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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 & Vaughan 2006; Allsopp et al. 2008). Bees are major insect pollinators, and recent declines in their population have raised questions about the security of the services they provide in agriculture (Gallai et al. 2009; Potts et al. 2010; Cameron et al. 2011). Approximately 70% of plants used directly for human consumption are dependent on pollinators, particularly insects (Klein et al. 2007). A complete loss of insect-mediated pollination services would, therefore, be devastating to the human population. In order to accurately assess and appropriately address the risk posed to humanity, we must have a complete understanding of pollination dynamics in agricultural systems. As such, it is important to indiscriminately explore the input of non-bee 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 studied thoroughly in recent yr. Other diurnal, non-bee, insect species also have been shown to positively contribute to crop pollination (Rader et al. 2016). Furthermore, pollinator diversity is essential for crop pollination (Kremen et al. 2002). Few studies, however, consider the contribution of nocturnal species to crop production, and even fewer studies focus specifically on nocturnal pollination in agroecosystems, generating a bias in understanding. There is an abundance of literature on the importance of nocturnal moths 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). It is important to understand the role of nocturnal moths in crop-pollination stability. However, recently moths have experienced declines in abundance and diversity similar to bees (Conrad et al. 2004, 2006; Mattila et al. 2006; Franzén & Johannesson 2007; Groenendijk & Ellis 2011; Fox 2013; Langevelde et al. 2018). We need current information on moth contributions to crop pollination in order to recognize effects, and to predict future effects associated with declining moth populations. Nocturnal moths 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 2 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, Arkansas, USA (peaches; Rosaceae) and the Arkansas Agricultural Experiment Station in Fayetteville, Arkansas, USA (muscadine grapes; Vitaceae). Redhaven peaches and 5 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 4 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 d. 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., Taichung, 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. The study began when we observed the first open flower, experimental or not. We visited plots 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 the 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. Experiments ceased following petal fall and deterioration of stigmas, which was based on individual samples. Once all samples had completed the experimentation phase, we allowed 1 wk for fruit to develop. Flowers then were revisited and developing fruit was counted. Developing fruit was determined by swelling of the ovaries combined with the strength of attachment to the stem (non-fertilized, remnant flowers fall off with little force). We used the ratio of developing fruit from each cluster to generate fruit set proportions (Fig. 1). Fruit set proportions were arcsine transformed (√ arcsin [proportion]) prior to analysis using ANOVAs (proc glm, SAS vers. 9.4,Cary, North Carolina, USA). Relationships were determined using Tukey's range tests (means Treatment/Tukey, SAS vers. 9.4, Cary, North Carolina, USA).

In addition to exclusion experimentation, we visited the experimental blocks during the evening h 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

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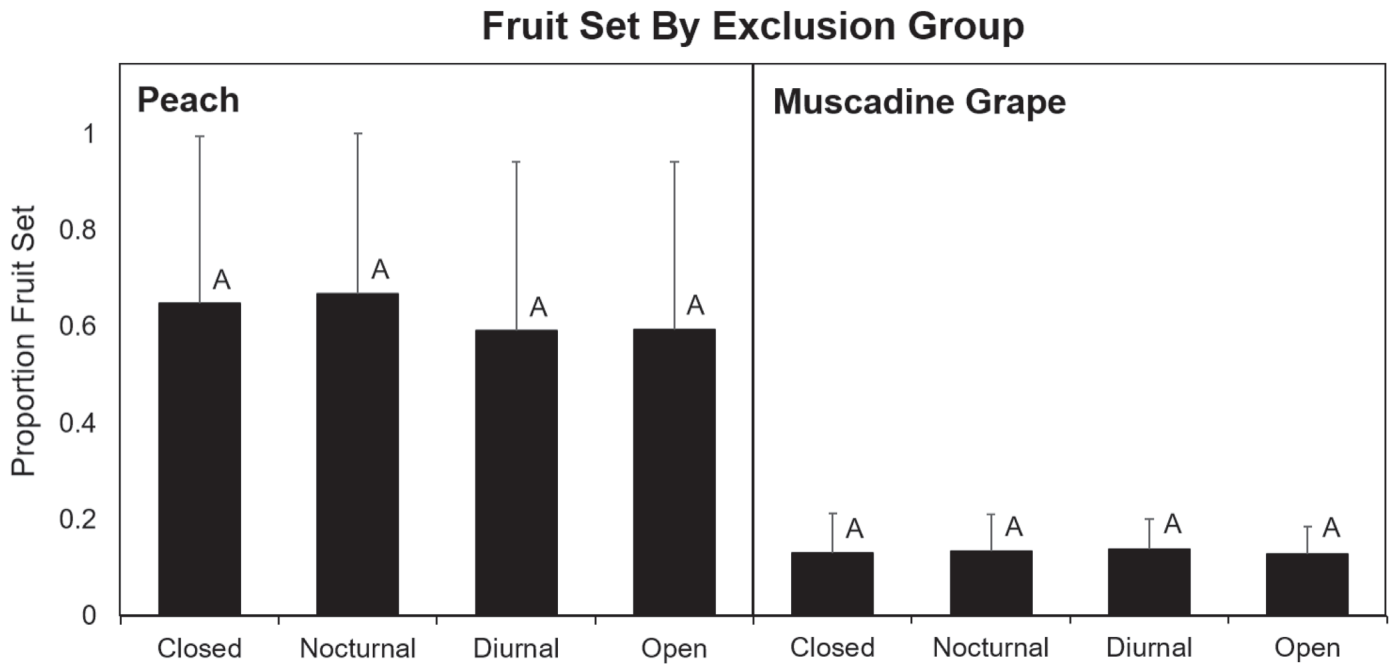

Fig. 1. Fruit-set proportions by treatment groups in Redhaven peaches and various muscadine grapes.

opened during the d 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 on fruit set among exclusion treatments in either crop (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 (± SD): 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 (± SD): 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 2 species of moths in the family Noctuidae, the true armyworm (*Mythimna unipunctata* [Haworth]; Lepidoptera: Noctuidae) and the variegated cutworm (*Peridroma saucia* Hübner; Lepidoptera: Noctuidae) (Fig. 2). Of the 275 flowers monitored for opening period, 197 (71.6%) opened during the d 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 distributed sparsely among the orchard. Solitary bees were present during peach bloom, but these species often were 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 also were present but were seldom observed among trees and rarely seen visiting flowers. Moths often were found on every tree. On nights when moth abundance was high, it was more common to find 5 or more individuals on 1 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 production, but to elucidate the meaning, if any, requires biodiversity and abundance assays in relation to environmental conditions.

We found noctuid moths vibrate their bodies prior to, during, and after nectar feeding and while the body was in contact with reproductive tissues. This behavior was reported during flower visits by 2 noctuid moths to *Struthiola ciliata* (Thymelaeaceae) in southern Africa (Makholela & Manning 2006), and the behavior may be more common than realized. Wing vibrations in moths are known to increase body temperature for flight (Dotterweich 1928; Krogh & Zeuthen 1941; Dorsett 1962). We believe it is possible that the vibration may enhance pollen transfer by encouraging the release of pollen from both the anther and then the body of the moth. Floral sonication by bees is reported to encourage nectar production and access in some flowers, and Veits et al. (2018) showed that flowers respond to moth (and bee) wing-beat frequency by increasing the concentration of sugars in the nectar. We did not attempt to measure any effect associated with this behavior, but the timing of the vibrations were compelling and occurred most frequently while visiting flowers. This in combination with fundamental differences observed in visitation patterns and behavior between the 2 common visiting moth species highlight the need for a focused behavioral study concerning floral visitation by moths.

Although we show that insects did not influence the fruit set of either self-fertile peaches or muscadine grapes, we observed moth numbers and behaviors that warrant further investigation. Moths contribute significantly to the fruit set of other crops (Luo et al. 2011; Cutler et al. 2012; Robertson et al. unpublished), though self-fertile varieties may not benefit from insect-mediated pollination. Further research is needed to understand nocturnal pollination in fruit agriculture.

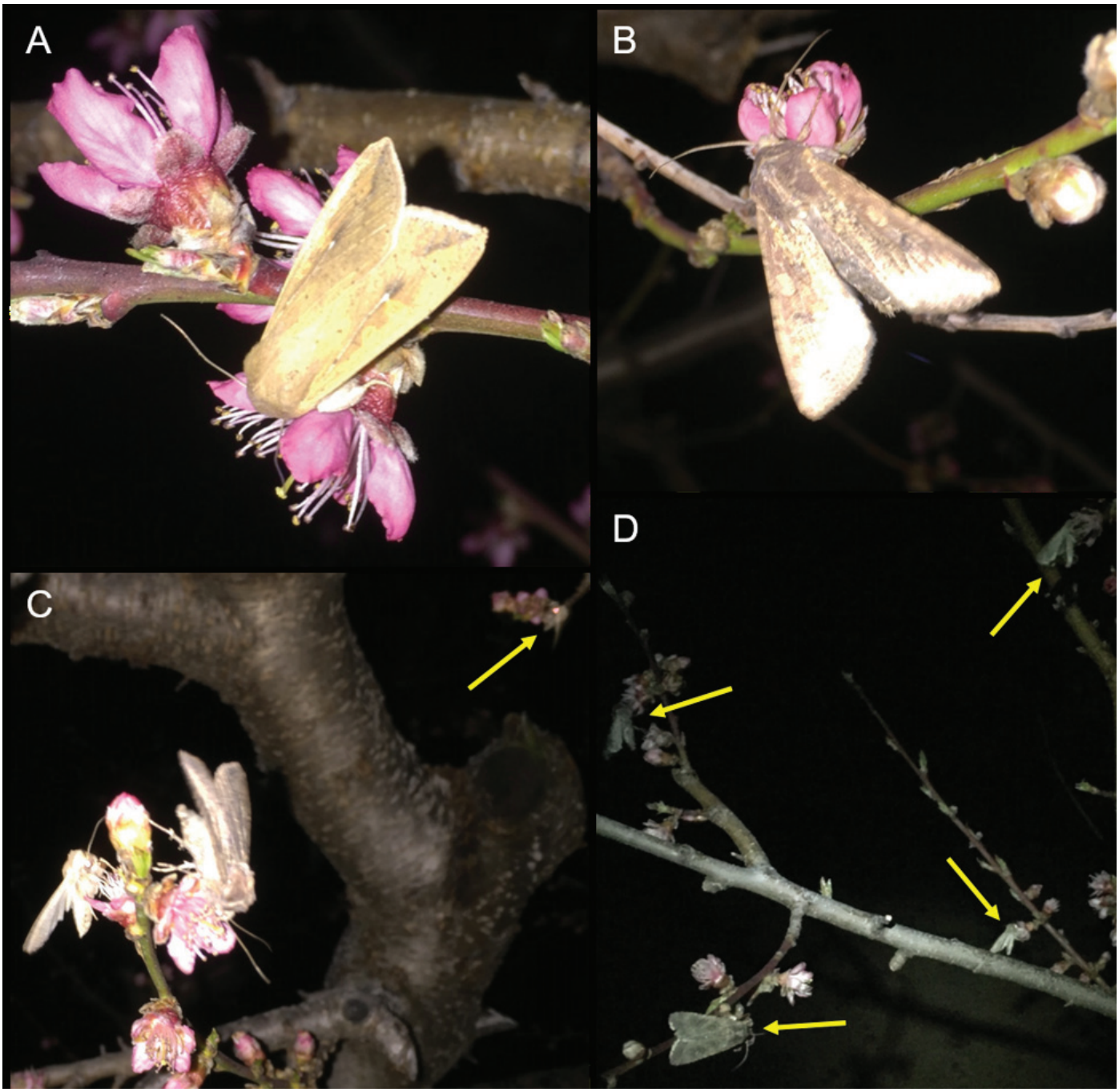

Fig. 2. Moth visitors to Redhaven peaches: (A) *Mythimna unipunctata*; (B) *Peridroma saucia* – note the blurred wings as an indication of vibration; (C) *P. saucia* (3×); (D) *P. saucia* (4×).

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 little or no 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.

Key Words: nocturnal pollinators; moths; pollination; peaches; muscadines

Sumario

Cada vez hay más pruebas de que las polillas nocturnas son importantes polinizadores de cultivos agrícolas. Sin embargo, la contribución de estos polinizadores a la producción de cultivos sigue siendo en gran medida desconocida. Exploramos la entrada de polinización que proporcionan los insectos nocturnos a variedades autofértiles de duraznos y uvas muscadinas. Nuestro objetivo fue determinar si estas variedades de fruta necesitan poco o ningún aporte de los polinizadores de insectos a como se creía anteriormente, o si la polinización es proporcionada por los polinizadores nocturnos de insectos que a menudo son ignorados. Se observaron las polillas anecdóticamente como el visitante floral dominante de los melocotones, diurnos o nocturnos. No se registraron visitantes florales en uvas muscadinas. Descubrimos que ningún grupo de polinizadores aumentó significativamente el conjunto de frutas de variedades autofértiles de ambas frutas, lo que sugiere que la contribución a la polinización proporcionada por los insectos era innecesaria. Curiosamente, observamos que los visitantes de las polillas comunes vibran constantemente sus alas durante las visitas florales. Se sabe que este comportamiento aumenta la temperatura corporal en las polillas para el vuelo, pero esta es la primera vez que el comportamiento de vibración se ha asociado con las visitas florales. Si bien las variedades de fruta examinadas no recibieron ningún beneficio de la polinización de insectos, las observaciones fueron importantes en resaltar nuestra falta de comprensión sobre la polinización nocturna y la necesidad de una investigación enfocada para dilucidar su impacto.

Palabras Claves: polinizadores nocturnos; polillas; polinización; melocotones; muscadinas

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