Effects of Different Organic Weed Management Strategies on the Physicochemical, Sensory, and Antioxidant Properties of Machine-Harvested Blackberry Fruits

George Cavender, Mingyang Liu, Deborah Hobbs, Balz Frei, Bernadine Strik, and Yanyun Zhao

Abstract: The effect of 3 different weed management strategies, nonweeding, hand weeding, and weed mat, were examined on physicochemical, sugar profile, and antioxidant properties of 2 cultivars of blackberry (*Rubus spp)*, "Marion" and "Black Diamond" harvested at 3 time intervals during the 2012 season. Sensory analysis on flavor intensity of 6 different descriptors by an experienced panel was also performed on "Black Diamond" berries harvested at the same interval during the 2013 season. While weed management had no effect on pH, titratable acidity, and total soluble solids of either cultivar ($P > 0.05$), it showed a marked effect on total phenolics (5.65 to 7.80 mg GAE/g FW), total monomeric anthocyanins (1.07 to 2.85 mg/g FW), ORAC (271.51 to 644.97 μMol TE/g FW), FRAP (408.56 to 719.10 μMol Fe^{2+}/g FW), sugar profile, and flavor intensity. Hand-weeding resulted in fruit antioxidant content and capacity as much as 30% greater, though the effect was not seen in the late harvest, where the nonweeded samples tended to have higher values. Overall, weed mat samples had the lowest antioxidant content and capacity in all harvests. Sugar profiling exhibited a greater variability based on cultivar and harvest, but overall, weed mat samples had lower sugar levels than fruit from the other 2 methods. Interestingly, the intensity of sensory attributes for "Black Diamond" appear to possibly be inversely related to phenolic and anthocyanin content, with the weed mat management strategy resulting in the highest values for virtually all sensory attributes. This study provided valuable information about the impact of organic production method on the quality of blackberries.

Keywords: blackberries, organic production, physicochemical quality, phytonutrients, sensory

Practical Application: Weed management is one of the largest costs associated with organic agriculture because of limited availability of approved herbicides. While much work has been done to evaluate the effect of different methods on plant growth and yield, few have determined the impact of weed management methods on fruit quality. This study investigated the impact of 3 common weed management strategies on physicochemical, sensory, and antioxidant properties of 2 organically grown blackberry cultivars. Given the widespread belief that organically grown products are of higher quality than conventionally grown ones, the information generated is particularly important for growers and consumers.

Introduction

Organic foods are growing in popularity, with a recent survey finding that over 75% of U.S. families choose at least some organic products, typically citing reasons such as the belief that organic products are of "higher quality" and that they are "healthier for me and my children" (OTA 2011). With this increase in popularity comes an increase in production, and while organic agriculture still represents a small portion of agricultural production, the increased consumer interest in organic food, as well as the premium price such food can command, has led to a sharp increase in production, with the total acreage of organic crops in the United States increasing over 75% in the 5 yr between 2002 and 2007 (USDA 2010).

Another trend in the market is an increase in demand for "superfoods"—fruits, nuts, and vegetables thought to aid in the prevention of significant health concerns by means of bioactive compounds like antioxidants and phytosterols. Among these are the antioxidant rich fruits like blackberries, cherries, and blueberries, the consumption of which have been linked to reduced risks of health concerns in cancers, coronary heart diseases, metabolic disorders, and inflammatory responses (Hagiwara and others 2001; Halvorsen and others 2002; Kang and others 2003; Srivastava 2009; Wang and others 2009; Obrenovich and others 2011). And given the previously mentioned focus that organic consumers place on health, it makes sense that they will also drive an increase in demand for organically produced "super foods."

In order to address these increased demands, farmers often explore new agricultural practices to increase yield such as new fertilizer regimes and alternative irrigation methods. One potential practice that can have a profound effect on yield is weed control. Weeds compete with crop plants for vital resources like water, nutrients, and even sunlight, and the annual cost of weed removal and control in the United States, across all crops, is estimated to be in excess of 6 billion dollars, with over 3.5 billion dollars of

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MS 20140663 Submitted 4/19/2014, Accepted 8/7/2014. *Authors Cavender, Liu, and Zhao are with Dept. of Food Science and Technology, Oregon State Univ., 100 Wiegand Hall, Corvallis, OR 97331, U.S.A. Authors Hobbs and Frei are with The Linus Pauling Institute, 307 Linus Pauling Science Center, Corvallis, OR 97331, U.S.A. Author Strik is with Dept. of Horticulture, Oregon State Univ., 4017 Agriculture and Life Sciences Building, Corvallis, OR 97331, U.S.A. Direct inquiries to author Zhao (E-mail: Yanyun.zhao@oregonstate.edu).*

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that sum being spent on chemical methods of control (PSU 2014). While these chemical methods can be quite effective, their use is forbidden in organic agriculture, meaning the farmers have to rely on more labor intensive methods of weed control, increasing the farmer's cost 200 fold, compared to conventional farming (Wood and others 2002; Gold 2007).

While agricultural practices, like weed management, can help address increased demand, it is important to also consider how such changes might affect fruit quality. Fruiting plants are living organisms, and will respond to the environmental stresses in different ways. Weeds are one such stress, and adverse effects on fruit quality due to their presence have been seen in diverse fruits such as apples, citrus fruits, cranberries, blackberries, and wine grapes (Jordan 1981; Jackson and Lombard 1993; Patten and Wang 1994; Marsh and others 1996). One limitation of these and other similar studies is that they tend to focus on fruit yield and plant health, and examine fruit quality from a limited point of view, typically only measuring fruit size, color, and possibly moisture and/or solids content. But when one is considering a fruit like blackberries (*Rubus spp.*), it is important to consider not only the quality factors that influence appearance, but also those that contribute to taste and those that influence the well-studied healthful antioxidant properties of the berries.

Blackberries contain high levels of anthocyanins and other phenolic compounds, all of which have differing antioxidant capacities (Siriwoharn 2001; Srivastava 2009). By measuring both the quantity of a given class of antioxidant, as well as the potential antioxidant capacity, it is possible to better understand and compare the healthful potentials of berries. The antioxidant studies of blackberries typically include the measures of total phenolic content, anthocyanin content and one or more measures of antioxidant capacity against a reference free radical (Siriwoharn 2001; Halvorsen and others 2002; Siriwoharn and others 2004; Fan-Chiang and Wrolstad 2005; Srivastava 2009). These antioxidant measures of blackberries have also been found to be affected by differences in cultivar, refrigerated storage, fertilizer, irrigation, and other factors, but the potential effect of weed management has remained unexplored (Bryant and others 1987; Iason and others 1993; Close and McArthur 2002; Wu and others 2010; Ali and others 2011; Veberic and others 2014).

The aim of this study was to examine the effects of different organically approved weed management strategies on the physicochemical, sensory, and antioxidant qualities of 2 cultivars of mechanically harvested blackberries ("Marion" and "Black Diamond"). Comparisons were not made between organically and conventionally grown and managed berries, as regulations for organic certification (CFR 7.205.C) prevent growing the 2 in close proximity, and if grown at separate sites it would be difficult, if not impossible to separate the effects of the agricultural systems from those of the location. While there have been studies of various fruits and vegetables that have attempted to do just that (Asami and others 2003; Zhao and others 2007; Györe-Kis and others 2012; Hallmann 2012; Heimler and others 2012), the validity of such comparisons and/or the practical significance of any measured differences have been called into question (Woese and others 1997; Brandt and Mølgaard 2001; Felsot and Rosen 2004).

Materials and Methods

Materials

All chemical reagents were analytical grade, except for the ultrapure (>18.2 MΩ cm) water used as a mobile phase in HPLC analysis of sugar profile, which was prepared *in situ* using a Millipore filtration system (Millipore Corp., Bedford, Mass., U.S.A.).

Two blackberry cultivars, "Marion" and "Black Diamond," were evaluated in this study, chosen for the fact that, together they account for a majority of blackberries grown for the processed market in the Pacific Northwest (Harkins and others 2013). All berries used in this study were grown in the certified organic plots of Oregon State Univ.'s North Willamette Research and Extension Center in Aurora, OR. Complete details of the growing conditions were described in the recent publication of Harkins and others (2013). In brief, berries were collected from randomly selected plots which all received the same rate of irrigation and fertilizer, and differed only in the methods used to manage weed growth. Weeds were either allowed to grow unmolested (except that the day prior to harvest they were mowed to prevent complications with the mechanical harvest) ("nonweeded"), removed by hand using a hoe ("hand weeded"), or inhibited through the use of "weed mat," a black water permeable woven polymer placed down the in-row area and around the base of the plant.

Berries were machine-harvested using an over-the-row rotary harvester (Littau Harvesters Inc., Stayton, Oreg., U.S.A.) 3 times at 7 d intervals during the 2012 and 2013 growing seasons, with an additional, nonexamined harvest being collected between each examined harvest. After collection, berries were sorted by hand to exclude molded, overly damaged, or otherwise unsuitable berries before being placed onto mesh trays for freezing in a forced air freezer at −25 °C overnight. Frozen berries for the physicochemical, antioxidant, and sugar profile assays were packed in polyethylene zip top bags (Bi-Mart Corp, Eugene, Oreg., U.S.A.) while those for the sensory study were placed in half-gallon glass canning jars with metal lids (Jarden Corp., Daleville, Ind., U.S.A.). All samples were then stored in the same −25 °C freezer for up to 9 mo.

Physicochemical assays

On the day of assay, frozen samples were removed from storage and pulverized under liquid nitrogen using a 1-L blender (Waring Laboratory Science, Torrington, Conn., U.S.A.) which had been modified to include a specialized lid allowing for pressure release while preventing sample loss. The resultant powdered samples were used to measure pH, titratable acidity (TA), and total soluble solids (TSS) after Fisk and others (2008). Briefly, 10 to 20 g of pulverized fruit samples were mixed with deionized (DI) water equal to 9 times the sample mass, then blended for 1 min using a homogenizer (Osterizer, Jarden Corp., Mexico). The resultant slurry was filtered to remove seeds, fruit pulp and other solids using a Buchner funnel and qualitative filter paper (Whatman Intl. Ltd., Maidstone, England). TSS of the filtrate was measured using an electronic refractometer (Model RA-250HE, Kyoto Electronics Manufacturing Co., LTD., Japan), while pH was measured with an electrolytic pH meter (Model 125, Corning Science Products, Medfield, Mass., U.S.A.). TA measurements were performed by titration of 10 mL aliquots of filtrate to an endpoint of 8.2 with 0.1N NaOH and calculated based on the assumption of malic acid as the predominant organic acid. TSS, TA, and pH measures were performed in triplicate on each assay date and mean values were reported based upon the mass of the berry sample used.

Sample preparation

Sample preparation for antioxidant assays. Aqueous phenolic extracts were prepared from samples of pulverized frozen berries using the ultrasound assisted procedure developed in our laboratory (Wu and others 2010). In brief, 3 sequential extractions using water/acidified acetone (0.1 mL/L HCl) solutions in concentrations of 0% water/100% acidified acetone (first extraction) and 30% water/70% acidified acetone (2nd and 3rd extractions) were performed on 15 g of pulverized sample. For all extractions, the solvent to sample ratio was 4:1, and each extraction involved a fixed time ultrasound treatment (90, 300, and 300 s, respectively) followed by centrifugation and decanting. Supernatants from each of the extractions were pooled together and partitioned with 150 mL of chloroform to remove any lipophilic components. The nonaqueous phase was then discarded and the aqueous phase was evaporated to remove residual volatile solvents using a rotary evaporator (Roto-vap, Brinkman Instruments, Westbury, N.Y., U.S.A.). Extract volumes were standardized to 150 mL using DI water, and 1.5 mL aliquots of the standardized solutions were stored at −80 °C until the time of assay.

Sample preparation for sugar profiling. Aqueous berry extracts were prepared. Briefly, approximately 35 g of pulverized berry powder were placed into a glass jar and mixed with a mass of boiling DI water equal to half mass of the berry sample. After fitting with lids, jars were subjected to 20 min thermal processing in boiling water bath to inactivate enzymes. After cooling, the jar contents were centrifuged to separate solids and then decanted into clean polypropylene bottles for storage at −25 °C until the time of assay.

Antioxidant content and capacity analysis

Total phenolic content (TPC). The Folin–Ciocalteu colorimetric method (Singleton and others 1999) was used to determine TPC. Briefly, the aqueous extracts were diluted to an appropriate absorbance value (<1.2 AU), and 0.5 mL aliquots of this diluted sample were taken to assay. These aliquots were combined with 0.5 mL of Folin–Ciocalteu reagent and 7.5 mL of DI water in a glass tube and vortexed to mix. After 10 min, 3 mL of 20% sodium carbonate solution was added and the solution was vortexed again. The tube was then immersed in a 40 °C water bath for 20 min, followed by chilling in an ice/water bath to rapidly bring them to room temperature. Aliquots of this solution were placed into cuvettes and examined using a spectrophotometer (Model UV160U, Shimadzu Corporation, Kyoto, Japan). The absorbance of the samples at 765 nm was used to calculate gallic acid equivalents using a standard curve constructed on the same day from absorbance measurements of gallic acid solutions of different concentrations (0, 150, 200, and 250 ppm). Assays were performed in triplicate, with values reported as mg gallic acid equivalents (GAE)/g fresh weight (FW).

Total monomeric anthocyanins (TMA). TMA was measured using the pH differential method (Giusti and Wrolstad 2001). Briefly, aliquots of a given extract were diluted with either a standardized sodium acetate buffer or a standardized potassium chloride buffer to alter the pH of the extract to either 4.5 or 1.0, respectively. After a 15-min rest period to allow for equilibration, the diluted samples were examined with the spectrophotometer. Absorbance at 700 and 510 nm, the former to account for haze, and the latter corresponding to the absorbance of cyanadin-3-glucoside, the predominant anthocyanin in blackberries (Siriwoharn and others 2004; Fan-Chiang and Wrolstad 2005) were used to calculate the concentration of monomeric anthocyanins in the fruit using the Beer–Lambert–Bouguer law, as shown in Eq. (1).

$$\mathrm{TMA}\left(\frac{\mathrm{mg}}{\mathrm{g\,FW}}\right)=\frac{\left[(A_{510\,\mathrm{nm}}-A_{700\,\mathrm{nm}})_{\mathrm{pH1.0}}-(A_{510\,\mathrm{nm}}-A_{700\,\mathrm{nm}})_{\mathrm{pH4.5}}\right]\times 449.2\,\frac{\mathrm{g}}{\mathrm{mol}}\times DF\times 1000\,\frac{\mathrm{mg}}{\mathrm{g}}}{26900\,\frac{\mathrm{L}}{\mathrm{cm\cdot mol}}\times 1\mathrm{cm}}\times\frac{1\mathrm{L}}{100\mathrm{gFW}}. \tag{1}$$

where DF was dilution factor. Each extract was assayed in triplicate, and values were reported as mg TMA/g FW.

Radical scavenging activity (RSA). The refined colorimetric assay method relying on the reduction of the stable free radical 2,2-diphenyl-1-picryhydrazyl (DPPH) (Brand-Williams and others 1995) was used to determine RSA. Briefly, a methanolic solution of DPPH was prepared by dissolving 9 g of DPPH in 100 mL of anhydrous methanol. Aliquots (1.5 mL) of this solution were added to 0.75 mL of diluted fruit extract, mixed by pipette and allowed to react at room temperature for 5 min before examination by spectrophotometer. Sample absorbance at 517 nm was used to calculate ascorbic acid equivalents (AAE) using a standard curve prepared from absorbance measurements of different concentrations of ascorbic acid solutions (0, 100, 200, 300, and 400 ppm) which had been taken the day of assay. Assays were performed in triplicate, and values were reported as mg AAE/g FW.

Oxygen radical absorbance capacity (ORAC). The fluorescent method described by (Cao and others 1993) adapted for use in a 96 well microplate fluorometer (SpectraMax Gemini XS, Molecular Devices, Foster City, Calif., U.S.A.) was used to determine ORAC. Briefly, 200 μL of a prewarmed β-phycoerythrin solution and 30 μL of a given extract (diluted as needed) were dispensed into the wells of a prewarmed microtiter plate. After 1 h of incubation at 37 °C, 70 μL of 2,2‘-Azobis(2-amidinopropane) dihydrochloride (AAPH) was added to initiate the reaction, and the fluorescence of β-phycoerythrin measured at 585 nm and induced by excitation at 485 nm was recorded every 2 min for 2 h. These data were then used to calculate the antioxidant capacity by comparing the positive changes of the area under the curve to a curve generated from a series of standardized Trolox solutions (0, 10, 20, or 40 μmol/L) using a proprietary software package (SoftMax Pro 5.4.5, Molecular Devices, LLC, U.S.A.). All extracts were assayed in triplicate, and results were expressed as μmol Trolox equivalent (TE)/g FW.

Ferric reducing antioxidant power (FRAP). The automated colorimetric method (Benzie and Strain 1996) was used to determine FRAP. Briefly, 40 μL aliquots of each extract and 300 μL of prewarmed FRAP reagent (a mixture of 83% 300 mmol/L acetate buffer, 3.5% 10 mmol/L tri(2-pyridil)-s-triazine, and 3.5% 20 mmol/L Iron (III) chloride) were dispensed into the wells of the pre-warmed microtiter plate. After incubation at 37 °C for 15 min, absorbance at 550 nm was recorded using a microplate absorbance reader (SpectraMax 190, Molecular Devices, Foster City, Calif., U.S.A.). These data were then used to calculate the antioxidant capacity based upon a standard curve generated from a series of standardized Trolox solutions (0, 62.5, 125, 250, or 500 mmol/L Trolox) using the same proprietary software package

Table 1–Physicochemical properties of 2 cultivars of blackberry fruit ("Marion" and "Black Diamond") in 2012 harvest.

		Early harvest	Middle harvest	Late harvest	All harvests*
pH†					
Marion	Nonweeded	3.13 ± 0.08	3.28 ± 0.06	3.40 ± 0.16	3.27 ± 0.15
	Hand weeded	3.04 ± 0.05	3.52 ± 0.36	3.35 ± 0.19	3.30 ± 0.29
	Weed mat	3.16 ± 0.17	3.18 ± 0.06	3.26 ± 0.19	3.20 ± 0.14
	All Treatments*	3.11 ± 0.11	3.33 ± 0.24	3.33 ± 0.17	
Black Diamond	Nonweeded	3.06 ± 0.02	3.59 ± 0.08	3.25 ± 0.23	3.30 ± 0.26
	Hand weeded	3.09 ± 0.01	3.20 ± 0.64	3.25 ± 0.19	3.18 ± 0.34
	Weed mat	3.24 ± 0.01	3.47 ± 0.16	3.14 ± 0.09	3.28 ± 0.17
	All Treatments*	3.13 ± 0.08	3.42 ± 0.37	3.21 ± 0.17	
Titratable acidity (%)†					
Marion	Nonweeded	1.44 ± 0.22	0.85 ± 0.28	0.93 ± 0.08	1.07 ± 0.33
	Hand weeded	1.53 ± 0.04	0.64 ± 0.16	1.01 ± 0.18	1.06 ± 0.41
	Weed mat	1.28 ± 0.42	0.85 ± 0.07	1.10 ± 0.32	1.08 ± 0.33
	All treatments*	1.42 ± 0.26a	0.78 ± 0.20b	1.01 ± 0.20c	
Black Diamond	Nonweeded	1.32 ± 0.17	0.63 ± 0.06	0.99 ± 0.36	0.98 ± 0.36
	Hand weeded	1.08 ± 0.16	0.77 ± 0.10	1.04 ± 0.41	0.96 ± 0.27
	Weed mat	1.13 ± 0.08	0.63 ± 0.14	1.23 ± 0.27	1.00 ± 0.32
	All Treatments*	1.18 ± 0.17a	0.67 ± 0.11b	1.08 ± 0.32a	
Total soluble solids (°Bx)†					
Marion	Nonweeded	12.50 ± 1.32	10.83 ± 1.26	10.67 ± 1.53	11.33 ± 2.38
	Hand weeded	12.83 ± 1.61	9.17 ± 0.29	12.00 ± 1.00	11.33 ± 1.91
	Weed mat	11.33 ± 0.58	9.33 ± 0.58	12.33 ± 0.58	11.00 ± 1.00
	All treatments*	12.22 ± 1.28a	9.78 ± 1.06b	11.67 ± 1.22a	
Black Diamond	Nonweeded	9.67 ± 2.08	10.50 ± 0.50	9.50 ± 0.87	9.89 ± 1.24
	Hand weeded	8.33 ± 0.58	7.83 ± 1.61	8.83 ± 0.29	8.33 ± 0.97
	Weed mat	9.00 ± 2.00	9.50 ± 0.87	10.00 ± 1.00	9.50 ± 1.27
	All treatments*	9.00 ± 1.58	9.28 ± 1.50	9.44 ± 0.85	

†Mean values ± SD, $n = 3$, unless otherwise noted.
*Mean values ± SD, $n = 9$.
Values in a given row with the different letters proceeding them are statistically different per ANOVA with LSD *post-hoc* testing at $\alpha \leq 0.05$.

as for ORAC measurement. All extracts were assayed in triplicate, and results were expressed as μmol Trolox equivalent (TE)/g FW.

Analysis of sugar profile

Sugar profiling was performed using the high pressure liquid chromatography (HPLC) method developed in our lab. Briefly, 2 mL aliquots of each juice extract were filtered using a 0.47 μm syringe filter, and placed into 2 mL screw cap autoloader vials. These vials were loaded into an HPLC system comprised of an auto-sampler, a quaternary pump, a solvent degasser, a column heater, and a temperature-controlled refractive index detector (Series 1200, Agilent Technologies, Santa Clara, Calif., U.S.A.). A 300-mm long ligand exchange column and an appropriate guard column (Hi-PLex Pb, Varian, Inc., Palo Alto, Calif., U.S.A.) were fitted to the system and maintained at 70 °C during analysis. Three 15 μL injections of each sample were analyzed using ultra-pure water as the mobile phase. Flow rate, detector temperature, and total run time were 0.7 mL/min, 35 °C, and 45 min, respectively. Concentrations of the 3 major sugars (fructose, glucose, and sucrose) were calculated based upon area using standard curves constructed from a series of pure solutions of each sugar (0.9375, 1.875, 3.75, and 7.5 g/100 mL).

Sensory analysis

Due to limited quantities of berries from the 2012 harvest, sensory analysis was performed on berries from the 2013 harvest. Since "Black Diamond" is a more recently developed and less studied cultivar than "Marion," it was chosen as the cultivar of study (Finn and others 2005; Du and others 2010). To prepare samples for evaluation, berries were first removed from frozen storage and allowed to thaw under refrigeration (4 ± 1 °C) for 48 h. Each of the 9 weed management/harvest time combinations were assigned 3 randomly generated 3-digit codes (to obscure the existence of replications from panelists) before being pureed individually using a blender (Waring Laboratory Science, Torrington, Conn., U.S.A.). Samples (approximately 100 mL) of each puree were placed into approximately 120 mL lidded polypropylene sample cups labeled on the top and sides with the appropriate code. Samples were allowed to come to room temperature prior to presenting to panelists.

A total of 22 sensory panelists (13 male, 9 female) were recruited from a pool of berry growers, researchers, and processors, all of whom had extensive experience with the quality attributes of blackberries, in order to ensure an experienced panel. This panel evaluated samples based on the intensity of 7 flavor descriptors (overall, blackberry flavor, fresh, cooked, sweet, sour, and astringent) using a 16 point scale. This scale was chosen to increase the sensitivity of the panelist response, since it is well known that panelists tend to score products toward the middle of a given scale, avoiding extreme values (Stone and Sidel 1993). Each panelist received all 27 samples, randomly presented across 5 tasting sessions with a minimum rest interval of 5 min between subsequent panels. Panelists were provided with sample spoons, an instruction sheet which included definitions of all flavor descriptors, a ballot, spring water and unsalted top crackers to use as palate cleansers between samples, as well as mozzarella cheese for use between sessions to eliminate any lingering astringency. Oversight for the use of human subjects was provided by the Oregon State Univ. Institutional Review Board and all procedures and materials used in the sensory study were accepted by the board prior to the beginning of the first sensory panel (Study ID: 5940–"Sensory evaluation of blackberry products").

Experimental design and statistical analysis

A completely randomized design was employed with the principle effects being weed management strategy and harvest date. Mean values and standard deviation were determined for all

Table 2–Initial total phenolic content and monomeric anthocyanins of 2 cultivars of blackberry fruit ("Marion" and "Black Diamond") in 2012 harvest.

		Early harvest	Middle harvest	Late harvest
TPC (mg GAE/g FW)†				
Marion	Nonweeded	5.65 ± 0.02	6.31 ± 0.19	6.66 ± 0.13
	Hand weeded	6.25 ± 0.04	6.53 ± 0.04	6.55 ± 0.16
	Weed mat	5.95 ± 0.12	6.34 ± 0.06	6.36 ± 0.11
Black Diamond	Nonweeded	6.86 ± 0.18	7.07 ± 0.29	7.80 ± 0.30
	Hand weeded	7.31 ± 0.06	7.49 ± 0.38	7.71 ± 0.08
	Weed mat	6.31 ± 0.06	7.30 ± 0.10	7.22 ± 0.05
TMA (mg/g FW)‡				
Marion	Nonweeded	1.07 ± 0.02	1.57 ± 0.01	1.77 ± 0.01
	Hand weeded	1.42 ± 0.02	1.62 ± 0.00	1.83 ± 0.02
	Weed mat	1.13 ± 0.02	1.46 ± 0.01	1.76 ± 0.03
Black Diamond	Nonweeded	2.21 ± 0.01	2.32 ± 0.14	2.85 ± 0.04
	Hand weeded	2.36 ± 0.01	2.58 ± 0.06	2.77 ± 0.02
	Weed mat	1.89 ± 0.04	2.22 ± 0.02	2.40 ± 0.04

†Mean values ± SD, $n = 3$; TPC: Total phenolic content, GAE: Gallic acid equivalents; FW: Fresh weight.
‡Mean values ± SD, $n = 3$; TMA: Total monomeric anthocyanins.

combinations of weed management strategy and harvest date and used to calculate coefficient of variation ($CV = \frac{\sigma}{\mu} CV$) as $CV = \frac{\sigma}{\mu}$. In accordance with the journal guidelines, statistical analysis was only performed in cased where both in-treatment CV exceeded 10% and the difference between treatment means was less than 3 standard deviations (IFT 2013). In those cases multiway analysis of variance (ANOVA) with least significant difference (LSD) *post hoc* testing as appropriate, was performed using SAS v9.2 (The SAS Institute, Cary, N.C., U.S.A.), and results were considered to be different if $\alpha \leq 0.05$.

Results and Discussion

Physicochemical properties

Table 1 presents the pH, TA, and TSS values for both cultivars. While no statistical difference was seen between the individual weed treatment and harvest combinations for either of the 3 measures, harvest date was a significant ($\alpha < 0.05$) factor for some. In both cultivars, harvest date influenced the TA, with mid-harvest fruit having a lower acidity than early- or late-harvest fruit. This result was somewhat expected as it is well known that berry fruits tend to have decreased acidity as ripening progresses (Basiouny 1995; Reyes-Carmona and others 2005; Tosun and others 2008). However, no difference was seen in pH and there was lack of a correlation between pH and TA ($R^2 = 0.48$). While not conclusive from our results, the most likely cause of this phenomenon was a change and/or difference in the predominant acid species in the berries. Blackberries can contain a variety of different organic acids, but the most common ones are malic, isocitric, and citric acids, and the relative amounts of these 3 have been known to vary with cultivar and year, and could be expected to also vary according to degree of ripeness and harvest time (Wrolstad and others 1980; Kafkas and others 2006; Fan-Chiang and Wrolstad 2010). Since TA calculations rely upon the equivalencies and formula weights of a presumed predominant acid, a change in predominance, particularly from a diprotic acid species, such as malic acid, to a triprotic species, like citric and isocitric, could easily affect the calculations and thus the end results.

Harvest date also affected TSS, but only in "Marion," which showed lower values in the middle harvest compared with the early and late. The reasons for this are unclear, but may be explained by metabolic concerns, such as differences in ripeness and berry maturity, by practical concerns, such as the influence of weather on berry moisture, or by the combination thereof. One such potential combination is the interaction of larger, softer fruits with the rigors of the mechanical harvest. Mechanical harvesting technologies rely upon shaking ripe berries free from the plant, allowing them to fall to near ground level and then conveying them to a central location (Given and Pringle 1985; Takeda and Peterson 1999). Blackberries are also known to become softer as they mature (Perkins-Veazie and others 2000), meaning that they should become more prone to damage during the harvest, resulting in loss of juice, and along with it some of the native sugars.

Antioxidant content and capacity

Table 2 presents the total phenolic and monomeric anthocyanin contents of the 2 cultivars. In general, for a given harvest, the berries from the hand-weeded plots had higher values ("Marion"—TPC: 6.24 to 6.55 mg GAE/g, TMA: 1.42 to 1.83 mg/g; "Black Diamond"—TPC: 7.31 to 7.70 mg GAE/g, TMA: 2.36 to 2.77 mg/g) than those from the nonweeded and weed mat plots ("Marion"—TPC: 5.64 to 6.66 mg GAE/g, TMA: 1.07 to 1.77 mg/g; "Black Diamond"—TPC: 6.31 to 7.80 mg GAE/g, TMA: 1.89 to 2.85 mg/g). This effect was less pronounced during the late harvest, with the TMA of berries from plants grown without weed control having the highest value in "Black Diamond," and the TPC values (6.55 to 6.66 mg GAE/g for "Marion" and 7.71 to 7.80 mg GAE/g for "Black Diamond") of both cultivars showing no difference between hand weeded and nonweeded samples, though both were greater than the weed mat grown samples (6.36 mg GAE/g for "Marion" and 7.22 mg GAE/g for "Black Diamond").

Similar trends were seen in the 3 measures of antioxidant capacity (DPPH, ORAC, and FRAP) which are presented in Table 3. Mean values across all 3 harvests (not shown) were the lowest in the samples from weed mat plots in all 3 measures (DPPH: 3.7 to 3.8 mg AAE/g; ORAC: 383.6 to 408.1 μMol TE/g; FRAP: 492.8 to 590.6 μMol Fe^{2+}/g) and these trends tended to remain even when the data were separated by harvest, excepting the DPPH values of early harvest "Marion" (weed mat had the second highest value), middle harvest "Black Diamond" (weed mat was equivalent with nonweeded for the highest value) and late harvest "Black Diamond" and the ORAC values of the middle harvest "Black Diamond" (weed mat had the highest value) and the late harvest "Marion" (weed mat was equivalent to nonweeded for the highest value).

Table 3–Initial antioxidant capacity of 2 cultivars of blackberry fruit ("Marion" and "Black Diamond") in 2012 harvest.

		Early harvest	Middle harvest	Late harvest
DPPH (mg AAE/g FW)*				
Marion	Nonweeded	3.49 ± 0.01	3.80 ± 0.01	3.82 ± 0.01
	Hand weeded	3.66 ± 0.02	3.77 ± 0.01	3.78 ± 0.01
	Weed mat	3.54 ± 0.01	3.76 ± 0.01	3.74 ± 0.02
Black Diamond	Nonweeded	3.79 ± 0.01	3.83 ± 0.00	3.82 ± 0.00
	Hand weeded	3.82 ± 0.01	3.82 ± 0.00	3.82 ± 0.00
	Weed mat	3.69 ± 0.02	3.83 ± 0.00	3.83 ± 0.00
ORAC (μMol TE/g FW)†				
Marion	Nonweeded	278.42 ± 12.80 a	398.42 ± 12.90 b	523.11 ± 39.48 ce
	Hand weeded	317.88 ± 7.11 a	477.91 ± 16.54 cd	406.83 ± 0.53 b
	Weed mat	282.95 ± 10.74 a	310.87 ± 47.97 a	557.10 ± 46.29 e
Black Diamond	Nonweeded	393.71 ± 6.97 b	401.21 ± 62.24 b	644.97 ± 77.11 f
	Hand weeded	414.97 ± 13.58 b	317.22 ± 11.56 a	616.38 ± 36.78 f
	Weed mat	271.51 ± 30.52 a	433.08 ± 27.93 bd	519.57 ± 12.36 ce
FRAP (μMol Fe^{2+}/g FW)‡				
Marion	Nonweeded	408.56 ± 9.46	565.80 ± 1.07	612.50 ± 3.56
	Hand weeded	502.37 ± 17.38	560.65 ± 22.26	554.24 ± 11.47
	Weed mat	408.69 ± 2.32	521.70 ± 0.17	548.13 ± 9.86
Black Diamond	Nonweeded	600.87 ± 5.59	621.77 ± 5.32	701.32 ± 2.13
	Hand weeded	635.39 ± 10.62	653.24 ± 2.66	719.10 ± 19.38
	Weed mat	477.28 ± 30.33	613.79 ± 14.11	680.85 ± 10.47

*DPPH: Radical scavenging activity by the 2,2-diphenyl-1-picryhydrazyl colorimetric method, mean values ± SD, $n = 3$; FW: Fresh weight.
†Oxygen radical absorbance capacity, mean values ± SD, $n = 3$; ORAC values with the same letters proceeding them are not statistically different per ANOVA with LSD *post hoc* testing at $\alpha \leq 0.05$.
‡Ferric reducing antioxidant power, mean values ± SD, $n = 2$.

While the range of both antioxidant content and capacity values fall within the ranges reported for conventionally grown blackberries (Fan-Chiang 1999; Sellappan and others 2002; Siriwoharn and others 2004; Reyes-Carmona and others 2005; Ali and others 2011), different patterns were observed due to weed management strategy. In particular the most effective method of weed management, weed mat (Harkins and others 2013), appeared to have the least positive effect on antioxidant content, but the least extreme, nonweeding, did not appear to have the opposite effect, except in late-harvested fruit. This pattern could be explained by the metabolic processes that lead to antioxidant production, in particular the use of reactive oxygen species (ROS) as signaling mechanisms for a variety of stresses (Dat and others 2000; Reddy and others 2004). In response to the increase in ROS, it was thought that the plant begins synthesizing phenolic compounds in order to quench them (Close and McArthur 2002). Among the types of stress known to elicit this signaling (and thereby the increased synthesis) are reduced availability of water and nutrients, 2 of the

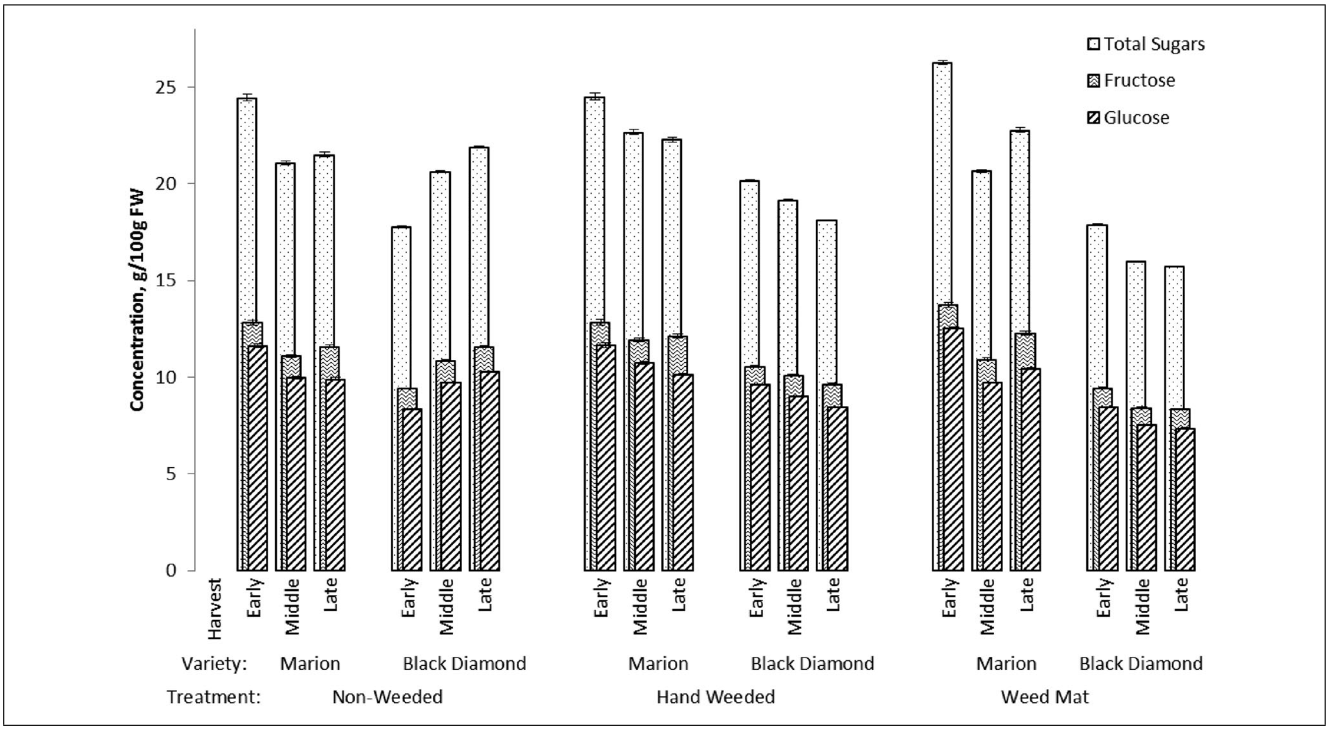

Figure 1–Sugar profile, expressed in total concentration, of 2 blackberry varieties ("Marion" and "Black Diamond") in 2012 harvest. Total sugar was calculated as the sum of all detected sugars.

Table 4–Flavor intensities of sensory attributes for cultivar 'Black Diamond', 2013 harvest.

		Blackberry flavor	Fresh	Cooked	Sweet	Sour	Astringent
Early Harvest	Nonweeded	7.08 ± 1.63 a	6.21 ± 1.60 a	3.86 ± 2.64 a	4.29 ± 1.73 a	6.84 ± 2.21 a	5.04 ± 3.10 abef
	Hand weeded	4.01 ± 0.67 b	3.50 ± 1.04 b	1.99 ± 1.15 b	2.35 ± 0.79 b	3.85 ± 1.14 b	2.43 ± 1.53 c
	Weed mat	6.98 ± 1.47 a	6.23 ± 1.89 ac	3.76 ± 2.64 a	4.84 ± 1.56 a	6.28 ± 2.17 ac	4.36 ± 2.77 ed
Middle Harvest	Nonweeded	8.62 ± 1.65 c	7.13 ± 2.16 d	5.47 ± 3.45 c	6.31 ± 1.76 c	7.04 ± 2.57 a	5.28 ± 3.45 aef
	Hand weeded	6.98 ± 1.84 a	5.73 ± 1.67 ae	4.48 ± 2.68 ad	4.78 ± 1.94 a	5.97 ± 2.11 cd	4.80 ± 3.17 ade
	Weed mat	8.49 ± 1.92 c	6.85 ± 2.60 cdf	5.53 ± 3.66 c	6.27 ± 2.16 c	6.63 ± 3.25 ad	5.07 ± 3.29 bef
Late Harvest	Nonweeded	4.15 ± 0.70 b	3.34 ± 1.00 b	2.49 ± 1.38 b	3.43 ± 0.90 d	2.83 ± 1.26 e	1.99 ± 1.30 c
	Hand weeded	7.07 ± 1.53 a	5.31 ± 2.07 e	4.91 ± 2.82 cd	5.84 ± 1.61 c	4.62 ± 2.46 b	3.89 ± 2.62 d
	Weed mat	7.91 ± 1.11 d	6.28 ± 1.90 af	5.05 ± 3.39 cd	6.21 ± 2.46 c	5.71 ± 2.72 c	4.21 ± 2.77 bd

Based on a 16 point intensity scale with posttest standardization applied and reported as mean values ± SD, $n = 66$. Within a given column, values with the same letters proceeding them are not statistically different per LSD *post hoc* testing with $\alpha \leq 0.05$.

resources for which weeds compete (Harkins and others 2013). Thus in the case of plants grown using weed mat, this absence of stress should correspond to lower levels of phenolic antioxidants, as was seen. In the case of hand weeding versus nonweeding, as could be expected, both tended to have higher levels of antioxidants and antioxidant capacity, while the differences in antioxidant contents, namely the higher levels of TPC and TMA in most of the hand-weeded samples, could be explained by the fact that the persistence of weeds in the nonweeded samples could have either deprived the plants of nutrients needed to synthesize the phenolic compounds, or could have resulted in additional signaling via ROS which would have degraded some of the antioxidant compounds.

Sugar profiles

The results of sugar profiling are shown in Figure 1. In all cases fructose was the predominant sugar (comprising 52.3% to 54.4% of total sugar), which agreed with some, but not all previously published data on conventionally grown blackberries (Wrolstad and others 1980; Fan-Chiang 1999; Kafkas and others 2006; Ali and others 2011). With the exception of Ali and others (2011) which only examined "Loch Ness" blackberries, all noted differences in relative sugar amounts between different cultivars. Kafkas and others (2006) and Wrolstad and others (1980) found higher levels of fructose than glucose in their studies, while Fan-Chiang (1999) reported the levels of the 2 sugars to be roughly even, particularly in "Marion." While the differences seen in the current work could be the result of differences in growth conditions, it is more likely an artifact of the mechanical harvest, which selected for more uniformly ripe fruits than hand harvest, but also could cause damage to the fruits (Given and Pringle 1985; Takeda and Peterson 1999). This would explain the lack of sucrose in any of the samples as blackberries typically have lower levels of sucrose as ripening progresses, due to increased enzymatic activity (Kafkas and others 2006) and fruits are well known to release these enzymes in their juice as they are damaged (Plowman and others 1989).

Examining the trends of sugar content reveals an interesting pattern, with most treatments showing decreases in both overall sugar levels and levels of individual sugars as the season progressed. The most notable exception was the fruit from the nonweeded "Black Diamond" which showed an increase in both individual and overall sugar content (% range) as harvest date progressed. "Marion" fruits also showed a slight deviation from the trend, with fructose levels experiencing a modest (2% to 13%) increase between the middle and late harvests across all weed management treatments, with this increase causing the total sugar value of the late harvest non-weeded samples to exceed those of the middle harvest by 2%. This trend also contributed to the higher overall sugar content of the weed mat "Marion" berries in the late harvest (22.8 g sugar/100g berries compared with 20.7 g sugar/100g berries), though those berries also showed a slight (8%) increase in glucose levels as well.

Comparing the sugar content from HPLC sugar profiling with the earlier reported values of TSS based on refractometry (Table 2) showed a marked discrepancy. While there are several potential explanations for this, the most likely was the fact that the TSS is based upon the refractive index of sucrose and water solutions, and the other dissolved compounds in juice have different refractive indices, which could necessarily affect the accuracy of the measurement.

Sensory results

Statistical analysis of sensory scores revealed variation among panelists to be an extremely significant effect ($\alpha < 0.0001$). This is hardly surprising since an experienced panel was used, rather than a trained panel, meaning that the panelists were not given formal training or standards, and it is well known that significant variability in flavor perception can exist between tasters (Miller 1987; Lundahl and McDaniel 1991; Gay and Mead 1992; Bett and others 1993; Prutkin and others 2000). Hence the scores for each weed management and harvest combination were standardized to the mean "overall" descriptor of each in order to minimize variations among the panelist using the method described by Bett and others (1993), as shown in Eq. (2):

$$\text{Adjusted Score}_{\text{sample}} = \text{Score}_{\text{sample}} \times \frac{\text{Mean Panel Overall Score}_{\text{sample}}}{\text{Overall Score}_{\text{sample}}}. \quad (2)$$

Table 4 presents these standardized sensory attribute scores, and shows an interesting pattern of effects. In the early and middle harvests, fruits from the hand weeded had lower scores in virtually all flavor attributes, ranging from 2.43 to 4.01 in the early harvest and 4.48 to 6.98 in the middle and those from the nonweeded and weed mat were not statistically different, ranging from 3.86 to 7.08 in the early harvest and 5.47 to 8.62 in the late harvest. The exceptions to this both occurred in the middle harvest where hand weeded and weed mat samples did not have statistically different sourness scores, and astringency scores did not vary significantly between all 3 treatments. This trend changed in the late harvest fruits, where the nonweeded fruits had the lowest scores in all flavor attributes (1.99 to 4.15), and the weed mat samples had either higher scores than the hand weeded samples as was the case in the "blackberry flavor," "fresh," and "sour" flavor descriptors

(5.71 to 7.91), or were statistically the same, as was the case for the "cooked," "sweet," and "astringent" descriptors (4.62 to 7.07).

Harvest time and weed treatment also influenced sensory quality individually, with the middle harvest having the highest values in all descriptors across the 3 weed treatments and the weed mat samples having higher values across the 3 harvests. The fact that the middle harvest had higher values was somewhat surprising, as conventional wisdom says that mechanical harvesting uniformly selects for optimal ripeness, based on the assumption that the strength of the receptacle is the best indicator of ripeness. However, studies have found variability in other indicators of ripeness among fruits from different harvests in a given system such as

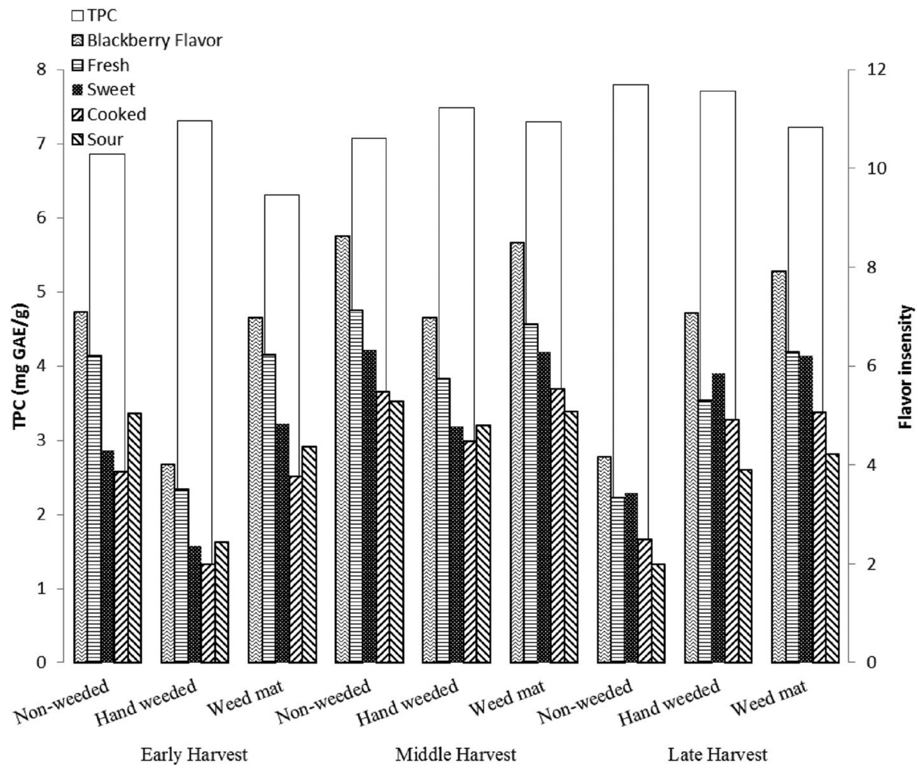

Figure 2–Relationship of total phenolic content (2012 data) with sensory attribute intensities (2013 data).

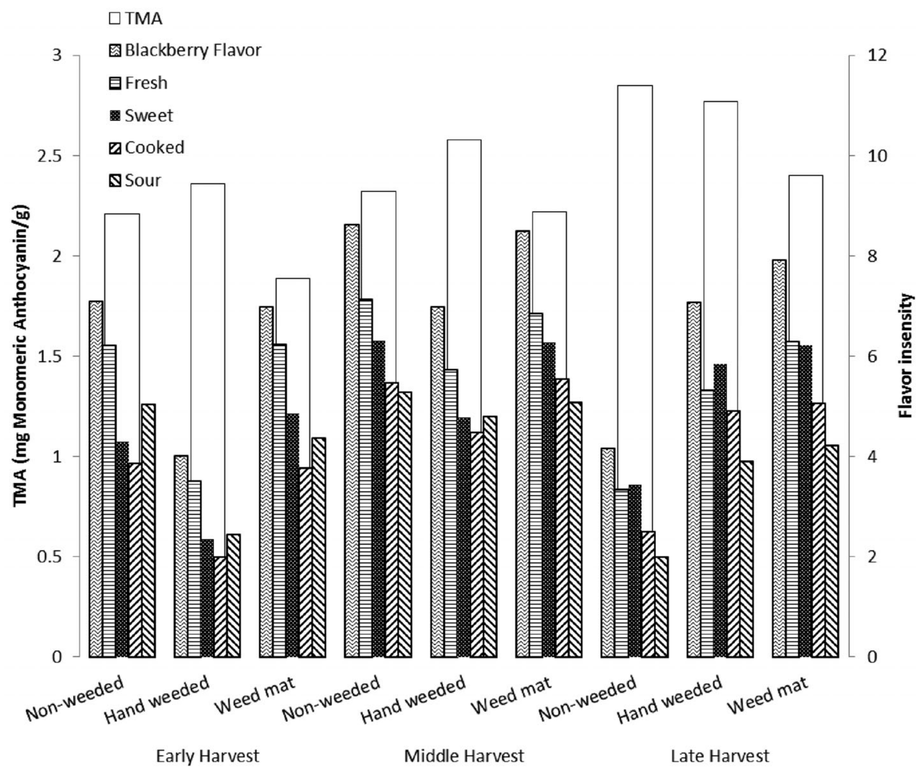

Figure 3–Relationship of total monomeric anthocyanin content (2012 data) with sensory attribute intensities (2013 data).

overall grade (Peterson and Takeda 2003) and acidity, carbohydrate content, and total anthocyanins (Given and Pringle 1985). Thus, it is possible that there is some variation in the degree of ripeness between individual fruit harvests, and if that is the case, one would expect that the middle harvest would have the most berries at the peak of ripeness, while the early and late harvests might have more overripe or barely ripe fruits. It is also possible that these differences are related to more complex phenomena such as seasonal/weather effects and plant physiology changes during the season. As for the higher values seen in the weed mat samples, it is likely related to the ability of the weed mat to prevent virtually all competition for resources, which would likely allow more nutrients to be available for the production of the metabolic products responsible for taste. What was more interesting was the manner in which the sensory data (from the 2013 harvest) related with the TPC and TMA measures (from the 2012 harvest), as seen in Figure 2 and 3, respectively. Specifically, the weed management strategies which resulted in the lowest intensity scores across the sensory attributes for a given harvest in 2013 (hand weeding in the early and middle harvests and nonweeding in the late) were the same treatments which resulted in the highest TPC and TMA values in 2012. While the differences in harvest year present challenges to making definitive relational determinations, measures of leaf nutrient levels have been shown to have similar responses to the 3 weed management strategies across multiple years (Harkins and others 2014), and when coupled with the degree of correlation seen between the 2013 sensory and 2012 antioxidant capacity measures, tends to reenforce the notion that resource competition likely led to increases in protective phenolics at the expense of other compounds.

Conclusion

Weed management strategies can have a marked effect on the quality characteristics of organically grown blackberry fruit. In particular, the sensory and antioxidant content of berries showed the most variability with treatment, and there was evidence that management strategies which resulted in increased levels of anthocyanins and phenolic compounds resulted in decreased intensity of the various flavor attributes and vice versa. While the variation in antioxidant content due to weed management ranged from 3% to 20%, previous studies have shown that it can have a much larger effect on total yield, with weed mat increasing yield by 20% to 100% while drastically reducing costs (Harkins and others 2013). This, coupled with the marked increase (22% to 102%) observed in the intensity of flavor characteristics, make a strong argument for the use of weed mat as the preferred weed management strategy in organic blackberry production. While there is no reason to believe that these phenomena are limited to organically grown blackberries, further study is needed to determine the degree of effect on different fruit crops and among different agricultural systems.

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Author Contributions

G. Cavender designed the study, performed HPLC analyses on sugar profile, performed the sensory study, and wrote the paper. M. Liu performed the assays of TSS, pH and Brix, TPC, TMA and RSA. D. Hobbs performed ORAC and FRAP assays. B. Strik developed and supervised the berry production trial and assisted with the recruitment of sensory panelists, B. Frei supervised the ORAC and FRAP assays. Y. Zhao supervised performance of the experiments and assisted with design and writing.

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