

# Higher Antioxidant Activity, Total Flavonols, and Specific Quercetin Glucosides in Two Different Onion (*Allium cepa* L.) Varieties Grown under Organic Production: Results from a 6-Year Field Study

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**ABSTRACT:** We carried out a 6-year study to assess the effect of conventional, organic, and mixed cultivation practices on bioactive compounds (flavonoids, anthocyanins) and antioxidant capacity in onion. Total flavonoids, total anthocyanins, individual flavonols, individual anthocyanins, and antioxidant activity were measured in two varieties ('Hyskin' and 'Red Baron') grown in a long-term split-plot factorial systems comparison trial. This is the first report of repeated measurements of bioactive content over an extensive time period in a single crop type within the same trial. Antioxidant activity (DPPH and FRAP), total flavonol content, and levels of Q 3,4' D and Q 3 G were higher in both varieties under fully organic compared to fully conventional management. Total flavonoids were higher in 'Red Baron' and when onions were grown under organic soil treatment. Differences were primarily due to different soil management practices used in organic agriculture rather than pesticide/ herbicide application.

**KEYWORDS:** onion (*Allium cepa* L.), organic, conventional, flavonoids, anthocyanins, antioxidant activity

## INTRODUCTION

Onion (*Allium cepa* L.) is one of the most important vegetable crops with a global production of 55 million tons per annum.<sup>1</sup> The bulbs are classified on the basis of their color into three types (yellow, red, and white) and on the basis of their taste as sweet or nonsweet.<sup>2</sup> White, yellow, and red types of onions are rich in flavonols including mainly quercetin (Q) and its sugar derivatives such as quercetin 3,4'-diglucoside (Q 3,4' D) and quercetin 4'-glucoside (Q 4' G).<sup>3</sup> Onions rank the highest in quercetin content among 28 vegetables and 9 fruits.<sup>4</sup> Levels of quercetin in red onions were 14-fold higher than in garlic and were 2-fold higher than in white onions.<sup>5</sup>

Few studies are available on the identification and quantification of specific beneficial compounds, such as Q, Q 3 G, Q 4' G, and Q 3,4' D under different agricultural practices.<sup>6</sup> Some authors have previously identified Q 3,4' D and Q 4' G as the major quercetin derivatives of the mature red onion bulb; these components account for about 93% of the total flavonols.<sup>7–9</sup> This is of interest from a human health perspective as it is well-known that quercetin derivatives have a pronounced preventive effect on allergies, asthma, arthritis, cancer, diabetic complications, gout, neurodegenerative disorders, and osteoporosis (for a review, see Cirillo et al.<sup>10</sup>).

Sales of organic food products have increased in recent years,<sup>11</sup> boosted by consumer perception that organic foods are healthier due to higher bioactive content and lower pesticide residues. Organic food is perceived to be more nutritious, better tasting, and environmentally friendlier compared to conventionally grown crops.<sup>12</sup> Organic crop production in Europe is controlled by EU Council Regulation No. 834/2007.<sup>13</sup> Organic certification enables producers to use an organic label indicating that the food was produced under interpretations of the guiding

EU legislation and inspected by national certification bodies, which may differ from country to country. Some studies have shown that organic cultivation directly affects the levels of secondary metabolites, mainly polyphenols, in fruits and vegetables,<sup>14–16</sup> although this has been disputed.<sup>17</sup> In addition to organic practices, the concentration of polyphenols in edible plants is affected by other factors such as cultivar and variety selection,<sup>18</sup> tissue maturity and damage at harvest, biotic stress (pathogen infection and pest attack),<sup>19</sup> climate and soil microenvironment, fertilizer regimen, temperature, irradiation, and postharvest treatment. Relative to conventional systems, organic systems may increase the exposure of crops to stresses, thus inducing the synthesis of secondary metabolites.<sup>20</sup>

The objective of this study was to compare the total flavonoid content, total flavonol content, individual flavonols, individual anthocyanins, and antioxidant activity in onions grown under organic, conventional, and mixed cultivation practices in a multiyear experiment.

## MATERIALS AND METHODS

**Field Trial.** The onions analyzed were from the Kinsealy systems field trial carried out at Teagasc, Kinsealy (53° 25' N, 6° 10' W), Dublin, Ireland. The trial design is described elsewhere.<sup>16,21</sup> Essentially, agricultural management was considered as consisting of two aspects—soil treatment (how the soil is fertilized and managed) and pest control (how biological pests such as weeds, insects, and microbial diseases are managed). This means that conventional agriculture consists of conventional soil (CS) treatment with

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conventional post control (CP), whereas organic agriculture consists of organic soil treatment (OS) with organic pest control (OP). Importantly, this also allows mixed practices (OS + CP, CS + OP), which allow exploration of subparts of crop management practices. There were four field replicates ( $n = 4$ ) for each of the four crop treatment combinations (OS + OP, CS + CP, OS + CP, CS + OP). Organic cultivation practices used were in compliance with EC1990/92<sup>22</sup> and EC834/2007<sup>13</sup> with pesticide application in accordance with national regulations.<sup>23</sup> Applied inputs for onion cultivation in 2009–2014 are shown in Table 1.

**Table 1. Specific Pest-Control and Soil Treatment Inputs Used in the Teagasc Kinsealy Systems Comparison Trial for Onion Cultivation 2009–2014**

Pest-Control Treatment	
organic pest control (OP)	mechanical weeding (hand hoeing) Serenade <sup>a,b</sup> (10 L ha <sup>-1</sup> )
conventional pest control (CP)	Proplant <sup>b,c</sup> (10 mL m <sup>2</sup> modular drench), Roundup <sup>d</sup> (4 L ha <sup>-1</sup> ), Stomp <sup>d</sup> (3.3 L ha <sup>-1</sup> ), CICP <sup>1</sup> (4.2 L ha <sup>-1</sup> ), Defy <sup>d</sup> (3.3 L ha <sup>-1</sup> ), Totril <sup>d</sup> (1.8 L ha <sup>-1</sup> ), Stratos Ultra <sup>d</sup> (4 L ha <sup>-1</sup> ), Penncozeb <sup>c</sup> (4.4 kg ha <sup>-1</sup> ), Folio Gold <sup>c</sup> (2 L ha <sup>-1</sup> ), Amistar <sup>c</sup> (1 L ha <sup>-1</sup> ).
Soil Treatment	
organic soil (OS)	previous crop – broccoli fertilizer (adjusted to) N 70 kg ha <sup>-1</sup> , P 20 kg ha <sup>-1</sup> , K 215 kg ha <sup>-1</sup> applied as Greenvale plant food (4.5:3:3) (pelleted chicken manure + calcified seaweed) and ProKali (3:0:14); a top dress equivalent to 35 kg ha <sup>-1</sup> N and contributing 25 kg ha <sup>-1</sup> P and 24 kg ha <sup>-1</sup> K was applied in June or July
conventional soil (CS)	previous crop – broccoli/carrot/lettuce fertilizer (adjusted to) N 70 kg ha <sup>-1</sup> , P 20 kg ha <sup>-1</sup> , K 215 kg ha <sup>-1</sup> applied as CAN (27% N), single superphosphate (7.8% P), and sulfate of potash (42% K); a top dress equivalent to 35 kg ha <sup>-1</sup> N, 25 kg ha <sup>-1</sup> P, and 24 kg ha <sup>-1</sup> K was applied in June or July

<sup>a</sup>Fungicide (certified organic). <sup>b</sup>Not applied in all years. <sup>c</sup>Fungicide. <sup>d</sup>Herbicide.

Onion bulbs were harvested at commercial maturity stage from the internal trial rows with guard rows excluded. Three onions of representative size were taken as a composite sample from each plot. Samples for analysis were immediately refrigerated and then frozen at  $-20^{\circ}\text{C}$  within 24 h of harvest. Frozen samples were freeze-dried in a large-scale freeze-dryer (Cuddon Frozen Dry, Blenheim, New Zealand). Once freeze-dried, samples were vacuum packed in polypropylene bags and kept at  $-20^{\circ}\text{C}$  until analysis.

**Chemicals.** Gallic acid, methanol, acetonitrile, ethanol, potassium acetate, aluminum chloride ( $\text{AlCl}_3$ ), acetate, ferric chloride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyrilidyl-s-triazine (TPTZ), hydrogen chloride (HCl), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and trifluoroacetic acid (TFA) were obtained from Sigma (Sigma-Aldrich, Arklow, Ireland). Quercetin 3-glucoside (Q 3 G), quercetin 4'-glucoside (Q 4' G), quercetin 3,4'-diglucoside (Q 3,4' D), quercetin (Q), and cyanidin 3-glucoside (C 3 G) standards were purchased from Extrasynthese (Geney Cedex, France).

**Preparation of Extracts from Dried Onions.** Freeze-dried onions were milled using a kitchen blender (Kenwood Limited, Havant, UK). The powdered onions (1 g) were mixed with 10 mL of 80% methanol and homogenized with an Omni-prep multisampling homogenizer (Omni International, Kennesaw, GA, USA) at 24,000 rpm. The homogenized sample suspension was shaken overnight with a V400 Multitude Vortexer (Alpha Laboratories, North York, ON, Canada) at 1500 rpm at room temperature. The sample suspension was then centrifuged for 20 min at 3000g (MSE Mistral 3000i, Sanyo

Gallenkamp, Leicestershire, UK) and filtered through  $0.22\ \mu\text{m}$  polytetrafluoroethylene filters. The extracts were kept at  $-20^{\circ}\text{C}$  for subsequent analysis. For this 6 year study total analyses were carried out on all stored samples from 2009 to 2014 determined as a single batch and using a single calibration curve.

**Analysis of Antioxidant Activity: Ferric Reducing Antioxidant Power (FRAP) Assay.** The FRAP assay was carried out according to the method of Stratil et al.<sup>24</sup> with slight modification. The FRAP solution was freshly prepared on the day of use, by mixing acetate buffer (pH 3.6), ferric chloride solution (20 mM), and TPTZ solution (10 mM TPTZ in 40 mM HCl) in a proportion of 10:1:1, respectively. Following this, the FRAP solution was heated, while protected from light, until it had reached a temperature of  $37^{\circ}\text{C}$ . Appropriate dilutions of onion methanolic extracts were prepared by diluting 10-fold in methanol. One hundred microliters of the diluted sample extract or, for blank (100  $\mu\text{L}$  of methanol) and for Trolox standard curves, 100  $\mu\text{L}$  of Trolox of appropriate concentration and 900  $\mu\text{L}$  of FRAP solution were added into a microcentrifuge tube. The tubes were vortexed and left at  $37^{\circ}\text{C}$  for exactly 40 min, and the absorbance was measured at 593 nm using spectrophotometer (Shimadzu UV-1700, Shimadzu Corp., Kyoto, Japan). The antioxidant activity of the samples was expressed in milligram Trolox equivalents per gram dry weight sample (Trolox mg g<sup>-1</sup> DW). All measurements were carried out in triplicate.

**Analysis of Antioxidant Activity: DPPH Antioxidant Power Assay.** The DPPH scavenging activity assay was performed using the method described by Goupy et al.<sup>25</sup> with a slight modification. DPPH was dissolved in methanol to a concentration of 0.238 mg mL<sup>-1</sup> in a conical flask. The reagent was prepared 2 h prior to use, to ensure that the DPPH had fully dissolved and stabilized. The flask containing DPPH solution was covered with aluminum foil to protect it from the light and stored in the refrigerator. For the actual measurement a 1:5 dilution of the DPPH stock was made using 10 mL of stock and making up to the 50 mL with methanol. Trolox (1–10  $\mu\text{g}$  mL<sup>-1</sup>) dissolved in methanol in appropriate dilution was used to make the standard curve. The experiment was carried out with three technical replicates for both samples and standard. In each replicate 500  $\mu\text{L}$  from the appropriately diluted sample extract was added with 500  $\mu\text{L}$  of DPPH solution. In the control, 500  $\mu\text{L}$  of methanol was added in place of sample extract with an equal volume of DPPH solution. For the blank, 500  $\mu\text{L}$  of sample extract was mixed with 500  $\mu\text{L}$  of methanol. The absorbance was measured at 515 nm by spectrophotometer (Shimadzu UV-1700, Shimadzu Corp., Kyoto, Japan). The radical scavenging activity was expressed in terms of milligram Trolox equivalents per gram of dry weight (Trolox mg g<sup>-1</sup> DW).

**Analysis of Total Flavonoids.** Total flavonoid content (TFC) was determined using the method described by Lin and Tang.<sup>26</sup> Briefly, 100  $\mu\text{L}$  of methanolic extract was mixed with 300  $\mu\text{L}$  of 95% ethanol, 40  $\mu\text{L}$  of 10%  $\text{AlCl}_3$ , 40  $\mu\text{L}$  of 1.0 M potassium acetate, and 520  $\mu\text{L}$  of distilled water. After incubation at room temperature for 40 min, absorbance of the reaction mixture was measured against blank at 415 nm using a spectrophotometer (Shimadzu UV-1700, Shimadzu Corp., Kyoto, Japan). Quercetin was used to develop a standard calibration curve and the TFC was expressed as milligrams of quercetin equivalents per gram dry weight (QE mg g<sup>-1</sup> DW).

**HPLC Analysis of the Extracts.** Reversed phase high-performance liquid chromatography (RP-HPLC) of the filtered sample extracts was carried out according to the method of Tsao and Yang<sup>27</sup> using an HPLC-DAD system (Shimadzu SPD-M10A). Flavonols and anthocyanins were separated on a ZORBAX SB-C18 column, 4.6 mm  $\times$  150 mm, 5  $\mu\text{m}$  particle size (Agilent Technologies, Santa Clara, CA, USA), and the target compounds were detected at 360 and 520 nm, separately. The mobile phase consisted of HPLC grade water with 0.05% trifluoroacetic acid (TFA) (solvent A) and acetonitrile with 0.05% TFA (solvent B). The gradient involved a linear increase in the amount of solvent B (%B), which was set as follows: 0–15 min, 12–21%; 15–25 min, 21–100%; and re-equilibrated to 12% B for the last 25–35 min at a flow rate of 1 mL min<sup>-1</sup>. Samples (10  $\mu\text{L}$ ) were injected and the separation of analytes achieved at  $30^{\circ}\text{C}$ . The data were processed using Shimadzu EZ Start version 7.3 software, and

**Table 2.** Significance of ANOVA for Each Measured Parameter in the Two Onion Varieties across 6 Years and Cultivation Systems<sup>a</sup>

treatment	mean bulb wt	TFC	TF	TAC	FRAP	DPPH	C 3,6 MG	C 3 G	C 3 LMB	C 3,6 MLMB	Q 3,4' D	Q 4' G	Q 3 G	Q
year	*	*	*	*	*	*	*	*	*	*	*	*	*	*
variety	*	*	*	*	*	*	*	*	*	*	*	*	*	*
soil	*	*	*	*	*	*	*	*	*	*	*	*	*	*
pest	NS	NS	NS	NS	*	*	NS	NS	NS	NS	NS	NS	NS	NS
year × variety	*	*	*	*	*	*	*	*	*	*	*	*	*	*
year × soil	NS	NS	*	NS	*	*	*	*	*	*	*	*	*	*
year × pest	NS	NS	*	NS	*	*	*	*	*	*	*	*	*	*
variety × soil	NS	NS	*	NS	NS	NS	NS	*	*	*	NS	*	*	*
variety × pest	NS	NS	*	NS	NS	NS	*	*	*	*	*	*	*	*
soil × pest	NS	NS	*	NS	NS	NS	*	NS	*	NS	*	NS	*	NS
year × variety × soil	NS	NS	*	NS	NS	NS	*	*	*	*	*	*	*	*
year × variety × pest	NS	NS	*	NS	NS	NS	*	*	*	*	*	*	*	*
year × soil × pest	NS	NS	*	NS	NS	NS	*	*	*	*	*	*	*	*
variety × soil × pest	NS	NS	*	NS	NS	*	*	*	*	*	*	*	*	*
year × variety × soil × pest	NS	NS	*	*	*	*	*	*	*	*	*	*	*	*
fully conventional (CS + CP) vs fully organic (OS + OP)	*	*	*	**	**	*	*	*	*	*	*	*	*	*

<sup>a</sup>TFC, total flavonoid content; TF, total flavonol content; TAC, total anthocyanin content; antioxidant capacity, FRAP and DPPH; C 3,6 MG, cyanidin 3-(6''-malonylglucoside); C 3 G, cyanidin 3-glucoside; C 3 LMB, cyanidin 3-laminaribioside; C 3,6 MLMB, cyanidin 3-(6''-malonylaminaribioside); Q 3,4' D, quercetin 3,4'-diglucoside; Q 4' G, quercetin 4'-glucoside; Q 3 G, quercetin 3-glucoside; Q, quercetin. NS, not significant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

concentrations of quercetin, quercetin glucosides, and individual and total anthocyanins were calculated against authentic calibration standards (Q 3 G, Q 4' G, Q 3,4' D, Q and C 3 G. Identification of cyanidin 3-laminaribioside (C 3 LMB), cyanidin 3-(6''-malonylglucoside) (C 3, 6 MG) and cyanidin 3-(6''-malonylaminaribioside) (C 3, 6 MLMB) were reached following their order of elution as described by Perez-Gregorio et al.,<sup>9</sup> and C 3 G standard curve was used for their quantification.

**Statistical Analysis.** Statistical analysis was carried out using SAS 9.1 (SAS Institute, Cary, NC, USA). To test the effect of factors (year of harvest, system of treatment, and variety) and their interactions on each measured parameter, the data were analyzed using the GLM model of analysis of variance (ANOVA). Significant differences were accepted at the minimum probability level of  $P < 0.05$ . The data for the yield and the total flavonoid, total flavonol, quercetin and its derivatives, total anthocyanins, individual anthocyanins, and antioxidant capacity (FRAP and DPPH) are reported as the mean values  $\pm$  standard error of the mean (SEM), and comparisons among the mean values were evaluated using Tukey's test. Correlations between variables and factors were also analyzed by principal component regression (PCR) using Unscrambler software, version 10.3 (CAMO ASA, Oslo, Norway) to achieve an overview of the correlation between variables and their contribution to the variation of year, treatment, and variety.

## RESULTS AND DISCUSSION

**Crop and Yield.** In crop production the main differences between organic and conventional systems involve the use of organic manures and crop rotations instead of inorganic mineral fertilizers and mechanical or biological methods (including naturally derived compounds) for pest control instead of synthetic pesticides. Analyses of onion mean bulb weight, antioxidant activity, and total and individual flavonoids are shown in Table 2. Year showed a significant ( $P < 0.01$ ) effect with some measures showing a significant year  $\times$  treatment interaction; therefore, data for each year were analyzed separately (Tables 3, 4, and 5).

Mean bulb weight in variety 'Hyskin' was generally significantly higher under fully conventional (CS + CP) management but in 'Red Baron' was higher under fully organic (OS + OP) management. This is of note as it indicates that some crop varieties are better suited to low-input organic cultivation practices. Conventional agriculture to date has focused on high-yield high-input crop cultivars that require chemical fertilizers and pesticides and, in some cases, irrigation to deliver high yields. Concern for this agricultural model is based on negative biological and environmental consequences and related long-term sustainability.

The effect of climate was clear with higher bulb weights recorded for both varieties in 2011 and 2014. Climate data for the trial site (Table 6) shows the growing seasons in year 2011 and 2014 were the two warmest and least humid, with the lowest rainfall of the 6 year trial period.

**Total Flavonoid Content.** 'Red Baron' is a deep red colored onion, whereas 'Hyskin' is a brown-skinned, white-fleshed onion. Thus, it is expected that 'Red Baron' would contain higher levels of anthocyanins than 'Hyskin'. This was reflected in the higher levels of total flavonoids in 'Red Baron' compared to 'Hyskin' (Table 3) and the statistically significant effect of variety (Table 2). Significant interactions between variety (V), soil treatment (S), and pest-control treatment (P) were observed but were not consistent across years (data not shown). In contrast, significant main effects for variety (V) and soil treatment (S) were observed in all years, with significant pest-control (P) treatment effects observed in some of the years. This shows that variety and soil treatment have a major influence on total flavonoid content in onion, with increased flavonoid levels found in the red variety 'Red Baron' and when onions are grown under the organic soil (OS) treatment.

In our study, equivalent amounts of nitrogen (N) were applied to both CS and OS treatments. However, mineral (conventional) fertilizer is more immediately available to the

**Table 3. Mean Bulb Weight, Total Flavonoid Content (TFC), Total Flavonol Content (TF), and Antioxidant Activity (FRAP and DPPH) in ‘Hyskin’ and ‘Red Baron’ under Different Management Systems in Each Year<sup>a</sup>**

year	variety	treatment	mean bulb wt (g)	TFC (QE mg g <sup>-1</sup> DW)	TF (mg g <sup>-1</sup> DW)	antioxidant capacity (Trolox mg g <sup>-1</sup> DW)	
						FRAP	DPPH
2009	Hyskin	OS + OP	152.3 ± 9.5c	3.65 ± 0.24bc	2.80 ± 0.28 cd	10.97 ± 0.1bc	4.06 ± 0.2c
		OS + CP	160.8 ± 12.3b	4.09 ± 0.15ab	3.08 ± 0.33bc	11.22 ± 0.14abc	4.66 ± 0.21ab
		CS + OP	187.0 ± 27.3a	3.05 ± 0.02d	2.32 ± 0.18d	11.06 ± 0.12bc	3.39 ± 0.22d
		CS + CP	186.7 ± 22.5a	3.25 ± 0.08 cd	2.61 ± 0.18 cd	10.86 ± 0.04bc	3.35 ± 0.25d
	Red Baron	OS + OP	125.0 ± 8.5e	4.37 ± 0.04a	3.62 ± 0.59a	11.80 ± 0.30a	5.06 ± 0.25a
		OS + CP	115.0 ± 9.3g	4.19 ± 0.15a	3.63 ± 0.48a	11.41 ± 0.02ab	5.14 ± 0.18a
		CS + OP	118.3 ± 12.2f	3.93 ± 0.41ab	3.4 ± 0.32ab	10.72 ± 0.13abc	4.42 ± 0.15bc
		CS + CP	148.0 ± 10.2d	4.06 ± 0.08ab	2.81 ± 0.43 cd	11.25 ± 0.31abc	5.05 ± 0.21a
2010	Hyskin	OS + OP	155.0 ± 9.3c	2.70 ± 0.03c	2.22 ± 0.82abc	7.70 ± 0.05e	2.87 ± 0.08 cd
		OS + CP	163.5 ± 7.2b	2.82 ± 0.07bc	1.92 ± 0.05d	9.32 ± 0.09c	3.80 ± 0.13a
		CS + OP	190.0 ± 5.2a	2.41 ± 0.33d	1.36 ± 0.13e	7.63 ± 0.12e	2.80 ± 0.09d
		CS + CP	189.2 ± 9.3a	2.69 ± 0.36c	2.34 ± 0.19ab	9.20 ± 0.16c	3.02 ± 0.12 cd
	Red Baron	OS + OP	150.8 ± 3.2d	2.82 ± 0.06bc	2.5 ± 0.13a	8.10 ± 0.13d	3.39 ± 0.05b
		OS + CP	117.5 ± 5.3g	3.17 ± 0.30a	2.14 ± 0.06bcd	10.40 ± 0.15a	4.03 ± 0.55a
		CS + OP	120.8 ± 6.2f	2.64 ± 0.20c	1.96 ± 0.12 cd	8.09 ± 0.08d	3.09 ± 0.04bc
		CS + CP	127.5 ± 9.1e	2.97 ± 0.50ab	2.16 ± 0.07bcd	9.90 ± 0.19b	3.02 ± 0.13 cd
2011	Hyskin	OS + OP	177.5 ± 8.7a	3.68 ± 0.08b	3.6 ± 0.14c	8.55 ± 0.47bcd	3.06 ± 0.29b
		OS + CP	183.3 ± 13.7a	3.56 ± 0.07bc	3.4 ± 0.15 cd	9.00 ± 0.15abc	2.85 ± 0.13bc
		CS + OP	181.7 ± 12.6a	3.27 ± 0.07 cd	2.9 ± 0.12ed	8.20 ± 0.17 cd	2.84 ± 0.03bc
		CS + CP	190.9 ± 14.2a	3.02 ± 0.04d	2.3 ± 0.12f	7.40 ± 0.26d	2.54 ± 0.12c
	Red Baron	OS + OP	186.7 ± 11.8a	4.70 ± 0.14a	4.46 ± 0.53a	9.81 ± 0.38ab	3.78 ± 0.43a
		OS + CP	151.7 ± 7.3b	4.65 ± 0.12a	4.24 ± 0.27b	10.10 ± 0.20a	2.97 ± 0.16bc
		CS + OP	139.1 ± 6.2b	4.58 ± 0.02a	4.37 ± 0.38b	8.50 ± 0.51bcd	2.96 ± 0.05bc
		CS + CP	149.2 ± 4.3b	4.64 ± 0.13a	2.78 ± 0.24ef	8.22 ± 0.19 cd	2.91 ± 0.02bc
2012	Hyskin	OS + OP	139.1 ± 10.4d	4.19 ± 0.23a	2.83 ± 0.1a	10.90 ± 0.18b	4.90 ± 0.05a
		OS + CP	155.1 ± 4.3b	3.92 ± 0.12a	2.1 ± 0.17b	10.40 ± 0.06d	3.90 ± 0.06c
		CS + OP	144.0 ± 5.6c	4.06 ± 0.39a	2.8 ± 0.14a	10.69 ± 0.34bcd	4.33 ± 0.19bc
		CS + CP	155.3 ± 8.2a	3.78 ± 0.36a	2.93 ± 0.11a	10.45 ± 0.43bc	3.93 ± 0.16c
	Red Baron	OS + OP	121.5 ± 11.3e	4.54 ± 0.05a	3.14 ± 0.17a	11.60 ± 0.22a	5.05 ± 0.06a
		OS + CP	107.5 ± 3.1h	4.26 ± 0.88a	2.20 ± 0.13b	10.92 ± 0.32bc	4.50 ± 0.41ab
		CS + OP	113.3 ± 5.2g	4.23 ± 0.11a	2.75 ± 0.12a	10.78 ± 0.42bcd	4.40 ± 0.15bc
		CS + CP	118.0 ± 1.9f	3.89 ± 0.12a	2.12 ± 0.24b	10.60 ± 0.33bcd	2.80 ± 0.18d
2013	Hyskin	OS + OP	153.8 ± 5.7c	3.69 ± 0.40 cd	2.84 ± 0.15bc	11.00 ± 0.55c	4.10 ± 0.10c
		OS + CP	161.4 ± 5.1b	4.15 ± 0.15abc	3.2 ± 0.33abc	11.80 ± 0.27bc	4.83 ± 0.14ab
		CS + OP	188.8 ± 8.7a	3.07 ± 0.25e	2.11 ± 0.28d	11.06 ± 0.09c	3.51 ± 0.21c
		CS + CP	187.0 ± 2.7a	3.29 ± 0.08de	2.67 ± 0.17 cd	10.86 ± 0.22ab	3.55 ± 0.31c
	Red Baron	OS + OP	149.6 ± 6.4d	4.48 ± 0.40a	3.66 ± 0.23a	12.10 ± 0.15a	5.12 ± 0.13a
		OS + CP	116.2 ± 7.5g	4.23 ± 0.35ab	3.69 ± 0.33a	11.96 ± 0.11ab	5.18 ± 0.09a
		CS + OP	119.6 ± 9.2f	3.99 ± 0.17bc	3.44 ± 0.24ab	11.00 ± 0.07c	4.52 ± 0.07bc
		CS + CP	126.3 ± 2.6e	4.15 ± 0.03abc	2.83 ± 0.25bc	11.61 ± 0.19b	5.10 ± 0.51a
2014	Hyskin	OS + OP	182.6 ± 3.5c	3.77 ± 0.20b	3.18 ± 0.22 cd	8.80 ± 0.71b	3.17 ± 0.17b
		OS + CP	176.3 ± 12.5e	3.67 ± 0.15bc	3.44 ± 0.28 cd	9.24 ± 0.77ab	2.90 ± 0.25b
		CS + OP	180.4 ± 8.4d	3.36 ± 0.16 cd	2.9 ± 0.33de	8.32 ± 0.68bc	2.87 ± 0.07b
		CS + CP	189.1 ± 6.3a	3.09 ± 0.13d	2.3 ± 0.45e	7.46 ± 0.53c	2.59 ± 0.24b
	Red Baron	OS + OP	185.4 ± 9.8b	4.78 ± 0.31a	4.50 ± 0.63a	10.07 ± 0.51a	3.83 ± 0.25a
		OS + CP	150.4 ± 13.2g	4.75 ± 0.29a	4.24 ± 0.68ab	10.34 ± 0.35a	3.08 ± 0.33b
		CS + OP	171.4 ± 4.9f	4.70 ± 0.27a	4.33 ± 0.80ab	8.76 ± 0.73b	2.98 ± 0.11b
		CS + CP	148.0 ± 11.3h	4.73 ± 0.18a	3.77 ± 0.24bc	8.44 ± 0.68bc	2.93 ± 0.05b

<sup>a</sup>Values are means ± standard error, *n* = 4, expressed on a dry weight basis. Means followed by different letters in the same column are significantly different according to Tukey's test in each year.

crop as no breakdown by soil microbes is required. The actual differences experienced by the crop between the CS and OS

treatments may include differences in plant-available N, P, and K; differences in the soil microbiome; and other unknown

**Table 4.** Concentration of Individual Quercetins in ‘Hyskin’ and ‘Red Baron’ under Different Management Systems in Each Year<sup>a</sup>

year	variety	treatment	Q	Q 3 G	Q 3,4' D	Q 4' G
2009	Hyskin	OS + OP	608.69 ± 14.06c	68.26 ± 3.96b	981.49 ± 15.93d	1138.48 ± 250.46d
		OS + CP	1021.64 ± 150.26a	38.84 ± 0.45c	840.66 ± 12.34e	1050.93 ± 166.98d
		CS + OP	710.99 ± 23.17b	65.23 ± 2.74b	497.33 ± 17.34f	1044.93 ± 135.32d
		CS + CP	612.72 ± 30.68d	38.56 ± 3.42c	352.28 ± 15.88g	1605.29 ± 132.13bc
	Red Baron	OS + OP	144.71 ± 17.21ef	106.74 ± 0.97a	1360.20 ± 290.9a	2007.46 ± 281.81ab
		OS + CP	172.59 ± 14.30e	72.40 ± 1.54b	1194.70 ± 270.56bc	2192.93 ± 191.96a
		CS + OP	92.56 ± 3.03f	32.40 ± 1.68c	1294.66 ± 180.52ab	1977.49 ± 110.27ab
		CS + CP	120.80 ± 14.74ef	24.37 ± 0.25c	1130.29 ± 300.10c	1511.65 ± 112.0c
2010	Hyskin	OS + OP	133.71 ± 14.88b	38.80 ± 1.06d	622.53 ± 21.48b	1275.61 ± 46.04c
		OS + CP	108.60 ± 11.74c	33.30 ± 0.90d	464.71 ± 13.05 cd	1317.35 ± 28.53c
		CS + OP	106.40 ± 12.34c	12.80 ± 0.19f	245.80 ± 7.58e	991.51 ± 106.65d
		CS + CP	179.97 ± 13.16a	19.19 ± 0.12e	451.30 ± 12.44d	1739.55 ± 158.97a
	Red Baron	OS + OP	39.70 ± 1.39e	80.40 ± 1.60a	694.70 ± 18.06a	1645.29 ± 79.80ab
		OS + CP	64.20 ± 2.79d	45.10 ± 0.64c	588.30 ± 12.69b	1447.66 ± 52.20bc
		CS + OP	28.10 ± 1.53e	45.90 ± 2.25c	508.20 ± 11.88c	1381.39 ± 86.51bc
		CS + CP	34.10 ± 1.07e	65.00 ± 1.43b	608.30 ± 13.81b	1458.33 ± 60.09bc
2011	Hyskin	OS + OP	242.78 ± 24.42c	36.90 ± 1.32d	686.70 ± 17.42d	2839.40 ± 109.65a
		OS + CP	93.96 ± 10.42ef	32.73 ± 3.07ed	485.70 ± 15.55e	2609.15 ± 112.09a
		CS + OP	287.30 ± 14.22a	28.80 ± 0.68ed	493.10 ± 27.74e	2236.90 ± 75.60ab
		CS + CP	105.80 ± 10.97e	12.60 ± 1.24e	302.40 ± 13.31f	1870.00 ± 95.80c
	Red Baron	OS + OP	76.25 ± 7.41f	277.50 ± 14.51a	2382.30 ± 238.02a	1596.02 ± 250.18a
		OS + CP	267.31 ± 14.82b	290.90 ± 12.17a	2123.40 ± 60.81b	1739.68 ± 196.5bc
		CS + OP	163.85 ± 11.14d	204.70 ± 11.84b	2195.40 ± 110.36b	1779.56 ± 241.90bc
		CS + CP	103.97 ± 11.54e	111.60 ± 14.95c	1117.40 ± 115.75c	1445.50 ± 106.37c
2012	Hyskin	OS + OP	40.60 ± 0.96ef	9.91 ± 0.25e	206.10 ± 12.22c	2487.02 ± 120.90a
		OS + CP	91.50 ± 1.44c	9.19 ± 0.68e	73.70 ± 1.65d	1902.80 ± 128.80b
		CS + OP	44.80 ± 0.91e	2.59 ± 0.12e	49.10 ± 2.06d	2692.64 ± 138.22a
		CS + CP	131.40 ± 1.59a	32.57 ± 1.57 cd	61.00 ± 1.36d	2897.34 ± 110.59a
	Red Baron	OS + OP	107.40 ± 5.97b	202.40 ± 15.23a	897.70 ± 19.12a	1937.70 ± 130.43b
		OS + CP	32.60 ± 0.82f	38.52 ± 0.43c	356.70 ± 13.79b	1764.45 ± 111.02b
		CS + OP	33.00 ± 1.16f	26.20 ± 1.03d	201.20 ± 12.49c	2490.62 ± 103.66a
		CS + CP	63.60 ± 1.89d	88.30 ± 1.07b	324.30 ± 10.58b	1711.29 ± 227.92b
2013	Hyskin	OS + OP	921.20 ± 14.06b	70.71 ± 3.96b	994.80 ± 85.93d	850.98 ± 50.46d
		OS + CP	1409.10 ± 105.26a	41.30 ± 0.45c	853.30 ± 52.34e	888.43 ± 166.98d
		CS + OP	798.50 ± 23.17b	67.73 ± 2.74b	497.50 ± 17.32f	741.93 ± 235.32d
		CS + CP	637.72 ± 13.68b	41.06 ± 3.42c	364.50 ± 15.08g	1618.00 ± 132.13bc
	Red Baron	OS + OP	157.20 ± 7.21c	106.70 ± 10.74a	1372.70 ± 129.90a	2019.96 ± 81.81ab
		OS + CP	197.80 ± 14.30c	74.90 ± 1.54b	1207.10 ± 127.56bc	2205.43 ± 191.96a
		CS + OP	105.10 ± 13.03c	34.90 ± 1.68 cd	1309.60 ± 118.52ab	1990 ± 110abc
		CS + CP	133.30 ± 4.74c	26.87 ± 0.25d	1142.70 ± 130.10c	1524.15 ± 115.34c
2014	Hyskin	OS + OP	245.28 ± 14.42b	39.37 ± 1.83d	698.95 ± 37.42e	2364.04 ± 169.65ab
		OS + CP	96.46 ± 1.42de	35.23 ± 5.07d	498.21 ± 25.55e	2634.15 ± 172.09a
		CS + OP	289.75 ± 24.22a	31.33 ± 0.93d	505.62 ± 37.7e	2261.99 ± 275.60abc
		CS + CP	108.30 ± 10.71d	15.10 ± 1.24d	315.27 ± 43.31f	1894.99 ± 395.80bcd
	Red Baron	OS + OP	78.75 ± 7.41e	279.92 ± 24.51a	2394.83 ± 138.02a	1580.02 ± 450.18d
		OS + CP	269.81 ± 24.82ab	293.46 ± 22.17a	2136.16 ± 260.81b	1714.68 ± 396.50 cd
		CS + OP	166.35 ± 17.14c	207.45 ± 21.84b	2208.14 ± 311.36b	1630.56 ± 441.90 cd
		CS + CP	106.47 ± 10.54d	114.26 ± 9.95c	1129.84 ± 115.75c	2420.50 ± 106.37ab

<sup>a</sup>Values are means ± standard error,  $n = 4$ , expressed on a dry weight basis (DW). Means followed by different letters in the same column are significantly different according to Tukey's test in each year. Q, quercetin; Q 3 G, quercetin 3-glucoside; Q 4' G, quercetin 4'-glucoside; Q 3,4' D, quercetin 3,4'-diglucoside. The data are expressed as  $\mu\text{g g}^{-1}$  sample.

differences that may be present. A number of other studies have shown total flavonoids decreased with increasing N application.

Groenbaek et al.<sup>28</sup> found a decrease in flavonoids with increased N for kale. Sander and Heitefuss<sup>29</sup> also reported

**Table 5. Concentration of Total and Individual Anthocyanins of 'Red Baron' under Different Management Systems in Each Year<sup>a</sup>**

year	treatment	C 3,6 MLMB	C 3 LMB	C 3 G	C 3,6 MG	TAC
2009	OS + OP	2.89 ± 0.58ab	2.14 ± 0.3a	27.20 ± 1.30a	40.15 ± 2.88ab	72.65 ± 3.64a
	OS + CP	3.45 ± 0.47a	1.45 ± 0.06b	23.90 ± 1.47b	43.89 ± 0.82a	72.38 ± 2.85a
	CS + OP	1.85 ± 0.39b	0.65 ± 0.14c	15.89 ± 1.27c	29.55 ± 0.43c	47.94 ± 4.18b
	CS + CP	2.42 ± 0.56ab	0.49 ± 0.06c	22.61 ± 1.51b	30.27 ± 0.72bc	55.7 ± 2.20b
2010	OS + OP	0.79 ± 0.06b	1.61 ± 0.06a	12.15 ± 0.55b	12.93 ± 0.3b	27.48 ± 3.00b
	OS + CP	1.29 ± 0.12a	0.90 ± 0.03c	11.77 ± 0.11b	28.97 ± 0.28a	42.97 ± 2.05a
	CS + OP	0.56 ± 0.06c	0.92 ± 0.09c	10.17 ± 0.08c	27.64 ± 0.34a	39.28 ± 3.51a
	CS + CP	0.68 ± 0.04bc	1.30 ± 0.06b	13.83 ± 0.32a	29.13 ± 0.20a	44.94 ± 2.25a
2011	OS + OP	48.56 ± 1.77b	55.50 ± 1.81a	76.30 ± 1.52b	37.02 ± 3.4b	217.5 ± 45.6b
	OS + CP	18.79 ± 0.57c	58.19 ± 4.87a	49.73 ± 2.9bc	47.68 ± 2.54b	174.7 ± 12.8b
	CS + OP	57.45 ± 1.69a	40.99 ± 0.74b	39.14 ± 0.5c	79.56 ± 1.63b	218.5 ± 18.2b
	CS + CP	21.16 ± 0.28c	22.35 ± 1.98c	223.40 ± 6.3a	289.50 ± 4.25a	556.0 ± 49.1a
2012	OS + OP	2.15 ± 0.24a	4.05 ± 0.09a	17.95 ± 0.77a	38.77 ± 0.35b	62.91 ± 2.93a
	OS + CP	0.65 ± 0.06c	0.77 ± 0.02c	7.14 ± 0.55b	35.25 ± 0.44b	43.85 ± 4.05c
	CS + OP	0.66 ± 0.07c	0.51 ± 0.04d	4.02 ± 0.05c	49.86 ± 0.49a	55.01 ± 3.83b
	CS + CP	1.27 ± 0.04b	1.77 ± 0.04b	6.5 ± 0.43b	32.43 ± 21.72b	43.76 ± 1.20c
2013	OS + OP	3.14 ± 0.29b	2.14 ± 0.03a	27.45 ± 1.96a	40.4 ± 3.27a	73.12 ± 4.0a
	OS + CP	3.95 ± 0.17a	1.49 ± 0.06b	24.14 ± 1.12b	24.13 ± 0.77b	53.70 ± 8.02b
	CS + OP	2.10 ± 0.12d	0.69 ± 0.07c	16.19 ± 0.74c	29.99 ± 4.41 b	48.79 ± 4.29b
	CS + CP	2.67 ± 0.19c	0.54 ± 0.01d	22.86 ± 1.20b	30.42 ± 0.45ab	56.5 ± 1.61b
2014	OS + OP	49.06 ± 2.72b	55.98 ± 2.25a	78.98 ± 4.48c	41.02 ± 3.89c	225.0 ± 47.6c
	OS + CP	19.29 ± 1.39c	58.69 ± 5.73a	127.23 ± 2.81b	292.68 ± 7.28b	498.1 ± 44.5b
	CS + OP	57.95 ± 2.45a	41.49 ± 1.35b	41.64 ± 0.9d	125.56 ± 3.51c	267.0 ± 45.5c
	CS + CP	21.66 ± 1.16c	22.85 ± 2.89c	225.98 ± 8.04a	481.50 ± 6.96a	754.6 ± 77.9a

<sup>a</sup>Values are means ± standard error, *n* = 4, expressed on a dry weight basis. Means followed by different letters in the same column are significantly different according to Tukey's test in each year. C 3 G, cyanidin 3-glucoside; C 3 LMB, cyanidin 3-laminaribioside; C 3,6 MG, cyanidin 3-(6"-malonylglucoside); C 3,6 MLMB, cyanidin 3-(6"-malonyllaminaribioside); TAC, total anthocyanin content. The data are expressed as  $\mu\text{g g}^{-1}$  sample.

**Table 6. Weather Conditions during Onion Crop Production between 2009 and 2014<sup>a</sup>**

year	T	TM	Tm	PP	V	RA/SN	H
2009	9.9	12.5	4.0	490.3	18.8	162	81.3
2010	10.0	12.4	4.1	465.5	17.4	153	81.9
2011	11.7	13.8	6.0	351.3	20.7	163	76.2
2012	11.2	12.9	5.7	560.0	19.9	156	76.9
2013	11.2	13.1	5.7	438.7	20.3	165	78.0
2014	12.1	16.3	7.5	341.6	19.7	140	76.0

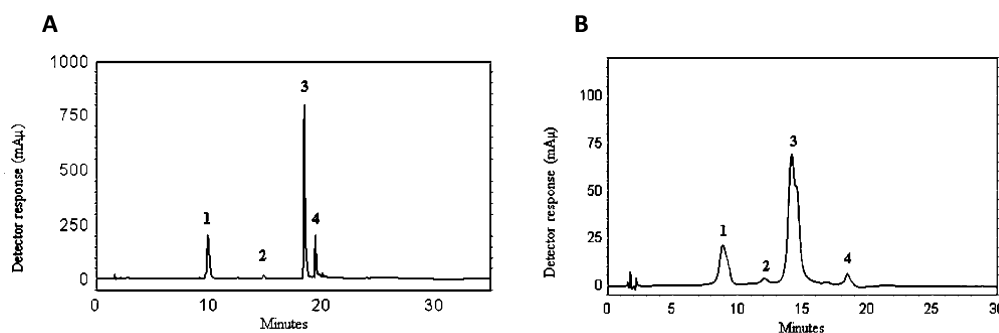
<sup>a</sup>T, mean temperature (°C); TM, mean maximum temperature (±); Tm, mean minimum temperature (°C); H, mean humidity (±); V, mean wind speed (Km/h); RA, daily indicator for occurrence of rain or drizzle (total days); PP, total monthly precipitation amount (mm); SN, indicator for occurrence of snow or ice.

that increasing mineral N fertilization resulted in reduced concentrations of phenolic compounds in wheat leaves. There is also evidence that differences in fertilizer regimens between organic and conventional production systems are associated with significantly higher phenolic concentrations in organic crops;<sup>30</sup> however, it is not clear if this is a nutrient stress effect or if other factors including effect of the soil microbiome or other factors are involved. In onion, an extensive previous study found that fertilizer type (mineral vs organic) and placement of fertilizer in onion had little effect on quercetin production.<sup>31,32</sup>

A previous study on the Kinsealy systems trial showed that soil treatment had a significant effect on the soil microbiome in a cultivated onion crop.<sup>21</sup> The types of changes in the soil microbiome described would be expected to be cumulative and to increase with the duration of time that land was cultivated under organic management. This offers an additional mechanism to explain significant differences seen between the OS and CS treatments in this study and more broadly in studies comparing organic and conventional produce in the literature.

The two onion varieties in this study showed a different quantitative response with regard to total flavonoid content under the same meteorological conditions over a 6-year trial period. Hallmann and Rembiałkowska<sup>33</sup> have demonstrated that red onion grown organically contained more flavonoids compared with conventional samples. Similarly, Ren et al.<sup>34</sup> have reported that organically grown Welsh onion had higher levels of flavonoids than conventionally farmed ones. However, Soltoft et al.<sup>35</sup> found no significant differences between conventionally and organically grown onions in the content of flavonoids. To our knowledge, no previous study has ever monitored the differences in a single crop type under organic compared to conventional management in the same trial over an extensive time period. Commonly, studies report on samples taken during one to two years or from shop-purchased samples.

'Red Baron' accumulated lower amounts of flavonoids in 2010, the year with the lowest temperature. Temperature is one



**Figure 1.** HPLC-DAD of onion extracts: (A) major flavonoids (peaks: 1, quercetin 3,4'-diglucoside (Q 3,4' D); 2, quercetin 3-glucoside (Q 3 G); 3, quercetin 4'-glucoside (Q 4' G); 4, quercetin (Q) at  $\lambda = 360$  nm); (B) anthocyanins (peaks: 1, cyanidin 3-glucoside (C 3 G); 2, cyanidin 3-laminaribioside (C 3 LMB); 3, cyanidin 3-(6''-malonylglucoside) (C 3,6 MG); 4, cyanidin 3-(6''-malonyllaminarinoside); C 3,6 MLMB at  $\lambda = 520$  nm).

of the most important factors affecting flavonoid accumulation in plants. Low temperature results in reduction of photosynthesis, which reduces the soluble sugar content of tissues and leads to a repression of genes that encode enzymes of the flavonoid biosynthetic pathway and to a reduction in substrates for flavonoid biosynthesis.<sup>36</sup> Our results showed that variety, soil management, and meteorological factors have a marked influence on the content of flavonoids in onions. Differences in onion total flavonoid content due to environmental conditions, in particular, temperature and humidity, have been reported in other studies.<sup>37</sup> In other crops studies in controlled growing environments have found that heat stress increases the total flavonoid content, with diverse results reported for low temperatures.<sup>38</sup> Drought stress seems to increase the total flavonoid content.<sup>39</sup> Chaves et al.<sup>40</sup> demonstrated that drought and high temperatures are correlated with an increase of the more methylated flavonoids in plants. In water-stressed plants, there is a general increase in the levels of phenolic compounds.<sup>41</sup> Wang and Zheng<sup>42</sup> found a strong correlation between temperature and production of phenolics in strawberry fruits. We propose that much of the conflicting results reported for bioactive content in studies comparing organic and conventional produce derive from limited time sampling and the influence of variation due to factors such as climate.

**Total and Individual Flavonols.** 'Hyskin' and 'Red Baron' had higher levels of total flavonols in 2011 and 2014 and the lowest levels in 2010 (Table 3). For example, the total flavonols in 'Red Baron' under OS + OP (fully organic) treatment increased by 38 and 39% in 2011 and 2014, respectively, compared to other growing years (2009, 2010, 2012, and 2013). The higher levels of flavonols observed in 2011 and 2014 are probably related to the higher global radiation and lower rainfall during the growing season. These meteorological conditions can increase secondary metabolism, favoring the synthesis of flavonols. In contrast, in the year with the lowest temperature, higher relative humidity, and higher rainfall (2010), onions accumulated less flavonol content. Mogren et al.<sup>31</sup> also found a high correlation between global radiation and levels of quercetin in onion bulbs. Similar results were obtained for apples, in which quercetin glycosides were found to be twice as high in light-exposed fruits.<sup>36</sup>

Our data show that organic farming improved flavonol content during the 6 years of analysis, particularly the levels of Q 3,4' D and Q 3 G in onion irrespective of cultivar type. A positive effect of organic farming (OS + OP treatment) was found on the content of bioactive compounds, with the flavonol

content increased by up to 20% compared to that of conventionally (CS + CP) grown onions.

Levels of quercetin and its glucosides in onion samples are shown in Table 4. Peaks from all samples were resolved and easy to identify (Figure 1A). In this study, the different concentration ( $\pm$  standard error,  $n = 4$ ) of major quercetins across production systems and over the 6-year period ranged from  $28.10 \pm 1.53 \mu\text{g g}^{-1}$  DW in 'Red Baron' to  $1409.10 \pm 105.26 \mu\text{g g}^{-1}$  DW in 'Hyskin' for Q; from 'Hyskin'  $2.59 \pm 0.12 \mu\text{g g}^{-1}$  to 'Red Baron'  $293.46 \pm 22.17 \mu\text{g g}^{-1}$  DW for Q 3 G; from 'Hyskin'  $744.93 \pm 13.5$  to 'Red Baron'  $2897.34 \pm 110.59 \mu\text{g g}^{-1}$  DM for Q 4' G; and from 'Hyskin'  $49.10 \pm 2.06 \mu\text{g g}^{-1}$  to 'Red Baron'  $2394.83 \pm 138.02 \mu\text{g g}^{-1}$  DM for (Q) 3,4' D, respectively. In the six seasons reported here, mean temperature, humidity, and wind speed were different; our data indicated levels of Q 3 G, Q 4' G, and Q 3,4' D were significantly higher ( $P < 0.05$ ) in 'Red Baron' than in 'Hyskin' across treatments and years (Table 4). Conversely, quercetin (Q) was significantly higher ( $P < 0.05$ ) in 'Hyskin' than in 'Red Baron' across treatments and years. The finding of consistently higher levels in 'Hyskin' is therefore of relevance from a health perspective. Levels of Q were lower in both varieties compared to the levels of Q 3 G, Q 4' G, and Q 3,4' D. The contents of flavonols were significantly influenced by the cultivation system. In particular, Q 3,4' D, and Q 3 G were positively influenced by organic cultivation in both varieties during the 6 years. Q 3,4' D, and Q 3 G contents were significantly ( $P < 0.05$ ) higher in samples grown under fully organic treatment (OS + OP) compared to samples grown under completely conventional treatment (CS + CP) in both varieties in every year with the single exception of Q 3 G in 'Hyskin' in 2012.

**Total and Individual Anthocyanins.** In the brown-skinned onion 'Hyskin', total anthocyanin levels were negligible and below the limit of detection (data not shown). Four anthocyanins were identified and found at quantifiable levels in 'Red Baron' (Figure 1B and Table 5). In general, total anthocyanins were considerably lower than reported by Donner et al.,<sup>43</sup> who found levels in the range of  $1090\text{--}2190 \text{ mg kg}^{-1}$  DW). HPLC chromatograms revealed one major anthocyanin, cyanidin 3-(6''-malonylglucoside) (C 3,6 MG), and cyanidin 3-glucoside (C 3 G) in all of the 'Red Baron' samples (Figure 1B); another acyl derivative (cyanidin 3-malonyllaminaribioside) is also an important component in concentration. Similar results for this anthocyanin profile were found by Rodrigues et al.<sup>37</sup> and Perez-Gregorio et al.<sup>9</sup> in cultivars of red onion. Total anthocyanin, C 3 G, and C 3,6 MG contents were highly



hypothesis (CNBH),<sup>51</sup> the cost-benefit hypothesis (CBH), and the resource availability hypothesis (RAH), also called the growth rate hypothesis (GRH).<sup>52</sup>

**Principal Component Regression.** PCR of the whole data set shows that the most common pattern in flavonoids reflects the differences between years (Figure 2A). The two onion varieties were placed in the diagonally opposite quadrant, indicating their opposite relationship of response to some parameters. Antioxidant capacity (DPPH and FRAP), 'Red Baron', and organic soil were located in the same quadrant of the plot and were considered positively correlated with each other, whereas they were negatively correlated with 'Hyskin' and conventional soil (CS), which were located in the diagonally opposed quadrant of the plot. 'Hyskin' with conventional soil treatment CS in 2010 tended to cluster separately on the left along PC1 due to the low content of the studied components. Individual flavonols (Q 3,4' D, Q 4' G), which were close to each other and 2011 and 2014 in the plot, had positive association with OP (organic pest control). Conventional pest control (CP) clustered with Q in the opposed quadrant of the plot. All detected compounds except quercetin showed higher values under OP treatment for the years 2011–2014. Despite the knowledge of the effect of environment on flavonol content, it is very difficult to generate precise predictive relationships between environment and flavonol concentration.<sup>37</sup> High direct correlations have been also reported between almost all of the identified flavonols, and this can be explained because of their common biosynthetic origin.<sup>53</sup> In our study, climatic variations had a strong effect on the flavonoid contents and the antioxidant capacity of the onions, particularly in the third year (2011) of analysis. This condition induced a higher flavonols content in 2011 and 2014, in which lower rainfall and higher temperature induced an increase in the flavonoid contents in different varieties of onion. It can be argued that onions cultivated in organic systems may be less affected in terms of phenolics content in the case of unfavorable climatic conditions. In fact, it seems that in conventional systems, in which nitrogen is readily available, the metabolism of plants changes toward the production of nitrogen-containing compounds such as free amino acids and alkaloids, which adversely affect the synthesis of bioactive compounds.<sup>50</sup>

The second PCR distinguished the anthocyanins with treatment and year in 'Red Baron'. PC1 and PC2 explained 75% variance of X-matrix and 20% variance of Y-matrix (Figure 2B). When the PCR was applied separately to all four different treatments in 'Red Baron', these differences between different years could easily be observed (Figure 2B). Differences between the organic and conventional treatments were clearly distinguishable on the biplot, and the total and individual anthocyanins showed some differences. As noted previously, some individual anthocyanins had higher concentrations in some years under fully conventional (CS + CP) treatment but had higher concentration in other years under organic (OS + OP) treatment; for example, the levels of total anthocyanins, C 3 G, and C 3,6 MG in CS + CP (2011, 2014) and the levels of C 3 LMB and C 3,6 MLMB in OS + OP (2011, 2014) both had highest concentrations in all 6 years. The biplot also shows correlation for total anthocyanins and individual anthocyanins with FRAP and DPPH, for which the outer ellipse and inner ellipse indicate 100 and 50% explained variance, respectively. Total anthocyanins and individual anthocyanins and antioxidant measurements were placed between the inner and outer

ellipses,  $r^2 = 0.5$  and  $1.0$ , respectively, indicating that antioxidant activity correlated well with total anthocyanin content, C 3 G, and C 3,6 MG.

Results presented here are robust, representing 6 year repeated sampling of two varieties of the same crop, grown in the same location in a replicated field trial with known agricultural inputs. Data showed that both varieties of onion had higher levels of total flavonols and quercetin glucosides Q 3,4' D and Q 3 G under organic cultivation (OS + OP) in all years. Antioxidant activity was generally higher under organic management (OS + OP) in both varieties and was consistently higher in the last 4 years for both varieties. Total flavonoid content was higher in the red onion variety and increased in 'Red Baron' under organic (OS + OP) cultivation, although total and individual anthocyanins did not show a consistent response to production system.

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### Notes

The authors declare no competing financial interest.

## ABBREVIATIONS USED

TPC, total phenolic content; TFC, total flavonoid content; TF, total flavonol content; Q 3 G, quercetin 3-glucoside; Q 4' G, quercetin 4'-glucoside; Q 3,4' D, quercetin 3,4'-diglucoside; Q, quercetin; C 3 G, cyanidin 3-glucoside; C 3 LMB, cyanidin 3-laminaribioside; C 3,6 MG, cyanidin 3-(6"-malonylglucoside); C 3,6 MLMB, cyanidin 3-(6"-malonyllaminaribioside); TAC, total anthocyanin content; TFA, trifluoroacetic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; MeOH, methanol; AlCl<sub>3</sub>, aluminum chloride; TPTZ, 2,4,6-triphenyl-1,3,5-triazine; HCl, hydrogen chloride; FRAP, ferric reducing antioxidant power; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

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