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Crop pests eaten by bats in organic pecan orchards

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ABSTRACT

Bats are generalist predators of night flying insects, including many crop pests. Attracting bats to agricultural areas using bat houses may reduce the numbers of these pests and, consequently, their economic impact. We use real time polymerase chain reaction of mitochondrial DNA found in the guano of bats living in bat houses on organic pecan orchards to document the consumption of pest moth species: pecan nut casebearer, *Acrobasis nuxvorella* Neunzig (Lepidoptera: Pyralidae), hickory shuckworm, *Cydia caryana* Fitch (Lepidoptera: Torticidae), and corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae). We also use direct sequencing of insect remains in bat fecal pellets to identify stink bugs consumed by bats in bat houses. Evidence that bats prey upon crop pests is the first step in showing that bats are beneficial to pecan farmers and provides incentives for bat conservation.

1. Introduction

Generalist predators can be effective biocontrol agents by reducing pest numbers, thereby reducing or preventing crop damage (Symondson et al., 2002). As native, generalist predators of night-flying insects, bats serve as significant agents for suppression of insect pests in agriculturally intensive areas (McCracken et al., 2012; Kunz et al., 2011). In particular, Brazilian free-tailed bats, *Tadarida brasiliensis* I. Geoffroy (Chiroptera: Molossidae), have been estimated to provide $741,000 annually in services by suppressing cotton bollworm (also known as corn earworm on corn plants), *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), in cotton fields in south-central Texas (Cleveland et al., 2006). Because of their consumption of crop pests, Boyles et al. (2011) cite bats as among the most overlooked, yet economically beneficial, non-domesticated animals in North America. However, predator–prey relationships can be challenging to document in bats, due to the difficulty of directly observing predation in fast-flying, nocturnal animals.

Broad-spectrum insecticides are commonly used to control insects, but these insecticides often have serious environmental and economic consequences. To encourage pest suppression by bats in lieu of pesticide use, some organic farmers install bat houses to attract bats to their farms and orchards. However, the consumption of crop pests by bats utilizing bat houses on organic farms has not previously been studied.

Pecan (*Carya illinoinensis*, Fagales: Juglandaceae) is a highly nutritious food product that is widely cultivated in the southern United States and is expensive to produce relative to other nut species (Mizell, 2003). The diversity of pests, the variation of insects among orchards, and the masting trait of pecans make it difficult to attribute crop damage and impact on yield to a particular insect pest (Dutcher et al., 2003). However, the most important pests of pecans include two moth species (pecan nut casebearer, *Acrobasis nuxvorella* Neunzig (Lepidoptera: Pyralidae), and hickory shuckworm, *Cydia caryana* Fitch (Lepidoptera: Torticidae)) and several stink bug species (Wood, 2003). In 2006 in Georgia alone, the costs of controlling *A. nuxvorella* and *C. caryana* on pecans were estimated at $520,000 and $650,000, respectively (Hudson and Dutcher, 2006).

Collecting and analyzing feces can provide a noninvasive and inexpensive method for obtaining genetic material for dietary analysis. Molecular analysis of fecal material allows identification of prey species to taxonomic levels that often are not feasible through traditional fecal analysis. Given the difficulty in capturing and monitoring bats in the wild, noninvasive molecular fecal analysis is particularly promising for studying predator-prey interactions in these volant mammals (Boston et al., 2012). Real time polymerase chain reaction (rtPCR; also known as quantitative PCR) is a highly sensitive method of amplifying a specific DNA sequence. When applied to fecal samples, rtPCR allows screening of many samples

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quickly for a specific prey item by using species-specific primers and fluorescent probes. This method is ideal for identifying soft-bodied insects, such as Lepidoptera, which are difficult to detect by traditional visual fecal analysis. Consumption of the agricultural pest *H. zea* by *T. brasiliensis* bats in the Winter Garden agricultural area of southern Texas was documented using rtPCR (McCracken et al., 2012).

Another molecular method for identifying prey DNA in fecal samples of bats involves using non-specific primers to sequence DNA extracted directly from insect fragments found in guano pellets (Clare et al., 2009, 2011). Hard-bodied insects are not as well masticated and digested by bats as soft-bodied insects. When bats consume hard-bodied insects such as stink bugs, the shell fragments left in guano tend to be distinctive from other insects under a dissecting microscope. From the fragments of these hard-bodied insects, DNA can be extracted and identified to the species level, which is not always possible using only a microscope.

The objective of this study is to use molecular techniques to examine the consumption of crop pests by bats inhabiting bat houses in two organic pecan orchards. There is substantial variation in bat house design, but the most common bat houses tend to be wooden structures approximately three feet in height, containing multiple panels separated by approximately 1-inch. Depending on the bat species, up to 50 bats per square foot can roost between panels (Tuttle et al., 2013). The bat houses we used in this study are mounted on poles 12–15 feet high in open areas within the orchards. By encouraging the presence of native, generalist predators, growers may reduce insect damage to their crops while reducing the costs and hazards associated with insecticides and also providing supplementary roosting habitat for a variety of insectivorous bat species. Here we analyze feces collected beneath bat houses for the presence of DNA from *A. nuxvorella*, *C. caryana*, *H. zea*, and stink bugs using both rtPCR and direct sequencing of amplified DNA products from insect fragments. Because both orchard owners reported reduced insect damage to pecan trees once bats began inhabiting the houses, we expected to document consumption of pecan pests by these bats on nights when these pests were most abundant in the orchards.

2. Materials and methods

2.1. Study sites

This study was conducted on two certified organic pecan orchards in the southern United States: a 27-acre orchard in Quitman, Georgia (approximately 30°47′5.45″N, 83°33′35.27″W), and a 100-acre orchard in San Saba, Texas (approximately 31°11′44.60″N, 98°43′5.15″W). Bat houses were first installed at the Georgia orchard in 1996 and are inhabited by up to an estimated 3500 bats, predominantly Brazilian free-tailed bats (*T. brasiliensis*) and evening bats (*Nycticeius humeralis*, Rafinesque Chiroptera: Vespertilionidae). Bat houses were first installed at the Texas orchard in 2003. Approximately 2500 bats live in the houses throughout the pecan growing season, predominantly *T. brasiliensis*, with fewer *N. humeralis* and potentially cave myotis (*Myotis velifer*, Allen Chiroptera: Vespertilionidae).

2.2. Insect monitoring

To document patterns of insect activity and to provide insects for genetic analysis, insects were collected using pheromone lures with sticky trap liners and using black light traps. In Georgia a single pheromone trap targeting mate-seeking *A. nuxvorella* male moths (Harris et al., 1997) was checked every 2–3 days throughout May, with counts averaged across those days. Pheromone traps targeting *A. nuxvorella* in Texas were checked daily with some gaps throughout the pecan-growing season. Three traps were used in 2008 and 5–6 traps in 2009, with counts averaged across traps. Pheromone traps for *C. caryana* were used in Texas only. Stink bugs and *H. zea* were collected in black light traps in Texas, but not Georgia. Corn and cotton are common crops surrounding the Georgia orchard, and *H. zea* and stink bugs are known to be prevalent in the area.

2.3. Fecal sample collection under bat houses

Guano was collected in 4 oz collection cups (Fisher Scientific) secured to wooden boards beneath the bat houses. The cups (10 in Georgia and 12 in Texas) were distributed to uniformly collect feces from bats roosting in different locations within the houses. The largest guano pellets were selected preferentially and put in 2 ml screw cap microtubes (Sarstedt) containing silica gel desiccant (4–10 mesh, Fisher Scientific) (Wasser et al., 1997) and stored at −20 °C following collections. In the Georgia orchard in 2008 and 2009 and Texas in 2008, feces from each collection cup were put into individual 2 ml tubes. In Texas in 2009, two fecal pellets from each collection cup were selected and each pellet was placed into an individual tube. Bats were not present in every bat house at every collection date, and collections were not made during rain.

In Georgia, cups were set out at ca. 0700 h and feces collected approximately 2 h later. In 2008, samples were collected weekly under 3 bat houses; in 2009, samples were collected twice a week under 2 houses. Persistent rain ended Georgia collections prematurely in 2009. In Texas, cups were placed under the most consistently occupied bat house at ca. 2100 h and feces collected approximately 12 h later. In 2008, feces were collected more frequently when insect activity was high, every 1–13 days; collections were made every 3–5 days in 2009. Sampling in 2008 was conducted throughout the summer, while sampling in 2009 was concentrated during the first generation of *A. nuxvorella* activity in May (Supplemental Information Table S1), which missed a fall spike in *A. nuxvorella* activity in Texas that was much larger than expected based on the previous year's data.

2.4. Fecal collections for positive controls

To provide positive controls for PCR, fecal samples were collected from known species of bats captured in mist nets within the orchards in Texas. Bats were held individually in cloth bags for up to 3 h immediately after capture, and feces were collected in individual 2 ml tubes containing silica gel. Two mm wing tissue biopsies were also collected and stored in 20% DMSO from 7 *N. humeralis* and 12 *M. velifer*.

To confirm insect-specific primers, positive fecal controls known to contain *A. nuxvorella* DNA were obtained by feeding adult moths to one adult male *N. humeralis* and one adult male *M. velifer*. Although *T. brasiliensis* is the dominant species at both sites, this species is difficult to hand feed and was therefore not included in controlled feedings. Bats were caught in mist nets, held for one day in a cloth bag at the site of collection and released the following evening. The bats were fed mealworms and as many moths as they would eat, with one bat eating half a moth and the other eating seven moths. Feces were collected and stored in silica gel.

2.5. DNA extraction and amplification

DNA was extracted from 26 *A. nuxvorella*, 12 *C. caryana*, several stink bug species and other insects from the study areas using DNeasy Tissue DNA Extraction kits (Qiagen) as described in