COVER SHEET

TITLE: Evaluating Nonstructural Carbohydrates in Cranberry (*Vaccinium macrocarpon* Ait.) Cultivars and their Role in Fruiting

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Introduction

Cranberry (*Vaccinium macrocarpon* Ait.) is a low-growing perennial vine that grows by sending out runners along the soil surface (Fig. 1). Fruit are borne on uprights, short vertical shoots that grow from axillary buds on runners. Uprights also continue growth from terminal buds that develop in the summer of the previous growing season and these buds may be vegetative or reproductive (Eck, 1990). Vegetative terminal buds contain just a vegetative meristem, while reproductive buds contain both a vegetative meristem and flower initials.

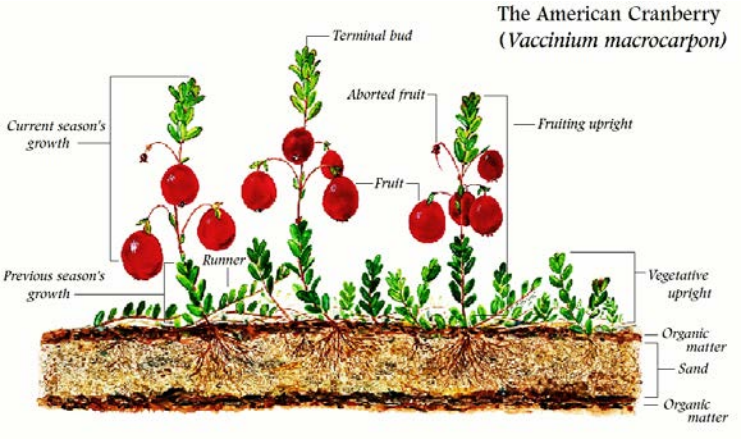

Figure 1: The classification and growth of uprights in a cranberry bed (wiscran.org).

Cranberries are native to the United States, originating in the northern areas fed by glacier runoff such as Wisconsin, Michigan, Washington, and New Jersey. They are a perennial fruit crop belonging to the Ericaceae family (Eck, 1990). Cranberries are the most economically important fruit crop in the state of Wisconsin, bringing in $300 million to the state economy on average each year from 2006 – 2008 (Keene, et al., 2010).

Despite the economic importance of this crop, we know very little about the specific factors involved in fruit production. The amount of photosynthesis-derived carbohydrates is believed to contribute to fruit production and total yield. Carbohydrate relationships, such as photosynthate partitioning and resource allocation, have long been recognized to impart a large influence on fruit set and yield (Gifford et al., 1984). Carbohydrate allocation patterns within an

upright could lead to fruit abortion and diminished fruit set if there are insufficient levels within the plant body. This mechanism is proposed to be the cause of biennial bearing in cranberry (Eaton, 1978; Roper et al., 1993). Biennial bearing occurs when reproductive uprights fail to form reproductive buds that would lead to fruit production the following growing season (Eaton, 1978; Roper et al., 1993; Elle, 1996). Vegetative uprights typically tend to produce reproductive buds that lead to fruit production the following growing season. This alternating pattern of bud formation and subsequent fruiting is the trend of biennial bearing.

Studies conducted on the movement and subdivision of carbohydrates show that the majority of carbohydrates produced from new leaves are allocated to fruit development (Birrenkott and Stang, 1990; Roper and Klueh, 1996). Similarily, Roper and Klueh (1994) demonstrated that the removal of new leaves at fruit set results in reduced fruit set, count, size, and yield, whereas the removal of one-year-old leaves or the removal of leaves after fruit set has less of an effect on yield relative to new leaf removal. These studies support the assertion that fruit development is a large carbohydrate sink. An earlier study proposed that biennial bearing may be affected by carbohydrate limitations (Strik et al., 1991). Wherby insufficient carbohydrate levels during fruit and bud development encourages formation of a vegetative bud over a reproductive bud. However, in several newer cultivars of cranberry return bloom is common and occurs when a fruiting upright produces a reproductive bud that leads to fruit production the following growing season. (DeVetter et al., 2013) These, new cultivars are able to maintain higher yields and bypass the trend of biennial bearing.

It has been shown in other plants that there are multiple factors that contribute to flowering and subsequent fruit production (Bernier et al., 1993). Aside from carbohydrates, factors that influence fruiting include other physiological factors (e.g. hormones and secondary

metabolites), environmental factors, and their interactions. Therefore, it is reasonable to hypothesize that there are other factors involved during the process of flower and fruit development in cranberry. This observation warrants further investigation as to the role of carbohydrates in fruit production of cranberry, particularly across different cultivars of cranberry.

This project sought to evaluate total nonstructural carbohydrates (TNSC), soluble sugars (SS), and starch concentrations in cranberry cultivars and assess their potential role in fruiting. The specific objective of this project was to analyze and compare TNSC over time in vegetative, reproductive, root, and fruit tissues across cultivars that differ in return bloom. This will be done in order to discern any relationship between TNSC within the surveyed tissues and return bloom. Information on these characteristics will be valuable as the industry seeks to explain and optimize the occurrence of return bloom.

Materials and Methods

MATERIAL COLLECTION. Samples were collected from a commercial cranberry marsh in Wood County, Wisconsin. All cultivar samples were collected from a single cultivar bed and received similar management. Cultivars collected and information regarding their respective dates of release, parentage, and geographical origin are presented in Table 1. The 2012 samples were utilized for protocol development and adjustment. During the 2013 growing season, six collections occurred every three weeks from 12 June to 30 October for each year. Collection of plant material corresponded to the following phenological stages with dates of collection for the 2013 season in parenthesis: 1) prebloom to roughneck (4 June), 2) full bloom (2 July), 3) late bloom/early fruit set (30 July), 4) fruit and bud development (27 August), 5) fruit harvest (19 September), and 6) postharvest (30 October). During each collection date, five cores were

randomly collected from each cultivar, each consisting of roots and accompanying uprights. The cores measured 11 cm in diameter and 19 cm in depth. Three cores were analyzed and the rest were stored in case further analyses were needed to compensate for variation in the samples. Additional years of data collection were not justified due to previous work by Hagidimitriou and Roper (1994), who found seasonal fluctuations in cranberry carbohydrates were similar across years. Fruits were collected from all reproductive uprights within three randomly placed 300 cm^2 quadrats per bed. The collection occurred a week prior to harvest for yield metrics and carbohydrate analysis.

SAMPLE PREPARATION. Collected plant material was immediately placed on ice and stored at 4^oC until processing and drying. Roots and upright tissues were sorted during processing and later dried at 80^oC until constant weight. Uprights with flowers or fruit at the time were classified as reproductive, whereas non-flowering/fruiting uprights were considered vegetative (as further described in DeVetter, et. al., 2013). Both soluble sugars and starch were extracted for TNSC via high-performance liquid chromatography (HPLC). Flowers and fruit were removed from the tissue and the sample was ground using a 40-mesh Wiley mill. Soluble carbohydrates included in the analysis were glucose, fructose, and sucrose and were extracted from 100 mg ground tissue using an 80%-ethanol extractant, as described by Botelho and Vanden Heuvel (2005). Starch was extracted from the remaining sample left over from soluble carbohydrate extraction. The same extraction procedure was repeated on the fruit collected. . Carbohydrates were compared across cultivars within a given sampling period and are presented as a percent concentration by dry weight. Modifications to the procedure by Botelho and Vanden Heuvel (2005) include: collected filtrates were passed through a sterile plastic syringe filter with a 4-mm membrane diameter and 0.2 μm pore size (Corning®, North Bend, OH) before injection

into the HPLC (Prominence UFLC; Shimadzu, Kyoto, Japan). A RezexTM monosaccharide column was used for separation of soluble sugars (Phenomenex; Torrence, CA). Runs were isocratic, with a mobile phase of 80°C HPLC-grade water and refractive index detector at 40°C. Data are presented as a concentration, which was determined by multiplying tissue dry weight by carbohydrate concentration determined by HPLC analysis.

STATISICAL ANALYSIS. Data were analyzed in SAS (SAS Version 9.2; SAS Institute, Cary, NC). A General Linear Model (GLM) was used and tests of significance were done at $\alpha = 0.05$ using a least-squares mean (lsmeans) option with a Tukey-Kramer adjustment for multiple comparisons.

Results and Discussion

In general, carbohydrate concentrations were greater in vegetative and reproductive tissues than in root tissue, and greater in vegetative tissue over reproductive (Fig. 1 to 3). Patterns of carbohydrate changes were similar among tissues with the exception of 'GH1' TNSC and SS concentrations around full bloom, which were greater than that of 'Stevens' and 'HyRed' in root and reproductive upright tissues (Figs. 1 to 3).

'GH1' vegetative uprights contained a greater concentration of TNSC and SS throughout the growing season, reaching the lowest concentration in July and September, respectively (Fig. 2A and B). In comparison, 'HyRed' reached its lowest concentration of TNSC and SS in July, while in 'Stevens' tissues it was in late August to early September (Fig. 2A and B). Starch concentration variability was less than in TNSC and SS, but a drop in concentration can be seen across all cultivars around late July, which corresponds to terminal but initiation (Fig. 2C).

In the reproductive uprights of all cultivars, TNSC concentrations are similar to vegetative tissues after the first sampling date and this time period corresponds to the transition

to full bloom (Fig. 3A). In ‘Stevens’ and ‘HyRed’ an increase in TNSC and SS can be observed following full bloom, while in ‘GH1’ these levels do not begin to rise until late August (Fig. 3A and B). Starch concentrations stay uniform across cultivars with the exception of the increase in starch in ‘GH1’ in early July, corresponding to late bloom (Fig. 3C).

No significant differences in carbohydrate concentrations were detected in the fruits, suggesting that carbon sink strengths do not differ across the three cultivars (data not presented). This illustrates that any carbohydrate differences between cultivars is due to the ability of the plants to generate, mobilize, and differentially partition carbohydrates within the vegetative plant body, not to the translocation of carbohydrates into fruit development. Out of the three cultivars tested, ‘GH1’ has the lowest return bloom potential (Table 2). In addition, reproductive uprights of ‘GH1’ had the lowest concentrations of TNSC and SS during August and September, which was not found in the vegetative uprights (Figs. 2 and 3). Comparatively, ‘Stevens’ and ‘HyRed’ had slightly greater or equal TNSC and SS concentrations in reproductive uprights compared to vegetative uprights during August and September. This evidence suggests that reproductive bud formation in ‘GH1’ may be inhibited by a reduction in carbohydrates during fruit and bud development and this could contribute to biennial bearing. In contrast to ‘GH1’, ‘Stevens’ and ‘HyRed’ had greater concentrations of TNSC and SS during August and September, and increased in concentration whereas ‘GH1’ decreased.

In addition to differing concentrations of TNSC, SS, and starch, the three cultivars analyzed showed differences in measured yield components, specifically upright density, fruit set, and mean berry weight (Table 2). ‘Stevens’ showed the greatest upright density at 236 uprights per unit area, while ‘HyRed’ showed the least density at 174 uprights per unit area. However, both ‘HyRed’ and ‘GH1’ showed the greatest fruit set with 2.2 fruits per upright. Note

that the fruit set values are reported to the tenth decimal position to numerically demonstrate statistical differences. Nevertheless, since the rounded mean fruit set is two berries per upright for all three cultivars, detection of a statistical difference may not be biologically significant in the context of this study. 'Stevens' was found to have the greatest mean berry weight, however, the floral induction, berry number, and overall yield were statistically the same across all three cultivars. Our findings of 'GH1' reproductive uprights with reduced carbohydrate concentrations relative to cultivars with a greater return bloom potential supports the role of carbohydrates as an important factor influencing biennial bearing. However, this evidence does not exclude the role of other possible physiological factors.

Note that the post harvest collection date was excluded from the presented figures. This was done deliberately as the TNSC and SS concentrations of 'GH1' exceeded 20% among upright tissues, which would have impacted data resolution due to scale changes. In the reproductive uprights of 'GH1' the TNSC concentration was 20.91 percent by dry weight, while in 'Stevens' and 'HyRed' TNSC was measured at 11.88 and 9.11 respectively. These increases in TNSC and SS concentrations are suspected to be due to the mobilization of carbohydrate reserves in the uprights after the stress of a five-day flood, which only happened to 'GH1.' Post harvest concentrations of TNSC, SS, and starch were not statistically different between 'Stevens' and 'HyRed'.

The results from this study support the conclusion that carbohydrate limitations during fruit set and development limits return bloom potential by inhibiting reproductive bud formation on reproductive uprights. Cultivar effects were observed, yet an explanation has not been found as to how cultivars with a greater return bloom potential are able to have greater carbohydrate concentrations during critical phenological stages. Some possible explanations may include a

greater photosynthetic capacity, a lower respiration rate, and a greater efficiency at partitioning carbohydrate resources. The implications of this study suggest that increased yields can be obtained by maximizing carbohydrate production in upright tissues, which would in turn favor the development of fruit and reproductive buds for subsequent growth cycles.

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Tables and Figures:

Table 1: Cranberry cultivars included in the study, their respective dates of release, parentage, and geographical origins.

Cultivar	Release Date	Parentage	Geographical Origin
Stevens	1950	McFarlin x Potter	New Jersey
HyRed	2003	Stevens x Ben Lear	Wisconsin
GH1*	1980	Searles x McFarlin	Wisconsin

* Gryglesky Hybrid 1

Table 2: Summary of measured data for three cultivars collected during the 2013 growing cycle. Values are means ± standard error (SE). Reprinted from DeVetter *el al.(*2014*).*

Cultivar	Upright density (no. uprights)	Floral induction (%)[z]	Fruit set (no./upright)	Berry no.	Yield (g)	Mean berry wt (g)	Potential return bloom (%)[y]
GH1	209 (20) ab[x]	44 (3.9)	2.2 (0.13) a	146 (23)	163 (25)	1.12 (0.01) b	1
Stevens	236 (10) a	40 (3.9)	1.7 (0.11) b	107 (20)	141 (22)	1.34 (0.05) a	20
HyRed	174 (3.1) b	39 (5.1)	2.2 (0.08) a	109 (8.4)	128 (12)	1.17 (0.02) b	19
Significance	0.043	0.67	0.042	0.3093	0.5281	0.0088	--

[z]Percentage of reproductive (flowering/fruiting) uprights per unit area.

[y]Potential for return bloom was determined as the percentage of current season's reproductive uprights with mixed terminal buds.

[x]Values are means determined from three 300-cm^2 quadrates per cultivar bed; means with the same letter within a column are not different at $P \leq 0.05$ using a Tukey-Kramer adjustment.

Figure 1: Total nonstructural carbohydrates (TNSC) (A), soluble sugars (B), and starch (C) concentrations in the roots of 'GH1', 'Stevens', and 'HyRed' cranberry during the 2013 growing season. Sample collection corresponded to the following phenological stages with dates of collection in parentheses: prebloom (4 June), full bloom (2 July), late bloom/early fruit set (30 July), fruit and bud development (27 August), and fruit harvest (19 September). Bars denote standard error and periods marked with an asterisk indicate concentrations were different within a sampling period at $P \leq 0.05$.

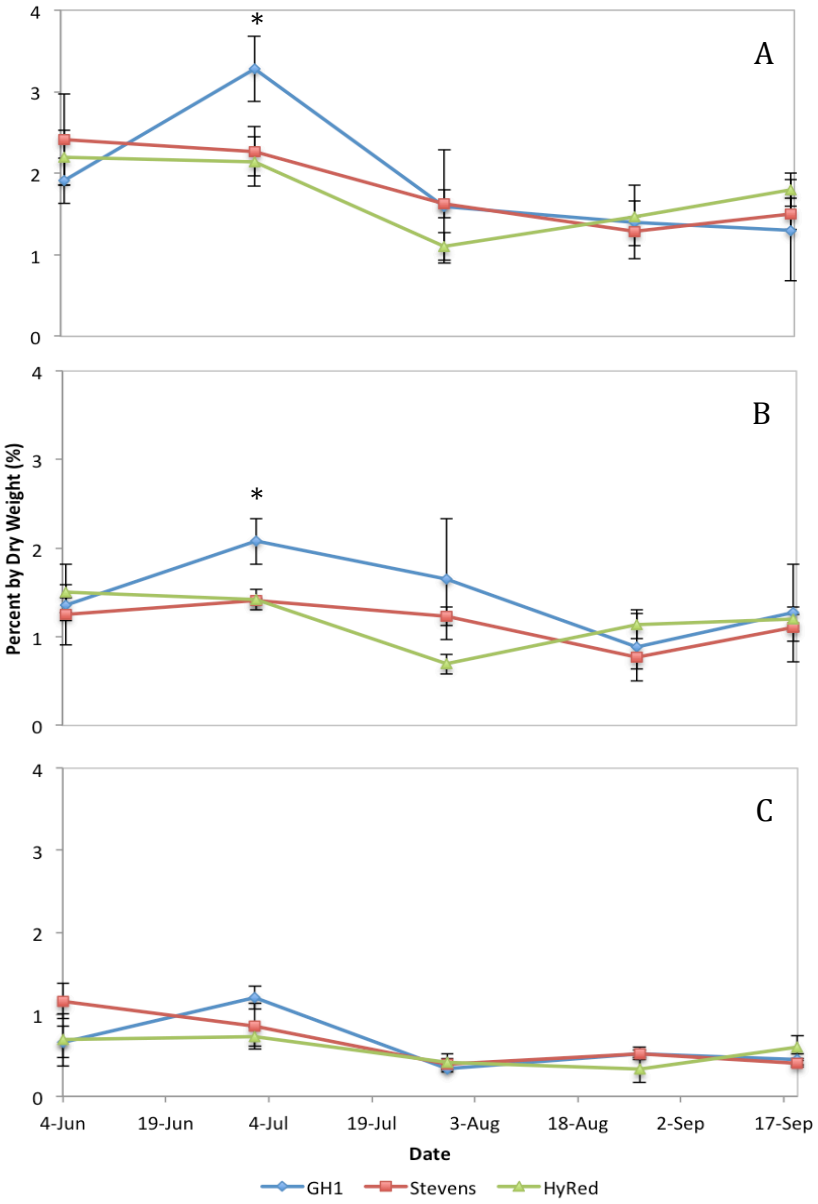

Figure 2: Total nonstructural carbohydrates (TNSC) (A), soluble sugars (B), and starch (C) concentrations in the vegetative uprights of 'GH1', 'Stevens', and 'HyRed' cranberry during the 2013 growing season. Sample collection corresponded to the following phenological stages with dates of collection in parentheses: prebloom (4 June), full bloom (2 July), late bloom/early fruit set (30 July), fruit and bud development (27 August), and fruit harvest (19 September). Bars denote standard error and periods marked with an asterisk indicate concentrations were different within a sampling period at $P \leq 0.05$.

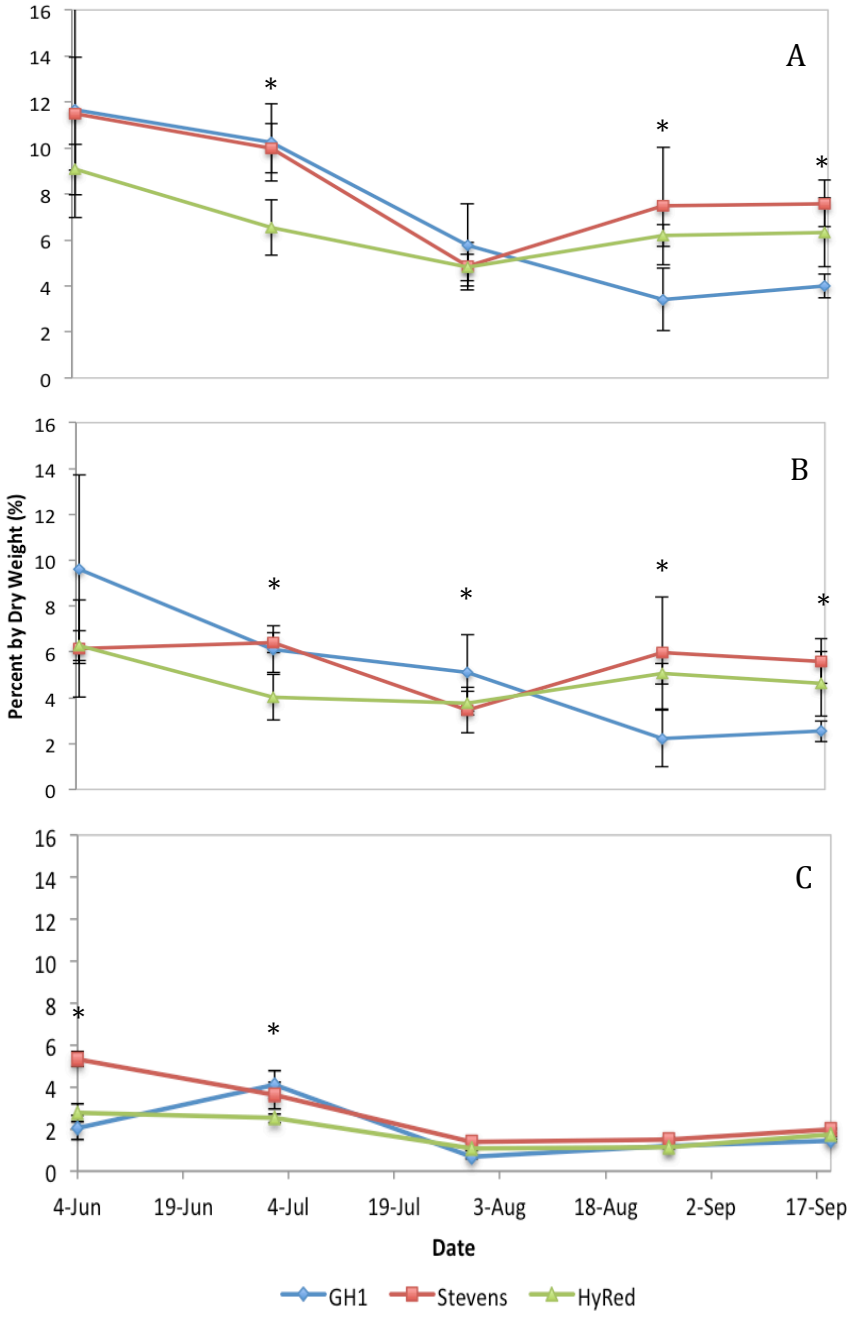

Figure 3: Total nonstructural carbohydrates (TNSC) (A), soluble sugars (B), and starch (C) concentrations in the reproductive uprights of 'GH1', 'Stevens', and 'HyRed' cranberry during the 2013 growing season. Sample collection corresponded to the following phenological stages with dates of collection in parentheses: prebloom (4 June), full bloom (2 July), late bloom/early fruit set (30 July), fruit and bud development (27 August), and fruit harvest (19 September). Bars denote standard error and periods marked with an asterisk indicate concentrations were different within a sampling period at $P \leq 0.05$.