Antioxidant Capacity and Flavonoid Content in Wild Strawberries

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ABSTRACT. Fruit of the cultivated strawberry (*Fragaria ×ananassa* Duchesne ex Rozier) are a good source of natural antioxidants, which play an important role in protecting human health. Antioxidant levels vary considerably among strawberry genotypes. The cultivated strawberry is a hybrid of two very different wild progenitor species: *F. virginiana* Mill. and *F. chiloensis* (L.) Mill. The progenitor species are valued by strawberry breeders as sources of novel traits, but have not been evaluated for antioxidant capacity or levels of antioxidant compounds. The objectives of this study are 1) to evaluate the antioxidant contents and antioxidant activities in representatives of *F. virginiana* and *F. chiloensis* in comparison with representatives of the cultivated strawberry species (*F. ×ananassa*), 2) to determine which strawberry compounds are most closely correlated with antioxidant capacity among these three species, and 3) to identify wild strawberry genotypes with high antioxidant activity and bioactive compounds for use in cultivar development. Fruit of 19 accessions from each of the three species, cultivated strawberry plus the two progenitor species (*F. ×ananassa*, *F. virginiana*, and *F. chiloensis*), were evaluated for levels of antioxidant capacity (oxygen radical absorbance capacity), total phenolics, total anthocyanins, ellagic acid, quercetin 3-glucoside plus quercetin 3-glucuronide, kaempferol 3-glucoside, kaempferol 3-rutinoside, *p*-coumaryl-glucose, pelargonidin 3-glucoside, pelargonidin 3-glucoside-succinate, cyanidin 3-glucoside, and cyanidin 3-glucoside-succinate. Fruit of the 13 accessions tested from the wild progenitor species *F. virginiana* had significantly higher antioxidant capacity, total phenolics, and total anthocyanins than did the fruit of three accessions tested from the cultivated strawberry *F. ×ananassa*, or the three accessions tested from the other wild progenitor species (*F. chiloensis*), and will be valuable as a source of parent material for developing more nutritious strawberry cultivars. The high correlation with antioxidant capacity, relative efficiency, and lack of genotype-by-year interaction in this study suggests that the measurement of total phenolics may be the better assay to use for the later selection stages in a strawberry cultivar development program. Of the evaluated *F. virginiana* accessions, NC 95-19-1, JP 95-1-1, CFRA 0982, NC 96-5-3, and RH 30 fruit were highest in antioxidant capacity and should prove useful toward development of strawberry cultivars with high antioxidant capacities.

Antioxidants are compounds that can delay or inhibit the oxidation of lipid or other molecules by inhibiting oxidizing chain reactions and they play an important role in health protection (Velioglu et al., 1998). In addition to being rich in folate, potassium, and fiber, fruit of the cultivated strawberry (*F. ×ananassa*) are a good source of a variety of natural antioxidants (Heinonen et al., 1998; Wang et al., 1996). Antioxidants in strawberry fruit include vitamin C and phenolic compounds such as phenolic acids and flavonoids, including flavonols and anthocyanins.

Important phenolic acids in strawberries are the ellagitannins and ellagic acid glucosides, which break down to pure ellagic acid, also present in the fruit (Cerdá et al., 2005). Ellagic acid is valuable to human health because it is antimutagenic and has anticarcinogenic activity against chemical-induced cancers (Okuda et al., 1989). Ellagitannins in strawberries are primarily

located in the achenes (Maas et al., 1991). The ellagic acid, glucosides, and pure ellagic acid are present in the red fleshy receptacle. Ellagic acid in pure form is poorly absorbed through the bowel and is mostly biologically unavailable whereas the ellagitannins and ellagic acid glucosides are easily available for absorption in mammalian systems (Okuda et al., 1989). In general, the content of ellagic acid glucoside of strawberry cultivars is higher than that of ellagic acid (Cerdá et al., 2005; Daniel et al., 1989).

Beneficial effects of flavonoids have been described for diabetes mellitus, allergies, cancer, viral infections, headache, stomach and duodenal ulcers, paradentosis, and inflammatory diseases (Kuehnau, 1976). Flavonoids in strawberry include the flavonols quercetin and kaempferol (Wang and Zheng, 2001). These and other polyphenols play a protective role in carcinogenesis by reducing the bioavailability of carcinogens (Starvic et al., 1992). Clegg and Morton (1968) reported that, of the flavonols, quercetin had the greatest antioxidant activity, followed by dihydroquercetin > kaempferol > quercitrin > chlorogenic acid = *p*-coumaric acid.

The anthocyanins are a group of flavonoids with exceptionally good scavenging activities. Pelargonidin-based anthocyanins such as pelargonidin 3-glucoside, pelargonidin 3-rutinoside, and pelargonidin 3-glucoside-succinate are the predominant anthocyanins in cultivated strawberry fruit (Gil et al., 1997; Wang and Zheng, 2001). Strawberry fruit generally

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contain much lower levels of cyanidin-based anthocyanins, cyanidin 3-glucoside and cyanidin 3-glucoside-succinate, than pelargonidin-based anthocyanins (Gil et al., 1997; Wang and Zheng, 2001). These anthocyanin compounds have shown protection against harmful free radicals and have been associated with lower incidence and mortality rates of cancer and heart disease in addition to a number of other health benefits (Ames et al., 1993; Dragsted et al., 1993; Velioglu et al., 1998; Wang et al., 1996). Antioxidant efficacy in preventing oxidation of human low-density lipoprotein among anthocyanins is as follows: delphinidin > cyanidin > malvidin > pelargonidin (Satué-Gracia et al., 1997).

The levels of antioxidants and antioxidant capacity in strawberry extracts from whole fruits vary considerably among genotypes (Wang and Lin, 2000; Zheng and Wang, 2003). This may be true partly because the cultivated strawberry (*F. ×ananassa*) is a hybrid of two very different wild species (Darrow, 1966). Accessions of the progenitor species are valued by strawberry breeders as sources of novel traits, especially pest resistance and abiotic stress tolerance. Because strawberry is a relatively new crop, dating to the 1700s (Darrow, 1966), as few as three backcrosses can yield selections of cultivar quality (J.F. Hancock, pers. comm.). Therefore, if found to be of value in improving antioxidant capacity or the balance of specific phenolic compounds, accessions from these progenitor species could be readily incorporated into a strawberry breeding program.

No accession from either of the progenitor species has been evaluated for antioxidant capacity or levels of phenolic compounds. A core subset of the *Fragaria* L. collection maintained at the U.S. Department of Agriculture National Clonal Repository, Corvallis, OR, has been constructed to contain a group of native *F. virginiana* and *F. chiloensis* thought to be of value to strawberry improvement. This core subset is being characterized for many horticultural traits useful to breeders (Hancock et al., 2001a, b), including resistance to black root rot, common foliar diseases, and nematodes (Hancock et al., 2001b, 2002; Pinkerton and Finn, 2005), but not for antioxidant activity.

There is some antioxidant information on another wild strawberry species, *F. vesca* L. This species is generally considered to be a progenitor of the genome of *F. virginiana* and *F. chiloensis*, and therefore *F. ×ananassa*. Sondheimer and Karash (1956) showed that the major pigments of *F. vesca* are the anthocyanins pelargonidin 3-monoglucoside and cyanidin 3-monoglucoside, and that the ratio of pelargonidin 3-glucoside to cyanidin 3-glucoside for this species was much lower (1 pelargonidin 3-glucoside : 1 cyanidin 3-glucoside) than for cultivated strawberries (1 pelargonidin 3-glucoside : 0.05 cyanidin 3-glucoside).

The objectives of this study are 1) to evaluate the antioxidant contents and antioxidant activities in representatives of *F. virginiana* and *F. chiloensis* in comparison with representatives of the cultivated strawberry species (*F. ×ananassa*), 2) to determine which strawberry compounds are more closely correlated with antioxidant capacity among these three species, and 3) to identify wild strawberry genotypes with high antioxidant activity and bioactive compounds for use in cultivar development.

Materials and Methods

GENOTYPES EVALUATED. Nineteen genotypes of three strawberry species (*F. chiloensis*, *F. virginiana*, and *F. ×ananassa*) (Table 1) were used in this study. Most of the accessions of wild strawberry, and one *F. ×ananassa*, were chosen because they are included in the core subset described by Hancock et al., (2002). Others were chosen based on past knowledge of their ability to thrive and produce fruit at Beltsville, MD. Three *F. chiloensis* accessions and 13 *F. virginiana* accessions were selected. The *F. ×ananassa* cultivar, Allstar, was selected for its relatively low antioxidant capacity, whereas 'Ovation' was selected for its relatively high antioxidant capacity among cultivars from the U.S. Department of Agriculture, Agricultural Research Service strawberry breeding program at Beltsville, MD (Lewers et al., 2004).

Table 1. *Fragaria* (strawberry) cultivars, selections, and wild genotypes grown on the north farm of the Henry A. Wallace Agricultural Research Center at Beltsville, MD, and evaluated for antioxidant activity, total phenolics, anthocyanin, and flavonoid content.

Species	PI no.	Common name	Origin
<i>F. ×ananassa</i> Duchesne ex Rozier	PI 551406	Allstar	Beltsville, MD
	PI 551929	CFRA 0638	Beltsville, MD
	PI 634800	Ovation	Beltsville, MD
<i>F. chiloensis</i> (L.) Mill.	PI 551735	CFRA 0368	near Juneau, AK
<i>F. chiloensis</i> ssp. <i>chiloensis</i> forma <i>patagonica</i> Staudt	PI 612317	2 TAP 4B	La Tapera, Aisen, Chile
<i>F. chiloensis</i> ssp. <i>pacifica</i> Staudt	PI 612488	CFRA 1267	British Columbia, Canada
<i>F. virginiana</i> Mill.	PI 612570	JP 95-1-1	Florida
	PI 612320	JP 95-9-6	Georgia
	PI 612495	LH 50-4	Montana
	PI 612325	NC 96-5-3	Jones County, NC
	PI 612323	NC 96-35-2	Greene County, AL
	PI 612324	NC 96-48-1	Chester County, SC
	PI 552275	CFRA 0982	near Ellsworth, ME
	PI 612486	NC 95-19-1	Monroe County, MS
<i>F. virginiana</i> ssp. <i>glauca</i> (S. Watson) Staudt	PI 612569	NC 95-21-1	Pontotoc County, MS
<i>F. virginiana</i> ssp. <i>grayana</i> (Vilm. ex J. Gay) Staudt	PI 616568	CFRA 1177	Allen County KY
<i>F. virginiana</i> ssp. <i>virginiana</i>	PI 612493	Frederick 9	Cochrane District, ON, Canada
	PI 612497	Montreal River 10	Algoma District, ON, Canada
	PI 612499	RH 30	Cook County, MN

EXPERIMENTAL DESIGN, FRUIT PRODUCTION, HARVEST, AND PROCESSING. The 19 strawberry genotypes were grown on the north farm of the Henry A. Wallace Agricultural Research Center at Beltsville, MD, using the advanced matted row production system (Black et al., 2002). For each of 2 years, genotypes were randomly assigned to field plots in two blocks; each genotype was represented once in each block. Plots were harvested individually. The fruits were hand harvested at a ripe stage; sorted to eliminate damaged, shriveled, and unripe fruit; and selected for uniform size and color. The fruit harvested from two plots of any genotype were kept separate for chemical analyses. From fruit of each plot, undamaged berries were sorted into three subsamples for separate analysis, frozen in liquid nitrogen, and then stored at -80°C until they were used for assays. The three subsamples were analyzed separately. After chemical analyses, the means of the subsamples were averaged to represent the fruit from each plot.

OXYGEN RADICAL ABSORBANCE CAPACITY (ORAC) ASSAY. The ORAC assay was carried out according to Huang et al. (2002) using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system. A microplate fluorescence reader (FL800; Bio-Tek Instruments, Winooski, VT) was used with 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; Bio-Tek Instruments). Sample dilution was accomplished using 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 (Huang et al., 2002). The standard curve was obtained by plotting Trolox concentrations against the average net AUC of the two measurements for each concentration. Final ORAC values were calculated using the regression equation between Trolox concentration and the net AUC, and were expressed as micromole Trolox equivalents per gram fresh weight (Huang et al., 2002).

TOTAL ANTHOCYANIN AND TOTAL PHENOLIC CONTENT. Strawberries were extracted with 80% acetone containing 0.2% formic acid using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). The homogenized samples from the acetone extracts were then centrifuged at $14,000 g_n$ for 20 min at 4°C . The supernatants were transferred to vials, stored at -80°C , and were later used for determination of total anthocyanins and total soluble phenolics.

Total anthocyanin contents in strawberry extract were determined using the pH differential method (Cheng and Breen, 1991). Absorbance was measured in a spectrophotometer (UV-160; Shimadzu Scientific Instruments, Columbia, MD) at 510 nm and 700 nm in buffers at a pH of 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{\text{pH } 1.0} (A_{510} - A_{700})_{\text{pH } 4.5}]$ with a molar extinction coefficient of pelargonidin-3-glucoside (22,400) for strawberry fruit juices. Results were expressed as milligrams of pelargonidin-3-glucoside equivalent, in the strawberry extract, per 100 g fresh weight.

Total soluble phenolics in the fruit extract were determined with Folin-Ciocalteu reagent by the method of Slinkard and Singleton (1997) using gallic acid as a standard. Results were expressed as milligrams gallic acid equivalent, in the strawberry extract, per 100 g fresh weight.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYSIS OF STRAWBERRY ANTHOCYANINS AND PHENOLIC

COMPOUNDS. High performance liquid chromatography was used to separate and determine individual anthocyanins and phenolic compounds in strawberry tissue samples. Fruit samples of 5 g were extracted twice with 15 mL acetone using a Polytron (Brinkmann Instruments) for 1 min. Extracts (30 mL) were combined and concentrated to 1 mL using a Buchler Evapomix (Buchler Instruments, Fort Lee, NJ) in a water bath at 35°C . The concentrated sample was dissolved in 10 mL acidified water (3% v/v of formic acid) and then passed through a C_{18} Sep-Pak cartridge (Waters Corp., Milford, MA), which was previously activated with methanol followed by water and 3% aqueous formic acid. Anthocyanins and other phenolics were adsorbed onto the column whereas sugars, acids, and other water-soluble compounds were eluted with 10 mL 3% aqueous formic acid. The anthocyanins and other phenolics were then recovered with 2.0 mL acidified methanol containing 3% formic acid. The methanolic extract was passed through a $0.45\text{-}\mu\text{m}$ membrane filter (Millipore, MSI, Westboro, MA), and 20 μL was analyzed by HPLC. The samples were analyzed using a Waters HPLC system equipped with two pumps (600 E system controller; Waters Corp.) coupled with a photodiode array detector (990 Series; Waters Corp.). Samples were injected at ambient temperature (20°C) onto 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 ; Sentry guard holder universal; Waters Corp.). The mobile phase was acidified water containing 2.5% formic acid (A) and acetonitrile (B) in a linear gradient from 5% to 20% B in the first 15 min, followed by a linear gradient from 20% to 30% B for 5 min, then an isocratic mixture for 5 min, followed by a linear gradient from 30% to 90% B for 5 min, then an isocratic mixture for 2 min before returning to the initial conditions. The flow rate was $1.0\text{ mL}\cdot\text{min}^{-1}$ and the wavelengths of detection were set at 320, 350, and 510 nm. Scanning between 240 and 550 nm was performed and data were collected using the Waters 990 3-D chromatography data system. Retention times and spectra were compared with pure standards. Results were expressed as microgram per gram fresh weight of phenolic acid or flavonol or expressed as microgram per gram fresh weight of cyanidin 3-glucoside or pelargonidin 3-glucoside.

STATISTICAL ANALYSIS. To determine differences between genotypes and genotype-by-year interactions, an analysis of variance was done using SAS (version 9.1.3; SAS Institute, Cary, NC). Genotypes and related interaction effects were considered fixed. Some of the genotypes were not tested in both years and some of the plots did not produce sufficient fruit, resulting in missing data. Therefore, the data were analyzed using the proc mixed command and the "pdmix800" subroutine. This analysis fits the data to a model and reports estimated means, based on the model. The model used included genotype, year, and genotype-by-year interactions as its sources of variance. Differences at $P = 0.05$ were considered significant. Because the proc mixed command does not generate estimates of mean squares for the sources of variance contributing to the model, the data also were analyzed using the proc glm command, and mean squares were reported from type III sums of squares.

To determine differences between species, and species-by-year interactions, a second analysis of variance was done using the same program, but this time the model included species, year, and species-by-year interactions as the sources

Table 2. Antioxidant activity, anthocyanin content, and total phenolic content in *Fragaria* (strawberry) cultivars, selections, and wild genotypes.

Genotype	Year	ORAC		Anthocyanin		Total phenolic	
		[TE (μmol·g ⁻¹ FW)] ^z		(mg·100 g ⁻¹ FW) ^y		(mg·100 g ⁻¹ FW) ^x	
<i>F. virginiana</i>	Average	45.65	a ^w	56.94	a	212.28	a
<i>F. chiloensis</i>	Average	35.94	b	44.65	b	160.06	b
<i>F. ×ananassa</i>	Average	32.32	b	46.12	b	144.86	b
Significance	df	Mean square		df	Mean square	df	Mean square
Species	2	704.9	**	2	836.5	**	19,297.5
Year	1	56.6	NS	1	299.8	*	2,245.8
Species × year	2	38.4	NS	2	41.4	NS	100.3
Error	52	44.3		52	61.4		549.5
<i>F. ×ananassa</i>							
Allstar	2004	—	—	—	—	—	—
Allstar	2005	26.19	m ^d	40.50	nop	123.24	m
CFRA 0638	2004	—	—	—	—	—	—
CFRA 0638	2005	32.06	jklm	48.69	jklm	159.99	ijkl
Ovation	2004	33.69	jklm	45.81	klmn	137.65	lm
Ovation	2005	34.58	ijklm	50.13	hijkl	173.09	fghij
<i>F. chiloensis</i>							
CFRA 0368	2004	26.84	m	36.17	p	146.45	jklm
CFRA 0368	2005	38.14	ghijkl	41.98	mno	142.66	klm
<i>F. chiloensis</i> ssp. <i>chiloensis</i> forma <i>patagonica</i>							
2 TAP 4B	2004	38.97	fghijk	44.21	lmn	170.90	hi
2 TAP 4B	2005	42.54	defghi	56.58	efgh	193.03	efgh
<i>F. chiloensis</i> ssp. <i>pacifica</i>							
CFRA 1267	2004	32.03	klm	38.22	op	139.58	lm
CFRA 1267	2005	37.12	ijkl	50.73	ijk	167.75	ij
<i>F. virginiana</i>							
JP 95-1-1	2004	46.12	defg	55.74	fghi	219.96	bcd
JP 95-1-1	2005	48.96	cd	57.33	efg	223.10	bc
JP 95-9-6	2004	42.49	defghij	46.67	jklmn	192.05	defghi
JP 95-9-6	2005	46.06	defg	61.17	def	233.69	b
LH 50-4	2004	49.26	bcd	63.37	cd	219.55	bcd
LH 50-4	2005	43.22	defghi	58.30	defg	209.67	bcde
NC 96-5-3	2004	—	—	—	—	—	—
NC 96-5-3	2005	57.22	ab	68.56	bc	227.30	b
NC 96-35-2	2004	40.46	efghij	46.89	klmn	181.34	fghi
NC 96-35-2	2005	46.06	defg	58.30	defg	212.15	bcde
NC 96-48-1	2004	46.16	defg	50.84	ijk	214.31	bcde
NC 96-48-1	2005	46.80	def	61.51	de	216.32	bcde
<i>F. virginiana</i> ssp. <i>glauca</i>							
CFRA 0982	2004	—	—	—	—	—	—
CFRA 0982	2005	61.69	a	74.83	A	259.98	a
<i>F. virginiana</i> ssp. <i>grayana</i>							
NC 95-19-1	2004	56.95	abc	73.59	ab	233.36	b
NC 95-19-1	2005	45.39	defgh	61.67	de	257.29	a
NC 95-21-1	2004	30.38	lm	33.90	P	169.11	hij
NC 95-21-1	2005	42.25	defghi	57.58	efg	198.99	cdefg
<i>F. virginiana</i> ssp. <i>virginiana</i>							
CFRA 1177	2004	45.22	defghi	48.47	jklmn	206.44	bcdef
CFRA 1177	2005	—	—	—	—	—	—
Frederick 9	2004	34.93	ijklm	45.53	klmn	170.83	ghijk
Frederick 9	2005	43.96	defghi	57.02	efgh	199.52	cdefg
Montreal River 10	2004	38.01	hijkl	52.70	ghij	182.67	fghi
Montreal River 10	2005	45.80	defgh	56.75	efgh	213.06	bcde
RH 30	2004	—	—	—	—	—	—
RH 30	2005	47.85	de	62.07	de	218.87	bcd
Significance	df	Mean square		df	Mean square	df	Mean square
Genotype	18	166.6	**	18	214.5	**	3337.4
Year	1	137.0	**	1	627.7	**	5428.7
Genotype × year	12	42.3	*	12	80.8	**	288.4
Error	26	15.2		26	7.7		140.4

^zData expressed as micromoles of Trolox (TE) equivalents per gram fresh weight (FW).^yData expressed as milligrams of cyanidin 3-glucoside equivalents per 100 g FW.^xData expressed as milligrams of gallic acid equivalents per 100 g FW.^wMean within same column followed different letter are significantly different at *P* = 0.05.NS, *, **Nonsignificant, significant at *P* = 0.05 and *P* = 0.01 respectively.

of variance. Species and related interaction effects were considered fixed. A third analysis was done that was identical to the second, except only three *F. virginiana* genotypes were included to confirm the analysis was not biased as a result of having 13 representatives of *F. virginiana* compared with only three representatives of either *F. chiloensis* or *F. xananassa*. The three genotypes selected were those with the lowest ORAC means according to the first analysis: LH 50-4, NC 96-35-2, and Frederick 9.

To determine which strawberry compounds are most closely correlated with antioxidant activity among these three species, correlation coefficients were calculated based on genotype mean averaged across plots within year for the antioxidant activities versus the concentration of each of the compounds measured. Correlation coefficients were calculated using Excel 2003 (Microsoft Corp., Redmond, WA), and are reported as R^2 values.

Results and Discussion

DIFFERENCES AMONG SPECIES. Fruit of the *F. virginiana* accessions tested had significantly higher ($P \leq 0.05$) antioxidant capacity, total phenolics, and total anthocyanins than did fruit of the *F. chiloensis* and *F. xananassa* accessions evaluated (Table 2). Levels of the phenolic acid, ellagic acid, were similar among representatives of the three species (Table 3), but levels of several flavonoids (the flavonols and anthocyanins) were significantly higher in fruit of the *F. virginiana* accessions compared with fruit of either the *F. chiloensis* or *F. xananassa* accessions evaluated (Table 4).

Of the anthocyanins, the levels of cyanidin 3-glucoside-succinate were highest in fruit of the 13 *F. virginiana* accessions, although not significantly different ($P \leq 0.05$) from those of the three *F. chiloensis* accessions evaluated (Table 4). Differences between the three species in the levels of this anthocyanin were not significant when only the three *F. virginiana* genotypes lowest in antioxidant capacity were used in the analysis. Fruits of the 13 *F. virginiana* accessions also had the highest levels of the anthocyanin pelargonidin 3-glucoside, although not significantly different from those in the three *F. xananassa* accessions tested. As has previously been reported with cultivated *F. xananassa* (Gil et al., 1997; Wang and Zheng, 2001), the levels, in accessions tested from both the wild progenitor species, of cyanidin 3-glucoside and pelargonidin 3-glucoside were higher than their succinate counterparts, and the pelargonidin-based anthocyanins were predominant over the cyanidin-based anthocyanins. As with *F. vesca* (Sondheimer and Karash, 1956), the ratios of pelargonidin 3-glucosate to cyanidin 3-glucosate were lower in fruits of both *F. virginiana* and *F. chiloensis* accessions tested compared with *F. xananassa* accessions tested, although it is not appropriate to test the significance of the differences between the ratios for the three species (Table 4). The lower ratios are thought to be desirable because cyanidin 3-glucoside has greater antioxidant capacity than that of pelargonidin 3-glucoside (Satué-Gracia et al., 1997).

Also supporting previous findings (Wang and Zheng, 2001) on strawberry fruit flavonols, the levels of quercetin-based flavonols were higher than those of the other flavonols present in strawberry fruit (Table 3). Once again, fruit of the 13 *F. virginiana* accessions tested contained higher levels of both these quercetin-based compounds and another flavonol, kaemp-

ferol 3-rutinoside. However, differences between the three species in the levels of the quercetin-based compounds were not significant when only the three *F. virginiana* genotypes lowest in antioxidant capacity were used in the analysis.

CORRELATIONS BETWEEN ANTIOXIDANT CAPACITY AND PHENOLIC COMPOUNDS. To determine which phenolic compounds were contributing the most to antioxidant capacity in the fruit of the wild strawberry, correlation coefficients were calculated between antioxidant capacity and the levels of each of the compounds tested. As with previous reports (Wang and Lin, 2000), significant correlations were found between antioxidant capacity and both total phenolics ($R^2 = 0.8093$) and total anthocyanins ($R^2 = 0.8037$). However, in this study, there was no correlation between antioxidant activity and any of the individual compounds tested. A study of the same genotypes found a strong positive correlation ($R^2 = 0.812$) between antioxidant capacity and vitamin C (S.Y. Wang, K.S. Lewers, L. Bowman, and M. Ding, unpublished).

Because ORAC was correlated only to total phenolics and total anthocyanins, any of these three measures will be useful in selecting genotypes for cultivar development. The choice of assay in different stages of selection will depend primarily on the balance of efficiency and reliability required at each stage. For example, visual selection for dark, bright fruit skin and flesh is a rapid and low-cost assay for anthocyanin levels. This may be the most efficient selection method to use on an individual plant basis in a seedling field. A more objective and quantifiable assay may be desirable for later selections from replicated evaluations at one or more locations. Previous studies have shown that antioxidant capacity, total anthocyanins, and total phenolics vary significantly in different growing environments (Wang and Lin, 2003; Wang and Zheng, 2001; Wang et al., 2002, 2003), so that it is highly desirable to make selections based on data from multiple replications and environments (years or locations). In this study, the genotype-by-year interactions for antioxidant capacity and total anthocyanins were significant, but the genotype-by-year interaction for total phenolic measurements was not (Table 2). The assays used to measure total phenolics or total anthocyanins were more simple and less expensive than the ORAC assay to measure antioxidant capacity. The high correlation with antioxidant capacity, relative efficiency, and lack of genotype-by-year interaction in this study suggests that the measurement of total phenolics may be the better assay to use for the later selection stages in a strawberry cultivar development program.

DIFFERENCES AMONG INDIVIDUAL GENOTYPES. Differences among genotypes were significant for each of the specific phenolic compounds measured (Tables 3 and 4). In determining differences among genotypes for antioxidant capacity and levels of the various phenolic compounds, significant genotype-by-year interactions were observed for all traits except total phenolics, so genotypic means are reported for each year (Tables 2–4).

The cultivar Ovation was tested with other *F. xananassa* genotypes only in 2005, and its higher ORAC value was not significantly different from that of the other two *F. xananassa* genotypes when tested in this group, even though Lewers et al. (2004) had compared it with several other *F. xananassa* cultivars and selections and found it to be significantly higher in antioxidant capacity. Genotypes with significantly higher ORAC values than that of 'Ovation' in both 2004 and 2005 were NC 95-19-1, JP 95-1-1, and NC 96-48-1. In 2004, CFRA

Table 3. Content of phenolic acids (ellagic acid) and flavonols (quercetin 3-glucoside plus quercetin 3-glucuronide, kaempferol 3-glucoside, kaempferol 3-rutinoside, and *p*-coumaryl-glucose) in fruit of *Fragaria* (strawberry) cultivars, selections, and wild genotypes.

Genotype	Year	Quercetin 3-glucoside plus quercetin						Kaempferol		<i>p</i> -Coumaryl-glucose			
		Ellagic acid (μg·g ⁻¹ FW) ^z		3-glucuronide (μg·g ⁻¹ FW) ^y		Kaempferol 3-glucoside (μg·g ⁻¹ FW) ^y		3-rutinoside (μg·g ⁻¹ FW) ^y		<i>p</i> -Coumaryl-glucose (μg·g ⁻¹ FW) ^x			
<i>F. virginiana</i>	Average	22.32		78.07	a ^w	11.23		19.82	a	20.12			
<i>F. chiloensis</i>	Average	34.98		48.94	b	6.53		11.98	b	24.53			
<i>F. × ananassa</i>	Average	19.88		40.81	b	8.37		10.94	b	11.42			
Significance		df	Mean square	df	Mean square	df	Mean square	df	Mean square	df	Mean square		
Species		2	309.6	NS	2	4,130.6	**	2	367.8	*	2	256.2	NS
Year		1	168.0	NS	1	2,129.3	*	1	665.8	*	1	112.5	NS
Species × year		2	24.3	NS	2	45.8	NS	2	31.4	NS	2	269.0	NS
Error		31	188.0		40	475.4		47	104.4		46	235.5	
<i>F. × ananassa</i>													
Allstar	2004	—	—	—	—	—	—	—	—	—	—	—	—
Allstar	2005	19.15	defg	46.10	ghij	3.53	ijklmn	17.11	defghij	15.12	efghij		
CFRA 0638	2004	—	—	—	—	—	—	—	—	—	—	—	—
CFRA 0638	2005	—	—	—	—	—	—	24.72	bcde	—	—	—	—
Ovation	2004	24.64	de	28.27	j	10.64	defgh	5.15	jk	8.30	j		
Ovation	2005	11.10	fg	60.59	defghi	9.56	efghi	8.40	fghijk	13.95	efghij		
<i>F. chiloensis</i>													
CFRA 0368	2004	—	—	70.65	cdefg	1.47	n	5.86	jk	10.67	hij		
CFRA 0368	2005	—	—	—	—	16.34	bc	13.71	defghijk	41.02	c		
<i>F. chiloensis</i> ssp. <i>chiloensis</i> forma <i>patagonica</i>													
2 TAP 4B	2004	38.32	bc	2.98	k	6.83	hijklm	3.28	k	56.98	b		
2 TAP 4B	2005	31.64	cd	56.15	fghi	7.57	ghijk	17.89	defgh	18.99	def		
<i>F. chiloensis</i> ssp. <i>pacifica</i>													
CFRA 1267	2004	—	—	51.58	ghi	2.59	lmn	9.85	ghijk	12.95	ghij		
CFRA 1267	2005	—	—	—	—	8.09	fghij	19.34	defg	16.02	efgh		
<i>F. virginiana</i>													
JP 95-1-1	2004	14.71	efg	70.50	cdefg	15.76	b	30.86	bc	16.27	efgh		
JP 95-1-1	2005	15.76	efg	86.30	bcd	16.09	bcd	34.63	abc	14.45	efghij		
JP 95-9-6	2004	—	—	85.66	bcde	8.59	efghijk	4.15	jk	7.47	ij		
JP 95-9-6	2005	—	—	139.91	a	10.13	efgh	24.04	cd	25.57	d		
LH 50-4	2004	15.86	efg	76.01	bcdef	11.38	bcdefg	31.22	bc	13.16	fghij		
LH 50-4	2005	13.34	fg	88.76	bc	3.91	ijklmn	36.03	ab	12.09	ghij		
NC 96-5-3	2004	—	—	—	—	—	—	—	—	—	—		
NC 96-5-3	2005	6.21	g	78.47	bcde	24.45	a	—	—	51.71	b		
NC 96-35-2	2004	55.59	a	41.47	ij	0.58	n	14.50	defghij	13.92	efghij		
NC 96-35-2	2005	31.41	cd	75.92	bcdef	25.50	a	5.11	jk	12.78	ghij		
NC 96-48-1	2004	19.96	ef	79.76	bcde	3.68	ijklmn	7.21	ijk	16.44	efgh		
NC 96-48-1	2005	36.80	bc	76.94	bcde	11.09	cdefgh	6.51	jk	12.84	ghij		
<i>F. virginiana</i> ssp. <i>glauca</i>													
CFRA 0982	2004	—	—	—	—	—	—	—	—	—	—		
CFRA 0982	2005	—	—	—	—	22.35	a	16.66	defghi	71.49	a		
<i>F. virginiana</i> ssp. <i>grayana</i>													
NC 95-19-1	2004	18.97	efh	93.92	b	13.76	bcde	17.56	defgh	8.83	ij		
NC 95-19-1	2005	17.98	efg	96.30	b	13.39	bcdef	22.63	cdef	—	—		
NC 95-21-1	2004	—	—	60.65	efghi	14.07	bcde	12.40	efghijk	23.92	d		
NC 95-21-1	2005	12.68	fg	85.13	bcd	12.58	bcdef	14.68	defghij	23.83	d		
<i>F. virginiana</i> ssp. <i>virginiana</i>													
CFRA 1177	2004	40.63	bc	82.36	bcde	8.11	fghijkl	5.70	hijk	7.17	j		
CFRA 1177	2005	—	—	—	—	—	—	—	—	—	—		
Frederick 9	2004	5.92	gh	43.12	hij	2.34	klmn	13.24	defghijk	15.68	efghi		
Frederick 9	2005	14.91	efg	85.32	bcd	3.32	klmn	33.01	abc	19.44	de		
Montreal River 10	2004	17.56	efh	41.57	ij	2.32	mn	15.06	defghij	15.27	efgh		
Montreal River 10	2005	49.71	ab	71.98	bcdefgh	2.72	lmn	42.42	a	17.05	efg		
RH 30	2004	—	—	—	—	—	—	—	—	—	—		

continued next page

Table 3. Continued.

Genotype	Year	Quercetin 3-glucoside plus quercetin 3-glucuronide										Kaempferol 3-rutinoside		<i>p</i> -Coumaryl-glucose	
		Ellagic acid (μg·g ⁻¹ FW) ^z		(μg·g ⁻¹ FW) ^y		Kaempferol 3-glucoside (μg·g ⁻¹ FW) ^y		(μg·g ⁻¹ FW) ^y		(μg·g ⁻¹ FW) ^x					
RH 30	2005	—	—	—	—	25.56	a	24.21	cd	16.48	efgh				
Significance		df	Mean square	df	Mean square	df	Mean square	df	Mean square	df	Mean square				
Genotype		12	386.7	**	15	1306.5	**	17	228.0	**	17	541.3	**		
Year		1	10.8	NS	1	6402.0	**	1	729.8	**	1	19.3	NS		
Genotype × year		8	223.1	**	10	300.1	*	12	88.4	**	11	207.3	**		
Error		15	25.4		19	94.9		22	23.8		22	8.2			

^zData expressed as micrograms of ellagic acid equivalents per gram fresh weight (FW).

^yData expressed as micrograms of quercetin 3-glucoside equivalents per gram FW.

^xData expressed as micrograms of *p*-coumaric acid equivalents per gram FW.

^wMeans within same column followed different letter are significantly different at $P = 0.05$.

NS, *, **Nonsignificant, significant at $P = 0.05$ and $P = 0.01$ respectively.

1177 also had a significantly higher ORAC value than 'Ovation', but it was not tested in 2005. Likewise, in 2005, CFRA 0982, NC 96-5-3, and RH 30 had higher ORAC values, but were not compared with 'Ovation' in 2004. The genotype-by-year interactions affected comparisons between 'Ovation' and JP 95-9-6, NC 96-35-2, and LH 50-4. These genotypes had significantly higher ORAC values than 'Ovation' in only 1 of 2 years tested. All the genotypes with significantly higher ORAC values than 'Ovation' were *F. virginiana* genotypes.

Genotypes with significantly higher total anthocyanin content than 'Ovation' also were all *F. virginiana* (Table 2). Genotypes with higher anthocyanin content in both years were NC 95-19-1, LH 50-4, and JP 95-1-1. Also higher in 2005 but not measured in 2004 were CFRA 0982, NC 96-5-3, and RH 30. The genotype-by-year interactions affected comparisons between 'Ovation' and Montreal River 10, NC 96-48-1, JP 95-9-6, NC 96-35-2, and NC 95-21-1, which had significantly higher total anthocyanin content than 'Ovation' in only 1 of 2 years tested.

The comparisons between 'Ovation' and the other genotypes were more consistent when comparing total phenolics (Table 2). The only genotypes significantly lower than 'Ovation' in total phenolics were CFRA 1267 and CFRA 0368 (both *F. chiloensis*) in 2004 and 2005, and also the other two *F. xananassa* genotypes ('Allstar' and CFRA 0638), which were added to the comparison in 2005. All other genotypes were higher in total phenolics, although the differences for NC 95-21-1, Frederick 9 (*F. virginiana*), and 2 TAP 4B (*F. chiloensis*) were not significant in 2005.

The two genotypes that were compared with 'Ovation' both years and were significantly higher in antioxidant capacity, total anthocyanins, and total phenolics were the *F. virginiana* genotypes NC 95-19-1 and JP 95-1-1. In addition, these two accessions are valued as strawberry parental material as potential sources of resistance to anthracnose crown rot incited by *Colletotrichum fragariae* A.N. Brooks (K.S. Lewers, W.W. Turechek, S.C. Hokanson, J.L. Maas, J.F. Hancock, S. Serge, and B.J. Smith, unpublished). JP 95-1-1 is also resistant to crown rot incited by *C. gloeosporioides* (Penz.) Penz. & Sacc. in Penz. [teleomorph *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk] (K.S. Lewers, W.W. Turechek, S.C. Hokanson, J.L. Maas, J.F. Hancock, S. Serge, and B.J. Smith, unpublished)

and to both the northern root knot nematode (*Meloidogyne hapla* Chitwood) and the root lesion nematode [*Pratylenchus penetrans* (Cobb) Filipjev & Shuurmans Stekhoven] (Pinkerton and Finn, 2005). NC95-19-1, a female, is also resistant to the root knot nematode (Pinkerton and Finn, 2005), and leaf scorch, *Diplocarpon earlianum* (Ellis & Everh.); tolerant to leaf blight *Phomopsis obscurans* (Ellis & Everh.) Sutton and powdery mildew *Sphaerotheca macularis* (Wallr.:Fr.) Jacz. f.sp. *fragariae* Peries (Hancock et al., 2001a, b, 2002); and has very good flavor (data not shown).

Three additional *F. virginiana* genotypes were higher for all three values but were compared with 'Ovation' only in 2005. These were CFRA 0982, NC 96-5-3, and RH 30. CFRA 0982 and RH 30 also are valued because they flower more than once per year (Hancock et al., 2001b). RH 30 also is resistant to root knot nematode (Pinkerton and Finn, 2005), black root rot incited by an unknown pathogen or group of pathogens, leaf scorch, and leaf spot incited by *Mycosphaerella fragariae* (Tul.) Lindau (Hancock et al., 2001a). NC 96-5-3 is of interest in strawberry breeding because it is resistant to both the northern root knot nematode and the root lesion nematode (Pinkerton and Finn, 2005), plus leaf scorch and powdery mildew is tolerant to leaf blight, and has upright peduncles (Hancock et al., 2001a, b; 2002) of value in keeping flowers and fruit off the ground and away from inoculum of fruit rotting pathogens.

Fruit of the 13 *F. virginiana* accessions had significantly higher ($P = 0.05$) antioxidant capacity, total phenolics, and total anthocyanins than fruit from the three *F. chiloensis* and the three *F. xananassa* accessions evaluated. Because we tested only a few *F. chiloensis* accessions, there may be other wild *F. chiloensis* accessions that are very high in antioxidants. In addition, high antioxidant content is a trait that can be selected for in cultivar development, so other *F. xananassa* genotypes may have fruit containing levels of antioxidants that are higher than the three *F. xananassa* accessions we tested, now or in the future. The best way to select these future cultivars may be to test for high total phenolics, because this was highly correlated with antioxidant capacity, was relatively efficient, and, in this study, lacked a genotype-by-year interaction. Accessions that should be valuable as parent material for developing more nutritious strawberry cultivars include NC 95-19-1, JP 95-1-1,

Table 4. Content and ratios of the prominent anthocyanins in *Fragaria* (strawberry) cultivars, selections, and wild genotypes.

Genotype	Year	Cyanidin 3-glucoside [CG ($\mu\text{g}\cdot\text{g}^{-1}$ FW)] ^z			Pelargonidin 3-glucoside [PG ($\mu\text{g}\cdot\text{g}^{-1}$ FW)] ^y			Cyanidin 3-glucoside-succinate [CG ($\mu\text{g}\cdot\text{g}^{-1}$ FW)] ^z			Pelargonidin 3-glucoside-succinate [PG ($\mu\text{g}\cdot\text{g}^{-1}$ FW)] ^y			PG-to-CG ratio
		df	Mean square		df	Mean square		df	Mean square		df	Mean square		
<i>F. virginiana</i>	Average		105.06			418.05	a ^z		38.04	a		98.72		5.07
<i>F. chiloensis</i>	Average		95.32			253.48	B		35.80	a		85.71		3.88
<i>F. ×ananassa</i>	Average		38.01			401.20	A		16.69	b		64.00		13.41
Significance		df	Mean square		df	Mean square		df	Mean square		df	Mean square		
Species		2	9,587.5	NS	2	116,303.8	**	2	970.7	*	2	2,921.7	NS	
Year		1	1,711.0	NS	1	2,338.3	NS	1	245.0	NS	1	527.9	NS	
Species × year		2	62.4	NS	2	3,074.2	NS	2	164.7	NS	2	611.2	NS	
Error		48	3,705.8		50	11,257.6		48	213.0		48	3,951.7		
<i>F. ×ananassa</i>														
Allstar	2004		—	—		—	—		—	—		—	—	—
Allstar	2005		16.29	p		361.48	hijklm		22.11	a		15.47	lmn	22.19
CFRA 0638	2004		—	—		—	—		—	—		—	—	—
CFRA 0638	2005		87.48	fg		375.79	ghijklm		4.22	ijk		36.56	efghi	4.30
Ovation	2004		30.03	nop		410.79	fghi		13.73	b		10.42	n	13.68
Ovation	2005		34.19	mnop		437.53	defgh		12.72	bc		16.85	klmn	12.80
<i>F. chiloensis</i>														
CFRA 0368	2004		68.25	ghij		300.90	mo		4.41	ij		31.37	hij	4.41
CFRA 0368	2005		58.77	ijkl		262.00	o		4.38	hijk		26.16	ijklm	4.46
<i>F. chiloensis</i> ssp. <i>chiloensis</i> forma <i>patagonica</i>														
2 TAP 4B	2004		27.75	op		161.82	p		5.85	fg		44.47	def	5.83
2 TAP 4B	2005		43.91	klmn		256.51	o		5.85	fg		69.14	a	5.84
<i>F. chiloensis</i> ssp. <i>pacifica</i>														
CFRA 1267	2004		172.14	c		262.72	o		1.52	n		20.95	jklmn	1.53
CFRA 1267	2005		179.85	c		275.36	o		1.54	n		16.15	mn	1.53
<i>F. virginiana</i>														
JP 95-1-1	2004		104.03	ef		300.26	mo		2.89	lm		49.73	cd	2.89
JP 95-1-1	2005		109.17	ef		302.08	lmno		2.69	lmn		45.40	cdefg	2.77
JP 95-9-6	2004		30.49	nop		295.59	lmo		9.61	e		26.72	hijklm	9.69
JP 95-9-6	2005		59.66	ijk		385.65	hijk		6.47	f		44.93	def	6.46
LH 50-4	2004		170.74	c		452.81	def		2.65	lm		37.40	efghi	2.65
LH 50-4	2005		139.16	d		374.56	hijkn		2.70	lm		39.79	defgh	2.69
NC 96-5-3	2004		—	—		—	—		—	—		—	—	—
NC 96-5-3	2005		—	—		813.44	a		—	—		—	—	—
NC 96-35-2	2004		68.72	ghij		394.84	fghij		5.75	fg		33.13	ghi	5.75
NC 96-35-2	2005		72.72	ghij		421.45	efgh		5.88	fg		37.42	efghi	5.80
NC 96-48-1	2004		65.87	hij		345.73	jklm		5.25	ghi		57.88	bc	5.25
NC 96-48-1	2005		60.85	ij		304.14	lmo		5.06	ghi		64.65	ab	5.00
<i>F. virginiana</i> ssp. <i>glauca</i>														
CFRA 0982	2004		—	—		—	—		—	—		—	—	—
CFRA 0982	2005		200.72	b		480.73	cde		2.40	lmn		46.13	def	2.40
<i>F. virginiana</i> ssp. <i>grayana</i>														
NC 95-19-1	2004		220.75	a		534.68	bc		2.43	lmn		47.70	cde	2.42
NC 95-19-1	2005		206.10	ab		392.49	fghij		1.91	mn		30.62	hij	1.90
NC 95-21-1	2004		78.69	gh		352.40	ijklm		4.49	i		31.31	hij	4.48
NC 95-21-1	2005		117.62	e		393.77	fghij		3.36	jkl		29.14	hijk	3.35
<i>F. virginiana</i> ssp. <i>virginiana</i>														
CFRA 1177	2004		32.31	nop		314.94	klmo		9.67	e		44.71	defg	9.75
CFRA 1177	2005		—	—		—	—		—	—		—	—	—
Frederick 9	2004		55.08	jklm		325.95	jklmo		5.84	fgh		16.59	klmn	5.92
Frederick 9	2005		75.30	ghi		364.22	hijkl		4.86	ghi		27.12	ijkl	4.84
Montreal River 10	2004		42.61	lmno		487.60	cd		11.50	cd		29.60	hijk	11.44
Montreal River 10	2005		43.26	lmno		451.96	defg		10.43	de		36.36	fghi	10.45
RH 30	2004		—	—		—	—		—	—		—	—	—
RH 30	2005		175.31	c		567.70	b		3.25	kl		11.51	n	3.24
Significance		df	Mean square		df	Mean square		df	Mean square		df	Mean square		
Genotype		17	11,282.0	**	18	40,454.1	**	17	596.2	**	17	10,974.5	**	
Year		1	275.9	*	1	1.2	NS	1	139.3	*	1	117.9	NS	
Genotype × year		12	317.2	**	12	4049.0	**	12	105.8	**	12	606.6	**	
Error		23	60.0		24	922.0		23	26.5		23	117.7		

^zData expressed as micrograms of cyanidin 3-glucoside equivalents per gram fresh weight (FW).^yData expressed as micrograms of pelargonidin 3-glucoside equivalents per gram FW.*Means within same column followed different letter are significantly different at $P = 0.05$.NS, *, **Nonsignificant, significant at $P = 0.05$ and $P = 0.01$ respectively.

CFRA 0982, NC 96-5-3, and RH 30, because these accessions were higher in antioxidant capacity, total anthocyanins, and total phenolics than the top-ranking *F. xananassa* accession tested.

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