Honey Bee (Hymenoptera: Apidae) Visits and Pollen Source Effects on Fruiting of 'Gulfcoast' Southern Highbush Blueberry

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ABSTRACT Bee visitation levels and pollen sources were varied in an effort to optimize fruit production (especially early ripening, fruit set, and berry weight) of southern highbush blueberry (low chill hybrids of *Vaccinium corymbosum* L.). Fruiting plants ('Gulfcoast') were enclosed in nylon-mesh cages with colonies of honey bees, *Apis mellifera* L., and pollinizer plants affording either intravarietal self-pollination, intervarietal crossing, or interspecific crossing with rabbiteye blueberries, *Vaccinium ashei* Reade. Newly opened blossoms were allowed 0, 1, 5, 10, or unlimited bee visits before being closed with a fine mesh bag. Significant improvements in fruiting characters were achieved between one and five visits and also usually between five and unlimited visits. Fruit set more than tripled between the fewest- and greatest-visit levels; set peaked near 70%. The pollination-to-harvest interval, a chief determinant of blueberry prices in the early season, was shortened by 5 d to 53 ± 0.5 (SE) d. Berry weight increased 28% from the 0- and 1-visit groups to 1.77 ± 0.05 g per berry with unlimited visits. Seed numbers increased 2.2-fold to 40 ± 1 seeds per berry. Sugar concentration of juice ranged from 11.0 to 12.9% and was lower at greater levels of bee visitation. Pollen source did not have a significant effect on any fruiting character measured in 'Gulfcoast'.

KEY WORDS *Apis mellifera*, *Vaccinium*, pollination

BLUEBERRY BREEDING PROGRAMS in the southeastern United States recently have developed southern-adapted hybrids derived from the northern highbush blueberry, *Vaccinium corymbosum* L. These southern highbush types are interspecific crosses of the tetraploid highbush and southern *Vaccinium* species of varying ploidy levels. Following three cultivar releases during the mid-1970s, cultivar availability has quadrupled since 1986.

A primary goal of breeding programs has been to provide low-chill requiring, early ripening cultivars (Draper et al. 1982, Lyrene & Sherman 1984). In southern climates, growers taking advantage of the shorter bloom-to-harvest interval of hybrids can have marketable fruit several weeks earlier than that produced by rabbiteye blueberries, *V. ashei* Reade (Lyrene & Sherman 1985), the major species currently grown in the southeast. Fresh quality blueberries marketed early in the season have highest value. In 1991, for example, a 5.7-liter (12 pint) flat sold for $41.80 on 10 May versus $12.57 on 31 May (USDA 1991).

Several aspects of pollination are crucial for fruit production in northern highbush blueberries. Adequate insect pollination is important (Eck 1988). Honey bees (*Apis mellifera* L.) are recognized as valuable pollinators, and colonies routinely are rented for commercial blueberry production (McGregor 1976, Robinson et al. 1989). Fruiting also can be influenced by pollen source. *V. corymbosum* is self-fertile (Merrill 1936), but intervarietal cross-pollination has been shown to increase fruit set and result in larger, earlier berries having more seeds (Meader & Darrow 1947, Marucci 1966, Brewer & Dobson 1969).

Relatively little is known of pollinator or pollen source requirements of southern highbush blueberries. The role of insect pollinators has not been studied, so it is not known if honey bees would provide commercially effective pollination. The value of honey bee pollination is controversial for rabbiteye blueberries (Cane & Payne 1990) and unknown for other self-infertile *Vaccinium* from which southern highbush are partially derived. The varying pedigrees of the southern hybrids may complicate pollinizer requirements; the self-fertile germplasm compo-

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nent (from *V. corymbosum*) of current cultivars ranges from 56% to 88% (Lang et al. 1990).

Determining the effects of self- versus cross-pollination is important for planting recommendations. An added consideration is the effectiveness of interspecific crossing with rabbiteyes, given the likelihood of mixed-species plantings as southern highbush are added to established orchards in the southeast. Gupton & Spiers (1991) found that southern highbush hand pollinated with rabbiteye or mixed rabbiteye–highbush pollen produced fewer seeds per berry but that fruit set was similar to that following intraspecific (intervarietal) cross-pollination. Effects of rabbiteye pollen on ripening interval and berry weight were inconsistent. Other studies showed that intervarietal crossing increased berry weight and seeds per berry and shortened pollination to harvest interval (El-Agamy et al. 1981, Gupton 1984, Lyrene 1989, Lang & Danka 1991). Fruit set results varied; sometimes fruit set increased (Lyrene 1989), sometimes it did not increase (Gupton 1984, Lang & Danka 1991), and sometimes results varied with cultivar (El-Agamy et al. 1981). Manual pollen transfer was used in all these tests except those of Lang & Danka (1991), who used honey bees to vector pollen.

This experiment was conducted to determine the effectiveness of honey bees as pollinators of 'Gulfcoast' southern highbush blueberry by quantifying the effect of varying numbers of bee visits on fruiting characters. The effects of increasing numbers of pollinator visits has not been measured for blueberries as it has for some crops (especially cucurbits [McGregor et al. 1965, Adlerz 1966, Collison & Martin 1967, Tepedino 1981]). The test also included measurements of effects of various pollen sources on fruiting characteristics. 'Gulfcoast', the fruiting cultivar of interest, contains ≈75% *V. corymbosum* (a tetraploid) and 25% *V. darrowi* (a diploid). This cultivar has performed better in south Louisiana than has the most widely planted southern highbush cultivar, 'Sharpblue' (G.A.L., unpublished observations).

Materials and Methods

Pollination was conducted from 25 March to 1 April 1991 at the campus Horticultural Farm of the Louisiana State University, Baton Rouge. Fruiting plants were nine 5-yr-old 'Gulfcoast', ≈1.5 m tall, grown in 42-liter (10 gal) pots. After chilling requirements had been met, plants were moved in early March into a dark, cool room (10°C) until studies were initiated. Plants were ranked according to total flower bud number and assigned so as to equalize flowering potential among three pollen source treatment groups.

Pollen sources were regulated by enclosing plants and honey bee pollinators in three nylon net cages (2.0 mm mesh; 6 by 3 by 2.5 m). 'Gulfcoast' was pollinated by either 'Gulfcoast', by a mixture of other southern highbush cultivars, or by a mixture of rabbiteye blueberry cultivars. Multiple pollinizer cultivars were used for crossing to avoid potential line-specific incompatibility problems (Gupton 1984). Pollinizers were arranged in a circle (≈2 m in diameter) around a target 'Gulfcoast' within each cage. For intravarietal selfing, four to six 'Gulfcoast' were used. For intervarietal crossing with southern highbush and interspecific crossing with rabbiteye, 4–15 plants of 4–6 cultivars of appropriate genotypes were used. Southern highbush pollinizers were 'Blue Ridge', 'Cape Fear', 'Cooper', 'Avonblue', 'O'Neal', and 'Georgiagem'; rabbiteye pollinizers were 'Baldwin', 'Tifblue', 'Climax', 'Brightwell', 'Briteblue', and 'Beckyblue'. Mean daily ratios of pollinizer to target 'Gulfcoast' flowers were 3.3:1.0 in the cage of 'Gulfcoast' × 'Gulfcoast', 2.6:1.0 in the cage of 'Gulfcoast' × southern highbush, and 3.4:1.0 in the cage of 'Gulfcoast' × rabbiteye.

Each cage was supplied with a small colony of honey bees consisting of an open-mated sister queen and her progeny. Colonies had equivalents of ≈6.5 combs covered with adult bees and 2.0 combs of brood. There typically were a few to a few dozen foragers actively visiting flowers in each cage during the pollination sessions.

The pollination scheme was to allow bee visits to one target 'Gulfcoast' per day in each cage. In each cage, two 'Gulfcoast' were used on each of 2 d and a third 'Gulfcoast' was used once, yielding a total of 5 d of pollination in the test. Three 'Gulfcoast' were taken from the cold room on the day before their use, and any open flowers were removed. These plants were excluded from pollinators until the next morning when 40–75 newly opened flowers were identified with individually numbered tags attached to the pedicels. During midmorning, after bees in the cages had been foraging for at least 1 h, target plants were taken into their assigned cages. Teams of two to four observers monitored bee visits to tagged blossoms, which were permitted to get 0, 1, 5, 10, or unlimited visits. After reaching a randomly assigned visit level of 1, 5, or 10, a flower was bagged to prevent further visitation. Bags were cylinders (1.5 cm diameter, 2.5 cm long) of fine nylon netting (1.0 mm mesh) with one end sealed. The open end of a bag was slipped over the corolla and sealed around the pedicel. Flowers in the 0-visit category were bagged before plants were taken into the pollination cages; unlimited-visit flowers were not bagged. In total, 957 flowers were observed; sample sizes for each of the five bee-visit levels ranged from n = 60–67 in each of the three pollen source cages. In a few cases, the 1-, 5-, and 10-visit categories included flowers that received 2 (n = 4), 6 (n = 3), and 9 (n = 5) visits, respectively.

Flowers were monitored at 2-d intervals following pollination. Bags were removed when corollas were shed. Fruit set was determined by retention or abscission of fruits at 2 wk after pollination. Fruits were harvested when completely blue at 1- to 4-d intervals from 13 May through 6 June; harvesters did not know the pollination treatment of fruits. Fruits were weighed and then stored frozen before measuring sugar concentration and seed content. Thawed berries were pierced several times to allow ≈100–200 μl of juice to be squeezed onto a digital refractometer (Model RFM 80, Bellingham-Stanley, Kent, England; calibrated to sucrose) for percent sugar content measurement (to ± 0.1%). To determine seed numbers, berries were softened by microwaving (625–720 watts) in 2 ml water for ≈10 s. Seeds were separated manually from the flesh and skin; seeds retained by a sieve (1.0 mm by 1.0 mm) after washing were counted. For the 5-, 10-, and unlimited-visit categories, ≥30 berries per visit level per cage were available to be analyzed for sugar and seeds, whereas for the 0- and 1-visit categories only 5–19 berries ripened and were analyzed.

Two-way analysis of variance (Proc ANOVA and Proc GLM; SAS Institute 1989) was used to compare effects of visit levels and pollen sources on fruiting characters. The experiment involved a randomized complete-block design with cages (i.e., pollen sources) as a blocking factor. Logarithmic transformations were made to help stabilize variances of fruit development period, sugar content, and seed number. Means were separated by least significant difference tests on either least-squares means or arithmetic means (for fruit set). Pearson's correlation and partial correlations (PROC CORR; SAS Institute 1989) were used to determine the strength of relationships between dependent variables.

Results and Discussion

The number of honey bee visits received per flower was the strongest and most consistent determinant of fruiting in 'Gulfcoast' southern highbush blueberry ($F \geq 4.68$; df = 4, 43 [4, 48 for fruit set]; $P \leq 0.003$ for visit effects for each of five dependent variables). Significant thresholds of improvement in fruiting characters were realized between one and five visits and usually realized between five and unlimited visits; larger improvements were found at the lower threshold than at higher thresholds (Figs. 1–5). Pollen source never had a significant effect on any fruiting character ($F \leq 2.60$; df = 2, 12; $P \geq 0.116$ for each of the five variables).

Fruit set increased more than three-fold from the 0-visit group to the mean of the 10- and unlimited-visit groups, which were statistically similar (Fig. 1). Fruit set in 'Gulfcoast' appeared to be maximized near 70%. This level is intermediate among levels of fruit set reported for self- and cross-pollinated southern highbush cultivars and breeding lines (Gupton 1984, Lyrene 1989, El-Agamy et al. 1981, Lang & Danka 1991).

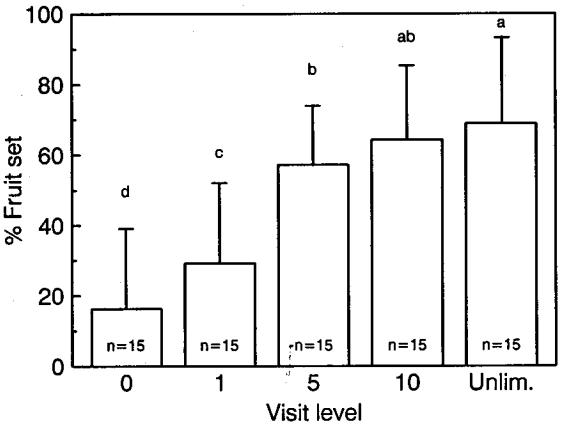

Fig. 1. Fruit set percentages in 'Gulfcoast' southern highbush blueberry as a function of level of honey bee visits. Sample sizes are based on fruit set percentages found on each of five plants in each of three pollen source cages. Data are mean + 1 SD. Bars of percentages not having the same letter differ at $P \leq 0.050$.

Increased numbers of bee visits shortened fruit development time from 58 d (mean of 0- and 1-visit groups) to 53 d (unlimited visits) (Fig. 2). Significant thresholds were reached between one and five visits and 10 and unlimited visits. A 5-d increase in maturation rate would have increased the value of blueberries marketed in May 1991 by an average of $6.96 (17–55%) per flat (USDA 1991).

Berry weight increased with visit level, but this result varied with cage. A statistical interac-

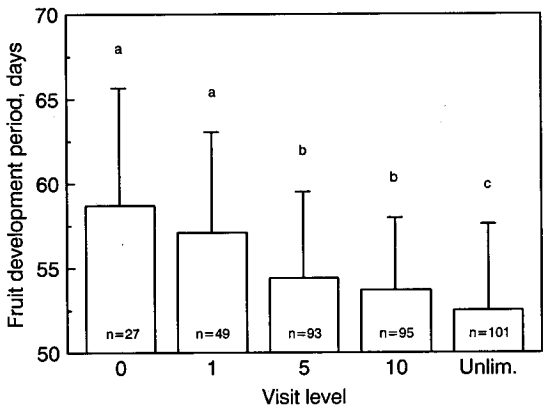

Fig. 2. Days from pollination to harvest of 'Gulfcoast' southern highbush blueberries as a function of level of honey bee visits. Sample sizes are based on all berries harvested from plants in each of three pollen source cages. Data are mean + 1 SD. Bars of development periods not having the same letter differ at $P \leq 0.037$.

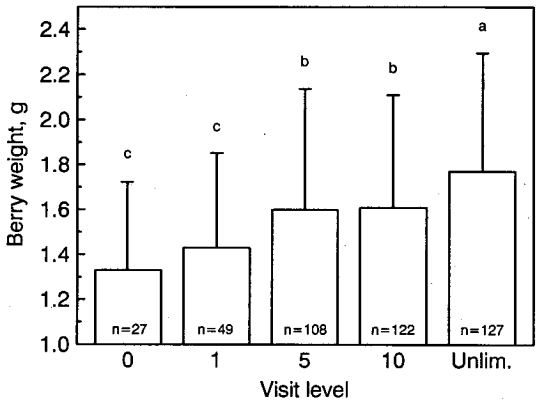

Fig. 3. Weights of 'Gulfcoast' southern highbush blueberries as a function of varying levels of honey bee visits. Sample sizes are based on all berries harvested from plants in each of three pollen source cages. Data are mean + 1 SD. Bars of weights not having the same letter differ at $P \leq 0.032$.

tion of visit and pollen source ($F = 2.15$; df = 8, 43; $P = 0.052$) occurred primarily because of varying trends in the 0- and 1-visit categories, which are represented by small sample sizes. Overall (Fig. 3), berry weights showed a 28% increase from the 0- and 1-visit group (mean = 1.38 g per berry) to the unlimited-visit group (mean = 1.77 g per fruit). Larger blueberries offer enhanced marketability and greater product volume and are especially beneficial if fruit set is maintained near maximum.

Sugar concentration in berry juice decreased as numbers of honey bee visits increased (Fig. 4). Mean concentrations ranged from 11.3% sugar (mean of 5, 10, and unlimited visits) to nearly

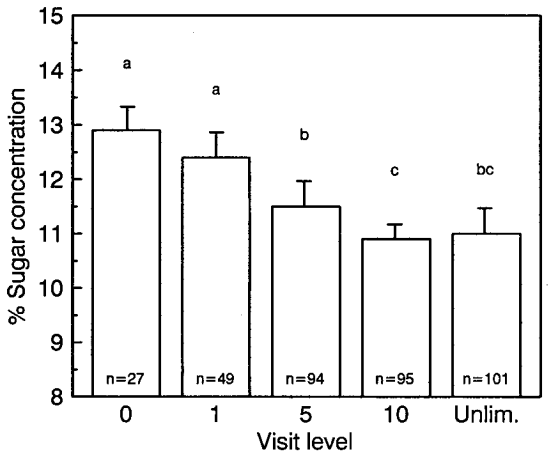

Fig. 4. Sugar concentrations of juice of 'Gulfcoast' southern highbush blueberries as a function of levels of honey bee visits. Sample sizes are based on berries harvested from plants in each of three pollen source cages. Data are mean + 1 SD. Bars of sugar concentrations not having the same letter differ at $P \leq 0.025$.

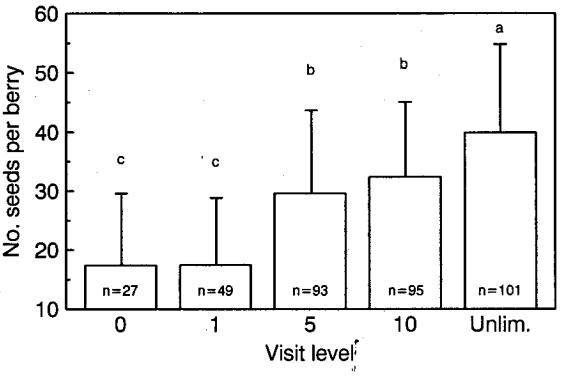

Fig. 5. Numbers of seeds per 'Gulfcoast' southern highbush blueberry as a function of levels of honey bee visits. Sample sizes are based on berries harvested from each of three pollen source cages. Data are mean + 1 SD. Bars of seed numbers not having the same letter differ at $P \leq 0.001$.

13% sugar (0 visits). This relationship may be attributable to bee visits increasing fruit size more than total sugar amounts, yielding lower sugar concentrations in larger berries of a given seediness (see results of correlation analyses). More detailed studies would be necessary to determine fully the relationships between pollination, carbohydrate partitioning, fruit size, and seed number.

Seeds per berry increased 2.2-fold as visits increased from 0 or 1 (mean = 18 seeds per fruit) to unlimited (mean = 40 seeds per fruit) (Fig. 5). Seed counts overall ranged from 3 to 75 per berry. It has been suggested that increased seed counts primarily are a benefit by hastening development time (Lang & Danka 1991); this hypothesis is supported by correlation trends (see *Results*).

Correlations among dependent variables indicated that all four characters measured for individual berries were correlated (most r values near 0.5 or −0.5, each $P < 0.001$, $n = 364–433$). The strongest relationship existed between seed count and development time ($r = -0.647$), suggesting that seed set is a primary determinant of economic return in the early blueberry market. The weakest relationship was between berry weight and sugar concentration ($r = -0.274$), which were not significantly correlated ($P \geq 0.711$) if effects of either seed number or development time were held constant through partial correlation analysis.

An important preliminary finding was the lack of any clear benefit of outcrossing to 'Gulfcoast'. This result suggests not only that providing intervarietal pollinizers may be unnecessary, but also that 'Gulfcoast' may be interplanted with rabbiteyes without loss of fruiting performance. However, previous research showed some fruiting characters to be reduced following hand pollinations of southern highbush with rabbiteye

and mixed-species pollen (Gupton & Spiers 1991). An additional consideration is that pollen source influence may vary substantially among southern highbush cultivars. 'Sharpblue', in which yields benefit from crossing (Lyrene 1989, Lang & Danka 1991), is only 56% *V. corymbosum* germplasm whereas 'Gulfcoast' is 75% of this self-fertile species. Further pollen source tests are necessary before recommending planting arrangements for southern highbush cultivars.

'Gulfcoast' fruiting benefited from five or more honey bee visits under the test conditions. The magnitude of bee visit effects furthermore may have been underestimated if inadvertent pollen transfer occurred when bagging blossoms; minor seed set probably would have more influence on fruiting characters in fewer-visit groups than in greater-visit groups (e.g., Lyrene 1989). Total visit numbers in the unlimited group were not measured, but several dozen visits within 3 d following anthesis seems likely given the foraging activity casually observed. The unrestricted visits led to greater fruit weight and seed number and hastened ripening compared with the 5- to 10-visit level, but may be difficult to achieve in commercial plantings. A future challenge would be to convert knowledge of bee visit benefits into practical, orchard-usable indices of pollination progress for commercial growers of southern highbush (and other) blueberries. One recognized obstacle to developing such a system is that foraging carpenter bees (*Xylocopa virginica* [L.]) may decrease pollination efficacy of honey bees by promoting illegitimate nectar collection through holes cut in the sides of the corolla (Dorr & Martin 1966). Field investigations of the interplay of pollination variables found in specific agroecosystems thus would be useful to help direct the growth of the southern highbush blueberry industry.

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