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Carbohydrate Reserve Concentrations and Flower Bud Density Effects on Vegetative and Reproductive Development in Southern Highbush Blueberry

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Abstract. **Vegetative budbreak, leaf area development, and fruit size in southern highbush blueberry (*Vaccinium corymbosum* L. interspecific hybrids) decrease as flower bud density increases. The effect on fruit size has been attributed to both insufficient carbohydrate reserves and reductions in current photoassimilates caused by decreased vegetative growth. Experiments were conducted with two southern highbush blueberry cultivars, 'Misty' and 'Sharpblue', to test the hypothesis that increased carbohydrate reserve concentrations can overcome the detrimental effects of high flower bud density by increasing vegetative budbreak, shoot development, and whole-canopy net CO_2 exchange rate (NCER), which in turn will increase fruit size. Fully foliated plants were placed in greenhouses with either ambient (AMB) CO_2 levels (≈360 μmol·mol^{-1}) or enriched (ENR) CO_2 levels (≈700 μmol·mol^{-1}) for 38 d during fall. Plants were then moved outdoors, hand defoliated, and flower bud density (flower buds/cm cane length) adjusted to range from 0.07 to 0.31. Root starch and whole plant carbohydrate concentrations increased in ENR compared with AMB plants of both cultivars. Vegetative budbreak (number per centimeter cane length), leaf area, and whole-canopy NCER decreased as flower bud density increased in AMB and ENR plants of both cultivars; however, ENR 'Sharpblue' plants had significantly greater vegetative growth and whole-canopy NCER at a given flower bud density compared with AMB 'Sharpblue'. Concomitant with this was an increase in fruit fresh weight in ENR compared to AMB 'Sharpblue'. This was not the case with 'Misty', where vegetative development and fruit size were similar in ENR and AMB plants. Thus, the hypothesis that increased carbohydrate reserves will increase vegetative development and subsequent fruit size may be true only in certain cultivars of southern highbush blueberry. Alternatively, the increased carbohydrate reserve concentrations in ENR compared with AMB 'Misty' plants may have been insufficient to affect subsequent vegetative or reproductive development.**

Flowering and fruit set begin before or concomitant with vegetative budbreak in southern highbush blueberry cultivars. Previous studies have shown that as flower bud density increases, the amount of vegetative budbreak and new shoot development decreases, resulting in a decrease in fruit size, as measured by fresh weight (FW) (Maust et al., 1999a). These effects of high flower bud density have been attributed to both an insufficient reserve carbohydrate supply, as well as a decrease in current assimilate supply due to reductions in leaf area and whole-canopy net CO_2 exchange rate (NCER) (Maust et al., 1999b).

Carbohydrate reserves clearly play a key role in supporting new spring growth in deciduous species. Numerous studies have documented mobilization of reserve carbohydrates into new spring growth (Darnell and Birkhold, 1996; Davis and Sparks, 1974; Hansen, 1971; Lockwood and Sparks, 1978; Quinlan, 1969) and changes in carbohydrate reserve levels during flowering and fruiting have been investigated extensively in several deciduous fruit crops (Loescher et al., 1990). The relationship between carbohydrate reserve levels and fruiting has also been studied in alternate bearing trees (Goldschmidt and Golomb, 1982; Nzima et al., 1997; Weinbaum et al., 1994; Wood, 1989). There are few studies, however, which document the effects of carbohydrate reserve levels on the amount of spring vegetative growth in woody, deciduous species and the subsequent effect on fruit growth. Late summer defoliation of sweet cherry (*Prunus avium* L.) decreased root starch reserves and resulted in tree death the following year (Loescher et al., 1990). Wilcox (1937) observed a positive correlation between reserve carbohydrate levels and shoot growth in apple [*Malus sylvestris* (L.) Mill var *domestica* (Borkh.) Mansf.]. These studies suggest that carbohydrate reserve levels may influence the extent of vegetative budbreak and subsequent shoot development in other species, such as southern highbush blueberry (*Vaccinium corymbosum* interspecific hybrids). Increased shoot development should, in turn, increase fruit development as leaf area to fruit and whole-canopy NCER to fruit ratios increase (Ballinger et al., 1963; Facteau et al., 1983; Ferree and Cahoon, 1987; Lakso et al., 1996; Roper and Loescher, 1987). The objective of the present study was to determine if increased reserve carbohydrate concentrations could overcome the detrimental effects of high flower bud density in southern highbush blueberry by increasing vegetative budbreak, shoot development, and fruit FW.

Materials and Methods

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 medium of 1 peat : 1 perlite (v/v), and grown outdoors. These two cultivars were chosen because they exhibit very different budbreak phenologies. 'Sharpblue' breaks floral and vegetative bud simultaneously, while vegetative budbreak in 'Misty' occurs several weeks after floral budbreak. The basis for the difference in budbreak patterns between these two cultivars is unknown. Plants were fertigated with 20N–8.8P–16.6K water-soluble fertilizer (Peters; Grace-Sierra, Milpitas, Calif.) at a rate of 1 g·L^{-1} twice a week until mid-September. On 15 Dec. 1996, while still fully foliated, plants of each cultivar were randomly separated into two equal groups and placed inside open-ended plastic tunnel

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greenhouses described by Sinclair et al. (1995) and grown under natural light conditions [photosynthetic photon flux (*PPF*) >1450 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on clear days]. One half of the plants of each cultivar were placed inside a tunnel greenhouse with ambient CO_2 concentrations (≈360 $\mu mol \cdot mol^{-1}$), while the other half was placed in a corresponding location in a similar tunnel greenhouse with CO_2 concentrations maintained at ≈700 $\mu mol \cdot mol^{-1}$ during the daytime by a computer-controlled CO_2 injection system (Sinclair et al., 1995). Temperatures were maintained at ambient temperatures ±1.5 °C except when ambient temperatures were <0 °C, then greenhouse temperatures were maintained >0 °C.

All plants were moved outdoors on 22 Jan. 1996 (≈5 weeks in treatment) and hand-defoliated. Total number of flower buds per plant were counted, total length of all canes and stems per plant were measured, and flower bud densities (flower bud number per centimeter cane length) were calculated for each plant. Different flower bud densities were randomly assigned to entire plants of each cultivar within each CO_2 treatment and densities were adjusted by hand, resulting in flower bud densities of 0.07 to 0.40 for 'Misty' and 0.07 to 0.27 for 'Sharpblue'. Flower buds were removed at evenly spaced intervals along the canes to decrease flower bud densities, or small stems with very low flower bud densities were removed to increase overall plant flower bud density. Plants were fertigated weekly with Peters 20N–8.8P–16.6K water-soluble fertilizer at 1 $g \cdot L^{-1}$.

Plant measurements. Floral and vegetative budbreak were recorded weekly and ripe fruit was harvested every 3 to 5 d. Fruit development period was calculated as the number of days from 50% bloom to 50% ripe fruit. Vegetative buds were considered breaking when they were extended at least 0.5 cm. Whole plants were randomly harvested from within each cultivar, CO_2 treatment, and across the range of flower bud densities at the following times: 1) removal from the greenhouse (end of dormancy, ≈20 d before bloom), 2) bloom [0 d after bloom (DAB)], 3) 4 weeks after bloom (28 DAB), and 4) fruit ripening. The number of plants harvested at each time varied, thus, sample number (n) for each measured parameter is given in the tables and figures. At each plant harvest, plants were divided into roots, previous years' canes, new stems, leaves, and flowers or fruit. Leaf area was measured using a portable area meter (LI-3000; LI-COR, Lincoln, Nebr.) and the leaves and current year's stems were dried to a constant weight at 70 °C to determine dry weights (DWs). Roots and canes were frozen and held at –30 °C until lyophilized and DWs measured. Lyophilized roots and canes were ground in a Wiley mill to pass a 20-mesh (1.27-mm) screen, and subsamples were analyzed for sugar and starch levels.

Carbohydrate analysis. Root and cane soluble sugars were extracted from 50 mg of tissue by boiling in 5 mL 80% ethanol for 2 min. Extracts were shaken for 20 min, centrifuged at 5,000 g_n, 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. Soluble sugars were assayed using the phenol-sulfuric acid method (Buysse and Merckx, 1993; Dubois et al., 1956). Tissue starch concentration was determined 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 (1118 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 at 5,000 g_n, the supernatant decanted, and final volume measured. 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 CO_2 exchange rate measurements. NCER of whole blueberry plant canopies within each cultivar, CO_2 treatment, and across flower bud densities was determined at the beginning of fruit ripening. Whole-canopy NCER was measured using an open flow system with an infrared gas analyzer (IRGA) (AR600R; Anarad Inc., Santa Barbara, Calif.). 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 gas sampling bag (Tedlar; Fisher Scientific, Pittsburgh, Pa.) sealed at the base of the canes. *PPF*, provided by a 400-W metal halide lamp (Sylvania Lumalux Lu400; Danvers, Mass.), was 1750 to 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the top of the canopy. The chamber was located in a laboratory illuminated with fluorescent light. Carbon dioxide concentrations of incoming ambient air, pumped from outside the building to the chamber, were 373 ± 10 $\mu mol \cdot mol^{-1}$. Water vapor pressure was measured with a dewpoint 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 below the light source 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 removed from the gas entering and leaving the chamber, respectively, and dried by passing through magnesium perchlorate before entering the IRGA for analysis. Differential CO_2 concentrations were recorded after they stabilized for at least 15 min. The NCER was calculated as the difference in inlet vs. outlet CO_2 concentration (mol CO_2/mol air) × air flow (mol air/s.).

Statistical analysis. Data within each cultivar were analyzed in a completely randomized design. 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 (flower bud density, plant harvest date, and CO_2 treatment) 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 account for differences in plant size. SAS software (SAS Institute, Inc., Cary, N.C.) was used for statistical analyses. PROC GLM was used for analysis of variance and regression analysis and PROC CORR was used to test correlations.

Results

Carbohydrates. Root starch and whole plant carbohydrate concentrations at the end of dormancy were higher in plants exposed to ENR CO_2 conditions compared with plants exposed to AMB CO_2 conditions in both 'Sharpblue' (Fig. 1) and 'Misty' (Fig. 2), across all flower bud densities. Increased concentrations were still apparent in ENR compared with AMB 'Misty' at bloom (0 DAB). No additional differences in root or cane carbohydrate concentrations were observed between ENR and AMB plants from bloom (0 DAB)

to fruit ripening (82 DAB). With the exception of root sugars in 'Sharpblue', root and cane carbohydrate concentrations decreased steadily between dormancy and 28 DAB in AMB and ENR plants of both cultivars. Between 28 DAB and fruit ripening, cane and root starch concentrations in 'Sharpblue' increased significantly. However, in 'Misty', only cane carbohydrate concentrations increased before fruit ripening, while root carbohydrate concentrations continued to decrease and remained low.

In general, cane and root carbohydrate concentrations decreased as flower bud density increased, especially in AMB plants (Tables 1 and 2). In AMB 'Misty' plants, cane and root carbohydrate concentrations at a given phenological stage (with the exception of dormancy) decreased 25% to 85% as flower bud density increased from 0.07 to 0.40 flower buds/cm cane length. Similar decreases in root starch concentration occurred in AMB 'Sharpblue' plants as flower bud density increased from 0.07 to 0.27, while root sugars and cane carbohydrate concentrations were less affected by flower bud density. Carbohydrate levels in ENR plants; however, were less influenced by flower bud density. In ENR 'Misty' plants, cane and root starch concentrations decreased as flower bud density increased at bloom, while in ENR 'Sharpblue' plants, carbohydrate concentrations at a given developmental stage were unaffected by flower bud density.

Plant growth and development. There was no visible shoot growth on any plants while in the CO_2 treatments. Cane and root DWs of AMB and ENR plants were similar within cultivars (Table 3) and were not affected by flower bud density. However, root and cane DWs changed during development. Root DW decreased between dormancy and bloom for both cultivars, then remained constant between bloom and fruit ripening. Cane DW decreased between dormancy and 28 DAB in both cultivars. Cane DW increased in AMB and ENR 'Sharpblue', but not in 'Misty' plants, at fruit ripening.

In 'Sharpblue', the amount of vegetative budbreak/cm cane length was greater in ENR compared with AMB plants (Table 3). However, in 'Misty', vegetative budbreak was similar between AMB and ENR plants. Vegetative budbreak decreased as flower bud density increased in AMB and ENR plants of both cultivars (data not presented), although flower bud density explained only ≈25% of the variability in vegetative budbreak. In both AMB and ENR 'Misty' plants, vegetative budbreak was reduced ≈90% at high (0.40) compared with low (0.07) flower bud density. Vegetative

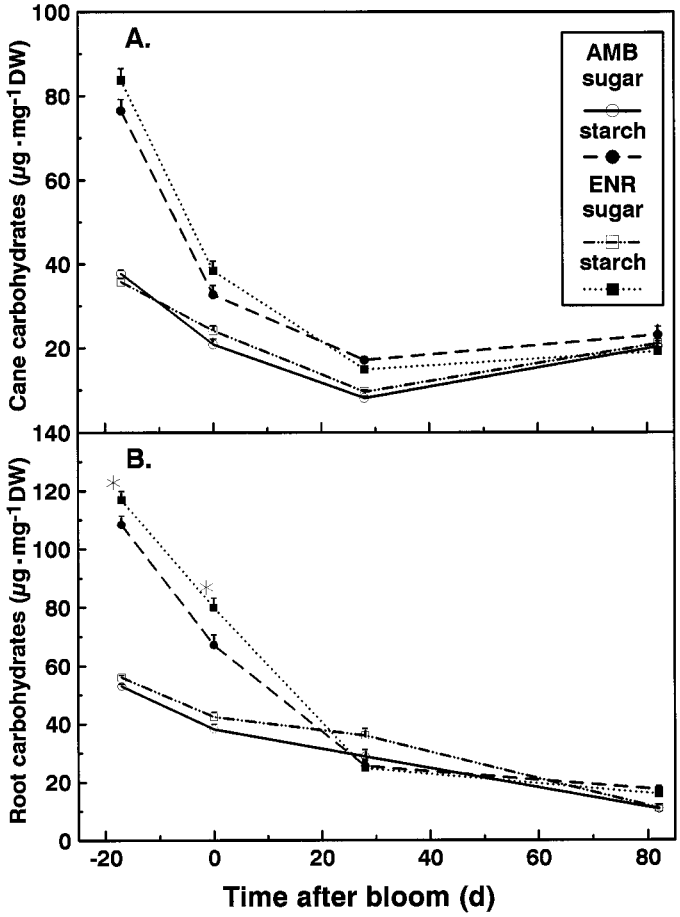


Fig. 1. (**A**) Cane and (**B**) root sugar and starch concentrations in AMB and ENR 'Sharpblue' southern highbush blueberry plants between dormancy (21 d before bloom) and fruit ripening [82 d after bloom (DAB)] (means ± SE, SE bars present only when larger than symbol). AMB = plants exposed to ambient (≈360 μmol·mol^{-1}) CO_2 concentrations for 5 weeks before defoliation in the winter. ENR = plants exposed to enriched (≈700 μmol·mol^{-1}) CO_2 concentrations for 5 weeks before defoliation in the winter. Means adjusted using flower bud density as a covariate. (Dormancy, n = 8; 0 DAB, n = 12; 28 DAB, n = 12; fruit ripening, n = 15). *Significant difference between root starch concentrations in AMB and ENR plants, $P < 0.05$.

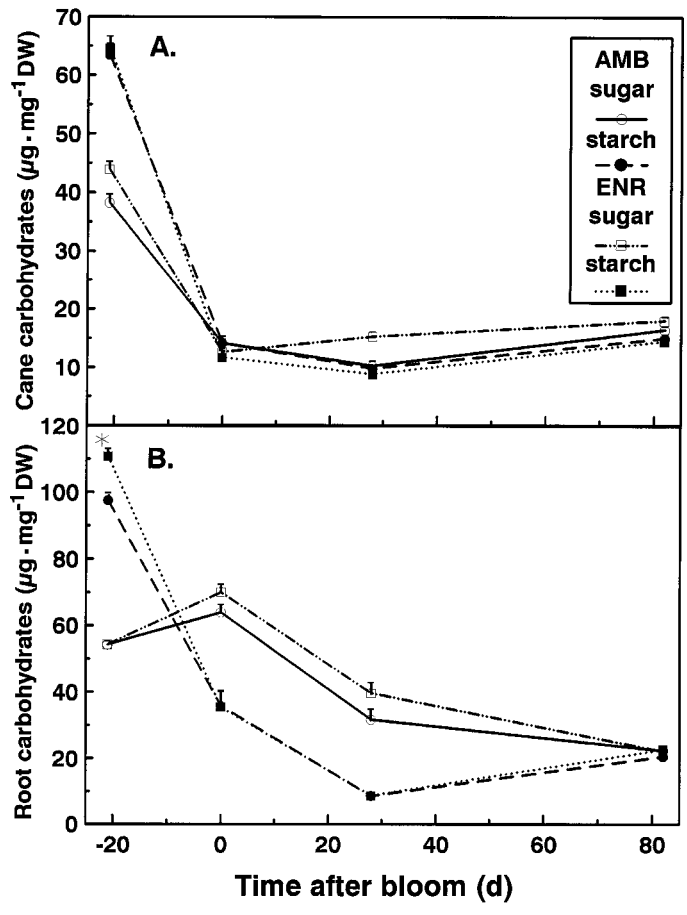


Fig. 2. (**A**) Cane and (**B**) root sugar and starch concentrations in AMB and ENR 'Misty' southern highbush blueberry plants between dormancy (17 d before bloom) and fruit ripening [82 d after bloom (DAB)] (means ± SE, SE bars present only when larger than symbol). AMB = plants exposed to ambient (≈360 μmol·mol^{-1}) CO_2 concentrations for 5 weeks before defoliation in the winter. ENR = plants exposed to enriched (≈700 μmol·mol^{-1}) CO_2 concentrations for 5 weeks before defoliation in the winter. Means adjusted using flower bud density as a covariate. (Dormancy, n = 8; 0 DAB, n = 12; 28 DAB, n = 12; fruit ripening, n = 8). *Significant difference between root starch concentrations in AMB and ENR plants, $P < 0.05$.

Table 1. Regression equations describing cane starch and root sugar and starch concentrations in 'Misty' southern highbush blueberry as related to flower bud density (FBD).

Phenological stage	AMB[z]	ENR
	Cane starch	
Dormancy	y = 76.4	y = 83.7
0 DAB (bloom)	y = 22.7 – 5.7 lnFBD – 15.3 $Date_1$ – 10.7 $Date_2$[y], R^2 = 0.74, P < 0.05	y = –3.3 – 23.6 lnFBD, r^2 = 0.42, P < 0.05
28 DAB	y = 22.7 – 5.7 lnFBD – 15.3 $Date_1$ – 10.7 $Date_2$, R^2 = 0.74, P < 0.05	y = 14.8
Fruit ripening	y = 22.7 – 5.7 lnFBD – 15.3 $Date_1$ – 10.7 $Date_2$, R^2 = 0.74, P < 0.05	y = 19.1
	Root sugar	
Dormancy	y = 53.0	y = 56.0
0 DAB (bloom)	y = 25.8 – 7.0 lnFBD – 8.9 $Date_1$ – 28.8 $Date_2$[y], R^2 = 0.78, P < 0.05	y = 42.2
28 DAB	y = 25.8 – 7.0 lnFBD – 8.9 $Date_1$ – 28.8 $Date_2$, R^2 = 0.78, P < 0.05	y = 36.0
Fruit ripening	y = 25.8 – 7.0 lnFBD – 8.9 $Date_1$ – 28.8 $Date_2$, R^2 = 0.78, P < 0.05	y = 10.9
	Root starch	
Dormancy	y = 108.4	y = 116.9
0 DAB (bloom)	y = 44.3 – 13.2 lnFBD – 41.2 $Date_1$ – 52.5 $Date_2$[y], R^2 = 0.91, P < 0.01	y = 7.5 – 41.1 lnFBD, r^2 = 0.56, P < 0.01
28 DAB	y = 44.3 – 13.2 lnFBD – 41.2 $Date_1$ – 52.5 $Date_2$, R^2 = 0.91, P < 0.01	y = 24.9
Fruit ripening	y = 44.3 – 13.2 lnFBD – 41.2 $Date_1$ – 52.5 $Date_2$, R^2 = 0.91, P < 0.01	y = 17.9

[z]AMB = ambient CO_2 concentrations (360 μmol·mol^{-1}); ENR = enriched CO_2 concentrations (700 μmol·mol^{-1}).
[y]Where $Date_1$ = 1 for 28 d after bloom (DAB) and = 0 otherwise and $Date_2$ = 1 for fruit ripening and = 0 otherwise.

Table 2. Regression equations describing cane starch and root sugar and starch concentrations in 'Sharpblue' southern highbush blueberry as related to flower bud density (FBD).

Phenological stage	AMB[z]	ENR
	Cane starch	
Dormancy	y = 63.5	y = 64.7
0 DAB (bloom)	y = –7.6 – 11.4 lnFBD, r^2 = 0.43, P < 0.05	y = 11.7
28 DAB	y = 9.8	y = 8.9
Fruit ripening	y = 3.9 – 5.9 lnFBD, r^2 = 0.30, P < 0.05	y = 14.5
	Root sugar	
Dormancy	y = 54.2	y = 54.3
0 DAB (bloom)	y = 15.3 – 25.6 lnFBD, r^2 = 0.53, P < 0.01	y = 70.0
28 DAB	y = 31.5	y = 39.7
Fruit ripening	y = 22.4	y = 22.1
	Root starch	
Dormancy	y = 97.5	y = 110.8
0 DAB (bloom)	y = 12.5 – 12.3 lnFBD + 2.5 $Date_1$ – 15.2 $Date_2$[y], R^2 = 0.57, P < 0.05	y = 35.5
28 DAB	y = 12.5 – 12.3 lnFBD + 2.5 $Date_1$ – 15.2 $Date_2$, R^2 = 0.57, P < 0.05	y = 8.7
Fruit ripening	y = 12.5 – 12.3 lnFBD + 2.5 $Date_1$ – 15.2 $Date_2$, R^2 = 0.57, P < 0.05	y = 22.7

[z]AMB = ambient CO_2 concentrations (360 μmol·mol^{-1}); ENR = enriched CO_2 concentrations (700 μmol·mol^{-1}).
[y]Where $Date_1$ = 1 for 28 d after bloom (DAB) and = 0 otherwise and $Date_2$ = 1 for fruit ripening and = 0 otherwise.

budbreak in AMB and ENR 'Sharpblue' was reduced ≈60% at high (0.27) compared with low (0.07) flower bud density.

Leaf areas were greater in ENR compared with AMB plants of 'Sharpblue', but not 'Misty' (Fig. 3). Leaf DW and new stem DW were also greater in ENR compared to AMB 'Sharpblue' plants but since leaf area, leaf DW, and stem DW were always highly correlated (r^2 >0.97), only leaf area is presented. As flower bud density increased, leaf area decreased in both cultivars, and the pattern of leaf area decrease was similar between AMB and ENR plants. The increase in leaf area in ENR compared with AMB 'Sharpblue' resulted in an increase in whole-canopy NCER in ENR 'Sharpblue' plants at fruit ripening (Fig. 4). As with leaf area, whole-canopy NCER decreased in a similar way in both AMB and ENR plants as flower bud density increased. Although leaf area of 'Misty' decreased as flower bud density increased, there was no concomitant decrease in whole canopy NCER in either ENR or AMB plants.

The timing of bloom was similar between AMB and ENR plants of both cultivars and was not affected by flower bud density in 'Sharpblue'. However, bloom was delayed ≈5 d as flower bud density increased in 'Misty' (data not presented). Fruit density (fruit/cm cane length) and total fruit number at ripening were similar between AMB and ENR plants, thus, leaf area to fruit and NCER to fruit ratios were lower in AMB 'Sharpblue' compared with ENR 'Sharpblue' plants, while there were no differences in leaf area to fruit or NCER to fruit ratios between AMB and ENR 'Misty'. Fruit density increased as flower bud density increased in 'Misty' (y = 2.84 + 0.88ln flower bud density, r^2 = 0.45, P < 0.001) and 'Sharpblue' (y = 2.95 + 0.88ln flower bud density, r^2 = 0.45, P < 0.001), thus leaf area to fruit and NCER to fruit ratios decreased as flower bud density increased in both cultivars. Since floral budbreak and fruit set overlapped with each other and fruit density was not adjusted in this study, it was not possible to separate the effects of flower bud density from fruit density and some of the effects of flower bud density are probably related to fruit density.

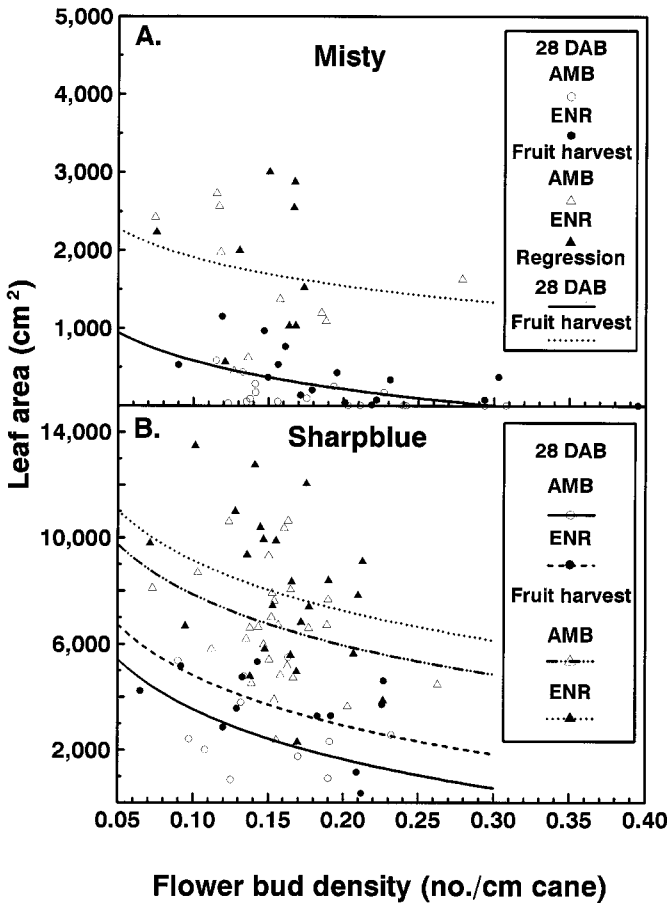
Average fruit FWs were greater in ENR plants compared to AMB plants in 'Sharpblue', but fruit FWs were similar between

Table 3. Reproductive and vegetative development of 'Misty' and 'Sharpblue' southern highbush blueberry as affected by CO_2 treatment or phenological stage.

Organ	Misty				Sharpblue			
and stage	AMB[z]	(n)	ENR	(n)	AMB	(n)	ENR	(n)
Root dry wt (g)								
Dormancy	23.5[y] a[x]A[w]	(8)	26.9 aA	(8)	52.0 aA	(8)	57.3 aA	(8)
0 DAB	20.3 aB	(12)	26.1 aB	(12)	44.2 aB	(12)	46.9 aB	(12)
28 DAB	19.3 aB	(13)	20.3 aB	(12)	45.2 aB	(12)	42.9 aB	(12)
Fruit ripening	16.6 aB	(8)	19.6 aB	(9)	45.2 aB	(17)	48.9 aB	(15)
Cane dry wt (g)								
Dormancy	50.7 aA	(8)	51.6 aA	(8)	71.0 aA	(8)	76.7 aA	(8)
0 DAB	45.3 aA	(12)	54.6 aA	(12)	63.8 aAB	(12)	67.9 aAB	(12)
28 DAB	42.1 aB	(17)	45.0 bB	(16)	65.9 aB	(12)	59.5 bB	(12)
Fruit ripening	41.2 aB	(10)	45.6 aB	(9)	73.7 aA	(28)	72.2 aA	(24)
Vegetative budbreak density (no./cm cane)								
0 DAB	0.019 a	(51)	0.025 a	(47)	0.041 b	(54)	0.067 a	(49)
14 DAB	0.022 a	(34)	0.030 a	(33)	0.069 b	(41)	0.088 a	(37)
28 DAB	0.024 a	(34)	0.031 a	(33)	0.081 b	(41)	0.104 a	(37)
Fruit FW (g)	1.13 a	(8)	1.03 a	(9)	0.90 b	(28)	1.02 a	(24)
Fruit developmental period (d)	86.5 a	(8)	89.3 a	(9)	81.5 a	(28)	78.6 a	(24)

[z]AMB = ambient CO_2 concentrations (360 μmol·mol^{-1}). ENR = enriched CO_2 concentrations (360 μmol·mol^{-1}).
[y]Values are means adjusted using flower bud density as a covariate within each cultivar.
[x]Lower case letters indicate mean separation between AMB and ENR plants within cultivars and whole plant harvest date by LSD, $P = 0.05$.
[w]Upper case letters indicate mean separation among whole plant harvest dates within columns and plant parts by LSD, $P = 0.05$.

ENR and AMB 'Misty' plants (Table 3). Fruit DWs increased as leaf area to fruit or whole-canopy NCER to fruit ratios increased in both cultivars (Fig. 5). The fruit development period was similar between AMB and ENR plants in both 'Misty' and 'Sharpblue' (Table 3) and the rate of fruit development decreased as leaf area to fruit ratio and whole-canopy NCER to fruit ratio increased ('Sharpblue': y = 24.1 – 9.8 ln NCER:fruit, $r^2 = 0.78$, $P < 0.01$; 'Misty': y = 38.0 – 7.6 ln NCER:fruit, $r^2 = 0.54$, $P < 0.01$).



Discussion

In both AMB and ENR plants of 'Sharpblue', root starch concentrations decreased ≈65% between dormancy and 0 DAB, indicating a strong mobilization of starch reserves into a readily translocatable form before vegetative and floral budbreak. This sharp decline in starch concentration between dormancy and bloom is similar to that found in 'Bonita' blueberry, a rabbiteye (*Vaccinium ashei* Reade) cultivar with a budbreak pattern similar to that of 'Sharpblue', i.e., simultaneous floral and vegetative budbreak (Darnell and Birkhold, 1996). In AMB and ENR 'Misty' plants; however, root starch concentrations decreased only ≈35% between dormancy and 0 DAB, a situation analogous to that observed in 'Climax' rabbiteye blueberry (Darnell and Birkhold, 1996), a cultivar that has a similar budbreak phenology as 'Misty'. Darnell and Birkhold (1996) suggested that the increased rate of starch depletion in 'Bonita' compared with 'Climax' during the period leading up to bloom resulted in an increased rate of vegetative development, which, in turn, increased the supply of current leaf carbohydrate to fruit development. The authors suggested that both the increase in the rate of reserve carbohydrate depletion between dormancy and 0 DAB and the increased supply of current carbohy-

Fig. 3. Relationship between leaf area and flower bud density (FBD) in AMB and ENR (**A**) 'Misty' and (**B**) 'Sharpblue' southern highbush blueberry plants. 'Misty': AMB and ENR (y = –630 – 526 ln FBD + 1329 Date, $R^2 = 0.66$, $P < 0.05$, where Date = 0 for 28 d after bloom (DAB) and Date = 1 for fruit ripening, n = 9. 'Sharpblue': AMB (y = –5287 – 2729 ln FBD + 4.96 length + 4312 Date, $R^2 = 0.56$, $P < 0.01$, where Date = 0 for 28 DAB and Date = 1 for fruit ripening, n = 12; ENR (y = –4001 – 2729 ln FBD + 4.96 length + 4313 Date, $R^2 = 0.61$, $P < 0.01$, where Date = 0 for 28 DAB and Date = 1 for fruit ripening, n = 12. AMB = plants exposed to ambient (≈360 μmol·mol^{-1}) CO_2 concentrations for 5 weeks before defoliation in the winter. ENR = plants exposed to enriched (≈700 μmol·mol^{-1}) CO_2 concentrations for 5 weeks before defoliation in the winter.

drate contributed to increased fruit yield in 'Bonita'. A similar situation may exist for the two southern highbush blueberry cultivars used in the present study, where the increased rate of starch depletion before bloom in 'Sharpblue' was associated with increased vegetative budbreak, leaf area development, and overall fruit yield (350 vs. 245 g FW/plant for 'Sharpblue' and 'Misty', respectively) compared with 'Misty'.

The increased rate and amount of leaf development in 'Sharpblue' relative to 'Misty' resulted in an increased source supply and likely contributed to the ability of 'Sharpblue' to replenish root carbohydrate reserves before fruit ripening. Previous studies with rabbiteye blueberry indicate that blueberry leaves become net exporters within 7 to 10 d of budbreak (Birkhold and Darnell, unpublished data). This carbohydrate reserve replenishment was not seen in 'Misty', where root carbohydrate concentrations continued to decrease throughout fruit development. Apparently, 'Misty' was unable to supply sufficient carbohydrate for both fruit development and replenishment of root reserves. This is similar to the situation in sweet cherry (Keller and Loescher, 1989), and probably reflects the high demand for carbohydrates elicited by simultaneous fruit and shoot growth, the short fruit development period, and the high harvest index in blueberry (Darnell and Birkhold, 1996). This would be especially true for cultivars such as 'Misty', in which vegetative budbreak and growth are delayed relative to floral budbreak.

Increased carbohydrate reserves in ENR 'Sharpblue' plants were associated with increased vegetative budbreak, leaf area development, and whole-canopy NCER compared to 'Sharpblue' plants with lower reserves (AMB). These results are similar to those found in sweet cherry, in which early fall defoliation reduced reserve carbohydrate concentrations and resulted in smaller leaves and less overall growth the following spring compared to control trees allowed to defoliate naturally (Loescher et al., 1990). Similarly, in apple, new shoot length was greater following a nonbearing year, which allowed carbohydrate levels to increase, than following a bearing year, which depleted carbohydrate levels (Wilcox, 1937). However, increased carbohydrate reserves did not lead to increased vegetative growth in 'Misty'. Thus, although practices that can elevate carbohydrate reserve levels may enhance canopy development the following year in some species or cultivars, this is not universally true.

The extent of reserve carbohydrate depletion in roots and shoots was correlated with flower bud density in AMB plants of both cultivars, and the correlation was much stronger in 'Misty' than in 'Sharpblue'. In ENR 'Sharpblue' plants, however, the correlation between high flower bud density and the extent of starch depletion was eliminated at all phenological stages. In contrast, the extent of

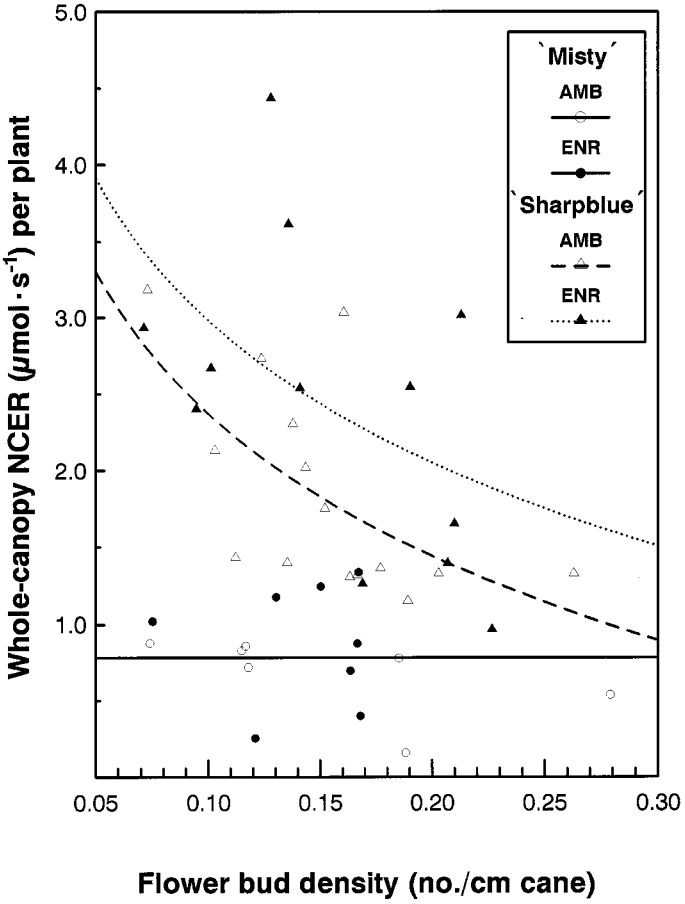


Fig. 4. Relationship between whole-canopy net CO_2 exchange rate (NCER) at fruit ripening and flower bud density (FBD) in AMB and ENR 'Misty' and 'Sharpblue' southern highbush blueberry plants. 'Misty': y = 0.78, n = 7. 'Sharpblue': (y = –2.60 – 1.34 ln FBD + 0.004 length + 0.61 CO_2 treatment, $R^2 = 0.44$, $P < 0.01$, where CO_2 treatment = 0 for AMB and CO_2 treatment = 1 for ENR, n = 15. AMB = plants exposed to ambient (≈360 μmol·mol^{-1}) CO_2 concentrations for 5 weeks before defoliation in the winter. ENR = plants exposed to enriched (≈700 μmol·mol^{-1}) CO_2 concentrations for 5 weeks before defoliation in the winter.

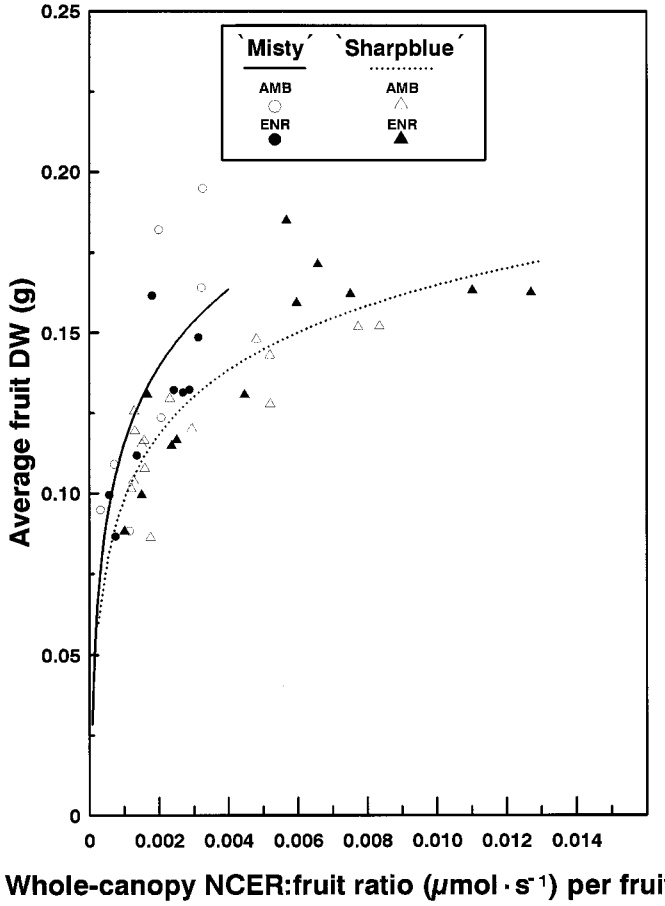


Fig. 5. Relationship between fruit DW and whole-canopy net CO_2 exchange rate (NCER) to fruit ratio in AMB and ENR 'Misty' and 'Sharpblue' southern highbush blueberry plants. 'Misty': AMB and ENR (y = 0.36 + 0.04 ln NCER:fruit, n = 7, $r^2 = 0.55$, $P < 0.002$); 'Sharpblue': AMB and ENR (y = 0.30 + 0.03 ln NCER:fruit, n = 12, $r^2 = 0.73$, $P < 0.001$). AMB = plants exposed to ambient (≈360 μmol·mol^{-1}) CO_2 concentrations for 5 weeks before defoliation in the winter. ENR = plants exposed to enriched (≈700 μmol·mol^{-1}) CO_2 concentrations for 5 weeks before defoliation in the winter.

cane and root starch depletion in ENR 'Misty' at 0 DAB were still significantly correlated with flower bud density. Thus, ENR 'Sharpblue' plants were able to use the higher concentrations of carbohydrate reserves at dormancy to increase vegetative budbreak, leaf development, and whole-canopy NCER, thus overcoming some of the competition from high flower bud density, and resulting in increased fruit size in ENR compared with AMB plants. However, ENR 'Misty' plants were not able to take equal advantage of their increased carbohydrate reserves during the critical period leading up to bloom. Since the majority of cell division in blueberry fruit is completed before anthesis (Cano-Medrano and Darnell, 1997) and cell number is a primary determinant of final fruit size in many crops (Cheng and Breen, 1992; Goffinet et al., 1995; Scorza et al., 1991), sufficient source availability before bloom is critical for blueberry fruit development. Although ENR 'Misty' plants had higher levels of root starch reserves compared with AMB plants, it was not sufficient to completely overcome the competition effects from high flower bud density at bloom. This may be a function of the inability to completely access these reserves, as evidenced by the slower rate of reserve depletion by 'Misty' compared with 'Sharpblue', or a function of insufficient reserves. If the latter were the case, additional increases in carbohydrate reserves may alleviate the competition from high flower bud density. Alternatively, there may be some other factor limiting canopy development in 'Misty', other than carbohydrate reserves.

Since the rate of carbohydrate reserve depletion was not affected by flower bud density in ENR 'Sharpblue' plants and vegetative budbreak and fruit FW were greater in ENR compared to AMB 'Sharpblue' plants, this would support the hypothesis that increased carbohydrate reserve levels may partially compensate for the competition from early flower and fruit development in some southern highbush blueberry cultivars, i.e., 'Sharpblue'. However, increased carbohydrate reserves were unable to completely overcome the effects of competition between reproductive and vegetative development since vegetative budbreak and leaf area decreased as flower bud density increased, even in ENR 'Sharpblue' plants. Thus, although increased carbohydrate reserves in ENR 'Sharpblue' plants eliminated the correlation between flower bud density and extent of starch depletion and resulted in increased fruit size, the correlation between flower bud density and vegetative growth was not eliminated. Greater increases in carbohydrate reserves than were obtained in the present study may further alleviate the correlation between flower bud density and vegetative growth, resulting in additional increases in fruit size. Alternatively, the correlation between flower bud density and vegetative growth may be an apical dominance phenomenon, thus substances other than carbohydrates (e.g., hormones) may play a role. Nonetheless, the benefit of increased reserve carbohydrate concentration appears to be cultivar-specific, since vegetative growth and fruit FW were similar in ENR and AMB 'Misty'.

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