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Effect of Water Deficit Irrigation and Inoculation with *Botrytis cinerea* on Strawberry (*Fragaria x ananassa*) Fruit Quality

LEON A. TERRY,* GEMMA A. CHOPE, AND JORDI GINÉ BORDONABA

Plant Science Laboratory, Cranfield University, Bedfordshire, MK45 4DT, U.K.

Deficit irrigation (DI) detrimentally affected berry size but had a profound effect on fruit physiology and biochemistry. Strawberry cv. Elsanta fruit from DI-treated plants had higher levels of abscisic acid (ABA). Dry matter content as a proportion of fresh weight was increased by a quarter in fruit from water-stressed plants as compared to fruit harvested from plants held at or near field capacity. Concomitant to this, the concentration of some taste-related (viz. monosaccharides and sugar/acid ratios) and health-related compounds/parameters (viz. antioxidant capacity and total phenolics) were generally much greater in DI-treated fruit. The effect of inoculation with *Botrytis cinerea* on fruit quality was also tested. Fruit derived from inoculated plants displayed symptoms of gray mold postharvest disease earlier than noninoculated fruit and had double the concentration of ABA. Inoculation had no significant effects on all other target analytes measured. There was no interaction between water treatment and inoculation. The possible mechanisms for increased synthesis of ABA and the different effects of pathogen-induced stress versus drought stress on fruit quality are discussed.

KEYWORDS: Abscisic acid; anthocyanins; FRAP; organic acids; sugars; total phenolics.

INTRODUCTION

The continued exploitation of valuable water resources in horticulture is of growing concern in terms of environmental risk. Growers are under increasing pressure to demonstrate that their water abstractions for irrigation are reasonable, justified, and environmentally sustainable. Given this climate, it is perhaps surprising that more research has not been conducted on elucidating the effects of deficit irrigation (DI) on strawberry fruit quality and biochemistry (*1*). Most previous studies have reported that conventional DI is difficult to manage and that berry size correlates positively with the amount of irrigation water applied during flowering and fruit development (*2–4*). Recently, it has been reported that DI reduced yield and berry weight as compared to fully irrigated strawberry cv. Honeoye plants (*5*). The authors concluded that, under the conditions imposed, DI was not commercially viable. This said, the effect of DI on other quality attributes and strawberry fruit biochemistry was not measured, and it is plausible to speculate that DI may lead to higher concentrations of specific taste-related (viz. sugars and organic acids; (*6*)), defense-related (*7*) and/or health-related compounds (viz. antioxidant capacity, ascorbate, phenylpropanoids *8–10*).

The aim of the present study was to elucidate whether or not the degree of DI affected strawberry cv. Elsanta fruit quality and biochemistry. Specific emphasis was given to quantifying the drought stress hormone abscisic acid (ABA), individual anthocyanins, antioxidant capacity (FRAP), nonstructural carbohydrates, total phenolics, and organic acids in both primary and secondary position fruit from the primary truss. In addition, the effect of inoculation with *Botrytis cinerea* at anthesis on these quality attributes and on disease incidence was recorded to attest whether *B. cinerea* affected fruit quality at harvest and whether there was an interaction between stress caused by pathogen load and water stress.

MATERIALS AND METHODS

Plant Material. Ninety cold-stored first-year A+ grade strawberry cv. Elsanta plants (R.W. Walpole, Cambs., UK) were grown during 2005 in a glasshouse in 1 L capacity pots containing compost. Plants were sprayed with chlorpyrifos (1.5 L ha^{-1}) and myclobutanil (1.5 L ha^{-1}) to control spider mites (*Tetranychus* spp.) and powdery mildew (*Podosphaera aphanis*, formerly *Sphaerotheca macularis* (Wallr.: Fr) Jacz f sp. *fragariae* Peries), respectively (*11*). The total nitrogen concentration of the compost substrate was 8.88 g N kg^{-1} as determined by Kjeldahl analysis. Flowers were initially hand-pollinated before inoculation treatment with a sable paintbrush to minimize the occurrence of misshapen fruit.

Inoculum Preparation. A single-spore isolate of *B. cinerea* (IMI 189121; CABI Bioscience, Surrey, UK) was cultured in 9 cm diameter Petri plates on ½ strength potato-dextrose-agar (PDA; 19.5 g PDA L^{-1}distilled water) (Oxoid Ltd., Berks., UK) at 22 ± 1 °C for 7 days. Streptomycin (1.0 mg mL^{-1}) was added to the PDA to inhibit any potential growth of bacteria (*12*). The *B. cinerea* colonies were exposed to diurnal (12 h day^{-1}) UV-A lighting to induce sporulation.

Preharvest Watering Regime, Inoculation, and Experimental Design. A split-plot design was adopted with each of 3 blocks (n = 3

* Telephone: +44-1525-863-275; Fax: +44-1525-863-277; E-mail: l.a.terry@cranfield.ac.uk.

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× 30) divided in half for cv. Elsanta plants receiving sprays with either a conidial suspension of *B. cinerea* (2×10^5 condia mL^{-1}) or sterile distilled water (control treatment) 8 weeks after planting ($n = 2 \times 45$) when the majority of primary, secondary, and tertiary flowers on the primary truss were at green stage 1, postanthesis, and white bud stages, respectively (*7*). Both the conidial suspension and the control contained Tween 80 (0.5% v/v) and were applied to plants until incipient runoff (ca. 10 mL per plant) with a thin layer chromatography sprayer connected to a N_2 gas cylinder (*13*). Within plots, a completely randomized design was adopted for three water stress treatments over an 8-week period. Distilled water was administered daily before 09:00 h at rates of either 50, 100, or 200 mL day^{-1} ($n = 30$). Plants ($n = 90$) were held at or near field capacity for four weeks prior to commencing water treatments.

Soil Moisture Content and Environmental Monitoring. Soil moisture content (m^3 water per m^3 of soil) was measured once each day (ca. 16:00 h) by time-domain-reflectometry (TDR) using a Thetaprobe (ThetaKit type TK3, Delta-T Devices, Cambs., UK). The water holding characteristics for the growing medium were determined, and the mV reading was converted to m^3 water per m^3 of soil using an equation derived from a calibration curve from a data set taking the mV readings (292–1037 mV) corresponding to the volume of water in a soil sample (0.1443–0.7086 m^3 per m^3 of soil). The equation was $y = 1.1766x + 190.42$ (R = 0.9565). Hourly temperatures within the glasshouse were recorded throughout the season using two Delta-T dataloggers, each shielded from solar radiation by a polystyrene cup.

Fruit Sampling and Disease Assessment. From each plant, three fruit were harvested from the primary truss; therefore, ninety fruit were harvested for each DI treatment ($n = 270$). Primary fruit and both secondary fruit were harvested at red stage (approximately 20–28 days after anthesis; DAA) (*7*, *14*). DAA was monitored following the tagging of flowers ($n = 270$) at anthesis (*7*). The objective color of each fruit was measured using a Minolta CR-400 colorimeter and DP-400 data processor (Minolta Co. Ltd., Japan) with an 8 mm light-path aperture. The instrument was calibrated with a Minolta standard white tile CR-400 ($Y = 93.5$, $x = 0.3114$, $y = 0.3190$). The mean of three readings at three equidistant points ($n = 9$) around the equatorial axis was recorded and the lightness (L*), chroma (color saturation; C*), and hue angle (H°) were automatically calculated (*15*).

The primary and the first secondary fruit from the primary truss (*14*) were weighed and individually prepared immediately for biochemical analysis. The remaining secondary fruit ($n = 30$ fruit per DI treatment) was assessed for disease during postharvest storage as previously described (*11*). Fruit were held in the dark at 5 °C in a Sanyo incubator (MLR 350HT, Sanyo, Japan) and 95–100% relative humidity (RH) in individual closed but vented polystyrene containers in a completely randomized design (*11*). Disease severity resulting from inoculation or natural infection was assessed as the percentage area of each fruit covered by gray mold and was recorded daily. A value of 10% disease severity was defined as the first instance disease was visible on surface of fruit (*7*).

Sample Preparation and Reagents. Strawberry fruit without calyxes were cut in half vertically and were immediately snap frozen in liquid nitrogen. Samples were stored briefly at −40 °C before being freeze–dried in an Edwards Modulyo freeze drier (W. Sussex, UK) for 3 days at 0.15 mBar. Lyophilized samples were then ground in a pestle and mortar, weighed, and returned to the freezer until use. All reagents were purchased from Sigma (Dorset, UK) unless otherwise stated.

Extraction and Quantification of Nonstructural Carbohydrates. Nonstructural carbohydrates (NSCs) were extracted as previously described (*16*). Briefly, freeze–dried samples (150 mg; $n = 60$ fruit per DI treatment) were mixed with 3 mL of 62.5% (v/v) aqueous methanol solvent, placed in a shaking waterbath at 55 °C for 15 min, vortexing the samples every 5 min to prevent layering, and then left to cool. The cooled samples were filtered through a 0.2 μm Millex-GV syringe driven filter unit (Millipore Corporation, MA), and the clear extract was analyzed.

The NSC content in strawberry extracts was determined using a HPLC system comprising a P580 pump, and GINA 50 autosampler (Dionex, CA) (*17*). For the analysis of soluble sugars, 20 μL of diluted strawberry extract (1:10), was injected onto a Rezex RCM monosaccharide Ca^+ column, 300 mm × 7.8 mm diameter, 8 μm particle size (Phenomenex, CA; Part no. 00H-0130-K0), with a Carbo-Ca^+ guard column, 4 mm × 3 mm diameter (Phenomenex, CA; Part no. AJ0–4493). The mobile phase was HPLC grade water at a flow rate of 0.6 mL min^{-1}. The column temperature was set to 75 °C using a Dionex STH column thermostat. Eluted NSCs were detected by an evaporative light scattering detector (ELSD 2420, Waters, MA) connected to the Dionex system using a UCI-50 universal chromatography interface. The presence and abundance of fructose, glucose, and sucrose were automatically calculated by comparing sample peak area to standards using Chromeleon version 4.6 software (Dionex). Assays ($n = 180$) were performed in triplicate.

Extraction and Quantification of Nonvolatile Organic Acids. Extracts for organic acids determination were prepared from freeze–dried strawberry samples (50 mg; $n = 60$ fruit per DI treatment) and dissolved into 3 mL of HPLC grade water. Samples were kept at room temperature (25 °C) for 10 min and then filtered as previously described.

L-ascorbic, citric, and malic acid contents in strawberry extracts were determined using the same Dionex HPLC system as described above. Strawberry extracts (20 μL) were injected into an Alltech Prevail Organic Acid column 250 mm × 4.6 mm diameter, 5 μm particle size (Alltech, CA; Part no. 88645), with an Alltech Prevail Organic Acid guard column, 7.5 mm × 4.6 mm diameter (Alltech, CA; Part no. 96429). The mobile phase was analytical grade KH_2PO_4 (25 mM) (BDH, Dorset, UK) in HPLC grade water. The pH was adjusted to 2.5 using phosphoric acid. The flow rate of the mobile phase was 1.5 mL min^{-1} at isocratic conditions for 10 min. Column temperature was set at 35 °C. Eluted organic acids were detected at 210 nm using a UVD 170S/340S (Dionex, CA). The presence and quantity of each acid was calculated by comparing the peak area obtained with the sample extracts to standards using Chromeleon software.

Extraction and Quantification of Individual Anthocyanins. Individual anthocyanins from fruits are commonly extracted using an acidified organic solvent. This type of solvent destroys the cell membrane at the same time that it dissolves and stabilizes the anthocyanins (*18*). Different solvent combinations, mainly water and methanolic solvents, were tested according to those previously reported (*18–20*). Results showed that 70% (v/v) methanol and 0.5% HCl (v/v) in HPLC-grade water was the best solvent for the extraction of these target analytes from freeze–dried strawberry samples (data not shown). Samples (150 mg; $n = 60$ fruit per DI treatment) were mixed with 3 mL of this solvent and were placed in a HAAKE SWB 20 water-bath (Thermo Scientific, Germany) at 35 °C for 1.5 h, mixing the samples every 15 min. Finally, the sample was filtered as previously described.

The anthocyanin profile of strawberries was determined as previously described (*9*) with slight modifications using an Agilent 1200 series HPLC system (Agilent, Berks., UK), equipped with an Agilent 1200 DA G1315B/G1365B photodiode array with multiple wavelength detector. Strawberry extracts (20 μL), were injected into an Alltech Allsphere ODS-1 column, 250 mm × 4.6 mm diameter, 5 μm particle size (Alltech, UK; Part no. 778357), with an Alltech Allsphere ODS-1 guard column, 7.5 mm × 4.6 mm diameter (Part no. 96402). The mobile phase used consisted of acetonitrile (A) and 1% (v/v) phosphoric acid (Acros Organics, Leics., UK) and 10% (v/v) acetic acid (Fischer Scientific, Leics., UK) in water (B). The program followed a linear gradient from 2 to 20% of A in 25 min and then from 20 to 40% of A in 15 min. The flow rate was 1 mL min^{-1}, and the temperature of the column was held at 40 °C. Eluted anthocyanins were detected at 520 nm. The presence and quantity of each anthocyanin was calculated by comparing the peak area obtained to that of external standards (viz. cyanidin-3-glucoside and pelargonidin 3-glucoside; Extrasyntheses, Lyon, France) using Agilent ChemStation Rev. B.02.01 software.

Extraction and Quantification of Abscisic Acid (ABA). ABA was quantified using a radio-immunoassay (RIA) as previously described (*21*) with some modifications (*22*). Only secondary fruit were analyzed ($n = 30$ fruit per DI treatment). Ground lyophilized tissue (50 mg) was extracted overnight in 1:20 parts tissue to sterile distilled water (SDW) at 4 °C in the dark on a suspension mixer (802/TW, Luckham

Ltd., Sussex, UK). Extracted samples were vortexed and then centrifuged at 3000 rpm (MSE Mistral 2000, Sanyo Gallenkamp, Leics., UK) for 10 min at 4 °C. A sample of the supernatant was removed and diluted 1 part supernatant to 9 parts SDW.

A solution of DL-cis, trans-[G-^{3}H] ABA (Amersham International, Bucks., UK) in 100% ethanol was diluted 10-fold in SDW and frozen at −20 °C in 500 μL aliquots. This stock solution was diluted further to 4.8 μL mL^{-1} in phosphate buffered saline (PBS; 50 mM NaH_2PO_4, adjusted to pH 6.0 with 50 mM Na_2HPO_4, and 100 mM NaCl) containing 5 mg mL^{-1} of bovine γ-globulin to act as a coprecipitant with the cell line supernatant monoclonal antibody for (+)-ABA, MAC252 (*23*). MAC252 was diluted 1:1000 in PBS containing 5 mg mL^{-1} of bovine serum albumin and 4 mg mL^{-1} of soluble polyvinylpyrrolidone (MW 40000) to enhance binding of the antibody. Incubations were carried out in duplicate. A 50 μL sample or ABA standard, 100 μL of ^{3}H ABA, 100 μL of MAC252, and 200 μL of 100% PBS were added to 2 mL microtubes with push-in caps (Sarstedt, Leics., UK), and the mixtures were incubated in the dark at 4 °C for 45 min. Then, to precipitate the antibody, 500 μL of saturated $(NH_4)_2SO_4$ was added. The tubes were closed, inverted, and incubated at room temperature for 30 min. The precipitated antibodies were pelleted by centrifugation for 4 min at 8800*g* (Eppendorf centrifuge 5413, Eppendorf UK Ltd., Cambs., UK). The pellet was washed with 1 mL of 50% (v/v) saturated $(NH_4)_2SO_4$ and then recentrifuged. One hundred microliters of SDW were added, and the mixture was left for 10 min before vortexing to resuspend the Pellet. EcoScint H (1.2 mL; National Diagnostics, E. Yorks., UK) was added to convert β-radiation emitted by the bound ^{3}H ABA into light. The tubes were placed inside 20 mL plastic screw-top scintillation vials (National Diagnostics), wiped with a damp cloth to remove static, and counted on a ^{3}H program in a liquid scintillation counter (LS 6000TA, Beckman Coulter Ltd., Bucks., UK). Concentrations of ABA were calculated from the radioactivity (counts per minute) present in the pellets. The calibration curve was produced from two replicates of five unlabeled ABA standards ranging from 62.5 to 2000 pg per tube assayed with each batch.

Extraction and Quantification of Total Phenolics. Freeze–dried strawberry samples (150 mg; n = 60 fruit per DI treatment) were dissolved in 3 mL of 80% aqueous ethanol solvent and were held in a water-bath for 2 h at 70 °C, with mixing every 20 min. The solution obtained was filtered as previously described, and the clear filtrate was analyzed.

Total phenolics were measured according to the Folin–Ciocalteu method (*24*), based on the reduction of a phosphowolframate–phosphomolibdate complex by phenolics to blue reaction products. Briefly, 20 μL of filtrate and 3.2 mL of distilled water were mixed with 200 μL of Folin–Ciocalteu's phenol reagent, followed by 600 μL of sodium carbonate (1.9 M). After 2 h of incubation at room temperature (20 °C) and in the dark, absorbance was measured at 760 nm using a Camspec M501 UV/vis spectrophotometer (Camspec Ltd., Cambs., UK). Phenol content was estimated from a standard curve of gallic acid, and the results were expressed as mg of gallic acid equivalents (GAE) 100 g^{-1} DW. It should be noted that both ascorbic acid and saccharides, which are both present in strawberry, can interfere with this assay (*25*)

Total Antioxidant Capacity. Antioxidant capacity was measured using the FRAP assay, as described previously (*26*), with modifications and is based on the reduction of a ferric–tripyridyltriazine complex to its ferrous, colored form in the presence of antioxidants. Samples were prepared as for individual anthocyanin analysis. A 50 μL aliquot of diluted sample extract (1:19; v/v) or Fe^{2+} ($FeSO_4 \cdot 7H_20$) standards (0–2.0 mM) was added to 3.6 mL of freshly prepared FRAP working solution (viz. 5 mL of 10 mM TPTZ (2,4,6-tripyridyl-2-triazine) in 40 mM HCl and 5 mL of 10 mM $FeCl_3$ in 50 mL of 300 mM acetate buffer). The reaction mixture was incubated at 37 °C for 10 min, and the absorbance was measured spectrophotometrically at 593 nm using a Camspec M501 UV/vis spectrophotometer. Antioxidant capacity was expressed as the concentration of antioxidants having a ferric reducing ability (mM Fe^{2+} g^{-1} DW).

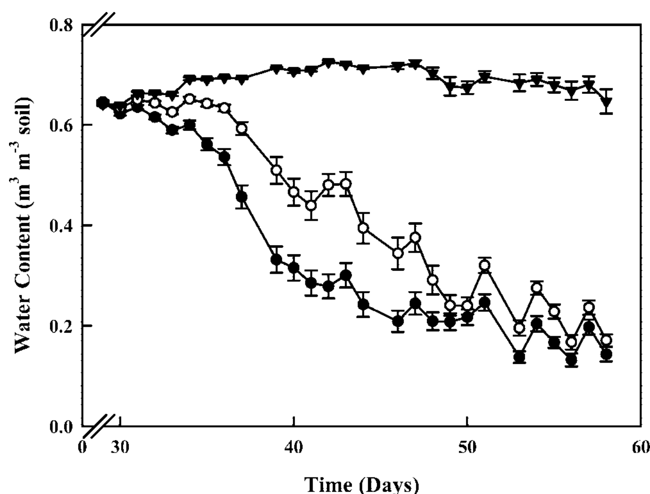

Figure 1. The water volume of the growing medium of strawberry plants watered daily before 09:00 h with rates of 50 (closed circles), 100 (open circles) or 200 mL of water per day (triangles) and measured at ca. 16: 00 h (4 p.m.). Mean time of anthesis was 32.2 days for the 100 and 50 mL per day water treatments and 33.0 days for the 200 mL per day water treatment.

DATA ANALYSIS

All statistical analyses were carried out using Genstat for Windows, Version 9.1.0.147 (VSN International Ltd., Herts., UK). Data were subjected to analysis of variance tests based on a split-plot design. Least significant difference values (LSD; $P = 0.05$) were calculated for mean separation using critical values of t for two-tailed tests. Tests for correlations between mean values for analyte concentrations were made using Spearman's Rank Correlation. Correlations are presented with the Spearman's Correlation Coefficient (r) and P value based on a two-tailed test. Unless otherwise stated, significant differences were $P < 0.01$. Means with different letters in tables are significantly different from one another ($P > 0.05$).

RESULTS

Volumetric water content of soil media (compost) was significantly different between all DI treatments (**Figure 1**). All treatments were held at or near field capacity (ca. 0.69 m^3 water per m^3 of soil) until 4 weeks after planting. Soil–water content for plants received 50 or 100 mL of water per day then progressively declined over a 30 day period to <0.2 m^3 water per m^3 of soil. The rate of decline in soil–water content was greatest for plants treated with 50 mL of water per day. Water content in the 50 and 100 mL of water per day treatments declined according to a Gompertz (asymmetrical s-shaped) curve with the equation $A + C \times \exp(-\exp(-B(X - M)))$. The parameters for 50 mL of water per day were B =0.1847, M = 12.449, $C = -0.4861$, and $A = 0.64922$, and the parameters for 100 mL of water per day were $B = -0.476$, $M = 4.96$, $C = -0.0642$, and $A = 0.69582$. Soil–water content for plants treated with 200 mL of water per day was maintained at 0.69 m^3 water per m^3 of soil throughout the trial. Accordingly, the degree of DI had a significant effect on almost all physiological and biochemical variables recorded. Mean temperature inside the glasshouse throughout the growing period was 23.0 °C.

Effect of DI on fruit physiology. Generally, fruit weight on both a fresh and dry weight basis was reduced in plants that received less water (**Table 1**). Fruit (mean of primary and secondary fruit) from plants treated with 200 mL of water per day (17.62 g) were nearly 1.3–1.6-fold heavier than fruit from

Table 1. Effect of water deficit treatment (mL of water per day) on weight characteristics of strawberry fruit.

	dry weight (g)		fresh weight (g)		proportion dry weight (g 100 g^{-1} FW)		calyx fresh weight (g)	
water treatment (mL)	primary fruit	secondary fruit	primary fruit	secondary fruit	primary fruit	secondary fruit	primary fruit	secondary fruit
50	1.867 b	0.984 a	13.95 c	8.40 a	13.31 d	11.79 c	0.61 a,b	0.13 a
100	1.976 b,c	1.161 a	16.92 d	11.07 b	11.62 c	10.52 b	0.62 a,b	0.17 a
200	2.188 b,c	1.235 a	21.88 e	13.36 b,c	9.80 a,b	9.38 a	1.12 b	0.37 a

Table 2. Effect of water deficit treatment (mL of water per day) on objective color ([a]) of strawberry fruit.

	L*			C*			H°		
water treatment (mL)	primary fruit	secondary fruit 1	secondary fruit 2	primary fruit	secondary fruit 1	secondary fruit 2	primary fruit	secondary fruit 1	secondary fruit 2
50	40.29 a,b	40.64 a,b	41.49 b	49.44 c	46.75 a,b	45.86 a	40.39 a,b,c	41.65 b,c,d	42.70 d
100	38.78 a	40.34 a,b	41.28 b	47.99 b,c	46.39 a,b	46.26 a,b	38.86 a	41.28 b,c,d	42.14 c,d
200	38.64 a	40.65 a,b	41.19 b	48.60 b,c	47.76 b,c	48.13 b,c	38.26 a	39.66 a,b	39.91 a,b

[a] L* is lightness, C* is chroma, and H° is the hue angle.

Table 3. Effect of water deficit treatment (mL of water per day) on the concentration of cyanidin 3-glucoside, pelargonidin glucoside derivative, pelargonidin 3-glucoside, total phenolics, and antioxidant capacity (FRAP) in strawberry fruit expressed per fresh weight (FW) and per dry weight (DW).

		cyanidin 3-glucoside[a] ($\mu g\ g^{-1}$)		pelargonidin glucoside derivative ($\mu g\ g^{-1}$)		pelargonidin 3-glucoside ($\mu g\ g^{-1}$)		total phenolics (mg GAE g^{-1})[a]		FRAP (mM Fe^{2+} g^{-1})	
	water treatment (mL)	primary fruit	secondary fruit	primary fruit	secondary fruit	primary fruit	secondary fruit	primary fruit	secondary fruit	primary fruit	secondary fruit
FW	50	1.83 a	2.42 b,c	32.73 a,b	39.68 c	113.99 a	157.98 c	3.46 d	3.35 d	9.55 b	11.07 b
	100	2.11 a,b	2.28 a,b,c	37.86 b,c	32.10 a,b	134.90 a,b	138.87 b,c	2.57 c	2.44 b,c	8.66 a,b	11.06 b
	200	2.50 b,c	2.72 c	34.39 b,c	27.49 a	129.09 a,b	128.17 a,b	2.20 a,b	2.07 a	8.53 a,b	7.40 a
DW	50	13.91 a	20.89 b,c	252.70 a	344.29 b	874.93 a	1369.06 b	26.14 b	28.54 b	71.01 a	95.02 c,d
	100	18.97 a,b	22.60 b,c	345.62 b	322.16 a,b	1239.78 b	1397.48 b	22.46 a	23.63 a	76.02 a,b	96.95 d
	200	25.25 c,d	28.81 d	352.38 b	301.00 a,b	1322.89 b	1406.88 b	22.24 a	22.10 a	87.66 b,c	79.77 a,b,c

[a] GAE = gallic acid equivalents

plants treated with 100 mL (13.99 g) or 50 mL of water per day (11.17 g). This said, dry weight as a proportion of fresh weight was significantly higher for fruit from plants treated with less water (**Table 1**). Fruits from plants treated with 50 or 100 mL of water per day contained almost 24 and 13%, respectively, more dry matter as a proportion of fresh weight than fruit kept at or near field capacity. In addition, both fresh weight (FW) and dry weight (DW) were significantly higher in primary fruit (17.58 and 2.011 g) as compared to secondary fruit (10.94 and 1.127 g). Similarly, calyx weight was also reduced by DI and was significantly higher in primary (0.78 g) versus secondary (0.22 g) fruit.

Fruit were only harvested from the primary truss. Anthesis occurred significantly earlier in primary fruit (30 days) than in first and second secondary position fruit (33 days). Similarly, primary fruit matured 54 days after planting, significantly earlier than the first secondary fruit, which took 55 days, and the second secondary fruit, which took 56 days. Fruit maturation was slower (not significantly) under DI, taking 23 days after anthesis when plants were treated with 50 mL of water per day, and 22 days at 100 and 200 mL of water per day.

All fruit were picked at the full red stage; however, significant differences in objective fruit color were observed (**Table 2**). Hue angle (H°) for fruit from plants treated with 50 mL day^{-1} was 41.58, indicating less red color as compared to 40.76 and 39.28 for 100 and 200 mL day^{-1} treatments, respectively. Similarly, the H° value for secondary fruit (40.86) was greater than for primary fruit (39.17). Fruit weight was greater with increased irrigation and in primary fruit as compared to secondary fruit; therefore, the lesser measured redness in smaller fruit may be an artifact of the objective colorimeter measurement system (8 mm aperture) used because there are greater distances between achenes on larger fruit. Water treatments started after anthesis. The number of achenes on fruit should have been similar between treatments. The more closely spaced achenes on DI-treated and smaller fruit may have contributed to the higher hue angle.

Table 4. Effect of inoculation and water deficit treatment (mL of water per day) on ABA concentration of secondary position strawberry fruit, expressed per fresh weight (FW) and per dry weight (DW).

	ABA concentration ($\mu g\ g^{-1}$)			
	FW		DW	
water treatment (mL)	noninoculated	inoculated	noninoculated	inoculated
50	6.40 a,b	10.19 b	57.06 a,b,c	89.68 b,c
100	3.66 a	7.55 a,b	31.85 a	72.80 b,c
200	3.33 a	8.30 b	36.66 a,b	75.41 b,c
column mean	4.46 x	8.68 y	41.86 x	79.30 y

Effect of DI on fruit biochemistry. Generally, DI had an effect on the concentrations of one individual anthocyanin, total phenolics, antioxidant capacity (**Table 3**), ABA (**Table 4**), nonstructural carbohydrates (**Table 5**), and nonvolatile organic acids (**Table 6**) for both primary and secondary fruit.

Anthocyanins, Total Phenolics, and Antioxidant Capacity. Neither pelargonidin 3-glucoside (Pg 3-gluc) nor pelargonidin 3-derivative (Pg der) were significantly affected by DI. However, cyanidin 3-glucoside (Cy 3-gluc) was significantly lower on a DW basis in fruit from plants treated with 50 (2.122 $\mu g\ g^{-1}$ FW, 17.40 $\mu g\ g^{-1}$ DW) and 100 (2.197 $\mu g\ g^{-1}$ FW, 20.79 $\mu g\ g^{-1}$ DW) than 200 mL of water per day (2.607 $\mu g\ g^{-1}$ FW, 27.03 $\mu g\ g^{-1}$ DW), respectively (**Table 3**). Both Pg 3-gluc and

Table 5. Effect of water deficit treatment (mL of water per day) on nonstructural carbohydrates of strawberry fruit, expressed per fresh weight (FW) and per dry weight (DW).

		sucrose (mg g^{-1})		fructose (mg g^{-1})		glucose (mg g^{-1})		(fructose + glucose)/sucrose		total sugars (mg g^{-1})	
	water treatment (mL)	primary fruit	secondary fruit	primary fruit	secondary fruit	primary fruit	secondary fruit	primary fruit	secondary fruit	primary fruit	secondary fruit
FW	50	28.54 b	25.43 a,b	36.10 e	26.98 c	29.87 d	21.34 b	2.51 b	2.25 a,b	94.51 d	73.76 b,c
	100	26.76 a,b	24.94 a,b	30.51 d	23.55 a,b	24.52 c	18.51 a	2.16 a,b	2.06 a,b	81.79 c	66.99 a,b
	200	23.00 a	23.58 a,b	26.39 b,c	22.54 a	21.15 b	18.01 a	2.24 a,b	1.99 a	70.54 a,b	64.13 a
DW	50	223.11 a	212.73 a	271.34 c	229.45 a,b	225.19 d	181.47 a,b			719.65 b	623.64 a
	100	228.16 a	231.74 a	257.86 c	220.07 a	209.13 c	175.38 a			695.20 b	627.11 a
	200	231.17 a	247.33 a	270.25 c	240.17 b	215.85 c,d	191.91 b			717.28 b	679.40 b

Table 6. Effect of water deficit treatment (mL of water per day) on nonvolatile organic acids of strawberry fruit, expressed per fresh weight (FW) and per dry weight (DW).

		ascorbate (mg g^{-1})		citrate (mg g^{-1})		malate (mg g^{-1})		total acids (mg g^{-1})		total sugar/total acid	
	water treatment (mL)	primary fruit	secondary fruit	primary fruit	secondary fruit	primary fruit	secondary fruit	primary fruit	secondary fruit	primary fruit	secondary fruit
FW	50	0.79 a	0.78 a	8.81 a	9.10 a	2.41 a	3.04 b	12.00 a	12.91 b	7.99 d	5.88 a,b
	100	0.78 a	0.76 a	9.16 a	9.19 a	2.24 a	2.79 b	12.18 a	12.74 b	6.94 b,c	5.35 a,b
	200	0.71 a	0.75 a	8.46 a	9.22 a	2.35 a	2.91b	11.52 a	12.88 b	6.16 b	5.04 a
DW	50	5.88 a	6.60 b	66.98 a	77.95 b	18.18 a	26.18 b,c	91.04 a	110.73 b,c		
	100	6.79 b,c	7.40 c,d	80.44 b,c	89.11 d	19.18 a	26.84 c	106.70 b	123.37 d		
	200	7.28 b,c	8.04 d	86.55 c,d	100.10 e	24.24 b	30.92 d	118.36 c,d	139.06 e		

Cy 3-gluc on a FW and DW basis were higher in primary fruit as compared to secondary fruit. There was a significant interaction between water treatment and fruit for Pg 3-gluc and Pg 3-der. The concentrations of Pg 3-gluc and Pg 3-der per FW increased with DI on a steady basis in secondary fruit, whereas the highest concentration in primary fruit was from plants treated with 100 mL of water per day. Per FW, there was little difference between fruit from plants treated with 100 or 200 mL of water day, with fruit from the lowest water treatment having the lowest concentration. There was no correlation between surface hue angle and concentration of anthocyanins in whole fruit tissue.

In contrast to that observed for anthocyanins, total phenolics on both a FW and DW basis significantly increased with greater degree of DI. Total phenolics, expressed as gallic acid equivalents (GAEs), were approximately 1.3- and 1.4-fold higher in fruit from plants treated with 50 mL of water per day (3.404 mg g^{-1} FW) as compared to 100 (2.504 mg g^{-1} FW) and 200 mL of water per day (2.135 mg g^{-1} FW), respectively (**Table 3**). Primary fruit contained higher ($P = 0.020$) total phenolics per FW (2.741 mg g^{-1} FW) as compared to secondary fruit (2.620 mg g^{-1} FW), but lower total phenolics per DW (primary fruit 23.61 mg g^{-1} DW, secondary fruit 24.76 mg g^{-1} DW). Like total phenolics content, antioxidant capacity per FW (as measured using the standard FRAP assay) increased with increasing DI; however, as with Cy 3-gluc, antioxidant capacity per DW decreased with DI (**Table 3**). Fruit from plants that received 50 mL of water per day (10.31 mM Fe^{2+} g^{-1} FW) had approximately 1.2-fold higher antioxidant capacity than fruit from plants treated with 200 mL water day^{-1} (7.96 mM Fe^{2+} g^{-1} FW). Again, there was an interaction between water treatment and fruit, with the greatest antioxidant capacity per FW being found in primary fruit from plants treated with 50 mL of water per day, with little difference between 100 and 200 mL of water per day. Conversely, in secondary fruit the greatest difference was between fruit from plants treated with 200 mL water day^{-1} and the DI treatments. The lowest antioxidant capacity per DW was in primary fruit from plants treated with 50 mL of water per day, with little difference between 100 and 200 mL of water per day. Similar to FW, in secondary fruit, the greatest difference was between fruit from plants treated with 200 mL of water per day and the DI treatments.

Abscisic Acid. The mean ABA concentration in secondary fruit from plants treated with 50 mL of water per day (73.4 μg g^{-1} DW, 8.30 μg g^{-1} FW) was substantially (but not significantly) higher than for 100 (52.3 μg g^{-1} DW, 5.61 μg g^{-1} FW) and 200 mL of water per day (56.0 μg g^{-1} DW, 5.81 μg g^{-1} FW) (**Table 4**).

Nonstructural Carbohydrates. Nonstructural carbohydrates were differentially affected by DI. Although sucrose concentrations were not affected by DI, monosaccharides (fructose and glucose) were both much higher in fruit from plants treated with either 50 or 200 mL of water per day as compared to 100 mL of water per day (**Table 5**). However, on a FW basis, the differences between treatments followed a more predictable pattern with glucose and fructose being ca. 1.2-fold higher in fruit from plants treated with 50 mL of water per day (25.67 and 31.89 mg g^{-1} FW) as compared to 100 (21.40 and 26.87 mg g^{-1} FW) and 200 mL of water per day (19.89 and 24.92 mg g^{-1} FW), respectively. Fructose and glucose were approximately 1.25-fold higher in primary fruit (31.36 and 25.31 mg g^{-1} FW) than secondary fruit (24.43 and 19.33 mg g^{-1} FW), respectively. There was a significant interaction between water treatment and fruit for glucose and fructose, where the concentration was greatest in secondary fruit from plants treated with 50 mL of water per day, with little difference between fruit from plants treated with 100 or 200 mL of water per day.

Nonvolatile Organic Acids. Nonvolatile organic acids per DW were differentially affected by DI (**Table 6**). On a DW basis, ascorbate, citrate, and malate were all significantly lower in fruit from plants treated with 50 mL of water per day (6.24, 72.46, 22.18 mg g^{-1} DW) as compared to 100 (7.09, 84.75, 23.16 mg g^{-1} DW) and 200 mL of water per day (7.66, 93.47, 27.58 mg

g^{-1} DW), respectively. There was no significant difference in acids per FW between the water treatments. In contrast to NSCs, ascorbate, citrate, and malate were significantly higher in secondary fruit versus primary fruit (DW). Crucially, sugar/acid ratios were significantly greater in fruit from plants treated with 50 mL of water per day (6.93) as compared to 100 (6.15) and 200 mL of water per day (5.60), respectively, with primary fruit (7.03) significantly higher than secondary fruit (5.42).

Effect of DI on Fruit Disease Resistance. Water stress had no significant effect on postharvest disease severity. All fruit (inoculated or not) eventually developed gray mold after harvest. Disease incidence occurred significantly sooner in fruit from plants that had been inoculated with *B. cinerea* at anthesis (ca. 24 days) as compared to controls (ca. 28 days). Inoculation had no significant effects on all target analytes measured apart from ABA. The ABA concentration in harvested fruit from plants that had been inoculated at anthesis (79.30 $\mu g\ g^{-1}$ DW, 8.68 $\mu g\ g^{-1}$ FW) with a conidial suspension (2×10^5 condia mL^{-1}) of *B. cinerea* was almost double that of fruit from noninoculated plants (41.86 $\mu g\ g^{-1}$ DW, 4.46 $\mu g\ g^{-1}$ FW). There was no interaction between water treatment and inoculation.

DISCUSSION

Deficit irrigation (DI) had profound effects on both fruit physiology and biochemistry and, for the first time, has been shown to generally increase the concentration of a number of target analytes that are either linked to taste (nonstructural carbohydrates) and/or ‘healthfulness’ (total phenolics and antioxidant capacity). Concentrations of target analytes measured were generally in agreement with that reported in the literature for strawberry fruit (viz. cv. Elsanta and other cvs) from non water-stressed plants (10, 14, 27–32). Critically, dry matter as a proportion of FW was increased with greater water stress, but to the detriment of overall fruit weight. Fruit from plants that received only 50 mL of water per day had nearly a quarter more dry matter per fruit as compared to fruit from plants that had been held at or near field capacity. Fruit from strawberry plants that received full irrigation have previously been reported to have a higher water content and individual berry fresh weight than those grown under drip irrigated or partial root drying (PRD) conditions (*5*). The authors concluded that PRD and DI treatments were not commercially viable because of reduced yield and decreased berry weight. Other authors have also reported that smaller fruit resulted from reduced irrigation (2–4, 33). However, the present study suggests that, despite DI (using the conditions imposed) reducing berry weight, other attributes that are related to fruit quality were increased.

Lower concentrations of sugars in fruit derived from plants that received more water was most probably caused by a ‘dilution effect’. A greater supply of irrigation-water generally resulted in a reduced concentration of flavor-related compounds, as described on a fresh weight basis (**Table 5**). However, the increased concentrations of many target analytes in DI-treated fruit may also have been elicited by drought stress. Antioxidant capacity (FRAP) and total phenolics were higher in DI-treated fruit, indicating that these fruit were more healthful; these characteristics are higher in achenes than in flesh (*9*). Therefore, it is likely that the higher proportion of achenes to flesh on the smaller DI-treated fruit contributed to this result. In contrast, it has been observed that overall antioxidant levels were reduced in strawberry cv. Elsanta fruit under drought stress conditions (*34*). However, only free radical content was measured rather than antioxidant capacity (FRAP), as in the present study.

The plant hormone ABA regulates various physiological reactions in plants, including induction of adaptive responses to water deficiency. Few works have quantified ABA in strawberry fruit (27, 35). ABA concentrations were approximately one-third higher than those reported for cv. Selva (*35*) using a similar RIA (*36*). No other studies to date, however, have quantified ABA concentration in strawberry fruit and shown that ABA concentration is affected by the degree of DI. This said, it has been reported that ABA concentration in strawberry cv. Selva fruit was higher when plants were grown in smaller pots with restricted roots (*35*). The authors also found that fruit maturity was advanced by root restriction. Moreover, a higher percentage of dry weight accompanied by a 1.3-fold higher concentration of reducing sugars and sucrose was found in fruit from plants that had restricted roots. Although the authors speculate that reduction of growth induced by root confinement was unrelated to conditions of water stress, the similarities between this study and the present one are evident because DI also increased ABA and dramatically increased the percentage dry matter of berries and the concentrations of both fructose and glucose by as much as 1.4-fold. Sucrose concentration was not affected by DI. Crucially, ref (*35*) did not measure soil moisture content, thus it is unknown whether strawberry plants grown in smaller pots were actually subjected to comparative water stress.

Others have shown that exogenously applied ABA can not only increase sugars when applied preharvest (*37, 38*), but that it also accelerates fruit color development, softening, and phenylalanine ammonia-lyase activity when applied as a post-harvest dip (*39*). Therefore, the increase in ABA in DI-treated fruit may be related to the increase in nonstructural carbohydrates and total phenolics in fruit seen in this study. The effect of DI on fruit quality is further complicated by differences between primary and secondary fruit on the primary truss. This suggests that for certain target analytes the degree of DI differentially affected resource partitioning in strawberry fruit.

ABA concentration tended not only to be higher in DI-treated fruit but was also doubled in fruit derived from inoculated plants. This is the first time that such a phenomenon has been reported for strawberry. Inoculation with *B. cinerea* at anthesis had generally no effect on any of the other target analytes measured. ABA has been reported to be produced by *B. cinerea* (*40*); thus, perhaps the higher levels of ABA in inoculated fruit originated not only from fruit as affected by the degree of DI but that ABA may also have been derived, or at least mediated, by the pathogen. Other evidence also suggests that *B. cinerea* can synthesize ABA, (*41*) and therefore it is possible that pathogen-derived ABA may have a function in the infection process and/or quiescence. Alternatively, *B. cinerea* may induce the fruit during ontogenetic development to synthesize ABA. ABA increases with strawberry fruit development (*27*). It is unknown whether there is a function for ABA in the strawberry/*B. cinerea* pathosystem; however, ABA may control the susceptibility of plant tissues to fungal infection by *B. cinerea* (*42*). It was previously demonstrated that exogenous treatment of roses cv. Mercedes with ABA doubled the susceptibility of petals to *Botrytis* blight (*43*). ABA was shown to have no effect on in vitro conidial germination or germ-tube elongation.

It is recognized that DI of strawberry plants can reduce berry size and yield. However, the present study has shown that DI can increase the concentration of many compounds that are related to taste and healthfulness. Because the promotion of strawberry sales is being increasingly based on ‘healthfulness’ and flavor rather than just berry size and yield, manipulating water delivery may be a viable prospect for increasing fruit quality.

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