Effect of Boron and Calcium Foliar Sprays on Pollen Germination and Development, Fruit Set, Seed Development, and Berry Yield and Quality in Lowbush Blueberry (*Vaccinium angustifolium* Ait.)

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ABSTRACT. In a managed field of native *Vaccinium angustifolium* Ait. clones, the effect of fall foliar sprays of B at 345 g·ha⁻¹ and/or Ca at 3,450 g·ha⁻¹ in remedying tissue deficiency of B varied among 12 clones, as seen in pollen germinability and on individual stems as seen in flower number, fruit set, and number of harvestable berries. With Ca applied alone, increased berry size did not overcome yield reduction due to fewer flowers and berries per stem. Berry diameter and mass correlated better to number of seeds of germinable size than to total number of seeds. Pollen germination averaged 17.4% on stigmata from untreated clones, and all three treatments (B, Ca, B + Ca) increased that average by 8%. More seeds per berry with the B-alone treatment implies more ovules fertilized when B deficiency is remedied. No relation was found between in vitro and in vivo pollen germination.

Although lowbush blueberry cultivars have been developed, the crop in Maine, the Canadian Maritimes, and Quebec is almost entirely produced by genetically diverse wild clones that have long lived a few m apart and may have originated from seeds that sprouted more than a century ago. *Vaccinium angustifolium* Ait. dominates, often interspersed by clones of *V. myrtilloides* Michx. Production is maximized by a biennial cycle with pruning to the ground level.

B deficiency is believed to be the most widespread of micronutrient deficiencies in plants (Sparr, 1970). Considering previous reports of improved fruit set and retention through B supplementation on cultivars of various tree fruit, *Fragaria*, *Vaccinium macrocarpon* Ait., and nuts (Batjer and Thompson, 1949; Bramlage and Thompson, 1962; Callan et al., 1978; DeMoranville and Deubert, 1987), we looked for possible benefits from B treatment of lowbush blueberry clones rated deficient in foliar B content. Linkage of genotype to B requirement is suggested by a large variation of both the requirement and tolerance of excess among crops and cultivars (Gupta, 1993).

The sufficiency of B in the reproductive tissues, the basis for its importance to fruit set, retention, and quality, requires more than sufficiency of the element in the substrate on which the plant grows. Uptake by roots demands a balance of solubility, which is favored by low pH, loss by adsorption on mineral particles, and a moisture level between drying and loss of solute through runoff. Once taken up by a dicotyledonous plant, some of the B is immobilized and may leave such fast-growing tissues as flowers deficient if overall B is low (Eck, 1988). According to recommendation to Maine growers, the B status of a blueberry field is best

assessed by foliar analysis at cessation of terminal growth (tip dieback) (Trevett et al., 1968).

Trevett (1972) proposed a foliar B standard of 24 mg·kg⁻¹ in the prune year and 30 mg·kg⁻¹ in the crop year. Only about half of Maine blueberry fields meet the prune-year standard (Smagula, 1993). Deficiency from this standard may be caused by leaching from the soil of B compounds solubilized at the pH of 4.5 to 5.2 characteristic of Maine blueberry land. This relatively low soil pH is otherwise beneficial by favoring ericoid mycorrhiza which delivers N from amino acids in the organic pad of the substrate through the action of extracellular acid proteinase (Leake and Read, 1990).

Need for B and Ca in the germination medium to optimize the initiation of the germination tube in vitro has been shown for various angiosperms (Brewbaker and Kwack, 1964; Thompson and Batjer, 1950). Boron is also required for stigma receptivity and pollen tube extension, possibly through formation of sugar-borate complexes that promote absorption, translocation, and metabolism of sugars in pollen, and through participation in synthesizing pectic material for the cell wall of the actively growing pollen tubes (Vasil, 1964). The subsequent development of seeds also requires B (Mozafar, 1993). Correlation found by Aalders and Hall (1961) between number of good (presumably germinable size) seeds per lowbush berry and size and mass of the berry further suggests that remedying B deficiency may increase yield.

Fruit quality and good yield require adequate B and Ca (Ballinger and Kushman, 1970; Smith et al., 1987; Woodruff et al., 1960), but in some crops adverse effects on the quality have resulted from B treatments (Bramlage and Thompson, 1962). It is now well established that when plants are unable to obtain adequate B from either the soil or from plant reserves to meet the requirement of young developing tissue, the B requirement can be effectively supplied in the form of B sprays (Batjer et al., 1953; Callan et al., 1978; Hanson, 1991; Shrestha et al., 1987).

Smagula (1993) reported that foliar application of B in fall of the noncrop year to lowbush blueberry clones that had assayed <20 mg·kg⁻¹ of B earlier in the year at tip dieback gave increases the following spring in blossoms per bud, but harvest of marketable fruit >5 mm was not significantly increased in two of the five

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clones treated even though foliar B deficiency in the crop year had been remedied in all five.

Here we report findings from 12 genetically diverse low-B (<20 mg·kg⁻¹) clones in a single field with both B and Ca fall foliar application, separately and together. Three experiments with supplemental B and/or Ca comprise this study: 1) in vitro assay of pollen germinability; 2) in vivo extension of pollen tubes from pollen deposited on stigmata; 3) effects in a blueberry field on fruit set, yield, and fruit quality.

Materials and Methods

Twelve discrete *Vaccinium angustifolium* clones in a commercial field in Washington County, Maine, were the source for all the plant material. These particular clones were selected for foliar B <20 mg·kg⁻¹ (well below the 24-mg·kg⁻¹ standard by more than the probable error in the analytical method) at tip dieback in July 1993 from 25 well-defined clones of which only two were >24 mg·kg⁻¹. Selection was based on leaf samples prepared according to the methods of Kalra and Maynard (1991) and analyzed by dry-ashing (Chapman and Pratt, 1961) using an inductively coupled Argon-plasma spectrophotometer (Jarrell-Ash, model 975, Atomcomp, Franklin, Mass.).

Within each clone four 2.4 × 2.4-m plots were defined. On 20 Sept. 1993 treatments were applied to plots with a handheld sprayer of high-density polyethylene (Craftsman model 786.15720, Sears Roebuck and Co., Chicago). Shields prevented spray from drifting between plots while spraying ≈500 mL of solution, to the point of dripping.

One plot in each clone received B at 400 mg·L⁻¹ (345 g·ha⁻¹ of B, prepared from Bortrac 1, 10.6% H₃BO₃, Shield-Brite, Kirkland, Wash.). A second plot received Ca at 4,000 mg·L⁻¹ (3,450 g·ha⁻¹ of Ca, prepared from Stopit-6, 12% CaCl₂, Shield-Brite). A third received the same volume spray containing both B and Ca at 400 mg·L⁻¹ and 4,000 mg·L⁻¹ from these products. The fourth plot in each clone received water as a control. Position of the four plots within the clones was randomized (randomized complete-block design).

For Expts. 1 and 2, two sod pieces were cut out of each treatment plot on 2 Nov. 1993 and kept in 11.2-cm plastic pots at 4 °C for 4 months to satisfy the bud dormancy requirement. To verify for the sod pieces and the test plots of which they were part that translocation of B and Ca had occurred from leaves to stems and buds, the apical 3.8 cm of 30 stems from each plot with their flower buds were cut two months after treatment, dried, and ground to pass through a 40-mesh sieve, and assayed as done earlier in selecting the low-B clones. Again at tip dieback in July 1994 leaves from 30 stems cut from each plot were assayed to determine to what extent the treatments of the previous season's leaves had increased B concentration and overcome presumed B deficiency.

EXPERIMENT 1. Pots were taken out of cold storage and kept in a greenhouse for two weeks at 25 °C to begin growth, then placed in a growth chamber (Conviron, model PGW 36, Controlled Environment Ltd., Winnipeg, Canada) for flowering under 16-h day length (34.2 W·m⁻²), 24 °C in light and 16 °C in darkness, and 54% to 62% relative humidity. The first flower was observed 4 d after transfer to the growth chamber.

The experiment used tetrads from six fully open flowers selected from a sod piece representing each treatment plot. They were dropped into an empty petri dish by rolling the flower between the thumb and the forefinger. Red nail polish on the outside of the dish gave visual contrast for picking up 15 tetrads, 5 at a time, with a single camel hair mounted (with nail polish) to

a glass rod. They were placed on a cover slip bearing a 1-mm-thick layer of the medium. The in vitro medium was 5% agar containing 12% lactose. Unlike sucrose, lactose provides osmolarity but is not utilized metabolically by pollen (Bishop, 1949). This eliminated influence on results by a factor extraneous to the pollen. In this medium we found from a trial with early pollen from the sod plugs that germination could be seen as soon as three h after deposit when kept at 21 °C in the incubator (model 818, GCA/Precision Scientific, Chicago). In the subsequent data collection, a characteristic four-grain pollen tetrad was counted as germinated if a tube protruding from it extended in 20 h by more than the tetrad diameter. The cover slip was inverted over a glass well slide (75 × 25 × 1.5 mm, 0.8-mm well depth, VWR Scientific Corp., South Plainfield, N.J.) with ≈1 μL of water in the well and sealed against drying with four evenly spaced drops of microscope immersion oil. After 20 h in the dark incubator at 21 °C the tetrads were scored at 2000× (model CH, Olympus Optical Co., Ltd., Tokyo) for evidence of germination. With sod plugs from clone 10 failing to yield adequate numbers of viable flowers, the experiment used 11 clones.

EXPERIMENT 2. Sod pieces from 4 of the 12 study clones—identified as numbers 1, 6, 9, and 12—provided the pollen for in vivo evaluation of germinability and pollen-tube extension. These were selected from results of the in vitro trials with early pollen available from the potted samples of the test plots that received no B or Ca (controls). Compared with ≈55% germination from clones 1, 9, and 12, the pollen from clone 6 showed ≈85% in vitro germinability but was used to avoid delaying Expts. 1 and 2 beyond usefulness of the potted plant material.

With the 16-h day length at 24 °C and 8-h darkness at 16 °C, stigmata remained sticky for 6 to 7 d after the corolla fully opened. Since *V. angustifolium* stigmata protrude beyond the corolla, selfing was not a problem as in most plant breeding work, and they were left and marked with ink, thus avoiding possible physiological effect from emasculation. Fifteen tetrads from one flower of a donor sod piece were placed with the camel hair on a receptive stigma; ten flowers from each treatment plot of a clone were thus pollinated. Clone 1 provided pollen on the stigmata of three clones, clone 9 on stigmata of three other clones, clone 12 on stigmata on three others, clone 6 on stigmata of one other clone. Two of the clones had too few flowers to be used as pollen receivers in Expt. 2. Only flowers from sod pieces from the control plots served as pollen donors. All pollination was done within 3 d of anthesis. In the growth chamber, germination on the stigmata was observed 2 d after pollination. After 3 d, pistils were collected individually with forceps from the marked flowers.

The technique of Martin (1959) was used to prepare the pistils for fluorescence microscopy to rate pollen germination on the stigma and observe extent of pollen-tube growth through the style. The pistils were fixed for 48 h in a bath of 3 parts ethanol and 1 part 1 N glacial acetic acid. They were then soaked for about 12 h in 8 N NaOH followed by five rinses in distilled water over 2 h and staining for 20 min. The stain was 0.1% water-soluble Aniline Blue in 0.7% K₃PO₄ buffer (pH 8.5). After a rinse in distilled water, the stained pistils were flattened with cover slips on glass slides and examined by fluorescence microscopy (model VBD, Nikon, Nippon Kogaku K.K., Tokyo). Excitation was at 365 to 510 nm and observation at 500× by emission filtered to pass 400 to 610 nm.

EXPERIMENT 3. In the field in the first week of May 1994, the crop year, two of the 12 study clones were found to have relatively few stems bearing exactly four fruit buds. In each treatment plot of the other 10 clones, 20 four-bud stems were tagged to be followed through the season for number of flowers from each bud, percent-

age of flowers setting fruit, and the number, size, mass, and color of each berry left at harvest. In July the number of fruit developing at each bud position on the tagged stems was recorded to calculate initial fruit set. In August, before fruit harvest, tagged stems with berries were cut and placed individually in plastic bags and transported on crushed ice to a freezer, and stored at -20°C .

Counting and sizing of each berry at each bud position on the 800 collected stems was automated with a scanning image analyzer (AgVision Monochrome System, Decagon Devices Inc., Pullman, Wash.). The berries from each bud were aligned on a light box, calyx end down, under the video camera of the analyzer system and the average radius of each berry determined and presented as average diameter. Each was then weighed and rated by eye as green, green pink, pink red, red blue, or blue.

For correlations between berry diameter, mass, and number of sized seeds per berry, only berries produced on the third bud from the tip on 10 randomly chosen stems in each plot were used, because a trial with fruit from an untreated low-B clone in the same field had found no effect of bud position on seed-size frequency distribution. Seed counting and sizing were done with the image analyzer. About 30 frozen berries per treatment from bud 3 from clones 2, 3, 4, 5, and 6 were each peeled with forceps to eliminate skin pigment that might lead to false images during subsequent scanning. The pulp was cut in half, and all seeds were washed out with several drops of distilled water and washed twice more to remove any remaining pigment. For each berry, seed number and transverse cross-sectional area of each seed were recorded.

All seeds of 20 berries from control plots were sized and germination-tested to ascertain germinable size for use in relating seed-size frequency to berry diameter and mass. For this test, seeds from each fruit were surface-sterilized with 10% Clorox for 30 s, then washed twice with distilled water. Seeds from fruit of the same plot were mixed, then grouped into nine size categories: <0.1 , 0.1 to 0.2 , 0.2 to 0.3 , 0.3 to 0.4 , 0.4 to 0.5 , 0.5 to 0.6 , 0.6 to 0.7 , 0.7 to 0.8 , and >0.8 mm². From all study clones 10 replicates consisting of one seed of each size category above the smallest, ordered by size on sterilized filter paper were sealed with Parafilm into petri dishes with 5 mL of sterilized water. After 10 weeks at 25°C , the replicates in randomized positions beneath two 60-W cool-white fluorescent tubes, were assessed for germination from the nine sizes.

For data on yield and fruit characteristics, all 48 treatment plots were harvested by hand rake and winnowed of leaves, stems, and smallest berries. Berries from each treatment plot were weighed in the field and ≈ 1500 g from each were transported under air conditioning and immediately sorted through two sets of parallel bars to a diameter between 6 and 9 mm.

After overnight storage at 4°C , three 35-g samples from each

treatment plot were tested for firmness with equipment that measures peak force used in crushing a layer of berries to 2 mm above their supporting surface (Instron model 1000, Instron Corp., Canton, Mass., equipped with extrusion cell, 500-kg weigh beam, crosshead speed $20\text{ mm}\cdot\text{min}^{-1}$). The remaining berries from each treatment plot were stored at -18°C for subsequent characterizing from purees.

Percentage of soluble solids in the supernatant from 2 g of puree from each of the field treatment plots was measured by refractometer (model A-300, Fisher Scientific Co., Medford, Mass.). Each sample was homogenized with 8 mL of distilled water in a polytron (Kinematica GMBH, Lucern, Switzerland) and centrifuged at $16,000\text{ g}_n$ for 1 min (Eppendorf Centrifuge 5415 C, Brinkman Instruments, Inc., Westbury, N.Y.).

Titrate acidity was measured by titrating 10 g of puree in 90 mL of water, for each of the 48 plots, with 0.1 N NaOH to pH 8.1 by pH meter (model 811 pH meter, Beckman Instruments, Inc., Fullerton, Calif.) and stated as percent citric acid.

To assay mineral content, 2-g samples of puree from each plot were dried at 105°C (Isotemp Oven, model 350, Fisher Scientific Co., Pittsburgh) for at least 2 h, ashed in a muffle furnace (model 293C, Barber-Colman Co., Rockford, Ill.) at 525°C for 5 h, weighed at room temperature, dissolved in 50% HCl, and brought to volume in a 100-mL volumetric flask for analysis by plasma spectrophotometer. Data were subjected to analysis of variance using the General Linear Model of SAS (Release 6.07, SAS Institute Inc., Cary N.C. 1992). Treatment effects were separated by Duncan's multiple range test at the 5% level. All percentage data were arcsin-transformed for statistical analysis, but presented as the original data.

Results and Discussion

Some clones gave positive effects from treatment, some negative, possibly reflecting differences in absorption and/or translocation to reproductive tissues of the nutrients applied to the low-B leaves of the previous year. Also, differences in inherent requirements of the reproductive tissues of specific clones might contribute to the difference in response. In all clones the control plots assayed higher for foliar B in July 1994 than those clones had assayed when selected for low B in July 1993 (Table 1), reflecting the difference between prune year and crop year standards (Trevett, 1972).

That foliar-applied B entered the plant is shown by the increase in stem, leaf, and fruit B concentrations over the controls (Tables 1 and 2). Among the 12 clones, the one that responded most to application of B alone showed an increase of foliar B from 17.1

Table 1. Boron and calcium concentrations in *Vaccinium angustifolium* stem, leaf, and fruit tissues as influenced by a $400\text{ mg}\cdot\text{L}^{-1}$ B and/or $4000\text{ mg}\cdot\text{L}^{-1}$ Ca foliar spray in September of the noncrop year.

Spray	B ($\text{mg}\cdot\text{L}^{-1}$)			Ca ($\text{mg}\cdot\text{L}^{-1}$)		
	Stem ²	Leaf ³	Fruit ⁴	Stem	Leaf	Fruit
Control	20.8 b ^w	26.0 b	8.5 b	5585 a	3949 b	1319 a
B	28.8 a	35.2 a	9.7 ab	5650 a	4174 a	1328 a
Ca	20.2 b	25.1 b	8.5 b	5552 a	3938 b	1282 a
B + Ca	26.9 a	34.2 a	10.7 a	5832 a	3961 b	1345 a

²Sampled in Nov. 1993, top 3.8-cm portion of stem from the tip, with fruit buds.

³Sampled in July 1994, the crop year.

⁴Intact fruit sampled in August 1994.

^wData represent means of 12 clones. Values within columns followed by different letters are significantly different by Duncan's multiple range test, 5% level.

Table 2. Comparison of clonal foliar concentrations in response to 400 mg·L⁻¹ B foliar spray in September of the noncrop year 1993.

Clone	July 1993 foliar B (mg·L ⁻¹)	July 1994 foliar B (mg·L ⁻¹)		July 1994 foliar B increase (mg·L ⁻¹)	
		Control	B treatment	Control	B treatment
1	17.1	29.5	41.6	12.4	24.5
2	16.1	26.1	32.5	10.0	16.4
3	12.6	21.2	31.6	8.6	19.0
4	16.0	24.7	38.3	8.7	22.3
5	15.3	27.0	39.0	11.7	23.7
6	15.4	24.8	30.2	9.4	14.8
7	17.0	27.9	34.8	10.9	17.8
8	12.9	24.6	32.8	11.7	19.9
9	17.2	28.4	36.8	11.2	19.6
10	17.8	30.5	37.5	12.7	19.7
11	15.7	23.9	38.1	8.2	22.4
12	15.3	22.8	29.4	7.5	14.1
Mean	15.7	26	35.1	10.3	19.4

Table 3. In vitro germination of pollen from 11 *Vaccinium angustifolium* clones as affected by 400 mg·L⁻¹ B and/or 4000 mg·L⁻¹ Ca foliar spray in September of the noncrop year.

Clone	Treatment				Mean
	Control	B	Ca	B+Ca	
	Pollen germination (%)				
1	57.8 a ^z	52.1 a	52.3 a	41.3 a	50.9
2	39.3 ab	21.0 b	61.5 a	56.4 a	44.6
3	95.6 a	51.0 b	83.4 a	84.9 a	78.7
4	67.1 a	22.4 c	36.2 b	31.0 bc	39.2
5	25.9 c	56.6 b	55.4 b	76.0 a	53.5
6	84.9 a	69.8 b	47.2 c	51.1 c	63.2
7	84.6 a	85.4 a	89.5 a	78.2 a	84.4
8	31.9 c	60.9 b	72.8 b	96.2 a	65.4
9	56.6 b	68.1 b	97.9 a	30.6 c	63.3
11	81.9 a	97.9 a	86.8 a	43.0 b	77.4
12	54.1 a	8.0 c	40.7 a	26.4 b	32.3
Mean	61.8 a	54.5 b	65.9 a	56.0 b	

^aEach value represents an average of 6 replications of 15 pollen tetrads. Values within rows followed by different letters are significantly different at the 5% level, according to Duncan's multiple range test.

mg·kg⁻¹ in July 1993 to 41.6 mg·kg⁻¹ in July 1994, while the one that responded least to application of B alone showed an increase from 15.3 to 29.4 mg·kg⁻¹ (Table 2), slightly below the 30 mg·kg⁻¹ standard for the crop year. The same two clones were respectively the highest and the lowest in their July 1994 foliar B concentrations resulting from application of B alone. This suggests an interclonal difference in B transport. No increased Ca concentration was found from Ca application in the November 1993 stems, July 1994 leaves, or the 1994 fruit (Table 1).

EXPERIMENT 1. In vitro pollen germination was significantly increased from two clones (5 and 8) by treatment with B alone, significantly decreased by the treatment from four clones (3, 4, 6, and 12), and not significantly affected from five other clones (Table 3). In two clones (3 and 12) Ca in the treatment overcame the negative effect of B applied alone. In one clone (4) Ca applied alone resulted in less depression of germinability of its pollen, than did application of B alone. In pollen from two clones (5 and 8), the combination of B and Ca was significantly more effective for in vitro germination compared to B or Ca alone, whereas in two others (9 and 11) the combination significantly depressed in vitro germination of the pollen compared to B or Ca alone. This diverse

response by a genetically diverse population (Table 3) contrasts with more consistent findings on in vitro germination by Montgomery (1951), Robbertse et al. (1990), and Vasil (1964), where nutrient was added directly to the pollen germination medium.

When our in vitro germination percentages were averaged over 11 clones, B alone or in combination showed a distinctly negative effect while Ca alone showed little effect (Table 3).

EXPERIMENT 2. No relationship was found between in vitro and in vivo germinability. Though the pollen source clones (1, 9, and 12) were chosen for similar in vitro germinability (~50%) without B or Ca treatment, pollen germination in vivo varied from 0% to >60% germination. This variation may represent degrees of genetic compatibility apart from that required for the mating of gametes when pollen tubes enter the ovary. Pollen from the control plot of one clone (6) that showed 85% in vitro germination (Table 3) gave only 13% to 33% in vivo germination on clone 8 stigmata (Table 4), depending on the treatment applied to the receiving clone.

The percentage of pollen tubes that had reached the base of the style 3 d after pollen transfer ranged from 0% to 34%. B treatment alone of the clones that supplied material for the female role did not

Table 4. In vivo 3-d pollen germination on the stigma and pollen tube growth through the styles of 10 *Vaccinium angustifolium* clones as affected by 400 mg·L⁻¹ B and/or 4000 mg·L⁻¹ Ca foliar spray in September of the noncrop year.

Clone	Treatment				Pollen source
	Control	B	Ca	B+Ca	
Pollen germination on stigma (%)					
1	61.3 a ^z	50.7 a	33.3 a	40.0 a	Clone 9
2	0.0 b	10.7 ab	16.7 ab	24.0 a	Clone 12
4	25.3 a	35.6 a	22.7 a	51.7 a	Clone 1
5	1.3 b	1.3 b	4.7 b	67.3 a	Clone 9
6	17.3 b	48.7 a	11.3 b	9.3 b	Clone 12
7	6.7 a	16.0 a	24.0 a	10.7 a	Clone 1
8	15.2 a	25.3 a	33.3 a	12.7 a	Clone 6
9	1.7 c	23.3 b	60.0 a	30.0 b	Clone 12
11	25.0 a	30.7 a	33.3 a	26.7 a	Clone 9
12	18.0 a	3.3 ab	16.7 ab	0.0 b	Clone 1
Pollen tubes reaching the base of the style ^y					
1	28.7 ab	34.0 a	12.5 b	24.4 ab	Clone 9
2	0.0 a	3.3 a	4.7 a	2.7 a	Clone 12
4	6.0 a	11.1 a	8.0 a	15.0 a	Clone 1
5	0.0 b	0.0 b	0.0 b	23.3 a	Clone 9
6	7.3 a	15.3 a	2.7 a	1.3 a	Clone 12
7	1.3 a	5.3 a	1.3 a	0.0 a	Clone 1
8	7.6 a	8.7 a	1.3 a	4.7 a	Clone 6
9	0.0 b	6.7 b	30.0 a	9.3 b	Clone 12
11	14.2 a	9.3 a	15.6 a	8.0 a	Clone 9
12	1.3 a	0.0 a	2.0 a	0.0 a	Clone 1

^zEach value represents an average germination of 10 replications of 15 pollen tetrads. Values within rows followed by different letters are significantly different at the 5% level, according to Duncan's multiple range test.

^yPollen tube extension to or beyond the lowest one-third of style.

Table 5. In vivo 3-d pollen germination and extent of pollen tube growth through styles of 10 *Vaccinium angustifolium* clones as affected by 400 mg·L⁻¹ B and/or 4000 mg·L⁻¹ Ca foliar spray in September of the noncrop year.

Spray	Germination (%)	To upper 1/3 (%)	To middle 1/3 (%)	To or beyond lowest 1/3 (%)
Control	17.4 b	7.0 b ^z	2.3 a	6.6 a
B	25.7 a	10.0 ab	4.0 a	9.9 a
Ca	25.5 a	13.8 a	3.3 a	7.6 a
B + Ca	26.6 a	9.6 ab	4.0 a	8.6 a

^zEach value represents an average of 10 clones and 10 replications of 15 pollen tetrads. Values within columns followed by different letters are significantly different at the 5% level, according to Duncan's multiple range test.

affect these percentages. Boron + calcium treatment of one clone (5), from which the pollen gained in vitro germinability with all treatments (Table 3), gave in the female role with clone 9 pollen an increase of in vivo germination from very low to 67%. This mating also resulted in an increase in percentage of tubes attaining complete traversal from 0% with no supplementation or with B or Ca applied alone to 23% with B + Ca treatment (Table 4). In clone 12, however, B + Ca treatment reduced in vivo germination of clone 1 pollen from moderate to zero. Tubes from what pollen germinated on clone 12 stigmata made little or no progress through the style. This was the clone with the lowest crop-year foliar B concentration and the least response to our B application.

The average in vivo germination and pollen tube extension through styles from the 10 clones and their respective treatments are presented in Table 5. On stigmata from clones receiving no treatment, germination averaged 17.4%; 6.6% of applied pollen tetrads produced tubes traversing to or beyond the lowest one-third of the style, 2.3% produced tubes that failed to reach beyond two-thirds of the style in 3 d, and 7.0% produced tubes that failed to

Fig. 1. In vivo pollen germination on the stigma of *Vaccinium angustifolium* (fluorescence micrograph at 500×, excitation at 365 to 510 nm and emission filtered to pass 400 to 610 nm).

Table 6. Average effects of 400 mg·L⁻¹ B and/or 4000 mg·L⁻¹ Ca sprays the previous year across 10 *Vaccinium angustifolium* clones on bud productivity in selected stems and 12 clones for total harvest yield per plot.

	Flowers/bud	Initial fruit no.	Initial fruit set (%)	Final fruit no.	Final fruit set (%)	Yield (kg·ha ⁻¹)
Control	4.80 a ²	3.92 a	81.3 a	3.32 a	67.8 ab	5847 a
B	4.68 a	3.86 ab	81.9 a	3.16 ab	66.8 b	5319 ab
Ca	4.53 b	3.73 b	81.8 a	3.12 b	68.3 ab	4278 b
B + Ca	4.69 a	3.91 a	83.4 a	3.30 a	70.4 a	5040 ab

²Mean separation within columns by Duncan's multiple range test, 5% level.

Table 7. Average berry yield per stem (g) among 10 *Vaccinium angustifolium* clones sprayed with 400 mg·L⁻¹ B and/or 4000 mg·L⁻¹ Ca the previous year.

Clone	Treatment			
	Control	B	Ca	B+Ca
1	2.48 a ²	2.27 a	2.51 a	2.92 a
2	3.31 a	3.13 a	3.31 a	2.70 a
3	1.73 a	1.65 a	1.93 a	2.23 a
4	1.41 a	1.47 a	1.51 a	1.12 a
5	2.07 a	2.34 a	2.18 a	2.76 a
6	3.47 a	3.22 a	3.60 a	3.45 a
7	3.28 a	2.67 a	2.96 a	2.59 a
8	2.05 a	2.14 a	2.17 a	2.42 a
9	3.90 a	4.53 a	5.13 a	4.20 a
10	3.13 a	3.19 a	2.40 a	2.78 a
11	2.65 a	2.88 a	2.23 a	2.27 a
12	4.46 a	3.64 a	4.21 a	3.73 a
Mean	2.83 a	2.78 a	2.85 a	2.76 a

²Values within rows followed by the same letters are not significantly different at the 5% level, according to Duncan's multiple range test. Each value represents an average yield per stem of 20 stems.

Table 8. Diameter and mass of *Vaccinium angustifolium* berries on 20 four-fruit-bud-stems receiving 400 mg·L⁻¹ B and/or 4000 mg·L⁻¹ Ca sprays the previous year.

Spray	Diam (mm)	Mass (g)
Control	7.37 ab	0.212 b
B	7.30 bc	0.209 b
Ca	7.44 a	0.227 a
B + Ca	7.20 c	0.198 c

²Values within columns followed by different letters are significantly different at the 5% level, according to Duncan's multiple range test. Each value represents an average from 20 stems with 4 fruit buds from 10 clones.

Table 9. Number of nonviable seeds, viable seeds and total seeds per berry of *Vaccinium angustifolium*, as affected by 400 mg·L⁻¹ B and/or 4000 mg·L⁻¹ Ca sprays the previous year.

Treatment	Seeds/berry	
	(no.)	(%)
Nonviable seeds^a		
Control	31.1 b ²	72.9 b
B	34.2 a	74.6 ab
Ca	34.9 a	77.0 a
B + Ca	31.5 b	74.1 ab
Viable seeds^a		
Control	11.9 a	27.1 a
B	11.9 a	25.4 ab
Ca	10.2 a	23.0 b
B+Ca	11.7 a	25.9 ab
Total seed		
Control	43.0 b	---
B	46.1 a	---
Ca	45.1 ab	---
B + Ca	43.2 b	---

^aNonviable seeds are seeds with longitudinal cross-sectional seed area <0.6 mm².

²Each value represents mean seed number averaged across 5 clones from berries of 20 stems with 4 fruit buds/stem. Values within columns in each section followed by different letters are significantly different at the 5% level according to Duncan's multiple range test.

^aViable seeds are seeds with longitudinal cross-sectional seed area >0.6 mm².

Table 10. Correlation of total number of seeds and different-size seeds per berry with berry diameter and mass of control plots of five *Vaccinium angustifolium* clones.

Seed size	Coefficient of correlation (r)	
	Berry diam	Berry mass
All seeds (all seed size classes combined)	0.358 ²	0.280
Area >0.6mm ²	0.499	0.434
Area >0.7mm ²	0.526	0.471
Area >0.8mm ²	0.572	0.528

²Pearson correlation coefficients (r), significant at the 5% level, between the number of seeds in a size category defined by transverse cross-sectional seed area and berry diameter or mass from five clones tested.

extend for more than one-third of the style in that time. Treatment with Ca alone, B alone, or B + Ca, raised in vivo germination, while treatment with Ca alone increased from 7% to 13.8% the percentage of germinated tetrads that extended into the upper one-third of the style within 3 d. It seems likely that more pollen tube extension would have been seen if style harvest were delayed beyond 3 d.

Fig. 2. *Vaccinium angustifolium* seed germination of controls 10 weeks after sowing as affected by seed size. Values represent germination of 960 seeds.

Table 11. Berry firmness and color^a of 12 *Vaccinium angustifolium* clones as affected by 400 mg·L⁻¹ B and/or 4000 mg·L⁻¹ Ca foliar sprays the previous year.

Spray	Firmness	Berries/stem (no.) ^z					
	(mN)	G	GP	PR	RB	B	Total
Control	79.6 a ^y	0.61 b	0.04 b	0.06 a	0.32 a	11.11 a	12.14 a
B	76.7 b	0.78 b	0.07 b	0.07 a	0.25 ab	10.46 ab	11.61 a
Ca	80.4 a	0.73 b	0.16 a	0.04 a	0.14 b	10.16 b	11.23 a
B + Ca	79.5 a	1.09 a	0.07 b	0.03 a	0.25 ab	10.68 ab	12.12 a

^aBerry color was rated as G = green, GP = green pink, PR = pink red, RB = red blue, or B = blue.

^yValues within columns followed by different letters are significantly different at the 5% level, according to Duncan's multiple range test. For number of berries per stem, each value represents an average number of berries on 20 stems with 4 flower buds/stem from 10 clones.

Figure 1 is an example of the images used to evaluate in vivo pollen germination and tube growth.

EXPERIMENT 3. Under the conditions of a commercial Maine blueberry field with its genetically diverse *V. angustifolium* population exposed to edaphic and microclimate differences with location in the field, foliar B applications the previous fall did not increase flowers per bud, initial or final fruit set, final fruit per bud, or bulk yield at harvest (Table 6). Berry retention was not affected by B or Ca treatment but a 13% to 15% decrease from initial to final fruit set was observed. Treatment with Ca alone significantly reduced the number of flowers per bud, the number of final fruit per bud, and the plot yield. Despite large differences found in Expts. 1 and 2 among clones and treatments in magnitude and direction of response by in vitro and in vivo pollen germinability, neither average percentage of fruit set (Table 6) nor berry yield per stem was affected (Table 7).

On average across the clones and treatments, the uppermost bud on four-bud stems produced the fewest flowers, initial fruit, and final fruit, while the third bud was the most productive by all three measures (data not shown). The counting and sizing of berries harvested from each bud position on 800 four-bud stems showed no effect on this relationship from treatment the previous autumn with Ca and/or B (data not shown). No statistically significant interaction was found between treatment and bud-position productivity.

While treatment with Ca alone did not on average across the clones affect berry yield per stem (Table 7), berries from plots receiving only Ca averaged larger (Table 8), but fewer per stem to an extent sufficient to depress yield from plots given that treatment. Seeds with an area <0.6 mm² (transverse cross sectional area) had 30% lower germination compared to larger seeds (Fig. 2). Ca applied alone increased the percentage of seeds per berry smaller than 0.6 mm², considered nonviable in this study, and decreased the percentage of seeds larger than that size (Table 9). That Ca-treated plots produced larger berries with more smaller and fewer larger seeds is in disagreement with Bell's (1957) finding that larger berries have a much greater proportion of large seeds. Berry diameter and mass were more closely correlated with the number of large seeds (Table 10) than with total seed number. More total seeds in a berry from B-treated plots implies more ovules fertilized and suggests that, under field conditions, despite equivocal results on in vitro and in vivo pollen germination in our Expts. 1 and 2, the B-alone treatment does increase pollen germination and tube traversal in B deficient clones. Since mixed pollination is to be expected in the field, fertilized ovules may fail to develop well because of poor genetic compatibility or other factors, resulting in undersized seeds.

Hanson et al. (1993), working with highbush blueberry, increased firmness by dipping picked fruit in CaCl₂ solution, but

preharvest Ca sprays failed to improve quality (Hanson 1995) as compared with *Fragaria* (Eaves and Leefe, 1962) and *Rubus* (Eaves et al., 1972), where in both crops preharvest Ca sprays did increase firmness. In the present study on lowbush blueberry, where B alone gave the softest fruit (Table 11), application of Ca alone the previous autumn delayed ripening as judged by more green pink and less red blue and blue berries, compared to the control. Ripening delay by Ca was demonstrated by Ferguson (1984) in *Malus × domestica* Borkh. and by Cheour et al. (1990) in *Fragaria*. Titratable acidity (0.51% to 0.52%), soluble solids (10.3% to 10.7%), and sugar-to-acid ratios (22.5 to 23.0) in our harvested lowbush blueberries were not affected by B, Ca, or B + Ca foliar sprays the previous autumn.

The benefit from fall foliar B treatment of 'Italian' prune found by Chaplin et al. (1977) and Hanson and Breen (1985), from spring foliar treatment of 'Barcelona' hazelnut by Shrestha et al. (1987), and from spring foliar B + Ca treatment of cranberries (*Vaccinium macrocarpon*) by DeMoranville and Deubert (1987) was not consistently achieved by our treatments of low-B managed wild lowbush blueberry clones in Maine.

Although no relationship was found between the effect of fall foliar treatments of B, Ca, or B + Ca on in vitro pollen germinability, these treatments did raise in vivo pollen germination without affecting the number of pollination tubes reaching the lower one-third of the style within 3 d.

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