

ABSTRACT

ROGERS, SHELLEY RENEE. Pollination Ecology of Highbush Blueberry Agroecosystems. (Under the direction of Hannah J. Burrack and David R. Tarpy).

Both managed and wild bee species provide pollination services to agroecosystems. However, our understanding of the relationship between bee community composition and agroecosystem functioning (productivity and stability of pollination) is still evolving. In highbush blueberry (*Vaccinium corymbosum*) agroecosystems, we evaluated (1) the relative contribution of different bee taxa to pollination, (2) the mechanisms underlying their contribution, (3) the importance of pollinator taxonomic diversity to crop productivity and stability, and (4) the influence of the pollinator community on bee foraging behavior. In 2010 and 2011, we surveyed the pollinator community (using transect observations and pan traps) during repeated visits to multiple blueberry farms in North Carolina. We assessed pollination (by measuring resultant seed set) from either a single bee visit or unrestricted visitation (i.e., open pollination) to flowers. We found that several bee taxa were consistently present and abundant flower visitors: honey bees (*Apis mellifera*), bumble bees (*Bombus* spp.), blueberry bees (*Habropoda laboriosa*), 'small native' bees (predominantly andrenids and halictids), carpenter bees (*Xylocopa virginica*), and horn-faced bees (*Osmia cornifrons*; in western NC only). These bee groups varied in their abundance at flowers, per-visit efficiency, and the degree to which their foraging behavior depended on weather. Despite a high density of managed pollinators (*A. mellifera*), we show that wild bee species contributed almost equally to pollination in the highbush blueberry agroecosystems that we observed. Additionally, blueberry pollinator taxa exhibited 'response diversity' to weather, thus stabilizing plant

visitation between inclement and optimal foraging conditions. In foraging experiments conducted in a controlled environment, we found that encounters between foraging bees influenced their subsequent foraging behavior. However, observations in more realistic field conditions suggest that these encounters and we found no impact of bee community composition on the per-visit efficiencies of *A. mellifera* and *H. laboriosa*. These studies represent the first formal documentation of the pollinator community in highbush blueberry agroecosystems. Further, while previous investigators have examined the importance of wild bee diversity to pollination in coffee, cucurbit, and sunflower crops, this work is the first thorough examination of this relationship in a temperate, perennial fruit crop.

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Pollination Ecology of Highbush Blueberry Agroecosystems

by
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DEDICATION

In loving memory of Wayne Rogers, and Frosty.

BIOGRAPHY

Shelley Rogers was born and raised in Statesville, North Carolina. She attended high school at the NC School of Science and Mathematics in Durham, and graduated from the University of North Carolina – Chapel Hill with a Bachelor's of Science in Environmental Science.

While at UNC, Shelley conducted research at the Highlands Biological Station, working with Patrick Brannon to study salamander populations in declining hemlock forests. After college, Shelley worked for the Morehead Planetarium and Science Center developing biodiversity-themed citizen-science programs and a traveling exhibit about hunter-gatherers in North Carolina 12,000 years ago. She spent several months working for Busy Bee Apiaries, helping to rear honey bee queens, just before beginning graduate school in July 2009. Shelley enjoys Appalachian-style clogging, gardening, beekeeping, and reading.

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INTRODUCTION

Animal pollination is essential to more than 35% of our global food supply (Klein et al. 2007). Bees (Hymenoptera: Apoidea: Anthophila), with an estimated 30,000 species (see Gaston 1991), are by far the most important animal pollinators (Klein et al. 2007). Managed pollinators, particularly honey bees, *Apis mellifera*, have long been a mainstay of agricultural pollination (Delaplane and Mayer 2000). However, concerns over potential declines in wild and managed pollinator populations (Biesmeijer et al. 2006, FAO 2010) have spawned a growing field of research around wild bees, pollination services, and agricultural landscapes (e.g., Lonsdorf et al. 2009, Garibaldi et al. 2011, Winfree et al. 2011).

Blueberry (*Vaccinium* spp.) is one pollinator-dependent crop grown throughout North America and the world. In North Carolina, highbush blueberry (*V. corymbosum*) and rabbiteye blueberry (*V. ashei*) are grown on > 2000 ha of land, with highbush blueberry accounting for the majority of commercial production (NCDA CS 2011). Blueberry flowers consist of a fused, narrow corolla with a constricted aperture (Lyrene 1994), a single stigma, and multiple poridically-dehiscent stamens that, upon vibration or ‘sonication’, release pollen tetrads through a terminal pore (Buchmann 1983). Blueberry flowers are protandrous (Brevis et al. 2006, MacKenzie 2009) with stigmas remaining receptive up to five days after anthesis (Brevis et al. 2006). Cultivars of *V. corymbosum* and *V. ashei* exhibit variable degrees of parthenocarpy and self-fertility, yet benefit from fertilization by intraspecific, outcrossed pollen (Gupton and Spiers 1994, Huang et al. 1997, Dogterom et al. 2000, Ehlenfeldt 2001), and rely on insect pollination for commercial production (Delaplane and Mayer 2000).

The bee community of blueberry agroecosystems is well-documented for a number of

geographical regions, including British Columbia (MacKenzie and Winston 1984, Ratti et al. 2008), Nova Scotia (Javorek et al. 2002), New York (MacKenzie and Eickwort 1996), Michigan (Tuell et al. 2009), southwest Virginia (Adamson 2011), and the southeastern United States (Cane and Payne 1993). Until the present study, there was no record of the wild-bee community in the highbush blueberry systems of North Carolina. The aforementioned pollinator surveys, taken together, show that several wild-bee taxa are common throughout blueberry systems: bumble bees (*Bombus* spp.), blueberry bees (*Habropoda laboriosa*; southeastern US, only), carpenter bees (*Xylocopa virginica*), andrenid bees, and halictid bees. Additionally, managed *A. mellifera*, commonly imported during bloom (2 to 10 colonies per hectare), are abundant in most blueberry agroecosystems.

Several questions are central to our understanding of pollination services in blueberry and agroecosystems generally:

- i. How do pollinator taxa vary in their contribution to the pollination of a given crop? Bees are a diverse taxonomic group, with differing morphologies (Thorp 1979, Krenn et al. 2005), life histories (Michener 1974), and foraging behaviors (Eickwort and Ginsberg 1980, Buchmann 1983, Chittka et al. 1999, Newman & Thomson 2005). As a result of this diversity, bees vary in their interaction with a crop and their contribution to its pollination. Several measures are important to evaluating a bee taxon's contribution to pollination: forager abundance, per-visit efficiency (or the amount of pollination contributed in a single flower visit), visitation rate, and time-, space-, or weather-dependent foraging activity (Inouye et al. 1994, Ne'eman et al.

2010, Tuell and Isaacs 2010). Understanding how different bee taxa contribute to pollination enables researchers to model predicted pollination services and evaluate the relative contribution of different bee groups to pollination (e.g., Winfree et al. 2007).

- ii. Is community-level bee diversity important to the quantity and stability of pollination services? The relationship between diversity and ecosystem functioning has been investigated in many biological systems (Balvanera et al. 2006). However, empirical work linking bee diversity and crop productivity in agroecosystems is sparse (Hooper et al. 2005). Bee diversity may enhance crop pollination through functional complementarity (Bluthgen and Klein 2011). For instance, Chagnon et al. (1993) found that *A. mellifera* and smaller wild bees visit different regions of a strawberry flower, so that flowers visited by both bee groups resulted in more fully-formed fruits. Bee diversity may also help to stabilize pollination services against declines in managed bee populations (Winfree et al. 2007) and environmental variability (Allen-Wardell et al. 2003, Winfree and Kremen 2009). By understanding how bee diversity impacts ecosystem functioning, we can better predict how agroecosystems will respond to disturbance.
- iii. How do community composition and individual pollinator performance interact? The composition of a bee community may affect the foraging behavior of individuals within that community. For instance, the presence of nectar-robbing bees (Inouye

1980) in a pollinator community can drastically alter visitation and floral-handling strategies of other bee species with respect to the same plant population (Maloof and Inouye 2000). Greenleaf and Kremen (2006) found another prime example of such non-linearity in the hybrid sunflower system: in more bee-diverse communities, *A. mellifera* were more efficient pollinators as a result of interspecific avoidance.

Accounting for these types of interactive effects is essential to valuing the pollination services provided by wild-bee communities.

In the following chapters and appendices, I attempt to answer these questions in the highbush blueberry crop, and contribute to a broader understanding of pollination services in agroecosystems.

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CHAPTER 1:
MULTIPLE CRITERIA FOR EVALUATING POLLINATOR PERFORMANCE IN
HIGHBUSH BLUEBERRY AGROECOSYSTEMS

Summary

1. Numerous bee species provide pollination services in agricultural ecosystems. Evaluating a pollinator's performance with regard to a crop is an important step in attributing pollination services and predicting how changes in bee community or foraging environment will affect those services.
2. We used multiple criteria to evaluate pollinators of North Carolina highbush blueberry *Vaccinium corymbosum* agroecosystems. For five groups of bees (*Apis mellifera*, *Bombus* spp. *Habropoda laboriosa*, 'small native' bees, and *Xylocopa virginica*), we measured forager abundance through timed transect observations, quantified per-visit efficiency as the viable seed set resulting from a single visit, and analyzed bee presence in different weather conditions. We also considered two other criteria affecting pollinator performance—visitation rate and community dynamics.
3. *Apis* was the most abundant group in the majority of our survey sites, yet they had a low per-visit efficiency and reduced foraging activity in inclement weather. 'Small native' bees were highly efficient pollinators, contributing nearly twice as many seeds as *Apis* or *Habropoda* in a single flower visit. *Bombus*, *Habropoda*, and 'small native' bees were more resilient to fluctuations in temperature, wind speed, and solar radiation.
4. Our results show that bee groups contribute to pollination in different ways. These differences may provide functional complementarity and stability of pollination services. Furthermore, the presence of certain species (such as nectar-robbing *Xylocopa*) within a bee community can affect the behavior and pollination performance of other individuals.
5. **'Synthesis and applications'** Multiple criteria are needed to evaluate a pollinator's

contribution to an agroecosystem. Honey bees are both highly abundant and amenable to augmentation. Though present in smaller numbers, other bee species also provide valuable pollination services by contributing more pollination in a single visit, visiting more flowers per unit time, or providing pollination during inclement weather conditions. Blueberry farms should be managed to support diverse bee communities that both contribute to and stabilize pollination services.

Key words: *Apis*, ecosystem service, *Habropoda*, honey bees, pollination, *Vaccinium*, wild bees

Introduction

Both wild and managed bees provide pollination services in agricultural systems. Evaluating the services provided by different bee groups (taxonomic or functional) has become increasingly common in an effort toward understanding the dynamic ecological processes underlying our food systems (Bos *et al.* 2007), developing a diversified and sustainable approach to agricultural pollination (Winfree *et al.* 2007; Rader *et al.* 2012), and conserving wild-bee species (Allen-Wardell *et al.* 1998).

Agroecosystems often host a rich community of pollinating bees from several families and many genera (Winfree *et al.* 2008; Tuell, Ascher & Isaacs 2009; Ngo, Mojica & Packer 2011; Watson, Wolf & Ascher 2011). These pollinators may vary in morphology (e.g., distribution and structure of hairs and proboscis length; Thorp 1979; Krenn, Plant & Szucsich 2005), life history (including colony size and phenology; Mitchell 1960, 1962; Michener 1974), and foraging behavior (such as floral constancy, visitation patterns, and floral handling strategies; Eickwort & Ginsberg 1980; Buchmann 1983; Chittka, Thomson & Waser 1999; Newman & Thomson 2005). These differences impart functional diversity and complementarity in an agroecosystem (Bluethgen & Klein 2011) and can affect the quantity of pollination services provided to a crop (Klein, Steffan-Dewenter & Tscharrntke 2003; Hoehn *et al.* 2008) and the stability of those services (Winfree *et al.* 2007, Winfree & Kremen 2009).

The community of bees visiting a crop is variable over time and space (Williams, Minckley & Silveira 2001), and accurately evaluating the pollination provided by the

constituents of this dynamic community is complex. A pollinator's contribution to a crop is often measured in terms of forager abundance, per-visit efficiency, or both (Ne'eman *et al.* 2010). However, bee community composition and foraging behavior may change in response to weather (Tuell & Isaacs 2010) or interactions between bees foraging at a common resource (Greenleaf & Kremen 2006; Lichtenberg, Imperatriz-Fonseca & Nieh 2010). We thus recommend assessing the contribution of a particular bee group to pollination with regard to multiple criteria (described in Table 1) including abundance, per-visit efficiency, activity patterns, visitation rate, and community dynamics. While it may not be appropriate or feasible to combine measurements of these five criteria, all are important in describing the multiple ways that different bee species contribute to the pollination of a crop. Plant biology may further complicate the evaluation of pollination services. For example, the per-visit efficiency of a bee may decrease in consecutive visits to a single self-sterile plant (Sanchez-Lafuente, Rodriguez-Girones & Parra 2012), flower age may limit fruit development (Brevis, NeSmith & Wetzstein 2006), differences in floral volatile profiles can alter pollinator attraction and visitation (Rodriguez-Saona *et al.* 2011), and pollinator contribution may depend on visitation sequence (Wenslauff & Lyrene 2001).

Blueberry *Vaccinium* spp. has a diverse and well-documented community of wild and managed pollinators (MacKenzie & Winston 1984; Cane & Payne 1993; MacKenzie & Eickwort 1996; Ratti *et al.* 2008; Tuell, Ascher & Isaacs 2009; Adamson 2011). The widely grown highbush blueberry *Vaccinium corymbosum* is self-fertile, but pollination improves fruit set, volume, weight, speed of ripening, and seed set (Huang *et al.* 1997; Dogterom, Winston & Mukai 2000) and is essential for agricultural production (Delaplane & Mayer

2000). Blueberry flowers have poridical anthers with pollen tetrads that are released through vibratile pollination, also known as buzz-pollination or sonication (Buchmann 1983). In North Carolina, highbush blueberry is visited by managed honey bees *Apis mellifera* and a number of wild-bee species, including both generalists and a reported specialist—the southeastern blueberry bee *Habropoda laboriosa*.

We evaluated common pollinators of the highbush blueberry agroecosystems of North Carolina with regard to three of the criteria from Table 1: (1) abundance, (2) per-visit efficiency, and (3) weather-related activity patterns. Additionally, we considered how visitation rates of blueberry pollinators, as reported in other published studies, and community composition may affect pollinator performance. While other researchers have assessed blueberry pollinators in different regions of North America (MacKenzie & Winston 1984; Dogterom 1999; Sampson & Cane 2000; Javorek, MacKenzie & Vander Kloet 2002; Ratti *et al.* 2008; Tuell, Ascher & Isaacs 2009; Adamson 2011), our study is the first to examine pollination in the highbush agroecosystems of North Carolina and to make use of these multiple criteria to evaluate pollinator contribution.

Materials and Methods

STUDY SYSTEM

We measured the bee community and pollination efficiency at four blueberry farms in North Carolina in 2010 and 2011. In the first year, we sampled two commercial farms in southeastern NC (each with at least 40 hectares in active production) and one 0.8-hectare farm in western NC. In 2011, we sampled a third commercial farm in southeastern NC. We

collected fruit data from *V. corymbosum* cultivar 'O'Neal' at all eastern sites, and 'Bluecrop' in western NC. We visited sites two- to four-times over the course of bloom, from 17-Mar-2010 to 23-Apr-2010 and 14-Mar-2011 to 18-Apr-2011.

We recorded hourly temperature measurements with WatchDog A150 data loggers (Spectrum Technologies, Inc., Plainfield, IL, USA) located in each planting. We obtained hourly solar radiation and wind speed recordings from NC Environment and Climate Observing Network weather stations located in either Whiteville (within 50 km of eastern sites) or Fletcher (13 km from western site). To assess plant phenology, we counted flower buds and total number of flowers (in bud, bloom, or petal-fall stage) on each of four plant branches. We calculated the proportion of flowers that had developed beyond the bud stage, then classified a planting as being in either an early (<0.33), middle ($0.34 < p < 0.66$), or late (>0.67) stage of bloom.

POLLINATOR ABUNDANCE

We sampled the bee community through timed observations in two transects per sample site. In 2010, we observed multiple 200-ft transects for 2 minutes each. We spaced transects five to six rows apart, each beginning at the first plant in a row. We repeated timed observations four times (at 900, 1100, 1300, and 1500 h) per site visit. In 2011, we reduced transect length to 100 ft, so to compare between years we doubled the 2011 bee counts.

We classified the observed bees into six groups that could be distinguished on the wing: *Apis mellifera*, *Bombus* spp., *Habropoda laboriosa*, *Osmia cornifrons*, 'small native' bees (comprised of Andrenidae, Halictidae, Colletidae, and Megachilidae), and *Xylocopa*

virginica; hereafter, all applicable bee groups will be referred to by their genus name only. For each group, we defined the observed floral handling strategy as 'legitimate' (if the bee probed the flower through the apical opening of the corolla) or 'robbing' (if the bee either created or reused a basal slit in the corolla to obtain nectar; Inouye 1980). We calculated the total number of bees observed from each pollinator group during each site visit.

POLLINATOR EFFICIENCY EXPERIMENTS

Per-visit efficiency is a measure of a pollinator's contribution to the stigmatic pollen load or to the plant's reproductive success in terms of seed production in a single visit (Inouye *et al.* 1994; Ne'eman *et al.* 2010). From a practical standpoint, pollen deposition is less difficult and time-intensive to assess than the resultant seed production. However, the relationship of pollen grains deposited to viable seed set can be asymptotic rather than linear (Cane & Schiffhauer 2003; Aizen & Harder 2007), and per-visit seed set more accurately quantifies a pollinator's contribution to fruit production (Westwood 1993). For these reasons, we used per-visit seed set (rather than pollen deposition) as our measure of per-visit efficiency.

We placed No-see-um[®] mesh (Denver Fabrics, Denver, CO, USA) cages on *V. corymbosum* branches with unopened flowers. Four to seven days later, we removed cages and continuously observed virgin flowers for bee visitation. Once a flower was visited by a bee, we loosely tied a short piece of embroidery thread around the pedicel and placed a small cage around the surrounding flower cluster to exclude further insect visits. The color of the thread indicated visitor identity (*Apis*, *Bombus*, *Habropoda*, *Osmia*, 'small native', or *Xylocopa*) and handling behavior (for nectar-robbing visitors). In 2011, we distinguished

Habropoda visitors as male or female, and as sonicating or non-sonicating. In addition to these single-visit treatments, we immediately recaged flowers in a similar stage of bloom as a pollinator-excluded control (five flowers per plant), or left them uncaged for open pollination (ten flowers per plant). Between 7 and 14 days after pollinator manipulations ended, we returned and placed cages on half of the open-pollinated flowers from each plant to test the effect of cage presence on fruit development.

Fifty days following the pollination event, we collected fruit samples and placed them in cold storage until they could be processed. At the time of collection, fruit development was incomplete but seeds had reached their mature size (Westwood 1993; W.O. Cline, personal communication). We weighed berries and measured each across three diameters for volume calculation (Westwood 1993). We then dissected each berry individually to remove seeds from pulp.

Individual *V. corymbosum* fruits produce around 100 seeds (Dogterom, Winston & Mukai 2000); of these, some are viable while others are small, un-developed, and non-viable. Historically, blueberry seed set has been quantified by the number of seeds that fit qualitative descriptions such as “plump,” “sound,” “large,” “dark,” and “apparently viable” (Eaton 1967; Krebs & Hancock 1991; MacKenzie 1997; Dedej & Delaplane 2004; Brevis, NeSmith & Wetzstein 2006; Isaacs & Kirk 2010). In 2010, we used such methods to visually classify all seeds as ‘small’, ‘medium’, or ‘large’. We counted only large seeds for viable seed set. However, many seeds did not fit clearly into one of these size categories, and thus in 2011 we employed a more quantitative approach. Under stereoscopic magnification (6.3x), we photographed (Q-Color 3, Olympus America, Center Valley, PA, USA) all seeds produced

by a single berry, and measured the long-axis of each seed using the image-processing software, ImageJ[®] (Abramoff, Magalhaes & Ram 2004). For each cultivar, we then generated a histogram of seed lengths from ≥ 100 open-pollinated fruits and applied a Gaussian mixture model (Nabney 2004) to determine the mean and standard deviation for each seed size class (presented in results, below). We calculated seed set as the total number of viable seeds per berry.

STATISTICAL ANALYSIS

To test for differences among bee groups in per-visit seed set, we used a generalized linear mixed model. Phenological stage, year, and bee group were included as fixed effects. Plants, nested within year, site, and visit, were treated as random effects. Site and a year by site interaction were included in the model, however their covariance parameters were negligible (<3% of the residual error), suggesting site homogeneity, and they were subsequently removed from the model. Analyses were conducted in SAS Proc GLIMMIX (SAS version 9.2, SAS Institute, Cary, NC, USA), and all statistics are reported as LSM means estimates \pm 95% CI unless otherwise noted. We conducted a power analysis for comparisons of *Habropoda* sex and sonication with the JMP Sample Size and Power platform (JMP version 9, SAS Institute, Cary, NC, USA).

We conducted a logistic regression with backward selection to model bee presence in response to weather. We analyzed each bee group separately, and only for the locations where it was present (e.g., data from the western NC site were not included in the analysis for *Habropoda*). *Osmia* presence was not modeled because observations were limited to a single

site and phenological stage. Our independent variables included data for weather (temperature, solar radiation, and wind speed), temporal variables (year, phenological stage, and time of day), and spatial variables (site, within-site location, and hive location – for *Apis* only). Year, site, within-site location, phenological stage, and hive position were categorical variables. We tested for correlation of bee presence with independent variables and retained all independent variables for $P \leq 0.25$ in the final model (see Bursac *et al.* 2007). For the analysis of ‘small native’ bee presence, we used Firth’s penalized likelihood to correct for quasi-complete separation of data points. All analyses were conducted in SAS Proc LOGISTIC (SAS version 9.2, SAS Institute, Cary, NC, USA).

Results

POLLINATOR ABUNDANCE

We observed 2,964 bees in transect walks repeated four times daily over 17 visits to sites in 2010 and 2011 (Table 2). Bee abundance data is based on two eastern site visits during early and late bloom and a single visit during mid bloom at an eastern and western site in 2010; and three eastern site visits during each stage of bloom and two visits to the western site during mid bloom only in 2011. In 2011, all sites were sampled at two different within-site locations.

At the three eastern sites, *Apis* was the most abundant flower visitor across all stages of bloom, and nectar-robbers made up 30 to 40% of all *Apis* visitors. The high and increasing abundance of *Apis* across the bloom period reflects the sequential importation of managed colonies (2 to 10 colonies ha⁻¹) into blueberry fields. *Habropoda* were also relatively

abundant, particularly in early- and mid-bloom. ‘Small native’ bees, *Xylocopa*, and *Bombus* queens were consistently present in low numbers that increased across the bloom period. Though only nectar-robbing *Xylocopa* were counted in transect observations, legitimately foraging (and sonicating) *Xylocopa* were observed on multiple occasions.

Our western site hosted a strikingly different community of flower-visiting bees. While *Apis* hives were present (10 colonies ha⁻¹), these bees were rarely observed in the focal crop. *Osmia*, however, were nearly twice as abundant as all other bees combined. *Habropoda* were neither present at this site nor observed in other sampling areas in western NC. Male and female *Xylocopa* were present but congregated (~10 to 20 bees) at one older blueberry plant at this site in both 2010 and 2011 and were therefore not observed in transect walks. *Bombus* and ‘small native’ bees were approximately 10 times more abundant in western than eastern sites.

VIABLE SEED LENGTH DETERMINATION

Both *V. corymbosum* ‘O’Neal’ and ‘Bluecrop’ seed length histograms exhibited a clear trimodal distribution. We generated the mean (m_i), and standard deviation (s_i^2) for each of the three seed size groups using a Gaussian mixture model. For ‘O’Neal’, parameter estimates for the three modes were: $m_1 = 0.380$ mm, $s_1^2 = 0.038$ mm; $m_2 = 1.005$ mm, $s_2^2 = 0.079$ mm; $m_3 = 1.606$ mm, $s_3^2 = 0.093$ mm (Fig. 1a). For Bluecrop, parameter estimates were: $m_1 = 0.630$ mm, $s_1^2 = 0.112$ mm; $m_2 = 1.048$ mm, $s_2^2 = 0.055$ mm; $m_3 = 1.629$ mm, $s_3^2 = 0.055$ mm (Fig. 1b).

Using seed size classes similar to our own distribution-based findings, Dogterom,

Winston, and Mukai (2000) demonstrated that only the largest size class contained viable seeds (85% germination). Based on this, we established a lower limit for viability at three standard deviations below the mean of the largest seed length mode, i.e. length of viable seed $\geq m_3 - 3s_3^2$, for each cultivar. We therefore used 1.3 mm and 1.45 mm as approximate minimum “viable” seed lengths for ‘O’Neal’ and ‘Bluecrop,’ respectively. Viable seed set was significantly correlated with fruit mass for both cultivars in both years (‘O’Neal’ 2010: $r^2 = 0.61$, $P < 0.0001$; ‘O’Neal’ 2011: $r^2 = 0.55$, $P < 0.0001$; ‘Bluecrop’ 2010: $r^2 = 0.26$, $P < 0.0001$; ‘Bluecrop’ 2011: $r^2 = 0.37$, $P < 0.0001$).

COMPARISON OF PER-VISIT SEED SET

We counted the viable seed set from fruits that were excluded from pollination (454), visited by a single bee (509), or open to pollination by multiple bees (675). Bee groups differed significantly in their per-visit seed set (Fig. 2; $F_{7,1585} = 198.20$, $P < 0.0001$). All bee groups except nectar-robbing *Xylocopa* (12 ± 6 seeds) significantly improved seed set over pollinator-excluded fruits (7 ± 2 seeds). Visits by ‘small native’ bees resulted in the numerically highest seed set (26 ± 3 seeds) of all bee groups, but were not significantly more efficient than *Bombus* (20 ± 4 seeds) or *Osmia* (19 ± 6 seeds) visitors. ‘Small native’ bees produced approximately twice as many seeds per visit as either *Apis* (12 ± 2 seeds) or *Habropoda* (15 ± 4 seeds).

In 2011, *Habropoda* visitors ($n = 30$) were distinguished as male or female, and sonicating or non-sonicating. *Habropoda* males (16) and females (14) did not differ from each other in per-visit seed set (males: 17 ± 4 seeds; females: 11 ± 4 seeds; $P = 0.25$).

Likewise, sonicating *Habropoda* visits (8) did not set more seeds than non-sonicating *Habropoda* (22) (sonicated: 13 ± 5 seeds; not sonicated: 15 ± 3 ; $P = 0.76$). These results suggest that neither bee sex nor sonication affect pollination efficiency, although our sample size is only effective in detecting differences of ≥ 15 seeds per visit at a power level of 0.8 and α of 0.05. We received a single legitimate visit from *Xylocopa* at our treatment plants. While strong inferences cannot be drawn from just one observation, it is worth noting that this legitimately foraging *Xylocopa* visit resulted in 38 seeds, more than three times the amount from nectar-robbing *Xylocopa* visits (12 ± 6 seeds, range 0 – 28 seeds).

Seed set differed between years (22 ± 3 and 15 ± 2 seeds in 2010 and 2011, respectively; $F_{1,167.5} = 17.02$, $P < 0.0001$). While climatic differences may be partially responsible for this interannual variation, we suspect differences in 2010 and 2011 seed set methodology are primarily responsible. Relative to the quantitative seed-set assessment in 2011, we may have overestimated the number of viable seeds per fruit in 2010 when classifying seeds as small, medium, or large. However, the relationship between bee groups was consistent in 2010 and 2011 (trt*year; $F_{6,1590} = 0.29$, $P < 0.9413$), which suggests that the less time-intensive method of classifying seeds may be just as effective for comparing pollinator groups in their per-visit contribution to plant fitness.

Phenology was a significant factor in seed set ($F_{2,158.8} = 14.45$, $P < 0.0001$). Seed set was low during early bloom (13 ± 3 seeds), compared with middle and late-stage bloom (both 21 ± 3 seeds). We suspect this difference is the result of increased pollen availability in middle and late stage bloom. Mesh cages had a small negative effect (-3.24 seeds, $F_{1,421.2} = 3.88$, $P = 0.050$) on seed set in open-pollinated flowers. We expect this caging effect

was consistent across all caged, single-visit treatments.

WEATHER AND FORAGER ACTIVITY

Weather varied throughout our bee observations (temperature, 8.9 – 30.8°C; wind speed, 1.5 – 21.8 km h⁻¹; solar radiation, 154 – 1093 Watts m⁻²), and was important in describing *Apis* and *Xylocopa* foraging activity (Table 3). *Apis* were significantly more likely to be present at flowers during higher temperature and solar radiation, and lower wind speed. *Xylocopa* were also more likely to be present at higher temperatures. Other significant factors in describing *Apis* presence included year (growers imported more colonies in 2011 than 2010), site (growers stocked sites at different densities), and hive position (some of our sampling areas were adjacent to colonies). Weather was not a significant factor affecting bee presence for *Bombus*, *Habropoda*, or ‘small native’ bees, though was descriptive ($P < 0.25$) for some bee groups (Table 3). All wild bees differed significantly between sites, and, except *Xylocopa*, were more present in 2010 than 2011.

Discussion

Our study demonstrates that different bees contribute to the pollination of a crop in different and potentially complementary ways. While some bees are highly abundant in the highbush blueberry system, others may contribute more pollination in a single visit, yet others may forage during inclement weather when most pollinators are inactive. These differences can provide for functional complementarity (Blüthgen & Klein 2011) and ecosystem stability (Hooper *et al.* 2005) in environments that experience variability in resource availability and

foraging conditions. Were we to compare pollinators with regard to only one or two criteria, we risk undervaluing their contribution to an ecosystem.

While *Apis mellifera* is an inefficient pollinator of blueberry (documented here and in Dogterom 1999; Javorek, MacKenzie & Vander Kloet 2002), its large social colonies can be easily managed, providing a readily available forager force. *Apis* were the most abundant pollinators at our sites in eastern NC. Though eight colonies were maintained perennially at our western site, we observed few *Apis* foraging at blueberry either year. Due to their relatively short tongues and inability to sonicate, *Apis* have difficulty extracting nectar and pollen from blueberry (Buchmann 1983; Dedej & Delaplane 2005) and may have preferentially foraged at more attractive co-flowering plants in the surrounding landscape. Another possibility is that *Apis* were outcompeted by the large population of *Osmia cornifrons* present at our western site.

Habropoda laboriosa is a surprisingly inefficient pollinator given its reputation as a blueberry specialist. Specialization in *Habropoda* has been overemphasized as this species forages on multiple plant families and genera (including *Azalea*, *Cercis*, *Gelsemium*, *Lonicera*, *Prunus*, and *Rubus*), sometimes in preference to blueberry (Pascarella 2007). Many of these plants are common constituents of southeastern blueberry agroecosystems (Roberts 2009), leading us to suggest that *Habropoda* evolved as a specialist of a particular community of plants that co-occur in time or space rather than a single plant genus within that community. *Habropoda's* relative lack of efficiency may be compensated for by its moderate abundance and ability to forage in windy and cool weather. Additionally, *Habropoda* have the lowest measured floral handling time (2.6 s flower⁻¹) of all blueberry

pollinators (Cane & Payne 1988; Dogterom 1999; Dedej & Delaplane 2005).

Though frequently neglected in pollination surveys, male bees can provide substantial levels of pollination to crops (e.g., Cane, Sampson & Miller 2011). Previously, *Habropoda* were evaluated only in terms of the female's contribution to pollination (Cane 1996). We observed many male *Habropoda* visiting blueberry in early stages of bloom, and our data suggest that males match females in their per-visit efficiency. Our results also indicate that sonicating *Habropoda* are no more efficient than non-sonicating conspecifics. As Wilson and Thomson (1991) demonstrated, pollen deposition may be disproportionate to pollen removal. We suspect sonicators are more effective at collecting pollen from flowers, but they may groom more frequently than nectar-collecting *Habropoda* and therefore deposit less of their total pollen load on the stigmatic surface.

Compared with other bees, *Bombus* are relatively rare in eastern North Carolina blueberry agroecosystems, yet they are moderately efficient pollinators and have a high floral visitation rate (Javorek, MacKenzie & Vander Kloet 2002). *Bombus* are also resilient to the range of weather fluctuations that occur within these ecosystems. Along with *Habropoda* and 'small native' bees, *Bombus* foragers may partially compensate for the reduced activity of *Apis* during high winds, cool temperatures, and 'April showers' (Vicens & Bosch 2000; Tuell & Isaacs 2010).

'Small native' bees were the most efficient pollinators on a per-visit basis. This group consisted of multiple bee families and genera and, though foraging behavior was superficially similar among bees, we suspect bee species differ in their per-visit contribution. We commonly observed one species, *Andrena bradleyi*, visiting and sonicating flowers, and

we believe this species was highly represented in our per-visit efficiency sampling. *A. bradleyi* was also the most common small native bee species collected in pan traps (Chapter 2) and is distributed throughout the eastern United States, where it is associated with *Vaccinium* culture (Mitchell 1960). Javorek, MacKenzie & Vander Kloet (2002) found that ‘small native’ *Andrena* and *Halictus* spp. visit fewer flowers per minute than either *Apis* or *Bombus*. Thus there may be a trade-off between per-visit efficiency and visitation rate as bee movement among plants (rather than within a single plant) may increase travel time but improve the transfer of xenogamous pollen (Sanchez-Lafuente, Rodriguez-Girones & Parra 2012).

Nectar-robbing *Xylocopa* are present at low densities and do not contribute significantly to per-visit seed set. However, these bees impacts other members of the pollinator community. In the presence of primary nectar robbers, more than 90% of legitimately-foraging *Apis* will shift to secondary robbing, reusing slits created by *Xylocopa* (Cane & Payne 1993; Dedej & Delaplane 2004). Because of this, *Xylocopa* have long been maligned as detrimental to blueberry pollination. However, in *Apis*, the shift to nectar robbing may be accompanied by a two-fold increase in visitation rate (Dedej & Delaplane 2005) with no reduction in stigmatic pollen deposition (Sampson, Danka & Stringer 2004). Furthermore, the presence of primary nectar-robbing slits may make blueberry a more attractive source of forage for *Apis*.

Single-visit efficiency is a potentially simplistic measure of a pollinator's per-visit contribution. Bees may have complementary patterns of floral handling, and pollinator efficiency may depend on the order of bee arrival at a flower. For instance, some bees may

be more effective in transferring pollen among flowers, while other bees may distribute previously-transferred pollen onto the stigmatic surface (Chagnon, Gingras & De Oliveira 1993; Greenleaf & Kremen 2006). While gathering efficiency data from sequential visits is logistically impractical, it is important to remember that per-visit efficiency comparisons (such as our own) neglect to account for these types of interactions.

Because pollinator performance varies with environmental context, our study underscores the importance of diverse, resilient bee communities to agricultural pollination. A pollinator community should be rich enough to ensure pollination across the normal range of variability experienced by agricultural landscapes. While managed *Apis* provide a sizeable fleet of foragers important to large-scale agriculture, less abundant, wild bees contribute to pollination through high per-visit efficiencies, visitation rates, and weather-independent foraging activity. Because many wild bees are active for months after blueberry bloom (Mitchell 1960, 1962), providing alternative sources of forage in these intervening months may be effective in growing wild bee populations (Williams & Kremen 2007).

Agricultural pollination should be evaluated from two perspectives. First, the pollinator community and its interaction with a crop should be assessed along multiple criteria, as done here. Based on these values, researchers can model expected pollination services and compare the relative importance of different bee groups to pollination (e.g., Winfree *et al.* 2007). Second, pollination should be evaluated by relating actual fruit (or seed) production back to the most influential factors in the agroecosystem (Chapter 2). Combining evaluations of both pollinator performance and pollination services enables us to understand how agroecosystems currently function, to predict how these systems will

respond to disturbance, and to improve management of pollination services.

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Table 1. Criteria for evaluating pollinator performance in an agricultural system.

Criteria	Description
Abundance	number of actively foraging bees in an agroecosystem (Winfree <i>et al.</i> 2008; Tuell, Ascher & Isaacs 2009)
Per-visit efficiency	amount of pollination provided by a bee in a single visit to a flower (Inouye <i>et al.</i> 1994; Ne'eman <i>et al.</i> 2010; Artz & Nault 2011)
Activity patterns	foraging activity that may be dependent on weather (Dogterom 1999; Tuell & Isaacs 2010), seasonal phenology (Cane & Payne 1993), and spatial aspects of bee foraging behavior (Dogterom 1999; Gathmann & Tschardtke 2002; Ratti <i>et al.</i> 2008)
Visitation rate	number of flowers (or plants) visited over a period of time (Cane & Payne 1988; Ne'eman <i>et al.</i> 2010; Artz & Nault 2011)
Community dynamics	interactions between bee groups that may reduce or enhance visitation rates or per-visit efficiency (Maloof & Inouye 2000; Greenleaf & Kremen 2006)

Table 2. Abundance of bees actively foraging on highbush blueberry counted in timed observations per site visit. All data has been adjusted to represent a sampling effort of two 200-ft transects observed four times daily. Floral handling behavior is defined as legitimate foraging (L) or nectar robbing (R).

	Floral Handling Behavior	Bee Visitation Frequency			
		Early Bloom	Mid Bloom		Late Bloom
		East	East	West	East
<i>Apis mellifera</i>	L	19.9	42.7	0.4	84.3
	R	8.5	29.3	0.0	45.3
<i>Bombus</i> spp.	L	0.1	1.1	11.0	1.4
<i>Habropoda laboriosa</i>	L	11.3	16.6	0.0	6.5
<i>Osmia cornifrons</i>	L	0.0	0.0	101.8	0.0
'Small native' bees	L	0.4	2.6	43.8	3.4
	R	0.0	1.3	0.4	1.6
<i>Xylocopa virginica</i>	R	0.9	2.4	0.0	4.8
Total bee abundance		41.0	96.0	157.4	147.1

Table 3. Importance of environmental factors to bee presence in highbush blueberry as analyzed through logistic regression with backward selection. All factors for $P < 0.25$ were included in the analysis and final model.

Bee group	Independent Variables†	Estimate	P-value	r^2 §
<i>Apis mellifera</i>	Temperature (°C)	1.04	0.003	
	Solar radiation (W m ⁻²)	0.01	0.008	
	Year (2010)	-3.18	0.009	
	Wind speed (km h ⁻¹)	-0.53	0.010	
	Hive position* (0)	-2.63	0.049	
	Final Model			0.834
<i>Bombus</i> spp.	Year (2010)	0.56	0.140	
	Phenological stage early vs late	-1.36	0.166	
	middle vs late	0.54		
	Final Model			0.497
<i>Habropoda laboriosa</i>	Year (2010)	0.24	0.028	
	Solar radiation (W m ⁻²)	0.002	0.175	
	Final Model			0.242
‘Small native’ bees	Phenological stage early vs late	-1.68	0.004	
	middle vs late	0.86		
	Year (2010)	0.73	0.035	
	Time of day	0.002	0.053	
	Wind speed (km h ⁻¹)	0.08	0.235	
	Final model			0.613
<i>Xylocopa virginica</i>	Temperature (°C)	0.48	0.001	
	Final Model			0.567

* Hive position was considered adjacent ‘1’ to the planting if within 150 m of bee observations; otherwise, hive position was non-adjacent ‘0’. †Pair-wise site and within-site location comparisons are not included. § Max-rescaled r^2 values.

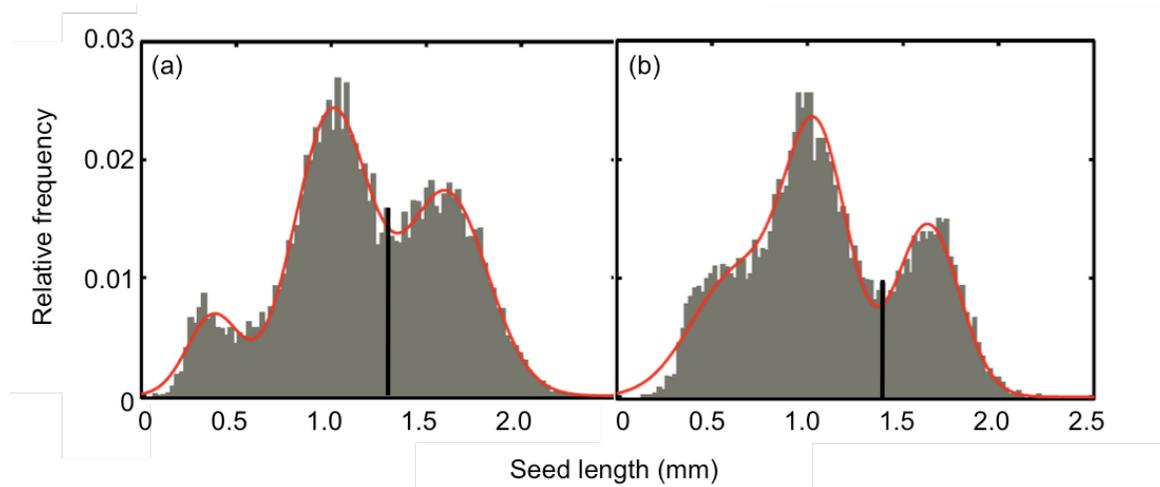


Figure 1. Relative frequency histogram of highbush blueberry seed length for (a) 'O'Neal' and (b) 'Bluecrop' cultivars. Curve represents the best fit for a trimodal distribution generated through a Gaussian mixture model; black line represents the lower limit for "viable" seed length.

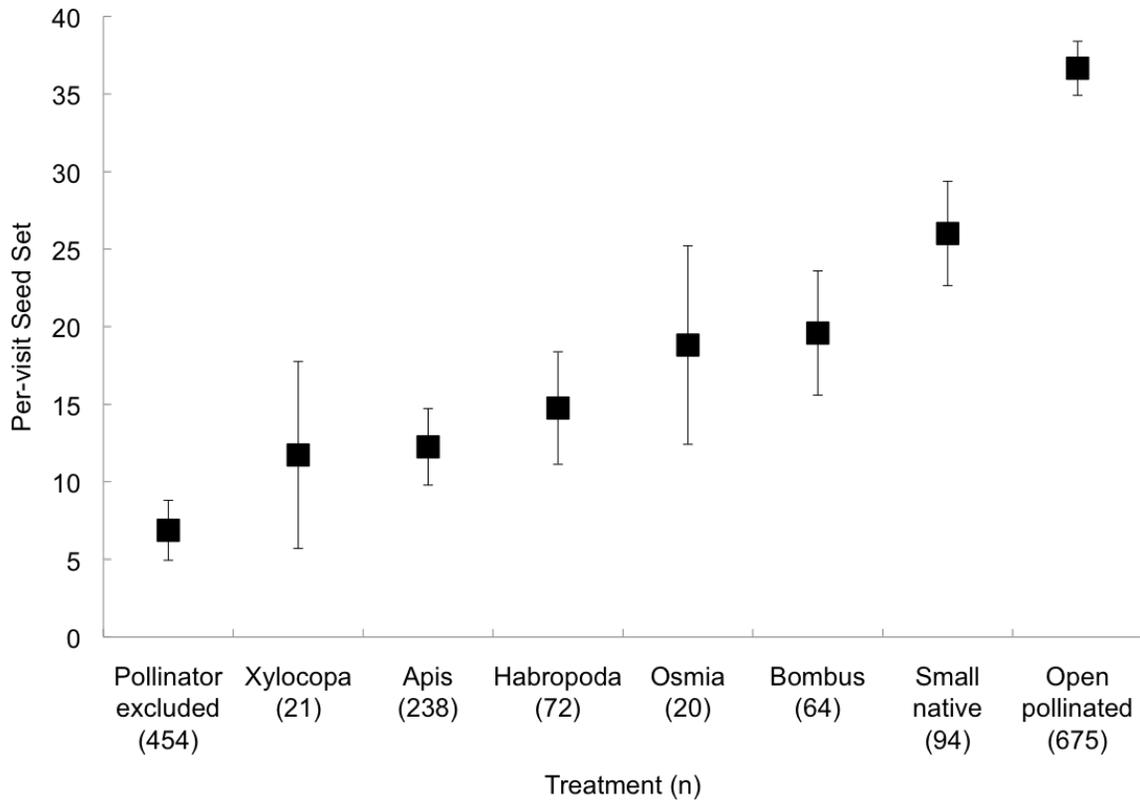


Figure 2. Seed set resulting from a single visit by *Xylocopa*, *Apis*, *Habropoda*, *Osmia*, *Bombus*, or a ‘small native’ bee to *V. corymbosum* flower. Pollinator-excluded and open-pollinated controls included for comparison. Values are based on seed set LSMeans estimates; error bars represent 95% CI.

CHAPTER 2:

BEE DIVERSITY ENHANCES CROP PRODUCTIVITY AND STABILITY

Summary:

Wild bees provide important pollination services to agroecosystems. However, the mechanisms underlying their contribution to ecosystem functioning are unclear. We evaluated the contribution of the wild-bee community to crop productivity, the stability of pollinator visitation, and the efficiency of individual pollinators in highbush blueberry agroecosystems. We surveyed the bee community (through timed observations and pan trapping) and measured pollination of both open- and singly-visited flowers. We found that the abundance of managed honey bees, *Apis mellifera*, and wild bee richness were equally important in describing resulting open pollination. Wild-bee richness was a better predictor of pollination than wild-bee abundance. We also find evidence suggesting pollinator visitation (and subsequent pollination) are stabilized through the differential response of bee taxa to weather (i.e., response diversity). Variation in the per-visit efficiency of *A. mellifera* and the southeastern blueberry bee, *Habropoda laboriosa*, was not associated with changes in the pollinator community.

Key words: biodiversity, ecosystem services, species richness, stability, wild bees

Introduction

Diversity is an important component of ecosystem functioning. Experimental reduction of species richness has been shown to result in productivity declines in many ecosystems (Tilman et al. 2001, Cardinale et al. 2006, Duffy et al. 2007). Species richness (i.e., the number of different species) is positively correlated with the density and diversity of links within species interaction webs (e.g., Ebeling et al. 2011) and can stabilize ecosystem services, such as pollination, in the face of environmental variability and disturbance (Hooper et al. 2005, Balvanera et al. 2006, Haddad et al. 2011).

In agricultural systems, bee diversity may benefit pollination services in at least three ways: (1) pollinator species richness may directly improve fruit and seed set (i.e., productivity), as demonstrated in coffee (Klein et al. 2003) and pumpkin (Hoehn et al. 2008); (2) diverse wild-bee communities may provide stability to pollination services, hedging against declines in managed pollinators (Allen-Wardell et al. 2003, Winfree et al. 2007); and (3) wild-bee richness may enhance the per-visit efficiency of individual pollinators within the community (Greenleaf and Kremen 2006).

Positive correlations between the richness of bee species and pollination may be explained by functional complementarity, sampling effects, or both. Within an ecosystem, functional complementarity exists "when species vary in their contribution to a collective function," such as pollination (Bluthgen and Klein 2011). For foraging bee species, this complementarity may be temporal (time of day or season; Hoehn et al. 2008) or spatial (within plants, or even flowers; Chagnon et al. 1993, Hoehn et al. 2008). Sampling effects, in

which species-rich communities are expected to host and be dominated by more efficient taxa, may also account for the diversity-productivity association (Cardinale et al. 2006). For example, bee species differ in their pollination efficiency (defined as an individual's contribution to pollination in a single flower visit) for a particular plant species (Chapter 1) and diverse bee communities are more likely to include the most efficient pollinators.

The 'insurance effect' is an often-invoked benefit of species diversity, particularly amid concerns over managed pollinator declines (e.g., Winfree et al. 2007). Several mechanisms have been used to predict and explain enhanced stability (or decreased variability) of ecosystem services with increasing biodiversity (e.g., Naeem 1998, Winfree et al. 2007). One such stabilizing mechanism is response diversity – the differential response of organisms to environmental variability (Elmqvist et al. 2003). Bees exhibit a diversity of ecological and life-history traits (Michener 1974), and, to a degree, these traits are predictive of a bee species' response to disturbance (Tuell & Isaacs 2010, Williams et al. 2010). This variability of responses suggests that diverse bee communities will be more resilient to environmental fluctuations. Indeed, Winfree and Kremen (2009) found evidence of response diversity in the pollinator communities of watermelon crops to the proportion of native flora in the landscape.

The composition of a bee community may also influence the foraging behavior of individuals within that community (Inouye 1978, Lichtenberg et al. 2010, Makino and Sakai 2005, Morse 1982, Pinkus-Rendon et al. 2005). Greenleaf and Kremen (2006) were the first to examine this relationship in the context of agricultural pollination and pollinator efficiency. In the hybrid sunflower system, in which male and female cultivars are grown in

alternating rows, they found that increasing wild bee abundance was positively correlated with per-visit seed set of honey bees, *Apis mellifera*, through an increased rate of interspecific encounter, avoidance, and movement of pollen-laden bees among plants (Greenleaf and Kremen 2006). This singular finding merits examination in other types of agroecosystems, particularly in crops where individual cultivars are monocropped rather than interspersed, such as highbush blueberry (*Vaccinium corymbosum*).

As Hooper et al. (2005) and others (e.g., Bluthgen and Klein 2011) have noted, theoretical work on biodiversity and ecosystem functioning has far outpaced empirical field research, particularly in agroecosystems. In response to this deficit, we conducted an observational study of bee diversity and pollination services in highbush blueberry, a crop that is widely grown throughout the United States and Canada and hosts a diverse, well-documented bee community (Adamson 2011, Cane and Payne 1993, MacKenzie and Eickwort 1996, MacKenzie and Winston 1984, Ratti et al. 2008, Tuell et al. 2009). Though self-fertile, *V. corymbosum* benefits from outcrossing (Dogterom et al. 2000, Huang et al. 1997) and relies on insect-pollination for agricultural production (Delaplane and Mayer 2000).

We analyzed the relationship of species richness to pollination services in highbush blueberry agroecosystems in North Carolina from each of the three perspectives described above: productivity, stability, and individual efficiency. We (1) constructed a descriptive model of pollination services to describe the relative contribution of highly-abundant managed *Apis* bees and wild bees to open-pollinated seed set; (2) tested the mechanism of response diversity in stabilizing pollinator visitation with respect to variability in foraging

conditions; and (3) evaluated the per-visit efficiency of two pollinator taxa (*A. mellifera* and the southeastern blueberry bee, *Habropoda laboriosa*), along a gradient of total and heterospecific bee abundance and bee species richness that exists among sampling locations and over the course of *V. corymbosum* bloom. We hypothesized that there would be a positive correlation between bee diversity and all three measures of pollination services.

Materials and Methods

Study system

We sampled the bee community and measured pollination in commercial blueberry farms in southeastern North Carolina. We selected two farms in 2010 and added a third site in 2011. Each farm contained at least 40 ha of blueberries in active production. We collected pollination data from *V. corymbosum* ‘Oneal’, a commonly grown cultivar with a long bloom period. Each site was visited multiple times over the course of bloom from 17-Mar-2010 to 5-Apr-2010 and 14-Mar-2011 to 23-Mar-2011.

In each planting, we placed WatchDog data loggers to record the hourly temperature. Hourly solar radiation and wind speed data were obtained through a local NC Environment and Climate Observing Network station. We quantified the percent bloom by counting all flowers (in either bud, bloom, or petal-fall) from four branches on separate plants per sampling location, and then calculated the proportion of flowers that had developed beyond the bud stage. Based on this proportion, we categorized the phenological stage of a planting as either early- (≤ 0.33), middle- ($0.33 < p \leq 0.66$), or late-bloom (> 0.66). We sampled each site at least once per bloom stage, with the exception of one site in the first year of the study.

Bee community

To assess the composition of the foraging bee community, we conducted timed observations along two transects per sampling location (two locations per farm in 2011). In 2010, we observed each 200-ft transect for two minutes, and repeated observations at 900, 1100, 1300, and 1500 h. In 2011, we observed 100-ft transects and have therefore doubled 2011 bee counts to normalize for sampling effort. We counted all actively foraging bees—including those engaged in nectar robbing (Inouye 1980)—and classified these into five groups that could be distinguished on the wing: *Apis mellifera*, *Bombus* spp., *Habropoda laboriosa*, ‘small native’ bees, and *Xylocopa virginica*; hereafter, bee groups will be referenced by genus name only. Based on transect counts, we calculated bee abundance and richness (number of species groups) for each site and within-site location per visit.

We employed pan trapping to complement our direct observations and to enable genus and species-level identification of bees. We constructed pan traps from plastic, 96-ml soufflé cups that we painted either white, fluorescent yellow, or fluorescent blue, then filled all pans halfway with soapy water (Droege 2010). Using Velcro, we attached pans atop 1.2-m step-in posts to elevate traps into the plant canopy (see Tuell and Isaacs 2009). In 2010, we placed two trap lines with 10 traps per line in the same cultivar block in which timed bee observations were conducted. Equal numbers of each color pan trap were randomly ordered and spaced 3 meters apart within rows (Droege et al. 2010). In 2011, we increased pan trap sampling in each cultivar block to three trap lines with 12 pan traps per line. We adjusted 2010 pan trap captures by a factor of 1.8 to account for differences in sampling effort. We separated trap lines from each other by three rows of plants, and from bee observation

transects by one row of plants. We established traps at 800 h and collected them at 1600 h during each site visit.

We identified pan-trapped specimens to genus and species level, as practical. We used both published and online guides—Michener et al. (1994), Mitchell (1960, 1962), and the Apoidea section of www.discoverlife.org—and the North Carolina State University Insect Museum reference collection to identify bees. Voucher specimens have been deposited in this same museum.

Blueberry pollination

We sampled fruits resulting from both open-pollinated flowers and flowers visited by a single bee. To determine per-visit pollinator efficiency, we placed No-see-um[®] mesh (Denver Fabrics, Denver, CO) cages on *V. corymbosum* branches without open flowers (which were removed as necessary) on four to eight plants per sampling location. During bloom, we returned, removed cages and observed virgin flowers for visitation by either *Apis* or *Habropoda*. Though other bees were common at flowers, *Apis* and *Habropoda* were particularly abundant across sites and years, and represent poly- and oligo-lectic pollinators, respectively, present in blueberry culture throughout the southeast (Cane and Payne 1993). Once visited by a bee, we marked a flower by tying embroidery thread around the pedicel. We distinguished flowers visited by *Apis* and *Habropoda* with different thread colors. We left flowers (five per plant) that were in a similar stage of bloom uncaged for the open pollination treatment. Pollination treatments were established on the same day that bee observations and pan trapping took place.

We collected fruit samples approximately 50 days following each site visit. We placed berries in cold storage until they could be dissected to count seeds. Blueberries produce both viable and non-viable seeds (Dogterom et al. 2000). We counted apparently-viable seeds to quantify seed set per fruit. In 2010, we counted only ‘large’ seeds as viable; in 2011, we counted seeds >1.3 mm in length as viable (for methods, see Chapter 1). Blueberry seed set is positively correlated with both fruit weight and volume (Chapter 1), but it is a more direct measure of pollination and less sensitive to cultural practices such as irrigation and pruning.

Statistical analysis

We calculated the number of bees from each group counted in a day’s worth of sampling effort (two transects, observed four times) per location. We then counted the number of different species groups present (species richness) in that same sampling period. We tested for correlation between transect and pan trap observations for each bee group using a multivariate analysis of variance in SAS Proc GLM (for all analyses: SAS version 9.2, SAS Institute, Cary, NC, USA). Our response variables were transect and pan trap counts; site and year were included as random effects. We also tested for correlation within and between species group densities (both *Apis* and wild bee groups) and community measures in Proc GLM.

We analyzed pollination through a generalized-linear mixed model (SAS Proc GLIMMIX). Our response variable was open-pollinated seed set per fruit. Due to correlation among the densities of wild-bee groups (*Bombus*, *Habropoda*, ‘small native’, and *Xylocopa*),

we combined their counts into one ‘wild bee’ group. Our predictor variables were *Apis* abundance, wild bee abundance and richness, and year. Though correlated, we included both wild bee abundance and richness in the model to determine which was more informative. Through simple bivariate comparisons we observed that *Apis* and wild bee abundance were logarithmically related to seed set, and thus log-transformed [$\log(x+1)$] both variables in our full model. Because of differences in the 2010 and 2011 seed count methods, we included year as a fixed effect for all models in which seed set was the response variable. *Apis* abundance was positively correlated with temperature and solar radiation and negatively correlated with wind speed. Thus these weather variables were not included as covariates in the model. We expect that the direct effect of these variables on pollination is negligible compared with their indirect effect on pollination via the bee community. Site and plant (nested within year, site, and visit) were treated as random effects. To account for differences in sample size (berries per sampling period), we used the Satterwhaite method to approximate degrees of freedom. Because a r^2 statistic cannot be obtained through maximum-likelihood estimation methods, we calculated a pseudo r^2 [$1 - (\text{sum of variance components})/(\text{total variance})$] to estimate the descriptive value of our model.

To test the hypothesis of response diversity of pollinator taxa to variation in weather, we evaluated the interaction between taxa and weather condition in a model describing pollinator visitation (number of bees per group counted in transect walks per day of sampling effort), a method first developed and employed by Winfree and Kremen (2009). Bee counts were square-root transformed to normalize the distribution of residuals. Pollinator taxa were *Apis*, *Bombus*, *Habropoda*, ‘small native’, and *Xylocopa*. Daily weather variables

(temperature, solar radiation, and wind speed) were calculated as the mean of hourly weather collected between 900 and 1700 hours (when foragers are active). Daily weather conditions were classified as ‘optimal’ if temperature $\geq 18.3^{\circ}\text{C}$, solar radiation $\geq 500 \text{ W m}^{-2}$, and wind speed $\leq 13 \text{ km h}^{-1}$. Daily weather that did not satisfy all of these conditions was classified as ‘inclement’. We conducted the analysis in SAS Proc GLIMMIX to allow for incorporation of random effects: site by year, and visit (nested within site and year).

We analyzed the per-visit efficiency of *Apis* and *Habropoda* along a gradient of both total and heterospecific bee abundance, and total bee species richness. Per-visit seed set was first arcsin-root transformed for normality. Solar radiation, which we suspected of affecting the physical extraction and dispersal of pollen, and year were included as fixed effects in all models. We retained variables all variables for which $p < 0.25$. We included site and plant (nested within year, site, and visit) as random effects in SAS Proc GLIMMIX and used the Satterwhaite approximation of degrees of freedom. From these analyses we present back-transformed estimates of per-visit seed set $\pm 95\%$ CI.

Results

Bee community

We observed a total of 2177 bees in transect sampling and captured 219 bees in pan traps in 23 days of sampling effort over the course of *V. corymbosum* bloom in 2010 and 2011 (table 1). From pan-trapped specimens, we identified five families (Andrenidae, Apidae, Colletidae, Halictidae, and Megachilidae) and 12 genera of bees. Two additional genera (*Osmia* and *Specodes*) were collected in traps placed around irrigation ponds at farms. One species,

Andrena bradleyi, was highly abundant at all stages of bloom, constituting 33 to 61% of all pan-trapped ‘small native’ bees. Pan trap captures were not correlated with transect observations for *Apis* ($r = 0.14, p = 0.53$), *Habropoda* ($r = 0.13, p = 0.57$), or ‘small native’ bees ($r = 0.11, p = 0.64$), and no *Xylocopa* were collected in pan traps. Pan trap and transect observations were correlated for *Bombus* ($r = 0.47, p = 0.03$).

Productivity

In biodiversity—productivity studies, species richness and total abundance are often positively confounded, making it difficult to distinguish their contribution to ecosystem services (Balvanera et al. 2006). Though we did not control pollinator density at our sites, bee abundance and species richness (total or wild bee) were not significantly correlated in our system (total richness, $r = 0.32, p = 0.13$; wild bee richness, $r = 0.25, p = 0.24$), enabling us to consider their contribution separately. We suspect the independence of these factors is due to the importation of managed *Apis*, as bee abundance was highly correlated with *Apis* abundance ($r = 0.96, p < 0.0001$), while spatial and temporal factors may drive species richness. Wild bee abundance and richness were correlated ($r = 0.77, p < 0.0001$), but neither measure was related to *Apis* abundance (wild bee abundance, $r = 0.12, p = 0.57$; wild bee richness, $r = 0.04, p = 0.86$).

Both *Apis* abundance (figure 1a) and wild bee richness (figure 1b) were significant factors describing pollination (seed set) in blueberry (table 2; pseudo $r^2 = 0.58$). Wild bee abundance was less informative than richness and was removed from the final model. However, if richness is excluded from the model, wild bee abundance is significantly

correlated with pollination (est \pm SE: 4.06 ± 1.24 , $F=10.80$, $p = 0.0014$). Seed set was significantly higher in 2010 than 2011; we suspect this reflects the difference in our seed count methods, rather than biological changes.

Response diversity

In 2010, weather conditions were ‘optimal’ on two days and ‘inclement’ on four days. In 2011, weather conditions were ‘optimal’ on three days and ‘inclement’ on six days. In both years, ‘inclement’ weather conditions were experienced during each bloom stage. The species groups present in our system exhibited response diversity with respect to weather, indicated by the significant interaction between taxa and weather condition (table 3).

Whereas *Apis* were three times less abundant in inclement than optimal weather conditions ($p = 0.009$, figure 2a), wild bee density did not differ ($p = 0.71$, figure 2b). On average, blueberry experienced reduced pollination (-12.9 seeds fruit⁻¹) during periods of inclement weather ($p < 0.0001$, figure 2c).

Community effects on per-visit efficiency

The per-visit efficiency of *Apis* ($n = 238$) was not affected by wild or total bee abundance ($p = 0.85$ and 0.47 , respectively) or richness ($p = 0.79$). *Habropoda* per-visit efficiency ($n = 72$) was not correlated with the abundance of heterospecific bees ($p = 0.21$) but trended negatively with total bee abundance (-0.03 ± 0.01 seeds visit⁻¹, $p = 0.08$) and richness (-0.32 ± 0.23 , $p = 0.06$).

Discussion

Diversity is an important component of ecosystem functioning (Hooper et al. 2005). Despite intensive management, farms function as ecosystems and are often situated in or near wild landscapes that provide ecosystem services (Lonsdorf et al. 2009, Garibaldi et al. 2011). In highbush blueberry, we find that wild bee diversity improves ecosystem functioning by enhancing the quantity of pollination services and the stability of pollinator visitation.

This study is the first to document the pollinator community of highbush blueberry agroecosystems in North Carolina. In addition to managed *Apis*, wild bees were common at blueberry flowers, accounting for nearly a fifth of all visits. *Habropoda laboriosa* and *Andrena bradleyi*, two *Vaccinium* oligoleges that sonicate flowers to release pollen tetrads, were abundant at all sampling locations. While *H. laboriosa* has been enthusiastically studied in southeastern blueberry systems (Cane and Payne 1988, Cane 1997, Pascarella et al. 2007), less is known about *A. bradleyi*. This latter species has been documented in *Vaccinium* crops, including cranberry, along the eastern US coast (Massachusetts; Loose et al. 2005; Maine; Stubbs et al. 1992; and Nova Scotia; Sheffield et al. 2003) but is absent in more interior (Michigan; Tuell et al. 2009) and western (British Columbia; Ratti et al. 2008) farms.

Pan traps have many benefits (Westphal et al. 2008), yet they may poorly sample the bee community when in competition with abundant floral resources (Cane et al. 2000) and under-represent large bee species such as *Apis* (Westphal et al. 2008) and *Bombus* (Roulston et al. 2007). Our findings in blueberry suggest that pan traps are indeed ineffective at documenting the relative abundance of bees visiting a mass-flowering crop. Pan-trap captures were not correlated with direct observations for *Apis*, *Habropoda*, 'small native' bees, or

Xylocopa. To our surprise, this was not the case for *Bombus* spp., though only a total of five bees were caught in two years of sampling. In the absence of direct observations or sweep netting, pan traps may mislead us as to the relative importance of different bee species in agricultural landscapes.

In our model of pollination services, *Apis* and wild bees were equally descriptive of seed set. Pollination increased logarithmically with *Apis* density, indicating relative saturation at higher densities. Because wild bee abundance and richness were highly correlated, we could not consider their contribution to pollination independently. However, wild bee richness was more informative of blueberry pollination. This is suggestive of two mechanisms: a sampling effect (species-rich bee communities may be more likely to host more efficient blueberry pollinators such as *Bombus* and ‘small native’ bees; Chapter 1) and functional complementarity (a species-rich community may host more functionally-diverse groups that visit plants in complementary ways; Hoehn et al. 2008).

In eastern North Carolina, *V. corymbosum* blooms for several weeks in early spring and is subject to variable weather conditions during this time. As weather influences the foraging behavior of bees (e.g., Vicens and Bosch 2000), it is an essential component of pollinator community dynamics in these agroecosystems. In Michigan blueberry systems, Tuell and Isaacs (2010) found that *Apis* were the dominant flower visitors during ‘good’ weather, while *Bombus* were dominant in ‘poor’ weather. Similarly, our analysis of response diversity shows that blueberry pollinators respond differently to changes in weather, with the number of *Apis* foragers dramatically reduced in inclement weather conditions. Wild bees were less affected by changes in weather and thus may reduce the temporal variability of

pollination services. Moreover, response diversity may act as a form of functional complementarity, enhancing ecosystem productivity, in addition to stability (Bluthgen and Klein 2011).

We found that *Apis* and *Habropoda* per-visit efficiencies were not significantly correlated with total or heterospecific bee abundance, or bee species richness. Taken together with those of Greenleaf and Kremen (2006), our findings suggest that community effects on per-visit efficiency are system dependent. Hybrid sunflower and highbush blueberry represent two very different cropping systems. In hybrid sunflower, male and female cultivars are alternated between rows. In highbush blueberry, a single, clonally-propagated cultivar is often planted over a large area (several hectares), so that the movement of bees between plants may not improve the transfer of xenogamous pollen (Sanchez-Lafuente et al. 2012). Hence cultural practices may constrain variation in per-visit efficiency in this system. Our understanding of the relationship between bee community and pollinator efficiency would benefit from future work in other pollinator-dependent systems (such as cucurbits and apples) with diverse flower morphologies and cropping practices.

We demonstrate two important ways that wild bees enhance pollination success; increased productivity and temporal stability. These findings add to a growing body of research showing that wild bee communities provide important pollination services to agroecosystems (Klein et al. 2003, Hoehn et al. 2008, Garibaldi et al. 2011). Aided by a better understanding of pollinator community composition, and the ability to conduct molecular ecology via genetic tools, we are hopeful that future research will elucidate how pollinator populations are affected by agricultural pressures within and among farms. *A.*

bradleyi, clearly an important component of blueberry agroecosystems along the eastern seaboard, provides an excellent opportunity for such work.

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Table 1. Bee species observed in transects and pan traps during early, middle, and late stages of blueberry bloom in eastern North Carolina. Transect and pan trap counts represent the total number of bees observed at three sites on multiple days (indicated by sampling effort) in 2010 and 2011. Bees observed at all sites (in either transects or traps) are indicated with an asterisk (*).

bloom stage	early		middle		late	
sampling effort (days)	(n = 8)		(n =7)		(n = 8)	
method	transect	pans	transect	pans	transect	pans
<i>Apis mellifera</i> *	227	65	504	34	1036	28
<i>Bombus</i> spp.*	1	1	8	2	11	1
<i>B. bimaculatus</i>		1		2		0
<i>B. impatiens</i>		0		0		1
<i>Habropoda laboriosa</i> *	90	2	116	2	52	2
'small native' bees*	3	31	27	33	40	18
<i>Agapostemon</i>		0		1		1
<i>splendens</i>						
<i>Andrena bradleyi</i> *		19		20		6
<i>Andrena</i> spp. *		6		8		6
<i>Augochlora pura</i> *		1		1		1
<i>Ceratina</i> spp.		1		0		0
<i>Colletes</i> spp.		1		0		1
<i>Halictus rubicundis</i>		0		1		0
<i>Lasioglossum</i>		2		0		3
(<i>Dialictus</i>) spp.						
<i>Lasioglossum</i>		0		1		0
(<i>Evyllaesus</i>) spp.						
<i>Nomada</i> spp.		1		1		0
<i>Xylocopa virginica</i> *	7	0	17	0	38	0
total bees	328	99	672	71	1177	49

Table 2. Descriptive model of pollination services (measured as open-pollinated seed set) provided by *Apis* and wild bees in highbush blueberry. Values reported are result of generalized-linear mixed models analysis with Type III test of fixed effects.

fixed effects	estimate \pm SE	<i>F</i>	<i>p</i> -value
Intercept	16.83 \pm 4.29	15.37	0.0012
<i>Apis</i> abundance*	2.64 \pm 0.84	9.95	0.0022
wild bee richness	3.66 \pm 1.06	11.91	0.0007
year †	-6.24 \pm 2.99	4.34	0.0391

*Data was log-transformed. †Estimate represents difference of 2011 from 2010.

Table 3. Analysis of response diversity of blueberry pollinators to ‘inclement’ and ‘optimal’ daily weather conditions. Response variable is number bees counted in transect walks (per taxonomic group). Bee counts were square-root transformed. Values reported are result of generalized-linear mixed model with Type III test of fixed effects.

source of variation	df	<i>F</i>	<i>p</i> -value
taxa*	4, 98	41.36	<0.0001
weather	1, 13	4.81	0.047
taxa × weather	4, 98	6.42	0.0001

*Bees were classified into five species groups (*Apis*, *Bombus*, *Habropoda*, ‘small native’, and *Xylocopa*).

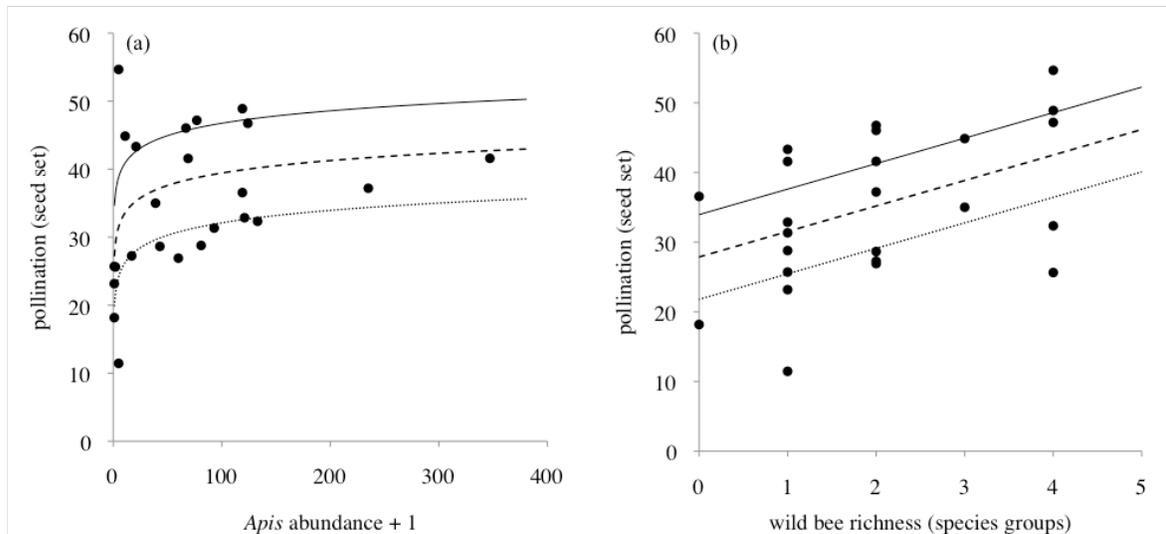
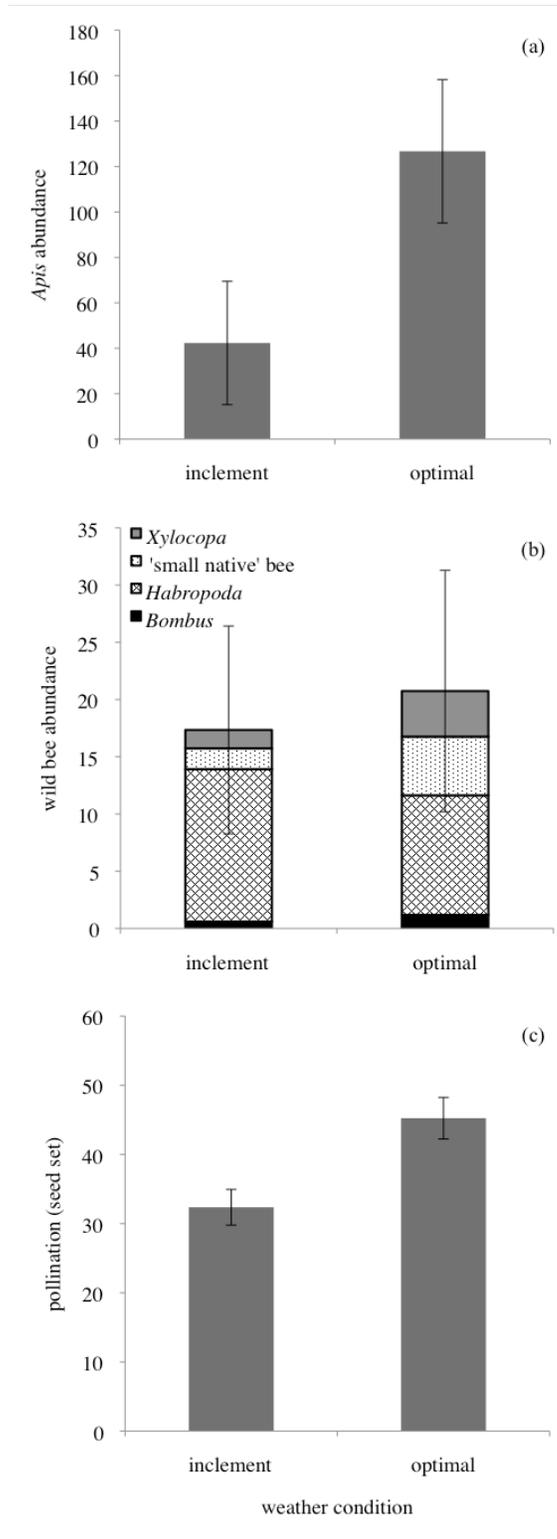


Figure 1. Relationship of pollination (open-pollinated seed set) to *Apis* abundance (a) and wild bee richness (b) in highbush blueberry. Each point represents the mean seed set per observational period (1 day). Trend lines are based on model estimates described in table 2 (using mean of year estimates) at different levels of *Apis* and wild bee richness. For (a), logarithmic fits represent wild bee richness of 0 (dotted line), 2 (dashed line), and 4 (solid line). For (b), linear fits represent *Apis* abundance of 2 (dotted line), 20 (dashed line), and 200 (solid line).

Figure 2. Mean estimates (with SE bars) of *Apis* abundance (*a*), wild bee abundance (*b*), and open-pollinated seed set (*c*) during 'inclement' and 'optimal' daily weather conditions in blueberry fields.



CHAPTER 3:
ENCOUNTERS BETWEEN BEES INFLUENCE FORAGING BEHAVIOR

Abstract

In both wild and agricultural ecosystems, multiple bee species may forage simultaneously at a common resource. Physical encounters between these bees may modify their subsequent foraging behavior and shape pollinator distribution and resource utilization in a plant community. We observed interactions between honey bees, *Apis mellifera*, and bumble bees, *Bombus impatiens*, visiting artificial flowers in a controlled, outdoor foraging arena. Both species were more likely to leave the feeder following an encounter with another bee, but *Apis* and *Bombus* differed in their responses to intra- and inter-specific encounters. Encounters with con- and hetero- specific individuals influenced *Apis* foraging behavior similarly. However, most *Bombus* that encountered *Apis* discontinued foraging at the observed station, but exhibited only a slight decrease in foraging following an intraspecific encounter. Interactions between bees that elicit changes in foraging behavior may have important implications for the pollination of wild and managed plants.

Keywords: *Apis*, *Bombus*, competition, interspecific avoidance, pollination

Introduction

Bees visit flowering plants for nectar and pollen resources. The community of bees foraging at a particular plant population is shaped by multiple interacting factors. These include biogeography and seasonal phenology (Mitchell 1960, 1962; Michener 1979), the availability and distribution of suitable nesting habitat in the landscape (Steffan-Dewenter et al. 2002; Williams et al. 2010), the relative attractiveness and accessibility of a plant population's nectar and pollen resources (variable with respect to different bee taxa; e.g., Dedej and Delaplane 2005), and the presence of co-flowering plants in the landscape which provide for vibrant bee communities (Westphal et al. 2003; Williams and Kremen 2007), which may either compete with or facilitate pollination in the target plant population (Feinsinger 1987; Feldman et al. 2004). Less understood is how encounters between con- and hetero-specific bees at floral resources influence foraging behavior.

Bees may alter their patterns of resource visitation and utilization in response to changes in their forager community. By manipulating forager composition, Inouye (1978) and Morse (1982) found that—in the presence of heterospecifics—some individual bumble bees (*Bombus* spp.) exhibited niche partitioning and shortened foraging bouts, while others remained unaffected. Though neither author reported physical encounters between foraging bees, these interactions have been observed in other systems (Eickwort and Ginsberg 1980) and may be particularly common (and impactful) in agricultural settings where the importation of managed bees (particularly honey bees, *Apis mellifera*) can result in a high density of foragers per floral resource. In squash and watermelon crops, for example, Pinkus-Rendon et al. (2005) found that contact between heterospecific bees often resulted in local

displacement, with certain species being displaced more often than others. Similarly, in sunflower, Greenleaf and Kremen (2006) found that *A. mellifera* were three times more likely to move between rows of male and female cultivars after interacting with a wild bee and, in doing so, enhanced the transfer of pollen and their per-visit pollination efficiency.

Still, relatively little is known about the impact of between-bee encounters on subsequent foraging behavior. In tropical stingless bees, Lichtenberg et al. (2010) showed that interspecific encounters mediated dominance of feeder stations by group-foraging species. Here, we examined the encounters between two bee species, *A. mellifera* and *Bombus impatiens*, which co-occur in wild- and managed-plant populations of eastern North America, in a controlled foraging environment with artificial plants to determine if bees were more likely to leave a resource following an encounter with another bee.

Materials and Methods

We established experimental foraging arenas in a hoop house covered with 70% shade cloth, then placed 10 feeding stations, designed to simulate plants, per arena (Fig. 1a). Each ‘plant’ consisted of a foraging platform with five ‘flowers’ (1.5 mL Eppendorf tubes) inserted in a florist foam block covered by a yellow card (Fig. 1b). Dark blue petals were printed beforehand on each card in a ring of four petals, with the Eppendorf tube being inserted in the center. We elevated foraging platforms on 1-m wooden dowels.

We placed one five-frame nucleus colony of *Apis* (~10,000 bees per colony) and between two and four *Bombus impatiens* colonies (depending on colony activity levels; ~ 30 to 40 bees per colony; Research Hive – Mini, Koppert Biological Systems, Inc., Howell, MI,

USA) in foraging arenas 12 to 24 hours before experiments. To train bees to feeders, we added ~0.5 mL of 2-M sucrose solution scented with 1% (v/v) peppermint oil to each flower. At the start of data collection, we replaced the training feeders with new foraging platforms. These new flowers contained 5- μ L, 2-M scented sucrose solution (a field-relevant volume of nectar; e.g., *Vaccinium ashei*; Dedej and Delaplane 2005). For 30 min following the placement of new feeders, we recorded foraging activity at three stations per arena using video cameras (Bloggie Touch Camera, Sony Corporation of America, New York, NY, USA). We conducted foraging observations five times: twice in the afternoon (12-4pm) on 15-Apr-2011, then twice in the morning (10am-12pm) and once during the afternoon of 2-May- 2011. We sampled different arenas (with different bee colonies) on the same day, and rotated colonies between foraging areas on different sampling days. Between sampling days, colonies were placed outside of the hoop house and allowed to forage normally.

We transcribed the sequence of bee activity at feeders from each video recording. We documented bee arrival and departure, encounters with another bee of either species (either physical contact or proximity <1 cm), foraging (entering flowers completely so that the apex of the metasoma was not visible above the lip of the Eppendorf tube), and activities related to flower inspection (such as circling and inspecting flowers without entering). We performed a Chi-squared analysis to determine if a physical encounter affected the likelihood of a bee's next recorded activity (either continuing to forage, leaving the feeder, or engaging in flower inspection at the same feeder).

Results

In 10 h of total recorded activity, we observed *Apis* engaged in 249 foraging events and 400 flower inspections and *Bombus* engaged in 225 foraging events and 366 flower inspections. Individuals of both species that interacted with another bee, regardless of its species, were less likely to continue foraging and more likely to leave the station compared with bees that had just foraged (*Apis*, $\chi^2_{3,384} = 23.8$, $P < 0.0001$, Fig. 2a; *Bombus*, $\chi^2_{3,258} = 22.9$, $P < 0.0001$, Fig. 2b). Flower inspection increased for *Apis* but not *Bombus*, following an interaction with another individual (Fig. 2a,b).

Apis and *Bombus* responded differently to intra- and inter-specific encounters. *Apis* that interacted with *Bombus* or other *Apis* were equally likely to leave the station, continue foraging, or inspect flowers ($\chi^2_{3,188} = 0.003$, $P = 1.0$, Fig. 2a). *Bombus*, however, tended to stop foraging at the observed station altogether after an encounter with *Apis*, while exhibiting only a small decrease in foraging after intraspecific contact ($\chi^2_{3,58} = 14.2$, $P = 0.003$, Fig. 2b).

Discussion

Our results show that when a foraging bee meets another bee, it is more likely to move from the foraging source than if it does not meet another bee. The frequency of this displacement depends on the identity of both bees involved in the interaction. *Bombus* exhibited drastic changes in foraging behavior following an interaction with *Apis*, while *Apis* responded similarly to both intra- and inter-specific interactions. Interference competition (physical blocking or behavioral aggression), exploitative competition (real or inferred resource depletion), or both may help to explain why bees are more likely to leave a plant after

meeting another bee. Following an encounter, *Apis* were more likely to engage in flower inspection, suggesting they were assessing resource depletion. However, *Bombus* reduced both flower inspection and foraging at the plant after meeting *Apis*, which suggests that they were more sensitive to interference competition. We did not observe any evident forms of behavioral aggression within or between species.

Because we used multiple colonies of *Bombus*, we anticipated finding higher levels of conspecific avoidance in *Bombus* than in *Apis* (with foragers originating from a common colony). However, our results show the opposite trend. While nestmate recognition has been documented in both *Apis* and *Bombus* (Breed 1983; Foster 1992) and influences bee activity at the nest entrance (Downs and Ratnieks 2000), it may have little or no impact on bees at floral resources. We know of only one report of intercolony aggression (between *Apis*) witnessed at feeding sites (Kalmus 1941).

To better understand the relative significance and impact of encounters between foraging bees, three questions must be answered:

- i. What is the frequency of bee encounters in more realistic, field conditions? By introducing a high density of bees into arenas with limited foraging options, we artificially elevated the frequency of such interactions. In an equal amount of video footage (10 hrs) from three blueberry fields (stocked with 2-10 *Apis* colonies ha⁻¹), we observed 67 instances of *Apis* foraging, with only one encounter between *Apis* (Appendix B). However, both Pinkus-Rendon (2005) and Greenleaf and Kremen (2006) observed many interactions between *Apis* and wild bees at cucurbits and sunflowers, respectively. Interaction frequency may vary in response to floral

morphology as the open morphology of cucurbits and especially sunflowers enables simultaneous visitation by multiple bees.

- ii. How important is physical contact between bees to foraging behavior, compared with other means of detection of other bees (e.g., an earlier forager's footprints)? *Bombus* may infer information about both resource profitability and depletion from the presence of conspecifics at feedings sites (Baude et al. 2011). Moreover, while bees may be present at flowers for less than a minute, chemical evidence of their visit may last up to 40 minutes (Stout and Goulson 2001). Both *Apis* and *Bombus* use these cues (detected while hovering over the surface) to avoid recently visited flowers and improve foraging efficiency (Stout and Goulson 2001; Gawleta et al. 2005; Witjes and Eltz 2007). A challenge for future studies is separating the response of bees to physical encounters from their response to chemosensory cues that are likely correlated with encounter rate.
- iii. How do bees respond to being displaced through encounters? Bees that move among plants of the same species may improve pollen dispersal and pollination (Greenleaf and Kremen 2006; Sanchez-Lafuente et al. 2012). However, if bees move to another plant species (Inouye 1978) or stop foraging altogether, pollination of the focal plant population may be reduced. The response of bees to physical encounters likely depends on both the plant and bee community (Inouye 1978; Potts et al. 2003; Raine and Chittka 2007; Baude et al. 2011).

Our study shows that between-bee encounters influence foraging behavior and may

shape resource utilization. However, several unknowns must be addressed before we can evaluate the impact of these encounters to foraging in both wild and agricultural settings. To address these challenges, we recommend that researchers track the movement of bees among plants in realistic field conditions, rather than monitor activity at individual feeder stations. It should become increasingly feasible to track foraging bees across large areas as the appropriate technology develops (e.g. Ohashi et al. 2010).

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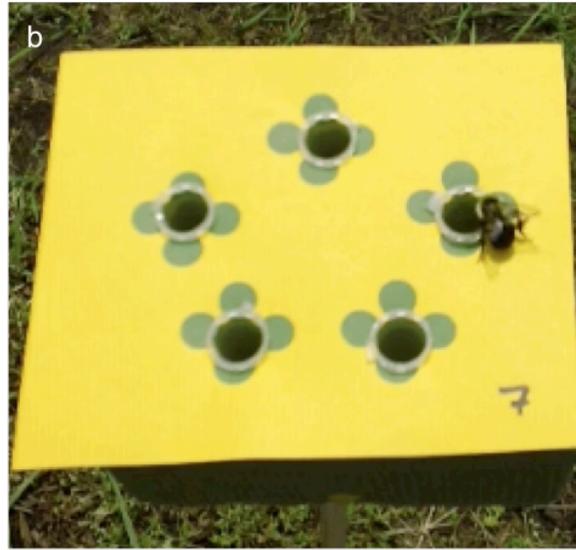
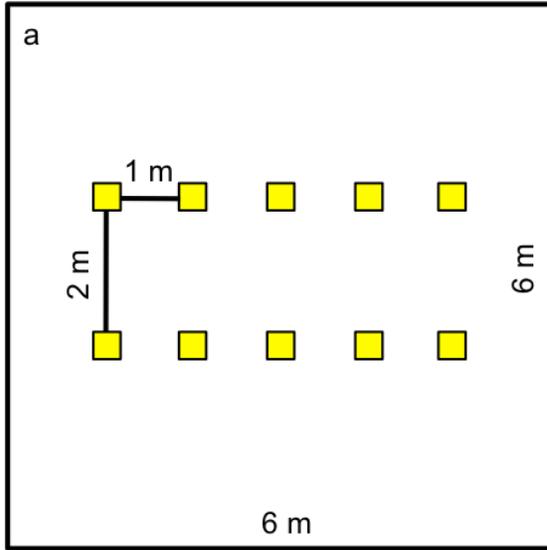


Figure 1. Foraging arena setup (a) with artificial plants. Surface of artificial plant (b) with *Bombus* inspecting 'flower'.

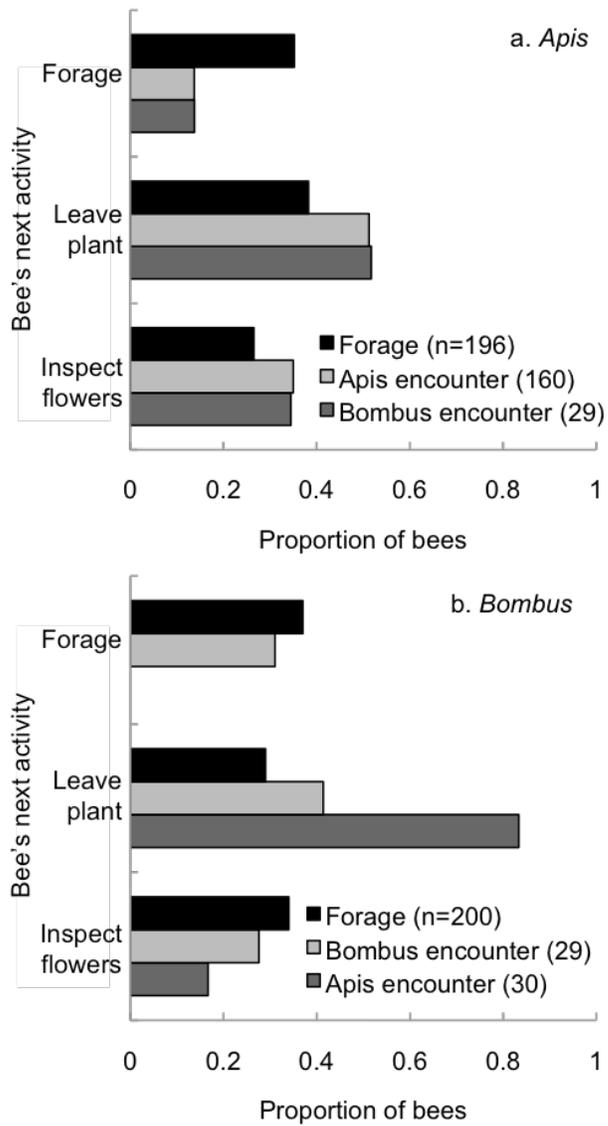


Figure 2. Proportion of (a) *Apis* and (b) *Bombus* that continued foraging, left the feeder, or engaged in another activity following either a foraging event or an intra- or inter-specific encounter.

APPENDIX

APPENDIX A:
FLOWER MORPHOLOGY SHAPES POLLINATOR COMMUNITY WITH
IMPLICATIONS FOR CROSS-POLLINATION: OBSERVATIONS IN RABBITEYE
BLUEBERRY, *VACCINIUM ASHEI*, CULTIVAR 'PREMIER'

Introduction

Rabbiteye blueberry (*Vaccinium ashei*) is commercially cultivated throughout the southeastern United States. Compared to other blueberry species, *V. ashei* flowers have a particularly long corolla with a narrow aperture (Fig 1a; Lyrene 1994). This constricted flower morphology is presumed to restrict shorter-tongued nectar foragers, particularly honey bees (*Apis mellifera*), from accessing nectaries, resulting in reduced visitation and pollination (e.g. Lyrene 1994; Ritzinger and Lyrene 1999). However, we are unaware of empirical evidence supporting this assertion.

Unlike other rabbiteye cultivars, ‘Premier’ often has abnormal flowers (Fig. 1b) in which the corolla is irregularly shortened, and, in some cases, does not extend beyond the calyx. Fittingly, Sampson and Cane (2000) described these flowers as “slipper-shaped.” This morphological mutation may provide easier access to nectaries, making them more attractive to *Apis*, which forage primarily for nectar in blueberry crops (Dogterom 1999). Rabbiteye cultivars are typically self-sterile and thus are intercropped with other rabbiteye cultivars to promote cross-pollination (Delaplane and Mayer 2000).

Observations

In 2009 and 2010, we observed bees visiting rabbiteye flowers at commercial blueberry farms in eastern North Carolina as part of a larger survey of blueberry pollinators (Chapter 2). While conducting transect observations in a planting of ‘Premier’ (Fig. 1b) intercropped with ‘Powderblue’ (Fig. 1a), we noted a distinct difference in the species and quantity of bees at these adjacent cultivars. Accordingly, we conducted four 15-min paired observations of

‘Premier’ and ‘Powderblue’ plants, using different plant pairs each time. Two investigators observed one plant each, counting all *Apis* and wild bees (predominantly *Bombus* spp. and *Habropoda laboriosa*) visiting flowers, and we alternated cultivars between observational periods so as to reduce observer bias. We then compared these data with 15-minute plant observations from 2009 from multiple sites (3) and sampling days (10) in which rabbiteye cultivars ‘Premier’ ($n = 15$), ‘Powderblue’ (9), and ‘Brightwell’ (28) were observed on their own.

We analyzed our data to determine if ‘Premier’ plants attracted a different community of bees than rabbiteye cultivars with normally-shaped flowers (‘Powderblue’ and ‘Brightwell’). Using a generalized linear mixed model, we tested for an interaction between cultivar and bee taxa (*Apis* or wild bee). We treated sites, nested within year, as random factors. For our analyses, we log-transformed bee-count data [$\log(x+1)$] for normality; however, because the relationship for raw and transformed data was the same, we present the untransformed means and standard errors below. To account for unbalanced sample sizes between sites and years, we employed the Satterwhaite method for estimating degrees of freedom. We conducted our analysis in SAS Proc GLIMMIX (SAS version 9.2, SAS Institute, Cary, NC, USA).

Results

Rabbiteye blueberry cultivars ‘Brightwell’ and ‘Powderblue’ did not differ from one another in bee visitation ($P = 0.39$) and were thus pooled for future analyses. There was variability among sites in the number of *Apis* and wild bees. We observed a mean of 3 to 39 *Apis* per

plant and 1 to 14 wild bees per plant among sites. There was a significant interaction between cultivar ('Premier' and other cultivars) and bee taxa ($F_{1,113} = 64.8$; $P < 0.0001$; Fig. 2).

'Premier' was visited by more *Apis* (19 ± 5) than other rabbiteye cultivars (5 ± 2 ; $P = 0.0001$) but received fewer visits from wild bees (1 ± 1) than the other cultivars (10 ± 2 ; $P < 0.0001$).

Discussion

We find that the rabbiteye blueberry cultivar 'Premier' attracted a pollinator community distinct from other cultivars. We expect that this difference is the result of the abnormal flower morphology of 'Premier' rather than among-cultivar differences in nectar volume, concentration, or volatile profiles (Rodriguez-Saona et al. 2011). We posit that *Apis* were abundant at 'Premier' flowers due to the ease of access to their nectaries. However, wild bees were more abundant at other rabbiteye cultivars than 'Premier'. Wild bees visit blueberry primarily for pollen, not nectar (Dogterom 1999), and may prefer flowers with a complete corolla, enabling more uniform handling—we commonly observed *Bombus* and *Habropoda* bracing themselves at the aperture of the corolla while sonicating flowers to release pollen. Alternately, wild bees may be avoiding interspecific competition with *Apis* at 'Premier' flowers (see Chapter 3). Our findings suggest a kind of within-crop niche partitioning that may in fact reduce cross-pollination in intercropped plantings: *Apis* may skip over less-attractive 'Brightwell' or 'Powderblue' plants to preferentially forage at 'Premier', and wild bees may do the opposite, avoiding 'Premier'. Despite the potential for reduced cross-pollination, Sampson and Cane (2000) found that *Apis* were efficient pollinators of 'Premier'.

The attraction of *Apis* to abnormal blueberry flowers is also worth considering in relation to the phenomenon of ‘nectar-robbing’ (Inouye 1980). The carpenter bee, *Xylocopa virginica* frequently robs nectar from blueberry flowers by creating a perforation in the base of the corolla. In the presence of these perforations, *Apis* readily switch to nectar-robbing (Cane and Payne 1993) as a more efficient way of extracting nectar from flowers (Dedej and Delaplane 2005). Abnormal ‘Premier’ flowers are effectively similar to *Xylocopa*-perforated blueberry flowers; both facilitate access to the floral nectaries. Thus, our findings, interpreted in this context, suggest that nectar-robbing by *Xylocopa* may actually increase blueberry visitation by *Apis*. If nectar-robbing *Apis* are equally efficient at pollinating blueberry flowers, as suggested by Sampson et al. (2004), nectar-robbing may actually enhance pollination in blueberry, as observed in other systems (Maloof and Inouye 2000). ‘Nectar-robbing’ has a negative connotation that may obscure our evaluation of the behavior and its consequences.

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Figure 1. (a) Flowers of a rabbiteye blueberry, *V. ashei*, cultivar ‘Powderblue’ have long, constricted corolla, typical of this species. (b) Flowers of *V. ashei* cv. ‘Premier’ are deformed, with shorted corollas.

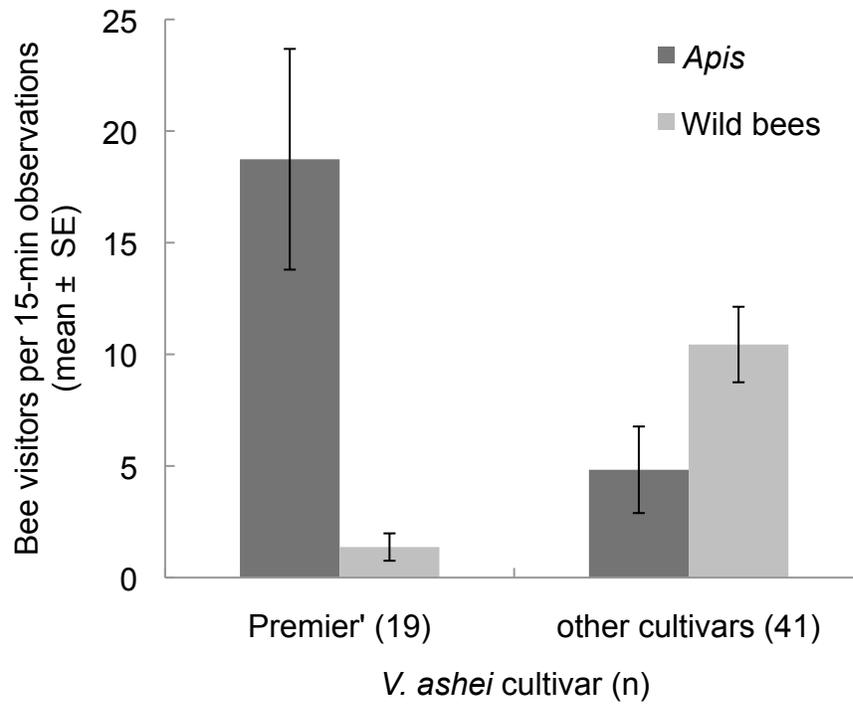


Figure 2. Number of *Apis* and wild bees observed visiting rabbiteye blueberry, *V. ashei*, cultivar 'Premier' or other, non-Premier cultivars, in 15-minute observational periods.

APPENDIX B:

VIDEO RECORDING OF BEE BEHAVIOR IN COMMERCIAL BLUEBERRY FIELDS

To determine the relative frequency of bee encounters in commercial highbush blueberry farms, we positioned cameras (Bloggie Touch Camera, Sony Corporation of America, New York, NY, USA) at plants to record bee activity. In 2011, we recorded activity from three farms, each stocked with 2-10 honey bee (*Apis mellifera*) colonies per hectare, during early-, middle-, and late-stage bloom (Chapter 2). During each farm visit, we placed a camera at each of four adjacent highbush blueberry plants, *Vaccinium corymbosum* cultivar ‘O’Neal’. At these plants, we recorded activity for 30-minute periods beginning at 1000 and 1100 hours. We then transferred our cameras to a second set of plants in a different location on the farm to repeat observations at 1300 and 1400 hours.

In the lab, we analyzed 10 hours of recorded video (approximately half of the total recording time), taking note of activity on approximately three flower clusters per plant (i.e., those that could be clearly observed). During this time, we counted 50 instances of *Apis* ‘legitimately’ foraging (through the terminal aperture of the corolla), and 17 instance of *Apis* poaching nectar (‘secondary nectar robbing’) through perforations first created by carpenter bees (*Xylocopa virginica*). On average, legitimately-foraging *Apis* spent 17 ± 2 seconds per flower (mean \pm SE) while nectar-robbing *Apis* spent 7 ± 1 sec flower⁻¹. We observed only one encounter between foraging *Apis*. Bee activity was evenly distributed amongst videos; however, bees were relatively absent from videos recorded early in the season (with less than 30% bloom). However, in only 2 hr 40 min of transect walks conducted during early bloom, we observed 227 foraging *Apis* (see Chapter 2).

Our results suggest that video cameras may be ineffective at documenting bee activity in the field. This supports works by Westphal et al. (2008) who found that single-plant observations were less effective than transect walks in describing bee activity. Our findings suggest that the frequency of encounters between bees visiting blueberry flowers may be fairly low.

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