

*DALEA AUREA, DALEA CANDIDA, DALEA MULTIFLORA AND DALEA
PURPUREA* HERBAGE AND ROOT NITROGEN AND DRY MATTER YIELD AS
INFLUENCED BY SOIL, PHOSPHORUS AMENDMENT AND *RHIZOBIUM*
INOCULANT

A Thesis

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ABSTRACT

Herbaceous, perennial, warm-season native North America legumes can contribute to agricultural objectives. *Dalea aurea* (golden prairie clover), *D. multiflora* (roundhead prairie clover), *D. purpurea* (purple prairie clover) and *D. candida* (white prairie clover) are native to drier prairies and hillsides of Northern Great Plains. Herbage dry matter yield (DMY), root DMY, herbage N DMY, root N DMY and herbage N content were evaluated in Stephenville, TX as a response to soil type, P amendment and *Rhizobium* inoculation. *Dalea aurea* yielded 69% more than *D. candida*, 238% more than *D. multiflora* and 193% more than *D. purpureum*, respectively ($p \leq 0.05$) in herbage DMY. *Dalea candida* produced 80% greater ($p \leq 0.05$) root DMY, than *D. purpurea* which yielded the least. *Dalea aurea* yielded 108% more than *D. candida*, 372% more than *D. multiflora* and 420% more than *D. purpurea* ($p \leq 0.05$) herbage N DMY, respectively. *Dalea candida* yielded 150% more than *D. multiflora*, 114% more than *D. purpurea* and 221% more than *D. aurea* ($p \leq 0.05$) root N DMY. *Dalea aurea* had 25% greater ($p \leq 0.05$) herbage N content than *D. multiflora*, which yielded the least. Overall, soil types affected herbage and root DMY as well as herbage and root N DMY. Sandy loam was the best for all *Dalea* species. Inoculation with commercial cowpea rhizobia and amending those soils with P are not recommended without further longterm investigation.

DEDICATION

I dedicate this thesis to my wonderful and supportive parents and brother. They were with me every step of this journey.

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NOMENCLATURE

ADF	Acid detergent fiber
ADL	Acid detergent lignin
CP	Crude Protein
N	Nitrogen
NDF	Neutral detergent fiber
DMY	Dry Matter Yield
DM	Dry Matter
USA	United States of America

TABLE OF CONTENTS

	Page
ABSTRACT	ii
DEDICATION.....	iii
ACKNOWLEDGEMENTS	iv
CONTRIBUTORS AND FUNDING SOURCES	v
NOMENCLATURE	vi
TABLE OF CONTENTS	vii
LIST OF TABLES	ix
1. INTRODUCTION	1
2. LITERATURE REVIEW	3
2.1. Restoration of Prairie and Rangeland.....	3
2.2. Importance of Forage	4
2.3. Forage Nutritive Value	5
2.4. Benefits of Legumes.....	6
2.5. Native Legumes in Livestock Systems	7
2.6. Soil pH and Legumes	9
2.7. Phosphorus and pH Response of Pasture Legumes	11
2.8. <i>Dalea</i> spp. Agronomy or Features of <i>Dalea</i> spp.	12
2.8.1. <i>Dalea aurea</i>	14
2.8.2. <i>Dalea candida</i>	16
2.8.3. <i>Dalea multiflora</i>	17
2.8.4. <i>Dalea purpurea</i>	18
2.9. Soil Types	20
2.9.1. Potting Soil.....	20
2.9.2. Sandy Soil	21
2.9.3. Sandy Loam Soil	21
2.9.4. Caliche Soil	22
2.10. Effects of Soil Types on <i>Dalea</i> spp.....	22
3. MATERIALS AND METHODS.....	26

3.1. Seed Sources	26
3.2. Greenhouse Method	26
3.3. Growing Conditions/ Study Site/ Experimental Design.....	26
3.4. Treatment (Factors).....	27
3.4.1. Phosphorus	27
3.4.2. <i>Rhizobium</i>	27
3.5. Soils	28
3.6. Harvesting Methods to Determine Plant Dry Matter Yield and Nutritive Value .	28
3.7. Laboratory Assays.....	29
3.8. Statistical Analysis	29
4. RESULT AND DISCUSSION	30
4.1. Herbage Dry Matter Yield	30
4.2. Root Dry Matter Yield	32
4.3. Root Dry Matter Yield Species x Rhizobium.....	34
4.4. Phosphorus.....	37
4.5. Nitrogen Content (%).....	41
4.6 Nitrogen Dry Matter Yield	41
5. CONCLUSIONS AND SUMMARY	44
REFERENCES	47
APPENDIX A	64

LIST OF TABLES

	Page
Table 1. Soil analysis before the experiment.	64
Table 2. Forage dry matter yield (g/plant) by species and soil types (species x soil type $p \leq 0.05$).....	64
Table 3. Root dry matter yield (g/plant) by species and soil types (species x soil type $p \leq 0.05$).	65
Table 4. Root dry matter (g/plant) response of species to <i>Rhizobium</i> inoculation (species x <i>Rhizobium</i> $p \leq 0.05$).....	65
Table 5. Soil analysis report after experiment (-P).....	66
Table 6. Soil analysis report after experiment (+P).....	67
Table 7. Nitrogen content (%) of herbage (g/plant) as affected by different species ($p \leq 0.05$).....	68
Table 8. Nitrogen dry matter of herbage (g/plant) as affected by different species and soil type ($p \leq 0.05$).....	68
Table 9. Nitrogen dry matter yield of root (g/plant) as affected by different species and soil type ($p \leq 0.05$).	68

1. INTRODUCTION

Finding legumes for a variety of land applications, for instance addition of diversity to indigenous, restored plant communities or addition of forage sources for wildlife and livestock is a developing interest. Applications of this kind could as well comprise cropland improvement, habitat restoration, prairie and rangeland restoration, and many more (Muir and Bow, 2008; Muir et al., 2008). This study's outcome could support the integration of these species for several applications. Warm-season perennial grasses in the southern United States of America (USA) offer major forage sources for livestock between early July and May, whereas poor growth through mid-September from mid-July (Evers, 2008; Rao et al., 2009).

Generally, legumes generally have greater CP (crude protein) digestibility and content, as well as less fiber and intakes when compared to grasses for ruminant nutrition (Ball et al., 2001). Native legumes are not being broadly integrated into rangeland reseedings or managed pastures at the present. More native legumes could offer important protection and food for local wildlife on top of enhancing the profits to grazing livestock if they were applied in complementing pastureland and range (Packard et al., 2004).

Normally, native species will be more advantageous as compared to introduced species or non-native and capable of better competing for resources. This study's objective was to evaluate *Dalea aurea* Nutt. ex Pursh, *D. candida* Michx. ex Willd., *D. purpurea* Vent. var. *purpurea*, and *D. multiflora* (Nutt.) Shinn's performance, as well as

development in four different potting soils: commercial potting soil, sand, sandy loam, and caliche in greenhouse conditions in Texas. In doing so, this study will further explore their forage nutritional values, growth, root dry matter and herbage dry matter yields (DMY).

2. LITERATURE REVIEW

2.1. Restoration of Prairie and Rangeland

There were approximated 162 million hectares of native prairie in the Great Plains of North America before European agricultural practices were introduced (Samson and Knopf, 1994). Today, areas covered by native prairies and rangeland have declined as a result of development, agricultural practices, overgrazing and recreation, though they still constitute about 61% of the United States land surface (Fuhlendorf and Engle, 2001; Samson and Knopf, 1994). Loss of range habitat and prairie often brings about reduction of wildlife diversity, augmented erosion and reduction of plant diversity (Fuhlendorf and Engle, 2001; Samson and Knopf, 1994). For total restoration to achieve, it can take centuries, making it a challenging task to accomplish.

The loss of rangeland and prairie environments have brought about rise in restoration struggles that regularly consist of reseeded damaged areas. When reseeding, it is important to take into consideration the native plant species to an area, whereby the seed is coming from and whether the seed species mix is capable of co-existing (Archer and Pyke, 1991). When reseeding these areas with native species, seeds that are locally native are preferred due to adaptation to edapho-climatic and biological conditions (Broadhurst et al., 2008; Archer and Pyke, 1991). The rationale is that local seeds are produced in environment comparable to the restoration location, are likely to have genes similar to the original plants, and will probably be better adapted to the conditions (Broadhurst et al., 2008). However, using genetic material from farther sources can

however have advantages for instance addition of genetic diversity that might make the plants more appropriate in persisting (Broadhurst et al., 2008). Distinctions in genomic manifestation in later life forms arise from environmentally prompted evolutionary adaptations of the organisms. Thus, according to Broadhurst et al. (2008), altering the environment can help to diversify gene presentation and improve the reproductive capacity of the population.

He also argued that the use of local seed only is probably going to underwrite restoration failure since inbreeding has an impact on the overall ability and fitness of a plant to fine-tune to changes in the environmental.

2.2. Importance of Forage

In accordance with Evers (2008), forage systems should satisfy ruminant nutritive requirements year-round to maximize fiber, meat or milk yields. In the southcentral USA, about 90% of agricultural land is rangeland or pasture, where much of the summer forage quality does not meet livestock nutritional needs(Greene, 1997). Forage quality can affect an animal's wellbeing and performance directly (Ball et al., 2001).

Forage supplies inexpensive nutrients for livestock as they reduce the necessity of offering supplementary feed, which can be expensive (Ball et al., 2001). In addition, the institution of high quality forage to the diet of an animal can improve the health of an animal, reproductive efficiency and resistance to disease and parasites (Ball et al., 2001; Kanani et al., 2006).

Maintaining and managing suitable forage systems can be more complicated compared to fiber and grain crops as a result of the necessity of managing numerous organisms, essentially the forage plants, including the animals that utilize the forage (Evers, 2008). The main challenge in managing forage for livestock is irregular forage quantity and quality distribution among seasons (Evers, 2008). Sometimes, this can be compensated for through the addition of new species for forage, especially high-protein, digestible legumes (Barnes et al., 2003; Pecetti et al., 2009). Warm-season perennial grasses in the southern USA offer a major forage sources and normally the highest produce between early May to July but subsequent growth is poor through mid-September from mid-July (Evers, 2008; Rao and Northup, 2009). Legumes can mitigate decreasing summer grass forage quantity and quality.

2.3. Forage Nutritive Value

Proper nutrition is important for the production and development of an animal. A approximation of the way a certain plant species is going to meet the nutritional needs of a ruminant can be achieved by measuring nutrient concentration (Ball et al., 2001). Knowing and measuring the nutritive values of forage species can assist in finding out if it is fitting for integration into a pasture that is meant for grazing by livestock, when the plant is at its highest nutritive value, as well as the way of managing the plant to maintain that value at peak levels.

Dry matter is the weight of the forage material following removal of all water via a drying process (Ball et al., 2001). Likening of dry weights enables more direct and

precise comparisons between forages and is applied in approximating total produce (Ball et al., 2001).

2.4. Benefits of Legumes

Legumes have been used by human beings in improving agricultural production as well as natural soil fertility, sources of food, and as animal feed (Abdin et al., 1997; Power, 1987; Rao and Northup, 2009; van Kessel and Hartley, 2000). In addition, legumes can be applied as grain production, for green cover and forage in order to reduce pollution, offer organic nitrogen, increase soil organic matter, reduce runoff and erosion and prevent the loss of soil nitrogen through leaching (Abdin et al., 1997; Power, 1987; Rao and Northup, 2009; van Kessel and Hartley, 2000).

Legumes are among the limited families of plant capable of utilizing atmospheric nitrogen and converting it to a plant-accessible form. This is possible through a symbiotic relationship with soil *Rhizobium* spp. that produces root nodules on the legume (Combs, 1936; Power, 1987; van Kessel and Hartley, 2000). Brady (1984) observes that nodules, which host surface organisms, form when the genus *Rhizobium* bacteria are injected from the environment into the cortical cells and root hairs. Legumes are able to fix N, in accordance with conditions and species that benefits other plants and increases soil nutrient values (Becker and Crockett, 1976; Power, 1987).

Biologically-captured atmospheric N can assist in decreasing consumption of industrial fertilizer for agriculture in regions where legumes can be present (Barnes et al., 2003; van Kessel and Hartley, 2000). In addition, adding N helps in increasing the

sustainability of grazing systems and provides higher protein forage to livestock (Pecetti et al., 2009).

Generally, legumes have better digestibility, CP concentrations, less fiber and intakes in comparison with grasses for ruminant nutrition (Ball et al., 2001). Similarly, legumes are capable of extending grazing seasons for both wildlife species and livestock (Barnes et al., 2003; Pecetti et al., 2009). Legumes can increase total forage quality and yield as well as provide a better distribution of forage all through the year when combined with other plant species like grasses (Sleugh et al., 2000; van Kessel and Hartley, 2000). Additionally, legume-grass mixtures can lessen weedy species encroachment (Sleugh et al., 2000).

Native legumes are presently not being integrated broadly into rangeland managed pastures or reseedings. In a situation where more native legumes were applied in complementing rangelands and pasture, they could offer vital food and protection for local wildlife apart from improving livestock grazing (Packard et al., 2004). Normally, native species will have benefits over introduced species or non-native and can regularly compete for resources more effectively.

2.5. Native Legumes in Livestock Systems

North America's native legumes are very important botanical components for grassland reclamation or rangeland reseeding. Normally, legumes increase the grass-legume combinations' protein matter applied for grazing (Schultz and Stubbendieck, 1983).

In north-central USA, the current major pasture system for beef production is founded on cool-season grasses like wheat, smooth brome grass and tall fescue that become unproductive and dormant during the months of summer. Bermudagrass and bahiagrass monocultures in southeastern USA are dominant and they show a summer quality slump (Barnes et al., 2003). In the south-central USA, a number of cattle producers rely on native warm season grasses, mainly on rangeland, that are neither of good quality during summer months nor productive. There is a common problem to all these livestock grazing systems: they are in need of high-protein warm-season, and actively-growing legumes to boost animal production during the hot summer months that are dry (Gerrish and Roberts, 1999).

Perennial legumes are uncommon constituents of woodlands and prairies native plants because the number of species have been reduced in overgrazing (Muir and Pitman, 2004). In North America's southern regions, there is potential for increasing native legumes into grass monocultures (Muir and Pitman, 2004).

Many native herbaceous legumes grow in the southern Great Plains grasslands of North America (Muir et al., 2015). A case in point is Diggs et al. (1999), who identified 50 species that are native in north-central Texas and some of their potential has been assessed (Muir et al., 2005; Muir et al., 2005). Genera that contain perennials include *Mimosa*, *Desmanthus*, *Dalea*, *Strophostyles*, *Desmodium* and *Lespedeza* (Muir et al., 2015). There is, however, very little known about their agronomic potential and very few are currently cultivated (Muir et al., 2018).

There are a number of challenges that these legumes face before being more widely used. These include seed production potential, seedling establishment, marketing, and competitiveness in ruminant ecosystems (Muir et al., 2015). In southern North America, there are very few domesticated, commercially available perennial warm-season legumes, introduced or native, which persists and establishes effectively in drier ecosystems like northcentral Texas (Muir et al., 2018). Native herbaceous species, comprising *Dalea* spp., could fill this niche (Muir et al., 2005).

2.6. Soil pH and Legumes

There are major features of soil that affect the development and adaptability of forage legume species, including nutrient levels, soil pH and texture or drainage (Evers, 2003). Forage legumes are more sensitive to soil pH as compared to grasses (Elliott et al., 1973). It is important to understand soil pH preferences so that species for specific places are selected and their establishment improved (Evers, 2003). Soil pH is among the important factors that affect development and growth of legume in part because Mn, Al, Mo, and P uptake are affected (Taylor and Quesenberry, 2010). There are a number of reports suggesting that pH may have an impact on development and growth of plant separately of other outcomes (Tanaka et al., 1984; Yokota and Ojima, 1995).

Soil pH has an impact on plant growth since it influences availability of nutrient as well as toxicity of a number of features for instance Mn and Al (Rengel, 2005). A point in case is high soil pH that may bring about Fe-deficiency chlorosis in forage legume species that grow on calcareous soils (Evers, 2003). Almost nothing is known about soil pH requirements of native, perennial herbaceous Texas legumes.

In soil with $\text{pH} \leq 5.2$, aluminum toxicity is observed (Rahman et al., 2018). In soil solution, excessive Al ions can bring about soil cation saturation exchange positions (Evers, 2003). In this situation, essential nutrients for plant growth are replaced by Al ions on clay surfaces or soil organic matter, greatly reducing their bioavailability. However, this consequent Al toxicity and sensitivity to pH, differs more extensively amongst warm-season (sub-tropical and tropical) legumes as compared to in legume species and temperate grass. Edmeades et al. (1991), for example, found that subterranean clover was most tolerant, whereas red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) were less tolerant to low soil pH.

Liming of acid soils has been a traditional approach to increase crops yields. On acid soils, poor legume growth results from solubility or activity of Mn and Al at low pH (Tisdale et al., 1985). Both Mn and Al can be openly toxic to plants and reduce uptake of Mg and Ca. In addition, zinc can reach levels of toxic on acidic soils (Marschner, 2012). In contradiction of this, Mo and P can become less accessible on acid soils as a result of their reaction with iron (Fe) and Al (Tisdale et al., 1985). According to Rice et al. (1977), nitrogen fixation in plants, which is dependent on specific strains of Rhizobium, is acid sensitive. Accordingly, extremely lower pH values (below 6.0) have a negative effect on legume rhizosphere and soil colonization. On some soils, a pH that is above 7.0 can constrain crop harvests. As levels of pH rise, all micronutrients obtainability apart from Mo is decreased (Tisdale et al., 1985). Acid soils that are highly weathered may be specifically vulnerable to over-liming. In the structure of these soils, liming can bring about deterioration and decrease some minerals availability (Kamprath, 1971).

2.7. Phosphorus and pH Response of Pasture Legumes

The main limiting nutrient for pasture production and crop in several environments is phosphorus (P). In soil, P is relatively immobile and frequently insufficient to a number of plant species (Vance et al., 2003).

Several studies have revealed that P is the most important nutrient for growth and establishment of legumes, despite the fact that P requirement of lines and species differ (Vadez and Drevon, 2001). Phosphorus deficiencies can negatively influence nodule function in legumes, host plant growth and symbiotic rhizobial establishment (Gutiérrez-Boem and Thomas, 1998; Israel, 1987). In dryland, soil phosphorus (P) and pH are usually low, whereby their impact on pasture legumes are negative (Maxwell et al., 2013). In literature on pH forbearing legumes and P efficient which could offer options to white clover, little facts are however existent.

According to Brown and Green (2003), farmers could benefit from a greater variety of annual and perennial legume species for their dryland farming systems. There is little available information on alternative species for upland environments when it comes to optimum nutrient or soil conditions for growth. At higher population concentrations, for legumes to compete with grasses and grow, available soil P is necessary (Caradus, 1980). For legume persistence and production, sufficient plant-available soil P is critical.

On the other hand, optimum soil P content for pasture legume species is rare. In addition, pasture legumes are sensitive to acidic (low pH) soil conditions (Rengel, 2005) especially when it comes to aluminum (Al) toxicity which affects legume establishment, persistence and production. Soil acidity and related aluminum (Al) toxicity therefore inhibit legume survival in grazed grasslands (Moir and Moot 2014). In agricultural systems, utilization of lime is applied in increasing soil pH. Native legumes capable of persisting and thriving in low pH soils, or with low inputs of lime and P, would be appropriate to this environment and may be advantageous to dryland farming. Comparable to plant P information, literature that report soil pH tolerance and optimum soil pH ranges for individual species of legume is limited. According to Dear and Ewing (2008), a probable benefit of novel perennial pasture species could be their adaptation to compounding environmental conditions such as acid soils, low nutrients, or low rainfall.

2.8. *Dalea* spp. Agronomy or Features of *Dalea* spp.

In herbaceous warm-season legume species, an extensive ecological range in rangeland reseeding and pasture species mixes usually brings about better sward establishment success in diverse specific ecosystem gaps, as determined by micro-climatic conditions and soil (Schellenberg and Biliget, 2015). Ecotype releases, as against broadly studied cultivars, offer a genetic basis to specific seed ecotype germplasm releases and selection that better address native reseeding objectives (Schellenberg and Biliget, 2015). My research will focus on soil adaptability of four *Dalea* spp.

According to Diggs et al. (1999), *Dalea* spp. are flowering herbaceous plants in the Fabaceae family with several endemic to northcentral Texas. These are commonly referred to as prairie clover in North America. Their centers of origin are generally in Mexico (115 species) and northern USA (23 endemic species) (Barneby, 1978). Prairie-clovers are small shrubs or perennial forbs that bear numerous spikes or flowering racemes that are densely crowded with small hermaphroditic plants between July and May (Cane et al., 2012).

Numerous *Dalea* spp., such as *D. Lanata*, *D. Candida*, *D. Formosa* and *D. Emarginata*, are good cattle forage as well as being utilized by native fauna. Purple prairie clover (*D. purpurea*) has an especially good forage nutritive value with condensed tannin (McGraw et al., 2004; Berard et al., 2011).

In grassland restoration seedings, the majority of *Dalea* spp. necessitate seed scarification before planting (Schellenberg and Biligetu, 2015). Usually, rate of germination linearly rises as temperature increases up to maximum, then it linearly decreases to a maximum temperature (Steinmaus et al., 2000; Bradford, 2002).

For *Dalea* spp., seed germination research has focused on seeds that are well-rounded and larger (Molano-Flores et al., 2011). The dissimilarities in seed shape and length of these species is possibly the outcome of environmental stressors that comprise moisture and temperature whereby plant-useful resource distributions have an impact on seed exceptional such as length of time, amount of endosperm or super pollination mechanisms for instance xenogamy, autogamy or geitonogamy (Molano-Flores et al., 2011).

Since the 1970s, there has been limited information and very little significant research available on rhizobial strains required for native Texas legume inoculation. Bushnell and Sarles (1937), came to the conclusion that the cowpea miscellany is comprised of *Chamaecrista* (previously *Cassia*) and *Desmodium*, together with *Lepedeza* rhizobia, but noted the difficulties involved in the study and classification of *Astragalus*, *Dalea* (previously *Petalastemum*) and *Amorpha* spp., as well as their rhizobia. Therefore, there is possible reliance on the general rather than specific rhizobial inoculants' selection for legumes to be successfully established (Thrall et al., 2005). So far, there is complete absence of knowledge on inoculation practices, as a survey of 38 tallgrass prairie managers tasked with the management of more than 12,000 ha in 11 states of the USA where no mention was made of rhizobial inoculation being a beneficial management for seeding native legumes (Rowe, 2010). In many cases, there is still lack of knowledge with regards to the species and even in the cases the genus of specific *rhizobia* for native legumes (Graham, 2005).

Dalea, a native plant in the Americas belong to the *Fabaceae* legume family, it is assertive that it has distinct species that include; *Dalea aurea*, *Dalea candida*, *Dalea purpurea* and *Dalea multiflora* among others.

2.8.1. *Dalea aurea*

Dalea aurea, commonly referred to as golden prairie clover, is a North American native plant that grows well in dry soils as well as in rock gardens. It is endemic from in eastern North America to western Texas (Diggs et al., 1999) and from the southern USA to the north. It is an erect, perennial plant that produces a cluster of unbranched stems

that are 20 to 90 cm long (Belofsky et al., 2006). Although the plant is green in color, it has fewer leaves compared to other plants that have considerably higher number of leaves. Its leaves are thin, facilitating its adaptation to low-moirture habitats (Ruthven, 2007).

Golden prarie clover inhabits gravelly prairies and, to a lesser degres, silty prairies usuually over limestone environments (Jin et al., 2015). Plant growth rate depends on the month of the year and July is often the month when *D. aurea* grows to its maximum (Carter and Lym, 2018) favorable conditions stimulate its growth. By contrast, it has a low growth rate from January to April. However, the growth picks up from the month of April steadily and reaches its maximum in July (Scott and Baer, 2019).

Dreesen and Harrington (1997), found that *D. aurea* seeds have a hard seed coat, a feature that impairs its germination. In ensuring the germination of the seeds, scarification before sowing improves both the germination rates as well as speed. Hot water scarification is accomplished by pouring hot water on the seeds, and soaking them in warm water for up to one day (Schellenberg and Biligetu, 2015). That allows the seeds to imbibe moisture and become swollen, thereby breaking the seed coat. While sowing in greenhouses is done in early spring, transplantation into their permanent positions is done in summer (Griffiths and Huxley, 1994). Due to their hard seed coats, *D. aurea* has a low germination rate without scarificatiioni and takes considerably longer periods to germinate where some may not germinate at all.

2.8.2. *Dalea candida*

Dalea candida is also referred to as white prairie clover and is native to various dry grasslands as well as hillsides in the Great Plains (Khanal et al., 2018). It may be considered an enduring species in the North America where it is commonly found. McGraw et al. (2004), indicated it is a Missouri native perennial found in rocky open woods, glades, as well as prairies across the state. The plants can flourish in average, medium, and well-drained soil exposed to full sun. It has a thick taproot that goes deep, enabling the plant to tolerate drought. During flowering that takes place in early summer, the plant is cross-pollinated by honey bees and wasps. Its native range extends west to the Rolling Hills of Texas (Diggs et al., 1999). White prairie clover inhabits semi-arid prairies and upland backwoods of North America with depleted sandy, gravelly to silty soils, circulated crosswise over Ontario, Manitoba, Saskatchewan, and Alberta in Canada and northern states in the United States of America (Salas-Luévano et al., 2017).

Throughout the growing season, white prairie clover biomass contains consolidate tannins that improves rumen protein usage and diminishes the danger of bloat in ruminants (Khanal et al., 2018). Moreover, consolidated tannins have antibacterial properties in some animal gastro-intestinal tracts (Khanal et al., 2018).

Recent research has examined white prairie clover seed germination, rhizobial inoculant conveyance, scavenge and seed yield, and rummage dietary quality (Khanal et al., 2018). Nonetheless, most of these investigations concentrated on a solitary wellspring of white prairie clover germplasm, 'Impala', which was released in 2000 from the North Dakota and Montana Plant Materials Centers in the USA (Khanal et al., 2018).

Seed germination of *D. candida*, like that of other *Dalea* spp., depends on the size of its seeds. These tolerate ecological stressors differently, including temperature and dampness among others (Khanal et al., 2018). Germination of *Dalea candida* increases from 3 to 70% following high-temperature scarification (Graham et al., 1999). According to Khanal et al. (2018), seed germination is one of the major constraints to the domestication of *D. candida* where the seed has a low emergence in greenhouse environment, unlike *D. purpurea*. Indeed, maximum seed germination was recorded at 41%, disclosing the plants' poor stand establishment (Molano-Flores et al., 2011). However, after germination, the plant is persistent for over three seasons after its establishment and its survival rate ranges between 64 to 88% (Khanal et al., 2018).

2.8.3. *Dalea multiflora*

Dalea multiflora, known as white or roundhead prairie clover, is a flowering herbaceous perennial legume associated with rocky limestone terrain that grows from 300 to 100 cm in height. (Everitt and Drawe, 1974; Springer and Thacker, 2017). According to Khanal et al. (2018), the native habitat of *D. multiflora* is in clear woodlands and meadows from central to southeast Texas, with a preference for clay and limestone soils. It requires less water and more sun, allowing it to perform best in areas with dry soil. It survives extreme cold as well as hot temperatures. Like other leguminous plants, it improves soil fertility and is a source of seeds for animals (Bird and Choi, 2017). At the onset of summer, between June and July, it produce white, spike-like flowers (Everitt and Drawe, 1974) leading to its seeds being dispersed by animals since it attaches itself on their bodies. Furthermore, the species is considered a

colonizing species in that it has the ability to grow and mature after normal dispersal of its seeds in favorable locations. The plant can sustain harsh climatic conditions such as soil moisture, cold climatic conditions and high heat levels (Flores de la Torre et al., 2018).

2.8.4. *Dalea purpurea*

Dalea purpurea is also referred to as purple prairie cover and it originates from a strong tap root that penetrates the soil up to a depth of six inches (Gustafson et al., 2002). It is a perennial forb, 20-90 cm tall, with a woody stem. Leaves are 1-4 cm long, with 3 to 7 flyers. The inflorescence is 1-7 cm spike situated at the closures of the branches. Branches are various, more often than not 3 per stem; however, some have upwards of 10 to 12. The developed purple prairie clover has a coarse, nonfibrous root framework with a solid woody taproot that can reach 1.7-2.0 m deep. The taproot support minutely spread sidelong roots. The pod is a 1-to-2-seeded case encased in bracts (Fenner et al., 2018). During its young stages, *D. purpurea* plants have single stems with no branches. However, as it matures, plants develop additional stems in a bushy vase - shaped (Wang et al., 2013). Upon its maturation, the plant is approximately 20 to 75 cm and develops woody stems. It has numerous leaves that have 3 to 7 leaflets (Mischkolz, 2013).

Bjugstand and Whitman (1982), utilized an assortment of forbs for recovery of strip-mined land and found that purple prairie clover demonstrated "astounding" germination and consequent "enthusiastic" development in the nursery. The capacity of purple prairie clover to recover vegetatively after herbivory is unknown. Meier and

Weaver (1997), indicated that purple prairie clover does not replicate abiogenetically. In any case, Towne and Knapp (1996), recommend that purple prairie clover grows from the root crown following above-ground burns. Further research is required on the capacity of purple prairie clover to recover from aboveground vegetation removal (Wolti et al., 2016).

Purple prairie clover is germinated at soil temperatures running from 15 to 30 °C (Cane, 2006). However, temperatures below 5 °C break seed dormancy, facilitating its germination (Cane, 2006). A review of local plant agriculture in Minnesota demonstrated low rates of germination of purple prairie clover. However, the germination of plant is improved by scarification, irritating litter as well as duff layers to uncover soil, and stratification (Flores de la Torre et al., 2018). When introduced to greenhouse conditions, purple prairie clover has a superior germination as well as a subsequent vigorous growth as compared to other varieties of forbs used for soil reclamation purposes. That is confirmed by Bjugstand and Whitman (1982), in their study of a variety of dalea species. When transplanted to reclaim various degraded areas, the purple prairie clover exhibited excellent growth as well as vigor. However, its ability to regenerate vegetatively is not clear because, as Meier and Weaver (1997), pointed out, the plant does not reproduce asexually. However, the plant sprouts from the root crown following top-kill by fire (Towne and Knapp, 1996).

2.9. Soil Types

Due to *Dalea* spp.'s distinct adaptations, different species require unique conditions to thrive. Soil is one of the primary aspects that defines the kind of plants that grow in various regions due to its structure and mineral composition. Changes in these conditions require plants to adapt, creating new features that allow their performance in the current conditions.

Each of these *Dalea* spp. is adapted to different soil types, necessitating the selection of the most appropriate species to match soil type in various regions during future ecosystem restoration efforts (Innes et al., 2004). Our hypothesis is that there are different soil types that support distinct *Dalea* spp. According to Peraltra et al. (2019), soil is an integral component of planting that supports the plants, giving them nutrients in any climate. Our research will test the efficacy of four distinct soils for these four *Dalea* spp.: commercial potting soil, sandy, sandy loam, and caliche soils.

2.9.1. Potting Soil

Potting soil is commercially formulated to be as similar to topsoil which is the uppermost as well as the outermost layer of soil. The organic matter-rich surface soil provides nutrients for plants while the thick dark layers increase its ability to hold water, which is needed for vegetation growth (Brady, 1984).

It is made of among other substances, mineral particles, organic matter and air. Nevertheless, the organic matter in the potting soil varies in different regions where some areas have more or distinct organic matter, an aspect that is dependent on vegetation cover (Peng et al., 2017). Potting soil in different areas holds varied levels of

productivity, an aspect that is further defined by the amount of moisture in the soil.

Potting soil is normally categorized into bulk and bagged where each has its distinct features (Tucker et al., 1995)

2.9.2. Sandy Soil

Unlike potting soil that contains more decomposed and organic materials, sandy soil is composed largely of coarser, small particles of -rock. It is light in texture, well-drained, and is often warmer than finer soils. Although it is the poorest kind of soil supporting the least plant cover due to its higher acidity and low nutrients, it is generally most favorable for *Dalea* species according to the literature (Diggs et al., 1999). That is because the plants are found in the dry prairies as well as hillsides of plains, an aspect that makes the sandy soil conducive for their growth. However, sandy soil has poor water holding capacity but is the most efficient in draining water. In improving their ability to support plants, sandy soil must often be amended with various nutrients as well as material that increases water holding capacity, especially in greenhouses (García-Arévalo, 2002).

2.9.3. Sandy Loam Soil

Sandy loam soil, on the other hand, is formed by a combination of sandy soil, silt, and clay. The combination results in soil with proper water storage capacity as well as drainage, hence, it is the most effective soil type in farming. Moreover, these soils often have larger quantities of nutrients, hence, its application for gardening purposes (Noah et al., 2012). Despite its ability to support different types of plant, according to the literature most *Dalea* spp. do not thrive in the sandy loam soil due to increased moisture.

However, the introduction of the legumes to the soil may improve its overall quality through reduced erosion and increased amounts N (Pigott and Taylor, 1964).

2.9.4. Caliche Soil

Caliche is commonly found in limestone-derived soils in the arid and semiarid southwestern USA (Hennessy et al., 1983). Generally, it is light colored; however, it can vary from white to pink and even reddish brown based on the types of impurities within a particular region. Caliche soil tends to have high pH and its particles can cemented together through lime, a carbonate. Semi-arid and arid soils tend to have a high concentration of caliche nodules, which are primarily made of Calcium carbonate (Hennessy et al., 1983). These high concentrations of calcium carbonate deposits have a negative effect on the ability of the soil to hold large amounts of water for prolonged periods. According to Hennessy et al. (1983), caliche deposits also result in the thinning of arid grasslands' soil, further affecting the ability of plants to access underground water. OThese hilltop soils can sometimes have less nutrients (besides Ca and Mg) due to P binding with resulting minimal vegetation cover.

2.10. Effects of Soil Types on *Dalea* spp.

Our hypothesis is that each *Dalea* spp. will respond differently to soil types. Research shows that native plants such as *Dalea* spp. can be very successful when grown in unamended soils. The reason for this is that legumes generally do not need N or P-rich soils having high organic contents (Wheaton et al., 2018). That does not mean they will not also perform very well when grown in soils having high organic content such as loam soil. However, according to a study conducted by (Barceló et al. 2017), many

native plants such as *Dalea* spp. thrive in clay soils. However, there are some species that need well-drained soils and thus might perform better in sandy soils.

In greenhouses, changes are made to soils in order to attain ideal texture and nutrient availability for effective growth of this particular plant. For instance, in order to make clay soils ideal for the growth of one *Dalea* spp., 10% compost and 15% small aggregate such as pea gravel in terms of volume is added to clay or clay loam and then incorporated into the root zone (Barneby, 1978). Therefore, planting on top can be helpful as a mechanism for improving drainage. On the other hand, when the soil type is excessively well drained and sandy or even rocky, greenhouses often add 3% compost by volume (Schellenberg and Biliget, 2015). It is important to test soil prior to actual planting in greenhouses in order to make adjustments.

Plants such as *Dalea* spp. do not usually require regular maintenance. However, the usual pruning of dead and infected materials is common (Schmer et al., 2017). Also, cutting back perennials in spring periods is also essential for proper development of this plant in different soils. Usually, in any kind of soil, they do not need fertilizer but require weeding and deadheading just like other types of plants that are nonnative.

These plants require regular soil moisture, especially the first season in the field and the first three weeks in greenhouse pots or the field. Once the plants have developed strong taproots, watering can be cut back gradually until they are fully grown (Barceló et al., 2017). Beyhaut et al. (2014), when growing herbaceous legumes, found that soil surface crusting has the potential of reducing emergence of crops on certain types of soils. They reported that soils having low organic matter can develop very strong surface

crusts and the emergence of seedlings for crops such as *Dalea* is significantly reduced as the strength of the crust increases. Such a challenge is rarely experienced among soil types having high degrees of organic matter (Beyhaut et al., 2014).

However, forage legumes such as *D. aurea* can minimize salinity challenges. The reason behind this is that it has slightly deep roots compared to the other species and thus gains high consumption of water and can thus effectively make use of excess water that drains to deeper soils (Beyhaut et al., 2014). *Dalea* spp. can therefore assume a central role in improving fodder nutritive value as well as in improving overall soil quality for other species. This could be applicable in reclaiming degraded lands. It also justifies the importance of developing native commercial herbaceous legume germplasm for grassland restoration, rangeland rehabilitation and pastures (Muir et al., 2018).

We hypothesize that the *Dalea* spp. will respond differently to soil types, cowpea inoculation and P soil amendment. Our overall goal is determining whether *D. multiflora* collected in northcentral Texas as well as other commercially-available *Dalea* spp. are useful as a native, warm-season herbaceous legume in native grassland re-establishment, cultivated pastures and rangeland reseeding mixtures. To achieve this, we must determine each species' soil preferences and agronomic features such as forage yields and nutritive value. Nothing has been published on these aspects of *D. multiflora*, so we propose comparing it to other *Dalea* spp. Our objective is to evaluate *D. aurea*, *D. candida*, *D. purpurea*, and *D. multiflora* performance, as well as, development in four different potting soils: commercial top soil, sandy, sandy loamy, and caliche in greenhouse conditions in Texas. In doing so, this study will further explore herbage and

root nitrogen and dry matter yield as influenced by soil, phosphorus amendment and *Rhizobium* cowpea inoculant.

3. MATERIALS AND METHODS

3.1. Seed Sources

Dalea multiflora seeds were collected from roadsides in Erath County, Texas USA. Seeds were collected by hand at each location from several randomly-selected plants representative of the local population in July 2017. *Dalea aurea*, *D. candida*, and *D. purpurea* seeds are acquired from Native American Seed, Junction, TX, USA. *Dalea aurea*, *D. candida*, *D. purpurea*, and *D. multiflora* species pots were set up with four different soil types, +/- cowpea rhizobium and +/- P soil amendments.

3.2. Greenhouse Method

Initially, seeds were mechanically scarified by nicking the seed coat with a scalpel to improve germination. Seeds were germinated in sterile petri dishes. After germination, seeds were planted in trial filled with commercial greenhouse tray mixture (medium soil). Plants were grown in a greenhouse environment, which included distilled water irrigation twice daily by an automatic system for a total of 10 mm d⁻¹. Once the plugs were root bound, a single plant was transferred to pots blocked by seedling development.

3.3. Growing Conditions/ Study Site/ Experimental Design

The experiment was conducted in a greenhouse at the Texas A&M AgriLife Research Center, Stephenville, TX, USA (32° 15'N, 98° 12'W, altitude 395 m). The four-factor experiment consisted of four *Dalea* spp., four soil types, with or without rhizobia, and two P soil-amendment levels. Each treatment combination was replicated

four times and arranged on four separate greenhouse tables as four blocks. Soils were placed in 20-cm diameter pots, each considered an experimental unit. Twice insect-killing soap concentrate insecticide (Safer Inc., Lititz PA, USA) was used to reduce or eliminate mealybugs and aphids within pots.

3.4. Treatment (Factors)

3.4.1. Phosphorus

Soil treatments were P amendment to 60 mg/kg⁻¹ and an untreated control. The recommended target P was a minimum 60 ppm. This required putting 60 mg P per kg soil or 264 mg KH₂PO₄ per kg of soil. The steps included dissolving 5.27 g KH₂PO₄ in water and diluting to 1 L. 50 ml of this solution was added to 1 kg soil by spraying or dripping onto soil as uniformly as possible. At the time of transplanting, half of all pots were applied with P. The soil was then mixed thoroughly and hand watered for establishment.

3.4.2. *Rhizobium*

At the time of transplanting, half of all pots were inoculated with 5 g of commercial cowpea inoculant (marketed by Visjon Biologics, Wichita Falls, TX, USA). Pots were 15-cm standard green plastic pots from BWI and 1 kg DM of each soil type was added.

The experiment was initiated on May 7, 2019 and terminated after 15 weeks. Average minimum and maximum temperatures of 26.5 and 43°C respectively, during the study. Pots were watered with dionized water as needed to maintain soil moisture at field capacity every third day.

3.5. Soils

Soil was collected from three local and one commercial sources: Windthorst fine sandy loam (Soil Survey Staff, 1973), sandy washout, caliche, which is described stony and gravelly soils that are shallow to very shallow over limestone, and a commercial potting soil purchased from Ace Top Soil (Ace Hardware Corp. in Oak Brook, Illinois, USA). The potting soil consisted of composted forest product, peat, and sand (up to 10%) with no additional nutrients added. The sandy washout soil was collected from a drainage area at the base of a Windthorst fine sandy loam field. We collected local soils at three locations in Erath County characterized by shallow, low-P soils with low water-holding capacity. Soils were taken from the upper 20 cm depth. Soil sub-samples were sent to the Texas A&M University Soil, Water and Forages Testing Laboratory for nutrient analyses (Table 1).

3.6. Harvesting Methods to Determine Plant Dry Matter Yield and Nutritive Value

Pots were harvested using hand clippers on June 25, 2019 in each replicate of *D. aurea* and *D. purpurea* and on 2 July, 2019 in each replicate of *D. candida* and *D. multiflora* were harvested when open flowers appeared (early flowering stage) to determine DMY and forage nutritive value. Clippings were bagged and dried at 55°C for 48 hr. For all harvests, individual plants were cut approximately 10 cm above the soil surface. Clipping was repeated every time the regrowth flowered up to the final harvest in which all the herbage was harvested at 3 cm above the soil. Clippings for each pot were batched by pot. Herbage DMY was determined by weighing all accumulated clipped material by pot. Soil samples were then collected from each pot for laboratory

analyses of pH and nutrients. Roots were harvested by washing the soil off all below-ground plant material and bagging individually by pot. These were dried in a forced-air dryer set at 55°C for 48 hr and then weighed to determine root DMY. Herbage and roots from individual pots were ground separately to pass a 1-mm screen for mineral analyses.

3.7. Laboratory Assays

Nitrogen (%) as well as N DMY by root and herbage was derived from multiplying root and herbage DMY by root and herbage N content. Nitrogen assays were assayed using a Leco CN828 C:N analyzer (Leco Corporation, St. Joseph, MI, USA). Representative dried and ground forage samples of 1 mg were placed in thin foil to form pellets which were then loaded in to the analyzer.

3.8. Statistical Analysis

Dependent variables were subjected to analysis of variance using SPSS statistical program (SPSS Inc., Chicago, IL). Species by soil by P by rhizobium application interactions were considered first. Four-way, three-way, two-way and simple effects were examined successively. Two-way and simple effects were considered where three-way or two-way interactions, respectively, were not significant. Independent variables included legumes species, soil types, phosphorus treatment and rhizobium treatment. Dependent variables included forage and root DMY. Multiple means were separated by least significant difference and differences identified by distinct letters in the tables. Values were considered significantly different at a $p \leq 0.05$.

4. RESULT AND DISCUSSION

4.1. Herbage Dry Matter Yield

There was a species x soil types interaction ($p \leq 0.05$) in shoot yield (Table 2). All *Dalea* spp. thrived more in sandy loam soils compared to other soils. *Dalea aurea*, especially, had the greatest DMY in this soil type with *D. multiflora* having the least yield. *Dalea aurea* produced 352% more DMY than *D. multiflora*, 246% more DMY than *D. purpurea* and 165% more than *D. candida*. When potting soil was used, herbage DMY was very low across all species. There was little difference between *D. candida*, which had the most yields, and *D. purpurea* which had the least yield. *Dalea aurea* had the greatest herbage DMY, 2.4 times greater than the next highest yield, *Dalea candida*, when grown in caliche. *Dalea multiflora* had very poor yields compared to the other species, regardless of the soil type. *Dalea aurea* and *D. candida* had high DMY compared to the other species, regardless of the soil used.

Dalea candida, commonly referred to as white prairie clover, is native to hillsides and grasslands in the Great Plains of North America (Khanal et al., 2018). It has a thick taproot and, as a result, is tolerant to drought. It also has the ability to thrive in sandy to gravelly soils (Taylor and O'Kennon, 2013). In my trial, it had consistently greater herbage DMY than the other prairie clovers, regardless of the soil type used.

Dalea aurea is found in gravelly prairie soils and occasionally limestone environments. The plant, however, rarely grows in silty soil (Jin et al., 2015). During the rainy season, the plant experiences maximum growth. *Dalea aurea* also has a hard seed

coat that impairs its germination. Germination involves the reactivation of the dominant metabolic processes in a seed, which necessitates uptake of optimal and often higher moisture levels (Taylor and O'Kennon, 2013).

Dalea purpurea has a strong tap root and can penetrate soil up to 15 cm deep. The plant grows well in nurseries where moisture is abundant and poorly in soils where drainage is poor (Israel, 1987). *Dalea purpurea* also has the ability to resprout from crowns after fire has damaged aboveground herbage. Taylor and O'Kennon (2013), described *Dalea reverchonii* that grows exclusively on limestone but only rarely in areas with thick soil. The solid crystalline sediments that result from the erosion of the bedrock have the capacity to support limited vegetation growth (Hartung et al., 2014). This species of *Dalea*, like the others, shows a preference for poor soil, and where the soil was more developed, grasses showed a tendency to outgrow them (Taylor and O'Kennon, 2013).

Restoration of degraded wetlands requires the consideration of several factors, especially if such areas are to support vegetation. For example, Thompson et al. (2006) observe that some of the issues that can hinder the reestablishment of *D. foliosa* in a restored wetland include a thin gravel layer and deeper soils. Another factor is the presence of pasture since certain species such as *Dalea* lack the capacity to compete for available nutrients (Muir et al., 2005). Also, in soils high in organic matter content, *Dalea* spp. fail to grow as expected and consequently the task of reintroducing them is challenging. These plants are close to extinction in their natural areas because of fire suppression which has favored competition from less fire-tolerant plant species and this

has made the genera scarce. Moreover, Thompson et al. (2006) argue that loss of habitation can also affect restoration of wetlands.

In my study *D. aurea* exhibited the earliest flowering dates compared with the other *Dalea* species. Thus, it may be a good candidate for revegetation in drier areas with shorter growing seasons limited by precipitation. The other species also flowered readily in greenhouse conditions while *D. multiflora* was the last to flower and produced the least inflorescences compared to the other species.

4.2. Root Dry Matter Yield

An interaction ($p \leq 0.05$) between species and soil types was also observed for root DMY (Table 3). In sandy soil, *D. candida* had the greatest root DMY compared to the other species. *Dalea candida* produced 56% more root DMY and 1.5 times greater than *D. purpurea*, when grown in sandy soil. *Dalea multiflora* had the lowest root DMY when grown in sandy soil and *D. candida* 3.97 times greater than *D. multiflora*. In sandy loam soil, *D. candida* had the greatest root DMY with *D. aurea* having the lowest yield. *Dalea candida* produced 340% more root DMY than *D. aurea*. The plants that grew in potting soil had the lowest yield compared to the other soils with *D. purpurea* yielding the least. *Dalea candida* still had the highest yield. This makes it one of the best candidate species in my experiment. The other two species had very low root DMY and were similar to *D. purpurea*.

Dalea candida again had the greatest root DMY when grown in caliche soil with *D. multiflora* having the lowest yields (Hartung et al., 2014). Overall, *D. multiflora* and *D. aurea* had very poor root DMY regardless of the soil type with *D. candida*

consistently having the greatest yields. The perennial legume, prairie Glover, is adapted to moderately to high drained areas with sandy soils (Taylor and O'Kennon, 2013).

However, for sandy soils to adequately support *Dalea* spp. growth, it has to be improved, especially its water holding capacity.

Dalea spp. also performed well in sandy loam soil. This seemed to be similar to those reported by Taylor and O'Kennon. This is largely because sandy loam soils often have larger quantities of N compared to sandy soils (Taylor and O'Kennon, 2013).

Caliche soils, on the other hand, have high pH and are often shallow, which limits roots from going downward to collect soil moisture during droughty periods. Caliche soils are formed by hardened calcium carbonate which binds silt, gravel and sand particles. These soils often have poor drainage and consequently are not suitable for most plant growth (Hartung et al., 2014). This in turn limits *Dalea* spp. from having high DMY.

All *Dalea* spp. in this trial performed poorly in potting soil. This can be attributed to high soil moisture retention which is not suitable for *Dalea* spp. (Taylor and O'Kennon, 2013). According to Franklin and Mills (2003), while the *Dalea* spp. lacks a desirable seedling vigor, the low productivity of the legume can be addressed by mixing it with rangeland reseeding species. Because most *Dalea* have unique adaptations, a small change in environmental conditions can adversely affect their populations, and when they fail to adapt, *Dalea* spp. can disappear from vast tracts of land that they previously occupied. *Dalea purpurea* is on the Great Plains and can be found in most soil types. Mainly, the occurrence of the legume in sandy soils should be a key consideration in the combination process since the deep taproots of *Dalea* spp. allow it

to collect water from deeper underground levels compared to other leguminous plants (Whittington et al., 2012; Hartung et al., 2014). As a result, they are abundant in areas where other plants cannot reach soil moisture, resulting in minimal competition. They can also be found on roadsides and pathways for similar reasons.

4.3. Root Dry Matter Yield Species x Rhizobium

There was an interaction ($p \leq 0.05$) between rhizobium inoculation x species for root DMY (Table 4). When rhizobium was introduced, *D. candida* had greater yield relative to other species. Its yield was 300% more than that of the least yielding plant, *D. aurea*. *Dalea purpurea* was the second greatest yielding species. In the absence of rhizobium, *D. candida* still had the greatest yield with *D. multiflora* having the lowest yield. *Dalea candida* improved its yield in the absence of commercial rhizobium inoculation. There was also little difference in *D. aurea* root DMY with and without rhizobium (Whittington et al., 2012). *Dalea multiflora* and *D. purpurea* increased their root DMY as a result of commercial rhizobium inoculation.

The interdependent association between Rhizobium bacteria and leguminous plants, according to Zahran (1999), facilitates the fixation of Nitrogen. As a result, it often increases plant N content and DMY. Also, when a plant does not rely on other species for N, they likely grow faster than their counterparts that must wait for plant litter decomposition to extract organic-matter N from the soil (Whittington et al., 2012). When the quantity of N in the soil increases, forage quality usually improves significantly, and the result can be seen in ruminants that feed on these plants. Kanani et al. (2006) maintain that the amount of N stored in plants is directly related to the health

of animals that feed on the vegetation. Khalsa et al. (2012) suggested that introducing legumes into pastures and rangeland improves forage quality, increased plant diversity and provide wildlife with added benefits. This can be observed from the results obtained after introducing rhizobia to the *D. candida*. Otherwise, inoculating with rhizobia did not improve *D. aurea*, *D. purpurea* and *D. multiflora* in my study.

Muir et al. (2005) described the benefit of using native legumes that are similar to the vegetation that is present in the southern Great Plains. The study found that this resulted in the plants doubling their herbage yields. This could be attributed to improved adaptation by plants to climate and soil conditions. Lafay and Burdon (1998) suggested that native legumes that share common habitats share similar rhizobia. Previous studies also reveal that legumes that inhabit the prairie regions may have unexpected specificities with other legumes that may share a common habitat. This may include microbial populations within different soils as influenced by heterogeneity (Franklin and Mills, 2003). Legume inoculation with commercially-available Rhizobia has been practiced to improve forage yields and, eventually, soil N. This is because legumes play a role in improving yields as well as soil nutrients. Ideally, commercial inoculants should attain longer shelf life, high rhizobial concentration and survivability. Also, cereals and other plants that grow alongside the inoculated plants are healthier and have greater yields. Positive results among the yield and growth of inoculated legumes including the cowpea have also been reported by Barceló et al. (2017). In their experiment, more than 80% of the forage yields increases were obtained compared to without general cowpea rhizobial inoculated treatment without N in the four potting soils. The forage yield and

nutritive levels of the response of the *Dalea* spp. to inoculation were related to the variables determined by with or without P amendment to the soil. Consequently, *Dalea* spp. that are inoculated may have an improved yield over the subsequent seasons if native rhizobial populations are not present (Lafay and Burdon, 1998). Although the symbiotic and growth characteristics of most rhizobia are dependent on environmental stress factors, others such as *Dalea* naturally have a slower rate of nitrogen fixation and nodulation (Nutman, 1962).

Differences in the pot soil from the beginning to the end of the study period demonstrated that soil pH (soil types), conductivity (cond.), nitrate-N (NO_3N), P, K, Ca, magnesium (Mg), sulfur (S) and sodium (Na) increased by the end of the study experiment (Tables 1, 5 and 6). However, soil nutrient richness increases that increase plant nutritive values does not necessarily correspond with an increase in the rhizosphere (rhizobium x root interaction) bacterial alpha diversity (Bird and Choi, 2017). Although all native soil types in my study had relatively low water-holding capacity and organic matter, the mean herbage DMY of unfertilized species differed. Prairie herbage DMY in different soil types is often different (Rice et al., 1977).

Different soils contain different soil pH, nutrients, drainage, and bacterial compositions. Soil fertility is determined by the mineral composition and the soil texture. Soil amendment by addition of legume residues, according to Peralta et al. (2019), alters its chemical composition by increasing soil pH. Consequently, it can be argued that the addition of organic residues or the soil amendment affects the soil mineralization and decomposition through the soil micro-organisms actions. According

to the Noah et al. (2012), the change of soil decomposition and mineralization change the soil pH thereby creating less acidic conditions that affects the release of the major soil nutrients such as the K, C, P, Mg, and N. However, the differences of results in nutritional levels obtained from the experiment could have been attributed by the differences in the dry matter during legume integration phases. Consequently, my results agree with García-Arévalo (2002), that claimed that environmental heterogeneity demonstrates a traditional explanation for the biodiversity seen in the soil nature and could be critical in structuring the soil type and soil microbial communities. Based on soil microbial approach, environmental heterogeneity is established by both the plant communities and by the soil type (Klabi et al., 2017).

4.4. Phosphorus

There is no effect interaction with and without P treatments. The results also can be visualized by evaluating the *D. aurea*, *D. candida*, *D. purpurea* and *D. multiflora* above- and below-ground DMY as well as forage nutritive value with and without P amendment to the soils.

The increases in the forage yield achieved with general cowpea rhizobia inoculation without P amendment to the soil indicated that the inoculation may be a preferable alternative to the P amendment to the soil type when any type of the soil is covered with the residue. The results are in conformity with Schmer et al. (2017) that explains that the availability of the P amendment to the soil is influenced or affected by the surface legume residues since it is dependent on the number of P amendments immobilized by the soil type microbes. Beyhaut et al. (2014) claims that the soil type

microbe population appears to significantly increase with the surface legume residues since without amendment soil type have higher levels of surface reduces compared to amended one for P immobilization, the negative impacts of the residue layers on the P availability may have limited or reduced the efficiency of the P applied to the soil type surface as demonstrated by Muir et al. (2018). Therefore, the P can be considered as one of the main factors influencing the nutritive levels and forage yields of the cowpea which is illustrated by the high response legumes to the application of inorganic P in the soil collected area.

According to Cane et al. (2012), established a conceptual basis for examining the interaction between combined phosphorus, rhizobium, the supply of mineral nutrients, and its impacts on growth. Similar to several possible interactions expressed in the study results, a completely negative interaction is expressed when mineral elements such as phosphorous are not required for growth of forage (plants) supplied by combined phosphorus but is always required for nodulated forage without phosphorus. However, an incompletely negative interaction is experienced when a lowly mineral nutrient soil type supply limits the growth of nodulated forage (crops) with or without phosphorus (Beyhaut et al., 2014). However, in this case, the growth of plants without phosphorous is thought to be limited to a greater extent at lower mineral nutrients supply than the growth with phosphorous. Besides, the growth of symbiotic crops without phosphorous is more responsive to an increase in the nutritional levels or mineral supply than the growth of crops combined with phosphorus (Foster et al., 2008). Consequently, positive interaction is likely to occur when the growth of the *Dalea spp.* with and without

phosphorous and rhizobium is restricted to a similar measure at lower nutrient supply or soil type and when the growth of the *Dalea* spp. with combined phosphorus and rhizobium is more responsive to increased nutrient supply as per the soil type than the growth without combined rhizobium and phosphorous (Tlusty et al., 2004).

According to Evers (2008), the timing of rhizobial inoculation in an interactive study is critical because there is always a lag between the period of inoculation and the initial infection, and the time the legumes nodule becomes functions in phosphorus fixation. Although not emphasized by the previous studies, it also critical to apply a rhizobial interactive root or strain that is highly efficient and effective in fixing phosphorus in symbiosis with the host crop at adequate nutritive levels (Noah et al., 2012; Mártir et al., 2007). However, the lack of attention to these critical matters could likely lead to false-positive interaction between the supply of nutrients and phosphorus. In the present studies, the interactive effects of phosphorus and general cowpea Rhizobium inoculation for the dry matter and growth are positive and highly significant (Packard et al., 2004). The mixed interactions are positive and highly significant in severely deficient to moderately deficient P concentration range (without phosphorus) to moderately deficient concentration range and negative in moderate to adequate phosphorus concentration range (Rao et al., 1996). Collectively, the results of this experiment with the *Dalea* spp. are consistent with a general conclusion that phosphorus plays significant and specific roles in nodules or roots initiation, functioning, and growth in the forage growth (Muir et al., 2008). However, other studies are also consistent with the findings and interpretations that increasing phosphorous supply increases other

minerals fixation such as nitrogen by stimulating the host crops growth instead of exerting specific impacts on the rhizobial growth on nodule growth, functioning or survival (Beyhaut et al., 2014). Consequently, an alternative explanation is consistent with the experiment result demonstrating that the interaction of phosphorus with other minerals fixation could be possible because of the rhizobium strains that are used as symbionts considering the low N₂ fixation abilities even at adequate phosphorus supply (Macharia et al., 2011).

Different soil types have different chemical constituents and impacts the growth of plants since they influence the availability of nutrients and toxicity of a number of features. Although phosphorus is relatively immobile and frequently insufficient to a number of plant species as demonstrated by the *Dalea* spp., the experiment alongside other studies confirms that phosphorus is an important nutritional element for growth and establishment of the legumes despite the differences in its requirements (Moir et al., 2016). Thus, the phosphorus deficiencies can negatively influence the forage yields and nutritive levels based on its significant impacts on the symbiotic rhizobial establishment, nodule functions, and growth (Lindgren, 1992). Besides, the soil type creates environmental stressors based on its differences in temperature, moisture, and pH levels resulting in dissimilarities in the length and shape of the crop species (*Dalea* spp.) evidenced by disparities in crop mass and growth. Therefore, the different *Dalea* spp. respond differently to different soil types, cowpea inoculation, and phosphorus soil amendment also considering that they have got different penetration abilities based on their tap root strength to different soil penetration. For example, the

D. purpurea originate from a strong taproot that penetrates the soil up to a depth of six inches while the *D. candida* has a slightly lower penetrative ability to different soil types and flourish effectively in an average, medium, and well-drained soil (Khanal et al., 2018).

4.5. Nitrogen Content (%)

There were no differences in root N content for any of the factors imposed. From the data obtained from the study, *D. aurea* registered the highest nitrogen content DMY followed by *D. candida* with a nitrogen content then *D. multiflora* and *D. purpureum* registered the lowest with a nitrogen content (Table 7). From the results obtained from the study, different species registered different values of nitrogen content of the herbage. According to a study by Gáborčík (2003) the N content varies between different species.

4.6 Nitrogen Dry Matter Yield

However, as a result of differences in herbage and root DMY, there were differences in herbage and root N DMY (Tables 8 and 9).

Dalea aurea had the greatest herbage N DMY of the herbage. *Dalea multiflora*, *D. purpurea* and *D. candida* yielded the least herbage N DMY while not being different from each other (Table 7). Results also indicated that soil affected herbage N DMY. Potting soil registered the lowest percentage (8%) to N DMY followed by sandy soil 25% then sandy loam with a 32% and then caliche with a 33% per species. Plant species affected herbage N DMY 76.7%. Soil type accounted for 45.3%.

From the results, *D. aurea* had the lowest N DMY, *D. candida* had the highest N DMY, then *D. purpureum* and *D. multiflora* had similar N DMY in the roots. Sandy

loam soil was the best in root N DMY for all species, followed by sandy soil and caliche and then potting soil had the lowest N DM yield of the roots. Computing data on species and soil type, the results showed that species accounted for 56.3% of root N DMY compared to soil type which has 36.6%.

Dry matter accumulation above the soil depends on some factors. Climatic conditions of the area, soil type, and species are some of the aspects that highly affect dry matter accumulation above the ground (Lamprey et al., 2017). Rasouli et al. (2014) further outline that Agro ecosystem yield is frequently inhibited by a low availability of nutrients and water. From the above data, as compared to all the other aspects that affects Nitrogen dry matter above the soil, species still affects the least. Phosphorus affects the highest due to its purpose of supporting growth of the plant. Soil type and rhizobium also has effects on the N DM yield above the ground. On the other hand, Nitrogen content above the ground is highly affected by soil type.

Soil highly affects nitrogen content in species in my study. On the contrary, species does not highly affect nitrogen in species above the sand. Other aspects like *rhizobium* and phosphorus affect the nitrogen content of species above the sand to. All these aspects combined can lead to great production in plants.

Nitrogen yields for legumes in this study were comparable to those of Power (1987). Power (1987) cites alfalfa (undetermined cultivar) yield of 114 to 223 kg N ha⁻¹ which is greater than all yield from this study. According to the Natural Resources Conservation Service (2001), P is used by plants to store and transfer energy produced

by photosynthesis for use in growth and reproductive processes. Habibzadeh (2015) further outlined that species use P for their growth. For DMY to increase in a plant, P plays a major role, especially in the soil. According to the NRCS (2001), the release of plant phosphorus, whose main function is to transfer stored energy, is dependent on environmental conditions and the decomposition rate of organic matter. Accordingly, higher matter decomposition rates coincide with increased release of P, especially in the presence of warm climates and highly oxygenated dry soils. Therefore, soil type and root type of a plant highly affect the rate at which P accumulates which triggers N DMY accumulation.

5. CONCLUSIONS AND SUMMARY

Dalea spp. responded differently to four soil types, cowpea rhizobia inoculation and P soil amendment generating different herbage and root forage DMY and nutritive values. In some soil types, commercial cowpea rhizobia inoculation increased herbage and N DMY. Sandy loam was the best for all *Dalea* spp. Thus, the impacts of the inoculated strains are relatively greater in soils with adequate P availability and response to P amendment occurred on low-P soils. Thus, the study demonstrated that *D. aurea*, *D. candida*, *D. purpurea*, and *D. multiflora* responded differently to four soil types, cowpea inoculation and P soil amendment and the application of inoculants together with P amendment result in positive interactions in some cases.

Dalea species have very specific requirements to grow well. My results as well as the literature indicate that they are unlike most plants which thrive in soils that are rich in nutrients and have good drainage. *Dalea* spp. can thrive on rocky outcrops such as limestone surfaces and sandy soils. Their thick taproots enable them to reach water that most other plants cannot get to. They require thin soils and where other plants exist; they cannot compete and are usually overtopped by grasses. *Dalea* spp. perform very poorly in potting soil where there is abundant moisture-holding capacity and organic matter. *Dalea* spp. perform well in sandy loam soils despite their low moisture-retaining capacity. They do this because these soils have more plant-available N. They also survived on caliche soils but not as well as they did in sandy and sandy loam soils.

When *Rhizobium* was introduced to the plants, *D. multiflora* and *D. purpurea* had greater herbage DMY. This increase was likely because the plants had more N which supported greater DMY. *Rhizobium* is normally a critical component in that it facilitates N fixation that these plants utilize to grow and ensures that subsequent yields increase. Their nitrogen-fixing capabilities are of crucial importance not just for the *Dalea* spp. but also to the plants that grow alongside the legumes. This particular feature has been key to revegetation programs where plant species are similar to the native plants such as is the case for *Dalea* spp. in northcentral Texas (Diggs et al., 1999). The lack of benefit to the other two *Dalea* spp. may indicate that they require more specific rhizobial associations or that the soils used had populations that satisfied their needs.

For high N DMY to be high, various aspects need to be considered. Phosphorus is one of the main nutrients needed by species for both growth and production. Some of the aspects that highly affect N DMY include species, soil type, *Rhizobium* and P. These aspects affect the P value in an ecosystem separately, so ensuring that all of them are sufficient will lead to increased N DMY both in the roots and herbage.

Herbage DMY was affected by species and the type of soil. The latter confirms the argument by Lamptey et al. (2017) that herbage yield is highly affected by the environment and climate of the area. In this case, the geographical location plays a vital role in the N content and DMY of these species both in the roots and herbage.

Therefore, for one to determine N DMY, species, type of soil, rhizobium and P must be taken into consideration. All species performed well during the establishment year and should be considered for possible use in forage systems. During the

establishment year, in all species, herbage and root DMY, herbage N DMY, herbage N content, and root N DMY did not respond to P amendment or *Rhizobium* inoculation. These species would probably not be recommended for use in cultivated lands, such as those used for row crops or hay production. However, due to their unique traits and soil preferences in the wild, research needs to determine the most favorable soil conditions necessary for *Dalea* spp. growth. My results indicate that, unlike most other legume species, they do not thrive in moist, nutritionally rich soils. This means that they should be used in reseeding efforts only where soils conditions mimic their original habitats.

In areas where *Dalea* spp. had previously colonized but have since been extirpated by overgrazing, competition with other vegetation and human activities, they should be reintroduced so that they can enrich the soil with nitrates. This ensures that other plants can thrive too because the *Rhizobium* will fix N and this benefits the entire ecosystem. This can affect neighboring plants as well as grazing or browsing insects, domestic ruminants or white-tailed deer by increasing herbage protein content. When animals feed on such plants, they improve their nutrition because of the nutritious nature of such plants. Further research should be conducted in order to make the inoculation process more effective across the different species of the *Dalea* spp. In a nutshell, more research should also be carried out to determine how to enable *Dalea* spp. to survive in areas where they are being introduced or reintroduced.

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APPENDIX A

Table 1. Soil analysis before the experiment.

Soil Types	pH	Cond	NO ₃ N	P	K	Ca	Mg	S	Na
		umhos /cm	-----ppm-----						
Sandy Loam	7.1	58	3	17	142	917	99	4	2
Sandy Potting Soil	5.6	54	0	23	47	627	40	5	1
Potting Soil	7.6	253	0	146	454	4	319	40	71
Caliche	8.2	82	0	1	70	41	138	12	23

Table 2. Forage dry matter yield (g/plant) by species and soil types (species x soil type $p \leq 0.05$).

Soil Type	Species			
	<i>Dalea candida</i>	<i>Dalea multiflora</i>	<i>Dalea purpureum</i>	<i>Dalea aurea</i>
Sandy Loam	1.14bA	0.54cA	0.76cA	1.88aA
Sandy	1.07bAB	0.48cAB	0.68cA	1.52aB
Potting Soil	0.37aC	0.21abB	0.03bB	0.36aC
Caliche	0.78bB	0.42cAB	0.47cA	1.87aA

*Means in the same line used different lower case letter differ at $p \leq 0.05$

*Means in the same column followed by different upper case letters differ at $p \leq 0.05$

Table 3. Root dry matter yield (g/plant) by species and soil types (species x soil type $p \leq 0.05$).

Soil Type	Species			
	<i>Dalea candida</i>	<i>Dalea multiflora</i>	<i>Dalea purpureum</i>	<i>Dalea aurea</i>
Sandy Loam	1.54aA	0.60cA	1.16bA	0.52cA
Sandy	1.68aA	0.42cAB	1.12bA	0.49cA
Potting Soil	0.83aB	0.28bB	0.11bC	0.12bB
Caliche	1.51aA	0.52bAB	0.67bB	0.55bA

*Means in the same line used different lower case letter differ at $p \leq 0.05$

*Means in the same column followed by different upper case letters differ at $p \leq 0.05$

Table 4. Root dry matter (g/plant) response of species to *Rhizobium* inoculation (species x *Rhizobium* $p \leq 0.05$).

Rhizobium	Species			
	<i>Dalea candida</i>	<i>Dalea multiflora</i>	<i>Dalea purpureum</i>	<i>Dalea aurea</i>
-	1.530938aA	0.38625cA	0.704688bA	0.437083cA
+	1.2425aB	0.525521cA	0.827917bA	0.408542cA

*Means in the same line used different lower case letter differ at $p \leq 0.05$

*Means in the same column followed by different upper case letters differ at $p \leq 0.05$

Table 5. Soil analysis report after experiment (-P).

Soil Type/Species	-P								
	pH	Cond	NO ₃ N	P	K	Ca	Mg	S	Na
		umhos/cm	-----ppm-----						
Sandy L./D.C	8.5	167	1	33	226	1660	261	35	90
Sandy L./D.M	8.4	302	0	21	239	2055	313	59	164
Sandy L./D.P	8.4	397	1	21	237	1756	296	54	154
Sandy L./D.A	8.3	559	1	31	293	1497	340	41	200
Sandy/D.C	8.6	348	0	49	167	957	311	51	81
Sandy/D.M	8.2	593	21	47	243	1431	305	63	176
Sandy/D.P	8.5	266	1	42	199	866	272	36	148
Sandy/D.A	8.1	438	2	44	217	1159	375	120	159
Potting Soil/D.C	8.1	727	1	138	632	3715	544	84	213
Potting Soil/D.M	7.9	855	1	161	892	3706	500	103	319
Potting Soil/D.P	8.1	849	0	141	858	3749	587	116	350
Potting Soil/D.A	7.9	1181	1	164	842	3732	553	136	351
Caliche/ D.C	8.1	949	20	3	125	34211	278	116	236
Caliche/ D.M	8.0	498	30	3	116	32544	273	69	188
Caliche/ D.P	8.0	493	34	4	128	32596	290	75	210
Caliche/ D.A	8.0	703	16	6	104	32090	265	108	250

*D.C mean is *Dalea candida*

*D.P mean is *Dalea purpurea*

*D.M mean is *Dalea multiflora*

*D.A mean is *Dalea aurea*

Table 6. Soil analysis report after experiment (+P).

Soil Type/Species	pH	+P							
		Cond umhos/cm	NO ₃ N	P	K	Ca	Mg	S	Na
Sandy L./D.C	8.5	358	0	27	225	1550	388	68	122
Sandy L./D.M	8.3	189	1	45	273	1313	259	63	114
Sandy L./D.P	8.4	373	1	47	252	1409	328	61	96
Sandy L./D.A	8.4	183	1	48	238	1426	335	39	93
Sandy/D.C	8.4	304	1	93	239	1044	264	32	119
Sandy/D.M	8.2	389	7	76	285	982	287	52	176
Sandy/D.P	8.2	399	1	83	271	838	246	48	161
Sandy/D.A	8.2	349	3	88	284	1138	346	110	208
Potting Soil/D.C	8.0	477	0	164	739	3764	479	53	220
Potting Soil/D.M	7.9	668	1	149	739	3537	436	80	207
Potting Soil/D.P	8.0	894	1	217	1053	3760	590	110	382
Potting Soil/D.A	7.9	898	1	201	988	3741	546	162	365
Caliche/ D.C	8.2	409	4	11	128	31498	263	49	184
Caliche/ D.M	8.0	616	39	12	122	31510	263	119	275
Caliche/ D.P	8.1	518	15	13	113	29258	273	80	235
Caliche/ D.A	8.2	821	10	14	117	31698	287	111	236

*D.C mean is *Dalea candida*

*D.P mean is *Dalea purpurea*

*D.M mean is *Dalea multiflora*

*D.A mean is *Dalea aurea*

Table 7. Nitrogen content (%) of herbage (g/plant) as affected by different species ($p \leq 0.05$).

Species			
<i>Dalea candida</i>	<i>Dalea multiflora</i>	<i>Dalea purpurea</i>	<i>Dalea aurea</i>
1.637ab	1.580b	1.192c	1.920a

*Means in the same line used different lower case letter differ at $p \leq 0.05$

Table 8. Nitrogen dry matter of herbage (g/plant) as affected by different species and soil type ($p \leq 0.05$).

Species			
<i>Dalea candida</i>	<i>Dalea multiflora</i>	<i>Dalea purpureum</i>	<i>Dalea aurea</i>
0.01380b	0.00649b	0.00599b	0.02853a
Soil Type			
Sandy Loam	Sandy	Potting Soil	Caliche
0.01791a	0.01397a	0.00484b	0.01809a

*Means in the same line used different lower case letter differ at $p \leq 0.05$

Table 9. Nitrogen dry matter yield of root (g/plant) as affected by different species and soil type ($p \leq 0.05$).

Species			
<i>Dalea candida</i>	<i>Dalea multiflora</i>	<i>Dalea purpurea</i>	<i>Dalea aurea</i>
0.02546a	0.01038b	0.01205b	0.007914c
Soil Type			
Sandy Loam	Sandy	Potting Soil	Caliche
0.01877a	0.01661ab	0.007057c	0.01337b

*Means in the same line used different lower case letter differ at $p \leq 0.05$