High Dissolved Oxygen Concentration of Floodwater Reduces Carbohydrate Concentration of Cranberry Uprights during Flooding

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Abstract. Cranberry production involves the use of flooding for several purposes during the growing season, including pest control, winter protection, and harvest. The effect of the dissolved oxygen concentration in floodwater on carbohydrate concentrations of uprights and roots during flooding was investigated using potted ‘Stevens’ cranberry (Vaccinium macrocarpon Ait.) vines. Pots were placed in large bins filled with water to simulate a spring pest control flood (called late water) over a 21-day period. Two treatments were applied: oxygenated (control) and nonoxygenated (treatment). Uprights and roots were collected every 3 days and prepared for HPLC analysis to quantify nonstructural carbohydrate concentration. Soluble sugar (sucrose, glucose, and fructose) and starch concentration, as well as total nonstructural carbohydrate (TNSC) concentration, decreased over the 3-week period in uprights but not roots regardless of treatment. Interestingly, the sucrose, glucose, fructose, and starch concentrations of uprights in the oxygenated treatment were lower than those of uprights in the control treatment throughout the experiment. This research indicates that vines in flooded bogs demonstrate a net carbon loss, resulting in reduced carbohydrate concentration available for growth and fruit production.

The American cranberry (Vaccinium macrocarpon Ait.) is a commercially grown fruit native to bogs of eastern North America (Eck, 1990). The low-growing, evergreen vine covers bog floors, forming a dense mat (Eck, 1990). Short vertical stems, known as uprights, arise from the vine or from older uprights (Eck, 1990). Individual uprights bear fruit biennially, but since cranberry bogs are populated by a large number of uprights, overall yield does not usually alternate (Roper and Vorsa, 1997). Optimal production appears to be dependent on an adequate number of flowering uprights (Eck, 1990). Baumann and Eaton (1986) demonstrated the most important components in cranberry yield, in decreasing order, were fruit set, berry size, and the number of flowers per centimeter of upright. Failure of upper fruit on an upright to develop is likely due to depletion of carbohydrate resources during the initial stages of fruit set (Birrenkott and Stang, 1990).

There are several indications that cranberries are under constant carbohydrate stress throughout the growing season: 1) fruit set is low, ranging from 30% to 40% (Roper and Vorsa, 1997); 2) uprights tend to bear biennially (Roper et al., 1993); and 3) carbohydrate concentration in uprights is lowest at the beginning of fruit set, suggesting that fruit compete for resources (Birrenkott et al., 1991).

A significant cause of carbohydrate stress may be prolonged periods of net respiration during flooding. Flooding is a common management tool used by growers to protect the plants from dry winter winds in cold climates (i.e., Massachusetts, New Jersey, and Wisconsin), to harvest fruit and remove fallen leaves, to control pests, and to reduce weed populations (DeMoranville, 1998). However, lack of research concerning the physiological effects of flood application and duration on cranberry vines has resulted in growers and extension personnel making educated guesses at best management practices.

Flood-stressed cranberry vines will typically show symptoms of leaf chlorosis, reddening, necrosis, and abscission (Crane and Davies, 1989). These symptoms are often anecdotally related to partial or complete oxygen deficiency in the floodwater (DeMoranville, 1998). It has been suggested that oxygen deficiency damage may occur when oxygen levels in the floodwater fall below 4 mg L⁻¹ [full oxygenation ≥ 10 mg L⁻¹] (Bergman, 1943). Although this effect has not been proven experimentally, it is the basis for current recommendations to growers. Vines flooded with water low in dissolved oxygen can convert to anaerobic respiration, prompting further carbohydrate depletion (Pezeshki, 1994; Schluter and Crawford, 2003). Carbohydrate reduction results in cranberry plants with diminished energy available for growth, which might ultimately affect yield.

Empirical data have shown that cranberry vines under ice-covered floods can replace oxygen photosynthetically as long as sunlight can penetrate through the ice (DeMoranville, 1998; Forsyth and Hall, 1967). If the ice becomes cloudy, snow-covered, or is exposed to an extended period of overcast conditions, however, the vines are thought to be unable to photosynthesize as efficiently as if exposed to full wintertime sunlight. Consequently, vines may be incapable of replenishing the oxygen to levels necessary for respiration (DeMoranville, 1998). If, indeed, vines are able to photosynthesize under water during the winter flood (Forsyth and Hall, 1967), they should be able to photosynthesize during other, warmer floods.

In present-day production, Massachusetts growers may remove the winter flood in March and then reflood the bog in late April for up to 30 days (known as late water) (DeMoranville, 1998). Late water can reduce fruit rot disease, suppress some insects and weeds, and improve fruit quality and yield after harvest (Averill et al., 1997). Occasionally, yields from late water bogs have been observed to be poor (Averill et al., 1997; DeMoranville, 1998). However, much of this information is anecdotal as there is a large amount of natural variation between bogs and it is difficult to properly replicate flooding treatments and the nonstructural carbohydrate concentration of cranberry vines and roots.

Materials and Methods:

Plant material. ‘Stevens’ uprights were harvested from State Bog, Massachusetts Cranberry Station, East Wareham, in June 2002. Uprights were placed in 4-inch pots containing 1 l sand : 1 peat (w/w) mix. Uprights were grown in a greenhouse under ambient temperature and light, but were moved into a darkened cooler during the winter to satisfy the chilling requirement (Rigby and Dana, 1972). Vines were then returned to the greenhouse.

Flooding treatments. Flooding was simulated using large, clear plastic bins (55 L capacity)
containing tap water. Dissolved oxygen levels were applied using three different models of aquarium-grade air pumps in order to obtain the three treatments (models DW96-2, DW24-2, and API50, high, medium, and low, respectively; Tetratec, Blacksburg, Va.). Uprights in the nonoxygenated control treatment were not exposed to an air pump. On 2 June 2003, pots were then carefully washed and bagged. The tissue was dried at 80 °C for a minimum of 5 d and weighed. Dissolved oxygen and water temperature of each flood bin were recorded using a dissolved oxygen meter (YSI 55; YSI Inc., Yellow Springs, Ohio) each morning at about 09:00 hr.

Pots of uprights were removed from the flood every 3 d, beginning with day zero (nonflooded control), for 3 weeks. At each removal date, two pots (eight uprights) were removed for each treatment level. One pot was placed on a greenhouse bench and was allowed to grow for the remainder of the season. The remaining pot of four uprights was processed for high-performance liquid chromatography (HPLC) analysis. The latter set of uprights was cut at the soil level and placed in paper bags. The roots were then carefully washed and bagged. The tissue was dried at 80 °C for a minimum of 5 d. Samples were then weighed and ground to 40 mesh using a Wiley mill.

Water temperature (°C) and dissolved oxygen (mg L–1) readings in each bin taken each day for the duration of the 21-d experiment showed that dissolved oxygen concentration decreased as temperature increased (Fig. 1). Floodwater temperature varied from about 17.5 to 22.5 °C over the course of the experiment (Fig. 2). Water temperature correlated well with ambient photosynthetic photon flux (PPF) in the greenhouse. Among the four treatments, no floodwater temperature differences were found (P > 0.05). There were no significant differences in dissolved oxygen concentration among the three oxygenation treatment levels (low, medium, and high) (Fig. 1). Each oxygenation treatment averaged 8.5 mg L–1 over the course of the experiment (Fig. 2); therefore, the three levels were combined into an oxygenated treatment and were compared to the control treatment (mean 6.3 mg L–1). These two treatments differed significantly (P = 0.0001). There was less variability in dissolved oxygen concentration in the oxygenated treatment, compared to the control (Figs. 1 and 2), likely due to the fact that depleted oxygen levels were constantly being replenished by the pumps in the oxygenated treatment, while the control treatment did not have an external supply of oxygen.

Carbohydrate data was analyzed on a concentration basis, though data were similar when calculated on a total carbohydrate per organ basis (data not shown). Uprights demonstrated a decrease in sucrose over the duration of the flood (Fig. 3A) in both the control and oxygenated treatments. Uprights in the oxygenated treatment demonstrated a loss of 25.5% of the initial sucrose concentration by the third day of flooding, and an overall loss of 98.8% over the 3-week period, while uprights in the control treatment had a 68.6% loss of sucrose over 3 weeks. Interestingly, sucrose concentration of uprights in the oxygenated treatment was lower than that of uprights in the control treatment throughout the flood. Fluctuations in sucrose concentration of the root tissue of both treatments were similar (Fig. 3A); however, roots in the control treatment had an overall decrease of 60.4%, while those in the oxygenated treatment decreased by 39.2% by the end of the experiment.

Shortly after the flood was applied, there was a large drop in glucose concentration (Fig. 3B) in the uprights of both treatments. Uprights in the control treatment had a loss of 55.0% of their initial glucose concentration by the third day of flooding, and an overall loss of 95.0%. Uprights in the oxygenated treatment had a loss of 65.7% in the first 3 d, and a total loss of 98.3%, resulting in uprights in the control treatment having a higher concentration of glucose than those in the oxygenated treatment at the end of the experiment. Roots in both treatments (Fig. 3B) had negligible amounts of glucose throughout the 3-week period.
Soon after the flood was applied, there was also a considerable drop in fructose concentration (Fig. 3C). Uprights in the control treatment had a loss of 62.2% of the initial fructose concentration by the third day of flooding, and an overall loss of 82.1%. Again, uprights in the oxygenated treatment contained lower levels of fructose than those in the control treatment. Uprights in the oxygenated treatments had a loss of 82.8% within the first 3 d of flooding, and an overall loss of 98.0%. Roots in both treatments (Fig. 3C) had negligible concentrations of fructose throughout the experiment.

Uprights in the control treatment increased in starch concentration by 22.6% (Fig. 3D) within the first 3 d of flooding but demonstrated an overall decrease of 34.9% after 21 days, while uprights in the oxygenated treatment increased 28.5% in the first 3 d, with an overall loss of 87.7% over 3 weeks. Roots in the control treatment (Fig. 3D) had an overall increase of 24.0%, while those in the oxygenated treatment lost 8.3% of their total starch concentration by the end of the experiment.

Within the first 3 d under flooded conditions, uprights in both treatments demonstrated a considerable decrease in soluble sugar concentration (sucrose, glucose, and fructose) and a simultaneous increase in starch concentration (Fig. 4). Both soluble sugar and starch concentrations decreased during the remainder of the experiment, with the exception of uprights in the control treatment, which demonstrated a slight increase in starch concentration approximately midway through the flood. The decrease in soluble sugar and concomitant increase in starch is an indication that uprights could be converting their soluble sugar into starch. Schluter and Crawford (2001) demonstrated that a slower initial use of starch under anoxic conditions is an indication of greater tolerance to oxygen deficiency. Since the flooded vines in both treatments demonstrated a rapid increase of starch during the first 3 d, they were likely tolerating the flood conditions well.

At the end of the 3-week flood, soluble sugar concentrations of uprights had decreased by 98.4% in the oxygenated treatment, and by 80.6% in the control treatment (Fig. 4), indicating that uprights used carbohydrates through respiration during the flooding period. During the first 3 d of the flood, glucose and fructose may have been used preferentially by the vine for respiration, as the decreases in these sugars were far greater than the decrease in sucrose. Following removal from the flood, sucrose accounted for 13.8% of TNSC in the uprights of the control treatment, but only 3.3% of TNSC in the oxygenated treatment. This may have resulted in the control uprights having more energy immediately accessible upon flood removal. Starch concentration in the uprights of the control treatment accounted for about 77.2% of the TNSC, while in the oxygenated treatment starch accounted for 88.5% of TNSC. It is noteworthy that the uprights had most of their carbohydrate reserves in the form of starch, given that it is more costly, in the form of ATP, for plants to access carbohydrates from starch (Bonna et al., 1995; Crawford, 1992). Over the duration of the 3-week flood, TNSC decreased by 57.6% in the control treatment and 93.0% in the oxygenated treatment (Fig. 3E).

Upright dry weight measurements were taken both in the spring as plants were removed from the flood for analysis and in autumn at the end of the growing season using plants held in the greenhouse. Dry weight of uprights in the control and oxygenated treatments were not significantly different during the flood (Fig. 5A); however, visual differences in growth habit were apparent when comparing plants in the control and oxygenated treatments. Uprights in the oxygenated treatment had new growth that bolted 2 to 3 cm, which has been observed in other flooding experiments involving uprights placed in warm water (J. Van-den Heuvel, unpublished data). Uprights in the control treatment showed a lesser degree of elongation. Dry weights of uprights harvested in autumn were not significantly different when treatment was tested as a main effect, though uprights in the control treatment tended to have greater dry weights than those in the oxygenated treatment (Fig. 5A), possibly due to increased soluble sugar and starch availability at the end of the flooding period (Fig. 4), (i.e., more energy for growth).

If these conditions exist in a bog situation, uprights exposed to flooding with high dissolved oxygen levels may be smaller at the end of the season by comparison to nonflooded uprights. This, in turn, could result in reduced freezing tolerance due to fewer carbohydrates in the vine.

Root dry weights of plants harvested during the spring flood were significantly different by date (Fig. 5B). About 2 weeks into the flood, roots of vines in the control treatment were significantly larger than roots in the oxygenated treatment. Roots harvested in autumn also differed significantly by date (Fig. 5B). Dry weights of nonflooded roots (day 0) in the oxygenated treatment were significantly larger than roots exposed to the flood on days 3 through 21. Dry weights of roots in the control treatment were slightly higher than those in the oxygenated treatment throughout most of the experiment, but were similar by the last day of the flood.

It is a possibility that the plants were translocating resources from aboveground to the uprights. This was also supported by visual observation: the longer period that the plant remained flooded, the less dense the root mass appeared. Root-to-shoot ratio often decreases as a result of environmental stress (Mooney and Winner, 1991). Concentration of TNSC in the root tissue was low throughout the experiment; this suggests that uprights are more important in supporting growth and development, as reported by Hagidimitriou and Roper (1994).

The reduced carbohydrate levels following flooding are a clear indication that net carbon exchange is negative in cranberry uprights subjected to flooding. If the vines were only respiring during the flood, however, dissolved oxygen levels should have fallen to very low concentrations in the control treatment. Since this did not occur, it would appear that the plants were not only respiring, but also photosynthesizing, thereby replenishing some of the dissolved oxygen in the water that was exhausted during respiration. Dissolved oxygen could also have been replenished from the ambient air; however, mixing at the water’s surface appeared to be minimal.
Dark respiration was likely greater in vines in the oxygenated treatments compared to the control. Oxygen is the required substrate for respiration, and vines in the oxygenated treatments had a greater amount of substrate available for dark respiration compared to the control vines, likely resulting in the rapid use of carbohydrates (Figs. 3 and 4). The exhaustion of carbohydrate supplies in the uprights would result in less energy being available for vine growth upon removal of the flood (Fig. 5).

Vines that were exposed to higher oxygen levels had lower carbohydrate concentrations upon removal from the flood. Though these uprights showed signs of growth, the elongation that occurred was atypical of normal growth seen on healthy vines and dry weights were not significantly different between treatments. Some species facing anoxia will maintain upward shoot elongation in an attempt to grow out of the oxygen deficient conditions (Schluter and Crawford, 2001); however, this growth pattern occurred to a greater degree in the uprights of the oxygenated treatment compared to those in the control.

**Conclusion**

This study provides the first quantification of carbohydrate losses in cranberry vegetative tissue during flooding. The data presented here indicates that the possibility exists for a direct negative relationship between flooding and yield. Maximizing dissolved oxygen levels in floodwater may have a harmful effect on the vines by providing increased substrate for respiration. While researchers currently recommend that growers be concerned with dissolved oxygen falling to warning levels of 5 mg·L⁻¹ in winter floods (DeMoranville, 1998), this study demonstrates that higher levels of oxygenation (about 8.5 mg·L⁻¹) may also have a detrimental effect during late water flooding of bog-grown uprights. Both uprights and roots experienced less growth when subjected to higher oxygen levels compared to those held at lower levels, but above 5 mg·L⁻¹. Uprights exposed to the oxygenated treatment experienced a 93.0% decrease in TNSC over a 3-week flooding period, while uprights in the control treatment only lost 57.6% of TNSC. If this phenomenon exists under bog conditions, recommendations to growers concerning dissolved oxygen levels during late water flooding may be incomplete. Further experimentation is required to determine whether the pattern of increased carbohydrate loss and subsequent reduced growth of vines held in floodwater with high dissolved oxygen levels is apparent at the whole-bog level.

**Literature Cited**

Averill, A.V., M.M. Sylvia, C.C. Kusek, and C.J. DeMoranville. 1997. Flooding in cranberry to minimize insecticide and fungicide inputs. Amer. J. Alt. Agr. 12:50–54.

Baumann, T.E. and G.W. Eaton. 1986. Competition among berries on the cranberry upright. J. Amer. Soc. Hort. Sci. 111:869–872.

Bergman, H.F. 1943. The relation of ice and snow cover on winter-flooded cranberry bogs to vine injury from oxygen deficiency, p. 3–24. In: H.J. Franklin, H.F.
Birrenkott, B.A. and E.J. Stang. 1990. Selective flower removal increases cranberry fruit set. HortScience 25:1226–1228.

Birrenkott, B.A., C.A. Henson, and E.J. Stang. 1991. Carbohydrate levels and the development of fruit in cranberry. J. Amer. Soc. Hort. Sci. 116:174–178.

Bouma, T.J., R. DeVisser, P.H. Van Leerwen, M.J. DeKock, and H. Lambers. 1995. The respiratory energy requirements involved in nocturnal carbohydrate export from starch-storing mature leaves and their contribution to leaf dark respiration. J. Expt. Bot. 46: 1185–1194.

Crane, J.H. and F.S. Davies. 1989. Flooding responses of Vaccinium species. HortScience 24:203–210.

Crawford, R.M.M. 1992. Oxygen availability as an ecological limit to plant distribution. Adv. Ecol. Res. 23:93-185.

DeMoranville, C.J. 1998. Flood management, p. 35–39. In: H.A. Sandler (ed.). Cranberry production—A guide for Massachusetts. Univ. Mass. Ext. Publ. SP-127.

Eck, P. 1990. The american cranberry. Rutgers Univ. Press, New Brunswick, N.J.

Forsyth, F.R. and I.V. Hall. 1967. Rates of photosynthesis and respiration in leaves of the cranberry with emphasis on rates at low temperatures. Can. J. Plant Sci. 47:19–23.

Hagidimitriou, M. and T.R. Roper. 1994. Seasonal changes in nonstructural carbohydrates in cranberry. J. Amer. Soc. Hort. Sci. 119:1029–1033.

Hagidimitriou, M. and T.R. Roper. 1994. Seasonal changes in nonstructural carbohydrates in cranberry. J. Amer. Soc. Hort. Sci. 119:1029–1033.

Mooney, H.A. and W.E. Winner. 1991. Partitioning response of plants to stress, p. 130–141. In: J.A. Mooney and W.E. Winner (eds.). Response of plants to multiple stresses. Academic Press, New York.

Pezeshki, S.R. 1994. Plant response to flooding, p. 289–321 In: R.E. Wilkinson (ed.). Plant-environment interactions. Marcel Dekker Inc., New York.

Rigby, B. and M.N. Dana. 1972. Rest period and flower development in cranberry. J. Amer. Soc. Hort. Sci. 97:145–148.

Roper, T.R., K.D. Patten, C.J. DeMoranville, J.R. Davenport, B.C. Strik, and A.P. Poole. 1993. Fruiting of cranberry uprights reduces fruiting the following year. HortScience 28:228.

Roper, T.R. and N. Vorasa. 1997. Cranberry: Botany and Horticulture. Hort. Rev. 21:215–249.

Schrüter, U. and R.M.M. Crawford. 2003. Metabolic adaptation to prolonged anoxia in leaves of American cranberry (Vaccinium macrocarpon). Physiol. Plant. 117:492–499.

Schrüter, U. and R.M.M. Crawford. 2001. Long-term anoxia tolerance in leaves of Acorus calamus L. and Iris pseudacorus. L. J. Expt. Bot. 52:2213–2225.

Stevens, N.E. and N.E. Thompson. 1942. Factors influencing injury to cranberry plants during flooding. Trans. Acad. Sci. Arts Lett. 34:73–81.