CENTIPEDEGRASS POLLEN: A RESOURCE FOR FORAGING BEES

by

OLUWATOMI DANIEL IBIYEMI

(Under the Direction of Shimat V. Joseph and David Jespersen)

ABSTRACT

This study aimed to investigate the role of centipedegrass (*Eremochloa ophiuroides*) as a pollen resource for bees and to explore bee foraging behaviors in centipedegrass lawns. When the pollen load composition of bees foraging on centipedegrass spikes was examined, bees, including *Bombus* spp., *Apis* spp., and *Lasioglossum* spp., predominantly collected Poaceae pollen, with significantly greater counts during early flowering in 2021. In 2022, there were no significant differences between early and late flowering phases. The pollen transfer to hives was examined, and data showed that bees transferred the collected Poaceae pollen to hives. When the centipedegrass pollen utilization was examined, the development of bumble bee larvae on centipedegrass pollen was similar to those developed on butterfly bush pollen, suggesting that centipedegrass pollen can support larval development. The foraging behaviors of bees were characterized by evaluating the video recordings, and data showed that Bombus spp. exhibited more crawling and tongue protrusion behaviors, whereas *Lasioglossum* spp. dangled on centipedegrass anther for pollen collection. Despite the availability of pollen from dicot plants with showy flowers, bees continued to forage on centipedegrass pollen. These findings underscore the ecological importance of centipedegrass in supporting pollinator populations and provide insights for turfgrass management and pollinator conservation efforts in urban, suburban, and rural landscapes.

INDEX WORDS: *Eremochloa ophiuroides*, centipedegrass pollen, pollinator ecology, bee foraging behavior, grassland ecosystems, turfgrass management, pollinator conservation, dicot flowering plants

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OLUWATOMI DANIEL IBIYEMI

B.TECH., FEDERAL UNIVERSITY OF TECHNOLOGY, AKURE, NIGERIA, 2014

M.S., AUBURN UNIVERSITY, ALABAMA, 2020

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OLUWATOMI DANIEL IBIYEMI

Major Professor:	Shimat V. Joseph David Jespersen
Committee:	Karen Harris-Shultz Michael Ulyshen Brian Schwartz

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Turfgrass is a perennial plant maintained at a low height, providing a contiguous ground cover. Turfgrass is planted and maintained at various sites, including low-maintenance sites, such as roadsides, to highly managed areas, such as golf courses, athletic fields, and recreational spaces (Larson et al., 2017). Turfgrasses is the largest crop in the United States, covering over 16.4 million ha (Milesi et al., 2005). They provide numerous environmental benefits, such as preventing erosion, filtering pollutants, cooling the environment, reducing glare and noise, heat reduction, and reducing carbon sequestration (Beard and Green, 1994; Milesi et al., 2005). Turfgrass utilization can be traced back to ancient civilizations, where its primary role was to enhance the aesthetic of ornamental gardens (Beard and Green, 1994).

Turfgrass breeding and genetics advancements have revolutionized the industry, giving rise to varieties with improved traits, such as enhanced disease resistance and drought tolerance (Carrow et al., 2010; Carey et al., 2012). The accelerated pace of research and development in the field has yielded extensive knowledge concerning optimal cultivation techniques, such as nutrient, pest, and disease management strategies (Turgeon, 2008; Carrow et al., 2010).

In warmer climates, warm-season turfgrasses are a notable component of landscapes, particularly in the southern United States. These turfgrasses are highly adaptable to high temperatures, low moisture levels, and variable soil conditions

(Turgeon, 2008). Among various warm-season turfgrass, centipedegrass is a low-maintenance, particularly suited for acidic soils (Hanna, 1995). It thrives in full sun to partial shade and is often recommended for low-fertility soils where other grasses may struggle. Centipedegrass forms a low, dense canopy, making it an excellent choice for erosion control in sloped landscapes (Braman and Pendley, 1993). The inflorescence of centipedegrass is a slender and slightly curved raceme, 3-10 cm long. Each raceme bears spikes. Centipedegrass prefers well-drained, sandy, and acidic soils, with optimal pH between 4.5 and 6.0 (Potter and Braman, 1991; Braman and Pendley, 1993). It exhibits moderate drought tolerance but will require supplemental irrigation during prolonged dry spells. In terms of maintenance, centipedegrass needs limited mowing operation and fertilization compared to other warm-season grasses (Hanna, 1995; Islam and Hirata, 2005).

Pollination

Pollination is the transfer of pollen from male to female reproductive organs, ultimately leading to fertilization and seed set. It is an important event for the survival of flowering plants as it impacts the function of ecosystems and food production (Roulston and Goodell, 2010; Ollerton et al., 2011). Plants have evolved various pollination strategies, including biotic, abiotic, or a combination of both. Biotic pollination involves the roles of organisms in transferring pollen from one flower to another. Insects are a major group of organisms that are passively involved in pollination, referred to as entomophily or insect pollination. Many insects, such as bees, flies, butterflies, and beetles, serve as insect pollinators. These insects are often attracted to flowers through visual cues and olfactory signals, which facilitate a mutualistic relationship where the plant offers nectar and sometimes pollen as a nutrition resource for foragers, which are used for their progeny in the nest (Potts et al., 2010; Ollerton et al., 2011).

Anemophily involves wind as an agent to disperse pollen grains and is a major abiotic pollination. Plants that have evolved with anemophily are often grasses and many trees, typically producing large quantities of lightweight, airborne pollen designed to be easily carried by wind currents (Culley et al., 2002; Friedman and Barrett, 2009). Ambophily is a blend of these pollination strategies, where a single plant species may use biotic and abiotic mechanisms to ensure successful reproduction. This dual strategy is often seen as an evolutionary adaptation to maximize the chances of fertilization under varying environmental conditions (Culley et al., 2002; Friedman and Barrett, 2009). Thus, pollinators involved in pollination services for plants are critically important as plants depend on plant reproduction, seed dispersal, and genetic diversity.

Understanding the strategies of pollinators and plant adaptations is important to conserve the pollinators. Their activities are integral to food web dynamics, biodiversity, and overall ecosystem health and resilience (Abrahamczyk et al., 2023; Stephens et al., 2023). Thus, the conservation of pollinators is necessary to ensure the survival and proliferation of many plant species, as the loss or decline in pollinators can have serious economic and ecological ramifications, affecting agriculture, forestry, and natural habitats (Potts et al., 2010; Ollerton et al., 2011).

Entomophily: Role of Bees in Pollination

Bees are crucial in pollinating flowering plants, ensuring the reproduction and survival of various plant species (Saunders, 2018; van Vierssen Trip et al., 2020). In agricultural ecosystems, bees are vital for pollination and producing fruits, nuts, and vegetables successfully. Bees contribute approximately 80% of insect pollination, making them crucial for crop production (Klein et al., 2006; Ollerton et al., 2011). In addition, bees also play a crucial yet often overlooked role in enhancing the biodiversity of many native plants, which support the overall health of ecosystems (Szawarski et al., 2019).

Bees have co-evolved with flowering plants for millions of years, developing specialized adaptations that efficiently gather nectar and pollen from flowers (Barbir et al., 2015). The mutualistic co-evolution between bees and angiosperm plants has shaped the intricate plant-pollinator network. Through morphological, physiological, chemical, and behavioral adaptations, bees and plants have developed mechanisms to promote successful pollination (Barbir et al., 2015; Stephens et al., 2023). Their hairy bodies and branched hairs, known as scopae, enable them to collect and carry large amounts of pollen. The specialized mouthparts of bees, such as their long tongues and proboscis, allow them to reach the nectar at the base of flowers (Barrios et al., 2016). These unique adaptations and behaviors of bees make them highly efficient and effective pollinators.

In recent years, there has been growing concern about the decline of bee populations and its potential implications on pollination and food security (Bencsik et al., 2015). The decline in bees, especially bumble bees, has threatened food production and the stability of the ecosystem (Goulson et al., 2008; Bencsik et al., 2015; Kovács-Hostyánszki et al., 2017). The intensification of agriculture and expanding urbanization have destroyed the nesting and foraging habitats of bees (Goulson et al., 2015). Losing floral richness and diversity is also an important factor impacting bee populations. The decline of wildflowers due to land conversion, monoculture, and urbanization reduces the availability of food resources for bees. The spread of parasites and diseases is a major issue contributing to the decline of bee populations. For instance, the varroa mite infestation weakens honey bee colonies and spreads diseases (Larson et al., 2017). The use of pesticides in agriculture has been linked to the decline of bees (Larson et al., 2017). Although most fruit, nut, and vegetable crops depend on bee pollination, the chemicals are applied to the crops for protection from pests that threaten the existence of bees. The decline in bee health and reproduction after neonicotinoid exposure is well documented (Woodcock et al., 2017). Climate change is another factor that aggravates bee decline. Changes in precipitation and temperature patterns disrupt the timing of blooming patterns, affecting the availability of pollen and nectar for the foraging bees (Kerr et al., 2015).

Losing these pollinators can seriously affect food security and directly impact human welfare. Global research has provided a range of evidence, both broad in scope and case-specific, to support the need for pollinator conservation (Goulson et al., 2008; Hobbs et al., 2009; Owen, 2016). Promoting policies that address bee decline by providing biodiverse floral resources for bees in farmland and reducing the use of pesticides can help. Moreover, increased awareness and education on the importance of bees in creating more green spaces that may serve as habitats for bees through urban planning is critical.

Anemophily (Wind Pollination)

Wind pollination, also known as anemophily, refers to the process of using wind to disperse pollen from the male part (anther) of a flower to the female part (stigma) of the same or other flowers, facilitating fertilization (Ackerman, 2000). Employed by approximately 12% of plant species worldwide, wind pollination is considered a common reproductive strategy, especially for plants with inconspicuous flowers or those in open habitats (Friedman and Barrett, 2009).

Wind-pollinated plants have evolved certain distinctive traits. They tend to have small, non-showy flowers because they do not need to attract pollinators. Instead, their flowers are structured to efficiently release and capture airborne pollen (Peeters and Totland, 2011). These plants also produce large quantities of light, dry pollen that the breeze can easily carry. Unlike insect-pollinated plants, most wind-pollinated species do not produce nectar since they do not need to attract insects (Ackerman, 2000). There are distinct examples of wind-pollinated plants in various ecosystems. Grasses and many tree species use wind pollination exclusively, a trait that opportunistically provides supplemental pollen as an alternative food resource for various bees (Severns and Moldenke, 2010).

Wind pollination also offers benefits, such as greater dispersal and adaptability to environments where insect pollinators are scarce, such as remote or high-altitude areas (Friedman and Barrett, 2009). However, it also has seen some limitations, as wind pollination requires producing large amounts of pollen because only a small fraction will reach a compatible female flower (Bolmgren et al., 2003). In addition, wind-pollinated plants are more susceptible to pollen wastage and competition, as the wind can carry pollen far away from its intended destination, and there may be a high level of overlap between the pollen grains of different plants (Saunders, 2018). Wind-pollinated plants may also face challenges in fragmented habitats. Habitat fragmentation can reduce pollination success for wind-pollinated species due to decreased pollen availability. This is because wind-pollinated plants typically have a high pollen-to-ovule ratio and limited pollen viability compared to animal-pollinated plants (Gong et al., 2016; Khan et al., 2023). As a result, wind-pollinated species may be pollen-limited, especially in fragmented habitats with fewer individuals within proximity for effective pollen transfer (Saunders, 2018).

Nonetheless, recent research has revealed interesting aspects of the relationship between wind-pollinated plants and bees (Jones, 2014; Saunders, 2018; Joseph et al., 2020). An intriguing proposition is that wind-pollinated plants could be incorporated into bee conservation strategies, potentially providing food resources when the pollen supply from traditional sources is lacking (Saunders, 2018). In particular, grass and certain tree species could be advantageous in this context because they produce large pollen volumes due to their wind-pollination mechanism.

Ambophily

The phenomenon of ambophily in plants—utilizing both biotic (often insect-mediated) and abiotic (usually wind-mediated) methods for pollination—represents an intriguing ecological and evolutionary development (Culley et al., 2002; Friedman and Barrett, 2009). This mixed mode of pollination has been recognized by scientists for over 130 years, but our understanding of ambophily is still limited and experimental data on this topic remain scarce (Ríos et al., 2014; Abrahamczyk et al., 2023; Khan et al., 2023). This section explores the mechanisms of ambophily, its evolutionary advantages, and its implications for the conservation of pollinators.

Pollination strategies typically bifurcate into biotic and abiotic mechanisms and have long been of interest in ecological and evolutionary biology (Ollerton et al., 2011). Entomophily and anemophily are well-studied paradigms (Friedman and Barrett, 2009;

Potts et al., 2010). However, some plant species employ a combination of these methods, known as ambophily, thereby optimizing reproductive success. Ambophily has been documented in various plant species from different families and geographical locations, indicating its wide occurrence in nature (Culley et al., 2002; Friedman and Barrett, 2009).

Ambophilous plants produce copious amounts of pollen and flowers adapted for entomophily or anemophily. For example, certain flowers may produce nectar to attract insect pollinators while simultaneously generating lightweight pollen that can be dispersed by wind (Culley et al., 2002). This dual strategy often appears as an evolutionary response to fluctuating environmental conditions, allowing plants to maximize their chances of successful reproduction (Friedman and Barrett, 2009).

One of the critical questions surrounding ambophily is whether it represents a transitional state in plant evolution (Culley et al., 2002). One hypothesis suggests that wind pollination might arise in combination with insect pollination, either sequentially or simultaneously, within a single growing season (Khan et al., 2023). This hypothesis suggests that ambophily may be a transitional stage between primarily insect-pollinated plants and primarily wind-pollinated plants. This transition could occur due to various factors, such as changes in environmental conditions or the availability of pollinator species. For example, in some ambophilous species, such as those from the Salicaceae and Arecaceae families, which were previously thought to be wind-pollinated, the relative frequency of insect and wind pollination can vary from predominantly insect-pollinated to mostly wind-pollinated (Ríos et al., 2014; Abrahamczyk et al., 2023). This variability in the relative frequency of pollination methods suggests that ambophily may represent a transitional state in plant evolution. There are counterarguments to the hypothesis that ambophily represents a transitional state. Ambophily may not necessarily indicate a transitional state but rather a strategy for maximizing pollination success in particular habitats (Friedman, 2011). For example, in habitats where both wind and insect pollinators are present, but their abundance or activity varies throughout the season, ambophily may allow plants to take advantage of both pollination methods to ensure successful reproduction (Abrahamczyk et al., 2023). Some species show a consistent intermediate frequency of wind and insect pollination, suggesting that ambophily may be stable for specific plants. Despite lacking definitive evidence, some researchers argue that ambophily may be a stable and adaptive plant strategy in variable environments (Ríos et al., 2014; Gong et al., 2016).

Ambophily offers unique advantages for bee pollinators regarding resource allocation and foraging efficiency. In plants employing ambophily, floral structures often produce nectar and other attractants as rewards for biotic pollinators, such as bees (Goodwillie et al., 2010; Potts et al., 2010). This suggests that bees can still access nectar sources, optimizing their foraging routes, and energy expenditure during low wind dispersal. Ambophily increases the chances of successful reproduction and the spread of genes, and makes plants more resilient to varying environmental conditions (Roulston and Goodell, 2010).

Bees participating in the pollination of ambophilous plants contribute to more significant genetic variability. While wind pollination can result in the mixing of alleles at a broader geographic scale, bees typically facilitate gene flow locally. This dual mechanism enhances genetic diversity, leading to more resilient plant populations that provide stable forage for bees across seasons (Culley et al., 2002; Goodwillie et al., 2010). This can be advantageous for plant populations in terms of increased adaptability to changing environmental conditions and resistance against diseases or pests.

The ambophilous pollination strategy indirectly buffers against environmental variables affecting bee populations, such as climate change and habitat fragmentation. During periods when climatic conditions make wind pollination more effective, there is reduced pressure on bee populations to serve as the exclusive agents of pollination. This adaptability allows bee populations to recover or stabilize during unfavorable conditions (Potts et al., 2010).

Understanding the ecological implications of ambophily is important for developing effective bee conservation strategies. Ambophily is an ecological insurance, offering a contingency that could alleviate pressure on declining bee populations. By conserving and promoting ambophilous plants in ecosystems, one could create more resilient foraging habitats for bees, thereby indirectly contributing to their conservation (Goodwillie et al., 2010; Saunders, 2018). The relative frequency of wind and insect pollination in ambophilous plants can vary, with some species exhibiting predominantly insect pollination and others mostly wind pollination (Culley et al., 2002). This variation in the frequency of ambophily can be influenced by several factors. Firstly, the characteristics of the plant species itself can play a role. Plant species with traits that make them more attractive to insect pollinators, such as brightly colored flowers or strong scents, may exhibit a higher frequency of insect pollination than wind pollination (Ackerman, 2000; Schulze-Albuquerque et al., 2020). Species with small, inconspicuous flowers and lightweight pollen may rely more on wind pollination (Schulze-Albuquerque et al., 2020). Secondly, environmental factors can also influence the frequency of ambophily. For example, plant species growing in wind-exposed but insect-poor sites may rely more on wind pollination. Those in sheltered habitats with high insect visitation rates may exhibit a higher frequency of insect pollination (Adams et al., 1981; Gong et al., 2016). Additionally, the availability and abundance of pollinators can influence the frequency of ambophily. Insect-pollinator populations can be highly variable, influenced by climate change, habitat fragmentation, and pesticide use. As a result, plant species may adjust their pollination strategy to maximize reproductive success.

The concept of ambophily still needs to be studied despite being acknowledged by scientists for over a century. While its existence has been inferred in various instances, there still needs to be comprehensive experimental data to fully understand this phenomenon. The characteristics and evolutionary significance of ambophilous plants are yet to be understood. However, ambophily may serve as an adaptation strategy for plants residing in environments with varying conditions that could either favor wind or insect pollination (Friedman, 2011; De Luca and Vallejo-Marín, 2013).

Common Bees in the Southeastern USA

The southeastern United States is a region rich in bee diversity, which is crucial for pollinating wild and cultivated plants, maintaining a healthy ecosystem, and achieving agricultural productivity.

1. Honey bees (*Apis* spp.) (Family: Apidae)

Taxonomy and Species Diversity. Honey bees (*Apis* spp.) are an important bee group in natural and agricultural ecosystems. European honey bee, *Apis mellifera* is the predominant species in the United States. It was introduced to the United States from Europe and is now naturalized across various states. Through breeding, subspecies *A.*

mellifera have emerged that thrive in various environmental conditions (Winston et al., 1987).

Morphology and Behavioral Adaptations. Honey bees are eusocial insects with well-developed social structures and hierarchical systems. They are distinguished from other bees with their barbed ovipositor, a wax-producing gland, and a proboscis adapted for nectar collection. They communicate with a unique 'waggle dance' to notify fellow workers about the direction and distance of food sources (Beekman and Ratnieks, 2000).

Lifecycle. Honey bees have a perennial colony where a single colony can survive for several years, led by one queen, thousands of workers, and males (drones). The mated queen constantly lays eggs, whereas workers perform essential tasks, such as foraging, brood care, and nest maintenance. Drones are primarily responsible for mating with a virgin queen (Hepburn and Radloff, 2011) through swarming in mid-air. The mated virgin queen forms a new colony (Winston et al., 1987).

Foraging Behavior and Pollination. Honey bees are exceptional pollinators as worker forage by travel far distances with floral constancy (Seeley, 1997). Honey bees produce honey by mixing the oral enzymes with nectar. They also beeswax, royal jelly, and propolis, and are used for various uses based on its unique properties (Crane, 1990).

2. **Bumble bees (*Bombus* spp.; Family: Apidae)**

Taxonomy and Diversity. Approximately 50 species of bumble bees have been documented in the United States, with distinct variations in size, coloration, and habitat preference. Bumble bees are relatively larger bees than other bees and have dense hair, which helps them with thermoregulation, an adaptation crucial for their survival and pollination efficiency (Heinrich, 1979). They can pollinate by “buzz pollination”, where they vibrate flight muscles to dislodge pollen from flowers representing various plant species (De Luca and Vallejo-Marín, 2013).

Lifecycle and Social Structure. Bumble bees are univoltine; queens mate before overwintering and initiate new colonies each spring. The colony size increases with time. Workers are involved in foraging for pollen and nest maintenance duties. By the fall, new males are produced, and they mate with new queens. The original colony ends and is not carried over to the following year (Goulson et al., 2008). The social structure within bumble bee colonies is characterized by a more flexible hierarchy compared to honey bees. Bumble bees communicate primarily using pheromones to complete tasks, such as locating food, instead of using 'waggle dance' as in honey bees.

Foraging Behavior and Conservation Concerns. Bumble bees are capable of utilizing a broad spectrum of floral resources. Flower selection is influenced by morphology, coloration, pollen and nectar availability (Bradshaw and Schemske, 2003). However, they face significant challenges from habitat loss and fragmentation, which suggest the need for effective conservation efforts (Goulson et al., 2008). In the United States, there is a decline in many bumble bee species, primarily due to habitat degradation, pesticide exposure, spread of viral pathogens and varroa mites (Goulson et al., 2008; Potts et al., 2010; Kerr et al., 2015; Williams et al., 2015). There is an urgent need to develop conservation strategies, such as habitat restoration and reduced pesticide use, etc (Cameron et al., 2011). A detailed understanding of their ecology and vulnerabilities will enhance our ability to develop effective conservation strategies.

3. Sweat bees (Family: Halictidae)

Halictids are commonly referred to as sweat bees, as they represent a diverse group within the bee fauna in the United States.

Taxonomic Diversity and Morphological Characteristics. Halictidae, one of the largest families of bees, encompasses a wide range of species with varied ecological roles. This family is represented by many important genera, including *Lasioglossum*, *Halictus*, *Agapostemon*, and *Nomia*. They are diverse with varied size, behavior, and habitat preference (Cameron et al., 2011). Halictid bees are generally small to medium-sized and often metallic in appearance. Their size and morphology vary considerably among species, adapted to specific ecological niches. These adaptations include variations in tongue length and body size, enabling them to forage effectively on a wide range of flowers (Gibbs, 2010).

Lifecycle. Halictid bees have a high degree of variability in social organization. Many species exhibit a solitary lifestyle, whereas others show varying degrees of sociality. They overwinter as adults or prepupae and they emerge in the spring or early summer. Female bees establish nests, oviposit, and provision their offspring with nectar and pollen. Some females function as workers and others as reproductive queens (Engel et al., 1997). Nesting habits of halictid bees range from ground-nesting to cavity nesting. The nest architecture varies significantly across species and is influenced by soil type, texture, moisture, compactness, and environmental factors (Gibbs et al., 2012). These bees are found in a variety of habitats, from forests and meadows to urban gardens, indicating their adaptability to different environmental conditions (Packer et al., 2003). Halictid bees are generalists, visiting a wide array of flowering plants for nectar and pollen. Their foraging behavior is crucial for the pollination of many wild and cultivated plants. The efficiency of halictids as pollinators is attributed to their diverse foraging strategies and adaptability to different floral resources (Cane and Sipes, 2006).

Rationale

The need for an in-depth exploration of the ecology of bees visiting turfgrasses, such as centipedegrass in urban and suburban lawns, is grounded in the evolving understanding of turfgrasses as ecological contributors rather than mere components of landscapes. Traditional perspectives have long dismissed related turfgrasses as exclusively wind-pollinated with negligible integrations with pollinators. Recent research showed that various bee species forage for turfgrass pollen, such as centipedegrass and bahiagrass (*Paspalum notatum* Flugge) (Jones, 2014; Saunders, 2018; Joseph et al., 2020; Joseph and Hardin 2022). The ubiquitous nature of where these grasses are grown, their hardiness, and low maintenance requirements make them promising candidates as alternative pollen sources for bee conservation.

Considering the alarming decline of bee populations, a paradigm shift is critical, with new strategies for bee conservation. Centipedegrass is known to flower heavily from July through October in Georgia, providing pollen resources. During mid to late summer, pollen resources begin to become scarce for foraging bees (Wood et al., 2018). This blooming period of centipedegrass can help bridge the gap in pollen availability for bees during critical periods of the year. Adopting turfgrasses in lawns and landscapes can enhance this ornamental landscape by not only adding aesthetic and recreational value but also contributing to the broader ecosystem by supporting pollinators during resource scarcity. The foraging behavior, pollen collection, and the reliance of bees on centipedegrass remain underexplored despite their potential implications for promoting biodiversity, improving conservation efforts, and providing ecosystem services to crops. This leaves a knowledge gap regarding bee-turfgrass interactions in residential and commercial lawns. This dissertation, therefore, aims to bridge this gap by investigating the types of pollen collected by bees on centipedegrass, the quantity and nutritional adequacy of this pollen, and how these factors, along with the presence of surrounding flora, influence bee foraging behavior. This research will provide comprehensive insights into the ecological dynamics at play in these man-made ecosystems, ultimately informing more sustainable landscape management practices that support both pollinator health and ecological diversity.

Research Objectives

Objective 1: ***To determine the role of centipedgrass pollen by understanding pollen collection, transfer to hives, and utilization of pollen for larval development of bees***

Sub-objective 1a: ***Determine the pollen load composition of bees foraging on centipedegrass spikes***

Sub-objective 1b: ***Determine if bees transfer of centipedegrass pollen to hives***

Sub-objective 1c: ***Determine if bees utilize the centipedegrass pollen for larval development***

This objective seeks to expand upon existing research indicating that bees forage on centipedegrass (Jones, 2014; Joseph et al., 2020). The primary aim is to identify and quantify the pollen types collected by bees on these lawns. However, it is unclear whether bee species forage on centipedegrass and collect pollen grains, effectively transfer collected grass pollen grains to their hives, and utilize them for larval development.

Objective 2: ***To characterize the foraging behaviors of bees on centipedegrass spikes***

Bees, including *Lasioglossum*, *Bombus* (bumble bees), and *Apis* (honey bees), have recently been reported to forage on centipedegrass spikes. However, the specific interactions between these different bee groups and the spikes during foraging have not been fully understood. This information is critical for optimizing pollination services and guiding bee conservation efforts in managed landscapes.

Objective 3: ***Impact of surrounding flora on bee foraging behavior on centipedegrass***

This study examines how the presence of other flowering plants, such as lavender, goldenrod, and butterfly bush known to be attractive to bees influence the foraging behavior of bees on centipedegrass pollen. This objective is crucial for understanding how diverse plant communities influence pollinator foraging patterns and preferences, thereby contributing to broader ecological and conservation strategies.

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CHAPTER 2

BEES FORAGE AND UTILIZE CENTIPEDEGRASS POLLEN

Daniel Ibiyemi, Karen-Harris Shultz, David Jespersen and Shimat Joseph

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ABSTRACT Bees are crucial for pollination, producing 35% of the world's fruits, nuts, and vegetables. However, the decline in bee populations due to habitat loss, monoculture farming, pesticide use, and urbanization threaten biodiversity and agricultural sustainability. Thus, it is critical to identify additional resources for pollinators. Centipedegrass (*Eremochloa ophiuroides*) is a common turfgrass in the southeastern United States, which can provide pollen resources to foraging bees. The objectives of this study were to determine 1) pollen load composition of bees foraging on centipedegrass spikes, 2) pollen transferred to hives, and 3) the utilization of centipedegrass pollen for larval development. In 2021 and 2022, foraging bees were collected from five centipedegrass lawns at 10-12 d and 14-16 d (early and late flowering phase) post-mowing and pollen loads were examined. Bees collected significantly more Poaceae than non-Poaceae pollen and their counts were significantly greater during early than late flowering in 2021, whereas in 2022, there were no significant differences in counts of centipedegrass pollen collected between early and late flowering phases. *Bombus* spp. foraged more in late summer than early or mid-summer. The pollen grains transferred to bee hives contained Poaceae pollen, which was significantly more when the lawn was mowed than when it was flowering. The no-choice experiment showed that similar numbers of bumble bee larvae developed feeding on centipedegrass pollen compared to those developed on butterfly bush pollen. Thus, the data suggests that centipedegrass pollen can offer resources for bees as they naturally collect and utilize them.

Keywords: Bees, centipedegrass, pollination, pollen load, turfgrass management, pollinator conservation.

Introduction

About 35% of fruits, nuts, and vegetables worldwide depend on bees (Klein et al., 2007). In recent years, bee populations worldwide have been declining (Goulson et al., 2008; Cameron et al., 2011), due to habitat loss caused by the intensification of agriculture with the expansion of monocultural farming and increased agricultural input, such as pesticide use and continued urbanization (Goulson et al., 2008; Potts et al., 2010). This decline in bee populations poses serious threats to the biodiversity of plants in the landscape and the sustainability of agriculture (Klein et al., 2006). The pollination services provided by bees are estimated at approximately $153 billion USD annually, which accounts for about 9.5% of the total value of agricultural production (Gallai et al., 2009). Thus, conservation efforts to preserve existing habitats and enhance potentially new habitats suitable for bees need more attention (Goulson et al., 2008; Potts et al., 2010).

Turfgrass is an important component of urban, suburban, and rural ornamental landscapes, especially in the eastern USA (Beard and Green, 1994; Adrian et al., 1996). Some of the major benefits of turfgrass include the prevention of soil erosion, sequestration of carbon, and providing habitats for diverse organisms, such as soil microorganisms, insects, birds, and small mammals (Beard and Green, 1994; Dean et al., 1996). However, turfgrasses are viewed as "ecological deserts" as they offer limited floral resources to foraging bees as all the turfgrasses are maintained at low heights with no or limited opportunity for them to produce spikes. If certain species of turfgrasses are allowed to produce spikes, bees will forage on them (Jones, 2014; Joseph et al., 2021;

Joseph and Hardin, 2022). A reduced or delayed mowing schedule allows weed plants to produce flowers with pollen and nectar (Threlfall et al., 2015; Larson et al., 2017; Jaiswal and Joseph, 2024). These studies suggest that more research is warranted to enhance our understanding of how turfgrass can contribute to bee ecology.

Exploring other potential avenues is critical to enhance bee conservation efforts. Centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.)] is a common turfgrass species in suburban and rural areas of the southeastern USA. It is adaptable to diverse soil types, including sandy and acidic soils (Islam and Hirata, 2005). Centipedegrass is compatible with a less aggressive mowing schedule during the summer, and minimal fertilizer needs, with 1-2 nitrogen applications annually (Trenholm and Unruh, 2009; Turgeon, 2012). Additionally, centipedegrass is tolerant of low fertility and infrequent irrigation, making it a low-maintenance and cost-effective option for homeowners and land managers (Beard and Green, 1994; Unruh et al., 2024). Thus, centipedegrass is an environmentally sustainable choice compared to other turfgrasses (Casler and Duncan, 2003). Bees were found foraging on centipedegrass spikes (Jones 2014; Joseph et al. 2020). When the pollen loads from honey bees, *Apis mellifera* L. foraging on centipedegrass lawns were examined, they mostly represented grass pollen (Jones, 2014). However, it is unclear whether other bee species forage on centipedegrass and collect pollen grains. It is unclear if they effectively transfer collected grass pollen grains to their hives and utilize them for larval development. Thus, the objectives of this study were to 1) evaluate the pollen load of foraging bees on centipedegrass spikes; 2) determine if bees transfer collected grass pollen grains to hives; and 3) utilize grass pollen grains for larval development.

Materials and Methods

Bees and pollen load

Study site

In 2021 and 2022, five centipedegrass lawns in Spalding County, Georgia were identified for sampling bees. These selected centipedegrass lawns were in residential areas under regular mowing and fertilization regimes. The lawns were surrounded by weeds, such as white clover (*Trifolium repens* L.), dandelions (*Taraxacum officinale* F.H. Wigg.), and buckhorn plantain (*Plantago lanceolata* L.), as well as wildflowers, and wooded pine areas (*Pinus* spp. L.). The selected lawn sites were approximately 10 km apart, ensuring minimum spatial distance between sampling sites. Centipedegrass initiated producing spikes from mid to late July, and thus, sampling was conducted from July to September when production of new spikes declined. The details of location, lawn size, weed species in the lawn, and surrounding vegetation are listed in S Table 2.1. Before the study in 2021 and 2022, some selected lawns were professionally managed for maintenance and pest control. In site 2 (S Table 2.1), carbaryl (Sevin dust, GardenTech, Palatine, IL) was applied for pest insects and chlorothalonil (Bravo, Syngenta Crop Protection, Greensboro, NC) fungicide on tomatoes to combat early blight in the surrounding garden plants. In site 5 (S Table 2.1), a combination product with dicamba (0.081%), pendimethalin (1.22%), and 2,4-D (1.22%) (Scotts Turf Builder Triple Action, The Scotts Company, Marysville, OH) was applied in June during both years.

Bee sampling

From July to September 2021 and 2022, bees foraging centipedegrass spikes were sampled. Centipedegrass lawns initiated flowering approximately 10 d following mowing. Sampling was conducted in two-time intervals: 1) 10-12 d (early) and 2) 14-16 d (late) post-mowing to ensure the presence of viable pollen for bees. Bees were sampled between 7 AM and 12 PM for 30 mins because they are most active during the morning. The sampling dates and abiotic factors during sampling day are listed in S Tables 2.2 and 2.3.

Bees were collected while foraging on a centipedegrass spikelet for at least 3 s. For bee sampling, a 166.9 mL clear plastic container with a white lid was used. The container was placed upside down on the spikelet while the bee foraged on it, and then the lid of the container was gently placed underneath to trap it. This method was less disruptive on bees and preserved pollen load on the bee's corbicula or scopal hairs. Collected bees were then transferred to a labeled plastic bag and stored in a cooler. The temperature and wind speed were recorded upon arrival at each site using a Mini Digital Anemometer (CO2322, Tech Instrumentation Inc., Elizabeth, CO).

Morphological identification

In the laboratory, pollen loads were carefully removed from one corbicula (one leg) of each bee using sterilized forceps for those species that stored pollen in corbiculae. The pollen on the other leg was saved for molecular analysis. For the species without corbiculae, each bee was vigorously shaken with 0.5 mL of 70% ethanol in a 1.5 mL microcentrifuge tube to dislodge pollen from its body. From the ethanol-pollen solution, 100 μL was pipetted and placed onto a 25 × 75 × 1 mm (length [L]: width [W]: height [H]) microscope slide (Fisher Scientific, Pittsburgh, PA) for each bee sample. To enhance the visibility of pollen grains under a light microscope, first, the slide was air-dried for 2 mins and then stained using glycerol jelly fuchsin dye (Basic Fuchsin, Sigma-Aldrich Inc.

St. Louis, MO; Kearns and Inouye, 1993). Centipedegrass pollen was examined under a compound microscope (400×; Olympus BX50, Hachioji, Tokyo, Japan) to differentiate between grass and non-grass pollen. Grass pollen is almost perfectly circular or spherical, whereas non-grass pollen has more varied shapes. Additionally, grass pollen grains are more uniform in size and have a single pore (monoporate), while non-grass pollen often has multiple pores or furrows and more complex surface textures. These morphological features were crucial in distinguishing between the two types of pollen. Reference collection for pollen was prepared using pollen identification guides (Hodges, 1984; Sawyer, 1988). The bees were identified to genera using Carril and Wilson (2021) and the Discover Life Bee Species Guide and World Checklist (Ascher and Pickering, 2015).

Molecular identification

Pollen samples were collected from the corbicula of one leg of each bee collected in 2021. Due to insufficient DNA quantities in the 2022 samples, they were excluded from the analysis. Each pollen sample was stored in 70% ethanol in 1.5 mL Eppendorf tubes and placed in a Thermo Scientific™ -80°C ultra-low-temperature freezer (Thermo Fisher Scientific, Waltham, MA) until further processing.

DNA was extracted using the Thermo Fisher GeneJET Plant Genomic DNA Purification Mini Kit. Pollen samples were centrifuged at 8000 revolutions per minute for 2 mins, and the ethanol supernatant was discarded. To extract DNA from the pollen grains, the samples were added to a prefilled tube with 0.5 mm zirconium beads, and the samples were mechanically disrupted using a bead beater for 30 s and for four cycles at a speed of 5. After each cycle, the tubes were placed on ice for one minute to prevent overheating and sample degradation. DNA was eluted in 30 µL of elution buffer for final analysis.

Two sets of primers from the internal transcribed spacer (ITS) region were used for qPCR analysis. The first set was a universal ITS primer (ITS2F: ATGCGATACTTGGTGTGAAT; ITS2R: GACGCTTCTCCAGACTACAAT) designed to amplify plant DNA broadly. The second primer was specific to centipedegrass (F: TATCTCGATCCACACGACTCT; R: CTGCGCCGAGAACAACTA), designed to amplify centipedegrass DNA. Primer validation was performed using PCR, followed by gel electrophoresis. The primers used for qPCR amplification demonstrated efficient and specific amplification of both the universal DNA and centipedegrass-specific DNA. Cycle threshold (CT) values were obtained for the universal primer and the centipedegrass-specific primer across all samples. The proportion of centipedegrass DNA in the total pollen DNA was quantified, with the centipedegrass-specific primer detecting varying amounts across samples. The CT values were consistent across replicates, confirming reliable amplification to confirm amplification of the target regions.

Quantitative PCR was conducted using the QuantStudio 3 Real-Time PCR System, with SYBR Green as the detection dye. The final reaction volume for each qPCR reaction was 20 µL, including the SYBR Green master mix, primers, and template DNA. The cycling conditions followed the QuantStudio system's default settings: initial denaturation, followed by 40 cycles of denaturation and annealing/extension. No-template controls (NTCs) were included in each run to ensure the absence of contamination.

Pollen transfer

Study site, insects, and experimental procedure

This study was conducted on four centipedegrass lawns in Griffin, Georgia, during September and October 2022 and 2023 (Table 2.4). Five-frame, 56 × 24 × 31.5 cm (L:W:H), *A. mellifera* nucleus boxes were used for this study. The hives had active colonies of honey bees. The bee hives were already exposed to various pollen resources before installation. The entry slit of hives was approximately 19 × 3.8 cm (W: H). The bee colony in each hive had approximately 5000-7000 workers. The hives were equipped with a screened bottom to ensure proper hygiene and aeration, which reduced the accumulation of droppings and mite infestation.

Two sampling periods were identified following mowing, 1) 0-5 d post-mowing when the centipedegrass had no spikes; and 2) 9-14 d post-mowing when 50% of centipedegrass lawn had spikes. In 2022 and 2023, four hives were deployed, with one hive in each centipedegrass lawn. An additional hive was added in 2023 and was placed in an area without centipedegrass within at least a 500 m radius.

A corrugated, 56 × 23 cm (L:W) plastic sheet was placed beneath the hive to sample pollen. The pollen grains or pellets drop on the sheet when the foraging bees try to enter the hive through the slit. This arrangement functioned as a pollen trap. The corrugated plastic sheets were deployed on each sampling day between 7:00 and 7:30 AM and recovered between 12:30 and 1:00 PM on the same day, and the sampling was conducted for 5 d during two sampling periods. The trapped pollen grains or pellets on the sheet were carefully transferred into 3.7 L plastic bags (Ziploc, Storage Bags, S. C. Johnson & Son, Inc., Racine, WI). and were labeled and stored at -20 °C in the laboratory until further processing. When pollen was collected, the sheets were removed from hives and redeployed the following day. This experiment lasted four weeks, and it was repeated once each year.

Pollen evaluation

For analysis, the entire pollen grains or pellets from each plastic bag, representing one sample, were transferred to 1 mL of 70% ethanol in a 1.5 mL Eppendorf tube. The tubes were centrifuged at 2000 revolutions per min for 5 mins. The samples were centrifuged to ensure proper mixing of the pollen with the ethanol. Following centrifugation, the mixture was stirred to homogenize it, and 100 µL was pipetted from the entire mixture onto a microscope slide rather than from the supernatant to ensure a representative sample of the pollen. Each slide was then stained with glycerin jelly containing fuchsin dye and warmed on a hot plate. The slides were then covered with coverslips to prevent contamination. The number of Poaceae and non-Poaceae pollen grains on each slide was quantified and averaged for each sample using a compound microscope (400× magnification; Olympus BX50, Hachioji, Tokyo, Japan). The pollen identification was achieved using the same method as described in the earlier experiment.

Utilization of centipedegrass pollen

Insects and plants

In September 2022 and 2023, a no-choice study was conducted at the University of Georgia, Griffin Campus, Griffin, GA. Eight eastern bumble bee, *Bombus impatiens* Cresson, colonies were purchased (Koppert Biological Systems Inc., Howell, MI). A colony was represented by a bee box. Upon arrival at Griffin Campus, pollen balls provided by the company were removed from every bee box to eliminate the pre-existing food source. Each colony had a 2.5 kg sugar solution, which was not removed. Before the start of the experiment, every bee colony was food deprived by denying access to any pollen source for a period of 24 h. The life cycle of the common eastern bumble bee is approximately 28-37 d from egg to adult at 28-32 °C (Goulson, 2003). The centipedegrass used in this study was obtained from a sod farm (Super Sod Farms, Fort Valley, GA). Upon reception, the centipedegrass was planted in plastic trays measuring 53.3 × 27.9 × 7 cm (L:W:H) (DSM&T Company Inc., Fontana, CA). The grasses were maintained in a greenhouse, where the average temperature was 30.6°C, with a high of 37.6°C and a low of 22.9°C. The relative humidity (RH) averaged between 67-71%. Maintenance of the centipedegrass included clipping the inflorescences every two weeks to a height of 2.5 cm. Fertilization was conducted using Scotts Turf Builder Starter 24-25-4 (Scotts Miracle-Gro Company, Marysville, OH), and the grasses were irrigated every other day using a water hose. In the field, centipedegrass was managed with similar practices, including mowing and irrigation as needed to maintain healthy growth and continuous availability of inflorescences for the foraging bees.

Bumble bees are attracted to butterfly bushes (*Buddleja* spp.) and thus, they were selected for the experiment as a control treatment. In Georgia, butterfly bushes typically flower from mid-summer to fall under full sun and well-drained soil conditions, ensuring abundant and vibrant flowers. The butterfly bush plants were purchased from a local ornamental retail nursery. Each butterfly bush had an average of seven to ten purple flowering cones. These plants were not exposed to any insecticides before purchase, ensuring their suitability for foraging by bees.

The butterfly bush and centipedegrass were used to create a realistic foraging environment to support the nutritional needs of bee colonies. The setup was designed to provide continuous floral pollen resources, although it was not confirmed if the pollen provided would entirely suffice for the colony. The centipedegrass trays and butterfly bush containers were replaced every three days to guarantee fresh and abundant pollen availability, supporting the foraging needs of the bee colonies throughout the study period.

Experimental procedures

In 2022 and 2023, *B. impatiens* colonies were exposed to centipedegrass and flowering butterfly bush plants in a no-choice setting. In 2023, the negative control treatment consisted solely of the sugar solution provided with the bumble bee colonies. However, the plant pollen provided by the company was entirely removed. The experimental unit was one bee box and two trays of centipedegrass with spikes or a 18.9 L flowering butterfly bush plant caged using a mesh cage (60 × 40 × 40 cm [L: W:H]; (Bug Dorm, MegaView Science Co., Ltd., Taichung, Taiwan). The treatments were: 1) caged centipedegrass and 2) caged butterfly bush. The bumble bee box was provided with sugar solution for both centipedegrass and butterfly bush treatments. Thus, in 2023, a third treatment with only sugar solution with no pollen source was added. The treatments were arranged in a randomized complete block design with four replications. The cages were arranged on shade house (50% light) benches for four weeks. After four weeks, the experiment was terminated by placing the bee boxes in a walk-in freezer at -15 ºC for 24 h. The four-week interval was sufficient time to let larvae or pupae present from the beginning of the study to complete adulthood. Thus, the larvae and pupae obtained after four weeks are newly developed individuals that utilized the centipedegrass or butterfly bush pollen.

Evaluation

The numbers of larvae and pupae in each bee box were documented. The colony weight difference during the experimental period was determined after calculating the pre- and post-experimental weights of bee boxes (UltraShip, My Weigh, Phoenix, AZ). Similarly, the sugar solution consumption was determined after calculating the pre- and post-weight of the sugar solution.

Statistical analyses

All data were analyzed using R Studio (RStudio Team 2020). Appropriate statistical methods were selected based on the data's structure and distribution. Where applicable, post hoc comparisons were performed using Tukey's Honestly Significant Difference (HSD) test.

Pollen load. To compare the pollen loads between bee genera (*Apis*, *Bombus*, and *Lasioglossum*) foraging on centipedegrass during July, August, and September of 2021 and 2022, two types of statistical tests were employed:

Kruskal-Wallis Test: This non-parametric test assessed the differences in foraging bee counts among the three genera for each sampling month and year. The fixed effect in this analysis was bee genus, and the response variable was the count of foraging bees.

Wilcoxon Rank Sum Test: This test was used to compare the proportions of Poaceae and non-Poaceae pollen collected by bees. It was applied for both the early and late flowering phases in 2021 and 2022, as the data did not meet parametric assumptions.

Molecular Identification

Relative quantification of centipedegrass pollen DNA was determined using the 2^(ΔCT) method. which calculates the relative expression of target DNA by comparing the difference in threshold cycle (CT) values between the target and a reference gene. This method is commonly used to assess the amount of DNA or RNA in qPCR experiments (Livak and Schmittgen, 2001). The universal ITS primer was used to estimate the total DNA content in the pollen samples, while the centipedegrass-specific primer quantified centipedegrass DNA. The proportion of centipedegrass DNA relative to the total DNA in each sample was calculated to assess the centipedegrass pollen load. The proportion of DNA from other plants was also recorded by subtracting the proportion of centipedegrass pollen DNA from 100. The specificity of amplification was confirmed via gel electrophoresis (Fig. 2.3). Each sample was run in two technical replicates, and a total of 32 pollen samples from bees collected in 2021 were analyzed.

Because data were not normally distributed, a Wilcoxon signed-rank test was performed to determine whether the centipedegrass DNA was significantly different from the total pollen DNA ($P < 0.05$).

Pollen transfer

Pollen transfer between bees and centipedegrass was analyzed in two phases. In 2022, a student's t-test was used to compare the mean quantities of Poaceae pollen between bees collected from flowering and mowed centipedegrass lawns. In 2023, a one-way ANOVA was conducted to examine the effect of mowing status (flowering vs. mowed) and a control plot (no centipedegrass within a 500 m radius) on the quantity of Poaceae pollen collected by bees. The fixed effect in this analysis was the mowing status and control treatment, with the locations (lawn sites) serving as replicates. The response variable was the quantity of Poaceae pollen collected, and the analysis was performed across multiple locations in 2023. Post hoc Tukey's HSD test was used to identify significant differences between the three treatments (flowering, mowed, and control).

Pollen utilization

The response variables analyzed for the pollen utilization study were larval and pupal development, colony weight changes, and nectar consumption.

1. In 2022, independent t-tests were used to compare the number of larvae and pupae between two treatments: centipedegrass and butterfly bush.
2. In 2023, a one-way ANOVA was employed to evaluate the effects of three treatments (centipedegrass, butterfly bush, and sugar solution as a negative control) on larval and pupal development. Colony weight changes and nectar consumption were also analyzed using one-way ANOVA, where the fixed effect was treatment. Colony weight was determined by measuring the difference between the pre-and post-experimental weights of each colony. Nectar consumption was calculated by weighing the sugar solution before and after the experimental period. Post hoc Tukey's HSD tests were performed to identify treatment-specific effects on larval development, colony weight changes, and nectar consumption.

Results

Bees and pollen load

The counts of foraging *Apis, Bombus*, and *Lasioglossum* collected from centipedegrass did not significantly differ in July 2021 ($H = 4.6$; df = 2; $p = 0.098$; Fig. 2.1A) and 2022

($H = 1.0$; df = 2; $p = 0.596$; Fig. 2.1B). In August 2021, there was no significant difference between bee genera collected ($H = 3.1$; df = 2; $p = 0.210$), whereas the number of *Bombus* was significantly greater than *Apis*, followed by *Lasioglossum* bees in 2022 ($H = 9.9$; df = 2; $p = 0.007$). The counts of *Bombus* were significantly greater than *Apis* and *Lasioglossum* bees in 2021 ($H = 20.5$; df = 2; $p < 0.010$) and 2022 ($H = 14.6$; df = 2; $p < 0.010$). A significantly greater proportion of Poaceae pollen was collected from bees than non-Poaceae pollen during the early (T = 3943; $p < 0.001$) and late flowering phases (T = 2432, $p < 0.001$) in 2021 (Fig. 2.1C), and early (T = 739; p < 0.001) and the late flowering phase (T = 553; $p < 0.001$) in 2022 (Fig. 2.1D).

The universal primer, amplifying the total pollen DNA, showed CT values ranging from 24.19 to 30.60, while the centipedegrass-specific primer had CT values between 24.39 and 34.57. The centipedegrass DNA proportions ranged from 6.39% to 86.65%, indicating substantial variation between samples. The average proportion of centipedegrass pollen DNA was 59.92% while the proportion of DNA from other plant pollen was 40.08%. The proportions of centipedegrass DNA and total pollen DNA, (W=157.0, p=0.044; Fig. 2.2) were significantly different. These results indicate that centipedegrass DNA forms a distinct and measurable portion of the total DNA, with significant variation across the samples. When compared with the amount of pollen recovered from the morphological data of the same samples used for the molecular identification, the proportion of Poaceae pollen was 98%, and the proportion of other flowering plants was 2%.

Pollen transfer

In 2022, of 47,116 pollen grains evaluated, 15,527 pollen grains were from Poaceae. Similarly, in 2023, of 41,153 pollen grains, 11,949 were identified as Poaceae pollen grains. In 2022, there was no significant difference in the number of Poaceae pollen grains recovered from bee hives between centipedegrass flowering and mowed lawns time periods ($t = 2.6$, df = 58; $p = 0.084$; Fig. 2.4A). In 2023, a significantly greater number of Poaceae pollen grains were recovered from bee hives when centipedegrass lawns were mowed than when centipedegrass lawns were flowering, or no centipedegrass was present in the area (control) ($F = 4.9$; df = 2, 58; $p = 0.011$; Fig. 2.4B).

Utilization of centipedegrass pollen

In 2022, there were no significant differences in number of bumble bee larvae ($t = 0.5$; df = 6; $p = 0.634$; Fig. 2.5A), pupae ($t = -1.7$; df = 6; $p = 0.150$; Fig. 2.5B), colony weight ($t = -0.1$; df = 6; $p = 0.925$; Fig. 2.5C), and nectar consumed ($t = -0.8$; df = 6; $p = 0.453$; Fig. 2.5D). In 2023, the number of bumble bee larvae was significantly greater for the centipedegrass and butterfly bush treatment than for the control (no plant) treatment ($F = 7.78$, df = 2, 9; $p < 0.001$; Fig. 2.6A). However, there was no significant difference for the larvae between centipedegrass and butterfly bush treatments (Fig. 2.6A). The number of pupae were not significantly different between treatments ($F = 0.6$; df = 2, 9; $p = 0.590$; Fig. 2.6B). The colony weight was significantly greater for the centipedegrass and butterfly bush treatments than for the control treatments ($F = 4.3$; df = 2, 9; $p = 0.05$; Fig. 2.6C). There was no significant difference for the colony weight between centipedegrass and butterfly bush treatments (Fig. 2.6C). For nectar consumed, no significant differences were observed between the treatments ($F = 0.1$; df = 2, 9; $p = 0.870$).

Discussions

When pollen loads of bees foraging on centipedegrass spikes were examined, more poaceae pollen grains were recovered than non-Poaceae pollen. This suggests that bees indeed collect pollen from centipedegrass spikes during foraging events. This result is consistent with observations by Jones (2014), who documented that bees were foraging on centipedegrass and collected 100% Poaceae pollen. Similarly, honey bee colonies predominantly collected Poaceae pollen, with pollen loads consisting of either unifloral loads (pollen from a single plant species) or multifloral loads (pollen from multiple plant species), comprising significant proportions of grass pollen (Sajwani et al. 2014). Click or tap here to enter text. In the current study, more counts of Poaceae pollen grains were recovered from *Bombus* and *Lasioglossum* bees. The relative quantification data revealed a significantly greater proportion of centipedegrass DNA in the samples, confirming that bees predominantly collected centipedegrass pollen. These collected pollens are likely transported to bee hives (Sajwani et al., 2014). When the pollen grains found on *A. mellifera* hives were examined when centipedegrass spikes were actively producing pollen, the pollen grains from Poaceae pollen grains comprised one-third of the total pollen collected. This suggests that centipedegrass pollen is actively transferred to the colony to support the development of larvae.

The timing of centipedegrass pollen availability is critical for pollinators, particularly for bumble bees, as their activity increases during August and September when other pollen sources, especially from the trees, decline (Williams et al., 2015). This is especially true in the southeastern US, including in Georgia, where centipedegrass blooms from mid-July through early October, when other floral resources may be scarce, providing bees with a crucial pollen source (Joseph et al., 2020). *Bombus* spp. is univoltine, with intense foraging activity in late autumn, and demands an abundant availability of pollen resources to sustain protein reserves for the queens emerging over the winter (Goulson et al., 2008; Goulson et al., 2015). This temporal activity of *Bombus* may strategically benefit if the Poaceae pollen, including centipedegrass and bahiagrass, becomes available and supplements the nutritional needs of *Bombus* in preparation for overwintering and late-season colony development (Ginsberg, 1983).

The consistency of Poaceae pollen collection across both the early and late flowering phases indicates a strong preference or ‘floral fidelity’ among bees, where they prioritize a single plant source despite the availability of other flowering species (Sajwani et al., 2014; Pangestika et al., 2017). However, it is important to note that bees are opportunistic over time, and their foraging behavior is flexible, allowing them to exploit multiple floral resources when necessary. This combination of fidelity and adaptability reflects a dynamic foraging strategy that enables bees to optimize pollen collection for colony sustenance (Sajwani et al., 2014). This behavior has important implications regarding phenology, as it underscores the role of grass pollen in supporting bee populations throughout different stages of the flowering season. The ability of bees to consistently exploit Poaceae pollen, regardless of the flowering phase, highlights the adaptability of their foraging strategies to the temporal dynamics of resource availability, ensuring the sustenance and development of their colonies during critical periods of the year.

In the current study, *A. mellifera* foragers transported Poaceae pollen back to their hives. This finding suggests that grass pollen is important in supporting the nutritional needs and development of *A. mellifera* colonies. Pollen is essential for bee health, providing carbohydrates, proteins and lipids for larval development and colony sustainability (Brodschneider and Crailsheim, 2010). In the current study, 2023 data showed that more pollen was collected when the centipedegrass was mowed, and pollen was not available close to hives. Although the exact reason is unknown, this could be due to a foraging behavioral response to sustain grass pollen when in scarcity in their immediate surroundings. *A. mellifera* can travel significant distances to compensate for pollen deficiencies, especially locally (Greenleaf et al., 2007). It is possible that *A. mellifera* traveled to where grass pollen is available, including from centipedegrass lawns. This behavior aligns with the general foraging strategy of *A. mellifera* to maximize pollen collection efficiency, mainly when preferred floral sources are scarce, ensuring they can meet the nutritional demands of their colonies (Pernal and Currie, 2001). This theory warrants further investigation to determine if *A. mellifera* is indeed persistent in collecting poaceae pollen and travels long distances when it temporarily becomes unavailable in the nearby landscapes.

The results of the no-choice study showed that the development of *Bombus* colonies with access to centipedegrass pollen and dicot pollen from butterfly bush was comparable as the larval and pupal counts, as well as colony and removed nectar weights, were similar for *Bombus* colonies developed on centipedegrass and butterfly bush pollen. This suggests that centipedegrass, despite being a monocot, can provide similar developmental support as a dicot. The absence of significant differences in crucial developmental metrics, such as larval and pupal abundance, particularly noted in the 2023 experiment that included a nectar-only control group, further reinforces the hypothesis that centipedegrass pollen can sustain *Bombus* colony development. The butterfly bush was selected as a standard in the current study because it can provide essential amino acids crucial for *Bombus* development (Drossart et al., 2017; Fussel and Sorbet, 1992). The nutritional profile of centipedegrass pollen is unknown, and more studies are needed to determine how the nutrients in centipedegrass impact the development of *Bombus* and other pollinators. The results from the current study underscore the adaptability of *Bombus* to alternative pollen sources and highlight the potential role of grass pollen in supporting pollinator populations, particularly during periods when traditional floral resources are scarce.

In 2023 data, no significant differences in pupal abundance across all treatment groups, including the control group fed only nectar, were observed. This result could be attributable to environmental factors, such as temperature, relative humidity, or stress, as they can delay or arrest the pupal development of adult bees (Lanterman and Goodell, 2017). For instance, high temperatures can accelerate metabolic rates, potentially leading to delayed pupation or even pupal mortality under prolonged exposure (Zhu et al., 2019). Additionally, stress factors, such as limited nutritional resources or environmental perturbations, can trigger developmental arrest in pupae, a mechanism that some species employ as a survival strategy under suboptimal conditions (Nijhout, 2003). These factors, coupled with potential variations in microclimatic conditions within the experimental setup, could have collectively masked any treatment effects, leading to the observed uniformity in pupal abundance (Lanterman and Goodell, 2017).

Given the ongoing decline of bee populations worldwide, exploring every possible resource to ensure bee conservation is critical. The results suggest that centipedegrass, a readily available and underutilized resource, could significantly drive conservation efforts of bees. *Bombus Apis* and *Lasioglossum* were observed foraging on centipedegrass even when other floral resources were available, especially during late summer and early autumn when other resources were scarce. *Bombus, Apis,* and *Lasioglossum* bees collected significant Poaceae pollen, particularly from centipedegrass, compared to non-Poaceae pollen when foraging on centipedegrass spikes. *Apis mellifera* actively transported Poaceae pollen to their hives. *Bombus* can utilize centipedegrass pollen for colony development as effectively as they use butterfly bush, indicating the potential of centipedegrass as a valuable resource for bee conservation. Future studies should explore the nutritional profile of centipedegrass pollen in greater detail to further understand its role in supporting bee health and development. Additionally, research should focus on the long-term impacts of integrating centipedegrass and other Poaceae species into managed landscapes on bee population dynamics and pollination services. By integrating grass species, such as centipedegrass into pollinator-friendly landscape management practices, a resilient ecosystem capable of supporting diverse and sustainable bee populations can be created. The current study showed the potential of centipedegrass for supporting bees, and this research can be expanded to other regions and grass species to determine how monocots can contribute to pollinator conservation, particularly in areas where traditional floral resources are declining.

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Table 2.1 Details of centipedegrass lawns selected for bee sampling in Spalding Co. Georgia (USA) in 2021 and 2022

Study site	Size (m^2)	Site coordinates	Weeds[a]	Surrounding vegetation[a]	Surrounding structure
Site 1	910.19	33.187680, -84.271065	White clover, dandelions	Pecan tree, pine tree, vegetable garden (tomatoes, peppers),	Barn, house
Site 2	214.77	33.204595, -84.241457	Blanket crabgrass	Vegetable garden (tomatoes, peppers, squash), ornamental flowers (spider flowers, roses, marigolds)	Wooden workshop, house, small pond, two bee boxes
Site 3	330.42	33.197109, -84.220127	Bushy aster, white clover, dandelions	Oak tree, pine trees, crapemyrtle tree, abelia, hydrangeas	House, dog kennel
Site 4	283.82	33.263934, -84.282609	White clover, carpetgrass	Turfgrasses, pecan trees, Crapemyrtle trees	Office building, car parks
Site 5	503.14	33.267245, -84.292229	Buckhorn plantain, white clover, blanket crabgrass, dallisgrass	pine tree, ornamental garden	Open field, large pond

[a]Scientific names of plants listed: White clover: *Trifolium repens*, Dandelions: *Taraxacum officinale*, Pecan tree: *Carya illinoinensis*, Pine tree: *Pinus spp.*, Tomato: *Solanum lycopersicum*, Pepper: *Capsicum spp.*, Blanket crabgrass: *Digitaria sanguinalis*, Squash: *Cucurbita spp.*, Spider flower: *Cleome hassleriana*, Rose: *Rosa spp.*, Marigold: *Tagetes spp.*, Bushy aster: *Symphyotrichum dumosum*, Oak tree: *Quercus spp.*, Crapemyrtle tree: *Lagerstroemia indica*, Abelia: *Abelia spp.*, Hydrangeas: *Hydrangea spp.*, Carpetgrass: *Axonopus fissifolius*, Buckhorn plantain: *Plantago lanceolata*, Dallisgrass: *Paspalum dilatatum*

Table 2.2 Details of sampling dates and weather conditions in 2021

Study site	Inflorescence Phase[a]	Sampling date	Temperature (°C)[b]	Wind speed (km per h)[b]	Overcast conditions
Site 1	Early	7/23/2021	27.0	4.8	Partly cloudy/smoky
	Late	7/26/2021	27.8	12.9	Partly cloudy
	Early	8/6/2021	27.2	3.2	Partly cloudy
	Late	8/10/2021	31.7	4.8	Partly cloudy
	Early	9/18/2021	25.6	6.4	Mostly cloudy
	Late	9/21/2021	26.3	8.0	Cloudy
Site 2	Early	7/23/2021	31.5	8.0	Mostly cloudy
	Late	7/26/2021	31.7	8.0	Partly cloudy
	Early	8/5/2021	23.6	0.0	Fair
	Late	8/10/2021	30.1	8.0	Partly cloudy
	Early	9/16/2021	21.7	12.9	Light rain
	Late	9/19/2021	22.8	0.0	Light rain
Site 3	Early	7/23/2021	28.6	6.4	Smoky

	Late	7/26/2021	32.8	5.6	Mostly cloudy
	Early	8/6/2021	24.0	0.0	Mostly cloudy
	Late	8/9/2021	30.3	8.0	Mostly cloudy
	Early	9/18/2021	28.2	11.3	Cloudy
	Late	9/21/2021	25.0	4.8	Cloudy
Site 4	Early	7/21/2021	25.6	8.0	Mostly cloudy
	Late	7/26/2021	32.1	9.7	Partly Cloudy
	Early	8/5/2021	24.6	8.0	Mostly cloudy
	Late	8/9/2021	28.9	9.7	Mostly cloudy
	Early	9/16/2021	23.4	8.0	Light Rain
	Late	9/19/2021	24.6	9.7	Cloudy
Site 5	Early	7/22/2021	28.3	8.0	Partly cloudy
	Late	7/28/2021	26.2	1.6	Partly Cloudy
	Early	8/5/2021	25.6	12.9	Partly cloudy
	Late	8/9/2021	29.6	9.7	Mostly cloudy
	Early	9/18/2021	28.9	9.7	Cloudy

	Late phase	9/21/2021	25.7	0.0	Cloudy

[a] Early phase, 10-12 d post-mowing; late phase, 14-16 days post-mowing; [b] Temperature and wind speed were recorded before each 30-min observation period.

Table 2.3. Details of sampling dates and weather conditions in 2022

Study site	Inflorescence Phase[a]	Sampling date	Temperature (°C)[b]	Wind speed (km per h)[b]	Overcast conditions
Site 1	Early	7/25/2022	28.4	11.3	Mostly cloudy
	Late	7/28/2022	29.7	12.9	Partly cloudy
	Early	8/11/2022	26.1	8.0	Mostly cloudy
	Late	8/15/2022	29.6	14.5	Partly cloudy
	Early	9/7/2022	27.3	9.7	Partly cloudy
	Late	9/10/2022	21.8	14.5	Cloudy
Site 2	Early	7/25/2022	30.4	12.9	Mostly cloudy
	Late	7/28/2022	30.7	12.9	Partly cloudy
	Early	8/3/2022	28.0	8.0	Mostly cloudy
	Late	8/7/2022	27.6	0.0	Mostly cloudy
	Early	9/9/2022	25.2	11.3	Mostly cloudy
	Late	9/12/2022	26.1	14.5	Mostly cloudy

Site 3	Early	7/25/2022	30.8	11.3	Mostly cloudy
	Late	7/28/2022	32.8	12.9	Mostly cloudy
	Early	8/8/2022	28.9	9.7	Mostly cloudy
	Late	8/10/2022	25.6	8.0	Mostly cloudy
	Early	9/9/2022	23.3	6.4	Cloudy
	Late	9/12/2022	28.7	9.7	Cloudy
Site 4	Early	7/25/2022	30.6	11.3	Mostly cloudy
	Late	7/28/2022	32.1	12.9	Mostly cloudy
	Early	8/11/2022	25.0	9.7	Mostly cloudy
	Late	8/14/2022	26.3	8.0	Mostly cloudy
	Early	9/7/2022	26.4	11.3	Partly cloudy
	Late	9/10/2022	21.0	9.7	Cloudy
Site 5	Early	7/25/2022	29.7	9.7	Mostly cloudy
	Late	7/28/2022	28.3	16.1	Partly cloudy
	Early	8/3/2022	24.1	9.7	Mostly cloudy
	Late	8/7/2022	26.1	8.0	Mostly cloudy

	Early	9/9/2022	26.2	4.8	Mostly cloudy
	Late	9/12/2022	27.4	14.5	Partly cloudy

[a] Early phase, 10-12 d post-mowing; late phase, 14-16 days post-mowing; [b] Temperature and wind speed were recorded before each 30-min observation period.

Table 2.4. Details of centipedegrass lawns used in pollen transfer study in Spalding Co. Georgia (USA) in 2022 and 2023

Study site	Size (m^2)	Coordinates	Weeds[a]	Surrounding vegetation[a]	Surrounding structure
Site 1	910.19	33.187680, -84.271065	White clover, dandelions	Pecan tree, pine tree, vegetable garden (tomatoes, peppers),	Barn, house
Site 2	214.77	33.204595, -84.241457	Blanket crabgrass	Vegetable garden (tomatoes, peppers, squash), ornamental flowers (spider flowers, roses, marigolds)	Wooden workshop, house, small pond, two bee boxes
Site 3	330.42	33.197109, -84.220127	Bushy aster, white clover, dandelions	Oak tree, pine trees, crapemyrtle tree, abelia, hydrangeas	House, dog kennel
Site 4	503.14	33.267245, -84.292229	Buckhorn plantain, white clover, blanket crabgrass, dallisgrass	pine tree, ornamental garden	Open field, large pond

[a] Scientific names of plants listed: White clover: *Trifolium repens*, Dandelions: *Taraxacum officinale*, Pecan tree: *Carya illinoinensis*, Pine tree: *Pinus spp.*, Tomato: *Solanum lycopersicum*, Pepper: *Capsicum spp.*, Blanket crabgrass: *Digitaria sanguinalis*, Squash: *Cucurbita spp.*, Spider flower: *Cleome hassleriana*, Rose: *Rosa spp.*, Marigold: *Tagetes spp.*, Bushy aster: *Symphyotrichum dumosum*, Oak tree: *Quercus spp.*, Crapemyrtle tree: *Lagerstroemia indica*, Abelia: *Abelia*

spp., Hydrangeas: *Hydrangea spp.*, Carpetgrass: *Axonopus fissifolius*, Buckhorn plantain: *Plantago lanceolata*, Dallisgrass: *Paspalum dilatatum*.

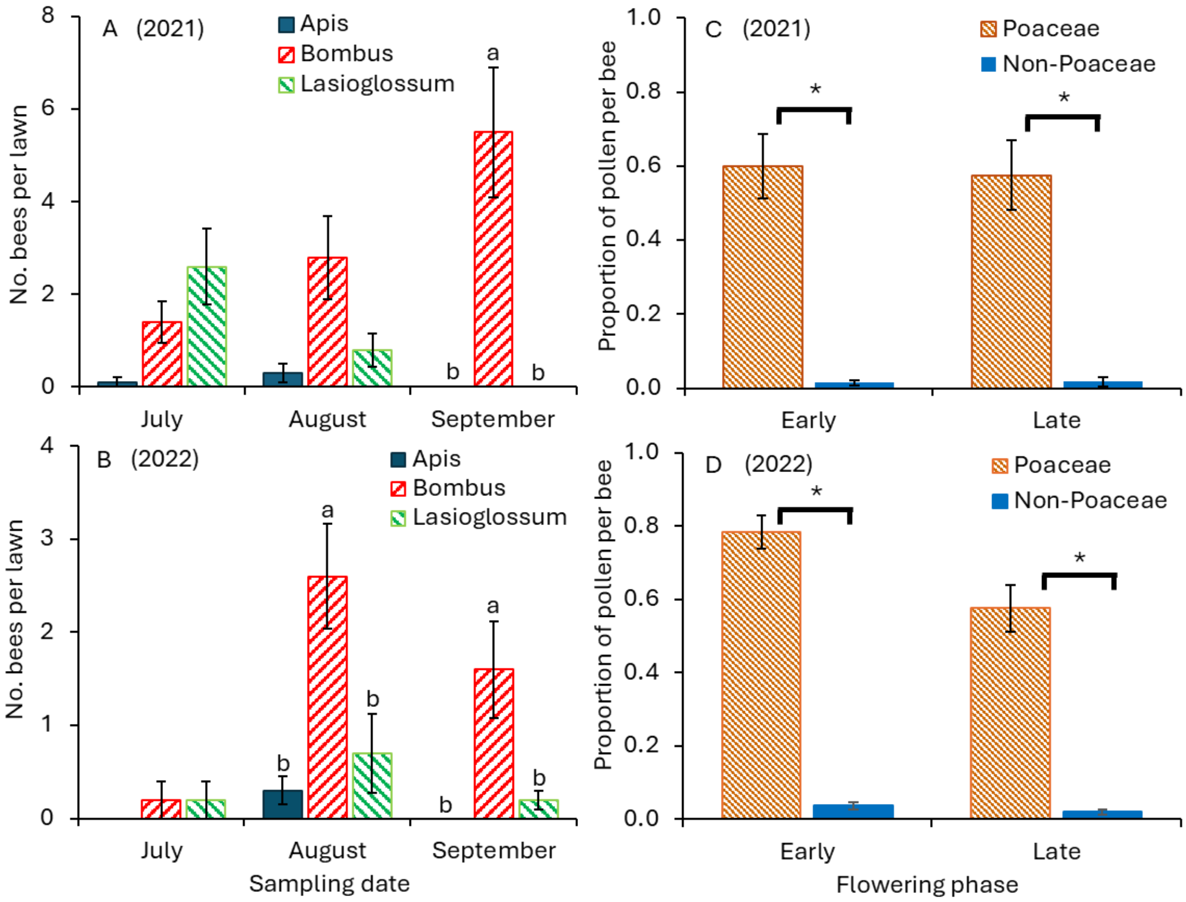

Fig. 2.1. Mean (±SE) number of bees by group sampled in July, August, and September in (A) 2021 and (B) 2022 and proportion of pollen during early and late centipedegrass flowering phases in (C) 2021 and (D) 2022. Same letters above bars indicate no significant difference within sampling dates (Kruskal-Wallis test, $P < 0.05$), and asterisks above pairs of bars indicate significant differences within the flowering phase (Wilcoxon rank sum test, $P < 0.05$). No letters on a bar denote no significant difference. Non-Poaceae pollen includes pollen from white clover (*Trifolium repens*), *Hibiscus* spp, dandelions (*Taraxacum officinale*), and others.

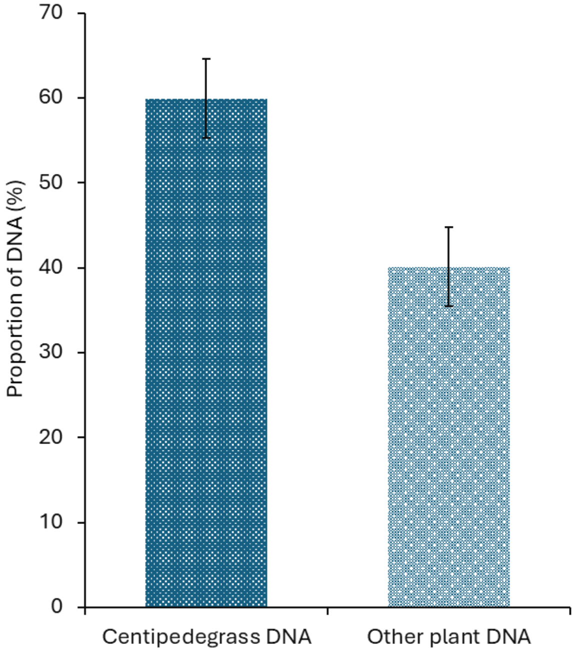

Fig. 2.2. Mean (±SE) proportion of DNA (%) from centipedegrass and other plants from pollen of bees collected in 2021. Asterisk (*) above the bars indicates a significant difference (Wilcoxon signed-rank test , $W < 0.05$).

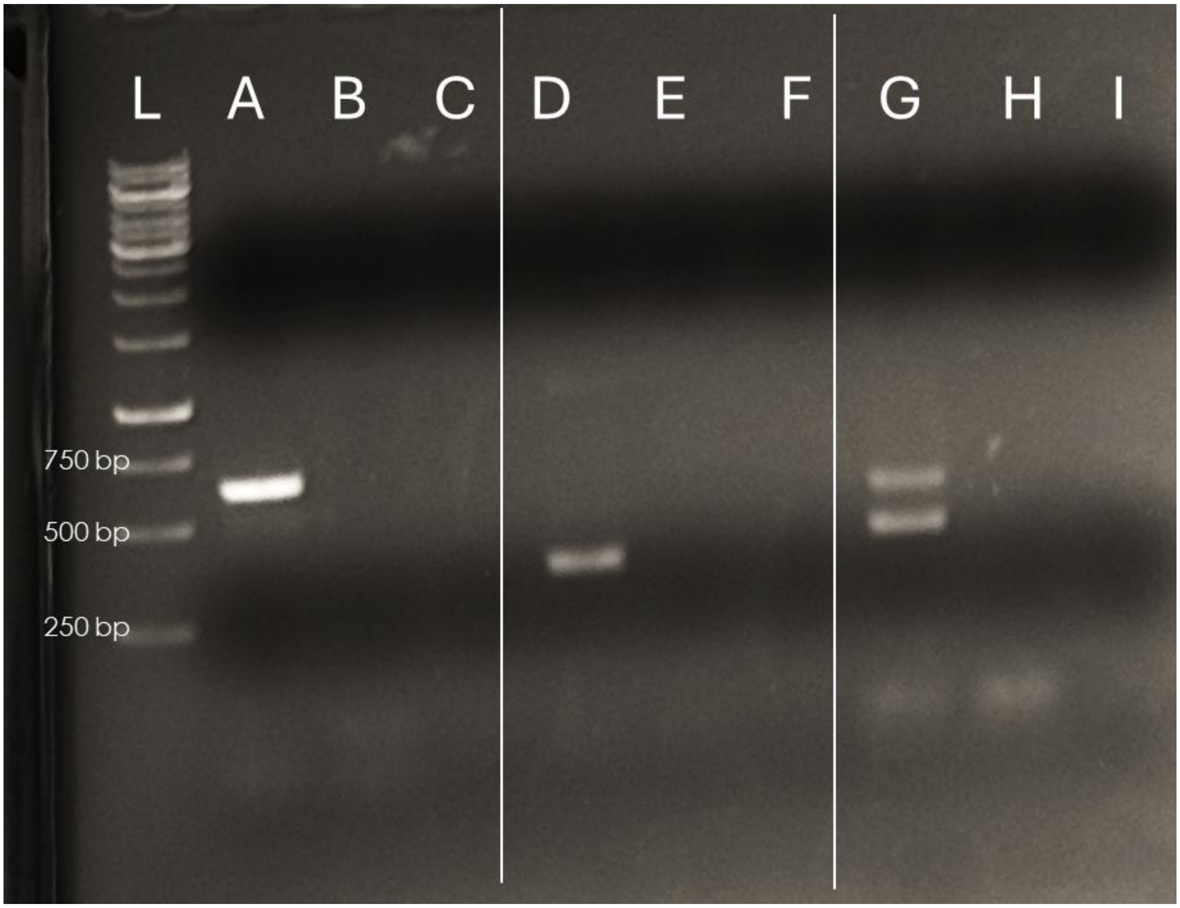

Fig. 2.3. Gel electrophoresis showing primer specificity confirmation. The sample was retrieved from a bee's corbicula foraging on centipedegrass near a buckhorn plantain patch. Well A contains Maturase K primers targeting monocots, Well D contains centipedegrass-specific primers from the ITS region, and Well G contains a universal ITS primer. Wells B, E, and H are no-template controls, while Wells C, F, and I are no-primer controls.

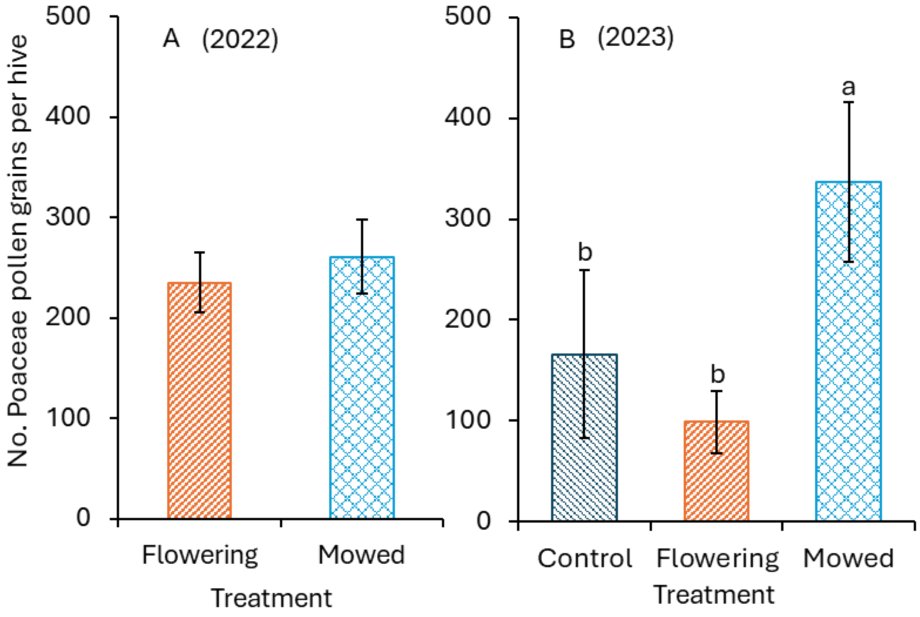

Fig. 2.4. Mean (±SE) number of Poaceae pollen grains collected from honey bee hives in (A) 2022 and (B) 2023. The same letters above the bars denote no significant difference (independent sample *t* test, and Tukey's test, $P < 0.05$). No letters on a bar indicate no significant difference.

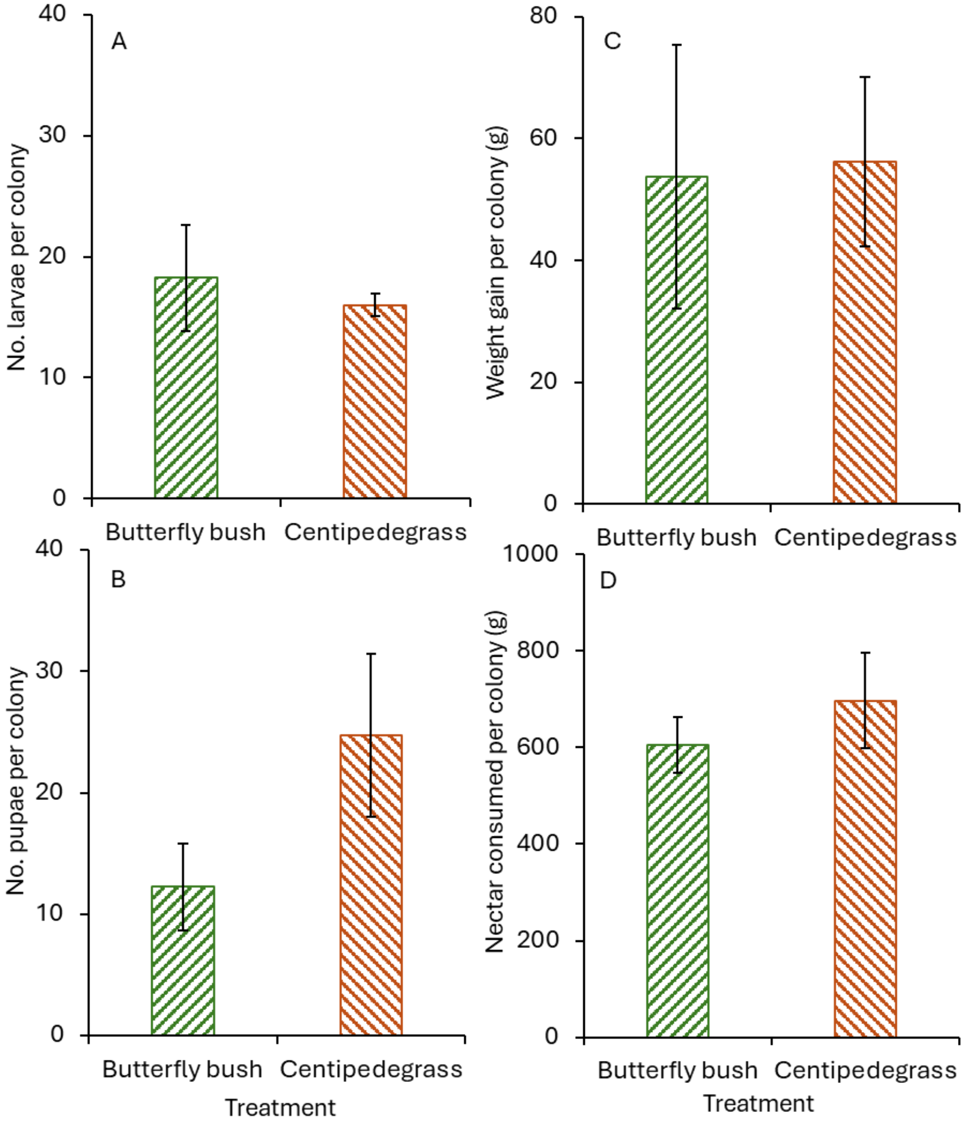

Fig. 2.5 Mean (±SE) number of bumble bee (A) larvae, (B) pupae, (C) colony weight gain, and (D) nectar consumed in 2022. No letters on a bar denote no significant difference (independent sample *t-test*, $P < 0.05$).

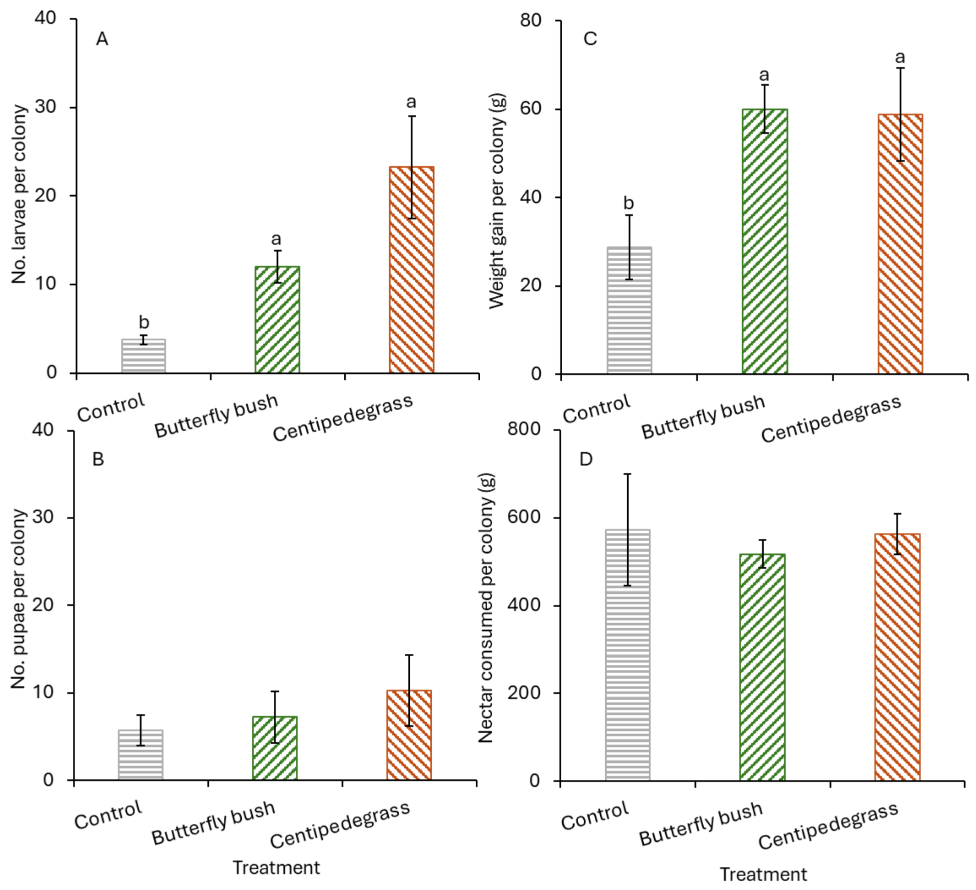

Fig. 2.6. Mean (±SE) number of bumble bee (A) larvae, (B) pupae, (C) colony weight gain, and (D) nectar consumed in 2023. No letters on a bar denote no significant difference. The same letters above the bars denote no significant difference (Tukey's test, *P* < 0.05).

CHAPTER 3

CHARACTERIZATION OF FORAGING BEHAVIORS OF BEES ON CENTIPEDEGRASS SPIKES

Daniel Ibiyemi, Karen-Harris Shultz, David Jespersen and Shimat Joseph

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ABSTRACT Bees, including *Lasioglossum* (sweat bees), *Bombus* (bumble bees), and *Apis* (honey bees), have been recently reported to forage on centipedegrass spikes. However, the specific interactions between these different bee groups and the spikes during foraging have not been fully understood. This information is critical for optimizing pollination services and guiding conservation efforts of bees in managed landscapes. Thus, the objective of this study was to characterize the foraging behaviors of bee species on centipedegrass spikes. A total of 181 video recordings collected during the summer of 2022 and 2023 were examined, focusing on behaviors such as crawling, clinging, dangling tongue protrusion, and wing buzzing. Bumble bees were observed to exhibit more frequent crawling behavior across spikes compared to honey bees and *Lasioglossum* bees. Clinging behavior, where bees held onto spikes with their legs to collect pollen, was more commonly observed in both bumble bees and honey bees than in *Lasioglossum* bees. *Lasioglossum* bees spent more time on individual inflorescences and visited fewer spikes than *Apis* and *Bombus*. *Lasioglossum* dangled on the spikes while foraging, but this behavior was not observed in other bee species. Bumble bees demonstrated more frequent tongue protrusion and wing buzzing than other bee groups. This suggests that various bee species exhibit unique or multiple behaviors when foraging on centipedegrass spikes and collecting pollen. Implications of these foraging behaviors on conservation strategies and pollination are discussed.

Keywords: Bee foraging, Centipedegrass, *Eremochloa ophiuroides*, turfgrass, conservation, pollinators.

Introduction

Pollinators, especially bees, play a critical role in maintaining the normal function of a healthy ecosystem (Larson et al., 2014). Bees pollinate approximately 75% of flowering plants and 35% of global food crops (Klein et al., 2006). Their activities are essential not only for seed and fruit production but also for enhancing the genetic diversity of plants essential for resilient ecosystems and agriculture (Gallai et al., 2009). Some food crops, such as almonds and apples, exclusively depend on bee pollination for fruit or nut production. In addition to fruit and seed sets, bees help to improve fruit size, quality (shelf life), and marketability (appearance) (Klatt et al., 2014). However, the widely reported decline in bee populations is a concern as various factors, such as habitat loss related to urbanization, excessive pesticide use, and climate change, likely contribute to this problem (Goulson et al., 2008; Potts et al., 2010; Cameron et al., 2011). In addition, bees provide invaluable pollination services to crops and plants in landscapes which support the biodiversity and sustainability of ecosystems (Kovács-Hostyánszki et al., 2017). Thus, conserving bee populations is crucial to consistently delivering these services for a healthy ecosystem.

Turfgrass is an important component of managed landscapes as it provides numerous ecological and societal benefits to the environment and human well-being. Turfgrass helps to reduce soil erosion as the root system stabilizes the soil and improves air quality as it traps dust and absorbs pollutants in the air (Beard and Green, 1994). Turfgrass also enhances water infiltration, reduces water runoff, and promotes groundwater recharge (Dean et al., 1996). Additionally, turfgrass contributes to enhancing urban cooling through evapotranspiration and mitigating heat island effects in urban areas (Qian and Follett, 2002). In suburban and urban areas, turfgrass provides recreational space to improve human well-being and habitats for many organisms, such as insects, birds, and small mammals (Beard and Green, 1994).

Centipedegrass (*Eremochloa ophiuroides* (Munro) Hack. (Poaceae) is a warm-season turfgrass common in suburban and rural areas of the southeastern US. It is adapted to sandy and acidic soils with minimal requirements for maintenance, and nutrient needs (Hanna, 1995; Islam and Hirata, 2005). Because of its high tolerance to low fertility and water, it is a cost-effective and environmentally sustainable option for homeowners in rural areas (Beard and Green, 1994; Unruh et al., 2024). In addition, bees forage on centipedegrass spikes (Joseph et al., 2020) and collected pollen grains when allowed to produce spikes (Jones, 2014).

The foraging behaviors of bees on angiosperms, such as sunflowers (*Helianthus annuus* L.; Asteraceae), clovers (*Trifolium* spp.; Fabaceae), and various fruit trees, such as apple (*Malus domestica* Borkh.; Rosaceae), cherry (*Prunus avium* L., Rosaceae), and almond (*Prunus dulcis* (Mill.) D.A. Webb; Rosaceae) (Klein et al., 2006; Ollerton et al., 2011) are well documented. The key behaviors studied were pollinator preferences, floral trait selection, and the effects of landscape composition on the diversity and abundance of pollinators (Potts et al., 2010). However, understanding of the foraging behavior of bees on turfgrass, such as centipedegrass spikes, is limited. Understanding behaviors associated with bee interactions will guide urban planners in selecting appropriate floral resources, including turfgrass spikes that enhance bee foraging in the landscape. Thus, the objective of this study was to characterize the foraging behavior of various bee groups on centipedegrass spikes.

Materials and Methods

Study site

In 2022 and 2023, the study was conducted in Griffin, GA, where five residential and open-field centipedegrass lawns were selected. Because centipedegrass produces spikes from July to September, the experiment was conducted from July to September. The size of the lawns used in the experiment varied from 214.8 to 910.2 m². The lawns were managed either by homeowners or by landscape installation and maintenance companies. The mowing schedule at each site was duly communicated to determine when to initiate the experiment each year. The selected lawns were sparsely infested with weeds. Those weeds were mainly white clover (*Trifolium repens* L.), dandelions (*Taraxacum officinale* F.H. Wigg.), blanket crabgrass (*Digitaria sanguinalis* (L.) Scop.), bushy aster (*Symphyotrichum dumosum* (L.) G.L. Nesom), carpetgrass (*Axonopus fissifolius* (Raddi) Kuhlm.), buckhorn plantain (*Plantago lanceolata* L.), and dallisgrass (*Paspalum dilatatum* Poir.). Most sites were irrigated with permanent irrigation installation. The cultivar and sodding or seed plant dates of centipedegrass in each site is unknown. Pesticides were applied in areas of certain sites. The pesticides used in this study included carbaryl (Sevin Dust, GardenTech, Walnut Creek, CA; 5% carbaryl) and chlorothalonil (Bravo Fungicide, Syngenta, Greensboro, NC; 54% chlorothalonil), which were applied to tomatoes at Site 2 to combat early blight. Additionally, at Site 5, a combination herbicide containing dicamba, pendimethalin, and 2,4-D (Scotts Turf Builder Triple Action, The Scotts Company LLC, Marysville, OH; 0.264% dicamba, 1.29% pendimethalin, and 0.734% 2,4-D) was applied. The GPS coordinates of the study sites were as follows: Site 1: 33.187680, -84.271065; Site 2: 33.204595, -84.241457; Site 3: 33.197109, -84.220127; Site 4: 33.263934, -84.282609; Site 5: 33.267245, -84.292229.

Devices and software

The study utilized two devices for video recording: a Sony FDR AX33 Digital 4k video camera recorder (Sony Corporation, Tokyo, Japan) with a frame rate of 50 frames per second (fps) and an iPhone 13 Pro Max (Apple Inc., Cupertino, CA, USA) with a triple-lens system: 12 Mega Pixels wide (26mm f/1.5), ultra-wide (13mm f/1.8), and telephoto (77mm f/2.8) lenses. The recordings were made in 4K resolution at 30 fps, utilizing the camera's ProRAW and ProRes features for high-quality capture.

The videos were analyzed using a VLC media player (VideoLAN Client, version 3.0.18, VideoLAN, Paris, France) for macOS. VLC was chosen for its user-friendliness, accessibility, and ability to slow video playback without compromising clarity and quality. These features were particularly useful for evaluating bee behaviors during foraging on centipedegrass spikes. Additionally, VLC is compatible with various video formats, including high-definition videos.

Video recording and analysis

Centipedegrass lawns were visited between 8:30 AM and 12:00 PM for video recording. During these visits, when a bee was spotted foraging on centipedegrass spikes, their videos were recorded until the bee left the spikelet. If a bee moved to a nearby spikelet, the recording was continued to the next spikelet. The videos of small-sized bees in the family Halictidae (sweat bees) were recorded at 5× zoom, whereas for large-sized bees, such as bumble bees or honey bees, videos were recorded at 3× zoom. The presence of dew on the spikes did not interfere with the foraging behavior of bees; however, rain affected the foraging behavior of bees, and videos were not recorded on those days. The recorded videos were analyzed using VLC video software. During analysis, the speed of videos was reduced by 4× to the original speed. This facilitated the careful characterization of behavior for various bee groups while they were foraging on centipedegrass spikes. The following parameters were documented from the recordings: 1) average time spent on each spikelet, and 2) number of spikes visited when a bee was spotted for every 10 s. The time spent by each bee on a spikelet was recorded, and the average visitation time was determined by the total amount of time foraging divided by the number of inflorescences visited in each video. To determine the number of floral visits, videos were restricted to 10 s. In addition to the time spent and the number of inflorescences visited, the bees were recorded when they landed on a spikelet, and their different foraging behaviors were observed. The frequency and duration of specific behaviors on each spikelet were documented.

Statistical analysis

Data were subjected to a Kruskal-Wallis test using Dunn's test as a post hoc analysis. The statistical computations were performed using R Studio software (RStudio, PBC, Boston, MA, USA). This analysis aimed to identify differences in foraging behaviors among various bee groups and to determine which bee group consistently exhibited specific behavior. The documented behaviors were crawling, clinging, and dangling on spikes. In addition, tongue protrusion and wing buzzing were recorded. The Kruskal-Wallis test was chosen for data analysis because it is a non-parametric method suitable for comparing more than two groups when the assumption of normality is not met ($\alpha = 0.05$).

Dunn's test was employed as a post hoc analysis to conduct pairwise comparisons between bee groups following a significant Kruskal-Wallis test result.

Results

Behavior when interacting with spikes

The major foraging behaviors recorded by bees were crawling, clinging, and dangling on the spikes (Fig. 3.1A). Other behaviors recorded were tongue protrusion and wing buzzing. When a bee landed on a spikelet, it crawled across the length of the inflorescence. The number of times this crawling behavior was exhibited per 30 s was significantly greater for the bumble bees than for honey bees or sweat bees ($\chi2= 20.1$; df = 2; $p < 0.001$; Fig. 3.2A). Some bees were clinging to the spikes. Clinging is defined as when a bee lands on a spikelet and uses its legs to hold onto it while collecting pollen. This behavior helped the bees to maintain an accessible position on the spikelet to reach the anthers and gather pollen. The number of clinging behaviors shown per 30 s was significantly greater for the bumble bees and honey bees than for sweat bees ($\chi^2 = 63.9$; df = 2; $p < 0.001$; Fig. 3.1B and 3.2B). Some bees dangled from the spikes. It is defined as dangling when a bee holds on to an anther on the spikelet and swings sideways to dislodge pollen onto its body. The number of dangling behaviors was significantly greater for the sweat bees than for the bumble bees or honey bees ($\chi^2 = 55.4$, df = 2, $p < 0.001$; Fig. 3.1C and 3.2C). This behavior may allow sweat bees to maneuver and extract pollen more effectively, potentially explaining the longer time spent on each flower.

Bumble bees rapidly vibrate their wings, referred to as wing buzzing to aid in dislodging pollen. This behavior was significantly greater for bumble bees than honey bees or sweat bees ($\chi^2 = 66.1$; df = 2; $p < 0.001$; Fig. 3.2D), where they did not elicit wing buzzing. Another behavior recorded when bees foraged on centipedegrass was tongue protrusion, defined as when a bee extends its tongue just before landing on a spikelet. The frequency of tongue protrusion is significantly greater for bumble bees than other bee groups ($\chi^2 = 35.5$; df = 2; $p < 0.001$; Fig. 3.1D and 3.2E).

Time and spikelet visit

Bumble bees visited significantly a greater number of spikes than sweat bees within 10 s ($\chi^2 = 56.9$; df = 2; $p < 0.001$; Fig. 3.3A). Bumble bees visited an average of three spikes and honey bees visited an average of two spikes within 10 s, while sweat bees visited less than one within the same time frame. Sweat bees spent significantly more time on centipedegrass inflorescence than bumble bees and honey bees ($\chi^2 = 59.1$; df = 2; $p < 0.001$; Fig. 3.3B) while bumble bees and honey bees spent an average of 3 seconds on an inflorescence, sweat bees extensively spent up to 35 seconds on an inflorescence.

Discussion

Bees are adapted to acquire pollen grains from anthers of diverse floral architecture present in nature (Armbruster, 2017). Centipedegrass has spike-like inflorescences (raceme) with flowers (spikes) arranged on them (Fig. 3.1A). Each spikelet comprises a gynoecium, androecium, lodicules, palea, and lemma (Kellogg, 2015). This arrangement of spikes on a raceme is designed for wind pollination. Results showed that bees exhibited many behaviors, such as crawling, clinging, and dangling on the spikes, tongue protrusion, and wing buzzing when foraging on the centipedegrass spikes. Crawling and clinging behaviors were exhibited in all the bee groups naturally foraging on spikes. Crawling through the spikes may suggest that bees are exploring the flowers to determine which anthers have pollen grains stored on them. Once they identify a region on the spikelet with pollen grain reserves, they tend to position themselves by clinging to the raceme to access the pollen. Although all the bee groups exhibited crawling and clinging behaviors, these behaviors were more frequently observed in bumble bees and honey bees than in sweat bees. Perhaps these behaviors are caused by the relative size of bees. Bumble bees and honey bees are larger than sweat bees, and thus, it is harder for them to pinpoint the region on the spikes where pollen is stored. Sweat bees are smaller than bumble bees and honey bees, and thus, they can land precisely where pollen is present on the spikelet. Bumble bees exhibited many behaviors associated with releasing pollen from anthers and efficiently collecting pollen grains after release. Foraging in bees is associated with species-specific traits that determine foraging efficiency and resource use (Forrest et al., 2015). Wing buzzing is a well-documented behavior exhibited by bumble bees (*Bombus spp.* Latreille, 1802), and it has been studied in detail in plants such as tomato (*Solanum lycopersicum* L.), blueberry (*Vaccinium corymbosum* L.), and eggplant (*Solanum melongena* L.) (De Luca and Vallejo-Marín, 2013). Wing buzzing behavior is a mechanism where bumble bees vibrate their flight muscles at a high frequency, which causes the anthers of flowers to release pollen (De Luca and Vallejo-Marín, 2013). However, this behavior has not been previously reported in grasses. This is the first report of wing buzzing behavior employed by bumble bees in releasing pollen from centipedegrass anthers. Once pollen grains are released into the environment, they must be gathered. Bees are adapted to gather pollen when released, as they have a dense covering of setae all over their body. The pollen grains are attached to branched setae present on their body and then later groomed using legs. The pollen is pressed and consolidated into scopa, which is present in the hind leg. In the current study, bumble bees protruded their tongue when foraging on centipedegrass spikes. Tongue protrusion occurred in bumble bees more frequently than other bee species, *Lasioglossum* spp, and *Apis* spp, which likely enhanced their ability to efficiently collect pollen from centipedegrass spikes. Tongue protrusion was not observed in sweat bees and honey bees in the current study. The crawling and clinging on the spikes enabled bumble bees to collect pollen, move quickly, and visit a number of spikes on centipedegrass.

Sweet bees spent more time on each spikelet than bumble bees and honey bees. They dangled from an anther and performed a dance to release pollen from the anther. The pollen grains released from anthers were collected on their abdomen. Bee species optimize their foraging strategies based on their morphological and physiological traits such as body size and tongue length, which affect their ability to exploit floral resources (Gathmann and Tscharntke, 2002). Sweat bees mostly have solitary habits, and they may not need to provision pollen for many larvae in their nest compared to social bees, such as bumble bees and honey bees (Michener, 2000). In addition, sweat bees are smaller than bumble bees and honey bees which makes it hard to apply strong force to break the anthers to release pollen grains. Thus, this unique behavior enabled them to acquire pollen from diverse sources, including grasses.

The diversity of foraging behaviors is indicative of functional diversity among bee species. For example, bumble bees exhibit complex behaviors, such as crawling, clinging, wing buzzing, tongue protrusion, etc., to access, release, and collect pollen from centipedegrass. These diverse behaviors enable them to forage on various floral resources representing various plant species, including grasses like centipedegrass, which is crucial for supporting their populations. The stability of pollination services decreases with increasing isolation from natural areas, and thus, habitat connectivity and floral diversity are essential in landscapes (Garibaldi et al., 2011). By recognizing and understanding the diverse foraging behaviors of bees, we can make more informed decisions about habitat management and conservation, ultimately contributing to the long-term sustainability of bee populations and the ecosystems they support.

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Fig. 3.1. (A), centipedegrass spikes, (B) *Apis* spp. bee exhibiting clinging, (C) *Lasioglossum* spp. bee exhibiting the dangling, and (D) *Bombus impatiens* exhibiting the tongue protrusion.

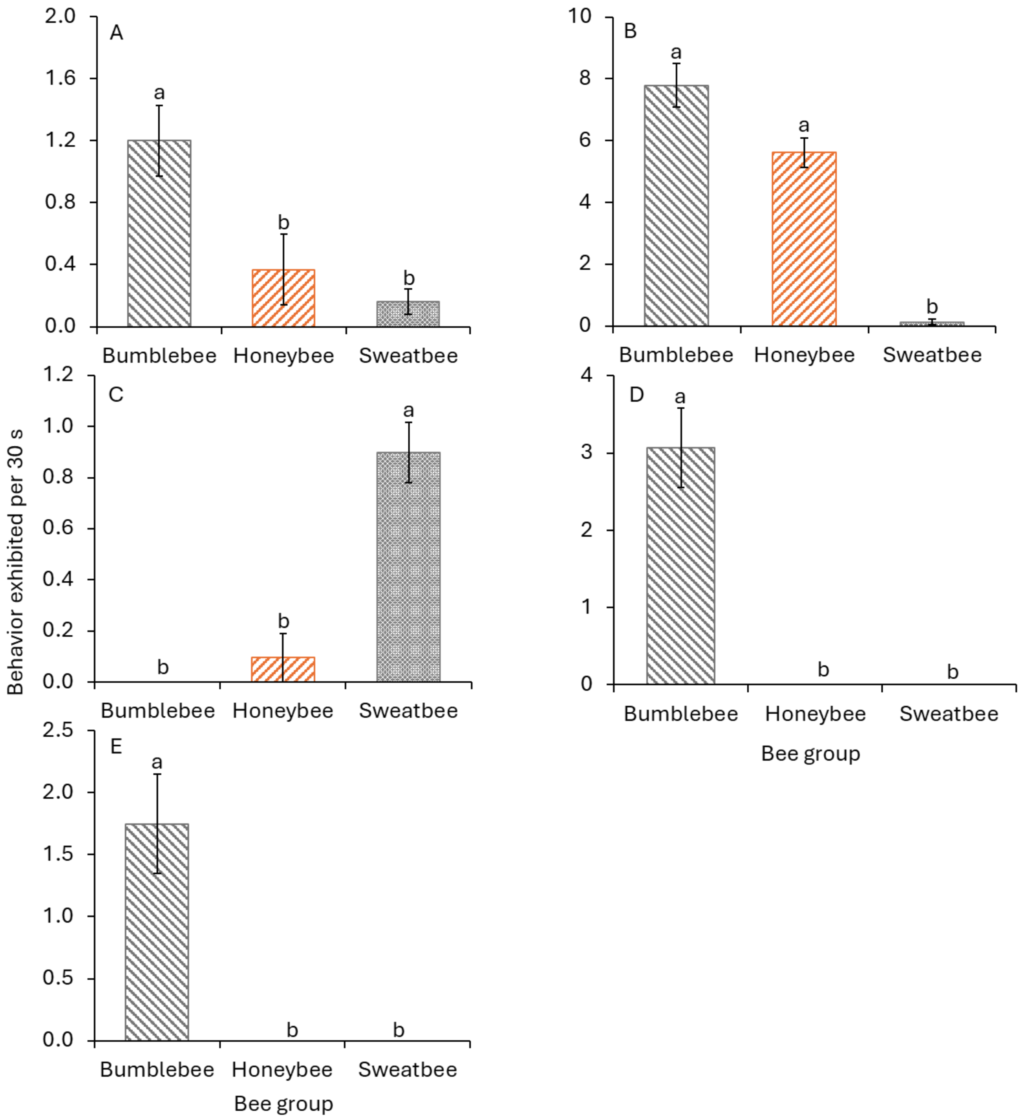

Fig. 3.2 Mean (±SE) numbers of times (A) crawling, (B) clinging, (C) dangling, (D) wing buzzing, and (E) tongue protrusion behaviors were exhibited per 30 s by various bee groups. The same letters above bars within a figure indicate no significant difference using the Kruskal-Wallis test at α =0.05.

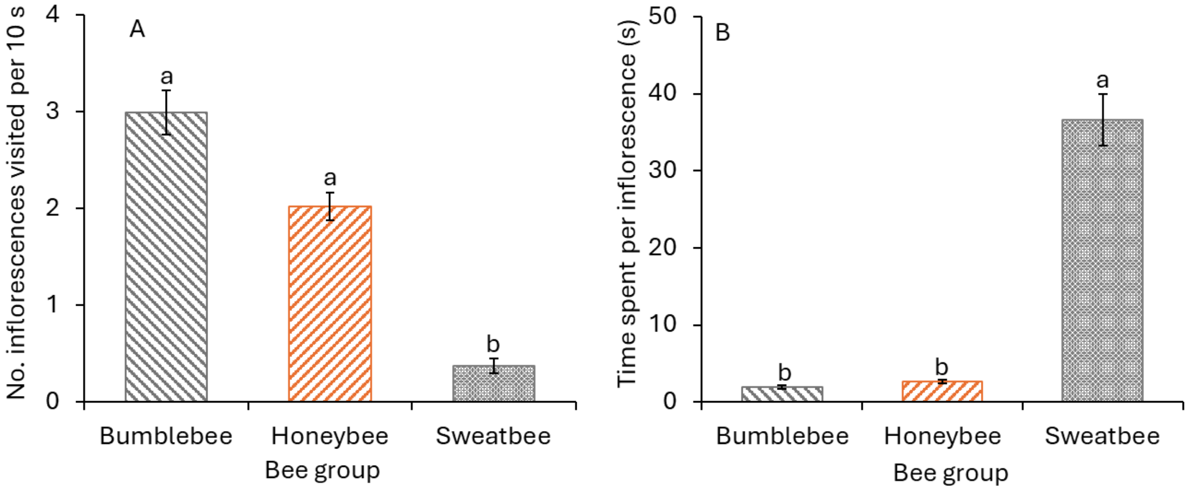

Fig. 3.3. Mean (±SE) (A) number of inflorescences visited by bees per 10 s, (B) time spent (s) on an inflorescence. The same letters above bars indicate no significant differences (Kruskal-Wallis test, α =0.05).

CHAPTER 4

PRESENCE OF DICOT FLOWERING PLANTS NEAR CENTIPEDEGRASS LAWNS DOES NOT AFFECT BEE VISITATIONS ON CENTIPEDEGRASS

Daniel Ibiyemi, Karen-Harris Shultz, David Jespersen and Shimat Joseph

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ABSTRACT Bees were found foraging on centipedegrass (*Eremochloa ophiuroides* [Munro] Hack.) lawns. However, it is unclear whether bees will choose to forage on flowers of dicot plants instead when those flowers are present over centipedegrass spikes. Thus, the objective of this was to determine the foraging behavior of bees when both flowering centipedegrass and dicot plants are available simultaneously. In 2023, three flowering plants, goldenrod (*Solidago canadensis* L.), butterfly bush (*Buddleja davidii* Franch.), and lavender (*Lavandula angustifolia* Mill.) containers were placed on flowering centipedegrass lawns producing pollen. Foraging bees were only visiting centipedegrass spikes and none visited the flowers of dicot plants. In 2024, butterfly bush and coneflower (*Echinacea purpurea* [L.] Moench) were placed on centipedegrass immediately after mowing. After 12 d post-mowing, the forging bees from centipedegrass spikes and flowers of dicot plants were collected. Results showed that a significantly greater number of bees were collected from centipedegrass spikes than from flowers of dicot plants. The major species collected were *Bombus impatiens* Cresson, *Apis* spp., *Lasioglossum* spp., *Halictus ligatus* Say, and *Melissodes bimaculatus* Lepeletier. This suggests that flowering centipedegrass is a valuable resource for foraging bees even when they have access to flowers of dicot plants

Keywords: Bee foraging behavior, Centipedegrass, Floral fidelity, Pollinator conservation, Grass pollen

Introduction

As urban areas are expanding and engulfing natural and rural landscapes, identifying and optimizing existing green spaces to enhance diverse pollinator resources is essential. Urbanization-mediated habitat degradation with a greater degree of landscape management practices, such as the use of excessive pesticides, increased impervious surfaces, and heat-island effects that follow, caused a decline in bee populations worldwide and continues to threaten plant biodiversity (Potts et al., 2010; Goulson et al., 2015). While pollinator conservation efforts focused on flowering plants, the role of grasses, such as centipedegrass (*Eremochloa ophiuroides* [Munro] Hack.), is overlooked as potential foraging resources for bees. Understanding how bees utilize these unconventional floral resources is critical for informing landscape management practices aimed at supporting a diverse pollinator population (Baldock et al., 2015).

Centipedegrass is a ubiquitous turfgrass species in the southeastern United States. It is a low-maintenance turfgrass and able to thrive in soils that are low in nitrogen and phosphorus, and it can tolerate acidic soils with a pH as low as 5.0, which makes it a popular choice in many areas (Islam and Hirata, 2005). Because turfgrass is mowed at least biweekly or sometimes twice or thrice a week at a low height (~5 cm tall) with limited opportunity for the grass to produce flowers, it is generally perceived as an ecological wasteland not directly benefiting pollinators (Beard and Green, 1994). This paradigm has changed as recent studies showed active bee foraging on centipedegrass when allowed to flower (Jones, 2014; Joseph et al., 2020). Thus, centipedegrass has the potential to contribute to the ecology of pollinators if managed adequately.

It is an established fact that dicot flowering plants are critical and contribute to the protein and energy needs of bees in many ecosystems (Vaudo et al., 2020; Stephen et al., 2024). Dicot flowering plants are common and found in most landscapes in the southern US. *Bombus impatiens* Cresson (1878) and *Apis mellifera* Linnaeus (1758) exhibit floral fidelity, often prioritizing specific plant types during foraging (Williams et al., 2010). However, recent studies also indicated that *B. impatiens* and *A. mellifera* forage on centipedegrass. Thus, it is uncertain whether bees will continue to forage on centipedegrass spikes or shift their focus to more diverse dicot flowers available when both centipedegrass and dicot flowering plant resources are available at the same time (Hicks et al., 2016; Garibaldi et al., 2013). It is critical to understand the behavior of bees when both grass and dicot floral resources are available simultaneously. The objective of this study was to determine the behavior of bees when pollen resources of both dicot flowering plants and centipedegrass are presented to bees at the same time. Understanding this behavior of bees is an important step toward expanding our knowledge of pollinator ecology in general landscapes when pollen from monocots and dicots are available at the same time to foraging bees.

Methods

Study site. In 2023 and 2024, a study was conducted on centipedegrass lawns in Griffin, Georgia (USA) from July to September. Four centipedegrass lawns were selected, and they were used in residential and non-residential (open-field) landscapes. Each site was approximately 10 km apart to minimize cross-visitation and ensure independent bee foraging activity, except for Site 2 and Site 4, which were approximately 2 km apart. Centipedegrass produced spikes from July to September in central Georgia. A 167.2 m^2

area was determined in each lawn site to conduct the study. Site 1 (residential) was managed by the homeowner, irrigated manually using a water hose, and was not fertilized or treated with pesticides. Weeds included white clover (*Trifolium repens* L.) and dandelions (*Taraxacum officinale* F.H. Wigg.) were present. Site 2 (non-residential) was located in the University of Georgia Griffin Campus, maintained by facility management, irrigated daily with a permanent sprinkler system, and was not treated with pesticides but contained similar weeds. Site 3 (residential) was irrigated using a water hose and mowed by a landscaping company, but no information was available regarding pesticide or fertilizer use. Site 4 (non-residential open field) was located in the University of Georgia Griffin Campus) and was irrigated every three days in 2023 but not in 2024. It was fertilized before the study with 32-0-4 (N-P-K) (Scotts Turf Builder Lawn Food (; Scotts Miracle-Gro Company, Marysville, OH) and treated with herbicide Celsius WG (8.7% thiencarbazone-methyl, 1.9% iodosulfuron-methyl-sodium, 57.4% dicamba; Bayer Environmental Science, Research Triangle Park, NC) in both years. The same sites were used in both years. The cultivars of centipedegrass used in these sites were unknown.

Plants. For various experiments, ‘Fireworks’ goldenrod (*Solidago rugosa* Mill.), butterfly bush (*Buddleja davidii* Franch.), ‘Royal velvet English’ lavender (*Lavandula angustifolia* Mill.) and ‘Cheyenne spirit’ coneflower (*Echinacea purpurea* [L.] Moench) were used because they attract bees, and their bloom periods overlap with centipedegrass. The plants used for the study were purchased from local ornamental nurseries. Goldenrod and lavender were purchased in 2023 and maintained in the shade house for six months before being used in experiments. Meanwhile, ‘Cheyenne spirit’ coneflower was purchased and maintained for three months in 2024. Butterfly bush was initially purchased in 2023 and reused in 2024. The goldenrod had bright yellow flowers, butterfly bush, and lavender both had purple/velvet-colored flowers, while the coneflowers displayed colors ranging from yellow, orange, and red. All the plants were in 11.4 L black plastic containers with a potting mixture comprised of peat moss, perlite, lime, and gypsum. After purchase, these ornamental plants were shipped to the Griffin Campus and were maintained in a shade house (50% light). Plant containers were irrigated daily and fertilized with 24-8-16 (NPK) (Miracle-Gro All Purpose Plant Food, Scotts Miracle-Gro Company, Marysville, OH). The echinacea plants were supplemented with Peter's Professional Peat-Lite Special fertilizer with 20-10-20 (NPK) (ICL Specialty Fertilizers, Dublin, OH). The plants were selected based on their attractiveness to various bee species, their bloom periods coinciding with centipedegrass flowering, and their representation of native and non-native species. goldenrod and coneflower are native to the United States. The butterfly bush and lavender are non-native (Goulson et al. 2008). These plants attract generalist bees, such as *A. mellifera* and *Bombus* spp. (Winfree et al. 2011). These plants are common in ornamental landscapes in the southeastern US.

Experiment 1

In 2023, the ornamental plant containers were moved to centipedegrass lawn sites on the day of the experiment. Six plant containers were used at each lawn site, comprising two containers each of goldenrod, butterfly bush, and lavender. All sites received distinct sets of plants, and the same set of plants was not used in different sites. The plant containers were randomly placed at least 6.1 m apart at each lawn site. When plants were introduced, the goldenrod, butterfly bush, and lavender had an average of fifteen, ten, and twelve flower heads per container, respectively. Lawns were mowed, and the experiment began 12 d post-mowing when centipedegrass began producing pollen and bees were actively foraging. The observation began 15 min after placing plant containers on centipedegrass lawns. This allowed sufficient time for the bees to recognize the newly introduced plants on 167.2 m² centipedegrass lawns. After 15 min of the acclimatization period, bees visiting the centipedegrass spikes and the introduced plant containers were observed for 30 min. During each visit, lawns were carefully scouted for foraging bees on both the centipedegrass spikes and on the flowers of introduced plant containers. When bees were spotted on centipedegrass and flowering plant containers, they were collected. The foraging bees were collected during each observation day between 8:00 am and 12:00 pm daily for 7 d. In general, bees did not forage on centipedegrass spikes past noon. This round of the experiment is referred to as trial 1. After 7 d of observation, the lawns were mowed, and all the spikes were removed. This experiment was repeated after 12 d post-mowing when the centipedegrass initiated pollen production again. The foraging bees on centipedegrass spikes and flowers of dicot plants were collected again daily for 7 d. The repeated trial is referred to as trial 2.

A container method was employed to collect foraging bees. When foraging bees remained on a centipedegrass spikelet or a flower of a dicot plant for at least 3 s, they were captured using a 166.9 mL clear plastic container with a white lid. The container was placed over the bee, and the lid was gently placed on the container to trap the bee. Collected bees were transferred to the laboratory. They were cleaned, washed, and dried before pinning. Bees were identified to genus and species using published keys (Ascher and Pickering, 2015; Carril and Wilson, 2021).

Experiment 2

In 2024, container plants were placed on the designated lawns immediately after centipedegrass lawns were mowed, and centipedegrass did not bear any spikes on them. In contrast, for experiment 1, six dicot plant containers were introduced to centipedegrass lawns on the same day the observation began when pollen was readily available to bees. Six plant containers of butterfly bush and coneflower, three each, were transferred to centipedegrass lawns. Lavender plants were not used in the 2024 experiment. Each butterfly bush had an average of eight purple flower heads, whereas coneflowers had an average of seven flower heads per container. They were randomly placed on centipedegrass lawns. The spacing between plant containers was the same as in experiment 1, which was conducted in 2023. The centipedegrass initiated the production of spikelet and pollen release in ~10-12 d post-mowing. This lag time provided sufficient time for newly placed flowering plants to integrate into the new landscape before the experiment was initiated. Once the centipedegrass spikes began to emerge, a 30 min observation, bee collection, curation of bees, and identification were conducted as described in experiment 1. This experiment was repeated and referred to as trials 3 and 4.

Statistical analyses

Data were subjected to a paired *t*-test using R Studio software (RStudio, PBC, Boston, MA, USA). The bee data for each trial in experiments 1 and 2 were subjected to paired *t*-tests as the data involved paired observations from the same sites, comparing two plant types (flowering centipedegrass and dicot plants). The lawn sites were the replications for each treatment. The statistical significances were determined $\alpha = 0.05$. Means and standard errors were calculated.

Results

Experiment 1

In 2023, a total of 130 bees were captured, where 75 *Bombus impatiens*, 24 *Apis* spp, 28 *Lasioglossum* spp., and 3 *Melissodes bimaculatus* were observed foraging on centipedegrass spikes, whereas no bees were recorded visiting goldenrod, butterfly bush, and lavender (Table 4.2). Forty-four observations were conducted in four centipedegrass lawn sites. Significantly greater numbers of foraging bees were collected from centipedegrass spikes than from dicot plants in trial 1 ($t = 5.7$; df = 19; $p < 0.001$; Fig. 4.1A) and trial 2 ($t = 6.2$; df = 23; $p < 0.001$; Fig. 4.1B).

Experiment 2

In 2024, a total of 149 bees were captured; 98 *Bombus impatiens*, 24 *Apis* spp, and 27 *Lasioglossum* spp., were observed foraging on centipedegrass lawns, whereas of the total of 13 bees; 10 *Halictus ligatus* and 3 *Lasioglossum* spp. were recorded visiting butterfly bush and coneflower (Table 4.3). Twenty-nine observations were conducted in four centipedegrass lawn sites. The number of bees collected from centipedegrass was significantly greater than from flowers of dicot plants in trial 3 ($t = 2.9$; df = 16.2; $p < 0.009$; Fig. 4.1C) and trial 4 ($t = 5.1$; df = 19.8; $p < 0.001$; Fig. 4.1D). Several bee species were observed foraging on centipedegrass spikes. The species recorded in both 2023 and 2024 included *Bombus impatiens* Cresson, *Apis* spp., *Lasioglossum* spp., *Halictus ligatus* Say, and *Melissodes bimaculatus* Lepeletier.

Discussion

The results showed that the foraging habits of bees centipedegrass pollen, especially *Bombus* spp. were not influenced by the presence of dicot flowering plants in the same lawn. The flowering plants, goldenrod, butterfly bush, Lavender, and coneflower, had more conspicuous and attractive flowers than centipedegrass spikes, but they did not prefer to forage on them. Clearly, this result challenges the common perception that grass lawns provide limited floral resources to pollinators. However, the maintenance of centipedegrass lawns should certainly be modified to allow the production of spikes. Previous (Jones, 2014; Joseph et al., 2020) and current studies have repeatedly shown that centipedegrass lawns begin to produce spikes and pollen 12 days after mowing.

It is unclear why foraging bees continue to forage on centipedegrass spikes when they can access more attractive floral resources in the same landscape. The dicot flowering plants offered to foraging bees had pollen and nectar resources. Centipedegrass spikes are devoid of nectar, but bees pursued centipedegrass pollen. There are some possible reasons for this behavior. First, it could be attributed to floral fidelity behavior, where bees tend to consistently forage on a specific plant species during a foraging trip on specific days (Williams et al. 2010), which is shown in social bees, such as *B. impatiens* and *Apis* spp. These species were the dominant bee species collected from centipedegrass spikes in the current study. Floral fidelity is tied to increased foraging efficiency, where bees can specialize in handling specific floral morphologies (Garibaldi et al., 2013). It is unclear how centipedegrass pollen provides sufficient rewards over more attractive flowers of dicot resources present in the landscape. Secondly, centipedegrass can produce abundant floral resources when available. This abundant resource may cost less energy for the foraging bees to move from spikelet to spikelet and collect all the pollen they need during a specific trip. Centipedegrass is a reliable and consistent pollen source for bees when present in the landscape, which provides a strong incentive to forage on them over flowers of dicot plants. More research is warranted to understand how the floral abundance hypothesis drives the foraging behavior of bees, especially bumble bees in the landscape. Thirdly, centipedegrass produces viable pollen in the morning hours and dries out before noon. Bees may strategize their foraging trips and forage on centipedegrass when present in the landscape and switch to other floral resources when centipedegrass pollen resources become unavailable. In the current study, the observations were conducted only when centipedegrass pollen was available. Bees might have utilized the dicot floral resources during afternoon periods or when the centipedegrass lawns were mowed. Finally, the knowledge of the nutritional content and quality of centipedgrass and dicot plants used in the study is limited. It is unclear how much variability in nutrient content existed among species in the current study and whether this existing variation influenced the foraging needs of bees, shaping their behavior. Thus, more research is warranted to understand how the nutrient content of pollen and bees' needs explain the foraging behaviors so that conservation efforts can be refined to increase ecosystem health.

Based on the results, lawn management practices for centipedegrass in urban and suburban environments should be modified to enhance pollen availability. In central Georgia, centipedegrass typically initiates pollen production from July to September. In central Georgia, centipedegrass typically initiates pollen production ~12 d post mowing. However, this is based on field observations rather than a study specifically designed to quantify the exact onset of pollen production. The pollen production lasts for 5-10 days. Once most flowers set seeds, bees stop foraging on the centipedegrass lawn. To ensure that bees have access to this pollen resource, the mowing interval should be adjusted to allow sufficient time for pollen to develop and become available for bee foraging. More educational events, demonstrations, and media involvement are needed to extend the new information, calling for subtle changes to lawn care practices to convert centipedegrass lawns to more pollinator-friendly ones. Similarly, factors that inhibit property owners' perception and willingness to modify mowing practices should be studied. These adjustments to lawn care practices will address the criticism that turfgrass is an ecological wasteland (Beard and Green, 1994). Furthermore, centipedegrass pollen production coincides with periods of pollen scarcity in other plants, such as trees (Schmidt, 2016). This suggests that centipedegrass could be a valuable floral resource for bees during late summer and early autumn (Williams et al., 2015). This is especially important for bumble bee colonies, as they require large amounts of pollen resources during the late summer to support the development of overwintering queens (Goulson et al., 2008).

In summary, data show that various species of bees, mainly *Bombus* spp. and *Apis*. spp. foraged on centipedegrass pollen when attractive dicot flowering plants were in the landscape. This suggests that grass-dominated landscapes can provide ecological value for enhancing urban pollinator conservation. The current study was conducted only in central Georgia, and thus, the more regional scale of this study, incorporating other geographical locations and expanding on other turfgrass species, such as zoysiagrass, would be beneficial. To date, bees are reported to forage on the pollen of two turfgrasses, centipedegrass (Jones, 2014; Joseph et al., 2020), and bahiagrass (Joseph and Hardin, 2022). Similarly, the long-term ecological benefits of flowering grasses, such as centipedegrass, on bee health and ecosystem services to crops are still not known in urban and suburban areas. Lawn care maintenance practices, such as amount, timing, and intervals of fertilizer and irrigation application on quality of pollen and abundance, are not completely understood, which can be improved with future research.

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Table 4.1. Details of centipedegrass lawns and dates of mowing, setup, and observation for trials in 2023 and 2024

Location	GPS coordinates	Trial							
		1	2	3			4		
		Observation started		Mowing	Plants deployed	Observation started	Mowing	Plants deployed	Observation started
Site 1	33.267245, -84.292229	26 Jul	14 Aug	1 Aug	2 Aug	12 Aug	17 Aug	19 Aug	26 Aug
Site 2	33.197109, -84.220127	25 Jul	14 Aug	2 Aug	5 Aug	12 Aug	16 Aug	19 Aug	26 Aug
Site 3	33.187680, -84.271065	25 Jul	14 Aug	1 Aug	2 Aug	12 Aug	16 Aug	19 Aug	26 Aug
Site 4	33.263934, -84.282609	25 Jul	14 Aug	1 Aug	2 Aug	12 Aug	17 Aug	19 Aug	26 Aug

Table 4.2. Details of bee species collected on flowering centipedegrass and dicot plants in 2023.

Location	Bee species on centipedegrass*
Trial 1	
Site 1	*Bombus impatiens* (10); *Apis* spp. (5); *Lasioglossum* spp. (7)
Site 2	*Bombus impatiens* (14); *Apis* spp. (6); *Lasioglossum* spp. (2)
Site 3	*Bombus impatiens* (6); *Apis* spp. (1); *Lasioglossum* spp. (2); *Melissodes bimaculatus* (1)
Site 4	*Bombus impatiens* (5); *Apis* spp. (1); *Lasioglossum* spp. (3)
Trial 2	
Site 1	*Bombus impatiens* (6); *Apis* spp. (4); *Lasioglossum* spp. (2)
Site 2	*Bombus impatiens* (17); *Apis* spp. (2); *Lasioglossum* spp. (7)
Site 3	*Bombus impatiens* (10); *Apis* spp. (4); *Lasioglossum* spp. (3); *Melissodes bimaculatus* (2)
Site 4	*Bombus impatiens* (7); *Apis* spp. (1); *Lasioglossum* spp. (2)

*no bees were collected from dicot flowering plants deployed in 2023. The parenthesis after bee genera or species indicate counts of specific bee type collected.

Table 4.3. Details of Bee species collected on flowering centipedegrass and dicot plants in 2024

Location	Bee species on centipedegrass	Bee species on dicot plants
Trial 1		
Site 1	none	none
Site 2	*Bombus impatiens* (21); *Apis* spp. (6); *Lasioglossum* spp. (4)	*Lasioglossum* spp. (2); *Halictus ligatus* (4)
Site 3	*Bombus impatiens* (5)	None
Site 4	*Bombus impatiens* (14); *Apis* spp. (2); *Lasioglossum* spp. (3)	*Halictus ligatus* (1)
Trial 2		
Site 1	*Bombus impatiens* (5); *Lasioglossum* spp. (1)	*Halictus ligatus* (3)
Site 2	*Bombus impatiens* (27); *Apis* spp. (5); *Lasioglossum* spp. (8)	*Lasioglossum* spp. (1); *Halictus ligatus* (1)
Site 3	*Bombus impatiens* (14); *Apis* spp. (5); *Lasioglossum* spp. (6)	*Halictus ligatus* (1)
Site 4	*Bombus impatiens* (12); *Apis* spp. (6); *Lasioglossum* spp. (5)	None

The parenthesis after bee genera or species indicate counts of specific bee type collected.

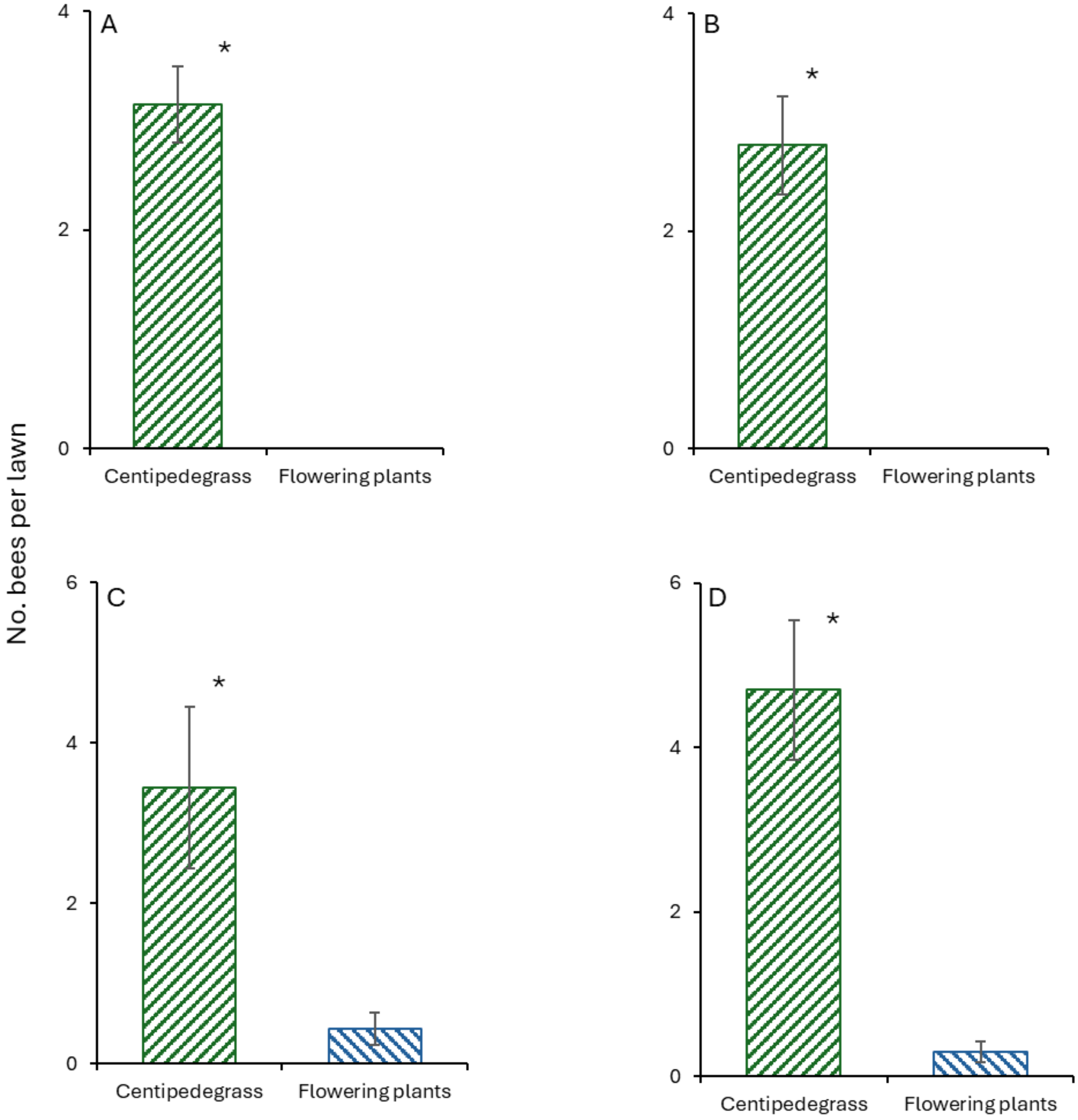

Fig. 4.1 Mean (±SE) number of bees collected on flowering centipedegrass and dicot plants in (A) trial 1, (B) trial 2 experiments in 2023, (C) trial 3, and (D) trial 4 in 2024. Asterisk (*) above the bars indicates a significant difference (Paired t-test, $P < 0.05$).

CHAPTER 5

SUMMARY

Bees produce 35% of the world's fruits, nuts, and vegetables. However, a decline in bee populations due to habitat loss, monoculture farming, pesticide use, and urbanization threatening biodiversity and agricultural sustainability have been widely reported. Identifying new resources for bees is essential to supporting their populations, particularly in managed landscapes. Centipedegrass (*Eremochloa ophiuroides*), a warm-season turfgrass, offers a potential pollen resource for bees in the southeastern United States, making it an important subject of study. This research aimed to assess the pollen load composition of bees foraging on centipedegrass spikes, examine the pollen transfer to hives, evaluate the utilization of centipedegrass pollen for colony development, characterize the foraging behaviors of bees on centipedegrass spikes, and assess the impact of surrounding flora on bee foraging behavior on centipedegrass inflorescence.

To understand the pollen composition collected by bees, sampling was conducted in 2021 and 2022 across five centipedegrass lawns. Sampling occurred at 10–12 days (early) and 14–16 days (late) post-mowing. Bees collected significantly more Poaceae than non-Poaceae pollen, with higher counts observed during the early flowering phase in 2021. However, in 2022, no significant differences were found between early and late flowering phases. Bumble bees (*Bombus* spp.) were more active in late summer, gathering predominantly Poaceae pollen. To assess the pollen transfer to bee hives, hives of *Apis mellifera* were deployed in centipedegrass lawns in 2022 and 2023. Pollen traps were placed to collect pollen from foraging bees, comparing 0–5 days post-mowing (no spikes) and 9–14 days post-mowing (50% spikelet coverage). Results showed that most of the pollen transferred to hives was Poaceae pollen. A no-choice experiment conducted in 2022 and 2023 evaluated the ability of bumble bee, *Bombus impatiens* Cresson larvae to develop on centipedegrass pollen compared to butterfly bush (*Buddleja* spp.) pollen. The study found no significant differences in larval development between bees fed on centipedegrass pollen and those fed on butterfly bush pollen, indicating that centipedegrass pollen provides sufficient resources for larval growth. These findings suggest that centipedegrass is a valuable pollen resource for bees during its flowering period, supporting pollen collection and pollen transfer to hives and bee larval development under controlled conditions.

Understanding the foraging behaviors of bees is critical for optimizing pollination services and supporting bee populations. The unique floral structure of centipedegrass spikes, which differs from more traditional floral resources, may present distinctive challenges or opportunities for bees during pollen collection. Thus, video recordings of bee activity were analyzed during the summers of 2022 and 2023 to characterize the foraging behaviors of bees on centipedegrass spikes. Bumble bees exhibited more crawling behaviors than honey bees and *Lasioglossum* spp., while clinging to spikes to collect pollen was common among both bumble bees and honey bees. *Lasioglossum* bees showed unique dangling behavior, which was not observed in other species, and spent more time on individual spikes. These findings demonstrate that different bee species exhibit varied behaviors when foraging on centipedegrass spikes, which may influence their efficiency in pollen collection.

The availability of diverse floral resources can influence bee foraging preferences, especially when monocot and dicot plants are in the same landscape. It is crucial to understand whether bees exhibit floral fidelity to centipedegrass when more attractive, pollen and nectar-producing dicot flowers are available, as this can inform landscape management and pollinator conservation strategies. In 2023 and 2024, experiments were conducted to determine whether bees would shift their foraging preference when dicot flowers (e.g., goldenrod, butterfly bush, coneflower) and centipedegrass pollen were present. Despite the availability of more conspicuous, showy dicot flowers, bees predominantly foraged on centipedegrass pollen. A significantly greater number of bees were collected from centipedegrass spikes than from dicot flowers, demonstrating a strong preference for centipedegrass pollen even when alternative floral resources were available. This finding suggests that centipedegrass can be a valuable pollen source for bees in landscapes with diverse plant species.

This study underscores the ecological significance of centipedegrass in supporting bee populations by providing Poaceae pollen, which they actively collect, transfer to hives, and utilize for larval development. The distinct foraging behaviors exhibited by different bee species on centipedegrass further emphasize the importance of this turfgrass in supporting pollinator populations. Even with the presence of alternate floral pollen resources, bees continued to forage on centipedegrass. These findings provide important insights for turfgrass management and pollinator conservation efforts, particularly in urban, suburban, and rural landscapes.