Fruit Varieties Journal 48(3):176-182 1994

Fruit and Seed Set of Half-High Blueberry Cultivars Following Repeated Pollinations at Varying Time Intervals

RICHARD C. HARRISON, JAMES J. LUBY AND PETER D. ASCHER[1]

Abstract

Self pollinations were detrimental to the fertility of the blueberry cultivars St. Cloud, Northsky, and Northcountry compared to outcross pollination with 'Northblue.' Multiple self pollinations resulted in higher fruit set than single self pollinations. For 'Northcountry,' initial self pollinations negatively affected seed set from subsequent outcross pollinations performed up to 24 h later. With 'St. Cloud' and 'Northsky,' outcross pollen applied up to 24 h following an initial self pollination was apparently 'outcompeting' or preferentially fertilizing ovules. Variation in seed set among cultivars may have been due, in part, to differences in number of available ovules and timing of their receptivity.

Introduction

In most blueberries, fruit set is positively correlated with seed set (1, 10, 16, 19 22). Significant reductions in seed set and fruit set following self pollination, as compared to outcross pollination, have been documented in several blueberry species (1, 4, 16, 20). Successful commercial production of many blueberry cultivars depends on the amount of seed set and pollen source in a given acreage.

The terms incompatibility and self incompatibility are often used to describe reduced reproductive fertility of blueberries following self pollination (2, 7, 8). By Mather's definition, self incompatibility is a pre-fertilization barrier (15). However, research in blueberry does not support the existence of a pre-fertilization barrier. Similar self and outcross pollen tube growth rates have been found (7) suggesting a lack of pre-fertilization control that would be expected with a self-incompatibility system. Self pollen tubes have also been observed entering (6, 13) and fertilizing (21) ovules even in self-sterile genotypes, indicating post-fertilization control over fruit and seed formation. Research further suggests that the reductions in self fertility observed in blueberries are a function of inbreeding depression (9, 11, 13). This evidence supports the hypothesis that a post-zygotic mechanism, rather than a pre-fertilization-self-incompatibility response, is causing the reductions in self fertility in *Vaccinium* spp.

One consequence of post-fertilization abortion would be a reduction in the number of viable seeds. In previous research, applications of self pollen reduced the number of potential receptive ovules available for fertilization in many cases (14). This suggests that, following an initial self pollination, fewer receptive ovules will be available for pollinations done hours later resulting in lower fruit and seed set for the double pollinations as compared to single outcross pollinations. The objective of this study was to determine whether initial self pollination would negatively affect the fertility of later self or outcross pollinations in several half-high blueberry cultivars reported to have reduced fertility in self pollinations compare to cross pollinations (10, 20).

Materials and Methods

The half-high cultivars St. Cloud, Northsky, and Northcountry were used as females and 'Northblue' was used as the outcross pollen source in all

Scientific Journal Series Paper Number 20,404 of the Minnesota Agricultural Experiment Station, St. Paul. We gratefully acknowledge the statistical assistance of Dr. Frank Martin and Mr. Harold Buck from the Dept. of Applied Statistics, Univ. of Minnesota.

[1]Research Assistant, Associate Professor, and Professor, respectively, Department of Horticultural Science, University of Minnesota, Saint Paul, MN 55108.

treatments. Several plants of each cultivar were forced into flower in a greenhouse following four months of chilling at approximately 7°C. Ten pollination treatments represented combinations of pollen sources (self and outcross) with, in some treatments, varying times between the initial and subsequent pollinations. There were four types of treatments: 1) a single self pollination at anthesis (self); 2) a single outcross pollination at anthesis (cross); 3) a self pollination at anthesis followed by a second self pollination (self+self) at 0 (anthesis), 6, 12 or 24 hours later; and 4) a self pollination at anthesis followed by an outcross pollination (self+cross) at 0 (anthesis), 6, 12 or 24 h later. Pollen was extracted by removing a flower and rolling it between the thumb and index finger. As the flower was rotated, the pollen was deposited onto the thumb nail of the other hand. The thumb nail was then touched to the stigmas to transfer pollen.

Berries were harvested on alternate days through the ripening period and the number of plump, brown seeds in each berry was counted. The experimental unit of this study was an inflorescence; however, data were taken on individual berries as they were collected. The berries from each inflorescence were used to calculate inflorescence means which were then used in the analysis. Data were taken for percent fruit set, seeds per berry, and seeds per pollinated flower. The number of seeds per pollinated flower was calculated as seeds per berry weighted by percent fruit set.

The data were analyzed as an incomplete block design. At anthesis, ten inflorescences, at a similar stage of development, were tagged as a block and randomly assigned the ten treatments. These inflorescences were judged developmentally similar when approximately one half of the flowers within an inflorescence were at anthesis. Each inflorescence was also thinned to a maximum of ten flowers. Three blocks of the ten treatments were assigned to each of the three cultivars for a total of nine blocks to be analyzed.

In the analysis of variance, cultivar and cultivar x treatment effects were tested by partitioning degrees of freedom from the block and block x treatment effects, respectively. Contrasts were included to study certain preplanned comparisons. Regression analysis was used to test linear and quadratic effects of the time between pollinations for both the self+self and self+cross time series treatments.

Results

The analysis of variance revealed significant cultivar and pollination treatment effects as well as cultivar x pollination interactions for seeds per berry and seeds per pollinated flower with all three cultivars included ($p \leq 0.01$; Table 1). Since the three cultivars responded differentially to the pollination treatments, separate analyses were also performed for each cultivar for these variables. Pollination effects were also significant ($p \leq 0.01$) for percent fruit set, but since cultivar x

Table 1. Analyses of variance for effect of pollination treatment on percent fruit set, seeds per berry, and seeds per pollinated flower for the blueberry cultivars 'St. Cloud,' 'Northsky,' and 'Northcountry.'

	Percent fruit set		Seeds per berry		Seeds per pollinated flower	
Source	df	Mean squares	df	Mean squares	df	Mean squares
Blocks	8	0.08	8	48.92	8	53.54
Cultivar	2	0.10	2	165.06**	2	170.98**
Residual	6	0.08	6	10.20	6	14.39
Treatment	9	0.38**	9	468.39**	9	502.57**
Block x Treatment	71	0.04	66	24.53	71	23.86
Cultivar x Treatment	18	0.04	18	46.56**	18	46.79**
Error	53	0.04	48	16.26	53	16.07

**, significant F tests ($p \leq 0.01$).

pollination interactions and cultivar effects were not significant, the percent fruit set data from the three cultivars were pooled. Responses for seeds per berry and seeds per pollinated flower were similar throughout this experiment, so, only seeds per pollinated flower will be presented. In general, cross pollination or some mixture of self and outcross pollen always resulted in greater fertility than self pollinations alone (Figure 1).

Percent fruit set. The length of time between the first and second pollinations had no significant effect on percent fruit set for either the self+self or self+cross time course treatments (regression coefficients were not significant, $p > 0.05$, data not presented). Single outcross pollinations at anthesis were also not significantly different ($p > 0.05$) from the self+cross treatments as a group (Table 2). Overall, the self+self treatments had lower fruit set than the self+cross and single outcross treatments (Figure 1) and the difference between the self+self and self+cross groups was significant ($p \leq 0.01$; Table 2). Single self pollinations at anthesis were also significantly lower ($p \leq 0.01$) than the self+self group (Table 2). Self pollinations followed immediately by cross pollinations (self+cross at anthesis) were not significantly different from single outcross pollinations at anthesis ($p \geq 0.05$), but significantly higher than single self pollinations at anthesis ($p \geq 0.01$). Single cross pollinations were also significantly higher than single self pollinations at anthesis ($p \leq 0.01$).

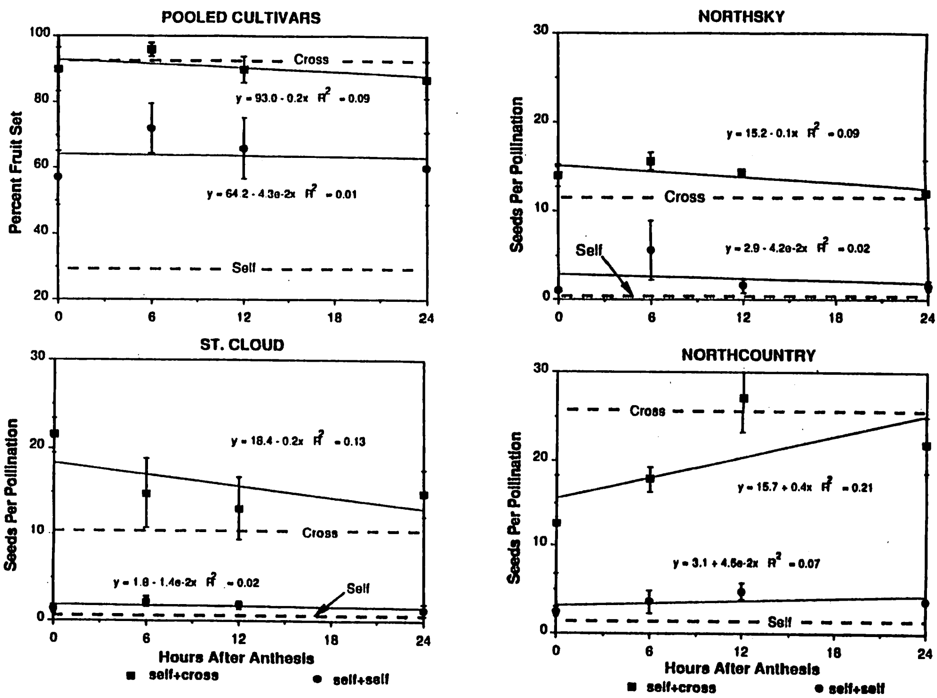

Figure 1. Linear regression (per observation basis) of percent fruit set (pooled) and seeds per pollinated flower on time between an a self pollination at anthesis followed by self (self+self) or cross pollinations (self+cross) applied 0, 6, 12 and 24 h later. Single self and cross pollinations at anthesis (0 h), are represented by dashed lines. Points and bars represent treatment means and standard errors, respectively.

Seed set. A single self pollination at anthesis produced significantly fewer seeds per pollination than a single cross pollination at anthesis for 'St. Cloud' ($p \leq 0.05$), 'Northsky' ($p \leq 0.01$) and 'Northcountry' ($p \leq 0.01$). The length of time between the first and second pollinations had no significant effect on seed set for the self+self time course treatments (regression coefficients were not significant, $p > 0.05$, data not presented). Likewise, time between pollinations had no effect ($p > 0.05$) on seeds per pollinated flower for the self+cross treatments in both 'Northsky' and 'St. Cloud.' In 'Northcountry' seeds per pollinated flower produced by the self+cross pollinations increased significantly ($p \leq 0.05$) with longer times between pollinations and was similar to the single cross pollination only when the time interval was 12 or 24 h (Figure 1).

The single self pollination was not significantly different ($p > 0.05$) from the multiple self pollination treatments as a group for seeds per pollinated flower in any cultivar (Table 2) and 'Northcountry' was the only cultivar which produced significantly more seeds per pollinated flower ($p \leq 0.05$) in the single outcross treatment compared to the self+cross treatments as a group (Table 2); the other cultivars did not differ for these treatments. In each of the three cultivars, the self+self treatments resulted in significantly fewer seeds per pollinated flower ($p \leq 0.01$) than the self+cross treatments (Table 2).

The three cultivars each had different responses to the pollination treatments applied only at anthesis. In 'Northcountry,' the single self pollination produced the fewest seeds followed by mixed pollination (self+cross at anthesis) and the outcross pollination resulted in most seeds ($p \leq 0.01$; Table 2 and Figure 1). In 'Northsky,' outcross and mixed pollinations were not significantly different ($p > 0.05$) while the self pollination produced significantly fewer seeds ($p \leq 0.01$; Table 2). 'St. Cloud' was different from the other cultivars in that it produced significantly more seeds per pollinated flower following mixed pollination at anthesis compared to the outcross pollination which, in turn, resulted in more seeds than in the self pollination ($p \leq 0.05$; Table 2).

Table 2. Means of pollination treatments at anthesis, means for self+self and self+cross treatments pooled over time after anthesis (0, 6, 12, 24h) and levels of significance for contrasts among pollination treatments.

	Seeds per pollination			% fruit set
Pollination treatments	'St. Cloud'	'Northsky'	'Northcountry'	Pooled cultivars
1. Self at anthesis	1.27	0.07	1.75	30.0
2. Cross at anthesis	10.39	11.57	26.26	94.0
3. Self+cross at anthesis	21.48	14.01	12.47	90.1
4. Pooled self+self	1.67	2.42	3.59	63.8
5. Pooled self+cross	16.03	14.01	19.81	90.6
Contrasts[1]				
1 vs 2	*	**	**	**
1 vs 3	**	**	**	**
1 vs 4	ns	ns	ns	**
2 vs 3	**	ns	**	ns
2 vs 5	ns	ns	*	ns
4 vs 5	**	**	**	**

[1]Single degree of freedom contrasts partitioned from the 9 treatment degrees of freedom.
ns, not significant; *, $p \leq 0.05$; **, $p \leq 0.01$.

Discussion

In all cases, self pollen alone, whether applied once or twice, resulted in inferior fertility compared to treatments that included outcross pollen. Multiple self pollinations resulted in higher percent fruit set than a single self pollination. The increased fruit set may have been due to the availability of ovules which were not yet receptive at the time of the initial self pollination but were fertilized with the second pollination and lead to increased fruit set. Multiple self pollinations by insect pollinators may explain why some monoculture plantings of blueberries maintain levels of fruit set that are similar to fruit set in mixed plantings (12, 17). If weather conditions are favorable for pollinator activity, a flower may be pollinated several times, increasing fruit set above that expected based on experimental single self pollination data.

The increase in percent fruit set with double self pollinations as compared to single self pollinations was accompanied by an increase in seeds per berry but not by an increase in seeds per pollination. However, even a small change in seeds per berry could determine the difference between fruit set and fruit abortion and, therefore, lead to significant differences in percent fruit set.

The gradual increase in seed set of 'Northcountry' as second pollinations were performed over the 24 hour time course was not consistent with the original hypothesis that ovules lost to zygote abortion from an initial self pollination would leave fewer receptive ovules available for pollinations performed hours later resulting in lower fruit and seed set for the double pollinations as compared to single outcross pollinations. This inconsistency may also be due to variation in ovule development. An inflorescence of up to ten flowers was initially pollinated when at least one half of the flowers reached anthesis. At this stage of development a number of receptive ovules are likely available for fertilization. Based on developmental studies of megagametogenesis in blueberry, as time passes more ovules continue to develop and become available for fertilization (3, 5, 17, 18). This may explain the gradual increase in seeds per pollinated flower as second pollinations were performed from 0 hours to 12 hours following the initial self pollination. Although the initial self pollination may have reduced the number of available receptive ovules, more ovules may have matured over time and compensated for those lost to the initial self pollination.

The seed set responses of 'Northcountry' to the pollinations performed at anthesis were consistent with the original hypothesis in that the presence of the self pollen reduced the fertility of the mixed pollination treatment as compared to the outcross treatment (Figure 1). However, the seed set responses of 'Northsky' and the pooled fruit set responses of the mixed and outcross pollinations did not differ suggesting that outcross pollen may be favored when in competition with self pollen or may preferentially fertilize ovules. Pollen competition was also suggested in a similar time-course experiment using *V. corymbosum* cultivars in which outcross pollen 'outcompeted' self pollen (14). The lack of change in seed set with increasing time between self and subsequent cross pollinations (Figure 1) and the difference between the self+cross treatments and the single outcross at anthesis (Table 2) further suggest that cross pollen may have an advantage over self pollen within the first twenty four hours following a self pollination for 'Northsky.'

In contrast to the other cultivars, mixed pollination of 'St. Cloud' at anthesis unexpectedly resulted in greater fertility than the outcross treatment. The various self+cross treatthe single outcross at anthesis (Table 2) suggesting that preferential fertilization or pollen competition may be a factor in this cultivar as well.

Beyond the hypothesized variation for ovule receptivity, variable total ovule numbers in the three cultivars may also account for their differing responses. For example, Northcountry, which appeared to have ovules maturing over a period of time following anthesis, may exhibit this response because of a larger total number of ovules. Ovule numbers were not counted for the three cultivars; however, examining the maximum observations for seeds per berry should give some relative indication of variation among the cultivars for total ovule number. 'Northcountry' had the highest (45.7 ± 4.6) followed by 'St. Cloud' (36.3 ± 3.2) and then 'Northsky' (29.3 ± 2.3). These responses seem to concur with the ovule receptivity observations.

In summary, the results from this study reinforce the observations that, compared to outcross pollinations, self pollinations are detrimental to the fertility of the Minnesota blueberry cultivars. Even though multiple self pollinations resulted in higher fruit set than single self pollinations, they always produced fruit or seed set levels lower than outcross pollination responses. Initial self pollinations did not negatively affect fertility when followed by outcross pollinations performed up to 24 h later. Outcross pollen applied up to 24 h following an initial self pollination appeared to be 'outcompeting' or preferentially fertilizing ovules in two of the three cultivars. In future studies of this kind data on variation among genotypes for timing of ovule receptivity may enhance understanding of variation among cultivars for fertility.

Literature Cited

1. Aalders, L. E. and I. V. Hall. 1961. Pollen incompatibility and fruit set in lowbush blueberries. Can. J. Genet. Cytol. 3:300-307.
2. Ballington, J.R. and G. J. Galletta. 1978. Comparative crossability of 4 diploid *Vaccinium* species. J. Amer. Soc. Hort. Sci. 103:554-560.
3. Bell, H. P. 1957. The development of the blueberry seed. Can. J. Bot. 35:139-153.
4. Coville, F. V. 1921. Directions for blueberry culture, 1921. U.S.D.A. Bul. 974.
5. Eaton, G. W. and A. M. Jamont. 1966. Megagametogenesis in *Vaccinium corymbosum* L. Can. J. Bot. 44:712-714.
6. El-Agamy, S. Z. A., W. B. Sherman, and P. M. Lyrene. 1980. Pollen germination and tube growth in blueberry. Proc. 4th North Amer. Blueberry Res. Workers Conf., 1979, Univ. of Arkansas, Fayetteville. p. 27-34.
7. El-Agamy, S. Z. A., W. B. Sherman, and P. M. Lyrene. 1981. Fruit set and seed number from self- and cross-pollinated highbush (4x) and rabbiteye (6x) blueberries. J. Amer. Soc. Hort. Sci. 106:443-445.
8. Garvey, E. J. and P. M. Lyrene. 1987. Self incompatibility in 19 native blueberry selections. J. Amer. Soc. Hort. Sci. 112:856-858.
9. Harrison, R. E., J. J. Luby and P. D. Ascher. 1993. Genetic characteristics of self fertility in highbush and half-high blueberries. Euphytica. 67:79-88.
10. Harrison, R. E., J. J. Luby and P. D. Ascher. 1993. Pollen source affects yield components and reproductive fertility of four half-high blueberry cultivars. J. Amer Soc. Hort. Sci. (in press).
11. Hellman, E. W. and J. N. Moore. 1983. Effect of genetic relationship to pollinizer on fruit, seed, and seedling parameters in highbush and rabbiteye blueberries. J. Amer. Soc. Hort. Sci. 108:401-405.
12. Johnston, S. 1943. Essentials of blueberry culture. Mich. St. Coll. Agr. Exp. Sta. Cir. Bul. 188.
13. Krebs, S. L. and J. F. Hancock. 1988. The consequences of inbreeding on fertility in *Vaccinium corymbosum* L. J. Amer. Soc. Hort. Sci. 113:914-918.
14. Krebs, S. L. and J.F. Hancock. 1990. Early-acting inbreeding depression and reproductive success in the highbush blueberry, *Vaccinium corymbosum* L. Theor. Appl. Genet. 79:825-832.
15. Mather, K. 1943. Specific differences in *Petunia*. I. Incompatibility. J. Genet. 45:215-235.
16. Meader, E. M. and G. M. Darrow. 1944. Pollination of the rabbiteye blueberry and related species. Proc. Amer. Soc. Hort. Sci. 45:267-274.
17. Merrill, T. A. 1936. Pollination of the highbush blueberry. Mich. Agr. Exp. Sta. Tech Bul. No. 151.
18. Moore, J. N. 1964. Duration of receptivity to pollination of flowers of the highbush blueberry and the cultivated strawberry. Proc. Amer. Soc. Hort. Sci. 85:295-301.

19. Morrow, E. B. 1943. Some effects of cross-pollination versus self-pollination in the cultivated blueberry. Proc. Amer. Soc. Hort. Sci. 42:469-472.
20. Rabaey, A. and J. Luby. 1988. Fruit set in half-high blueberry genotypes following self and cross pollination. Fruit Var. J. 42:126-129.
21. Vander Kloet, S. P. 1991. The consequences of mixed pollination on seed set in *Vaccinium corymbosum*. Can. J. Bot. 69:2448-2454.
22. White, E. and J. H. Clark. 1938. Some results of self-pollination of the highbush blueberry at Whitesbog, New Jersey. Proc. Amer. Soc. Hort. Sci. 36:305-309.

Fruit Varieties Journal 48(3):182-184 1994

'Splendor' and 'Regal' Lingonberry—New Cultivars for a Developing Industry

ELDEN J. STANG, J. KLUEH AND G. WEIS

Abstract

'Splendor' and 'Regal' lingonberry are named and released by the University of Wisconsin-Madison for use in breeding programs or for commercial production. Both are derived as selections from open pollinated seed obtained from southwest Finland. 'Splendor' and 'Regal' are precocious, moderately vigorous with some frost tolerance displayed in fruit buds and flowers. Fruits are medium to large in size, high in anthocyanin pigment and are comparable to other lingonberry genotypes in ascorbic acid content and juice pH.

Introduction

The lingonberry, *Vaccinium vitis-idaea* L. is a woody, evergreen, low growing shrub of the Ericaceae, widely distributed in temperate, boreal and arctic regions of the northern hemisphere. Fernald (1) described the species as consisting of two botanical varieties including the larger European plant *V.* var. *vitis-idaea* L. and the more diminutive North American type *V.* var. *minus* Lodd. The lingonberry fruit, a small red berry up to 1.2 cm in diameter is widely consumed in Northern Europe, Asia and Canada. Principal uses include sauce, juice, jams, wines or liqueurs and as a component of baked dessert products. Until recently, fruits were solely obtained from the wild. Limited areas of commercial plantings have been established within the past decade in Germany and Sweden.

In 1987, under the auspices of a Fulbright research grant, extensive collections of lingonberry germplasm including seed and plants were made in Finland by the senior author. In 1988, a project was initiated at the University of Wisconsin-Madison to assess the adaptability of lingonberry to northern U.S. conditions and to determine fundamental cultural management requirements of this potential new crop (2).

One of the objectives of this project is the testing and release of germplasm adapted for commercial production. 'Splendor' and 'Regal' are the first of a potential series of lingonberry cultivar releases from this program.

Origin

'Splendor' (WI102) and 'Regal' (WI-108) originated from open-pollinated seed collected in August, 1987 from different lingonberry clones growing in the wild near the village of Lieto in southwest Finland. Plants from seed were planted at the Hancock Experiment Station, Hancock, Wisconsin in the spring of 1988. Initial plant se-

Research supported in part by a Fulbright Research Grant and by the College of Agricultural and Life Sciences, University of Wisconsin-Madison, Wisconsin Hatch Project No. 3215.
Professor, Assistant Researcher, and Professor Emeritus, respectively, Department of Horticulture, University of Wisconsin-Madison, Wisconsin 53706.