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Dry Leaf and Steviol Glycoside Productivity of *Stevia rebaudiana* in the Western United States

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***Abstract*. Stevia (*Stevia rebaudiana* Bertoni) is a perennial herbaceous plant native to Paraguay, where it was used by the native Guarani peoples for centuries. Although steviol glycosides from stevia are powerful natural noncaloric sweeteners, stevia has been cultivated and commercially available only for the past 50 years. Cultural practices are still in development, and productivity potential in the United States is largely unknown. Currently commercial growers and processors worldwide are seeking to maximize the productivity of rebaudioside A, a steviol glycoside. The trials reported here examined the effects of location, harvest strategy, and cultivar on stevia dry leaf yield, steviol glycoside content, and steviol glycoside yield. Six or seven stevia cultivars were grown for ≈5 months at four western U.S. locations, with an irrigation criterion of 20 kPa. Stevia at every location was subjected to two harvest strategies: either one harvest at the end of the season or two harvests, one midseason and another at the end of the season. The main plots at each location were the stevia cultivars, and the split plots were the harvest strategies in a randomized complete block, split-plot design with four replicates. Dry leaf yield, leaf steviol glycoside content, and leaf steviol glycoside yield varied by cultivar, location, and cultivar by location, but not by harvest strategy or interactions of harvest strategy with location or cultivar. Dry leaf yield averaged 4.12 Mg·ha^{-1} with significant differences by cultivar and location. One of the steviol glycosides, rebaudioside A yield averaged 300 kg·ha^{-1} with significant differences by cultivar and by interactions of location with cultivar. Leaf productivity was greater at Ontario, OR, than at Hanford, CA, Indio, CA, or Yuma, AZ. Dry leaf yield greater than 7 Mg·ha^{-1} and rebaudioside A yields greater than 500 kg·ha^{-1} were observed at Ontario. Stevia perenniated at Hanford and Indio, providing the option of multiyear harvests from a single planting.**

Stevia (*Stevia rebaudiana*) is an herbaceous perennial, endemic to a semitropical region in the highlands of Paraguay, where it adapted to small niche environments between wetter marshlands and drier vegetated areas (Shock, 1982a, 1982b). The acidic, sandy soil is relatively infertile with an underlying shallow water table that provides a continuous source of water without surface soil saturation.

Stevia is among many species of economic importance from the Asteraceae or Compositae family, including sunflower, lettuce, and herbal teas. Stevia usually grows as an herbaceous perennial but can be cultivated in northern latitudes or other areas, such as China, as an annual. Stevia is an obligate short-day plant that has a critical daylength of less than 13 h for flower initiation, although daylength sensitivities are subject to varietal differences (Metivier and Viana, 1979; Valio and Rocha, 1977; Zaidan et al., 1980).

Paraguay's native Guarani peoples have used stevia leaves for centuries as a sweetening agent for normally bitter medicinal teas (Madan et al., 2010; Ramesh et al., 2006). The sweet flavor, reportedly up to 300 times sweeter than sugar, comes from noncaloric steviol glycoside compounds found in the leaf (Brandle et al., 1998). The two most prominent steviol glycosides are stevioside and rebaudioside A.

Sugar consumption in the United States is projected to decline 10%, from $2.18 billion in 2013 to $1.95 billion by 2018 (Sprinkle, 2014). In 2014, 48% of the sweetener market consisted of sugar and 17% consisted of all noncaloric sugar substitutes. Within the noncaloric sugar substitutes, steviol glycosides comprised 29% of the market compared with 45% for sucralose. Over the previous 2 years, 33% of the U.S. adults increased their consumption of steviol glycosides, 55% maintained their consumption, and 12% reduced their consumption. Manufacturers of diet soft drinks are using steviol glycosides (principally rebaudioside A) as sweeteners, which reduces sugar content and associated costs, while marketing a potentially healthier alternative to soda sweetened with sugar or high-fructose corn syrup.

Currently, the U.S. Federal Drug Administration (FDA) regulations limit stevia use in food products to processed combinations of stevioside, rebaudioside A, rebaudioside D, rebaudioside M, enzyme-modified steviol glycosides, and other steviol glycosides, and they must be at least 95% pure (U.S. Food and Drug Administration, 2015). The FDA allows stevia leaf use as a dietary supplement but not as a dietary ingredient, a sweetener, or flavoring agent. Industrial extraction of steviol glycosides from stevia leaves is currently only performed overseas. The U.S. market for domestically produced leaf may be limited by shipping costs until leaf processing facilities are built.

Commercial cultivation of stevia was reported in Paraguay and Japan during the early 1960s (Madan et al., 2010; Yadav et al., 2011). Today, stevia has been introduced for crop production in many countries including China, India, Brazil, Mexico, Canada, parts of Europe, and Africa, and to a lesser extent, the United States.

Efficient production of stevia will require efficient cultivars and sound cultural practices. Planting densities, fertility requirements, and pest management practices have been reviewed by several authors (Brandle et al., 1998; Madan et al., 2010; Ramesh et al., 2006), yet there are many unsolved problems in stevia production. Shock (1982a, 1982b) studied stevia yield for possible cultivation in California. The field study at the University of California, Davis, CA, produced dry leaf yield up to 9.2 Mg·ha^{-1} in small plots in a single growing season with a density of 191,400 plants/ha, but most yields were lower. The stevia was grown with frequent, shallow irrigations.

Over the past decade, several stevia trials have examined stevia dry leaf yields, and in some instances, steviol glycoside content on single cultivars from several regions of the world. In Paraguay, where stevia occurs naturally, the Cooperativa Colonias Unidas, Obligado, Itapúa, Paraguay, compared microsprinkler and drip irrigation with or without patchouli mulch in areas of high annual rainfall (2019 mm), low soil fertility, and high erosion (Prieto et al., 2010). Stevia dry leaf yields were compared at various plant densities over multiple sequential harvests. After the initial planting, any dead plantlets were replaced with live stevia plants to maintain densities. Dry leaf yield over 3 years improved from 4.12 Mg·ha^{-1} per year with no irrigation to 4.6 Mg·ha^{-1} per

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Table 1. 2014 *Stevia rebaudiana* S&W Seed Company yield trials performed on seven cultivars at four western U.S. sites.

Site location	Indio, CA	Yuma, AZ	Hanford, CA	Ontario, OR
Soil type	Sandy loam	Clay loam	Silt loam	Silt loam
Field type	Agricultural research	Agricultural research	Private organic	Agricultural research
Herbaceous growth	Perennial	Perennial	Perennial	Annual
Irrigation	Drip	Furrow	Drip	Drip
Cultivar	1090, 1108, SW 107, SW 129, SW 201, Candy	1090, 1108, SW 107, SW 129, SW 201, Candy	1049, 1090, 1108, SW 107, SW 129, Candy	1049, 1090, 1108, SW 107, SW 129, SW 201, Candy
Planting date	8 Apr. 2014	9 Apr. 2014	12 Apr. 2014	5 May 2314
Split-plot A, first harvest date	10 July 2014	12 July 2014	17 July 2014	24 July 2014
Split-plot A, second harvest date	4 Sept. 2014	8 Sept. 2014	12 Sept. 2014	3 Oct. 2014
Split-plot B, single harvest date	4 Sept. 2014	8 Sept. 2014	12 Sept. 2014	3 Oct. 2014

year with microsprinkler irrigation, and 5.22 $Mg{\cdot}ha^{-1}$ per year with drip irrigation at a planting density of 111,000 plants/ha. With mulch and replanting, using microsprinkler irrigation, harvest yields increased to 5.08 $Mg{\cdot}ha^{-1}$ per year. Before these advancements by Prieto et al. (2010), Ramesh et al. (2006) reported typical stevia leaf yields in Paraguay in the range of 1.5–2.5 $Mg{\cdot}ha^{-1}$ per year.

Fronza and Folegatti (2003) studied stevia in San Piero, Italy (43° N, 11° E; 5 m above sea level), where the climate is Mediterranean. Stevia leaf yield was 4.37 $Mg{\cdot}ha^{-1}$, stevioside content was 6.49%, and rebaudioside A and total steviol glycosides were not reported. In southwestern Belavatagi, India (15°34′ N, 75°21′ E, 578 m above sea level), Aladakatti et al. (2012) studied flood-irrigated stevia with various irrigation regimes and planting densities. Seedlings were planted at densities of 74,000, 83,000, 111,000, and 167,000 plants/ha. Harvest was initiated after a 90-d interval from planting date and subsequent harvests were performed on 70-d intervals totaling five harvests per year for 2 years. Yields as high as 10.54 $Mg{\cdot}ha^{-1}$ per year were obtained.

In Bhubaneswar, Odisha, India, Behera et al. (2013) grew stevia with various drip irrigation and fertigation regimes at 55,600 plants/ha. Stevia dry leaf yield of 2.74 $Mg{\cdot}ha^{-1}$ was obtained. Lavini et al. (2008) studied stevia water consumption, leaf yield, and steviol glycoside content in the Mediterranean climate of southern Italy with 50,000 plants/ha and two harvests per season. Yearly dry leaf yield reached 4.6 $Mg{\cdot}ha^{-1}$.

In the United States, the S&W Seed Company, Fresno, CA, had an interest in stevia leaf production in California and planted stevia from seed purchased from China and a patented clonal cultivar PC1 (Alvarez, 2012) in 2010, 2011, and 2012 at Chowchilla, CA. Stevia leaf yields were below 1 $Mg{\cdot}ha^{-1}$ in 2011 and 2012, but the leaf steviol glycoside analyses were favorable.

Stevia leaf and steviol glycoside productivity need to be better understood in the western United States. Limited information is available to growers who are interested in stevia production. Stevia seedling vigor is very low, so crop establishment currently depends on transplants from established seedlings or vegetatively propagated rooted cuttings. Major stevia production costs include labor for plant propagation and transplanting, weed control, irrigation, leaf harvest, and drying. Those investing in stevia production have little available information as to leaf yield and quality that could be expected. The stevia cultivar trials reported here were conducted at four western locations to obtain an understanding of the effects of location, harvest strategy, and cultivar on stevia dry leaf yield, steviol glycoside content, and steviol glycoside yield.

Table 2. Soil analysis comparison during preplant preparations at four locations selected for S&W Seed Company *Stevia rebaudiana* yield trials.

Soil analysis	Indio, CA	Yuma, AZ	Hanford, CA	Ontario, OR
pH	7.9	7.6	7.3	7.1
Cation exchange capacity, meq/100g	17	20	13	10
Organic matter, %	NA	1.6	NA	2
Nitrate, $\mu g{\cdot}g^{-1}$	40	48	11	31
Ammonium, $\mu g{\cdot}g^{-1}$	NA	6	NA	4
Phosphorus, $\mu g{\cdot}g^{-1}$	11	25	12	34
Potassium, $\mu g{\cdot}g^{-1}$	123	380	242	524
Calcium, $\mu g{\cdot}g^{-1}$	2,760	6,475	2,190	2,785
Magnesium, $\mu g{\cdot}g^{-1}$	128	415	105	553
Sodium, $\mu g{\cdot}g^{-1}$	212	332	22	242
Sulfur, $\mu g{\cdot}g^{-1}$	146	48	22	23
Zinc, $\mu g{\cdot}g^{-1}$	2.2	0.9	1.7	4.4
Iron, $\mu g{\cdot}g^{-1}$	3.7	21	6	13
Copper, $\mu g{\cdot}g^{-1}$	1.1	3	1.9	1.4
Manganese, $\mu g{\cdot}g^{-1}$	0.9	5	12	6
Boron, $\mu g{\cdot}g^{-1}$	0.6	2	0.2	1.1

Materials and Methods

In 2014, stevia yield trials were conducted for S&W Seed Company at four locations with various latitudes in the western United States, including Indio, CA, Yuma, AZ, Hanford, CA, and Ontario, OR (Table 1). The sites differed in soil type and irrigation practices. Three sites were university agricultural research facilities and one was a private organic farm. Soils were analyzed for preplant nutrient levels and fertilized accordingly (Table 2).

Six improved S&W stevia lines and cultivars '1049', '1090', '1108', 'SW 107', 'SW 129', and 'SW 201' were selected for their plant vigor, leaf taste, and steviol glycoside content and were compared with a seedling control cultivar for their leaf yield, steviol glycoside composition, and steviol glycoside yields (Shock and Parris, 2015, 2016a, 2016b). Stevia cuttings were rooted and increased during the 2013 Fall and Winter. The control was the cultivar Candy (Genesis Seed Ltd., Ashalin, Israel) grown from seed. Candy seedlings were grown at the same time as cuttings were rooted.

The stevia cultivars were planted at each location in a randomized complete block, with split-plot designs. The in-row transplanting distances varied by bed width at each site and were adjusted to 40,000 plants/acre (99,000 plants/ha). The cultivars were the main plots, and two harvest strategies were the split plots. Each main plot of each cultivar contained 208 plants in 21 m^2. One split plot was harvested twice, the first harvest at midseason and second at the end of the season. The other split plot was harvested only once at the end of the season (Table 1). At Yuma, Indio, and Hanford, 24 main plots were measured, staked, and numbered to accommodate six cultivars with four replicates. At Ontario, 28 main plots accommodated seven cultivars and four replicates. All seven cultivars would have been planted at the four locations if there had been sufficient plant material.

At Indio, Yuma, and Ontario, each main plot consisted of two beds with two rows of stevia plants on each bed and with one drip line buried at 10-cm depth at Indio and Ontario. To reduce border effects, the harvested areas used for yield estimates were from the adjoining interior plant rows of each bed. At Hanford the main plots consisted of four rows of plants on single bed with two surface drip lines. Two rows of stevia were planted on opposite sides of each drip line in an alternate manner creating four rows per plot, and the measured harvest areas were from the middle two rows of plants on the four-row bed.

Stevia was grown with drip irrigation at Indio, Hanford, and Ontario and furrow irrigation at Yuma. The soil water tension (SWT) was measured using five Watermark soil

moisture sensors (Irrometer Company, Inc., Riverside, CA) and a temperature sensor installed at 20-cm depth and connected to a Watermark Monitor data-logger (Irrometer Company, Inc.). Watermark soil moisture sensors had been previously calibrated to SWT using tensiometers with pressure transducers (Shock et al., 1998). Supervised by local management teams, irrigations were initiated when the average SWT of the five Watermark soil moisture sensors was close to 20 kPa (Shock and Wang, 2011; Shock et al., 2013).

Nutrient supplements consisted of 56 kg·ha^{-1} of nitrogen as solution 32 (urea ammonium nitrate) and 0.012 kg·ha^{-1} of iron as Sprint 138 6% iron chelate (Becker Underwood, Inc., Ames, IA) applied 2 weeks after transplanting and after the first harvest (Table 3). The Hanford site was fertilized organically with a preplant blood, bone, and feather meal (8-4-4) (Phyta-Grow Salinas Veggie-Mix; California Organic Fertilizers, Inc., Fresno, CA). Fish emulsion (4-3-4) (Westbridge Agricultural Products, Vista, CA) was applied monthly at Hanford.

Entrust (Dow AgroSciences Canada Inc., Calgary, Alberta, Canada), an Organic Materials Review Institute–approved control of *Bacillus thuringiensis*, was used once to control beet armyworm (*Spodoptera exigua*) at Indio. No other controls of insects or leaf diseases were needed at these arid or semiarid sites. Weeds were controlled by cultivation and hand weeding at Hanford, Indio, and Yuma. No mechanical cultivation was performed at Ontario, only light hand weeding.

Before each harvest, plant observations (average plant height, percent flowering, and subjective observations) were recorded for all split plots. Harvested plant material was collected from the two innermost rows of each four-row plot. The outside rows of plants in each plot and the first and last plants on the inside rows were designated border plants and were not used to estimate leaf yield. At each harvest, for steviol glycoside analysis, stevia branches from inside each split-plot border row were cut at a 20-cm height, bagged, and dried. After drying, the

Table 3. Fertilizer application rates applied to selected field locations for S&W Seed Company *Stevia rebaudiana* cultivars for 2014 yield trial studies.

Location	Date	Nitrogen (kg·ha^{-1})	Iron (kg·ha^{-1})	Copper (kg·ha^{-1})	Zinc (kg·ha^{-1})
Indio, CA	13 May 2014	56	0.012		
	16 July 2014	56	0.012		
Yuma, AZ	2 June 2014	56	0.012	0.005	0.005
	21 July 2014	56	0.012	0.005	0.005
Hanford, CA	3 Mar. 2014	90			
(preplant)	7 Apr. 2014	5	0.005		
	5 May 2014	5	0.005		
	2 June 2014	5	0.005		
	7 July 2014	5	0.005		
	4 Aug. 2014	5	0.005		
Ontario, OR	30 May 2014	56	0.012		
	28 July 2014	56	0.012		

Table 4. *Stevia rebaudiana* leaf yield, steviol glycoside content, and steviol glycoside yield from the first harvest at four locations in 2014.

		First harvest								
		Yield	Stev	Reb A	Reb C	TSG	Stev	Reb A	Reb C	TSG
Location	Cultivar	Mg·ha^{-1}	%				kg·ha^{-1}			
Indio, CA	1090	2.06	4.46	13.35	1.40	20.23	91.8	274.5	28.7	416.1
	1108	2.95	7.11	6.39	1.60	15.37	209.7	188.7	47.1	453.6
	SW 107	2.36	6.46	10.94	1.52	19.43	152.7	258.4	36.0	459.0
	SW 129	1.11	2.42	13.97	1.51	18.43	26.8	154.7	16.7	204.1
	SW 201	2.47	6.06	13.12	1.87	21.21	149.8	324.1	46.3	523.9
	Candy	1.98	6.42	6.85	1.22	14.71	127.2	135.6	24.2	291.2
	Average	2.15	5.49	10.77	1.52	18.23	126.3	222.7	33.2	391.3
Yuma, AZ	1090	1.79	5.81	7.69	1.30	14.89	103.9	137.6	23.2	266.2
	1108	1.79	6.03	6.90	1.65	14.57	108.1	123.7	29.5	261.3
	SW 107	2.04	3.97	11.42	1.49	17.39	81.0	233.0	30.4	354.7
	SW 129	0.95	2.77	12.08	1.16	16.49	26.3	114.5	11.0	156.3
	SW 201	2.16	4.20	14.26	1.70	20.16	90.8	308.6	36.7	436.1
	Candy	1.58	6.95	6.79	1.49	15.56	109.9	107.3	23.5	246.0
	Average	1.72	4.96	9.86	1.46	16.51	86.7	170.8	25.7	286.8
Hanford, CA	1049	1.98	7.40	7.32	1.39	16.10	146.7	145.1	27.5	319.3
	1090	1.72	3.85	12.85	1.28	18.39	66.4	221.3	22.0	316.7
	1108	1.67	6.02	7.83	1.63	15.59	100.7	130.9	27.3	260.7
	SW 107	1.79	4.07	10.60	1.27	16.25	72.7	189.5	22.8	290.6
	SW 129	1.07	1.33	16.27	1.39	19.60	14.3	174.3	14.9	210.0
	Candy	1.46	7.43	7.80	1.27	16.69	108.3	113.6	18.5	243.1
	Average	1.62	5.02	10.44	1.37	17.10	84.8	162.5	22.2	273.4
Ontario, OR	1049	2.35	6.35	5.36	1.06	12.77	149.1	125.7	24.9	299.6
	1090	1.97	3.61	10.28	0.95	15.26	71.1	202.6	18.8	300.7
	1108	1.70	5.33	4.74	1.01	11.08	90.8	80.7	17.2	188.7
	SW 107	2.98	5.44	10.72	1.35	17.84	162.3	320.0	40.2	532.4
	SW 129	2.77	1.97	11.98	1.02	15.49	54.6	332.1	28.4	429.1
	SW 201	2.09	4.71	10.50	1.29	16.49	98.4	219.4	26.9	344.7
	Candy	1.98	4.89	5.96	0.91	11.87	96.7	117.9	18.1	234.7
	Average	2.26	4.61	8.51	1.09	14.40	103.3	199.8	24.9	332.8
Average	1049	2.17	6.87	6.34	1.22	14.43	147.9	135.4	26.2	309.5
	1090	1.88	4.43	11.04	1.23	17.19	83.3	209.0	23.2	324.9
	1108	2.03	6.12	6.46	1.47	14.15	127.3	131.0	30.3	291.1
	SW 107	2.29	4.99	10.92	1.41	17.73	117.2	250.2	32.3	409.1
	SW 129	1.47	2.12	13.58	1.27	17.50	30.5	193.9	17.8	249.9
	SW 201	2.24	4.99	12.63	1.62	19.29	113.0	284.0	36.6	434.9
	Candy	1.75	6.42	6.85	1.22	14.71	110.5	118.6	21.1	253.7
Overall average		1.88	4.81	9.46	1.30	15.84	96.5	182.1	25.4	309.2
LSD (0.05), location		0.23	0.49	NS	NS	NS	14.8	21.6	3.50	38.2
LSD (0.05), cultivar		0.34	1.05	1.29	0.16	1.85	20.6	33.2	5.04	58.2
LSD (0.05), location × cultivar		0.67	NS	NS	NS	NS	41.2	66.5	10.1	116

Stev = stevioside; Reb A = rebaudioside A; Reb C = rebaudioside C; TSG = total steviol glycosides; LSD = least significant differences; NS = nonsignificant.

leaves were separated from the stems and ground in a Wiley No. 4 Mill (Thomas Scientific, Swedesboro, NJ) using a 2-mm screen. The ground samples were placed in labeled manila coin envelopes for leaf steviol glycoside analysis.

Steviol glycoside analyses using high-performance liquid chromatography. Steviol glycoside standard kits (JECFA Standards Kit) were purchased from ChromaDex (Irvine, CA). Water was obtained from a Milli-Q purification system (Millipore, North Ryde, NSW, Australia). Acetonitrile and methanol were purchased from EMD (Gibbstown, NJ) of high-performance liquid chromatography (HPLC) grade.

Stevia leaf powders were dried using a vacuum oven at 70 °C for 17 h before analysis. A 0.5-g sample of dried stevia leaf powder was weighed into a 125-mL flask and 75 mL of milli-Q water was added. The flask was placed on a hot plate, and the sample was boiled for 1 h. The flask was swirled every 20 min during heating. After cooling to room temperature, the sample was poured through filter paper (VWR NO. 417, Radnor, PA); the filtrate was collected in a 100-mL volumetric flask and brought to volume using deionized water. A C18 cartridge (500 mg, J.T.Baker®; Avantor Performance Materials, Center Valley, PA) was conditioned with 3 mL of methanol and 3 mL of milli-Q water, and 2 mL of sample was applied slowly to the cartridge. After washing with 2 mL of milli-Q water and 2 mL of acetonitrile:water (20:80, v/v), the cartridge was dried under vacuum. The glycosides were eluted with 2 mL of acetonitrile:water (50:50, v/v).

The purified stevia glycosides sample (20 µL) was injected to an HPLC instrument with a SUPELCOSIL™ LC-NH_2 column (25 cm × 4.6 mm, 5 µm; Supelco Inc., Bellefonte, PA). The eluents were 86% acetonitrile in milli-Q water (v/v) (solvent A) and 60% acetonitrile in milli-Q water (v/v) (solvent B). Total flow rate was 2 mL·min^{-1}. The following binary gradient system was employed: 0–10 min (0% to 5% B); 10–25 min (5% to 40% B); 25–35 min (40% to 50% B); 35–40 min (50% B); 40–45 min (50% to 0% B); and 45–55 min (0% B). Absorbance at wavelength of 210 nm was recorded.

The peaks from the sample solution were identified by comparing the retention time with the peaks from the mixture of nine steviol glycosides (stevioside, rebaudioside A, rebaudioside C, rebaudioside B, rebaudioside D, rebaudioside F, dulcoside A, rubusoside, and steviolbioside) standard solution. For calibration, a stock solution of stevioside and rebaudioside A was diluted sequentially in water to yield final concentrations ranging from 0.5 to 5.0 g/L. Calibration curves were constructed by plotting the peak area of the target compound against the concentration, using linear regression. Dulcoside A, rubusoside, and steviolbioside were calculated as stevioside equivalents. Rebaudioside C, rebaudioside D, rebaudioside B, and rebaudioside F were calculated as rebaudioside A equivalents. The leaf contents of dulcoside A, rubusoside, steviolbioside, rebaudioside D, rebaudioside B, and rebaudioside F were small and were only reported as part of the total steviol glycosides.

Statistical procedures. Differences in stevia dry leaf yields, steviol glycoside concentrations, and steviol glycoside yields were evaluated over locations, cultivars, and harvest

Table 5. *Stevia rebaudiana* leaf yield, steviol glycoside content, and steviol glycoside yield from the second harvest at four locations in 2014.

		Second harvest								
		Yield	Stev	Reb A	Reb C	TSG	Stev	Reb A	Reb C	TSG
Location	Cultivar	Mg·ha^{-1}	%				kg·ha^{-1}			
Indio, CA	1090	1.63	2.80	8.53	0.93	12.46	44.5	138.4	14.9	200.7
	1108	1.87	4.80	4.33	0.87	10.02	90.1	80.5	16.2	187.2
	SW 107	1.74	3.84	7.37	0.88	12.23	66.0	128.7	15.3	212.4
	SW 129	1.15	1.31	9.27	0.86	12.00	15.0	106.3	9.8	137.5
	SW 201	1.69	4.37	8.44	1.02	14.20	75.9	148.2	17.7	248.5
	Candy	1.67	6.03	3.87	0.87	10.95	100.3	61.5	13.8	178.4
	Average	1.62	3.86	6.97	0.90	11.98	65.3	110.6	14.6	194.1
Yuma, AZ	1090	0.83	1.84	8.59	0.71	11.28	14.6	68.2	5.8	90.0
	1108	1.04	3.84	4.39	0.90	9.21	40.4	46.7	9.4	96.9
	SW 107	1.98	2.60	7.76	0.87	11.45	53.9	157.8	18.0	235.2
	SW 129	1.00	0.78	7.06	0.57	8.48	7.8	73.3	5.8	87.8
	SW 201	1.93	2.90	6.89	0.88	10.74	56.4	132.8	16.9	207.3
	Candy	0.65	4.02	4.28	0.68	9.27	24.5	30.9	4.4	61.7
	Average	1.24	2.66	6.50	0.77	10.07	32.9	85.0	10.1	129.8
Hanford, CA	1049	2.42	4.64	5.26	0.83	10.83	112.1	127.3	19.7	260.8
	1090	2.16	1.86	8.02	0.86	10.89	40.3	175.6	19.0	238.2
	1108	2.95	3.99	4.56	0.85	9.40	116.5	132.8	24.6	273.9
	SW 107	3.12	3.74	8.93	1.08	14.38	116.1	279.8	33.7	449.2
	SW 129	1.71	1.33	9.86	0.92	12.57	21.4	181.6	17.2	229.3
	Candy	2.05	5.56	3.24	0.72	10.12	118.9	66.2	15.3	214.0
	Average	2.40	3.52	6.65	0.88	11.36	87.5	160.5	21.6	277.6
Ontario, Ore.	1049	4.73	5.50	3.33	0.63	9.67	261.9	157.8	29.5	459.6
	1090	3.39	4.64	5.86	0.74	11.64	157.3	205.1	22.3	398.9
	1108	3.63	4.26	2.78	0.54	7.62	155.2	100.9	19.4	275.8
	SW 107	4.42	4.58	5.89	0.68	11.57	204.2	251.2	27.5	501.7
	SW 129	3.64	1.72	7.90	0.76	10.96	68.0	297.4	24.9	410.1
	SW 201	2.70	4.43	6.96	0.72	12.63	151.1	235.2	24.3	427.8
	Candy	3.35	5.94	1.60	0.31	8.14	205.3	50.2	9.8	276.6
	Average	3.70	4.44	4.91	0.62	10.32	171.8	185.4	22.5	392.9
Average across locations	1049	3.57	5.07	4.30	0.73	10.25	187.0	142.6	24.6	360.2
	1090	2.00	2.79	7.75	0.81	11.57	64.2	146.8	15.5	231.9
	1108	2.37	4.22	4.01	0.79	9.06	100.6	90.2	17.4	208.5
	SW 107	2.81	3.69	7.49	0.88	12.41	110.1	204.4	23.6	349.6
	SW 129	1.87	1.29	8.52	0.78	11.00	28.0	164.7	14.4	216.2
	SW 201	2.11	3.90	7.43	0.87	12.52	94.5	172.1	19.7	294.5
	Candy	1.93	5.39	3.25	0.64	9.62	112.2	52.2	10.8	182.7
Overall average		2.21	3.51	5.96	0.76	10.49	89.1	132.1	16.7	244.6
LSD (0.05), location		NS	NS	NS	NS	NS	35.8	NS	NS	NS
LSD (0.05), cultivar		0.58	0.70	1.09	0.12	1.08	29.2	54	4.40	72.3
LSD (0.05), location × cultivar		NS	NS	NS	NS	NS	NS	NS	NS	NS

Stev = stevioside; Reb A = rebaudioside A; Reb C = rebaudioside C; TSG = total steviol glycosides; LSD = least significant differences; NS = nonsignificant.

strategies using general linear model analysis of variance (Hintze, 2007). Locations were the main treatment effects, and cultivars were evaluated as split plots within locations. When considering differences within harvest strategies, we assigned harvest strategies as split-split plots. Least significant differences (0.05), were calculated only where treatment or interaction effects had significant F values.

Results and Discussion

Leaf yield. Leaf production for the first harvest (midseason) had significant differences between locations; Indio (2.15 Mg·ha^{-1}) and Ontario (2.26 Mg·ha^{-1}) had greater leaf yield than Yuma (1.72 Mg·ha^{-1}) and Hanford (1.62 Mg·ha^{-1}) (Table 4). For the second harvest and the single harvest strategy, yield production was not significantly affected by location (Tables 5 and 6). Averaged over harvest strategies and cultivars, season-long leaf yields were higher at Ontario (5.88 Mg·ha^{-1}) than at the other three locations (Table 7).

Averaged over locations, there were significant differences among cultivars in dry leaf yield at all harvests. For the first harvest, 'SW 107' (2.29 Mg·ha^{-1}) and 'SW 201' (2.24 Mg·ha^{-1}) had significantly higher dry leaf yields than the Candy (1.75 Mg·ha^{-1}) and 'SW 129' (1.47 Mg·ha^{-1}). For the second harvest, 'SW 107' (2.81 Mg·ha^{-1}) and '1049' (3.57 Mg·ha^{-1}) had significantly higher dry leaf yields than all other cultivars. The single-harvest dry leaf yield for cultivars '1049' (6.46 Mg·ha^{-1}), 'SW 107' (5.01 Mg·ha^{-1}), 'SW 201' (4.91 Mg·ha^{-1}), and '1108' (4.65 Mg·ha^{-1}) were significantly higher than the dry leaf yields of '1090' (3.64 Mg·ha^{-1}), the Candy (3.53 Mg·ha^{-1}), and 'SW 129' (3.28 Mg·ha^{-1}). Significant interactions of location by cultivar on leaf yield occurred at the first harvest and for the overall average yields. Averaged over harvest strategies, the cultivars 1049 (7.95 Mg·ha^{-1}) and SW 107 (7.41 Mg·ha^{-1}) grown at Ontario had the highest leaf yields. Although the stevia leaf yields reported here resulted from relatively brief production seasons, they are similar to those found in many studies (Fronza and Folegatti, 2003; Lavini et al., 2008; Moraes et al., 2013; Prieto et al., 2010; Shock 1982a, 1982b), greater than those reported by others (Behera et al., 2013; Serfaty et al., 2013; Vasilakoglou et al., 2016; Xu et al., 2013), but less than those observed by Aladakatti et al. (2012) in India over a long growing season with five harvests per year.

Leaf steviol glycoside content. The predominant steviol glycosides in the leaves were rebaudioside A, stevioside, and rebaudioside C, with small amounts of other steviol glycosides. Leaf steviol glycoside content had few significant differences among locations except for stevioside (Tables 4–6). For the first harvest, leaf stevioside content was significantly higher at Indio (5.49%) than at Ontario (4.61%) and Yuma (4.96%), but not at Hanford (5.02%). For the second harvest, there were no significant differences in leaf steviol glycoside content between locations. For the single harvest, leaf stevioside content was significantly higher at Ontario (4.11%) and Indio (4.09%) than at Hanford (3.33%) or Yuma (2.8%). There were no significant interactions between locations and cultivars in leaf steviol glycoside content.

Table 6. *Stevia rebaudiana* leaf yield, steviol glycoside content, and steviol glycoside yield from the single harvest at four locations in 2014.

		Single harvest								
		Yield	Stev	Reb A	Reb C	TSG	Stev	Reb A	Reb C	TSG
Location	Cultivar	Mg·ha^{-1}	%				kg·ha^{-1}			
Indio, CA	1090	3.72	3.06	9.85	1.07	14.12	105.1	352.1	40.1	500.3
	1108	5.45	5.13	5.49	1.35	12.15	264.7	298.8	73.3	643.6
	SW 107	4.33	4.13	8.35	1.15	14.22	174.0	354.6	47.7	601.4
	SW 129	3.32	1.27	10.68	1.10	13.69	42.7	355.8	36.7	457.6
	SW 201	4.65	3.71	8.99	1.20	14.00	166.3	406.2	57.4	635.5
	Candy	4.46	7.26	4.37	1.08	12.99	313.1	203.5	50.9	581.4
	Average	4.32	4.09	7.96	1.16	13.53	177.6	328.5	51.0	570.0
Yuma, AZ	1090	2.34	2.17	7.84	1.21	11.66	50.8	171.9	26.4	259.9
	1108	4.14	4.17	4.90	1.03	10.25	176.1	202.2	41.9	426.8
	SW 107	4.49	2.57	8.35	1.14	12.30	114.8	376.1	51.9	554.1
	SW 129	2.02	1.22	11.25	1.19	13.95	24.1	222.1	22.1	272.9
	SW 201	5.00	2.58	8.67	1.14	12.70	127.4	427.7	57.5	628.2
	Candy	2.94	4.10	6.15	0.98	11.49	121.6	170.9	27.3	329.7
	Average	3.49	2.80	7.86	1.11	12.06	102.5	261.8	37.9	411.9
Hanford, CA	1049	4.09	4.87	5.38	0.87	11.28	201.2	223.2	36.4	468.0
	1090	3.72	1.87	8.70	0.94	11.71	69.6	323.2	34.9	435.4
	1108	3.85	3.78	6.10	1.02	11.13	141.4	231.9	38.5	419.8
	SW 107	3.78	3.48	7.19	0.90	11.84	123.9	282.2	34.3	452.7
	SW 129	2.60	0.92	11.29	1.03	13.96	23.5	290.3	26.6	359.2
	Candy	2.56	5.05	3.75	0.74	9.81	123.2	99.5	18.7	248.3
	Average	3.43	3.33	7.07	0.92	11.62	113.8	241.7	31.6	397.2
Ontario, OR	1049	8.82	6.15	4.69	0.68	11.71	538.6	409.2	58.4	1022.4
	1090	4.80	3.07	7.08	0.60	10.96	146.7	329.5	30.8	519.5
	1108	5.14	5.03	3.32	0.48	8.82	258.6	164.0	24.2	446.8
	SW 107	7.42	4.69	6.12	0.69	11.81	353.6	460.2	52.8	890.3
	SW 129	5.20	1.28	9.33	0.60	11.44	67.6	478.8	32.4	593.6
	SW 201	5.07	3.99	7.51	0.76	12.26	220.7	413.6	42.2	676.5
	Candy	4.16	4.59	3.23	0.41	8.27	189.7	135.0	16.7	343.0
	Average	5.80	4.11	5.90	0.60	10.75	253.6	341.5	36.8	641.7
Averages across locations	1049	6.46	5.51	5.04	0.78	11.49	369.9	316.2	47.4	745.2
	1090	3.64	2.54	8.37	0.95	12.11	93.0	294.1	33.0	428.8
	1108	4.65	4.53	4.95	0.97	10.59	210.2	224.2	44.5	484.3
	SW 107	5.01	3.72	7.50	0.97	12.54	191.6	368.2	46.7	624.6
	SW 129	3.28	1.17	10.64	0.98	13.26	39.5	336.7	29.5	420.8
	SW 201	4.91	3.43	8.39	1.03	12.99	171.4	415.8	52.4	646.8
	Candy	3.53	5.25	4.38	0.80	10.64	186.9	152.2	28.4	375.6
Overall average		4.16	3.47	6.87	0.90	11.48	159.2	283.9	37.7	491.0
LSD (0.05), location		NS	0.48	NS	NS	NS	61.6	NS	NS	NS
LSD (0.05), cultivar		0.90	0.56	0.83	0.10	1.03	39.2	69.3	8.6	108
LSD (0.05), location × cultivar		NS	NS	NS	NS	NS	78.4	NS	17.3	216

Stev = stevioside; Reb A = rebaudioside A; Reb C = rebaudioside C; TSG = total steviol glycosides; LSD = least significant differences; NS = nonsignificant.

Table 7. *Stevia rebaudiana* leaf yield in response to harvest strategy for cultivars grown at four locations in 2014.

		Harvest strategy, yield		
Location	Cultivar	First + second harvest	Single harvest	Avg of harvest strategies
Indio, CA	1090	3.69	3.72	3.70
	1108	4.82	5.45	5.14
	SW 107	4.10	4.33	4.22
	SW 129	2.26	3.32	2.79
	SW 201	4.16	4.65	4.40
	Candy	3.65	4.46	4.05
	Average	3.78	4.32	4.05
Yuma, AZ	1090	2.62	2.34	2.48
	1108	2.83	4.14	3.48
	SW 107	4.02	4.49	4.26
	SW 129	1.95	2.02	1.98
	SW 201	4.10	5.00	4.55
	Candy	2.24	2.94	2.59
	Average	2.96	3.49	3.22
Hanford, CA	1049	4.40	4.09	4.25
	1090	3.88	3.72	3.80
	1108	4.62	3.85	4.24
	SW 107	4.91	3.78	4.34
	SW 129	2.78	2.60	2.69
	Candy	3.51	2.56	3.04
	Average	4.02	3.43	3.73
Ontario, OR	1049	7.08	8.82	7.95
	1090	5.36	4.80	5.08
	1108	5.34	5.14	5.24
	SW 107	7.40	7.42	7.41
	SW 129	6.41	5.20	5.81
	SW 201	4.79	5.07	4.93
	Candy	5.33	4.16	4.74
	Average	5.96	5.80	5.88
Averages across locations	1049	5.74	6.46	6.10
	1090	3.89	3.64	3.77
	1108	4.40	4.65	4.52
	SW 107	5.11	5.01	5.06
	SW 129	3.35	3.28	3.32
	SW 201	4.35	4.91	4.63
	Candy	3.68	3.53	3.61
Overall average		4.09	4.16	4.12
LSD (0.05), location			NS	0.90
LSD (0.05), cultivar			0.90	0.63
LSD (0.05), location × cultivar			NS	1.26
LSD (0.05), harvest strategy		NS		
LSD (0.05), location × harvest strategy		NS		
LSD (0.05), cultivar × harvest strategy		NS		
LSD (0.05), location × cultivar × strategy		NS		

LSD = least significant differences; NS = nonsignificant.

Cultivars had significant differences in leaf steviol glycoside content at all harvests (Tables 4–6). 'SW 129' had significantly lower stevioside content compared with the other cultivars at each harvest. 'SW 129' had significantly higher rebaudioside A content than the other cultivars with the single harvest strategy, and had the highest rebaudioside A content at the first and second harvest. At the first harvest, rebaudioside A content of 'SW 129' (13.58%) was similar to 'SW 201' (12.63%), and significantly greater than that of '1090', 'SW 107', the 'Candy', '1108', and '1049', at 11.04%, 10.92%, 6.85%, 6.46%, and 6.34%, respectively. At the second harvest, 'SW 129' rebaudioside A content (8.52%) was similar to that of line '1090' (7.75%) and 'SW 107' (7.49%), and significantly greater than that of 'SW 201', '1049', '1108', and the 'Candy', at 7.43%, 4.30%, 4.01%, and 3.25%, respectively. When comparing harvest strategies of two vs. one harvest, there were no significant differences in steviol glycoside content between the harvest strategies, in the interaction of location-by-harvest strategy, in the interaction of cultivar-by-harvest strategy, or even the interaction of location-by-cultivar-by-harvest strategy (Table 7).

The cultivars 'SW 107', 'SW 129', and 'SW 201', and the line '1090' consistently contained more rebaudioside A than stevioside, while the other plant materials had roughly similar amounts of rebaudioside A and stevioside. These plant compositions are similar to those reported by Xu et al. (2013) in China, but differ from the plant composition in many other trials where there was substantially more stevioside than rebaudioside (Behera et al., 2013; Moraes et al., 2013; Pal et al., 2015; Serfaty et al., 2013; Vasilakoglou et al., 2016).

Steviol glycoside yields. Steviol glycoside yields were most often influenced by cultivar and interactions of locations by cultivar (data not shown except for rebaudioside A, Table 8). Harvest strategy as used in these trials was never a significant factor in any of the individual or total steviol glycoside yields (data not shown except for rebaudioside A, Table 8). Over all locations, cultivars, and harvest strategies, rebaudioside A yield averaged 300 kg·ha^{-1} with significant differences by cultivar and differences by interactions of location with cultivar. Average rebaudioside A yields greater than 500 kg·ha^{-1} were observed at Ontario. The 554.2 kg·ha^{-1} of rebaudioside A produced by 'SW 129' at Ontario was statistically greater than any other combination of location and cultivar except for 'SW 107' that yielded 525.3 kg·ha^{-1} at Ontario, and 'SW 201' that produced 456 kg·ha^{-1} of rebaudioside A at Indio. Averaged over all locations and harvest strategies, 'SW 201' and 'SW 107' had the highest rebaudioside A yields, 444.8 kg·ha^{-1} and 413.8 kg·ha^{-1}, respectively. The Candy cultivar had by far the lowest rebaudioside A yield, averaging only 161.5 kg·ha^{-1}.

Few trials achieved the high amounts of steviol glycosides as did these trials. Xu et al. (2013) recorded up to 287 kg·ha^{-1} of rebaudioside A. The best results of Moraes et al. (2013) were 398.8 kg·ha^{-1} of rebaudioside A and 512.2 kg·ha^{-1} of stevioside. Their results, although similar to the current trial, had plant composition with a less favorable ratio of rebaudioside A to stevioside. Among the highest rebaudioside A yield in the current trial were 'SW 107' (525.3 kg·ha^{-1}) and 'SW 129' (554.2 kg·ha^{-1}) at Ontario, given the significant interaction of cultivar with location.

Previous cultivar performance. Cultivar SW 107 was selected for vigor and its total steviol glycoside content, 'SW 129' was selected principally for its rebaudioside A content and high ratio of rebaudioside A to stevioside, and 'SW 201' was selected for its leaf flavor (Shock and Parris, 2015, 2016a, 2016b). All of the six new lines tested in this trial were judged to have less bitterness and aftertaste than the plant population from which they were selected. The cultivar Candy was convenient to include since it could be started from seed, whereas the other cultivars had the inconvenience of requiring vegetative propagation by cuttings.

Harvest strategies. In both Mississippi and Israel, a single harvest produced more leaf yield and steviol glycosides than two or three annual cuttings, results were similar to this trial (Moraes et al., 2013; Serfaty et al., 2013). In India, however, three harvests per year produced the greatest leaf yield, and two harvests per year resulted in higher steviol glycoside content (Pal et al., 2015). In the current trial, one or two harvests per season were not significant factors in leaf yield or rebaudioside A yield.

Commercial considerations. Practical considerations for commercial plantings of stevia go considerably beyond leaf and steviol glycoside productivity reported here; costs and product value were not studied in this trial. Results from single-year trials favored Ontario, but the stevia plantings at Hanford and Indio overwintered and could be productive for

Table 8. *Stevia rebaudiana* leaf rebaudioside A yield (kg·ha^{-1}) in response to harvest strategy at four locations in 2014.

	Harvest strategy, rebaudioside A yield			
Location	Cultivar	First + second harvest	Single harvest	Avg of harvest strategies
Indio, CA	1090	412.9	352.1	382.5
	1108	269.2	298.8	284.0
	SW 107	387.1	354.6	370.8
	SW 129	260.9	355.8	308.4
	SW 201	505.8	406.2	456.0
	Candy	197.1	203.5	200.3
	Average	338.9	328.5	333.7
Yuma, AZ	1090	204.3	171.9	188.1
	1108	170.4	202.2	186.3
	SW 107	390.8	376.1	383.4
	SW 129	187.8	222.1	205.0
	SW 201	441.4	427.7	434.6
	Candy	138.2	170.9	154.6
	Average	255.5	261.8	258.7
Hanford, CA	1049	272.4	223.2	247.8
	1090	396.9	323.1	360.0
	1108	263.6	231.9	247.8
	SW 107	469.3	282.2	375.7
	SW 129	355.9	290.3	323.1
	Candy	179.7	99.5	139.6
	Average	323.0	241.7	282.3
Ontario, OR.	1049	283.6	409.2	346.4
	1090	407.6	329.5	368.6
	1108	181.6	164.0	172.8
	SW 107	590.4	460.2	525.3
	SW 129	629.5	478.8	554.2
	SW 201	474.1	413.6	443.8
	Candy	168.1	135.0	151.5
	Average	390.7	341.5	366.1
Yield, overall locations	1049	278.0	316.2	297.1
	1090	355.4	294.1	324.8
	1108	221.2	224.2	222.7
	SW 107	459.4	368.2	413.8
	SW 129	358.6	336.7	347.6
	SW 201	473.8	415.8	444.8
	Candy	170.8	152.2	161.5
Yield average, overall		316.9	283.9	300.4
LSD (0.05), location		NS	NS	NS
LSD (0.05), cultivar		62.9	69.3	53.2
LSD (0.05), location × cultivar		126	NS	106
LSD (0.05), harvest strategy			NS	
LSD (0.05), location × harvest strategy			NS	
LSD (0.05), cultivar × harvest strategy			NS	
LSD (0.05), location × cultivar × strategy			NS	

LSD = least significant differences; NS = nonsignificant.

a second year or multiple years, as has been observed for stevia in tropical, subtropical, and Mediterranean climates: Paraguay (Prieto et al., 2010), India, Mississippi (Moraes et al., 2013), and Italy (Lavini et al., 2008). The planting at Yuma did not persist well because of root diseases that may have been aggravated by cycles of more extreme wetting and drying than at Indio and Hanford. The stevia at Ontario was killed as expected by cold winter temperatures. The plantings at both Indio and Hanford persisted well during a second year of production, and second year outcomes are not reported here.

Conclusions

Averaged over the four western U.S. locations, seven cultivars, and two harvest strategies, stevia yielded 4.12 Mg·ha^{-1} of dry leaf over about a 5-month season from planting to harvest. Dry leaf yields at Ontario, 5.88 Mg·ha^{-1}, were greater than the other three locations. Two cultivars at Ontario exceeded 7 Mg·ha^{-1}. Rebaudioside A was the predominant steviol glycoside in four of the seven cultivars tested. Rebaudioside A in excess of 500 kg·ha^{-1} and total steviol glycosides in excess of 1000 kg·ha^{-1} were achieved with specific combinations of cultivars and locations. Harvesting stevia either once or twice during the growing season at 20-cm height had little effect on the total annual leaf yield or steviol glycoside yields in these trials.

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