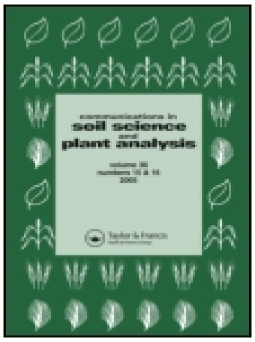

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Impact of Irrigation Thresholds on Total Anthocyanin Content in Cranberries

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The impact of irrigation thresholds based on soil water potentials on total anthocyanin content (TAcy) of cranberry fruit was studied at two sites. Two methods based on spectrophotometric measurements were compared: the acidic solvent extraction (ASE) method and the pH-differential method. TAcy was greater by 21–29 percent when irrigation was managed using a wetter threshold, −5.5 and −6.0 kPa, in comparison with a drier threshold, from −6.5 to −10.0 kPa. The pH-differential method resulted in significantly greater TAcy measurements than the ASE method, although the ASE method was faster and simpler to use.

Keywords Anthocyanin, cranberry, irrigation, soil water potential, tensiometer

Introduction

Cranberries are known for their health benefits, in which anthocyanin, of the phenol chemical family, plays an important part. Total anthocyanin content (TAcy) is one of the criteria used by buyers to determine the price of the cranberry crop. In the United States, cranberries are grown mainly in the state of Wisconsin, where around 220,000 tons of berries were harvested on almost 8000 ha in 2012. In Canada, the province of Québec is the largest producer, where more than 80,000 tons of cranberries were harvested on about 3000 ha in 2012.

Environmental and horticultural factors can influence total anthocyanin (TAcy) content in cranberries (Sapers et al. 1986), but little information is available on the impact of water availability on TAcy in cranberries. In grapevines, however, it is well established that TAcy can be increased by inducing water deficit. Bucchetti et al. (2011) reported greater anthocyanin concentrations under water deficit. Results appear to be inconsistent, however, as Zarrouk et al. (2012) found TAcy to be lower in nonirrigated vines than in irrigated ones.

A number of methods are now available to measure TAcy in cranberries. Chromatographic measurements, such as liquid chromatography, are among the more

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recent methods, but these require costly material and apparatus, experienced technical staff, and time-consuming sample preparation. Spectrophotometric measurements, on the other hand, are usually faster and easier to use than chromatography and hence are the methods generally employed to quantify polyphenols (Ignat, Volf, and Popa 2011). Among them are the acidic solvent extraction (ASE) method and the pH-differential method. Lee, Rennaker, and Wrolstad (2008) showed a strong correlation ($R \geq 0.925$, $P \leq 0.05$) between high-performance liquid chromatography and the pH-differential method.

The objective of the current study was twofold: first to determine if irrigation thresholds influence TAcy in berries and second to compare the ASE method and the pH-differential method for determining TAcy.

Material and Methods

Field Setup

One cranberry bed was located in Québec, Canada, at 46° 17′ N, 71 °59′ W (site 1), and a second one was in Wisconsin, in the United States, at 44° 05′ N, 90° 28′ W (site 2). Stevens cultivar was used at site 1 and Grygleski 1 was used at site 2. Irrigation treatments were managed so as to maintain soil water potentials above predetermined thresholds. At site 1, the threshold used by the producer corresponded to the conventional threshold for cranberry irrigation in Québec (−6.5 kPa) and was compared to a wetter threshold (−5.5 kPa) and a drier threshold (−10.0 kPa). At site 2, the producer threshold (−6.0 kPa) was compared to a drier (−7.5 kPa) threshold. The irrigation treatment setup was repeated in nine blocks at site 1 and eight blocks at site 2. Soil water potential was measured using HXM-80 tensiometers (Hortau, Lévis, Qc) installed at a depth of 10 cm below the soil surface, corresponding to the active root zone in cranberry production. The design of the field study is described in greater detail by Pelletier, Gallichand, and Caron (2013). The volume of water used was reduced by 21–93 percent when irrigation was managed according to dry thresholds ranging between −7.0 and −10.0 kPa while a wet threshold of −5.5 kPa required 54–186 percent more water. Those thresholds did not significantly impact yields except in the driest treatment at −10.0 kPa.

Laboratory Analyses

At harvest in 2012, one composite sample was hand-raked in each experimental unit at both sites and an additional sample was taken in the wet treatment units at site 2. Each sample consisted of approximately 250 g of berries from all levels in the plant canopy. The samples were frozen at −20 °C and kept for subsequent TAcy analysis. Berries from the samples were first analyzed to determine TAcy using the ASE method, as previously described in Fuleki and Francis (1968), but with filtration omitted. Each cranberry sample was homogenized in a commercial blender with acidic ethanol (85 V of ethanol, 15 V of hydrochloric acid 1.5 N), and the solution was left to settle in two test tubes in the dark for 2 h. The tubes were then shaken and once sedimentation was complete, a 1-mL subsample was diluted in 13 mL of the same acidic ethanol solution described previously. This procedure was carried out once for site 2 and twice for site 1. Each test tube was analyzed using a Genesys 6 spectrophotometer (Thermo, Rochester, USA). The TAcy of each experimental unit was calculated as the average of all measurements for that unit.

Next, in site 1 samples only, TAcy was analyzed using pH-differential spectrophotometry, with certain modifications made to the original methods published by Giusti, Rodríguez-Saona, and Wrolstad (1999). The pH-differential method is based on the effect of pH on anthocyanin forms: at pHs of 3 and lower, anthocyanins exist mainly in their colored flavylium forms, whereas at greater pHs, from 3 to around neutrality, they are mainly found in the colorless carbinol form (Brouillard 1988). For this analysis, the cranberries were first freeze dried and then pulverized. A study done by De Torres et al. (2010) found that freeze drying maintained the phenolic and volatile contents of grape skins. A total of 250 mg of cranberry powder was dissolved in 100 mL of distilled water and sonicated for 15 min. Four 1-mL aliquots of this solution were then taken: two were dissolved in pH 1 buffer and the other two were dissolved in pH 4.5 buffer. A Genesys 6 spectrophotometer (Thermo, Rochester, USA) was used to take two spectrophotometric readings, one at 700 nm and one at 510 nm, for each of the cranberry/buffer solutions. The reading at 700 nm was used to correct for turbidity while the reading at 510 nm corresponds to the maximum absorbance of cyanidin-3-glucoside. Absorbance was calculated as the difference between the 510-nm reading and the 700-nm reading in pH 1 buffer minus the same difference in pH 4.5 buffer. TAcy was then calculated from absorbance using the Lambert-Beer's law (Giusti, Rodríguez-Saona, and Wrolstad 1999).

Statistical Analysis

The data for sites 1 and 2 were treated separately due to differences in experimental design and cultivar. First, for site 1, a two-way analysis of variance (ANOVA), with treatment and method as factors, was performed using SAS 9.2 (SAS Institute, Cary, NC). Then, for both sites 1 and 2, a one-way ANOVA with treatment as the factor was performed using SAS 9.3 (SAS Institute, Cary, NC). All statistical analyses were done with the Bonferroni adjustment and a 5 percent level of significance.

Results and Discussion

Effect of Irrigation Treatment on TAcy

At site 1, TAcy as determined with the pH-differential method was greater in the wettest treatment (Table 1). When data from both methods were combined, TAcy was still greater in the wet treatment compared to grower practice. Contrast analysis (data not shown) revealed a significant quadratic relationship between irrigation treatment and TAcy at this site. At site 2, TAcy was also greater in the wetter treatment. In this study, cranberries appeared to produce more anthocyanin under stress. Excess irrigation in the wet treatments may have reduced oxygen availability, thus creating an anthocyanin-inducing stress. On the other part of the quadratic curve (site 1), berries under slight hydric stress also produced more anthocyanins. Our results are consistent with those of Zarrouk et al. (2012), who reported that TAcy was 20 percent less in nonirrigated grapevines, and those of Bucchetti et al. (2011), who found that TAcy was 53–61 percent greater under water deficit.

In the year following the treatment application, the number of fruiting uprights was significantly less by 37 percent in the −5.5 kPa at site 1 (unpublished data). Hence drier treatments allow considerable water savings without affecting yields (Pelletier, Gallichand, and Caron 2013). Moreover, other techniques have been shown to be more effective in increasing TAcy than overwatering plants. The application of a growth regulator in the field increased TAcy by 28 percent to 54 percent in relation to a control (Farag, Palta, and Stang 1992). In a study that included sixteen cultivars, TAcy varied from 13 to 43 mg /

Table 1
TAcy in each treatment for each method

Site	Treatment (kPa)	TAcy (mg per 100 g of fresh cranberries ± SE)	
		Acidic solvent method	pH-differential method
1	Wet (−5.5)	38.2 ± 1.8 a	48.8 ± 3.7 a
	Producer (−6.5)	33.4 ± 1.8 a	37.9 ± 3.7 b
	Dry (−10.0)	37.0 ± 1.8 a	40.4 ± 3.7 ab
	Mean for each method	36.2 ± 1.3 b[a]	42.4 ± 1.3 a
2	Producer (−6.0)	22.8 ± 1.4 a	—
	Dry (−7.5)	18.9 ± 1.6 b	—

Note. For each treatment and each method, mean ± standard error values with a same letter are not significantly different ($P < 0.05$) from one another.
[a]Comparison between methods, as the treatment × method, was not significant in the ANOVA.

100 g of fresh cranberries (Sapers et al. 1986). This variation among cultivars may explain why TAcy was lower at site 2 than at site 1.

More research is needed to fully understand the impact of water management on TAcy in cranberries in order to obtain an optimal anthocyanin content with sustainable water use. It would also be useful to study the impact of water availability on other fruit quality parameters such as sugar content, titratable acidity, and pH of the juice.

Effect of Detection Method on TAcy

The interaction between treatments and methods (Treat × Meth) was not significant and conclusions on irrigation treatments are therefore not method dependent. However, at site 1, the least square mean for the ASE method (TAcy of 36.2 mg / 100 g of fresh cranberries) was significantly less than the least square mean for the pH-differential method (TAcy of 42.4 mg / 100 g of fresh cranberries). This difference highlights the importance of deciding on an analytical method to be adopted and used by all producers and industry stakeholders. In this study, the pH-differential method was found to be more time consuming because it requires freeze drying the fruit prior to analysis. The ASE method, on the other hand, was quick and simple but generated more chemical waste due to the use of solvent. Also, ASE measurements are made at a wavelength that corresponds to maximum absorbance for a wide variety of anthocyanins while the pH-differential method assumes that cyanidin-3-glucoside is representative of the anthocyanins contained in cranberries and it probably is not uniformly representative among different cultivars (Sapers and Hargrave 1987).

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References

Brouillard, R. 1988. Flavonoids and flower colour. In *The flavonoids: Advances in research since 1980*, ed. J. B. Harborne. London: Chapman and Hall.

Bucchetti, B., M. A. Matthews, L. Falginella, E. Peterlunger, and S. D. Castellarin. 2011. Effect of water deficit on Merlot grape tannins and anthocyanins across four seasons. *Scientia Horticulturae* 128: 297–305. doi:10.1016/j.scienta.2011.02.003.

De Torres, C., M. C. Díaz-Maroto, I. Hermosín-Gutiérrez, and M. S. Pérez-Coello. 2010. Effect of freeze-drying and oven-drying on volatiles and phenolics composition of grape skin. *Analytica Chimica Acta* 660: 177–82. doi:10.1016/j.aca.2009.10.005.

Farag, K. M., J. P. Palta, and E. J. Stang. 1992. Ethanol enhances the effectiveness of ethephon on anthocyanin production in cranberry fruits in the field. *HortScience* 27:411–12.

Fuleki, T., and F. J. Francis. 1968. Quantitative methods for anthocyanin: Extraction and determination of total anthocyanin in cranberries. *Journal of Food Science* 33: 72–77. doi:10.1111/j.1365-2621.1968.tb00887.x.

Giusti, M., L. E. Rodríguez-Saona, and R. E. Wrolstad. 1999. Molar absorptivity and color characteristics of acylated and non-acylated pelargonidin-based anthocyanins. *Journal of Agricultural and Food Chemistry* 47: 4631–37. doi:10.1021/jf981271k.

Ignat, I., I. Volf, and V. I. Popa. 2011. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chemistry* 126: 1821–35. doi:10.1016/j.foodchem.2010.12.026.

Lee, J., C. Rennaker, and R. E. Wrolstad. 2008. Correlation of two anthocyanin quantification methods: HPLC and spectrophotometric methods. *Food Chemistry* 110: 782–86. doi:10.1016/j.foodchem.2008.03.010.

Pelletier, V., J. Gallichand, and J. Caron. 2013. Effect of soil water tension threshold for irrigation on cranberry yield and water productivity. *American Society of Agricultural and Biological Engineers* 56:1325–32.

Sapers, G. M., and D. L. Hargrave. 1987. Proportions of individual anthocyanins in fruits of cranberry cultivars. *Journal of the American Society for Horticultural Science* 112:100–4.

Sapers, G. M., S. B. Jones, M. J. Kelley, and J. G. Phillips. 1986. Breeding strategies for increasing the anthocyanin content of cranberries. *Journal of the Amercan Society for Horticultural Science* 111:618–22.

Zarrouk, O., R. Francisco, M. Pinto-Marijuan, R. Brossa, R. R. Santos, C. Pinheiro, J. M. Costa, C. Lopes, and M. M. Chaves. 2012. Impact of irrigation regime on berry development and flavonoids composition in Aragonez (Syn. Tempranillo) grapevine. *Agricultural Water Management* 114: 18–29. doi:10.1016/j.agwat.2012.06.018.