Environmental Effects on Blueberry Flower Size and Shape Are Minor

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Abstract. The effects of environmental factors, including chilling duration during dormancy and temperature during flower bud expansion, were studied on the following blueberry flower parameters: corolla length, corolla aperture diameter, stigma location relative to the apex of the corolla tube, position of the anthers relative to the stigma and to the apex of the corolla, and style length. Flowers on plants that were chilled over 1400 hours differed little from those that received only 310 chill units. Flowers that developed under warmer temperatures had significantly wider corolla apertures. In one experiment but not the other, corolla length and style length increased under warmer temperatures. For nearly every parameter in each of three experiments, there were significant environment $\times$ clone interactions. Overall, however, it appeared that neither lack of chill units during dormancy nor warm temperatures during flower development changed flower morphology enough to affect fruit set.

Flowers of the cultivated blueberry species (*Vaccinium angustifolium* Ait., *V. corymbosum* L., and *V. ashei* Reade) have evolved for cross pollination by insects. The most effective of these are the sonicating bees, which extract blueberry pollen by positioning themselves at the aperture of the pendent corolla tube and vibrating their flight muscles (Buchmann, 1983; Cane and Payne, 1990). The resulting vibration causes pollen to pour out of the poricidally dehiscent anthers onto the bee's face. The stigma of the cultivated blueberry flower is normally slightly exserted from the corolla tube, but in some cases may be about flush with the end of the corolla tube or slightly inserted. Honeybees (*Apis* spp.) do not sonicate flowers (Cane et al., 1993). Reports on their effectiveness in pollinating commercial blueberry plantings are variable. Domesticated honeybee colonies are normally placed in commercial plantings of lowbush (*V. angustifolium*) and highbush (*V. corymbosum*) blueberries to supplement native populations of *Bombus* and other sonicating bees. In most cases, honeybees appear to improve fruit set, although in some years they abandon the blueberry fields for more attractive pollen sources.

The reputation of the honeybee as a pollinator of the rabbiteye blueberry (*V. ashei*) in the southeastern United States is even more tenuous. Flowers of rabbiteye blueberry are normally longer and have a smaller corolla tube aperture than flowers of highbush and lowbush blueberries. Furthermore, carpenter bees (*Xylocopa* spp.) often cut holes in the base of the rabbiteye corolla tube to extract nectar, and honeybees quickly learn to search for carpenter bee slits rather than to attempt to get nectar from the natural corolla aperture (Cane and Payne, 1988).

Low fruit set (percentage of the flowers that form mature berries) has been a problem in many commercial rabbiteye blueberry fields in Florida and Georgia during the past decade (Lyrene and Crocker, 1983). Small plantings tend to have higher fruit set than large plantings, which may indicate insufficient populations of native sonicating bees to provide good pollination on large farms. Another pronounced trend has been for fruit set problems to worsen as a standard group of cultivars is planted along a line from Wilmington, N.C. (colder winters), to Orlando, Fla. (warmer winters). In north Florida, fruit set on rabbiteye blueberries appears

to be best after cold winters and cool springs (Lyrene and Crocker, 1983).

In tomato (*Lycopersicon esculentum* Mill.), high temperatures during flowering reduce fruit set by causing the style to elongate beyond the anther cone, reducing the possibility of pollen deposition on the stigma (Charles and Harris, 1972). This suggested the possibility that high temperatures during flower development might change some structural feature of the blueberry flower in such a way as to decrease the effectiveness of bee pollination.

Eck and Mainland (1971) studied morphological factors in the blueberry flower that might be related to insect pollination efficiency. Flower measurements from 35 cultivars of highbush blueberry were compared to data that estimated the reliability of fruit set in these cultivars. The ideal flower from the standpoint of maximum fruit set appeared to be one with a short corolla that widened at the middle to >8 mm and then narrowed at the base.

The purpose of this study was to determine whether any structural features of the blueberry flower that could affect pollination or fruit set are highly sensitive to change by environmental factors such as duration of chilling during dormancy and temperature during flower bud expansion.

Materials and Methods

Three experiments were conducted. In the first experiment, ten 3-year-old blueberry plants, each grown from a rooted cutting of a different cultivar, were dug from field plots at the Univ. of Florida Horticultural Unit in Gainesville, Fla., on 20 Dec. 1992. These were potted and placed in an unlighted refrigerator at 5C for 1300 h. The plants had been fertilized only lightly during the fall and were nearly leafless when placed in the cooler. They had already received some chilling ($\approx$ 100 h below 7C) in November and December before they were placed in the refrigerator. Five plants were rabbiteye, two were rabbiteye $\times$ *V. constablaei* Gray F₁ hybrids, three were highbush, and one was a synthetic hexaploid hybrid (three rabbiteye, two highbush, and one *V. darrowi* Camp genomes). On 12 Feb. 1993, the plants were moved to a greenhouse where temperature varied from 5 to 27C. On 24 Mar. 1993, 10 flowers from each plant were harvested several days after anthesis and placed in 70% ethanol. On 30 Mar., flowers were collected from the same clones growing in field nurseries at the Univ. of Florida Horticultural Unit. The mean temperature from 1 Dec. 1992 through 30 Jan. 1993 at the Horticultural Unit was 15C,

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which equates to a winter with about 310 chill units (CUs) (Sherman et al., 1987), according to the relationships developed by Weinberger (1956) and Sharpe (1969).

Six structural features were measured for each flower: *Corolla length*, measured from the pedicel attachment point to the end of the corolla tube. The pedicel attachment point was chosen as the point from which to measure the corolla length even though the base of the corolla does not extend quite that far, because the actual position of the base of the corolla is hidden by the calyx. *Corolla aperture*, the widest diameter was measured. *Corolla position vs. stigma*, the distance between the tip of the stigma and the innermost sinuses of the corolla tube. Negative values were assigned where the corolla did not reach to the stigma apex; i.e., the stigma extended outside of the corolla sinuses. *Anther length vs. corolla*, the distance from the end of the longest anther in the flower to the sinuses of the corolla tube. Negative values were recorded when the anthers did not extend as far as the corolla sinuses. *Anther length vs. stigma*, the distance from the end of the longest anther to the apex of the stigma. Negative values were assigned when the anthers did not extend as far as the stigma apex. *Style length*, measured from the point of attachment with the ovary to the apex of the stigmatic surface. Except for the first two characteristics, all measurements were made using a 20× stereo microscope.

Analysis of variance (ANOVA) was performed as follows. A sampling error variance was obtained by pooling over plants the variances among the 10 flowers within plants. A two-way ANOVA was then performed to find the clone variance, the environmental variance, and the variance for clone × environment interaction. The interaction variance was used to test the significance of the clone and environment variances, and the significance of the interaction was tested against the sampling error variance.

In the second experiment flowers were gathered from four rabbiteye cultivars ('Powderblue', 'Tifblue', 'Beckyblue', and 'Bluebelle') and one southern highbush cultivar ('Sharpblue') growing in field plots at two locations (Gainesville and Dover, Fla.). The flowers were gathered shortly after anthesis, in Gainesville on 30 Mar. 1993 and in Dover on 26 Apr. 1993. The mean daily temperature in Gainesville for the 30 days before sampling was 16C and the mean temperature in Dover for the 30 days before sampling was 20C. The mean temperature for Dover from 1 Dec. 1992 through 30 Jan. 1993 was 19C (≈25 CUs by the Weinberger-Sharpe relationship) compared to a mean temperature of 15C (310 CUs) for Gainesville for the same period. Flower measurements and statistical analysis of the data were made as in Expt. 1.

In the third experiment, branches bearing flower buds were gathered from nine native rabbiteye blueberry clones growing in a forest near Crestview, Fla., on 28 Mar. 1993. The mean temperature for 1 Dec. 1992 through 30 Jan. 1993 at Crestview was 14C (480 CUs). Only strong branches with 1 m of 2-year-old wood attached were used. All larger flowers and all but a few flower buds were removed from the branches. The remaining flowers had just begun to emerge from the flower buds, and had corollas <5 mm long. Two branches for each clone were placed in a flower-forcing solution consisting of 0.11 g citric acid, 0.12 g 8-hydroxy-quinoline hemisulfate salt, plus 50 g sucrose per liter of water. The branches were then placed in dark growth chambers, one at 5C and the other at 20C. The ends of the branches were re-cut and the forcing solutions were changed weekly throughout the forcing period. The first 3 to 10 flowers that opened on each branch were measured as in Expt. 1. The mean value for each flower parameter was calculated for each branch. A two-way ANOVA was conducted to partition the variances due to environment, clone, and environment × clone interaction. The significances of the environ-

ment and clone effects were tested with F tests, with the interaction variance as the denominator.

Results and Discussion

In Expt. 1, increased CUs during dormancy, (>1400 compared to ≈400) had little effect on any flower parameter when plants that flowered in the field in Gainesville were contrasted with those that were potted from the field, chilled in a refrigerator, and then placed in a greenhouse for flowering. The only statistically significant change in overall means was that corolla aperture diameter was slightly higher in the highly chilled plants that were placed in the greenhouse (3.2 mm vs. 2.9 mm, Table 1). The clonal variance component and the clone × environment interaction were significant to highly significant for all parameters measured (Table 2). There was no indication that insufficient chilling received in the field changed flower morphology in a way that would have reduced fruit set.

Two factors differed significantly for flowers that received 40 CUs and opened under warmer field conditions compared to plants that received 310 CUs and opened under cooler field conditions (Dover, collected 26 Apr. vs. Gainesville, collected 30 Mar.). The corolla aperture diameter averaged 3.2 mm over all cultivars under the warmer conditions compared to 2.2 mm under the cooler conditions (Table 3). The only other feature for which the means over all cultivars differed significantly between the two environments was anther length vs. corolla length; in the cooler environment, the anthers were recessed deeper into the corolla (Table 3). As in Expt. 1, the location × clone interaction was significant for every parameter measured (Table 4), and there was significant

Table 1. Average flower shape parameters for 10 blueberry clones flowering in a greenhouse following 1400 chill units and in the field at Gainesville, Fla., following 310 chill units.

Trait	Mean (mm)		F test
	Greenhouse	Field	
Corolla length	11.9	11.7	NS
Corolla aperture	3.2	2.9	*
Corolla length vs. stigma	-1.5 ^z	-1.4	NS
Anther length vs. corolla	-1.7	-1.5	NS
Anther length vs. stigma	-2.6	-2.4	NS
Style length	9.6	9.2	NS

^zNegative values indicate that the first item is shorter than the second.

NS,*Nonsignificant or significant at $P = 0.05$ according to F test with numerator = environments (1 df) and denominator = environment × clone interaction (9 df).

Table 2. Analysis of variance for various traits for 10 clones (C) in two environments (E) (see Table 1), mean squares and significance test.^z

Trait and df	Environments	Clones	E × C	Error ^y
df	1	9	9	180
Corolla length	1.80 ^{NS}	32.44 ^{**}	3.72 ^{**}	0.21
Corolla aperture	5.12 [*]	6.48 ^{**}	0.62 ^{**}	0.23
Corolla length vs. stigma	0.41 ^{NS}	2.69 [*]	0.80 ^{**}	0.12
Anther length vs. corolla	0.91 ^{NS}	16.08 ^{**}	0.86 ^{**}	0.19
Anther length vs. stigma	0.25 ^{NS}	20.58 ^{**}	2.43 ^{**}	0.10
Style length	7.41 ^{NS}	17.69 ^{**}	1.60 ^{**}	0.11

^zE × C interaction was tested against the error variance with an F test.

^yVariance among flowers within cultivars within locations.

NS,*Nonsignificant or significant at $P = 0.05$ or 0.01, respectively. E and C variance were tested against the E × C variance.

Table 3. Average flower shape parameters for five blueberry clones in field plantings at two Florida locations (Dover and Gainesville). Flowers collected 26 Apr. at Dover and 30 Mar. in Gainesville.

Trait	Mean (mm)		F test
	Dover	Gainesville	
Corolla length	11.6	11.5	NS
Corolla aperture	3.2	2.2	**
Corolla length vs. stigma	-1.3 ^z	-1.6	NS
Anther length vs. corolla	-2.2	-1.4	*
Anther length vs. stigma	-2.9	-2.3	NS
Style length	9.8	9.4	NS

^zNegative values indicate that the first item is shorter than the second.
^{NS,*,**}Nonsignificant or significant at $P = 0.05$ or 0.01 , respectively, according to F test with locations (1 df) the numerator and location $\times$ clone interaction (4 df) the denominator.

Table 4. Analysis of variance for various traits for five clones (C) in two Florida locations (L) (Dover vs. Gainesville). Mean squares and significance test.^z

Trait and df	L	C	L $\times$ C	Error ^y
df	1	4	4	90
Corolla length	0.20 ^{NS}	11.52*	0.98*	0.34
Corolla aperture	28.09**	7.76*	0.74**	0.17
Corolla length vs. stigma	2.11 ^{NS}	7.59 ^{NS}	4.69**	0.08
Anther length vs. corolla	14.82*	5.15 ^{NS}	1.02**	0.16
Anther length vs. stigma	8.53 ^{NS}	13.61*	1.66**	0.14
Style length	5.43 ^{NS}	17.46*	2.31**	0.16

^zMean squares for L and C were tested against L $\times$ C; L $\times$ C was tested against error.
^yVariance among flowers within cultivars within locations.
^{NS,*,**}Nonsignificant or significant at $P = 0.05$ or 0.01 , respectively.

Table 5. Average flower shape parameters on cut branches for nine rabbiteye blueberry clones forced at 20 or 6C.

Trait	Mean (mm)		F test
	20C	5C	
Corolla length	9.64	9.26	*
Corolla aperture	1.86	1.26	**
Corolla length vs. stigma	-1.83 ^y	-1.51	NS
Anther length vs. corolla	-0.15	-0.08	NS
Anther length vs. stigma	-1.80	-1.39	NS
Style length	8.65	7.94	**

^yNegative values indicate that the first item was shorter than the second.
^{NS,*,**}Nonsignificant or significant at $P = 0.05$ or 0.01 , respectively. Denominator of F test was clone $\times$ environment interaction.

clonal variation for corolla length, corolla aperture diameter, style length, and anther length compared to stigma length.
For the flowers that developed on cut branches forced in dark growth chambers, three parameters differed significantly between the two temperatures (Tables 5 and 6). As in Expt. 2, the corolla aperture was significantly wider under the warmer conditions. In addition, both the corollas and the styles were slightly longer when they developed under warmer conditions. It is not apparent how either change could markedly reduce fruit set under the warmer conditions.

Table 6. Analysis of variance for various flower characteristics for nine rabbiteye blueberry clones (C) with flowers developing in warm and cold environments (E) (see Table 5). Means squares and significance tests.^z

Trait and df	E	C	E $\times$ C
df	1	8	8
Corolla length	0.648*	0.844**	0.070
Corolla aperture	1.670**	0.144 ^{NS}	0.043
Corolla length vs. stigma	0.448 ^{NS}	0.345 ^{NS}	0.143
Anther length vs. corolla	0.016 ^{NS}	0.639**	0.102
Anther length vs. stigma	0.753 ^{NS}	0.686*	0.179
Style length	2.224**	0.160 ^{NS}	0.088

^zE and C mean squares tested against the E $\times$ C interaction.
^{NS,*,**}Nonsignificant or significant at $P = 0.05$ or 0.01 , respectively.

Clone $\times$ environment interactions were significant for most parameters (Tables 2 and 4). Even for parameters where means over all clones did not differ significantly between environments, means for individual clones often did differ significantly between environments.
However, for some characteristics, the magnitude, and sometimes even the direction of the environmentally induced changes, were inconsistent from one clone to another.
Taken as a whole, the data in these three experiments give little support to the idea that either duration of winter chilling or temperatures during flower development changes the structure of the blueberry flower enough to have a major effect on flower fertility. Although the corolla apertures are wider at higher temperatures, this would seem to improve rather than interfere with cross pollination by affording better bee access to the anthers and stigma. Chill unit accumulation and/or temperatures during flower development could influence fruit set in rabbiteye blueberries by some other mechanism, but their effects on important flower morphology parameters seem minor.

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