

Seasonal growth dynamics and carbon allocation of the wild blueberry plant (*Vaccinium angustifolium* Ait.)

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Kaur, J., Percival, D., Hainstock, L. J. and Privé, J.-P. 2012. Seasonal growth dynamics and carbon allocation of the wild blueberry plant (*Vaccinium angustifolium* Ait.). Can. J. Plant Sci. 92: 1145–1154. Field studies were conducted at the Wild Blueberry Research Station, Debert, NS, to examine the carbon allocation dynamics within the wild blueberry (*Vaccinium angustifolium* Ait.). This was achieved with biweekly measurements of dry weight, soluble sugar and starch levels of the rhizomes, roots, stems/leaves and berries of plants in the vegetative (i.e., sprout phase) and cropping phases of production. Non-structural carbohydrate levels were determined using high-performance liquid chromatography (HPLC). Growth parameters included phenology, stem height, dry weights of the above-ground vegetation (stems and leaves), berries, rhizomes and roots. Interestingly, root growth was observed prior to upright shoot emergence and dry weight for rhizome remained higher compared with stems and leaves. The rhizomes acted as a carbohydrate source during stem and root growth. The developing berry crop appeared to be a strong sink for photo-assimilates, as berries were found to import sucrose and convert it to fructose and glucose during maturation, and HPLC studies further confirmed the increasing levels of fructose and glucose. Given the phenology of the wild blueberry, the results exemplify the importance of the rhizomes as a strong carbohydrate source, especially in the early stages of a growing season when the carbohydrate production is limited.

Key words: Vegetative/sprout phase of production, cropping phase of production, high-performance liquid chromatography, phenology

Kaur, J., Percival, D., Hainstock, L. J. et Privé, J.-P. 2012. Dynamique de la croissance saisonnière et répartition du carbone chez le bleuet sauvage (*Vaccinium angustifolium* Ait.). Can. J. Plant Sci. 92: 1145–1154. Des études entreprises sur le terrain à la station de recherche sur le bleuet sauvage de Debert devaient préciser la dynamique de la répartition du carbone chez le bleuet sauvage (*Vaccinium angustifolium* Ait.). Pour cela, on a mesuré le poids sec, la concentration de sucres hydrosolubles et la teneur en amidon des rhizomes, des racines, des tiges/feuilles et des fruits toutes les deux semaines pendant les phases culturales correspondant à la phase végétative (à savoir, germination) et à la phase de croissance. La concentration d'hydrates de carbone non structurels a été déterminée par chromatographie en phase liquide à haute pression (HPLC). Parmi les paramètres de croissance figuraient la phénologie, la hauteur de la tige ainsi que le poids sec des organes aériens (feuilles et tiges), des baies, des rhizomes et des racines. Fait intéressant, on a observé la croissance des racines avant la levée verticale des pousses et un poids sec constamment plus élevé dans les rhizomes que dans les tiges et les feuilles. Les rhizomes servent de source d'hydrates de carbone pendant la croissance des tiges et des racines. Les baies en développement semblent constituer un important puits de photo-assimilats, car elles importent du sucrose pour le transformer en fructose et en glucose durant la maturation. Les études avec la HPLC ont confirmé cette augmentation de la teneur en fructose et en glucose. Compte tenu de la phénologie du bleuet nain, ces résultats illustrent bien l'importance des rhizomes en tant qu'abondante source d'hydrates de carbone, surtout au début de la saison de croissance quand la production de glucides est limitée.

Mots clés: Phase végétative/germinative de la production, phase de croissance de la production, HPLC, phénologie

The wild blueberry (*Vaccinium angustifolium* Ait.) is a vital crop to the Canadian Maritime provinces, Quebec and Maine, with berry production and farm gate values presently exceeding 110 million kg yr⁻¹ and \$150 million, respectively. The demand for wild blueberries has increased in recent years due to the international attention being drawn to the desirable taste and extensive health benefits of eating the fruit, which is very high in antioxidants that play roles in maintaining eye health, combating degenerative eye diseases, and memory loss (McCord 1999). Individual farm acreage varies greatly, but land devoted to wild blueberry production

totals in excess of 44 000 ha in Maine, Quebec, and the Maritime provinces of Canada (Yarborough 2007).

The majority of wild blueberry plants are commercially produced on a 2-yr production cycle and all plant tissue above ground is burnt and/or mowed down to ground level following harvest or the next spring to stimulate new growth and resume the production cycle (Kinsman 1993; Jordan and Eaton 1995). The perennial growth habit of the wild blueberry plant (Kender and

Abbreviations: CPP, cropping phase; HPLC, high performance liquid chromatography; SPP, sprout phase

Egger 1966), the high degree of inherent variability from a natural ecosystem (Hepler and Yarbrough 1991), and the fact that over 80% of the plant is below ground (Kinsman 1993) offer challenges in exploring successional plant growth dynamics. Rhizomatous tissue can be an important source of stored carbohydrates (Loescher et al. 1990) and may also be important for carbon sequestration in the soil (Xiong and Kätterer 2010). In the sprout phase of wild blueberry production, stored reserve carbohydrates in below-ground tissue are necessary for development of upright shoots due to the removal of all above-ground tissue (Townsend et al. 1968). As different parts of the plant become more active or dormant, the need for carbohydrates will also change, which has an effect on the strength of the sink in terms of total carbon sequestered (Fernandez and Pritts 1994; Privé et al. 1994; Dennis et al. 1997). Ultimately, an examination of plant growth and source-sink dynamics allows for more effective crop management (Hall et al. 1971; Fernandez and Pritts 1994).

Changes in the seasonal allocation and composition of carbohydrates also occur within the wild blueberry tissues. Results from a greenhouse study indicate that the leaves normally had higher concentrations of sucrose and reducing sugars compared with rhizomes, which were higher in starch (Townsend et al. 1968). In the rhizomes, there was a general decrease in starch and sucrose with an increase in reducing sugars until June (Townsend et al. 1968). Reducing sugars continuously increased in the leaves from July to September. Although past studies have focused on greenhouse trials using selected clonal material to examine the carbohydrates in stems and rhizomes of fruiting (Townsend et al. 1968) and recent studies have explored the impact of drought stress and supplemental irrigation on growth and development of the wild blueberry under field conditions (Glass 2000), there is limited understanding of the seasonal and production phase changes in: (1) dry weight, (2) vegetative and reproductive yield components, (3) relative levels of soluble sugars and starch, and (4) the specific soluble sugars of stems, berries, roots, and rhizomes for the wild blueberry plant.

MATERIALS AND METHODS

Site Location

Trial plots were established for the 1999 and 2000 growing seasons in two adjoining wild blueberry fields in the sprout phase (SPP) and cropping phase (CPP) at the Wild Blueberry Research Centre (WBRC) in Debert, Nova Scotia (lat. 45°27'N, long. 62°36'W). Having both phases of production available within a growing season allowed each year to be used as a blocking factor. The WBRC controlled the management of the fields, in terms of mowing, burning, harvesting, and agrochemical applications.

Treatments and Experimental Design

A completely randomized experimental design was used with the SPP and CPP of the production cycle considered as the two treatment levels. There were four plots, each measuring 8 m × 9 m, which were used for each phase of production. The plots formed a long narrow band across the field to ensure sampling occurred across the area. Year of the study was used as a blocking factor to account for variability in growing conditions that occurred between 1999 and 2000.

Weather Data

For the 1999 and 2000 growing season, a LI-1000 and a CR10X datalogger (LI-COR Inc., Lincoln, NE) were attached to an atmospheric weather station respectively to record an hourly average of incident solar radiation (200X pyranometer, Campbell Scientific Corp., Logan, UT), wind speed (014A anemometer, LI-COR Inc.), rainfall (CS700 tipping bucket, Campbell Scientific Corp.), air temperature (CS500 thermistor, Campbell Scientific Corp.), and relative humidity (CS500 thermistor, Campbell Scientific Corp.).

Dry Matter Partitioning

Three whole plant samples per plot for each phase of production were randomly collected at 2-wk intervals from May to August, and then increasing intervals from September to November. Metal forms measuring 16.5 × 16.5 × 16.5 cm (l × w × h) were used to collect the samples and were randomly placed within each plot. Within the form, stems were first cut at ground level and placed into paper bags. With stems in the cropping phase of production, berries were separated from the stem and leaf tissue and placed into paper bags. Extraction of the roots and rhizomes occurred by driving the metal form into the ground and removing the entire core. After extracting the whole plant sample, the soil with rhizomes and roots was placed into plastic bags and transported back to the Nova Scotia Agricultural College. Both 1999 and 2000 plant samples were treated similarly. All plant samples, including above-ground tissues, were randomly placed in two drying ovens (Boekel Industries Inc. Model 107801, Philadelphia, PA, Fisher Isotemp Oven Model 230G, Nepean, ON) at temperatures of 100°C until core temperatures reached 70°C (Ojima and Isawa 1967) to denature the invertase enzymes and prevent the degradation of sucrose (Bonvehí et al. 2000).

The above-ground plant tissues were placed in a Precision Scientific Inc. mechanical convection oven (Chicago, IL) for at least 2 wk at 65°C and dry weights obtained on a DeltaRange Mettler PM4600 digital scale (Mettler Instrument AG, Zürich, Switzerland). The total number of stems per sample area was also recorded. In the cropping field, berries were separated and included in the study.

The below-ground plant tissues were spread out in the greenhouse to air dry for 5 d. The air-dried below-ground

tissue was then shifted from the soil, washed, and separated into roots and rhizomes. The roots and rhizomes were then re-dried in paper bags at 65°C for at least 2 wk in a Precision Scientific Inc. mechanical convection oven (Chicago, IL) (until constant weights were achieved) and dry weights obtained on a digital scale.

Phenol-Sulphuric Acid Determination of Carbohydrates

The whole plant samples from the growth rate experiment were finely ground using a Wiley Model no. 3 mill (Arthur H. Thomas Co., Philadelphia, PA) with a 1-mm sieve. The replicates from each plot were combined to create four samples for each production phase per date. For each samples, 0.25 g of stem tissue and 0.5 g of root and rhizome tissues, were used for analysis. Soluble sugars were assayed using the phenol-sulphuric acid method (Maust et al. 2000). This method consisted of initially submersing the samples in 8 mL of 80% ethanol (vol/vol) in centrifuge tubes and heated to 55.0°C for 1 h. The samples were then centrifuged and washed twice with 5 mL of 80% ethanol (vol/vol). The pellet was used for starch analysis, while the supernatants were combined for soluble sugar analysis.

For the soluble sugars analysis, 0.06 g of activated charcoal was added to supernatant then filtered with Whatman no. 2 filter paper. From the filtrate, 0.5 mL was pipette into a 13 × 100-mm disposable borosilicate glass culture tube (Fisher Scientific, Pittsburg, PA). One millilitre of 5% phenol (vol/vol) followed by 5 mL of concentrated sulphuric acid was added to the culture tube and vortexed. The solution was allowed to react for 15 min before reading, but could remain stable for 1 h (Buysse and Merckx 1993). Solutions of sucrose (99+ % ACS reagent-Sigma-Aldrich Canada) were used as standards. Solutions were measured using a Beckman DU-70 spectrophotometer (Fullerton, CA) at a wavelength of 490 nm. Any readings above 1 were diluted and re-measured accordingly.

For starch analysis, the starch was hydrolysed into glucose according to methods of Maust et al. (2000) with slight modifications. The pellet was re-suspended in 2 mL of 0.20 M KOH and boiled on a hotplate for 30 min. After removing from the heat, pH was adjusted to 4.5 with 1 mL of *Rhizopus* acetate buffer (pH 5) was added and incubated in a 37°C TBVS Hetromix shaking water bath with an AT 110 water heater (InterScience Inc., Markham, ON) for 24 h. The tubes were then centrifuged, decanted and washed twice with 2 mL of distilled water. The supernatant was mixed with 0.06 g of activated charcoal and followed the same procedure as the soluble sugar analysis, except with glucose (99+ % ACS reagent, Sigma-Aldrich Canada) used for the standard solutions.

High-performance Liquid Chromatography Determination of Carbohydrates

Analysis of carbohydrates composition was completed on samples collected during the 2000 season with the use of high-performance liquid chromatography (HPLC). The three ground dry weight samples from within each plot were combined and thoroughly mixed for a total of four replicates per phase of production per sample date. From this mixture, 0.5-g samples were used for stems, berries, roots, and rhizomes. The tissue samples were suspended in 10 mL of 80% ethanol at 55°C for 1 h. The samples were centrifuged, decanted, and washed twice with 3 mL of deionized water. Starch content was extracted from the pellet following the same methods as outlined in the phenol-sulphuric acid method.

Supernatant was transferred into 50-mL beakers and placed on a hotplate at 55°C until all fluid was evaporated (approximately 20 to 30 min). The sugars were re-suspended in 6 mL of deionized water. C18 sep-pak filters (Waters Limited, Mississauga, ON) were prepared with 2 mL acetonitrile and 4 mL of deionized water before filtering samples. Samples were then filtered through a Millex-FG13 millipore filter (0.2 µm, Millipore Products division, Bedford, MA) attached to a 5 cc luer-lok sterile syringe (Becton Dickinson and Company, Rutherford, NJ) into 2-mL Varian glass vials and sealed with a polypropylene cap and PTFE/Silicone septa (Varian, Walnutcreek, CA). Samples were randomly placed into the Varian 9100 autosampler (serial #2560, Walnutcreek, CA). A 9010 solvent delivery system was set at a flow rate of 0.80 mL min⁻¹. The autosampler was set for 40 µL sample injections, and a Varian 9050 UV-VIS detector was set at 193 nm, and controlled by the Varian Star Integrater software with a run time of 20 min. In addition, a Zorbax NH2 P4 guard column (Chromatographic Specialties Inc., Brockville, ON) was used before the Zorbax carbohydrate analysis column (4.6 mm i.d. × 250 mm, Chromatographic Specialties Inc., Brockville, ON). The guard column was used to protect the analysis column.

Statistical Analysis

All parameters measured were analyzed as repeated measures throughout the growing season (i.e., Julian date) using the Proc Mixed procedure of SAS (Version 9, SAS Institute, Inc., Cary, NC). Normality and constant variance were evaluated for using the Proc Univariate procedure of SAS software. The least significant means (LSmeans) were generated for the cycle and date interactions and significance was determined at the 5% level (*P* value < 0.05). Significant differences were represented as alphabetical letters to signify LSmeans for graph of transformed data, whereas vertical error bars are used on graphs of data that did not require transformation.

Attempts to analyze the data using growing degree-days with a base temperature of 5°C were unsuccessful, resulting in the presentation of results on a Julian date basis. However, substantial variances in

phenological development were observed presenting results by Julian date.

RESULTS AND DISCUSSION

Dry Weight Partitioning

Growth Dynamics

There were significant effects of year, production phase, Julian date, and the interaction of production phase and Julian date (P values < 0.001) on wild blueberry stem length (Fig. 1). Peak SPP lengths (Julian dates 189 and 195, respectively) were 27% significantly higher in 1999 than in 2000 (Fig. 1). Cropping phase stem lengths were variable throughout the seasons (Fig. 1). This occurred at an earlier stage than proposed by Townsend et al. (1968) and is consistent with what has been observed in other fruit-bearing plants e.g., highbush blueberry (Maust et al. 2000) peach (DeJong 1999), cherry (Flore and Layne 1999), and apple (Lakso et al. 1999).

Rhizome Dry Weights

There was no significant difference in rhizome dry weights between years (P value = 0.381). However, within the growing seasons, a significant effect of Julian date (P value = 0.003) on rhizome dry weight (Fig. 2a–d) was observed. The rhizome dry weights in both the SPP and CPP had significant drops at the beginning of the seasons (Fig. 2a–d). On comparing rhizome dry weights from mid-May to mid-June, the combined (i.e., 1999 and 2000) SPP rhizome dry weights decreased 37% versus a decline of only 8% in CPP rhizome dry weights (Fig. 2a–d). However, by midsummer, all rhizome dry weight levels recovered to original levels and remained relatively constant until the end of the season (Fig. 2a–d). The 1999 rhizome dry weights were initially much higher than in 2000 (Fig. 2a–d), as the drought stress of the plants in 1999 could have resulted in a decrease in the amount of photoassimilates the plant was able to produce for storage for the 2000 growing season. Similar decreases

in rhizome non-structural carbohydrate levels were observed in asparagus (*Asparagus officinalis* L.) when grown in drought conditions (Ernst and Krug 1998). Continued respiration within the rhizomes might have been occurring as a result of the plant not yet entering dormancy by the final sampling date (Loescher et al. 1990). Reserve levels of carbohydrates within the wild blueberry rhizomes served as a carbohydrate supply in support of new shoot and root growth.

The changes in rhizome dry weights observed in this study illustrate the importance of the rhizomes as a carbohydrate reservoir. This feature enhances winter survival and also enables the early resumption of growth the following growing season. This is particularly important considering most above-ground plant tissue is pruned and removed following the CPP. Thus, the remobilization of carbohydrates into the rhizomes for storage during the winter is needed to ensure the long-term survival of the plant. The ability of the wild blueberry to develop an extensive root system is believed to be the reason for its observed drought tolerance (Hicklenton et al. 2000; Percival et al. 2003). The lower sink strength of the 1999 stems for carbohydrates could also partially explain the observed later increase in stem and leaf dry weights in 1999 compared with 2000 (Fig. 2a–d). The dry weights of the rhizomes in the SPP became more constant (Fig. 2a–d), as the need for reserve carbohydrates was reduced with the establishment of full development of the wild blueberry uprights, which resulted in the termination of vertical growth allowing for the lateral development of reproductive nodal primordial (Barker and Collins 1963; Hall and Ludwig 1961).

Root Dry Weights

There was a significant influence of year on root dry weight (P values < 0.001). Root dry weights were generally significantly higher in the first half of the 1999 growing season compared with 2000 within both phases

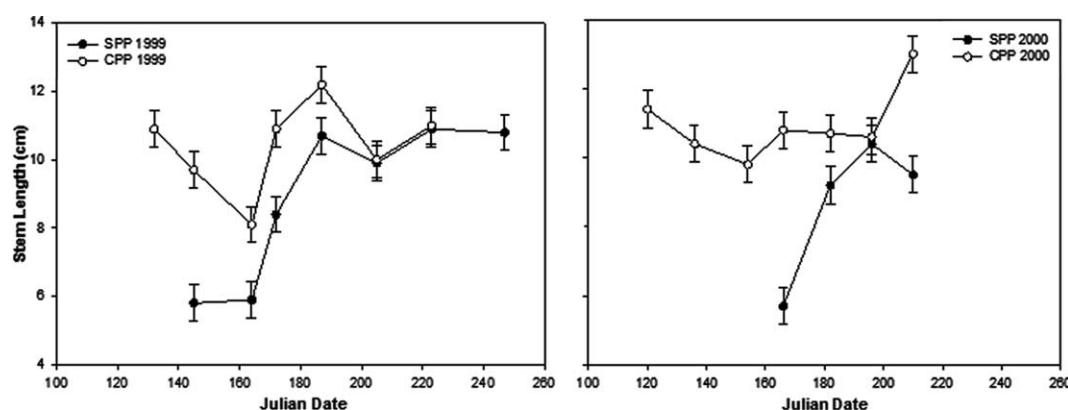


Fig. 1. Changes in vegetative and fruiting stem lengths for wild blueberry plants (vertical bars represent standard error at 5% significance).

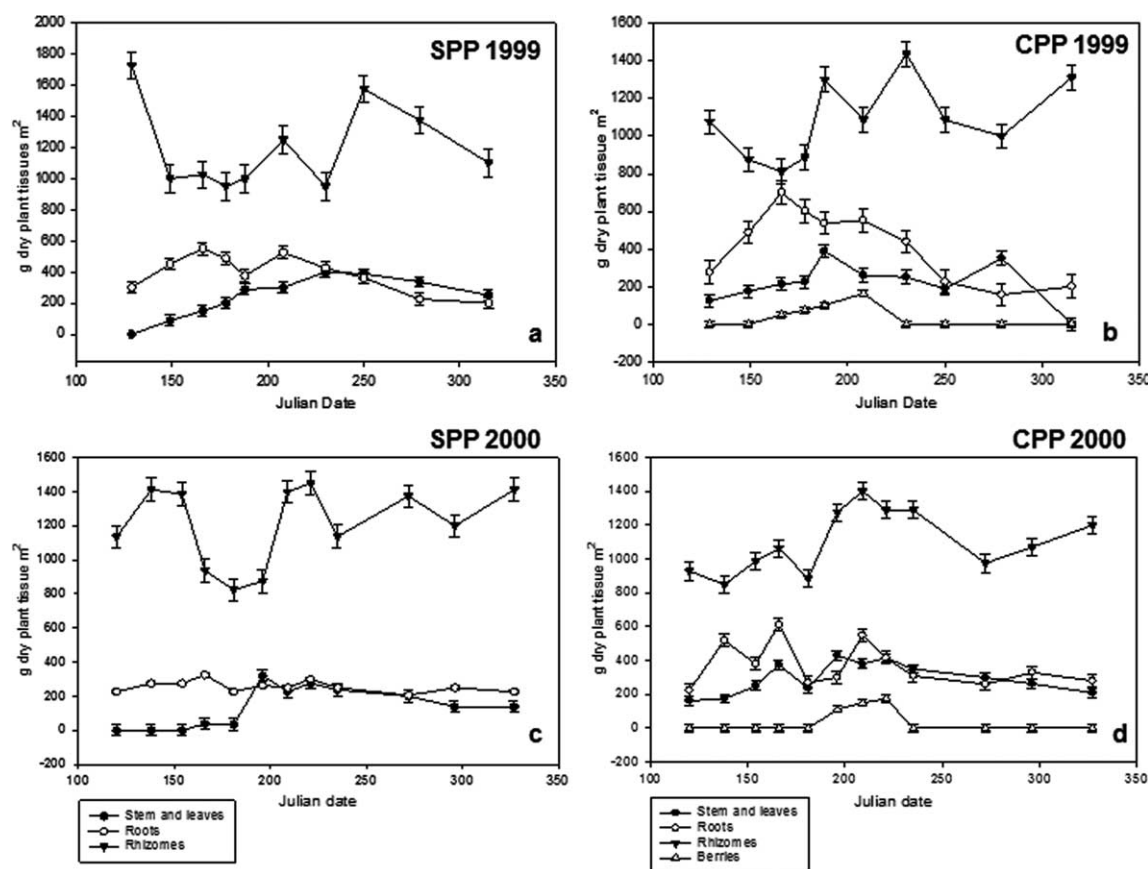


Fig. 2. (a–d) Dry weights of stems/leaves, roots, rhizomes and berries in vegetative and cropping phase of production during 1999 and 2000 plants (vertical bars represent standard error at 5% significance).

of production (Fig. 2a–d). The wild blueberry roots appeared to initiate growth early in the season when air temperatures were low and shoot growth had not yet commenced (Fig. 2a–d). This is in sharp contrast to other berry crops with raspberry primocane shoots being observed to emerge before apparent root growth and also at a stage when sufficient photoassimilates could be provided to the developing roots (Fernandez and Pritts 1994).

Upright Stem and Leaf Dry Weights

There were significant differences in upright stem and leaf dry weights between years (P value = 0.004) (Fig. 2a–d). Within each year, there was a significant effect of production phase, Julian date, and the interaction between the two factors (all P values < 0.001). The 1999 and 2000 SPP upright stem and leaf dry weights remained constant until the first week of October (Julian date 275 and 280, respectively), but declined significantly to levels similar to those observed at the beginning of the 1999 and 2000 CPP (Fig. 2a–d). The 1999 and 2000 CPP upright stem and leaf dry weight decreased significantly, 55 and 20%, within the same time period following harvest. However, the 1999 CPP upright stem and leaf

dry weights then increased similar by 53% on Oct. 06 (Julian date 280), whereas the 2000 levels continued to decrease significantly till the end of the season (Fig. 2a–d). The stems and roots of plants contain actively growing meristematic tips that require significant amounts of carbohydrates (Loescher et al. 1990; Fernandez and Pritts 1994; Dennis et al. 1997). Therefore, it appears that strong competition existed between these two areas of the plant for the limited carbohydrate quantities (Fernandez and Pritts 1994) and that might be the reason for dry weight fluctuations in two seasons.

The developing crop depended heavily on carbohydrate supplies throughout the season and, thus, greatly affected the overall plant source/sink dynamics in terms of carbohydrate allocation. As air temperature increased with the progression of spring, the floral buds further developed into reproductive structures and acted as strong sinks for resources, similar to other fruits crops (Fig. 2a–d) (Lockwood and Sparks 1978; Jiao et al. 1989; Privé et al. 1994). Accordingly, it is possible that the dip in the 1999 CPP rhizome dry weights and lag increased stem dry weights (Fig. 2a–d) were due to the input of reserve carbohydrates into growth and development of the floral structures lost through respiration

and petal drop (Darnell and Birkhold 1996; Eaton and Ormrod 1970; Davis and Sparks 1974). The same drop occurred at the end of June 2000 (Julian date 181) in rhizome, root, and stem dry weights (Fig. 2a–d), which might be due to reduced levels of reserve carbohydrates in the rhizomes from the drought stress in 1999 to create sources from other areas of the plant such as the roots to meet the demand of the developing inflorescences.

As the berries began to develop, the roots declined in dry weight and the balance of carbon allocation appeared to shift to the rest of the plant, with peak levels of stem and rhizome dry weights achieved in mid-July (Julian dates 189 and 195 for 1999 and 2000, respectively) (Fig. 2a–d). In late July, floral primordia development on the CPP stems was also initiated, while crop development continued (Hall and Ludwig 1961). The presence of the developing floral primordial and fruit meristematic tissue would create very strong cumulative sinks for photoassimilates being moved within the stems (Privé et al. 1994; Darnell and Birkhold 1996). A similar trade-off was found to exist between raspberry stems and roots (Fernandez and Pritts 1994) and highbush blueberry plants (Swain and Darnell 2001) as the strength of the sinks changed. However, the

spreading and long-term survival of wild blueberry plants is highly dependent on the growth of healthy rhizomes, thus, the rebuilding of the rhizomes would also have created a strong competitive sink with the stems for photoassimilates (Kender and Eggert 1966). In many woody plants, the restoration of reserve carbohydrate levels often occurred following their depletion with interruptions during the period of fruit ripening (Loescher et al. 1990). A slight, but non-significant decrease occurred in rhizome dry weights with the ripening of 1999 berries and 2000 berries, which might be similar to the interruption noted in other woody plants (Fig. 2a–d).

Phenol–Sulphuric Acid Colorimetric Determination of Carbohydrates

Rhizome Soluble Sugar and Starch Concentration

Rhizome soluble sugars increased an average of 57% for SPP and CPP from the end of May (Julian date 152) to mid-June (Julian date 166) (Fig. 3b and d). Despite no overall differences between production phases, only the rhizome soluble sugar levels of the SPP reached similar peak soluble sugar levels again at the end of July (Julian date 209) (Fig. 3b and d). Rhizomes in the CPP experienced minimal soluble sugar levels the first week

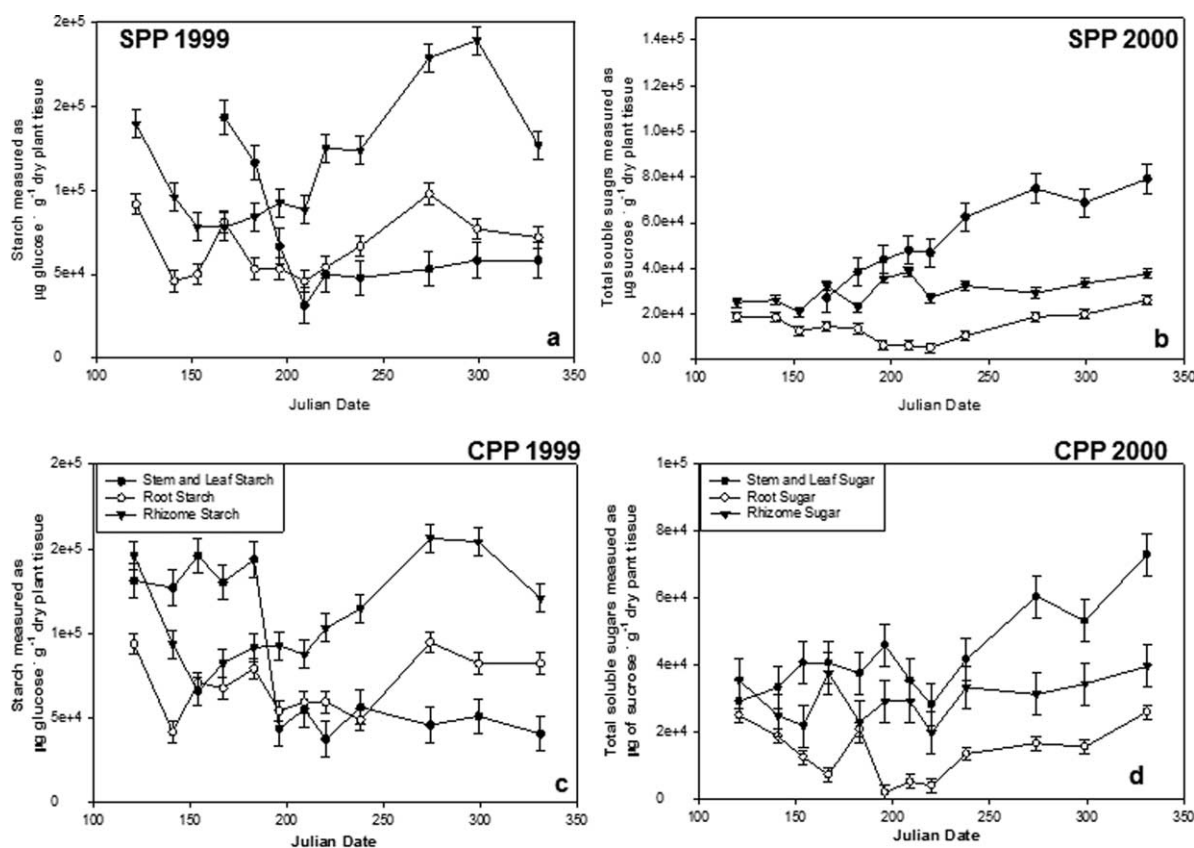


Fig. 3. (a–d). Seasonal changes in nonstructural carbohydrates determined by the phenol-sulphuric acid method for the sprouting and cropping phase of production plants (vertical bars represent standard error at 5% significance).

of August (Julian date 221) then increased 74% 19 d later (Fig. 3b and d) back to similar levels as the rhizomes in the SPP (Fig. 3b and d). Both production phases remained at the same levels until the end of the season (Fig. 3b and d). The presence of the rhizomes allows the wild blueberry to be unique in that rhizome carbohydrate reserves allow roots to develop before SPP stem growth. (Fig. 3b and d).

At the beginning of the 2000 SPP growing season, starch was reallocated from the rhizomes and roots to provide carbohydrates to the developing root and upright shoot tissues (Fig. 3a and c) (Townsend et al. 1968; Darnell and Birkhold 1996). The drop in root and upright shoot starch levels from Julian date 175 to 225 appeared to be quickly converted to sugar as the roots and upright shoot rapidly increased in dry weights with growth and development (Fig. 3a and c).

As occurs in other perennial woody plant species, the absence of a developing crop as a strong sink for photoassimilates resulted in the redirection of the photoassimilates for use in other parts of the plant (Lockwood and Sparks 1978; Fernandez and Pritts 1994). There was then an indication that the rhizome sugar was converted into starch, as the SPP and CPP rhizomes increased in starch and dry weight levels for the first part of August (Fig. 3a–d).

Root Soluble Sugar and Starch Concentration

The combined root soluble sugar levels of SPP and CPP plants decreased by 50% from the beginning of the season until mid-June (Julian date 166) (Fig. 3b–d). Following the decline, there was a combined increase of 102% in SPP and CPP root soluble sugar levels at the end of June (Julian date 181) (Fig. 3b–d). By mid-July (Julian date 195), root soluble sugar levels for the SPP and CPP returned to the low levels present before the peak (Fig. 3b–d). The root soluble sugar levels remained low until after the harvest period, when they increased to levels present at the beginning of the season (Fig. 3b–d).

The root starch levels of plants in the SPP and CPP were also significantly affected by Julian dates (P value, 0.001) (Fig. 3a–c). The starch levels of the SPP and CPP roots were quite variable throughout the season (Fig. 3a–c). The SPP and CPP root starch levels remained low from April until Sep. 29 (Julian date 273) when the levels increased back to the original concentrations observed at the beginning of the season (Fig. 3a–c). In October, the root starch levels decreased to 75% of peak levels and maintained at these levels until the end of the growing season (Fig. 3a–c).

From the beginning of October to the last sampling date in November, there was a drop in rhizome and root starch levels for plants in the SPP (Fig. 3a), which occurred in the absence of a corresponding drop in dry weight of either roots or rhizomes. There is no accounting for the loss of carbohydrates due to reallocation, as the stem dry weights also decreased with constant stem

soluble sugar and starch levels (Fig. 3a–b). It is possible that the wild blueberry plant was still active, as the ground had not yet frozen, and might have been moving sugars within the plants to create osmotic protection of the plant and especially the reproductive structures against frost damage (Konakahara and Sakai 1967; Smith 1968), or the loss of sugars could also be due to respiration (Salisbury and Ross 1992). Despite the final drop in storage carbohydrates, starch and soluble sugar levels were still significantly higher than mid-season levels, which would be in agreement with work reported on cranberries that indicated an increase in soluble sugars and starch as the plant entered dormancy (Hagidimitriou and Roper 1994). Despite these final changes within the rhizomes, the dry weight, starch, and sugar levels were similar to the relatively high levels present at the beginning of the season (Fig. 3a–d).

Upright Stems And Leaves Soluble Sugar and Starch Concentration

Stem soluble sugar levels were significantly influenced (P values <0.001) by Julian date and the interaction of production phase and Julian date (Fig. 3b–d). There was no significant effect (P value = 0.218) of production phase on stem soluble sugar levels (Fig. 3b–d). Excluding the later sampling date of SPP stems, the stem soluble sugar concentrations for both phases of production remained at constant levels from the beginning of sampling, until mid-July (Julian date 195) when the CPP stems experienced a significant increase of 159% in soluble sugar levels (Fig. 3b–d). The soluble sugar levels of CPP stems quickly returned to previous levels following the peak (Fig. 3b–d). From the first week of August (Julian date 221) until the end of September (Julian date 273), the plants in the SPP and CPP both experienced a total increase in stem soluble sugar levels of 61 and 175%, respectively, and remained at these levels until the end of the season (Fig. 3b–d).

Julian dates (P value <0.001) significantly influenced the stem starch levels in the SPP and CPP (Fig. 3a–c). There was no significant effect of production (P value = 0.921) or the interaction of production phase and Julian date (P value = 0.471) (Fig. 3a–c). The combined SPP and CPP stem starch levels decreased until Jul. 13 (Julian date 195) from initial levels by 75% (Fig. 3a–c). The stem starch levels for the SPP and CPP remained at the lower levels for the rest of the growing season (Fig. 3a–c). Thus, it was most likely that the presence of the reproductive structures in the CPP that caused these differences in stem and leaf carbohydrate levels between the phases of production and that carbohydrate supply is critical for fruit set along with the growth and development of the wild blueberry. Similar observations were noted in cranberries, where vegetative uprights were found to have higher total non-structural carbohydrate concentrations compared with fruiting uprights due to the depletion of starch levels during flowering and berry

development (Hagidimitriou and Roper 1994). The rapid conversion of carbohydrates also agrees with the conclusion that the supply of carbohydrates during the early part of fruit development was vital in the determination of yield and size potential of Empire apple cultivars (*Malus × domestica* Borkh.) due to the occurrence of rapid cell division (Lakso et al. 1998).

Despite the heavy demands for carbohydrates throughout the plants, there was no difference in the rhizome starch or sugar levels between phases of production (Fig. 3a–d). Thus, the vital role of rebuilding of rhizome reserves for long-term plant survival resulted in the rhizomes maintaining a stronger sink for carbohydrates following harvest as compared with the rest of the plant. In a burning tolerance study with *V. myrtilloides* Michaux (Vander Kloet 2000), it was found that plant spread and yield were highest for plants burnt every 3 yr compared with burning every 2 yr or no burning. The reduced number of burnings in the Vander Kloet (2000) study probably resulted in reserve accumulation instead of depletion caused by the heavy requirements of redeveloping all the above-ground tissue, as happens in the SPP of wild blueberries. However, in order to maintain higher yields, burning will still be required every third year to remove old upright growth that is no longer productive (Kinsman 1993).

High-performance Liquid Chromatography

Fruit Fructose, Sucrose, Glucose, and Starch Levels

There were significant changes (P value < 0.001) in fructose, glucose, and sucrose concentrations as the berries matured (Fig. 4). The fructose and glucose continued to increase in concentration throughout berry development to peak levels that were 30 and 78% higher, respectively, on Aug. 08 (Julian date 221) than the initial levels measured on Jul. 13 (Julian date 195) (Fig. 4). Similarly, sucrose increased by 574% from Jul. 13 to Jul. 27 (Julian dates 195 to 209), but then dropped

to negligible concentrations by Aug. 08 (Fig. 4). There was a small amount of starch detected in some samples of the first sampling date (Julian date 195), but not enough to run a statistical analysis for any of the berry samples.

It is possible that the sugars tested were not the main forms of carbohydrates present within the wild blueberry plant. Mannitol was originally included in the standards run with the HPLC trials, but did not consistently elute at the same time for each run. Standard elutions of soluble sugars were reported by Martin-Villa et al. (1982), which show mannitol being eluted around the same time as fructose and glucose. Mannitol is known to be present in the Rubiaceae family (Loescher et al. 1990), thus it is possible that mannitol was naturally present in the wild blueberry plant tissues, which would explain the erratic results obtained for the roots, rhizomes, and stems.

Other carbohydrates, such as fructans, that have been found in monocots (Ojima and Isawa 1967) and dicot plants (Points and del Campillo 1985) and play important roles in temperate plants that overwinter and may have been present in the wild blueberry plant (Points and del Campillo 1985). An inverse relationship has been found between fructan content and temperatures where fructans can act as “osmotic buffers” in plants that experience cold or dry periods (Ojima and Isawa 1967). These compounds have been found in rhizomes of Irises (Pontis and del Campillo 1985) and can make up to 50% of dry weight of some storage tissue (Kandler and Hopf 1980).

Despite the failure to obtain results with the HPLC, the phenol-sulphuric acid method was still useful for determining relative changes within the partitioning of dry matter (Dubois et al. 1956; Buysse and Merckx 1993; Maust et al. 2000). It was possible that interfering compounds could be present in the samples, but not in the standard curves, which would have caused an inflated result. It was assumed that any possible interference would also be present in all the samples, thus the overall trend of the sugars was analysed instead of the absolute concentrations (Penner 1994).

SUMMARY AND CONCLUSIONS

The results of this study illustrate the important role the rhizomes serve as a carbohydrate reservoir to the wild blueberry, and the ability of the wild blueberry to use and replenish these carbohydrates over the course of a growing season. The roots had their own stored starch reserves for spring growth, but also acted as a sink that competed with the upright vegetation for other sources. As the plant entered dormancy at the end of the seasons, the SPP and CPP roots began to rebuild soluble sugar levels and converted them into starch for overwintering. Sink strength differences between the two phases of production appeared to have the greatest significant effect on the stem soluble sugar levels, which were reflected of the SPP and CPP stem dry weights.

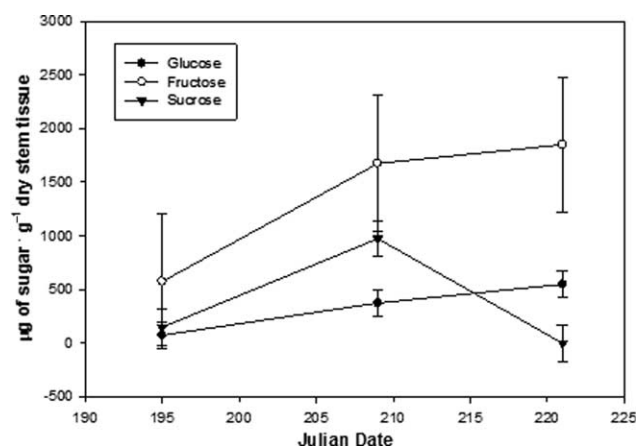


Fig. 4. Sucrose, fructose and glucose composition of wild blueberries determined by HPLC.

The importance of carbohydrates in the below-ground plant tissue for over-winter survival was further supported by the observation that carbohydrate levels within the plant were still fluctuating into November and it was possible that the plant might have been active in the spring before above-ground temperatures are above freezing. Therefore, results from this study have indicated that the wild blueberry is a unique temperate fruit crop well adapted to cool root zone temperatures. The early onset of wild blueberry growth below-ground before other plants were able to begin development would offer the wild blueberry a competitive advantage against faster growing plants for acquiring space and resources.

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