The Effects of Flood Initiation Timing and Water Temperature During Flooding on Nonstructural Carbohydrate Concentration and Anatomy of Cranberry

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Additional index words. *Vaccinium*, respiration, yield potential

Abstract. The objective of this research was to determine the effect of water temperature during spring and fall floods on nonstructural carbohydrate concentration and anatomy/morphology of ‘Stevens’ and ‘Early Black’ cranberry vines. Potted vines of each cultivar were subjected to either a simulated 1-month late water (LW) flood in the spring at either 11 or 21 °C or a simulated 1-week harvest flood in the fall at either 12 or 20 °C. Higher water temperature resulted in decreased total nonstructural carbohydrate concentration (TNSC) during the LW flood in both uprights (i.e., vertical shoots) and roots of ‘Early Black’ and ‘Stevens’. The effect of water temperature was much less during the harvest flood than during the LW flood, but flooding at either temperature during the harvest flood had an impact on TNSC, whereas for LW floods, high water temperature was more influential than low water temperature. Clumping of chloroplasts in the palisade layer and occlusion of vascular tissues was observed in the leaves of both cultivars as a result of LW flooding. Some epidermal erosion and formation of a fungal mat was apparent on the upper epidermis of some flooded leaves. Senescence in some fine roots was visible after harvest flooding, more so in vines flooded at 20 °C than at 12 °C. Stems and major roots showed no influence of flooding on tissue senescence.

Native to bogs of eastern North America, the American cranberry (*Vaccinium macrocarpon* Ait.) is a low-growing, vine-like woody perennial that forms a dense mat on the soil surface (Eck, 1990). Short vertical stems, known as uprights, arise from the vine or from older uprights (Eck, 1990) and bear fruit biennially (Strik et al., 1991). There are several indications that cranberry vines are under significant carbohydrate stress, including low fruit set (Roper and Vorsa, 1997),

biennial bearing of uprights (Birrenkott et al., 1991; Strik et al., 1991), and low carbohydrate concentrations at the beginning of fruit set (Hagidimitriou and Roper, 1994).

Another significant cause of carbohydrate stress in vines may be prolonged periods of net respiration during flooding (Botelho and Vanden Heuvel, 2005, 2006). Flooding is a common management tool used by cranberry growers for several purposes, including protection from dry winter winds in cold climates. Late water (LW) and flash floods are used for weed reduction and pest control in the spring (Averill et al., 1997; Cockfield and Mahr, 1992; Marucci and Moulter, 1971), whereas harvest floods are used to harvest fruit and can be extended to control insects and weeds (DeMoranville et al., 2005). Botelho and Vanden Heuvel (2006) determined that floods applied in the fall (i.e., harvest floods) reduced total nonstructural carbohydrate concentration (TNSC) of cranberry uprights more than in spring floods (i.e., LW or flash floods). Factors that affect vine respiration rate such as water temperature and dissolved oxygen concentration (Botelho and Vanden Heuvel, 2005) have been noted to affect vine TNSC concentration during flooding (Botelho and Vanden Heuvel, 2006),

although how these factors specifically affect the vine (e.g., biology, physiology, function) is not clear.

We hypothesized that flooding detrimentally affects cranberry tissue and that this would be reflected in changes in organ structure. Based on previous findings that increased water temperature reduced TNSC concentration (Botelho and Vanden Heuvel, 2006), elevated water temperatures may similarly affect tissue structure more negatively than would cooler temperatures. However, evidence for such tissue responses to flooding has not been published for cranberry. The objective of this research was to determine the effect of water temperature during spring and fall floods on TNSC and anatomy/morphology of ‘Stevens’ and ‘Early Black’ cranberry vines.

Materials and Methods

Experimental setup for the 2 years of the study is summarized in Table 1.

Year 1 (2004)

Plant material. Actively growing ‘Stevens’ cranberry uprights were collected from the University of Massachusetts Amherst Cranberry Experiment Station research bog (State Bog), E. Wareham, MA (lat. 41°45′N, long. 70°40′W) in May 2003. Four non-rooted cuttings, 10 cm in length, were planted in a 10-cm-diameter pot containing 50 : 50 (by volume) of sand (collected from State Bog) and ‘Berger’ peatmoss (R.F. Morse, Wareham, MA). Pots were placed in a greenhouse at ambient temperature and irrigated daily using overhead mist. Osmocote slow-release fertilizer (14N–6P–11K) (Scotts, Marysville, OH) was applied to the surface of the soil mix ≈4 weeks after planting. Flowers were removed to prevent fruit production. Pots were stored in the dark over the winter at 3 °C (±1) for a minimum of 16 weeks to meet chilling requirements. Pots used for harvest flooding were moved into the greenhouse for the growing season. Pots used for LW flooding treatment were kept in the cooler until just before the simulated flood when they were moved outside to a shaded location. After 48 h, they were moved into the flooding bins.

Flooding treatments. Four large rectangular plastic containers (115 L) were fitted with an inlet and outlet, filled with water, and connected to a Neslab water circulator to control water temperature (one of model RTE-221, RTE-211, or RTE111; Thermo Electron Corp., Waltham, MA). Circulators ran continuously to maintain water temperature. Approximately 700 μmol·m⁻²·s⁻¹ photosynthetic photon flux was supplied to the vines using two HPS greenhouse lamps hung above the bins.

Two floods were simulated on the potted vines: a 4-week LW flood (Botelho and Vanden Heuvel, 2006) under a 13-h daylength (to mimic daylength during LW flooding on a commercial cranberry farm) and a 1-week harvest flood (Botelho and Vanden

Received for publication 12 Sept. 2007. Accepted for publication 2 Oct. 2007.

This research was supported by USDA/CSREES, the Massachusetts Agr. Expt. Sta. Project MAS00875, the Cape Cod Cranberry Growers’ Association, Ocean Spray Cranberries Inc., and the Cranberry Experiment Station.

We gratefully acknowledge the technical assistance of Mary Jean Welser, Amber Awad, and Michelle Salvas and thank Hilary Sandler and Joan Davenport for their assistance with this manuscript.

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Table 1. Summary of cultivars, flooding dates, replications, sampling times, and flood conditions for simulated floods conducted over a 2-year period on potted cranberry vines.

Year	Cultivar	Flood	Dates of flood initiation	No. of complete replications ^z	Times of sampling for carbohydrate quantification ^y	Conditions during flood
2004	S	LW	2004	11 °C—3 reps	0 (preflood)	1 mo
			14 May	21 °C—2 reps +	1 week	H ₂ O temp:
			15 June	3 weeks of 3rd rep	2 weeks	11 or 21 °C
			14 July		3 weeks	Daylength: 13 h
					4 weeks (end)	
2004	S	H	2004	12 °C—4 reps	2 weeks postflood	
			17 Sept.	20 °C—4 reps	22 weeks postflood (harvest)	
			24 Sept.		0 (preflood)	1 week
			1 Oct.		3 d	H ₂ O temp: 12 or 20 °C
					7 d (end)	Daylength: 10.5 h
2005	S, EB	LW	2005	11 °C—3 reps	2-weeks postflood	
			22 Apr.	21 °C—4 reps	0 (preflood)	1 mo
			24 May		2 weeks	H ₂ O temp: 11 or 21 °C
					4 weeks (end)	Daylength: 13 h
					2 weeks postflood	
2005	S, EB	H	2005	12 °C air control—2 reps	0 (preflood)	1 week
			20 Sept.	20 °C air control—2 reps	3 d	Temp: 12 or 20 °C floods; 12 or 20 °C air controls
						Daylength: 10.5 h
			30 Sept.	12 °C water—4 reps	7 d (end)	
				20 °C water—4 reps	2 weeks postflood	

^zBrief power interruptions often caused noncontinuous maintenance of water temperature leading to lost replicates.

^yPreflood and end flood samples were examined for anatomical anomalies of flooding in 2005.

S = Stevens; EB = Early Black; LW = late water flood; H = harvest flood.

Heuvel, 2006) at 10.5-h daylength. For the LW flood, water temperature was maintained at either 11 or 21 °C covering the approximate reported temperature range for LW floods (Botelho and Vanden Heuvel, 2006). The experiment was performed in three blocks (over time) with two replications of each temperature treatment in each block. Floods were initiated 14 May, 15 June, and 14 July 2004. Two pots were removed from each bin at day 0 (before flooding), 1 week, 2 weeks, 3 weeks, and 4 weeks (i.e., end of flood). After the flood, four subsample pots per replication were placed on a greenhouse bench to simulate vines returning to normal conditions on a bog. Two subsample pots from each replication were destructively harvested at 2 weeks postflood, and the remaining two pots per replication were harvested on 4 Nov. 2004. A complete set of data could not be collected for some blocks because brief power interruptions resulted in noncontinuous maintenance of water temperature control. Complete data sets were obtained for three replications of the 11 °C treatment and two replications of the 21 °C treatment as well as the first three sample dates of the third replication of the 21 °C treatment.

For the harvest flood, pots were removed in Mar. 2004 from the cooler and placed in a greenhouse under ambient conditions until the beginning of the flood. Very few flowers formed on the uprights and so all flowers were removed. Floods were initiated 17 Sept., 24 Sept., and 1 Oct. 2004. Water temperature was either 12 or 20 °C, covering the approximate range of those recorded in harvest floods on Massachusetts bogs (Botelho and Vanden Heuvel, 2006). The experiment was performed in three blocks (over time) resulting in complete data sets for four replications of each temperature treatment. Two subsam-

ple pots were removed from each bin at day 0 (before flooding), day 3, and day 7 (i.e., end of flood). After the flood, two pots per replication were placed in the greenhouse to simulate vines returning to normal conditions on a bog and destructively harvested at 2 weeks postflood.

Carbohydrate analysis. All vines in a pot dedicated for carbohydrate analysis were combined, destructively harvested, prepared, and carbohydrate concentration quantified according to the protocol described by Botelho and Vanden Heuvel (2005) with the exception of a change to Empower software (Waters Corp., Milford, MA). When two pots were harvested, carbohydrate analysis was performed separately on the vines in each pot, and then the concentrations of the two subsamples were averaged.

Year 2 (2005)

Plant material. In 2005, vines of 'Stevens' and 'Early Black' were grown according to protocols described for the Year 1 study, although vine cuttings were planted in the greenhouse in 2004.

Flooding treatments. Water temperature treatments for both the LW and harvest flood were the same as in Year 1; however, dates of flood initiation and the sampling protocol changed. Pots of both 'Stevens' and 'Early Black' were used in each bin.

For the LW flood, two subsample pots of each cultivar were removed from each bin at day 0 (before flooding), 2 weeks, and 4 weeks (i.e., end of flood). After the flood, four subsample pots of each cultivar per replication were placed on a greenhouse bench. Two subsample pots from each replication were harvested at 2 weeks postflood; however, the vines placed in the greenhouse to be destructively harvested in the fall showed symptoms of a fungal disease and were not analyzed.

Floods were initiated on 22 Apr. and 24 May with two replications of each temperature in each of the two blocks resulting in four complete replications of the 21 °C treatment and three complete replications of the 11 °C treatment.

For the harvest flood, pots were removed in Mar. 2005 from the cold storage facility and placed in a greenhouse under ambient conditions until the beginning of the simulated flood. Water temperature was maintained at either 12 or 20 °C. Daylength during the flood was 10.5 h. In addition to the flooding treatments, pots were held in Percival growth chambers (PGC-10; Percival Scientific, Perry, IA) at the same temperature as the water treatments (i.e., 12 or 20 °C) to provide a comparison between flooded and unflooded vines at a given temperature. The experiment was performed in two blocks (over time) with samples collected at day 0 (preflood), day 3 (midflood), day 7 (end of flood), and day 21 (2 weeks postflood). Floods were initiated on 20 Sept. and 30 Sept., resulting in four complete replications of each flooding treatment and two replications of each air treatment. After the flood, two pots per replication were placed in a growth chamber at 20 °C under full light conditions and destructively harvested at 2 weeks postflood. Carbohydrate analysis occurred as in Year 1 of the experiment.

Anatomical methods. At the time vines were destructively harvested for carbohydrate analysis in the second year of the study, a portion of the potted vines was used to provide samples of leaves, roots, and internodes of uprights for anatomical analysis both before and after flooding. Roots were divided into three classes for anatomical assessment. In order of decreasing diameter, we prepared and examined fine, very fine, and ultrafine root cross-sections. Segments of each organ

were excised and preserved in a killing fixative solution of formalin–acetic acid–ethanol. Subsequently, they were rinsed, dehydrated, embedded in TissuePrep (Fisher Scientific, Fair Lawn, NJ), sectioned at 8 to 10 μm , mounted on microscope slides, dewaxed, stained, and a coverslip applied using methods in Ruzin (1999). Stains were either safranin and fast green (Ruzin, 1999) or tannic acid–iron alum with safranin and orange gold (Sharman, 1943). The Sharman protocol was also used for starch (amyloplast) localization within our sections, because starch stains bluish black with the tannic acid–iron alum in this stain combination. Sections were viewed and photographed with an Olympus BX60 microscope (Olympus America, Melville, NY) fitted with a Q-Capture digital camera (Quantitative Imaging Corp., Burnaby, British Columbia, Canada) linked to a laptop computer. We specifically looked for the effects of treatments and cultivar on tissue changes that would reflect loss of tissue integrity and, therefore, loss of physiological function.

Statistical analysis. Least square means and SES of the means were calculated using Proc GLM in SAS (SAS Institute, Cary, NC). As a result of the incomplete data set (detailed in Table 1, as a result of the loss of temperature control in the flooding bins), significant differences among treatments were determined by differences between SES of the means.

Results

Year 1 ('Stevens')

Data from Year 1 (2004) are not presented because carbohydrate declination patterns during flooding were very similar to those seen for 'Stevens' in the Year 2 study (2005).

Year 2 ('Early Black' and 'Stevens')

Late water flood. Soluble carbohydrate concentration declined in uprights of 'Early Black' (Fig. 1A) and 'Stevens' (Fig. 1D) during the LW flood. By the end of the flood, greater reductions in soluble carbohydrate concentration occurred in uprights of both cultivars flooded at 21 °C (63% and 61% in 'Early Black' and 'Stevens', respectively) than uprights flooded at 11 °C (21% and 27% for 'Early Black' and 'Stevens', respectively). Four weeks of flooding had little effect on starch concentration of 'Early Black' (Fig. 1B) and 'Stevens' (Fig. 1E) flooded at 11 °C, but starch concentration was 40% lower in uprights of 'Early Black' subjected to flooding at 21 °C. TNSC concentration was generally unaffected after 4 weeks of flooding in 'Early Black' at 11 °C but was reduced by 54% when vines were flooded at 21 °C. In 'Stevens', TNSC concentration was reduced by 21% in the uprights flooded at 11 °C and by 56% in uprights flooded at 21 °C.

At 2 weeks postflood, soluble carbohydrate concentration of 'Early Black' uprights flooded at 11 °C was 85% greater than that of uprights flooded at 21 °C (Fig. 1A). Starch (Fig. 1B) concentration was 95% greater and

Fig. 1. Cranberry uprights in 4-week late water flood: the effect of water temperature during a simulated flood at 11 and 21 °C on the carbohydrate concentration ($\pm$ SE) of 'Early Black' (A–C) and 'Stevens' (D–F) uprights in 2005. Arrows indicate time of removal of vines from the water after imposition of the flood. Week 6 samples were collected at 2 weeks postflood.

TNSC was 86% greater (Fig. 1C) in the uprights flooded at 11 °C than in uprights flooded at 21 °C. In 'Stevens' uprights, soluble carbohydrate concentration was 36% greater at 2 weeks postflood in uprights flooded at 11 °C than in uprights flooded at 21 °C; however, there were no differences between treatments with regard to starch concentration or TNSC concentration at that time.

Soluble carbohydrate concentration in roots of both cultivars was unaffected by 4 weeks of flooding at 11 °C, but concentration was reduced by 51% in 'Early Black' (Fig. 2A) and by 57% in 'Stevens' (Fig. 2D) roots

flooded at 21 °C. Starch concentration was greatly reduced in 'Early Black' roots of both treatments (Fig. 1B) (approximate reduction of 44%), but starch concentration in 'Stevens' root tissue was unaffected (Fig. 2E). TNSC concentration was reduced by 30% in 'Early Black' roots flooded for 4 weeks at 11 °C (Fig. 1C) and by 51% in roots flooded at 21 °C. TNSC concentration in 'Stevens' roots was unaffected by flooding at 11 °C, but concentration was reduced by 30% in roots flooded at 21 °C (Fig. 2F).

At 2 weeks postflood, 'Early Black' root tissue subjected to flooding at different

Fig. 2. Cranberry roots in 4-week late water flood: the effect of water temperature during a simulated flood at 11 and 21 °C on the carbohydrate concentration ($\pm$ SE) of 'Early Black' (A–C) and 'Stevens' (D–F) roots in 2005 at 11 and 21 °C. Arrows indicate time of removal of vines from the water after imposition of the flood. Week 6 samples were collected at 2 weeks postflood.

temperatures did not differ with respect to concentration of soluble carbohydrates (Fig. 2A), starch (Fig. 2B), or TNSC at the two temperatures (Fig. 2C); however, 'Stevens' roots flooded at 11 °C had significantly greater concentrations of soluble carbohydrates (81%) (Fig. 2D), starch (44%) (Fig. 2E), and TNSC (61%) (Fig. 2F) than did roots flooded for 4 weeks at 21 °C.

Harvest flood. For this flood, we conducted air (i.e., nonflooded) controls during the experiment at the same temperatures as those in the flood treatments. As in 2004 (data not shown), flooding at both temper-

atures caused a large decrease in nonstructural carbohydrate concentration (Fig. 3A–C). During a 1-week flood, concentrations of soluble carbohydrates, starch, and TNSC decreased, but concentrations of each of these parameters did not differ for 'Early Black' uprights flooded at different temperatures (Fig. 3A–C). During the flooding period, vines placed in air at either temperature demonstrated an increase in nonstructural carbohydrate concentration compared with flooded vines (Fig. 3A–C). Vines in air at 12 °C had a 28% increase in soluble carbohydrate concentration over that of vines in air at

20 °C (Fig. 3A). As a result, uprights subjected to flooding for 1 week had TNSC concentrations ranging from $\approx$ 2.2 to 2.5 mg/100 mg, whereas uprights in air at similar temperatures during the same period had TNSC concentrations ranging from $\approx$ 5.4 to 5.8 mg/100mg (Fig. 3C). Similar to 'Early Black', there were no significant differences in nonstructural carbohydrate concentration in 'Stevens' vines subjected to flooding at 12 and 20 °C (Fig. 3D–F). After the 1-week harvest flood, upright TNSC concentration ranged from $\approx$ 3.2 to 3.5 mg/100 mg, whereas concentration in uprights in air at similar temperatures ranged from $\approx$ 6.2 to 6.5 mg/100 mg (Fig. 3F).

Roots of 'Early Black' vines subjected to 1 week of simulated flooding at 20 °C had lower (33% or more) soluble carbohydrate concentrations than did roots flooded at 12 °C and roots of plants kept in air at similar temperatures (Fig. 4A). There were no apparent differences in starch concentration among treatments (Fig. 4B); however, vines flooded at 20 °C had slightly lower TNSC concentrations in the roots compared with those in air (Fig. 4C). Roots of 'Stevens' vines subjected to flooding contained lower soluble carbohydrate concentrations than those grown at similar temperatures in air for a 1-week duration (Fig. 4D). Roots of vines grown in air at 20 °C contained more starch and TNSC than did vines subjected to flooding at either temperature (Fig. 4E–F).

At 2 weeks postflood (i.e., 2 weeks after removal from the floods), there were no significant differences in the concentration of soluble carbohydrate, starch, or TNSC of uprights among treatments in either 'Early Black' or 'Stevens' (Fig. 3A–F). There were also no differences among treatments with respect to concentration of nonstructural carbohydrates in root tissue (Fig. 4A–F) with the exception of starch concentration in roots of 'Early Black' flooded at 20 °C, which contained significantly lower starch than did roots subjected to other treatments (Fig. 4B).

Anatomical Studies (2005)

Leaf anatomy. After 4 weeks of LW flooding at either 11 or 21 °C, some leaves of both cultivars showed signs of clumping of chloroplasts and plugging of vascular elements in fine vascular bundles (Fig. 5). The upper, but not lower, leaf surface was often matted with fungi (Fig. 5C), which in some instances appeared to compromise the upper epidermis. This was not a consistent problem and, often, leaves subjected to 4 weeks of warm water did not have any internal structural problems compared with leaves before flooding. After 1 week of harvest flooding at either 12 or 20 °C, we had difficulty discerning any structural differences between leaves collected pre- and postflood in either cultivar.

Woody stems and major roots. The permanent woody organs sampled from cranberry plants after the LW flood or harvest flood yielded no clearly defined influence of water temperature on tissue structure. Internodes were well protected from either desiccation

Fig. 3. Cranberry uprights in 1-week flood or air controls: the effect of air and water temperature at 12 and 20 °C on the carbohydrate concentration ($\pm$ SE) of 'Early Black' (A–C) and 'Stevens' (D–F) uprights in 2005. Arrows indicate time of removal of vines from the water after imposition of the flood. Day 21 samples were collected at 2 weeks postflood.

or waterlogging by the production of a heavy layer of waxy periderm tissue, which covered the phloem and xylem. This was so, even though the stem surface was often coated with a fungal mat after 4 weeks of LW flooding (data not shown).

Large roots before and after the LW and harvest floods were similar in anatomical characteristics (data not shown). As in stems, these large roots were also well protected by a heavy layer of waxy periderm.

Conductive elements in both xylem and phloem were well organized and little to no breakdown could be seen, even after flooding for 4 weeks, as in the case of LW. A fungal mat was rare on flooded roots. All cranberry roots examined anatomically showed good starch deposition (based on staining of amyloplasts) only in the periderm, even in the nonflooded specimens, with occasional specimens showing significant deposits in the wood rays.

Anatomy of small roots. Cranberry develops a mass of fine roots and fibrous roots that make up most of the volume of the nutrient-absorbing system of cranberry. Fine roots did not show definitive negative impact after 4 weeks of LW flooding. Fine roots had a central core of xylem vessels, fibers, and parenchyma cells, including large ray cells, with a thin band of surrounding phloem that, in turn, was covered by a periderm. Very fine and ultrafine root cross-sections had very small amounts of xylem and phloem surrounded by a single endodermal layer that was covered by either a thin cortex layer or directly by the epidermal cell layer (data not shown). Very fine roots often contained mycorrhizal fungi in their epidermal cells, but ultrafine roots always appeared to contain such symbionts.

Very fine and ultrafine roots of the two cultivars in the harvest flood treatments appeared to be in a constant state of root "turnover." There was always a significant proportion of the root population in a senescent state, even in plants not subjected to flooding (data not shown). Neither 'Early Black' nor 'Stevens' showed increased fine-root senescence during the 1-week harvest flood at 12 °C (data not shown). Depending on the particulars of the sample, more or fewer roots would be found in the senescent state. Nevertheless, very fine and ultrafine roots did not appear to fare as well at the 20 °C flooding temperature as they did at 12 °C. We often saw increased numbers of dark materials in such roots flooded at this temperature and higher proportions of these roots appeared to be senescing (Fig. 6).

Discussion

Higher water temperature resulted in decreased concentrations of soluble carbohydrates, starch, and hence TNSC during the LW flood in both 'Early Black' and 'Stevens'. These findings confirm a previous negative relationship between water temperature and TNSC concentration noted by Botelho and Vanden Heuvel (2006) when studying flooded commercial cranberry beds. The detrimental effect of water temperature was still apparent at 2 weeks postflood in 'Early Black'. Two weeks after a LW flood (i.e., early June in Massachusetts) is when resources are accumulating in the vine (Botelho and Vanden Heuvel, 2006) in preparation for fruit set. Although TNSC concentration did not differ between treatment temperatures in 'Stevens' vines harvested at 22 weeks postflood in Year 1 of the study (data not shown), partitioning between soluble carbohydrates and starch varied in the LW uprights in Year 2 with 67% and 53% of TNSC existing as soluble carbohydrates in uprights flooded at 11 and 21 °C, respectively. This difference in carbon partitioning may have a long-term effect on productivity of the bog.

The impact of water temperature on TNSC was much less during the harvest flood than during the LW flood. However, flooding

Fig. 4. Cranberry roots in 1-week flood or air controls; the effect of air and water temperature at 12 and 20 °C on the carbohydrate concentration ($\pm$ SE) of 'Early Black' (A–C) and 'Stevens' (D–F) roots in 2005. Arrows indicate time of removal of vines from the water after imposition of the flood. Day 21 samples were collected at 2 weeks postflood.

at either temperature during the harvest flood had an impact on TNSC, whereas high water temperature was more influential than low water temperature for LW floods. The detrimental effects of fall flooding have also been noted in field studies of cranberry (Botelho and Vanden Heuvel, 2006). Flooding during the fall, even at cool temperatures, severely diminished TNSC concentration, particularly of uprights.

The ability of cranberry vines to recuperate after a detrimental flooding event in the

fall remains unclear. Increases in TNSC concentration in uprights and roots during the fall are mostly the result of an increase in soluble carbohydrate concentration (Botelho and Vanden Heuvel, 2006). Two weeks after the harvest flood, both soluble carbohydrate and starch concentrations were higher compared with the concentrations in the vines sampled at the end of flooding in both years and both cultivars. Both uprights and roots were able to replace respired carbohydrates, so that at 2 weeks postflood, few differences

existed between flooded and nonflooded treatments, perhaps as a result of the optimal conditions in the growth chamber. It is possible that conditions for photosynthesis (and replacement of lost carbohydrate reserves) may not be as optimal on a commercial cranberry bog. The opportunity for carbohydrate accumulation during the fall may be lost during periods of flooding for various pest management and horticultural reasons.

Anatomical examination of stems of uprights and of the large and fine roots showed that these persistent structures were minimally affected by flooding. This was the case for both cultivars, both flood water temperatures, and for both the LW and harvest floods that we imposed. In both cultivars, phloem and xylem tissues appeared similar in both flooded and nonflooded woody organs taken from vines exposed to LW or harvest floods. There was little evidence of xylem vessel plugging or loss of integrity of phloem-conductive elements and supporting cell types.

Crane and Davies (1989) reported little external evidence of deterioration of leaves of root-flooded cranberry plants (i.e., leaf chlorosis, reddening, necrosis, and leaf abscission). In our whole-plant immersion studies, we also saw few negative effects of flooding on external leaf morphology or on leaf abscission in the timespan of the simulated floods. Leaf structure appeared negatively affected by flooding only in some examined leaves after the LW flood. The most obvious problems were clumping of chloroplasts in the palisade layer and browning (and presumably occlusion) of vascular elements in the conductive bundles. However, these characteristics were not universal with flooding. Leaves under harvest flooding had few anomalies, perhaps because of the short (1-week) flood duration or perhaps because they were approaching dormancy at the time the flood was initiated.

We did not see the severe changes in leaf structure reported for root-flooded highbush blueberry plants by Abbott and Gough (1987). Their observations included greatly shortened palisade cells, a highly disorganized spongy layer with increased intercellular spaces, and a thinned epidermal cell layer. Such tissue changes are likely a result of developmental changes in cell organization and cell-to-cell connectivity that occur as leaves finish growth. Such changes are highly unlikely in flooded mature cranberry leaves such as the middle leaves on uprights that we studied. Our mature leaves were also more likely to be in a stable or declining physiological state and, so, less affected by flood water conditions. The stomates of cranberry may not adjust rapidly to varying conditions of light, temperature, and moisture (Sawyer, 1932), which might be a factor in the resistance to flooding seen in the mature leaves.

Current-season leaves in cranberry are almost wholly responsible for providing carbon for fruit growth (Roper and Klueh, 1996). We have conducted some preliminary work

Fig. 5. Cross-sections of cranberry leaves taken before and after a 4-week late water flood. (A) 'Stevens' before flooding. Palisade cells have well-defined chloroplasts, as do spongy cells. Vascular bundle has a small group of xylem and phloem elements. (B) 'Stevens' after 4 weeks of flooding at 21 °C shows clumping of chloroplasts (arrow) and occluded vascular tissues (arrowhead). (C) 'Early Black' after 4 weeks of flooding at 21 °C has fungal mat on upper epidermis, possible epidermal erosion (arrow), and possible vascular occlusion (arrowhead).

Fig. 6. (A) 'Early Black' fine cranberry roots before flooding. (B) 'Early Black' fine roots after 1-week harvest flood at 20 °C. Note the darker, more senescent roots after flooding.

on the impact of flooding treatments on tissue organization of very young leaves and internodes within the shoot apical region, but thus far we have not found obvious detrimental effects of the treatments in these organs or their tissues. Future work on effects of flooding on shoot tissue injury should include more work on these younger organs to determine the range of vulnerability to flooding stress along the upright's gradient in organ production and maturation.

Abbott and Gough (1987) described young white roots initiated under root flooding as developing cortical aerenchyma. However, we rarely saw new white root tips in our flooding experiments and few also in our nonflooded control plants over the course of the harvest flood, suggesting that few roots are initiated late in the season (DeMoranville, 1992). Thus, the roots we sectioned were

roots that were already in place for some time, including the fine and very fine roots. Because aerenchyma develops during primary growth of root tips under flooding, we did not see aerenchyma.

This investigation contributes the first evidence that physiological degradation of cranberry plants under seasonal flooding may in part be caused by direct tissue senescence, at least in the fine roots. Fine, very fine, and ultrafine roots of cranberry were characterized by anatomical features that could foster susceptibility to flooding and desiccation stresses, namely, large surface-to-volume ratios, fewer cell layers providing a barrier against anoxia and temperature stress, a greater rate of natural root senescence, and (for very fine and ultrafine roots) the critical symbiotic occupation of outer root cells by beneficial, symbiotic mycorrhizal fungi.

Warm harvest flood water may predispose the finest roots to premature senescence more so than cooler water. In addition, the clumping of chloroplasts and occlusion of vascular tissues noted in some leaves may reduce the ability of the vine to recover after removal of the flood.

The anatomical impacts of warm flood water combined with the detrimental impact on TNSC during both LW and harvest flooding suggests that water temperature should be closely monitored by cranberry growers during floods. If effective pest control can be obtained, shorter flood durations should be considered. This work supports the recommendations of Botelho and Vanden Heuvel (2006) with regard to flood duration and temperature.

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