

Influence of Environmental Parameters, Pinching, and Ethephon Application on Growth and Branching of Potted Stevia

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Abstract. *Stevia (Stevia rebaudiana)* is an herb grown commercially for the extraction of intensely sweet-tasting, non-caloric, steviol glycosides produced primarily in the leaves and used as a sugar substitute. While most stevia production occurs as an industrial field crop, more recently, consumer demand for stevia for home gardens and patio containers has increased. Research on how environmental inputs impact growth, branching, and flowering of stevia under greenhouse conditions for potted plant production is currently lacking. A series of experiments was conducted to quantify how methods to promote branching, fertilizer concentration, photoperiod and temperature impact branch production, growth and development, and flowering of stevia. Both manual decapitation and ethephon application increased lateral branch production, though hard pinching (cutting plants back to leave four nodes) yielded a more desirable plant architecture. Neither temperature nor fertilizer concentration impacted the number of branches produced by plants given a hard pinch. Shoot dry biomass was similar at fertilizer concentrations (applied at each watering) of 50, 100, and 200 mg·L⁻¹ N, but decreased at 300 or 400 mg·L⁻¹ N. Stevia responded to photoperiod as a facultative short-day plant, with earliest flowering occurring, both in days to flower and the number of nodes produced before flowering, at photoperiods <13 hours. The number of nodes produced on the longest branch increased as temperature increased from 17 to 26 °C. Plant height and longest branch length were shorter at 17 °C than at higher temperatures. The results of these studies indicate that for potted plant production, stevia should be grown under a photoperiod of 14 hours or longer with moderate nutrient levels, a minimum temperature of 20 °C, and plants should receive one or more manual pinches to promote branching.

Stevia (Stevia rebaudiana) is an herb grown for the extraction of sweet-tasting steviol glycosides from the leaves for use as a sugar substitute (Yadav et al., 2011). More recently, stevia has become a popular herb with home gardeners. However, cultural information for producing greenhouse-grown stevia as a transplant or potted plant is lacking. As with many herbs grown for commercial sales in pots, it is desirable to produce a full, well-branched plant that is sold during the vegetative stage of development.

Maintaining vegetative development is desirable for stevia, as flowering reduces plant growth and steviol glycoside concentrations (Bondarev et al., 2003), and because the senesced inflorescences do not abscise, reducing visual appeal. Flowering of stevia is photoperiod-sensitive, and stevia has been categorized as a short-day plant (Valio and Rocha, 1977).

Stevia vegetative growth and morphological responses to irradiance and nutrient levels are only partially understood, and information on the influence of temperature on stevia growth and development in controlled environments is lacking. Increasing daily light integral generally decreased plant height and individual leaf area (Evans et al., 2015). For field-grown plants, increasing nitrogen (N) application rate from 30 to 90 kg·ha⁻¹ (the highest level evaluated) increased branch number, dry leaf yield, and dry stem yield (Pal et al., 2015). Ucar et al. (2018) determined that increasing N application rate from 0 to 100 kg·ha⁻¹ increased dry leaf yield, but further increasing N application rate to 150 or 200 kg·ha⁻¹ did not further increase the yield. However, these studies have primarily been conducted on field-grown plants and cannot be directly applied to greenhouse production

in soilless media with continuous fertigation using water-soluble fertilizers.

Lateral branch production is critical for producing attractive potted plants, particularly for plants such as stevia where lateral branching is strongly inhibited during the young plant stage. Promotion of axillary meristem release from apical dominance is often achieved through shoot decapitation, or “pinching” (Larson, 1985), or by application of chemical growth regulators, including benzyladenine (Latimer and Freeborn, 2010; Rezzazadeh et al., 2015), dikegulac sodium (Grossman et al., 2013; Sun et al., 2015), and ethephon (Currey and Erwin, 2012; Hayashi et al., 2001). Removal of apical buds of field-grown stevia plants increased branch number at harvest by 48% to 72% while foliar applications of KNO₃ and Ca(NO₃)₂ did not impact branch number (Pal et al., 2013).

Most research on stevia growth and development has been conducted either in field soils or under in vitro conditions. The objectives of the current work were to quantify the effects of temperature, fertilizer application rate, photoperiod and branching-induction method on branch production, growth, and development of stevia grown in soilless media under greenhouse conditions.

Materials and Methods

Plant materials and culture. A series of experiments were conducted to determine the effects of fertilizer application rate, photoperiod, branching-induction method, and temperature on growth, branching, and flowering of stevia. For all experiments, stevia synthetic cultivar MSU17-02 seed (derived from the free intercrossing of four parental lines) were sown in 128-cell (cell volume 12 mL) trays in soilless media containing 70% peatmoss, 21% vermiculite, and 9% perlite (Suremix; Michigan Grower Products, Galesburg, MI) and placed in a greenhouse compartment under intermittent mist at 23.1 ± 0.9 °C (24-h average temperature ± SD) for seed germination. After 14 d, seedling trays were removed from mist and grown on capillary matting in a greenhouse at 23 °C under a 16-h photoperiod, provided by ambient irradiance plus 85 ± 15 μmol·m⁻²·s⁻¹ photosynthetic photon flux density (400–700 nm) at plant level provided by high-pressure sodium lamps when outdoor irradiance dropped below 440 μmol·m⁻²·s⁻¹ between 0600 and 2200 HR, and irrigated as needed by saturating the capillary matting. When plants had developed ca. four nodes, they were transplanted into 10-cm round plastic containers (480-mL volume) filled with the same medium and grown under the same greenhouse conditions. After transplanting, plants were irrigated as needed with reverse osmosis treated water supplemented with (mg·L⁻¹): 125 nitrogen, 13 phosphorus, 125 potassium, 15 calcium, 1 iron, 0.5 manganese, zinc and copper, and 0.1 boron and molybdenum (MSU Special; GreenCare Fertilizers, Kankakee, IL). For the photoperiod experiment (Expt. 3), plants were immediately placed into treatments after transplanting. For the three other experiments, plants were moved

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Table 1. Effect of manual decapitation (soft pinch and hard pinch) or ethephon application on lateral branch number, length of the longest branch, number of nodes on the longest branch, and plant height of *Stevia rebaudiana* (n = 20).

Treatment	Branches (no.)	Longest branch (cm)	Nodes on longest branch (no.)	Ht (cm)
Control	0.2 a ^z	4.0 a	2.7 a	46.6 b
Soft pinch	1.6 b	28.8 c	13.2 c	45.7 b
Hard pinch	2.2 b	26.3 c	13.8 c	30.8 a
Ethephon				
250 mg·L ⁻¹	1.0 ab	3.9 a	3.2 a	34.3 ab
500 mg·L ⁻¹	4.0 b	10.0 b	9.0 b	32.2 a
750 mg·L ⁻¹	1.7 b	10.4 b	6.8 ab	35.1 ab
Significance	**	***	***	***

^zValues followed by different letters within a column represent statistically significant differences ($P \leq 0.05$) based on Tukey's honestly significant difference.

, *Significant at $P < 0.01$ or 0.001 , respectively.

into treatments when seedlings had an average of eight nodes.

Expt. 1. Methods to promote lateral branching. Plants were grown at 23 °C under a 16-h photoperiod as described above. Shielded Type E thermocouples (Omega Engineering, Stamford, CT) connected to a data logger (CR1000; Campbell Scientific, Logan, UT) recorded the air temperature in each compartment every minute, and hourly averages were stored. Actual air temperatures were (24-h average \pm sd) 22.6 ± 1.2 °C. At the initiation of the experiment, plants received either manual decapitation (soft or hard pinch) at the initiation of the experiment or were treated with spray applications of ethephon at 0 (control), 250, 500, or 750 mg·L⁻¹ of ethephon (Florel; Monterey Lawn and Garden, Fresno, CA) on day 1 and again on day 15 of the experiment. A soft pinch consisted of removing the shoot apex and partially unfolded leaves, leaving seven nodes. A hard pinch consisted of cutting plants back to leave only the first four nodes. After 42 d, the number of lateral branches >4 cm, length of the longest branch, number of nodes on the longest branch, and plant height (from the media surface to the highest point) were determined.

Expt. 2. Fertilizer rate responses. Plants were cut back to four nodes at the initiation of the experiment and grown in a greenhouse compartment under the light and temperature conditions described for Expt. 1. Plants were irrigated with reverse osmosis water supplemented with the MSU Special fertilizer blend described above with rates adjusted to provide 50, 100, 200, 300, or 400 mg·L⁻¹ N (with proportional concentrations of the other nutrients in the fertilizer blend). After 42 d in treatments, the number of lateral branches >4 cm, length of the longest branch, number of nodes

on the longest branch, and plant height were determined. Plants were then cut at the media line, and whole shoot biomass was determined after drying plant material at 70 °C for 72 h.

Expt. 3. Photoperiod responses. Plants were grown under a 9-h photoperiod of ambient irradiance, supplemented with high-pressure sodium lamps as described above. Plants were covered with blackout curtains daily from 1700 to 0800 HR. Daylengths were extended to the desired photoperiod with 3 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR (400–700 nm) provided by incandescent lamps beginning at 1700 HR. When the first flower opened, the date, the number of nodes below the first flower, and plant height were recorded.

Expt. 4. Temperature responses. Cuttings were cut back to four nodes and placed in glass-covered greenhouse compartments set to 17, 20, 23, or 26 °C under a 16-h photoperiod as described above. Actual air temperatures were 17.3 ± 1.5 , 20.8 ± 1.0 , 22.6 ± 1.4 , and 25.8 ± 1.9 °C. After 42 d in temperature treatments, the number of nodes on the longest branch, the length of the longest branch, branch number, and plant height were determined. Development rate was calculated by dividing node number on the primary branch by 42 d and is expressed in nodes/day.

Statistical analysis. Expts. 1–3 employed a completely randomized complete block design with 10 plants per treatment in each of two blocks. Expt. 4 consisted of two blocks of 10 plants each within each temperature compartment. Data were analyzed in SPSS (IBM SPSS Statistics 27; IBM, Chicago, IL). Mean separation was conducted using Tukey's honestly significant difference. Block was not significant for any variable, so data from both blocks were pooled for analysis. For Expt. 4, linear regression analysis was performed on development

rate (DRate) using the actual 24-h average daily temperatures (ADT), where $\text{DRate} = b_0 + b_1 \times \text{ADT}$. The base temperature (T_{base}) for DRate was determined by setting DRate to 0 nodes d⁻¹ and solving for ADT.

Results

Expt. 1. Branching induction technique influenced branch number, length of the longest branch, node numbers on the longest branch, and plant height (Table 1). Ethephon application at 500 mg·L⁻¹ resulted in the greatest number of branches. However, a soft or hard pinch resulted in the longest branches and greatest number of nodes on the longest branch. Branches induced by ethephon application developed higher on the primary shoot and tended to be short (Table 1). A hard pinch or application of 500 mg·L⁻¹ ethephon resulted in reduced plant height compared with control or soft pinch treatments.

Expt. 2. Fertilizer concentration >200 mg·L⁻¹ N reduced shoot dry biomass compared with lower concentrations (Table 2). Similarly, plant height was greater at 50 mg·L⁻¹ N than at 300 or 400 mg·L⁻¹ N. Branch number, length of the longest branch, and the number of nodes on the longest branch were not impacted by fertilizer concentration.

Expt. 3. Stevia exhibited a facultative short-day response to photoperiod for flowering. Plants flowered earliest in time when photoperiod was 12 h or less, latest under 14 h, and intermediate under a 13-h photoperiod (Table 3). Node number at first flowering and plant height at flowering were greater under a 14-h photoperiod compared with the other photoperiod treatments, but similar among the photoperiods <14 h.

Expt. 4. Temperature influenced plant height, length of the longest branch, and node number on the longest branch, but not the number of branches produced (Table 4). Plant height was shorter at 17 °C compared with other temperatures. Similarly, the length of the longest branch was shortest at 17 °C compared with other temperatures. Vegetative development rate (in nodes/day) on the longest branch was greatest at 26 °C, lowest at 17 °C, and intermediate at 20 and 23 °C. The response of development rate to temperature fit a linear response with a regression equation of $\text{DRate} = -0.116 + 0.017 \times \text{Temperature (°C)}$ ($P \leq 0.001$; $R^2 = 0.497$). From this linear equation, the base temperature for stevia development is calculated as 6.8 °C.

Table 2. Influence of nitrogen concentration on shoot biomass production, length of the longest branch, node number on the longest branch, and plant height of *Stevia rebaudiana* (n = 20).

N (mg·L ⁻¹)	Shoot dry biomass (mg)	Branches (no.)	Ht (cm)	Longest branch (cm)	Nodes on longest branch (no.)
50	281 b ^z	3.2	34.9 b	29.3	12.6
100	249 ab	2.3	32.3 ab	25.8	10.1
200	305 b	2.2	30.6 ab	24.4	12.3
300	203 a	2.7	23.8 a	20.5	11.1
400	200 a	2.9	25.9 a	21.6	11.9
Significance	**	NS	*	NS	NS

^zValues followed by different letters within a column represent statistically significant differences ($P \leq 0.05$) based on Tukey's honestly significant difference.

NS, *, **Nonsignificant or significant at $P < 0.05$ or 0.01 , respectively.

Table 3. Influence of photoperiod on time to flower, node number below the first inflorescence and plant height at flowering for *Stevia rebaudiana* (n = 20).

Photoperiod (h)	Time to flower (d)	Nodes (no.)	Ht (cm)
9	26.5 a ²	12.6 a	43.5 a
10	26.5 a	11.8 a	46.5 a
11	27.5 a	12.5 a	50.7 a
12	28.8 a	13.6 a	51.8 a
13	32.5 b	13.0 a	51.9 a
14	46.8 c	20.8 b	73.6 b
Significance	***	***	***

²Values followed by different letters within a column represent statistically significant differences ($P \leq 0.05$) based on Tukey's honestly significant difference.

***Significant at $P < 0.001$.

Discussion

The results presented here provide insight into crop management strategies for potted plant production of stevia. For commercial sales, potted stevia should be vigorous, well-branched, and sold during the vegetative phase of development. Stevia seedlings and rooted vegetative cuttings exhibit strong apical dominance during the early developmental period that would coincide with potted plant production (R. Warner, personal observation). Decapitation of primary and secondary apical buds of field-grown stevia 45 and 75 d after transplant increased branch numbers by 48 and 72% in a 2-year study (Pal et al., 2013). In the current study, both manual decapitation and ethephon application at 500 or 750 mg·L⁻¹ increased lateral branch production. However, branches induced by ethephon application were shorter than those induced by decapitation (Table 1) and emerged from the middle of the canopy, resulting in undesirable plant architecture. While soft and hard pinches produced similar numbers of lateral shoots, a hard pinch produced a more desirable plant architecture. For both pinching treatments, lateral branches emerged from nodes just below the decapitation point. Therefore, a soft pinch resulted in branching higher in the plant canopy while leaving several centimeters of a single stem at the base of the plant, while the hard pinch resulted in branching much closer to the media line. A soft pinch may be effective if performed on younger plants. Otherwise, pinching plants back to 3–4 nodes is recommended. Lateral branch production by stevia grown in the field exhibited considerable variability across genotypes (Huber and Wehner, 2021; Vallejo and Warner, 2021). Therefore, screening more germplasm may aid in identifying genotypes that produce

lateral branches earlier in development that are suitable for greenhouse production.

Previous studies evaluating nutritional requirements for stevia have focused on field production and pre-plant soil-incorporated fertilizers (Karimi and Moradi, 2018; Pal et al., 2015; Rashid et al., 2013; Ucar et al., 2018). Information on appropriate levels of water-soluble fertilizer for continuous fertigation of stevia produced in soilless media is not available. The results presented here indicate that stevia has a relatively low nutritional requirement, with biomass production decreasing as N concentration exceeded 200 mg·L⁻¹ N (Table 1). In a greenhouse study of stevia grown in field soil, Karimi and Moradi (2018) reported that as incorporated urea rate increased from 0 to 30 or 60 kg·ha⁻¹, plant height, and leaf and stem dry mass increased. However, leaf and stem dry mass were lower at 150 kg·ha⁻¹ urea compared with 60 kg·ha⁻¹. Ucar et al. (2018) reported similarly that as N application rate increased from 0 to 100 kg·ha⁻¹, fresh and dry leaf yield and total biomass yield of field-grown stevia increased, but further increasing N to 150 or 200 kg·ha⁻¹ did not further increase any of these yield parameters. Fertilizer concentration did not impact branch number in this study. Ucar et al. (2018) reported branch numbers were similar for N application rates of 0, 50, 100, or 150 kg·ha⁻¹ but branch number was higher at 100 than 200 kg·ha⁻¹ N.

Stevia responded to photoperiod as a facultative short-day plant for flowering. Valio and Rocha (1977) initiated photoperiod treatments at two stages of plant development, 4–5 nodes or 10–12 nodes, with plants growing under continuous light up to those stages. In both cases, photoperiods of 14 h or longer inhibited flowering for the duration of the experiment, which was 65 d for the 4–5 node plants, and 50 d for the 10–12 node plants. In contrast, plants in the current study did flower

under a 14-h photoperiod, though significantly later than under shorter photoperiods (Table 2). Valio and Rocha did not report the temperature that plants were grown under, or the number of nodes produced during the experiment or at flowering. Therefore, it is not possible to directly compare the results of plants grown at 14-h photoperiod, or to know if those plants would have eventually flowered if the duration of the experiments was increased. Regardless, our results support the previous findings that the critical photoperiod for stevia appears to be between 12 and 13 h.

The calculated T_{base} for stevia development rate of 6.8 °C is intermediate to T_{base} values for development rates of other greenhouse crops (Blanchard and Runkle, 2011; Warner, 2010). Blanchard and Runkle (2011) determined T_{base} for progress toward flowering of 17 species of annual bedding plants, with values ranging from 1.1 °C for *Tagetes patula* 'Janie Flame' to 9.9 °C for *Angelonia angustifolia* 'Serena Purple'. Cultivars within the same species can also exhibit considerable variation in T_{base} for developmental responses. For example, T_{base} for rate of progress toward flowering on 20 *Petunia* cultivars varied from 0.15 °C for 'Damask Purple' to 7.1 °C for 'Wave Purple' (Warner, 2020).

Studies on the influence of temperature on stevia are limited to seed germination and early seedling growth. Seed germination percentage increased as temperature increased from 15 to 25 °C (Shahverdi et al., 2019; Ucar et al., 2016). Further increasing temperature from 25 to 30 °C reduced germination percentage and increased time to germination, suggesting that the optimal temperature (T_{opt}) for germination is around 25 °C. T_{opt} for rate of progress toward flowering varied from 19.1 °C for *Dahlia* 'Figaro Mix' to >30 °C (the highest temperature evaluated) for five of the 17 species evaluated (Blanchard and Runkle, 2011). In the current study, development rate increased following a linear function between 17 and 26 °C, indicating that T_{opt} for vegetative development rate is greater than 26 °C and stevia should be grouped with other warm-tolerant species for greenhouse production.

The results presented here indicate that stevia should be grown under a photoperiod of greater than 14 h to delay or prevent undesired flowering. Given that manual decapitation produced better lateral outgrowth than ethephon applications, but still only produced about two branches from the axillary meristems directly subtending the pinch point, either multiple pinches may be necessary, or multiple plants per pot may be needed to produce commercially viable potted stevia. Stevia has modest nutrient requirements, and fertigation solutions should not exceed 200 mg·L⁻¹ N for continuous feed programs. Additionally, stevia growth is tolerant of warm temperatures and temperatures less than 20 °C should be avoided, as growth was severely compromised.

Table 4. Influence of air temperature on plant height, lateral branch production, length of the longest branch, and the number of nodes on the longest branch of *Stevia rebaudiana* (n = 20).

Temperature (°C)	Ht (cm)	Branches (no.)	Longest branch (cm)	Development rate (nodes/day)
17	15.7 a ²	2.3	10.0 a	0.164 a
20	33.5 b	2.5	27.9 b	0.238 b
23	26.8 b	2.6	21.8 b	0.236 b
26	32.3 b	2.1	27.9 b	0.333 c
Significance	***	NS	***	***

²Values followed by different letters within a column represent statistically significant differences ($P \leq 0.05$) based on Tukey's honestly significant difference.

NS, ***Nonsignificant or significant at $P < 0.001$, respectively.

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