumes, whether analyzed as a fresh or stored forage.

The threat of acute cyanide toxicity in ruminants occurs when CNG concentration in consumed feed exceeds 220 $\mu\text{g/g}$ on a wet weight basis and 500 $\mu\text{g/g}$ on a dry weight

basis (Cope, 2021). In a report from Purdue University Extension specifically related to sorghums, the threat of acute cyanide toxicity is increased when grazing new growth, grazing following a frost, over-fertilizing with nitrogen, and during drought conditions (Gruss et al., 2022). In order to minimize the threat to ruminant health, these conditions and circumstances must be accounted for when planning management practices. The threat of acute cyanide toxicity can be greatly reduced by providing the forage as green chop, hay, or silage, as this reduces cyanogenic potential through cell disruption and degradation of cyanogenic compounds. The threat of toxicity for ruminants can also be reduced in fresh forages by allowing animals to graze other forages known to be safe or by providing a grain ration prior to being allowed to graze plants containing CNGs. This prevents ruminant from overconsuming the forage and thereby reduces the g/kg of body weight that is consumed (Gruss et al., 2022).

A 2021 article in the journal *Molecules* examined the cyanogenic potential of American elderberry (Appenteng et al., 2021). This study was directly related to cyanogens contained in the ripe and unripe berries, seeds, skin, pulp, and juice, and did not analyze the leaf, rachis, or petiole material of the plant. The Appenteng et al. study chose to analyze for the cyanogens amygdalin, linamarin, and dhurrin. The study also analyzed prunasin, which is a diastereomer of sambunigrin (also referred to as (S)-prunasin). The Metabolomics Center at the University of Missouri used ultra-high-performance liquid chromatography (UHPLC) to examine the elderberry leaf biomass and was unable to differentiate between sambunigrin and prunasin in their analysis. The Appenteng et al. study identified the same issue of differentiating between sambunigrin and prunasin in their UHPLC analysis (Appenteng et al., 2021). The study showed that

CNG concentrations of various fruit tissues ranged from 0.12 to 6.38 $\mu\text{g/g}$, with the highest content found in the stems ($37.43 \pm 9.19 \mu\text{g CN}^- \text{ eq./g}$) and green berries ($25.6 \pm 5.07 \mu\text{g CN}^- \text{ eq./g}$).

One study examined nine populations of wild, “roadside” American elderberry for the presence of cyanogenic compounds (Buhrmester et al., 2000). Seven of the nine populations did not reveal the presence of cyanogens when tested with Feigl-Anger paper strips (Feigl & Anger, 1966). Two of the populations tested positive for cyanogens. One population showed a distribution of readings between not present, weak, medium, and strong. The other population showed the present cyanogens as strong, which is classified as greater than 50 mg/kg HCN. Additional analysis with gas chromatography identified the cyanogen as sambunigrin. This study suggested considerable variability in cyanogenic compounds among uncultivated populations.

Statistical analysis of elderberry leaf biomass completed for the present study shows that a maximum aggregated mean of the highest individual plot readings for amygdalin (35.7 $\mu\text{g/g}$), linamarin (10.7 $\mu\text{g/g}$), dhurrin (19.5 $\mu\text{g/g}$), and sambunigrin (54.5 $\mu\text{g/g}$) has a potential maximum cyanogenic concentration of 117.4 $\mu\text{g/g}$. The average aggregated concentration of amygdalin (1.4 $\mu\text{g/g}$), linamarin (0.5 $\mu\text{g/g}$), dhurrin (5.4 $\mu\text{g/g}$), and sambunigrin (4.8 $\mu\text{g/g}$) was an order of magnitude lower at 12.1 $\mu\text{g/g}$. The observed spikes in cyanogenic glycoside concentrations could potentially be attributed to the drought that occurred at the HARF location in the summer of 2022. The months of June, July, August, and September saw rainfall totals 55-65% lower than the 30 year average (Missouri Department of Natural Resources, 2024; University of Missouri Extension, 2024; USDA, 2024). With the threat of acute cyanide toxicity being realized

above 500 $\mu\text{g/g}$ on a dry weight basis (Cope, 2021), the analysis in this study did not detect toxic levels of CNGs in cultivated American elderberry biomass grown under typical Missouri conditions. When compared with the previous work on wild populations, this study suggests that cultivated American elderberry presents a relatively low risk of toxicity for ruminant livestock.

Conclusion

The primary goal of this study was to determine the viability of utilizing American elderberry leaf biomass as a supplemental feed source for ruminant livestock. In order to qualify its viability for this purpose, the nutrient concentration and concentration of toxic compounds in American elderberry leaf biomass was thoroughly analyzed. The nutrient analyses in this study showed that the concentrations of dry matter, organic matter, neutral detergent fiber, acid detergent fiber, acid detergent lignin, and crude protein in elderberry biomass were equitable with other known and proven tree fodders used in ruminant livestock production. American elderberry biomass also compared favorably with other traditional cool and warm season grass and legume fresh and stored forages. Chemical analysis of the examined cultivars of American elderberry revealed the expected presence of CNGs. While the consumption of CNGs can be detrimental to ruminant health, the concentrations reported in this study suggest that American elderberry leaf biomass grown under the study conditions likely presents a low risk of toxicity if utilized as a ruminant feed supplement.

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Tables

Table 1: Mean monthly temperatures in °C for sampling dates at HARF and SWC in 2021-2022, with 10 year average monthly temperatures for comparison.

Mean Temperature 2021	May	June	July	August	September
HARF¹	16.3	23.5	24.3	24.5	20.8
SWC²	16.0	23.1	23.9	24.7	21.8
Mean Temperature 2022	May	June	July	August	September
HARF	19.0	23.6	25.7	24.2	19.3
SWC	18.4	23.6	27.8	24.9	20.3
10 Year Avg Temperature	May	June	July	August	September
HARF	18.2	23.4	24.9	23.6	20.5
SWC	18.0	23.4	25.4	24.0	21.1

¹Horticulture and Agroforestry Research Farm (HARF), New Franklin, Missouri. ²Southwest Research Center (SWC), Mount Vernon, Missouri.

Table 2: Total year and monthly precipitation in mm for sampling dates at HARF and SWC in 2021-2022, with 30 year average monthly and yearly precipitation for comparison.

Total Precip. 2021	May	June	July	August	Sept	Yr. Total
HARF	121.7	297.7	109.2	42.9	58.9	1223.6
SWC	184.2	136.4	89.7	45.7	32.0	1132.6
Total Precip. 2022	May	June	July	August	Sept	Yr. Total
HARF	125.5	42.7	47.8	40.4	34.5	739.4
SWC	258.3	41.7	9.1	112.0	60.7	1051.8
30 Year Avg Precip.	May	June	July	August	Sept	Yr. Avg.
HARF	126.7	127.0	111.0	118.1	99.1	1059.9
SWC	132.3	139.2	92.7	92.2	122.7	1162.8

¹Horticulture and Agroforestry Research Farm (HARF), New Franklin, Missouri. ²Southwest Research Center (SWC), Mount Vernon, Missouri.

Table 3: Organic matter (OM)% three-way interaction between location, date, and cultivar of American elderberry at two Missouri locations in 2021-22 across the growing season.

Fixed Effect	Kelly 714					
	May	Jun	Jul	Aug	Sep	<i>P</i> value
HARF ¹	91.70 ^{cd}	92.97 ^{bc}	91.71 ^{cd}	91.60 ^{cd}	91.49 ^{cd}	0.017
SEM	0.38	0.41	0.41	0.41	0.41	
SWC ²	93.04 ^{bc}	94.05 ^a	92.69 ^{bc}	92.32 ^{bc}	92.53 ^{bc}	
SEM	0.41	0.41	0.41	0.47	0.41	
	Wyldeewood					
	May	Jun	Jul	Aug	Sep	<i>P</i> value
HARF	92.00 ^{bc}	92.90 ^{bc}	92.05 ^c	92.25 ^{bc}	92.55 ^{bc}	0.017
SEM	0.35	0.41	0.41	0.41	0.41	
SWC	93.80 ^a	94.27 ^a	92.59 ^{bc}	92.73 ^{bc}	92.61 ^{bc}	
SEM	0.35	0.41	0.41	0.41	0.41	
	Pocahontas					
	May	Jun	Jul	Aug	Sep	<i>P</i> value
HARF	91.12 ^{cd}	90.86 ^d	90.09 ^{de}	89.83 ^e	89.64 ^e	0.017
SEM	0.35	0.41	0.41	0.41	0.41	
SWC	93.88 ^a	92.11 ^{bc}	89.74 ^e	91.70 ^{bc}	91.59 ^{cd}	
SEM	0.35	0.41	0.41	0.41	0.41	
	Bob Gordon					
	May	Jun	Jul	Aug	Sep	<i>P</i> value
HARF	92.44 ^{bc}	91.16 ^{cd}	90.05 ^{de}	89.34 ^e	90.21 ^{de}	0.017
SEM	0.35	0.41	0.41	0.41	0.41	
SWC	93.50 ^a	94.14 ^a	91.88 ^{cd}	92.93 ^{bc}	91.97 ^c	
SEM	0.38	0.56	0.41	0.47	0.41	
	Ozark					
	May	Jun	Jul	Aug	Sep	<i>P</i> value
HARF	93.09 ^b	93.46 ^a	92.61 ^{bc}	90.69 ^d	90.90 ^d	0.017
SEM	0.38	0.41	0.41	0.41	0.41	
SWC	93.86 ^a	93.65 ^a	92.34 ^{bc}	92.05 ^c	91.66 ^{cd}	
SEM	0.35	0.41	0.41	0.41	0.41	
	Rogersville					
	May	Jun	Jul	Aug	Sep	<i>P</i> value
HARF	92.87 ^{bc}	91.24 ^{cd}	90.32 ^{de}	90.39 ^{de}	90.00 ^{de}	0.017
SEM	0.38	0.41	0.41	0.41	0.41	
SWC	94.07 ^a	93.58 ^a	91.96 ^c	92.78 ^{bc}	92.06 ^c	
SEM	0.35	0.41	0.41	0.41	0.41	

¹Horticulture and Agroforestry Research Farm (HARF), New Franklin, Missouri. ²Southwest Research Center (SWC), Mount Vernon, Missouri.

Figures

Figure 1: Dry matter (DM)% two-way interaction between location and date from six cultivars of American elderberry at two Missouri locations in 2021-2022 across the growing season.

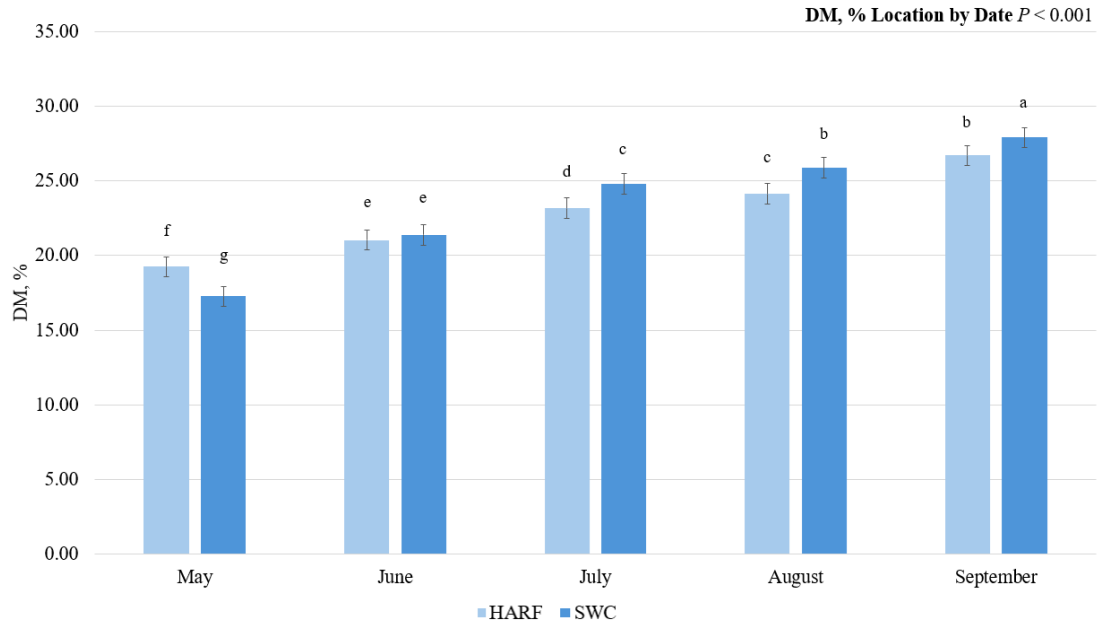


Figure 2: Dry matter (DM)% two-way interaction between location and cultivar in American elderberry at two Missouri locations in 2021-2022 across the growing season.

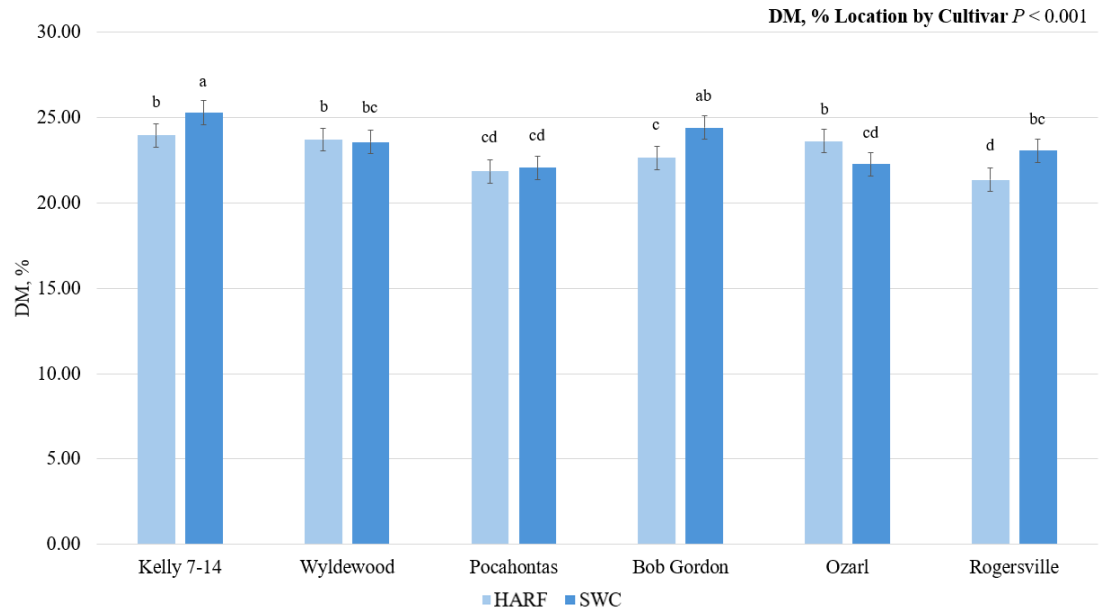


Figure 3: Neutral detergent fiber (NDF)% simple means by date from six cultivars of American elderberry at two Missouri locations in 2021-2022 across the growing season.

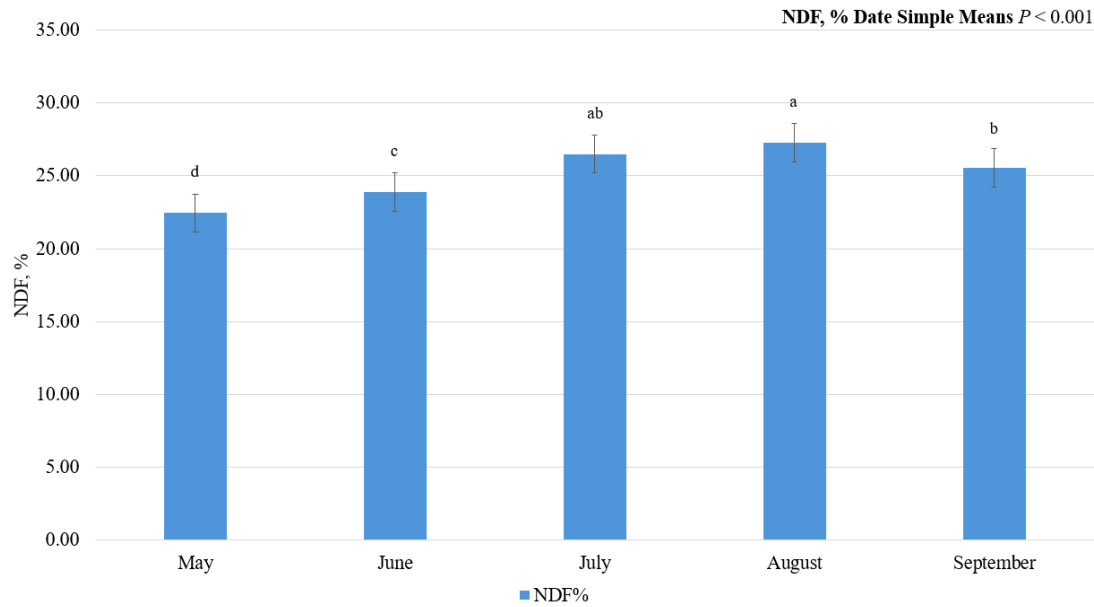


Figure 4: Acid detergent fiber (ADF)% simple means by date from six cultivars of American elderberry at two Missouri locations in 2021-2022 across the growing season.

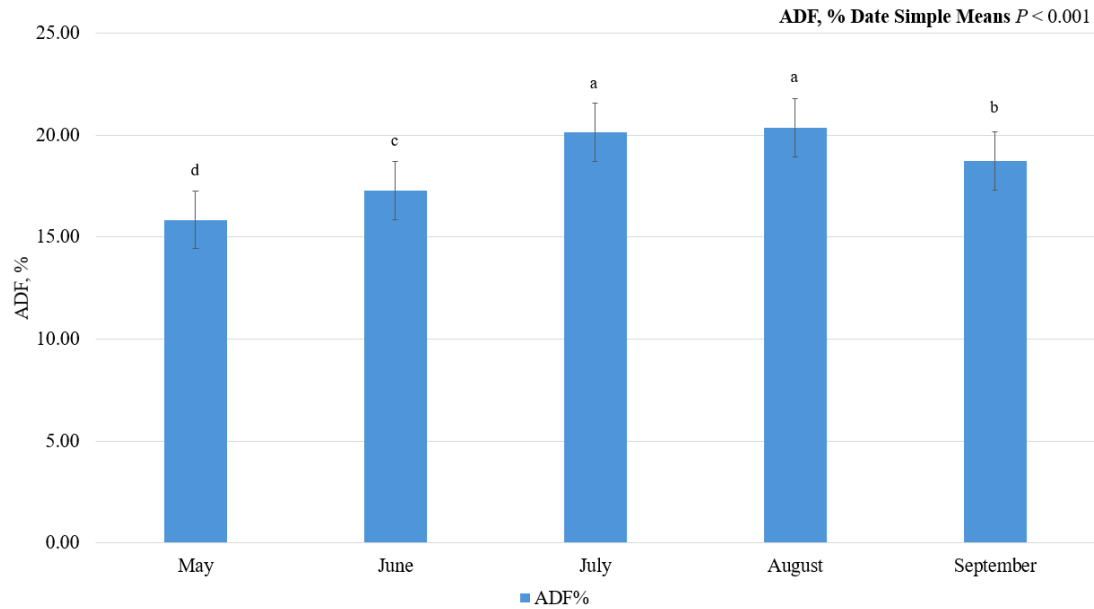


Figure 5: Acid detergent lignin (ADL)% simple means by date from six cultivars of American elderberry at two Missouri locations in 2021-2022 across the growing season.

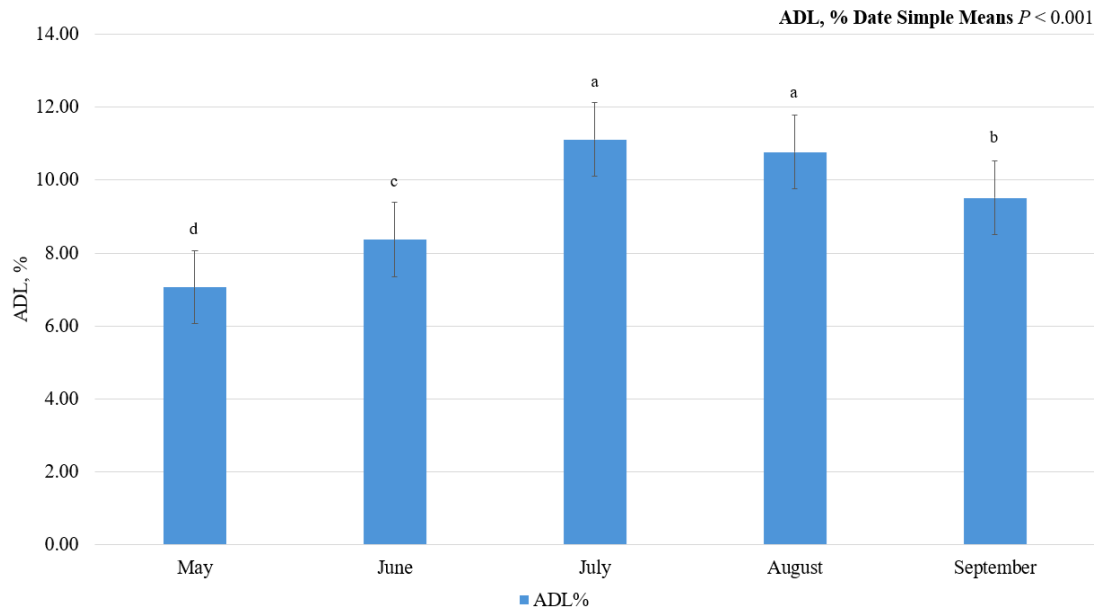


Figure 6: Neutral detergent fiber (NDF)% simple means by cultivar in American elderberry at two Missouri locations in 2021-2022 across the growing season.

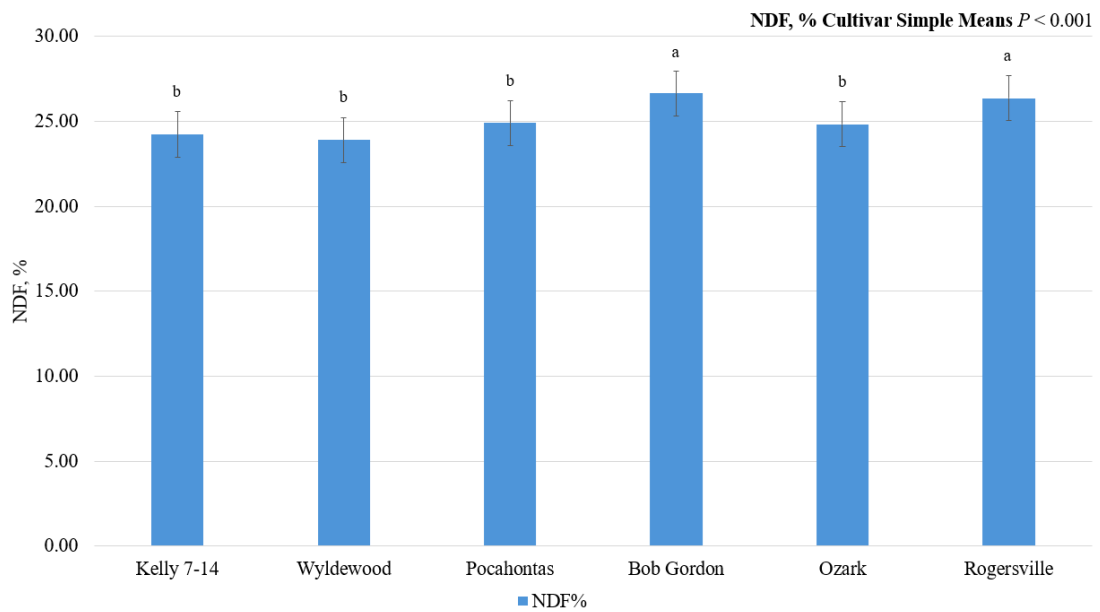


Figure 7: Acid detergent fiber (ADF)% simple means by cultivar in American elderberry at two Missouri locations in 2021-2022 across the growing season.

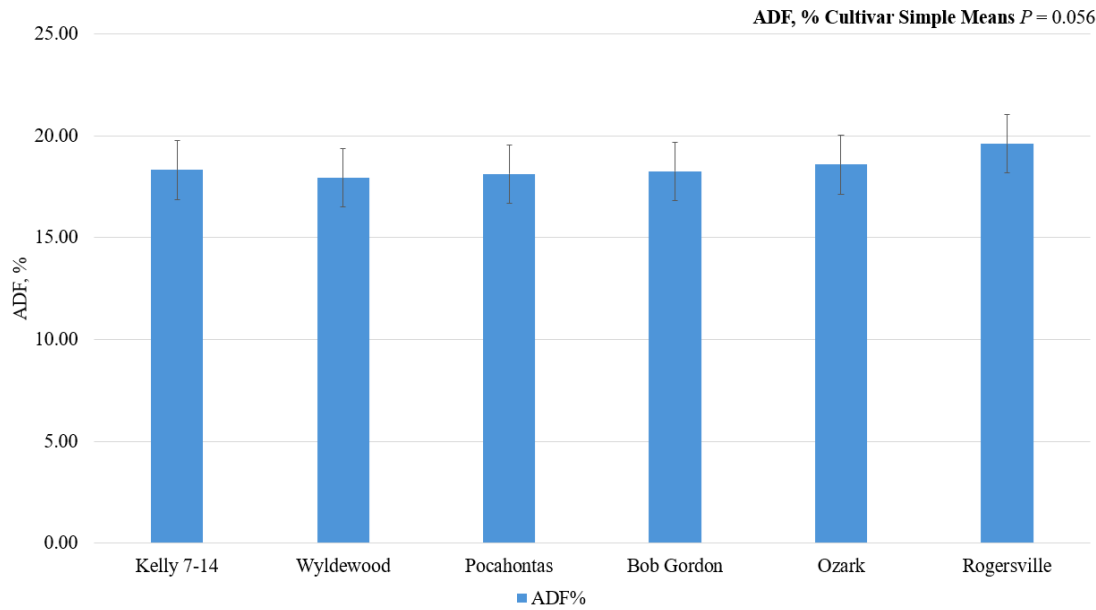


Figure 8: Acid detergent lignin (ADL)% simple means by cultivar in American elderberry at two Missouri locations in 2021-2022 across the growing season.

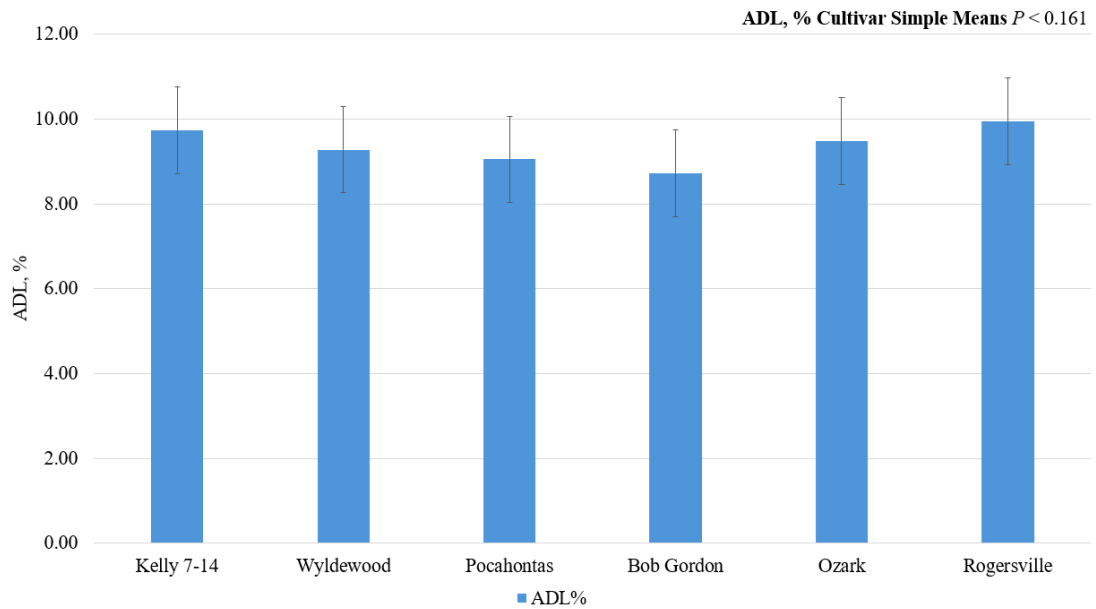


Figure 9: Crude protein (CP)% simple means by cultivar in American elderberry at two Missouri locations in 2021-2022 across the growing season.

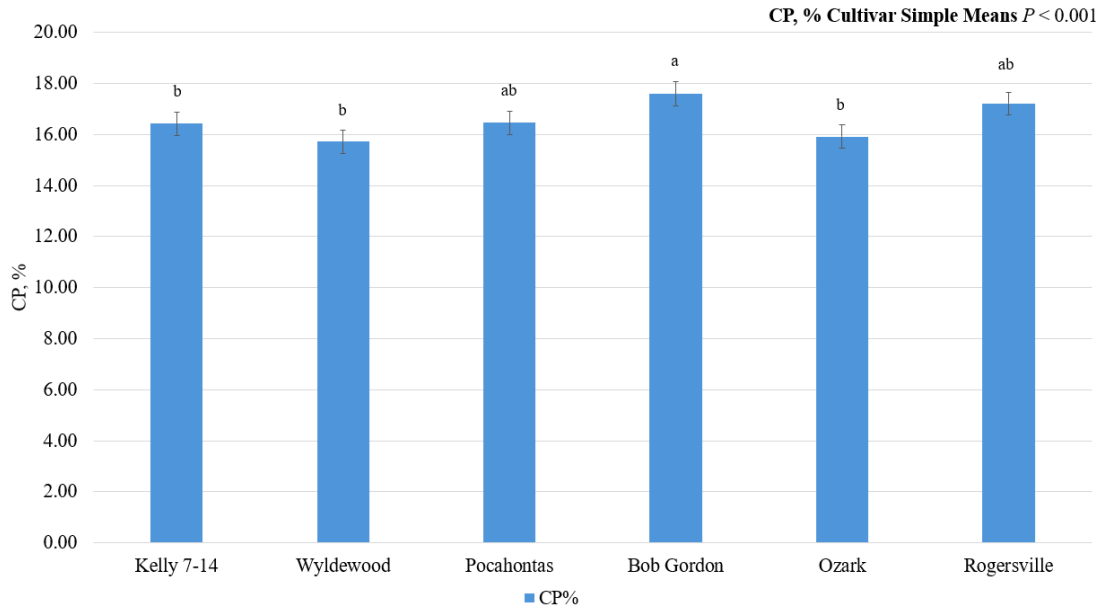


Figure 10: Neutral detergent fiber (NDF)% simple means by location from six cultivars of American elderberry at two Missouri locations in 2021-2022 across the growing season.

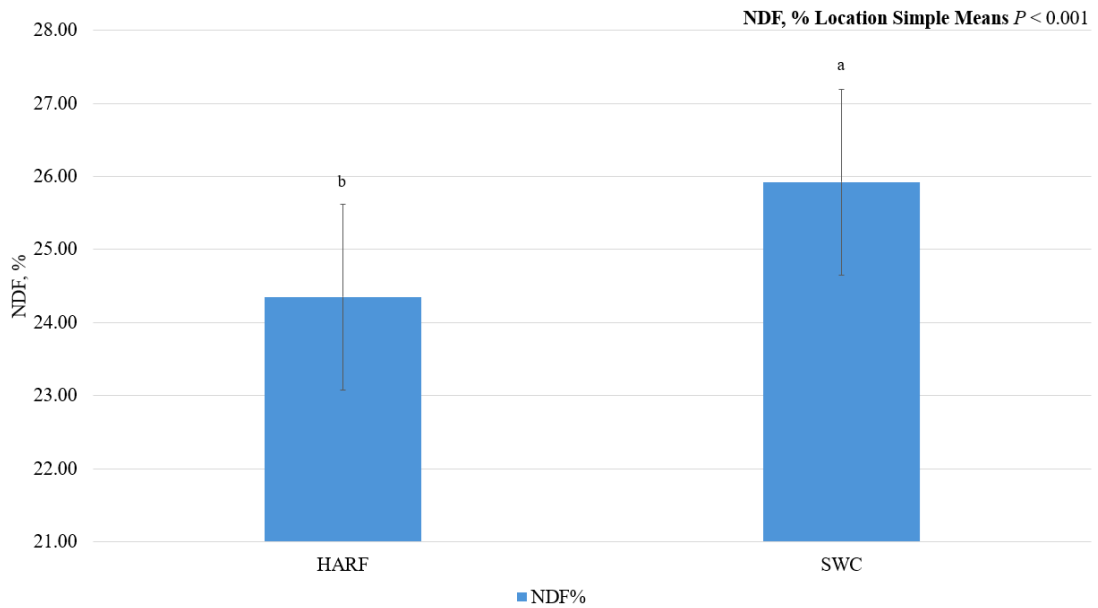


Figure 11: Acid detergent fiber (ADF)% simple means by location from six cultivars of American elderberry at two Missouri locations in 2021-2022 across the growing season.

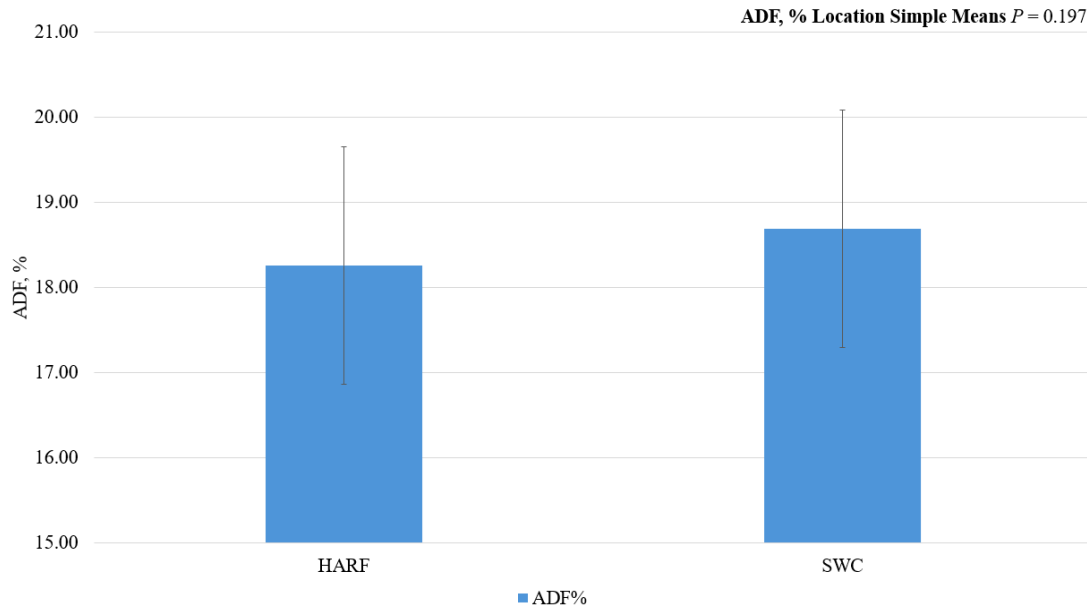


Figure 12: Acid detergent lignin (ADL)% simple means by location from six cultivars of American elderberry at two Missouri locations in 2021-2022 across the growing season.

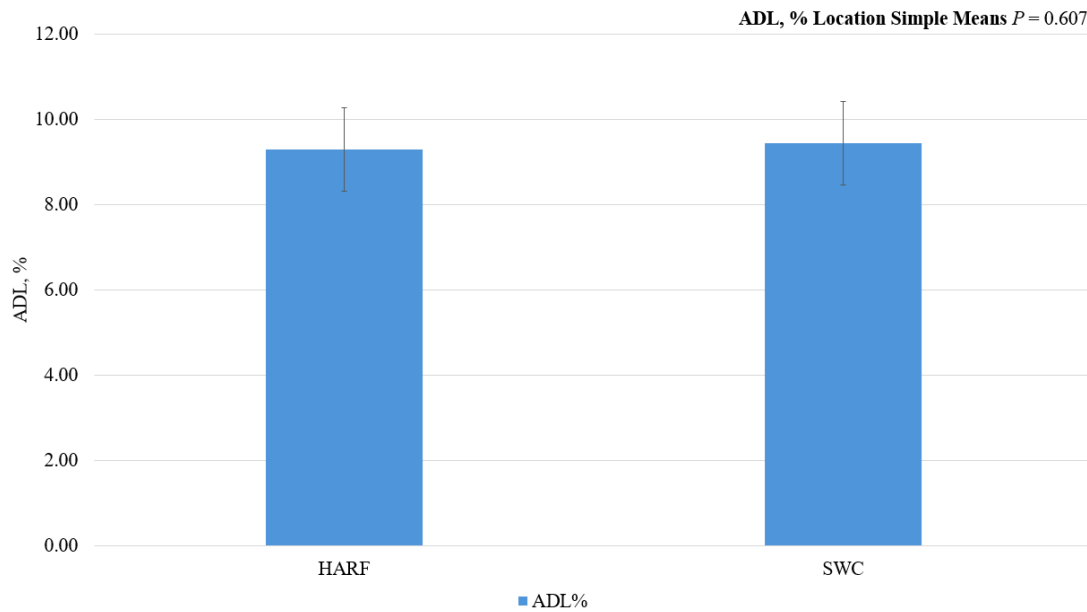


Figure 13: Crude protein (CP)% two-way interaction between location and date from six cultivars of American elderberry at two Missouri locations in 2021-2022 across the growing season.

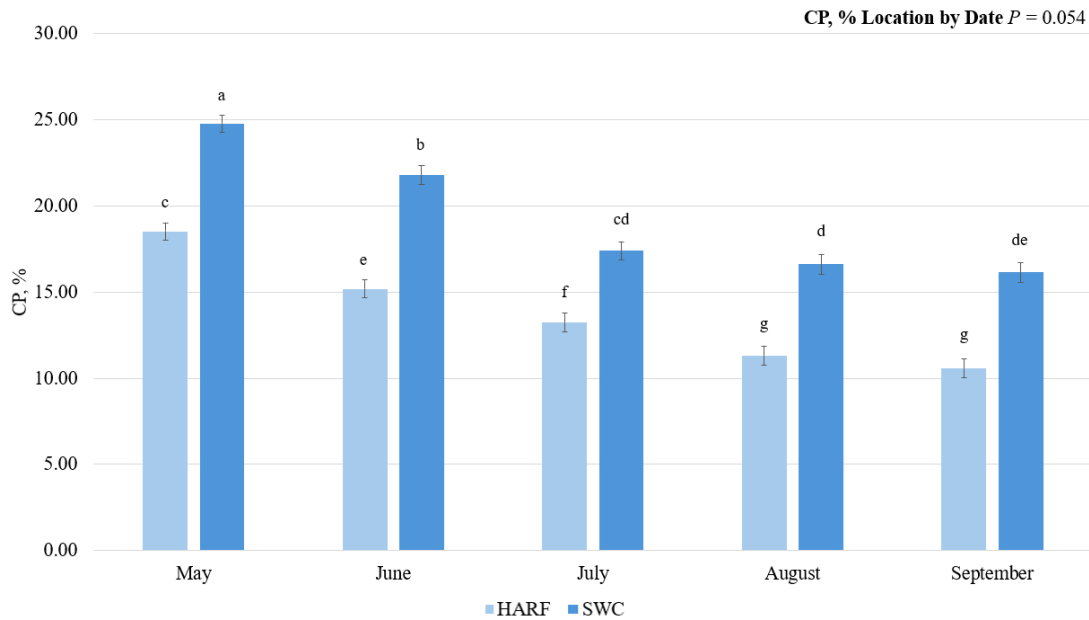


Figure 14: Dhurrin cyanogenic glycoside two-way interaction between location and cultivar in American elderberry at two Missouri locations in 2021-2022 across the growing season.

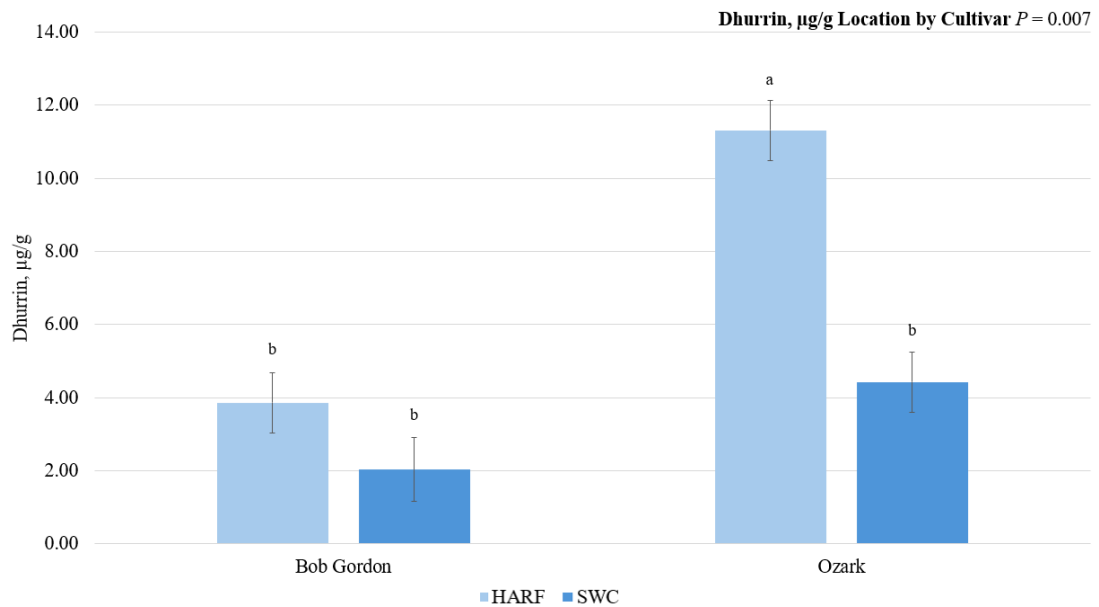


Figure 15: Amygdalin cyanogenic glycoside simple means by date from two cultivars of American elderberry at two Missouri locations in 2021-2022 across the growing season.

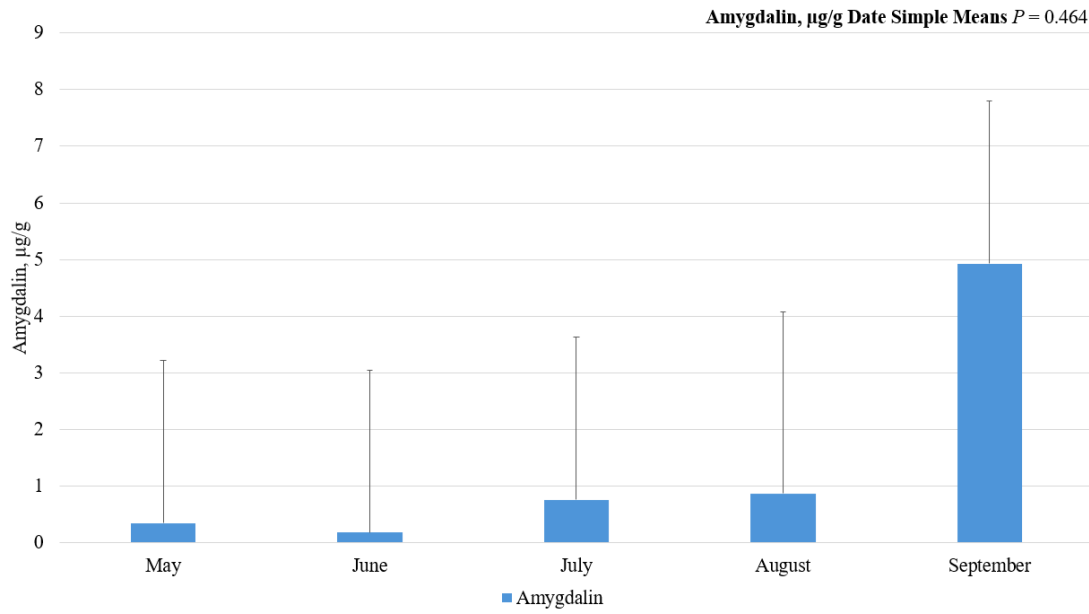


Figure 16: Linamarin cyanogenic glycoside simple means by date from two cultivars of American elderberry at two Missouri locations in 2021-2022 across the growing season.

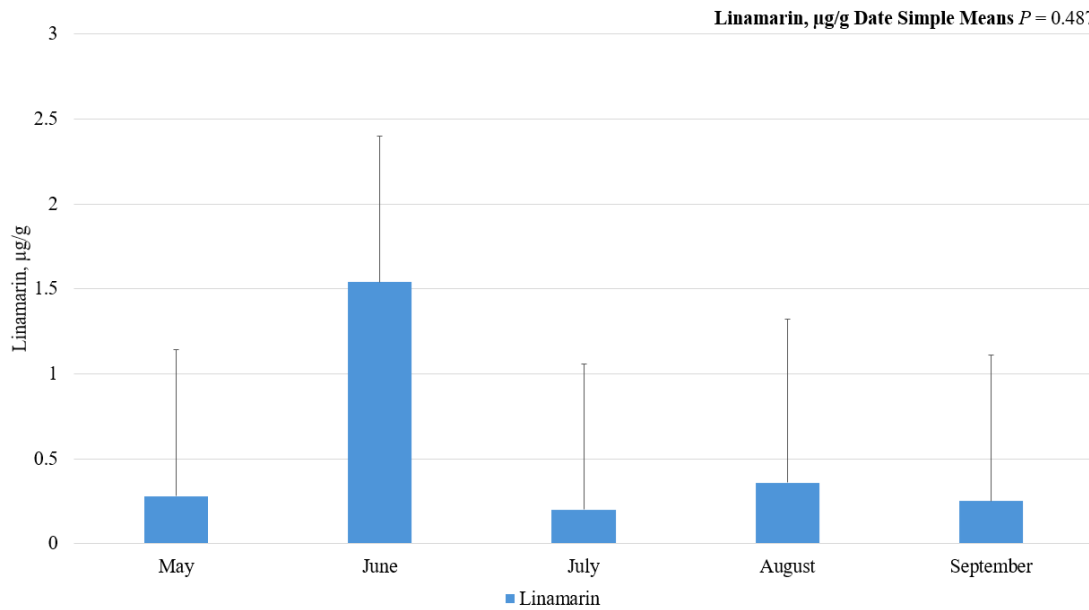


Figure 17: Sambunigrin cyanogenic glycoside simple means by date from two cultivars of American elderberry at two Missouri locations in 2021-2022 across the growing season.

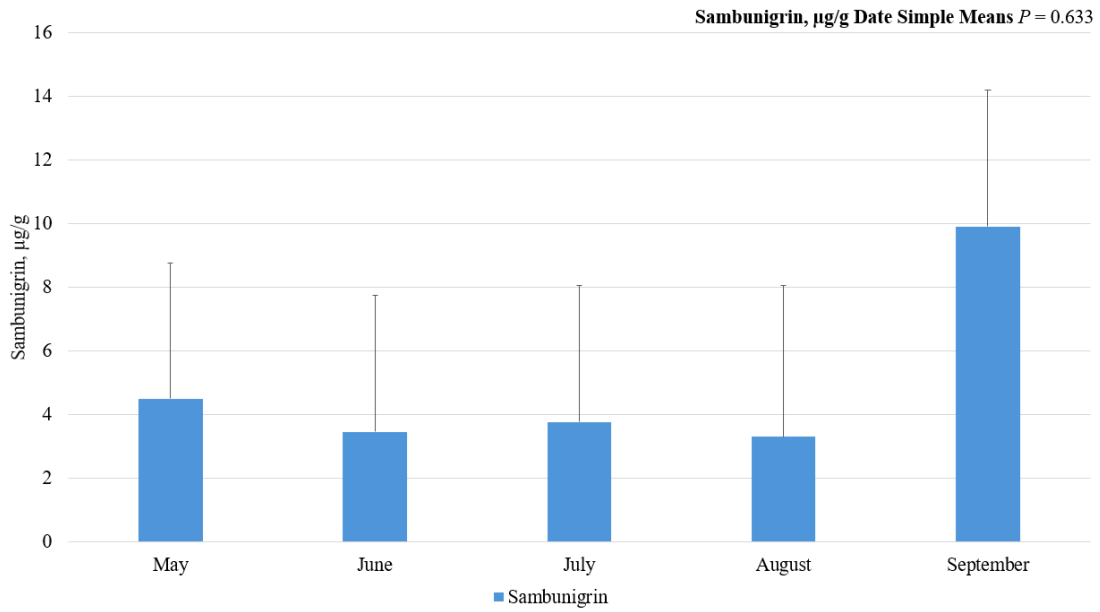


Figure 18: Amygdalin cyanogenic glycoside simple means by cultivar in American elderberry at two Missouri locations in 2021-2022 across the growing season.

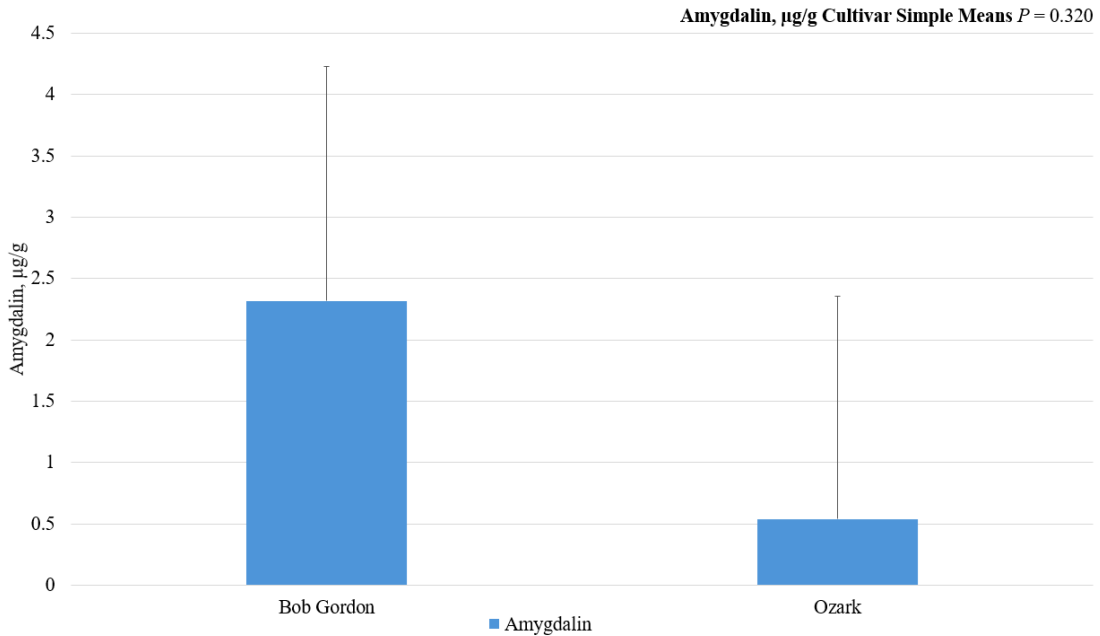


Figure 19: Linamarin cyanogenic glycoside simple means by cultivar in American elderberry at two Missouri locations in 2021-2022 across the growing season.

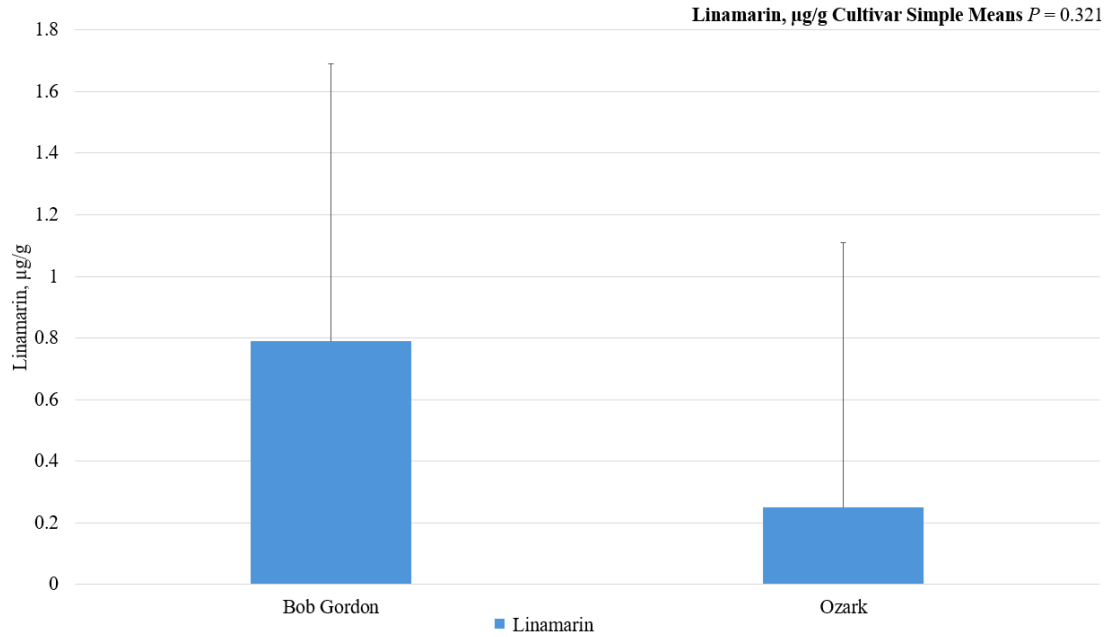


Figure 20: Sambunigrin cyanogenic glycoside simple means by cultivar in American elderberry at two Missouri locations in 2021-2022 across the growing season.

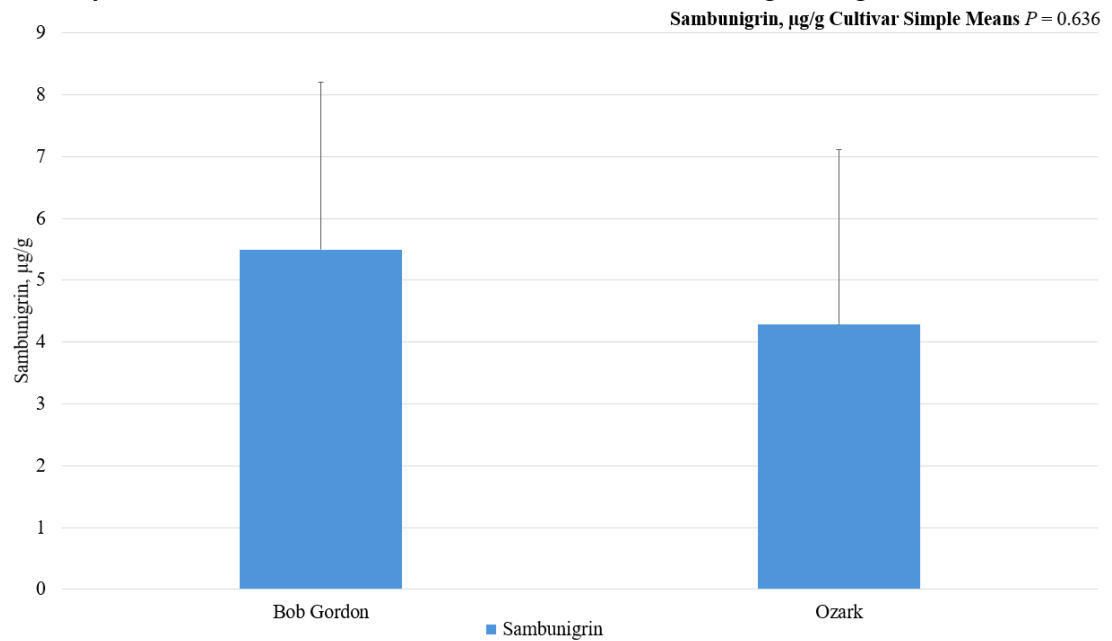


Figure 21: Amygdalin cyanogenic glycoside simple means by location from two cultivars of American elderberry at two Missouri locations in 2021-2022 across the growing season.

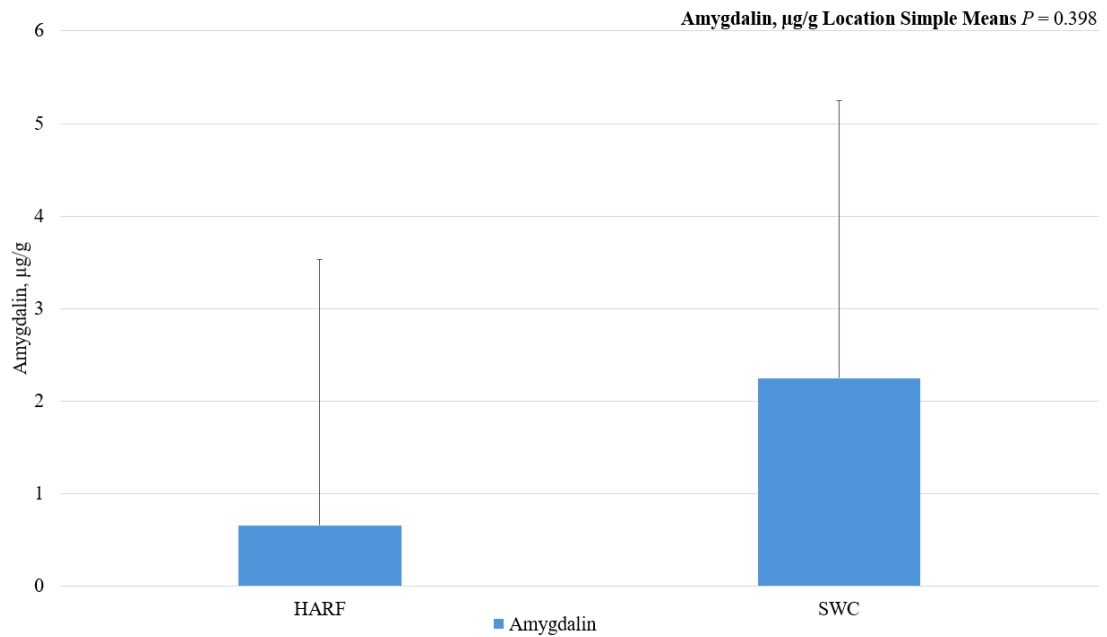


Figure 22: Linamarin cyanogenic glycoside simple means by location from two cultivars of American elderberry at two Missouri locations in 2021-2022 across the growing season.

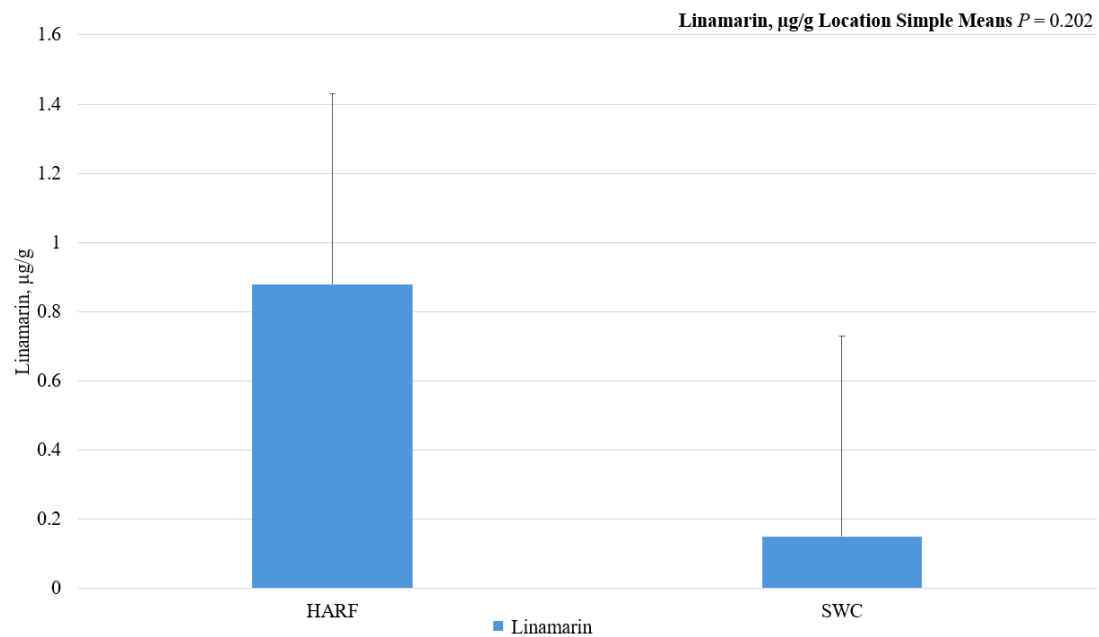


Figure 23: Sambunigrin cyanogenic glycoside simple means by location from two cultivars of American elderberry at two Missouri locations in 2021-2022 across the growing season.

