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I am submitting herewith a thesis written by Kimberly Shawn Kennard entitled "The Effects of Prey Abundance and *Bt* (*Bacillus thuringiensis*) Crops on Bat Activity in South-Central Texas Agroecosystems." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology and Evolutionary Biology.

Gary F. McCracken, Major Professor

We have read this thesis
and recommend its acceptance:

Thomas G. Hallam

Nathan Sanders

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the
Graduate School

(Original signatures are on file with official student records.)

THE EFFECTS OF PREY ABUNDANCE AND
BT (*BACILLUS THURINGIENSIS*) CROPS ON BAT ACTIVITY IN
SOUTH-CENTRAL TEXAS AGROECOSYSTEMS

A Thesis
Presented for the
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Degree
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Kimberly Shawn Kennard
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I dedicate this thesis to the staff at Selah Bamberger Ranch Preserve for inspiring me, my parents for always being proud of me, and Jonathan for believing in me.

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Abstract

Agroecosystems produce insects in great abundance, with episodic irruptions in time, and patchy distributions in space. In the industrial scale agroecosystems of south-central Texas, millions of Brazilian free-tailed bats (*Tadarida brasiliensis*) consume these insect pests. In the past decade, growers in Texas have planted transgenic Bt (*Bacillus thuringiensis*) crops on a large scale, which may reduce populations of target insect species by up to 95%. To investigate potential impacts of this evolving agricultural landscape on insectivorous bats, I examined the response of foraging bats to emergences of insects from replicate Bt and non-Bt fields of corn and cotton in the Winter Garden region of south-central Texas. I quantified bat activity using ultrasonic detectors deployed simultaneously in Bt and non-Bt fields. I measured insect activity using pheromone traps and video imaging. Professional crop consultants scouted fields to determine dates of insect emergence. We recorded 92% more echolocation calls, 62% more AnaBat files, and 257% more feeding buzzes over agricultural fields during periods of local insect emergence. During these insect emergence periods, bat activity was correlated with the abundance of moths and negatively related to the distance between foraging sites and roosting sites. In general, Bt crops did not have a measurable impact on the activity of bats except at one site where moths were more abundant over non-Bt crops versus Bt crops. Foraging bats showed a delayed response to moth abundance, which is consistent with the hypothesis that roosts serve as information centers that enhance foraging efficiency. The ability of millions of bats to exploit localized patches of prey across a large area provides further evidence of their pest control service. This economically important pest control service extends beyond growers in Texas, as the populations of moths produced in agroecosystems in Texas influence agricultural production on a continental scale.

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Introduction

Bats are receiving increased attention for their important roles in ecosystems. Approximately 75% of the more than 1,000 extant species of bats are insectivorous (Jones and Rydell 2003) and are the main predators of night-flying insects (Altringham 1996). Many insectivorous bats are opportunistic foragers and show adaptations for feeding on insects that form abundant, but patchy and ephemeral, food sources (Fenton and Morris 1976; Gould 1978; Anthony and Kunz 1977; Brigham and Fenton 1991; Rydell 1992; Griffin 1958; Lee and McCracken 2002). This opportunistic feeding behavior has been demonstrated experimentally by manipulating patches of prey for several species of bats (Bell 1980; Fukui et al. 2006).

Agroecosystems often produce insects in great abundance, with episodic irruptions in time and patchy distributions in space (Fitt 1989). In northern Mexico and the southern United States, industrial agricultural production of corn and cotton functions as an insectary, producing billions of noctuid moths (Raultson et al. 1986). Multiple generations of these moths disperse locally or over long distances (Harstack et al. 1982; Raulston et al. 1990; Wolf et al. 1990; Westbrook et al. 1995). Growing evidence documents the role of Brazilian free-tailed bats (*Tadarida brasiliensis*) as important predators of these noctuid moths in south-central Texas (Lee and McCracken 2005).

Brazilian free-tailed bats in south-central Texas

Each spring Brazilian free-tailed bats migrate from their wintering grounds in Mexico into the southwestern U.S., forming maternity colonies that are among the largest known assemblages of vertebrates in the world (Cockrum 1969; McCracken 2003). During the summer, south-central Texas is home to an

estimated 100 million bats, the majority of which are reproductive females (McCracken 2003). Females typically give birth to one pup between mid- and late June (Davis et al. 1962) and nurse their young for about 6-7 weeks (McCracken and Gustin 1991). Energetic demands are considerable for bats due to their small body size, high metabolic rates, and the costs associated with flight (Thomas 1975). During pregnancy and lactation, the feeding rates of individual female free-tailed bats can double or even triple, and individual bats may consume up to two-thirds of their body mass every night during peak lactation (Kunz et al. 1995). A substantial resource base is necessary to support these dense seasonal aggregations of Brazilian free-tailed bats in south-central Texas.

Links between Brazilian free-tailed bats and agricultural pests

The timing of the migration and arrival of corn earworms (*Helicoverpa zea* (Bodie 1850)) and other insect pests into south Texas from Mexico closely correlates with the flight patterns, colony locations, and migration of Brazilian free-tailed bats (Lee and McCracken 2005). The temporal and seasonal variation in moth consumption in the diet of Brazilian free-tailed bats is strongly correlated with the availability of migratory moth populations (Lee and McCracken 2005; Whitaker et al. 1996). At times, moths make up over 80% of the bats' diet (Whitaker et al. 1996; Lee and McCracken 2005). Fecal DNA analysis has recently confirmed the presence of corn earworm (McCracken et al. submitted), beet armyworm (*Spodoptera exigua*) (Whitworth 2006), tobacco budworm (*Heliothis virescens* F.), and fall armyworm (*S. frugiperda*) (G. F. McCracken, personal communication) moths in the diet of Brazilian free-tailed bats.

The economic benefit of this pest control service warrants recognition because losses in the U.S. due to damage from and control for corn earworms and tobacco budworms often exceed \$1 billion annually (Johnson et al. 1986). Models of the consumption of noctuid moths by foraging bats indicate that this pest control service reduces the number of pesticide applications needed to

control pests, lowers the number of moths emigrating from crops, and reduces damage to crops (Cleveland et al. 2006, Federico et al. 2008).

Dynamics of agricultural pest populations

Each year, over the course of several weeks in early June, three to four billion corn earworm and fall armyworm moths emerge at dusk from agricultural fields in the lower Rio Grande Valley of northeastern Mexico (Raulston et al. 1992). Assisted by northerly winds at altitudes of up to 800 m, these moths can travel 400 km in one night and arrive in agricultural regions of south-central Texas the morning after their emergences in the Lower Rio Grande Valley (Wolf et al. 1990). Following their arrival, females lay eggs mainly on corn crops, which are the preferred host and are silking at this time (Fitt 1989). Development from egg to adult takes about 3-4 weeks (Fitt 1989), and the next generation of moths emerges from senescing corn between late June and early July. Some moths ascend to high altitudes and continue their northward migration. Others disperse locally, mainly to nearby cotton crops. The next generation of moths emerges from cotton fields, typically in late July to early August. The seasonal pattern of abundance of noctuid moths is highly variable and is influenced by crop availability, temperature, and indirectly by rainfall, which influences the development, abundance, and quality of host plants (Fitt 1989). The multiple generations of noctuid moths that occur through the summer in south-central Texas create a mosaic of rich but ephemeral and patchy food resources for foraging bats (J.K. Westbrook and E.H. Gillam, unpublished data).

Modern Agricultural Pest Management: Transgenic Bt Crops

Traditionally, farmers have controlled insect pest populations by applying broad-spectrum synthetic pesticides that can be dangerous to human health and the environment. With the isolation of microbial toxins from the soil bacterium

(*Bacillus thuringiensis*), Bt sprays became available that were organically derived and targeted specific taxa. Still, Bt sprays were more expensive and less practical than conventional insecticides, and made up less than 2% of the global insecticide market until transgenic Bt crops became available in 1995 (Shelton et al. 2002). Modified forms of the *Cry* gene from *B. thuringiensis* are now engineered directly into crop plants. These plants produce δ -endotoxins that are toxic when ingested by susceptible species. In Texas, Bt crops offer some protection against damage by the larval stages of corn earworms, tobacco budworms (USDA-ERS 2007), and to some extent, fall armyworms (Chilcutt et al. 2007). Susceptibility to Bt toxins varies for these insect species (Tabashnik et al. 2008). The level of expression of toxins also differs between varieties of Bt crops (Adamczyk and Gore 2004), and differs throughout the anatomy of the plant itself (Adamczyk and Sumerford 2001). Additional insecticides are often applied to cotton crops, resulting in ~95% mortality rates for target species (Johnson et al. 2002). To date, adult female corn earworm moths show no significant changes in oviposition behavior between Bt and non-Bt host plants (Torres and Ruberson 2006), and it is the larval stage that experiences higher mortality after feeding on plant tissues containing Bt toxins. Therefore, Bt crops are expected to have no effect on moth densities until locally established populations emerge.

Despite only 13 years on the commercial market, Bt crops have become widespread. In 2007, 49% of all corn and 59% of all cotton planted in the U.S. were transgenic for Bt (USDA-ERS 2007). In the Winter Garden agricultural region of south-central Texas, the percentage of Bt crops is even higher, with reports of Bt crops accounting for up to 50% of all corn and 95% of all cotton planted in 2005 (Federico et al. 2008).

The adoption of Bt crops has resulted in decreased use of pesticides (Carpenter 2001), and increased yields for growers (USDA-ERS 2007). However, there are several potential risks associated with the large-scale adoption of Bt crops. These risks include direct effects on non-target species and indirect

effects resulting from impacts on beneficial predators of the targeted pest. Due to their selective impact on target organisms, direct toxicological effects on non-target species are generally considered to be minimal (but see Rosi-Marshall et al. 2007; Lang and Vojtech 2006). In fact, non-target species are often more abundant in Bt crops than in sprayed conventional crops due to the effects of broad-spectrum insecticides (Marvier et al. 2007). Indirect effects can occur due to a reduction in the abundance of prey populations targeted by Bt, or due to lower quality prey reared on Bt plants (e.g. Hilbeck et al. 1998 for green lacewing insects (*Chrysopa oculata*)). Recently, more attention has been paid to indirect effects of Bt crops, but with a focus on arthropods (for a review, see O'Callaghan et al. 2005). Despite their importance as insectivores in agroecosystems, we know very little about potential impacts on vertebrate predators, such as birds and bats. Recognizing this disparity in both the scientific literature and data submitted during government registration of Bt crops, several research advisory groups, including the National Research Council and Scientific Advisory Panels for the Federal Insecticide, Fungicide, and Rodenticide Act under the EPA, have called for broader ecological assessments of risks to non-target species (see also Marvier 2001; Obrycki et al. 2001; Naranjo et al. 2005).

Another major concern over the large-scale use of Bt crops is the development of resistance to Bt by target species. Resistance to Bt has been documented in the field for corn earworms (Tabashnik et al. 2008) and the diamondback moth (*Plutella xylostella* Linnaeus) (Tang et al. 1999). Additionally, several target pests have evolved resistance in laboratory selection experiments (Tabashnik et al. 1994; Gould et al. 1995). Concerns over the evolution of insect resistance to Bt have led to the “high dose/refuge strategy” (US EPA 1998). This strategy requires that plants are engineered to produce doses of toxins sufficient to kill heterozygote carriers of resistance alleles. In addition, growers are required to plant refuges of conventional crops near Bt fields to maintain populations of susceptible insects and promote the dilution of resistance alleles in populations

of target species. This regime of planting Bt and conventional fields in close proximity allows for direct comparison of the abundances of insects and insectivores in different crop types while eliminating potentially confounding variables such as local climate, soil characteristics, and geographic location.

Brazilian free-tailed bats provide important pest control services at the “continental-scale” (Lee and McCracken 2005; Cleveland et al. 2006; Federico et al. 2008). In south-central Texas, Brazilian free-tailed bats consume an estimated 9×10^5 kg of insects nightly (Lee and McCracken 2005). These bat populations overwinter in Mexico, where noctuid moths are also important components of their diets (López and Medellín 2007). The year-long consumption of moths by bats in Mexico and Texas reduces the number of emigrating moths that would otherwise infest crops across the central U.S. (Federico et al. 2008). Yet, no studies have investigated their foraging habits in response to ephemeral irruptions of prey at the field crop level. Furthermore, despite the rapid and widespread adoption of transgenic crops, their potential impact on the foraging activity of bats or any other vertebrate insectivore has not been explored.

This study examines the activity of bats in relation to the asynchronous emergence of moths in conventional and transgenic Bt crops in the Winter Garden region of south-central Texas (Figure 1). I hypothesized that the foraging activity of Brazilian free-tailed bats will increase over crop fields during local emergences of insect pests. I predicted that moth and bat activity will be the similar over Bt and non-Bt crops prior to local emergences. However, survival of insects from larvae to adult should be reduced in Bt fields. As a result of this reduced moth survival in Bt crops, I predicted that bat activity will be greater over non-Bt crops versus Bt crops during periods of local insect emergences. Finally, I predicted that bat activity will be greater over corn versus cotton fields because cotton matures later in the summer and is less attractive to noctuid moths that prefer corn as their host plant (Fitt 1989).

Methods

Study sites

I monitored bat and insect activity from 13 May to 11 August 2006 at four locations in Uvalde and Medina Counties in the Winter Garden agricultural region of South-central Texas (Figure 1). The croplands in these counties are mostly cultivated cotton, corn, sorghum, and wheat (USDA-NASS 2007), and are surrounded by ranches and isolated small towns. Several known Brazilian free-tailed bat roosts are located within foraging distance of the farm sites, including Frio Cave, Ney Cave, and Seco Creek bridge (Figure 1). Although historical estimates of colony sizes are much larger, current census results indicate that about 1,000,000 bats roost at Frio Cave and 400,000 bats roost at Ney Cave (Betke et al. 2008). Colony size at Seco Creek Bridge during the summer of 2006 was estimated to be ~250,000 bats (T. H. Kunz, personal communication). I estimated the linear distances between farms and roost sites using GPS coordinates obtained at each site.

Uvalde County receives an average of 5 cm of precipitation each year (NOAA 2007) summer, the average low temperature is 21.4 °C and the average high temperature is 35.7 °C. The rest of south-central Texas, including Medina County, experiences similar climatic conditions.

In early summer, I monitored bat and insect activity at two sites, both containing fields of Bt and non-Bt corn. In mid to late summer, I monitored at two other sites, both containing fields of Bt and non-Bt cotton (Table 1). Crop types in each site were verified by sampling plant tissue from every ten rows and testing for the presence or absence of transgenic Bt proteins using the QuickStix Kit for Cry1Ab/Cry1Ac Leaf and Seed (EnviroLogix, Inc., Portland, Maine, USA). All fields were subject to standard agronomic management practices, and Bt and non-Bt fields were treated equally in this respect. Corn crops did not receive

pesticide applications prior to or during the course of my sampling, but were treated with herbicides at planting. Cotton fields were treated with ultra low volume (ULV) Malathion (12 oz/ac) (Fyfanon®, 96.5% AI) (Cheminova Inc., Wayne, NJ), an organophosphate pesticide used to combat populations of boll weevil (*Anthonomus grandis* (Coleoptera: Curculionidae)) when pest infestation reached economic thresholds based on scouting data. This occurred at Uvalde South on 24 July and 4 August, and at Uvalde West on 26 July and 8 August. Cotton fields were also treated with Orthene, a foliar organophosphate pesticide used to control thrips (typically *Frankliniella occidentalis*, *Frankliniella fusca*, and *Thrips tabaci*). Both Malathion and Orthene are broad-spectrum insecticides, possibly affecting Lepidopteron species, and were applied equally to both Bt and non-Bt cotton fields at the monitored sites.

Bat activity

To survey relative bat activity over corn, I used broadband AnaBat II ultrasonic bat detectors (Titley Electronics, Ballina, New South Wales, Australia) to record the echolocation calls of free-flying bats. Ultrasonic detectors transform the echolocation signals of bats to audible frequencies (Fenton 1988), and are widely used to study habitat use by bats (Barclay 1984; Humes et al. 1999; Krusic et al. 1996; Vaughn et al. 1997; Walsh and Harris 1996; Lee and McCracken 2002; Wickramasinghe et al. 2003). The Anabat II bat detector system is a durable broadband detector that is coupled with a “zero-crossings analysis interface module” (ZCAIM) that transforms echolocation calls into digital data that are stored on a compact flash card for later analysis. The Anabat II bat detector system can be programmed for automated operation, allowing for simultaneous sampling by multiple detectors, thereby increasing comparability among sites (Hayes 2000). Although previous studies show that spatial variation within sites may be considerable (Britzke 2003), few studies of habitat use by bats involve the use of more than one or two detectors at a site. When monitoring

activity over corn, I used 12 detectors recording simultaneously, with six in each crop type (Bt and non-Bt) on each night of sampling. Two detectors malfunctioned during the monitoring over cotton, so ten detectors were divided equally among Bt and non-Bt fields at a site. Activity was recorded from 2100 h until 2300 h for 2-3 consecutive nights at each site before rotating to the alternate site.

At each monitoring station, a high-frequency microphone attached to the Anabat II detector was oriented NE to face away from the typical direction of prevailing weather. Microphones were housed in a waterproof PVC shroud with a 45° reflector (Messina 2004), mounted one meter above the crop canopy (Figure 2). Sampling stations were fixed within fields (Figure 3), and assignment of individual detectors to stations was randomized. Detectors were tuned at a sensitivity of “7” and frequency division ratio of 16. The sensitivity setting and detection range were estimated using an omnidirectional ultrasonic speaker to broadcast previously recorded echolocation calls of Brazilian free-tailed bats at 100 dB 10 cm from the microphone (Avisoft 60401, Avisoft Bioacoustics, Berlin, Germany). At this signal intensity, the detectors had an average detection range of 16.0 ± 1.8 m with the speaker placed at the same height as the microphone. Observations in the field suggest a somewhat larger detection range for signals broadcast from bats flying at higher altitudes. In the field, sampling locations were a minimum of 50 m apart to avoid overlap in sampling of the same calls.

Recorded files were analyzed using the software program AnaLookW (version 3.3q, Titley Electronics) and applying a custom-designed filter to detect signals that are characteristic of the echolocation calls of Brazilian free-tailed bats. Typically, bat activity is measured as numbers of bat “passes” defined as two or more pulses recorded as a bat flies through the airspace sampled by the detector’s microphone (Fenton 1970). However, very high levels of bat activity, such as those encountered in this study, preclude the use of this index because multiple bats echolocating in the same airspace often produce nearly continuous

and overlapping call sequences. Therefore, bat activity was quantified as 1) the number of files containing calls per two hour sampling period, and 2) the numbers of calls per two hour sampling period. These two indices of bat activity have been employed in previous studies using AnaBats (Britzke et al. 1999; Tibbels and Kurta 2003). In the AnaBat system, “files” are saved automatically when five seconds passed without detecting a signal or after fifteen seconds of continuous recording. The use of both files and calls as relative measures of bat activity is warranted because files vary in the number of calls they contain. Nightly activity patterns are presented as the mean number of files and calls across multiple detectors in a field to provide a robust measurement of bat activity during the two-hour sampling period. By using the mean activity levels, I avoid issues of pseudoreplication that could arise from using observations from multiple detectors located in the same fields.

In addition to the use of AnaBats, I also used a single Pettersson D230 detector (Pettersson Elektronik AB, Uppsala, Sweden) set on heterodyne mode to record echolocation calls over each Bt and non-Bt field. When in pursuit and capture of insects, bats emit terminal phase known as “feeding buzzes” that have lower amplitude, shorter call duration, and higher call repetition rates (Griffin 1958). Narrowband Pettersson heterodyne detectors are more sensitive than frequency division recorders, and thus are better suited than AnaBats for detecting feeding buzzes (G. F. McCracken, personal communication). Pettersson detectors were set to monitor a frequency of 25 kHz, which is typical of the peak echolocation call frequency of Brazilian free-tailed bats (Gillam and McCracken 2007). The Pettersson detectors were mounted on tripods and signals were recorded through the audio input of digital video cameras that provided simultaneous video recordings of the activity of moths and bats. I reviewed alternating ten minute periods within each 2100-2300 h sampling period for feeding buzzes and used the number of feeding buzzes detected during this two hour period to characterize the foraging activity per night over a given field.

As in all acoustic monitoring studies, bat activity quantified as files, calls, feeding buzzes, and bat passes are relative indices of activity and not estimates of numbers of bats in a given area (Thomas and LaVal 1988).

I did not sample on rainy nights because rainfall both decreases bat activity (Erkert 1982) and interferes with ultrasonic detection systems. Additionally, sites were not sampled when central pivot irrigation systems that might damage equipment were operating. I also suspended sampling in cotton for three days following the application of ULV Malathion.

Insect activity

Recognizing that all insect sampling methods are biased with regard to species, size, or flight patterns of different insects (Kunz 1988; Whitaker 1994), insect activity was measured in three ways: pheromone trapping, insect scouting, and video imaging.

Pheromone-baited traps

The abundances of adult male moths of four species (corn earworm-cotton bollworms, fall armyworms, beet armyworms, and tobacco budworms) was monitored at each farm using species-specific pheromone traps. All four species are important agricultural pests and are important prey of Brazilian free-tailed bats (Lee and McCracken 2005). Pheromone traps were emptied at least bi-weekly, and daily when possible. The abundance of each species was estimated as the average daily number of moths captured.

Although pheromone traps provide information on the abundances of moths, the origin of these moths is unknown, because pheromone traps may attract moths over an area of approximately 20 ha (or 0.2 km²) (Schneider 1999). Thus, differences in moth abundance in the Bt and non-Bt fields sampled in this study cannot be determined from pheromone trap data. Because pheromone traps attract males looking for mates in the local vicinity, they also may

underestimate insect abundance for populations emerging and dispersing long distances (Raulston et al. 1990).

Insect scouting to determine emergence dates

Bt and non-Bt fields were surveyed bi-weekly by professional crop consultants for eggs and larvae of corn earworms and tobacco budworms, which are indistinguishable prior to the adult stage. Dates of emergence of adult moths were estimated by determining a “biofix” date associated with the peak occurrence of large larvae for each site. Degree-day accumulations were then used to adjust the biofix date to estimate the timing of subsequent peaks in adult moth abundance. Cumulative heat units were calculated from hourly air temperature values measured by thermocrons at each site. Cumulative heat units were defined as the temperature minus a lower threshold of 12.6 °C, the temperature below which insect development terminates (Harstack et al. 1976). An upper threshold was set at 33.3 °C, the temperature above which further increases in rates of insect development do not occur (Harstack et al. 1976). Because insect scouting was not performed for the cotton fields at Uvalde West, emergence dates could not be estimated for this site.

Video imaging

Moth activity within Bt and non-Bt fields was surveyed concurrently with ultrasonic monitoring for bats. To record moths over crops, Sony DCR-TRV11 digital video cameras set within fields of Bt and non-Bt crops were mounted at a height of 1.5 m in corn, or 1 m in cotton, and aimed upward to film activity directly above the crop canopy. Cameras were focused on a test pattern target resulting in an approximately 1.3 x 1.7-m viewing area centered at 2.5 m above the camera. Cameras were operated in NightShot mode at a shutter speed of 1/30 seconds. Long-play mode was used to extend the tape recording time to two hours. The camera’s field of view was illuminated using two infrared (IR)/ red LED strobe lights placed 2 m to either side of each camera and pointed to intersect at 2.5 m above the camera. The LED strobe lights were powered by a

12 VDC battery and pulsed by a controller circuit for 6 ms at a rate of 30 Hz to enhance the illumination of targets. Although most insects do not have red photoreceptors, this trait has evolved multiple times in Lepidopterans (Briscoe and Chittka 2001). However, the attraction of moths to IR radiation has been questioned (Hsiao 1972). The IR lights were strobed to reduce the likelihood of affecting moth behavior, and it was assumed that any effects of IR lights were equally distributed across treatments and through time.

Films were reviewed using Windows Media Player at 70% contrast to aid in moth image detection (Microsoft Corp., Redmond, WA, USA). Moths flying into the field of view were counted over alternating 10 minute sampling periods to obtain a total of one hour of data from each two hour recording. Video imaging provides information on the relative flight activity of moths in Bt and non-Bt fields, but does not allow for identification of moths to species.

Statistical analysis

I performed analyses of variance using mixed models in SAS v. 9.1 (PROC MIXED, SAS Institute, Cary, NC). I used log- or square-root transformations when necessary to improve normality and homoscedasticity. When I obtained significant results for factors with more than two levels, I used Tukey's pairwise comparison tests (or Tukey-Kramer for unequal sample sizes) to determine which levels were significantly different. Means $\pm$ 1 SE are presented, unless otherwise indicated. I developed general models (using data from multiple sites) and site specific models as described below.

General models

To determine if crop type (corn versus cotton) had a significant effect on bat activity, I tested for crop, treatment (Bt versus non-Bt), and their interaction term as fixed effects with site nested within crop as a random effect, and individual nights as repeated measures. Because crop was not significant in

explaining bat activity in these models, this factor was ignored in subsequent models.

Using data from the two corn sites and one cotton site for which insect emergence data were available, I then categorized nightly bat activity as occurring “pre-” or “post-emergence” relative to the estimated onset of insect emergence within each field. I calculated overall mean activity across nights in both periods for each site. I analyzed mean bat activity in the pre- and post-insect emergence periods using an ANCOVA model with fixed treatment (Bt versus non-Bt) and time (pre- versus post- insect emergence) effects, a treatment x time interaction effect, and random site and site x treatment effects. Time was treated as a repeated measure. Because Bt was predicted to affect bat activity during periods of local insect emergences, I used the time x treatment interaction term as in a Before-After Control-Impact (BACI) design (McDonald et al. 2000). I used the number of moths detected with video imaging and the distance to closest known bat roost as covariates in separate models.

I performed analyses of variance (ANOVA) on all measures of bat activity for the fixed effects of time, treatment, and a time x treatment interaction, with site as a random effect, and excluding covariates. As in the ANCOVA models, sampling nights were not used as replicates. Instead, nightly data on bat activity were averaged across the pre- and post- insect emergence periods. Because these tests are highly conservative with small sample sizes, and there was considerable variation between sites, I used a significance level of $\alpha = 0.10$ to minimize Type II errors.

Site by site analysis

For each AnaBat sampling night, I tested for differences in bat activity in number of calls over non-Bt and Bt fields for each site using AnaBat stations within fields as replicates in Wilcoxon Rank Sum tests. (PROC NPAR1WAY, SAS Institute, Cary, NC).

For each site where pheromone trap data were available, I tested for correlations between numbers of feeding buzzes and numbers of moths captured in pheromone traps. To test for a delayed response from bats, I also examined correlations based on one-, two-, and three- day time lags in bat activity relative to moth abundance. All correlations were performed using JMP Data Analysis Software (Version 7.0, SAS 2007).

A Randomized Intervention Analysis (RIA) was also used to investigate the interaction of crop type (Bt versus non-Bt) and insect emergence (pre- versus post-) on bat activity measured with numbers of calls for each site. RIA can be used to detect a non-random change in a manipulated ecosystem relative to a control reference system (Carpenter et al. 1989). This analysis is an extension of BACI analysis (Stewart-Oaten et al. 1986), but results are less affected by non-normality, heterogeneous variances, and autocorrelations in time series data (Carpenter et al. 1989). This analysis tests for a difference in a response variable in control and manipulated sites that exceeds the spatial and temporal variation occurring before the manipulation or “impact”. For this study, Bt crops are considered as the manipulated system and non-Bt as the control system, with insect emergence as the “impact” (i.e. the time during which Bt is predicted to affect bat activity). Differences between the numbers of calls recorded over non-Bt versus Bt crops were determined for each sampling night. Then, the mean differences in numbers of calls were determined for the pre- and post-emergence periods. As in Carpenter et al. (1989), the absolute value of the difference between the mean pre-emergence difference and the mean post-emergence difference is the test statistic. To estimate the probability of generating the observed test statistic, random permutations using 1000 Monte Carlo simulations were performed by shuffling the nightly differences among the pre- and post-emergence periods (Matlab R, Mathworks Inc, Massachusetts). Large values for the test statistic indicate large differences in bat activity over non-Bt compared to Bt fields during periods of local insect emergences.

Results

General bat activity

Data from AnaBat monitoring were obtained for a total of 44 nights yielding 1004 hours of recordings (Table 1). The data collected include 77,656 files containing a total of 2,574,317 echolocation calls. Concurrent video imaging and recording with Pettersson detectors occurred on 33 of these nights, producing a total of 132 hours of video containing over 14,784 moth images and recordings of 3,622 feeding buzzes. On average, per two hour recording period, I recorded 157.2 ± 94.3 AnaBat files containing echolocation calls, 5174.4 ± 93.3 calls, and 86.2 ± 59.4 feeding buzzes. There was considerable variation in bat activity within and between sites (Figure 4).

When nights were treated as independent observations, mean numbers of files were correlated with mean numbers of calls ($r = 0.8$, $P < 0.0001$, $n = 84$). The numbers of feeding buzzes were also correlated with mean numbers of files ($r = 0.7$, $P < 0.0001$, $n = 42$) and mean numbers of calls ($r = 0.7$, $P < 0.0001$, $n = 42$).

Effect of crop type (corn vs. cotton) on bat and moth activity

Twelve times as many moths were detected during filming over corn versus cotton ($P < 0.001$), but bat activity did not differ between corn and cotton (all $P > 0.1$; Table 2). Because crop type (corn vs. cotton) is not a significant predictor of bat activity in this model, this factor is ignored in subsequent models.

Effects of insect emergence, prey abundance, and location on bat activity

Using data from the three sites for which insect emergence dates could be determined, bat activity measured as files, calls, and feeding buzzes, increased after insect emergences began, ($P < 0.1$ in all cases, Table 3, Figure 5). After insect emergences began, all measures of bat activity were positively correlated with the number of moths detected during filming ($P < 0.1$, Table 4, Figure 6, a-c). Bat activity and numbers of moths were not correlated prior to local emergences ($P > 0.1$, Table 4, Figure 6, a-c). Similarly, after local insect emergences began, bat activity was inversely related to the distance to the closest known roost (files: $P = 0.109$, calls and feeding buzzes: $P < 0.1$, Table 4, Figure 6, d-f).

Effect of Bt crops on bat activity during insect emergence

ANOVA and ANCOVA models

Data from the three sites for which emergence dates could be determined show that the presence of Bt crops (treatment) and the interaction of Bt and insect emergence period (time by treatment interaction) are not significant predictors of bat activity (all $P > 0.1$, Table 3, Figure 5). Likewise, Bt is not a significant predictor of bat activity in the ANCOVA models using distance to roost or numbers of moths as covariates ($P > 0.1$, Table 4).

When the effect of Bt versus non-Bt crops was examined on a site-by-site basis, there was some evidence of reduced bat activity over Bt crops versus non-Bt crops during periods of local insect emergences from corn at Uvalde North and cotton at Uvalde South. However, the available data suggest that Bt crops did not affect bat activity at the other two sites.

Wilcoxon Rank Sum tests

More calls were recorded over non-Bt versus Bt crops on six of the eight nights of sampling after the onset of insect emergence from corn at Uvalde North ($P < 0.05$, Figure 7a) and on two of four nights of sampling after the insect emergence from cotton at Uvalde South ($P < 0.05$, Figure 7c). More calls were recorded over Bt corn versus non-Bt corn on three sampling nights at Uvalde North, but only prior to the estimated insect emergence. At Hondo East and Uvalde West, there were no significant differences between number of calls recorded over Bt versus non-Bt crops on any of the sampling nights (all $P > 0.05$, Figures 7b and 7d).

Randomized Intervention Analysis

RIA showed that during the local insect emergence period, bat activity was significantly higher over non-Bt crops compared to Bt crops at Uvalde North ($P < 0.0001$), but not at Hondo East or Uvalde South ($P > 0.05$, Figure 8). At Uvalde South, there was a trend toward higher bat activity over non-Bt compared to Bt crops during the insect emergence period, but this trend was not significant. This analysis could not be performed for Uvalde West because information on emergence period was lacking.

Foraging activity vs. pheromone trap captures of moths

At all three sites where pheromone trap data were available, the numbers of feeding buzzes were positively correlated with daily captures of corn earworm moths using a one, two, or three day lag in bat activity relative to moth abundance (all $P \leq 0.05$, Table 5). The number of feeding buzzes was also correlated with the combined totals of all four insect species that were monitored when using the same time delays (all $P < 0.05$, Table 5). Numbers of feeding buzzes were not correlated with insect pheromone trap data in the absence of a time lag (Table 5).

Moth activity: emergence, video imaging, and pheromone traps

Each method of insect sampling provides different information on insect activity. Video imaging provides information on relative moth activity over Bt and non-Bt fields; whereas pheromone trap data provide information on the abundances of insect by species, but not on the origin(s) of captured moths.

Twice as many moths were detected during filming over non-Bt crops versus Bt crops at Uvalde North (Figure 10), where bat activity was also significantly higher over non-Bt crops versus Bt crops (Figure 8). At this site, peaks in moth activity from filming corresponded to peaks shown in pheromone trap captures and coincided with the estimated date of insect emergence. The numbers of moths detected during filming were less influenced by Bt crops at the other sites where bat activity was also similar between the crop types. However, at Uvalde South, video recording did not occur during the major peak of corn earworm captures in pheromone traps (Figure 11). There were twice as many moths filmed over Bt versus non-Bt on 1 Aug at this site. Although video imaging does not allow identification of moths to species, this observation of more moths over Bt versus non-Bt corresponds to a peak in pheromone trap captures of fall armyworm moths. The number of moths detected during filming was similar between non-Bt and Bt fields at both Hondo East (Figure 12) and Uvalde West (Figure 13). Large numbers of moths were detected with filming at Hondo East prior to the estimated insect emergence at this site, whereas the emergence date could not be estimated at Uvalde West.

Discussion

The levels of bat activity reported here are higher than levels observed in other systems (e.g. Crampton and Barclay 1996; Patriquin and Barclay 2003), with up to 231 feeding buzzes per two hour sampling period, or close to two buzzes per minute. It is likely that feeding buzzes are underestimated due to the difficulty of discerning buzzes amid the call sequences from multiple bats during periods of very high bat activity. The call sequences recorded in this study are presumed to be from Brazilian free-tailed bats. Hoary bats (*Lasiurus cinereus*) echolocate at similar frequencies as Brazilian free-tailed bats, and their calls could be confused using heterodyne or frequency division bat detectors. However, hoary bats are relatively rare in south-central Texas during the summer, compared to the highly abundant Brazilian free-tailed bat.

The central hypothesis in this study is that moth activity drives bat activity. Bat activity was higher over agricultural fields during periods of insect emergence. Bt crops affected bat activity at sites where moth activity was reduced over Bt crops compared to non-Bt crops. Contrary to the observed reduction in moth abundance in cotton fields, we observed similar levels of bat activity over corn and cotton. Other factors were found to influence bat activity, including the location of foraging sites and time-delayed responses to prey abundance which may be related to group foraging behavior in Brazilian free-tailed bats.

Response of bats to insect emergences

The hypothesis that insect abundance drives bat activity is supported by the significant increase in all measures of bat activity over agricultural fields during periods of insect emergence (Figure 5). These results complement previous work showing that moth consumption by Brazilian free-tailed bats

increased in mid-summer, corresponding to the mass emergences of noctuid moths from corn (Whitaker et al. 1996; Lee and McCracken 2005). Brazilian free-tailed bats are known to exploit other localized concentrations of insects around lights in urban areas (Lee and McCracken 2002), and experimentally placed UV lights in desert habitats (Bell 1980). Opportunistic feeding behavior has also been documented for other bat species that take advantage of temporarily available swarming insects (Fenton and Morris 1976; Fukui et al. 2006).

Effects of local insect emergences: Do Bt crops matter?

The potential impact of transgenic Bt crops on the foraging activity of bats has not been explored previously. Current evidence supports the assumption that moths do not distinguish between Bt and non-Bt plants when laying eggs (Jackson et al. 2003), which is required for Bt technology to be effective in suppressing insect populations. For example, Torres and Ruberson (2006) compared corn earworm egg densities between Bt and conventional cotton over several growing seasons and concluded that oviposition behavior has not been affected by Bt despite a decade of widespread planting of Bt varieties. Although they found more eggs on Bt plants during the second oviposition peak of one year, insecticide applications may have confounded these results. Adult females emerging later in the season may prefer to lay eggs on Bt plants if Bt plants are less damaged, and thus more attractive than non-Bt plants. Furthermore, other insect species, including tobacco budworms, may be attracted to higher concentrations of secondary volatile compounds that are produced in Bt plants, but more work is needed to address this (Ramaswamy et al. 1987). Oviposition behavior of noctuid moths on Bt and non-Bt crops was not a focus of this study, but is important to consider with regards to maintaining the efficacy of Bt technology, and my expectations regarding the impact of Bt on insect production and the foraging activity of bats.

Despite the expectation that bat activity would be reduced during periods of insect emergence over Bt crops compared to non-Bt crops, the results of this study suggest that, in general, the presence of transgenic Bt crops did not affect bat activity during insect emergences (Figure 5). Unfortunately, the small number of sites and the considerable variation in bat activity I observed among sites limit the inferential power from statistical tests.

Of the three study sites where data on insect emergence were available, Bt crops account for variation in moth and bat activity at one site, Uvalde North, where moth and bat activity were reduced over Bt corn versus conventional corn during the insect emergence (Figures 8a and 11). However, the conventional corn at this site was sweet corn, whereas the Bt variety was field corn. Sweet corn produces more carbohydrates than field corn, which may differentially attract female moths selecting plants for oviposition, and consequently impact insect emergence. Whatever factors are responsible for increasing prey density at this site over non-Bt corn, these results support the hypothesis that moth abundance drives bat activity. Video recordings were not made during much of the emergence period at Uvalde South, making it difficult to adequately test for a Bt effect. Still, there appears to be a trend toward more bat activity over non-Bt versus Bt cotton at this site as well, where the plant varieties differed only by the presence or absence of Bt expression (Figure 8c).

At the other sites, the number of moths detected during filming was similar between Bt and non-Bt fields (Figures 13 and 14). Thus, similar bat activity over Bt and non-Bt crops at these sites is not surprising. Several factors may explain the observation of similar numbers of moths detected over Bt and non-Bt fields at these sites. First, noctuid moths disperse considerable distances. During migration, noctuids can move several hundred kilometers (Westbrook et al. 1995), and during local dispersal, when habitat selection may be important, they often move tens of kilometers (Schneider 1999). The distance between Bt and non-Bt fields for the sites in this study ranged from a few meters to several

hundred meters (Figure 2). The distances between fields were determined by the growers without regards to this study, and were beyond our control. As a result, moths reared on nearby non-Bt plants may be detected flying over fields of Bt plants compared in this study. The distances between Bt and non-Bt fields seems to be related to the Bt effect measured by RIA analysis (where the site with the largest test statistic had the greatest separation between Bt and non-Bt fields). This hypothesis cannot be adequately tested based on the current sampling effort. At Uvalde North, where the activity of moths and bats was significantly reduced over Bt corn during the insect emergence, the Bt and non-Bt fields were separated by approximately 400 m. Furthermore, the bat detectors in Bt and non-Bt fields at this site were separated by a minimum of 800 m, by far the greatest separation of crop types of any site in this study. At all other sites, Bt and non-Bt crops were planted in adjoining fields. Weak evidence for a Bt effect at Uvalde South also was evident, where detectors in Bt and non-Bt fields had the next greatest separation of a minimum distance of 220 m. At Hondo East, where there was no measurable Bt effect, detectors in Bt and non-Bt fields were separated by only 170 m. At Uvalde West, emergence data are unavailable. Moth and bat activity were similar between Bt and non-Bt fields on all sampling nights at this site.

In addition to the potential dispersal of moths between nearby fields, other factors may be important for explaining the observation of similar numbers of moths in Bt and non-Bt fields. This study was conducted during the warmest summer on record for Texas and precipitation was below normal (NOAA 2006). The harsh environmental conditions may have reduced survivorship of larvae in both Bt and non-Bt crops (J.K. Westbrook, personal communication). Potential increases in mortality due to climatic conditions could make the differences in larval densities in Bt and non-Bt crops less pronounced.

Because the video recordings in this study do not permit the identification of moths to species, many of the moths detected over Bt crops may have been

non-target organisms of Bt toxins or organisms that are less susceptible to these toxins, such as fall armyworms (Chilcutt et al. 2007). This is consistent with the observation of more moths flying over Bt crops than non-Bt crops at Uvalde South on 1 August, which coincides with an increase in pheromone trap captures of fall armyworm moths at this site (Figure 12). Alternatively, moths detected flying over Bt crops may have survived on those plants, indicating the presence of resistant individuals of target species. With the available video data, I cannot distinguish between these two alternatives. The evolution of resistance to Bt toxins has been documented in field and laboratory populations of target species, including corn earworms (Tabashnik et al. 2008). Also, the presence of DNA fragments and associated endotoxins from Bt transgenes have been documented in fecal samples collected from Brazilian free-tailed bats in the Winter Garden region (Hallam et al., unpublished). The presence of moths and foraging bats over Bt fields in this study is consistent with the possibility that Bt crops produce some moths that are eaten by bats. As Bt acreage increases and new varieties of Bt genes are discovered and implemented in agroecosystems, more work will be necessary to address their potential effects on foraging bats.

Bat activity over corn and cotton

I detected twice as many moths flying over corn in early summer than cotton in late summer, which is consistent with typical patterns of insect abundance in this region (J. Westbrook, personal communication). However, bat activity was as great over cotton in late summer as over corn earlier in the summer (Table 2). This is unexpected based on previous results from dietary analysis, which show a decline in moth consumption from both morning and evening fecal samples collected in mid- to late-July (Lee and McCracken 2005). One explanation for the presence of high levels of bat activity over both corn and cotton involves the distance between foraging and roosting sites. The cotton fields sampled in this study were very close to the large colony of Brazilian free-

tailed bats at Frio Cave (Figure 1). In contrast, one of the corn fields, Hondo East, showed the lowest levels of bat activity of all the sites, and was substantially farther away from any known bat colonies of comparable size in south-central Texas. Additionally, there is also evidence that other bat colonies are located close to the cotton fields at Uvalde West, which had the highest levels of bat activity among those sampled in this study. Anecdotal reports suggest that bats are using abandoned mines just 25 km west of this site. Observations from the National Weather Service's Doppler radar system (NEXRAD) are consistent with these reports, revealing plumes of bats emerging close to this site (NCDC 2008). The life history of these bats may also be important in explaining seasonal variation in activity. Pups are sufficiently mature to begin their nightly foraging bouts in mid-late July, which dramatically increases the number of foraging bats in the region (McCracken and Gustin 1991). Also, bats may increase their foraging rates during the late summer to prepare for fall migration. Just prior to migration, bats may forage disproportionately over cotton fields despite relatively low prey availability because of the high lipid content of migratory moths (Angelo and Slansky 1984). Finally, weather can be increasingly hot and dry in mid-late summer and irrigated crops, even of Bt varieties, may appear as “oases” to both insects and foraging bats.

Group foraging in Brazilian free-tailed bats

In addition to the emergence of prey and the presence of Bt crops, other factors help explain variation in the foraging activity of Brazilian free-tailed bats. Bat activity was higher at sites closer to roosts and increased with increasing numbers of moths, but only during periods following insect emergences (Figure 6). Correlations of bat activity with distance to the nearest bat roost are not present prior to mass emergences of insects. This suggests that the distance from the roost as well as patch quality may influence where bats forage, and that

bats forage at particular sites where there is a minimum threshold of insect abundance that occurs during mass emergence periods.

The results from pheromone trap collections from this study are consistent with previous work showing that moths in agricultural systems occur in rich, but ephemeral and localized patches (Raulston et al. 1990). Although the region as a whole produces an abundance of insects during the growing season, no single site provides a continuous source of prey. The unpredictable nature of pest population dynamics may present a challenge to insectivores in this region.

Several studies suggest that highly colonial animals that may benefit from information shared at common assembly sites, which may serve as “information centers” (Ward and Zahavi 1973; Brown 1986). Colonial animals may enhance foraging efficiency by exchanging information at the roost regarding the location of abundant, but ephemeral and patchy food sources (Ward and Zahavi 1973), such as patches of insects produced in agroecosystems. Previously unsuccessful foragers would benefit by following successful foragers to profitable foraging sites, especially when food sources are unpredictable and often located far from the assembly site. Although much field work has been conducted to test the information center hypothesis as it relates to birds’ roosts, some of the most promising evidence has come from studies of bats (Galef and Giraldeau 2001).

Among bats, the transfer of information at roosts to enhance foraging success has been demonstrated for colonies of insectivorous evening bats (*Nycticeius humeralis*) (Wilkinson 1992) and frugivorous greater spear-nosed bats (*Phyllostomus hastatus*) (Wilkinson and Boughman 1998). Brazilian free-tailed bats exhibit several features that are prerequisites for improved foraging efficiency via information transfer. Brazilian free-tailed bats form large colonies, often exceeding one million individuals, and forage over large areas. Their estimated foraging range of 400 km² (Williams et al. 1973) is likely an underestimate. Brazilian free-tailed bats have also been documented foraging over an altitudinal gradient of up to 1,200 m above the ground (McCracken et al.

2008, in press). Despite high encounter rates between conspecifics while foraging, Brazilian free-tailed bats rarely show signs of aggressive or territorial behavior (G.F. McCracken, personal communication). Though unpredictable in space and time, ephemeral food patches are rich. The existence of prey in great abundance may reduce the likelihood of competition between foraging bats (Gillam 2007). In fact, Brazilian free-tailed bats are documented to eavesdrop on the feeding buzzes of conspecifics, and this behavior has been described as an example of passive information transfer rather than parasitism (Gillam 2007). However, echolocation calls, and feeding buzzes in particular, can only be detected within 100 m (Griffin 1971), so eavesdropping is beneficial only when bats are foraging in close proximity to one another. Group foraging behavior that includes the exchange of information at both the roost and at foraging sites would allow large aggregations of bats to quickly locate high quality patches over a large area. Information transfer could occur at multiple times prior to foraging, including when bats form “circling mills” (Davis et al. 1962) prior to exodus from the roost.

I found significant correlations between the foraging activity of Brazilian free-tailed bats and the abundance of moths captured in pheromone traps at each site, but only when inserting a time-delay of 1-3 days between moth activity relative to bat activity (Table 5). This suggests that bats return to high quality patches on successive nights, after returning to the roost. Foraging activity is not correlated with the abundance of moths captured on the same night. A two-day lag in moth abundance relative to bat activity produced significant correlations for data from two sites. Only one site produced a significant correlation using a three-day lag in moth abundance. These findings are consistent with the information center hypothesis. In the agroecosystems studied here, rich patches of prey may persist for several days, so that bats successful in locating high quality patches may return on successive nights. Bats would increase their foraging efficiency by sharing information regarding the location of abundant but

localized patches of prey. Rich patches do not persist long in agricultural fields, however, so patch models suggest that consumers in such an environment might benefit from limiting the number of nights they return to the same patch (Giraldeau and Caraco 2000). This is illustrated in the declining number of significant correlations as the time delay is increased from one to two to three days. While the foraging activity of Brazilian free-tailed bats is consistent with the predictions of the information center hypothesis, observing the movements of such large numbers of bats between foraging and roosting sites covering sizeable areas presents a challenge to understanding temporal and spatial associations of foraging bats. Such information might become accessible using remote sensing techniques such as Doppler Radar, especially as dual-polarization radar becomes available in the next few years, in which resolution may be improved as a result of the use of two beams instead of one (NOAA 2008). With sufficient financial and logistic support, miniature radio-transmitters attached to individual bats could be used to determine if bats take turns leading and following to distant foraging sites when leaving roosts for their nightly foraging.

Implications for pest management and conservation of bats

The ability of large numbers of bats to exploit episodic irruptions of agricultural pests may significantly reduce crop damage, associated costs of pest control, and subsequent populations of injurious insects (Federico et al. 2008; Cleveland et al. 2006). Noctuid moths typically begin mating the night following emergence and females begin laying eggs on the night after mating (Callahan 1958). In her lifetime, a single female corn earworm can lay over 1000 eggs (Fitt 1989). The abundance of later generations of corn earworms is closely related with the timing and extent of infestation of populations reared in corn, which is the most important early season host plant (Fitt 1989). The populations of moths produced in cornfields in Texas influence agricultural production on a local and

interregional scale extending into the southern and central U.S. and possibly farther north (Harstack et al. 1982; Raulston et al. 1990; Wolf et al. 1990; Westbrook et al. 1995; Lingren et al. 1994; Matis et al. 1995). Therefore, the harvesting of insects emerging from corn may be the most economically important benefit to growers in Texas and beyond. The value of this pest control service is likely to increase with the projected increase in acreage and market value of corn for ethanol production (Westcott 2007).

Bats foraging near Bt crops may provide an additional service to growers by harvesting Bt resistant insects. Recent evidence shows that corn earworms have evolved resistance to Cry1Ac in the field, particularly in those areas with less acreage devoted to conventional crops (Tabashnik et al. 2008). Models indicate that predation can affect the rate at which pest populations become resistant (Gould et al. 1991). The consumption of resistant individuals by bats may also delay the need for new insecticides (Federico et al. 2008). The lack of widespread crop failures as a result of resistant populations of corn earworms has been attributed to the augmentation of Bt technology with chemical insecticides (Tabashnik et al. 2008). The role of natural predators, such as millions of insectivorous bats, largely has been overlooked (but see Federico et al. 2008).

The economic benefit of Brazilian free-tailed bats in south-central Texas warrants conservation efforts for declining bat populations (McCracken 1986). Compared to similar sized mammals, bats exhibit several characteristics that may place them at risk for population decline when faced with loss of habitat or resources (Soule 1986). These include long lifespans, low fecundity, and long periods of infant care (Findley 1993). Bat populations in Great Britain have declined as a result of agricultural intensification, which is characterized by the use of agrochemicals and habitat modification (Wickramasinghe et al. 2003). Ultimately, the potential reduction in prey abundance due to the large-scale adoption of transgenic Bt crops in the U.S. must be weighed against the

consequences of traditional pest management practices involving heavy application of pesticides, some of which may have reduced populations of Brazilian free-tailed bats in the past through poisoning (Clark 2001; Geluso and Altenbach 1976). Additional studies are needed to address the impacts of Bt technology on resource availability to insectivorous bats and the potential role of bats in reducing crop failures as insect evolve resistance to Bt.

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Appendix

Table 1. Site information, crop types, monitoring dates, and data collection for farm sites monitored in the Winter Garden agricultural region of south-central Texas. HE: Hondo East, UN: Uvalde North, US: Uvalde South, UW: Uvalde West.

Farm site	Crop	Variety	Bt toxin	Irrigation	Field size (ha)	Planting date	Row spacing (cm)	Begin monitoring	End Monitoring	Total nights	Total bat detector hours*
HE	Non-Bt Corn	DKC69-72	-	Pivot	16.2	10 Mar	76.2	3 Jun	6 Jul	12	144
HE	Bt Corn	DKC69-71 RR2/ YGCB	Cry1Ab	Pivot	16.2	10 Mar	76.2	3 Jun	6 Jul	12	144
UN	Non-Bt Corn	Asgrow 959	-	Furrow	40.5	10 Mar	76.2	31 May	9 Jul	16	198
UN	Bt Corn	B&H 8913	Cry1Ab	Pivot	60.7	10 Mar	76.2	31 May	9 Jul	16	198
US	Non-Bt Cotton	CT 210	-	Pivot	14.6	16 Mar	16.2	16 Jul	1 Aug	7	70
US	Bt Cotton	DP 455 BG/RR (Bollgard®)	Cry1Ac	Pivot	14.6	16 Mar	16.2	16 Jul	1 Aug	7	70
UW	Non-Bt Cotton	CT 210	-	Pivot	20.2	12 Apr	16.2	29 Jul	11 Aug	9	90
UW	Bt Cotton	PHY 372 (WideStrike®)	Cry1Ac & Cry1F	Furrow	12.1	12 Apr	16.2	29 Jul	11 Aug	9	90

*Total bat detector hours = recording hours for AnaBats x number of detectors x number of night.

Table 2. Means and statistics associated with a mixed model ANOVA for the fixed effects of crop (corn vs. cotton) and treatment (Bt vs. non-Bt) on numbers of files, calls, feeding buzzes, and moths detected with video imaging from 2100-2300 h.

	Files	Calls	Feeding Buzzes	Moths
Means^a				
Corn, Non-Bt	115.8 ± 64.7	2277.7 ± 22.6	80.1 ± 35.2	446.1 ± 3.7
Corn, Bt	113.4 ± 64.7	1850.6 ± 20.4	74.6 ± 35.2	379.4 ± 3.4
Cotton, Non-Bt	219.3 ± 65.5	4916.6 ± 34.1	105.1 ± 36.3	40.0 ± 1.3
Cotton, Bt	211.3 ± 65.5	4333.7 ± 32.0	97.6 ± 36.3	27.5 ± 1.1
Statistics^b				
Crop	2.39 _{1,4} (0.198)	1.24 _{1,4} (0.327)	0.45 _{1,4} (0.538)	75.24_{1,38} (<0.001)
Treatment	0.01 _{1,4} (0.941)	0.05 _{1,4} (0.830)	0.03 _{1,4} (0.864)	0.85 _{1,38} (0.361)
Trt x Crop	0.00 _{1,4} (0.968)	0.00 _{1,4} (0.958)	0.00 _{1,4} (0.978)	0.13 _{1,38} (0.716)

^aValues are means ± 1 SE (analyses on calls and moths were performed on log-transformed data, with back transformed means and SE presented).

^bValues are *F* statistic with probability in parentheses and degrees of freedom as subscript, for two-way ANOVA on each of four response variables, with crop (corn vs. cotton) and treatment (Bt vs. non-Bt) as fixed effects and site nested within crop as a random effect (site not shown). When the 'Trt x Crop' interaction term is removed, all non-significant fixed effects remain non-significant.

Table 3. ANOVA results for the fixed effects of treatment (Bt vs. non-Bt), time (pre- vs. post- insect emergence) and the interaction on the number of files, calls, and feeding buzzes averaged across emergence periods for each site. Time was treated as a repeated measure and site nested within treatment was a random effect. Trt: Bt vs. non-Bt; Time: pre- vs. post- insect emergence.

Factor	Files		Calls ^a		Feeding Buzzes	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Treatment	0.05	0.838	0.46	0.535	0.00	0.989
Time	55.24	0.002	5.44	0.080	20.82	0.002
Treatment x time	1.42	0.299	1.01	0.371	0.02	0.894

^aCalls were square-root transformed prior to analysis.

Notes: Values in boldface indicate *P*-values < 0.10.

For each test, degrees of freedom were 1,4.

Table 4. ANCOVA results testing relationships between bat activity and a) number of moths from video imaging and b) distance to closest known bat colony, for Bt and non-Bt crops before and after periods of local insect emergences. Trt: Bt vs. non-Bt; Time: pre- vs. post- insect emergence.

Factor	Files		Calls		Feeding buzzes	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
a)						
Trt	1.08 _{1,2}	0.405	0.01 _{1,1}	0.940	0.32 _{1,5}	0.594
Time	483.88 _{1,1}	0.029	0.10 _{1,2}	0.781	7.62 _{1,5}	0.040
Trt x Time	49.52 _{1,1}	0.090	1.25 _{1,2}	0.362	0.81 _{1,5}	0.410
Moths	89.65 _{1,1}	0.067	8.06 _{1,4}	0.045	15.65 _{1,5}	0.011
Moths x Trt	29.53 _{1,1}	0.115	0.15 _{1,2}	0.733	0.00 _{1,5}	0.990
Moths x Time	65.55 _{1,1}	0.078	21.48 _{1,2}	0.040	29.09 _{1,5}	0.003
b)						
Trt	0.04 _{1,1}	0.868	4.21 _{1,5}	0.096	0.03 _{1,5}	0.862
Time	26.00 _{1,3}	0.015	9.28 _{1,5}	0.028	28.60 _{1,5}	0.003
Trt x Time	2.89 _{1,3}	0.188	2.40 _{1,5}	0.182	0.06 _{1,5}	0.813
Dist	7.38 _{1,1}	0.225	15.94 _{1,5}	0.010	11.25 _{1,5}	0.020
Dist x Trt	0.00 _{1,1}	0.993	2.74 _{1,5}	0.159	0.03 _{1,5}	0.862
Dist x Time	5.12 _{1,3}	0.109	5.62 _{1,5}	0.064	10.13 _{1,5}	0.024

Notes: Values in boldface indicate *P*-values < 0.10.

Degrees of freedom are shown as subscripts with associated *F* statistics.

Degrees of freedom were insufficient to test for three-way interactions.

Table 5. Correlations (Pearson's r) between daily capture of moths in pheromone traps and number of feeding buzzes recorded from 2100-2300 h at each site using sampling nights as observations. All correlations are positive. CEW: Corn earworm, ALL: combined number of corn earworm, tobacco budworm, fall armyworm, and beet armyworm moths. Lag days refer to number of days prior to date of feeding buzz recording (i.e. "0" indicates concurrent bat and moth data, "1" indicates a one-day delay in bat activity relative to the number of moths captured in pheromone traps).

Correlation between feeding buzzes and daily capture of moths from pheromone traps						
Species/# lag days	UN ($n = 12$)		HE ($n = 12$)		US ($n = 7$)	
	r	P	r	P	R	P
CEW/0	0.11	0.720	0.19	0.550	0.09	0.812
CEW/1	0.60*	0.039	0.57	0.054	0.86**	0.002
CEW/2	0.04	0.901	0.58*	0.049	0.94***	<0.001
CEW/3	0.16	0.611	0.09	0.777	0.88***	<0.001
ALL/0	0.12	0.708	0.18	0.566	0.00	0.998
ALL/1	0.64*	0.025	0.58*	0.047	0.90***	<0.001
ALL/2	0.05	0.871	0.62*	0.032	0.92***	<0.001
ALL/3	0.17	0.586	0.27	0.405	0.76*	0.011

Notes: Values in boldface type indicate correlations with $P < 0.05$.

* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

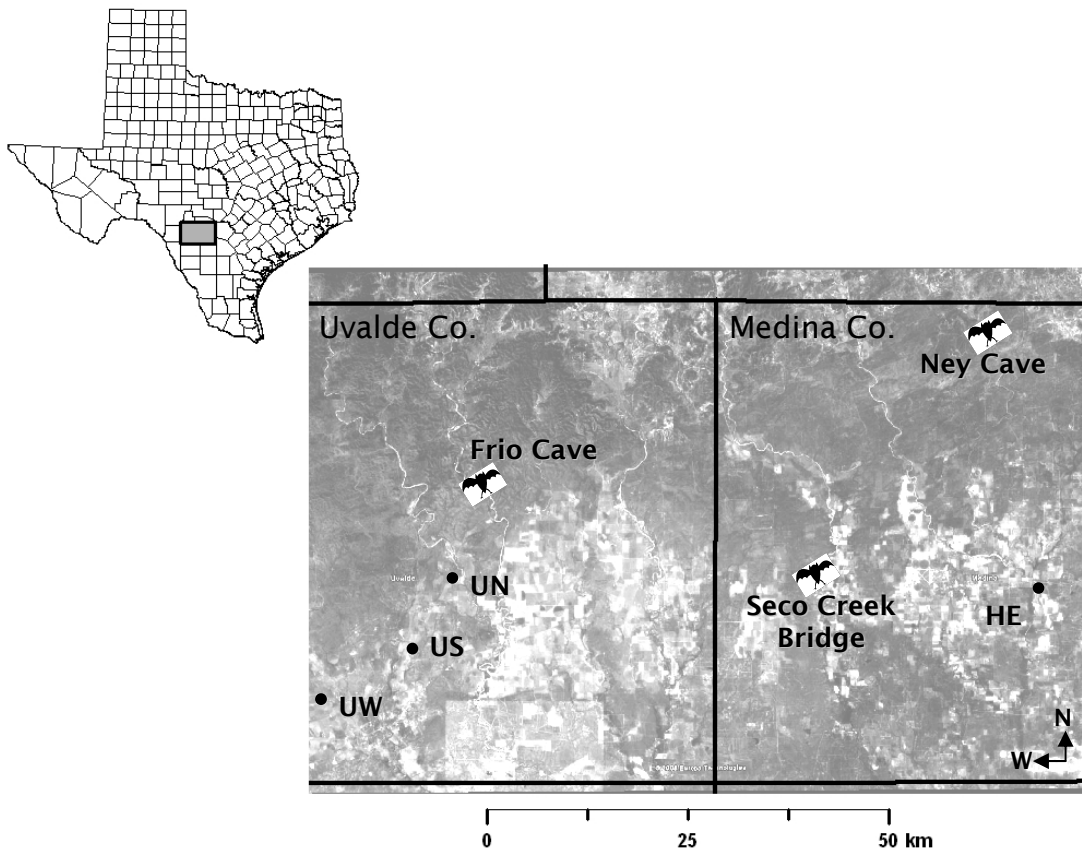


Figure 1. Field sites in Uvalde and Medina Counties, Texas. Farm sites include Bt and non-Bt corn at Uvalde North (UN) and Hondo East (HE), Bt and non-Bt cotton at Uvalde South (US), and Uvalde West (UW). Large bat colonies are located at Frio Cave, Ney Cave, and Seco Creek Bridge. Satellite imagery provided by Google Earth (<http://earth.google.com/>).

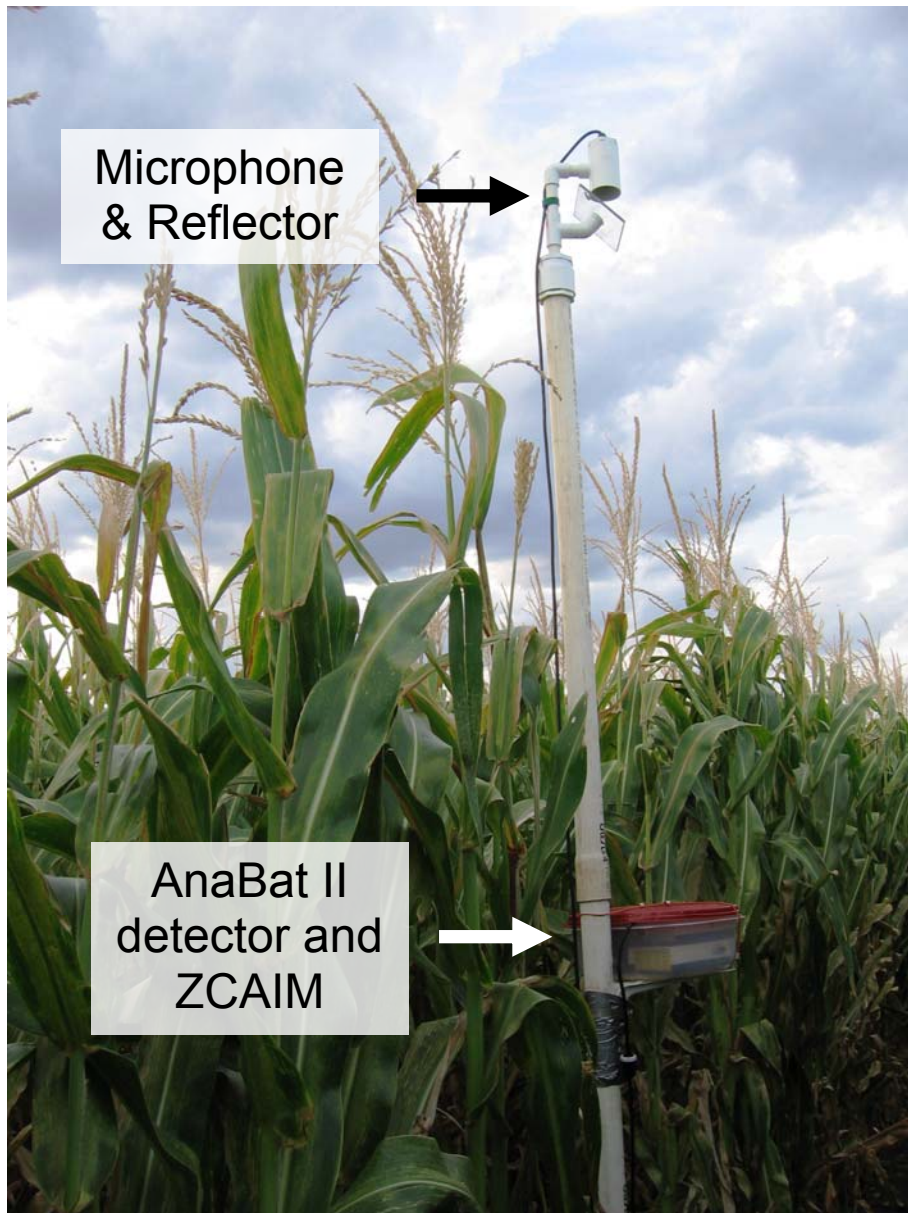


Figure 2. Configuration of AnaBat II detector, microphone, and reflector.

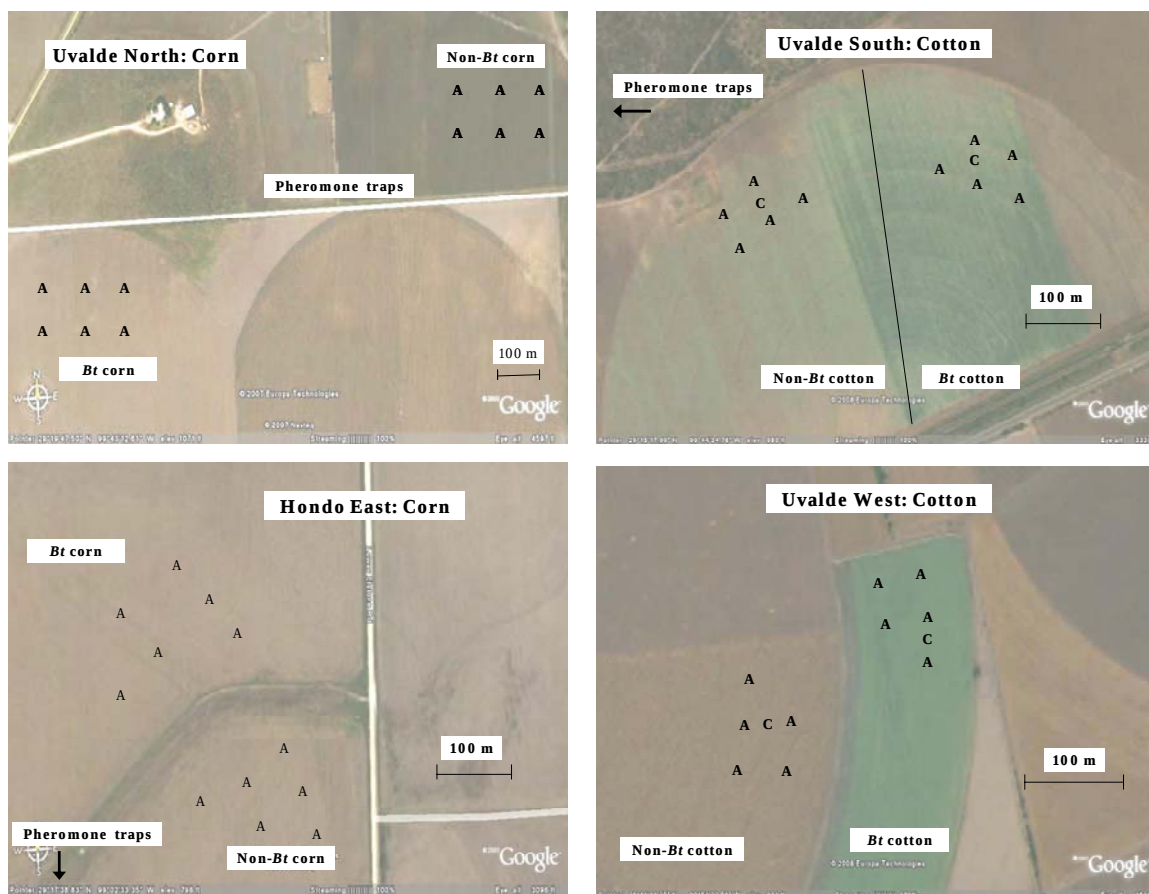


Figure 3. Anabat (A) and camera (C) deployment in corn at Uvalde North and Hondo East and cotton at Uvalde South and Uvalde West. All satellite imagery accessed from Google Earth (<http://earth.google.com/>). Satellite images are not from the period of the field study.

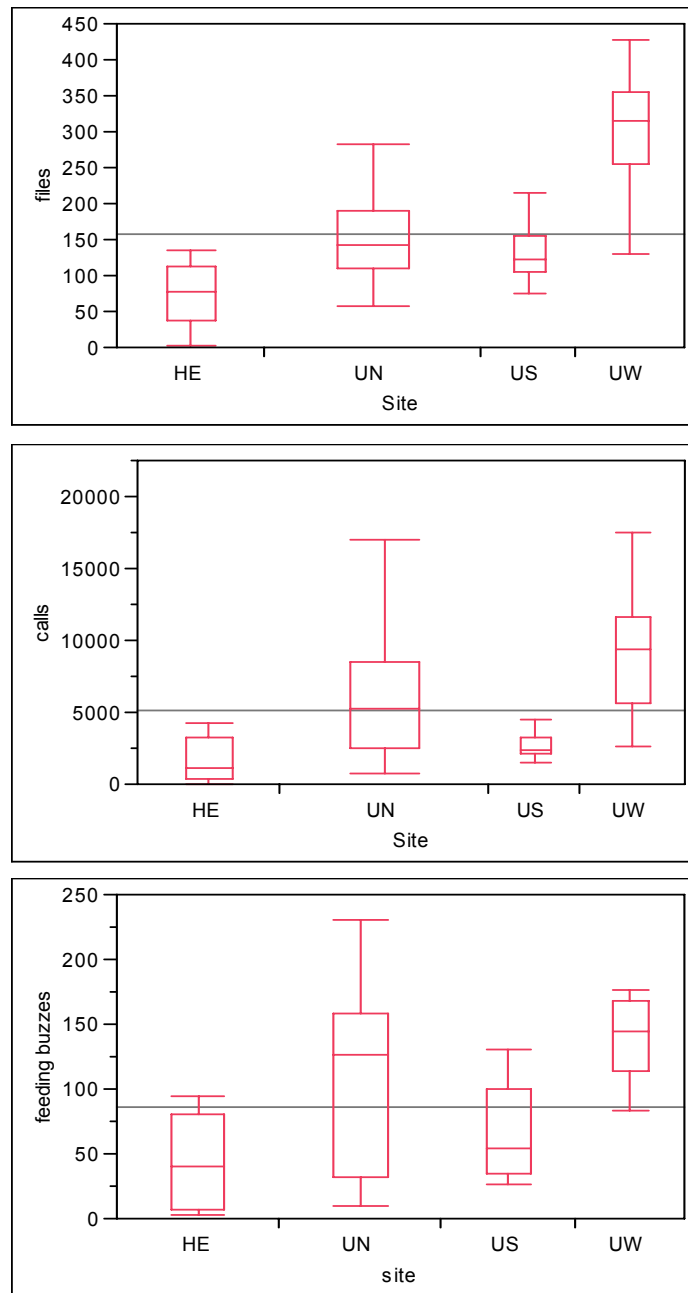


Figure 4. Boxplots showing variation in bat activity among sites, measured as numbers of files, calls, and feeding buzzes. Boxes show the middle 50% of the data and whiskers show the range of values for each site. Median activity for each site is shown as the line within each box, while the solid gray lines indicate the overall mean across sites for each variable.

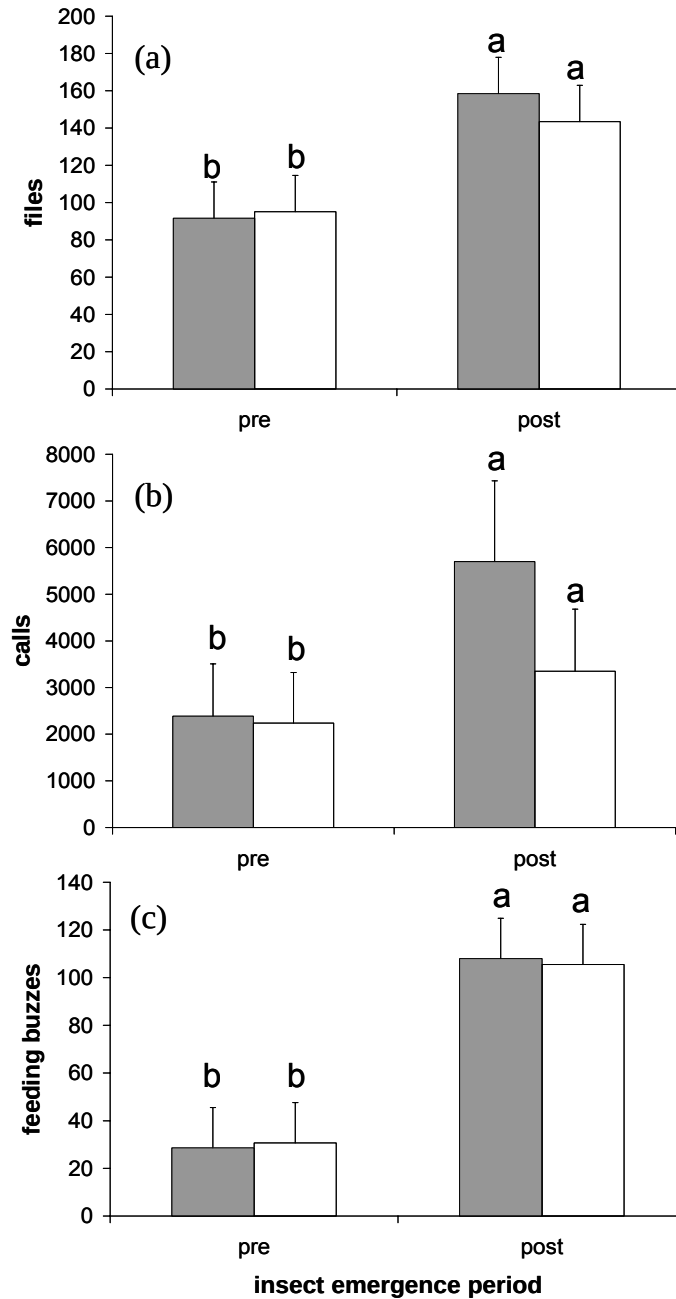


Figure 5. Effects of treatment (Bt vs. non-Bt) and time (pre- vs. post- insect emergence) on bat activity using a mixed model ANOVA (for statistics see, Table 3). Bat activity is shown as mean numbers of a) files, b) calls, and c) feeding buzzes recorded from 2100-2300 h. Bars represent mean ± 1 SE. Gray bars represent activity over non-Bt crops; white bars represent activity over Bt crops. Different letters indicate significant differences in mean bat activity. Calls were square-root transformed for analysis; back transformed means are presented.

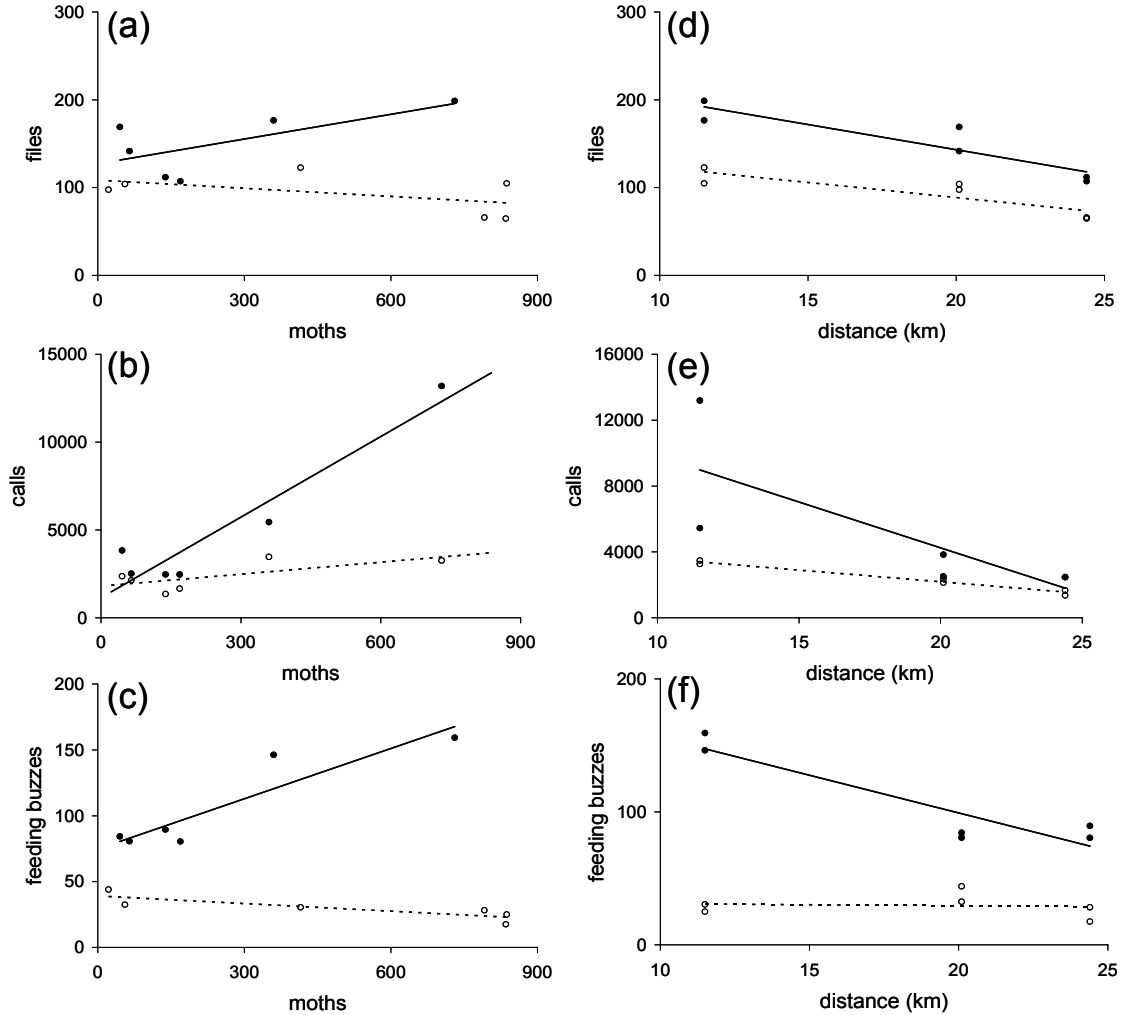


Figure 6. Relationships between bat activity and number of moths detected with video imaging and distance to closest known roost prior to insect emergence (---) and post insect emergence (—). Bat activity is shown as numbers of files, calls, and feeding buzzes recorded from 2100- 2300 h, aggregated across treatment combinations for each site (see text). Each index of bat activity is positively correlated with numbers of moths detected with video imaging (a-c) and negatively correlated with the distance to roost (d-f), but only after the onset of insect emergence.

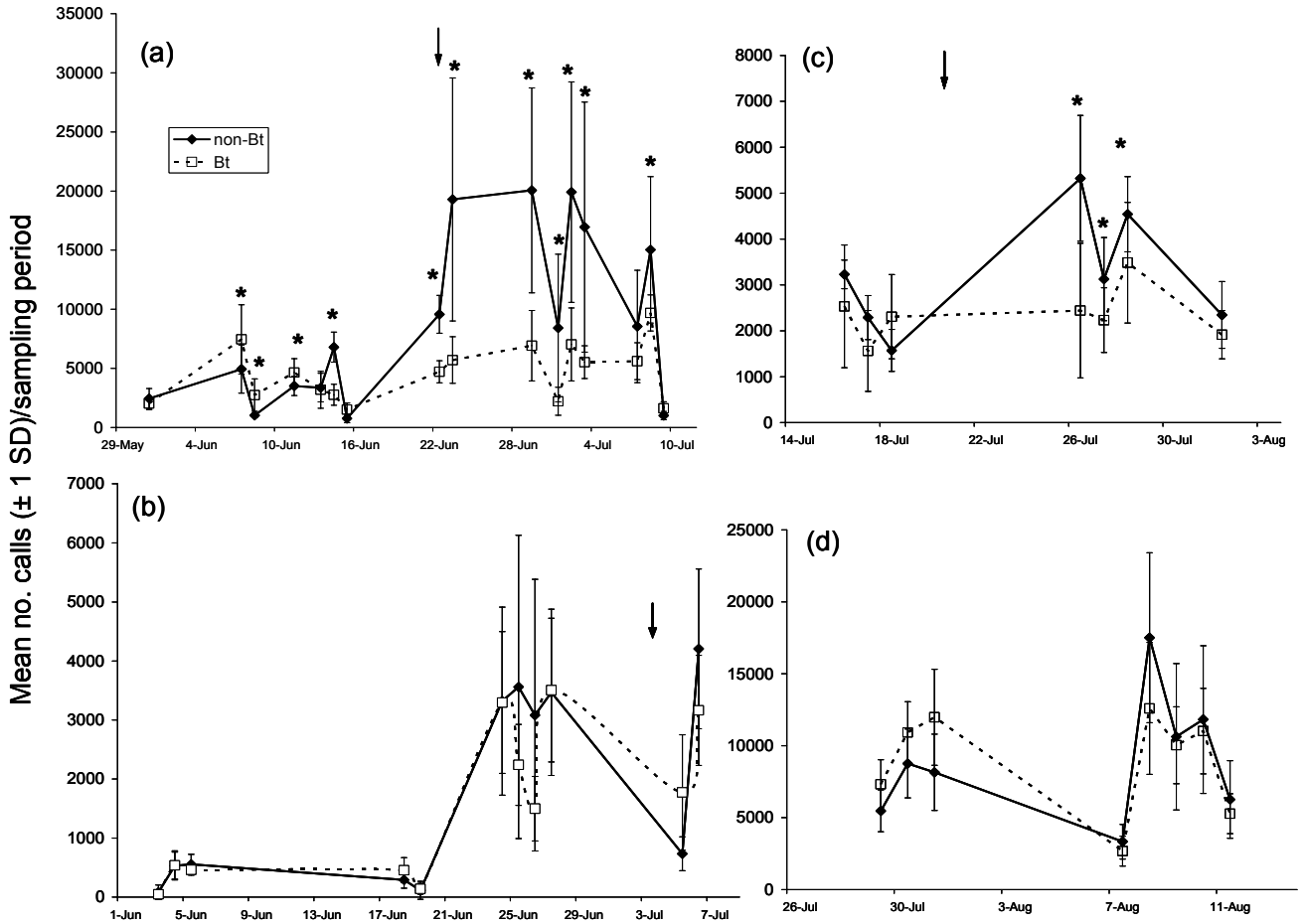


Figure 7. Bat activity measured by number of calls (mean $\pm$ 1 SD) recorded from 2100-2300 h over corn at a) Uvalde North and b) Hondo East, and cotton at c) Uvalde South and d) Uvalde West. Arrow indicates estimated peak of adult insect emergence. * Indicates a significant difference between the number of calls recorded over Bt vs. non-Bt (Wilcoxon Rank Sum, $P < 0.05$). Note that both the x and y scales differ between sites.

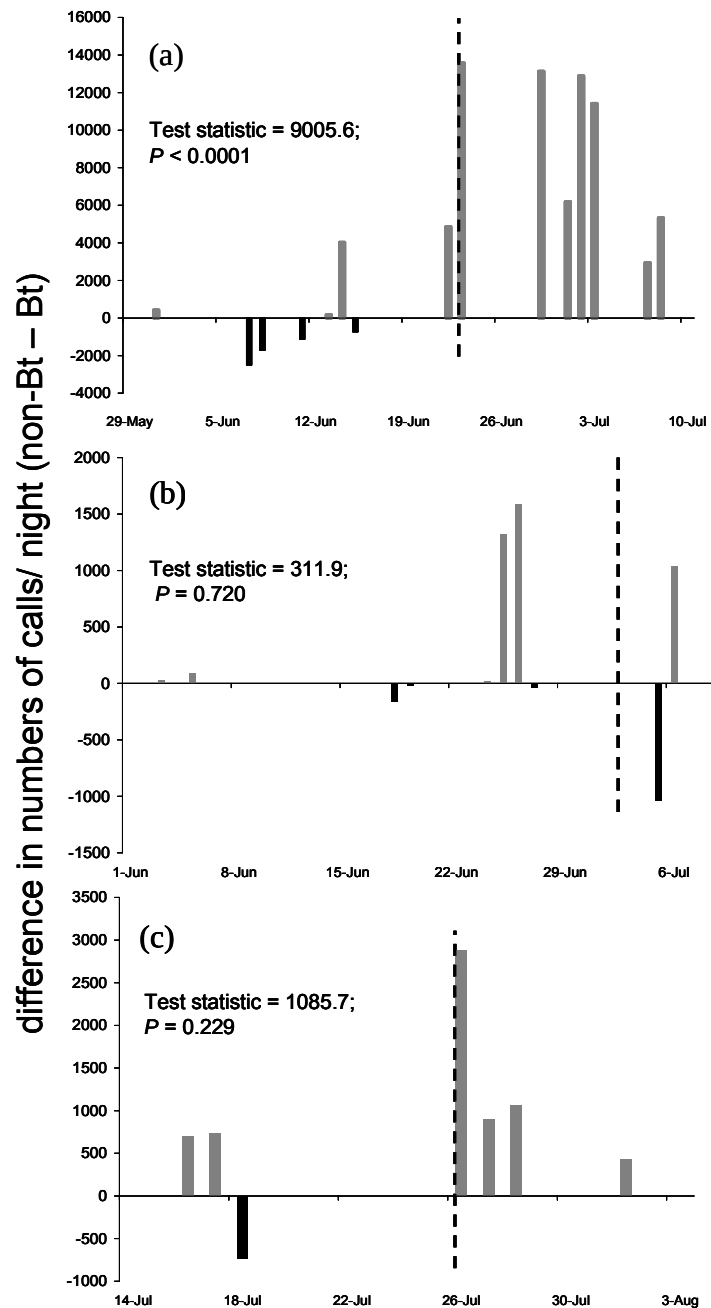


Figure 8. Nightly differences in numbers of calls recorded over Bt and non-Bt corn at a) Uvalde North and b) Hondo East, and cotton at c) Uvalde South. Black bars indicate more calls recorded over Bt than non-Bt; gray bars indicate more calls recorded over non-Bt than Bt. Dashed lines indicate estimated onset of insect emergence at each site. Test statistics and associated probability values from RIA analyses are shown (see text).

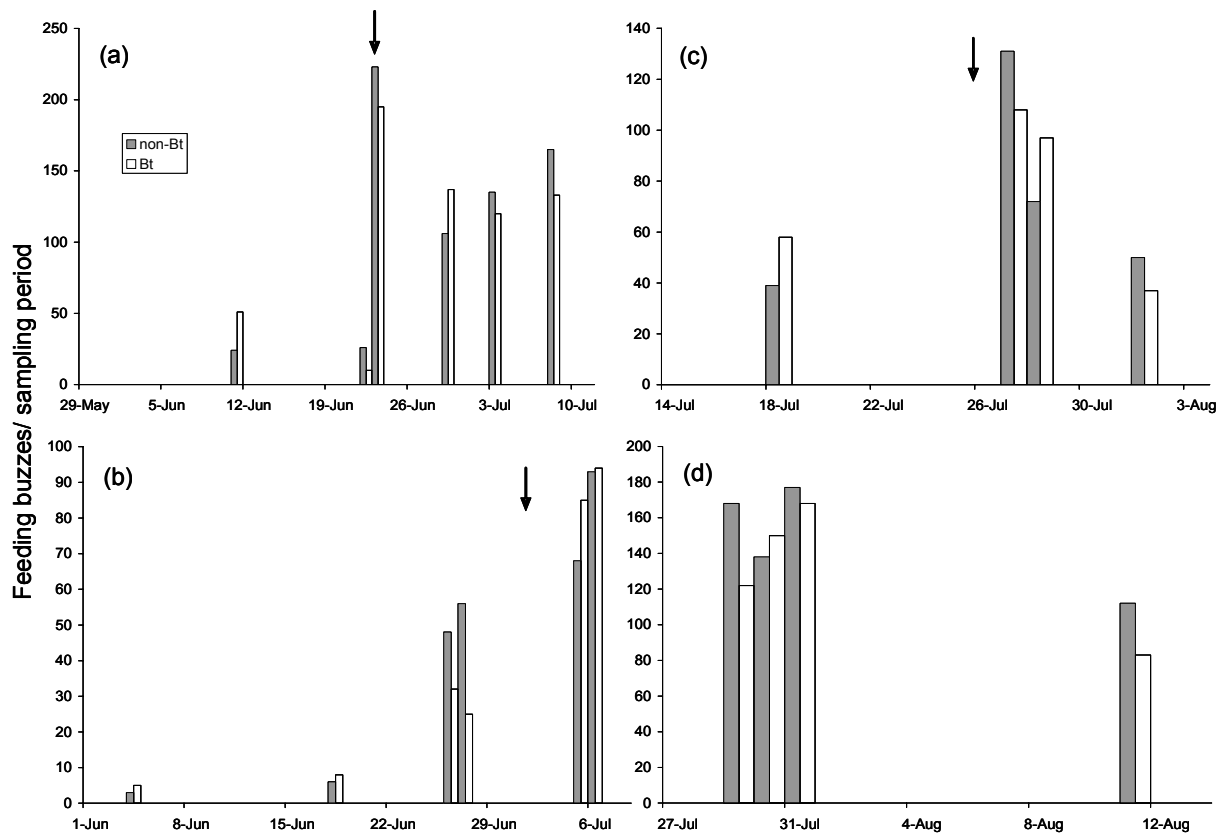


Figure 9. Number of feeding buzzes recorded from 2100-2300 h over corn at a) Uvalde North and b) Hondo East, and cotton at c) Uvalde South and d) Uvalde West. Arrow indicates estimated peak of adult insect emergence. Note that both the x and y scales vary between sites.

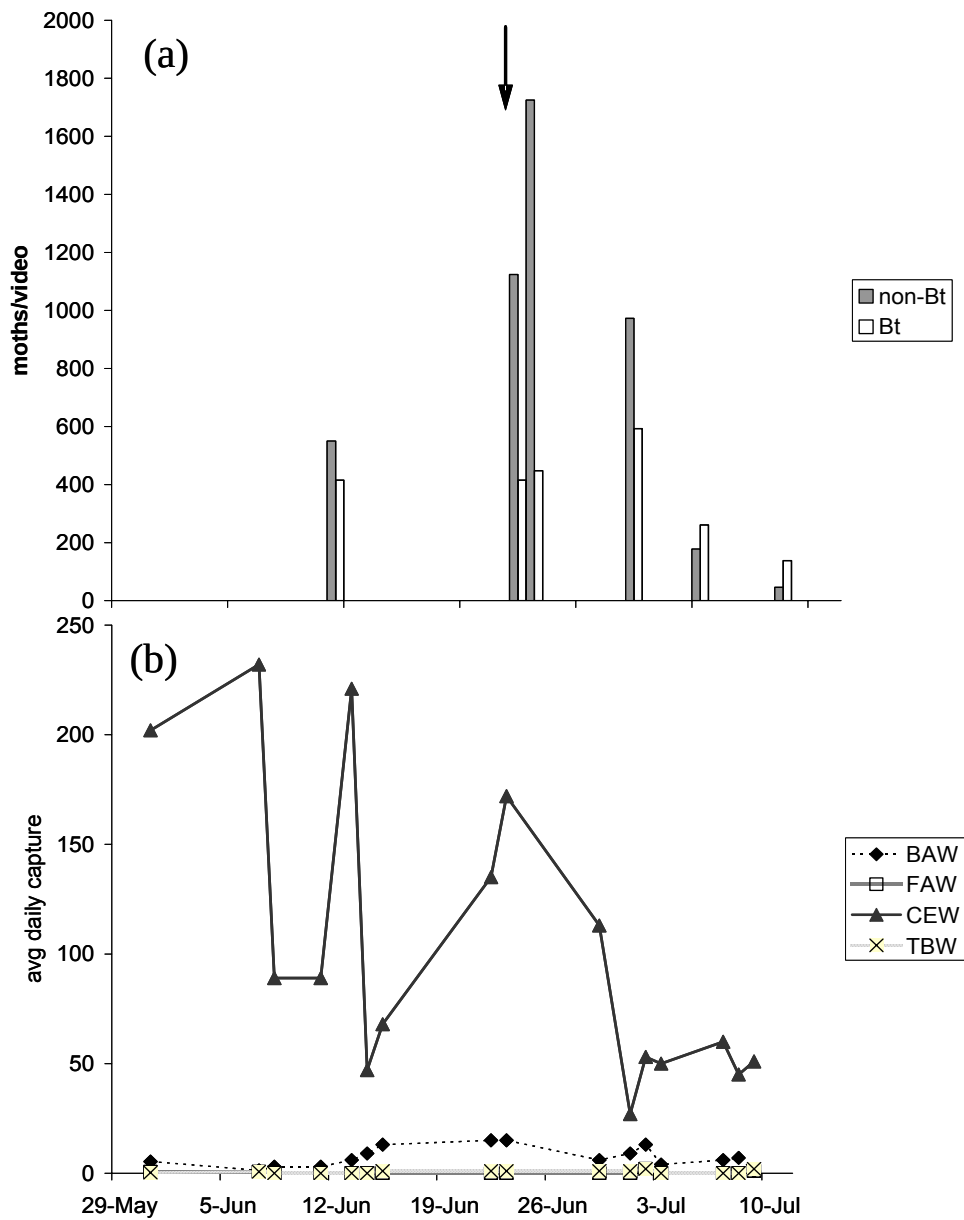


Figure 10. Numbers of moths (a) detected with video imaging and (b) captured in pheromone traps at Uvalde North. BAW: Beet armyworm, FAW: Fall armyworm, CEW: Corn earworm, TBW: Tobacco budworm. Arrow indicates estimated peak of adult insect emergence.

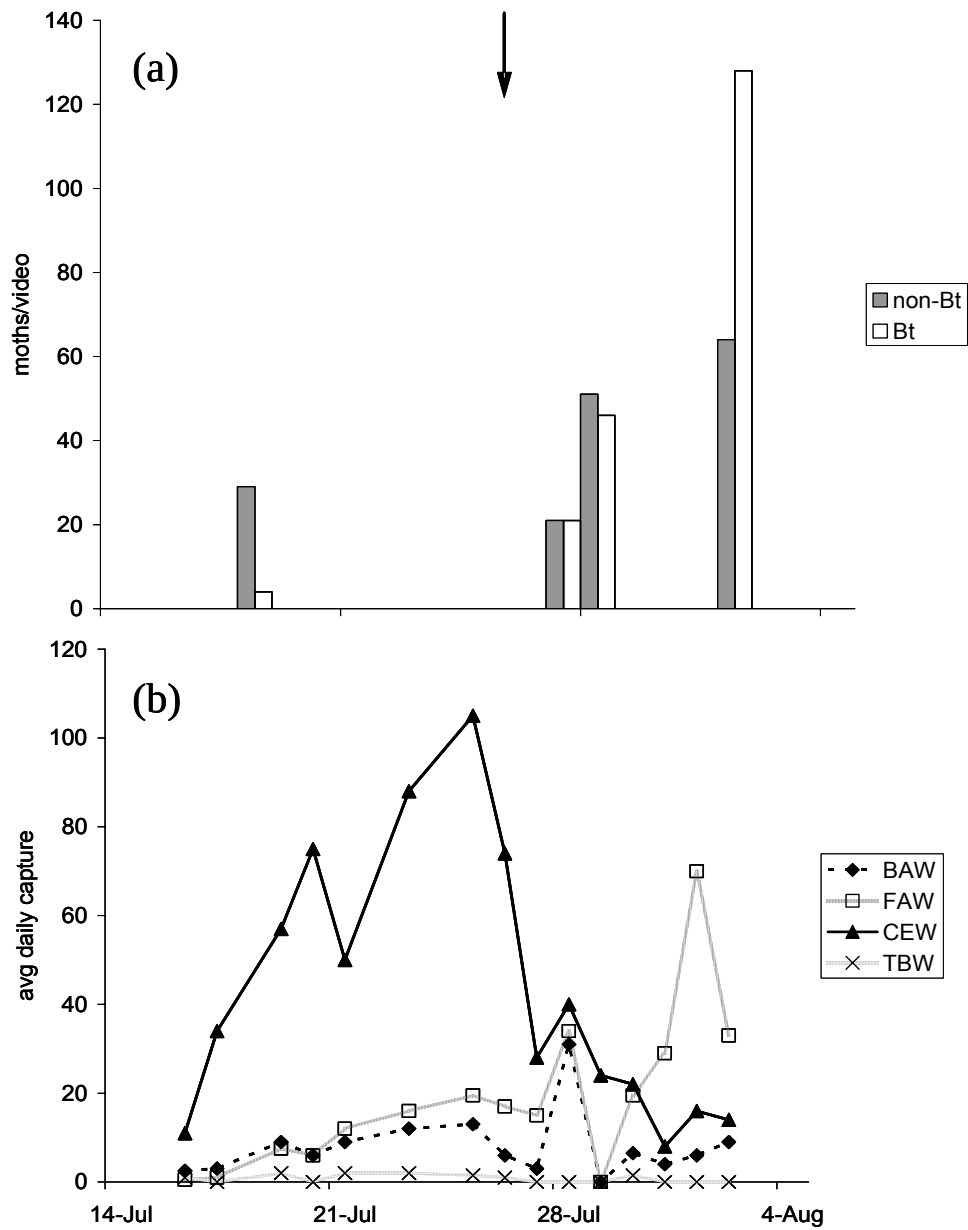


Figure 11. Numbers of moths (a) detected with video imaging and (b) captured in pheromone traps at Uvalde South. BAW: Beet armyworm, FAW: Fall armyworm, CEW: Corn earworm, TBW: Tobacco budworm. Arrow indicates estimated peak of adult insect emergence.

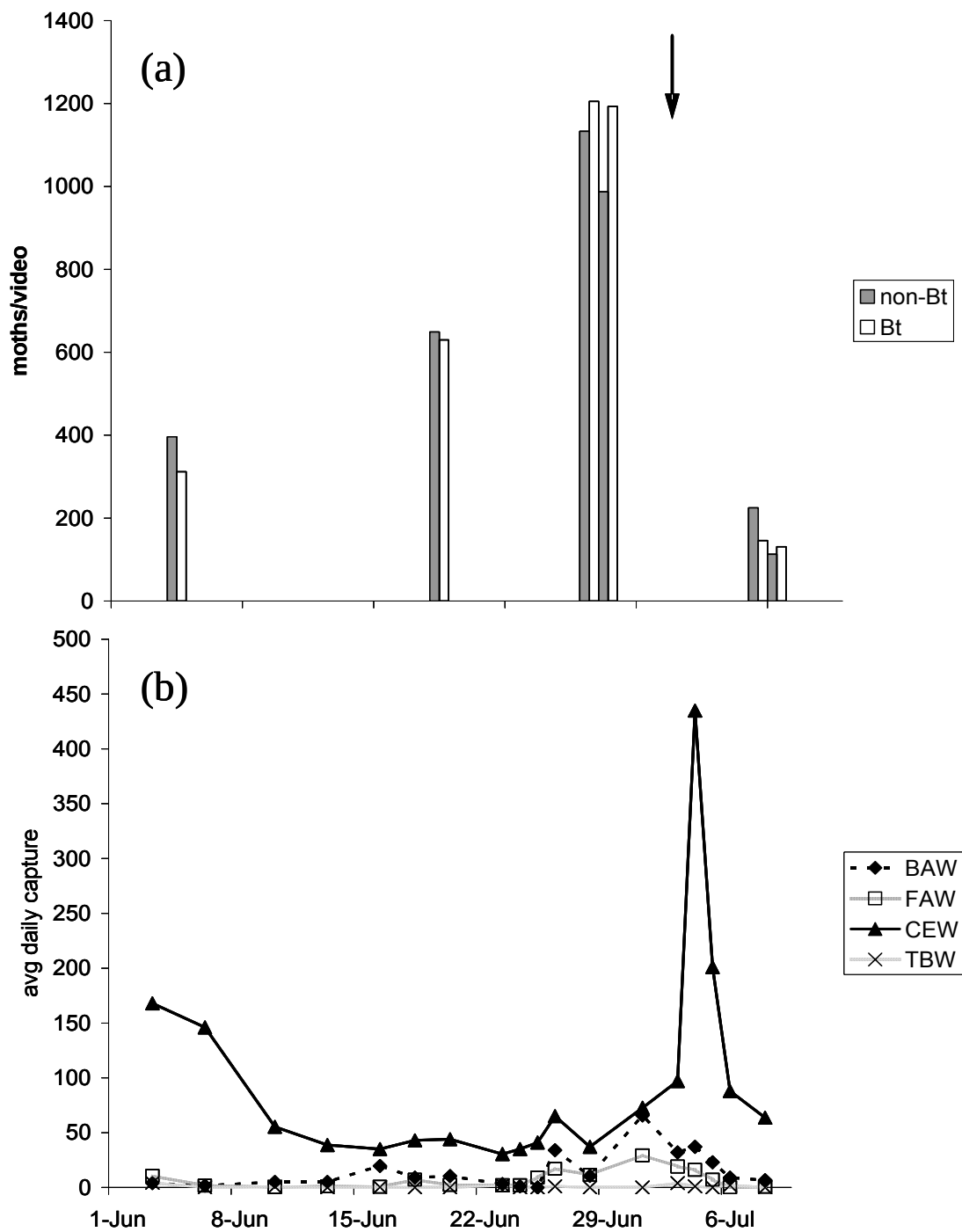


Figure 12. Numbers of moths (a) detected with video imaging and (b) captured in pheromone traps at Hondo East. BAW: Beet armyworm, FAW: Fall armyworm, CEW: Corn earworm, TBW: Tobacco budworm. Arrow indicates estimated peak of adult insect emergence.

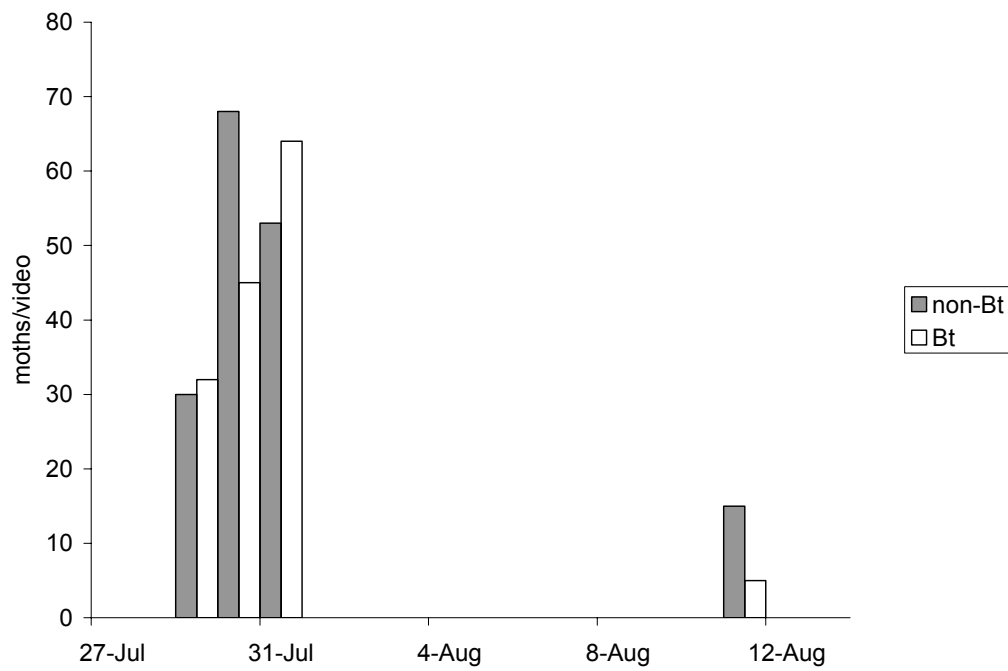


Figure 13. Number of moths detected with video imaging over cotton at Uvalde West.

Vita

Kimberly Shawn Kennard was born on March 10, 1977, in Miamisburg, Ohio. She graduated from Miamisburg High School in 1995. She graduated *Summa cum laude* from the University of Tennessee with a major in Ecology and Evolutionary Biology in 2003. After developing her research skills in a variety of laboratory and field settings, she entered the graduate program at UT and earned a M.S. in Ecology and Evolutionary Biology in 2008. Kimberly plans to pursue a career in science education to share the wonder of the natural world with young people.