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Author(s): Faye E. Benjamin and Rachael Winfree

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Lack of Pollinators Limits Fruit Production in Commercial Blueberry (*Vaccinium corymbosum*)

FAYE E. BENJAMIN¹ AND RACHAEL WINFREE

Department of Ecology, Evolution and Natural Resources, 14 College Farm Road, New Brunswick, NJ 08901

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ABSTRACT Modern agriculture relies on domesticated pollinators such as the honey bee (*Apis mellifera* L.), and to a lesser extent on native pollinators, for the production of animal-pollinated crops. There is growing concern that pollinator availability may not keep pace with increasing agricultural production. However, whether crop production is in fact pollen-limited at the field scale has rarely been studied. Here, we ask whether commercial highbush blueberry (*Vaccinium corymbosum* L.) production in New Jersey is limited by a lack of pollination even when growers provide honey bees at recommended densities. We studied two varieties of blueberry over 3 yr to determine whether blueberry crop production is pollen-limited and to measure the relative contributions of honey bees and native bees to blueberry pollination. We found two lines of evidence for pollen limitation. First, berries receiving supplemental hand-pollination were generally heavier than berries receiving ambient pollination. Second, mean berry mass increased significantly and nonasymptotically with honey bee flower visitation rate. While honey bees provided 86% of pollination and thus drove the findings reported above, native bees still contributed 14% of total pollination even in our conventionally managed, high-input agricultural system. Honey bees and native bees were also similarly efficient as pollinators on a per-visit basis. Overall, our study shows that pollination can be a limiting factor in commercial fruit production. Yields might increase with increased honey bee stocking rates and improved dispersal of hives within crop fields, and with habitat restoration to increase pollination provided by native bees.

KEY WORDS pollen limitation, honey bee, *Apis mellifera*, native bee, agriculture

Pollination services provided by insects, especially bees, are essential to the production of many food crops; globally, about one-third of the leading crop plants require pollination for marketable fruit production (Klein et al. 2007, Potts et al. 2010). Recent research raises concerns about whether pollinator populations can keep pace with increasing food production worldwide (Garibaldi et al. 2009, Ellis et al. 2010, Potts et al. 2010). Analyses of long-term production trends suggests that agricultural intensification of pollinator-dependent crops may not increase production and may in fact decrease stability of yields (Deguines et al. 2014), whereas similar intensification has a positive effect on crops that are not dependent on pollination (Aizen et al. 2008, Garibaldi et al. 2009). However, whether fruit production increases with increased pollination (i.e., whether crop production is pollination limited) has been directly measured in field experiments for only a few crops (Chacoff et al. 2008, Holzschuh et al. 2008, Aizen et al. 2009, Groeneveld et al. 2010).

Honey bees (*Apis mellifera* L.) are the single most common agricultural pollinator, but native, wild bees

(hereafter native bees) are also important, particularly in less intensive agricultural settings. For many crop plants, honey bees are less effective pollinators; studies that have quantified per-visit effectiveness have found that many native bees deposit more pollen per visit than honey bees (Javorek et al. 2002, Kremen et al. 2002, Klein et al. 2003, Winfree et al. 2007, Nicodemo et al. 2009, Cane et al. 2011). In addition, a recent synthesis of 41 crop systems worldwide showed that the presence of native, wild pollinators increases fruit production in many crops, even when honey bees are present (Garibaldi et al. 2013). Although the mechanism behind this finding is not known, one possibility is that native bees may increase outcrossing rates and thereby fruit production because they tend to move between plants more frequently than honey bees while also stimulating honey bees to move between flowers more frequently (Greenleaf and Kremen 2006, Carvalheiro et al. 2011, Garibaldi et al. 2011, Brittain et al. 2013). Further work is needed to fully understand the relative contribution of honey bees and native bees to the pollination of most crops.

Like many crops, highbush blueberry (*Vaccinium corymbosum* L.) is pollinated primarily by bees, including both honey bees and native bees. Blueberry is

¹ Corresponding author, e-mail: fayeb@rutgers.edu.

visited by many bee species native to eastern North America, including *Habropoda laboriosa* F., *Bombus* spp., *Osmia* spp., *Colletes* spp., and *Andrena* spp. (Cane and Payne 1988, Tuell et al. 2009, Benjamin et al. 2014). Native bees are generally thought to be more efficient pollinators of blueberry than domesticated honey bees (Dogterom et al. 2000, Javorek et al. 2002) because of their ability to sonicate the poricidal *Vaccinium* anther, thus resulting in more effective pollen release (Buchmann 1983, Cane and Payne 1988, Javorek et al. 2002, Ratti et al. 2008). Honey bees are unable to sonicate anthers and therefore may not release or transfer as much pollen per visit. However, native bee abundance in crop fields decreases with increasing agricultural intensity (Chacoff et al. 2008, Ricketts et al. 2008, Isaacs and Kirk 2010, Holzschuh et al. 2012), and therefore as blueberry cultivation has expanded, reliance on commercial, domesticated colonies of honey bees has increased. Concern about the continued health and availability of honey bee hives is stimulating interest in whether blueberry crops are receiving sufficient pollination and whether native bees are a reliable alternative or supplement to honey bees.

The goal of our study was to answer the following questions: 1) Is blueberry fruit production pollen-limited in a nationally leading region for commercial blueberry production? and 2) What are the relative contributions of honey bees and native bees to pollination in this same system? To answer the first question, we used two approaches: experimental manipulations to measure the extent of pollen limitation in the field, and investigation of the relationship between bee visitation rate to flowers and open-pollinated fruit mass. To answer the second question, we measured both the visitation rate and the per-visit pollination contribution of honey bees and native bees to estimate the total pollination provided by each type of bee.

Materials and Methods

Our study system included 18 conventionally managed highbush blueberry (*Vaccinium corymbosum*) farms in southern New Jersey. Southern New Jersey is an area of intense blueberry production, and New Jersey ranks first in the United States for used fresh blueberry production (U.S. Department of Agriculture [USDA] 2013). Reliable pollination in highbush blueberry is important for maximum production because fruits of maximum mass are only developed with pollination by insects (Mackenzie 1997, Klein et al. 2003). Specifically, berry mass in highbush blueberry is strongly correlated with the number of pollen grains deposited on the stigma (Dogterom et al. 2000); increased fruit growth is stimulated by hormones produced by the developing seeds (Suzuki et al. 1998). Although the relationship between pollination and fruit mass can vary considerably by cultivar, with some cultivars showing greater parthenocarpy than others (Cane and Payne 1988, Ehlenfeldt and Martin 2010), even varieties that achieve high fruit set without pol-

linators show increased fruit mass with pollination (Isaacs and Kirk 2010).

We selected our study fields such that they were separated by at least 1 km (range: 1–37 km). All farms used conventional agricultural methods but followed integrated pest management guidelines to reduce frequency of chemical insecticide and fungicide application to six sprays or fewer during fruit development. All study farms were monoculture stands of either 'Duke' or 'Bluecrop', which are widely planted in New Jersey (Ehlenfeldt and Martin 2010). Both cultivars will readily set fruit of marketable mass with self pollen (Mackenzie 1997), and their bloom periods overlap within the growing season. All sites stocked domesticated honey bees at recommended densities of 2.5–7.5 hives ha⁻¹, as recommended by Brewer (1970, cited in Eck, 1988). At each site, we established a 200-m transect consisting of four 50-m segments of crop row. We used the same location for each transect in all 3 yr, although not all experiments were conducted in all 3 yr.

Pollen Limitation Experiments. Pollen limitation experiments were conducted at all 18 field sites in 2 of the 3 yr of the study (2011 and 2012). On the day before beginning our experiments, we haphazardly chose clusters of flowers, each on a different bush, within two adjacent crop rows, and covered these clusters with organza bags as pollinator exclosures (YourOrganzaBag.com, white 6 × 9" bags). The following day, when flowers had opened, we randomly assigned a subset of pollinator-excluded clusters to one of the two treatments: open-pollinated or hand-pollinated. In 2011, we assigned six clusters to each treatment, and in 2012 we assigned four clusters to each treatment. One to three open flowers per cluster were labeled, treated with the same treatment, and followed through development. Open-pollinated flowers were left open to ambient pollination in the field. Hand-pollinated flowers had their stigmas saturated with pollen collected from several flowers from a different bush of the same variety within the same field and were then left open.

Flowers were allowed to develop normally throughout the bloom and berry development periods. Experimental berries ripened on the bush for 42 d (± 1 d) in 2011 and 50 d (± 1 d) in 2012, at which time they were harvested and brought into the laboratory. Berry collection in both years was timed such that our samples were collected just before the first harvest by growers; cold weather during the 2012 bloom period extended the ripening interval compared with 2011. We weighed each berry to an accuracy of 0.001 g on the day of collection. We calculated the mean mass for each cluster of berries as a data point for analysis because individual berries within a cluster could not be considered independent replicates. On rare occasions (23 of the 858 labeled flowers) we were unable to recover a berry, and we assumed that fruit set failed to occur and the label fell off the pedicel after flower drop. We included these zeros in our calculation such that our measures of fruit mass were the product of the

Table 1. Median pollen deposition (\pm interquartile range) by species groups and species composition of groups

Species group	Bee species	N (no. of single-visit experiments)	Pollen grains deposited per visit (median, interquartile range)	
<i>Apis</i>	<i>Apis mellifera</i> L.	85	7.0 (0.5–25.0)	
	<i>A. mellifera</i> (nectar foraging)	38	18.5 (7.0–37.0)	
	<i>A. mellifera</i> (pollen foraging)	11	24.0 (10.0–73.0)	
	<i>A. mellifera</i> (2° nectar robbing)	36	0.5 (0.0–4.0)	
<i>Bombus</i>	<i>Bombus bimaculatus</i> Cresson	80	23.5 (9.5–45.0)	
	<i>Bombus griseocollis</i> DeGeer			
	<i>Bombus impatiens</i> Cresson			
<i>Colletes</i>	<i>Bombus perplexus</i> Cresson			
	<i>Colletes inaequalis</i> Say	28	15.0 (8.0–25.5)	
	<i>Colletes thoracicus</i> Smith			
<i>Habropoda</i>	<i>Colletes validus</i> Cresson			
	<i>Habropoda laboriosa</i> L.	38	27.5 (10.0–39.0)	
Large <i>Andrena</i>	<i>Andrena carlini</i> Cockerell	23	9.0 (2.5–14.5)	
	<i>Andrena vicina</i> Smith			
Medium <i>Andrena</i>	<i>Andrena banksi</i> Malloch	60	11.5 (2.5–32.0)	
	<i>Andrena barbara</i> Bouseman & Laberge			
	<i>Andrena bradleyi</i> Viereck			
	<i>Andrena carolina</i> Viereck			
	<i>Andrena cressonii</i> Robertson			
	<i>Andrena fenningeri</i> Viereck			
	<i>Andrena ilicis</i> Mitchell			
	<i>Andrena imitatrix</i> Cresson			
	<i>Andrena mandibularis</i> Robertson			
	<i>Andrena morrisonella</i> Viereck			
	<i>Andrena scrypteropsis</i>			
	Small bees	<i>Augochlora pura</i> Say	8	7.0 (0.5–15.5)
		<i>Augochlorella aurata</i> Smith		
<i>Ceratina calcarata</i> Robertson				
<i>Lasioglossum acuminatum</i> McGinley				
<i>Lasioglossum coeruleum</i> Robertson				
<i>Lasioglossum fuscipenne</i> Smith				
<i>Lasioglossum leucomum</i> Lovell				
<i>Lasioglossum oblongum</i> Lovell				
<i>Lasioglossum pilosum</i> Smith				
<i>Lasioglossum versatum</i> Robertson				
<i>Lasioglossum weemi</i> Mitchell				
<i>Xylocopa</i>	<i>Lasioglossum zephyrum</i> Smith			
	<i>Xylocopa virginica</i> L.	34	2.5 (0.0–11.0)	

Group species composition was based on identified netted specimens collected within transects where observations were made (see Benjamin et al. 2014 for details).

binary category of fruit set (0 or 1) and fruit mass per set fruit.

Honey Bee and Native Bee Flower Visitation Rate. We collected data on honey bee and native bee flower visitation rate at each site on three separate days per year in all 3 yr of the study, 2010–2012. Data collection took place during peak bloom, which was between 13 April and 15 May in all years. All observations were made on days with conditions favorable for bee activity: $>14^{\circ}\text{C}$ before 1030 hours and $>17^{\circ}\text{C}$ by 1200 hours, with clear to partly cloudy skies. To minimize observer bias, data collectors were rotated between sites within each year and the same observer never collected data more than once per site.

During each site visit, field technicians conducted three observation periods at standardized times throughout the day. In each observation period, observers walked the transect at a pace of 10 m min^{-1} for 20 min, recording all flower visits by honey bees and native bees along one side of the row. Native bees were recorded to one of the seven identification groups (hereafter, species groups): *Bombus*, *Colletes*, *Habropoda*, *Xylocopa*, large *Andrena*, medium *An-*

drena, and other small bees. Each group includes 1–14 species of similar size and shape, which are indistinguishable from each other in the field (Table 1). Immediately following each round of observation, observers collected flower-visiting native bees with a hand net during a second 20-min transect walk, so that we could obtain species-level identifications for each species group. Honey bees were not collected but are readily identified to species by eye. Netted specimens were identified to the species level by Dr. John Ascher (National University of Singapore) and Dr. Jason Gibbs (Michigan State University).

Single-Visit Pollen Deposition. Single-visit pollen deposition data were collected in 2011 and 2012 using protocols similar to those used in other studies measuring pollinator effectiveness (Kremen et al. 2002, Thomson and Goodell 2002, Cariveau et al. 2013, King et al. 2013). Data were collected at each site, although the availability of each species group and therefore the number of single visits collected varied between sites and years. To determine the number of blueberry pollen grains deposited per flower visit for each bee species group, we prepared unpollinated (pollinator-

excluded) flowers in the same way described above for the pollen limitation experiments. The next day, when buds had opened, the cluster was cut from the bush. The cluster of virgin flowers was offered to bees foraging on the blueberry crop row by hand. A bee was allowed to visit one of the flowers on the cluster in its usual manner. We used only one experimental flower per cluster because multiple flowers would not have been independent. When the bee departed, the visited flower was marked with red ink and the bee's species group, sex, duration of the visit, and foraging behavior (nectar foraging, pollen foraging, or nectar robbing) were recorded. The cluster was then recovered with an organza bag and maintained indoors in water at constant temperature for 48 h to allow sufficient time for pollen tube growth (Daily 1997, Dogterom et al. 2000, Mayer et al. 2011). After 48 h, the stigma of the visited flower and stigmas from up to four control flowers from the same cluster were collected into 70% ethanol for storage until processing.

To process stigmas for pollen grain and pollen tube visualization, stigmas were removed from the ethanol and soaked in 1 M NaOH for 24 h to soften the tissue. Stigmas were then stained in 0.01% aniline blue buffered in K_3PO_4 for 48 h (after Kearns and Inouye 1993). Pollen grains with visible pollen tubes, both those attached to the stigma and elsewhere on the slide, were counted at 100 \times magnification. We counted only pollen with tubes because pollen tubes indicate both pollen viability and pollen-stigma adhesion during the original field experiment (as distinguished from pollen contamination that occurred later during flower processing; blueberry flowers are bisexual and are technically capable of within-flower pollen transfer, although floral morphology makes this unlikely to occur).

Honey Bee Hive Counts. We collected two different forms of data on honey bee hive stocking density at each farm. First, we asked farm managers for the overall density of honey bees that they stocked at the farm, calculated as the total number of hives rented divided by the area of the farm. Second, in 2012, we used satellite images of the farm and surveyed the area within a 300-m radius of the observation transect for honey bee hives, and calculated the hive density within this 28.3-ha area. This second measure gives us a more accurate measure of the likely honey bee density available within our study transect; although honey bees can fly several kilometers to forage (Greenleaf et al. 2007), when confronted with a super-abundant resource such as a blooming crop, they tend to forage over much shorter distances.

Analyses. All analyses were done using the default stats package in the statistical analysis software R (R Core Team 2014) and with *lsmeans* (Lenth 2014). Graphs were generated using the default package and with *ggplot2* (Wickham 2009).

*Is Blueberry Crop Production Pollen-Limited?*² Our investigation of pollen limitation in blueberry used two analyses, one experimental and one associational, to assess whether current total pollination is sufficient for maximum fruit production. In all analyses below,

berry mass was square root-transformed to meet the assumptions of normality.

First, in the experimental study of pollen limitation, we compared mean berry mass for both treatments (hand-pollinated and open-pollinated) using linear mixed-effect models with function *lmer* in package *lme4* (Bates et al. 2014) in R (R Core Team 2014). We constructed a global model as follows: our outcome variable was square root of mean berry mass; our fixed effects were treatment, cultivar, and year; and our random effect was farm. We also included all possible two-way interaction terms, but not the three-way interaction term. We then used the dredge function in package *MuMIn* (Bartoń 2013) to compare all subset models and the global model. Models with $\Delta AICc < 2$ were retained for model averaging, which was done using the *AICcmodavg* package (Mazerolle 2013).

In our second, associational analysis, we determined the relationship between bee flower visitation and fruit mass again using linear mixed-effect models using the *lme4* package. We constructed a global model as follows: our outcome variable was square root of mean berry mass; our fixed effects were honey bee visitation rate, native bee visitation rate, year, and cultivar; and our random effect was farm. We used year as a fixed effect because our berry collection methods varied slightly between years (Winter 2013). We also include interactions between cultivar and all other fixed effects because response to pollination is known to vary between cultivars (Ehlenfeldt 2001). We included a year-by-cultivar interaction because varying weather between years may also affect the cultivars differently, but we did not include any other two-, three-, or four-way interactions. We again used the dredge function to generate the set of all possible models using subsets of the fixed effects of the global model. Models with $\Delta AICc < 2$ were retained for model averaging. This analysis used visitation data from only 2011 and 2012, as we did not have fruit weight data from 2010.

We repeated this analysis using summed honey bee and native bee visits to assess whether total bee visitation was sufficient for maximum fruit mass at all sites, which would indicate a complementary relationship between honey bee and native bee visitation rates across sites.

*What Are the Relative Contributions of Honey Bees and Native Bees to Pollination?*² To calculate aggregate pollination function by honey bees, we multiplied median pollen deposited per flower visit for each type of visit (nectar-collecting, pollen-collecting, or secondary nectar robbing through punctures made by *Xylocopa virginica* L.) by the number of visits of each type. To calculate aggregate pollination function by each type of native bee, we multiplied median pollen deposited per flower visit by the number of flower visits for each bee species group. We did not separate native bee visits by behavior because we were unable to target specific behaviors for any species other than honey bees; therefore, we made the assumption that our random sample of visits reflected the typical frequency of each visit type by each group. We then

pooled the function data across all native bee species groups and compared aggregate native bee pollination to the pollination provided by honey bees. As a form of sensitivity analysis, this process was repeated using the mean, and using the first and third quartiles from the single-visit pollen deposition distributions of each species group in place of the median. Results of this analysis were qualitatively similar to those obtained using the median, indicating that our choice of the median did not strongly affect our results.

Does Hive Density Predict Honey Bee Visitation Rate?

To assess whether hive stocking density predicts honey bee visitation rate, we compared both the grower's reported honey bee hive density and our own measurements of hive density to the total number of honey bee visits recorded at that farm in 2012. We used a standard linear regression in R (*lm* function, R Core Team 2014) with either our estimated density or the grower-provided density as the predictor and total honey bee visits recorded as our response variable.

Results

In 2011, our data set for the pollen limitation experiments included 96 open-pollinated and 104 hand-pollinated clusters; in 2012, we used 66 open- and 68 hand-pollinated clusters. Sample sizes were smaller than planned in the study design (planned sample sizes were 108 clusters per treatment in 2011, and 72 clusters per treatment in 2012) because some clusters were lost to weather damage each year. In the 3 yr of the study (2010–2012) used to calculate total relative pollination function provided by honey bees and native bees, we observed 14,066 visits from honey bees and 1,379 visits from native bees. Of these, the subset of data from 2011 and 2012, including 9,878 visits from honey bees and 944 visits from native bees, were used in the analysis of pollen limitation because those were the 2 yr in which we did pollination limitation experiments. Over 2 yr (2011 and 2012), we conducted 368 single-visit flower experiments, including 85 from honey bees and 281 from native bees. Sample sizes for each species group ranged from 8 visits from small bees to 80 visits from bumblebees (see Benjamin et al. 2014 for more details). Honey bees deposited a median of 18.5 (interquartile range: 7.0–37.0) grains of pollen during a nectar-collecting visit, 24.0 (10.0–73.0) grains during a pollen-collecting visit, and 0.5 (0.0–4.0) grains during a secondary nectar-robbing visit. Median values for pollen deposition by native bees ranged from 7.0 to 27.0 grains per visit; single-visit pollen deposition data for each species group are given in Table 1.

Is Blueberry Crop Production Pollen-Limited? In our experimental analysis of pollen limitation, our mixed-effect model selection protocol retained five models for model averaging. Treatment and year were retained as the most important fixed effects: they were retained in all models and had a relative importance of 1.0. Cultivar was retained in only three models, with a relative importance of 0.48. The interactions of treatment–year and cultivar–year were each retained in

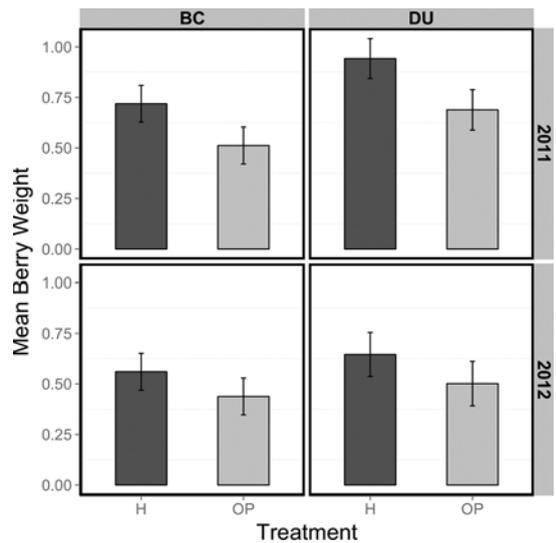


Fig. 1. Comparison of hand-pollinated (H, dark gray) and open-pollinated (OP, light gray) berry masses for Bluecrop (left column) and Duke (right column) berries in 2011 (top) and 2012 (bottom). Bar heights are the least-squared means based on the mixed-effect model estimates (Lenth 2014). Error bars are SEs.

two models, with relative importance of 0.38 and 0.35, respectively. Open-pollinated berries were smaller than hand-pollinated berries in both years (Fig. 1), indicated by the negative model-averaged coefficient for open-pollinated treatment; however, the positive value for the open-pollinated treatment–year interaction indicates that this difference was smaller in 2012 (Table 2a).

In our analysis of observational data, honey bee abundance, year, and cultivar had the most important effects on berry weight (Fig. 2). Our model selection protocol retained seven models for model averaging (Table 2b). Honey bee visitation rate, cultivar, and year were the most important fixed effects and were retained in all models (relative importance = 1.0). Native bee abundance and the interaction terms of cultivar–honey bee and cultivar–native bee were also retained in several models each (relative importance: 0.86, 0.73, 0.61, respectively), while the cultivar–year interaction was only retained in two models and had a relative importance of 0.33. The importance of the interaction effects indicates that the effect of year and bee visitation on berry weight may have varied between cultivars. Results for combined honey bee and native bee visitation were qualitatively similar to those for honey bees alone: bee abundance, cultivar, and year were equally important predictors (relative importance of 1.0) of mean berry mass, and the interactions were retained in some models.

What Are the Relative Contributions of Honey Bees and Native Bees to Pollination? We found that honey bees performed a mean of 86% of the visits to blueberry flowers (range, across farms: 55–100%). Native bees contributed 14% of the visits (range, across

Table 2. Model-averaged coefficients and relative variable importance for (a) experimental analysis of berry weight by treatment group, and (b) observational analysis of berry weight by bee activity

	Estimate of model-avg coefficient (with shrinkage)	SE	z value	Pr (> z)	Relative variable importance
(a) Experimental					
(Intercept)	0.8548	0.0444	19.1960	<2.0E-16***	
Cultivar	0.0856	0.0736	1.1610	0.2456	0.64
Treatment	-0.1267	0.0240	5.2550	1.0E-07***	1.00
Year	-0.1030	0.0334	3.0800	0.0021**	1.00
Cultivar-year	-0.0332	0.0460	0.7200	0.4714	0.44
Treatment-year	0.0194	0.0342	0.5680	0.5699	0.40
Cultivar-treatment	-0.0010	0.0114	0.0850	0.9325	0.08
(b) Observational					
(Intercept)	0.5174	0.0588	8.7500	<2E-16***	
Cultivar	0.1735	0.1098	1.5750	0.1153	1.00
Honey bee visits (HB)	0.0008	0.0002	4.5210	6.1E-6***	1.00
Native bee visits (NB)	-0.0001	0.0010	0.0640	0.9492	0.86
Year	-0.1388	0.0370	3.7260	0.0002***	1.00
Cultivar-NB	0.0025	0.0020	1.2140	0.2246	0.73
Cultivar-year	-0.0270	0.0499	0.5390	0.5896	0.34
Cultivar-HB	-0.0002	0.0003	0.9170	0.3593	0.61

"Estimate" is the model-based estimate for the coefficient for each parameter without weighting; "model-avg coefficient" is the model-averaged coefficient, with shrinkage, for each variable. "Relative variable importance" is the relative importance of each parameter in the average model, calculated by summing the AICc for each model included in model averaging that includes the parameter.

$P \leq 1$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

farms: 0–44%). When we translated the visitation rate into aggregate pollination function, we found that native bees also contributed a mean of 14% (range, across farms: 0–58%) of the pollination function across farms.

Does Hive Density Predict Honey Bee Visitation Rate? We found that the grower-provided density of honey bee hives did not predict our measurement of honey bee activity ($R^2 = 0.05$; $P = 0.4$). Our local measurement of hive density was a better, although not strong, predictor of honey bee activity ($R^2 = 0.24$; $P = 0.03$; Fig. 3).

Discussion

Pollen limitation, or reduced fruit or seed production based on insufficient pollen transfer to a flower, is a well-known phenomenon for many wild plant species (Ashman et al. 2004, Knight et al. 2005). Surprisingly, however, pollen limitation has not been well-studied for many crop plants, possibly because growers have assumed they can provide sufficient pollination from honey bees by stocking hives at the recommended densities. There are several indications from global-scale studies that pollen limitation in crops could occur soon if it is not occurring already. Several studies have recently examined this issue using a correlational analysis of Food and Agricultural Organization data (Aizen et al. 2008; Garibaldi et al. 2009, 2011). The earliest analyses (Aizen et al. 2008) found that global yields (in tons per hectare) of all pollinator-dependent crops as a group and nondependent crops have increased at similar rates in the past five decades, likely because of improved agricultural techniques, and are not detectably constrained by pollinator shortages. However, they also they found that the proportion of land devoted to pollinator-dependent crops has increased in that time frame, making

global food production more reliant on pollinators and making continued expansion of pollinator-dependent crops increasingly difficult if pollinators are in short supply. Later studies that separated pollinator-dependent crops into categories based on levels of pollination dependence (based on Klein et al. 2007) found that pollen limitation of crops may already be occurring in the crops that are most dependent on pollinators; Garibaldi et al. (2011) found that crops with greater pollinator dependence also had slower yield growth, greater yield variability, and greater variability in yield growth over time. In a regional study based on French agricultural and land use data, Deguines et al. (2014) found that yield of more pollinator-dependent crops did not benefit from continually increasing agricultural intensification (including increased honey bee hives), suggesting that lost natural pollination services were not offset by increased honey bee inputs. Although recent studies of several individual crops, including grapefruit, cherry, and cacao (Chacoff et al. 2008, Holzschuh et al. 2008, Groeneveld et al. 2010), have demonstrated pollen limitation with pollen supplementation experiments, pollen limitation is still generally considered a "wild plant problem" because many crop systems supplement native pollinators with domesticated honey bees, and are therefore considered to be at reduced risk for pollen limitation. Here, we show that a valuable fruit crop, highbush blueberry, may be pollen-limited under current honey bee stocking levels, and that native bees do not provide enough pollination to compensate for either current honey bee health problems (e.g., weaker colonies than those used in the past) or potential future declines in honey bees (Potts et al. 2010).

Is Blueberry Crop Production Pollen-Limited? Both our experimental and our associational studies indicate that commercial highbush blueberry produc-

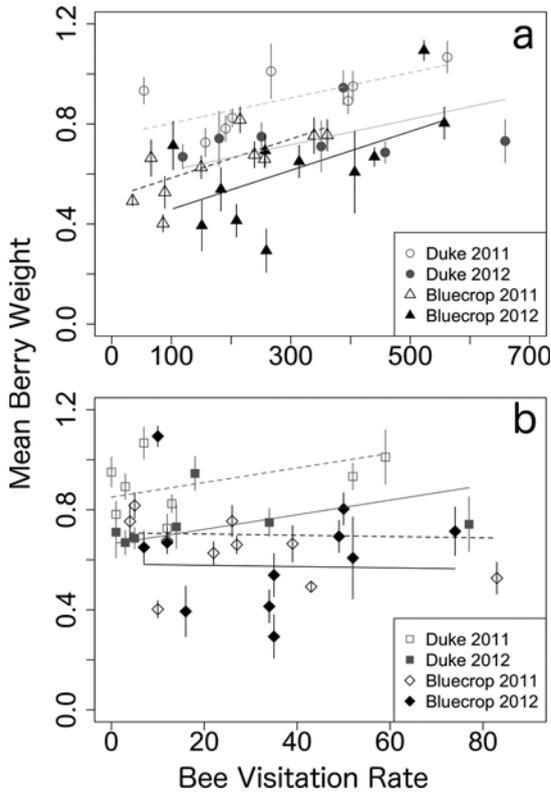


Fig. 2. Comparison of honey bee (top) and native bee (bottom) visitation rate to the mean mass (\pm SE) of open-pollinated berries at the same site. Honey bee visitation rate was an important predictor of berry mass; native bee visitation was less important. Each point represents one site, with honey bee or native bee visitation summed over three observation dates during the bloom period. Lines are drawn using model-averaged estimates of the slope and intercept values from the mixed-effect model. Gray lines and symbols indicate Duke; black lines and symbols indicate Bluecrop. Open symbols and dashed lines are 2011 data, while filled symbols and solid lines are 2012 data.

tion in New Jersey is experiencing pollen limitation. First, we found that in pollen limitation experiments, hand-pollinated berries were heavier than open-pollinated berries (Fig. 1; Table 2). Second, we found that fruit mass increased with honey bee visitation rate and did not reach an asymptote (Fig. 2a), indicating that further increase in fruit mass with greater pollination might be possible. Together, our findings indicate that fruit production might be limited by pollination insofar as greater numbers of honey bee visits might increase fruit mass even further.

Contrary to our findings for honey bees, we did not find a strong relationship between native bee visitation and berry mass (Fig. 2b). This was the opposite of our expectations: a recent global synthesis showed that native pollinators enhance fruit production regardless of honey bee presence or abundance (Garibaldi et al. 2013), and other studies have found that native species transfer more pollen per visit to blue-

berry flowers than do honey bees (Dogterom 1999, Javorek et al. 2002). However, the mean total contribution of native pollinators across all farms in our study was still only 14%. Thus, despite the efficiency of some species as pollinators, native bees may not be abundant enough in our conventionally managed, intensive production system to have a strong effect on fruit mass. Further, one of the possible explanations for greater fruit production in the presence of native bees in other study systems is that the study crops receive greater outcrossing, which is necessary for many crops (Klein et al. 2007). In conventional blueberry agriculture, there is little opportunity for outcrossing because most commercial blueberry is self-fertile enough to plant in large, monoculture blocks, thus limiting the potential benefits of native bees for fruit production.

There are two possible, but we believe unlikely, alternative interpretations of our results regarding pollen limitation. First, for our associational study, it is possible that farms with greater fruit production are using more inputs of all kinds—including greater numbers of honey bee hives, larger amounts of fertilizer, or more consistent watering regimens, all of which may contribute to berry mass. This “high input farm” hypothesis could explain why some farms have both more honey bee visitation and greater fruit production without requiring the two to be linked by pollen limitation. However, the results of our analysis of honey bee stocking densities suggest that the overall number of hives at a farm may not be a sufficient indicator of honey bee visitation. We found that the number of hives in the immediate (28 ha) area around our transects was a better predictor of honey bee activity than the number of hives per hectare reported by growers for the entire farm, most of which are hundreds of hectares in size. Further, none of our graphs showed fruit mass clearly reaching an asymptote, suggesting that there is still room for improvement in honey bee abundance. This interpretation is consistent with the results of Isaacs and Kirk (2010) who also found a nonasymptotic relationship between bee visitation rate and berry mass, even though their study farms stocked bees at the high end of the range of densities used in New Jersey.

An alternative interpretation of our experimental studies of pollen limitation is that individual hand-pollinated berries may grow larger because of unequal resource allocation by the bush favoring well-pollinated berries. If this were the case, then berry-level pollen limitation might not translate into whole-plant pollen limitation because increased pollination to the entire plant would not cause resource allocation changes (Knight et al. 2005). However, our consistent results indicating a steady increase in berry mass with increasing pollination seen in both our experimental and correlational analyses suggest that pollen limitation may be a factor at least in some sites and years.

Taken together, our data indicate that even at farms on the high end for bee activity and berry mass, the recommended hive stocking densities for New Jersey of 2.5–7.5 hives ha^{-1} , which may have been in use

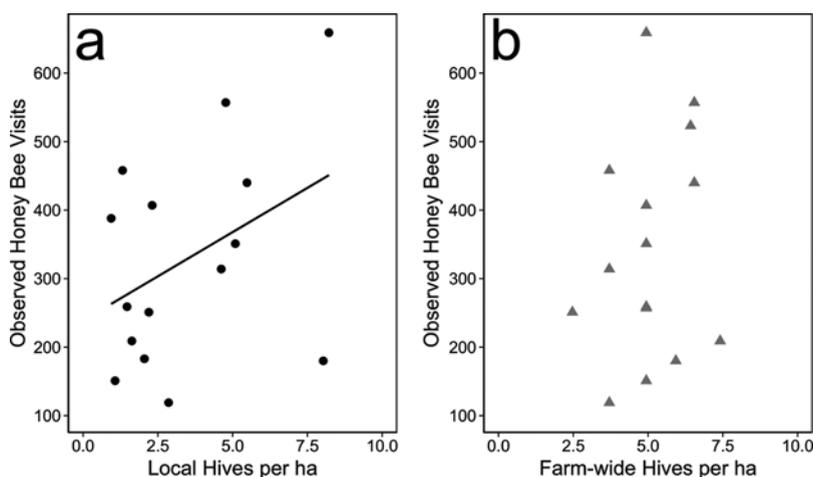


Fig. 3. Comparison of (a) our estimate of local honey bee hives per hectare and (b) reported farm-wide stocking density of honey bee hives per hectare with observed honey bee visits in 2012. Local hive abundance was moderately correlated with honey bee visitation rate ($R^2 = 0.24$; $P = 0.06$) and was a better predictor than the farm-wide stocking densities ($R^2 = 0.05$; $P = 0.4$). Each point represents one site, with honey bee visitation summed over three observation dates.

since at least 1970 (Brewer, 1970, cited in Eck, 1988), may no longer be adequate. These guidelines may reflect an earlier agricultural climate in which native bees and feral honey bee colonies were a reliable source of supplementary pollination in blueberry; expansion in blueberry production in recent decades may have reduced native pollinator abundance on blueberry to levels that do not provide sufficient supplementary pollination, and declines in feral honey bees largely owing to *Varroa* mites may have reduced their viability as alternative pollinators (Ellis et al. 2010). These guidelines also do not specify the distribution of honey bee hives around a farm. We found that our transects with higher density of hives at a very local scale had higher visitation rates, suggesting that despite their well-studied ability to fly long distances to find food (Beekman and Ratnieks 2000), honey bees in our system may stay within a short distance of their hives. Furthermore, advances in other areas of agricultural production such as improved fertilization, flower density, and pest control may have removed some limitations on production, making pollination more likely to be the rate-limiting step (Rufus Isaacs, personal communication).

What Are the Relative Contributions of Honey Bees and Native Bees to Pollination? Although native bees were frequent flower visitors at particular sites, contributing up to 44% of flower visits and 58% of pollination, across all sites pollination in our system is primarily performed by honey bees. This is not surprising given that New Jersey is a major blueberry producer for the fresh fruit market, and virtually all growers place apiaries throughout their production fields. In addition, blueberry bloom occurs relatively early in the season, coinciding with emergence of a number of groups of spring-flying bees but also with the spring emergence of overwintered bumblebee (*Bombus* spp.) queens that have not yet established

nects. Because these queen bees do not yet have established colonies of worker bees, blueberry crops cannot take advantage of the abundance of *Bombus* workers that pollinate later-blooming crops in the region (Winfree et al. 2008, Cariveau et al. 2013), and therefore there are far fewer bumblebees available to visit flowers. Further, much of our study region is an area of intensive agricultural production, and we have previously found that farms surrounded primarily by cropland had fewer native bees than sites with extensive noncrop vegetation nearby (Benjamin et al. 2014). However, at some sites with large populations of native bees, we did find that visitation by native bees accounted for almost half of all flower visits, while pollination by native bees, especially the highly efficient native pollinators (e.g., *Bombus* and *Habropoda*), was greater than suggested by their abundance alone. Taken together, the early bloom period and the increasing agricultural development of the region may limit the number of native bees available to pollinate blueberry fields, making honey bees the most abundant and therefore most important pollinator in our study system at most, but not all sites.

To increase blueberry production by decreasing pollen limitation, two possible, and not mutually exclusive, solutions exist. The first is to increase the recommendation of the number of honey bee hives per acre to thoroughly saturate blueberry bloom with pollinators. In addition to increasing the number of hives, hives may need to be dispersed throughout the farm more evenly than current practice. Second, although we conducted our study in a conventionally managed agricultural system stocked with honey bees, we found that native bees still contributed an average of 14% of pollination service to blueberry flowers and up to 58% at individual farms; therefore, planning pollinator habitat restoration to maintain or increase their numbers may ensure that this service continues

at current levels or even rises. Although native bee visits were not correlated with fruit mass in our study, their contributions to pollination, which may be valued at 14% of the cost of renting honey bee hives, cannot be ignored. However, many native bee species in this study region respond negatively to increasing agricultural intensification, most likely because of loss of habitat patches in the landscape. We have previously found that local, farm field-scale habitat patches may mitigate declines in pollinator populations (Benjamin et al. 2014). Increasing available pollinator habitat at the field scale, which can be done by individual growers, may be a way to increase native pollinator populations that does not rely on landscape-wide shifts in land use.

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