Flower bud initiation in southern highbush blueberry cv. O’Neal occurs twice per year in temperate to warm-temperate conditions

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Abstract
In Argentina, southern highbush blueberry (Vaccinium sp.) exhibits two periods of vegetative growth in the same year, the first one in spring (spring growth, SpG), arising from vegetative buds on one-year-old wood, and the second in summer, from vegetative buds formed on spring growth, just after harvest (summer growth, SmG). Histological studies confirmed that flower bud initiation (FBI) occurred at the end of December on SpG and at the end of March on SmG. On SmG, FBI occurred under an 8 h photoperiod, and shortening daylength. However, on SpG, FBI was observed under increasing daylength (up to 15 h) and an average temperature of 22.5 ºC. Basal florets in apical floral buds were always in a more advanced reproductive stage on SpG than on SmG during the season. The two peaks in volume of fruit harvested were likely a result of differences in the ontogeny of buds on SpG and SmG shoots in the previous year.

Key words: Vaccinium sp., southern highbush blueberry, flower bud initiation, flower bud differentiation, day length, temperature

Introduction
In Argentina, commercial blueberry production began in the 1990s on a small scale, but increased rapidly to over 4,200 ha in 2009 (CAPAB, 2009). Southern highbush blueberry (Vaccinium sp.) is the most important type grown in this country in warm-temperate or temperate regions.

Blueberry plants in Argentina usually begin flowering from the end of June to the beginning of August (average daily temperature of 10.4 ºC) with bloom lasting for 4 to 6 weeks, depending on cultivar, growing region, and climatic conditions (mainly temperature). Fruit harvest occurs from October to December (spring), with peak production in October-November. After fruit harvest, at the beginning of summer, plants remain vegetative until fall, when they enter dormancy (Fig. 1).

Most southern highbush blueberry plants in this region, exhibit two periods of vegetative growth. The first period of vegetative growth occurs in spring (spring growth = SpG). Vegetative bud break takes place in September (monthly average temperature: 13.8 ºC), several weeks after flowering; these shoots originate from vegetative buds on one-year-old wood. The second period of growth occurs in summer (SmG), starting after fruit harvest, usually in January (average temperature of 23.9 ºC); these shoots develop from buds on spring growth (Bañados et al., 2007; Pescie and Lovisolo, 2005; Fig.1). Although this second period of growth (SmG) occurs naturally, it can be increased through tipping of shoots in spring (Bañados et al., 2009; Pescie and Lopez, 2007).

The period of flower bud initiation (FBI) is well documented in northern highbush (Vaccinium corymbosum L.) and lowbush (V. angustifolium Ait.) blueberry, occurring in general, the year preceding flowering and fruiting (Aalders et al., 1964; Bañados and Strik, 2006; Bell and Burchill, 1952; Gough et al., 1978). FBI does not begin until cessation of shoot growth occurs (Aalders and Hall, 1964; Bañados and Strik, 2006).

There is limited information on the interaction between photoperiod and temperature on FBI in blueberry. In southern blueberry, FBI was reduced at 28 ºC compared with 21 ºC under SD (Spann et al., 2004). Also, flowers that developed at the higher temperature remained in a dormant-like state, showing no further development, or abscised. They hypothesized that higher temperatures not only inhibited FBI in southern highbush, but impaired FBD as well, resulting in failure to open. In lowbush
blueberry, flower buds were larger when plants were exposed to warmer temperatures during FBI (21 to 26°C rather than 10 to 16°C; Hall and Ludwig, 1961; Hall et al., 1970). In early, mid-season, and late-season northern highbush blueberry cultivars, FBI only occurred under SD (at constant temp 22 °C) with the number of flower buds correlated to the length of exposure. Plants grown under SD for 4 or more weeks ceased growth and entered endo-dormancy, whereas those grown under LD conditions had continuous growth and did not initiate flower buds or go dormant (Bañados and Strik, 2006).

Plant and fruit quality are also affected by photoperiod and temperature in lowbush and highbush blueberry. Plants grown under long days (16-h photoperiod) and 21 °C had more rapid vegetative growth and produced longer shoots than those grown at 10 °C and short days (8-h photoperiod; Hall and Ludwig, 1961; Hall et al., 1963). Similar results were found by Spann et al. (2003) in ‘Misty’ and ‘Sharpblue’. In northern highbush blueberry, fruit set, fruit size, and rate of fruit development were positively influenced by warm temperature (16 to 27 °C) under greenhouse conditions (Knight and Scott, 1964). Although, Hall and Aalders (1968) found a better rate of fruit development at 21°C day temperature, there was no relationship between temperature and fruit set or berry weight.

Southern highbush blueberry cultivars are hybrids of *V. corymbosum* L. and *V. darrowii* Camp with minor contributions from *V. angustifolium* Aiton, *V. virgatum* Aiton (syn. *V. ashei* Reade), and *V. tenellum* Aiton (Lang, 1993; Spann et al., 2003). Little is known about the conditions under which FBI and differentiation occur in southern highbush blueberry grown under field conditions in temperate to warm temperate areas such as the main production regions of Argentina, where two periods of vegetative growth occur.

The objectives of this study were to characterize flower bud development of southern highbush blueberry grown under field conditions in a warm temperate climate, in particular: 1) ascertain the period of flower bud initiation in spring and summer growth; and 2) to determine if there is a different degree of development of flower buds on spring and summer growth, as they develop under different photoperiod and temperature conditions.

### Materials and methods

The experiment was carried out in a commercial blueberry farm in Northeastern Buenos Aires Province (34° lat S; 59° long W), on 10-year-old plants of ‘O’Neal’. Plants grown on raised soil beds (pH about 5.0) were fertigated from bud break through the growing season, and otherwise maintained according to standard commercial practice.

One hundred spring shoots (SpG) and summer shoots (SmG) were randomly selected and tagged at the beginning of September (soon after bud break) and in mid-January, respectively, on 20 plants (5 SpG and 5 SmG shoots per plant). Shoots were tagged on all sides of the bush (N, S, E, and W) to minimize variance due to possible differences in light exposure (Hall, 1958; Yáñez et al., 2009). The length of the tagged shoots was measured every two weeks from 25 Oct. 2005 in SpG and from 27 Feb. 2006 in SmG to determine time of shoot growth cessation. Five to six-node shoot tips were excised from 10 tagged shoots collected per day, at 10-12 d intervals (totaling approximately 100 tips). On SpG, shoot tips were collected from October until a clear terminal bud was visible and there was microscopic evidence of floral differentiation. On SmG, shoot tips were collected from March through April and analyzed for evidence of floral bud differentiation in the terminal bud under a microscope. After leaf senescence and presumed dormancy, 10 shoots each from the remaining SpG and SmG tagged shoots were collected at 12-15 d intervals from 15 May to bud break in July, 2006.

FBI (presence or absence) and degree of flower bud differentiation (on the basal floret in the inflorescence) was determined for the terminal bud, of each sampled shoot, according to Huang et al. (1997), at each sampling date, totaling 100 analyzed shoots. Terminal buds excised from the shoots, were fixed in formalin-acetic-alcohol, and treated with 25-50% hydrofluoric acid for 24-48 h to soften the tissue. The buds were then embedded in paraffin wax and sections made using standard techniques. The sections were stained in a safranin-fast green combination (D’Ambrogio, 1986), observed under a light microscope, and pictures taken with a digital camera. Buds were considered to have started FBI when the apical dome was flattening (Huang et al., 1997).

Daily, low, high, and average air temperature data were recorded from November 2005 to July 2006 (INTA San Pedro Meteorological Services).

### Results and discussion

Vegetative bud break occurred around 18 September, 2005 on one-year-old shoots. However, floral bud break occurred on 8 August, 40 days before vegetative bud break, when there were no leaves present. This type of growth pattern is typical in this region (Fig. 1). It has been reported that vegetative buds require more chilling hours than reproductive buds in blueberry (Darnell and Davies, 1990; Maust et al., 1999).

The SpG shoots grew from bud break (~18 September) until about mid-December and stayed in an apparent vegetative state (no visible floral buds). However, there was microscopic evidence that terminal buds on SpG shoots showed flattening of the apical dome with floral organs beginning to develop in late spring to early summer (mid-December, Table 1). By 9 January 2006, 100% of the terminal buds were in some stage of flower bud differentiation.

Summer shoots (SmG) developed from axillary buds on SpG later in the season after fruit harvest was complete. In general, two or three SmG shoots arose from each of the more vigorous SpG shoots (Fig. 2). The SmG shoots started growing at the beginning of January and stopped growing by the end of March.

#### Table 1. Temperature, photoperiod, and percentage of apical buds confirmed as flower buds (FBI) on spring (SpG) and summer (SmG) shoots, n=10 per sample date, 2005-2006

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2005</th>
<th>2006</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17 Dec.</td>
<td>27 Dec.</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>25.9</td>
<td>26.6</td>
</tr>
<tr>
<td>Photoperiod (d)</td>
<td>15.4</td>
<td>15.3</td>
</tr>
<tr>
<td>FBI, SpG (%)</td>
<td>30</td>
<td>60</td>
</tr>
</tbody>
</table>
Apical buds on SmG shoots showed evidence of FBI on 2 April with all of the apical buds sampled being floral by 15 Apr. 2006 (Table 1). Flower bud initiation thus occurred in both types of shoots after growth cessation, as has been found in other types of blueberry (Bañados and Strik, 2006).

Yield on the studied plants averaged 11,500 kg ha⁻¹, a good commercial yield for this region and cultivar. Fruit harvest occurred from 17 October to 24 November, 2006. We observed that the fruit from the buds on SpG were ripe during the early part of the fruiting season, whereas the fruit from SmG shoots matured later in the season.

Under controlled conditions, FBI in highbush, lowbush, and rabbiteye blueberries was stimulated by SD photoperiod (8 h) for 4 or more weeks with an average temperature of 21 ºC. FBI and further differentiation was reduced with increasing photoperiod, with no development at a 16 h daylength (Bañados and Strik, 2006; Hall et al., 1963; 1970; Spann et al., 2003; 2004). Spann et al. (2003) found no FBI occurred in southern highbush blueberry plants exposed to 16-h photoperiods. Some authors have suggested that 5 to 6 weeks of shortening daylength are needed to induce FBI in highbush and rabbiteye blueberry (Darnell, 1991; Hall and Ludwig, 1961; Hall et al., 1963). However, although conditions of short daylength were present in our study in April when FBI occurred in SmG shoots (12 h photoperiod and 18 ºC average temperature), these conditions were not present in December when FBI occurred on SpG shoots.

In December, the photoperiod increased slightly from 15.2 to

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**Table 2. Stages of flower bud differentiation in spring (SpG) and summer growth (SmG) from May to flowering.**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Stage of flower bud differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 May</td>
<td>MMc before meiosis</td>
</tr>
<tr>
<td>27 May</td>
<td>MMc in meiosis stage</td>
</tr>
<tr>
<td>9 June</td>
<td>Tetrahedrals</td>
</tr>
<tr>
<td>29 June</td>
<td>Free microspores</td>
</tr>
<tr>
<td>14 July</td>
<td>Mature pollen grains</td>
</tr>
<tr>
<td>Spring growth</td>
<td>Flowering</td>
</tr>
<tr>
<td>(SpG)</td>
<td></td>
</tr>
</tbody>
</table>

**Summer growth (SmG)**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primordia of all</td>
<td>Sporogenous tissue</td>
</tr>
<tr>
<td>floral parts</td>
<td>MMc before meiotic division</td>
</tr>
<tr>
<td></td>
<td>Tetrahedral tetrads</td>
</tr>
<tr>
<td></td>
<td>Free microspores cells / mature pollen grain</td>
</tr>
</tbody>
</table>

**MMc=Microspore mother cells**

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Fig. 1. Approximate time of reproductive and vegetative development of cv. O’Neal plants in Buenos Aires, Argentina (southern hemisphere)

Fig. 2. Location of spring (SpG) and summer (SpG) shoots sampled from cv. O’Neal plants

Fig. 3. Immature anther at stage: A. tetrahedral tetrads (tt) and B. free microspore (fm) in the basal floret of the apical bud on spring growth of cv. O’Neal, 9 June, 2006. (40x)
Flower bud initiation in southern highbush blueberry occurs twice per year in temperate to warm-temperate conditions. The daylength began to shorten after 22 Dec. 2005 (INTA San Pedro, Meteorological Information Center). Our results thus show that FBI in field-grown southern highbush blueberry can also occur under long-day conditions. In our study, FBI began when photoperiod was increasing and finished when plants had been exposed to two weeks of shortening daylength. Aalders and Hall (1964), while comparing flower bud development in lowbush blueberry plants grown in the greenhouse under controlled temperature and photoperiod to plants grown under field conditions, observed that under a constant 16 h photoperiod, plants did not develop flower buds. However, flower bud development did occur in the field, where plants were exposed to a 16 or 15-h photoperiod during June and July, a period when FBI usually occurs under field conditions, thus agreeing with our observations in O’Neal southern highbush blueberry.

We speculate that FBI on SpG shoots could be stimulated by carbohydrate status in the plant or a combination of factors such as carbohydrates, temperature, and light exposure, rather than photoperiod. In most fruit trees, plant carbohydrate status is associated with flower bud formation. DeJong and Day (1991) found a positive correlation between the dry matter content per unit leaf area and the number of flower buds in peach. In apple, as in blueberry, FBI occurs after cessation of shoot growth, and excessive shoot growth reduces or prevents FBI (Buban and Faust, 1982). In rabbiteye and southern highbush blueberry, there was a positive correlation between leaf number and flower bud number (Lyrene, 1991; Williamson and Miller, 2000). Furthermore, a decrease in carbohydrate concentration was associated with a decrease in FBI in southern highbush blueberry grown under controlled conditions of high temperature (28 ºC) and SD photoperiod (Spann et al., 2004). Studies done in growth chambers or greenhouses, using potted plants, often involve non-fruiting or young plants with little yield. Under field conditions, using mature, fruiting plants, FBI is likely affected by different source-sink relationships than existing in young, potted plants. In our study, by the time FBI occurred on SpG shoots, they had stopped growing and fruit harvest had finished, thus eliminating or reducing the vegetative (shoot growth) and fruit sinks. Air temperature during the FBI period of SmG shoots was within the favorable range found in controlled studies (Spann et al., 2004).

In northern Argentinean regions, and in some regions of Australia, Mexico and southern California (USA), a second flowering period is often observed on southern highbush cultivars in summer (Strik, personal observation). In Tucumán (26° lat S; 65° long O), northern Argentina, O’Neal plants produce a small second harvest at the end of summer—beginning of fall (Díiguez, personal communication), likely from flower buds on SpG shoots that bloom in the same season. However when the same cultivar is grown in the Buenos Aires region, the flower buds induced in December on SpG shoots do not bloom in the same season and there is no second harvest. In Tucumán, the average monthly temperature increased from 12 ºC in July to 25 ºC in January with 1969 heat units (10 year average, base 10 ºC; INTA San Pedro, Meteorological Information Center), with a frost-free period from October to May. However in Buenos Aires, the average monthly temperature increases from 10 ºC in July to 23 ºC in January, but the accumulated heat units are lower (1594) and the frost-free period is shorter, from November to March. Hall et al. (1970) found a positive relationship between flower bud size and temperature, associating bud size with the level of flower bud differentiation. In Tucumán, the higher temperature and accumulation of heat units than in Buenos Aires, likely advance flower bud differentiation and promote a second bloom period in this region.

Differences in the rate of flower bud development were observed between flower buds on SpG and SmG shoots in our study. Flower buds on SpG were consistently at a more advanced developmental stage than on SmG on all sampling dates (Table 2). The following season, flowering began on 28 June 2006. On 9 June, reproductive cells in the anthers of SpG flower buds were more advanced in development than those of SmG flower buds. The basipetal florets on SpG flower buds, had tetrahedral tetrads visible (Fig. 3A) and free microspores (Fig. 3B) were observed, while the anthers of SmG flower buds were at an earlier stage, microspore mother cells before meiotic division (Fig. 4). In lowbush blueberry, flower buds required four to five weeks to develop from the microspore mother cells stage to tetrads of uninucleate pollen grains (Bell and Burchill, 1952). Huang et al. (1997) observed that southern highbush blueberry cv. Sharpblue grown in Louisiana (USA), took about 15 d from microspore mother cells to tetrads of uninucleate pollen grains. Based on these results, it can be assumed that in our study by 9 June, there were maturity differences of at least 15 d between the basal florets of flower buds on SpG and SmG shoots.

We observed that FBI occurred more than 3 months later on SmG shoots than on SpG shoots. On SpG shoots, the flower bud differentiation period was from the end of December to bloom in July, under temperature conditions for rapid flower bud development. According to Hall et al. (1970), the number of primordial meristems and the degree of floret primordial development is enhanced by warmer temperatures. Spann et al. (2004) found that more flower buds were developed at 21 ºC than at 28 ºC when ‘Misty’ was exposed to either 4 or 8 weeks at an 8 h photoperiod in a greenhouse.

The higher average temperature during the period of flower bud development on SpG shoots (Table 1) may have lead to better flower development and fruit set than found on SmG shoots, perhaps a result of greater photosynthesis or carbohydrate

![Fig. 4. Immature anther in a stage of microspore mother cells (mmc) before meiosis showing callosa residues in anther wall well-differentiated, in basal floret of an apical bud on summer growth in cv. O’Neal (9 June) (20x)](image-url)
status. In addition, the occurrence of two periods of FBI and the consequent differences in time of flower bud development likely prolonged the fruit harvest season. Two peaks in volume of fruit harvested were observed (Pescie and Lovisolo, 2005), likely resulting from differences in the ontogeny of buds on SpG and SmG shoots. When O’Neal were summer pruned in Chile, tipping SpG shoots increased SmG shoots and caused a higher second harvest peak in the following season (Bañados et al., 2009).

In summary, FBI in the southern highbush blueberry cv. O’Neal, grown in temperate to warm-temperate conditions, occurred twice during the growing season under different photoperiods, long for SpG shoots and short for SmG shoots. The rate of flower bud development was greater for SpG than for SmG shoots. Studies are underway to clearly establish the relationship between shoot type, rate of flower bud development and yield and time of fruit harvest the following season and the impacts of cultural practices on these relationships.

References

Received: December, 2010; Revised: December, 2010; Accepted: January, 2011