

Effects and Sustainability of Clover Inclusion within Warm-Season Turf Swards

by

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Abstract

Efforts to decrease supplemental nitrogen (N) applications to turfgrass justify alternative fertility strategies such as legume inclusion. Legumes such as clovers (*Trifolium* spp.) are present within many turfgrass scenarios. Legume persistence is partly due to an ability to biologically fix atmospheric N, which is incorporated into the plant as proteins and other compounds. N is subsequently shared with associated grasses through the decomposition of legume -roots and -foliage. For this reason, turf health is often improved rather than diminished.

There are very few guidelines for white clover (*T. repens*) establishment and maintenance within warm-season turfgrasses. In fact, much of what we know is from clover inclusion within forage and pasture scenarios. Research was conducted to answer serious knowledge gaps preventing the implementation of white clover inclusion within warm season turf swards. Four studies were conducted to evaluate seeded white clover establishment within dormant bermudagrass (*Cynodon* spp.) turf as affected by 1) pre-seeding mechanical surface disruption, 2) establishment timing, 3) seeding rate, and 4) companion grass species. White clover establishment was improved by scalping prior to October seeding, but these effects were not further enhanced by the addition of verticutting or hollow tine aerification. Un-scalped turfgrass yielded nearly 50% lower white clover densities than those scalped prior to seeding, possibly due to decreased seed to soil contact and increased bermudagrass competition. January and February establishment dates generally yielded the lowest spring clover densities, while October timing yielded superior establishment. Clover densities resulting from six seeding rates (0 to 6.0 g live seed m⁻²) were fit to the linear model $y = y_0 + ax^b$, where y equals trifoliate leaves m⁻² and x is

equal to initial seeding rate. An important feature of this model was that it accurately represented the diminishing response of increasing seeding rate. Clover establishment was negatively correlated with companion grass densities, with the largest densities occurring when planted with tall fescue and the smallest when planted with annual ryegrass.

Weed control within turf-clover swards is often hampered by the lack of effective herbicides that are safe on clovers. Furthermore, differential tolerance of legume species to common row-crop and pasture herbicides has previously been reported. Field and greenhouse studies conducted in Auburn, AL indicate varying herbicide tolerances of *Trifolium* species to common turf herbicides. In field experiments, imazaquin controlled hop clover 91% but controlled white clover only 50%. Imazaquin reduced hop clover height 87%, which far exceeded height reductions measured among other clovers (< 46%). Although visual estimates of 2,4-DB control (35%) did not differ due to species, differential height reductions were significant. 2,4-DB failed to reduce the height of crimson and ball clovers, while white clover was almost 50% shorter than the non-treated. In contrast to field experiments, 2,4-DB control during greenhouse experiments was less than all other clovers (3% versus > 40% for other clovers). These differential herbicide tolerances are novel but must be refined in order to be adapted in real-world scenarios. On a practical level, our results demonstrate potential herbicide options for maintaining biodiverse turf-legume swards. Candidate herbicides include bentazon, MCPA, 2,4-DB, imazaquin, and imazethapyr. The relative tolerance of clover species to these candidate herbicides is further evidence of their utility within certain mixed turf scenarios.

Little is known of the N contribution and carbon (C) sequestration from decaying clover foliage. An *in situ* decomposition study was conducted in Auburn, AL to quantify C and N - release from the decomposition of white clover (*T. repens* L.) foliage within a bermudagrass

lawn. Fresh white clover was applied during March, June, and December and was retrieved periodically after application. Four parameter double exponential decay models were used to describe clover mass as well as N and C -loss. These models reveal important features of white clover decomposition; mainly that white clover is composed of a quickly decaying labile fraction. White clover litter applied at $0.5 \text{ kg fresh weight (FW) m}^{-2}$ potentially contributed from 2.9 to 4.2 g N m^{-2} , with more than half available for mineralization between 10 and 73 days after application, depending upon time of year. Given that clover populations are regenerative, litter deposited during mowing events may be considered a viable N source to sustain healthy turf. Knowledge of the decomposition of clover within turf swards will enable turfgrass- researchers and professionals to more accurately predict nutrient contribution to associated grasses and help optimize supplemental fertilizer recommendations.

A 3-year study evaluated the effects of white clover inclusion within a hybrid bermudagrass lawn. Supplemental N (0, 0.5, 1, 2, 4, and 8 g N m^{-2}) was applied monthly, April to August, in order to evaluate the effects of supplemental N upon biomass composition, N fixation, N transfer, and soil carbon. Mixed grass plus clover swards yielded higher clipping biomass than grass-alone swards, which was evidence of enhanced bermudagrass growth due to biological N fixation. Likewise, grass biomass of mixed swards was increased relative to that of grass-alone swards at supplemental N rates $\leq 10 \text{ g N m}^{-2} \text{ year}^{-1}$ but was decreased at higher supplemental N rates. N fixation was estimated to be $6.6 \text{ g m}^{-2} \text{ year}^{-1}$ during the 3-year study, with an apparent increase in fixation as years progressed. Results indicate that N fixation was suppressed at the lower and upper extremes of supplemental N rates. N transfer to the associated bermudagrass sward was estimated to be 24% during the latter two years of the study. Soil carbon levels were similar among treatments.

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Literature Review

Turfgrass Sustainability

The sustainability of urban environments is among the foremost issues facing humanity. More than 80% of the United States population resides within urban or suburban environments, and it is estimated that greater than 90% will reside within urban centers by the year 2050 (United Nations, 2009, CIA World Fact Book, 2013). Urban ecology has become a central concern for residents, designers, and ecologists, alike. In much of the U.S., rooftops, parking lots, busy city streets, and home lawns are quickly replacing native flora. Civilization, for better and for worse, has changed the way we interact, build, and perceive our environment. Turfgrass is just one result of these changes.

Turfgrass has been a mainstay of U.S. urban ecology since the mid-20th century, during which large tracts of land were developed to accommodate growing urban populations. Turfgrass comprises 163,800 km² ($\pm 35,850$ km²) of the contiguous United States (Milesi et al. 2005), an area roughly the size of Florida. Turfgrass occupies approximately 1.9% of U.S. surface area and by some estimates is the largest irrigated crop within the contiguous U.S. Turfgrasses and their definitive uses vary around the world. However, in the U.S., turf is frequently utilized for transportation right-of-way, golf courses, sports-pitch, and commercial- and residential- lawns. In fact, it has been estimated that roughly 80% of U.S. cultivated turf inhabits residential lawns (Roberts and Roberts, 1987).

Benefits of turf are well documented and include: recreational health, erosion control, increased water infiltration, reduced nutrient leaching, aesthetics, carbon (C) sequestration, and mediation of the 'heat-island' effect (Beard and Green 1994, Qian and Follett, 2002). Yet the ecological impact of turf is often questioned, due in part to nutrient and water requirements as

well as its often-unsustainable monoculture cultivation (Milesi et al. 2005, Robbins and Birkenholtz 2003, Robbins et al. 2001). Turfgrass is often managed using repeat applications of synthetic fertilizers and pesticides, which are costly and may be detrimental to the environment (Robbins and Birkenholtz 2003, Robbins et al. 2001). Nitrogen (N) is essential to turf health and quality (Beard 1973, Turgeon 2002). Commercial-lawn N requirements vary with species and environmental conditions, but within the southern U.S. common rates range from less than $5 \text{ g m}^{-2} \text{ year}^{-1}$ for bahiagrass (*Paspalum notatum*) and centipedegrass (*Eremochloa ophiuroides*) to almost $30 \text{ g m}^{-2} \text{ year}^{-1}$ for hybrid bermudagrass (*Cynodon dactylon* \times *C. transvaalensis*; Duble 1996).

Improper N fertilization leads to negative environmental effects. Nitrogen loss from turf contributes to surface water eutrophication, leads to elevated nitrate (NO_3) levels in drinking water, and contributes to rising global temperatures by emitting the potent greenhouse gas nitrous oxide (N_2O ; Robbins and Birkenholtz 2003, Robbins et al. 2001, Wu and McGechan 1999). In addition, resources currently used to maintain turfgrass would arguably be more efficiently allocated if used in food-production.

Nitrogen application often leads to a lush monoculture turfgrass sward that favors plant-feeding arthropods by influencing bottom-up effects on nutritional quality and chemical defenses of their hosts (Busey and Snyder 1993, Davidson and Potter 1995, Salminen et al. 2003) and by reducing harborage and alternative resources for natural enemies (Braman et al. 2002, Frank and Shrewsbury 2004). Furthermore, pesticides required to support these conditions can disrupt ecosystem services, leading to soil compaction and excessive thatch accumulation, pest resurgence, or secondary pest outbreaks (Lopez and Potter 2000, Peck 2009, Potter 1993).

An equally important consequence of turfgrass cultivation may be its impact upon insect habitat -loss and -fragmentation (Gels et al. 2002). As urban areas expand, managed landscapes replace natural insect habitat. Furthermore, the aesthetic standards for manicured turfgrass, such as that found upon golf courses and home lawns, result in significant insecticide use to control foliage-feeding insects, which further disrupts ecosystem stability.

Clover Inclusion

Since the advent of herbicides, efforts in the turf industry have often focused on maintaining monocultures for aesthetics and increased playability. For this reason, a biologically diverse turf sward, with mixed species of grasses and broadleaves, is sometimes classified as weedy and therefore undesirable for home lawns and golf courses. However, for many scenarios, the environmental impact of biodiversity may outweigh those of monoculture.

Inclusion of leguminous species, which biologically fix N and provide pollinator habitat, is a proposed means of increasing the sustainability of certain low maintenance turfgrass scenarios. White clover (*Trifolium repens* L.) is well suited for use within warm-season turfgrasses and is already a common feature within bermudagrass pastures of the southeastern U.S. (Brink and Fairbrother, 1991). White clover increases turfgrass greenness by contributing N to associated grasses and has been reported to increase turfgrass color ratings within cool-season turfgrass (Sincik and Acikgoz, 2007) as well as increase vegetative cover within dormant bermudagrass (Dudeck and Peacock, 1983). Estimates of white clover N fixation within three cool-season turfgrasses are greater than 25 g N m⁻² year⁻¹, with 4.2 to 13.7% of total N contributed to the associated turfgrasses (Sincik and Acikgoz, 2007).

Other pertinent research concerning white clover inclusion has been conducted in forage scenarios where white clover was grazed or harvested for animal fodder. Estimates of N fixation

for grass-white clover pastures range from nil to $40 \text{ g N m}^{-2} \text{ year}^{-1}$, though most are from 10 to $25 \text{ g N m}^{-2} \text{ year}^{-1}$ (Ledgard and Steele, 1992; McNeil and Wood, 1990; Whitehead, 1995). Using the ^{15}N transfer method, McNeil and Wood (1990) estimated N fixation by white clover within perennial ryegrass was approximately $15.5 \text{ g N m}^{-2} \text{ year}^{-1}$, with 28% of the total fixed N having been transferred to associated ryegrass.

Clover Habitat

Various clovers (*Trifolium* spp.) occur throughout the world and are found within a wide range of habitats. They are commonly found within areas that receive high solar irradiation, and they rarely tolerate low light conditions. Clovers are frequently cultivated as livestock forage and as green manure within rotational cropping systems. In fact, it is theorized that certain clovers have co-evolved with foraging animals and relied upon them for their maintenance and transmittance (U.S. Fish and Wildlife, 2007). Clovers may also have been aided in development by pollinators such as bees (*Apis*, *Bombus*, and other spp.).

Insect Habitat

There are many reasons that warrant further research into new, likely biodiverse, turfgrass swards. As urban areas expand, turfgrass continues to supplant and augment natural insect habitat. Incorporating nectar-producing plants, such as legumes, into turf habitats has been shown to attract and sustain pollinating insects, such as *Apis* spp., and predatory arthropods, such as *Tiphia vernalis* and *Larra bicolor* (Abraham et al. 2010, Rogers and Potter 2004). Diversification of turfgrass ecosystems to conserve and augment natural enemies is increasingly recognized as compatible with golf course and home lawn maintenance (Held and Potter 2011).

A negative consequence, however, may be that insecticides are applied to turf areas with flowering weeds that attract honeybees and native pollinators. Lawn care professionals,

homeowners, and golfcourse superintendants routinely apply insecticides to lawns with flowering weeds (Potter 1998, Racke and Leslie 1993, Racke 2000). Foliar feeding pests are typically controlled with applications of organophosphate, carbamate or pyrethroid insecticides, with residues allowed to dry on stems (Potter 1998). Exposure to these insecticides has been associated with bee poisonings in food crops (Kevan 1975, Johansen 1977, Kearns et al. 1998). Such compounds may intoxicate pollinators through direct contact, exposure to residues, or spray contamination of nectar and pollen (e.g., Burgett and Fisher 1980, Johansen et al. 1983).

***Trifolium* Taxonomy**

According to the USDA's Plant Database (2013), the order Fabales contains only one family - Fabaceae. Similarly, the Cronquist System places only the family Fabaceae within the order Fabales (Cronquist, 1981). However, the Angiosperm Phylogeny Group (APG) has rather convincingly listed Fabaceae as well as Quillajaceae, Surianaceae, and Polygalaceae families as part of the order Fabales (Stevens, 2001).

Quillajaceae has previously been included within Rosaceae (Takhtajan, 1997) or within Spiraeaoideae as Quillajeae (Robertson, 1974). Members of this family are small evergreen trees that contain saponins within their bark. The only apparent economically important species from this family is the soapbark tree (*Quillaja saponaria*). A native of the temperate climes of central Chile, this tree has many uses. Most notably, its saponins have application as adjuvant within certain anti-viral medicines (Dalsgaard, 1978; Takahashi et al., 1990). The extracts obtained from its bark are also commonly used as food additives for their foaming characteristic (Eastwood et al., n.d.).

Surianaceae has previously been included within Rosales by Cronquist (1981) and in Rutales by Takhtajan (1997). It is synonymous with Stylobasiaceae. Plants range from small

shrubs to tall trees (Stevens, 2001). Stevens (2001) also notes that this family is quite variable in vegetative description and that members of this family have not been studied extensively for their chemical properties.

Polygalaceae are widely distributed throughout the world. The family has previously been grouped within its own order, Polygalales, by Cronquist (1981) and includes many perennial or annual herbs, shrubs, and trees. Taxonomic features vary. Polygalaceae include the *Polygala* genus, commonly referred to as milkworts. These plants have numerous medicinal properties.

Fabaceae, sometimes called the pea family or bean family, is the third largest angiosperm family. The family is characterized by compound leaf structure. Its flowers are highly variable; though, fruit of these plants are characteristically legumes. Plants of this family are known for their symbiotic relationships with rhizo-bacteria, an end result of which is fixation of atmospheric nitrogen (Sprent, 2001).

Fabaceae has traditionally been divided into three subfamilies, Mimosoideae, Caesalpinioideae, and Papilionoideae (Polhill and Raven, 1981). There has been considerably recent molecular phylogeny (since the mid 1990's) that has reasoned successfully for grouping these three subfamilies as a monophyletic family (Doyle et al., 2000; Kajita et al., 2001). Yet the placement of several subfamilies is still unresolved (e.g., Cercideae and Detarieae). Evidence of legume nodulation is lacking in the Cassieae sub-family. This includes Detarieae and Cercideae (Sprent, 2006).

Trifolium is, by broad taxonomic means, most closely related to other genera within first, its sub-tribe (Trifolieae, somewhat synonymous with Vicioid clade), secondly, its subfamily (Papilionoideae, sometimes called Faboideae), and more generally, within its family (Fabaceae).

For brevity, I mention genera that fall within Trifolieae. These include, but are not necessarily limited to: *Medicago*, *Melilotus*, *Ononis*, *Parochetus*, and *Trigonella*. This subject is reviewed in-depth within Ellison et al. (2006). Currently accepted taxonomy is shown within the Inset of Figure 4.

A phylogenetic approach places *Trifolium* most closely linked to *Trigonella* and *Melilotus*. The genus *Melilotus* includes many important plants. Yellow sweet clover (*Melilotus officinalis*) or alfalfa plants within this genus are best known for use in forage production. Like many members of this family, they are a source of nectar for honey bees (USDA, n.d.). There are more than 30 species recognized within the genus *Trigonella*. Of note is fenugreek. *T. foenum-graecum* is both an herb and spice, often found in Indian and South Asian cuisine (Katzner, n.d.; USDA, n.d.). It is also a source of animal fodder (USDA, n.d.).

Origins and Evolution

Estimates place Fabaceae diversification within the Early Tertiary, approximately 60 Mya (Herendeen et al., 1992). The fossil record of Fabaceae is abundant and diverse, according to Wojciechowski et al. (2000). It includes many fossil legume fruits and flowers as well as early indications of nodulation and symbiotic relationships. Legumes first appeared during the late Paleocene, circa 56 Mya (Herendeen, 2001; Herendeen and Wing, 2001; Wing et al., 2004). Diversification into the currently accepted subfamilies, Caesalpinioids, Mimosoids, and Papilionoids began around 50 to 55 Mya (Herendeen et al., 1992). It is interesting to note that a diverse assemblage of taxa were located upon the Mississippi Embayment of North America during the upper Eocene (55 to 34 Mya during the emergence of modern mammals; Herendeen et al., 1992).

Lavin et al. (2005) and Schrire et al. (2005) suggests we consider the diversification of Fabaceae in terms of the success of biomes rather than geographic regions, as the North Atlantic land bridge would have been aiding in the trans continent dispersal of early legumes. There are an abundance of trans continent disjunctions within Fabaceae, most of them no older than 22 Mya (Schrire et al. 2005).

Fossil evidence indicates diversification of Papilionoids 59 to 39 Mya (Lavin et al., 2005). Based upon multiple sources the Papilionoideae can be divided into several major clades. Hologalegina is the name given to the largest of these well-supported major clades, which, based upon its center of diversity, originated in Eurasia. Wojciechowski et al. (2000) suggests Hologalegina, the major clade containing *Trifolium*, originated approximately 50 Mya. Hologalegina lacks an early Eocene fossil record; though, its origin is estimate at 51 Mya (Lavin et al., 2005).

Based upon phylogenetic analyses, strong evidence has emerged for two subclades of the Hologalegina - that of the Robinioids and the Inverted Repeat-lacking clade (IRLC). Unlike the Robinioid clade, which contains species such as birdsfoot trefoil (*Lotus corniculatus*), the IRLC lacks one copy of a large inverted repeat (25 kb) that encodes a duplicate set of ribosomal RNA genes. This mutation is remarkable for its rarity - with few exceptions, it is conserved throughout green algae and land plants (Palmer et al. 1988). The IRLC centers of greatest species diversity lie within Eurasia and Northern America (Polhill and Raven, 1981; Polhill, 1994). The clade includes many economically important crops, such as alfalfa (*Medicago sativa*), garden pea (*Pisium sativum*), and the genus *Trifolium*.

The IRLC contains several yet unresolved genera, including *Afgekia*, *Calerya*, *Wisteria*, and *Glycyrrhiza*, as well as three well-supported sub-clades, including the Hedysaroid, Galegeae

and Vicioid. The latter contains many of the agriculturally important crops mentioned previously, most notably *Trifolium*.

Trifolium is estimated to have originated from other Fabaceae in the Early Miocene, 16 to 23 Mya (Lavin et al., 2005; Ellison et al., 2006). Current bioinformatics such as mapping of chloroplast DNA and known species diversity lead to a fairly well substantiated center of origin within the Mediterranean basin. Ellison et al. (2006) go into incredible detail during their review of clover phylogenetics. A summary of which is that the dispersal of *Trifolium* species has led to more than 275 individual species, many of them considered native to North and South America. Rather than displaying a mix of lineages, these groups can be distinguished by their monophyletic qualities, meaning that they are singly cladistic in origin.

Numerous accounts of hybridization and reticulate evolution are present. Ellison et al. (2006) highlight the likely introgression of cytoplasmic chloroplast DNA between *T. campestre* and *T. dubium* (two very similar hop-clovers endemic to the southeastern U.S.). They also attempt to identify the origins of *T. repens* (white clover) and come short of concluding that *T. occidentale* and *T. pallescens* are likely its diploid progenitors.

Nitrogen Fixation

Estimates place Fabaceae diversification approximately 60 Mya. (Herendeen et al., 1992). All nitrogen-fixing, flowering plants fall within the Eurosoid clade. Scattered throughout this clade are numerous plants that nodulate with filamentous bacterium, such as *Frankia*. More confined, however, are the plant species that nodulate with unicellular rhizobia. The evolution of symbiotic soil-borne bacteria has paralleled the origins of modern legumes. Early legume nodulation occurred roughly 58 Mya and were inviting habitats for soil-borne life to develop. Many single-celled organisms may not have been beneficial to early plants. Rather, legume-

rhizobia relationships developed gradually and resulted in highly specific pairings. In fact, they only occur within the order Fabales. Furthermore, with only one exception, Ulmaceae, these plants fall within the family of Fabaceae (Soltis et al., 2000).

Legume root nodulation occurs due to at least three known genera of “rhizo-bacteria”, including: *Rhizobium*, *Bradyrhizobium*, and *Azorhizobium*. Certain anatomical features typify legume root nodulation, primarily induction of a new plant meristem that develops as an invitation to (or possibly as a result of interspecific signaling from) bacterial infection (Rolfe and Gresshoff, 1988; Schultze and Kondorsori, 1998). This is induced by the initial colonization of the root surface by the bacteria. Prior to infection, lectin-receptors on the host plant must specifically recognize the potential pathogen. Coordination and communication between the symbiotes is required throughout the initial and subsequent stages of infection and are highly specific for both host and pathogen.

Rhizobia near the surface of host plants respond to flavonoids, such as luteolin, by expressing *nod* genes (Brewin, 1991; Schultze and Kondorsori, 1998). Expression leads to the production of return signals, sometimes called Nod -signals or -factors (Schultze and Kondorsori, 1998). For *Trifolium* spp. these Nod factors are lipochito-oligosaccharides and are specific to *Rhizobium* species. They initiate root-hair curling and consequently nodule primordia (Figures 5 and 6). Subsequently invasion occurs as the infection thread penetrates the epidermis then moves into the inner cortex. The spread of infection between cells is aided by regular planes of cell division in young meristamatic tissue as well as pre-infection orientation (reviewed by Brewin, 1991; and Buchannan et al. 2000).

During this infection, bacteria are engulfed by plant cells forming organelle-like structures that some have termed symbiosomes (Roth and Stacey, 1989; Buchannan et al., 2000).

Nodules are populated by roughly dozens of bacteria, which in many cases cease to propagate after two or three rounds. Proteins involved in transport of substrate, as well as metabolism of carbon and nitrogen, are manufactured within these structures. These furnish the machinery necessary to “fix” atmospheric nitrogen and share that nitrogen with the associated plants as NH_3 .

The primary structure responsible for nitrogen fixation is nitrogenase. Nitrogenase enzyme is actually two separate protein structures – dinitrogenase and dinitrogenase reductase. Dinitrogenase binds N_2 while dinitrogenase reductase provides electrons to reduce N_2 resulting in 2 NH_3 molecules. An important note about dinitrogenase reductase: it not only reduces N but also reduces acetylene to ethylene. This provides a useful assay to assess nitrogenase activity.

Nitrogenase activity is inhibited at oxygen concentrations greater than roughly 1% (Brewin, 1991). Therefore, it is important, that uninfected parenchyma cells function as barriers to oxygen. In addition, leguminous plants produce the oxygen-binding protein leghemoglobin, which serves to reduce oxygen concentrations near the site of nitrogenase activity (Buchanan et al., 2000).

As an exchange for the nitrogen fixed by rhizobia, plant hosts provide photosynthate. This carbon source enters nodules as sucrose. Evidence suggests that mono- and di-saccharides are not directly transported into bacterioids; rather, the sugars are converted into dicarboxylic acids such as malate and oxaloacetate via a process similar to fermentation (Buchanan et al., 2000).

Nitrogen Fixation within Mixed Grass Clover Swards

Even in persistent stands of legumes biological N fixation varies, largely due to the relative composition of turfgrass swards and soil N availability (Crush et al., 1982). Fixation is

highly dependent upon the relative level of nodulation occurring in root tissues and activity of the bacteria within. Most research indicates that high soil N concentrations inhibit nodule growth and development. Macduff et al. (1996) observed that the ratio of root to nodule dry-weights was 6:1 in white clover without NO₃ treatment but increased with applications of NO₃. It is well documented that increasing N fertilization decreases clover density and allows the grass portion of the sward to outcompete clover (Frame and Boyd 1987; Pederson 1995; Sincik and Acikgoz 2007).

Other factors affecting biological N fixation include absorption of photosynthetically active radiation, C- assimilation rates, and allocation of photosynthate to roots (Lie 1971). White clover leaves have a higher photosynthetic capacity at low N levels than do competing perennial ryegrass; however, at higher N levels the opposite is true (Faurie et al. 1996). Increased light interception at low N levels can be attributed to a greater leaf area index in the upper canopy of the grass-clover sward as well as clover's ability to avoid shade by increasing petiole length (Davies and Evans 1990; Faurie et al. 1996; Woledge et al. 1992).

White clover persistence varies greatly due to soil conditions. In their review of N fixation of grass-legume pastures, Ledgard and Steele (1992) report that fixation is greatly reduced due to dry soil conditions, acid soils, and the "pest/disease complex." Another major factor affecting N fixation is soil temperature. Frame and Newbould (1986) found that a minimum temperature of 9°C was necessary for active N fixation by *Rhizobium*. It has also been reported that temperatures for nitrogenase activity range from 13 to 26°C (Halliday and Pate 1976).

Decomposition of Clover Biomass within Turf

There is no doubt that root nodule decomposition is a significant source of N for associated grasses, as reported N concentration of root nodules ranges from 4.8 to 9.0% of root dry matter (Chu and Robertson, 1974; Wardle and Greenfield, 1991). However, root nodules are not the sole source of N transfer, as above ground white clover dry matter has been reported to be 9.1 to 24.2% protein, depending upon harvest date (about 1.5 to 4.0% N; Burton and DeVane, 1992). Unlike forage scenarios, turfgrass systems differ in that they are not grazed; rather, they are mown frequently to maintain utility and aesthetics. Mown clippings are returned to the turf surface, potentially contributing a mineralizable source of N.

Polyculture lawns of grass and white clover are historically common, yet little is known of the N-contribution and C-flux from decaying clover foliage. The rates of decomposition, N mineralization, and C deposition would be useful information for future research regarding this subject as well as when assigning nutrient credits to white clover-culture in warm and cool season turfgrass. Such information would be highly dependent upon a multitude of factors, including time of year, litter composition, soil and climactic -conditions, as well as soil fauna. For these reasons, it may not be possible to control all factors *in situ*.

Organic residues decompose in two phases. Soil microbes rapidly consume the labile fraction, which is composed of sugars, starches, and proteins, leaving behind a recalcitrant fraction composed of cellulose, fats, waxes, lignin, and tannins (Wieder and Lang, 1982). This slowly decomposing fraction helps to develop soil organic matter. Due to the two-step nature of decomposition, a double exponential decay model is often implemented to describe litter decay (Wieder and Lang, 1982). Double exponential decay equations are of the form $Y = Ae^{-k_1t} + Be^{-k_2t}$, where Y = response, A and B are initial concentrations approximating the labile and

recalcitrant portions, k_1 and k_2 are rate constants fitted to the data, and t equals time in days after application (DAA). Such models have been used successfully to describe quickly decaying legume litter in Alabama (Mulvaney et al., 2010) as well as the decomposition and N release of hedgerow species in Haiti (Isaac et al., 2000). Modeling white clover decomposition may enable turfgrass researchers and professionals to more accurately predict nutrient contribution to associated grasses and help optimize supplemental fertilizer recommendations.

Clover Establishment

Legumes such as clovers are present within many turfgrass scenarios in the temperate climates of the southeastern United States as both weeds and amenity plants. As amenity plants, clover species may provide important ecosystem services, such as nitrogen fixation (Ledgard and Steele 1992, McNeil and Wood 1990, Whitehead 1995) and insect habitat (Abraham et al. 2010, Rogers and Potter 2004).

Clover has been, and continues to be, included in grass mixtures for roadsides as well as other maintained turfgrass areas and has proven useful for slope stabilization (Roberts and Bradshaw 1985). In particular, white clover (*Trifolium repens* L.) thrives within home lawns and golf courses because it can flower and produce seed at mowing heights as low as 6 mm (Sincik, and Acikgoz 2007; Watschke et al. 1995). Other clover species are also common within maintained turf swards. Prominent amongst Auburn, AL flora are small hop clover (*T. dubium* Sibth.), crimson clover (*T. incarnatum* L.), and ball clover (*T. nigrescens* Viv.).

Little has been written about the establishment and maintenance of mixed turf-clover swards; though, similarities can be drawn between those of mixed grass-legume forage systems as well as examples provided by overseeding practices common within the transition zone, where warm and cool season grasses grow equally as well.

Proper white clover establishment is key to maximizing stand uniformity as well as N contribution to associated grasses (Frame and Newbould, 1986). However, there are currently no guidelines for establishment within warm-season turfgrass scenarios common to the southeastern U.S. Furthermore, unlike pasture systems, managed turfgrass scenarios may offer unique opportunities to manipulate turfgrass height and density, as well as soil characteristics, in favor of white clover establishment.

There are several agronomic practices used to improve overseeded grass establishment within maintained turf scenarios. Scalping is among the most common techniques and refers to the excessive removal of living tissue at any one mowing occurrence (Turgeon, 2002). Though scalping often results in turfgrass injury, it is a means of exposing bare soil and eliminating turfgrass competition, which is essential to overseeded grass establishment. Verticutting, or vertical mowing, is another mechanical method often used to remove accumulated thatch or to elevate decumbent turfgrass prior to overseeding. Verticutting is performed by passing a rapidly rotating horizontal shaft with vertically oriented knives over affected turfgrass (Turgeon, 2002). Vertical mowing is often used in addition to scalping in order to prepare warm-season turfgrass for overseeding. Hollow tine aerification is less commonly used for fall overseeding but is an agronomic practice used to improve soil characteristics by removing cores of soil from turfgrass. Core sizes may vary, but the desired result is much the same. That is, the cores are removed to alleviate compaction by decreasing soil bulk density, accelerate drying, and increase infiltration of water and gasses. Once performed, cores are often collected or scattered, and the remaining holes are either filled with sand or left open.

Hypothetically, scalping alone or scalping in combination with vertical mowing and aerification may be a means of improving seeded white clover establishment, via improved seed-

to-soil contact, and by limiting competition effects from associated turfgrasses. Soil aerification may also alleviate competition but has the added benefit of providing holes in which white clover may find more adequate soil conditions for initial establishment. It is therefore reasonable that it too should be tested as a means of improving white clover establishment.

Other variables that affect white clover establishment are establishment timing and seeding rate. Recommended establishment dates for white clover in the southeastern U.S. are largely anecdotal. For instance, establishment timing is often recommend from 2 to 6 weeks prior to historical first frost (approximately November 1st in Auburn, AL). Previous research in Florida recommends September planting dates (Dudeck and Peacock, 1983), while others have recommended spring seeding to avoid hard freeze in more northern climates (Frame and Newbould, 1986). These dates are highly variable and dependent upon locations and climate. Further, they may not account for nuances of a maintained turf sward, which may insulate young white clover seedlings from effects of frost or hard freeze. Anecdotal to our own research, proper stand density is highly dependent upon seeding rate, yet it does not appear to be a linear response, perhaps due to intra-species competition.

White clover establishment within cool-season grass swards has largely been dictated by seed mixtures of cool-season grass blends containing roughly 3 to 10% white clover by weight (Sincik and Acikgoz, 2007). Yet, these rates have not been evaluated in existing warm season turf swards. Likewise, information about interaction effects of white clover and companion grass species is absent from the scientific literature. Alternative, grass-white clover mixtures for turfgrass are commercially available in much of Europe and the United States; however, they have not been evaluated for winter overseeding of dormant warm-season grasses.

Weed Control in Mixed Grass-Clover Swards

Like forage systems, broadleaf weeds and sedges are problematic during sward establishment. Herbicidal weed control is often required as seedling legumes are not competitive with many weeds and grasses (Carlisle et al. 1980; Evers et al. 1993; Young et al. 1992). In addition to competition effects, weedy species often negatively affect aesthetics and reduce the utility of certain mixed swards.

Weed control within turf-clover swards is often hampered by the lack of effective herbicides that are safe on clovers. Few herbicides are labeled for postemergence application to various clover species, and most are restricted to states where the respective species are grown for seed production. Furthermore, differential tolerance of legume and cultivars within species to common row-crop and pasture herbicides has previously been reported (Beran et al. 1999; Bowran 1993; Young et al. 1992). These results have shown that individual species exhibit different reactions to various broad-leaf herbicides, including differential reductions in seed yield, biomass, and nitrogen input for subsequent crops (Bowran 1993).

For these reasons, research is needed to evaluate differential herbicide responses of *Trifolium* species, which are commonly included within mixed turf scenarios. It is also increasingly important to identify common turf herbicides that are tolerated legume plants of biodiverse swards.

Kingdom: Plantae – Plants
Subkingdom: Tracheobionta – Vascular plants
Superdivision: Spermatophyta – Seed plants
Division: Magnoliophyta – Flowering plants
Class: Magnoliopsida – Dicotyledons
Subclass: Rosidae (Figure 2)
Order: Fabales
Family: Fabaceae – Pea family
Subfamily: Papilionoideae
Tribe: Trifolieae
Genus: *Trifolium* L. – Clover

Figure 1. *Trifolium* Taxonomy (Plants Database, 2012)

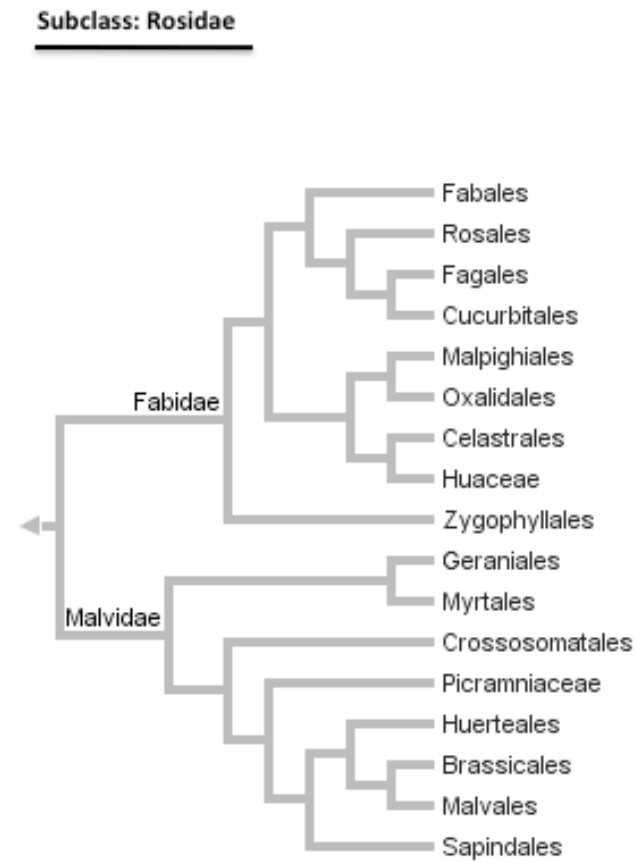


Figure 2. Rosids (or Eurosids) clade according to Wang et al. (2009) and Worberg et al. (2009).

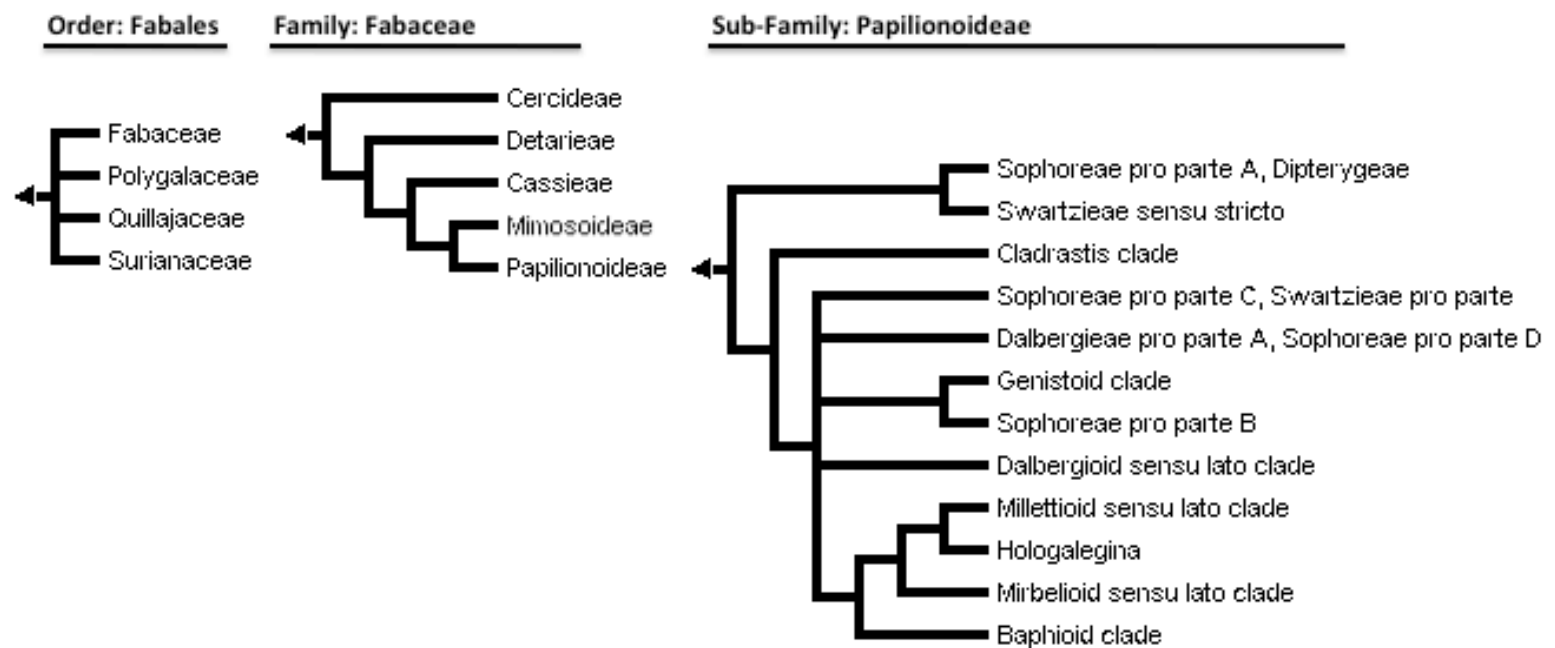


Figure 3. Papilionoideae (Wojciechowski et al., 2000) is the largest of the three subfamilies of Fabaceae.

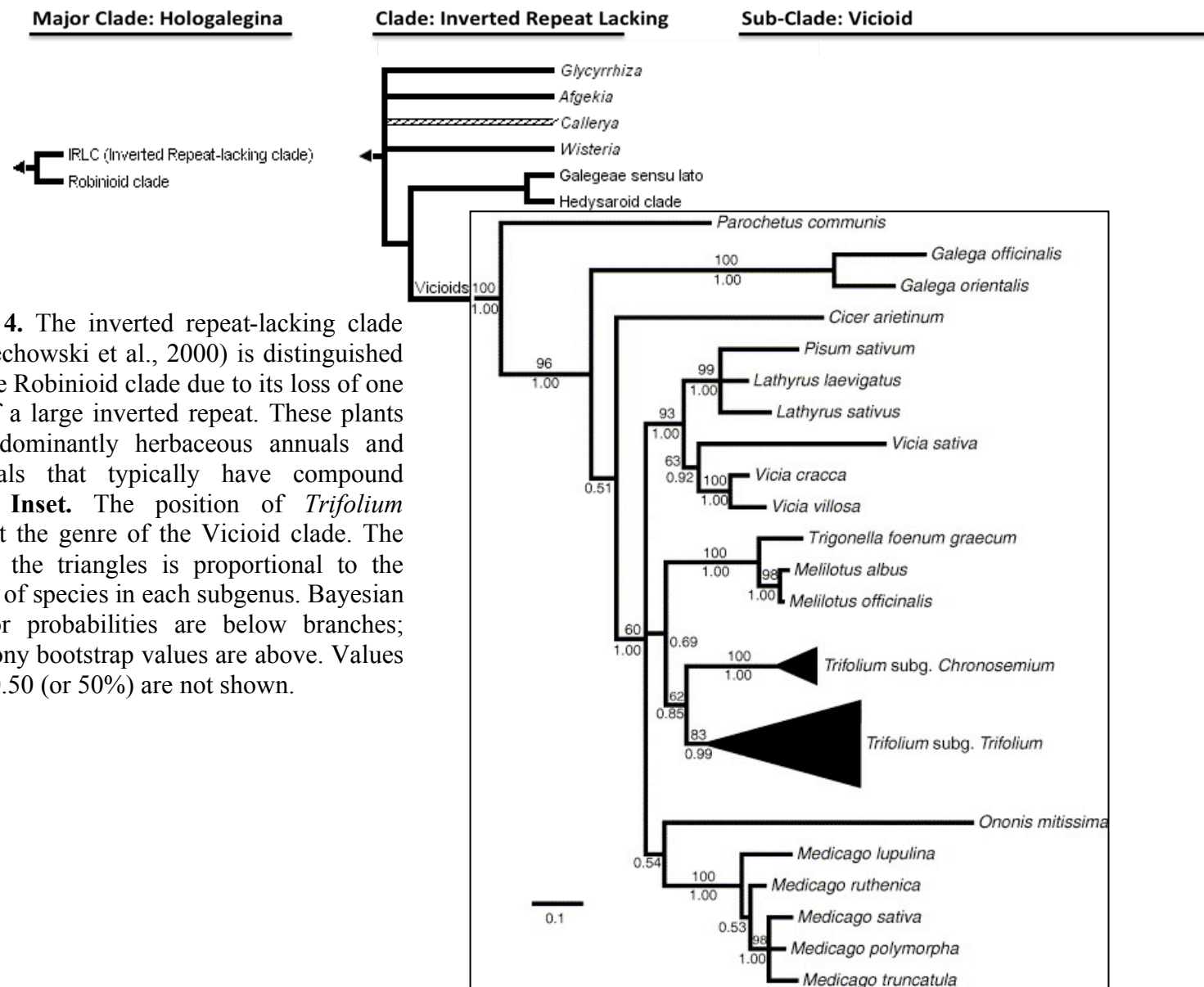
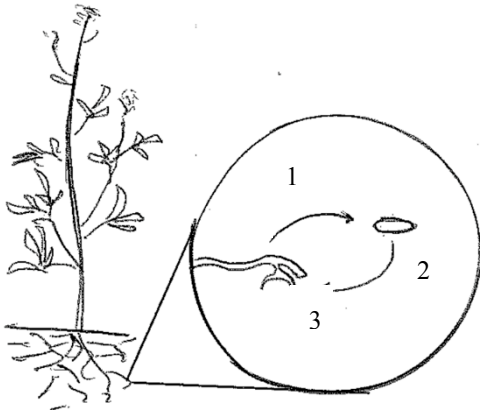


Figure 4. The inverted repeat-lacking clade (Wojciechowski et al., 2000) is distinguished from the Robinioid clade due to its loss of one copy of a large inverted repeat. These plants are predominantly herbaceous annuals and perennials that typically have compound leaves. **Inset.** The position of *Trifolium* amongst the genre of the Vicioid clade. The area of the triangles is proportional to the number of species in each subgenus. Bayesian posterior probabilities are below branches; Parsimony bootstrap values are above. Values below 0.50 (or 50%) are not shown.

Figure 5. Legume root nodulation process (based upon Buchanan et al., 2000).



1. Plant roots release elicitors of *Nod* gene expression
2. Bacterium releases Nod factor.
3. Plant root is infected and undergoes nodule morphogenesis.

Figure 6. Root hair curling and invasion.

1. Nod factors initiate root-hair curling.
2. Invasion occurs as an infection thread penetrates the epidermis then the inner cortex.

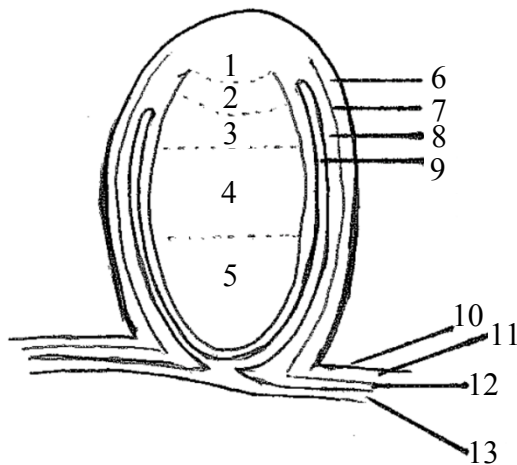


Figure 7. Nodule meristem and zones of infection.

1. Nodule meristem
2. Zone of infection thread growth and cell penetration
3. Zones of expanding infected cells
4. Mature bacteroid-containing tissue
5. Senescent bacteroid-containing tissue
6. Outer cortex
7. Nodule endodermis.
8. Inner cortex
9. Nodule vascular bundle.
10. Root epidermis
11. Root cortex
12. Root endodermis
13. Root xylem and phloem elements.

White Clover (*Trifolium repens*) Establishment within Dormant Bermudagrass (*Cynodon dactylon*) Turf

INTRODUCTION

Benefits of turf are well documented and include: recreational health, erosion control, increased water infiltration, reduced nutrient leaching, aesthetics, carbon (C) sequestration, and mediation of the ‘heat-island’ effect (Beard and Green, 1994; Qian and Follett, 2002). Yet the ecological impact of turf is often questioned, due in part to nutrient and water requirements (Milesi et al., 2005; Robbins et al., 2001; Robbins and Birkenholtz, 2003) as well as often-unsustainable monoculture cultivation, which contributes to insect habitat -loss and -fragmentation (Gels et al., 2002). For these reasons, the turfgrass industry is experiencing new demands for ecologically and economically -sustainable maintenance options.

Inclusion of leguminous species, which biologically fix N and provide pollinator habitat, is a proposed means of increasing the sustainability of certain low maintenance turfgrass scenarios. However, little is known about inclusion of legumes in maintained turfgrass. Since the advent of herbicides, efforts in the turfgrass industry have often focused on maintaining monocultures for aesthetics and increased playability. Thus, a biologically diverse turfgrass sward with mixed species of grasses and broadleaf plants is sometimes classified as weedy and therefore undesirable for scenarios such as golf-course and sports pitch. However, for many scenarios, such as home lawns, roadsides, or other “unimproved” turfgrass areas, the environmental benefits of biodiversity may outweigh those of monoculture.

White clover inclusion within maintained turfgrass has mainly been limited to cool-season turfgrass scenarios. Important research by Sincik and Acikgoz (2007) reported increased color

ratings in three cool-season turfgrass-white clover (*T. repens* L.) mixtures and that white clover fixed greater than 25 g N m⁻² year⁻¹ and contributed between 4.2 to 13.7% of that total N to the associated turfgrass. Additional information concerning white clover inclusion within maintained turf is absent. However, information about the benefits of white clover inclusion within pasture systems is fairly abundant but mainly focuses on perennial ryegrass (*Lolium perenne* L.) -white clover pastures. These mixed systems supply high-quality grazing for animals while simultaneously improving soil fertility (Lampkin, 2002). Estimates of N fixation for grass-white clover pastures range from nil to 40 g N m⁻² year⁻¹, though most are roughly 10 to 25 g N m⁻² year⁻¹ (Ledgard and Steele, 1992; McNeil and Wood, 1990).

Yet white clover is well suited for use within warm-season turfgrasses and is already a common feature within bermudagrass pastures of the southeastern U.S. (Brink and Fairbrother, 1991). Proper white clover establishment is key to maximizing stand uniformity as well as N contribution to associated grasses (Frame and Newbould, 1986). However, there are currently no guidelines for establishment within warm-season turfgrass scenarios common to the southeastern U.S. Furthermore, unlike pasture systems, managed turfgrass scenarios may offer unique opportunities to manipulate turfgrass height and density, as well as soil characteristics, in favor of white clover establishment.

Our objectives were to test standard overseeding methods, cultural practices, seeding rates, and companion grass combinations for their effects upon spring white clover establishment within a maintained bermudagrass lawn. We hypothesized that white clover establishment is comparable to overseeding dormant warm-season turfgrass with cool-season grasses such as perennial ryegrass. However, unlike perennial ryegrass, recommended white clover establishment rates are much lower [from 3 to 5 kg white clover seed ha⁻¹ recommended by Frame and Newbould (1986)].

There are several agronomic practices used to improve overseeded grass establishment within maintained turf scenarios. Scalping is among the most common techniques and refers to the excessive removal of living tissue at any one mowing occurrence (Turgeon, 2002). Though scalping often results in turfgrass injury, it is a means of exposing bare soil and eliminating turfgrass competition, which is essential to overseeded grass establishment. Verticutting, or vertical mowing, is another mechanical method often used to remove accumulated thatch or to elevate decumbent turfgrass prior to overseeding. Verticutting is performed by passing a rapidly rotating horizontal shaft with vertically oriented knives over affected turfgrass (Turgeon, 2002). Vertical mowing is often used in addition to scalping in order to prepare warm-season turfgrass for overseeding. Hollow tine aerification is less commonly used for fall overseeding but is an agronomic practice used to improve soil characteristics by removing cores of soil from turfgrass. Core sizes may vary, but the desired result is much the same. That is, the cores are removed to alleviate compaction by decreasing soil bulk density, accelerate drying, and increase infiltration of water and gasses. Once performed, cores are often collected or scattered, and the remaining holes are either filled with sand or left open.

Hypothetically, scalping alone or scalping in combination with vertical mowing and aerification may be a means of improving seeded white clover establishment, via improved seed-to-soil contact, and by limiting competition effects from associated turfgrasses. Soil aerification may also alleviate competition but has the added benefit of providing holes in which white clover may find more adequate soil conditions for initial establishment. It is therefore reasonable that it too should be tested as a means of improving white clover establishment.

Other variables that affect white clover establishment are establishment timing and seeding rate. Recommended establishment dates for white clover in the southeastern U.S. are largely anecdotal. For instance, establishment timing is often recommend from 2 to 6 weeks prior

to historical first frost (November 1st in Auburn, AL). Previous research in Florida recommends September planting dates (Dudeck and Peacock, 1983), while others have recommended spring seeding to avoid hard freeze in more northern climates (Farne and Newbould, 1986). These dates are highly variable and dependent upon locations and climate. Further, they may not account for nuances of a maintained turf sward, which may insulate young white clover seedlings from effects of frost or hard freeze. Anecdotal to our own research, proper stand density is highly dependent upon seeding rate, yet it does not appear to be a linear response, perhaps due to intra-species competition.

White clover establishment within cool-season grass swards has largely been dictated by seed mixtures of cool-season grass blends containing roughly 3 to 10% white clover by weight (Sincik and Acikgoz, 2007). Yet, these rates have not been evaluated in existing warm season turf swards. Likewise, information about interaction effects of white clover and companion grass species is absent from the scientific literature. Alternative, grass-white clover mixtures for turfgrass are commercially available in much of Europe and the United States; however, they have not been evaluated for winter overseeding of dormant warm-season grasses.

Due to the many knowledge gaps limiting the utility of white clover inclusion within warm-season scenarios, experiments were conducted to test the effects of pre-seeding mechanical surface disruption, establishment timing, seeding rate, and companion grass species on establishment of two commercially available white clover populations within dormant bermudagrass turfgrass. White clover was chosen as a model species for a variety of reasons, but specifically because turf-compatible white clover varieties are commercially available, and due to white clover prevalence in maintained turfgrass as a weed species (Watschke et al. 1995). Here we present results that may influence future scientific studies and the utility of white clover inclusion within warm and cool season turf scenarios.

MATERIALS AND METHODS

Studies were designed as randomized complete blocks with four replications. Blocking considerations were mowing direction and return of clippings. Studies were initiated 14 October 2010 and 1 October 2011 at the Auburn University Turfgrass Research Unit (32°34'40" N, 85°29'57" W; elevation 185 m) in Auburn, AL. Research was conducted within a maintained 'Tifway' hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burt Davy] lawn on a Marvyn sandy loam (fine-loamy, kaolinitic, thermic Typic Kanhapludult) soil with an average pH of 6.3 (1:1 soil:H₂O). Turfgrass was maintained at a height of 3.8 cm; all clippings were returned to the turfgrass surface. Plots received 3 cm supplemental irrigation on a weekly basis between March and September of 2011 and 2012. The area was fertilized (5 g N m⁻²) 15 February 2011 and 20 February 2012.

Four studies were conducted to evaluate the effects of pre-seeding mechanical surface disruption, establishment timing, seeding rate, and companion grass species on establishment of two commercially available white clover populations, Dutch White (Main Street Seed and Supply, Bay City, MI) and DLF Microclover (DLF-International Seeds, Halsey, Oregon). Seed were drop seeded through a stainless steel device, which contains 5 seed dispersion screens (6.4 mm² mesh openings) oriented horizontally to evenly scatter small grass and broadleaf seeds. With the exception of the seeding rate study, all clover were seeded at 1.5 g live seed m⁻². Trifoliate leaves were counted within three 730 cm² sub-samples per 1.0 m² experimental unit on 20 April, 2011 and 2012 as a means of quantifying spring clover density (trifoliate leaves m⁻²). Companion grass plants were quantified using similar subsampling methods during January of 2011 and 2012 when bermudagrass was completely dormant.

Mechanical Disruption Study.

This study evaluated common pre-overseeding cultural practices, such as verticutting, aerification, as well as scalping, and their ability to enhance seeded clover establishment relative to normally mown, non-scalped turfgrass. Treatments were arranged as a factorial to test the effects of four common cultural practices upon the establishment of two commercially available clover populations. Treatments were intended to mechanically disrupt the soil surface as well as eliminate bermudagrass competition and included: scalping (6 mm mowing height), scalping plus vertical mowing (6 mm below soil level), and scalping plus hollow tine aerification (6 mm hollow tines; 3.8 cm depth; 15.2 cm spacing). Treatments also included a non-scalped control maintained at 3.8 cm mowing height. Clippings were removed from scalped surfaces, and clover was drop seeded as previously described.

Timing Study

Treatments were arranged as a factorial to test the effects of seeding time (October through February) upon establishment of two commercially available clover populations. Plots were scalped at each seeding date, as previously described, and were blown free of clippings. Clover was seeded at monthly intervals beginning in October and ending in February.

Seeding Rate

A seeding rate trial was arranged as a factorial to estimate the effects of seeding rate upon establishment of two commercially available clover populations. Plots were scalped and blown free of clippings. Clover was seeded at 0, 0.4, 0.8, 1.5, 3.0, and 6.0 g live seed m⁻².

Companion Grasses

Treatments were arranged as a factorial to test the effects of seeding companion grass species in combination with one of two commercially available clover populations. Companion species were: annual ryegrass (*Lolium multiflorum* Lam.) perennial ryegrass (*L. perenne* L.),

creeping bentgrass (*Agrostis stolonifera* L), red fescue (*Festuca rubra*), and *Poa trivialis* L. (See Table 1 for rates and sources).

Statistical Analysis

All data were subject to analysis of variance (ANOVA) within SAS procedure GLIMMIX using mixed model methodology (SAS[®] Institute v. 9.2, Cary, North Carolina, USA). Treatment was considered a fixed effect in the model. Year, replication (nested within year), and iterations containing these effects were considered random in the model (Carmer et al. 1989; Hager et al. 2003). Basic model assumptions were confirmed. Means were separated based upon adjusted 95% confidence intervals, which allows for multiple comparisons by protecting family-wise error rate (Littell et al. 2006).

Least squares estimates for linear models were determined for rate-response studies using the Marquardt-Levenberg algorithm to provide the best fit (SPSS Inc., Sigma Plot v. 11.2, Chicago, Illinois, USA). R^2 values were used to determine ‘goodness of fit’ for the selected equations. Initial parameter ranges were selected with a maximum of 200 fits and 200 iterations. The relationship of clover density (trifoliate leaves m^{-2}) to the clover seeding rates investigated in this trial were described using the linear model $y = y_0 + ax^b$, where y equals trifoliate leaves m^{-2} , y_0 equals the y-intercept (held constant at 0), a serves as a scaling factor (moving the values of x^b up or down), x is equal to initial seeding rate (g live seed m^{-2}), and b is the scaling exponent that determines the function’s rate of growth or decay. Correlation between companion grass density and clover establishment were described using Pearson product moment within SigmaPlot 11.2.

RESULTS

Analysis of variance (Table 2) indicated that results for all studies differed due to replication year. For this reason, 2010-2011 and 2011-2012 (season 1 and 2, respectively) results are presented separately for all studies. However, with few exceptions, treatment separations were

similar across years and are used in support of our main conclusions. It is possible that the earlier initiation date of season 2 (October 1 rather than October 14) had some affect upon clover establishment, as bermudagrass dormancy was much more delayed during season 2 relative to season 1.

Mechanical canopy disruption methods

Results were generally similar across seasons, with scalping alone and in combination with other methods having enhanced spring white clover establishment (Table 3). When established in 2010, scalping alone and in combination with verticutting or aerification yielded 499, 502, and 513 trifoliate leaves m^{-2} , respectively. Normally maintained turfgrass (3.8 cm mowing height) yielded significantly lower white clover densities (279 trifoliate leaves m^{-2}). Varietal differences were not detected during the season 1 replication of this study.

Season 2 establishment densities were generally lower than those of season 1 (Table 3). Scalping in combination with aerification yielded higher spring establishment levels (204 trifoliate leaves m^{-2}) than those of normally maintained turfgrass (6 trifoliate leaves m^{-2}) and scalping alone treatments (73 trifoliate leaves m^{-2}). Scalping in combination with verticutting yielded 108 trifoliate leaves m^{-2} , which was greater than normally maintained turfgrass. Varietal differences were apparent during season 2, with the Dutch variety having greater spring white clover density than Microclover (130 and 86 trifoliate leaves m^{-2} , respectively).

Establishment timing

During season 1, October timing yielded superior spring white clover densities (199 trifoliate leaves m^{-2}) relative to all other seeding dates (Table 4). November timing resulted

in 68 trifoliate leaves m^{-2} , which was less than October timing but greater than the reduced stand densities of December, January, and February timings (22, 17, and 4 trifoliate leaves m^{-2} , respectively). Varietal differences were detected during season 1, with the Dutch variety having yielded nearly double the white clover density of Microclover across establishment timings (80 versus 44 trifoliate leaves m^{-2} , respectively).

Season 2-spring white clover establishment differed due to timing by variety interaction ($P = 0.0401$). This interaction was largely due to exaggerated varietal differences of the December timing, perhaps due to delayed seedling emergence of Microclover (Data not shown). When established in December, the Dutch variety yielded 166 trifoliate leaves m^{-2} , whereas Microclover yielded only 50 trifoliate leaves m^{-2} . Similar trends were seen throughout the experiment; however, no other establishment timing yielded significant varietal differences. These trends are again manifest by varietal main effects (Data not shown), with the Dutch variety having yielded more than double the white clover density of Microclover across establishment timings (90 versus 29 trifoliate leaves m^{-2} , respectively).

Establishment timing main effects were again evident during season 2 (Table 4). January and February timing clearly diminished spring white clover establishment levels, with February timing having yielded only 9 trifoliate leaves m^{-2} . However, unlike the previous season, October timing did not result in superior spring white clover densities (51 trifoliate leaves m^{-2}) and was in fact equal to levels from November, January and February timings (91, 39, and 9 trifoliate leaves m^{-2} , respectively). Rather, December timing yielded the highest spring white clover density (108 trifoliate leaves m^{-2}), which was equaled only by November establishment (91 trifoliate leaves m^{-2}).

Seeding rate.

As anticipated, April observed white clover densities increased proportionally to October seeding rate (Figure 8). For this reason, data were fit to the linear model $y = y_0 + ax^b$, where y equals trifoliate leaves m^{-2} and x is equal to initial seeding rate (g live seed m^{-2}). An important feature of this model is the diminished response of increasing seed yield. This characteristic highlights an important feature of white clover overseeding. That is, as white clover-seeding rate increases beyond a certain point, competition effects may begin to reduce yield response. We acknowledge that these functions do not account for seasonable variability. In fact, there are many variables that may affect white clover establishment, including soil and air temperature as well as moisture availability. Ideally, these equations could be used to estimate spring white clover densities and demonstrate the diminishing nature of seeded white clover yields.

Companion grass study

Grass species affected spring white clover establishment during both seasons (Table 5). However, no differences due to white clover variety were observed. Season 1 spring white clover establishment was largest when seeded with tall fescue (383 trifoliate leaves m^{-2}) and smallest when seeded with annual ryegrass (68 trifoliate leaves m^{-2}). Creeping bentgrass, yielded 262 trifoliate leaves m^{-2} , which was less than white clover density observed within tall fescue but greater than that of other companion grasses. Perennial ryegrass yielded 165 trifoliate leaves m^{-2} , which equaled the white clover density observed within *Poa trivialis* (109 trifoliate leaves m^{-2}), but was greater than that of annual ryegrass (68 trifoliate leaves m^{-2}).

White clover establishment was generally lower during season 2 (Table 5), but like the season prior, white clover establishment was largest when seeded with tall fescue (65

trifoliate leaves m^{-2}). When seeded with creeping bentgrass, white clover density was 49 trifoliate leaves m^{-2} , which was similar to those of tall fescue and annual ryegrass (11 trifoliate leaves m^{-2}). Clover densities were lower than those of tall fescue and creeping bentgrass when seeded with *Poa trivialis* and perennial ryegrass (7 and 5 trifoliate leaves m^{-2} , respectively) but equal to that of annual ryegrass.

Companion species were quantified during January of each season while bermudagrass was dormant, because it was not possible to distinguish between the leaves of bermudagrass and companion species without introducing bias at other times of the year. During season 1 and 2, spring white clover densities were negatively correlated with companion grass densities (Pearson correlation coefficients = -0.485 and -0.231, respectively; Figure 2). Season 1-grass densities were inverse those of spring white clover, with the highest grass density observed within *Poa trivialis* (15,113 plants m^{-2}). Annual ryegrass and perennial ryegrass densities (11,302 and 9,429 plants m^{-2} , respectively) were similar to one another but less than that of *Poa trivialis*, while creeping bentgrass and tall fescue densities (3,810 and 2,647 plants m^{-2} , respectively) were lowest among companion grass species.

Season two grass densities were also inverse those of spring white clover densities, with the highest grass density observed within *Poa trivialis* (15,007 plants m^{-2} ; Figure 2). Perennial ryegrass, annual ryegrass, and creeping bentgrass densities (8,626, 8,491, and 6,264 plants m^{-2} , respectively) were less than that of *Poa trivialis* but similar to one another, while tall fescue density (2,454 plants m^{-2}) was lowest among companion grass species.

DISCUSSION AND IMPLICATIONS

White clover spring seedling density was enhanced via scalping of the bermudagrass sward as well as scalping in combination with other mechanical surface disruption methods. White clover seed germination occurred eight to ten days after October seeding; however, white clover remained in cotyledon stage for at least one to two weeks and was visibly more advanced in growth stage when plots were scalped or when plants were grown on the fringe of un-scalped plots. Long-term viability of mixed turf-white clover swards depends upon proper establishment. Previous research confirms that the availability of photosynthetically active radiation is critically linked to N fixation (Chu 1974; Lie 1971). Furthermore, reducing turfgrass height may increase the ability of white clover to avoid shade by increasing petiole length (Davies and Evans 1990; Faurie et al. 1996; Woledge et al. 1992).

White clover seeding rate is undoubtedly important for proper establishment; however, recommendations for white clover seeding rates are scarce. Dudeck and Peacock (1983) recommended 1500 pure live seed (PLS) m^{-2} be sown in September after verticutting Florida bermudagrass. However, these researchers did not report seed count per unit weight, as is often overlooked within overseeding studies. Logically, N contribution to associated grasses will be positively correlated with white clover density. However, too much white clover, and the benefits of turfgrass may be voided (i.e.: white clover may not withstand traffic or erosive forces as well as turfgrass). From our own research, as well as prior literature, we feel that 0.5 to 1.0 g m^{-2} is an appropriate white clover-seeding rate given available soil moisture and adequate time prior to first frost. However, seed counts per unit weight do differ with variety. For instance, in our own studies, Microclover

contained 1209 seed g⁻¹ (\pm a standard deviation of 21 seed), while the Dutch variety contained 1510 g⁻¹ (\pm 24 seed). These differences, though slight, do not explain all of the varietal differences observed within our own research. An argument could be made that Microclover is a less aggressive white clover, unable to compete for light within a dense bermudagrass turf. Though undocumented, we also observed lower spring flower densities amongst the micro variety, which may decrease self-seeded propagation in following years.

Soil temperatures were not directly correlated with observed trends in establishment timing. Though inconclusive, it is clear that establishment-timing recommendations must be adapted to a wider range of warm-season turfgrass climates. Largely the best indicator within our own region is historical first frost, due to predictability and its timing with the onset of bermudagrass dormancy. Furthermore, existing turfgrass cover may be an important consideration when seeding white clover, as green turf cover may buffer surface temperatures as well as affect soil drying and seedling desiccation. Similarly, existing turfgrass may remain too dense if soil temperatures remain high. As with any case of seeded establishment, proper agronomic principles should be employed. That is, the decision must take into account: forecast precipitation, cooling events, remaining turf-vigor, availability of supplemental water, and whether cultural methods may be employed to disrupt or change any of these variables.

Warm-season turfgrass is often overseeded with a cool-season alternative in the fall or early winter. This overseeding event offers a unique opportunity to simultaneously establish legumes. Cool-season legumes may contribute soil N for a sustainable overseeded sward as well as soil N for warm-season turfgrass breaking dormancy in the spring. Overseeding with a companion grass species may further the aesthetic value of mixed grass-white clover swards by

providing year round green turf and may help to better synchronize N mineralization for warm-season turf demands. Since cool and warm -season grasses differ in growth cycles, an additional benefit of companion species may be that cool-season grasses act as a “catch crop” for fixed N, only to later contribute that N to the associated warm-season turf via decomposition of above and below ground plant parts. Inter cropping with companion species is a means of capturing excess soil nutrients, such as N and Phosphorus, and thus prevent them from entering ground and surface waters (Hauggaard-Nielsen et al. 2012; Martinez and Guiraud, 1990).

Our research demonstrates cool-season turfgrass options and compatibility with white clover. However, future research must fully evaluate white clover-seeding rate by companion grass species interactions. The negative correlation of grass versus white clover density detected within our own studies would be much better detected if companion grass seeding rates were treated as a planned treatment variable. Subsequent trials should evaluate the effects of winter grass cover on soil temperature and soil moisture, as both may vary with overseeded cover and subsequently affect white clover establishment.

Future research should also evaluate N fixation and transfer to associated bermudagrass swards. Previous research has shown white clover inclusion to be a viable means of providing N to associated turfgrass. However, It is difficult to predict whether seeded white clover establishment of mixed turfgrass swards will be widely adopted by the turfgrass industry. In summary, legume inclusion within warm-season turfgrass may play a role in sustaining low maintenance scenarios. However, legume inclusion offers unique challenges for turfgrass agronomists during seeded establishment.

Table 1. Companion grasses planted 14 October, 2010 and 1 October, 2011 with two commercial white clover varieties (1.5 g live seed m⁻²).

Common Name	Species	Variety	Rate (g m⁻²)
Perennial Ryegrass	<i>Lolium perenne</i>	Top Gun II ^a	20
Annual Ryegrass	<i>Lolium multiflorum</i>	Marshall ^b	30
Creeping Bentgrass	<i>Agrostis stolonifera</i>	G-2 ^c	10
Tall Fescue	<i>Festuca arundinacea</i>	Rebel IV ^d	30
Rough Stalk Bluegrass	<i>Poa trivialis</i>	Havana ^a	50

^a Jacklin Seed, Post Falls, Idaho

^b Wetsell Seed, Harrisonburg, Virginia

^c Tee-2-Green, Hubbard, Oregon

^d Pennington Seed, Madison, Georgia

Table 2. Analysis of variance (ANOVA) for 2010-2011 and 2011-2012 white clover establishment trials. Replication year was significant in all studies; therefore, analysis was performed separately.

Study	Effect	F-value	P > F	F-value	P > F
		2010-2011		2011-2012	
<i>Mechanical disruption study</i>	Method	7.91	<0.0001	9.49	<0.0001
	Variety	3.30	0.0721	4.00	0.0495
	Method × variety	2.18	0.0766	0.66	0.6191
<i>Establishment timing</i>	Time	53.97	<0.0001	8.65	<0.0001
	Variety	15.77	0.0001	24.53	<0.0001
	Time × variety	1.60	0.1808	2.66	0.0401
<i>Seeding rate</i>	Rate	64.96	<0.0001	13.30	<0.0001
	Variety	2.59	0.1111	1.51	0.2227
	Rate × variety	2.16	0.0789	0.31	0.8735
<i>Companion grass</i>	Grass	32.37	<0.0001	7.85	<0.0001
	Variety	3.43	0.0670	0.01	0.9390
	Grass × variety	0.84	0.5043	0.62	0.6491

All data were subject to analysis of variance (ANOVA) within SAS procedure GLIMMIX using mixed model methodology. Treatment was considered a fixed effect in the model. Year, replication (nested within year), and iterations containing these effects were considered random in the model

Table 3. April observed, spring white clover density as affected by mechanical surface disruption methods.

Season	Method	Trifoliate leaves m ⁻²	± 95% CI
2010-11	Aerification	513 a ^a	75
	Verticut	502 a	75
	Scalp	499 a	75
	Non-treated	279 b	77
2011-2012	Aerification	204 A	49
	Verticut	108 AB	49
	Scalp	73 BC	49
	Non-treated	6 C	49

^a Means were separated by 95% confidence intervals (CI).

Table 4. White clover density as affected by seeded establishment timing.

Season	Time	Trifoliolate leaves m ⁻²	± 95% CI
2010-11	October	199 a ^a	23
	November	68 b	20
	December	22 c	19
	January	17 c	19
	February	4 c	19
2011-2012	October	51 BC	27
	November	91 AB	28
	December	108 A	27
	January	39 BC	27
	February	9 C	27

^a Means were separated by 95% confidence intervals (CI).

Table 5. Companion grass densities along side affected white clover densities.

Year	Grass	Trifoliolate leaves m ⁻²	± 95% CI	Grass (plants m ⁻²)	± 95% CI
2010-2011	Tall fescue	383 a ^a	47	2648 c	1410
	Creeping bentgrass	262 b	42	3810 c	1410
	Perennial ryegrass	165 c	46	9429 b	1410
	<i>Poa trivialis</i>	109 cd	42	15113 a	1411
	Annual ryegrass	68 d	42	11302 b	1410
2011-2012	Tall fescue	65 A	20	2454 C	1452
	Creeping bentgrass	49 AB	20	6465 B	1452
	Perennial ryegrass	11 BC	21	8491 B	1483
	<i>Poa trivialis</i>	7 C	20	15007 A	1516
	Annual ryegrass	5 C	20	8626 B	1483

^a Means were separated by 95% confidence intervals (CI).

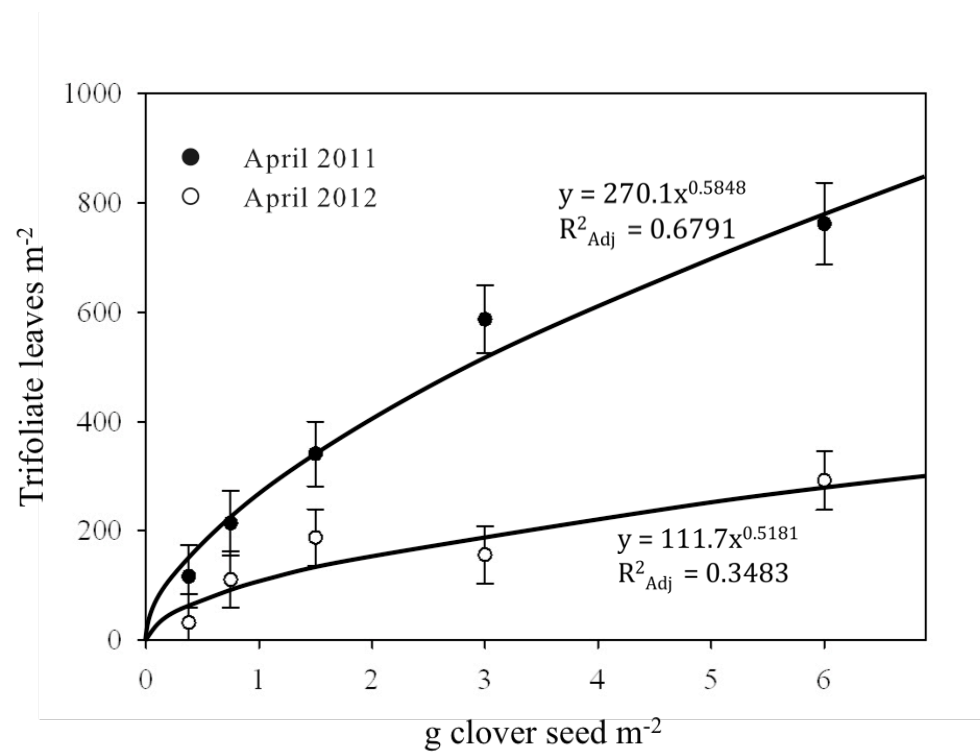


Figure 8. April observed white clover density as a function of five rates of October seeded white clover. Error bars represent 95% confidence intervals about the mean.

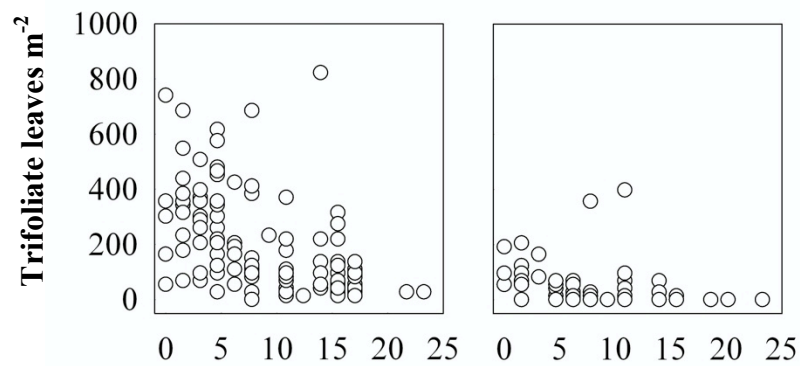


Figure 2. Correlation of December grass density and April white clover density during seasons one and two of companion grass experiments, where r = Pearson's Correlation Coefficient, and P = probability that r is different from 0. Correlations were only significant when white clover data were combined across companion grass species.

Differential Response of Four *Trifolium* Species to Common Broadleaf Herbicides: Implications for Mixed Grass-Legume Swards.

INTRODUCTION

Clovers (*Trifolium* spp.) are routinely included within pastures and low-maintenance turf as utility plants. These legumes provide important ecosystem services, such as nitrogen (N) fixation (Ledgard and Steele 1992, McNeil and Wood 1990, Whitehead 1995) and insect habitat (Abraham et al. 2010, Rogers and Potter 2004). Clovers, like many legumes, increase forage yields and quality as well as decrease N fertilizer requirements (Hoveland 1989; Rao et al. 2007). When included within low maintenance turf, clovers improve sward color by contributing N to associated grasses (Sincik and Acikgoz 2007) and have proven useful for maintaining roadside slopes maintained as turf (Roberts and Bradshaw 1985).

Herbicidal weed control is critical to maximizing forage yields (DiTomaso 2000; Seefeldt et al. 2005) and is often required during clover establishment, as seedlings are not competitive with many weeds and grasses (Carlisle et al. 1980; Evers et al. 1993; Young et al. 1992). Weeds compete with desirable species for nutrients and resources and are often toxic to grazing animals (Carlisle et al. 1980; Marten and Andersen 1975; Vengris et al. 1953).

Selective weed control in grass-clover swards is hampered by the lack of effective herbicides that are tolerated by clovers. Many effective broadleaf herbicides are reported to control clover, including 2,4-D, carfentrazone, clopyralid, dicamba, and triclopyr (MacRae et al. 2005, Neal 1990, Neal and Mascianica 1988, Willis et al. 2007). Yet few herbicides are labeled for postemergence application to various clover species, and most are restricted to states where clovers are cultivated for seed production or forage.

Furthermore, differential herbicide tolerance of legume -cultivars and -species has previously been reported, including differential reductions in seed yield, biomass, and N input for subsequent crops (Beran et al. 1999; Bowran 1993; Young et al. 1992). Understanding differential herbicide treatment effects upon clover species may advance efforts for selective weed control within grass-clover swards as well as increase clover control options within grass monocultures.

Experiments were conducted to identify herbicides tolerated by utility clovers and to evaluate the potential for differential clover response to common herbicide treatments. Due to previous reports of differential herbicide tolerance amongst other legume species, researchers postulated that clover response to herbicides would differ by species. Emphasis was placed upon determining herbicide tolerance of four clover species endemic amongst the local flora, including: white clover (*T. repens* L.), small hop clover (*T. dubium* Sibth.), crimson clover (*T. incarnatum* L.), and ball clover (*T. nigrescens* Viv.). We report differential responses of these species to a range of broadleaf herbicides.

MATERIALS AND METHODS

Field and greenhouse experiments were repeated for two years to evaluate clover response to a range of common broadleaf herbicides. Field experiments were conducted during 2010 and 2011 at the Auburn University Turfgrass Research Unit (32°34'40" N, 85°29'57" W) in Auburn, AL.

Cool-season legumes (Table 6) were collected to a depth of 7.6 cm using a 10.8 cm diameter golf-green cup-cutter (Par Aide Product Company, Lino Lakes, Minnesota, USA) between 19 to 22 January 2010 and 1 to 18 February 2011. Plants were collected at a single site from a Marvyn sandy loam (fine-loamy, Kaolinitic, thermic Typic Kanhapludult) soil with pH

6.3 (1:1 soil:H₂O) and were allowed to mature in a greenhouse setting until subject to selection for uniform size and maturity.

Plants were transplanted into field conditions 10 February 2010 or 15 to 21 February 2011. The transplant site was a hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burt-Davy) sward maintained at 5 cm mowing height without supplemental fertility. Soil at the transplant site was a Marvyn sandy loam soil similar to that found at the collection site where plants originated. The site was not mown or fertilized during studies, but was hand watered to prevent clover wilt. Plants were clipped with shears to identical height (8 cm) and diameter (11 cm) two days prior to treatment. Further information concerning collection date, stage of growth, and transplant date is presented in Table 6.

The field study was conducted as a split-plot design with the four clover species as randomized sub-units within herbicide main plots (3 replications). Herbicide treatments and application rates (Table 7) included commonly applied broadleaf herbicides or were chosen based upon labeling for leguminous crops. Treatments included a non-treated control. All treatments included a 0.25% v v⁻¹ non-ionic surfactant (Induce, Helena Chemical Company, Collierville, TN). Herbicides were applied at 280 L ha⁻¹ spray volume on 10 March 2010 or 22 February 2011 via a CO₂ pressurized back-pack sprayer equipped with four TeeJet XR8002 flat fan nozzles (Spraying Systems Co., Wheaton, Illinois, USA).

During field experiments, clover control was visually assessed 6 weeks after treatment (WAT) relative to the non-treated control, where 100% control equaled complete plant death. Control was based upon a combination of herbicide injury and plant health. Control assessments did not account for height reductions. However, plant height from the soil surface was sampled

twice by lifting the two tallest foliar meristems, whether inflorescence or leaf, and measuring to the uppermost point.

Supplemental greenhouse experiments were conducted during 2011 and 2012 at the Auburn University Weed Science Greenhouse, (32°35'12" N, 88°29'15" W) in order to evaluate herbicide effects upon clover biomass. Plants were collected 1 to 18 February 2011 and 13 to 20 January 2012 (Table 6) identically to those of the field experiments. To prevent sample erosion and to facilitate sample randomization, greenhouse plants were placed in pots (11 cm diameter, 730 cm³ volume). Greenhouse air temperature was maintained between 23 and 25 °C. Plants were subject to normal daytime irradiance (less than 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at foliage height) and were watered via over-head mist irrigation twice daily. Herbicide treatments were identical to those applied in field experiments (Table 7). Treatments were applied in an enclosed research spray cabinet applying 280 L ha⁻¹ through a single TeeJet TP8002EVS nozzle (Spraying Systems Co., Wheaton, Illinois, USA). The study was conducted as a completely randomized design with three replications and one pot per experimental unit. Plants were randomized daily to account for variations within the greenhouse microclimate. Foliage was harvested at the soil surface and oven dried at 50°C for 72 hours to ascertain above ground biomass.

Height and biomass responses are based upon percent reduction relative to the non-treated control. All data were subject to analysis of variance (ANOVA) within SAS procedure GLIMMIX using mixed model methodology (SAS[®] Institute v. 9.2, Cary, North Carolina, USA). Field and greenhouse data were analyzed separately. Treatment was considered a fixed effect in the model. Year, replication (nested within year), and iterations containing these effects were considered random in the model and were non-significant for all response variables (Carmer et al. 1989; Hager et al. 2003). Basic model assumptions were confirmed. Means were separated

based upon adjusted 95% confidence intervals, which allows for multiple comparisons by protecting family-wise error rate (Littell et al. 2006).

RESULTS AND DISCUSSION

Analysis of variance indicated that year and year by treatment interactions were not significant ($P > 0.05$; Table 8). Therefore, experiments were pooled across years with respect to growing condition (field or greenhouse). Precedence was given to field data, with greenhouse biomass reductions presented separately. Of the field data, priority was given to percent control, with relative height discussed as supporting evidence. Studies indicated varying control and height reductions due to species by herbicide interactions. Interaction effects were given precedence to main effects.

Field experiments. ANOVA (Table 8) indicated significant herbicide by species interaction effects upon control and height data of field experiments (Table 9). 2,4-D control did not differ due to species and was $\geq 88\%$ for all clovers. However, 2,4-D reduced small hop clover height greater than that of white clover (97% versus 41%) and reduced ball and crimson clover heights 64 and 63%, respectively. Herbicide effects on plant height are likely of biological importance to plant survival and stand resilience. However, reductions in size may be linked to more than just herbicide induced plant injury. Fletcher and Raymond (1956) first demonstrated that phenoxy-herbicides, like 2,4-D, reduced the success of *Rhizobium trifolii* to form symbiotic relationships with white clover, subsequently reducing N fixation. More recent studies have demonstrated that various herbicides directly damage both host plant and symbiotic rhizobium (Clark and Mahanty 1991). Herbicide effects upon rhizobium, nodulation, and N fixation were not examined within these experiments. However, future research should focus upon plant competitiveness, rather than simply plant survival.

Since the 1950's legume tolerance to butyric acid compounds, such as 2,4-DB and MCPB, has been linked to reduced beta-oxidation within tolerant species (Wain and Wightman 1954). Within our own experiments, 2,4-DB was moderately tolerated by all clovers, and control did not differ due to species ($\leq 58\%$ control; Table 9). However, 2,4-DB did affect clover heights differently. 2,4-DB did not affect crimson and ball clover heights (+2% and 12%, respectively) relative to the non-treated control; however, 2,4-DB did reduce small hop clover height 27%, which was similar to ball and white clover height reductions but greater than that of crimson clover. 2,4-DB reduced white clover height 50%, which was greater than ball and crimson clover height reductions and similar to height reductions observed due to 2,4-D. Differential response to 2,4-DB in leguminous pasture species has previously been reported. Mulholland et al. (1989) demonstrated differential *Medicago* species responses, while Young et al. (1992) reported that *M. aculeata* and *T. subterraneum* were more tolerant of 2,4-DB than *M. truncatula*.

MCPA is applied alone and in commercially available herbicide mixtures for pasture and rangeland management but may lack selectivity for many pasture legumes (Conrad and Stritzke 1980; Evers et al. 1993). Our experiments demonstrated this lack of tolerance amongst four clover species. MCPA controlled clovers between 56 and 86% and reduced heights between 11 and 67%. An alternative to MCPA not included amongst our treatments was the butyric acid compound MCPB, which has utility within leguminous crops (Senseman 2007) and has previously been demonstrated safe upon white clover (Elliot 2006).

Clopyralid and dicamba effectively controlled all clovers ($\geq 95\%$; Table 9) and completely reduced heights across species. Triclopyr control was similar to that of clopyralid ($\geq 81\%$); however, triclopyr affected clover heights differently. Triclopyr failed to reduce ball clover height relative to the non-treated and reduced crimson clover height only 22%. Small hop

clover height was reduced 61%, which was similar to reductions in crimson clover height but greater than that of ball clover. Triclopyr reduced white clover height 91%, which was greater than ball and crimson clover height reductions. It is noteworthy that herbicides from the same family (e.g., clopyralid and triclopyr) did not exhibit similar efficacy in this experiment.

Atrazine effectively controlled all clovers ($\geq 98\%$) and reduced clover heights $\geq 86\%$ (Table 9). On the contrary, bentazon was well tolerated by all clover species ($\leq 15\%$ control and $\leq 17\%$ height reduction). In fact, a 30% increase in white clover height was observed due to bentazon application. Other researchers have previously reported similar responses to bentazon. Ceballos et al. (2004) reported increases in red clover (*T. pretense*) plant height (70 and 48% for 12 and 24 g 100 m⁻² rates) at the expense of roots, which were reported to have decreased 42% by 20 days after treatment. Root biomass was not measured during our experiments.

Only imazaquin resulted in differential clover control. Imazaquin controlled small hop clover greater than white clover (91% versus 50%; Table 9). Ball and crimson clover control (80 and 62%, respectively) were similar to that of other clovers. Imazaquin reduced small hop clover height 88%, which exceeded height reductions measured among other clovers ($\leq 47\%$). Differential soybean-cultivar responses to imazaquin have been reported (Kent et al. 1988). More recently, differential responses to acetolactate synthase (ALS) inhibitors, such as imazaquin, have been attributed to resistance mechanisms (Tranel and Wright, 2002). However, ALS resistance has not been confirmed amongst *Trifolium* spp. (International Survey of Herbicide Resistant Weeds, 2012).

Imazethapyr was well tolerated by all clover species. Imazethapyr controlled clovers $\leq 15\%$ (Table 9). Crimson clover height (+9%) did not differ from that of the non-treated. Small hop and white clovers were reduced in height 33 and 45%, respectively, while ball clover height

was reduced 12%. Previous research has demonstrated imidazolinone herbicides, such as imazethapyr, can be utilized for promoting the establishment of certain legumes within tall-grass prairies (Beran et al. 1999).

Metsulfuron and trifloxysulfuron herbicides are highly effective against many broadleaf weeds found within mixed grass swards, yet knowledge of differential tolerance among legume species is limited. Our results did not suggest differential tolerance, with metsulfuron and trifloxysulfuron having controlled and reduced heights similarly across clovers. Metsulfuron controlled all clover species $\geq 88\%$ and reduced clover heights $\geq 78\%$ (Table 9). Similarly, trifloxysulfuron controlled clovers $\geq 80\%$ and reduced clover heights $\geq 45\%$.

Greenhouse experiments. Supplemental greenhouse experiments evaluated biomass harvests (Table 10). Biomass reductions differed due to herbicide treatment as well as clover species but did not differ due to herbicide by species interaction. Biomass reductions are important considerations when managing mixed grass-clover swards for forage.

Clopyralid and atrazine reduced clover biomass 98%, similar to 2,4-D (85%), dicamba (92%), triclopyr (89%), and metsulfuron (84%), but greater than those of all other treatments (Table 10). Imazaquin reduced clover biomass 73%, similar to 2,4-D, dicamba, triclopyr, metsulfuron, and trifloxysulfuron (68%). MCPA reduced clover biomass 50%, similar to 2,4-DB (45%), bentazon (36%), and imazethapyr (28%).

White clover biomass was reduced less than crimson and hop clovers (58% versus 72%), but equal to that of ball clover (61%; data not shown). Species main effects are important in several contexts. Foremost, labels do not always clearly define species for which herbicides are tolerated. These results suggest that clovers vary in herbicide susceptibility. Secondly, labels may ambiguously emphasize hop clover control. Yet there are at least three *Trifolium* spp. that

are generically called “hop clovers” (Plants Database, 2013; WSSA, 2013), some of which differ dramatically in phylogeny (Ellison et al. 2006).

IMPLICATIONS FOR MANAGEMENT

On a practical level, our results demonstrate potential herbicide options for maintaining mixed grass-clover swards. Candidate herbicides include bentazon, 2,4-DB, and imazethapyr. These herbicides are commonly labeled for use within leguminous crops as well as forage and rangeland legumes. Bentazon and 2,4-DB have proven to be moderately tolerated by subterranean- (*T. subterranean*) and arrowleaf- (*T. vesiculosum*) clovers (Hawton et al. 1990; Smith and Powell, 1979). The relative tolerance of clover species to these candidate herbicides is further evidence of their value within certain scenarios. Yet, it is difficult to foresee herbicide applicators choosing these herbicides without further evidence of weeds controlled, costs, and effects upon mixed swards. There are undoubtedly many herbicides that are tolerated by clover species, yet questions remain about application rates and timing.

Our experiments suggest varying tolerances amongst clover species and common broadleaf herbicides. This agrees with previous research of differential herbicide tolerance amongst other pasture and forage legumes (Bowran 1993; Mulholland et al. 1989; Young et al. 1992). However, to our knowledge, this is the first report of differential tolerance solely amongst *Trifolium* spp. This supposition has broad impacts within agronomic scenarios. Pasture and rangeland managers have long sought herbicidal weed control without harming utility clover species, with limited success. Clover seed producers may benefit from the knowledge that certain clovers may be preferentially favored by differential herbicide responses. Additionally, legumes such as clovers have application within mixed turf swards. Legume species and varieties continue to be developed and improved for various agronomic applications (Rajeev et al. 2009).

However, herbicide labels often fail to clearly define the clover species for which an herbicide is intended (whether for selective weed control or for tolerance). As the number of species, varieties, and uses of clovers increase, label statements must more precisely scrutinize species tolerance in order to increase the viability and profitability of biodiverse agricultural scenarios.

Table 6. Four clover (*Trifolium*) species and their respective harvest and transplant dates. Plants were harvested and allowed to mature in a greenhouse setting. Plants were then subject to selection for uniform size and maturity followed by random assignment to either field or greenhouse experiments.

Year	Clover	Harvest date	Growth cycle	Transplant date	Treatment date	Flowering stage at treatment ^a	Leaves per plant at treatment
2010	white (<i>T. repens</i>)	19 January	Perennial	10 February	10 March	vegetative	10 to 20
	small hop (<i>T. dubium</i>)	19 January	Annual	10 February	10 March	early-flowering	20 to 30
	crimson (<i>T. incarnatum</i>)	20 January	Annual	10 February	10 March	early-flowering	10 to 20
	ball (<i>T. nigrescens</i>)	19 January	Annual	10 February	10 March	early-Flowering	15 to 25
2011 ^b	white (<i>T. repens</i>)	11 February	Perennial	15 February	22 February	vegetative	10 to 20
	small hop (<i>T. dubium</i>)	11 February	Annual	15 February	22 February	early-flowering	20 to 30
	crimson (<i>T. incarnatum</i>)	10 February	Annual	15 February	22 February	early-flowering	10 to 20
	ball (<i>T. nigrescens</i>)	10 February	Annual	15 February	22 February	early-flowering	20 to 30
2012 ^c	white (<i>T. repens</i>)	11 February	Perennial	15 February	22 February	early-flowering	10 to 20
	small hop (<i>T. dubium</i>)	11 February	Annual	15 February	22 February	late-flowering	20 to 30
	crimson (<i>T. incarnatum</i>)	10 February	Annual	15 February	22 February	early-flowering	20 to 30
	ball (<i>T. nigrescens</i>)	10 February	Annual	15 February	22 February	mid-flowering	20 to 30

^a Flowering stage is indicated as either early (blooms present but remaining un-opened or slightly opened), mid (having bloomed but no signs of flower senescence), late (flower keels having more than roughly 25% discoloration due to senescence).

^b 2011 dates refer to both field and greenhouse studies.

^c 2012 dates refer to greenhouse studies only.

Table 7. Herbicide rates and formulations applied in field and greenhouse experiments to four clover (*Trifolium*) species. All treatments included a 0.25% v v⁻¹ non-ionic surfactant. Herbicides were applied at 280 L ha⁻¹ spray volume. Experimental rates were chosen based upon common labeled rates and unpublished studies where legume tolerance had been observed.

Mechanism of Action ^a	Common name	Trade name	Formulation	Rate 100 m ⁻²	Manufacturer	City, State	Website
synthetic auxins	2,4-D	Amine 400	dimethyl amine salt	15.8 g ae	PBI Gordon	Kansas City, MO	www.pbigordon.com
	2,4-DB ^{b,c}	Butyrac 200	dimethyl amine salt	15.8 g ae	Albaugh	Ankeny, IA	www.albaughinc.com
	dicamba	Banvel	dimethyl amine salt	11.2 g ae	Arysta LifeScience	Cary, NC	www.arystalifescience.com
	MCPA ^b	MCPA Ester 4	ethylhexyl ester	5.2 g ai	Albaugh	Ankeny, IA	www.albaughinc.com
	clopyralid	Lontrel Turf and Ornamental	monoethanola mine salt	4.2 g ai	Dow AgroSciences	Indianapolis, IN	www.dowagro.com
	triclopyr	Turflon Ester Ultra	butoxyethyl ester	5.6 g ai	Dow AgroSciences	Indianapolis, IN	www.dowagro.com
photosystem II inhibitors	atrazine	AAtrex 4L	--	22.4 g ai	Syngenta Crop Protection	Greensboro, NC	www.syngenta.com
	bentazon ^{b,c}	Basagran	sodium salt	11.2 g ai	Arysta LifeScience	Cary, NC	www.arystalifescience.com
acetolactate synthase inhibitors	imazaquin ^{b,c}	Scepter 70 DG	free acid	5.6 g ai	BASF	Research Triangle Park, NC	www.basf.com
	imazethapyr ^{b,c}	Pursuit	ammonium salt	0.7 g ai	BASF	Research Triangle Park, NC	www.basf.com
	metsulfuron-methyl	MSM Turf	--	0.2 g ai	FarmSaver	Raleigh, NC	www.farmsaver.com
	trifloxysulfuron	Monument 75 WG	sodium salt	0.3 g ai	Syngenta Crop Protection	Greensboro, NC	www.syngenta.com

^a According to Senseman (2007).

^b Commonly labeled for use within forage and pasture legumes.

^c Labeled for use within soybean production (*Glycine max*).

Table 8. ANOVA results and source sum of squares (SS) relative to the total SS for field and greenhouse experiments 6 WAT.

Experiment	Field ^a		Greenhouse ^b
Source	Control ^c	Height ^d	Biomass ^d
Herbicide	0.0001 ^e	0.0001	0.0028
Species	0.5752	0.0001	0.0001
Herbicide × Species	0.0081	0.0001	0.0695

^a Field experiments were conducted during winters 2010 and 2011 and did not include biomass analysis.

^b Supplemental greenhouse experiments were conducted during winters 2011 and 2012 and evaluated biomass.

^c Control was visually assessed on a percent scale 6 WAT relative to the non-treated control.

^d Height and biomass responses were calculated based upon percent reduction relative to the non-treated control 6 WAT.

^e $P > F$ values obtained within SAS Proc MIXED.

Table 9.1. Control and height reductions of four clover (*Trifolium*) species measured 6 weeks after treatment (WAT) in field studies. Effects were restricted to $P \leq 0.05$ level of significance. Effects were combined across years. Model validity ($P > F$) is provided for significant species by herbicide interaction.

Herbicide	Clover	% Control ^a			% Height reduction ^b		
		Mean ^c	± 95% CI ^d	$P > F$	Mean	± 95% CI	$P > F$
2,4-D	ball	88	8	NS ^d	-64 ab	34	0.049
	crimson	91	8		-63 ab	27	
	small hop	95	8		-97 a	27	
	white	91	8		-41 b	27	
2,4-DB	ball	18	28	NS	-12 bc	12	< 0.001
	crimson	30	28		+2 c	12	
	small hop	58	26		-27 ab	12	
	white	28	28		-50 a	12	
dicamba	ball	99	1	NS	-100	0	NS
	crimson	100	1		-100	0	
	small hop	100	1		-100	0	
	white	100	1		-100	0	
MCPA	ball	86	31	NS	-23	32	NS
	crimson	58	27		-11	32	
	small hop	56	25		-67	32	
	white	78	25		-51	39	
clopyralid	ball	100	3	NS	-100	0	NS
	crimson	100	3		-100	0	
	small hop	95	3		-100	0	
	white	100	3		-100	0	
triclopyr	ball	88	11	NS	-17 c	21	< 0.001
	crimson	81	12		-22 bc	21	
	small hop	92	12		-61 ab	21	
	white	88	11		-91 a	21	

Continued in Table 9.2 on the following page.

^a % Control was visually assessed 6 WAT relative to the non-treated control.

^b % Height and biomass reductions are relative to the non-treated control. Negative numbers indicate height reduction.

^c Mean separations were performed using 95% confidence intervals. Overlapping intervals signify a lack of difference between means of the same herbicide treatment. Letters are presented as a method of easily distinguishing significant differences amongst herbicide treatment.

^d Abbreviations: 95% CI, 95% confidence interval; NS, non-significant.

Table 9.2. Continued from Table 9.1: Control and height reductions of four clover (*Trifolium*) species measured 6 weeks after treatment (WAT) in field studies. Effects were restricted to $P \leq 0.05$ level of significance. Effects were combined across years. Model validity ($P > F$) is provided for significant species by herbicide interaction.

Herbicide	Clover	% Control ^a			% Height reduction ^b		
		Mean ^c	± 95% CI ^d	$P > F$	Mean	± 95% CI	$P > F$
atrazine	ball	100	1	NS	-100	21	NS
	crimson	100	1		-100	17	
	small hop	100	1		-100	17	
	white	98	1		-86	17	
bentazon	ball	9	11	NS	-17	22	NS
	crimson	15	10		-2	22	
	small hop	4	11		-3	22	
	white	5	10		+30	27	
imazaquin	ball	80 ab	19	0.033	-47 b	20	0.012
	crimson	62 ab	19		-38 b	24	
	small hop	91 a	21		-88 a	20	
	white	50 b	19		-36 b	20	
imazethapyr	ball	7	13	NS	-12	26	NS
	crimson	15	13		+9	26	
	small hop	10	13		-33	26	
	white	10	13		-45	32	
metsulfuron	ball	90	12	NS	-79	24	NS
	crimson	93	11		-78	24	
	small hop	93	11		-97	24	
	white	88	11		-82	24	
trifloxysulfuron	ball	92	14	NS	-84	35	NS
	crimson	95	14		-70	29	
	small hop	80	14		-91	29	
	white	89	15		-45	35	

^a % Control was visually assessed 6 WAT relative to the non-treated control.

^b % Height and biomass reductions are relative to the non-treated control. Negative numbers indicate height reduction.

^c Mean separations were performed using 95% confidence intervals. Overlapping intervals signify a lack of difference between means of the same herbicide treatment. Letters are presented as a method of easily distinguishing significant differences amongst herbicide treatment.

^d Abbreviations: 95% CI, 95% confidence interval; NS, non-significant.

Table 10. Herbicide main effects upon clover (*Trifolium*) biomass reductions measured 6 weeks after treatment (WAT) during greenhouse experiments.

Herbicide	Greenhouse	
	% Biomass reduction	
	Mean ^a	± 95% CI
2,4-D	-85 abc	9
2,4-DB	-45 ef	10
MCPA	-50 ed	9
dicamba	-92 ab	9
clopyralid	-98 a	9
triclopyr	-89 ab	9
atrazine	-98 a	9
bentazon	-36 f	9
imazaquin	-73 bc	9
imazethapyr	-28 f	9
metsulfuron	-84 abc	9
trifloxysulfuron	-68 cd	9

^a Mean separations were performed using 95% confidence intervals. Overlapping intervals signify a lack of difference between means of the same herbicide treatment. Letters are presented as a method of easily distinguishing significant differences amongst herbicide treatment.

Dynamics of White Clover (*Trifolium repens*) Decomposition in a Southeastern Bermudagrass Lawn

INTRODUCTION

The ecological impact of turfgrass is frequently questioned, due in part to nutrient and water requirements as well as often-unsustainable monoculture cultivation (Milesi et al., 2005; Robbins and Birkenholtz, 2003; Robbins et al., 2001). Nitrogen (N) is essential to turfgrass health and quality (Beard, 1973; Turgeon, 2002). Commercial-lawn N requirements vary with species and environmental conditions, but within the southern United States, common rates range from less than 5 g N m⁻² year⁻¹ for bahiagrass (*Paspalum notatum* Flueggé) and centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.) to almost 30 g N m⁻² year⁻¹ for bermudagrass (*Cynodon dactylon* (L.) Pers.; Duple, 2004).

Clover (*Trifolium* spp.) inclusion within maintained turfgrass is a proposed means of increasing turfgrass sustainability (Dudeck and Peacock, 1983; Sincik and Acikgoz, 2007). Clover has been included in grass mixtures for roadsides as well as other maintained turfgrass areas and has proven useful for slope stabilization (Roberts and Bradshaw, 1985). In particular, white clover (*T. repens* L.) thrives within home lawns and golf courses because it can flower and produce seed at mowing heights as low as 6 mm (Watschke et al., 1995).

White clover increases turfgrass greenness by contributing N to associated grasses and has been reported to increase turfgrass color ratings within cool-season turfgrass (Sincik and Acikgoz, 2007) and increase vegetative cover within dormant bermudagrass

(Dudeck and Peacock, 1983). Estimates of white clover N fixation within three cool-season turfgrasses are greater than $25 \text{ g N m}^{-2} \text{ year}^{-1}$, with 4.2 to 13.7% of total N contributed to the associated turfgrasses (Sincik and Acikgoz, 2007).

Other pertinent research concerning white clover inclusion has been conducted in forage scenarios where white clover was grazed or harvested for animal fodder. Estimates of N fixation for grass-white clover pastures range from nil to $40 \text{ g N m}^{-2} \text{ year}^{-1}$, though most are from 10 to $25 \text{ g N m}^{-2} \text{ year}^{-1}$ (Ledgard and Steele, 1992; McNeil and Wood, 1990; Whitehead, 1995). Using the ^{15}N transfer method, McNeil and Wood (1990) estimated N fixation by white clover within perennial ryegrass was approximately $15.5 \text{ g N m}^{-2} \text{ year}^{-1}$, with 28% of the total fixed N having been transferred to associated ryegrass.

Transfer of N from legumes to associated turfgrass occurs indirectly through excreted N and decomposition of nodules, roots, and foliage (Brophy et al., 1987; Dubach and Russelle, 1994; Jensen, 1996; Wardle and Greenfield, 1991). Decomposition of root nodules is a significant source of N. Reported N concentration of root nodules ranges from 4.8 to 9.0% of root dry matter (Chu and Robertson, 1974; Wardle and Greenfield, 1991). However, root nodules are not the sole source of N transfer, as above ground white clover dry matter has been reported to be 9.1 to 24.2% protein, depending upon harvest date (about 1.5 to 4.0% N; Burton and DeVane, 1992). Unlike forage scenarios, turfgrass systems differ in that they are not grazed; rather, they are mown frequently to maintain utility and aesthetics. Mown clippings are returned to the turf surface, potentially contributing a mineralizable source of N.

Polyculture lawns of grass and white clover are historically common, yet little is known of the N-contribution and C-flux from decaying clover foliage. The rates of

decomposition, N mineralization, and C deposition would be useful information for future research regarding this subject as well as when assigning nutrient credits to white clover-culture in warm and cool season turfgrass. Such information would be highly dependent upon a multitude of factors, including time of year, litter composition, soil and climactic -conditions, as well as soil fauna. For these reasons, it may not be possible to control all factors *in situ*.

Organic residues decompose in two phases. Soil microbes rapidly consume the labile fraction, which is composed of sugars, starches, and proteins, leaving behind a recalcitrant fraction composed of cellulose, fats, waxes, lignin, and tannins (Wieder and Lang, 1982). This slowly decomposing fraction helps to develop soil organic matter. Due to the two-step nature of decomposition, a double exponential decay model is often implemented to describe litter decay (Wieder and Lang, 1982). Double exponential decay equations are of the form $Y = Ae^{-k_1t} + Be^{-k_2t}$, where Y = response, A and B are initial concentrations approximating the labile and recalcitrant portions, k1 and k2 are rate constants fitted to the data, and t equals time in days after application (DAA). Such models have been used successfully to describe quickly decaying legume litter in Alabama (Mulvaney et al., 2010) as well as the decomposition and N release of hedgerow species in Haiti (Isaac et al., 2000).

Modeling white clover decomposition may enable turfgrass researchers and professionals to more accurately predict nutrient contribution to associated grasses and help optimize supplemental fertilizer recommendations. Our objectives were to 1) explore the use of double exponential decay models as a method to predict C and N

contributions of white clover litter applied at different times throughout the year, and 2) quantify white clover litter decomposition, as well as C and N -release rates.

MATERIALS AND METHODS

An *in situ* decomposition study was conducted at the Auburn University Turfgrass Research Unit (32°34'40" N, 85°29'57" W; elevation 185 m) in Auburn, AL, on a Marvyn sandy loam (fine-loamy, kaolinitic, thermic Typic Kanhapludult) soil with pH 6.3 (1:1 soil:H₂O). Treatments (application date by retrieval timing) were arranged in a completely random design with four replicates. White clover litter was applied Mar 1, 2010; Jun 1, 2010; and Dec 1, 2010 (March, June and December -applications, respectively). Retrieval timings were 0, 1, 4, 7, 14, 28, 56, and 112 DAA. Time 0 DAA samples were truly replicated in the field.

Samples for decomposition studies were harvested from a stand of commercially available white clover, 'Dutch' white clover (Main Street Seed and Supply, Bay City, MI), which had been established in previous experiments unrelated to this research. The population is an intermediate growth-type marketed for grazing and wildlife habitat. White clover was maintained at 7.6 cm mowing height with supplemental irrigation applied as needed and no supplemental fertilization. Soil moisture was greater than 25% at each harvest date, ensuring that the clover stand was fully turgid prior to harvesting foliage. Leaves of the harvest area were patted dry with paper towels prior to harvest. In order to mimic a standard mowing occurrence, white clover foliage was harvested 4 cm above soil level using hand-held shears. Litter was transported on ice in order to preserve samples during preparation and field placement. Within two hours of harvest, litter was mixed thoroughly, and contaminants such as grass and dead or necrotic tissue were

removed. Clover foliage, excluding flowers, was placed into nylon bags measuring 10 × 20 cm with 50 to 60 µm openings on a fresh rate (FW) basis at 10.0 g bag⁻¹ (500 g FW m⁻²).

Individual litterbags were placed within a 50 × 50 cm experimental area to prevent possible bag-to-bag interference. In preparation for litterbag placement, 10 × 20 cm areas of ‘Tifway’ hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burt Davy) were denuded to the soil level using a gas-powered string trimmer. Steel sod staples 20 cm in length were used to secure each of the four corners of the sealed litterbag to the soil layer. The study area was maintained at a height of 3.8 cm; all clippings were returned to the turfgrass surface. Plots received 3 cm supplemental irrigation on a weekly basis between May 31 and September 10, 2010, and resuming March 14, 2011. Prior to study initiation, the area was fertilized (49 kg N ha⁻¹) Feb 15, 2010 and received no supplemental fertility for the duration of the study.

Retrieved litter was air-dried at 60°C for 48 hours and weighed for dry-matter determination. Litter was then ground to pass a 16-mesh sieve and analyzed for total C and N by LECO TruSpec CN (Leco Corp, St. Joseph, MI). To account for possible soil contamination of litterbag contents, all data were converted to an ash-free dry weight (AFDW) basis by ashing 5 g of sample in a muffle furnace at 400°C for 12 hours (Cochran, 1991). Soil temperature was recorded via TidbiT[®] v2 Temp loggers (Onset Computer Corp, Pocasset, MA) buried 10 cm below soil level. Air temperatures at 1.5 m above ground level were obtained from a nearby weather station (32°36’00” N, 85°30’00” W; elevation 199 m) in Auburn, AL (AWIS, 2013).

Analysis of variance was conducted. Means, standard errors, and statistical significance of treatments were determined at the 95% confidence level using mixed models procedures within Proc Glimmix (SAS Institute, 2004). Least squares estimates for nonlinear models were determined within SigmaPlot 11 using Marquardt-Levenberg algorithm to provide the best fit (Systat Software, 2008). Initial parameter ranges were selected with a maximum of 200 fits and 200 iterations.

Double exponential decay models served as the basis for comparison of mass, N, and C loss between application dates. In most cases, double exponential decay models minimized residual sums of squares and produced comparatively lower residual mean squares, standard errors, and PRESS statistics as well as better coefficients of determination (R^2_{adj}) than single exponential decay models. For brevity, comparisons of single and double exponential models are omitted. However, instances where double exponential decay models could be collapsed into single exponential models are generally indicated by the presence of k_2 values close to zero. Days to 50% decomposition (D50) values were estimated based upon double exponential decay equations to compare and contrast regression estimates.

RESULTS AND DISCUSSION

Double exponential decay models

Analysis of variance indicated a significant application-date by retrieval-time interaction for all response variables (discussed separately below). Parameters fit to the double exponential decay curve are shown in Table 11. All regression equations were significant ($p < 0.0001$) and were good approximations of the data (R^2_{adj}). It is convenient to represent decay patterns on a percent of original material basis such that one can

extrapolate for hypothetical amounts of residue in field conditions. Table 11 shows residue persistence normalized to 100% of initial AFDW. Normalized equations offer an approximation of labile (A) and recalcitrant (B) litter on a percent basis. Generally, models revealed two mass, C, and N pools for all application dates. Across all response variables, initial decomposition of litter occurred more rapidly during June application relative to March and December applications. These trends are visually evidenced by steeper slopes (Figure 11, 12, 13) as well as greater k_1 and k_2 values (Table 11) during the decay of labile and recalcitrant portions. Differences in the rate of decay are apparent by comparing k_1 and k_2 values from each equation. Decay constants are similar, whether presented on a percent remaining or area (data not shown) -basis.

Mass remaining.

White clover mass decreased fastest when applied in June (Figure 11). This was visually evidenced by steeper slopes and greater decay constants (Table 11). The labile decay constant of June-applied litter (0.1056) was nearly three times greater than that of March-applied litter (0.0367) and more than 6 times that of December-applied litter (0.0166). The effects of application date upon the decay of recalcitrant portions were more pronounced. The recalcitrant decay constant of June-applied litter (0.0043) was more than 10 orders of magnitude greater than that of March and December-applied litter. The relatively quick decay of June-applied litter is typical of warmer soil temperatures (Figure 1) and the increased microbial activity involved in decomposition.

For all application dates, labile portions were greater than 80%, and recalcitrant portions were $\leq 25\%$ (Table 11). Mass predictions based upon % remaining data generally over estimated 0 DAA mass, which is likely due to 3 and 7 DAA data having

been abnormally greater than predicted levels (Figure 11). This phenomenon may be in large part due to an initial resistance to decay during application of fresh litter. This lag is not reflected by the double exponential decay curve but may be more adequately considered a sigmoidal response. Others have suggested that litter deposited during climactic periods unfavorable for decomposition, such as winter conditions, may be best explained by a sigmoidal curve (Swift et al., 1979). We suggest that the initial delay is linked to litter having been applied as fresh material, rather than dried material. Yet it could also be argued that fresh litter had the moisture necessary to drive microbial activity. Our goal was simply to simulate actual occurrences under field conditions, and it is not known what effect foliage moisture had on initial decay.

Table 12 contains predicted mass persistence expressed as 95% confidence intervals ranging from 0 to 112 DAA. Applications varied upon rapidness to reach 50% of original material (Figure 11). June-applied litter mass halved in 12.1 d, decreasing from an initial equivalent of 104.4 g m^{-2} to 52.2 g m^{-2} (data not shown). March-applied litter was slightly slower, taking 29.0 d to halve in remaining mass from 71.9 g m^{-2} to 36.0 g m^{-2} , while December-applied litter took an estimated 57.4 d to decrease from 72.1 g m^{-2} to 36.1 g m^{-2} . Due to the nature of depositing fresh material rather than dry material, seasonal applications differed in dry matter equivalence placed upon an area basis. These differences may have been due to foliage moisture content despite precautions to minimize differentials. To what extent this may have influenced decay is not explored within this analysis.

Carbon remaining.

Carbon composition of initial clover foliage differed slightly among application date. June-applied litter C ($44.0\% \pm 95\% \text{ CI} = 0.3\%$) was slightly greater than that of March ($42.7\% \pm 95\% \text{ CI} = 0.1\%$) and December ($42.1\% \pm 95\% \text{ CI} = 0.6\%$) -applied litter. C loss models (Figure 12) were comparable to those of mass loss. This is attributed to mass lost through microbial respiration of C, which is lost as CO_2 to the atmosphere (Wood and Edwards, 1992).

White clover C decreased fastest when applied in June (Figure 12). The labile decay constant of June-applied litter (0.1061; Table 11) was three times greater than that of March-applied litter (0.0354) and more than seven times that of December-applied litter (0.0143). The recalcitrant decay constant of June-applied litter (0.0045) was nearly six times greater than that of March-applied litter (0.0008) and nearly eight orders of magnitude greater than December-applied litter ($6.7 \text{ E-}13$).

When percent remaining data were analyzed, four parameter exponential decay models revealed two C pools for application dates (Table 11). Labile portions of all applications were greater than 79%, and recalcitrant portions were $\leq 26\%$. Carbon predictions based upon % remaining data generally over estimated 0 DAA C, which is likely due to 3 and 7 DAA data having been abnormally greater than predicted levels (Figure 12). This phenomenon may be in large part due to an initial resistance to decay during application of fresh litter.

Applications varied upon rapidness to reach 50% of original material (Figure 12). June-applied litter C halved in 11.0 d, decreasing from an initial equivalent of 44.6 g C m^{-2} to 22.3 g C m^{-2} . March-applied litter was slightly slower, taking 27.8 d to halve in

remaining C from 31.6 g C m⁻² to 15.8 g C m⁻², while December-applied litter took an estimated 60.5 d to decrease from 30.3 g C N m⁻² to 15.2 g C m⁻².

Nitrogen remaining.

Nitrogen composition of June-applied litter (4.8% ± 95% CI = 0.4%) was slightly greater than that of March (4.1% ± 95% CI = 0.1%) and December (4.0% ± 95% CI = 0.2%) -applied litter. However, these data agree with the range of 4.1 to 4.9% N reported by Sincik and Acikgoz (2007). Variation of N content within clover stands is likely due to environmental factors affecting symbiotic N fixation within plant roots, which were not accounted for within this study, as well as N availability within cooler soils. In their review of N fixation of grass-legume pastures, Ledgard and Steele (1992) report that fixation is greatly reduced due to dry soil conditions, acidic soils, and the “pest/disease complex.” Another factor affecting N fixation is soil temperature. Frame and Newbould (1986) found that a minimum temperature of 9°C was necessary for active N fixation by *Rhizobium*. It has also been reported that temperatures necessary for nitrogenase activity range from 13 to 26°C (Halliday and Pate, 1976). Soil and air temperatures may not have been compatible with active N fixation prior to harvest of March and December plant material.

Loss of N from decomposition of white clover was quickest when applied in June (Figure 13). The labile decay constant of June-applied litter (0.0938; Table 11) was more than three times that of March-applied litter (0.0271) and nearly 10 times that of December-applied litter (9.970 E-3) suggesting that temperatures during June were far more conducive to microbial decomposition of litter; however, climactic conditions are not modeled within decomposition equations. Decay constants of the recalcitrant portions

(k2) were smaller when litter was applied in March (1.2947E-12; Table 11) rather than June (0.0036) and December (0.0100), indicating that the nature of the recalcitrant decay was slower when applied in June than March or December. It is also possible that the lack of sampling dates beyond 112 DAA did not allow for accurate prediction of recalcitrant decay within March and December applications. Had sampling dates extended further, N remaining would have been more likely to approach 0%.

When percent remaining data were analyzed, four parameter exponential decay models revealed two N pools for application dates, each having similar size. The labile portions of all applications were greater than 80% (Table 11), and recalcitrant portions were less than 25%. These sizes are slightly greater for the faster decaying labile portions than the approximately one to one ratios of *Lespedeza cuneata* (Dum. Cours.) G. Don, *Albizia julibrissin* Durazz., and *Glycine max* (L.) Merr. reported by Mulvaney et al. (2010). Predictions generally over estimated 0 DAA N, which is likely due to 3 and 7 DAA data having been abnormally greater than predicted levels (Figure 13). This abnormality is not exceptional, and may be due to N immobilization from surrounding sources. Similar faults in non-linear models fit to litter decay curves have been attributed to N immobilization, though these were for higher C/N ratio wheat straw (Mulvaney et al., 2010). Similarly, rainfall or irrigation during these months could be sources of immobilized N.

Application dates varied markedly in their rapidness to reach 50% of original material (Figure 13). June-applied litter N concentrations halved in 10.9 d, decreasing from an initial equivalent of 3.4 g N m⁻² to 1.7 g N m⁻². March-applied litter was slightly slower, taking 37.0 d to halve in remaining N from 4.2 g N m⁻² to 2.1 g N m⁻², while

December-applied litter took an estimated 73.6 d to decrease from 2.9 g N m⁻² to 1.5 g N m⁻². After 112 d, predicted N remaining from March, June, and December-applied litter had reduced to 22.7, 14.6, and 34.2% of that applied, respectively (Table 12). Caution should be used when making predictions beyond the length of the study due to the variable nature of litter decay over seasons. Furthermore, predicted decay is rapid given suitable conditions. Therefore, any extrapolations would be minute in comparison to initial nutrient release.

C/N ratios.

The nature of clover decomposition is similar to that of other legumes. That is, legumes contain a relatively high concentration of N, allowing for very rapid initial decay. Clover samples had C/N ratios ($10.1 \pm \text{S.D. } 0.8$), which were similar across all application intervals. C/N composition of remaining litter according to predicted decay equations are shown in Table 14. C/N ratios are frequently used to describe a residue's propensity to mineralize or immobilize soil inorganic N. However, associated chemical analysis of decomposable fractions (e.g., the labile and recalcitrant fractions) may be a better means of determining a residues' effect upon soil N concentrations (Hadas et al., 2004). What is not well understood is the persistence of recalcitrant fractions beyond initial decay. With C/N ratios of nearly 10 to 1 throughout decomposition (Table 12), these fractions are likely long-term N contributors when surface applied. Also not understood are the effects of multiple applications of litter upon the soil surface. It may be that increased clover litter contributes to residual soil N pools. Though we have not gone so far as to use presented data for such estimations.

CONCLUSIONS

This research demonstrates important aspects of white clover decomposition, mainly that clovers are composed of a quickly decaying labile fraction. Given that clover populations are regenerative, N from white clover decay may be adequate to maintain associated turfgrasses. Modeling white clover decomposition may enable turfgrass researchers and professionals to more accurately predict nutrient contribution to associated grasses. Such information could be used to formulate an integrated N fertility program that includes both biologically fixed and synthetic N sources. The underpinnings of such a program have largely been overlooked by mainstream turfgrass research. Questions remain as to the consequences of synthetic N applications upon mixed turfgrass-legume swards. For instance, how would N application effect clover populations and levels of biologically fixed N?

Future research should evaluate different leguminous species and their inherent decay patterns. White clover is not the only *Trifolium* species capable of cultivation within maintained turfgrass. Others include *T. incarnatum*, *T. dubium*, *T. nigrescens*, *T. campestre*, and *T. aureum*. Likewise, there are many leguminous species already present within low-maintenance turf, including *Medicago* and *Kummerowia* spp. Results of future studies may enable more appropriate species selections that sustain associated turfgrasses with much needed N, while simultaneously contributing other ecosystem services, such as pollinator habitat. Furthermore, research should evaluate periodicity of legume N contribution to warm and cool season grasses as well as possible ways to synchronize litter deposition with turfgrass N needs. All of these topics serve to advance

the effectiveness of alternative turf scenarios but also apply broadly to other sectors of conservation agriculture.

Table 11. Double exponential decay equations regressed on time (days) for mass, carbon (C), and nitrogen (N) -loss from white clover incubated in litter bags under field conditions. Double exponential decay equations are of the form $Y = Ae^{-k_1t} + Be^{-k_2t}$, where Y = response, A approximates the labile portion, B approximates the recalcitrant portion, k1 and k2 are rate constants fitted to the data, and t = time in days after application. Percent remaining data were normalized to initial day 0 applications to facilitate approximations of labile and recalcitrant portions.

% Remaining	Equation	P > F [†]	R ² _{adj}	Syx [‡]
Mass				
<i>March</i>	$Y = 84.2842e^{-0.0367t} + 19.4689e^{-2.8495EE-12t}$	<0.0001	0.9641	7.3424
<i>June</i>	$Y = 80.6503e^{-0.1056t} + 25.0928e^{-0.0043t}$	<0.0001	0.9754	5.2408
<i>December</i>	$Y = 88.1369e^{-0.0166t} + 16.0402e^{-9.9901E-13t}$	<0.0001	0.9695	4.6932
C				
<i>March</i>	$Y = 83.8157e^{-0.0354t} + 19.4509e^{-0.0008t}$	<0.0001	0.9531	7.0082
<i>June</i>	$Y = 79.3461e^{-0.1061t} + 26.3596e^{-0.0045t}$	<0.0001	0.9744	5.3148
<i>December</i>	$Y = 93.8594e^{-0.0143t} + 10.5685e^{-6.9172E-12t}$	<0.0001	0.9704	4.6338
N				
<i>March</i>	$Y = 84.464e^{-0.0271t} + 18.6349e^{-9.7045E-14t}$	<0.0001	0.9668	5.5337
<i>June</i>	$Y = 81.7382e^{-0.0938t} + 21.7853e^{-0.0036t}$	<0.0001	0.9807	4.6019
<i>December</i>	$Y = 52.2190e^{-9.9700E-3t} + 52.2280e^{-9.9690E-3t}$	<0.0001	0.9596	5.0713

[†] Significance of fit.

[‡] Standard error of the estimate of Y on X.

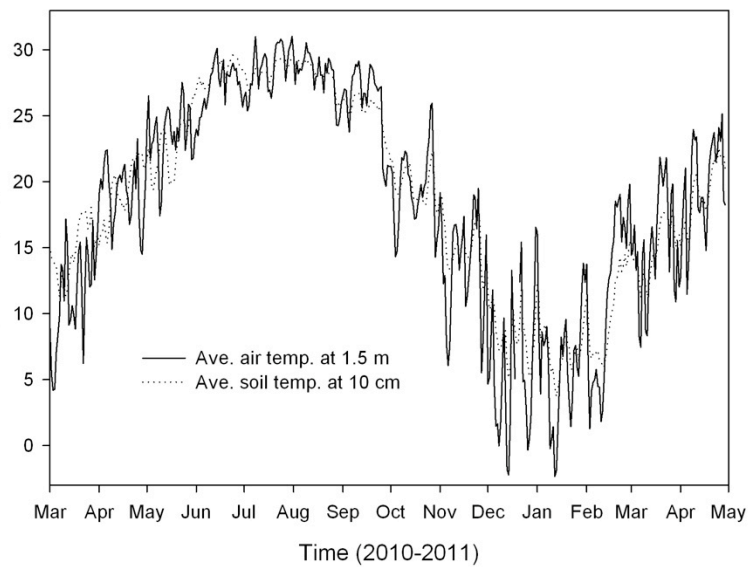


Figure 1. Average daily soil temperatures at 10 cm depth at the study site and average daily air temperature at 1.5 m near the Auburn, AL study site.

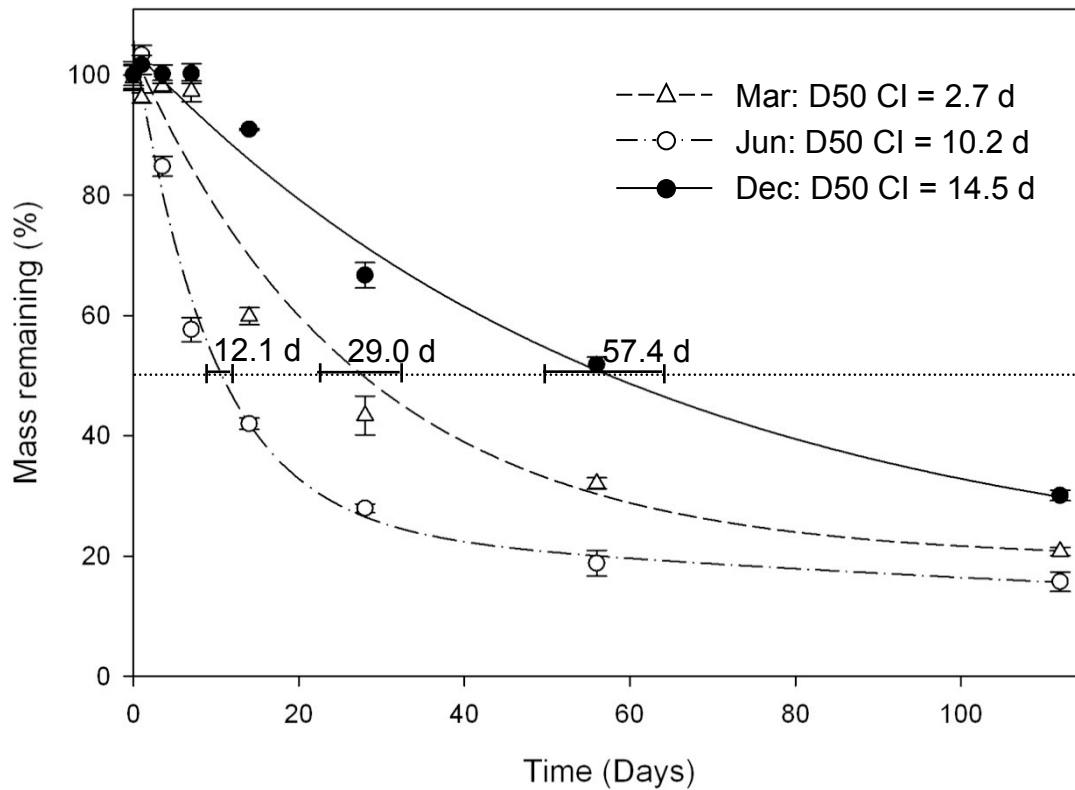


Figure 11. Percent mass remaining from surface incubated white clover residue. Shapes represent mean \pm 95% confidence intervals (CI's) . Residue persistence was normalized to 100% ash free dry weight of initial Day 0 applications. Days to 50% decomposition (D50) values are presented on the horizontal axis with adjusted 95% CI's as a means of comparing residue persistence across application date.

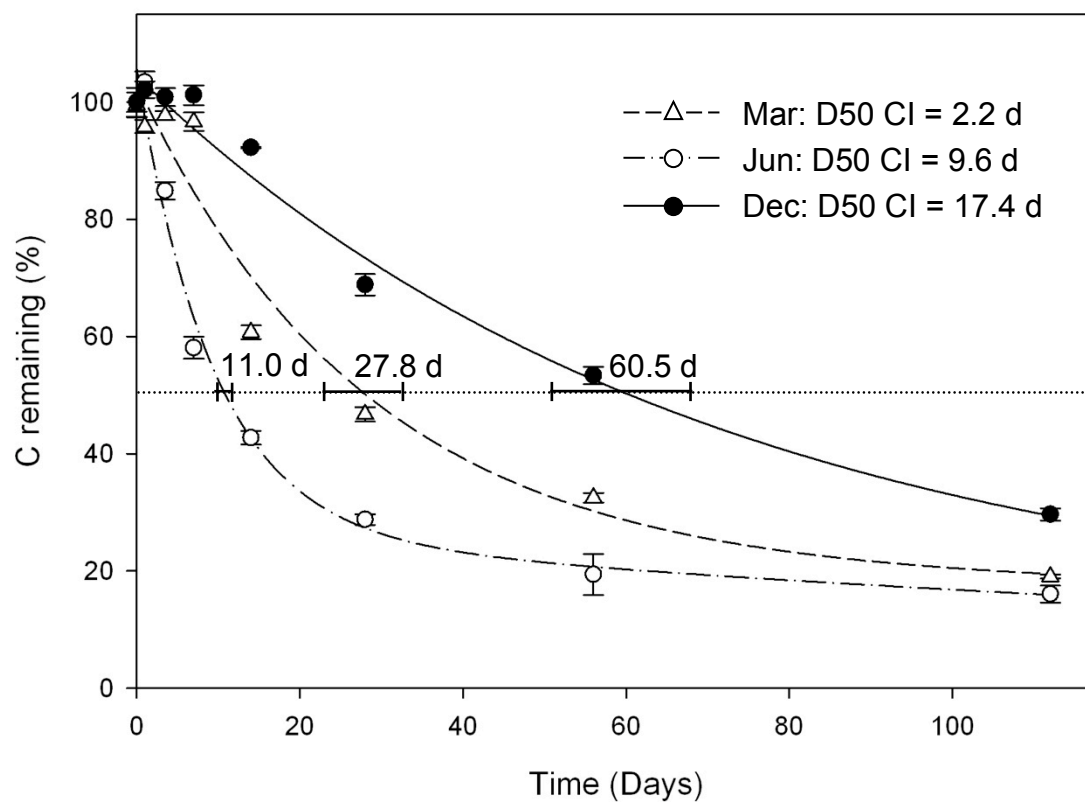


Figure 12. Percent carbon (C) remaining from surface incubated white clover residue. Shapes represent mean \pm 95% confidence intervals (CI's) . Residue persistence was normalized to 100% ash free dry weight of initial Day 0 applications. Days to 50% decomposition (D50) values are presented on the horizontal axis with adjusted 95% CI's as a means of comparing residue persistence across application date.

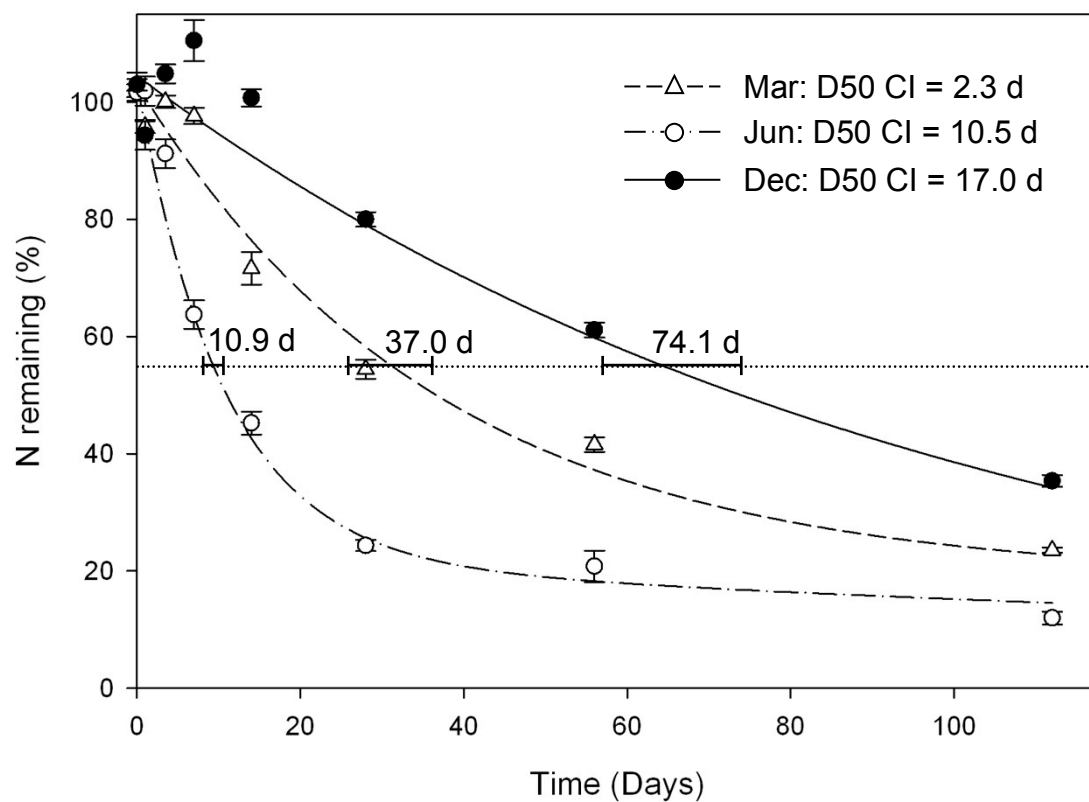


Figure 13. Percent nitrogen (N) remaining from surface incubated white clover residue. Shapes represent mean \pm 95% confidence intervals (CI's). Residue persistence was normalized to 100% ash free dry weight of initial Day 0 applications. Days to 50% decomposition (D50) values are presented on the horizontal axis with adjusted 95% CI's as a means of comparing residue persistence across application date.

Table 12. Persistence of white clover litter based upon predicted 95% confidence bands of double exponential decay equations (Table 11) regressed on time (days) for nitrogen (N), mass, and carbon (C) -remaining. Residue persistence was normalized to 100% ash free dry weight of initial day 0 applications.

Date [†]	Days [‡]	Mass		Carbon		Nitrogen		C/N
		% Remaining						Remaining
		Mean	± 95% CI	Mean	± 95% CI	Mean	± 95% CI	
1 Mar. 2010	0	103.8	6.7	103.3	5.0	103.1	4.6	10.4
8 Mar. 2010	7	84.7	3.8	84.8	4.4	88.5	2.9	10.0
15 Mar. 2010	14	69.9	5.6	70.3	4.8	76.4	4.4	9.6
29 Mar. 2010	28	49.6	5.5	50.1	5.5	54.2	4.7	9.6
26 Apr. 2010	56	30.3	7.0	30.1	6.8	37.2	5.4	8.4
21 Jun. 2010	112	20.9	7.7	19.4	7.0	22.7	5.6	8.9
1 Jun. 2010	0	105.7	3.1	105.7	3.5	103.5	2.1	9.4
8 Jun. 2010	7	62.9	3.6	63.3	3.6	63.6	3.1	9.1
15 Jun. 2010	14	42.0	3.3	42.7	3.3	42.7	3.0	9.2
29 Jun. 2010	28	26.4	4.0	27.3	4.3	25.6	3.7	9.8
27 Jul. 2010	56	19.9	4.3	20.7	4.4	18.2	4.2	10.4
21 Sep. 2010	112	15.5	6.1	15.9	6.1	14.6	5.3	10.0
1 Dec. 2010	0	104.2	4.9	104.4	7.0	104.4	3.7	10.5
8 Dec. 2010	7	94.5	3.9	95.5	4.0	97.4	3.5	10.3
15 Dec. 2010	14	85.9	6.4	87.4	5.2	90.8	4.1	10.1
29 Dec. 2010	28	71.4	4.1	73.5	4.8	79.0	4.1	9.8
26 Jan. 2010	56	50.8	4.4	52.7	5.6	59.8	4.4	9.3
23 Mar. 2010	112	29.8	4.9	29.5	4.7	34.2	5.1	9.1

[†] Date of litter retrieval from field conditions. Day 0 litter was truly replicated and placed within the field.

[‡] Days after initial application.

White Clover Inclusion within a Bermudagrass Lawn: Effects of Supplemental Nitrogen upon Botanical Composition and Nitrogen Cycling

INTRODUCTION

Total turfgrass cultivation within the contiguous United States occupies 163,800 km² ($\pm 35,850$ km²; Milesi et al., 2005), an area nearly the size of the state of Florida. Turfgrass is frequently criticized for its negative environmental effects, due in part to the use of agrichemicals and often-limited natural resources (Milesi et al., 2005; Robbins and Birkenholtz 2003; Robbins et al., 2001). However, there are many turfgrass scenarios, that when managed properly, can be a sustainable asset to modern communities. Benefits of turfgrass are well documented and include: erosion control, increased water infiltration, reduced nutrient leaching, aesthetics, and carbon sequestration (Beard and Green 1994).

Nitrogen (N) is essential to turfgrass health and quality (Beard 1973; Turgeon 2002), with commercial-lawn N requirements within the southern United States ranging from less than 5 g m⁻² year⁻¹ for bahiagrass (*Paspalum notatum* Fluegge) and centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.] to almost 30 g m⁻² year⁻¹ for hybrid bermudagrass [*Cynodon dactylon* \times *C. transvaalensis*; Duple 1996]. Improper N fertilization can lead to negative environmental effects. N loss from turfgrass may contribute to the eutrophication of aquatic and terrestrial ecosystems as well as lead to elevated nitrate (NO₃) levels in drinking water (Robbins and Birkenholtz 2003; Robbins et al., 2001). For these reasons, and due to the economic costs associated with labor and materials, reducing N application to certain turfgrass scenarios is desirable.

White clover (*Trifolium repens* L.) inclusion within turfgrass is a proposed means of increasing the sustainability of certain low maintenance turfgrass scenarios due to its ability to

biologically fix N and transfer it to associated grasses (Dudeck and Peacock, 1983; Sincik and Acikgoz, 2007; McCurdy et al., 2013). Little is known about white clover N contribution to maintained turfgrass swards. Sincik and Acikgoz (2007) reported that white clover fixed greater than $25 \text{ g N m}^{-2} \text{ year}^{-1}$ and contributed between 4.2 to 13.7% of that total N to three associated cool season turfgrass species, resulting in improved color ratings.

Beyond these estimates, most are limited to cool season pasture scenarios. Historically, grass plus clover mixtures have proven to be important pasture systems that supply high-quality grazing for animals while simultaneously improving soil fertility (Lampkin 2002). Estimates of N fixation for grass plus clover pastures are roughly $10 \text{ to } 25 \text{ g N m}^{-2} \text{ year}^{-1}$ (Ledgard and Steele 1992; McNeil and Wood 1990; Whitehead 1995). Using the ^{15}N transfer method, McNeil and Wood (1990) estimated white clover N fixation to be $15.5 \text{ g N m}^{-2} \text{ year}^{-1}$, with 28% having been transferred to associated ryegrass. N transfer occurs indirectly through excreted N and mineralization of nodules, roots, and foliage (Brophy et al., 1987; Dubach and Russelle 1994; Jensen 1996; Wardle and Greenfield 1991). Previous research indicates that root nodules are a significant N source in mixed swards; with root nodule N concentrations ranging from 4.8 to 9.0% of root dry matter (Chu and Robertson 1974; Wardle and Greenfield 1991). Decomposition of foliage is another means of N transfer, as above ground white clover dry matter has been reported to be 4.0 to 4.9% N (McCurdy et al., 2013; Sincik and Acikgoz 2007). White clover foliage is composed of a quickly decaying labile fraction; however, decay is highly dependent upon time of year, presumably due to soil temperature and microbial decay mechanisms (McCurdy et al., 2013).

Even in persistent stands of legumes, biological N fixation varies, largely due to the relative composition of turfgrass swards and soil N availability (Crush et al., 1982). Fixation is

highly dependent upon the relative level of nodulation occurring in root tissues and activity of the bacteria within. Most research indicates that high soil N concentrations inhibit nodule growth and development. Macduff et al. (1996) observed that the ratio of nodule to root dry-weights was 1:6 in white clover without NO₃ treatment but decreased with applications of NO₃. Similarly, it is well documented that increasing N fertilization decreases clover density and allows the grass portion of the sward to outcompete clover (Frame and Boyd 1987; Pederson 1995; Sincik and Acikgoz 2007).

Other factors affecting biological N fixation include absorption of photosynthetically active radiation, carbon assimilation rates, and allocation of photosynthate to roots (Lie, 1971). At low N levels, white clover leaves have a higher photosynthetic capacity than do competing perennial ryegrass; however, at higher N levels the opposite is true (Faurie et al., 1996). Increased light interception at low N levels can be attributed to a greater leaf area index in the upper canopy of the grass plus clover sward as well as clover's ability to avoid shade by increasing petiole length (Davies and Evans 1990; Faurie et al., 1996; Woledge et al., 1992). Another factor affecting N fixation is soil temperature. Frame and Newbould (1986) found that a minimum temperature of 9°C was necessary for active N fixation by *Rhizobium*. It has also been reported that temperatures required for nitrogenase activity range from 13 to 26°C (Halliday and Pate 1976).

Our research was prompted by the numerous knowledge gaps pertaining to white clover inclusion within warm-season turf scenarios. We sought to evaluate the effects of white clover inclusion within a maintained hybrid bermudagrass lawn. Our objectives were 1) quantify the effects of clover inclusion upon sward biomass, 2) evaluate the effects of long term supplemental

N upon clover establishment and sward composition, 3) estimate clover N fixation and N transfer to associated turfgrass.

MATERIALS AND METHODS

Study Design and Field Conditions

A 3-year study was conducted to evaluate the effects of white clover (*Trifolium repens* L.) inclusion within a hybrid bermudagrass (*Cynodon dactylon* × *C. transvaalensis*) lawn. The study was conducted at the Auburn University Turfgrass Research Unit, (32°34'40" N, 85°29'57" W) in Auburn, AL, on a Marvyn sandy loam (fine-loamy, Kaolinitic, thermic Typic Kanhapludult) soil with pH 6.3 (1:1 soil/H₂O). Treatments were arranged as randomized complete blocks (4 replicates). Treatment factors were clover inclusion and supplemental N rate. Plot size was 2 m². White clover [c.v. *Dutch White* (Main Street Seed and Supply, Bay City, MI)] was seed-established October 2009, 2010, and 2011 (1.5 g pure live seed m⁻²). Supplemental N (0, 0.5, 1, 2, 4, and 8 g N m⁻²) was applied monthly, April to August, during 2010, 2011, and 2012 as CaNO₃, with Ca applied to uniformity via CaSO₄. During 2011 and 2012, the area received 1 cm of supplemental overhead irrigation twice per week from March to September in order to insure adequate clover and bermudagrass growth. Air temperature at a 1.5 m height and soil temperature at a 10 cm depth were obtained from a nearby weather station (32°36'00" N, 85°30'00" W; elevation 199 m) in Auburn, AL (AWIS, 2013).

Clipping Biomass and Sampling

Trifoliate leaves were counted within three 730 cm² sub-samples per 2.0 m² experimental unit on 20 April 2010, 2011 and 2012 as a means of quantifying spring clover density (trifoliate leaves m⁻²). Plots were harvested and collected at a 2.5 cm mowing height 1 month after fertilization (May to September) via reel mower. Biomass was air-dried at 60°C for 1 wk and

was weighed for dry-matter determination. Botanical composition of mixed swards was determined by partitioning three, 3 g sub-samples into their constituent grass or clover parts. A 20 g sample of whole harvest biomass from each plot was ground to pass a 16-mesh sieve for N analysis by LECO TruSpec CN (Leco Corp, St. Joseph, MI, USA). Remaining biomass was returned to the turfgrass surface in order to mimic normal turfgrass mowing practices. However, due to processing constraints, biomass could not be returned immediately and was instead returned to respective experimental units one month later following the subsequent harvest or one wk after the last harvest of the year.

Nitrogen Fixation and Transfer

Nitrogen fixation was calculated using the difference method, by subtracting N-yield of grass-alone plots from the total N-yield of grass plus clover mixtures. Furthermore, the apparent N transfer was estimated as the difference between grass-alone N-yield of mixtures and that of grass-alone monocultures. Grass N-yield of mixtures was calculated based upon estimates of grass-clover composition, with error propagated throughout.

Statistical Analysis

Analysis of variance (ANOVA) was conducted. Means, standard errors, and statistical significance of treatments were determined at the 95% confidence level using mixed models procedures within Proc Glimmix (SAS Institute, Raleigh, NC, USA). Means were separated based upon adjusted 95% confidence intervals, which allowed for multiple comparisons by protecting family-wise error rate (Littell et al., 2006). Overlapping limits indicated lack of significant difference between responses. Least squares estimates for nonlinear models were determined within SigmaPlot 11 (Systat Software, Chicago, IL, USA) using the Marquardt-

Levenberg algorithm to provide the best fit. Initial parameter ranges were selected with a maximum of 200 fits and 200 iterations.

Data were fit to the quadratic model $y = ax^2 + bx + c$, where a is the quadratic coefficient, b is the linear coefficient, c is the estimated response at $0 \text{ g N m}^{-2} \text{ year}^{-1}$, and x is $\text{g N m}^{-2} \text{ year}^{-1}$. In most cases, quadratic models minimized residual sums of squares and produced comparatively lower residual mean squares, standard errors, and PRESS statistics as well as better coefficients of determination (R^2_{adj}) than linear models. For brevity, comparisons of quadratic and linear exponential models are omitted. However, instances where quadratic models could be collapsed into linear models are generally indicated by the presence of a values near zero.

RESULTS AND DISCUSSION

Clover Establishment

Spring clover densities decreased as the study progressed, with $875 > 605 > 451$ trifoliate leaves m^{-2} for 2010, 2011, and 2012, respectively. This apparent trend may be one of chance or may be due to the effects of long term clover cultivation. Previous research indicates that clover persistence varies greatly due to soil conditions. In their review of N fixation of grass-legume pastures, Ledgard and Steele (1992) report that fixation was reduced due to dry soil conditions, acid soils, and the “pest/disease complex.” October seeding rates were equal throughout the study; however, in simultaneous research at a similar location, we also noticed declining clover establishment during this study. These effects were likely due to delayed bermudagrass dormancy during years two and three. Similarly, a delayed spring in 2011 likely had similar effects (Figure 14). When regressed across supplemental N rates applied during the previous

season, 2011 and 2012 clover establishment did not differ. This implies that seeded clover establishment was not suppressed by prior supplemental N applications.

Clipping Biomass

During 2010, grass biomass ($80.3 \text{ g m}^{-2} \text{ year}^{-1}$) was smaller than that of 2011 or 2012 (484 and $526 \text{ g m}^{-2} \text{ year}^{-1}$, respectively; Table 13). Similarly, 2010 grass plus clover biomass ($105 \text{ g m}^{-2} \text{ year}^{-1}$) was smaller than that of 2011 ($690 \text{ g m}^{-2} \text{ year}^{-1}$) and 2012 ($791 \text{ g m}^{-2} \text{ year}^{-1}$), with 2012 biomass having been the largest of the three years. First year biomass was much smaller than that of subsequent years due to a lack of supplemental water prior to, and during, the 5-month harvest period. Supplemental irrigation was applied twice weekly during the following 2011 and 2012 seasons in order to produce adequate harvests to determine botanical composition.

Within years, biomass differed due to date by supplemental N rate interactions (data not shown). However, for simplicity, we present annual effects of supplemental N by sward type (grass alone or grass plus clover). Sward types responded differently to supplemental N (Figure 15). Generally, grass plus clover biomass was greater than that of grass alone plots; however, 2011 and 2012 grass plus clover biomass were equal to that of grass alone swards at the highest supplemental N rate (Figure 15). This trend was consistent across date of harvest (Data not shown).

Biomass data for both grass and mixed swards was regressed with supplemental N levels using a quadratic model (Table 14). The quadratic model, $y = ax^2 + bx + c$, provides an estimate of sward biomass when no supplemental N is applied (c) and estimates the response due to increasing supplemental N (b). Unlike a simple linear model, the quadratic model's quadratic coefficient (a) may provide modest insight into the diminishing returns due to increasing N

levels by way of its parabolic shape. The x -coordinate of the parabola's vertex is predicted by the equation $x = -b / 2a$, which estimates the theoretical N level at which maximum biomass may be realized. In cases where the parabola is upward opening, c values are positive. In contrast, where the parabola is downward opening, c values are negative, indicating a plateau effect or deleterious effect of N application beyond a certain point. We present means corresponding to treatment effects as well as theoretical N levels corresponding to maximum biomass.

2010 Biomass

The quadratic response of 2010 biomass to increasing N levels is less pronounced than other years, presumably due to the aforementioned lack of supplemental irrigation during the first season of the study. When 0 to 20 g N m⁻² year⁻¹ was applied, grass biomass was ≤ 85 g m⁻² year⁻¹ (Figure 15; Table 13). Grass biomass was greater (137 g m⁻² year⁻¹) when 40 g N m⁻² year⁻¹ was applied. A plateau effect was not evident within the quadratic model of grass-alone biomass, as the upward opening parabola implies that N, even at the highest supplemental rate, may have been limiting to bermudagrass growth (Figure 15). In this instance, the relatively low quadratic coefficient (Table 14) indicates that a simple linear model would have been adequate. Grass plus clover biomass generally increased with increasing N levels. However, grass plus clover biomass appeared to plateau (169 g m⁻² year⁻¹) at the 20 to 40 g N m⁻² year⁻¹ level, which was confirmed by the theoretical estimate 33.9 g N m⁻² year⁻¹ provided by the quadratic model.

2011 Biomass

Grass alone biomass increased from 281 to 948 g m⁻² year⁻¹ with supplemental N (Table 13; Figure 15). The quadratic model estimates a maximum biomass at the 64 g N m⁻² year⁻¹ rate (Table 14). Grass plus clover biomass was larger than grass alone biomass, presumably due to mixed sward composition and fixed N. Grass plus clover biomass increased from 458 to 965 g

$\text{m}^{-2} \text{ year}^{-1}$, with the highest biomass due to the $40 \text{ g N m}^{-2} \text{ year}^{-1}$ rate; although this level was equaled by that of the $20 \text{ g N m}^{-2} \text{ year}^{-1}$ rate ($864 \text{ g m}^{-2} \text{ year}^{-1}$). Similarly, the quadratic response indicates a theoretical maximum biomass at $35 \text{ g N m}^{-2} \text{ year}^{-1}$.

2012 Biomass

Grass alone biomass increased from 271 to $964 \text{ g m}^{-2} \text{ year}^{-1}$ with increasing supplemental N (Figure 15; Table 13). The quadratic model estimates a maximum biomass at $48 \text{ g N m}^{-2} \text{ year}^{-1}$ (Table 14). Grass plus clover biomass was again larger than that of grass alone swards, increasing from 572 to $990 \text{ g m}^{-2} \text{ year}^{-1}$. The quadratic response indicates a maximum biomass at $33 \text{ g N m}^{-2} \text{ year}^{-1}$.

Biomass Composition

The composition of grass plus clover swards was quantified throughout 2011 and 2012 seasons by subsampling total clippings. Clover biomass of 2011 ($180 \text{ g m}^{-2} \text{ year}^{-1}$) and 2012 studies ($231 \text{ g m}^{-2} \text{ year}^{-1}$) accounted for 23 and 29% of 2011 and 2012 biomass, respectively (Table 13). Contrary to previous reports, clover biomass was not seriously affected by increasing N rate. Only 2011 clover biomass was affected by supplemental N rate, with the highest rate of supplemental N having reduced clover biomass relative to the $20 \text{ g N m}^{-2} \text{ year}^{-1}$ rate. Furthermore, grass biomass of grass plus clover swards (data not shown) was greater than that of grass-alone plots at supplemental N rates $\leq 10 \text{ g N m}^{-2} \text{ year}^{-1}$. However, grass of mixed swards was reduced relative to grass alone plots at 20 and $40 \text{ g N m}^{-2} \text{ year}^{-1}$ rates.

Nitrogen Fixation

Similar to Elgersma et al. (1998), white clover-derived N was calculated using the difference method, by subtracting N-yield of grass-alone plots from the total N-yield of grass plus clover mixtures. During the 3-year study, N fixation was estimated to be $6.6 \text{ g m}^{-2} \text{ year}^{-1}$

regardless of supplemental N rate. However, N fixation differed due to study years (0.9, 8.0, 10.9 g N m⁻² year⁻¹ for 2010, 2011, and 2012, respectively), with an apparent increase in fixation as years progressed (Table 15). This observation is most likely due to the relatively slow soil organic matter degradation and lagging N availability between years (i.e. clover residue mineralized N at a pace detected only by multiple sampling years). We have previously reported that, when applied at 500 g fresh weight m⁻² (equivalent to approximately 75 to 100 g dry weight m⁻²), more than half of available N was mineralized between 10 and 73 days after application, depending upon application timing (McCurdy et al., 2013). However, in the present study, effective clover biomass accumulation exceeded the dry weight equivalents of our previous work and suggests that some residue remains within the system acting as a long term N contributor.

The authors acknowledge several limitations pertaining to the N difference method. A basic assumption of the N difference method is that litter mineralization and subsequent N immobilization are the same for all treatment scenarios (Hauck and Bremner, 1976). However, due to the “priming effect,” it is often reported that fertilized plots (whether through biological fixation or applied N) have increased N availability beyond the levels of that applied (Rao et al., 1992). This may be due to a number of factors, including: increased microbial activity (Westerman and Kurtz, 1973), acid hydrolysis of soil organic matter (Turchin, 1964), and increased root growth in fertilized plots (Olson and Swallow, 1984) possibly increasing nutrient access. In instances where such faults cannot be accounted for, the difference method may significantly over estimate apparent N fixation.

On the contrary, the difference method may also underestimate apparent N fixation, in part due to unaccounted loss through volatility and leaching. The method may also underestimate N fixation when soil N is sufficient to meet bermudagrass needs without additional fertilizer

(Varvel and Peterson, 1990). During preparations for this study, plots were maintained at considerably lower N levels than recommended for actively growing bermudagrass, yet turfgrass cover was at no time diminished in 0 g N m⁻² year⁻¹ treated plots. This may indicate adequate soil N and mineralizable N sources within the turfgrass canopy that, in this instance, led to an underestimate of N fixation within clover-included plots. The method also assumes that bermudagrass and white clover take up soil N at the same rate, which is unlikely, as bermudagrass is considered an almost voracious N consumer while white clover most often abounds without supplemental N.

2010 N Fixation

During 2010, N fixation was 0.9 g m⁻² year⁻¹ (Table 15). N fixation was generally suppressed at the high and low extremes of supplemental N rate (Figure 15), which is evidenced by estimates of maximum N fixation at the supplemental N rate of 24 g N m⁻² year⁻¹ (Table 14). N fixation generally decreased as the summer progressed (data not shown), presumably because early season harvests measured N fixation that had occurred prior to the five month long harvest season. Another explanation may be decreased clover populations and increased competitiveness of bermudagrass as the harvest season progressed.

2011 N Fixation

During 2011, N fixation was 8.0 g m⁻² year⁻¹, which was significantly larger than that of 2010 (Table 15). However, like 2010, N fixation was generally suppressed at the high and low extremes of supplemental N rate (Figure 15). This is again evidenced by estimates of maximum N fixation at the supplemental N rate of 17 g N m⁻² year⁻¹ (Table 14). Like that of the previous season, N fixation waned towards the latter summer months; although, May N fixation was equivalent to that of August and September (data not shown).

2012 N Fixation

During 2012, N fixation was $10.9 \text{ g m}^{-2} \text{ year}^{-1}$, which was significantly larger than that of previous years (Table 15). Like that of previous years, N fixation was suppressed at high supplemental N rates (Figure 15), evidenced by a regression model decreasing across application rates and a maximum estimate of N fixation at the supplemental N rate of $-10.0 \text{ g N m}^{-2} \text{ year}^{-1}$ (Table 14). Like that of previous seasons, N fixation waned towards the latter summer months.

N Transfer

Nitrogen transfer from white clover to associated bermudagrass was estimated during 2011 and 2012 harvest periods (Table 15). During the two-year period, N transfer was $2.3 \text{ g m}^{-2} \text{ year}^{-1}$ regardless of supplemental N rate, which amounted to 24% of N fixed during those two harvest years. Our estimate appears to be similar to that reported by McNeil and Wood (1990) who estimated 28% N transfer from white clover to associated perennial ryegrass. However, it is slightly higher than the estimated 4.2 to 13.7% N transfer from white clover to three cool season turfgrasses reported by Sincik and Acikgoz (2007). As with fixation estimates, we admit that our results may overestimate N transfer due to a lag between early season N fixation before bermudagrass green-up. Simultaneously, the complex nature of N availability from fixed N in the relatively cool soil temperatures is poorly understood. We have previously reported slower N availability from winter and spring applied clover biomass relative to that of summer-applied biomass (McCurdy et al., 2013).

2011 Nitrogen Transfer

Nitrogen transfer to associated bermudagrass was $3.9 \text{ g m}^{-2} \text{ year}^{-1}$ during 2011, which is equivalent to 49% of fixed N (Table 15). The quadratic model of 2011 N transfer was upward opening (Figure 15), seemingly due to the somewhat lower N transfer estimate of the 20 g N m^{-2}

year⁻¹ rate (Table 15). Despite this phenomenon, we propose that N transfer was less affected by increasing N rate than N fixation during 2012. This is evidenced by the nearly overlapping N fixation and transfer estimates at the highest level of supplemental N during 2011. N transfer did, however, differ due to supplemental N rate within harvest months (Table 15). Yet unlike N fixation, N transfer generally did not decrease throughout the harvest period (data not shown). This may be due to a priming effect from supplemental N and the resulting N mineralization from accumulated biomass, as well as the delayed release of fixed N from organic matter within the gradually warming soils.

2012 Nitrogen Transfer

Nitrogen transfer to associated bermudagrass was only 0.6 g m⁻² year⁻¹ during 2012, which is equivalent to 6% of fixed N (Table 15). However this estimate was disproportionately affected by a single highly negative estimate (-10.3 g m⁻² year⁻¹) at the 40 g N m⁻² year⁻¹ rate. It is not clear how such a negative estimate can exist. If this estimate was removed, the average transfer amounted to more than 25%. In spite of this, N transfer appears to have been suppressed at higher supplemental N rates (Figure 15). This was further evidenced by the quadratic model, which estimates a maximum N transfer at a supplemental N rate of 1.25 g N m⁻² year⁻¹ (Table 14).

CONCLUSIONS

Year one results were severely impacted by the lack of supplemental irrigation, which suggests the utility of white clover inclusion as a means of sustaining low maintenance turfgrass may be limited in drier climates. However, our results demonstrate that white clover inclusion is a viable option for sustainably supplementing the N requirements of warm-season grass swards. Grass plus clover swards yielded higher clipping biomass than grass-alone swards during

irrigated study years, which was evidence of enhanced bermudagrass growth due to biological N fixation. Likewise, grass biomass of mixed swards was increased relative to that of grass-alone plots at supplemental N rates $\leq 10 \text{ g N m}^{-2} \text{ year}^{-1}$. N fixation was estimated to be $6.6 \text{ g N m}^{-2} \text{ year}^{-1}$ during the 3-year study, with an apparent increase in fixation as years progressed. This estimate is considerably lower than the roughly 10 to $25 \text{ g N m}^{-2} \text{ year}^{-1}$ reported in various cool season scenarios (Ledgard and Steele 1992; McNeil and Wood 1990; Whitehead 1995). N transfer to the associated bermudagrass sward was estimated to be 24% across the latter two years of the study, which is comparable to previous estimates within cool-season pastures and larger than those reported within cool-season turfgrass by Sincik and Acikgoz (2007).

Unlike previous research, our results indicate that N fixation was suppressed at low supplemental N rates. These results may indicate the N demands of clover establishment and that small amounts of supplemental fertility are needed to maximize N fixation. However, it is also possible that supplemental N increased soil organic matter decay and N uptake by associated bermudagrass, effectively biasing estimates of N fixation within fertilized plots. This is again a liability associated with the N difference method. Upper extremes of supplemental N were also deleterious to N fixation, which agrees with previous indications of clover decline in the presence of high soil N levels.

Our research was limited to white clover inclusion; however, other legumes should be evaluated for their utility within warm-season turfgrasses. Multiple *Trifolium* species are common amongst pasture and turfgrass scenarios of the southeastern U.S. Alternatives include: *T. incarnatum*, *T. dubium*, *T. nigrescens*, *T. campestre*, and *T. aureum*. Likewise, warm-season legumes, such as *Kummerowia* and *Arachis*, may provide more timely N release to associated warm-season turfgrasses.

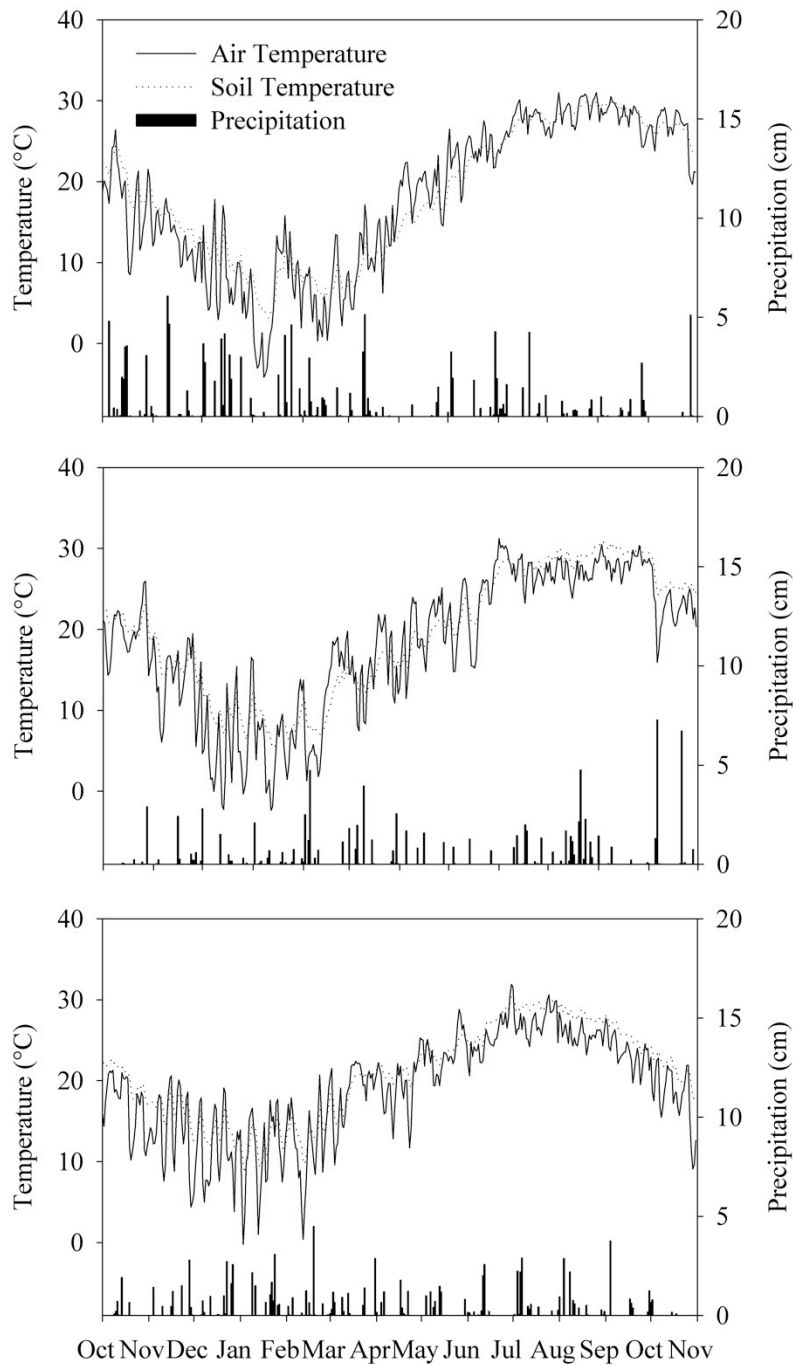


Figure 14. Average daily air and soil temperature as well as average daily precipitation for the 2009-2010, 2010-2011, and 2011-2012 bermudagrass-white clover growing seasons.

Table 13. Grass alone and grass plus white clover biomass, as well as clover portion of the mixed sward, \pm 95% confidence interval (CI) as affected by supplemental N. Means \pm CI are presented to allow treatment separation among similar response variables.

Supplemental N [†] g m ⁻² year ⁻¹		Total Biomass (g m ⁻² year ⁻¹)						Clover portion (% Total Biomass)			
		2010		2011		2012		2011		2012	
		Mean	± 95% CI	Mean	± 95% CI	Mean	± 95% CI	Mean	± 95% CI	Mean	± 95% CI
Grass plus Clover	0.0	49 c	20	458 e	48	572 e	56	23 ab	6	31 a	9
	2.5	72 bc	20	710 c	53	723 cd	56	25 ab	6	35 a	9
	5.0	78 bc	20	587 d	53	682 de	58	24 ab	6	36 a	9
	10.0	103 b	20	774 bc	52	836 bc	56	25 ab	6	28 a	9
	20.0	169 a	20	864 ab	54	927 ab	56	29 a	6	25 a	9
	40.0	169 a	20	965 a	52	990 a	56	13 b	6	19 a	9
Yearly Total		105 C [‡]	53	690 B	39	791 A	38	23 <i>B</i>	2	29 <i>A</i>	2
Grass alone	0.0	60 b	23	281 d	48	271 e	56				
	2.5	66 b	23	309 d	48	291 de	56				
	5.0	56 b	23	347 d	48	364 d	58				
	10.0	85 b	23	473 c	48	503 c	56				
	20.0	78 b	23	739 b	53	752 b	56				
	40.0	137 a	23	948 a	51	964 a	56				
Yearly Total		80 B	65	484 A	46	526 A	38				

[†] Supplemental N was applied during five consecutive months (April to August).

[‡] Yearly totals are comparable amongst years, respective to response variables.

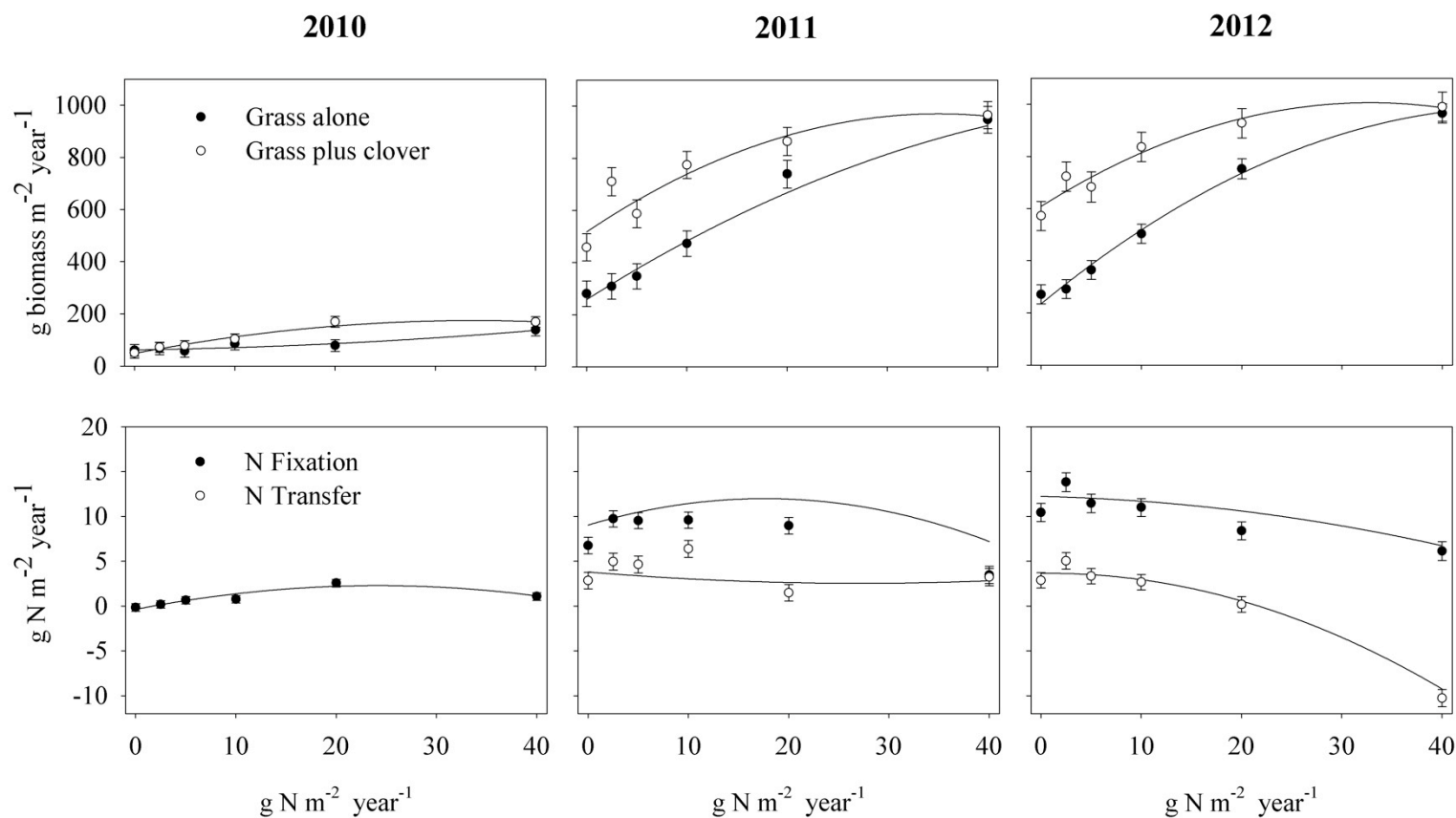


Figure 15. Grass alone and grass plus clover biomass, as well as estimated biological N fixation and N transfer to associated grasses, regressed upon yearly supplemental N levels. Transfer was not calculated in 2010. Data were fit to the quadratic model $y = a + bx + cx^2$, where a is the estimated response at 0 g N m⁻² year⁻¹, b is the linear coefficient, c is the quadratic coefficient, and x is g N m⁻² year⁻¹ applied over five months of active bermudagrass growth from April to August as CaNO₃. Means ± 95% confidence intervals are presented to allow treatment separation among similar response variables.

Table 14. Grass alone and grass plus clover biomass, as well as estimated biological N fixation and N transfer to associated grasses, regressed upon yearly supplemental N levels. Transfer was not calculated in 2010. Data were fit to the quadratic model $y = a + bx + cx^2$, where a is the estimated response at 0 g N m⁻² year⁻¹, b is the linear coefficient, c is the quadratic coefficient, and x is g N m⁻² year⁻¹ applied over five months of active bermudagrass growth from April to August as CaNO₃.

	Response	Equation	P > F[†]	R²_{adj}	Syx[‡]	Maximum response[§]
2010	G biomass	$61.65 + 0.54x + 0.03x^2$	< 0.0001	0.1993	51.17	-9.0 [¶]
	G + C biomass	$47.76 + 7.45x - 0.110x^2$	< 0.0001	0.3331	62.15	33.9
	N fixation	$-0.37 + 0.22x - 0.045x^2$	< 0.0001	0.1394	2.06	24.4
2011	G biomass	$257.84 + 24.34x - 0.191x^2$	< 0.0001	0.3040	338.73	63.7
	G + C biomass	$517.22 + 25.84x - 0.369x^2$	< 0.0001	0.1577	363.43	35.0
	N fixation	$9.03 + 0.34x - 0.010x^2$	< 0.0001	0.0278	9.11	17.0
	N transfer	$3.80 + 0.09x + 0.002x^2$	0.0498	0.0019	7.88	-22.5
2012	G biomass	$234.51 + 31.67x - 0.333x^2$	< 0.0001	0.3676	333.21	47.6
	G + C biomass	$607.99 + 24.25x - 0.370x^2$	< 0.0001	0.2129	265.29	32.8
	N fixation	$8.72 - 0.06x - 0.003x^2$	< 0.0001	0.0819	8.02	-10.0
	N transfer	$3.64 + 0.02 - 0.008x^2$	< 0.0001	0.2520	7.72	1.25

[†] Significance of regression fit.

[‡] Standard error of the estimate of Y on X.

[§] N rate (g m⁻² year⁻¹) to obtain theoretical maximum response.

[¶] Negative numbers represent a minimum response due to an upward facing parabola.

Table 15. Nitrogen Fixation and Nitrogen Transfer \pm 95% confidence interval (CI) as affected by supplemental N. Means \pm CI are presented to allow treatment separation among similar response variables.

Supplemental N \dagger g m ⁻² year ⁻¹	Nitrogen Fixation (g m ⁻² year ⁻¹)						Nitrogen Transfer (g m ⁻² year ⁻¹)			
	2010		2011		2012		2011		2012	
	Mean	\pm 95% CI	Mean	\pm 95% CI	Mean	\pm 95% CI	Mean	\pm 95% CI	Mean	\pm 95% CI
0.0	-0.2 d	0.4	6.7 b	0.1	10.4 b	1.0	2.8 cd	0.9	2.9 b	0.9
2.5	0.2 cd	0.4	9.7 a	0.1	13.8 a	1.1	5.0 ab	0.9	5.0 a	0.9
5.0	0.7 bcd	0.4	9.5 a	0.1	11.5 b	1.0	4.6 abc	0.9	3.3 ab	0.9
10.0	0.8 bc	0.4	9.6 a	0.2	11.0 b	1.0	6.4 a	0.9	2.7 b	0.9
20.0	2.6 a	0.4	9.0 a	0.1	8.4 c	1.0	1.5 d	0.9	0.2 c	0.9
40.0	1.1 b	0.4	3.5 c	0.2	6.1 d	1.0	3.2 bcd	1.0	-10.3 d	1.0
Yearly Total	0.9 C [‡]	0.7	8.0 B	0.4	10.9 A	0.4	3.9 A	0.3	0.6 B	0.3

[†] Supplemental N was applied during five consecutive months (April to August).

[‡] Yearly totals are comparable among years, respective to response variable.

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Appendices

Appendix 1:
Effects of Nitrogen and *Rhizobium* Inoculation on White Clover Characteristics and Control

INTRODUCTION

Understanding the effects of inoculation and nitrogen fertilization upon clover morphology may lead to a more integrated approach of managing clover within maintained turfgrass swards. Most research indicates that high concentrations of soil N inhibit nodule growth and development within leguminous plants. Macduff et al. (1996) observed that the ratio of root to nodule dry-weights was 6:1 in white clover (*Trifolium repens* L.) without NO₃ treatment, but increased with applications of NO₃. It is well documented that increasing N fertilization decreases clover density and allows the grass portion of the sward to out compete clover (Frame and Boyd 1987; Pederson 1995; Sincik and Acikgoz 2007). However, little is known about how this interference manifests itself. Still in question are effects of fertilization on clover not in competition with grass. Additionally, little is known about effects of seed inoculation in native soils and whether an interaction is present between inoculation and nitrogen application.

MATERIALS AND METHODS

A study was conducted at Auburn University, Auburn, AL (32°35'N, 85°29'W, elevation 198 m), in an environmentally controlled greenhouse. Experiments were conducted as a completely random design with a two-by-six factorial treatment arrangement. Factorial levels were seed treatment (inoculated or not) by N-rate (0, 1.8, 3.6, 7.8, 14.4, 28.8 g N m⁻²) applied as CaNO₃. Experiments were initiated 5 April, 7

June, and 9 August of 2010. Temperatures were monitored and maintained between 25 and 32°C.

Fungicide-treated seeds of Dutch white clover (*T. repens*; Main Street Seed and Supply Co., Bay City, MI) were inoculated with N-Dure (INTX Microbials, LLC, Kentland, IN) which contains the clover specific inoculant *Rhizobium leguminosarum biovar trifolii*. Inoculant was applied dry directly to seeds according to specimen label. Inoculated seeds (approximately 25) were sown into 90 cm² plastic pots containing a Wickham sandy loam soil (fine-loamy, siliceous, subactive, thermic Typic Hapludult). It was the goal of the researchers to choose a soil from the Auburn area that represented a new- or newly renovated- turf site that had previously been maintained as a monoculture. The soil for this study was excavated at 5 to 20 cm depth from a centipede grass (*Eremochloa ophiuroides* (Munro) Hack.) site that had been fumigated with methyl bromide three years prior and had no recent (within three years) history of legume growth. Soil was mixed thoroughly and was screened through a 4.75 mm sieve to remove grass roots.

One month after germination, clover seedlings were thinned to five seeds per pot and were fertilized with CaNO₃ at six different N rates: 0, 0.6, 1.2, 2.4, 4.8, 9.6 g N m⁻². All pots were fertilized with a modified 6x, N free, Hoagland's solution, including minors, to ensure that there would be no nutrient deficiencies. Beginning two weeks after initial fertilization, plants were mown with a rotary mower at 5.1 cm mowing height. Mowing continued on a bi-weekly basis at the same height until two weeks before final harvest. Plants received overhead mist irrigation daily and supplemental irrigation when

needed. Greenhouse temperatures were monitored and maintained between 25°C and 35°C.

Plants were fertilized monthly for three months. One month after final fertilizer treatment, foliar growth was harvested at soil level, and roots were gently shaken free of soil. Soil of individual pots was dried, sieved at 2 mm particle size, and analyzed for total Carbon (C) and N by LECO (?). Roots were washed free of excess soil and patted dry with paper towels. Only foliar- fresh weights were recorded, as root samples were placed within sealed plastic bags and were frozen for later analysis. Plant foliage was dried in a plant press, and leaf area of pressed and dried foliage was determined using a Licor 3100C leaf area meter (LICOR BioSciences, Lincoln, NE). Foliar-dry weight, total number of trifoliate leaves, and the length of three randomly sub-sampled petioles of individual pots were recorded. Upon thawing, root nodules were removed, counted, and weighed. After removing nodules, root-alone-fresh weight was recorded. Total root weights were the sum of nodule- and root-alone- fresh weight. Data were analyzed using PROC Mixed within SAS. Data were normally distributed. Differences were determined by “Type 3 Tests of Fixed Effects,” with *p*-value less than 0.05 indicating a significant effect.

RESULTS AND DISCUSSION

Neither inoculation by N-rate interaction or inoculation main effect was observed. Only root DW differed due to N-rate, increasing from 250 to nearly 500 mg/pot as rate increased (Figure A1). Foliar DW as well as petiole length, leaf -area, -count, and -size were unaffected by N-rate. Percent C and N of roots, nodules, and foliage were similar to those reported within previous literature.

Future research should quantify soil born Rhizobia within common lawn soils and determine if these numbers are adequate for legume nodulation *in situ*. Our study, however, is reasonable evidence to conclude that clover-seed inoculation may not be necessary upon the planting of new or over-seeded lawns within the immediate geographical region. We feel that the size and length of this sole experiment may limit its conclusiveness; however, it appears that N-rate has a limited effect upon acute clover phenology, including many important leaf characteristics. These and other studies like them will enable more appropriate agronomic decisions for managing clover with maintained lawn scenarios.

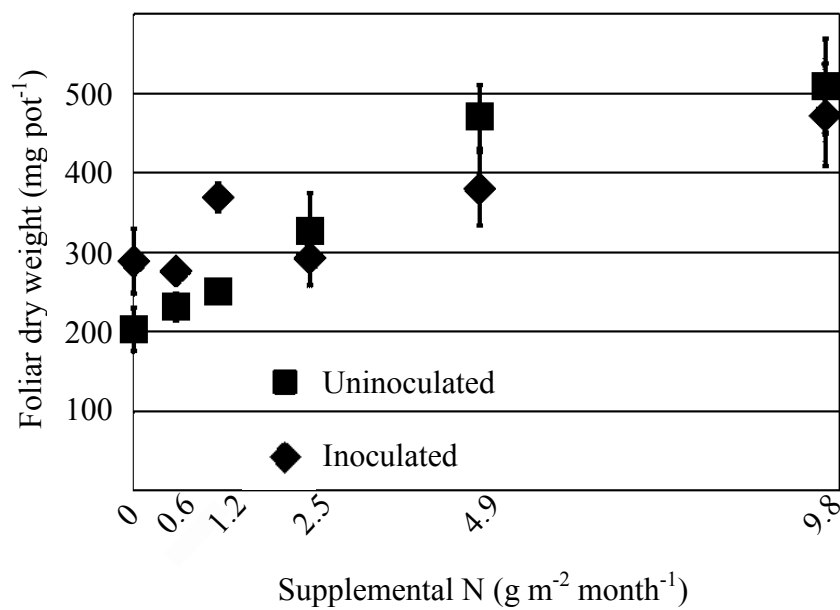


Figure A1. Root dry weight response to supplemental N applied as CaNO_3 . Effects of inoculation are displayed, but they are insignificant ($P > 0.05$).