Winter Hardiness and Spring Regrowth of Four Varieties of *Stevia rebaudiana* (Bertoni) in Eastern North Carolina

Carlette Sykes Coggin

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Dr. Gregory E. Welbaum, School of Plant and Environmental Sciences

Dr. Maria Balota, School of Plant and Environmental Sciences

Dr. Wade Thomason, School of Plant and Environmental Sciences

Virginia Polytechnic Institute and State University

Blacksburg, VA 24016

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Contents

Abstract

Stevia (*Stevia rebaudiana*) is attracting attention in the United States in response to consumer demand for healthier low-calorie sweeteners. Farmers are looking at stevia as a valuable alternative crop to add to their rotations not only for its high value but because it is perennial and can be repeatedly harvested reducing annual establishment costs. S&W Seed Company is testing stevia varieties across the southeastern United States at locations in North and South Carolina, Georgia, and Alabama. This study evaluated four stevia varieties grown at two locations near Nashville and Roseboro in North Carolina. S&W is focused on developing cultivars suited to the climate of the southeastern United States. Research was conducted for eight months in fields near Nashville and Roseboro North Carolina to assess winter survival, spring regrowth, and leaves per stem count of four top performing varieties in plots contracted by S&W with local farmers. All four varieties overwintered without significant winter kill and spring regrowth began from crowns in April and May. Weed interference in the Nashville location negatively affected percentage of regrowth in the spring. In Roseboro with lower weed pressure, regrowth occurred a month earlier with increases of 43% for SW-1005, 50% for SW-2267, and 114% for SW-2647. SW-2427 had no difference in percentage of survival between locations. Plants in Roseboro had greater mean plant heights and leaves per stem. Stevia can be grown as an alternative perennial crop in coastal North Carolina although there was significant winter die back and spring regrowth was slow.

Literature Review

Stevia (*Stevia rebaudiana*), also commonly called candyleaf, belongs to the Asteraceae (aka Compositae) family, which includes many diverse species such as lettuce, daises, and sunflowers (Gantait, Das, & Banerjee, 2018). It was discovered in its native country of Paraguay by Dr. Moisés S. Bertoni in 1901, and has been the focus of many studies into its use as a non-caloric sugar alternative (Kinghorn & Hardman, 2002). Along the Ypane River in Paraguay, stevia grows in sandy, infertile, acidic soils. Stevia does not compete well with weeds, grow well in saline soils, or tolerate drought. Stevia is a perennial herb reaching a height of up to 3 feet if there is no weed competition (Shock, 1982). Stevia growth habit is bushy with heavy branching becoming semi-woody with age (Ramesh, Singh, & Megeji, 2006). Flowering is induced by short days in the late summer though fall whereas greater leaf and steviosides production occur during long days (Shock, 1982). Flowers on stevia plants are self-incompatible and most likely cross-pollinated by insects (Ramesh, Singh, & Megeji, 2006).

Germination from dispersed seed tends to be poor especially in cool weather (Shock, 1982). Stevia seeds and plants collected by Dr. Clinton Shock in Paraguay by the Ypane River were used for a study on germination and overwintering survival at the University of California - Davis (Shock, 1982). The seeds were planted in the greenhouse, and most germination occurred on the sixth day but the total germination percentage was low. Two hundred plants were transplanted into a field and only 18 lines survived the moderate winter in the southern Sacramento Valley Northwest of San Francisco. One line remained green through the winter after a light frost while others died back to the crown. The crowns began to regrow in March but accelerated growth was not observed until

April. Transplants were used for field establishment because of poor seed germination, slow seedling growth, and to reduce competition with weeds (Shock, 1982).

The realization by Bertoni that stevia leaves taste sweet led early researchers to discover many sweetening compounds (steviol glycosides) including: stevioside, rebaudiosides A – E, dulcoside, and steviolbioside. In fact, it is the only plant in the Stevia genus that produces high concentrations of sweetening compounds (Kinghorn & Hardman, 2002). Steviol glycosides can be at least 300 times sweeter than sucrose and make up 3-10% of dry leaf weight (Shock, 1982). Stevioside, although 100-300 times sweeter than sucrose depending upon concentration, leaves a bitter aftertaste. Rebaudiosides, however, display a range of sweetness 50-450 times that of sucrose but do not leave the bitter aftertaste of stevioside. The most widely used rebaudioside is Rebaudioside A, which has the most pleasant taste and is more water soluble than other compounds (Kinghorn & Hardman, 2002). Greater leaf to stem ratios are desirable for the crop since the leaves contain these sweetening compounds (Ramesh, Singh, & Megeji, 2006). One focus of the S&W Seed Company's breeding program is to enhance the levels of other rebaudioside molecules in stevia to improve flavor of sweetening molecules (Klingenberg, 2019).

Stevia has been used medicinally in many countries across Central America to treat a variety of conditions from pain, inflammation, burns, bites, scratches, colds, fevers, and even as a contraceptive and in baths for women after childbirth (Kinghorn & Hardman, 2002). Stevioside sweeteners became popular in the mid-1970s in Japan when synthetic sweeteners were banned. Stevioside sweeteners retained nearly half of the sweetener market in Japan until the late 1980s when aspartame, an artificial on-saccharide sweetener 200 times sweeter than sucrose, was developed. The United States and the European Union were not as receptive to stevioside sweeteners as Japan. In fact, importation of stevia leaves was banned by the United States until 1991. The Dietary Supplement Health and Education Act was passed in 1994 enabled the import and use of stevia in the US. At that time, stevia leaves and extract were only permitted for import if they were labeled as a Dietary Supplement (Kinghorn & Hardman, 2002). In 2008, stevia was approved by the US Food and Drug Administration as a food sweetener (Hart, 2014).

Alternative sweeteners such as stevia are trending in the United States in response to obesity and other health problems associated with the increased use of sugar in recent years. America ranks 6th for per capita sugar intake at 130 pounds of sugar per person every year. In the last twenty years, the percentage of obese adults in the United States has doubled from 15% to 30% with the number of severely obese adults tripling since 1986 (Welbaum & Lambert, 2018). Children are increasingly obese and may not outlive their parents. The annual health-care cost in America for obesity and its associated health issues is $147 billion, which is more than the cost of health issues associated with smoking. Health costs for obesity have risen 25% in the last 15 years. Obesity is the underlying factor in heart disease and an important factor causing diabetes type 2. Americans have increased their rate of snacking with high sugar foods between meals by 240% in the last twenty years (Welbaum & Lambert, 2018).

Stevia appeals to tobacco farmers as a rotational crop because some of the same equipment can be used for production. Stevia seeds are started in the same float trays used to grow tobacco transplants, which are set in the field when they are 8-12 weeks old in late April to early May. The tobacco hydroponic transplant greenhouses can also produce stevia transplants. Field planting recommendations for stevia have yet to be established in North Carolina but generally vary in a range with row spacings from 30-42" with plants spaced 6-7 inches and populations from 22,000-44,000 plants per acre (Koehler, 2018; Carroll, 2019). The first-year harvest occurs in September using a hay combine to cut and windrow plants for initial drying in the field. Alternatively, stevia can be field cut and blown or conveyed into bin wagons or trucks for transport. Plants are dried in tobacco bulk barns or steel mesh-walled bin wagons similar to those used for peanuts or cotton harvest at 130ºF for 2-3 days." The dried leaves are baled and shipped to a processor for sweetener extraction. Established plants can be harvested twice, once in June or July and a second time in September (Koehler, 2018). If plants can overwinter in eastern North Carolina, seasonal production can occur for three to five consecutive years without reestablishment.

The first stevia crop in North Carolina was planted in 2011 in Bertie county. In the next few years, stevia production in North Carolina expended to 400 acres across ten North Carolina counties including Bertie, Halifax, Nash, Johnston, Chatham, Hartnett, Hoke, Robeson, Moore, and Randolph (Hart, 2014). Research at North Carolina State University has focused on investigating diseases associated with stevia and herbicides for weed management. White mold (*Sclerotinia sclerotiorum* (Lib.) DeBary) was found on "second year plants in multiple counties in 2013" (Koehler & Shew, 2014; Koehler & Shew, 2017a). Charcoal rot (*Macrophomina phaseolina* (Tassi) Goid.) was found on stevia plants in Lenoir and Edgecombe counties in 2016 (Koehler & Shew, 2018b). Pythium root rot (*Pythium spp.*) was observed in commercial float systems in Harnett County in 2015 and 2016 (Koehler & Shew, 2017c). In 2015, in greenhouse research at North Carolina

State University, stevia plants began to display “virus-like symptoms,” which testing revealed to be Tomato spotted wilt virus (genus Tospovirus; family Bunvaviridae) (TSWV) possibly transmitted by thrips (*Frankliniella occidentalis* (pergande)) (Koehler, Brown, Huber, Wehner, & Shew, 2016). Leaf spot (*Septoria steviae*) is also a concern for stevia producers and was first observed in a greenhouse production in 2015 (Koehler & Shew, 2018a).

Currently, North Carolina is the northern most geographical region of the USA where stevia roots and crowns have over-wintered and grown back in spring (Koehler & Shew, 2019). Overwintering is further complicated by stem rot (*Sclerotinia rolfsii* (Sacc. infection)) (Koehler & Shew, 2017b). Also known as southern blight, *S*. *rolfsii* is also an important fungal disease affecting peanuts, tomatoes, peppers, grasses, and many flower genera (Mullen, 2001). Research on prevention of stem rot in stevia at two eastern North Carolina locations, Rocky Mount and Kinston, identified azoxystrobin and tebuconazole as effective fungicides (Koehler & Shew, 2017b). These fungicides also control *S*. *rolfsii* in peanuts (Koehler & Shew, 2017b). Overwintering percentages during these stevia trials ranged from 16.8-88.1% and 58.4-94.2% for the Kinston and Rocky Mount locations, respectively (Koehler & Shew, 2017b).

The purpose of this study was to investigate if the four selected stevia varieties would overwinter and regrow the following spring in the two northern most plots in a series of growth and performance trials conducted by S&W Seed Company in grower fields. These trials were located in eastern North Carolina while other plots in a coordinated regional study of 16 varieties developed by S&W Seed Company were located South Carolina, Georgia, and Alabama. Height of the plants was recorded in April and May. The leaves per stem counts were also analyzed for each plant in May. Stevia is a promising alternative profitable rotational crop for tobacco farmers in the mid-Atlantic region. This study will help assess the potential of stevia in the southeastern United States.

Methods

Plot Locations and Design

Both trials were located in eastern North Carolina's inner coastal plain along the fall line that separates the coastal plain from the piedmont region. The coastal plain of North Carolina is characterized by "sandy soil" that is "the state's best farmland" (Anonymous, 2012). A wide variety of crops are grown in the coastal plain including tobacco, soybeans, wheat, sweet potatoes, corn, cotton, peanuts, watermelon, cucumbers, and blueberries (USDA, 2019). Nashville, North Carolina is located in Nash County and Roseboro is located in Sampson County. It should be noted that although the town of Roseboro is located in Sampson County, the Roseboro experiment was actually in eastern Cumberland County. A map of plot locations is located in the Appendix (Figure 1). The experiment design for both plots was a randomized complete block with four replications containing a total of sixteen (16) varieties developed by S&W Seed Company of Longmont, Colorado to be evaluated by a regional stevia breeding and development representative. This study was completed within those plots by recording data from only four varieties. Four plants of the four chosen varieties were tagged within all four replications at each location. This was 64 plants at both locations for the sample size. The stevia plants were propagated in float trays in a greenhouse in Tifton, Georgia, shipped by truck, and then established in plots by transplanting in May 2019.

The Nashville plot was located at 35.9275 latitude, -78.0112 longitude. The entire plot measured 480 feet (146.3 meters) by 125 feet (38 meters). Stevia transplants were planted in two beds per row with beds spaced 16 inches (40.6 cm) apart and rows spaced 42 inches (106.7 cm) apart. Plants were 12 inches (30.5 cm) within the rows. The rows were 75 feet (22.9 m) long and there were 60-inch alleys (152.4 cm) between replications. The soil type of this plot contained equal proportions of NrB (Norfolk, Georgeville, and Faceville soils) with 2-8% slopes and Ra (Rains sandy loam) with 0-2% slopes (NCDA-NRCS, 2020). A picture of this plot layout is labeled as Figure 2 in the Appendix.

The Roseboro plot was located at 34.8763 latitude, -78.5724 longitude. The entire plot measured 225 feet (68.5 meters) by 400 feet (122 meters). Stevia transplants were planted in four beds per row with beds spaced 32 inches (81.3 cm) apart and rows spaced 32 inches (81.3 cm) apart. Plant spacing was 12 inches (30.5 cm) apart within the rows. The rows were 52.5 feet (16 m) long and there were 60-inch alleys (152.4 cm) between replications. The soil type of this plot is Ra (Rains sandy loam) with 0-2% slopes (NCDA-NRCS, 2020). A picture of this plot layout is labeled as Figure 3 in the Appendix.

Plant Selection and Identification

The four varieties chosen for this study were chosen by the S&W Seed Company regional stevia breeding and development representative to be more favorable performing varieties thus far in the S&W trials. The sample population of each variety was made up of 4 plants from each of the 4 replications for a total of 16 sample plants at each location. This was 64 plants at each location. Identification codes were assigned to individual sample plants using the first two digits and last four digits of the variety identification code from S&W Seed Company followed by a letter and numerical identification number for each individual plant pertinent to this project (e.g. “SW-1005 A1”). Loop plant tags were made up for all 128 plants (64 at each location). The tags were marked with permanent ink and wrapped in packing tape to protect them from weather and irrigation, so the ink would not wash off or fade. Plants were tagged in October at both locations. The plants were randomly selected and evenly distributed within each replication, bed, and row.

Data Collection

This study took place over the course of eight months from October 2019 to May 2020. Monthly observations of plant height, flowering, lodging, diseases, and insects were recorded in a notebook on a prepopulated paper chart in the field. All data from the field logs was transferred into Microsoft Excel to analyze by ANOVA. Data was not collected in February and March at either plot because plants were dormant and because of unforeseen events and scheduling conflicts. Plant stems were collected from Roseboro on May 22 and from Nashville on May 23 to calculate leaves per stem count. Ten stems were planned to be collected from each plant but this was not possible on some plants because they had fewer than ten stems. Paper bags were used for each individual plant that had regrown in May. Each bag was labeled with each of the individual plant identification codes with permanent ink. The paper bags were then punched six times (three on each side) with a handheld hole punch while the bag was folded as it was from the package to produce a total of 24 holes. The cut stems were placed into paper bags in the field as they were removed from the plant. The bags were folded over once and stapled shut so no samples were lost. The bags were placed into laundry hamper to contain them for transport. Once the samples had been brought back to my home, each bag was unfolded to prevent samples from molding until counts of leaves and stems could be recorded 24-72 hours after harvest. Leaves on each stem were counted and recorded on a paper chart. Counts were then transferred to Excel for data analysis.

Statistical Analysis

Because of unequal population sizes among varieties at both locations, standard errors rather than ANOVA and LSD were used for mean separation.

Results and Discussion

The plants were mowed back by the S&W Seed Company regional stevia breeding and development representative in September at the Roseboro location and in October at Nashville, so no growth data could be collected for fall 2019 as planned. The plants displayed some growth on old stems in October and November but this died back in December. The growth on the old stems also flowered at a rate of 10% judged visually. It is unclear from this study if plants were physiologically dormant over the winter after growth stopped in December or quiescent because lower temperatures prevented growth. Some studies suggest that dormancy is induced in lateral buds of stevia by certain environmental conditions (Pal, Prasad, & Pathania, 2013). The lengthy period with no significant growth from November to April supports this dormancy hypothesis. Reducing dormancy either through chemical applications or breeding may enable vegetative growth for longer periods increasing biomass production. Very few disease symptoms or insects were observed on the plants in October. Lodging, flowering, diseases, and insects were very minimal and the few observations made were on plants before dormancy was induced and did not affect spring regrowth. Varieties were not statistically compared for differences in the percentage of plants that lodged, flowered, were diseased, or were attacked by insects during the fall. We planned to include stevioside profiles for the varieties but that data was not available at the time of this report.

Spring Regrowth

Spring regrowth from the crowns of plants began in April at the Roseboro location and May in Nashville. At both locations, not every plant regrew in spring but other factors caused plant mortality besides winter kill. Some plants were mechanically damaged by farm equipment and others were out competed by weeds. There was no visible indication of possible over-winter plant loss caused by disease. Regrowth showed no disease symptoms in April or early May at either location when heights were measured. However, when stems were collected on May 22nd in Roseboro and May 23rd in Nashville, visual symptoms of stem rot and leaf spot began to show. The Nashville location had great weed interference and some plants were damaged by farm machinery. The Roseboro location had one plant disrupted by farm machinery. Part of this location was also plowed under after heights were recorded in early May because of poor stand establishment. This led to two replications of one variety not available for the leaves per stem count. These differences in the locations and plant numbers led to data being analyzed separately.

Weather data was gathered from the National Weather Service (NWS) to compare the lowest recorded temperature of the 2019-2020 winter to the 30-year historical low (1981-2010) recorded for both locations. The weather data for the locations was determined using the closest major city NWS listed which was Raleigh/Durham (RDU) for Nashville and Fayetteville (FAY) for Roseboro. The NWS recorded the lowest temperature for Raleigh/Durham at 24ºF (-4.4ºC) in January 2020 and the historical low is 30.6ºF (-0.8ºC) in January. The National Weather Service recorded the lowest temperature for Fayetteville at 33ºF (0.6ºC) in January and the historical low is 32.8ºF (0.4ºC) (NOAA, 2020). Thus, the winter of 2020 was typical in terms of temperatures for both locations. Additional testing is needed to determine how well stevia would survive when winter temperatures are colder than average.

The percentage of overwintering plants suggests that stevia is adapted to these areas of North Carolina, based on a single, typical-year observation. Spring regrowth is directly impacted by weed pressure in the field. The highest percentage of regrowth at Nashville was 62.5% among varieties SW-2267 and SW-2427. The highest percentage of regrowth in Roseboro was nearly 94% among SW-2267 and SW-2647. Variety SW-2267 showed the best regrowth at both locations. The significant difference in regrowth of SW-2647 at Nashville and Roseboro suggest this variety is especially impacted by weed interference. The lack of change in regrowth by SW-2427 between locations suggests weed interference was not the major factor in lack of spring regrowth. ANOVA was not performed on these data because of unequal population sizes. The percentage of plants that survived the winter were calculated for each variety at both locations by dividing those that began to regrow by the total number in the trial which was 16 except when farm equipment damage occurred or there was weed interference. The number of plants available for calculation, regrowth percentages and standard error values are summarized in Table 1 below.

Table 1: Percentage of Survival in May of Varieties at Both Locations for Spring 2020

	Nashville			Roseboro		
	S / T	%	Std. Error	S / T	%	Std. Error
SW-1005	7 / 15	46.7%	±0.54	10 / 15	62.5%	±0.73
SW-2267	10 / 15	66.7%	±0.29	15 / 16	93.8%	±0.34
SW-2427	10 / 16	62.5%	±0.63	10 / 16	62.5%	±1.04
SW-2647	7 / 16	43.8%	±0.40	15 / 16	93.8%	±0.95

S: Number of plants that survived

T: Number of plants not impacted by farm equipment

The greatest average plant height at Nashville was measured for SW-2427 at 7.3 inches (18.5 cm). The remaining varieties fell well below this at just over 4 inches (10.2 cm) to just over 4.5 inches (11.4 cm) each. The greatest average in plant height in Roseboro was measured for SW-2427 at 13 inches (33 cm). The remaining varieties fell just below this at 10.5 inches (26.7 cm) to 11.5 inches (29.2 cm) each. The average heights for each variety at Nashville and Roseboro are summarized in Table 2 below.

Table 2: Average Height of Varieties in May 2020 at Nashville and Roseboro

	Nashville		Roseboro	
	Height (in.)	Std. Error	Height (in.)	Std. Error
SW-1005	4.64	±0.54	10.50	±0.72
SW-2267	4.50	±0.29	11.47	±0.40
SW-2427	7.30	±0.63	13.00	±1.04
SW-2647	4.21	±0.40	11.13	±0.95

Leaves per Stem

Stems were collected from each plant that regrew in the spring to calculate the leaves per stem. The goal was to collect ten stems from each of these plants, but some plants had fewer than ten stems. Some of the plants at the Nashville location were not available because of damage caused by farm machinery and major weed interference. Part of the Roseboro location was plowed away at the direction of S&W Seed Company because of poor stand establishment. Four plants across two replications of variety SW-1005 were in this area and not available for stem collection. The percentage of plants available at each trial location for stem collection is shown in Table 3 below.

Table 3: Percentage of Plants Available for Stem Collection in Nashville and Roseboro

	Nashville			Roseboro		
	L / T	%	Std. Error	L / T	%	Std. Error
SW-1005	6 / 15	37.5%	±4.66	5 / 11	45.5%	±7.18
SW-2267	10 / 15	62.5%	±2.22	15 / 16	93.8%	±3.76
SW-2427	9 / 16	56.3%	±4.10	10 / 16	62.5%	±5.07
SW-2647	6 / 16	37.5%	±9.16	15 / 16	93.8%	±3.29

L: Number of plants that survived

T: Number of plants not impacted by farm equipment

The greatest leaf per stem count at Nashville was SW-2647 which averaged 52.2 leaves per stem even though only 6 plants were available for stem collection. The second highest leaf per stem count was SW-2427 which averaged 41.4 leaves per stem among 9 plants that regrew. Variety SW-1005 averaged 36 leaves per stem with only 6 plants available. There were 10 plants available for variety SW-2267 but this variety averaged the least leaves per stem at 26 leaves per stem. The greatest leaves per stem count at Roseboro was SW-2427 which averaged 58.9 leaves per stem with 10 plants available. The second highest leaves per stem count was SW-1005 at 49.6 leaves per stem even though only 5 plants were available. Variety SW-2267 followed close behind with 49.3 leaves per stem with 15 plants available. Variety SW-2647 wasn't far behind with 41.8 leaves per stem with 15 plants available. The leaf to stem counts and standard error values are summarized in Table 4 below.

Table 4: Leaves per stem count of Varieties at Nashville and Roseboro

	Nashville		Roseboro	
	Leaf / stem	Std. Error	Leaf / stem	Std. Error
SW-1005	36.00	±4.66	49.56	±7.18
SW-2267	26.04	±2.22	49.32	±3.76
SW-2427	41.43	±4.10	58.92	±5.07
SW-2647	52.24	±9.16	41.78	±3.29

Conclusions

The steviol glycosides found within the leaves of *Stevia rebaudiana* produce a range of sweetness profiles that are 100-400 times sweeter than that of sucrose. Stevia has been used in other countries as a food additive and for medicinal remedies for the last century. After delayed legal acceptance as a food sweetener in the United States, stevia is being promoted as an alternative sweetener to aid in combating the obesity issue and other obesity associated health issues. Stevia shows promising potential to over-winter in the locations of this study. There is still ongoing research with pesticides to control weeds and mitigate fungal diseases that affect spring regrowth.

The results of this study based on single year study confirms stevia will grow in eastern North Carolina as a perennial crop. Unfortunately, the field was harvested in fall before data could be collected. Therefore, data for a second harvest date was not possible. Weed interference at Nashville seriously impacted regrowth in the spring. This impact on regrowth along with unequal population sizes complicated data analysis. We were unable to decide on the variety best suited for that area. In Roseboro, SW-2427 performed best with greatest average height and the highest leaves per stem count even though just over half the plants were available to calculate this data. We were unable to acquire lab results on the steviol glycoside profiles of each variety to make conclusions on the most desirable variety for the alternative sweetener market.

The fact that stevia will overwinter in eastern North Carolina with limited mortality suggest that it may also grow further north in coastal Virginia. Perennial stevia may be a suitable alternative crop for tobacco growers in Southside Virginia and a potential crop to grow in rotation with peanut or soybean in southeast Virginia. However, significant regrowth was not observed until late March or early April in our studies in North Carolina. Combined with the onset of dormancy in November, stevia plants remained dormant with little vegetative growth for almost 5 months out of the year. Therefore, annual stevia production may be the most beneficial practice that could expand the range of production and should be compared with perennial production. What is clear from this study is that stevia is very slow growing and doesn't perform well in cool or cold conditions. To improve seedling growth and establishment, various seed enhancer treatments should be investigated to speed germination and early seedling growth. Fertilization practices may also stimulate growth after establishment and spring regrowth in perennial production. No serious disease or insect pests were observed, however, in one location weeds were a serious problem that negatively impacted stevia growth and development. Growing stevia on plastic mulch would reduce weed competition and warm soils to improve early season growth.

Despite the problems in this study, stevia shows promise as a high value alternative crop for the mid-Atlantic. This study should be replicated in more locations, with multiple harvests. Yield should be calculated as pounds per acre yield and a laboratory analysis of the steviol glycoside profiles for each line or cultivar yield can be expressed as pounds of steviol glycoside per unit production are to adequately access productivity.

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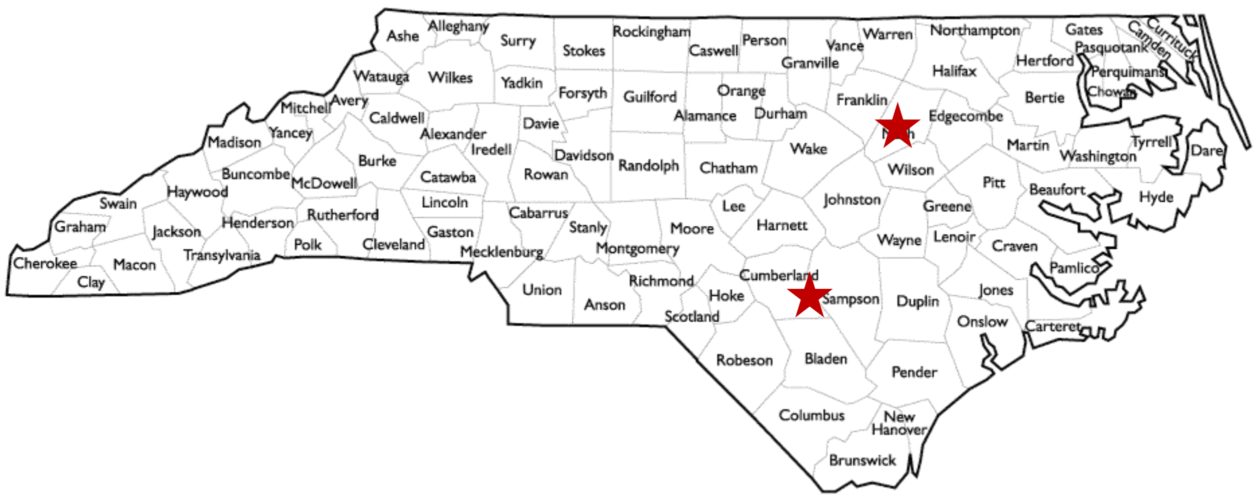
Appendices

Figure 1: Approximate locations of test plots in eastern North Carolina.



(https://www.worldatlas.com/webimage/countrys/namerica/usstates/counties/nccountymap.htm)

Nashville (Nash County):	(35.9276, -78.0111)
Roseboro (Cumberland County):	(34.8763, -78.5724)

	10										
Rep 4	9		**1**	**7**	**3**	**4**	**16**	**6**	**11**	**13**	
	8		**9**	**15**	**14**	**5**	**12**	**10**	**2**	**8**	
Rep 3	7		**2**	**9**	**5**	**14**	**8**	**16**	**12**	**10**	
	6		**15**	**7**	**3**	**13**	**1**	**6**	**4**	**11**	
Rep 2	5		**5**	**11**	**10**	**2**	**6**	**1**	**16**	**4**	
	4		**12**	**13**	**9**	**15**	**8**	**7**	**14**	**3**	
Rep 1	3		**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	
	2		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
16'	1										
Bed		1	2 3	4 5	6 7	8 9	10 11	12 13	14 15	16 17	18
Row		B	1	2	3	4	5	6	7	8	

Figure 2: Research Plot Map at Nashville, NC, for the coordinated regional trial (not to scale). Highlighted cells show plants where data in the current report was collected:

1: SW-1005   7: SW-2267   10: SW-2427   11: SW-2647

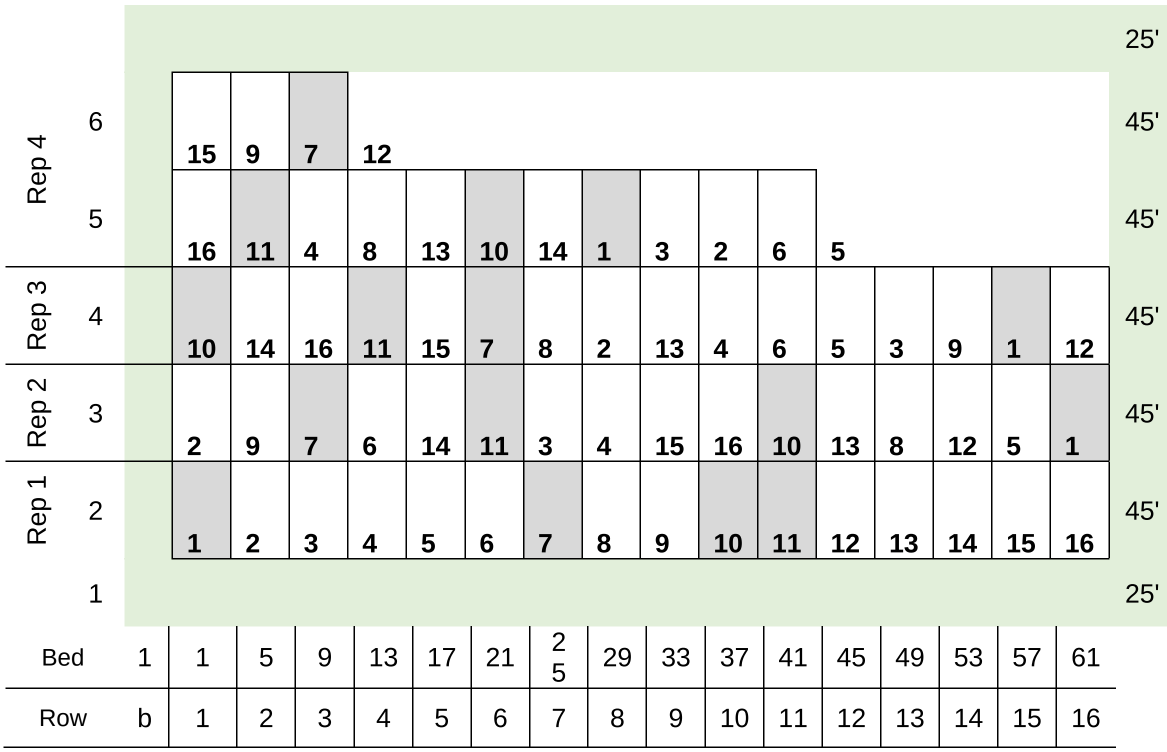


Figure 3: Research Plot Map for Roseboro, NC, for the coordinated regional trial (not to scale) Highlighted cells showing plants where data was collected:

1: SW-1005 7: SW-2267 10: SW-2427 11: SW-2647

Pictures of Plots and Plants

Figure 4: Stevia float trays at Nashville site in May 2019

Figure 5: Stevia being planted at Nashville location May 2019.

Figure 6: Weed interference at the Nashville location in May 2020 that impacted the data

Figure 7: Roseboro plot in March 2020

Figure 8: Roseboro plot April 2020

Figure 9: Roseboro plot May 2020

Figure 10: SW-1005 Replication 1 in Roseboro

Figure 11: SW-1005 Replication 4 in Roseboro

Figure 12: SW-2267 Replication 1 in Roseboro

Figure 13: SW-2267 Replication 2 in Roseboro

Figure 14: SW-2267 Replication 3 in Roseboro

Figure 15: SW-2267 Replication 4 in Roseboro

Figure 16: SW-2427 Replication 1 in Roseboro

Figure 17: SW-2427 Replication 2 in Roseboro

Figure 18: SW-2427 Replication 3 in Roseboro

Figure 19: SW-2427 Replication 4 in Roseboro

Figure 20: SW-2647 Replication 1 in Roseboro

Figure 21: SW-2647 Replication 2 in Roseboro

Figure 22: SW-2647 Replication 3 in Roseboro

Figure 23: SW-2647 Replication 4 in Roseboro

Figure 24: Plant F13 of SW-2427 at Roseboro in December

Figure 25: Plant F13 of SW-2427 at Roseboro in April

Figure 26: F13 of SW-2427 in May in Roseboro

Figure 27: Plant H8 of SW-2647 at Roseboro in December

Figure 28: Plant H8 of SW-2647 at Roseboro in April

Figure 29: H8 of SW-2427 in May in Roseboro