Effects of Flower Bud Density on Vegetative and Reproductive Development and Carbohydrate Relations in Southern Highbush Blueberry

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ABSTRACT. Vegetative budbreak and subsequent canopy development in some southern highbush blueberry (*Vaccinium corymbosum* L. interspecific hybrid) cultivars are delayed and/or reduced as flower bud density increases. This delay/reduction in vegetative growth has been correlated with decreased weight and soluble solids of individual fruit. In the present study, the effects of flower bud density (FBD) on vegetative budbreak and canopy development, starch reserves, and whole-canopy net CO₂ exchange rate (NCER) were assessed to determine how FBD affected the source supply for fruit development. A range of flower bud densities was established in two cultivars of containerized southern highbush blueberry during dormancy. Vegetative budbreak was delayed and vegetative budbreak, leaf area, and leaf area to fruit ratios decreased as FBD increased. In general, increasing FBD increased the rate of root and cane starch depletion during the first four weeks after bloom. Whole-canopy NCER was similar across the range of FBD during early fruit development, but between four weeks after bloom and fruit ripening, NCER decreased as FBD increased. Although FBD explained only a small proportion of the variability observed in carbohydrate concentration and NCER, the data suggest that both the rapid depletion of starch reserves early in fruit development and the decrease in whole canopy NCER later in fruit development contribute to the detrimental effects of increased FBD on fruit development.

In many deciduous fruit crops, vegetative budbreak and leaf development precede floral budbreak, thus, initial vegetative development does not compete with developing fruit. For example, ≈20% of the total canopy in apple (*Malus ×domestica* Borkh.) is present at full bloom, and the canopy is completely developed by 3 weeks after bloom (Ferree, 1980; Forshey et al., 1983). In grape (*Vitis vinifera* L.), anthesis does not occur until ≈8 weeks after vegetative budbreak (Williams et al., 1995). Similarly, floral budbreak in northern highbush blueberry (*V. corymbosum* L.) occurs after new shoot expansion begins (Bell, 1950). In many southern highbush blueberry cultivars; however, floral budbreak is either concomitant with or precedes vegetative budbreak, in some cases by as much as four weeks. Field experiments indicate that heavy flower and fruit loads reduce vegetative budbreak and canopy development and decrease fruit fresh weight and soluble solids in some southern highbush blueberry cultivars (Maust et al., 1999). These detrimental effects of high flower bud density (FBD) suggest that there is insufficient source (usually carbohydrate) supply for the amount of sink demand.

Carbohydrate sources for fruit development include fruit photosynthesis, current leaf photosynthesis, and reserves from the previous year(s). In blueberry, fruit photosynthesis supplies ≈15% of the total fruit carbohydrate (Birkhold et al., 1992); the remainder must come from leaf photosynthesis or reserves. The detrimental effects of high FBD on vegetative and fruit development in southern highbush blueberry (Maust et al., 1999) may be due to decreases in whole plant canopy net CO₂ exchange rates (NCER), which are not fully compensated for by reserve sources. Leaf NCER is often higher in fruiting compared with defruited or nonfruiting plants of various species (Gucci et al., 1995; Hansen,

1970; Schaffer et al., 1986a, b); however, whole-canopy increases in NCER are not necessarily observed because total leaf area is often reduced in heavily fruiting plants (Maust et al., 1999; Schaffer et al., 1986a, Wibbe et al., 1993).

Cultural/management and/or breeding approaches may be useful in addressing the problem posed by high FBD on growth and development of southern highbush blueberry. First, however, the physiological basis for the detrimental effects must be established. Information on the effects of FBD on reserve carbohydrate levels and NCER in blueberry is lacking; and little information is available on the relationship of both reserve carbohydrates and NCER to fruit development. Additionally, studies in most fruit crops compare fruiting vs nonfruiting plants, and there is little information available on the effects of varying crop load on reserve carbohydrate levels, whole-canopy NCER and fruit development for any species. The objective in the present study was to determine the physiological basis for FBD effects on fruit development in southern highbush blueberry by assessing the effects of varying FBD on growth, leaf and whole-canopy NCER and reserve carbohydrate levels.

Materials and Methods

Experiments on two southern highbush blueberry cultivars, ‘Misty’ and ‘Sharpblue’, were conducted in 1995 and repeated with slight modifications in 1996. These two cultivars were selected because they have different budbreak phenologies. ‘Sharpblue’ typically breaks floral and vegetative buds simultaneously, while floral budbreak in ‘Misty’ precedes vegetative budbreak by several weeks.

1995 Experiment

PLANT MATERIAL AND TREATMENTS. Two-year-old ‘Misty’ and ‘Sharpblue’ plants were obtained from a commercial grower in Aug. 1994, transplanted into 12-L containers using a 1 peat : 1 perlite mix (v/v), and grown outside. Throughout the growing season, plants were fertigated twice weekly with a 20N–8.8P–

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16K water-soluble fertilizer (Peters, Grace-Sierra Horticultural Products Co., Milpitas, Calif.) at 14 mm N. Fertigation was stopped in early October and plant growth was allowed to slow before entering dormancy. On 24 Jan. 1995, plants were hand-defoliated and placed in coolers at 6 °C for 31 d to ensure adequate chilling for breaking endodormancy. After the chilling period, plants were again moved outside and placed in alternating rows of 'Misty' and 'Sharpblue' oriented north-south. Total flower bud number was determined, total lengths of all canes and stems were measured, and flower bud densities (flower bud number/cm cane length) were calculated for each plant. Plants of each cultivar were grouped in three blocks according to cane length, with 24 plants per block. Different FBDs were randomly assigned and flower bud number per cm cane length was adjusted by hand, resulting in an FBD range of 0.05 to 0.39 for 'Misty' and 0.05 to 0.31 for 'Sharpblue'. Flower buds were removed at evenly spaced intervals along the canes to decrease FBD, or stems with very low FBD were removed to increase overall plant FBD. Weekly fertigation at the previous rate was resumed.

PLANT MEASUREMENTS. Floral and vegetative budbreak were measured on a weekly basis and ripe fruit were harvested every 3 to 5 d. The fruit development period was calculated as the number of days from 50% bloom to 50% ripe fruit. Vegetative buds were denoted as breaking when they were at least 0.5 cm extended.

Immediately after the chilling period (dormancy, 24 d before 50% bloom), five 'Misty' plants, ranging in FBD from 0.09 to 0.22, and four 'Sharpblue' plants, with FBD ranging from 0.06 to 0.21, were harvested and separated into component parts. Subsequently, two plants of each cultivar were randomly harvested from each treatment level and block at each of the following times: 14 d after bloom (DAB), 42 DAB, and fruit ripening ($\approx$ 67 DAB). The 14 DAB plant harvest occurred at 80% bloom, but since whole plants were harvested in the 1996 experiment relative to 50% bloom, results from the 1995 experiment are also reported in terms of 50% bloom. At each whole plant harvest, plants were divided into roots, previous years' canes, new stems, leaves, and flowers or fruit (when present). Leaf area was measured using a portable leaf area meter (LI-3000; LI-COR, Lincoln, Neb.) and the leaves and current year's stems were dried at 70 °C to determine dry weight. The roots and canes were frozen and held at -30 °C until lyophilized and dry weight was measured.

NET CO₂ EXCHANGE RATES. Net CO₂ exchange rates of whole 'Misty' blueberry plant canopies were determined immediately after chilling ($n = 5$), 14 DAB ($n = 8$), and at the beginning of fruit ripening ($n = 9$). Whole-canopy NCER of 'Sharpblue' plants were measured immediately after chilling ($n = 4$), 14 DAB ($n = 20$), 42 DAB ($n = 19$), and at the beginning of fruit ripening ($n = 20$). Whole-canopy NCER was measured using an open flow system with an Anarad AR600R infrared gas analyzer (IRGA) (Anarad Inc., Santa Barbara, Calif.). Whole plants were enclosed in a 1 × 1 × 1 m plexiglass chamber covered on the inside with Propafilm C (ICI Films, Wilmington, Del.). Roots were enclosed in a Tedlar gas sampling bag (Fisher Scientific) sealed at the base of the canes. Photosynthetic photon flux (PPF), provided by a 400-W metal halide lamp (Sylvania Lumalux Lu400), was 1750 to 2000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the top of the canopy ($\approx$ 50 cm). The chamber was located in a laboratory illuminated with fluorescent light. CO₂ concentration of incoming ambient air, pumped from outside, was $373 \pm 10 \mu\text{mol}\cdot\text{mol}^{-1}$. Vapor pressure was measured with a dew point hygrometer (1100DP; General Eastern Corp., Watertown, Mass.). Vapor pressure deficit was maintained at <1

kPa (Moon et al., 1987). Air flow into the chamber was regulated using flowmeters (36-546-305; Manostat, New York, N.Y.). Fans built into the chamber circulated the air and the temperature was held at 25 ± 1 °C using a water-filled reservoir between the light source and the chamber and a water-cooled heat sink inside the chamber. Leaf and chamber temperatures were monitored using copper-constantan thermocouples and a digital thermometer (model AD2036, Analog Devices, Norwood, Mass.). Reference and sample gas subsamples were pulled from the gas entering and leaving the chamber, respectively, and dried by passing through magnesium perchlorate before entering the IRGA for analysis. Differential CO₂ concentration was recorded after it stabilized for at least 15 min.

1996 Experiment

PLANT MATERIAL AND TREATMENTS. Two-year-old 'Misty' and 'Sharpblue' southern highbush blueberry plants were obtained from a commercial grower in July 1995, transplanted into 12-L containers using a 1 peat : 1 perlite mix (v/v), and grown outside. Plants were fertigated until the middle of September as previously described. On 15 Dec. 1995, plants were placed in an open-ended tunnel greenhouse. PPF was $>1450 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ on clear days. The plants were protected from freezes and the greenhouse temperatures averaged 1.5 °C warmer than the outside temperatures. On 22 Jan. 1996, plants were transferred back outside and placed in alternating rows of 'Misty' and 'Sharpblue' oriented north-south. Total flower bud number was counted, total cane and stem lengths were measured, and FBD was calculated for each plant. Different FBDs were randomly assigned and flower bud number per cm cane length was adjusted by hand, resulting in an range of 0.07 to 0.31 for 'Misty' and 0.07 to 0.26 for 'Sharpblue'. FBD was adjusted as previously described.

PLANT MEASUREMENTS. Measurement of floral and vegetative budbreak and calculation of fruit development period were done as previously described. Ripe fruits were harvested every 3 to 5 d until 50% of the fruit were ripe. Four to six plants of each cultivar were randomly selected from within each treatment range, and whole plant harvests were done at each of the following times: immediately after removal from the greenhouse (dormancy, 18 d before 50% bloom); 50% bloom (0 DAB); 28 DAB; and 50% ripe fruit (fruit harvest, $\approx$ 84 DAB). At each plant harvest, plants were divided into roots, previous years' canes, new stems, leaves, and flowers or fruit. Total leaf area and dry weight of all plant organs were determined as described previously. Lyophilized roots and canes were ground in a Wiley mill to pass a 20-mesh screen, and subsamples were analyzed for sugar and starch levels.

CARBOHYDRATE ANALYSIS. Root and cane soluble sugars were extracted from 50 mg dried tissue by boiling in 80% (v/v) ethanol for 2 min. Extracts were shaken for 20 min and centrifuged, the supernatant decanted, and the pellet reextracted twice. The supernatants were combined, and final volumes were measured. Sample pigment was removed by adding 35 mg activated charcoal, and soluble sugars were assayed using the phenol-sulfuric acid method (Buysse and Merckx, 1993; Dubois et al., 1956). Tissue starch content was determined as previously described (Darnell and Birkhold, 1996), by suspending the insoluble fraction from the 80% ethanol extraction in 2.0 mL 0.2 N KOH and boiling for 30 min. After cooling, pH was adjusted to 4.5 with 1.0 mL 1.0 M acetic acid, and 1.0 mL of *Rhizopus* amyloglucosidase (118 units/mL) (Sigma Chemical Co., St. Louis, Mo.) in 0.2 M calcium acetate buffer (pH 4.5) was added. Samples were incubated in a shaking water bath for 24 h at 37 °C. After incubation, samples

were centrifuged and the supernatant decanted. Sample pigment was removed by adding 35 mg activated charcoal, and glucose liberated from starch hydrolysis was quantified by the phenol-sulfuric acid method.

NET CO₂ EXCHANGE RATES. Whole-canopy NCER of 'Misty' plants was determined at 0 (n = 14) and 28 DAB (n = 15), and at the beginning of fruit ripening (≈ 58 DAB) (n = 7) as previously described. Whole-canopy NCER of 'Sharpblue' plants was measured at the beginning of fruit ripening (≈ 55 DAB) (n = 15).

Single leaf NCER measurements were taken on five recently matured leaves on randomly selected 'Misty' (n = 7) and 'Sharpblue' (n = 15) plants to determine the influence of FBD on photosynthetic rates of individual leaves. A portable closed gas exchange system equipped with a one liter cuvette (LI-6200, LI-COR, Lincoln, Neb.) was used. Measurements were taken outside on clear days between 11 to 17 April 1996 when the fruit was beginning to ripen. Measurements were taken under ambient temperatures ($28 \pm 2^\circ\text{C}$), relative humidity ($20\% \pm 5\%$), CO₂ concentrations ($371 \pm 11 \mu\text{mol}\cdot\text{mol}^{-1}$) and PPF ($>1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Three measurements were taken per leaf and averaged.

STATISTICAL ANALYSIS. The 1995 experiment was set up as a RCBD design; however, block was never significant. Thus, data for both years were analyzed as completely randomized designs. SAS (SAS Institute, Cary, N.C.) was used for statistical analyses. Regression analysis was used to evaluate relationships within the data, and the regression model (linear, quadratic, or log) with the best fit was used. When interactions between different levels of a variable (FBD, cultivar, plant harvest date) were not significant, multiple regression analysis was used. In those cases, the regression equation gives the slope, which is the same for all levels of a variable, and the y-intercept for the level that was assigned value = 1. All other levels were assigned value = 0. To determine the y-intercepts for the other levels of a given variable, that level is assigned value = 1 and all other levels assigned value = 0. When interactions between different levels of a variable were significant, these were analyzed separately using simple regression. Total cane length was used as a covariate to adjust for differences in plant size. Cane lengths averaged over both cultivars were 178 cm in 1995 and 475 cm in 1996. PROC GLM was used for analysis of variance and regression analysis, PROC CORR was used to test correlations, and PROC MIXED was used to analyze for consistent patterns in vegetative budbreak regression slopes.

Results

In 1995, 50% bloom (0 DAB) occurred ≈ 24 d after removal from chilling for both 'Misty' and 'Sharpblue', while in 1996, 50% bloom occurred ≈ 18 d after removal from the greenhouses. The bloom period was similar for the two cultivars in both years, averaging 3 to 4 weeks. The fruit development period for both cultivars averaged ≈ 67 and 84 d in 1995 and 1996, respectively. The general patterns of vegetative growth and canopy NCER were similar in both years for a given cultivar; thus, primarily 1996 data are presented.

Since floral budbreak and fruit set overlapped and fruit density was not adjusted in this study, it was not possible to separate the effects of FBD from the effects of fruit density. Fruit density increased as FBD increased in both years. In 1995, fruit density was similar for both cultivars, increasing from 0.1 to 0.8 fruit/cm cane length as FBD increased from 0.07 to 0.30 ($y = -0.05 + 2.88 \text{ FBD}$, $r^2 = 0.41$, $P < 0.001$). Fruit density in 1996 was higher for both cultivars compared to 1995, ranging from 0.8 to 1.9 fruit/cm

cane length as FBD increased from 0.07 to 0.30 ('Misty' $y = 0.38 + 5.14 \text{ FBD}$, $r^2 = 0.79$, $P < 0.001$; 'Sharpblue' $y = 0.64 + 4.62 \text{ FBD}$, $r^2 = 0.16$, $P < 0.01$). The number of plants harvested in both cultivars and both years varied with harvest date. This was due to *Botryosphaeria dothidea* infection and subsequent removal of plants from the experiment. In 1995, both 'Misty' and 'Sharpblue' were infected at all treatment levels. However, more 'Misty' plants were affected, thus no 'Misty' plants were harvested at 42 DAB and 14 plants (across the range of FBD) remained at fruit ripening. In 1996, *Botryosphaeria dothidea* was observed only in 'Misty', which began showing decline ≈ 4 to 5 weeks after bloom.

VEGETATIVE DEVELOPMENT AND BIOMASS ALLOCATION. As FBD increased, the number of vegetative buds that broke and grew per unit cane length decreased for both cultivars in both years (data not shown). However, FBD explained only $\approx 15\%$ of the total variability in vegetative budbreak. In 'Misty', vegetative budbreak at 28 DAB was reduced by $\approx 60\%$ (1995) and $\approx 75\%$ (1996) at high (0.31) compared to low (0.07) FBD. In 'Sharpblue', vegetative budbreak was reduced by $\approx 50\%$ (1995) and $\approx 65\%$ (1996) at high (0.26) compared to low (0.07) FBD. Vegetative budbreak density in 'Sharpblue' was greater than in 'Misty' by 28 DAB, and this trend continued through final fruit harvest.

Total leaf area, leaf dry weight, and current year's stem length and dry weight were highly correlated at all sampling dates ($r > 0.85$) and responses to FBD were similar, therefore only the relationship between FBD and leaf area is presented (Fig. 1). Leaf area decreased with increasing FBD for both cultivars in 1996, but in 1995, a relationship similar to that found in 1996 occurred

Fig. 1. Effect of FBD on leaf area in 'Misty' and 'Sharpblue' southern highbush blueberry in 1996. 'Misty': $y = -892 - 593 \ln \text{FBD} + 1334 \text{ date}$, where date = 0 for 28 DAB and date = 1 for fruit harvest, $R^2 = 0.74$, $P < 0.05$, (n = 27); 'Sharpblue': $y = -5973 - 2790 \ln \text{FBD} + 6.5 \text{ length} + 3972 \text{ date}$, where date = 0 for 28 DAB and date = 1 for fruit harvest, $R^2 = 0.56$, $P < 0.05$, (n = 40). The regression lines and data points shown indicate adjustment at the average value of the cane length covariate.

Table 1. Organ dry weight (g) of 'Misty' and 'Sharpblue' southern highbush blueberry at various stages of development in 1996.

Developmental stage	Dry wt	
	'Misty' ^z	'Sharpblue'
Root		
Dormancy	23.5 a ^y	52.0 a
0 DAB	20.3 ab	44.2 a
28 DAB	19.3 bc	45.2 a
Fruit harvest	16.6 c	45.2 a
Cane		
Dormancy	50.7 a	71.0 a
0 DAB	45.3 ab	63.8 a
28 DAB	42.1 b	65.9 a
Fruit harvest	41.2 b	73.7 a
Flower		
Dormancy	3.36 c	4.16 c
0 DAB	6.17 bc	6.83 bc
28 DAB	9.31 b	14.5 b
Fruit harvest	33.9 a	54.5 a
Stem		
Dormancy	0	0
0 DAB	0.01 b	0.05 b
28 DAB	0.07 b	1.52 b
Fruit harvest	1.93 a	12.4 a
Leaf		
Dormancy	0	0
0 DAB	0.34 b	0.74 c
28 DAB	0.74 b	12.0 b
Fruit harvest	12.0 a	49.4 a

^z'Misty': n = 8, 12, 17, and 10 at dormancy, 0 DAB, 28 DAB, and fruit harvest, respectively. 'Sharpblue': n = 8, 12, 12, 28 at dormancy, 0 DAB, 28 DAB, and fruit harvest, respectively.

^yMean separation within groups within columns by LSD, P = 0.05. Values are means adjusted using flower bud density and cane length as covariates.

only for 'Sharpblue' (data not shown). The lack of significant response in 'Misty' in 1995 was probably a result of the small sample number, since the incidence of *Botryosphaeria dothidea* was severe. The decrease in leaf area as FBD increased was evident by 14 DAB in 1995 and 28 DAB in 1996. In 1996, total leaf area at fruit harvest was reduced by $\approx 55\%$ in both cultivars at high compared to low FBD; however, leaf area of 'Misty' throughout fruit development averaged only 25% of the leaf area of 'Sharpblue'. The reduction in leaf area with increased FBD also resulted in decreases in leaf area to fruit ratios in both cultivars in 1996 ($y = 15.2 - 10.0 \ln \text{FBD} - 23.3 \text{ cultivar}$, where cultivar = 0 for 'Misty' and cultivar = 1 for 'Sharpblue', $R^2 = 0.44$, $P < 0.01$).

Flower bud density did not affect root or cane dry weight. However, root and cane dry weight differed with various developmental stages. In general, root and cane dry weight decreased between dormancy and 28 DAB in 'Misty', while root and cane dry weight in 'Sharpblue' remained the same from dormancy until fruit harvest (Table 1, data for 1996 only).

Dry weight allocation to the fruit was low in 1995, accounting for 4% to 9% of the total plant dry weight at fruit harvest for both cultivars (data not shown). This is probably a reflection of the low fruit density in that year. As a consequence, leaves and new stems were the major sink for dry weight gain throughout fruit development for both cultivars. In 1996, fruit biomass accounted for 32% and 23% of the total plant dry weight at fruit harvest in 'Misty' and 'Sharpblue', respectively, while leaf and new stem dry weight at

fruit harvest averaged 13% in 'Misty' and 26% in 'Sharpblue' (Table 1).

CARBOHYDRATES. Cane sugar concentrations were similar at all FBD in both 'Misty' and 'Sharpblue' within each sampling date in 1996, decreasing from ≈ 40 to $10 \mu\text{g}\cdot\text{mg}^{-1}$ dry weight between dormancy and 28 DAB, before increasing to $\sim 20 \mu\text{g}\cdot\text{mg}^{-1}$ dry weight at fruit harvest. Cane starch, root sugar, and root starch concentrations decreased with increased FBD at bloom, 28 DAB, and at fruit harvest in 'Misty' (Fig. 2A–C). A similar, but weaker correlation was evident in 'Sharpblue' (Fig. 3A–C), except that decreased root sugar concentrations with increased FBD were observed only at bloom.

Root and cane carbohydrate concentrations changed with development. Root starch concentrations decreased by 37% and 63% for 'Misty' and 'Sharpblue', respectively, between dormancy (18 d before bloom) and bloom (0 DAB) (Figs. 2C and 3C). Root starch concentrations continued to decrease with development and by 28 DAB, concentrations were reduced by 75% and 90% in 'Misty' and 'Sharpblue', respectively, relative to concentrations in dormant plants. Root starch concentrations in 'Sharpblue' increased between 28 DAB and fruit harvest, while

Fig. 2. Cane starch concentrations (A) and root sugar (B) and starch (C) concentrations (dry weight basis) in 'Misty' southern highbush blueberry as related to flower bud density in 1996. (A) Cane starch: $y = 22.7 - 5.7 \ln \text{FBD} - 15.3 \text{ date}_1 - 10.7 \text{ date}_2$, where $\text{date}_1 = 1$ for 28 DAB and $= 0$ otherwise and $\text{date}_2 = 1$ for fruit harvest and $= 0$ otherwise, $R^2 = 0.74$, $P < 0.05$, ($n = 33$). (B) Root sugar: $y = 25.8 - 7.0 \ln \text{FBD} - 8.9 \text{ date}_1 - 28.8 \text{ date}_2$, where $\text{date}_1 = 1$ for 28 DAB and $= 0$ otherwise and $\text{date}_2 = 1$ for fruit harvest and $= 0$ otherwise, $R^2 = 0.78$, $P < 0.05$, ($n = 33$). (C) Root starch: $y = 44.3 - 13.2 \ln \text{FBD} - 41.2 \text{ date}_1 - 52.5 \text{ date}_2$, ($n = 33$), where $\text{date}_1 = 1$ for 28 DAB and $= 0$ otherwise and $\text{date}_2 = 1$ for fruit harvest and $= 0$ otherwise, $R^2 = 0.91$, $P < 0.01$, ($n = 33$).

in 'Misty', root starch concentrations continued to decrease until fruit harvest. Cane starch concentrations decreased until 28 DAB in both cultivars before exhibiting a slight increase by fruit harvest (Figs. 2A and 3A).

LEAF AND WHOLE-CANOPY NCER. In 'Sharpblue', leaf NCER at fruit ripening increased from 3.8 to 10.9 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ as FBD increased from 0.07 to 0.26, although the relationship was variable ($r^2 = 0.30$, $P < 0.002$); however, there was no effect of FBD on leaf NCER in 'Misty' ($y = 7.9 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

Whole-canopy NCER was not affected by FBD between dormancy and 14 DAB (1995) or 28 DAB (1996). Respiration was greater than photosynthesis during dormancy and bloom, with whole-canopy NCER averaging $-0.17 \mu\text{mol}\cdot\text{s}^{-1} \text{CO}_2$ per plant during both measurement periods. Whole-canopy NCER was positive ($0.25 \mu\text{mol}\cdot\text{s}^{-1} \text{CO}_2$ per plant) by 14 DAB (1995) or 28 DAB (1996), indicating net carbon gain in the experimental conditions used. In 1995, whole-canopy NCER decreased with increased FBD between 14 and 42 DAB and this effect of FBD on NCER continued through fruit ripening in both cultivars (data not shown). In 1996, whole-canopy NCER decreased with increased FBD between 28 DAB and fruit ripening for 'Misty' and by fruit ripening for 'Sharpblue' (Fig. 4).

Fig. 3. Cane starch concentrations (A) and root sugar and starch concentrations (dry weight basis) (B) and (C) in 'Sharpblue' southern highbush blueberry as related to flower bud density in 1996. (A) Cane starch: dormancy $y = 63.5$, $P = \text{NS}$ ($n = 8$); 0 DAB $y = -7.6 - 11.4 \ln \text{FBD}$, $r^2 = 0.43$, $P < 0.05$, ($n = 12$); 28 DAB $y = 9.8$, $P = \text{NS}$, ($n = 12$); fruit harvest $y = 3.9 - 5.9 \ln \text{FBD}$, $r^2 = 0.30$, $P < 0.05$ ($n = 17$). (B) Root sugar: dormancy $y = 54.2$, $P = \text{NS}$ ($n = 8$); 0 DAB $y = 15.3 - 25.6 \ln \text{FBD}$, $r^2 = 0.53$, $P < 0.01$, ($n = 12$); 28 DAB $y = 31.5$, $P = \text{NS}$ ($n = 12$); fruit harvest $y = 22.4$, $P = \text{NS}$ ($n = 17$). (C) Root starch: $y = 12.5 - 12.3 \ln \text{FBD} - 27.5 \text{ date}_1 - 15.2 \text{ date}_2$, where $\text{date}_1 = 1$ for 28 DAB and $= 0$ otherwise and $\text{date}_2 = 1$ for fruit harvest and $= 0$ otherwise, $R^2 = 0.57$, $P < 0.05$, ($n = 41$).

FRUIT DEVELOPMENT. In both years and for both cultivars, average fruit fresh and dry weights at fruit ripening decreased as whole-canopy NCER to fruit ratio decreased (Fig. 5, data shown for 1996 only) and as leaf area to fruit ratio decreased. The patterns for all were similar, thus only the fruit dry weight relationship to the NCER to fruit ratio is shown. Leaf area to fruit and whole-canopy NCER to fruit ratios also affected the fruit development period, which increased with decreased leaf area to fruit and whole-canopy NCER to fruit ratios in both years and for both cultivars (1996: $y = 35.1 - 7.8 \ln \text{NCER:fruit}$, $r^2 = 0.63$, $P = 0.001$, cultivar effect nonsignificant). Thus, the decrease in canopy development and carbohydrate supply as FBD increased resulted in a delay in fruit harvest.

Discussion

The decrease in the number of vegetative buds that broke and grew per unit cane length as FBD increased explained only a small proportion (12% to 18%) of the variability in vegetative budbreak. The correlation between increased FBD and decreased leaf area was stronger, although also variable, and was evident early in fruit development. The reduction in leaf area and leaf area to fruit ratios with increased FBD is similar to that found in field grown 'Misty' and 'Sharpblue' (Maust et al., 1999) and is analogous to the situation found in other crops, in which final leaf area of fruiting plants was less than that of nonfruiting plants (Maggs, 1963; Schaffer et al., 1986b; Schechter et al., 1994). The lower overall leaf area of 'Misty' compared to 'Sharpblue', which was also reflected in the decreased percent dry weight allocation to 'Misty' leaves, probably reflected the different budbreak phenologies of these two cultivars. 'Sharpblue' typically breaks floral and vegetative buds simultaneously. Although some veg-

Fig. 4. Effect of FBD on whole-canopy NCER at fruit ripening in 'Misty' and 'Sharpblue' southern highbush blueberry in 1996. $y = -2.85 - 0.88 \ln \text{FBD} + 0.004 \text{ length} + 0.19 \text{ cultivar}$, where cultivar = 0 for 'Misty' and cultivar = 1 for 'Sharpblue', $R^2 = 0.69$, $P < 0.05$ ($n = 22$). The regression lines and data points shown indicate adjustment at the average value of the cane length covariate.

etative budbreak occurred in 'Misty' at anthesis, the majority of buds did not break during the first 28 DAB, and in fact, many buds did not break at all. Previous work with blueberry found that fruit photosynthesis supplied $\approx 85\%$ of the total carbon used for fruit growth during the first 10 d after bloom (Birkhold et al., 1992). By this time, the earliest formed leaves are nearing full expansion and have already converted from import to export status (Birkhold and Darnell, unpublished data). This suggests that there may be less competition for carbohydrates between fruit and vegetative growth in cultivars that break bud simultaneously compared with cultivars in which floral budbreak occurs prior to vegetative budbreak, since new vegetative growth begins exporting carbohydrate before the import demand by the fruit increases.

FBD did not affect root and cane dry weight in either cultivar, although it did affect reserve carbohydrate concentration. The rapid decline in root and cane starch concentrations between dormancy and bloom, particularly as FBD increased, indicates a strong demand on carbohydrate reserves during flower bud development. Cano-Medrano and Darnell (1997) reported that 80% of the cells present in ripe rabbiteye blueberry fruit were already present prior to bloom. Thus, the majority of cell division occurs preanthesis in blueberry fruit, and may represent a significant sink demand.

The rapid decrease in cane and root starch concentrations during the first four weeks of fruit development in both cultivars indicates continued use of carbohydrate reserves, and is similar to findings in rabbiteye blueberry (Darnell and Birkhold, 1996). The

observation that increased FBD (and therefore increased fruit density) resulted in faster depletion of starch reserves during this time differs from work done in apple (Ferree and Cahoon, 1987) and sweet cherry (Roper et al., 1988) where no differences were seen in root or shoot carbohydrate concentrations at fruit ripening for different crop loads. The high harvest index in blueberry observed in the 1996 experiment, coupled with concomitant fruit and vegetative growth may explain the different effects of FBD on starch use observed in blueberry vs apple and sweet cherry.

The observation that whole-canopy NCER did not decrease at high compared with low FBD between dormancy and 14 DAB (1995) or 28 DAB (1996, data from 'Misty' only), while the rate of carbohydrate reserve usage did, indicates that early blueberry fruit development (i.e., at least the first 4 to 5 weeks after bloom) depends more on reserve carbohydrates than current photosynthates, and agrees with work in rabbiteye blueberry (Darnell and Birkhold, 1996). The subsequent decrease in whole-canopy NCER with increased FBD at fruit ripening is similar to that found in fruiting vs nonfruiting strawberry (Schaffer et al., 1986b), where fruiting plants exhibited lower whole-canopy NCER rates than nonfruiting plants, but contrary to results found in grape (Edson et al., 1993) and apple (Francesconi et al., 1996), where no differences in whole-canopy NCER were detected due to crop load variation. Those results suggest that grape and apple are able to compensate for the reduction in leaf area to fruit ratios through increases in leaf NCER. Although 'Sharpblue' leaf NCER increased as FBD increased at fruit ripening, it was apparently not enough to fully compensate for the decrease in leaf area. The lack of a similar effect on leaf NCER in 'Misty' may be due to the fact that all the 'Misty' plants had low leaf area to fruit ratios and so sink demand relative to source supply was always high. Lower leaf area to fruit ratios in 'Misty' compared to 'Sharpblue' may partially explain the overall higher leaf NCER (across all FBD) in 'Misty' ($7.9 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) compared to 'Sharpblue' ($5.6 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

The increase in final fruit fresh and dry weight as whole canopy NCER to fruit ratio increased is consistent with results found in apple where final fruit size was positively related with whole-canopy NCER to fruit ratio (Lakso et al., 1996). Generally, correlations are drawn between fruit weight and leaf area to fruit ratios (Ferree and Cahoon, 1987; Roper and Loescher, 1987; Weinberger, 1931); however, a variety of factors that influence whole canopy NCER (and therefore carbohydrate production) may have little influence on total leaf area. These factors include variations in light, temperature, and/or CO_2 concentrations within the canopy, which would affect leaf development and assimilatory capacity, as well as CO_2 assimilation and loss rates for the entire plant.

The physiological basis for the effect of FBD on fruit development appears to involve effects on both carbohydrate reserves and current photoassimilates. Early in fruit development, and perhaps as early as the beginning of flower bud initiation, the supply of carbohydrate reserves plays the major role, in terms of both supply to the flower/fruit and the supply to the developing vegetative buds and leaves. The stronger correlation between starch concentrations and FBD in 'Misty' compared to 'Sharpblue' is probably due to the longer time that 'Misty' fruit must rely on reserves before sufficient leaf area is available. In the final stages of fruit development, the FBD effect in both cultivars appears to be due primarily to effects on whole-canopy NCER via effects on leaf area.

Practices such as flower bud thinning should increase fruit size by increasing vegetative budbreak, which in turn would increase

Fig. 5. Effect of whole-canopy NCER to fruit ratio on fruit dry weight at ripening in 'Misty' and 'Sharpblue' southern highbush blueberry in 1996. 'Misty' $y = 0.08 + 31.40 \text{ NCER:fruit}$, $r^2 = 0.70$, $P < 0.05$ ($n = 7$); 'Sharpblue' $y = 0.10 + 6.55 \text{ NCER:fruit}$, $r^2 = 0.68$, $P < 0.001$ ($n = 15$).

the photosynthetic leaf area and canopy NCER. Other practices that increased or advanced vegetative budbreak, either cultural or chemical, should also increase fruit size. Additionally, factors that increased carbohydrate reserve levels, such as maintaining healthy leaves through the previous summer and fall, may also result in larger, earlier ripening fruit, although this aspect was not tested here.

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