

Early-season Air Temperature Affects Phenolic Production in ‘Early Black’ Cranberry Fruit

Justine E. Vanden Heuvel^{1,2}

University of Massachusetts Amherst, Cranberry Experiment Station, 1 State Bog Road, P.O. Box 569, East Wareham, MA 02538

Wesley R. Autio

Department of Plant, Soil, and Insect Sciences, University of Massachusetts Amherst, Amherst, MA 01003

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Abstract. Anecdotal information has linked cool air temperatures before harvest with increased phenolic production in cranberry; however, there is little information available on the effect of temperature on phenolic production in cranberry fruit. The objective of this study was to determine the effect of air temperature throughout the growing season on the concentration of total anthocyanins, total flavonols, and total phenolics at harvest in fruit from seven ‘Early Black’ bogs in southeastern Massachusetts. Contrary to the anecdotal information available, correlations of temperature to fruit composition indicated that warmer temperatures early in the season (around bloom and fruit set) had the most positive impact on total anthocyanins and total flavonols. Total phenolic concentration in the harvested fruit was impacted by air temperature in the preharvest period; however, that relationship was positive.

In recent years, cranberries (*Vaccinium macrocarpon* Ait.) have been recognized as a major source of phenolic compounds in the human diet. Research is accruing that demonstrates the positive health benefits of cranberries (Neto et al., 2005, and references therein). Phenolic compounds of medicinal interest in cranberry consist primarily of flavonoids, which can be induced by environmental factors such as light (Onayemi et al., 2006) and fungal infection (Vvedenskaya et al., 2004). Flavonoids can further be classified into smaller subgroups such as anthocyanins, flavonols, and proanthocyanidins.

Little information exists concerning the development of secondary metabolites in cranberry fruit. There are variations in the flavonoid content among different cultivars of cranberry (Vvedenskaya and Vorsa, 2004;

Wang and Stretch, 2001), and berry size (surface to volume ratio) affects anthocyanin content (Sapers et al., 1986; Vorsa and Welker, 1985). Improved light interception by the fruit can improve total anthocyanins (TAcy) (Boulanger and Singh, 1997; Zhou and Singh, 2002) as well as total flavonols (TF) (Onayemi et al., 2006). Production of TAcy and TF in the fruit appears to be regulated independently of each other in cranberry (Onayemi et al., 2006; Vvedenskaya and Vorsa, 2004) and is likely affected by berry environment as well as the whole plant environment.

Cranberry growers have anecdotally linked cool night temperatures before harvest

to high TAcy concentration; however, with the exception of some early work with detached berries (Hall and Stark, 1972), there is no information available on the effect of temperature on phenolic production in cranberry fruit. Information detailing environmental impacts on phenolic production is sparse in other fruits as well, but in grapes, increased heat summation between fruit set and veraison has been associated with an increase in proanthocyanidin content (Pastor del Rio and Kennedy, 2006), whereas high night temperatures were demonstrated to hinder expression of anthocyanin biosynthetic genes at an early stage of ripening (Mori et al., 2005).

As a result of the interest in cranberry as a source of phytonutrients, and the reduced price growers receive from handlers for fruit low in TAcy, investigations into development of phenolic compounds in cranberry are highly warranted. The objective of this study was to determine the effects of air temperature throughout the growing season on production of TAcy, TF, and total phenolics (TP) in ‘Early Black’ cranberries.

Materials and Methods

Seven bogs of ‘Early Black’ cranberries intended for dry harvest were selected before bloom in 2005 for the 2-year experiment. Dry-harvested bogs were selected so that upright density would be similar among bogs, because dry harvesters prune uprights as they are removing fruit if a Furford or Western picker is used (DeMoranville and Sandler, 2000). All sites were located in southeastern Massachusetts (Table 1) and were owned by a single cranberry company and managed similarly. Latitudes and longitudes of bog locations were: Rochester: 41.73°N, 70.82°W; West Wareham: 41.79°N, 70.75°W; and Carver: 41.88°N, 70.76°W.

Hobo dataloggers (Onset Computer Corp., Bourne, MA) containing thermocouples with radiation shields were nestled into the canopy of uprights at a randomly selected vine location on each bog beginning on

Table 1. Total flavonols, total anthocyanins, and total phenolics measured in fruit harvested during 2005 and 2006 from seven southeastern Massachusetts farms planted with ‘Early Black’ cranberry vines (n = 3)^a.

Year	Location	Bog	Total flavonols (mg/100 g FW)	Total anthocyanins (mg/100 g FW)	Total phenolics (GA equiv. mg·g ⁻¹ DW)
2005	West Wareham, MA	1	11.59 c	7.63 c	7.07 b
	West Wareham, MA	2	22.73 a	20.54 a	10.33 a
	West Wareham, MA	3	15.82 bc	10.85 bc	7.07 b
	Rochester, MA	4	20.61 ab	14.87 abc	11.50 a
	Carver, MA	5	22.27 ab	19.93 ab	9.93 ab
	Carver, MA	6	18.15 ab	16.53 ab	9.07 ab
	Carver, MA	7	21.34 ab	17.68 ab	7.35 b
2006	West Wareham, MA	1	14.88 b	12.58 c	4.03 a
	West Wareham, MA	2	20.30 ab	29.07 a	6.93 a
	West Wareham, MA	3	17.33 ab	13.06 bc	4.57 a
	Rochester, MA	4	20.42 a	22.01 abc	6.11 a
	Carver, MA	5	18.55 ab	24.55 ab	4.92 a
	Carver, MA	6	17.73 ab	24.26 abc	7.25 a
	Carver, MA	7	18.32 ab	28.87 a	5.23 a

^aValues within column and year followed by similar letters are not significantly different according to Tukey’s honestly significant difference ($P = 0.05$).

FW = fresh weight; GA = gallic acid; DW = dry weight.

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¹Current address: Department of Horticultural Sciences and Department of Horticulture, Cornell University, Ithaca, NY.

²To whom reprint requests should be addressed; e-mail jev32@cornell.edu

Table 2. Average night temperature (AN), average day temperature (AD), daily minimum temperature (MIN), daily maximum temperature (MAX), and daily average temperature (AVG) in °C through bloom, fruit set, fruit growth, and preharvest periods for 2005 and 2006 on seven southeastern Massachusetts farms planted with 'Early Black' cranberry vines.

Year	Bog	Bloom			Fruit set			Fruit growth			Preharvest		
		AN	AD	MIN	MAX	AVG	AN	AD	MIN	MAX	AVG	AN	AD
2005	1	12.9	20.8	9.5	28.3	17.2	18.3	25.1	14.7	31.0	22.0	18.5	25.2
	2	13.5	20.5	10.7	26.8	17.3	19.1	25.0	16.5	30.4	22.3	19.6	26.1
	3	13.1	20.7	9.9	27.1	17.2	18.6	25.5	15.3	31.5	22.2	18.8	26.0
	4	13.3	20.2	10.2	27.0	17.0	18.8	25.3	16.0	31.5	22.3	19.3	25.8
	5	13.9	20.2	11.2	26.7	17.3	19.6	24.8	17.2	29.9	22.4	20.5	26.6
	6	13.4	20.2	10.6	26.4	17.1	19.5	26.1	16.6	32.0	23.0	19.7	26.9
	7	13.5	20.7	10.3	27.4	17.3	19.0	25.2	16.0	31.2	22.3	19.7	26.5
2006	1	— ^z	—	—	—	—	18.2	25.4	16.3	30.9	22.7	16.9	26.2
	2	—	—	—	—	—	18.5	25.5	16.7	30.7	22.9	17.3	26.1
	3	—	—	—	—	—	17.8	25.5	15.7	31.2	22.6	16.6	25.9
	4	—	—	—	—	—	18.9	25.8	17.3	31.4	23.2	18.3	25.8
	5	—	—	—	—	—	19.0	25.7	17.5	30.9	23.2	18.0	26.1
	6	—	—	—	—	—	18.4	25.0	16.8	30.2	22.5	17.6	24.8
	7	—	—	—	—	—	17.8	25.6	16.1	31.6	22.7	16.5	26.0

^zBloom temperature data were not collected in 2006 as a result of equipment malfunction.

Table 3. Growing degree day accumulations (base 4.4 °C) through bloom, fruit set, fruit growth, and preharvest periods for 2005 and 2006 on seven southeastern Massachusetts farms planted with 'Early Black' cranberry vines.

Year	Bog	Bloom	Fruit set	Fruit growth	Preharvest	Total (fruit set through harvest)
2005	1	535	460	579	264	1,303
	2	534	479	622	291	1,392
	3	519	474	592	280	1,346
	4	523	482	613	293	1,388
	5	536	478	631	292	1,401
	6	520	496	652	298	1,446
	7	529	476	598	282	1,356
2006	1	— ^z	459	593	287	1,339
	2	—	463	591	300	1,354
	3	—	456	582	295	1,332
	4	—	477	603	312	1,392
	5	—	475	609	311	1,394
	6	—	457	572	301	1,330
	7	—	466	602	305	1,372

^zBloom temperature data were not collected in 2006 as a result of equipment malfunction.

20 May 2005 and 26 June 2006. Air temperature readings were initiated at midnight and then recorded every 3 h for the duration of the season. Loggers were downloaded as needed.

To determine the impact of air temperature for different periods during the growing season, the season was divided into the following phenological stages: bloom (20 May to 25 June 2005; bloom data not collected in 2006 as a result of equipment malfunction), fruit set (26 June to 20 July 2005; 26 June to 20 July 2006), fruit growth (21 July to 20 Aug. 2005; 21 July to 20 Aug. 2006), and preharvest (21 Aug. to 5 Sept. 2005; 21 Aug. to 10 Sept. 2006). Within each of the four phenological periods (bloom, fruit set, fruit growth, and preharvest), data from each bog within each year were used to calculate daily average, average high, average low, average night, and average day temperatures as well as the average difference between the low and the high temperature for each 24-hr period (Table 2). Growing degree day (GDD) accumulations (base 4.4 °C) were calculated for each bog during each phenological stage (Table 3).

Fruit were harvested from all bogs on the same date (6 Sept. 2005; 11 Sept. 2006), which was determined by the first commercial harvest date at one of the sites. Three random samples of ≈500 g each were picked from throughout the canopy (i.e., top through bottom) using a hand scoop within 3 m of the datalogger. Fruit were sorted to remove rotted berries, and a random subsample of ≈150 g was frozen in liquid nitrogen for further analysis. Quantification of TAc, TF, and TP followed the method of Onayemi et al. (2006); however, TP was calculated as gallic acid equivalents as opposed to catechin equivalents, and a Hitachi U-1100 (Hitachi America Ltd., Brisbane, CA) spectrophotometer was used.

All temperature means were correlated using PROC CORR (SAS Institute, Cary, NC) with TAc, TF, and TP concentrations measured for each bog in each year with data combined across bogs and years. Differences in TAc, TF, and TP among bogs were determined by analysis of variance (using SAS PROC MIXED), and means were separated with Tukey's honestly significant difference ($P = 0.05$).

Results

TAc and TF differed among bogs in both years, and TP differed among bogs in 2005 but not 2006 (Table 1). TAc ranged from 7.63 to 20.54 mg/100 g fresh weight in 2005 and from 12.58 to 29.07 mg/100 g fresh weight in 2006. TF ranged from 11.59 to 22.73 mg/100 g fresh weight in 2005 and from 14.88 to 20.42 mg/100 g fresh weight in 2006. TP ranged from 7.07 to 11.50 mg·g⁻¹ dry weight in 2005 and did not differ significantly in 2006 ranging from 4.03 to 7.25 mg·g⁻¹ dry weight. In general, trends were similar between years with Bog 1 and Bog 2 producing fruit with the lowest and highest TAc and TF concentrations, respectively. TAc was positively correlated with TF ($r = 0.506$, $P = 0.036$), but not with TP ($P = 0.801$). TF and TP were not significantly correlated ($P = 0.064$).

Average night temperature through bloom (Table 2) was positively correlated with TAc concentration in the harvested fruit (Fig. 1A). Similar to TAc, TF in harvested fruit was positively correlated with average night temperature through bloom and fruit set (Fig. 1B), whereas average night temperature through fruit set, fruit growth, and preharvest was positively correlated with TP concentration in harvested fruit. The only significant correlation between fruit chemistry and average day temperature occurred with TP in harvested fruit (Fig. 1C).

Air temperature over a 24-h period (midnight to midnight) (Table 2) through the growing season impacted fruit composition at harvest. TAc concentration in harvested fruit was positively correlated with daily minimum temperature and negatively correlated with temperature differential through bloom and fruit set (Fig. 2A). Average daily temperature was positively correlated with TAc concentration through fruit set (Fig. 2A). After fruit set, temperature had no effect on TAc (Fig. 2A). Daily minimum temperature through bloom, fruit set, and fruit growth was positively correlated with TF in harvested fruit, whereas temperature differential through bloom and fruit set was negatively correlated with TF at harvest (Fig. 2B). Average daily temperature during fruit

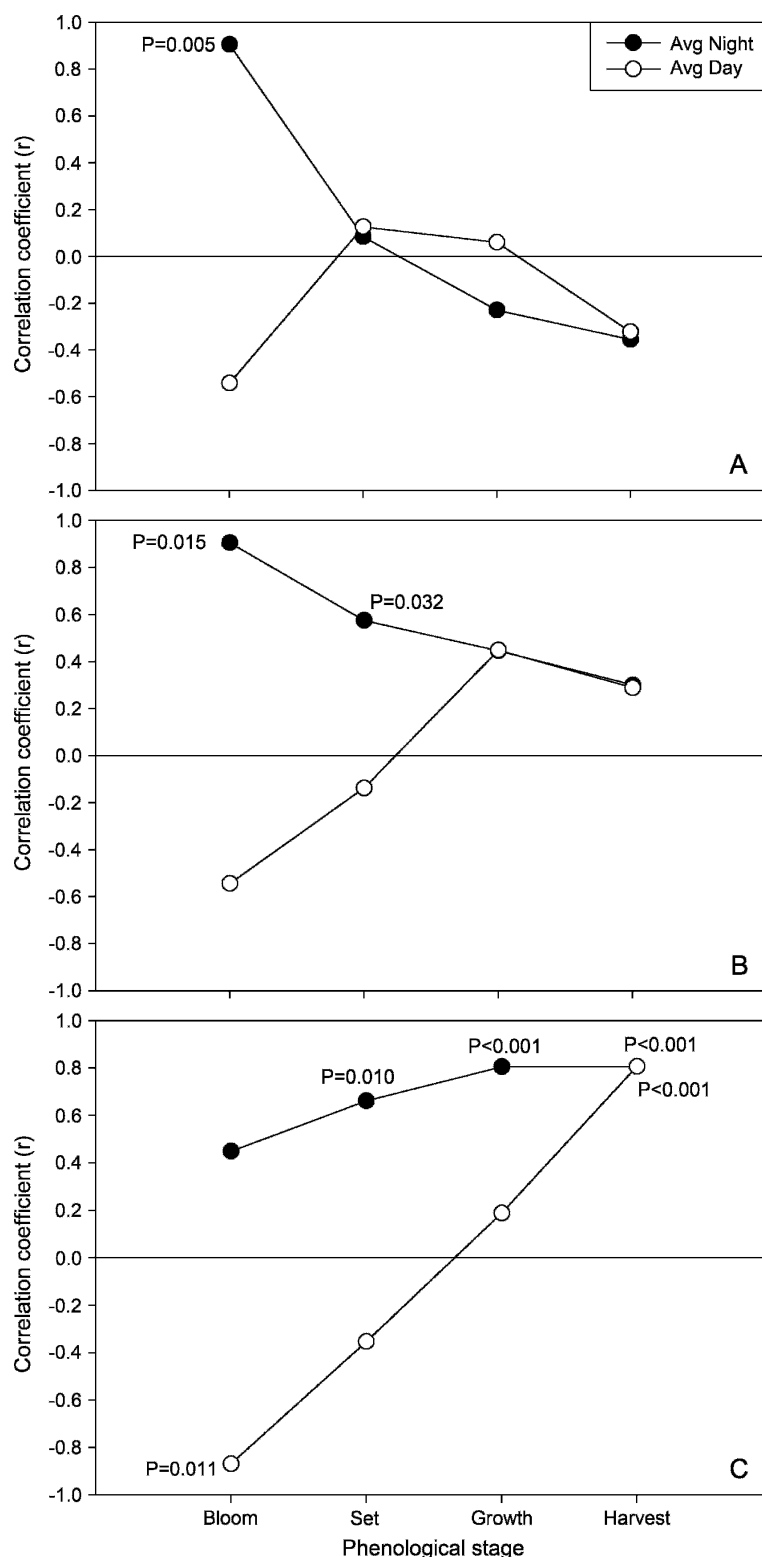


Fig. 1. Correlation coefficients of the relationship of average night and average day temperature at four phenological periods to (A) total anthocyanins, (B) total flavonols, and (C) total phenolics in 'Early Black' cranberry fruit collected at harvest. Three subsamples of fruit were collected from seven southeastern Massachusetts cranberry farms over a 2-year period ($n = 42$ for each point except bloom where $n = 21$). Significant P values are positioned next to the relevant phenological data point.

growth was positively correlated with TF measured in harvested fruit (Fig. 2B).

Although TAc and TF concentrations were most affected by temperatures early in the growing season, TP was most affected later in the season. Daily minimum temper-

ature for the fruit growth and preharvest periods was positively correlated with TP in harvested fruit, and daily maximum temperature and average temperature late in the season were positively correlated with TP in harvested fruit (Fig. 2C).

Discussion

The data suggest a large variation in phenolic concentration of 'Early Black' cranberries growing in Massachusetts within a given season, even on bogs located geographically close to one another and grown using similar production practices. Interestingly, Bog 1 was generally lowest in phenolic concentration; however, it was the first bog harvested by the company in both years of the study. Commercial harvest occurred in early September in both years before significantly cold temperatures on the bog, thereby limiting our ability to determine the impact of cold fall temperatures and mild frost events on phenolic concentration of berries.

There are several possible explanations for differences in fruit phenolic concentrations among bogs, the most likely being microclimatic differences resulting from a number of factors, including proximity to the ocean, elevation, and surrounding topography. Although the furthest distance between bogs was ≈ 20 km, the microclimates at each bog differed a great deal in temperature with average air temperatures through a given period differing by as much as 3.0°C (Table 2), whereas at any given time, temperatures differed by as much as $\approx 6^{\circ}\text{C}$ (data not shown). Growing degree day accumulations during the defined phenological stages also varied (Table 3). Although not measured in this study, it is likely that light microclimate in the canopy was similar among bogs, because dry harvest machines prune the uprights to a given density (DeMoranville and Sandler, 2000), and the same dry harvest machines were used on all bogs in the study.

Differences in phenolic concentration of fruit among bogs may be the result of differences in fruit size, which was not recorded in this study. Fruit size impacts phenolic concentrations when quantified on a whole-berry basis through changes in the surface-to-volume ratio (Sapers et al., 1986; Vorsa and Welker, 1985), and fruit size can be impacted by air temperature (DeMoranville et al., 1996). Another possible explanation for the wide variation noted in phenolic production could be the existence of differing genotypes (both on a single bog and among bogs), because studies have revealed significant genotypic variation within the same cranberry cultivar (Novy and Vorsa, 1995; Novy et al., 1996). However, all fruit were visually determined to have 'Early Black' features and ripened according to the characteristics associated with the cultivar (DeMoranville, 2004).

Anecdotal information regarding cranberry suggests that cooler night temperatures (or large temperature differentials or a light frost) before harvest increase TAc concentrations, but this study suggests otherwise. Average night temperatures through bloom were positively correlated to TAc in harvested fruit (Fig. 1A) suggesting that higher temperatures, not lower temperatures, actually improve TAc (as well as TF and, later in the season, TP). Temperature had no effect

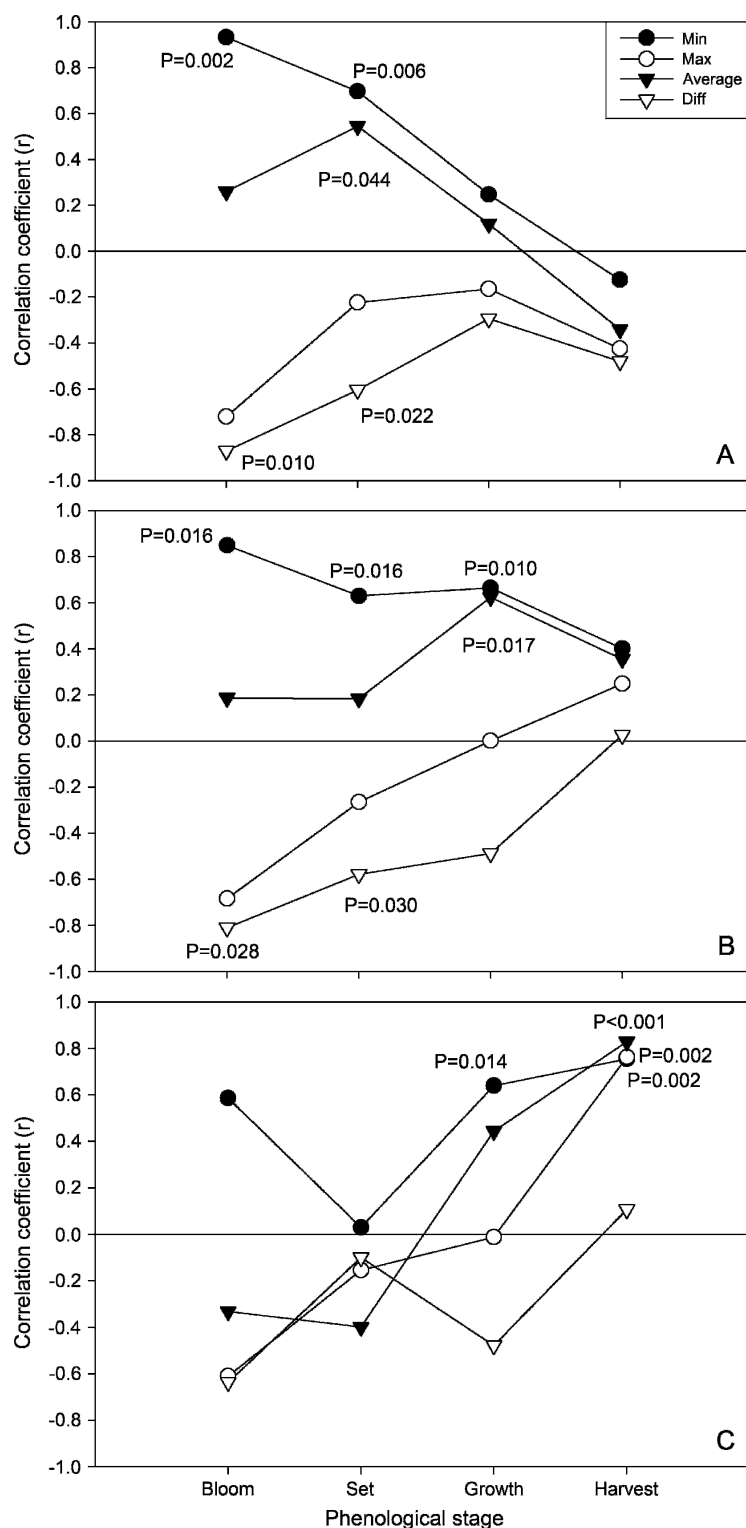


Fig. 2. Correlation coefficients of the relationship of daily maximum temperature, daily minimum temperature, daily average temperature, and daily differential temperature (maximum–minimum) at four phenological periods to (A) total anthocyanins, (B) total flavonols, and (C) total phenolics in ‘Early Black’ cranberry fruit collected at harvest. Three subsamples of fruit were collected from seven southeastern Massachusetts cranberry farms over a 2-year period ($n = 42$ for each point except bloom where $n = 21$). Significant P values are positioned next to the relevant phenological data point.

on TAcY and TF later in the growing season (Fig. 1), indicating that high temperatures before harvest do not detrimentally impact TAcY in ‘Early Black’ cranberries as they do in grapes (Spayd et al., 2002). It is not clear how warmer temperatures through bloom are

positively influencing TAcY and TF in harvested fruit, because the fruit are not yet formed at that time. One hypothesis is that higher temperatures through bloom accelerated plant development (Table 3), resulting in advanced fruit maturity at those locations.

This hypothesis is supported by the GDD data demonstrating that Bog 1, which was lowest in TF, TAcY, and TP in both years when compared with the other bogs, had the lowest GDD accumulations through fruit set, growth, and preharvest in 2005. Additionally, the correlation between high temperatures at bloom and increased phenolic production could be a function of temperature impacting photosynthesis and hence carbon availability in vegetative tissues for precursors. The relationship between carbon availability and phenolic production in cranberry is not clear, because TAcY has been negatively correlated with nonstructural carbohydrate concentrations in the uprights, whereas TF has been positively correlated with nonstructural carbohydrate concentrations (Onayemi et al., 2006). In addition, the effects of temperature on photosynthesis of cranberry are unclear, and high temperatures have demonstrated little detrimental impact on carbon exchange (Kumudini, 2004; Vanden Heuvel and Davenport, 2005). Another possibility is that higher temperatures may lead to increased fungal pressure resulting in formation of phenolic compounds (or their precursors) as a stress response (Vvedenskaya and Vorsa, 2004) early in the season.

Minimum daily temperature was also positively correlated to TAcY and TF early in the season and to TP later in the season, suggesting that low temperatures may detrimentally impact phenolic production. Temperature differential (maximum–minimum) was negatively correlated to TAcY and TF through bloom and through fruit set, again in contrast to the anecdotal information existing in the industry. This may again be a function of carbon availability or a stress response for formation of anthocyanin or flavonol precursors. Studies have suggested that low temperature induces anthocyanin accumulation by inducing expression of anthocyanin biosynthetic genes in apple (Ubi et al., 2006) and that high night temperatures hinder expression of anthocyanin biosynthetic genes in at an early stage of ripening in grape (Mori et al., 2005), although the low temperatures in both of those studies were not nearly as low as those recorded in this study on the bogs.

The correlation coefficients between temperatures and TAcY, TF, or TP often changed dramatically through the growing season (for example, the relationship between minimum temperature and TAcY demonstrated in Fig. 2A). Numerous potential explanations exist for this observation, the most likely being that the chemistry of the berry changes throughout the season, and so temperature could have impacted fruit composition through indirect avenues (i.e., multiple precursors). Additionally, influences on plant development may be confounding the results as well.

This study confirms the observation by Onayemi et al. (2006) that production of anthocyanins and flavonols can be regulated independently of one another and proposes that anecdotal information suggesting the relationship of cool night temperatures

before harvest on increased anthocyanin production in cranberry is misleading. Warmer temperatures early in the season (around bloom and fruit set) had the most positive impact on phenolic production in 'Early Black' growing in southeastern Massachusetts, although it is not clear why. This phenomenon may be cultivar-dependent.

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