ABSTRACT

SMART, PAIGE KENNEDY. Comparing the Effectiveness of Establishment Between Feeding and Frost Seeding Red Clover in Tall Fescue Pasture. (Under the direction of Dr. Matt Poore).

Incorporating legumes into pastures may increase weaning weight, average daily gain, and reproductive performance in cattle grazing endophyte-infected tall fescue. Constraints to the limited legume establishment adoption by producers include high seed cost, lack of education, and the greater management required. A number of producers feed seed to cattle, by means of a free choice mineral or supplement, in an attempt to easily sow clovers in their pastures through distribution of seed in fecal matter. A total of four studies were conducted two years (2014-2015, 2015-2016) to test the efficacy of this method, termed endozoochorous, on establishing red clover (RC) in pastures. These included: the impact of seed contact with minerals, viability of the seed following passage through the ruminant digestive tract, and competition after passage with existing grasses and fecal pats. The effect of a limestone/inoculant coating was also tested in each study. The mineral contact study confirmed that prolonged exposure to loose minerals reduced the viability of red clover seeds. Coated seeds (C) showed a more rapid decline in seed viability (14 days) than uncoated seeds (U) (28 days). The coating enhanced moisture collection near the seeds, which likely decreased the time necessary for salt toxicity to occur. Fecal samples were removed from cattle consuming RC seeds in free choice mineral, and recovered seeds were germinated. Coated seeds had lower viability (5%) than U (14%), likely due to the coating holding rumen fluids close to the seed. The changes in pH and the cellulolytic bacteria throughout the digestive tract likely caused the reduction in viability.

A small plot study in Bahama, North Carolina tested the efficacy of endozoochorous under the assumption that all seeds survived passage. A comparison was made of C and U RC seeds established in ‘KY-31’ tall fescue pastures via either frost-seeded (FS) or mixed in a fecal pat (F). A dragging treatment [with (D) or without (ND)] was applied to quantify impact of this management on improving seedling survival and distribution. Frost seeding resulted in the highest RC seedling densities and experienced no improvements from dragging. Dragging significantly improved establishment in F treatments. This study suggests that dragging improves establishment of RC seeds that are present in fecal pats. The field study [treatment groups: F (C and U, D and ND) and FS (C and U, D and ND)], determined that dragging was not significant in year one but did improve the FS-U treatment in year two. There was no difference in the number of established RC seedlings for the C or FS-U treatments in year one, but the FS-U treatment had more seedlings in year two than the other FS treatments. Both the F and negative control treatments had little clover in both years. Endozoochorous was not a successful method of RC establishment in ‘KY-31’ Fescue pastures, whereas frost seeding of either coated or uncoated seed was successful.

Comparing the Effectiveness of Establishment Between Fed and Frost Seeded Red Clover into Perennial Pasture

by
Paige Kennedy Smart

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APPROVED BY:

Dr. Matt Poore Committee Chair	Dr. Ben Goff
Dr. Carrie Pickworth	Dr. Lori Unruh Snyder

DEDICATION

This thesis is dedicated to all of the producers and farmers who strive year after year to improve their operation. May their ability to take risks by trying new things pay off in the long run. This includes my grandfather, Alton Earl Smith, father, David Wayne Kennedy, and brother, David Ryan Kennedy, for working so tirelessly to keep the family farm running. Their efforts to improve the operation despite the struggles of farming are nothing short of an inspiration.

BIOGRAPHY

Paige Kennedy Smart was raised on a row crop farm in Gray's Creek, North Carolina. She quickly found her place in agriculture during her involvement with the FFA in high school. Her college career began at the University of North Carolina-Wilmington, but following a year without fellow "aggies" in her life, she chose to transfer to North Carolina State University in the Animal Science program. Her love for cattle began on a trip to Costa Rica where she was involved with a team that vaccinated, dewormed, and administered vitamins to herds of Brahman cows. Upon arriving back to the states, she quickly became involved with the Animal Science club and took a job at the university dairy farm to further her experience with cattle. An opportunity arose for Paige as a sophomore to work in a ruminant nutrition lab, which stemmed into a four-year journey working for Dr. Matt Poore. During this time, she was given the opportunity to lead on-farm Amazing Grazing workshops focused around soil health and summer annuals on beef cattle operations for two summers. These opportunities, accompanied by her growth of knowledge during those four years, lead Paige to her first job out of college working as a regional manager for Southeast Agriseeds, LLC.

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CHAPTER 1: Introduction

'KY- 31' Tall Fescue (*Festuca arundinacea*) is the most utilized grass by beef cattle producers in the Southeastern United States (Aiken and Strickland, 2015). It was discovered that this particular variety of tall fescue contains a fungal endophyte (*Epichloë coenophiala*) that causes production losses, including average daily gain, of growing cattle (Ball et al., 2015). Establishing clovers (*Trifolium* sp.) into pastures is a method of minimizing some of these losses associated with tall fescue toxicosis (Beck et. al., 2012). Legumes also fix atmospheric nitrogen, which becomes available to the soil after the plant decays and through animal excreta, thus reducing the need for nitrogen fertilizer application in pastures (Dobson and Beaty, 1977).

Frost seeding and no-till drilling are the most common methods utilized by producers to establish these legumes into existing sod. There is conflicting literature concerning which method is more effective: some work favors no-till drilling (Mueller and Chamblee, 1984) while others show each method is equally effective (Schlueter, 2012). Producers without access to the equipment or with rough terrain cannot utilize no-till drilling. Broadcast seeders can present the same limitations as a no-till drill, and hand seeders may be unrealistic for large acreage. With both methods, the ideal window of time to seed becomes an issue for part-time farmers. Weather and/or off-farm obligations may reduce the likelihood of a part-time farmer being able to successfully establish clovers into their fields. In a well-managed system, cattle generally have access to minerals or supplemental feeds every day and could easily consume any seeds mixed in with these substances. If seeds remain viable after passage and are adequately distributed throughout the pasture, feeding seed could be an economical means of establishing red clover into pastures. This method would require little producer input and increases the likelihood of clover being seeding into their pastures. It is known that various types of seeds from grazed forages pass through the ruminant digestive tract ending with a wide array of viability (Simao et al. 1987, Tjelele et. al 2014, Whitacre et. al. 2006).

The objective of this thesis was to determine feasibility of establishing red clover by feeding seed with minerals. Three experiments were used to determine the viability of red clover (*Trifolium pratense L.*) at different points in this feeding process: after coming in contact with loose mineral, after passage through the bovine digestive tract, and following excretion from the animal. The culmination of these experiments was also examined in field by grazing cattle with *ad libitum* access to mineral-red clover seed mixture on KY-31 tall fescue pastures. Additionally, both coated (limestone and inoculant) and uncoated seeds were subjected to all conditions to determine if coating offered an advantage in frost seeding or feeding scenarios.

CHAPTER 2: Literature Review

Introduction to Legumes

Legumes are plants that are classified within the Fabaceae family, produce a pod as fruit, and can have a symbiotic relationship with rhizobia bacteria which create nodules on the roots in which nitrogen fixation occurs. They possess many unique characteristics that give them particular importance for grazing animals including higher nutritive value than grasses or forbs, reduction or elimination of the need for nitrogen fertilization in mixed pastures, extension of the grazing season, and dilution of the toxic effects of endophyte infected tall fescue. Forage legumes are planted on 20 million hectares annually, with the majority in alfalfa (*Medicago sativa* L.), white (*Trifolium repens* L.) and red clover (*Trifoliom pretense* L.), or birdsfoot trefoil (*Lotus corniculatus* L.) (Graham and Vance, 2003). It is estimated that alfalfa alone, which is regarded as the most economically important legume, generates $8.1 billion dollars in hay sales each year (Bouton, 2006).

Despite having a substantial amount of research showing the benefits of including legumes in pasture systems, adoption by farmers is suboptimal. This low adoption rate in recent decades is believed to be due to a lack of producer education and seed costs (Graham and Vance, 2003). Additionally, legumes do not tolerate grazing pressure as well as grasses; therefore, it is important to alter management to ensure their longevity. The stocking rate and residual grazing height must be changed to favor legume growth. This is due to the erect growth habits of alfalfa and red clover, which require a longer residual height after grazing or cutting for long-term persistence. White clover is better suited for grazing at shorter residual heights due to its lateral growth by means of stolons. It has also been reported that this growth habit will reduce weed pressure in pastures by quickly and effectively shading out weed seedlings (Andrae, 2014); however, if not managed properly, the disappearance of clover plants will result in increased weed presence.

Clovers in particularly are sensitive to soil pH as well as potassium and phosphorous concentrations in the soil (Andrae, 2014). Red clover thrives well between a pH of 6.1 and 6.7. It is considered a short-lived perennial crop with the second year being the most productive (Taylor et al., 1976). When added to a pasture system, it is recommended to fertilize to meet the needs of the clover so as not to give a competitive advantage to grasses. For example, nitrogen fertilization should be minimized because not only does this introduction of nitrogen halt the nitrogen fixation by legumes, but grasses are more efficient at using nitrogen and will become more competitive to the legume stand. This will give the grasses a greater boost in growth than legumes and may work to shade them out (Andrae, 2014). Several legumes also offer excellent reseeding ability. In a study by Freeman et al. (2013) hairy vetch, crimson clover, and arrowleaf clover were found to reseed between 20.6 and 35.1% of the pasture area the following growing season. This offers producers the opportunity to build up the seed bank of legumes and reduce seeding costs in subsequent years.

Nitrogen Fixation. Legumes are capable of fixing atmospheric nitrogen that is present in the soil through a symbiotic relationship with a strain of bacteria. Clovers require the presence of the bacteria *Rhizobium trifolii*. In a review about biological nitrogen fixation in mixed grassland pastures, Ledgard and Steele (1992) summarized the sensitive infection process referred to as nodulation. Its success is dependent on the proper environmental conditions. The appropriate Rhizobium strains for nodulation of the specific legume must be able to survive in the soil and successfully colonize. These colonies must then recognize the legume root and will begin to expand populations near the root surface. The process of curling must then occur, which is described as root-hair deformation. The Rhizobia then digest the root-hair walls and enter the root. This is referred to as infection of the legume root hair. Excess nitrates in the soil will reduce the frequency of this process, which is another reason limited nitrogen fertilization is recommended when legumes are present. Nodule initiation is the passage of the Rhizobia into the cortical cells of the root. The infection initiates cell division, which forms the nodule itself. The bacteria then multiply within the nodule and work to fix atmospheric nitrogen. The amount of nitrogen produced by each plant is affected by the genetics of both the Rhizobia and the legume itself. It has also been reported that coating seeds with calcium carbonate (lime) will improve the nodulation process due to a localized increase in soil pH. However, most coated legume seeds also contain various strains of rhizobia. These introduced strains will often compete with any existing colonies.

In the same review (Ledgard and Steele,1992) the authors go on to explain that the production of nitrogen by legumes is heavily dependent on several factors including: soil nitrogen status, legume persistence and yield, and competition with grasses. As previously mentioned, the presence of nitrogen will reduce the infection rate of Rhizobium, which will consequently reduce the amount of nitrogen fixed. The nitrogen availability of a soil will also influence the legumes ability to compete with grasses. With lower soil nitrogen, legumes can still thrive whereas grass production suffers. This results in the dominance of legumes until soil nitrogen is increased. It is then that legume percentage within the pasture will be reduced due to increased grass yield. Changes in soil nitrogen can occur due to the weather, with lowest nitrogen fixation being during hot or dry conditions, or due to the presence of urine or feces from grazing animals. Low soil moisture will reduce the microbial activity, slow the inorganic nitrogen uptake from other plants, and reduce photosynthesis. These factors all work to reduce the persistence of leguminous plants. Improper soil pH will reduce legume persistence, subsequently reducing nitrogen fixation.

Grazing animals can also reduce legume persistence due to preferential grazing. Intensive grazing, however, has shown to increase the amount of biological nitrogen fixation over time (Ledgard and Steele, 1992). Several studies have found that the amount of nitrogen fixed is directly correlated with legume dry matter yield for both annuals and perennials. Legume and grass mixed crops tend to have higher values of nitrogen fixation than legumes alone at a fixed dry matter yield. This is due to the more competitive uptake of soil nitrogen by grasses, which forces the legumes to fix greater amounts of nitrogen for their use (Carlsson and Huss-Danell, 2003).

Nitrogen Cycling. Transfer of fixed nitrogen to grasses in the pasture can occur both above ground by means of a grazing animal or below ground via excretions by the root or microbial decomposition. Above ground occurs when an animal grazes the legumes and returns 65-

90% of the nitrogen in the feces or urine. Grasses can then take up this nitrogen as it is returned to the soil. Below ground transfer will occur when bacteria in the soil decompose legume tissue or if roots excrete the nitrogen into the soil. Nearby grass roots to either of these processes can then benefit from the nitrogen release (Ledgard and Steele, 1992). Nitrogen transfer from legumes to nearby grasses is suggested to be facilitated by mycorrhizal fungi and through nitrogen release into the soil by leaky roots. Only small amounts of nitrogen are excreted compared to the amount of atmospheric nitrogen that the legumes themselves fix. Decomposition of dead roots and nodules is suggested as the greatest method of nitrogen transfer in non-grazed systems. In a study by Dubach and Russelle (1994), alfalfa fine roots release more nitrogen when decomposing than active nodules. In Birdsfoot trefoil, the decaying nodules provided more nitrogen than decaying fine roots. However, neither of these processes could account for the amount of nitrogen that other studies have found transferred between living legumes and nearby grasses. This points to other mycorrhizal fungi actions that have not yet been determined.

Nitrogen Contributions by Clover. Due to the variation in factors that impact it, the amount of nitrogen added to a system by leguminous plants are difficult to determine. Estimated nitrogen fixation values range for the various legume species. Alfalfa has the highest estimated potential, with a range of 56 to 336 kilograms of nitrogen per hectare. White clover can produce a range of nitrogen anywhere from 34-280, red clover 56-280, and crimson clover from 34-168 kilograms of nitrogen per hectare (Hancock et al., 2014). In a study conducted by Dobson and Beaty (1980), grasses were sown with or without white clover and then fertilized with 3 different rates of nitrogen. The nitrogen content of the grasses was then measured and analyzed. As expected, nitrogen fertilization did increase plant tissue nitrogen across all treatments. However, those treatments sown with white clover with no exogenous nitrogen had nitrogen concentrations equal or greater to the highest level of nitrogen fertilization in the grass-only treatments. Phosphorous concentration was not significantly different between treatments planted with or without clover and plant tissue calcium concentration was higher in those treatments with white clover than those without. This improvement of nitrogen and calcium concentrations had greater significance in warm-season perennial grasses than cool-season perennial grasses.

Further research by Dobson and Beaty (1977) has followed the influence of including white clover on the dry matter yield of five grasses: orchardgrass, tall fescue, coastal bermudagrass, common bermudagrass, and dallisgrass. The dry matter yield of each of these grasses was measured after the addition of 0, 37, 112, or 336 kilograms of nitrogen per hectare as both a monoculture and as a mixed grass/white clover pasture. As expected, as fertilizer rates increased, dry matter yield increased, regardless of the presence of clover. The amount of forage produced with the presence of clover and no nitrogen fertilization was equal to the amount of forage produced when fertilized with 96 kg of nitrogen per hectare. This means the inclusion of clover increased forage production by an average of 3,284 kg/ha (219%). The average percent of clover for each grass/clover treatment decreased as nitrogen fertilizer increased and also decreased over time between the months of June and September. The percent dry matter in the sward from clover ranged from 57% in June to 9% in

September without nitrogen fertilizer. At the highest rate of fertilization, percent clover dry matter in June was 40% and was 5% in September. The resulting increase of pasture dry matter yield without the addition of exogenous nitrogen resulted in a more economical production of beef for cattle producers. In a study following steers fall grazing a nitrogen-fertilized wheat-ryegrass and a wheat-ryegrass-white clover pasture had the same average daily and body weight gains. Although animal grazing days was much lower for the pasture with white clover, cost to gain averaged about $0.26 less in the pastures with clover than those without. This reduction was due to the cost of nitrogen fertilizer (Beck et al., 2012a).

Improved Livestock Performance. The improved cost to gain ratio seen by livestock producers is not the only reason to graze legumes. The nutritive value of legumes compared to grasses is generally much higher at the same stage of growth. In a study that compared the nutritive value of different legumes, red clover had low crude protein values, but produced the greatest dry matter yield after the establishment year as compared to alfalfa and birdsfoot trefoil. Alfalfa was generally higher in neutral detergent and acid detergent fiber than both red clover or birdsfoot trefoil (Cassida et al., 2000). Bermudagrass pastures, which are dormant during the fall and winter months, are often overseeded with cereal grains to increase cool-season grazing. A study looked at the impacts of adding crimson clover to a typical cereal rye overseeded pasture. The calf gains per hectare increased 91% compared to no overseeding at all, with the clover component attributing 40% of the increase. The researchers also compared cereal rye alone to crimson clover alone, and found that the calf average daily gain increased 24% on clover alone. Cow and calf gains per hectare averaged

785 kilograms for the clover only overseedings, whereas the mixture of cereal rye and clover averaged 897 kilograms per hectare. Although the grazing season was shorter for those pastures overseeded with clover alone, Hoveland et al. (1978) concluded that including clovers into overseeded pasture mixes is critical for cow and calf performance through winter. In a comparison of dry matter yield of three annual legumes for overseeding, crimson clover produced the greatest dry matter yield as compared to hairy vetch or arrowleaf clover. All three legumes reseeded the following fall, and crimson clover had the lowest impact on subsequent bermudagrass productivity (Freeman et al., 2015). A study conducted in Oklahoma evaluated the potential of several cool- and warm-season legumes to improve bermudagrass pastures and found very little success in increasing dry matter yield. Overseeding of hairy vetch, black medic, or Korean lespedeza increased herbage mass in the establishment year, but decreased significantly the following two years. White clover was slow to establish and only ever marginally improved the overall dry matter yield of the pasture. However, this study was conducted in Oklahoma, where environmental conditions vary greatly from the southeastern United States. Seeding was also done using hand broadcasting, which may not provide the most uniform distribution of seeds. Overseeding bermudagrass pastures with crimson clover and cereal rye will result in the highest dry matter yield and calf gain per hectare (Bartholomew, 2010).

Negation of the Impacts of Endophyte Infected Fescue. Kentucky 31 Tall Fescue is the predominant cool-season grass in the southeastern United States. This grass was first planted in the United States commercially in the 1940s, and it was discovered 30 years later that the grass often contained a fungus living between the cells in the plant. This fungus, referred to as an endophyte (*Epichloë coenophiala*), produces ergot alkaloids that negatively impact the growth and performance of grazing livestock. Ball et al. (2015) discussed this endophyte in a review. The most dramatic and obvious sign of fescue toxicity is fescue foot. This relatively rare syndrome is characterized by the loss of tail switches, the tips of ears, lameness and can even result in sloughing off of hooves. This condition is most apparent in extremely cold weather conditions. While this condition can be most often attributed to fescue toxicity, there are a number of production losses that are more difficult to directly attribute to grazing the endophyte infected grass that are collectively referred to as Fescue Toxicosis. These symptoms include reduced feed intake, decreased weight gain, lower milk production, increased respiration rate and body temperature, retention of winter hair coat, low serum prolactin, and lower reproductive performance in cattle. Endophyte infected tall fescue can also cause abortions, prolonged pregnancy, and dystocia in horses that have grazed during the third trimester of pregnancy (Ball et al., 2015). It is estimated that one billion dollars in losses are associated with fescue toxicosis annually (Klotz, 2015). The most obvious strategy to eliminate these issues is to remove the endophyte infected fescue and replace it with a forage that does not contain the endophyte (termed endophyte free) or with an infected fescue that contains a fungus not toxic to grazing livestock, termed novel endophyte fescue. There has been producer hesitation with this model because it is estimated to cost up to $600 per hectare just to destroy endophyte infected fescue pastures and then replant with an endophyte free or novel endophyte fescue (Kallenbach, 2015). The addition of legumes, such as white clover, to endophyte infected fescue pastures has been shown to significantly reduce the negative effects on grazing livestock. The addition of ladino clover to endophyte infected fescue pastures resulted in significantly greater gain per acre in the spring than those pastures with only fescue, with some reporting as much a 0.1 kg increase per day in growing animals (Clover, 1981; Ball, 1991). However, in a recent review by Gadberry et al. (2015) it was determined in a meta-analysis that these increases in cattle average daily gain were only substantial during the spring and summer. The authors also concluded that interseeding legumes into infected fescue did not decrease fescue toxicosis or impart the same benefits as grazing non-toxic fescue. They suggested interseeding legumes results in an additive response in tall fescue and non-toxic fescue pastures because benefits to animal performance are similar in non-toxic fescue pastures as they are in toxic fescue pastures.

The addition of white clover reduced the carrying capacity of an endophyte-infected fescue-based pasture in a study by Beck et al. (2012a) as compared to those that had nitrogen fertilization and no clovers added. Despite the reduction in grazing days, there was no significant difference between BW or ADG of steers at the time of removal. After grazing these pastures again in the spring, those cattle that grazed the clover pastures had a higher final body weight despite seeing a reduction in grazing days again. These researchers found a 0.2 kilogram per day increase in average daily gain with the added clover. They did not see a total elimination of fescue toxicosis; however, the amount of clover that was in the pasture was not reported, so it is possible that clover stands were too low to achieve a reduction of symptoms (Beck, 2012b).

It was previously believed that the addition of clovers simply reduces the amount of toxins consumed during a day and acts as a dilutant, but it appears that animal performance improvement is likely due to the overall improvement of the pasture's nutritive value (Gadberry et al., 2015). However, an exciting new study out of Kentucky suggests that some clovers may actually be doing more. Many legumes produce small molecules referred to as isoflavones. These compounds have estrogenic effects and can cause hindrance to reproductive success, particularly in small ruminants. However, these isoflavones may also provide benefits that we were previously unaware of. In a study by Aiken et al. (2016), red clover extracts were used to test the impact of the isoflavone biochanin A on rumen function and artery dilation in steers fed toxic fescue. This study determined that the extract from red clover was successful in dilating blood vessels that were constricted due to the ergot alkaloid present in endophyte infected fescue as well as reducing populations of gram positive ruminal bacteria, similar to the response seen when feeding ionophores. This study warrants further research to determine the dosage required to see these beneficial effects, and concentrations found in various species and varieties of legumes, but are certainly exciting for the future of the importance that legumes have in fescue-based grazing systems.

Lack of Legume Acreage. Although the benefits of legumes have been well studied, their presence is not well represented in pastoral systems. Some researchers attribute this to their intolerance to grazing pressure, pests, pasture herbicides, and grass competition in the southeastern United States specifically (Graham and Vance, 2003). Although these all contribute greatly to reduced longevity of legumes, difficulty or confusion with establishment is just another barrier producer's face when seeding legumes into perennial pasture systems.

Methods of Establishment

No-Till Drilling. No-till drilling was first done in New Zealand around 1958 (Baker, 1978). This method of seeding involves running a drill through the pasture that only disturbs a small area of the surface. A blade cuts into the soil to the set depth, a seed is dropped into the slit, and a small wheel runs behind to push the soil back over the seed. For sowing clover seed, this method can be done in either fall or spring. In a study where fall and winter no-till drillings were compared, alfalfa and ladino clover seeds were planted into 10 inch rows. The population every week for four weeks was counted. It was found that plant density at all counts post planting was highest for February (winter) sowing as compared to the October and November (autumn) (Rogers et al., 1983).

No-till drills can also be used for another method of seeding referred to as strip-seeding. Strip-seeding is planting all of the recommended amount of seed in a quarter of the area. In a study conducted in Arkansas, the efficacy of strip seeding as opposed to traditional no-till drilling was compared. The thinking behind this method is that since white clover spreads by stolons, increasing the rate in a small area allows for greater establishment and would warrant significant spreading from the stolons over time. The hope is that this would result in a larger quantity of white clover established overall. To study this, researchers used a no-till drill and seeded Patriot white clover into tall fescue pasture that had been closely grazed to a residual height of 5 cm. Strip seeded treatments were conducted by drilling 100% of the recommended seeding rate over 25% of the area. Depending on the size of the treatment area, this was generally done in three strips across the pasture averaging 50 feet wide. Counts were conducted monthly at 15 random locations throughout the pasture between April and September using an eight-by-eight cm frame and recording the percent of clover in each. Strip seeded clover average 65% of the total area as compared to 42% for solid no-till drilling. This same trend was seen in the second year, with 84% clover stand in strip seeding versus 61% for solid no-till drilling. After two growing seasons, 92% of the strip seeded field was covered with white cover as opposed to 70% with solid no-till drilling. The authors attributed this to seed dispersal by cattle as well as stolon growth from white clover plants (Jennings and Simon, 2010).

Frost Seeding. Frost seeding refers to a method of planting in which seeds are broadcast throughout the pasture in mid- to late-winter. The idea behind this method is that the freezing and thawing action of the soil incorporates the seeds better than if they were broadcast in other periods during the year. It has been reported to work best in loam or clay soils that have greater soil moisture leading into summer. Currently, recommendations include close grazing of pastures prior to frost seeding and seeding about 45 days prior to grass growth. Following seeding, the grass should grow to about 20 centimeters in height and then be lightly grazed in order to open up the canopy for reduced seedling competition (Leep, 1989). Comparison between broadcasting in the fall or in the late winter have also been conducted for both red and white clover. It was determined that red clover established better than Ladino white clover when seeded in the fall, whereas there was no significant difference between the two species when seeded in late winter. It was noted that red clover was more productive on a dry matter basis than Ladino white clover. Although no yield or establishment differences were observed between seasons, the authors suggested that due to cricket pressure during the fall, seeding clover during late winter should be the preferred method (Smith et al., 2012). A separate study conducted in North Carolina observed a difference between seeding alfalfa in mid-February compared to mid-March. They found no difference between the dates in year one or two, but in year three the alfalfa seedlings were denser from the February frost seeding date (Zarnstorff et al., 1990).

Aerial Seeding. Seeding by broadcasting from an airplane or helicopter into a pasture, cropland, or logged areas is becoming of interest. In logged areas, aerial seeding is preferred because large and otherwise unpassable areas can be seeded to prevent soil erosion. It also allows for the application of cover crops when it is not possible to do so from the ground in row crop and possibly even pasture scenarios. A loose soil with sufficient cracks or residual cover will allow for greater establishment. Soil moisture is critical and these conditions should be used for broadcasting success. As with frost seeding, large seeded legumes will not perform well in this system. Seeding rates should be adjusted to account for a 25% loss. Research has suggested that helicopter applications may provide a slight advantage as compared to airplane seeding due to the turbulence causing the vegetation below to shake, which reduces the amount of seed caught in the stand canopy. Helicopters are also more maneuverable, improving distribution in irregularly shaped pastures. Regardless of the aircraft used, the maximum height to drop seed is 50-60 feet above the canopy (Brooke, 1988).

Comparing No-Till Drilling to Frost Seeding. Some research has been done to compare the establishment success between no-till drilling and frost seeding legumes into perennial pasture. Schlueter and Tracy (2012) studied the impact of no-till drilling versus broadcasting in winter with low seeding rates of just 2.2 kg/ha for white clover and 4.5 kg/ha of red clover. No significant differences were found in this study between the establishment methods. However, a study by Mueller and Chamblee (1984) compared February and March no-till and frost-seeded establishment. They found that in that the first year, the no-till drill treatments resulted in significantly higher seedling counts (71% coverage versus 33% coverage within a 20 x 30 cm frame). In the following three years of the experiment, the authors concluded that acceptable stands resulted from frost seeding (21%, 62%, and 25%). Again, February seedings were more successful than March seedings. Authors attributed this to lowered grass competition (Mueller and Chamblee, 1984). Interestingly, a study out of Arkansas determined that no-till drilling resulted in more clover seedlings than broadcasting. However, this study was done in the fall (Smith et al., 2012). Further work needs to be done to compare no-till drilling in the fall and frost seeding in mid- to late-winter.

Seed and Seedling Competition with Perennial Vegetation. After sowing, clover seedlings have much competition to overcome. Perhaps the largest source of competition is with grasses for light, moisture, and nutrients. There have been many studies exploring how to best reduce this competition to achieve successful establishment. Many have looked at the impact of applying sub-lethal levels of herbicide, such as glyphosate or paraquat, throughout the perennial pasture. This sets the grass back, but does not completely kill it. A study compared the two herbicides and their impact on seedling establishment. Glyphosate was found to be more effective at killing the sod stand than paraquat. However, treatment with either herbicide resulted in greater seedling densities. Alfalfa seedling density was increased with fall herbicide control, however, successful stands of alfalfa were still established without herbicide control (Zarnstorff et al., 1990).

Numerous studies have found a negative relationship between grass residual biomass and legume seedling establishment (Schlueter and Tracy, 2012; Smith et al., 2012; Zarnstorff et al., 1990; Dowling et al., 1972). In a study by Smith et al. (2012), legumes were seeded into recently grazed paddocks, averaging 5 cm in height or 15 cm height. Those paddocks left tall were grazed for 5 days after seeding. They found no difference in establishment between treatments grazed before or after sowing, indicating that competition was not hindering seeds for up to 5 days after seeding. Contrary to the mass of results, one study found no significance between the frequency of cutting nor residual grass biomass on clover seedling establishment. They did find, however, that both measures impacted the number of leaves per plant at 9, 12, and 15 weeks post-cutting. The lowest cutting height, 3cm, resulted in more leaves per plant than a cutting height of 7 cm. The number of stolons present was also increased by 50% in the 3 cm cutting height treatments (Boatman and Haggar, 1985). Reducing competition by decreasing residual grass biomass is seen as a simple way to reduce competition for emerging seedlings for producers.

Animal treading after sowing seeds was studied by Sithamparanathan et al. (1986) as a method of improving seed-soil contact and decreasing competition from existing vegetation. Five treading intensities were applied to treatments after sowing for a 24-hour period, upon which the livestock were removed. For both red clover and alfalfa, a positive response was seen with increasing treading intensity.

Seed Coating. It is currently widely believed that coating of clover seed with bacterium, *Rhizobia trifolii* and limestone (calcium carbonate) improves establishment, nodulation, and yield. It has also been suggested that the limestone coating protects seeds during times of moisture stress and improves localized pH. As previously mentioned, clovers are particularly sensitive to pH, and a localized increase in soil pH is thought to improve clover establishment (Lowther and Kerr, 2011). Dowling et al. (1971) studied the impact of coating on moisture stress and found that seeds coated with limestone had improved germination in a controlled environment than those without coating. Those coated seeds were better able to hold moisture during periods of dryness than seeds without coating. They were also able to reach higher moisture contents that uncoated seeds. However, this study was conducted in a lab setting.

Studies conducted in field suggest little improvement from the presence of coating. In a study out of New Zealand, Adams and Lowther (1970) sought to determine if there were yield or nodulation differences between coated seeds versus uncoated seed sown into a field that had previously had varying rates of lime applied to the surface. They determined that the limestone coating had no significant effects on clover yield or nodulation even if the field had not had lime applied by broadcasting. They also found that coating seeds that had been inoculated reduced the viability of the added *Rhizobium*. Similarly, Sithamparanathan et al. (1986) were not able to find significant differences in seed coating and establishment success in a field setting. In a review by Lowther and Kerr (2011), they determined that due to the rapid spread of clovers throughout New Zealand, inoculation was not beneficial except in three specific times: when sowing into undeveloped grasslands, virgin pastureland after cleared woods, or areas that had been used to grow corn only for a decade or longer. The authors concluded that there was no significant research to conclude that inoculation improved clover nitrogen fixation in areas that already had the *Rhizobia* spp. necessary for nodulation. Despite research that suggests little to no benefit from inoculation or coating with limestone, it has become an industry standard to market seeds with these additives.

Summary. The wide array of climates and variation in data has made it difficult to determine an absolute recommendation for establishing clover in perennial pastures. From the current literature, it appears that no-till drilling in the fall or broadcasting in mid- to late-winter (frost seeding) will yield the best results. Those producers challenged with topography or lack of equipment, or that stockpile autumn growth for winter grazing should consider frost seeding rather than drilling. Given the amount of conflicting data, it is understandable why producers may become confused about which methods to use, so better outreach materials on the establishment and management of clovers in pasture-based systems is warranted.

Endozoochory

Introduction. Perceived lack of time or equipment limitations when broadcasting or no-till drilling into perennial pasture has led some producers to try a unique method of seeding. Endozoochorous is described as the dispersal of seeds by animals through ingestion and is an established folk practice for establishing clover in perennial pastures. Producers who use this method will mix seeds, typically clover, into the mineral or concentrate that is fed to cattle daily. The hope is then that the cattle will walk throughout the pasture and the seeds will germinate in the fecal pats, and eventually, populate the pasture. Researchers have taken particular interest in this phenomenon in regards to weeds and weed management. It has been found that some weed species have seeds that survive the ruminant digestive tract, and use this and their primary method of distribution. Endozoochory has also been shown to be a method of dispersal for more desirable pasture species. Jones et al. (1991) sought to discover how many and how often seeds were found in cattle fecal pats. They found that seed content of feces was higher during the summer and fall than other seasons, which follows the seasonal patterns of seed head emergence. They also found a positive correlation between stocking rate of the pasture and the fecal pat seed density. This was due to an interaction with stocking rate and grazing pressure. Those pastures with higher stocking densities were overgrazed and up to 20 times the number of seeds were found in those fecal pats as opposed to the lightly grazed pastures with a lower stocking rate. It was also determined that fecal pat distribution was uneven across pastures, with a tendency to have dung concentrated near lounging areas. This would result in uneven seed distribution, which would cause localized spreading of pasture plant species.

Seed Viability After Passage. Despite cattle consuming significant amounts of seed, either by grazing or through introduced seed, very few end up as viable. The seeds encounter severe conditions within the ruminant digestive tract, including contact with microbes the rumen and digestive enzymes in the small intestine, each of which can negatively impact viability. This reduction is likely due to drastic changes in pH as well as cellulolytic and saccharolytic microorganisms within the rumen (Kiley, 2014). Furthermore, increased gut retention decreases viability (Whitacre and Call, 2006), although this factor has been suggested to be more severe with some species than others (Doucette et al., 2001). A large percentage of seeds recovered after passage were found to be swollen, have removed or broken seed coats, or have begun germination (Doucette et al., 2001; Gardener et al., 1993; Whitacre and Call, 2006; Blackshaw and Rode, 1991). Blackshaw and Rode (1991) reported that the relation between gut retention time and viability was not gradual. The seed viability remained strong for up to 8 hours post ingestion for some species and then drastically declined.

Passage Rate and Seed Viability. Passage rate can be directly correlated with diet quality. Tjelele et al. (2014) determined that the nutritive value of the diet greatly affects seed recovery and survival after passage. Forage with higher nutritive value allows for increased seed passage, resulting in decreased seed coat damage. Additionally, Simao et al. (1987) concluded that both animal species and weight greatly impact passage rate. Seeds ingested by cattle had greater recovery than that of sheep and goats. The authors concluded it is likely due to the size differences in the digestive tract. Cattle did not digest seeds as completely as sheep and goats, which may be due to initial mastication or the mere size difference of the digestive tracts. The passage of digesta into the omasum is determined by particle size, so the larger reticulo-omasal area in cattle allows for greater particle size passage, and therefore, the decrease in damaged seed passage is to be expected. This is conflicting with data reported by Russi et al. (1992), who found sheep to have the highest total seed recovery. This discrepancy may be related to physiological differences in diet and passage rate.

Seed Characteristics. Seed characteristics such as size, weight, shape, and hardness all play a key factor in the passage rate and subsequent viability after passage through the ruminant digestive tract. Doucette et al. (2001) determined that smoother, denser seeds had increased passage rates when compared to those seeds that were lighter and rougher. Whitacre and Call (2006) concluded this was due to ease of separation with digesta. The lighter seeds float and enter the ruminal mat in the dorsal rumen, while denser seeds will quickly enter the reticulum and from there pass through to the omasum. Other researchers have found small seed is a key factor in successful passage with some viability remaining (Russi et. al, 1992; Tjelele et al., 2014). Bruun and Poschlod (2006) did not find this correlation between seed size and survival. They did, however, find a correlation between the number of seeds fed and viability. The fewer number of seeds fed, the greater the total recovery and survival. Tjelele et al. (2014) also made this conclusion, but Whitacre and Call (2006) suggested that this was only important in some species.

Seed hardness was determined to be a key factor in the achievement of an endozoochorous seed dispersal strategy. Doucette et al. (2001) asserted that cattle dispersing hard seed would result in successful seed delivery to the pasture soil surface. Gardener et al. (1993) found that hard legume seeds passed twice as fast as grass seed, supporting the previous findings. They reported that hard seeded legumes would thrive well under this dispersal technique. Simao et al. (1987) also determined a more positive outlook for hard seeded legumes, but asserted that using this method for commercial seed was likely not worth the effort.

Competition with Fecal Pats. Gökbulak (2009) conducted two separate studies to determine if emergence of viable seeds from the fecal pats was a barrier to establishment. In a greenhouse experiment, they planted seeds to specific depths with uniform fecal pats. They sought to determine if location within the fecal pat, distance to the soil surface, moisture, and surface crusting impacted seedling establishment. Gökbulak (2009) determined that smaller seeded species had lower seedling emergence than larger seeded species, regardless of the planting depth. This causes difficulty for the endozoochorous strategy considering smaller seeded species were able to have higher viable seeds after passage. He determined that surface crusting of the feces was the largest influence on failure to emerge. As a result, he simulated a dry and a wet spring to see if conditions of moisture improved seedling establishment of three species: bluebunch wheatgrass [Psuedoroegneria spicata (Pursh) A. Love], sandberg bluegrass (Poa secunda L.), and hycrest crested wheatgrass [Agropyron desertorum (Fisch. Ex Link) Schult. X Agropyron cristatum (L.) Gaert.] (Gökbulak, 2008).

He concluded that simulated "wet springs" improved seedling emergence, which he suspected to be due to maintaining dung pat moisture content and reducing surface crusting. Location within the feces also impacted establishment. Those seeds planted near the surface of thin dung pats, residing no taller than 0.5 cm from the soil, had the greatest seedling emergence at 32%. Seeds in this same dung pat that were planted in the center had poor establishment with near 5% emergence average for each species. This result was similar to seeds planted at any depth throughout patties residing at 2.0 or 3.5 cm tall. He concluded that given the specific fecal pat conditions needed, this method was unlikely to work in field.

Conclusion. Legumes provide well established benefits in perennial pasture systems, but there is still a lack of effort to increase populations across the southeastern United States. Producers who may have time, equipment or topography constraints have turned to an unproven method of seeding. While researchers have investigated the establishment potential in portions of the process of endozoochory, there has yet to be a comprehensive study to determine if this is a comparable method of legume establishment to the already suggested methods.

Therefore, a study was warranted to determine if endozoochorous was as effective at establishing clover in perennial pasture as frost seeding. Legume seeds had greater survivability in the digestive tract of ruminants, particularly in cattle (Simao et al., 1987). Due to the findings of Gökbulak (2008), the implementation of dragging to reduce fecal pat depth should be tested in this system. Additionally, further research determining the impact of seed coating on establishment, both in the endozoochory and the frost seeding scenarios, is needed.

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CHAPTER 3: Mineral and Seed Contact Experiment

Design. An in-lab study was conducted in the North Carolina State University Crop Science facility. The goal was to determine the impact that mineral contact has on seed viability over time on both coated (limestone/inoculant) and uncoated red clover seed (*Trifolium pratense* L., variety not stated). A germinator was set to 4.5 °C with a relative humidity of 71.6% (averages during November through January in 2014-2015; Bahama, NC). This was an effort to simulate in-field conditions. The germinator was turned on three days prior to the experiment so it could reach equilibrium. In order to generate a trend line of percentage germination, incubation periods of two, seven, 14, 28, and 56 days were tested on both coated and uncoated seed that had been in contact with a loose Piedmont high-magnesium free-choice mineral (Camp Chemical; Roxboro, NC).

Mineral to Seed Ratio. The seed to mineral ratio was one ounce of mineral to 6.8 g of uncoated seed or 9.1 g of coated seed. This was determined by the average cattle consuming 4 ounces of mineral per day at the seeding rate of 11.2 kg/ha of red clover seed.

Application of Treatments. Glass beakers were used to house the mineral and seeds in the germinator. Seeds and mineral were mixed together using tongue depressors for even contact. At the appropriate time, the treatment beaker, along with a control beaker for both coated and uncoated seeds, was removed from the germinator. Using tweezers and a magnifying lamp, seeds were removed from mineral. Although some of the mineral tended to get shaken off, no other efforts were taken to remove mineral from seeds in fear interfering with the germination process. A total of 400 seeds were removed from each treatment and divided evenly into four replications. Seeds were placed on germination paper (Anchor Paper Company, SD3.5, 38#) which was wetted until saturation and placed into petri dishes. The petri dishes were placed into a germinator at 20 °C for seven days with germination counts taken on days zero, four and seven. The petri dishes were checked daily and water was added as needed. At day seven, rotting or germinated seeds were removed and recorded. Any hard seeds were left in the germinator for an additional five days. After a total of 12 days, all seeds were terminated and recorded as either germinated, rotted, or hard seeds.

Statistical Analysis. The GLIMMIX procedure of SAS (SAS Institute, Cary, NC) was used with a model statement including replicate, time, treatment, and the time by treatment interaction. A p-value of less than 0.05 was used to determine significance. Tendencies were considered with a p-value < 0.10.

Results. A time by treatment interaction ($p = 0.0001$) was observed for germination. The coated and uncoated treatments that came in contact with mineral both had reduced viability over time, unlike both control treatments ($p = 0.001$). At two days (d) and seven d, treatment did not differ in the total number of germinated seedlings. However, at 14, 28, and 56 d, there were significant differences ($p = 0.0001$). At day 14, the coated seeds that came in contact with the mineral had a significant decline in viable seeds, whereas both controls and the uncoated seeds did not. After 28 d of contact, both the coated and the uncoated seeds had reduced germination compared to control. However, the number of viable coated seeds was significantly less than the number of viable uncoated seeds. After 56 d of mineral contact, both seed types had very few viable seeds remaining, whereas the control seeds had high viability (Figure 1).

Discussion. The high viability of both sets of control seed indicates that conditions prior to the initiation of germination did not cause any negative effects. There was no impact on either coated or uncoated seeds with up to seven days of mineral contact. However, after 14 days, the coated seeds showed declines in seed viability, whereas the uncoated seed did not. It should be noted that at 14 days, a fair amount of condensation accumulated within the beakers. This is likely due to the relative humidity, set at 74.6% to match average field conditions. As Dowling et al. (1971) determined, the limestone coating on the seed increases its ability to hold moisture. The condensation likely contained salts from the free choice mineral, which could pass through the seed coat and damage the seed embryo. Given that the seed coating holds moisture, those seeds were likely exposed to greater concentrations of salts. This supports the subsequent data that shows yet another rapid decline in coated seed viability at 28 and 56 days, when nearly all seeds (99%) rotted. The uncoated seeds, however, did not show this steady decline until 28 days of contact, where the viability was reduced to 47.5%. It is suspected that if moisture were present before 14 days, the decline in seed viability over time may also occur sooner. Further research is warranted to determine if this is the case.

CHAPTER 4: Viability After Passage Experiment

Design. Approximately 450 mL of feces were obtained via rectal palpation from heifers consuming red clover seeds that had been mixed with loose mineral. Treatment groups included coated and uncoated seeds. The feces of nine (2014-2015) or six (2015-2016) heifers per treatment were compiled and immediately transported to a lab where the fecal material was sieved through sieve numbers 35, 18, and 12 following the Jones and Bunch (1988) method. Tweezers were used to remove seed from feces and, following rinsing, were then placed onto germination paper. As many seeds as possible were removed with a maximum of 400 per treatment. This was replicated four times in each year. Treatment averages were used for statistical analysis.

Statistical Analysis. The GLIMMIX procedure of SAS (SAS Institute, Cary, NC) was used with a significance of $P < 0.05$, and including year, treatment and their interaction in the model statement. Tendencies were considered with a p-value < 0.10.

Results. There was no effect of coating, year, or year by coating in the number of seeds that germinated. There was a higher percentage of coated seed that rotted compared to the uncoated seeds ($p = 0.0956$).

Discussion. Viability of both coated and uncoated seeds was reduced in recovered seeds that had passed through the cattle's digestive tract as compared to those germinated directly form the seed bag. Although there was a slight treatment effect (95% rotted coated seeds, 86% rotted uncoated seeds), both had severe reduction in viability. The ability of the coated seed to hold moisture may account for the reduced viability compared to the uncoated seeds (Dowling et al., 1971). If the coating absorbed ruminal fluid more readily or maintained the fluid closer to the seeds than that of the uncoated seed, it is possible that the cellulolytic bacteria in the rumen had a longer period of exposure for the coated seed. Although this data cannot conclusively determine where viability was lost, Kiley and Unruh-Snyder (2014) suggest that microbes in the rumen degrade the seed coat and seed embryo. The enzymes present in the small intestine as well as the drastic pH changes throughout the digestive tract likely contribute to the loss of seed viability as well. These results were unexpected, as it is understood that denser particles pass through the ruminant digestive tract more quickly (Poore et al., 1993). However, the seeds weigh so little that the difference in their weight may not have been enough to see differences in passage rate. Results from this study suggest that it may be beneficial to coat seed with rumen protected proteins if passage of viable seed is desired.

CHAPTER 5: Small Plots Experiment

Design

Small Plot Layout. A small plot study was conducted at Butner Beef Cattle Field Labs in Bahama, North Carolina in an ungrazed KY-31 Tall Fescue (*Festuca arundinacea*) field in order to quantify the competition seedlings encountered for moisture, light, and nutrients with other grasses and fecal pats. A randomized complete block design was used with a total of four replications per treatment. The area was prepared by mowing to 7.6 cm height. Each treatment area was measured to 0.914-meters by 0.914-meters to create 0.84-square meter plots. An allotment of a 0.61-meter buffer between rows and a 1.5-meter row between columns was given so that adjacent plots would not be disturbed while taking measurements. Treatments were randomly assigned a color, which was spray painted on four small wooden stakes used to mark the edges of the treatment area. Each replicate included two of the following treatments, one which was subjected to a simulated dragging: negative control, frost seeded coated seed, frost seeded uncoated seed, simulated fed coated seed, and simulated fed uncoated seed. Red clover seed was from the same lot for both the coated and uncoated treatments. The variety was a variety not stated (VNS) in year one and Kenland in year two.

Fecal Pat and Seeding Rate Determination. Wet feces were added to the center of each small plot. This amount, 567 g, was calculated using the total digestible nutrients for the grass, the water content of the feces, and the expected consumption of heifers for an 0.8

square meter area. Feces were collected rectally from heifers grazing KY-31 tall fescue (year 1), and a KY-31 tall fescue and corn silage diet (year 2). All feces were weighed out and placed in pie tin-pans. Red clover seeds were applied to the appropriate treatments at a 11.2 kg/hectare rate and scaled down to the small plot size. The same number of coated and uncoated seeds were applied, so the percentage inert material of the coated seed was used to adjust the rate. This equated to 0.95 g of uncoated seed and 1.27 g of coated seed per 0.8 square meters. These seeds were weighed out and kept in Whirl-Pack bags until the time of treatment application.

Application of Treatments. Small plots treatments were applied in early February to correspond with the commonly recommended dates for the Piedmont of NC (year 1: February 9th, 2015; year 2: February 10th, 2016). A contractor's flag was placed in the center of the plot for reference. For application of fed treatments, the Whirl-Pack of seed was poured directly into the fecal pat minutes prior to application in the small plot area. A wooden tongue depressor was then used to mix seeds thoroughly and a flexible spatula was used to scrape out the fecal pat onto the center of the plot area. For frost seeded treatments, a tarp was laid across outside the treatment area to catch seeds inadvertently placed outside the designated plot. The seeds were poured into the researcher's hand from the whirl-pack and then evenly distributed across the treatment area. Dragging was simulated on necessary treatments using a spatula. This was done by spreading from the center of the patty towards the edge of the plot in each direction. To better simulate the disturbance to the established grasses that a drag harrow may have, a hand-held three-point cultivator was then used to disturb the area after fecal pat spreading. Any debris from this process was removed from the plot. An electric deer fence was set up surrounding the small plot area in an effort to reduce wildlife disturbance. Small plots were clipped at eight and ten weeks post-seeding to 7.6 centimeters using a hedge clipper. Debris was removed as part of the effort to simulate managing for clover establishment.

Grid Count Apparatus. In order to quantify the number of red clover seedlings established, counts were conducted a total of 4 times each year. Counts were conducted using a 0.84-square meter grid with 36 total cells. The frame was built using wood that was 2.54-cm thick and 15.24-cm tall. Metal braces were used at each of the four corners to create extra support for the wooden frame. Small screws were evenly spaced on the wood and kite string of a vibrant color was stretched between the screws to create the grid frame with 15.24 x 15.24-cm cells. The wood frame was slipped over the colored wooden stakes to clearly discern the treatment area from the buffer.

Counting Procedure. A trained researcher looked directly down at each cell and counted the number of red clover seedlings in each of the 36 cells. A second researcher recorded the values and ensured the counter was in the correct grid. Red clover seedlings were counted at six, eight, 10, and 14 weeks after seeding.

Statistical Analysis. The number of seedlings was totaled across the 36 cells and statistically analyzed using the GLIMMIX procedure of SAS (SAS Institute, Cary, NC). The model included replicate, year, establishment method, dragging, and date along with all interactions.

A p-value of 0.05 was considered significant. Tendencies were considered with a p-value < 0.10.

Results. Year x establishment method (p = 0.0001), year by drag (p = 0.084), est by drag (p = 0.0001), year by date (p = 0.0001), and est by date interactions (p = 0.0007) were observed. The year by date interaction resulted because year two had more red clover seedlings established at each count date than year one (Figure 2). The year by est interaction resulted because more red clover seedlings were observed in year two than in year one for each treatment, respectively (Figure 3). The year by drag interaction resulted because while dragging increased the establishment of red clover seedlings each year, in year two the effect was greater than in year one, regardless of the treatment (Figure 4). The est by date interaction denoted changes between each treatment over time (Figure 5). In positive control treatments, red clover seedling counts increased over time. However, in the uncoated fed treatment there was an overall decrease in red clover seedling count. The coated fed treatment statistically remained the same throughout the study. The fed treatments, regardless of the presence or absence of coating, remained significantly lower in seedling counts than the positive control treatments. An establishment by drag interaction was also observed (p = 0.0001). While dragging increased the number of red clover seedlings within each treatment the degree of change between those dragged and not dragged treatments varied by establishment method (Figure 6).

Discussion. It is critical to remember that seeds used in this experiment never passed through a ruminant digestive tract, but were only mixed with feces at the time they were deposited on pasture. This study aimed to understand the establishment potential for seeds that passed intact through the digestive tract. Competition experienced by seeds and seedlings during establishment only. The year interactions seen with count date, establishment method, and dragging were likely due to environmental conditions or seed variety differences. This includes an increase in monthly average rainfall (Figure 12) and monthly average temperature for clover germination throughout the growth period (Figure 11) in year two as compared to year one. As a result, year two resulted in higher seedling counts for all treatments at all counting dates than in year one.

Dragging did significantly increase the number of clover seedlings in all treatments except the negative control, which is likely due to a reduction in competition and better soil-seed contact. For the fed treatments, this reduction in competition is largely because of the fecal pat itself. As determined by Gökbulak (2009), the thinner the fecal pat, the greater the seedling emergence and survival. Dragging reduced the thickness of the fecal pat; therefore, it dragging decreased the competition the seeds experiences. Frost seeded treatments likely had increased germination due to increased seed to soil contact after dragging. By dragging, most of the seeds caught in the sward of the established grasses were removed and landed on the soil surface.

While counting treatment groups that were fed and not dragged, we observed a high number of seedlings within the cell or cells where the fecal pat was placed, with some upwards of 40 seedlings in one cell. These seedlings would slowly die off over time, which was represented in the establishment by date interaction. This effect was more dramatic in the first year than in the second, suggesting that existence in the fecal pat caused the seedlings to be more subjected to environmental conditions. With reduced rainfall in year one, the fecal pat did not maintain a high moisture content, and was observed to elevate above the sward as grass started to grow; therefore, many seedlings died around the third and fourth counting. In year two, while this reduction in seedlings was seen only in the third count of the undragged uncoated treatment, the undragged coated seeds experienced no significant reduction in seedling numbers. This is attributed to higher rainfall in year two, which not only kept the fecal pats at a higher moisture, it also dissipated the feces and allowed seedlings to establish roots in the soil as opposed to within the fecal pat itself. Fed treatments that were dragged did have significantly higher establishment than those that had not been dragged. However, the establishment was still less successful than frost seeded treatments. The number of seedlings in both frost seeded treatments, regardless of coating, increased across count dates, which indicates that the competition was less severe for these treatments than the fed treatments. Coating did impact the establishment potential of the frost seeded treatments. The uncoated-dragged treatment had a significantly higher number of red clover seedlings. The coated treatments were impacted by dragging, with the dragged treatment averaging a higher number of seedlings. However, it did not differ from the uncoated-not dragged treatment. This indicates that the uncoated seed may have an advantage for establishment in this system when dragged. Despite the significant differences between the treatments, the fed treatments that were dragged were considered successful stands.

CHAPTER 6: Large Plot Experiment

Design

Animal Management and Treatments. The large plot study was conducted two years (2014-2015 and 2015-2016) in KY-31 Tall Fescue pastures located at Butner Beef Cattle Field Labs (Bahama, NC). A total of 36 two-year-old heifers in year one and 20 one-year-old heifers in year two were split into four uniform groups based on initial body weight and assigned to treatments: positive control (frost seeding: coated and uncoated), negative control, fed coated-seed, and fed uncoated-seed. The groups were strip grazed (year 1: December 8th, 2014-January 29th, 2015; year 2: December 7th, 2015-February 1st, 2016) with 3 moves per week (using a three-day, two-day, two-day fence-moving schedule) following a one-week adjustment period. This allotment schedule was used to improve forage utilization efficiency and manure distribution compared to continuous grazing. The area was back-fenced every seven days to keep animals off the previously grazed areas. Both water and mineral feeders were moved with the heifers weekly. Each group remained in a paddock for 14 days before being moved to the next plot. Each plot received each treatment according to a Latin Square design. Each group of animals remained on their assigned treatment for the entirety of the study. Every 14-days during the grazing period, each animal was weighed, body condition scored, and approximately 450 mL of feces was collected via rectal grab samples for determination of seed viability (experiment 1).

Mineral Handling and Management. Mineral and seed mixtures were added to mineral feeders in accordance with the fence moving schedule. The mineral and seed were weighed out and bagged separately, then mixed after placement in the mineral feeder. Every 7 days (at the time of back-fencing or movement to a new plot), any previously unconsumed mineral or seed was removed and weighed, and was replaced with fresh mineral.

Pasture Measurements. The nutritive value of the upcoming grazing area for each group was sampled along with the post-grazing available biomass using the falling plate meter. The nutritive value samples were taken to determine if differences in diet quality were experienced between groups or between years. The sward and drop heights of the previously grazed areas were taken in an attempt to quantify the height and amount (in kilograms per hectare) of residual forage biomass the seeds and seedlings were competing with for establishment in the pasture.

Seed Management and Sowing. Seeds from the small plot study (experiment 3) were also used in the large plot study (experiment 4). Frost seeded treatments were grazed without introduction of red clover seed during the grazing period and were broadcasted shortly after termination of grazing at the time (year 1: February 11th, 2015; year 2: February 4th, 2016). A hand broadcast spreader (Scotts Handy Green II, The Scott's Company LLC) was used to broadcast the seeds. The spread of the broadcaster was measured to be 4.6-meters for both coated and un-coated seed. Wooden stakes were placed in the middle of this 4.6-meter spread on each side within each treatment area so the trained individual could easily achieve an even broadcasting over the area to accommodate for the 4.6-meter broadcasting width. There were two frost seeded treatments: coated and uncoated seed. Negative control treatments were grazed and never sowed with red clover seed. The two fed treatments each received red clover seed mixed in with the loose free choice mineral. One group received coated seed, while the other group received uncoated seed mixed in the mineral. To ensure each group of animals received the same total number of seeds, the coated group's allotment was adjusted for the percent of weight made up of inert material. The amount of seed added was determined by adjusting the seeding rate to the acreage the cattle were grazing following the fence-moving schedule. Using a 11.21 kilogram per hectare seeding rate, this amounted to 3.5 kilograms for the uncoated seed and 4.3 kilograms for the coated seed for each 14-day period.

Dragging Implementation. Half of each treatment plot was dragged immediately following seeding using a section of chain link fence pulled by a Gator (John Deere Gator XUV590i) in year one (frost seeded and negative control) or by hand (fed treatments). A drag harrow (Tarter ATV 4-foot x 4-foot chain harrow with 4-foot drawbar) pulled with a Gator was used in year two for all treatments. Fed treatments were dragged immediately following the movement of cattle groups to new plots every 14 days, whereas the frost seeded and negative control treatments were dragged immediately following the time of seeding. Both the drag harrow and gator were washed between treatments so as to not cross-contaminate any treatment areas with the dragging instruments.

Seedling Counts. In order to quantify the average number of clover seedlings emerged, contractor's flags were placed in a grid fashion in each treatment area approximately 10 weeks after frost seeding. At 15 (year one) and 13 (year two) weeks after frost seeding, a trained researcher placed a square 0.25-meter frame at each flag and recorded the number of red clover seedlings present. After placing the frame on the ground and prior to seedling counting process, researchers visually estimated forage mass within the frame on a scale of one to five. A score of one represented very little competition with almost bare ground, and five represented an area that had significant amounts of biomass where no light would be able to penetrate the sward. Researchers also noted if there was fecal material present within the frame area. The number of flags in each plot ranged from 20 to 40, depending on the size of the plot. The number of flags was determined by ensuring that no flags were more than twice the distance in length as they were in width from another flag. Following the counting process, a total of ten quarter-meter square frames were clipped to the ground and dried for 48 hours to determine biomass at each competition score. Two of each score (1-5) were clipped.

Statistical Analysis. Red clover seedling counts were averaged across the plot and statistical analysis was completed using the SAS procedure GLIMMIX (SAS Institute Cary, NC). The model included replicate, year, establishment method, dragging, and all interactions. A p-value of 0.10 was considered significant. Tendencies were considered with a p-value < 0.15. This p-value was chosen due to the greater number of uncontrolled variables seen in this study, such as environmental variability.

Results

Competition Score. There was no significant difference in competition score as a result of replicate (rep), year, establishment method (est), year by est, year by drag, or year by est by drag. Dragging, however, did result in a slightly lower competition score ($p = 0.0391$) (3.5260 for dragged versus 3.6932 for not dragged).

Residual Grass. A year x date effect was observed for sward height ($p = 0.0106$). As seen in Figure 7, year two had a higher residual sward height (cm) than in year one. The residual biomass was measured in kilograms per hectare (kg/ha) and also had a year x date effect ($p = 0.0783$). At all sampling dates except date four, the residual biomass in year two was almost twice as large as year 1 (Figure 8).

Seed Refusal. There was no statistical effect of treatment or year x treatment for total mineral weighback, but a year effect was observed ($p = 0.0003$). The total number of seeds refused did not differ significantly between years, by treatment, or their interaction.

Forage Nutritive Value. In year one, there was a slight significant difference between pastures in the crude protein content of the forage ($p = 0.0530$), but no difference was seen in the total digestible nutrient content (TDN). In year two, there was no significant difference for crude protein or TDN between pastures.

Treatments. Year by treatment interaction for total stand count was significant, so the years were analyzed separately. In year one, there were no replicate, drag, or establishment method by drag interactions observed. Establishment method effect was significant ($p = 0.0001$). Neither fed treatment differed in red clover seedling count from the negative control, whereas the frost-seeded treatments had higher clover counts than the negative control but did not differ from each other (Figure 9). In year two, there was no effect of replicate, but there was an establishment by drag interaction ($p = 0.0337$). Both fed treatments and the negative control had similar values of red clover seedling counts (range of 0 to 2.15 clovers per square meter). Dragging did not affect the coated frost-seeded treatment ($p = 0.14$), whereas dragging increased the red clover seedling count in the frost-seeded uncoated treatment ($p = 0.0006$) (Figure 10).

Discussion. In both years, the fed treatments performed no differently than the negative control, regardless of coating or dragging. The effective seeding rate in year one was nine kg/ha and eight kg/ha in year two due to some mineral refusal by the cattle. Although this seeding rate was slightly lower than what was applied via frost seeding (11.2 kg/ha), this is not believed to contribute to the poor establishment in the fed treatments. Instead, the previous experiments offer plausible explanation for the stand failure. In experiment two, the seed viability was tested after passage through the ruminant digestive tract. On average, uncoated and coated seeds remained only 14% or 5% viable, respectively. Pair this reduction in seed viability with the competition experienced with dragged or not dragged fed treatments in the small plot experiment (3), and it comes as no surprise that the fed treatments did not perform well in-field. Contact with mineral is not suspected to have any impact on this study since seeds were in contact with the mineral for no longer than seven days. Dragging did not impact the negative control, fed coated, or fed uncoated treatments in either year. In year one, no treatments were impacted by dragging. This was also the case in year two for the coated treatment; however, the uncoated treatment was positively impacted by dragging in year two. This is likely due to the differences between the residual grass sward height and biomass between years. In year one, the average residual sward height was 4.0 cm with 1462 kg/ha in residual biomass. This was nearly doubled in year two with an average residual sward height of 8.1 cm and 2752 kg/ha in residual biomass. Coated seeds for this experiment were about 30% heavier than the uncoated seeds, which likely enabled the coated seeds to reach the soil surface better than uncoated seeds at initial frost seeding. When dragged, uncoated seeds that were caught in the sward of the perennial grasses were able to reach the soil surface. This accounts for the increase in establishment in uncoated seed that was not see in coated seed after dragging.

CHAPTER 7: Implications

The practice of endozoochory is a seed dispersal strategy that is commonly found in nature and has been mimicked by producers for many years. Although several studies have observed one sector of this establishment method (passage through the ruminant digestive tract), no studies to date have investigated the entire process. Seeds subjected to endozoochorous are exposed to loose mineral (or in some cases, supplemental feedstuffs), passage through the ruminant digestive tract, and then compete with fecal pats and established grasses after elimination for establishment. This study sought to quantify the impact of these three sectors of feeding red clover seed to cattle individually, as well as combined in a field setting. Additionally, this study sought to determine if inoculation and coating was necessary for the successful establishment of legumes in perennial pastures.

As a result of this study and others, it can be concluded that it is unlikely that feeding red clover seed to cattle can serve as an effective means of establishment in perennial pasture. A combination of factors that reduce seed viability, including contact with mineral for extended periods of time, passage through the ruminant digestive tract, as well as competition after passage with fecal pats and existing grasses, deem this method not beneficial for producers. Further research to be conducted in order to increase the effectiveness of this method could include seeds coated with a rumen bypass protein or seeds with a higher percentage of hard seed. Due to the similar establishment of coated and uncoated red clover seed after frost seeding, further research is warranted to determine if the coating is necessary in perennial pasture systems under a variety of environmental conditions. Producers looking for a simple and and inexpensive method of establishing clovers in perennial pasture should use the frost seeding method. In situations where the residual grass height after grazing is taller than 3.8 centimeters, dragging should be considered in order to achieve greater establishment. Further research to better understand how coating, and sward conditions including biomass and sward height influence the effectiveness of dragging following frost seeding should be conducted.

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FIGURES

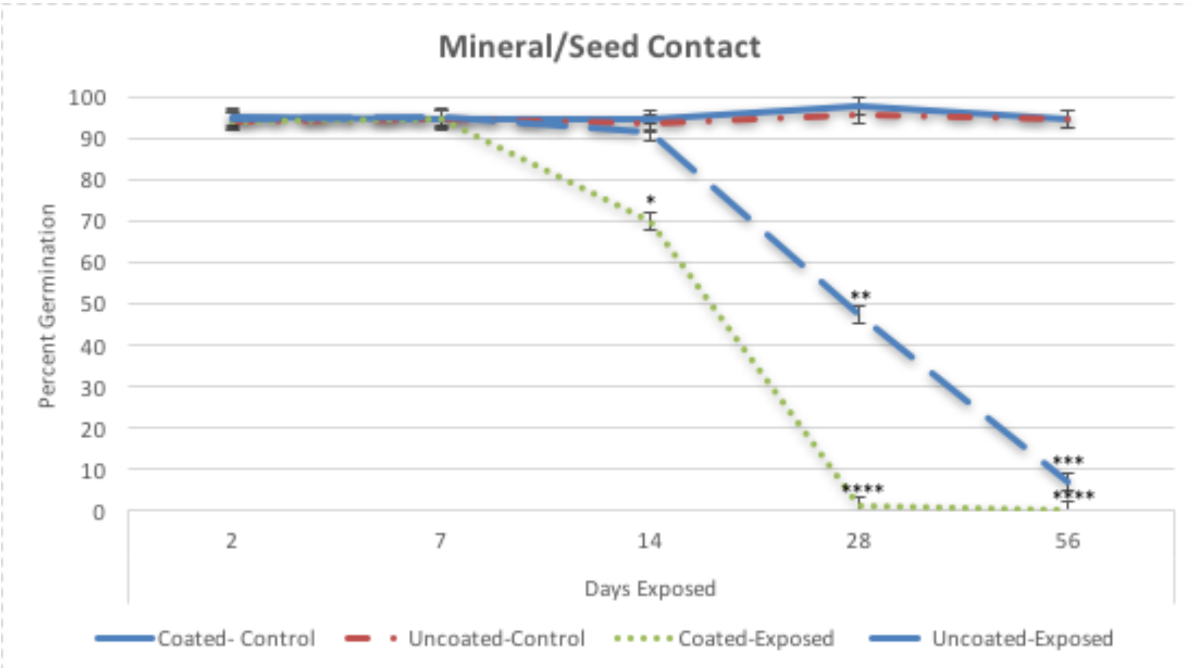

Figure 1. Average seed germination after contact with free choice mineral at certain intervals of time (averages with the same number of asterisks are not significantly different, $p < 0.10$). Conducted in 2015 in germination chambers, Raleigh, North Carolina.

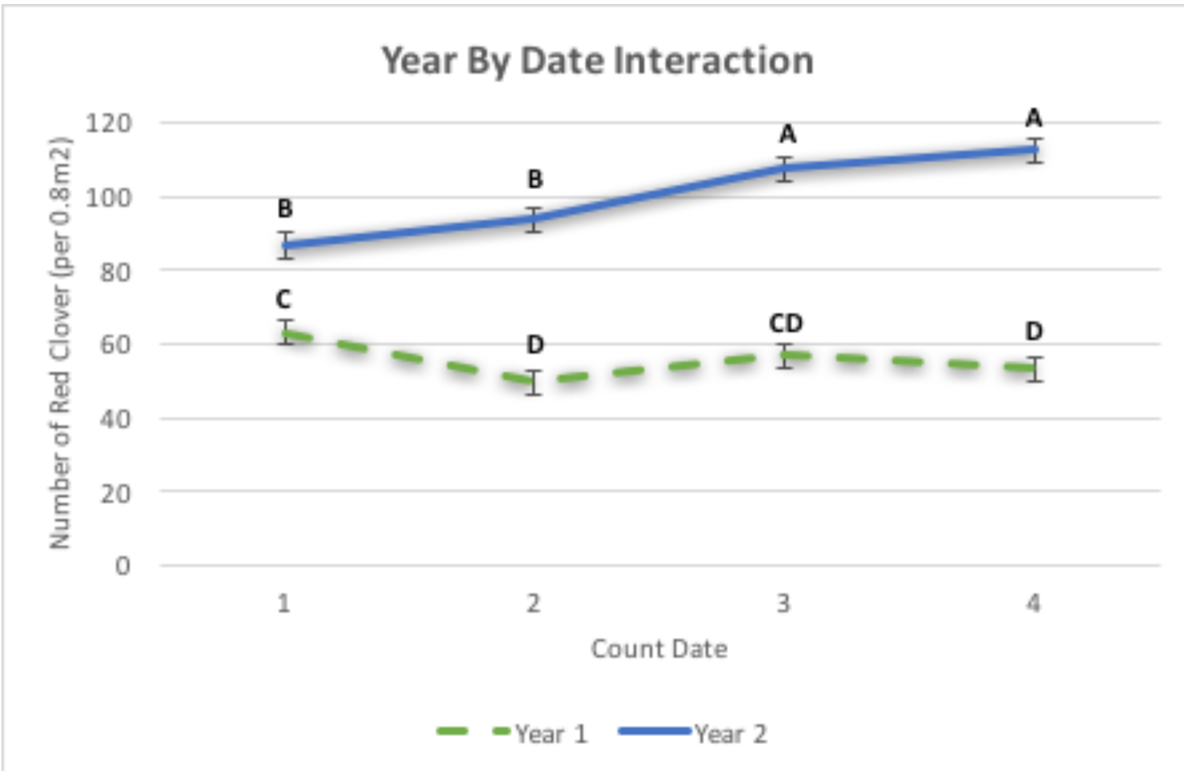

Figure 2. Average number of red clover seedlings per 0.8 square meters at each counting date by year (averages with the same letter are not significantly different, $p < 0.05$). Seeding occurred in February with counts beginning in late March of 2015 and 2016. Conducted in Bahaman, North Carolina.

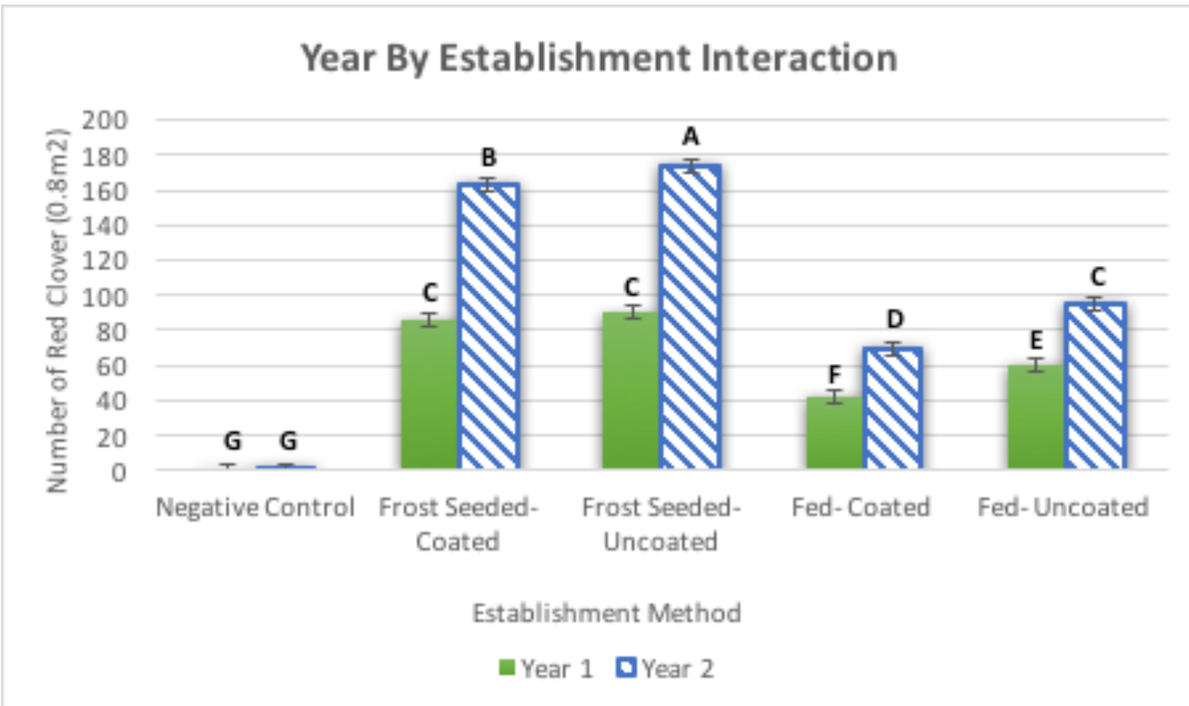

Figure 3. Average number of red clover seedlings per 0.8 square meters by each establishment method each year (averages with the same letter are not significantly different, $p < 0.05$). Seeding occurred in February with counts beginning in late March of 2015 and 2016. Conducted in Bahaman, North Carolina.

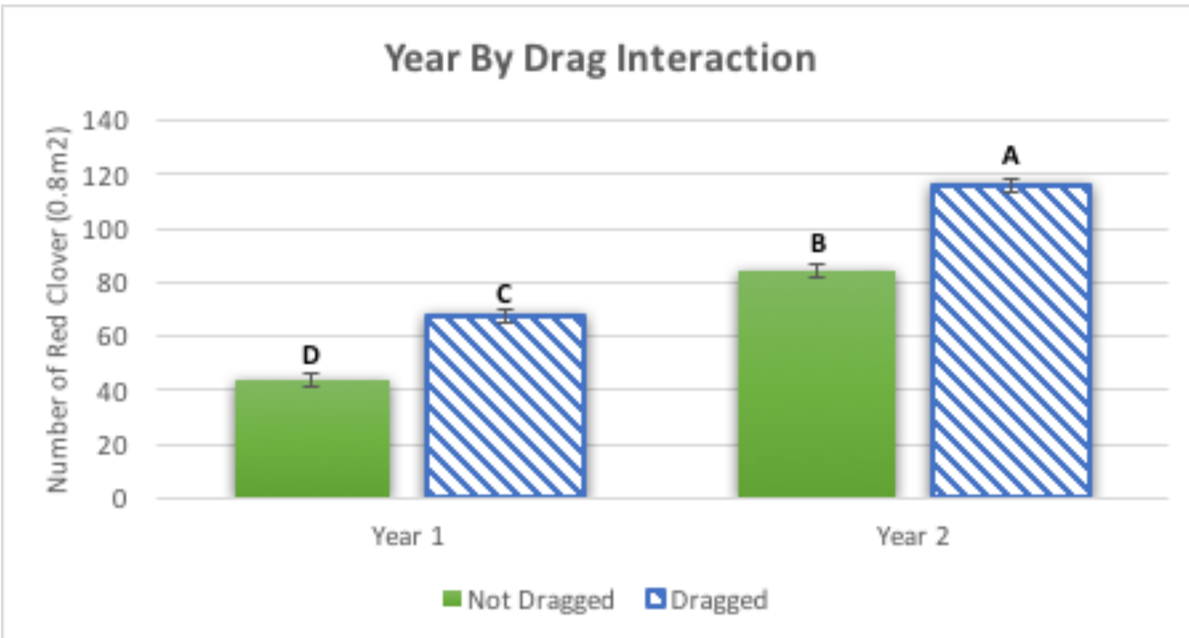

Figure 4. Average number of red clover seedlings per 0.8 square meters by year as impacted by dragging (averages with the same letter are not significantly different, $p < 0.05$). Seeding occurred in February with counts beginning in late March of 2015 and 2016. Conducted in Bahaman, North Carolina.

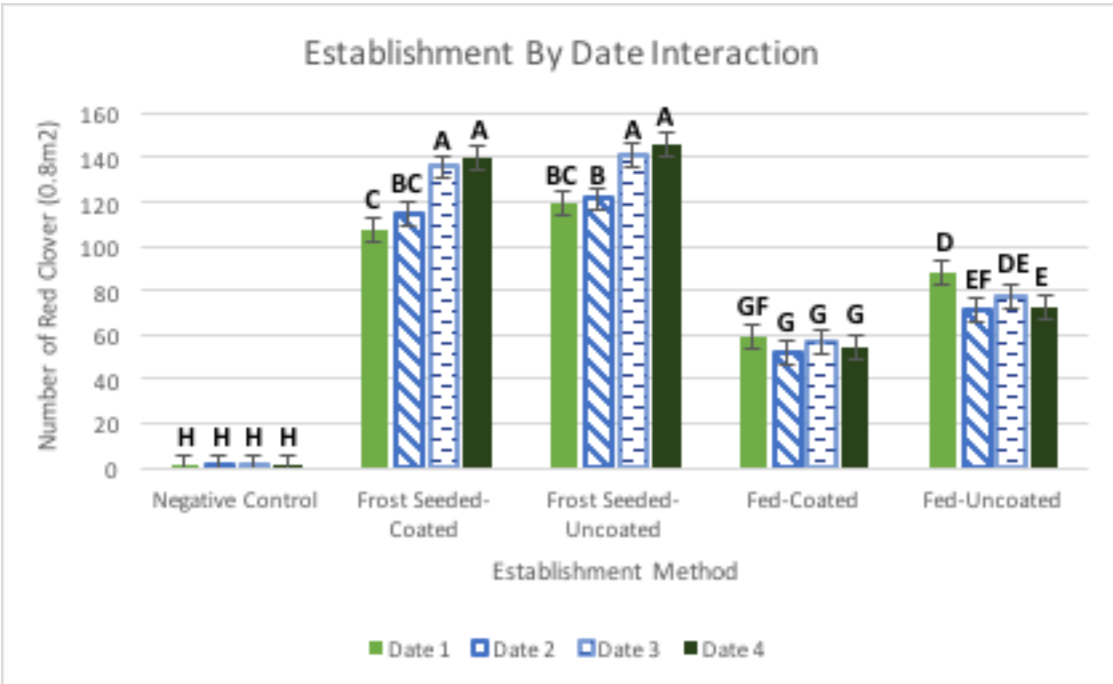

Figure 5. Average number of red clover seedlings per 0.8 square meters by establishment method at each count date (averages with the same letter are not significantly different, p < 0.05). Seeding occurred in February with counts beginning in late March of 2015 and 2016. Conducted in Bahaman, North Carolina.

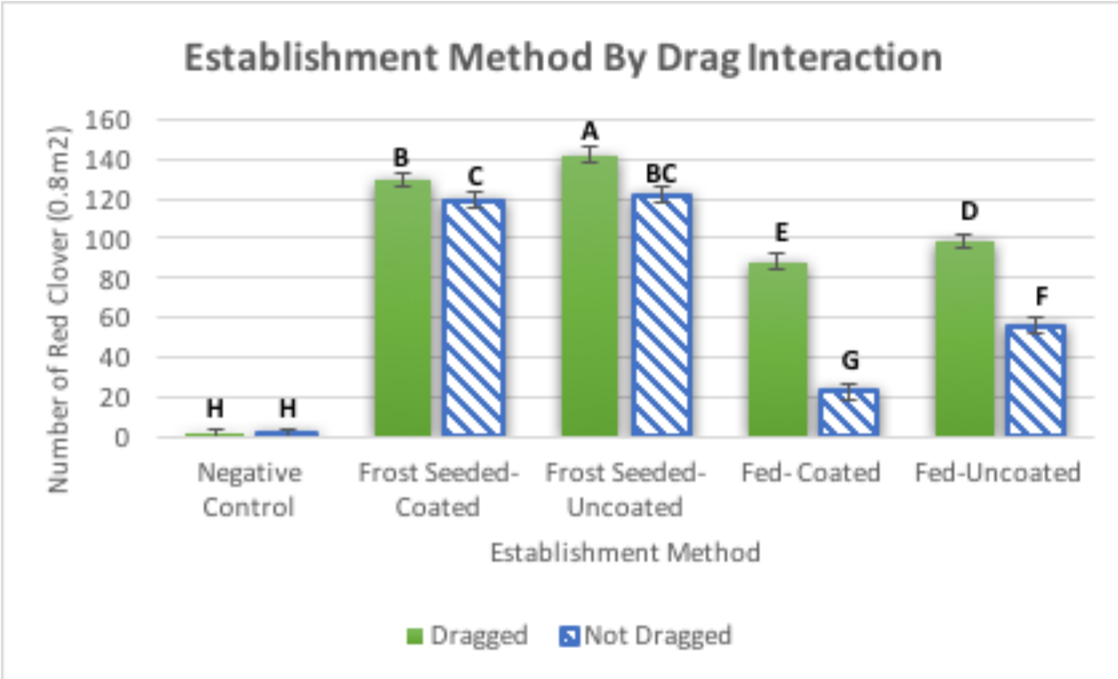

Figure 6. Average number of red clover seedlings per 0.8 square meters by each establishment method as impacted by dragging (averages with the same letter are not significantly different, $p < 0.05$). Seeding occurred in February with counts beginning in late March of 2015 and 2016. Conducted in Bahaman, North Carolina.

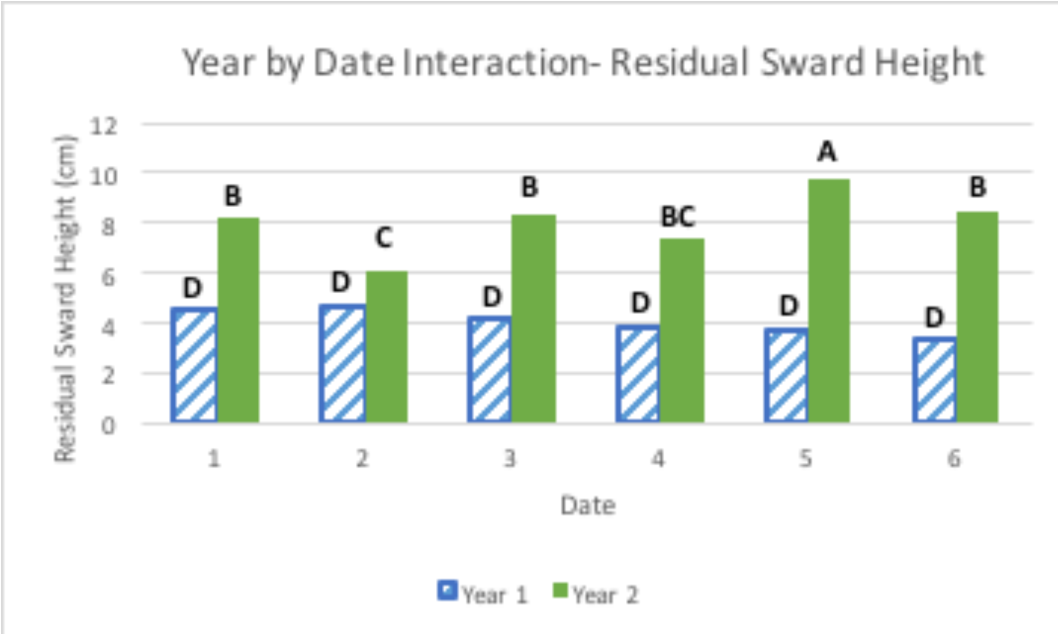

Figure 7. Average residual sward height in cm by date for each year (averages with the same letter are not significantly different, $p < 0.05$). Year 1 ran spanned from December 2014 through January 2015, and year 2 spanned from December 2015 through January 2016.

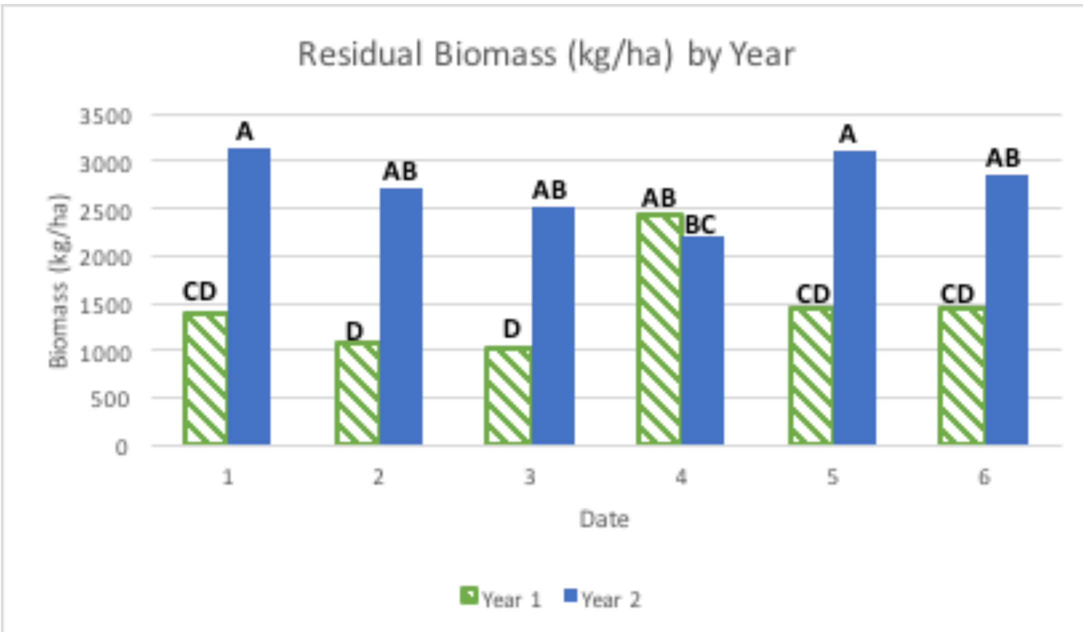

Figure 8. Average residual biomass in kg/ha by date for each year (averages with the same letter are not significantly different, $p < 0.05$). Year 1 ran spanned from December 2014 through January 2015, and year 2 spanned from December 2015 through January 2016.

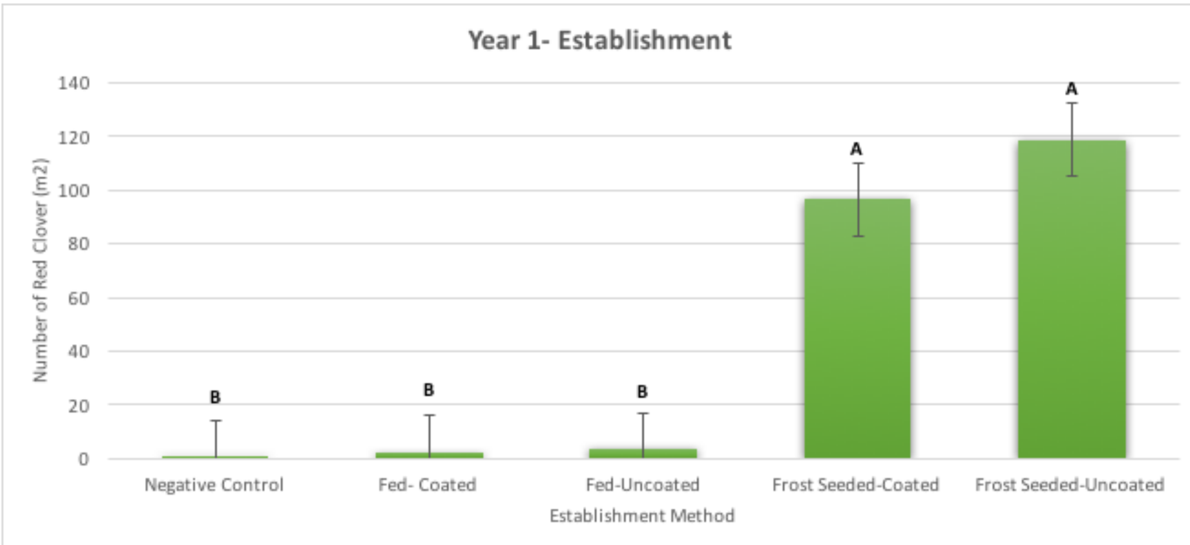

Figure 9. Average number of red clover seedlings per square meter by establishment method in year one (averages with the same letter are not significantly different, $p < 0.10$). Counting was conducted in early to mid-May in 2015 and 2016.

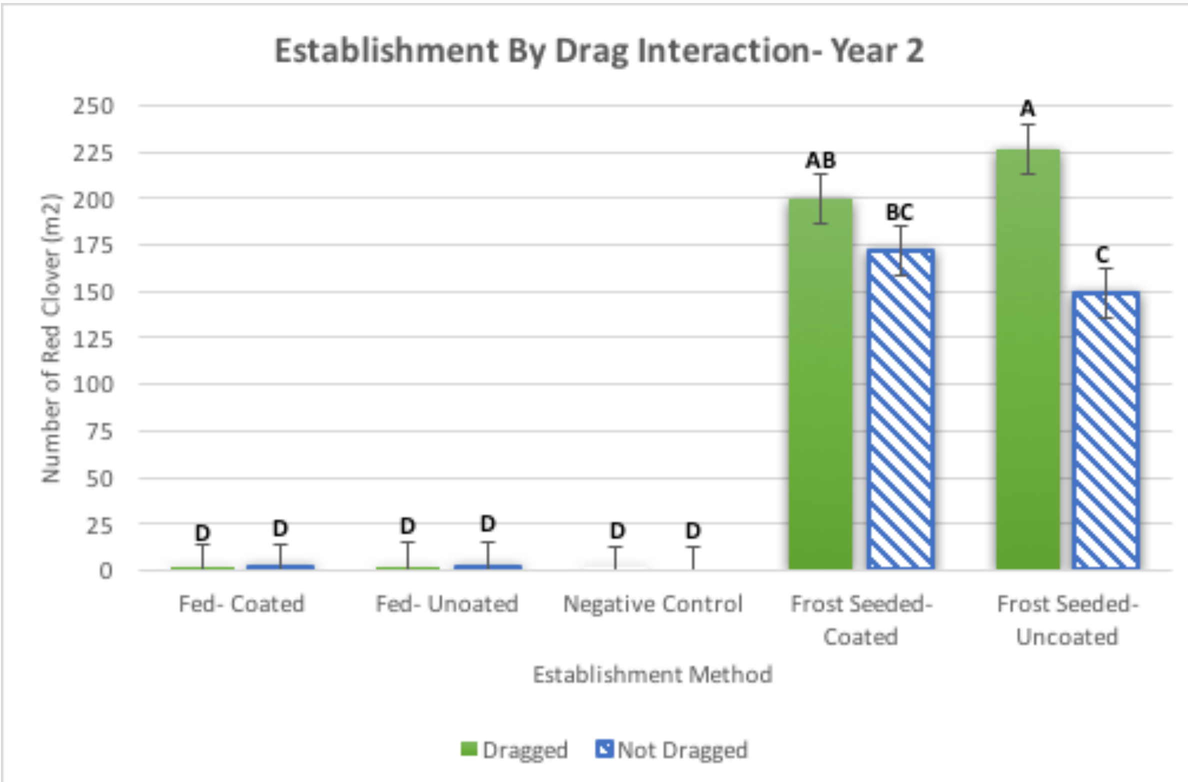

Figure 10. Average number of red clover seedlings per square meter for each establishment method as affected by dragging in year two (averages with the same letter are not significantly different, p < 0.10). Counting was conducted in early to mid-May in 2015 and 2016.

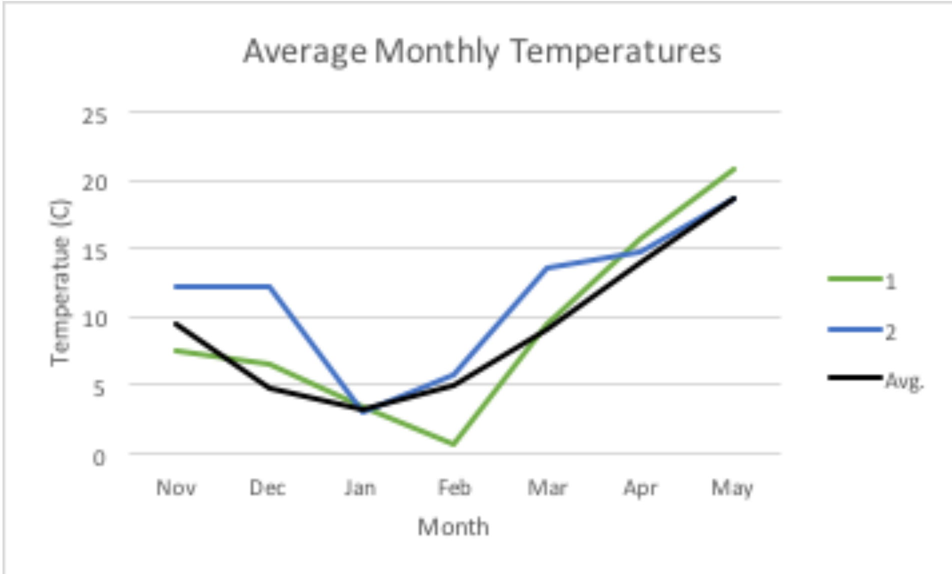

Figure 11. Average monthly temperature, based on average daily temperature, for months of research trial, years one and two, along with the 30-year average. Temperatures reflect average conditions in Bahama, North Carolina for year 1 (2015) and year 2 (2016) retrieved from the CRONOS database.

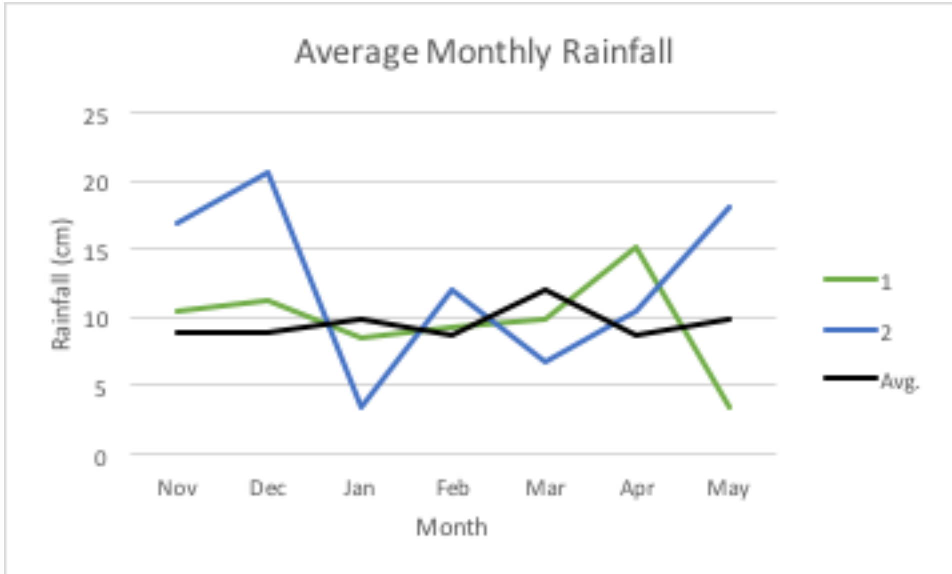

Figure 12. Average monthly precipitation, based on average daily precipitation, for months of research trial, years one and two, along with the 30-year average. Rainfall data reflects average conditions in Bahama, North Carolina for year 1 (2015) and year 2 (2016) retrieved from the CRONOS database.

APPENDICES

Appendix 1- ***Passage Through Ruminant Digestive Tract Study.*** **Average seed germination after exposure to free choice mineral at certain intervals in time. Treatment C indicates coated seeds; treatment U indicates uncoated seeds.**

Year	Treatment	Total # of Seeds Recovered	Date	Total Germ Day 7	Rotted Seeds	Hard Seeds	Total Percent Germ
1	C	153	1	0	141	12	0.00
1	C	417	2	3	399	9	2.16
1	C	400	3	0	383	7	2.50
1	C	395	4	0	395	0	0.00
2	C	65	1	3	60	2	4.62
2	C	112	2	1	108	4	0.89
2	C	94	3	2	89	3	2.13
2	C	10	4	0	9	1	0.00
1	U	150	1	0	139	11	0.00
1	U	400	2	0	398	1	0.25
1	U	417	3	4	297	0	28.78
1	U	416	4	0	390	23	0.72
2	U	158	1	54	96	7	34.18
2	U	34	2	0	31	3	0.00
2	U	86	3	2	79	5	2.33
2	U	20	4	0	17	3	0.00

Appendix 2- ***Large Plot Study.*** **Nutritive value analysis for each pasture throughout the grazing trials, years one (December 2014-January 2015) and two (December 2015-January 2016).**

YEAR	DATE	PASTURE ID	DM (%)	NDF (%)	ADF (%)	TDN (%)
2	12/7/15	1A	24.88	47.88	28.06	70.16
2	12/7/15	1B	26.85	48.97	28.31	69.97
2	12/7/15	1C	25.88	46.09	25.94	71.85
2	12/7/15	1D	28.86	47.6	26.95	71.04
2	12/21/15	1A	34.19	47.06	27.47	70.63
2	12/21/15	1B	35.03	50.26	29.11	69.33
2	12/21/15	1C	31.58	50.59	28.85	69.54
2	12/21/15	1D	32.82	51.19	30.17	68.49
2	1/4/16	1A	23.58	57.46	33.66	65.7
2	1/4/16	1B	25.87	56.57	32.19	66.87
2	1/4/16	1C	25.36	58.61	33.83	65.56
2	1/4/16	1D	22.99	55.05	31.34	67.55
2	1/18/16	1A	20.34	57.11	33.23	66.05
2	1/18/16	1B	30.07	59.98	33.82	65.58
2	1/18/16	1C	25.27	55.9	31.35	67.54
2	1/18/16	1D	26.67	55.53	31.15	67.7
2	1/23/16	HAY	95.545	58.405	32.11	66.94
1	12/8/14	2A	30.5	26.48	71.42	0.42
1	12/8/14	2C	32.6	26.6	71.33	0.46
1	12/8/14	3A	31.54	27.54	70.58	0.41
1	12/8/14	3C	33.8	27.64	70.5	0.45
1	12/22/14	2A	20.89	22.49	74.6	0.38
1	12/22/14	2C	21.02	26.52	71.39	0.35
1	12/22/14	3A	19.88	27.84	70.34	0.42
1	12/22/14	3C	18.2	27.07	70.95	0.39
1	1/5/15	2B	30.72	30.84	67.95	0.41
1	1/5/15	2D	29.68	31.72	67.25	0.43
1	1/5/15	3B	25.61	23.41	73.87	0.44
1	1/5/15	3D	27.83	30.12	68.52	0.37
1	1/19/15	2B	27.59	30.53	68.19	0.34
1	1/19/15	2D	30.97	31.18	67.68	0.31
1	1/19/15	3B	27.06	31.55	67.39	0.37
1	1/19/15	3D	24.77	30.56	68.17	0.4

Appendix 3- ***Large Plot Study.*** **Mineral/seed mixture and total seed refusal for fed treatment groups throughout the grazing trials, years one (December 2014-January 2015) and two (December 2015-January 2016).**

Year	Date	Treatment	Total Refusal (g)	Seed Refusal (g)
1	12/8/14	Uncoated	1199.3	235.577
1	12/15/14	Uncoated	77.1	15.145
1	12/22/14	Uncoated	1000.9	196.605
1	12/29/14	Uncoated	3738	734.250
1	1/5/15	Uncoated	2124.2	417.254
1	1/12/15	Uncoated	4514.1	886.698
1	1/19/15	Uncoated	325.4	63.918
1	1/26/15	Uncoated	617.4	121.275
2	12/14/15	Uncoated	3468.7	447.184
2	12/21/15	Uncoated	3237.7	417.404
2	12/28/15	Uncoated	3972.7	512.160
2	1/4/16	Uncoated	3001.6	386.966
2	1/11/16	Uncoated	3666.7	472.710
2	1/18/16	Uncoated	3225.4	415.818
2	1/25/16	Uncoated	3497.6	450.910
2	2/1/16	Uncoated	2846.3	366.945
1	12/8/14	Coated	551.3	137.825
1	12/15/14	Coated	2042.7	510.675
1	12/22/14	Coated	3015.9	753.975
1	12/29/14	Coated	3787	946.750
1	1/5/15	Coated	176.2	44.050
1	1/12/15	Coated	1163.6	290.900
1	1/19/15	Coated	0	0.000
1	1/26/15	Coated	1071.4	267.850
2	12/14/15	Coated	3993.5	668.908
2	12/21/15	Coated	3369.7	564.422
2	12/28/15	Coated	3899.3	653.130
2	1/4/16	Coated	2663	446.050
2	1/11/16	Coated	2601.3	435.716
2	1/18/16	Coated	2845.1	476.552
2	1/25/16	Coated	2676.3	448.278
2	2/1/16	Coated	2534.2	424.476

Appendix 4- ***Small Plot Study.*** **Average number of seedlings per small plot in years one (2015) and two (2016) for each treatment. Located in Bahama, North Carolina.**

	Number of Seedlings	
Establishment Method	Year 1	Year 2
Negative Control	0.09375	0.5
Frost Seeded-Coated	86.25	162.47
Frost Seeded-Uncoated	90.875	173.19
Fed- Coated	41.625	69.2188
Fed- Uncoated	59.7187	94.9375

Appendix 5- ***Small Plot Study.*** **Average number of seedlings for each treatment type at each count date, conducted in Bahama, North Carolina in 2015 and 2016.**

	Number of Seedlings			
Establishment Method	Date 1	Date 2	Date 3	Date 4
Negative Control	0.1875	0.25	0.4375	0.3125
Frost Seeded- Coated	107.31	114.5	135.75	139.87
Frost Seeded- Uncoated	119.69	121.31	140.75	146.37
Fed-Coated	59.125	51.625	56.1875	54.75
Fed-Uncoated	88.5	70.875	77.0625	72.875

Appendix 6- ***Small Plot Study.*** **Average number of red clover seedlings for each treatment with dragging or no dragging. Conducted in Bahama, North Carolina in 2015 and 2016.**

	Number of Seedlings	
Establishment Method	Dragged	Not Dragged
Negative Control	0.47	0.125
Frost Seeded- Coated	129.44	119.28
Frost Seeded- Uncoated	142.28	121.78
Fed- Coated	88.25	22.6
Fed-Uncoated	98.53	56.13

Appendix 7- ***Small Plot Study.*** **Total number of red clover seedlings in years one (2015) and two (2016) for dragged and not dragged treatments. Located in Bahama, North Carolina.**

	Number of Seedlings	
Treatment	Year 1	Year 2
Not Dragged	43.86	84.09
Dragged	67.55	116.04

Appendix 8- ***Large Plot Study.*** **Average number of red clover seedlings within a square meter in years one (2015) and two (2016) for each treatment method. Conducted in Bahama, North Carolina.**

	YEAR	
ESTABLISHMENT METHOD	1	2
NEGATIVE CONTROL	0.05	0
FED- COATED	2	0.48
FED-UNCOATED	3.2	1.8
FROST SEEDED- COATED	96.47	186.25
FROST SEEDED- UNCOATED	118.87	187.64

Appendix 9- ***Large Plot Study.*** **Average number of red clover seedlings within a square meter in year two (2016) with or without dragging. Conducted in Bahama, North Carolina.**

	Treatment	
Establishment Method	Dragged	Not Dragged
Negative Control	0	0
Fed-Coated	0.6	0.35
Fed- Uncoated	2.15	1.45
Frost Seeded- Coated	200.22	172.27
Frost Seeded- Uncoated	226.67	148.61

Appendix 10- ***Large Plot Study.*** **Residual sward and biomass for each plot, and the average, for both years (December 2014-January 2015, December 2015-January 2016). Conducted in Bahama, North Carolina in KY-31 Fescue pastures.**

YEAR	PLOT ID	DATE	AVERAGE SWARD HEIGHT (CM)	AVERAGE BIOMASS RESIDUAL (KG/HA)
1	3A	12/22/14	4.95	1414.11
1	3C	12/22/14	4.00	1342.89
1	3A	1/5/15	4.76	1084.87
1	3C	1/5/15	4.51	1045.91
1	2B	1/19/15	4.70	1067.19
1	2D	1/19/15	3.75	978.87
1	2B	1/29/15	4.25	1470.46
1	2D	1/29/15	3.30	3392.27
1	2A	2/16/15	2.92	1568.59
1	2C	2/16/15	4.50	1717.34
1	3B	2/16/15	3.86	1657.17
1	3D	2/16/15	2.94	1570.09
AVERAGE			4.04	1525.81
2	1C	12/21/15	8.32	3392.27
2	1D	12/21/15	8.033	2874.78
2	1C	1/4/16	5.27	3681.03
2	1D	1/4/16	6.10	2937.85
2	1A	1/21/16	8.48	2519.40
2	1B	1/21/16	8.22	2536.52
2	1A	2/1/16	7.78	2368.79
2	1B	2/1/16	6.92	2036.40
2	1A	2/4/16	10.54	3303.62
2	1B	2/4/16	9.08	2892.46
2	1C	2/4/16	7.78	2607.82
2	1D	2/4/16	9.18	3098.04
AVERAGE			7.97	2854.08

Appendix 11- ***Mineral/Seed Contact Study.*** **Percent germination for each treatment after a specified number of days exposed to free choice mineral.**

	Days Exposed				
Treatment	**2**	**7**	**14**	**28**	**56**
Coated- Control	95.5	95	94.5	98	95
Uncoated-Control	94.25	95	93.5	95.75	94.75
Coated-Exposed	94.25	94.5	70.25	1	0
Uncoated-Exposed	95	95.5	91.75	47.5	7.25

Appendix 12- Average air temperature, humidity, precipitation, and soil temperature for each month in experiment years one (2014-2015) and two (2015-2016). Data reflects average conditions in Bahama, North Carolina as retrieved from the CRONOS database.

	YEAR 1			YEAR 2		
MONTH	Temp (Celsius)	Humidity (%)	Precip (cm)	Temp (Celsius)	Humidity (%)	Precip (cm)
NOV	7.6	67	10.5	12.2	73	16.9
DEC	6.5	74	11.2	12.2	80	20.7
JAN	3.5	64	8.5	3	63	3.5
FEB	0.8	58	9.3	5.8	61	12
MAR	9.5	62	9.9	13.5	62	6.7
APR	15.7	64	15.1	14.8	57	10.5
MAY	20.9	67	3.4	18.6	74	18.1