

Floral Enrichment of Turf Lawns to Benefit Pollinating Insects

A Thesis
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
MASTER OF SCIENCE IN ENTOMOLOGY

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May 2016

Acknowledgements

I would first like to acknowledge the researchers and assistants of both the bee and turf labs, without whose patient support would have made this work impossible. Similarly the guidance of many plant restoration professionals from both public and private institutions was critical to the formation and implementation of this work, and for which I am especially grateful. Lastly I would like to acknowledge the friends, family, and mentors who have contributed to my intellectual and personal growth over the last decade. Their time and efforts have formed me into the person I am, and to them I owe everything.

Dedication

I dedicate this work to those reading who seek knowledge to become better stewards of the land and the animals in it.

Abstract

Turf lawns are a common landscape modification in many anthropogenic habitats, and often the largest contributor to “green space” in urban landscapes. Despite the ubiquitous nature of lawns their function is largely subjective, based on the aesthetic values of its owner. This flexibility offers unique opportunities to develop cultural practices and planting strategies to meet alternative goals that land managers may have, such as habitat enhancement for pollinating insects. Within this thesis I explore a range of establishment and management techniques of various turf and forb species, with the goal of providing recommendations to lawn managers for maximizing bloom of bee friendly flowers. The results from these studies are a significant first step in the creation and management of flowering lawns, and will provide a basis for future work as the value of flowering lawns to bees continues to grow.

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Chapter 1

Thesis introduction

Bees and their Importance

Anthophila is the clade of insects commonly known as bees (Michener et al. 1994). Bees are a highly diverse group comprised of nine families and an estimated 20,000 species worldwide. In the last published survey Minnesota was home to 325 bee species (Wolf and Ascher 2008), but this is likely an underestimate. Recent review and databasing of the University of Minnesota insect collection has revealed at least 400 distinct bee species collected in the state over the last 100 years (unpublished data). While bees as a clade have diverse forms and functions, they are most commonly known for their ecologically and economically important role in facilitating plant pollination.

It is estimated that 78% of plant species in temperate areas and up to 94% of plant species in tropical areas depend on animals for pollen transfer (Ollerton et al. 2011).

While a wide diversity of taxa play a role in pollen transport, bees are acknowledged as being one of the most frequent in their floral visitation, and diverse in their floral relationships (Willmer 2011). The importance of bees to pollination is derived from their complete reliance on floral products for all stages of their development. Up to 35% of world crop production receives at least some benefit from pollination (Klein et al. 2007), and this service is estimated to be worth up to \$173 billion worldwide annually (Gallai et al. 2009).

The economic benefit of providing honey bee colonies (*Apis mellifera*) for pollination to crops has been well documented, and the value of this service alone is

estimated to be between \$1.5-\$5 billion (Southwick and Southwick 1992). While honey bees provide yield benefits to crop production, there is increasing evidence that native bees (indigenous and unmanaged) contribute greatly to these pollination services as well. The recent valuation of services provided exclusively by native bees has been valued at approximately \$3 billion (Losey and Vaughan 2006). One recent study revealed that even when honey bees are at their highest visitation rate in agricultural fields, increasing visitation by native bees continued to increase crop yields (Garibaldi et al. 2013). This study is especially important as it suggests that honey bees alone do not achieve maximum pollination but are supplementary to native pollinators. In fact, there is good evidence that the best pollination outcomes occur when both honey bees and native bees are present (Chagnon et al. 1993, Greenleaf and Kremen 2006, Brittain, Williams, et al. 2013, Garibaldi et al. 2014, Rogers et al. 2014).

While the benefit of having high abundances of both honey bees and native bees for crop production seems clear, the benefit of native bee species diversity to agriculture is still not fully understood. Some studies have found positive relationships between bee community richness and fruit set for agricultural crops (Klein et al. 2003, Mallinger and Gratton 2015), but it has been argued recently that it is the abundance of common visitors that drives crop pollination (Kleijn et al. 2015, Winfree et al. 2015). What seems most likely is that pollination services are driven by a combination of the two, with bee species richness providing benefits when there is complementarity between species visiting the same floral host, such as differential foraging strategies (Hoehn et al. 2008, Frund et al. 2013). In addition to floral interactions, there is building evidence that bee community

richness buffers pollination through differential responses to environmental factors (Winfree and Kremen 2009), such as weather related factors (Brittain, Kremen, et al. 2013, Rogers et al. 2014), and in the flowering phenology shifts caused by climate change (Bartomeus, Park, et al. 2013).

While pollination is most commonly studied in the context of benefits to agriculture, it is also very important to natural systems as well. The persistence of plant communities relies in large part on seed production (Grubb 1977) which, for flowering plants, often requires adequate pollination. The prevalence and effect of pollen limitation on seed set in plant communities has been explored in-depth, often with complicated and varied results (Burd 1994, Knight et al. 2005). General conclusions are that pollen limitation is higher than previously thought in flowering plants, while its effect on plant reproduction is generally negative. This negative impact typically varies based on resources available to the plant and species context, so is often hard to study in nature. One such study of the pollination of Midwestern forb *Echinacea angustifolia* found a strong case for pollen limitation in natural habitats with negative consequences for plant seed set (Wagenius 2011), though the role of variation in pollinator visitation and diversity is unclear. One of the few studies to link pollen limitation to pollinators found that one of the primary factors restricting range expansion of California plant *Clarkia xantiana* was a decline in pollinator visitor abundance at the geographic edge of its current distribution (Moeller et al. 2012).

Bee Decline

Honey Bees

Honey bees have benefited from nationwide surveys performed by the Bee Informed Partnership that detail annual honey bee colony health measures. According to their most recent published survey (Lee et al. 2015) average annual mortality for commercial beekeepers is at 32.6%, a number regarded as unsustainable by beekeepers. Many causes have been implicated in high colony mortality, including introduced pests such as varroa mites and *Nosema ceranae*, pesticides such as neonicotinoids, and the reduction of foraging habitat in the landscape (Goulson et al. 2015). While no single factor has been able to adequately explain these losses, the role of nutrition seems to be central to how sensitive honey bees are to other stressors (Huang 2012).

Increased honey bee immune function has been linked to pollen feeding (DeGrandi-Hoffman et al. 2010), pollen diversity (Alaux et al. 2010), and pollen quality (Di Pasquale et al. 2013). Pollen feeding and pollen quality have also been linked to reduced pesticide sensitivity (Wahl and Ulm 1983), and pollen feeding was observed to improve pesticide detoxification (Schmehl et al. 2014). While these studies offer hints into the importance of nutrition in mediating other stresses in honey bees, a recent meta-analysis linked increased colony mortality with decreases in open land (theoretical foraging area) in the United States (Naug 2009). A recent study of the relationship between land use surrounding apiaries and honey bee health found that higher proportions of land that contain foraging resources result in bees with better nutritional

status, lower varroa mite levels, and lower overwintering mortality, supporting the link between better nutrition and resistance to environmental stress (Smart et al. 2016). One study conducted in the U.K. found that increasing proportions of arable land and coniferous forest around honey bee colonies had negative impacts on the protein content of pollen provisions (Donkersley et al. 2014). These studies quantify the intuitive link between foraging areas and nutrition.

Native Bees

In the United States, usage of the term ‘native bee’ has come to refer to any non-*Apis* bee species in the United States. This typically includes solitary bees, which nest in the soil or hollow cavities, and species in the genus *Bombus* that are both social and parasitic. While the term native is relative to location, it has a tendency to include solitary non-native bees that have colonized North America, while specifically excluding honey bees. For the purposes of this thesis, native bee refers only to those bees with historical occurrence in the geographic location of the study area, though the studies listed below may define them separately.

The most recent evidence for declines of native bees in the Midwestern U.S. has come from Carlinville, Illinois. Approximately 100 years ago the naturalist Charles Robertson made detailed records of the bees visiting different species of plants, and the resources they collected there. These observations formed an important historical data set that would allow Laura Burkle and colleagues to quantify the change in community dynamics since Robertson’s time (Burkle et al. 2013). Burkle’s study was careful to

replicate Robertson's collection techniques to make accurate comparisons between the two time periods. This paper also focused on the important spring flower *Claytonia virginica*, looking at how visitor richness and pollen loads of collected bees have changed over this time. The results were alarming: over half of the bee species collected by Robertson were not found in the Burkle et al survey. The data collected also allowed investigations of the plant-pollinator interactions between the two data sets, and found that a third of the interactions that existed during Robertson's time had disappeared due to bee species extirpations. They found similarly negative trends for the pollination of *C. virginica*, which had half as many bee visitors, and a corresponding decline in *C. virginica* pollen being carried by visiting bees.

While Burkle's study provides good evidence for pollinator community changes in the Midwest, a large body of literature exists on the status of *Bombus* (bumble bees) species specifically. One nationwide study found evidence of declines in three species in the northeastern part of the U.S., and one species in the western U.S. (Cameron et al. 2011) in comparison to historical records. These results have been confirmed by a different study of the northeastern U.S. which found a similar negative trend for *Bombus* species over time (Bartomeus, Ascher, et al. 2013). Many potential drivers for bumble bee declines have been investigated, and include: transfer of *Nosema bombi* to wild bumble bee populations from colonies mass reared for green houses (Colla et al. 2006, Cameron et al. 2011, Graystock et al. 2013, Lim et al. 2013); reductions in genetic diversity (Cameron et al. 2011); and exposure to environmental insecticides (Gill et al. 2012, Whitehorn et al. 2012, Larson et al. 2013)

There is mounting evidence that bee species are declining in Europe as well. Region-specific losses of bumble bee species have been documented across the continent (Kosior et al. 2007, Goulson et al. 2008). Declines in solitary bee species have similarly been observed in the United Kingdom and Netherlands, with 50%-62% of the areas investigated having a reduced number of species compared to historical records (Biesmeijer et al. 2006). These losses prompted the European Union to initiate a study to determine the status of Europe's bee species. The results of this study have been dubbed, "the European Red List for Bees", and indicate that about 14% of Europe's bee species are threatened and at risk. The study also concluded that there was insufficient data to evaluate 56% of known species (Nieto et al. 2015). This report highlights the severe lack of monitoring resources being provided for the evaluation of bee communities.

Finding causes for native bee species losses is not an easy task. Patterns of decline have been largely inconsistent for bee communities as a whole, and many species appear to be more prevalent compared to historical data. To help explain this trend of increasing and decreasing richness of bee communities and the relative abundance of a given species in a region, researchers have begun using functional traits (biological variation between species that dictate how they interact with the environment) to disentangle species-specific patterns of decline. In the northeast U.S. a study conducted by Bartomeus, Ascher, et al. (2013) used historical specimens housed in museums across the region to investigate how pollinator communities have changed. Their study found that declines differed among taxa, and that there were specific ecological traits that helped predict if a species was likely to be in decline. Traits that predict a potential decline include

oligolecty (pollen specialization), increasing body size, phenological timing, and northern range. A similar decline in species richness was discovered in the U.K. and The Netherlands (Biesmeijer et al. 2006) for traits relating to oligolecty, habitat specialization, and seasonality. Using traits to help explain patterns of biodiversity has been applied in a variety of ways, and promises to be an invaluable tool for uncovering drivers of bee decline (Biesmeijer et al. 2006, Williams et al. 2010, Hopfenmüller et al. 2014) .

Many of these functional traits are related to a given bee species' ability to cope with increasing amounts and types of landscape disturbances. Most plant and animal communities follow predictable populations trends based on habitat size and continuity: as habitat becomes confined to increasingly smaller areas, so too does the community of species occupying that habitat decline (Rosenzweig 1995, Fahrig 2003). This relationship applies to bees as well, with richer communities measured in larger habitat patches (Neame et al. 2013, Hopfenmüller et al. 2014), which could be due to larger patch sizes having a higher probability of containing more types of habitat features. These features could be specific to a given bee species, such as a suitable nesting site, or to a specific forage plant on which a given bee depends. Bee diversity also relies heavily on floral abundance and diversity (Potts et al. 2003, Hines and Hendrix 2005, Grundel et al. 2010), which are also negatively impacted by reduction and fragmentation of natural areas (Aguilar et al. 2006).

Conservation Efforts

Conservation efforts for bees have taken two forms: those for ecosystem service delivery, and those for the preservation of biological diversity (Isaacs et al. 2009, Murray et al. 2009). The positive relationship between plant species richness and bee species richness has been well established (Potts et al. 2003, Ebeling et al. 2008), and all forms of bee conservation employ some form of floral species enhancement. While managing bees for ecosystem services takes a similar approach, the end goal of increasing species richness vs. abundance is debated (Kleijn et al. 2015). In looking for opportunities to conserve bee communities people have, knowingly or not, employed a form of conservation termed Reconciliation Ecology (Rosenzweig 2003).

The framework of Reconciliation Ecology differs from traditional conservation mainly in the role of people in preserving biodiversity. Traditional conservation focuses primarily on removing land from anthropogenic use, and returning it to a more natural state that is free of human disturbance. Reconciliation Ecology views people as an essential part of habitat restoration, and seeks ways of modifying anthropogenic areas to serve both human and natural functions in tandem.

Floral enhancements in a variety of human habitats have been investigated for their ability to support bee communities. Modifying crop field borders (Morandin and Kremen 2013, Blaauw and Isaacs 2014), roadside margins (Noordijk et al. 2009, Hopwood et al. 2010), green roofs (Braaker et al. 2014), and neighborhood gardens (Pawelek et al. 2009) are all examples of how human functionality can be retained while also enhancing habitat. As we look for new ways to preserve bee biodiversity,

reconciliation ecology is a useful framework for investigating new methods of habitat enhancement, and core to the ideology put forth in the studies undertaken in my thesis.

The Turf Lawn

One common human landscape modification is the cultivation of turfgrass for the creation of lawns. Turfgrass lawns perform a variety of functions including: providing durable playing surfaces, erosion control, and aesthetical appeal. While management varies by function and individual manager, turfgrass is typically characterized by three primary cultural practices: mowing, fertilizing, and irrigation (Turgeon 1999). These practices are intended to favor turf species and, when properly applied in tandem with proper turf species selection and establishment techniques, result in stands of uniform and aesthetically pleasing turf. Despite these common guidelines, turf stands are prone to pest problems such as invasion by weeds and attack by insect and pathogen pests.

Lawns cover roughly 2% of the continental United States (Milesi et al. 2005), mostly in highly urbanized areas. Turf is, by land coverage, the most cultivated landscape under irrigation in the United States. The management of lawns as uniform stands of grass is likely not helpful to bees, but there is evidence that lawns can provide forage for bees when the turf is not a uniform grass species. Lawn cover in Paris, France, was positively associated with pollinator richness (Shwartz et al. 2013), which is likely due to flowering weeds growing within the lawn. Similarly in Sussex, England, many lawn weeds attracted a variety of insects, including bees (Garbuzov et al. 2014). In Lexington, Kentucky, European lawn weeds such as dandelion (*Taraxacum officinale*) and white

clover (*Trifolium repens*) have been shown to support a large variety of native bee visitors (Larson et al. 2014).

Only a few studies have analyzed the relationship among lawn management practices and floral abundance. In Paris, France, low mowing heights were found to negatively impact flowering plant richness in lawns (Shwartz et al. 2013). Infrequent mowing regimes in park lawns in Sussex, England similarly resulted in both higher forb species richness and correspondingly more insect visitation (Garbuzov et al. 2014). Conversely, another study conducted in Sheffield, England found no relationship between management variables such as cutting height and lawn plant richness (Thompson et al. 2004). These contrasting studies and a focus on European lawns underline the need for broader and more focused research into the factors that support floral richness in managed lawns.

While these studies looked at natural colonization of local plants, there has also been some interest in seeding forbs directly into lawns. In the U.S. this has mostly centered on the common lawn weed white clover (*Trifolium repens* L.). Interest in white clover incorporation largely revolves around its nitrogen benefit for turfgrasses (Sincik and Acikgoz 2007), though authors also acknowledge its benefit as a ground cover and pollinator plant. One study done in Mississippi looked at different methods of disrupting turf lawns in order to incorporate clover and cool season turfgrasses to compliment warm season Bermudagrass commonly used in the southern U.S. while another study in Kentucky used cool season grasses. Both studies focused on the effects of scalping (mowing of grass below 1.5 inches), aeration, and vertical mowing to the establishment

of white clover, and found that scalping resulted in the highest levels of establishment (McCurdy et al. 2013, Sparks et al. 2015). The study at Mississippi also looked at the effects of cool season companion grass seeding, though this was done into an existing stand of warm season grass typical of golf courses in warm climates. They found significant differences in clover establishment based on turf species (McCurdy et al. 2013), suggesting that turfgrass species may play an important role in the establishment of white clover.

Based on the documented decline of bees and heightened public awareness and willingness to do something to help bees, there is a great opportunity to further investigate how lawns could be used to support more diverse pollinator communities. To better investigate how lawns potential role in pollinator conservation could be realized, I have undertaken a series of studies designed to explore different aspect of turf lawns, and how they might be modified to a) improve floral metrics such as abundance and richness, and b) measure if improving those metrics will have the desired benefit of increasing the number of pollinator species capable of using lawns as a foraging resource.

When considering the planting of a flowering lawn, the first question to arise was which turfgrass would be most amenable to companion forb planting. I hypothesized that grass competition would be a significant limiting factor in future plant trials, and that research into turfgrass/forb dynamics was an important first step. To that end I chose Kura clover (*Trifolium ambiguum*), a slow growing flower species with low seedling vigor, that could serve as a model for other flowering species with conserved growth habits to test this hypothesis. I found significant effects of grass species on measurements

of Kura clover success, which informed the establishment of trials designed to identify forb species for flowering lawns.

To identify new forb species with high value to bees, I selected eight flowering plants test in turf lawns. These plants were primarily native, as native plants are likely better resources for the conservation of native bees (Frankie et al. 2005, Pardee and Philpott 2014). Using results from my competition study, I established a planting of hard fescue (*Festuca brevipila*) from seed in tandem with my selected forb species. These forb species were seeded concurrently with hard fescue so that germination and establishment would occur in tandem with the turfgrass, but contained to separate plots to avoid competition from one another. I hypothesized that the establishment and flowering of these forbs would be impacted by both soil environment and mowing practice, thus establishment of forbs was done as two separate locations with dramatically different soil textures. Forb plantings were arranged into randomized blocks where low and high mowing heights could be applied in a systematic way. Of the eight flowering plants investigated, four successfully established in trials. The four species that established abundance and flowering were affected by both location and mowing height.

To facilitate next steps for flowering lawns I conducted a study to determine if three forb species could be established in mature Kentucky bluegrass lawns (*Poa pratensis*) in Minnesota. Adoption of flowering lawns will likely be contingent on cost and effort, and establishing forbs into mature lawns will be more economical in both time and money. I applied pre-seeding treatments of scalping, aeration, scalping + aeration, and an untreated control to lawns at two locations before drop seeding forbs into plots.

Pre-seeding treatments improved establishment of forbs, but this improvement was largely based on lawn management. Replication of this study is needed to confirm results.

In anticipation of future work on the conservation effectiveness of lawns enhanced with flowering plants, I conducted surveys of bee species found on the common lawn weed, white clover (*Trifolium repens*), in lawns of Minneapolis parks. Parks were chosen in a spatially explicit way to ensure even sampling across the geographic area. This survey identified 37 species of bees utilizing white clover, with an estimated visitation of 52 species. Results from this survey will be used to test hypotheses on how enhancing floral diversity will change the abundance and richness of bees using lawns as a foraging resource in Minneapolis lawns.

This work represents a significant first step toward increasing our knowledge about the creation of flowering lawns. I identified grass and forb species, management, and establishment practices that could be combined to create and optimize a flowering lawn. In addition I have established community composition of floral visitors to current urban lawns, which will allow the ability to evaluate lawns enhanced with flowers impact on the bee community. Future work should revolve around two avenues: identifying additional native plants that will persist in mowed lawns; and evaluating how the combination of multiple flowering species perform in managed lawns.

Chapter 2

Flowering Lawns: How turfgrass species and seeding rate affect establishment and bloom of a model forb, *Trifolium ambiguum*

Introduction

Bees (Hymenoptera: Anthophila) provide pollination services that are important to the seed set of flowering plants in both agricultural and natural landscapes (Grubb 1977, Southwick and Southwick 1992, Losey and Vaughan 2006, Ollerton et al. 2011). Evidence is accumulating that honey bees (*Apis mellifera*) and native bees are facing a suite of interacting stressors (Goulson et al. 2015) that threaten both honey bee colony survival (Huang 2012, van Dooremalen et al. 2013, Schmehl et al. 2014, Lee et al. 2015, Sánchez-Bayo et al. 2016) and the richness and persistence of native bee communities (Biesmeijer et al. 2006, Kosior et al. 2007, Cameron et al. 2011, Bartomeus, Ascher, et al. 2013, Burkle et al. 2013). One stressor that is at the heart of many conservation efforts is the loss of floral foraging habitat from the landscape. The positive relationship between floral diversity and bee community diversity has been well established (Potts et al. 2003, Ebeling et al. 2008), and has become the focus of most bee conservation strategies. These strategies have been targeted at florally enhancing and diversifying human dominated landscape features, including crop field borders (Morandin and Kremen 2013, Blaauw and Isaacs 2014), roadside margins (Noordijk et al. 2009, Hopwood et al. 2010), rooftops (Braaker et al. 2014), and neighborhood gardens (Pawelek et al. 2009). This focus on anthropogenic landscape features is in the line with the practice of reconciliation ecology

(Rosenzweig 2003), a conservation practice that seeks to maintain human functionality of a landscape while improving its ability to support biodiversity.

One anthropogenic landscape feature that could be improved to support the goals of reconciliation ecology is the turf lawn. Lawns are estimated to cover roughly 2% of the continental United States (Milesi et al. 2005), mostly in highly urbanized areas. While management of lawns varies by function and individual manager, it is typically characterized by three primary cultural practices: mowing, fertilizing, and irrigation (Turgeon 1999). These practices are intended to favor turf species and, when applied in tandem with proper establishment techniques, result in stands of uniform turf.

Despite lawns being currently managed as uniform monocultures, they are often host to flowering plants that provide foraging resources for bees. A recent insect survey of park lawns hosting dandelion (*Taraxacum officinale* F.H. Wigg.) and white clover (*Trifolium repens* L.) conducted in Lexington, Kentucky (Larson et al. 2014) found 37 species of bees associated with these two forbs. These species are typically considered weeds and are eliminated through the use of broad leaf herbicides; however, lawns intentionally managed for forb abundance and richness would likely have a beneficial impact to local foraging bee communities. Such management goals would necessitate the reduction in use of other lawn inputs and would further the goal of increased sustainability. Mowing is often negatively correlated with plant species richness in lawns (Bertoncini et al. 2012, Shwartz et al. 2013, Garbuzov et al. 2014, Smith and Fellowes 2015), and managing for floral lawns could lead to less intensive mowing regimes. The inclusion of legumes such as white clover has shown to increase turf quality through

nitrogen fixation (Sincik and Acikgoz 2007), and would potentially reduce fertilizer inputs into lawns.

While floral lawns hold great promise in improving biodiversity in urban areas, guidelines into usable forbs, establishment methods, and management practices are needed to encourage adoption. Some effort has been made into developing seeding strategies for white clover into established lawns to benefit pollinators and soil nitrogen (McCurdy et al. 2013, Sparks et al. 2015). White clover is an established agronomic crop that is widely associated with pasture agriculture, and is well adapted to the grazing systems under which it evolved (Leffel and Gibson 1973). Pasture systems and lawns are similar in many ways, (e.g., compaction and cutting by animals or humans) and it is no surprise that white clover does well in both environments.

At the other extreme of flowering lawns, researchers at the University of Reading in the UK have abandoned the use of turfgrass altogether, and have been developing species lists and management practice guidelines for purely floral lawns (Smith and Fellowes 2015) that have been found to have benefits to flower-visiting insects (Smith et al. 2014). These lawns may provide benefits to flower visiting insects, but they are not meant to be walked on or utilized for recreation. One potential challenge of these plantings is the reliance on pre-cultivation and installation, which can be costly on a large scale, and the potential need for more specialized care. In addition, there is still a strong cultural connection with turf lawns (Harris et al. 2013) and an intermediate flowering lawn, that combines turfgrass and floral resources, may have broader and more practical appeal, especially for recreational use. For these reasons we have chosen to focus on

grass-forb mixes established from seed that can provide quick ground cover, are economical, are relatively easy to maintain, and can be utilized as a traditional lawn for recreation.

Toward the goal of identifying cultural practices that will help lawn owners adopt practices that favor floral abundance in lawns, this study tests the hypothesis that slower growing turfgrasses and higher forb seeding rates will favor the establishment of slower growing and less competitive forbs. Establishing both seeding rates and turf species amenable to introduction of slow growing forbs is an important first step for more detailed explorations of plant material for flowering lawn conservation plantings.

Methods

Species

To test the effect of turf species on forb establishment, we selected four commonly used cool-season turfgrasses: ‘Moonlight SLT’ an elite cultivar of Kentucky bluegrass (*Poa pratensis*); ‘Beacon’ hard fescue (*Festuca brevipila*); ‘Grande II’ tall fescue (*Festuca arundinacea*); and ‘Apple GL’ perennial ryegrass (*Lolium perenne*). Species were selected based on their proven performance as cool season turfgrasses and to capture a range of growth characteristics (Table 1).

To represent a slow growing forb we selected Kura clover (*Trifolium ambiguum*). Kura clover is a rhizomatous perennial plant, originating from Eastern Europe/Western Asia, and has been investigated as a cold tolerant forage plant for pasture cattle. Kura clover is not commonly cultivated across Minnesota, but can be found infrequently

growing in semi-natural areas. One challenge for Kura clover cultivation is its low seedling vigor (DeHaan et al. 2001), which limits its adoption as a pasture based crop (Seguin et al. 1999). The slow growth and establishment characteristics make Kura clover an excellent model plant for investigating competitive effects of turfgrass species which we could then compare to other slow growing forbs, especially native plants with low germination under lawn management. Although seeding rates for Kura clover have been recommended for pasture systems, we chose to experimentally manipulate the seeding rate to better infer appropriate rates for lawn mixtures.

Experimental Design

This study was conducted at the Turfgrass Research, Outreach, and Education Center (TROE) on the University of Minnesota St. Paul campus. Two trials were conducted, the first starting in 2013 and the second in 2014, each in different locations within TROE. Aggregate soil samples were collected at the conclusion of the trials to characterize the soil environment through standard soil testing at the University of Minnesota Soil Testing Lab. The soil measurements for phosphorous, potassium, and soil organic matter were nearly identical in the two trials, with the exception of pH (trial one pH = 6.5 and trial two, 6.1). Trials were organized as a complete random factorial design, with four grass species and three Kura clover seeding rates as experimental factors. Each grass/seeding rate combination was replicated three times for a total of 36 observational units per trial, and combinations were randomly arranged into a 6-plot by 6-plot grid. Individual plot dimensions were 1 m by 1.5 m and separated by a 0.15 m border.

The first trial was seeded on June 20, 2013, and the second on July 2, 2014. Temperatures during establishment averaged a high of 29.5° C and low of 20.2° C in 2013 for trial one, and a 27.2° C high and 16.3° C low in 2014 for trial two. Sites were prepared by application of glyphosate to clear undesirable plants, rototilling to break up rooting structures, and power raking directly before seeding. Grass species were seeded at rates recommended by Turgeon (Turgeon 1999) and converted to our plot dimensions (Table 1). Kura clover seeding rate treatments were 0.57 g·m⁻², 0.85 g·m⁻², and 1.1 g·m⁻². Trial plots were given a broadcast fertilizer application of EC grow[®] greens grade fertilizer with an analysis of 10N–7.9P–18.3K at time of seeding, applied at a rate of 30g·m⁻². Plots were irrigated twice daily for 20 minutes during the first two weeks to assist establishment. Plots were mowed to 7.6 cm if any plot was higher than 11.4 cm. Grass clippings were left on site. Data collection began in June of the year following seeding.

Data Collection and Analysis

Data were collected once per month for three months starting in June each year, and always preceded a mowing event to avoid introducing a cutting bias on plant metrics. A 1 m × 1.5 m quadrat with nylon wire grid lines was used to help visually break plots into 12 mm² square sections to facilitate counting of Kura clover trifoliolate leaves and blooms, which were summed over the entire plot, thereby providing an absolute abundance of trifoliolate leaves and blooms. To estimate the amount of plot area covered by Kura clover leaves, grid lines were used to visually estimate the total number of

squares covered by trifoliate leaves. The number of squares covered was divided by 117 (the total number of squares in the quadrat), and a percentage of Kura clover cover was obtained for each plot. Leaf cover was measured to determine if Kura clover would produce leaves of differing size in response to turf species competition.

Response variables used to measure the establishment of Kura clover included trifoliate leaf count, bloom number, and plot leaf coverage. Data were analyzed with the R statistical program (v3.2.3) utilizing the 'nlme' and 'multcomp' packages. All three response variables were first tested for correlation with one another using Pearson's product-moment correlation coefficient. Results were compared by first examining the ANOVA of a linear mixed effects model (except in the case of blooms), with Kura clover seeding rate and turf species specified as the interaction term and trial for fixed effects. Plot number was used as a random effect to account for repeated measures throughout the sampling season, and a Tukey means separation protocol was used if F statistics were below an $\alpha = 0.05$ to test for significant differences among factor levels. The bloom response variable was analyzed with a linear regression model without mixed effects due to blooms only occurring during one time point in the year (July). Assumptions of homoscedasticity and normality were verified by inspection of residual plots. Square root transformations were sufficient to meet assumptions for all analyses except for the bloom response variable in trial 1, in which case a log transformation was used.

Results

Trifoliate leaf count and percent cover of Kura clover were highly correlated ($r = 0.937$, $t_{(214)} = 39.2607$, $p\text{-value} < 0.001$), indicating a low amount of variability in the amount of cover provided by individual trifoliate leaves; that is, trifoliate leaf size was relatively constant. Due to the high degree of correlation, only trifoliate leaf counts were used in the analysis, which had less bias during data collection compared to the estimation of leaf cover. The relationship between trifoliate leaves and bloom number was strong ($r = 0.712$, $t_{(70)} = 8.4764$, $p\text{-value} < 0.001$). Despite the strong correlation, analysis of bloom was included because it is more relevant to the goal of providing foraging resources for pollinating insects.

Due to differences in establishment time and success, trials were analyzed separately. In both trials, turf species significantly affected the numbers of trifoliate leaves and blooms ($p \leq 0.05$) (Table 2.). The seeding rate of Kura clover did not significantly affect leaf or bloom counts in any of the plots in either trial, and no significant interaction between turf species and seeding rate was detected. Hence all pairwise comparisons between trifoliate leaves and blooms in different grass treatments were averaged over Kura clover seeding rate. Statistics for all pairwise comparisons are provided in Table 3.

Trial One

The number of Kura clover trifoliate leaves in Kentucky bluegrass and hard fescue plots were significantly higher than in both tall fescue and perennial ryegrass plots (Fig. 1A). This pattern was also the same for the number of blooms counted, with

Kentucky bluegrass and hard fescue both having significantly higher bloom counts than tall fescue and perennial ryegrass.

Trial Two

The number of trifoliolate leaves in Kentucky bluegrass plots was significantly higher than the number in both tall fescue and perennial ryegrass plots (Fig. 2B). This result was mirrored for bloom data, with the number of blooms in Kentucky bluegrass being significantly higher than blooms in perennial ryegrass and tall fescue.

Discussion

Establishment and bloom of Kura clover was significantly higher in Kentucky bluegrass both years than in perennial ryegrass and tall fescue. Despite the differences in forb establishment between trials, we did observe higher establishment of Kura clover trifoliolate leaves and blooms in the slower growing turfgrass species, supporting our hypothesis that slower growing turfgrasses are better suited for flowering lawns.

One factor that likely contributed to the higher establishment of Kura clover in Kentucky bluegrass was the limited fertilizer regime used in this study. Elite cultivars of Kentucky bluegrass and perennial ryegrass both have recommended nitrogen fertilization rates of between 15.1 and 19.4g·m⁻² in a given year, which is higher than that of tall fescue and hard fescue where a nitrogen fertilizer application of between 9.7 and 15.1 g·m⁻² is recommended. This reduction in fertilizer use compared to recommended rates may have resulted in slower growth of Kentucky bluegrass, and given Kura clover more opportunity to establish. However, the fertilizer requirements for grass establishment in

our plots were likely on the low side of recommended rates due to the loamy soil, conservative cutting regime, the legume companion forb, and the fact that clippings were left on the field. These practices are key in the creation of sustainable floral lawns.

There was lower establishment of Kura clover in trial two compared to trial one despite efforts to keep establishment conditions (i.e. nutrient availability and soil moisture content) consistent. This difference could be related to differential climatic conditions between years, with average temperatures during the first week of establishment being notably higher during trial one than trial two. Other factors that may have contributed to the differences are reductions in seed germination, as the same seed lot was used for planting of both trials. Trial location differences could have played a role, with higher soil pH in trial one (6.5) than trial two (6.1). The field space where trial two was established was prepared for planting in 2013 through tilling and leveling, but was left fallow for one year without further cultivation. While direct measurements of weed pressure were not taken as a part of this study, it is possible this fallow period without further cultivation might have led to a larger weed seed bank, and thus greater weed pressure on plot establishment in trial two (Roberts and Dawkins 1967, Froud-Williams et al. 1983). Our study also failed to find any difference between Kura clover seeding rates in either study within the range we selected. While seeding rate likely plays a role in Kura clover establishment, our selected rates may not have varied enough to allow for detectable differences.

Other studies have found similar effects of turfgrass competition, though in different contexts. One study in which an Australian flower bulbine lily (*Bulbine bulbosa*

R. Br.) was given differing amounts of bare ground within two different types of grass plantings found that higher amounts of competition-free space was found to be more important to bulbine lily establishment in the aggressive tall fescue patches than it was for the less aggressive native grass patches (Hitchmough et al. 1996). Another study involving *Trifolium repens* co-seeded with companion grasses into dormant Bermudagrass [*Cynodon transvaalensis* Burt- Davy 3 *C. dactylon* (L.) Pers.] found clover produced more trifoliolate leaves in tall fescue, compared to the faster growing varieties with more seedlings·m⁻² such as annual ryegrass (McCurdy et al. 2013). A similar study used three species of turfgrass co-seeded with birdsfoot trefoil (*Lotus corniculatus* L.), and found that Kentucky bluegrass and red fescue typically had higher yields of birdsfoot trefoil than perennial ryegrass (Laskey and Wakefield 1978). These studies generally support the hypothesis that slow growing grasses are more amenable to the growth of companion forbs, though there is also evidence that tall fescue is partially alleopathic to species in the clover genus (*Trifolium*) (Springer 1996). While our study was constrained to upper midwestern United States, the number of studies with similar results span many climatic zones, suggesting this relationship could be applicable in a range of systems.

Finding commercially available flowers that meet land owners' needs, the foraging requirements of bees, can tolerate lawn management, and are aesthetically acceptable by lawn owners are some of the largest issues facing the creation of diverse lawn plantings. Mowing and public use have been found to negatively impact lawn community diversity (Bertoncini et al. 2012, Shwartz et al. 2013) but the species of

turfgrass used in lawns also has an important impact on whether flowers can establish and continue to bloom in lawns. This study combined with other previous research suggests that slow growing turfgrasses such as Kentucky bluegrass and hard fescue are good candidates for future lawn forb trials aimed at identifying new species for flowering lawn mixes.

Table 1: List of turfgrass species used and their relevant growth traits

Species	Growth	Observed Establishment Rate in Study	Seeding Rate
<i>Poa pratensis</i>	Rhizotemous	Slow	6.8 g·m ⁻²
<i>Festuca brevipila</i>	Bunch type	Slow	22.7 g·m ⁻²
<i>Festuca arundinaceae</i>	Bunch type/short rhizomes	Fast	27.2 g·m ⁻²
<i>Lolium perenne</i>	Bunch type	Fast	27.2 g·m ⁻²

Table 2. Results of ANOVA testing the effect of grass species, seeding rate, and their interaction on trifoliolate and bloom counts for trials 1 and 2.

Factor	Degrees of Freedom	F	P
Trial 1			
<i>Trifoliolate Leaves:</i>			
Grass Species	3,24	21.73	< 0.001*
Seeding Rate	2,24	2.05	0.151
Grass Species x Seeding Rate	6,24	0.83	0.557
<i>Blooms:</i>			
Grass Species	3,24	2.97	< 0.001*
Seeding Rate	2,24	0.19	0.133
Grass Species x Seeding Rate	6,24	0.14	0.302
Trial 2			
<i>Trifoliolate Leaves:</i>			
Grass Species	3,24	4.03	0.019*
Rate	2,24	2.68	0.089
Grass Species x Seeding Rate	6,24	0.87	0.529
<i>Blooms:</i>			
Grass Species	3,24	6.95	0.002*
Rate	2,24	0.29	0.748
Grass Species x Seeding Rate	6,24	0.89	0.515

(*) = Denotes significant result

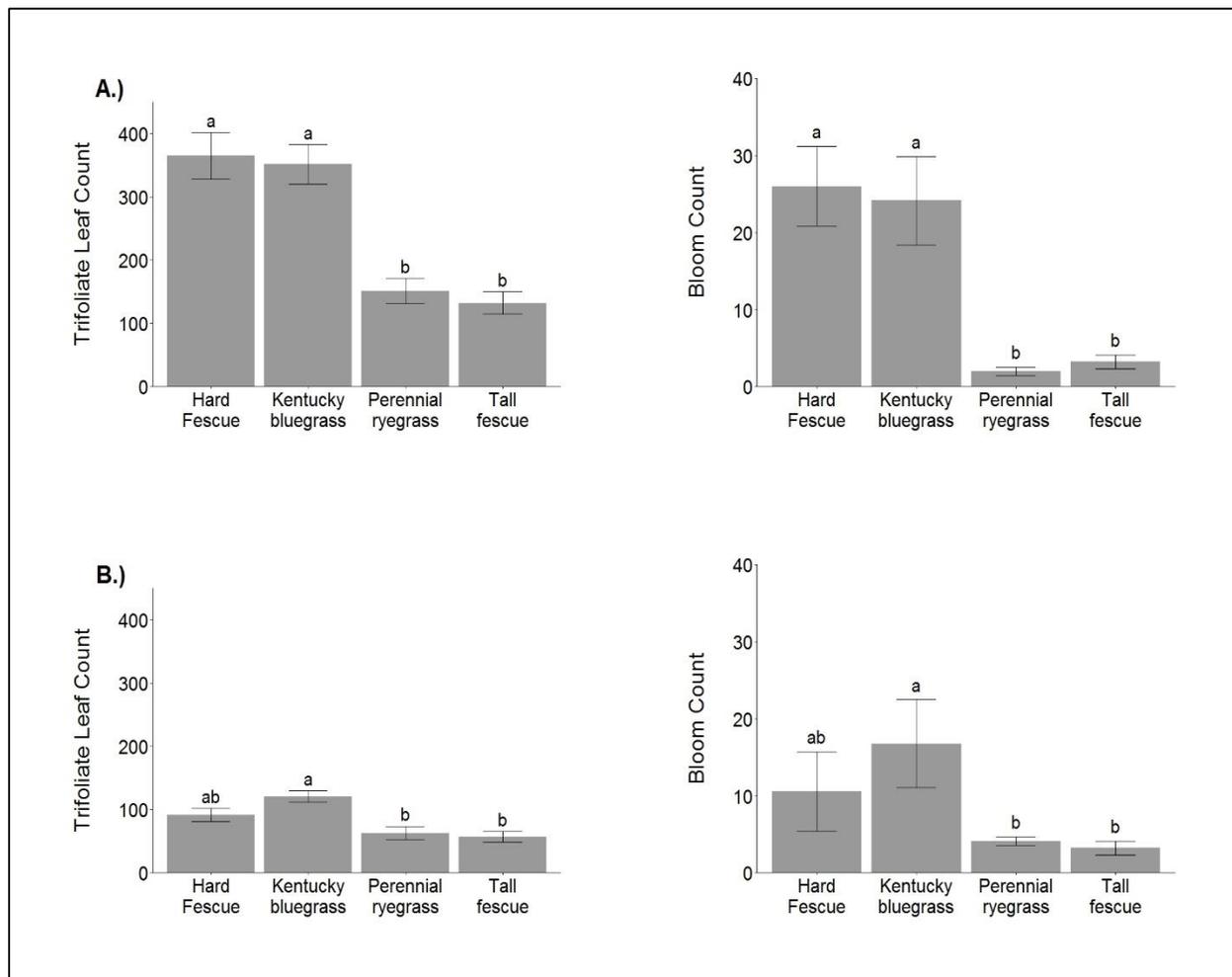


Figure 1: Mean seasonal *Kura clover* bloom and trifoliolate leaves for trial 1 in 2014 (**A**) and trial 2 in 2015 (**B**) in four different grass species. Error bars represent standard error and letters represent statistical differences as determined by pairwise comparisons using Tukey's mean separation protocol with $\alpha = 0.05$.

Chapter 3

Establishment of eight pollinator-friendly forbs in mowed lawns of hard fescue (*Festuca brevipila*)

Introduction

Pollination is an important ecosystem service that is valuable to both agriculture (Southwick and Southwick 1992, Losey and Vaughan 2006, Rader et al. 2015) and natural systems (Grubb 1977, Ollerton et al. 2011). One group of pollinators, bees (Hymenoptera: Anthophila), have been of particular concern due to rapid declines of many species over the past 40 years (Goulson et al. 2015). These declines have generated increased public concern and an interest in their conservation.

One of the primary methods of conserving pollinators is through the addition of forbs that provide attractive pollen and nectar resources in the landscape (Murray et al. 2009). This method has proven effective in increasing the abundance and species richness in a variety of land use types, including farm borders (Morandin and Kremen 2013, Blaauw and Isaacs 2014), roadside verges (Noordijk et al. 2009, Hopwood et al. 2010), green roofs (Braaker et al. 2014), and neighborhood gardens (Pawelek et al. 2009). Despite this progress, modifying our landscapes to meet both anthropogenic goals and biodiversity conservation remains an important area of research, and has been coined reconciliation ecology (Rosenzweig 2003).

Reconciliation ecology is a method of dealing with the seemingly antagonistic goals of natural habitat preservation and economic development. Achieving these disparate goals is increasingly important given that nearly 50% of U.S. land has been

converted to some form of anthropogenic use (crops, pasture, urban, roads) (Nickerson et al. 2007). While urban areas occupy roughly 3% of total US land area, the urban landscape is the fastest growing land use category, having quadrupled its land cover since 1945. The challenges and opportunities of conserving nature in urban habitats have been well reviewed (McKinney 2002, Goddard et al. 2010), and will become increasingly important as 66% of the world's population is projected to live in cities by 2050 (United Nations, 2014). Reconciliation ecology seems particularly well suited for urban environments where human land use is most intense.

One urban landscape feature that has multifunctional conservation potential is the turf lawn. Lawns are estimated to cover roughly 1.9% of the continental U.S., and represent a large proportion of area considered to be urbanized (Milesi et al. 2005). Lawns are classically managed as monocultures of turfgrass species, but can host a variety of flowering forbs and grasses that are often considered weeds. In the American Midwest, these plants are predominantly of European origin (Whitney 1985).

Efforts to utilize lawns as areas for enhancing biodiversity have already received attention in Europe. In Paris, France, gardening practices have been incentivized and designed to improve biodiversity through a program known as the 'Differential Management' program. This program aims to modify a number common of landscaping practices to benefit biodiversity, such as mowing and pesticide use. Shwartz et al. (2013) evaluated the program's success in a study that found gardens certified as 'biodiversity friendly' housed a greater diversity of pollinators, birds, butterflies, and wild plants. The number of species found for groups studied were generally positively affected by lawn

cover, which housed 69% of wild flowering plants in their study. One research project in Reading, UK developed completely floral lawns that hosted high abundances of flower-visiting insects compared to regular turf (Smith et al. 2014).

One central hypothesis of these studies is that mowing factors, such as height and frequency, impact floral diversity of lawns. In Shwartz's study of Paris' lawns, the results were mixed; there was no effect of mowing frequency on floral diversity, and higher mowing heights benefited floral richness in smaller home lawns but not in large park lawns. In both the completely floral lawns and conventional turf in Reading, more intense mowing frequencies negatively impacted floral visitors, but floral richness was favored by intermediate mowing frequencies (Smith and Fellowes 2014). Shwartz's study seems to refute an earlier finding that found mowing frequency, among other factors, negatively impacted lawn diversity (Bertoncini et al. 2012). Conversely another observational study of lawns in Sheffield, England found that mowing frequency had little effect on plant richness (Thompson et al. 2004). In Saltdean, England where mowing frequencies were experimentally controlled, an increase in floral abundance was observed, with a corresponding increase in floral visitor abundance under less intense mowing regimes (Garbuzov et al. 2014). These studies highlight the temperamental role of mowing on plant community metrics in lawns, but seem to point to mowing height and frequency as important factors affecting the ability of many species to persist.

To better design floral lawns with benefits to pollinators in the American Midwest, forb species and management practices need to be identified and developed that are specific to the region. Europe seems unique in that many of the non-turf lawn flora

are considered indigenous to the region. In England, 83%-94% of non-turf plants found in lawns were considered to be native (Thompson et al. 2004), while in Paris an average of 91% of non-turf plants were considered native (Bertoncini et al. 2012). While flowering lawn weeds such as white clover (*Trifolium repens*) attract a variety of insect visitors in the U.S. (Larson et al. 2014), native plants have been found to attract greater quantities and, in some cases, numbers of species than non-native hosts (Frankie et al. 2005, Pardee and Philpott 2014, Smith et al. 2014).

Since native plants are not common in Midwestern lawns, we hypothesize that the typical lawn is generally unfavorable for region-specific indigenous forbs. In order to modify lawns to support native forbs, we manipulated three common lawn factors. First we selected the turfgrass species fine fescue (*Festuca brevipila*) as the companion grass for a flowering lawn planting. Previous studies have indicated that fine fescues have slow growing and low input qualities that make them amenable to grass/forb mixtures (Laskey & Wakefield 1978, Lane chapter 2). Secondly we chose a subset of mostly native forbs that, with consultation from nursery operators, thought to have potential for establishing in lawns. To investigate how different sites and practices might affect the success of these forbs, our study locations and soil conditions differed dramatically. The flower species selected for this study came from a range of habitats, and soil conditions likely favored some species but not others. Due to the impact of mowing on the diversity and flowering of plants, we manipulated cutting height to better understand how mowing affects establishment and bloom of the selected species. We predicted that native forb species

would establish and bloom in fine fescue depending on site and soil conditions, and on mowing heights.

Methods

Site Characteristics

Two study sites were established in November of 2013, one at the Turf Research, Outreach, and Education Center (TROE) at the University of Minnesota St. Paul campus and the other at the University of Minnesota Sand Plains Research Farm (SPRF) located in Becker, Minnesota. Aggregate soil samples were taken over the entire study area at each site. Texture was determined using the ‘hydrometer method’ and organic matter content was determined through the ‘loss of ignition method’ (LOS) at the University of Minnesota Soil Testing Laboratory. The TROE site was a silty clay loam (7.5% sand, 61.3% silt, 31.3% clay) with an organic matter content of 4.3%. The SPRF site was a sandy clay loam (68.8% sand, 8.2% silt, and 22.5% clay) with an organic matter content of 1.7%. Sites were prepared for planting through an application of glyphosate, rototilling, and soil leveling to prepare an adequate soil bed.

Species Selection and Establishment

Eight forb species were selected (Table 3) for co-establishment in turf, based on recommendations from native plant specialist, known growth height characteristics, and perceived value as a forage plant for bees; that is, a flower that provides floral nectar or pollen for bee pollinators. ‘Beacon’ hard fescue (*Festuca brevipila*) was chosen as the turf species for its slow growth habit and its low water and fertilizer input needs.

Sites were established by first broadcast seeding *F. brevipila* at a rate of $0.06 \text{ kg}\cdot\text{ha}^{-2}$ over a total area of 0.26 ha^2 . One m^2 plots were then delineated in a 10×8 plot grid in the center of the *F. brevipila* seed bed. Forb species were hand seeded into delineated plots the same day as grass seeding at a standardized rate of 241 seed per plot, with only a single species being seeded to each plot. Species were seeded in a random order such that all eight species were represented in each row, forming 10 blocks containing eight plots, with a border two meter border of hard fescue separating each plot at each study location. On June 16th 2014, Sustane©, a slow release starter fertilizer (15-3-9, N-P-K analysis), was applied at a rate of $0.007 \text{ kg N}\cdot\text{ha}^{-2}$ to assist with planting establishment. Species blocks were paired and randomly assigned a mowing height treatment of 6.4 cm or 8.9 cm for the course of the study, resulting in a complete random block design with five experimental blocks at each location.

Mowing height treatments were maintained using height-based criteria to initiate a mowing event. If any plot in a mowing treatment exceeded its assigned height by $1/3$ (9.5 cm for the 6.4 cm treatment group and 10.8 cm for the 8.9 cm treatment group), all plots in that treatment group were mowed to its designated height. This scheme is based on the botanical principle in which no more than $1/3$ of turf biomass should be pruned at a given time (Turgeon 1999). This mowing scheme resulted in a fairly conservative mowing schedule, which ranged between 3 to 4 (2014) and 5 to 7 (2015) mowing events for the SPRF and TROE locations respectively. The SPRF location received fewer mowing treatments due to slower growth rates, presumably due to nutrient and water limitations imposed by the low organic matter content and the high proportions of sand in

the soil. This assumption is in part supported by excessive yellowing of grass blades in late spring, which led us to apply a slow release fertilizer, Sustane© 0.05 kg N/ha² (18-1-10 analysis), in July of 2014 and May of 2015. The SPRF location was provided supplemental irrigation to avoid confounding effects of water drift from nearby and unrelated studies, and had a total of 17.8 cm and 22.2 cm of water applied in 2014 and 2015, respectively.

Data Collection and Analysis

The success of forb establishment was measured by counting the number of “vegetative structures” or blooms within a plot. Vegetative structures varied by species, but were considered any structure that arose from the ground that could be reasonably counted over the entire plot. These structures included trifoliate leaves, basal rosettes, and stems. Data for vegetative structures were collected in September of each year prior to a final mowing event to avoid introducing a cutting bias in the data. Since each species in this study had a different flowering phenology, plots were surveyed for the onset of flowering before each mowing event. If flowering was detected, blooms within a plot were counted for all plots of that species at that location. Data for each forb were considered separately during analysis, and only once blooming had begun at that location. A bloom was counted only if it contained at least one un-senesced floret.

The R statistical environment (v. 3.2.3) was used to analyze location and treatment effects on vegetation and bloom parameters for each forb species. First, a mixed-effects analysis of variance (ANOVA), utilizing the ‘*nlme*’ package, was used to

look for significant interactions between mowing treatment and location before carrying out a means separation procedure. The interaction between mowing treatment and location was considered a fixed effect, while plot number was specified as a random effect to account for repeated measures. Model assumptions of homoscedasticity and normality were evaluated by inspection of residual plots. If assumptions were violated, square root transformations were applied and residual plots re-evaluated. If assumptions were still not met through square root transformations, a log transformation was applied and residual plots were reevaluated. In all cases transformations were sufficient to accommodate model assumptions. In many cases, one level of a category would contain data while the opposing category would have none. For example, *A. crassicaarpus* leaves were observed at the SPRF location, but not at the TROE location. In these cases ANOVA was not possible, and instead factors were tested individually as described below.

Factor levels were compared using a ‘Tukey’ means separation protocol for pairwise comparisons utilizing the ‘*multcomp*’ package in the R statistical environment. If the mowing-treatment by location interaction term in the ANOVA was not significant, means were averaged over terms for purposes of means separation. In cases where there were no data for one level of a category (as described in the previous paragraph), a Student’s *t*-test was used to determine if the mean count of the category was different from zero with an $\alpha = 0.05$.

Results

Vegetative Establishment

The establishment of hard fescue at both sites was not quantified in this study, but was subjectively adequate for a homeowner lawn. No evidence of vegetative establishment or blooms of *C. virginica*, *A. patens*, *O. lambertii*, or *E. compositus* were observed in plots throughout this study. Thus our analysis was constrained to *T. repens*, *P. vulgaris*, *A. crassicaarpus*, and *T. serpyllum*. ANOVA indicated that none of the observed forb vegetation showed a significant interaction between mowing treatment and location or between mowing treatments within location. All forbs except *T. serpyllum* showed a significant effect of location (Table 4). Mean trifoliolate leaf counts of *T. repens* were significantly higher at SPRF (mean = 385 trifoliate/plot) than at the TROE location (= 208) (Fig. 2). The reverse was true for mean counts of *P. vulgaris* basal rosettes, which were significantly higher at TROE (mean 76 rosettes/plot) than the SPRF location (= 20). Leafing units of *A. crassicaarpus* were only found at the SPRF location, with a *t*-test indicating that mean stem counts at the SPRF location (mean = 3.25 stems/plot) were significantly different from zero. While no significant effect was detected for *S. serpyllum*, stem counts were higher at SPRF (mean = 106 stems/plot) than at TROE (= 70).

Bloom

T. repens was the only species to bloom at both locations, and neither location nor bloom count were affected by location or mowing treatment (Tab. 4). *P. vulgaris* blooms were only found at the TROE site, and a *t*-test indicated that mean bloom counts were significantly higher than zero (mean = 8.7 blooms/plot) (Fig. 4). *P. vulgaris* bloom counts

were also significantly higher in the 8.9 cm mowing treatment (= 13.2 blooms/plot) compared to the 6.4 cm mowing treatment (= 4.1). *T. serpyllum* blooms were only found at the SPRF location, with a *t*-test indicating that mean bloom counts were significantly higher than zero (= .65 blooms/plot), and in the 8.89 cm mowing treatment, with a *t*-test indicating that mean bloom counts (= 1.3) were significantly higher than zero. *A. crassicaarpus* blooms were only observed in one plot at the SPRF location, and thus analysis was not possible.

Discussion

Research site played an important role in vegetative and flowering measurements, confirming our hypothesis that site-specific conditions were important in forb establishment, and that forb response to site conditions varied by species. Of the four forbs that established in our study, the SPRF location had greater vegetative establishment of *T. repens* and *A. crassicaarpus*. While *T. serpyllum* vegetation was not significantly affected by site, it only achieved flowering at the SPRF location. Conversely, *P. vulgaris* ssp. *lanceolata* had higher vegetative establishment at TROE, the only location where it bloomed.

While mowing had a negligible role on vegetative establishment, it did affect two of the four forbs that flowered during our study. Both *P. vulgaris* ssp. *lanceolata* and *T. serpyllum* were negatively impacted by the low-height mowing scheme, partially supporting our hypothesis that low mowing height negatively impacts forb flowering. The third forb that achieved flowering in our study was *T. repens*, which showed no

relationship between mowing and bloom. This is perhaps not surprising, as *T. repens* is adapted to intense mowing regimes, under which it has been observed to flower. During the course of the study, *A. crassicaarpus* flowered in only one plot at the SPRF, so it was not possible to infer the effects of study location and mowing.

Our results suggest that forb species *P. vulgaris* ssp. *lanceolata*, *A. crassicaarpus*, and *T. serpyllum* have potential value as components of a flowering lawn seed mix intended to benefit pollinators when paired with sites that match their specific growing requirements and mowed at higher heights. Of these plants, *P. vulgaris* ssp. *lanceolata* had the most promise, as it established quickly and bloomed profusely under low cutting heights. While *P. vulgaris* ssp. *lanceolata* did poorly at SPRF, it excelled at the TROE location, which represents a more typical lawn environment with high organic matter. Observations of *P. vulgaris* ssp. *lanceolata* suggest this plant is an excellent foraging resource for bumble bees and many species of solitary bees (Lane, unpublished data). While *A. crassicaarpus* established consistently at SPRF, it was relatively sparse compared to other forbs. This suggests that higher seeding rates may be necessary and that it would only be suitable to sandy sites. *A. crassicaarpus* only achieved flowering in its final year and only in one plot, though establishment and bloom may increase over time given its slow growth rate.

While these results are encouraging, only two of the four plants that established (*P. vulgaris* ssp. *lanceolata* and *A. crassicaarpus*) were native in origin. It is possible that our growing sites were poor environments for establishment of the other forbs. *C. virginica* has been known to establish in lawns (Schemske et al. 1978), but is typically

found in more shaded environments and its seeds are dispersed by ants. *A. patens* has also been found in association with close grazing and mowing (Wildeman and Steeves 1982), but low seed germination rates (Greene & Curtis 1950, Lane unpublished data) and a preference for high soil pH may have made it uncompetitive in our plantings. *O. lambertii* is a low growing forb, but is more characteristic in dry environments (Whitman and Stevens 1952, Wheeler et al. 1992). *E. compositus*, while a native to the central U.S., is more characteristic of rocky sites in montane habitats very different from our planting sites.

Perhaps the largest barrier to developing and studying flowering lawns with native forbs is a lack of seed stock available from local nurseries. Many native plants have traits desirable of companion plantings with turfgrass, such as low and competitive growth habits, but seeds are not available in appreciable quantities. This is potentially due to the difficulty in harvesting plant seeds from low growing plants as well as a lack of demand from the public for plants with these qualities. Future directions with flowering lawns utilizing native plants should seek partners in the native plant seed industry to facilitate the exploration and production of candidate plant seeds for use by the public.

Table 3. Forb species tested and their relevant growth traits

Species	Common Name	Height(cm)	Habitat	Bloom Time
<i>Anemone patens</i>	Pasque flower	7.6 – 45.7	Dry – sunny	March - May
<i>Claytonia virginica</i>	Spring beauty	7.6 – 12.7	Moist – Shady	April - June
<i>Oxytropis lambertii</i>	Purple locoweed	10.2 – 40.64	Dry – sunny	April - June
<i>Astragalus crassicaarpus</i>	Ground plum	10.2 - 61	Dry – sunny	May-June
<i>Erigeron compositus</i>	Cutleaf daisy	15.2	Dry – sunny	May - July
<i>Trifolium repens</i>	Dutch white clover	20.3	Moist – sunny	June-October
<i>Prunella vulgaris</i> ssp. <i>lanceolata</i>	Lanceleaf self-heal	7.6 – 30.5	Moist – sunny	June-August
<i>Thymus serpyllum</i>	Creeping thyme	20.32	Dry – sunny	July - September

Table 4. Results from ANOVA testing the effect of height, location, and their interaction (where possible) on leaf and bloom counts for all plant species established in plots.

Factor	Degrees of Freedom	<i>F</i>	<i>p</i> -value
<i>Trifolium repens</i>			
<i>Trifoliolate leaves:</i>			
Height	1,17	0.86	0.367
Location	1,19	5.15	0.035*
Height x Location	1,17	0.01	0.926
<i>Blooms:</i>			
Height	1,16	0.46	0.509
Location	1,16	0.07	0.792
Height x Location	1,16	1.04	0.324
<i>Prunella vulgaris</i>			
<i>Basal Rosettes:</i>			
Height	1,17	2.08	0.168
Location	1,19	95.05	< 0.001*
Height x Location	1,17	2.09	0.166
<i>Blooms:</i>			
Height	1,8	5.43	0.048*
<i>Thymus serpyllum</i>			
<i>Stems:</i>			
Height	1,18	0.11	0.393
Location	1,18	3.72	0.07
Height x Location	1,18	0.003	0.959
<i>Blooms:</i>			
Height	1,8	8.72	0.018*
<i>Astragalus crassicaarpus</i>			
<i>Stems:</i>			
Height	1,8	0.03	0.875

(*) = Denotes significant result

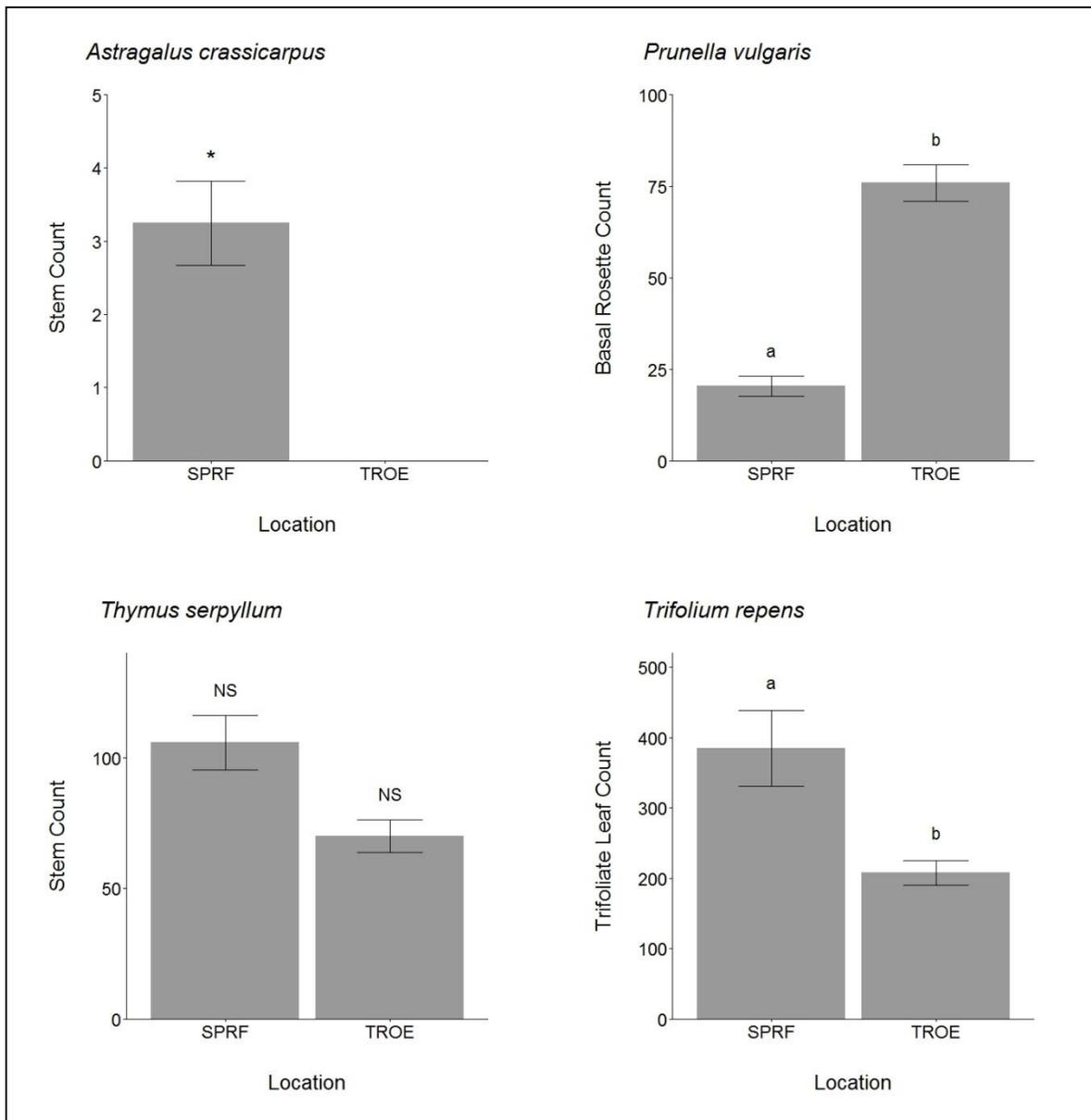
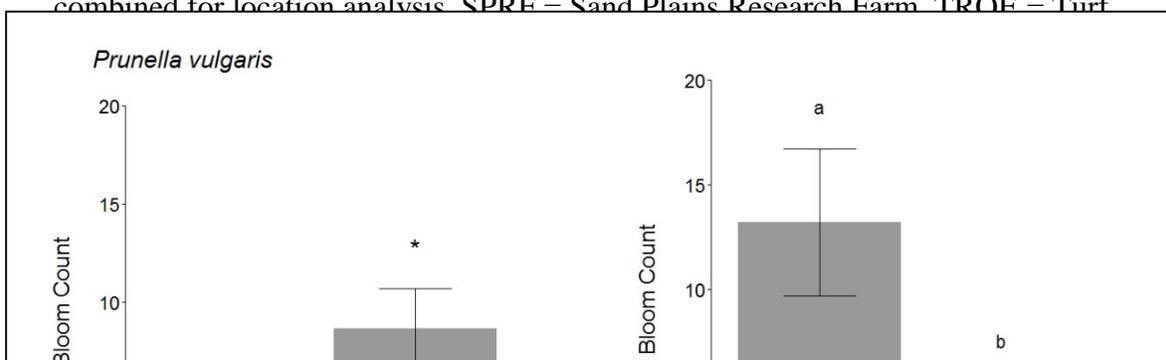


Figure 2. Mean leafing unit counts for forbs seeded with fine fescue. Since forb vegetation was affected by location, but not by mowing height, mowing height treatments were combined for location analysis. SPRF = Sand Plains Research Farm, TROE = Turf



Chapter 4

Pre-Planting Disruption of Mature Kentucky Bluegrass (*Poa pratensis*) Lawns to Establish Flowering Lawns for Pollinators

Introduction

Pollination is an important ecosystem service that is valuable to agriculture (Southwick and Southwick 1992, Losey and Vaughan 2006, Rader et al. 2015) and natural systems (Grubb 1977, Ollerton et al. 2011). One group of pollinators, bees (Hymenoptera: Anthophila), have been of particular concern due to rapid declines of many species over the past 40 years (Goulson et al. 2015). These declines have generated increased public concern and an interest in their conservation.

One of the primary methods of conserving pollinators is through the addition of forbs that provide attractive and nutritious pollen and nectar resources in the landscape (Murray et al. 2009). This method has proven effective in increasing the abundance and species richness in a variety of land use type: including farm borders (Morandin and Kremen 2013, Blaauw and Isaacs 2014), roadside margins (Noordijk et al. 2009, Hopwood et al. 2010), green roofs (Braaker et al. 2014), and neighborhood gardens (Pawelek et al. 2009). Despite this progress, modifying our landscapes to meet both anthropogenic goals and biodiversity conservation in tandem remains an important area of research, and has been coined reconciliation ecology (Rosenzweig 2003).

Reconciliation ecology is a method of dealing with the seemingly antagonistic goals of natural habitat preservation and economic development. Achieving these disparate goals is increasingly important, given that nearly 50% of U.S. land has been converted to some form of anthropogenic use (crops, pasture, urban, roads) (Nickerson et al. 2007). While urban areas occupy roughly 3% of total US land area, the urban landscape is the fastest growing land use category, having quadrupled its land cover since 1945. The challenges and opportunities of conserving nature in urban habitats have been well reviewed (McKinney 2002, Goddard et al. 2010), and will become increasingly important as 66% of the world's population is projected to live in cities by 2050 (United Nations, 2014). Reconciliation ecology seems particularly well suited for urban environments where human land use is most intense, and permanent nature preserves seem unlikely.

One urban landscape feature that has a multifunctional opportunity is the turf lawn. Lawns are estimated to cover roughly 1.9% of the continental U.S., and represent a large proportion of area considered to be urbanized (Milesi et al. 2005). Lawns are classically managed as monocultures of turfgrass species, but can host a variety of flowering forbs and grasses that are often considered weeds. In the American Midwest, these plants are predominantly of European origin (Whitney 1985), but have been found to support a wide variety of bee species (Larson et al. 2014). While lawn cultural practices typically aim to remove flowering plants, there is evidence that public tolerance for some classic lawn weeds is high (Dahmus and Nelson 2013).

The survey of flowering plants in lawns by Larson et al (2014) highlights an increasing interest in non-traditional landscapes such as flowering lawns, but cultural methods for establishing and managing such areas are lacking. While some work has focused on creating new plantings of turf together with forbs (Laskey & Wakefield 1978, Lane chapters 2 & 3), uniformly establishing new flowering species into mature lawns can be difficult due to competition with the turf (Ross and Harper 1959). There have been recent efforts by researchers to evaluate techniques for introducing *Trifolium repens* (white clover) into mature lawns for the benefit of pollinators (McCurdy et al. 2013) the nitrogen provided to turfgrasses through nitrogen fixation (Sincik and Acikgoz 2007, Sparks et al. 2015). In the study done by Sparks et al., *T. repens* was overseeded into lawns that had been treated with scalping (mowing below a height of 3.8 cm), core aeration (removal of a multiple cores of soil to allow for oxygen infiltration into the root zone), and vertical mowing (a process in which rotating blades cut into soil to remove thatch buildup) to test if any of these lawn practices would improve establishment. The purpose of these treatments is to disrupt the turf canopy to allow more *T. repens* seed to achieve adequate soil contact and sun exposure needed for germination and establishment. Their finding suggest that these treatments all improved establishment of *T. repens* over taking no action, with the best results coming from scalping treatments.

While the results are encouraging, these types of establishment methods need to be evaluated for other species of forbs with the goal of creating florally diverse plantings. Floral diversity is one of the key factors organizing pollinator species composition (Potts et al. 2003, Ebeling et al. 2008), with native plants often being the most beneficial

(Frankie et al. 2005, Isaacs et al. 2009, Pardee and Philpott 2014). To further develop establishment methods for the integration of flowering plants into lawns, we selected three flowering species to incorporate into mature *Poa pratensis* (Kentucky bluegrass) stands, a common lawn species in the Midwestern United States. These species include *Trifolium repens*, *Prunella vulgaris* ssp. *lanceolata*, and *Thymus serpyllum*, which have all shown the ability to establish and bloom in lawns in Minnesota (Lane chapter 3). *P. vulgaris* ssp. *lanceolata* and *T. serpyllum* are both in the Lamiaceae with commercially available seed, but little work has been done relating to cultural practices for their use in lawns. *P. vulgaris* ssp. *lanceolata* is indigenous to the United States and may have value as a pollinator plant, but it has not been tested in a turf setting. There is also evidence that management factors (i.e., mowing height and nutrient availability) could impact the establishment of these plants (Lane chapter 3). To that end we predicted that pre-planting disruption treatments such as scalping, aeration, or a combination of the two would improve forb establishment by reducing grass competition during establishment

Methods

Experimental design and establishment

We chose two sites with extant *Poa pratensis* lawns for this study: the Victory Links golf course in Blaine, Minnesota and StoneMill Farms North Park in Woodbury, MN. The sites were selected primarily because of their large continuous tracts of *P. pratensis* lawns, and cooperation from lawn managers.

On May 21, 2015, each site was subjected to three types of disruption: 1) scalping (mowing of the lawn below 3.8 cm); 2) hollow tine aeration; and 3) a combination of both scalping and aeration. Additional areas were left untreated during seeding to serve as a control group. Disruption treatments and forb species were arranged into a split-block design for ease of applying disruption treatments and subsequent over-seeding of forbs. Each block of the experimental design contained parallel strips, 1.22m wide, running from north to south, of the three disruption treatments and an untreated control strip (Fig. 1).

After disruptions were applied, forb species were added in a given block of treatments through drop seeding in a 1.22m wide strip perpendicular (east to west) to the treatment strips. Forb strips included a 0.3m border separating parallel species strips to avoid species spillover, and the strips were seeded immediately following disruption. Each forb species was seeded at a rate of 1,346 seeds·m⁻², or 2000 seeds in a plot. Drop seeding was conducted by combining seed of a given species for the entire study site with 4.65 kg of fertilizer (7N-3.1P-5.8K) to facilitate even and accurate distribution of seed across study plots. The amount of nitrogen added during seeding was extremely low, amounting to a total of 0.83 g of nitrogen·m⁻² at each study site.

Disruption treatment and species treatment blocks were repeated four times for a total of 16 experimental blocks, each containing nine 1.5 m² plots with a unique combination of disruption and forb species per block (Fig 1).

Site Conditions and Management

Soil properties were derived from aggregate soil samples taken over the entire study area at each site by the University of Minnesota Soil Testing Laboratory. Texture was determined using the 'hydrometer method', and organic matter content was determined through the 'loss of ignition method' (LOS).

Experimental plots located at Victory Links golf course were situated within a large area maintained as a sod nursery for fairway repairs. This area, including study plots, was under management by the golf course for the entire growing season and was subject to more intense management techniques (i.e. fertilizing, mowing, and irrigation) to keep turf quality high. Inputs of 0.005 kg of nitrogen·m⁻² (25-0-5 analysis) and 20 cm of supplemental irrigation were applied over the course of the growing season. The site was mowed every week to a height of 6.3 cm, as is typical in golf course lawn management. Soil texture at Victory Links was a sandy clay loam (70% sand, 8.8% silt, 21.3% clay), with a soil organic matter (SOM) content of 6.3% and a pH of 7.3.

The other experimental plots were located at StoneMill North Park, managed by the Woodbury parks and recreation service. The park service halted input use and mowing of the research area during the course of this experiment, which was assumed by us at the start of this study. The site received no supplemental fertilizer or irrigation, and was typically mowed once every two weeks to a height of 7.62 cm. Soil texture at StoneMill North is a clay loam (28.8% sand, 38.8% silt, 32.5% clay), with a SOM content of 2.7% and a pH of 6.8.

Data Collection and Analysis

Data were collected from both sites over a two-day period during the first week of September, 2015. A meter-by-meter quadrat with plastic grid wires subdividing the quadrat into twelve 40 mm² sections used to subsample plots. Vegetative structures of the target species were counted within nine squares that crossed the quadrat from corner to corner forming an 'x' shape. Subsampling was used due to the high densities of vegetative structures that occurred in plots, including trifoliolate leaves for *T. repens*, basal rosettes for *P. vulgaris* ssp. *lanceolata*, and stems for *T. serpyllum*. The numbers of broadleaf weeds in the quadrat were also counted to determine if disruption treatments affected weed establishment.

Forb species were analyzed separately and mixed effects ANOVA was used to test for interactions between site and treatment. Mixed effects ANOVA was conducted in the R statistical environment using the package 'nlme', with the treatment by site interaction considered a fixed effect, and block considered a random effect to account for error due to spatial arrangement. If interaction terms were found to be significant at the $\alpha = 0.05$ level, a separate analysis was conducted of that species at each site. A Tukey's mean separation procedure using the R package 'multcomp' was used to determine significant differences between treatments at the $\alpha = 0.05$ level.

Results

Location, treatment, and the interaction terms all had significant effects on forb species density (Tab. 1); thus sites were considered separately for all species. Forb

establishment was higher at StoneMill North than at Victory Links (Fig. 1), regardless of treatment.

Forb establishment after different disruption treatments varied by both species and location. Although forb establishment at Victory Links was relatively low overall, *T. repens* and *P. vulgaris* ssp. *lanceolata* (Fig. 2) established in significantly higher amounts in both scalping and scalp/aeration treatments than in aeration treatments or no disruption (control). Aeration alone seemed to have little impact on establishment of these forb species when compared to undisrupted control plots. For *T. serpyllum*, the scalp/aeration disruption was the only treatment that led to significantly higher establishment than in the undisrupted control (Fig. 2), indicating a potential benefit of aeration when combined with scalping for this forb. Forb establishment responses to treatments at Victory Links golf course typically supported our hypothesis that pre-seeding disruption of turf can reduce turf competition on newly seeded forbs, resulting in a higher rate of floral establishment. However, the highly managed (fertilized and mowed) golf course did not provide acceptable conditions for establishment of any forb species we tested.

Forb establishment at StoneMill North was generally higher, and varied among species. *T. repens* established at higher amounts in StoneMill North than at Victory Links, and the response to disruption treatments was similar. Scalped and scalped/aerated treatments yielded higher trifoliolate leaf counts compared to aeration alone or no disruption (Fig. 2). In contrast, *P. vulgaris* ssp. *lanceolata* had a significantly higher basal rosette counts in the control plots than in the scalp and scalp/aeration treatments, with aeration having an intermediate effect. In *T. serpyllum* plots, there were significantly

higher stem counts in the scalping treatment compared to scalping/aeration or aeration alone, but disruption treatments did not result in increased establishment of creeping thyme compared to control plots. The pattern of establishment of white clover at the StoneMill North was similar to that of Victory Links, but this was not the case for *P. vulgaris* ssp. *lanceolata* or *T. serpyllum*.

Discussion

My results indicate that *T. repens* established at higher rates in disruption treatments that involved scalping at both research locations. Additionally, *P. vulgaris* ssp. *Lanceolata* establishment was aided by treatments that involved scalping, and *T. serpyllum* establishment was improved by the combination of scalping and aeration at the Victory Links site. The differences in establishment between sites were likely due to differences in management history and management during establishment, with Victory Links receiving supplemental nitrogen and irrigation, and had more frequent mow schedule. Previous studies have found negative relationships between increasing nitrogen application rates and establishment of non turfgrass plants (Busey 2003, Kohler et al. 2004, Busey and Johnston 2006). While this seems to be the case with many species and nitrogen fertilizer, high amounts of soil nutrients, such as potassium, can actually favor the establishment of some species (Tilman et al. 1999). While our results and previous research support that increasing nutrient applications in lawns negatively impacts non-turf plant establishment, this finding does not apply to all species.

Mowing height and frequency also affect the establishment of flowers in turf.

While the impact of mowing on non-turf plant species varies by study and by plant (Busey 2003, Abu-Dieyeh and Watson 2005), most studies demonstrate that intensive mowing (either frequency, height, or both) is detrimental to lawn community richness (Thompson et al. 2004, Shwartz et al. 2013, Garbuzov and Ratnieks 2014). None of the forbs used in the present study (Lane, Chapter 3) were significantly impacted by mowing heights of 6.35 cm or 8.89 cm when mowed on a height-based schedule designed to never exceed 1/3 their assigned height. This schedule resulted in mowing about once every 2.5 weeks, substantially less than the weekly mowing conducted at Victory Links. These results are encouraging as reducing inputs (i.e. mowing and fertilizer) and increasing the value of lawns to wildlife is one the primary goals of promoting flowering lawns for conservation.

Responses to pre-planting disruption at Victory Links supported our hypothesis that disruption would aid forb establishment. These results also support research conducted by Sparks et al. (2015), which suggested that scalping aids the establishment of *T. repens* in cool season lawns in Lexington, KY. Results from Victory Links also support research on over-seeding *T. repens* into dormant warm-season grasses in Auburn, AL (McCurdy et al. 2013) where treatments that included scalping improved *T. repens* establishment.

Establishment in response to pre-planting disruption at StoneMill North had mixed results. While *T. repens* had higher establishment at StoneMill North than Victory Links, the response to disruption was similar, with higher average trifoliolate counts in

scalped and scalped + aerated plots. This finding supports our hypothesis that we would see higher establishment in plots with some form of disruption, but this was not the case with *P. vulgaris* ssp. *lanceolata* and *T. serpyllum*, for which the untreated control plots had average plant counts that were similar to any of the disruption treatments.

In the case of *P. vulgaris* ssp. *lanceolata*, we saw significantly higher establishment in control plots than in scalping and scalping+aeration plots. It is unclear why *P. vulgaris* ssp. *lanceolata*, but not *T. repens*, would be detrimentally impacted by scalping treatments at StoneMill North, as both plants have seeds of similar size and similar growing environments. It is possible that differences in biology and growing habit of these plants may have an unforeseen interaction with lawn scalping. For example, growing habits of *T. repens* may have provided earlier ground cover and prevented excessive evaporation from scalped plots. Scalped plots would potentially be more prone to water loss due to the more open canopy and lack of shade. *Prunella vulgaris* ssp. *lanceolata*'s high rate of establishment at StoneMill North suggest that management and competition for space may have been less a limiting factor than at Victory Links. Our disruption treatments were intended to reduce competition and create opportunities for seedling establishment not present in dense lawn stands. Results suggest that when management and turfgrass competition is less intense, such as the case for StoneMill North, there is no benefit to pre-seeding disruption (and it may even be detrimental) to *P. vulgaris* ssp. *lanceolata* establishment.

Our results indicated that pre-seeding disruption might also negatively impact the establishment of *T. serpyllum*. Establishment of *T. serpyllum* at StoneMill North was high

compared to Victory Links, but establishment response to pre-seeding disruption was very different between sites. There was no discernable pattern of establishment at StoneMill North except that treatments involving aeration had lower establishment. This finding differed from Victory Links, where only the treatment combining aeration and scalping had any impact on establishment when compared to the untreated control. The results suggest that for *T. serpyllum*, as with *P. vulgaris* ssp. *lanceolata*, pre-seeding disruption is effective only under conditions with high management and turfgrass density, but neutral to deleterious under low maintenance conditions.

Overall, these results indicate that pre-seeding disruption is not as important in the establishment of forbs in turf as is management intensity of the turf. Reducing lawn management, combined with proper establishment techniques, should result in a flowering lawn that requires substantially less input (i.e. fertilizer, mowing, and irrigation) and provides more ecological value than a typical turf lawn. Future work could include: trials designed to test the effect of management intensity and history on forb establishment. Additionally, our data represent only one field season of data, and thus further replication and longer-term studies would help to interpret our findings.

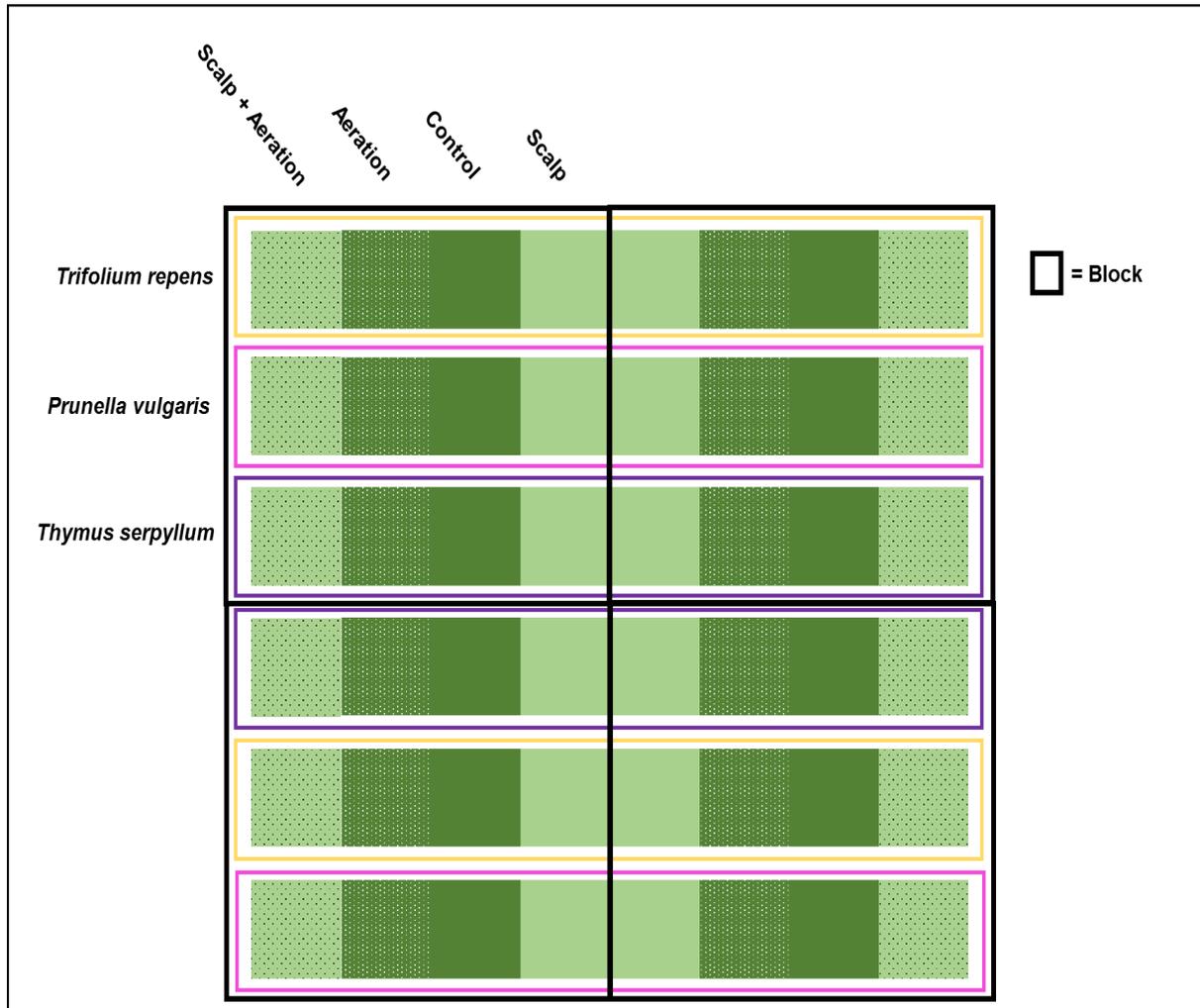


Figure 4: Graphical representation of part of the experimental set-up. Species were seeded in strips from East to West and randomized within blocks from top to bottom with a 0.03 m border between each species strip. Disruption treatments were applied from North to South and randomized within blocks from left to right. This figure depicts four blocks, but each study had a total of 16 blocks.

Table 5. Results from ANOVA testing the effect of study location, pre-seeding disruption treatment, and their interaction for the three forbs used in this study.

Factor	Degrees of Freedom	<i>F</i>	<i>p</i> -value
<i>White clover</i>			
Location	1,30	131	< 0.001*
Treatment	3,90	59.1	< 0.001*
Treatment x Location	3,90	5.4	0.002*
<i>Lanceleaf self-heal</i>			
Location	1,30	27.6	< 0.001*
Treatment	3,90	5.1	0.003*
Treatment x Location	3,90	23.3	< 0.001*
<i>Creeping thyme</i>			
Location	1,30	131	< 0.001*
Treatment	3,90	7.3	< 0.001*
Treatment x Location	3,90	5.7	0.001*

(*) = Denotes significant result at $\alpha = 0.05$

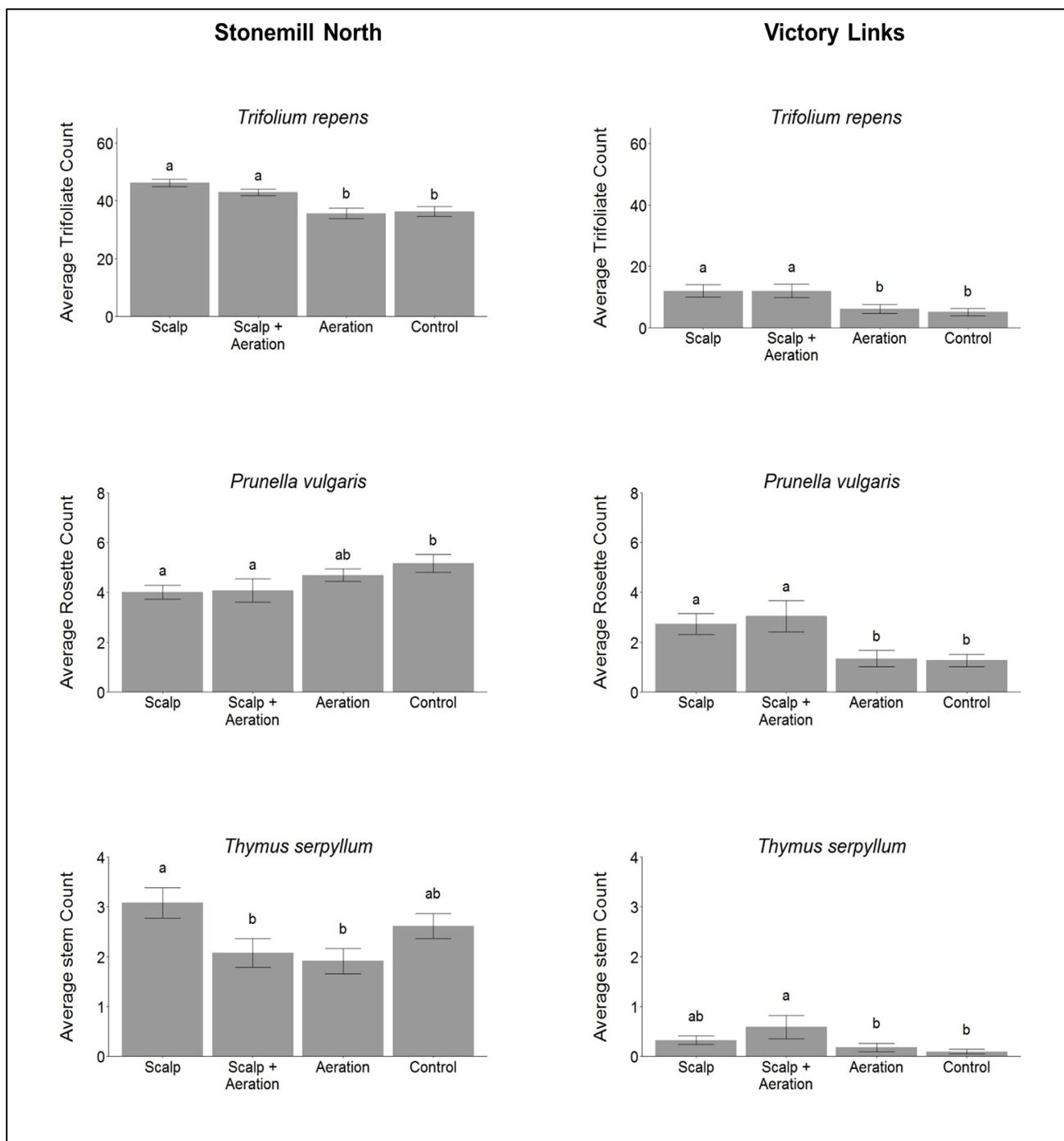


Figure 5: Average vegetative count per sub-sample square for each pre-seeding disruption treatment for: *T. repens*, *P. vulgaris* ssp. *lanceolata*, and *T. serpyllum* at StoneMill North (Left column) and Victory Links (Right Column). Error bars represent standard error of the mean, and letters indicate significant differences between disruption treatments.

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Appendix A

A1: List of parks in Minneapolis, MN where bees were collected from *Trifolium repens* blooming in turf

Park	Quadrant	Park Area (ha)	Number of Surveys
Windom	North East	3.3	8
Logan	North East	4.2	7
Audubon	North East	2.3	6
Matthews	South East	4.0	7
Longfellow	South East	3.3	7
Powderhorn	South East	26.7	1
Bancroft Meadows	South East	1.8	4
Washburn Fair oaks	South West	3.1	8
Painter	South West	1.2	7
Kenwood	South West	13.3	6
Farview	North West	8.5	8
Willard	North West	0.5	6
Hall	North West	2.4	6

A2: Number and species of bees collected during surveys of *Trifolium repens* blooming in turfgrass lawns. All lawns were located in the neighborhood parks of Minneapolis, MN.

Family	Species	Number	Native(Y/N)
Andrenidae	<i>Andrena commoda</i>	1	Y
	<i>Andrena dunningi</i>	1	Y
	<i>Andrena nivalis</i>	1	Y
	<i>Andrena vicina</i>	4	Y
	<i>Andrena wilkella</i>	157	N
	<i>Andrena wilmattae</i>	4	Y
	<i>Calliopsis andreniformis</i>	125	Y
Apidae	<i>Apis mellifera</i>	570	N
	<i>Bombus bimaculatus</i>	9	Y
	<i>Bombus fervidus</i>	43	Y
	<i>Bombus griseocollis</i>	11	Y
	<i>Bombus impatiens</i>	328	Y
	<i>Bombus rufocinctus</i>	29	Y
	<i>Bombus ternarius</i>	1	Y
	<i>Bombus vagans</i>	2	Y
	<i>Nomada articulata</i>	1	Y
	<i>Nomada luteoloides</i>	1	Y
Halictidae	<i>Agapostemon sericeus</i>	23	Y
	<i>Agapostemon texanus</i>	12	Y
	<i>Agapostemon virescens</i>	9	Y
	<i>Augochlorella aurata</i>	10	Y
	<i>Halictus confusus</i>	23	Y
	<i>Halictus ligatus</i>	1	Y
	<i>Halictus rubicundus</i>	63	Y
	<i>Lasioglossum admirandum</i>	3	Y
	<i>Lasioglossum coriaceum</i>	1	Y
	<i>Lasioglossum forbesii</i>	2	Y
	<i>Lasioglossum lineatulum</i>	2	Y
	<i>Lasioglossum</i>	2	Y
	<i>Lasioglossum paraforbesii</i>	1	Y
	<i>Lasioglossum pilosum</i>	1	Y

	<i>Lasioglossum pruinosum</i>	1	Y
	<i>Lasioglossum tegulare</i>	1	Y
Megachilidae	<i>Anthidium oblongatum</i>	23	N
	<i>Megachile frigida</i>	1	Y
	<i>Megachile latimanus</i>	4	N
	<i>Megachile rotundata</i>	7	N
