Honey Bee (Hymenoptera: Apidae) Pollination of Rabbiteye Blueberry *Vaccinium ashei* var. 'Climax' is Pollinator Density-Dependent

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ABSTRACT   In a 2-yr field study, mature orchard plants of rabbiteye blueberry (*Vaccinium ashei* Reade variety 'Climax'), plus potted pollenizers ('Premier') were caged with varying densities of honey bees (0, 400, 800, 1,600, 3,200, 6,400, or 12,800 bees plus open plot) during the bloom interval. The rate of legitimate flower visits tended to increase as bee density increased within a range of 400–6,400 bees; there were more legitimate visits in cages with 6,400 bees than in those with ≤1,600 bees. Similarly, within a range of 400–6,400 bees there was a trend for a corresponding increase in fruit-set with means ranging from 25.0 to 79%. Fruit-set was higher in cages with 6,400 or 3,200 bees than in those with ≤800 bees. Regression analyses showed that fruit-set increased linearly with the rate of legitimate bee visits. Mean weight of berries was unaffected by bee density but varied significantly between years. Within a range of 0–3,200 bees/cage the average seeds per berry tended to increase with increasing bee density; there were more seeds in open plots than in cages with 12,800 honey bees or ≤1,600 bees. Sucrose content ranged from 12.1 to 16.7% and fruits tended to have more sugar in cages with lower bee densities. Speed of ripening tended to be higher in cages with higher bee densities. Earlier work has shown that the effectiveness of *Apis mellifera* L. as a pollinator of rabbiteye blueberry is variety-dependent. Our data indicate that the effectiveness of *A. mellifera* is also bee density-dependent.

KEY WORDS   pollination, rabbiteye blueberry, *Vaccinium ashei*, *Apis mellifera*, bee density

INSECT POLLINATION IS BENEFICIAL for blueberry production, especially for rabbiteye cultivars (*Vaccinium ashei* Reade) that are generally self-incompatible and require cross-pollination with another rabbiteye cultivar (Delaplane and Mayer 2000). Rabbiteye blueberries often have low fruit-set and unacceptable commercial yields (Lyrene and Crocker 1983), problems usually associated with poor pollination (Filmer and Marucci 1963).

Honey bees (*Apis mellifera* L.) are the most numerous bee visitors of blooming rabbiteye blueberries in south Georgia, followed in descending order of abundance by bumble bee queens (*Bombus* spp.), bumble bee workers, carpenter bees (*Xylocopa* spp.), and southeastern blueberry bees (*Habropoda laboriosa* [F.]) (Delaplane 1995). Based on single-bee flower visits, *H. laboriosa* and *Bombus* queens were determined to be the most efficient pollinators of rabbiteye variety 'Tifblue,' and honey bees the least efficient (Cane and Pane 1990). This disparity is due in part to the catholic foraging habits of honey bees, their inability to sonicate (Delaplane and Mayer 2000), and the comparative inaccessibility of the 'Tifblue' flower to short-tongued honey bees. Despite these disadvantages, honey bees are widely recognized as a valuable resource for blueberry growers (Dorr and Martin 1966) to ensure adequate blossom pollination (Eck 1988). They are easily managed and available in large numbers all year long. Moreover, the success with honey bees realized by Sampson and Cane (2000) with the rabbiteye variety 'Climax' suggests that the pollination efficacy of honey bees on *V. ashei* is variety-dependent.

We were further interested in whether the efficacy of honey bees is bee density-dependent. It is conceivable that an individually inefficient pollinator species, as measured by single-bee flower visits, may perform satisfactorily if it is able to field a forager force large enough to increase the rate of legitimate flower visits. Hence, in this 2-yr study we evaluated the effects of different honey bee densities on the rate of legitimate bee flower visits, rabbiteye blueberry fruit-set, berry weight, number of seeds, percent sucrose content of juice, and speed of ripening.

Methods

Plants used for this experiment were part of a permanent orchard at the Horticulture Farm of the University of Georgia, Oconee County, GA. The experi-

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ment was replicated in both 2000 and 2002 and consisted of caging plants with varying densities of honey bees during the bloom interval and then measuring certain characters of fruits. Each caged plot contained four rabbiteye blueberry plants (two mature 'Climax' plants with two potted 'Premier' as pollenizers). Cages were $1.8 \times 1.8 \times 1.8$ m frames covered with Lumite screen (Bioquip, CA). For each year, each $1.8 \times 1.8 \times 1.8$ m plot was assigned one of eight experimental treatments: caged with one honey bee colony containing either 400, 800, 1,600, 3,200, 6,400, or 12,800 bees, containing zero bees, or left open as a positive control. Bees were added to the plots (10 March in 2000, 22 March in 2002) when advanced buds at stage five were still unopened (Spiers 1978). Target bee populations were reached by the gravimetric methods of Delaplane and Hood (1997). Open plots were not provided with potted pollenizers because the orchard was already planted in alternating rows of 'Climax' and 'Premier' plants. Because the apiary of University of Georgia was near the experimental field, honey bees, as well as other bee species, were able to freely visit the open plots. Plants were irrigated as we deemed necessary.

Bee colonies were fed regularly with sugar syrup and socially stabilized with synthetic queen mandibular pheromone (QMP) (one queen equivalent of Bee-Boost [Phero Tech, BC, Canada]) (Currie et al. 1994). QMP was used in lieu of a queen to eliminate confounding effects of differential brood production resulting from variable bee populations.

After bloom was finished, the honey bee colonies were removed and final bee populations determined as before. The Lumite screens were removed to minimize shade effects and all plots netted with poultry fencing to protect fruit from animals and unauthorized harvesting.

The average density of honey bees ([beginning + ending] ÷ 2) for each cage was determined to be 245, 468, 1,008, 2,273, 4,186, and 8,468 (for the 400, 800, 1,600, 3,200, 6,400, and 12,800 bee groups, respectively) for the year 2000 and 286, 609, 1,359, 2,228, 4,490, and 10,656 for 2002. These values were later used for regression analyses of continuous effects, but for convenience we discuss the discrete effects in terms of initial cage densities. The number of unopened florets per raceme was determined for 40 tagged racemes on the 'Climax' plants in each plot during 8–9 March 2000 and 18–20 March 2002 before inserting bee colonies into cages at the stage four and five of blooming (Spiers 1978). To compensate for wind loss of tags, we marked twice as many racemes (80) in the open plots in 2002.

On several days during the bloom period (early March through early April), we measured for each plot (excepting the 0 bee plots) the rate of legitimate honey bee flower visits (number of legitimate flower visits per 2 min) during normal flight hours (1100–1600). Visits during which honey bees probe the terminal aperture of the flower, presumably effecting pollination, were considered legitimate whereas those realized by probing lateral robbery holes in corollae made by carpenter bees [*Xylocopa virginica* (L.)] were considered illegitimate (Faegri and Van der Pijl 1979). *X. virginica* is ubiquitous in the Southeast and with *V. ashei* it invariably engages in nectar thievery. Honey bees are incapable of making robbery holes but readily visit holes made by *X. virginica* and thus act as secondary nectar thieves (Delaplane and Mayer 2000).

Harvest of fruits started at the beginning of June and finished about the middle of July depending on the year. The following dependent variables were measured for each recovered raceme: percent fruit-set, number of seeds per fruit, berry weight (g), speed of ripening, and sucrose content of juice. Percentage fruit-set ([no. fully formed fruit ÷ no. unopened florets] × 100) was determined for each raceme with ripe fruit (2000) or full-sized green fruit (2002). Number of seeds per fruit was determined by expressing berry contents and counting the number of fully-formed seeds. Speed of ripening was calculated for only 1 yr as the percentage of fruits ripe on one arbitrarily-chosen date: 1 June 2002. Percent sucrose content of juice was determined with a bench-top refractometer (Fisher, PA).

Because independent variables used in this study were both discrete (bee density) and continuous (rate of legitimate flower visits, log average bee density), we used both analysis of variance (ANOVA) and regression models. The effects of bee density on rate of legitimate bee visits, fruit-set, berry weight, seeds per berry, and percent sucrose content were determined with a completely randomized ANOVA blocked on year and recognizing density × year interaction as test term. Speed of ripening was measured only 1 yr, so its analysis employed residual error as test term and was not blocked. Means were separated by Duncan's test, and differences were accepted at the $\alpha \leq 0.05$ level (SAS Institute 1992). The relationships of fruit-set and seeds per berry with log-transformed average bee population ($\log_{10}$ [avg. population + 1]) as well as the relationships of fruit-set and seeds per berry with rate of legitimate bee visits, and g per berry with seeds per berry were analyzed with regression models testing for linear, quadratic, and cubic effects (SAS Institute 1992).

Results and Discussion

Bee Density Effects on Flower Visitation. There were bee density effects for the rate of legitimate bee visits ($F = 4.4$; df = 6,6; $P = 0.0463$), but no year effects ($F = 0.1$; df = 1,6; $P = 0.736$). Rate of visitation tended to increase with increasing bee density up to 6,400 bees (Table 1). These results are important because they support the general assumption that increases in *A. mellifera* density correspond to actual increases in flower visitation.

Fruit-Set. Fruit-set was affected by bee density ($F = 5.2$; df = 7,7; $P = 0.0223$), but not year ($F = 3.0$; df = 1,7; $P = 0.1284$). Within a range of 400–6,400 honey bees, there was a trend for increasing fruit-set as bee number increased (Table 1). The highest fruit-set was

Table 1. Bee flower visitation and fruit characteristics of 'Climax' rabbiteye blueberry as affected by honey bee density in cages (ca. 2 × 2 m)

| Honey bee density	Legitimate bee visits/2 min	Fruit set (%)	Mature seeds/berry	Berry weight (g)	Sucrose content of juice (%)	Speed of ripening (%)
Open plot	2.3 ± 0.8 (21) cd	68.9 ± 3.1 (98) ab	23.1 ± 1.3 (63) a	1.2 ± 0.1 (64) a	12.0 ± 0.2 (63) c	27.2 ± 3.5 (67) b
No bees	NA	32.4 ± 3.5 (67) c	0.2 ± 0.1 (43) c	0.8 ± 0.05 (43) a	15.9 ± 0.3 (43) ab	11.5 ± 4.8 (25) c
400	0.5 ± 0.3 (22) d	25.0 ± 3.3 (68) c	1.0 ± 0.5 (34) c	0.9 ± 0.05 (33) a	16.7 ± 0.5 (34) a	9.9 ± 3.7 (18) c
800	4.9 ± 1.4 (21) bcd	48.7 ± 4.1 (78) bc	6.9 ± 1 (57) bc	1.1 ± 0.1 (57) a	16.0 ± 0.4 (57) ab	28.8 ± 5.4 (35) b
1600	7.8 ± 2.4 (21) bcd	52.4 ± 3.5 (70) abc	8.1 ± 0.8 (60) bc	0.9 ± 0.04 (61) a	13.2 ± 0.4 (59) c	8.0 ± 2.5 (32) c
3200	20.3 ± 2.9 (21) ab	79.1 ± 3.3 (63) a	14.2 ± 0.9 (62) ab	1.2 ± 0.1 (62) a	13.1 ± 0.3 (62) c	49.3 ± 5.1 (36) a
6400	25.5 ± 3.4 (20) a	79.0 ± 2.9 (71) a	14.1 ± 0.8 (64) ab	1.2 ± 0.1 (66) a	13.8 ± 0.3 (65) bc	37.1 ± 4.8 (36) ab
12800	16.4 ± 2.1 (22) abc	52.2 ± 4.0 (67) abc	6.5 ± 0.6 (57) bc	1.1 ± 0.1 (57) a	13.7 ± 0.4 (56) bc	38.2 ± 5.5 (33) ab

Data are pooled for years 2000 and 2002 except for speed of ripening which was determined only for 2002. Values are means ± standard errors, with n in parentheses. Means within a column followed by the same letter are not significantly different at the $\alpha = 0.05$ level.

achieved in the open plots and in cages with ≥1,600 honey bees. When fruit-set was analyzed as a response variable related to log-transformed average bee density, the best fit was achieved with a quadratic model (Fig. 1). Declining fruit-set at upper bee density levels is best explained as an artifact of experimental conditions; bees in the 12,800 plots frequently exhibited aberrant flight behavior (flying against the screens) and it is possible that the comparatively high bee : flower ratio reduced the average pollen load of individual foragers. When fruit-set was analyzed as a response variable related to rate of legitimate bee visits (Fig. 2), the resulting best-fit model was linear, indicating no leveling off at upper rates of visitation. It remains to be determined the rate of flower visitation beyond which no additional benefit to fruit-set is realized.

The effectiveness of *A. mellifera* as a pollinator of rabbiteye blueberry is partly variety-dependent. Honey bees were demonstrated to be inefficient pollinators of 'Tifblue' (Cane and Payne 1990) but effective for 'Climax' (Sampson and Cane 2000) based on assays of single-bee flower visits. Our results support those of Sampson and Cane (2000) and confirm that *A. mellifera* is an effective pollinator of *V. ashei* variety

'Climax.' Our data further indicate that the effectiveness of *A. mellifera* is bee density-dependent. More broadly, our results underscore the need to consider the pollinator densities achievable with candidate pollinator species. It is possible that a relatively inefficient pollinator, as determined by a lack of specialized behaviors or phenologies, may nevertheless be effective if it can field a forager force large enough to increase the rate of legitimate flower visitation.

The general manageability of honey bees, considered with their demonstrated variety-specific efficacy as pollinators of *V. ashei* (present results, Sampson and Cane 2000) argue for renewed attention to the plant side of the pollination management syndrome. It is plausible to select for floral morphology in *V. ashei* that is conducive to honey bee pollination. Lyrene (1994) suggested that comparatively short and wide corollae, large apertures, and short distances between stigmata and anthers are favorable characters that would make the blueberry flower more amenable to honey bee pollination. Flowers of *V. ashei* in general exhibit the unfavorable end of these spectra, although there is inter-varietal variation. Indeed, the difference in honey bee efficacy noted between 'Tifblue' (Cane and Payne 1990) and 'Climax' (present results, Sampson

$$y = -501.9 + 334.2x - 48.7x^2$$
$$r^2 = 0.75$$
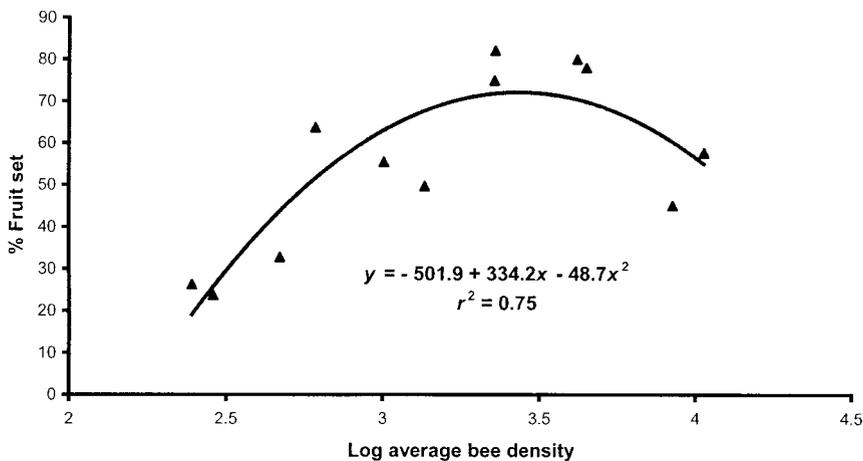
Fig. 1. Regression of fruit set in 'Climax' rabbiteye blueberry with honey bee density in field cages (expressed as log average density). Observed values are mean fruit set (▲), and the line connects predicted values from the quadratic model.
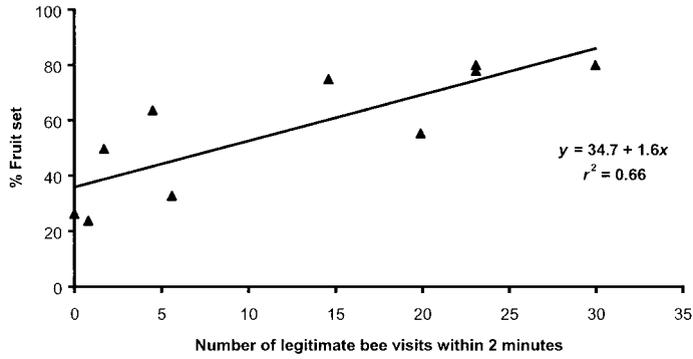
Fig. 2. Regression of fruit set in 'Climax' rabbiteye blueberry in relation to the number of legitimate bee visits within two minutes in field cages. Observed values are mean fruit set (▲), and the line connects predicted values from the linear model.

and Cane 2000) are likely traced to the comparative shortness of the 'Climax' flower. A genetic solution to this problem was intimated by Ritzinger and Lyrene (1999) who were able to show that F_1 hybrids between *V. ashei* and a wild subspecies expressing desirable flower characteristics, *V. constablaei*, displayed flower characteristics intermediate between the two. Such genetic plasticity suggests that plant breeders could select for *V. ashei* phenotypes that are more conducive to honey bee pollination.

Seed Number. The number of fully developed seeds per berry was affected by bee density ($F = 7.2$; df = 7,7; $P = 0.0093$), but not year ($F = 0.3$; df = 1,7; $P = 0.6223$). The number of seeds is a good indicator of the effectiveness of the pollinator as well as a measure of female fertility if compatible pollen is abundant (Ritzinger and Lyrene 1998). In our study the number of seeds per berry was highest in the open plots and plots with 3,200 or 6,400 honey bees; among caged plots there was a tendency for increasing seed number as bee density increased (Table 1). These results are consistent with previous studies that found that blue-

berry fruits produce few seeds when insect pollinators are excluded (Lang and Danka 1991, Froborg 1996, Sampson and Cane 2000).

When seed number was analyzed as a response variable to log-transformed average bee density, the best fit was achieved with a quadratic model (Fig. 3). Declining seed numbers at upper bee density levels is best explained as an artifact of experimental conditions as described above for fruit-set. When seed number was analyzed as a response variable to rate of legitimate bee visits (Fig. 4), the resulting best-fit model was linear, indicating no leveling off at upper levels of visitation, similar to the results for fruit-set. Indeed, one of the most notable features of our study is the general congruence of trends for fruit-set and seed number (compare, e.g., Figs. 1 and 3 with Figs. 2 and 4).

One possible exception to this trend for congruence is the conspicuously high seed numbers achieved in the open plots (Table 1). One explanation for this is the likelihood that flower visitation by a diversity of bee species optimizes seed-set. *Vaccinium* spp. are
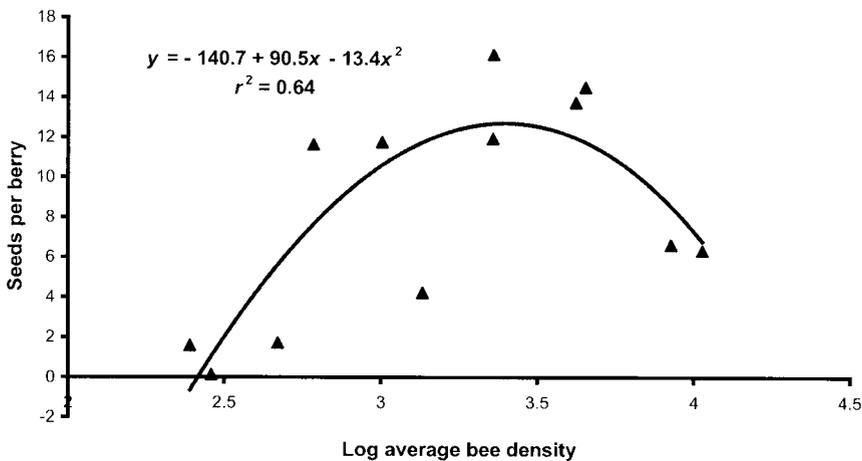

Fig. 3. Regression of number of seeds per berry in 'Climax' rabbiteye blueberry with honey bee density in field cages (expressed as log average density). Observed values are mean seeds per berry (▲), and the line connects predicted values from the quadratic model.
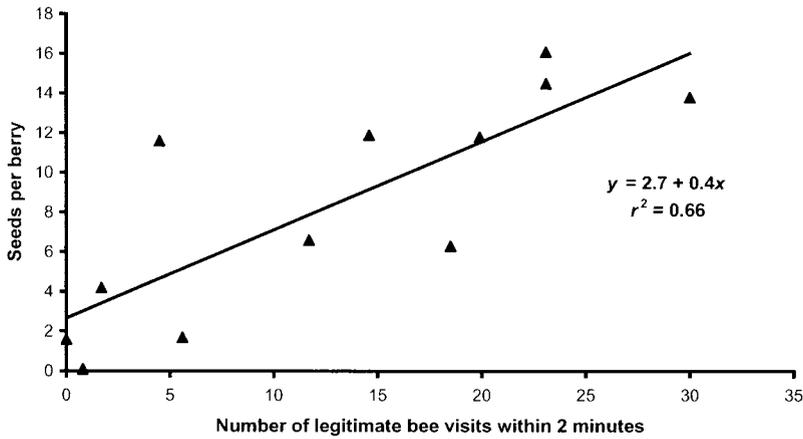
Fig. 4. Seed number in 'Climax' rabbiteye blueberry in relation to the number of legitimate bee visits within two minutes in field cages. Observed values are mean seeds per berry (▲), and the line connects predicted values from the linear model.

responsive to sonicating pollinators such as *Bombus* spp. and *H. laboriosa* (Delaplane and Mayer 2000), and we observed sonicating *Bombus* spp. visiting open plots during this study. Another explanation is the relative abundance of pollenizer pollen in open plots. The high fruit-set achieved in caged plots with relatively intermediate seed numbers (Table 1), however, suggests a partial physiological independence between seed-set and fruit-set in *V. ashei* variety 'Climax.'

Berry Weight. Average berry weight was affected by year ($F = 23.0$; df = 1,7; $P = 0.002$), but not bee density ($F = 0.9$; df = 7,7; $P = 0.564$). Average weight (g) of berries was higher in 2002 (1.35 ± 0.03, $\bar{x} \pm$ SE, $n = 240$) than in 2000 (0.75 ± 0.02, $n = 203$). When berry weight was tested as a response variable against seed number the best fit was achieved with a quadratic model (Fig. 5) wherein berry weight responded positively with seed number but leveled off and declined at upper levels. A positive relationship between the

two is expected in *Vaccinium* spp. (Filmer and Marucci 1963, Dorr and Martin 1966, Brewer and Dobson 1969, Moore et al. 1972) but is not universal, as demonstrated by MacKenzie (1997), who detected no association between seed number and fruit weight in highbush blueberry. With rabbiteye there appears to be a correspondence between small fruit size and low seed number in plants treated with the growth regulator gibberellic acid (NeSmith et al. 1995). Our data implicate a physiological limit to the responsiveness of fruit development to seed set in rabbiteye.

Sucrose Content. The percent sucrose content of juice was affected by bee density ($F = 5.4$; df = 7,7; $P = 0.0201$) and year ($F = 13.3$; df = 1,7; $P = 0.0082$). Fruit juice tended to have more sucrose in plots with fewer bees and correspondingly lower fruit-set (Table 1). Fruits from plants experiencing poor fruit-set are invested with a comparatively higher fraction of available carbohydrates. The percentage sucrose was higher in 2002 ($14.8 \pm 0.2\%$) than 2000 ($13.3 \pm 0.2\%$).
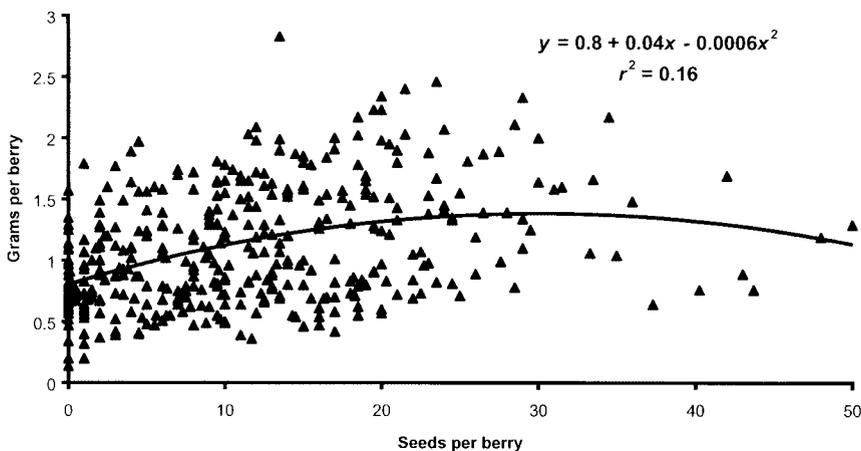

Fig. 5. Berry weight (g) in 'Climax' rabbiteye blueberry in relation to number of fully-formed seeds. Observed values are berry weight (▲), and the line connects predicted values from the quadratic model.

The most notable climatic difference between years was greater rainfall in 1 January through 31 July 2002 (Σ = 586.5 mm) than for the same period in 2000 (451.4 mm).

Speed of Ripening. Speed of fruit ripening was affected by honey bee density (F = 9.1; df = 7, 274; $P <$ 0.0001). Fruits ripened more quickly in plots with $\geq$3,200 bees than in plots with 1,600 bees or $\leq$400 (Table 1). Speed of ripening was higher in the 3,200-bee plot than in the open plot. Cross-pollination is known to improve speed of ripening in highbush blueberries (MacKenzie 1997); the present data indicate a similar benefit when pollination is optimized in rabbiteye.

Conclusion. Earlier work has shown that the effectiveness of *Apis mellifera* as a pollinator of rabbiteye blueberry is variety-dependent. Our data indicate that *V. ashei* variety 'Climax' responds positively to increases in honey bee density as measured by fruit-set, seed number, and speed of ripening. We conclude that honey bee pollination of *V. ashei* variety 'Climax' is also pollinator density-dependent.

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