Nocturnal Pollination in Fruit Agriculture

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Nocturnal Pollination in Fruit Agriculture

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Entomology

by

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Abstract

Insect pollination in agriculture provides as much as 35% of the global food supply and contributes hundreds of billions of dollars to the global economy each year. In the past 30 years, reports of declining populations of managed and wild bees, notably the western honey bee (*Apis mellifera*) and a wide array of bumble bees (*Bombus* spp.), have raised concerns about the stability and outlook of agriculture. At the same time, agricultural dependence on insect pollinators is increasing as greater percentages of land being converted to pollinator-dependent crops, such as soy and oil palm, than pollinator-independent crops, such as grains and oats. Current knowledge of animal-mediated pollination in agriculture is focused on diurnal pollinators, with particular attention given to bees. Nocturnal insects, especially moths, represent a significant source of pollinator diversity, even greater than that of all diurnal pollinators combined. They are also well-known for their pollination services outside of agriculture. As such, these insects could offer valuable pollination services to agriculture, potentially providing additional stability and security to production. In this collection of works, I examine the roles of nocturnal-insect pollinators to fruit agriculture. The primary question was whether or not nocturnal pollinators offer any benefit to the production of selected fruits. I then examined which insects may be responsible for the observed pollination services. I found that nocturnal pollinators do not provide significant increases to the production of peaches or muscadines. However, nocturnal pollinators significantly increased apple fruit set by comparison to a negative control, and nocturnal pollination levels were similar to those of diurnal pollinators. The most likely nocturnal pollinators were moths. These results are unprecedented and provide a new, potentially greatly underestimated, pollination system that demands immediate study.
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Chapter 1: Introduction

Nocturnal-insect pollinators may be an undervalued source of pollination services in agriculture. These insects are active outside of typical human activity periods, which has limited observations which may indicate a role in pollinating agricultural crops. Researchers have given little attention to nocturnal pollinators in agriculture as a result. Some researchers have even suggested that nocturnal pollinators are likely unimportant for their pollination-services in agricultural crops without specific testing (Blanche et al. 2006, Hahn and Brühl 2016). A lack of evidence, however, does not necessarily indicate a lack of importance. Literature concerning their participation in crop pollination is scant and has, to date, focused on crops of relatively low impact to the global food supply and economy, like *Jatropha curcas* L. (Euphorbiaceae), a tree whose nuts are used to generate biofuel (Luo et al. 2011), and four cucurbits (Cucurbitaceae) grown regionally in southern Asia, grown for use as containers (gourds) and food vegetables (Lu et al. 2021). Results are mixed among studies examining the inputs of nocturnal pollinators. However, most studies find that nocturnal pollinators significantly contribute to the production of examined crops (Tasen et al. 2009, Luo et al. 2011, Cutler et al. 2012) and one identified nocturnal pollinators as the most important group of pollinators to crop production (Lu et al. 2021).

Approximately 80% of the world’s leading crops depend on insect-mediated pollination and generate upwards of 35% of the global food supply (Klein et al. 2007). Additionally, these crops are important to human health, providing key sources of essential vitamins and nutrients that are not readily available from other sources in the human diet (Eilers et al. 2011, Smith et al. 2015). Because these crops are dependent on pollination services provided by insects, recent declines in distribution, abundance, and diversity of pollinating insects, such as bees and flies,
have raised concerns about the stability and outlook of agriculture (Aizen et al. 2008, 2009, Garibaldi et al. 2011). Meanwhile, the rate of increase of land devoted to pollinator-dependent crops is nearly double that of pollinator-independent crops (Aizen et al. 2019). As agriculture becomes increasingly dependent on insect-pollination, it also become less stable (Garibaldi et al. 2011). Furthermore, increases in pollinator-dependent crops are exceeding increases in production of commercial honey bees, potentially creating a gap between the pollination needs of growers and the pollination provisions able to be provided by pollinators (Aizen and Harder 2009).

At the start of this series of projects, I observed an unexpected abundance and diversity of moths visiting the flowers of peaches, blueberries, and blackberries, suggesting that moths may provide pollination services to these pollinator-dependent crop plants. Considering that 1) pollinator diversity is valuable component to the production of most pollinator-dependent crops, 2) a number of diurnal pollinators are declining in abundance and diversity, and 3) nocturnal pollinators are relatively unknown in agricultural pollination, it seemed prudent to examine their roles in a variety of fruit crops. The overall goal of this research was to establish if, to what extent, and which nocturnal pollinators contribute to fruit production. As moths were found to be the most common nocturnal pollinators throughout my studies and is the most studied nocturnal pollinator, much of my focus throughout my project are focused on moths.

In the second chapter I review literature relevant to nocturnal pollination performed by moths. To do so, I compiled indirect evidence for the importance of nocturnal moths as pollinators in agriculture using evolution, morphology, behavior, and perception. I also review direct evidence for their importance in pollination networks in a variety of ecosystems, the pollination of various wild plant species, and the pollination of few agricultural crops. The third
and fourth chapters are dedicated to exploring the roles and identities of moths in apple 
production, using exclusion experiments and a rigorous trapping protocol. The fifth chapter is 
focused on identifying the inputs of nocturnal moths to self-fertile peaches and muscadine 
grapes. Finally, chapter six summarizes my findings.

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Chapter 2: The potential of moths as pollinators in agriculture: a literature review

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**Introduction**

Insect pollinators are critical to agriculture. Approximately 80% of the leading global crop plants are dependent on insect-mediated pollination and contribute as much as 35% to the global food supply (Klein et al. 2007). Although most of the global food supply is generated by pollinator-independent crops, such as corn, rice, and wheat (Klein et al. 2007), these crops are poor sources of essential nutrients, such as vitamin A, folic acid, and calcium (Eilers et al. 2011). In fact, diets absent of fruit and vegetable products generated by pollinator-dependent crops would result in substantial increases to malnutrition-related diseases and deaths (Smith et al. 2015). Pollination services provided to agriculture by insects is valued at over 50 billion USD per year in the United States alone (Calderone 2012) and as much as 152 billion USD worldwide (Bauer and Wing 2016). As such, losses of pollination services provided by insects would have severe repercussions to human food, health, and economy.

Insect pollinators have experienced widespread declines in abundance and distribution in recent history, with declines reported in the major pollinating orders Diptera (flies) (Powney et al. 2019), Hymenoptera (bees, ants, and wasps) (Centrella 2019, Cameron and Sadd 2020, Soroye et al. 2020), and Lepidoptera (moths and butterflies) (Macgregor 2015, Fox et al. 2021). Causes of declines are predominantly associated with habitat loss but also include climate change, pathogens and parasites, pesticides, and various human-driven causes (e.g., artificial lighting on moth populations), which have deleterious effects by themselves but can also act synergistically with other factors (Goulson et al. 2015, Steinhauer et al. 2018, Powney et al. 2019, Sánchez-Bayo and Wyckhuys 2019, Goulson et al. 2015, van Langevelde 2018, Soroye et al. 2020). Because of agricultural dependence on insect pollinators, losses in important pollinating insect groups have raised concern about the future productivity and stability of

The most studied pollinators in agriculture are bees, a monophyletic clade of seven families of Hymenoptera called Anthophila. Bees have been identified as floral visitors of 73% of pollinator-dependent crops (Nabhan and Buchman 1997, Kremen and Chaplin-Kramer 2007). Bee pollinators of crops include managed bees, such as the western honey bee (Apis mellifera L. Apidae) and the common eastern bumble bee (Bombus impatiens Cresson Apidae), and wild bees, such as the orchard mason bee (Osmia lignaria Say Megachilidae) and a variety of native bumble bee (Bombus spp.). Managed bees are often transported to or reared in agricultural regions that lack a sufficient supply of pollination services from wild insect populations, which exist in these areas outside of human control (Ahn et al. 2012). Population declines have been reported for both managed (van der Zee 2012, Oberreiter 2020) and wild bees over the past 30 years (Centrella 2019, Cameron and Sadd 2020, Soroye et al. 2020). For example, managed populations of western honey bees have experienced increased annual colony losses in Europe (Morawetz et al. 2018, Potts et al. 2010), South America (Antúnez et al. 2017, Requier et al. 2018, Castilhos et al. 2019), North America (Currie et al. 2010, Kulhanek et al. 2017), and Australasia (Brown et al. 2018). Declines in populations of wild bees, such as bumble bees
Bee declines and the threat these declines pose to agricultural production have prompted explorations into alternative, non-bee insect taxa as crop pollinators. It has been shown that a variety of non-bee insects participate in crop pollination (Rader et al. 2016, Rader et al. 2020). For example, flies (Diptera) are adept pollinators in agriculture, being both abundant floral visitors and adept at transferring pollen (Orford et al. 2015, Stavert et al. 2018, Cook et al. 2020). Butterflies and moths (Lepidoptera) also provide significant crop-pollination services in some crops, like apples, cucurbits, and lowbush blueberry (Cutler et al. 2012, Lu et al. 2021, Robertson et al. 2021). Importantly, many studies into non-bee pollination of crops find that these alternative taxa provide complimentary pollination services to crops, wherein non-bee species (as well as different bee species) pollinate fundamentally different sets of flowers, adding to crop yield irrespective of bee abundance (Rader et al. 2016, Rader et al. 2020). In fact, it is now understood that pollinator diversity is a key component of crop pollination (Hoehn et al. 2008, Martins et al. 2015). However, expansion of research into non-bee pollinators of crops has been slow and predominantly focused on diurnally-active species.

Nocturnally-active moths are estimated to be the most diverse group of pollinators (Ollerton 2017). Most crops are angiosperms, and early moth evolution and radiation coincides with that of angiosperms (Kawahara et al. 2019). Furthermore, moths maintain physical
characters, sensory capabilities, and behaviors that enable them to adeptly locate and pollinate a wide variety of plants (Ala-Laurila 2016, Powers et al. 2020). However, studies into their roles as crop pollinators remain limited. One important factor for the general absence of moths in crop research appears to be their nocturnal activity, which is outside of typical observation periods in research and grower activity periods. This greatly reduces potential observations from which to pose questions. Furthermore, moth larvae are notorious pests of many crop plants, capable of causing significant losses to crop yield and quality (Steinkraus and Mueller 2003, Maish 2019). Factors such as these likely influence scientists to ignore moths in crop-pollination research and lead to interpretations that they are unimportant to crop pollination. However, a lack of evidence due to an absence of research is not necessarily evidence that these insects do not play a valuable role in agricultural production, and nocturnal-moth contributions to crop pollination should be considered independent of their pestilent status. In this review, I use available literature concerning moth-pollination roles in both crop and non-crop plants to postulate that moths are likely among the most valuable pollinators in agriculture. As pollinator populations are in decline, including moth populations, I emphasize the immediate need for broad-scale investigations into the role of moths in agricultural production.

**Evolutionary relationship between moths and angiosperms**

Modern angiosperms are the dominant form of plant life on Earth, representing about 79% of extant plant species (Christenhusz and Byng 2016). Approximately 80% of modern angiosperms are dependent on pollinators for reproduction (Ollerton 2011), including most of the leading crop plants (Klein et al. 2007). The relationship between angiosperms and pollinators is believed to be an important factor in their rise, success, and subsequent radiations (Kawahara et al. 2019). In fact, the key feature of angiosperms is their fragrant and colorful flowers, which
house their reproductive systems and are adapted to recruit appropriate animal pollinators for visitation. Angiosperms are a relatively new plant lineage, with the oldest confirmed fossil dating to 132 Ma in the Early to Middle Cretaceous Period (Friis et al. 2006) and fossilized angiosperm-like pollen dating ca. 245 Ma in the Middle Triassic (Hochuli and Feist-Burkhardt 2013). However, fossil records for angiosperms are incomplete, making inferences about their early origins difficult (Foster et al. 2017, Silvestro et al. 2021). Using modern phylogenetic approaches and access to complete genomes of extant angiosperms has allowed researchers to more accurately estimate their origins. Current estimates place the most common ancestor of all modern angiosperms (stem group) to somewhere from ~330 Ma (Magallón et al. 2013) to 355 Ma (Salamo et al. 2017), with a general consensus that the split from gymnosperms occurred in the Early- to Mid-Carboniferous Period (~350-325 Ma) (Foster et al. 2017). The crown group of angiosperms is estimated to have originated in the Triassic or Permian periods, somewhere between 194 Ma (Magallón et al. 2013) and 284 Ma (Salamo et al. 2017), with a general consensus that some angiosperm lineages were present >300 Ma (Foster et al. 2017, Barba-Montoya 2018, Sauquet and Magallón 2018). Interestingly, the crown group of bees is believed to have originated sometime between 113 and 132 Ma with the origin and diversification of eudicots, the most diverse extant plant group (Cardinal and Danforth 2013). By these estimations, angiosperms predate bees by 62 to 171 million years. While bees are believed to be the most important group of pollinators in modern times, this gap between the origins of angiosperms and bees suggests that the success of early angiosperms was owed to a different group of pollinators.

A recent phylogenetic analysis placed the most recent common ancestor of crown Lepidoptera to the Late Carboniferous, about 300 Ma (Kawahara et al. 2019). At this early stage
in moth evolution, moths were mandibulate (having mandibles as adults) and believed to feed on early, nonvascular plants, called bryophytes (Powell et al. 2013, Kawahara et al. 2019). This is still true of the extant and ancient mandibulate-moth lineage Micropterigoidea (Powell et al. 1998), which is the sister taxon to all Lepidoptera (Banizet et al. 2017, Kawahara et al. 2019). The evolution of Lepidoptera is believed to have followed plant evolution, with the earliest plant associations originating with bryophytes prior to the transition to vascular plants and then angiosperms (Powell et al. 2013, Kawahara et al. 2019). In fact, Heterobathmioida, a group of mandibulate moths, is thought to have been one of the first moth lineages to feed on angiosperms (Angiospermivora), an association that now represents more than 99% of extant moths (Kawahara et al. 2019). Modern adult heterobathmioids use their mandibles to feed on pollen from Nothofagus (Fagales) (Kristensen 1998), an ancient genus of angiosperms with few extant species. This association between the reproductive mechanisms of angiosperms and moths offers evidence that moths were among the first pollinating insects of angiosperms.

One of the most recognizable characters of most modern moths is the adult proboscis (also called haustellum). Proboscis-bearing moths, Glossata, represent more than 99% of extant moth species (van Nieukerken et al. 2011). Eriocranioidea, the sister group to all other extant Glossata, was one of the first groups of moths to have proboscises as adults and are known to use this structure to drink water and feed on plant sap (Kristensen 1998). The primary function of the proboscis in more derived species of moths is feeding on floral nectar from angiosperms (Scoble 1992). Interestingly, nectar-feeding moths first appeared about 241 Ma during the Middle Triassic, which conspicuously overlaps with the early diversification of angiosperm crown groups (Foster et al. 2017, Kawahara et al. 2019). This moth-angiosperm relationship may have contributed to the superradiation of angiosperm crown groups in the Early Cretaceous and likely
promoted subsequent superradiations of lepidopteran superfamilies in the Early to Middle Cretaceous (Kawahara et al. 2019). These diversifications of angiosperms and Lepidoptera occurred just before and during, respectively, proposed timing of the origin of bee crown groups (Cardinal and Danforth 2013, Kawahara et al. 2019).

Given the long-term and close relationship between Lepidoptera and angiosperms and the sheer species diversity of angiosperms (~295,000 described species) (Christenhusz and Byng 2016), it is no surprise that Lepidoptera is one of the most speciose groups of animals on the planet. Currently, there are approximately 158,000 described extant species of Lepidoptera (Van Nieukerken et al. 2011, Goldstein 2017), with estimates of the total diversity being as high 500,000 (Gaston 1991). Although the primary relationship between angiosperms and lepidopterans is associated with larval feeding habits (herbivores of angiosperms) and not all adult Lepidoptera feed on nectar or pollen, Lepidoptera are by far the most speciose pollinators, with an estimated 141,600 pollinating species, compared to Coleoptera with 77,300 species and Hymenoptera with 70,000 (Wardhaugh 2015, Ollerton 2017). Given the striking difference in these estimates, the long-term relationship between Lepidoptera and angiosperms, and the importance of pollinator diversity, it is conceivable that, contrary to popular belief, Lepidoptera are the most important global pollinators.

**Nocturnal adaptations which may enhance pollination**

Approximately 75-85% of extant lepidopteran species, primarily moths, are nocturnal as adults (Kawahara et al. 2018), and as such, moths are well-adapted to the lower temperatures and light conditions presented by a nocturnal diel-activity period. Moths are the hairiest of insects, owing to the abundance of their namesake scales (Roquer-Beni et al. 2020). These scales act insulate moths, reducing heat loss and playing an essential role in thermoregulation (May 1979,
Heinrich 1995). Interestingly, hairiness is considered an important trait for pollination, as the increased surface area and electrostatic forces allow pollen to readily adhere to hairier bodies (Roquer-Beni 2020). Moths also vibrate their wing muscles in cold temperatures in a pre-flight warmup, generating the metabolic energy to increase their thoracic temperature to support flight (Krogh and Zeuthen 1941, Heinrich 1995). Wing vibration has been observed while moths are visiting flowers (Makholela and Manning 2006, Robertson et al. 2020, Robertson et al. 2021). Many bees are known to express a similar behavior, called sonication or buzz-pollination, during floral visits (Cardinal et al. 2018). Sonication encourages the release of pollen from plants with poricidal anthers (De Luca and Vallejo-Marín 2013, Vallejo-Marín 2019), which include important pollinator-dependent crops, such as tomatoes and potatoes (Buchmann 1983, Proença 1992). Furthermore, the vibration frequency generated by bees during flight and sonication increases sugar concentrations in *Oenothera drummondii* Hook (Onagraceae) (Veits et al. 2019).

While there has been no previous suggestion that moths participate in buzz-pollination for plant species with poricidal anthers, it possible that their thoracic vibration while in contact with flowers may result in higher quantities of pollen to be released from anthers and may also promote nectar production.

Moths have exceptional sensory capabilities that allow them to overcome low-light conditions present during the night. The hawkmoths *Deilephila elpenor* L. (Sphingidae), *Hyles lineata* Fabricius (Sphingidae), and *H. gallii* Rottemburg (Sphingidae) have been shown to be capable of color vision in starlight (near complete dark) conditions (Kelber et al. 2002, Kelber et al. 2003). These moths were trained to differentiate nectar rewards based on the color (eight shades of grey, blue, and yellow) of artificial flowers (Kelber and Roth 2006). Furthermore, some hawkmoths exhibit a process called neural summation for optical resolution in dark
conditions, wherein they delay optical-sensory processing through space and time to allow the limited number of photons present at night to summate into comprehensible images (Ala-Laurila 2016, Stöckl et al. 2016). The extent and phylogenetic basis of these adaptations is currently unknown, but considering the prevalence of nocturnality in moths, they may be common throughout this group (Johnsen et al. 2006). Such visual adaptations may allow moths to identify flowers that provide high-quality nectar rewards and improve foraging efficiency.

**Currently described nocturnal-moth to flower relationships**

Moths exhibit unique floral-visitation patterns that benefit ecosystem functionality and individual plant reproduction. As an example of a mechanism, moths carry pollen further than diurnal pollinators (Young 2002, Skogen 2019), often being more prone than bees to visit flowers between floral patches rather than within (Barthelmess 2006). This visitation behavior is valuable to gene flow, potentially connecting disparate populations (Barthlemess 2006, Skogen 2019), and enhancing the reproduction of plants that restrict fertilization by genetically similar pollen (Benning 2015). In fact, pollen loads carried by moths have been used to identify flight origin and pathways of moth seasonal migrations in temperate regions, and show that moths can carry pollen extremely long distances (as far as 1,600 km) (Hendrix III et al. 1987, Hendrix III et al. 1992, Warrant et al. 2016, Chang et al. 2018). By comparison, bee-foraging ranges are considerably smaller, being generally less than 6 km (and as great at 14 km) for the western honey bee (Visscher and Seely 1982, Beekman and Ratnieks 2000), less than 800 m for multiple bumble-bee species (Knight et al. 2005), and generally far less 1 km for many solitary bees (Gathmann and Tscharntke 2002). Although no relationship between moth migration and pollination has been established, moths are known to migrate during the spring, when the highest number of plants, including most agricultural plants, bloom. Furthermore, migrating moths feed
on a wide variety of available nectar resources (Warrant et al. 2016, Chang et al. 2018) and migration pathways often carry them through agricultural epicenters (Rui-Lu 1989, Chang et al. 2018, Wu et al. 2021). Regardless of the potential association with migration, it is clear that moth pollination can act to exchange genetic information at greater distances than bees, potentially reducing the effects of genetic bottlenecking.

Nocturnal-moth pollinators are important to the normal functioning for a variety of ecosystems. Many studies have examined broad interactions between pollinators and plants across ecosystems, yet approaches often restrict observations on nocturnal moth-plant interactions (Kato and Kawakita 2004, Kato et al. 2008). For example, Kato et al. (2008) and Kato and Kawakita (2004) observed floral visitors in tropical monsoon forests in Southeast Asia and on New Caledonia in the South Pacific, respectively, only devoting observation efforts to nocturnal visitors when no diurnal pollinators were found. This approach and approaches that make observations only during daylight hours bias pollinator importance towards diurnal species and could lead to underestimating the contributions of nocturnal species (Chamorro et al. 2012). This is evident in plant species that are most frequently visited by diurnal pollinators but are most effectively pollinated by nocturnal pollinators, such as *Inga sessilis* (Vellozo) Martius (Fabaceae) (Amorim et al. 2013), *Agave vriginica* L. (Asparagaceae) (Groman and Pellmyr 1999), and *Asclepias syriaca* L. (Apocynaceae) (Jennerston and Morse 1991). Studies specifically focusing on moth-pollination networks are limited but increasing in prevalence (Devoto et al. 2011, Banza et al. 2015, MacGregor et al. 2019, Wonderlin et al. 2019, Walton et al. 2020). These studies have invariably concluded that moths are valuable components for ecosystem pollination, exhibiting complex networks of moth-plant interactions that resemble diurnal networks in nestedness and connectedness.
Nocturnal moths are also important to the reproduction of specific plants. The classic example of Darwin’s star orchid *Angraecum sesquipedale* Thouars (Orchidaceae) and the hawkmoth *Xanthopan morganii* Walker (Sphingidae) highlights an extreme specialization of plant and pollinator, wherein features of the pollinator were predicted using only the morphological traits of the plant. However, this is only one such example of the importance of moths as pollinators of specific plants. Other plants are also dependent on moth pollinators for reproduction, only reproducing with moths as the pollen vectors, like *Struthiola ciliata* L. (Thymelaeaceae) (Makholela and Manning 2006) and *Oenothera suffrutescens* Wagner and Hoch (Onagraceae) (Clinebell et al. 2004). Other plants are attractive to and visited by diurnal pollinators but receive the greatest pollination inputs from nocturnal moths, like *Lyonia lucida* Lamarck (Ericaceae) (Benning 2015), *Inga sessilis* Martius (Fabaceae) (Amorim et al. 2013), and *Agave macroacantha* Zucc. (Asparagaceae) (Arizaga et al. 2000). In some cases, moths have been shown to be the most efficient pollinators, achieving similar or greater levels of pollination than diurnal pollinators with fewer visits, as in *Asclepias syriaca* L. and *A. verticillata* L. (Apocynaceae) (Bertin and Wilson 1980).

Historically the association between nocturnal moths and pollination of agricultural crops has been little studied, but studies on this topic are growing in prevalence. In the last decade (2011 – present), there have been five studies examining the inputs of nocturnal pollinators to nine different crops (Luo et al. 2011, Cutler et al. 2012, Robertson et al. 2020, Lu et al. 2021, Robertson et al. 2021). For comparison, only four studies had explored the roles of nocturnal pollinators to nine crop species in previous years (Heard 1993, Pelletier et al. 2001, Soehartono and Newton 2001, Tasen et al. 2009). In total, the role of nocturnal moths in pollination has been examined for 17 species of crops from seven plant families, including Cucurbitaceae, Ericaceae,
Euphorbiaceae, Proteaceae, Rosaceae, Thymelaceae, and Vitaceae, with *Aquilaria crasna* Pierre (Thymelaeaceae) being examined multiple times. Results vary by species, with all but one crop species, *Macadamia integrifolia* Maiden and Betche (Proteaceae) (Heard 1993), receiving significant increases to some metric of production from pollination by nocturnal insects. Most of the crop species examined are specialty crops not commonly grown and do not have major impacts on global agriculture. The plant species with the most significant contribution to global agriculture, apple (*Malus domesticus* Borkhausen Rosaceae), was shown to receive similar levels of pollination by diurnal and nocturnal pollinators in self-infertile varieties (Robertson et al. 2021). It is highly likely that other crops also benefit from nocturnal pollination. Even if diurnal pollinators supply the majority of pollination services, as has been a common conclusion, the additional provisions offered by nocturnal pollinators could be valuable for production stability. As pollinator populations experience declines and to better understand the impact of moth pollinators to agriculture, it is essential to examine the roles of nocturnal pollinators in a broader set of crop plants.

**Declines in moth populations**

Declines in moth abundance and diversity have been reported throughout Europe and North America over the last 20 years. Fox et al. (2021) reported that 41% of moths in Britain had experienced declines in abundance and 32% had experienced declines in distribution. European monitoring and historical records are far more complete than in other continents, with some databases dating back centuries (Matilla et al. 2006, Matilla et al. 2008, Groenendijk and Ellis 2011). Most examinations include only resident species of macromoths, with collection and survey data for micromoths being sparse and migratory species removed from analyses (Groenendijk and Ellis 2011, van Langevelde 2018, de Miranda et al. 2019). These are often
necessary exclusions as micromoths are difficult to study, with identification being complicated and limited by expertise and for which historical data is often absent, and because migratory species are affected by factors that lie outside of study areas. Furthermore, there is evidence of continental bias, with the greatest concentration of studies focused on moth populations coming from European regions, like Great Britain (Fox et al. 2021), Portugal (de Miranda et al. 2019), Scotland (Fuentes-Montemayor et al. 2012), the Netherlands (Groenendijk and Ellis 2011), Finland (Matilla et al. 2008), and Sweden (Franzen and Johannesson 2007). In fact, most studies directly examining changes in moth abundance and diversity in Europe arise from Britain (Conrad et al. 2004, Conrad et al. 2006, Fox et al. 2013, Fox 2014, Fox et al. 2021) and from the northeastern United States in North America (Wagner 2012, Schweitzer et al. 2014).

Nonetheless, there are consistencies in population changes that are common across all regions where studies have been performed and proposed causes are ubiquitous with human habitation. While significant regional variation exists, e.g. southern Britain is experiencing greater declines than northern Britain (Fox et al. 2021), all reviewed studies report larger percentages of moth species in decline than those that appear stable or are increasing (citations). These losses are of serious concern because moths are central features in healthy ecosystems, being critical food sources for many bird species and predatory arthropods, herbivores of plant species, and pollinators of a variety of flowering plants (Wagner 2012, Schweitzer et al. 2014).

Several physical and life-history traits have been linked to declines in moth distribution abundance and an increased risk of extinction. Capture rates and observations of large-bodied moths, such as Saturniidae, Sphingidae, and *Catacola* spp. (Erebidae), by hobbyists in North America have reportedly declined in areas known to harbor an abundance of those moths, leading some researchers to suggest that body size is an important factor in declines (Hessel...
However, there is a known observation bias in human observations of large moths in comparison to others. Empirical studies in Europe that examine factors associated with declines in abundance and distribution within groups have demonstrated that body size does not significantly explain declines (Matilla et al. 2006, Matilla et al. 2008). Instead, studies on European moths revealed that life-history characters, such as larval host specificity, length of flight period, and overwintering stage, are most closely associated with declines in distribution and abundance (Matilla 2006, Franzén and Johannesson 2007, Matilla 2008). In general, it appears that moths that are less capable of responding to changing conditions (i.e., specialist species, moths with short flight periods and dispersal ability, and moths that overwinter in stages unable to fly) are significantly more likely to experience declines in distribution and abundance.

Moth declines have also been associated with a variety of environmental factors. The most commonly cited factor associated with moth decline is habitat changes (Hessel 1976, Summerville and Crist 2004, Fuentes-Montemayor et al. 2012, Wagner et al. 2012, Schweitzer et al. 2014, Fox et al. 2021). Habitat specialists are particularly susceptible to declines. Franzén and Johannesson (2007) found that 70% of habitat specialists in Sweden had been lost, and moth species restricted to non-forested habitats suffering the greatest losses. However, declines appear to be context specific. Schweitzer et al. (2014) showed that moths that feed on understory plants in forested habitats of western New Jersey were more likely than other moth species to be in decline unless they fed on ericaceous shrubs. This was due to understory-browsing preferences by large populations of white-tailed deer *Odocoilus virginianus* Zimmermann (Cervidae), which avoid ericaceous shrubs.

Another potential factor influencing moth declines are growing numbers of artificial light sources at night and increasing usage of light-emitting diodes, which produce light at broader
spectrums and generate more intense light (Macgregor et al. 2015). For example, increased artificial night lighting has been cited by numerous authors as a potential source of population declines (Hessel 1976, Wagner 2012, Macgregor et al. 2015). Van Langevelde (2018) found that populations of nocturnal moths that are attracted to light sources were more likely to experience declines in the Netherlands than other moth groups. Additionally, artificial lighting has been linked to behavioral changes that likely effect moth populations, such as reductions in larval foraging (van Geffen et al. 2014), adult dispersion (Brown 1984, Pfrimmer et al. 1955), and reproduction (Nemec 1969, Sower et al. 1970). Artificial lights also attract nocturnal predators exploiting higher prey densities (Frank 2006) and interfere with anti-predatory behaviors in moths (Svensson and Rydell 1998, Acharya and Fenton 1999).

Conclusions

Moths are abundant, diverse, and integral components of many ecosystems, serving as important food sources for predators, major herbivores of plants, and as pollinators (Fox et al. 2021). Their presence has even been used as an indication of ecosystem health (Summerville et al. 2004, Nakamura et al. 2015). Moths are the most speciose group of pollinators (Ollerton 2017), evolved with angiosperms (Kawahara et al. 2019), have been associated with a wide array of plants that include important crops, and are experiencing wide-ranging declines in distribution and abundance. Their roles in agriculture remain poorly understood, yet evidence for their importance to agricultural production is growing.

As pollinator declines continue, and the threat to agricultural production becomes more evident (Reilly et al. 2020, Lipert et al. 2021), studies examining the pollination inputs moths provide to agriculture are important. It is possible that moths add stability to agricultural systems, being an excellent source of pollinator diversity, and because moths are in decline in regions
where monitoring has been performed, there is a sense of urgency in understanding their roles in pollination. This urgency is exacerbated as agricultural dependence on pollinators grows. Moths are potentially the unsung heroes of agricultural production, and expanding the scope of agricultural pollination to include moths is vital to obtain a realistic assessment of the effects of pollinator declines on the production of human food.

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Chapter 3: Nocturnal pollinators significantly contribute to apple production

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Abstract.
Agricultural dependency on insect-mediated pollination is increasing at the same time that
pollinator populations are experiencing declines in diversity and abundance. Current pollinator
research in agriculture focuses largely on diurnal pollinators, yet evidence for pollination by
moths and other nocturnal pollinators is growing. Apples are one of the most valuable and
important fruits produced globally, and apple production is dependent on insect-mediated cross-
pollination to generate a profitable crop. We examined contributions to apple production
provided by nocturnal insects via an exclusion experiment. We compared the relative
contributions to apple production provided by nocturnal and diurnal pollinators using fruit set,
likelihood of cluster pollination, and seed set. We found nocturnal pollinators capable of
facilitating the production of as many apples at similar levels of pollination as diurnal
pollinators. We further found evidence that nocturnal and diurnal pollinators pollinate
synergistically, with pollination contributions being additive in one year of our study. Our
research identifies significant contributions to apple production provided by nocturnal
pollinators, which may interact with diurnal pollinators in ways that are currently unrecognized.
Expansions of this research into additional pollinator-dependent crops and focused investigations on specific nocturnal insects will provide more accurate assessments of nocturnal-pollinator roles in agriculture and improve our overall understanding of pollination in agriculture.

**Key Words:** non-bee, diversity, night, evening
Introduction

Most crops, including many fruits, nuts, and oilseeds, require or benefit from animal-mediated pollination (Klein et al. 2007). A recent analysis of global land use and pollination for 114 crops showed that the cultivated area of pollinator-dependent (hereafter PD) crops has increased faster than area devoted to pollinator-independent (PI) crops, such as cereals and grains (Aizen et al. 2019). Even though PI crops (e.g., grass crops that are wind pollinated) represent 67% of global crop land, the amount of land devoted to PD increased by an average of 2.1% annually between 1961 and 2016 (Aizen et al. 2019)—double the rate of expansion of PI crops during the same period. PD crop growth was largely dominated by greater cultivation of oil-seed crops, such as soybean, rapeseed, sunflower, and oil palm (Aizen et al. 2019).

Increases in land devoted to PD crops are associated with increased dependence on pollinators (Aizen et al. 2008, Aizen et al. 2009, Aizen et al. 2019), which is occurring at a time when pollinators, particularly bees, are in decline (Arbetman et al. 2017, Powney et al. 2019). Declines of pollinators have been noted for decades, with losses recorded in both native bees (Jacobson et al. 2018, Centerella 2019, Vega-Hidalgo 2020) and managed honey bees (Antúnez et al. 2017, Chen et al. 2017, Kulhanek et al. 2017, Oberreiter 2020). Native bee declines have been attributed to loss of habitat and resources, climate change, pesticide exposure, pathogens, and competition with non-native pollinators (Potts et al. 2010, Thomson 2016, Cameron and Sadd 2019, Soroye et al. 2020). Honey bee declines have been linked to similar factors, as well as parasites, such as the Varroa mite (Steinhauer et al. 2018). Each of these potential drivers for bee losses has been shown to affect bee health independently of and synergistically with other factors (Steinhauer et al. 2018, Cameron and Sadd 2019).
As the concern for pollinator health and the maintenance of pollination services continues, agricultural research has focused largely on diurnal pollinators, including bees and non-bee insects (Rader et al. 2016). Nocturnal pollinators are regarded as important pollinators outside of agricultural systems (Bawa 1990, LeCroy et al. 2013, Banza et al. 2016, Hahn and Brühl 2015, Wonderlin et al. 2019), and have received attention for their roles in the transport of wild pollen in agricultural landscapes (MacGregor et al. 2019, Walton et al. 2020). Furthermore, nocturnal pollinators have been demonstrated to be significant contributors towards the production of cloudberry (Rubus chamaemorus L.) (Pelletier et al. 2001), resinous trees in Southeast Asia (Aquilaria crassna Pierre) (Tasen et al. 2009), trees grown for biofuel (Jatropha curcas L.) (Luo et al. 2011), and lowbush blueberry (Vaccinium angustifolium Aiton) (Cutler et al. 2012) and were noted as prominent visitors or peaches (Robertson et al. 2020). Regardless, little attention has been given to understanding nocturnal pollination of crop plants. In fact, common methods used to study pollinators in agricultural systems, such as survey and observation times and trapping methods (Joshi et al. 2015, 2016), may exclude nocturnal insects (e.g., Bhardwaj and Srivastava 2012, Thakur and Mattu 2014, Mattu and Baghat 2015). Even studies that intend to include nocturnal pollinators may use methods that fail to account for variables important to their pollination activity (e.g., study time and duration), thus missing target nocturnal pollinators (Keys et al. 1995). As a result, current understanding of crop pollination may not accurately include contributions by nocturnal pollinators.

Apples are one of the world’s most valuable fruits, being a primary fruit crop grown in China, the European Union, and the United States (USDA 2019a), and are a significant source of global food and nutrition (Lee and Mattick 1989; Hyson 2011). Commercial apple sales and exports also contribute billions of dollars annually to the global economy (USDA 2019a,
Shahbandeh 2020). In 2018, apples were the third most produced fruit in the world by weight behind bananas and watermelons (Shahbandeh 2020), and generated $85 billion USD in revenue (IndexBox 2020). Commercial apples generated $3.0 billion USD in 2018 in the United States alone (USDA 2019b).

Apples are a well-studied, PD crop with over 6,000 cultivars grown worldwide (Ramírez and Davenport 2013). Most apple cultivars are self-infertile and require cross-pollination with a different variety to produce fruit. Even cultivars that are partially self-fertile (limited capability of being fertilized and producing fruit from pollen of the same variety) require cross-pollination to produce a profitable crop (Dennis 2003). Apple pollen is heavy and not readily carried by the wind and, as such, cross-pollination in apples relies on pollen transfer mediated by insects (Dennis 2003, Pardo and Borges 2020). We identified insects from three orders (Diptera, Lepidoptera, and Neuroptera) visiting apple flowers at night in an observational study, with the most common visitors being moths from the family Noctuidae. Yet, nocturnal pollinators are absent from modern literature concerning apple pollination (Pardo and Borges 2020). The goal of this research was to determine if nocturnal pollinators contribute to apple pollination and, if so, to compare their inputs with those of diurnal pollinators.

**Materials and Methods**

Experiments were conducted in two apple plots at the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR (36.10 N, 94.17 W). The apple plot used in 2017 was a breeding plot with multiple experimental varieties (lineages unknown) consisting of ten rows occupying approximately 0.40 hectares. Some trees in this plot were reported to be self-fertile, but we were unable to learn whether individual trees used were self-fertile or infertile. The apple plot used in 2018 consisted of two rows of Enterprise apples (self-infertile) flanked on either side
by a row of guard/pollenizer apple varieties (one side: experimental AR 127, 124; the other side: Goldrush) on approximately 0.14 hectares. Plots were separated by about 400 m. Adjacent areas were largely devoted to field-crop research, and the habitat surrounding the research station was predominantly residential/urban with some mixed deciduous greenways.

We used 20 x 30 cm nylon-mesh, pest-exclusion bags to control pollinator visitation. We used Agfabric Bug Net Bag Garden Netting Against Insects Birds Barrier Bags in 2017, which were of a heavier nylon construction and had 0.76 mm openings. In 2018 we switched to BugDorm exclusion bags (BioQuip Products Inc.) made with lighter nylon and 0.3 mm openings. In the closed position, bags covered entire apple flower clusters and were tied around the branch at the proximal end with strings sewn into bags to exclude pollinator visitation (Fig. 1). Bags in the open position were hung by their strings behind clusters to allow pollinator access to flowers.

We generated four experimental treatments, including a permanently closed treatment excluding all pollinators (negative control) and a permanently open treatment permitting access to all pollinators (positive control). The nocturnal treatment was open from sunset to sunrise (~2000 hours to 0700 hours) and closed from sunrise to sunset (~0700 hours to 2000 hours) to allow only nocturnal pollinators access to flower clusters. The diurnal treatment was open from sunrise to sunset and closed from sunset to sunrise to allow only diurnal pollinators access to flower clusters.

We used a randomized complete block design for this experiment. Trees were selected based on flower availability (favoring greater numbers and even distributions), perceived health (e.g., intact trunks, absence of fire blight, vertical alignment, symmetry, etc.), and stage of floral development (varied in experimental trees only in 2017). Clusters on trees within the same block, being the set of all four treatments, were selected from similar heights (within 1 m of each other).
In order to avoid the effect hanging bags may have had on pollinator visitation, we separated treatments into one of four quadrants projecting at approximately 45° angles from rows. We rolled a die to randomize the placement of treatments into quadrants. Experimental treatments were equally represented on each tree. In 2017, we used 18 trees with each having no more than three blocks (one block: n = 3; two blocks: n = 7; three blocks: n = 8). When a single tree receive multiple blocks, blocks were stratified vertically (1 m strata separated by approximately 0.5 m) and the placement of treatments in each stratum was randomized by quadrant as described above. In 2018, 51 trees were used and had only one block per tree.

We counted the number of developing flowers on each sample cluster and covered all sampled clusters with exclusion bags prior to flower opening. Bag manipulations began at the start of bloom, which was identified by the first opened flower in plots. Bag positions (closed or open) for experimental groups were switched at both sunrise and sunset based on treatment. At sunset, bags for control groups were placed in the alternate position and immediately returned to the original position (covered to uncovered back to covered or vice versa) to account for any pollination that might result from bag manipulations.

We examined clusters and flowers as bags were manipulated. Bags covering clusters with broken stems or severely damaged flowers (indicated by large portions of petals and/or the reproductive tissues of at least one flower being necrotic) were switched to undamaged clusters with unopened flowers when possible. Damage to flowers and stems in 2017 combined with unavailable alternate flower clusters resulted in different sample sizes (n_{closed} = 41; n_{nocturnal} = 40; n_{diurnal} = 38; n_{open} = 40). Following a late freeze in 2018 that damaged at least one flower in all replications, all experimental clusters were successfully replaced with apparently (adjacent unopened flowers were bisected to estimate condition) healthy clusters bearing unopened
flowers. Exclusion bags were removed from clusters once all styles (female receptive tissue) began to wither in those clusters. Manipulations were stopped when all clusters were uncovered and bags were removed.

Fruit set is the stage of fruit development during which fertilized ovaries (located below petals in apples) swell as fruit begins to form and serves as a metric to estimate the overall pollination success. We used a dual approach to verify individual set fruit. Swollen ovaries were visually identified and then prodded with an index finger. If fruit remained attached to the stem after prodding, it was counted as a set fruit. Numbers of set fruit were collected two weeks after all bags had been removed.

Fruit-set data was collected as the ratio of set fruit to the original number of flowers in respective apple-flower clusters (with the dividend of paired numbers being the proportion of fruit set per cluster). We separated each ratio into their 2x2 factorial treatment levels describing the position of bags during the day and night, such that during each time periods bags were either open or closed (e.g., nocturnal-pollinator treatment: day = closed and night = open; diurnal-pollinator treatment: day = open and night = closed, etc.). In this way, our four treatment levels are represented by the interaction of these terms (day*night). Each fruit-set ratio was paired with their blocks (tree identity and strata in 2017 only tree identity in 2018).

While proportions of fruit set are useful to compare the overall contributions of functional pollinator groups, they do not necessarily provide useful information for apple growers. Apple trees naturally drop some developing fruit, and growers typically reduce the number of developing fruit to one per cluster to ensure tree resources are devoted to fewer apples, thus generating higher quality and more-valuable fruit. To provide a measure of pollinator contribution relevant to growers, we compared treatments for the likelihood that at
least one flower would be pollinated. We used the numbers of clusters that set (pollinated) and did not set fruit (not pollinated) to estimate the likelihood that a cluster would be pollinated by respective pollinator groups.

Seed set is the number of seeds produced in a single fruit and represents the number of ovaries that were fertilized by pollen granules. In this way, seed set is a direct proxy of the level of pollination. Apples were harvested in late July in 2017 and in early June in 2018, prior to full development but late enough that seeds could easily be counted. Only one fruit could be harvested from the closed treatment in 2017 (none in 2018), which had no developing seeds, so seed-set data from the closed treatment were left out of analyses. We collected five, three, and seven apples from the nocturnal, diurnal, and open treatments, respectively, in 2017. In 2018, we collected three, fourteen, and seven apples from the same treatments, respectively. Harvested fruit were bisected laterally to expose seeds, and the number of seeds from each apple were counted. We used seed set to compare levels of pollination among treatments.

Statistical Analyses

Collected data are influenced by plant reproductive biology in addition to levels of pollination. As such, significant changes in the experiment between years (e.g., difference in tree variety and change in exclusion bags) rendered between-year comparisons and combinations of data inappropriate. All statistical analyses were performed on within-year data with SAS software using the SAS Studio interface through SAS On Demand for Academics (SAS Institute Inc. 2021).

We programmed SAS to calculate fruit-set proportions using collected fruit-set ratios. Proportions derived from a discrete number of successes (e.g., number of set fruit) out of a number of trials (e.g., number of original flowers) result in a binomial distribution (Douma and
We used generalized linear mixed models (PROC GLIMMIX) to model fruit-set proportions. In 2017, we used bag positions during day and night and the vertical strata as our fixed effects variables, with individual trees and strata within individual trees as random effects. Because experimental clusters were not stratified in 2018, we used only the bag positions during day and night as our fixed effects and individual trees as a random effect variable. We used a binomial ANOVA to determine the effect level of each fixed-effect variable and their interaction terms. We then generated least-square means for each significant effect variable and each interaction between day and night bag positions (four treatment levels).

We compared the likelihood that a cluster would be pollinated based on treatment within years using chi-squared analyses on 4x2 contingency tables (treatment by pollination status). Significant differences in the likelihood clusters would be pollinated across all treatments were further explored with sequential chi-squared tests comparing treatments in pairs using a Bonferroni correction (three paired post-hoc chi-squared tests in each year; \( \alpha = 0.017 \)) to establish relationships among treatments. Within-year seed sets were compared among treatments using an ANOVA (PROC GLM) and means were grouped using Tukey HSD.

Results

In 2017, the number of flowers per cluster ranged from three to thirteen (\( \bar{x} = 5.3 \pm 1.3; n = 159 \)), with the most (93/159) having five flowers. In 2018, the number of flowers per cluster ranged from three to seven (\( \bar{x} = 5.0 \pm 0.6; n = 204 \)), with most (151/204) having five flowers. Numbers of flowers per cluster were similar among treatment groups in both years (2017: \( F = 0.15; df = 3, 155; p = 0.93 \); 2018: \( F = 2.04; df = 3, 200; p = 0.11 \)).

Neither vertical strata nor its interactions with treatments significantly affected fruit set in 2017 (strata: \( F_{2, 24.4} = 1.18, p = 0.32 \); strata*day: \( F_{2, 147} = 0.56, p = 0.57 \); strata*night: \( F_{2, 147} = \))
Fruit set was significantly influenced by treatments in both 2017 and 2018. In 2017, bag positions during the day and night significantly contributed to fruit set (day: $F_{1, 147} = 60.58, p < 0.0001$; night: $F_{1, 147} = 8.32, p = 0.0045$) but the interaction between these terms did not (day*night: $F_{1, 147} = 2.89, p = 0.091$). LS mean fruit sets for treatment levels were significantly different in 2017 (LS means ± S.E.: closed = 0.08 ± 0.03; nocturnal = 0.17 ± 0.05; diurnal = 0.36 ± 0.08; open = 0.42 ± 0.08) (means and relationships shown in Fig. 2A). Each fixed effect used in 2018 significantly contributed to fruit set (day: $F_{1, 200} = 9.88, p = 0.0019$; night: $F_{1, 200} = 5.83, p = 0.017$; day*night: $F_{1, 200} = 16.06, p < 0.0001$). LS mean fruit sets for treatment levels were also significantly different in 2018 (LS means ± S.E.: closed = 0.04 ± 0.01; nocturnal = 0.14 ± 0.02; diurnal = 0.16 ± 0.03; open = 0.11 ± 0.02) (means and relationships shown in Fig. 2B).

The likelihood a cluster would be pollinated varied by treatment in both years (2017: $\chi^2 = 38.59; p < 0.0001$; 2018: $\chi^2 = 18.02; p = 0.0004$) (Fig. 2C and D). In 2017, clusters in the nocturnal treatment were nearly twice as likely to be pollinated (57.5%) as clusters in the closed treatment (29.3%) ($\chi^2 = 6.58, p = 0.01$), but were less likely to be pollinated than clusters in the diurnal (84.2%) ($\chi^2 = 6.67, p = 0.01$) treatment. Clusters in the diurnal treatment were similarly likely to be pollinated as clusters in the open treatment (87.5%) ($\chi^2 = 0.17, p = 0.68$). In 2018, clusters in the nocturnal treatment were more likely to be pollinated (11.3%) than the closed treatment (3.4%) ($\chi^2 = 12.09, p = 0.0005$) and were similarly likely to be pollinated as both the diurnal (12.8%) ($\chi^2 = 0.35, p = 0.55$) and open (10.8%) ($\chi^2 = 0.04, p = 0.84$) treatments.

Average seed set ranged between 5.8 and 6.3 seeds per fruit in 2017 and between 4.0 and 5.3 in 2018. In 2017, nocturnal treatments resulted in an average of 5.8 (S.E. ± 0.72) seeds per fruit, while diurnal and open treatments resulted in 6.3 (± 0.87) and 6.1 (± 0.72) seeds per fruit,
respectively. Nocturnal treatments resulted in an average of 5.3 (± 0.87) seeds per fruit in 2018, with diurnal and open treatments generating 5.3 (± 0.72) and 4.0 (± 0.83) seeds per fruit, respectively. Seed set means were similar among nocturnal, diurnal and open treatments in both 2017 ($F_{2, 12} = 0.10, p = 0.91$) and 2018 ($F_{2, 21} = 0.70, p = 0.51$) (Fig. 2E and F).

Discussion

We found that nocturnal pollinators significantly contributed to apple pollination in both years of our experiment. In 2017, nocturnal pollinators set more fruit than the negative control, increasing fruit set from 8% to 17%, and were nearly twice as likely (29.3% to 57.5%) to pollinate a cluster when diurnal pollinators were excluded. Additionally, fruit generated by nocturnal pollinators bore a similar number of seeds as fruit generated by diurnal pollinators and both pollinator groups acting together. Relationships concerning fruit set from 2017 resemble results obtained in similar experiments in other fruit crops, where nocturnal pollinators increased fruit set from the negative control but did not achieve the same levels of pollination as diurnal pollinators or both pollinator groups combined (Pelletier 2001, Luo et al. 2011, Cutler et al. 2012). This might give the impression that nocturnal pollinators are less effective than diurnal pollinators across all (or most) agricultural fruit. However, nocturnal pollinator contributions were more pronounced in 2018, when they increased fruit set from 4% to 14% and increase the likelihood a cluster would be pollinated from 3.4% to 11.3%. These contributions were greater than the negative control and similar to both diurnal pollinators and the positive control across all measured pollination variables. This implies that nocturnal pollinators may be as efficient in pollinating apples as their diurnal counterparts. Perhaps there are annual variations, as have been demonstrated in other systems (Devoto et al. 2011) that are not accounted for in other, single-
year, agricultural studies. Regardless, our results provide the first evidence that nocturnal pollinators contribute to apple production.

Diurnal pollinators significantly increased fruit set and the likelihood of cluster pollination even when in the presence of nocturnal pollinators in 2017, but not in 2018. In fact, pollination by diurnal pollinators was similar to the effect of both nocturnal and diurnal pollinators acting together in both years. Superficially, this suggests that nocturnal pollinators, while participatory in apple pollination, do not enhance apple pollination when acting in concert with diurnal pollinators. However, studies have shown that apple pollination receives substantial benefits from a diverse set of pollinators, including bee and non-bee species (Park et al. 2016, Sapir et al. 2017, Pardo and Borges 2020). For example, diverse communities of wild bees increase seed set and decrease pollen limitation among apples in the presence of western honey bees (*Apis mellifera* L.) (Blitzer et al. 2016), a species that is often imported at high cost to ensure adequate levels of pollination. Although seed set was not improved, we found that nocturnal-pollinator contributions to fruit set were additive to those of diurnal pollinators in 2017, even in the presence of a diverse diurnal community (observed western honey bees, common eastern bumble bees *Bombus impatiens* Cresson, solitary bees from the families Andrenidae and Halictidae, and flies from the family Syrphidae). These results suggest complimentary pollination between nocturnal and diurnal pollinators, wherein members of these groups visited different subsets of flowers (Blüthgen and Klein 2011). Contributions were not additive in 2018, possibly due to between-year difference in experimental design or environmental effects, which may have changed the effectiveness of pollination or composition of the pollinator communities. Nonetheless, nocturnal pollinators may provide an important
source of stabilization in apple production as pollinator populations experience broad-scale declines.

While we found evidence for nocturnal pollination of apples, we were unable to determine which nocturnal insects were responsible. Moths from the family Noctuidae were the most commonly observed insects visiting apple flowers at night, with the two most common being true armyworm (*Mythimna unipuncta* Haworth) and variegated cutworm (*Peridroma saucia* Hübner) moths, the same species Robertson et al. (2020) reported in peaches. Floral visitation does not mean these insects pollinated apple flowers. Given the importance of pollinator diversity, efforts to identify prominent nocturnal pollinators of apples are warranted.

Current understanding of and approaches to managing apple pollination are centered on diurnal pollinators (Rader et al. 2016), but management may benefit from considering nocturnal pollinators.

There were important caveats to our data in both years. Different apple varieties have different pollination demands (Garratt et al. 2016). High fruit set, fruit set variance, and likelihood of cluster pollination in 2017 were likely the result of using a breeding plot of apples containing multiple varieties, some reportedly self-fertile. Exclusion bags used in 2017 did not offer complete exclusion. *M. unipuncta* were observed nectaring from flowers through exclusion bags on two occasions in that year. This behavior may have affected outcomes of treatments designed to exclude nocturnal pollinators in 2017. Flowers are killed by freezing temperatures, and those in later stages are more susceptible to damage (Longstroth 2013). A late freeze in 2018 killed most nearby pollinizer blooms (later stage of bloom than experimental trees), which could have limited viable-pollen availability and resulted in lower-than-normal levels of pollination. Nonetheless, a late freeze represents a real-life scenario with which growers must contend. While
changes to the experiment were necessary and environmental conditions not uncommon, results should be considered with respect to these limitations.

Although experimental design and results changed between years, to our knowledge, this is the first study to demonstrate that nocturnal pollinators are capable of providing comparable pollination levels to diurnal pollinators in any cultivated crop. The implications of such a finding should not be undervalued. Nocturnal insects represent a previously unrecognized and diverse group of pollinators that may offer stability to apple and other fruit production. Their contributions to agriculture require further study, as their inclusion may fundamentally change our interpretation of the threat landscape associated with declining pollinators and agricultural stability. As such, it is important to establish which crops receive pollination benefits from nocturnal pollinators and to identify the nocturnal-insect species that are responsible. Such studies have the potential to alter our understanding of agricultural production.

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Figures

Figure 1. Photographs of exclusion bags used in 2017 (left) and 2018 (right) covering flower clusters.
Figure 2. Results from each analysis for both experimental years. (A) and (B) show the LS means of the proportion of flowers that were pollinated for each treatment group flanked by upper and lower 95% confidence intervals. (C) and (D) display the numbers of pollinated and not-pollinated clusters that were used to compare the likelihood a cluster would be pollinated. (E) and (F) are distributions of seed-set data with means represented by diamonds. Letters above data indicate relationships among groups.
Chapter 4: Moths and other nocturnal flower-visiting insects of apples in Northwest Arkansas

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Abstract. Apples are a fruit crop of economic and nutritional importance that require cross-pollination by insects for sustainable production. We recently showed that nocturnal pollinators are capable of contributing as much to apple pollination as diurnal pollinators. To date, no identities for potential nocturnal pollinators of apples exist. We surveyed nocturnal moths present in an apple orchard during apple bloom and observed their visitation to apple flowers over a three-year study period. We captured 1087 individual moths from at least 68 species in 12 moth families and made 154 observations on floral visitors by 15 species from five families. Most of the captured individuals (674) were species observed visiting apple flowers. Noctuidae was the most abundant and diverse family captured, and noctuids were also the most commonly observed visitors to apple flowers. Two noctuid species, *Mythimna unipuncta* and *Peridroma saucia*, were particularly common in both captures and observations and were, thus, concluded as the most likely nocturnal pollinators. Overall, we found nocturnal moths to be sufficiently diverse, abundant, and active at flowers to account for previously recorded contributions to apple pollination.
Introduction

Apples are one of the world’s most important agricultural fruits. A fruit crop grown primarily in China, the European Union, and the United States (USDA 2019a), apples are a significant source of global food and nutrition (Lee and Mattick 1989; Hyson 2011). Commercial apple sales and exports contribute billions of dollars annually to the global economy (USDA 2019a, Shahbandeh 2020). In 2018, apples were the third most produced fruit in the world by weight, behind bananas and watermelons (Shahbandeh 2020), and generated $85 billion USD in revenue (IndexBox 2019). In the United States alone, commercial apples generated $3.0 billion USD in 2018 (USDA 2019b).

Apple production depends on pollination provided by insects (Dennis 2003). Although some cultivars are partially self-fertile and have a limited capability of being fertilized by pollen of the same variety, producing a profitable crop requires cross-pollination (Dennis 2003). Western honey bees (Apis mellifera L. Apidae: Hymenoptera) are considered the most important pollinators of apples (Ramírez and Davenport 2013). These insects are managed on large scales for their pollination services and are often transported to apple orchards to augment pollination (Ahn et al. 2012). However, western honey bees are not always the most efficient pollinators of apples (Russo et al. 2017). In fact, adding more A. mellifera to apple orchards does not necessarily improve pollination (Blitzer et al. 2016). In some cases, apple pollination is more complete and cost effective by enhancing pollinator diversity through management practices that support a variety of pollinators (Russo et al. 2017, Sapir 2017).

We recently showed that nocturnal pollinators were capable of generating as many apple fruit of similar quality as diurnal pollinators, including the western honey bee (Robertson et al. 2021). During that study, moths were the most-frequent nocturnal visitors to apple flowers (pers.
obs.). We used those observations to direct our efforts to trap moths and observe moths visiting apple flowers during apple bloom. Here we report the identities of moths and their diversity, abundances, and potential relationships with apples.

**Materials and Methods**

Trap sampling and observational surveys were conducted between sunset and sunrise in an apple plot at the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, Arkansas (36.10 N, 94.17 W), during apple bloom in 2018, 2019, and 2020. Apple bloom varied in initiation and duration by year (2018: 12 April to 30 April; 2019: 8 April to 28 April; 2020: 31 Mar to 13 April). Sunset occurred between 1938 and 2003 hours, and sunrise occurred between 0623 and 0703 hours. The 0.14 ha plot consisted of two rows of Enterprise apples flanked by guard/pollinizer apple varieties, on one side by a row of Goldrush and the other side by experimental AR 124 and 127 varieties. Areas adjacent to the plot were devoted to fruit- and field-crop research, and the habitat around the research station was mostly residential with mixed deciduous greenways.

We sampled nocturnal moths from 2000 hours to 0700 hours on eight clear (no precipitation) nights in each of the three years for a total of 264 trap hours. We used two black-light bucket traps (BioQuip #2851A) with the lights secured horizontally with bungee cords across the top of the traps. Each trap contained an open, wide-mouthed jar (approximately 0.5 L) of ethyl acetate containing a paper towel extending beyond the mouth to serve as a wick. Traps were placed five meters inside each end of the middle row. Trap lights were turned on at 2000 hours, and trap samples were collected every hour until 0700 hours. Specimens from both traps were combined into press-seal bags, labeled with date and hour, and stored in a freezer for further processing. Moths were removed from frozen samples, thawed, pinned, spread, and
identified to the lowest taxonomic level possible, given the condition of the specimen, using external morphological features. Specimens that could not be identified beyond family were left out of diversity measures, except when families were unique, but were kept for abundance measures. Capture data was used to generate presence profiles by hour for the top five common moths. Collected specimens are stored at the University of Arkansas Entomological Museum.

Observations to identify nocturnal insects visiting apple flowers were performed on the same nights as black-light trapping in all three study years. In 2018, observations were also made on nights that sampling did not occur. Observations were initiated at 2015 hours and recurred every half hour until 0645 hours. During observations, the apple plot was traversed for no less than two minutes, and insects in physical contact with apple flowers (stems, petals, or reproductive tissues) were identified, on site, to the lowest taxonomic level possible. Observations of individual floral visitors were made over no less than three minutes when positive identity could be assured. Unrecognized insects were photographed for later identification. The primary goal was to identify floral visitors and, to that end, this protocol was not strictly followed, allowing observers to make unprompted and/or lengthier observations. As such, efforts by night and year were unequal. To further explain the presence of moths in trap samples, we determined which taxa are known to use apple as a larval host plant. Taxa that were not identified below family level were not included in counts for either floral visitors or apple feeders.

**Results**

We captured 1087 moths representing at least 68 species from 12 families over the three-year period. In 2018, we captured 216 individuals representing 38 species from 10 families (Fig. 1). In 2019, we captured 414 individuals representing 42 species, including 18 previously
unrecorded, from 10 families. In 2020, we captured 457 individuals representing 47 species, including 12 previously unrecorded, from nine families. Nineteen species were captured in all three years and represented 80.3% (874 individuals) of the total number captured. Nine sampling nights (one night in 2018 and four nights in each 2019 and 2020) resulted in more than 50 captured individuals. Combined, these nine productive nights produced 836 captured individuals (76.8% of the total) for an average of 92.9 individuals and 20.5 species per night. The remaining 15 sample nights averaged 16.8 individuals and 7.0 species per night.

Noctuidae, Erebidae, Geometridae, and Tortricidae accounted for 56 of the 68 species captured (Table 1, Fig. 2). We captured 17 species from the family Noctuidae, 16 species from the family Erebidae, 14 species from the family Geometridae, and 9 species from the family Tortricidae. Noctuids were the most diverse in 2019, when we captured 13 species. Erebids, geometrids, and tortricids were the most diverse in 2020, when we captured 12, 9, and 7 species, respectively. Three families, Attevidae, Plutellidae, and Sphingidae, were represented by a single species. The families Gracillariidae, Pyralidae, and Tineidae were represented by 5, 10, and 1 specimens, respectively, none of which were identified below family level.

The families Noctuidae, Crambidae, Tortricidae, and Erebidae accounted for 83.6% of all moths captured (Table 1, Fig. 2). We captured 461 noctuid moths (42.4% of total abundance), which represented 28.0%, 47.8%, and 43.9% of moth abundance in 2018, 2019, and 2020, respectively. Four noctuid species, *Peridroma saucia* Hübner, *Mythimna unipuncta* Haworth, *Elaphria grata* Hübner, and *Galgula partita* Guenée, were among the five most captured moths overall. These four species comprised 78.7% of noctuids captured and 33.4% of the total abundance. We captured 192 individuals (17.7% of total moth abundance) from the family Crambidae. Crambids were the most numerous in 2019, when we captured 123 individuals.
accounting for 29.7% of the moths captured in that year. We captured 172 *Udea rubigalis* Guenée (Crambidae) individuals, which was the most abundant moth overall. We trapped 148 moths (13.6% of total moth abundance) from the family Tortricidae. Most tortricids were caught in 2018, when 83 individuals accounted for 38.4% of all moths captured that year. Individuals of three species of Tortricidae, *Clepsis peritana* Clemens, *C. virescana* Clemens, and *Grapholita packardi* Zeller, accounted for 75.0% of the tortricids collected. Moths in the family Erebidae were the fourth most common family collected, with 109 individuals captured (10.0% of total individuals). Erebids were most abundant in 2020, representing 15.1% of moths captured in that year. Two erebids, *Caenurgina erechtea* Cramer and *Hypena scabra* Fabricius, accounted for 57.8% of the erebids collected. Five families were represented by 10 or fewer individuals. Most captured taxa (51/68) were represented by 10 or fewer individuals and accounted for 15.2% (165 individuals) of the total abundance.

We observed 154 moths from 15 species, representing the families Crambidae, Erebidae, Geometridae, Noctuidae, and Sphingidae, visiting apple flowers (Table 2). Seven of these species were photographed during visitation to apple flowers (Fig. 3). Species of Noctuidae were the most observed floral visitors, accounting for 118 (76.6%) of the observations. The five most commonly observed floral visitors were *Mythimna unipuncta* (n = 61), *Peridroma saucia* (n = 28), *Udea rubigalis* (n = 14), *Agrotis ipsilon* Hufnagel (Noctuidae; n = 12), and *Hypena scabra* (Geometridae; n = 8). Both *Eupithecia* sp. (Geometridae) and *Hyles lineata* Fabricius (Sphingidae) were observed at flowers more frequently than they were captured (5:3 and 4:3, respectively). All species observed visiting flowers were represented in black-light samples. All 15 species observed visiting flowers were captured in the first year, and ten of these taxa were captured in all three sample years.
Eleven species from four families—Gelechiidae, Geometridae, Noctuidae, and Tortricidae—captured in black-light traps are known to use apples as a larval host plant. Those taxa represented 278 individuals (25.6%) of all moths captured. The four most-numerous species—*Peridroma saucia* (Noctuidae) and three tortricids, *Clepsis peritana*, *C. virescana*, and *Graphlita packardi*—represented 21.9% of all moths captured. Although gelechiids and tortricids represented 16.7% of the total abundance (181 individuals), no species from either family were observed visiting flowers.

Combined, 24 species were either observed visiting flowers or are known to use apple as a larval host plant. These species accounted for 74.9% (814 individuals) of the total captured abundance. Two species of noctuid (*Balsa malana* Fitch and *Peridroma saucia*) were recognized in both categories. One captured species, *Idia aemula* Hübner (Erebidae), is known to feed on dead leaves as larvae, but only one individual from this species was captured over the study period. Most of the captured taxa (43) were not recognized in either category. Uncategorized taxa accounted for 17.0% (185 individuals) of the total captured abundance, and most of these taxa (40) were represented by 12 or fewer individuals.

We observed five non-moth insects visiting flowers in addition to moths (Fig. 4). Two species were beetles (Coleoptera), *Atalantycha bilineata* Say (Cantharidae) and a *Phyllophagus* sp. *A. bilineata* was commonly observed nectaring from flowers. While the *Phyllophagus* sp. was often seen visiting apple flowers, this beetle was only observed eating the petals. Two flies (Diptera) were also observed visiting flowers, *Culex* sp. and a tipulid. However, only one observation was made of each visitor. Finally, a green lacewing of either *Chrysopa* or *Chrysoperla* sp. (Neuroptera) was routinely observed visiting flowers. These insects visited anthers, spending entire observation periods apparently eating pollen. While the neuropteran
visitors were especially common, these insects were never observed visiting more than one flower during observations.

**Discussion**

Although most recognized taxa were captured in the first year, including all taxa observed visiting flowers, the raw abundance and diversity were lowest in that year. This was likely due to fewer nights of high capture rates (more than 50 individuals captured) in 2018 when compared to 2019 and 2020 (1:4:4, respectively). Conditions that favored the great abundance and diversity of moths on specific nights are not known. However, the timing coincided with spring migration for a number of moth species (Westbrook et al. 2016, Wang et al. 2017), and a number of species captured (e.g., *Agrotis ipsilon*, *Helicoverpa zea* Boddie, *Mythimna unipuncta*, *Plutella xylostella* L., etc.) are known to migrate (Hendrix et al. 1987, Sappington and Showers 1992, Hendrix and Showers 1992, Chapman et al. 2002). Perhaps moths are most abundant and diverse among apples during bloom when conditions, like wind direction (Chapman et al. 2008, Chapman et al. 2010), favor moth migration. Whatever conditions influenced moth presence, targeting those conditions for sampling could reduce effort while capturing much of the annual moth abundance and diversity. Nonetheless, the species accumulation curve (Fig. 1) indicates that accurate estimates of species in the habitat require multiple sampling dates and, possibly, years.

We cannot readily explain most of the moth diversity captured among apples during apple bloom, as the majority of taxa were not observed visiting flowers nor are known to use apple as a larval host. However, considering the long association between humans and apples as a crop and our abundant knowledge of apple pests, it is not likely that captured moths had not been previously recognized to feed on apple as larvae. It is more likely that many of the moth
taxa that were not observed visiting flowers actually did visit flowers, but because their abundances were so low, observing this behavior was less likely to occur. It is also possible that the presence of moths with no identified relationship with apples in traps was merely coincidence, being attracted to black lights while searching adjacent areas for appropriate food sources or simply flying through the habitat. Nonetheless, moth diversity appeared to be greatest on productive trap nights. The seemingly random appearance of captured taxa with low abundance may be indicative of prevailing environmental conditions favoring vagrants.

Most of the captured moth abundance was from taxa found to or known to have a relationship with apple plants. Apple nectar appeared to be a predominant explanatory factor, as 62.0% (674 individuals) of the total captured abundance were from taxa observed visiting apple flowers. Moths that are known to use apple as a larval host accounted for only 25.6% (278 individuals) of the total abundance, which includes 138 individuals from two noctuid species (*Peridroma saucia* and *Balsa malana*) also observed visiting apple flowers. This is an intuitive result, as apples produce thousands of flowers per tree in a two- to three-week period and, in an orchard, this creates dense concentrations of available resources for nectar feeding species. Additionally, most apple tissues commonly fed on by larvae of moth pests (e.g., leaves and fruit) are, if present, in low abundance and early stages of development during the periods we sampled, thus generating a resource landscape that is potentially less attractive than later in the year.

Based on our data, we conclude that moths in the family Noctuidae are the most likely nocturnal insects to participate in apple pollination. This family was prevalent in traps and had the most species observed visiting flowers. The species *Mythimna unipuncta* and *Peridroma saucia* are particularly suspect as pollinators. Both species were abundant in captures, were consistently captured in all three sample years, and were the most commonly observed floral
visitors. Moths from the families Erebidae and Geometridae may also pollinate apples. Only five species of these families (three erebids and two geometrids) were observed visiting flowers. However, no fewer than 30 species (16 erebids; 14+ geometrids) from these families were captured in traps. Given the small capture numbers of most of those species, a lack of observations of floral visitation is neither surprising nor indicative of a lack of pollination. It is possible that minimal inputs from many species, including the rare captures such as *Hyles lineata* (Sphingidae), could add to be much more than any one species.

Interestingly, noctuid moths have also been recorded and captured visiting apple flowers during diurnal sampling in India (Thakur and Mattu 2014, Mattu and Bhagat 2015). Species captured in those studies included the black cutworm moth *Agrotis ipsilon*, which was also captured in our sampling. A number of the moths we captured, including *Mythimna unipuncta* and *Peridroma saucia*, have a very broad distribution (these two species occupy all continents except Antarctica). Perhaps their wide distribution makes them more valuable to apple pollination than this regional study can conclude.

It is impossible to rule out other insects as potential pollinators of apples. Although moths were by far the most observed floral visitors, a few non-moth insects were also observed. These insects were rare visitors, and not all of them appeared to visit floral nectaries or make contact with pollen-bearing anthers. Additionally, only *Atalantycha bilineata* was observed visiting multiple flowers during observations. Cantharids have been identified as floral visitors in a variety of systems (Pérez-Hernández 2018), and this species may provide additional, even if minor, pollination inputs to apples. The importance of non-moth insects to apple production should be considered as studies increase.
Ours is the first known report detailing the diversity of nocturnal insects to visit apple flowers. The results can be used to focus study efforts in order to enhance our understanding of nocturnal pollination in agriculture. It further identifies apple-visiting species in northwest Arkansas, allowing for population monitoring efforts of potential moth-floral visitors and regional diversity comparisons. We hope this information is used to quickly expand the breadth of information available on nocturnal pollination in agriculture.

**Acknowledgements**

We extend gratitude to Vaughn Skinner and the staff at the Milo J. Shult Agricultural Research and Extension Center in Fayetteville for their efforts to maintain the apple orchards during the project. We thank the University of Arkansas Department of Horticulture for permissions to use apple orchards under their purview. We further thank Richard Brown for his expertise in moth identifications. Finally, we would like to acknowledge the numerous individuals who assisted with the collection of data and Robert N. Wiedenmann for his efforts to hone this manuscript.

**Literature Cited**


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Tables

Table 1. Numbers of individual moths and species (in parentheses) of the twelve lepidopteran families captured by black light traps in an apple orchard for each of three sample years and overall total. Families with at least one species that visited apple flowers are denoted by *. Families with at least one captured taxon known to feed on apple tissues as larvae are denoted by +.

<table>
<thead>
<tr>
<th>Family</th>
<th>2018</th>
<th>2019</th>
<th>2020</th>
<th>Total</th>
</tr>
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<td>Attevidae</td>
<td>2 (1)</td>
<td>3 (1)</td>
<td>0</td>
<td>5 (1)</td>
</tr>
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<td>Crambidae*</td>
<td>26 (2)</td>
<td>123 (2)</td>
<td>43 (2)</td>
<td>192 (2)</td>
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<td>17 (6)</td>
<td>68 (12)</td>
<td>108 (16)</td>
</tr>
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<td>14 (4)</td>
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<td>33 (4)</td>
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<td>20 (8)</td>
<td>35 (9)</td>
<td>63 (14)</td>
</tr>
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<td>0</td>
<td>5 (1)</td>
</tr>
<tr>
<td>Noctuidae*,+</td>
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<td>198 (13)</td>
<td>201 (10)</td>
<td>461 (17)</td>
</tr>
<tr>
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<td>1 (1)</td>
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<td>10 (1)</td>
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<td>39 (1)</td>
<td>58 (1)</td>
</tr>
<tr>
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<td>0</td>
<td>3 (1)</td>
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<td>0</td>
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<td>1 (1)</td>
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<td>83 (5)</td>
<td>17 (5)</td>
<td>48 (7)</td>
<td>148 (9)</td>
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<td><strong>Total</strong></td>
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<td><strong>414 (42)</strong></td>
<td><strong>458 (47)</strong></td>
<td><strong>1087 (68)</strong></td>
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Table 2. Species of moths from five lepidopteran families observed visiting apple flowers, total number of visitations recorded, and total number of species collected in blacklight traps (in parentheses), in an apple orchard in each of three years and the overall total. Two species whose larvae feed on apple tissues are denoted by *.

<table>
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<th>Family</th>
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<th>2019</th>
<th>2020</th>
<th>Total</th>
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<td>2 (10)</td>
<td>12 (23)</td>
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<td>54 (88)</td>
<td>59 (309)</td>
<td>45 (277)</td>
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Figures

Figure 1. Cumulative numbers of new species collected versus the numbers of individuals captured. Vertical lines indicate the end of each sampling year, and the numbers of individuals and new species captured in each year are provided in parentheses.
**Figure 2.** Pie graphs displaying the distribution of the abundance (left) and diversity (right) of trap catches by moth families.
Figure 3. The five most captured moths that were observed visiting apple flowers and their capture profiles. (a) *Udea rubigalis* Guenée (Crambidae); (b) *Peridroma saucia* Hübner (Noctuidae); (c) *Mythimna unipuncta* Haworth (Noctuidae); (d) *Elaphria grata* Hübner (Noctuidae); (e) *Galgula partita* Guenée (Noctuidae). Bars in red, orange, and yellow indicate the three capture times in which each moth was most commonly captured. Picture from (d) was taken from en.wikipedia.org/wiki/Elaphria_grata.
Figure 4. Non-moth nocturnal insects photographed visiting apple flowers. (a) and (b) are beetles from the order Coleoptera. (b) is a mosquito from the order Diptera. (d) is a green lacewing from the order Neuroptera. Species (or lowest level of identification) and families (in parentheses) are provided below the photographs.
Chapter 5: Nocturnal vs diurnal pollination of self-fertile peaches and muscadine grapes

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Pollination of agricultural crops is a multi-billion dollar ecosystem service primarily provided by insects (Losey and Vaughan, 2006; Allsopp et al. 2008). Bees are major insect pollinators, and their population declines in recent years have brought into question the security of these services in agriculture (Potts et al. 2010, Cameron et al. 2011, Gallai et al. 2009). A complete collapse in insect-mediated pollination services would be devastating to the human population. As such, it is important to indiscriminately explore the input of alternative insect pollinators to agricultural production.

There is a growing and fundamental need to study the role of nocturnal pollinators in crop production. Pollination of agriculturally important crops by different species of diurnal bees has been well studied in recent years. Other diurnal, non-bee, insect species have also been shown to positively contribute to crop pollination (Rader et al. 2016). Few studies, however, consider the contribution of nocturnal species to crop production, and even fewer studies focus specifically on nocturnal pollination in these systems, generating a bias in understanding. Literature is abundant with examples of nocturnal moth importance as pollinators in a wide range of ecosystems (Bawa 1985, Arizaga et al. 2000, Clinebell et al. 2004, Alarcón et al. 2008, Kato et al 2008, Travers et al. 2011, LeCroy et al. 2013, Banza et al. 2015, Rhodes et al. 2017). In fact, growing evidence suggests nocturnal-moths are more important as pollinators of crops than previously believed (Luo et al. 2011, Cutler et al. 2012, MacGregor et al. 2019). Moths have recently exhibited similar declines in abundance and diversity as bees (Conrad et al. 2004, 2006, Mattila et al. 2006, Franzén and Johannesson 2007, Groenendijk and Ellis 2011, Fox 2013, Langevelde et al. 2018), emphasizing the need for research. Nocturnal insects are critical to assessing crop-pollination stability and require immediate, focused research in order to understand the full breadth of pollination services insects provide to human agriculture.
In this study, we examined the difference of diurnal and nocturnal insect-mediated pollination input in two self-fertile varieties of muscadine grapes and peaches. The goal was to determine if these crops, which are suggested to receive little benefit from insect-mediated pollination, are significantly pollinated by nocturnal insects. The findings here have implications for other self-fertile crops.

A field study was performed during the 2019 bloom season at the Fruit Research Station in Clarksville, AR (peaches) and the Arkansas Agricultural Experiment Station in Fayetteville, AR (muscadine grapes). Redhaven peaches and five varieties of self-fertile muscadine grapes (Granny Val, Noble, and the experimental AM-26, AM-70, and AM-77) were used to examine pollination inputs of diurnal and nocturnal insects.

We generated four experimental groups for each fruit crop. Flowers that were to receive no input from pollinators (“Closed”) were bagged at all times. Flowers receiving only nocturnal pollination (“Nocturnal”) were bagged only during the day. Flowers receiving only diurnal pollination (“Diurnal”) were bagged only during the evening. “Open” flowers received pollination inputs from both groups and were left unbagged at all times. We used micromesh (300 μm aperture), insect rearing bags (BugDorm, MegaView Science Co., Ltd., No. 656-2, Fuya Rd, Talchung, Taiwan) to exclude pollinators. For both fruit crops, bags were placed over developing flower clusters after flower heads were counted prior to their opening. We used 49 samples for each treatment in peaches and 12 samples for each treatment in muscadine grapes, for total of 196 samples in peaches and 48 in muscadine grapes. Fruit plots were visited once daily to monitor flower opening. Experimentation began when the first opened flower, experimental or not, was observed. Plots were visited every morning at sunrise and every evening at sunset, with the exception of thunderstorms, to switch the state of bags for the
Nocturnal and Diurnal groups. To account for any pollination input manipulating bags may have caused, Closed and Open groups received mock treatments, wherein we removed bags (or put them on) and immediately put them back on (or removed them) once daily. Experimentation ceased based on individual samples, which occurred following petal fall and obvious deterioration of stigmas. Once all samples had completed experimentation phase, we allowed one week for fruit to develop. Flowers were then revisited and developing fruit were counted. Developing fruit were determined by swelling of the ovaries combined with the strength of attachment to the stem (non-fertilized, remnant flowers fall off with little force). Developing fruit from each cluster were used to generate fruit set proportions (Fig. 1). Fruit set proportions were arcsine transformed (√ arcsin [proportion]) prior to analysis using ANOVAs (SAS 9.4, proc glm). Relationships were determined using Tukey’s range tests (SAS 9.4, means Treatment / Tukey).

In addition to exclusion experimentation, we visited the experimental blocks during the evening hours periodically throughout bloom to observe floral visitation. Floral visitors were photographed when possible. Identifications to the lowest taxonomic level possible are provided for most visitors. To determine if peach flowers predominantly opened during the day or night, which can be an indication of pollination preference, separate flower clusters were monitored for opening during each visit.

There was no significant effect among exclusion treatments in either fruit (Peaches: F = 0.430; p = 0.728; Muscadine grapes: F = 0.070; p = 0.977). Peach fruit set average across all groups was 0.626 or 62.6%. In order from greatest to least peach fruit set mean (± s.d.): Nocturnal (0.670 ± 0.333), Closed (0.650 ± 0.346), Open (0.595 ± 0.348), and Diurnal (0.594 ± 0.351). The mean fruit set across all groups for muscadine grapes was 0.132 or 13.2%. In order
from greatest to least muscadine fruit set mean (± s.d.): Diurnal (0.138 ± 0.062), Nocturnal (0.134 ± 0.076), Closed (0.130 ± 0.082), and Open (0.128 ± 0.056).

We found no evidence to suggest that insects are important to the pollination and fruit production in either Redhaven peaches or self-fertile muscadine grapes. However, it is important to note that both peaches and muscadines have self-infertile varieties. In fact, muscadine grapes are naturally dioecious, with perfect flowers and self-fertile varieties being relatively recent developments in agricultural production. We suspect that the differences observed in the present experiments would be more pronounced between pollination groups in varieties that are self-incompatible, owing to the increase of dependence on pollen transfer mechanisms. We believe this to be an important supposition requiring further investigation.

Peach flowers were observed being visited primarily by two species of moths in the family Noctuidae, the True armyworm (Mythimna unipunctata) and the Variegated cutworm (Peridroma saucia) (Fig. 2). Of the 275 flowers monitored for opening period, 197 (71.6%) opened during the day and 78 (28.4%) opened during the evening. No nocturnal insects were observed visiting muscadine flowers.

Moths visited the Redhaven peach flowers at a greater frequency than any other observed group. Honey bees were sparsely distributed among the orchard. Solitary bees were present during peach bloom, but these species were often concentrated in a small area, with the entire group (save for a few individuals) visiting few trees (mostly outside of the experimental block) in close proximity. Flies were also present but were seldom observed among trees and rarely seen visiting flowers. Moths were often found on every tree. On nights when moth abundance was high, it was more common to find five, or more, individuals on one peach tree than to find no visitors at all. The visitation frequency may explain the increase, however insignificant, in
peach fruit set in the nocturnal group. These observations may be crucial to understanding moth pollination in agricultural fruit, but to elucidate the meaning, if any, requires biodiversity and abundance assays in relation to environmental conditions.

We also report evidence for moth sonication behavior for the first time. We found moths to vibrate flight muscles, vibrating their entire bodies, while in contact with plant reproductive tissues at a more than coincidental rate (estimated more than half the visits). This behavior in moths is known to be associated with increasing body temperature for flight. However, floral sonication by bees is reported to encourage nectar production and access in some flowers. We do not know if there is any enhancement to the effectiveness of moth pollination through this behavior. However, the timing of the vibrations were compelling, occurring most frequently while visiting flowers. This in combination with fundamental differences observed in visitation patterns and behavior between the two common visiting moth species highlight the need for a focused behavioral study concerning floral visitation by moths.

Although we show that no insect group influences the fruit set of either self-fertile peaches or muscadine grapes, we observed moth numbers and behaviors that warrant further investigation. Moths likely contribute to the fruit set of other crops, though self-fertile varieties may be free of insect dependence. Further research is needed to understand nocturnal pollination in fruit agriculture.

Summary

There is growing evidence that nocturnal moths are important pollinators of agricultural crops. However, the contribution of these pollinators to the production of crops remains largely unknown. We explored the pollination input nocturnal insects provide to self-fertile varieties of peaches and muscadine grapes. Our goal was to determine if these fruit varieties need no or little
input from insect pollinators, as previously believed, or if pollination is provided by the oft-
ignored nocturnal-insect pollinators. Moths were anecdotally observed to be the dominant floral
visitor in peaches, diurnal or nocturnal. No floral visitors were recorded in muscadine grapes.
We found that the fruit set of self-fertile varieties of both fruits was not significantly increased by
any pollinator group, suggesting that pollination contribution provided by insects was
unnecessary. Interestingly, we observed both common moth visitors to consistently vibrate their
wings during floral visits. This behavior is known to increase body temperature in moths for
flight, but this is the first time the vibration behavior has been associated with floral visitation.
While the examined fruit varieties received no benefit from insect pollination, important
observations highlight our lack of understanding concerning nocturnal pollination and require
focused research to elucidate.

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Figures

**Figure 1.** Fruit-set proportions by treatment groups in Redhaven peaches and various muscadine grapes.
Figure 2. Moth visitors to Redhaven peaches. (A) *Mythimna unipunctata*; (B) *Peridroma saucia*—note the blurred wings as an indication of vibration; (C) *P. saucia* (x3); (D) *P. saucia* (x4).
Chapter 6: Conclusions

This dissertation covered nocturnal pollination in fruit agriculture. In chapter two, I reviewed literature pertinent to the potential of moths to serve as pollinators in agriculture, where they have rarely been considered, in order to exemplify the need for the studies found in this dissertation. In that chapter, I presented evidence that moths 1) have a long-standing evolutionary relationship with angiosperms, 2) maintain attributes that are known to be valuable towards pollination, 3) have been demonstrated to provide important pollination services to a number of ecosystems, wild plants, and cultivated crops, and 4) are experiencing dramatic declines in abundance, distribution, and diversity. In chapter three, I, along with my coauthors, demonstrate that moths are capable of producing apples in similar numbers and quality as diurnal pollinators. Because of the global crop value of apples, this chapter indirectly highlights the high value of moth-pollination services in agriculture. Chapter four expands on chapter three, identifying the potential pollinators of apples based on observational evidence and a rigorous, three-year survey of moth species present in apples during bloom. The fourth chapter identifies target species associated with apple pollination that can be used for comparison to other regions and fruit, species monitoring, species conservation, behavioral studies, and studies examining the interaction between moths and other pollinators and moths and crop plants. The fifth chapter furthers the examination of fruit pollination by nocturnal pollinators by incorporating both peaches and muscadine grapes. This chapter expands the number of crops for which nocturnal pollinators have been associated and highlights the need to include a broad variety of crops while considering the pollination needs of each crop species.

The cumulative evidence from these collected studies suggest that nocturnal pollinators, particularly moths, are likely highly valuable for pollination services in fruit crops. While
nocturnal pollinators did not enhance the production of either self-fertile peaches or muscadine grapes, moths were observed visiting the flowers of peaches in high numbers and with surprising diversity. Furthermore, results in apples suggest that a diverse set of moths are capable of producing apples at similar levels as diurnal pollinators. These results add to the growing evidence that moths are important contributors to pollination in agriculture. As pollinator populations continue to experience declines in distribution, diversity, and abundance, understanding the roles of all participating pollinating insects is critical. It is possible, even likely, that moths add a previously unrecognized source of stability to fruit agriculture and, perhaps, other pollinator-dependent crops.

Future studies should focus on broadening the scope of nocturnal pollination in agriculture. A variety of pollinator-dependent crops may receive significant benefits from the presence of moths and other nocturnal pollinators, and such information could prove invaluable to assessments on crop stability. Moth behaviors observed during these studies and inferred from knowledge concerning the natural history of moths collected during these studies, such as their visitation patterns, vibration behavior while in contact with floral reproductive tissues, and the migratory behavior of a number of collected species, would be interesting for study and could add important dynamics of crop pollination. Studies should also seek to identify the interactions between diurnal and nocturnal pollinators, as such interactions could influence crop pollination. Declines in resident moth populations in regions of North America and throughout Europe hasten the need for understanding their roles in agriculture. Given the high abundance and diversity of migrating species collected among apples, it would also be wise to begin examinations on the statuses of migratory-moth populations. Finally, examinations into the factors that influence the presence of nocturnal pollinators among crop plants could provide
recommendations to growers that would bolster the pollination provisions they receive from wild pollinating species.