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Effect of Different Cultural Systems on Antioxidant Capacity, Phenolic Content, and Fruit Quality of Strawberries (*Fragaria* × *aranassa* Duch.)

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The effect of cultivation practices for controlling strawberry black root rot (BRR) on fruit quality, antioxidant capacity, and flavonoid content in two strawberry cultivars Allstar and Chandler (*Fragaria* × *ananassa* Duch.) was evaluated. Strawberry fruits used in this study were from plants grown in soils which had a prior history of BRR and red stele, and had not been fumigated during the seven years prior to the study. Results from this study showed that fruit from plants grown in compost socks had significantly higher oxygen radical absorbance capacity (ORAC), flavonoids, anthocyanins, soluble solids content (SSC), titratable acid (TA), fructose, glucose, sucrose, malic acid, and citric acid than fruit produced in the black plastic mulch or matted row systems. Cultivar Chandler surpassed cv. Allstar in sugar content, acid content, and flavonoid content regardless of preplanting vinegar drenching and various culture treatments. However, preplanting vinegar treatment increased cyanidin-based and pelargonidin-based anthocyanins but decreased sugar content in fruits of both cultivars.

KEYWORDS: ***Fragaria* × *ananassa* Duch.; antioxidant activity; anthocyanins; compost socks; total phenolics; flavonoids; sugars; organic acids; cultural systems**

INTRODUCTION

Black root rot (BRR) is the general name for several root disorders which produce similar symptoms. This disease is caused by a complex interaction of fungi, nematodes, and environmental factors, and its nature is not clearly understood. Several fungi including *Rhizoctonia* spp., *Pythiumspp*., and *Fusarium* spp., and the lesion nematode *Pratylenchus penetrans* are implicated in the disease (*1*, *2*). Black root rot has been found in every strawberry-growing area of the United States. Strawberry plants infected with BRR have small root systems with black lesions or completely blackened roots, and in addition, their shoots, crowns, and leaves lack vigor, exhibit poor runner growth, and produce small and few berries (*2*).

Soil fumigation with methyl bromide (CH_3Br, MeBr) has been used to control this soil borne plant disease, but the ban on methyl bromide has prompted growers and researchers to evaluate various chemical, biological, and cultural alternatives (*2*−*4*). Growers usually use a matted row system to grow perennial (three to five years) strawberries. Strawberry growers also found high profitability in utilizing the raised bed, plastic mulch (*5*, *6*). Himelrick (*7*) showed that plants grown with black plastic mulch (BPM) produce a higher number of runners and strawberry fruit than those in clear or white plastic mulches, and total fruit weight was increased by the use of black plastic mulch compared to that with bare soil. Strawberry plants growing in black plastic mulch were also found to have less BRR compared to those grown in matted rows (*2*). Incorporating compost into the soil can suppress this plant disease activity and can improve soil quality in organic as well as in conventional production systems (*8*−*11*). A single drench of vinegar (20% acetic acid) provided 100% destruction of *Rhizoctonia solani* and *Pythium* spp. and also aid in weed control (*2*). Millner (*2*) used a novel, raised-bed growing method that used 100% mature compost socks that lie directly on top of nonfumigated BRR-infested soil with or without a preplant soil drench of 20% vinegar as an alternative to fumigation and found that BRR symptoms were significantly reduced in all compost sock treatments. Compost sock treatment also produced significantly ($P < 0.05$) greater yields, healthier plant roots, and greater plant coverage in the rows than matted row or black plastic mulch systems, regardless of vinegar treatment (*2*). Vinegar alone was ineffective in significantly preventing plant disease. However, the effect of these treatments on strawberry fruit quality and antioxidant capacity has not yet been evaluated. The objectives of this investigation were to determine if cultural practices and alternatives to control BRR in strawberry production could influence fruit quality, anthocyanin and phenolic content, and antioxidant activity as well as flavonoid content in two strawberry cultivars (Allstar and Chandler).

MATERIALS AND METHODS

Chemicals. 2′,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA Inc., (Richmond VA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and

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fluorescein disodium were obtained from Aldrich Chemical Co. (Milwaukee, WI). Acetonitrile, methanol, acetone, and water were of HPLC grade and were purchased from Baxter (Muskegon, MI). All anthocyanins and aglycons were obtained from Indofine Chemical Co., Inc. (Somerville, NJ). Other authentic standards were obtained from Sigma Chemical Co. (St. Louis, MO) and Fisher Scientific (Suwanee, GA).

Plant Material and Field Experiments. Field plots were established with standard farm tillage equipment and practices in August at a commercial strawberry farm in Germantown, Maryland, with two strawberry cultivars (Allstar and Chandler). The soils were loam-sandy soil. The experiment sites had a prior history of red stele, caused by *Phytophthora fragariae* Hickman, and BRR, and had not been fumigated during the seven years prior to the study. Two strawberry cultivars (Allstar and Chandler) were grown on matted rows, compost socks, or black plastic mulch with or without preplant soil drench with 20% apple cider vinegar (Musselman's, Biglersville, PA). The randomized complete block plot plan was used with the three cultural systems as main plots and vinegar supply for soil disinfection as subplots.

Vinegar Drench. Fields were prepared using standard farm tillage equipment and practices in mid-August and in late August. Designated subplots (5 m long × 5 m wide, with 0.6 m wide rows) were treated with 20% (v/v) apple cider vinegar drench once prior to the placement of compost, transplants, or black plastic mulch (BPM). Vinegar was applied with manual pump-style sprayers at a rate equivalent to 1.2 L per 1-m band of soil. No additional vinegar was applied for the remainder of the experiment. Soil disinfection drenches were randomized in a complete block design with four replicates per treatment at each site.

Plastic Mulch. After seven days without rainfall or irrigation, 1 mil smooth, black plastic mulch (Robert Marvel Plastic Mulch, Annville, PA) was placed on the designated rows covering the previously vinegar-treated and untreated areas. The BPM was stretched across the soil surface after the drip irrigation tape was placed and was operational; film edges were buried in soil, and the BPM was applied on a raised bed.

Compost Socks. Mature, leaf-yard-trimming compost (Leafgro, Millersville, MD) was used to fill 20-cm-diameter compost socks (Filtrexx Inc., Grafton, OH) using a pneumatic blower system attached to a flexible hose. Leaf-grass-poultry (layer) manure compost produced at the USDA Composting Facility, Beltsville, MD, and polyethylene-mesh socks were also used. A drip irrigation system (Berry Hill Irrigation, Buffalo Junction, VA) with emitters spaced 30.5 cm apart and an emitter flow rate of 0.055 L-min per linear m (4.5 gal-min per 1000 linear ft) of row was placed on bed centers (on top of compost socks) and secured with metal landscape pins.

Transplants. Container-grown, soilless plug plants produced from gutter-propagated parent−plant runner tips (Davoncrest Nursery, Hurlock, MD) of cultivars Allstar and Chandler were transplanted 7 days after the vinegar drench. Plants were initially irrigated for 1 h immediately after transplanting and for 2 h each in the morning and afternoon on each of the following 7 days. They were subsequently fertigated with NH_3NO_3 (600 g N per acre/week) for five weeks in the fall and spring. Plant and row spacing was 30.5 cm within-row (single) × 30.1 m long on 1.5-m centers. Wheat straw provided winter protection (grower practice). Full details of this experimental plan were described in a previous publication (*2*).

Soluble Solids Content (SSC), and Titratable Acid (TA). The SSC of the fruit was determined on a digital refractometer Palette 100 PR-100 (ATAGO-Spectrum Technologies, Plainfield, ILL.) standardized with distilled water. Titratable acid (TA) was determined by diluting each 5 mL aliquot of strawberry juice to 100 mL with distilled water, then titrating to pH 8.2 using 0.1 N NaOH. Acidity was expressed as g citric acid per 100 mL juice (%).

Sugars and Organic Acids. Three 5 g subsamples of strawberries were extracted twice with 15 mL of imidazole buffer (20 mM, pH 7.0) using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). The extraction, purification, and derivatization procedures for nonstructural sugars and organic acids have been described previously (*12*). A Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector and a fused silica capillary column (dimethylsilicone fluid, 12.5 m × 0.2 mm) was used for separation of sugars and organic acids. Sugars and organic acids were quantified by comparing peak areas with those of standards.

Total Anthocyanin and Total Phenolic Content. Three 5 g subsamples of fresh berries were extracted with 25 mL of 80% acetone containing 0.2% formic acid. Total anthocyanin content in fruit extracts was determined using the pH differential method (*13*). Absorbance was measured using a Shimadzu Spectrophotometer (Shimadzu UV-160U, Columbia, MD) at 510 nm and 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}]$ with a molar extinction coefficient of cyanidin 3-glucoside (29,600). Results were expressed as milligrams of cyanidin 3-glucoside equivalent per 100 g of fwt (mg 100 g^{-1} fwt). Total soluble phenolics in the berry fruit extracts were determined with Folin−Ciocalteu reagent by the method of Slinkard and Singleton (*14*) using gallic acid as the standard. Results were expressed as mg gallic acid equivalent (GAE) per 100 g fwt (mg GAE 100 g^{-1} fwt).

HPLC Analysis of Berry Anthocyanins and Phenolic Compounds. High-performance liquid chromatography (HPLC) was used to separate and determine individual anthocyanins and phenolic compounds in berry tissue samples. The supernatants (15 mL) from the above extracts were concentrated to dryness using a Buchler Evapomix (Fort Lee, NJ) in a water bath at 35 °C and were dissolved in 4 mL of acidified water (3% formic acid) and then passed through a C_{18} Sep-Pak cartridge (Waters, Milford, MA), which was previously activated with methanol followed by water and then 3% aqueous formic acid. Anthocyanins and other phenolics were adsorbed onto a column while sugars, acids, and other water-soluble compounds were eluted with 10 mL of 3% formic acid. Anthocyanins and other phenolics were then recovered with 2.0 mL of acidified methanol containing 3% formic acid. The methanol extract was passed though a 0.45-μm membrane filter (Waters, Milford, MA), and 20 μL was analyzed by HPLC. The samples were determined using a HPLC (Waters, Milford, MA) system coupled with a photodiode array detector (Waters 2996 Series) and equipped with two pumps (600E system controller). Samples were injected at ambient temperature (20 °C) into a reverse phase NOVA-PAK C_{18} column (150 × 3.9 mm, particle size 4 μm) with a guard column (NOVA-PAK C_{18}, 20 × 3.9 mm, particle size 4 μm) (Waters, Milford, MA). The mobile phase consisted of 5% aqueous formic acid (A) and HPLC grade acetonitrile (B). The flow rate was 1 mL min^{-1}, with a gradient profile consisting of mobile phase A with the following proportions (v/v) of B: 0−1 min, 4%, 1−10 min, 4−6% B; 10−15 min, 6% B; 15−35 min, 6−18% B; 35−40 min, 18−20% B; 40−42 min, 20−45% B; 42−45 min, 45−100% B; 45−50 min, 100% B. The phenolic compounds in fruit extracts were identified by their UV spectra, recorded with a diode array detector and by chromatographic comparison with authentic markers (*15*−*21*). Retention times and spectra were compared to those of the pure standards, and the results were confirmed by coinjection with authentic standards. Individual phenolic acid, flavonols, and anthocyanins were quantified by comparison with an external standard of ellagic acid, p-coumaroyl-glucose, quercetin 3-glucoside, and cyanidin 3- glucoside, pelargonidin 3-glucoside, pelargonidin 3-rutinoside, and pelargonidin 3-glucoside-succinate. Each standard was dissolved in methanol at a concentration of 1 mg mL^{-1}, and five dilute solutions from these stock solutions were used to prepare calibration curves of each standard. Recoveries were measured by extracting the recovered amounts of pure substances added to strawberries before the experiment. Three replicates from each sample were used for HPLC analyses. Scanning between 250 and 550 nm was performed, and data were collected by the Waters 990 3-D chromatography data system.

Oxygen Radical Absorbance Capacity (ORAC) Assay. The supernatants from the above extracts were used for the ORAC assay. The ORAC assay was carried out using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system (*22*) with a FL800 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT) and fluorescence filters for an excitation wavelength of 485 ± 20 nm and an emission wavelength of 530 ± 25 nm. The plate reader was controlled by software KC4 3.0 (revision 29). Sample dilution was accomplished by a Precision 2000 automatic pipetting system managed by precision power software (version 1.0) (Bio-Tek Instruments, Inc.). The ORAC values were determined by calculating the net area under the curve (AUC) of the standards and samples (*22*). The standard curve was obtained by plotting Trolox concentrations against the average net AUC of the two measurements for each concentration. Final ORAC

Table 1. Soluble Solid Content (SSC) and Sugar (Fructose, Glucose, and Sucrose) in Fruits of Two Strawberry Cultivars Chandler and Allstar in Field Tests with and without Vinegar in Three Cultural Systems (Matted Row, Black Plastic Mulch, and Compost Socks)[a]

				sugar (%)		
culture system	treatment	cultivar	SSC (%)	fructose	glucose	sucrose
matted row	vinegar	Allstar	6.41 ± 0.07 e	1.87 ± 0.13 def[b]	1.76 ± 0.08 ef	0.78 ± 0.02 f
matted row	vinegar	Chandler	5.52 ± 0.06 g	1.60 ± 0.10 h	1.50 ± 0.01 h	0.66 ± 0.01 h
matted row	no vinegar	Allstar	6.72 ± 0.07 d	2.11 ± 0.11 bc	1.96 ± 0.02 c	0.97 ± 0.03 a
matted row	no vinegar	Chandler	5.80 ± 0.05 f	1.69 ± 0.12 gh	1.60 ± 0.02 g	0.71 ± 0.00 g
black plastic mulch	vinegar	Allstar	7.21 ± 0.07 bc	2.02 ± 0.07 bcd	2.06 ± 0.05 b	0.83 ± 0.01 de
black plastic mulch	vinegar	Chandler	6.42 ± 0.04 e	1.84 ± 0.02 fgh	1.74 ± 0.06 f	0.77 ± 0.00 f
black plastic mulch	no vinegar	Allstar	7.32 ± 0.11 b	2.52 ± 0.05 a	2.18 ± 0.04 a	0.85 ± 0.02 cd
black plastic mulch	no vinegar	Chandler	6.73 ± 0.08 d	1.97 ± 0.06 cd	1.84 ± 0.01 de	0.82 ± 0.03 e
compost socks	vinegar	Allstar	7.34 ± 0.10 b	2.14 ± 0.00 b	1.91 ± 0.04 cd	0.86 ± 0.03 cd
compost socks	vinegar	Chandler	6.72 ± 0.07 d	1.96 ± 0.10 cd	1.85 ± 0.02 de	0.82 ± 0.02 e
compost socks	no vinegar	Allstar	7.64 ± 0.11 a	2.62 ± 0.12 a	2.23 ± 0.07 a	0.92 ± 0.05 b
compost socks	no vinegar	Chandler	7.12 ± 0.08 b	2.06 ± 0.11 bc	1.96 ± 0.02 c	0.87 ± 0.02 c
			significance[c]			
culture system (cs)			*	*	*	*
treatment (trt)			*	*	*	*
cultivar (c)			*	*	*	*
cs × trt			ns	ns	ns	*
cs × c			ns	ns	*	*
trt × c			ns	*	*	*
cs × trt × c			ns	ns	ns	*

[a] Data expressed as the mean ± SD (n = 3). [b] Means within the same column followed by different letters were significantly different at $p < 0.05$. [c] * ns, significant or nonsignificant at $p < 0.05$.

values were calculated using the regression equation between Trolox concentration and the net AUC, and were expressed as micromole Trolox equivalents per gram of fresh weight (*22*).

Statistical Analysis. Data were subjected to analysis of variance using SPSS (version 17) (*23*). Values of SSC, TA, sugars, organic acids, ORAC, total phenolics, total anthocyanin, and flavonoids were evaluated by the Duncan's test. Differences at $P < 0.05$ were considered significant. Correlation coefficients between antioxidant activity (ORAC), total phenolics, and total anthocyanins were calculated using Excel (Microsoft Corp., Redmond, WA) and are reported as R^2 values.

RESULTS AND DISCUSSION

The SSC and sugar (fructose, glucose, and sucrose) content of fruit grown in compost socks was higher than that of fruit grown in black plastic mulch or matted rows (**Table 1**). The general flavor selection criteria for strawberries have been high sweetness and high acidity. Therefore, strawberries grown in compost socks were considered to be of high quality. Addition of the vinegar treatment showed decreased SSC. Galletta et al. (*24*) reported that SSC in strawberry fruit generally is in the range of 7% to 12% depending on genotype. Shaw (*25*) reported that genotypic SSC ranges for two sets of California strawberry seedling selections (n = 13; 12) were 5.28−8.74% and 6.06−8.73%. Fruits of cv. Allstar had higher content of SSC compared to those of cv. Chandler grown in compost socks, black plastic mulch, or matted rows. SSC in two strawberry cultivars showed no interaction between culture system × treatment, culture system × cultivar, vinegar treatment × cultivar, and culture system × treatment × cultivar (**Table 1**).

Fructose, glucose, and sucrose were found to be the three major sugars, comprising >65% of the total SSC in fruits of strawberry (**Table 1**). The amounts of these sugars in the fruit were different between two cultivars and treatments. In general, strawberry fruit contained lower sucrose concentrations compared to that of fructose and glucose. The low sucrose content in the fruit is probably due to enzymatic hydrolysis after translocation from the leaves (*26*). The proportions of fructose, glucose, and sucrose are important in the perception of fruit quality since fructose is 1.8 times sweeter than sucrose (*27*), while the sweetness of glucose is only 60% of that sucrose (*28*, *29*). When plants were grown in compost socks, fruit from cv. Allstar and cv. Chandler had higher fructose and glucose content than fruit grown in other cultural systems. A comparison between sugar (fructose, glucose, and sucrose) content of strawberry fruit produced in two mulch systems revealed that the growth in black plastic mulch led to higher total sugar content compared to that from the growth in matted rows. There were significant differences in fructose, glucose, and sucrose content with and without vinegar treatments. A significant interaction between vinegar treatment and cultivar for fructose, glucose, and sucrose was found. An interaction of culture system × cultivar for glucose and sucrose was also evidenced (**Table 1**).

The TA and organic acids (citric and malic) content of fruit grown in three culture systems are shown in **Table 2**. Additional vinegar treatment showed no significant difference in TA, citric, and malic content of all culture systems and cultivars. Citric acid was the major organic acid found in strawberries compared to malic acid (**Table 2**). Organic acids are minor components of strawberry fruit, but they are important attributes of flavor that, in combination with sugars, have an impact on the sensory quality of strawberry fruit. Total organic acid level was positively correlated with titratable acidity (R^2 = 0.9432). There were distinct differences in TA and organic acid content in two cultivars examined. Fruit of cv. Allstar had lower content of TA compared to that of cv.Chandler. Citric and malic acid contents of cv. Allstar and cv. Chandler fruits were generally higher when plants were grown in compost socks, compared to those grown in black plastic mulch or in matted row. Strawberries from plants grown in black plastic mulch generally had higher citric and malic acids, compared to those grown in matted rows (**Table 2**). A significant interaction between culture system × vinegar treatment on TA was found (**Tables 2**). There were also significant interactions between culture system × cultivar and culture systems × cultivar × vinegar treatment for citric acid (**Table 2**).

Table 2. Titratable Acid (TA) and Organic Acid (Malic Acid and Citric Acid) Contents in Fruits of Two Strawberry Cultivars Chandler and Allstar in Field Tests with and without Vinegar in Three Cultural Systems (Matted Row, Black Plastic Mulch, and Compost Socks)[a]

				organic acids (%)	
culture system	treatment	cultivar	TA (%)	malic acid	citric acid
matted row	vinegar	Allstar	0.59 ± 0.02 ef[b]	0.10 ± 0.01 cd	0.43 ± 0.02 fg
matted row	vinegar	Chandler	0.61 ± 0.01 de	0.11 ± 0.02 bcd	0.47 ± 0.03 ef
matted row	no vinegar	Allstar	0.56 ± 0.02 f	0.09 ± 0.00 d	0.40 ± 0.01 g
matted row	no vinegar	Chandler	0.64 ± 0.01 cd	0.10 ± 0.02 cd	0.43 ± 0.02 fg
black plastic mulch	vinegar	Allstar	0.64 ± 0.02 cd	0.10 ± 0.00 cd	0.51 ± 0.01 cde
black plastic mulch	vinegar	Chandler	0.75 ± 0.03 b	0.13 ± 0.01 ab	0.56 ± 0.03 b
black plastic mulch	no vinegar	Allstar	0.63 ± 0.02 cd	0.10 ± 0.01 cd	0.5 ± 0.01 de
black plastic mulch	no vinegar	Chandler	0.72 ± 0.02 b	0.12 ± 0.01 abc	0.52 ± 0.02 bcd
compost socks	vinegar	Allstar	0.74 ± 0.03 b	0.12 ± 0.01 abc	0.55 ± 0.02 bc
compost socks	vinegar	Chandler	0.84 ± 0.03 a	0.14 ± 0.02 a	0.61 ± 0.03 a
compost socks	no vinegar	Allstar	0.67 ± 0.02 c	0.11 ± 0.01 bcd	0.47 ± 0.04 ef
compost socks	no vinegar	Chandler	0.83 ± 0.03 a	0.13 ± 0.02 ab	0.61 ± 0.03 a
			significance[c]		
culture system (cs)			*	*	*
treatment (trt)			ns	ns	ns
cultivar (c)			*	*	*
cs × trt			*	ns	ns
cs × c			*	ns	*
trt × c			*	ns	ns
cs × trt × c			ns	ns	*

[a] Data expressed as the mean ± SD (n = 3). [b] Means within the same column followed by different letters were significantly different at $p < 0.05$. [c] * ns, significant or nonsignificant at $p < \leq 0.05$.

Table 3. Total Anthocyanins, Total Phenolics, and Antioxidant Activity (ORAC) in Fruits of Two Strawberry Cultivars Chandler and Allstar in Field Tests with and without Vinegar in Three Cultural Systems (Matted Row, Black Plastic Mulch, and Compost Socks)[a]

culture system	treatment	cultivar	total anthocyanins[b] (mg/100 g fwt)	total phenolics[c] (mg/100 g fwt)	ORAC[d] (μmol TE/g fwt)
matted row	vinegar	Allstar	36.8 ± 1.6 f[e]	97.9 ± 4.5i	26.19 ± 0.31 h
matted row	vinegar	Chandler	55.1 ± 2.3 c	157.1 ± 3.0 f	41.26 ± 0.43 e
matted row	no vinegar	Allstar	32.0 ± 1.5 g	89.7 ± 5.8 k	23.64 ± 0.29 i
Matted row	no vinegar	Chandler	47.8 ± 1.9 ef	161.8 ± 2.7 e	40.73 ± 0.62 e
black plastic mulch	vinegar	Allstar	46.8 ± 1.8 d	98.5 ± 3.0 i	28.31 ± 0.23 g
black plastic mulch	vinegar	Chandler	60.1 ± 2.1 b	174.7 ± 3.1 c	45.98 ± 0.10 c
black plastic mulch	no vinegar	Allstar	44.5 ± 1.6 e	93.9 ± 1.9 j	26.85 ± 0.15 h
black plastic mulch	no vinegar	Chandler	58.4 ± 2.3 c	170.1 ± 1.8 d	44.03 ± 0.12 d
compost socks	vinegar	Allstar	47.7 ± 2.0 d	121.2 ± 4.3 g	32.82 ± 0.12 f
compost socks	vinegar	Chandler	68.2 ± 2.1 a	205.1 ± 2.9 a	53.14 ± 0.20 a
compost socks	no vinegar	Allstar	45.4 ± 1.1 e	114.6 ± 5.8 h	30.35 ± 0.13 g
compost socks	no vinegar	Chandler	62.4 ± 2.6 b	195.1 ± 2.3 b	50.09 ± 0.21 b
			significance [f]		
culture system (cs)			*	*	*
treatment (trt)			*	*	*
cultivar (c)			*	*	*
cs × trt			ns	ns	ns
cs × c			ns	*	*
trt × c			ns	ns	ns
cs × trt × c			ns	ns	ns

[a] Data expressed as the mean ± SD (n = 3). [b] Data expressed as milligrams of cyanidin 3-glucoside equivalents per 100 g of fresh weight. [c] Data expressed as milligrams of gallic acid (GAE) equivalents per gram of fresh weight. [d] Data expressed as micromoles of Trolox equivalents per gram of fresh weight. [e] Means within the same column followed by different letters were significantly different at $p < 0.05$. [f] * ns, significant or nonsignificant at $p < 0.05$.

The effects of cultural systems in two cultivars of strawberry Allstar and Chandler on total anthocyanins, total phenolics, and ORAC were significant (**Table 3**). The highest anthocyanins, total phenolics, and ORAC were observed in strawberries grown in compost socks, while the lowest ORAC were found in fruits from plants grown in a matted row system. Vinegar treatment in the culture system also showed s significant effect, generally increasing total anthocyanins, total phenolics, and ORAC values (**Table 3**). Cultivar Chandler exhibited higher values of anthocyanins, total phenolics and antioxidant capacity (ORAC) than cv. Allstar (**Table 3**). The interaction between culture system × vinegar treatment, vinegar treatment × cultivar, and culture system × vinegar treatment × cultivar showed no significant difference for total anthocyanins, total phenolics, and ORAC. However, the interaction between culture system × cultivar was shown significant difference for total phenolics and ORAC (**Table 3**).

Ellagic acid, ellagic acid glucoside, *p*-coumaroylglucose, quercetin 3-glucoside and quercetin 3-glucuronide, kaempferol 3-glucoside, and kaempferol 3-glucuronide were detected in strawberries

Table 4. Ellagic Acid, *p*-Coumaroyl-glucose, Ellagic Acid Glucoside, Quercetin 3-Glucoside and Quercetin 3-Glucuronide, Kaempferol 3-Glucoside, and Kaempferol 3-Glucuronide Contents (Micrograms per Gram of Fresh Weight) in Fruits of Two Strawberry Cultivars Chandler and Allstar in Field Tests with and without Vinegar in Three Cultural Systems (Matted Row, Black Plastic Mulch, and Compost Socks)[a]

culture system	treatment	cultivar	ellagic acid[b]	ellagic acid glucoside[b]	*p*-coumaroyl-glucose[c]	quercetin 3-glucoside and quercetin 3-glucuronide[d]	kaempferol 3-glucoside and kaempferol 3-glucuronide[d]
matted row	vinegar	Allstar	8.3 ± 0.3 h[e]	19.7 ± 1.1 e	23.9 ± 1.2 e	8.8 ± 0.5 f	3.5 ± 0.2 e
matted row	vinegar	Chandler	13.5 ± 0.4 e	31.9 ± 1.9 c	38.7 ± 2.3 c	14.2 ± 0.8 d	5.6 ± 0.3 c
matted row	no vinegar	Allstar	7.8 ± 0.2 h	18.3 ± 0.8 e	22.5 ± 1.0 e	8.4 ± 0.3 f	3.7 ± 0.2 e
matted row	no vinegar	Chandler	13.9 ± 0.3 de	32.6 ± 2.0 bc	39.5 ± 2.2 bc	14.6 ± 0.7 cd	5.8 ± 0.1 bc
black plastic mulch	vinegar	Allstar	8.4 ± 0.3 h	19.9 ± 1.0 e	24.1 ± 0.8 e	8.9 ± 0.7 f	3.5 ± 0.1 e
black plastic mulch	vinegar	Chandler	14.8 ± 0.4 c	35.3 ± 2.2 b	42.7 ± 1.7 b	15.8 ± 0.6 c	6.2 ± 0.4 b
black plastic mulch	no vinegar	Allstar	7.9 ± 0.1 h	18.9 ± 0.9 e	22.8 ± 0.7 e	8.4 ± 0.5 f	3.3 ± 0.2 e
black plastic mulch	no vinegar	Chandler	14.4 ± 0.3 cd	34.3 ± 1.7 bc	41.5 ± 2.3 bc	15.4 ± 0.3 cd	6.1 ± 0.3 b
compost socks	vinegar	Allstar	10.3 ± 0.5 f	24.5 ± 1.2 d	29.6 ± 1.5 d	10.9 ± 0.5 e	4.3 ± 0.2 d
compost socks	vinegar	Chandler	17.4 ± 0.6 a	41.4 ± 2.1 a	50.1 ± 2.7 a	18.5 ± 1.2 a	7.3 ± 0.4 a
compost socks	no vinegar	Allstar	9.7 ± 0.1 g	23.1 ± 1.3 d	28.0 ± 1.4 d	10.4 ± 0.6 e	4.4 ± 0.0 d
compost socks	no vinegar	Chandler	16.5 ± 0.4 b	39.4 ± 2.5 a	47.7 ± 2.6 a	17.6 ± 1.7 b	7.0 ± 0.3 a
				significance[f]			
culture system (cs)			*	*	*	*	*
treatment (trt)			*	ns	ns	ns	ns
cultivar (c)			*	*	*	*	*
cs × trt			ns	ns	ns	ns	ns
cs × c			*	ns	*	ns	*
trt × c			ns	ns	ns	ns	ns
cs × trt × c			ns	ns	ns	ns	ns

[a] Data expressed as the mean ± SD ($n = 3$). [b] Data expressed as micrograms of ellagic acid equivalents per gram of fresh weight. [c] Data expressed as micrograms of *p*-coumaroyl-glucoside equivalents per gram of fresh weight. [d] Data expressed as micrograms of quercetin 3-glucoside equivalents per gram of fresh weight. [e] Means within the same column followed by different letters were significantly different at $P < 0.05$. [f] * ns, significant or nonsignificant at $p < 0.05$.

(**Table 4**). Ellagic acid is a naturally occurring phenolic constituent of many plant species (*30*) and has shown promising antimutagenic and anticarcinogenic activity against chemical-induced cancers (*31*, *32*). In nature, ellagic acid may occur in free form but is more commonly present as esters of the diphenic acid analogue on glucose. Strawberries contained both ellagic acid and ellagic acid glucoside, and large differences in levels have been found among species, cultivars, tissues, and environmental conditions (*33*, *34*).

Fruits of cv. Allstar and cv. Chandler grown in compost socks had higher amounts of ellagic acid, ellagic acid glucoside, *p*-coumaroylglucose, quercetin 3-glucoside, and quercetin 3-glucuronide than fruits grown in the other two culture systems, while vinegar treatment showed no effect on these constituents (**Table 4**). The content of ellagic acid, ellagic acid glucoside, *p*-coumaroylglucose, quercetin 3-glucoside, and quercetin 3-glucuronide was higher in cv. Chandler than in cv. Allstar. In general, the content of these flavonols were significantly higher than that of other flavonols, such as kaempferol 3-glucoside and kaempferol 3-glucuronide (**Table 4**). All of these flavonols are effective antioxidants (*35*). Kaempferol and quercetin are potent quenchers of $ROO^{\bullet}$, $O_2^{\bullet -}$, and $^1O_2$ (*36*). Quercertin and other polyphenols have been shown to play a protective role in carcinogenesis by reducing the bioavailability of carcinogens (*37*). Quercetin, with 3′,4′-dihydroxy substitution in the B-ring and conjugation between the A- and B-rings, has higher antioxidant potential against peroxyl radicals compared to that of kaempferol (*38*). The antioxidant capacities measured by the ORAC assay for quercetin and kaempferol were 3.29 and 2.67 μM Trolox equivalents, respectively (*39*). Clegg and Morton (*40*) also reported that quercetin had the greatest antioxidant activity, followed by dihydroquercetin > kaempferol > quercetin > chlorogenic acid = *p*-comaric acid. Flavones in general have higher antioxidant activity compared to that of anthocyanidins with the same hydroxylation patterns as measured with ORAC assay (*41*).

The anthocyanins are a group of flavonoids with exceptionally good scavenging activities. The antioxidant capacity of anthocyanins may be one of their most significant biological properties (*42*). It has been shown that anthocyanins are strong antioxidants with free radical scavenging properties attributed to the phenolic hydroxyl groups attached to ring structures. Different hydroxylations and glycosylation may modulate their antioxidative properties (*43*−*46*). The anthocyanin, cyanidin, with 3′,4′-dihydroxy substitution in the B ring and conjugation between the A- and B-rings, possesses high antioxidant activity (*38*, *47*, *48*) and has antioxidant potentials four times that of Trolox (*44*). The antioxidant capacities measured by the ORAC assay for cyanidin 3-glucoside and pelargonidin 3-glucoside were found to be 2.24 and 1.54 μM Trolox equivalents, respectively (*38*, *39*). The bright red color of strawberry fruits is due to their anthocyanin content. Pelargonidin 3-rutinoside was found only in cv. Chandler fruit (**Table 5**). Two anthocyanidin glycosides, pelargonidin 3-glucoside and cyanidin 3-glucoside, are almost exclusively responsible for the red color of strawberries (*49*). Strawberry fruit contained four major anthocyanins: cyanidin 3-glucoside, pelargonidin 3-glucoside, cyanidin 3-glucoside-succinate pelargonidin 3-glucoside-succinate, and a pelargonidin derivative. The content of cyanidin-based anthocyanins in strawberry fruit was much lower than that of pelargonidin-based anthocyanins (**Table 5**). Cultivar Chandler generally had higher values of these components compared to those of the fruits of cv. Allstar (**Tables 4** and **5**).

Strawberries grown in all three cultural systems (matted row, black plastic mulch, and compost socks) had the same main phenolic compounds, including anthocyanin pigments. However, there were significant quantitative differences. In general, phenolic acid and flavonol content as well as cyanidin-based and pelargonidin-based anthocyanins and total flavonoids were generally greatest in the compost sock system. The mean values of the anthocyanins cyanidin 3-glucoside, pelargonidin 3-glucoside,

Table 5. Cyanidin 3-Glucoside, Pelargonidin 3-Glucoside, Pelargonidin 3-Rutinoside, Cyanidin 3-Glucoside-succinate, and Pelargonidin Derivative Contents (Micrograms per Gram of Fresh Weight) in Fruits of Two Strawberry Cultivars Chandler and Allstar in Field Tests with and without Vinegar in Three Cultural Systems (Matted Row, Black Plastic Mulch, and Compost Socks)[a]

culture system	treatment	cultivar	cyanidin 3-glucoside[b]	pelargonidin 3-glucoside[b]	pelargonidin 3-rutinoside[b]	cyanidin 3-glucoside-succinate[b]	pelargonidin 3-glucoside-succinate[b]	pelargonidin derivative[b]
matted row	vinegar	Allstar	21.2 ± 0.3 h[c]	421.0 ± 2.4 j		15.7 ± 1.2 h	53.4 ± 2.3 h	2.1 ± 0.2 ef
matted row	vinegar	Chandler	31.7 ± 0.4 d	629.6 ± 7.2 e	30.3 ± 1.5 d	23.4 ± 0.5 d	79.8 ± 2.7 d	3.1 ± 0.3 bc
matted row	no vinegar	Allstar	19.4 ± 0.2 i	369.3 ± 5.1 k		14.6 ± 0.7 i	49.3 ± 2.1 i	1.9 ± 0.1 f
matted row	no vinegar	Chandler	27.5 ± 0.5 e	546.6 ± 1.4 f	31.8 ± 1.3 d	20.4 ± 0.6 e	69.3 ± 1.8 e	2.7 ± 0.2 cd
black plastic mulch	vinegar	Allstar	27.0 ± 0.3 ef	535.4 ± 5.7 g		19.9 ± 0.6 ef	67.9 ± 1.6 ef	2.6 ± 0.1 d
black plastic mulch	vinegar	Chandler	34.6 ± 2.5 bc	687.0 ± 5.8 c	41.6 ± 2.4 c	25.6 ± 0.7 bc	87.1 ± 2.5 bc	3.4 ± 0.3 ab
black plastic mulch	no vinegar	Allstar	25.6 ± 0.8 f	509.1 ± 4.2 h		19.0 ± 0.5 f	64.5 ± 2.1 f	2.5 ± 0.2 de
black plastic mulch	no vinegar	Chandler	33.6 ± 1.2 c	667.5 ± 6.5 d	42.8 ± 2.3 c	24.9 ± 0.2 c	84.6 ± 2.8 c	3.3 ± 0.4 b
compost socks	vinegar	Allstar	27.4 ± 0.3 e	544.9 ± 1.8 f		20.3 ± 0.7 e	69.1 ± 0.8 e	2.6 ± 0.2 d
compost socks	vinegar	Chandler	39.3 ± 1.1 a	780.1 ± 4.6 a	49.1 ± 2.8 b	29.0 ± 0.1 a	98.9 ± 2.4 a	3.8 ± 0.4 a
compost socks	no vinegar	Allstar	23.8 ± 0.6 g	473.5 ± 3.4 i		17.6 ± 0.1 g	60.2 ± 1.5 g	2.3 ± 0.1 def
compost socks	no vinegar	Chandler	36.2 ± 1.5 b	713.8 ± 6.9 b	53.2 ± 2.2 a	26.6 ± 0.2 b	90.5 ± 2.3 b	3.5 ± 0.3 ab
				significance[d]				
culture system (cs)			*	*	*	*	*	*
treatment (trt)			*	*	*	*	*	*
cultivar (c)			*	*		*	*	*
cs × trt			*	*	ns	*	*	*
cs × c			*	*		*	*	ns
trt × c			ns	ns		ns	ns	ns
cs × trt × c			ns	*		ns	ns	ns

[a] Data expressed as the mean ± SD ($n = 3$). [b] Data expressed as micrograms of cyanidin 3-glucoside equivalents per gram of fresh weight. [c] Means within the same column followed by different letters were significantly different at $P < 0.05$. [d] * ns, significant or nonsignificant at $p < 0.05$.

pelargonidin 3-rutinoside, cyanidin 3-glucoside-succinate pelargonidin 3-glucoside-succinate, and the pelargonidin derivative for the fruit grown from compost socks were higher than those from matted row and black plastic mulch systems. Flavonol content (kaempferol and quercetin derivatives) in strawberries was also significantly greater in fruit from plants grown in compost socks than fruit grown in matted row and black plastic mulch systems. Strawberry fruit from plants grown with additional vinegar treatments showed higher cyanidin-based and pelargonidin-based anthocyanins than fruit grown without vinegar treatment (**Tables 4** and **5**). There were significant interactions between culture system × cultivar for ellagic acid, *p*-coumaroylglucose, kaempferol 3-glucoside, and kaempferol 3-glucuronide, cyanidin 3-glucoside, pelargonidin 3-glucoside, cyanidin 3-glucoside-succinate, and pelargonidin 3-glucoside-succinate (**Tables 3** and **4**). Significant interactions between culture system × vinegar treatment for cyanidin 3-glucoside, pelargonidin 3-glucoside, cyanidin 3-glucoside-succinate, pelargonidin 3-glucoside-succinate, and pelargonidin derivative were also found (**Tables 3** and **4**).

There was a linear correlation between total flavonoids and scavenging capacity of the ROO$^{\bullet}$ radical (ORAC) for strawberries. The correlation coefficient between total anthocyanins and ORAC values was 0.8980, and the correlation coefficient between total phenolics and the ORAC values was 0.9932. We found that sugars and organic acids in strawberries showed no antioxidant activity with the ORAC assay (data not shown). Gil et al. and Lea (*50*, *51*) also found that sugars and organic acids showed no antioxidant activities with DPPD, FRAP, and TEAC in pomegranate and apple juice.

Collectively, the results from this study indicate that different cultural systems (matted row, black plastic mulch, and compost socks) and different cultivars (Allstar and Chandler) significantly affected strawberry fruit quality, antioxidant capacity, and flavonoid content. Strawberries grown in the compost sock system had significantly higher soluble solids content, total sugars, fructose, glucose, sucrose, malic acid, citric acid, and flavonoid content, and antioxidant capacities than fruit grown in two other culture systems. Cultivar Chandler also had higher content of these components than cv. Allstar. Preplant vinegar treatment increased cyanidin-based and pelargonidin-based anthocyanins but decreased sugar content in fruits of both cultivars.

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