Inflorescence Bud Initiation, Development, and Bloom in Two Southern Highbush Blueberry Cultivars

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ABSTRACT. Blueberry (*Vaccinium* spp.) production is increasing worldwide, particularly in subtropical growing regions, but information on timing and extent of inflorescence bud development during summer and fall and effects on bloom the next season are limited. The objectives of this study were to determine time of inflorescence bud initiation, describe internal inflorescence bud development, and determine the relationship between internal inflorescence bud development and bloom period the next spring in two southern highbush blueberry [SHB (*Vaccinium corymbosum* interspecific hybrids)] cultivars. ‘Emerald’ and ‘Jewel’ SHB buds were collected beginning in late summer until shoot growth cessation in late fall for dissection and identification of organ development. Inflorescence bud frequency and number, vegetative and inflorescence bud length and width throughout development, and bloom were also assessed. Inflorescence bud initiation occurred earlier in ‘Emerald’ compared with ‘Jewel’. Five stages of internal inflorescence bud development were defined throughout fall in both cultivars, ranging from a vegetative meristem to early expansion of the inflorescence bud in late fall. ‘Emerald’ inflorescence buds were larger and bloomed earlier, reflecting the earlier inflorescence bud initiation and development. Although inflorescence bud initiation occurred earlier in ‘Emerald’ compared with ‘Jewel’, the pattern of development was not different. Timing of inflorescence bud initiation influenced timing of bloom with earlier initiation resulting in earlier bloom.

Blueberry is a crop that has been increasing in importance worldwide (Strik and Yarborough, 2005) with areas expanding in non-traditional subtropical areas (Bañados, 2009). In 2012, there were ≈84,000 ha of cultivated blueberry worldwide with the majority located in the United States and Canada and yield exceeded 399,000 tons (Food and Agriculture Organization of the United Nations, 2014). Flower or inflorescence bud number is the most important component of yield in many crops, including blueberry (Salvo et al., 2011) and is determined by the extent of floral bud initiation and development that occurs in late summer and early fall. The extent and timing of inflorescence bud initiation and development in blueberry may affect bloom time the next spring (Spann et al., 2004), which plays a critical role in yield. Early bloom may be subject to damage by spring freezes with resultant crop losses, whereas late bloom may delay harvest.

There have been few studies examining inflorescence bud differentiation in blueberry. Bell and Burchill (1955) described the formation of individual flowers within the inflorescence bud in lowbush blueberry (*V. angustifolium*). Gough et al. (1978b) described the development of the inflorescence in field-grown northern highbush blueberry (*V. corymbosum*) using only floral buds from the fourth node from the shoot apex. Both studies described the histological formation of flowers, but information on the formation of the inflorescence was limited. Tamada (1997) described differentiation of the inflorescence in terminal buds in field-grown highbush and rabbiteye (*V. virgatum*) blueberry but did not examine differentiation of more proximal

buds. Huang et al. (1997) evaluated floral development in SHB, but only after flowers were already present.

Previous assessments of floral development in blueberry are limited and have focused on single inflorescence buds rather than the development and distribution of floral buds in a shoot, even though there are timing differences in development of apical vs. more proximal inflorescence buds (Huang et al., 1997; Lyrene, 1984). Furthermore, the relationship between timing and extent of blueberry inflorescence bud development in the fall and subsequent bloom progression in the spring has not been determined. The objectives of the present study were to: 1) describe the internal development of blueberry inflorescence buds; 2) compare the timing and node location of inflorescence bud initiation in two cultivars of SHB; and 3) determine the relationship between timing and extent of internal inflorescence bud development with the bloom period the next spring in the same two cultivars.

Materials and Methods

PLANT MATERIAL. Data were collected from 8-year-old ‘Emerald’ and ‘Jewel’ SHB plants, at the University of Florida’s Institute of Food and Agricultural Sciences Plant Science Research and Education Unit in Citra, FL. ‘Emerald’ and ‘Jewel’ are currently the major cultivars grown in Florida (Williamson et al., 2014). ‘Emerald’ is considered to be one of the highest yielding cultivars in Florida and produces large, high-quality fruit. ‘Jewel’ yields less than ‘Emerald’ and has slightly smaller fruit but is still considered to be high-yielding. Inflorescence buds of ‘Emerald’ are visually apparent in late summer, whereas buds of ‘Jewel’ are not apparent until later in the fall. The plants were grown on raised pine bark beds with a plant density of 3987 plants/ha. Fertilizer was applied at an

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annual rate of 239 kg·ha⁻¹ nitrogen, 35 kg·ha⁻¹ phosphorus, and 132 kg·ha⁻¹ potassium. Irrigation was applied with microsprinklers as needed, and overhead sprinklers were used for frost protection. Hydrogen cyanamide (1.5%) was applied in late December of each year to aid in fulfilling the chilling requirement. Recommended practices were used to control pests, diseases, and weeds. A randomized complete block design was used with six replications comprised of six two-plant subsamples per cultivar.

INFLORESCENCE BUD INTERNAL DEVELOPMENT SCALE. One shoot per subsample per cultivar (six shoots per cultivar per replication) was collected biweekly and stored at 4 °C for 1 week or less before evaluation. Shoots were randomly selected from the upper plant canopy and consisted of all of the current season's growth. The starting dates for the sampling were 20 Oct. 2011, 9 Aug. 2012, and 14 Aug. 2013 for 'Emerald' and 20 Oct. 2011, 4 Oct. 2012, and 26 Sept. 2013 for 'Jewel'. The starting dates in 2012 and 2013 were earlier compared with 2011 because inflorescence bud initiation had already started at the first shoot collection date in 2011. Starting from the shoot apex, bud scales and leaf primordia were removed from individual buds until the apical and lateral meristems were visible under a stereomicroscope (Leica MZ12.5; Leica Microsystems, Wetzlar, Germany).

Light microscopy was used in addition to stereoscopy to evaluate development of structures within 'Emerald' buds, because the most advanced stages of floral development occurred more frequently in this cultivar. Buds were fixed in formalin-acetic-alcohol [70% ethanol:glacial acetic acid:commercial formalin (9:0.5:0.5)] for 2 d and then stored in 70% ethanol. The samples were then dehydrated in a graded tert-butyl alcohol and ethanol series (70%, 85%, 100% alcohol) for ≈24 h in each solution. The samples were then placed in a vial containing slightly solidified low melting point paraffin (LMP), and a 1:1 tert-butyl alcohol:paraffin oil mixture was used to complete the vial, which was placed in a paraffin oven. The mixture was replaced with pure LMP three times with 6 h between changes. The samples were cast in paraffin blocks and sectioned at 12 μm using a rotary microtome. The sections were heat-mounted on a slide and stained with hematoxylin (0.5%) and safranin (1%). Sections were photographed using a light microscope (Ortholux; Leica Microsystems) with a 1.3-megapixel camera (Moticam 1000; Motic, Hong Kong, China) attached.

Observation of internal development was used to create a scale describing inflorescence bud development. The scale ranged from 1 (completely vegetative) to 5 (complete formation of all floral organs) and development of the scale is fully described in the presentation of Figure 1. A vegetative bud was identified by the presence of only one meristem. The early stages of floral initiation were identified by the formation of lateral meristems in the bud, as described by Tamada (1997) for northern highbush blueberry.

INFLORESCENCE BUD INITIATION, DEVELOPMENT, AND BLOOM. Vegetative shoot growth in blueberry occurs in multiple flushes (Gough et al., 1978a); therefore, each growth flush from the current season's shoot growth was collected and position of inflorescence buds within each growth flush was recorded. Frequency of inflorescence buds in each position was obtained using SAS PROC FREQ (Version 9.2; SAS Institute, Cary, NC) and compared between cultivars on each date. Because the majority of inflorescence buds are localized in the apex of the shoot, growth flushes were counted basipetally, as number of growth flushes may vary. Thus, the most recent or distal flush

Fig. 1. Dissected buds of 'Emerald' southern highbush blueberry viewed with a stereomicroscope; stages of internal development 1 to 5 (Stages I-1 through I-5). Stage I-1: vegetative bud (A) showing a single vegetative meristem (Vm). Stage I-2: multiple meristems in an early developing inflorescence bud (B), terminal floral meristem (TFm), two lateral floral meristems (LFm) are visible, and bracts (Br) are developing. Stage I-3: differentiated flowers are visible (C) and sepals (Se) are beginning to develop. Stage I-4: developed inflorescence bud (D), petals (Pe), and sepals (Se) are visible after removal of bract (Br). Stage I-5: semiexpanded bud (E), sepals and petals are visible after removal of bract, pedicel (Pd), and peduncle (P) have expanded; bar = 1 mm.

was designated flush 1, the penultimate flush was designated flush 2, etc. Inflorescence buds were considered initiated for a cultivar whenever the frequency of buds containing floral structures was significantly different from 0% on a given date. Each inflorescence bud was rated for internal bud development using the scale described, and stage of development was averaged within each date for both cultivars. Data were analyzed using PROC GLIMMIX, and cultivar means for each date were considered different when there was a significant effect for cultivar in the analysis of variance. The number and percentage of inflorescence buds and total number of buds per growth flush were obtained from the shoots collected on the last date in December of each year. Shoot length, number, and percentage of inflorescence buds and total number of buds per growth flush were analyzed using PROC GLIMMIX, and means were separated using Tukey's honestly significant difference (HSD) at $P \leq 0.05$.

In Fall 2013, two shoots per subsample per cultivar (12 shoots per replication per cultivar) were selected for bud length

and width measurements. On the most distal growth flush of each shoot, the third and fourth buds from the distal end downward and third and fourth buds from the proximal end of the most recent growth flush upward were measured using a caliper. These buds were chosen to avoid the vigor of the most apical bud and because both vegetative and reproductive buds would be selected. The starting dates were 11 Sept. and 17 Oct. 2013 for 'Emerald' and 'Jewel', respectively, and buds were measured biweekly until leaf abscission. By the end of the growing season, the buds were categorized as vegetative or reproductive by external appearance and the measurements separated. Data were analyzed using PROC GLIMMIX, and means were separated using Tukey's HSD at $P \leq 0.05$.

Inflorescence bud external development (Spiers, 1978) and bloom progression (measured as percent bloom) were estimated visually for the whole plant in Spring 2012 and 2013. For each cultivar, six two-plant subsamples per replication were rated biweekly from 10 Jan. to 7 Mar. 2012 and 14 Jan. to 18 Mar. 2013. Bloom progression data, as cumulative bloom, were fit into the general logistic equation using PROC NLIN (modified from Kirk and Isaacs, 2012): percent bloom = $1 / 1 + e^{-(\beta_0 + \beta_1 \times \text{GDD})}$, where GDD stands for growing degree-days and parameters β_0 and β_1 are the intercept and slope of the linear combination of the logit function, respectively, generated by PROC NLIN. Accumulated GDD $\{\Sigma \text{GDD} = [(T_{\max} - T_{\min}) / 2] - T_{\text{base}}\}$ (where $T_{\max}$, $T_{\min}$, and T_{base} are the maximum and minimum daily temperatures and base temperature for blueberry, respectively) were calculated for each date using weather data provided for the location by Florida Automated Weather Network starting on 1 Jan. of each year (Kirk and Isaacs, 2012), and a general base temperature of 7 °C was used for both cultivars. The difference between cultivars was evaluated using a sum of squares reduction Test, and fit of the non-linear regression was evaluated using Efron's pseudo- R^2 . Inflorescence bud external development was analyzed using PROC GLIMMIX and means were separated using Tukey's HSD at $P \leq 0.05$.

Results

INFLORESCENCE BUD INTERNAL DEVELOPMENT SCALE. Five stages of internal bud development from vegetative to reproductive were identified using the scale described previously. These stages were assigned from observations with a stereoscope and further supported by observation with light microscopy. The stages were similar in appearance between cultivars. Buds were considered to be in Stage 1 of internal development (Stage I-1) when there was a single vegetative meristem present (Figs. 1A and 2A) after removal of all bud scales (modified leaves that protect the bud) and/or leaf primordia surrounding the meristem (Fig. 2A). Buds were considered to have reached Stage I-2 when lateral floral meristems were apparent (Figs. 1B and 2B). Broadening or doming of the apical meristem before the formation of multiple flower meristems, which is often observed during initial stages of floral development, was not observed at this stage.

The third stage of development (Stage I-3) was designated by the early development of sepals (Figs. 1C and 2C). At early Stage I-3, only a ridge on the meristems was visible in both the stereoscope and the light microscope, whereas at later Stage I-3, triangular shapes identified the sepals. Flower development occurred acropetally within the bud (data not shown). As flowers developed, the two bracts concealing individual flowers

Fig. 2. Light micrographs of 'Emerald' southern highbush blueberry buds; stages of internal development 1 to 5 (Stages I-1 through I-5). Stage I-1: vegetative bud (A), showing a single vegetative meristem (Vm). Stage I-2: multiple meristems in an early developing inflorescence bud (B), terminal floral meristem (TFm) and lateral floral meristem (LFm) are visible. Stage I-3: differentiated flower is visible (C), encased by two bracts (Br), sepals (Se) are beginning to develop. Stage I-4: developed inflorescence bud (D), flower is encased by bracts; petals (Pe), sepals, anthers (An), and pistil (Pi) are visible. Stage I-5: semiexpanded bud (E), bracts are no longer tight around flower, sepals, petals, anthers, and pistil have expanded; bar = 100 μm .

were separated and flowers were visible without the removal of the bracts. At this stage, the transition of the apical meristem to a flattened dome was clearly observed. In some meristems, the dome then became an uneven surface as the petal primordia arose.

Stage I-4 was marked by the clear development of petals, the second and last whorl visible with the stereoscope before bloom (Fig. 1D). Newly formed petals were differentiated in color from the other organs with a slightly white color on the tips. The two bracts had grown and concealed individual flowers at this stage and had to be removed for observation. This stage coincides with Stage 1 of external inflorescence bud development as described by Spiers (1978), where there is no visible swelling of the bud and the inflorescence is completely enclosed by the bud scales. With light microscopy, it was possible to visualize the formation of the anther and the pistil, still compacted within the unexpanded flower (Fig. 2D). Most inflorescence buds of both cultivars reached Stage I-4 in late fall, at which point further development was suspended until the next spring. However, some apical buds, especially of 'Emerald', reached Stage I-5, where the peduncle of the inflorescence, the pedicels, and flowers were semiexpanded (Fig. 1E). At this stage, stamens and pistils had expanded, although they were encased by the petals (Fig. 2E). This stage corresponds with Stage 2 of external bud development (Spiers, 1978).

INFLORESCENCE BUD INITIATION, DEVELOPMENT, AND BLOOM. The first signs of floral transition (Stage I-2) were detected in

early August in ‘Emerald’ and late September in ‘Jewel’. However, the frequency of occurrence of inflorescence buds was not different from 0% until late August for ‘Emerald’ and early October for ‘Jewel’ (Table 1). The decreasing frequency of buds containing floral structures from the apex of the shoot downward in both cultivars clearly demonstrates basipetal inflorescence bud initiation in blueberry. Starting in late August, ‘Emerald’ had significantly greater frequency of inflorescence buds in nodes 1 and 2, increasing to nodes 1 through 8 in early October, compared with ‘Jewel’. In late October, the frequency of inflorescence buds in node 1 was not different between ‘Emerald’ and ‘Jewel’, after which time significant differences in inflorescence bud frequency between cultivars shifted to more basipetal buds. By early December, at the end of the growing season, ‘Emerald’ had a greater frequency of inflorescence buds compared with ‘Jewel’ in nodes 5 through 9. Vegetative buds remained in Stage I-1 from August through December. The average internal development of inflorescence buds increased along with the frequency of inflorescence buds

from early August through mid-November in ‘Emerald’ and from late September through early December in ‘Jewel’ (Table 1). For a given date, inflorescence bud development in ‘Jewel’ was delayed compared with ‘Emerald’. Shoots ceased growth by the time inflorescence buds were initiated in both ‘Emerald’ and ‘Jewel’ (Table 1).

Inflorescence buds were found in the two most distal growth flushes (i.e., growth flush 1 and 2), whereas there were no inflorescence buds in flush 3 in either cultivar. ‘Emerald’ had significantly more inflorescence buds in growth flush 1 than ‘Jewel’, whereas there were no differences in flush 2 or 3 (Table 2). There were no differences between ‘Emerald’ and ‘Jewel’ in percent of inflorescence buds within a given growth flush. For both cultivars, 97% of all inflorescence buds occurred in growth flush 1. Total bud number in ‘Emerald’ was greater in growth flush 1 and less in growth flush 3 compared with ‘Jewel’, whereas there were no differences in the number of buds in growth flush 2 between cultivars (Table 2). Total bud number was similar for all growth flushes of ‘Jewel’, whereas

Table 1. Frequency of buds containing floral structures in a given position, inflorescence bud inner development, and shoot length in ‘Emerald’ and ‘Jewel’ southern highbush blueberry.

Bud position (node no.)	Buds containing floral structures (%)							
	Early Aug.	Late Aug.	Early Sept.	Late Sept.	Early Oct.	Late Oct.	Mid Nov.	Early Dec.
Emerald								
1	9.7 ^z	29.2* F ^y	74.1*	93.5*	98.1*	98.1	99.1	98.1
2	4.2	15.3*	53.7*	75.9*	85.9*	89.8*	92.6	98.1
3	0.0	4.2	31.5*	40.7*	63.7*	72.2*	82.4	77.8
4	1.4	1.4	9.3*	17.6*	34.1*	38.8*	57.4*	50.0
5	1.4	0.0	7.4	14.8*	34.4*	44.4*	58.3*	48.1*
6	0.0	1.4	2.8	9.3*	16.4*	29.6*	40.7*	30.6*
7	0.0	0.0	0.9	4.6	13.1*	14.8*	25.9*	22.2*
8	0.0	0.0	1.9	1.9	7.6*	12.0*	21.3*	15.7*
9	0.0	0.0	0.9	1.9	5.7	9.3*	10.2*	10.2*
10	0.0	1.4	0.9	0.0	3.4	5.6	6.5	7.4
11	0.0	0.0	0.0	0.0	1.1	2.8	2.8	5.6
Jewel								
1	0.0	0.0	0.0	1.9	49.1F	92.6	96.3	97.2
2	0.0	0.0	0.0	0.0	28.7	72.2	92.6	88.9
3	0.0	0.0	0.0	0.0	13.9	35.2	72.2	74.1
4	0.0	0.0	0.0	0.0	6.5	10.2	29.6	35.2
5	0.0	0.0	0.0	0.0	3.7	5.6	26.9	28.7
6	0.0	0.0	0.0	0.0	2.8	1.9	5.6	8.3
7	0.0	0.0	0.0	0.0	0.9	0.9	1.9	3.7
8	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.9
9	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.9
10	0.0	0.0	0.0	0.0	0.9	0.9	0.0	0.0
11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Avg inflorescence bud internal development (1–5)^x</i>								
Emerald	2.3**	2.5*	2.9*	3.5*	3.5*	3.4*	3.7*	3.7*
Jewel	1.0	1.0	1.0	2.0	2.6	2.8	3.3	3.5
<i>Avg shoot length (cm)</i>								
Emerald	15.3 b	18.7 ab	19.2 a	18.9 a	18.9 a	19.1 a	19.2 a	18.3 ab
Jewel	—	—	—	22.9 a	23.9 a	21.2 a	22.5 a	22.2 a

^zMeans were averaged over 3 years. Means followed by asterisks indicate significant differences between cultivars in the frequency of floral structures at a given node ($P \leq 0.05$).

^yF indicates date of inflorescence bud initiation for each cultivar.

^xInternal development refers to Stages I-1 through I-5 of internal development where the stage number (1 to 5) was averaged for each date.

**Means were averaged over 3 years. Asterisks indicate significant differences between cultivars within dates ($P \leq 0.05$).

Table 2. Number and percent of inflorescence buds and total number of buds per growth flush in ‘Emerald’ and ‘Jewel’ southern highbush blueberry.

Treatment	Inflorescence buds per flush (no.)			Total inflorescence buds per flush (%)			Total buds per flush (no.)		
	Growth flush no.			Growth flush no.			Growth flush no.		
	1 ^z	2	3	1	2	3	1	2	3
Emerald	4.6 a ^y	0.2 c	0.0 c	97.4 a	3.2 b	0.0 c	10.4 a	8.8 ab	5.7 c
Jewel	3.4 b	0.1 c	0.0 c	96.8 a	2.6 bc	0 c	8.4 b	9 ab	7.9 b

^zGrowth flushes were counted basipetally from the apical end.

^yMeans were averaged over 3 years. Means followed by the same letter within a variable are not significantly different by Tukey’s honestly significant difference test at $P \leq 0.05$.

Fig. 3. Width (A), length (B), and width:length ratio (C) of vegetative and reproductive buds in ‘Emerald’ and ‘Jewel’ southern highbush blueberry in 2013. Letters indicate significant differences among means within each date at $P \leq 0.05$.

bud number in growth flush 3 of ‘Emerald’ was less than in flush 1 and 2.

There was no difference in length or width between vegetative and reproductive buds in ‘Emerald’ in early September (Fig. 3). However, for ‘Jewel’ on the first measurement

A

B

C

date (17 Oct.), reproductive buds were already wider and longer compared with vegetative buds, although other external differences were not observed. Reproductive buds continued to increase in width and length in both cultivars compared with vegetative buds, which did not change in size. Reproductive buds of ‘Emerald’ were always significantly larger than ‘Jewel’, whereas vegetative buds of ‘Emerald’ were wider but not longer than those of ‘Jewel’. The width:length ratio remained the same throughout the growing season for ‘Emerald’ vegetative and reproductive buds and ‘Jewel’ vegetative buds, and there were no differences between the ratios for these buds. The width:length ratio in ‘Jewel’ reproductive buds decreased throughout development during the growing season.

Percentage of bloom as a function of GDD was described by non-linear logistic curves for both cultivars and cultivar differences were significant ($P < 0.0001$). ‘Emerald’ bloomed earlier than ‘Jewel’ with ‘Jewel’ requiring ≈ 75 GDDs more to reach the same percent bloom (Fig. 4). Peak bloom (50% bloom) for ‘Emerald’ occurred at 225 GDDs, whereas peak bloom occurred at 302 GDDs for ‘Jewel’. ‘Emerald’ also reached each stage of inflorescence bud development (as described by Spiers, 1978) significantly earlier than ‘Jewel’ (Fig. 4), but at approximately the same percent bloom.

Discussion

To our knowledge, this is the first work describing the influence of timing, extent, and distribution of blueberry floral development during the fall on bloom the next spring. Although

Fig. 4. Percentage of bloom non-linear logistic curves and stage of inflorescence bud outer development (Spiers, 1978) in 'Emerald' and 'Jewel' southern highbush blueberry. For 'Emerald', the relationship of percentage of bloom to growing degree-days (GDD) was described by the equation: percent Bloom = $1 / 1 + e^{-(3.3740 + 0.0150 \times \text{GDD})}$. For 'Jewel', the relationship of percent Bloom to GDD was described by the equation: percent Bloom = $1 / 1 + e^{-(4.6172 + 0.0153 \times \text{GDD})}$. Pseudo- r^2 s = 0.88 and 0.91 for 'Emerald' and 'Jewel', respectively. The points where the dashed lines cross the percent Bloom curves is where Spier's inflorescence bud development stage occurred for each cultivar (Stage 2 = visible swelling of bud; 3 = bud scales separated with flower apices visible; 4 = individual flowers distinguishable; 5 = individual flowers separated, unexpanded corollas are closed; 6 = expanded corollas are open; 7 = corollas dropped).

for many species, increase in diameter of the apical meristem followed by the doming of the meristem is the first evidence of floral commitment (Foster et al., 2003), vegetative buds of northern highbush blueberry have been described as "domed" (Gough et al., 1978a). Tamada (1997) noted that the raceme inflorescence of blueberry can hinder the observation of the dome stage during floral development. However, it was possible to see the formation of lateral meristems, identifying the early formation of inflorescence buds, as observed in lowbush blueberry by Bell and Burchill (1955) and in northern highbush and rabbiteye blueberry by Tamada (1997). We considered single apical meristems with little development as vegetative based on previous work in hydrangea [*Hydrangea macrophylla* (Orozco-Obando et al., 2005)] and multiple meristems within a bud as floral, similar to previous work in cranberry [*Vaccinium macrocarpon* (DeVetter et al., 2013)]. By the time inflorescence buds were initiated there was no further shoot growth, concurring with Bañados and Strik (2006) and Tamada (1997), who found that terminal bud formation and shoot growth cessation precedes inflorescence bud formation in northern highbush and rabbiteye blueberry.

Internal differentiation of each meristem within an inflorescence bud occurred acropetally regardless of the basipetal differentiation of inflorescence buds on individual canes. Acropetal differentiation of flowers within an inflorescence bud has also been described in northern highbush (Gough et al., 1978b; Tamada, 1997) and rabbiteye blueberry (Tamada, 1997), and acropetal development of floral meristems within a flower bud can be observed in micrographs of cranberry (DeVetter et al., 2013). The acropetal differentiation observed within blueberry inflorescence buds differs from apple [*Malus domestica* (Foster et al., 2003)], in which differentiation within each bud progresses basipetally. The acropetal development further demonstrates the raceme type of open inflorescence (Claßen-Bockhoff and Bull-Hereñu, 2013; Tamada, 1997) in blueberry. Stage I-5 (partial expansion of peduncle,

pedicel, and flowers), which corresponds to Stage 2 by Spiers (1978), was not reached by all inflorescence buds by the time bud development ceased in late fall. However, some inflorescence buds, especially in 'Emerald', did reach this stage before winter. Buds at this stage have decreased cold-hardiness compared with buds at earlier stages (Spiers, 1978), which could result in freeze damage in cultivars such as Emerald.

Inflorescence bud formation occurred earlier and to a greater extent in 'Emerald' than in 'Jewel'. Cultivar differences in inflorescence bud development were also found by Tamada (1997) between northern highbush and rabbiteye blueberry. Inflorescence bud formation in the fall can be negatively affected by early defoliation in short-day plants such as blueberry; therefore, cultivars that form inflorescence buds earlier could be less impacted by defoliation (Lyrene, 1992). 'Jewel' is more prone to foliar diseases that lead to defoliation than 'Emerald' (Williamson et al., 2012), which may partially explain the decreased number of inflorescence buds in 'Jewel'. The increased frequency of inflorescence buds at nodes 5 to 9 in 'Emerald' compared with 'Jewel' reflected the greater number of inflorescence buds in growth flush 1 in that cultivar. The majority of inflorescence buds in both cultivars were in growth flush 1, demonstrating that basipetal development occurs not only within a growth flush, but also among growth flushes. Lyrene (1984) also concluded that the majority of inflorescence buds in a shoot were located at the distal end—presumably in the most recent growth flush (i.e., growth flush 1)—although growth flushes were not noted in his work.

Although inflorescence bud external appearance is often not visible until later in the fall (Gottschalk and Nocker, 2013), in the present study, reproductive buds were significantly wider compared with vegetative buds by late September for 'Emerald' and mid-October for 'Jewel'. Greater width of cranberry flower buds was also observed by DeVetter et al. (2013) in mid- or late fall, although bud development throughout the season was not evaluated in that study. Gough et al. (1978b) looked at width and length of northern highbush blueberry, but only after inflorescence buds were formed and during the period of dormancy establishment. In the present study, bud length was also a predictor of the transition to reproductive development. Evaluation of width:length ratios demonstrated that 'Emerald' buds increase proportionally in size regardless of their reproductive status. However, 'Jewel' had a more pronounced increase in length of reproductive buds compared with vegetative, resulting in a smaller ratio. Therefore, it is possible that using length as a predictor for reproductive buds in some blueberry cultivars such as 'Jewel' is more accurate than using width, whereas either bud length or width is similarly effective in predicting whether buds are reproductive or vegetative in cultivars such as 'Emerald'.

The increased size of 'Emerald' reproductive buds compared with 'Jewel' was probably the result of earlier inflorescence bud initiation and consequently greater internal development in 'Emerald' buds on a given date compared with 'Jewel'. This difference could also be the result of increased flower number in 'Emerald' inflorescence buds compared with 'Jewel', although this was not assessed in the present study.

Our study found that bloom phenology in SHB was consistent across years and was clearly described by GDD accumulation. Considering the low-chilling requirement of these cultivars (Williamson et al., 2014) and previous work indicating that GDD accumulation can occur during the latter stages of chilling (Campoy et al., 2011; Couvillon and Erez,

1985), it is likely that the chilling requirement of both cultivars had been met before the date at which GDD calculation began. Additionally, the use of hydrogen cyanamide helps satisfy the chilling requirement, although other effects of this chemical include increased rate of flower bud opening in the spring (Williamson et al., 2002). However, this latter effect would affect both cultivars similarly. Although consistent across years, the correlation between phenological stages and GDD accumulation was cultivar-dependent. These results are similar to conclusions reached by Kirk and Isaacs (2012) working with northern highbush blueberry cultivars, although those authors were looking at percentage of bloom as number of open flowers at a given time. Earlier bloom in 'Emerald' could be the result of the earlier formation of inflorescence buds compared with 'Jewel' and increased internal development by the end of the fall, leading to earlier accumulation of chilling units (Davies, 1986). This is in agreement with Spann et al. (2004), who speculated that earlier bloom was a result of increased internal floral development in plants subjected to longer inductive periods. However, correlations between timing of inflorescence development and bloom period in other blueberry cultivars is necessary to determine if this is universal. Although peak bloom in 'Emerald' occurred at a lower GDD accumulation compared with 'Jewel', both cultivars had their peak bloom at similar GDDs as northern highbush blueberry cultivars studied by Kirk and Isaacs (2012). The significantly later blooming found in 'Jewel' compared with 'Emerald' can be a beneficial freeze-avoidance mechanism, considering that early blooming cultivars have decreased cold-hardiness (Arora et al., 2004). Progression of 'Emerald' inflorescence buds through external developmental stages (Spiers, 1978) occurred at lower accumulated GDDs compared with 'Jewel', but at similar percentage of bloom. This suggests that percent bloom values could be assigned for each external stage of development to facilitate bloom phenology analyses.

There were no differences in the development of individual inflorescence buds between the two cultivars studied, and the development was similar to other species. Although only five stages of internal flower development were described in this work, the events identifying the beginning of each stage are very important in floral development, easily identified on fresh material, and can be used in the interpretation of gene expression and other studies involving floral development patterns in blueberry.

Cultivar differences were found in the timing of internal and external development of inflorescence buds. Internal inflorescence bud development in 'Emerald' occurred earlier in the fall than in 'Jewel', which led to larger, more developed inflorescence buds that broke and bloomed earlier in the spring. The greater number of inflorescence buds in growth flush 1 of 'Emerald' was likely a result of this earlier development of inflorescence buds. Our data indicate that measuring buds as early as late September and mid-October for 'Emerald' and 'Jewel', respectively, can be used to identify inflorescence buds regardless of the external appearance.

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